Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Synthesis of Sydnonimines from Sydnones and their use for bioorthogonal release of isocyanates in cells.

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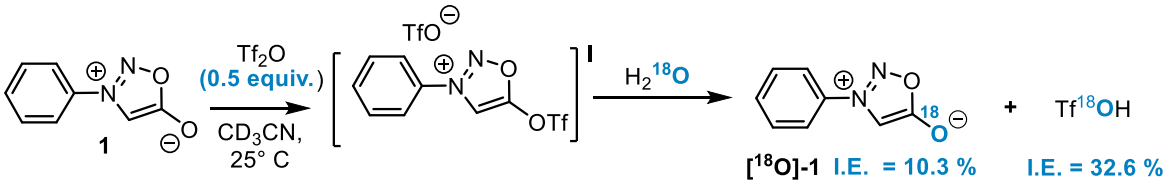
I. Material and equipment

All chemical products commercially available were purchased from Sigma-Aldrich, Acros and Fluka and used without further purification. Anhydrous solvents: 1, 2-dichloroethane, acetonitrile, DMF, DMSO were purchased in anhydrous form and used without further purification. THF was dried from sodium/benzophenone under nitrogen. Dichloromethane was distilled form calcium hydride under nitrogen.

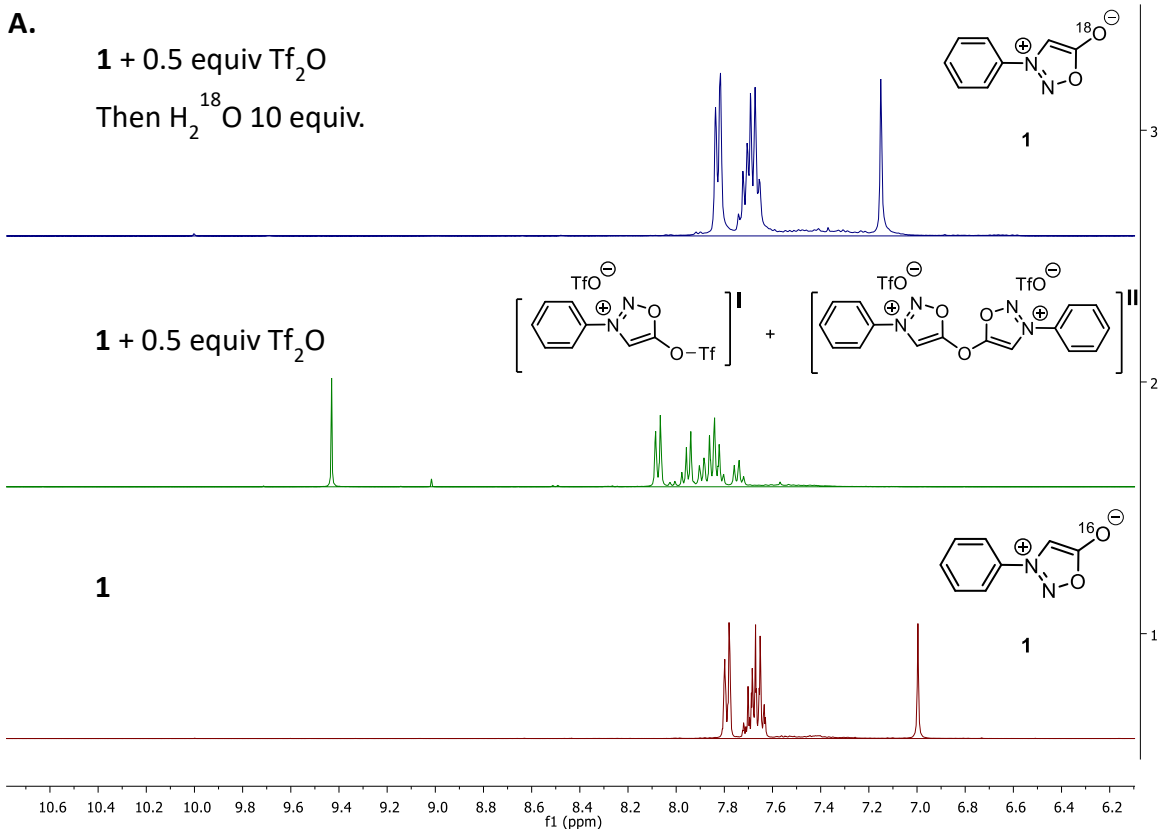
Reactions were monitored by TLC carried out on silica 0,25 mm (60 F254, Merck) using UV light as visualizing agent and basic aqueous permanganate as developing agent.
^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), ^{19}F NMR (376 MHz) were measured on a Brucker Avance 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from residual solvents peaks and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), broad singlet (br. s), doublet (d), triplet (t), quartet (q), quintet (quint), heptuplet (hept), multiplet (m). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m).
LC-MS mass spectra were recorded using a single quadrupole mass spectrometer (SQD 2, Waters) with electrospray source coupled to Ultra-High Performance Liquid Chromatography (Acquity UPLC H-Class, Waters) equiped with PDA eλ detector.
LC-UV analysis were performed on the same LC system.
High resolution mass spectra offinal compounds were obtained using a Xevo® G2-XS Q-Tof mass spectrometer equiped with ESI source.
Infrared spectra (IR) were obtained on a Perkin Elmer system 2000 FT-IR spectrophotometer or a Perkin Elmer UATR TWO FTIR spectrophotometer and are reported as wavelength numbers (cm-1).
Melting points (Mp) were obtained on a BÜCHI Melting Point B-545 and are reported in °C.
Absorbances were measured on a UV JASCO V-750 equipped with an injection module (GSP-909) and a Peltier (EHCS-760).
Steady state spectra were reported on an Edinburgh FS5 spectrofluorometer with SC20 module.
Purification on reversed phase chromatography were done with a Puriflash® XS520Plus (Columns: BIOTAGE® SNAP Cartridge KP-C18-SH-120g or KP-C18-SH-60g).

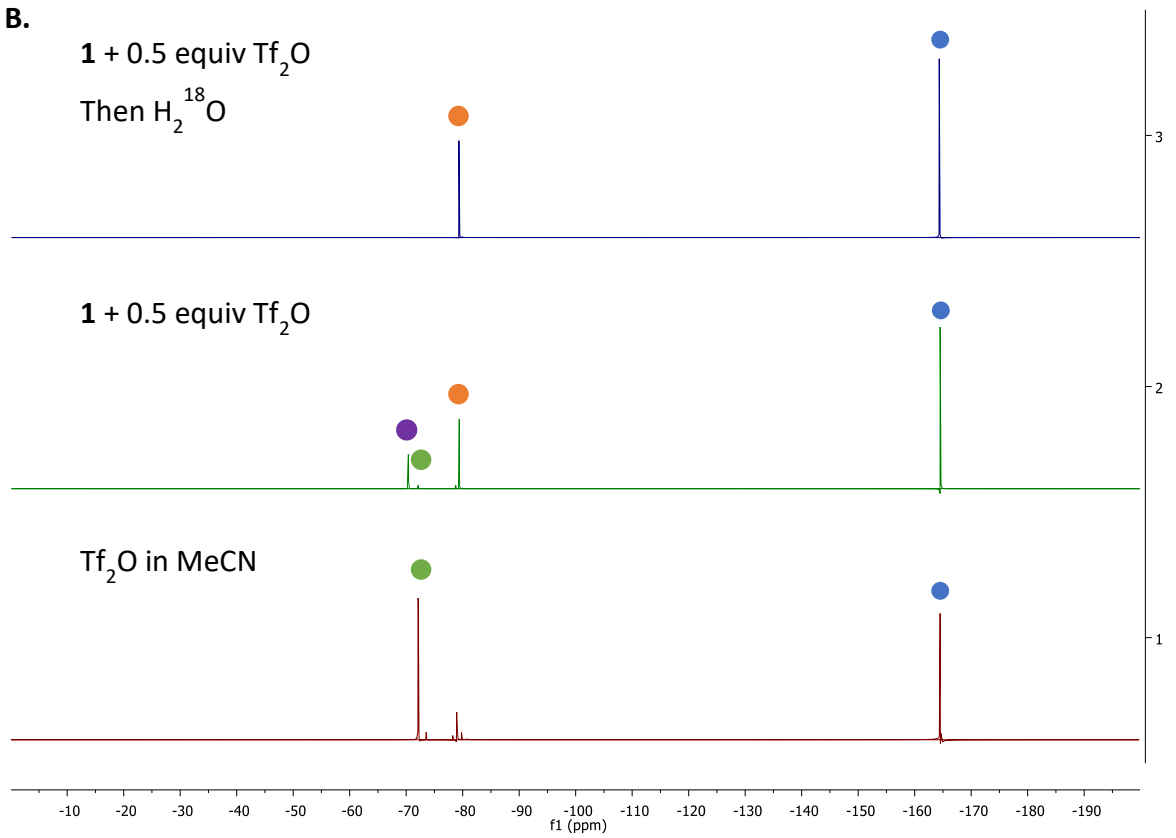
II. Synthesis of sydnonimines from sydnones

A. Mechanistic investigations



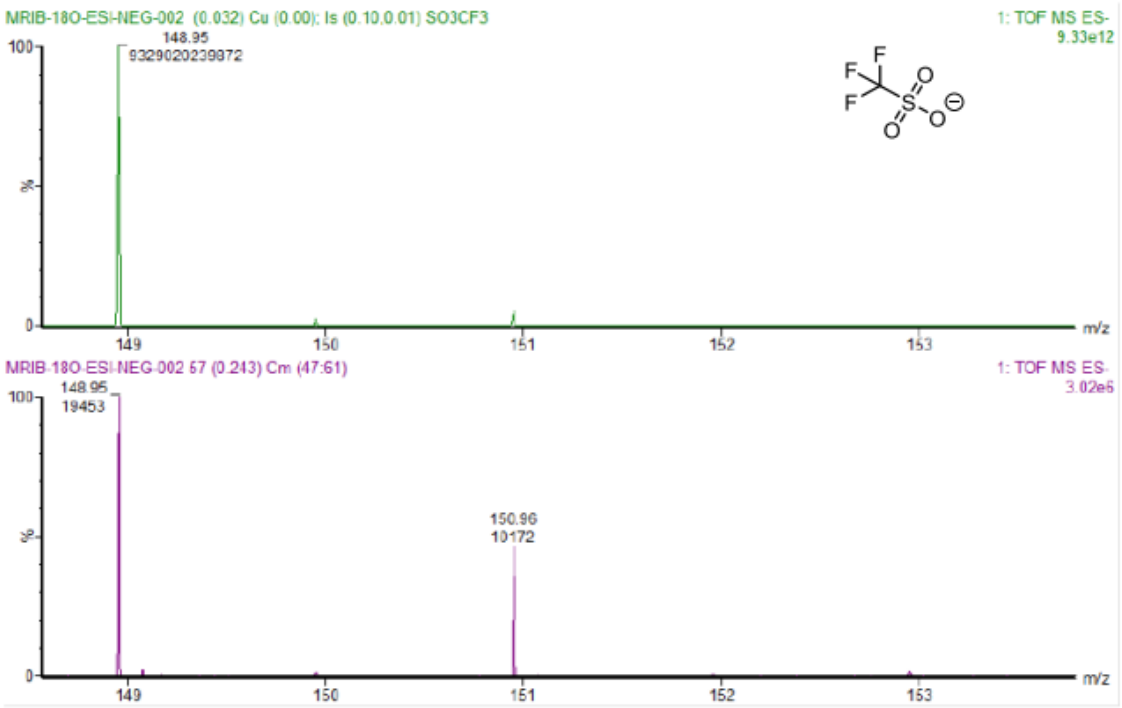
1 was dissolved in dry MeCN-d_3 (0.1 M) under an argon atmosphere in a dry NMR tube and Tf_2O (0.5 equiv.) was added to the solution at room temperature. After 5 min, ^1H NMR was first recorded and then a solution of $H_2{}^{18}O$ (10.0 equiv.) in dry MeCN-d_3 was added to the mixture. A second ^1H NMR was recorded.





- Hexafluorobenzen as internal standard.
- Triflic acid
- Triflic anhydride
- Intermediate **I**

Figure S1. NMR monitoring of the reaction. A) ^{1}H-NMR monitoring; B) ^{18}F-NMR monitoring.



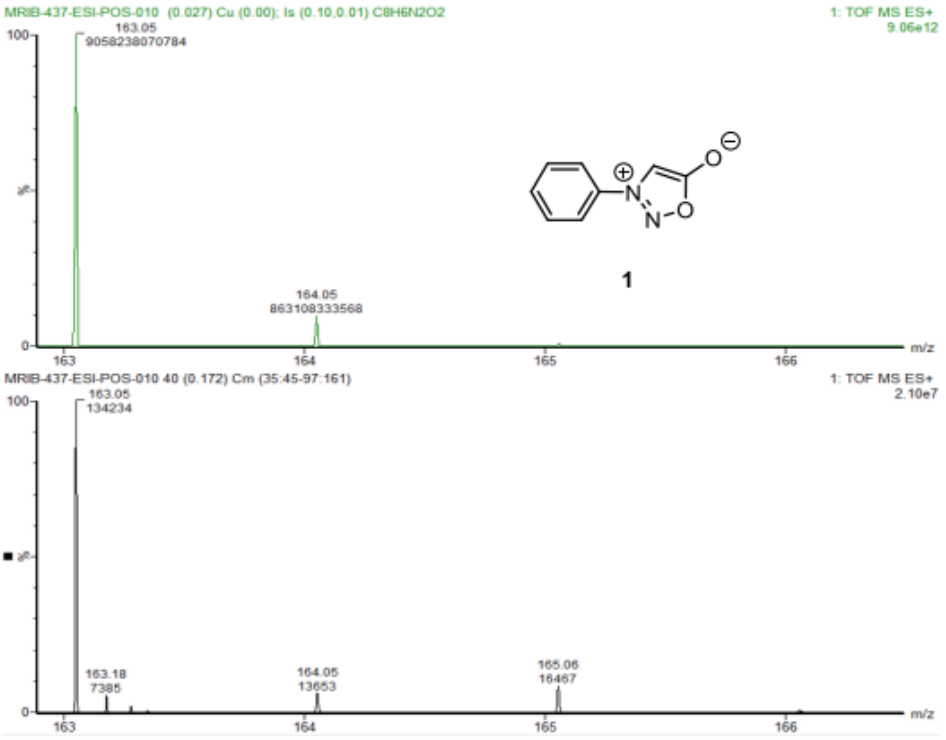
theorical isotopic distribution

	M	M+1	M+2	M+3	M+4	M+5	M+6	M+7
m/z	149	150	151	152	153	154	155	156
%	100	1,99	5,04	0	0	0	0	0

Enrichment calculation

Isotopomer	m/z	Area	natural isotope correction	Corrected area	Isotopic purity (%)
0	149	192020	0	191948	68,93
1	151	100486	9501	90776	32,60
2	153	84	0	-4489	-1,61
3	155	0	0	226	0,08
4	157	0	0	-11	0,00
5	159	0	0	1	0,00
6	161	0	0	0	0,00
7	163	0	0	0	0,00
Total		292590		278449,59	100,00

% Isotopic enrichment ^{18}O: 32,6



theorical isotopic distribution

	M	M+1	M+2	M+3	M+4	M+5	M+6	M+7
m/z	163	164	165	166	167	168	169	170
%	100	9,6	0,8	0	0	0	0	0

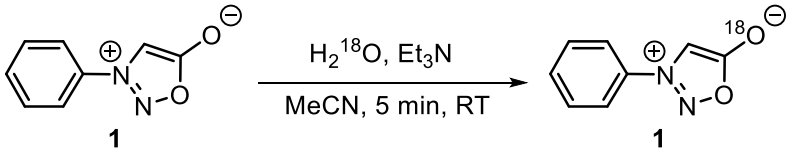
Enrichment calculation

Isotopomer	m/z	Area	natural isotope correction	Corrected area	Isotopic purity (%)
0	163	134307	0	133091	89,65
1	165	16467	982	15262	10,28
2	167	226	0	103	0,07
3	169	0	0	-1	0,00
4	171	0	0	0	0,00
5	173	0	0	0	0,00
6	175	0	0	0	0,00
7	177	0	0	0	0,00
Total		151000		148454,93	100,00

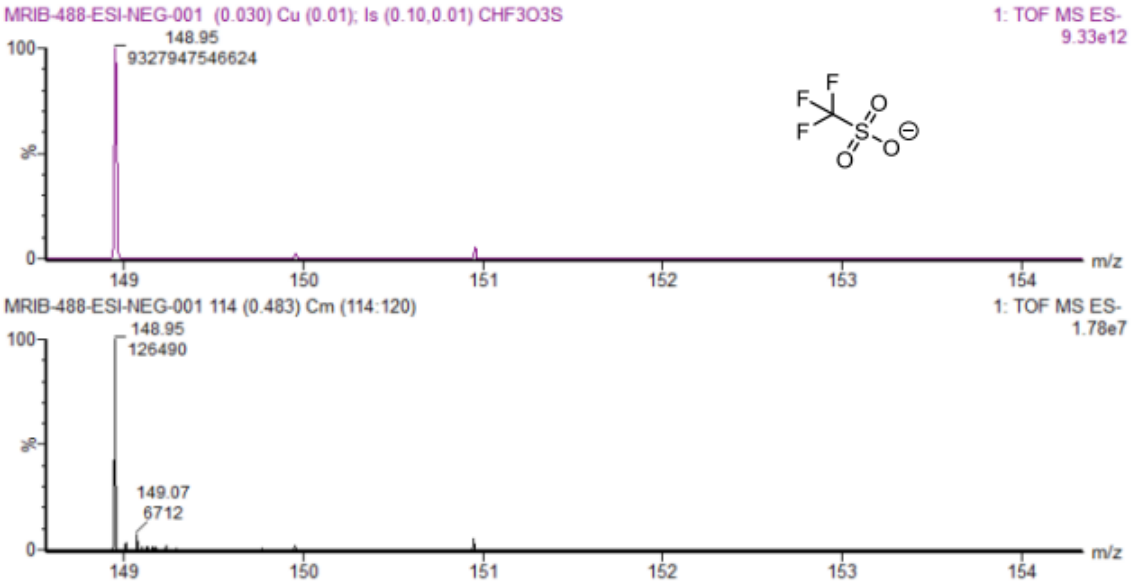
% Isotopic enrichment ^{18}O: 10,3

Figure S2. HRMS isotopic enrichment calculation of the ^{18}O-labeled products.

Blank experiments:



EI : 0.0%



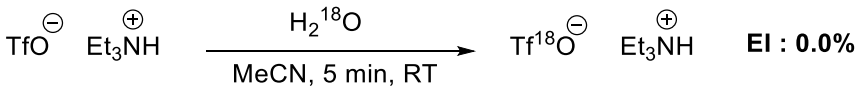
theorical isotopic distribution

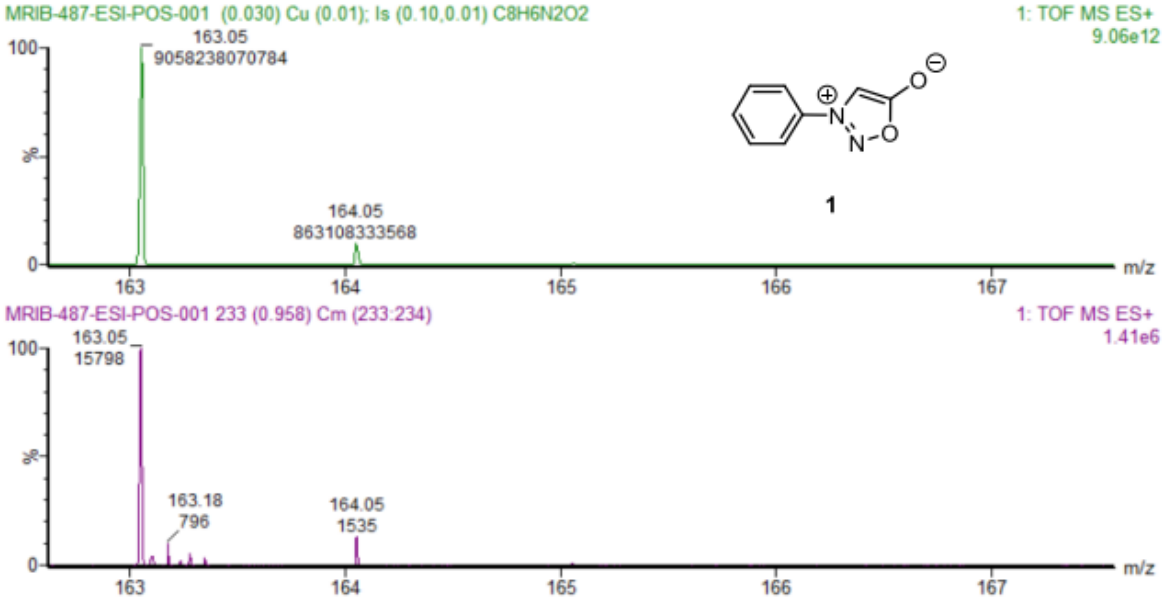
	M	M+1	M+2	M+3	M+4	M+5	M+6	M+7
m/z	149	150	151	152	153	154	155	156
%	100	1,99	5,04	0	0	0	0	0

Enrichment calculation

Isotopomer	m/z	Area	natural isotope correction	Corrected area	Isotopic purity (%)
0	149	126490	0	126442	100,00
1	151	6313	6261	-60	-0,05
2	153	66	0	69	0,05
3	155	0	0	-3	0,00
4	157	0	0	0	0,00
5	159	0	0	0	0,00
6	161	0	0	0	0,00
7	163	0	0	0	0,00
Total		132869		126447,94	100,00

% Isotopic enrichment ^{18}O: 0,0





theorical isotopic distribution

	M	M+1	M+2	M+3	M+4	M+5	M+6	M+7
m/z	163	164	165	166	167	168	169	170
%	100	9,6	0,8	0	0	0	0	0

Enrichment calculation

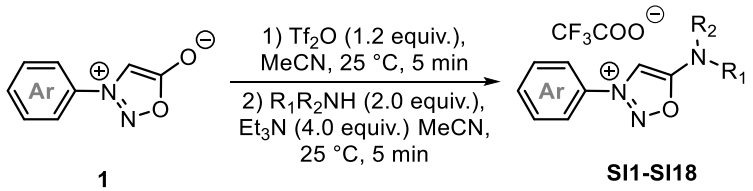
Isotopomer	m/z	Area	natural isotope correction	Corrected area	Isotopic purity (%)
0	163	15798	0	15663	100,04
1	165	119	116	-6	-0,04
2	167	0	0	0	0,00
3	169	0	0	0	0,00
4	171	0	0	0	0,00
5	173	0	0	0	0,00
6	175	0	0	0	0,00
7	177	0	0	0	0,00
Total		15917		15657,05	100,00

% Isotopic enrichment ^{18}O: 0,0

Figure S3. HRMS isotopic enrichment calculation of control experiments.

B. Synthetic procedure and analytical data

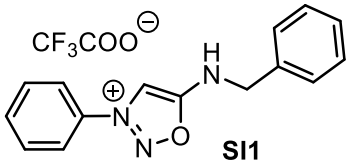
General procedure A for the nucleophilic aromatic substitution:



3-arylsydnone (1.0 equiv.) was dissolved in dry MeCN (0.1 M) under an argon atmosphere in a dry schlenck tube and Tf_2O (1.2 equiv.) was added to the solution at room temperature. After 5 min, a solution of the amine (2.0 equiv.) and triethylamine (4.0 equiv.) in dry MeCN (0.1 M) was added to the mixture. After 30 min, volatiles were removed under reduced pressure and the crude mixture was rapidly purified by flash chromatography on normal phase (DCM to DCM/MeOH 90/10) in order to remove the excess of amine and the unreacted sydnone. Finally, the sydnonimine was purified again by reversed phase chromatography on automatized Puriflash® (Water/TFA 99/01 to MeCN/TFA 99/01 over one hour) to give the desired trifluoroacetate 6-*N*-aryl sydnone imine salt.

Due to the limited scale of the reactions and moderated amount of products, the ^{13}C signal of trifluoroacetate is not observed in ^{13}C-NMR in most of the cases.

5-(benzylamino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI1**



SI1

$C_{17}H_{14}N_3O_3F_3$
MW: 400.76 g.mol-1
Beige solid
40 %

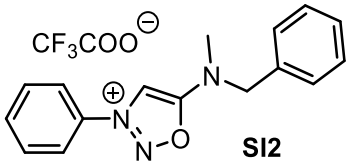
Starting from phenylsydnone **1** using general procedure A, 45 mg of compound **SI1** were obtained in 40% yield.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.61 (s, 1H), 7.81 (d, *J* = 7.4 Hz, 2H), 7.74 (t, *J* = 7.4 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 2H), 7.47 (d, *J* = 7.0 Hz, 2H), 7.39-7.28 (m, 3H), 4.72 (d, *J* = 6.0 Hz, 2H), NH unobserved.

The data were in accordance with the literature.[1]

[1] M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A. Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. Alexandre Champagne, G. Pieters, D. Audisio, and F. Taran. *J. Am. Chem. Soc.* **2023,** *145*, 4, 2219–2229

5-(benzyl(methyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI2**



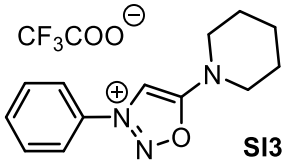
SI2

$C_{18}H_{16}F_3N_3O_3$
MW: 379.3 g.mol^{-1}
Yellow sticky solid
20 %

Starting from phenylsydnone **1** using general procedure A, 23.6 mg of compound **SI2** were obtained in 20% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.00 – 7.95 (m, 2H), 7.82 – 7.76 (m, 1H), 7.75 – 7.69 (m, 2H), 7.44 – 7.30 (m, 5H), 4.81 (s, 2H), 3.24 (s, 3H), proton in position C4 not observed.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 169.9, 163.8-162.8 (q, J_{C-F} = 35.1 Hz, TFAO-), 135.2, 134.7, 134.6, 131.8 (2C), 130.3 (2C), 129.9, 129.3 (2C), 123.4 (2C), 122.4-113.7 (q, J_{C-F} = 291.9 Hz, TFAO-), 97.8, 56.7, 36.9.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.18.
IR (cm^{-1}): 3390, 3067, 1671, 1455, 1201, 1134, 800.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 266
HRMS (ESI): calculated for $[C_{16}H_{16}N_3O]^+$: 266.1293; found : 266.1291

3-phenyl-5-(piperidin-1-yl)-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate– **SI3**



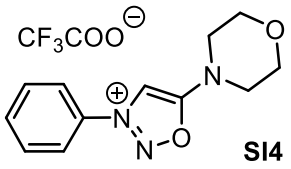
SI3

$C_{15}H_{16}F_3N_3O_3$
MW: 343.3 g.mol^{-1}
Yellow sticky solid
38 %

Starting from phenylsydnone **1** using general procedure A, 40 mg of compound **SI3** were obtained in 38% yield.

^{1}H NMR (400 MHz, Acetone-d_6, δ ppm): 8.95 (s, 1H), 8.11 (d, J = 7.8 Hz, 2H), 7.90 (t, J = 7.3 Hz, 1H), 7.83 (t, J = 7.6 Hz, 2H), 3.91 – 3.84 (m, 4H), 1.88 – 1.76 (m, 6H).
^{13}C NMR (100 MHz, Acetone-d_6, δ ppm): 168.4, 134.9, 134.2, 131.7 (2C), 123.22 (2C), 103.2, 49.2 (2C), 25.6 (2C), 23.6 (Carbons of trifluoroacetate missing).
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.04.
IR (cm^{-1}): 3090, 2954, 1748, 1670, 1448, 1303, 1207, 1183, 1133, 799.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 230
HRMS (ESI): calculated for $[C_{13}H_{16}N_3O]^+$: 230.1293; found : 230.1293

5-morpholino-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI4**



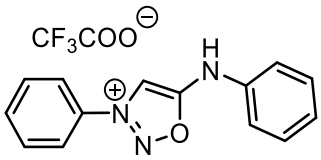
SI4

$C_{14}H_{14}F_3N_3O_4$
MW: 345.3 g.mol^{-1}
Yellow solid
42 %

Starting from phenylsydnone **1** using general procedure A, 45 mg of compound **SI4** were obtained in 42% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.68 (s, 1H), 7.93 – 7.88 (m, 2H), 7.76 – 7.70 (m, 1H), 7.69 – 7.62 (m, 2H), 3.83 (t, J = 4.8 Hz, 4H), 3.74 (t, J = 4.8 Hz, 4H).
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 169.2, 135.2, 134.5, 131.9 (2C), 123.4 (2C), 103.2, 66.4 (2C), 47.9 (2C). (Carbons of trifluoroacetate missing).
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.12.
IR (cm^{-1}): 3386, 3072, 2870, 1672, 1451, 1289, 1201, 1115, 1031, 901.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 232
HRMS (ESI): calculated for $[C_{12}H_{14}N_3O_2]^+$: 232.1087; found : 232.1088
Mp: 187 – 190 °C.

3-phenyl-5-(phenylamino)-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI5**



SI5

$C_{16}H_{12}F_3N_3O_3$
MW: 351.28 g.mol-1
Yellow solid
44%

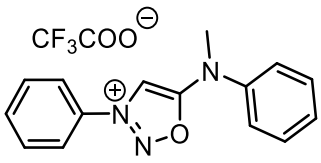
Starting from phenylsydnone **1** using general procedure A, 48 mg of compound **SI5** were obtained in 44% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.94 (s, 1H), 8.13 – 8.06 (m, 2H), 7.92 – 7.85 (m, 1H), 7.84 – 7.78 (m, 2H), 7.56 (ddd, *J* = 9.2, 5.6, 1.9 Hz, 2H), 7.51 – 7.46 (m, 2H), 7.42 – 7.34 (m, 1H), NH unobserved.

