Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Development of a fluorous trapping reagent for rapid detection of electrophilic reactive metabolites

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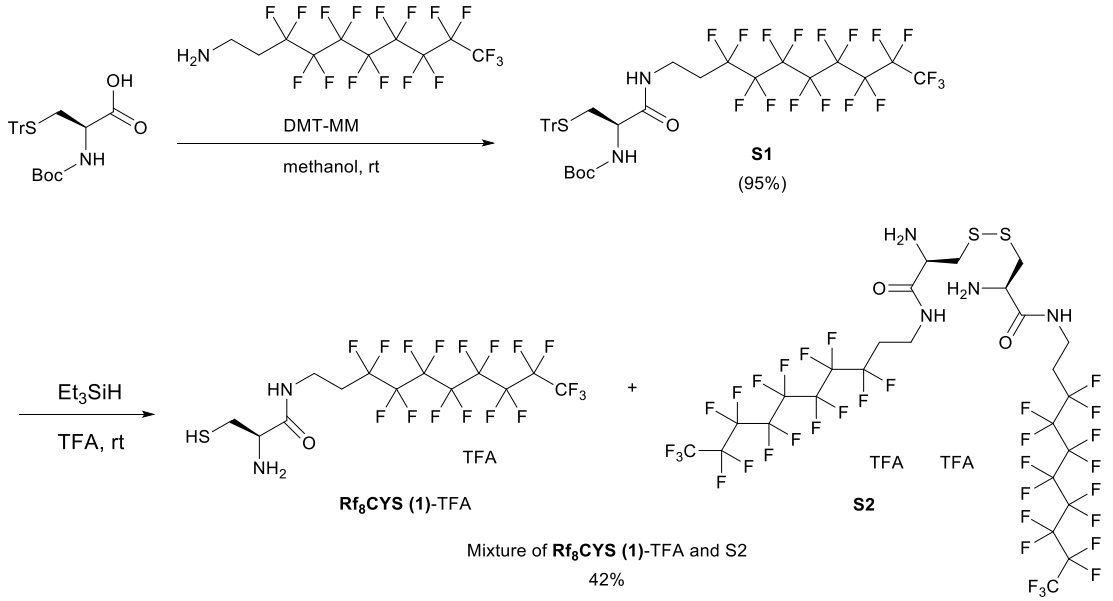
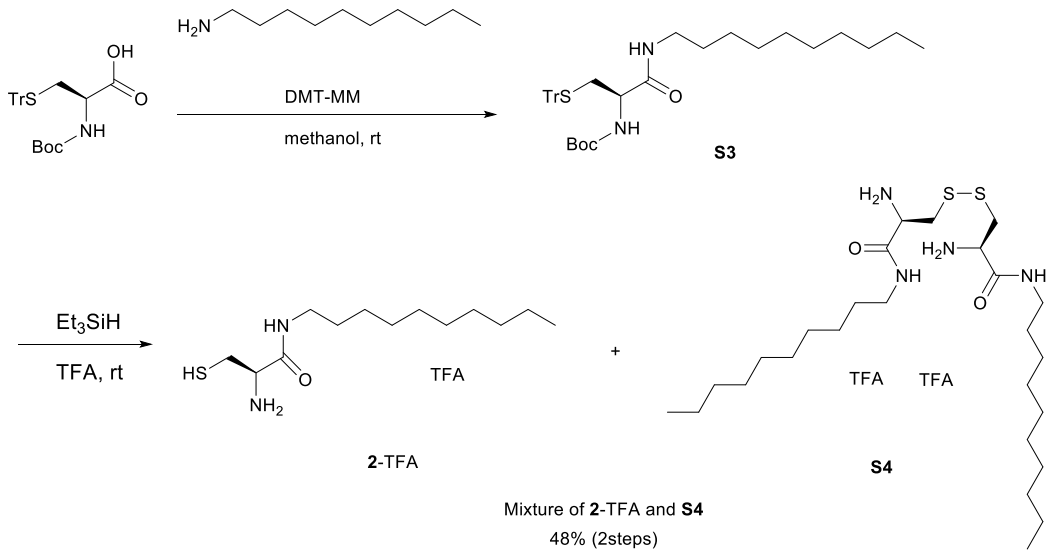
Table of Contents

1. Supplementary figures



Scheme S1 Synthesis route of Rf_8CYS (**1**)-trifluoroacetic acid (TFA).

Rf_8CYS-TFA was synthesized from *N*-(*tert*-butoxycarbonyl)-*S*-trityl-L-cysteine and was obtained as a mixture with the dimer structure **S2**. Rf_8CYS was converted to **S2** during storage in a refrigerator. Reduction of **S2** with tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) was performed before the use of Rf_8CYS-TFA.

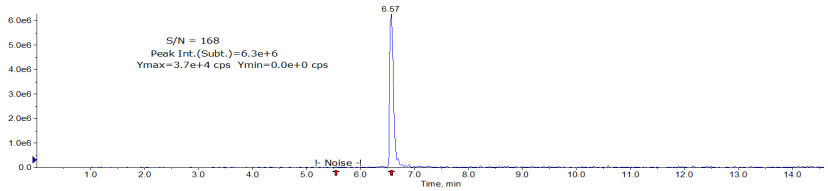


Scheme S2 Synthesis route of **2**-TFA.

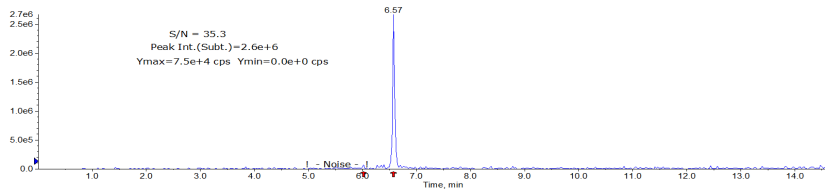
2-TFA was synthesized from *N*-(*tert*-butoxycarbonyl)-S-trityl-L-cysteine and was obtained as a mixture with the dimer structure **S4**. **2** was converted to **S4** during storage in a refrigerator. Reduction of **S4** with TCEP-HCl is performed before the use of **2**-TFA.

(A) MS chromatogram of Rf_8CYS

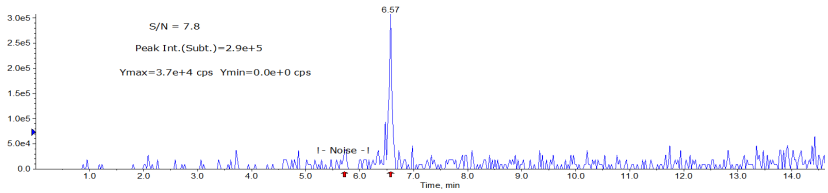
1.5 mmol/L



0.15 mmol/L

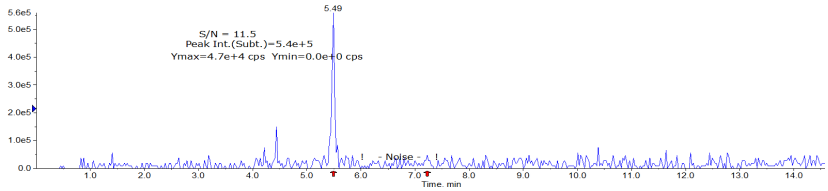


0.015 mmol/L

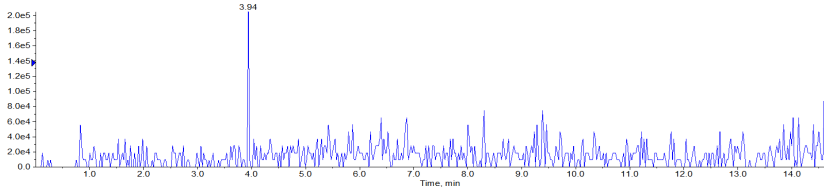


(B) MS chromatogram of **2**

1.5 mmol/L



0.15 mmol/L



(C)

	Rf_8CYS (MS chromatogram MS 567)		**2** (MS chromatogram MS 261)	
Concentration (mmol/L)	S/N ratio	Peak int., cps	S/N ratio	Peak int., cps
1.5	168	$6.3e^6$	11.5	$5.4e^5$
0.15	35.3	$2.6e^6$	-	ND
0.015	7.8	$2.9e^5$	-	-

Figure S1 Comparison of sensitivity in LC/MS between Rf_8CYS and **2**.

MS chromatogram of (A) Rf_8CYS (*m/z* 567) at concentration of 1.5, 0.15 and 0.015 mmol/L, (B) **2** (*m/z* 261) at the concentrations of 1.5 and 0.15 mmol/L. (C) Signal-to-noise (S/N) ratio and peak intensity at each concentration were compared between Rf_8CYS and **2**.

The S/N ratio and peak intensity of Rf_8CYS were more than 10-fold higher than those of **2** at 1.5 mmol/L. Rf_8CYS was detectable at an S/N ratio of 7.8 even at a concentration of 0.015 mmol/L, whereas **2** was not detectable at a concentration of 0.15 mmol/L.

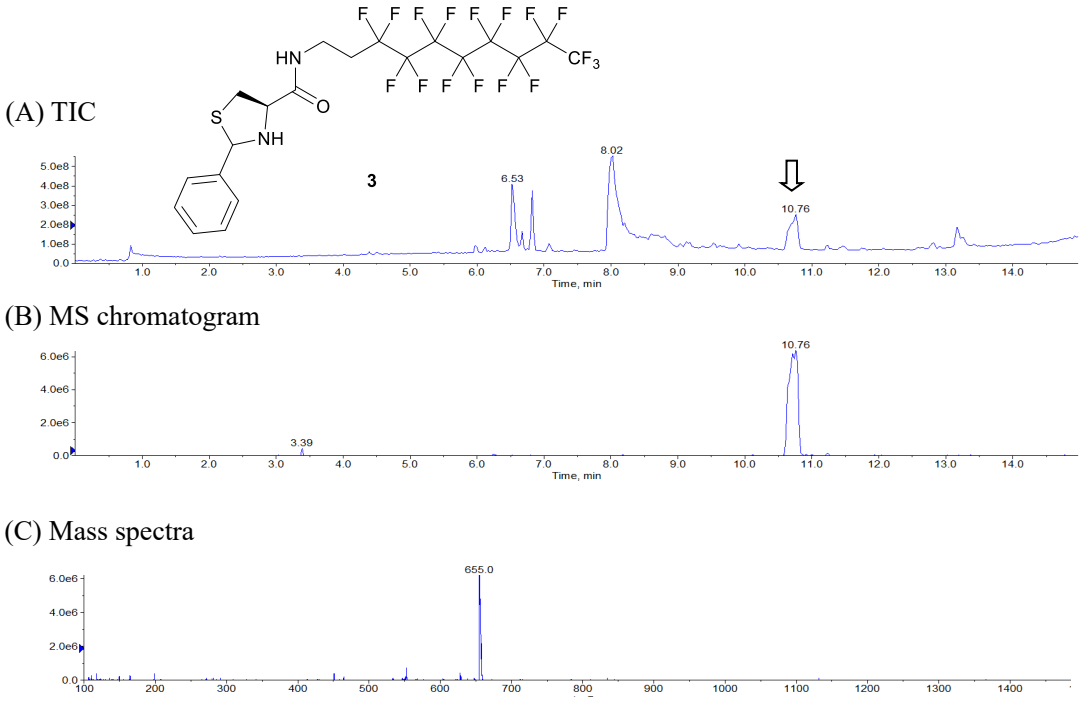


Figure S2. Chromatogram, proposed adduct structure and mass spectra of the reaction with Rf_8CYS and benzaldehyde in phosphate-buffered saline (PBS)/methanol=100/1 (v/v). (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 655), (C) mass spectra of the adduct **3** (retention time 10.76 min).

The structure of **3** was determined by comparing the retention time with that of the product synthesized by another method.

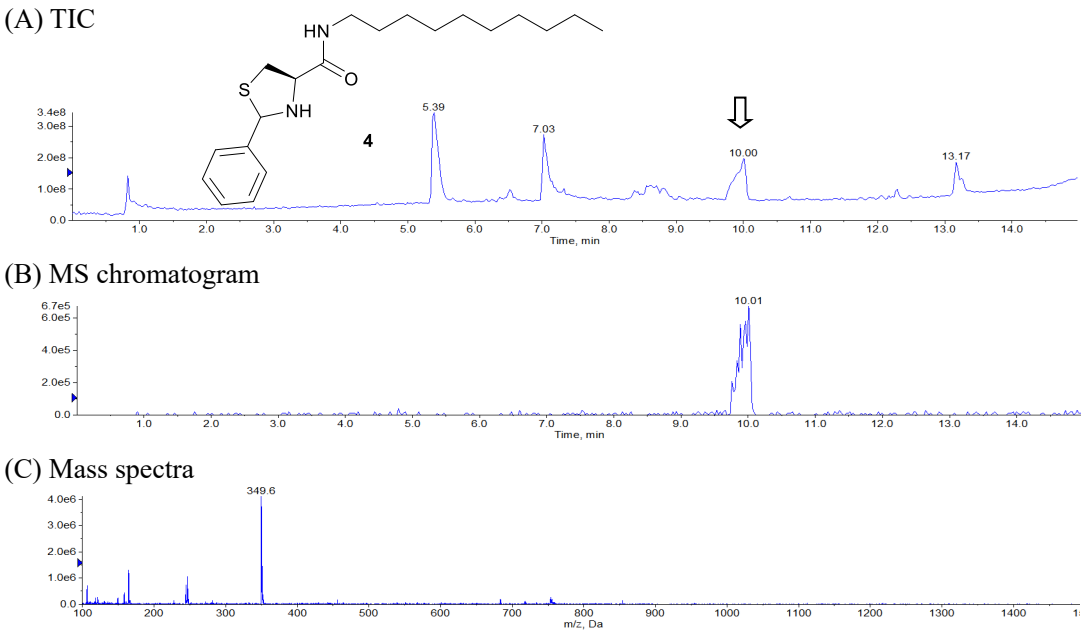


Figure S3. Chromatogram, proposed adduct structure and mass spectra of the reaction with **2** and benzaldehyde in PBS/methanol=100/1 (v/v). (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 349), (C) mass spectra of the adduct **4** (retention time 10.00 min).

