Fluorogenic detection of cyanide ions in pure aqueous media through intramolecular crossed-benzoin reaction: limitations unveiled and possible solutions

Vincent Gaumerd,[a,b] Yoan Capello,[a] Quentin Bonnin,[a] Pierre-Yves Renard[c] and Anthony Romieu[*a]

[a]*Institut de Chimie Moléculaire de l'Université de Bourgogne, UMR 6302, CNRS, Université de Bourgogne, 9, Avenue Alain Savary, 21000 Dijon, France*
E-mail: anthony.romieu@u-bourgogne.fr; lab homepage: http://www.icmub.com

[b]*French Environment and Energy Management Agency, 20, avenue du Grésillé - BP 90406, 49004 Angers Cedex 01 France*

[c]*Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Normandie Univ, COBRA UMR 6014, INC3M FR 3038, 76000 Rouen, France - 1, Rue Tesnière, 76821 Mont-Saint-Aignan Cedex, France*

Supporting Information

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Abbreviations

The following abbreviations are used throughout the text of the ESI file: @, at; Abs, absorption; Ac_2O, acetic anhydride; AFU, arbitrary fluorescence unit; Ag_2O, silver(I) oxide; 7-AMC, 7-amino-4-methylcoumarin; aq., aqueous; a.m.u, atomic mass unit; Ar, argon; ATR, attenuated total reflectance; $CDCl_3$, deuterated chloroform; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; CTRL, control (*i.e.*, incubation of probe in buffer alone); δ, chemical shift; 2D, two dimensional; d, doublet; dd, doublet of doublet; DAD, diode array detector/detection; DIEA, *N*,*N*-diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; DMSO-d_6, deuterated dimethylsulfoxide; Em, emission; equiv., equivalent; ESI, electrospray ionization; EtOAc, ethyl acetate; EtOH, ethanol; Et_2O, diethyl ether; Ex, excitation; FA, formic acid; FC, flash-column chromatography; F.I., fluorescence intensity; FT, Fourier transform; HATU, *O*-(7-aza-1H-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; H_2O, water; HPLC, high-pressure liquid chromatography; HRMS, high-resolution mass spectrum; IC, ion chromatography; IR, infrared; *J*, coupling constant; KCN, potassium cyanide; K_2CO_3, potassium carbonate; $KHSO_4$, potassium hydrogenosulfate; LOD, limit of detection; LRMS, low-resolution mass spectrum; m, multiplet; MeCN, acetonitrile; MeOH, methanol; min, minutes; MS, mass spectrometry; MsCl, mesyl chloride; $NaHCO_3$, sodium bicarbonate; Na_2CO_3, sodium carbonate; NaI, sodium iodide; Na_2SO_4, sodium sulfate; $Na_2S_2O_3$, sodium thiosulfate; NMR, nuclear magnetic resonance; n, refractive index; O/N, overnight; PACSMUB, Plateforme d'Analyse Chimique et de Synthèse Moléculaire de l'Université de Bourgogne; PBS, phosphate buffered saline; PBr_3, phosphorus tribromide; PHBA, *para*-hydroxybenzyl alcohol; PhMe, toluene; PMT, photomultiplier tube; ppm, part per million; quant., quantitative; RP, reversed-phase; RT, room temperature; s, second; s (NMR context), singlet; SIM, selected ion monitoring; sol., solution; std dev., standard deviation; t, triplet; td, triplet of doublet; $TBAHSO_4$, tetrabutylammonium hydrogenosulfate; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; t_R, retention time; UV, ultraviolet; vis, visible; vs., versus.

S1 Instruments and methods

Safety note: all samples containing aq. solutions of KCN, from fluorescence assays and RP-HPLC-MS analyses, and rinsing waters (cleaning of quartz cells and HPLC glass vials) were pooled and incubated with bleach (2.6% active chlorine) for several days, then transferred into a special tank devoted to the sole storage of cyanide wastes. Thereafter, this tank will be collected and treated by a specialist firm (SUEZ France).

General

Unless otherwise noted, all commercially available reagents and solvents were used without further purification. TLC was carried out on Merck Millipore or Supelco® DC Kieselgel 60 F-254 aluminum sheets (Merck group). The spots were directly visualized or through illumination with a UV lamp (λ = 254/365 nm). The majority of purifications by flash-column chromatography were performed on coarse granulometry silica gel (60-200 μm) from VWR. Cyanide-responsive probes **IND-1**, **1** and **3** were purified by flash-column chromatography over "small" granulometry silica gel (40-63 μm, Geduran® Si 60) from Merck. HPLC-grade MeCN, and toluene were dried over alumina cartridges immediately prior to use (water

content for CH_2Cl_2: 50 ppm; MeCN: 12.5 ppm, determined by Karl Fischer titration[1]), using a solvent purification system PureSolv PS-MD-5 model from Innovative Technology. DMF and THF were purchased from Fisher Chemical (>99%, lab reagent grade) and AcrosOrganics (99.5%, extra dry, stabilized, AcroSeal®, water <0.005%) respectively, stored over activated 3Å molecular sieves and kept under Ar atmosphere. *N*,*N*-Diisopropylethylamine (DIEA) and triethylamine (TEA) were purified/dried by distillation over KOH and stored over KOH pellets and kept under Ar atmosphere. HPLC-gradient grade MeCN and MeOH were obtained from Fisher Chemical. All aqueous buffers used in this work and aqueous mobile-phases for RP-HPLC were prepared using water purified either with a PURELAB Ultra system or a Chorus PURELAB system (ELGA, VEOLIA, purified to 18.2 MW.cm).

Instruments

Freeze-drying operations were performed with a Christ Alpha 2-4 LD plus. Centrifugation steps were performed with a Sprout® Plus Mini Centrifuge instrument (Heathrow Scientific). ^{1}H-, ^{13}C- and ^{19}F- NMR spectra were recorded on a Bruker Avance Neo 500 MHz (equipped with a 5 mm BBOF iProbe. NMR spectroscopy chemical shifts are quoted in parts per million (δ) relative to TMS (for ^{1}H, and ^{13}C) or $CFCl_3$ (for ^{19}F). For ^{1}H and ^{13}C spectra, calibration was made by using residual signals of partially deuterated solvent summarized in 2010 by Fulmer *et al.*[2] For all other nuclei, SR value obtained after zero-calibration of the corresponding reference was applied. *J* values are expressed in Hz. IR spectra were recorded with a Bruker Alpha FT-IR spectrometer equipped with a universal ATR sampling accessory. The bond vibration frequencies are expressed in reciprocal centimeters (cm^{-1}). RP-HPLC-MS analyses were performed on a Thermo Scientific Vanquish™ Flex instrument (pump + autosampler at 20°C + column oven at 25°C) equipped with a UV-visible DAD and ISQ-EM single quadrupole mass spectrometer. Purifications by semi-preparative HPLC were performed on a Thermo-Dionex Ultimate 3000 instrument (semi-preparative pump HPG-3200BX) equipped with an RS Variable Detector (VWD-3400RS, four distinct wavelengths within the range 190-800 nm). Ion chromatography analyses (for the determination of TFA mass content in freeze-dried samples) were performed using a Thermo Scientific Dionex ICS 6000 ion chromatograph equipped with a conductivity detector CD (Thermo Scientific Dionex) and a conductivity suppressor ADRS 600 2 mm (Thermo Scientific Dionex), and according to a method developed by the PACSMUB staff[3]. LRMS analyses were achieved with a Thermo Scientific ISQ-EM single quadrupole mass spectrometer equipped with an electrospray (ESI) source. HRMS analyses were achieved with an Orbitrap Exploris 240 mass spectrometer (Thermo Scientific) equipped with an electrospray ionization source (HESI), by one member of the PACSMUB staff. By default, the samples are injected *via* the HPLC Vanquish™ system composed of a binary pump, an autosampler (25 °C), and a column compartment (25 °C). The following MS source parameters were used in HPLC mode if no further specification is mentioned: ion transfer tube temperature: 320°C, gas flow: sheath 40 / aux 5 / sweep 0, spray Voltage: 3.5 kV, spray current: 0.75 µA, capillary temperature: 275°C, resolution (*m/z* = 200): 240 000. Mass calibration in the 100-2000 Da mass range was operated using the commercially available Pierce Flexmix calibration solution (Thermofisher Scientific, #15988796), every month; a one-point mass calibration

[1] A. S. Meyer and C. M. Boyd, *Anal. Chem.*, 1959, **31**, 215-219.

[2] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176-2179.

[3] G. Dejouy, M. Laly, I. E. Valverde and A. Romieu, *Dyes Pigm.*, 2018, **159**, 262-274.

(fluoranthene) was operated for control every week. The following HPLC conditions were used: (Kinetex C_8 SecurityGuard cartridge, AJ0-4290) with LCMS grade MeOH and 0.1% aq. formic acid (FA) as eluents [isocratic: 98% MeOH- 2% FA (6 min)] at a flow rate of 0.2 mL/min. Typically, a 10^{-5} M solution of sample (sample preparation: stock solution in DMSO was diluted by a factor of 21 in H_2O-MeCN 1:1 (v/v) except for **1** which was diluted in MeCN alone) was then analysed *via* the RP-HPLC-HRMS.

HPLC separations

Several chromatographic systems were used for the analytical experiments and the purification steps:

System A: RP-HPLC-MS with Vanquish™ Flex instrument (Phenomenex Kinetex C_{18} column, 2.6 µm, 2.1 × 50 mm) with MeCN (+0.1% FA) and 0.1% aq. formic acid (aq. FA, pH 2.1) as eluents [5% to 100% (5 min) of MeCN, then 100% MeCN (3 min)] at a flow rate of 0.5 mL/min. UV-visible detection was achieved at 220, 260, 350 and 500 nm (+ DAD 190-800 nm allowing extraction of any single wavelength channel within this spectral range). Low resolution ESI-MS detection (ISQ-EM detector) in the positive/negative mode with the following parameters: full scan, 100-1000 a.m.u., spectrum type: centroid, dwell or scan time: 1s, source CID voltage: 20 V, vaporizer temperature: 282 °C, ion transfer tube temperature: 300 °C, source voltage positive ions: 3 kV, source voltage negative ions: 2 kV, sheet gas pressure: 49.9 psig (3.4 bars), aux gas pressure: 5.7 psig (0.35 bar) and sweep gas pressure: 0.5 psig (0.035 bar).

System A': system A with (Phenomenex Kinetex C_8 column, 1.7 µm, 2.1 × 30 mm) and UV-visible detection achieved at 220, 260, 350 and 650 nm (+ DAD 190-800 nm allowing extraction of any single wavelength channel within this spectral range).

System A'': system A with the following detection parameters for LRMS (ESI+/-): full scan, 100-1000 a.m.u.; SIM(-), *m/z* 225.0 ± 0.5 for **S2**; SIM(+), *m/z* = 422.0 ± 0.5 for **IND-1**; SIM(+), *m/z* = 371 ± 0.5 for **1**, SIM(+), SIM(+), *m/z* = 214.0 ± 0.5 for **resorufin**, SIM(+), *m/z* = 163.0 ± 0.5 for **umbelliferone**, SIM(+), m/z = 209.0 ± 0.5 for **9,10-phenanthrenequinone**, SIM(+), *m/z* = 236.0 ± 0.5 for cyanohydrin **5**.

System B: semi-preparative RP-HPLC (SiliCycle SiliaChrom C_{18} column, 10 µm, 20 × 250 mm) with MeCN and aq. 0.1% TFA (pH 1.9) as eluents [10% MeCN (5 min), followed by a linear gradient from 10% to 30% MeCN (10 min), then 30% to 100% MeCN (70 min)] at a flow rate of 20.0 mL/min. Quadruple UV detection was achieved at 220, 260, 330 and 350 nm.

System C: semi-preparative RP-HPLC (SiliCycle SiliaChrom C_8 column, 10 µm, 20 × 250 mm) with MeCN and aq. 0.1% TFA (pH 1.9) as eluents [40% MeCN (5 min), followed by a gradient from 40% to 60% MeCN (10 min), then 60% to 100% MeCN (80 min)] at a flow rate of 20.0 mL/min. Quadruple UV detection was achieved at 220, 260, 320 and 340 nm.

System D: semi-preparative RP-HPLC (SiliCycle SiliaChrom C_8 column, 10 µm, 20 × 250 mm) with MeCN and H_2O (pH 6.4) as eluents [40% MeCN (5 min), followed by a linear gradient from

40% to 100% MeCN (80 min)] at a flow rate of 20.0 mL/min. Quadruple UV detection was achieved at 220, 260, 310 and 320 nm.

Table S1. Data table related to the preparation of batches of probes

Probe	**IND-1**	**1**	**3**	**4**
V batch (mL)	27.0	27.0	27.0	27.0
V PBS (mL)	26.730	26.730	26.730	26.730
V DMSO (*q.s.* 1%) (µL)	170.0	141.4	161.9	156.2
V probes (µL)	100.0	128.6	108.1	113.8

Table S2. Conditions map of fluorescence time-course assays (concentration: 10 µM)

Entry	Quartz cell position	[KCN] N (µM)	$V_{(KCN\ solution)}$ N+1 (µL)	$V_{solution}$ N (µL)	$V_{(KCN\ addition)}$ (µL)	Final [KCN] in quartz cell (µM)	KCN equiv.
1	10	100000	/	300	5	200	20
2	9	75000	112.5	150		150	15
3	8	50000	100	150		100	10
4	7	37500	112.5	150		75	7.5
5	6	25000	100	150		50	5
6	5	20000	120	150		40	4
7	4	15000	112.5	150		30	3
8	3	10000	100	150		20	2
9	2	5000	75	150		10	1
10	1					0	0 (control)

Table S3. Parameters used for time-course assays

Probe	**IND-1**	**1**	**3**	**4**
Parameters for Kinetics mode				
Ex wavelength (nm)	565	330		
Em wavelength (nm)	595	450		
PMT voltage (V)	848	655		
Bandwidths Ex/Em (nm)	1.5/1.5	2/2		
Parameters for Scan mode				
Ex wavelength (nm)	540	330		
Em wavelength (nm)	555-800	350-650		
PMT voltage (V)	439	391		
Bandwidths Ex/Em (nm)	5/5	5/5		

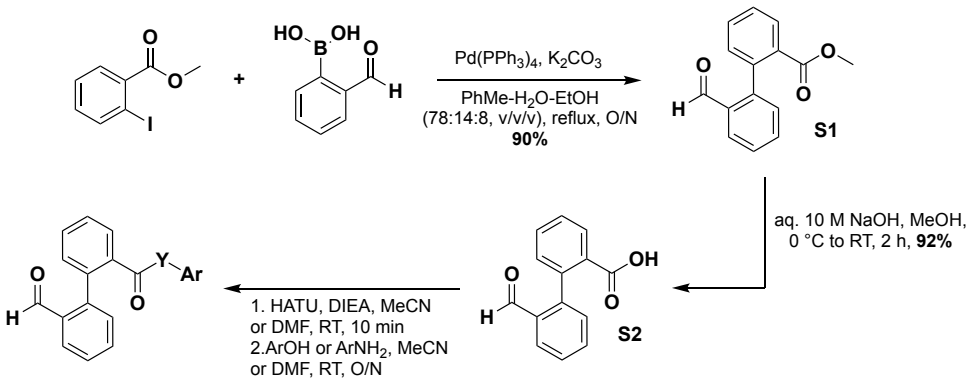
Table S4. Conditions map of fluorescence time-course assays conducted with 1.0 µM and 10 µM concentrations

Entry	1	2	3	4	5	6	7	8
Quartz cell position	1	2	3	4	5	6	7	8
Probe **IND-1** concentration	1.0 µM			10 µM				
Resorufin (sodium salt)							1.0 µM	10. 0 µM
KCN	**0 equiv.** (control)	**10 equiv.** (2.5 µL of 0.01 M)	**20 equiv.** (5 µL of 0.01 M)	**0 equiv.** (control)	**10 equiv.** (2.5 µL of 0.1 M)	**20 equiv.** (5 µL of 0.1 M)		

S2 Synthetic procedures

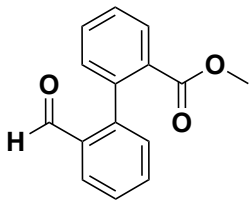
Please note: atropoisomerism was observed in some biphenyl derivatives described below, leading to duplication of NMR signals and/or peak splitting during RP-HPLC analyses of the corresponding pure compounds. This kind of chirality leads also to the split of some CH_2 into AB systems.

Scheme S1. Synthetic route towards probes IND-1, 1 and 2



IND-1, Y = O, ArOH = resorufin (yield after FC (SiO_2) **51%**)
1, Y = O, ArOH = umbelliferone (yield after FC (SiO_2) **59%**)
2, Y = NH, $ArNH_2$ = 7-amino-4-methylcoumarin (yield after RP-HPLC purification **12%**)

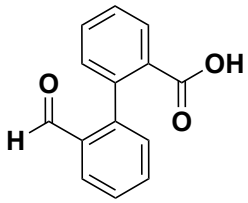
Methyl 2'-formyl-[1,1'-biphenyl]-2-carboxylate S1 [16231-67-7][4]



[4]M. Penhoat, S. Leleu, G. Dupas, C. Papamicaël, F. Marsais and V. Levacher, *Tetrahedron Lett.*, 2005, **46**, 8385-8389.

To a solution of methyl-2-iodobenzoate (2.18 g, 8.32 mmol, 1 equiv.) in toluene (125 mL) and absolute EtOH (12.5 mL), were added 2-formylphenylboronic acid (1.25 g, 8.32 mmol, 1 equiv.) and aq. K_2CO_3 solution (7.7 g in 22 mL of H_2O). The reaction mixture was degassed with an Ar flow for 15 min. After adding $Pd(PPh_3)_4$ (481 mg, 5 mol%), the reaction mixture was degassed with an Ar flow for further 15 min. The mixture was stirred at reflux under Ar atmosphere overnight and then cooled to RT. The solution was filtered through a plug of dicalite and toluene was evaporated under reduced pressure. Then, Et_2O (100 mL) was added to the resulting aqueous phase. After phase separation, the organic layer was washed with deionized water (50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure, and the resulting residue was purified by column chromatography over silica gel (bed size: 3 cm × 20 cm, VWR 50-200 µm, eluent: step gradient of EtOAc in heptane from 0% to 25%) to afford biphenyl-type compound **S1** as a yellow oil (1.80 g, 7.5 mmol, yield 90%). *Please note: two distinct signals are visualized on ^{1}H NMR spectrum attributed to the aldehyde proton (δ = 9.80 ppm); we assumed that they are assigned to two distinct atropoisomers*. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.80 (1 H), 8.05 (1 H, dd, *J* 7.8, 1.5), 8.01 (1 H, dd, *J* 7.8, 1.5), 7.59 (2 H, qd, *J* 7.6, 1.5), *please note: signals at 7.59 ppm appear as one qd but are actually two td on top of each other*, 7.55-7.48 (2 H, m), *please note: signals at 7.55-7.48 ppm appear as m but are actually two td on top of each other*, 7.30 (1 H, dd, *J* 7.6, 1.4), 7.25 (1 H, dd, *J* 7.7, 1.2), 3.61 (3 H, s) ppm; HPLC (system A): t_R = 4.0 min (purity >95% at 220 nm, purity >95% at 260 nm); LRMS (ESI+, recorded during RP-HPLC analysis): *m/z* 241.1 $[M + H]^+$ (7.5), calcd for $C_{15}H_{13}O_3^+$ 241.1.

2'-Formyl-[1,1'-biphenyl]-2-carboxylic acid S2 [6720-26-9][4]



To a solution of methyl ester **S1** (1.80 g, 7.5 mmol, 1 equiv.) in MeOH (43 mL), was added aq. 10 M NaOH (4.6 mL, 46 mmol, 6.1 equiv.) at 0°C. The resulting reaction mixture was stirred at RT for 2 h. Then, MeOH was evaporated under reduced pressure. Deionized H_2O (30 mL) was added and the resulting aq. layer was washed with CH_2Cl_2 (30 mL). After treatment with aq. 1.0 M HCl (pH ca. 3-4), the aqueous layer was extracted with EtOAc (2 × 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford carboxylic acid **S2** as a white solid (1.56 g, 6.9 mmol, yield 92%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.77 (1 H, s), 8.10 (1 H, dd, *J* 7.8, 1.4), 7.97 (1 H, dd, *J* 7.8, 1.4), 7.59 (2 H, dtd, *J* 13.0, 7.5, 1.5), *please note: signals at 7.59 ppm appear as one dtd but are actually two td on top of each other*, 7.53-7.48 (2 H, m), *please note: signals at 7.53-7.48 ppm appear as m but are actually two td on top of each other*, 7.27 (1 H, d, *J* 7.5), 7.22 (1 H, d, *J* 7.4) ppm; HPLC (system A): t_R = 3.5 min (purity >99% at 220 nm, purity >99% at 260 nm); LRMS (ESI+, recorded during RP-HPLC analysis): *m/z* 227.1 $[M + H]^+$ (100), calcd for $C_{14}H_{11}O_3^+$ 227.1; LRMS (ESI-, recorded during RP-HPLC analysis): *m/z* 225.0 $[M - H]^-$ (100), 293.0 $[M + FA + Na - 2H]^-$ (7.5) and 473.0 $[2M + Na - 2H]^-$ (5), calcd for $C_{14}H_9O_3^-$ 225.1.

Resorufin-based probe IND-1 [1705596-76-4][5]

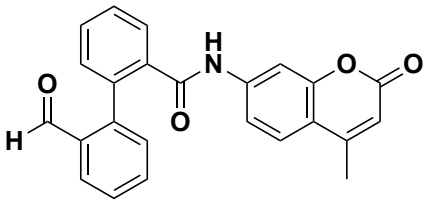
Synthetic protocol adapted from that published by Lee *et al.*[5] To a solution of carboxylic acid **S2** (50 mg, 0.22 mmol, 1 equiv.) in dry MeCN (2.2 mL), was added DIEA (75 μL, 0.44 mmol, 2 equiv.) and HATU (92 mg, 0.24 mmol, 1.1 equiv.). The reaction mixture was stirred at RT for 10 min and the formation of HOAt active esters was checked by RP-HPLC-MS (system A). Then, sodium salt of resorufin (65.5 mg, 0.31 mmol, 1.4 equiv.) and a catalytic amount of DMAP (2.7 mg, 0.022 mmol, 0.1 equiv.) were sequentially added. The reaction mixture was stirred at RT for 4 h. Thereafter, MeCN was evaporated under reduced pressure, and deionized H_2O (30 mL) and brine (20 mL) were added to the residue. The aqueous phase was extracted with CH_2Cl_2 (4 × 50 mL). Combined organic phases were then washed with deionized H_2O (100 mL), dried over anhydrous Na_2SO_4 and finally evaporated to dryness, under reduced pressure. The resulting residue was purified by flash-column chromatography over silica gel (bed size: 2 cm × 20 cm, Merck 40-63 μm, eluent: CH_2Cl_2-EtOAc 90:10, v/v) to afford resorufin-based probe **IND-1** as a yellow solid (47.6 mg, 0.11 mmol, yield 51%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.90 (1 H, s), 8.25 (1 H, dd, *J* 7.8, 1.4), 8.01 (1 H, dd, *J* 7.8, 1.4), 7.74-7.69 (2 H, m), 7.67-7.61 (2 H, m), 7.55 (1 H, t, *J* 7.6), 7.40 (2 H, d, *J* 9.6), 7.34 (1 H, dd, *J* 7.6, 1.2), 6.92 (1 H, d, *J* 2.4), 6.86 (2 H, m), 6.30 (1 H, d, *J* 2.0) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.6, 186.4, 164.6, 153.3, 149.4, 148.5, 144.5, 144.4, 140.5, 135.3, 134.9, 134.0, 133.6, 133.0, 132.0, 131.4, 131.2, 130.3, 129.1, 128.7, 128.6, 128.4, 119.1, 109.6, 107.4 ppm; HPLC (system A): t_R = 4.4 min (purity >99% at 220 nm, purity >99% at 260 nm, purity >99% at 355 nm, purity >99% at 445 nm); LRMS (ESI+, recorded during RP-HPLC analysis): *m/z* 422.2 $[M + H]^+$ (65), calcd for $C_{26}H_{16}NO_5^+$ 422.1.

Umbelliferone-based probe 1

Synthetic protocol adapted from that published by Lee *et al.*[5] To a solution of carboxylic acid **S2** (50 mg, 0.22 mmol, 1 equiv.) in dry MeCN (2.2 mL), was added DIEA (41 μL, 0.24 mmol, 1.1 equiv.) and HATU (92 mg, 0.24 mmol, 1.1 equiv.). The reaction mixture was stirred at RT for 10 min and the formation of HOAt active esters was checked by RP-HPLC-MS (system A). Then, umbelliferone (35.5 mg, 0.22 mmol, 1 equiv.) was added. The reaction mixture was stirred at RT for 4 h. Thereafter, MeCN was evaporated under reduced pressure, and CH_2Cl_2 (20 mL) was added. The organic phase was washed with deionized H_2O (2 × 20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 and finally evaporated to dryness, under reduced pressure. The resulting residue was purified by flash-column chromatography over silica gel (bed size: 2 cm × 20 cm, Merck 40-63 μm, eluent: CH_2Cl_2-EtOAc 96:4, v/v) to afford coumarin-based probe **1** as a white solid (48.2 mg, yield 59%). IR (ATR): ν = 3057, 2838, 2752, 1726, 1694, 1621, 1594, 1567, 1497, 1472, 1443, 1424, 1397, 1278, 1251, 1238, 1198, 1149, 1127, 1100, 1080, 1039, 1003, 983, 887, 827, 757, 702 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.89 (1 H, s), 8.24 (1 H, dd, *J* 7.8, 1.4), 8.00 (1 H, dd, *J* 7.8, 1.4), 7.69 (1 H, td, *J* 7.6, 1.4), 7.66-7.60 (3 H, m), 7.54 (1 H, t, *J* 7.6), 7.42-7.36 (2 H, m), 7.33 (1 H, dd, *J* 7.6, 1.2), 6.86-6.79 (2 H, m), 6.36 (1 H, d, *J* 9.6) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.6, 164.8, 160.4, 154.7, 153.0, 144.6, 142.9, 140.3, 134.0, 133.6, 132.8, 132.0, 131.2, 130.3, 129.4, 128.6, 128.6, 128.5, 128.4, 118.3, 116.8, 116.3, 110.3 ppm; HPLC (system A): t_R = 4.3 min (purity >98% at 220 nm, purity >99% at 260 nm, purity >99% at 304 nm); HRMS (ESI+): *m/z* calculated 371.09140 for $[M + H]^+$, found 371.09112 and 393.07334 for $[M + Na]^+$, found 393.07314.

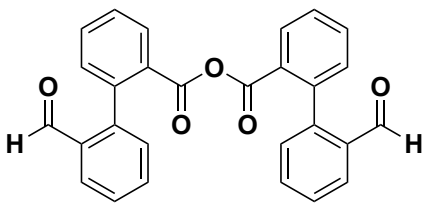
[5] J. H. Lee, J. H. Jang, N. Velusamy, H. S. Jung, S. Bhuniya and J. S. Kim, *Chem. Commun.*, 2015, **51**, 7709-7712.

7-Amino-4-methylcoumarin-based probe 2



Synthetic protocol adapted from that published by Lee *et al.*[5] To a solution of carboxylic acid **S2** (50 mg, 0.22 mmol, 1 equiv.) in dry DMF (2.0 mL), was added DIEA (42 μL, 0.25 mmol, 1.1 equiv.) and HATU (88 mg, 0.23 mmol, 1.1 equiv.). The reaction mixture was stirred at RT for 10 min and the formation of HOAt activate esters was checked by RP-HPLC-MS (system A). Then, 7-amino-4-methylcoumarin (39 mg, 0.22 mmol, 1 equiv.) was added. The reaction mixture was stirred at RT for 16 h. Thereafter, DMF was evaporated under reduced pressure and the resulting residue was directly purified by semi-preparative RP-HPLC (system B, t_R = 39.0-41.0 min). The product containing fractions were lyophilized to give coumarin-based probe **2** as a white amorphous powder (10.3 mg, 0.027 mmol, yield 12%). *Please note: the presence of a minor amount of TFA in freeze-dried sample (TFA mass content <1.5%) was confirmed by IC analyses*. IR (ATR): ν = 3306, 3280, 2921, 2849, 1694, 1669, 1616, 1579, 1527, 1474, 1444, 1437, 1417, 1391, 1368, 1290, 1264, 1197, 1156, 1088, 1068, 1019, 887, 872, 847, 828, 813, 769, 685 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 10.67 (1 H, s), 9.79 (1 H, s), 7.85 (1 H, dd, *J* 7.8, 1.4), 7.77-7.71 (1 H, m), 7.68-7.58 (4 H, m), 7.56-7.52 (1 H, m), 7.50 (1 H, t, *J* 7.6), 7.46-7.43 (1 H, m), 7.37 (2 H, d, *J* 7.9), 6.24 (1 H), 2.35 (3 H, s) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 191.1, 167.4, 159.9, 153.4, 153.0, 143.4, 142.1, 137.0, 136.0, 133.5, 133.4, 131.3, 130.7, 130.0, 128.3, 128.2, 127.5, 126.7, 125.8, 115.5, 115.4, 112.5, 106.0, 18.0 ppm; HPLC (system A): peak splitting due to atropoisomerism was observed, <u>minor peak</u>: t_R = 3.8 min (purity 20.5% at 220 nm, purity 24.1% at 260 nm, purity 31.5% at 314 nm, purity 18.6% at 328 nm); LRMS (ESI+, recorded during RP-HPLC analysis): *m/z* 384.2 $[M + H]^+$ (100), 447.3 $[M + MeCN + H]^+$ (7.5) and 789.1 $[2M + Na]^+$ (35), calcd for $C_{24}H_{18}NO_4^+$ 384.1; LRMS (ESI-, recorded during RP-HPLC analysis): *m/z* 381.8 $[M - H]^-$ (100), calcd for $C_{24}H_{16}NO_4^-$ 382.1; <u>major peak</u>: t_R = 4.0 min (purity 79% at 220 nm, purity 76% at 260 nm, purity 85% at 314 nm, purity 81% at 328 nm); LRMS (ESI+, recorded during RP-HPLC analysis): *m/z* 384.1 $[M + H]^+$ (100), 447.1 $[M + MeCN + H]^+$ (7.5) and 789.1 $[2M + Na]^+$ (35), calcd for $C_{24}H_{18}NO_4^+$ 384.1; LRMS (ESI-, recorded during RP-HPLC analysis): *m/z* 382.0 $[M - H]^-$ (100), calcd for $C_{24}H_{16}NO_4^-$ 382.1.

2'-Formyl-[1,1'-biphenyl]-2-carboxylic anhydride S3

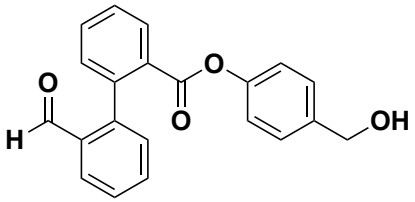


Synthetic protocol adapted from that published by Liao *et al.*[6] To a solution of carboxylic acid **S2** (500 mg, 2.2 mmol, 1.7 equiv.) in dry THF (2.3 mL) was added MsCl (103 μL, 1.33 mmol, 1

[6]W. Liao, Jr., S.-Y. Lin, Y.-S. Kuo and C.-F. Liang, *Org. Lett.*, 2022, **24**, 4207-4211.

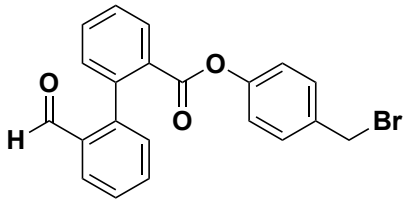
equiv.) and a solution of TEA (770 µL, 5.5 mmol, 4.2 equiv.) in dry THF (0.7 mL) at 0 °C. The resulting reaction mixture was stirred at RT overnight. Thereafter, the reaction mixture was evaporated under reduced pressure. Deionized H_2O (50 mL) and CH_2Cl_2 (50 mL) were added; after extraction/decantation, the organic phase was sequentially washed with aq. saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and finally evaporated to dryness to give symmetrical anhydride **S3** as a brown viscous oil (471 mg, 1.1 mmol, quantitative yield). IR (ATR): ν = 3061, 2845, 2749, 1785, 1725, 1689, 1594, 1568, 1495, 1471, 1441, 1395, 1249, 1197, 1114, 1057, 1015, 981, 888, 849, 826, 754, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.76 (1 H, s), 9.73 (1 H, s), 7.91 (2 H, dt, *J* 7.8, 1.6), 7.81 (1 H, dd, *J* 7.9, 1.3), 7.76 (1 H, dd, *J* 7.9, 1.4), 7.65-7.59 (2 H, m), 7.59-7.53 (2 H, m), 7.50-7.43 (4 H, m), 7.27 (2 H, d, *J* 1.3), *please note: the second part of the dd at 7.27 ppm is masked by the residual $CHCl_3$ in $CDCl_3$*, 7.20 (2 H, dd, *J* 7.4, 5.9) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.4, 161.8, 144.1, 143.9, 141.0, 140.8, 133.8, 133.6, 133.6, 133.4, 133.2, 133.2, 132.2, 131.6, 131.4, 130.4, 130.4, 128.7, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2 ppm; HPLC (system A): t_R = 4.6 min (purity >99% at 220 nm, purity >98% at 260 nm); LRMS (ESI+, recorded during RP-HPLC analysis): 457.2 [M + Na]$^+$ (100), calcd for $C_{28}H_{18}NaO_5^+$ 457.1.

4-(Hydroxymethyl)phenyl 2'-formyl-[1,1'-biphenyl]-2-carboxylate S4



Synthetic protocol adapted from that published by Liao *et al.*[6] To a solution of symmetrical anhydride **S3** (236 mg, 0.54 mmol, 1 equiv.) in dry DMF (1.5 mL), $Na_2S_2O_3$ (17 mg, 0.11 mmol, 0.2 equiv.), PHBA (135 mg, 1.08 mmol, 2 equiv.) and Na_2CO_3 (57.2, 0.54, 1 equiv.) were sequentially added. The resulting reaction mixture was stirred at RT overnight. The reaction was checked for completion by RP-HPLC-MS (system A), then, the mixture was evaporated under reduced pressure. The resulting residue was re-suspended in Et_2O (50 mL), and this organic phase was washed with aq. 5% K_2CO_3 (3 × 50 mL), dried over anhydrous Na_2SO_4, and finally evaporated under reduced pressure. The resulting residue was purified by column chromatography over silica gel (bed size: 3 cm × 20 cm, VWR 50-200 µm, eluent: step gradient of EtOAc in heptane from 0% to 30%; *please note: isocratic elution with heptane-EtOAc 85:15 (v/v) was also tried but a larger amount of impure compound was recovered even if this latter procedure is less time-consuming*) to afford PHBA ester derivative **S4** as a white amorphous solid (78 mg, 0.23 mmol, yield 44%). IR (ATR): ν = 3369, 3062, 2921, 2850, 2750, 1732, 1689, 1595, 1506, 1442, 1395, 1274, 1238, 1190, 1162, 1115, 1063, 1038, 1013, 1004, 877, 827, 756, 704 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.89 (1 H, s), 8.23 (1 H, dd, *J* 7.9, 1.4), 7.98 (1 H, dd, *J* 7.8, 1.4), 7.66 (1 H, td, *J* 7.6, 1.5), 7.64-7.57 (2 H, m), 7.50 (1 H, t, *J* 7.6), 7.37 (1 H, dd, *J* 7.5, 1.3), 7.33 (1 H, dd, *J* 7.5, 1.2), 7.29 (2 H, d, *J* 8.3), 6.83 (2 H, d, *J* 8.5), 4.63 (2 H, s) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.7, 165.5, 150.0, 145.0, 140.0, 138.7, 134.0, 133.5, 132.4, 131.9, 131.0, 130.3, 130.1, 128.5, 128.2, 128.1, 128.1, 121.5, 64.9 ppm; HPLC (system A): t_R = 4.0 min (purity >98% at 220 nm, purity >98% at 260 nm); LRMS (ESI+, recorded during RP-HPLC analysis): 355.1 [M + Na]$^+$ (50), calcd for $C_{21}H_{16}NaO_4^+$ 355.1.

4-(Bromomethyl)phenyl 2'-formyl-[1,1'-biphenyl]-2-carboxylate S5



Synthetic protocol adapted from that published by Jenni *et al*.[7] PBr_3 (24 μL, 0.25 mmol, 1 equiv.) was added to a cooled solution of benzyl alcohol **S4** (84 mg, 0.25 mmol, 1 equiv.) previously dissolved in dry THF (3 mL). The resulting reaction mixture was stirred at RT for 30 min and the reaction was checked for completion by ^{1}H NMR. Thereafter, CH_2Cl_2 (50 mL) was added and the organic phase was sequentially washed with aq. 1.0 M $KHSO_4$ (60 mL) and brine (60 mL), dried over anhydrous Na_2SO_4, and finally evaporated under reduced pressure. The resulting residue was dried under vacuum to afford benzyl bromide derivative **S5** as a red oil (92 mg, 0.23 mmol, yield 92%). This product was used in the next *O*-alkylation step without further purification to avoid its premature degradation. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.88 (1 H, s), 8.22 (1 H, dd, *J* 7.8, 1.5), 7.99 (1 H, dd, *J* 7.8, 1.5), 7.67 (1 H, td, *J* 7.5, 1.5), 7.61 (2 H, m), 7.51 (1 H, t, *J* 7.5), 7.37 (1 H, dd, *J* 7.7, 1.4), 7.31 (3 H, d, *J* 8.6), 6.81 (2 H, d, *J* 8.5), 4.43 (2 H, s) ppm.

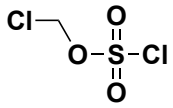
Three-component umbelliferone-based probe 3

Benzyl bromide derivative **S5** (46 mg, 0.12 mmol, 1 equiv.) was dissolved in dry MeCN (1.2 mL). Anhydrous K_2CO_3 (32 mg, 0.23 mmol, 2 equiv.) was added and the reaction mixture was stirred at RT for 15 min. Then, umbelliferone (19 mg, 0.12 mmol, 1 equiv.) was added and the reaction mixture was stirred at RT overnight. The reaction was checked for completion by RP-HPLC-MS (system A or A'). Thereafter, solvents were removed by evaporation under reduced pressure. Then, CH_2Cl_2 (10 mL) was added and the resulting organic layer was washed sequentially with aq. 1.0 M $KHSO_4$ (10 mL) and brine (2 × 10 mL). After drying over anhydrous Na_2SO_4, the organic phase was evaporated under reduced pressure. The resulting residue was purified by column chromatography over silica gel (bed size: 2 cm × 20 cm, Merck 40-63 μm, eluent: step gradient of CH_2Cl_2 in heptane from 80% to 100%, followed by a second step gradient of EtOAc in CH_2Cl_2 from 0% to 1%) to afford coumarin-based probe **3** as white solid (6.8 mg, 14.6 μmol, yield 12%). *Please note: the poor isolated yield was explained by partial degradation of compound on silica gel stationary phase as supported by the recovery of a significant amount of starting umbelliferone whereas the conversion rate of this O-alkylation reaction was found to be almost quantitative; the use of semi-preparative RP-HPLC (system C, t_R = 27.0-29.0 min) was identified as a valuable alternative purification method and enabled isolation of **3** with a higher yield (20 mg, 42 μmol, yield 28%)*. *Please note: the presence of a minor amount of TFA in freeze-dried sample (TFA mass content = 1.9%) was confirmed by IC analyses*. *Sample purified by column chromatography was solely used for recording IR spectrum*. IR (ATR): ν = 3063, 2995, 2924, 2853, 2748, 1782, 1728, 1692, 1610, 1557, 1508, 1463, 1400, 1275, 1230, 1191, 1163, 1119, 1062, 1037, 1004, 884, 830, 756, 703 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.89 (1 H, s), 8.23 (1 H, dd, *J* 7.8, 1.4), 7.99 (1 H, dd, *J* 7.8, 1.4), 7.70-7.57

[7] S. Jenni, F. Ponsot, P. Baroux, L. Collard, T. Ikeno, K. Hanaoka, V. Quesneau, K. Renault and A. Romieu, *Spectrochim. Acta Part A*, 2021, **248**, 119179.

