# Electronic Supplementary Information

## Readily functionalizable phosphonium-tagged fluorescent coumarins for enhanced detection of bioconjugates by mass spectrometry

Antoine Lizzul-Jurse, Laetitia Bailly, Marie Hubert-Roux, Carlos Afonso, Pierre-Yves Renard, Cyrille Sabot*

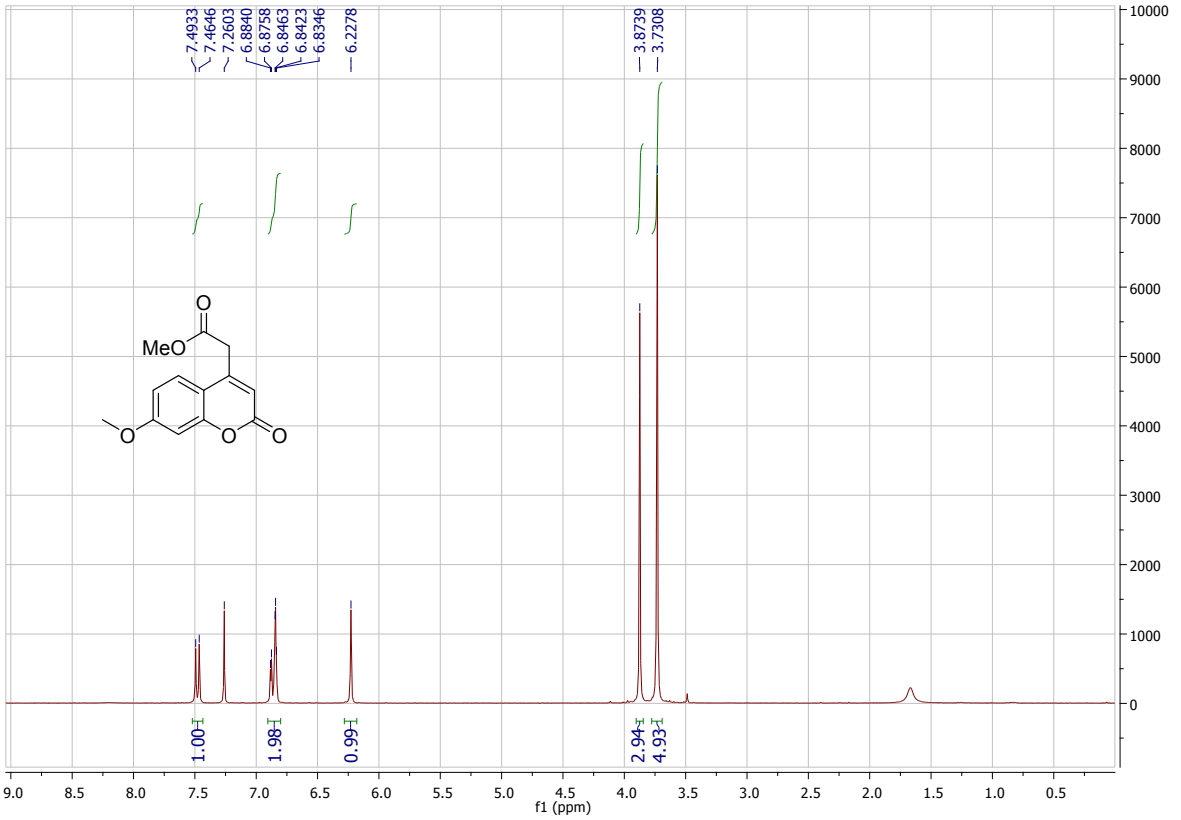
Normandie Univ, COBRA, UMR 6014 & FR 3038; Univ Rouen; INSA Rouen; CNRS, 1 rue Tesnière 76821 Mont-Saint-Aignan, Cedex (France).
E-mail : cyrille.sabot@univ-rouen.fr

## Table of contents

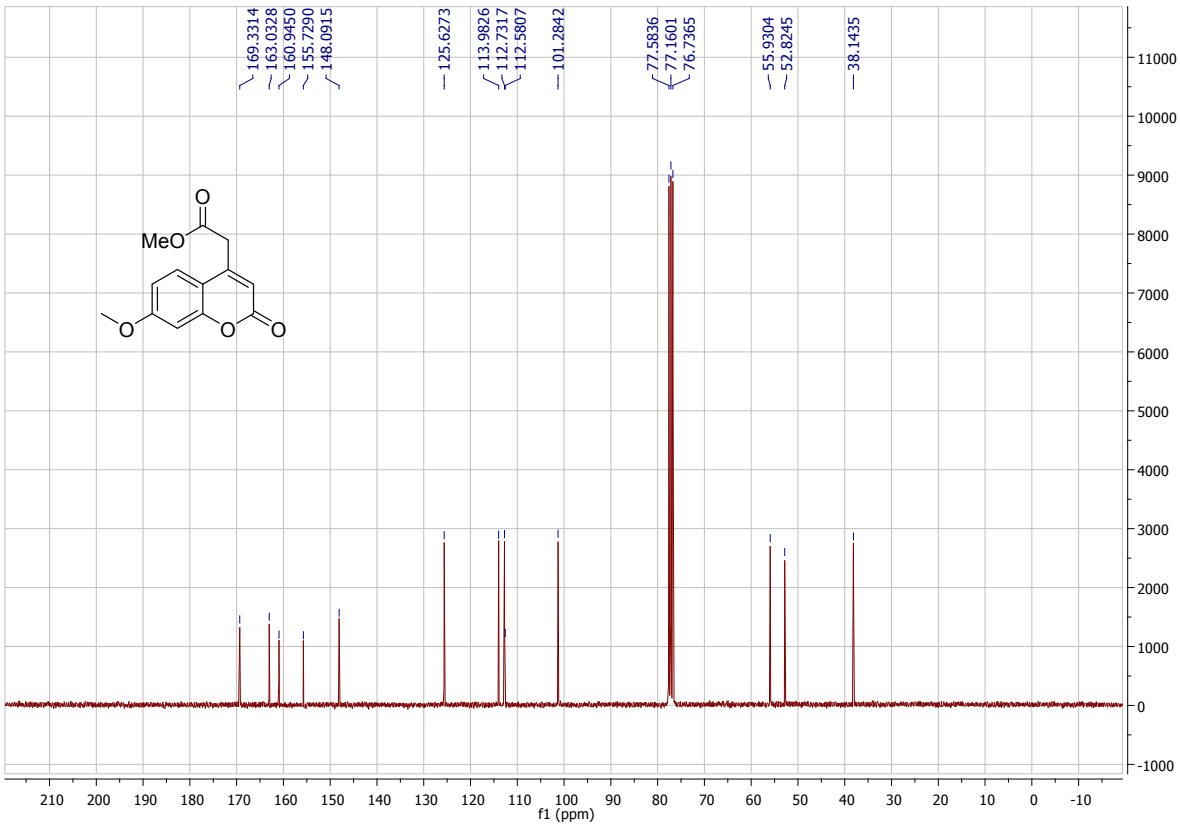
# 1) Copies of $^{1}$H NMR, $^{13}$C NMR spectra

**Methyl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate 1**

$^{1}$H NMR spectrum in $CDCl_3$ (300 MHz)

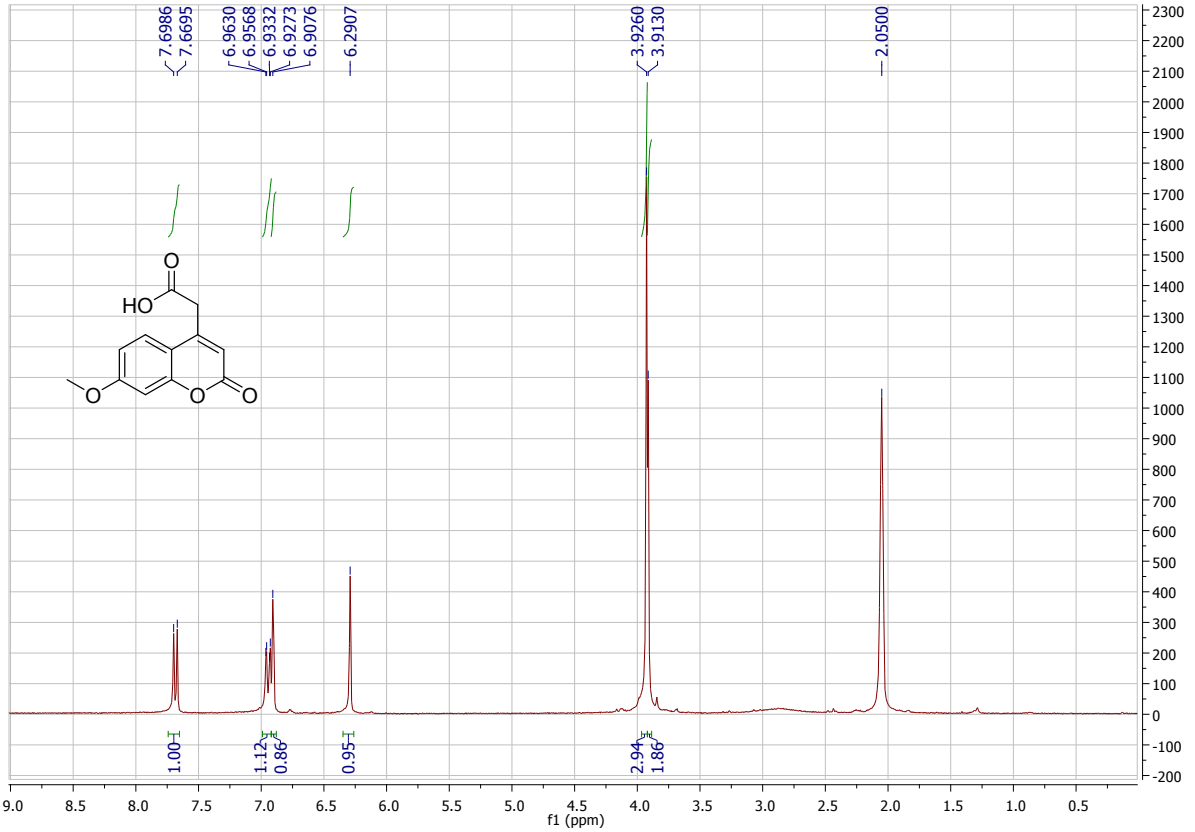



$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)



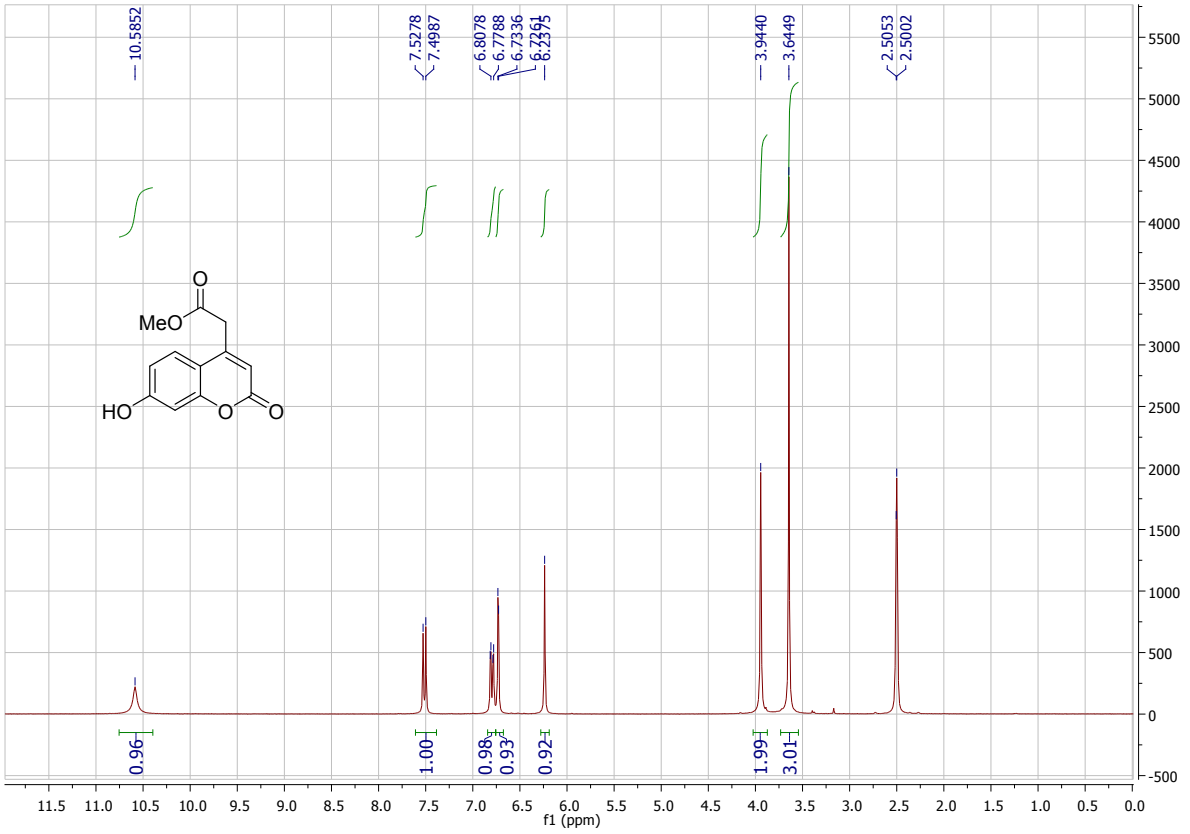
**2-(7-Methoxy-2-oxo-2H-chromen-4-yl)acetic acid 2**

$^1$H NMR spectrum in $(CD_3)_2CO$ (300 MHz)



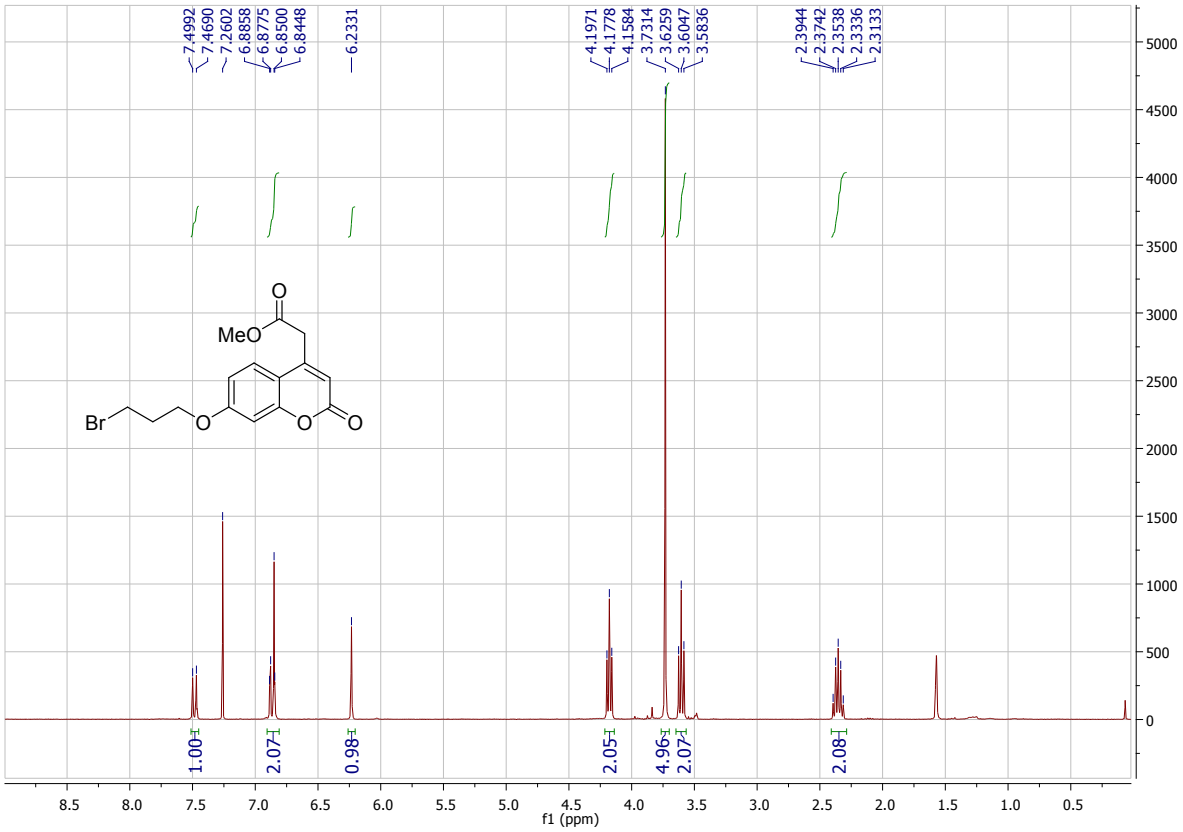
**Methyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate 3**

$^1$H NMR spectrum in $(CD_3)_2SO$ (300 MHz)

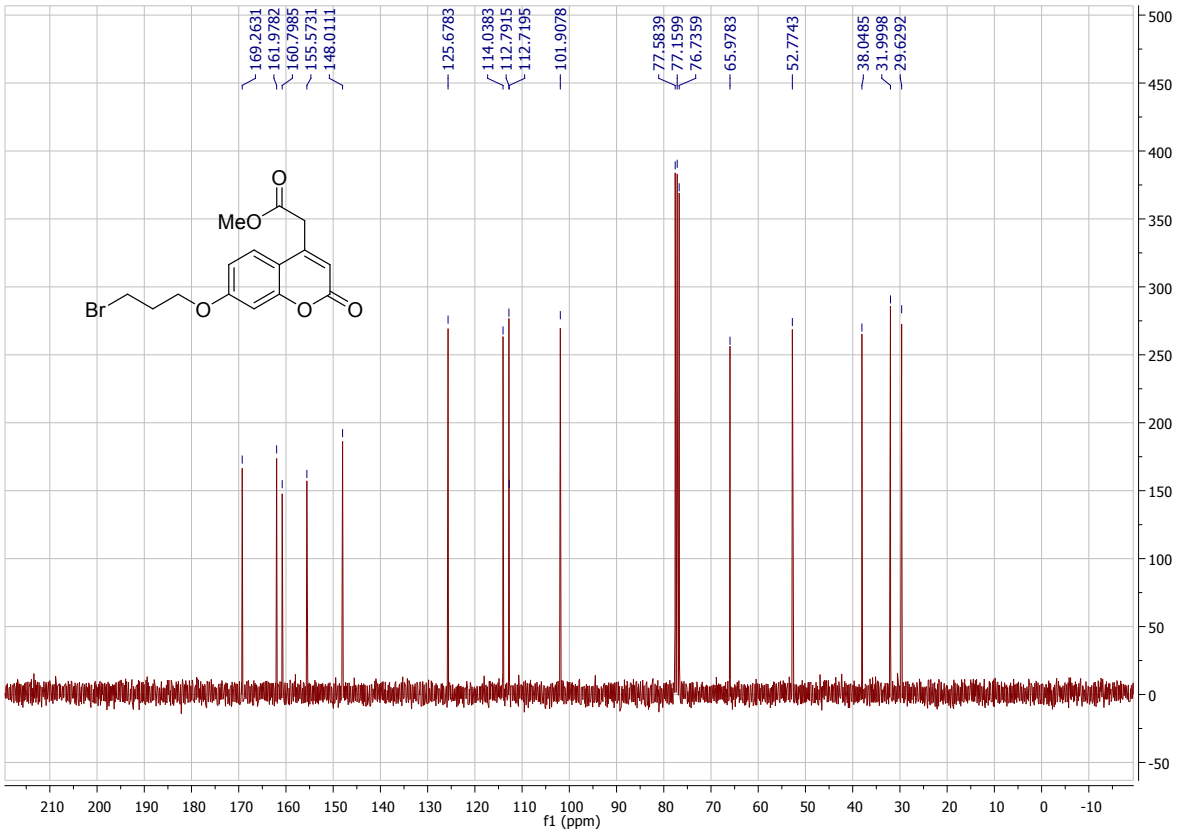


**Methyl 2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)-acetate 4**

$^{1}$H NMR spectrum in $CDCl_3$ (300 MHz)

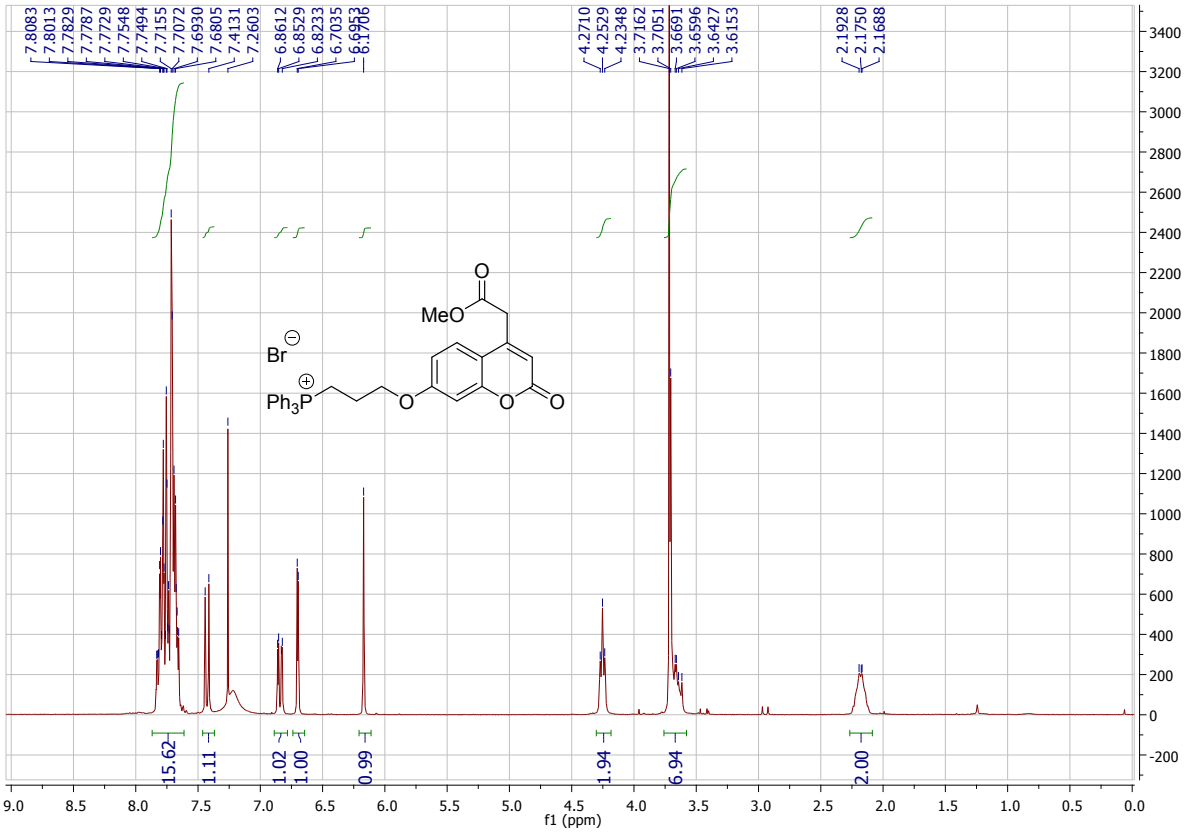



$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)

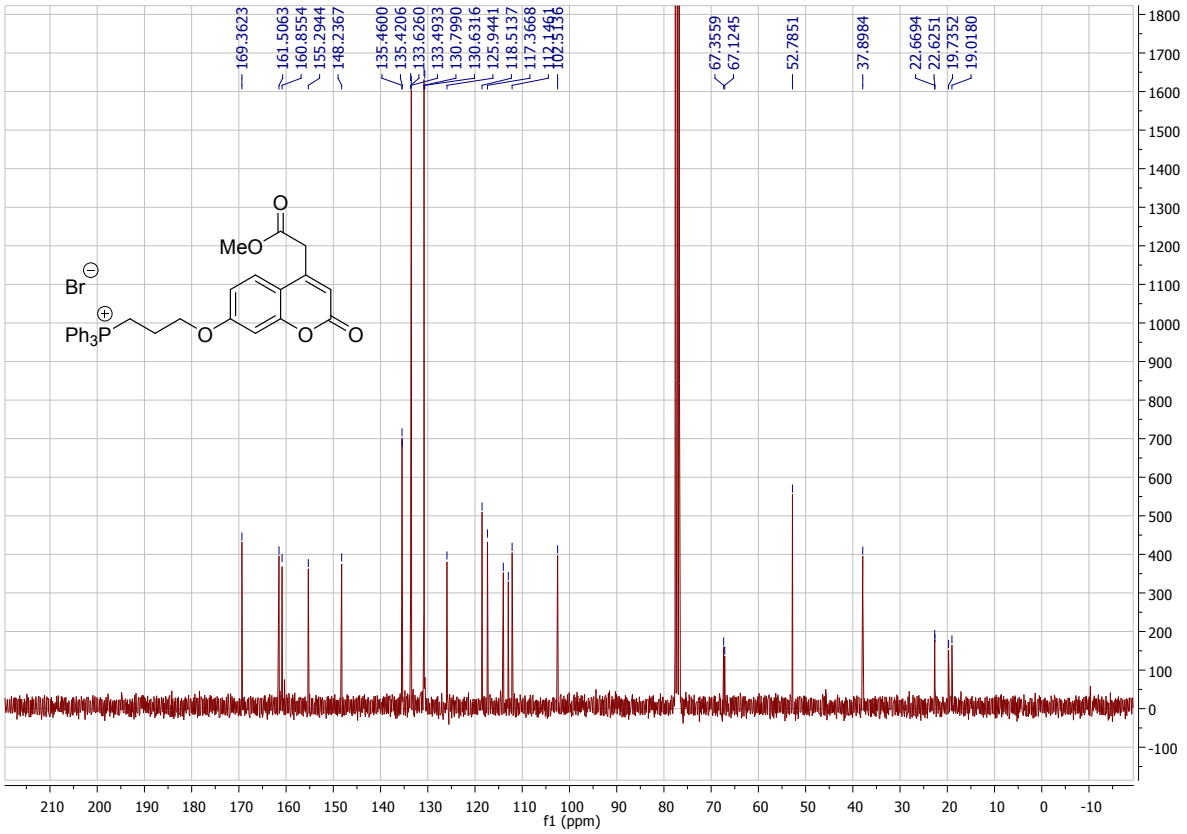


**(3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7yl)oxy)-propyl)triphenylphosphonium bromide 5**

$^{1}$H NMR spectrum in $CDCl_3$ (300 MHz)

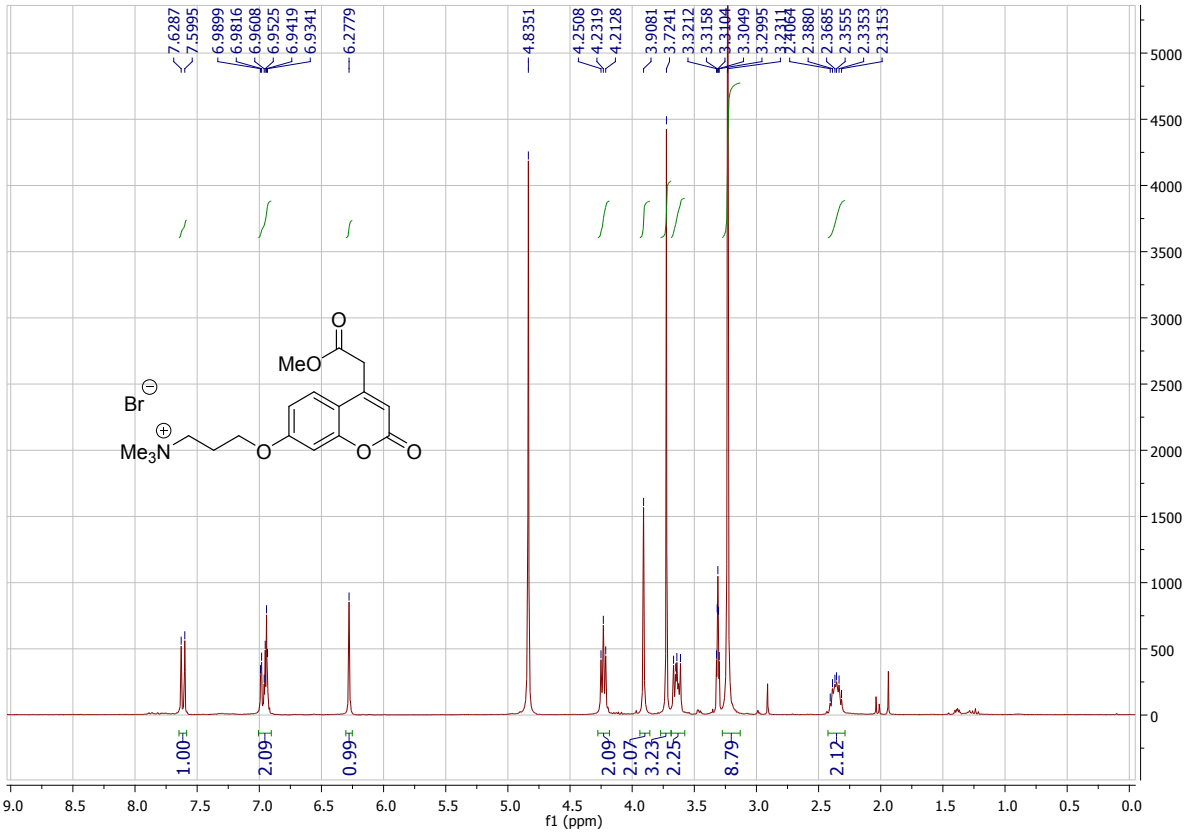



$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)

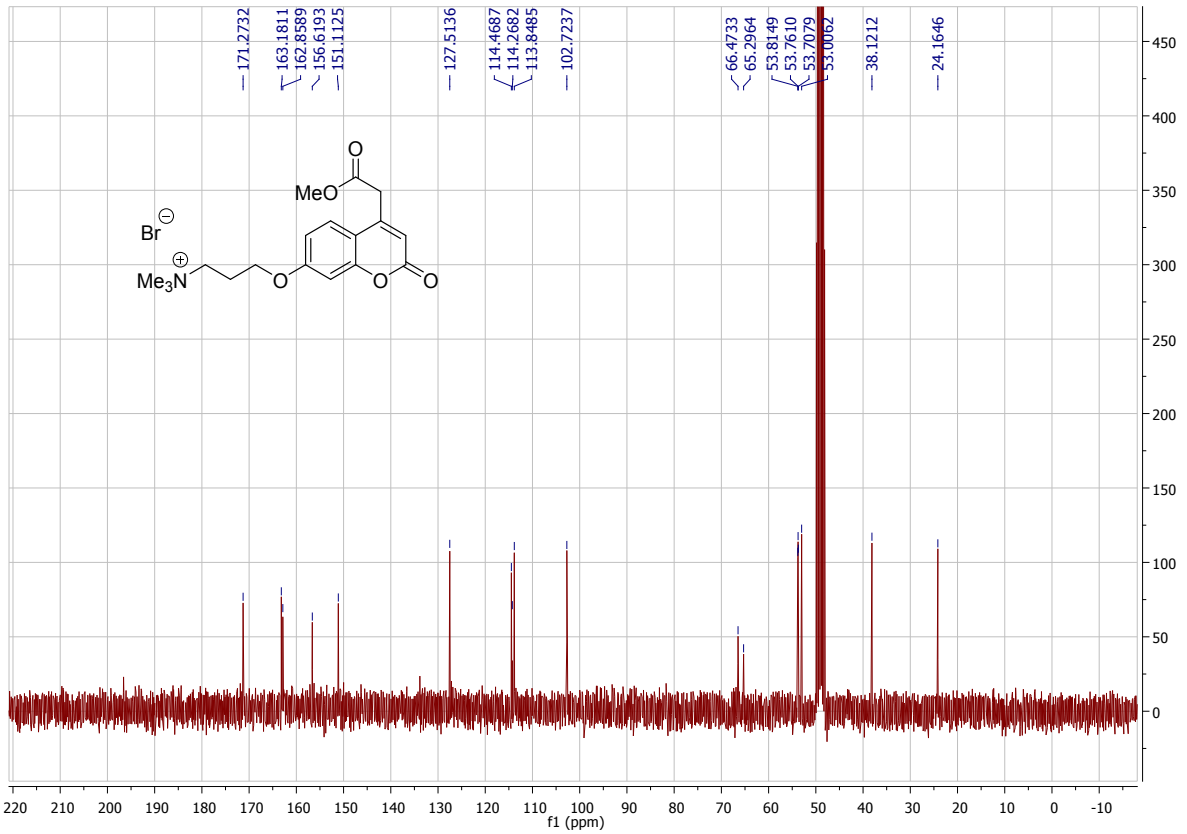


**3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)-N,N,N-trimethylpropan-1-aminium bromide 6**

$^{1}$H NMR spectrum in $CD_3OD$ (300 MHz)