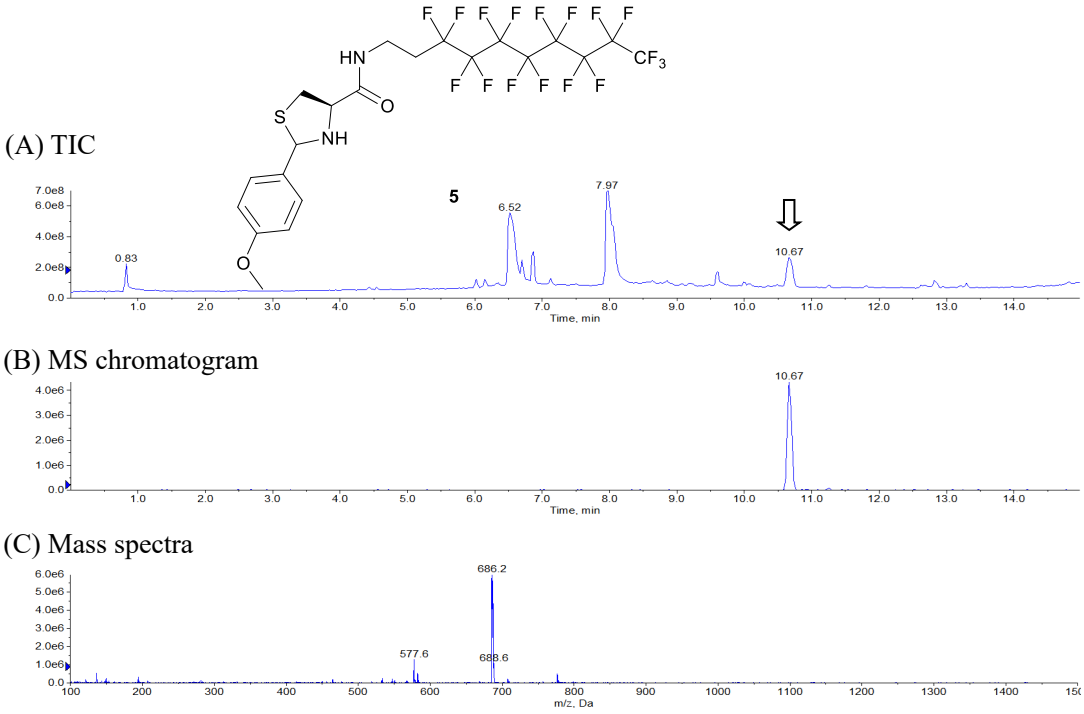


Figure S4. Chromatogram, proposed adduct structure and mass spectra of the reaction with Rf_8CYS and *p*-methoxybenzaldehyde in PBS/methanol=100/1. (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 685), (C) mass spectra of the adduct **5** (retention time 10.67 min).

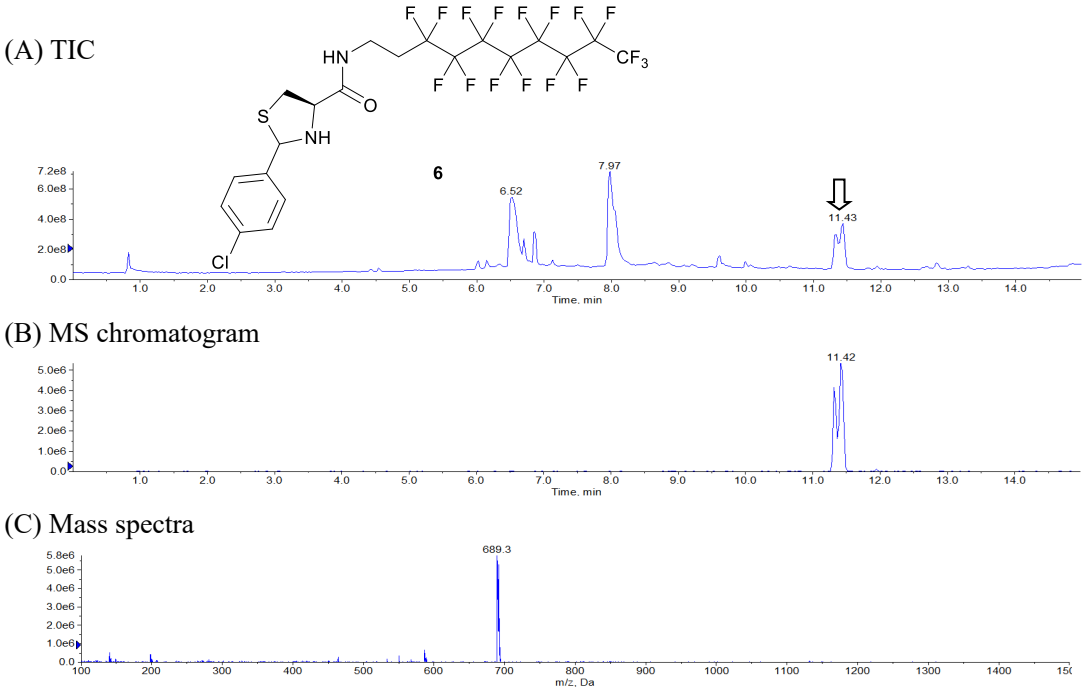


Figure S5. Chromatogram, proposed adduct structure and mass spectra of the reaction with Rf_8CYS and *p*-chlorobenzaldehyde in PBS/methanol=100/1. (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 689), (C) mass spectra of the adduct **6** (retention time 11.42 min).

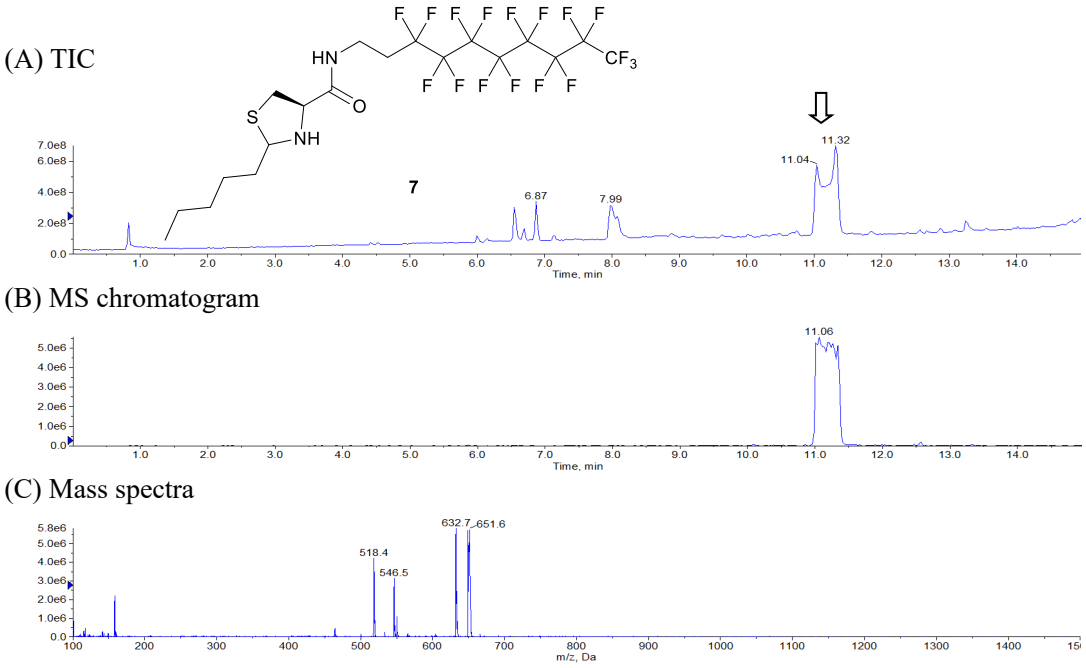
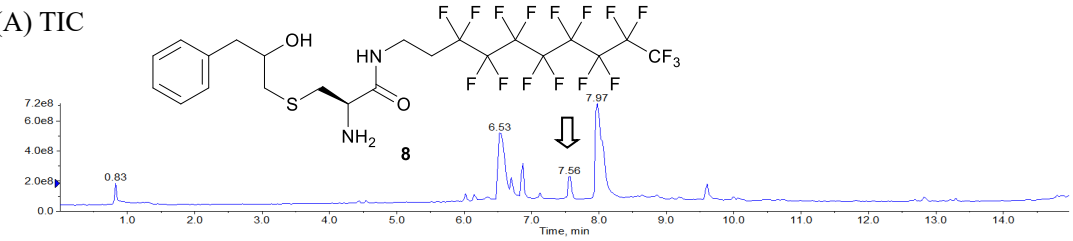
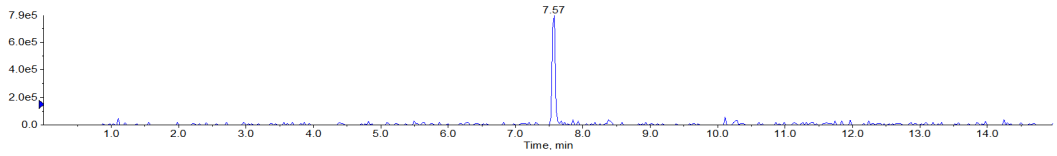


Figure S6. Chromatogram, proposed adduct structure and mass spectra of the reaction with Rf_8CYS and hexanal in PBS/methanol=100/1. (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 649), (C) mass spectra of the adduct **7** (retention time 11.06 min).

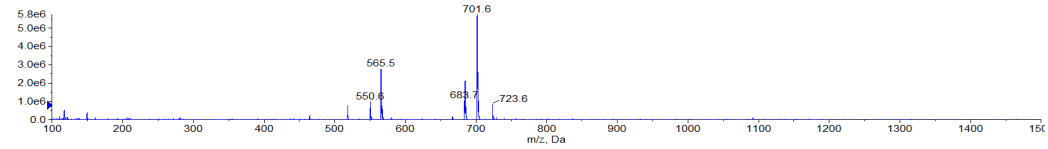
(A) TIC



(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS)

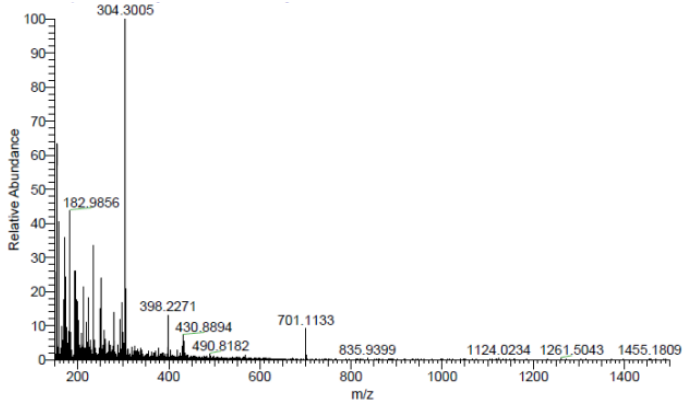
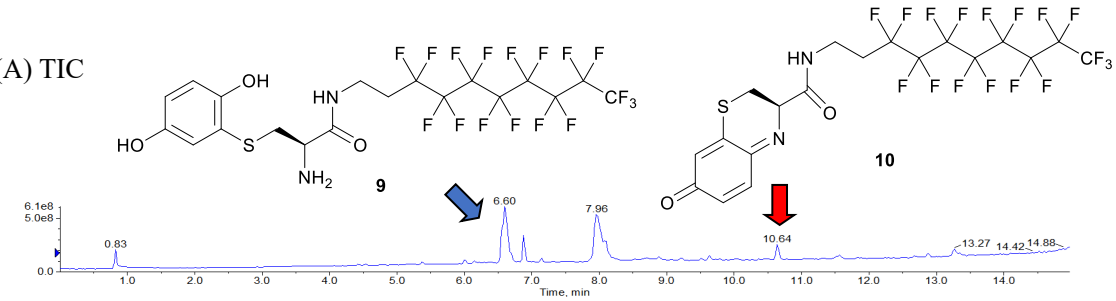


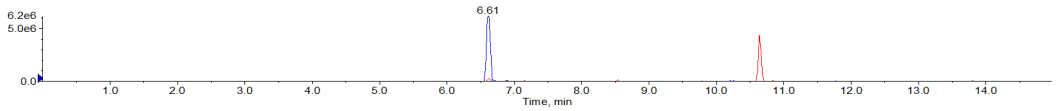
Figure S7. Chromatogram, proposed adduct structure and mass spectra of the reaction with Rf_8CYS and (2,3-epoxypropyl)benzene in PBS/methanol=100/1. (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (*m/z* 701), (C) mass spectra of the adduct **8** (retention time 7.57 min). (D) HRMS (ESI) spectra of the adduct **8** $[M+H]^+$ calculated for $C_{22}H_{22}O_2N_2F_{17}S$ 701.1131; found 701.1133.

8 decomposed during purification and could not be isolated. The structure of **8** was proposed from the structure of a derivative **S8** synthesized by another method.

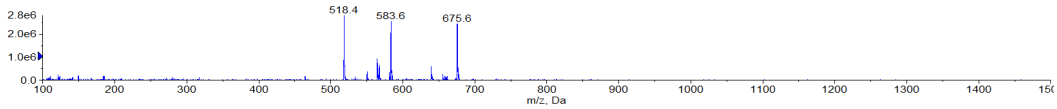
(A) TIC



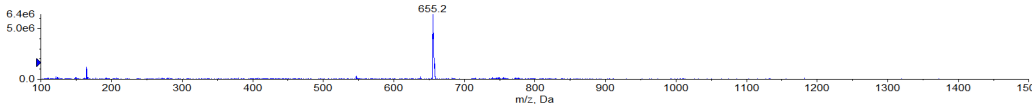
(B) MS chromatogram



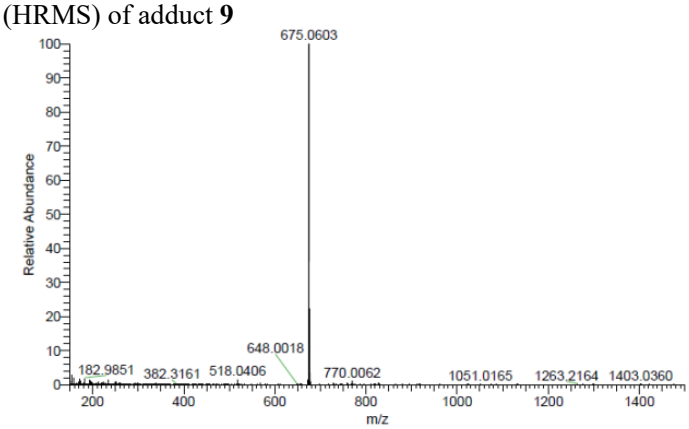
(C) Mass spectra (6.61 min)



(D) Mass spectra (10.64 min)



