Supplementary Material

A method for the efficient evaluation of substrate-based cholinesterase imaging probes for Alzheimer's disease

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(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate

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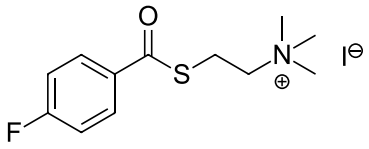
A. Synthetic Procedures and Spectroscopic Data:

Aryl Thiocholine Derivatives

(5) 2-[(*p*-Fluorobenzoyl)thio]-1-(trimethylammonio)ethane Iodide:



Synthesis:

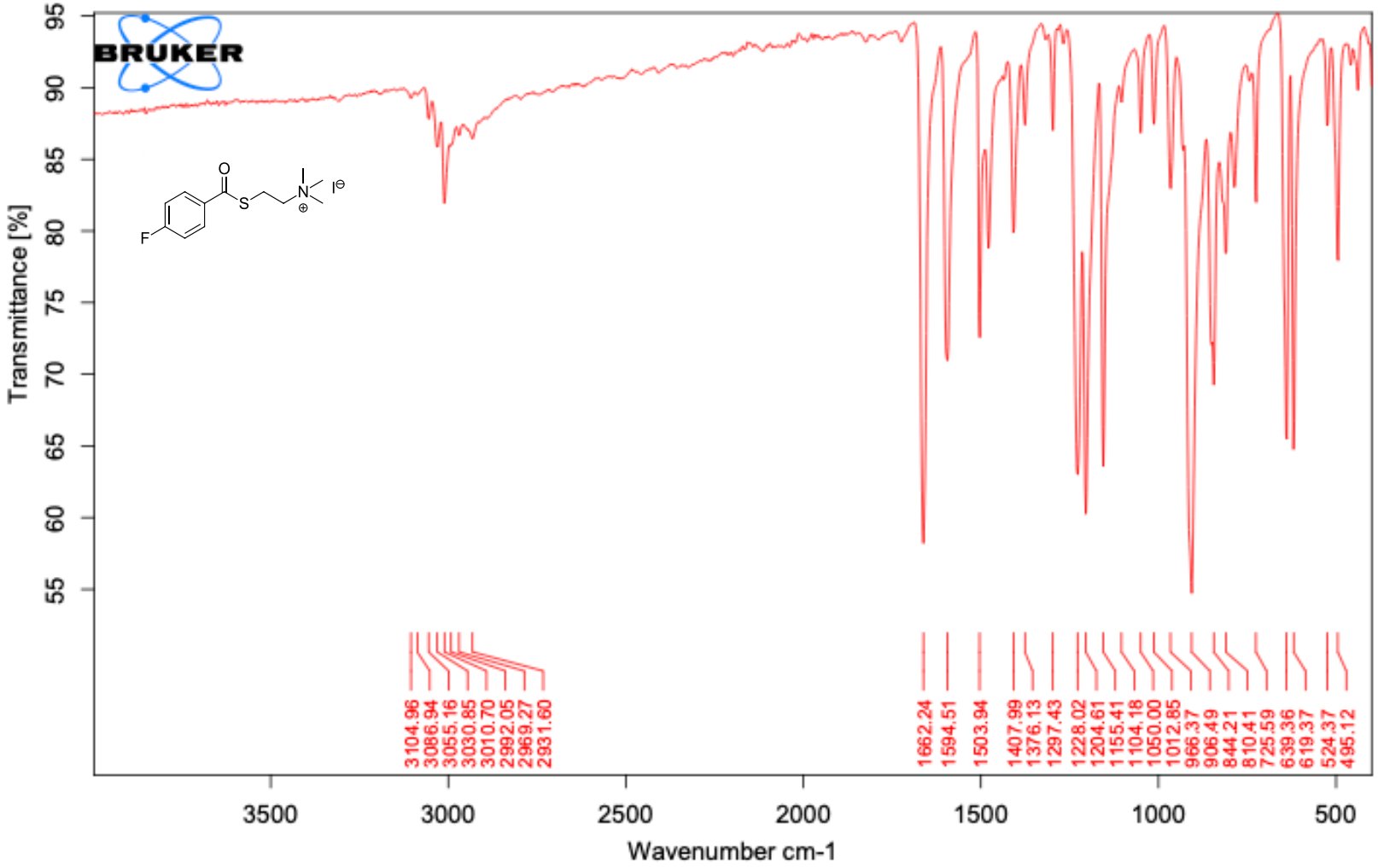
2-[(*p*-Fluorobenzoyl)thio]-1-(trimethylammonio)ethane Iodide: 2-(Dimethylamino) ethanethiol hydrochloride (1.23 g, 8.66 mmol) was suspended in anhydrous dichloromethane (17 mL) and cooled in an ice bath. Triethylamine (3.0 mL, 21.7 mmol) was added dropwise; followed by 4-dimethylaminopyridine (0.106 g, 0.866 mmol) and 4-fluorobenzoyl chloride (1.2 mL, 10.4 mmol). The mixture was warmed to room temperature and stirred for 16 hrs where a white precipitate formed. After this stirring time, the reaction was gravity filtered to remove the precipitate, and the filter cake was washed with dichloromethane (2 × 10 mL). The filtrate was washed with 0.04 *N* $HCl_{(aq)}$ (50 mL), saturated $NaHCO_{3(aq)}$ (50 mL), and H_2O (50 mL). The organic layer was dried over Na_2SO_4, gravity filtered, and the filtrate was concentrated *in vacuo* to afford 1.58 g of an off-white solid. This material (1.51 g, 6.65 mmol) was used immediately without purification. It was dissolved in anhydrous THF (10 mL) and the reaction was cooled to 0^oC using an ice bath. Iodomethane (0.50 mL, 7.98 mmol) was added dropwise to the above solution resulting in the formation of a white precipitate. The mixture was warmed to room temperature and stirred for 2 hrs at this temperature. The precipitate was then collected via suction filtration, and the filter cake was washed with Et_2O (100 mL). The filter cake was

transferred to a round bottom flask and placed under a vacuum (1mm Hg) for 5 hrs to afford 2-[(*p*-fluorobenzoyl)thio]-1-(trimethylammonio)ethane iodide (1.92 g, 60% yield) as a white solid.

Spectroscopic data:

$MP_{(THF)}$: 197−199°C; IR(ATR) 3105, 3087, 3011, 2969, 2932, 1662, 1595, 1205, 906 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.06-8.02 (m, 2H), 7.46-7.42 (m, 2H), 3.55-3.45 (m, 4H), 3.17 (s, 9H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 188.8 (0), 165.6 (d,$^{1}J_{C,F}$ = 253.5 Hz, 0), 132.3 (d, $^{4}J_{C,F}$ = 2.8 Hz, 0), 130.1 (d,$^{3}J_{C,F}$ = 9.7 Hz, 1), 116.5 (d,$^{2}J_{C.F}$ = 22.3 Hz, 1), 63.7 (2), 52.3 (3), 21.6 (2); LRMS (ESI^{+}): 242.1 (M^{+}); ^{19}F NMR (376.6 MHz, DMSO-*d6*) δ -103.8; HRMS (ESI^{+}): calculated for $C_{12}H_{17}FNOS^{+}$: 242.1009 amu; found for $C_{12}H_{17}FNOS^{+}$: 242.1011 amu; HPLC purity at 230 nm (25% CH_3CN : 75% CH_3OH, retention time: 1.837 mins): 99%.

(5) 2-[(*p*-Fluorobenzoyl)thio]-1-(trimethylammonio)ethane Iodide IR(ATR)



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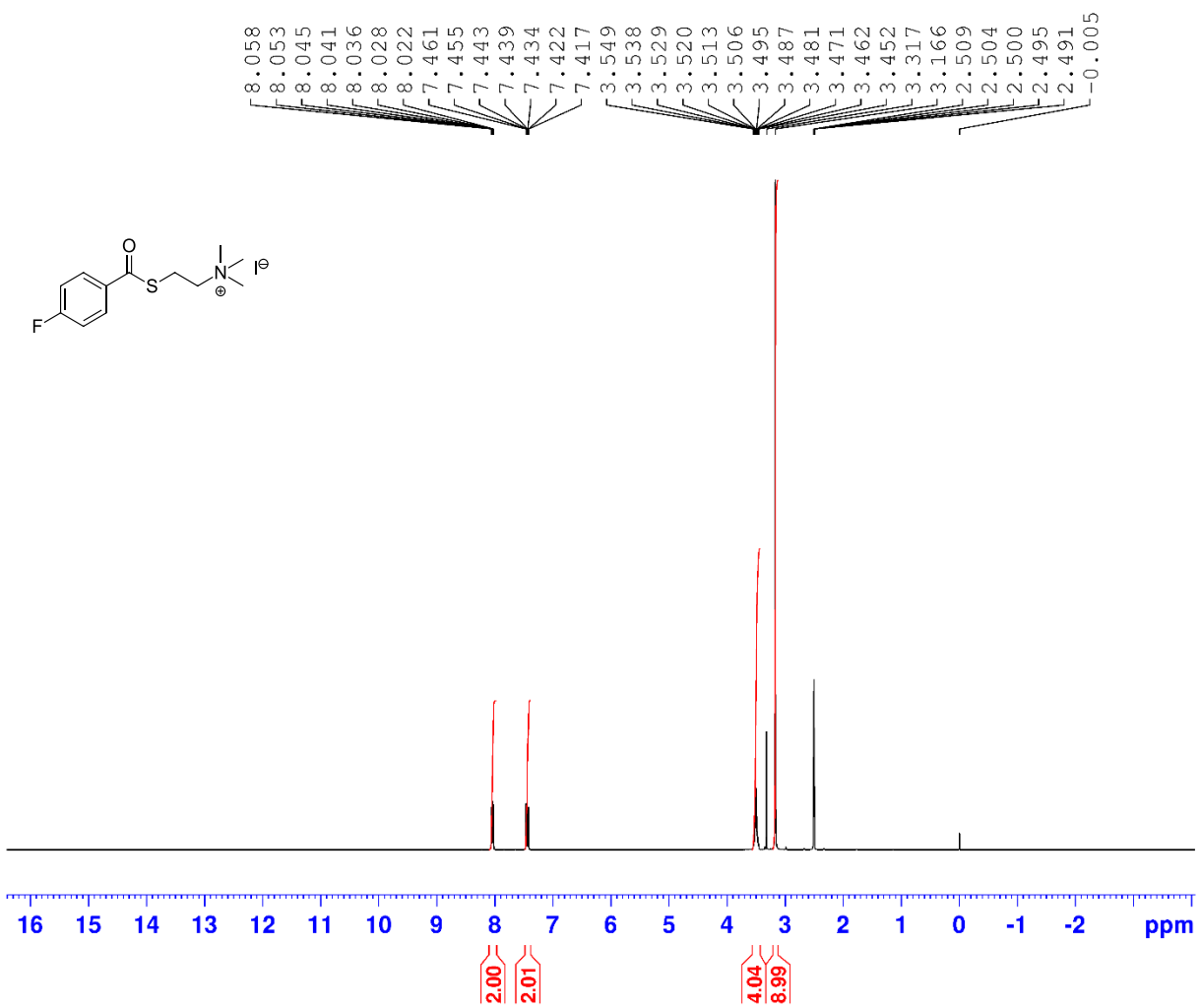


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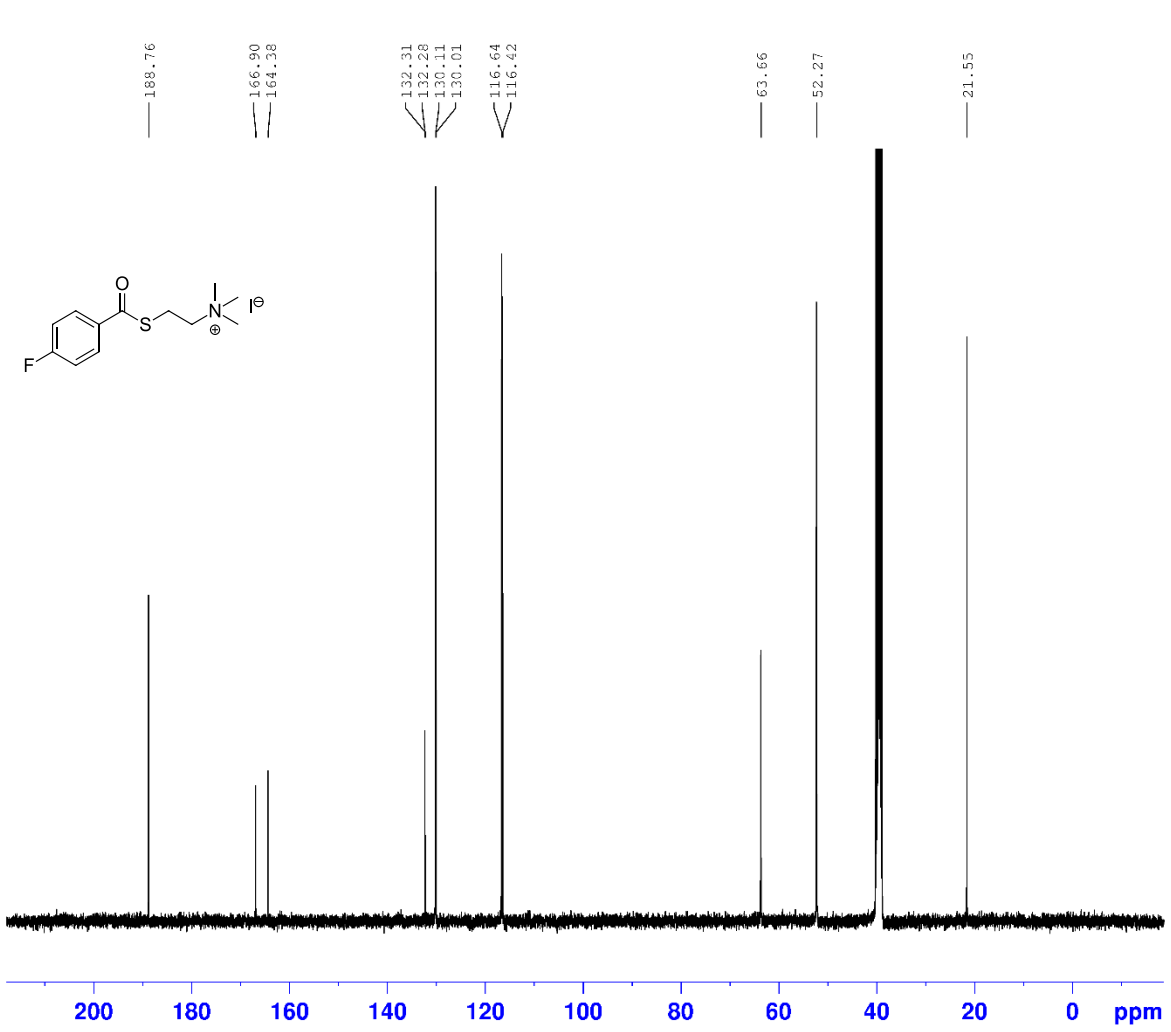

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NUC2                   1H
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(5) 2-[(*p*-Fluorobenzoyl)thio]-1-(trimethylammonio)ethane Iodide High Resolution Mass Spectroscopy (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

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Comment			

Acquisition Parameter

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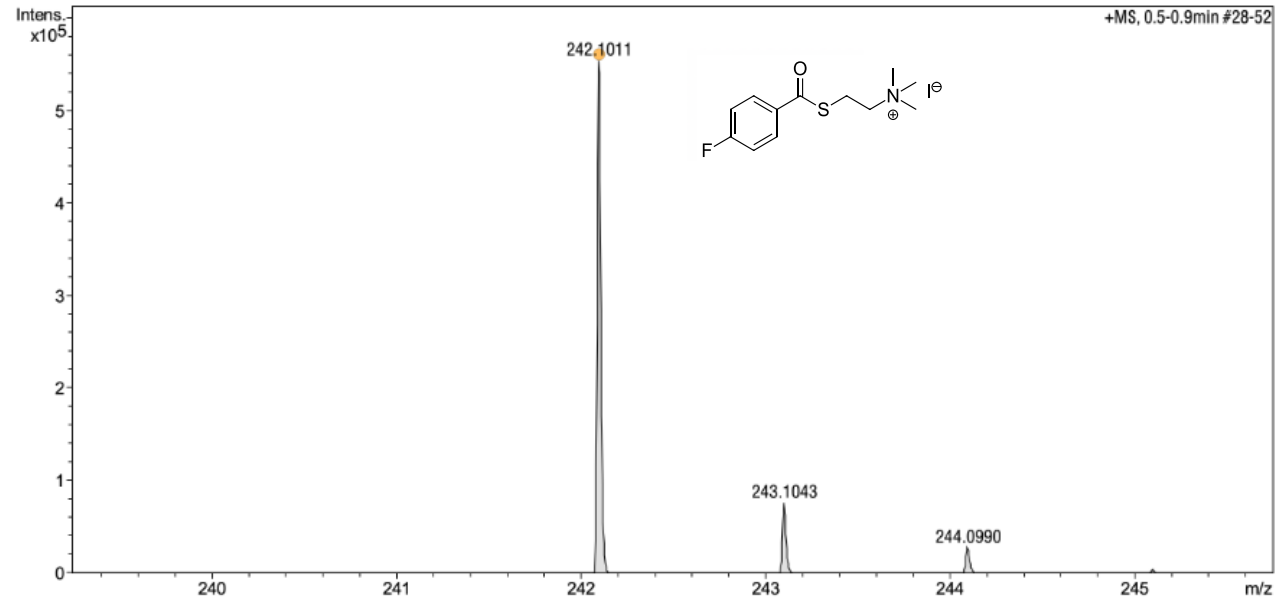

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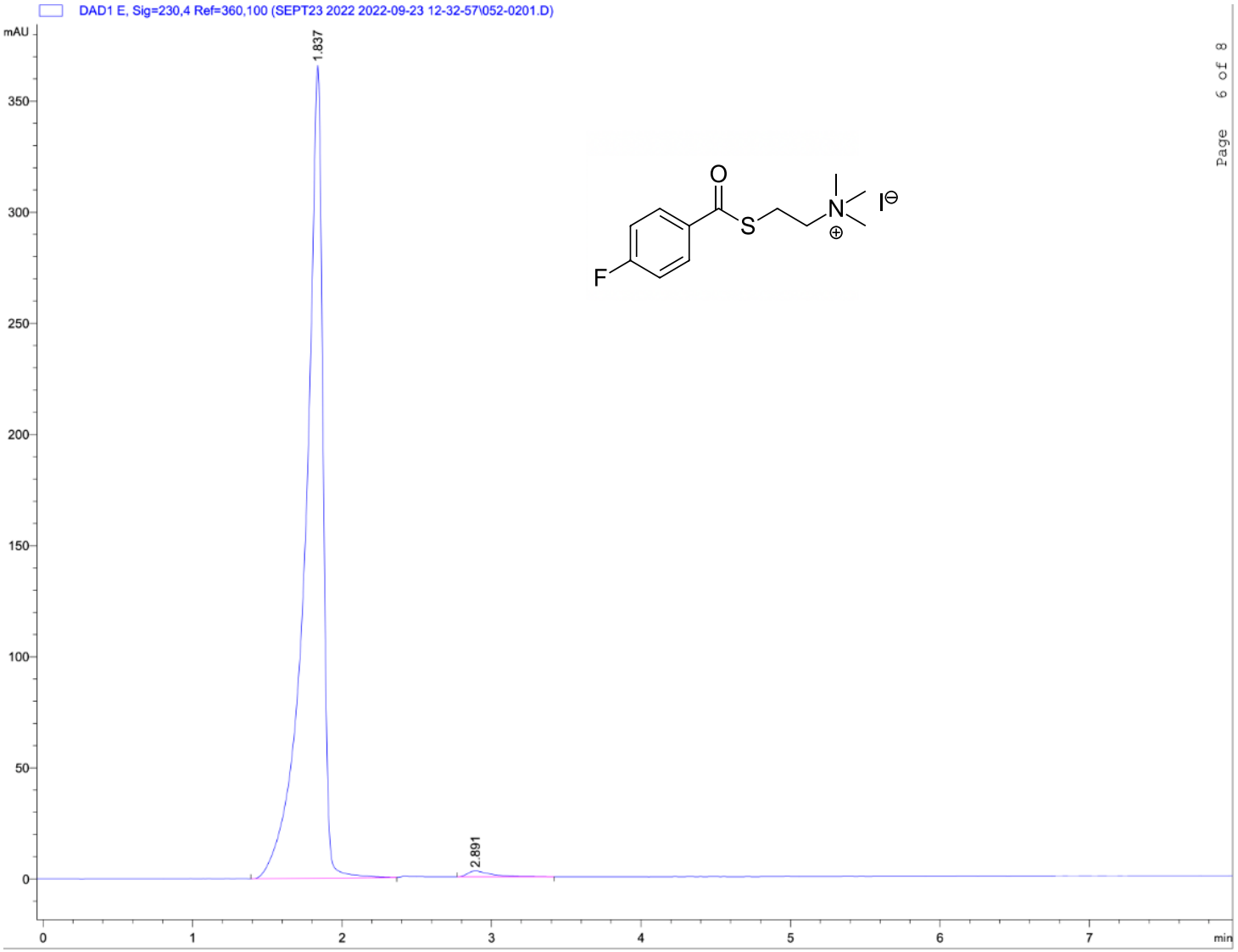
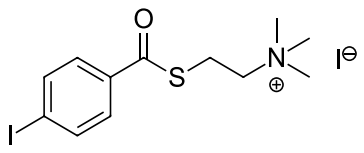
(5) 2-[(*p*-Fluorobenzoyl)thio]-1-(trimethylammonio)ethane Iodide HPLC Trace



(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide:



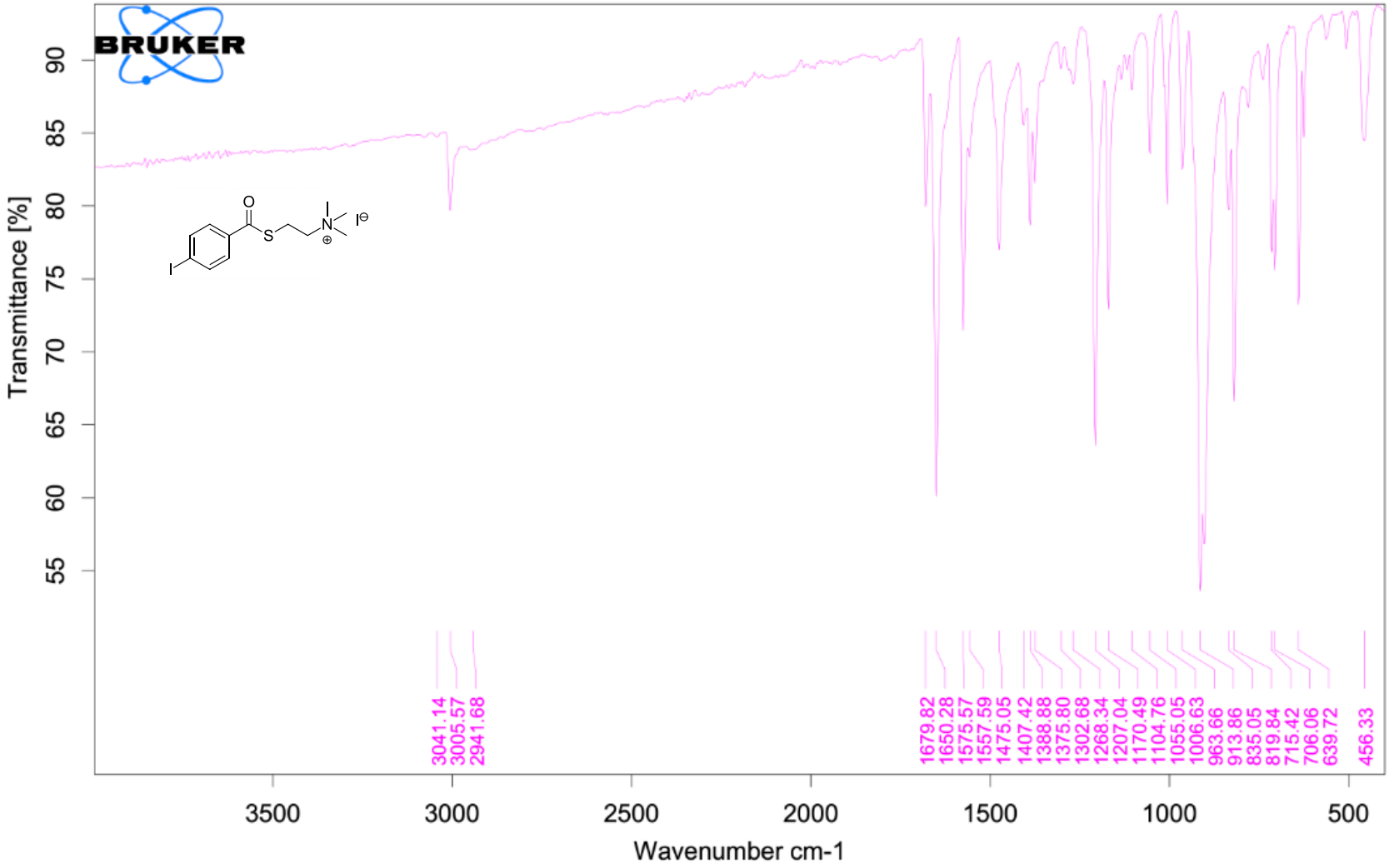
Synthesis:

2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide: 2-(Dimethylamino)ethanethiol hydrochloride (1.16 g, 8.20 mmol) was suspended in anhydrous dichloromethane (16 mL) and cooled in an ice bath. Triethylamine (2.9 mL, 20.8 mmol) was added dropwise; followed by 4-dimethylaminopyridine (0.100 g, 0.82 mmol) and 4-iodobenzoyl chloride (2.62 mL, 9.84 mmol). The mixture was warmed to room temperature and stirred for 16 hrs where a white precipitate formed. After this, the reaction was gravity filtrated to remove the precipitate, and the filter cake was washed with dichloromethane (2 × 10 mL). The filtrate was washed with 0.04 N $HCl_{(aq)}$ (50 mL), saturated $NaHCO_{3(aq)}$ (50 mL), and H_2O (50 mL). The organic layer was dried over Na_2SO_4, gravity filtered, and the filtrate was concentrated *in vacuo* to afford 2.33 g of an off-white solid. This material (2.33 g, 6.95 mmol) was used immediately without purification. It was dissolved in anhydrous THF (10 mL) and cooled in an ice bath. Iodomethane (0.52 mL, 8.34 mmol) was added dropwise to the above solution resulting in the formation of a white precipitate. The mixture was warmed to room temperature and stirred for 2 hrs at this temperature. The precipitate was then collected *via* suction filtration, and the filter cake was washed with Et_2O (100 mL). The filter cake was transferred to a round bottom flask and placed under a vacuum (1 mm Hg) for 5 hrs to afford 2-[(*p*-iodobenzoyl)thio]-1-(trimethylammonio)ethane iodide (3.21 g, 82% yield) as a white solid.

