SuFEx click chemistry enabled late-stage drug functionalization

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1. EXPERIMENT SECTION

CHEMISTRY

General

^{1}H NMR spectra were recorded at 400 MHz on Bruker AV-400 NMR spectrometers; ^{13}C NMR were recorded at 151 MHz on Bruker AV-600. ^{19}F NMR were recorded at 376 MHz on Bruker AV-400. All chemical shifts (δ) are quoted in ppm; coupling constants (*J*) in hertz. Tetramethylsilane was used as international reference for ^{1}H and ^{13}C NMR. Trichlorofluoromethane was used as international reference for ^{19}F NMR. Abbreviations are: s, singlet; d, doublet; t, triplet; q, quartet, p, pentet; br s, broad singlet; m, multiplet. LC-MS was performed on an Agilent 1260 LC/MSD with an Agilent 6120 quadrupole mass spectrometer (electrospray ionization, ES) eluting with 0.05% trifluoroacetic acid in H_2O and 0.05% trifluoroacetic acid in CH_3CN. Precoated Merck F-254 silica gel plates were used for thin layer analytical chromography (TLC) and visualized with short wave (254 nm) UV light or by potassium permanganate stain. Column chromatography was performed using Silicycle Silica Gel 60 (40-63 μm). All phenol compounds were purchased from Selleck chemicals. Sulfuryl Fluoride (SO_2F_2) gas was a gift from Dow AgroSciences™. Phenolic compound library (10 mM in DMSO) were purchased from Selleck Chemicals (http://www.selleckchem.com).

Preparation of Sulfuryl Fluoride (SO_2F_2) or Thionyl Tetrafluoride (SOF_4) Solution in Organic Solvent

A glass vial of organic solvent (5 mL) was evacuated *in vacuo* and a balloon containing SO_2F_2 gas or Thionyl Tetrafluoride (SOF_4) gas was connected with the glass vial allowing it is filled with gas. Then the organic solvent was vigorously stirred for 30 min to make the stock solution of sulfuryl fluoride or thionyl tetrafluoride.

Condition Screening For *in situ* SuFEx

A solution of SO_2F_2 in organic solvent (CH_3CN, DCM or THF, 100 μL) and base (triethylamine (TEA) or *N*,*N*-Diisopropylethylamine (DIPEA), 1 μmol in 10 μL corresponding organic solvent) were added in an Eppendorf tube containing compound **3** (0.1 μmol in 10 μL DMSO). The tube was sealed by parafilm and left at room temperature for 3 hours before the product and yield was evaluated by LC-MS.

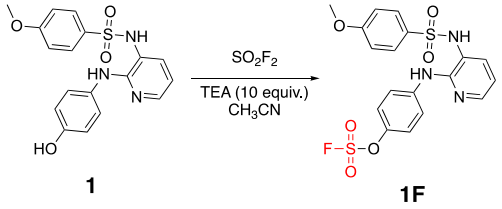
General Procedure I for the gas/liquid based SuFEx method on a 96-well plate

CH_3CN (100 μL) and TEA (1 μmol in 10 μL CH_3CN) were added in each well of a 96-well plate containing phenol compounds (0.1 μmol in 10 μL DMSO). The plate was left without lid in a vacuum desiccator containing SO_2F_2 (~1 atm) at room temperature overnight as shown in **Figure S1B**, before the products and yields were evaluated by LC-MS.

General Procedure II for the liquid based SuFEx method on a 96-well plate

A solution of SO_2F_2 in CH_3CN (~4 mg/mL, 100 μL) and TEA (1 μmol in 10 μL) were added in each well of a 96-well plate containing phenol compounds (0.1 μmol in 10 μL DMSO). The plate was left tightly covered by a solvent resistant sealing mat (Corning® 96 well storage system) at room temperature overnight as shown in **Figure S1C**. Then trimethylsilanol (2 μmol in 10 μL CH_3CN) was added to each well and left for 0.5 hours before the plate was left *in vacuo* overnight. The resulting crudes were dissolved in DMSO (10 μL, ~10 mM) before biological assays. The products and yields were determined by LC-MS.

Synthesis of 1F, 11F and 25F in milligram scale for IC_{50} evaluation

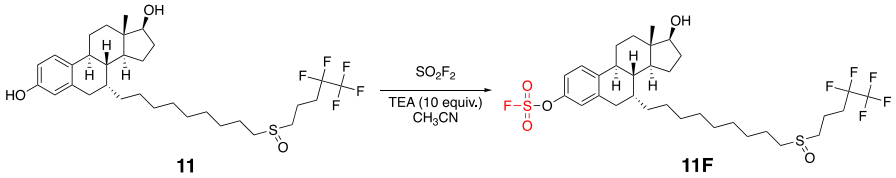


1 **1F**

4-((3-((4-Methoxyphenyl)sulfonamido)pyridin-2-yl)amino)phenyl sulfurofluoridate **1F**

TEA (27.2 mg, 0.27 mmol) was added in a solution of compound **1** (10 mg, 0.027 mmol) in CH_3CN (0.5 mL). After evacuation, SO_2F_2 gas was back-filled in the flask and the reaction mixture was left with stirring at room temperature for 12 hours until TLC (hexane/ethyl acetate, 1:1) showed the full conversion of starting material **1** to **1F** (R_f 0.70). Then volatiles were removed *in vacuo* and the resulting crude product was purified by flash column chromatography (hexane/ethyl acetate, 2:1 to 1:2) to obtain fluorosulfate **1F** (11.3 mg, 0.025 mmol, 93%) as a light red solid.

m.p. 158 °C– 160 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 8.15 (dd, 1H, *J* = 4 Hz, 8 Hz), 7.67 (t, 4H, *J* = 8 Hz), 7.26 (d, 2H, *J* = 8 Hz), 6.94 (d, 2H, *J* = 8 Hz), 6.81 (dd, 1H, *J* = 4 Hz, 8 Hz), 6.63 (dd, 1H, *J* = 4 Hz, 8 Hz), 3.85 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$): 164.0, 153.0, 147.4, 144.6, 140.8, 136.8, 130.2 (2C), 129.5, 121.6 (2C), 120.5 (2C), 118.1, 115.8, 114.7 (2C), 56.0; ^{19}F NMR (376 MHz, $CDCl_3$): 36.1; ESI (m/z): 454 $[M + H]^+$.

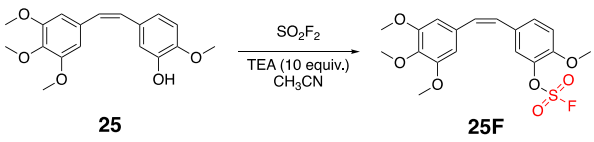


11 **11F**

(7*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-17-Hydroxy-13-methyl-7-(9-((4,4,5,5,5-pentafluoropentyl)sulfinyl)nonyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl sulfurofluoridate **11F**

TEA (41.5 mg, 0.41 mmol) was added in a solution of compound **11** (25 mg, 0.041 mmol) in CH_3CN (0.5 mL). After evacuation, SO_2F_2 gas was back-filled in the flask and the reaction mixture was left with stirring at room temperature for 1 hours until TLC (hexane/ethyl acetate, 1:1) showed the full conversion of starting material **11** to **11F** (R_f 0.40). Then volatiles were removed *in vacuo* and the resulting crude product was purified by flash column chromatography (hexane/ethyl acetate, 5:1 to 1:2) to obtain fluorosulfate **11F** (25.5 mg, 0.037 mmol, 90%) as a colorless foam.

^{1}H NMR (400 MHz, $CDCl_3$): 7.37 (d, 1H, *J* = 8 Hz), 7.09 (d, 1H, *J* = 8 Hz), 7.03 (s, 1H), 3.76 (t, 1H, *J* = 12 Hz), 2.93 (dd, 1H, *J* = 8 Hz, 16 Hz), 2.82-2.59 (m, 5H), 2.40 - 2.13 (m, 7H), 1.95 (d, *J* = 12 Hz), 1.80-1.72 (m, 3H), 1.65-1.59 (m, 4H), 1.53-1.19 (m, 20H), 0.98 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): 148.4, 140.8, 139.0, 128.3, 121.9, 118.0, 82.2, 53.0, 51.3, 46.8, 43.6, 41.8, 38.6, 37.1, 34.9, 33.3, 30.9, 30.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 27.4, 28.4, 26.0, 23.0, 22.92, 22.91, 15.0, 11.4; ^{19}F NMR (376 MHz, $CDCl_3$): 37.2, -85.7, -118.05 to -118.64 (m); ESI (m/z): 689 $[M + H]^+$



25 **25F**

(*Z*)-2-Methoxy-5-(3,4,5-trimethoxystyryl)phenyl sulfurofluoridate **25F**

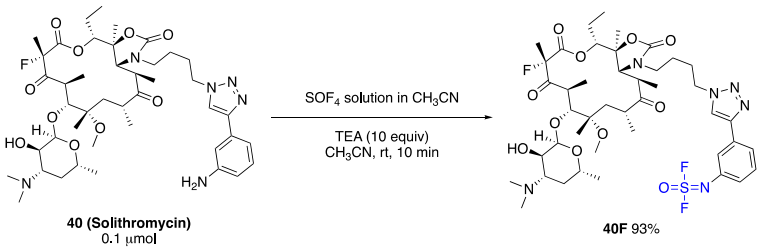
TEA (79.9 mg, 0.79 mmol) was added in a solution of compound **25** (25 mg, 0.079 mmol) in CH_3CN (0.5 mL). After evacuation, SO_2F_2 gas was back-filled in the flask and the reaction mixture was left with stirring at room temperature for 2 hours until TLC (hexane/ethyl acetate, 1:1) showed the formation of product **25F** (R_f 0.86). Then solvent was removed *in vacuo* and the resulting crude product was purified by flash column chromatography (hexane/ethyl acetate, 5:1 to 1:1) to obtain fluorosulfate **25F** (28.7 mg, 0.072 mmol, 91%) as a light-yellow syrup.

^{1}H NMR (400 MHz, $CDCl_3$): 7.26 (d, 1H, *J* = 4 Hz), 7.24 (d, 1H, *J* = 4 Hz), 7.19 (br s, 1H), 6.93 (d, 1H, *J* = 8 Hz), 6.57 (d, 1H, *J* = 12 Hz), 6.46 (d, 1H, *J* = 12 Hz), 6.43 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.70 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$): 153.6, 150.4, 150.3,

138.9, 138.8, 137.8, 132.3, 131.3, 130.8, 130.6, 127.8, 123.0, 113.3, 106.0 (2 x C), 61.3, 56.6, 56.2 (2 x C); ^{19}F NMR (376 MHz, $CDCl_3$): 39.5; EI (m/z): 399 $[M + H]^+$

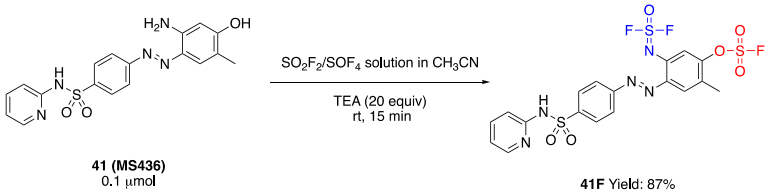
The reaction of amines with SOF_4 solution in CH_3CN

40 to 40F



A solution of SOF_4 in CH_3CN (40 μL) and TEA (1 μmol in 10 μL CH_3CN) was added in one Eppendorf tube containing phenol compound **40** (0.1 μmol in 10 μL DMSO). The tube was left tightly sealed by parafilm at room temperature. LC-MS indicated the formation of **40F** in a yield of 93% after 10 min.

41 to 41F



A solution of SO_2F_2 in CH_3CN (100 μL), a solution of SOF_4 in CH_3CN (200 μL) and TEA (1 μmol in 10 μL CH_3CN) was added in one Eppendorf tube containing phenol compound **41** (0.1 μmol in 10 μL DMSO). The tube was left tightly sealed by parafilm at room temperature. LC-MS indicated the formation of **41F** in a yield of 87% after 15 min.

BIOLOGY

Reagents and Software

All culture media are purchased from GIBCO®. Charcoal-stripped FBS, PolarScreen™ ERα competitor assay kit (Green) and CellTiter-Glo assay kit are purchased from Life Technologies. Mouse monoclonal anti-tublin-FITC antibody (DM1A) is from AbCam Inc. Rabbit monoclonal UGT1 antibody is from Cell Signalling Technology. Rabbit monoclonal ERα antibody is from AbCam Inc. Experimental data were processed by Prism 7 and western blot images were analyzed by ImageJ 1.50i.

Cell Culture

The MCF-7, A549, SKBR3 and HT-29 cells were originally purchased from ATCC. The T47D and ER$^-$ MCF-7 cells were kind gifts from Christopher K. Glass group (UCSD). All cancer cell lines were routinely maintained in DMEM (+Gluta™ MAX) supplement with 10% FBS and 1% peneciline/streptomycin in 5% carbon dioxide at a temperature of 37 °C.

Two-Dose Cell Viability Assay to Compare the Cytotoxicities of Fluorosulfurylation Products (1F to 39F) and Phenol Parents (1 to 39)

All cancer cells for the anti-cancer assay were grown in DMEM (+Gluta™ MAX) supplement with 5% FBS. MCF-7 and A549 cancer cells were inoculated into 96-well plates in 100 μL medium at a plating density of 5,000 cells/well. After cell inoculation, the plates were incubated at 37° C and 5 % CO_2. After 24 hours, all wells were refreshed with medium containing DMSO (0.2%, as vehicle control), phenols (20 μM or 500 nM) or *in situ* generated fluorosulfurylation products (20 μM or 500 nM). Then cancer cells were maintained at 37° C and 5 % CO_2 for 72 hours before cell viability ($V_{control}$, V_{phenol} and $V_{fluorosulfurylation\ product}$) were evaluated by

CellTiter-Glo Assay following manual of protocol. Cell viability percentage relative to vehicle control is defined as $V_{phenol}/V_{control}$ * 100% or $V_{fluorosulfurylation\ product}/V_{control}$ * 100%. **The cytotoxicity difference between a fluorosulfurylation product and its phenol precursor is quantified as ($V_{phenol}/V_{control}$ * 100%) - ($V_{fluorosulfurylation\ product}/V_{control}$ * 100%)**.

Cancer Cell Growth Inhibition Assay

Compounds Dilution

Experimental compounds were dissolved in DMSO at 500-fold of the desired final maximum test concentration. Then it was 10-fold serially diluted to other concentrations in DMSO. All aliquots were frozen prior to use. At the time of test, the aliquots of frozen concentrates are thawed and diluted to the desired final test concentrations with DMEM (+GlutaTM MAX) supplement with 5% FBS.

Growth Inhibition of MCF-7, A549, SKBR3 and HT-29 by **1** and **1F**

All cancer cells for the anti-cancer assay were grown in DMEM (+Gluta^{TM} MAX) supplement with 5% FBS. Cancer cells were inoculated into a 96-well plate in 100 μL medium at a plating density of 5,000 cells/well. Another same plate of cells was inoculated for the evaluation of time 0. After cell inoculation, the plates were incubated at 37 °C and 5 % CO_2 for 24 hours. Then the viabilities of cancer cells for time 0 were evaluated by CellTiter-Glo Assay following manual of protocol to obtain V_0. And another plate of cancer cells was refreshed with medium containing either DMSO (0.2%, as control) or test compounds (**1** or **1F**) in 7 different concentrations from 20 μM. After incubation for 72 hours, cell viabilities were evaluated by CellTiter-Glo Assay to obtain $V_{control}$ and V_{test}. The growth inhibition percentage is defined as $(V_{test} - V_0) / (V_{control} - V_0)$ * 100%. IC_{50} value were obtained from dose-response curves. All test included 3 repeats.

Growth Inhibition of MCF-7, T47D, ER$^-$ MCF-7 and A549 by **11** and **11F**

All cancer cells for the anti-cancer assay were grown in DMEM (+Gluta^{TM} MAX) supplement with 5% charcoal-stripped FBS and 0.01 nM 17β-estradiol. Cancer cells were inoculated into a 96-well plate in 100 μL medium at a plating density of 3,000 cells/well. Another same plate of cells was inoculated for the evaluation of time 0. After cell inoculation, the plates were incubated at 37 °C and 5 % CO_2 for 24 hours. Then the viabilities of cancer cells for time 0 were evaluated by CellTiter-Glo Assay following manual of protocol to obtain V_0. And another plate of cancer cells was refreshed with medium containing either DMSO (0.2%, as control) or test compounds (**11** or **11F**) in 5 to 8 different concentrations from 20 μM (2 μM for MCF-7 and T47D cells). Then cells were incubated for 6 days with one change of medium containing test compounds on day 3, before cell viabilities were evaluated by CellTiter-Glo Assay to obtain $V_{control}$ and V_{test}. The growth inhibition percentage is defined as $(V_{test} - V_0) / (V_{control} - V_0)$ * 100%. IC_{50} value were obtained from dose-response curves. All test included 3 repeats.

Growth Inhibition of MCF-7, A549, SKBR3 and HT-29 using **25** and **25F**

All cancer cells for the anti-cancer assay were grown in DMEM (+Gluta^{TM} MAX) supplement with 5% FBS. Cancer cells were inoculated into a 96-well plate in 100 μL medium at a plating density of 5,000 cells/well. Another same plate of cells was inoculated for the evaluation of time 0. After cell inoculation, the plates were incubated at 37 °C and 5 % CO_2 for 24 hours. Then the viabilities of cancer cells for time 0 were evaluated by CellTiter-Glo Assay to obtain V_0. And another plate of cancer cells was refreshed with medium containing either DMSO (0.2%, as control) or test compounds (**25** or **25F**) in 7 to 9 different concentrations from 20 μM (2 μM for MCF-7 cells). Then cells were incubation for 72 hours, before cell viabilities were evaluated by CellTiter-Glo Assay to obtain $V_{control}$ and V_{test}. The growth inhibition percentage is defined as $(V_{test} - V_0) / (V_{control} - V_0)$ * 100%. IC_{50} value were obtained from dose-response curves. All test included 3 repeats.

ER Binding Assay

Estrogen receptor binding assays were preformed using a PolarScreen^{TM} ERα competitor assay kit (Green) from Life Technologies following manual of protocol. This method uses recombinant ER and competition with a fluoromone ligand. The experiment included

7 concentrations of test compounds from 10^4 nM to 10^{-2} nM and three repeats for each concentration. Relative EC_{50} were obtained from dose-response curves.

Western Blot for ERα Downregulation

MCF-7 cells were inoculated in a 6-well plate at a seeding density of 10,000 cells/well in DMEM (+Gluta™ MAX) supplement with 5% FBS. After incubation for 24 hours, cells were exposed to either **11** or **11F** at the concentrations of 0 nM (0.2% DMSO), 3 nM, 9 nM and 27 nM for 5 days with daily changes of media containing test compounds. Then cells were lysed and stored at –80 °C until western blot for ERα. After cell lysis, denaturing and centrifuge at 20,000 rpm for 20 min, protein samples in equal volume were subjected to western protocol. Membranes were blocked and then incubated with 1:400 dilution of ERα antibody at 4 °C overnight followed by 1:5000 dilution of secondary antibody for 1 h at room temperature. GAPDH was detected as loading control. They were then imaged on a ChemiDoc™ imaging system (Bio-Rad).

Western Blot for UGT1 expression on A549, MCF-7 and HT-29 cells

A549, MCF-7 and HT-29 cells were inoculated in a 6-well plate at a seeding density of 100,000 cells/well in DMEM (+Gluta™ MAX) supplement with 5% FBS. After incubation for 24 hours, cells were incubated for 24 hours before cells were lysed and stored at –80 °C until western blot for UGT1. After cell lysis, denaturing and centrifuge at 20,000 rpm for 20 min, protein samples in equal volume were subjected to western protocol. Membranes were blocked and then incubated with 1:500 dilution of UGT1 antibody at 4 °C overnight followed by 1:5000 dilution of secondary antibody for 1 h at room temperature. GAPDH was detected as loading control. They were then imaged on a ChemiDoc™ imaging system (Bio-Rad).

Immunofluorescence Microscopy of HT-29 Microtubule Disruption induced by 25 and 25F

HT-29 were inoculated in a 4-well chamber at a seeding density of 10,000 cells/well in DMEM (+Gluta™ MAX) supplement with 5% FBS. After incubation at 37 °C for 24 hours, cells were treated with media containing DMSO (0.2%), **25** (1 μM, 0.1 μM) or **25F** (1 μM, 0.1 μM, 0.01 μM) for 24 hours. Then cells were gently washed in PBS, fixed for 20 min with 4% paraformaldehyde in PBS and permeabilised in 0.5% Triton X-100. Following washes in PBS containing 0.1% Tween (PBST), cells were blocked in 5% bovine serum albumin diluted in PBST. Then cells were incubated with mouse monoclonal anti-tublin-FITC antibody (DM1A) (1:100 in PBS) for 3 hours. After washing with PBST, cells were incubated with DAPI (1:2000) for 30 min and mounted in PBS for confocal analysis. Images were captured by Nikon spinning disk confocal microscopy. All images in each experiment were collected on the same day using identical parameters.

2. SUPPLEMENTARY FIGURES

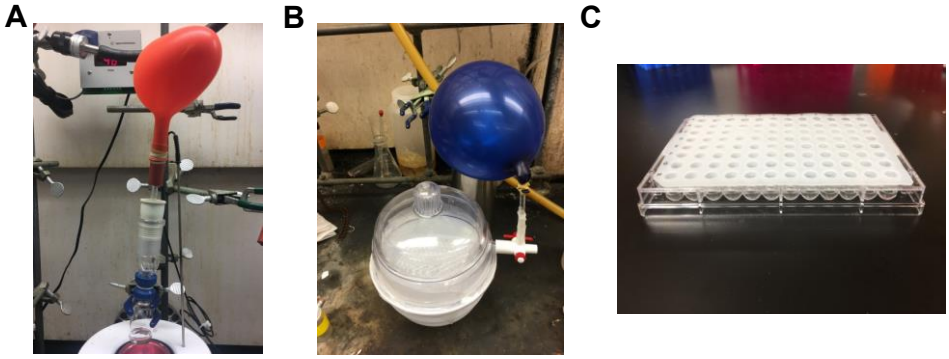
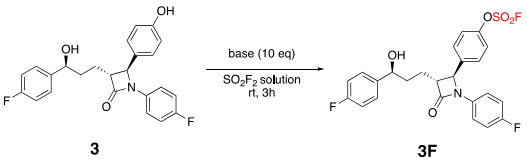


Figure S1. Photographs of gas-based and liquid-based SuFEx systems. (A) Gas-liquid based standard chemical synthesis using a flask. (B) Gas-liquid based synthesis using a 96 well plate and a vacuum dessicator. (C) Liquid based synthesis using a 96 well plate.



3 3F

	Yields in different SO_2F_2 SOLVENTS*		
BASES	CH_3CN	DCM	THF
TEA (10 eq)	quant**	<50%	<50%
DIPEA (10 eq)	quant	<50%	<50%

*: All yields were determined by LC-MS
**: quantitative yield

Figure S2. Solvent/base screening for *in situ* SuFEx protocol.