(E) Mass spectra (HRMS) of adduct **9**



(F) Mass spectra (HRMS) of adduct **10**

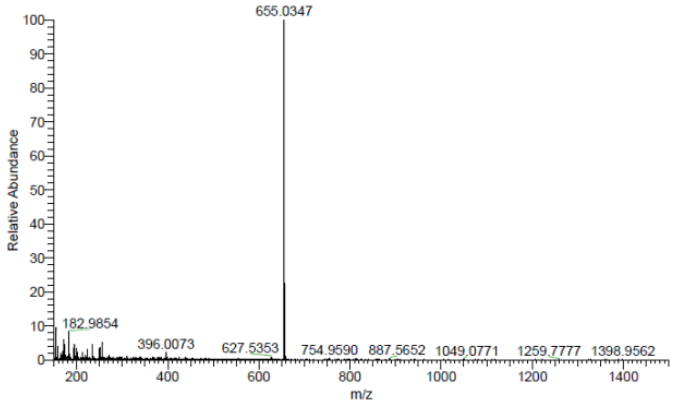


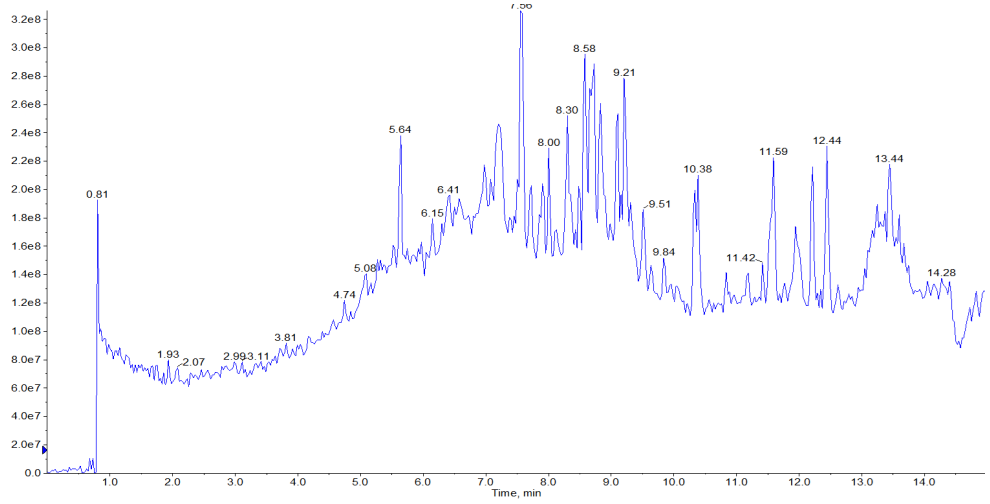
Figure S8. Chromatogram, proposed adduct structures and mass spectra of the reaction with Rf_8CYS

and *p*-benzoquinone in PBS/methanol=100/1 (v/v). (A) Total ion chromatogram (*m/z* 100 to 1500), (B) MS chromatogram (blue line: *m/z* 675, red line: 655, (C) mass spectra of the adduct **9** (retention time 6.61 min), (D) mass spectra of the adduct **10** (retention time 10.64 min).

(E) HRMS (ESI) spectra of the adduct **9** $[M+H]^+$ calculated for $C_{19}H_{16}O_3N_2F_{17}S$ 675.0610; found 675.0603. (F) HRMS (ESI) spectra of the adduct **10** $[M+H]^+$ calculated for $C_{19}H_{12}O_2N_2F_{17}S$ 655.0348; found 655.0347.

The structures of **10** were determined by comparing their retention times with those synthesized by other methods. **9** was eliminated over time and could not be isolated.

(A) TIC of control sample before F-SPE



(B) TIC of control sample after F-SPE

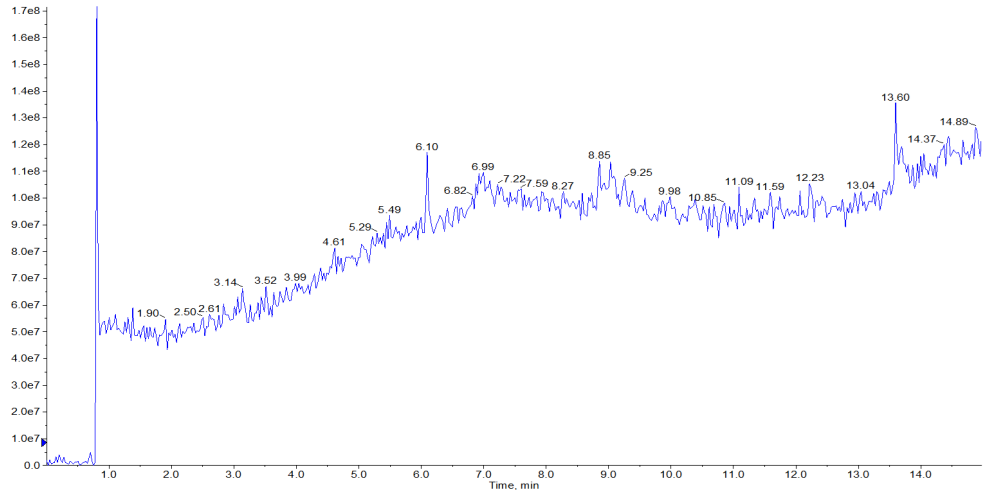
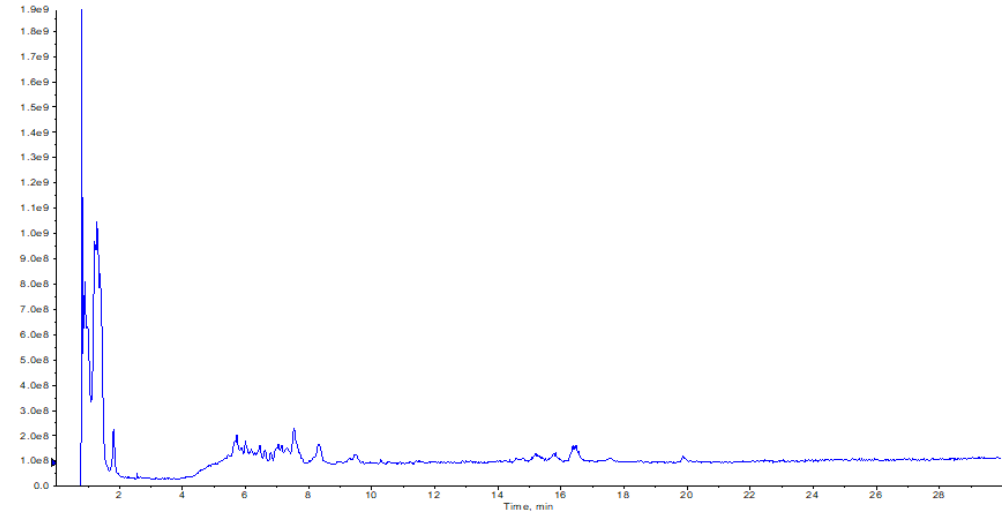
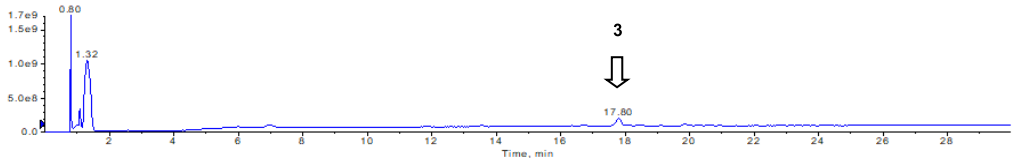


Figure S9. Total ion chromatogram (*m/z* 100 to 1500) of a control sample (human liver microsomes (HLM), (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min) (A) before purification and (B) after purification using fluorous solid-phase extraction (F-SPE) method.

(A) TIC of control sample



(B) TIC of adduct **3**



(C) MS chromatogram TIC of adduct **3**

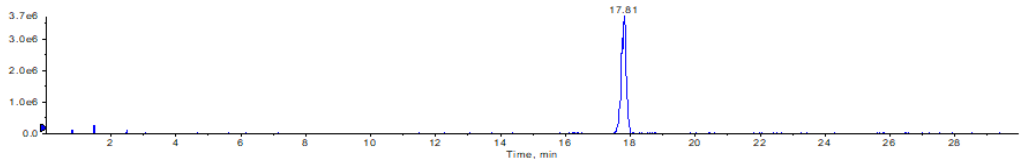


Figure S10. (A) Total ion chromatogram *(m/z* 100 to 1500) of a control sample (HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min) using a direct injection method. (B) Total ion chromatogram (*m/z* 100 to 1500) and (C) MS chromatogram (*m/z* 655) of benzaldehyde adduct **3** (retention time 17.81 min) using a direct injection method.

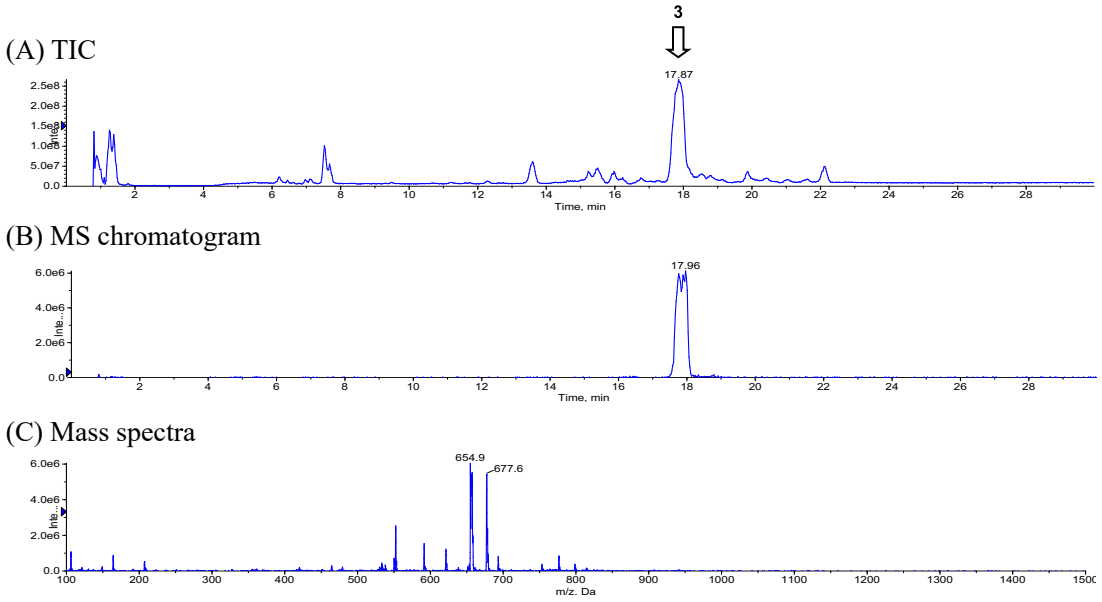


Figure S11. Chromatogram and mass spectra of the reaction with Rf_8CYS and benzaldehyde in human liver microsome condition (Rf_8CYS (150 µM), benzaldehyde (150 µM), HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min). (A) Total ion chromatogram (*m/z* 600 to 700), (B) MS chromatogram (*m/z* 655), (C) mass spectra of the adduct **3** (retention time 17.86 min) using a direct injection method.

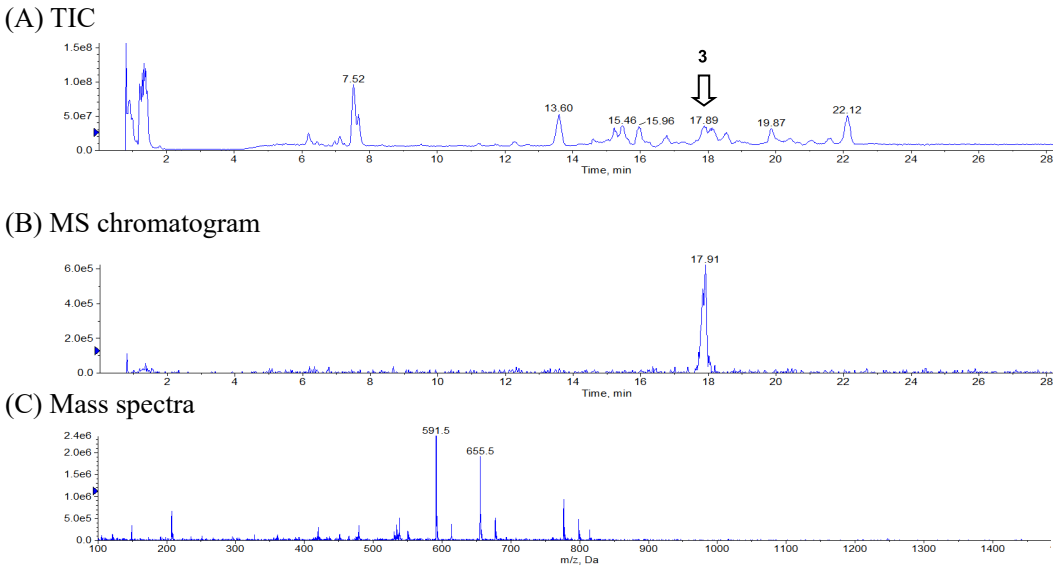


Figure S12. Chromatogram and mass spectra of microsomal incubations with benzyl alcohol and Rf_8CYS. Benzyl alcohol (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 600 to 700), (B) MS chromatogram (*m/z* 655), (C) mass spectra of the adduct **3** (retention time 17.91 min) using a direct injection method.

(A) TIC of control sample (without $NADPH^+$)

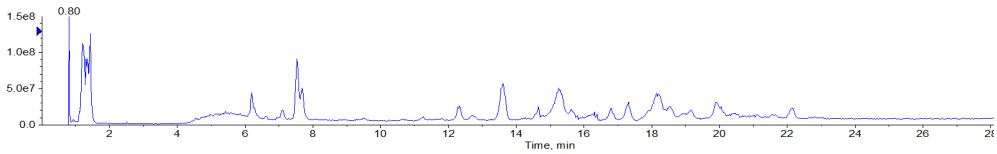


Figure S13. Total ion chromatogram (*m/z* 600 to 700) of control sample (benzyl alcohol (150 µM), Rf_8CYS (150 µM), HLM (1.0 mg/mL) without a $NADPH^+$ generating system at 37°C for 60 min) using a direct injection method.

(A) TIC of control sample (without benzyl alcohol)

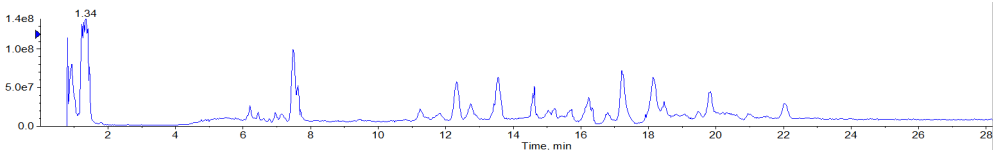


Figure S14. Total ion chromatogram (*m/z* 600 to 700) of a control sample (Rf_8CYS (150 µM), HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min) using a direct injection method.