The data were in accordance with the literature.[2]

[2] M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A. Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. Alexandre Champagne, G. Pieters, D. Audisio, and F. Taran. *J. Am. Chem. Soc.* **2023,** *145*, 4, 2219–2229

5-(methyl(phenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI6**



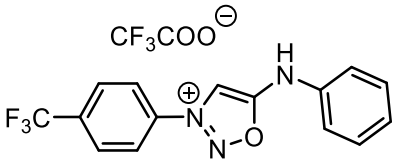
SI6

$C_{17}H_{14}F_3N_3O_3$
MW: 365.31 g.mol^{-1}
Yellow sticky solid
40%

Starting from phenylsydnone **1** using general procedure A, 46.7 mg of compound **SI6** were obtained in 40% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.71 (s, 1H), 8.05 – 7.99 (m, 2H), 7.83 (d, J = 7.5 Hz, 1H), 7.76 (dd, J = 8.5, 6.9 Hz, 2H), 7.64 – 7.50 (m, 5H), 3.76 (s, 3H).
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 169.8, 142.0, 135.2, 134.5, 131.8 (2C), 131.7 (2C), 130.5, 125.7, 125.7, 123.5 (2C), 104.4, 40.8.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.13.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 252
HRMS (ESI): calculated for $[C_{15}H_{14}N_3O]^+$: 252.1137; found : 252.1132

5-(phenylamino)-3-(4-(trifluoromethyl)phenyl)-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI7**



SI7

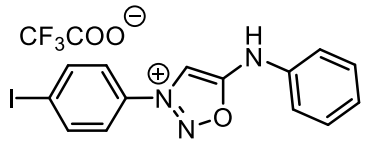
$C_{17}H_{11}F_6N_3O_3$
MW: 419.3 g.mol^{-1}
Yellow solid
42%

Starting from CF_3-phenylsydnone **2** using general procedure A, 50 mg of compound **SI7** were obtained in 42% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 9.03 (s, 1H), 8.29 (d, J = 8.4 Hz, 2H), 8.12 (d, J = 8.5 Hz, 2H), 7.57 – 7.50 (m, 2H), 7.49 – 7.44 (m, 2H), 7.39 – 7.32 (m, 1H), NH unobserved.

^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.5, 137.0, 136.8 (m) 131.3 (3C), 129.1-129.0 (q, $J_{C\text{-}F}$ = 3.8 Hz), 128.0, 125.0 (2C), 121.5 (2C), 121.4 (2C), 105.2.
^{19}F NMR (376 MHz, CD_3OD, δ ppm):-77.00, -64.69.
IR (cm^{-1}): 3070, 1676, 1592, 1324, 1181, 1134, 1069, 848.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 306
HRMS (ESI): calculated for $[C_{15}H_{11}F_3N_3O]^+$: 306.0858; found : 306.0855
Mp: 128-130 °C

3-(4-iodophenyl)-5-(phenylamino)-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI8**



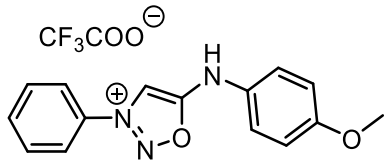
SI8

$C_{16}H_{11}F_3IN_3O_3$
MW: 477.18 g.mol^{-1}
Yellow sticky solid
13%

Starting from *p*-iodo-phenylsydnone **3** using general procedure A, 18.5 mg of compound **SI8** were obtained in 13% yield (18.5 mg, 39 µmol, 13%).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.94 (s, 1H), 8.18 – 8.13 (m, 2H), 7.86 – 7.81 (m, 2H), 7.56 – 7.49 (m, 2H), 7.48 – 7.44 (m, 2H), 7.38 – 7.33 (m, 1H), NH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.14, 141.11 (2C), 137.03, 131.21 (2C), 127.79, 125.04 (2C), 121.31 (2C), 121.29, 104.52, 101.80.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.25.
IR (cm^{-1}): 3059, 2923, 2852, 1664, 1590, 1498, 1424.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 364
HRMS (ESI): calculated for $[C_{14}H_{11}IN_3O]^+$: 363.9948; found : 363.9952

5-((4-methoxyphenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI9**



SI9

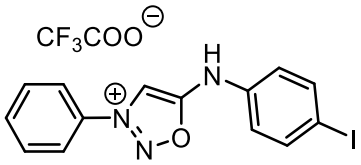
$C_{17}H_{14}F_3N_3O_4$
MW: 381.31 g.mol-1
Yellow solid
33%

Starting from phenylsydnone **1** using general procedure A, 38.8 mg of compound **SI9** were obtained in 33% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): **)**: 8.68 (s, 1H), 7.99 – 7.95 (m, 2H), 7.77 (t, *J* = 7.5 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 2H), 7.34 – 7.31 (m, 2H), 7.01 – 6.97 (m, 2H), 3.76 (s, 3H), NH unobserved.

^{13}C NMR (100 MHz, CD_3OD, δ ppm): 167.7, 159.3, 134.4, 133.8, 131.0 (2C), 128.9, 122.9, 122.9 (2C), 115.5 (2C), 103.1, 55.3, 54.0.
^{19}F NMR (376 MHz, CD_3OD, δ ppm):-78.80.
IR (cm^{-1}): 3423, 1723, 1666, 1514, 1455, 1204, 1146, 801, 725.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 268
HRMS (ESI): calculated for $[C_{15}H_{14}N_3O_2]^+$ 268.1087; found 268.1086
Mp: 204-206 °C

5-((4-iodophenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI10**



SI10

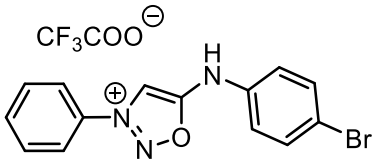
$C_{16}H_{11}F_3IN_3O_3$
MW: 477.2 g.mol^{-1}
Yellow solid
15%

Starting from phenylsydnone **1** using general procedure A, 21.5 mg of compound **SI10** were obtained in 15 % yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): δ 8.96 (s, 1H), 8.12 – 8.05 (m, 2H), 7.91 – 7.84 (m, 3H), 7.83 – 7.76 (m, 2H), 7.29 – 7.22 (m, 2H), NH unobserved.

The data were in accordance with the literature.[3]

5-((4-bromophenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI11**



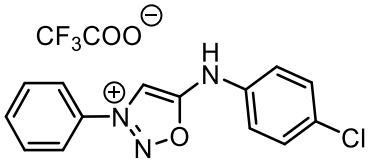
SI11

$C_{16}H_{11}F_3BrN_3O_3$
MW: 430.2 g.mol^{-1}
Yellow sticky solid
11%

Starting from phenylsydnone **1** using general procedure A, 15 mg of compound **SI11** were obtained in 11 % yield.
^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.95 (s, 1H), 8.06 (dd, *J* = 8.6, 1.2 Hz, 2H), 7.88 – 7.83 (m, 1H), 7.81 – 7.73 (m, 2H), 7.69 – 7.65 (m, 2H), 7.38 (d, *J* = 8.9 Hz, 2H), NH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.0, 136.4, 135.4, 134.2 (2C), 131.8 (2C), 131.4, 123.7 (2C), 123.0, 122.7, 120.5, 104.9.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.42.
IR (cm^{-1}): 3077, 1677, 1492, 1205, 1139.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 316
HRMS (ESI): calculated for $[C_{14}H_{11}BrN_3O]^+$ 316.0085 ; found 316.0092.

[3] M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A. Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. Alexandre Champagne, G. Pieters, D. Audisio, and F. Taran. *J. Am. Chem. Soc.* **2023,** *145*, 4, 2219–2229

5-((4-chlorophenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI12**



SI12

$C_{16}H_{11}F_3ClN_3O_3$
MW: 385.7 g.mol^{-1}
Yellow sticky solid
7%

Starting from phenylsydnone **1** using general procedure A, 8.42 mg of compound **SI12** were obtained in 7% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.96 – 7.93 (m, 2H), 7.77 – 7.67 (m, 3H), 7.34 – 7.30 (m, 2H), 7.23 (d, J = 8.8 Hz, 2H). NH and C4 proton missing.

^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.1, 135.9, 135.4, 134.5, 133.0, 131.9 (2C), 131.3 (2C), 123.7 (2C), 122.9 (2C), 104.8.

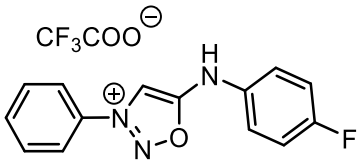
^{19}F NMR (376MHz, CD_3OD, δ ppm): -77.36.

IR (cm^{-1}): 3081, 1677, 1587, 1496, 1199, 1136, 831, 763.

LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 272

HRMS (ESI): calculated for $[C_{14}H_{11}ClN_3O]^+$ 272.0591 ; found 272.0596.

5-((4-fluorophenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI13**



SI13

$C_{16}H_{11}F_4N_3O_3$
MW: 369.3 g.mol^{-1}
Yellow sticky solid
9%

Starting from phenylsydnone **1** using general procedure A, 10.5 mg of compound **SI13** were obtained in 9% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): δ 8.86 (s, 1H), 8.04 (d, J = 7.8 Hz, 2H), 7.83 (d, J = 7.3 Hz, 1H), 7.76 (t, J = 7.7 Hz, 2H), 7.51 – 7.45 (m, 2H), 7.26 (t, J = 8.7 Hz, 2H), NH unobserved.

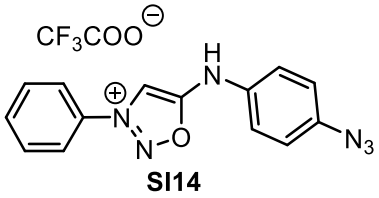
^{19}F NMR (376MHz, CD_3OD, δ ppm): -116.96, -77.12.

IR (cm^{-1}): 3416, 1669, 1513, 1204, 1147, 801, 726.

LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 256

HRMS (ESI): calculated for $[C_{14}H_{11}FN_3O]^+$ 256.0886; found 256.0888

5-((4-azidophenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI14**



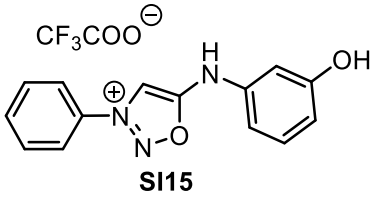
SI14

$C_{16}H_{11}F_3N_6O_3$
MW: 392.3g.mol^{-1}
Yellow solid
13%

Starting from phenylsydnone **1** using general procedure A, 15 mg of compound **SI14** were obtained in 13% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.89 (s, 1H), 8.09 – 8.04 (m, 2H), 7.89 – 7.83 (m, 1H), 7.81 – 7.75 (m, 2H), 7.51 – 7.46 (m, 2H), 7.25 – 7.20 (m, 2H), NH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.1, 140.0, 135.3, 133.9, 131.8 (2C), 123.7 (2C), 123.2 (2C), 123.1, 121.6 (2C), 104.6.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.35.
IR (cm^{-1}): 3364, 2119, 1672, 1591, 1507, 1285, 1200, 1131, 835, 799, 720.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 279
HRMS (ESI): calculated for $[C_{14}H_{11}N_6O]^+$ 279.0995; found 279.0994
Mp: Degardation at 180 °C

5-((3-hydroxyphenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate **– SI15**



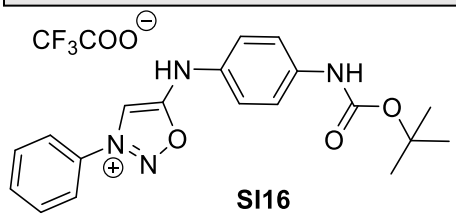
SI15

$C_{16}H_{12}F_3N_3O_4$
MW: 367.3 g.mol^{-1}
Yellow sticky solid
40%

Starting from phenylsydnone **1** using general procedure A, 45 mg of compound **SI15** were obtained in 40% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.76 (s, 1H), 7.99 – 7.92 (m, 2H), 7.77 – 7.71 (m, 1H), 7.66 (dd, *J* = 10.5, 4.9 Hz, 2H), 7.20 (t, *J* = 8.1 Hz, 1H), 6.80 (ddd, *J* = 9.9, 6.2, 2.1 Hz, 2H), 6.65 (ddd, *J* = 8.3, 2.2, 0.7 Hz, 1H), NH and OH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 168.0, 160.2, 138.0, 135.2, 134.5, 132.0, 131.7 (2C), 123.7, 114.7, 111.9, 108.3, 104.5.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.48.
IR (cm^{-1}): 3421, 1660, 1461, 1199, 1146, 852, 800, 726.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 254
HRMS (ESI): calculated for $[C_{14}H_{12}N_3O_2]^+$ 254.0930; found 254.0938

5-((4-((tert-butoxycarbonyl)amino)phenyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI16**



SI16

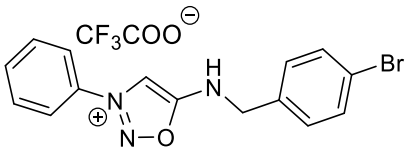
$C_{21}H_{21}F_3N_4O_5$
MW: 466.4 g.mol^{-1}
Orange solid
20%

Starting from phenylsydnone **1** using general procedure A, 28.7 mg of compound **SI16** were obtained in 20% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.82 (s, 1H), 8.09-8.04 (m, 2H), 7.84 (dd, *J* = 4.9, 3.8 Hz, 1H), 7.81-7.75 (m, 2H), 7.57 (d, *J* = 9.0 Hz, 2H), 7.40-7.34 (m, 2H), 1.53 (s, 9H), both NH unobserved.

The data were in accordance with the literature.[4]

5-((4-bromobenzyl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI17**



SI17

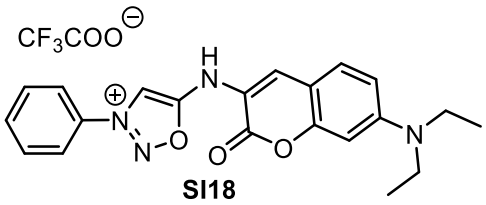
$C_{17}H_{13}BrF_3N_3O_3$
MW: 444.2 g.mol^{-1}
Orange solid
16%

Starting from phenylsydnone **1** using general procedure A, 21.9 mg of compound **SI17** were obtained in 16% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.62 (s, 1H), 8.03-7.99 (m, 2H), 7.84 (m, 1H), 7.79-7.73 (m, 2H), 7.60-7.56 (m, 2H), 7.43-7.38 (m, 2H), 4.69 (s, 2H),NH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 170.4, 136.1, 135.1, 134.6, 133.2 (2C), 131.8 (2C), 130.9 (2C), 123.5 (2C), 123.4, 102.6, 48.3.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -76.93
IR (cm^{-1}): 2970, 1671, 1489, 1407, 1351, 1199, 1131, 1072, 1011.
LCMS (ESI) *m/z*: [M-CF_3COO]$^+$ 330
HRMS (ESI): calculated for **[**$C_{15}H_{13}BrN_3O$]$^+$ 330.0242; found 330.0240.

[4] M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A. Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. Alexandre Champagne, G. Pieters, D. Audisio, and F. Taran. *J. Am. Chem. Soc.* **2023,** *145*, 4, 2219–2229

5-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)amino)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI18**



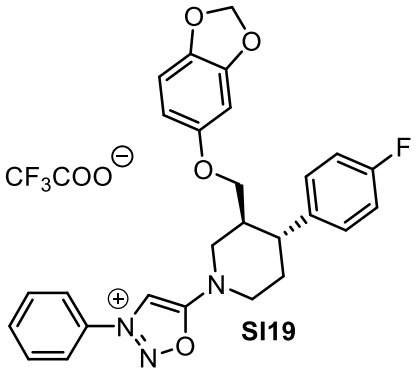
SI18

$C_{23}H_{21}F_3N_4O_5$
MW: 490.4 g.mol^{-1}
Red sticky solid
8%

Starting from phenylsydnone **1** using general procedure A, 12.3 mg of compound **SI18** were obtained in 8% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.77 (s, 1H), 8.10 – 7.99 (m, 3H), 7.84 (ddd, J = 6.6, 3.8, 1.2 Hz, 1H), 7.80 – 7.72 (m, 2H), 7.47 (d, J = 9.0 Hz, 1H), 6.80 (dd, J = 8.9, 2.5 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1H), 3.49 (q, J = 7.1 Hz, 4H), 1.20 (dd, J = 11.4, 4.3 Hz, 6H) ,NH unobserved.
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 195.9, 168.3, 168.1, 156.2, 152.5, 135.3, 131.9 (3C), 130.7, 123.6 (2C), 111.3, 108.5, 105.4, 98.1, 54.8, 45.8 (2C), 12.7 (3C).
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -77.21.
IR (cm^{-1}): 3400, 1671, 1204, 1138, 800, 723.
LCMS (ESI) *m/z*: [M-CF_3COO]$^+$: 377
HRMS (ESI): calculated for $[C_{21}H_{21}N_4O_3]^+$ 377.1615 ; found 377.1616.

5-((3S,4R)-3-((benzo[d][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidin-1-yl)-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate– **SI19**



SI19

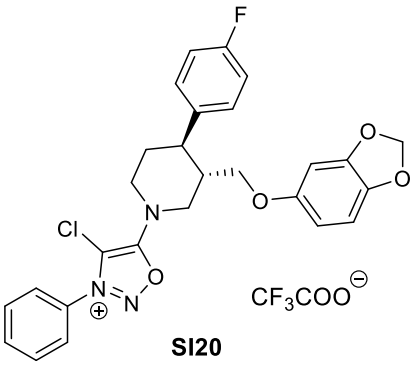
$C_{29}H_{25}F_4N_3O_6$
MW: 587.5276 g.mol^{-1}
Yellow sticky solid
20 %

Starting from phenylsydnone **1** using general procedure A, 36.2 mg of compound **SI19** were obtained in 20% yield.

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 8.83 (s, 1H), 8.06 – 8.02 (m, 2H), 7.89 – 7.83 (m, 1H), 7.82 – 7.75 (m, 2H), 7.35 – 7.28 (m, 2H), 7.05 (t, *J* = 8.8 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.43 (d, *J* = 2.5 Hz, 1H), 6.22 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.86 (dd, *J* = 1.8, 1.1 Hz, 2H), 4.39 (dd, *J* = 13.4, 3.0 Hz, 1H), 4.23 (d, J = 13.2 Hz, 1H), 3.72 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.66 – 3.55 (m, 3H), 3.05 (td, *J* = 11.5, 4.4 Hz, 1H), 3.00 – 2.83 (m, 1H), 2.50 – 2.38 (m, 1H), 2.17 – 2.01 (m, 1H).
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 169.0, 164.5, 162.1, 155.5, 149.7, 143.4, 139.5 (d, J = 3.2

Hz), 135.2, 134.6, 131.9 (2C), 130.4 - 130.3 (d, J = 7.9 Hz, 2C), 123.4 (2C), 116.6 - 116.4 (d, J= 21.4 Hz, 2C), 108.8 - 106.7 (d, J = 211.9 Hz), 103.1, 102.5, 98.9, 69.4, 51.5, 49.1, 43.8, 42.6, 33.7.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -117.62, -79.98, -76.93.
IR (cm^{-1}): 3072, 2924, 1665, 1488, 1469, 1181, 1125, 1030.
LCMS (ESI) *m/z*: $[M\text{-}CF_3COO]^+$: 474.6
HRMS (ESI): calculated for $[C_{27}H_{25}FN_3O_4]^+$: 474,1829; found : 474,1831

5-((3S,4R)-3-((benzo[d][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidin-1-yl)-4-chloro-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI20**



SI20

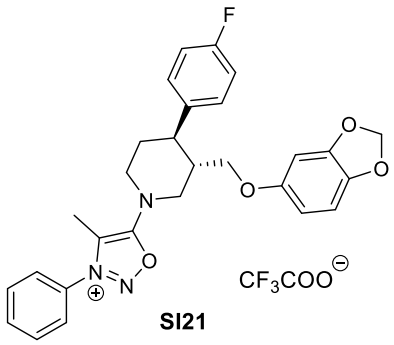
$C_{29}H_{24}ClF_4N_3O_6$
MW: 621.97 $g.mol^{-1}$
Orange solid
Yield: 13%

10 mg of **SI20** were obtained in 13% yield from 4-chloro-3-phenylsydnone synthesized according to previously described protocol,[5] using the general procedure. Purification was carried out by reverse phase chromatography on automatized Puriflash® (100% Water + 0,1% TFA to 100% MeCN + 0,1% TFA over one hour).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.95 – 7.89 (m, 1H), 7.88 – 7.83 (m, 4H), 7.36 – 7.29 (m, 2H), 7.09 (t, *J* = 8.8 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 1H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.21 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.86 (m, 2H), 4.82 (m, 1H), 4.63 (m, 1H), 3.73 (dd, *J* = 10.0, 3.1 Hz, 1H), 3.69 – 3.60 (m, 3H), 3.1 – 3.03 (m, 1H), 2.54 – 2.43 (m, 1H), 2.16 – 2.06 (m, 2H).
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 163.5, 155.3, 149.8, 143.5, 139.3, 135.4, 132.2, 131.9 (2C), 130.3 (2C), 126.6 (2C), 116.7, 116.5, 108.9, 106.7, 105.9, 102.5, 102.4, 98.8 (2C), 69.2, 51.2, 43.9, 43.0, 34.2. Carbone CF_3COO not observed.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -117.47, -76.93.
IR (cm^{-1}): 2924, 1666, 1510, 1489, 1469, 1185, 1136, 1037.
HRMS (ESI-TOF) *m/z*: calcd for $[C_{27}H_{24}ClFN_3O_4]^+$ 508.1440, found 508.1440.

[5] L. Plougastel, O. Koniev, S. Specklin, E. Decuypere, C. Créminon, D-A. Buisson, A. Wagner, S. Kolodych, F. Taran. *ChemComm*, **2014**, *50 (66)*, 9376 – 9378.

5-((3S,4R)-3-((benzo[d][1,3]dioxol-5-yloxy)methyl)-4-(4-fluorophenyl)piperidin-1-yl)-4-methyl-3-phenyl-1,2,3-oxadiazol-3-ium 2,2,2-trifluoroacetate – **SI21**



SI21

$C_{30}H_{27}F_4N_3O_6$
MW: 601.55 g.mol^{-1}
Colourless solid
Yield: 19%

14 mg of **SI21** were obtained in 19% yield from 4-methyl-3-phenylsydnone synthesized according to previously described protocol,[5] using the general procedure. Purification was carried out
by reverse phase chromatography on automatized Puriflash® (100% Water + 0,1% TFA to 100% MeCN + 0,1% TFA over one hour).

^{1}H NMR (400 MHz, CD_3OD, δ ppm): 7.91 – 7.73 (m, 5H), 7.39 – 7.28 (m, 2H), 7.12 – 7.01 (t, , *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 8.5 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 6.20 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.86 – 5.84 (m, 2H), 4.59 – 4.53 (m, 1H), 4.43 – 4.36 (m, 1H), 3.71 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.66 – 3.51 (m, 3H), 3.02 (td, *J* = 11.7, 4.1 Hz, 1H), 2.48 (s, 4H), 2.18 – 2.00 (m, 2H).
^{13}C NMR (100 MHz, CD_3OD, δ ppm): 166.7, 164.5, 162.1, 155.4, 149.7, 143.4, 139.6, 139.5, 134.7, 132.8, 131.8 (2C), 130.4, 130.3, 126.7 (2C), 116.6, 116.4, 114.8, 108.9, 106.6, 102.5, 98.8, 69.3, 54.8, 51.4, 44.1, 43.0, 34.2, 10.6.
^{19}F NMR (376 MHz, CD_3OD, δ ppm): -115.22, -77.49.
IR (cm^{-1}): 2925, 1690, 1657, 1510, 1489, 1469, 1269, 1186, 1031
HRMS (ESI-TOF) *m/z*: calcd for $C_{28}H_{27}FN_3O_4$ $[M]^+$ 488.1985, found 488.1984.

C. Crystallographic data

The data collection for **SI4** was performed at 100(2) K on a Bruker D8 Quest diffractometer using an Incoatec Microfocus Source (IμS 3.0 Mo) and a PHOTON III area detector, and operated with APEX3.[6] The data were processed with SAINT,[7] and an empirical absorption correction (multi-scan) was made with SADABS.[8] The structure was solved by intrinsic phasing with SHELXT,[9] and refined by full-matrix least-squares on F^2 with SHELXL,[10] using the ShelXle interface.[11] Two-component twinning was detected with TwinRotMat in PLATON,[12] and the refined batch scale factor was 0.30. The hydrogen atoms were introduced at calculated positions and were treated as riding atoms with an isotropic displacement parameter equal to 1.2 times that of the parent atom. The drawing was made with ORTEP-3.[13]

Crystal data for **SI4**: $C_{18}H_{14}CaF_9N_3O_8$, M = 611.40, monoclinic, space group $P2_1/n$, a = 24.1213(14), b = 8.1633(4), c = 24.2668(17) Å, β = 98.981(2)°, V = 4719.8(5) Å^3, Z = 8. Refinement of 704 parameters on 8929 independent reflections out of 76528 measured reflections (R_{int} = 0.077) led to $R1$ = 0.083, $wR2$ = 0.235, S = 1.107, $\Delta\rho_{min}$ = –0.61, $\Delta\rho_{max}$ = 1.20 e Å^{-3}.

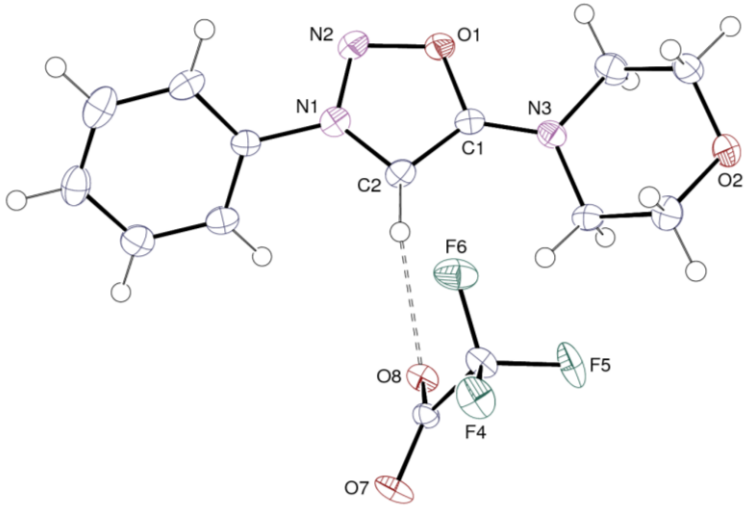


Figure S4 (a) View of one of the two independent molecules in **SI4** with displacement ellipsoids shown at the 50% probability level. Only one CF_3COO^- anion of the $Ca(CF_3COO)_3^-$ coordination polymer is shown. The hydrogen bond is shown as a dashed line.

[6] *APEX3*, *ver. 2019.1-0*, Bruker AXS, Madison, WI, 2019

[7] *SAINT*, *ver. 8.40A*, Bruker Nano, Madison, WI, 2019.

[8] (*a*) *SADABS*, *ver. 2016/2*, Bruker AXS, Madison, WI, 2016; (*b*) L. Krause, R. Herbst-Irmer, G. M. Sheldrick and D. Stalke, *J. Appl. Crystallogr.*, 2015, **48**, 3.

[9] G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 2015, **71**, 3

[10] G. M. Sheldrick, *Acta Crystallogr.*, *Sect. C*, 2015, **71**, 3

[11] C. B. Hübschle, G. M. Sheldrick and B. Dittrich, *J. Appl. Crystallogr.*, 2011, **44**, 1281

[12] A. L. Spek, *Acta Crystallogr., Sect. D*, 2009, **65**, 148.

[13] (*a*) M. N. Burnett and C. K. Johnson, *ORTEPIII*, Report ORNL-6895; Oak Ridge National Laboratory: TN, 1996; (*b*) L. J. Farrugia, *J. Appl. Crystallogr.*, 2012, **45**, 849.

III. Kinetic studies

A. Kinetic studies with SI5 and SI6

Reactions were followed by measuring the decrease of the maximum absorbance signal of iminosydnones at different wavelength depending on the media.

30 µL of 6-*N*-aryl sydnonimine at 10 mM in DMSO (UV-spectroscopy grade) was diluted in 2960 µL of desired media. The absorbance spectrum of the solution was first measured during 100 sec. until equilibration of the solution and then 10 µL of DBCO-Acid (30 mM in UV-spectroscopy grade DMSO; 1.0 equiv.) were added. The kinetic of the reaction was followed by the absorbance of the solution at the wavelength where the disparition of the starting material can be observed. Each experiments was done in duplicate.
Correspondence between absorbance and concentrations were established by plotting the calibration curve obtained by measuring the absorbance at the appropriate wavelength at different concentrations.