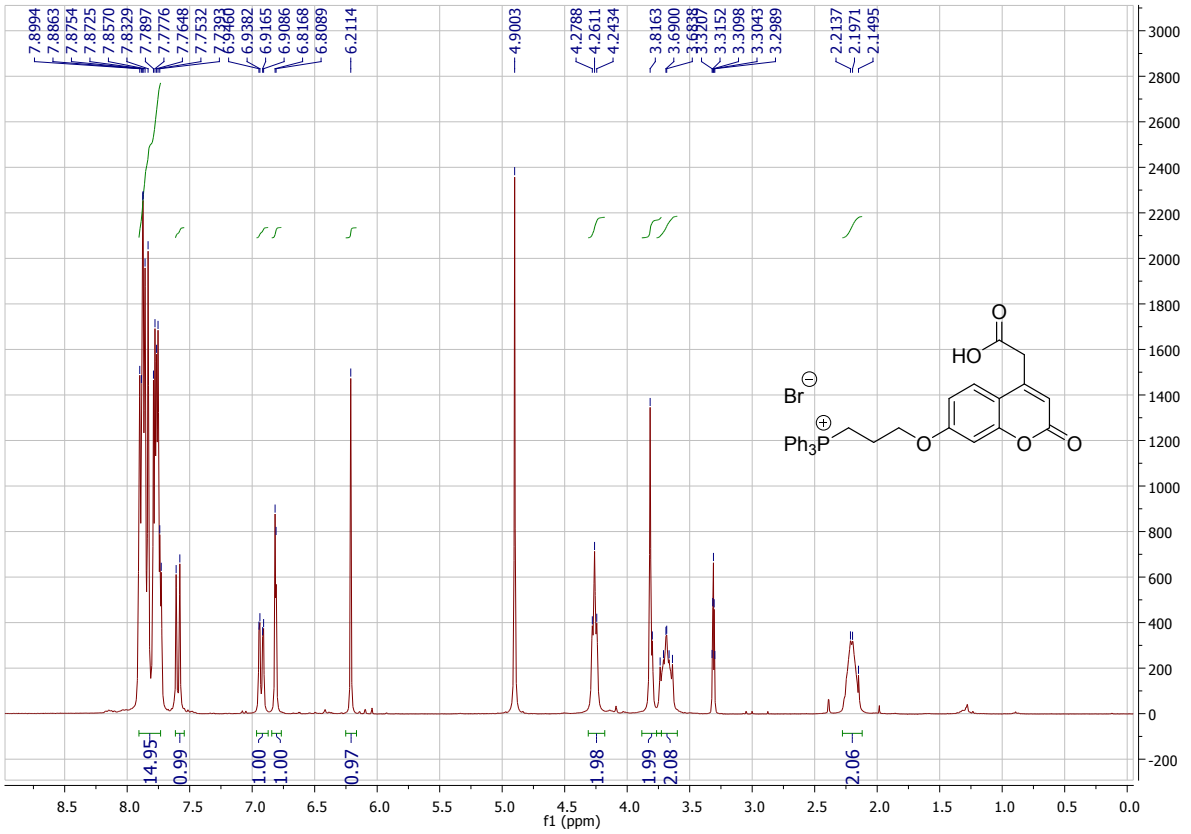



$^{13}$C NMR spectrum in $CD_3OD$ (75 MHz)

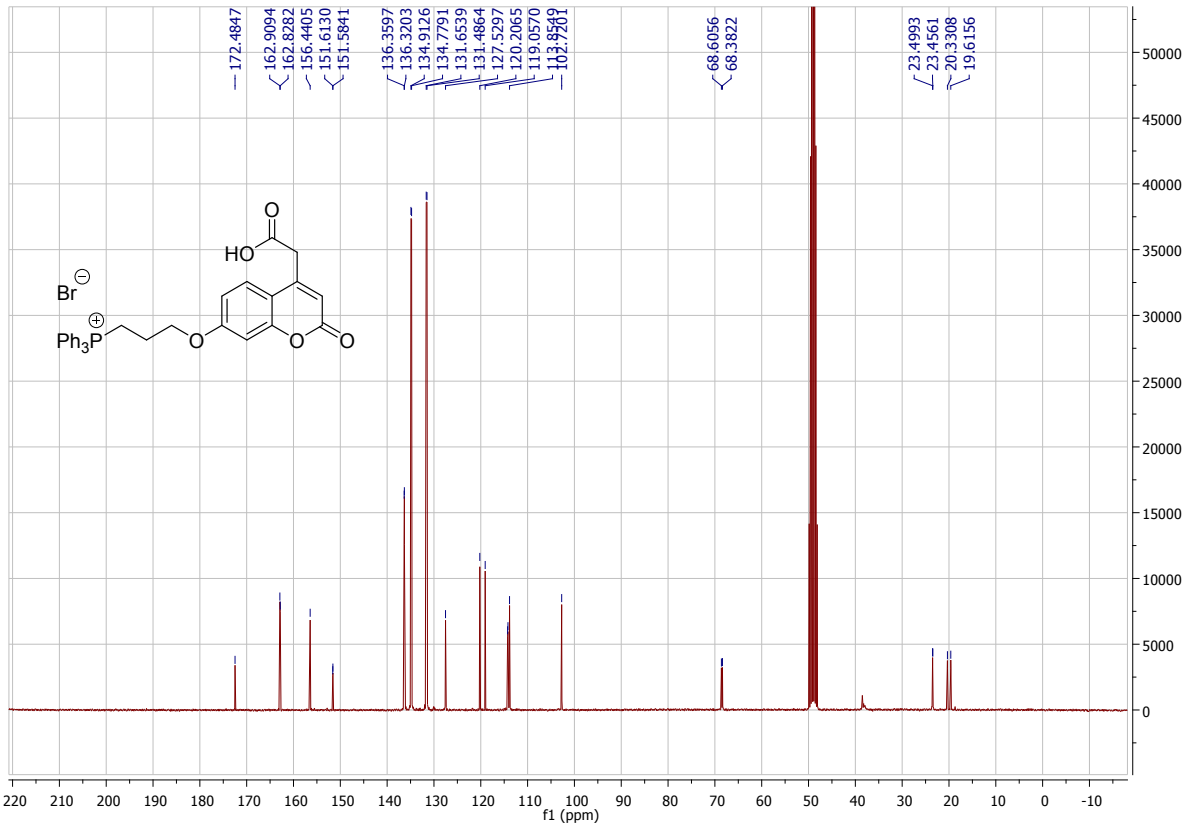


**(3-((4-(carboxymethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-triphenylphosphonium bromide 7**

$^{1}$H NMR spectrum in $CD_3OD$ (300 MHz)

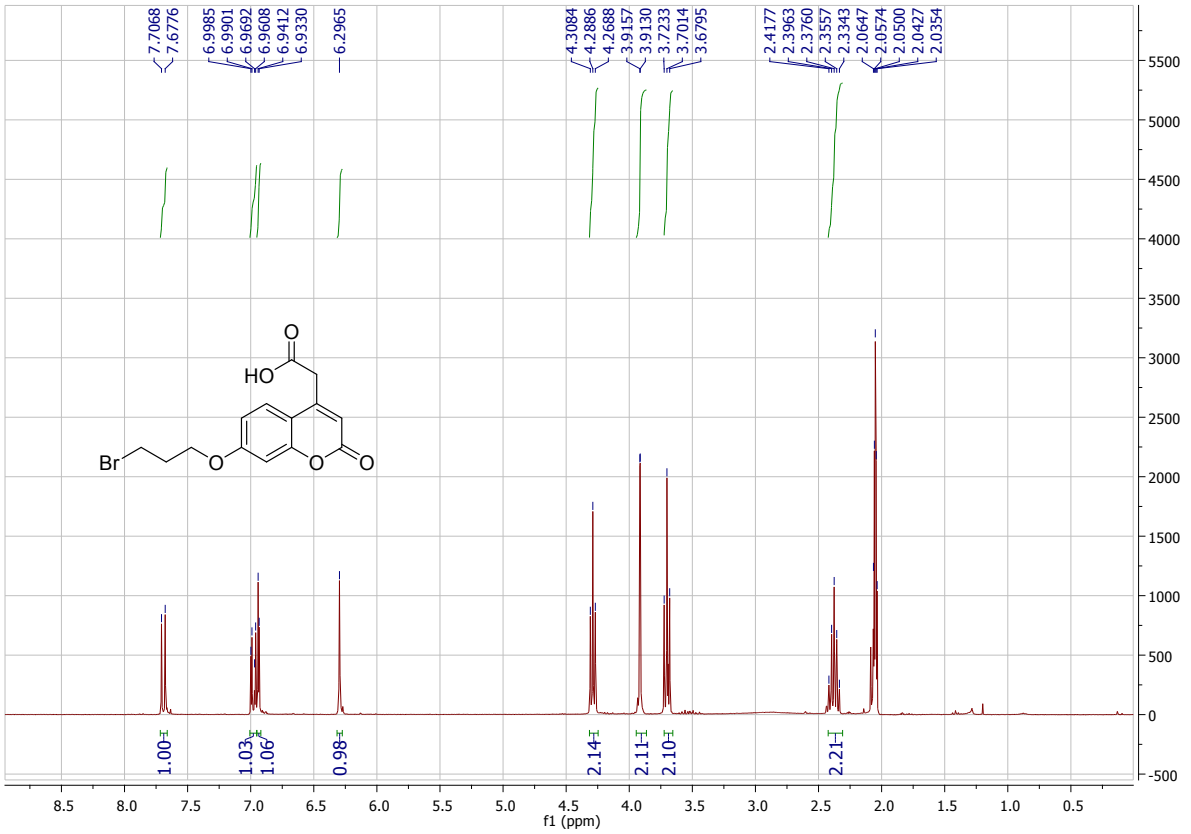



$^{13}$C NMR spectrum in $CD_3OD$ (75 MHz)

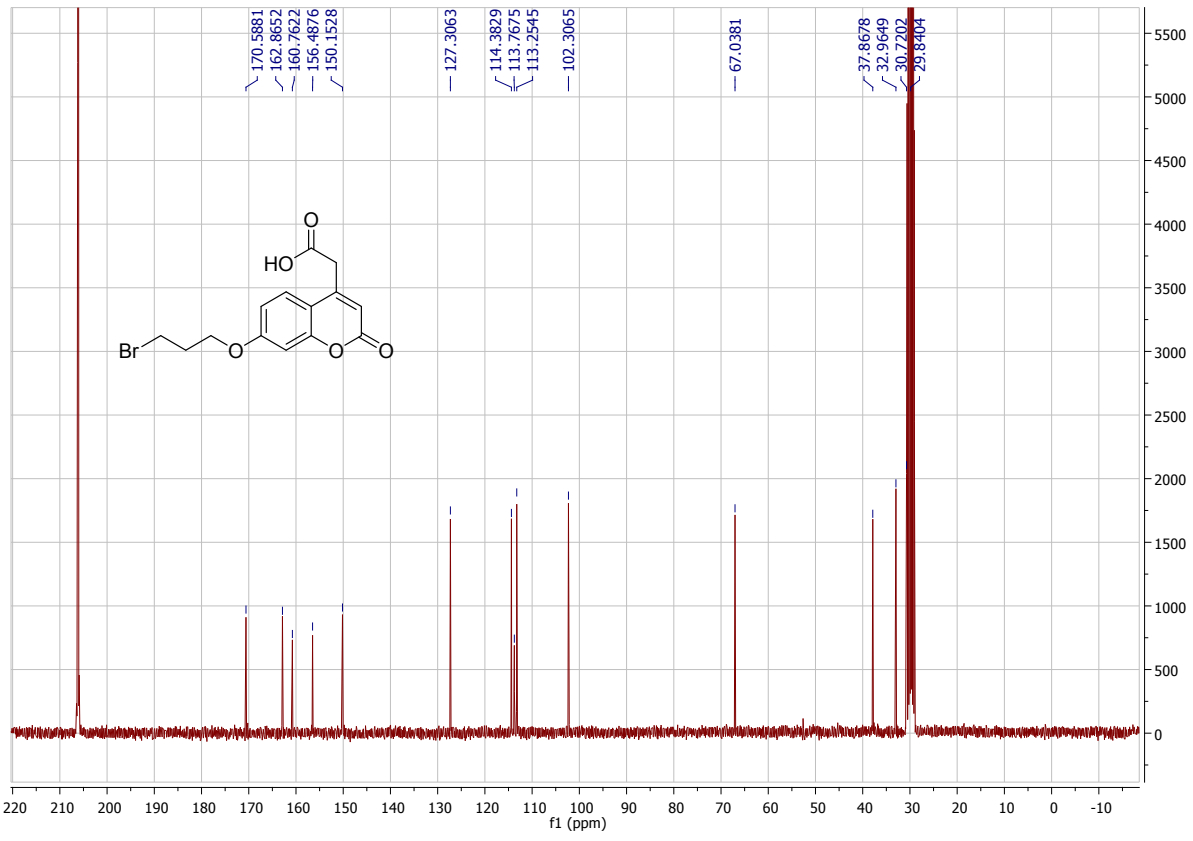


**2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)acetic acid 8**

$^{1}$H NMR spectrum in $(CD_3)_2CO$ (300 MHz)

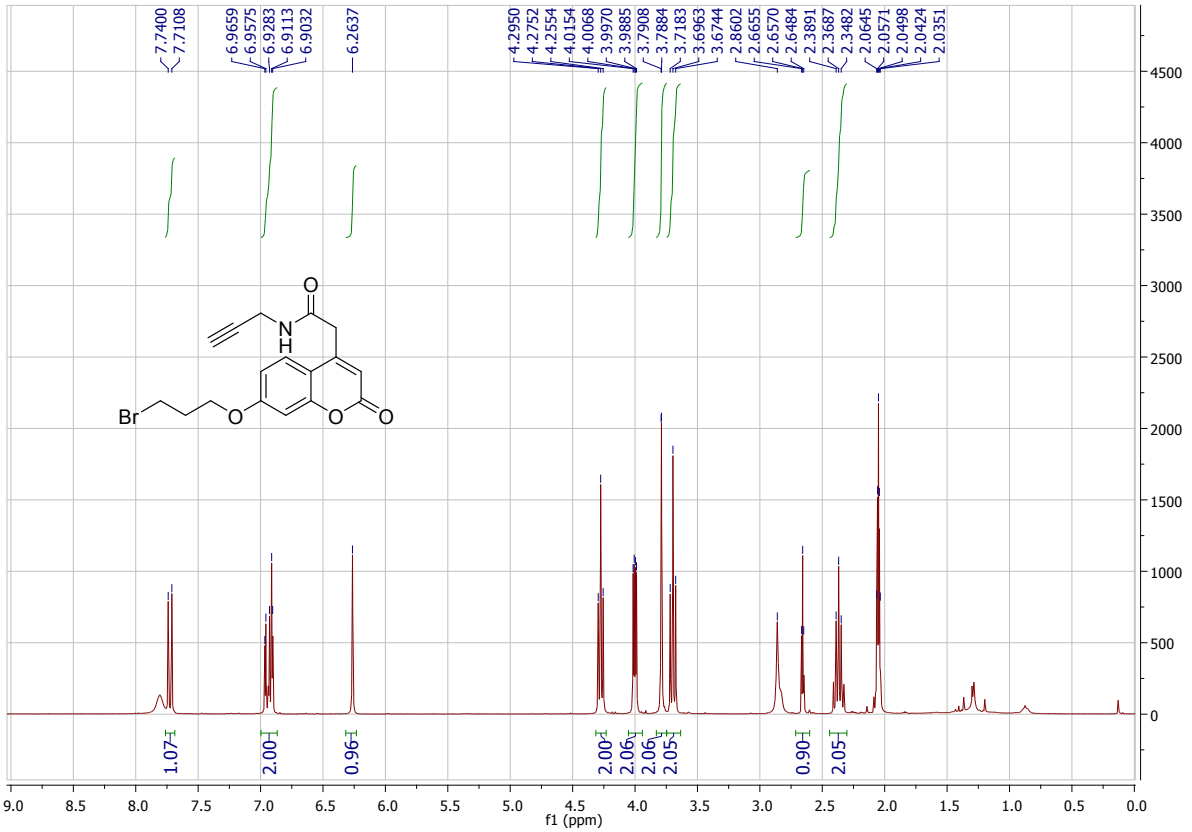



$^{13}$C NMR spectrum in $(CD_3)_2CO$ (75 MHz)

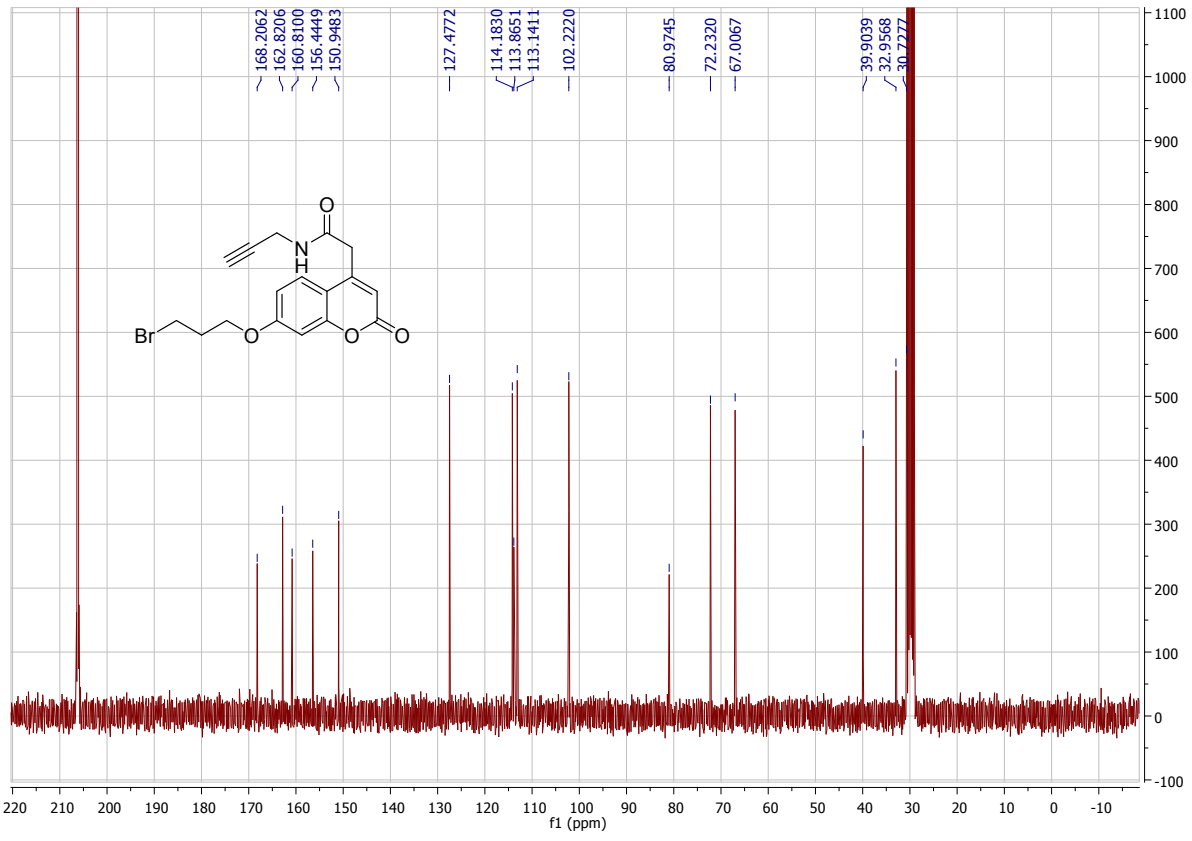


**2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)-N-(prop-2-yn-1-yl)acetamide 9**

$^{1}$H NMR spectrum in $(CD_3)_2CO$) (300 MHz)

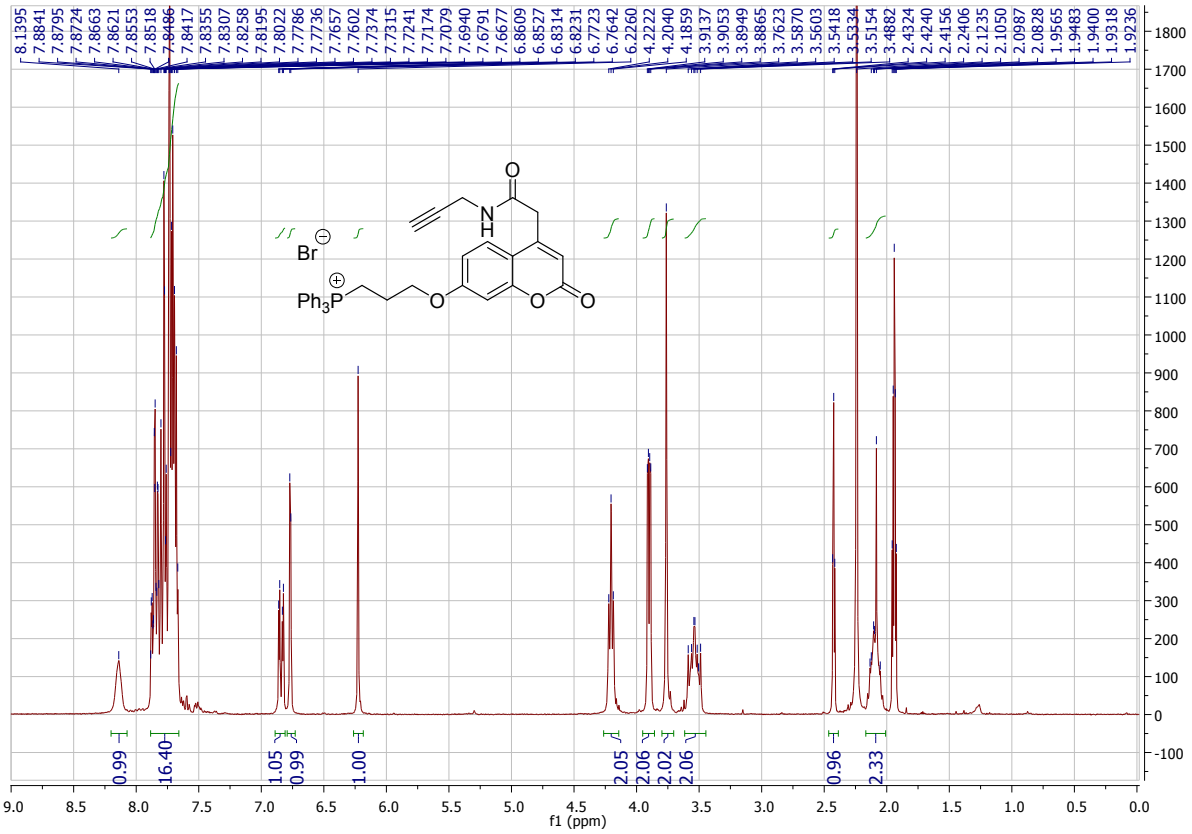



$^{13}$C NMR spectrum in $(CD_3)_2CO$) (75 MHz)

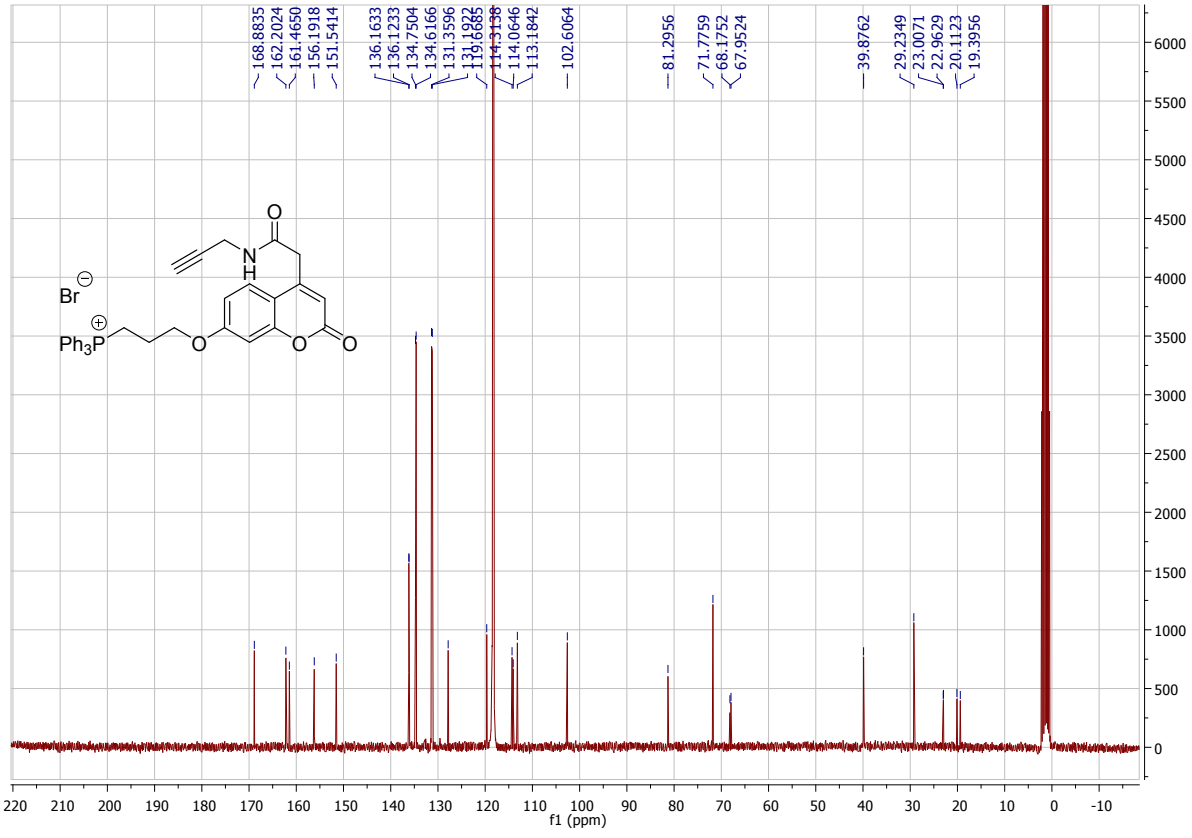


**(3-((2-oxo-4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium bromide 10**

$^{1}$H NMR spectrum in $CD_3CN$ (300 MHz)

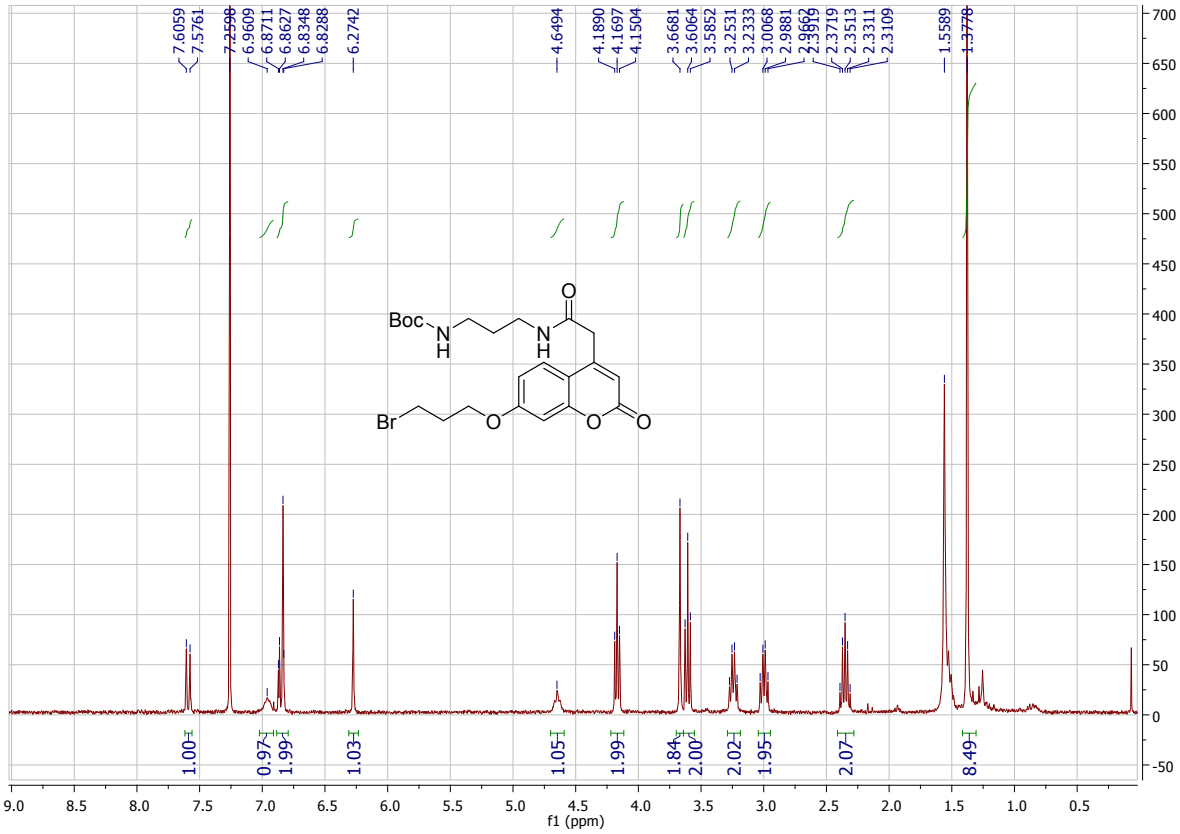



$^{13}$C NMR spectrum in $CD_3CN$ (75 MHz)

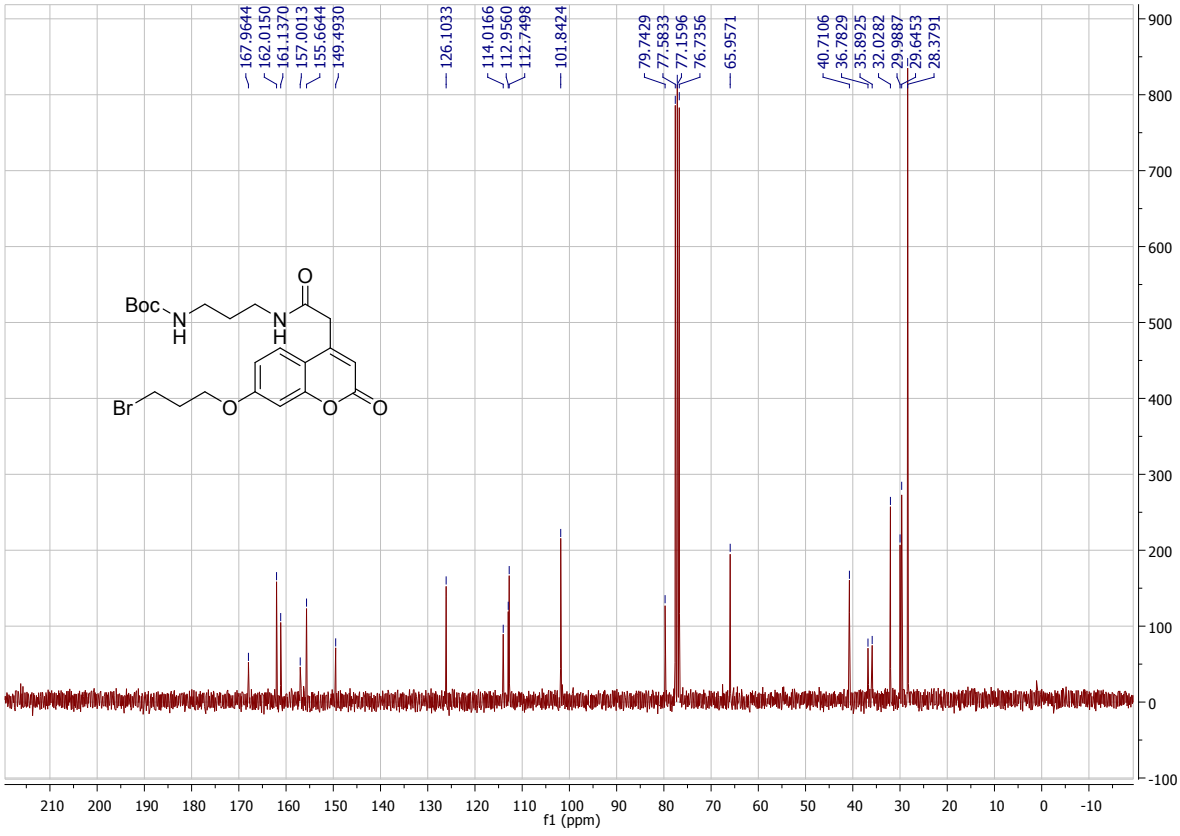


**Tert-butyl (3-(2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)acetamido)propyl)carbamate 11**

$^{1}H$ NMR spectrum in $CDCl_3$ (300 MHz)