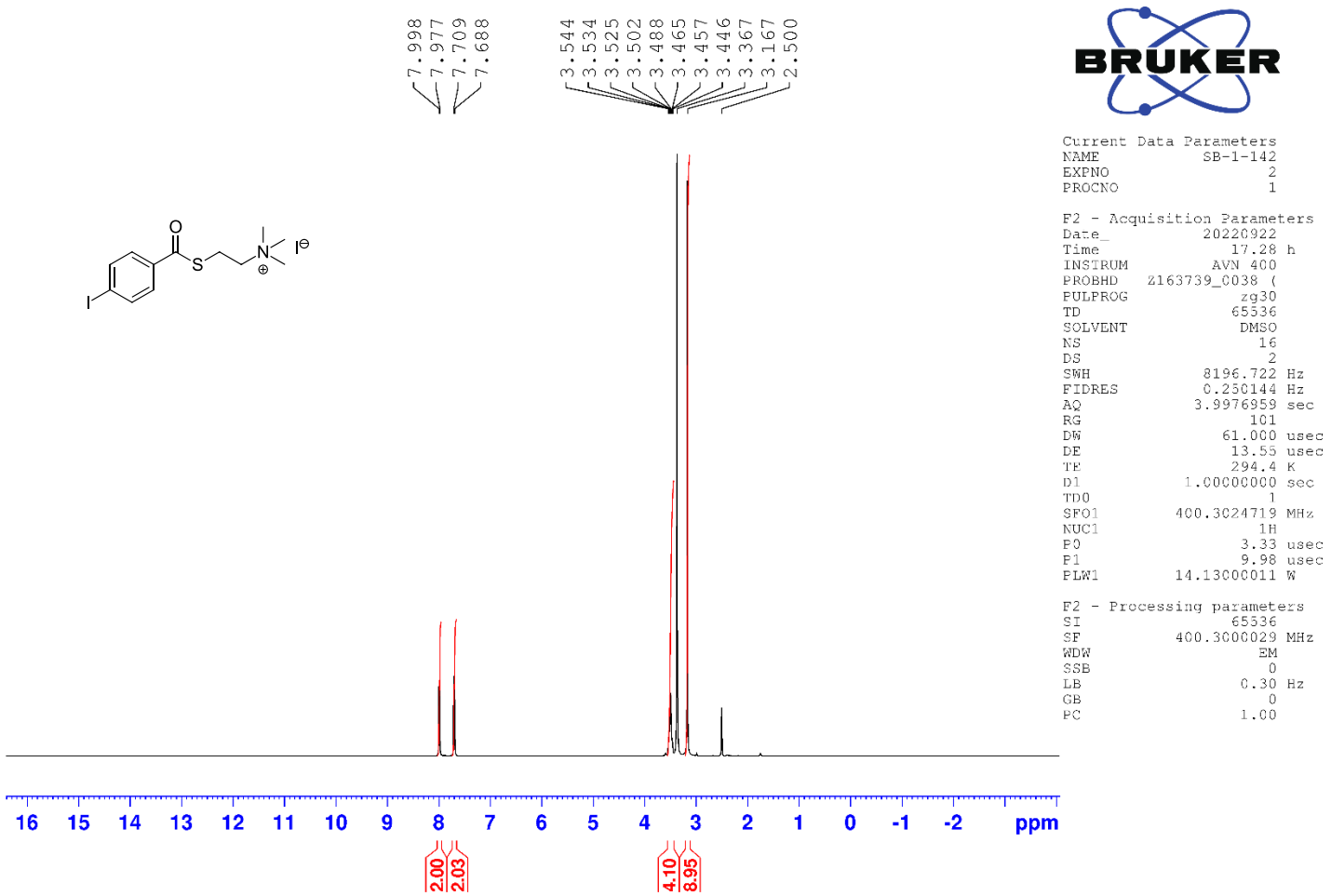
Spectroscopic data:

$MP_{(THF)}$: >250°C; IR(ATR) 3041, 3006, 2942, 1680, 1650, 1576, 1207, 913, 835, 820 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.00-7.98 (m, 2H), 7.71-7.69 (m, 2H), 3.54-3.45 (m, 4H), 3.17 (s, 9H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 189.7 (0), 138.3 (1), 134.9 (0), 128.5 (1), 103.5 (0), 63.6 (2), 52.3 (3), 21.5 (2); LRMS (ESI^+): 350.0 (M^+); HRMS (ESI^+): calculated for $C_{12}H_{17}INOS^+$: 350.0070 amu; found for $C_{12}H_{17}INOS^+$: 350.0068 amu; HPLC purity at 230 nm (25% CH_3CN : 75% CH_3OH, retention time: 1.790 mins): 98%.

(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide IR(ATR)



(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide ^{1}H NMR 400 MHz (DMSO-*d6*)

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P1                       9.98 usec
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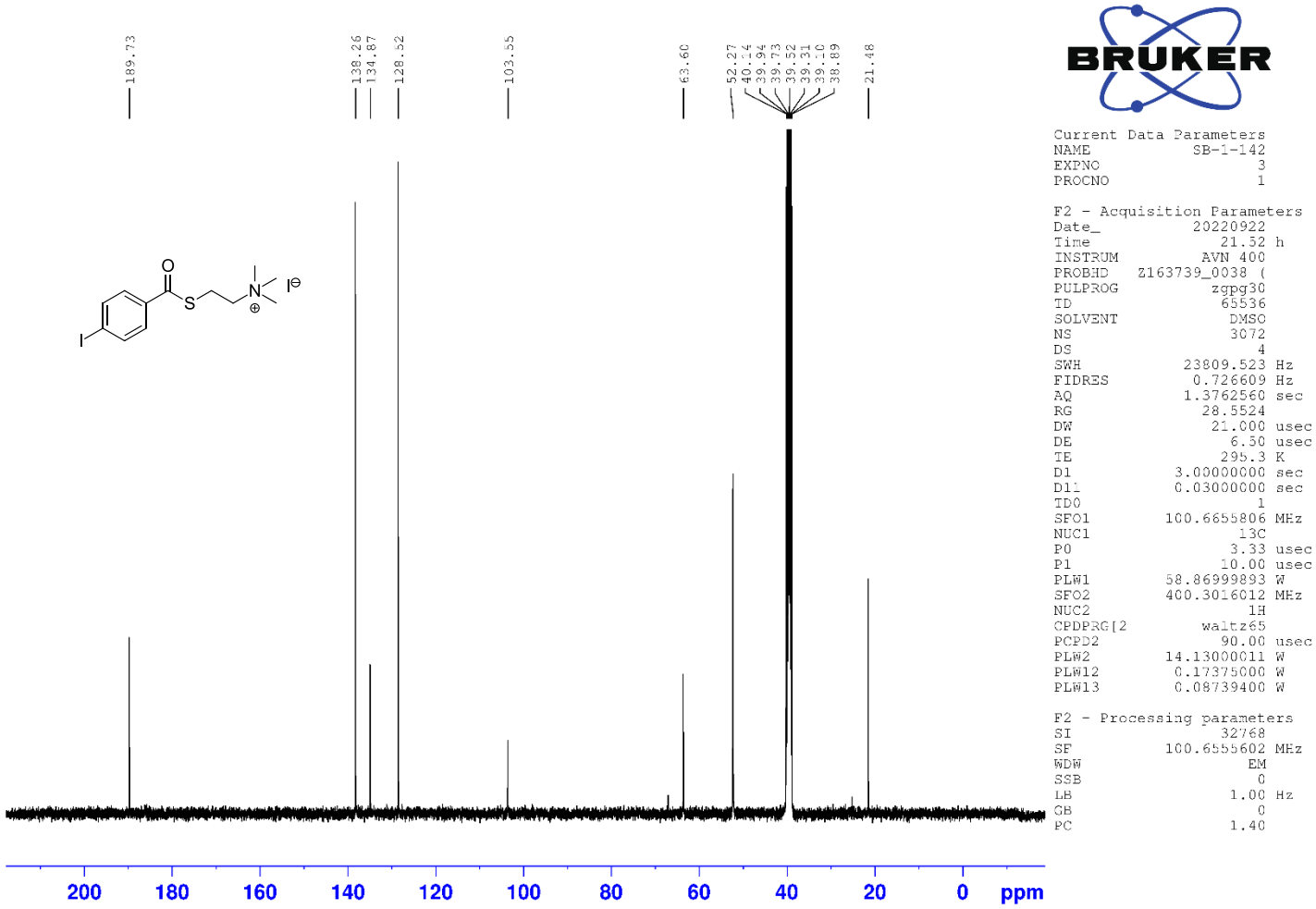
(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide ^{13}C NMR 100 MHz (DMSO-*d6*)

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RG                     28.5524
DW                      21.000 usec
DE                       6.50 usec
TE                      295.3 K
D1                   3.00000000 sec
D11                  0.03000000 sec
TD0                          1
SFO1               100.6655806 MHz
NUC1                        13C
P0                       3.33 usec
P1                      10.00 usec
PLW1               58.86999893 W
SFO2               400.3016012 MHz
NUC2                        1H
CPDPRG[2                waltz65
PCPD2                   90.00 usec
PLW2               14.13000011 W
PLW12               0.17375000 W
PLW13               0.08739400 W

F2 - Processing parameters
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SF                 100.6555602 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
PC                       1.40
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(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	TRV 6022	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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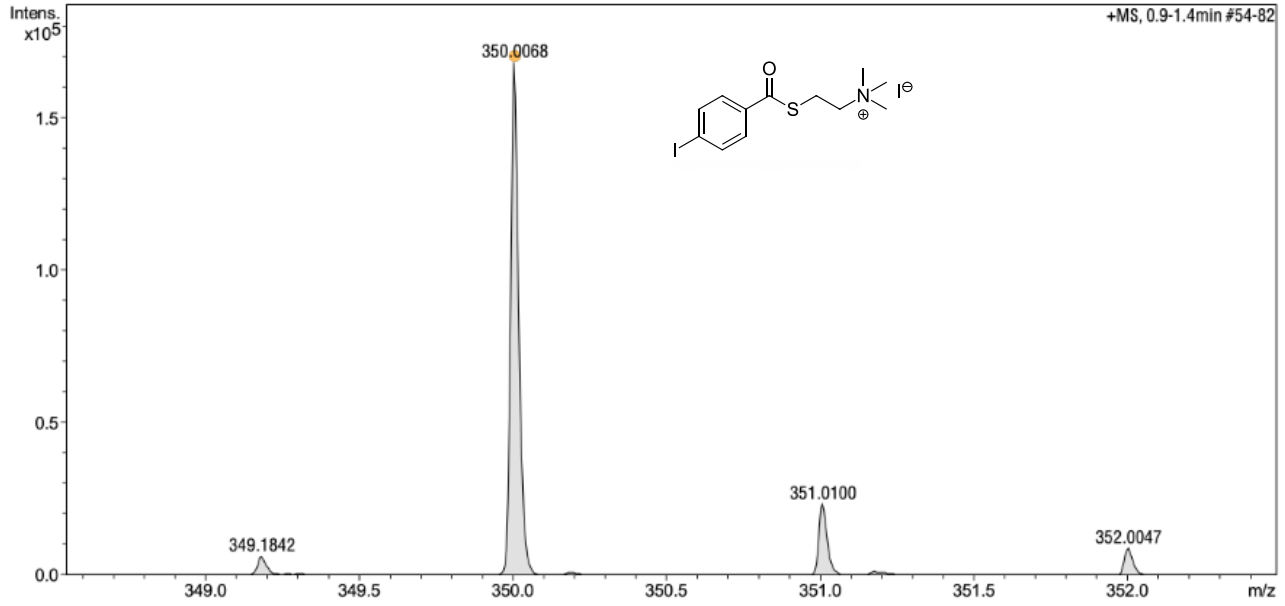

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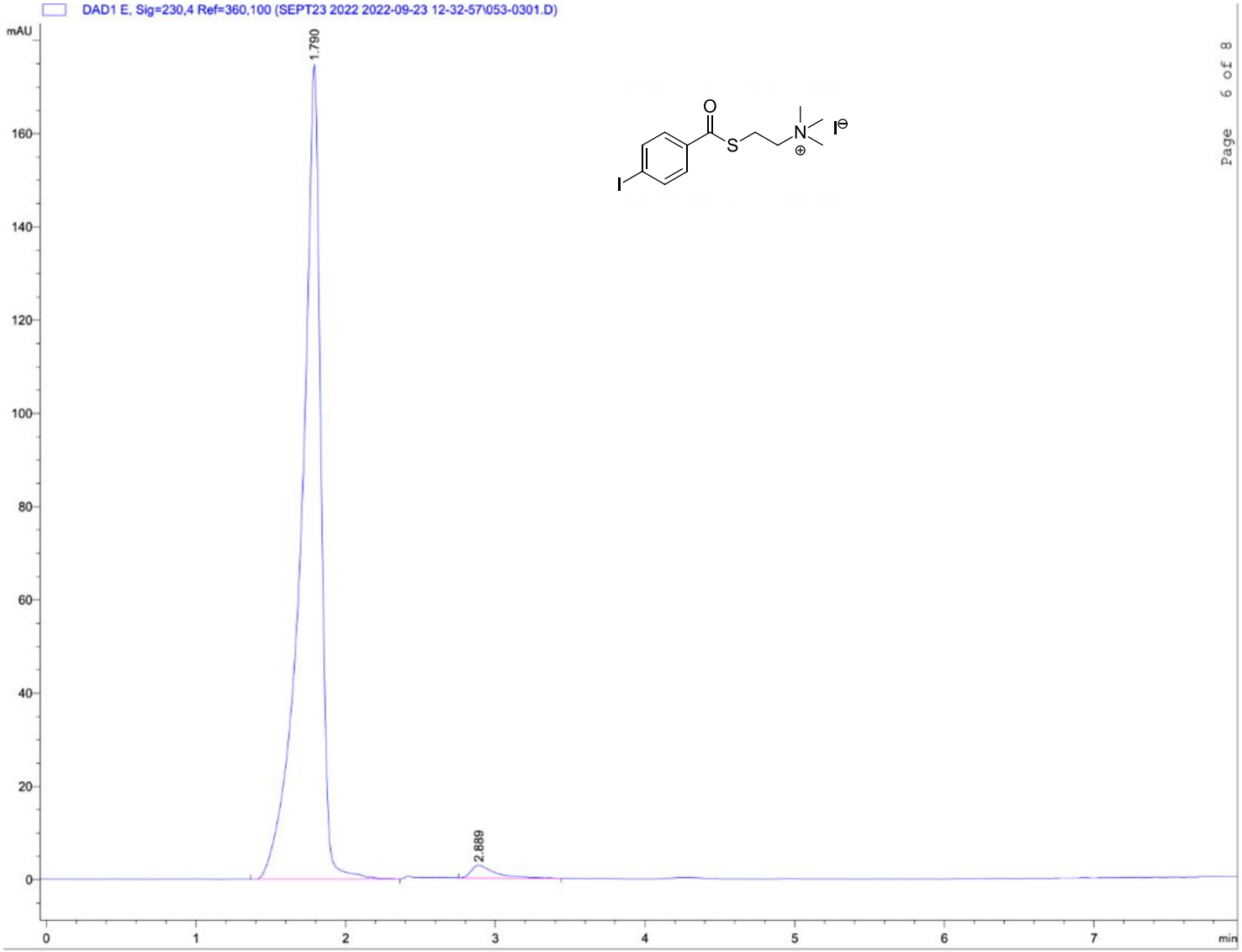
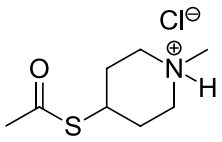
(6) 2-[(*p*-Iodobenzoyl)thio]-1-(trimethylammonio)ethane Iodide HPLC



Alkyl N-Methylpiperidinyl Derivatives

(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride



Synthesis:

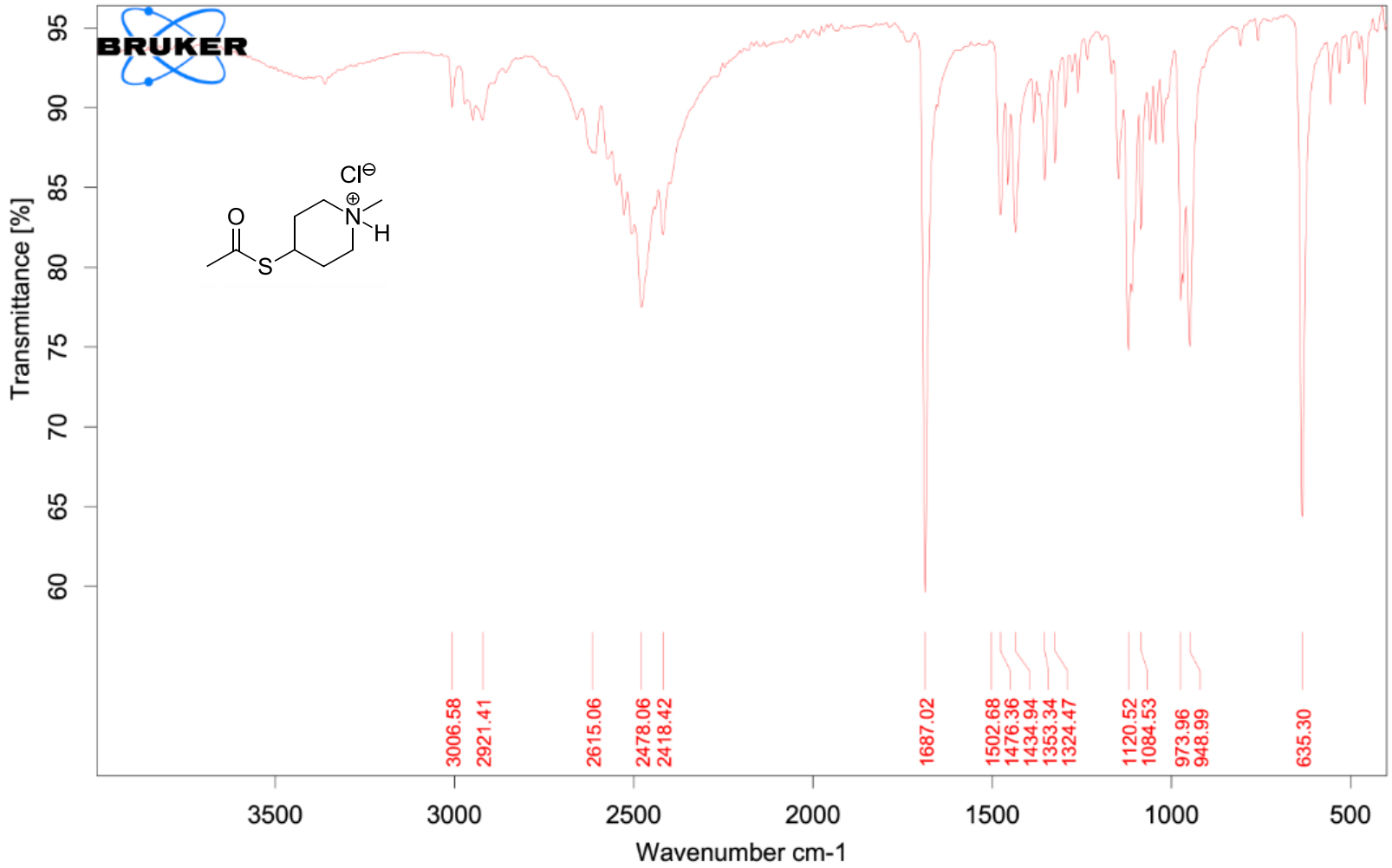
***S*-(1-Methylpiperid-4-yl) Ethanethioate**: *N*-Methyl-4-piperidinethiol (0.50 mL, 4.2 mmol) was dissolved in anhydrous dichloromethane (5 mL), cooled to 0°C with an ice bath and stirred under an argon atmosphere. Acetyl chloride (0.895 mL, 12.6 mmol) was added dropwise at 0°C. The ice bath was removed after 5 mins, and reaction was stirred for 2 hrs at room temperature. After this time, saturated $NaHCO_{3(aq)}$ (10 mL) was added to the reaction and the layers were separated. The organic layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_4, gravity filtered and the filtrate was concentrated *in vacuo* to produce a clear colourless oil *S*-(1-methylpiperid-4-yl) ethanethioate (0.303 g, 42%). The analytical data was similar to previously published data.[1]

***S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride**: *S*-(1-Methylpiperid-4-yl) ethanethioate (0.245 g, 2.78 mmol) was dissolved in anhydrous diethyl ether (28 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 2.11 mL, 2.11 mmol) was added dropwise and stirred for 1 hr. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (3 × 20 mL). Residual diethyl ether was removed *in vacuo* to produce *S*-(1-methylpiperid-4-yl) ethanethioate hydrochloride (0.198 g, 67% yield) as a pair of isomers (25:75).

