

Supporting Information

Small-molecule optical probes for live-cell imaging of protein sulfenylation and its application to monitor cisplatin induced protein oxidation

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1. Experimental details for the synthesis of all compounds reported in the paper

Chemical Synthesis. *General Procedures and Materials.* Unless otherwise stated, all reagents were purchased from chemical suppliers and used without further purification, room temperature corresponds to ambient temperature and yields refer to spectroscopically and chromatographically pure compounds. Reactions were monitored by thin layer chromatography (TLC) performed on commercially available glass plates pre-coated with Merck silica gel 60 F254 or Merck silica gel 60 RP-18 F254s. Visualisation was by the quenching of UV fluorescence (λ_{max} = 254/365 nm) and by staining with iodine or potassium permanganate. All flash chromatography was carried out using slurry packed Merck 9325 Keisegel 60 or Aldrich C₁₈-reverse phase silica gel. Melting points were obtained using a Gallenkamp Melting Point apparatus and are uncorrected.

NMR spectra were recorded using an internal deuterium lock at ambient probe temperatures on a Bruker DRX-400 (400 MHz) instrument. Chemical shifts (δ) are quoted in ppm, to the nearest 0.01 ppm (for ¹H NMR spectra), or 0.1 ppm (for ¹³C NMR spectra), and are referenced to the residual non-deuterated solvent peak. Coupling constants (J) are reported in Hertz (Hz) to the nearest 0.5 Hz. Data are reported as follows: chemical shift, multiplicity (br= broad; s= singlet; d= doublet; t= triplet; m= multiplet, or as a combination of these, e.g. dd, dt, etc.), integration, assignment and coupling constant(s). Assignments were determined either on the basis of unambiguous chemical shift or coupling pattern, and analogy to fully interpreted spectra for related compounds. Diastereotopic protons are assigned as CH-*H*.

Liquid chromatography–mass spectrometry (LC-MS) experiments were performed on Waters Aquity UPLC I-CLASS coupled with Waters LCT Premier (operating in ES⁺ or ES⁻ mode). High resolution masses (HR-MS) for accurate mass determination were performed on the same equipment and samples referenced against Leucine Enkephalin or Sulfadimethoxine. For analytical HPLC, a Waters BEH Acquity C18 (50mm x 2.1mm) column was used and the mobile phase was composed of solvent A (99.9% Water, 0.1% Formic Acid) and solvent B (99.9% Acetonitrile, 0.1% Formic Acid) used in a linear gradient (time= 0 min, 95%A and 5%B; time= 3.2 min, 5%A and 95%B; time= 3.5 min, 95%A and 5%B; total run time 4 min). The sample solutions were prepared at a concentration of 0.1 mg/1 mL. The injection volume was 10 μ L, the flow rate was 0.5mL/min, the column temperature was 40 °C, and the UV detector wavelength was fixed at 210 to 280 nm. The values of retention time (t_R) are given in minutes. Electron spray ionisation (ESI) conditions were as follow: 2kV (ES⁺) and 2.5kV (ES⁻) capillary voltage; 30 V (ES⁺) and 150 V (ES⁺) sample cone voltage;

2.1kV MCP Voltage; 350 °C desolvation temperature; 120 °C source temperature; 10 L/h cone gas flow (N₂); 400 L/h desolvation gas flow (N₂). Mass values are reported within the error limits of ±5 ppm mass units.

UV-vis spectra of the probes were recorded using a Perkin-Elmer Lambda 25 UV-vis spectrophotometer. Fluorescence emission spectra were obtained on a Varian Cary Eclipse fluorescence spectrofluorimeter using 0.05-0.1 mg/mL sample solutions and 1.0 cm path-length quartz cuvettes (1.0-3.0 mL) at 25°C. Spectra were not corrected for light intensity or detector sensitivity. Data were recorded on-line and analysed by Excel software.

N-succinimidyl ester of 7-methoxycoumarin-3-carboxylic acid (**4**) and 3-methoxy-5-oxocyclohex-3-ene-1-carboxylic acid (**9**) were prepared following literature procedures [1,2].

Synthesis of tert-butyl-[4-(7-methoxy-2-oxo-2H-chromene-3-carboxamido)butyl]carbamate, 6. DMAP (13.2 mg, 0.11 mmol), **4** [1] (327.0 mg, 1.03 mmol) and DIPEA (0.38 mL, 2.16 mmol) were sequentially added to a stirred solution of **5** (0.21 mL, 1.08 mmol) in 8 mL of anhydrous CH₂Cl₂ and the reaction was carried out under N₂ for 5 h at room temperature. After evaporation of the solvent, the crude mixture was purified by flash column chromatography using CH₂Cl₂/MeOH/NH₄OH 95:5:0.5 as eluent. Yield = 400.0 mg, 1.02 mmol, 99.5%; mp = 153-54°C. ¹H NMR (400 MHz, CDCl₃) δ 8.74 (s, 1H, Ar), 8.71 (exch br t, 1H, NH), 7.51 (d, 1H, Ar, J = 8.8 Hz), 6.86 (dd, 1H, Ar, J = 6.4 Hz, J = 2.4 Hz), 6.78 (d, 1H, Ar, J = 2.4 Hz), 4.71 (exch br s, 1H, NH), 3.85 (s, 3H, OCH₃), 3.40 (q, 2H, NCH₂, J = 6.4 Hz), 3.11 (q, 2H, NCH₂, J = 6.0 Hz), 1.50-1.62 (m, 4H, NCH₂(CH₂)₂), 1.37 (s, 9H, Boc). LC-MS (ESI): *m/z* calcd. for C₂₀H₂₆N₂O₆ 390.4 (MW), found 391.2 [M+H]⁺, 413.2 [M+Na]⁺; *t*_R = 2.1. HR-MS (ESI): *m/z* calcd. for C₂₀H₂₇N₂O₆ 391.1869 [M+H]⁺, found 391.1872.

Synthesis of N-(4-aminobutyl)-7-methoxy-2-oxo-2H-chromene-3-carboxamide, 7. Intermediate **6** (400.0 mg, 1.02 mmol) was dissolved in CH₂Cl₂ (3.0 mL) and 1.0 mL of TFA was slowly added. The reaction was stirred at room temperature for 1.5 h and evaporated under vacuum. Excess of TFA was removed by five consecutive co-evaporations with CH₂Cl₂ to recover pure product **7** as TFA salt. Yield = 400.0 mg, 0.99 mmol, 96.8%; mp = 229-31°C. ¹H NMR (400 MHz, CD₃OD) δ 9.11 (exch br t, 1H, NH), 8.81 (s, 1H, Ar), 7.76 (d, 1H, Ar, J = 8.8 Hz), 7.04 (dd, 1H, Ar, J = 6.4 Hz, J = 2.4 Hz), 7.01 (d, 1H, Ar, J = 2.4 Hz), 3.94 (s, 3H, OCH₃), 3.48 (q, 2H, NCH₂, J = 4.0 Hz), 2.99 (t, 2H, NCH₂, J = 6.8 Hz), 1.70-1.75 (m, 4H, NCH₂(CH₂)₂). LC-MS

(ESI): m/z calcd. for $C_{15}H_{18}N_2O_4$ 290.3 (MW), found 291.1 $[M+H]^+$, 313.1 $[M+Na]^+$; t_R = 1.1. HR-MS (ESI): m/z calcd. for $C_{15}H_{19}N_2O_4$ 291.1345 $[M+H]^+$, found 291.1352.

Synthesis of 7-methoxy-N-[4-(3-methoxy-5-oxocyclohex-3-ene-1-carboxamido)butyl]-2-oxo-2H-chromene-3-carboxamide, 10. HOBt (134.0 mg, 0.99 mmol), Et_3N (0.19 mL, 1.39 mmol) and EDC (0.24 mL, 1.39 mmol) were sequentially added to a solution of **9** [2] (236.5 mg, 1.39 mmol) in anhydrous DMF (4.0 mL). A solution of **7** (TFA salt, 400.0 mg, 0.99 mmol) in anhydrous DMF (8 mL) was drop-wise added and the reaction was stirred under N_2 at room temperature for 24 h. DMF was evaporated and the crude mixture was purified by flash column chromatography using $CH_2Cl_2/MeOH$ (gradient 95:5→9:1) as eluent, to afford **10** as a white solid. Yield = 438.0 mg, 0.99 mmol, 99.9%; mp = 207–09°C. 1H NMR (400 MHz, CD_3OD) δ 8.80 (s, 1H, Ar), 7.75 (d, 1H, Ar, J = 8.4 Hz), 7.00–7.04 (m, 2H, Ar), 5.41 (s, 1H, $CHCOCH_3$), 3.94 (s, 3H, $PhOCH_3$), 3.76 (s, 3H, CH_2COCH_3), 3.44 (t, 2H, NCH_2 , J = 6.4 Hz), 3.24 (t, 2H, NCH_2 , J = 6.8 Hz), 2.95–3.01 (m, 1H, $NHCOCH$), 2.71–2.78 (m, 1H, $COCHCH-H$), 2.42–2.58 (m, 3H, $COCHCH_2 + COCHCH-H$), 1.57–1.67 (m, 4H, $NCH_2(CH_2)_2$). LC-MS (ESI): m/z calcd. for $C_{23}H_{26}N_2O_7$ 442.5 (MW), found 443.2 $[M+H]^+$, 465.2 $[M+Na]^+$; t_R = 1.5. HR-MS (ESI): m/z calcd. for $C_{23}H_{27}N_2O_7$ 443.1818 $[M+H]^+$, found 443.1807.

Synthesis of N-[4-(3-hydroxy-5-oxocyclohex-3-ene-1-carboxamido)butyl]-7-methoxy-2-oxo-2H-chromene-3-carboxamide, 1. LiOH (21.6 mg, 0.90 mmol) was added to a stirred solution of **10** (100.0 mg, 0.22 mmol) in THF/MeOH/ H_2O (4:1.6:1.6 mL). After 16 h at room temperature, solvents were evaporated. The crude mixture was suspended in H_2O and acidified to pH = 4 using 6N HCl. Lastly, H_2O was evaporated and the residue purified by RPh column chromatography using MeOH/ H_2O 1:1 as eluent, to obtain **1** as a yellowish solid. Yield = 89.0 mg, 0.21 mmol, 94.4%; mp = 99–101°C. 1H NMR (400 MHz, CD_3OD) δ 8.92 (exch br s, 1H, NH), 8.79 (s, 1H, Ar), 7.75 (d, 1H, Ar, J = 8.8 Hz), 7.59 (exch br s, 1H, NH), 7.03 (dd, 1H, Ar, J = 6.4 Hz, J = 2.4 Hz), 7.00 (d, 1H, Ar, J = 2.4 Hz), 5.41 (s, 1H, $CHCOH$), 3.94 (s, 3H, OCH_3), 3.44 (t, 2H, NCH_2 , J = 6.8 Hz), 3.24 (t, 2H, NCH_2 , J = 6.4 Hz), 2.97–3.01 (m, 1H, $NHCOCH$), 2.70–2.78 (m, 1H, $COCHCH-H$), 2.46–2.55 (m, 3H, $COCHCH_2 + COCHCH-H$), 1.58–1.67 (m, 4H, $NCH_2(CH_2)_2$). ^{13}C NMR (100 MHz, CD_3OD) δ 180.2 (C), 174.9 (C), 166.8 (C), 164.3 (C), 162.9 (C), 158.2 (C), 149.4 (CH), 132.5 (CH), 120.5 (C), 115.6 (C), 115.2 (CH), 113.6 (C), 102.2 (CH), 101.3 (CH), 56.8 (CH_3), 41.3 (CH), 40.3 (CH_2), 40.1 (CH_2), 39.8 (CH_2), 32.6 (CH_2), 27.8 (CH_2), 27.7 (CH_2). LC-MS (ESI): m/z calcd. for $C_{22}H_{24}N_2O_7$ 428.4 (MW), found 429.2 $[M+H]^+$,

451.1 [M+Na]⁺; *t*_R = 1.5. HR-MS (ESI): *m/z* calcd. for C₂₂H₂₅N₂O₇ 429.1662 [M+H]⁺, found 429.1671. UV-vis (H₂O): λ/nm 348. Fluorescence (H₂O): λ_{max}(ex) 348 nm, λ_{max}(em) 403 nm.

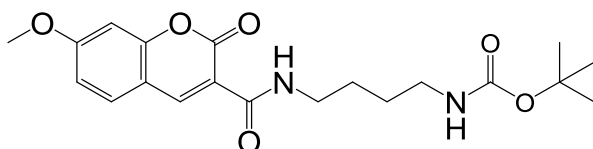
Synthesis of 5-[[2-(3-methoxy-5-oxocyclohex-3-ene-1-carboxamido)ethyl]amino] naphthalene-1-sulfonic acid, 12. HOBt (200.5 mg, 1.48 mmol), Et₃N (0.29 mL, 2.12 mmol) and EDC (0.37 mL, 2.12 mmol) were added to a stirred solution of **9** [2] (360.0 mg, 2.12 mmol) in 5 mL of anhydrous DMF under N₂. A suspension of **11** (394.0 mg, 1.48 mmol) in anhydrous DMF (5 mL) was subsequently added and the reaction was carried out for 2 days at room temperature. After removal of the solvent under vacuum, the crude mixture was purified by two consecutive flash column chromatography (eluent: CH₂Cl₂/MeOH/ AcOH 8:2:0.2 and CH₂Cl₂/MeOH 8:2), to obtain **12** as a yellowish solid. Yield = 590.0 mg, 1.41 mmol, 95.3%; mp= 270-73°C (130 °C, dec). ¹H NMR (400 MHz, CD₃OD) δ 8.11-8.18 (m, 3H, Ar), 7.37-7.42 (m, 2H, Ar), 6.68 (d, 1H, Ar, J= 7.6 Hz), 5.39 (d, 1H, COCHCO, J= 1.2 Hz), 3.71 (s, 3H, OCH₃), 3.51-3.63 (m, 2H, NCH₂), 3.41 (t, 2H, NCH₂, J= 6.4 Hz), 2.98-3.06 (m, 1H, CH₂CH), 2.71-2.78 (m, 1H, COCH-H), 2.44-2.61 (m, 3H, COCH-H + CH₂COCH₃), 1.93 (exch br s, 1H, OH). ¹³C NMR (100 MHz, CD₃OD) δ 200.6 (C), 180.1 (C), 175.9 (C), 145.5 (C), 142.0 (C), 131.6 (C), 128.6 (CH), 126.8 (CH), 125.7 (C), 125.4 (CH), 123.7 (CH), 116.5 (CH), 105.1 (CH), 102.3 (CH), 56.9 (CH₃), 45.0 (CH₂), 41.4 (CH), 40.2 (CH₂), 39.8 (CH₂), 32.7 (CH₂). LC-MS (ESI): *m/z* calcd. for C₂₀H₂₂N₂O₆S 418.5 (MW), found 419.2 [M+H]⁺; *t*_R = 0.9.