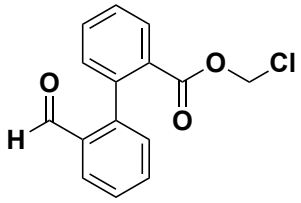
(4 H, m), 7.51 (1 H, t, *J* 7.6), 7.40-7.35 (1 H, m), 7.38-7.31 (4 H, m), 6.90-6.83 (4 H, m), 6.26 (1 H, d, *J* 9.4), 5.06 (2 H, s) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.7, 165.4, 161.8, 161.3, 155.9, 150.5, 144.9, 143.5, 140.1, 134.0, 133.6, 133.5, 132.5, 131.9, 131.0, 130.3, 130.0, 129.0, 128.8, 128.6, 128.2, 128.2, 121.8, 113.5, 113.3, 113.0, 102.1, 70.0 ppm; *please note: batch purified by column chromatography:* HPLC (system A) : t_R = 4.8 min (purity >95% at 220 nm, purity >96% at 260 nm, purity >96% at 320 nm); *please note: batch purified by semi-preparative RP-HPLC:* HPLC (system A'): t_R = 3.2 min (purity >99% at 220 nm, purity >99% at 260 nm, purity >99% at 320 nm); HRMS (ESI+): *m/z* calculated 477.13326 for $[M + H]^+$, found 477.13303.

Chloromethyl chlorosulfate S6 [49715-04-0][8]



A mixture of bromochloromethane (32.5 mL, 0.5 mmol, 1 equiv.) and chlorosulfonic acid (66.5 mL, 1 mmol, 2 equiv.) was heated under reflux for 3 h. Thereafter, the reaction mixture was poured into ice and the resulting aq. suspension was extracted with CH_2Cl_2 (2 × 100 mL). Combined organic phases were washed with deionized H_2O (3 × 150 mL) and dried over anhydrous Na_2SO_4. Volatiles were evaporated under reduced pressure and the resulting oily residue was purified by vacuum distillation (boiling point 25 °C at 1 mbar) to give chloromethyl chlorosulfate **S6** as a colourless low-viscosity syrup (7.6 g, 0.046 mol, yield 9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.97 (2 H, s) ppm.

Chloromethyl 2'-formyl-[1,1'-biphenyl]-2-carboxylate S7



Synthetic protocol adapted from that published by Tian *et al.*[9] To a biphasic solution of $TBAHSO_4$ (45 mg, 0.13 mmol, 0.1 equiv.) and K_2CO_3 (735 mg, 5.3 mmol, 4.0 equiv.) in deionized H_2O (1.6 mL) and CH_2Cl_2 (1.6 mL) were sequentially added carboxylic acid **S2** (300 mg, 1.33 mmol, 1.0 equiv.) and a CH_2Cl_2 solution of freshly distilled chloromethyl chlorosulfate **S6** (0.2 mL, 2.0 mmol, 1.5 equiv., in 0.8 mL dry CH_2Cl_2). The resulting reaction mixture was stirred vigorously (600 rpm) at rt for 1 h. Thereafter, the mixture was diluted by adding deionized H_2O and CH_2Cl_2 (final volumes of both phases ca. 5-6 mL) and the layers were separated. The aq. layer was extracted with CH_2Cl_2 (2 × 10 mL) and combined organic phases were dried over anhydrous Na_2SO_4, and finally evaporated under reduced pressure to afford **S7** as red viscous oil (364 mg, 1.33 mmol, quantitative yield). *Please note: **S7** was used in the next O-alkylation step without further purification and assumed to be pure for calculation of isolated yield. Please note: two distinct signals are clearly visualized on ^{1}H NMR spectrum attributed to the*

[8]H. Ouyang, R. T. Borchardt, T. J. Siahaan and D. G. Vander Velde, *J. Peptide Res.*, 2002, **59**, 183-195.
[9]L. Tian, Y. Yang, L. M. Wysocki, A. C. Arnold, A. Hu, B. Ravichandran, S. M. Sternson, L. L. Looger and L. D. Lavis, *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 4756-4761.

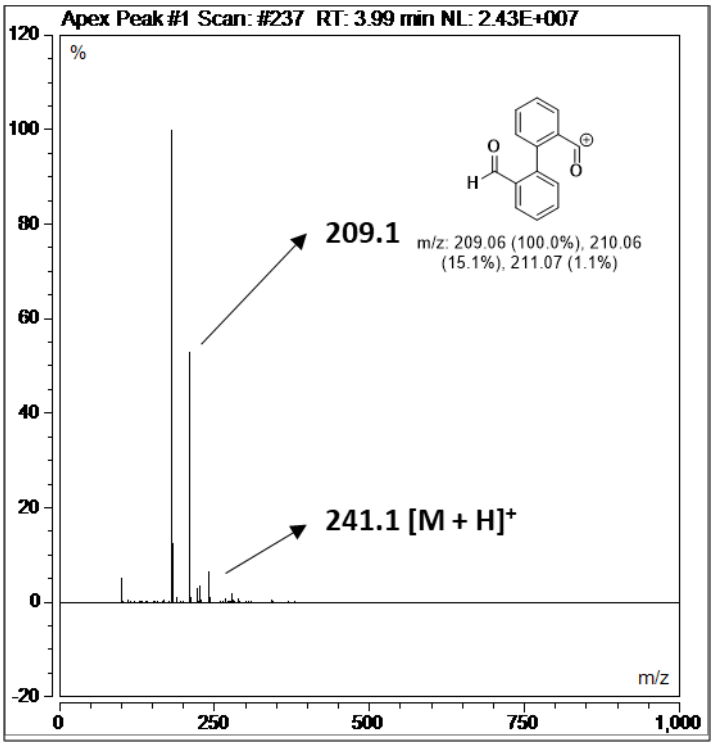
CH_2 (δ = 5.67-5.64 ppm); we assumed that they are assigned to two distinct atropoisomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.80 (1 H), 8.11 (1 H, dd, *J* 7.9, 1.4), 8.01 (1 H, dd, *J* 7.8, 1.4), 7.63 (2 H, m), 7.55 (2 H, m), 7.33 (1 H, dd, *J* 7.6, 1.3), 7.24 (1 H, d, *J* 1.3), *please note: the second part of the dd at 7.24 ppm is masked by the residual signal of $CHCl_3$*, 5.67 (1 H, d, *J* 6.0), 5.64 (1 H, d, *J* 6.1) ppm. *Please note: signals at 5.67 and 5.64 correspond to the CH_2 as AB system induced by axial chirality of ortho-substituted biphenyl*; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.6, 164.7, 144.4, 140.5, 133.9, 133.5, 132.8, 132.0, 131.0, 130.4, 128.9, 128.5, 128.3, 128.2, 69.1 ppm. HPLC (system A): t_R = 4.2 min (purity 82% at 220 nm, purity 88% at 260 nm). *Please note: we failed to record LRMS or HRMS of this compound due its very poor ability to be ionized through ESI mode.*

Three-component umbelliferone-based probe 4

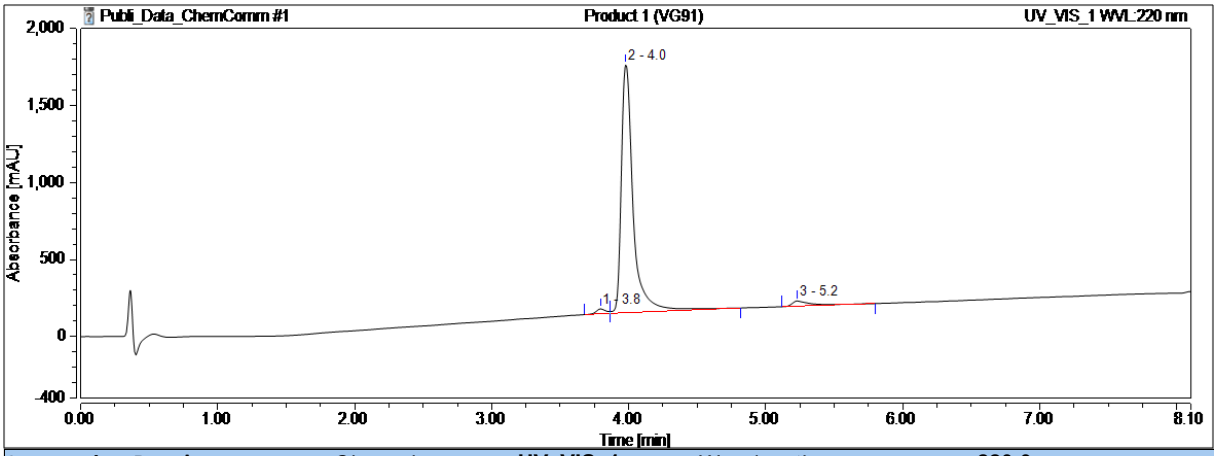
Synthetic protocol adapted from that published by Tian *et al.*[9] To a solution of chloromethyl derivative **S7** (364 mg, 1.33 mmol, 1 equiv.) in dry MeCN (3 mL) was added NaI (199 mg, 1.33 mmol, 1 equiv.). The mixture was stirred at RT for 12 h. Afterwards, umbelliferone (215 mg, 1.33 mmol, 1 equiv.) and Ag_2O (286 mg, 1.33 mmol, 1 equiv.) were sequentially added and the resulting reaction mixture was stirred at RT for 10 min. Thereafter, the crude reaction mixture was filtered over dicalite pad (to remove insoluble Na^+ and Ag^+ salts), washed with CH_2Cl_2 (ca. 150 mL). This organic filtrate was washed with deionized H_2O (2 × 100 mL), dried over anhydrous Na_2SO_4 and finally evaporated under reduced pressure. The resulting residue was purified by semi-preparative RP-HPLC (system D, t_R = 28.0-30.0 min). The product containing fractions were partly evaporated to remove MeCN and aq. suspension was extracted with CH_2Cl_2 (2 × 25 mL). Combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Drying under high vacuum afforded coumarin-based probe **4** as a white solid (43.4 mg, 0.11 mmol, yield 8%). *Please note: the lack of TFA in freeze-dried sample of **4** was confirmed by ^{19}F NMR analysis*. IR (ATR): ν = 3062, 2919, 2849, 2749, 1727, 1690, 1613, 1561, 1506, 1471, 1441, 1397, 1347, 1228, 1194, 1162, 1118, 1098, 1020, 983, 946, 891, 835, 754, 731, 710 cm^{-1}; *please note: two distinct sets of signals are clearly visualized on both ^{1}H and ^{13}C NMR spectra; we assumed that they are assigned to two distinct atropoisomers*. *Only signal pattern of major atropoisomer is described. Inaccuracy on integration values may be due to partial overlapping of signals of both atropoisomers.* ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.77 (1 H, s), 8.09 (1 H, dd, *J* 7.9, 1.4), 7.91 (1 H, dd, *J* 7.8, 1.4), 7.65 (1 H, d, *J* 9.5), 7.61 (1 H, dd, *J* 7.5, 1.5), 7.55 (2 H, m), 7.45 (1 H, t, *J* 7.6), 7.36 (1 H, d, *J* 8.3), 7.29 (1 H, dd, *J* 7.6, 1.3), 7.23 (1 H, dd, *J* 7.6, 1.2), 6.78-6.74 (2 H, m), 6.32 (1 H, d, *J* 9.5), 5.79 (1 H, d, *J* 6.8), 5.75 (1 H, d, *J* 6.8) ppm. *Please note: signals at 5.79 and 5.75 correspond to the CH_2 as AB system*; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 191.5, 165.4, 160.9, 159.6, 155.6, 144.7, 143.2, 140.2, 133.8, 133.5, 132.7, 132.1, 131.0, 130.2, 129.1, 129.1, 128.5, 128.2, 128.1, 114.5, 114.2, 113.4, 103.2, 85.1 ppm; HPLC (system A): t_R = 4.3 min (purity >99% at 220 nm, purity >99% at 260 nm, purity >99% at 310 nm); HRMS (ESI+): *m/z* calculated 401.10196 for $[M + H]^+$, found 401.10166 and 423.08391 for $[M + Na]^+$, found 423.08352.

S3 Analytical data of synthesised compounds

ESI+ mass spectrum (low resolution) of S1

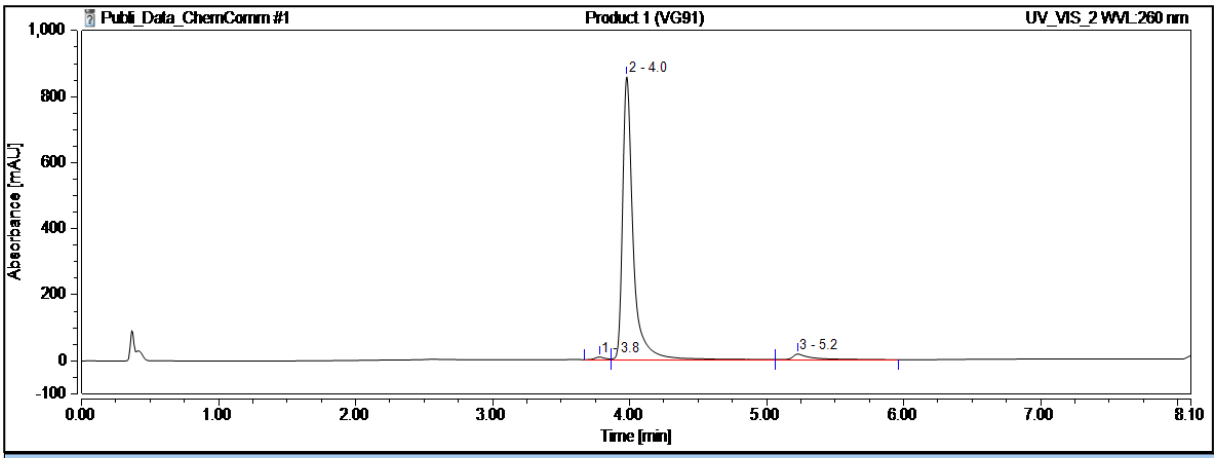


RP-HPLC elution profile of S1 (system A, detection at 220 nm)



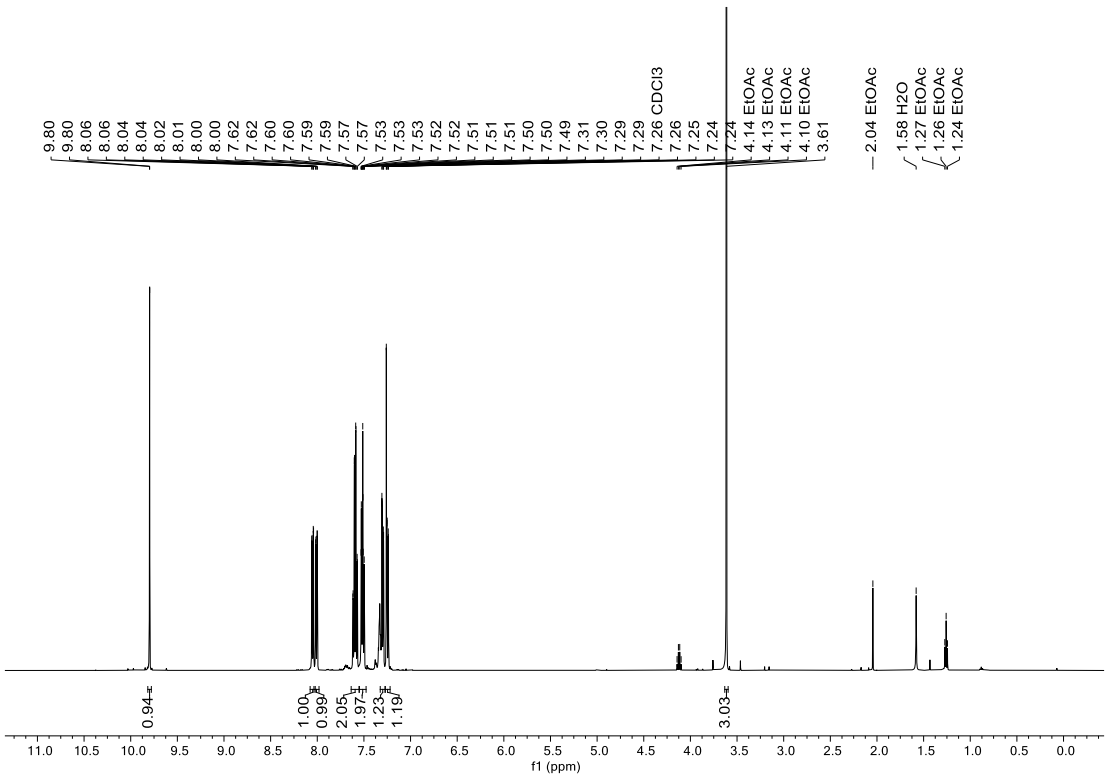
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.79	28.1	2.662	32.749	1.57	1.95
2	3.98	1402.5	161.660	1613.066	95.31	96.03
3	5.23	52.4	5.291	33.884	3.12	2.02
Total:			**169.613**	**1679.699**	**100.00**	**100.00**

RP-HPLC elution profile of S1 (system A, detection at 260 nm)

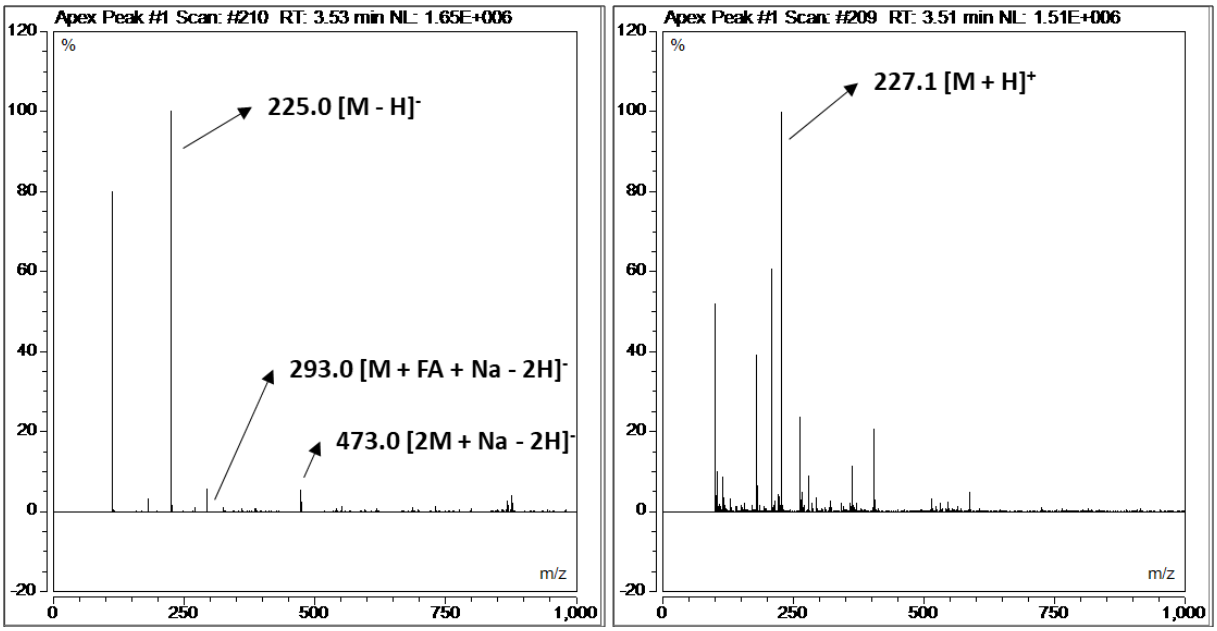


Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.78	5.7	0.666	7.829	0.83	0.89
2	3.98	719.4	76.837	856.422	95.64	97.15
3	5.23	10.4	2.839	17.250	3.53	1.96
Total:			**80.343**	**881.500**	**100.00**	**100.00**

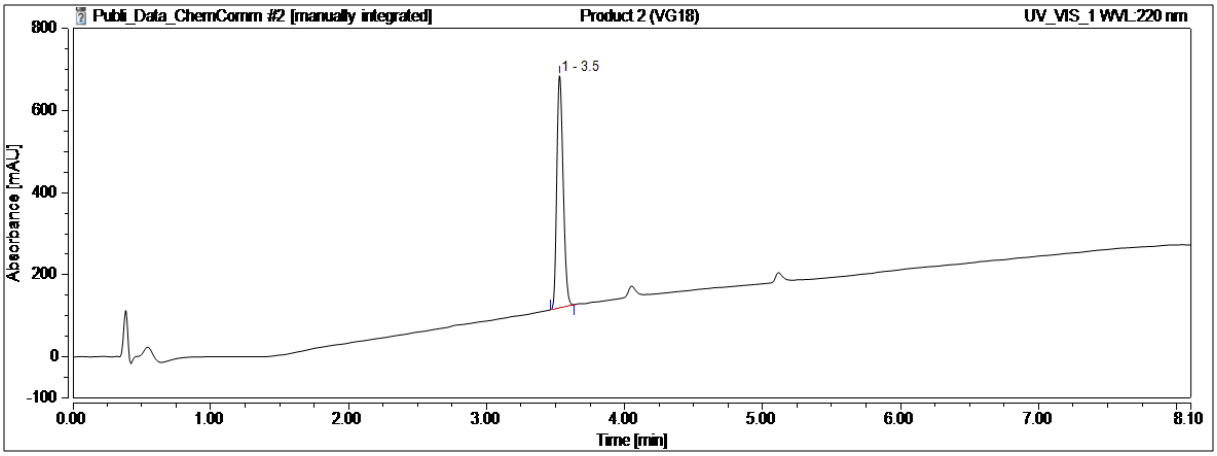
^{1}H NMR spectrum of S1 in $CDCl_3$ (500 MHz)



ESI- (left) and ESI+ (right) mass spectra (low resolution) of S2



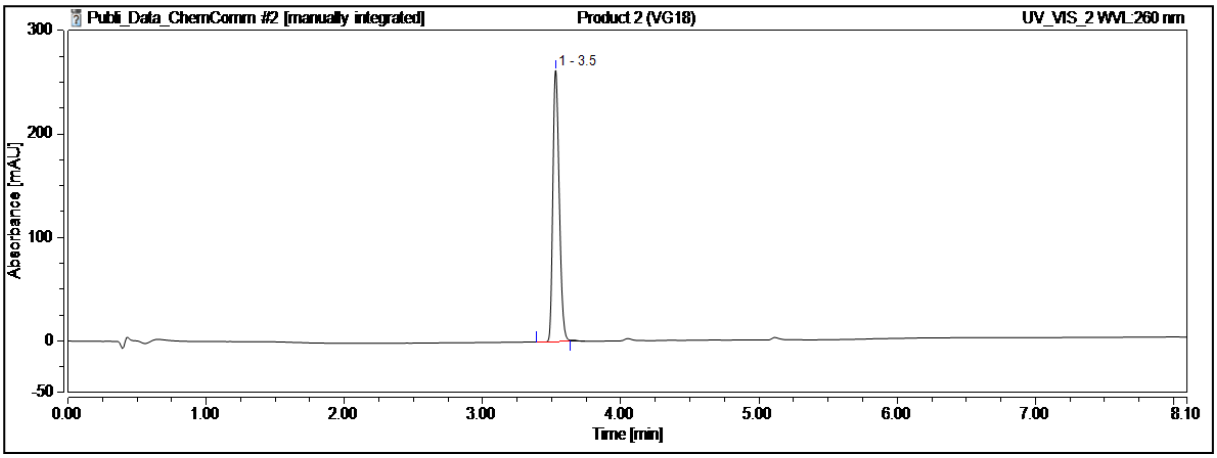
RP-HPLC elution profile of S2 (system A, detection at 220 nm)



Please note: peaks at ca. t_R = 4.0 and 5.1 min found in blank run.

Integration Results	*Channel:*	**UV_VIS_1**	*Wavelength:*	**220.0**	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.53	37.9	30.343	565.427	100.00	100.00
Total:			**30.343**	**565.427**	**100.00**	**100.00**

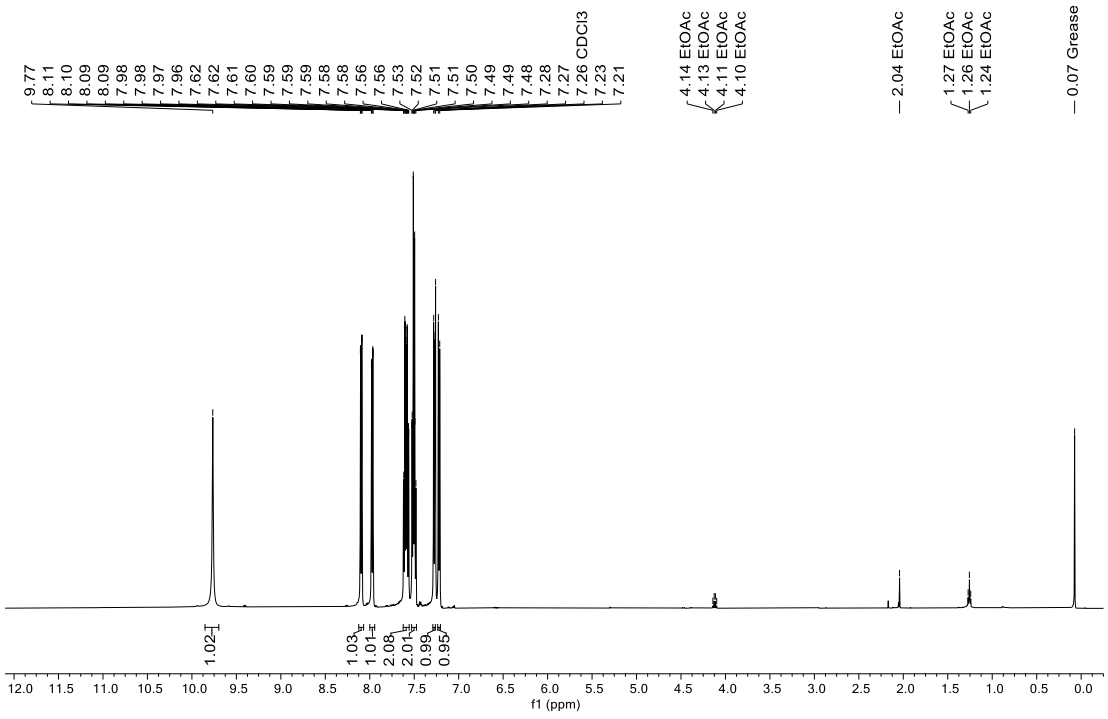
RP-HPLC elution profile of S2 (system A, detection at 260 nm)



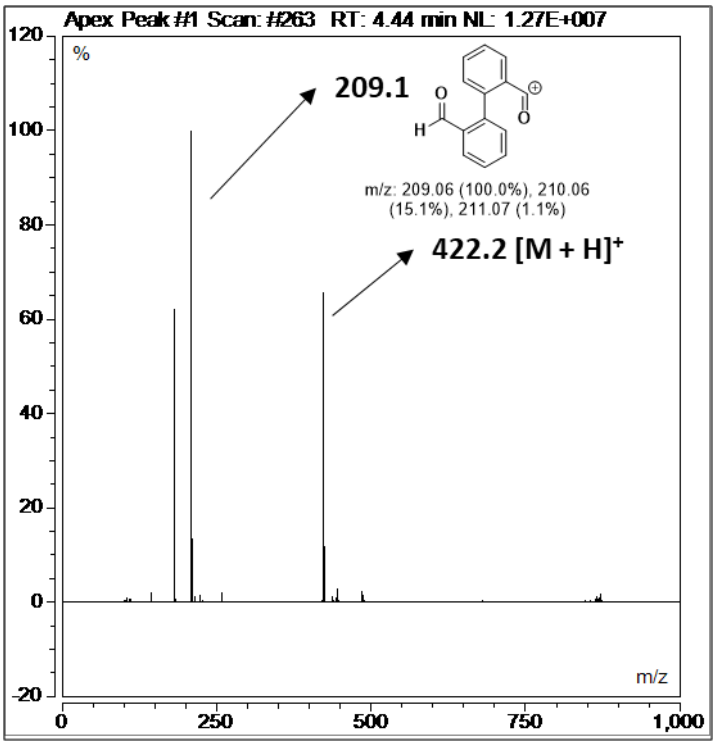
Please note: peaks at ca. t_R = 4.0 and 5.1 min found in blank run.

Integration Results		*Channel:*	**UV_VIS_2**	*Wavelength:*	**260**	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.53	2741.7	14.055	262.045	100.00	100.00
Total:			**14.055**	**262.045**	**100.00**	**100.00**

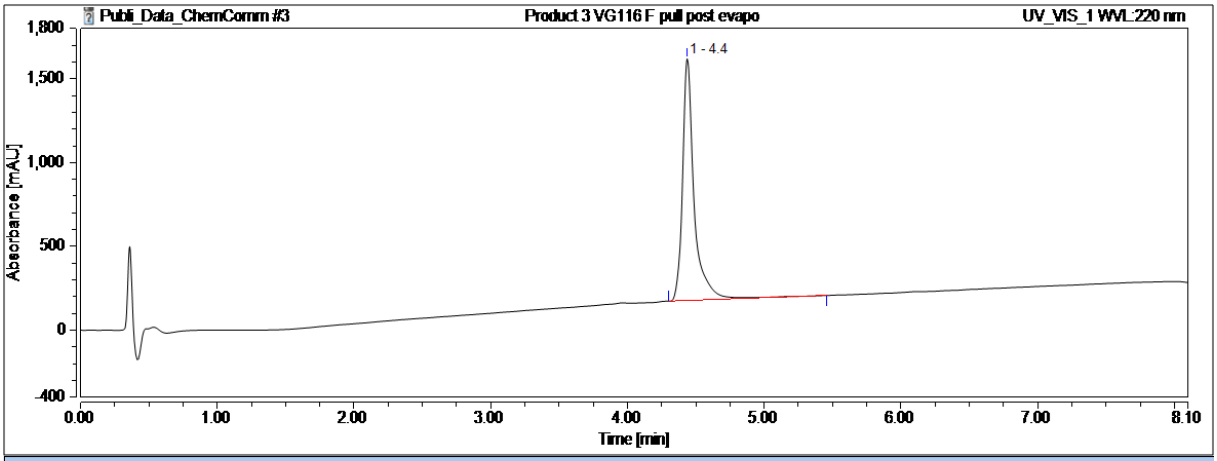
^{1}H NMR spectrum of S2 in $CDCl_3$ (500 MHz)



ESI+ mass spectrum (low resolution) of IND-1

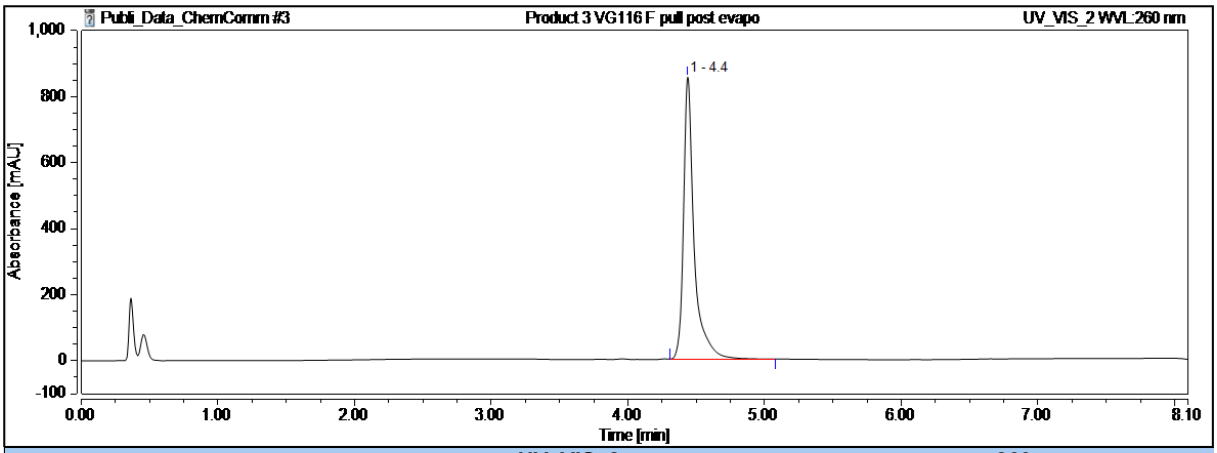


RP-HPLC elution profile of IND-1 (system A, detection at 220 nm)



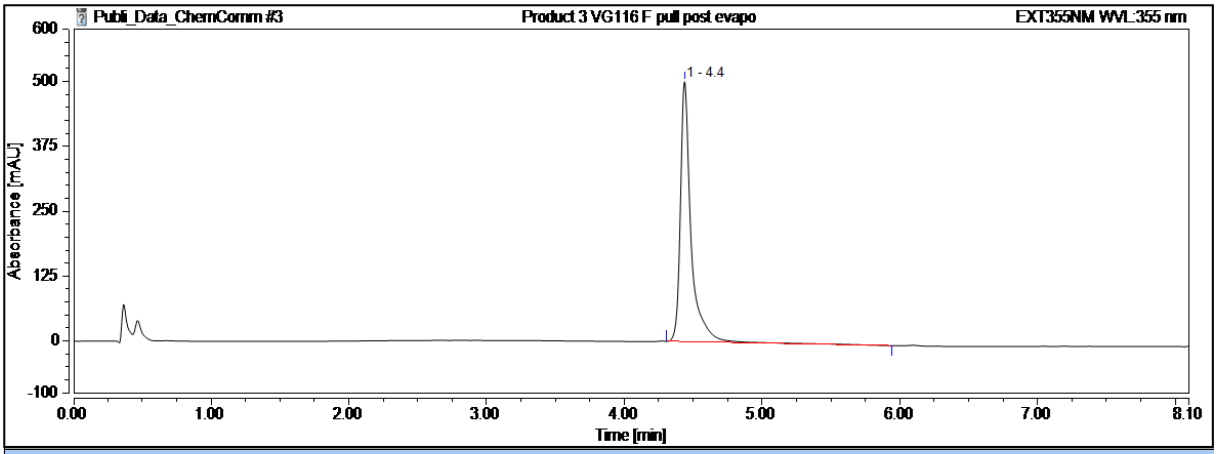
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.44	257.1	141.504	1443.606	100.00	100.00
Total:			**141.504**	**1443.606**	**100.00**	**100.00**

RP-HPLC elution profile of IND-1 (system A, detection at 260 nm)



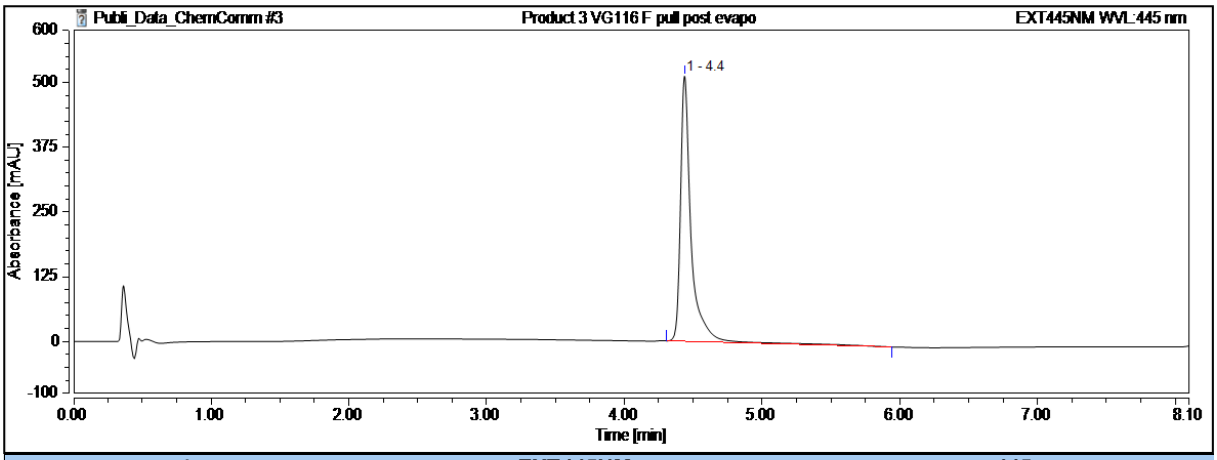
Integration Results	Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.44	647.4	76.570	854.304	100.00	100.00
Total:			**76.570**	**854.304**	**100.00**	**100.00**

RP-HPLC elution profile of IND-1 (system A, detection at 355 nm)



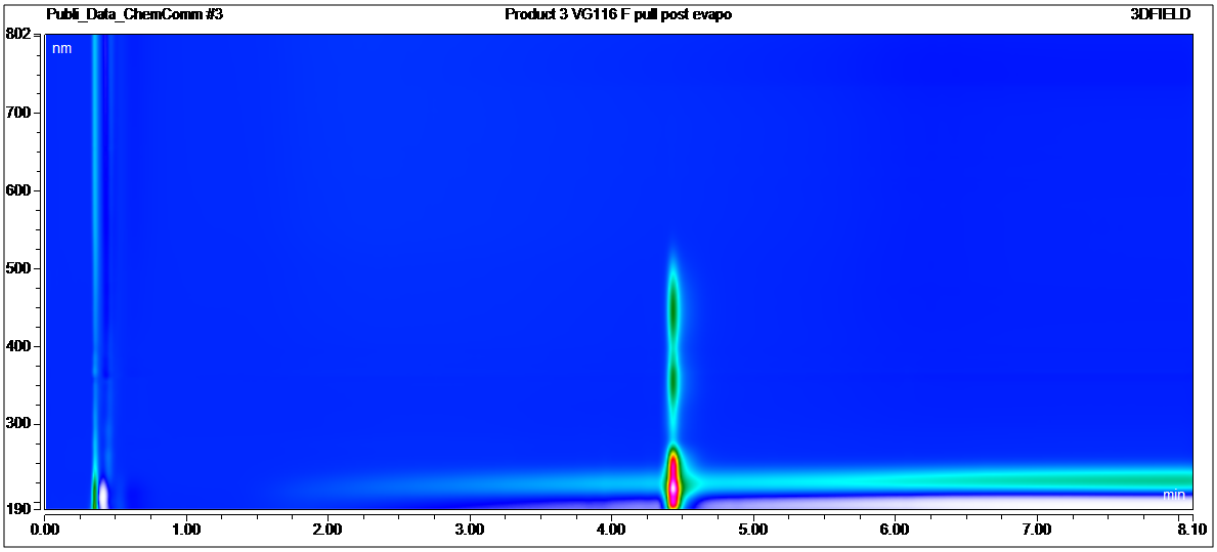
Integration Results	Channel:	EXT355NM	Wavelength:	355	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.44	691.5	45.386	500.783	100.00	100.00
Total:			**45.386**	**500.783**	**100.00**	**100.00**

RP-HPLC elution profile of IND-1 (system A, detection at 445 nm)