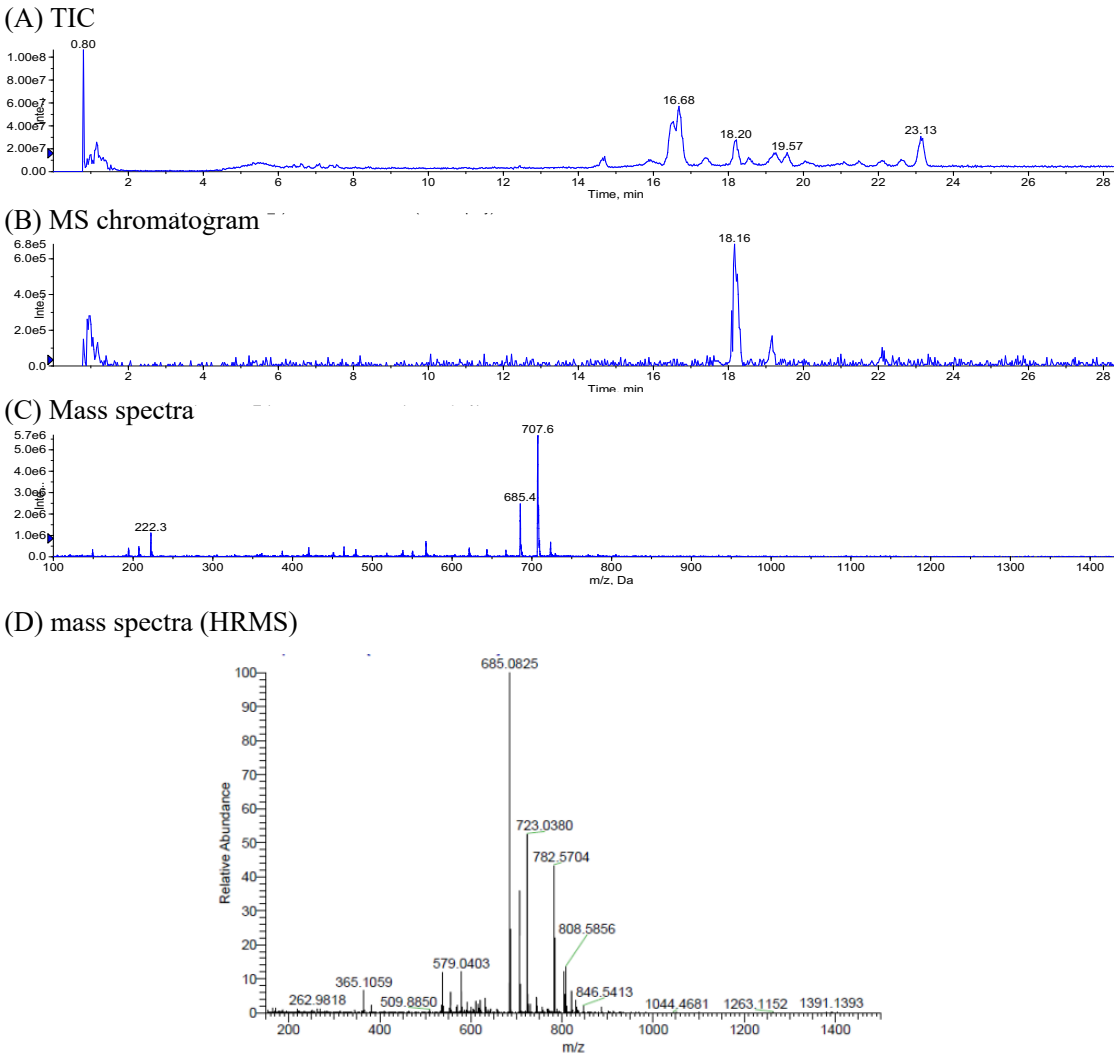
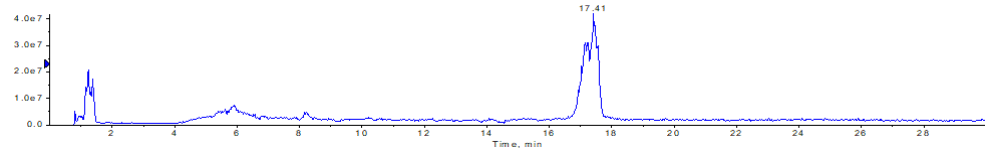
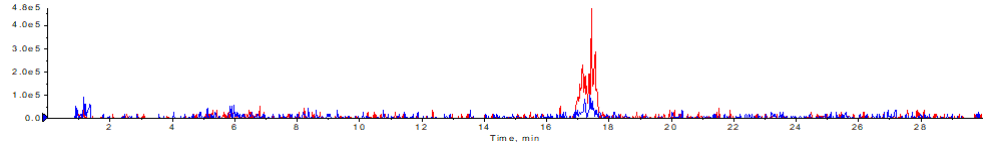


Figure S15. Chromatograms and mass spectra of microsomal incubations with ethynylbenzene and Rf_8CYS. Ethynylbenzene (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 650 to 700), (B) MS chromatogram (*m/z* 685), (C) mass spectra of the adduct (retention time 18.16 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{21}H_{18}O_2N_2F_{17}S$ 685.0812; found 685.0825.

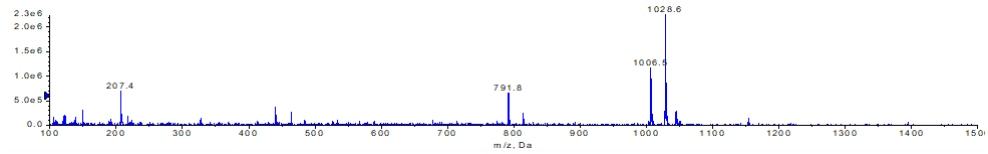
(A) TIC



(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS)

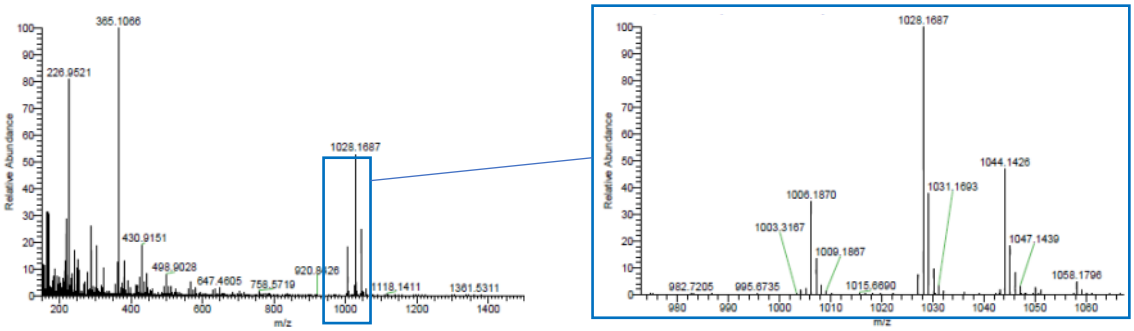
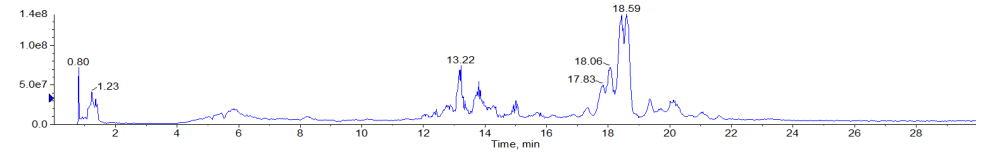
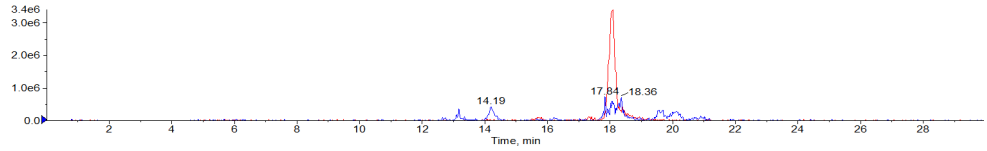


Figure S16. Chromatograms and mass spectra of microsomal incubations with troglitazone and Rf_8CYS. Troglitazone (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 1000 to 1050), (B) MS chromatogram (blue line: *m/z* 1006, red line: 1028), (C) mass spectra of the adduct (retention time 17.41 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{37}H_{37}O_6N_3F_{17}S_2$ 1006.1852; found 1006.1870 and $[M+Na]^+$ calculated for $C_{37}H_{36}O_6N_3F_{17}NaS_2$ 1028.1672; found 1028.1687.

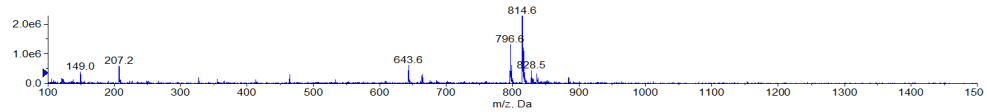
(A) TIC



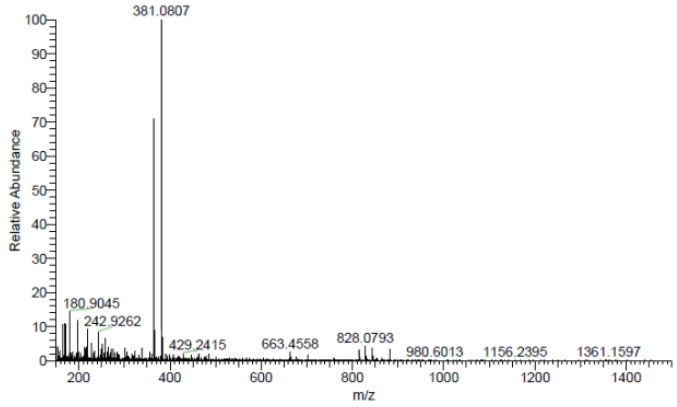
(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS) of adduct (MS 828)



(E) Mass spectra (HRMS) of adduct (MS 814)

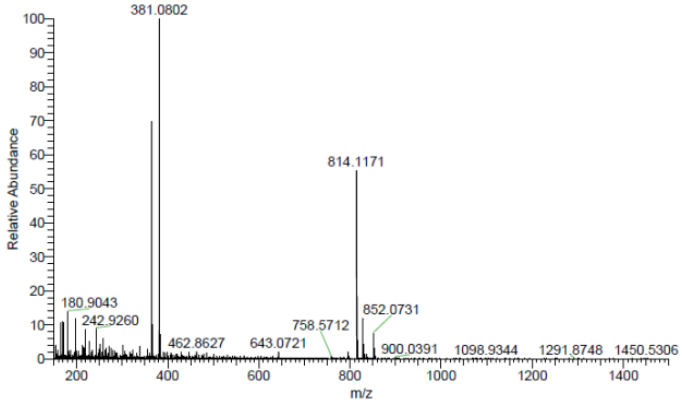
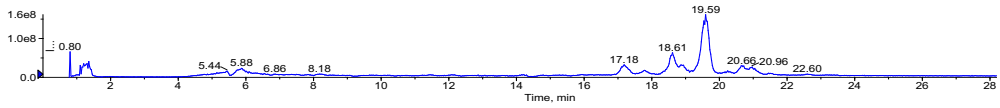


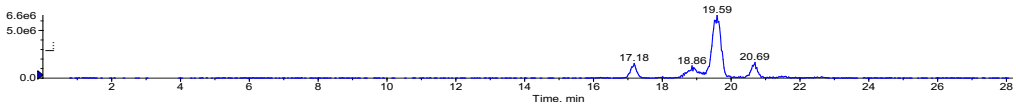
Figure S17. Chromatograms and mass spectra of microsomal incubations with ticlopidine and Rf_8CYS. Ticlopidine (150 μM) was incubated with Rf_8CYS (150 μM) in HLM (1.0 mg/mL) fortified with $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 800 to 900),

(B) MS chromatogram (blue line: *m/z* 828, red line: 814), (C) mass spectra of the adduct (retention time 18.14 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{27}H_{24}ON_3ClF_{17}S_2$ 828.0778; found 828.0793 and (E) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{27}H_{26}O_2N_3ClF_{17}S$ 814.1163; found 814.1171.

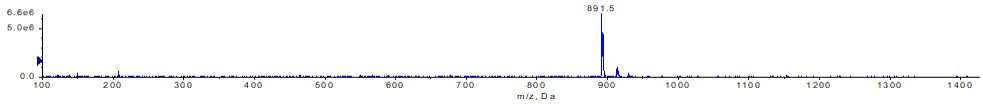
(A) TIC



(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS)

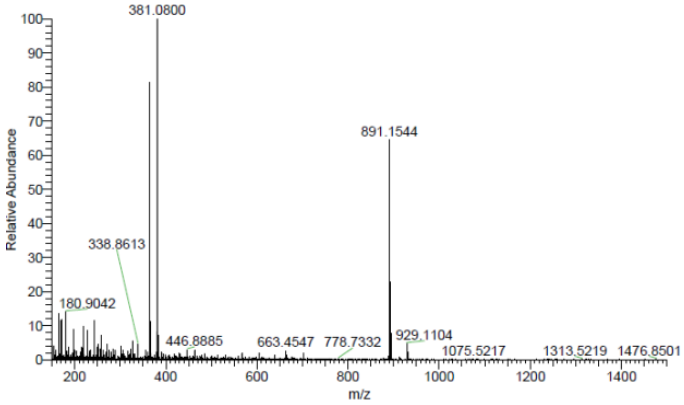
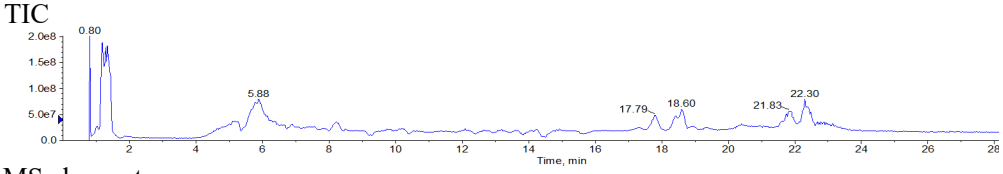
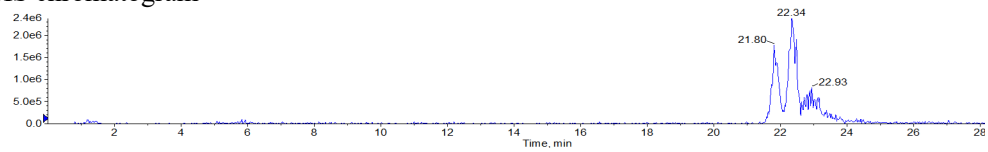


Figure S18. Chromatograms and mass spectra of microsomal incubations with clozapine and Rf_8CYS. Clozapine (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 850 to 950), (B) MS chromatogram (*m/z* 891), (C) mass spectra of the adduct (retention time 19.59 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{31}H_{29}ON_6ClF_{17}S$ 891.1541; found 891.1544.