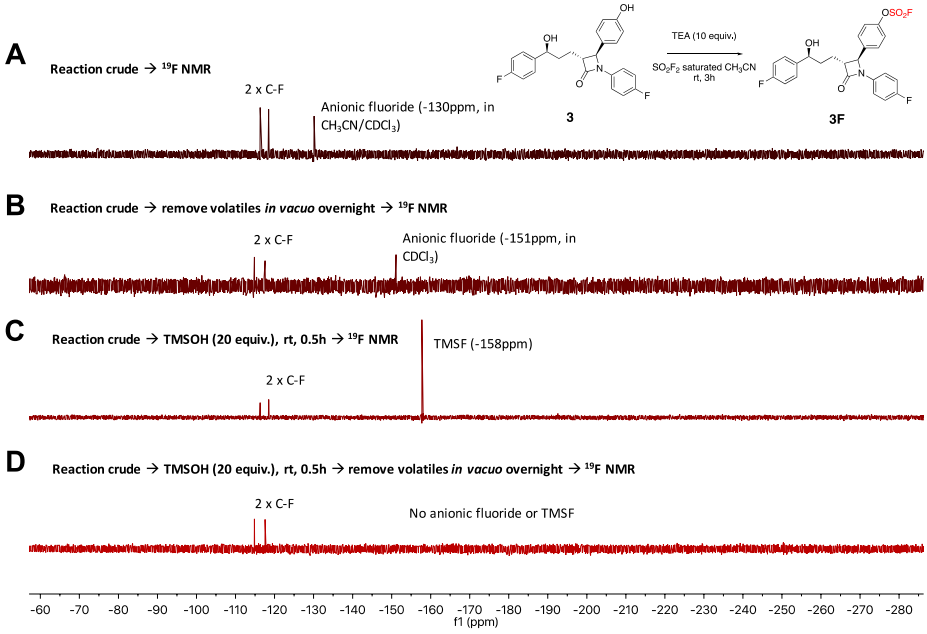
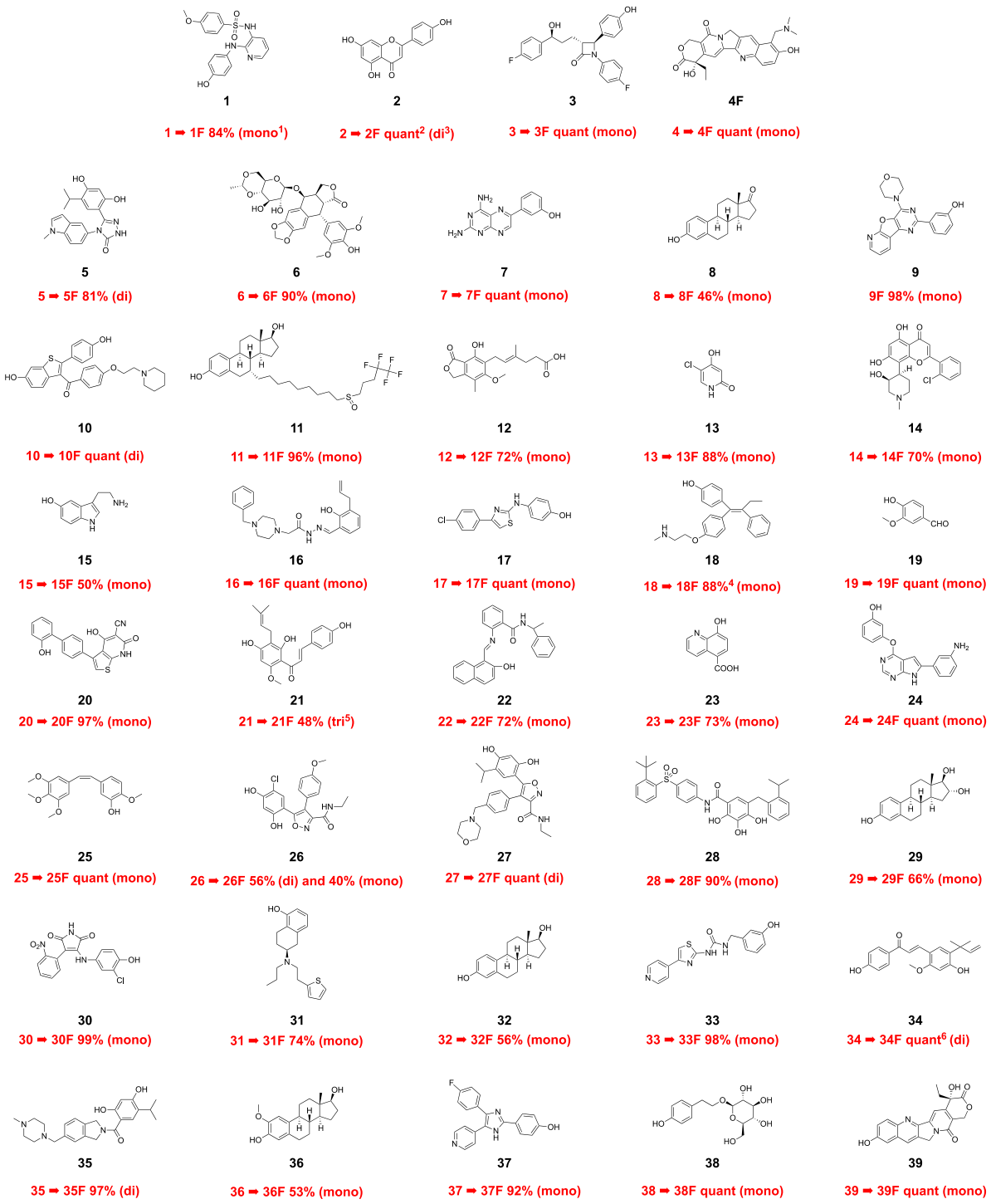


Figure S3. Demonstration of fluoride ions removal through TMSOH work-up. (A) ^{19}F NMR showed the reaction mixture contains anionic fluoride (-130 ppm, in $CH_3CN/CDCl_3$); (B) Volatiles in the reaction mixture were removed *in vacuo* overnight, ^{19}F NMR showed the presence of anionic fluoride (-151 ppm, in $CDCl_3$); (C) After reaction, the reaction mixture was treated with TMSOH (2 μmol, 20 eq) for 30 min and ^{19}F NMR showed the formation of TMSF (-158 ppm, in $CH_3CN/CDCl_3$); (D) After treated with TMSOH, volatiles in the reaction mixture were removed *in vacuo* overnight and ^{19}F NMR showed no presence of anionic fluoride or TMSF. Full ^{19}F spectra are showed in NMR section.



1. One -SO_2F was installed according to LC-MS.
2. Quantitative yield.
3. Two -SO_2F were installed according to LC-MS.
4. **18F** is a mixutre of E/Z isomers since starting material **18** contains both E/Z isomers.
5. Three -SO_2F were installed according to LC-MS.
6. **34F** is a mixture of E/Z isomers since starting material **34** contains both E/Z isomers.

Figure S4. Thirty-nine phenol compounds for *in situ* SuFEx. The thirty-nine parent phenolic compounds are all selected from anti-cancer compound libraries of *Selleckchem* as a solution in DMSO (10 mM), which are named compound **1** to **39**. They were transformed to

corresponding crude products **1F** to **39F** by *in situ* SuFEx protocol introduced above. The yields and numbers of fluorosulfates moieties installed are showed below each structure (red).

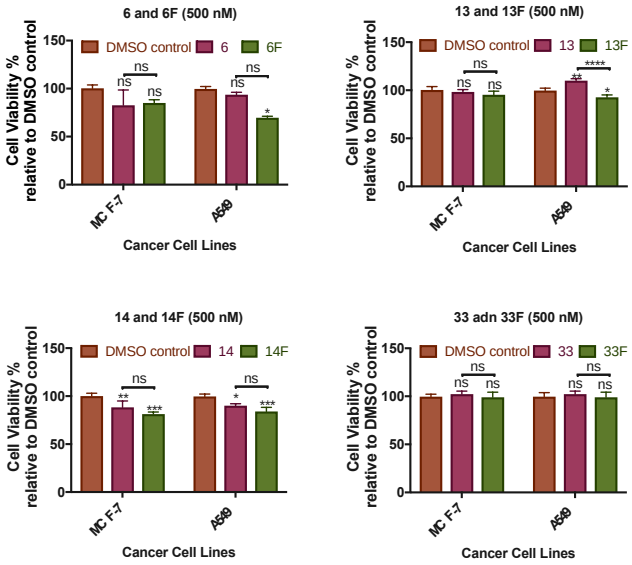
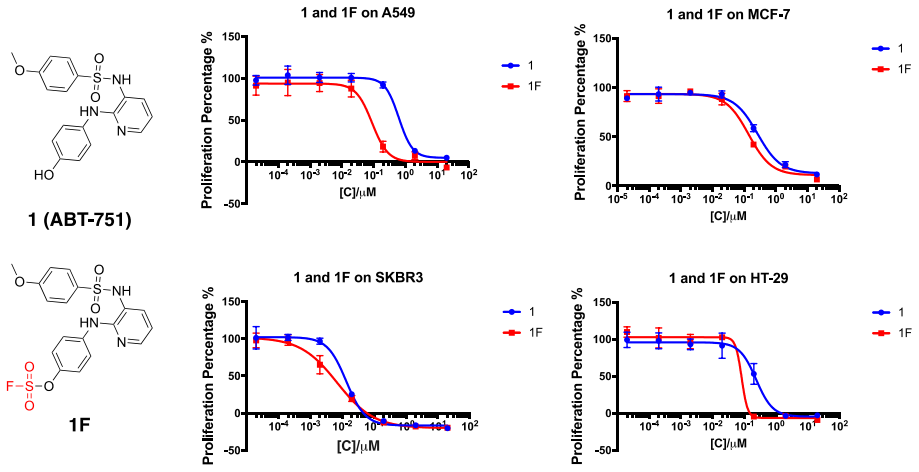


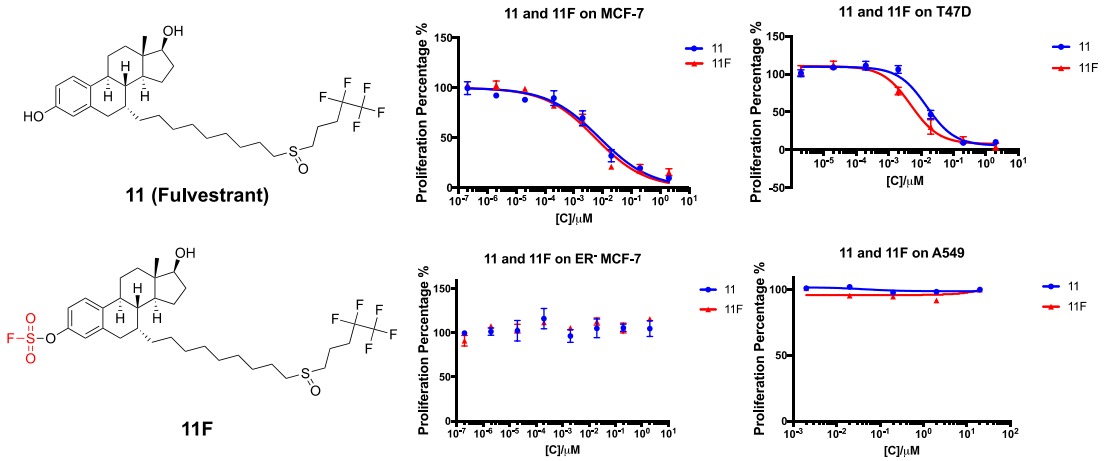
Figure S5. Representative examples of cancer cell viability assay results of the *in situ* SuFEx generated **6F**, **13F**, **14F** and **33F** and their phenol precursors (500 nM). P values were calculated using two-way ANOVA. Error bars represent the mean ± SEM (n = 3); ns: P≥0.05; *: P≤0.1; **: P≤0.01; ***: P≤0.001; ****: P≤0.0001.



	IC$_{50}$ (nM) on Different Cancer Cell Lines			
	A549	**MCF-7**	**SKBR3**	**HT-29**
1 (ABT-751)	632	266	13.5	236
1F	**89**	**134**	**6.9**	**82**

* NI : No inhibition (less than 50% inhibition at 10000 nM)

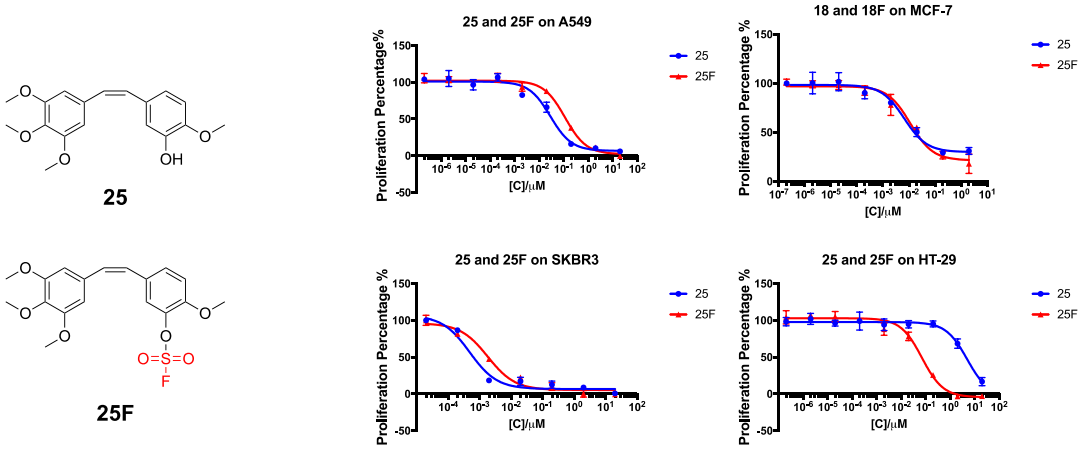
Figure S6. Proliferation inhibition curves of **1** and **1F** on different cancer cell lines. Error bars represent the mean ± SEM (n = 3).



IC_{50} (nM) on Different Cancer Cell Lines				
	A549	MCF-7	ER⁻ MCF-7	T47D
11 (Fulvenstrant)	NI*	7.7	NI	14.8
11F	NI	5.5	NI	4.8

* NI : No inhibition (less than 50% inhibition at 10000 nM)

Figure S7. Proliferation inhibition curves of **11** and **11F** on different cancer cell lines. Error bars represent the mean ± SEM (n = 3).



IC_{50} (nM) on Different Cancer Cell Lines				
	A549	MCF-7	SKBR3	HT-29
25 (Combretastatin A4)	28	6.6	0.48	4947
25F	113	15.6	1.8	70

* NI : No inhibition (less than 50% inhibition at 10000 nM)

Figure S8. Proliferation inhibition curves of **25** and **25F** on different cancer cell lines. Error bars represent the mean ± SEM (n = 3).

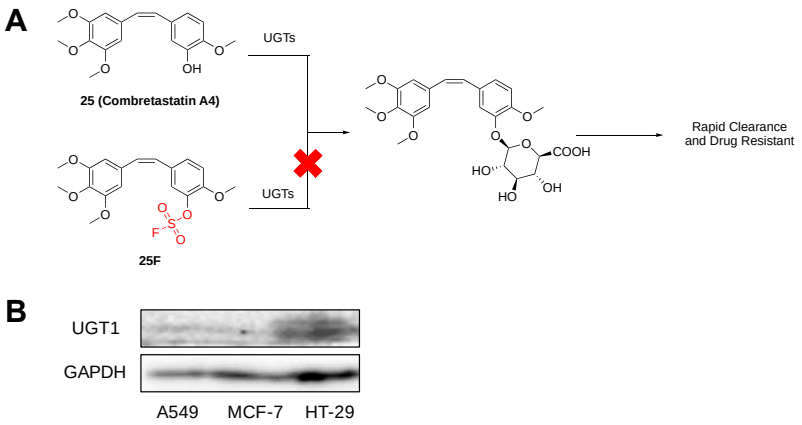


Figure S9. Combretastatin drug resistance on HT-29. (A) Mechanism of Combretastatin A4 resistance by UDP-glucuronosyltransferases (UGTs) in HT-29 colon cancer cells; (B) UGT1 expression levels in A549, MCF-7 and HT-29 cells.

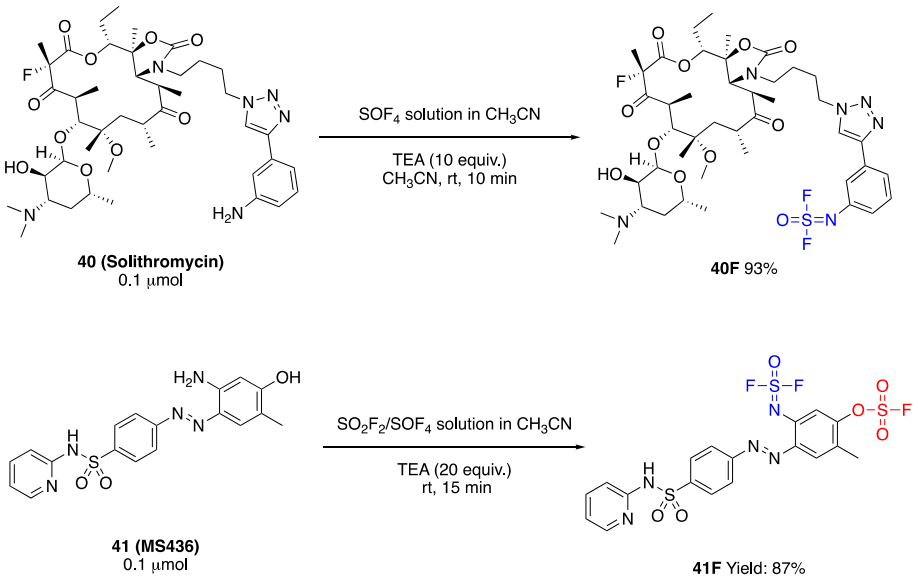
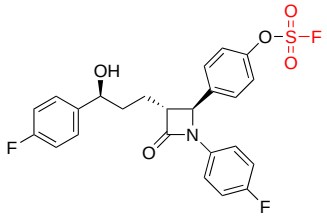


Figure S10. Expansion of liquid-based SuFEx protocols. (A) Selective functionalization of amine moiety to tetrahedral iminosulfur oxydifluorides in Solithromycin. (B) Simultaneous and selective generations of fluorosulfates and tetrahedral iminosulfur oxydifluorides on a bioactive compound.

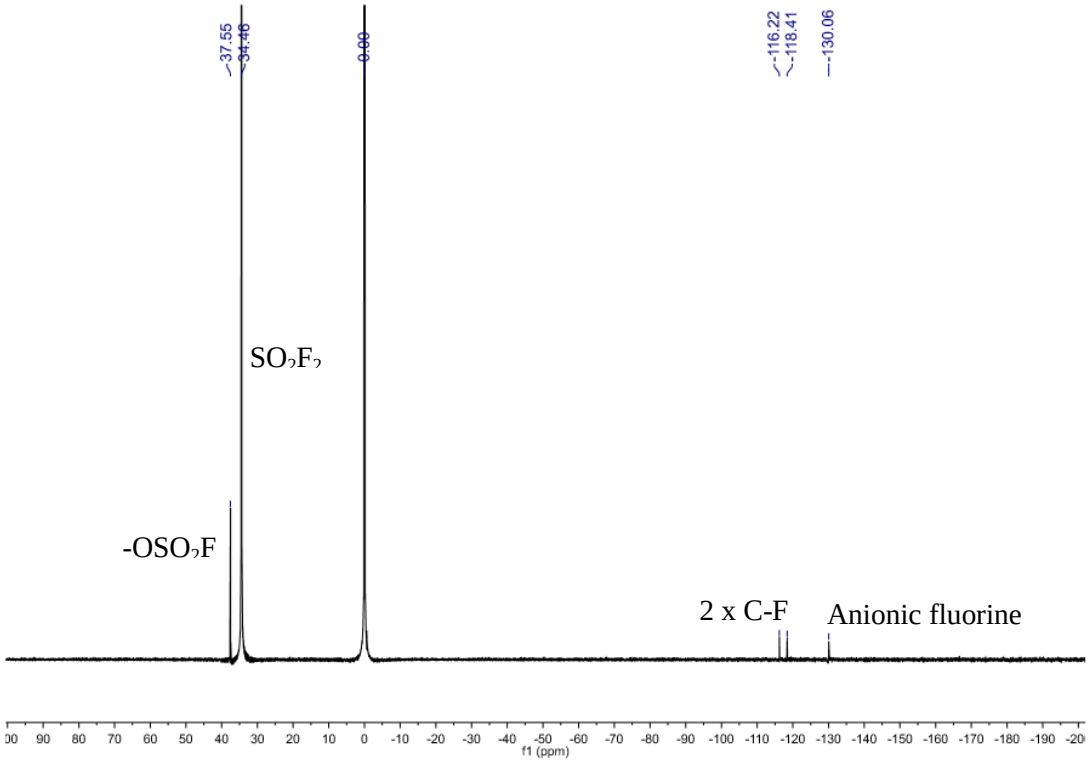
3. NMR SPECTRA

1. Full ^{19}F NMR spectra of crude 3F by different work-up procedures described in Figure S3

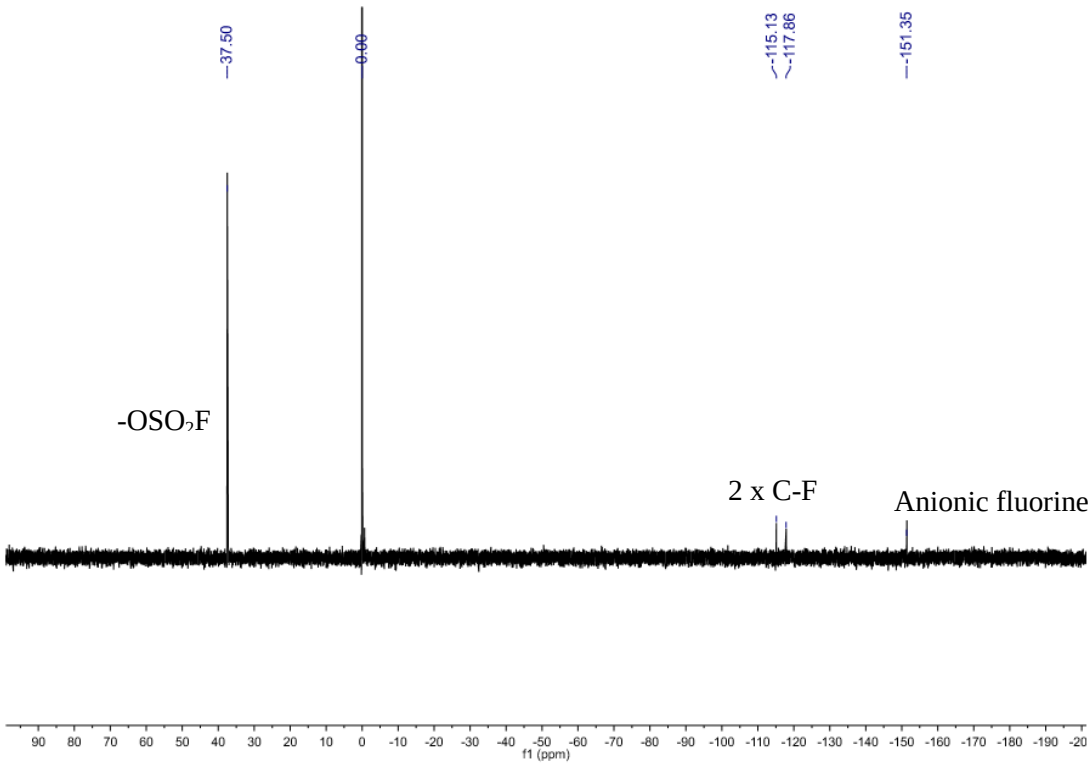


3F

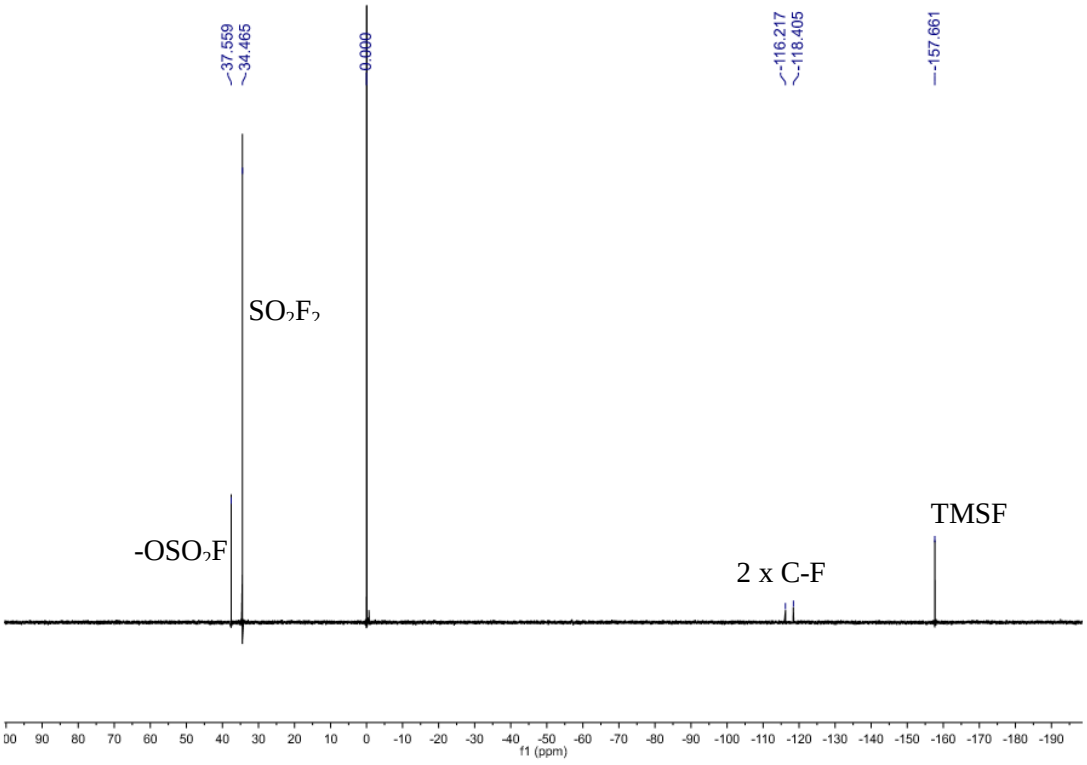
A. Reaction crude → ^{19}F NMR in $CH_3CN/CDCl_3$