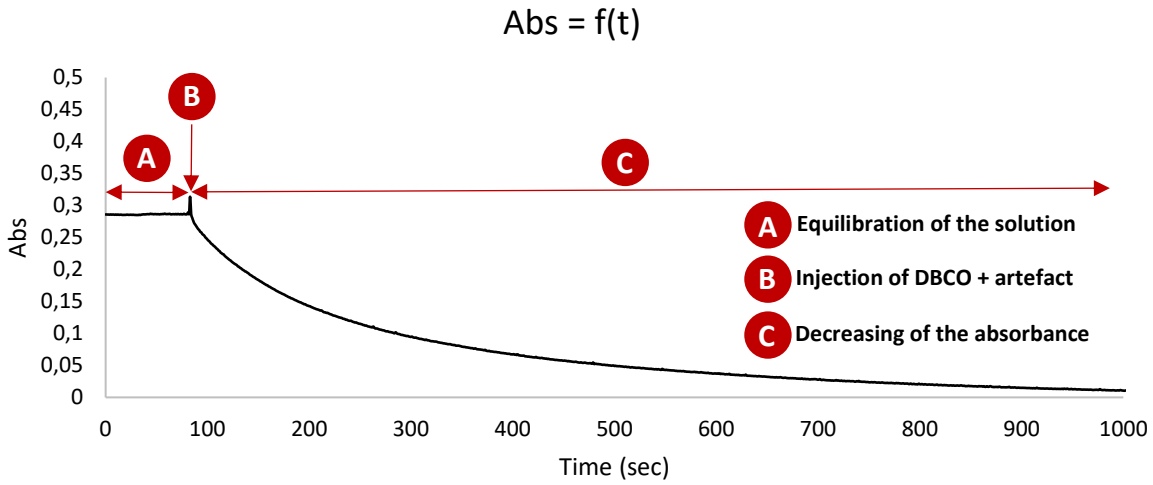


Figure S5. Example of recorded spectrum.

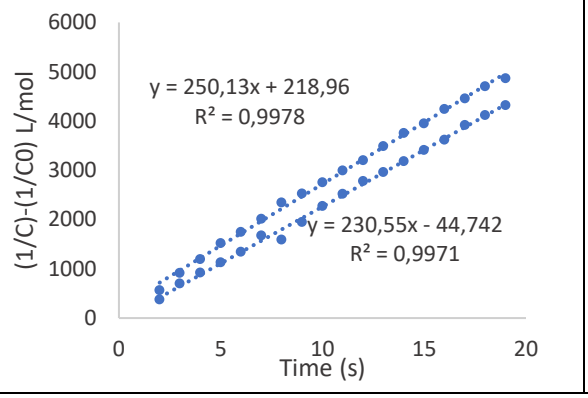
All the kinetics were calculated after the decreasing of the artefact due to the DBCO injection (B in **Figure S5**). The monitoring of the solution was performed after stabilization of the temperature (± 0.1 °C). The injection was performed after 100 seconds of monitoring in order to wait the equilibration of the solution (A in **Figure S5**). The corresponding rate constant was determined by plotting $(1/C)-(1/C_0)$ = f(t) between 0% to 30% of conversion. All the experiments were done in duplicate. Rate constants are reported as the mean of the two measurements taken, with an error interval corresponding to the standard deviation of the two measurements.

Table S1. Result of the calibration curves for compound **SI5** and **SI6.**

Compound	**SI5**	**SI5**	**SI6**	**SI6**
Media	PBS	DMSO	PBS	DMSO
Wavelength	380 nm	380 nm	340 nm	340 nm
Calibration curve	A = 4778.9C	A = 3795.7C	A = 4204.6C	A = 3762,4C

Table S2. Kinetics of the reaction between **SI5** and DBCO in PBS

100 µM in PBS, **SI5** + DBCO (1.0 eq.)
$k_{app\text{-}moy}$ = 240 ± 14 L mol^{-1} s^{-1}



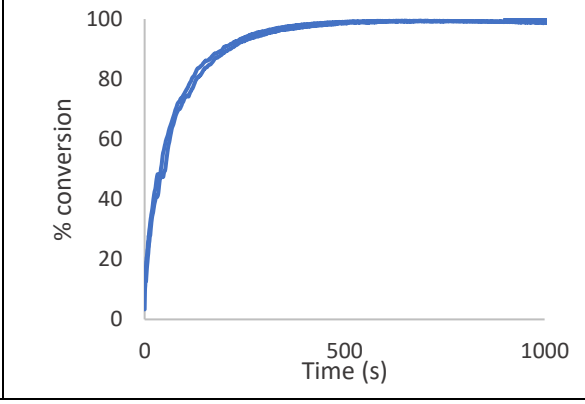
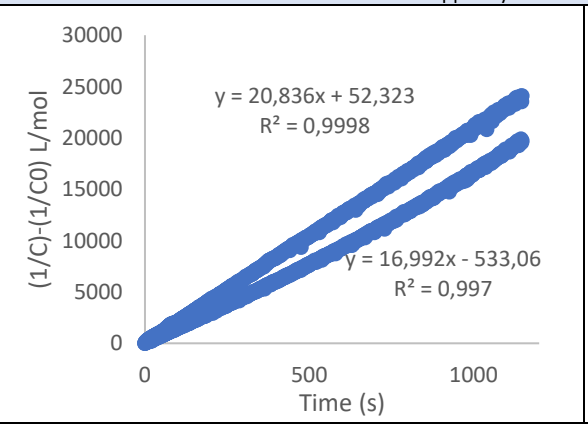


Table S3. Kinetics of the reaction between **SI5** and DBCO in DMSO.

100 µM in DMSO, **SI5** + DBCO acid (1.0 eq.)
$k_{app\text{-}moy}$ = 19 ± 3 L mol^{-1} s^{-1}



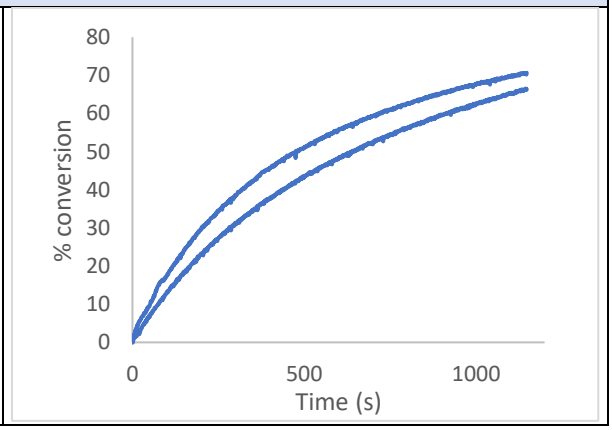


Table S4. Kinetics of the reaction between **SI6** and DBCO in PBS

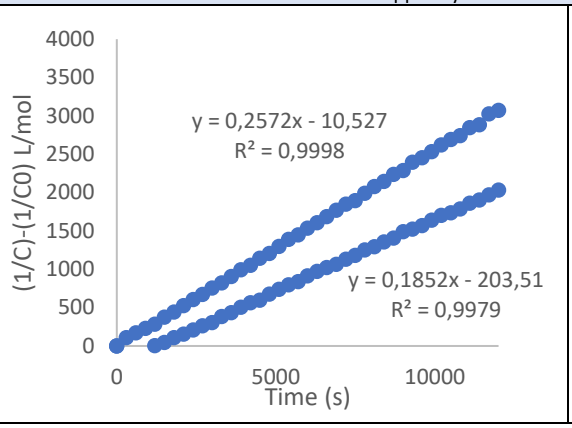
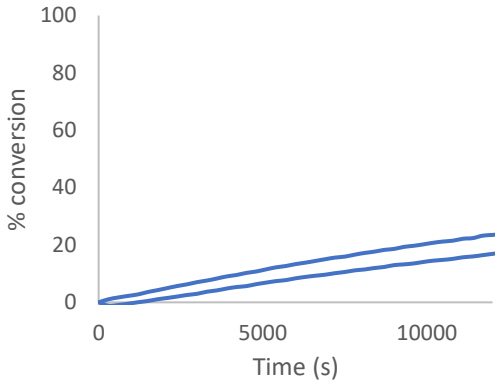
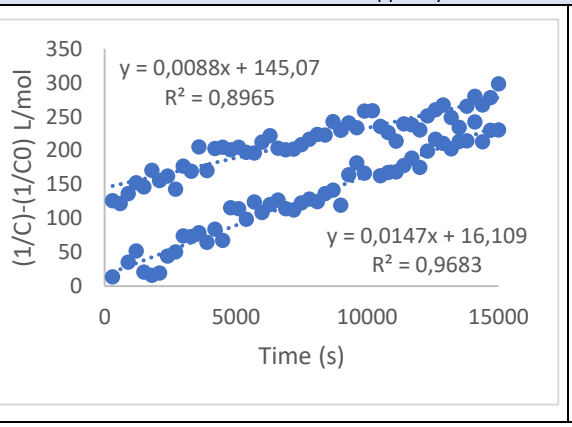
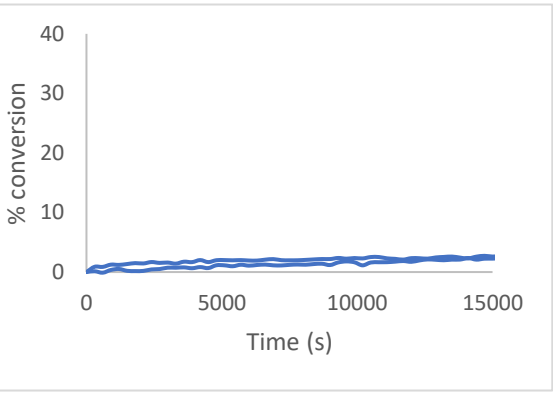
100 µM in PBS, **SI6** + DBCO acid (1.0 eq.) $k_{app\text{-}moy}$ = 0.22 ± 0.05 L mol^{-1} s^{-1}	
	

Table S5. Kinetics of the reaction between **SI6** and DBCO in DMSO

100 µM in PBS, **SI6** + DBCO acid (1.0 eq.) $k_{app\text{-}moy}$ = 0.012 ± 0.004 L mol^{-1} s^{-1}	
	

B. Kinetic studies with probe SI18

1) Fluorescence properties of **SI18**

Absorbance spectrum of the probe **SI18** was performed at 100 µM in PBS containing 1% DMSO. Two maximums of absorbance were determined: 260 nm and 404.4 nm (Figure S6). λ_{ex} = 404 nm was selected as excitation wavelength of the probe for fluorescence monitoring. Fluorescence spectrum of the probe **SI18** was performed at 10 µM in PBS 1% DMSO, λ_{ex} = 404.4 nm. A maximum of fluorescence was found at 510 nm (Figure S7).

Absorbance and fluorescence spectra of compound **SI18**	

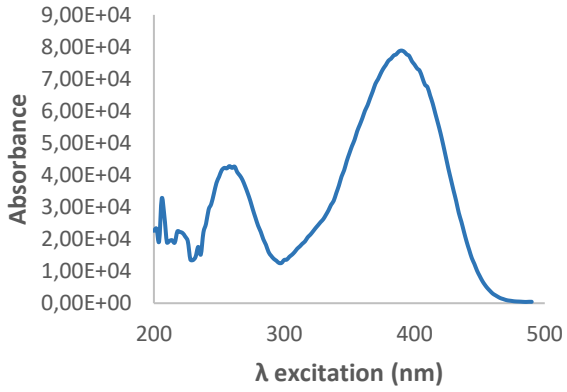
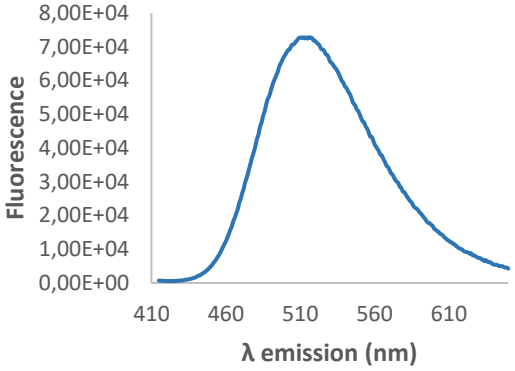
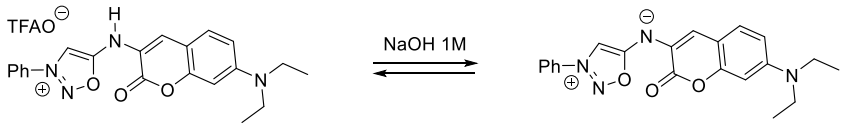

	

Figure S6. Absorbance spectrum of compound **SI18** at 100 µM in PBS 1% DMSO	**Figure S7.** Fluorescence spectrum of compound **SI18** at 10 µM in PBS 1% DMSO, λ_{ex} = 404 nm

2) Protocol for pKa determination of compound **SI18**



To a 100 µM solution of the sydnonimine **SI18** in AcOH (0.1 M) was progressively added NaOH (1 M). The pH of the solution was measured after each addition and the absorbance spectrum was recorded for each point (from pH 3.2 to pH 12.2).
The pKa of the compound **SI18** was determined by plotting the absorbance characteristic of the protonated compound (410 nm) versus pH.

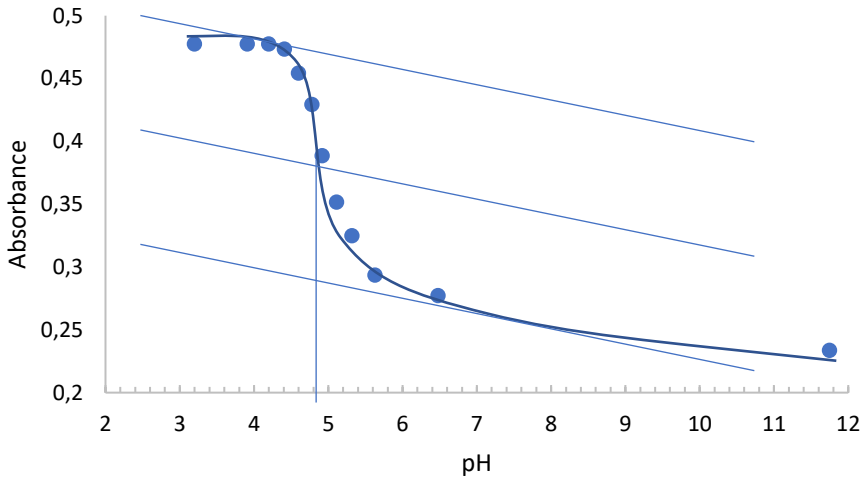


Figure S8. Absorbance as a function of the pH of **SI18** at 410 nm

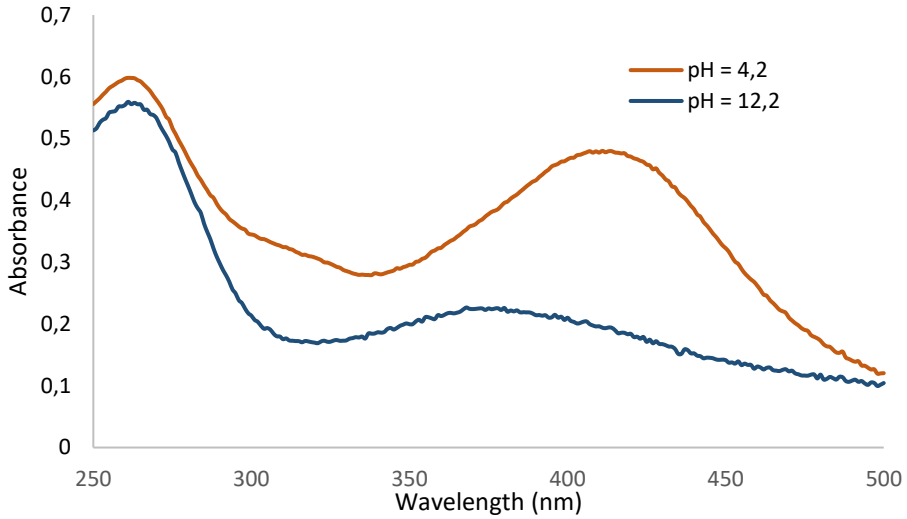


Figure S9. Absorbance as a function of the Wavelength of SI18 at pH = 4,2 and pH = 12,2

In a second experiment, the pKa of the compound **SI18** was also determined by fluorescence. To a 100 µM solution of the iminosydnone **SI18** in AcOH (0.1 M) was progressively added NaOH (1 M). The pH of the solution was measured after each addition and the fluorescence spectrum was recorded for each point (from pH 3.2 to pH 12.2) with an excitation of 410 nm.
The pKa of the compound **SI18** was determined by plotting the fluorescence characteristic of the protonated compound (excitation: 410 nm, emission: 510 nm) versus pH.

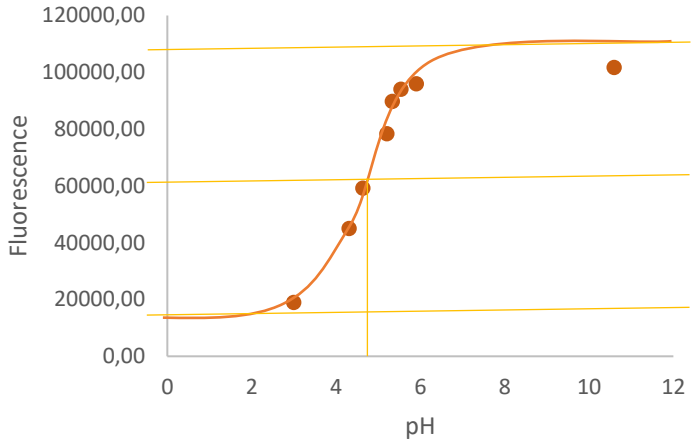


Figure S10. Fluorescence of the compound **SI18** in function of the pH, λ_{ex} = 404.4 nm, λ_{em} = 510 nm

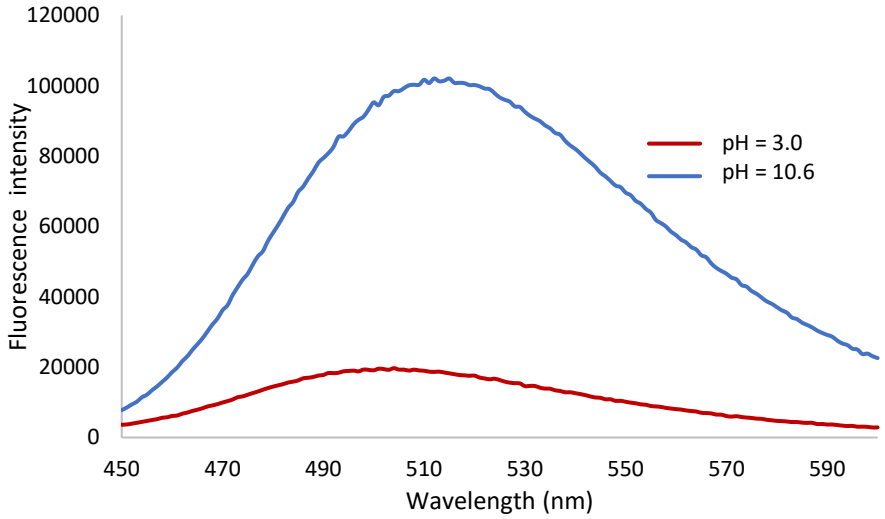


Figure S11. Fluorescence intensity as a function of the Wavelength of **SI18** at pH = 3.0 and pH = 10,6.

The pKa of **SI18** was found at 4.8 ± 0.1.

3) Kinetic study of the reaction between **SI18** and DBCO acid

Reaction kinetics were monitored by measuring the fluorescence signal at 510 nm with an excitation of 404 nm according the following protocol: in a vial was added 30 µL of **SI18** (1 mM in DMSO) in 2.925 mL of PBS, final concentration: 10 µM. To this solution were added 45 µL of a DBCO solution (1mM in DMSO, 1.5 eq.). Fluorescence of the solution (λ_{ex}: 404 nm, λ_{em}: 510 nm) was recorded under stirring.

Second order reaction rates were determined by plotting $-\ln([A]/[B])/([A]_0 - [B]_0)$ versus time and analyzed by linear regression. Second order rate constants correspond to the determined slopes.

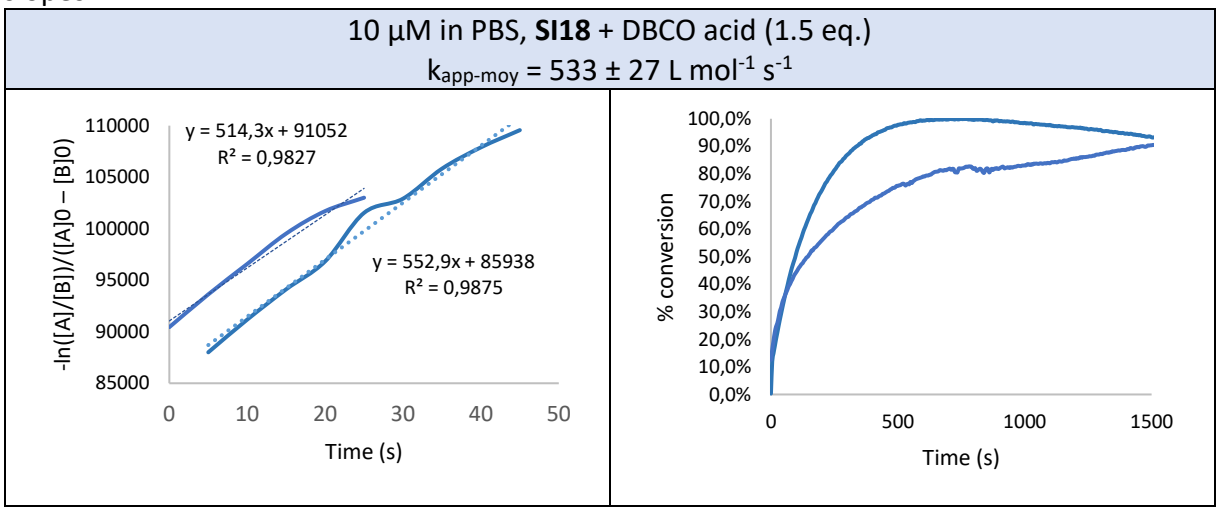


Figure S12. Reaction kinetics between **SI18** and DBCO.

A kinetic constant of k = 533 ± 27 L mol^{-1} s^{-1} was found.

C. Kinetic studies with caged paroxetine compounds SI19 and SI20

1) Determination of rate constants

Stock solutions were first prepared separately:
- sydnonimines **SI19** or **SI20** (1 mg in 100 µL of DMSO, 17 mM)
- internal standard ditertbutylbiphenyl (1 mg in 100 µL of DMSO, 37.5 mM)
- DBCO-acid (1 mg in 100 µL of DMSO, 30 mM).

To a mixture of PBS/DMSO (950 µL/ 19.3 µL) were added 18 µL of stock solution **SI19** or **SI20** and 2.7 µL of the internal standard. A first LC-UV chromatogram was obtained at t_0. Then, 10 µL of stock solution DBCO-acid were added and the reaction was stirred at room temperature and followed by LC-UV. The final concentrations of **SI19** or **SI20** and DBCO was 300 µM and the final concentration of internal standard was 100 µM.

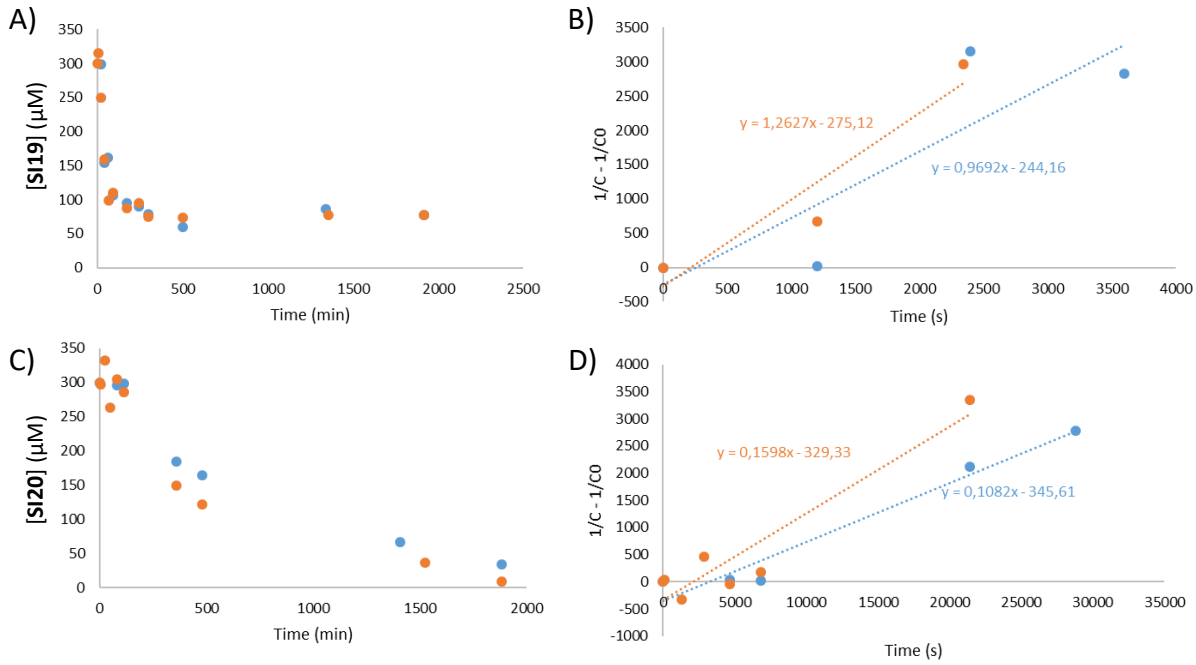


Figure S13. A and B) Reaction of **SI19** with DBCO; C and D) Reaction of **SI20** with DBCO.

2) Paroxetin release : Protocol for LC-UV-MS study of the reaction between **SI19** or **SI20** and DBCO acid in PBS

In a vial was dissolved the compound **SI19** or **SI20** (0.12 mg, 200 nmol, 1 eq.) in PBS/DMSO (3:1) and 2-fluoro-4-nitrophenol was added as an internal standard (0,047 mg, 200 nmol, 1 eq.). A first LC-UV chromatogram was obtained at t0. Then, DBCO acid (0.10 mg, 300 nmol, 1 eq.) was added and the reaction was stirred at room temperature and followed by LC-UV. The final concentration of the compound in solution in PBS/DMSO (3:1) is 200 µM.

Release of paroxetine was followed by LC-UV. A calibration curve was obtained by plotting the ratio of UV-peaks area of paroxetine and internal standard versus paroxetin concentration in PBS/DMSO (3:1) and with 200 nmol internal standard (Figure S14).

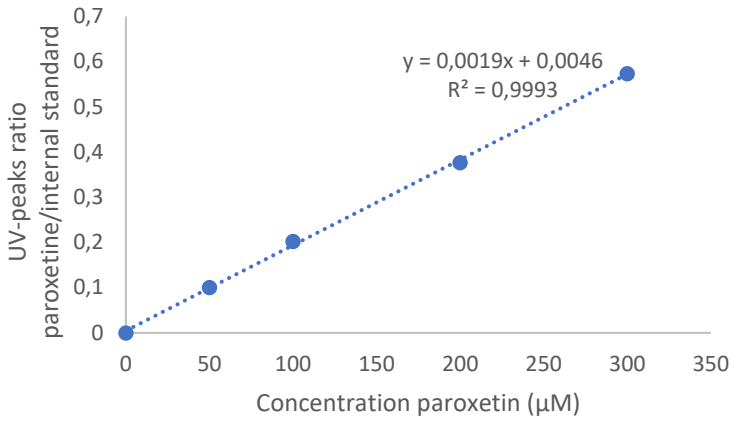


Figure S14. Calibration curve for paroxetine in PBS/DMSO (3:1)

The concentration of paroxetine was determined and plotted versus time.

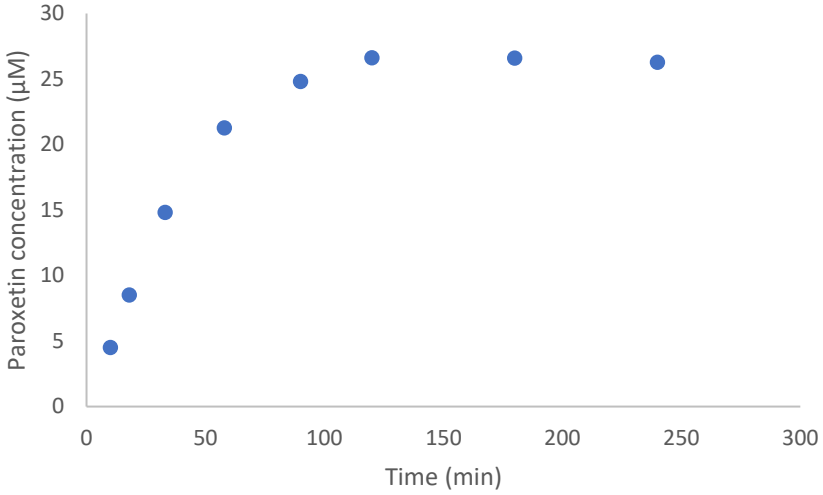


Figure S15. Concentration of paroxetine over time during the reaction between **SI19** and DBCO, 200 µM in PBS.

A final concentration in paroxetine of 27 µM was measured, corresponding to a 14% conversion of **SI19**.