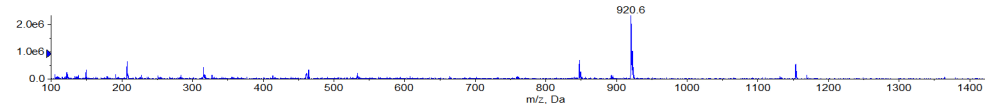
(A) TIC



(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS)

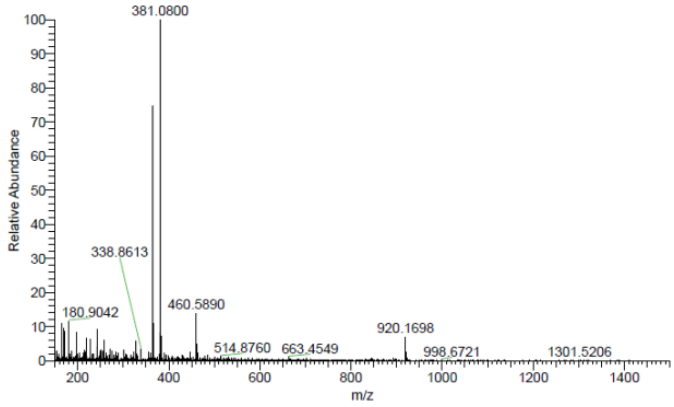
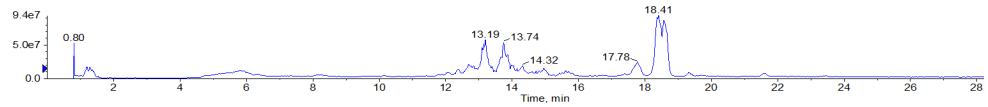
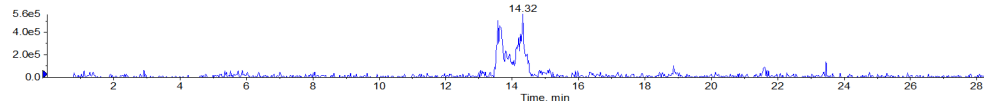


Figure S19. Chromatograms and mass spectra of microsomal incubations with amodiaquine dihydrochloride dihydrate and Rf_8CYS. Amodiaquine dihydrochloride dihydrate (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 830 to 1300), (B) MS chromatogram (*m/z* 920), (C) mass spectra of the adduct (retention time 22.34 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{33}H_{32}O_2N_5ClF_{17}S$ 920.1694; found 920.1698.

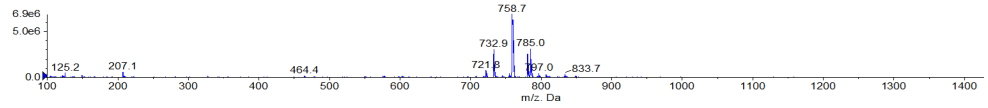
(A) TIC



(B) MS chromatogram



(C) Mass spectra



(D) Mass spectra (HRMS)

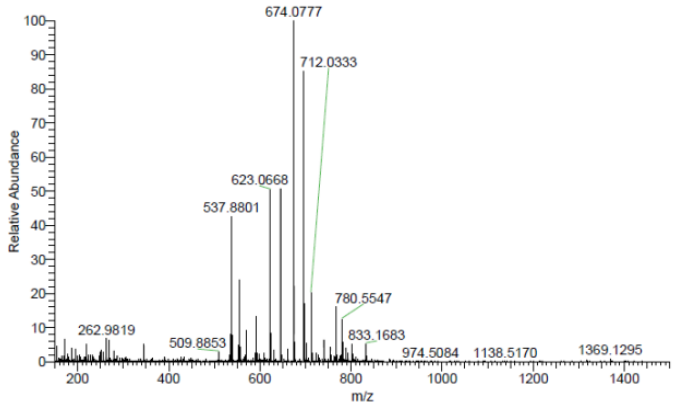
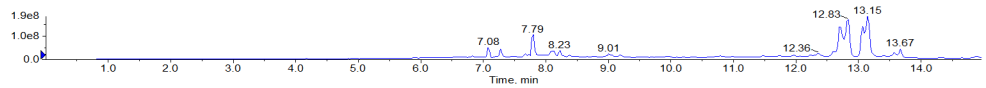
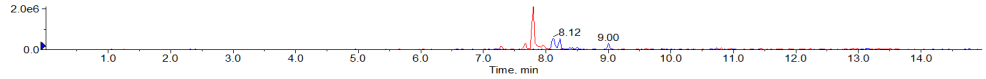


Figure S20. Chromatograms and mass spectra of microsomal incubations with abacavir and Rf_8CYS. Abacavir (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 800 to 850), (B) MS chromatogram (*m/z* 833), (C) mass spectra of the adduct (retention time 14.32 min) using a direct injection method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{27}H_{26}ON_8F_{17}S$ 833.1679; found 833.1683.

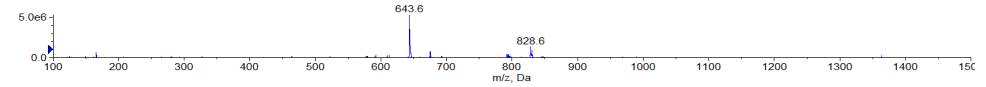
(A) TIC



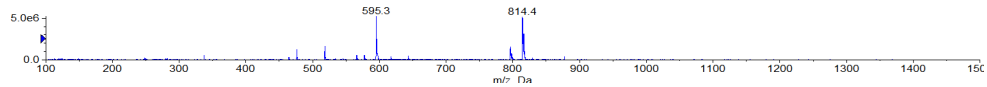
(B) MS chromatogram



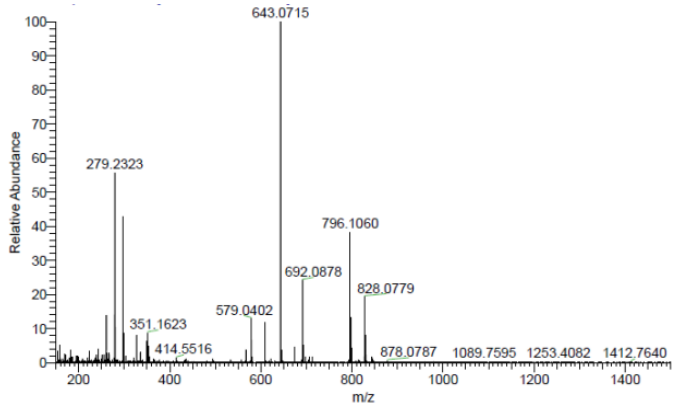
(C) Mass spectra (8.12 min)



(D) Mass spectra (7.79 min)



(E) Mass spectra (HRMS) of adduct (MS 828)



(F) Mass spectra (HRMS) of adduct (MS 814)

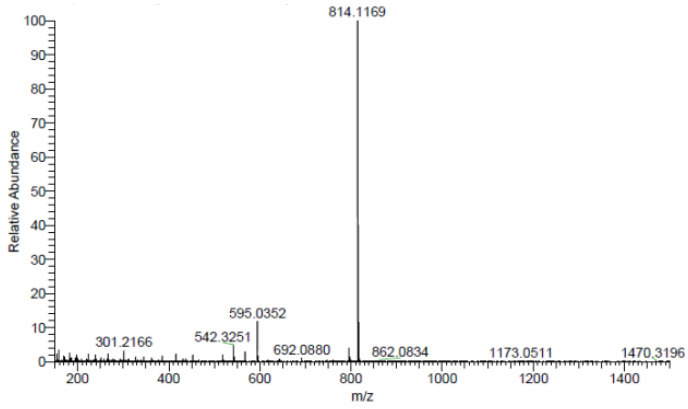
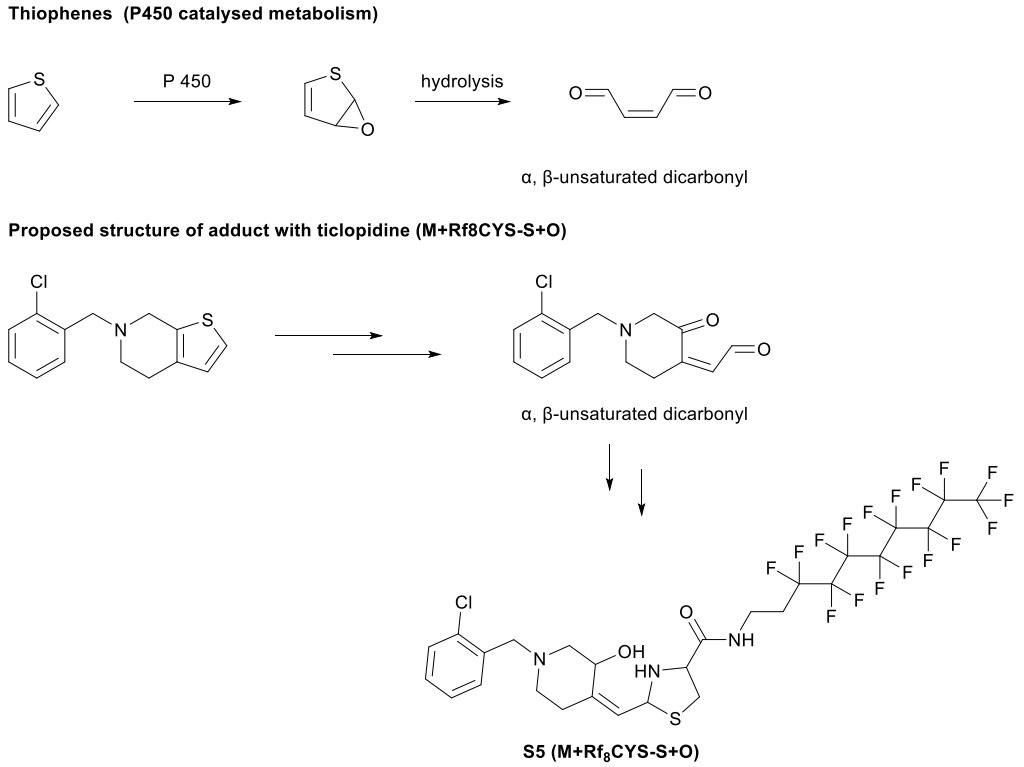


Figure S21. Chromatograms and mass spectra of microsomal incubations with ticlopidine and Rf_8CYS. Ticlopidine (150 µM) was incubated with Rf_8CYS (150 µM) in HLM (1.0 mg/mL) fortified with a $NADPH^+$ generating system at 37°C for 60 min. (A) Total ion chromatogram (*m/z* 800 to 900),

(B) MS chromatogram (blue line: *m/z* 828, red line: 814), (C) mass spectra of the adduct (retention time 8.12 min) (D) mass spectra of the adduct (retention time 7.79 min) after purification using F-SPE method. (D) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{27}H_{24}ON_3ClF_{17}S_2$ 828.0778; found 828.0779, (E) HRMS (ESI) spectra of the adduct $[M+H]^+$ calculated for $C_{27}H_{26}O_2N_3ClF_{17}S$ 814.1163; found 814.1169.



Scheme S3. Metabolic pathway of thiophene and proposed structure of adduct (M+Rf_8CYS-S+O) Thiophene is bio-activated to form an electrophilic α,β-unsaturated dicarbonyl.[1] Given the proposed metabolite of thiophene, the structure of (M+Rf_8CYS-S+O) was determined to be **S5**.

MS/HRMS spectra of adduct (M+Rf$_8$CYS-S+O)

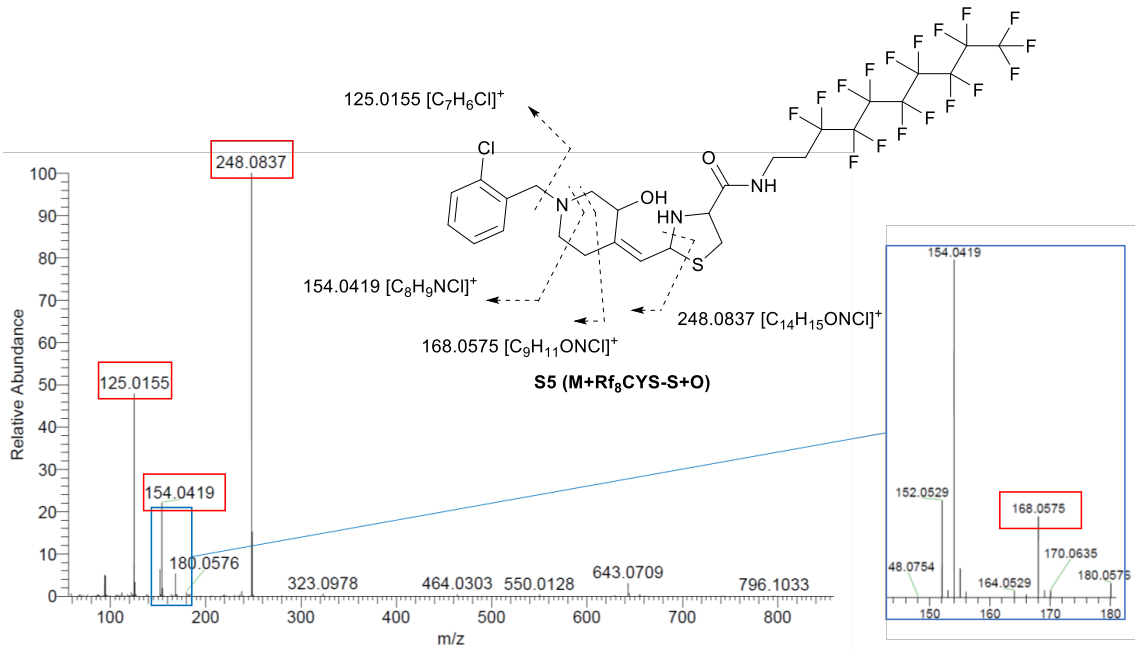


Figure S22. Tandem mass spectra of the positive enhanced product ion scanning of *m/z* 814 of microsomal incubations with ticlopidine and Rf$_8$CYS. Ticlopidine (150 μM) was incubated with Rf$_8$CYS (150 μM) in HLM (1.0 mg/mL) fortified with a NADPH$^+$ generating system at 37°C for 60 min. Product ions of (M+Rf$_8$CYS-S+O) was found; [M+H]$^+$ calculated for C_7H_6Cl 125.0153; found 125.0155, calculated for C_8H_9NCl 154.0418; found 154.0419, calculated for $C_9H_{11}ONCl$ 168.0575; found 168.0575, calculated for $C_{14}H_{15}ONCl$ 248.0837; found 248.0837. The $C_{14}H_{15}ONCl$ fragment was considered to be formed by a cleavage of the thiazolidine ring.[2] [3] All assigned fragments were consistent with the proposed structure of **S5**.