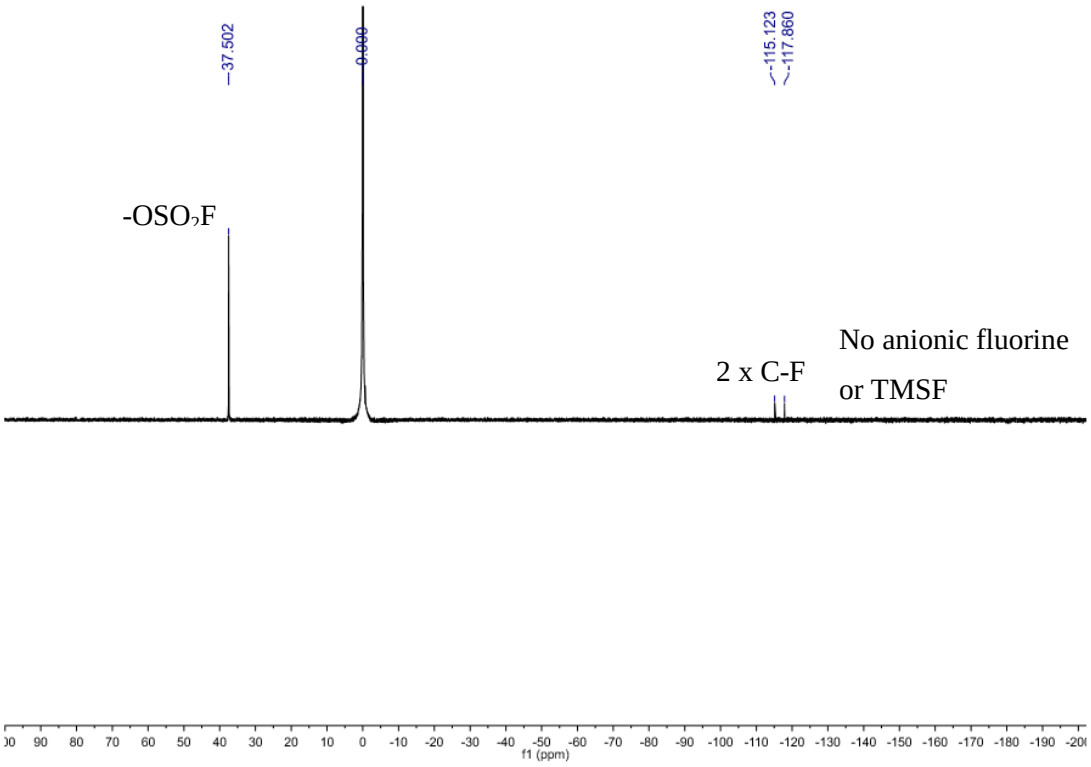
B. Reaction crude → remove solvent *in vacuo* overnight → ^{19}F NMR in $CDCl_3$



C. Reaction crude → TMSOH (20 equiv.), rt, 0.5 h → ^{19}F NMR in $CH_3CN/CDCl_3$

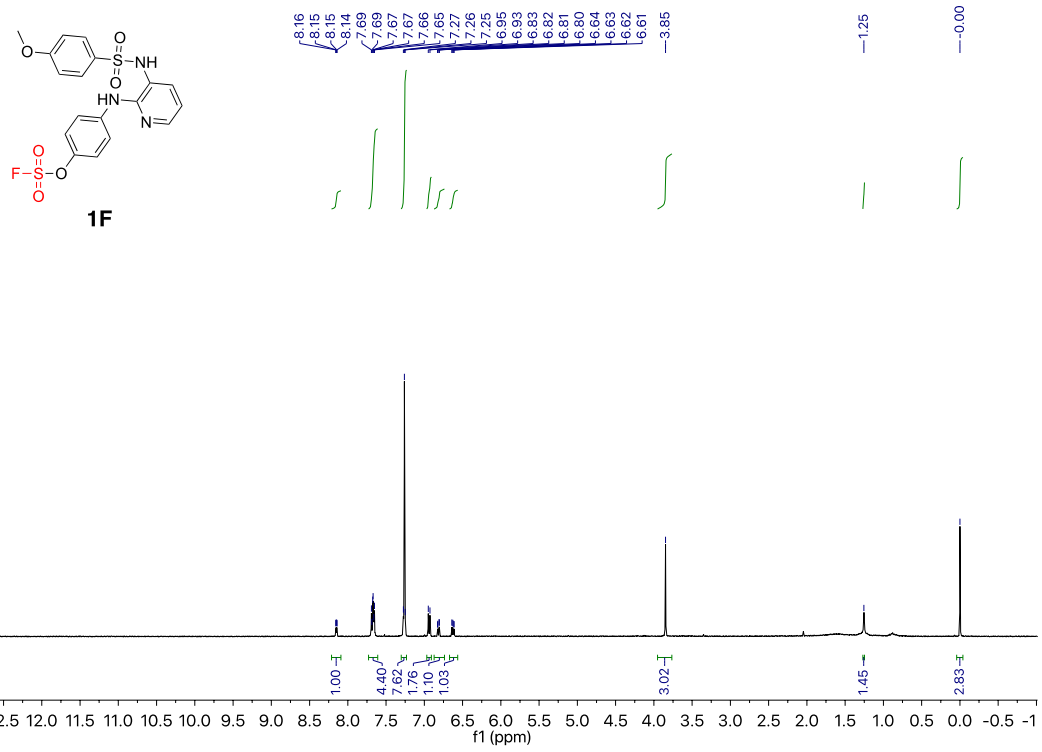


D. Reaction crude → TMSOH (20 equiv.), rt, 0.5 h → remove solvent *in vacuo* overnight → ^{19}F NMR in $CDCl_3$

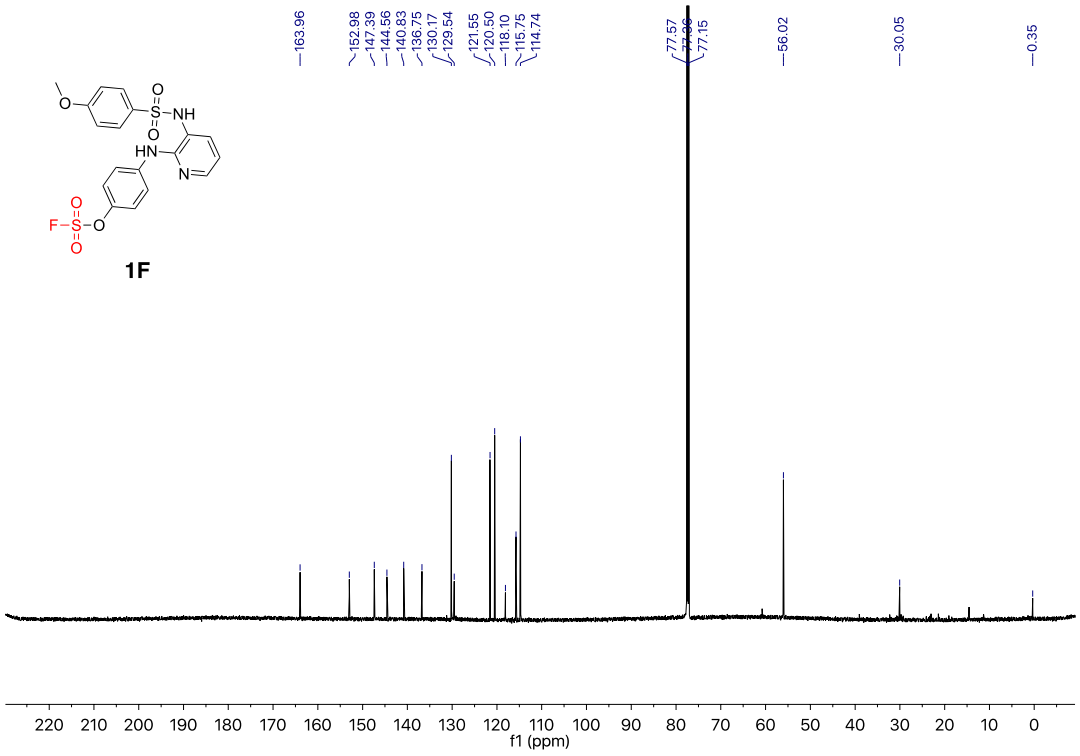


2. NMR spectra for pure 1F, 11F and 25F

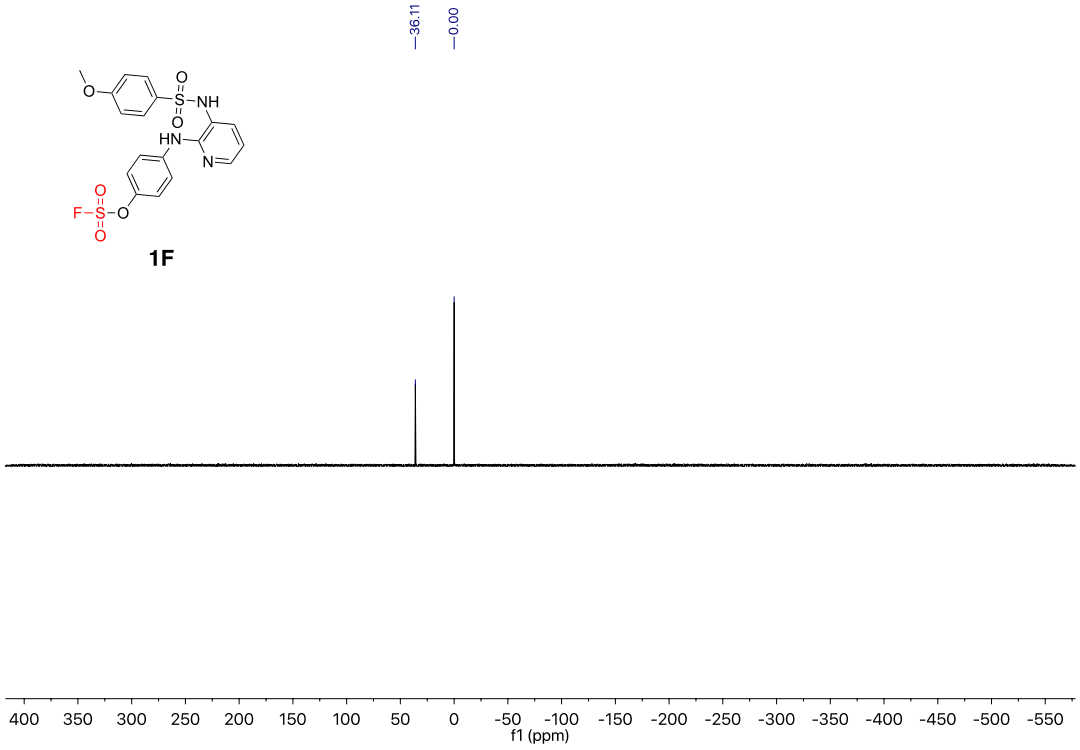
^{1}H NMR spectrum for **1F** (400 MHz, $CDCl_3$)



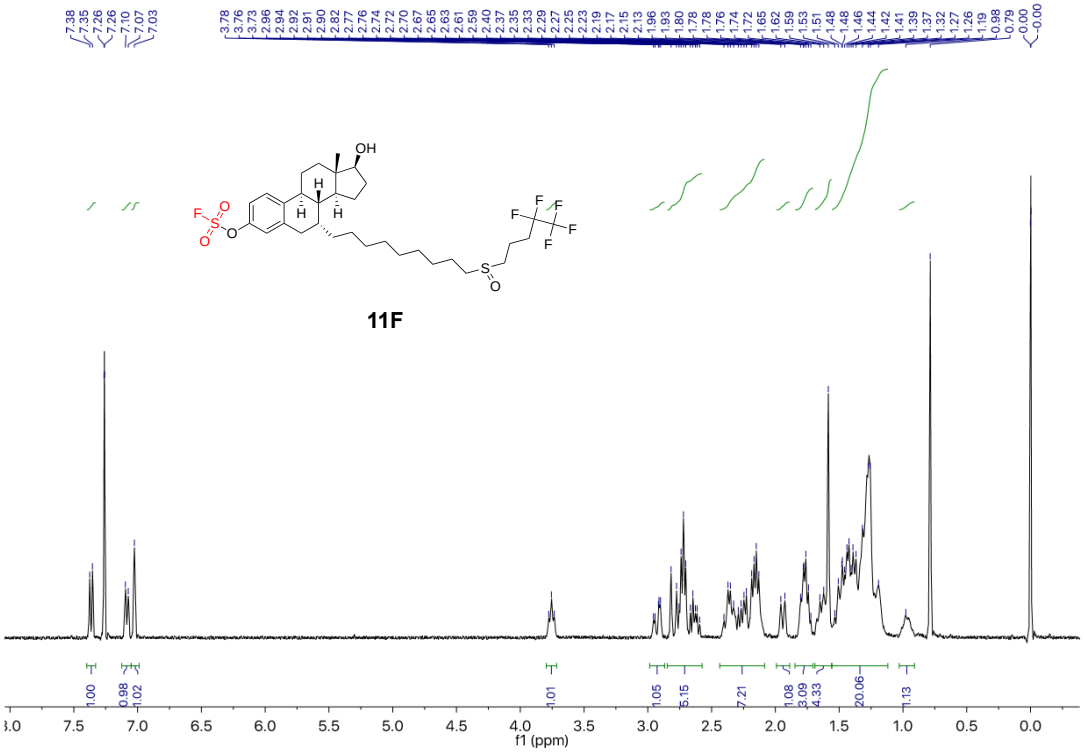
^{13}C NMR spectrum for **1F** (151 MHz, $CDCl_3$)



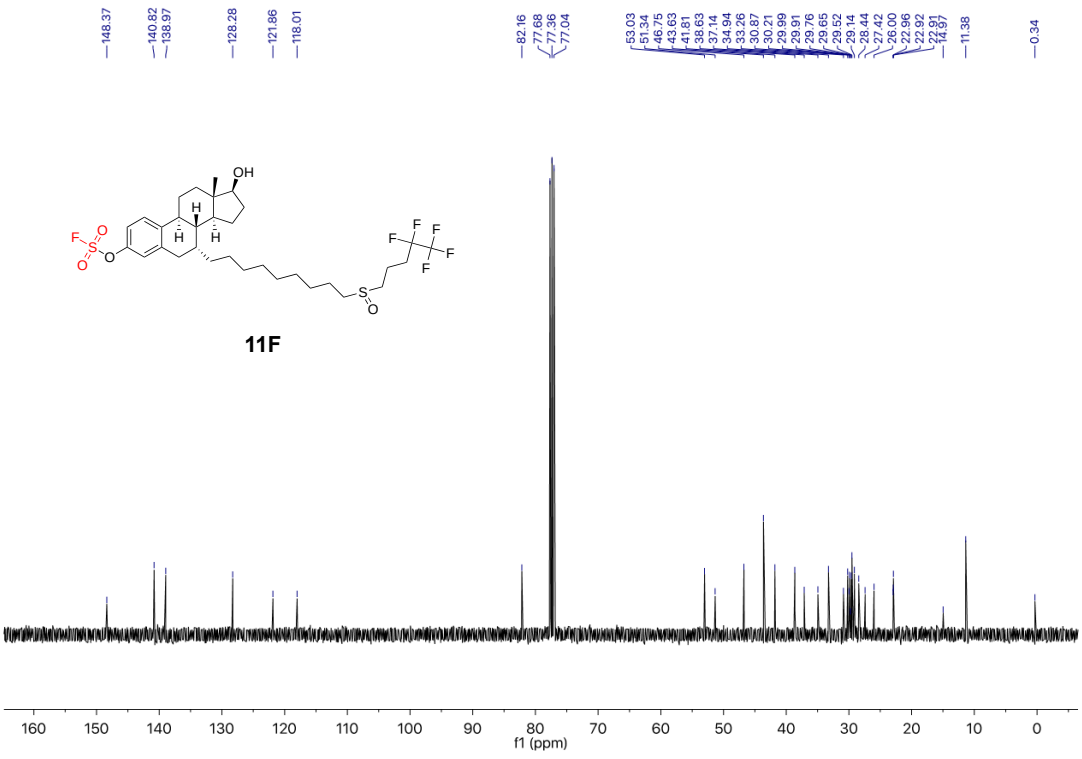
^{19}F NMR spectrum for **1F** (376 MHz, $CDCl_3$)



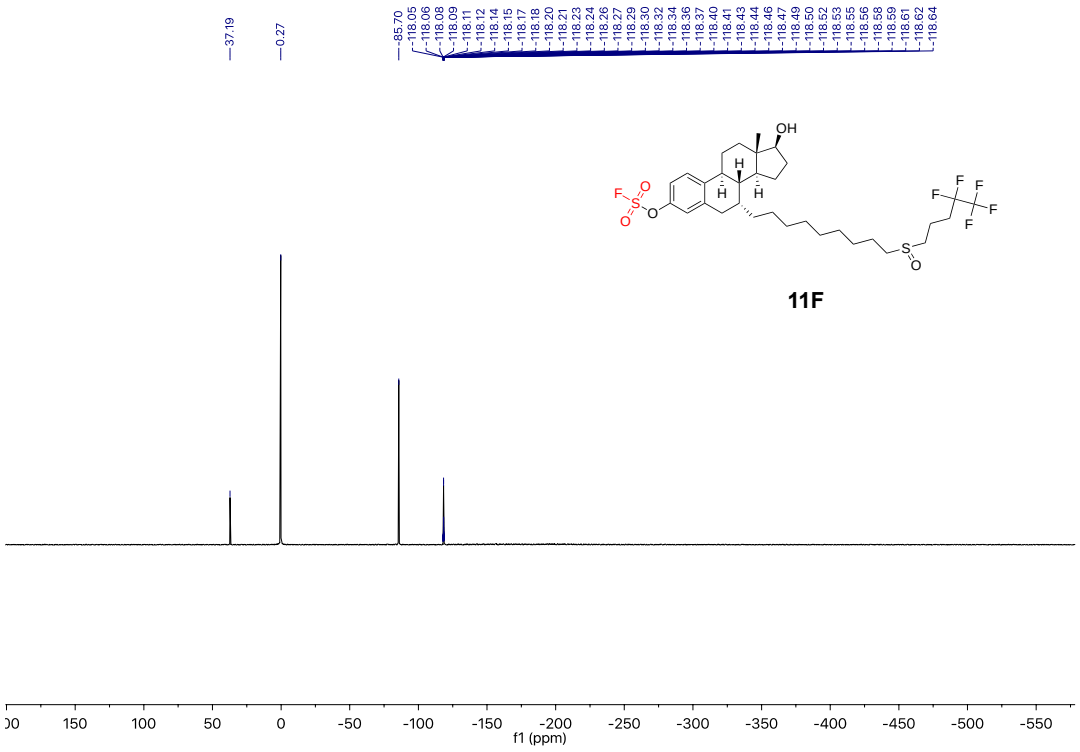
^{1}H NMR spectrum for **11F** (400 MHz, $CDCl_3$)



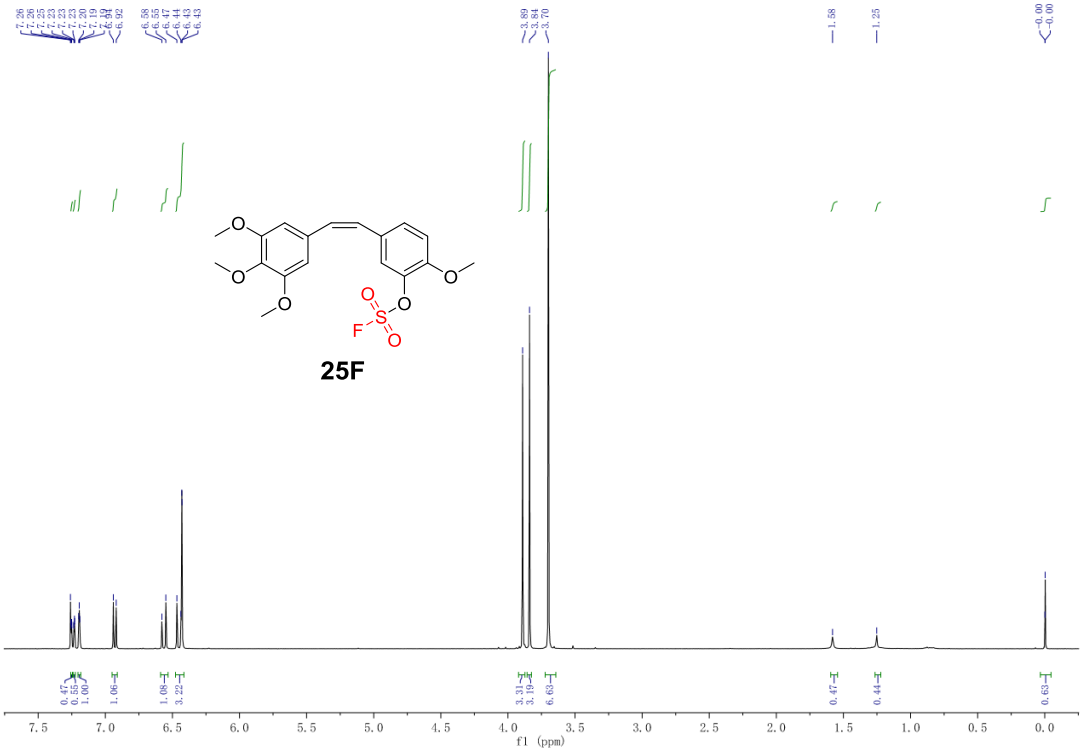
^{13}C NMR spectrum for **11F** (151 MHz, $CDCl_3$)



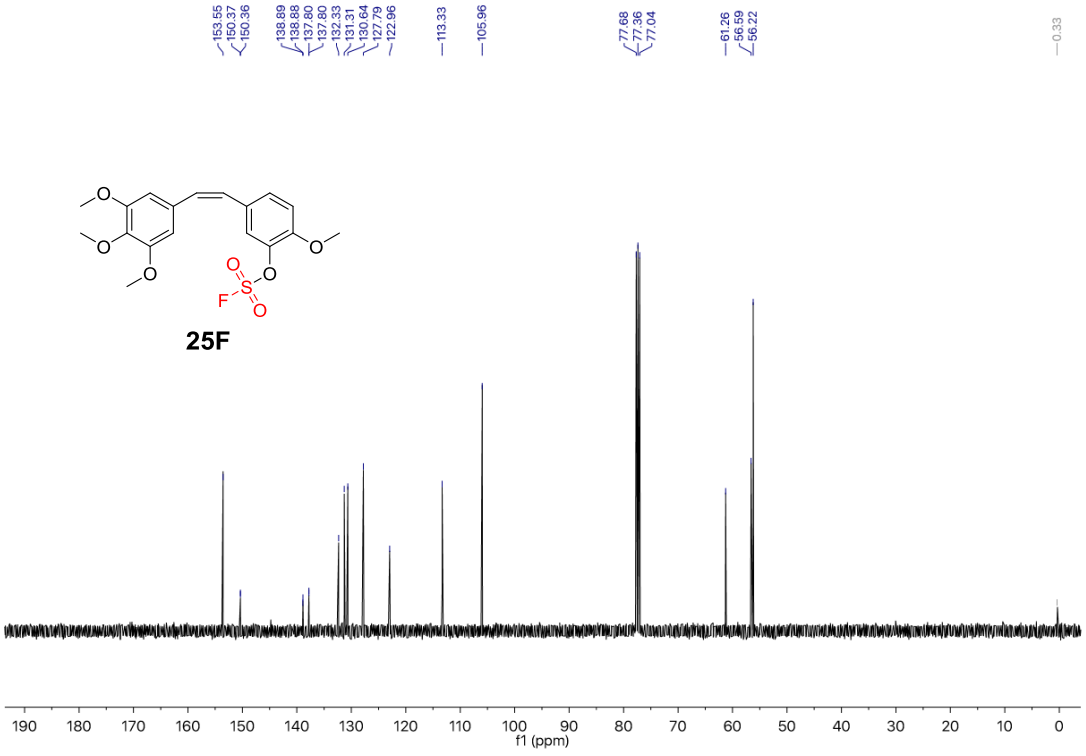
^{19}F NMR spectrum for **11F** (376 MHz, $CDCl_3$)