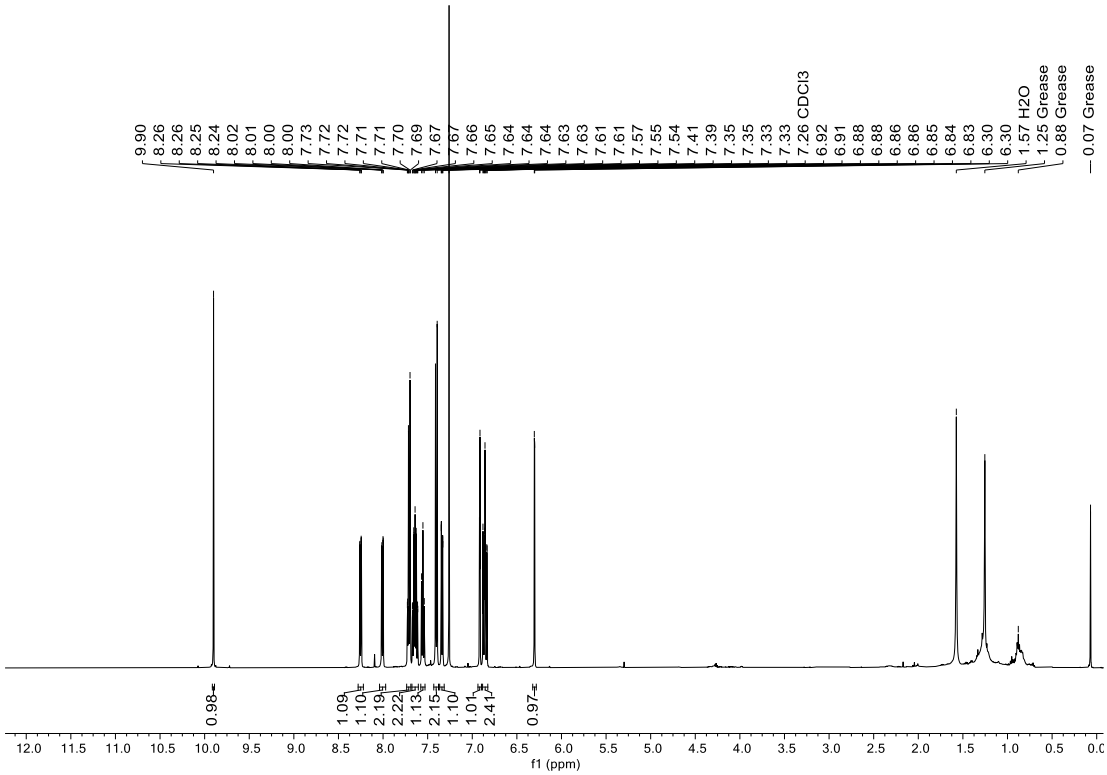


Integration Results		Channel:	EXT445NM	Wavelength:	445	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.44	829.2	46.386	511.901	100.00	100.00
Total:			**46.386**	**511.901**	**100.00**	**100.00**

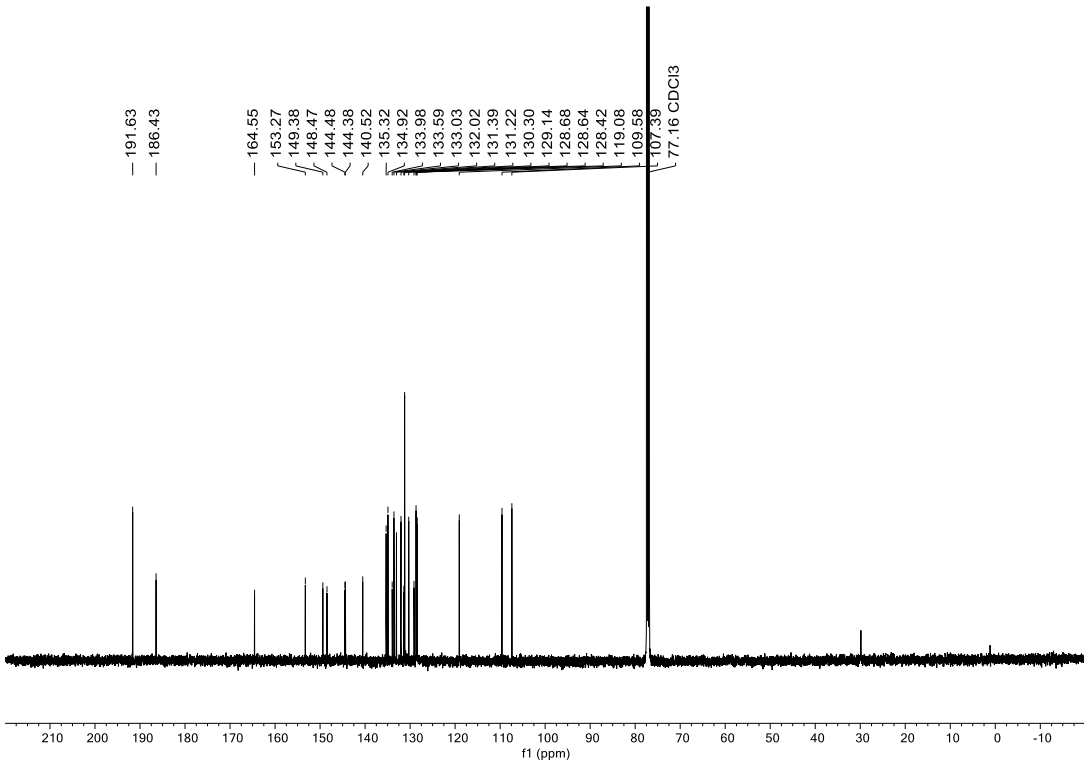
2D map of DAD of IND-1 (system A)



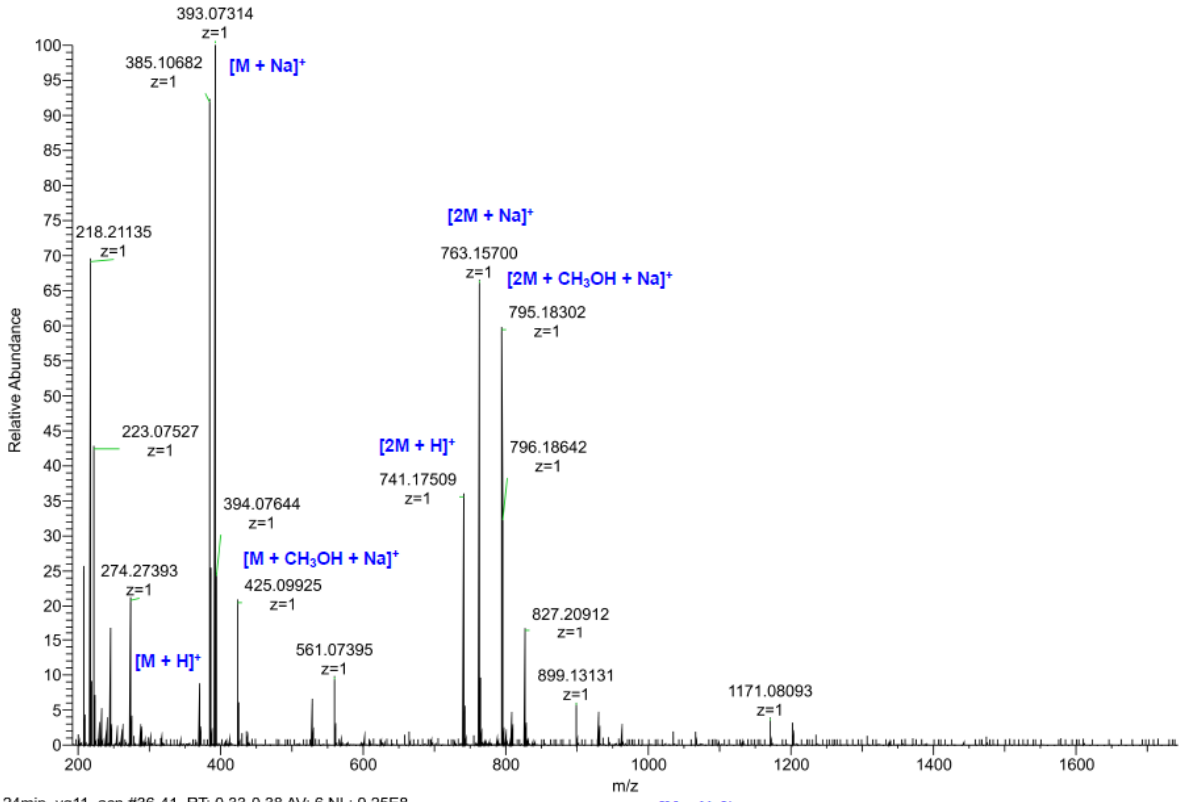
^{1}H NMR spectrum of IND-1 in $CDCl_3$ (500 MHz)

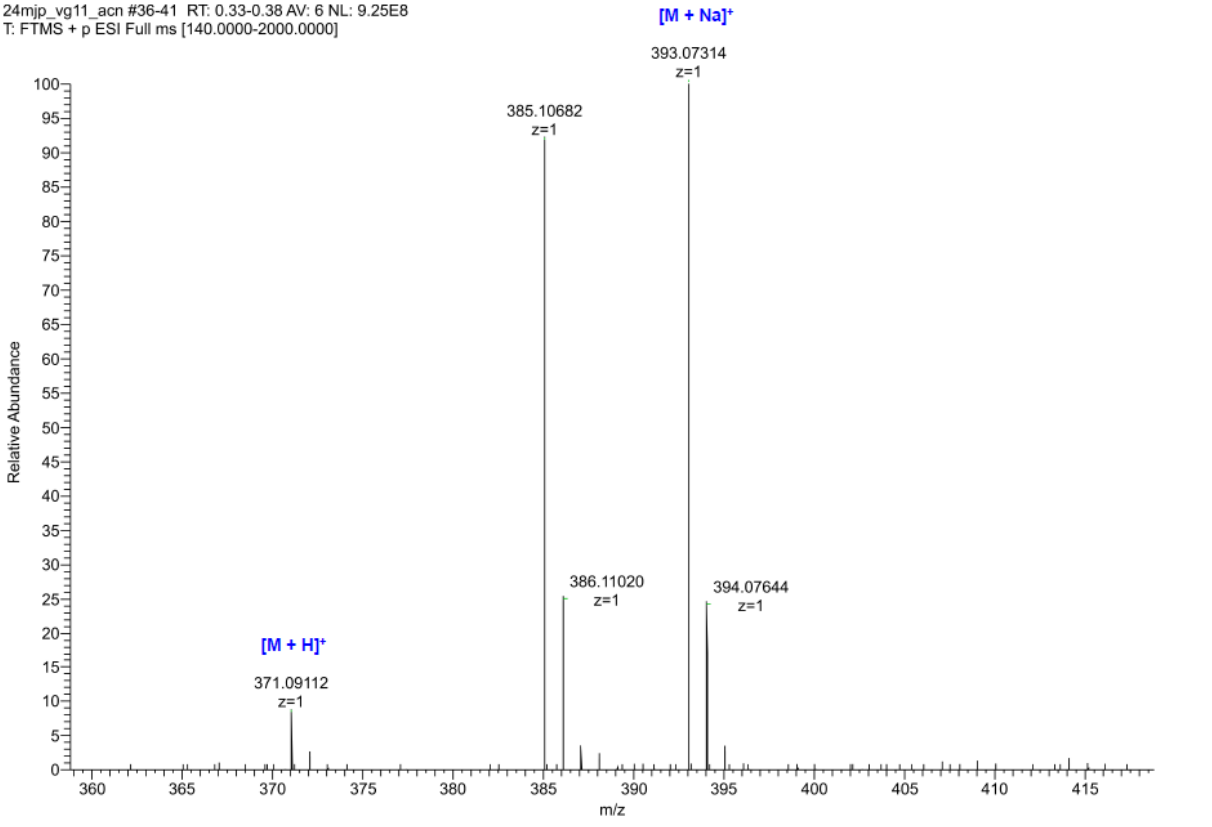


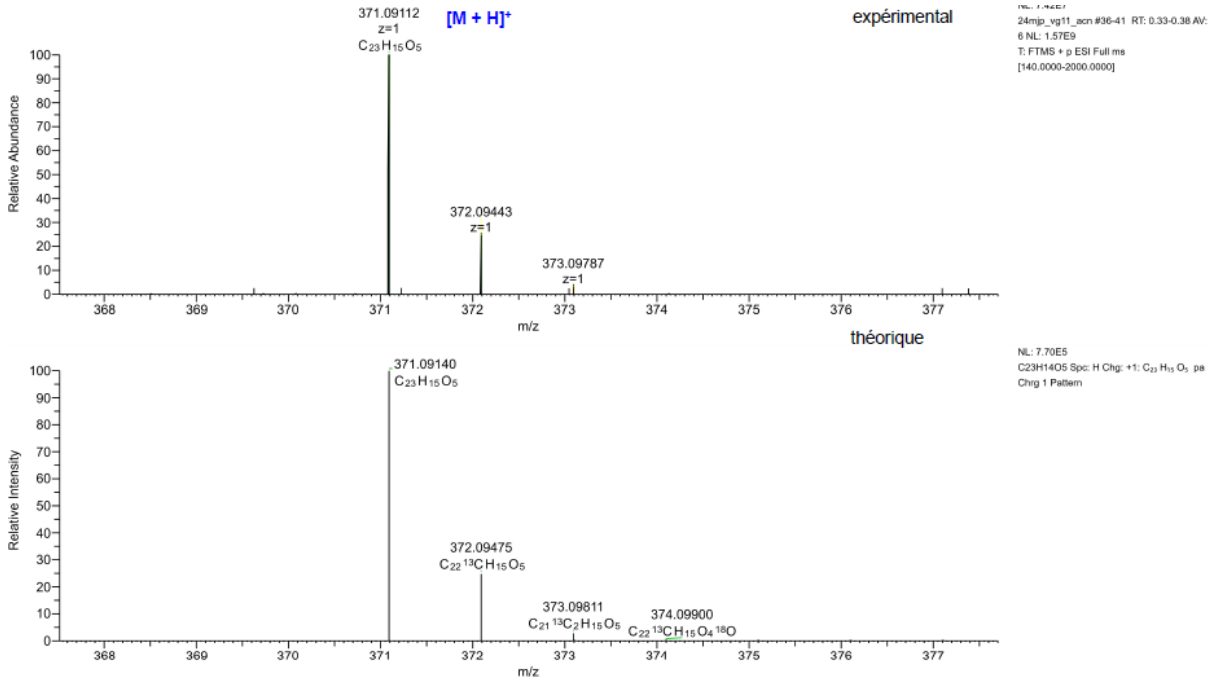
^{13}C NMR spectrum of IND-1 in $CDCl_3$ (126 MHz)



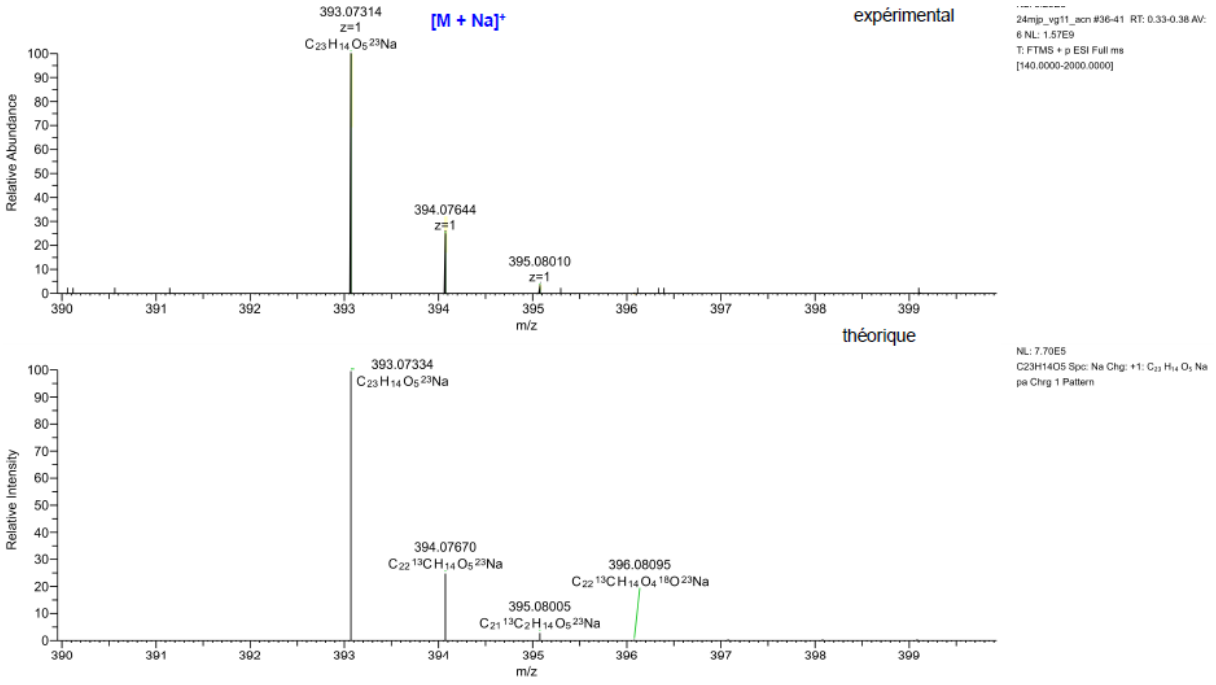
ESI+ mass spectrum (high resolution) of 1





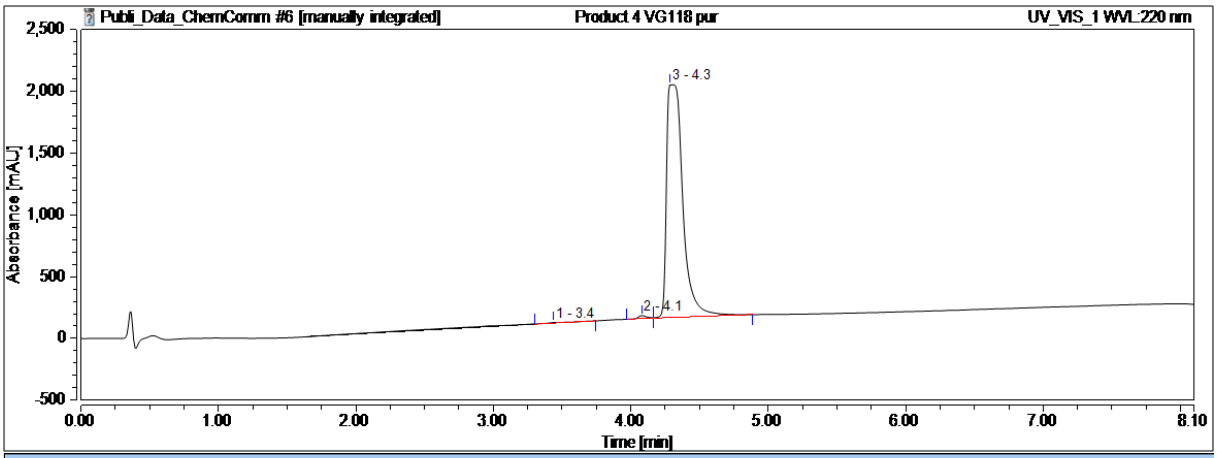


Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]	MSMS Matched Fragm...
371.09112	$C_{23}H_{15}O_5$	16.50	-0.76	371.09140	1	97.65	(Collection)



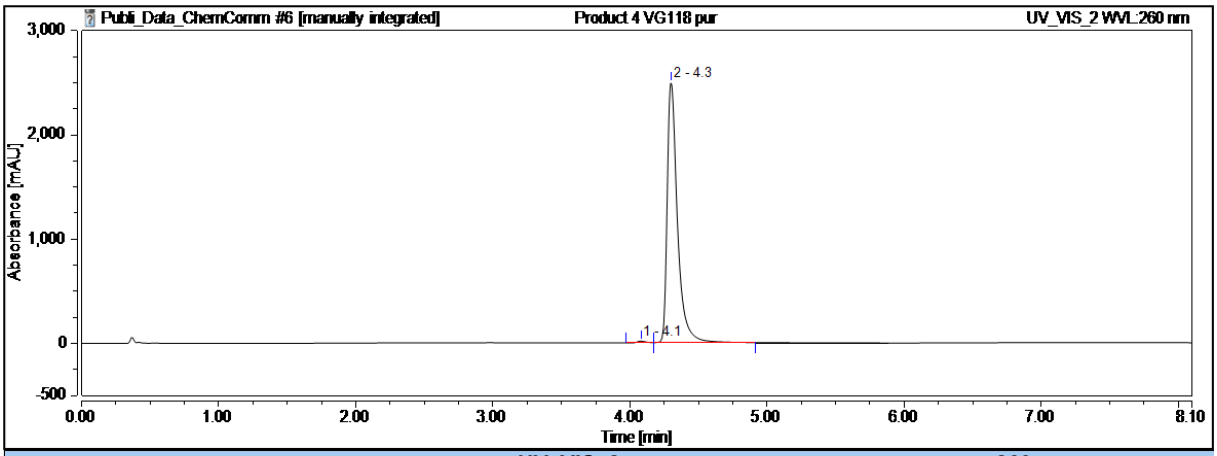
Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]	MSMS Matched Fragm...
393.07314	$C_{23}H_{14}O_5{}^{23}Na$	16.50	-0.52	393.07334	1	99.61	(Collection)

RP-HPLC elution profile of 1 (system A, detection at 220 nm)



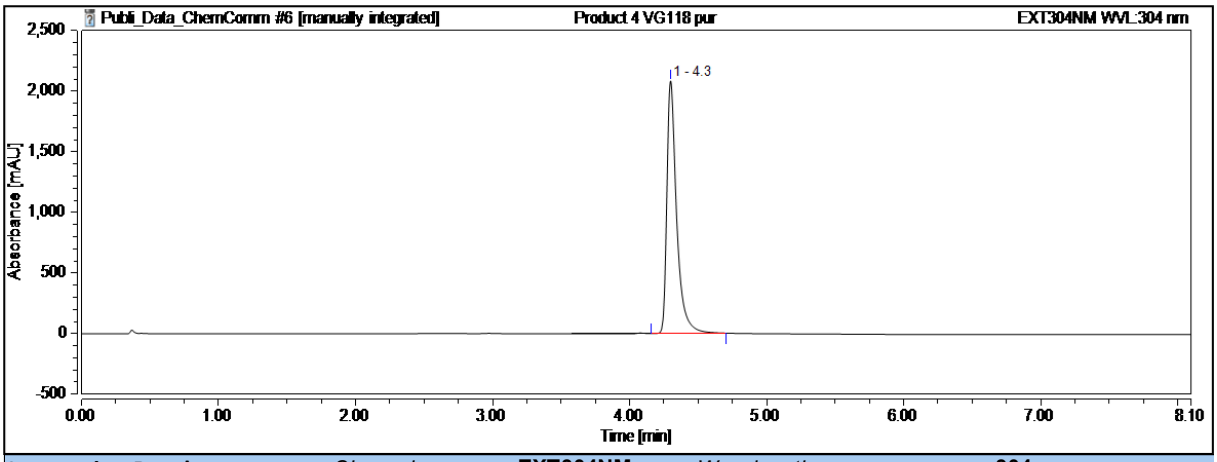
Integration Results	Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.44	6.0	1.032	5.843	0.40	0.30
2	4.08	8.8	1.833	24.956	0.70	1.30
3	4.29	598.6	257.512	1887.695	98.90	98.39
Total:			**260.377**	**1918.494**	**100.00**	**100.00**

RP-HPLC elution profile of 1 (system A, detection at 260 nm)



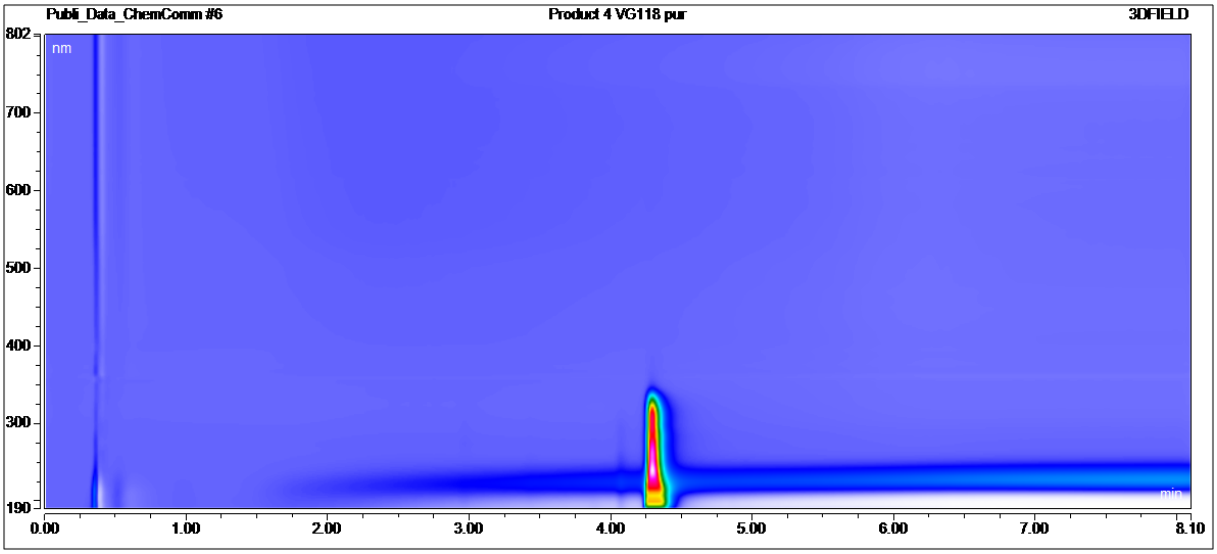
Integration Results	Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.08	18.1	1.343	17.459	0.61	0.69
2	4.30	2536.2	217.944	2499.260	99.39	99.31
Total:			**219.287**	**2516.719**	**100.00**	**100.00**

RP-HPLC elution profile of 1 (system A, detection at 304 nm)

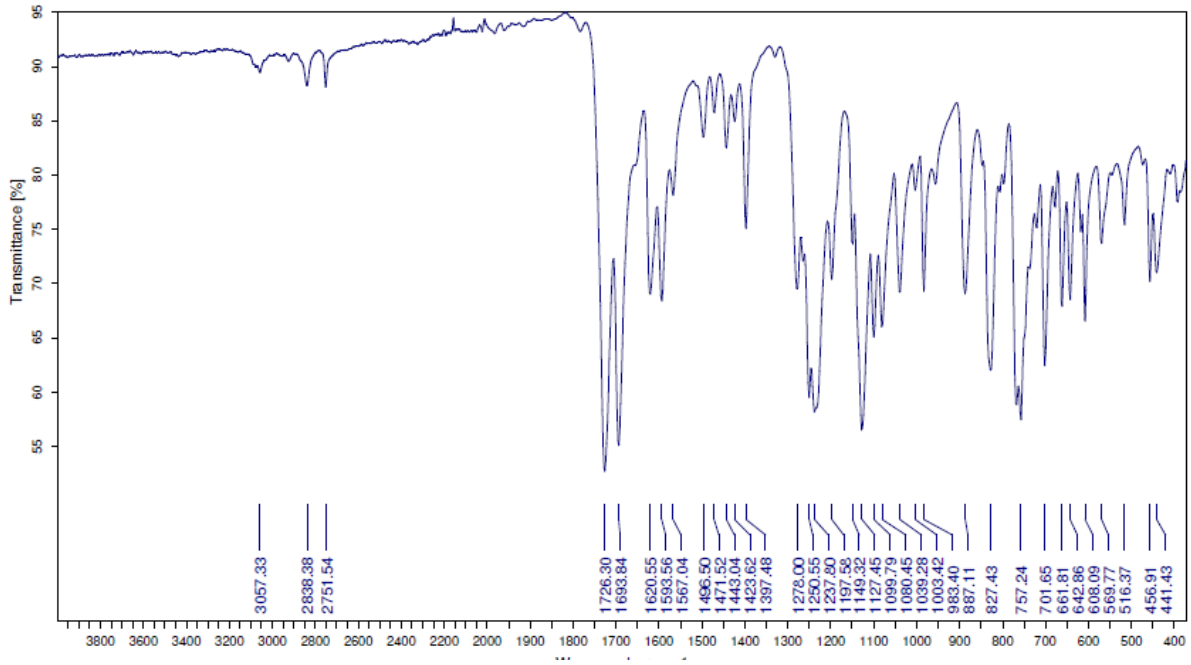


Integration Results	*Channel:*	EXT304NM	*Wavelength:*	304	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.30	1463.0	171.449	2083.347	100.00	100.00
Total:			**171.449**	**2083.347**	**100.00**	**100.00**

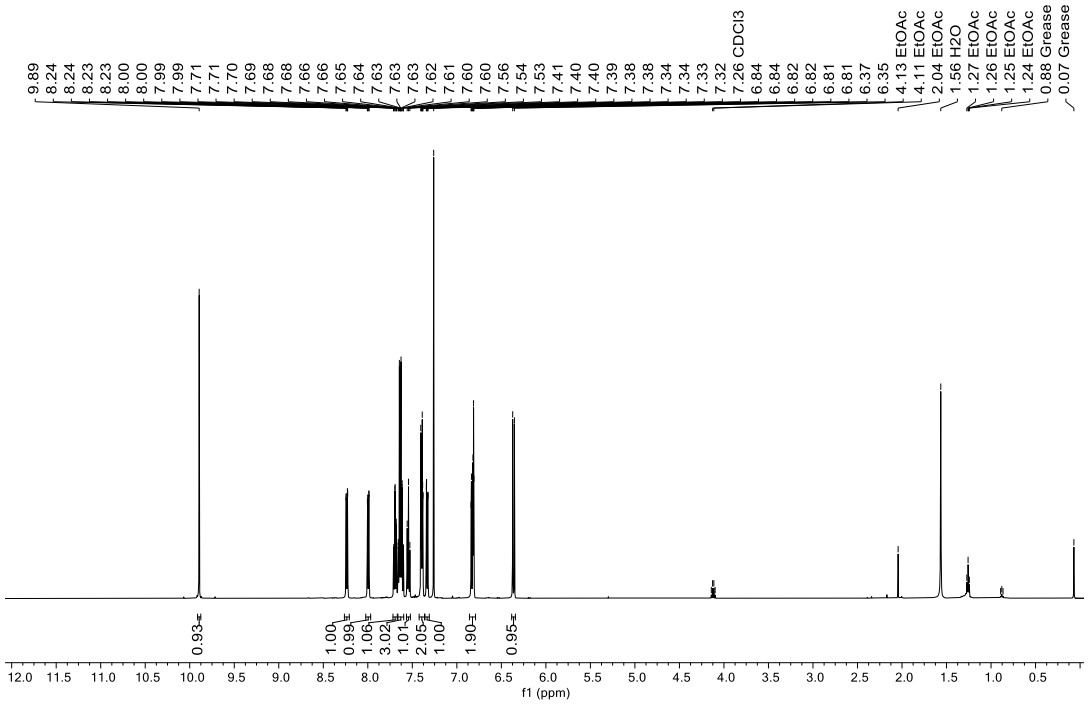
2D map of DAD of 1 (system A)



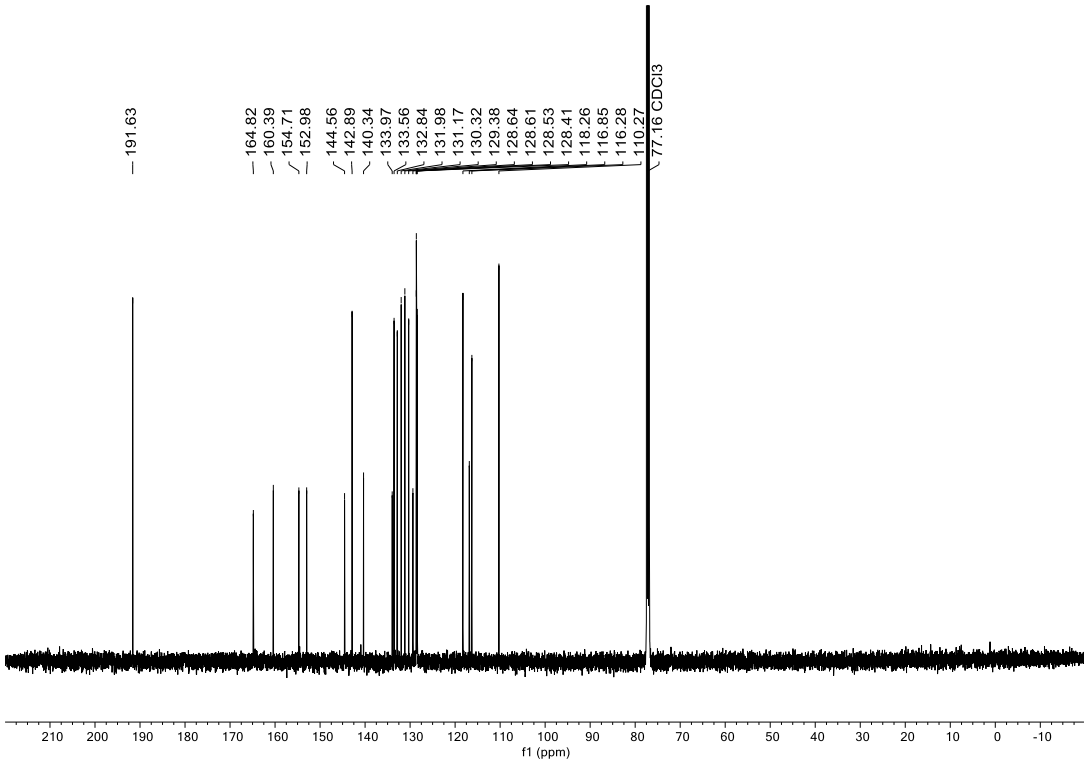
IR-ATR spectrum of 1



^{1}H NMR spectrum of 1 in $CDCl_3$ (500 MHz)

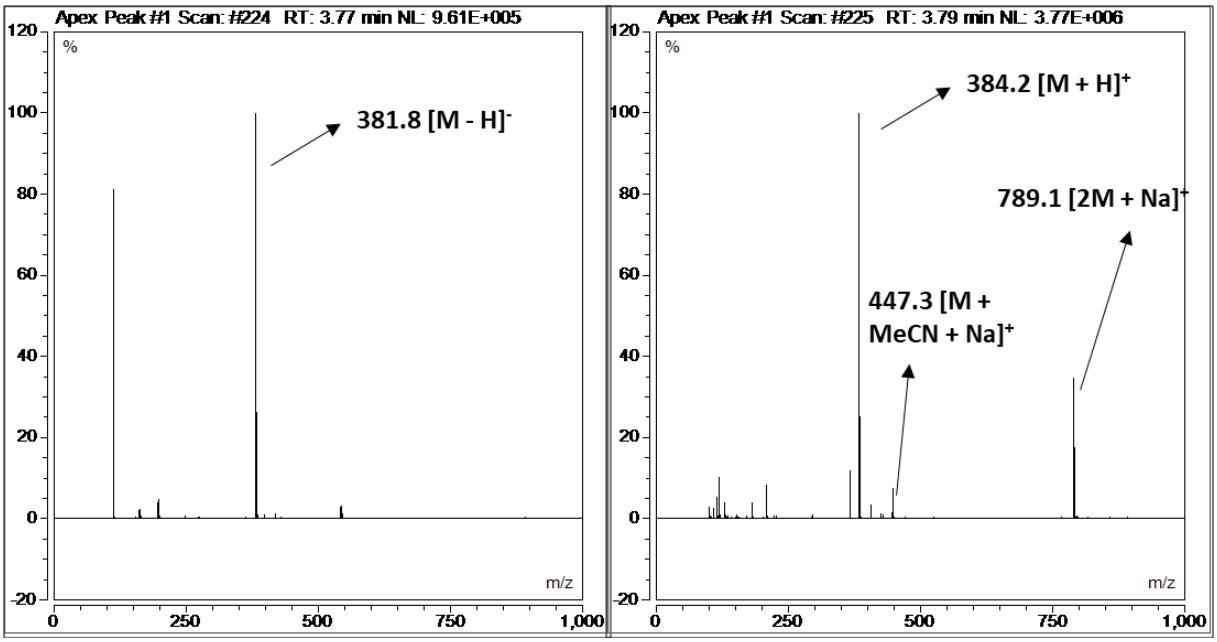


^{13}C NMR spectrum of 1 in $CDCl_3$ (126 MHz)

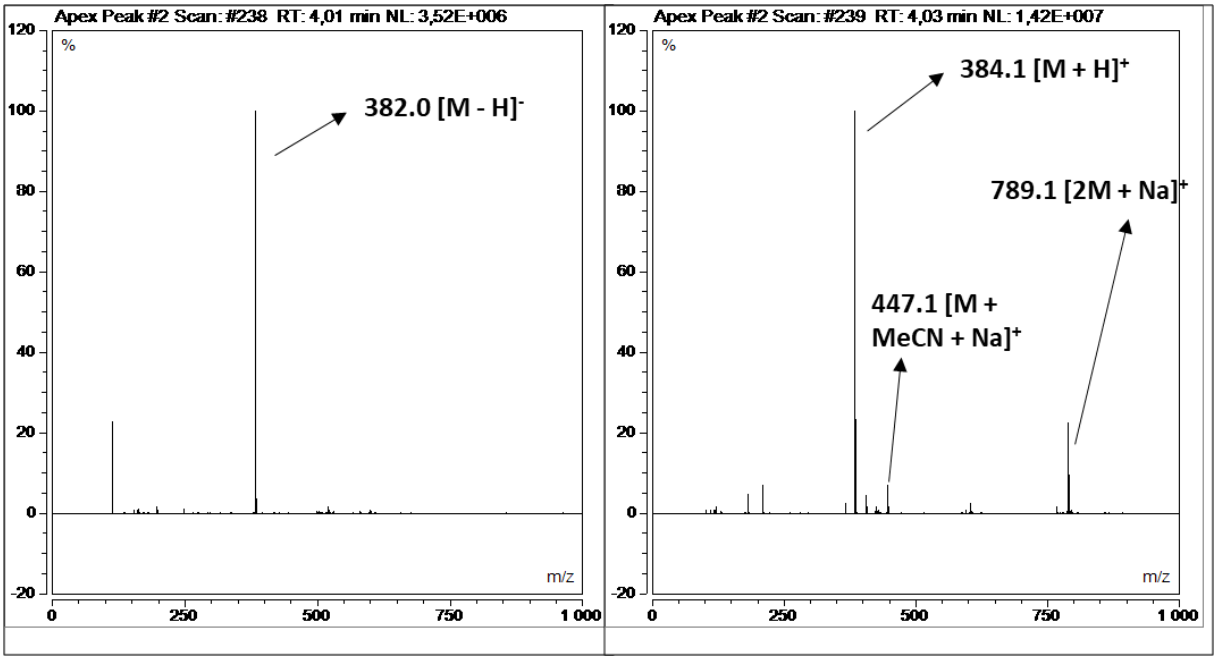


ESI- (left) and ESI+ (right) mass spectra (low resolution) of 2

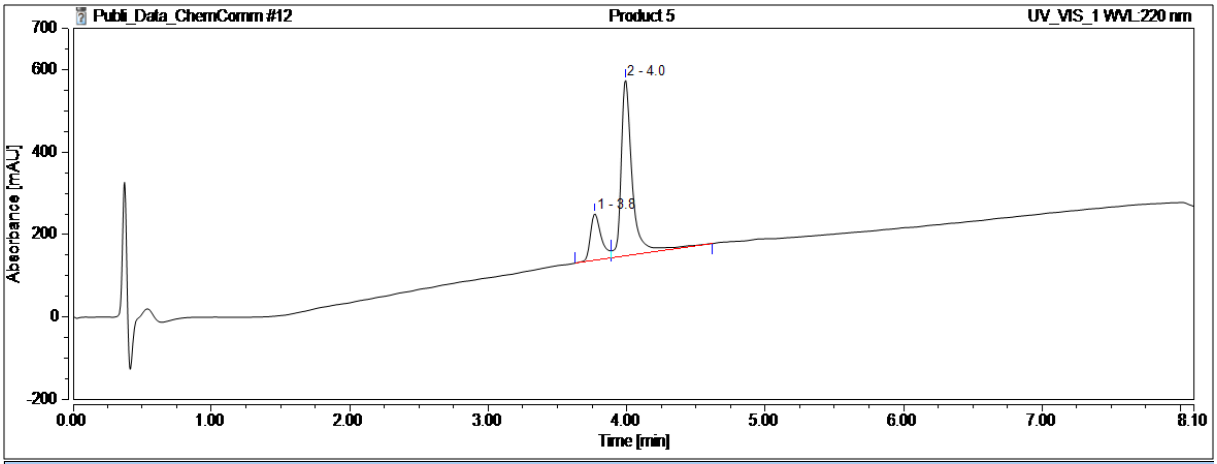
Peak 1 (t_R = 3.8 min)