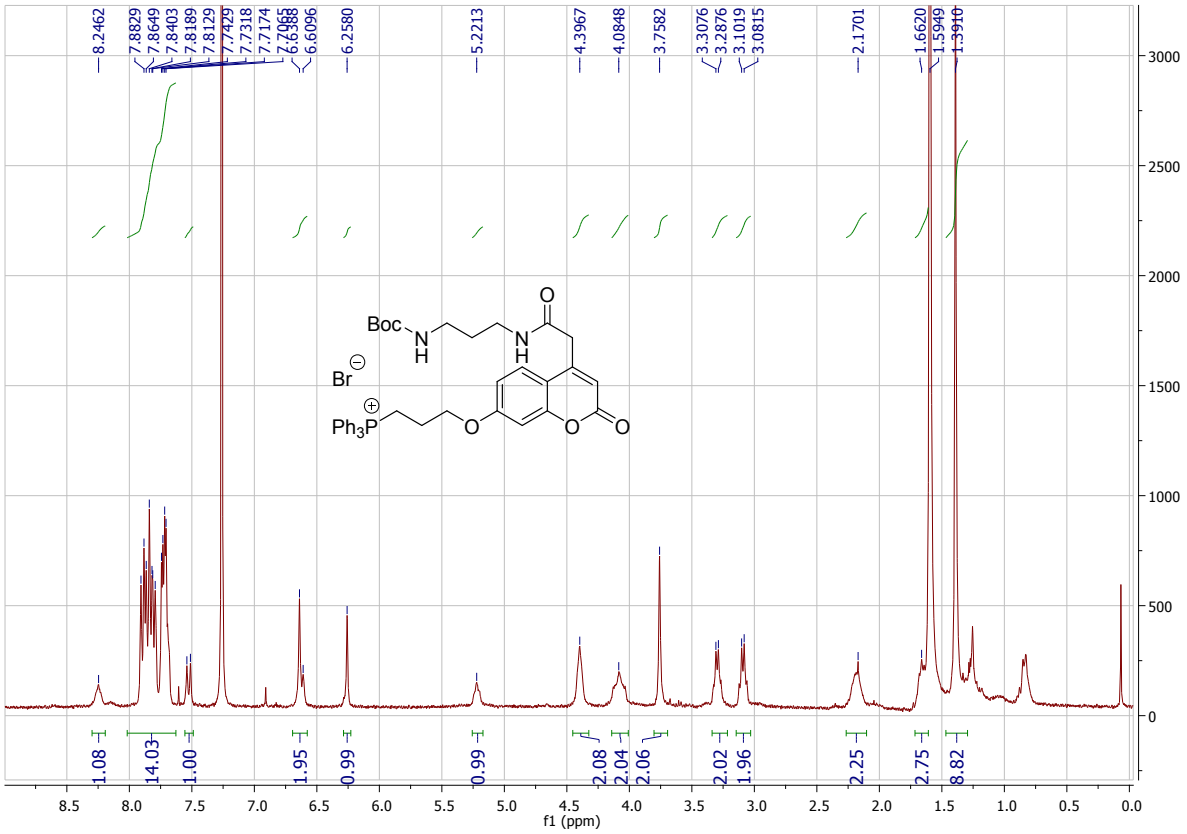



$^{13}C$ NMR spectrum in $CDCl_3$ (75 MHz)

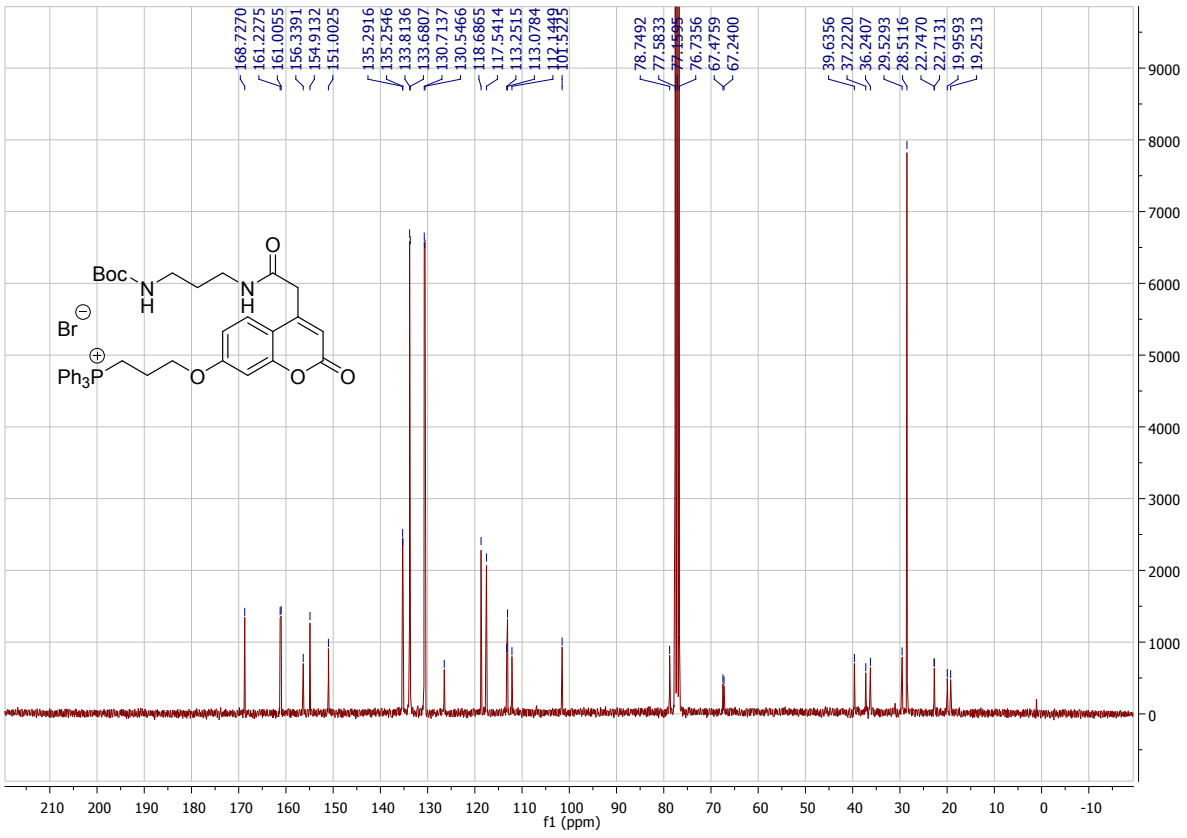


**tert-butyl (3-(2-(2-oxo-7-(3-(triphenyl-l4-phosphanyl)propoxy)-2H-chromen-4-yl)acetamido)propyl)carbamate 12**

$^{1}$H NMR spectrum in $CDCl_3$ (300 MHz)

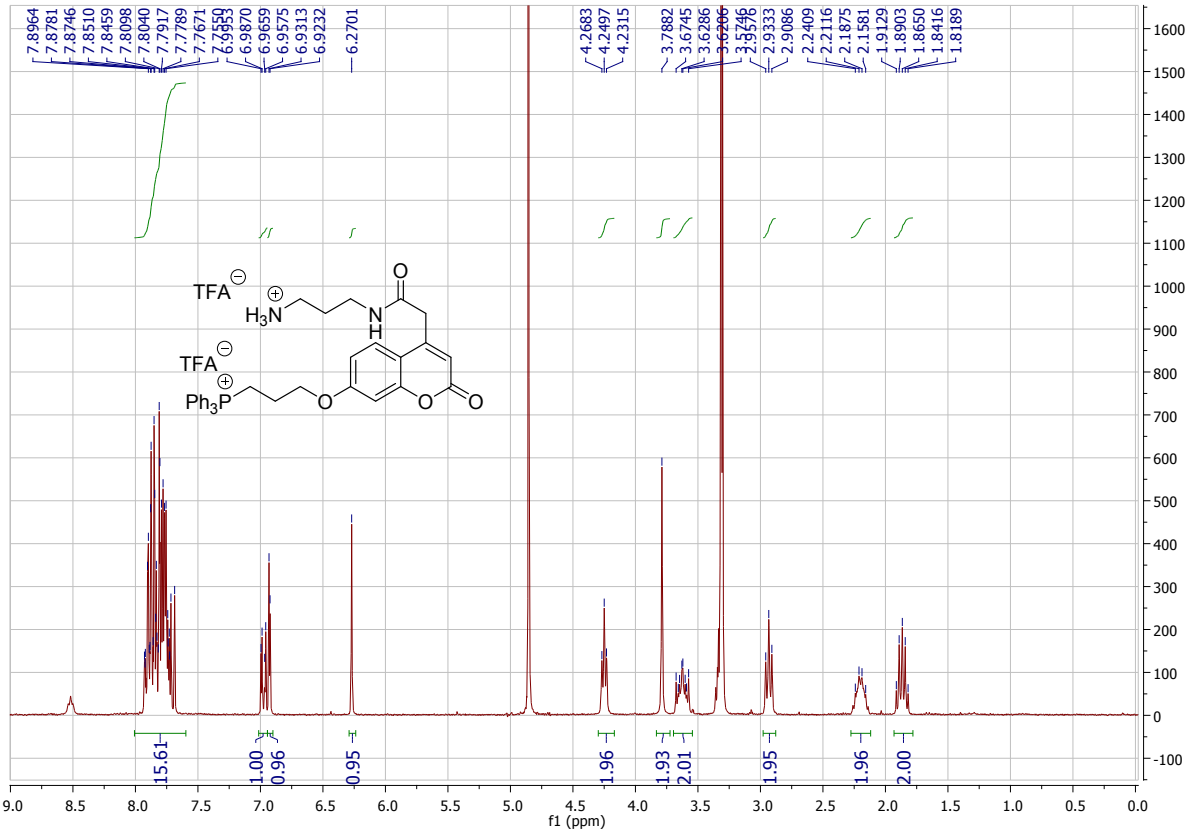



$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)

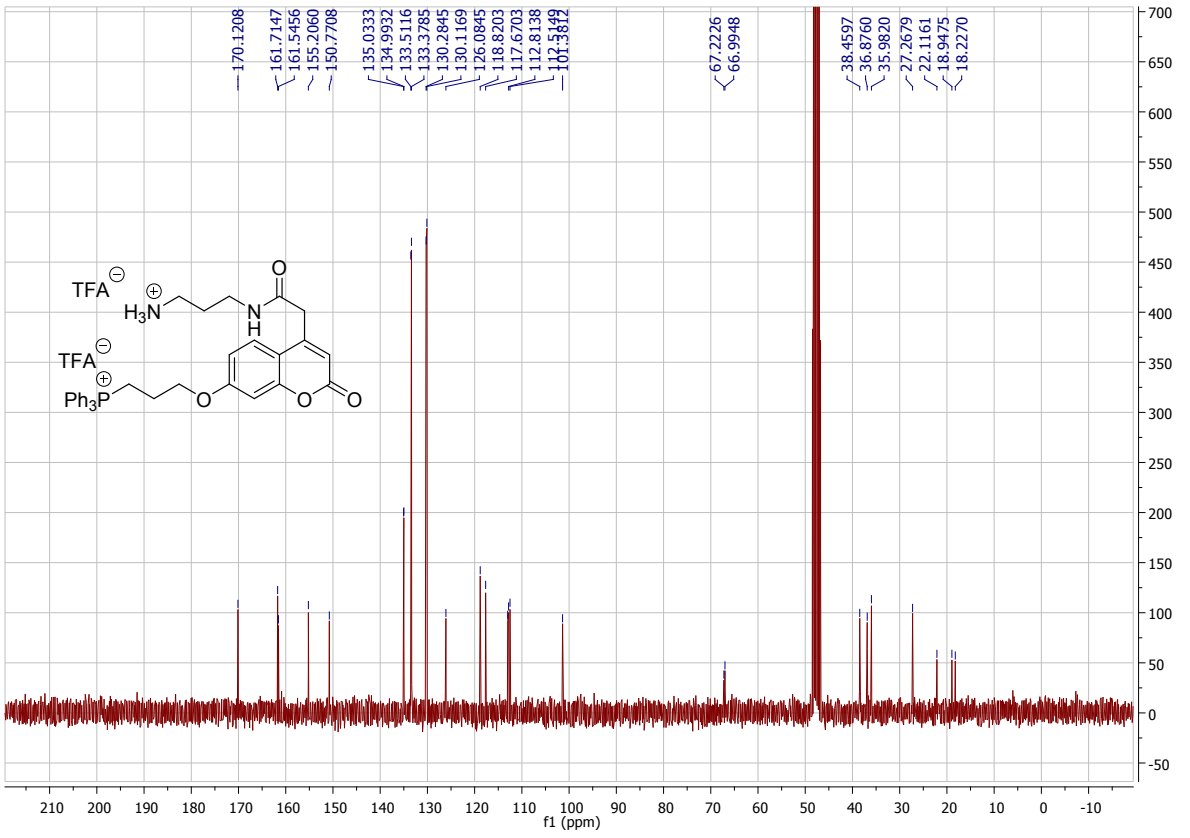


**(3-((4-(2-((3-aminopropyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyltriphenyl-phosphonium bromide 13**

$^{1}$H NMR spectrum in $CD_3OD$ (300 MHz)

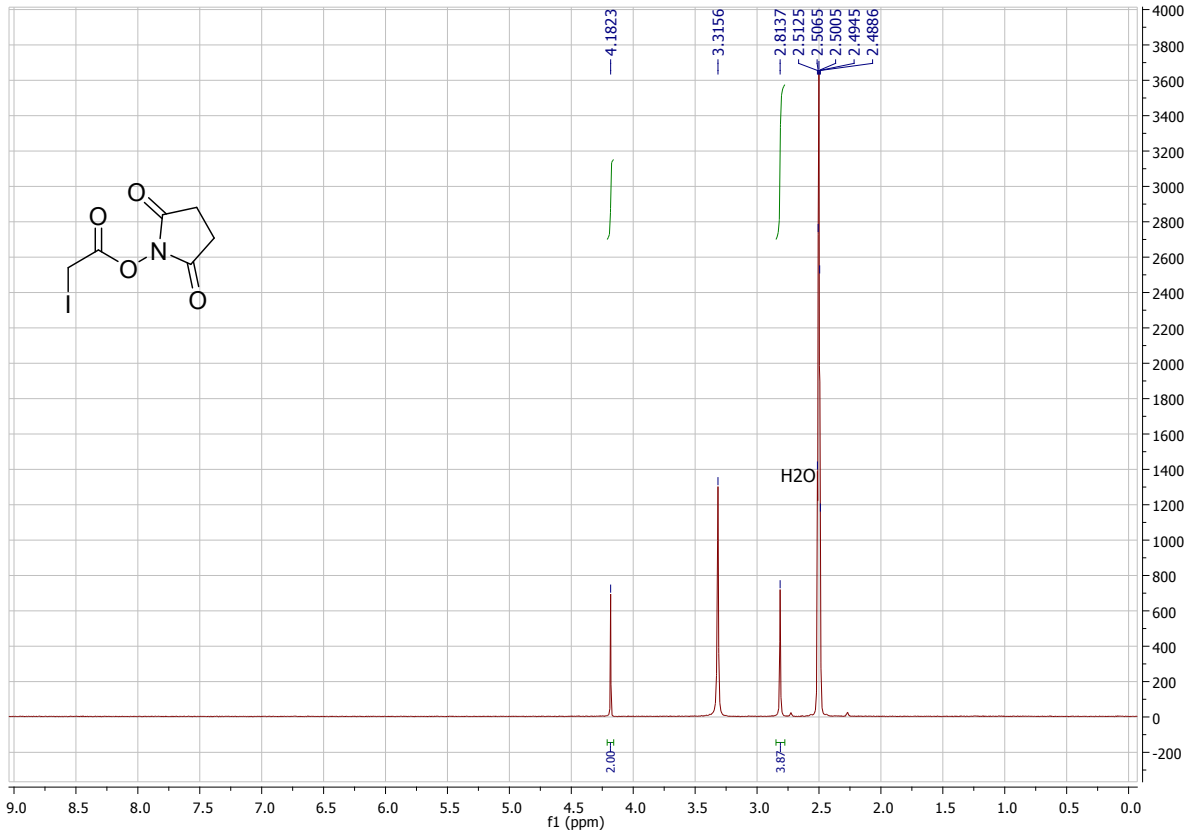



$^{13}$C NMR spectrum in $CD_3OD$ (75 MHz)



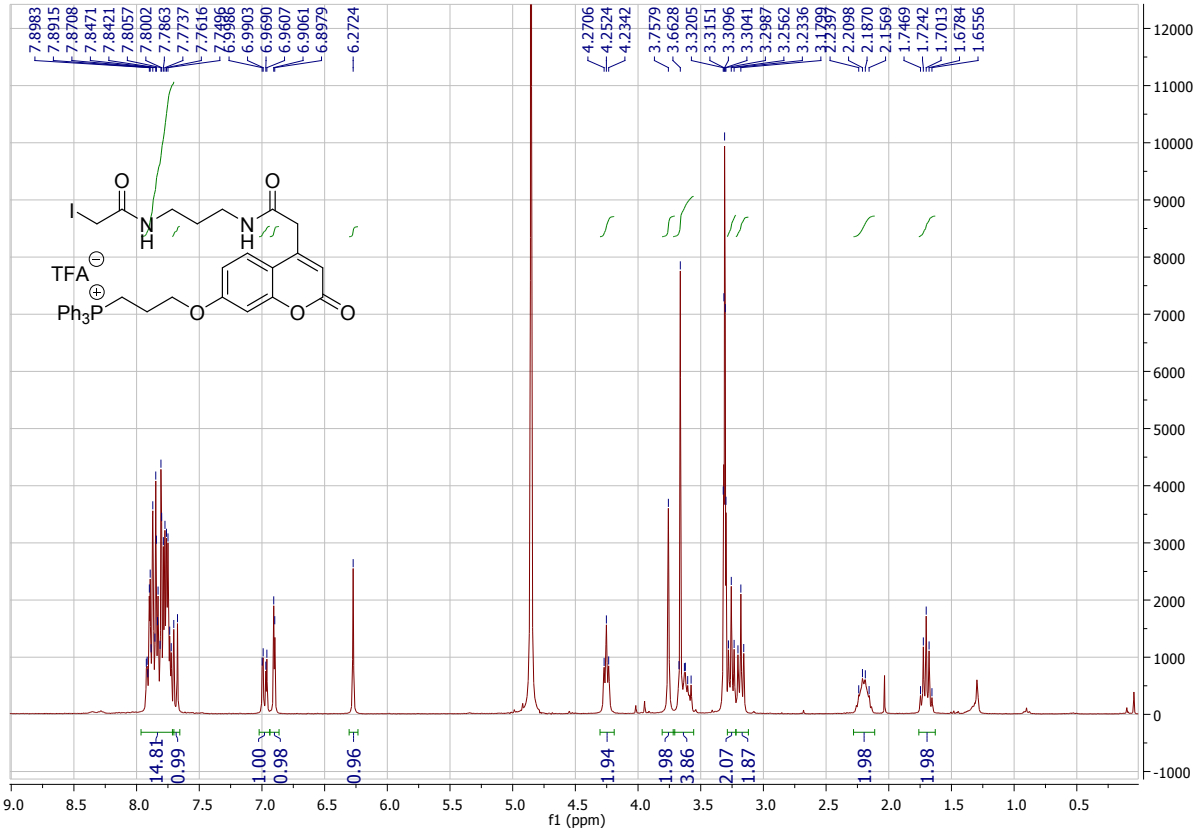
## 2,5-Dioxopyrrolidin-1-yl 2-iodoacetate

*$^1$H NMR spectrum in $(CD_3)_2SO$ (300 MHz)*

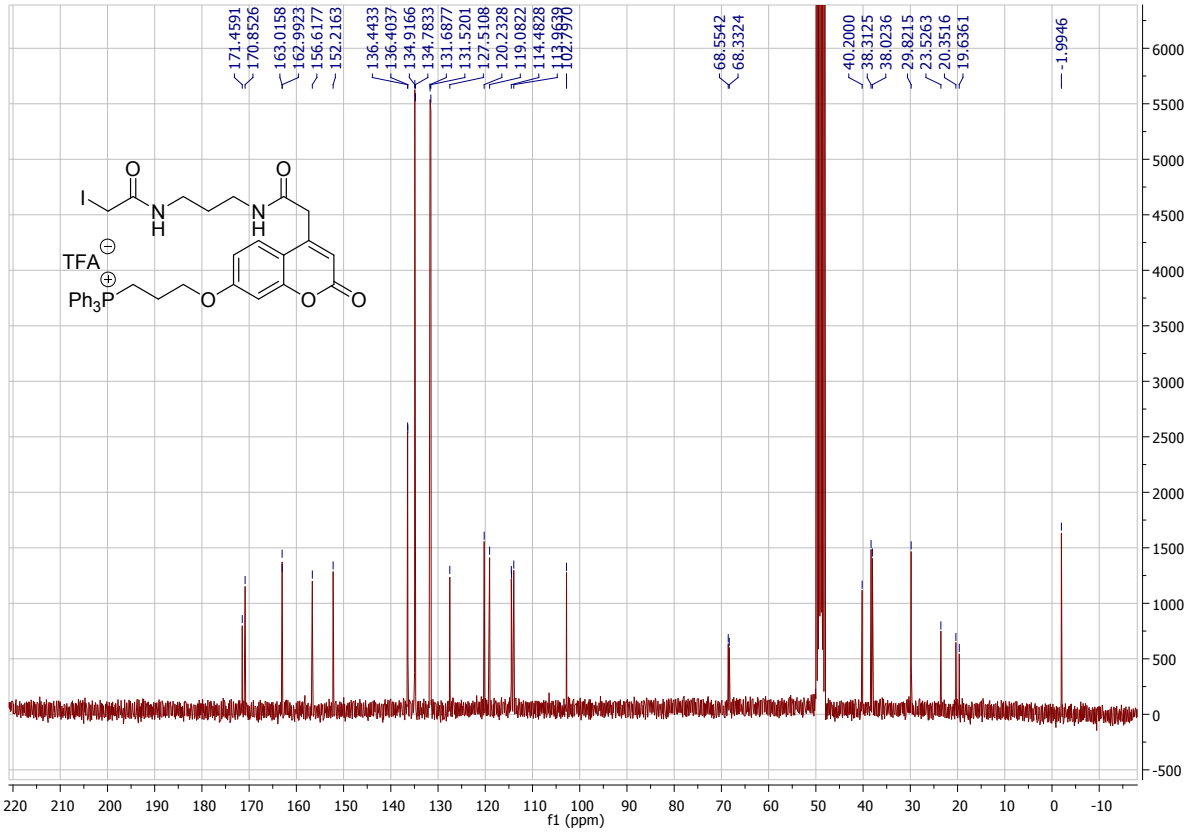


**(3-((4-(2-((3-(2-iodoacetamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenyl-phosphonium 2,2,2-trifluoroacetate 14**

$^{1}$H NMR spectrum in $CD_3OD$ (300 MHz)

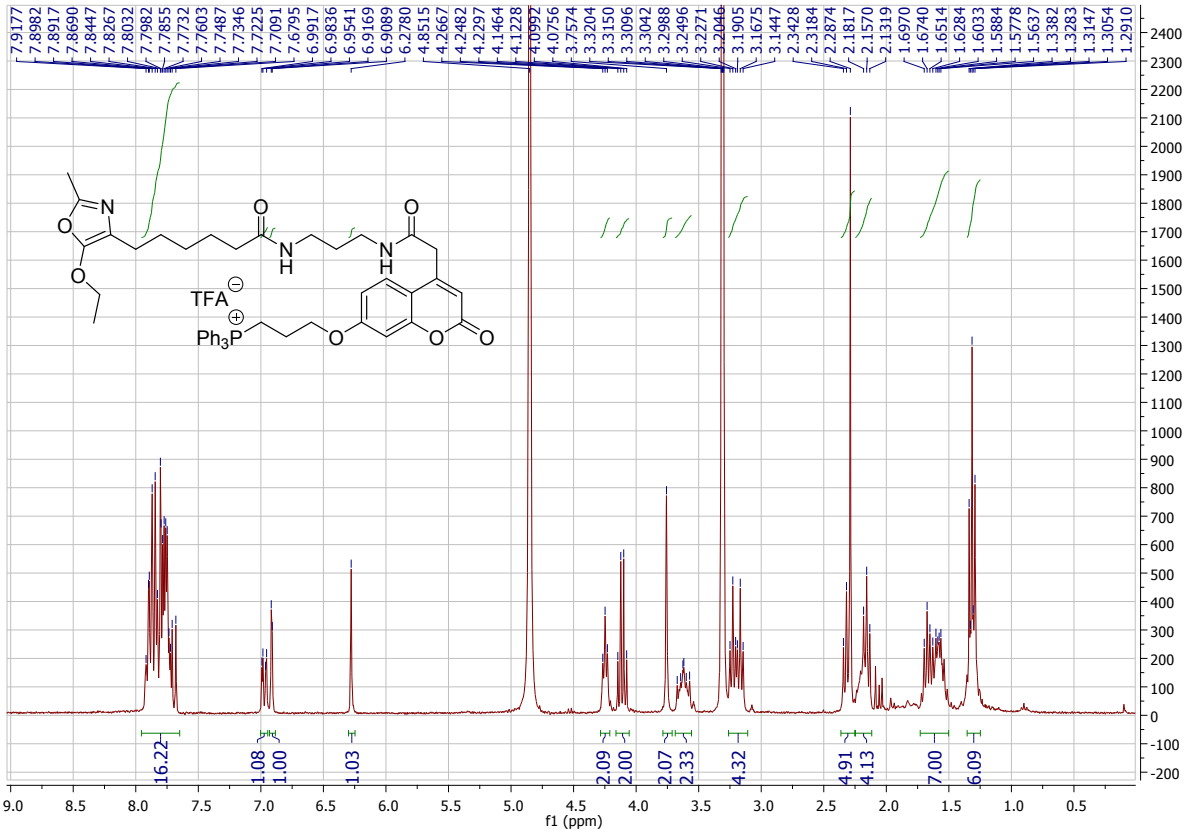



$^{13}$C NMR spectrum in $CD_3OD$ (75 MHz)

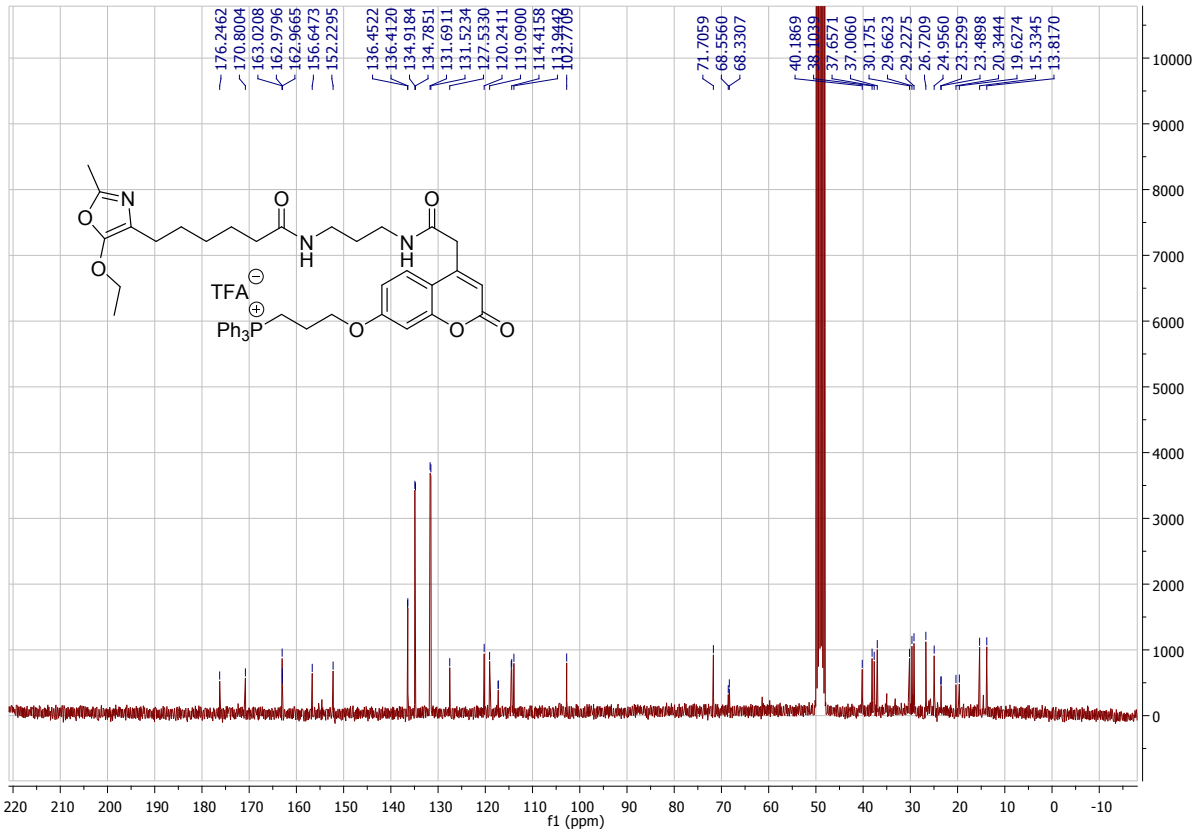


**(3-((4-(2-((3-(6-(5-ethoxy-2-methyloxazol-4-yl)hexanamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 15**

$^{1}$H NMR spectrum in $CD_3OD$ (300 MHz)

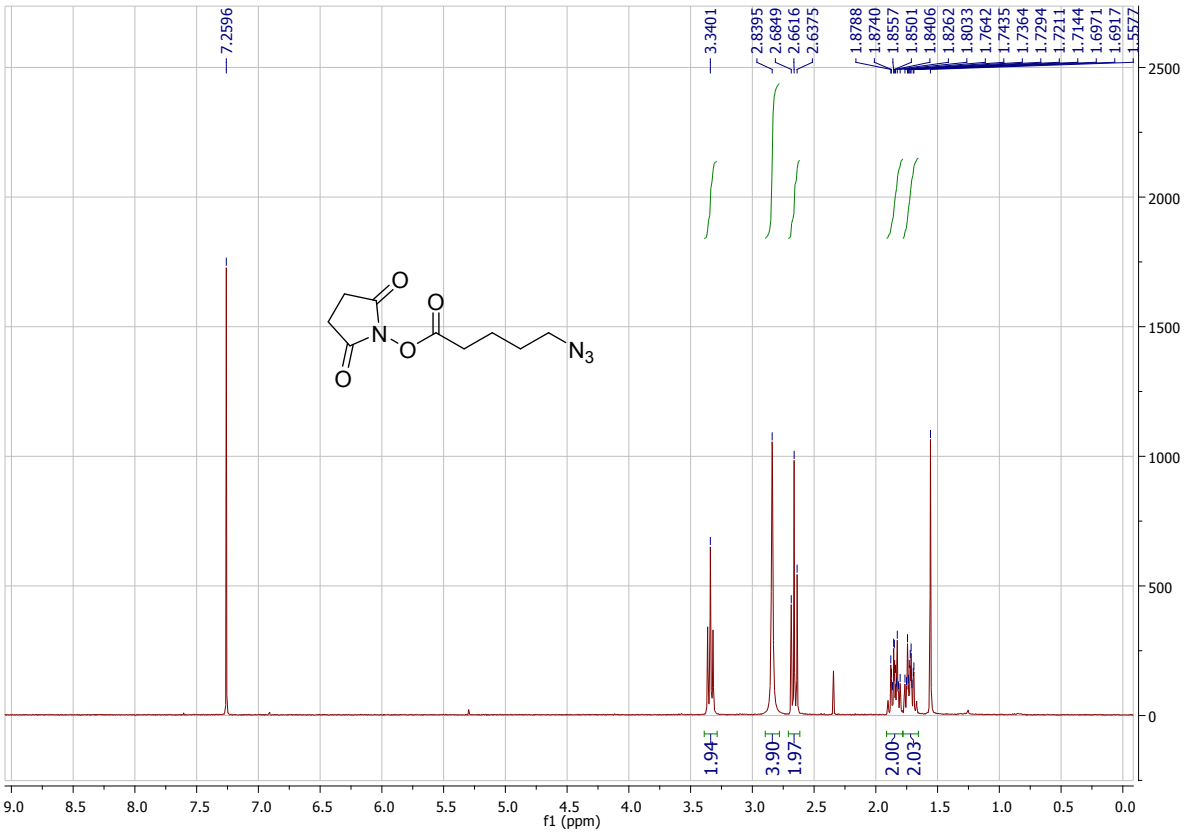



$^{13}$C NMR spectrum in $CD_3OD$ (75 MHz)