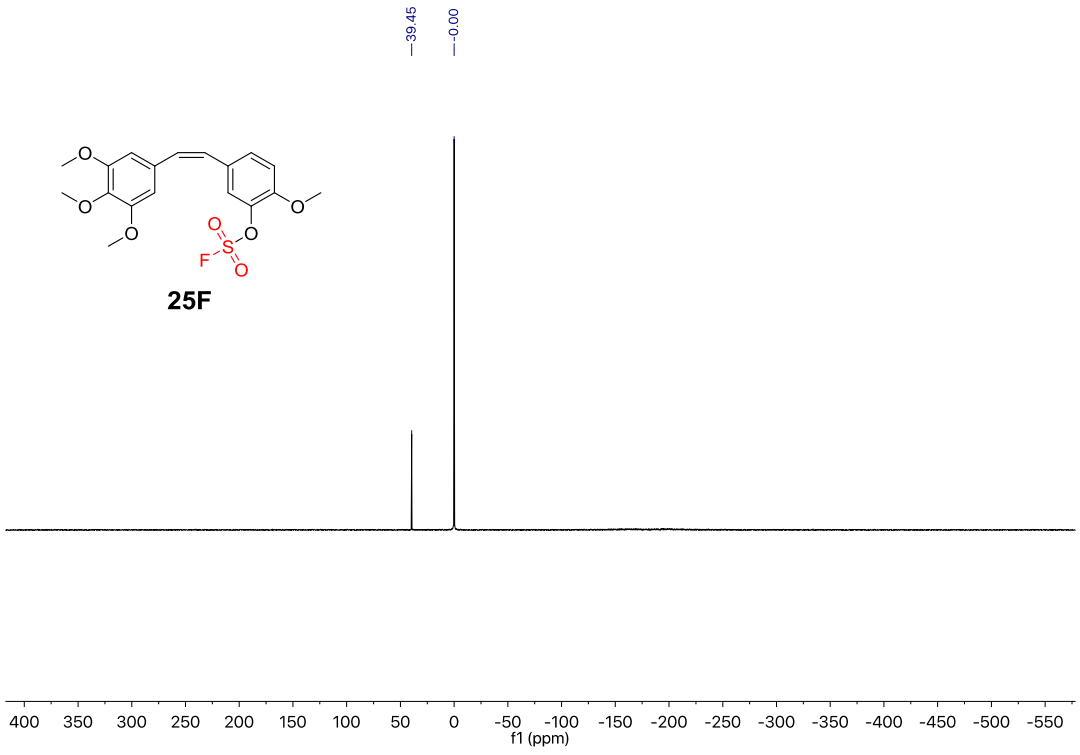
^{1}H NMR spectrum for **25F** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum for **25F** (151 MHz, $CDCl_3$)



^{19}F NMR spectrum for **25F** (376 MHz, $CDCl_3$)



4. LC TRACES

LC Method I

Solvent A: H_2O; Solvent B: CH_3CN

TIME (MIN)	A (%)	B (%)	FLOW [ML/MIN]	MAX. PRESSURE LIMIT [BAR]
0	100	0	0.550	400.00
3	10	90	0.550	400.00
4	10	90	0.550	400.00
5	100	0	0.550	400.00
5.5	100	0	0.550	400.00

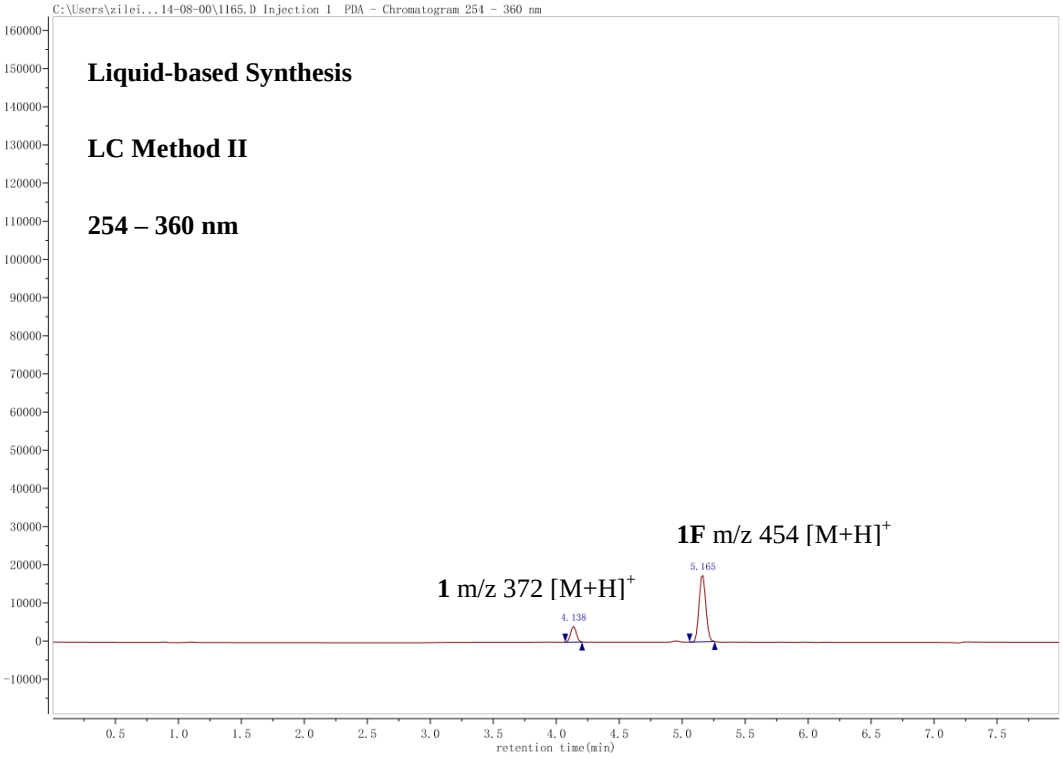
LC Method II

Solvent A: H_2O; Solvent B: CH_3CN

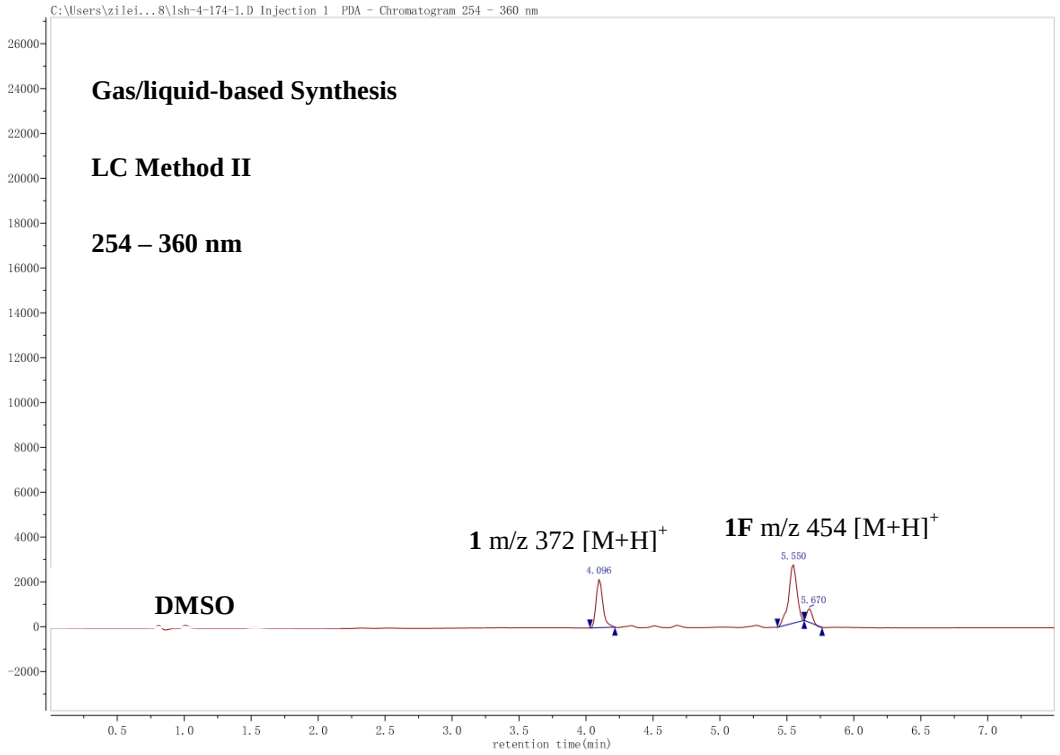
TIME (MIN)	A (%)	B (%)	FLOW [ML/MIN]	MAX. PRESSURE LIMIT [BAR]

