Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# A Novel Sulfonated Prosthetic Group for [$^{18}$F]-Radiolabelling and Imparting Water Solubility of Biomolecules and Cyanine Fluorophores

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## Supporting Information

## Abbreviations

The following abbreviations are used throughout the text of the ESI file: $Boc_2O$, di-*tert*-butyl dicarbonate; BSA, bovine serum albumin protein; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; ESI, electrospray ionisation; PBS, phosphate buffered saline; RP-HPLC, reversed-phase high performance liquid chromatography; TFA, trifluoroacetic acid; TLC, thin-layer chromatography.

## Experimental Section

Pyridine was dried through distillation over KOH and stored over BaO and under an Ar atmosphere. Flash column chromatography purifications of cyanine derivatives were performed on Geduran® Si 60 silica gel (63-200 μm) from Merck.

### Instruments and methods.

$^1H$, $^{13}C$ and $^{19}F$ spectra were recorded either with a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France) or with a Bruker AC 200. Chemical shifts are expressed in parts per million (ppm) from $CDCl_3$ ($\delta_H = 7.26$, $\delta_C = 77.16$) or $CD_3OD$ ($\delta_H = 3.31$, $\delta_C = 49.0$)[1]. *J* values are expressed in Hz. $^{13}C$ substitutions were determined with DEPT-135 experiments, differentiating signals of methyl and methine carbons pointing "up" (+) from methylene carbons pointing "down" (-). Analytical HPLC was performed on a Thermo Scientific Surveyor Plus instrument equipped with a PDA detector. Semi-preparative HPLC was performed on a Thermo Scientific SPECTRASYSTEM liquid chromatography system (P4000) equipped with a UV-visible 2000 detector. Low-resolution mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source. High-resolution mass spectra (HRMS) were recorded on a LTQ Orbitrap Elite (Fisher Scientific). UV-visible absorption spectra were obtained on a Varian Cary 50 scan spectrophotometer by using a rectangular quartz cell (Varian, standard cell, Open Top, 10 × 10 mm, 3.5 mL). Fluorescence spectroscopic studies (emission/excitation spectra) were performed on a Varian Cary Eclipse spectrophotometer with a semi-micro quartz fluorescence cell (Hellma, 104F-QS, 10 × 4 mm, 1400 μL). The absorption spectra of cyanine dyes were recorded (400-900 nm) in DMSO, PBS and PBS + 5% BSA (concentration: 1.0-10.0 μM) at 25 °C. Excitation/emission spectra were recorded under the same conditions after emission/excitation at the corresponding wavelength (760/600 nm, excitation and emission filters: auto, excitation and emission slit = 5 nm). All fluorescence spectra were corrected. Relative quantum yields were measured at 25 °C by a relative method using symmetrical sulfobenzoindocyanine dye Cy 5.5 (Cy5.205) as a standard ($\Phi_F$ = 23% in PBS)[2]. The following equation was used to determine the relative fluorescence quantum yield:

$$\Phi_F(x) = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2 \Phi_F(s)$$

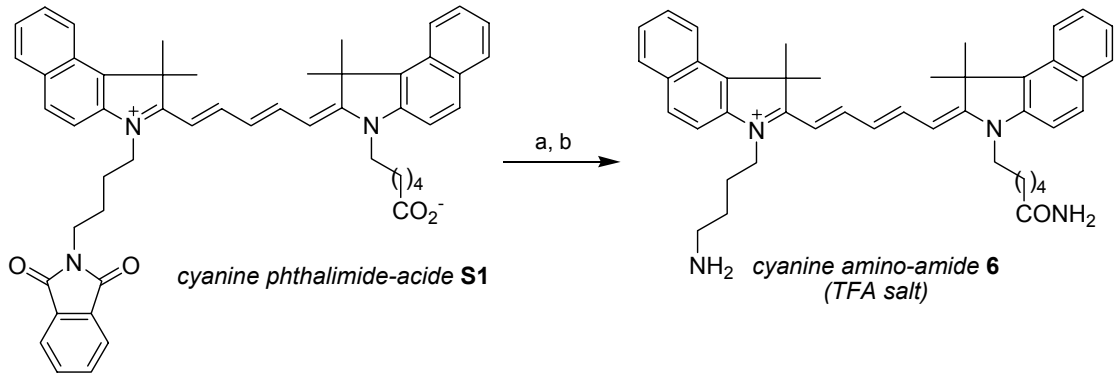
Where A is the absorbance (in the range 0.01-0.1 A.U.), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements, and the subscripts s and x represent standard and unknown, respectively. The following refractive index values were used: 1.479 for DMSO and 1.337 for PBS and PBS + 5% BSA.

[1] 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.
[2] S. R. Mujumdar, R. B. Mujumdar, C. M. Grant and A. S. Waggoner, *Bioconjugate Chem.*, 1996, **7**, 356-362.

**HPLC separations.**
The following chromatographic system was used for the analytical experiments:

- System S1: RP-HPLC (Thermo Hypersil GOLD $C_{18}$ column, 5 µm, 2.1 × 100 mm) with $CH_3CN$ and aq. 0.1% trifluoroacetic acid (aq. TFA 0.1%, pH 2.0) as eluents [80% TFA (5 min), then linear gradient from 20 to 40% (5 min) and 40 to 100% (50 min) of $CH_3CN$] at a flow rate of 0.25 mL $min^{-1}$. UV-vis detection with the "Max Plot" (*i.e.*, chromatogram at absorbance maximum for each compound) mode (220-798 nm).

**Cyanine amino-amide (6):**



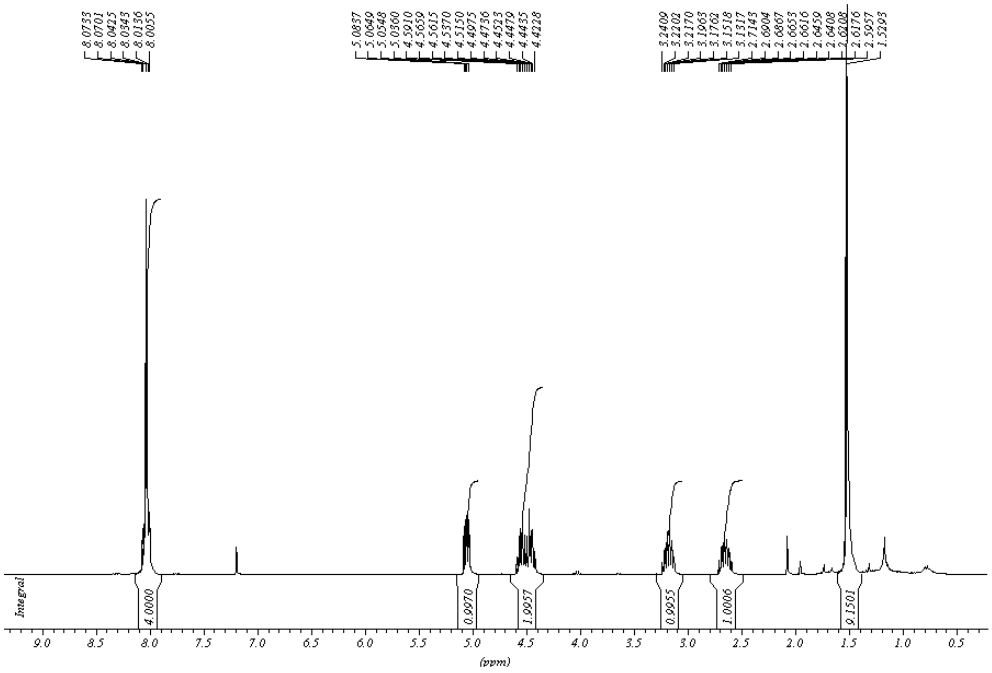

**Scheme S1** *Reagents and conditions*: (a) $Boc_2O$ (3 equiv.), $NH_4HCO_3$ (3 equiv.), pyridine, DMF, rt, overnight, 89%; (b) $CH_3NH_2$ (6 equiv.), $CH_3OH$-$H_2O$ (88 : 12, v/v), rt, 24 h, RP-$C_{18}$ flash-chromatography, 37%.

*Amide formation*: Cyanine phthalimide-acide[3] **S1** (596 mg, 0.77 mmol, 1 equiv.) was dissolved in dry DMF (26.7 mL) and mixed with an heterogeneous DMF solution (14.3 mL) containing $NH_4HCO_3$ (185.6 mg, 2.35 mmol, 3 equiv.), dry pyridine (187 µL, 2.32 mmol, 3 equiv.) and $Boc_2O$ (512 mg, 2.35 mmol, 3 equiv.). The resulting reaction mixture was protected from light (with aluminum foil) and stirred at rt overnight. The complete conversion into the carboxamide derivative was confirmed both by TLC ($CH_2Cl_2$-$CH_3OH$, 9 : 1, v/v) and ESI mass spectrometric analyses. Thereafter, the reaction mixture was evaporated to dryness and the resulting oily residue was purified by flash-chromatography on a silica gel column with a step gradient of $CH_3OH$ (0-1%) in $CH_2Cl_2$ (stabilised with 1% ethanol) as the mobile phase. The desired cyanine phthalimide-amide was obtained as a blue amorphous solid (525 mg, yield 89%). Its purity was checked by RP-HPLC (system S1) and this product was directly engaged in the next step without further purification. $R_f$ 0.34 ($CH_2Cl_2$-$CH_3OH$, 9 : 1, v/v); HPLC (system S1): $t_R$ = 32.6 min, purity 98%; MS (ESI+): *m/z* 769.40 $[M]^{+\bullet}$, calcd for $C_{51}H_{53}N_4O_3^+$ 769.41.
*Removal of phthalimide*: Phthalimide derivative (442 mg, 0.57 mmol, 1 equiv.) was dissolved in $CH_3OH$ (20 mL) and 35% aq. methylamine solution (2.75 mL, 3.4 mmol, 6 equiv.) was added; the resulting reaction mixture was protected from light (with aluminum foil) and stirred at rt for 24 h. The complete conversion into the amino-amide derivative was confirmed both by TLC ($CH_2Cl_2$-$CH_3OH$, 9 : 1, v/v) and ESI mass spectrometric analyses. Thereafter, the reaction mixture was evaporated to dryness; the resulting oily residue was dissolved in a

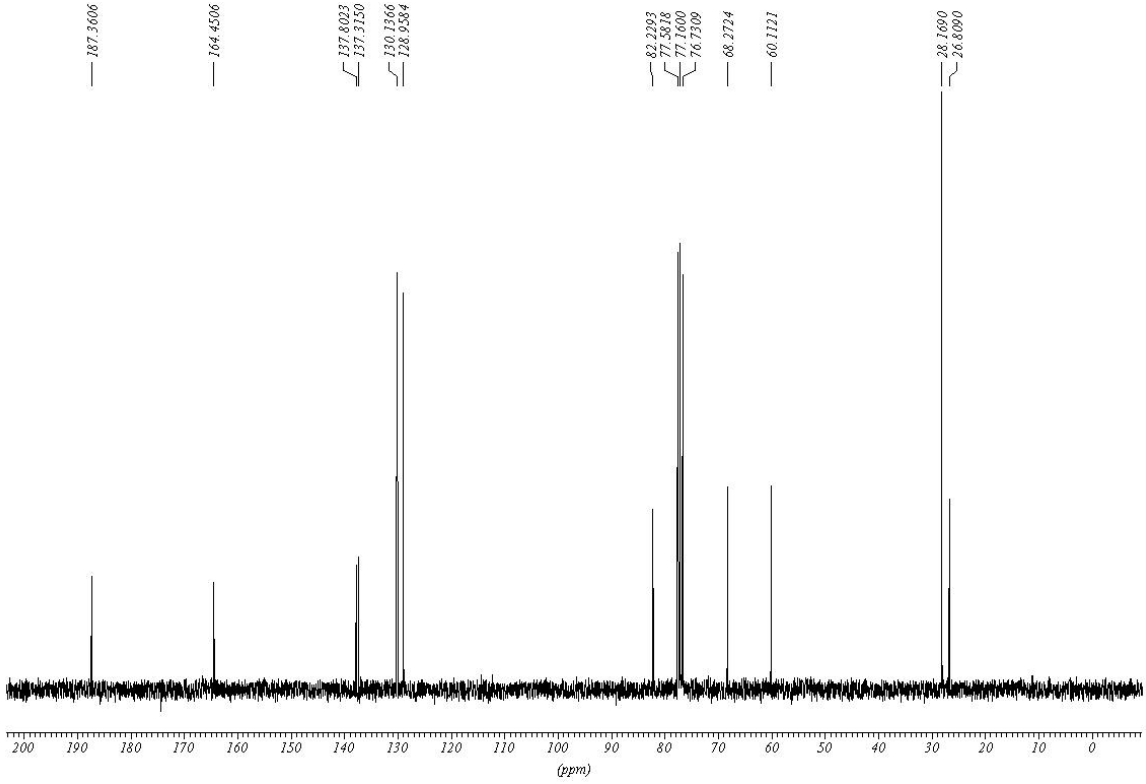
[3] B. Chipon, G. Clavé, C. Bouteiller, M. Massonneau, P.-Y. Renard and A. Romieu, *Tetrahedron Lett.*, 2006, **47**, 8279-8284. Corrigendum, 2007, **48**, 501.