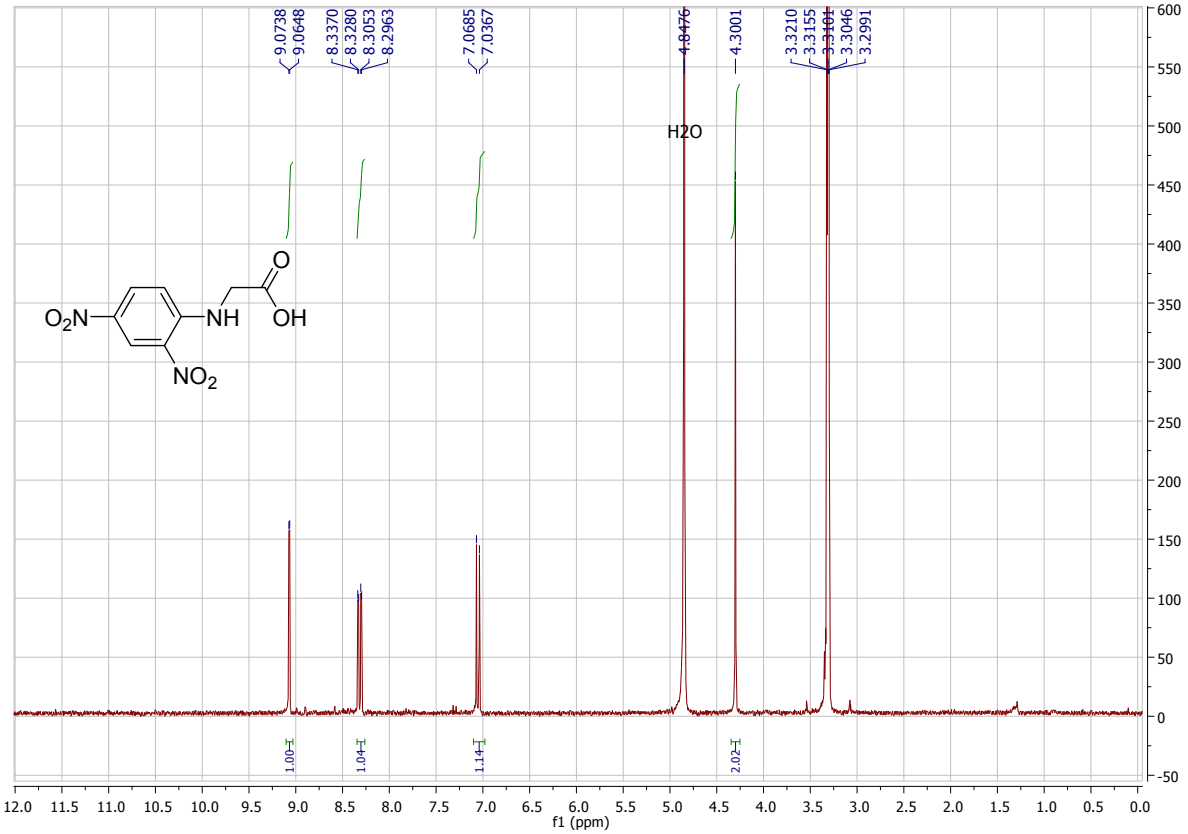
**2,5-dioxopyrrolidin-1-yl 5-azidopentanoate**

*$^1$H NMR spectrum in $CDCl_3$ (300 MHz)*



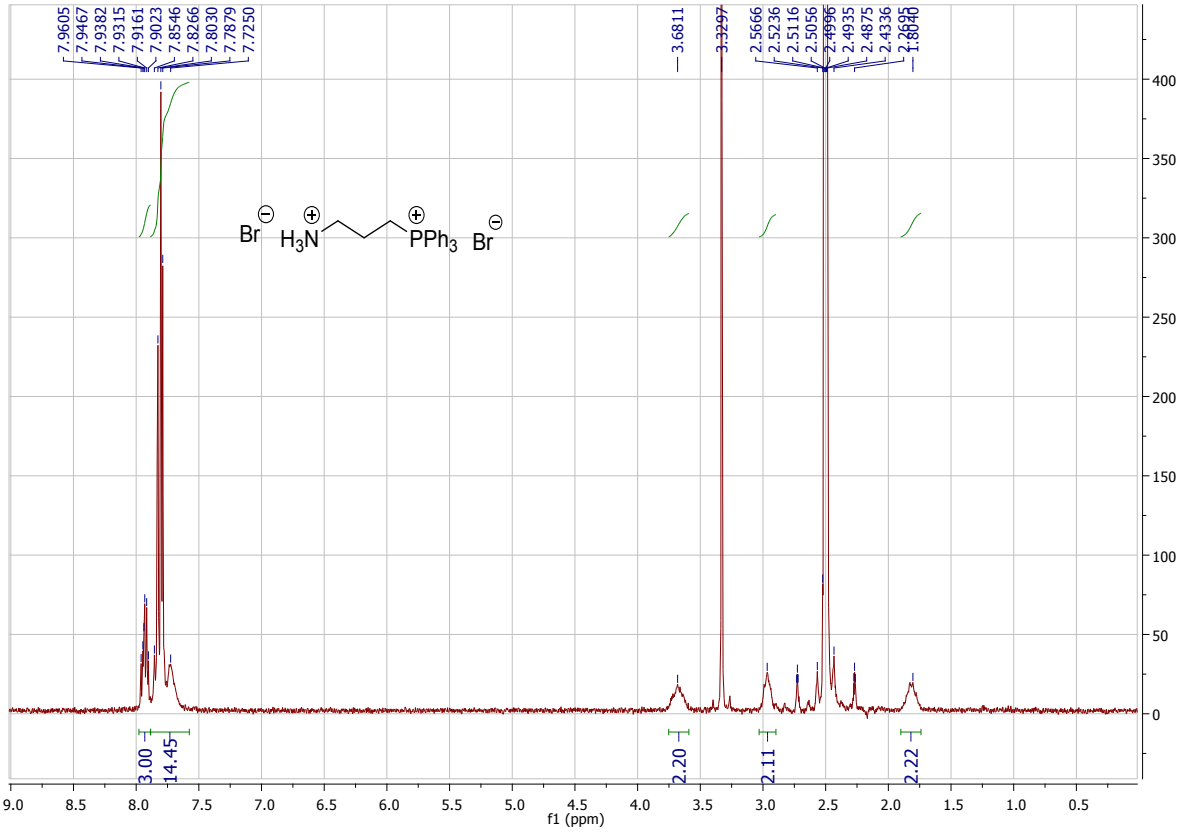
**(2,4-dinitrophenyl)glycine**

*$^1$H NMR spectrum in $CD_3OD$ (300 MHz)*



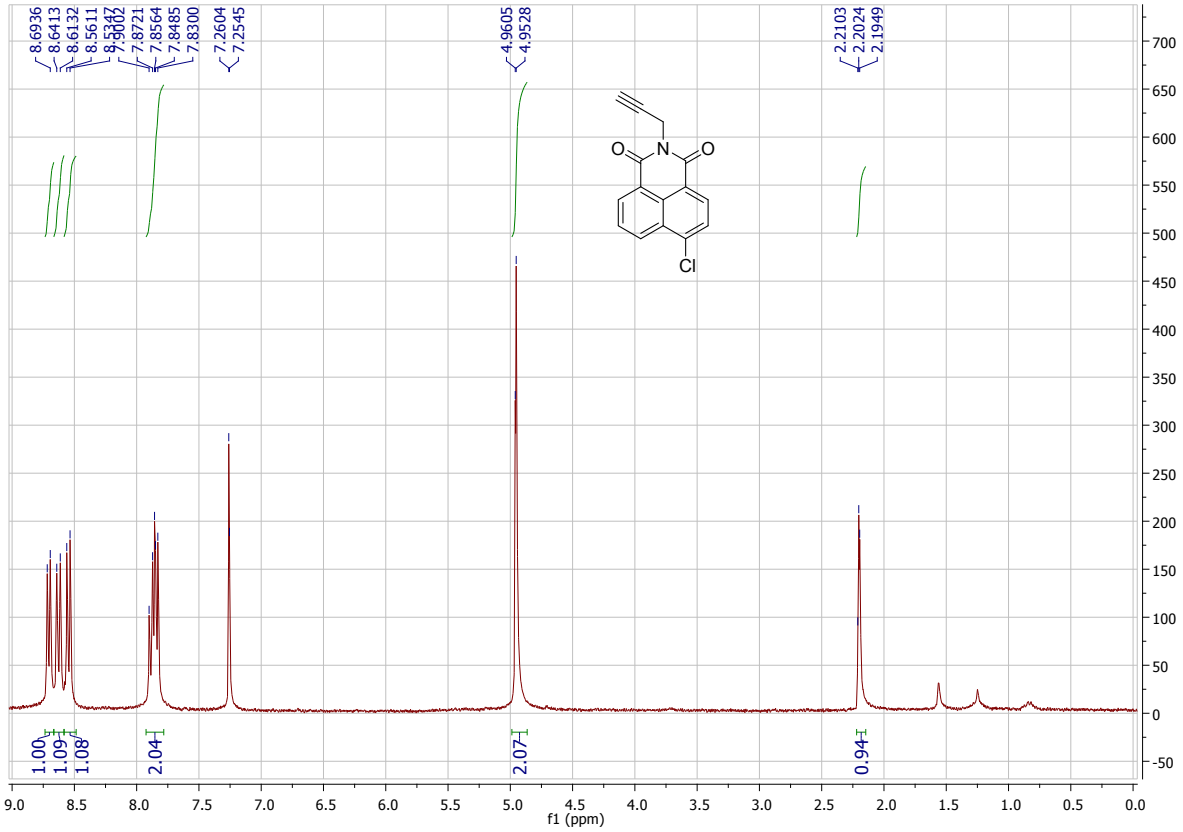
**(3-ammoniopropyl)triphenylphosphonium bromide**

*$^1$H NMR spectrum in $(CD_3)_2SO$ (300 MHz)*



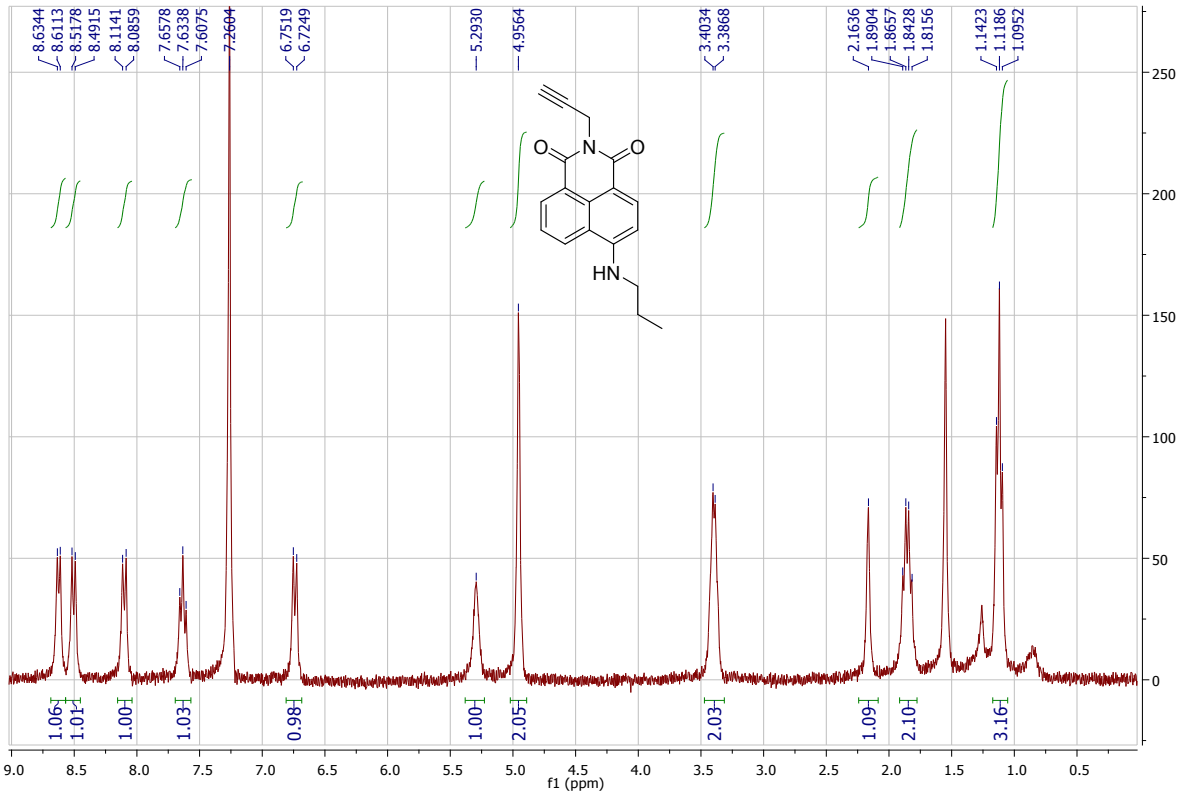
**4-Chloro-(N-(2-propyn-1-yl))-1,8-naphthalimide 27**

*$^1$H NMR spectrum in $CDCl_3$ (300 MHz)*

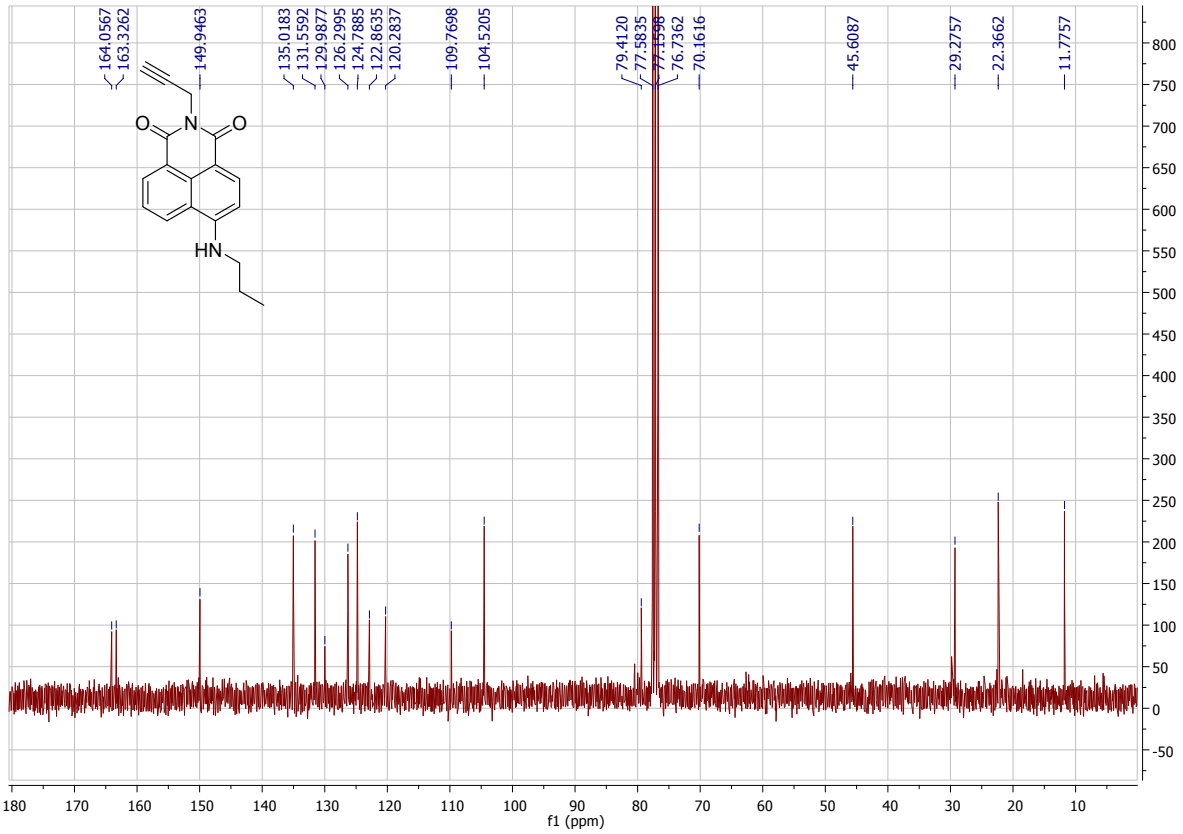


**2-(prop-2-yn-1-yl)-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione 28**

*$^1$H NMR spectrum in $CDCl_3$ (300 MHz)*

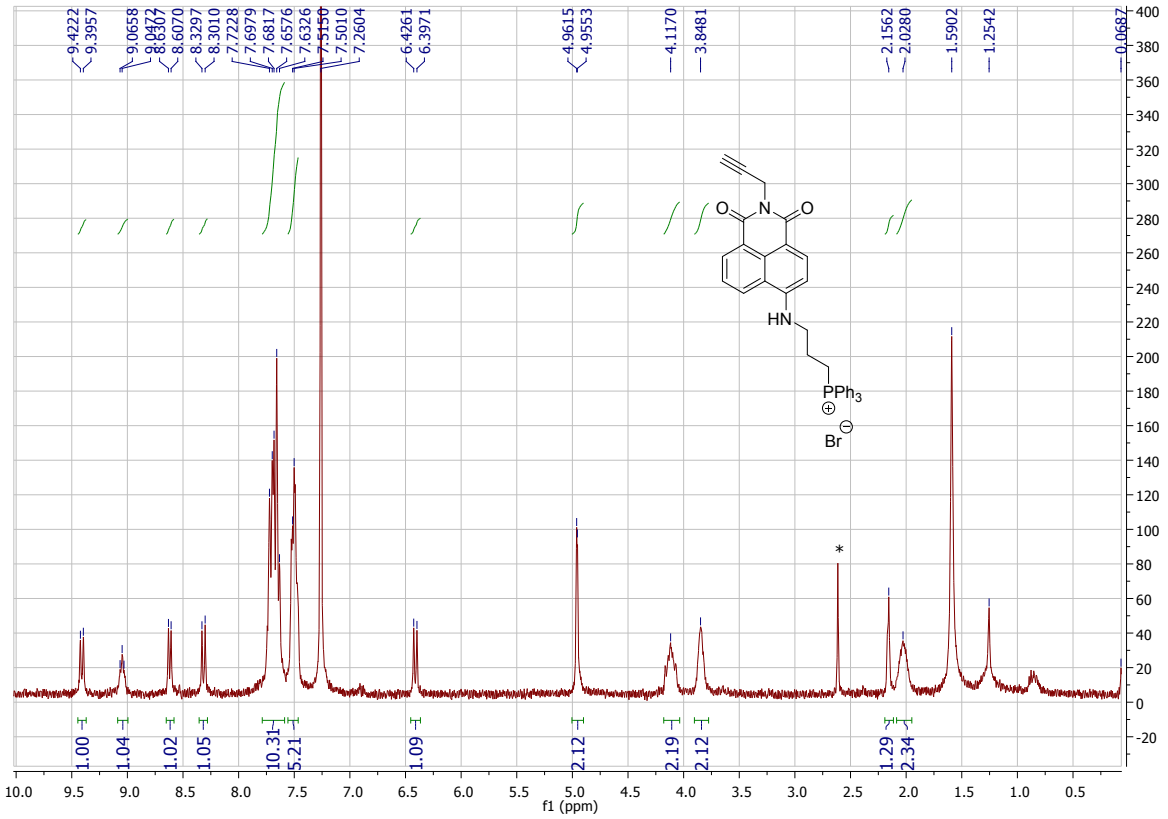



$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)

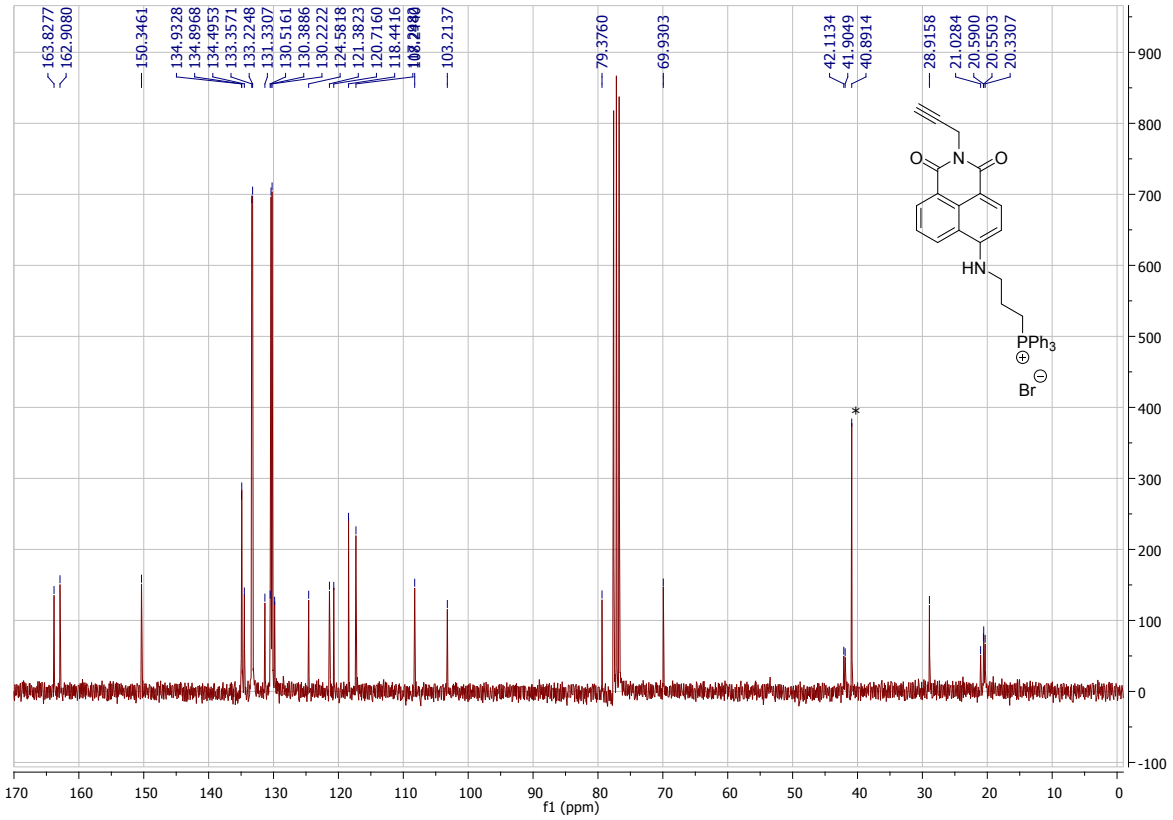


**(3-((1,3-dioxo-2-(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)propyl)triphenylphosphonium bromide 29**

*$^1$H NMR spectrum in $CDCl_3$ (300 MHz)*




$^{13}$C NMR spectrum in $CDCl_3$ (75 MHz)

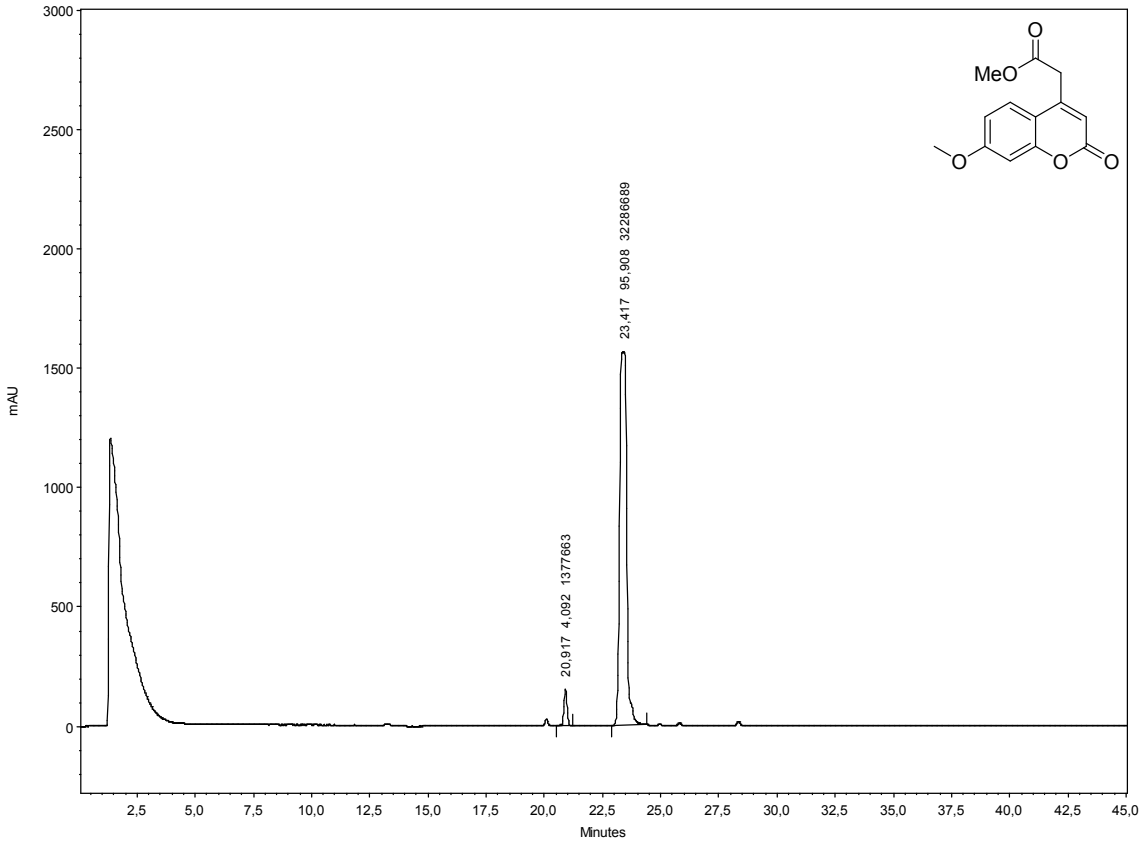



* DMSO residual peak

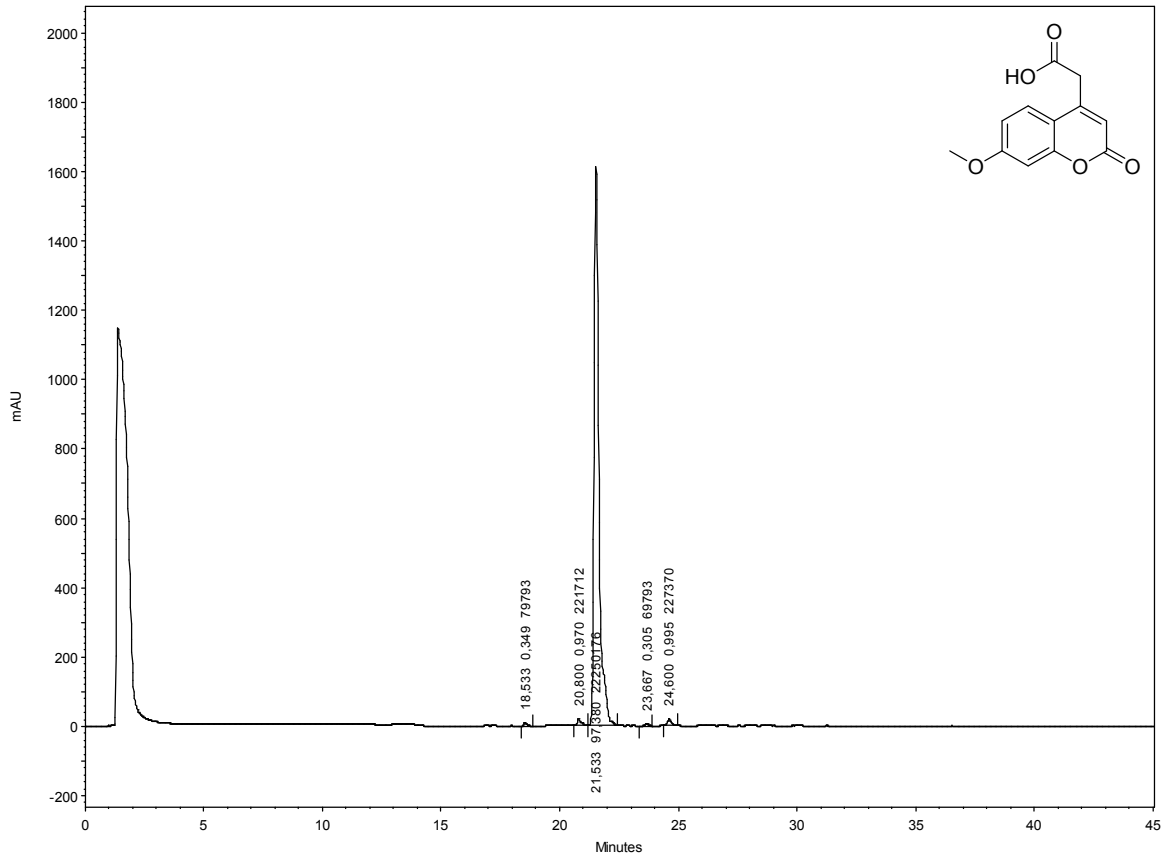
# 2) RP-HPLC elution profiles

The following analytical RP-HPLC were performed using the System QC: (Thermo Hypersyl GOLD C18 column, 5 µm, 2.1 x 100 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min) followed by linear gradient from 0% to 100% (40 min) of ACN] at a flow rate of 0.25 mL/min.