0	90	10	0.550	400.00
4	0	100	0.550	400.00
7.5	0	100	0.550	400.00

1F: Mol. Wt. 453

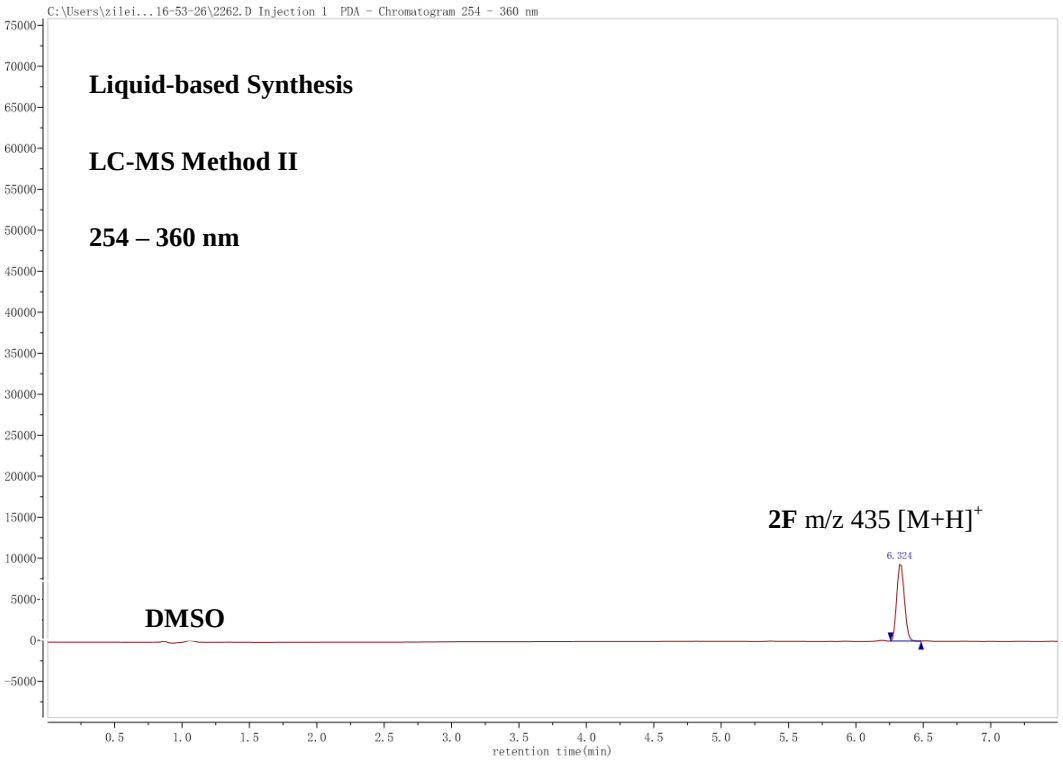


Retention Time (min)	Area %
4.138	**16**
5.165	**84**

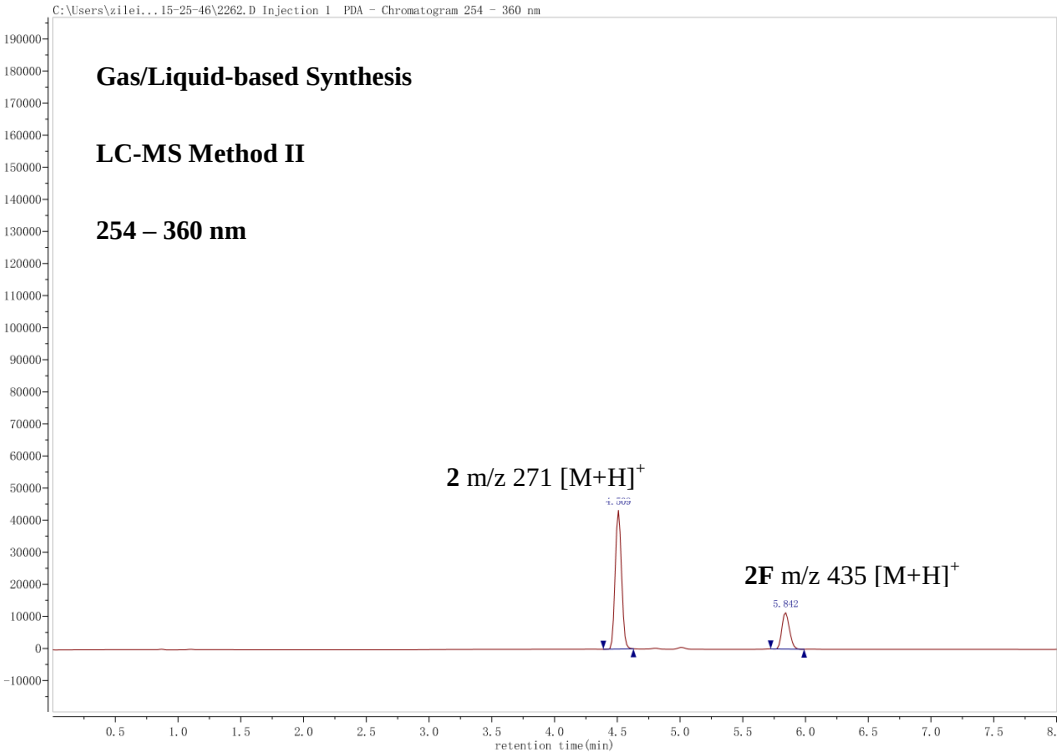


Retention Time (min)	Area %
4.096	**37**
5.550	**55**
5.670	**8**

2F: Mol. Wt. 434

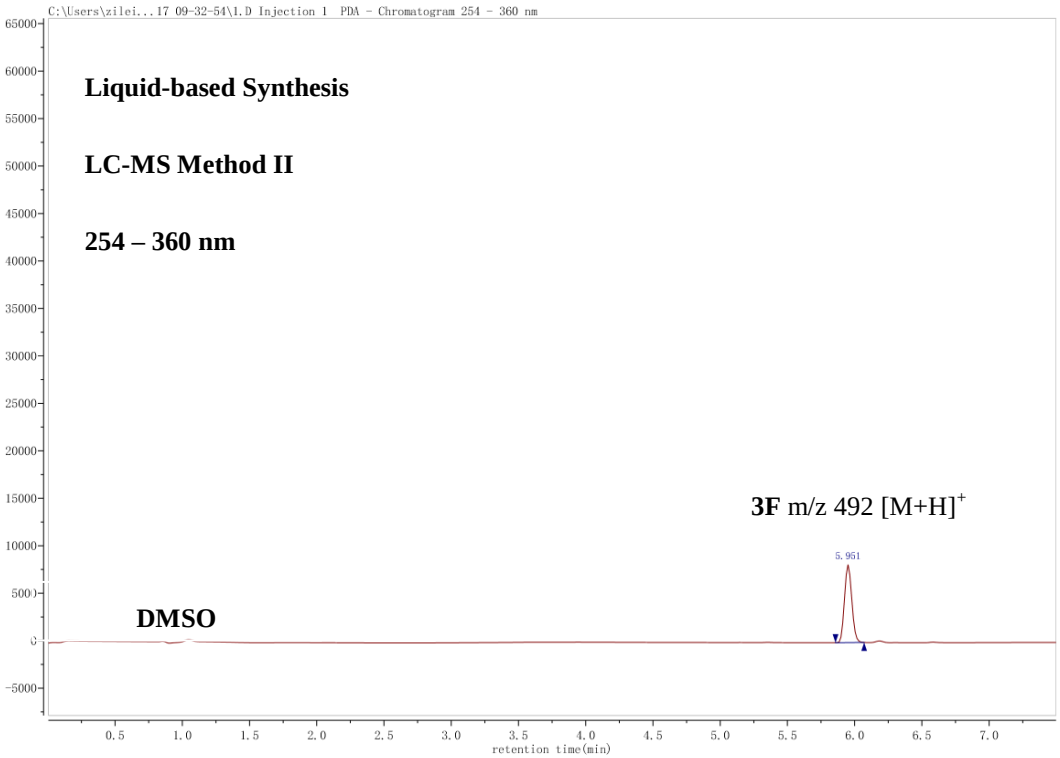


Retention Time (min)	Area %
6.324	**100**

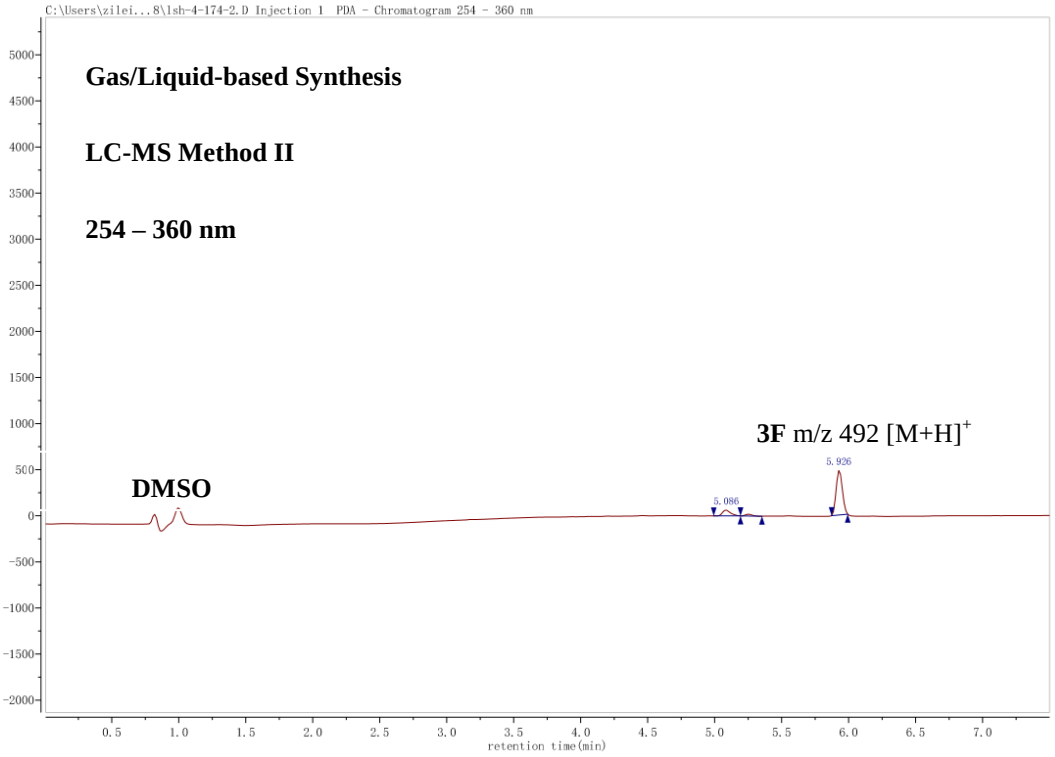


Retention Time (min)	Area %
4.509	**76**
5.842	**24**

3F: Mol. Wt. 491

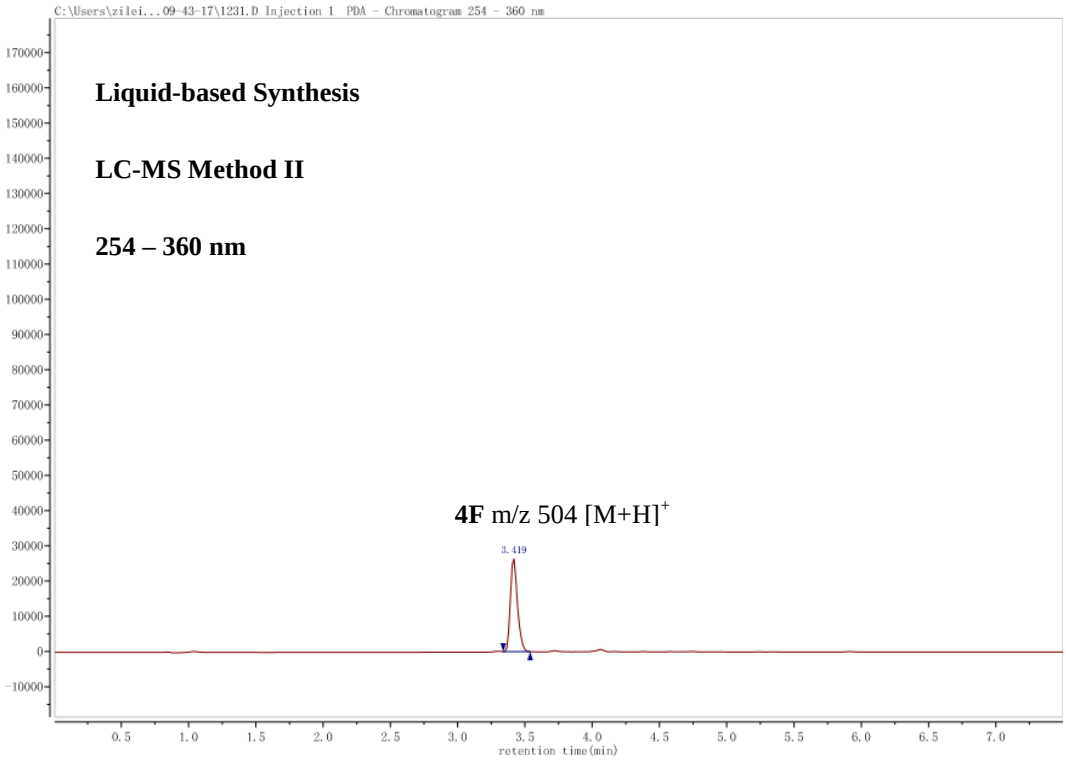


Retention Time (min)	Area %
5.951	**100**

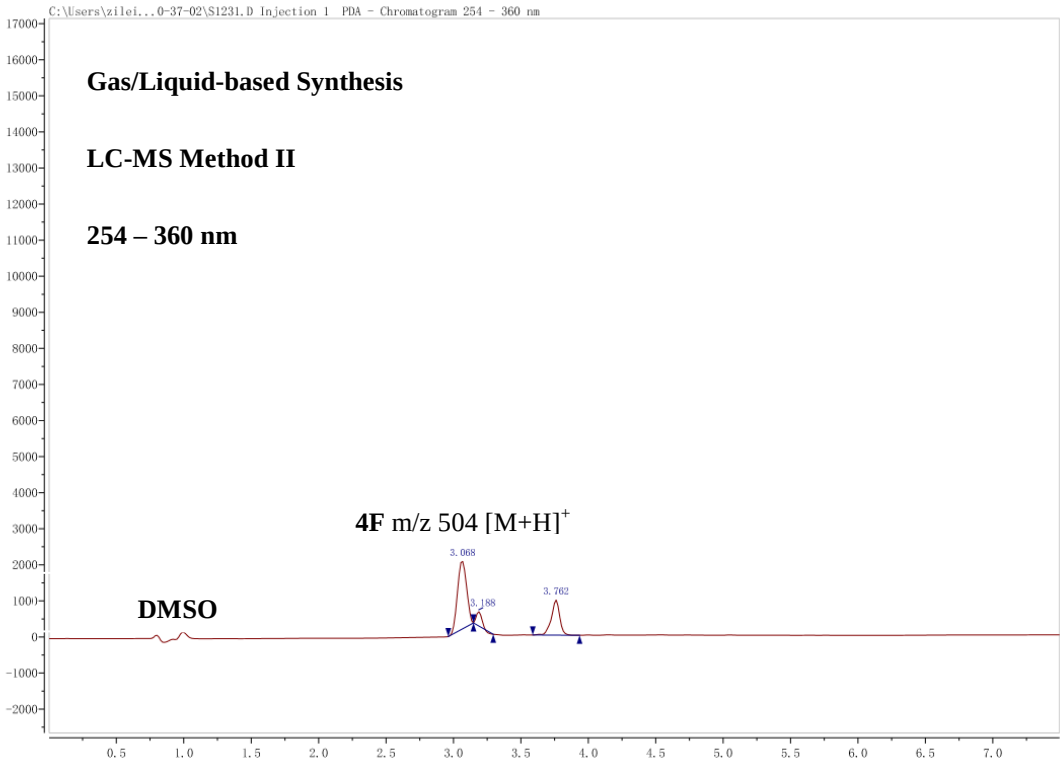


Retention Time (min)	Area %
5.086	**14**
5.246	**3**
5.926	**83**

4F: Mol. Wt. 503

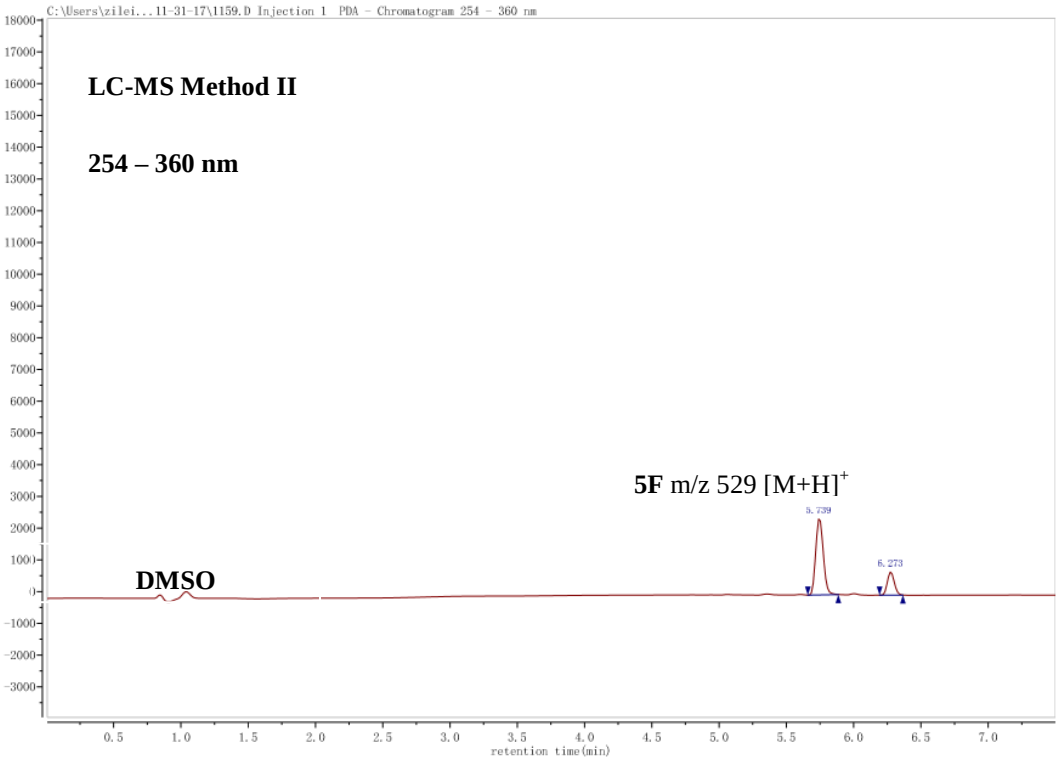


Retention Time (min)	Area %
3.419	**100**



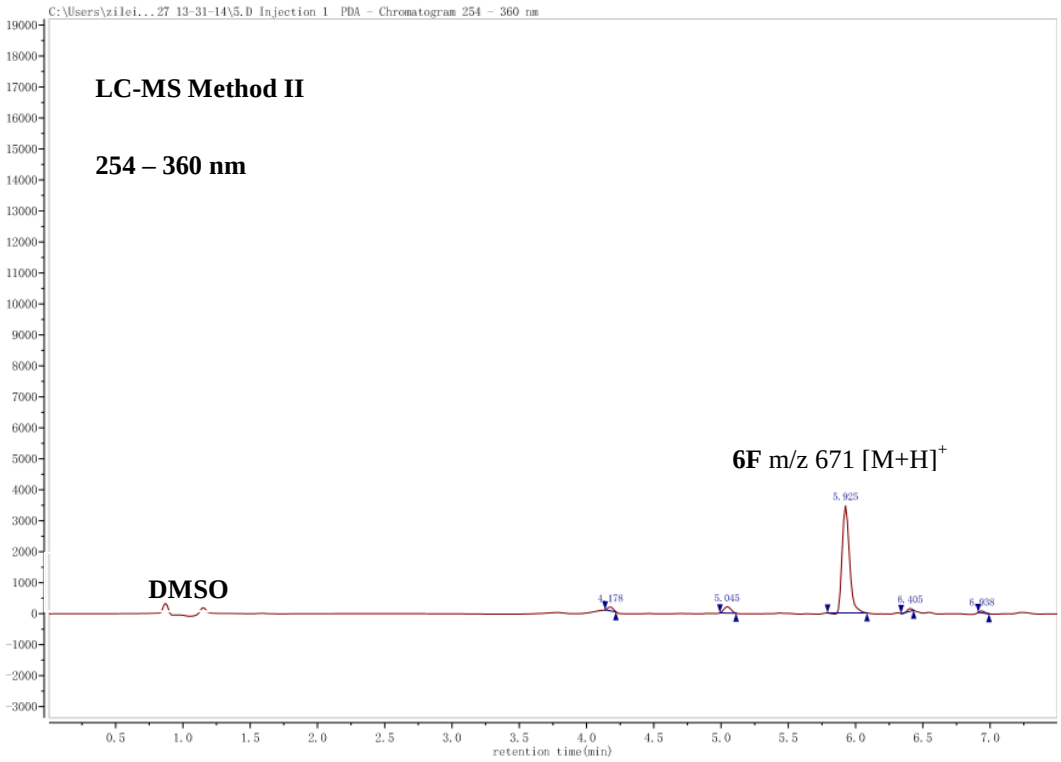
Retention Time (min)	Area %
3.068	**63**
3.188	**8**
3.068	**29**

5F: Mol. Wt. 528



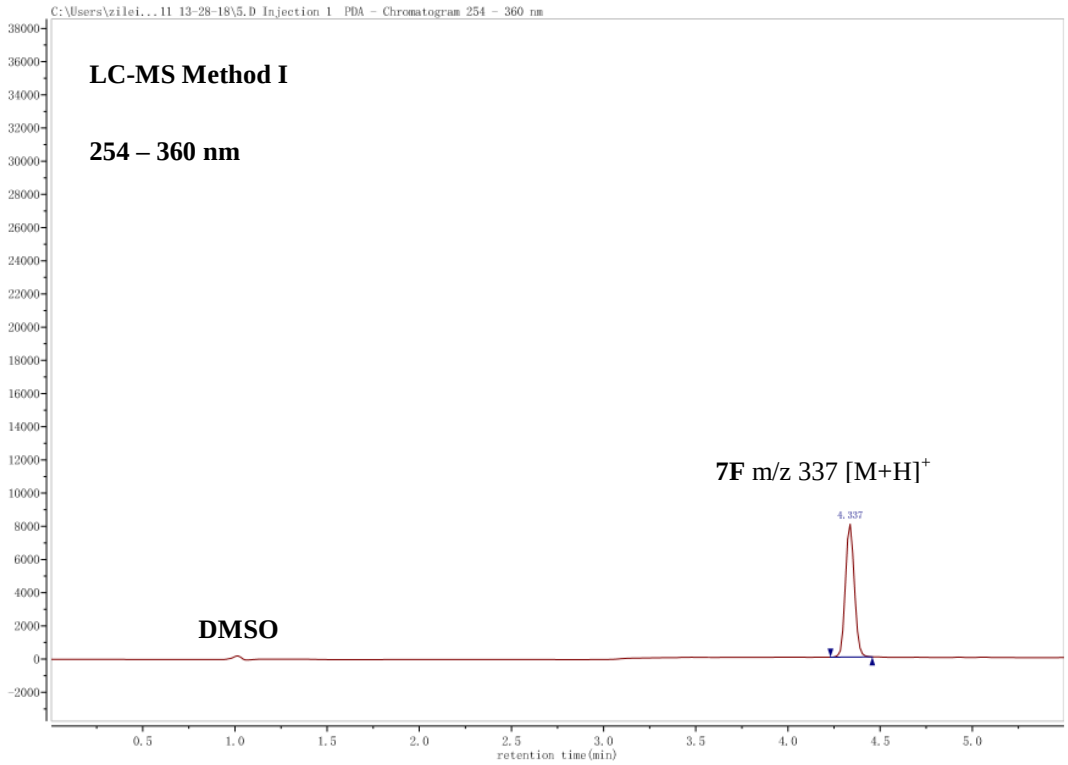
Retention Time (min)	Area %
5.739	**81**
6.273	**19**

6F: Mol. Wt. 670



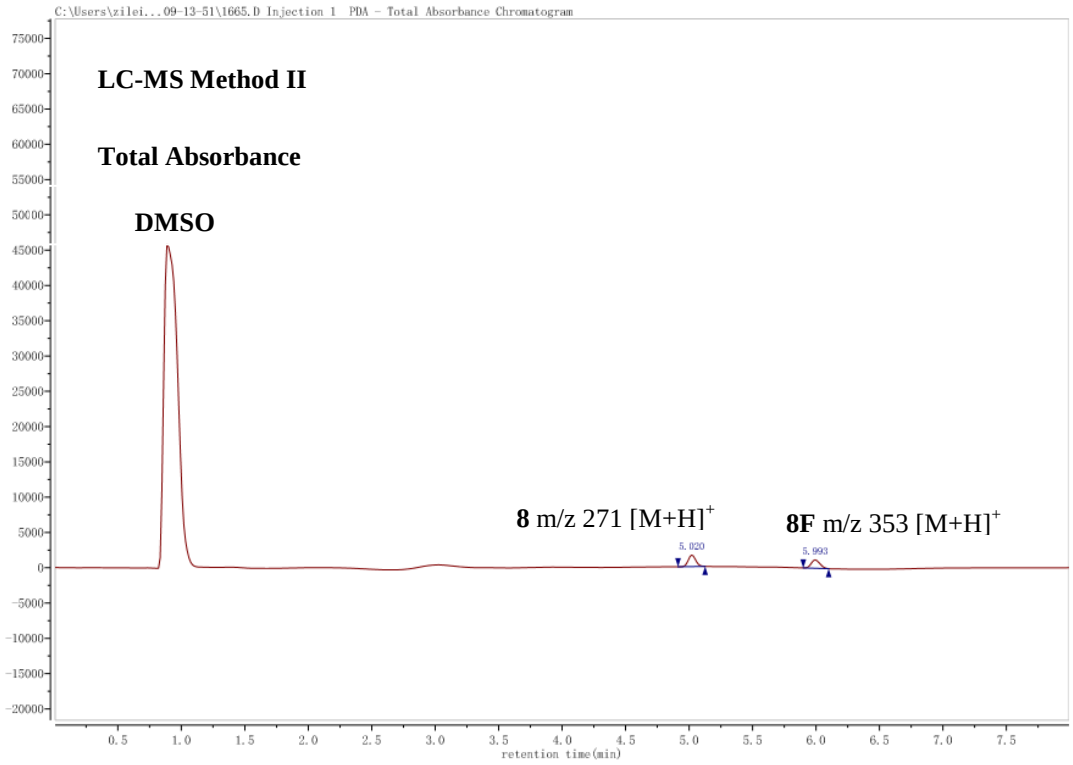
Retention Time (min)	Area %
4.178	**3**
5.045	**5**
5.925	**90**
6.405	**1**
6.938	**1**

7F: Mol. Wt. 336



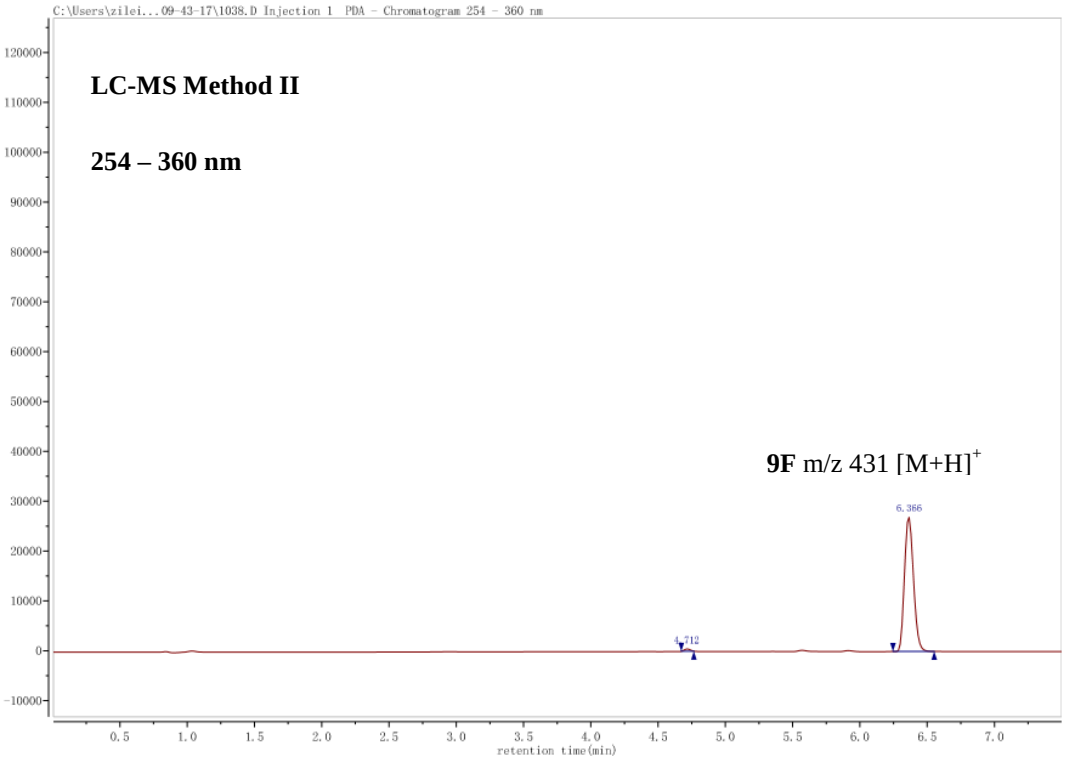
Retention Time (min)	Area %
4.337	**100**

8F: Mol. Wt. 352



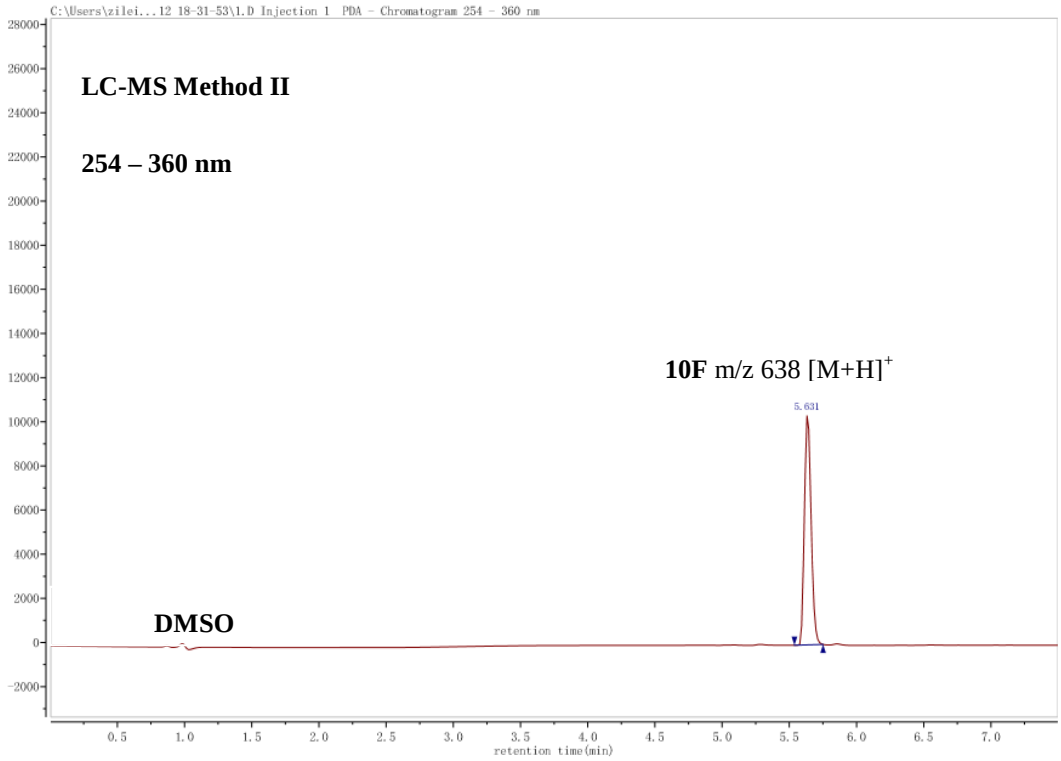
Retention Time (min)	Area %
5.020	**54**
5.993	**46**

9F: Mol. Wt. 430



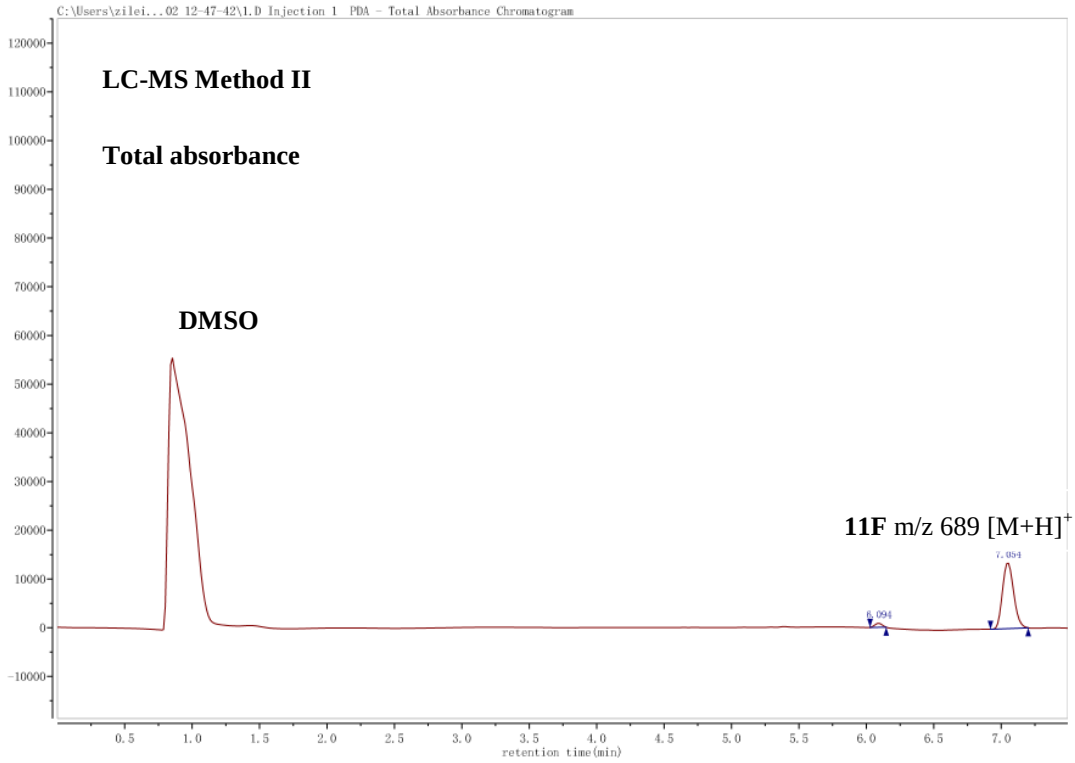
Retention Time (min)	Area %
4.712	**2**
6.366	**98**

10F: Mol. Wt. 637



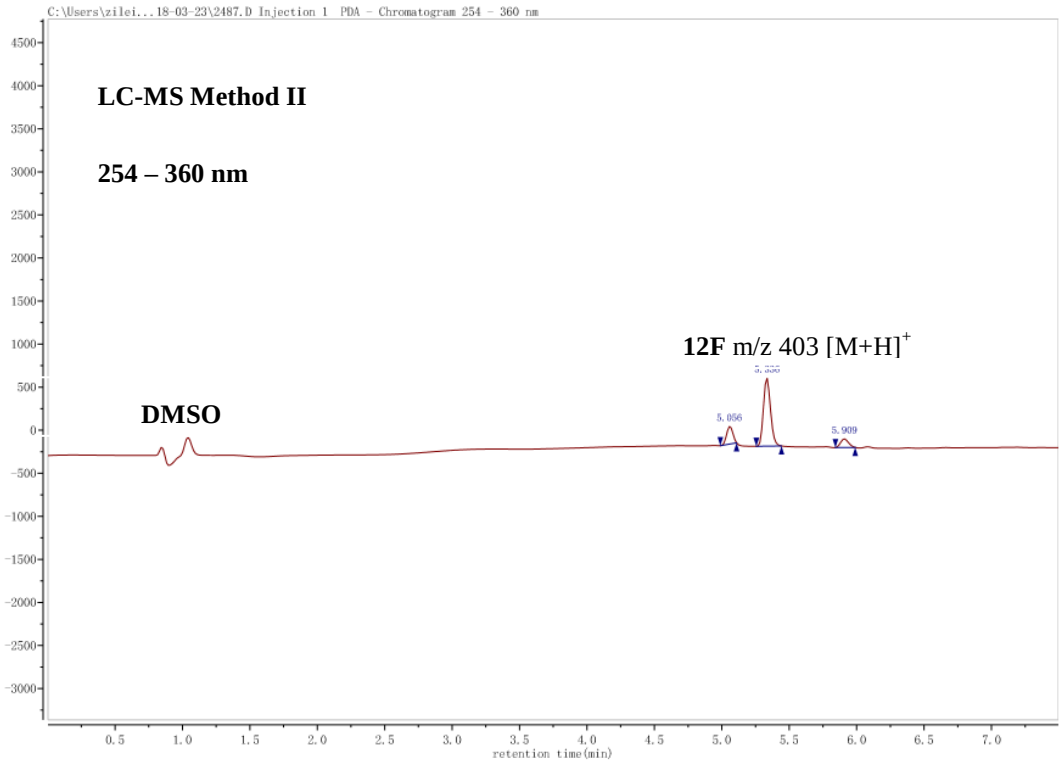
Retention Time (min)	Area %
5.631	**100**

11F: Mol. Wt. 688



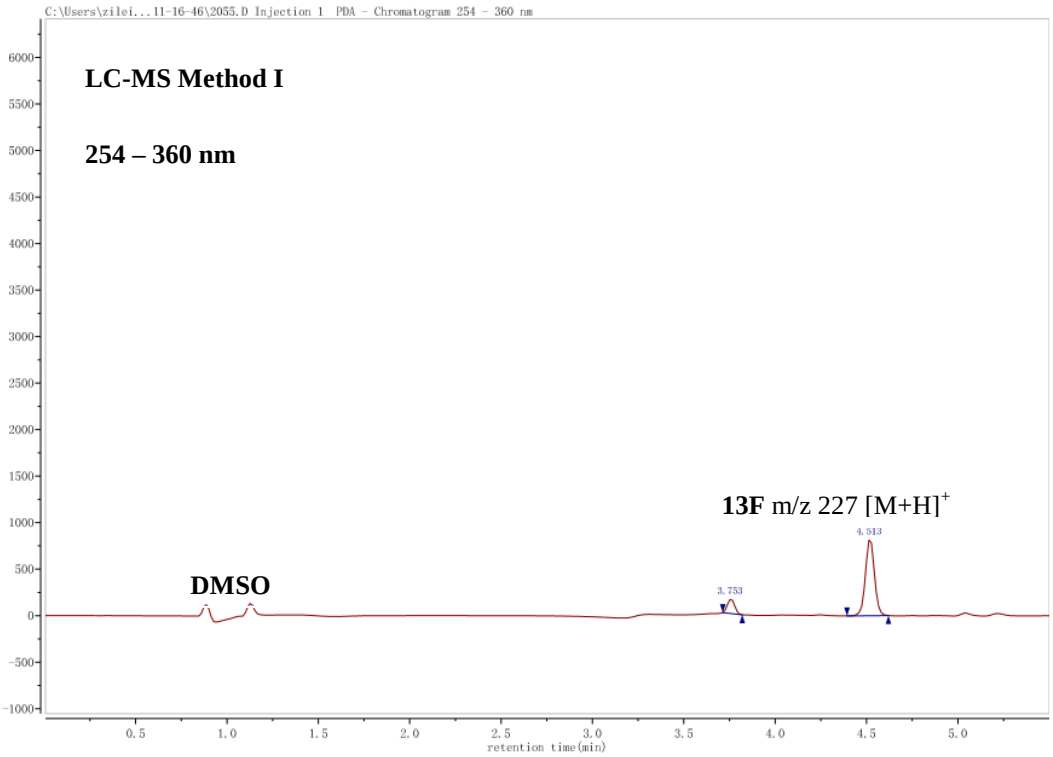
Retention Time (min)	Area %
6.094	**4**
7.054	**96**