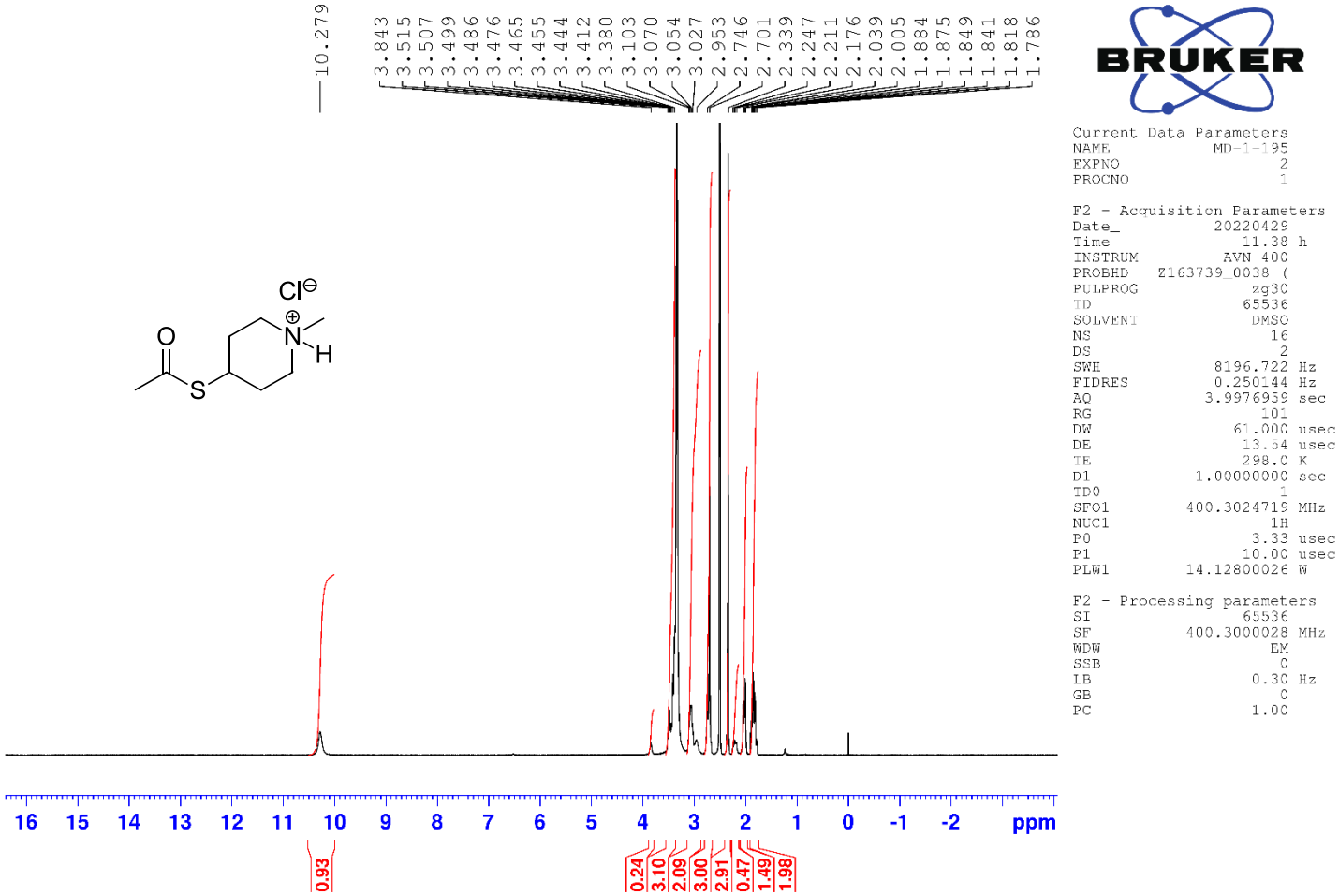
Spectroscopic data:

$MP_{(diethyl\ ether)}$: 157–159°C; IR(ATR) 3007, 2921, 2478, 2418, 1687, 1120, 973, 635 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 10.29 (br s, 1H), 3.51-3.38 (m, 3H), 3.10-3.03 (m, 2H), 2.70 (s, 3H), 2.33 (s, 3H), 2.04-2.01 (m, 2H), 1.88-1.79 (m, 2H), isomer B: 10.29 (br s, 1H), 3.84 (br s, 1H), 3.41-3.38 (m, 2H), 2.98-2.92 (m, 2H), 2.75 (s, 3H), 2.33 (s, 3H), 2.25-2.18 (m, 2H), 1.88-1.79 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ isomer A: 194.6 (0), 52.9 (2), 42.4 (3), 36.7 (1), 30.7 (3), 28.8, isomer B: 194.7 (0), 50.1 (2), 41.8 (3), 36.3 (1), 28.8 (2), 27.2 (3); LRMS (ESI^+): 174.1 (M^+); HRMS (ESI^+): calculated for $C_8H_{16}NOS^+$: 174.0947 amu; found for $C_8H_{16}NOS^+$: 174.0946 amu; HPLC purity at 254 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 3.685 mins): 98.7%.

(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride IR(ATR)



(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride ^{1}H NMR 400 MHz (DMSO-*d6*)



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PROCNO 1

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SOLVENT DMSO
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FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.54 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.3024719 MHz
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P0 3.33 usec
P1 10.00 usec
PLW1 14.12800026 W

F2 - Processing parameters
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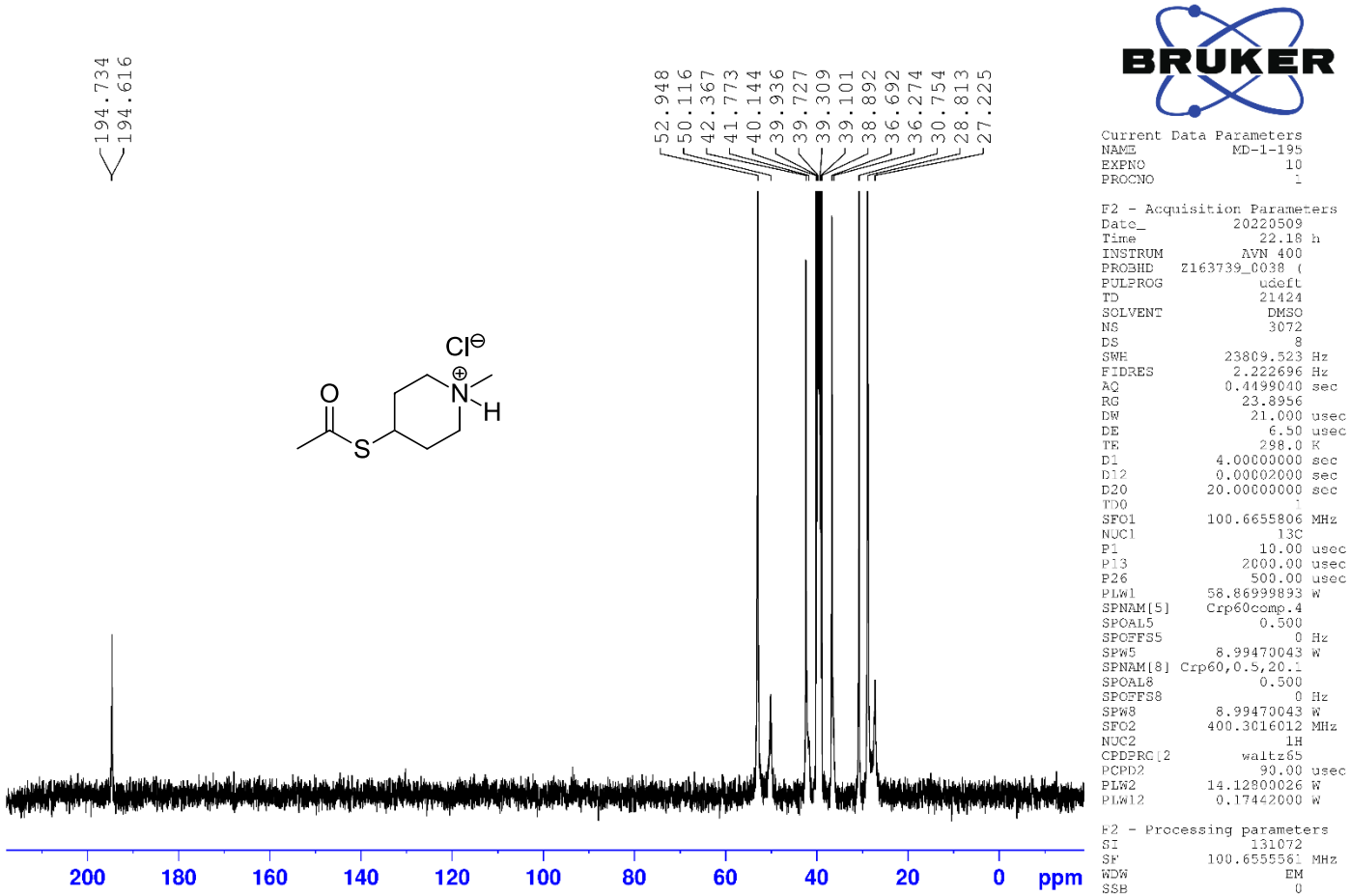
SF 400.3000028 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride ^{13}C NMR 100 MHz (DMSO-*d6*)

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TD                       21424
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DE                         6.50 usec
TE                        298.0 K
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SFO1                100.6655806 MHz
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SPNAM[8] Crp60,0.5,20.1
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SFO2                400.3016012 MHz
NUC2                        1H
CPDPRG[2                waltz65
PCPD2                     90.00 usec
PLW2                14.12800026 W
PLW12                0.17442000 W

F2 - Processing parameters
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WDW                          EM
SSB                           0
LB                         2.00 Hz
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PC                         1.40
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(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000002.d	Acquisition Date	7/7/2022 9:07:46 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6008 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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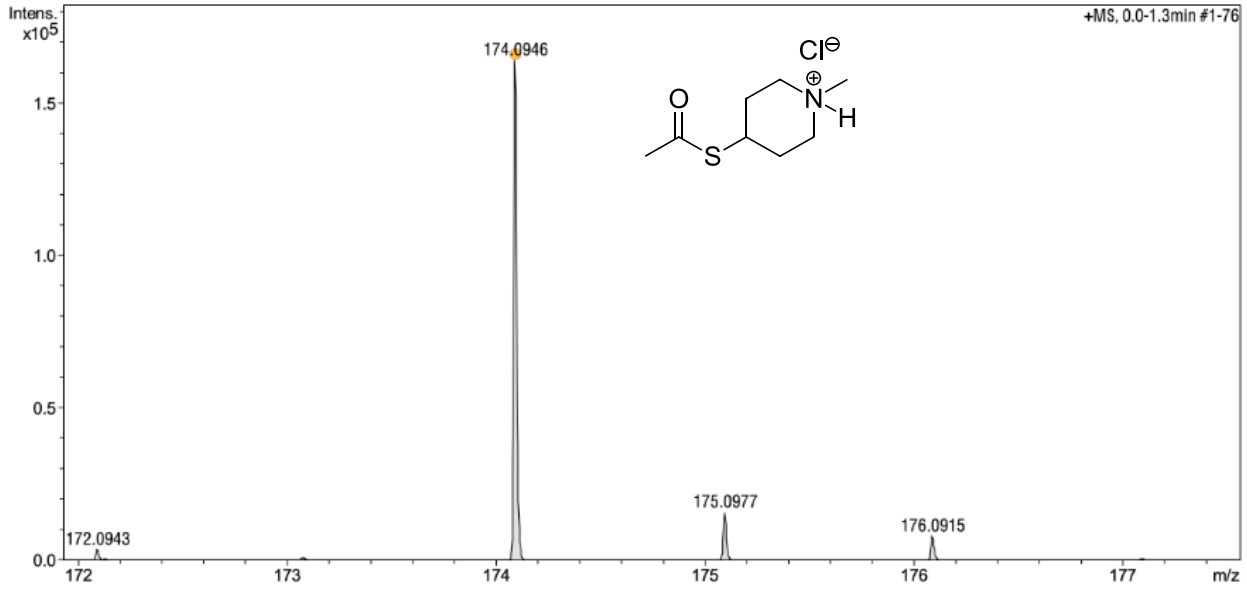

July 07 2022000002.d

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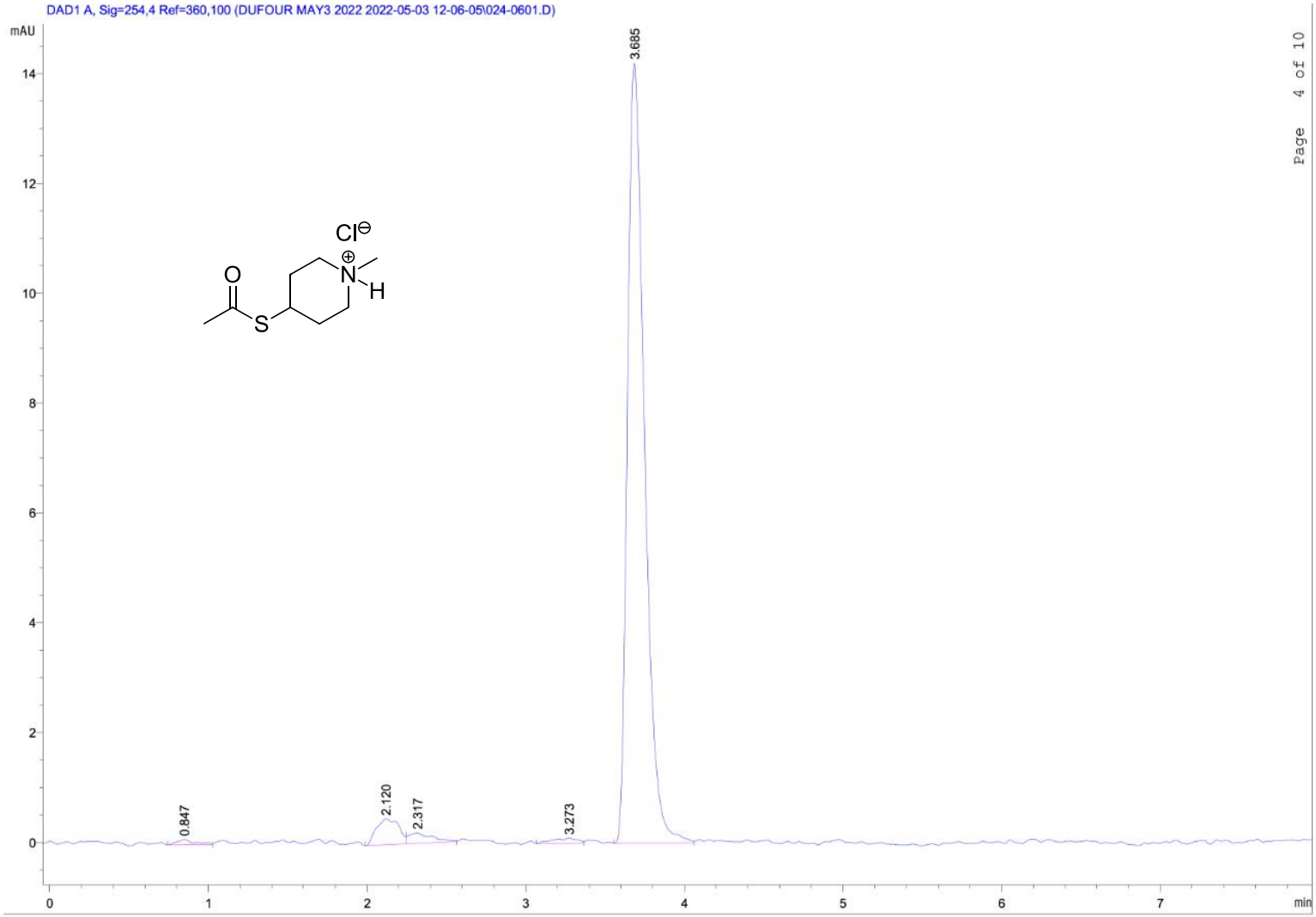
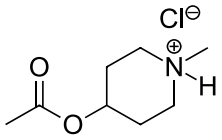
(7) *S*-(1-Methylpiperid-4-yl) Ethanethioate Hydrochloride HPLC



(8) 4-Acetoxy-1-methyl-1-piperidinium Chloride



Synthesis:

1-Methyl-4-piperidyl Acetate: A slightly modified procedure published by Bormans *et al.*[2] was used to produce 1-methyl-4-piperidyl acetate. Briefly, *N*-methyl-4-piperidinol (1.52 g, 9.80 mmol) was dissolved in anhydrous dichloromethane (26 mL) and stirred under an argon atmosphere. Triethylamine (2.73 mL, 19.6 mmol) was added, and the reaction mixture was cooled to 0°C with an ice bath. Acetyl chloride (0.906 mL, 12.74 mmol) was added dropwise to the reaction mixture at 0°C. The ice bath was removed after 5 mins, and reaction was stirred for 2 hrs at room temperature. After this time, brine (20 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 15 mL), and combined organic layers were dried over Na_2SO_3 then gravity filtered. The filtrate was concentrated *in vacuo* to produce an orange oil, 1-methyl-4-piperidyl acetate (1.54 g, 97%). The analytical data was similar to previously published data.[2]

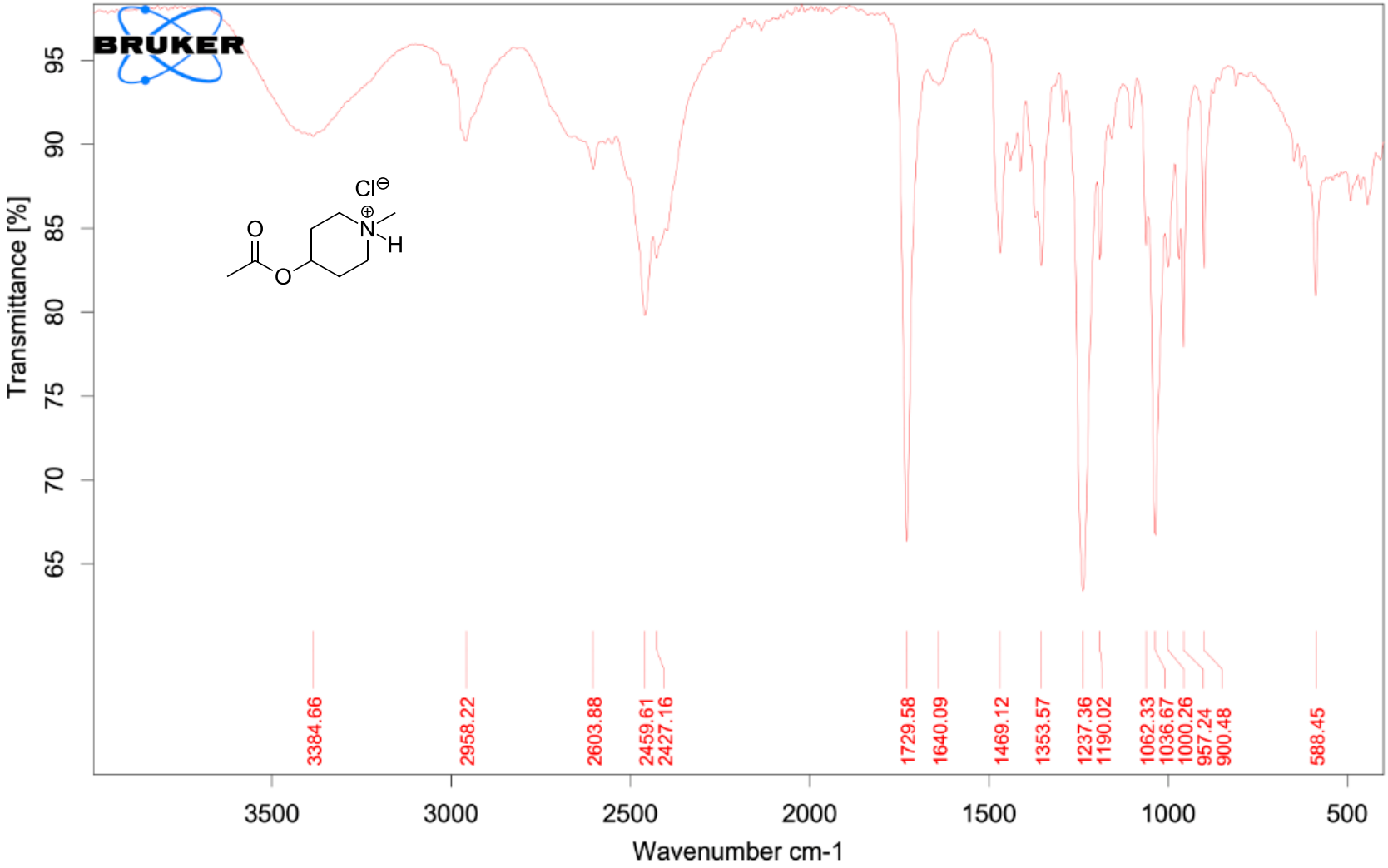
4-Acetoxy-1-methyl-1-piperidinium Chloride: To a flask charged with 1-methyl-4-piperidyl acetate (0.436 g, 2.78 mmol), anhydrous diethyl ether (55 mL) was added until complete dissolution under an argon atmosphere. HCl dissolved in diethyl ether (1 N - 4.16 mL, 4.16 mmol) was added dropwise to the 1-methyl-4-piperidyl acetate solution and stirred for 1h at room temperature. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (3 × 20 mL). Residue diethyl ether was removed *in vacuo* to produce 4-acetoxy-1-methyl-1-piperidinium chloride (0.538 g, 99%) as a pair of isomers

(46:54).

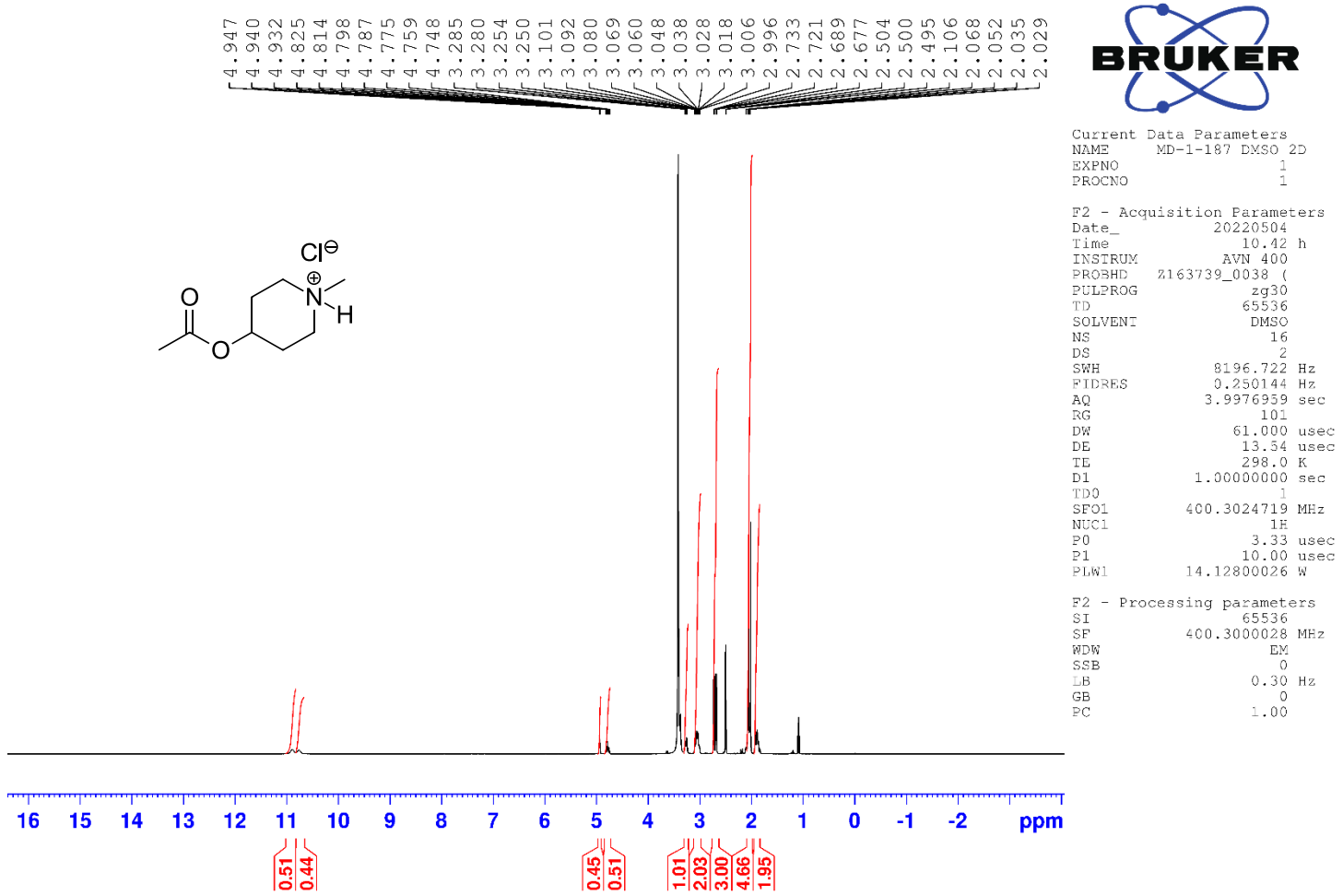
Spectroscopic data:

$MP_{(diethyl\ ether)}$: 82-87°C; IR(ATR) 2958, 2603, 2460, 2427, 1730, 1237, 1037 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 10.90 (br s, 1H), 4.83-4.75 (m, 1H), 3.45-3.43 (m, 1H), 3.28-3.25 (m, 1H), 3.10-3.00 (m, 2H), 2.68 (d, J = 4.8 Hz, 3H), 2.08-2.01 (m, 2H), 2.01 (s, 3H), 1.92-1.86 (m, 2H), isomer B: 10.77 (br s, 1H), 4.95-4.93 (m, 1H), 3.45-3.43 (m, 1H), 3.28-3.25 (m, 1H), 3.10-3.00 (m, 2H), 2.73 (d, J = 4.9 Hz, 3H), 2.08-2.01 (m, 2H), 2.05 (s, 3H), 1.92-1.86 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ isomer a: 169.8 (0), 67.3 (1), 48.7 (2), 41.9 (3), 27.8 (2), 21.0 (3), isomer B: 169.0 (0), 63.7 (1), 51.4 (2), 42.4 (3), 26.5 (2), 21.0 (3); LRMS (ESI^{+}): 158.1 (M^{+}); HRMS (ESI^{+}): calculated for $C_8H_{16}NO_2^{+}$: 158.1176 amu; found for $C_8H_{16}NO_2^{+}$: 158.1176 amu; HPLC purity at 210 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 3.333 mins): >99%. Product was confirmed through comparison of key peaks reported from ^{1}H NMR in the literature.[3]

(**8**) 4-Acetoxy-1-methyl-1-piperidinium Chloride IR(ATR)



(**8**) 4-Acetoxy-1-methyl-1-piperidinium Chloride ^{1}H NMR 400 MHz (DMSO-*d6*)



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PROCNO                   1

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TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8196.722 Hz
FIDRES         0.250144 Hz
AQ             3.9976959 sec
RG                  101
DW               61.000 usec
DE               13.54 usec
TE               298.0 K
D1            1.00000000 sec
TD0                  1
SFO1         400.3024719 MHz
NUC1                1H
P0                3.33 usec
P1               10.00 usec
PLW1         14.12800026 W

F2 - Processing parameters
SI                65536
SF           400.3000028 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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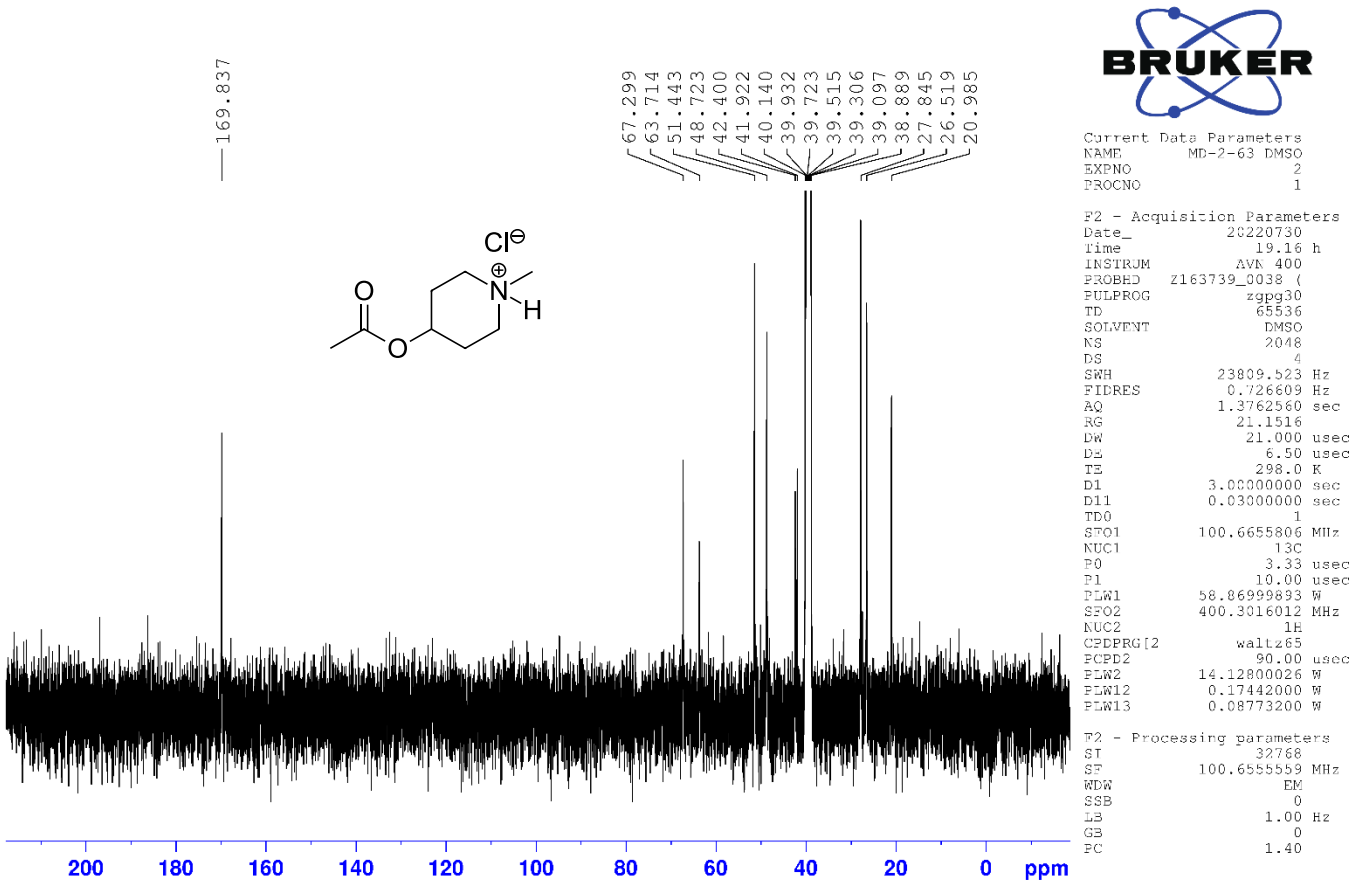
(8) 4-Acetoxy-1-methyl-1-piperidinium Chloride ^{13}C NMR 100 MHz (DMSO-*d6*)



(8) 4-Acetoxy-1-methyl-1-piperidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000008.d	Acquisition Date	7/7/2022 9:38:26 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6014 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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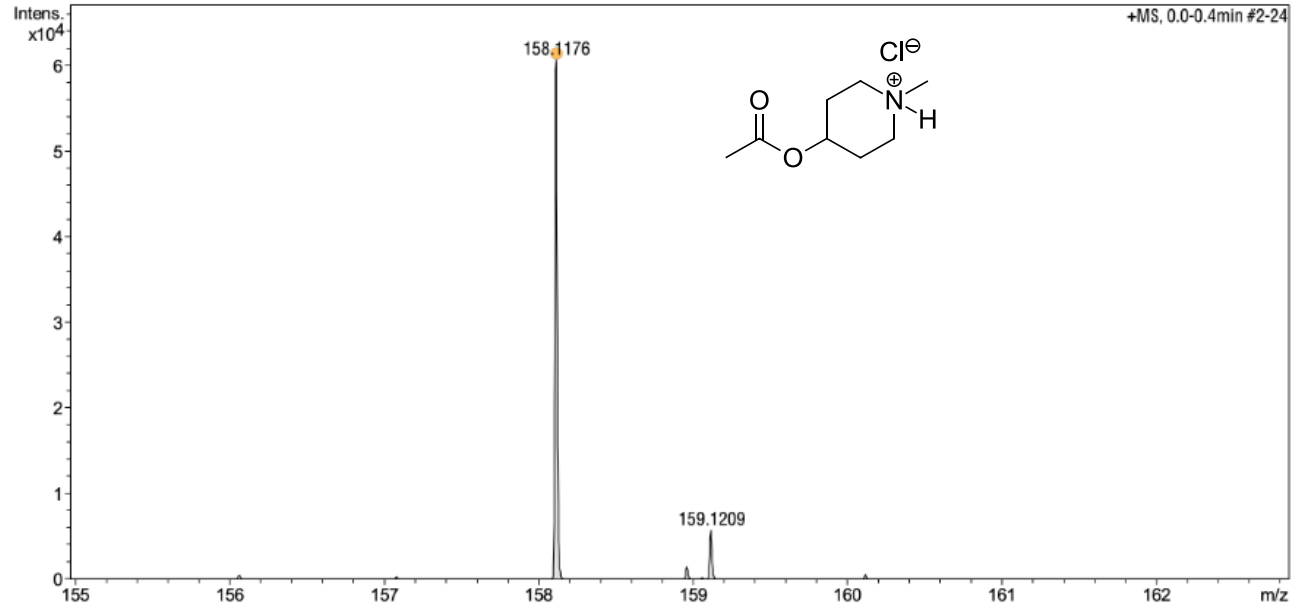

July 07 2022000008.d

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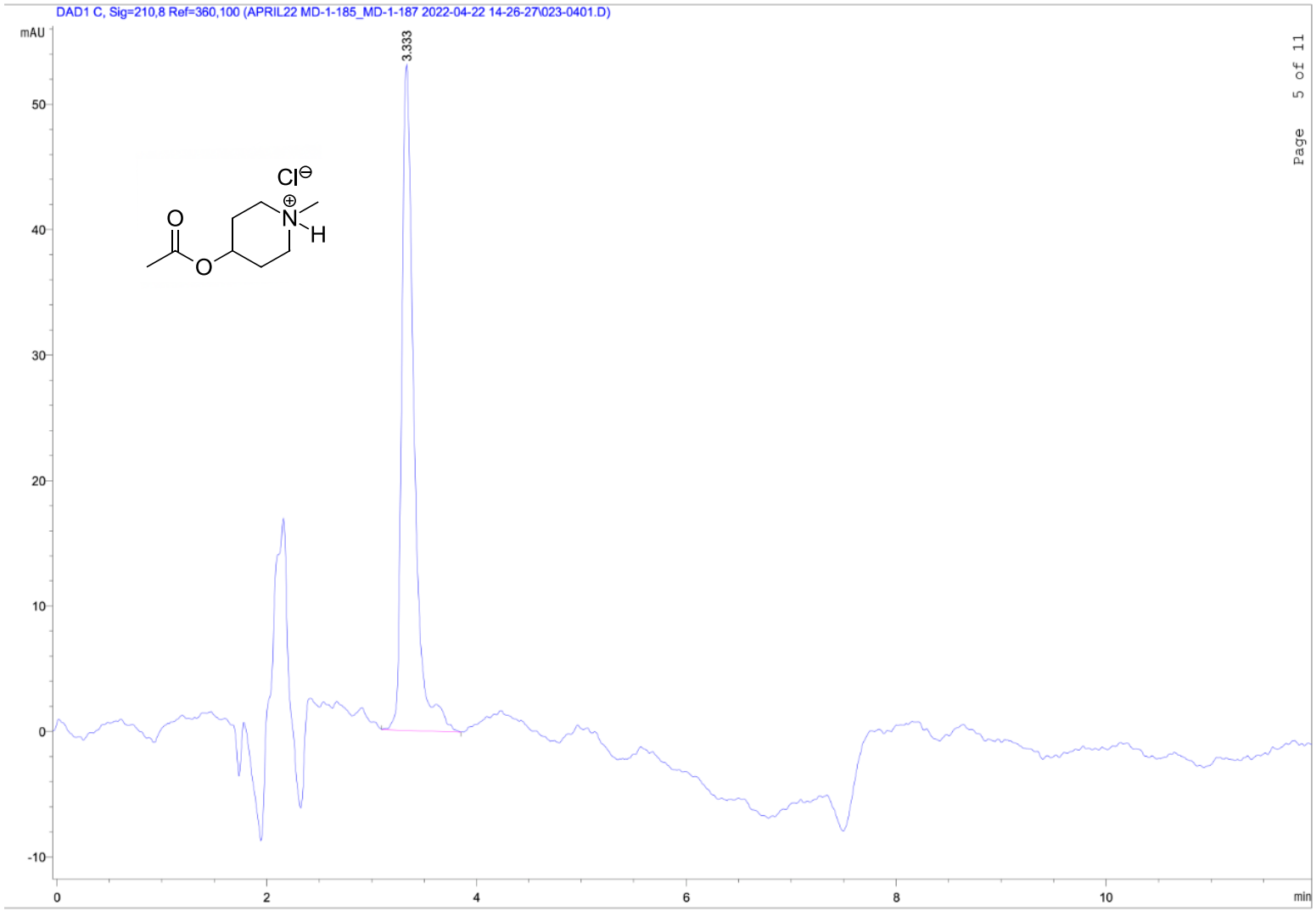
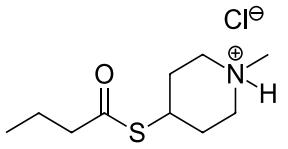
(8) 4-Acetoxy-1-methyl-1-piperidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 3.333 min.



(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride



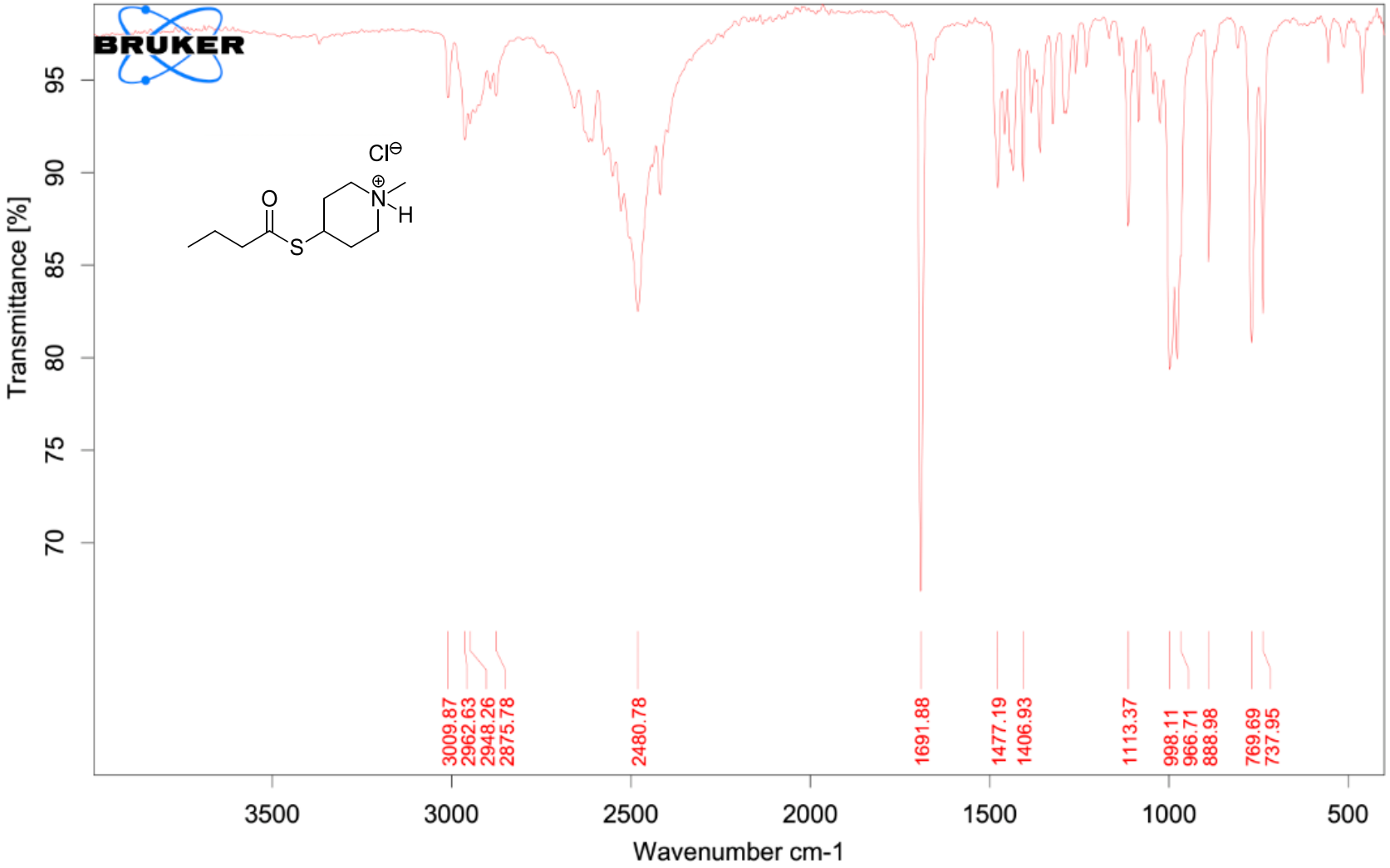
Synthesis: ***S*-(1-Methylpiperid-4-yl) butanethioate**: To a solution of *N*-methyl-4-piperidinethiol (0.50 mL, 4.2 mmol) in anhydrous dichloromethane (5 mL) cooled in an ice bath was added butyryl chloride (1.30 mL, 12.6 mmol) dropwise *via* syringe under an argon atmosphere. After 15 mins, the ice bath was removed and the mixture was allowed to stir for 16 hrs, until TLC indicated complete consumption of starting material. The mixture was diluted with dichloromethane (20 mL). The organic layer was washed with saturated $NaHCO_{3(aq)}$, water and dried over Na_2SO_4. The mixture was gravity filtered and the filtrate was concentrated *in vacuo* to afford 0.846 g of a colorless liquid. Purification *via* flash chromatography (5 % MeOH / DCM) afforded 0.5373 g (64% yield) of **10** as a translucent colorless oil. The analytical data was similar to previously published data.[1]

***S*-(1-Methylpiperid-4-yl) butanethioate, hydrochloride (10)**: *S*-(1-Methylpiperid-4-yl) butanethioate (0.172 g, 0.854 mmol) was dissolved in anhydrous diethyl ether (17 mL) under an argon atmosphere. To this solution, HCl dissolved in diethyl ether (1 N - 1.28 mL, 1.28 mmol) was added dropwise and stirred for 1 hr. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (20 mL), and solvent was removed from the solid with a pipet. The process was repeated twice. The compound was dried *in vacuo* to produce **10** (0.180 g, 89%) as a white solid.

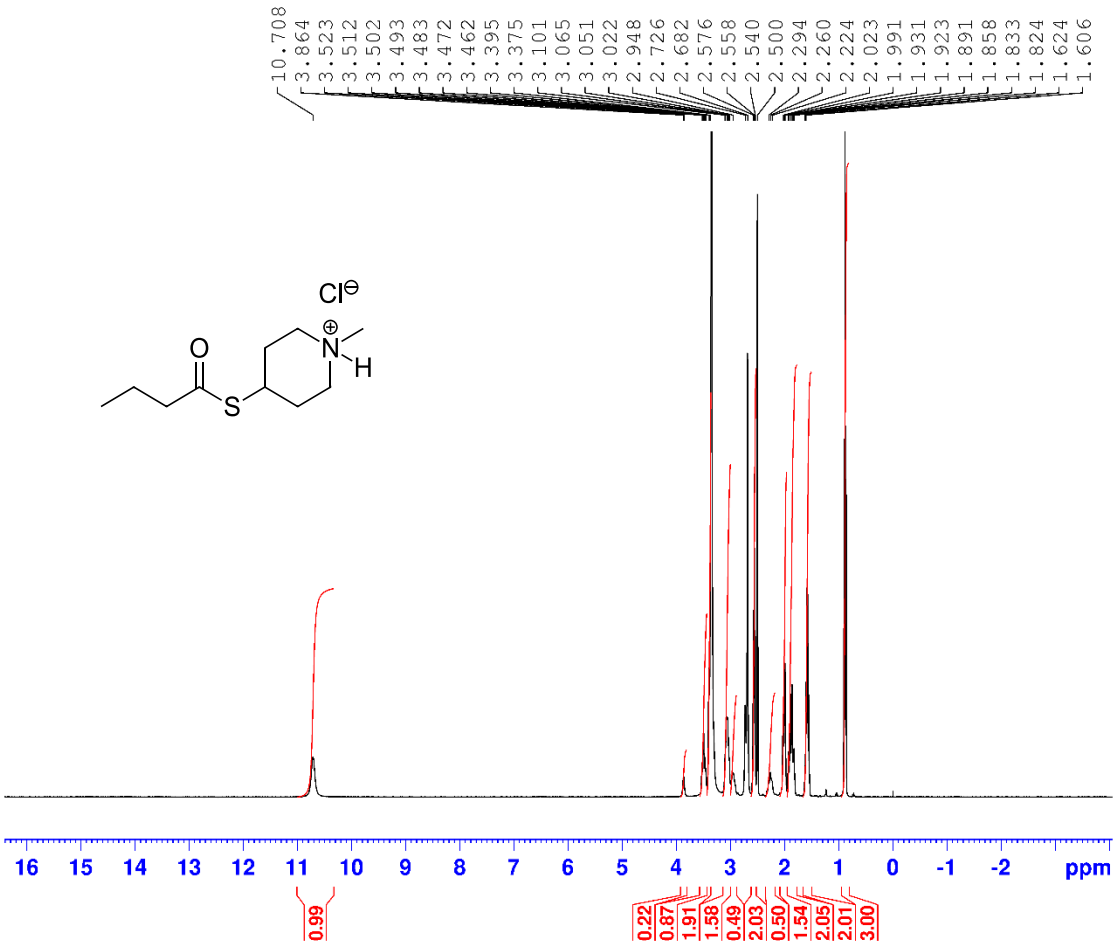
Spectroscopic data: $MP_{(diethyl\ ether)}$: 142−146°C; IR (ATR) 3010, 2963, 2948, 2481, 1691, 1113, 998, 770; ^1H NMR (400 MHz, DMSO-*d6*) d Isomer A: 10.71 (br s, 1H), 3.52-3.46 (m, 1H),

3.40-3.37 (m, 2H), 3.10-3.02 (m, 2H), 2.68 (s, 3H), 2.56 (t, J = 7.2 Hz, 2H), 2.02-1.99 (m, 2H), 1.93-1.82 (m, 2H), 1.58 (sext, J = 7.3 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H), isomer B: 10.71 (br s, 1H), 3.86 (app br s, 1H), 3.40-3.37 (m, 2H), 2.97-2.90 (m, 2H), 2.73 (s, 3H), 2.56 (t, J = 7.2 Hz, 2H), 2.29, 2.22 (m, 2H), 1.93-1.82 (m, 2H), 1.58 (sext, J = 7.3 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) d isomer A: 198.0 (0), 53.1 (2), 45.3 (2), 42.4 (3), 36.4 (1), 28.9 (2), 18.6 (2), 13.2 (3), isomer B: 198.0 (0), 50.2 (2), 45.6 (2), 41.7 (3), 35.8 (1), 27.3 (2), 18.6 (2), 13.2 (3); LRMS (ESI$^+$): 202.1 (M+); HRMS (ESI$^+$): calculated for $C_{10}H_{20}NOS^+$: 202.1260; found for $C_{10}H_{20}NOS^+$: 202.1261; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 5.212 mins): 99.1%.