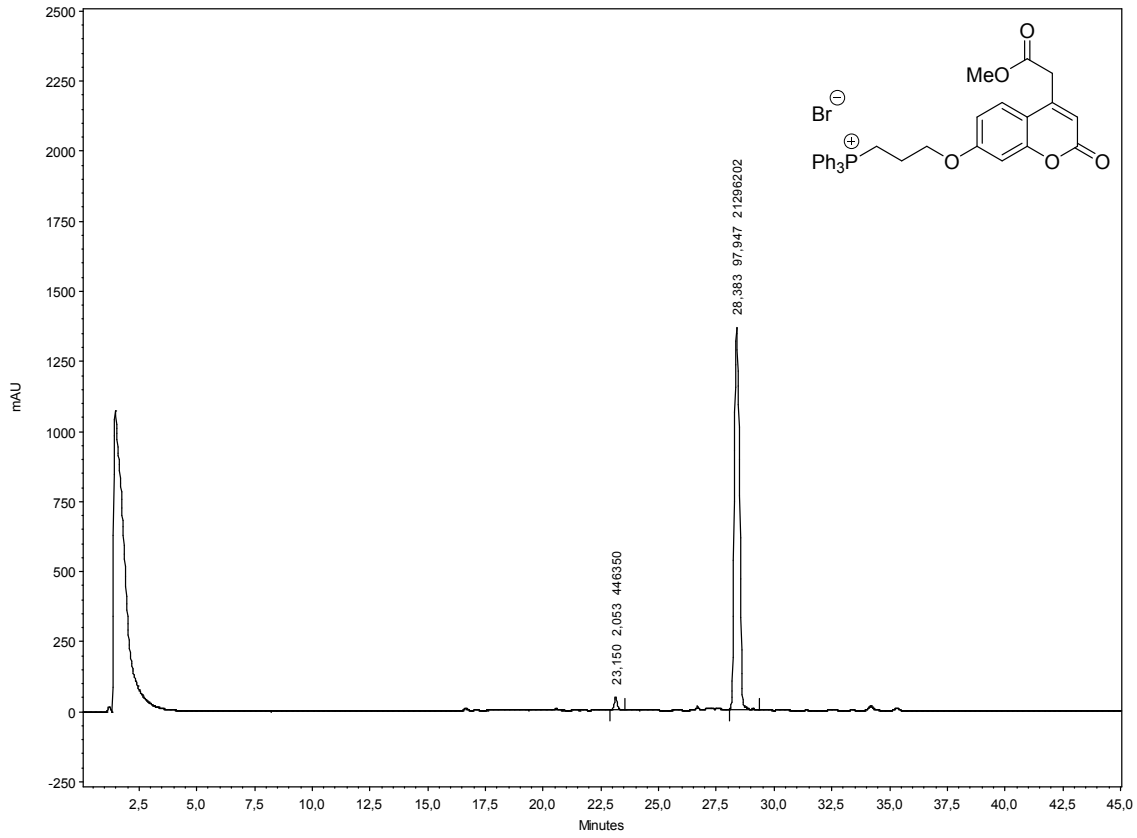
**Methyl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate 1**



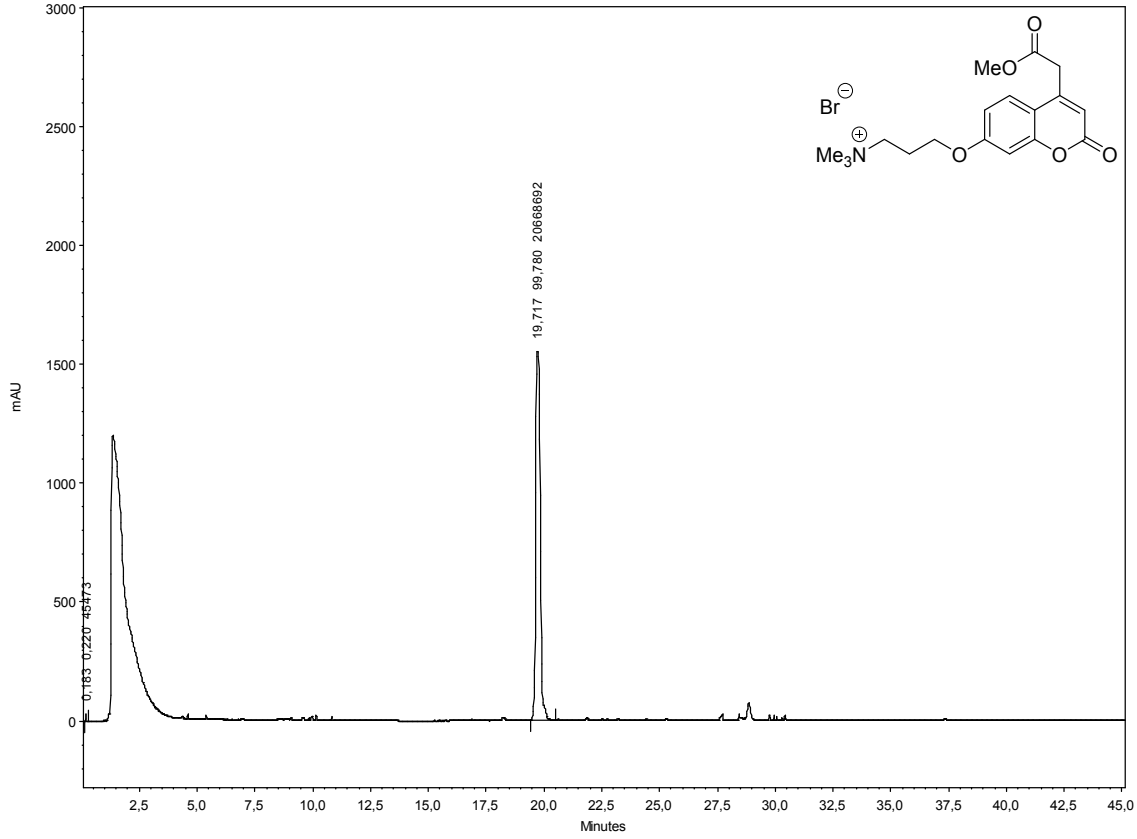
**2-(7-Methoxy-2-oxo-2H-chromen-4-yl)acetic acid 2**



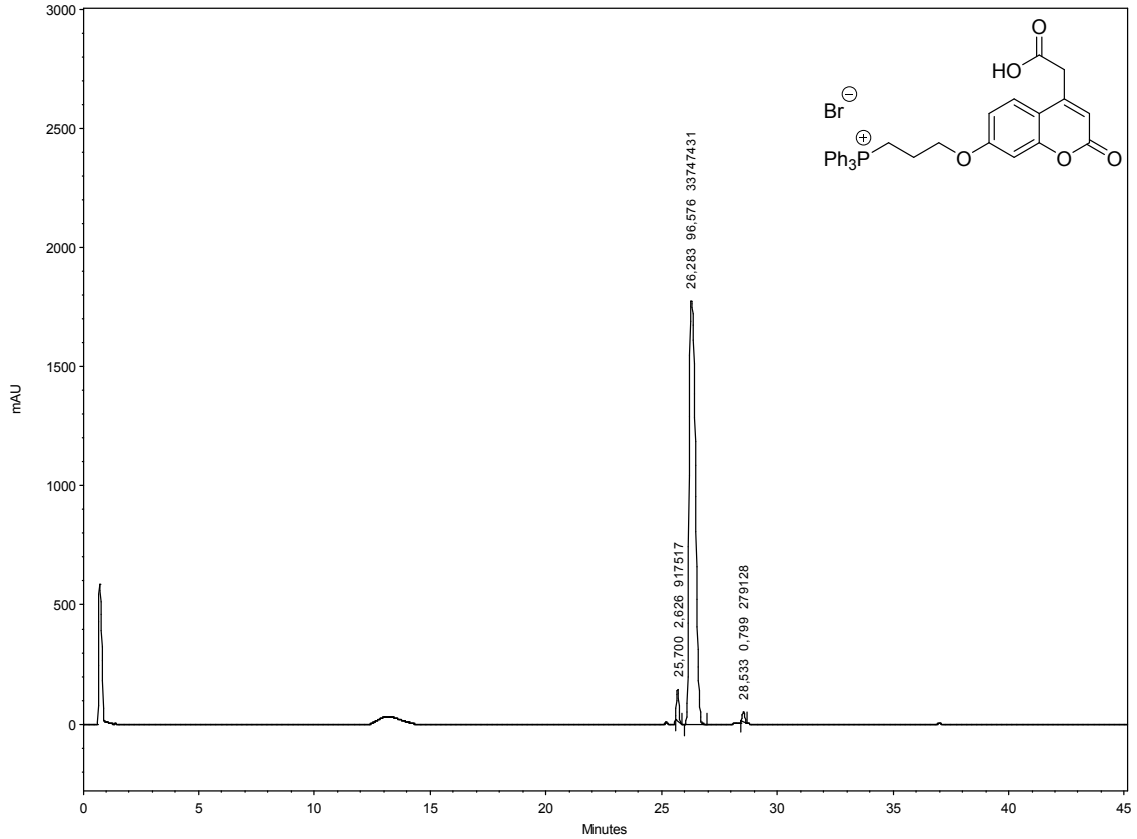

**(3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7yl)oxy)-propyl)triphenylphosphonium bromide 5**



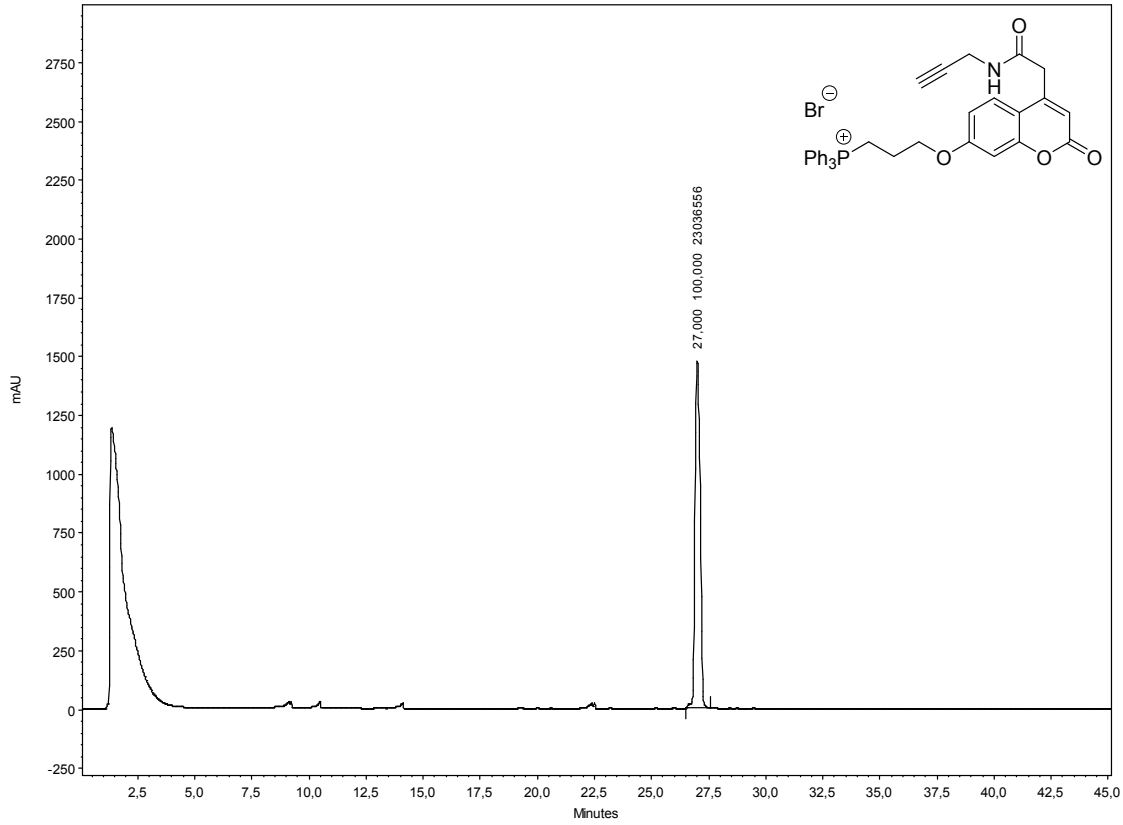
**3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)-N,N,N-trimethylpropan-1-aminium bromide 6**



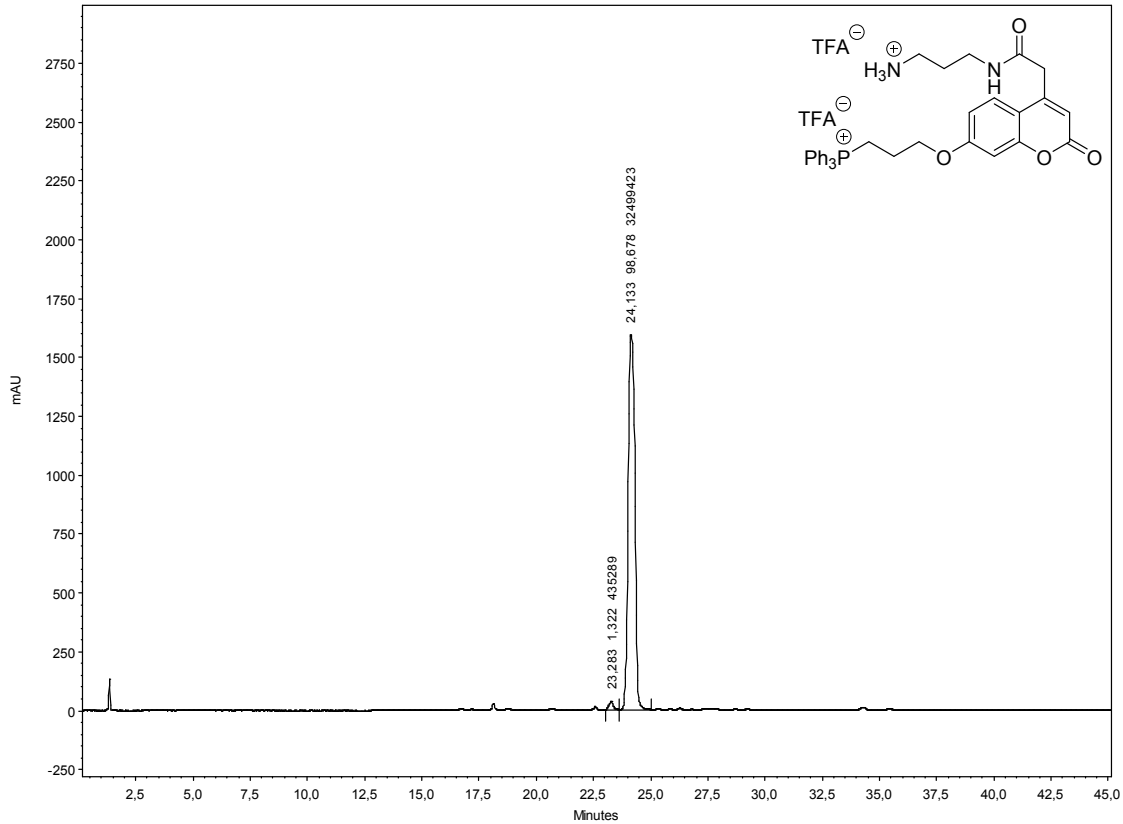

**(3-((4-(carboxymethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-triphenylphosphonium bromide 7**



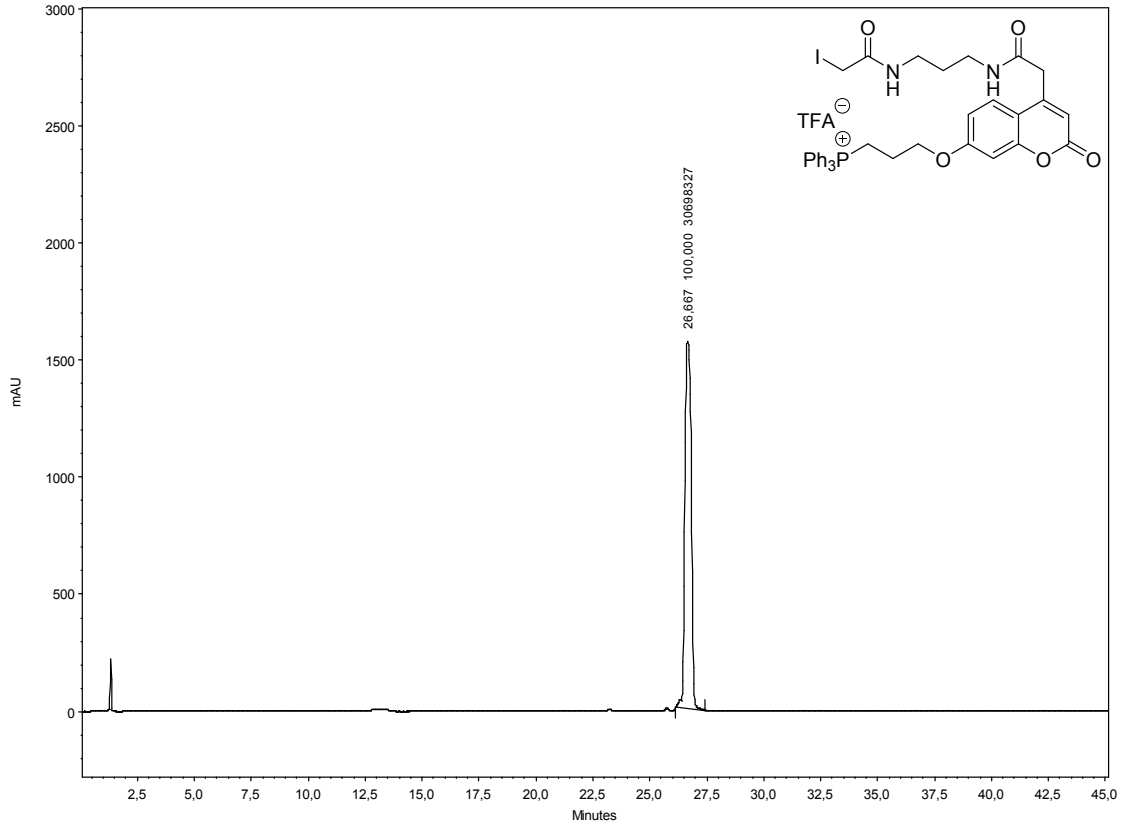
**(3-((2-oxo-4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-2H-chromen -7-yl)oxy)propyl)triphenyl-phosphonium bromide 10**



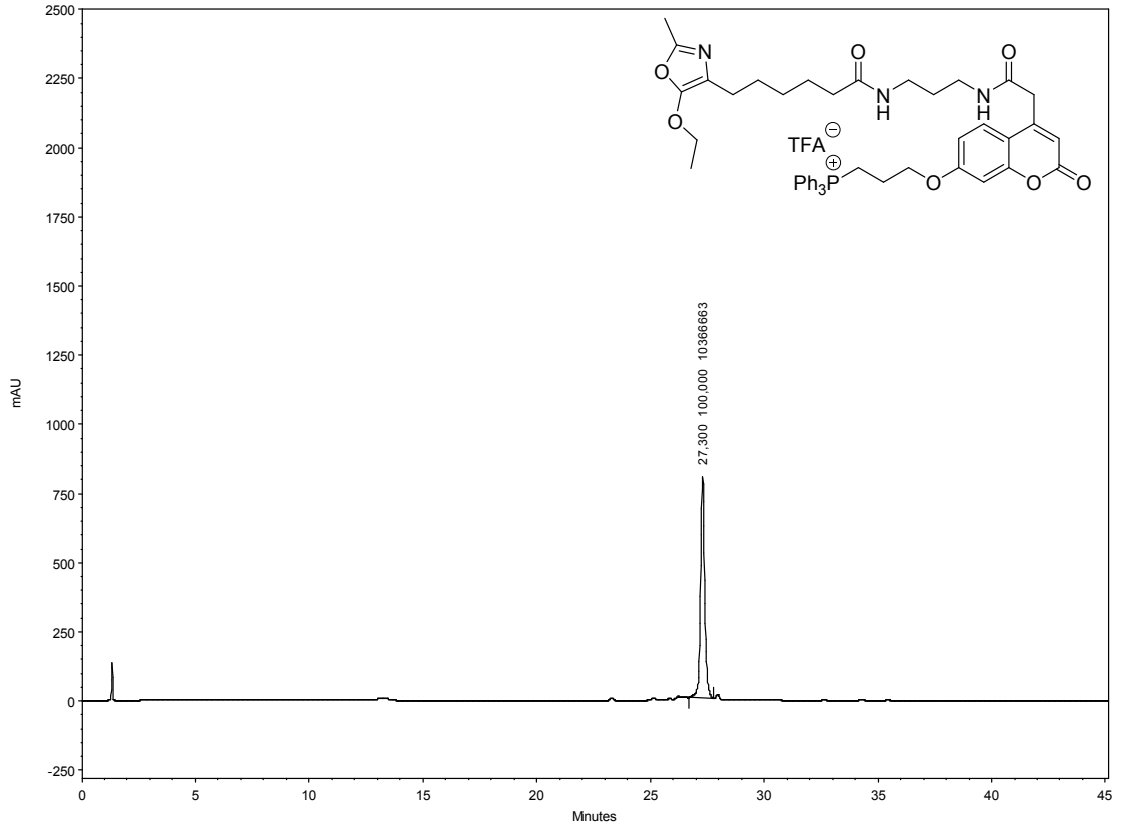

**(3-((4-(2-((3-aminopropyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyltriphenyl-phosphonium bromide 13**



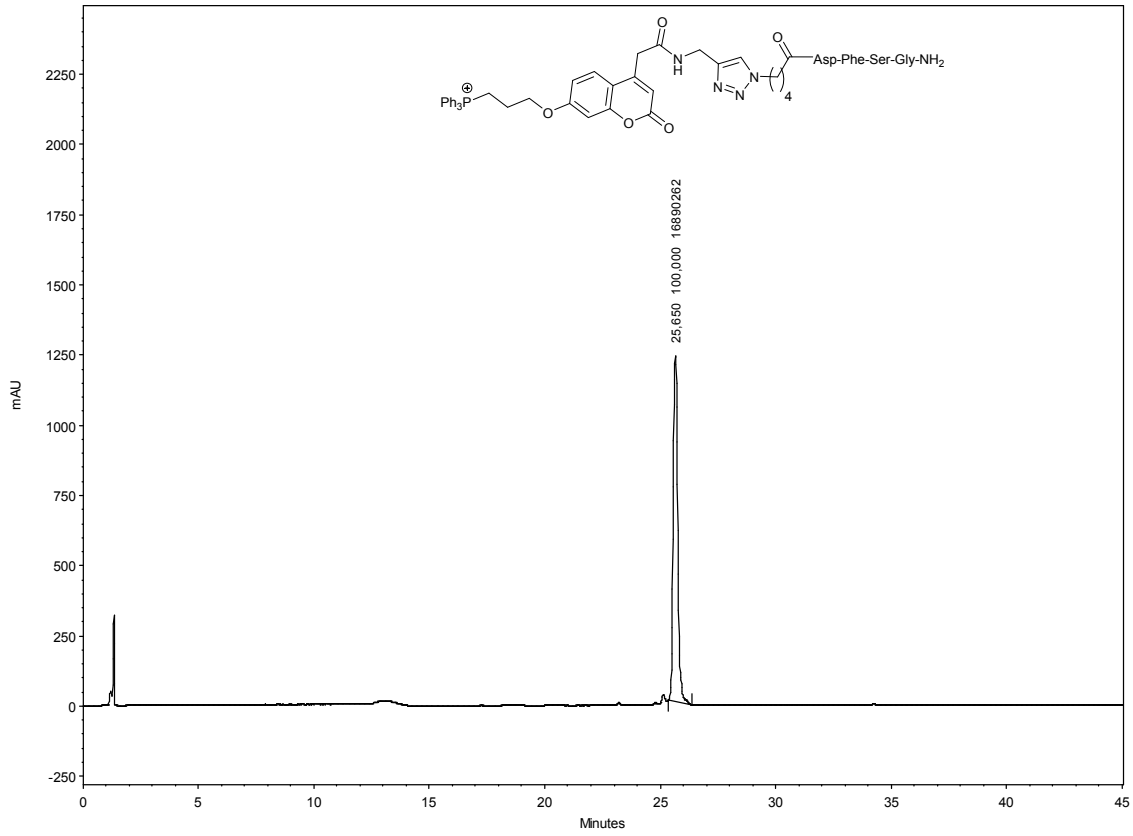
**(3-((4-(2-((3-(2-iodoacetamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 14**



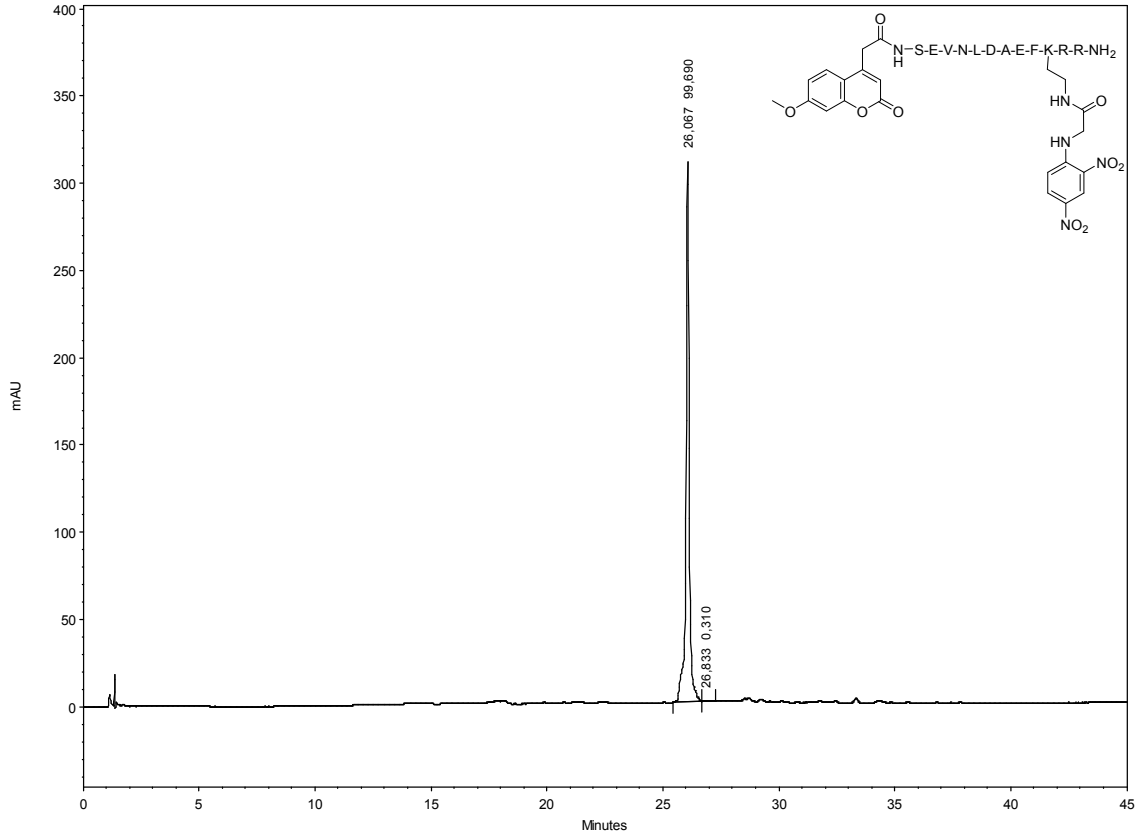

**(3-((4-(2-((3-(6-(5-ethoxy-2-methyloxazol-4-yl)hexanamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 15**



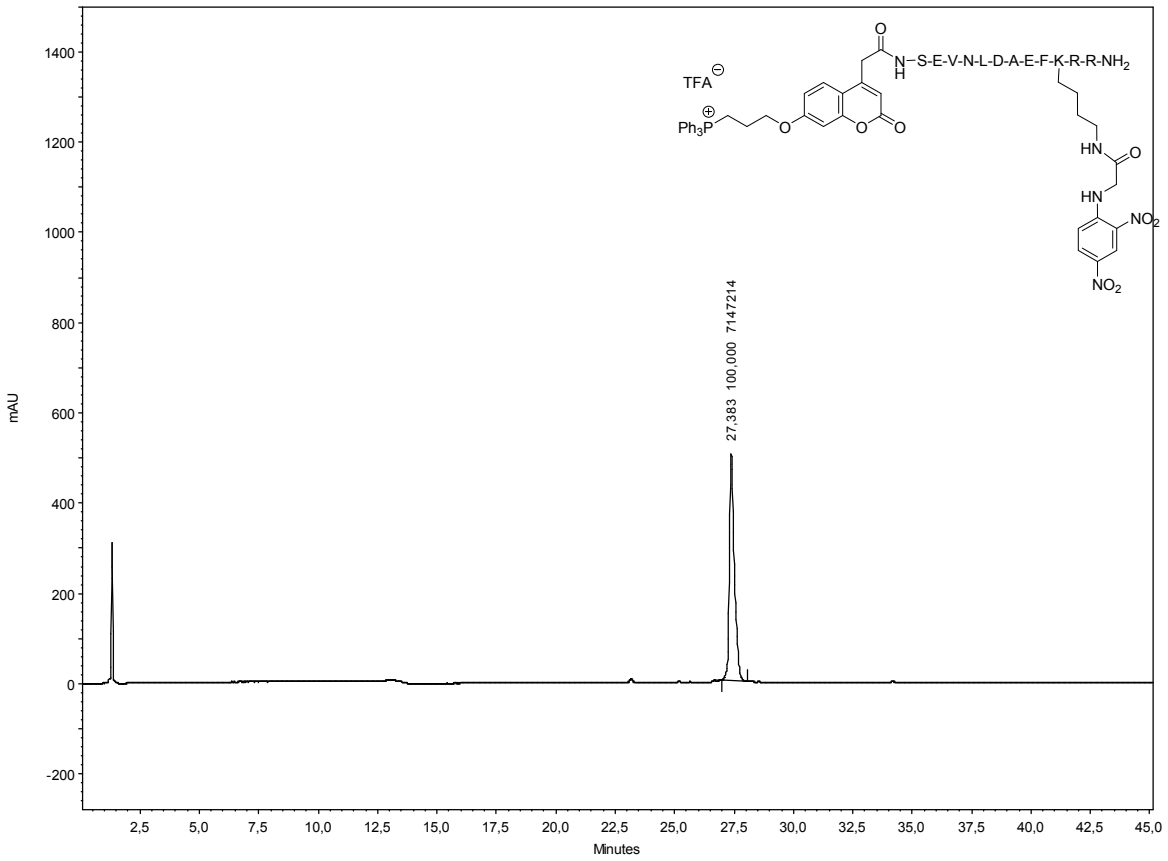
**Phosphonium-tagged tetrapeptide 17**



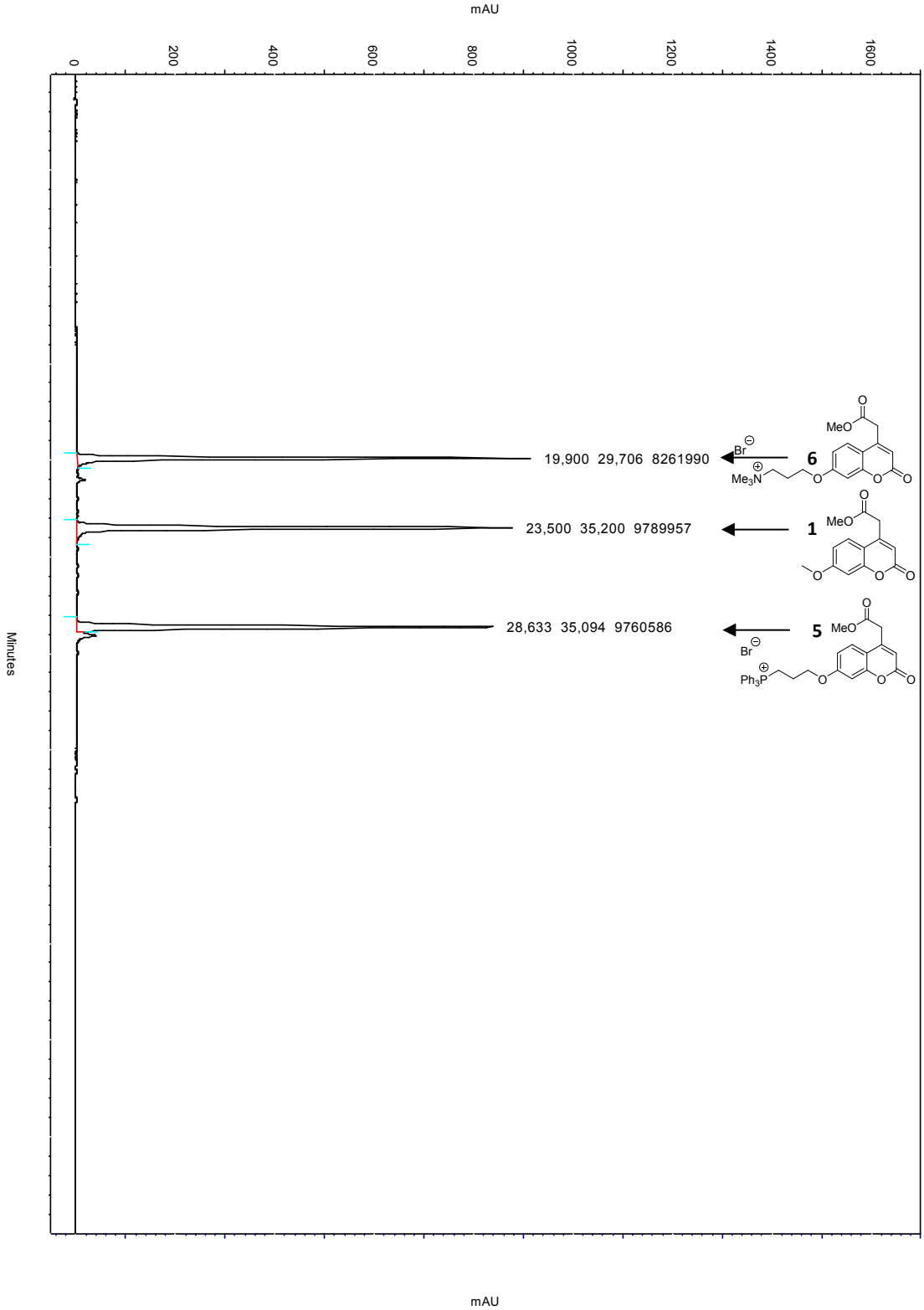

**(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19**



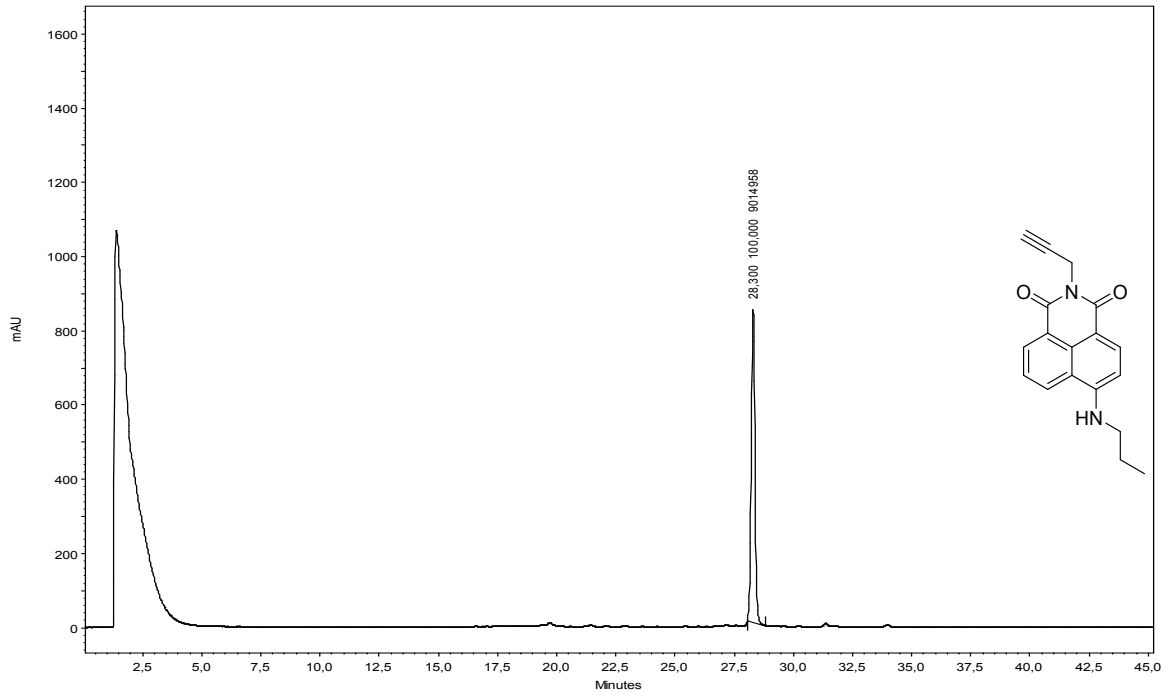
**(PPh$_3$C)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20**