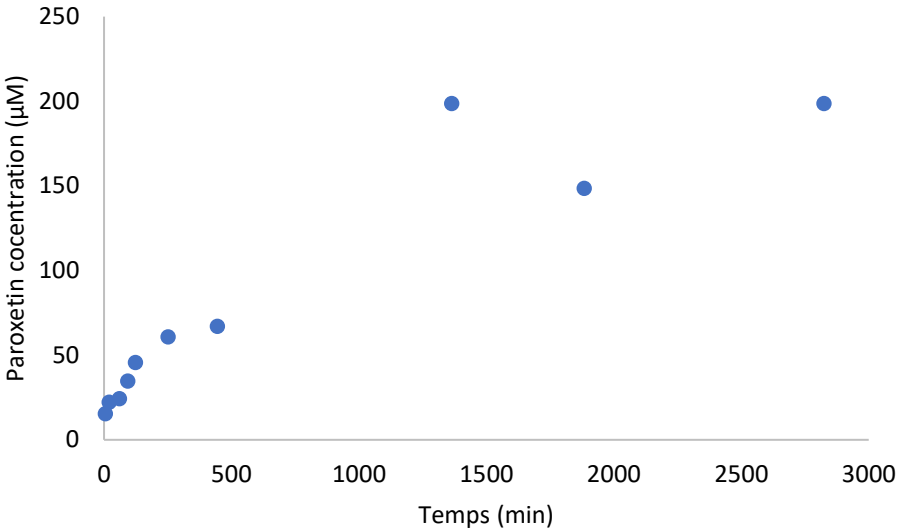


Figure S16. Concentration of paroxetine over time during the reaction between **SI20** and DBCO, 300 µM in PBS.

A final concentration in paroxetine of 198 µM was measured, corresponding to a 66% conversion of **SI20**.

IV. Experiments with cells

A. Cell Culture

Cell lines were obtained from the American type Culture Collection (Rockville, USA) and were cultured according to the supplier's instructions. A459 cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and were split every 3 or 4 days at a time when enough confluence was obtained.

B. Fluorescence imaging with probe SI19

An Ibidi® µ-Slide 8 Well high Glass Bottom plate was seeded with 15 000 cells/well and then maintained at 37 °C in a humidified atmosphere containing 5% CO_2 for 24h to 48h.

*Incubation of A549 Cells with **SI18***

After adequate confluence was obtained, the medium was removed and replaced by 300 µL of 50 µM solution of probe **SI18**, prepared in RPMI medium starting from a stock solution of fluorophore in DMSO (final concentration in DMSO < 1.0%). Please note that no solubility issue was noted. The cells were incubated with the probe SI18 for 2h at 37 °C in a humidified atmosphere containing 5% CO_2. The **SI18** solution was then removed and the cells were washed once with pre-warmed PBS (1X). Finally, RPMI medium was added before imaging.
When washing steps were required, the cells were washed three additional times with pre-warmed PBS (3 x 5 min).

In cell activation with DBCO

After incubation with the compound **SI18** and PBS washing steps, a 250 µM DBCO solution (300 µL with final concentration of DMSO = 1.0%) was added. The cells were incubated for an appropriate period of time at 37 °C in a humidified atmosphere containing 5% CO_2.
When washing steps were required, the DBCO solution was then removed and the cells were washed once with pre-warmed PBS (3 x 5 min). Finally, an appropriate medium was added before imaging.

Protocol for NucSpot® live 610 staining:

The staining of the nucleus was performed following the instructions given by the furnisher. Briefly, The 1X nucleus staining agent NucSpot® live 610 solution was obtained by dilution of the commercial 1000X solution in DMSO supplemented with 50 nm of Verapamil. The cells were then incubated for 30 minutes at 37 °C with the NucSpot® solution. The imaging experiments were then directly conducted without washing step

Wide Field Microscopy

Fluorescence images were acquired using a Nikon Eclipse TE2000-E wide field microscope. Excitation was performed using a super high-pressure Mercury lamp and UV filter was used for blue channel (Leica, 50 mW).

Confocal Microscopy

Fluorescence images were acquired using a Leica SP8-X inverted confocal microscope with a 63× oil immersion objective (HC PL APO CS2 Leica). Excitation was performed using a white laser pulsed at 80MHz set at the desired excitation wavelength or with a Diode 405 nm (Leica, 50 mW). Detection was carried out by using GaAsP Hybrid (Hamamatsu) collecting photons over the appropriate emission wavelength window.

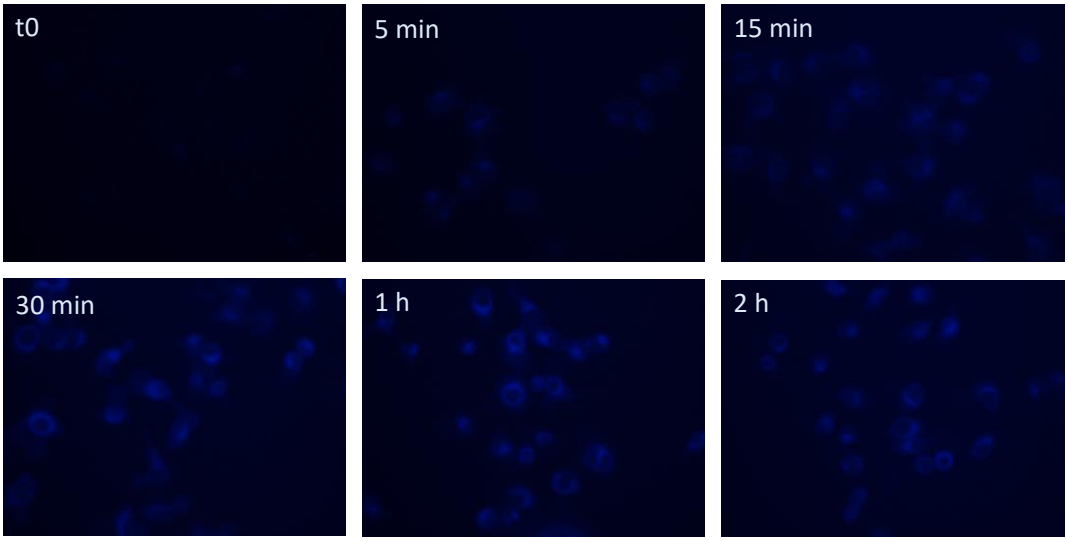


Figure S17: Monitoring of turn-ON over time. Wide field microscopy of live A549 cells treated with probe **SI18** at 50 µM and DBCO 250 µM. Images were recorded using the blue channel at different time and different spots using X40 objective.

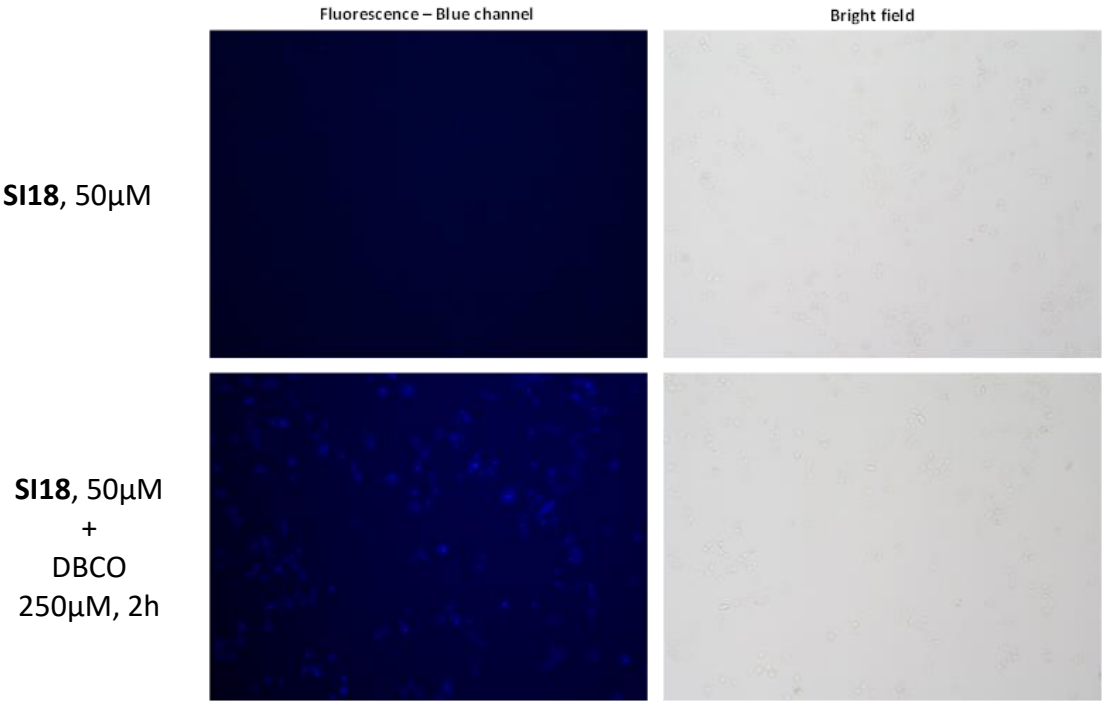


Figure S18: Visualization of Turn-ON effect. Wide field microscopy of live A549 cells treated with probe **SI18** at 50 µM (top line) and after treatment for 2 h with DBCO 250 µM (bottom line). Images were recorded using the blue channel (left) and bright field (right) using X10 objective.

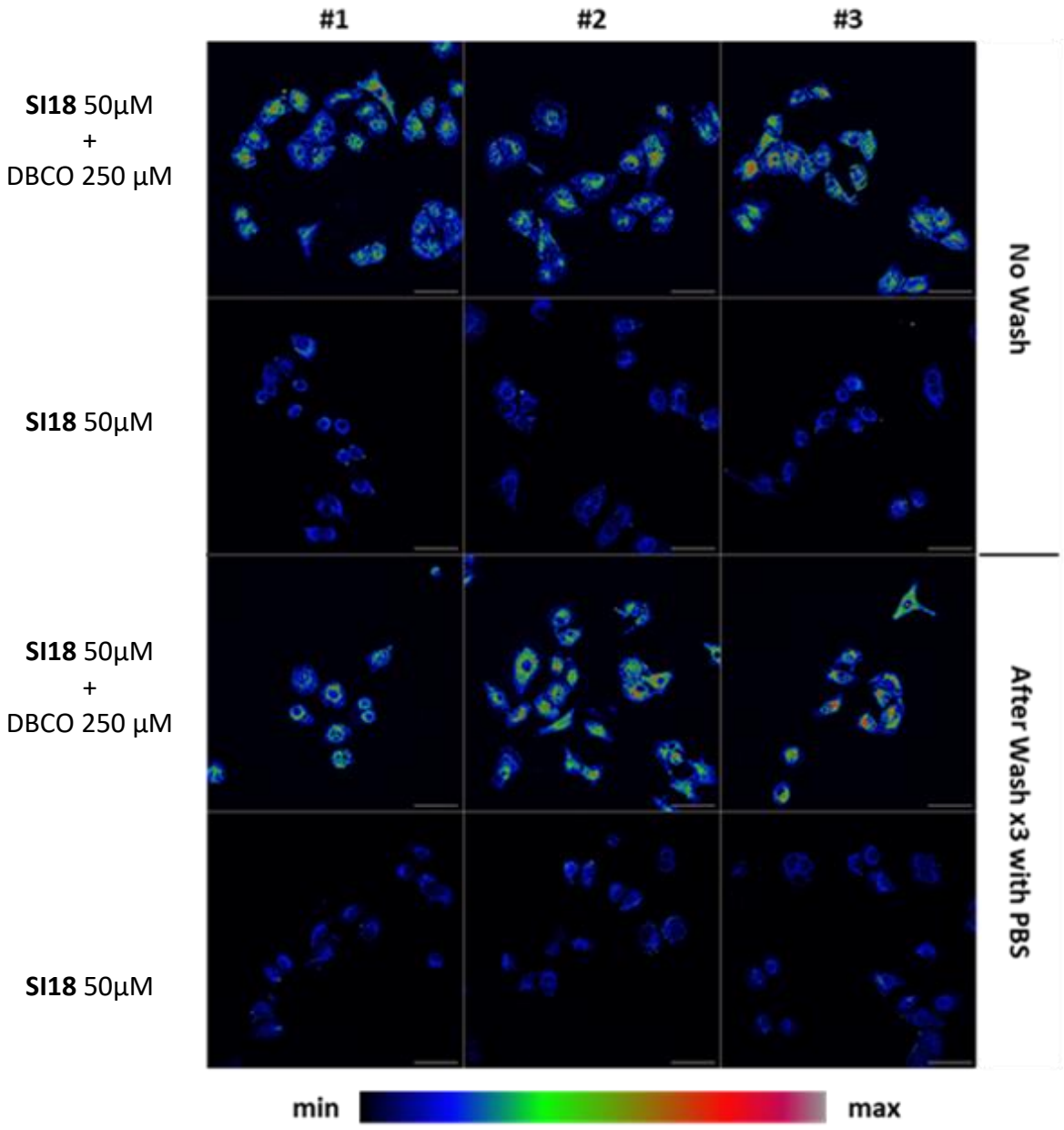
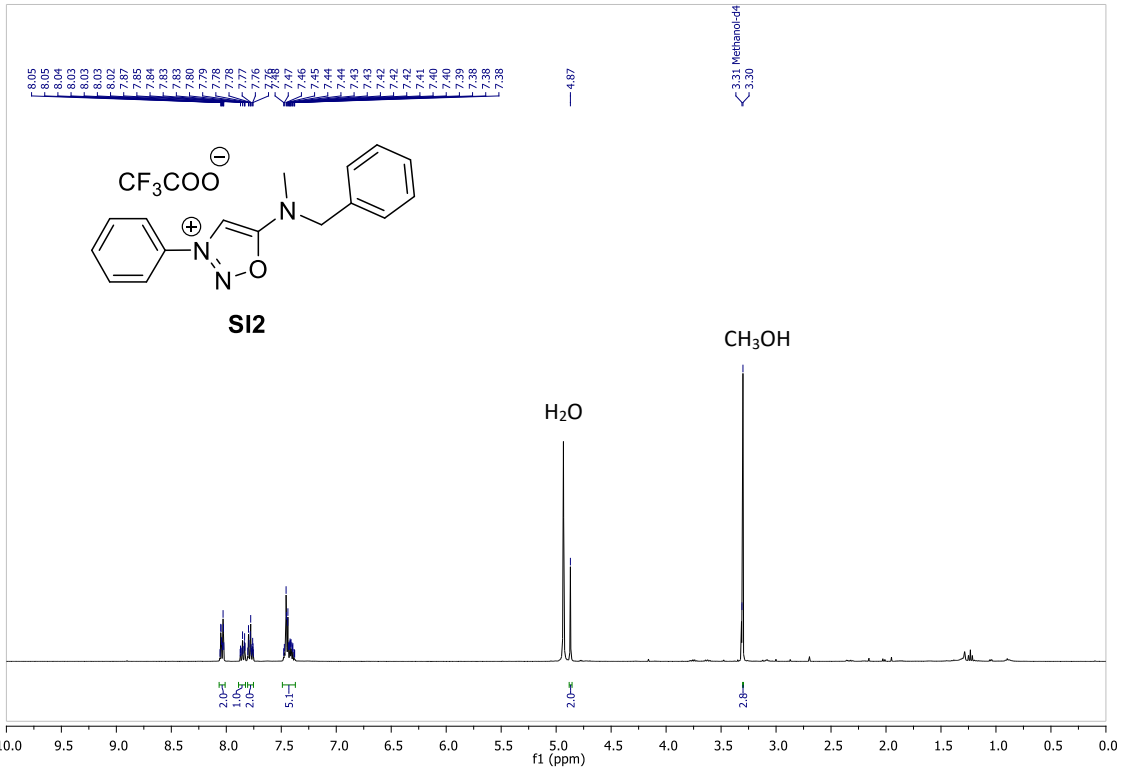


Figure S19: **Influence of PBS washing steps**. Confocal microscopy images of live A549 cancer cell treated **SI18** at 50 µM at 37 °C with our without treatment with DBCO (250µM) using a 63× oil immersion objective. λExc: 405 nm, λEm: 450 to 600 nm. Scale Bar 50 µm.

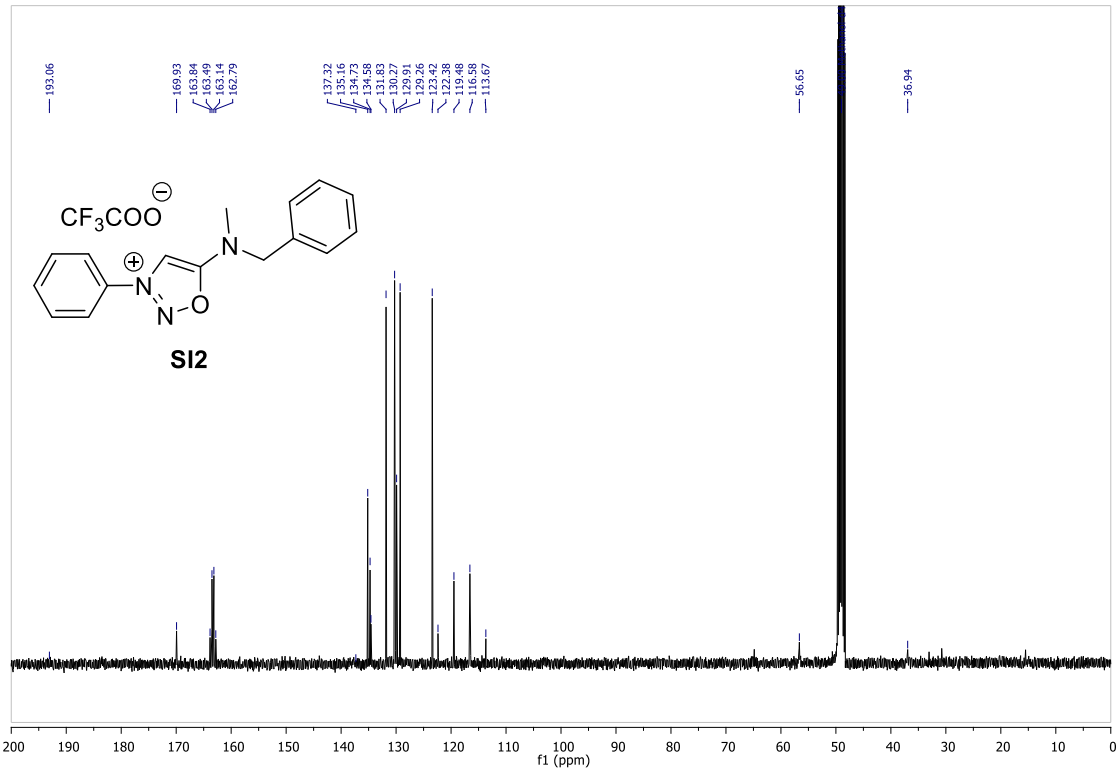
V. NMR Spectra

NMR spectra of compounds **SI1**, **5**, **11**, **17** and **18** were in accordance with literature.[14]

^{1}H NMR compound **SI2** (400 MHz, CD_3OD)

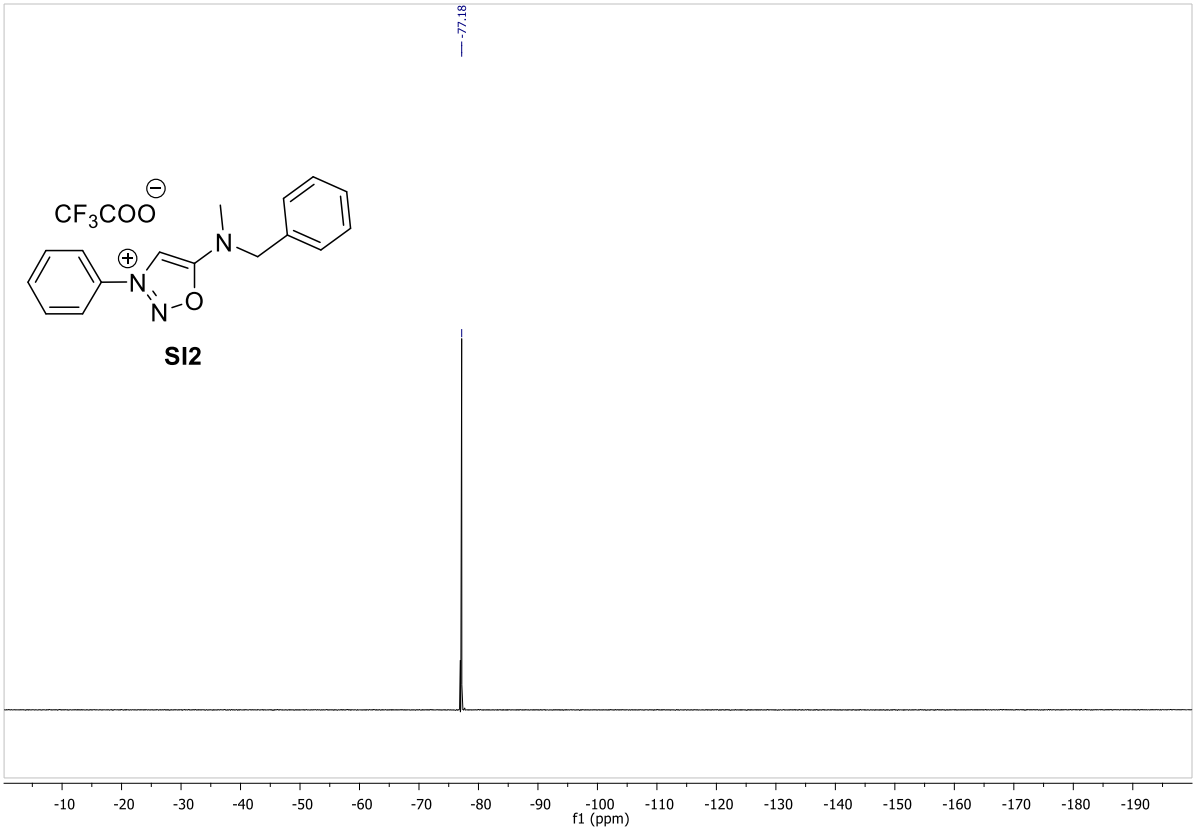


^{13}C NMR compound **SI2** (100 MHz, CD_3OD)

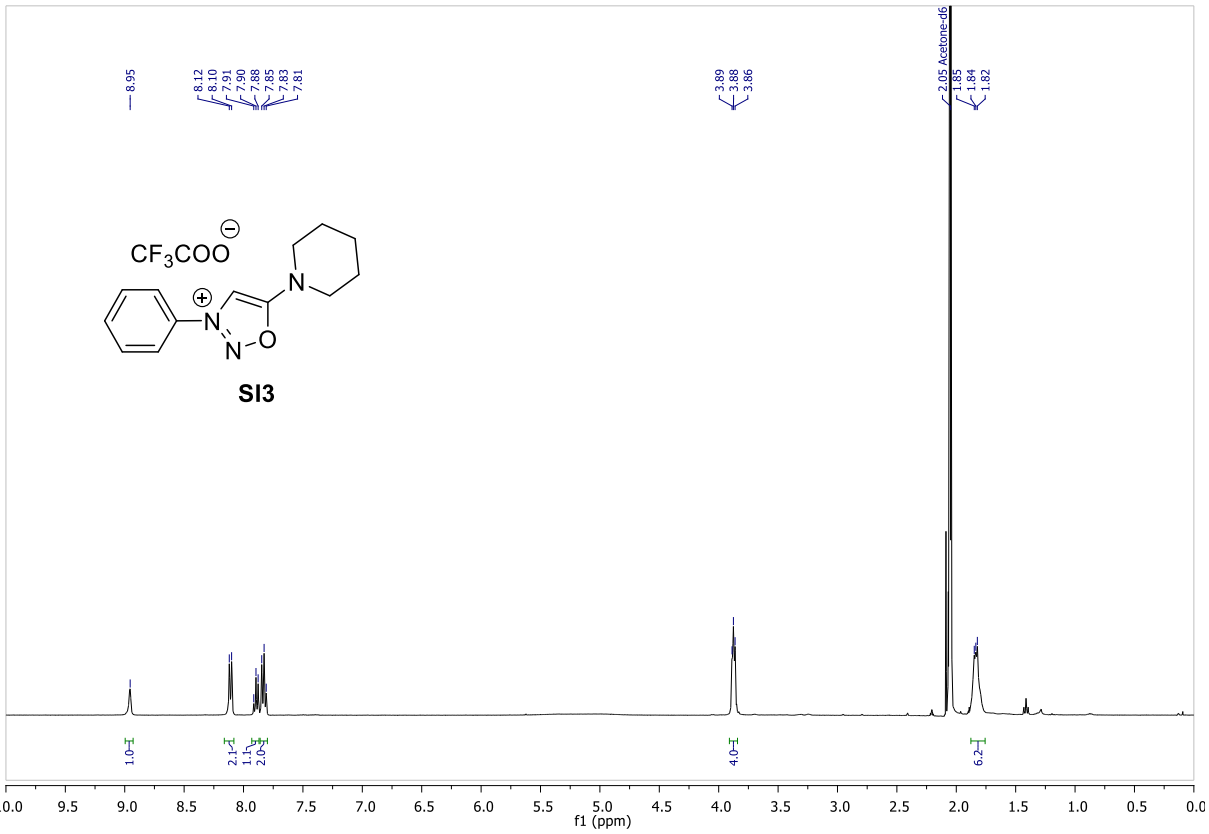


[14] M. Ribéraud, K. Porte, A. Chevalier, L. Madegard, A. Rachet, A.Delaunay-Moisan, F. Vinchon, P. Thuéry, G. Chiappetta, P. Alexandre Champagne, G. Pieters, D. Audisio, F. Taran. *J. Am. Chem. Soc.* **2023,** *145*, 4, 2219-2229.