Peak 2 (t_R = 4.0 min)

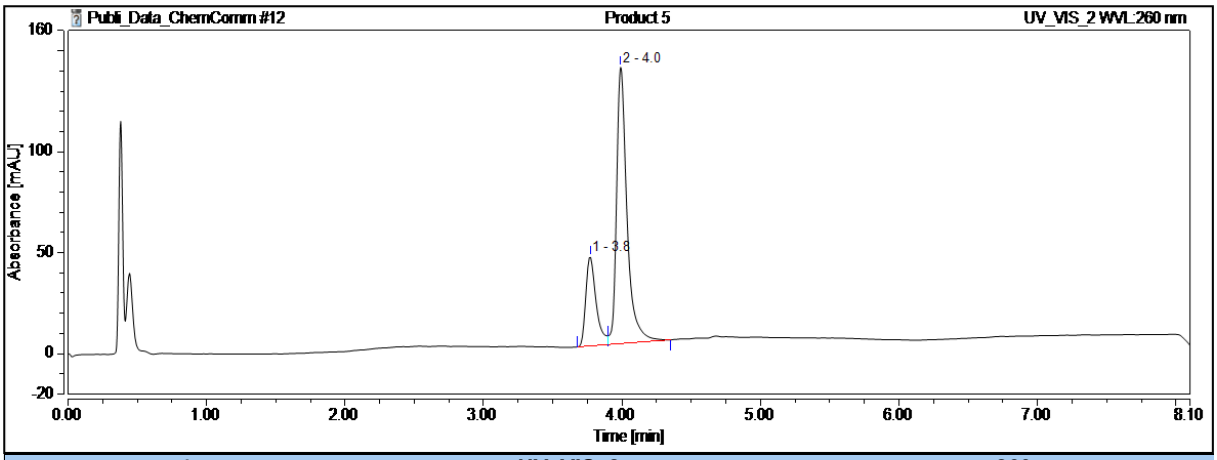


RP-HPLC elution profile of 2 (system A, detection at 220 nm)



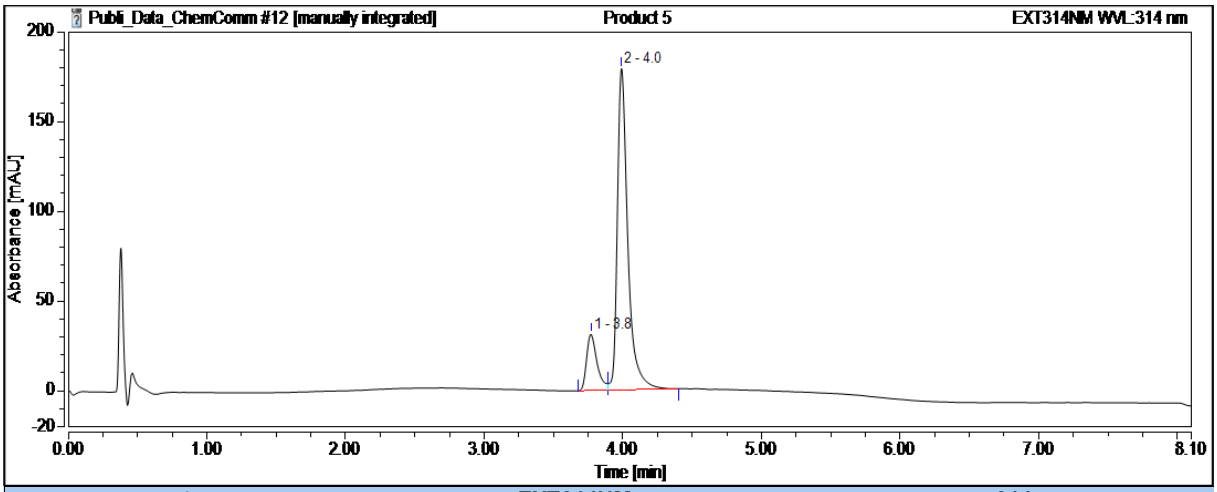
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.77	71.5	10.217	113.098	20.53	20.93
2	3.99	254.1	39.545	427.354	79.47	79.07
Total:			**49.762**	**540.452**	**100.00**	**100.00**

RP-HPLC elution profile of 2 (system A, detection at 260 nm)



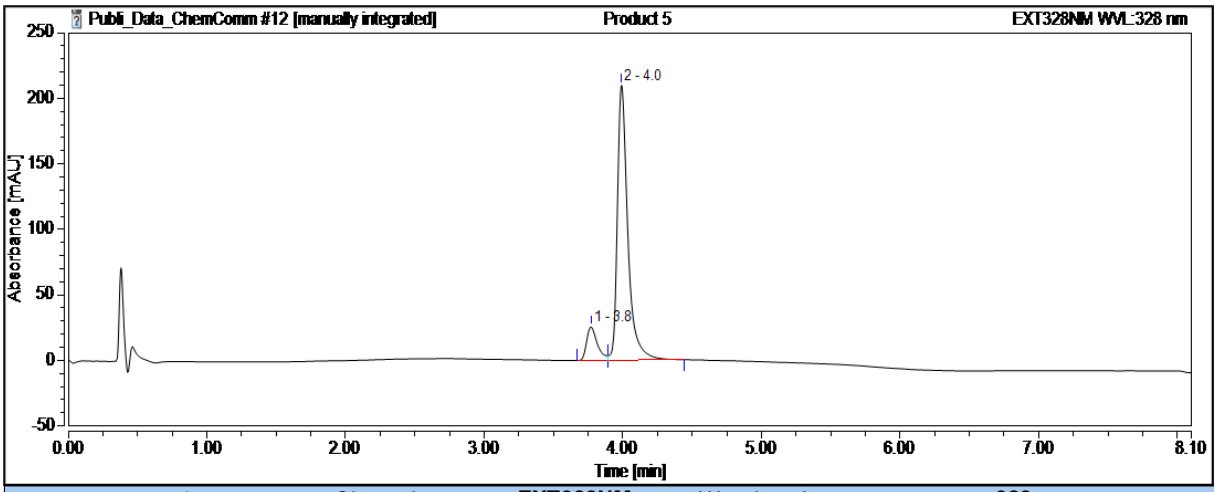
Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.77	45.0	3.812	44.293	24.10	24.44
2	3.99	141.0	12.006	136.934	75.90	75.56
Total:			**15.819**	**181.227**	**100.00**	**100.00**

RP-HPLC elution profile of 2 (system A, detection at 314 nm)



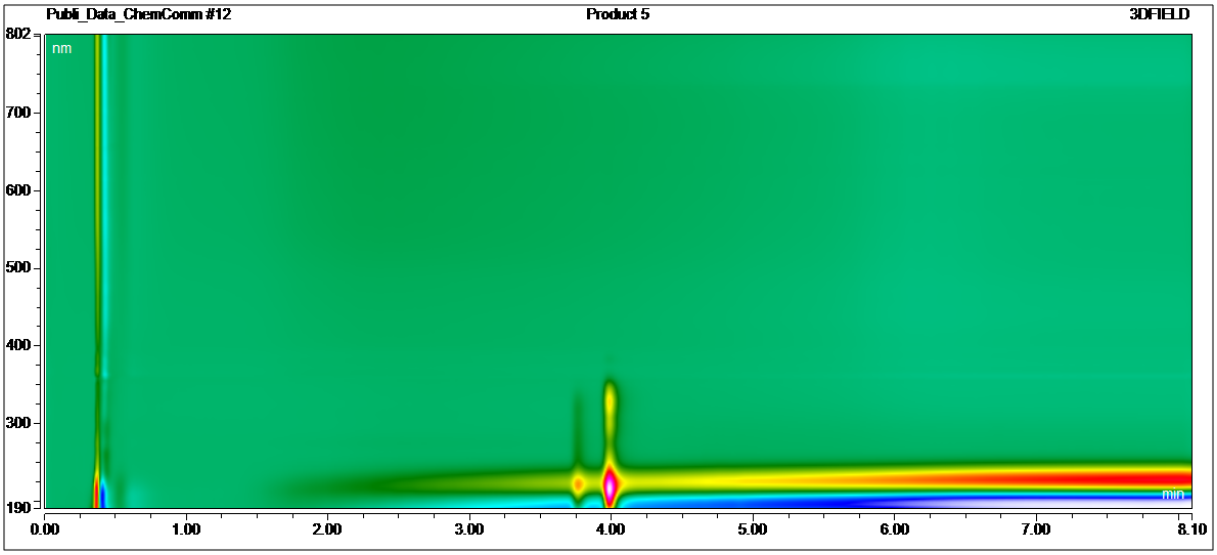
Integration Results		Channel:	EXT314NM	Wavelength:	314	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.77	35.8	2.766	31.472	15.06	14.95
2	3.99	245.6	15.598	179.089	84.94	85.05
Total:			**18.364**	**210.561**	**100.00**	**100.00**

RP-HPLC elution profile of 2 (system A, detection at 328 nm)

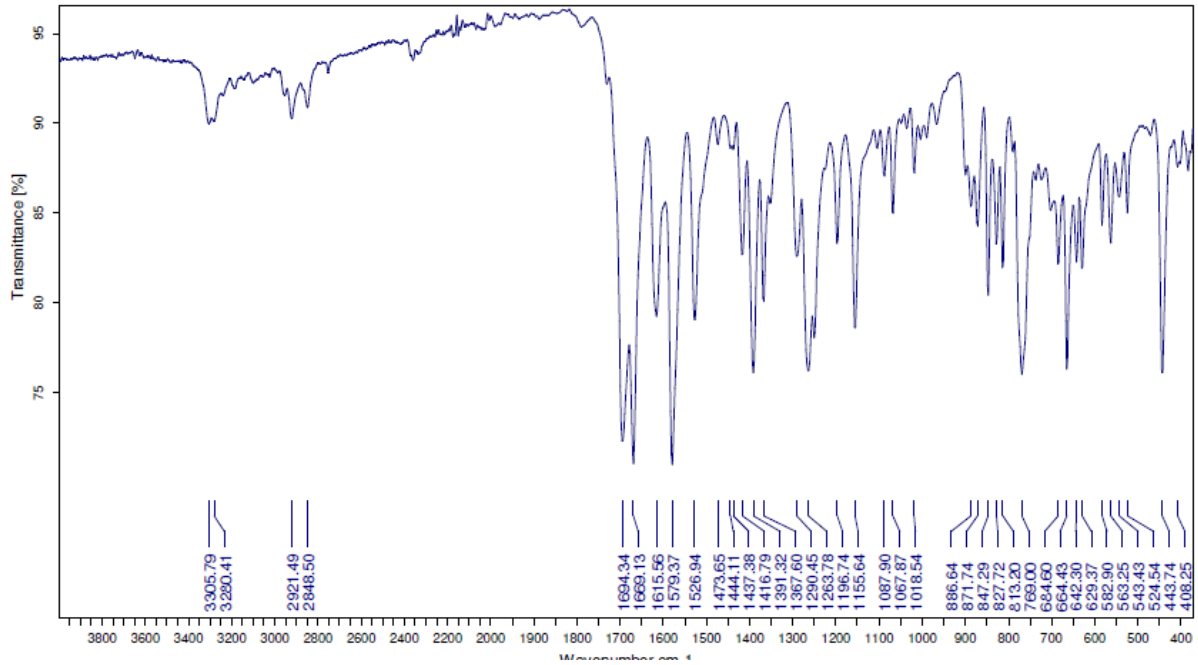


Integration Results		Channel:	EXT328NM	Wavelength:	328	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.77	29.5	2.309	25.678	11.21	10.91
2	3.99	284.1	18.282	209.777	88.79	89.09
Total:			**20.591**	**235.454**	**100.00**	**100.00**

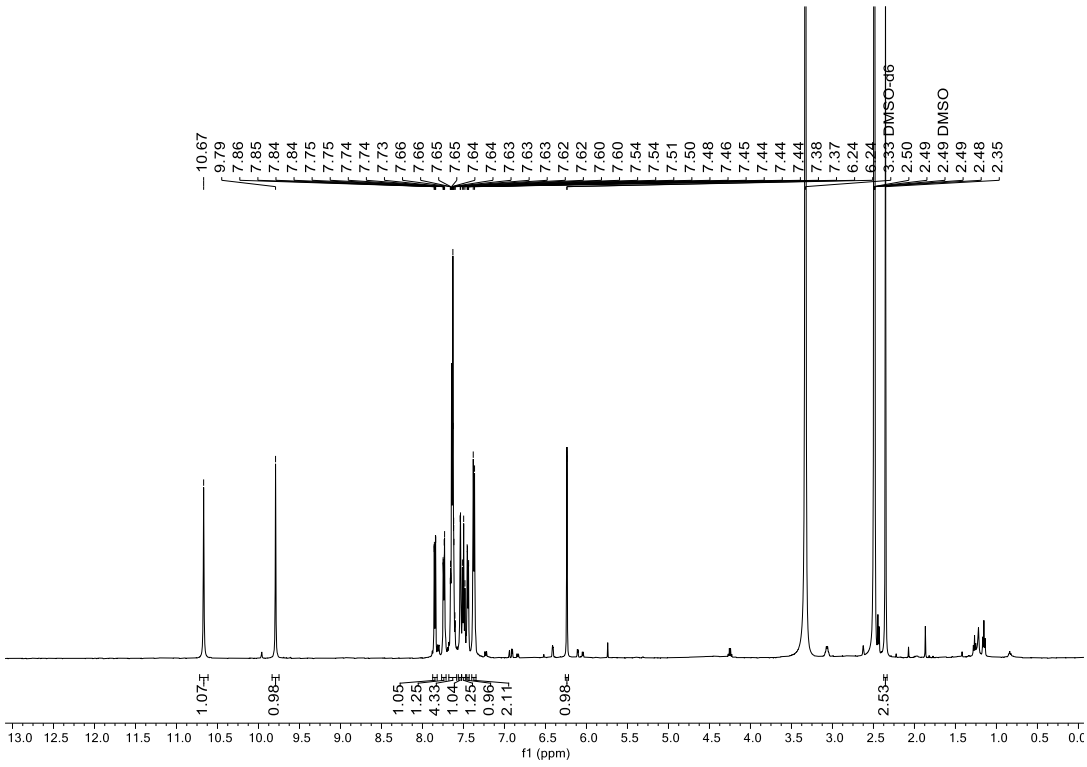
2D map of DAD of 5 (system A)



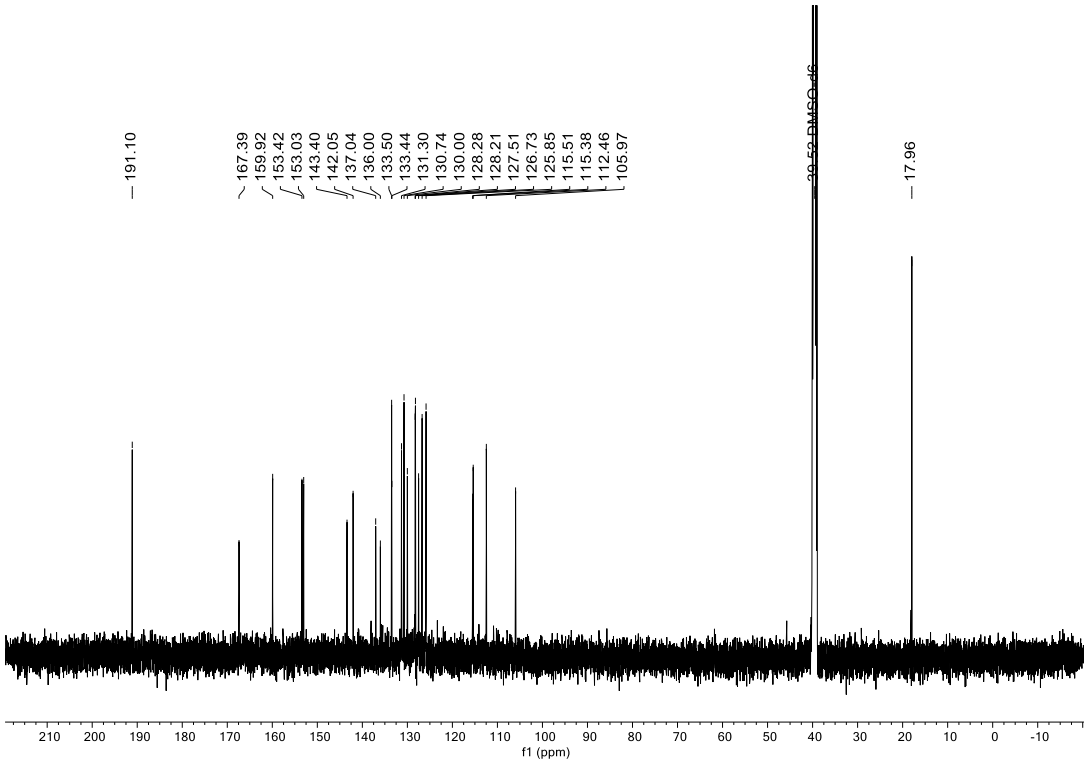
IR-ATR spectrum of 2



^{1}H NMR spectrum of 2 in DMSO-d_6 (500 MHz)



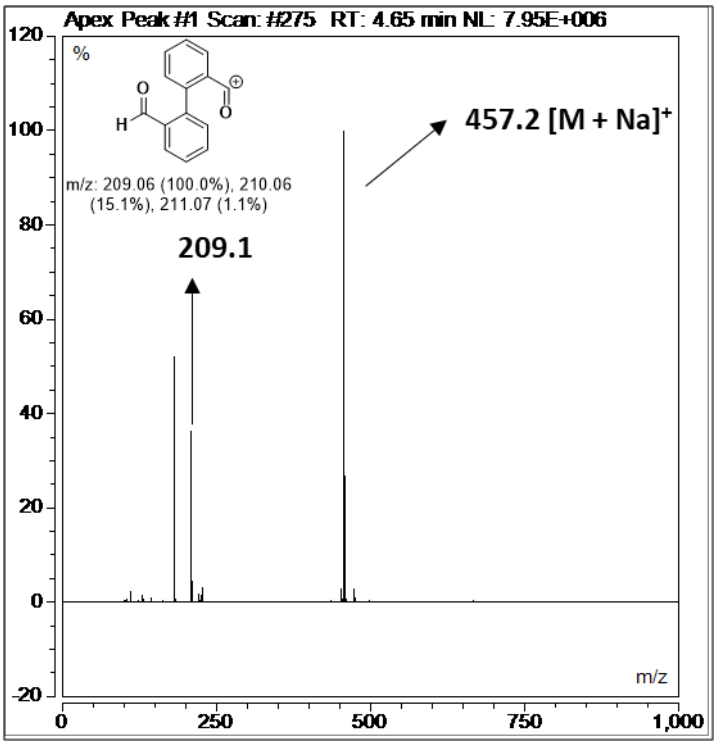
^{13}C NMR spectrum of 5 in DMSO-d_6 (126 MHz)



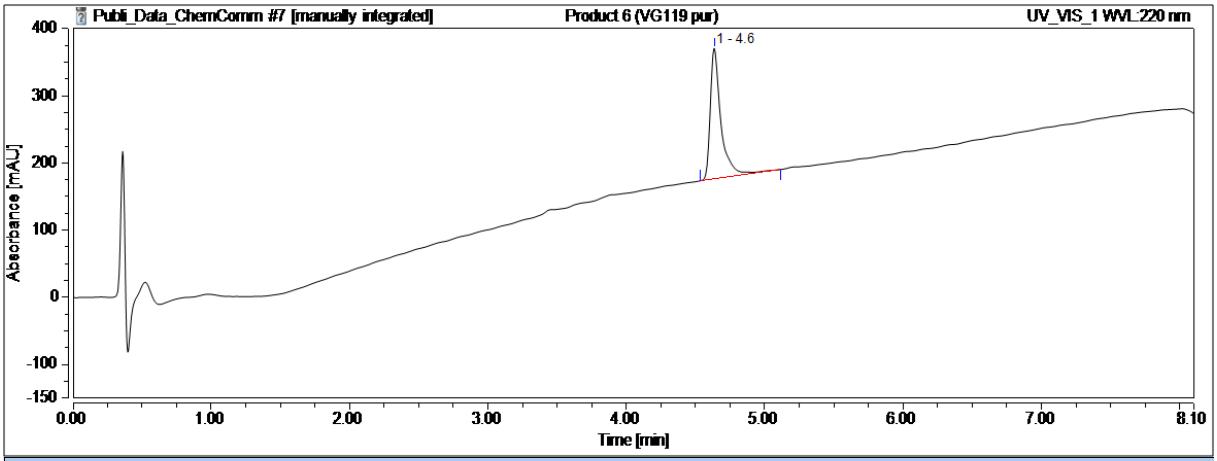
Determination of TFA content in sample of probe 2, by IC

Concentration (stock sol. **2**)	1.0 mg/mL in DMSO
Sample dilution factor	10
Raw data ppm	1.5000
Content in wt %	1.50
Average content in wt %	<1.50

ESI+ mass spectrum (low resolution) of S3

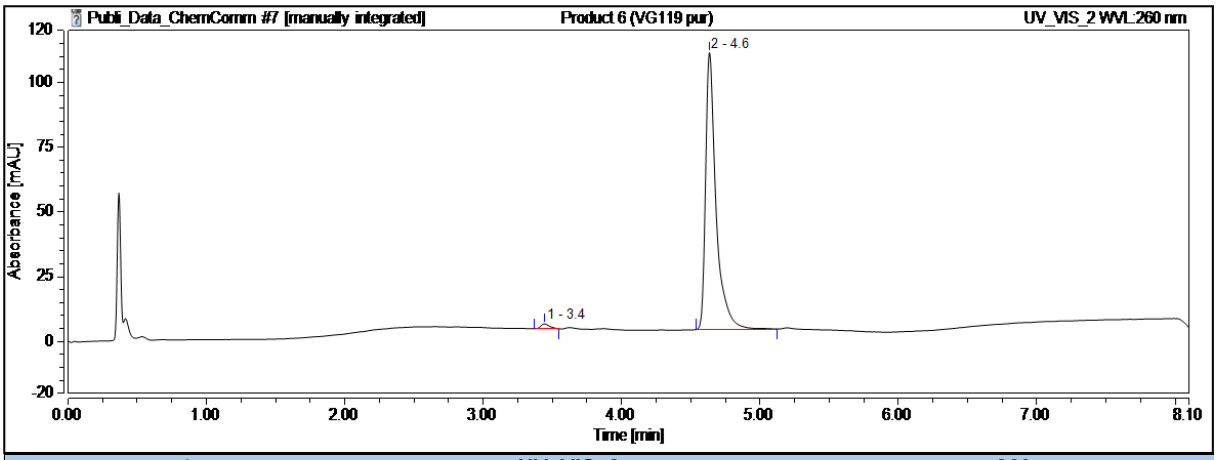


RP-HPLC elution profile of S3 (system A, detection at 220 nm)



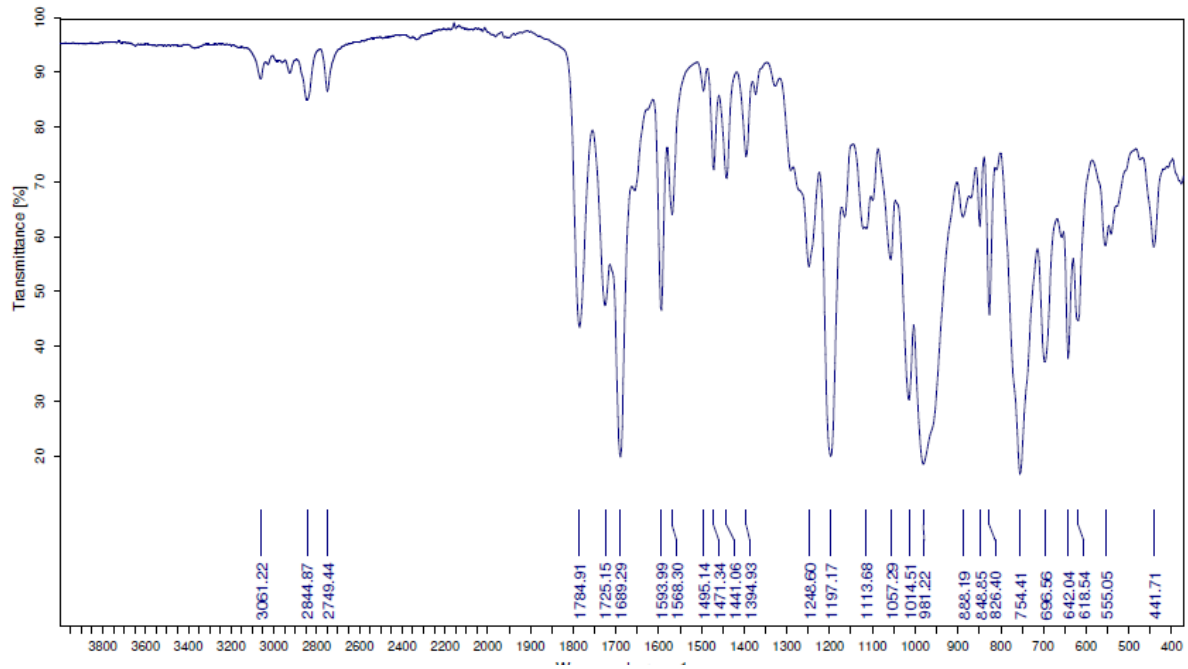
Integration Results		*Channel:*	UV_VIS_1	*Wavelength:*	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.64	40.0	17.323	194.114	100.00	100.00
Total:			**17.323**	**194.114**	**100.00**	**100.00**

RP-HPLC elution profile of S3 (system A, detection at 260 nm)

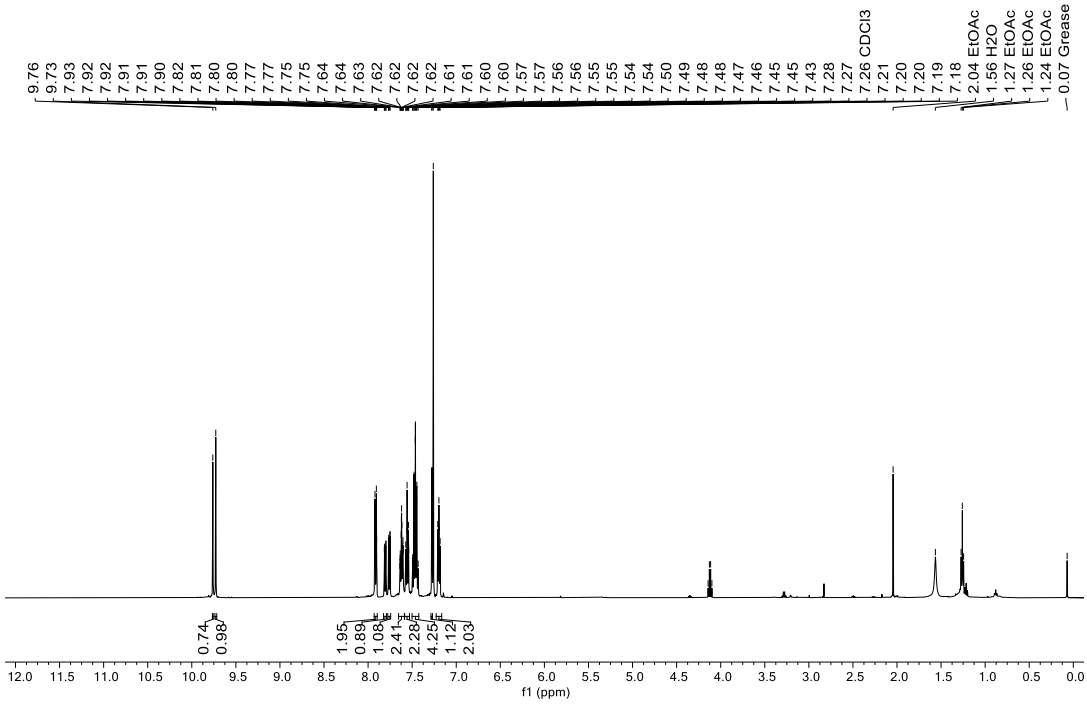


Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.44	1.7	0.140	1.902	1.47	1.75
2	4.63	143.8	9.344	106.724	98.53	98.25
Total:			**9.484**	**108.626**	**100.00**	**100.00**

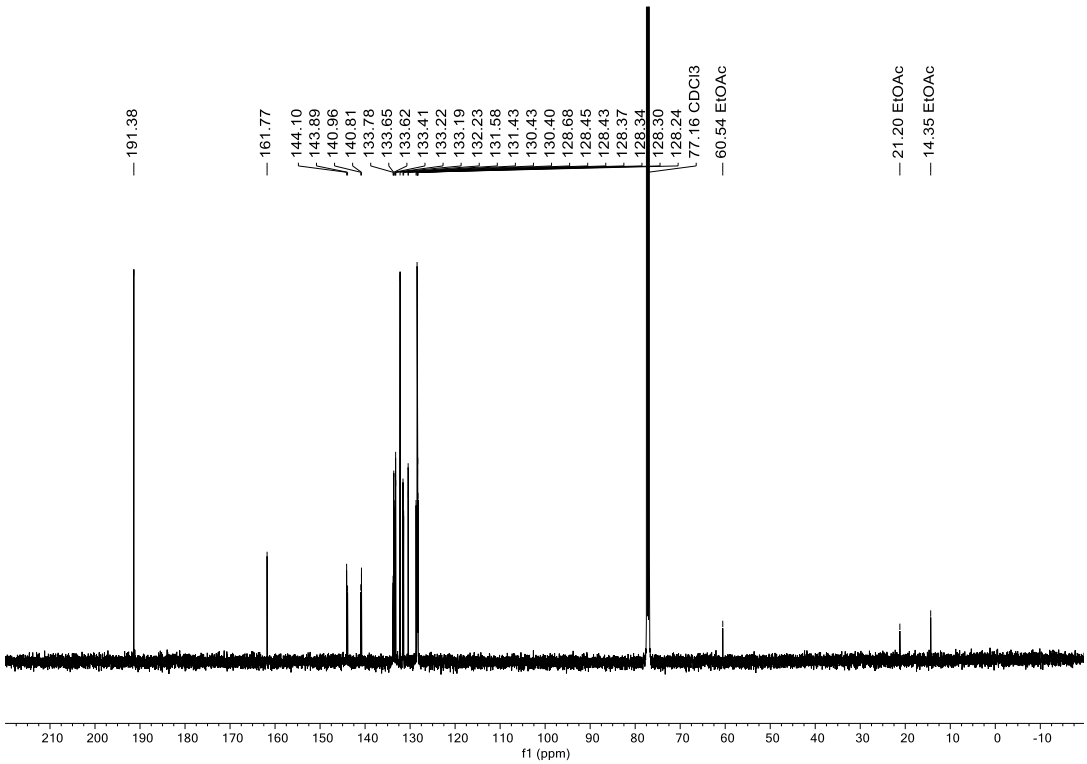
IR-ATR spectrum of S3



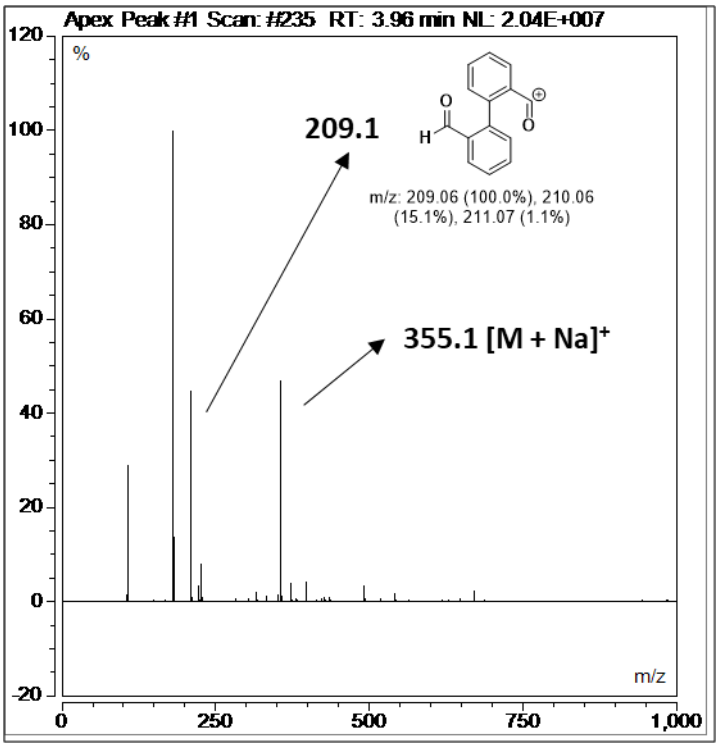
^{1}H NMR spectrum of S3 in $CDCl_3$ (500 MHz)



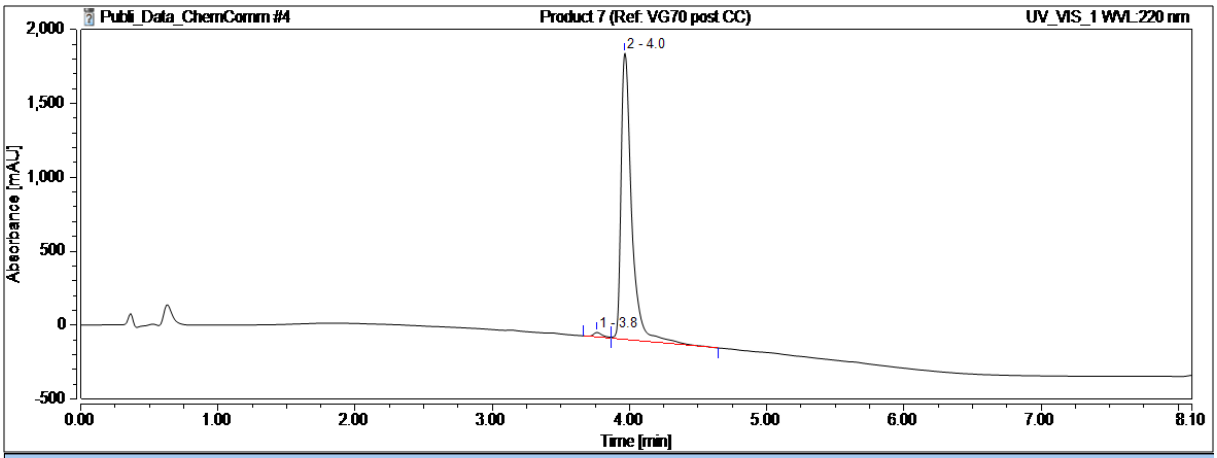
^{13}C NMR spectrum of S3 in $CDCl_3$ (126 MHz)



ESI+ spectrum (low resolution) of S4

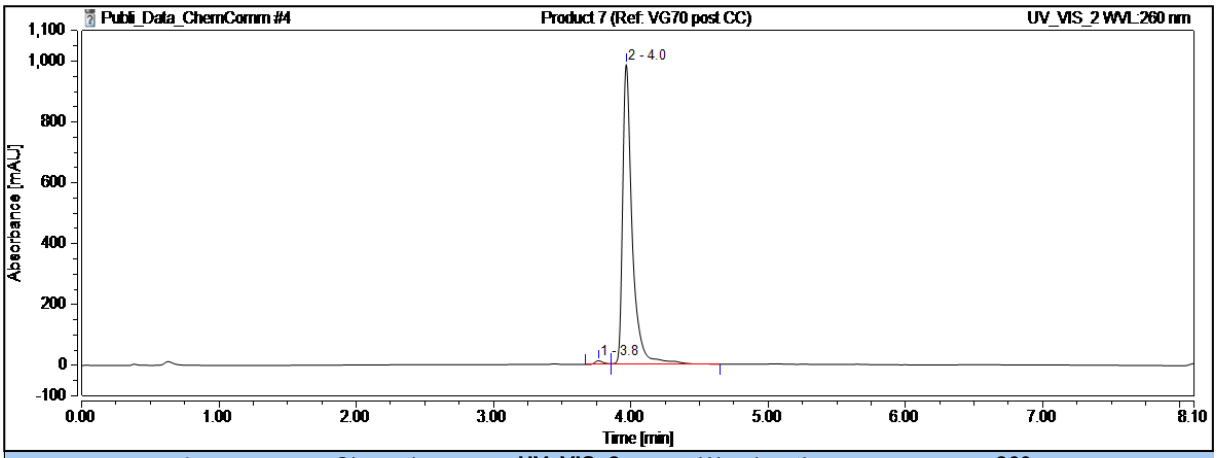


RP-HPLC elution profile of S4 (system A, detection at 220 nm)



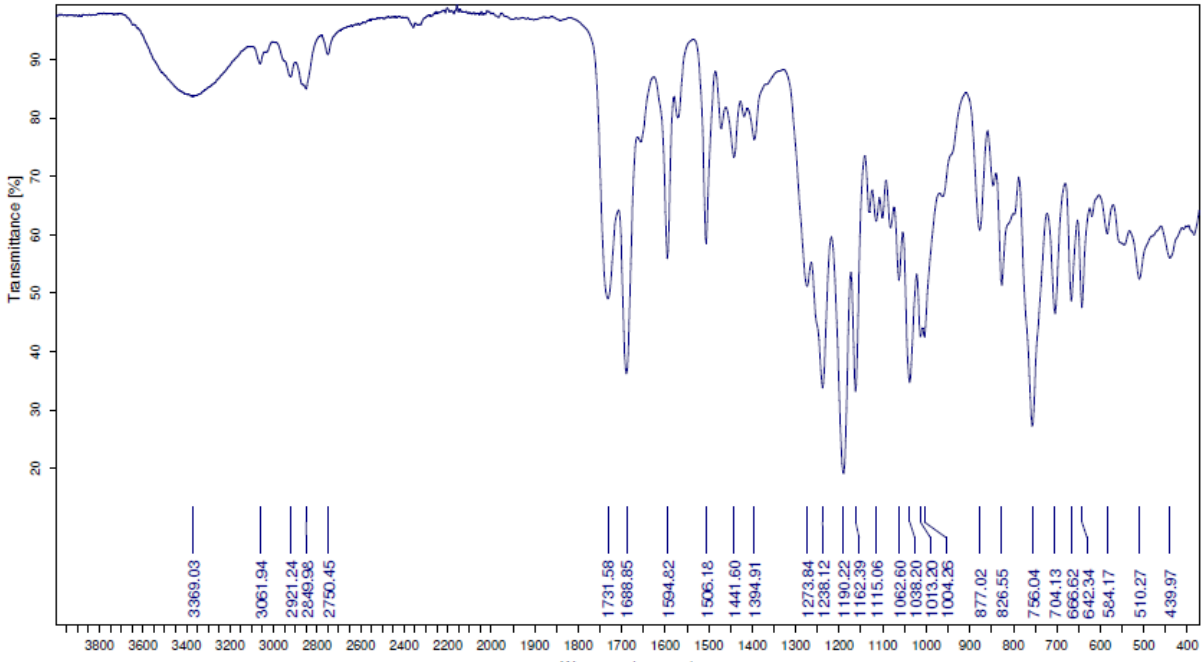
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.76	5.7	2.534	31.197	1.43	1.58
2	3.97	332.2	174.628	1938.950	98.57	98.42
Total:			**177.162**	**1970.147**	**100.00**	**100.00**