Synthesis of 5-[[2-(3-hydroxy-5-oxocyclohex-3-ene-1-carboxamido)ethyl]amino] naphthalene-1-sulfonic acid, 2. Ammonium cerium(IV) nitrate (CAN, 14.4 mg, 0.03 mmol) was added to a solution of **12** (110.0 mg, 0.26 mmol) in CH₃CN/H₂O 1:1 (8.0 mL) and the reaction was stirred for 6 h at reflux (100 °C). After cooling, the solvent was evaporated under vacuum and the crude was purified through flash column chromatography using CH₂Cl₂/MeOH/NH₄OH (eluent: gradient 8:2:0.2 → 7:3:0.3) to obtain **2** as a yellow solid. Yield = 51.5 mg, 0.13 mmol, 49.0%; mp= 114-16°C. ¹H NMR (400 MHz, CD₃OD) δ 8.12-8.18 (m, 3H, Ar), 7.39 (t, 2H, Ar, J= 7.6 Hz), 6.68 (d, 1H, Ar, J= 7.6 Hz), 5.19 (s, 1H, COCHCO), 3.61-3.65 (m, 1H, NCH-H), 3.47-3.53 (m, 1H, NCH-H), 3.42 (t, 2H, NCH₂, J= 5.6 Hz), 2.82-2.93 (m, 1H, CH₂CH), 2.56-2.63 (m, 1H, COCH-H), 2.42-2.49 (m, 1H, COCH-H), 2.23-2.35 (m, 2H, CH₂COH), 1.95 (exch br s, 1H, S-OH). ¹³C NMR (100 MHz, CD₃OD) δ 197.3 (C), 176.5 (C), 171.3 (C), 145.8 (C), 142.1 (C), 131.7 (C), 128.7 (CH), 126.9 (CH), 125.9 (C), 125.6 (CH), 123.8 (CH), 116.5 (CH), 105.3 (CH), 45.1 (CH₂), 42.4 (CH), 40.1 (CH₂), 39.1 (CH₂), 32.0 (CH₂). IR *v*_{max}

(neat)/cm⁻¹: 3325 cm⁻¹ (OH), 2923 cm⁻¹ (NH), 2854 cm⁻¹ (NH), 2823 cm⁻¹ (NH), 1641 cm⁻¹ (C=O), 1533 (C=O), 1365 (S=O). LC-MS (ESI): *m/z* calcd. for C₁₉H₂₀N₂O₆S 404.4 (MW), found 404.2 [M]⁺; *t_R* = 0.8. UV-vis (H₂O): λ nm 337. Fluorescence (H₂O): $\lambda_{\text{max}}(\text{ex})$ 335 nm, $\lambda_{\text{max}}(\text{em})$ 500 nm.

2. Full spectral characterization for intermediates 6-10 and optical probe 1

tert-Butyl-[4-(7-methoxy-2-oxo-2H-chromene-3-carboxamido)butyl]carbamate, **6**



C₂₀H₂₆N₂O₆
Exact Mass: 390,2
Molecular Weight: 390,4

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

471 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

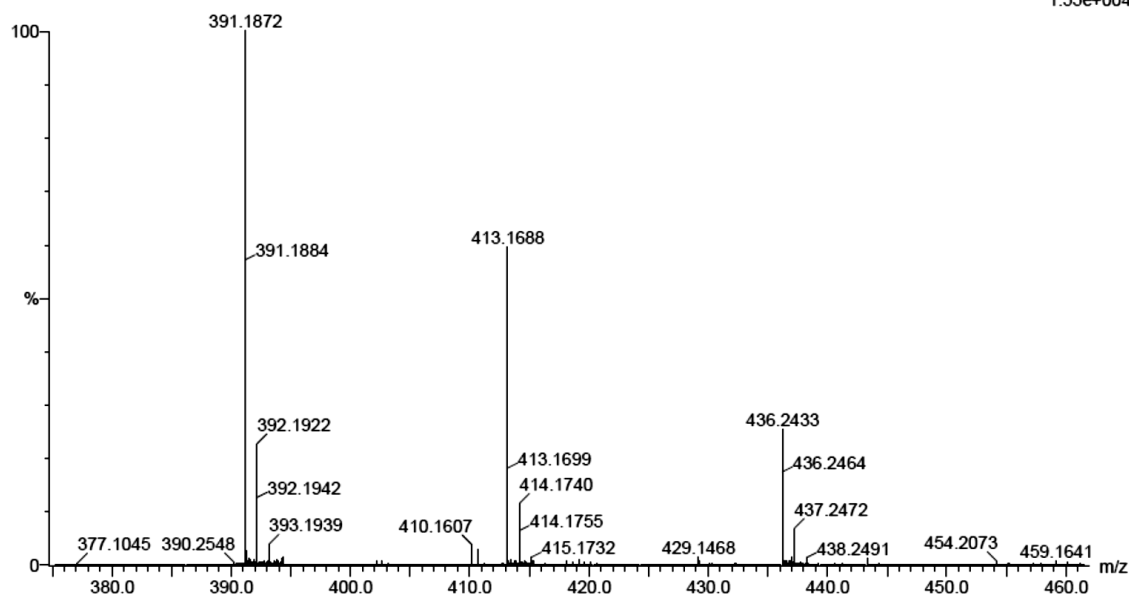
Elements Used:

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A.CILIBRIZZIAC123

MS16801A 175 (2.114) Cm (175:180)

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1.55e+004



Minimum:

Maximum:

5.0

10.0

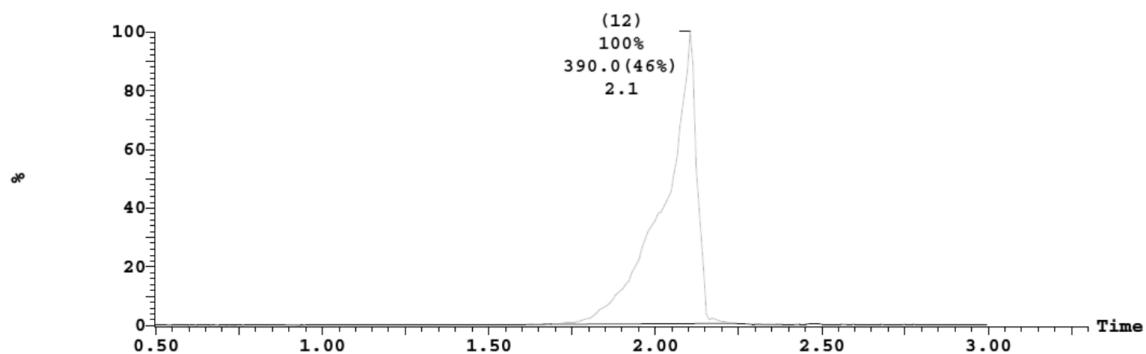
-1.5

50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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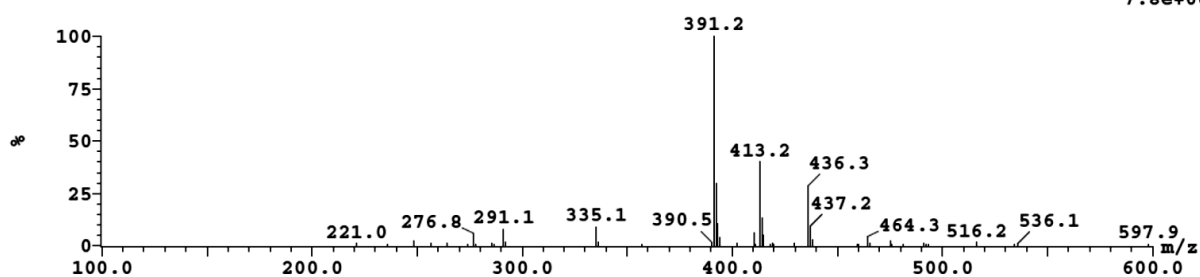
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2.3e+004

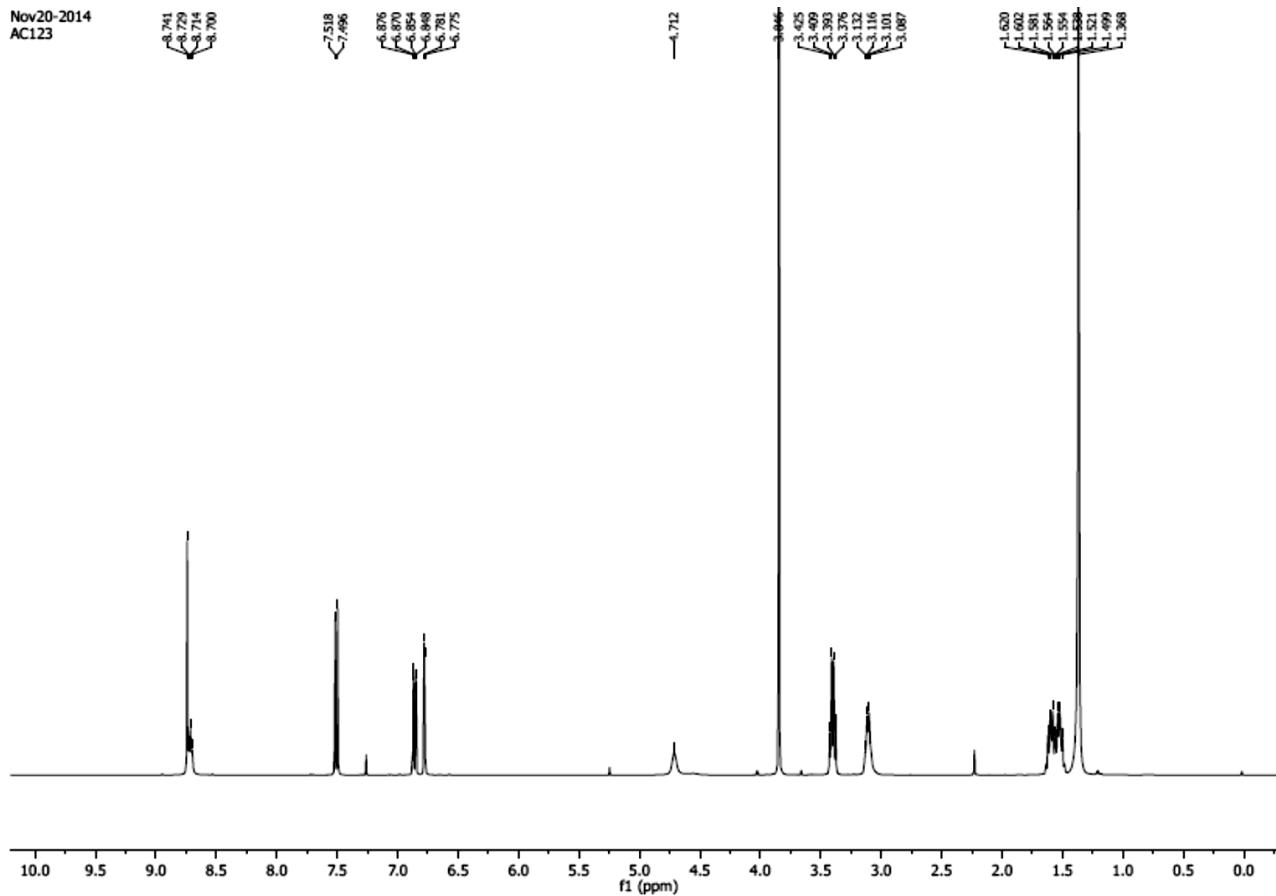


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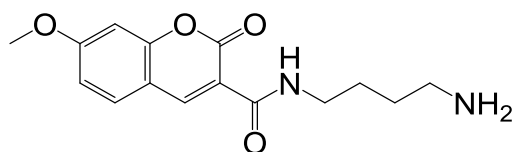
1:TOF MS ES+
7.8e+004



Nov20-2014
AC123



N-(4-Aminobutyl)-7-methoxy-2-oxo-2H-chromene-3-carboxamide, **7**



Chemical Formula: C₁₅H₁₈N₂O₄

Exact Mass: 290,1

Molecular Weight: 290,3

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

447 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

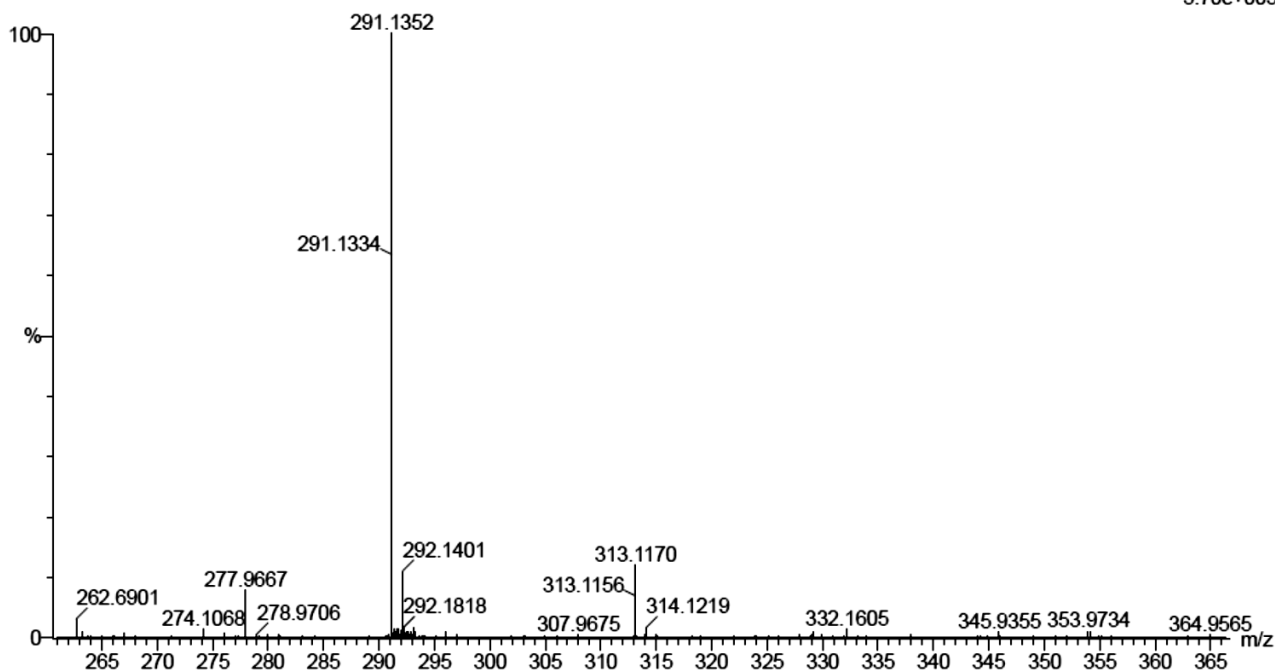
Elements Used:

C: 15-15 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 Cl: 0-1

A.CILIBRIZZI AC125

MS16847 86 (1.042) Cm (86:88)

1: TOF MS ES+
5.70e+003

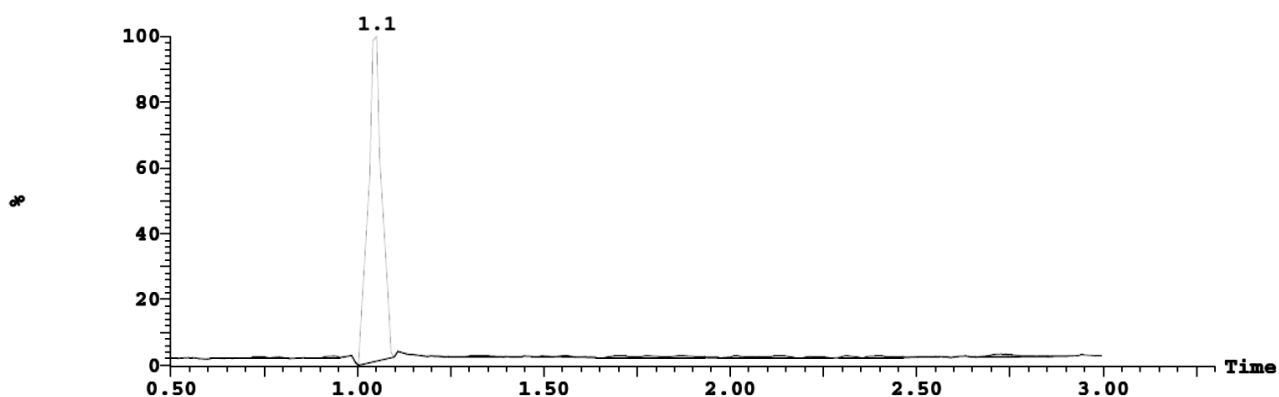


Minimum: -1.5
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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	291.1376	-2.4	-8.2	7.5	486.4	4.9	C15 H20 N4 Cl

1: TOF MS ES+ :313+291

4.0e+003

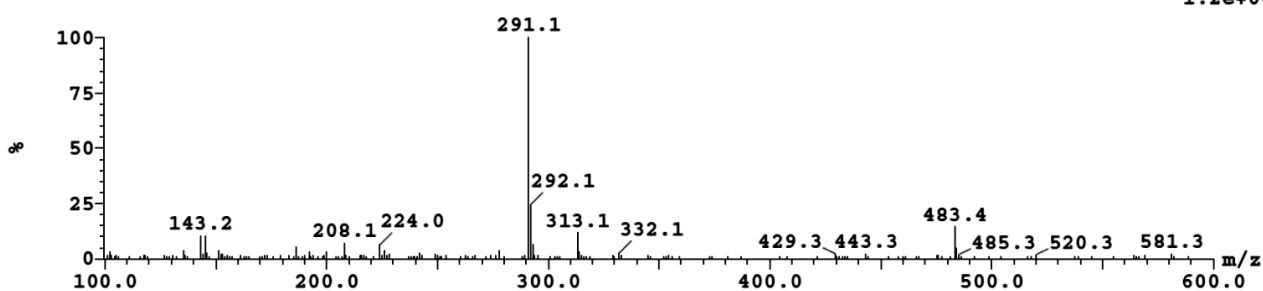


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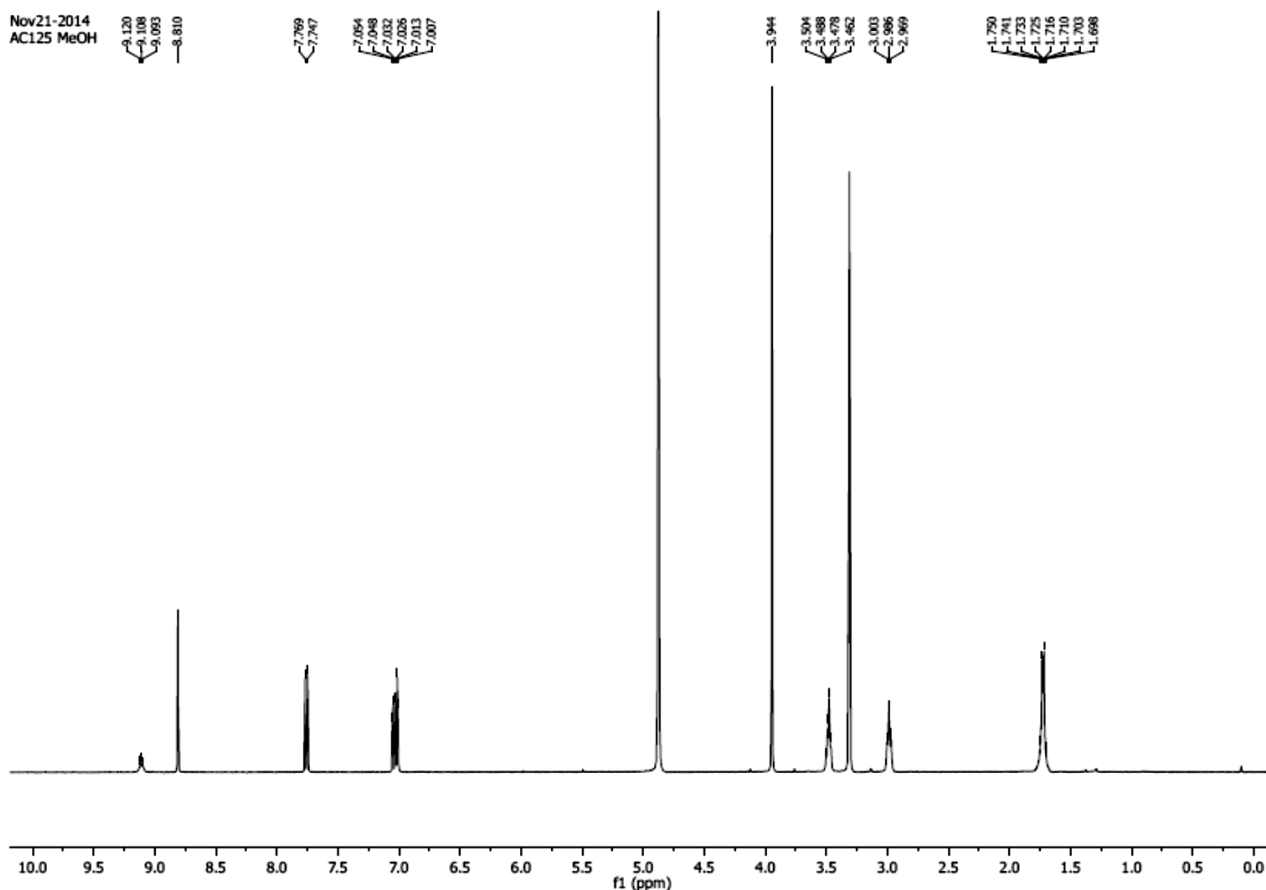
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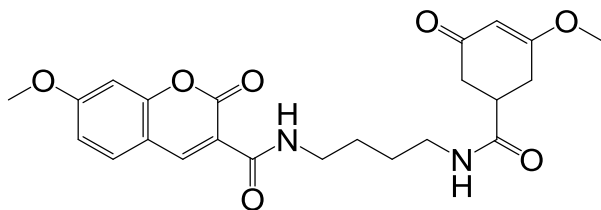
1.2e+004



Nov21-2014
AC125 MeOH



7-Methoxy-N-[4-(3-methoxy-5-oxocyclohex-3-ene-1-carboxamido)butyl]-2-oxo-2H-chromene-3-carboxamide,
10



Chemical Formula: $C_{23}H_{26}N_2O_7$

Exact Mass: 442,2

Molecular Weight: 442,5

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

236 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

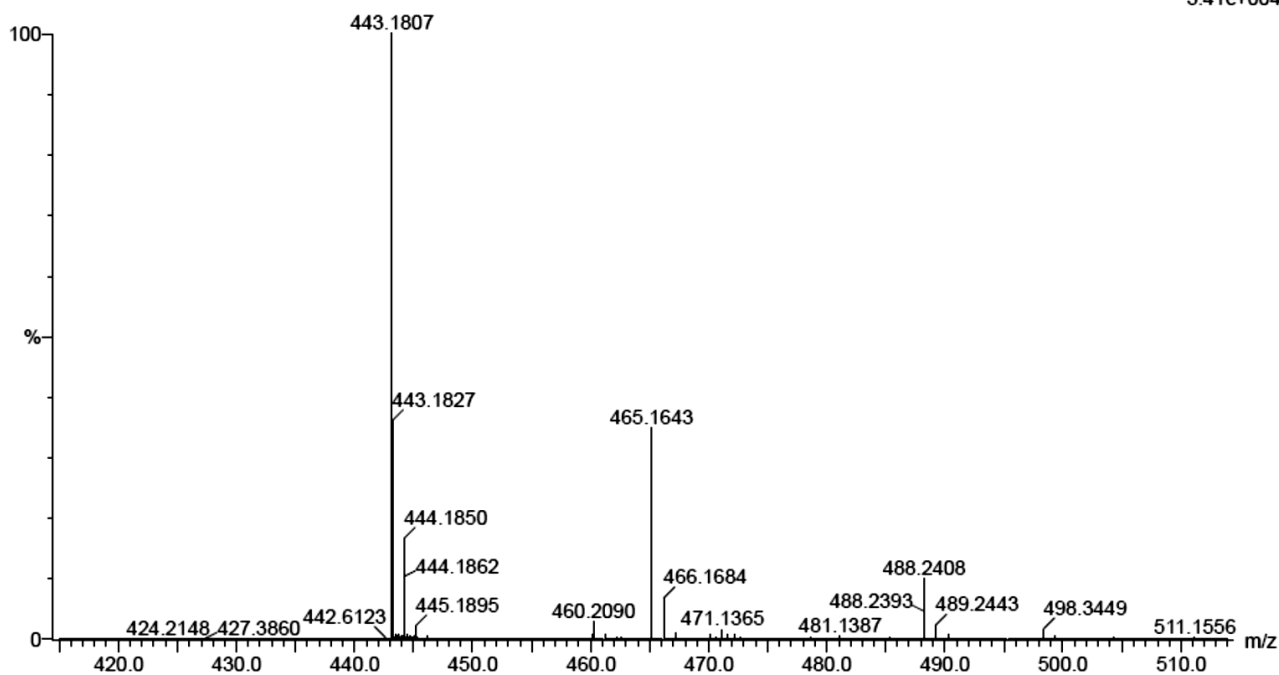
Elements Used:

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A.CILIBRIZZI AC126 FLASH

ms17223 124 (1.497) Cm (120:126)

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3.41e+004

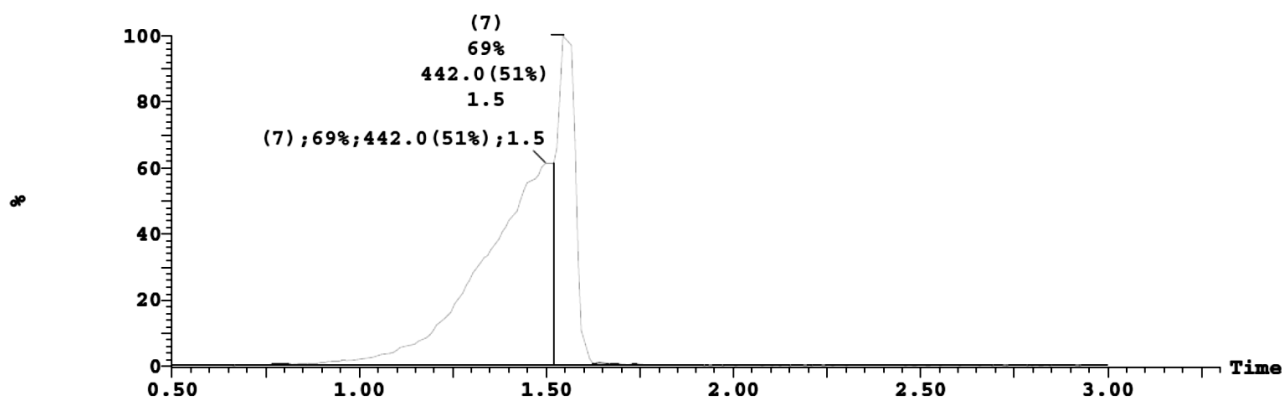


Minimum: -1.5
Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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1.5e+004