(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride IR(ATR)



(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride ^{1}H NMR 400 MHz (DMSO-*d6*)

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TD                      65536
SOLVENT                  DMSO
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DS                          2
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FIDRES                0.250144 Hz
AQ                    3.9976959 sec
RG                         101
DW                      61.000 usec
DE                      13.54 usec
TE                      298.0 K
D1                    1.00000000 sec
TD0                          1
SFO1                400.3024719 MHz
NUC1                        1H
P0                       3.33 usec
P1                      10.00 usec
PLW1                14.12800026 W

F2 - Processing parameters
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WDW                        EM
SSB                         0
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GB                          0
PC                       1.00
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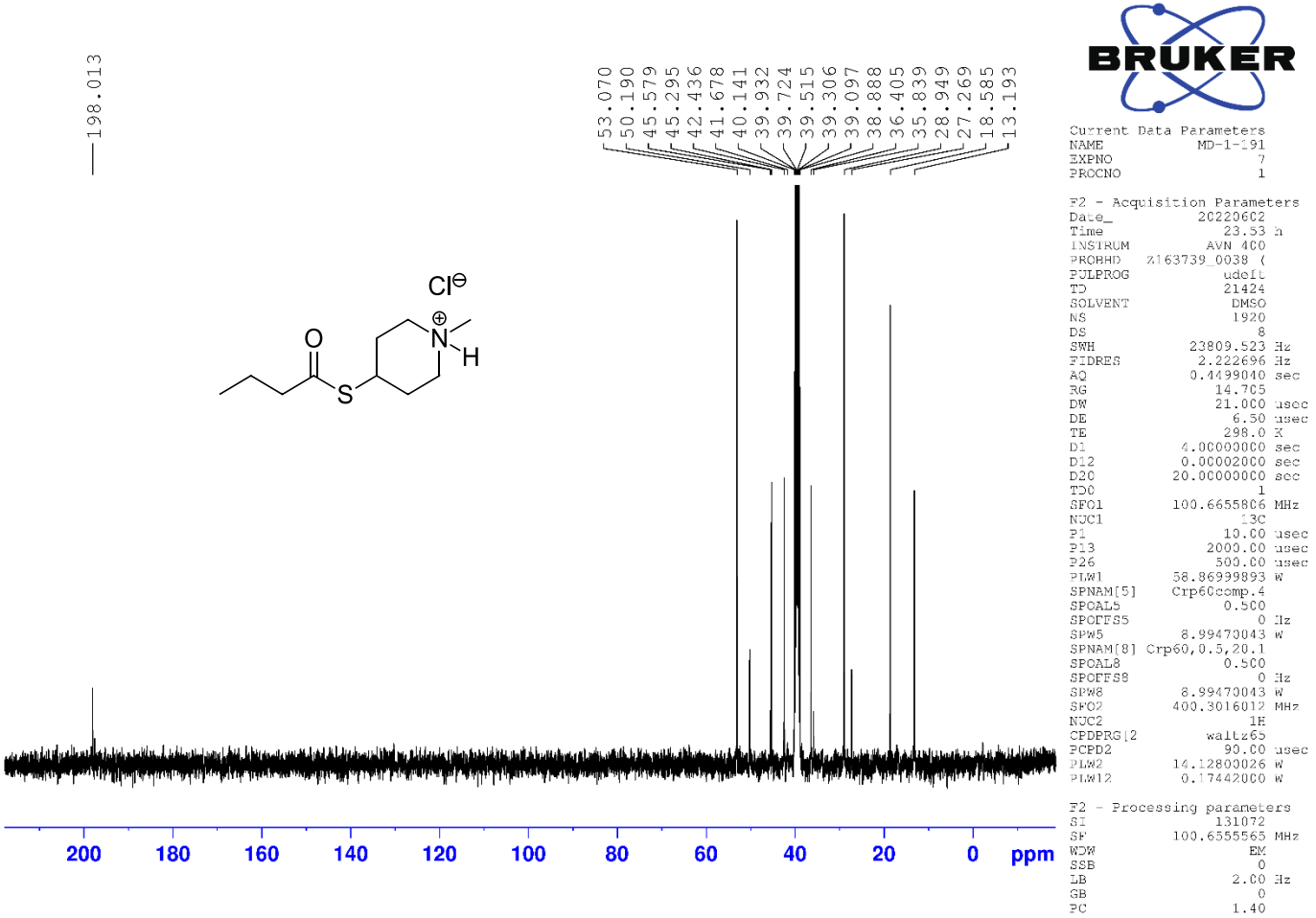
(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride ^{13}C NMR 100 MHz (DMSO-*d6*)



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PROCNO                       1

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TD                     21424
SOLVENT                 DMSO
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FIDRES              2.222696 Hz
AQ                  0.4499040 sec
RG                     14.765
DW                     21.000 usec
DE                      6.50 usec
TE                     298.0 K
D1                  4.00000000 sec
D12                 0.00002000 sec
D20                20.00000000 sec
TD0                          1
SFO1               100.6655806 MHz
NUC1                       13C
P1                       10.00 usec
P13                    2000.00 usec
P26                     500.00 usec
PLW1               58.86999893 W
SPNAM[5]           Crp60comp.4
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SPOFFS5                      0 Hz
SPW5                8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8                   0.500
SPOFFS8                      0 Hz
SPW8                8.99470043 W
SFO2               400.3016012 MHz
NUC2                        1H
CPDPRG[2               waltz65
PCPD2                    90.00 usec
PLW2               14.12800026 W
PLW12               0.17442000 W

F2 - Processing parameters
SI                      131072
SF                 100.6555565 MHz
WDW                        EM
SSB                         0
LB                       2.00 Hz
GB                          0
PC                       1.40
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(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000004.d	Acquisition Date	7/7/2022 9:17:31 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6010 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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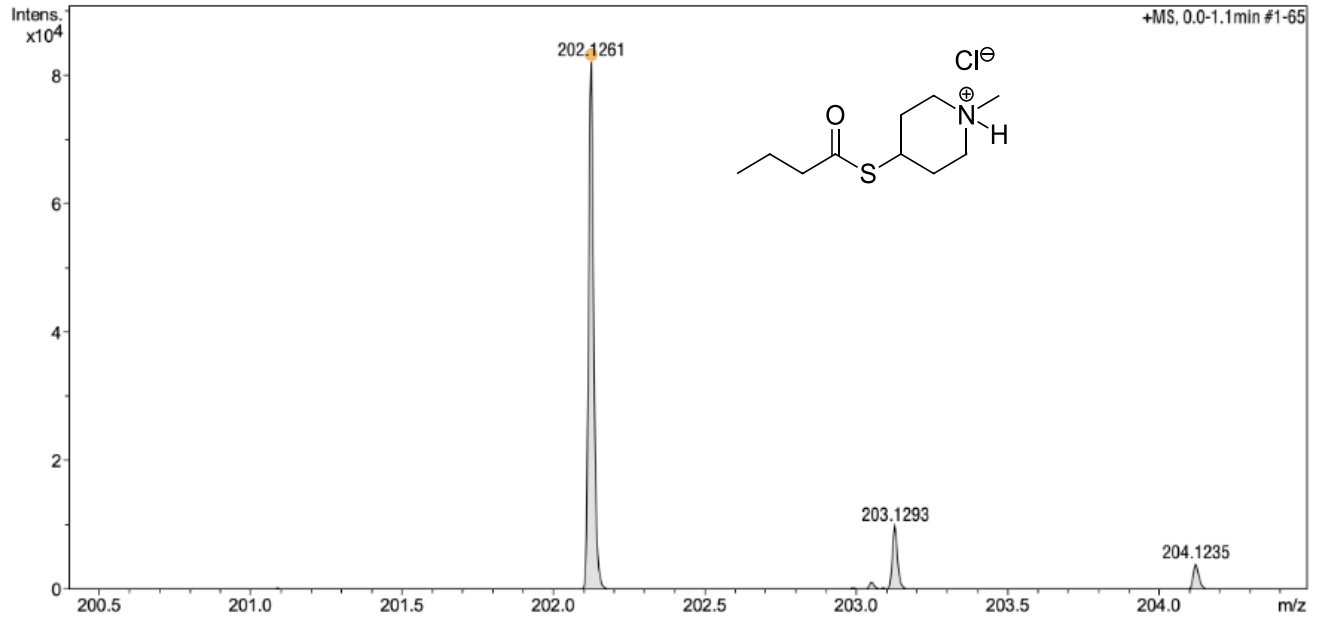

July 07 2022000004.d

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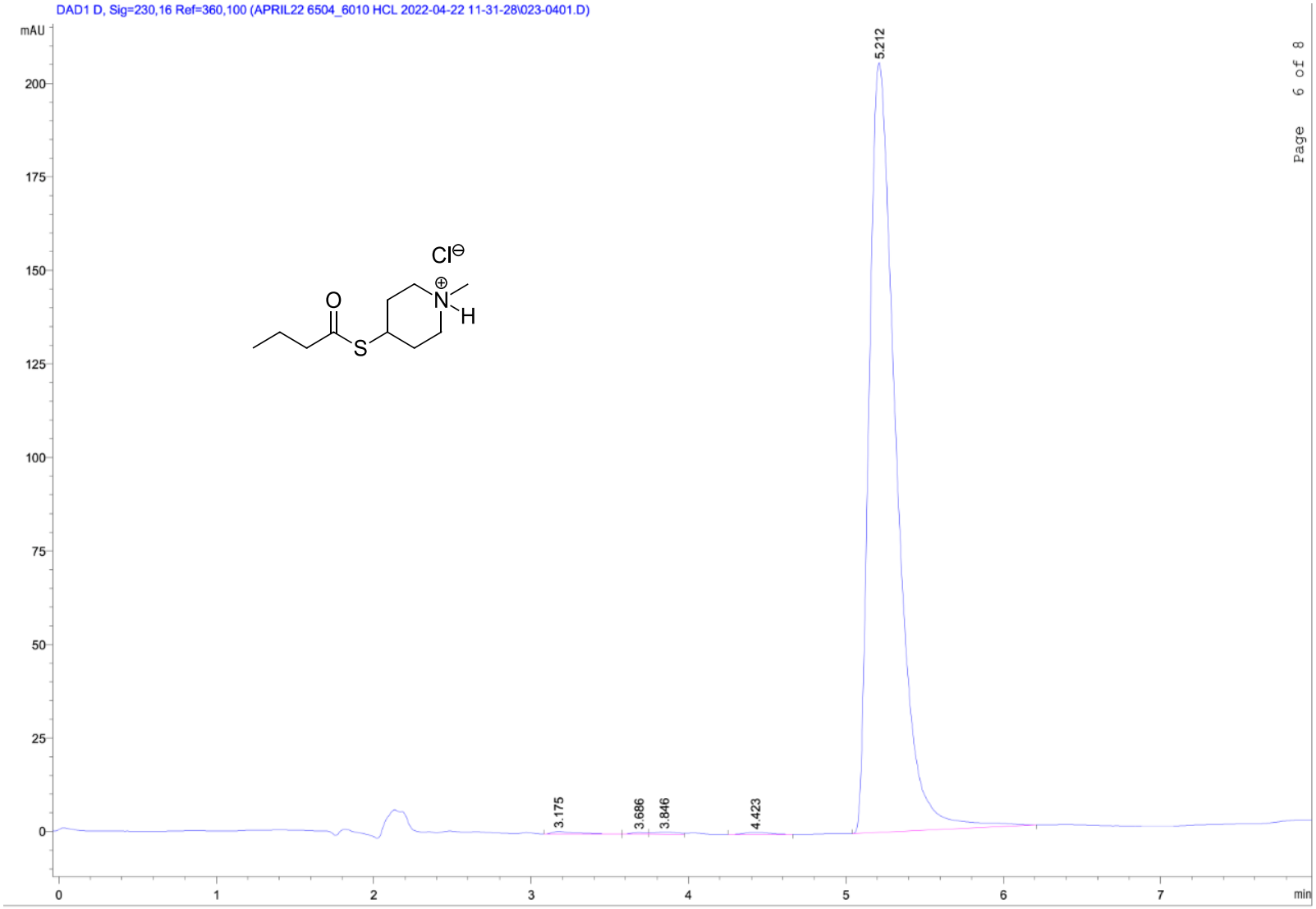
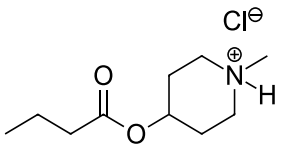
(9) *S*-(1-Methylpiperid-4-yl) Butanethioate Hydrochloride HPLC



(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride



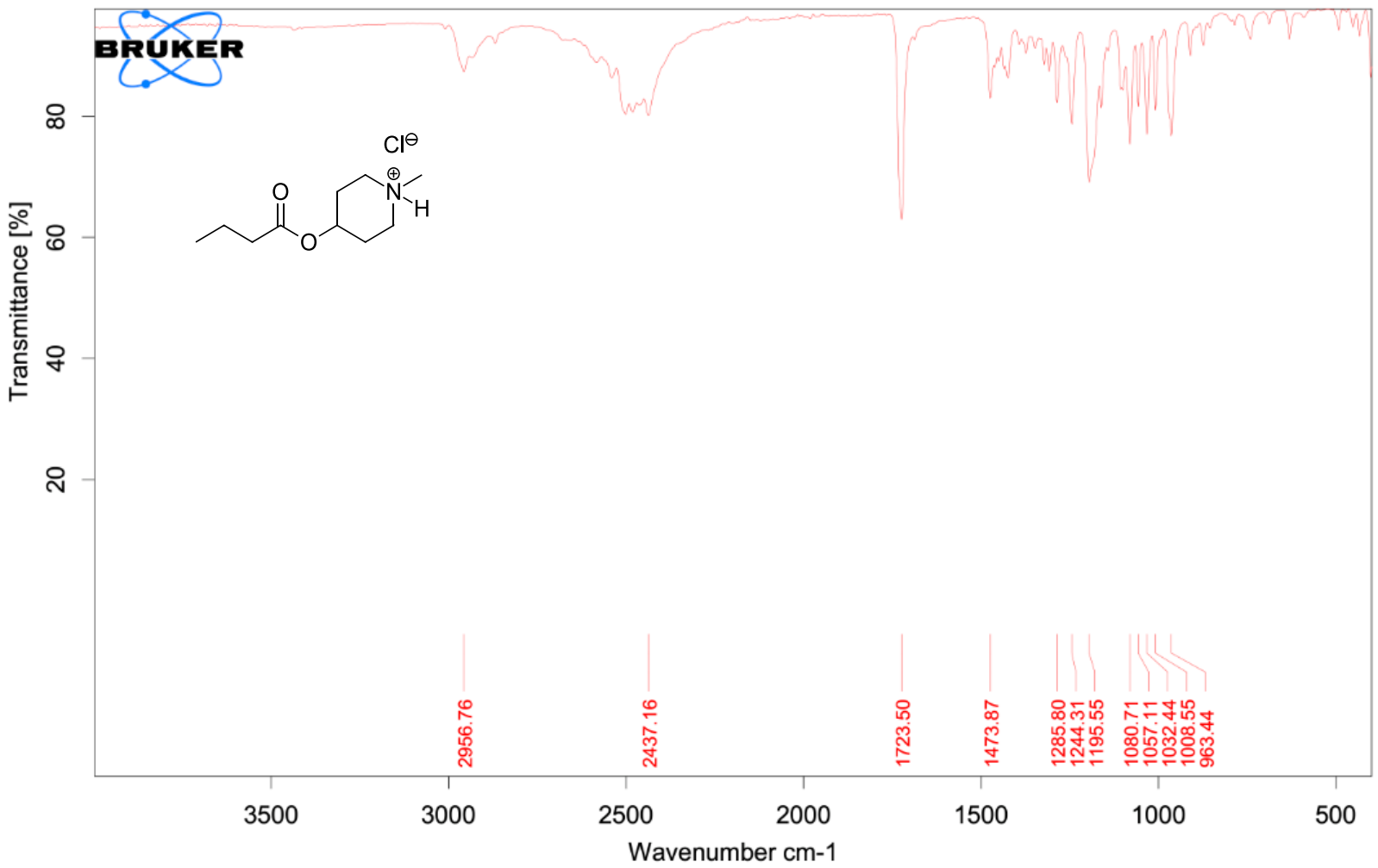
Synthesis:

***N*-Methylpiperidin-4-yl Butyrate**: *N*-Methyl-4-piperidinol (0.803 g, 5.18 mmol) was dissolved in anhydrous dichloromethane (10 mL) and stirred under an argon atmosphere. Triethylamine (1.44 mL, 10.4 mmol) was added, and the reaction was cooled to 0°C with an ice bath. Butyryl chloride (0.699 mL, 6.73 mmol) was added dropwise at 0°C. The ice bath was removed after 5 mins, and reaction was stirred for 3 hrs. The reaction was quenched with brine (20 mL), extracted with dichloromethane (3 × 15 mL) and dried over Na_2SO_3. The solvent was removed *in vacuo* to produce a yellow oil. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to produce a yellow oil, *N*-methylpiperidin-4-yl butyrate (0.954 g, 99%). The analytical data was similar to previously published data.[4]

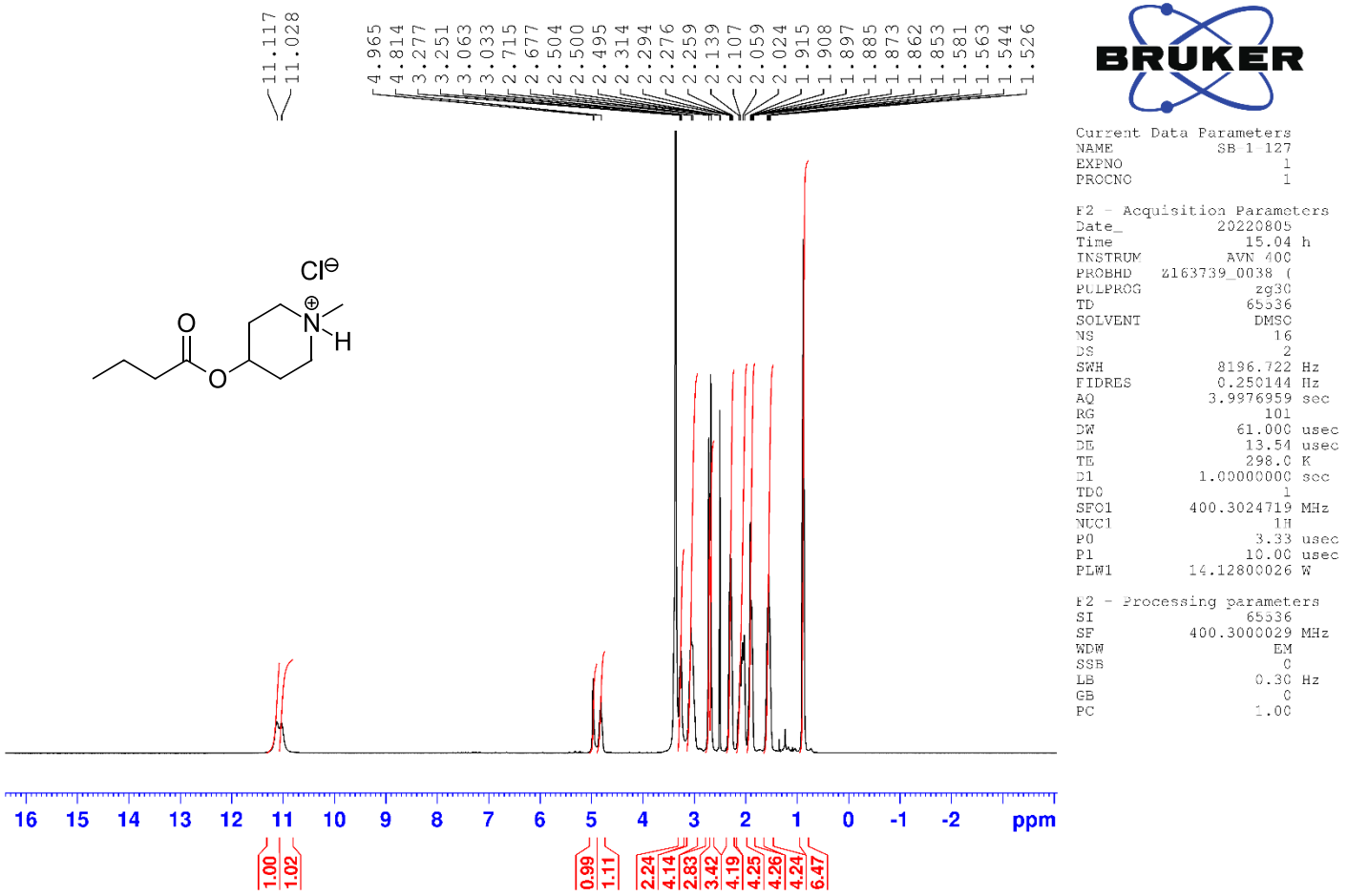
4-Butyroxy-1-methyl-1-piperidinium chloride (9): To a flask charged with *N*-methylpiperidin-4-yl butyrate (0.48 g, 2.59 mmol) anhydrous diethyl ether was added until complete dissolution (52 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 3.88 mL, 3.88 mmol) was added dropwise and stirred for 1 hr. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (20 mL), and solvent was removed from the solid with a pipet. This process was repeated two more times. The compound was dried *in vacuo* to produce 4-butyroxy-1-methyl-1-piperidinium chloride (0.583 g, 90%) as a white solid as a pair of isomers (46:54).

Spectroscopic data: $MP_{(diethyl\ ether)}$: 110–113°C; IR(ATR) 2957, 2437, 1724, 1244, 1195, 963 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 11.12 (br s, 1H), 4.98-4.95 (m, 1H), 3.41-3.77 (m, 2H), 3.10-3.00 (m, 2H), 2.72 (s, 3H), 2.33-2.26 (m, 2H), 2.14-2.06 (m, 2H), 1.92-1.85 (m, 2H), 1.60-1.51 (m, 2H), 0.89-0.86 (m, 3H) isomer B: 11.03 (br s, 1H), 4.84-4.79 (m, 1H), 3.27-3.25 (m, 2H), 3.10-3.00 (m, 2H), 2.68 (s, 3H), 2.33-2.26 (m, 2H), 2.06-2.2.02 (m, 2H), 1.92-1.85 (m, 2H), 1.60-1.51 (m, 2H), 0.89-0.86 (m, 3H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ isomer a: 172.0 (0), 63.5(1), 48.6 (2), 42.3 (3), 35.6 (2), 26.5 (2), 17.9 (2), 13.4 (3), isomer B: 172.2 (0), 67.2 (1), 51.3 (2), 41.8 (3), 35.4 (2), 27.8 (2), 17.9 (2), 13.3 (3); LRMS (ESI^{+}): 186.1 (M^{+}); HRMS (ESI^{+}): calculated for $C_{10}H_{20}NO_2^{+}$: 186.1488 amu; found for $C_{10}H_{20}NO_2^{+}$: 186.1492 amu; HPLC purity at 210 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.406 mins): >99%.