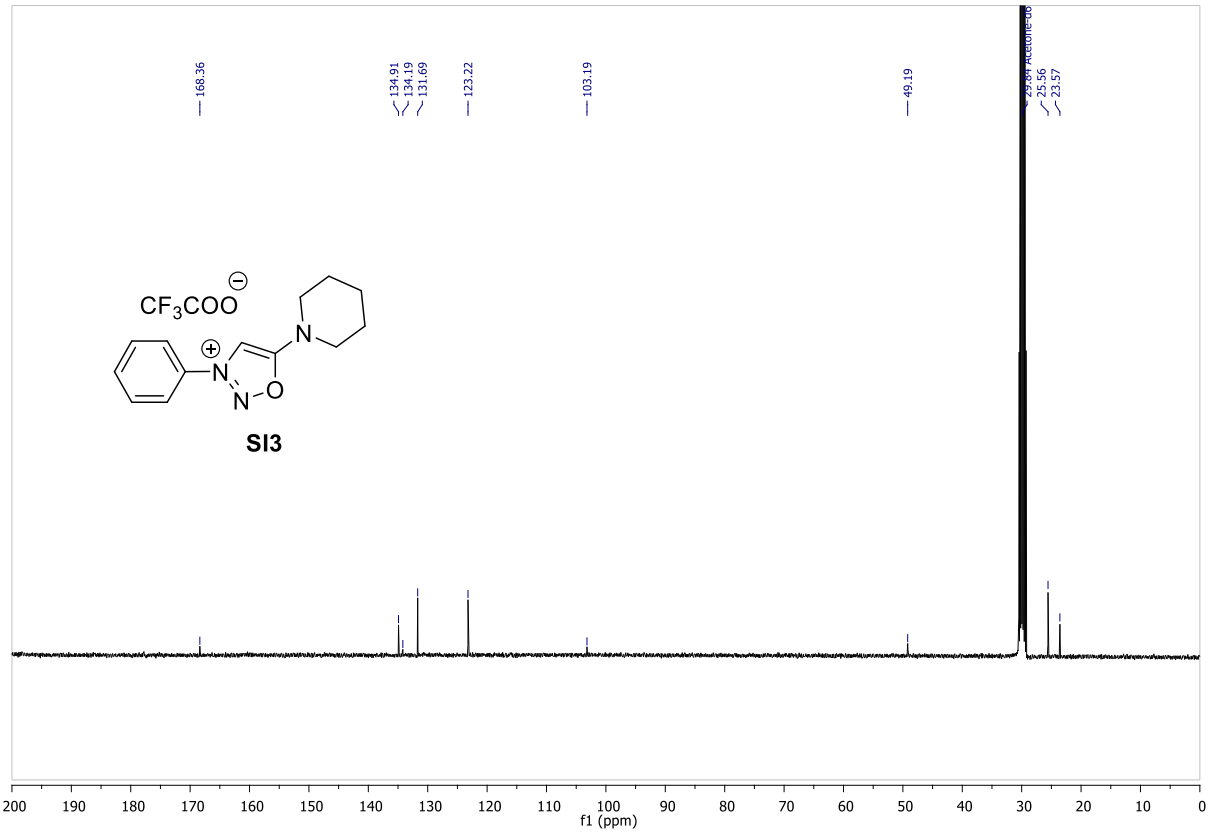
^{19}F NMR compound **SI2** (376 MHz, CD_3OD)



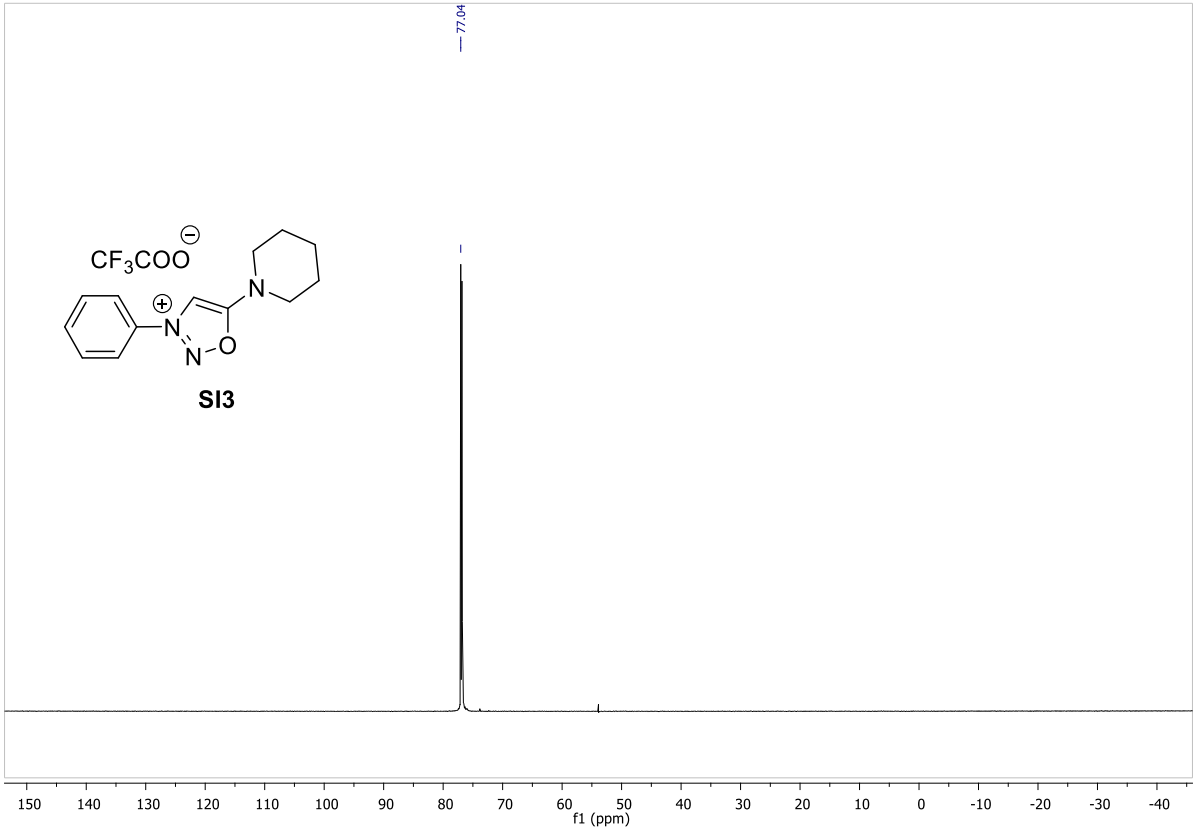
^{1}H NMR compound **SI3** (400 MHz, Acetone-d_6)



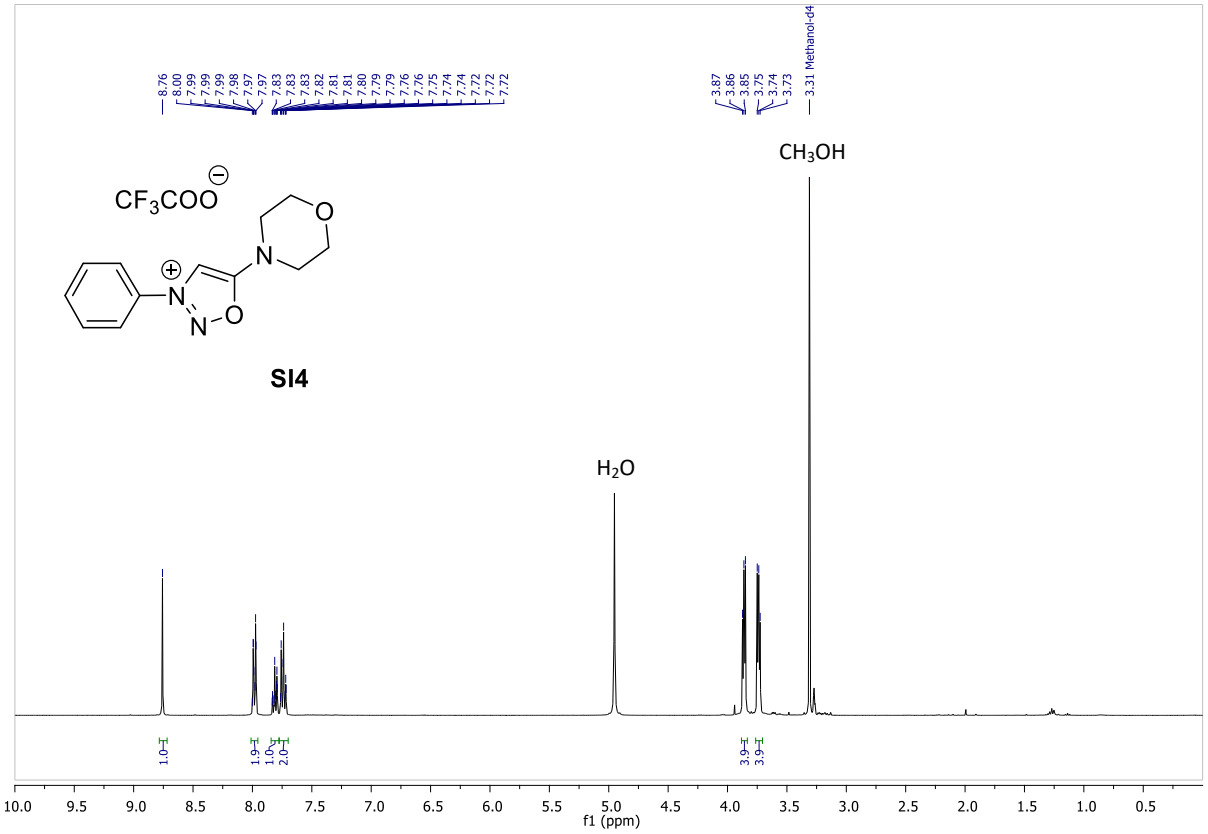
^{13}C NMR compound **SI3** (100 MHz, Acetone-d_6)



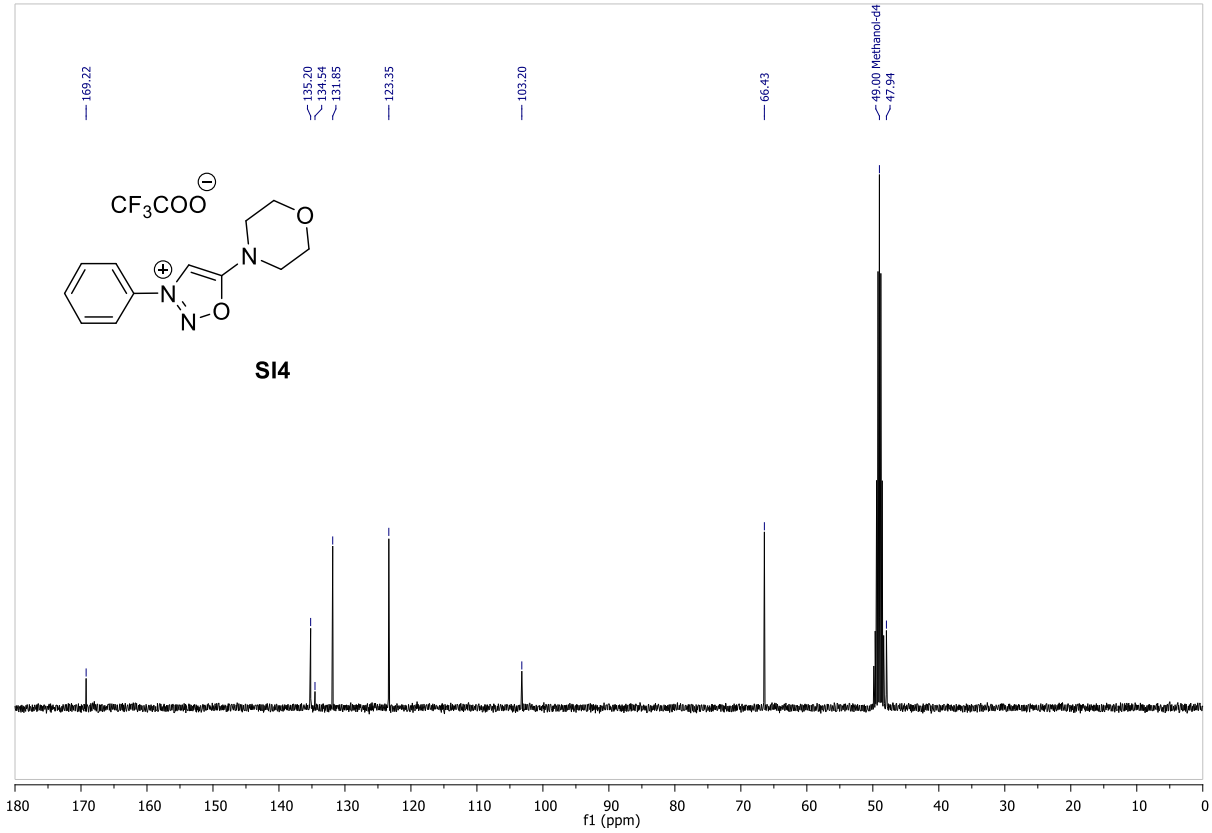
^{19}F NMR compound **SI3** (376 MHz, Acetone-d_6)



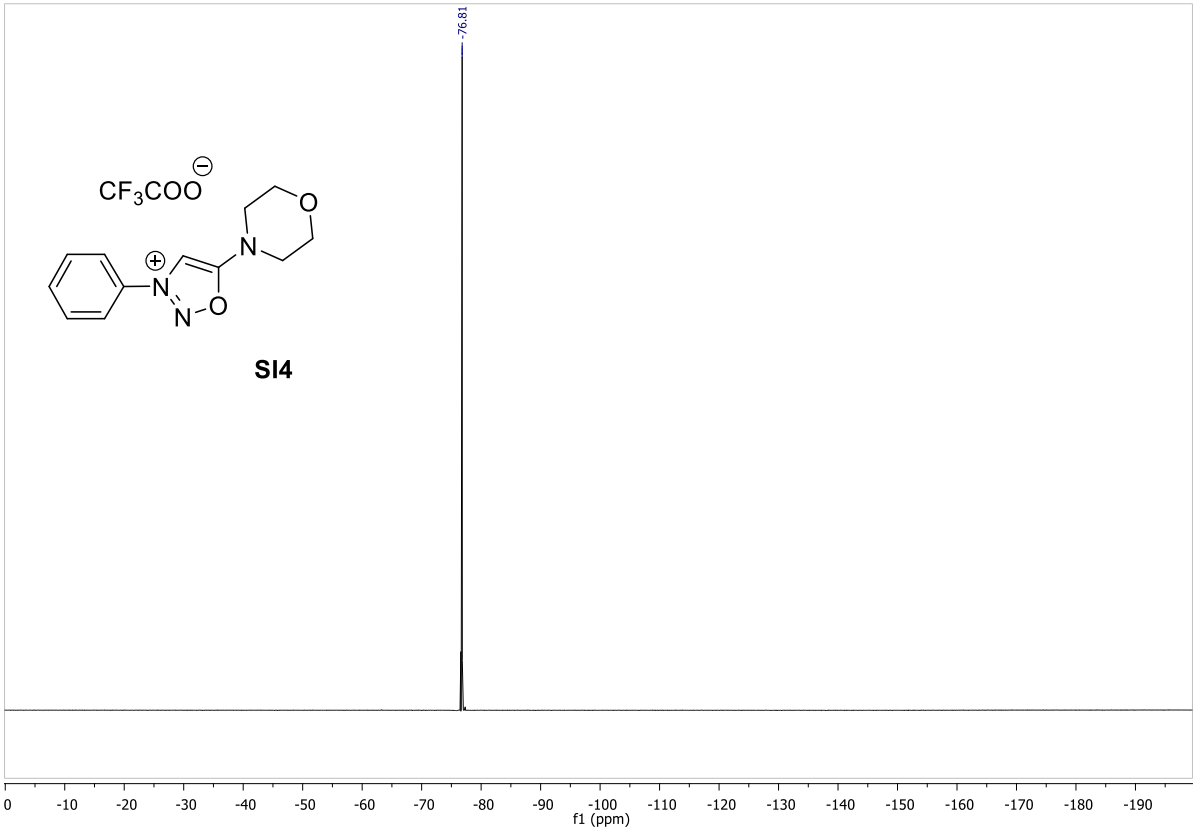
^{1}H NMR compound **SI4** (400 MHz, CD_3OD)



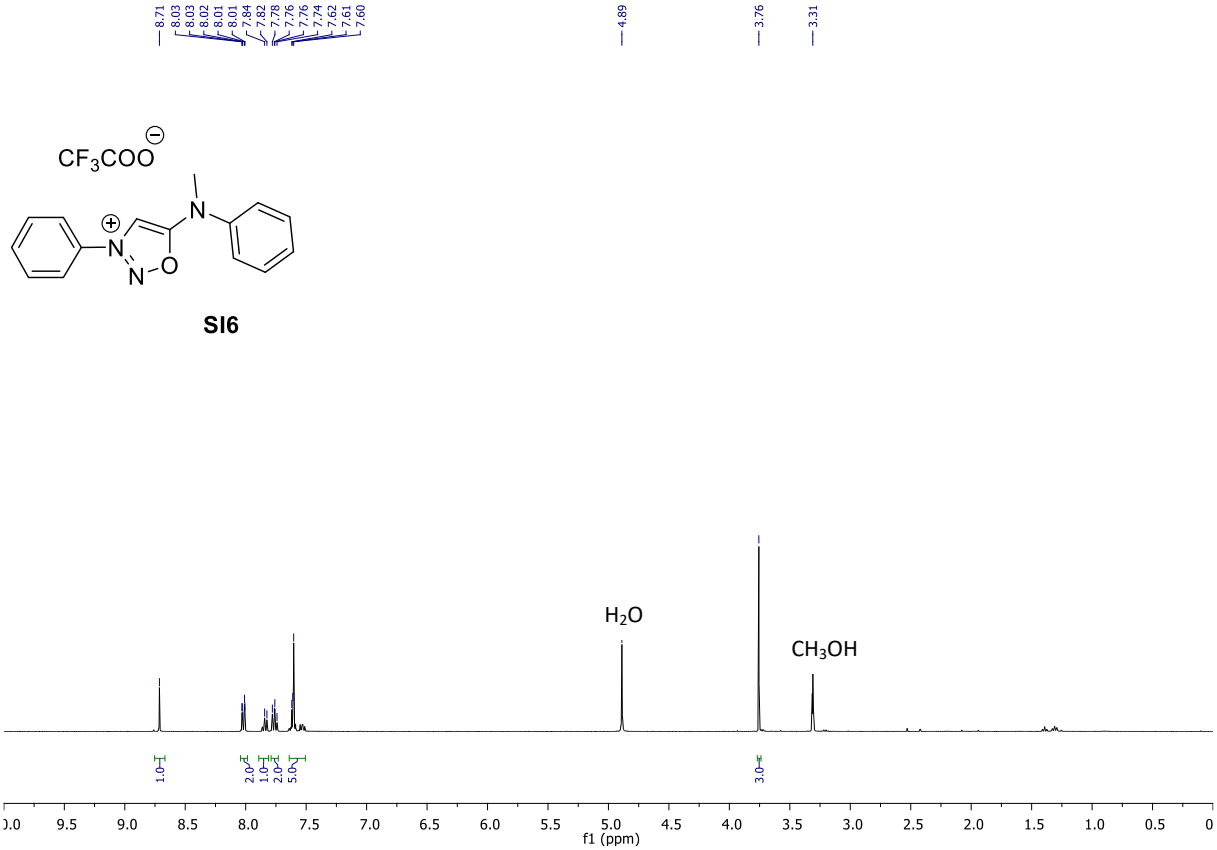
^{13}C NMR compound **SI4** (100 MHz, CD_3OD)



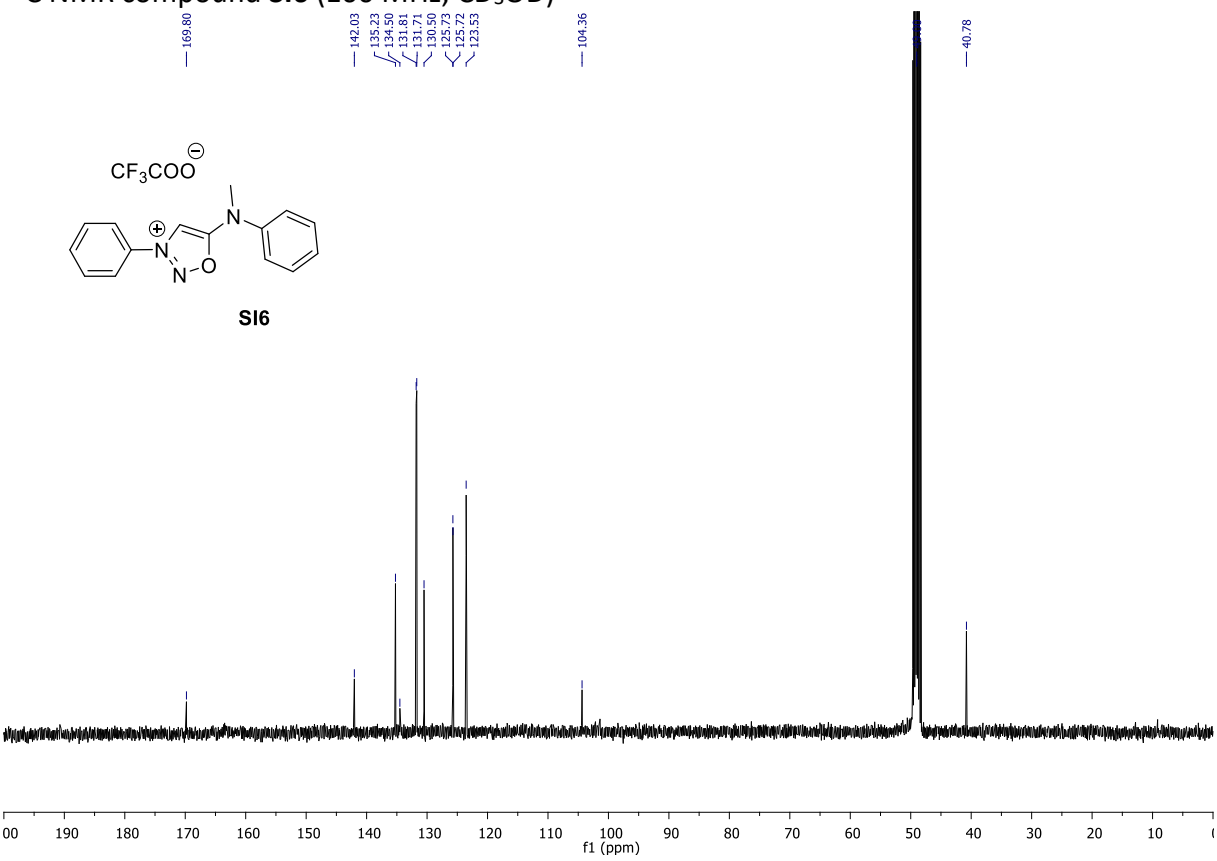
^{19}F NMR compound **SI4** (376 MHz, CD_3OD)



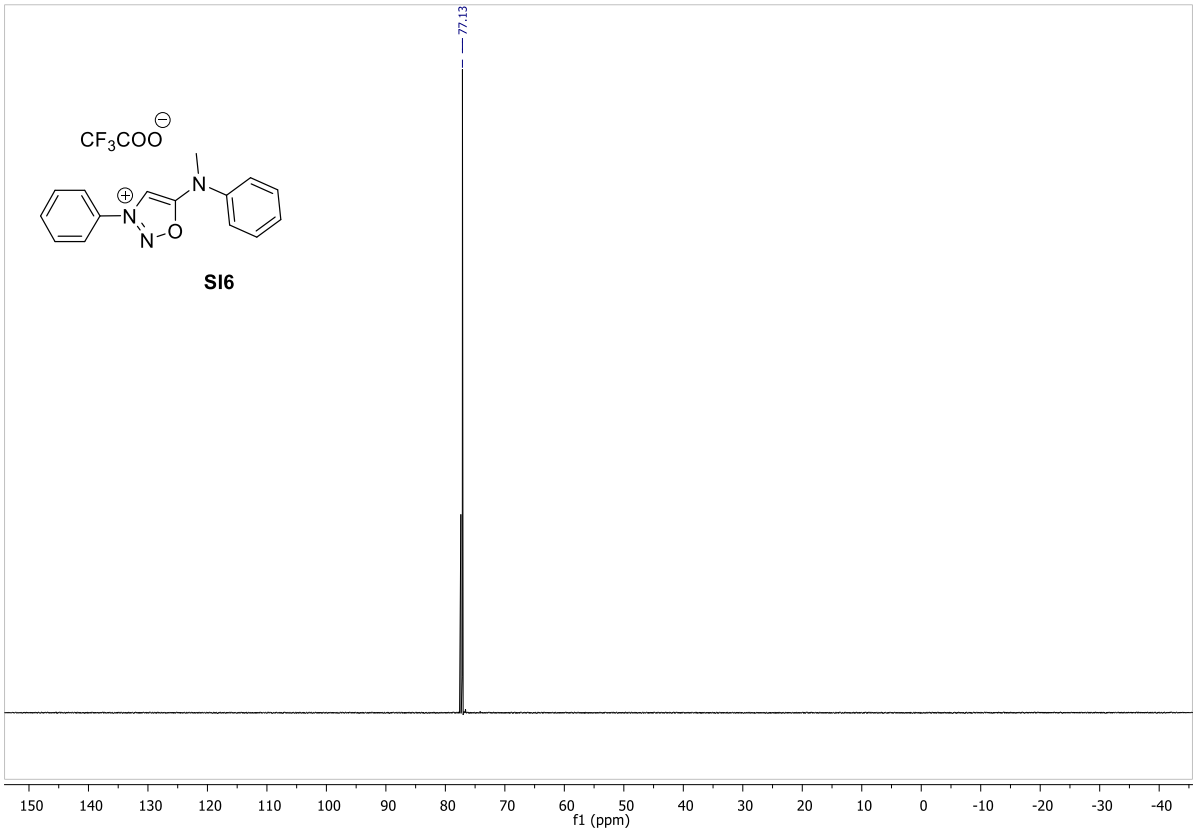
^{1}H NMR compound **SI6** (400 MHz, CD_3OD)



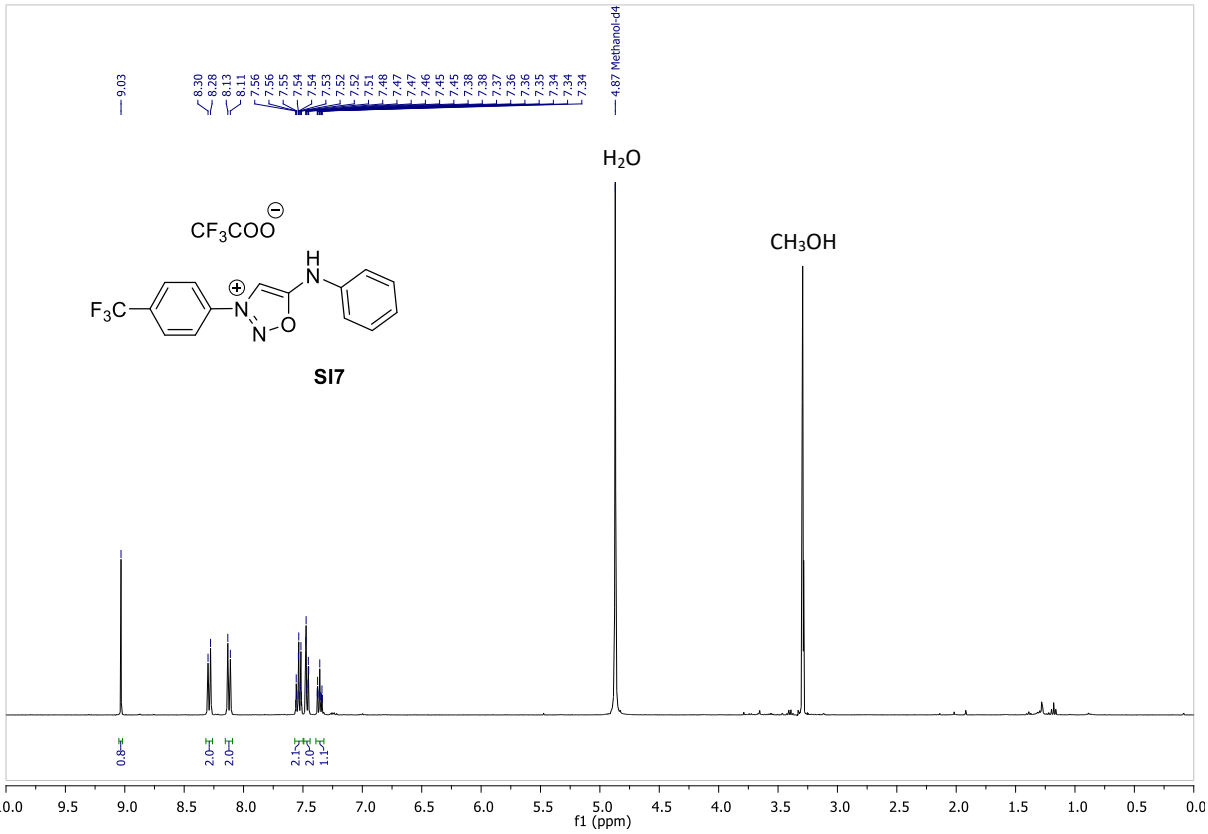
^{13}C NMR compound **SI6** (100 MHz, CD_3OD)



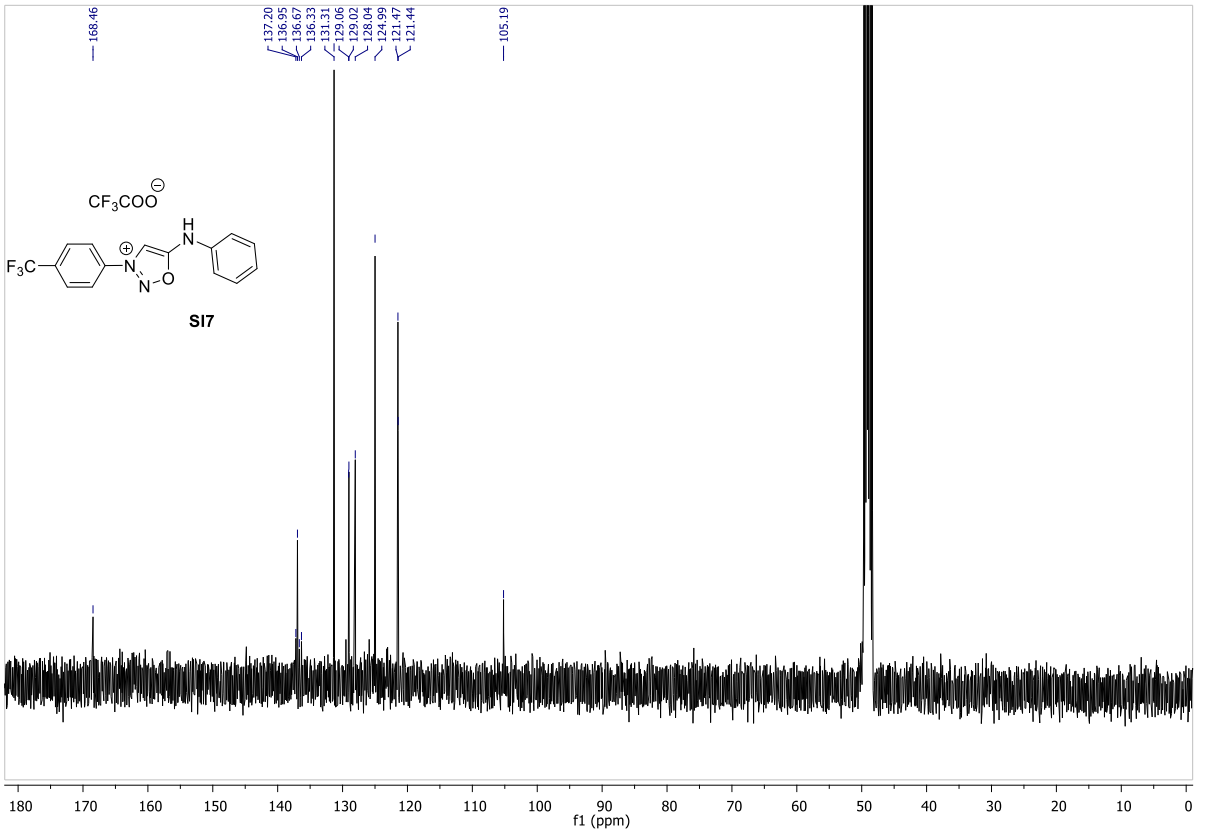
^{19}F NMR compound **SI6** (CD_3OD)