mixture of $CH_3CN$ and aq. TFA 0.1% and purified on a KP-C18-HS SNAP cartridge (30 g, Biotage®) by means of an automated flash purification system (Biotage® Isolera One), and by using a linear gradient from 0 to 75% (80 min) of $CH_3CN$ in aq. TFA 0.1% as the mobile phase (flow rate 25 mL $min^{-1}$ and UV detection at 254/280 nm). The product-containing fractions were lyophilised to give the TFA salt of cyanine amino-amide **6** as a blue amorphous powder (206 mg, 0.21 mmol, yield 37%). *Please note: Hydrazine monohydrate can be used as alternative deblocking reagent. Reaction kinetics is faster but traces of diimide ($N_2H_2$) may cause partial or complete degradation of the cyanine core through reduction of its polymethine chain especially if the crude reaction mixture is slightly heated (ca. 40 °C) during the evaporation step.* $R_f$ 0.0 ($CH_2Cl_2$-$CH_3OH$, 9 : 1, v/v); $\delta_H$(200 MHz, $CDCl_3$) 8.42 (bs, 3H, N$H_3^+$), 8.06-7.31 (14H, m, Ph-benzoindole & 2 × CH=C*H*-CH=C), 6.65 (1H, t, *J* 12.4, C*H*=CH-CH=C), 6.18 (2H, pt, *J* 14.0, 2 × C*H*=CH-CH=C), 4.03 (4H, bm, 2 × *N*(benzoindole)-C$H_2$), 3.09 (2H, bm, C$H_2$-$NH_3^+$), 2.25 (2H, bm, C$H_2$-$CONH_2$), 1.92-1.25 (22H, m, 4 × C$H_3$(benzoindole) & 5 × -C$H_2$-); $\delta_C$(75.5 MHz, $CDCl_3$) 24.8 (C$H_2$) 24.9 (C$H_2$), 25.2 (C$H_2$), 26.4 (C$H_2$), 27.4 (C$H_2$), 27.7 (4 × C$H_3$), 35.4 (C$H_2$), 39.4 (C$H_2$), 43.9 (C$H_2$), 44.3 (C$H_2$), 51.2 (2 × C($CH_3$)$_2$), 103.4 (2 × CH), 110.7 (2 × CH), 116.8 (1C, *J* 292.3, C$F_3$-TFA), 122.3 (2 × CH), 125.2 (CH), 125.3 (CH), 126.4 (CH), 127.9 (2 × CH), 128.0 (2 × Cq), 130.3 (2 × CH), 131.0 (2 × CH), 132.0 (2 × Cq), 133.8 (1 × Cq), 134.0 (1 × Cq), 139.4 (2 × Cq), 152.1 (2 × CH), 161.5 (1C, q, C$O_2$H-TFA), 174.0 (1 × CH), 174.1 (1 × CH), 177.7 (1 × Cq, C$ONH_2$); HPLC (system S1): $t_R$ = 21.3 min, purity 96%; MS (ESI+): *m/z* 320.27 $[M + H]^{2+}$, 639.40 $[M]^{+\bullet}$, calcd for $C_{43}H_{51}N_4O^+$ 639.40; HRMS (ESI+): *m/z* 320.2061 $[M + H]^{2+}$, calcd for ($C_{43}H_{52}N_4O^{2+}$)/2 320.2070; $\lambda_{max}$(DMSO) nm 688 (ε/$dm^3$ $mol^{-1}cm^{-1}$ 195 000), $\lambda_{max}$ em (DMSO) nm 712 ($\Phi_F$ 0.45); $\lambda_{max}$(PBS + 5% BSA) nm 695 (ε/$dm^3$ $mol^{-1}cm^{-1}$ 162 000), $\lambda_{max}$ em (PBS + 5% BSA) nm 710 ($\Phi_F$ 0.34).

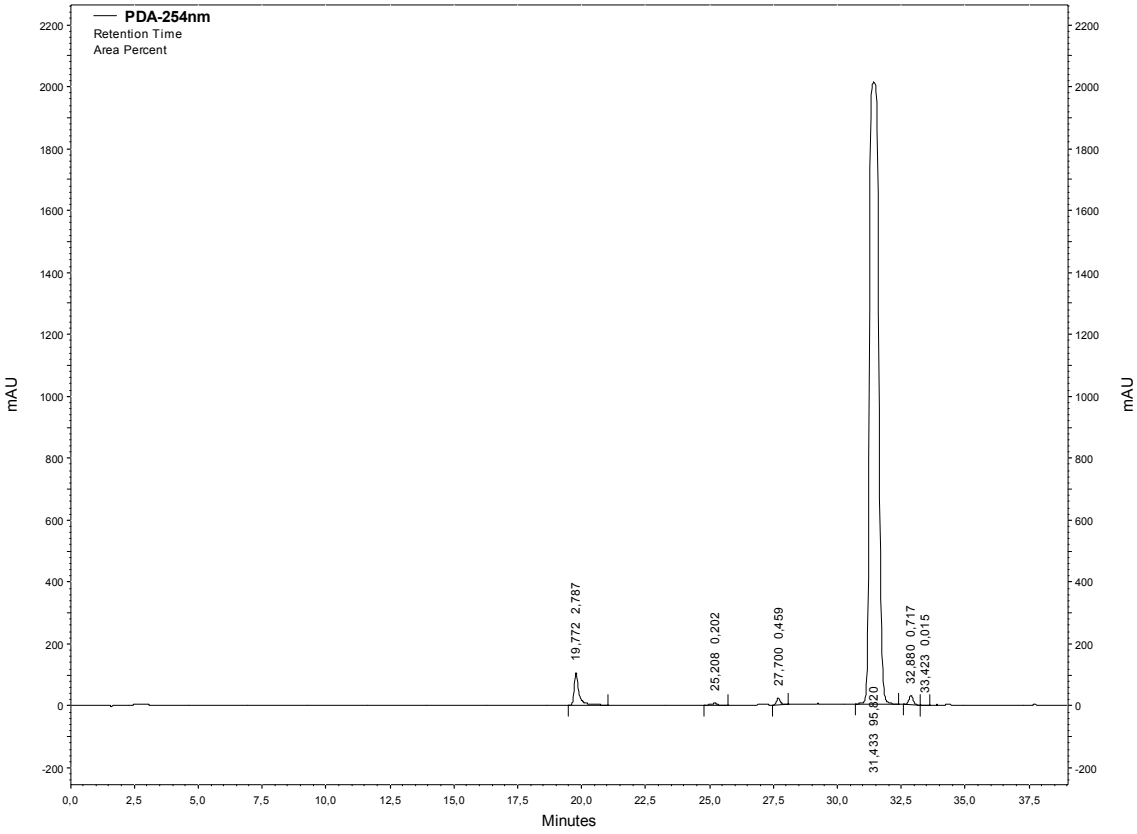
**$^{1}$H NMR spectrum of sultone-benzoic acid, *tert*-butyl ester 2 recorded in $CDCl_3$ at 300 MHz.**



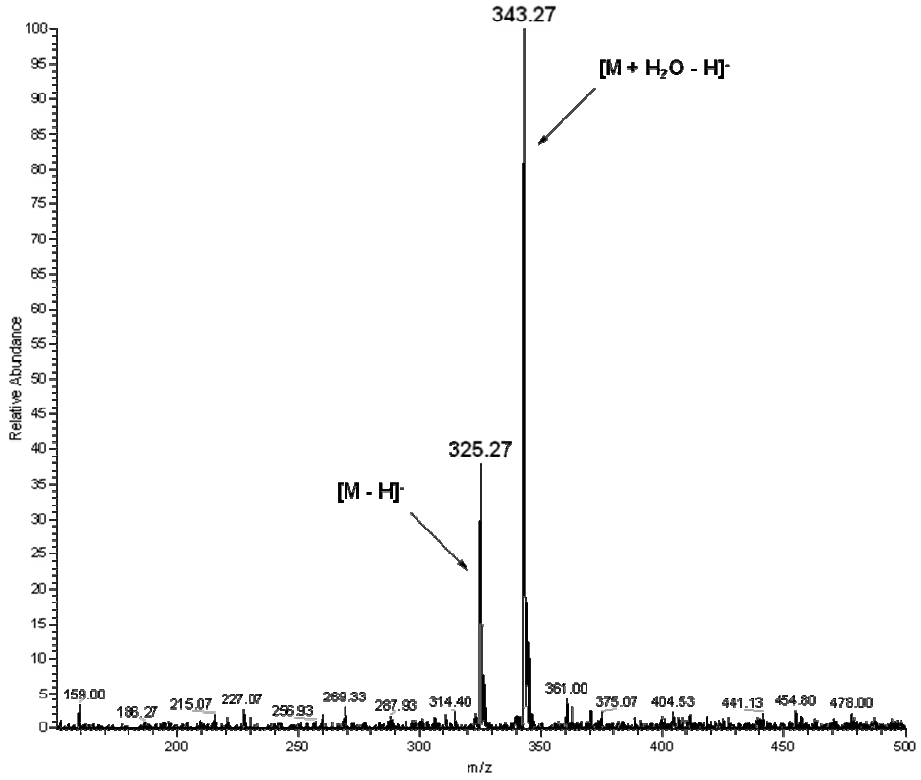

**$^{13}$C NMR spectrum of sultone-benzoic acid, *tert*-butyl ester 2 recorded in $CDCl_3$ at 75.5 MHz.**



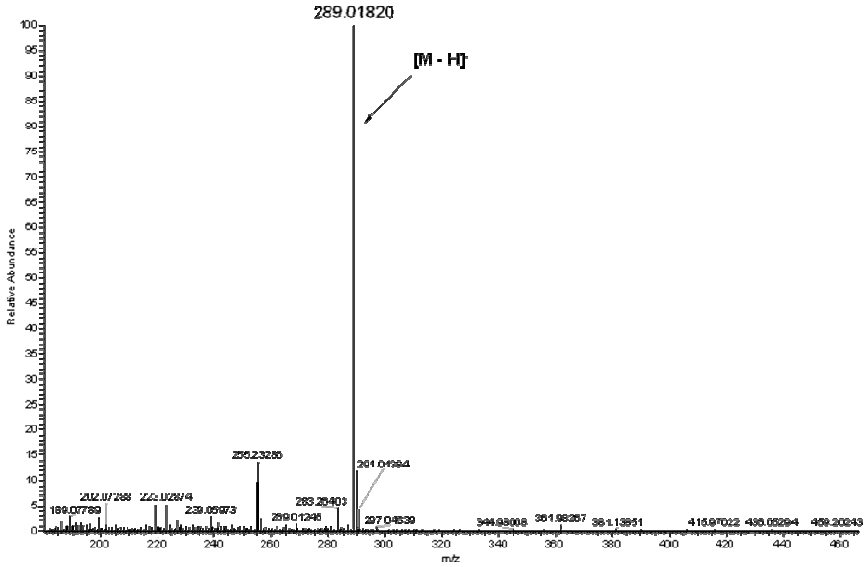
**RP-HPLC elution profile (system A) of sultone-benzoic acid, *tert*-butyl ester 2.**