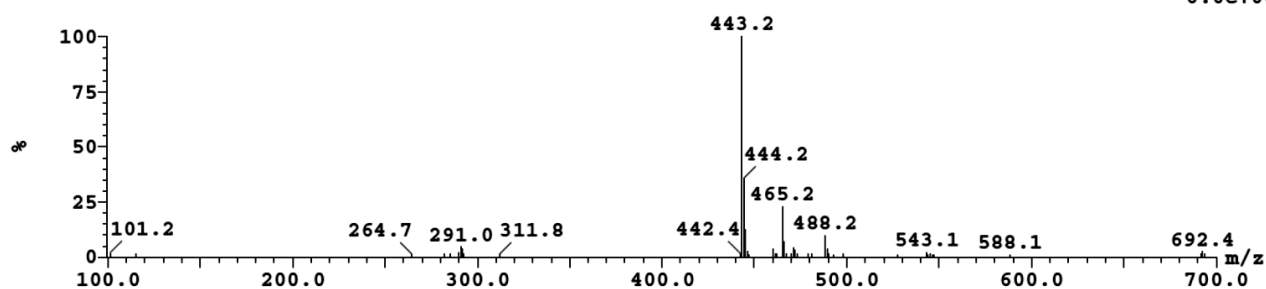


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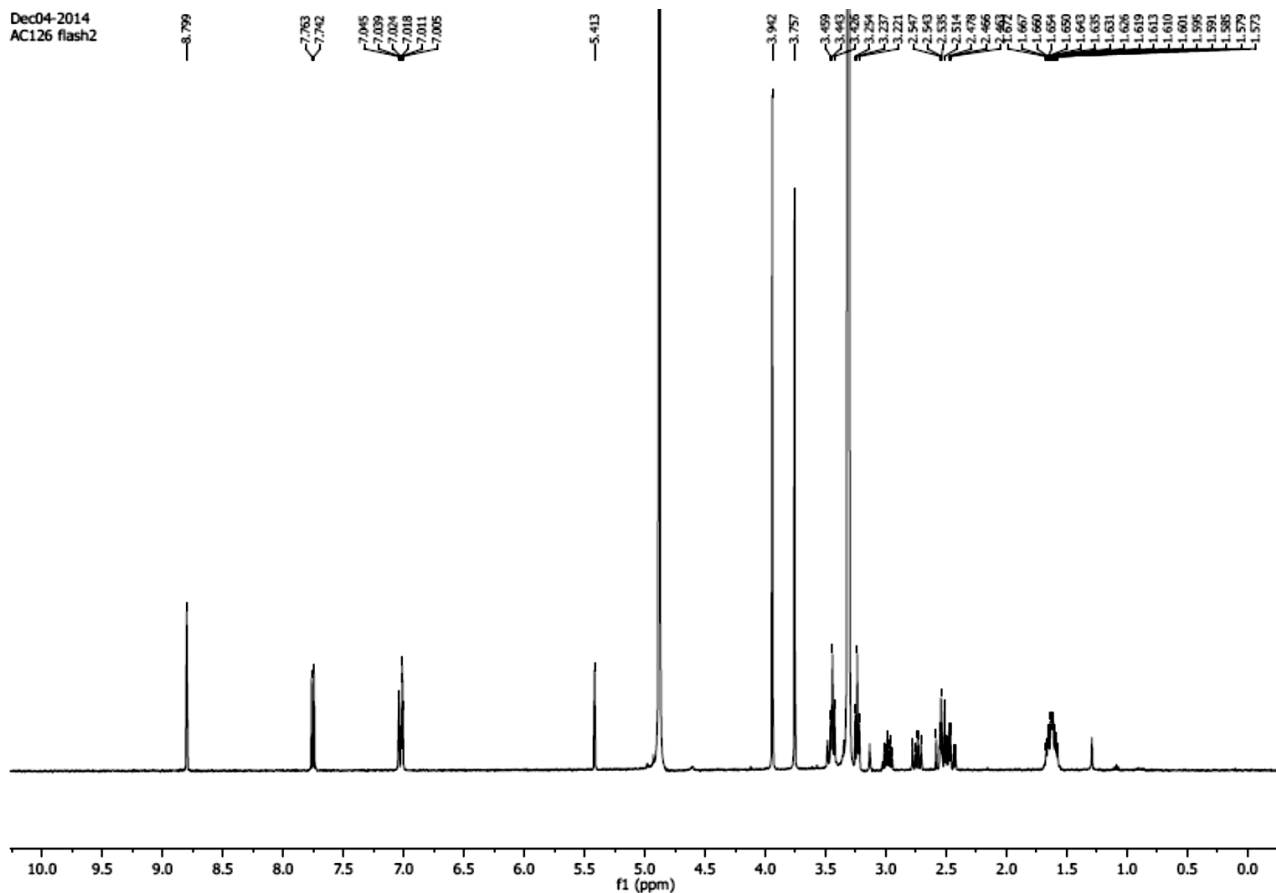
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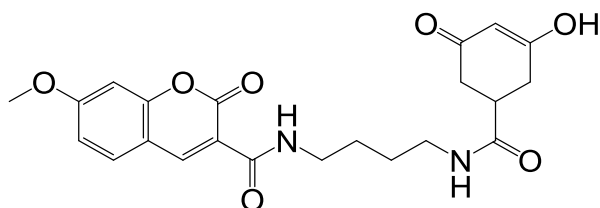
6.0e+004



Dec04-2014
AC126 flash2



N-[4-(3-Hydroxy-5-oxocyclohex-3-ene-1-carboxamido)butyl]-7-methoxy-2-oxo-2*H*-chromene-3-carboxamide,**1**



Chemical Formula: C₂₂H₂₄N₂O₇

Exact Mass: 428,2

Molecular Weight: 428,4

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

445 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

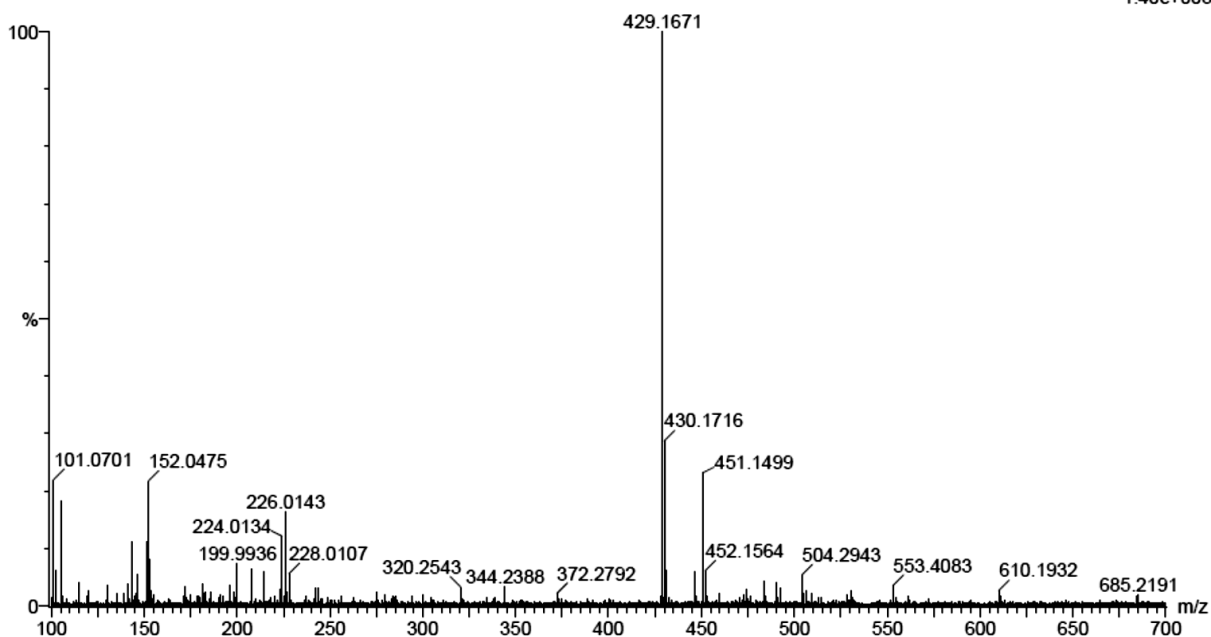
Elements Used:

C: 22-22 H: 0-200 N: 0-10 O: 0-10 Na: 0-1 Au: 0-5

A.CILIBRIZZI AC129

ms17154 121 (1.470)

1: TOF MS ES+
1.40e+003



Minimum:

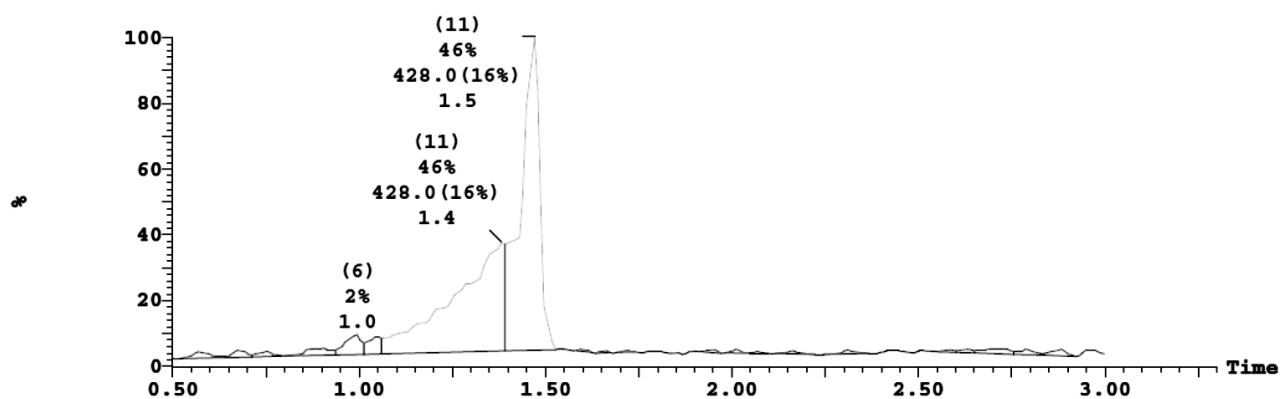
Maximum: 5.0 10.0 -1.5

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
------	------------	-----	-----	-----	-------	--------------	---------

429.1671	429.1662	0.9	2.1	11.5	89.9	0.0	C ₂₂ H ₂₅ N ₂ O ₇
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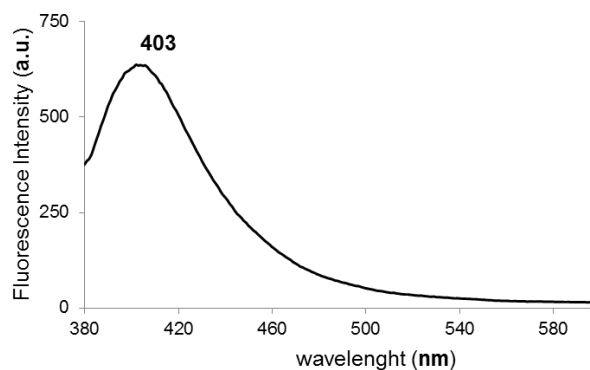
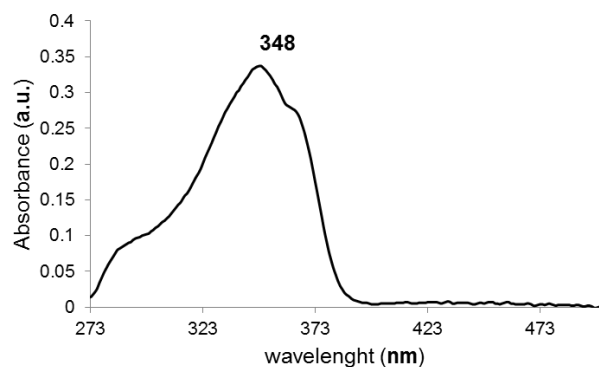
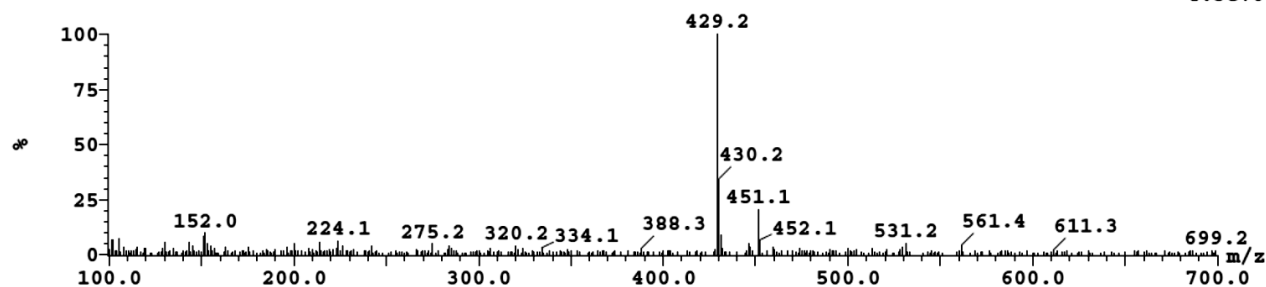
1: TOF MS ES+ :451+429