2. Experimental procedures

Preparation of Rf$_8$CYS solution

A mixture of Rf$_8$CYS-TFA and **S2** in methanol or dimethyl sulfoxide (DMSO) (31.5 mmol/L as monomer) and TCEP-HCl aqueous solution (200 mmol/L) was mixed in the ratio of 19/1 (v/v). After 60 min, the solution was used as the Rf$_8$CYS solution (30 mmol/L).

Preparation of 2 solution (for Figure S1 and S3)

A mixture of **2**-TFA and **S4** in methanol (31.5 mmol/L as monomer) and TCEP-HCl aqueous solution (200 mmol/L) was mixed in the ratio of 19/1 (v/v). After 60 min, the solution was used as **2** solution (30 mmol/L).

Reaction method in aqueous solution (for Figure S1–S8)

The substrate (150 μM) was mixed with Rf_8CYS or **2** (150 μM) in PBS (100 mmol/L, pH 7.4). Although the substrate and trapping reagent were prepared using methanol or DMSO, the organic solvent concentration was less than 1% in the reaction mixture. The total volume was 202 μL. After 60 min at 37°C, 600 μL of 0.1% formic acid aqueous solution /acetonitrile=1/2 (v/v) was added to the mixture and the sample was analyzed by a liquid chromatography or F-SPE then performance liquid chromatography.

Trapping assay in HLM condition (for Figure S9–S21)

The incubation mixture contained the individual substrates (150 μM), HLM (1.0 mg/mL), G6P (3.3 mmol/L), G6P-DH (0.45 unit/mL), $MgCl_2$ (3.3 mmol/L), $NADP^+$ (1.3 mmol/L) and Rf_8CYS (150 μM) at the final concentrations in 100 mmol/L potassium phosphate buffer (pH 7.4). The total volume was 202 μL. Although the substrate and Rf_8CYS were prepared using DMSO, the concentration was less than 1% in the reaction mixture. After 60 min incubation at 37°C, 200 μL of 0.1% formic acid containing acetonitrile was added to quench the reaction. After centrifugation for 5 min at 4°C and 10,000 rpm, the supernatant was analyzed by liquid chromatography using a fluorous LC column directly (direct injection method) or using a non-fluorous LC column after F-SPE (F-SPE method).

Method for F-SPE (for Figure S9 and S21)

Fluorous silica gel, 500 mg was loaded in a spin column and prewashed by 1 mL of methanol three times followed by 1 mL of 80% methanol/water (v/v) three times. 300 μL of the sample was then slowly loaded on the spin column. After washing with 700 μL of 80% methanol/water three times, the fluorous compound was eluted by 2.1 mL of methanol. The sample was then dried by SpeedVac and reconstituted in 300 μL of 50% acetonitrile/water (v/v) for LC/MS analysis.

Instrumentation and conditions

A Shimadzu (Japan) LC system comprising two LC-20AD pumps as well as a high-pressure gradient unit, a DGU-20A5 on-line degasser, an SIL-20AC autosampler, and a CTO-20AC column oven were used. Injections of 10 μL samples were carried out automatically.

LC condition using a non-fluorous column (for Figure S1–S8 and S21)

CAPCELL CORE AQ, (2.7 μm, 2.1 ID×50 mm OSAKA SODA CO., LTD, Japan) was used. Solvent A (purified water/formic acid=1000/1, v/v) and solvent B (acetonitrile/formic acid=1000/1, v/v) were used as the mobile phases for the gradient elution (gradient curve: 0-1 min, 10% B; 1-15 min, linear change from 10 to 95% B; 16-17 min, linear change from 95 to 10% B; run-time, 20 min). The flow

rate was set at 0.6 mL/min, and the column oven temperature was set at 40 °C. The eluent from the LC column was directly introduced into the ion source of the mass spectrometer.

LC condition using fluorous column (for Figure S10–S20)

A Wakopak Fluofix-II 120E column (5 µm, 2.0 ID×150 mm., WAKO, Japan) was used. Solvents A (1 M ammonium acetate aqueous solution /acetic acid/water=10/1/1000, v/v/v) and solvent B (1 M ammonium acetate aqueous solution /acetic acid/methanol/2-propanol = 10/1/800/200, v/v/v/v) were used as the mobile phases for the gradient elution (gradient curve: 0-1 min, 10% B; 1-3 min, linear change from 10 to 70% B; 3-25 min, linear change from 70 to 90% B; 35.0-35.1 min, linear change from 90 to 10% B; run-time, 37 min). The flow rate was set at 0.3 mL/min, and the column oven temperature was set at 40°C. The effluent from the LC column was directly introduced into the ion source of the mass spectrometer.

An API 4000 Qtrap tandem mass spectrometer (Sciex, USA) was used for MS measurements. For the identification of the adducts, the full scan mode was operated in the positive ion spray ionization mode. The full scan over the *m/z* range of 100-1500 was performed under the following the operating conditions: an ion spray voltage of 3500 V, a source temperature of 750°C, curtain gas of 10, an ion source gas 1 pressure of 70, and ion source gas 2 setting of 80.

A Q Exactive mass spectrometry (Thermo Fisher Scientific Inc, Germany) was used for High resolution mass spectra (HRMS). For the identification of the adducts, the full scan mode was operated in the positive ion spray ionization mode. The full scan over the *m/z* range of 100-1500 was performed under the following the operating conditions: a spray voltage of 3.5 kV, capillary temperature of 275 ºC, aux gas heater temperature of 450 ºC, S-Lens RF level of 50, sheath gas of 55, auxiliary gas of 15, and sweep gas setting of 3.

In addition, structural characterization of the ticlopidine RMs-adduct (M+Rf8CYS-S+O) using the MS/HRMS fragments obtained in the product ion mode using the respective identified precursor ion with positive mode.

General information

All solvents and reagents were purchased from commercial sources and used without additional purifications. 1H,1H,2H,2H-Perfluorodecylamine was obtained from Manchester Organics Limited (UK). TFA, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), clozapine, amodiaquine dihydrochloride dihydrate and abacavir were obtained from Tokyo Chemical Industry (Japan). N-(tert-butoxycarbonyl)-S-trityl-L-cysteine, *p*-benzoquinone, benzaldehyde, decylamine, triethylsilane, ethynylbenzene and (2,3-epoxypropyl)benzene were obtained from Sigma

Aldrich (USA). LC/MS grade 2-propanol, formic acid, acetic anhydrite (Ac_2O), dipotassium hydrogenphosphate, TCEP-HCl, troglitazone, and potassium dihydrogenphosphate were obtained from Fujifilm Wako (Japan). Benzyl alcohol was obtained from Nacalai Tesque (Japan). 2-Phenyl-1,3-thiazolane-4-carboxylic acid was obtained from Matrix Scientific (USA). 3,4-Thiazolidinedicarboxylic acid and 2-phenyl-,3-(1,1-dimethylethyl) ester were obtained from Amatek Chemical (China). XTreme 200 mixed gender human liver microsomes - 0.5mL (20 mg/mL) was obtained from Xeno Tech (USA). LC/MS grade acetonitrile, Tetrahydrofuran (THF) triethylamine, ethyl acetate and HPLC grade methanol were obtained from Kanto Chemical (Japan). Corning gentest $NADPH^+$ system solution A and Corning gentest $NADPH^+$ system solution B were obtained from Corning (USA). Ultrapure water was produced using a Milli-Q.

^1H, ^{13}C and ^{19}F NMR spectra were recorded on Agilent Technologies DD2 500 MHz FT-NMR spectrometer and JEOL ECZ600. CD_3OD, DMSO-d6 and $CDCl_3$ were used as a solvent. TMS was used as an internal standard. The chemical shifts were reported in ppm downfield (δ) from TMS, the coupling constants *J* are given in Hz. The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet. TLC was carried out on SiO_2 (silica gel 60 F254, Merck, Germany), and the spots were located with UV light. F-SPE was carried out on fluoroSep-RP Octyl (particle size 5um pore Size 60, ES INDUSTRIES, USA).

Method for the synthesis of Rf_8CYS-TFA

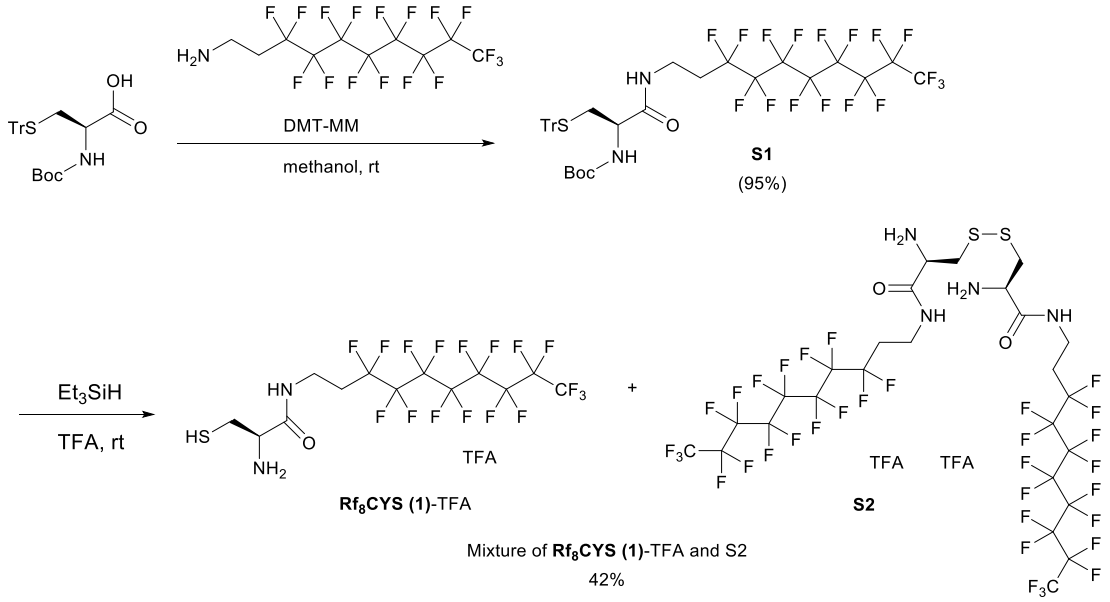
To a stirred solution of N-(tert-butoxycarbonyl)-S-trityl-L-cysteine (2.6 mmol, 1.2 g) in acetonitrile (35 mL), 1H,1H,2H,2H-perfluorodecylamine (2.2 mmol, 600 μL) and DMT-MM (4.4 mmol, 1.2 g) were added and the reaction was allowed to stir at room temperature for 18 h. After completion (monitored by TLC), 8 mL of water was added to reaction mixture and all the mixture were loaded to a fluorous silica gel cartridge. After washing by 120 mL of 80% methanol/water, **S1** was eluted by ethyl acetate to afford as a white solid in (1.88 g, 95% yield).

To a stirred solution of **S1** (1.1 mmol, 1 g) in THF (4.5 mL), triethylsilane, (1 mL) and TFA (4.5 mL) were added and the reaction was allowed to stir at room temperature for 22 h. After concentration in vacuo, the resulting residue was purified using a Waters Prep System (Waters Corporation, USA) equipped with an XTerra Prep MS C18 OBD 5 μm 30ID×50 mm (Waters Corporation) to afford the mixture of Rf_8CYS-TFA and **S2** as a white gam in (301 mg, 41% yield). Solvents A (TFA /water=1/1000, v/v) and solvent B (TFA/acetonitrile = 1/1000, v/v) were used as the mobile phases for the gradient elution (gradient curve: 30-70% B for 8 min, followed by 100% B for another 2 min (run-time, 10 min)). The flow rate was set at 40.0 mL/min. Rf_8CYS-TFA was dimerized to disulfide form **S2** during refrigerated storage.

Rf_8CYS-TFA; δ_H (500 MHz, CD_3OD); 3.99 (1H, dd, J = 8.0, 11.2 Hz), 3.68-3.52 (3H, m), 2.99 (2H,

ddd, J = 11.2, 24.0, 34.4 Hz), 2.57-2.42 (4H, m) δ_C (126 MHz, CD_3OD); 168.68, 168.51, 56.11, 52.91, 39.01, 33.07, 31.39, 31.22, 31.05, 26.16, -0.05; δ_F (470 MHz, CD_3OD); –82.32, –82.48, –115.12, –115.48, –122.57, –122.69, –122.92, –123.06, –123.65, –123.877, –123.87, –124.53, 124.64, 124.74, 127.20, 127.41; HRMS (ESI) $[M+H]^+$ calcd. for $C_{13}H12ON_2F_{17}S$ 567.0399; found 567.0412.

S2; δ_H (600 MHz, CD_3OD); 4.21 (2H, dd, *J* = 7.2, 11.2 Hz), 3.60−3.54 (6H, m), 3.34 (4H, ddd, *J* = 8.0, 19.2, 156.0 Hz), 2.47−2.41 (8H, m); HRMS (ESI) $[M+H]^+$ calcd. for $C_{26}H_{21}O_2N_4F_{34}S_2$ 1131.0558; found 1131.0553.



Method for the synthesis of 2-TFA

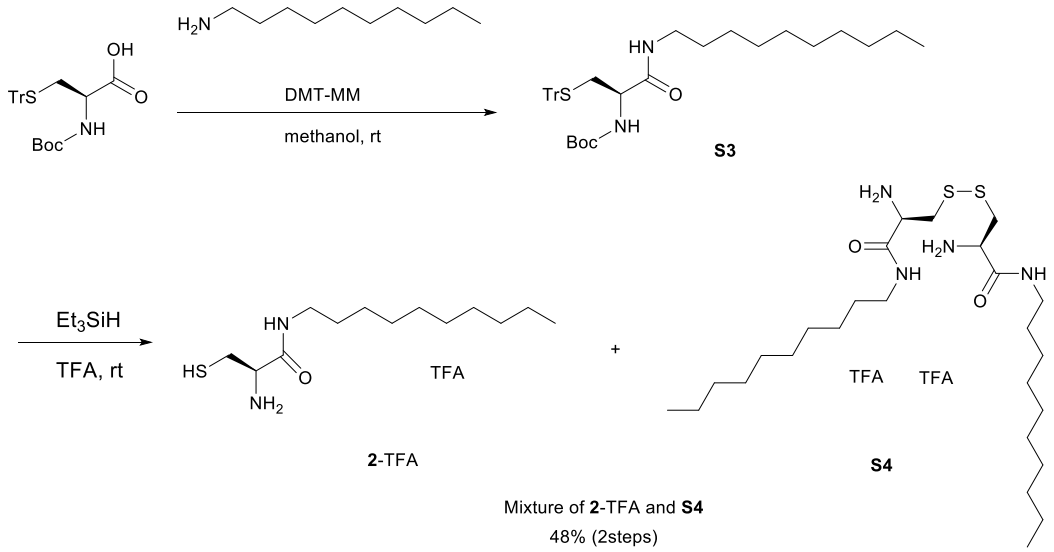
To a stirred solution of *N*-(*tert*-butoxycarbonyl)-*S*-trityl-L-cysteine (1 mmol, 464 mg) in acetonitrile (10 mL), decylamine (1.2 mmol, 240 μL) and DMT-MM (1.2 mmol, 332 mg) were added and the reaction was allowed to stir at room temperature for 24 h. After completion (monitored by TLC), 10 mL of water was added to reaction mixture. It was extracted with ethyl acetate (20 mL). The organic layer was washed with water, concentrated in vacuo to give the crude product of **S3** as a yellow gum in (633 mg) and used for the next reaction without further purification.