12F: Mol. Wt. 402



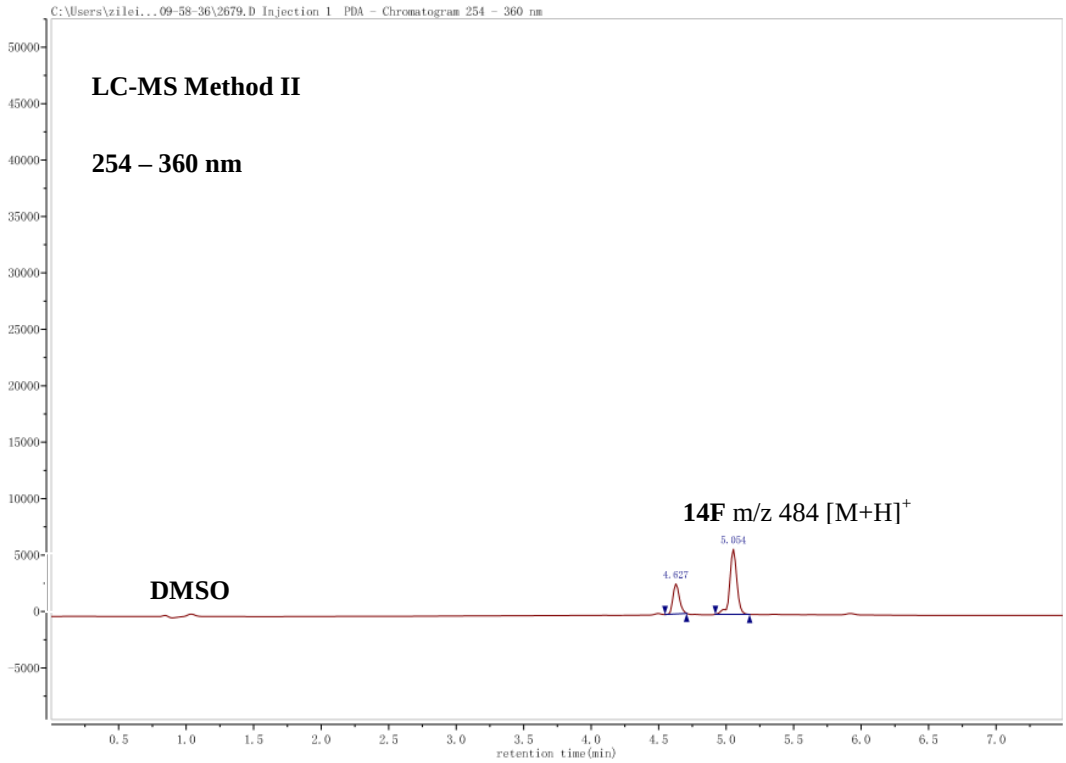
Retention Time (min)	Area %
5.056	**20**
5.366	**72**
5.909	**8**

13F: Mol. Wt. 226



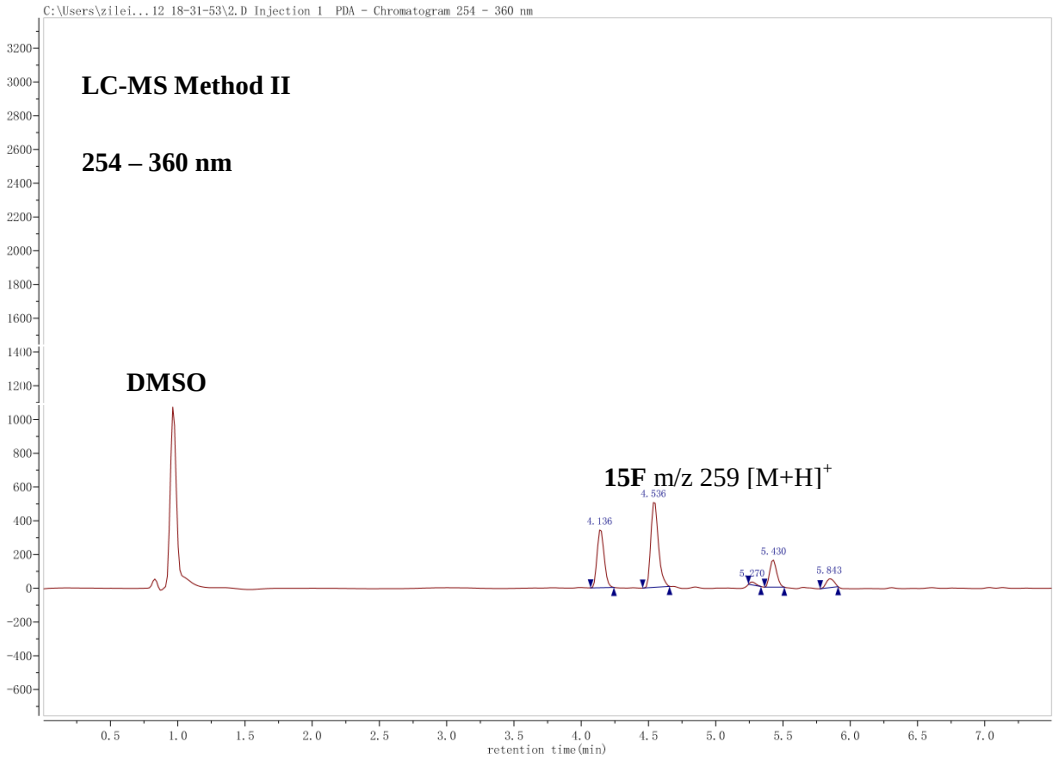
Retention Time (min)	Area %
3.753	**12**
4.513	**88**

14F: Mol. Wt. 483



Retention Time (min)	Area %
4.627	**30**
5.054	**70**

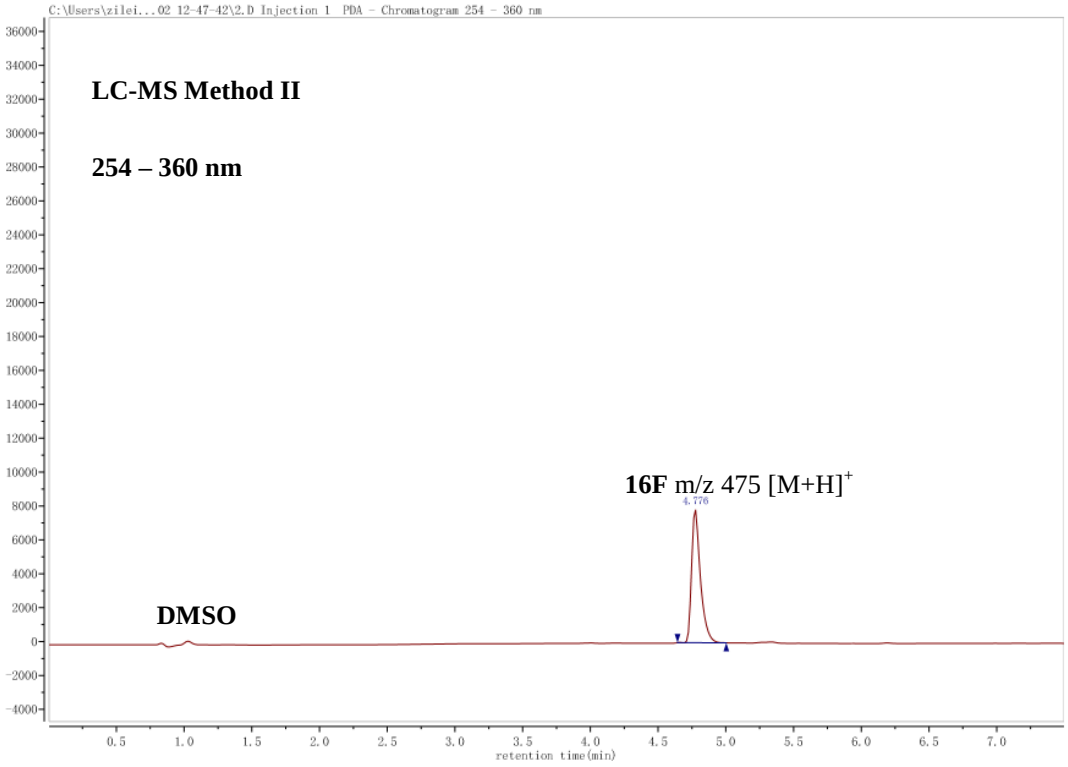
15F: Mol. Wt. 258



Retention Time (min)	Area %
4.136	**30**

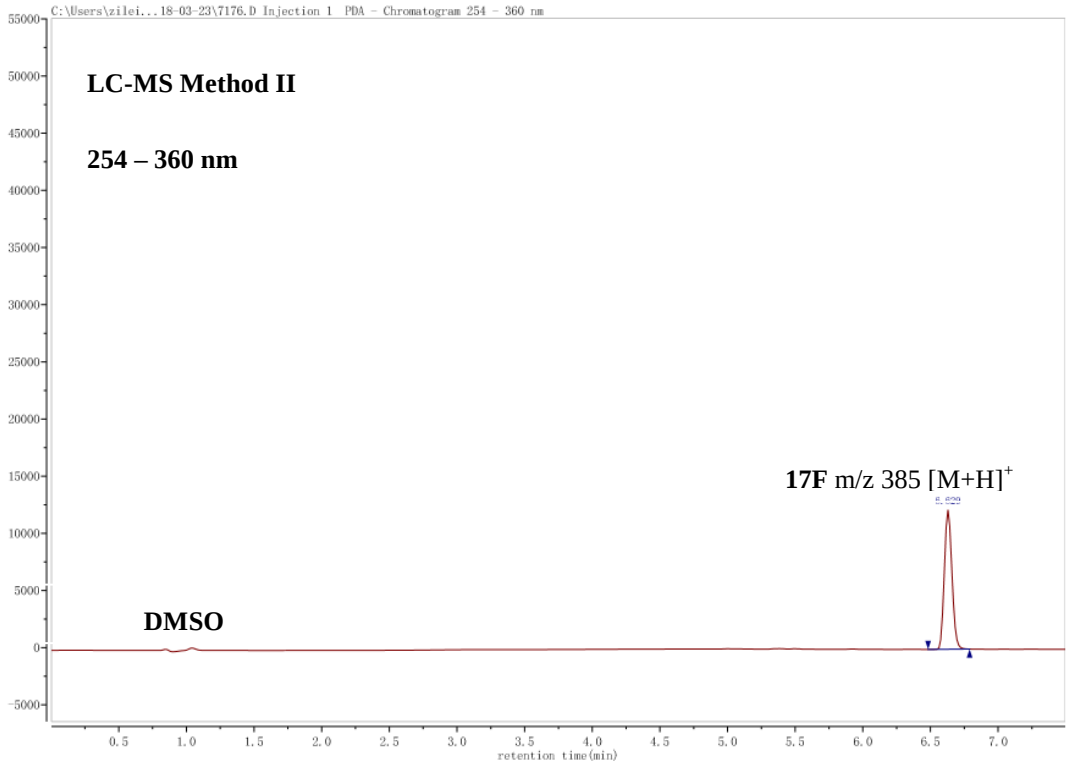
4.536	**50**
5.270	**1**
5.430	**14**
5.843	**5**

16F: Mol. Wt. 474



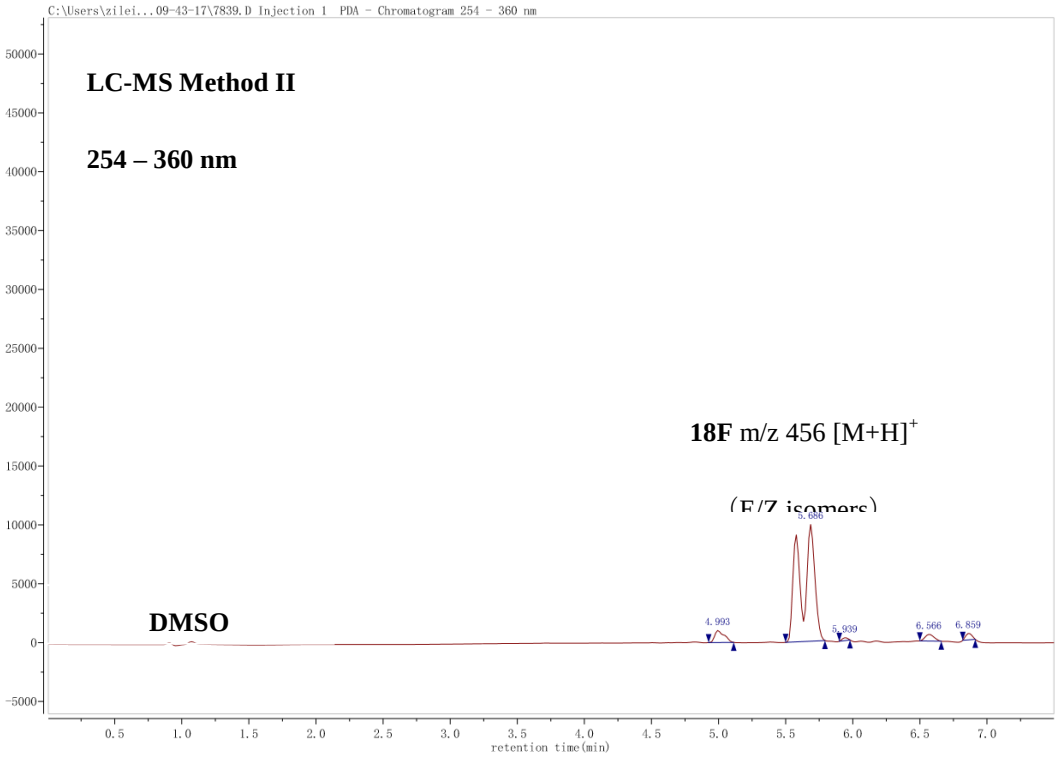
Retention Time (min)	Area %
4.776	**100**

17F: Mol. Wt. 384



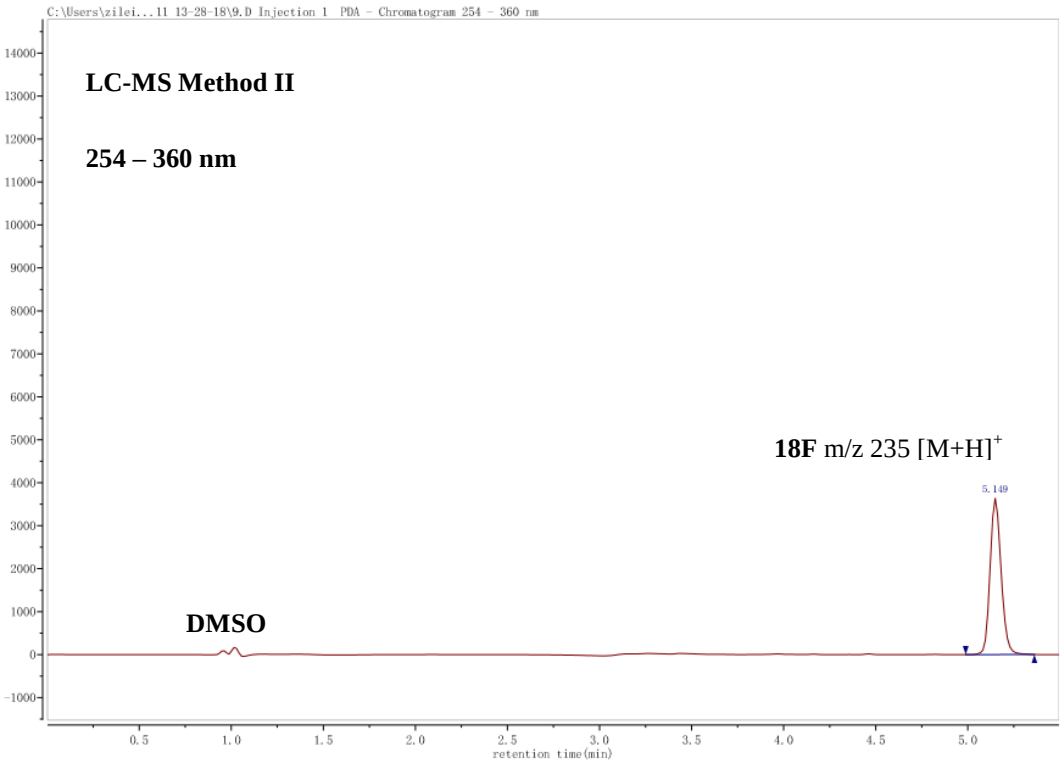
Retention Time (min)	Area %
6.629	**100**

18F: Mol. Wt. 455



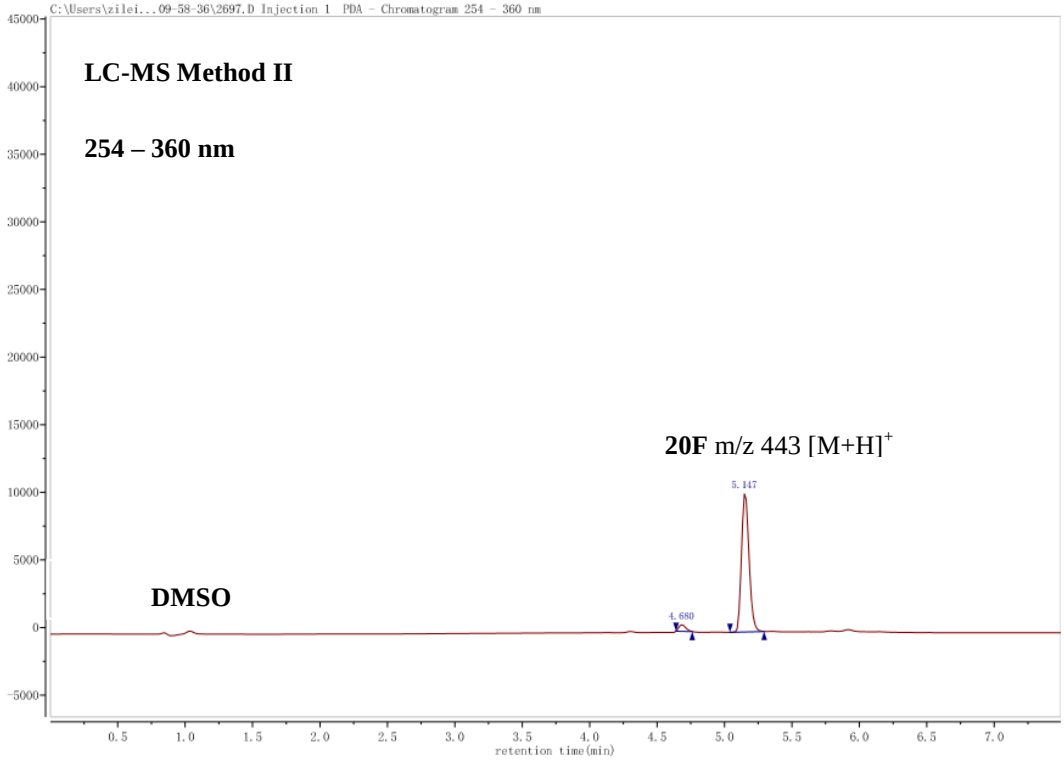
Retention Time (min)	Area %
4.993	**6**
5.686	**88**
5.939	**1**
6.566	**3**
6.859	**2**

19F: Mol. Wt. 234



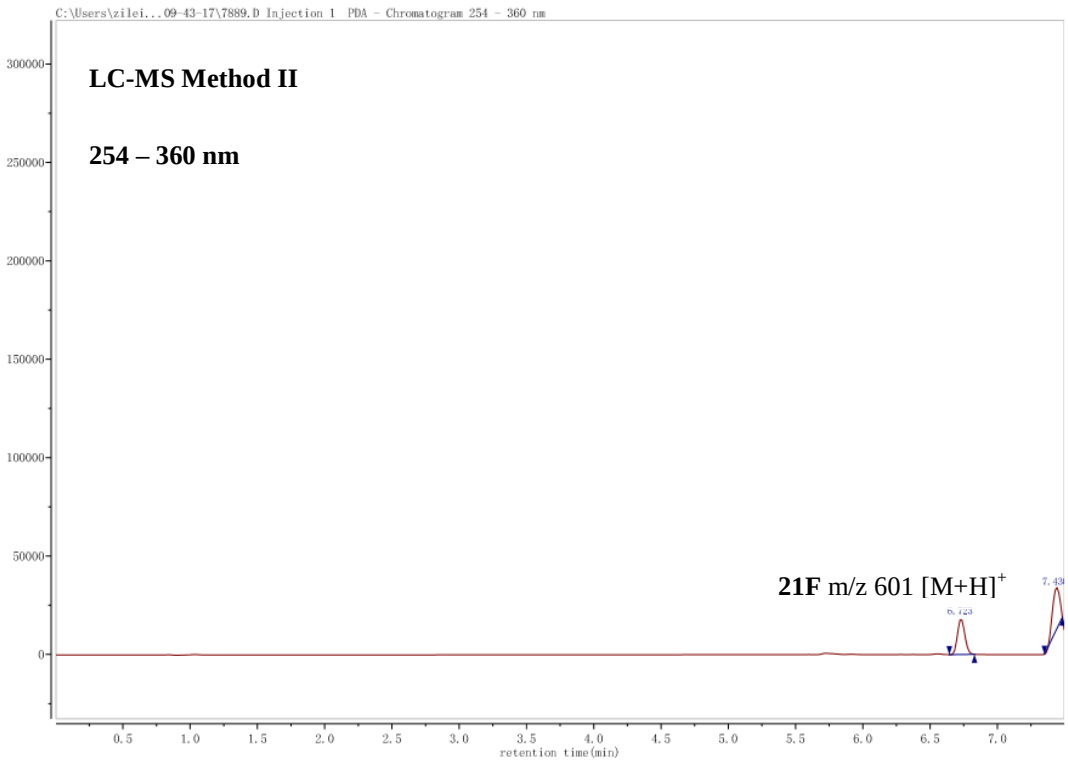
Retention Time (min)	Area %
5.149	**100**

20F: Mol. Wt. 442



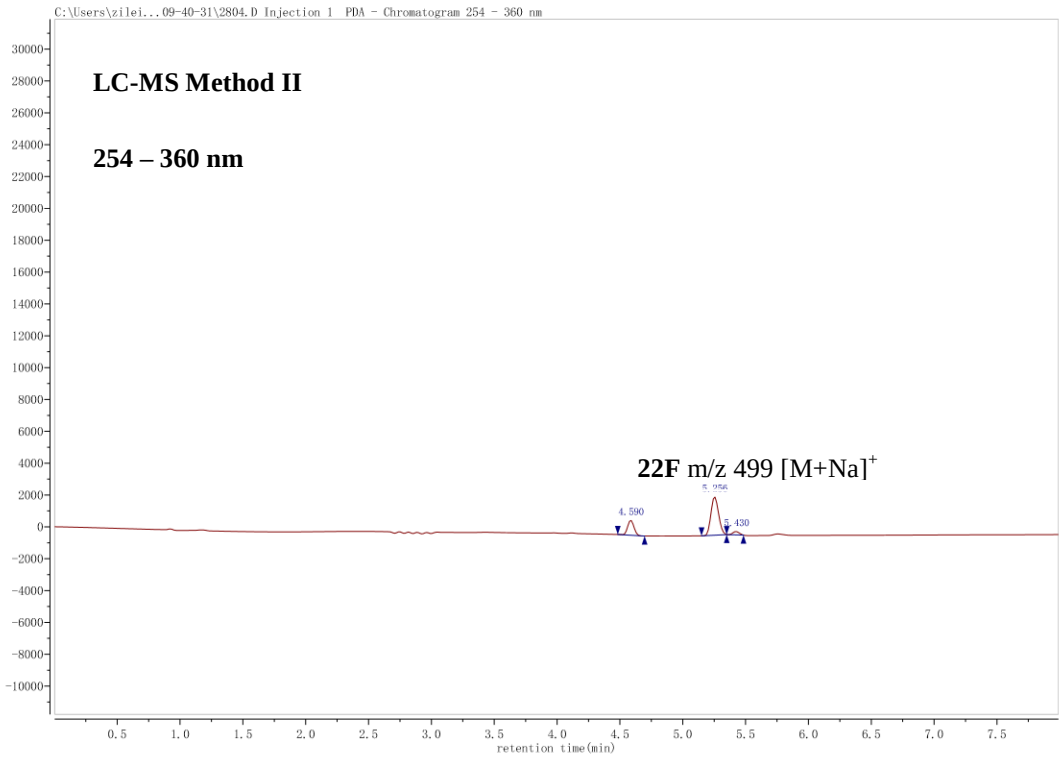
Retention Time (min)	Area %
4.680	**3**
5.147	**97**

21F: Mol. Wt. 600



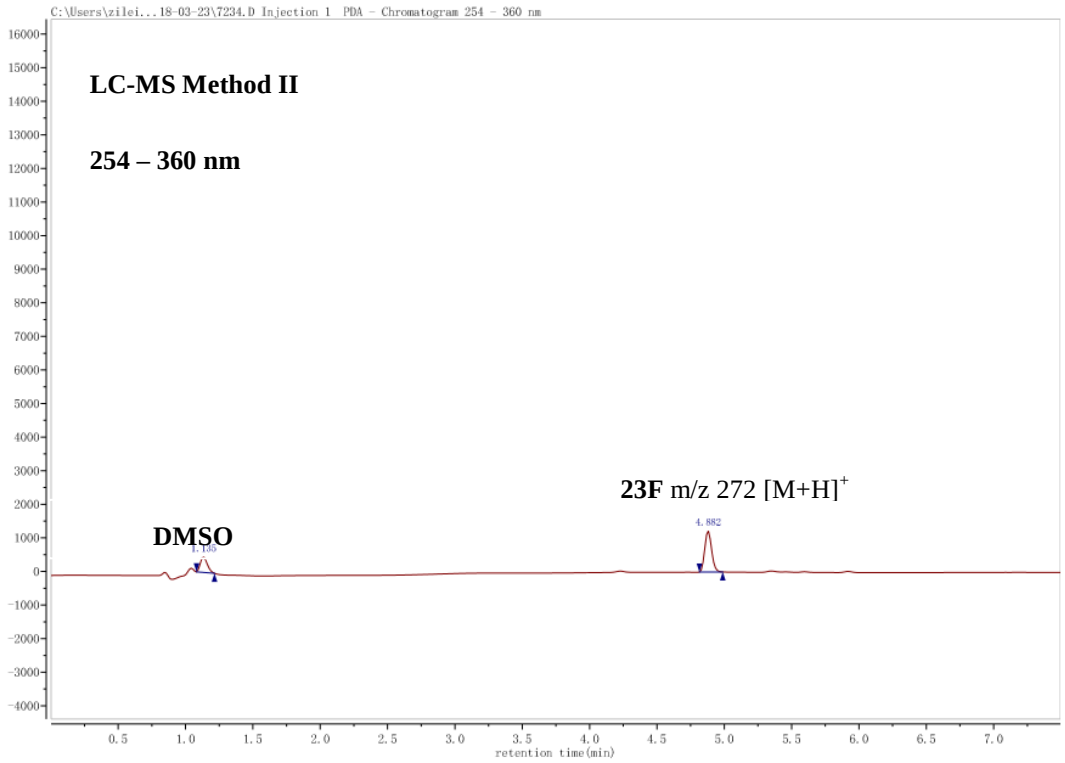
Retention Time (min)	Area %
6.723	**48**
7.435	**52**