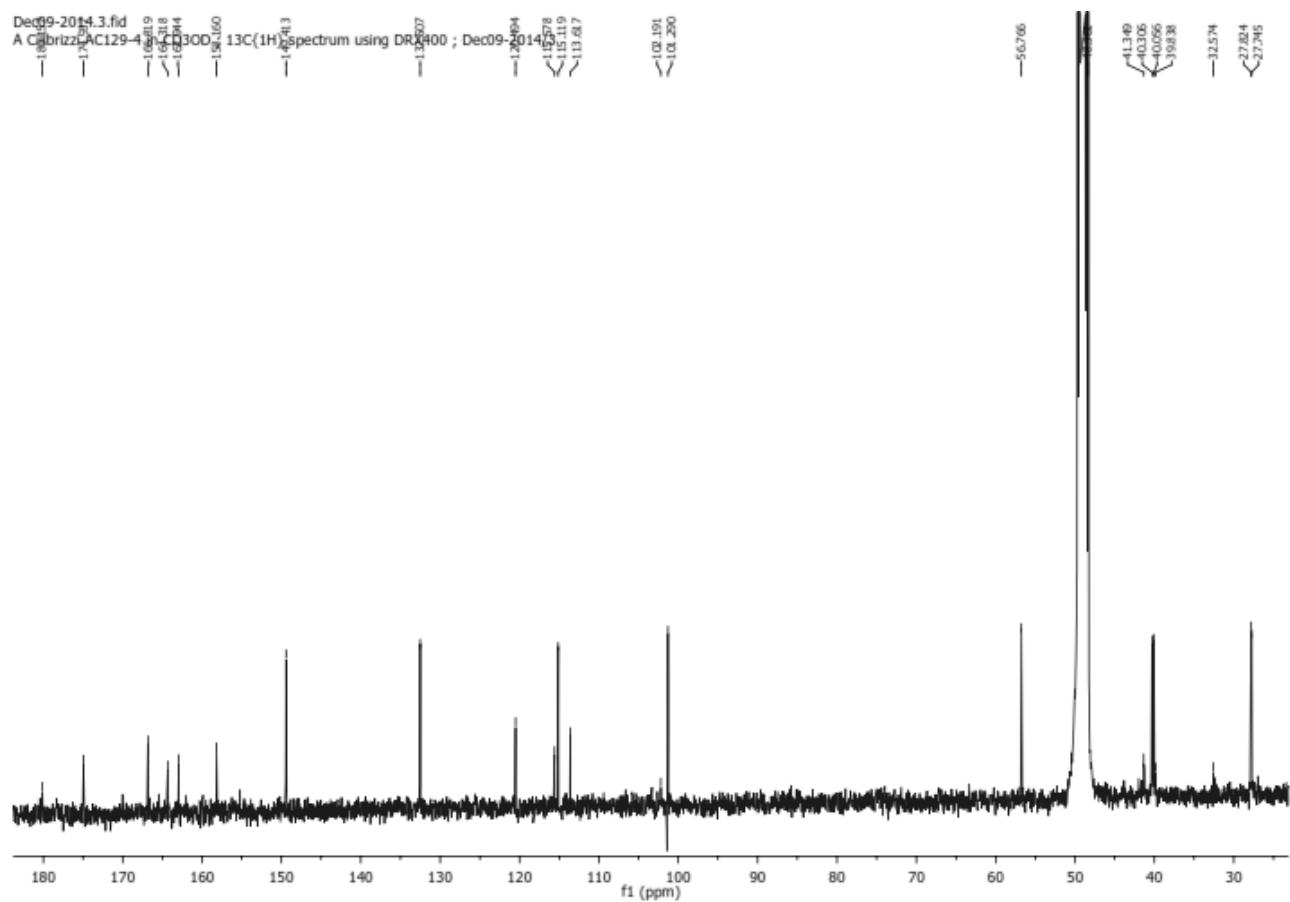
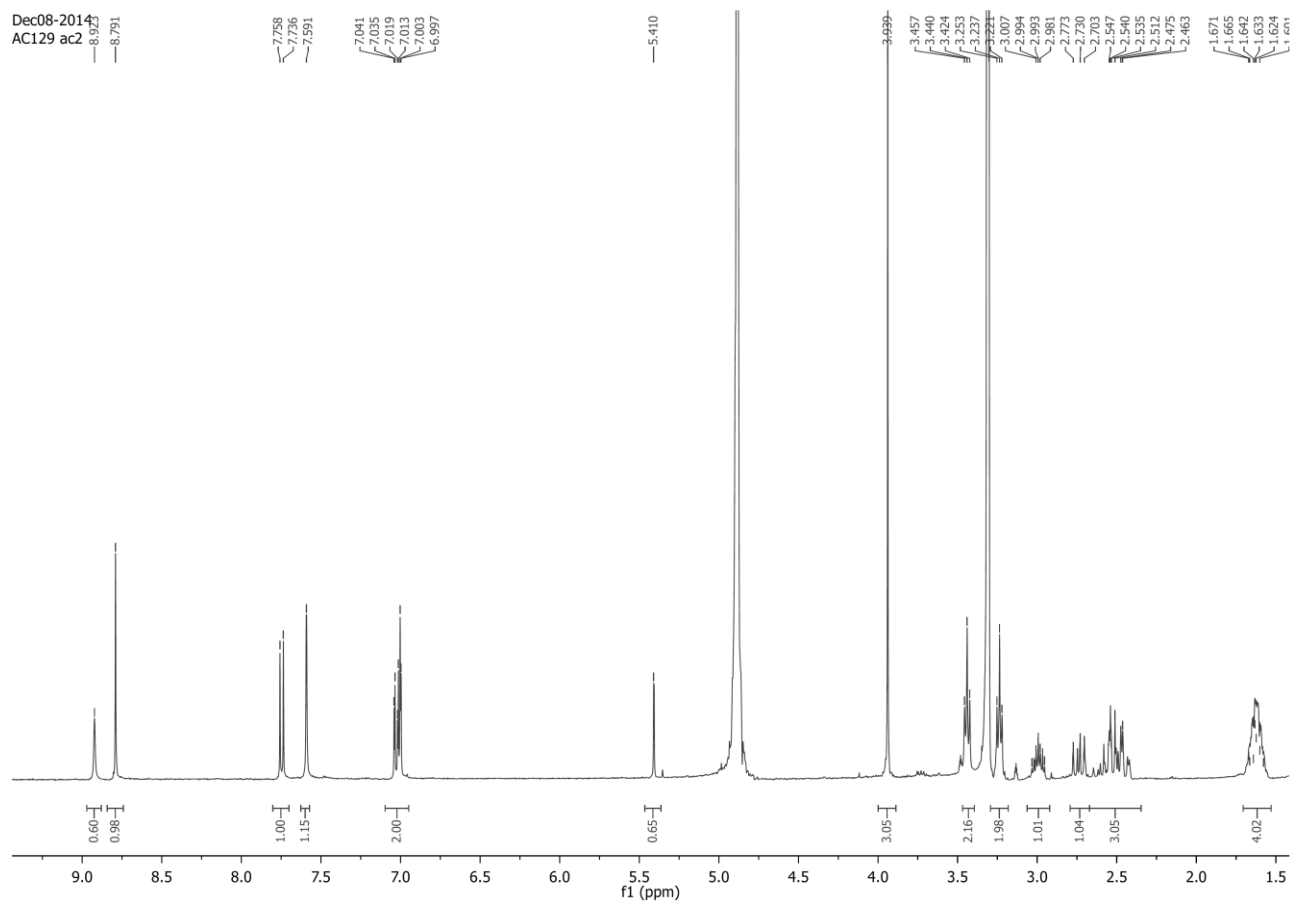
1.5e+003

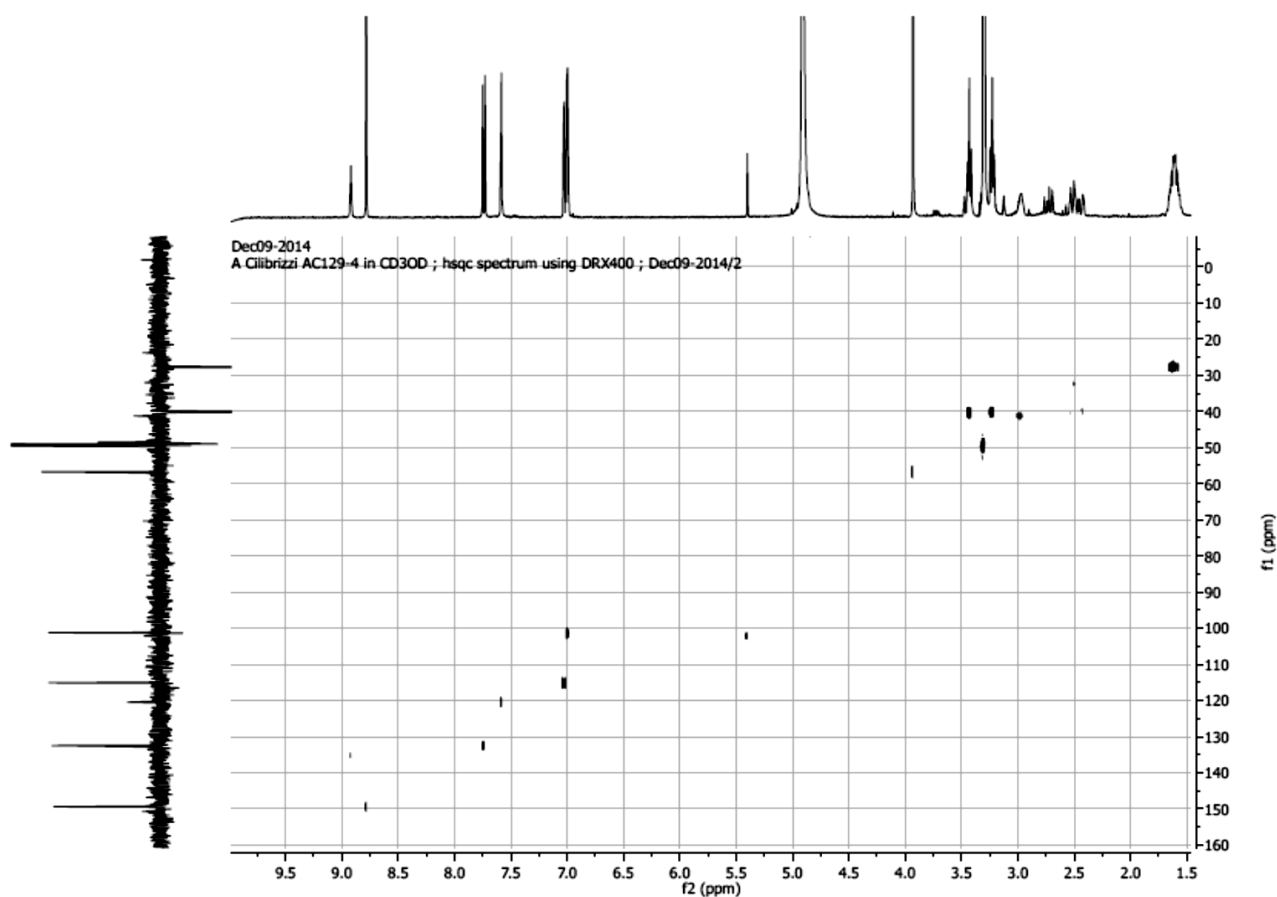


Peak ID Compound Time Mass Found
 11 Found 1.47 451.00,429.00
 11: (Time: 1.47) Combine (117:125-(105:108+135:138))

1: TOF MS ES+
 4.3e+003

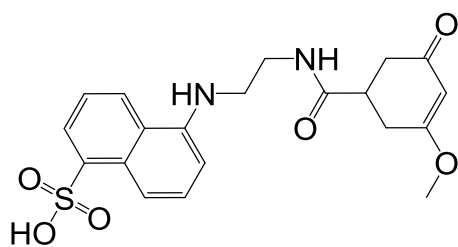






3. Full spectral characterization for intermediate 12 and optical probe 2

5-[[2-(3-Methoxy-5-oxocyclohex-3-ene-1-carboxamido)ethyl]amino]naphthalene-1-sulfonic acid, **12**



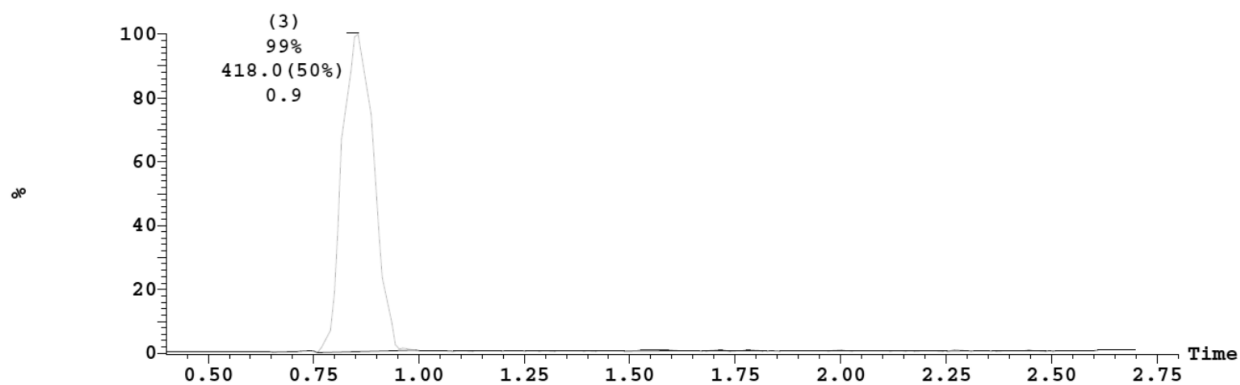
Chemical Formula: $C_{20}H_{22}N_2O_6S$

Exact Mass: 418,1

Molecular Weight: 418,5

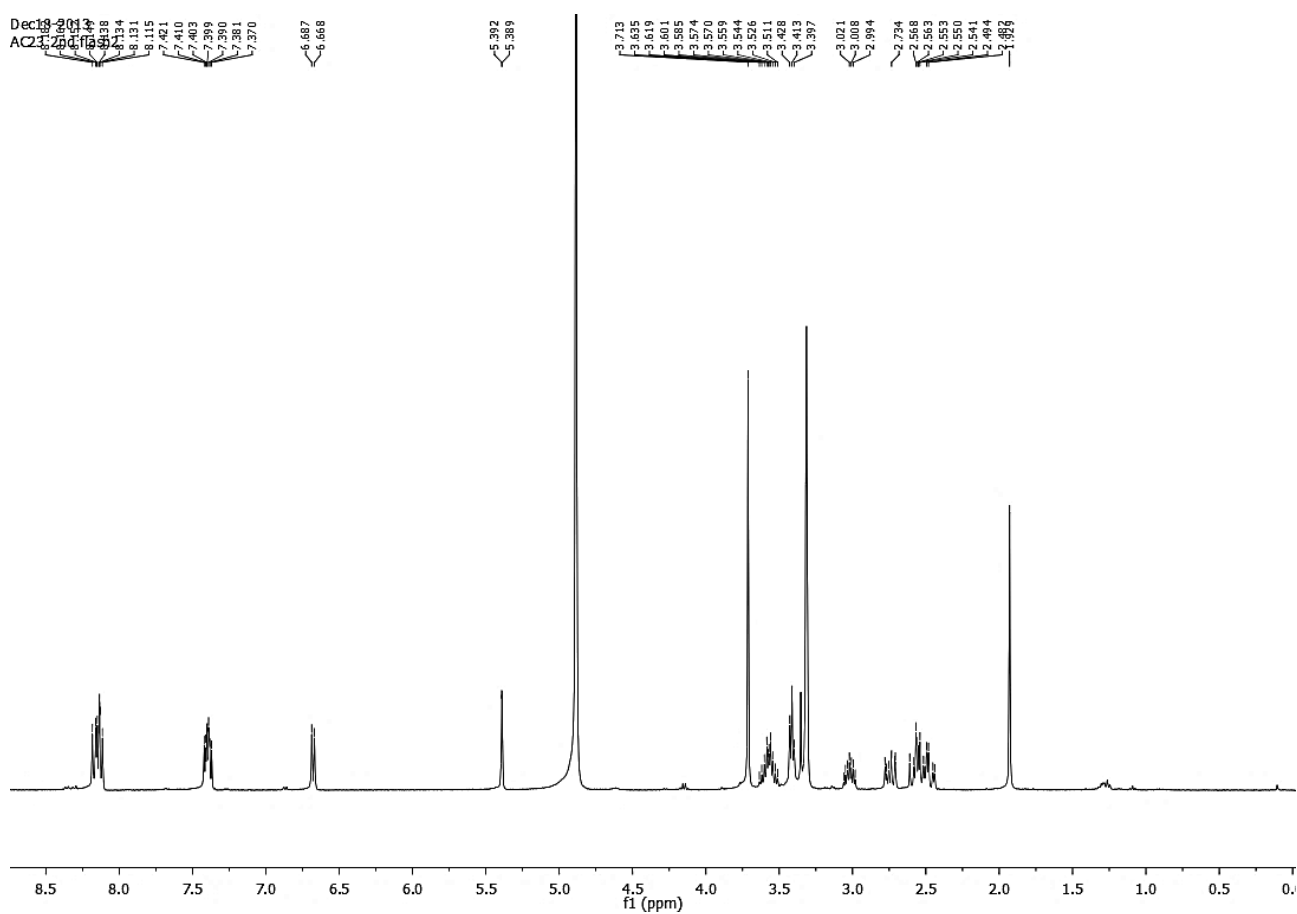
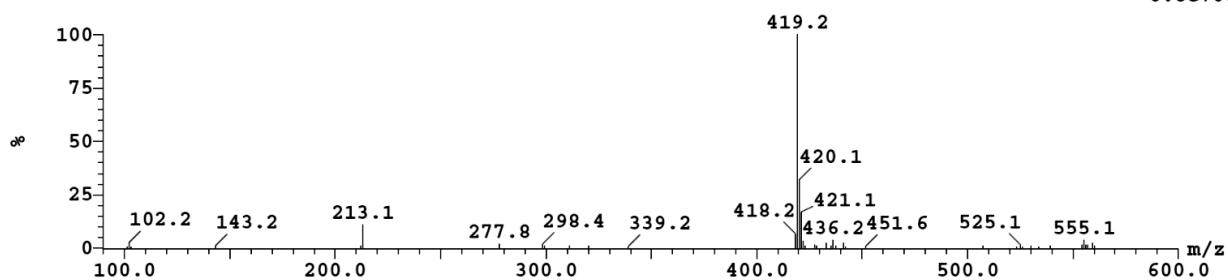
1: TOF MS ES+ :441+419