To a stirred solution of crude product of **S3** (633 mg) in THF (2.8 mL), triethylsilane (0.63 mL) and TFA (2.8 mL) were added and the reaction was allowed to stir at room temperature for 22 h. After concentration in vacuo, the resulting residue was purified using a Waters Prep System equipped with an XTerra Prep MS C18 OBD 5 μm 30ID×50 mm to afford the mixture of **2**-TFA and **S2** as a yellow oil in (204 mg, 48% yield). Solvents A (TFA /water=1/1000, v/v) and solvent B (TFA/acetonitrile = 1/1000, v/v) were used as the mobile phases for the gradient elution (gradient curve: 25-65% B for 8 min, followed by 100% B for another 2 min (run-time, 10 min)). The flow rate was set at 40.0 mL/min.

2-TFA was dimerized to disulfide form **S4** during refrigerated storage.

2-TFA; δ_H (500 MHz, CD_3OD); 3.93 (1H, dd, J = 8.8, 10.4 Hz), 3.35−3.20 (3H, m), 2.97 (2H, m, J = 8.0, 23.2, 72.8 Hz), 1.54 (2H, t, J = 11.2 Hz), 1.34−1.30 (16H, m), 0.90 (3H, t, J = 10.4 Hz); δ_C (126 MHz, CD_3OD); 168.17, 33.09, 30.77, 30.74, 30.71, 30.47, 30.41, 30.33, 28.10, 28.02, 26.40, 23.76, 14.45; HRMS (ESI) $[M+H]^+$ calcd. for $C_{13}H_{29}ON_2S$ 261.1995; found 261.1997.

S4; δ_H (500 MHz, CD_3OD); 4.11 (2H, dd, J = 8.8, 12.8 Hz), 3.34−3.06 (8H, m), 1.54 (4H, dt, J = 10.4, 22.4 Hz), 1.32−1.28 (32H, m), 0.88 (6H, t, J = 10.4 Hz), 14.46, 0.00; HRMS (ESI) $[M+H]^+$ calcd for $C_{26}H_{55}O_2N_4S_2$ 519.3761; found 519.3762.

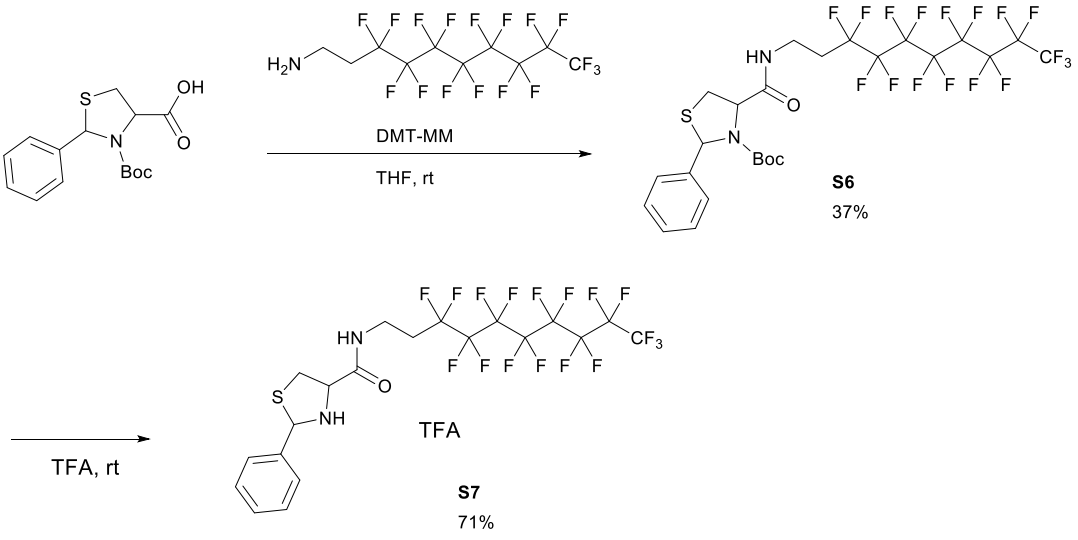


Method for the synthesis of S7

To a stirred solution of 3-(tert-butoxycarbonyl)-2-phenylthiazolidine-4-carboxylic acid (0.1 mmol, 30 mg) in THF (1 mL), 1H,1H,2H,2H-perfluorodecylamine (0.2 mmol, 39 μL) and DMT-MM (0.2 mmol, 54 mg) were added and the reaction was allowed to stir at room temperature for 18 h. After completion (monitored by TLC), 0.5 mL of water was added to the reaction mixture and all of the mixture was loaded to a fluorous silica gel spin column. After washing with 5 mL of 80% methanol/water, the crude product was eluted with methanol. The crude product of **S6** was purified by Preparative thin-Layer chromatography to afford 26.8 mg as a yellow oil in (26.8 mg, 37% yield).

To **S6** (0.0068 mmol, 5.1 mg), TFA (0.1 mL) were added, and the reaction was allowed to stir at room temperature for 2 h. The solution was concentrated in vacuo to give the product of **S7** as yellow oil in (3.6 mg, 71% yield).

δ_H (600 MHz, CD_3OD); 8.25 (1H, s), 7.61 (2H, dd, J = 1.2, 4.8 Hz), 7.46 (2H, m), 5.63 (1H, s), 4.92 (1H, d, J = 6.4 Hz), 4.75 (1H, br), 3.67−3.49 (5H, m), 2.40−2.34 (2H, m); HRMS (ESI) $[M+H]^+$ calcd. For $C_{20}H_{16}ON_2F_{17}S$ 655.0712; found 655.0710.

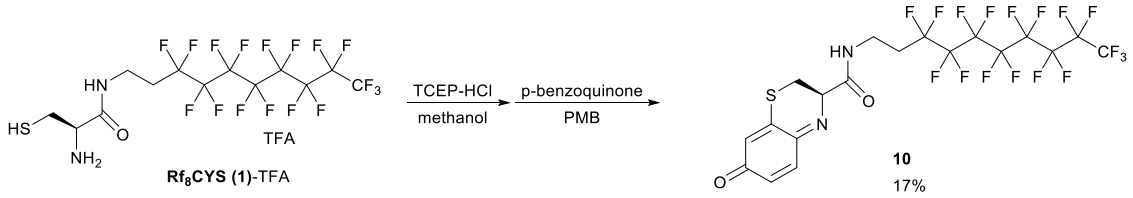


Method for the synthesis of 10

To a stirred solution of Rf_8CYS (0.0046 mmol, 3 mg) in methanol (1 mL), TCEP-HCl (0.021 mmol, 6 mg) were added, and the reaction was allowed to stir at room temperature for 1 h. To the reaction mixture, PBS (100 mmol/L, pH 7.4) and *p*-benzoquinone (0.044 mmol, 4.8 mg) were added, and the reaction was allowed to stir at room temperature for 3 h. The resulting residue was purified using a Shimadzu LC system equipped with an CAPCELL CORE C18, (2.7 μm, 4.6 ID×50 mm OSAKA SODA CO., LTD) to afford **10** as a yellow solid in (0.5 mg, 17% yield). Solvents A (water) and solvent B (acetonitrile) were used as the mobile phases for the gradient elution (gradient curve: 65% B for 1 min, 65-95% B for 2.5 min, 95% B for 1.1 min, 95-65% for 0.1 min followed by 65% B for another

1.3 min (run-time, 6 min)) The flow rate was set at 2.4 mL/min.

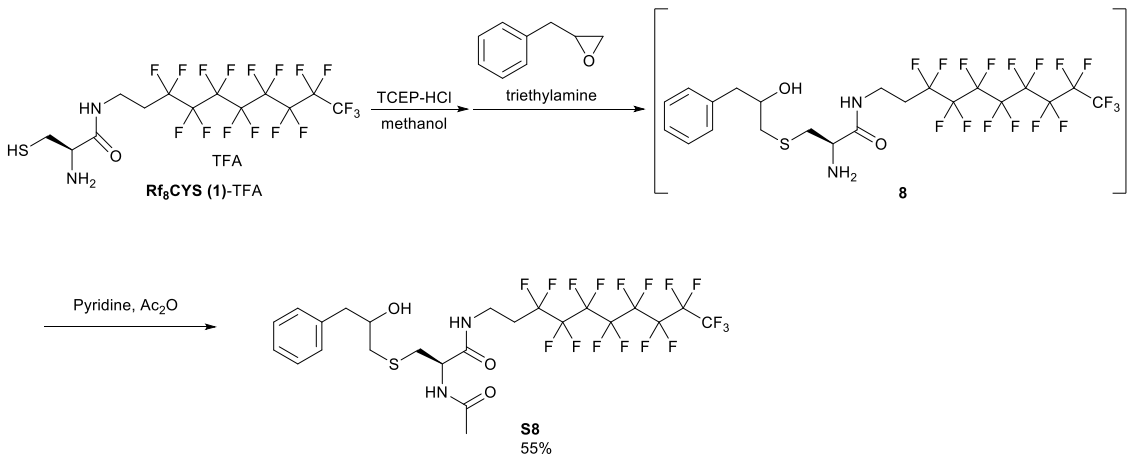
δ_H (600 MHz, $CDCl_3$); 7.80 (1H, dd, J = 4.8,4.8 Hz), 7.28 (1H, d, J = 5.6 Hz), 6.76 (1H, d, *J* = 2.0 Hz), 6.69 (1H, dd, *J* = 2.0, 5.6 Hz), 5.16 (1H, s), 3.75 (2H, t, *J* = 4.4 Hz), 3.65 (2H, s), 2.47–2.41 (2H, m); HRMS (ESI) $[M+H]^+$ calcd. for $C_{19}H_{12}O_2N_2F_{17}S$ 655.0348; found 655.0351.



Method for the synthesis of S8

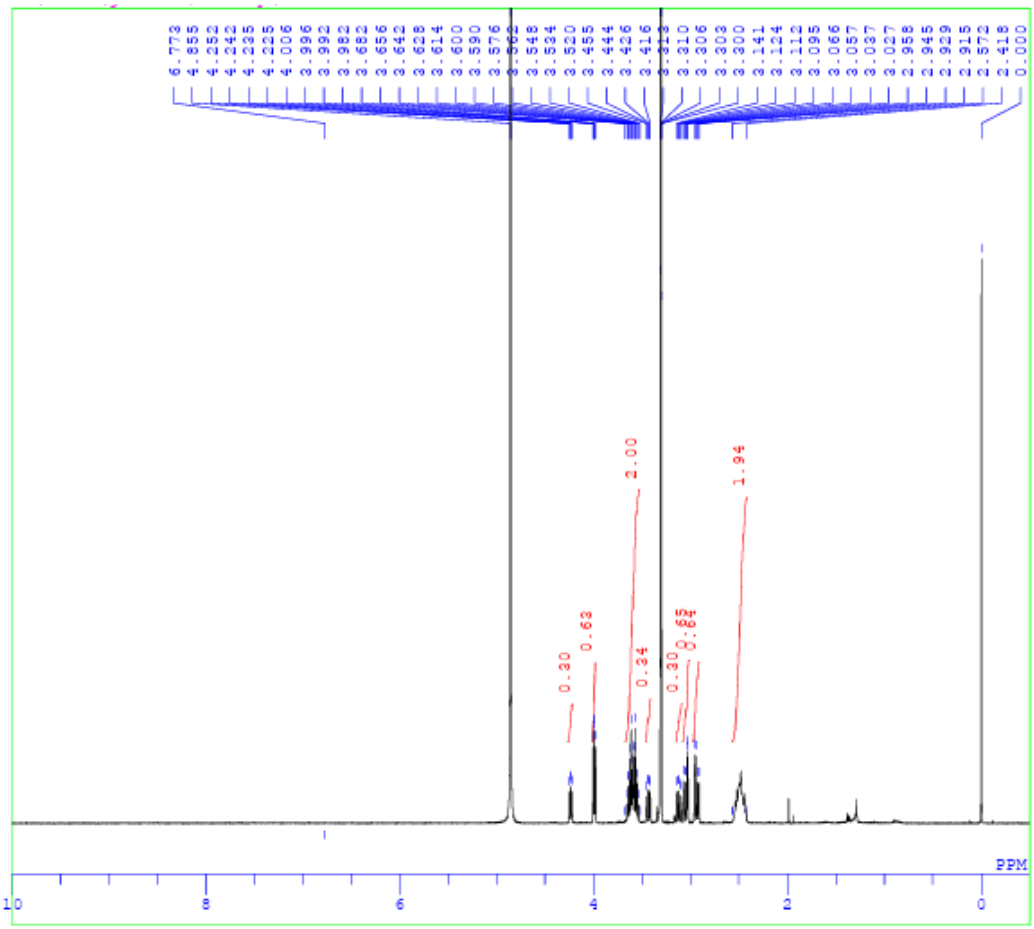
To a stirred solution of Rf_8CYS (0.013 mmol, 8.8 mg) in methanol (200 µL), TCEP-HCl (0.033 mmol, 9.5 mg) was added, and the reaction was allowed to stir at room temperature for 1 h. To the reaction mixture, triethylamine (0.246 mmol, 34 µL) and (2,3-epoxypropyl)benzene (0.123 mmol, 16.2 µL) were added, and the reaction was allowed to stir at room temperature for 3 h. To the reaction mixture, pyridine (0.246 mmol, 20 µL) and Ac_2O (0.246 mmol, 23 µL) were added, and the reaction was allowed to stir at room temperature for 24 h. The resulting residue was purified using a Shimadzu LC system equipped with an CAPCELL CORE C18, (2.7 µm, 4.6 ID×50 mm) to afford **10** as a white solid (5.7 mg, 55% yield). Solvents A (water) and solvent B (acetonitrile) were used as the mobile phases for the gradient elution (gradient curve: 65% B for 2 min, 65-95% B for 0.1 min, 95% B for 0.4 min, 95-65% for 0.5 min (run-time, 6 min)) The flow rate was set at 2.4 mL/min.