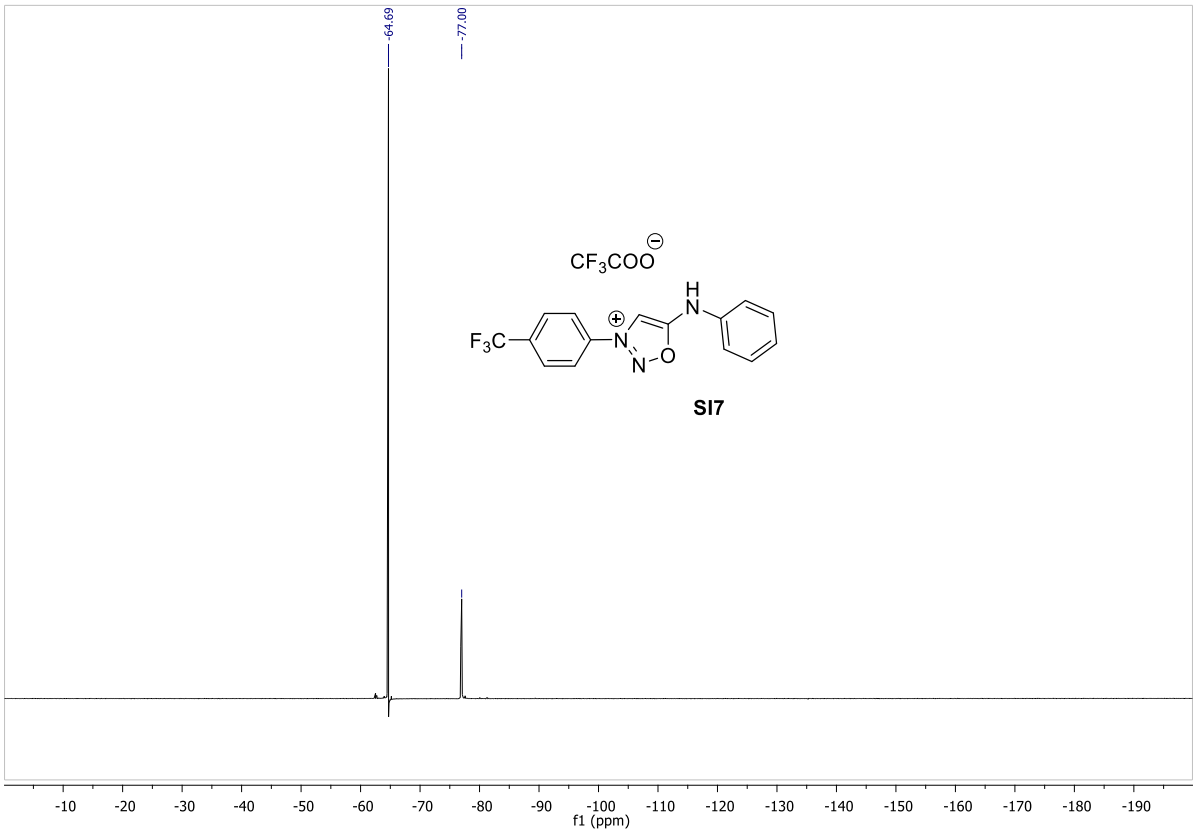
^{1}H NMR compound **SI7** (400 MHz, CD_3OD)



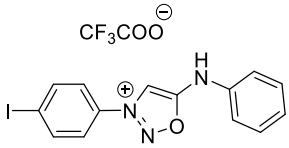
^{13}C NMR compound **SI7** (100 MHz, CD_3OD)



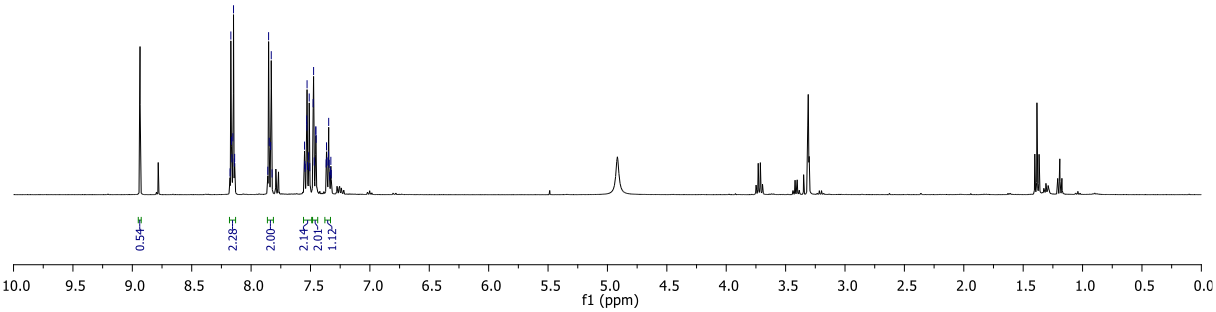
^{19}F NMR compound **SI7** (376 MHz, CD_3OD)



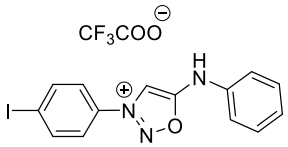
^{1}H NMR compound **SI8** (400 MHz, CD_3OD)



SI8

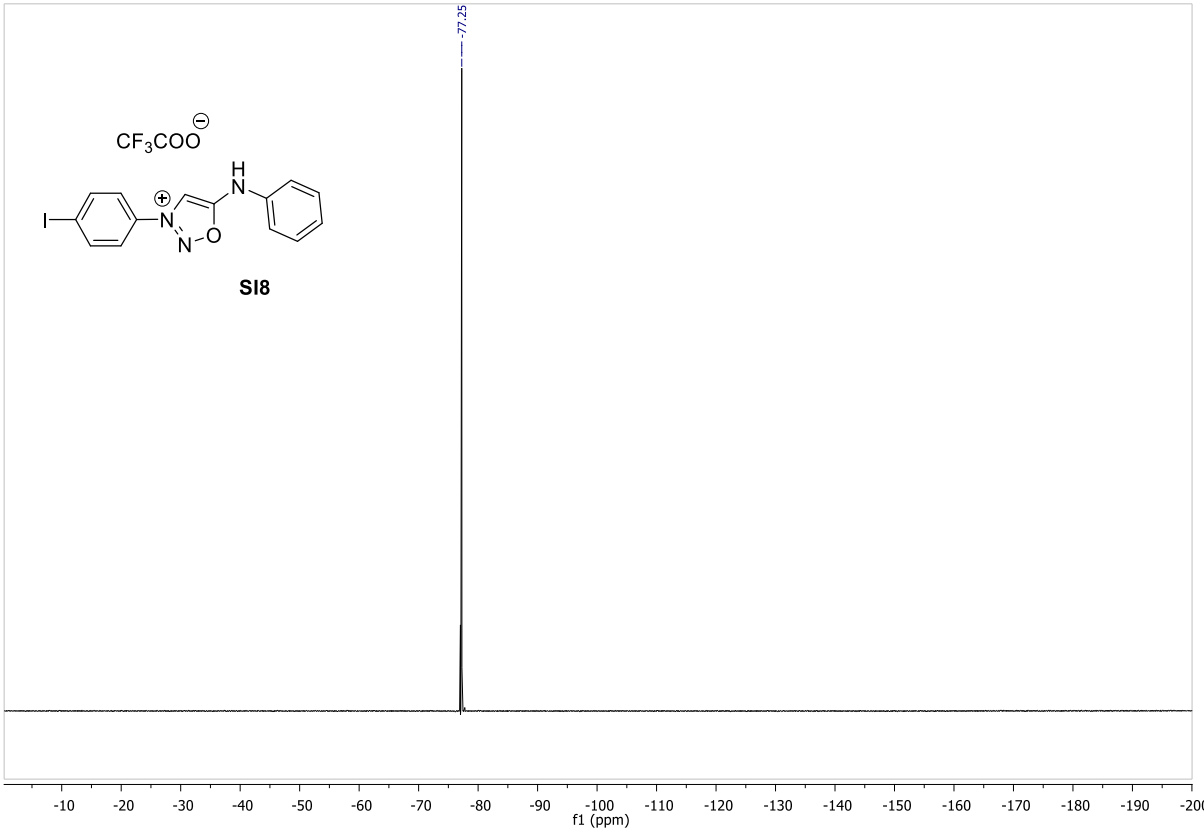


^{13}C NMR compound **SI8** (100 MHz, CD_3OD)

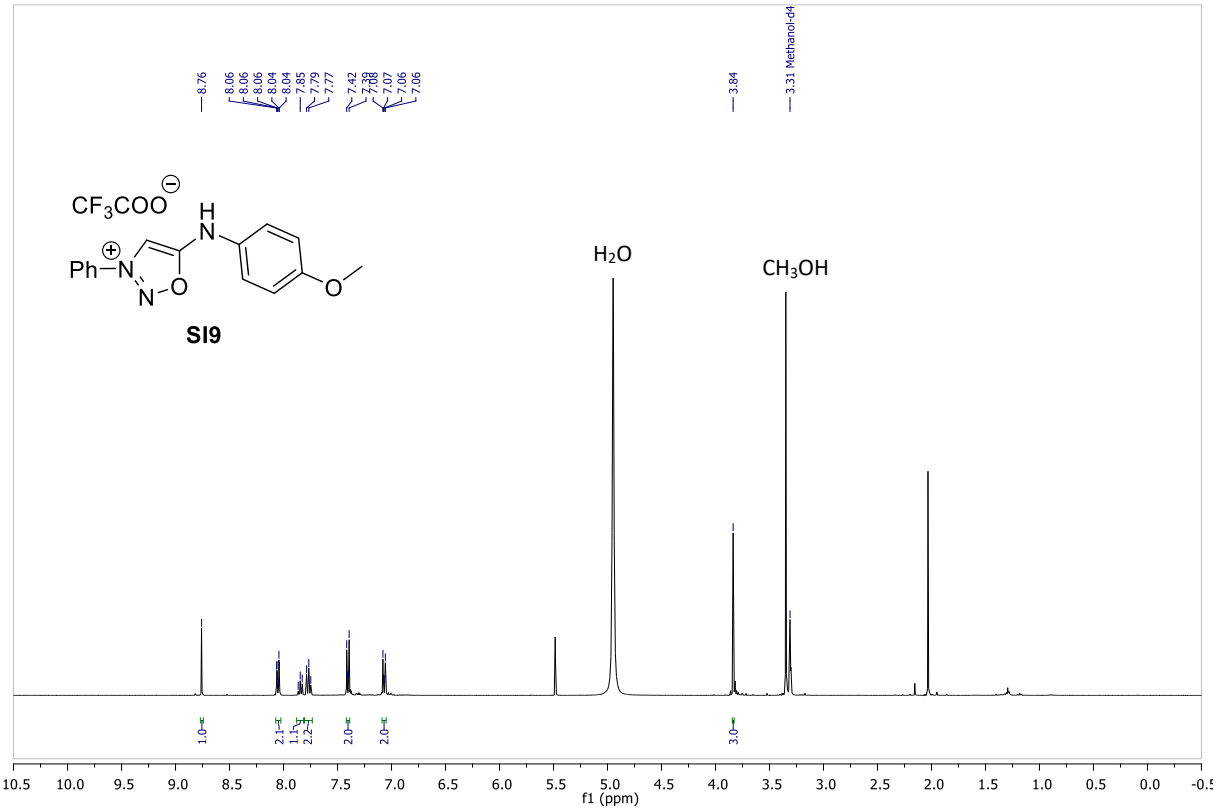


SI8

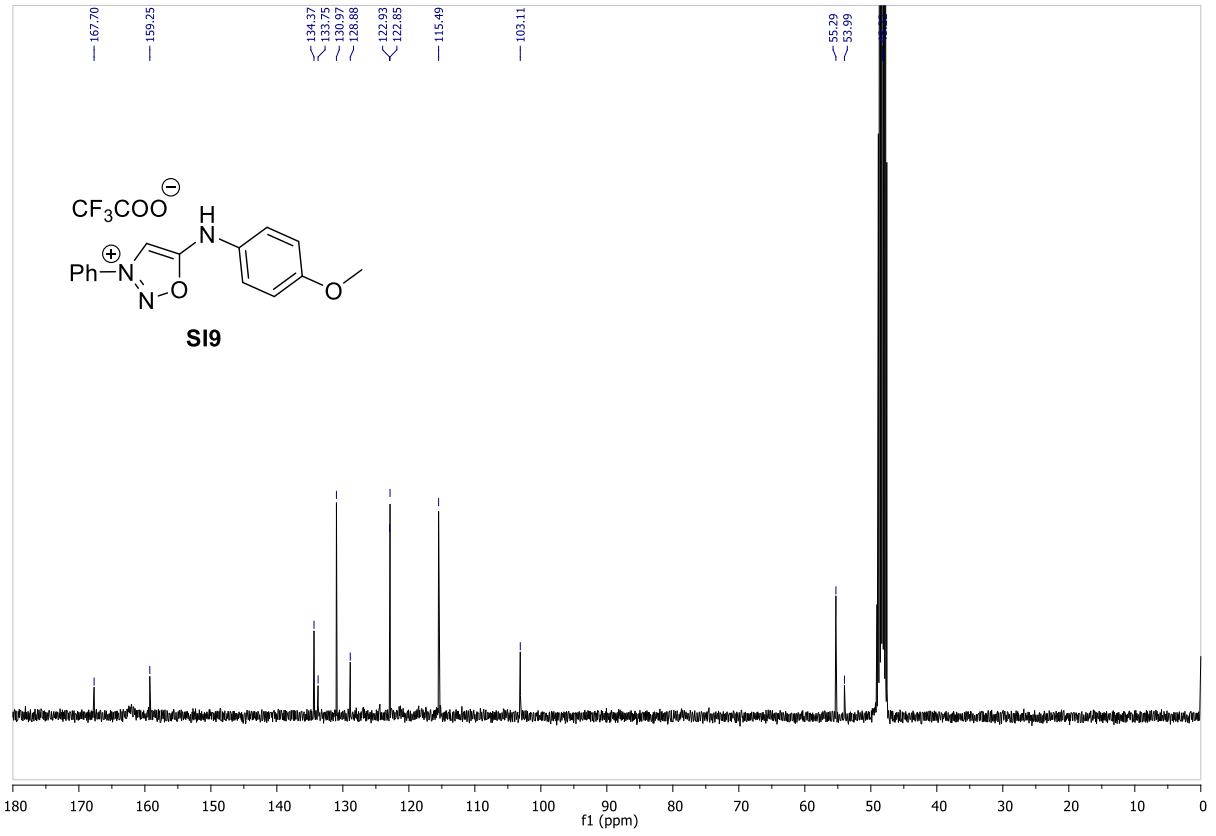
^{19}F NMR compound **SI8** (300 MHz, CD_3OD)



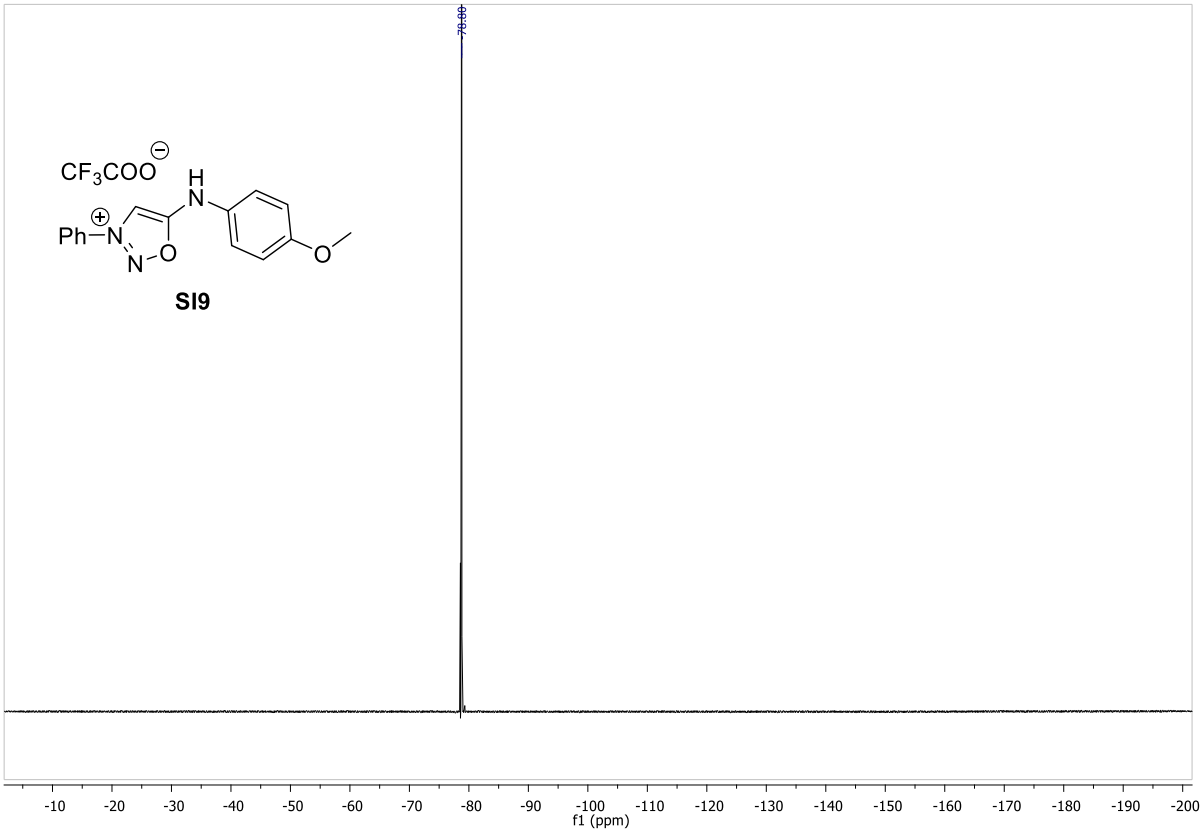
^{1}H NMR compound **SI9** (400 MHz, CD_3OD)



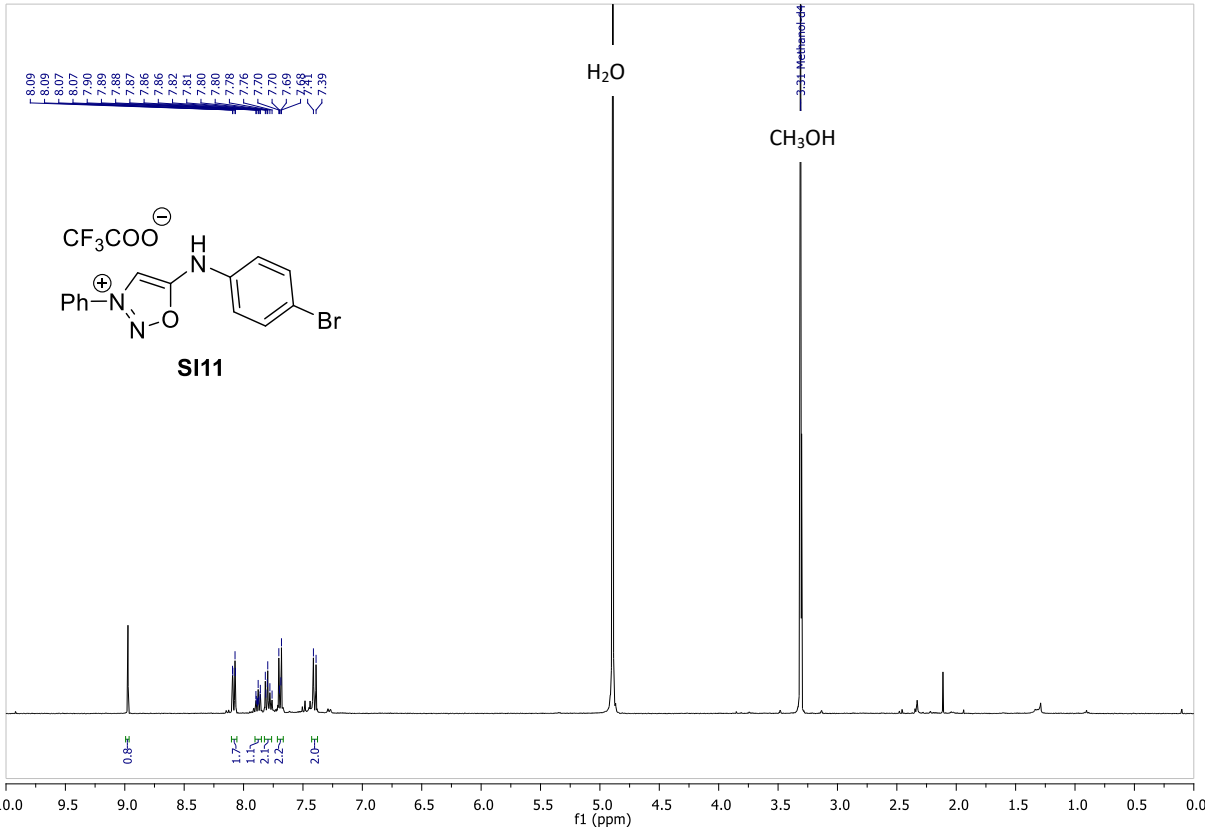
^{13}C NMR compound **SI9** (100 MHz, CD_3OD)



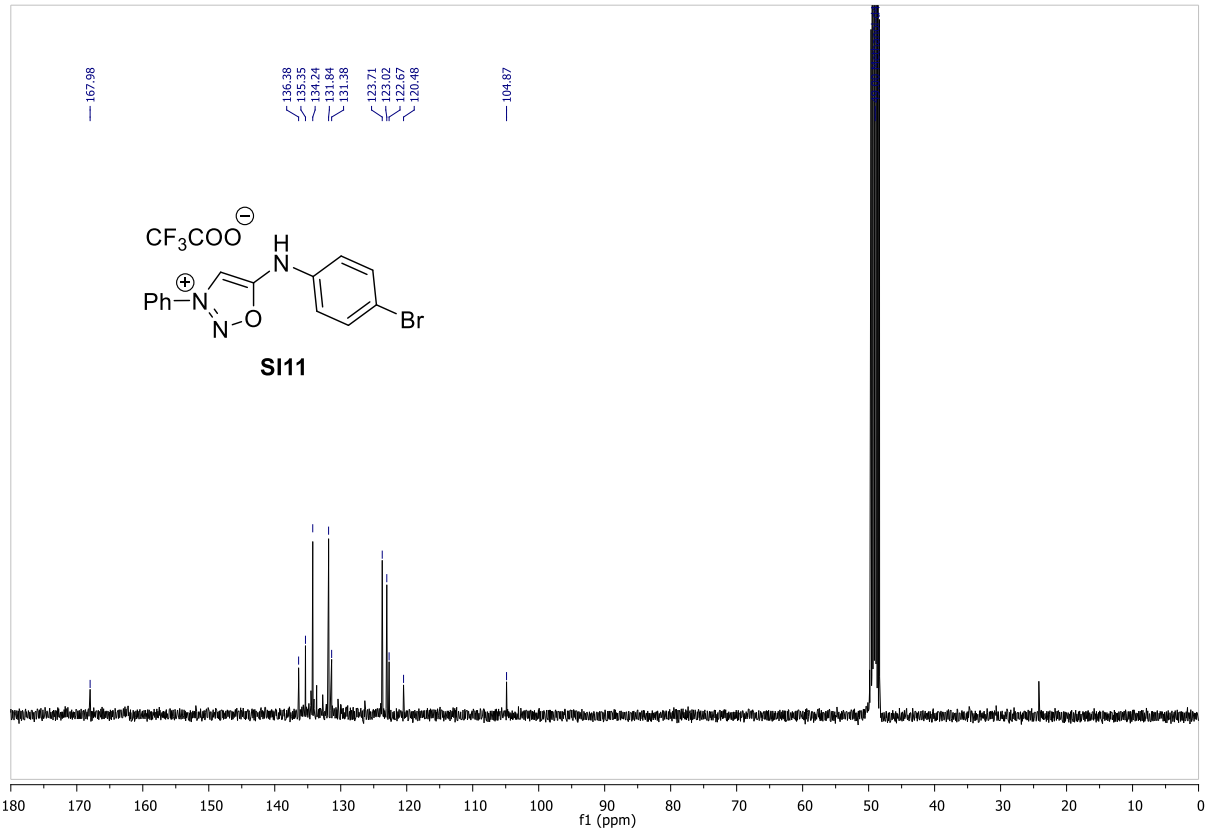
^{19}F NMR compound **SI9** (376 MHz, CD_3OD)



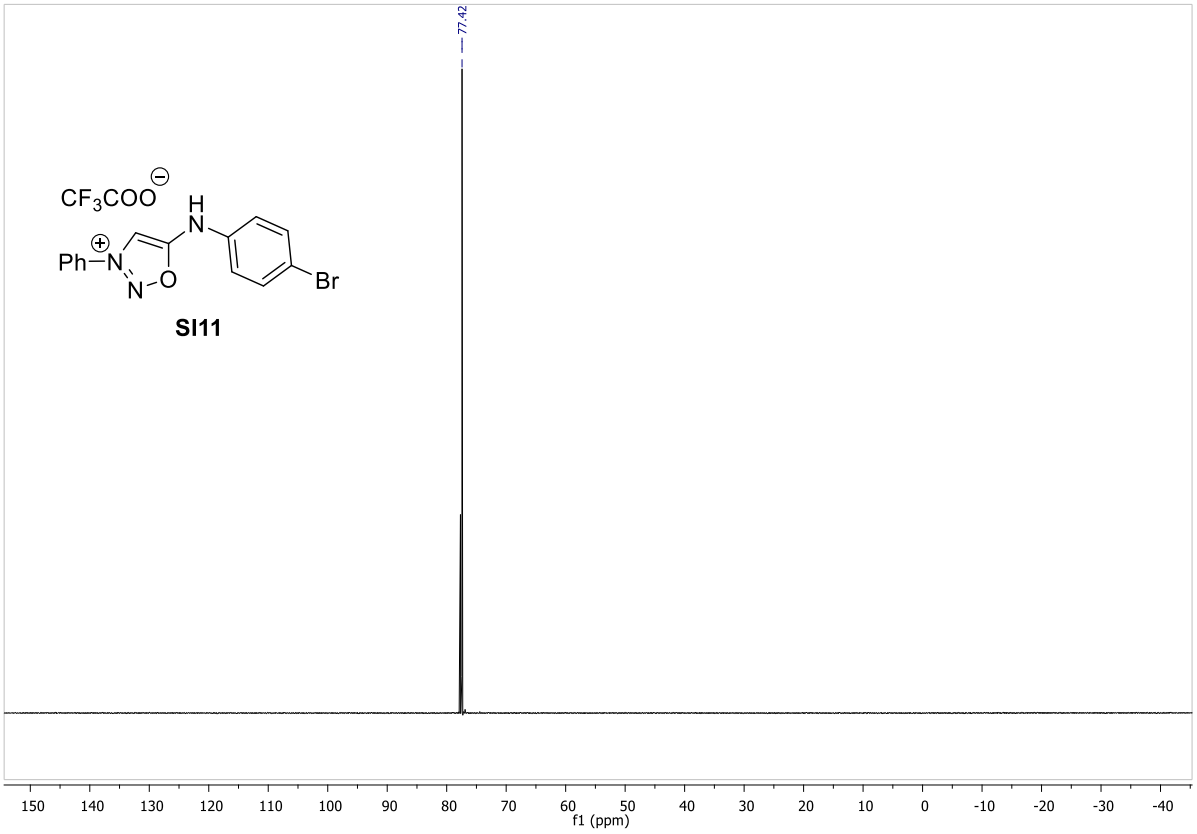
^{1}H NMR compound **SI11** (400 MHz, CD_3OD)



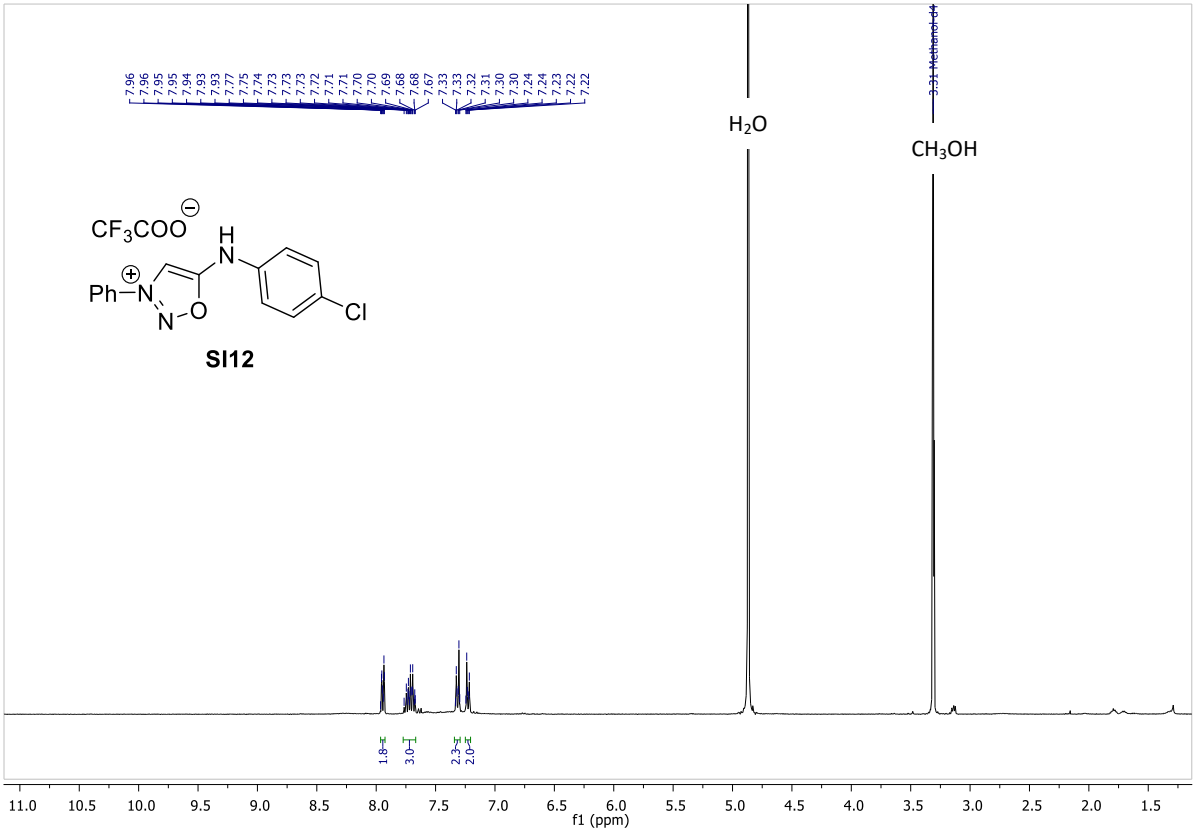
^{13}C NMR compound **SI11** (100 MHz, CD_3OD)