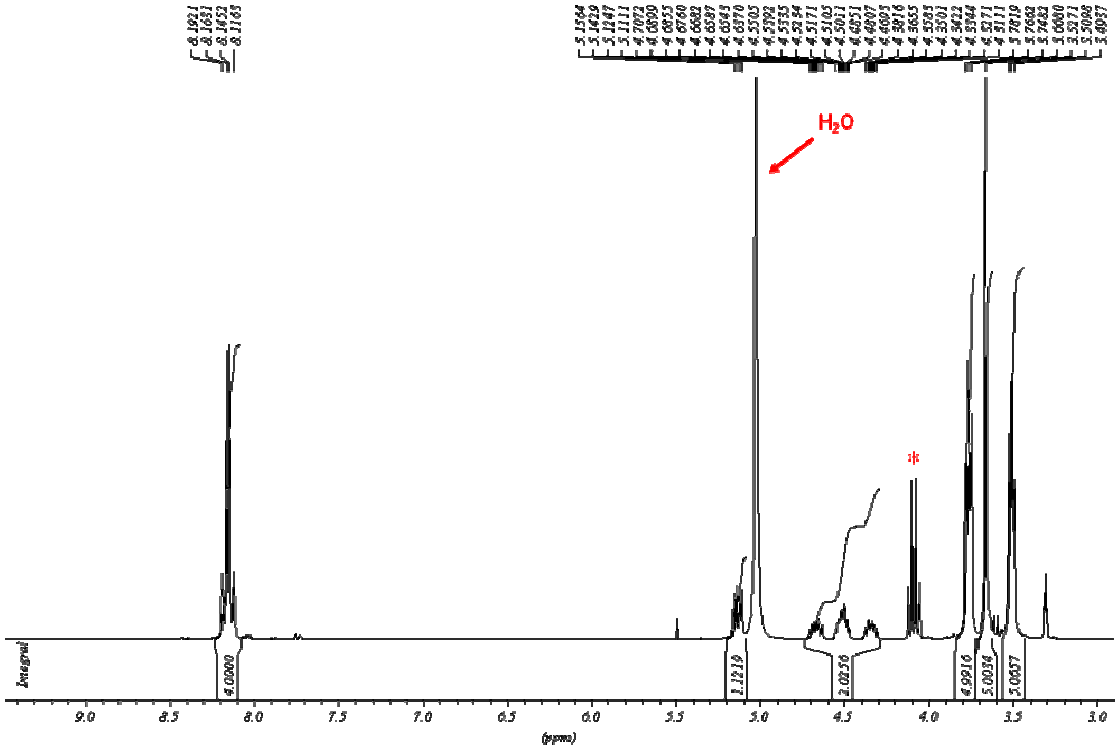

**(ESI-) mass spectrum of sultone-benzoic acid, *tert*-butyl ester 2.**

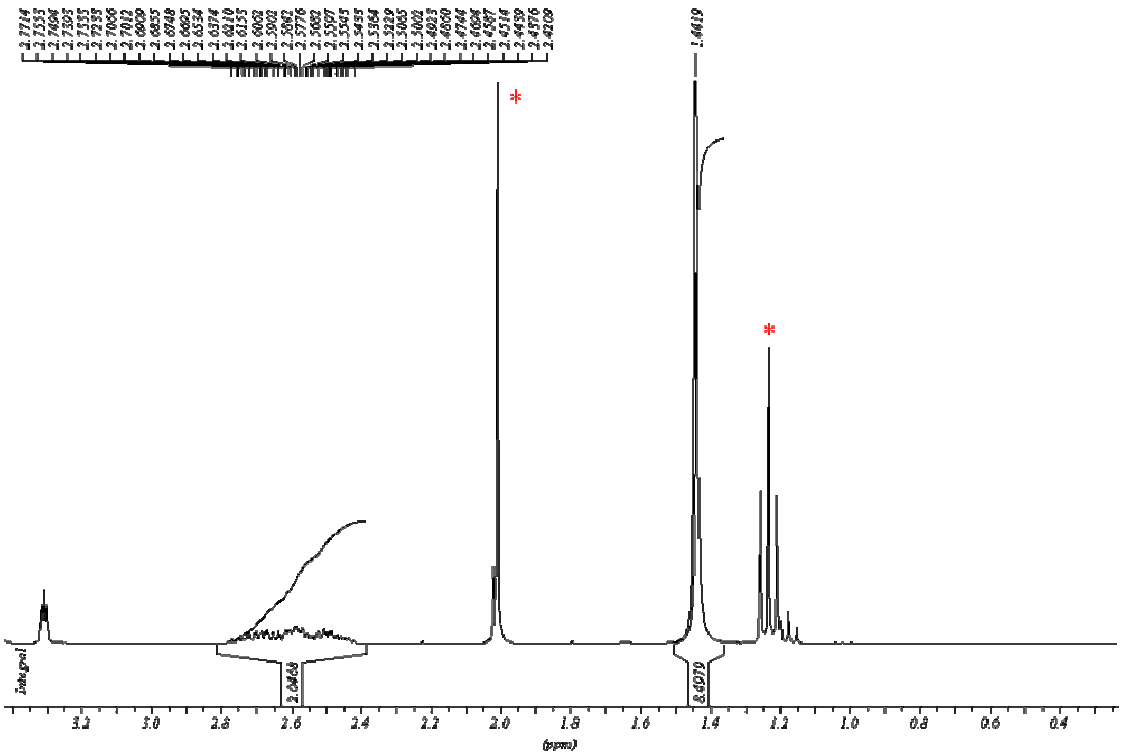


**(ESI-) mass spectrum (high resolution) of sultone-benzoic acid, *tert*-butyl ester 2.**



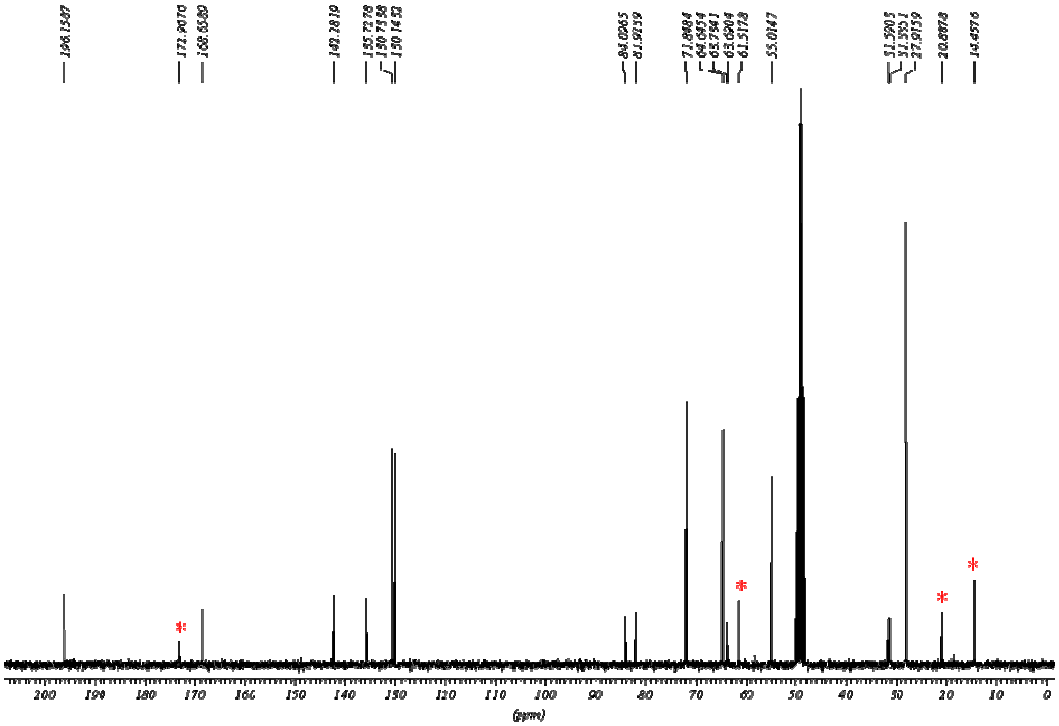

**$^1$H NMR spectrum of mono-fluoro sulfonated *tert*-butyl ester 3 recorded in $CD_3OD$ at 300 MHz.[a]**





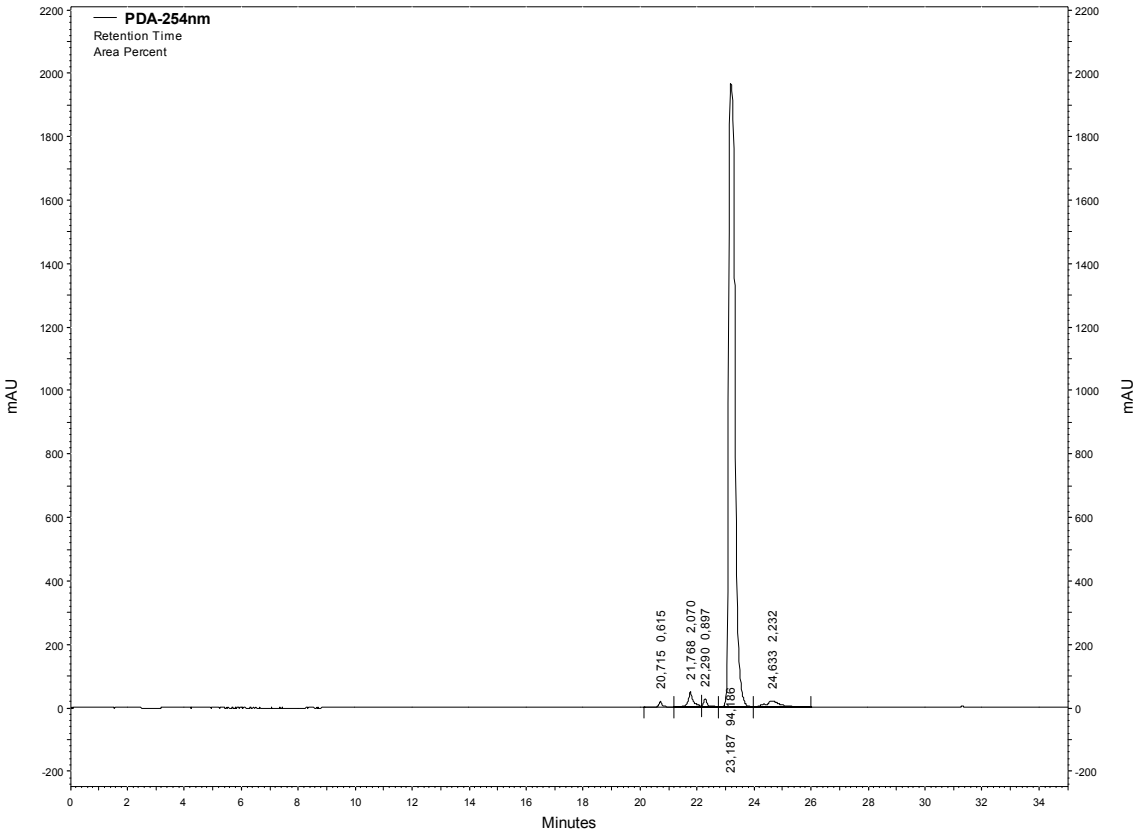
*peaks assigned to residual ethyl acetate.
[a]This compound was partially obtained as the trialkylammonium (Kryptofix[K222]) salt (**3**-[K222], 1 : 0.4), 3.79-3.51 ppm: peaks assigned to Kryptofix.

**$^{13}$C NMR spectrum of mono-fluoro sulfonated *tert*-butyl ester 3 recorded in $CD_3OD$ at 75.5 MHz.[a]**

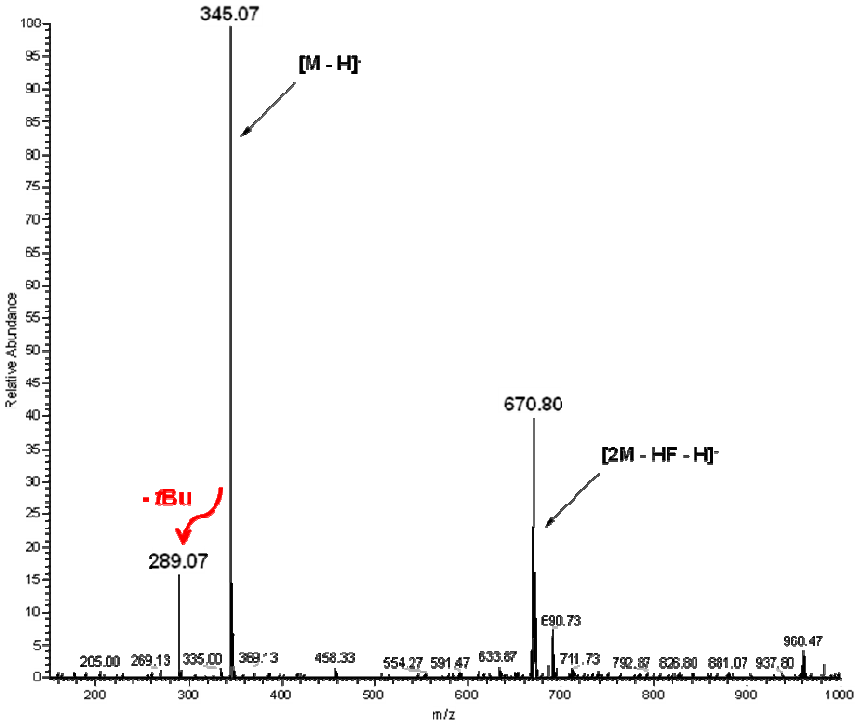


*peaks assigned to residual ethyl acetate.
[a]This compound was partially obtained as the trialkylammonium (Kryptofix[K222]) salt (**3**-[K222], 1 : 0.4).

**RP-HPLC elution profile (system A) of mono-fluoro sulfonated *tert*-butyl ester 3.**

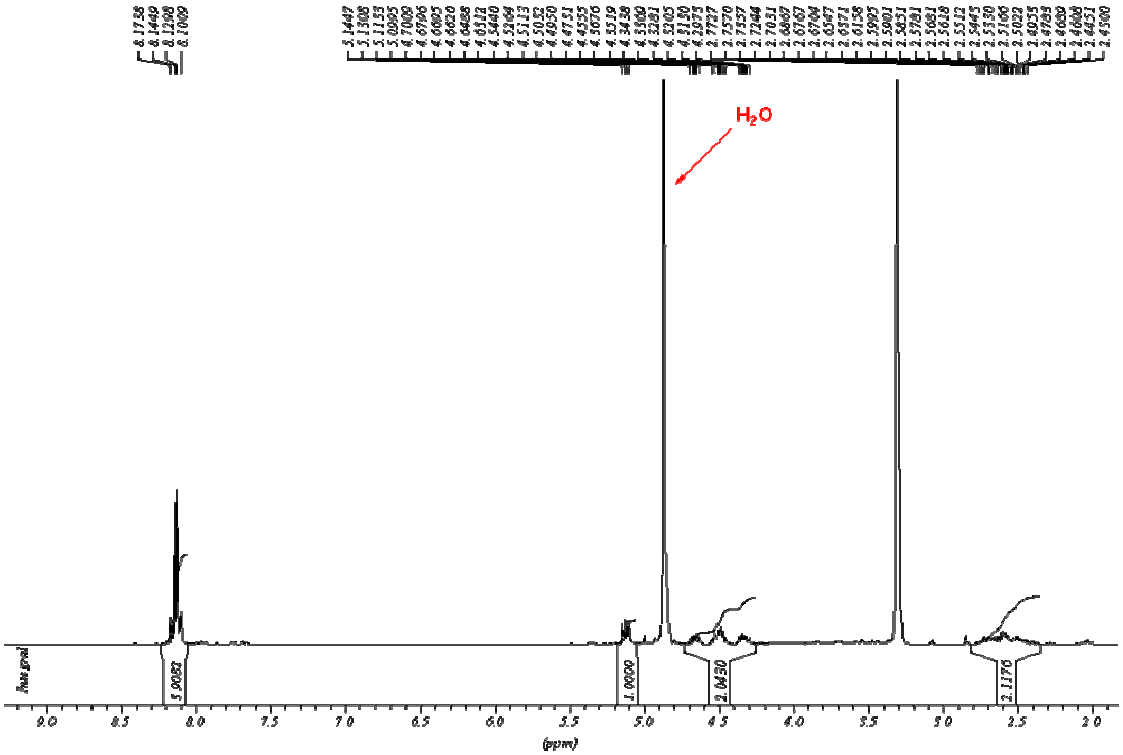



**(ESI-) mass spectrum of mono-fluoro sulfonated *tert*-butyl ester 3.**

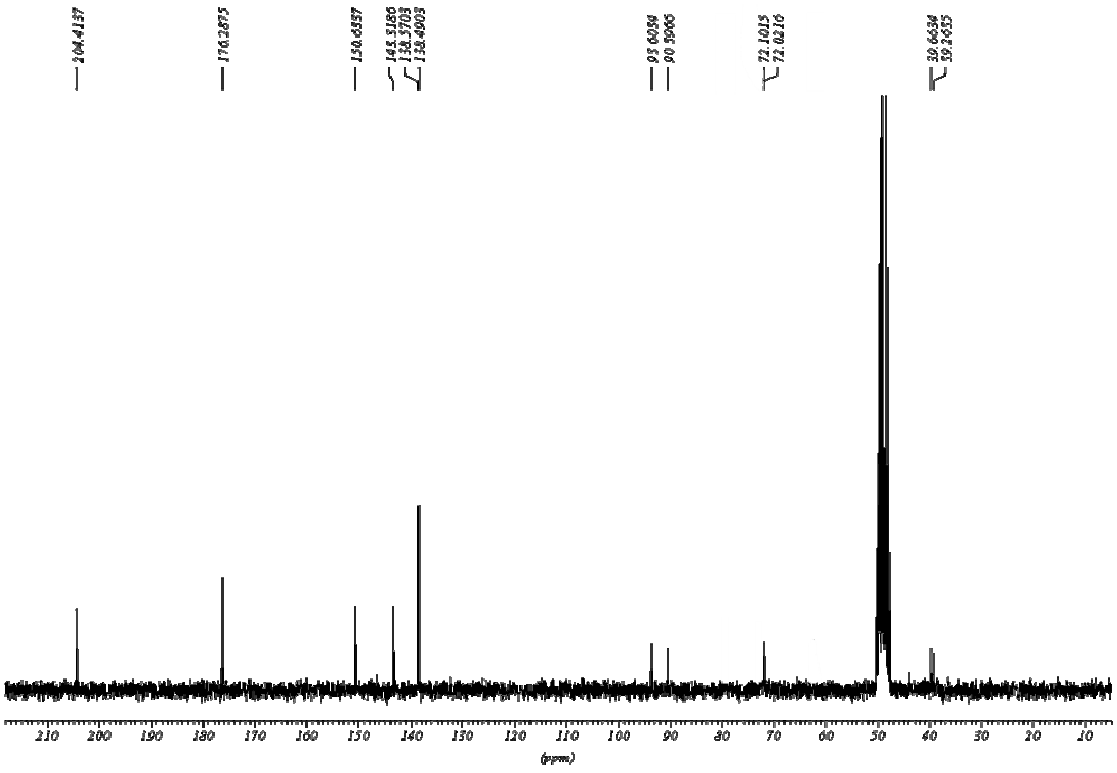



*The peak at m/z = 289.07 was assigned to the free carboxylic acid derivative whose formation occurred during the ionisation process.*