δ_H (600 MHz, $CDCl_3$); 7.39–7.15 (5H, m), 6.78-6.67 (1H, m), 4.65–4.55 (1H, m), 4.41–3.95 (1H, m), 3.61–3.55 (2H, m), 3.15–2.58 (8H, m), 2.42–2.27 (2H, m), 2.02 (3H, s); HRMS (ESI) $[M+H]^+$ calcd. for $C_{24}H_{24}O_3N_2F_{17}S$ 743.1231; found 743.1237.

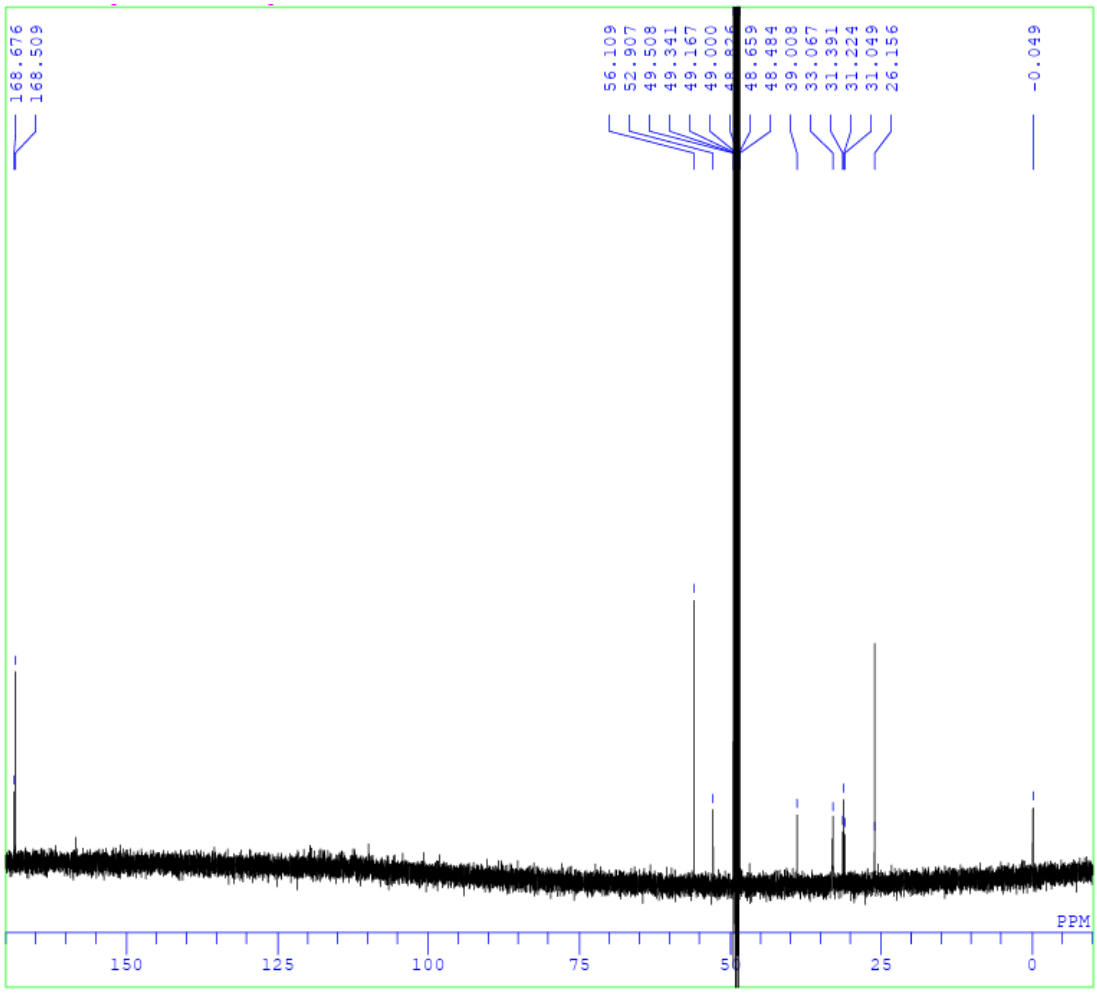


3 . Copies of ^{1}H, ^{13}C and ^{19}F NMR spectra

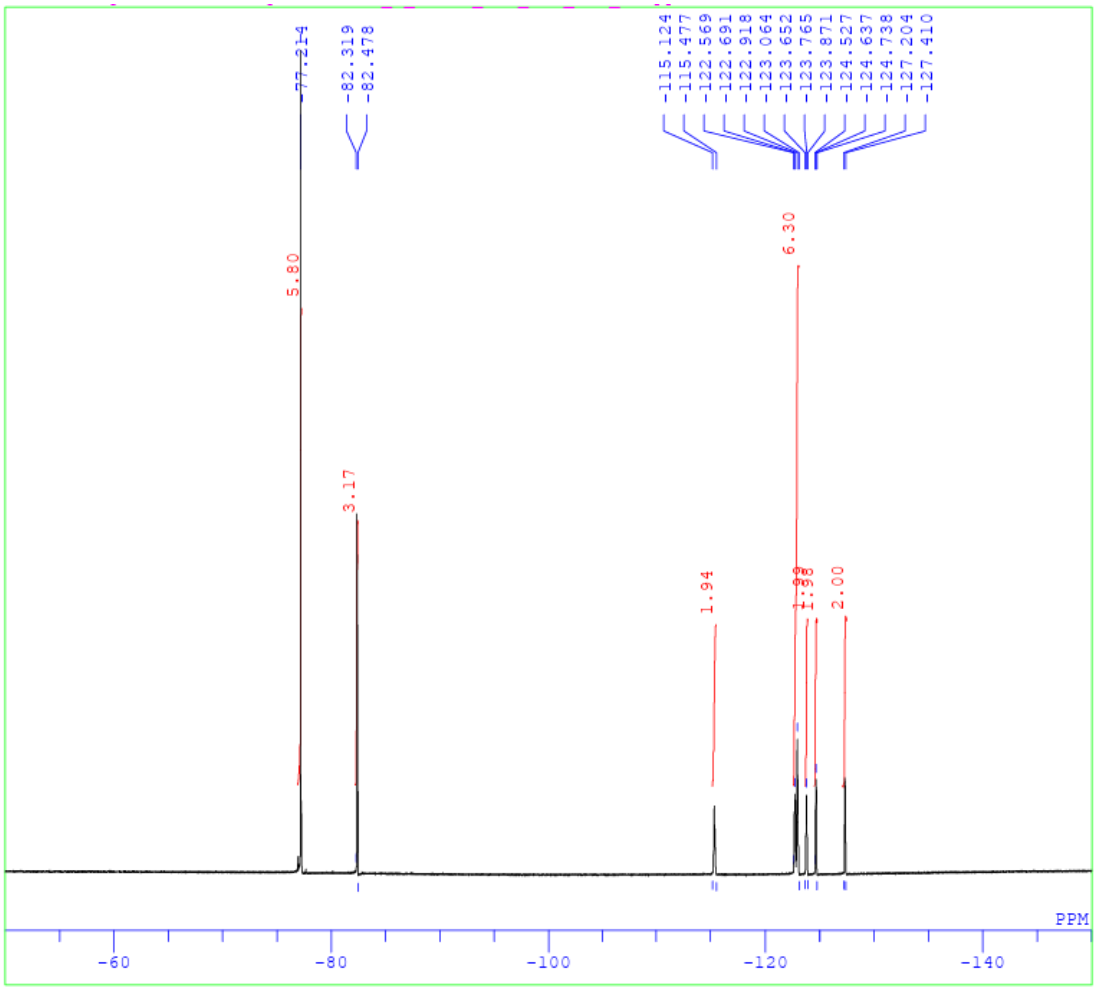
^{1}H NMR of mixture (Rf_8CYS-TFA and **S2**)



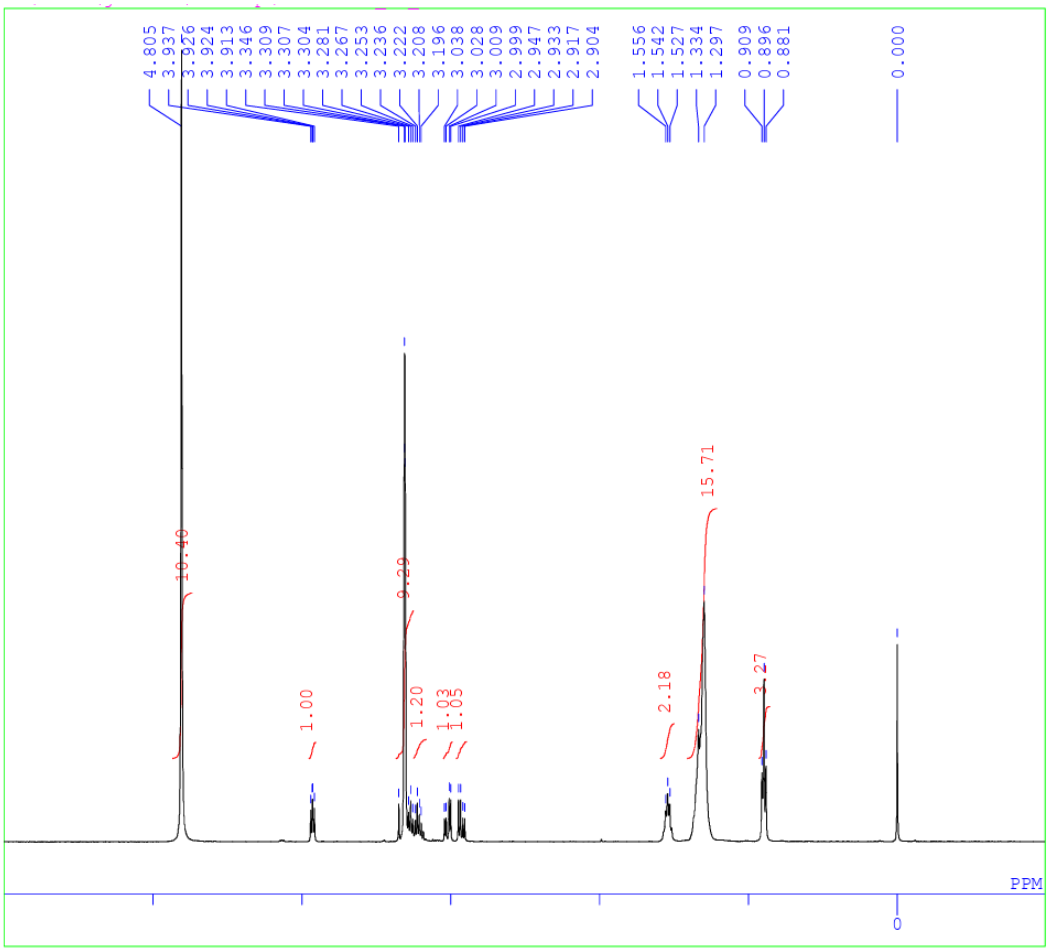
^{13}C NMR of mixture (Rf_8CYS-TFA and S**2**)



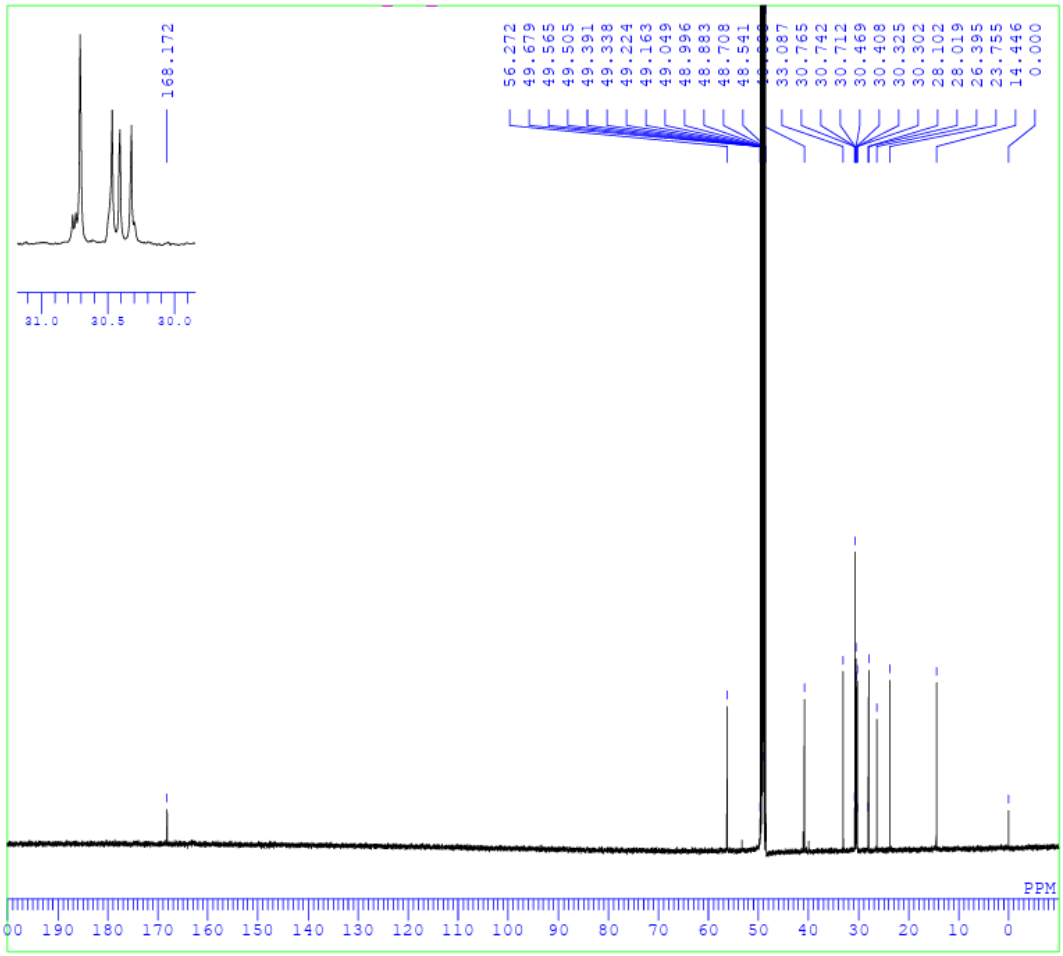
^{19}F NMR of mixture (Rf_8CYS-TFA and **S2**)



^{1}H NMR of **2**-TFA



^{13}C NMR of **2**-TFA



4. Additional references

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3. E. M. Lenz, S. Martin, R. Schmidt, P. E. Morin, R. Smith, D. J. Weston and M. Bayrakdarian, *Chem. Res. Toxicol.*, 2014, **27**, 968-980.