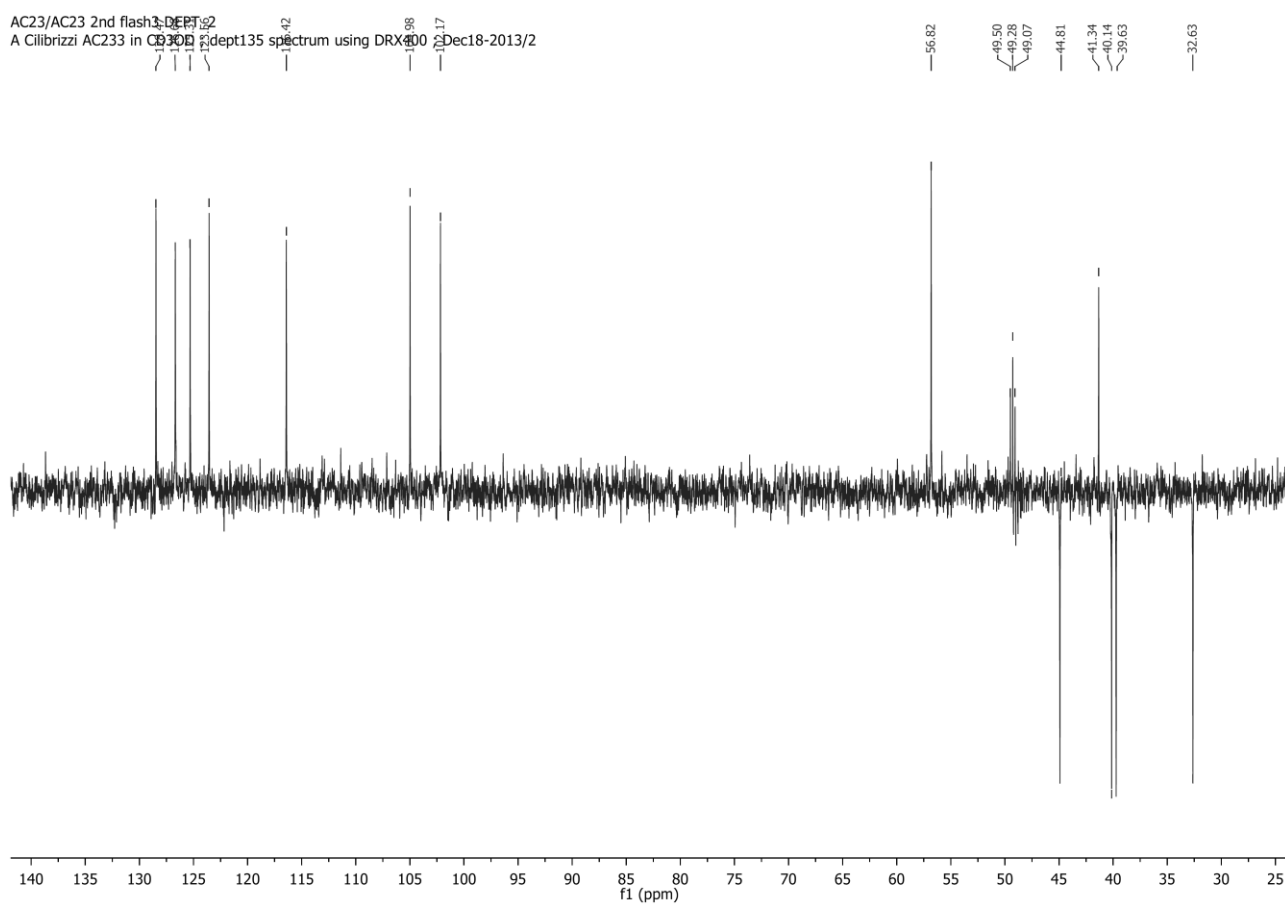
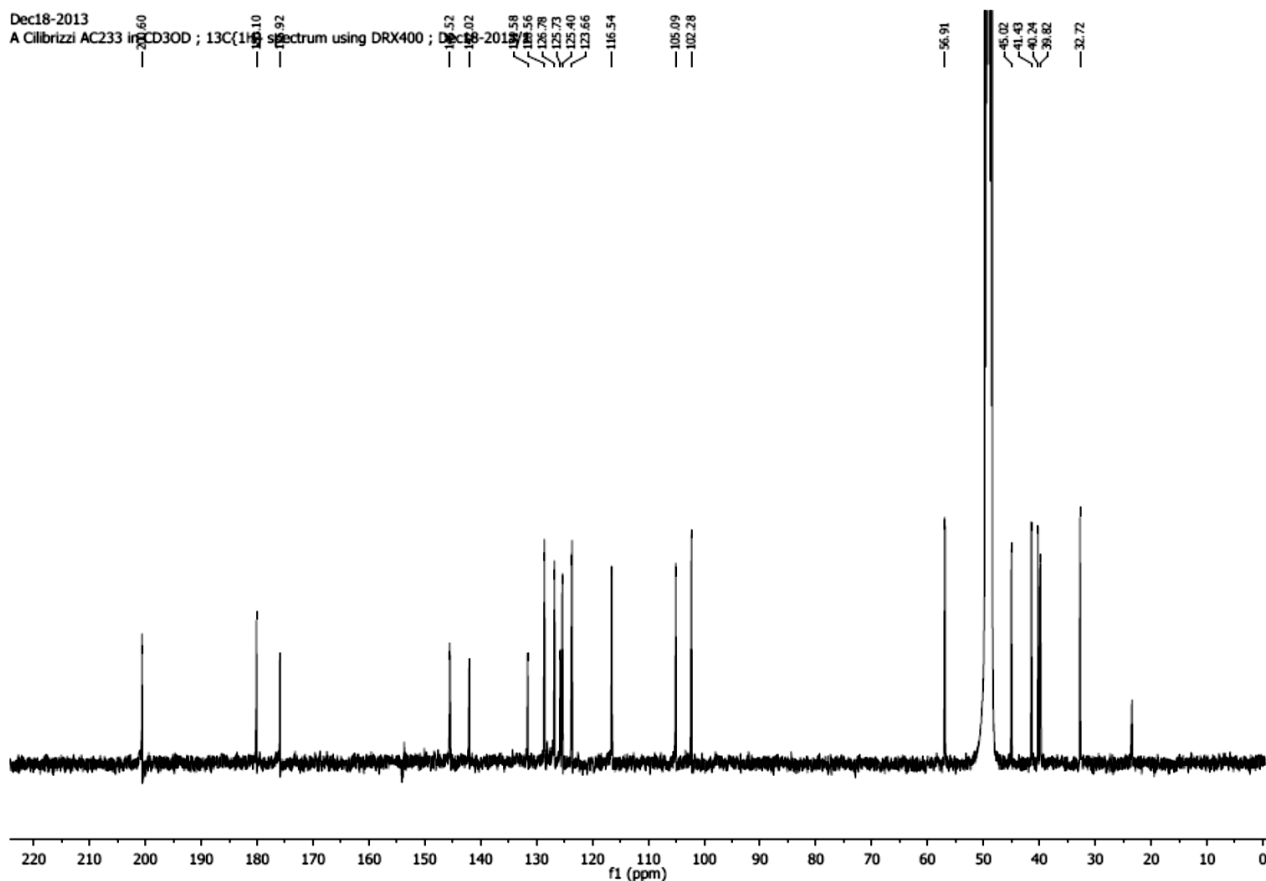
1.0e+004



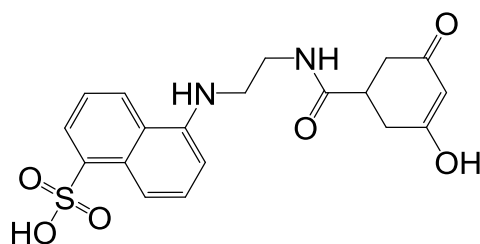
Peak ID Compound Time Mass Found
 3 Found 0.86 441.00,419.00
 3: (Time: 0.86) Combine (67:75- (54:57+85:88))

1:TOF MS ES+
 6.8e+004





5-[[2-(3-hydroxy-5-oxocyclohex-3-ene-1-carboxamido)ethyl]amino] naphthalene-1-sulfonic acid, **2**



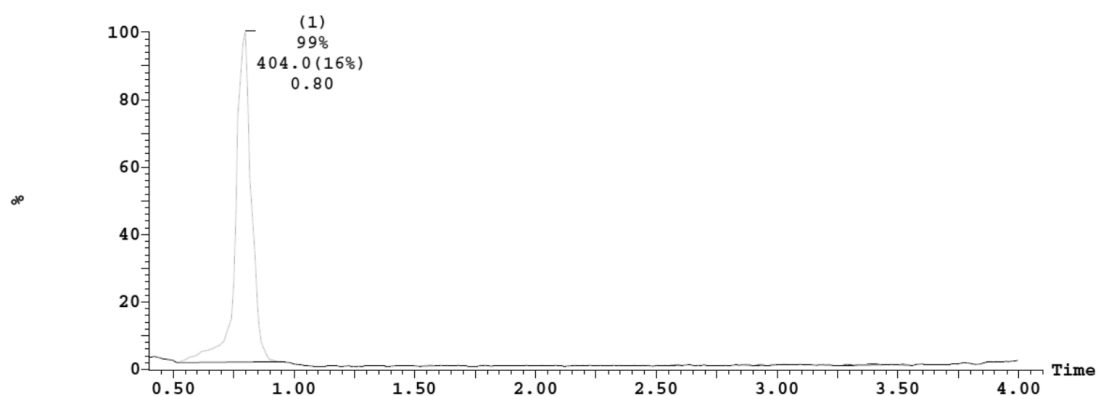
Chemical Formula: $C_{19}H_{20}N_2O_6S$

Exact Mass: 404.1

Molecular Weight: 404.4

1: TOF MS ES+ :427+405 Smooth (SG, 2x2)

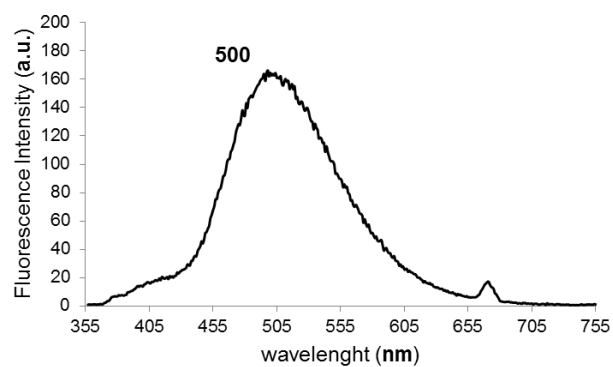
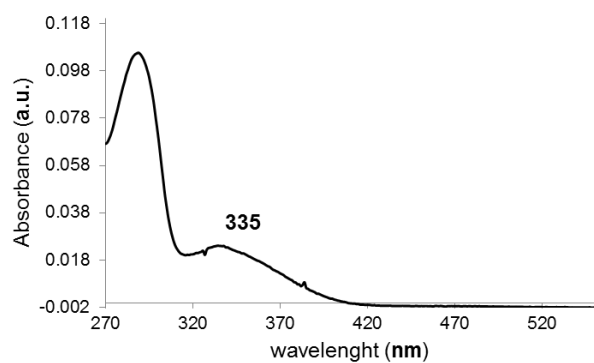
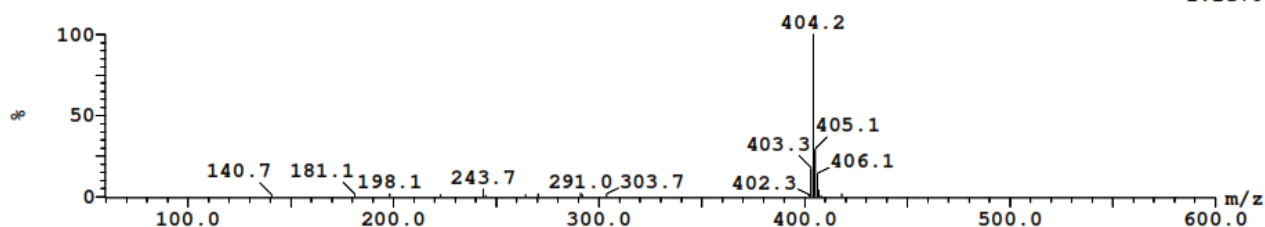
7.0e+003