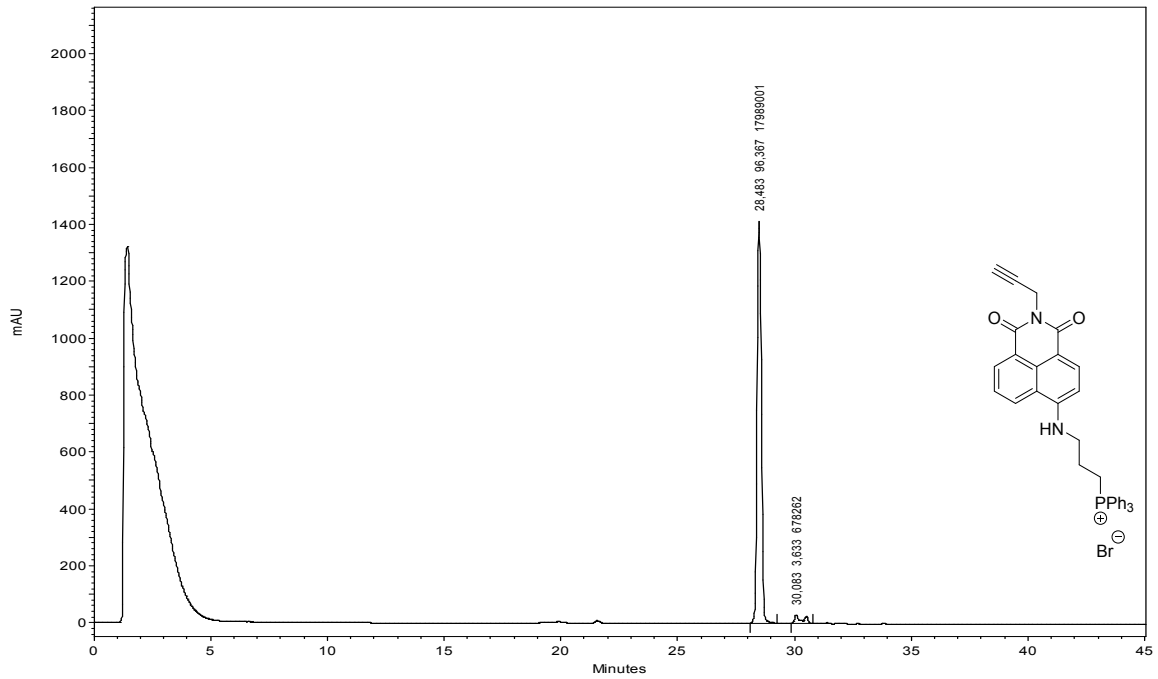
**RP-HPLC analysis of a 2.17 x $10^2$ µM solution of coumarins 1 - 5 - 6 in a 1:1:1 molar ratio.**



**2-(prop-2-yn-1-yl)-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione 28**



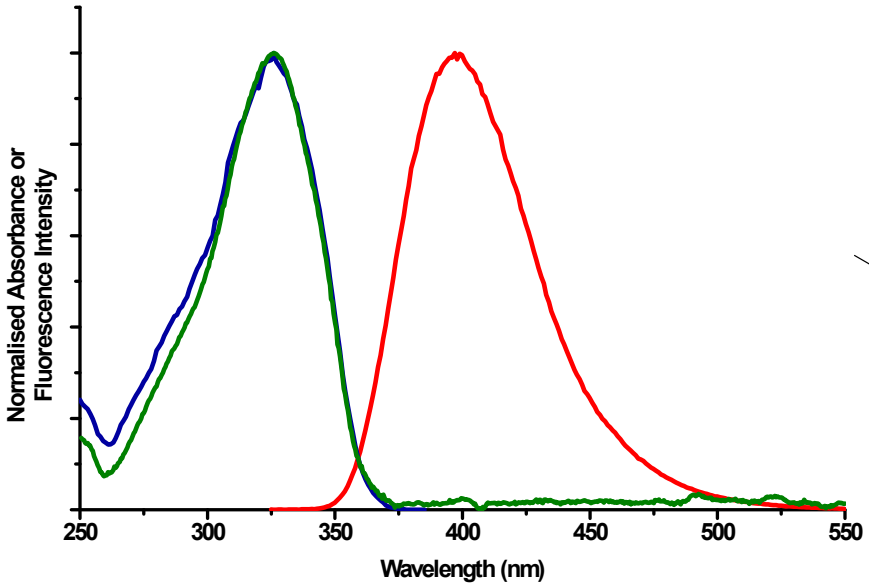

**(3-((1,3-dioxo-2-(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)propyl)triphenylphosphonium bromide 29**

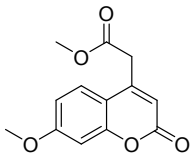


# 3) Spectroscopic data

## 1) Absorption, excitation, emission spectra of coumarin derivatives

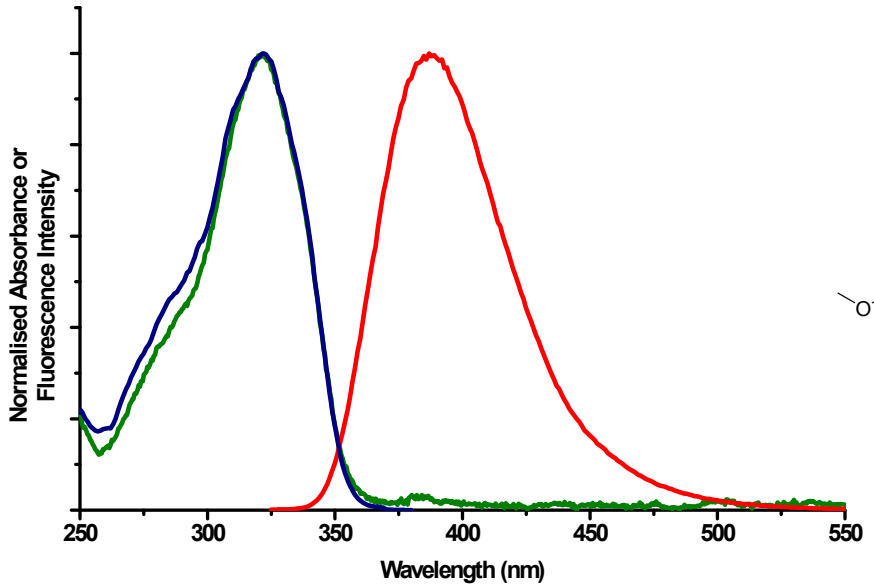
**Methyl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate 1**

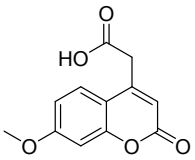



Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

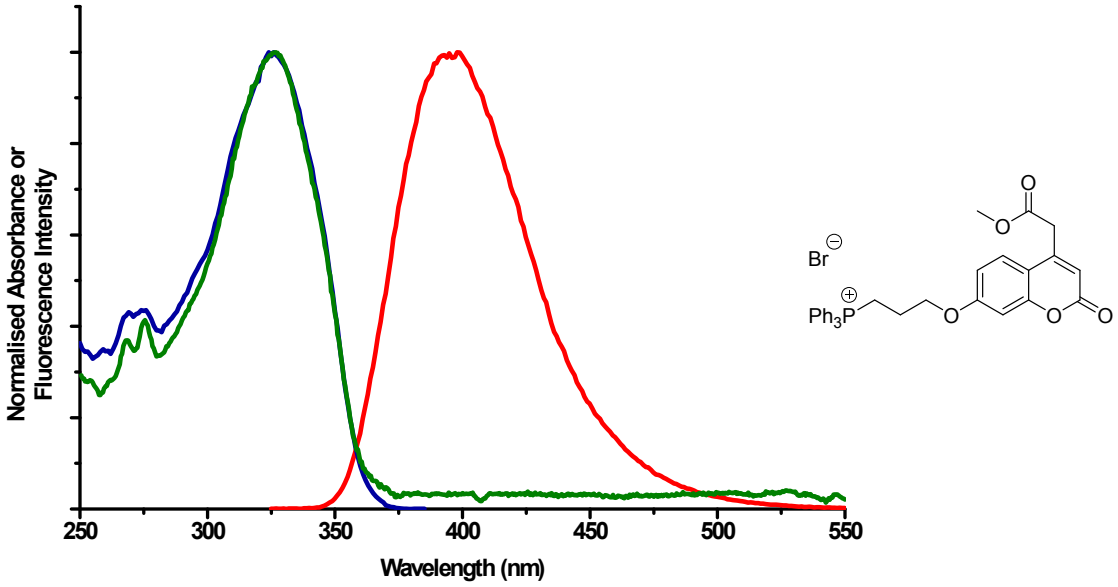
**2-(7-Methoxy-2-oxo-2H-chromen-4-yl)acetic acid 2**



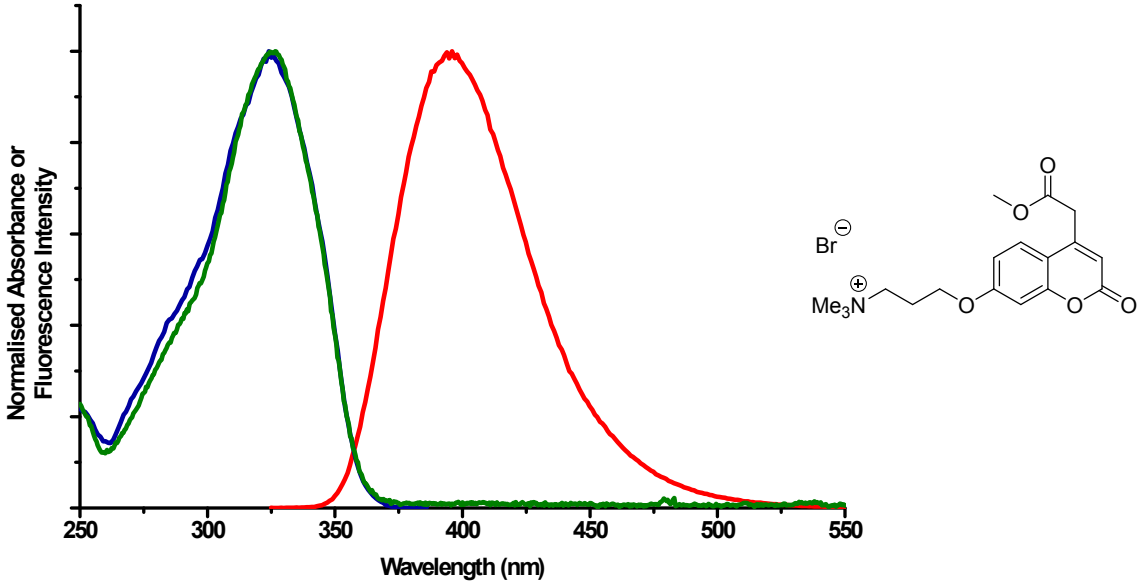

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7yl)oxy)-propyl)triphenylphosphonium bromide 5**



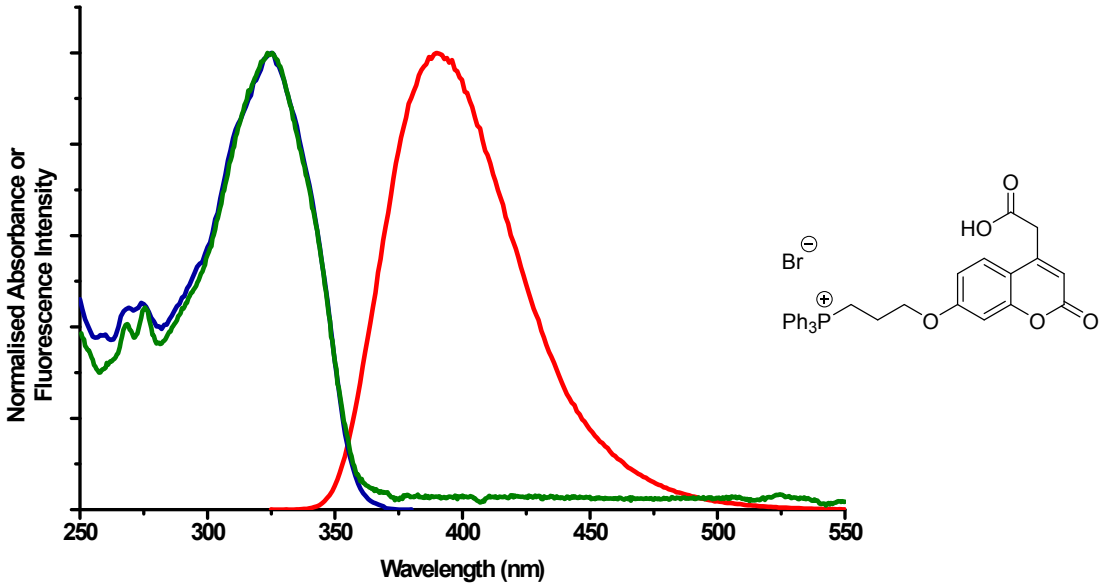

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)-N,N,N-trimethylpropan-1-aminium bromide 6**



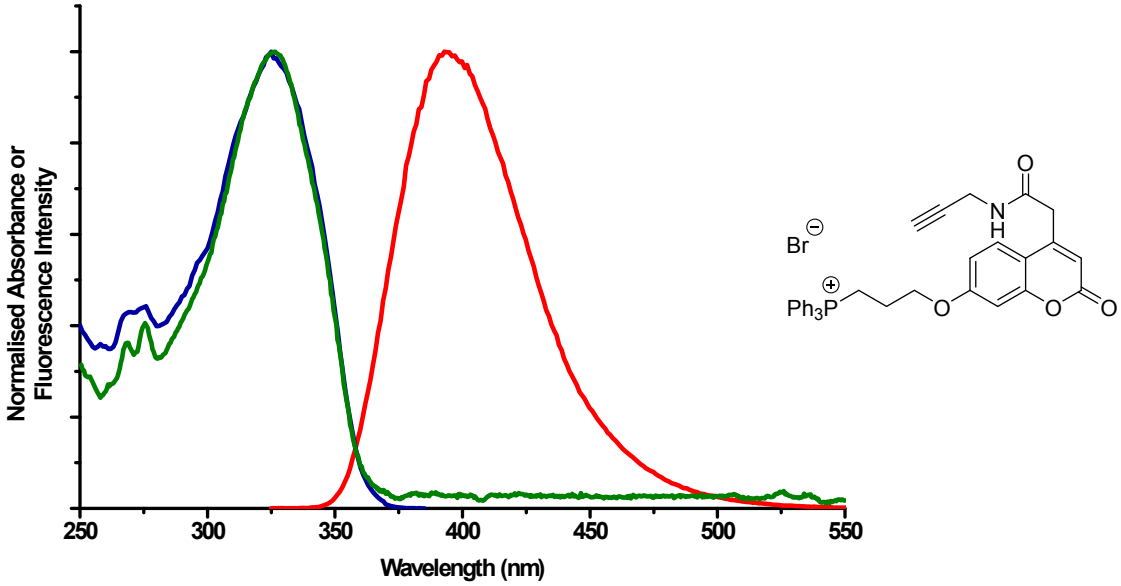

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((4-(carboxymethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-triphenylphosphonium bromide 7**



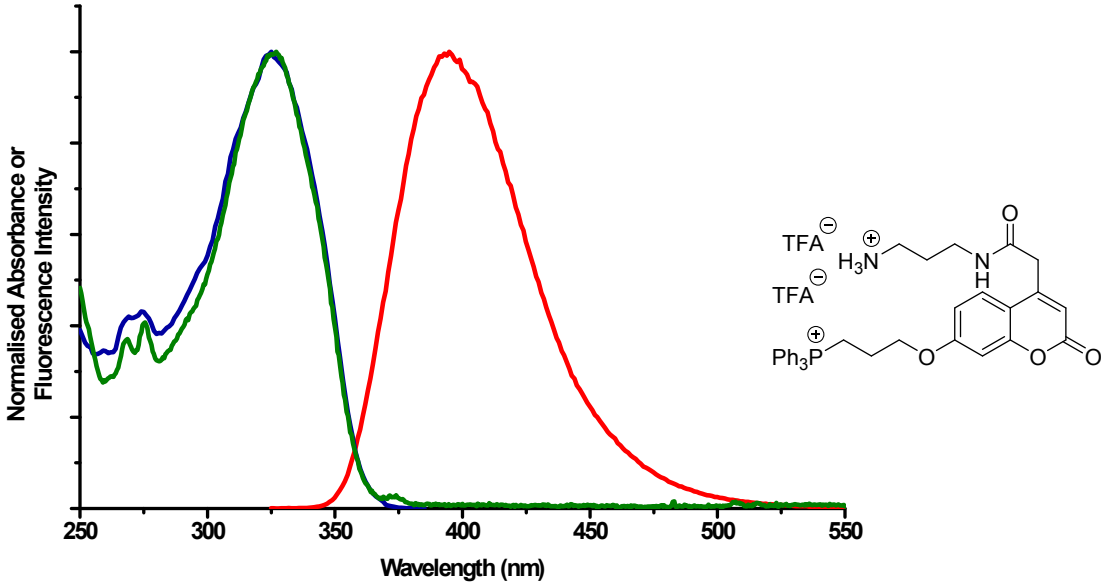

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((2-oxo-4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium bromide 10**



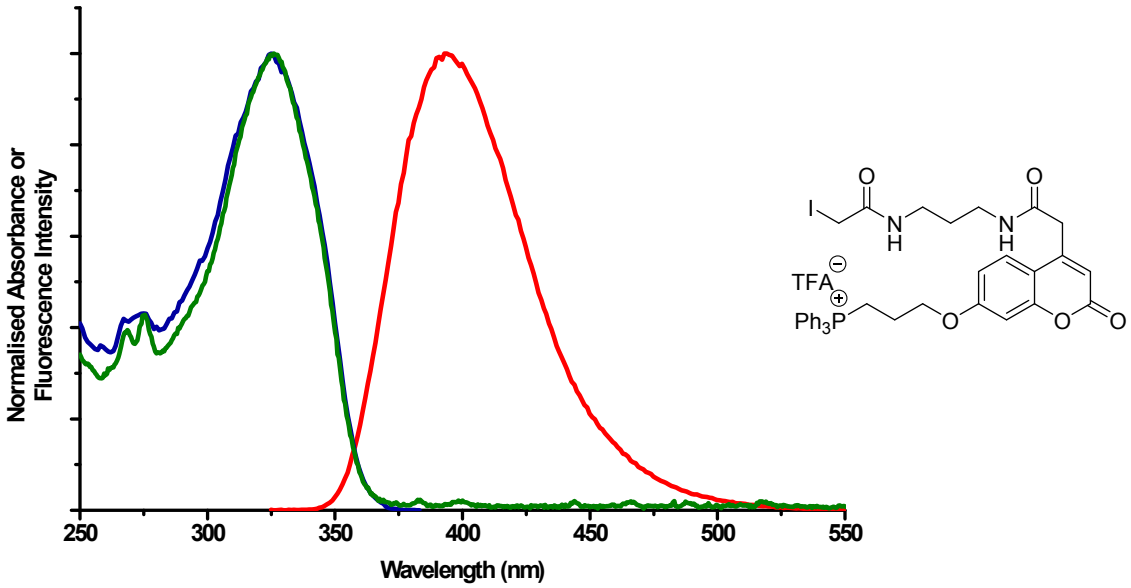

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((4-(2-((3-aminopropyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyltriphenyl-phosphonium bromide 13**



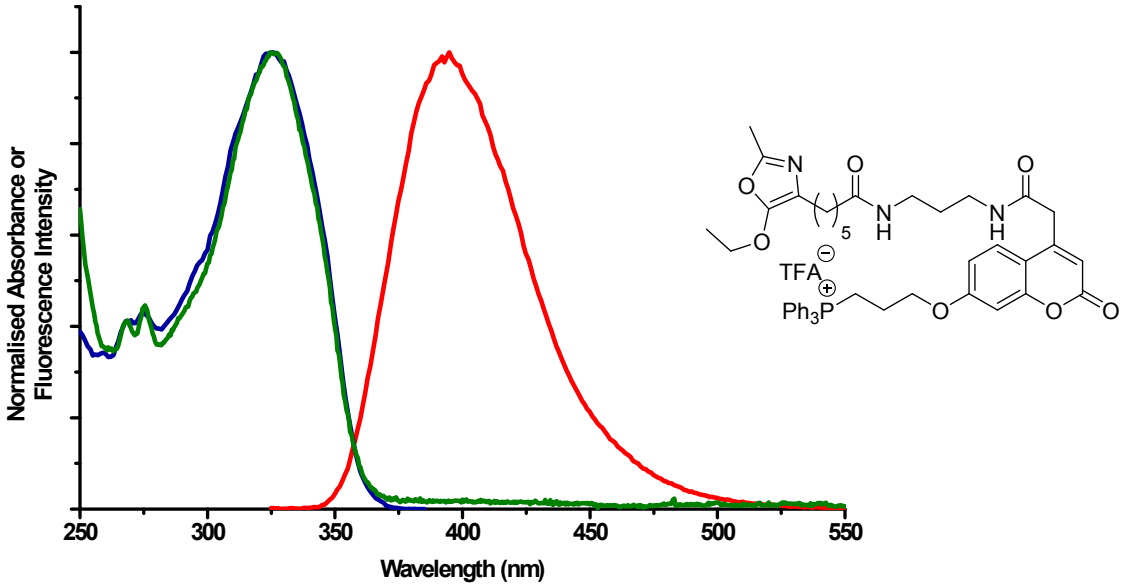

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((4-(2-((3-(2-iodoacetamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenyl-phosphonium 2,2,2-trifluoroacetate 14**



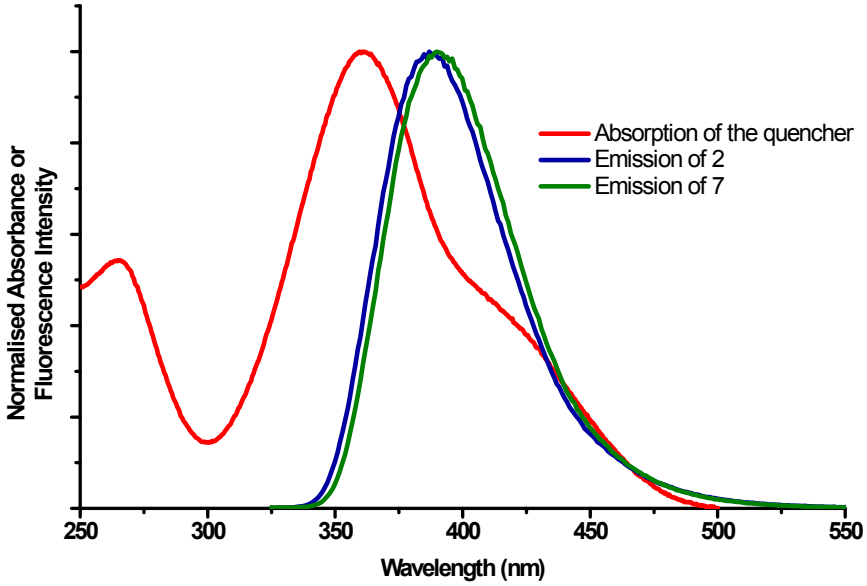

Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

**(3-((4-(2-((3-(6-(5-ethoxy-2-methyloxazol-4-yl)hexanamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 15**




Absorption (green), excitation (blue, $\lambda_{em}$ = 390 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

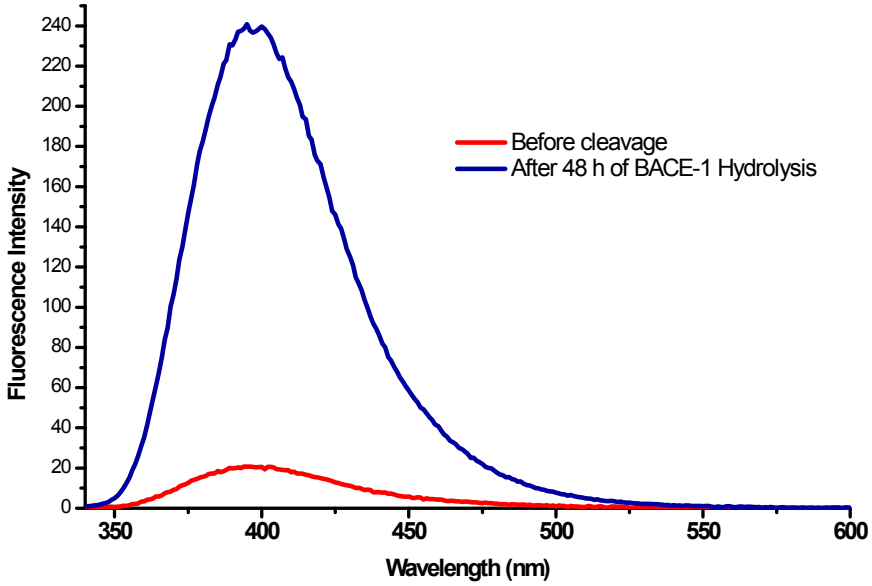
## 2) Absorption of the quencher, emission of coumarins 2 and 7 spectra superimposition




Quencher (2,4-dinitrophenyl)glycine absorption (red), coumarin **2** emission (blue, $\lambda_{ex}$ = 315 nm), coumarin **7** emission (green, $\lambda_{ex}$ = 315 nm), spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

## 3) Quenching efficiency and fluorescence exaltation of probes 19 and 20 after BACE-1 hydrolysis

**(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19**

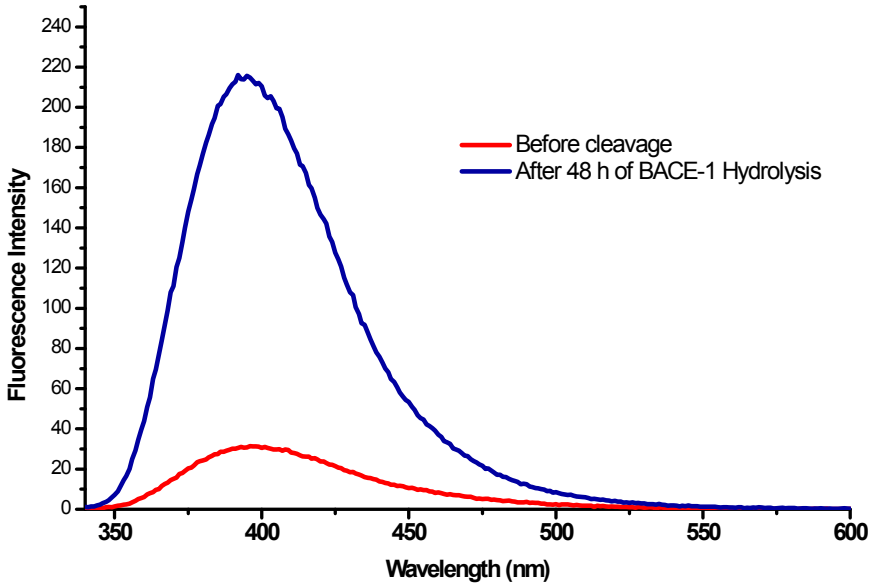



Emission ($\lambda_{ex}$ = 328 nm) spectra in acetate buffer (pH 4.48, 0.1 M) at 25 °C.

Quenching Efficiency = 91 %

Fluorescence Exaltation = 11

**($PPh_3C$)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20**



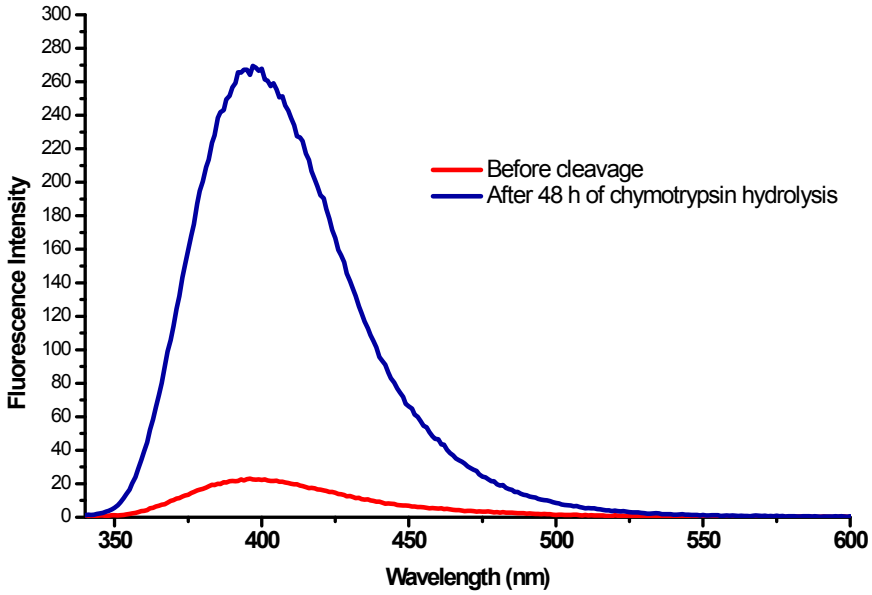

Emission ($\lambda_{ex}$ = 328 nm) spectra in acetate buffer (pH 4.48, 0.1 M) at 25 °C.