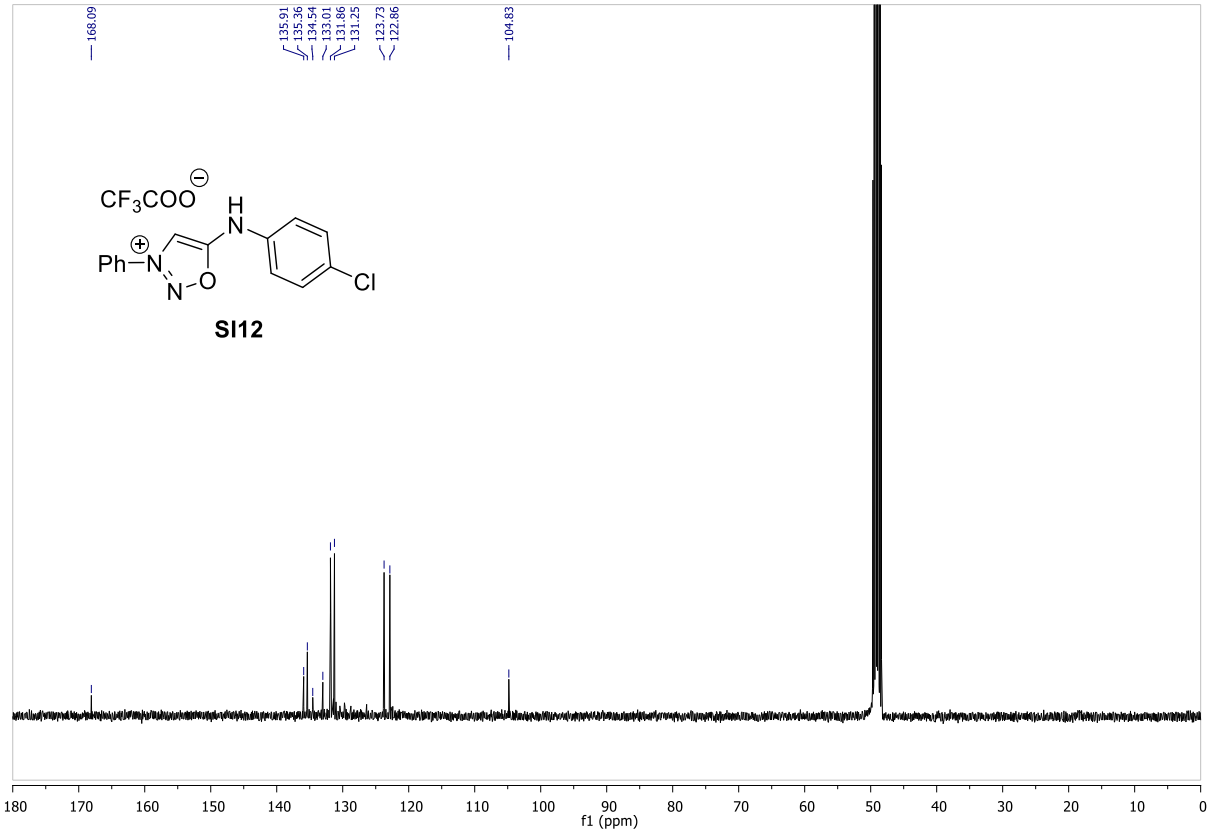
^{19}F NMR compound **SI11** (CD_3OD)



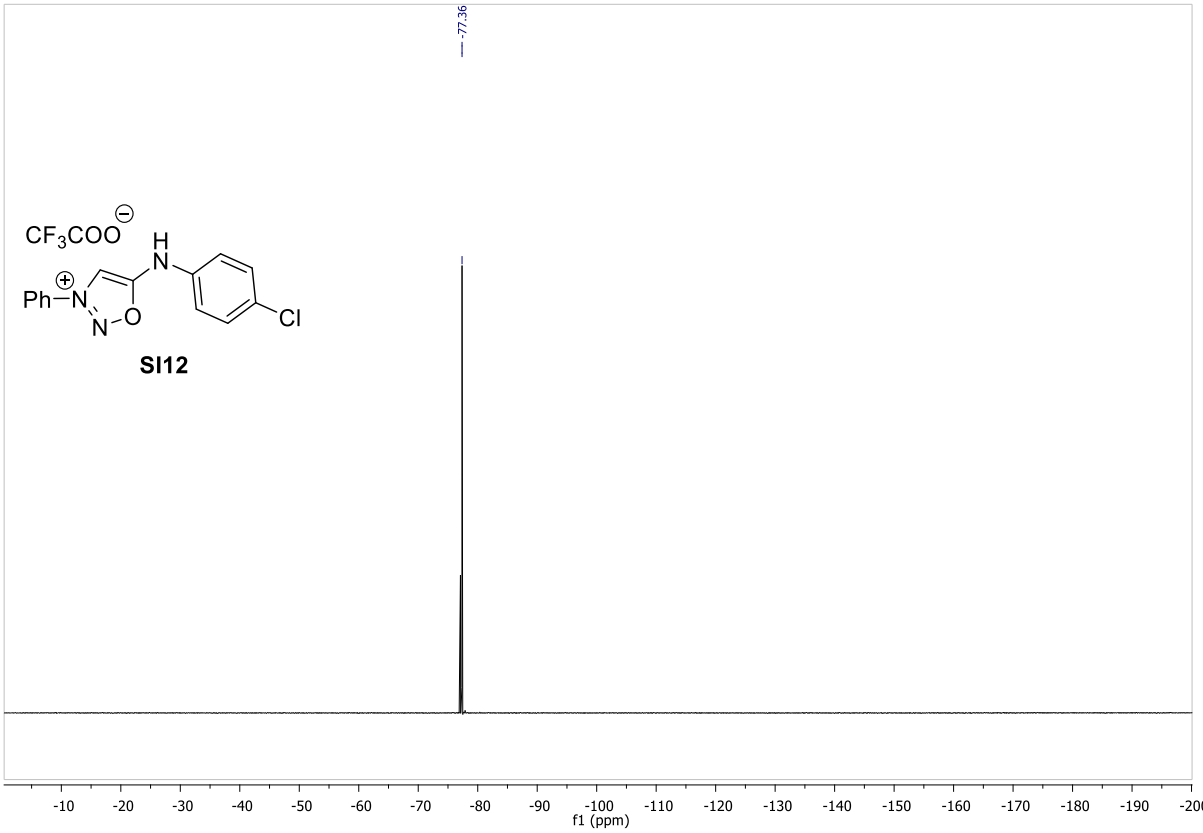
^{1}H NMR compound **SI12** (400 MHz, CD_3OD)



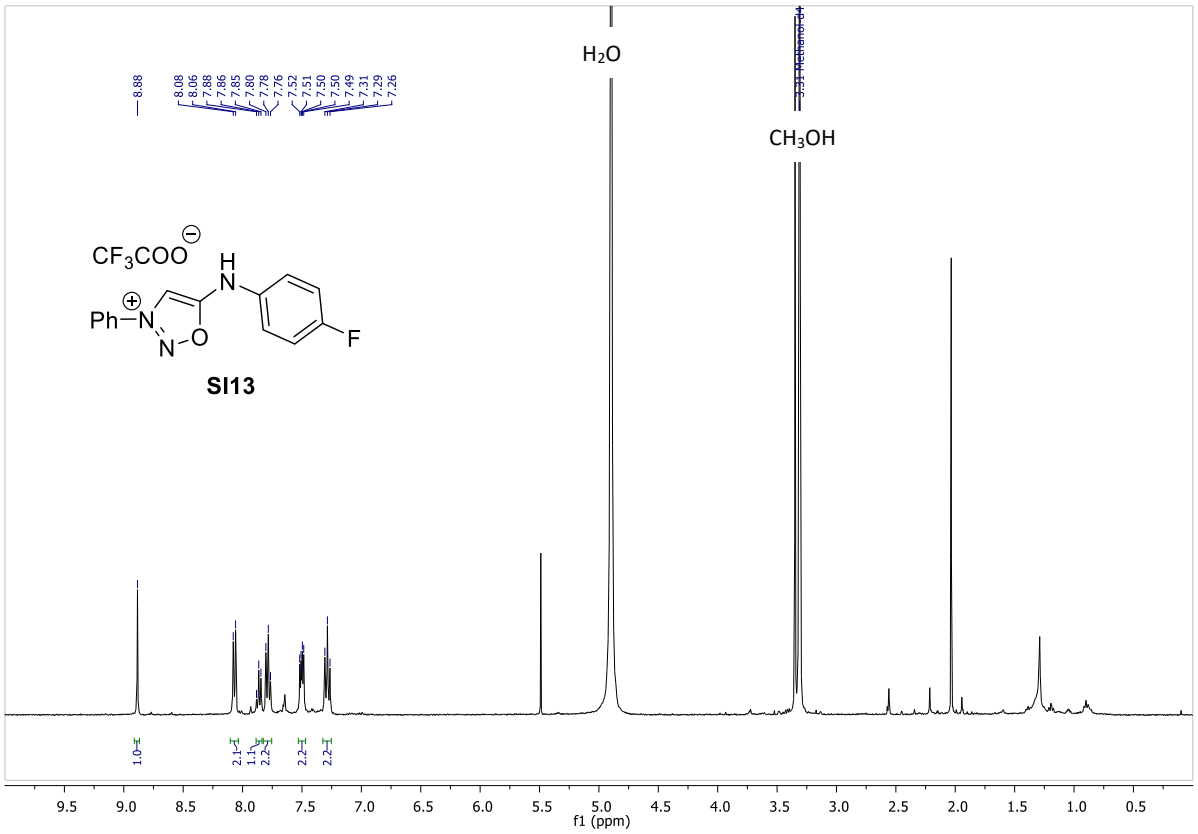
^{13}C NMR compound **SI12** (100 MHz, CD_3OD)



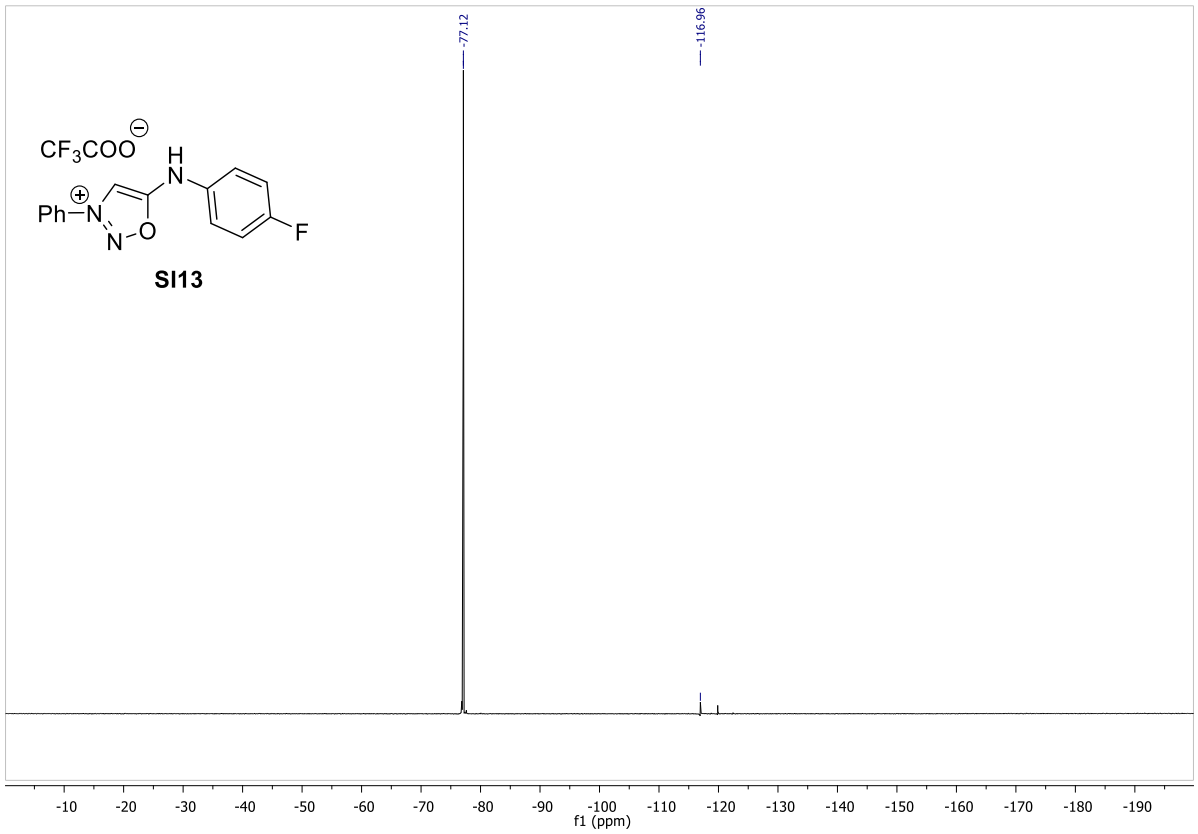
^{19}F NMR compound **SI12** (376 MHz, CD_3OD)



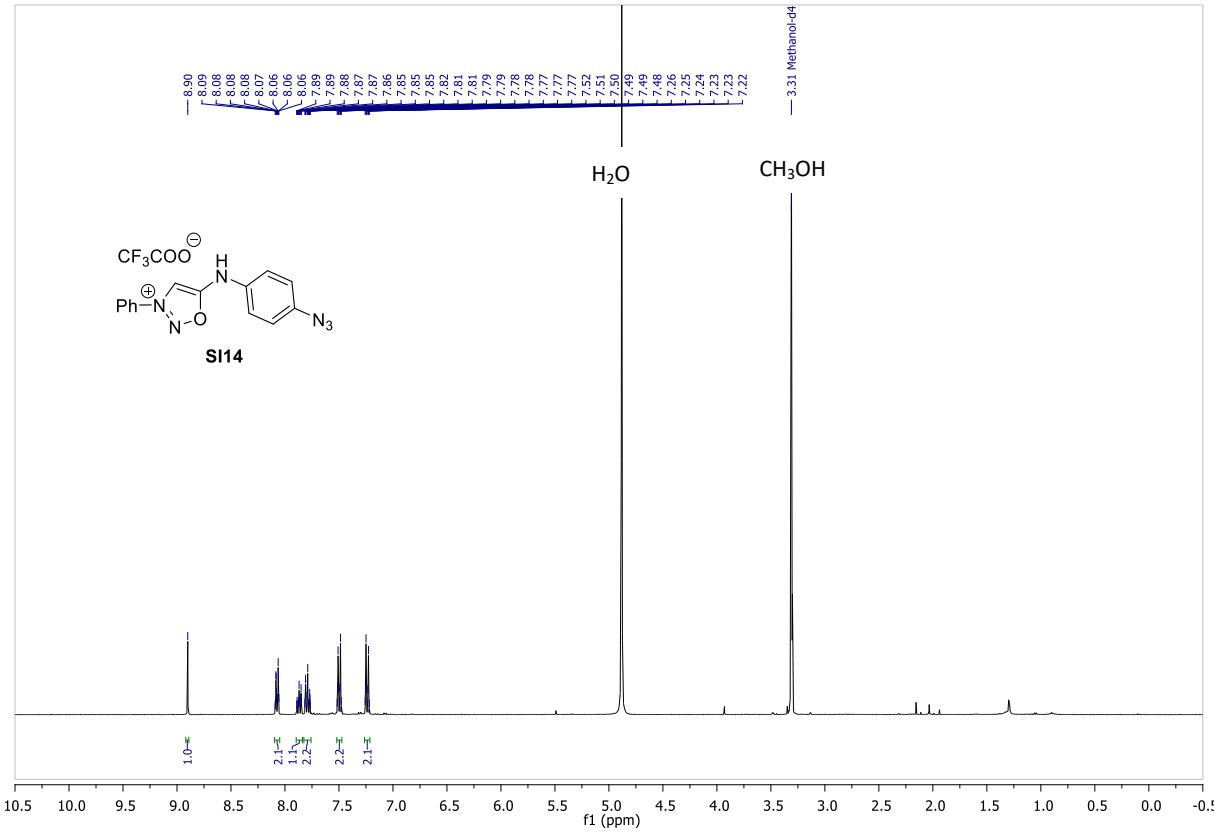
^{1}H NMR compound **SI13** (400 MHz, CD_3OD)



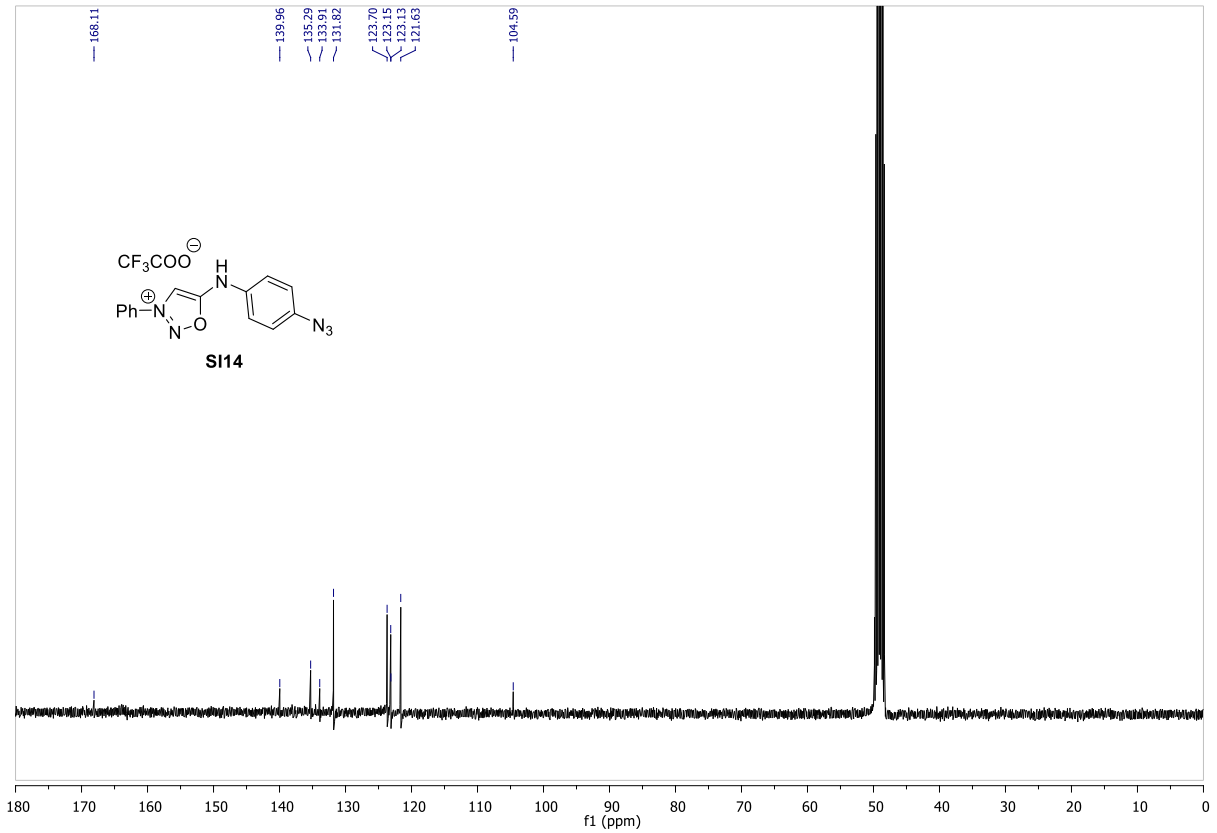
^{19}F NMR compound **SI13** (376 MHz, CD_3OD)



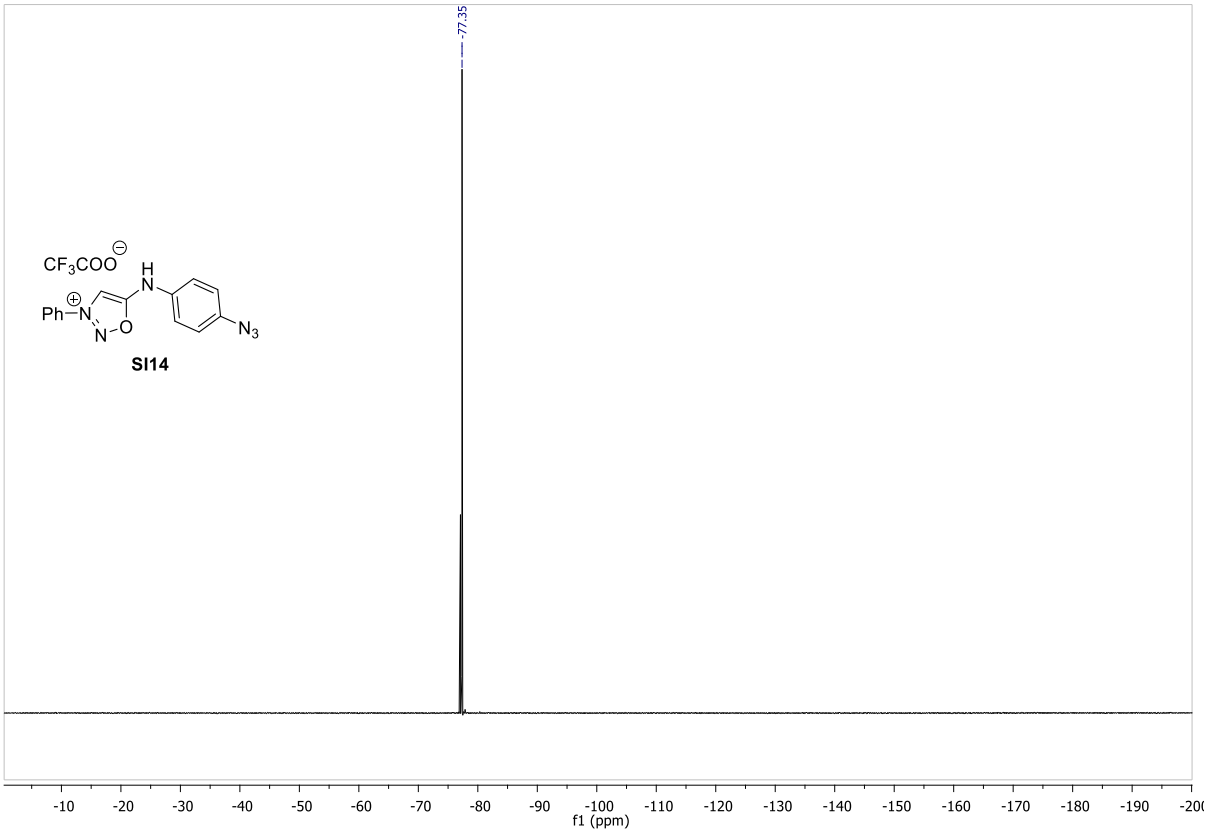
^{1}H NMR compound **SI14** (400 MHz, CD_3OD)



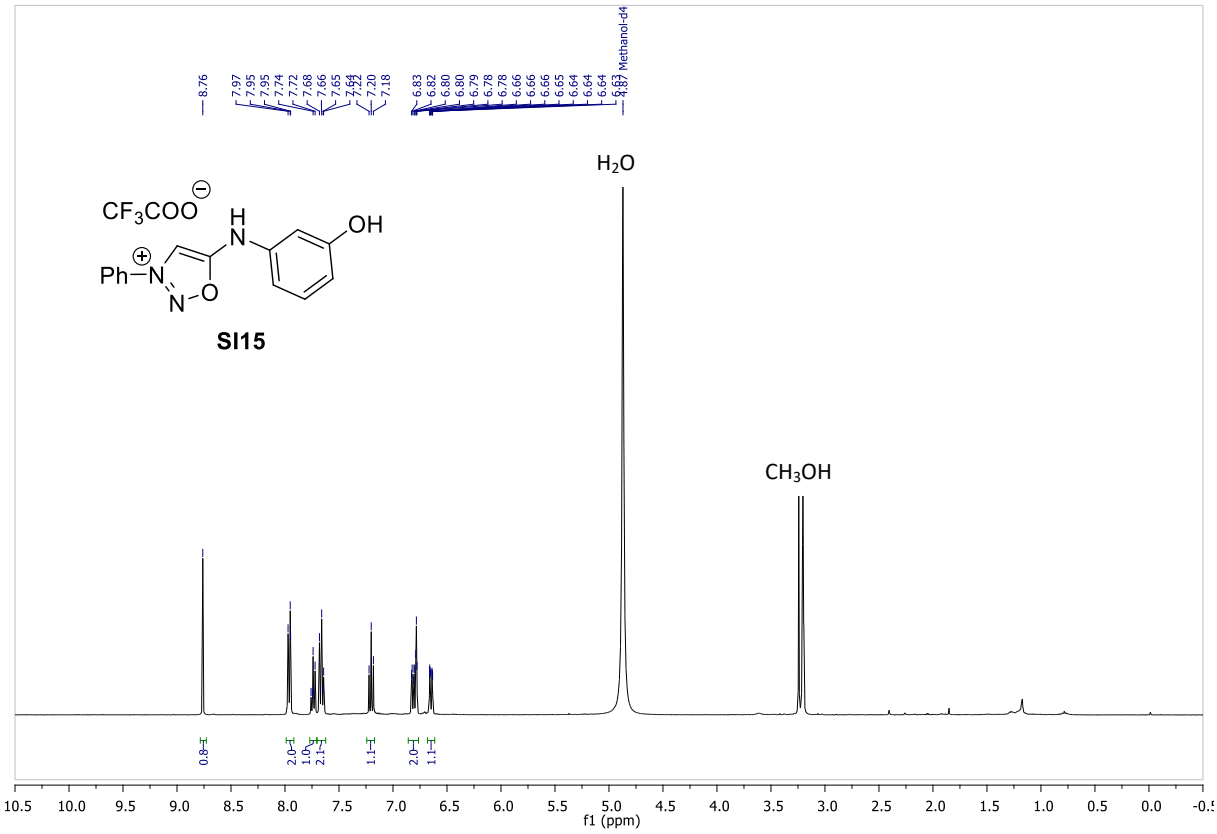
^{13}C NMR compound **SI14** (100 MHz, CD_3OD)



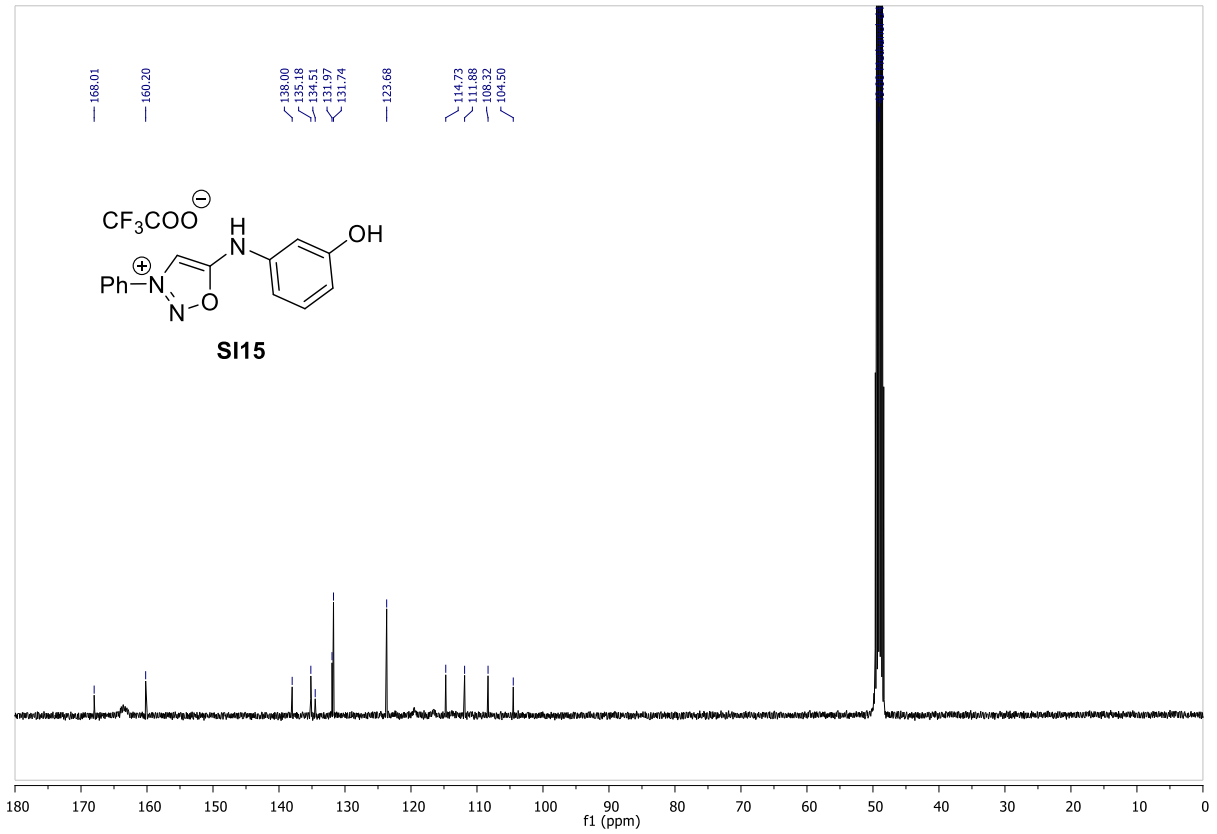
^{19}F NMR compound **SI14** (376 MHz, CD_3OD)