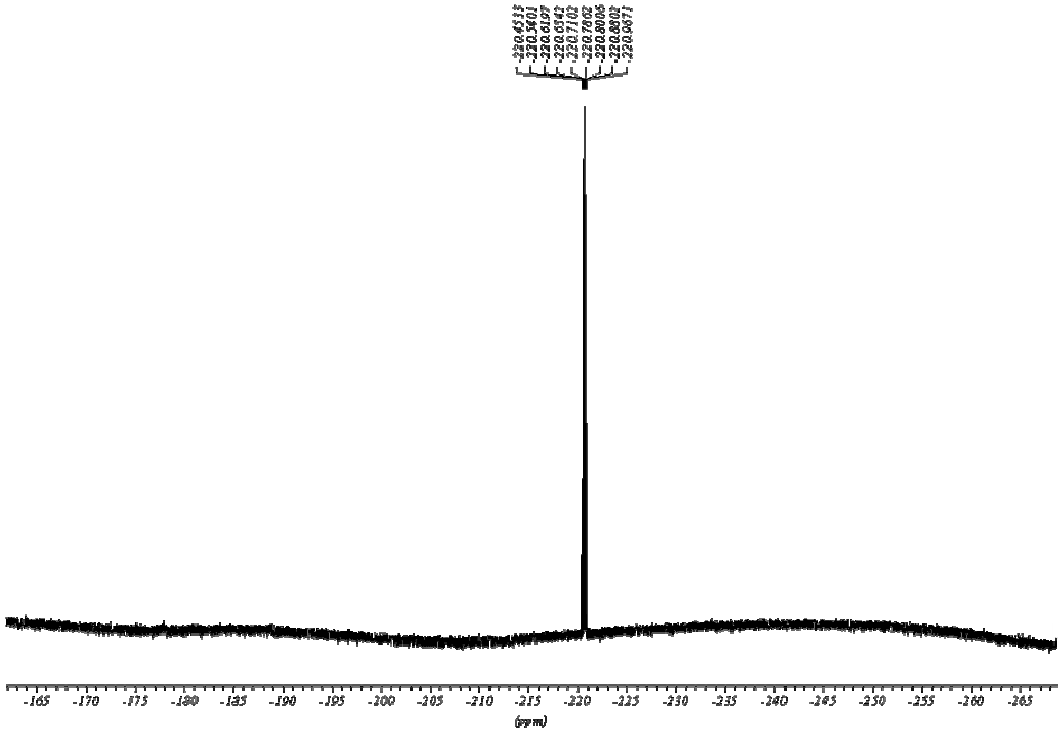
**$^{1}$H NMR spectrum of mono-fluoro-sulfonated benzoic acid 4 recorded in $CD_3OD$ at 300 MHz.**



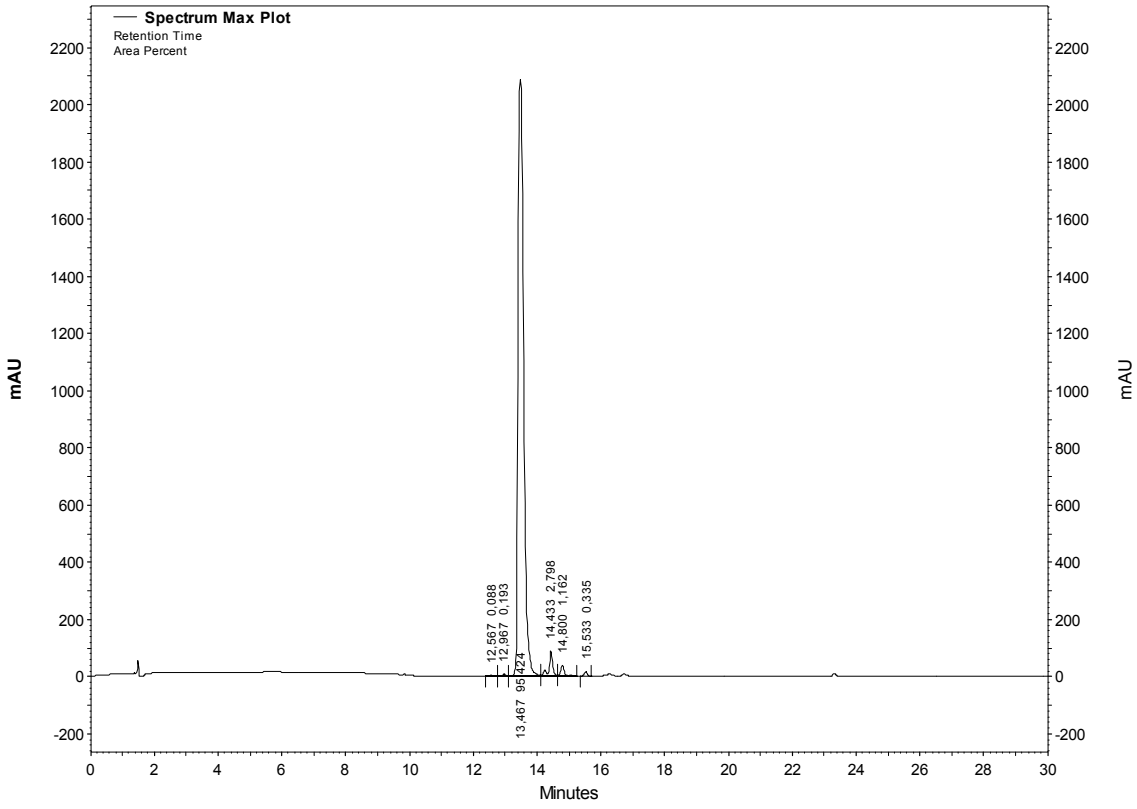

**$^{13}$C NMR spectrum of mono-fluoro-sulfonated benzoic acid 4 recorded in $CD_3OD$ at 75.5 MHz.**



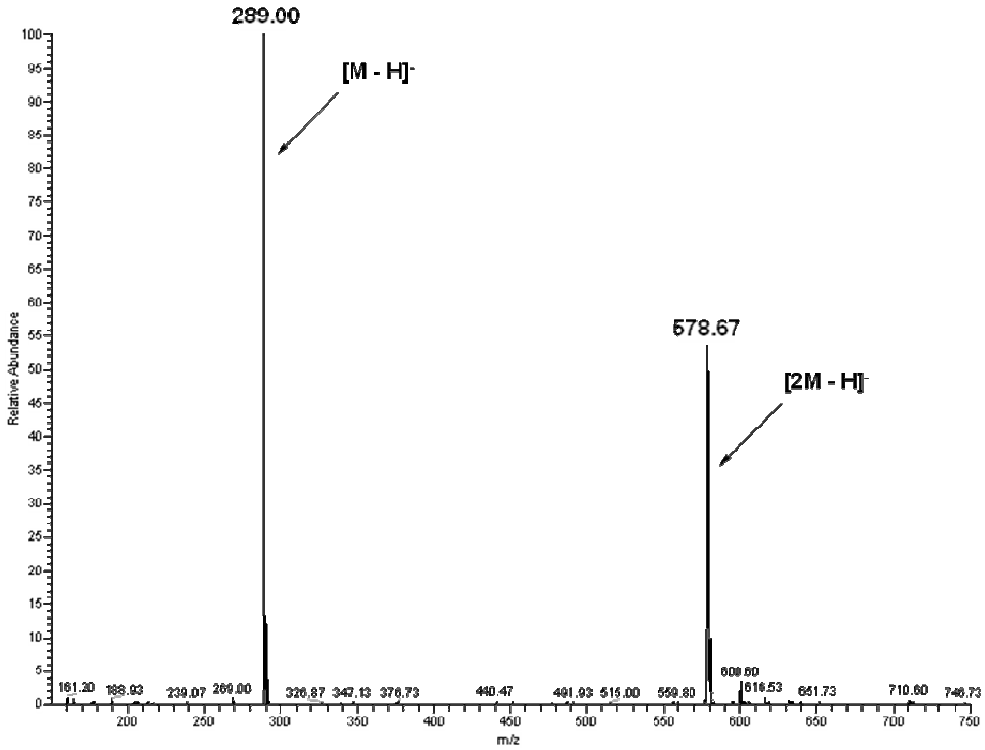
**$^{19}$F NMR spectrum of mono-fluoro-sulfonated benzoic acid 4 recorded in $CD_3OD$ at 282.5 MHz.**



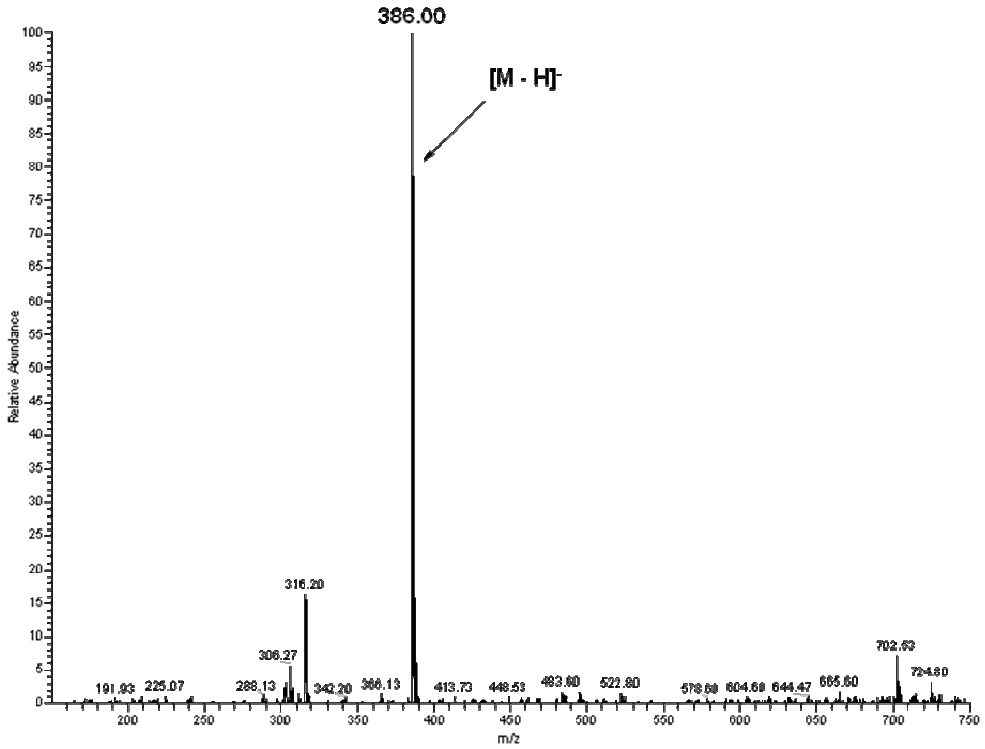

**RP-HPLC elution profile (system A) of mono-fluoro-sulfonated benzoic acid 4.**



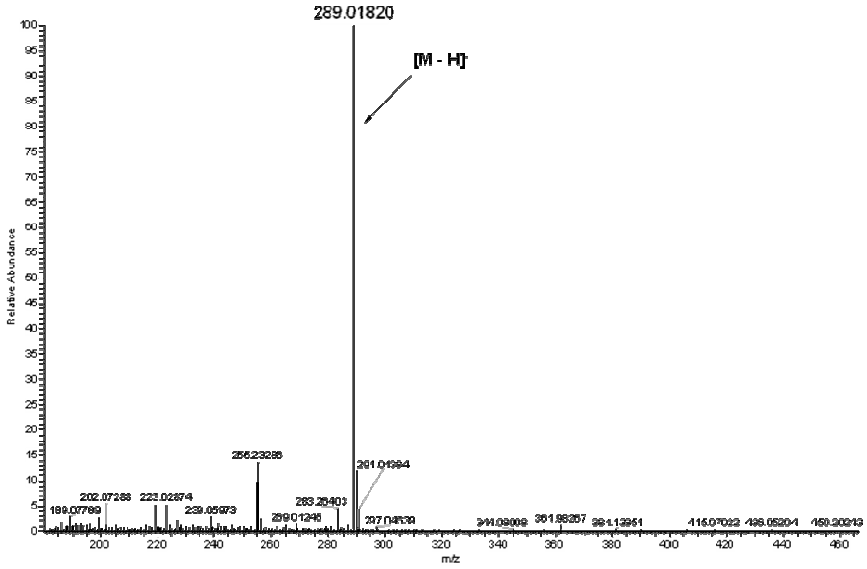
**(ESI-) mass spectrum of mono-fluoro-sulfonated benzoic acid 4.**