Quenching Efficiency = 84 %

Fluorescence Exaltation = 6.2

## 4) Quenching efficiency and fluorescence exaltation of probes 19 and 20 after α-Chymotrypsin hydrolysis

**(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19**

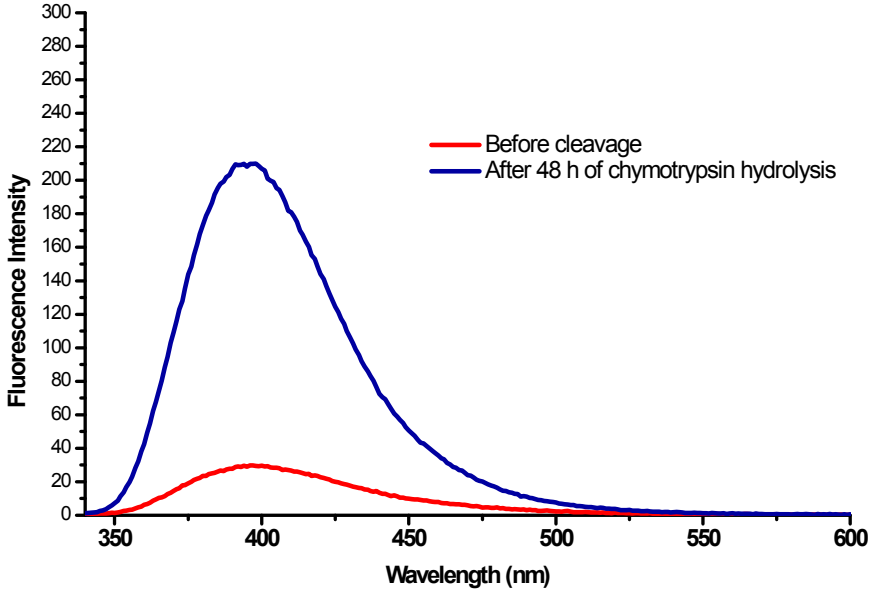



Emission ($\lambda_{ex}$ = 328 nm) spectra in Tris buffer (pH 7.8, 0.1 M Tris + 10 mM $CaCl_2$) at 25 °C.

Quenching Efficiency = 91 %

Fluorescence Exaltation = 10.7

**($PPh_3C$)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20**



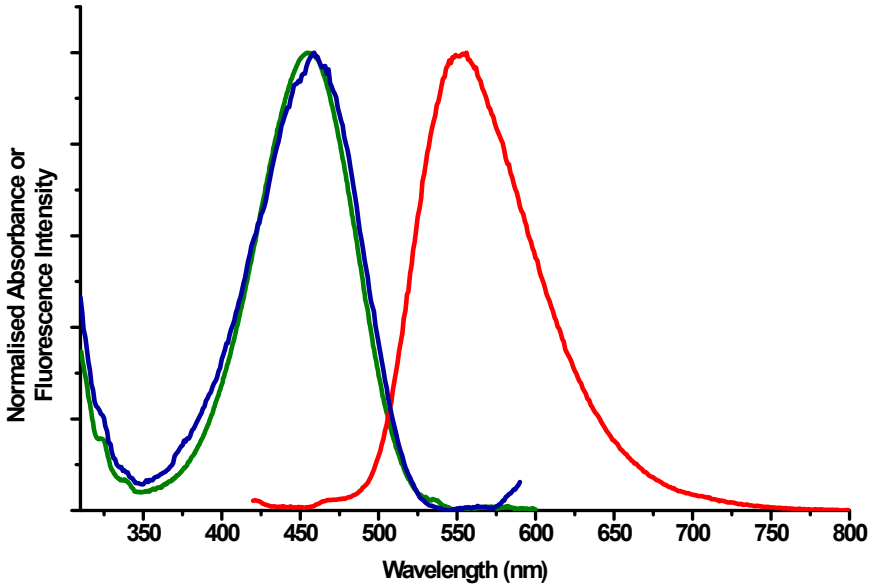

Emission ($\lambda_{ex}$ = 328 nm) spectra in Tris buffer (pH 7.8, 0.1 M Tris + 10 mM $CaCl_2$) at 25 °C.

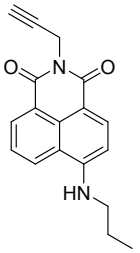
Quenching Efficiency = 84 %

Fluorescence Exaltation = 6.3

## 5) Absorption, excitation, emission spectra of naphthalimide derivatives

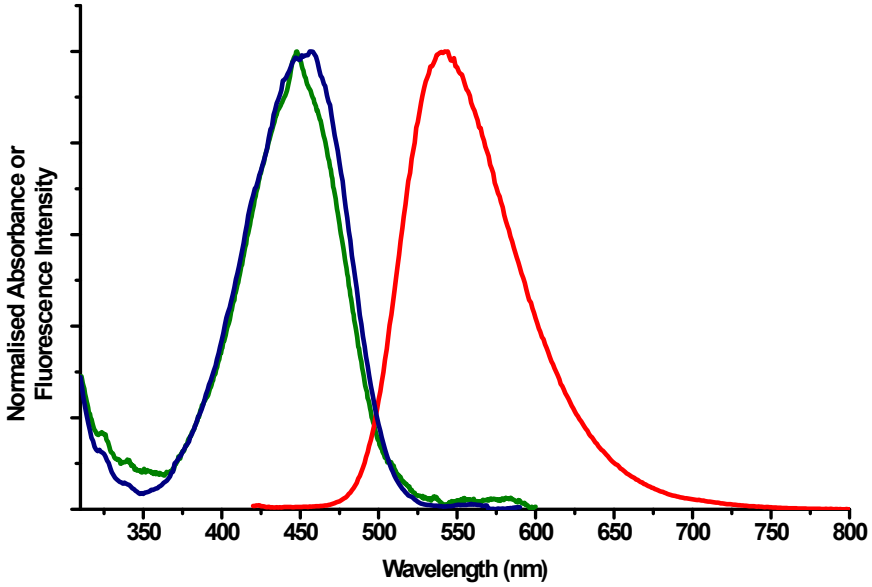
**2-(prop-2-yn-1-yl)-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione 28**

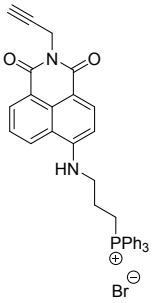



Absorption (green), excitation (blue, $\lambda_{em}$ = 600 nm), emission (red, $\lambda_{ex}$ = 410 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

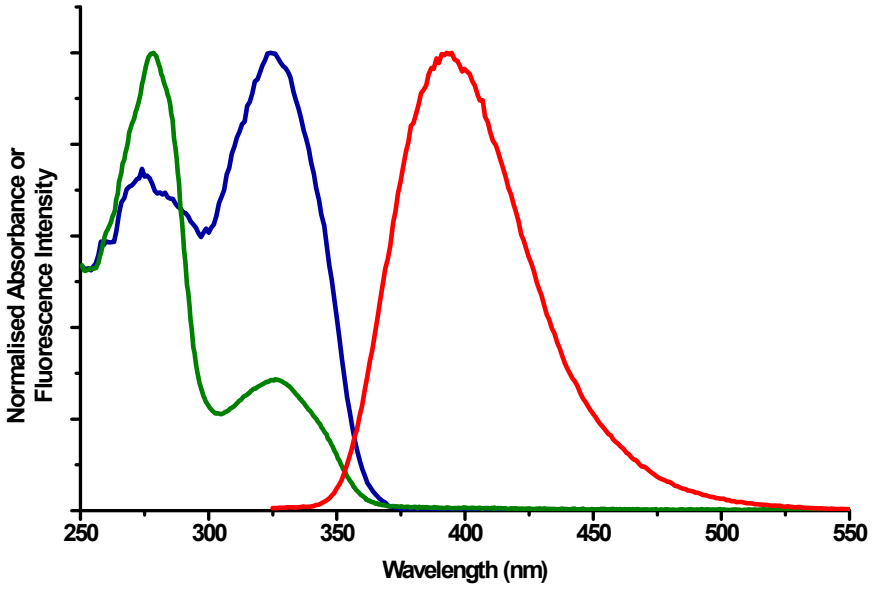
**(3-((1,3-dioxo-2-(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)propyl)triphenylphosphonium bromide 29**



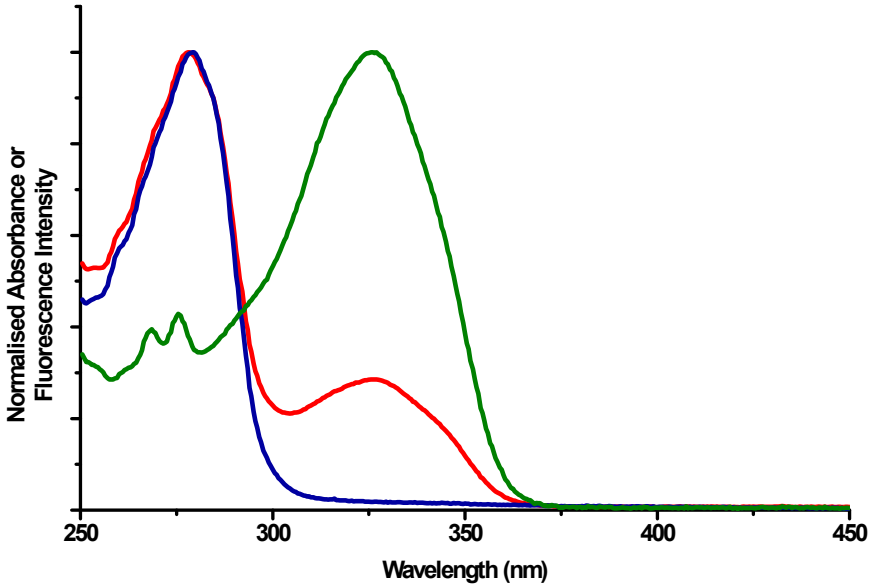

Absorption (green), excitation (blue, $\lambda_{em}$ = 600 nm), emission (red, $\lambda_{ex}$ = 410 nm) spectra in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

## 6) Absorption, excitation, emission spectra of labeled BSA




Absorption (green), excitation (blue, $\lambda_{em}$ = 425 nm), emission (red, $\lambda_{ex}$ = 315 nm) spectra in milliQ water at 25 °C.

## 7) Determination of *F/P* ratio of labeled BSA




Absorption spectra of unlabeled BSA (blue), Absorption spectra of **14** (green), absorption spectra of labeled BSA (red) in PBS (pH 7.4, 0.1 M phosphate + 0.15 M NaCl) at 25 °C.

The degree of labeling of the BSA protein, *i.e.* the fluorophore:protein (*F/P*) molar rations, were estimated from the relative intensities of protein and dye absorption, according to the following equation:

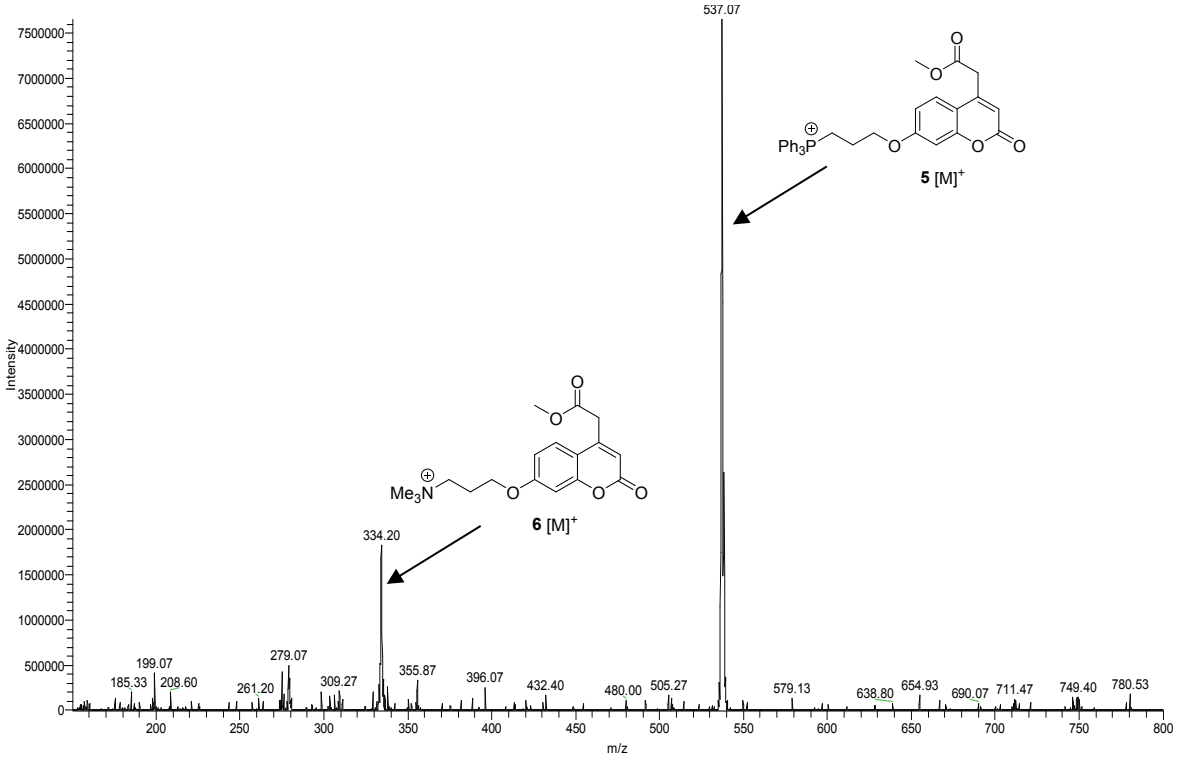
$$\frac{F}{P} = \frac{\mathrm{Cf}}{\mathrm{Cp}} = \frac{\varepsilon \mathrm{P},280\ \mathrm{A326}}{\varepsilon \mathrm{F},326\ (\mathrm{A280}\ -\ (f \times\ \mathrm{A326}))}$$

The molar absorption coefficient of the BSA protein $\varepsilon_{p,280}$ was determined to be 45551 $M^{-1}$ $cm^{-1}$ at 280 nm in milliQ water at 25 °C. The molar absorption coefficient of **14** $\varepsilon_{F,326}$ was determined to be 12252 $M^{-1}$ $cm^{-1}$ at 326 nm in milliQ water at 25 °C. The correction factor *f* equal to the ration of fluorophore absorbance at 280 and 326 nm was calculated to be 0.3469.

For labeled BSA protein, $A_{280}$ = 0.4077 and $A_{326}$ = 0.1186; allowing *F/P* = 1.2.

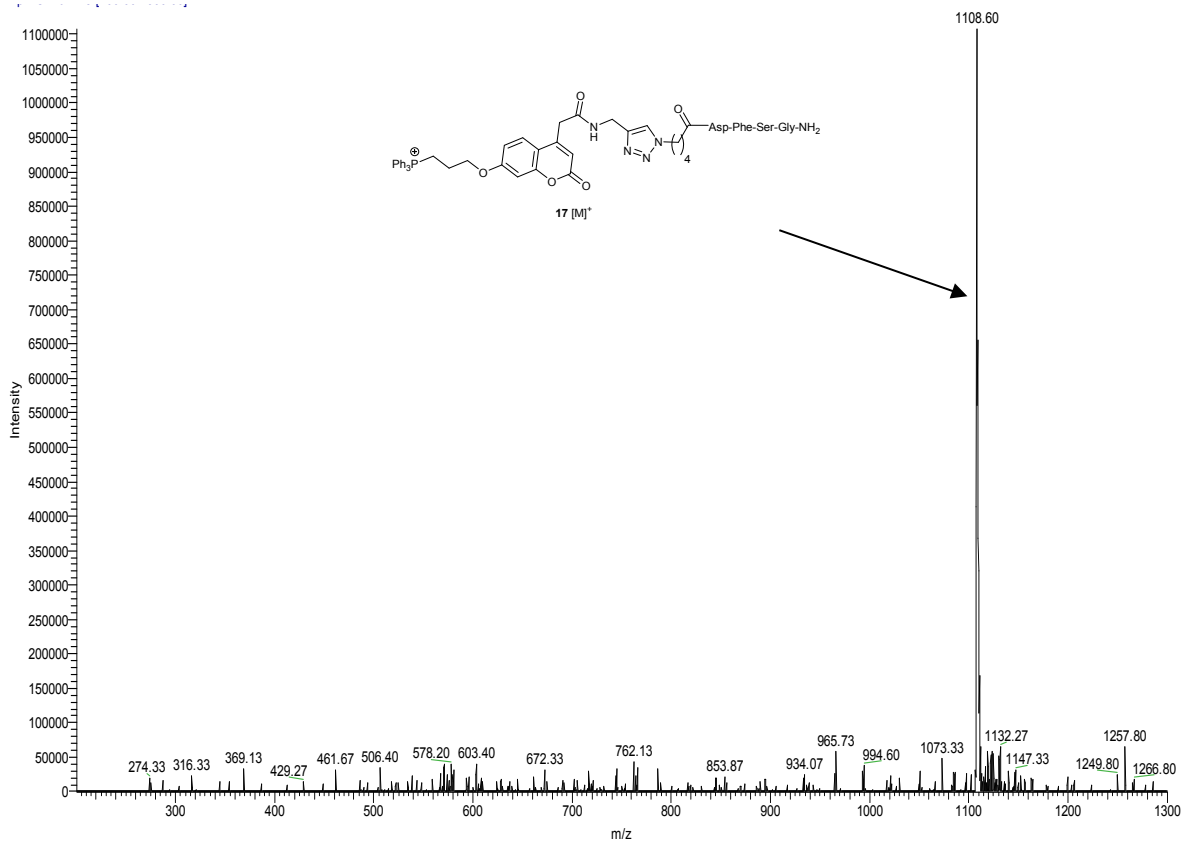
# 4) Mass spectrometry analyses

## 1) ESI-MS analysis of a 2.17 x $10^2$ µM solution of coumarins 1 - 5 -6 in a 1:1:1 molar ratio.



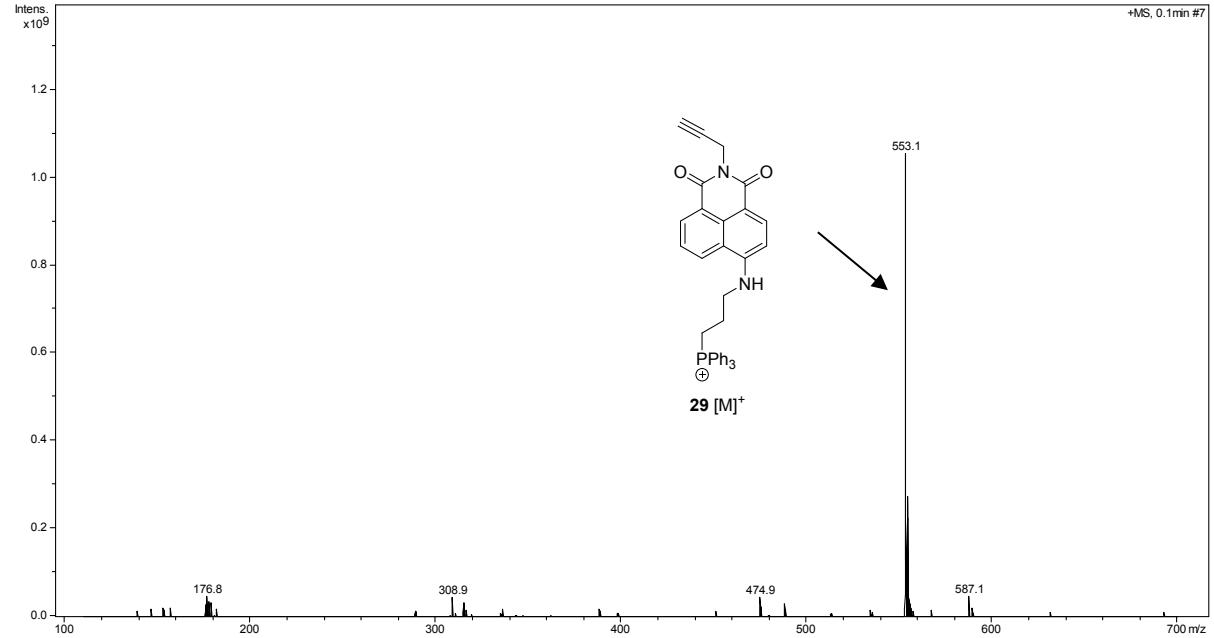

ESI-MS parameters : $ESI^+$; Full scan mode; Sheath Gas Flow Rate (arb): 20; Aux/Sweep Gas Flow Rate (arb): 0; I Spray Voltage (kV) I: 5.4; Capillary Temp (°C): 220; Capillary Voltage (V): 36; Tube Lens Offset (V): 50; Injection flow rate (ml/min): 0.25

## 2) ESI-MS analysis of a 5 x $10^2$ µM solution of peptide 16-17 in a 1:1 molar ratio.




ESI-MS parameters : ESI$^+$; Full scan mode; Sheath Gas Flow Rate (arb): 20; Aux/Sweep Gas Flow Rate (arb): 0; I Spray Voltage (kV) I: 5.4; Capillary Temp (°C): 220; Capillary Voltage (V): 36; Tube Lens Offset (V): 50; Injection flow rate (ml/min): 0.25

## 3) ESI-MS analysis of a 2.17 x $10^2$ µM solution of 28 and 29 in a 1:1 molar ratio.

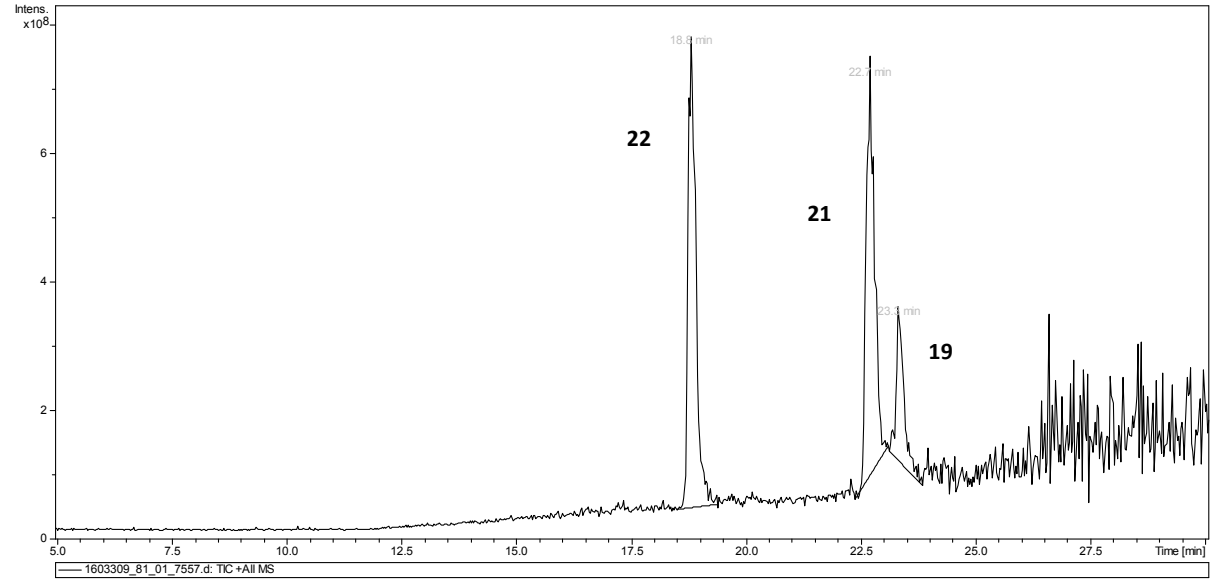



ESI-MS parameters (HCT Ultra ETD II mass spectrometer): $ESI^+$; Full scan mode; Nebulizer Gas Flow Rate (Psi): 20; Dry Gas Flow Rate (L/min.): 5; Dry Temp (°C): 300; Capillary Voltage (V): -3000; Skimmer (V): 40; Injection flow rate (ml/min): 0.25.

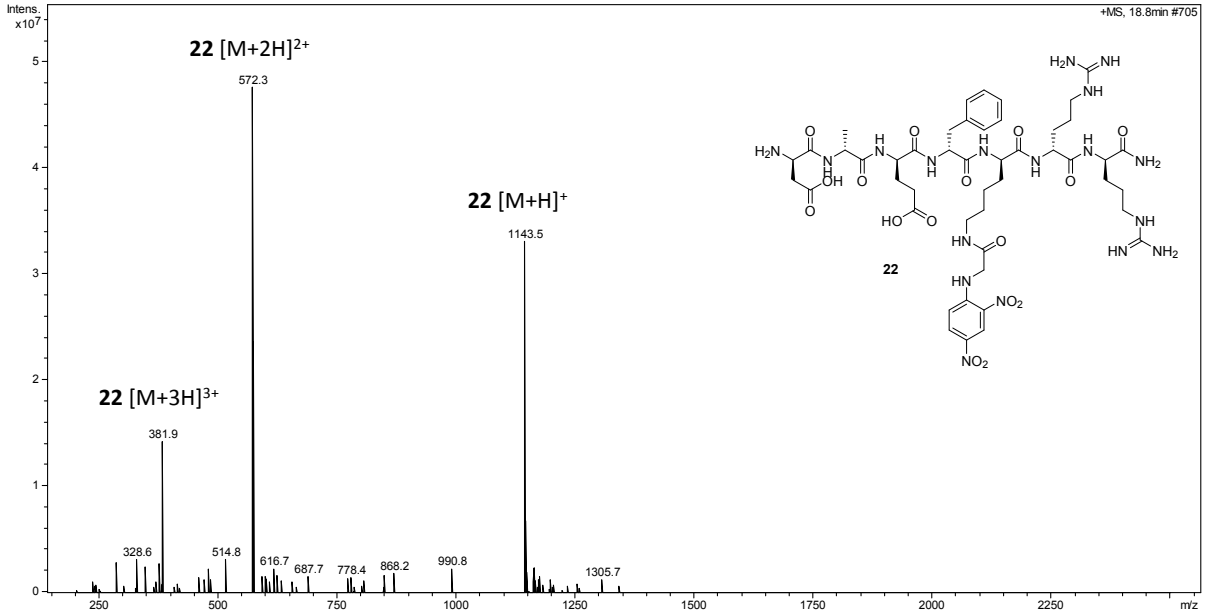
## 4) LC -MS analysis of the crude BACE1-mediated hydrolysis (solution of 19 and 20 at 25 µM).

**(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19**

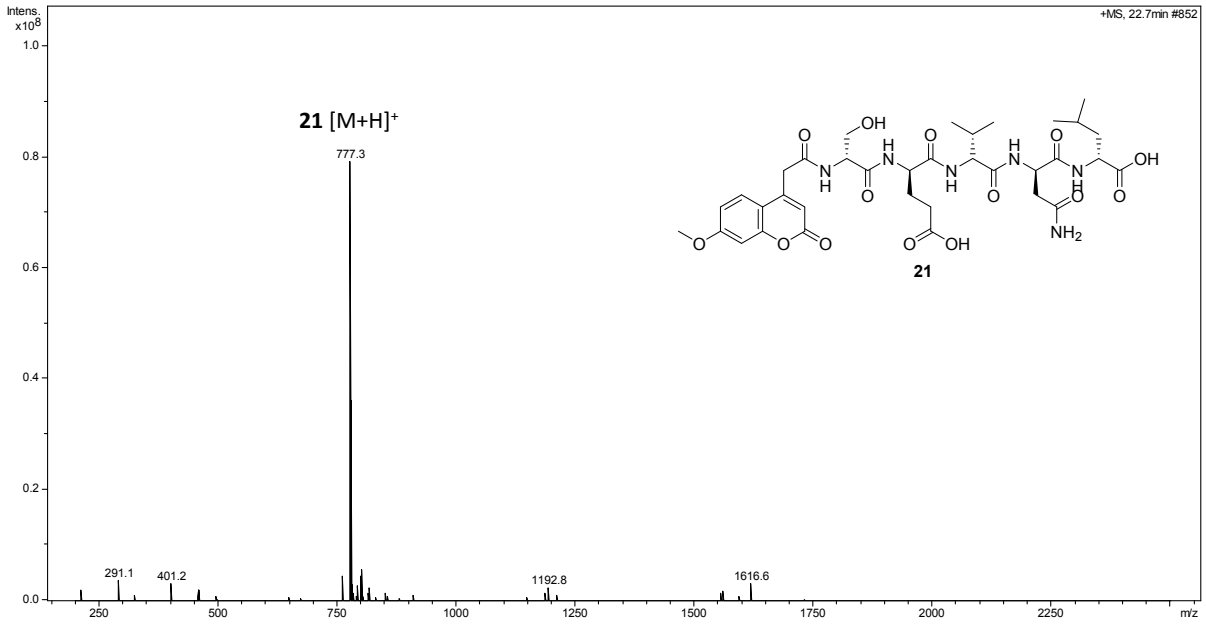
Total ion current (TIC) chromatogram:



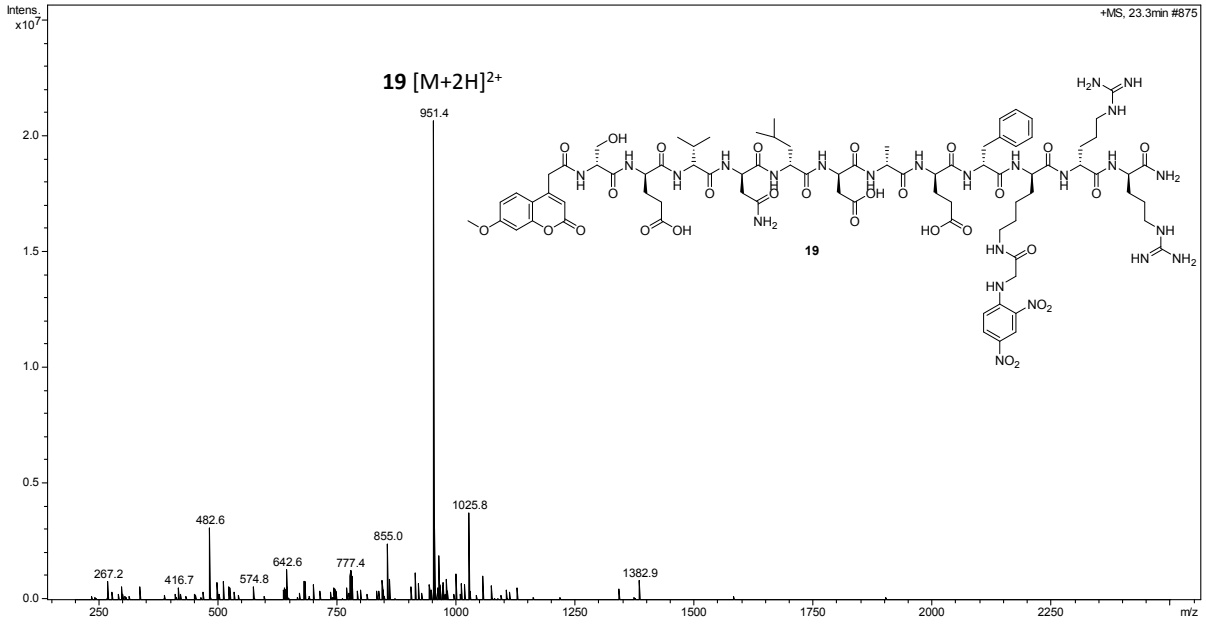

ESI mass spectrum of the compound **22** ($t_R$ = 18.8 min).



ESI mass spectrum of the compound **21** ($t_R$ = 22.7 min).