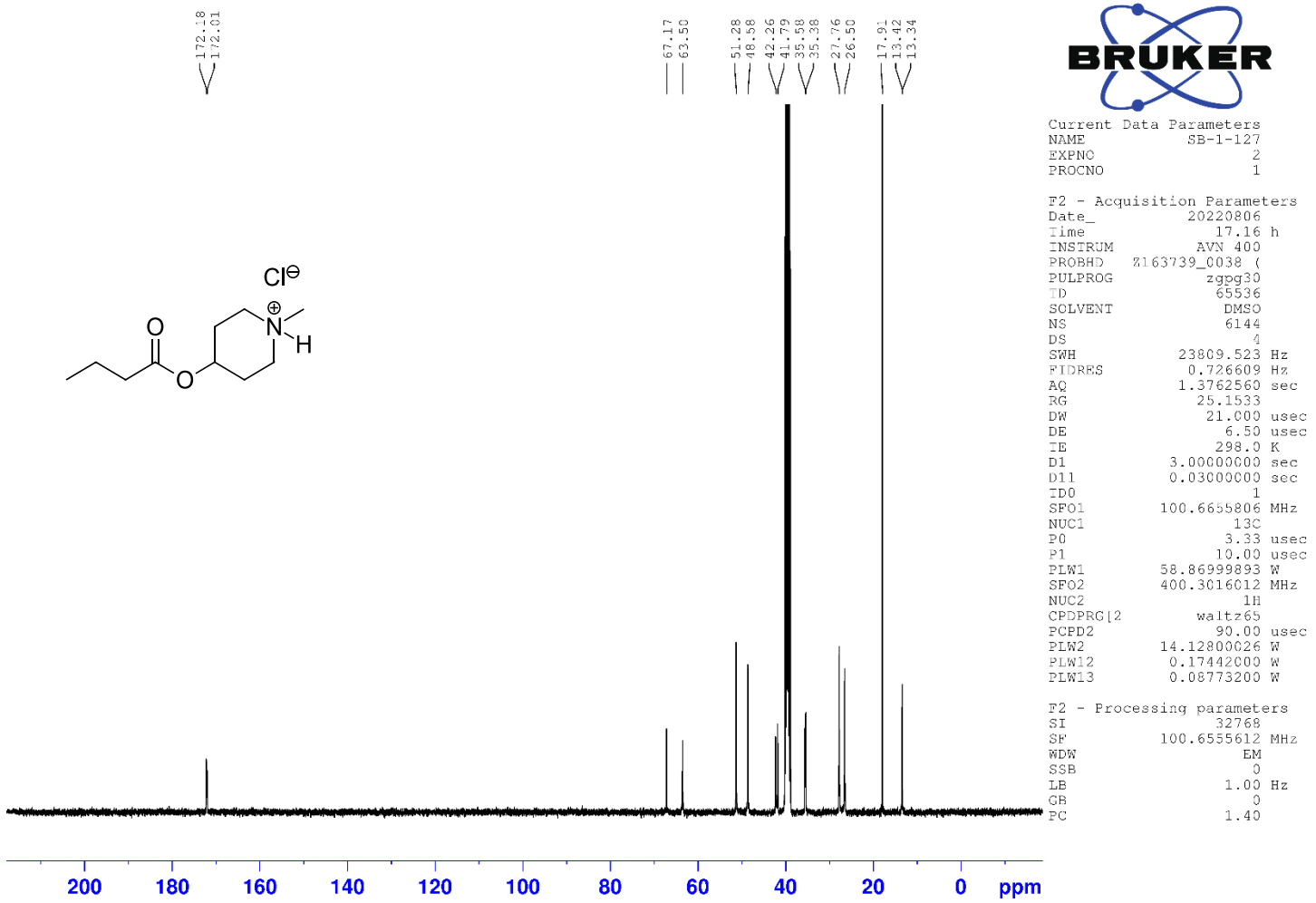
(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride IR(ATR)



(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride ^{1}H NMR 400 MHz (DMSO-*d6*)



(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride ^{13}C NMR 100 MHz (DMSO-*d6*)

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DE                        6.50 usec
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NUC1                       13C
P0                        3.33 usec
P1                       10.00 usec
PLW1               58.86999893 W
SFO2               400.3016012 MHz
NUC2                       1H
CPDPRG[2                waltz65
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PLW12               0.17442000 W
PLW13               0.08773200 W

F2 - Processing parameters
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GB                           0
PC                        1.40
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(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	TRV 6013 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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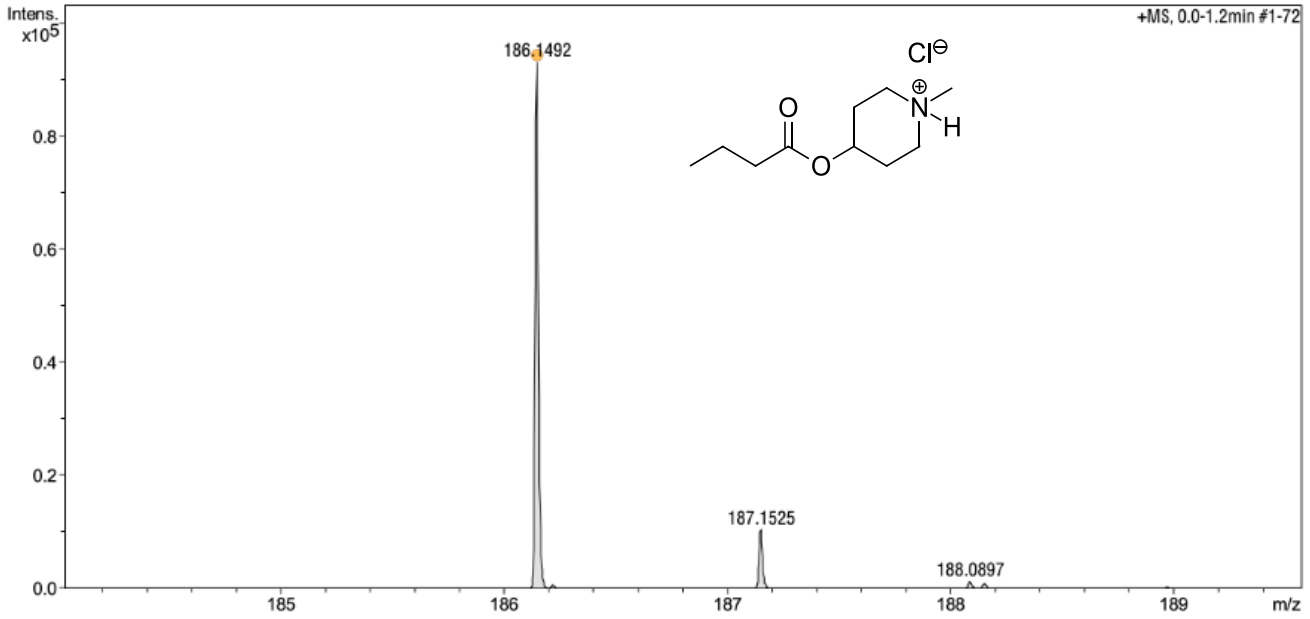

July 07 2022000006.d

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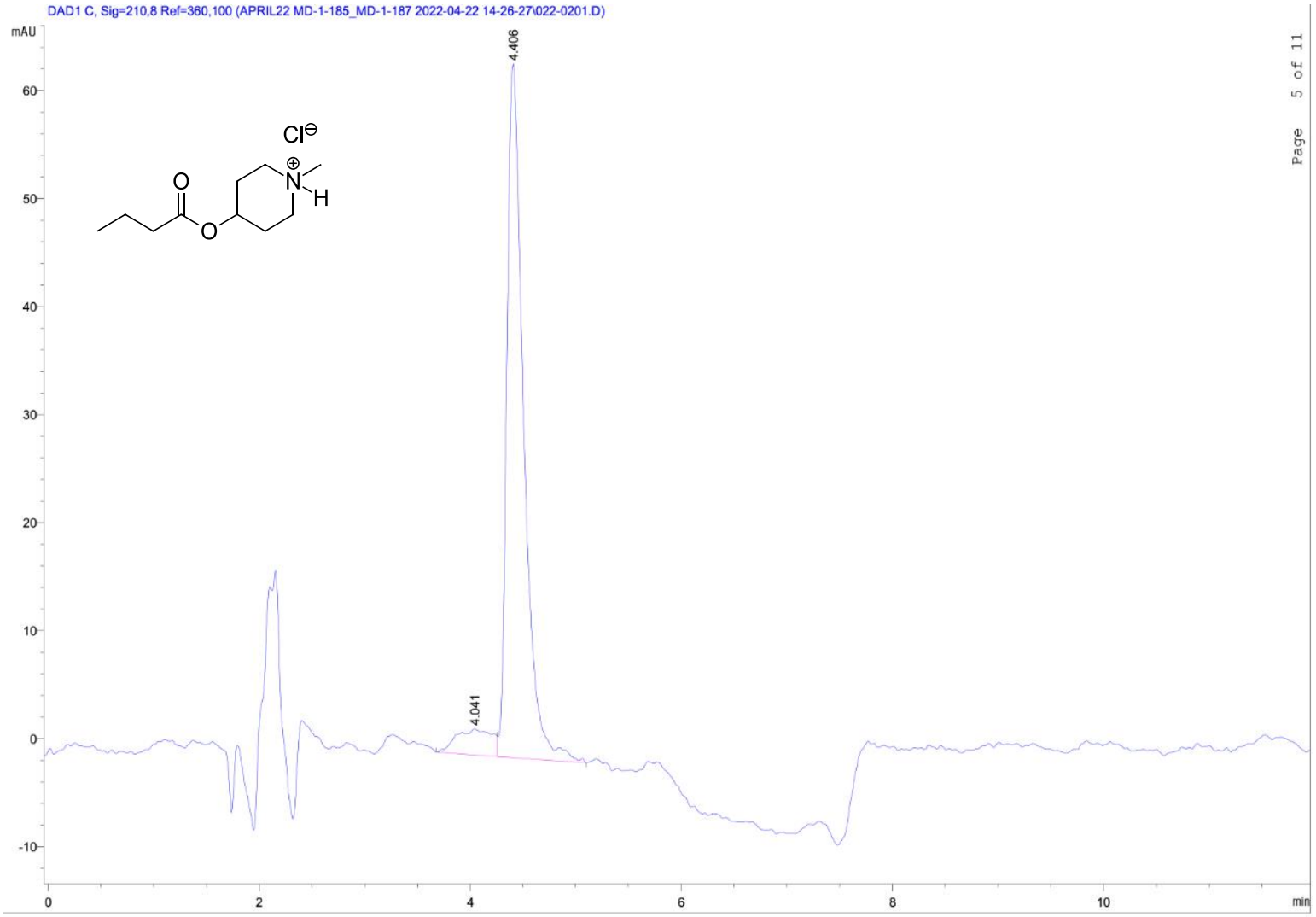
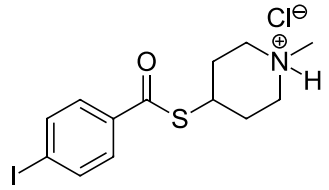
(10) 4-Butyroxy-1-methyl-1-piperidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 4.406 min.



Aryl N-Methylpiperidinyl Derivatives

(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride



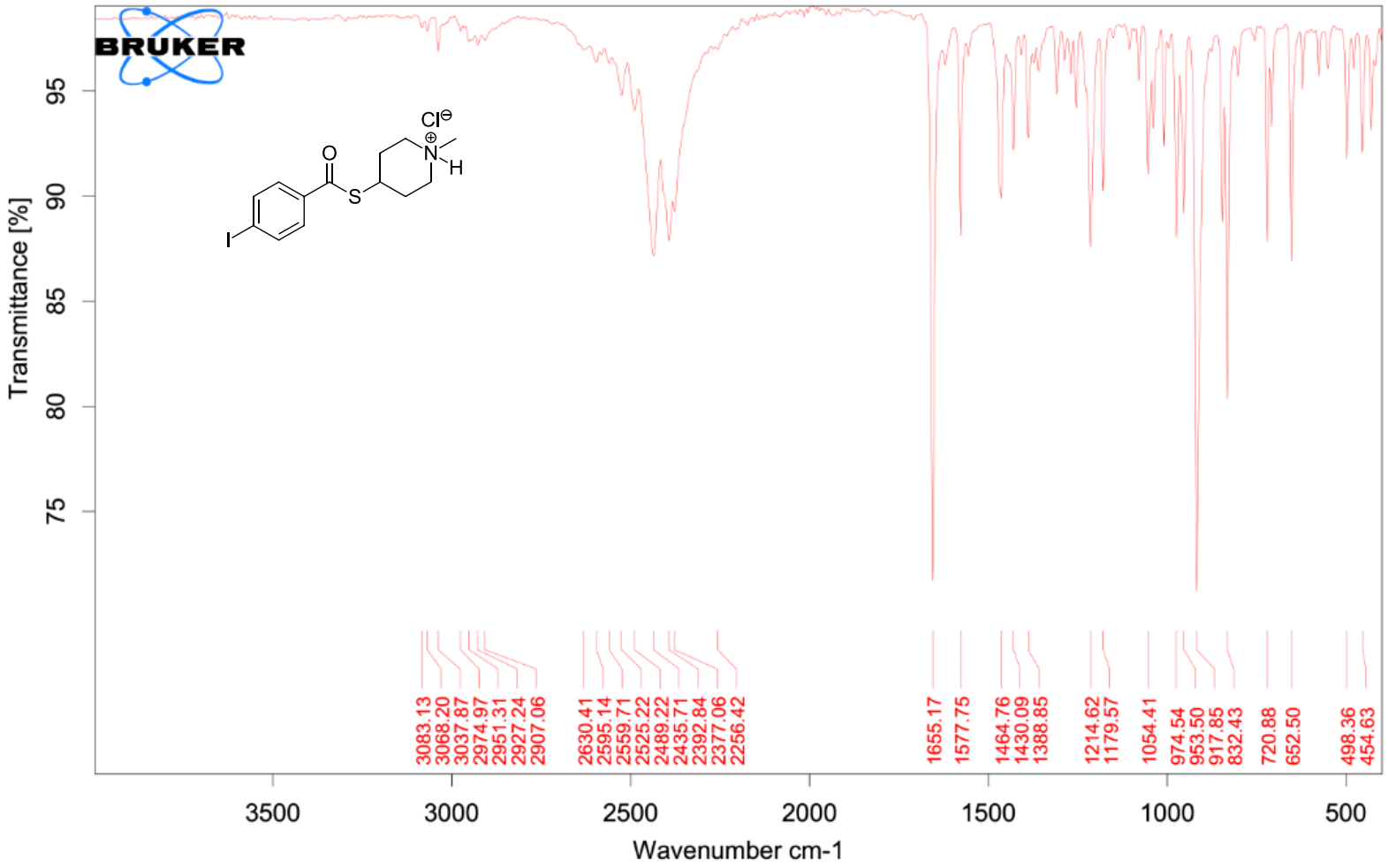
Synthesis: **(1-Methylpiperidin-4-yl) *p*-iodobenzenecarbothioate**: *N*-methyl-4-piperidinethiol (0.538 g, 4.1 mmol) and triethylamine (0.90 mL, 6.2 mmol) were dissolved in anhydrous dichloromethane (10 mL) under an argon atmosphere. 4-Iodobenzoyl chloride (1.31 g, 4.9 mmol) was added at 0°C. The ice bath was removed after 5 mins and the reaction was stirred for 16 hrs at room temperature. The reaction was quenched with brine (20 mL), extracted with dichloromethane (3 × 15 mL), and the combined organic layers were dried over Na_2SO_3. After gravity filtration, the solvent was removed *in vacuo* to produce 1.49 g of a brown solid. The crude mixture was purified by silica gel column chromatography (step gradient: 5% MeOH/DCM to 10 % MeOH/DCM) to produce a white solid, (1-methylpiperidin-4-yl) *p*-iodobenzenecarbothioate (0.644 g, 43% yield). The analytical data was similar to previously published data.[1]

(1-Methylpiperidin-4-yl) *p*-iodobenzenecarbothioate hydrochloride (12):
(1-methylpiperidin-4-yl) *p*-iodobenzenecarbothioate (0.47 g, 1.3 mmol) was dissolved in anhydrous diethyl ether (86 mL) under an argon atmosphere. HCl in diethyl ether (1 N solution, 2.0 mL, 2.0 mmol) was added dropwise resulting in the immediate formation of a white precipitate. The reaction was stirred for an additional 60 mins. The white solid was collected *via*

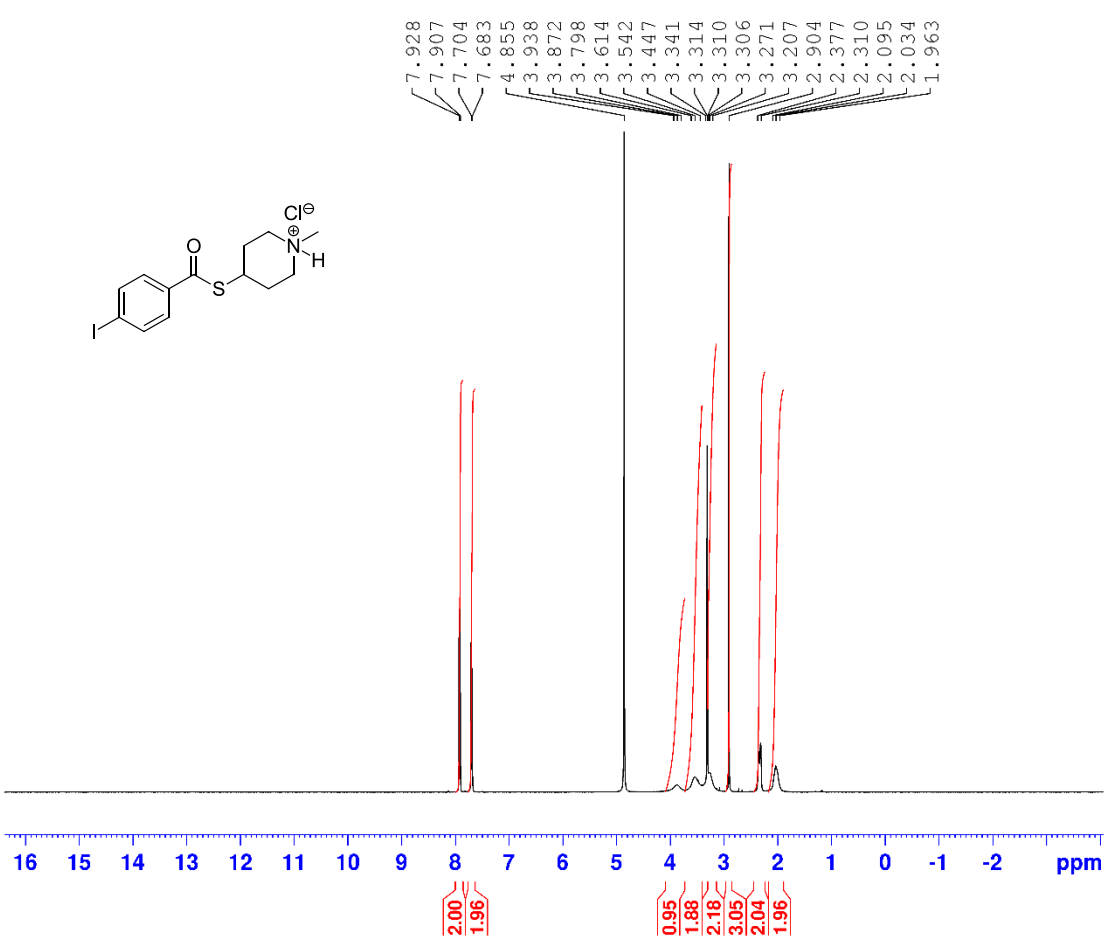
suction filtration, washed with Et_2O (2 × 30 mL), to afford (1-methylpiperidin-4-yl) *p*-iodobenzenecarbothioate hydrochloride, (0.450 g, 87% yield) as a white solid.

Spectroscopic data: $MP_{(diethyl\ ether)}$: >250°C; IR(ATR) 3083, 3068, 3038, 2975, 2951, 2436, 2393, 1655, 1578, 1465, 918; ^1H NMR (400 MHz, CD_3OD) δ 7.93-7.91 (m, 2H), 7.70-7.68 (m, 2H), 3.94-3.80 (m, 1H), 3.62-3.47 (m, 2H), 3.34-3.18 (m, 2H), 2.90 (s, 3H), 2.34-2.31 (m, 2H), 2.09-1.98 (m, 2H); ^{13}C NMR (100.7 MHz, CD_3OD) δ 191.4, 139.5, 137.3, 129.5, 102.5, 55.4, 43.8, 37.9, 30.6; LRMS (ESI^+): 362.0 (M^+); HRMS (ESI^+): calculated for $C_{13}H_{17}INOS^+$: 362.0070 amu; found for $C_{13}H_{17}INOS^+$: 362.0073 amu; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 11.364 mins): 98.2 %.

(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride IR(ATR)



(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride ^{1}H NMR 400 MHz (CD_3OD-*d4*)



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TE 298.0 K
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P0 3.33 usec
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PLW1 14.12800026 W

F2 - Processing parameters
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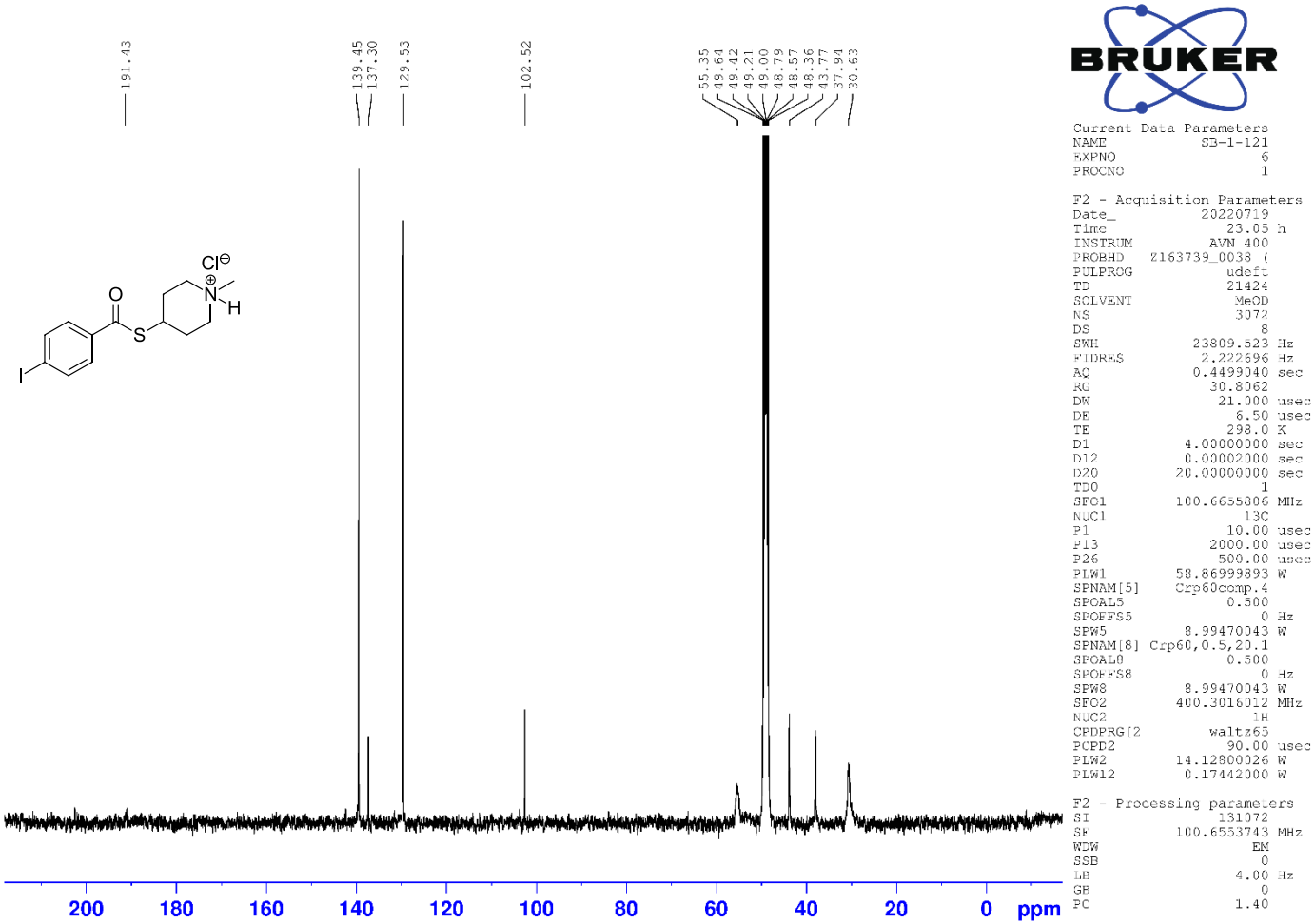
(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride ^{13}C NMR 100 MHz (CD_3OD-*d4*)

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SFO2               400.3016012 MHz
NUC2                        1H
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PCPD2                    90.00 usec
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PLW12               0.17442000 W