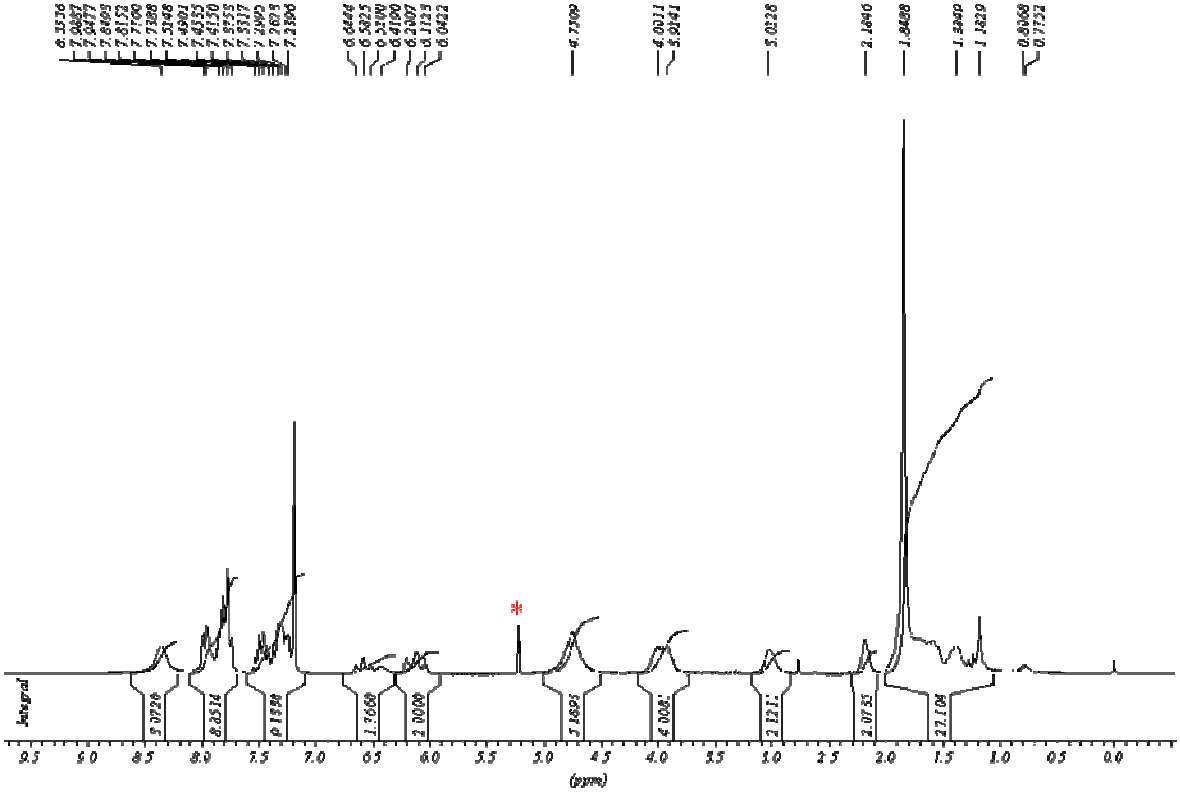

**(ESI-) mass spectrum of [$^{19}$F]-prosthetic group 1.**



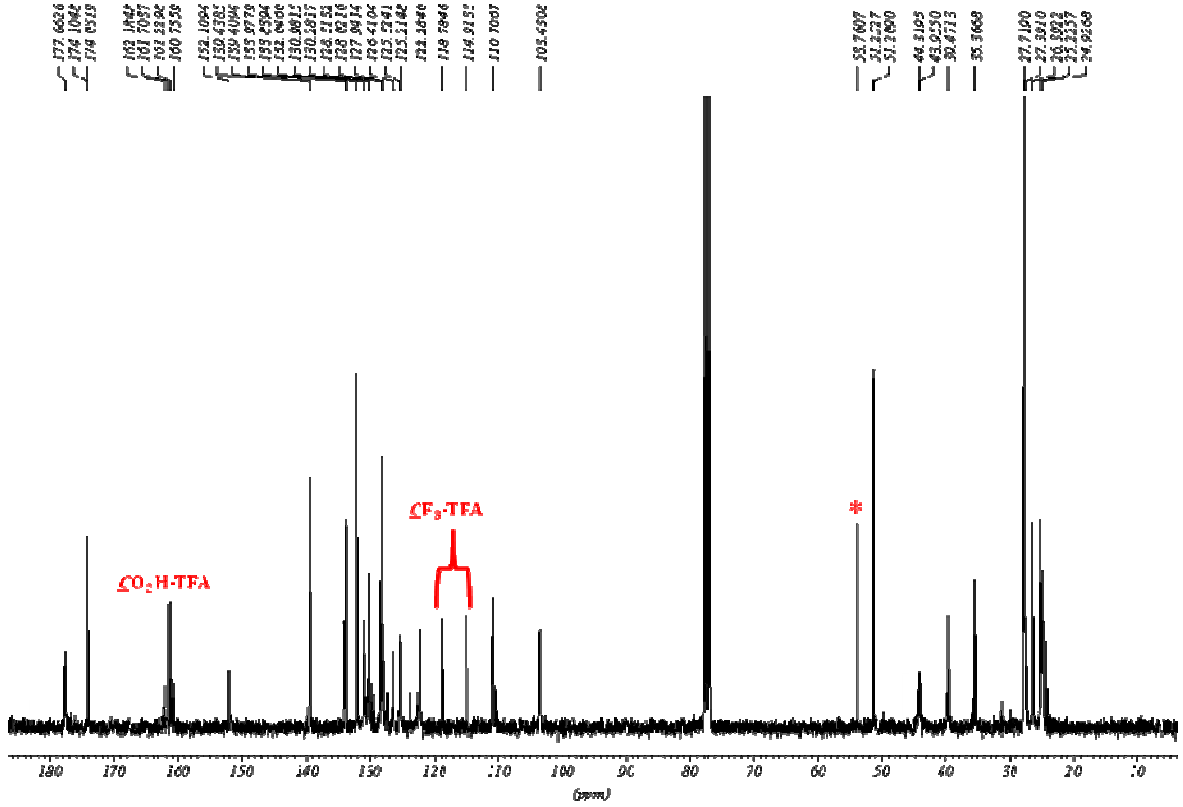
**(ESI-) mass spectrum (high resolution) of mono-fluoro sulfonated benzoic acid 4.**




**$^1$H NMR spectrum of cyanine amino-amide 6 (TFA salt) recorded in $CDCl_3$ at 200 MHz.**

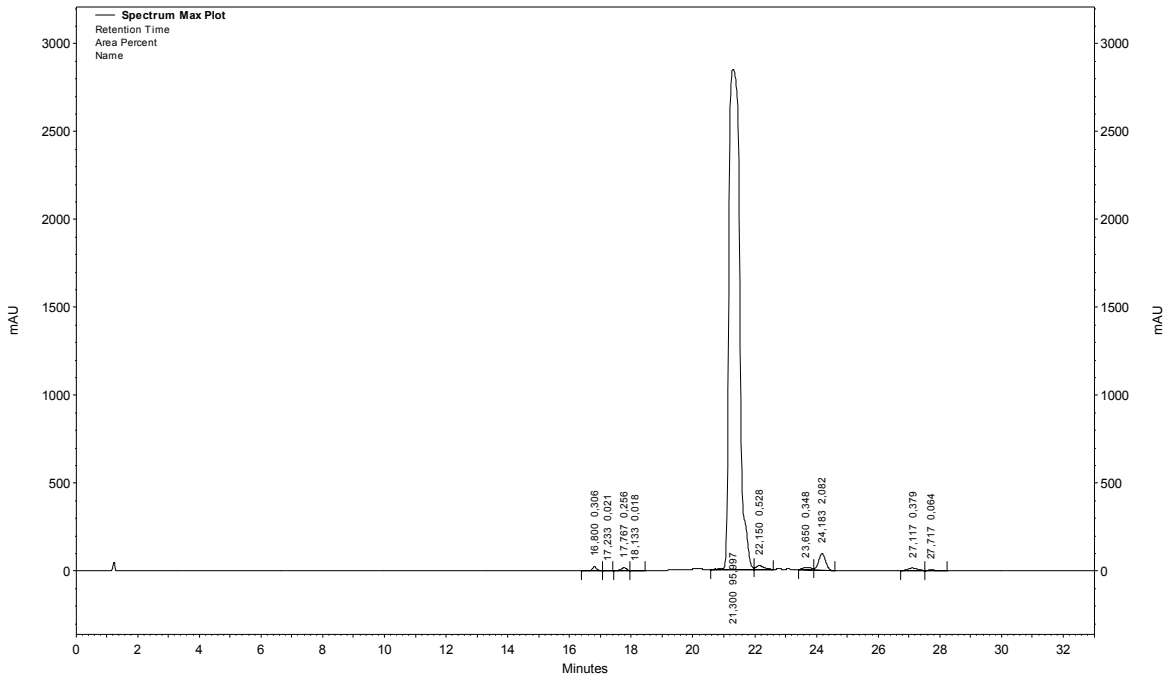


**$^{13}$C NMR spectrum of cyanine amino-amide 6 (TFA salt) recorded in $CDCl_3$ at 75.5 MHz.**

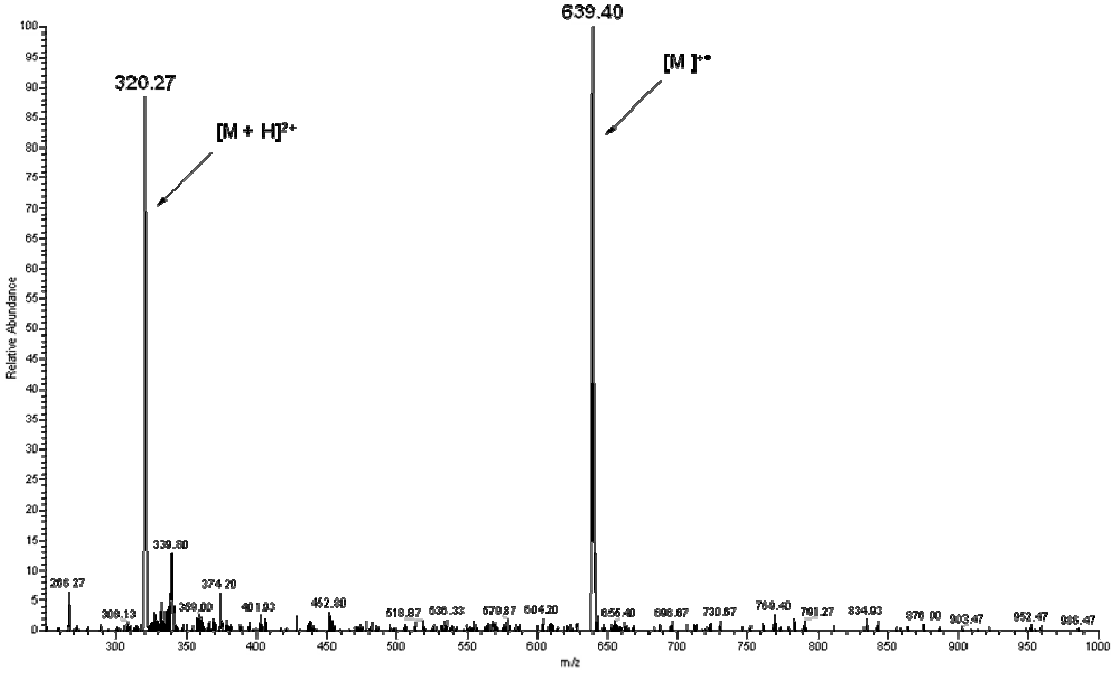



**peaks assigned to residual $CH_2Cl_2$.*

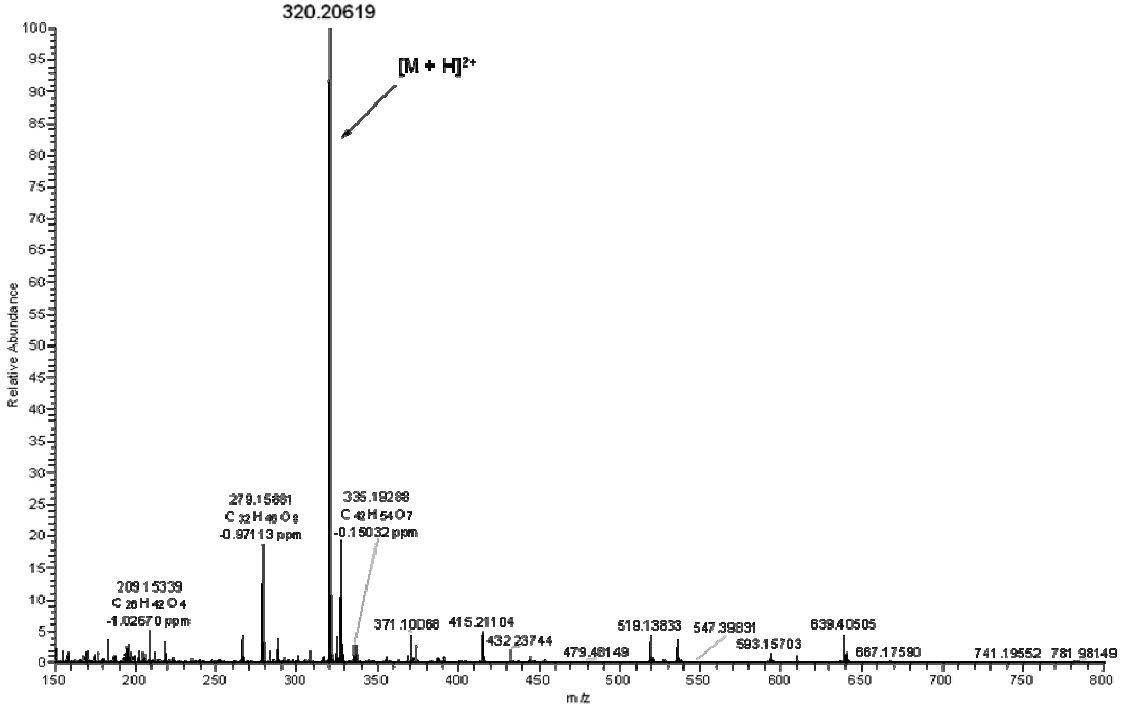
**RP-HPLC elution profile (system S1) of cyanine amino-amide 6.**