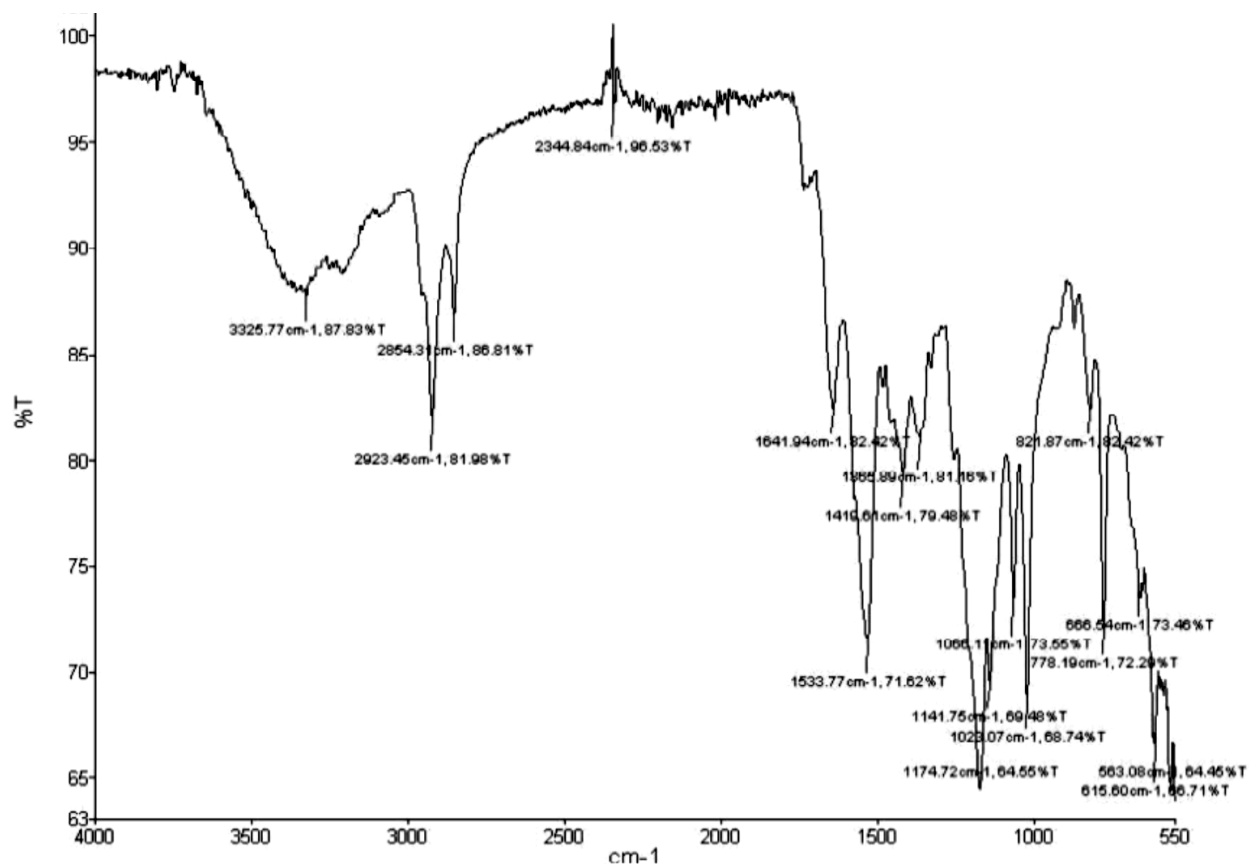


Peak ID	Compound	Time	Mass Found
1	Tentative	0.80	405.00

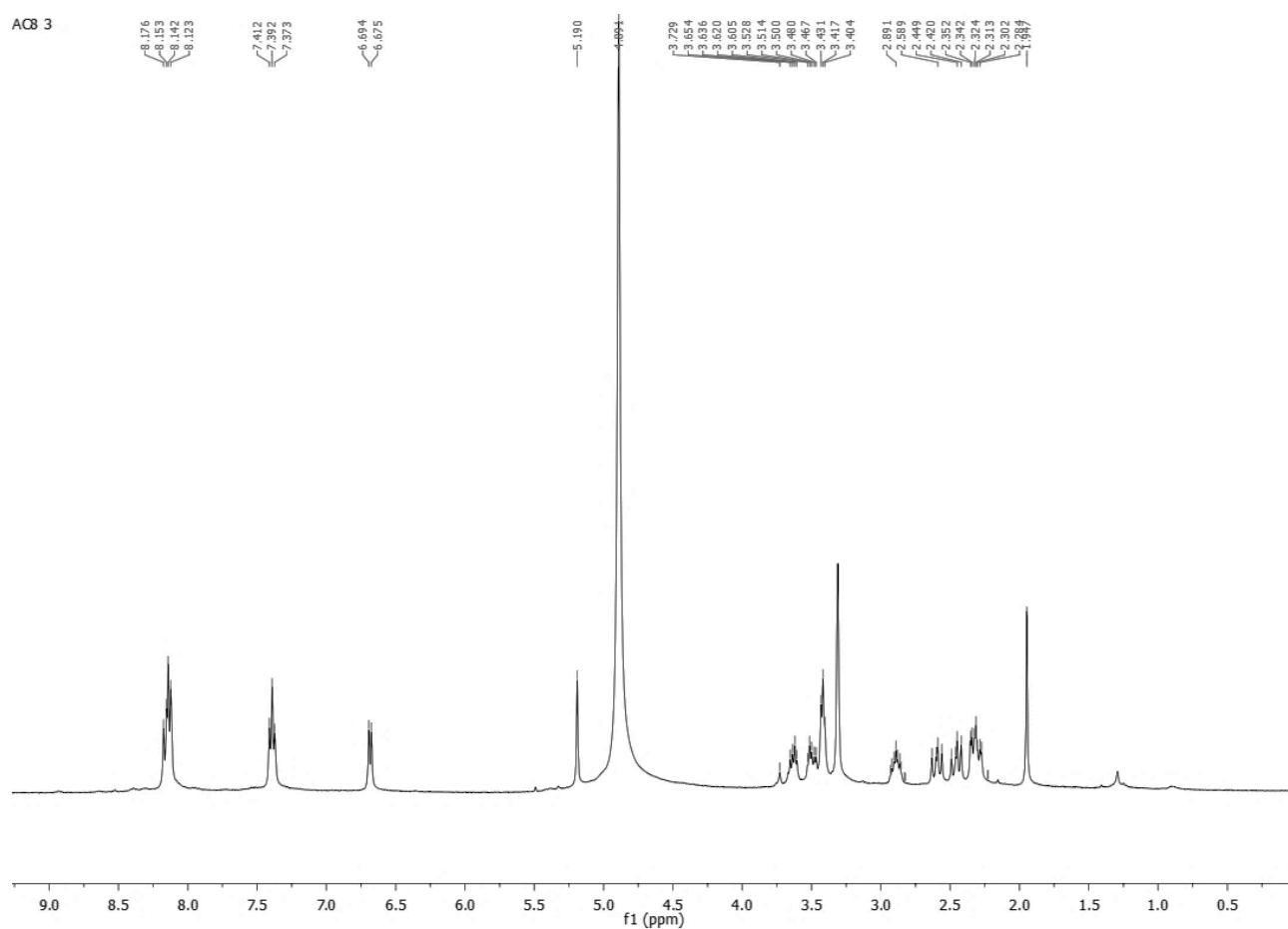
1: (Time: 0.80) Combine (62:70-(49:52+79:83))

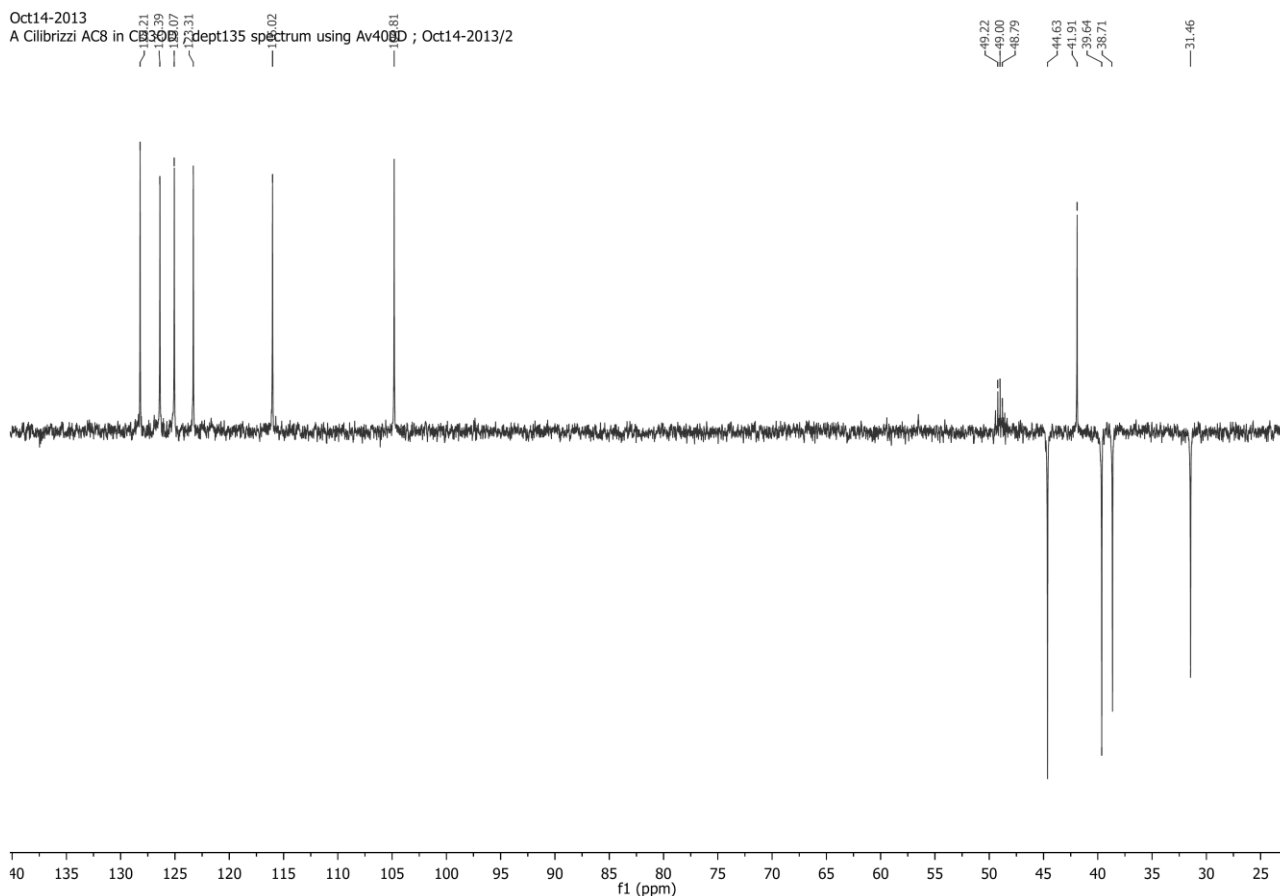
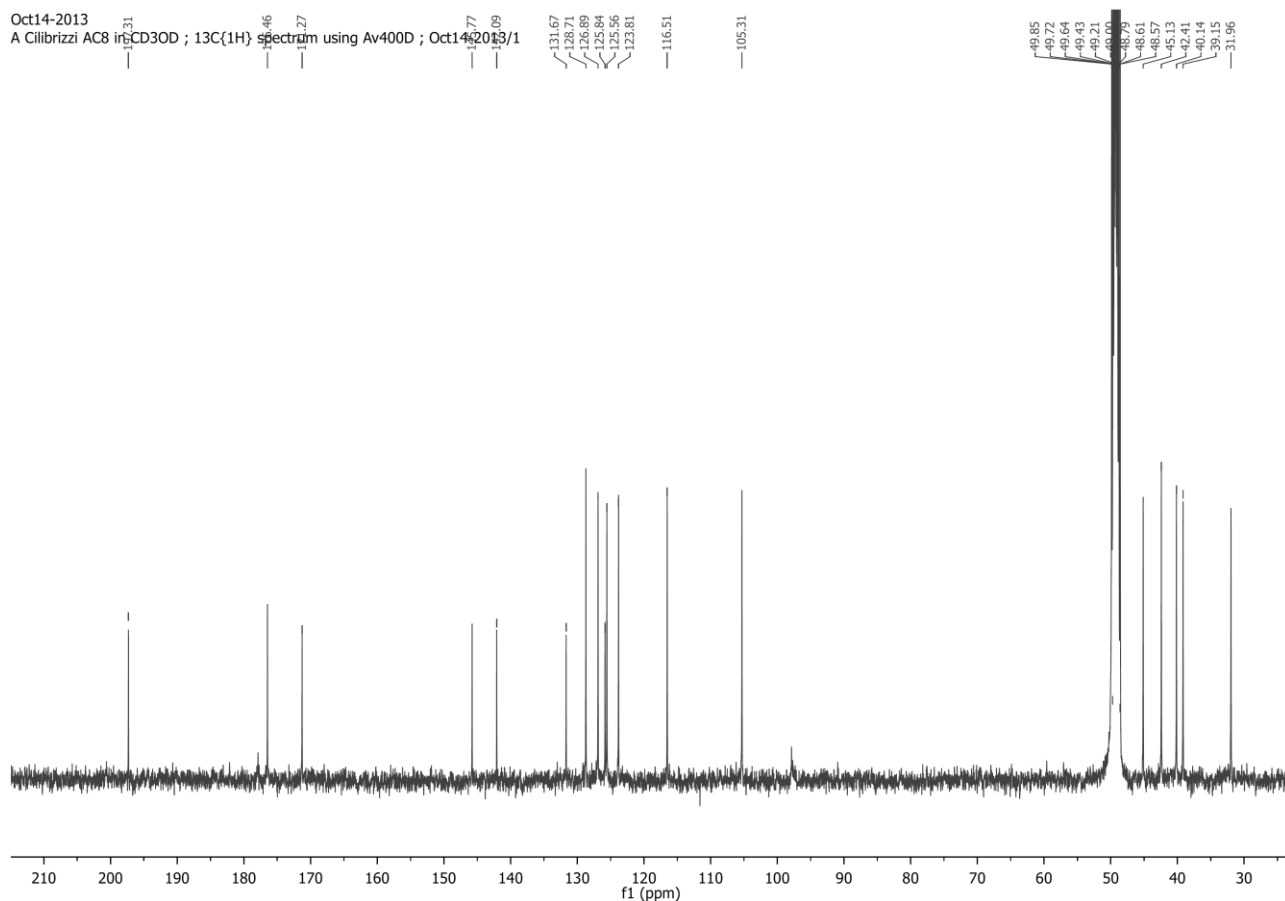
1: TOF MS ES+
1.1e+005





ACS 3





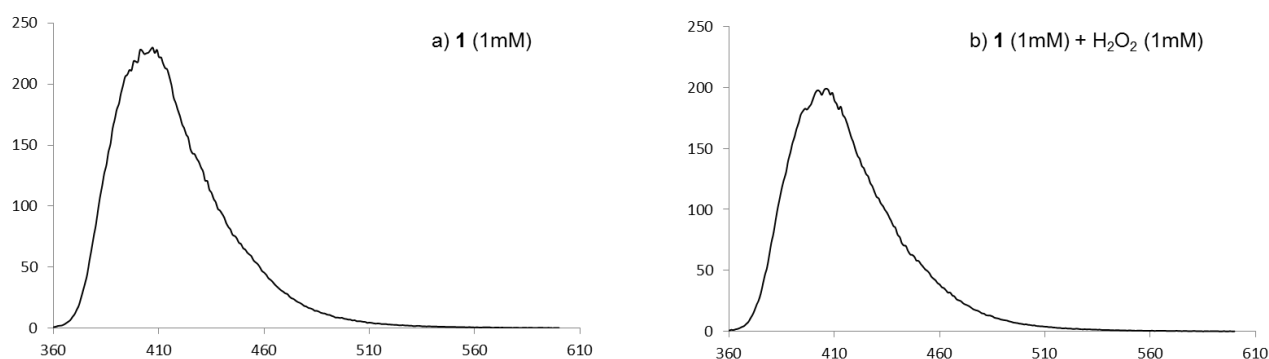


Figure S1. Emission spectra of probe **1** in the absence/presence of H₂O₂ (30 min. incubation). a) Fluorescence emission of **1** (1mM) in DPBS. b) Fluorescence emission of **1** (1mM) + 30% H₂O₂ (1mM) in DPBS after 30 minutes.