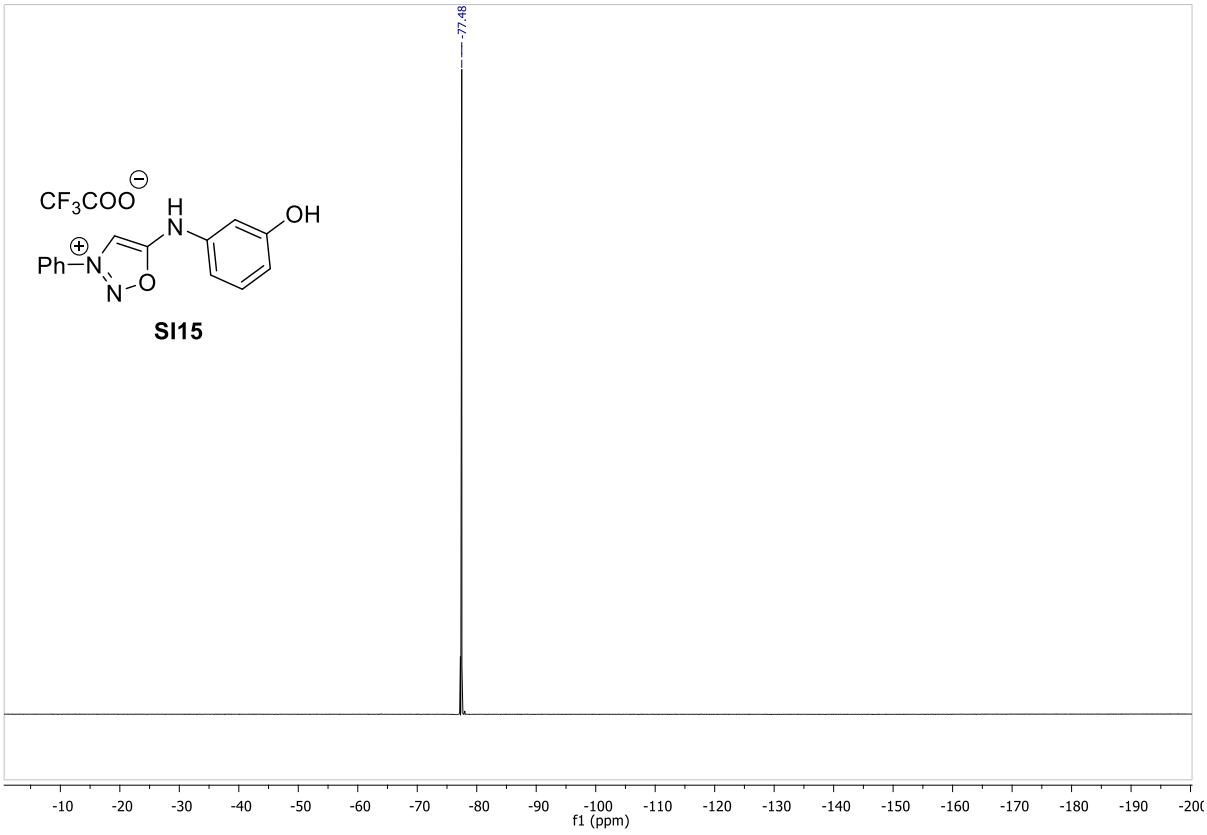
^{1}H NMR compound **SI15** (400 MHz, CD_3OD)



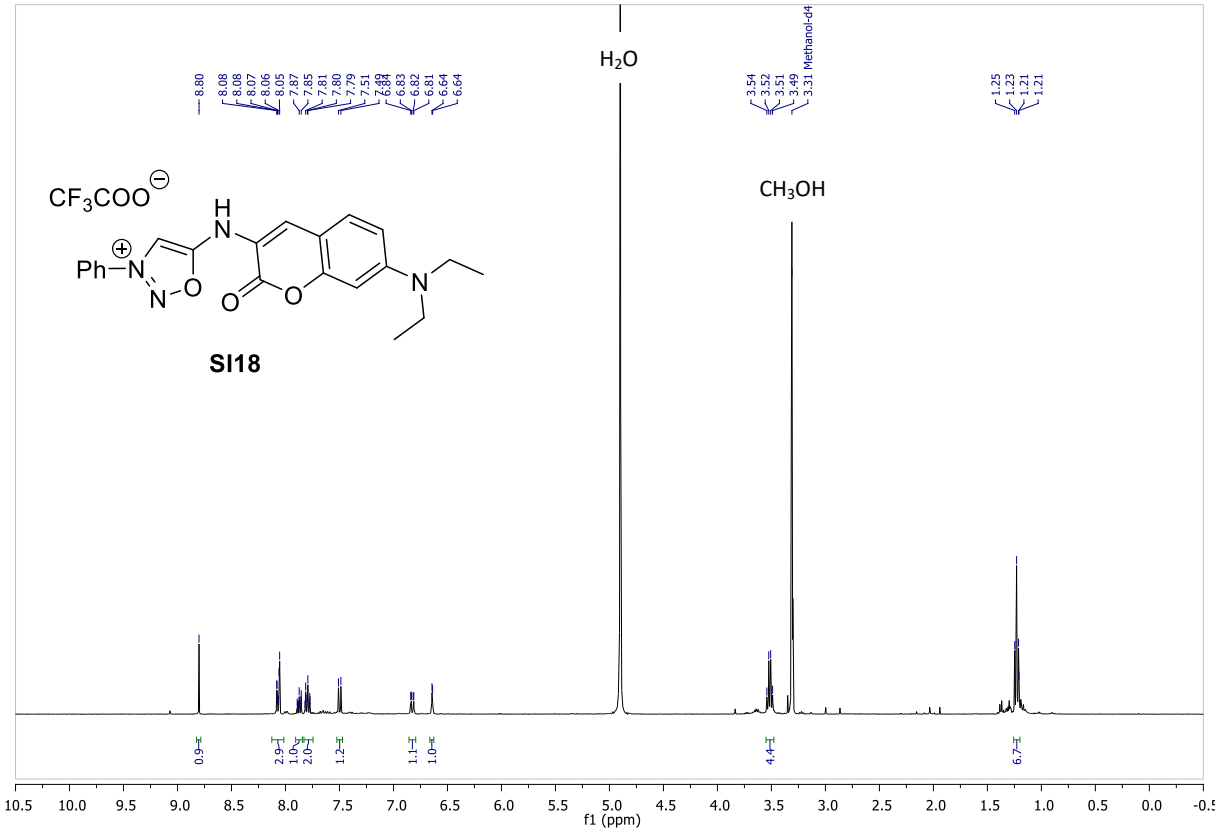
^{13}C NMR compound **SI15** (100 MHz, CD_3OD)



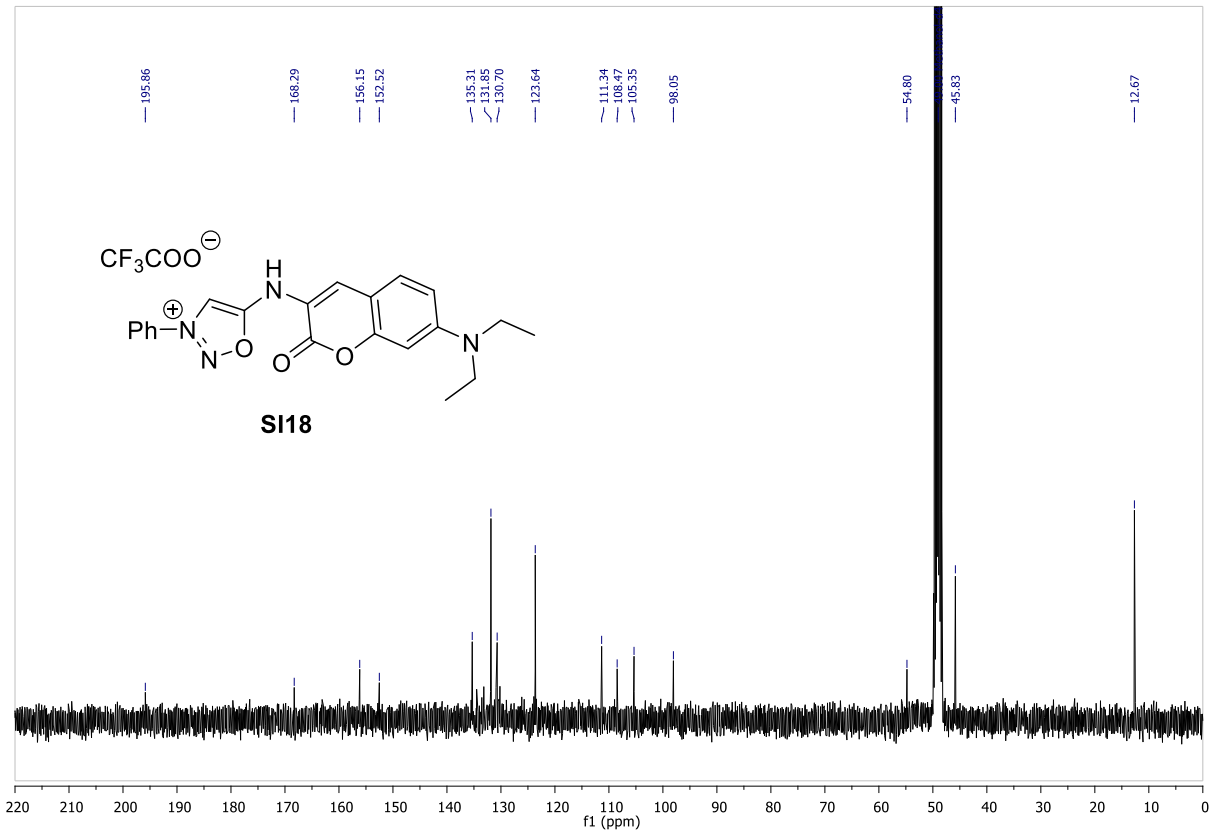
^{19}F NMR compound **SI15** (376 MHz, CD_3OD)



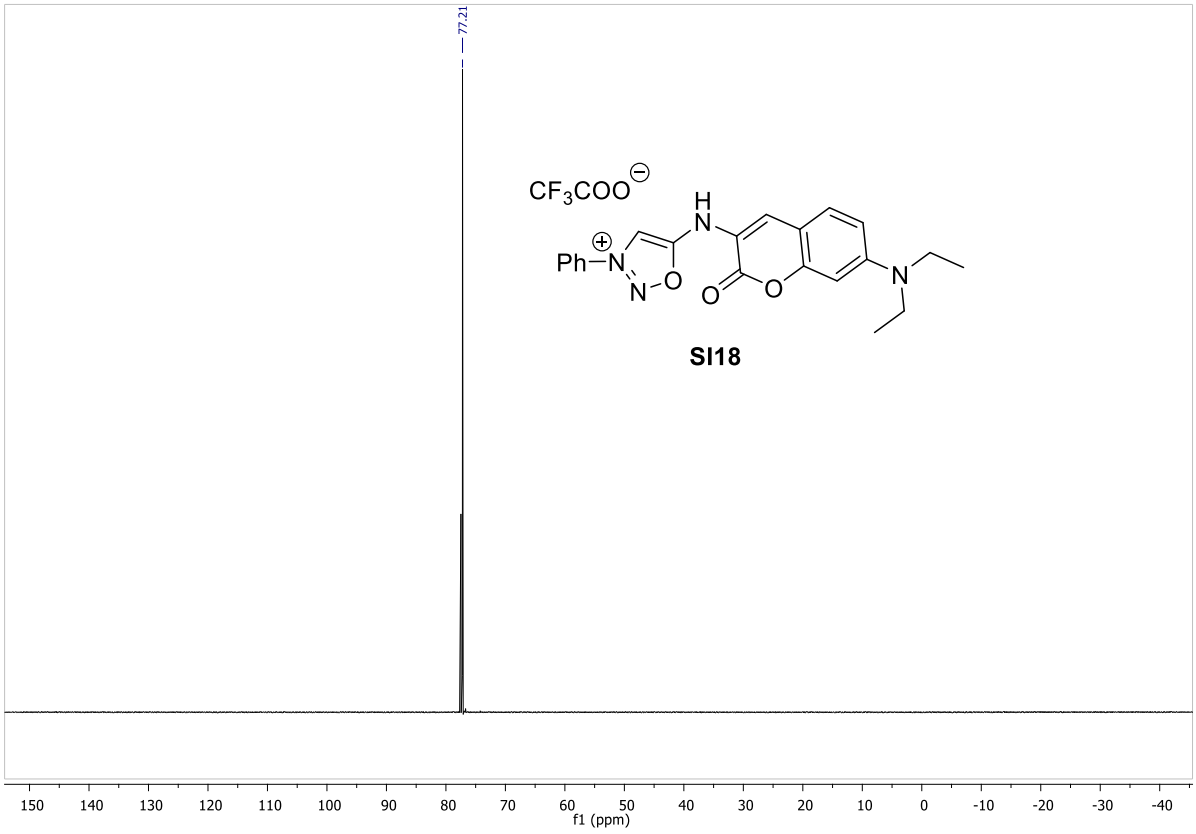
^{1}H NMR compound **SI18** (400 MHz, CD_3OD)



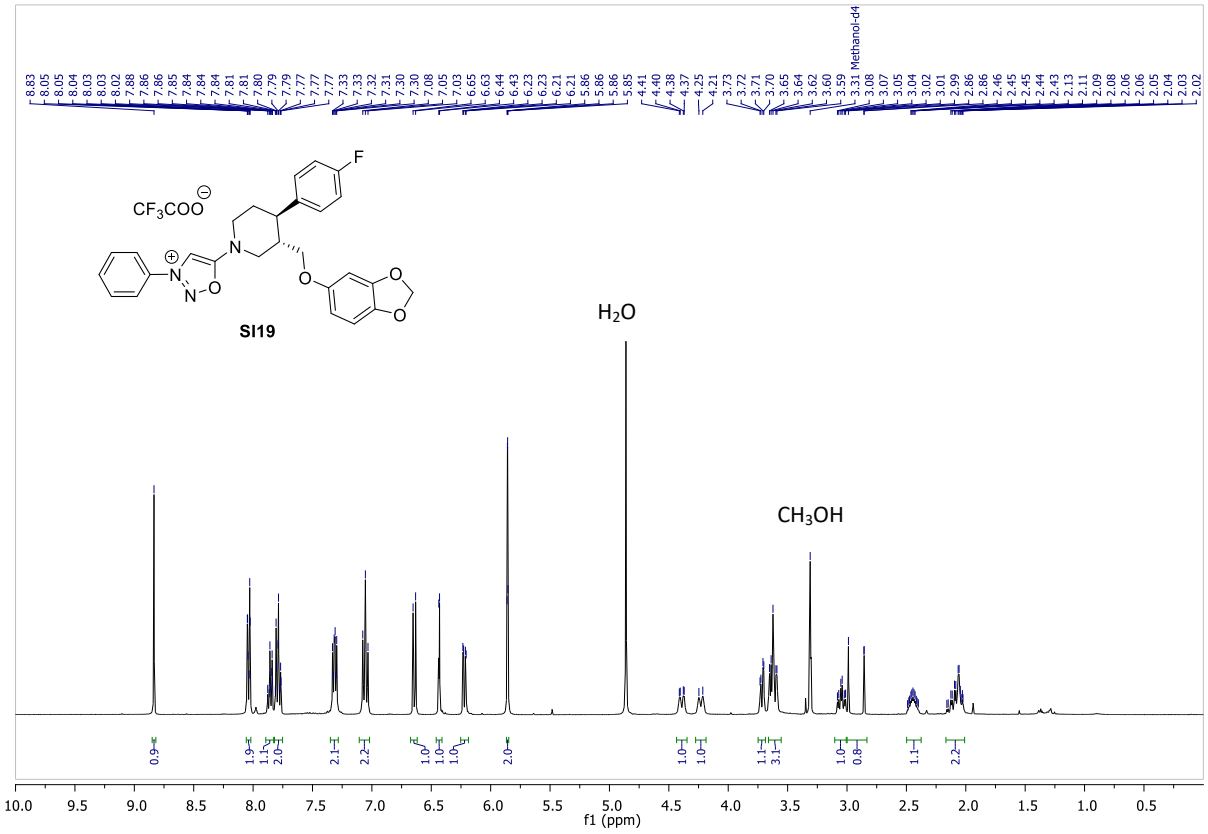
^{13}C NMR compound **SI18** (100 MHz, CD_3OD)



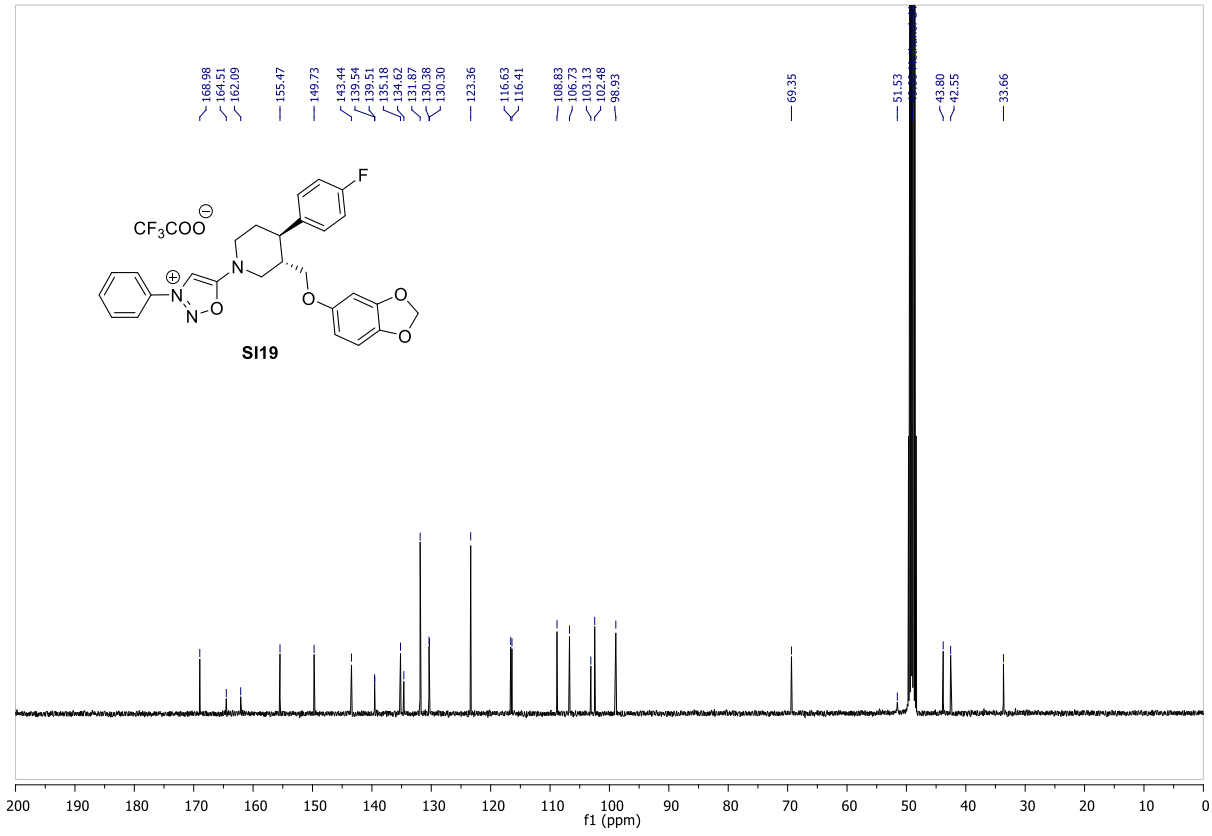
^{19}F NMR compound **SI18** (376 MHz, CD_3OD)



^{1}H NMR compound **SI19** (400 MHz, CD_3OD)



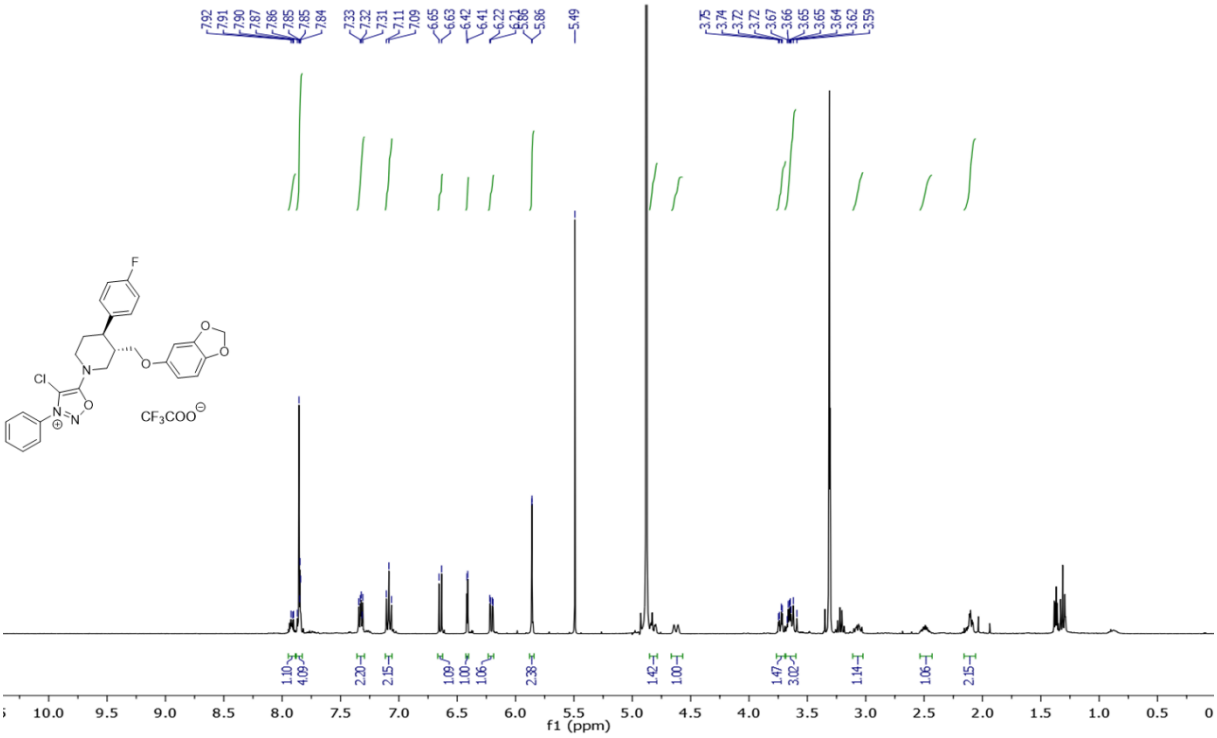
^{13}C NMR compound **SI19** (100 MHz, CD_3OD)



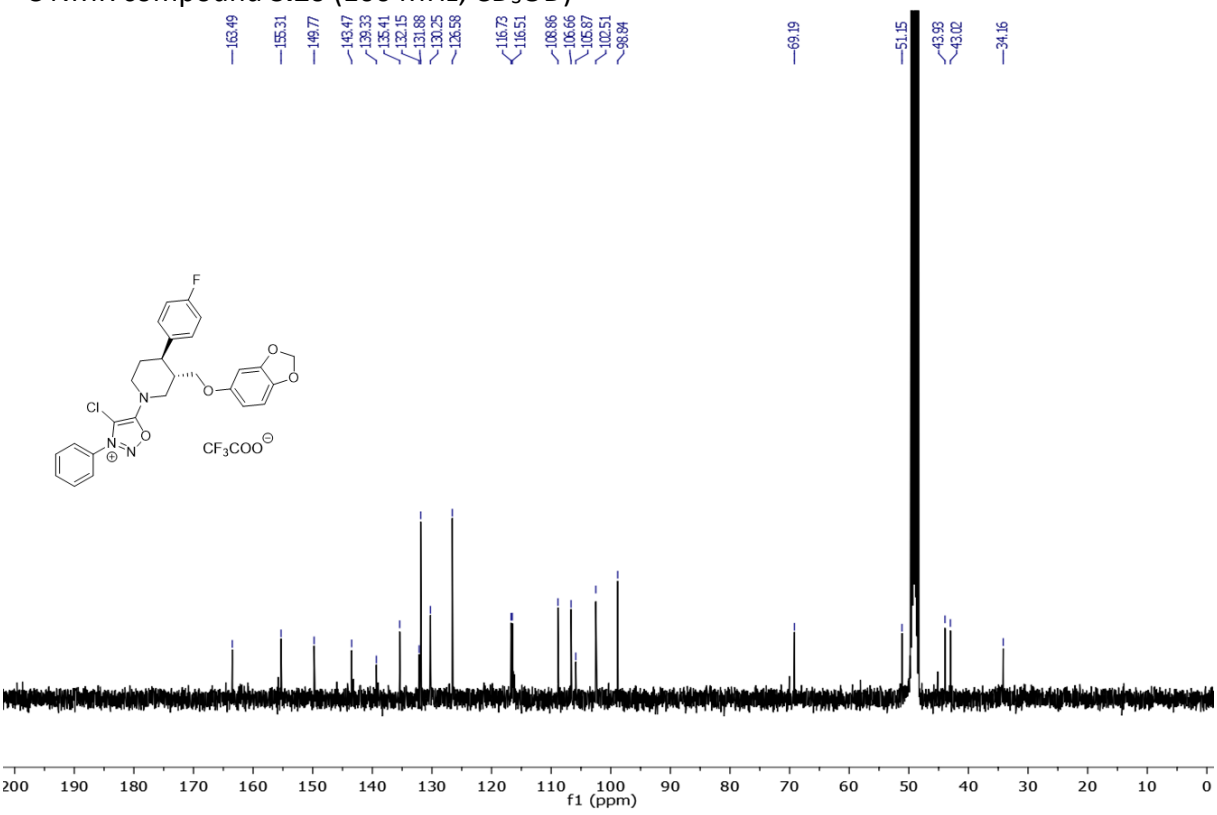
^{1}H NMR compound **SI20** (400 MHz, CD_3OD)

CH_3OH

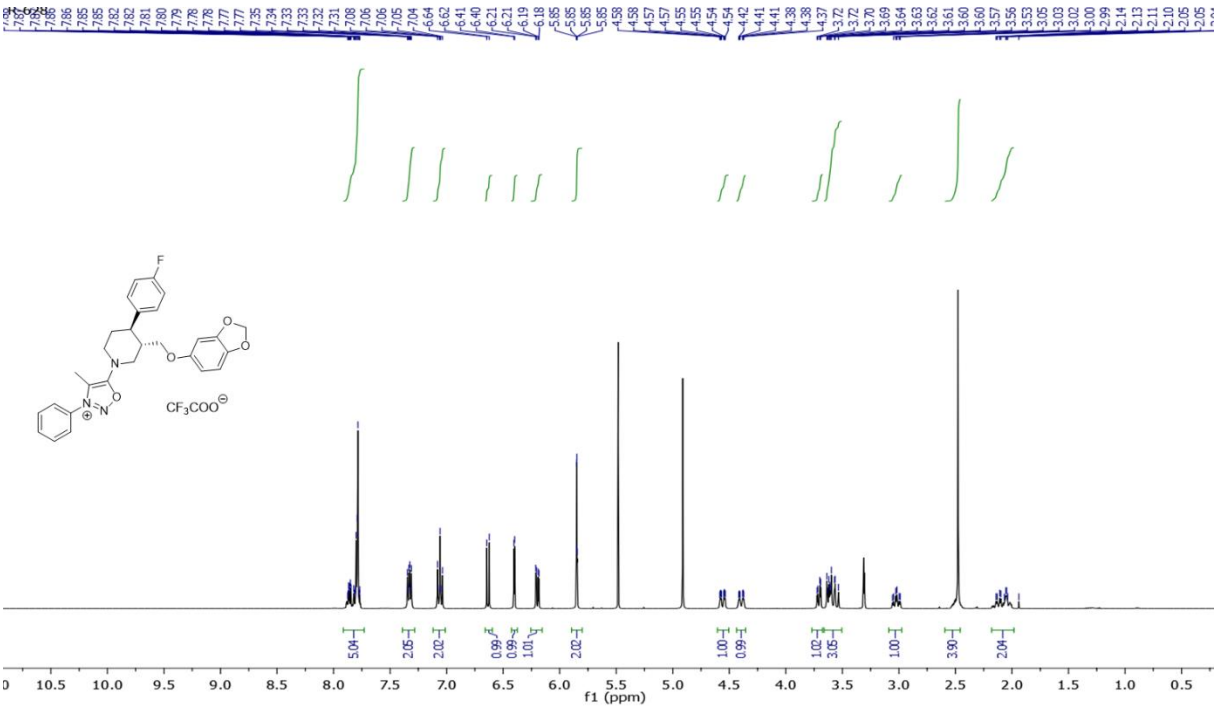
H_2O



^{13}C NMR compound **SI20** (100 MHz, CD_3OD)



^{1}H NMR compound **SI21** (400 MHz, CD_3OD)



^{13}C NMR compound **SI21** (100 MHz, CD_3OD)