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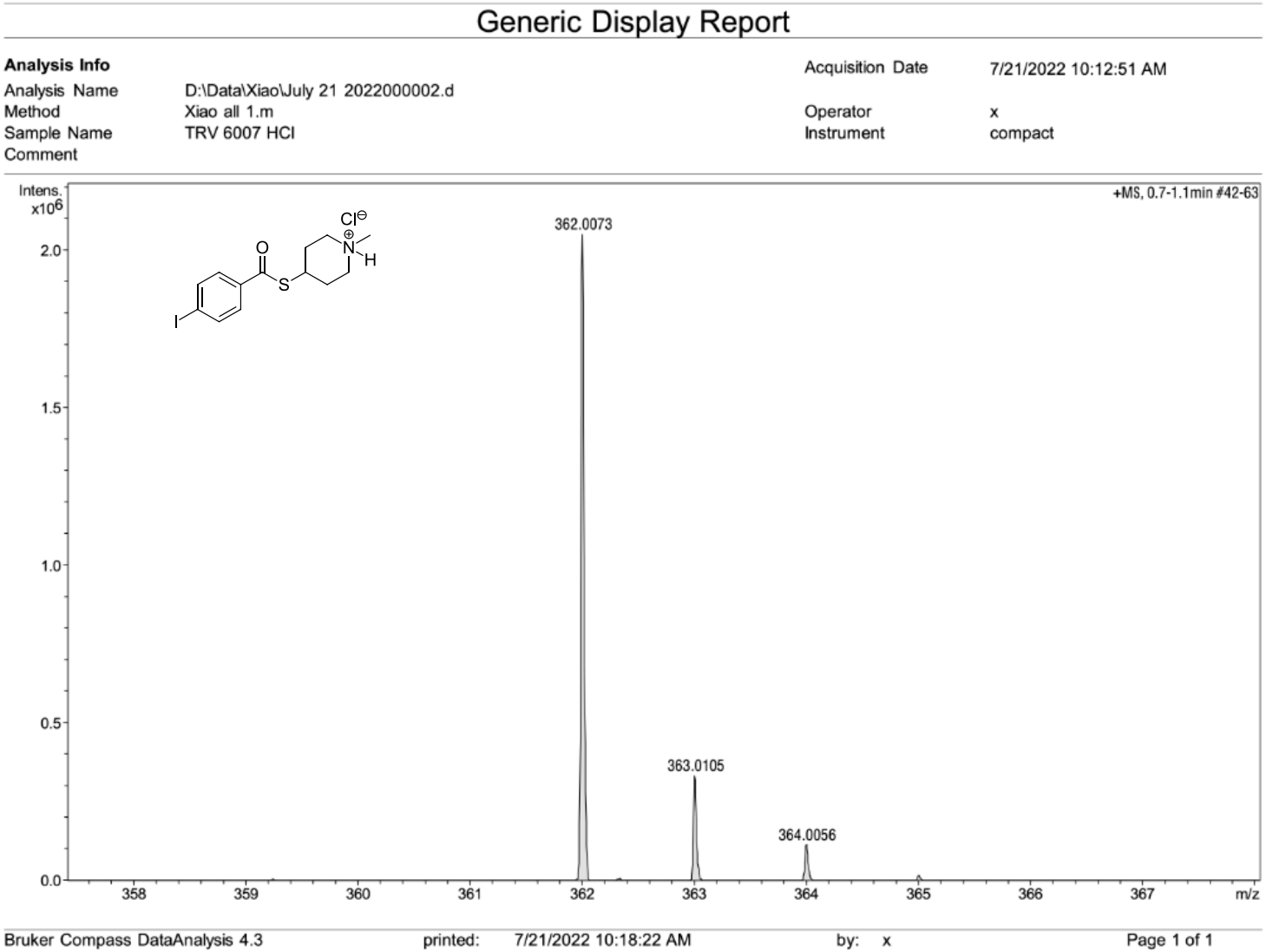
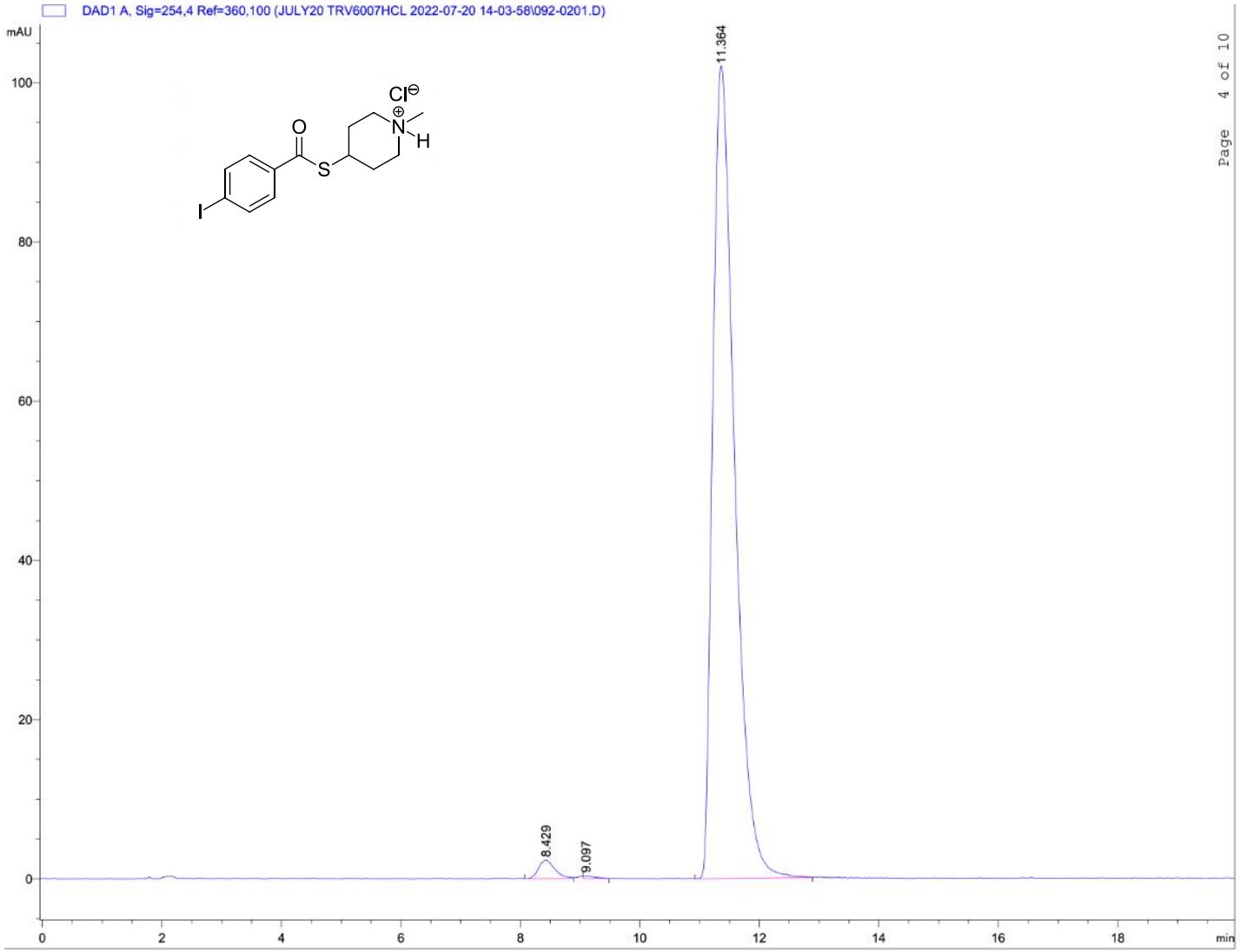
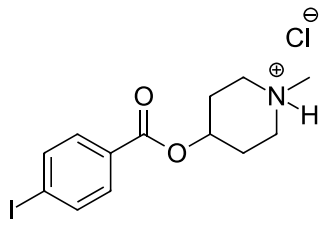
(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride High Resolution Mass Spectrum (ESI, positive mode)



(11) (1-Methylpiperidin-4-yl) *p*-Iodobenzenecarbothioate Hydrochloride HPLC



(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride



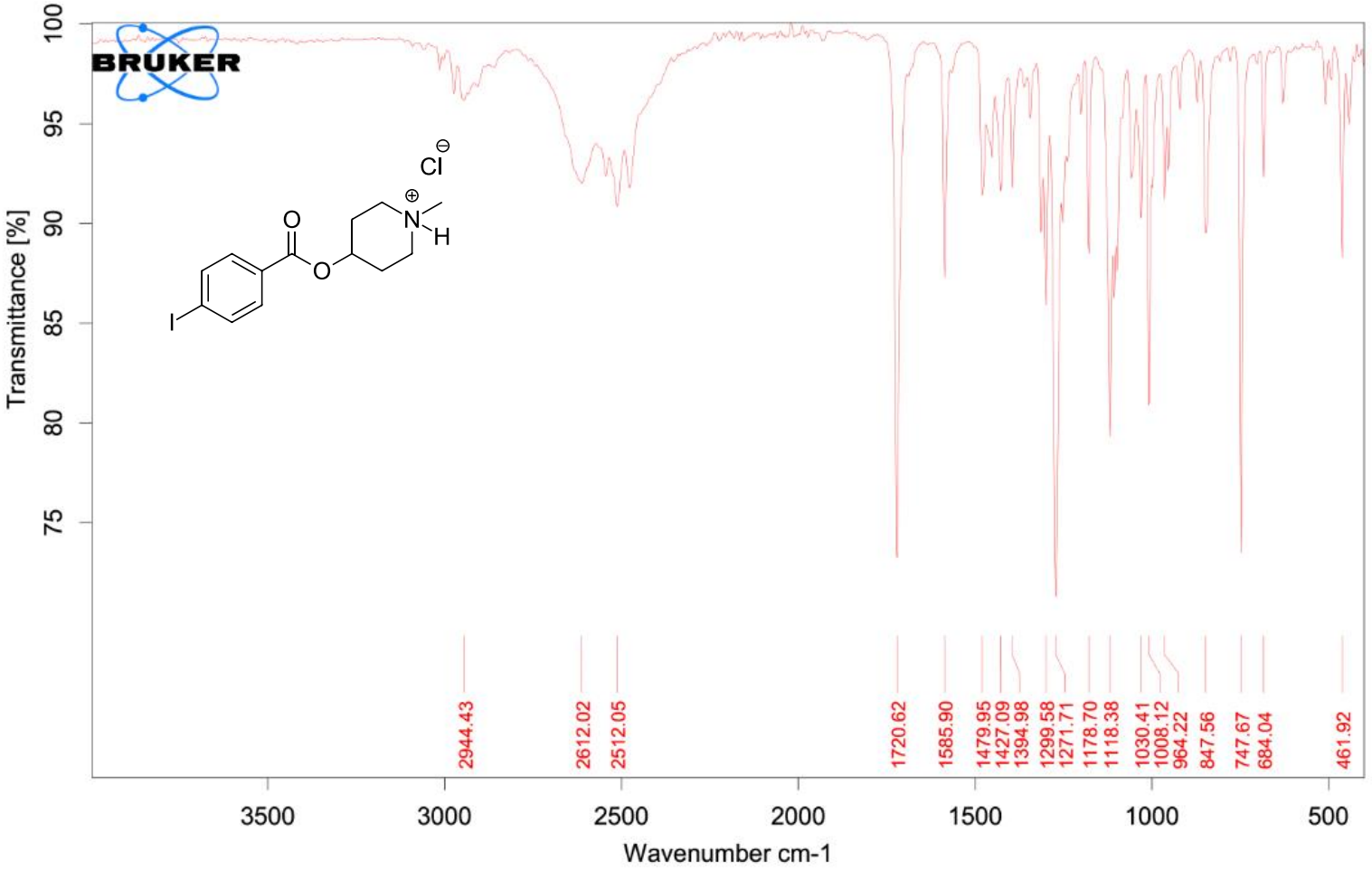
Synthesis: **1-Methylpiperidin-4-yl 4-iodobenzoate**: A sample from a previous study was obtained from our lab and the analytical data was similar to previously published data.[1]

4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium chloride (**11**): *N*-methylpiperidin-4-yl 4-iodobenzoate (0.4032 g, 1.2 mmol) was dissolved in anhydrous diethyl ether (39 mL) under an argon atmosphere. HCl in diethyl ether (1 N solution, 1.8 mL, 1.8 mmol) was added dropwise resulting in the immediate formation of a white precipitate. The reaction was stirred for an additional 60 mins. The precipitate was collected via suction filtration, washed with Et_2O (2 x 15 mL), to afford 4-(*p*-iodobenzoyloxy)-1-methyl-1-piperidinium chloride (0.200 mg, 44 % yield) as a white solid and a mixture of isomers (59:41).

Spectroscopic data: $MP_{(diethyl\ ether)}$: >250°C(Lit $MP_{(acetone/EtOH/H2O)}$:267-268.5°C);[5] IR(ATR) 3014, 3003, 2944, 2612, 2512, 1721, 1586, 1272, 1118, 1008, 748; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 10.88 (br s, 1H), 7.94-7.92 (m, 2H), 7.73-7.71 (m, 2H), 5.11-5.02 (m, 1H), 3.33-3.30 (m, 2H), 3.17-3.07 (m, 2H), 2.73 (s, 3H), 2.21-2.15 (m, 2H), 2.06-2.00 (m, 2H), isomer B: 10.69 (br s, 1H), 7.94-7.92 (m, 2H), 7.86-784 (m, 2H), 5.23 (app br s, 1H), 3.47-3.44 (m, 2H), 3.25-3.17 (m, 2H), 2.79 (s, 3H), 2.21-2.15 (m, 2H), 2.06-2.00 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ isomer A: 164.7 (0), 137.8 (1), 131.0 (1), 128.9 (0), 102.14 (0), 68.4 (1), 51.2 (2), 41.8 (3), 27.7 (2), isomer B: 164.6 (0), 137.6 (1), 131.3 (1), 129.2 (0), 102.07 (0), 64.8 (1), 48.7 (2), 42.4 (3), 26.7 (2); LRMS (ESI^+): 346.0 (M^+); HRMS (ESI^+): calculated for $C_{13}H_{17}INO_2^+$:

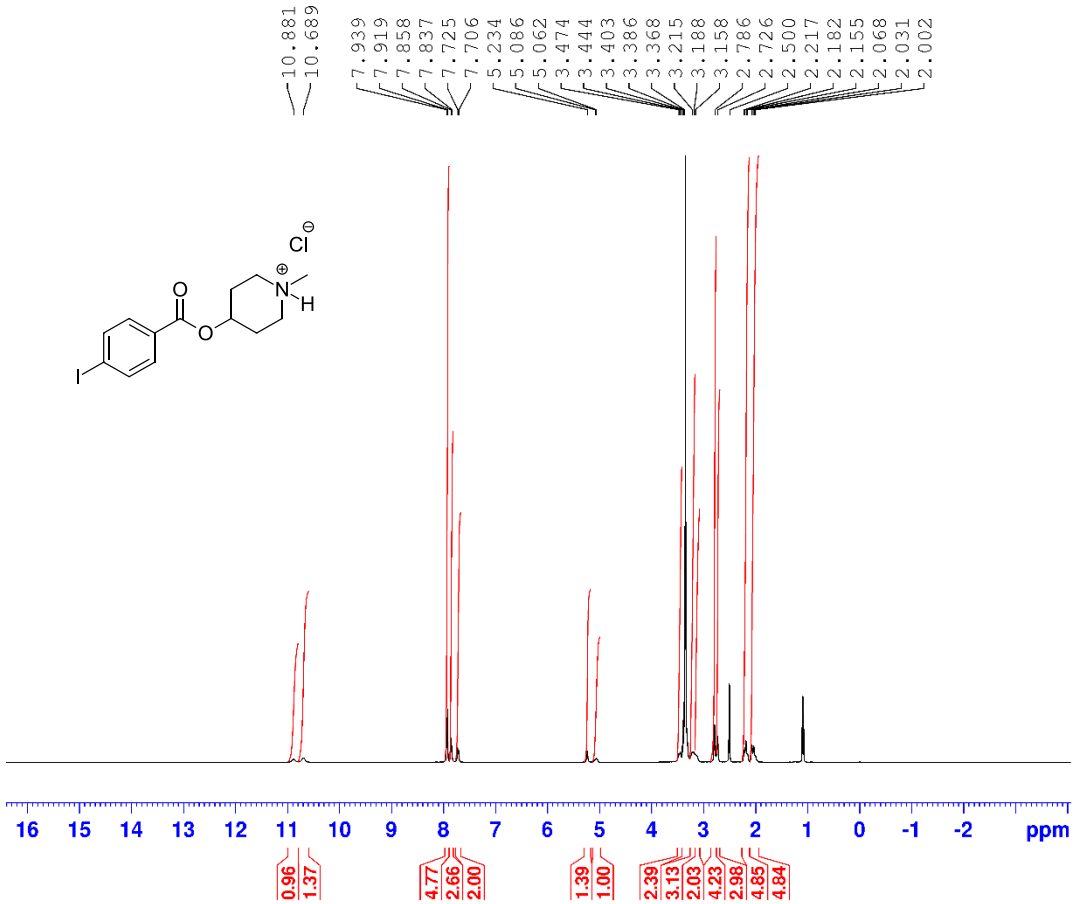
346.0298; found for $C_{13}H_{17}INO_2^+$: 346.0296; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 7.883 mins): 99.3%.

(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride IR(ATR)



C:\Users\Administrator\Documents\Bruker\OPUS_7.5.18\DATA\MEAS\TRV6001 HCl.0	TRV6001 HCl	Instrument type and / or accessory	7/4/2022

(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride ^{1}H NMR 400 MHz (DMSO-*d6*)

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F2 - Processing parameters
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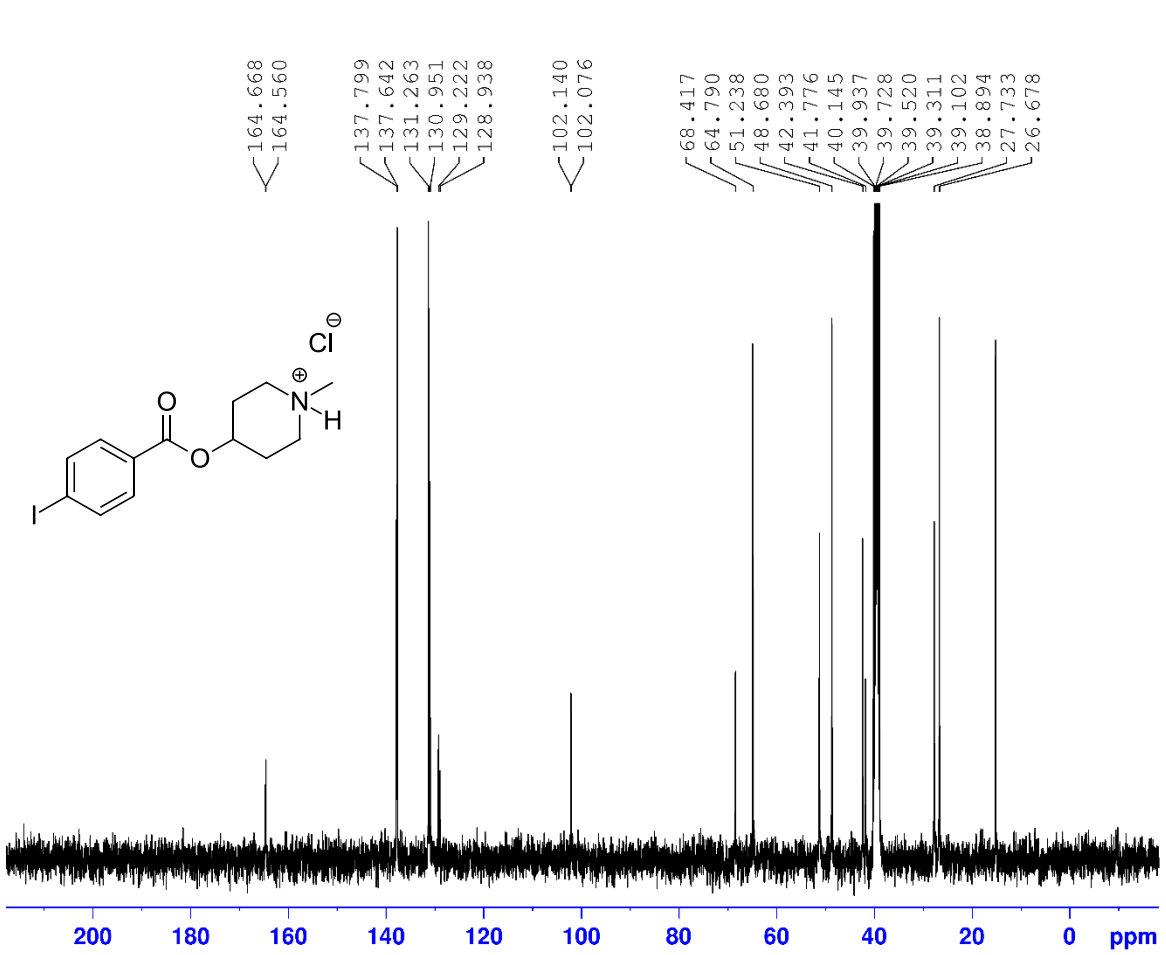
(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride ^{13}C NMR 100 MHz (DMSO-*d6*)



BRUKER

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PLW12                0.17442000 W

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GB                             0
PC                        1.40
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(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

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Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

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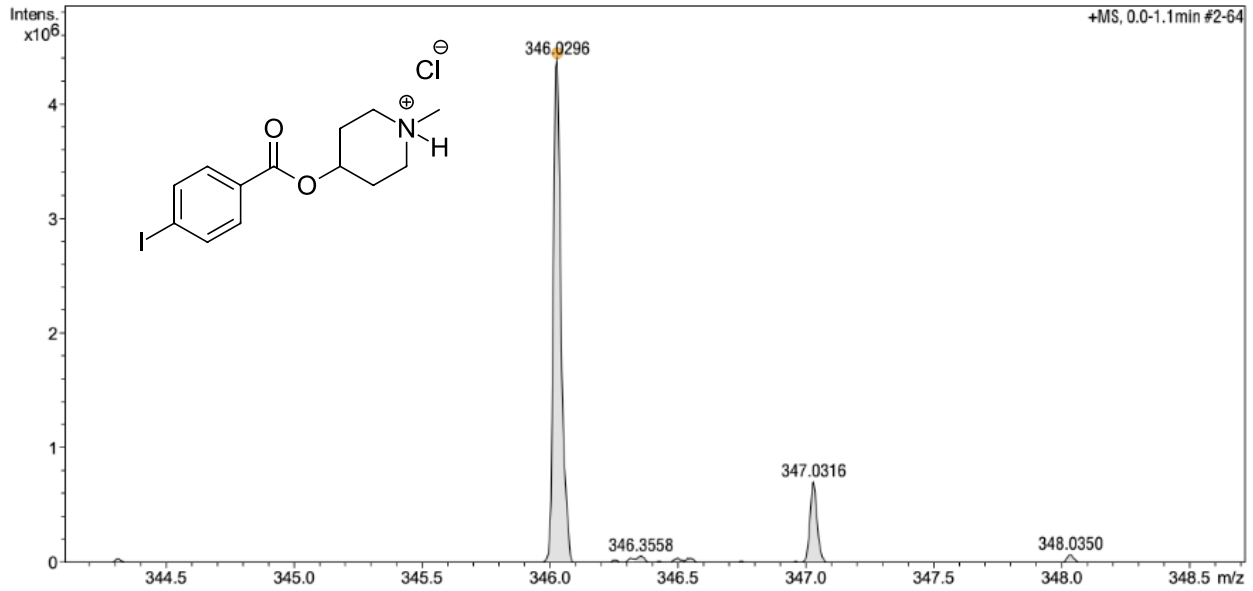

July 06 2022000008.d

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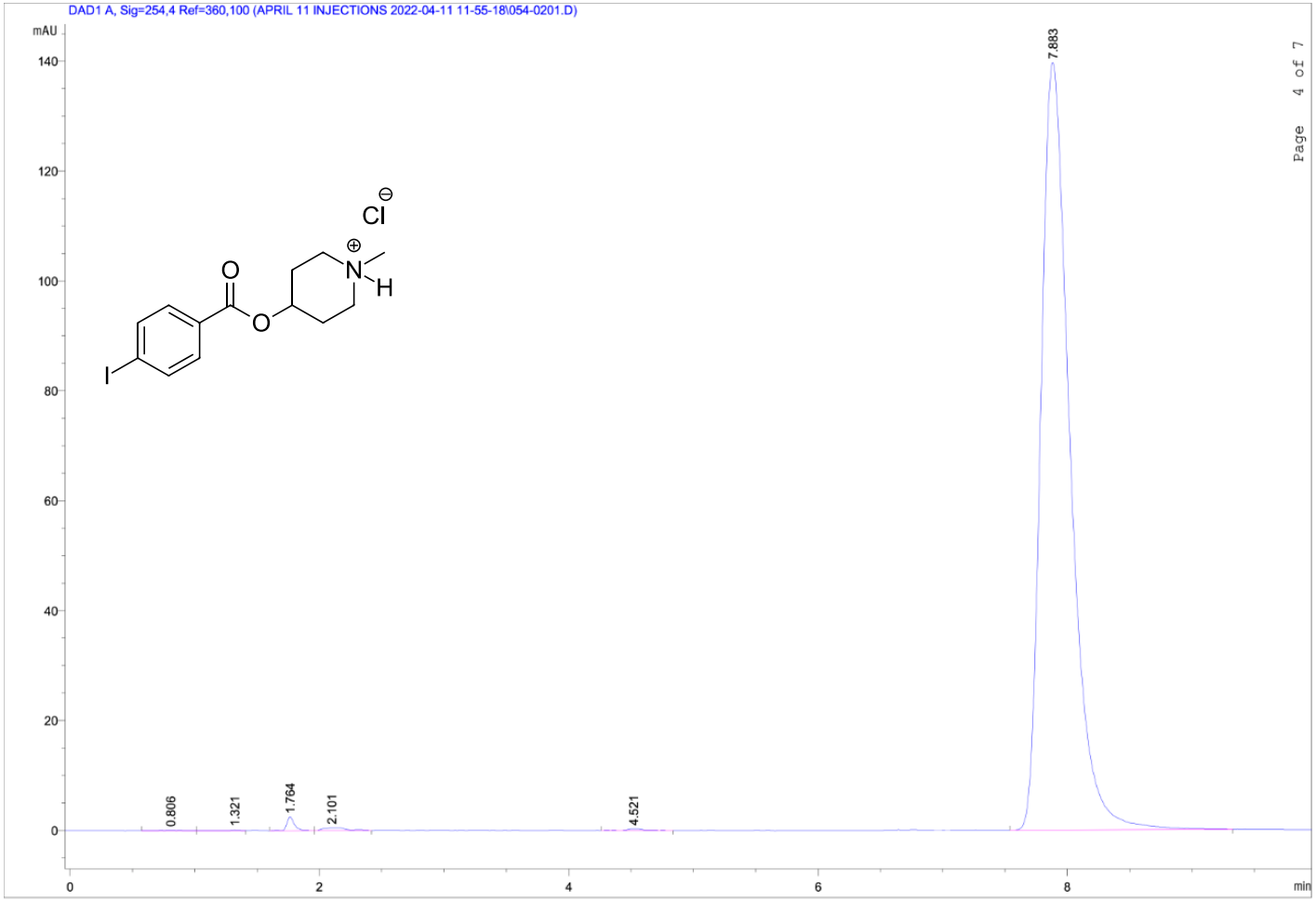
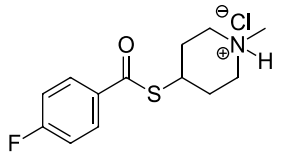
(12) 4-(*p*-Iodobenzoyloxy)-1-methyl-1-piperidinium Chloride HPLC