RP-HPLC elution profile of S4 (system A, detection at 260 nm)

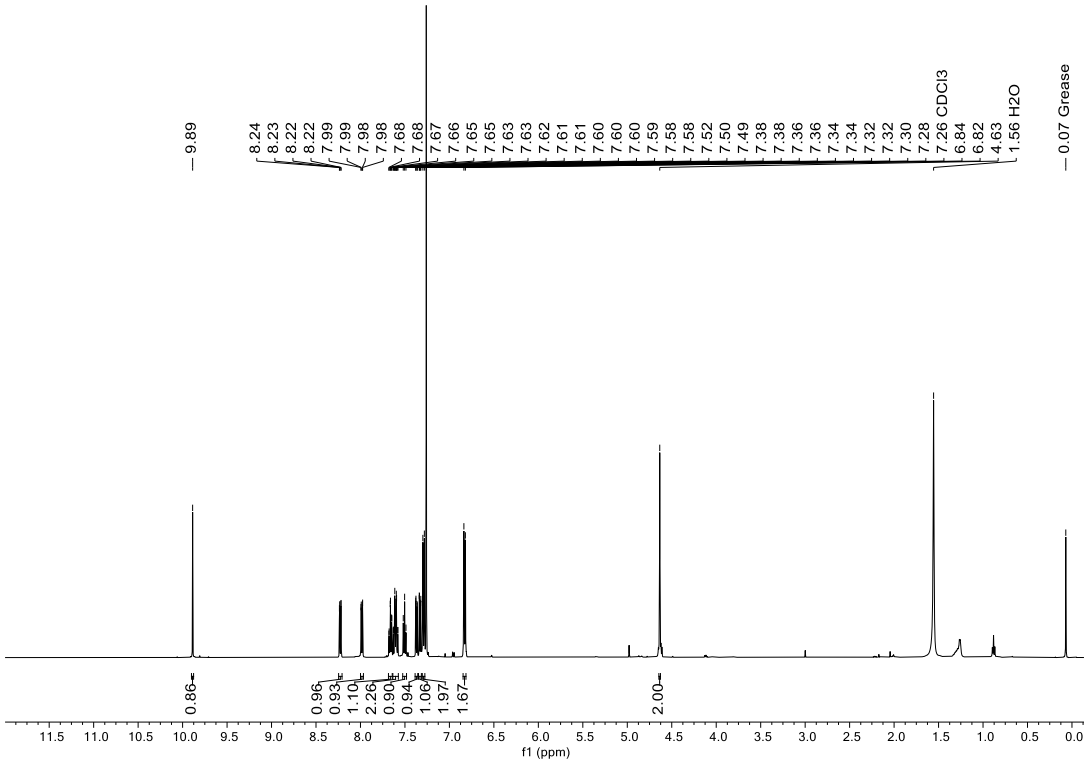


Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.76	10.8	0.918	12.148	1.13	1.22
2	3.97	878.6	80.084	986.081	98.87	98.78
Total:			**81.002**	**998.229**	**100.00**	**100.00**

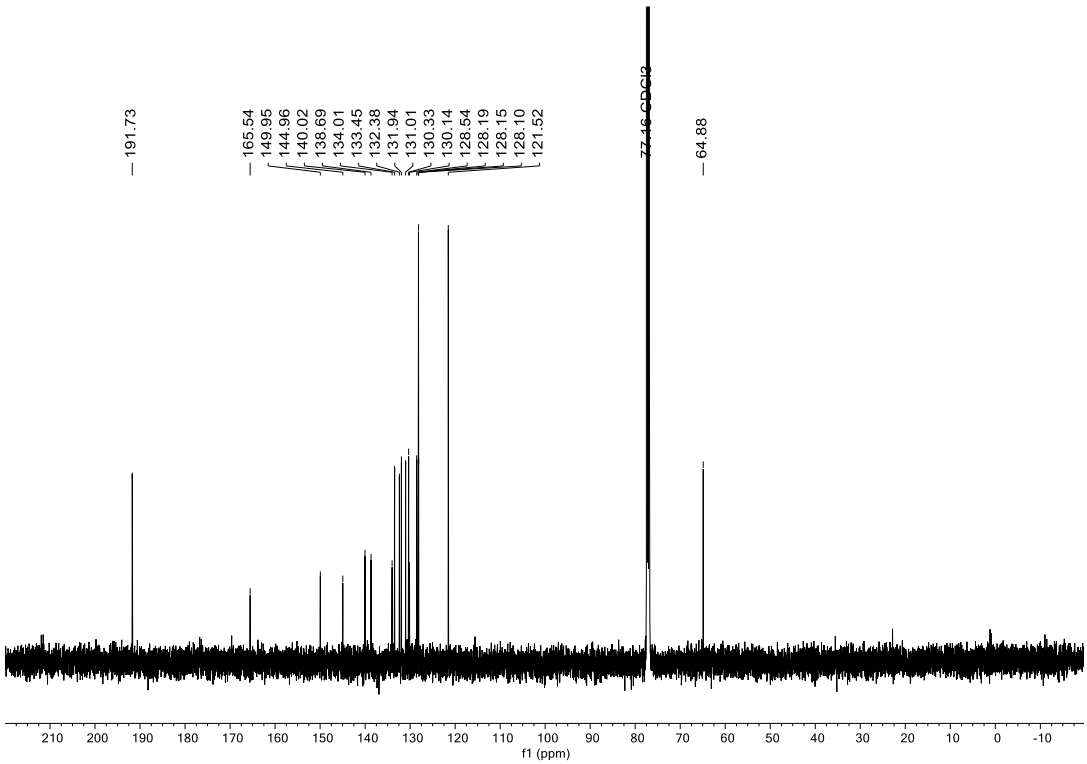
IR-ATR spectrum of S4



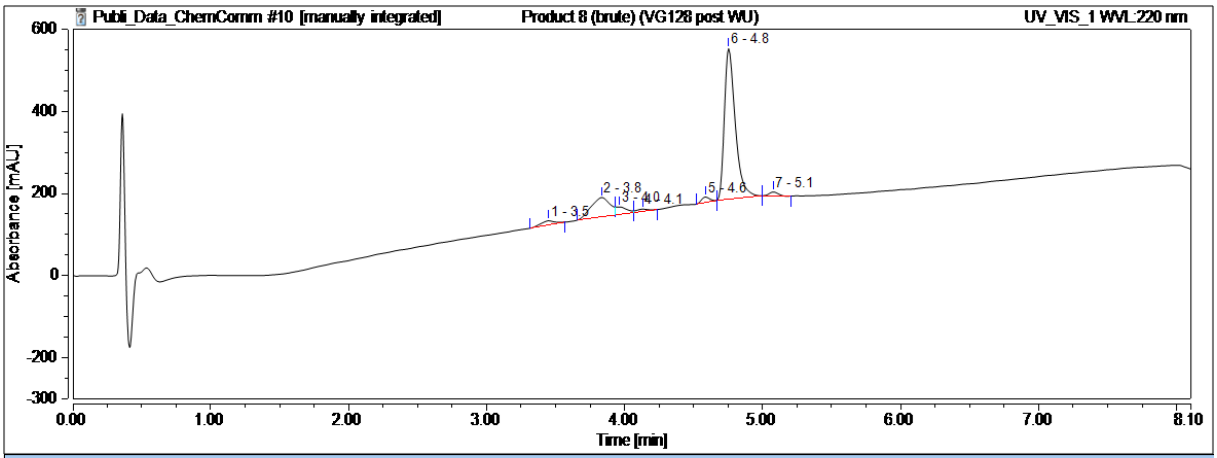
^{1}H NMR spectrum of S4 in $CDCl_3$ (500 MHz)



^{13}C NMR spectrum of S4 in $CDCl_3$ (126 MHz)

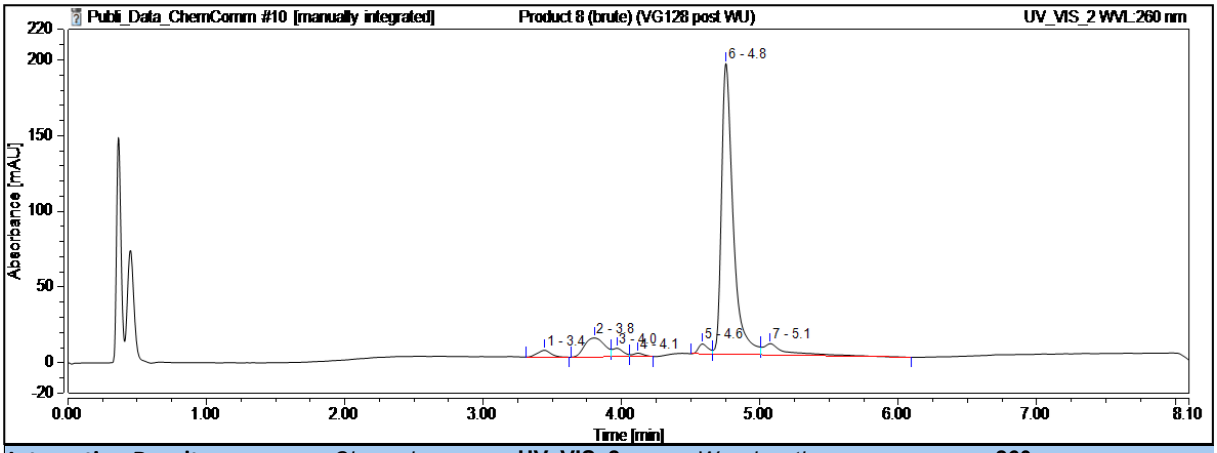


RP-HPLC elution profile of S5 (crude product) (system A, detection at 220 nm)



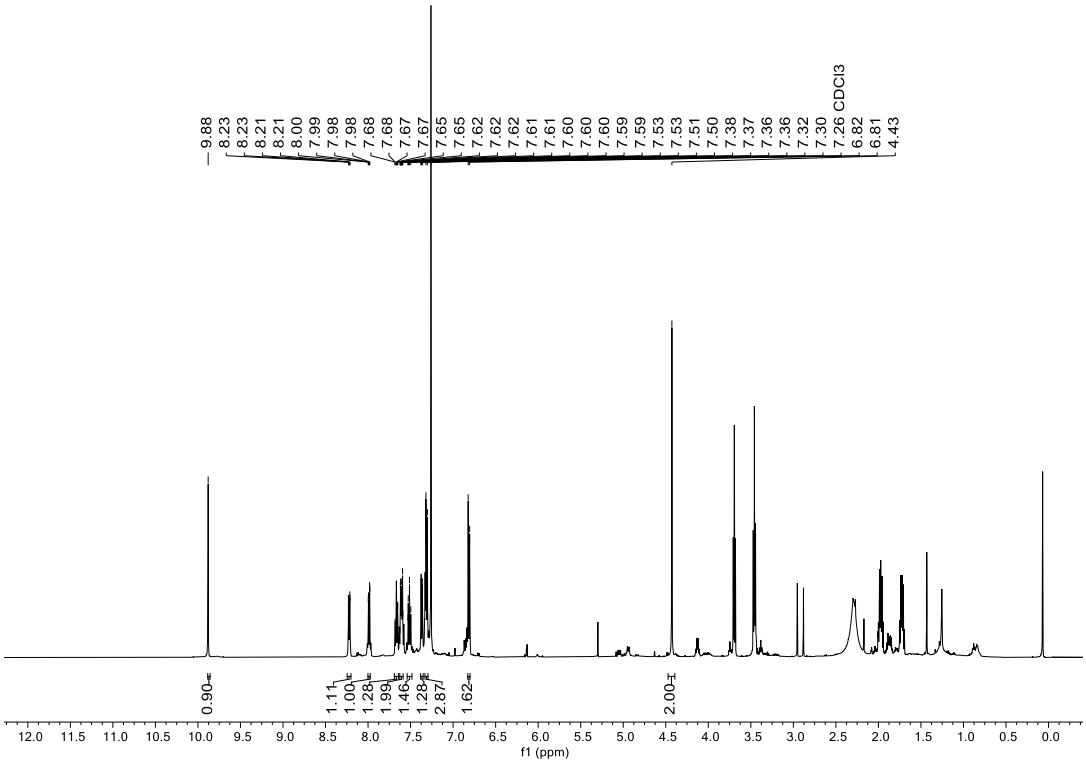
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.45	0.9	1.200	10.180	2.60	2.16
2	3.83	3.0	7.411	47.368	16.09	10.04
3	3.96	n.a.	1.732	18.566	3.76	3.93
4	4.13	n.a.	0.561	5.709	1.22	1.21
5	4.59	6.0	0.908	13.266	1.97	2.81
6	4.75	165.3	33.599	367.574	72.95	77.88
7	5.08	4.2	0.648	9.292	1.41	1.97
Total:			**46.060**	**471.956**	**100.00**	**100.00**

RP-HPLC elution profile of S5 (crude product) (system A, detection at 260 nm)

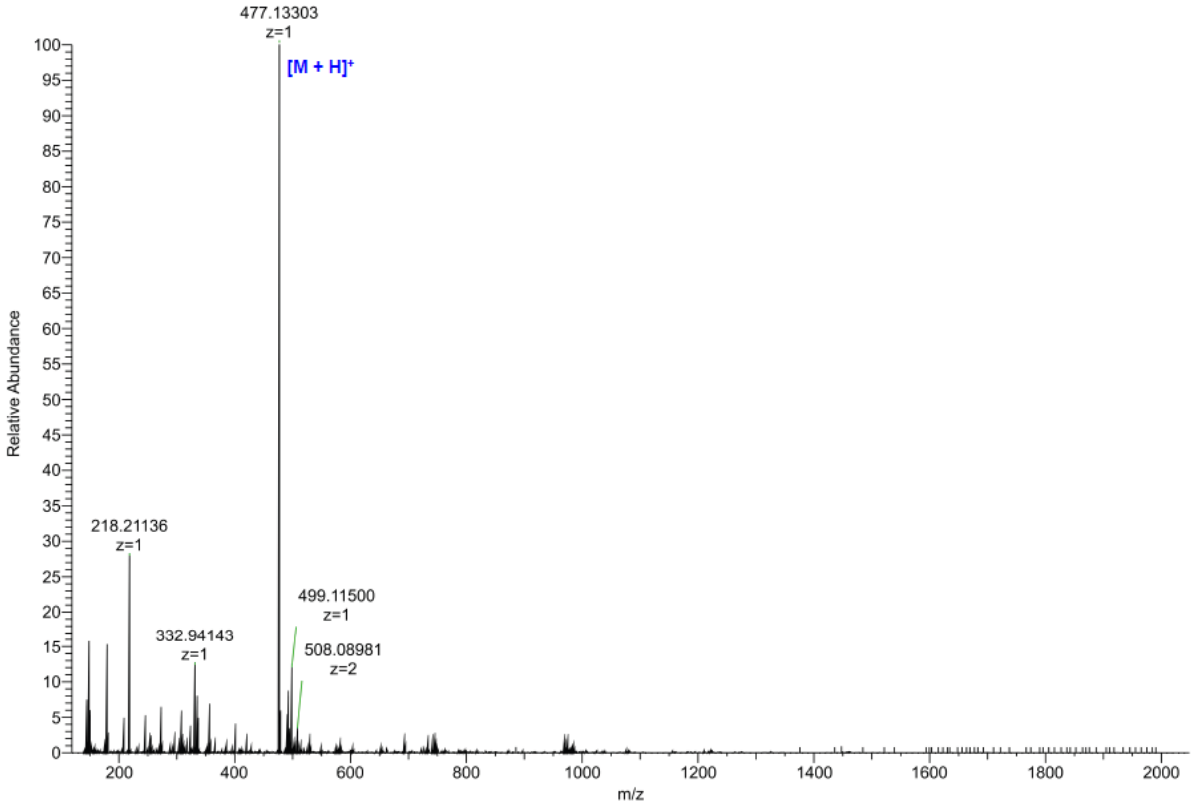


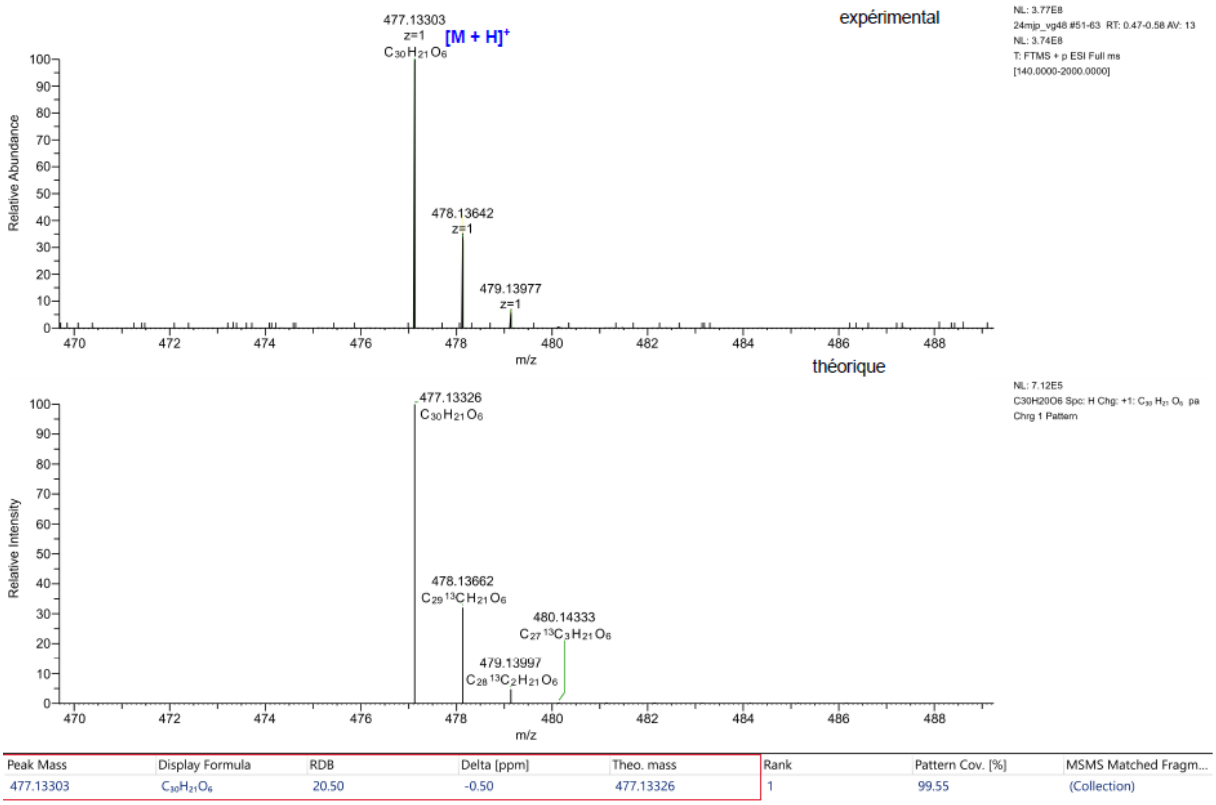
Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.44	3.3	0.512	4.549	2.12	1.97
2	3.80	9.9	2.108	12.477	8.72	5.40
3	3.97	n.a.	0.514	5.643	2.13	2.44
4	4.12	n.a.	0.205	2.223	0.85	0.96
5	4.58	12.9	0.545	6.573	2.26	2.84
6	4.75	375.9	18.691	192.087	77.31	83.12
7	5.07	n.a.	1.601	7.530	6.62	3.26
Total:			**24.176**	**231.082**	**100.00**	**100.00**

^{1}H NMR crude spectrum of S5 (crude product) in $CDCl_3$ (500 MHz)



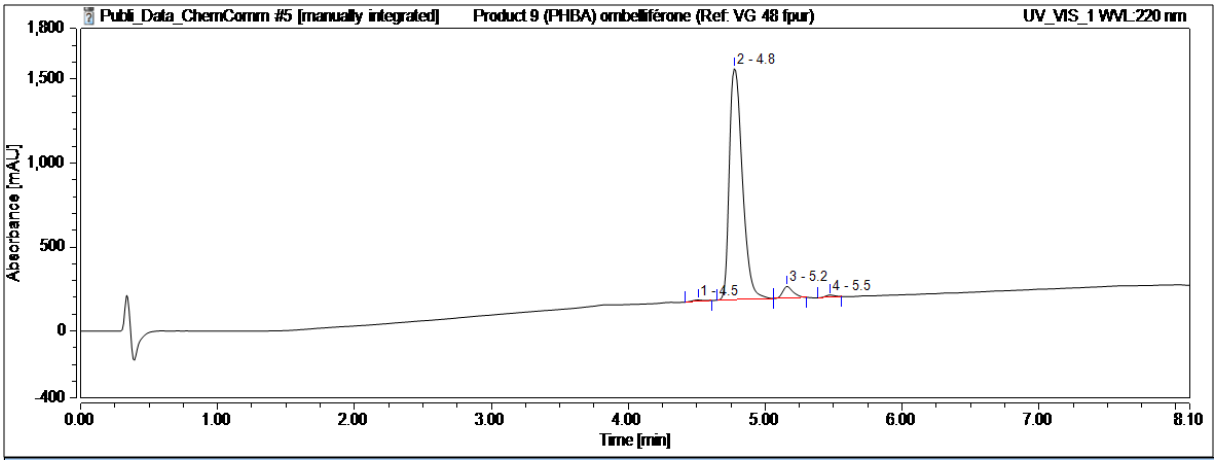
ESI+ mass spectrum (high resolution) of 3





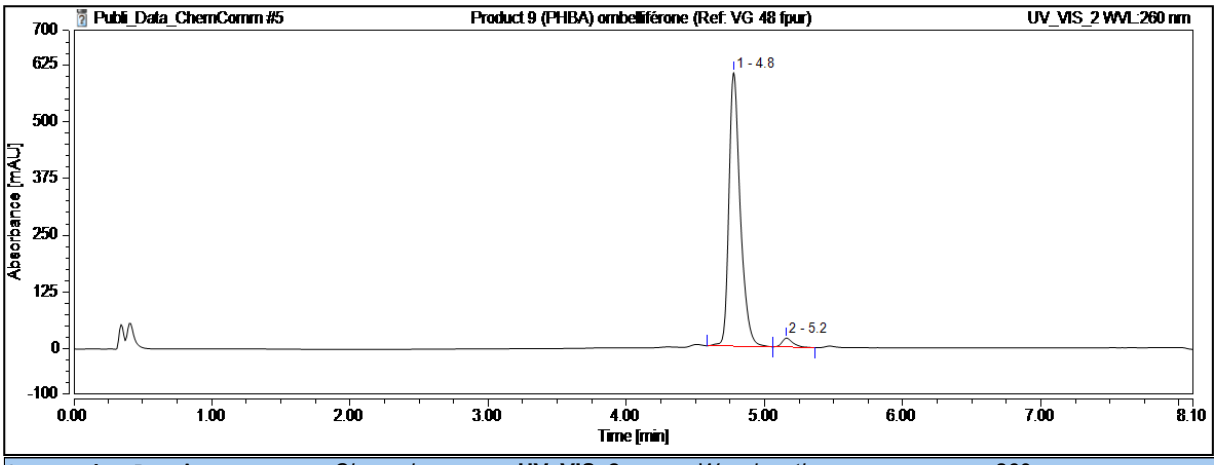
Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]	MSMS Matched Fragm...
477.13303	$C_{30}H_{21}O_6$	20.50	-0.50	477.13326	1	99.55	(Collection)

RP-HPLC elution profile of 3 (system A, purified by FC (SiO_2), detection at 220 nm)



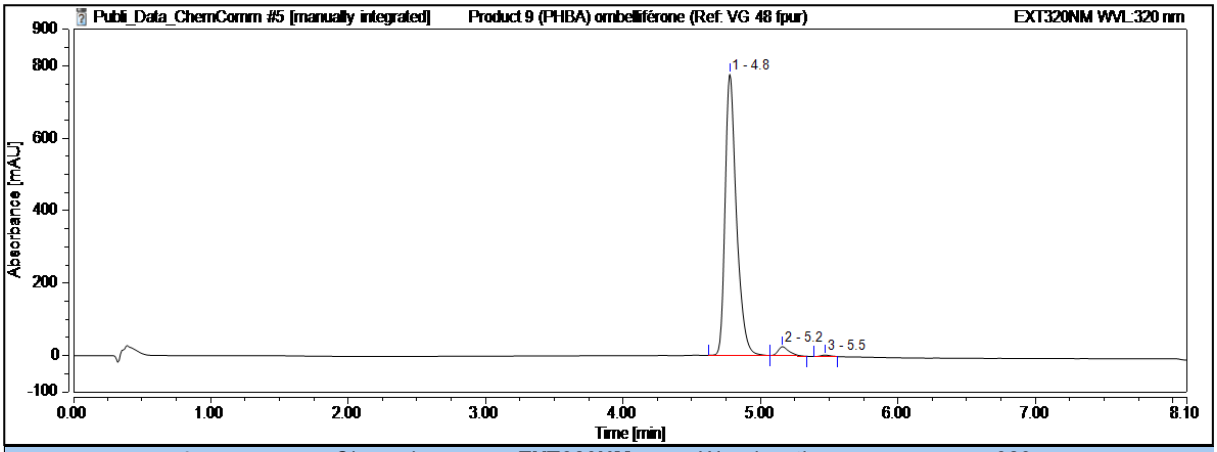
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.51	0.8	0.695	7.902	0.43	0.54
2	4.78	134.3	152.175	1377.119	95.19	93.91
3	5.16	7.6	6.055	69.065	3.79	4.71
4	5.47	9.0	0.934	12.341	0.58	0.84
Total:			**159.859**	**1466.426**	**100.00**	**100.00**

RP-HPLC elution profile of 3 (system A, purified by FC (SiO_2), detection at 260 nm)



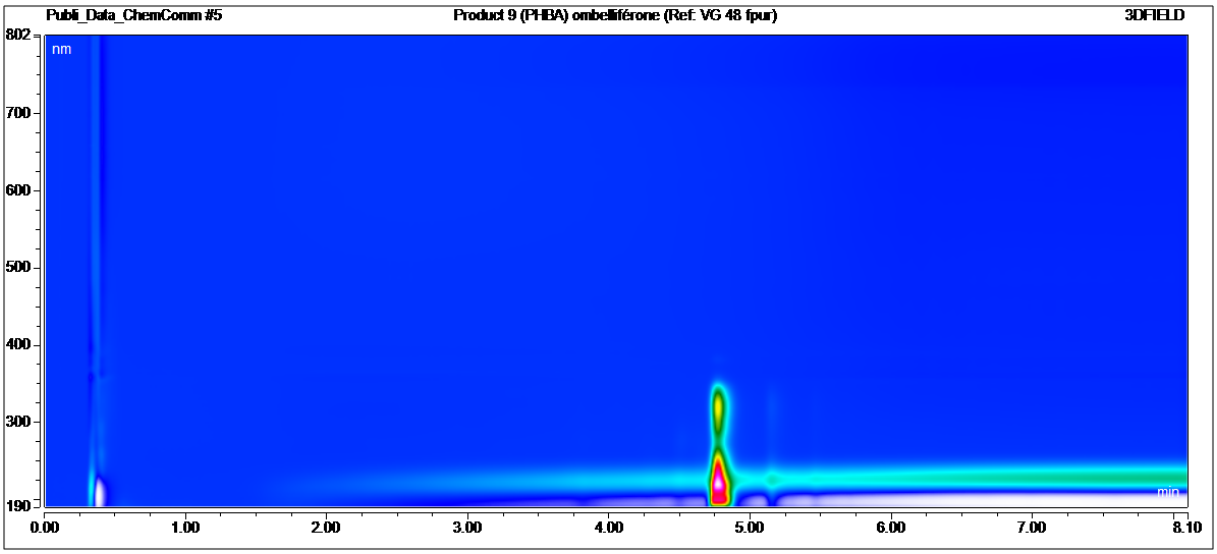
Integration Results	Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.78	193.0	57.282	602.135	96.91	96.81
2	5.16	6.4	1.825	19.857	3.09	3.19
Total:			**59.107**	**621.992**	**100.00**	**100.00**

RP-HPLC elution profile of 3 (system A, purified by FC (SiO_2), detection at 320 nm)

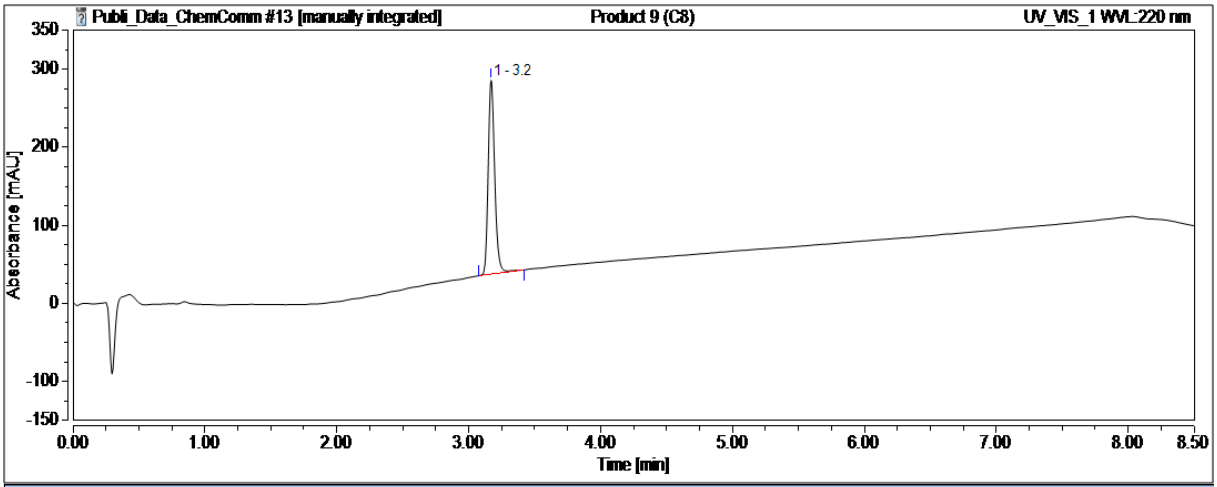


Integration Results	Channel:	EXT320NM	Wavelength:	320	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.78	1561.2	74.004	776.052	96.53	96.39
2	5.16	50.6	2.357	25.150	3.07	3.12
3	5.47	7.8	0.307	3.901	0.40	0.48
Total:			**76.668**	**805.103**	**100.00**	**100.00**

2D map of DAD of 3 (system A, purified by FC (SiO_2))

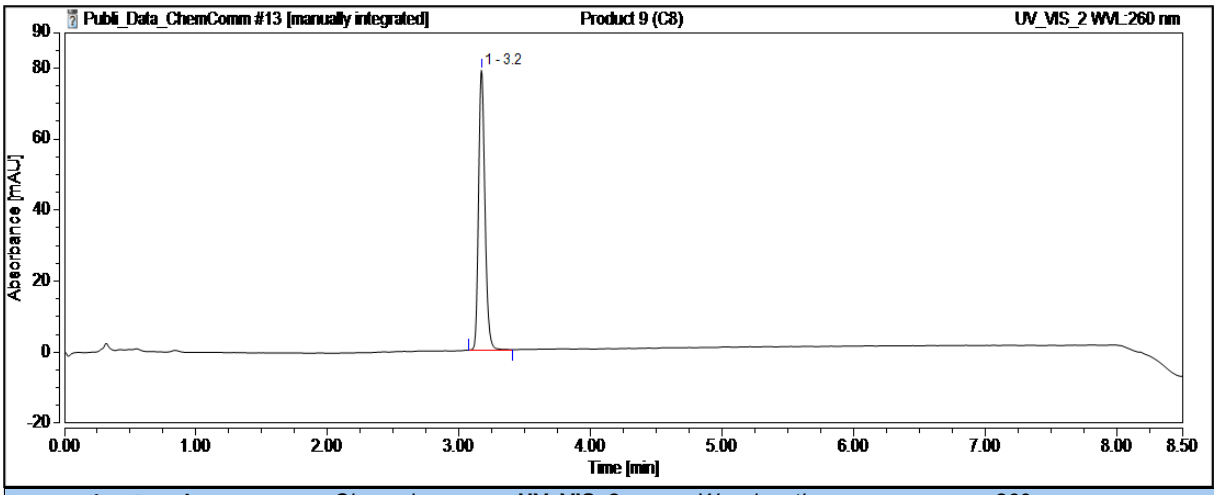


RP-HPLC elution profile of 3 (system A', purified by semi-preparative RP-HPLC, detection at 220 nm)



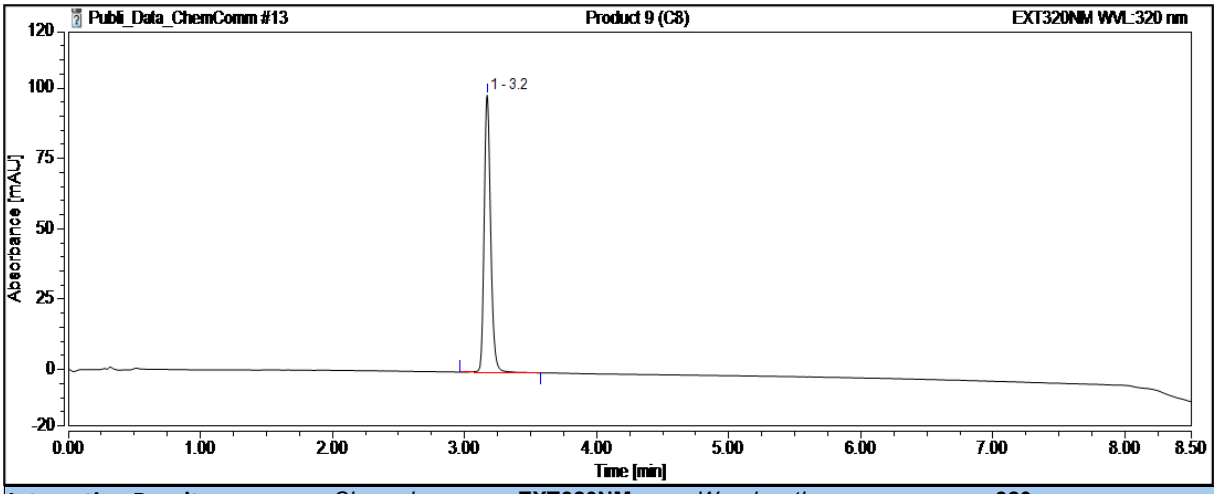
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.17	318.4	14.152	248.391	100.00	100.00
Total:			**14.152**	**248.391**	**100.00**	**100.00**

RP-HPLC elution profile of 3 (system A', purified by semi-preparative RP-HPLC, detection at 260 nm)



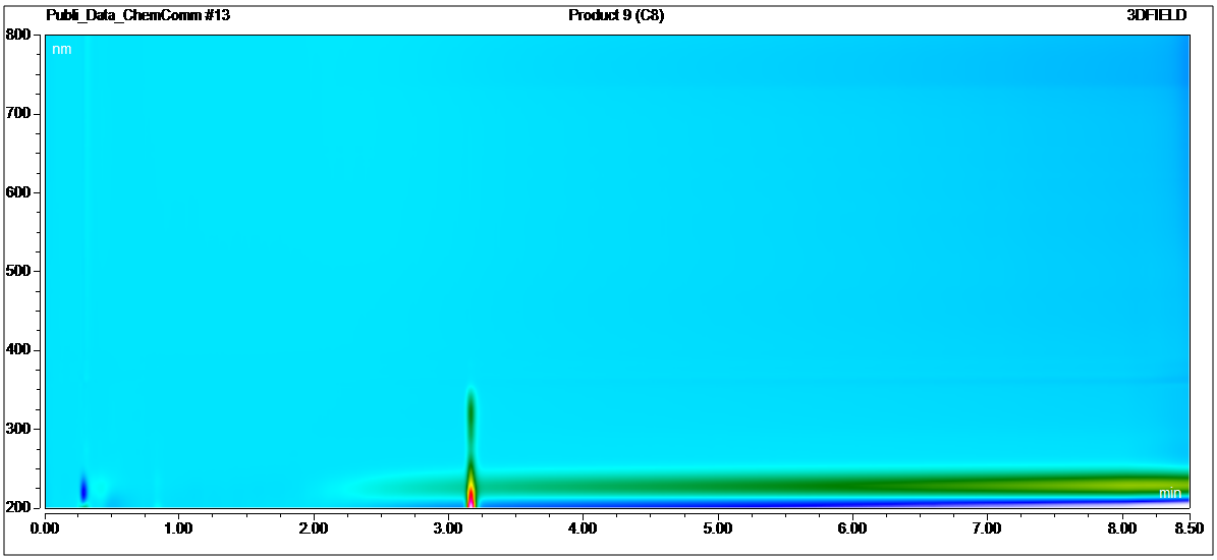
Integration Results	Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.17	705.9	4.427	78.778	100.00	100.00
Total:			**4.427**	**78.778**	**100.00**	**100.00**

RP-HPLC elution profile of 3 (system A', purified by semi-preparative RP-HPLC, detection at 320 nm)

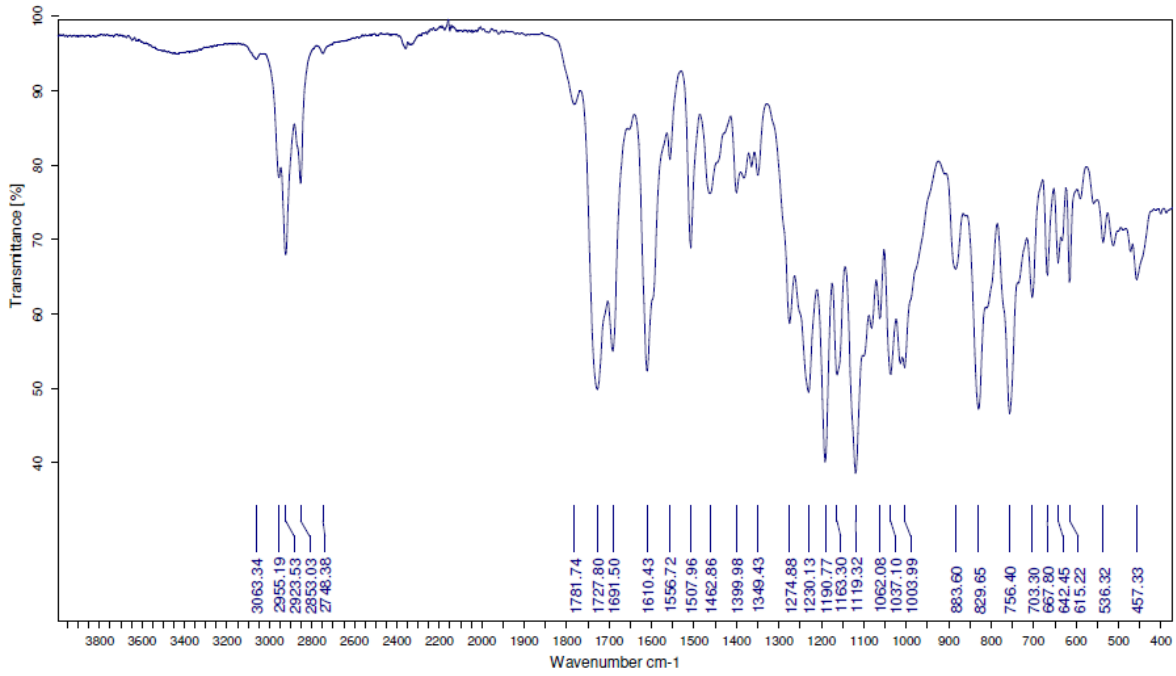


Integration Results	Channel:	EXT320NM	Wavelength:	320	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.17	1332.2	5.636	98.541	100.00	100.00
Total:			**5.636**	**98.541**	**100.00**	**100.00**