22F: Mol. Wt. 476



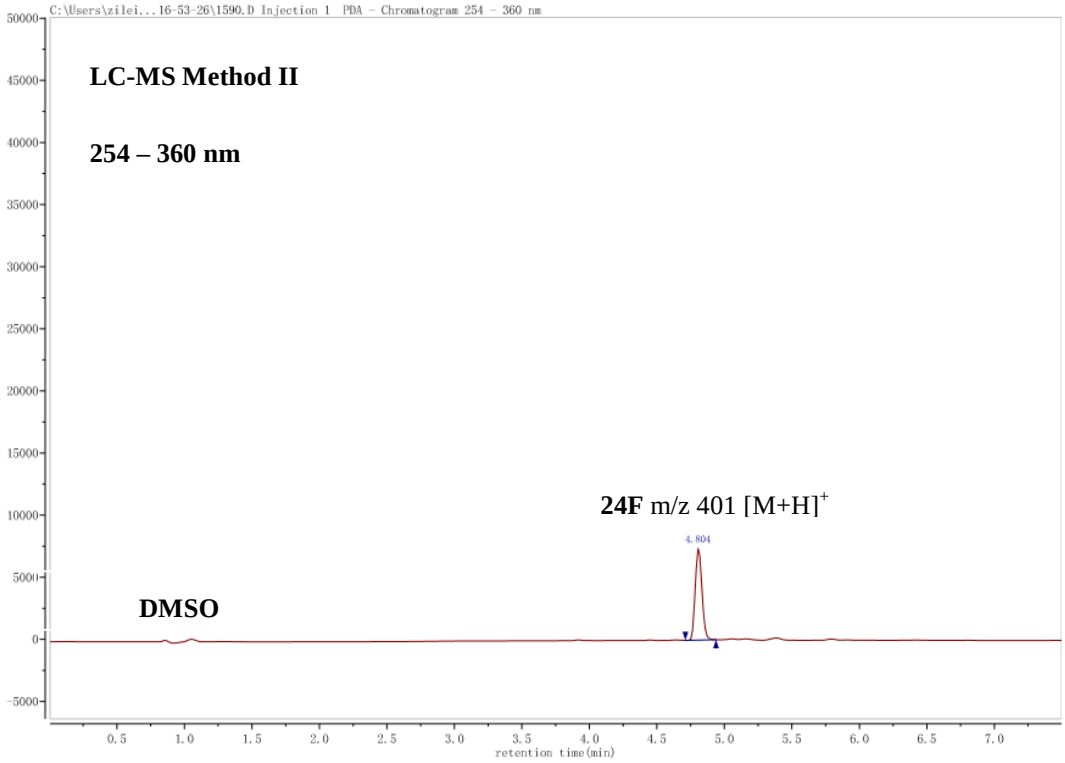
Retention Time (min)	Area %
4.590	**23**
5.256	**72**
5.430	**5**

23F: Mol. Wt. 271



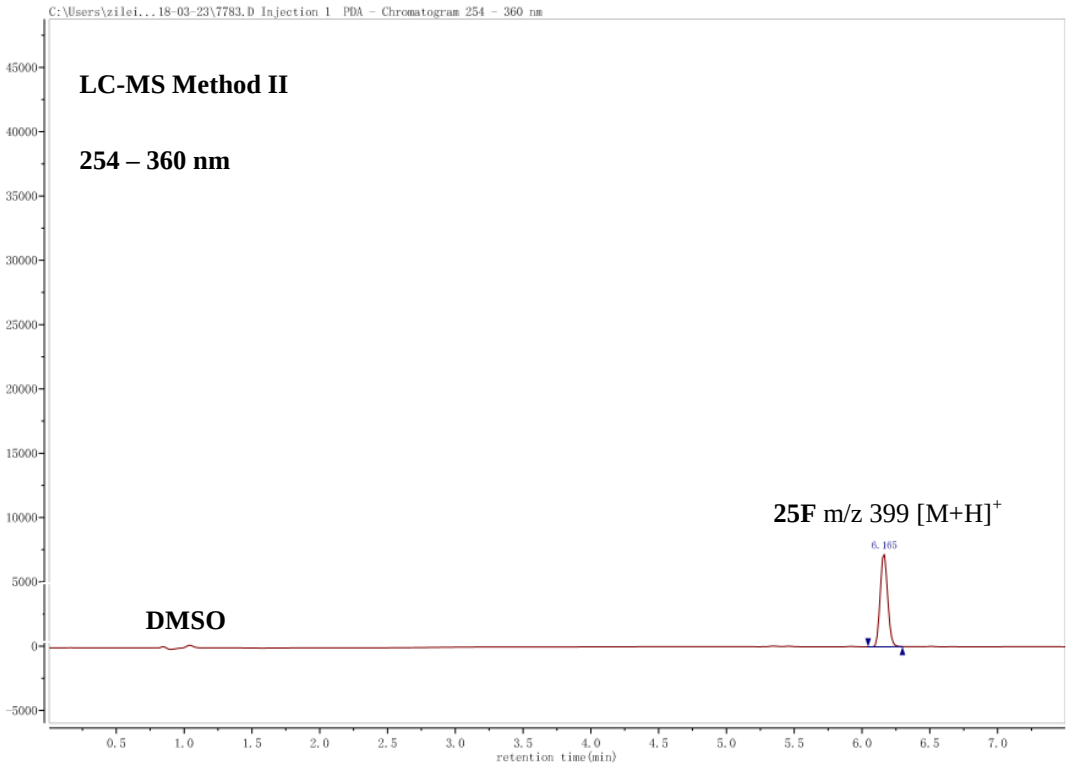
Retention Time (min)	Area %
1.135	**27**
4.882	**73**

24F: Mol. Wt. 400



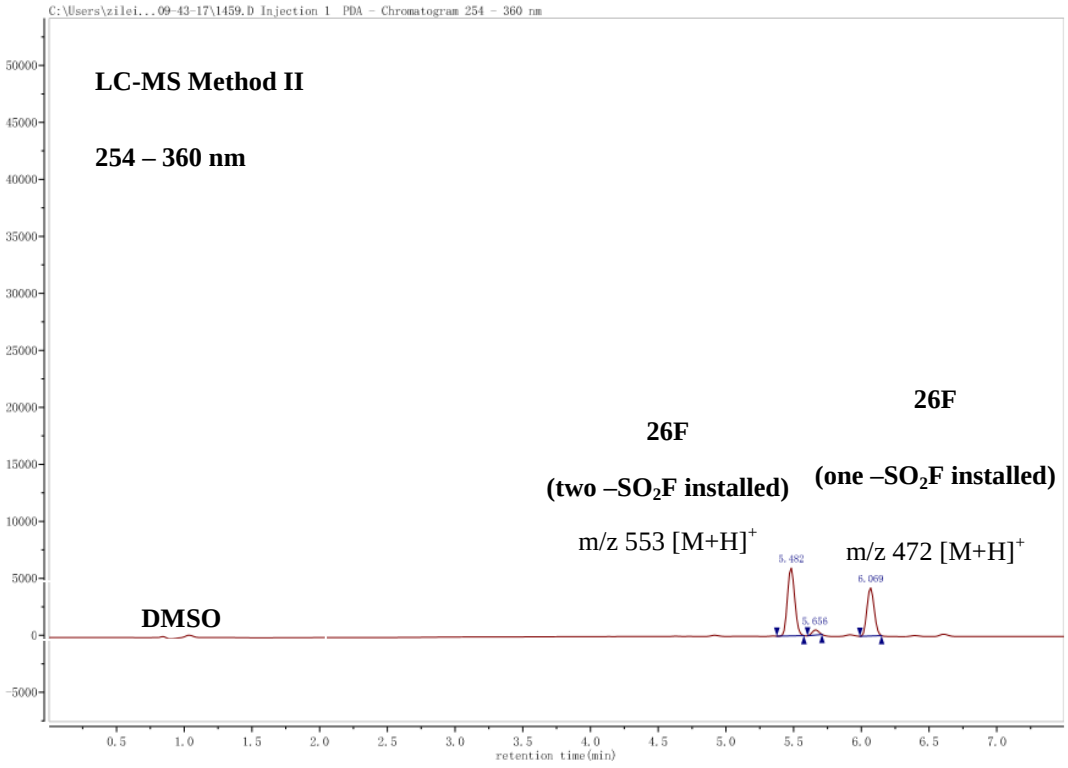
Retention Time (min)	Area %
4.804	**100**

25F: Mol. Wt. 398



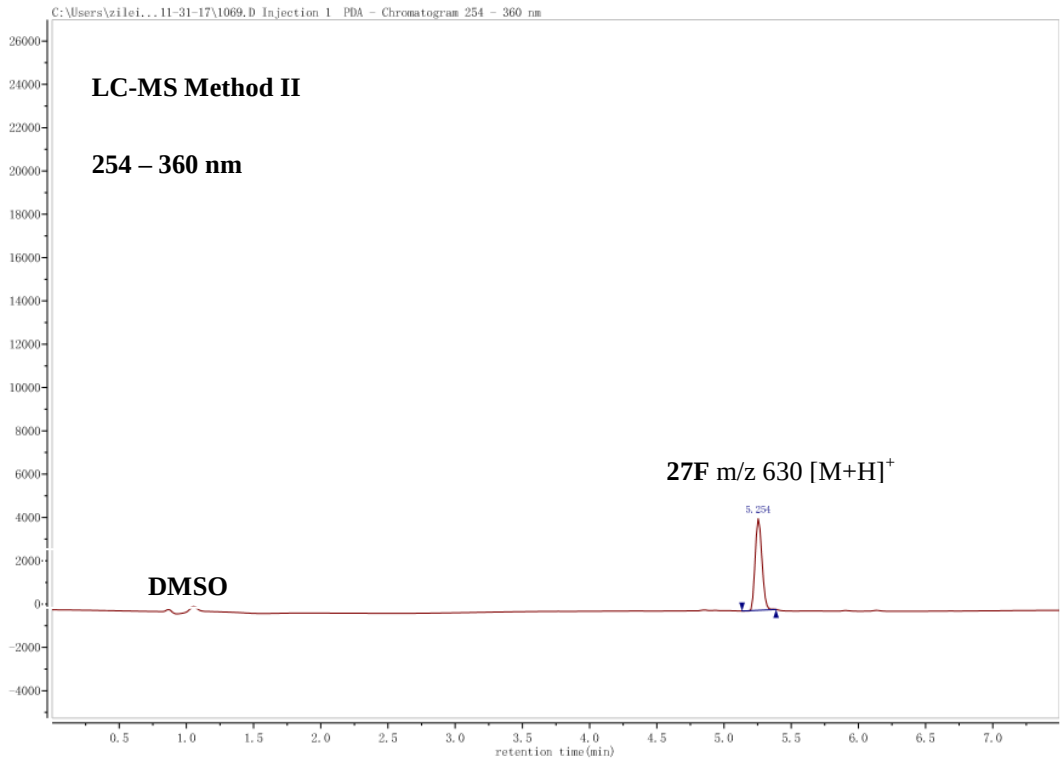
Retention Time (min)	Area %
6.165	**100**

26F: Mol. Wt. 552;



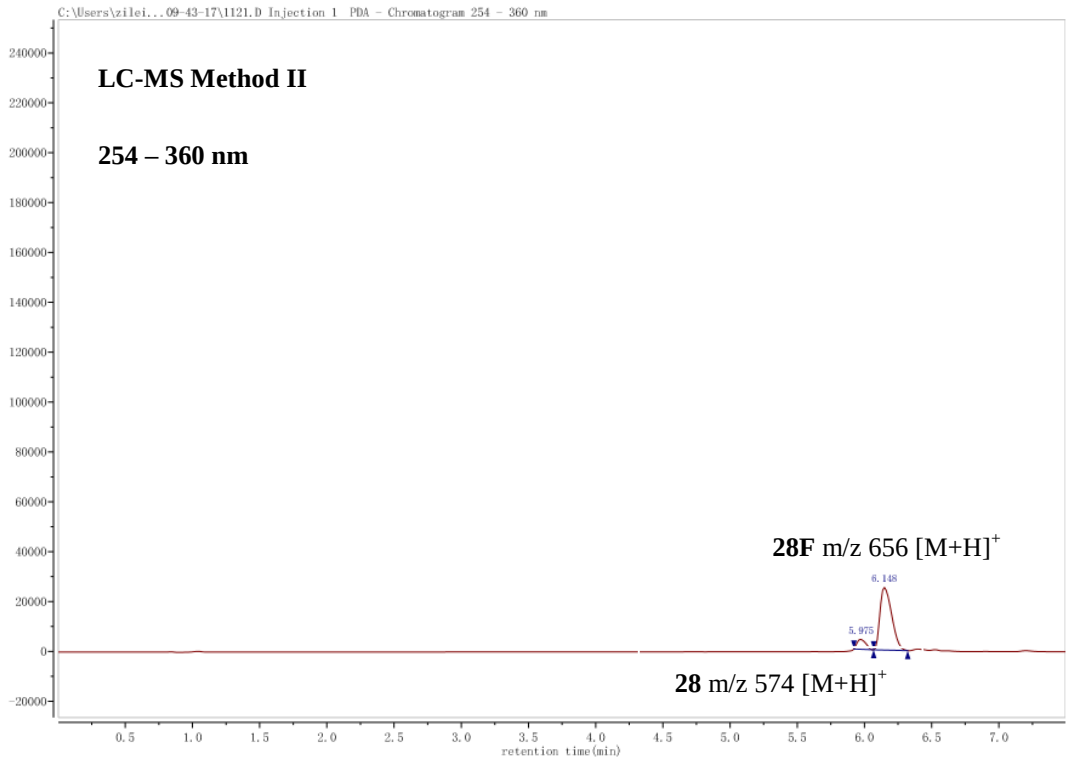
Retention Time (min)	Area %
5.482	**56**
5.656	**4**
6.069	**40**

27F: Mol. Wt. 629



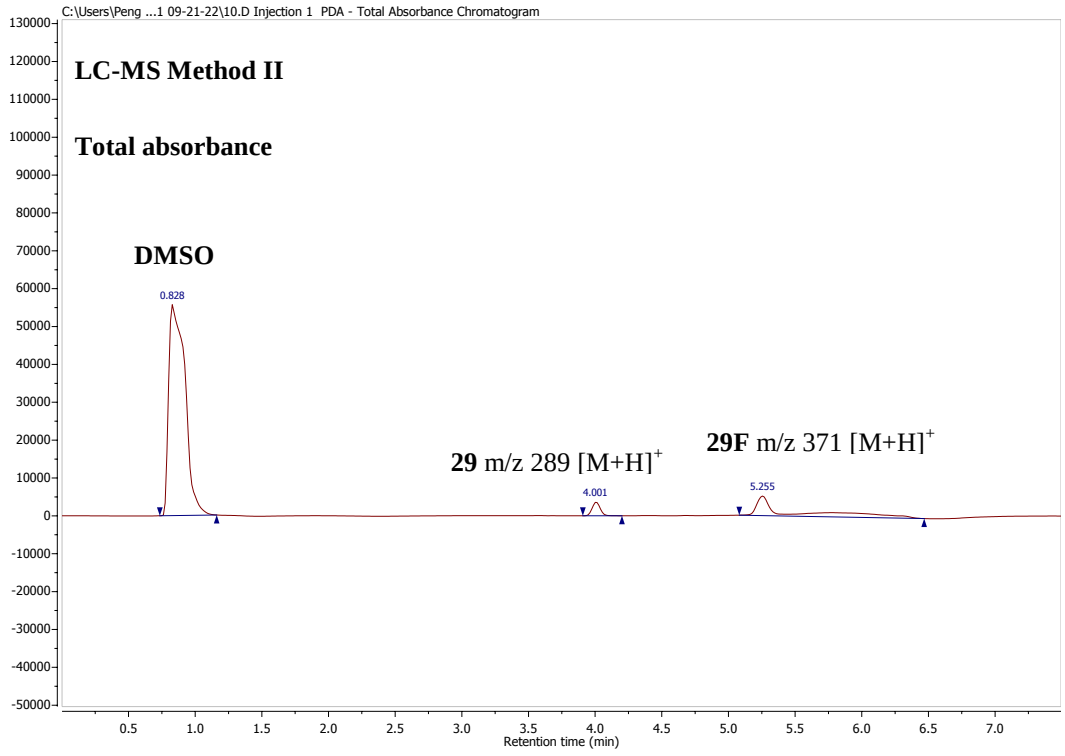
Retention Time (min)	Area %
5.254	**100**

28F: Mol. Wt. 655



Retention Time (min)	Area %
5.975	**10**
6.148	**90**

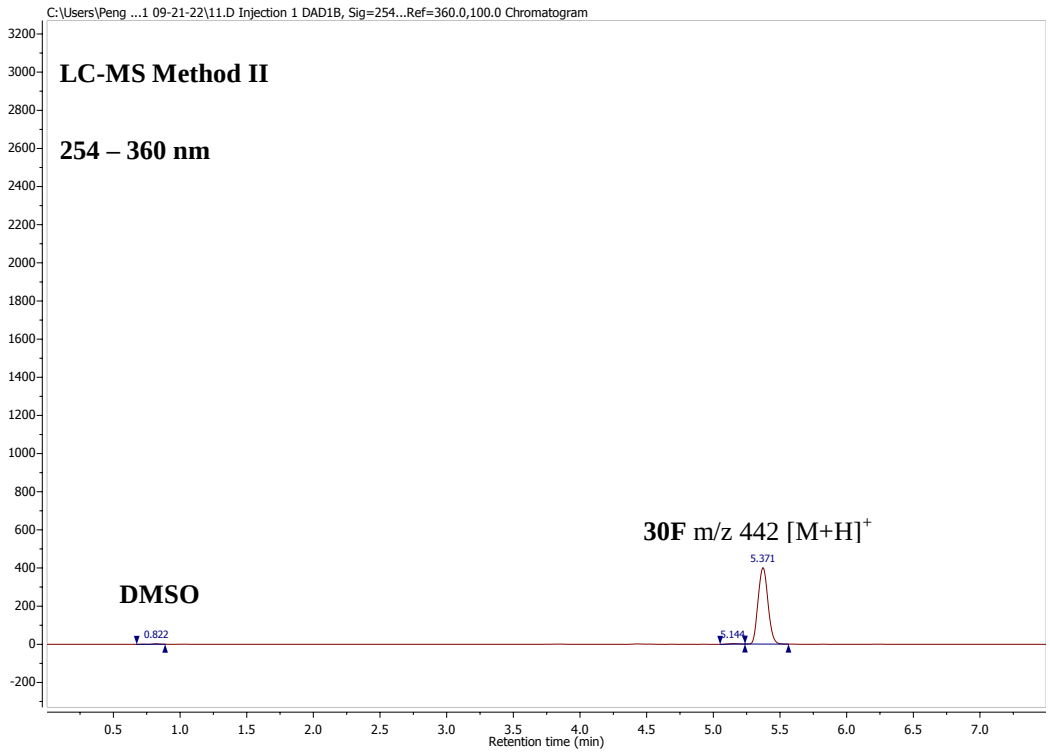
29F: Mol. Wt. 370



Retention Time (min)	Area %

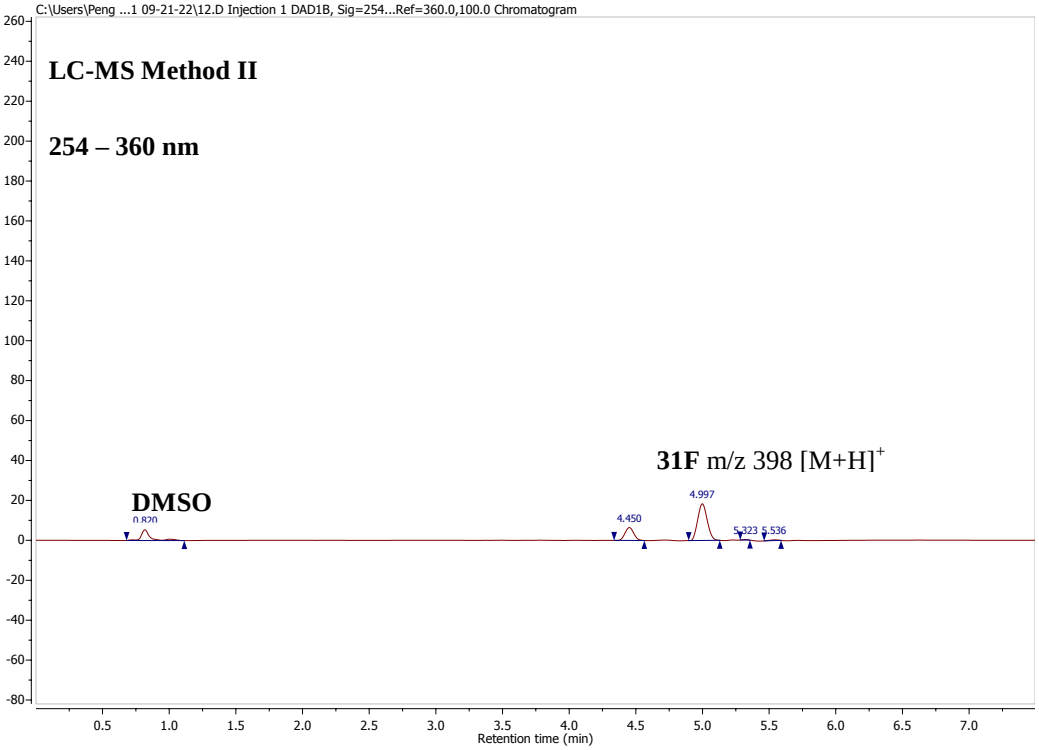
4.001	**34**
5.255	**66**

30F: Mol. Wt. 441



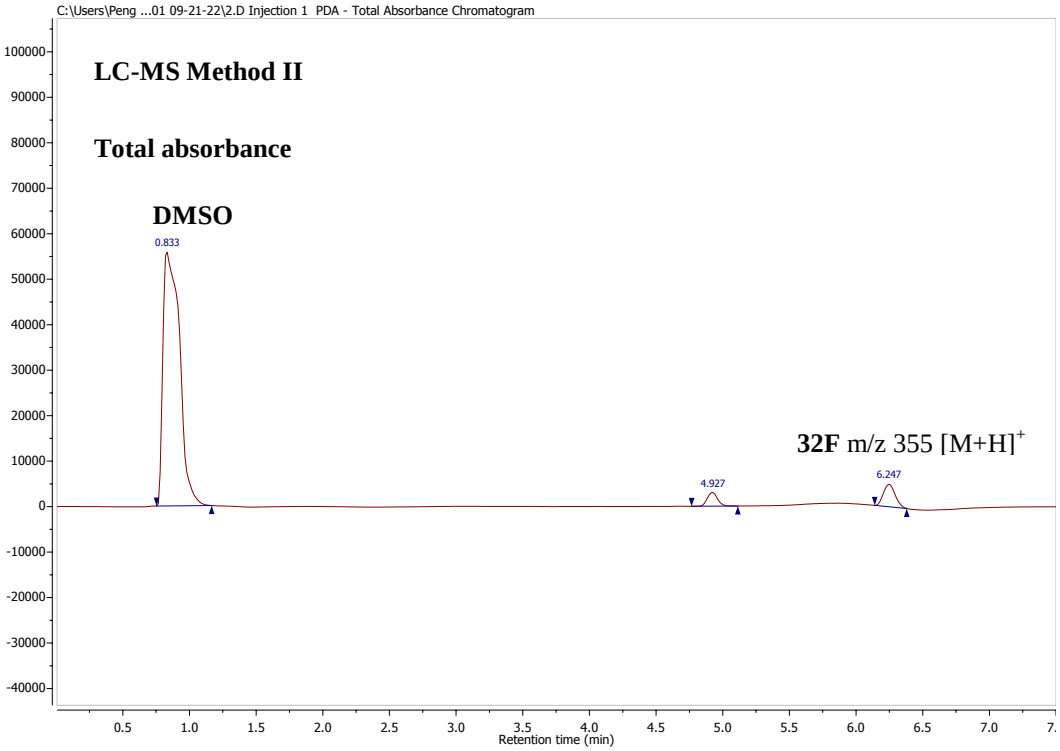
Retention Time (min)	Area %
5.144	**0.8**
5.371	**99.2**