4. Cell culture protocols

MCF7 and MDA-MB-231 were obtained from American Type Culture Collection and were grown following the manufacturer's instruction in complete DMEM-high glucose (Sigma-Aldrich, #D6429) supplemented with 10 % FBS (Sigma-Aldrich, #F6178) (DMEM+) in a 5% CO₂ humidified atmosphere at 37 °C. Cells were typically sub-cultured every two days or around 80 % confluence with a split ratio of 1:4 to 1:6. For the microscopy experiments, cells were seeded at ≈ 50 % confluence on glass coverslips in a 96-well plate format (Greiner Sensoplate™, borosilicate glass, thickness=175 μM, black polystyrene plate, Sigma-Aldrich, #M4187) and allowed to adhere for at least 15 hours in DMEM⁺ in a humidified incubator with a 5 % CO₂ atmosphere at 37 °C. 96-well plate glass bottom was pre-treated overnight at 4 °C with 20 μg mL⁻¹ of fibronectin from bovine plasma (Sigma-Aldrich, #F1141) in Dulbecco Phosphate Buffer Saline with Ca²⁺/Mg²⁺ (DPBS, Sigma-Aldrich, #D8662). The fibronectin solution was removed immediately prior to cell seeding; the coverslips were washed twice with DPBS and pre-filled with DMEM⁺. All the experiments to assess the sulfenylation in live cells were performed at 37 °C in a humidified incubator with a 5% CO₂ atmosphere.

5. Image analysis

Acquired images were analysed for quantitative single cell signal intensity with an in-house developed script in Fiji software (National Institute of Health, USA; <http://imagej.nih.gov/ij/>; v 1.49m) [3], following the protocol described in the Results and Discussion section (Figure S2 and 'macro run code' files, Supporting

Information). The threshold value for the identification of the cells above the background is defined by $(mean_{bkg} + (2 \times stdev_{bkg}))$ where the $mean_{bkg}$ is the mean signal intensity of the area without cells and $stdev_{bkg}$ is the standard deviation of the mean signal intensity measured for the area without cells. From the mask of identified cell by the Analyze particle tool in Fiji, the signal from each individual cell is integrated to obtain the mean pixel intensity ($IntMean_i = \frac{\sum Area_i IntPx}{Area_i}$; where $IntMean_i$ is the mean intensity of a specific single cell "i", $Area_i$ is the area in square pixels identified for the single cell "i" and $\sum Area_i IntPx$ is the total sum of pixel intensities in the $Area_i$ for the single cell "i").

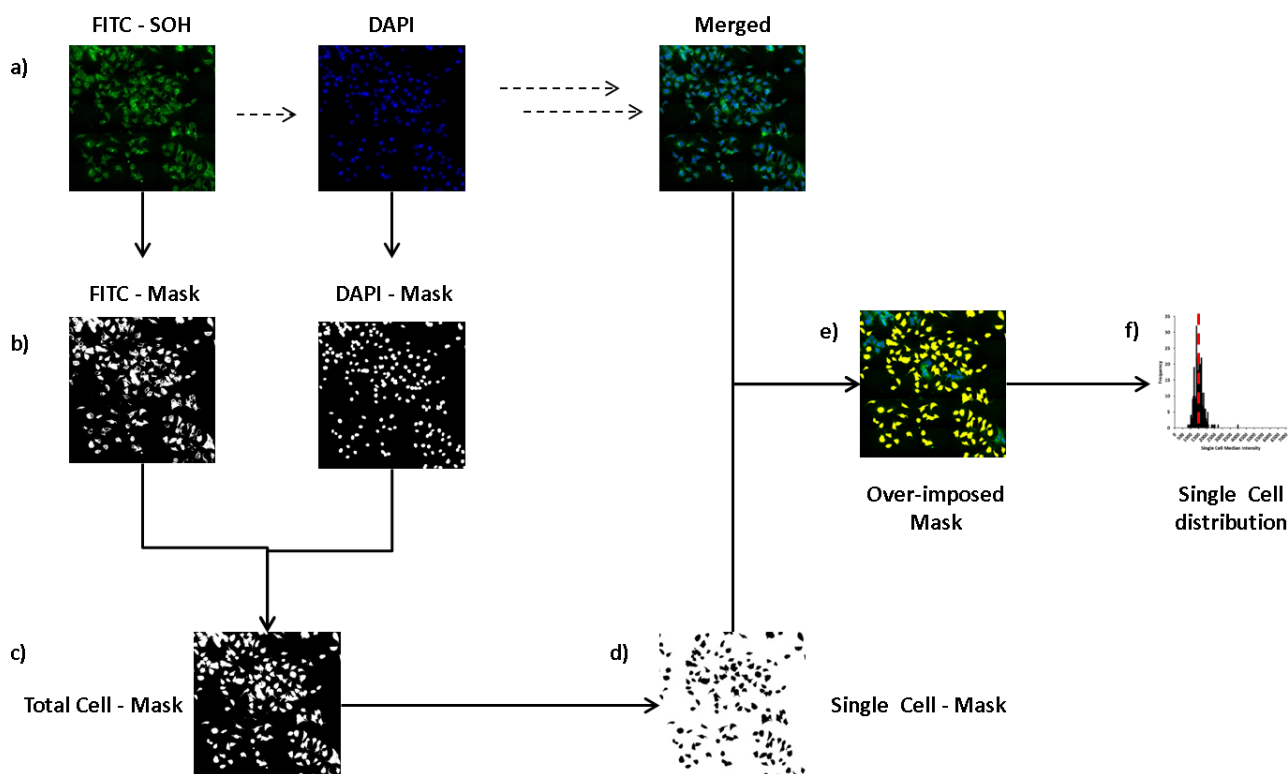


Figure S2. Schematic representation of the Fiji (free to download at <http://imagej.nih.gov/ij>) developed protocol for the quantification of single cell signal intensity in the channel of interest. As an example, the FITC and DAPI channel acquisition is shown in the scheme, although the script can handle single (e.g. FITC only) or multiple (e.g. DAPI, FITC, Cy5) channels for the identification of cell boundaries and further quantitative analysis. a) Images acquired in the channels of interest at 16-bit (DAPI and FITC for nuclei and sulfenic acid (probe 2) are flattened and background subtracted; b) from every fluorescent channel, a 8-bit mask with white objects above black background above a defined threshold is obtained; c) the masks from every channel are combined to obtain the mask for the total cell surface and submitted to particle identification algorithm in Fiji; d) an output mask of the identified single cell with black objects above the white background is obtained; e) the latter mask is over-imposed to the 16-bit images in every channel and the signal from each individual cell is integrated to obtain the mean pixel; f) mean pixel intensities obtained for single cells in the channel of interest, e.g. FITC for sulfenic acid measured with probe 2, are plotted as frequency plots for the evaluation of the mean signal across the cell population (dash red line).

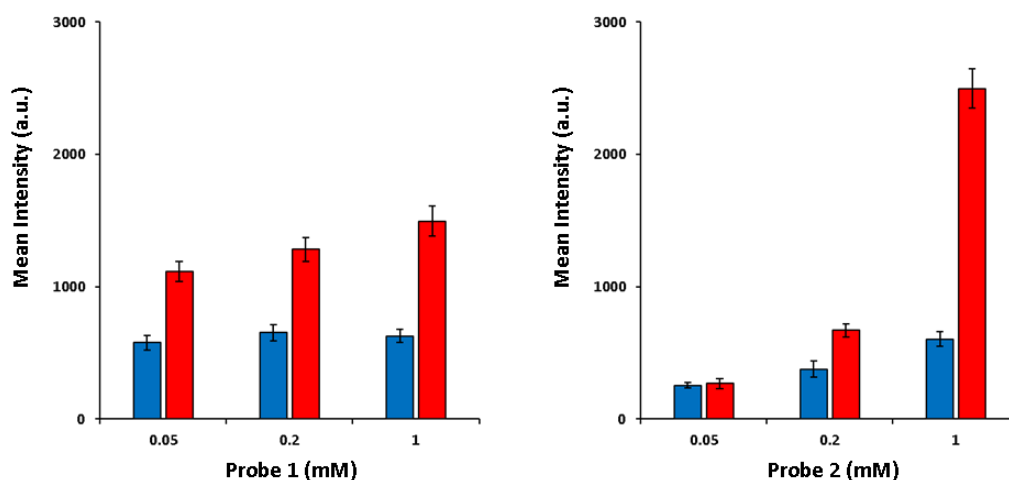


Figure S3. Mean intensity signal from MCF7 cells treated with increasing concentrations (i.e. 0.05, 0.2 and 1 mM) of probes **1** (DAPI channel) and **2** (FITC channel) with auto-fluorescence removed in all the relevant channels. Blue bars represent the basal levels of SOH measured and the red bars the SOH levels after treatment with 1 mM H₂O₂ in DPBS for 30 minutes at 37°. Error bars are the standard errors of the mean.

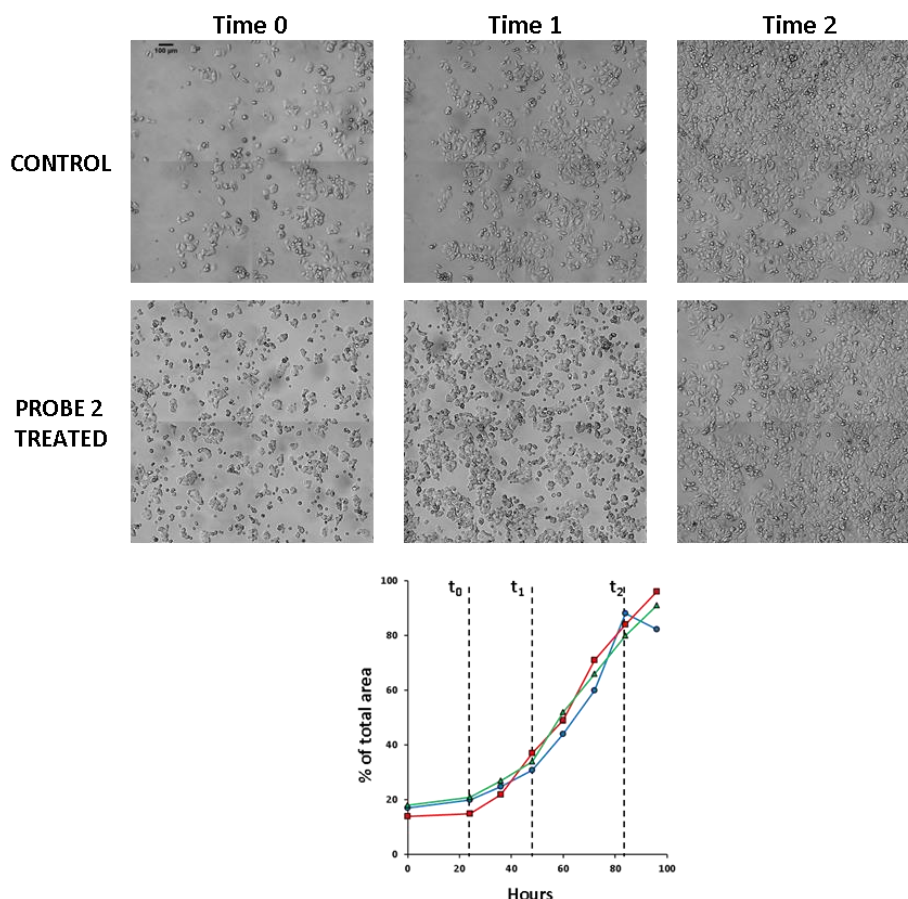


Figure S4. Evaluation of the MCF7 cell's proliferation before and after the treatment with probe **2** (1 mM). **Top:** representative bright field images obtained with a 10x objective corresponding to the times indicated on the plot at treatment (t₀), 24 hours after treatment (t₁) and 60 hours after treatment (t₂) for the control (top row) and the probe **2** treated (bottom row) samples. **Bottom:** cell's growth profile obtained by measuring the percentage of area occupied by adhered cells in respect to the total area observed. Treated (blue line for probe **1** and green line for probe **2**, 1 mM) and control cells (red line) were prepared in triplicate and imaged every 12 hours in five random locations for each experiment. Cells were allowed to adhere for 24 hours before incubation with probe **2** was carried out (time zero = t₀). Standard deviation at each time point for the percentage of area occupied by cells was ≤10%

References

- [1] Bardajee, G.R., Winnik, M.A., and Lough, A.J. (2007) Succinimidyl 7-methoxy-2H-chromene3-carboxylate, *Acta Cryst.* E63, o1513–o1514.
- [2] Reddie, K.G., Seo, Y.H., Muse III, W.B., Leonard, S.E., and Carroll, K.S. (2008) A chemical approach for detecting sulfenic acid-modified proteins in living cells, *Mol. BioSyst.* 4, 521–531.
- [3] Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. (2012) Fiji: an open-source platform for biological-image analysis, *Nat. Methods* 9, 676-682