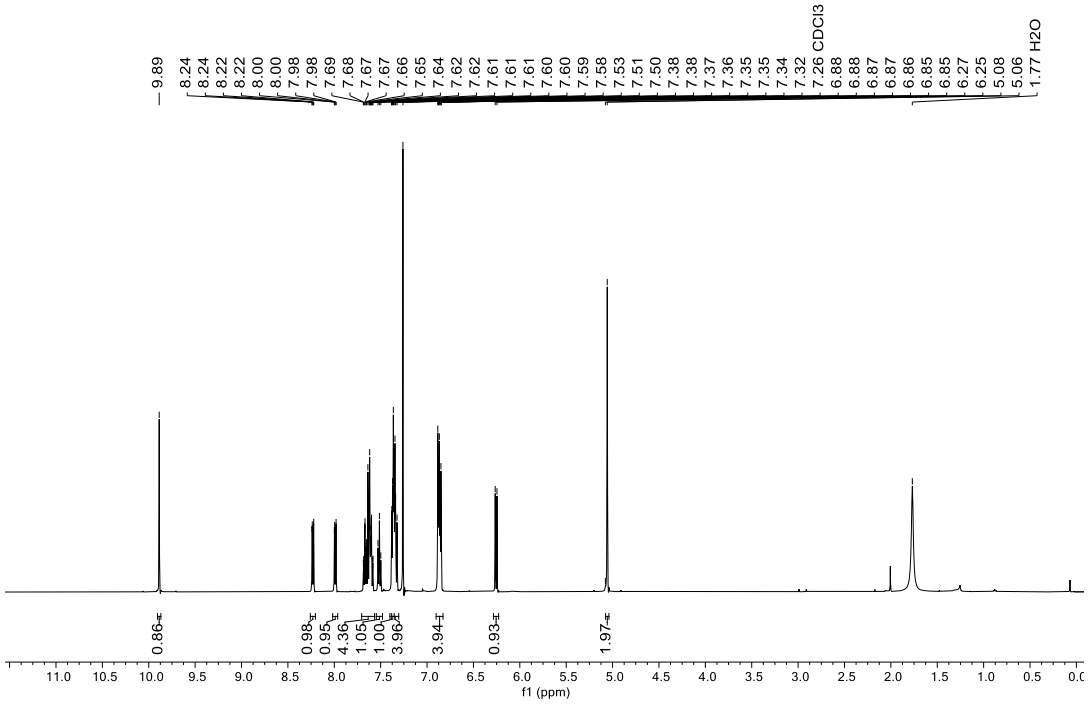
2D map of DAD of 3 (system A', purified by semi-preparative RP-HPLC)



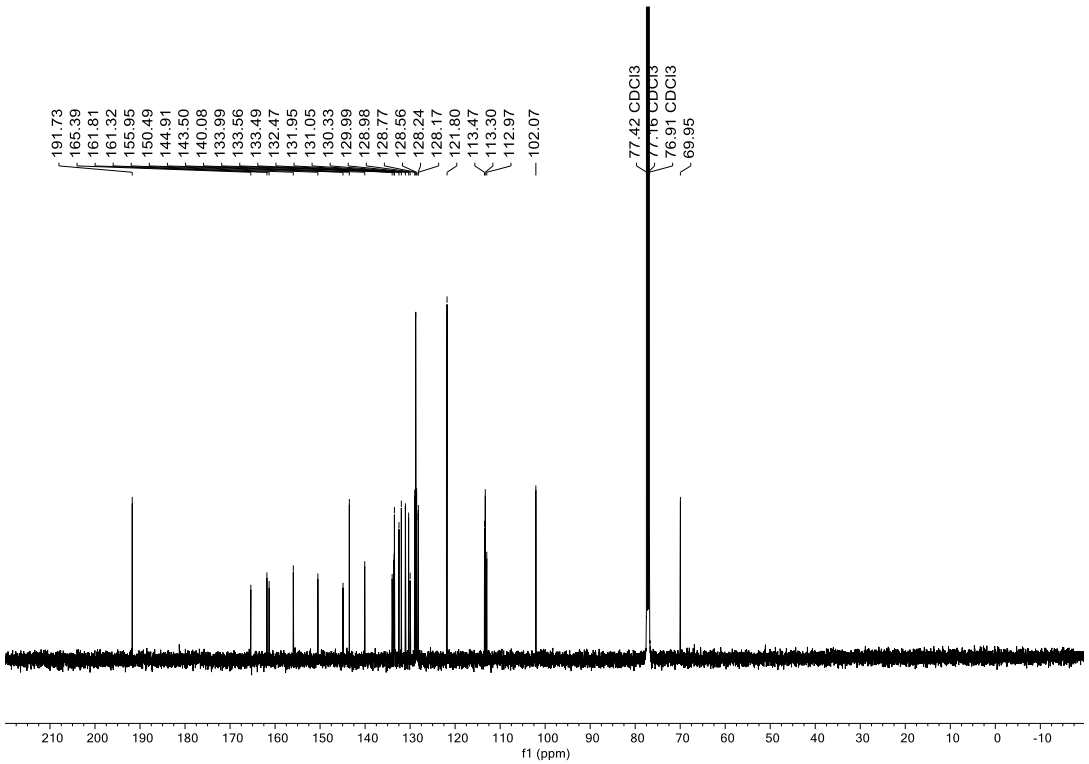
IR-ATR spectrum of 3 (purified by FC (SiO_2))



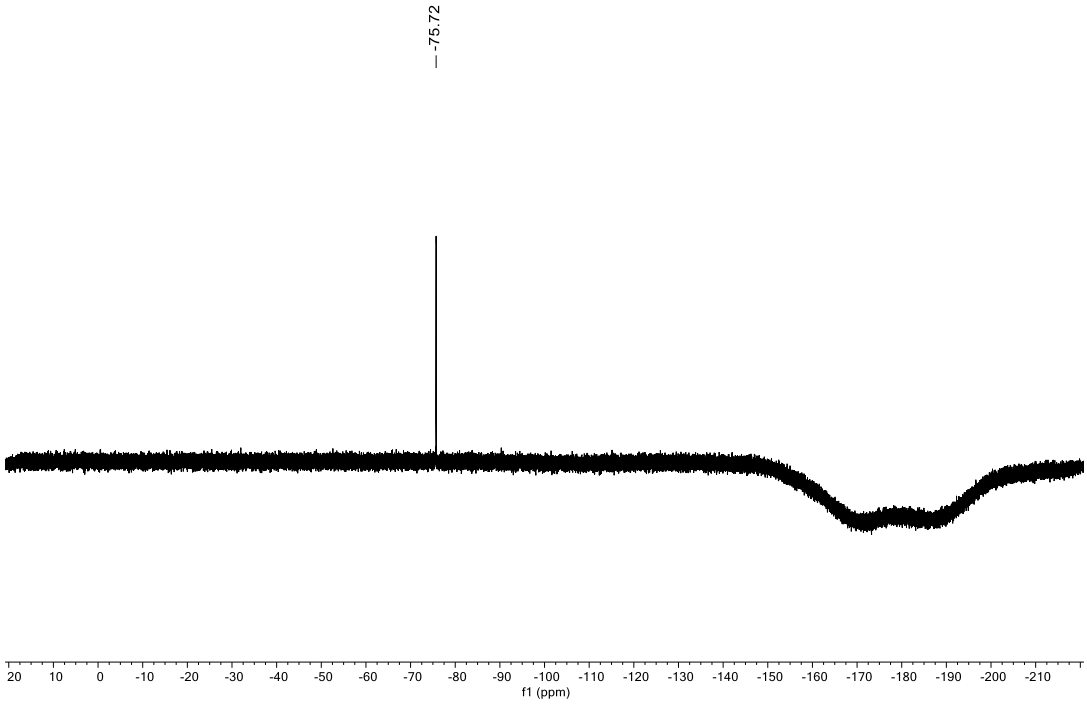
^{1}H NMR spectrum of 3 in $CDCl_3$ (500 MHz), (purified by semi-preparative RP-HPLC)



^{13}C NMR spectrum of 3 in $CDCl_3$ (126 MHz), (purified by semi-preparative RP-HPLC)



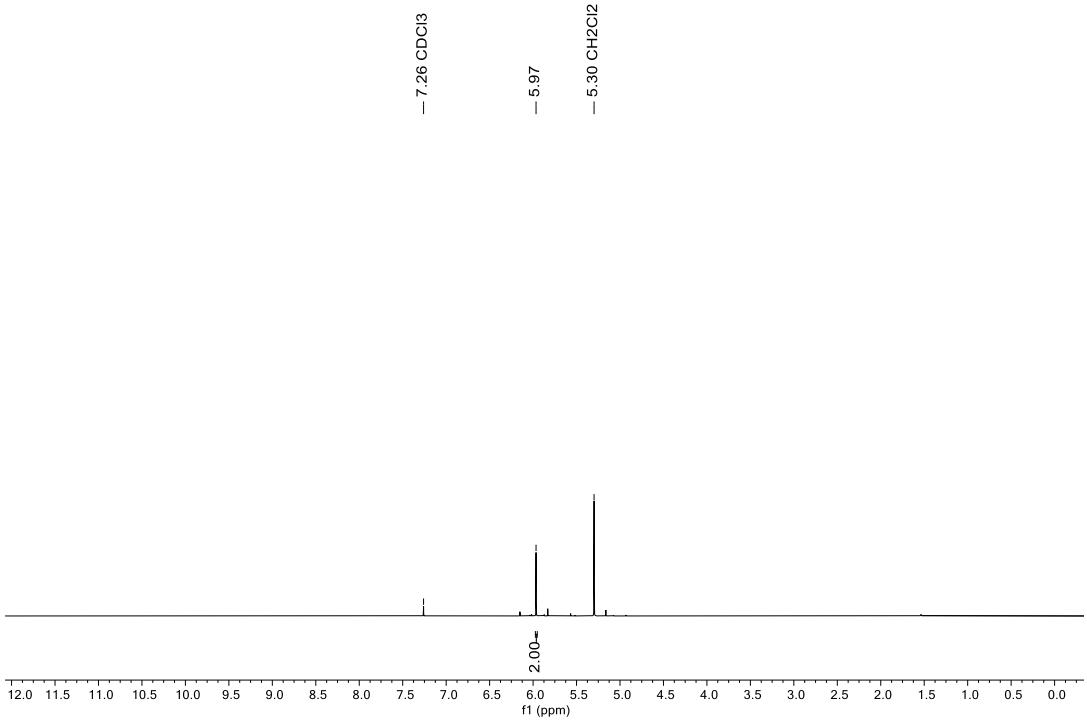
^{19}F NMR spectrum of 3 in $CDCl_3$ (470 MHz), (purified by semi-preparative RP-HPLC)



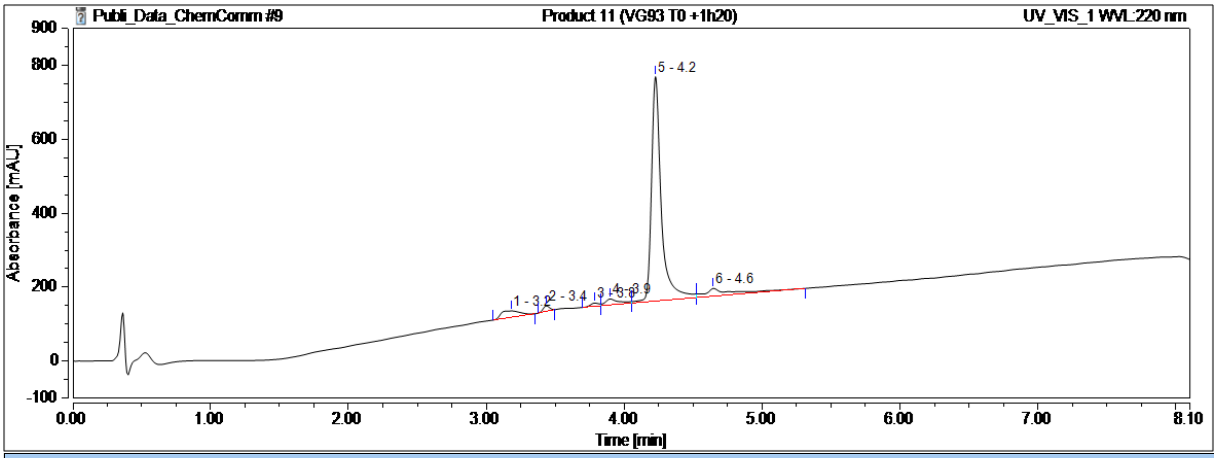
Determination of TFA content in sample of probe 3, by IC

Concentration (stock sol. **3**)	1.0 mg/mL in DMSO
Sample dilution factor	10
Raw data ppm	1.9173
Content in wt %	1.92
Average content in wt %	1.92

^{1}H NMR spectrum of S6 in $CDCl_3$ (500 MHz)

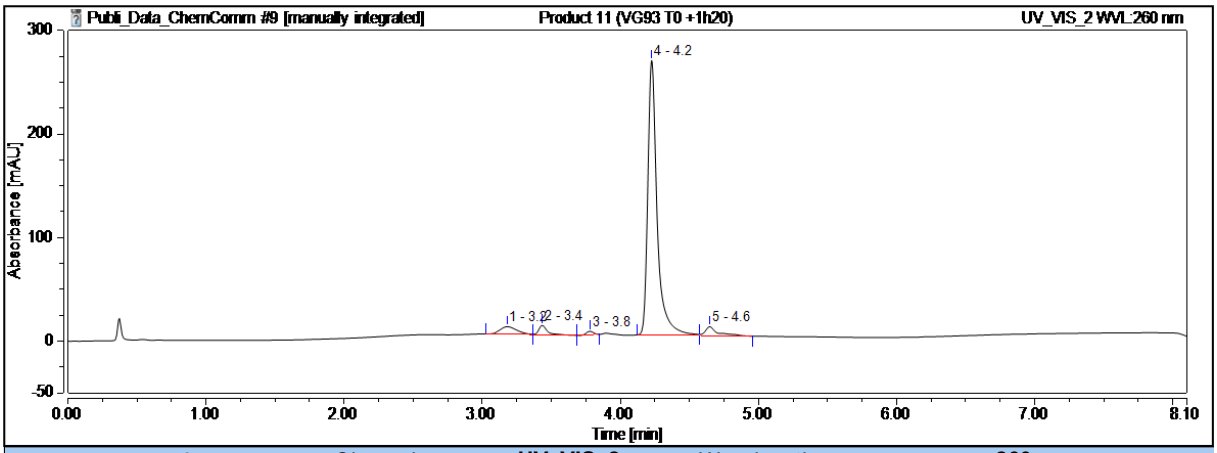


RP-HPLC elution profile of S7 (system A, detection at 220 nm)



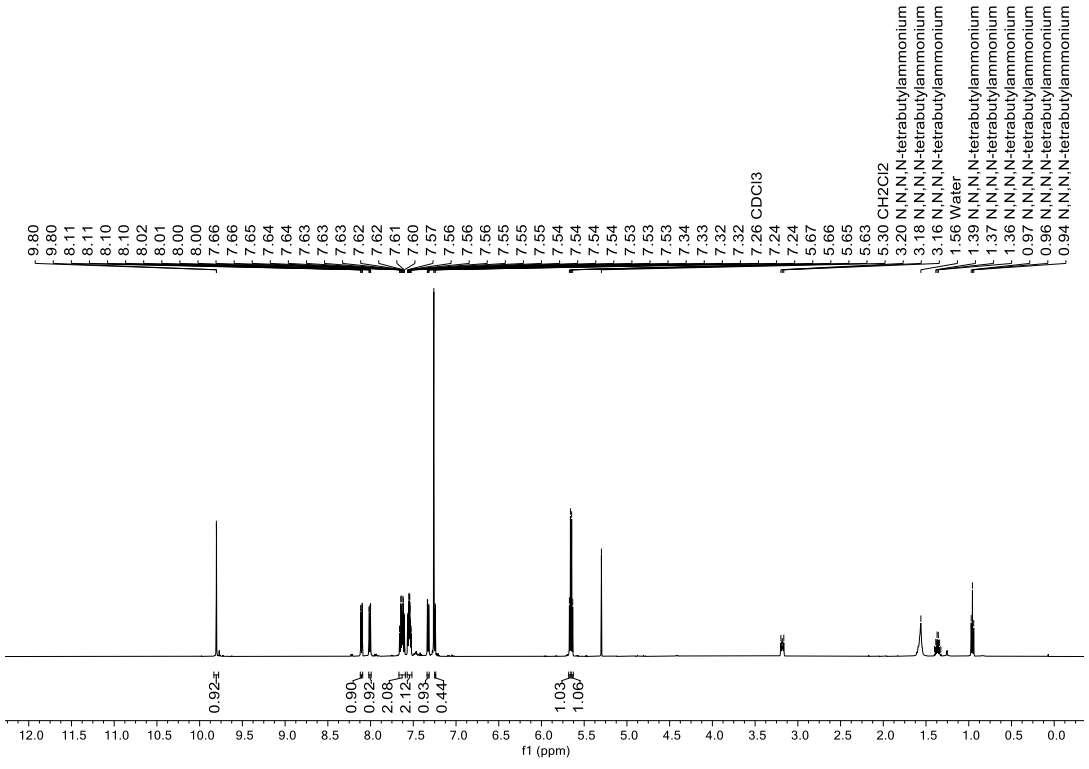
Integration Results		Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.18	1.0	2.867	17.363	4.71	2.52
2	3.44	10.5	0.809	15.002	1.33	2.18
3	3.79	n.a.	0.626	9.094	1.03	1.32
4	3.90	18.7	1.928	16.694	3.17	2.43
5	4.23	681.2	50.066	608.198	82.32	88.43
6	4.65	24.0	4.522	21.399	7.43	3.11
Total:			**60.818**	**687.750**	**100.00**	**100.00**

RP-HPLC elution profile of S7 (system A, detection at 260 nm)

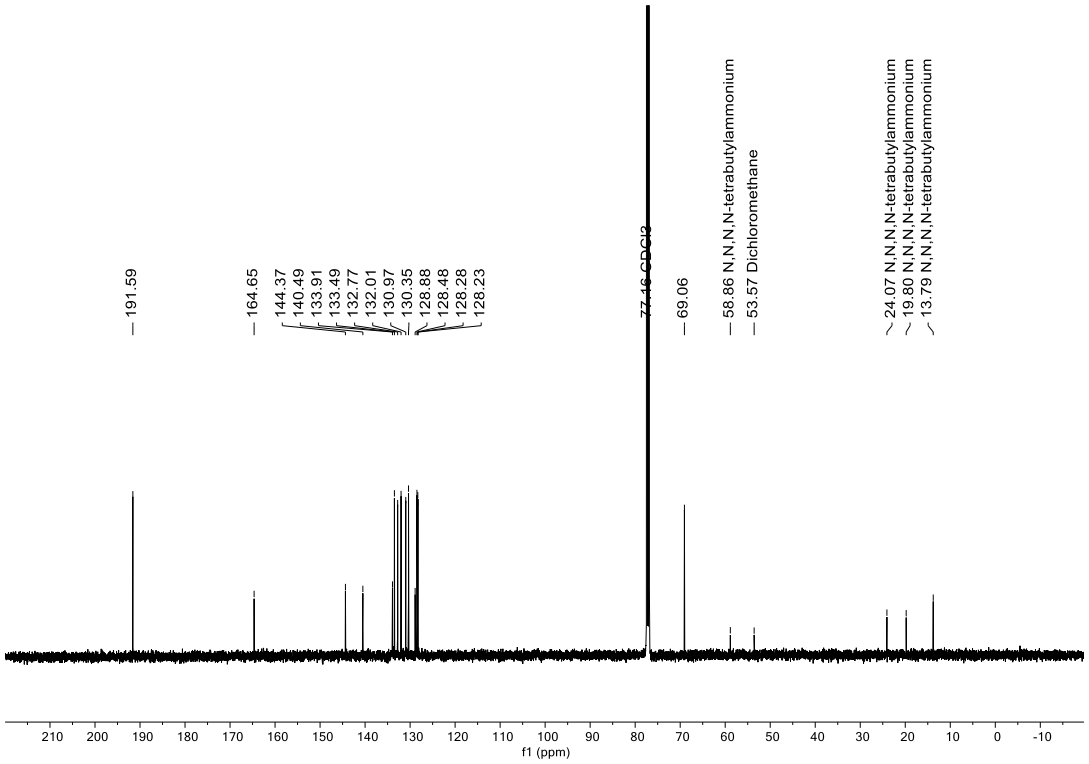


Integration Results		Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	3.18	5.6	1.040	7.303	4.41	2.49
2	3.43	12.6	0.654	9.133	2.77	3.11
3	3.78	4.7	0.193	3.393	0.82	1.15
4	4.23	365.4	20.771	265.346	88.03	90.29
5	4.65	11.9	0.939	8.717	3.98	2.97
Total:			**23.595**	**293.893**	**100.00**	**100.00**

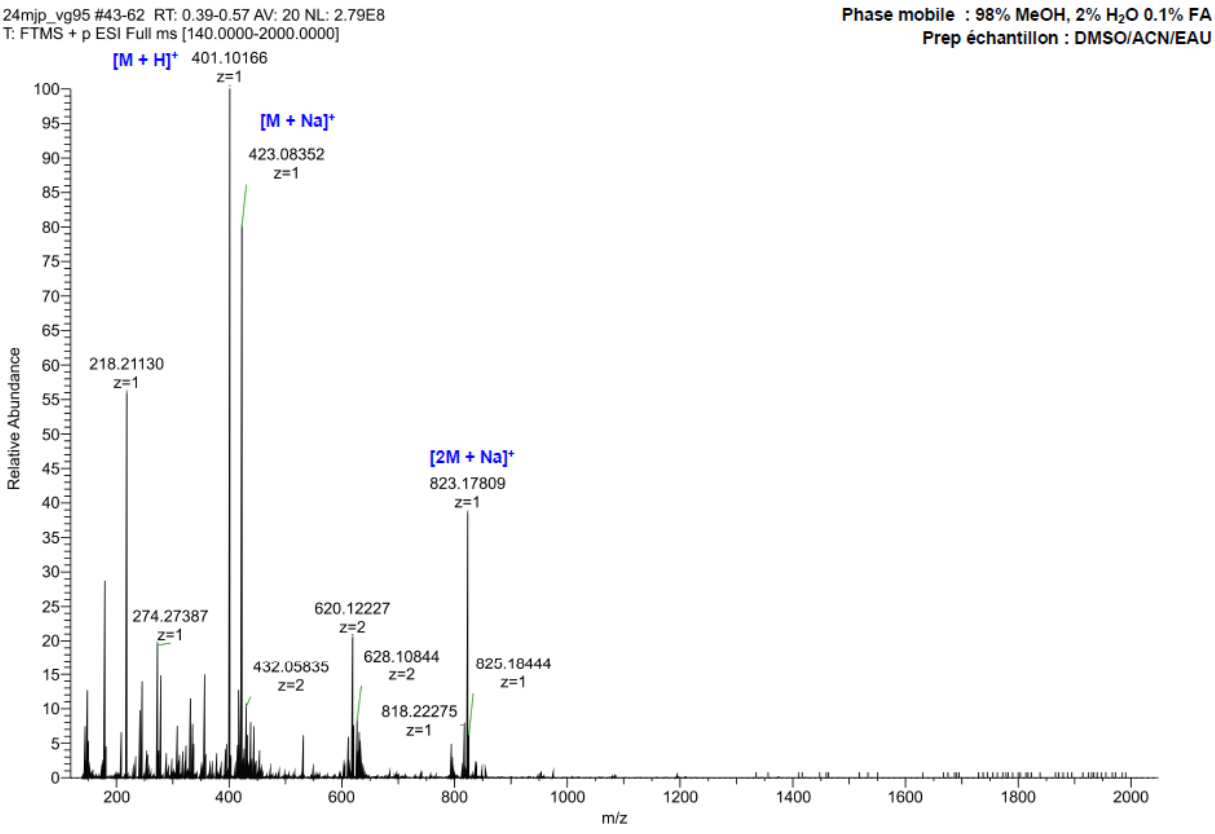
^{1}H NMR spectrum of S7 in $CDCl_3$ (500 MHz)

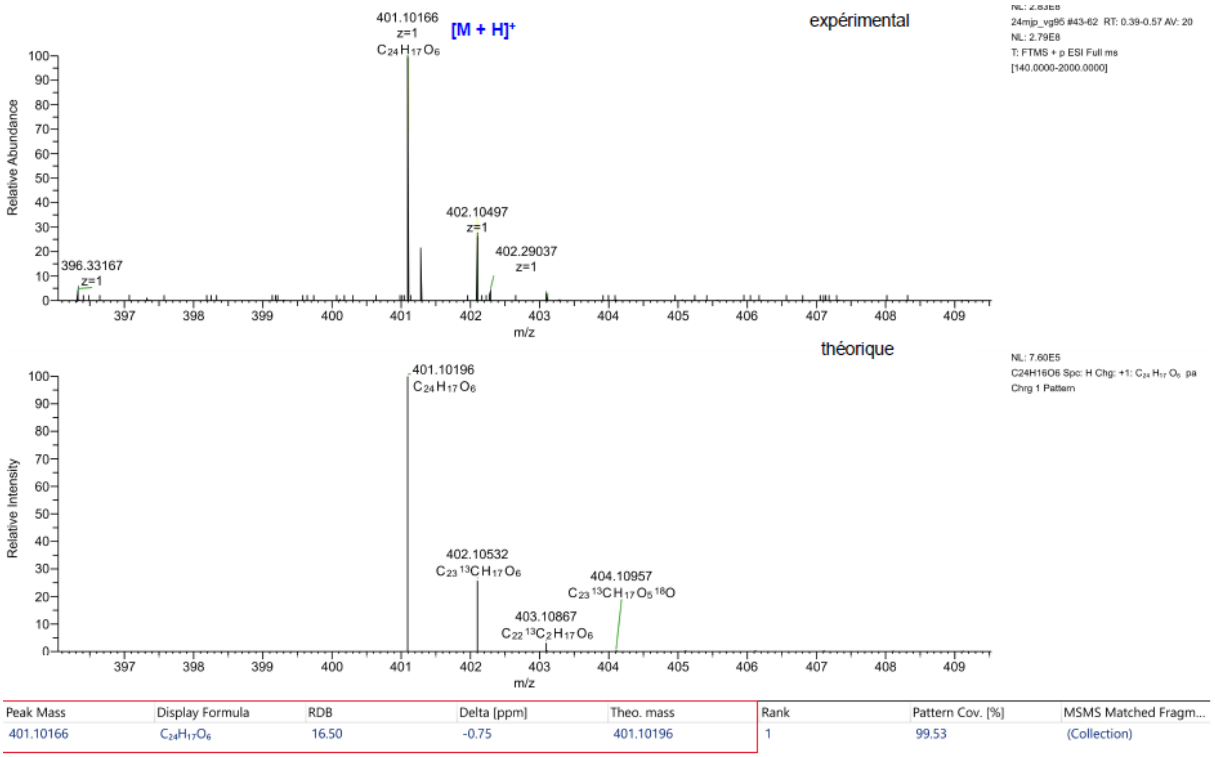


^{13}C NMR spectrum of S7 in $CDCl_3$ (126 MHz)

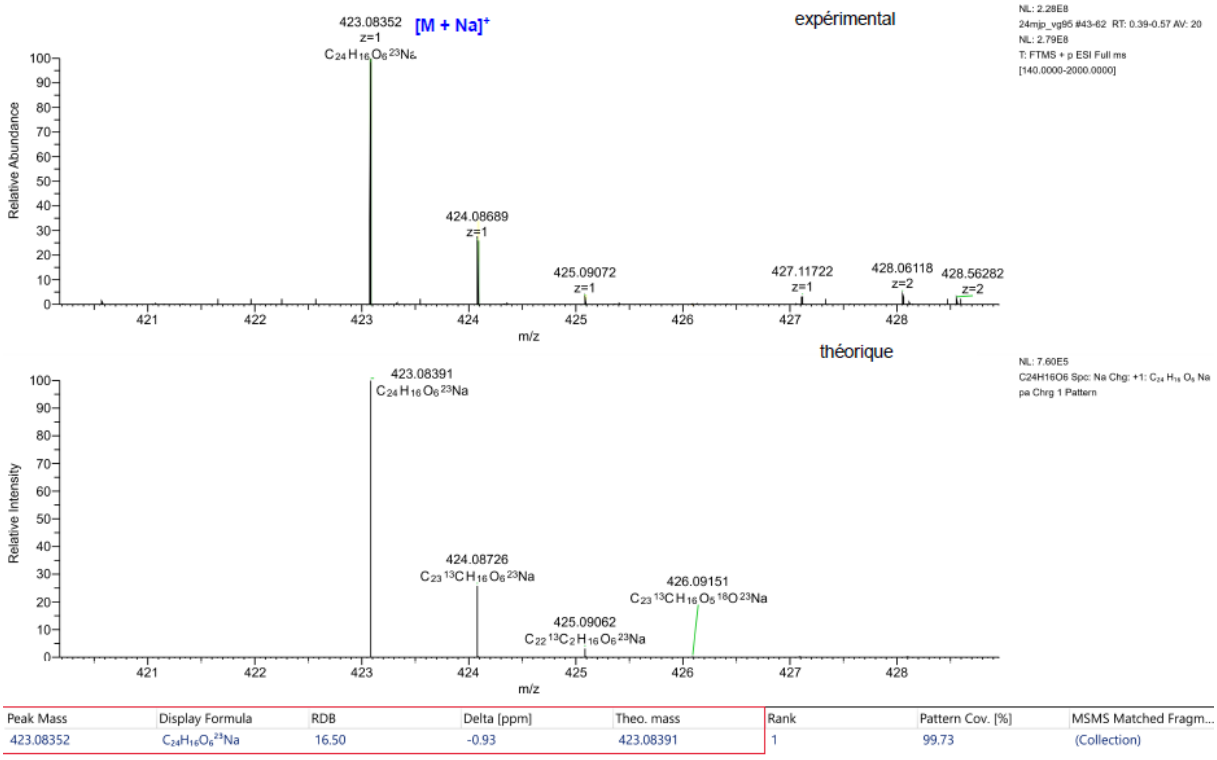


ESI+ mass spectrum (high resolution) of 4



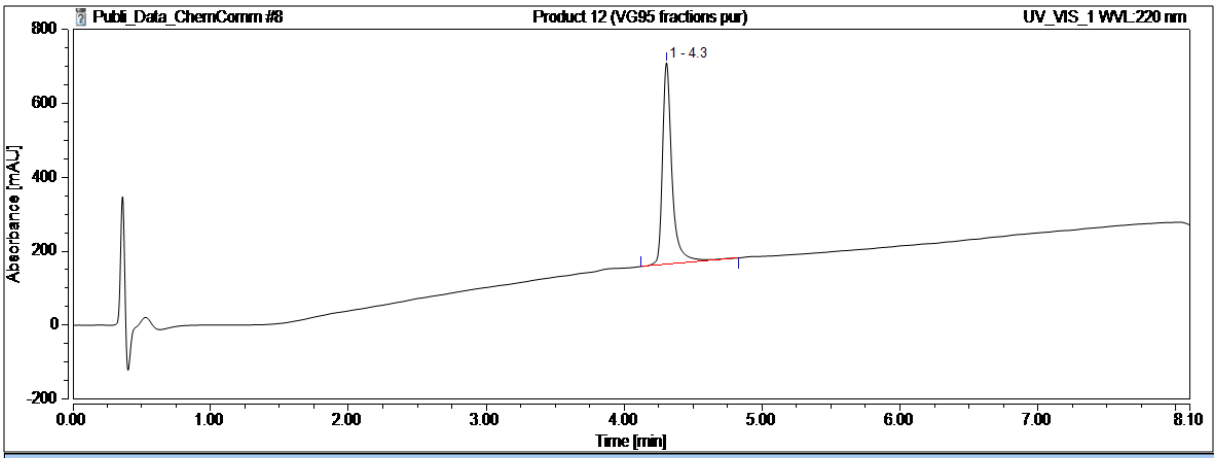


Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]	MSMS Matched Fragm...
401.10166	$C_{24}H_{17}O_6$	16.50	-0.75	401.10196	1	99.53	(Collection)



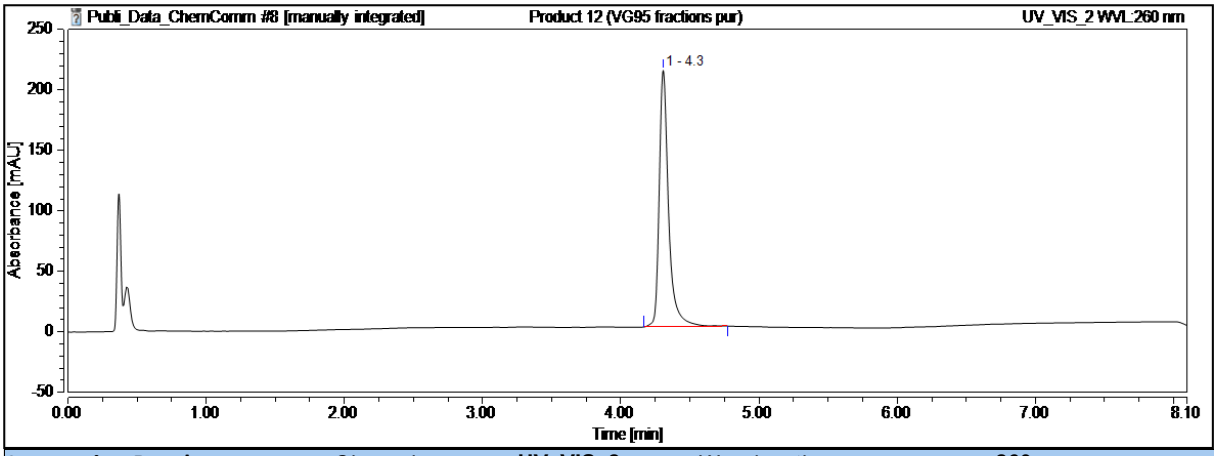
Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]	MSMS Matched Fragm...
423.08352	$C_{24}H_{16}O_6{}^{23}Na$	16.50	-0.93	423.08391	1	99.73	(Collection)

RP-HPLC elution profile of 4 (system A, detection at 220 nm)



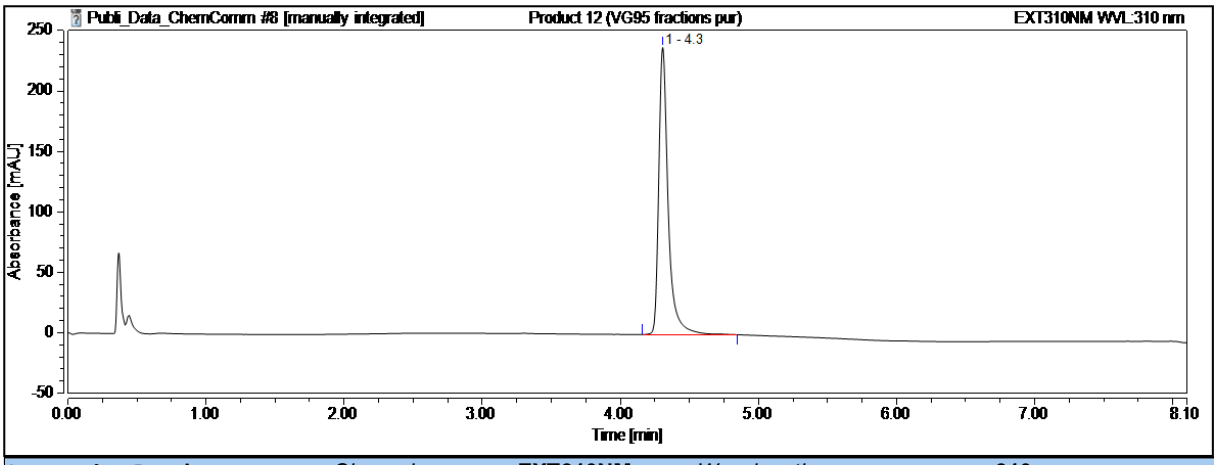
Integration Results	Channel:	UV_VIS_1	Wavelength:	220.0	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.31	167.3	43.518	545.762	100.00	100.00
Total:			**43.518**	**545.762**	**100.00**	**100.00**

RP-HPLC elution profile of 4 (system A, detection at 260 nm)



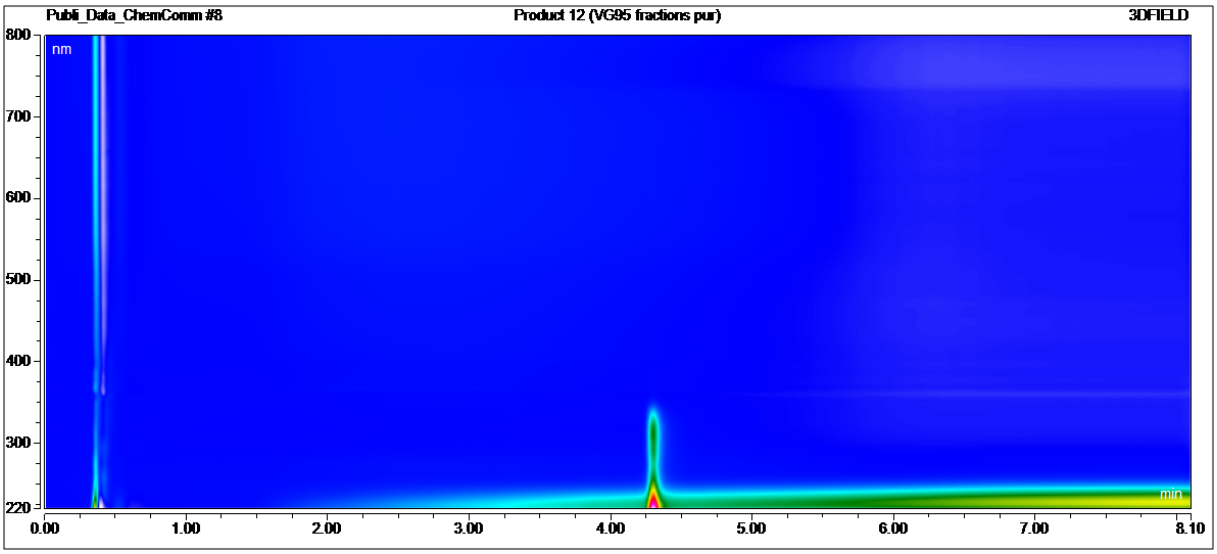
Integration Results	Channel:	UV_VIS_2	Wavelength:	260	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.31	433.3	16.652	212.080	100.00	100.00
Total:			**16.652**	**212.080**	**100.00**	**100.00**

RP-HPLC elution profile of 4 (system A, detection at 310 nm)