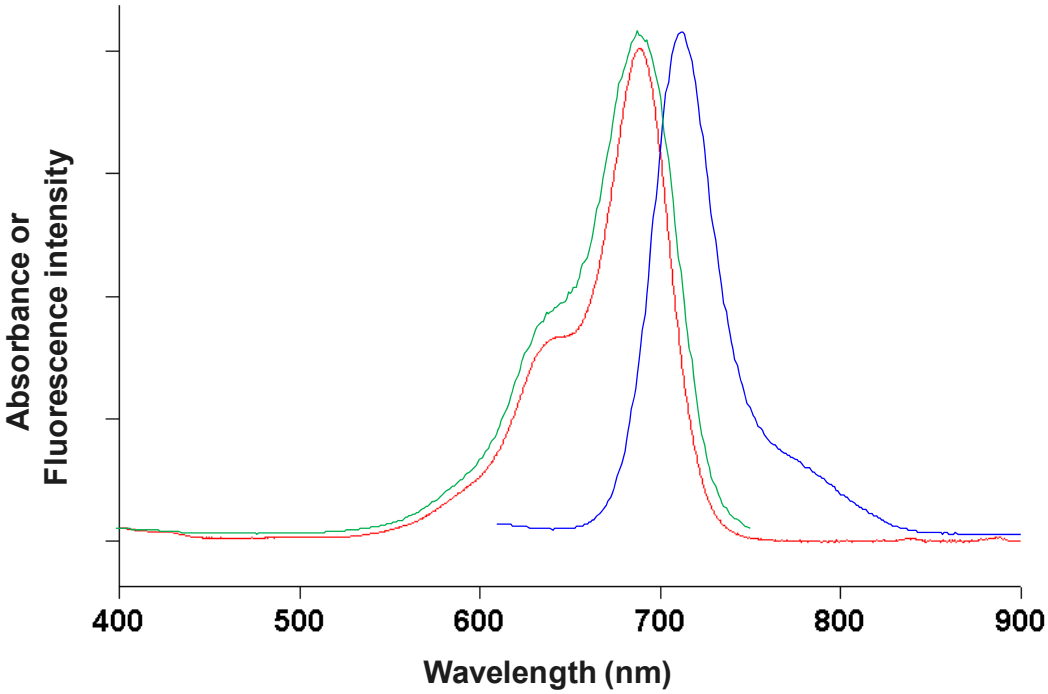
**(ESI+) mass spectrum of cyanine amino-amide 6.**



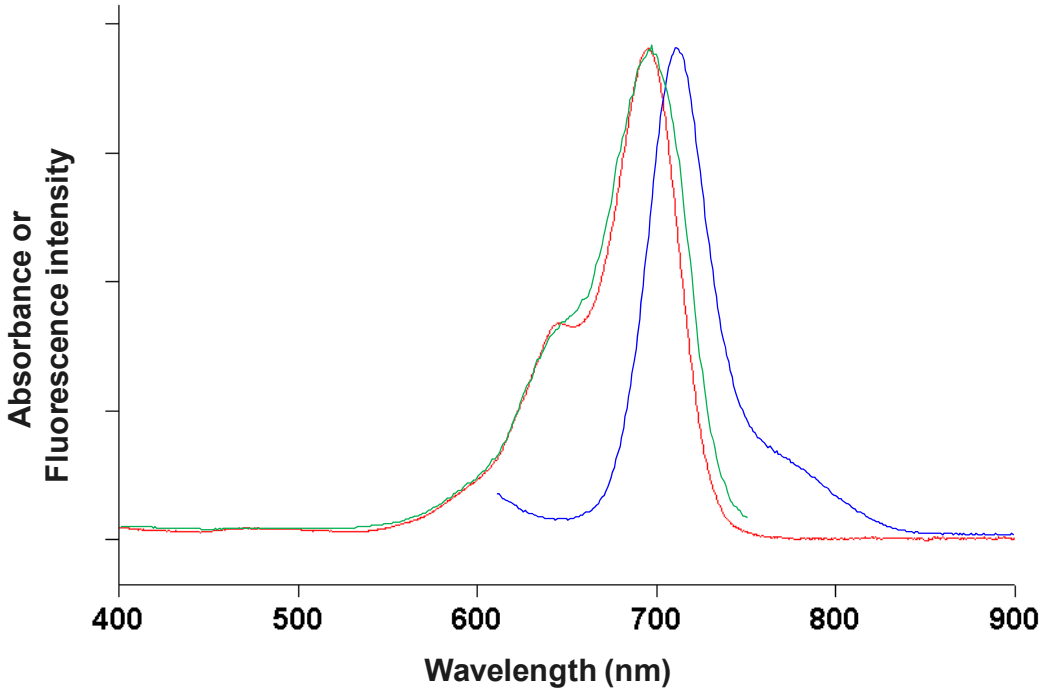

**(ESI+) mass spectrum (high resolution) of cyanine amino-amide 6.**



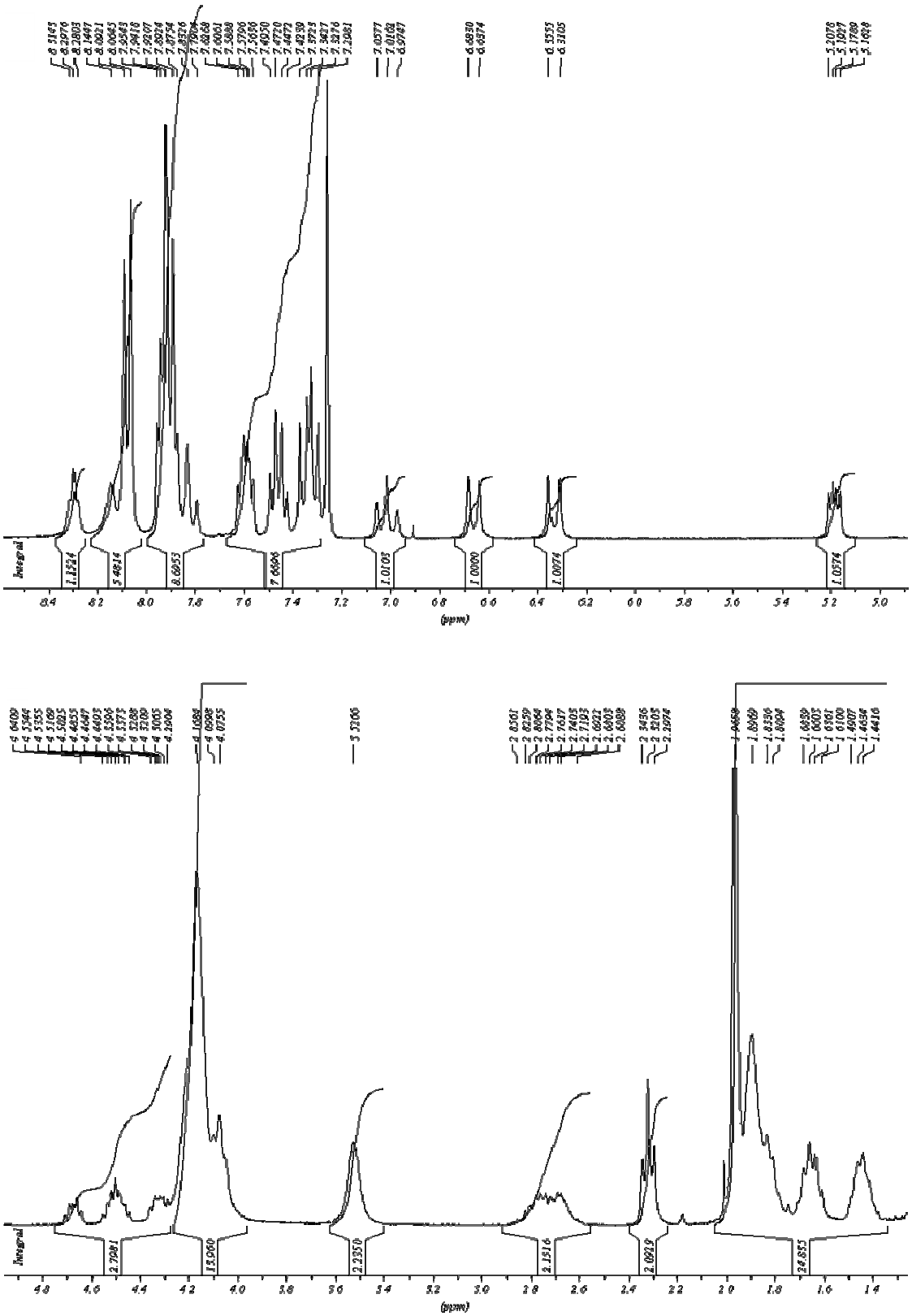
**Normalised absorption (—), emission (—) (Ex. 600 nm) and excitation (—) (Em. 760 nm) spectra of cyanine amino-amide 6 in DMSO at 25 °C.**



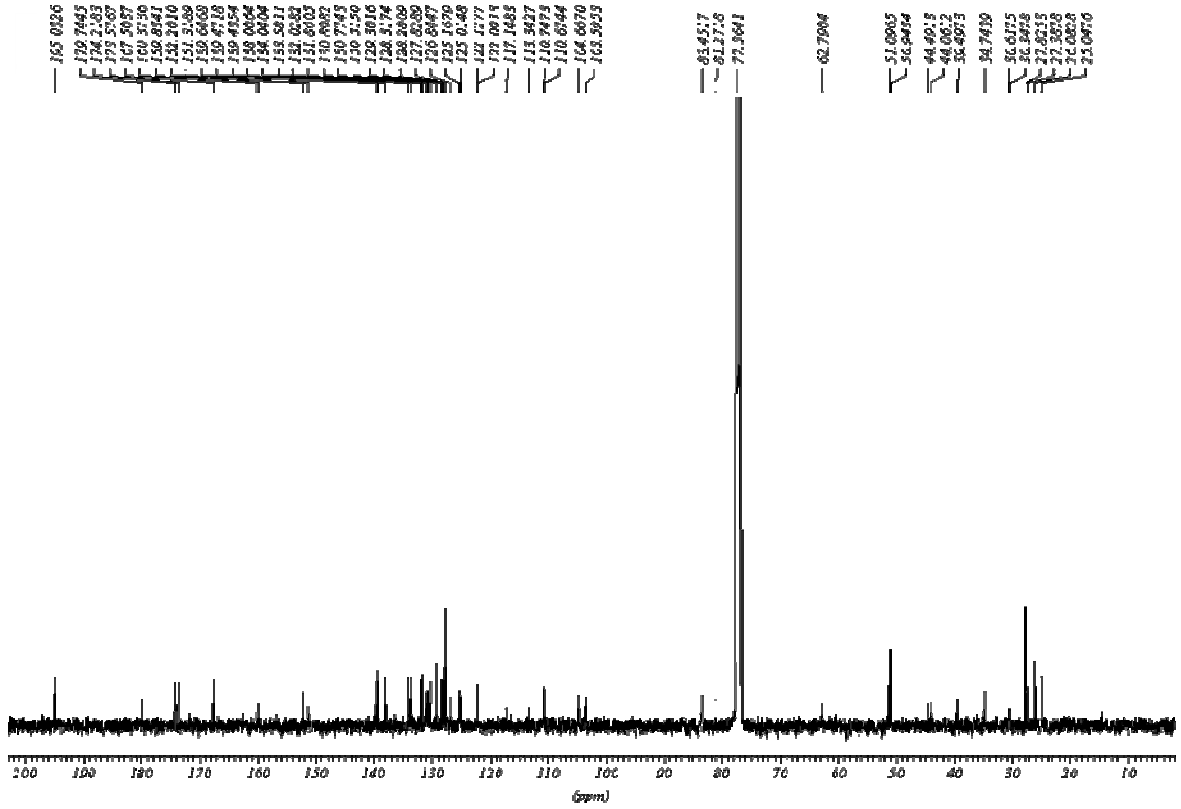

**Normalised absorption (—), emission (—) (Ex. 600 nm) and excitation (—) (Em. 760 nm) spectra of cyanine amino-amide 6 in PBS + 5% BSA at 25 °C.**



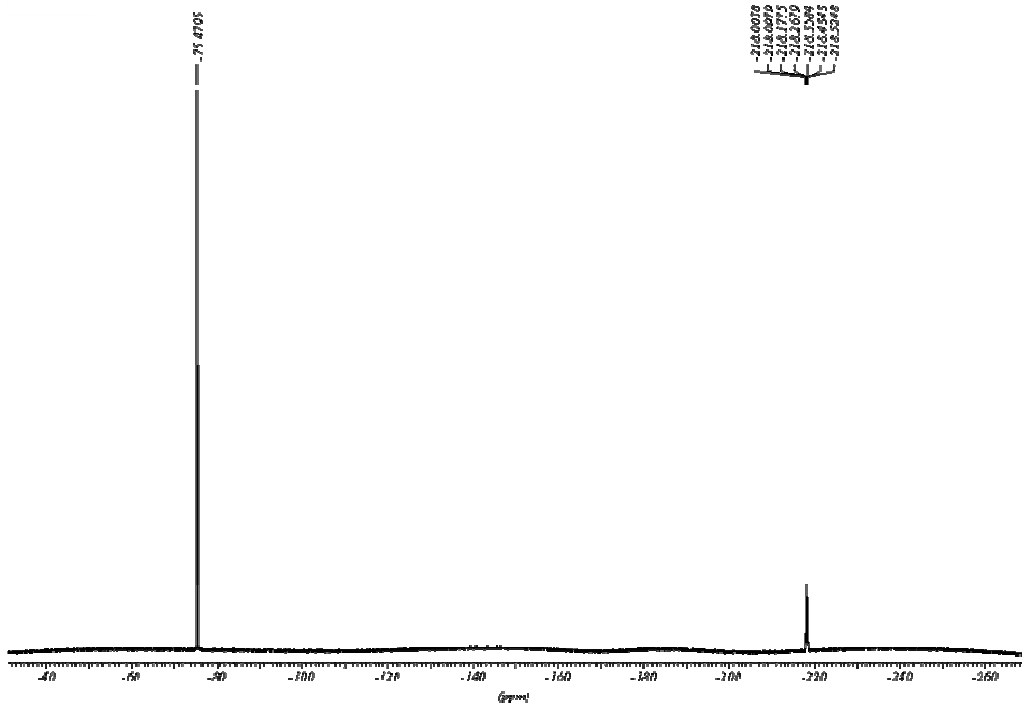
**$^1$H NMR spectrum of fluoro-monosulfonated cyanine "cold" reference 7 (TFA salt) recorded in $CDCl_3$ at 300 MHz.**