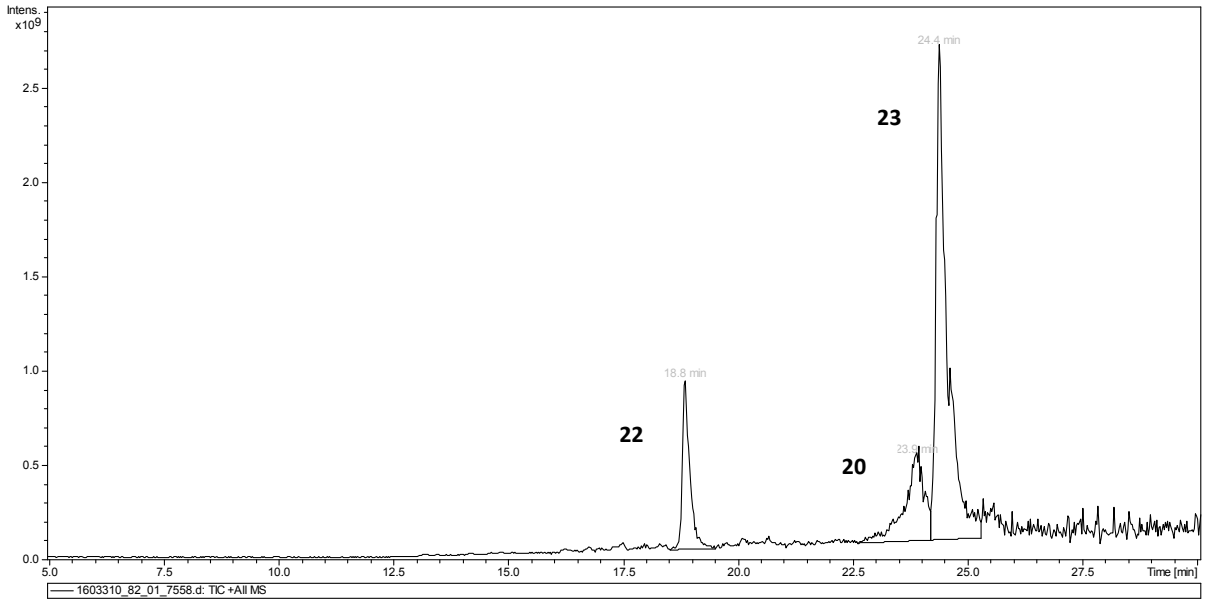



ESI mass spectrum of the compound **19** ($t_R$ = 23.3 min).

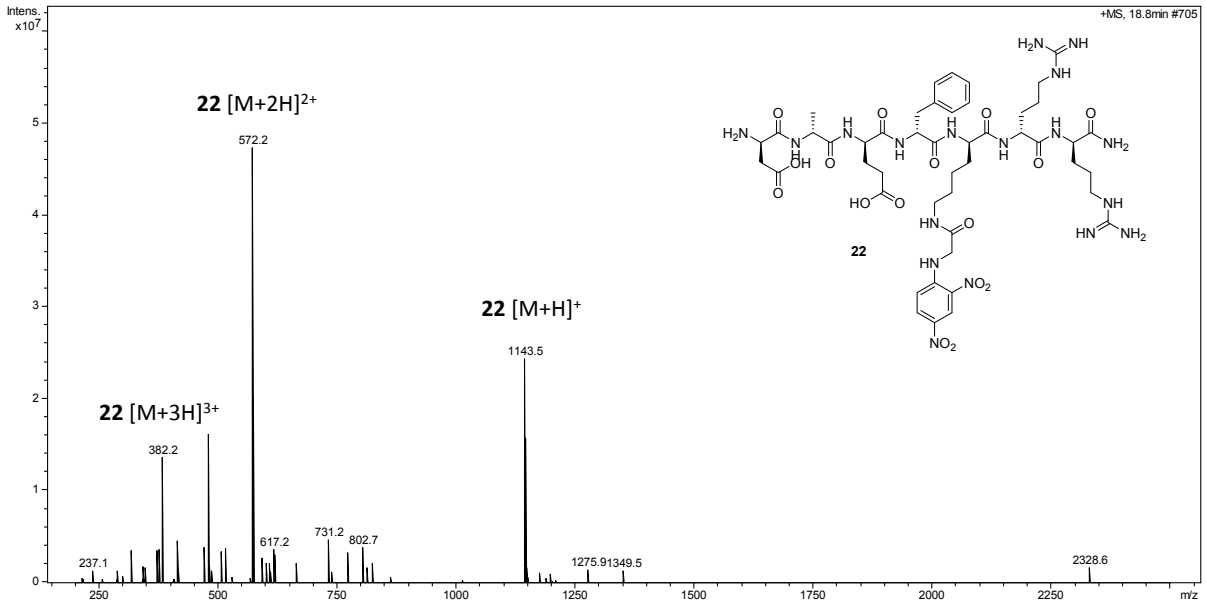


**(PPh$_3$C)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20**

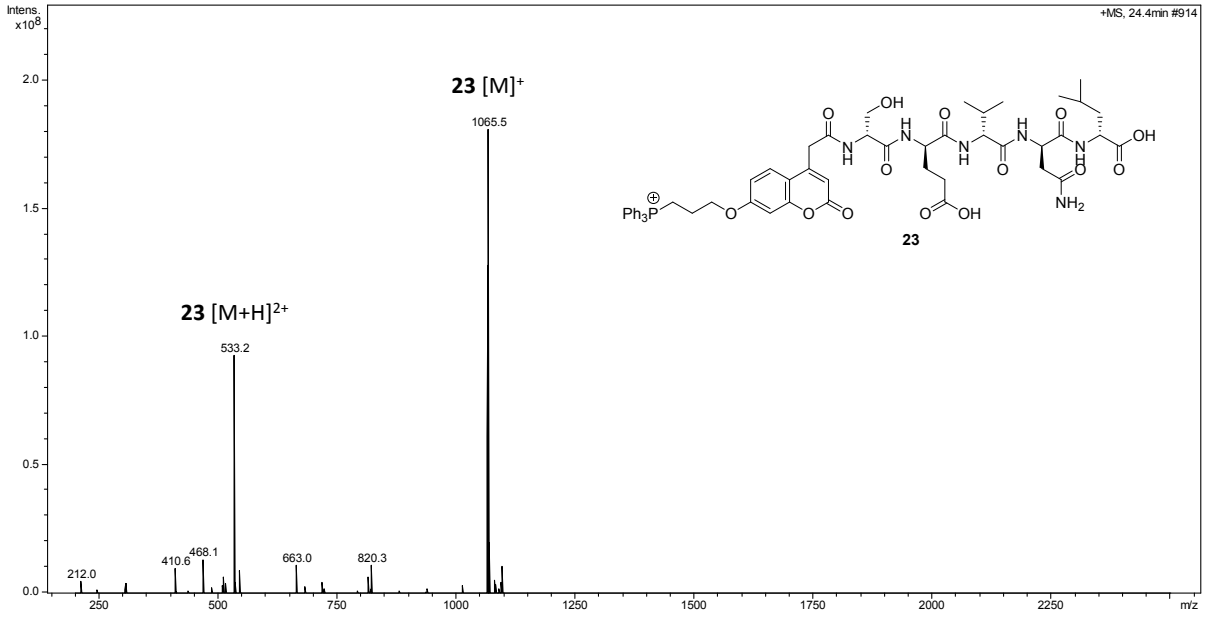
Total ion current (TIC) chromatogram:



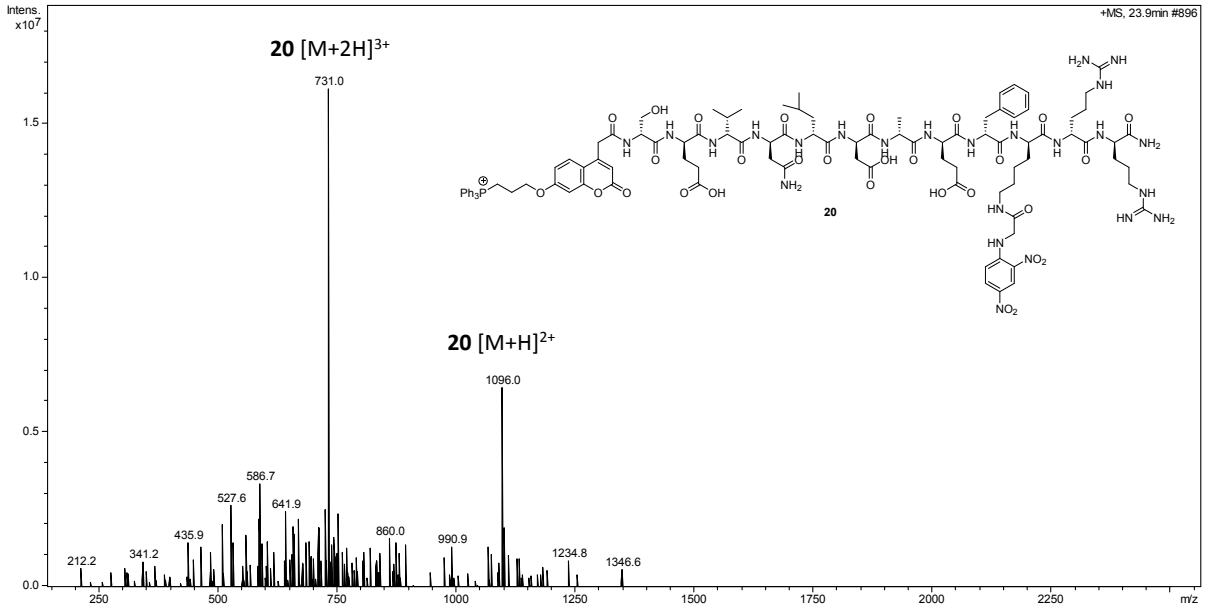

ESI mass spectrum of the compound **22**. ($t_R$ = 18.8 min)



ESI mass spectrum of the compound **23**($t_R$ = 24.4 min).



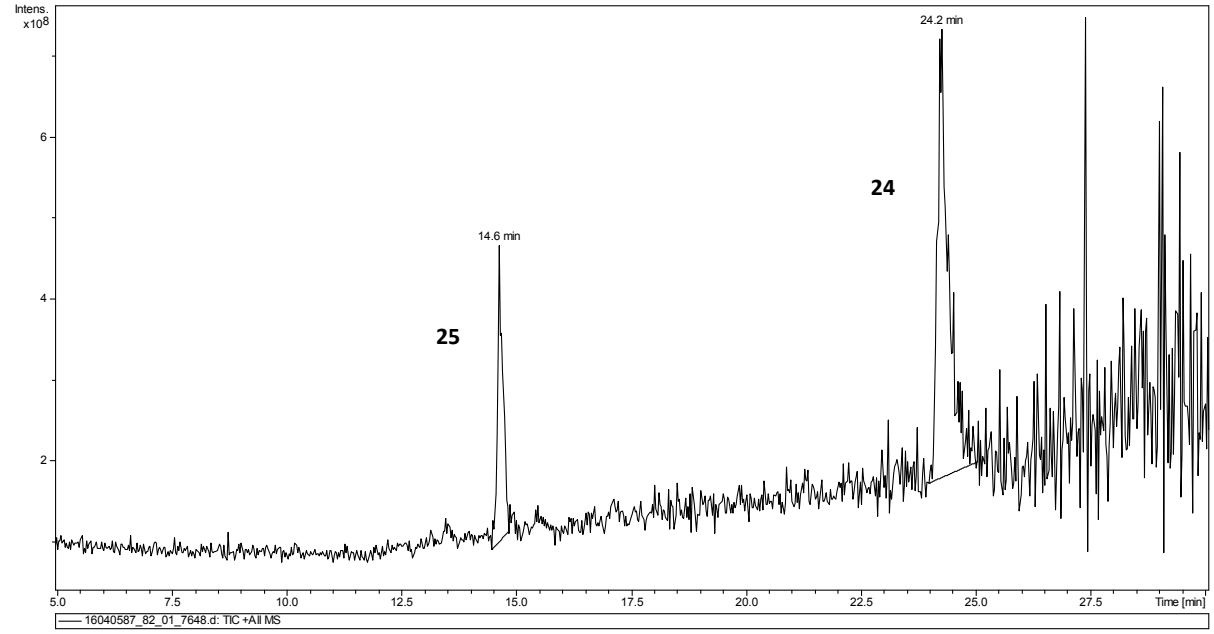

ESI mass spectrum of the compound **20** ($t_R$ = 23.9 min).



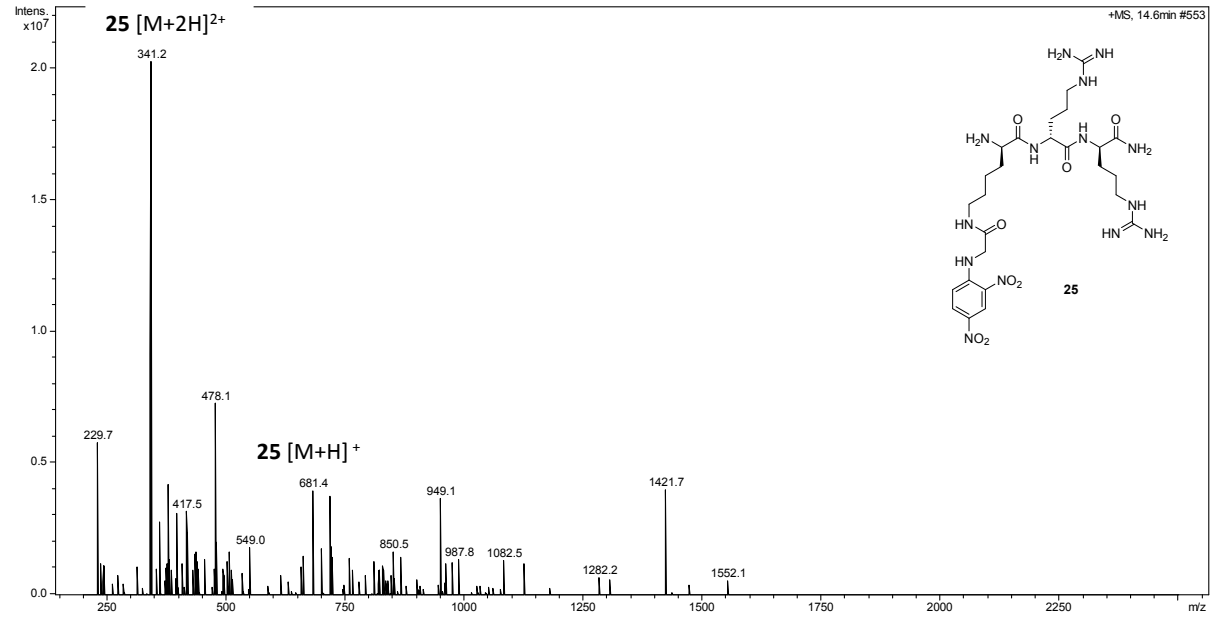
## 5) LC -MS analysis of the crude α-Chymotypsin-mediated hydrolysis (solution of 19 and 20 at 25 µM).

**(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19**

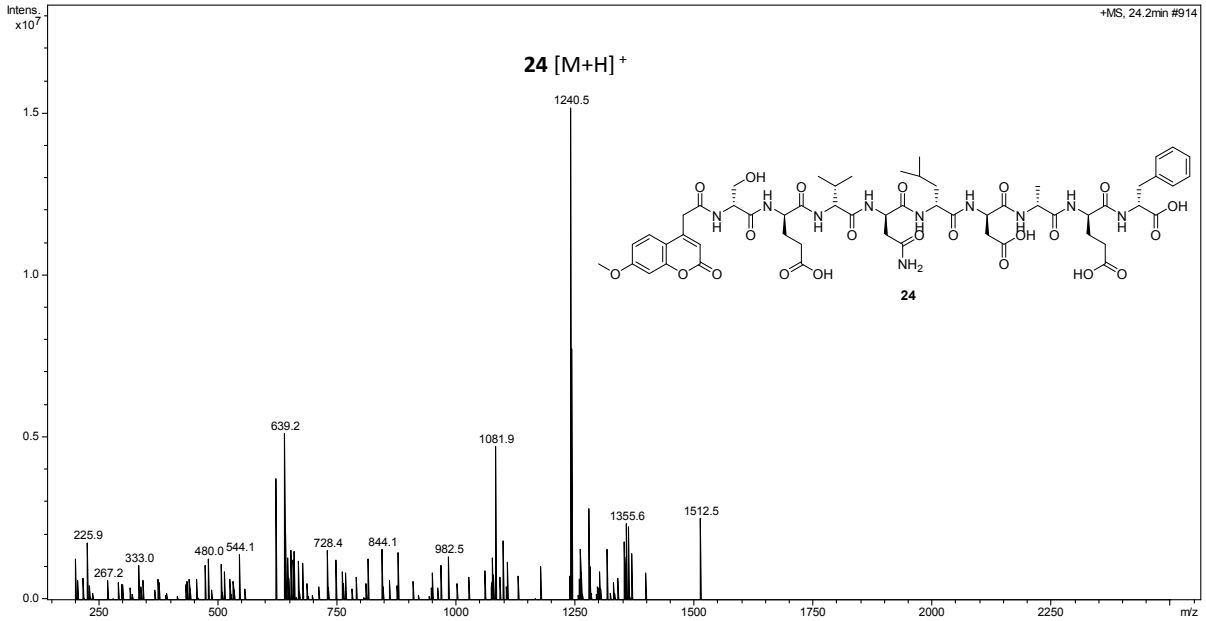
Total ion current (TIC) chromatogram:




ESI mass spectrum of the compound **25** ($t_R$ = 14.6 min).

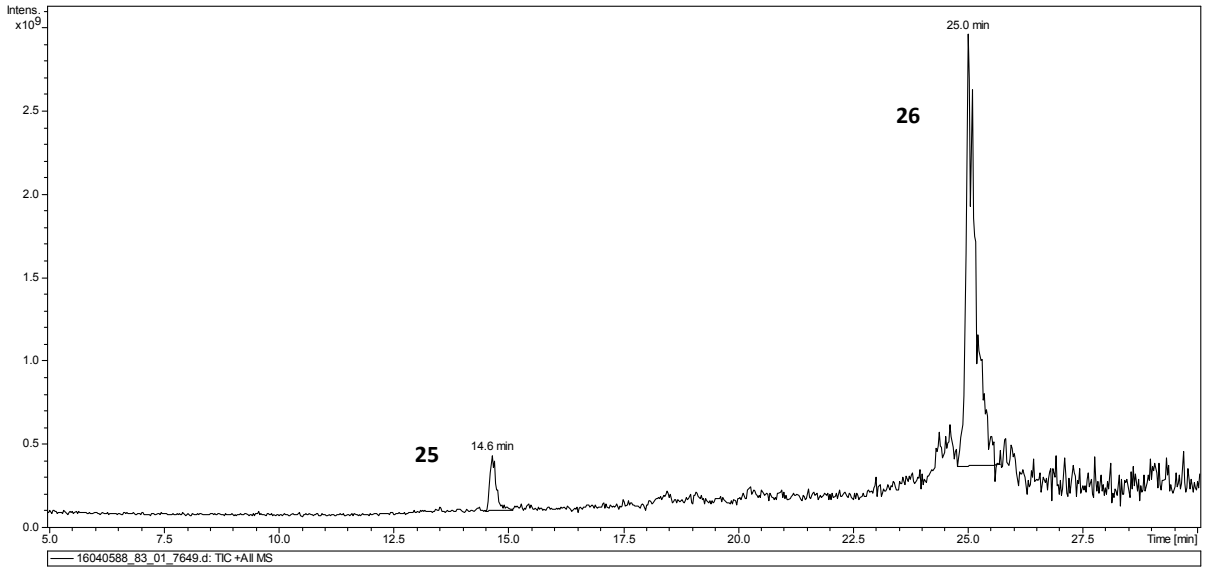


ESI mass spectrum of the compound **24** ($t_R$ = 24.2 min).

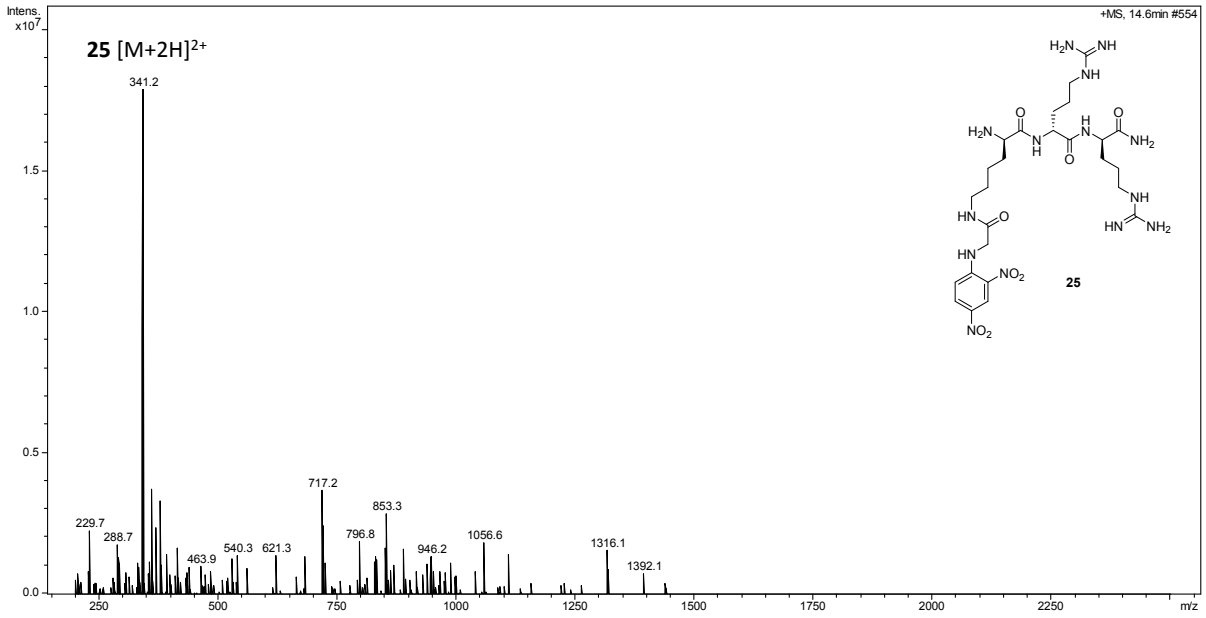



**(PPh$_3$C)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20**

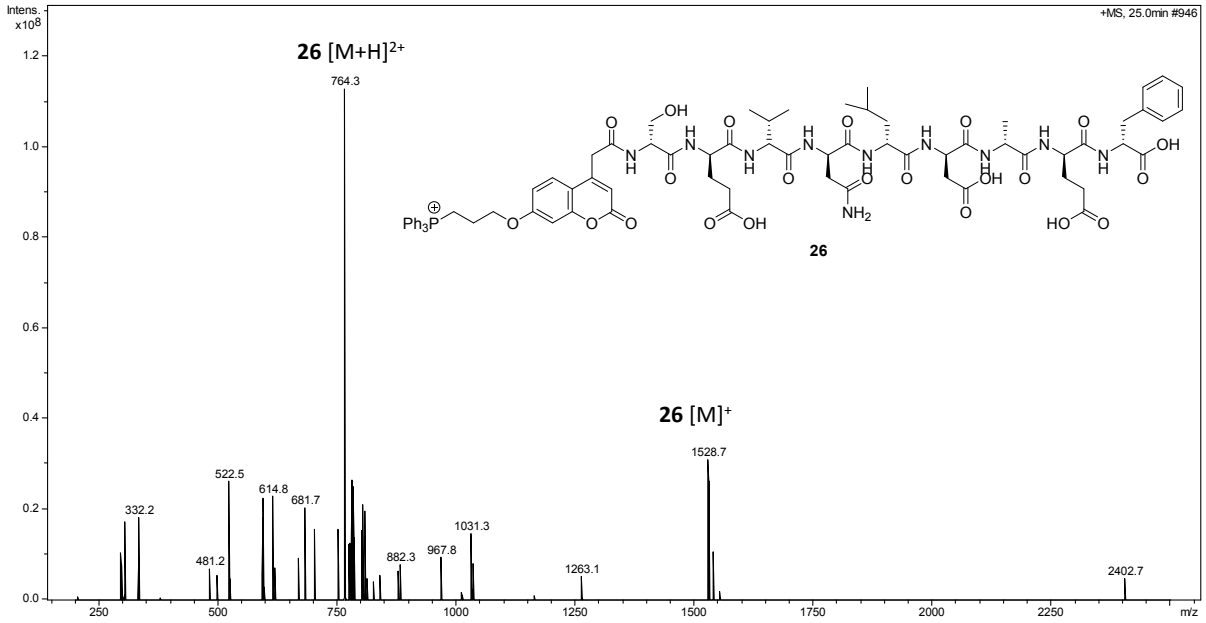
Total ion current (TIC) chromatogram:



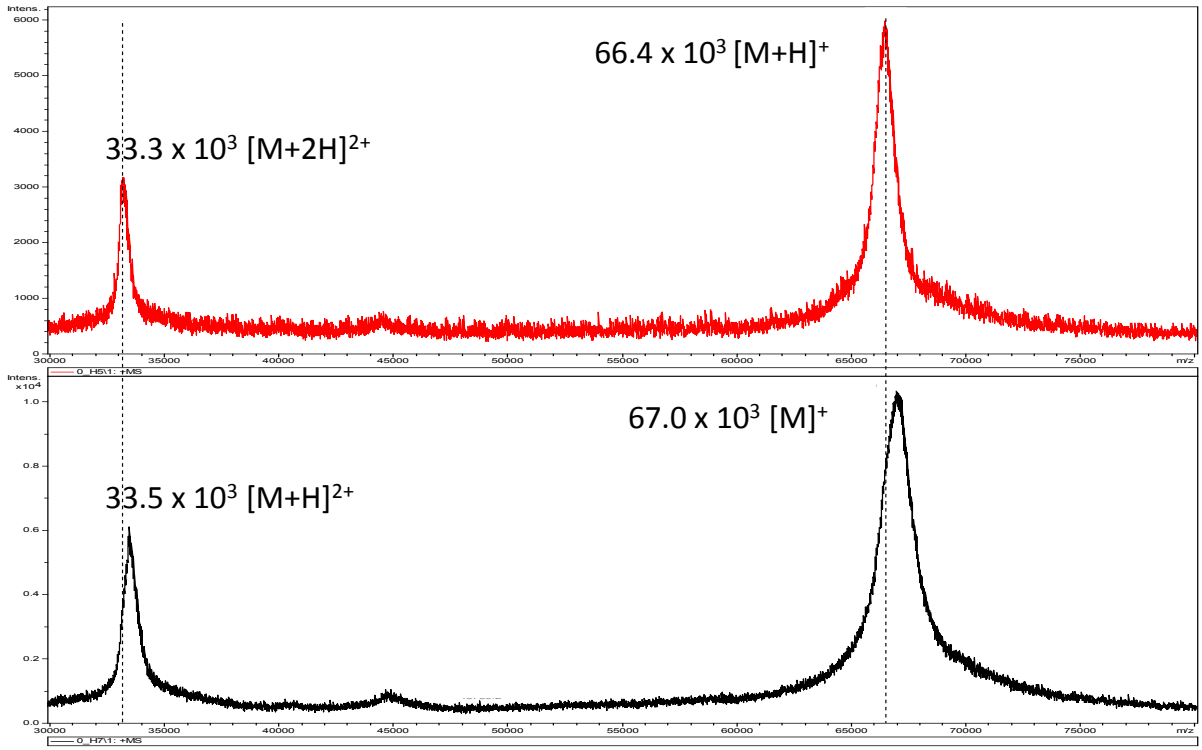
ESI mass spectrum of the compound **25** ($t_R$ = 14.6 min).




ESI mass spectrum of the compound **26** ($t_R$ = 25.0 min).

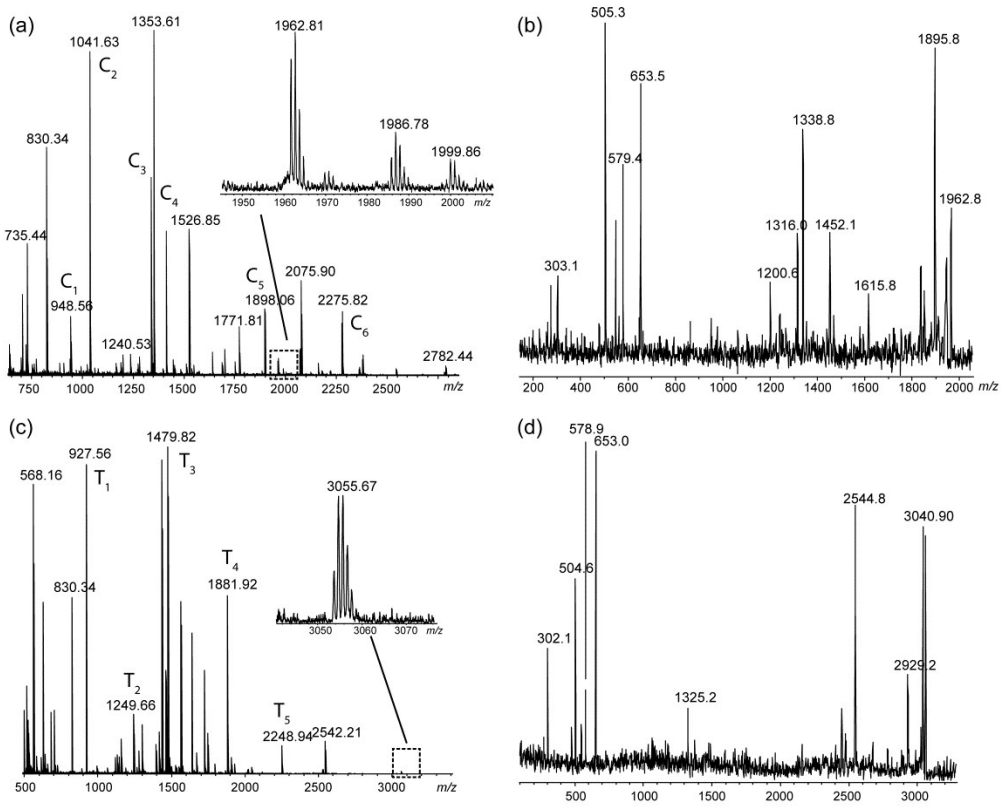


# 6) MALDI-TOF analysis of unlabeled and labeled BSA protein.

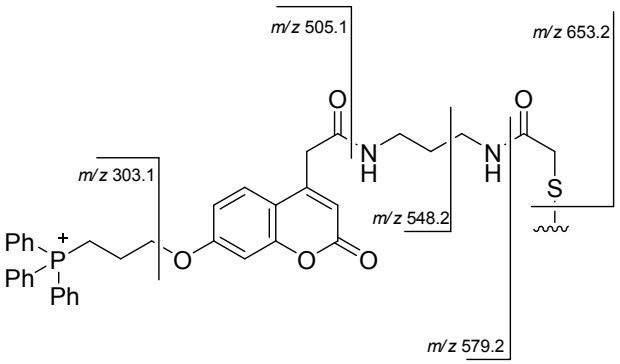



MALDI-TOF analysis of unlabeled BSA (red) and labeled (black) protein.

## 7) MALDI mass spectra of Chymotrypsin and Trypsin digests of modified BSA.




**a)** MALDI mass spectrum of chymotrypsin digest of modified BSA (CHCA matrix - Cn : chymotryptic peptides.) The enlargement of the *m/z* 1950-2010 range showing the chymotryptic peptide at *m/z* 1961.82 containing the fluorophore group is on the top right of the figure. **b)** MALDI-MS/MS spectrum of the precursor ion at *m/z* 1961.82. The fragment ions at *m/z* 303.1, 505.3, 579.4 and 653.5 are specific of the fluorophore group. **c)** MALDI mass spectrum of trypsin digest of modified BSA (CHCA matrix - Tn : tryptic peptides.) The enlargement of the *m/z* 3040-3070 range showing the tryptic peptide at *m/z* 3053.67 containing the fluorophore group is on the top right of the figure. **d)** MALDI-MS/MS spectrum of the precursor ion at *m/z* 3053.67. The fragment ions at *m/z* 302.1, 504.6, 578.9 and 653.0 are specific of the fluorophore group.




Fragment ions of the fluorophore group.