31F: Mol. Wt. 397



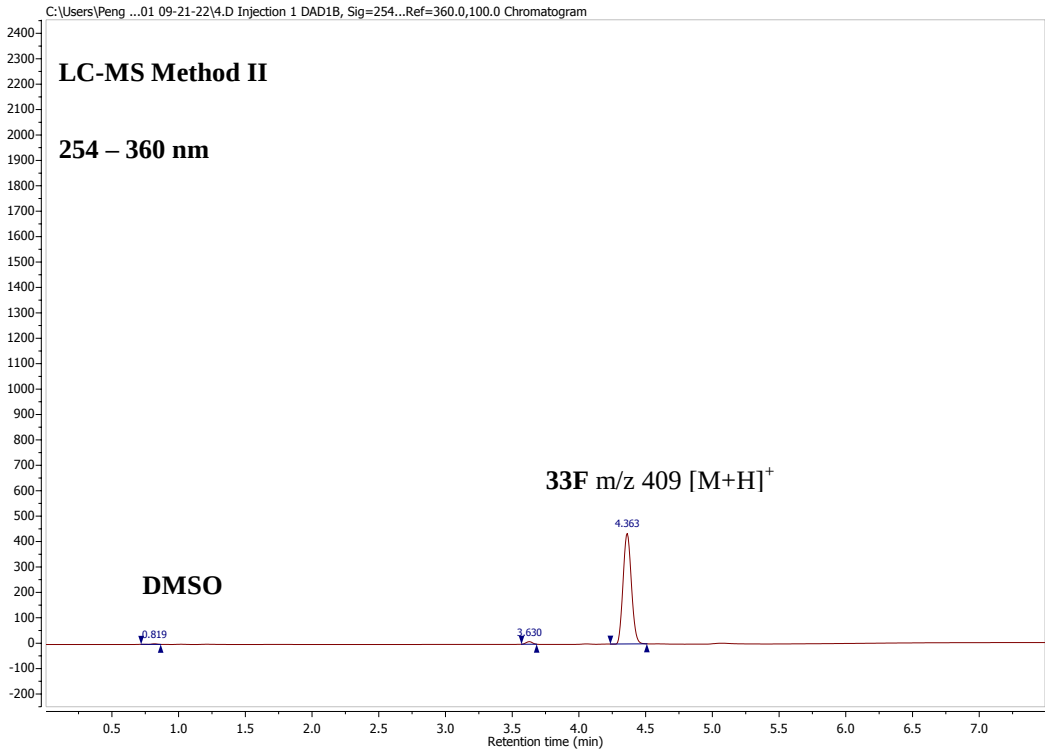
Retention Time (min)	Area %
4.450	**24**
4.997	**74**
5.323	**0.5**
5.536	**1.5**

32F: Mol. Wt. 354



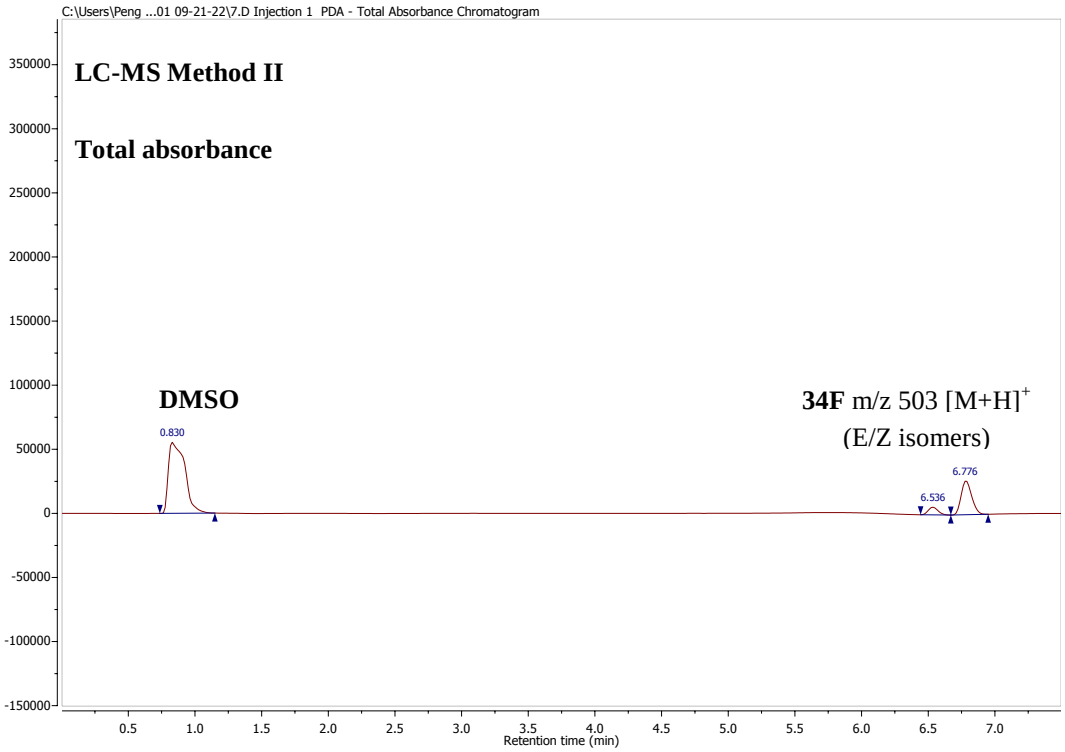
Retention Time (min)	Area %
4.927	**44**
6.247	**56**

33F: Mol. Wt. 408



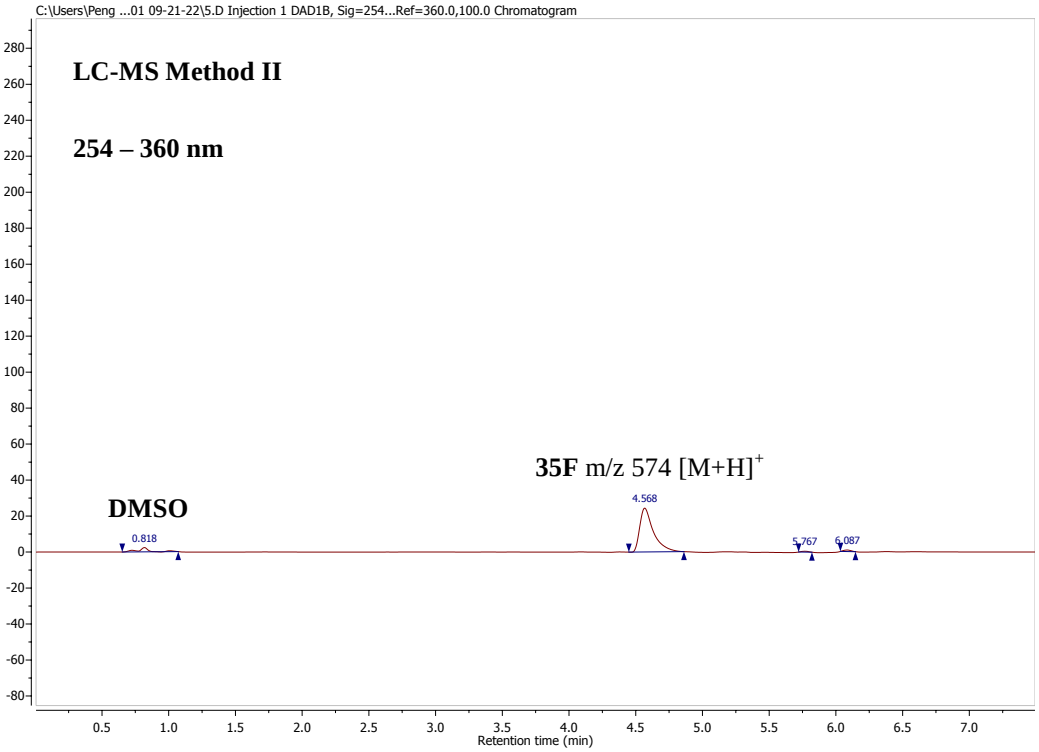
Retention Time (min)	Area %
3.630	**1.7**
4.363	**98.3**

34F: Mol. Wt. 502



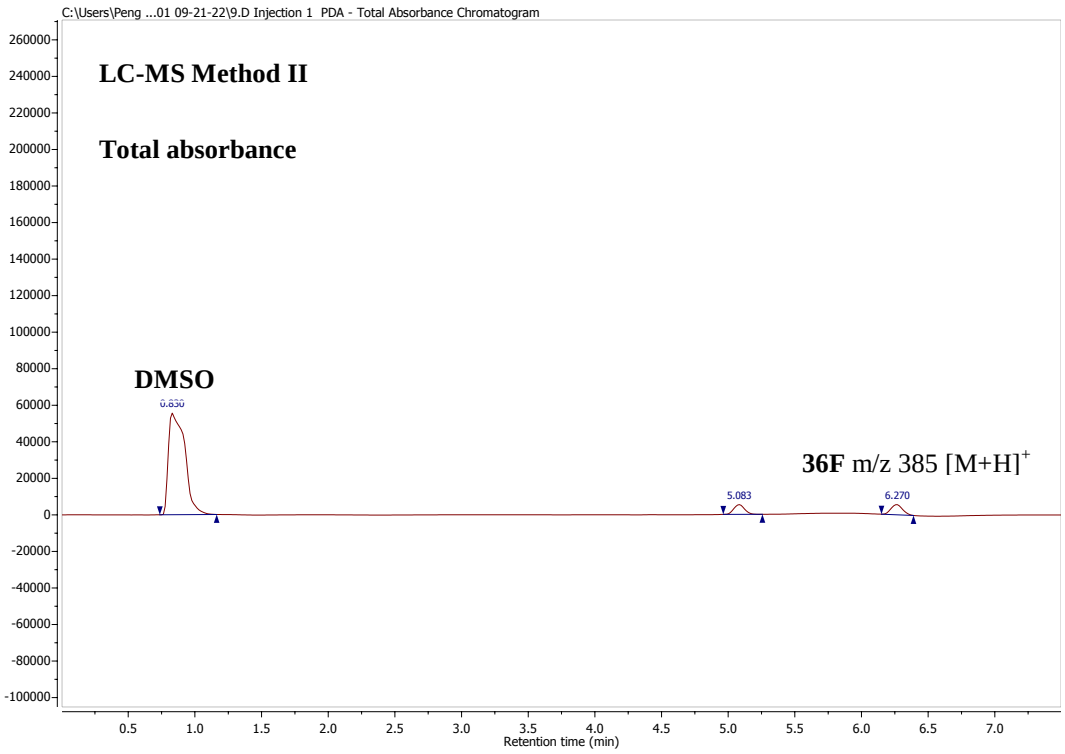
Retention Time (min)	Area %
6.536	**17**
6.776	**83**

35F: Mol. Wt. 573



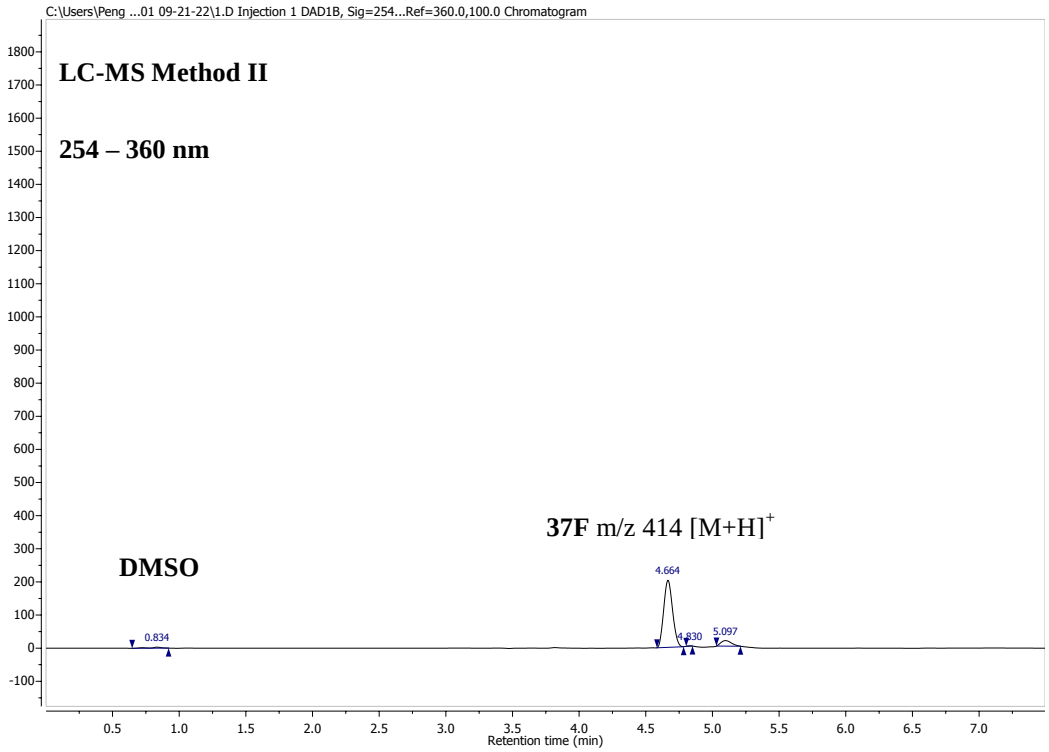
Retention Time (min)	Area %
4.568	**97**
5.767	**1**
6.087	**2**

36F: Mol. Wt. 384



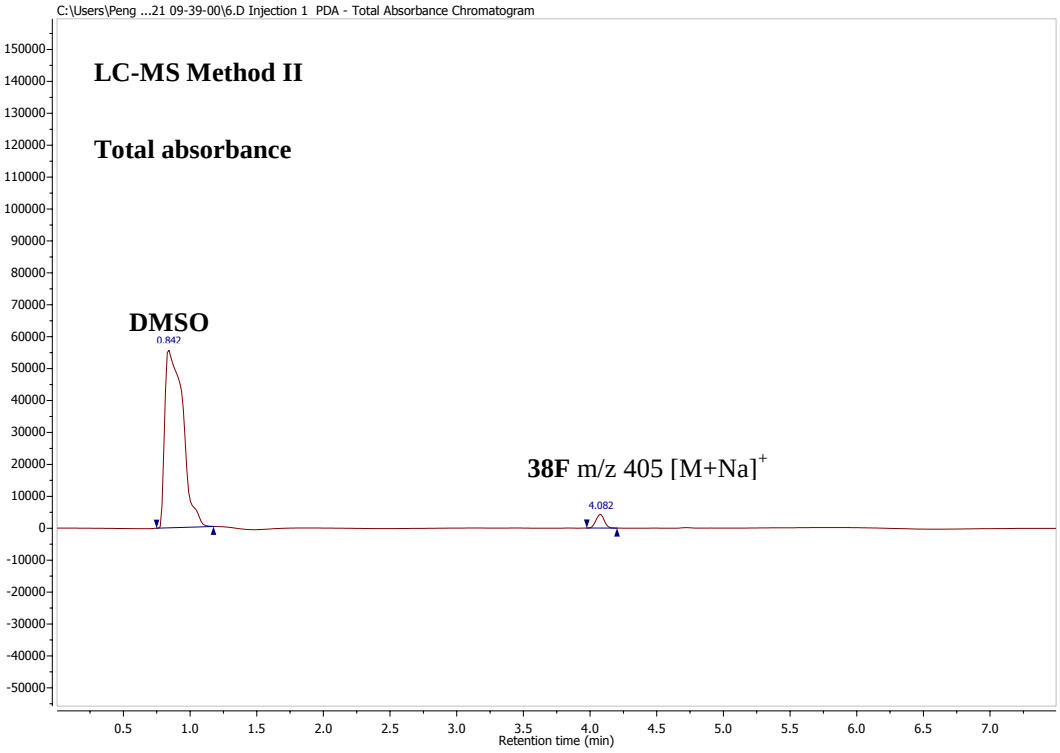
Retention Time (min)	Area %
5.083	**47**
6.270	**53**

37F: Mol. Wt. 413



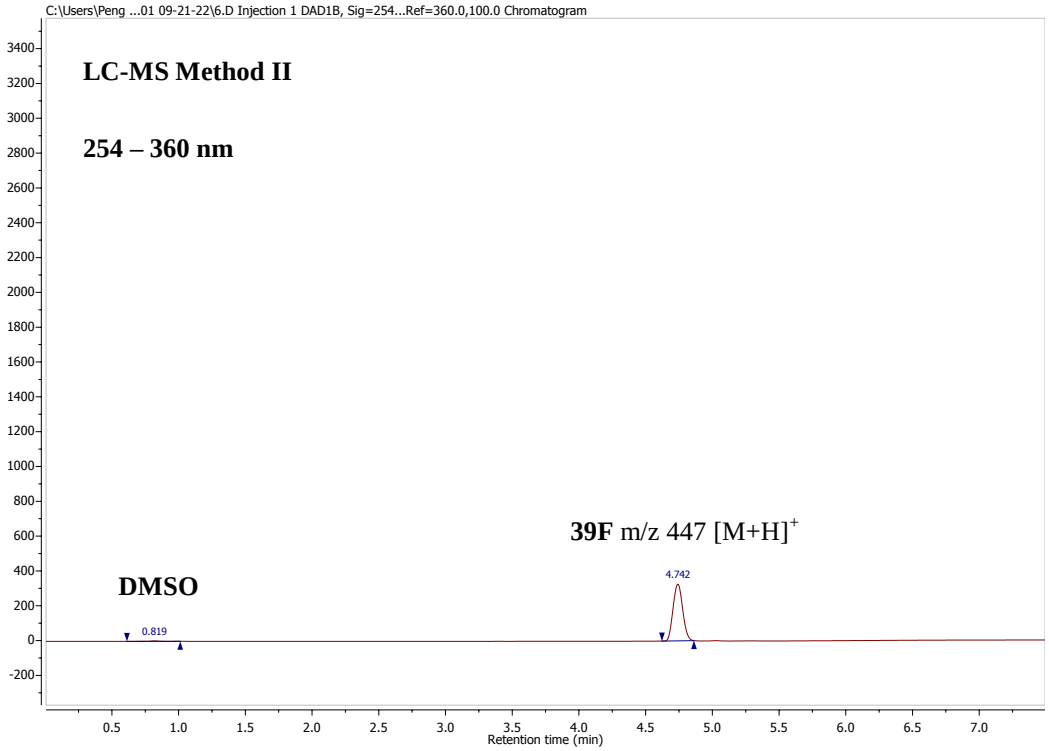
Retention Time (min)	Area %
4.664	**92**
4.830	**1**
5.097	**7**

38F: Mol. Wt. 382



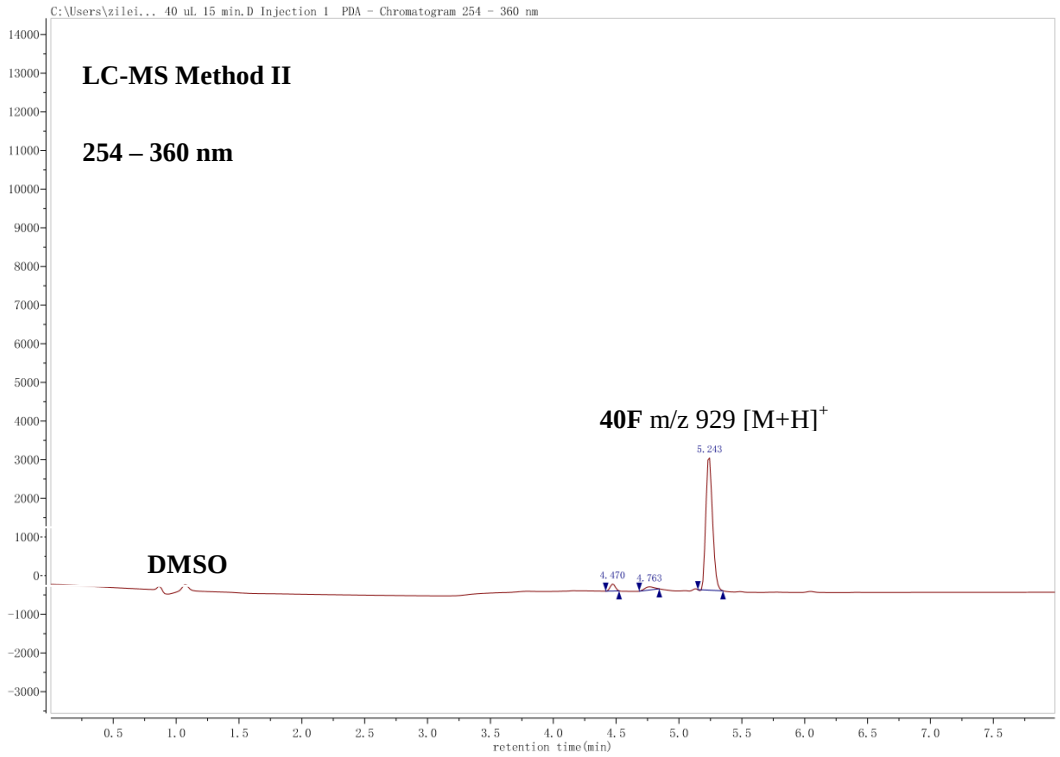
Retention Time (min)	Area %
4.082	**100**

39F: Mol. Wt. 446



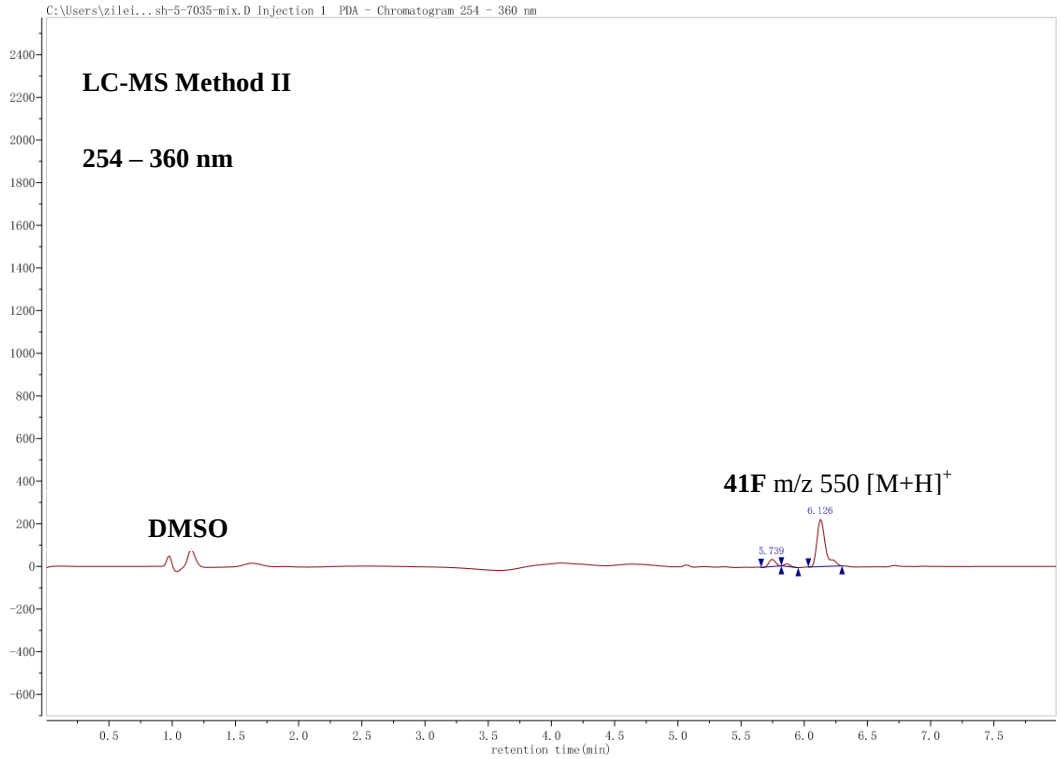
Retention Time (min)	Area %
4.742	**100**

40F: Mol. Wt. 928



Retention Time (min)	Area %
4.470	**4**
4.763	**3**
5.243	**93**

41F: Mol. Wt. 549



Retention Time (min)	Area %
5.739	**10**
5.859	**3**
6.126	**87**