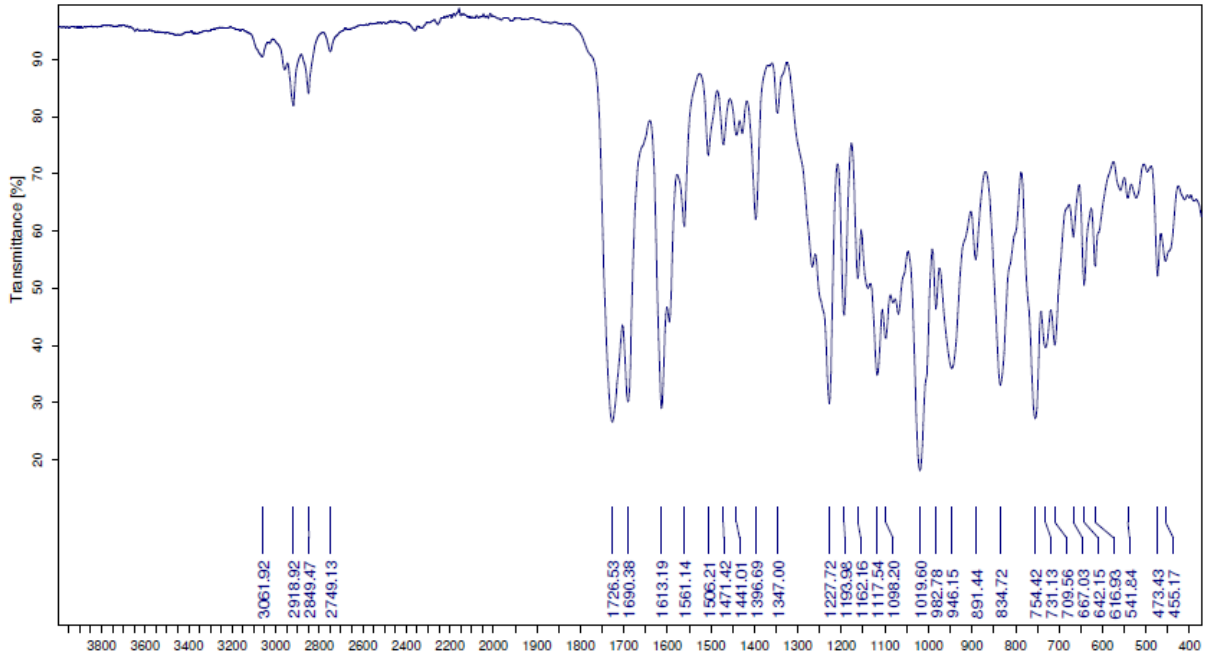


Integration Results	*Channel:*	**EXT310NM**	*Wavelength:*	**310**	
No.	Retention min	S/N	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1	4.31	751.8	18.824	237.545	100.00	100.00
Total:			**18.824**	**237.545**	**100.00**	**100.00**

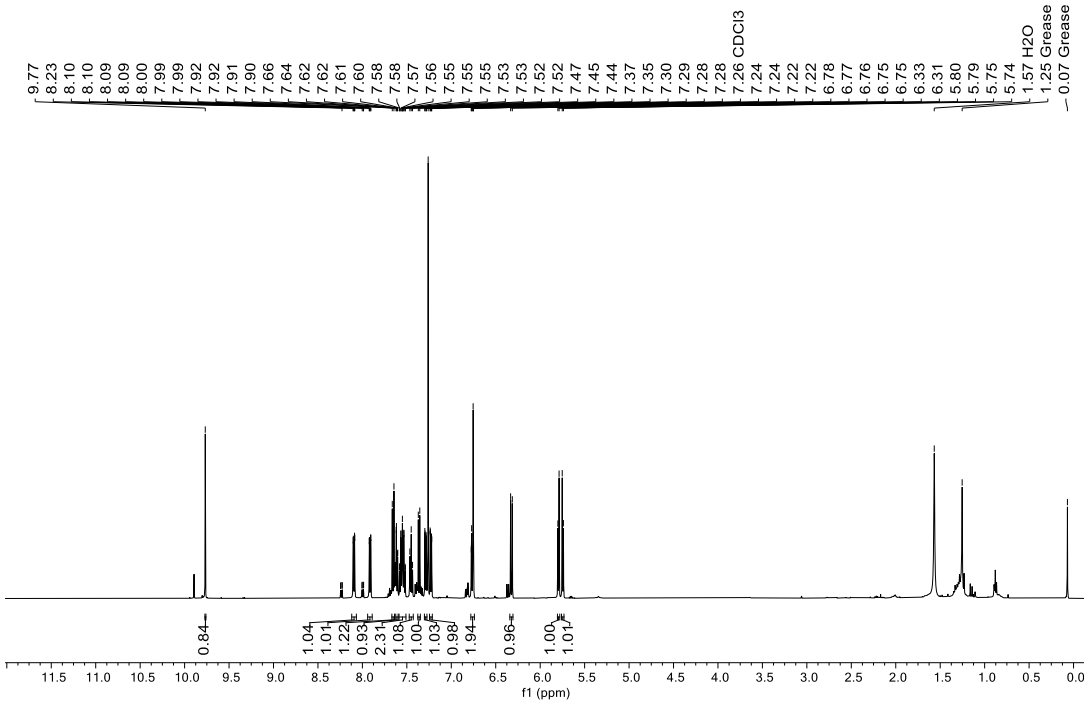
2D map of DAD of 4 (system A)



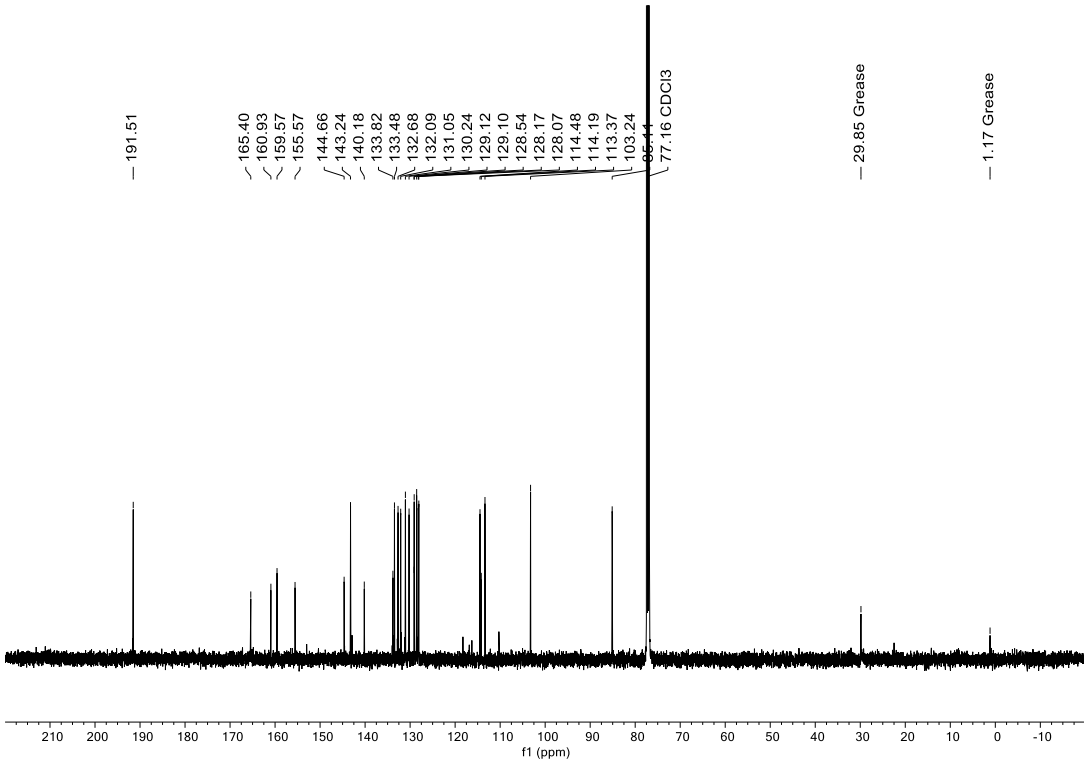
IR-ATR spectrum of 4



^{1}H NMR spectrum of 4 in $CDCl_3$ (500 MHz)

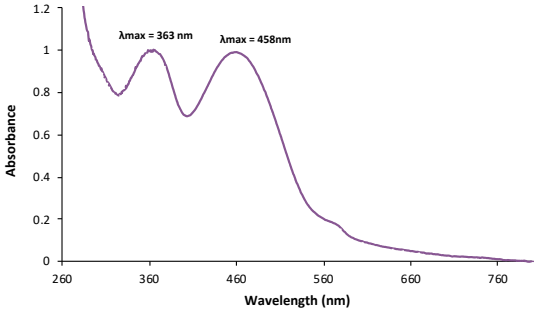


^{13}C NMR spectrum of in 4 $CDCl_3$ (126 MHz)



S4 UV-vis absorption spectrum (left) & molar extinction coefficient determination (right) of cyanide-responsive fluorogenic probes

Fig. S1 Resorufin-based probe IND-1



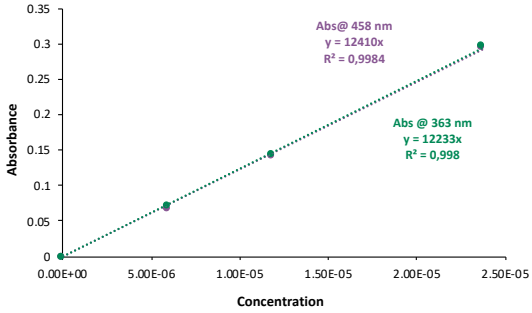
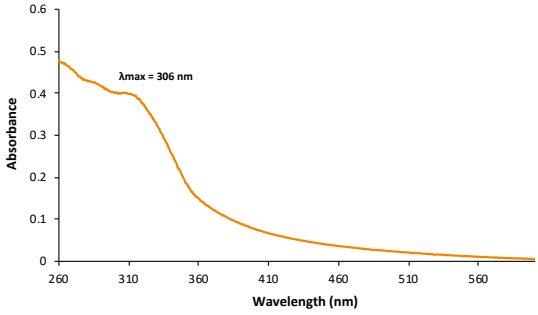


Fig. S2 Umbelliferone-based probe 1



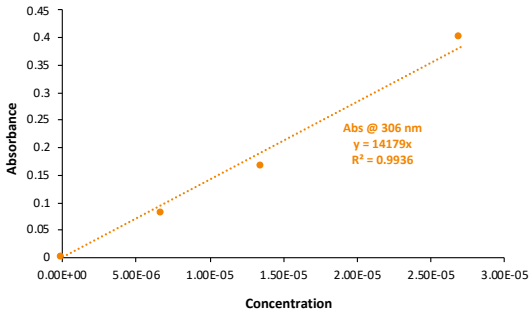
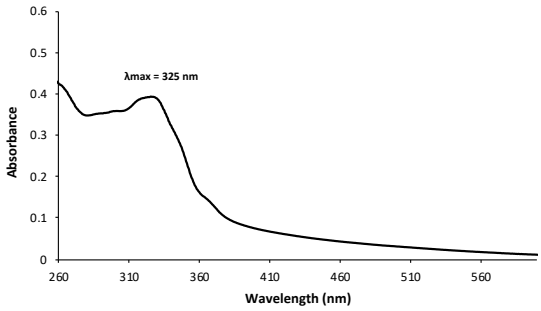


Fig. S3 7-Amino-4-methylcoumarin-based probe 2



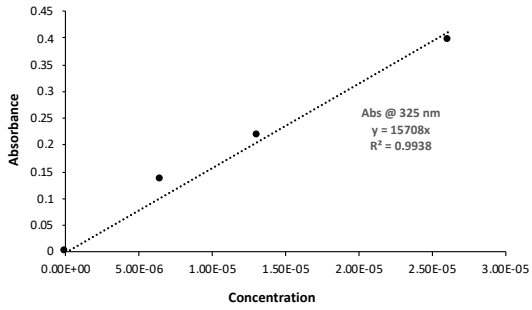


Fig. S4 Three-component umbelliferone-based probe 3

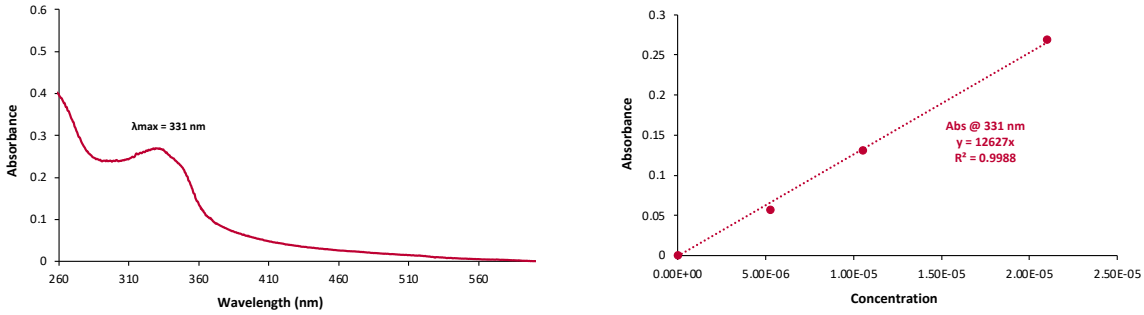
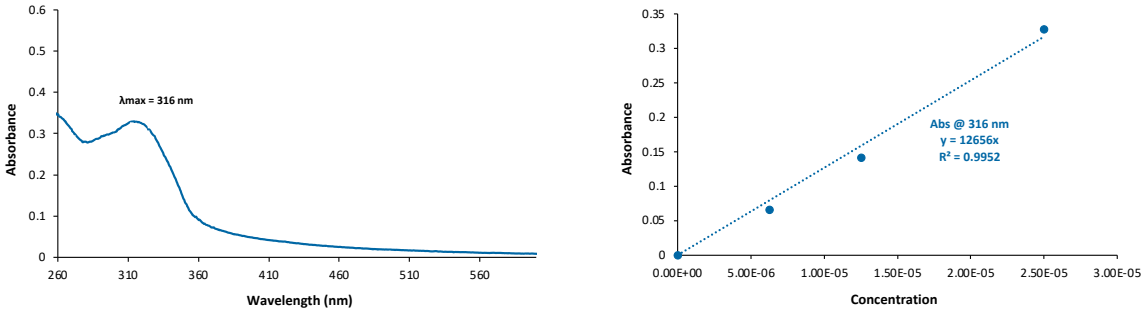
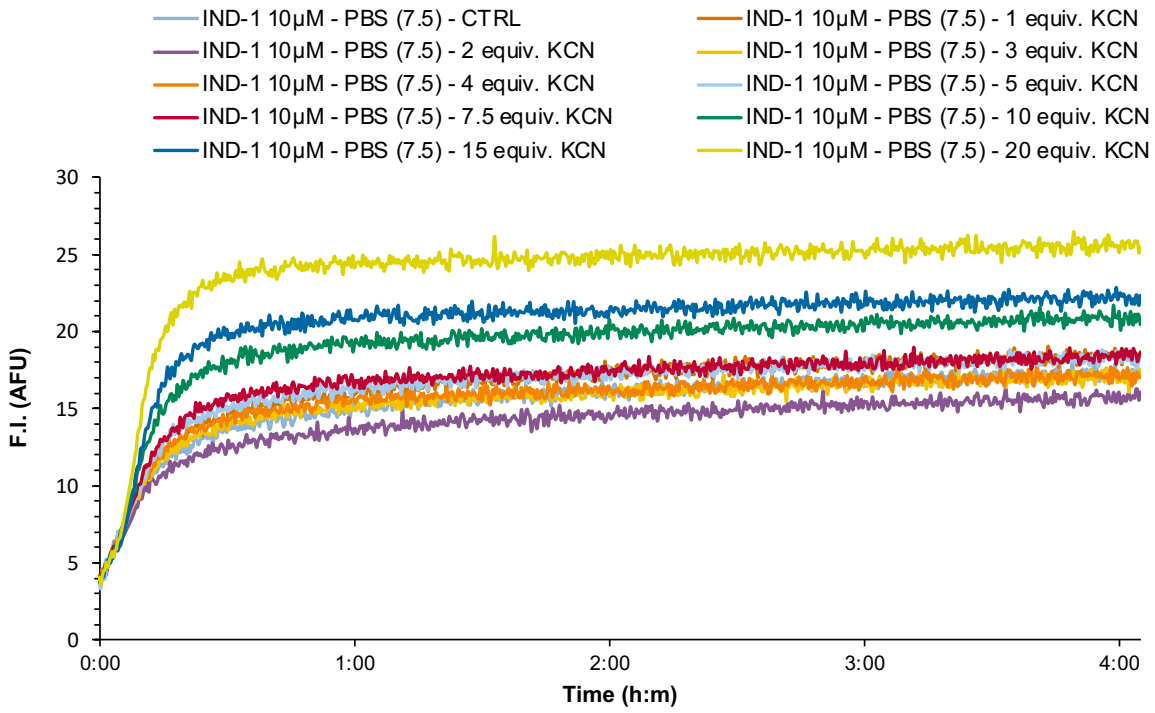


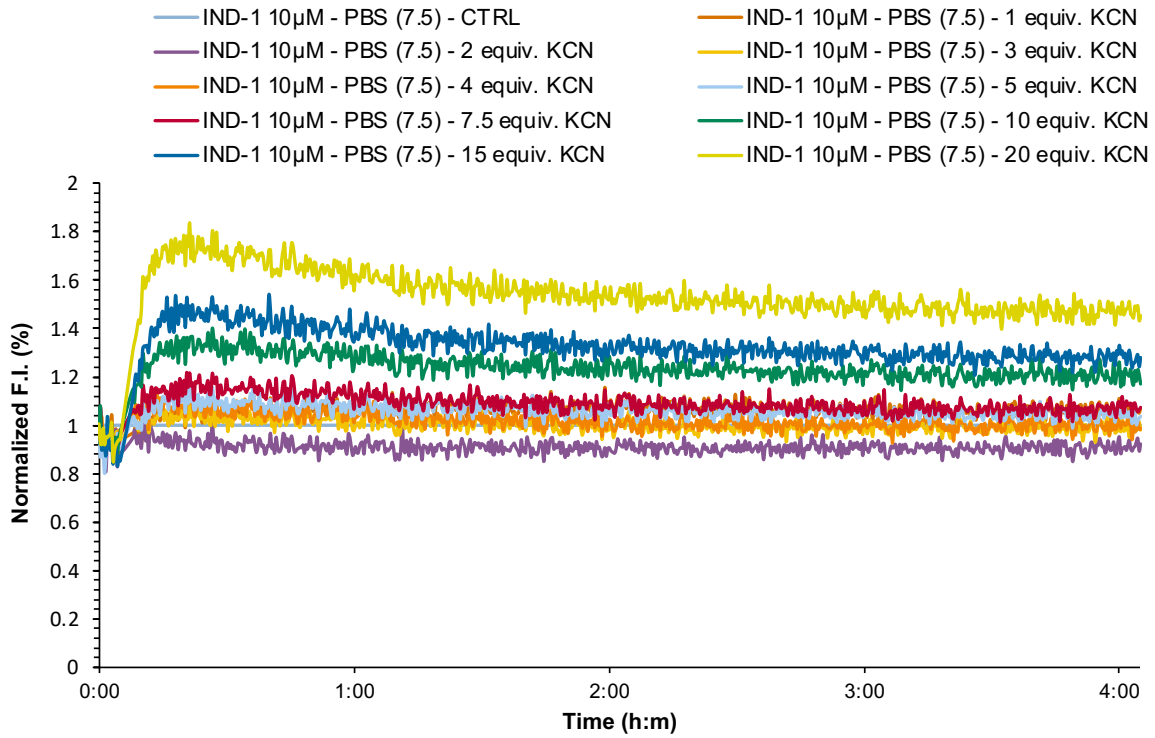
Fig. S5 Three-component umbelliferone-based probe 4



S5 Fluorescence-based assays

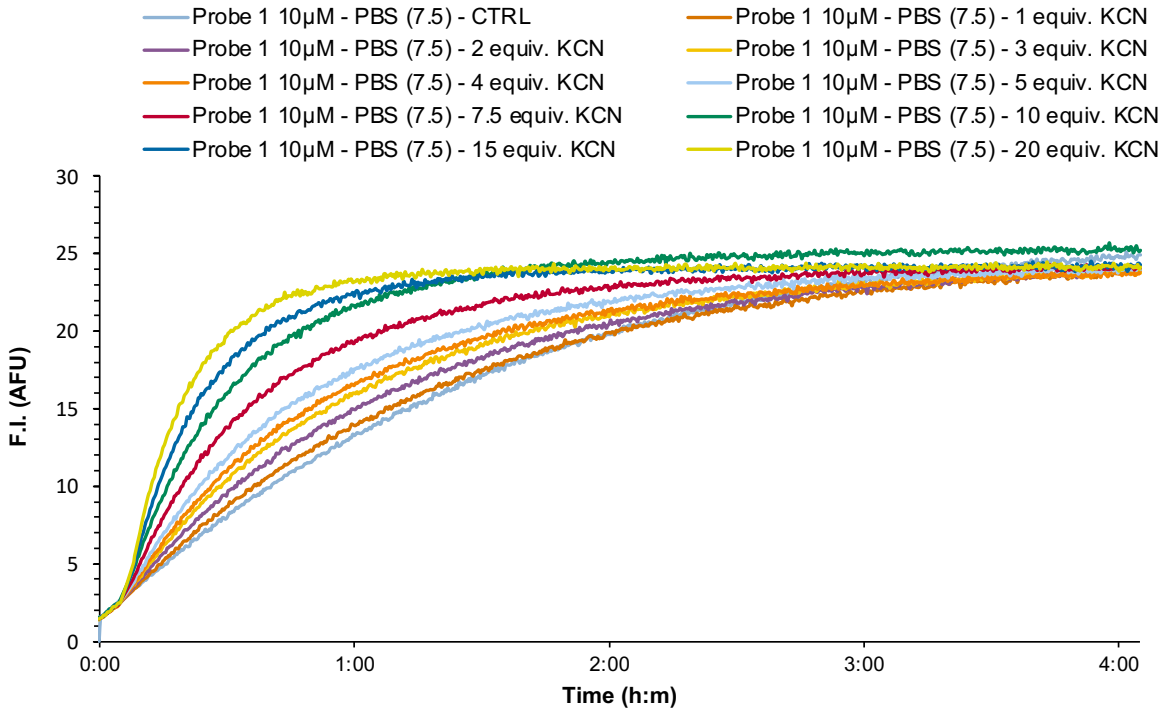
Fig. S6 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probe IND-1 (concentration: 10 μM)

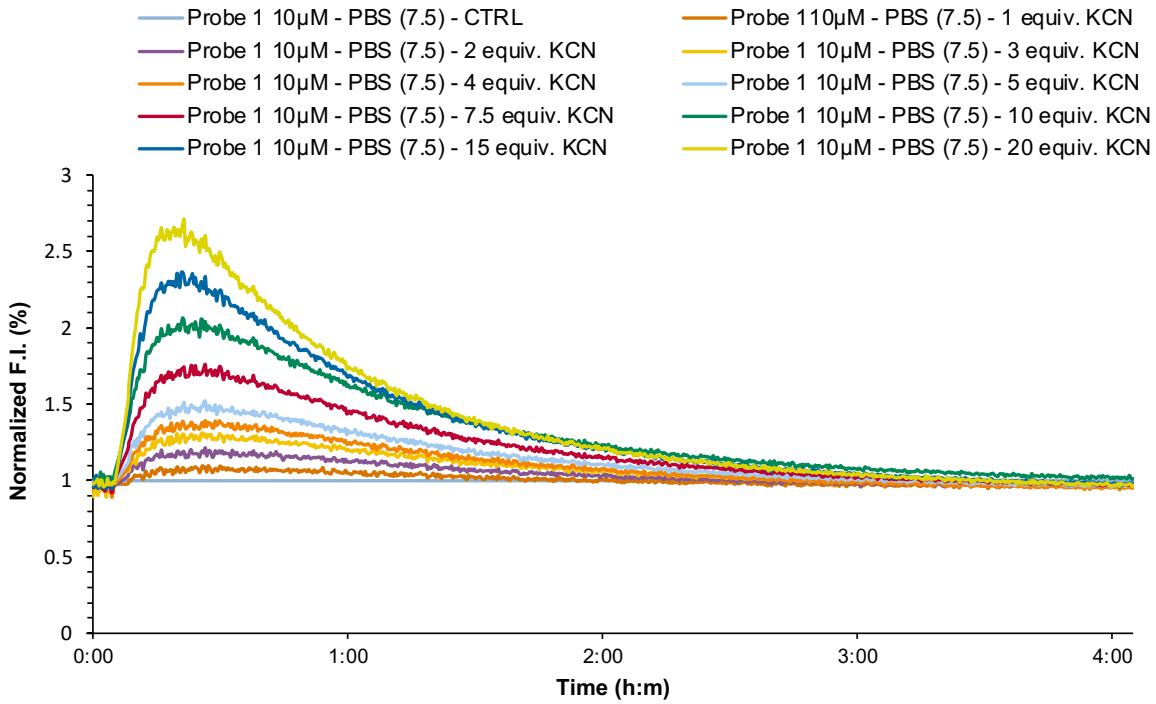




Please note: data are normalized to the control condition.

Fig. S7 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probe 1 (concentration: 10 µM)





Please note: data are normalized to the control condition.

Fig. S8 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probe 2 (concentration: 10 µM)

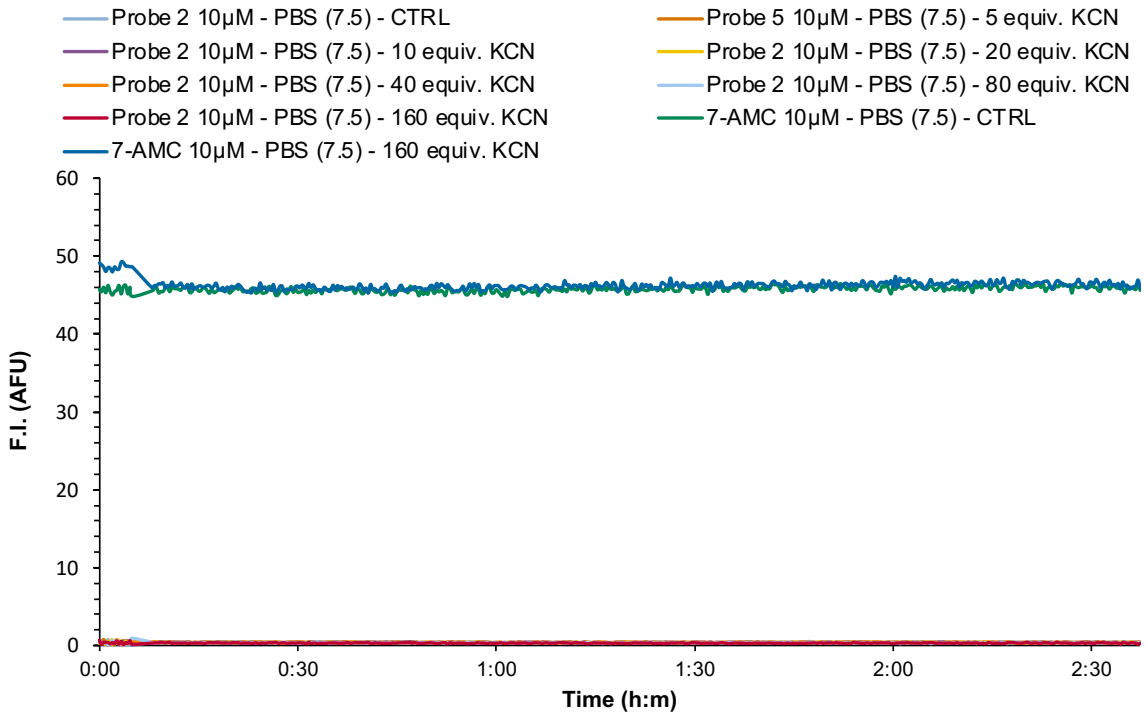
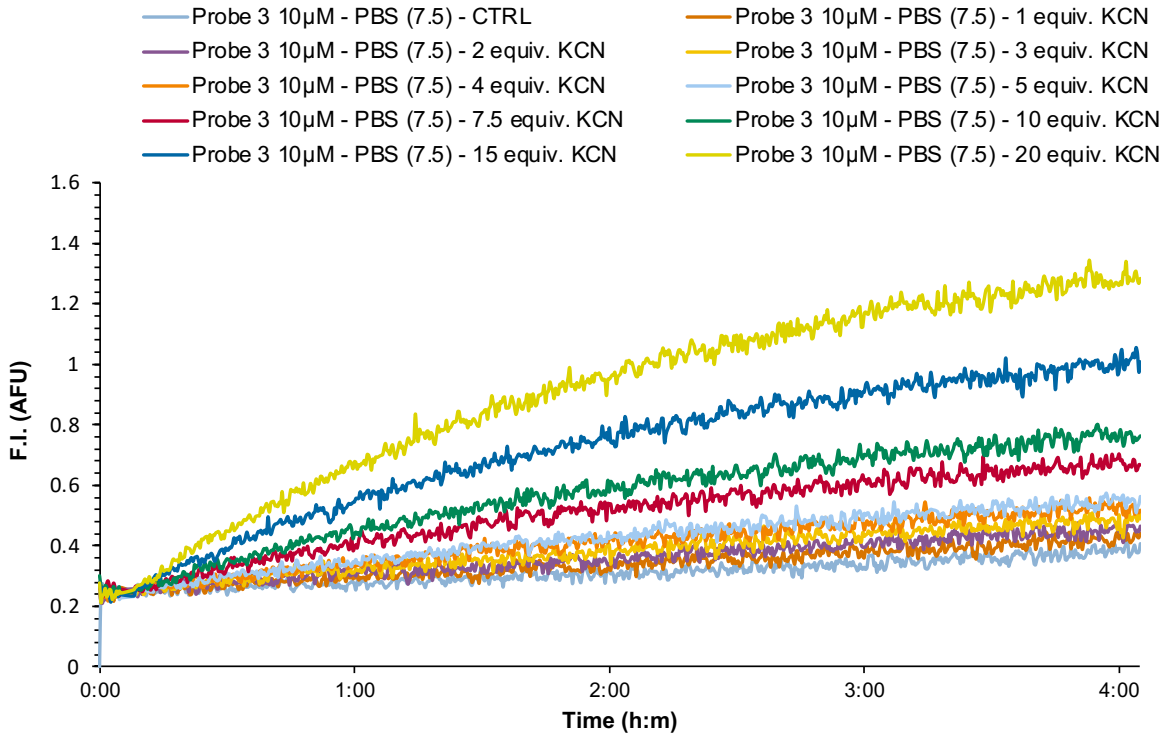
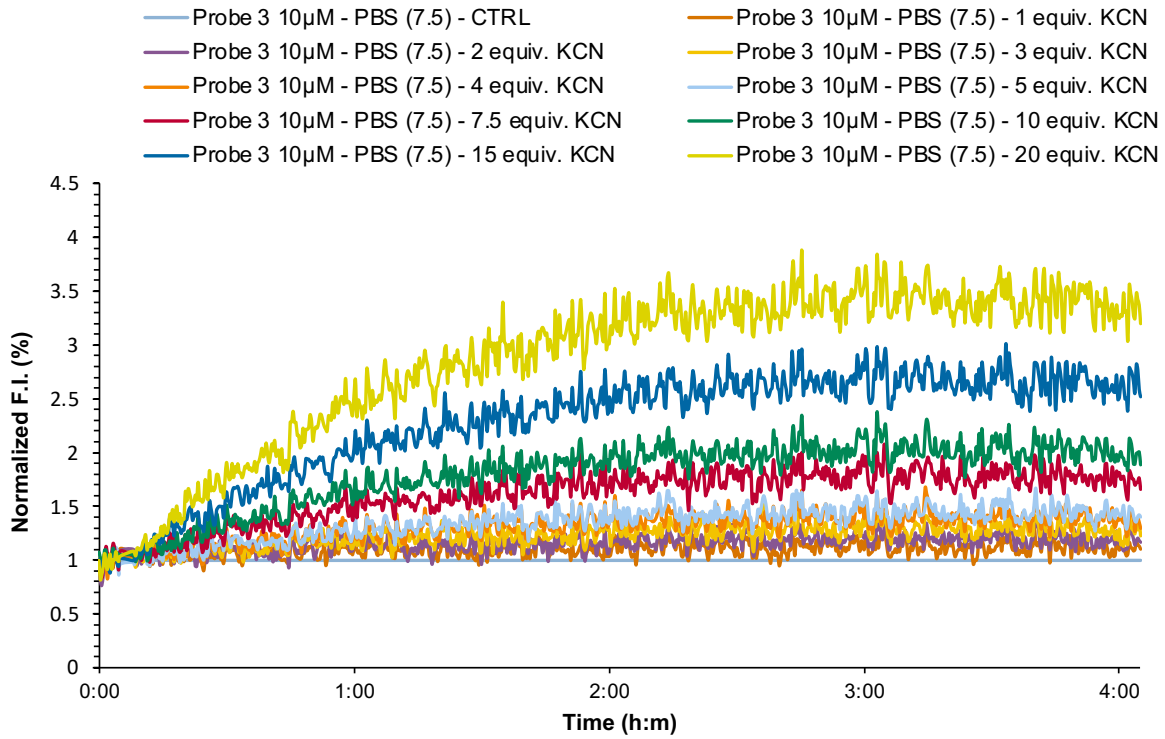


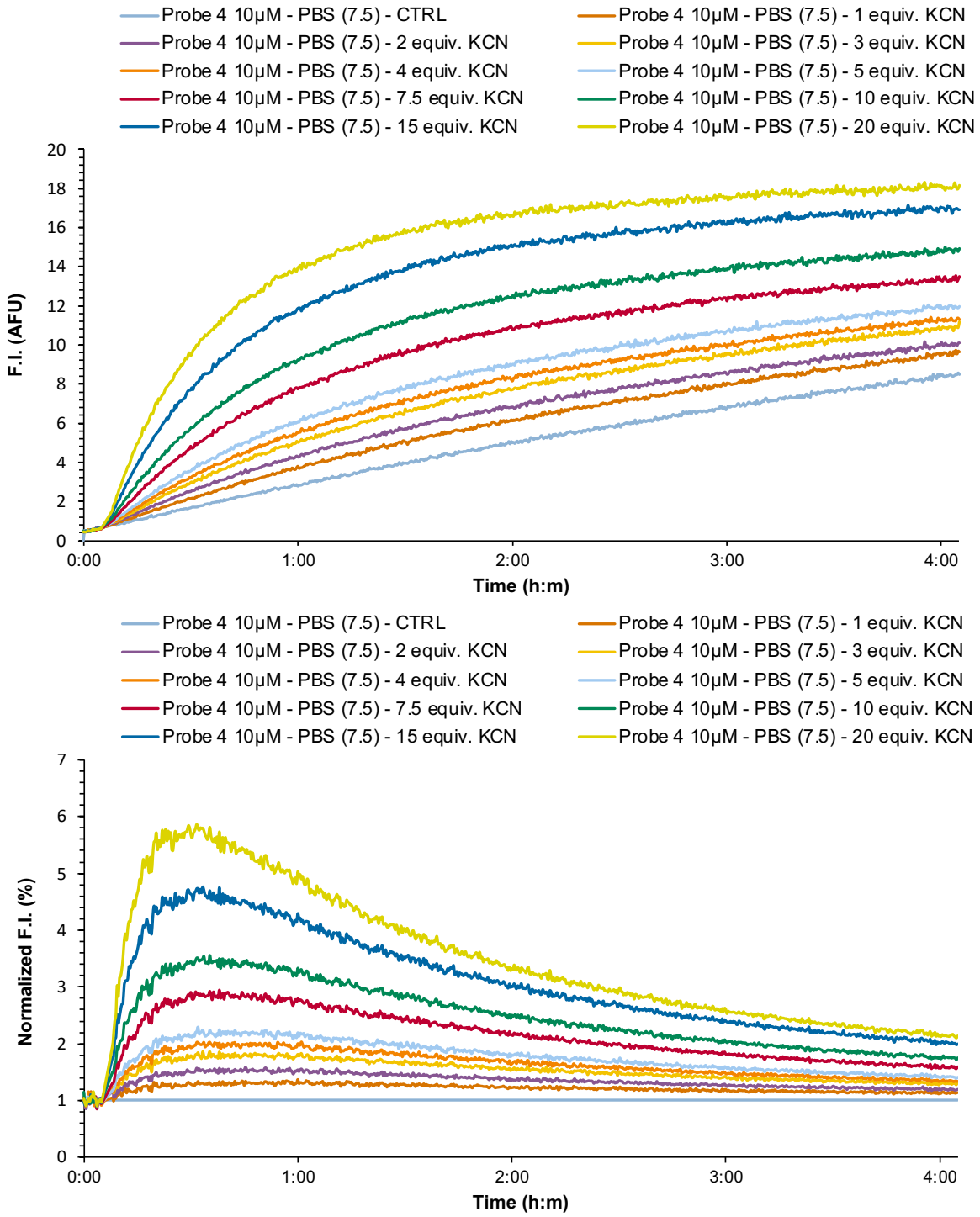
Fig. S9 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probe 3 (concentration: 10 μM)





Please note: data are normalized to the control condition.

Fig. S10 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probe 4 (concentration: 10 μM)



Please note: data are normalized to the control condition.