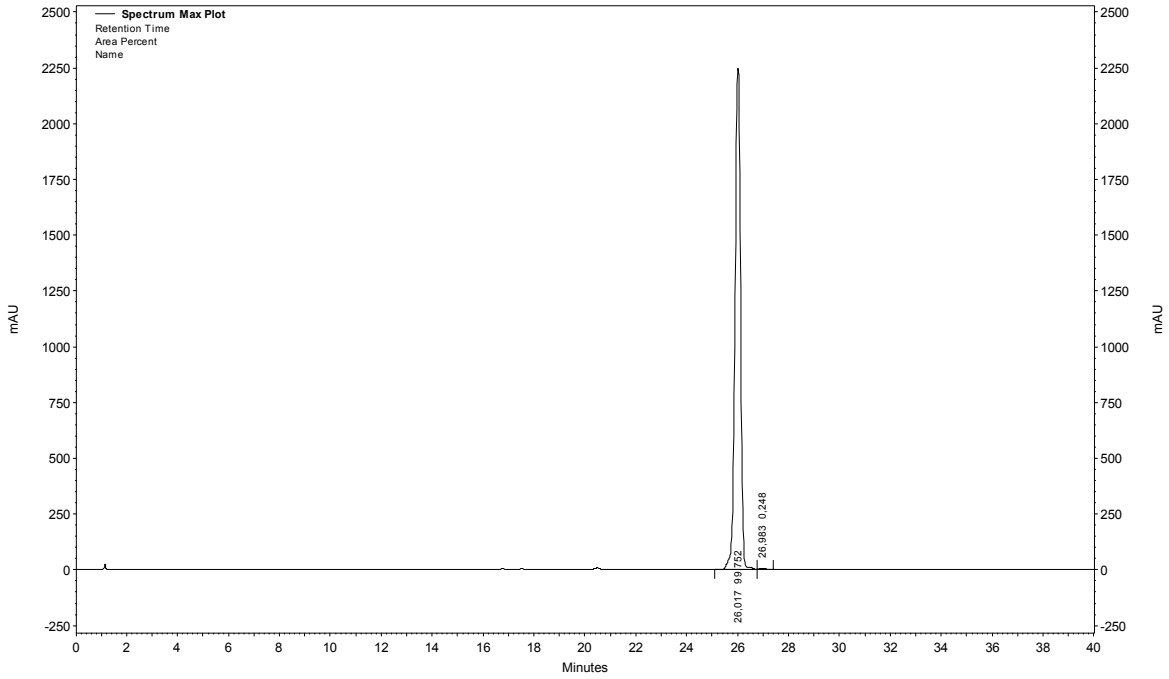
**$^{13}$C NMR spectrum of fluoro-monosulfonated cyanine "cold" reference 7 (TFA salt) recorded in $CDCl_3$ at 75.5 MHz.**



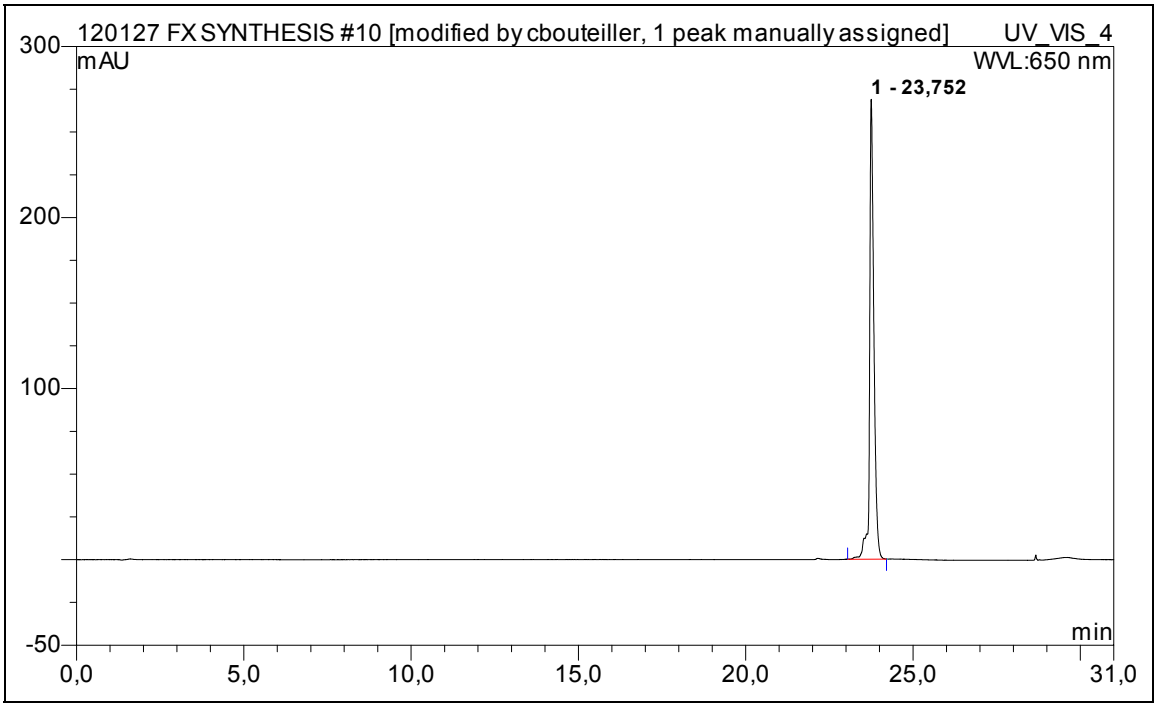
**$^{19}$F NMR spectrum of fluoro-monosulfonated cyanine "cold" reference 7 (TFA salt) recorded in $CDCl_3$ at 282.5 MHz.**



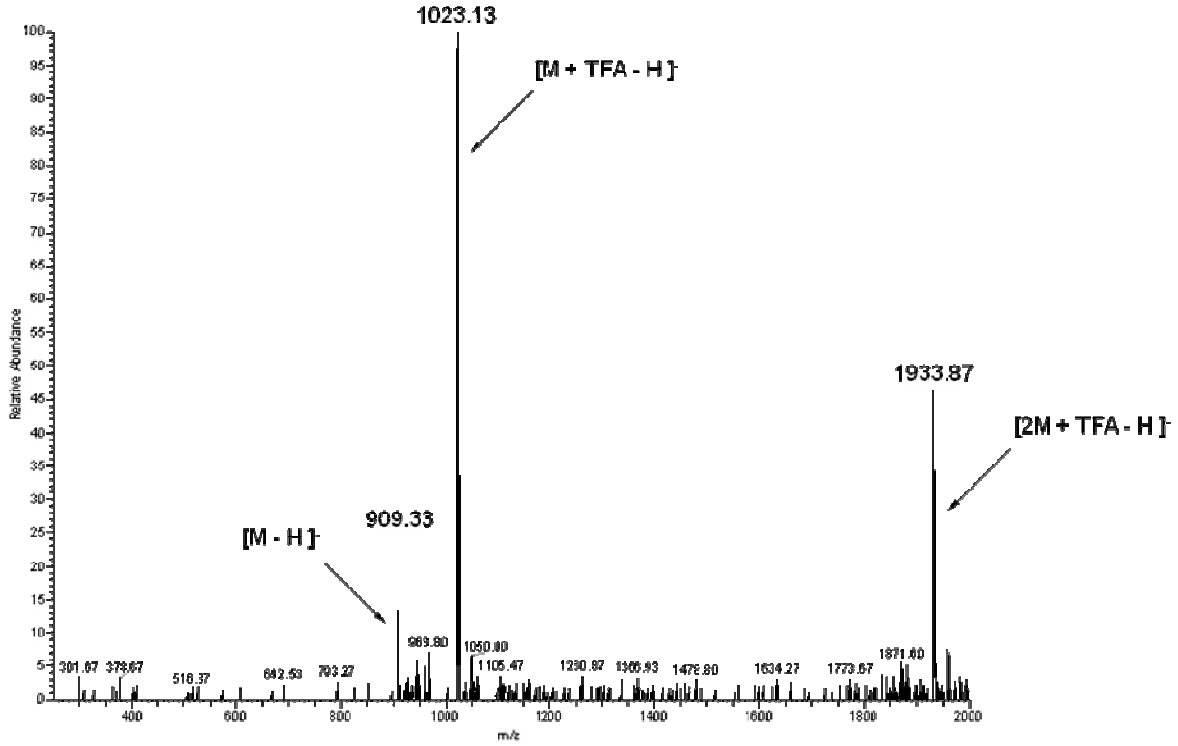
**RP-HPLC elution profile (system B) of fluoro-monosulfonated cyanine "cold" reference 7.**



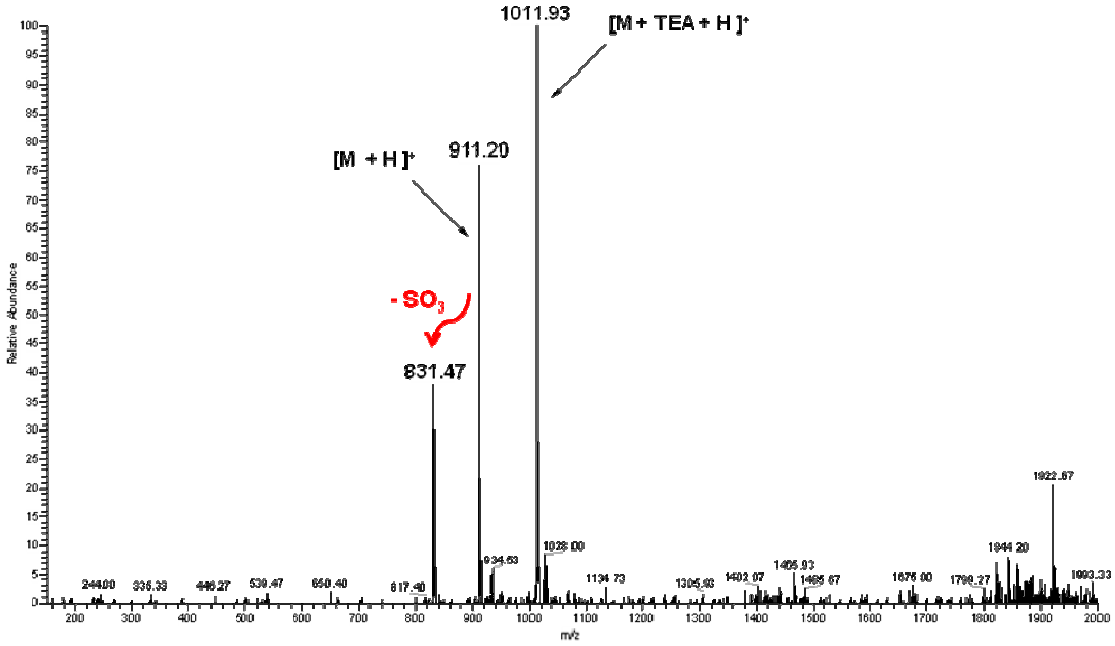

**RP-HPLC elution profile (system I) of fluoro-monosulfonated cyanine "cold" reference 7.**



**(ESI-) mass spectrum of fluoro-monosulfonated cyanine "cold" reference 7.**

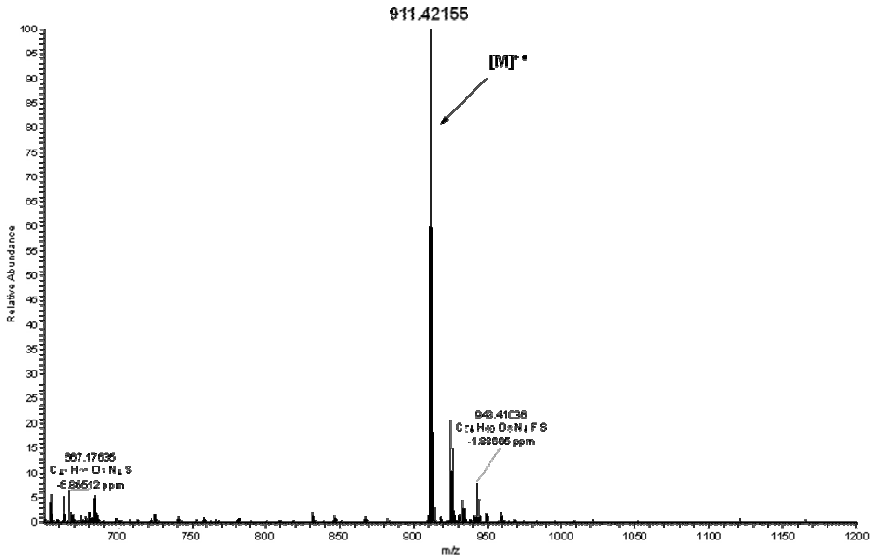



**(ESI+) mass spectrum of fluoro-monosulfonated cyanine "cold" reference 7.**

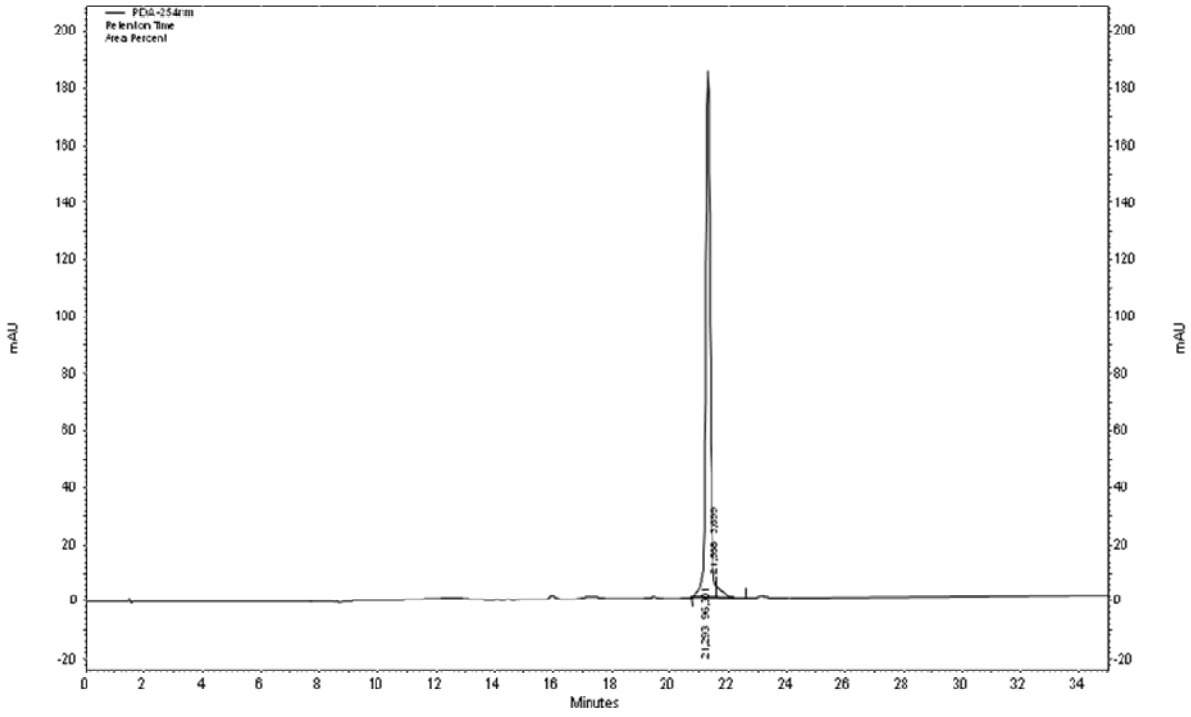



*The peak at m/z = 831.47 was assigned to the desulfonation derivative whose formation occurred during the ionisation process (ESI+).*