Fig. S11 Fluorescence intensity vs. concentration of resorufin (sodium salt) in PBS (pH 7.5) at 25 °C

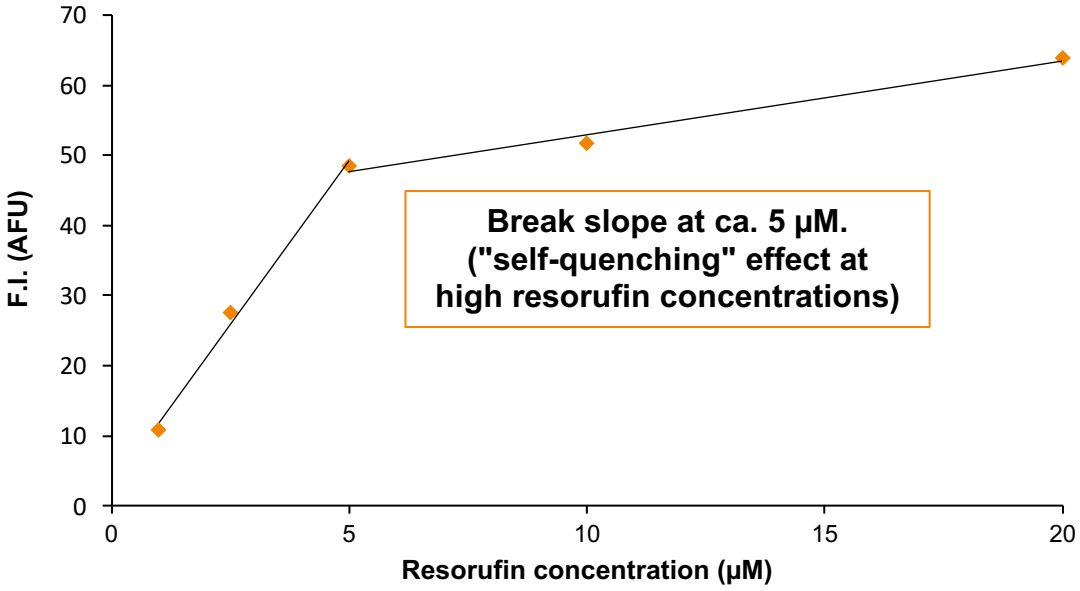


Fig. S12 Fluorescence emission time-course assay of resorufin-based probe IND-1 - Influence of probe concentration (1.0 μM (top) and 10 μM (bottom))

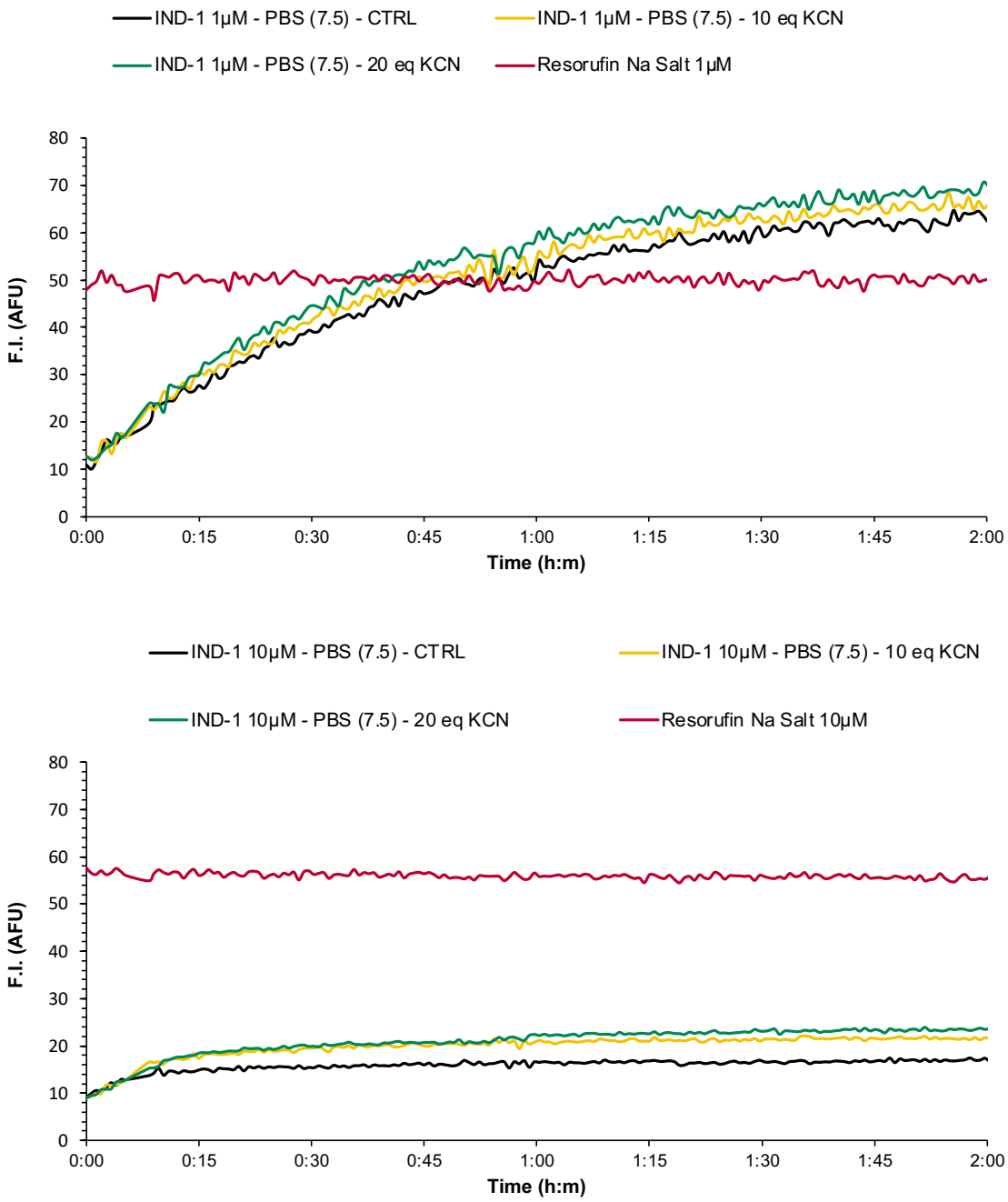
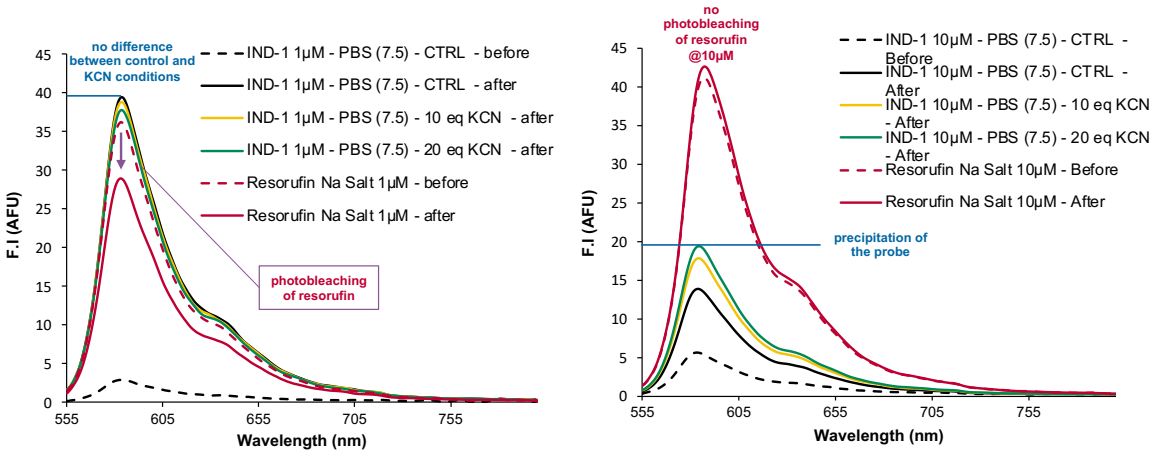


Fig. S13 Overlay of fluorescence emission spectra (Ex at 540 nm, bandwidths 5/5 nm) recorded before and after time-course assays (1.0 μM (left) and 10 μM (right))



Picture of 10 μM solution of **IND-1** in PBS (pH 7.5) - visualisation of precipitation:

S6 Determination of the limit of detection (LOD)

Hartley test: table of critical value for $\alpha = 0.05$

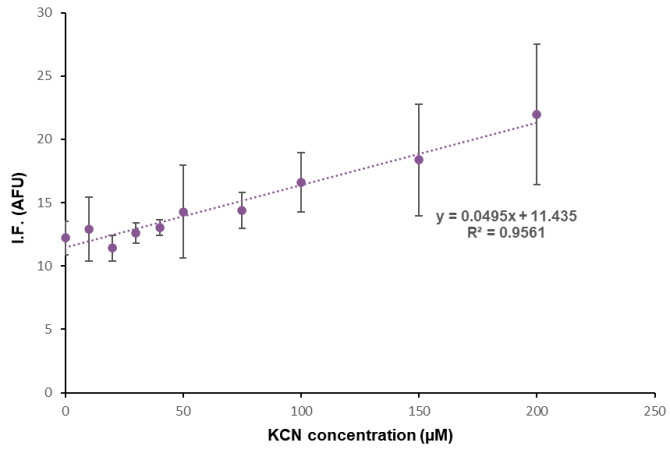
Level of significance $\alpha = 0.05$

	k									
n - 1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
2	39.0	87.5	142	202	266	333	403	475	550	626	704
3	15.4	27.8	39.2	50.7	62.0	72.9	83.5	93.9	104	114	124
4	9.6	15.5	20.6	25.2	29.5	33.6	37.5	41.1	44.6	48.0	51.4
5	7.15	10.8	13.7	16.3	18.7	20.8	22.9	24.7	26.5	28.2	29.9
6	5.82	8.38	10.4	12.1	13.7	15.0	16.3	17.5	18.6	19.7	20.7
7	4.99	6.94	8.44	9.70	10.8	11.8	12.7	13.5	14.3	15.1	15.8
8	4.43	6.00	7.18	8.12	9.03	9.78	10.5	11.1	11.7	12.2	12.7
9	4.03	5.34	6.31	7.11	7.80	8.41	8.95	9.45	9.91	10.3	10.7
10	3.72	4.85	5.67	6.34	6.92	7.42	7.87	8.28	8.66	9.01	9.34
12	3.28	4.16	4.79	5.30	5.72	6.09	6.42	6.72	7.00	7.25	7.48
15	2.86	3.54	4.01	4.37	4.68	4.95	5.19	5.40	5.59	5.77	5.93
20	2.46	2.95	3.29	3.54	3.76	3.94	4.10	4.24	4.37	4.49	4.59
30	2.07	2.40	2.61	2.78	2.91	3.02	3.12	3.21	3.29	3.36	3.39
60	1.67	1.85	1.96	2.04	2.11	2.17	2.22	2.26	2.30	2.33	2.36
∞	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Kanji, Gopal K. **100 Statistical Tests**. London : SAGE Publication Ltd., 1993.

Fig. S14 LOD of resorufin-based probe IND-1

Concentration (μM)	0	10	20	30	40	50	75	100	150	200
I.F. (AFU)	12.2114667	12.9091667	11.4056	12.5867	13.0334	14.2957333	14.3709667	16.6094667	18.3723667	21.9524333
STD DEV	1.32264695	2.5453451	1.03741864	0.80661895	0.62221442	3.65720994	1.40366117	2.32290344	4.39128959	5.55111108
VAR	1.74939494	6.47878166	1.07623744	0.65063413	0.38715079	13.3751845	1.97026469	5.39588037	19.2834243	30.8148342
Var max	30.8148342				n-1=2	k=10	alpha=0.05			
Var min	0.38715079		Fmax	79.5938819	Critical value	550		Variance is homogeneous		
					Fmax<Crit Val			No correlation		

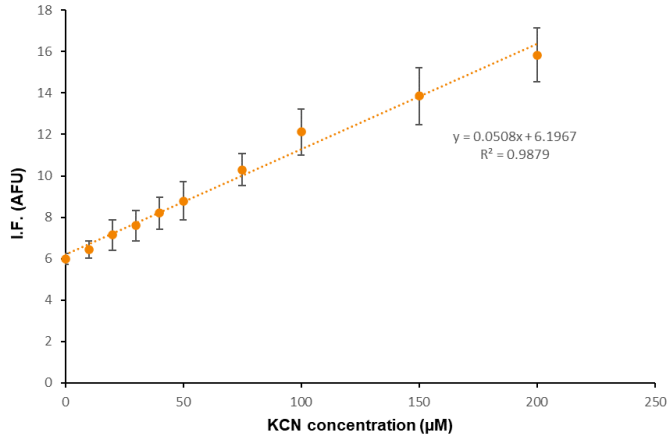


	a	b	
Value	0.0495	11.4353	
std dev	0.0037	0.3428	
r^2	0.9561	0.7313	S(y.x)

LOD (S(y.x))	48.8	μM
LOD (intercept)	22.9	μM

Fig. S15 LOD of umbelliferone-based probe 1

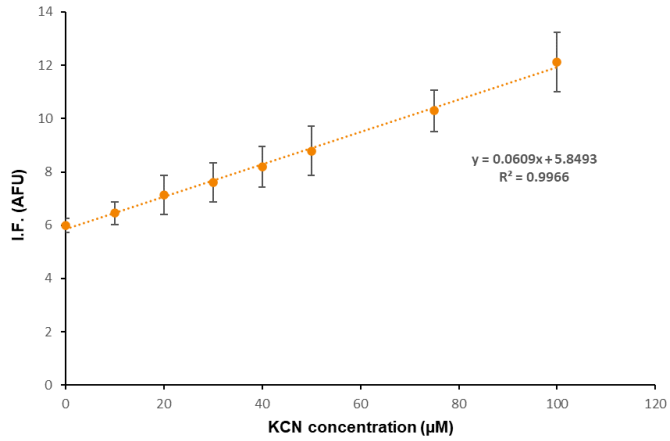
Concentration (μM)	0	10	20	30	40	50	75	100	150	200
I.F. (AFU)	5.98487667	6.44958667	7.14625667	7.59863333	8.19216667	8.79051	10.29284	12.1258	13.8475333	15.8404333
STD DEV	0.26874762	0.41669372	0.73514522	0.73890733	0.75687005	0.92405396	0.77162378	1.10972167	1.3779254	1.30217072
VAR	0.07222528	0.17363366	0.54043849	0.54598404	0.57285227	0.85387573	0.59540325	1.23148219	1.89867841	1.69564857
Var max	1.89867841				n-1=2	k=10	alpha=0.05			
Var min	0.07222528		Fmax	26.2882795	Critical value	550		Variance is homogeneous		
					Fmax<Crit Val			No Correlation for 0-200μM		



	a	b	
Value	0.0508	6.1967	
std dev	0.0020	0.1818	
r²	0.9879	0.3879	S(y.x)

LOD (S(y.x))	25.2	μM
LOD (intercept)	11.8	μM

Concentration (μM)	0	10	20	30	40	50	75	100	150	200
I.F. (AFU)	5.98487667	6.44958667	7.14625667	7.59863333	8.19216667	8.79051	10.29284	12.1258	13.8475333	15.8404333
STD DEV	0.26874762	0.41669372	0.73514522	0.73890733	0.75687005	0.92405396	0.77162378	1.10972167	1.3779254	1.30217072
VAR	0.07222528	0.17363366	0.54043849	0.54598404	0.57285227	0.85387573	0.59540325	1.23148219	1.89867841	1.69564857
Var max	1.23148219				n-1=2	k=8	alpha=0.05			
Var min	0.07222528		Fmax	17.0505693	Critical value	403		Variance is homogeneous		
					Fmax<Crit Val			Correlation for 0-100μM		



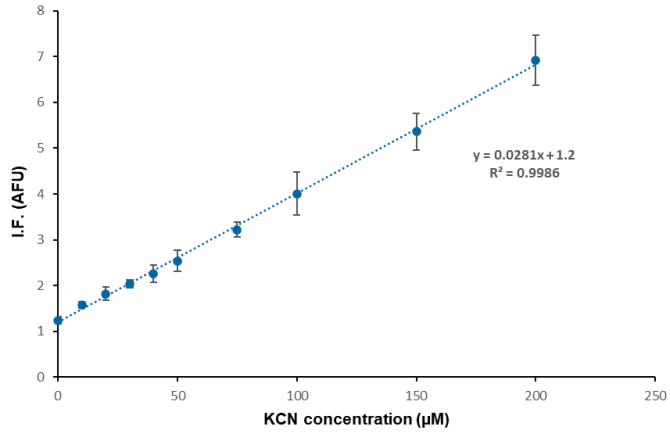
	a	b	
Value	0.0609	5.8493	
std dev	0.0015	0.0747	
r²	0.9966	0.1293	S(y.x)

LOD (S(y.x))	7.0	μM
LOD (intercept)	4.0	μM

Please note: we excluded the larger concentration to plot the calibration function.

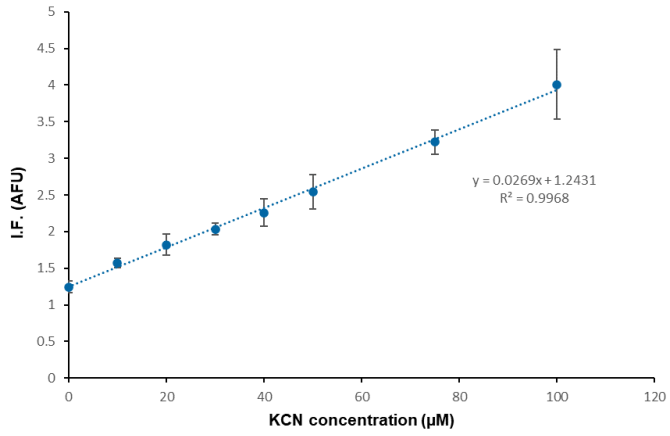
Fig. S16 LOD of three-component umbelliferone-based probe 4

Concentration (µM)	0	10	20	30	40	50	75	100	150	200
I.F. (AFU)	1.24078667	1.57343333	1.82027667	2.03361	2.25919667	2.539283333	3.22172	4.00649	5.36439	6.91849667
STD DEV	0.07996727	0.06369051	0.14414151	0.08167952	0.18766116	0.236534147	0.16931231	0.4738293	0.40171395	0.54964522
VAR	0.00639476	0.00405648	0.02077677	0.00667154	0.03521671	0.055948403	0.02866666	0.2245142	0.1613741	0.30210987
Var max	0.30210987				n-1=2	k=10	alpha=0.05			
Var min	0.00405648		Fmax	74.475842	Critical value	550		Variance is homogeneous		
					Fmax<Crit Val			Correlation for 0-200µM		



	a	b	
Value	0.0281	1.2000	
std dev	0.0004	0.0339	
r²	0.9986	0.0723	S(y.x)
LOD (S(y.x))	8.5	µM	
LOD (intercept)	4.0	µM	

Concentration (µM)	0	10	20	30	40	50	75	100	150	200
I.F. (AFU)	1.24078667	1.57343333	1.82027667	2.03361	2.25919667	2.539283333	3.22172	4.00649	5.36439	6.91849667
STD DEV	0.07996727	0.06369051	0.14414151	0.08167952	0.18766116	0.236534147	0.16931231	0.4738293	0.40171395	0.54964522
VAR	0.00639476	0.00405648	0.02077677	0.00667154	0.03521671	0.055948403	0.02866666	0.2245142	0.1613741	0.30210987
Var max	0.2245142				n-1=2	k=8	alpha=0.05			
Var min	0.00405648		Fmax	55.3470304	Critical value	403		Variance is homogeneous		
					Fmax<Crit Val			Correlation for 0-100µM		



	a	b	
Value	0.0269	1.2431	
std dev	0.0006	0.0320	
r²	0.9968	0.0554	S(y.x)
LOD (S(y.x))	6.8	µM	
LOD (intercept)	3.9	µM	

Please note: we excluded the larger concentration to plot the calibration function.

S7 Study of activation mechanism (for fluorogenic intramolecular crossed-benzoin reaction) based on RP-HPLC-MS analyses

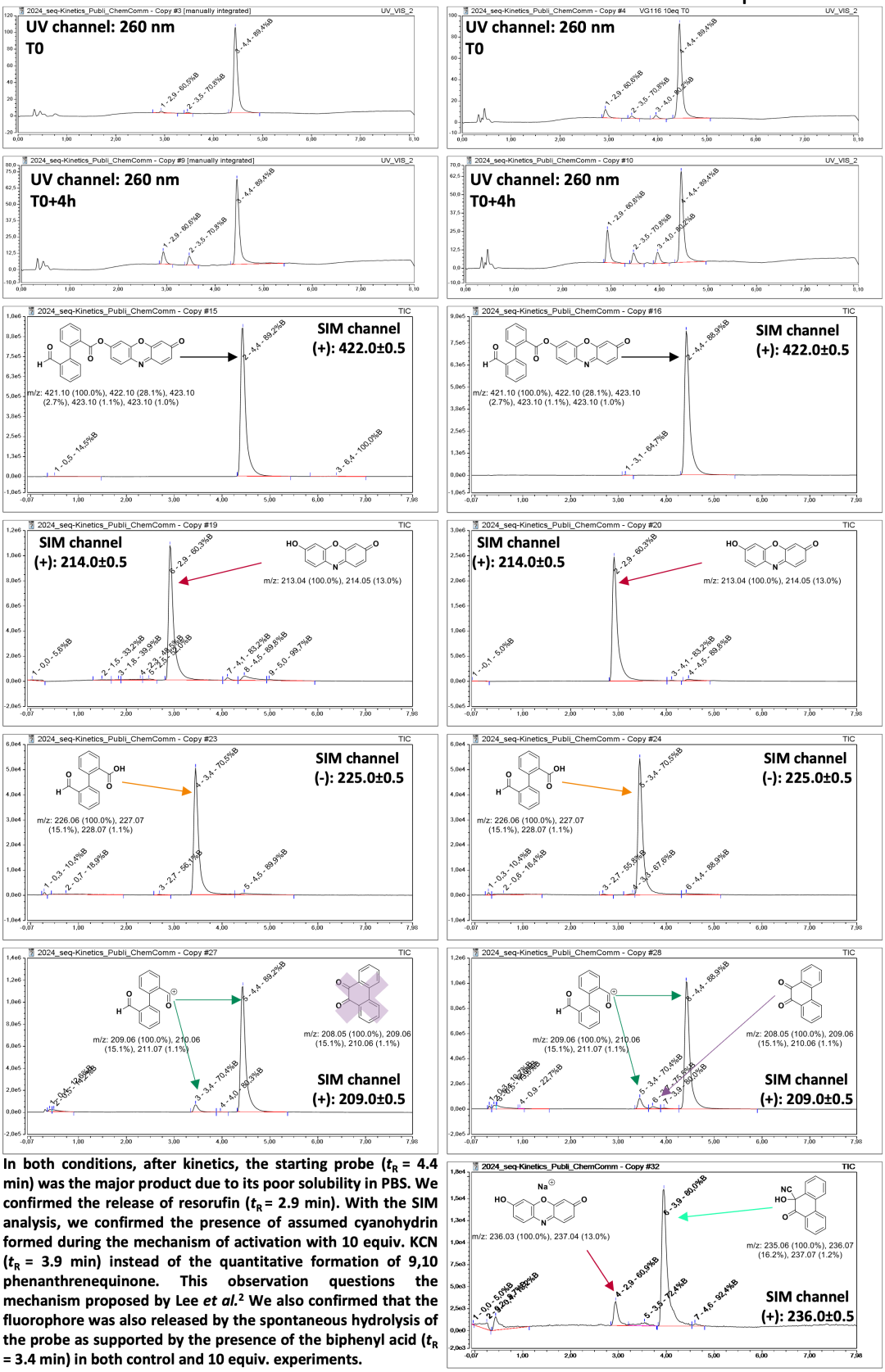
Fig. S17 RP-HPLC-MS analyses (system A'') of cyanide-mediated activation of fluorogenic probes IND-1 and 1 (concentration: 10 µM)

2 mL HPLC vials were independently prepared using stock solutions of probes **3** and **4** to reach a final concentration of 10 µM in PBS (pH 7.5) +1% DMSO (v/v), volume = 1 mL. RP-HPLC

elution profiles with full scan mass detection were obtained using system A. RP-HPLC elution profiles with SIM mass detection were obtained using system A''.

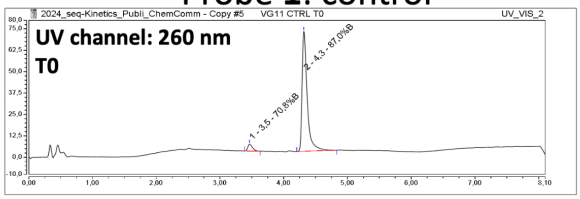
Probe **IND-1**: control

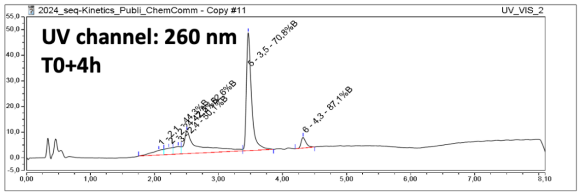
Probe **IND-1**: 10 equiv. KCN

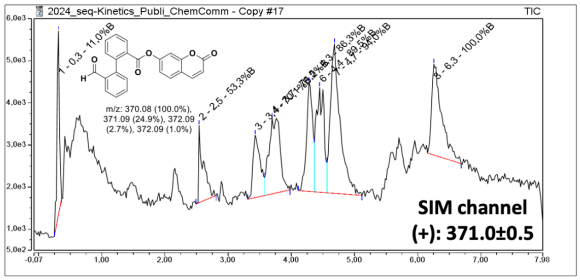


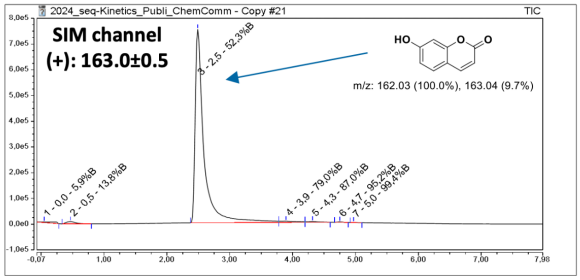
In both conditions, after kinetics, the starting probe (t_R = 4.4 min) was the major product due to its poor solubility in PBS. We confirmed the release of resorufin (t_R = 2.9 min). With the SIM analysis, we confirmed the presence of assumed cyanohydrin formed during the mechanism of activation with 10 equiv. KCN (t_R = 3.9 min) instead of the quantitative formation of 9,10 phenanthrenequinone. This observation questions the mechanism proposed by Lee *et al.*[2] We also confirmed that the fluorophore was also released by the spontaneous hydrolysis of the probe as supported by the presence of the biphenyl acid (t_R = 3.4 min) in both control and 10 equiv. experiments.

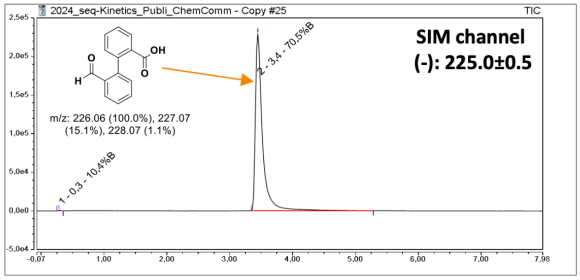
Probe **1**: control

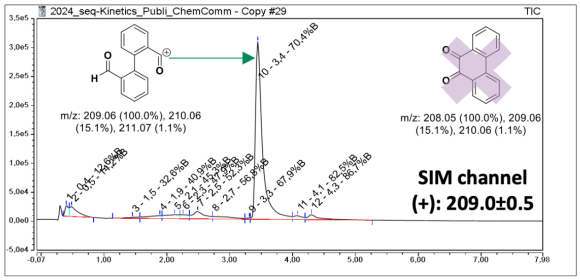




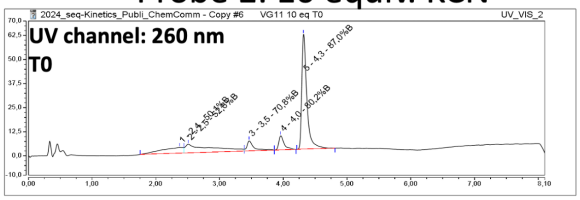


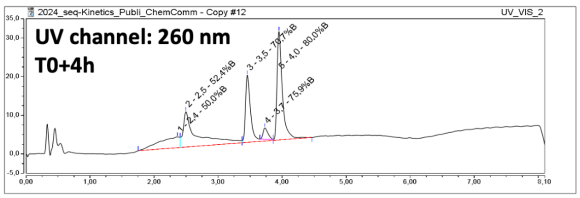


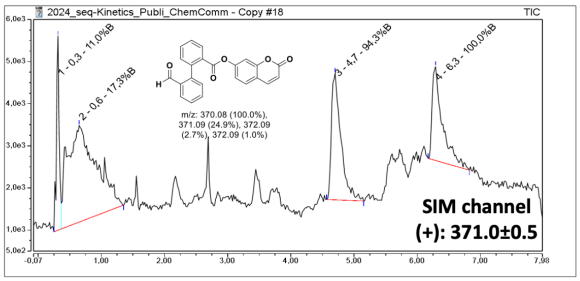


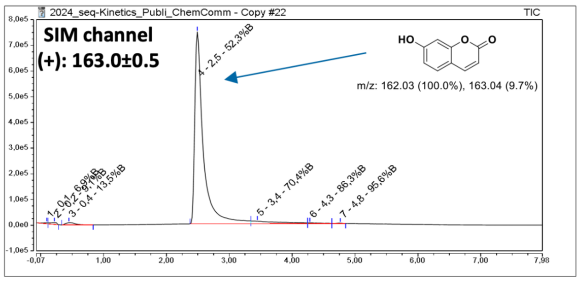


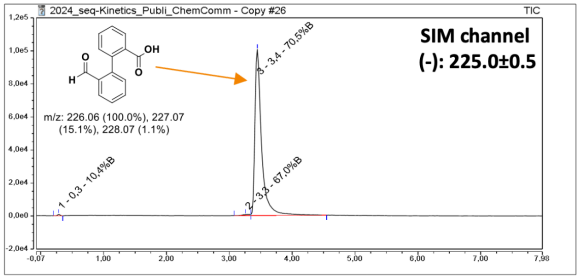
Probe **1**: 10 equiv. KCN

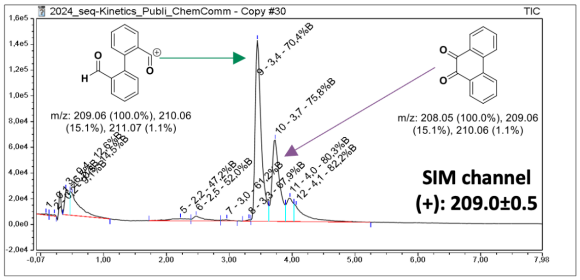












In both condition, after kinetics, the starting probe (t_R = 4.4 min) is fully consume (total hydrolysis in control condition) We confirm the liberation of the umbelliferone (t_R = 2.5 min) in both conditions. With the SIM analysis, we confirm the presence of a supposed cyanohydrin formed during the mechanism of activation with 10 equiv. KCN (t_R = 3.9 min). We also observe the liberation of the fluorophore due to the spontaneous hydrolysis by the presence of the biphenyl acid (t_R = 3.4 min) with 10 equiv. of KCN.

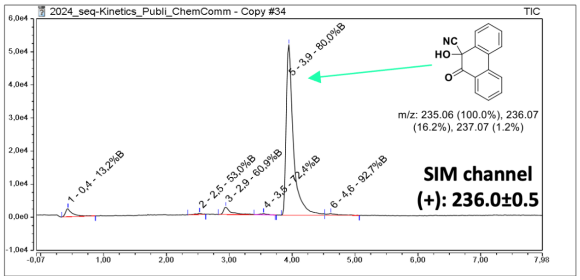
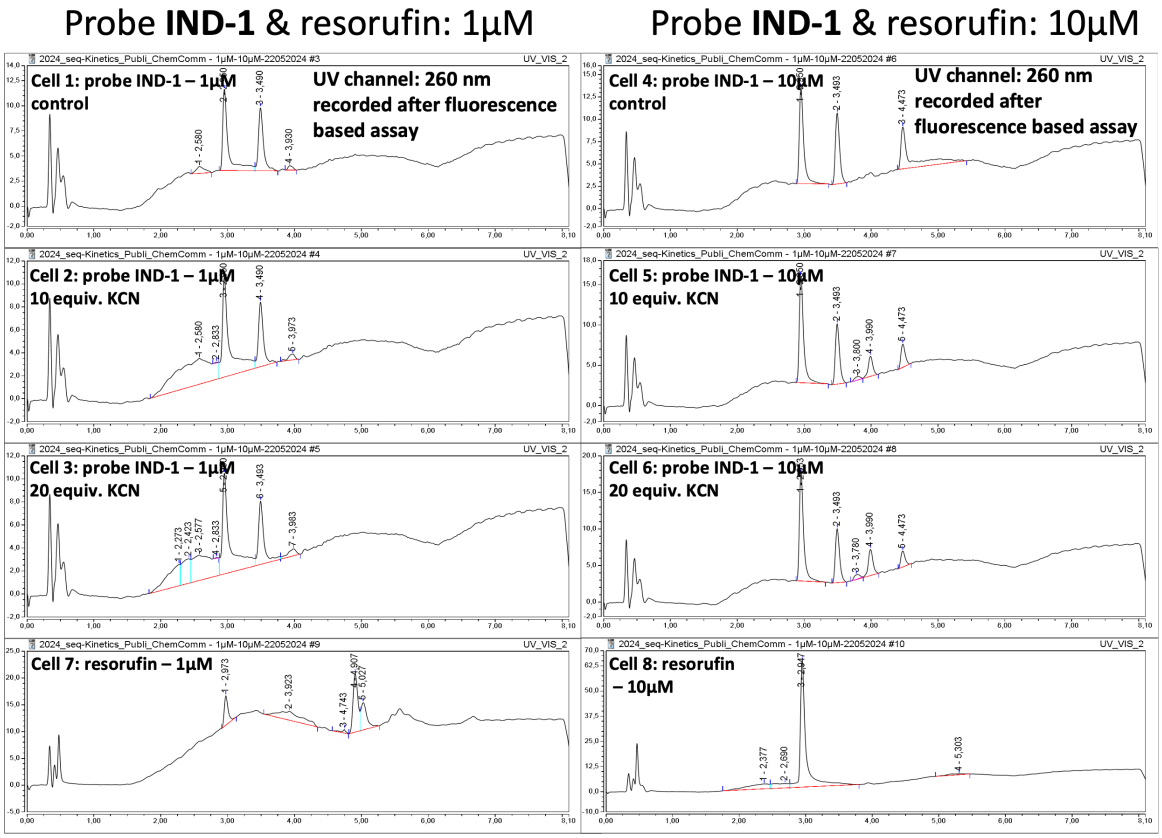


Fig. S18 RP-HPLC-MS analyses (system A) of cyanide-mediated activation of fluorogenic probe IND-1 - Influence of probe concentration (1.0 μM and 10 μM)

2 mL HPLC vials were filled with solutions arising from Table S4 (entries 1-8). *Please note: for solutions of probe **IND-1** at 10 μM, only supernatant was sampled.* RP-HPLC elution profiles with full scan mass detection were obtained using system A.

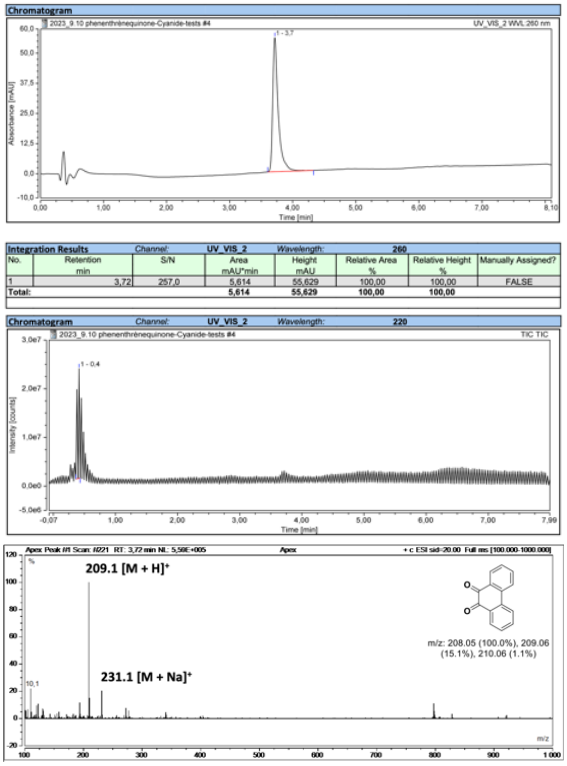


Resorufin-based probe **IND-1** was readily hydrolysed in PBS alone, at a concentration of 1.0 μM, regardless of the amount of KCN added, confirming our first hypothesis of a major drawback related to poor aq. stability of the ester linkage of this cyanide-responsive fluorescent probe.

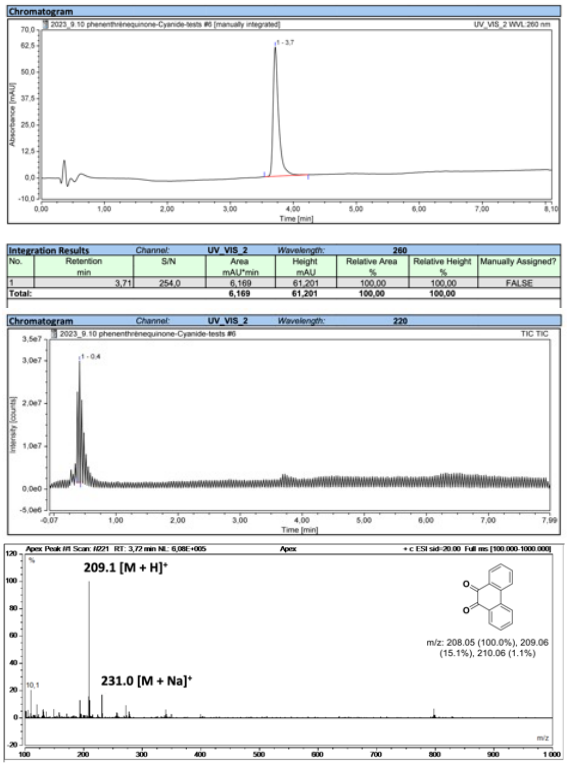
Fig. S19 RP-HPLC-MS analyses (system A'') of control reaction between 9,10-phenanthrenequinone and KCN

The same methodology than that used to perform cyanide-mediated activation of fluorogenic probes **IND-1** and **1-4**, was used by replacing the 10 µM solution of probe by a 10 µM solution of 9,10-phenanthrenequinone. Incubation with KCN was directly performed in 2 mL HPLC vials.

Commercial 9,10-phenanthrenequinone
10 µM, incubation with 50 equiv. KCN
T0

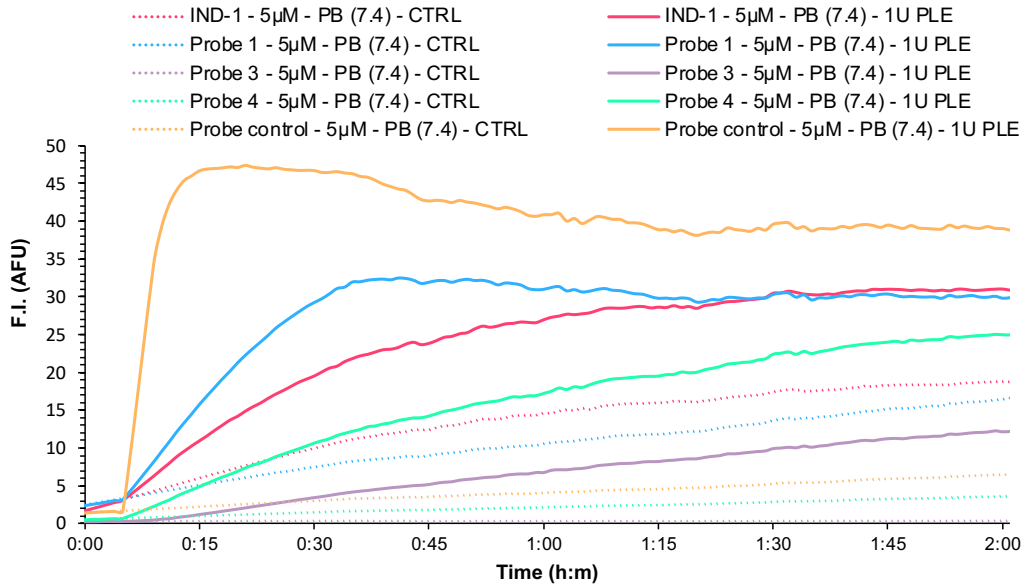


Commercial 9,10-phenanthrenequinone
10 µM, incubation with 50 equiv. KCN
T0 + 48 h



S8 Study of the reactivity of cyanide-responsive reaction-based probes IND-1, 1, 3 and 4, towards pig liver esterase (PLE)

Fig. S20 Fluorescence emission time-course assay of cyanide-responsive fluorogenic probes (concentration: 5 μM) in the presence or absence of PLE (1 U) in PB (0.1 M, pH 7.4) at 25 °C



*please note: (1) probe control corresponds to 7-acetoxycoumarin [10387-49-2], synthesised by 7-O-acetylation of umbelliferone with Ac_2O and pyridine in dry CH_2Cl_2[10]; (2) PLE (1 U) was after 5 min of incubation in PB alone; (3) for **IND-1**, detection of the released resorufin using the following parameters: Ex/Em 565/595 nm, slits 5 nm, PMT voltage = 417 V; (4) for other probes, detection of the released umbelliferone using the following parameters: Ex/Em 330/450 nm, slits 5 nm, PMT voltage = 430 V.*

[10]P. Stocker, M. Cassien, N. Vidal, S. Thétiot-Laurent and S. Pietri, *Talanta*, 2017, **170**, 119-127.