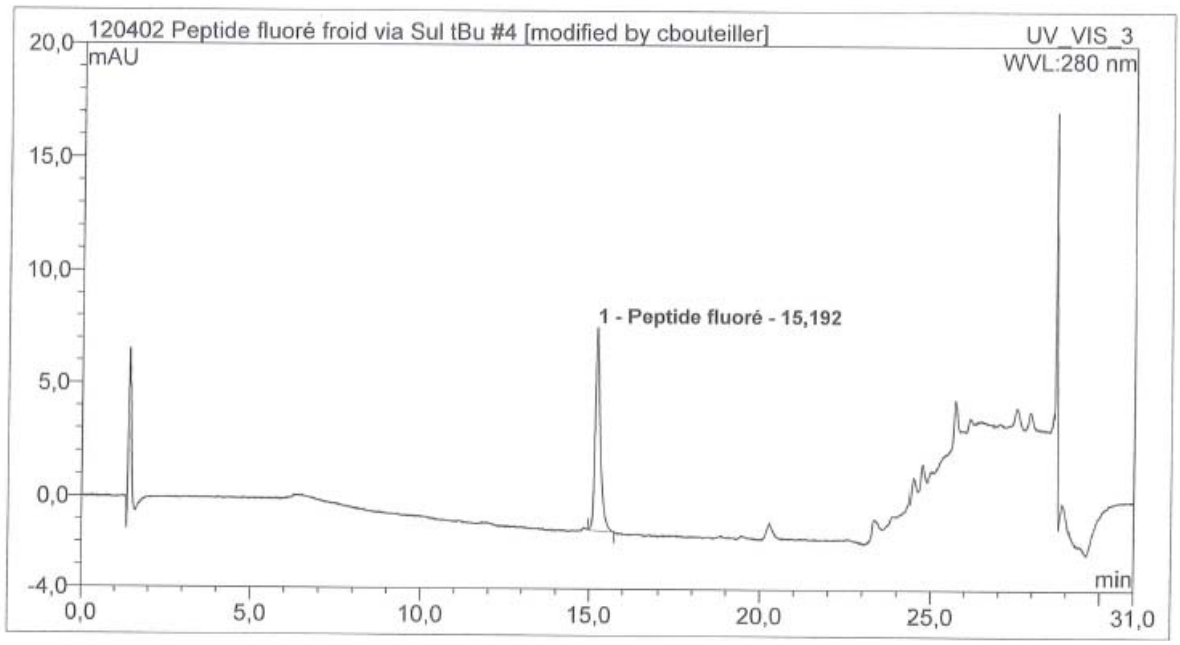
**(ESI+) mass spectrum (high resolution) of fluoro-monosulfonated cyanine "cold" reference 7.**



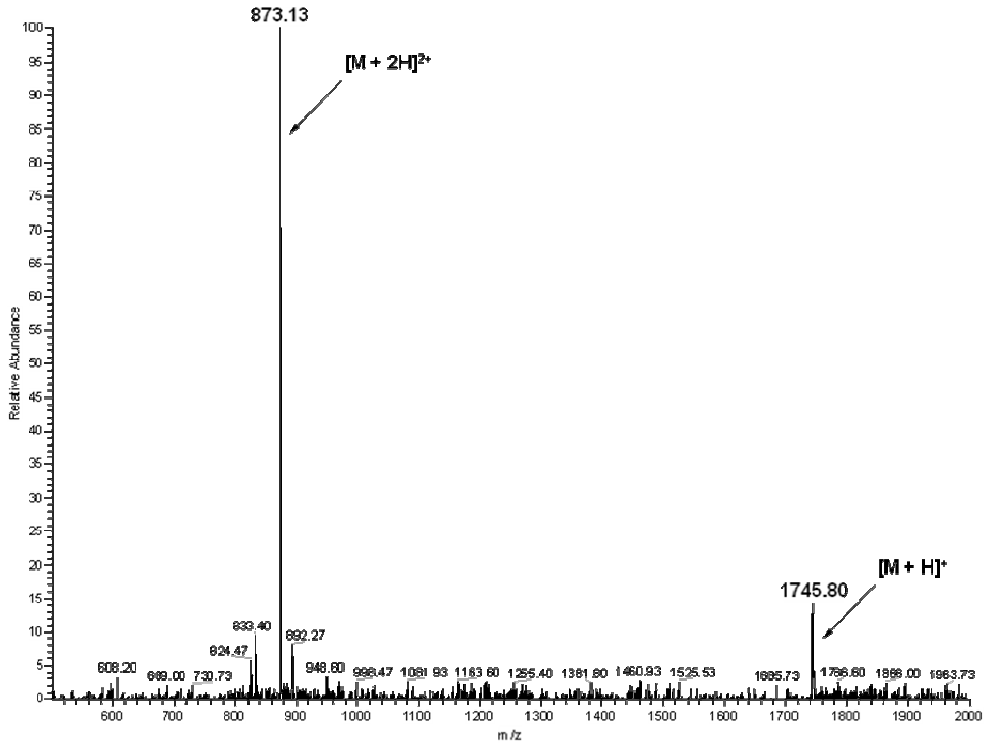

**RP-HPLC elution profile (system A) of fluoro-monosulfonated dodecapeptide "cold" reference 8.**



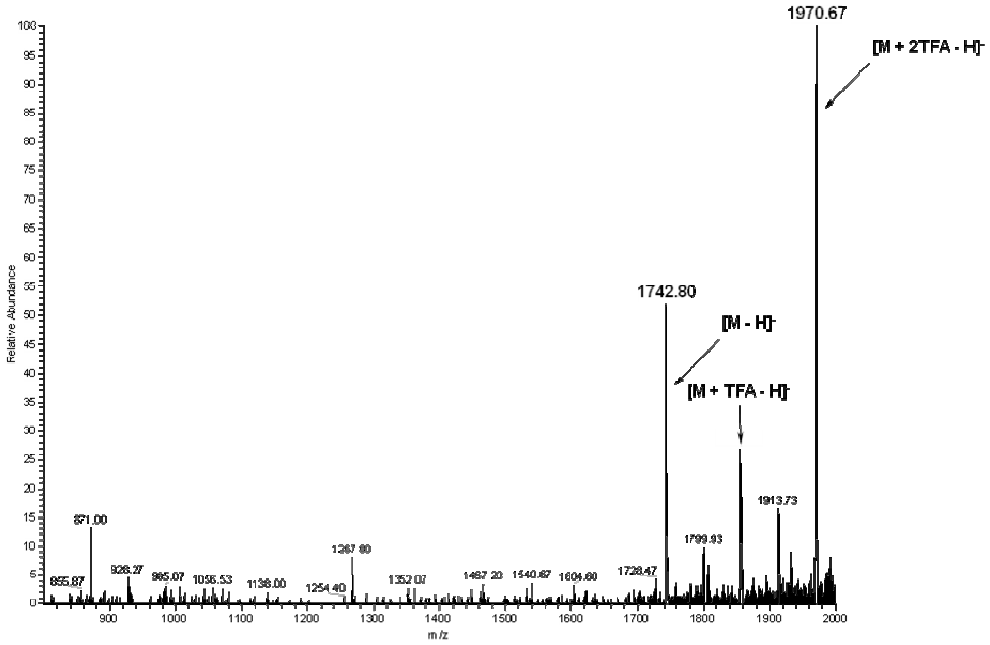
**RP-HPLC elution profile (system I) of fluoro-monosulfonated dodecapeptide "cold" reference 8.**



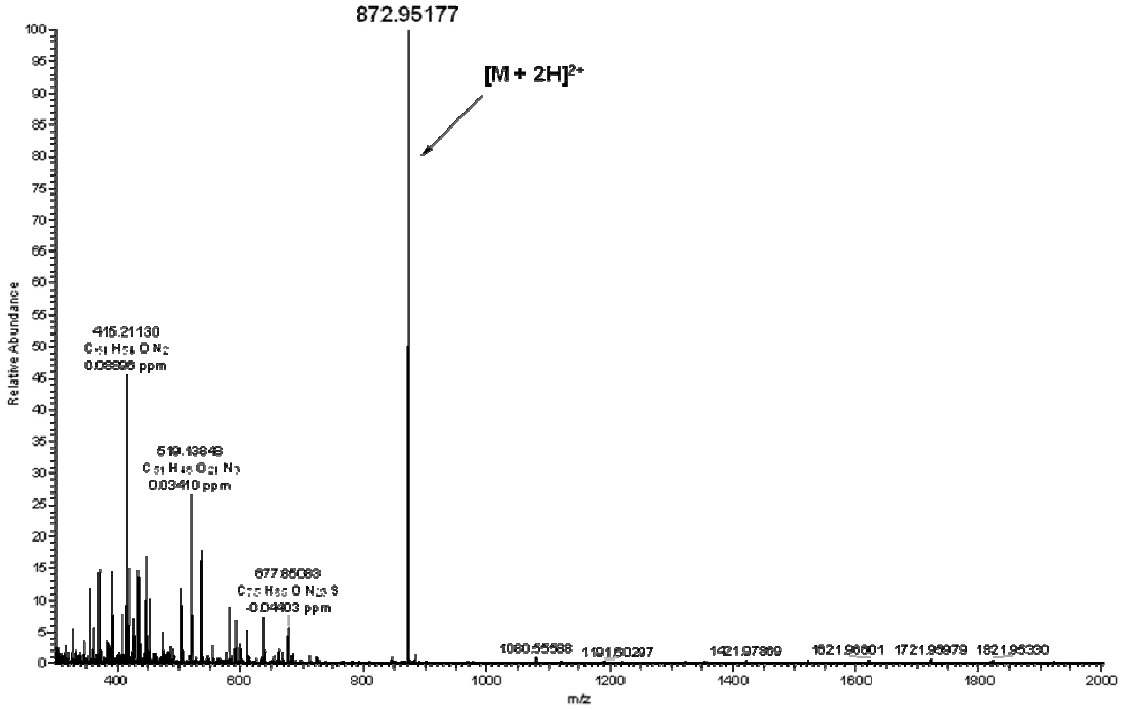

**(ESI+) mass spectrum of fluoro-monosulfonated dodecapeptide "cold" reference 8.**



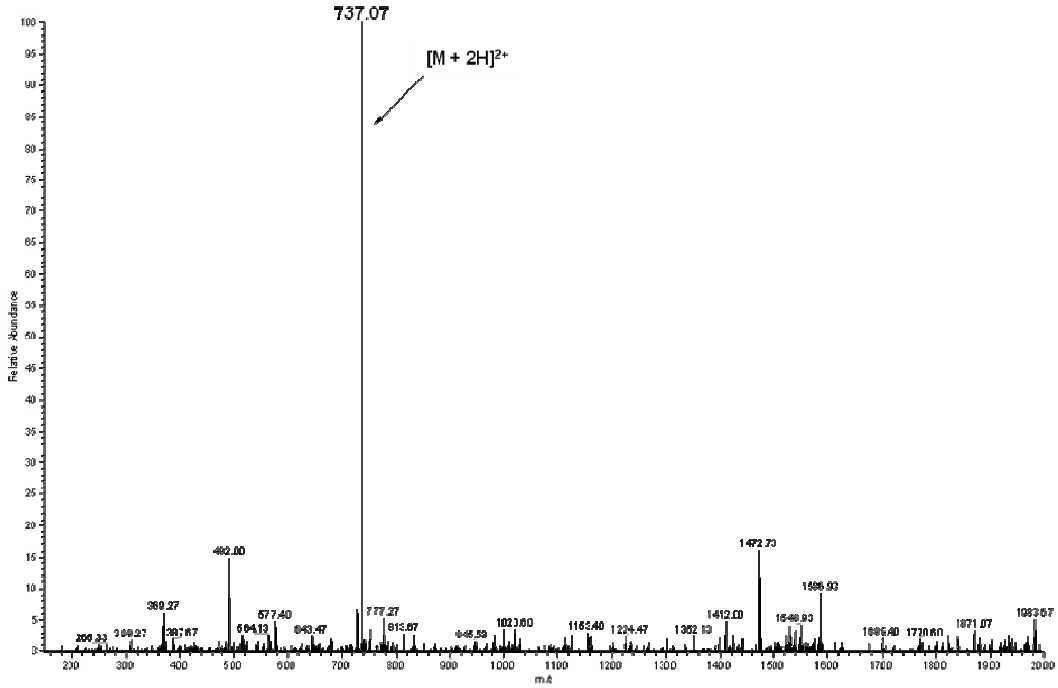
**(ESI-) mass spectrum of fluoro-monosulfonated dodecapeptide "cold" reference 8.**




**(ESI+) mass spectrum (high resolution) of fluoro-monosulfonated dodecapeptide "cold" reference 8.**



**(ESI+) mass spectrum of dodecapeptide before derivatisation with [$^{19}$F]-prosthetic group 1[a].**




[a]The mass difference between this peptide and the "cold" reference **8** (*vide supra*) is 272 Da that corresponds to the derivatisation of its lysine residue with [$^{19}$F]-prosthetic group **1**.