(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride

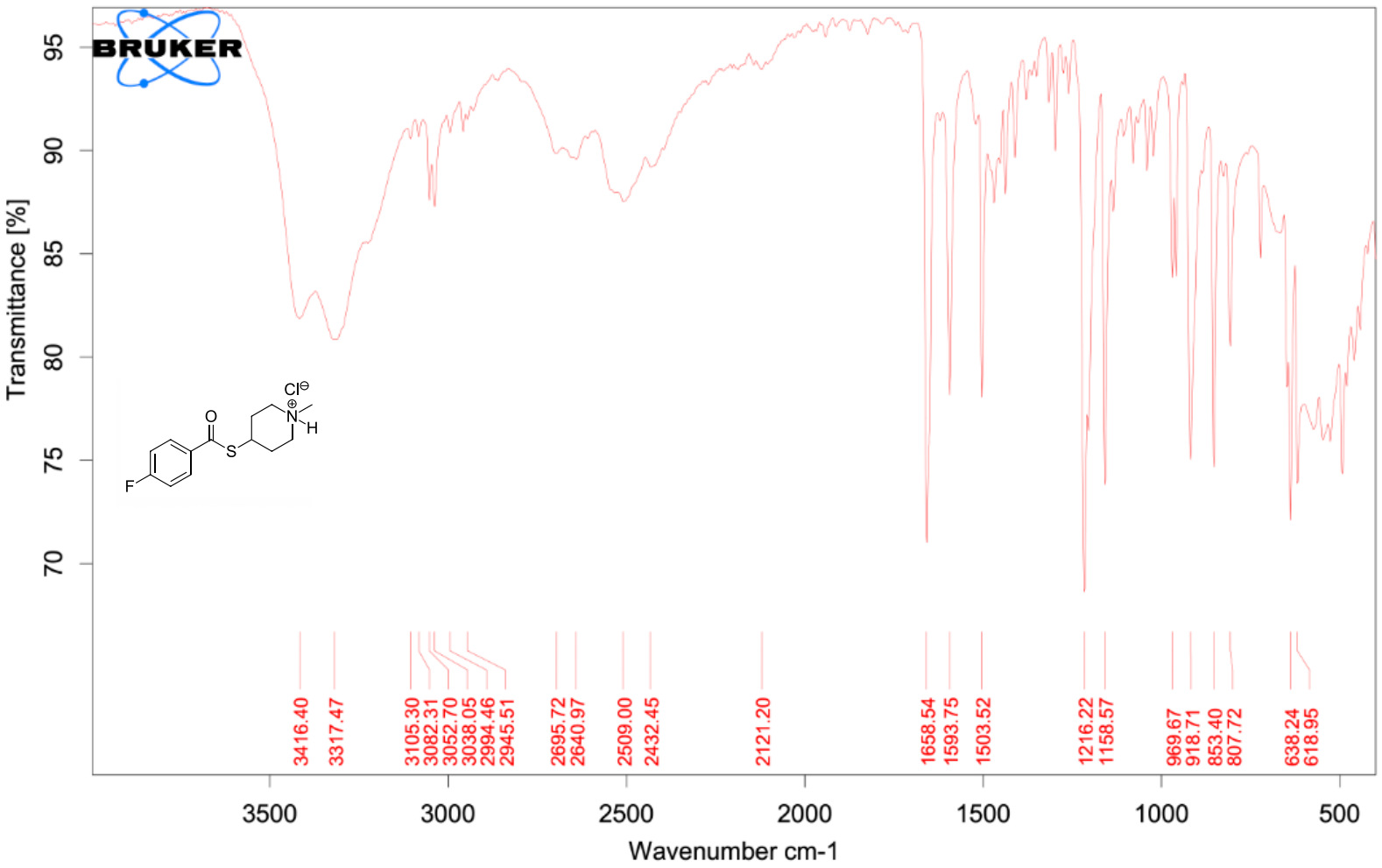


Synthesis: ***S*-(1-Methylpiperidin-4-yl) *p*-fluorobenzenecarbothioate**: To a solution of *N*-methyl-4-piperidinethiol (0.4987 g, 3.8 mmol) and triethylamine (0.80 mL, 5.7 mmol) in anhydrous dichloromethane (20 mL) was added 4-florobenzoyl chloride (0.55 mL, 4.6 mmol) dropwise via syringe under an argon atmosphere. The mixture was heated to reflux for 3 hrs until TLC indicated complete consumption of starting material. Upon cooling to room temperature, the mixture was diluted with DCM (20 mL). The organic layer was washed with saturated $NaHCO_{3(aq)}$ (20 mL), water (20 mL) and dried over Na_2SO_4. The mixture was gravity filtered and concentrated to afford the crude material. Purification *via* flash chromatography (5 % MeOH / DCM) afforded *S*-(1-methylpiperidin-4-yl) *p*-fluorobenzenecarbothioate **(**0.448 g, 47 % yield) as an off-white solid. The analytical data was similar to previously published data.[1]

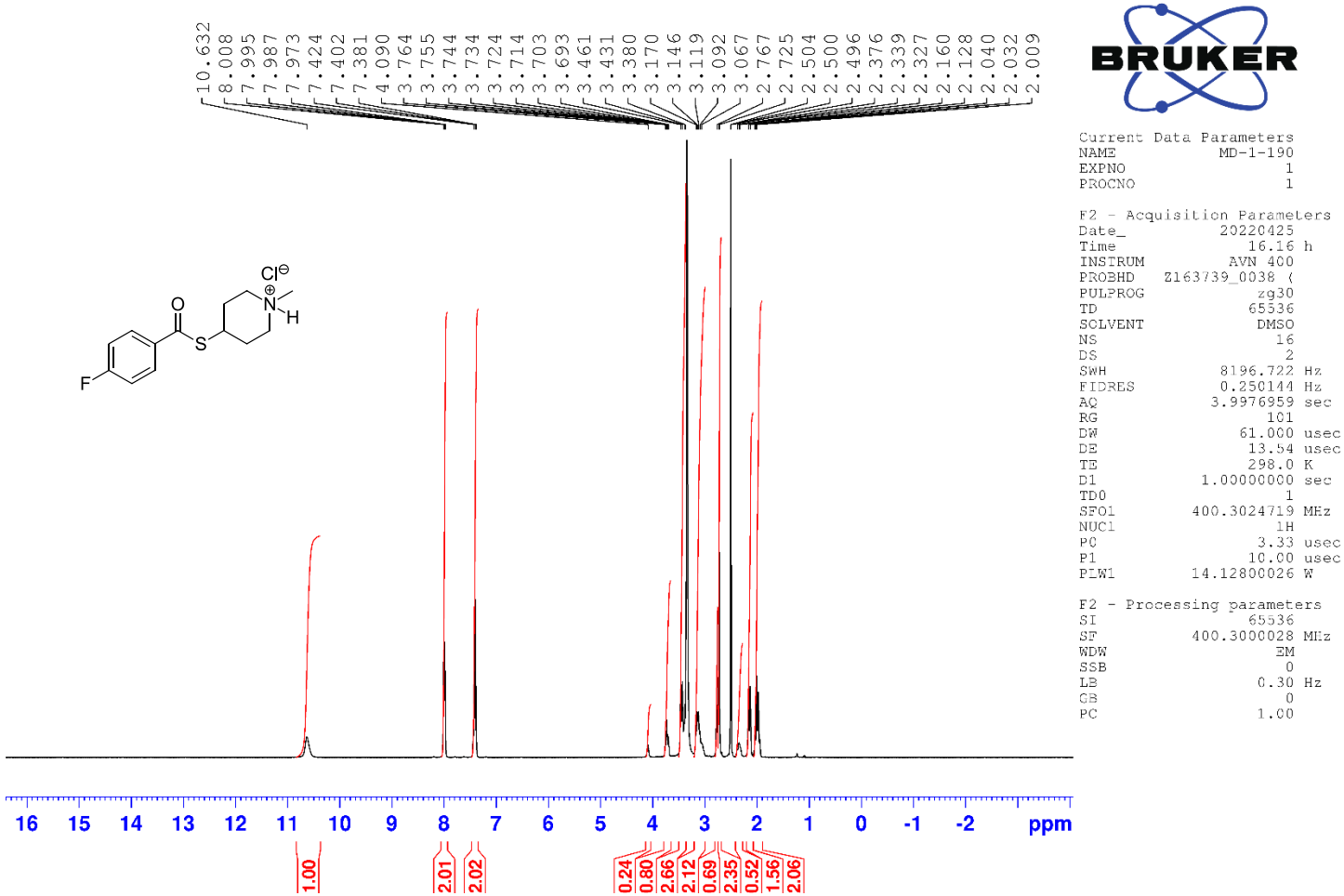
***S*-(1-Methylpiperidin-4-yl) *p*-fluorobenzenecarbothioate hydrochloride (14):** To a flask charged with *S*-(1-methylpiperidin-4-yl) *p*-fluorobenzenecarbothioate (0.206 g, 0.815 mmol) anhydrous diethyl ether was added until complete dissolution (16 mL) under an argon atmosphere. HCl in diethyl ether 1 N - 1.22 mL, 1.22 mmol) was added dropwise and stirred for 1hr. The solvent was removed *in vacuo* resulting in a white solid. The product was triturated three times with diethyl ether (20 mL), solvent was removed with a pipet and compound was dried to produce *S*-(1-methylpiperidin-4-yl) *p*-fluorobenzenecarbothioate hydrochloride (0.230 g, 97%).

Spectroscopic data: $MP_{(diethyl\ ether)}$: 209-211°C, IR(ATR): 3416, 3317, 3105, 3082, 2994, 2946, 2696, 2509, 2432, 1659, 1594, 1504, 1216, 919, 638 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 10.63 (br s, 1H), 8.01-7.97 (m, 2H), 7.42-7.38 (m, 2H), 4.09 (br s, 1H), 3.40-3.38 (m, 2H), 3.09-3.07 (m, 2H), 2.77 (s, 3H), 2.38-2.33 (m, 2H), 2.04-1.94 (m, 2H); isomer B: 10.63 (br s, 1H), 8.01-7.97 (m, 2H), 7.42-7.38 (m, 2H), 3.76-3.69 (m, 1H), 3.46-3.43 (m, 2H), 3.17-3.10 (m, 2H), 2.73 (s, 3H), 2.17-2.13 (m, 2H), 2.04-1.94 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ isomer A: 188.6, 164.3 (d, $^{1}J_{C,F}$ = 252.9 Hz), 132.9 (d, $^{4}J_{C,F}$ = 2.7 Hz), 129.9 (d, $^{3}J_{C,F}$ = 9.7 Hz), 116.4 (d, $^{2}J_{C,F}$ = 22.4 Hz), 50.3, 41.7, 36.6, 27.3, isomer B: 188.8, 166.8 (d, $^{1}J_{C,F}$ = 252.9 Hz), 132.9 (d, $^{4}J_{C,F}$ = 2.7 Hz), 129.9 (d, $^{3}J_{C,F}$ = 9.7 Hz), 116.4 (d, $^{4}J_{C,F}$ = 22.4 Hz), 53.1, 42.5, 37.1, 29.0; LRMS (ESI^{+}): 254.1 (M^{+}); HRMS (ESI^{+}): calculated for $C_{13}H_{17}FNOS^{+}$: 254.1010 amu; found for $C_{13}H_{17}FNOS^{+}$: 254.1010 amu; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 5.912 mins): 98.9 %.

(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride IR(ATR)



(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride ^{1}H NMR 400 MHz (DMSO-*d6*)

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DE                      13.54 usec
TE                      298.0 K
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P1                      10.00 usec
PLW1               14.12800026 W

F2 - Processing parameters
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WDW                         EM
SSB                          0
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GB                           0
PC                        1.00
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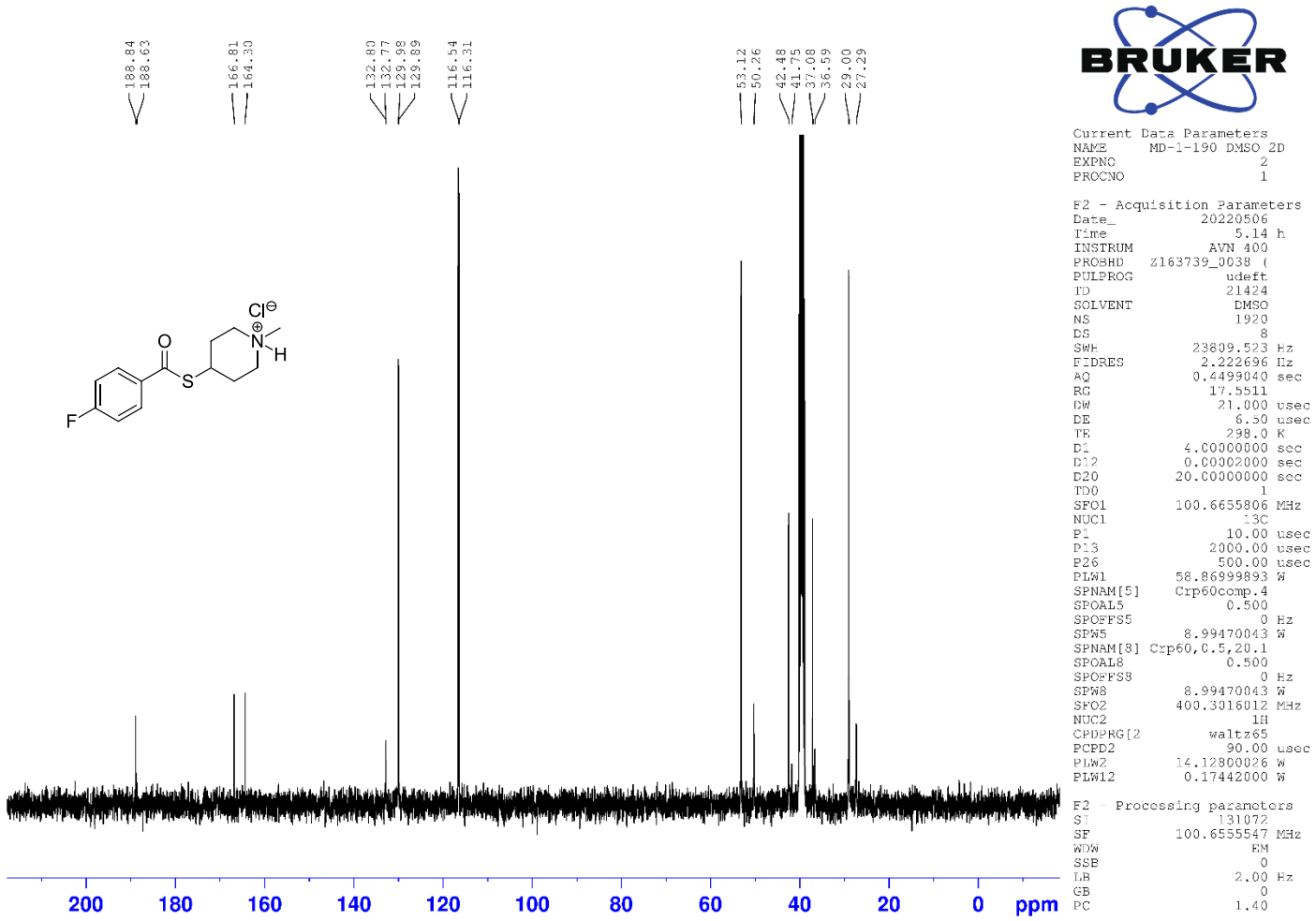
(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride ^{13}C NMR 100 MHz (DMSO-*d6*)

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TD                  21424
SOLVENT              DMSO
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RG                17.5511
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TE                298.0 K
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D12            0.00002000 sec
D20           20.00000000 sec
TD0                     1
SFO1          100.6655806 MHz
NUC1                  13C
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P26               500.00 usec
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SPNAM[8] Crp60,0.5,20.1
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SPOFFS8                 0 Hz
SPW8           8.99470043 W
SFO2          400.3016012 MHz
NUC2                  1H
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PLW2          14.12800026 W
PLW12          0.17442000 W

F2 - Processing parameters
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WDW                   EM
SSB                    0
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PC                  1.40
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(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

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Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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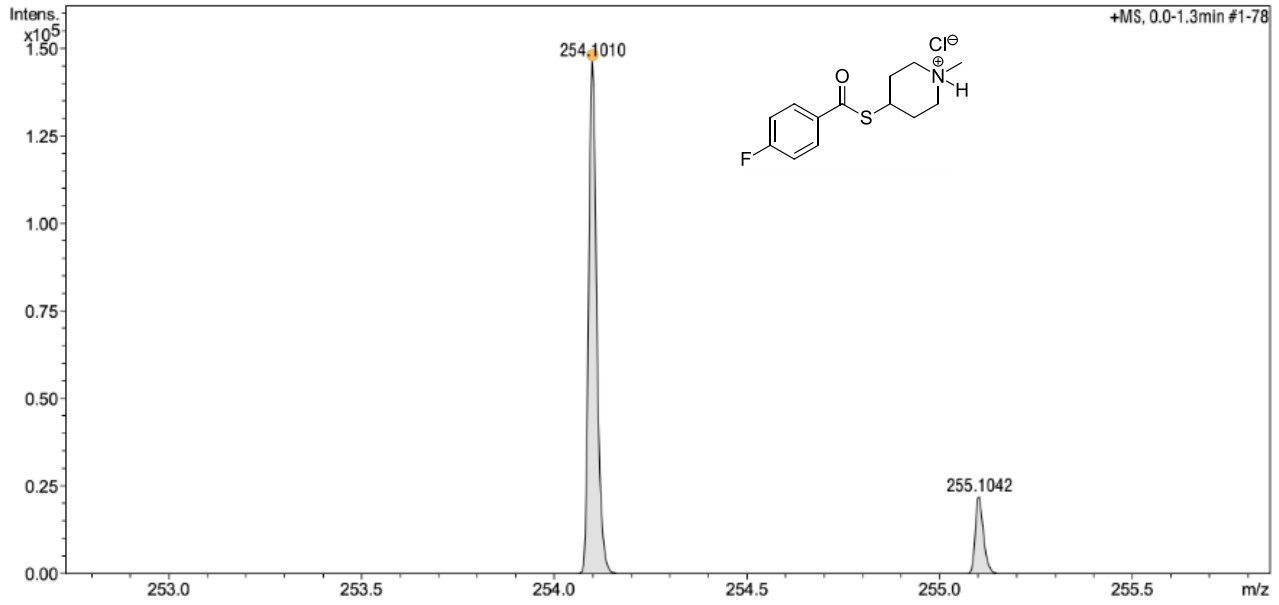
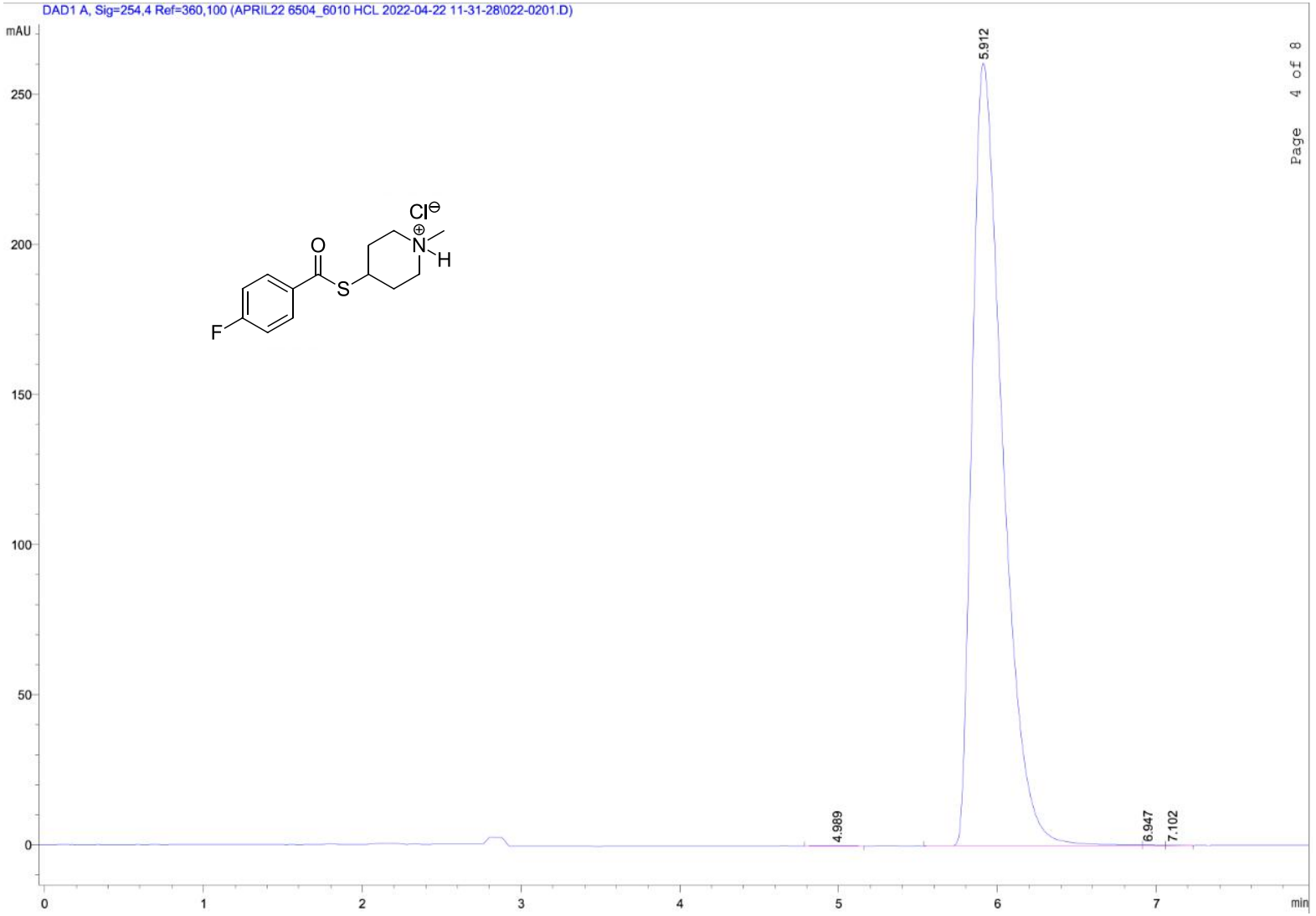


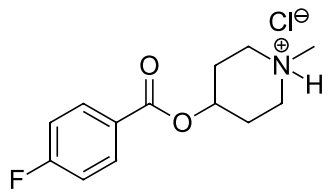
July 07 2022000015.d

Bruker Compass DataAnalysis 4.3 printed: 7/7/2022 10:15:30 AM by: x Page 1 of 1

(13) *S*-(1-Methylpiperidin-4-yl) *p*-Fluorobenzenecarbothioate Hydrochloride HPLC



(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride

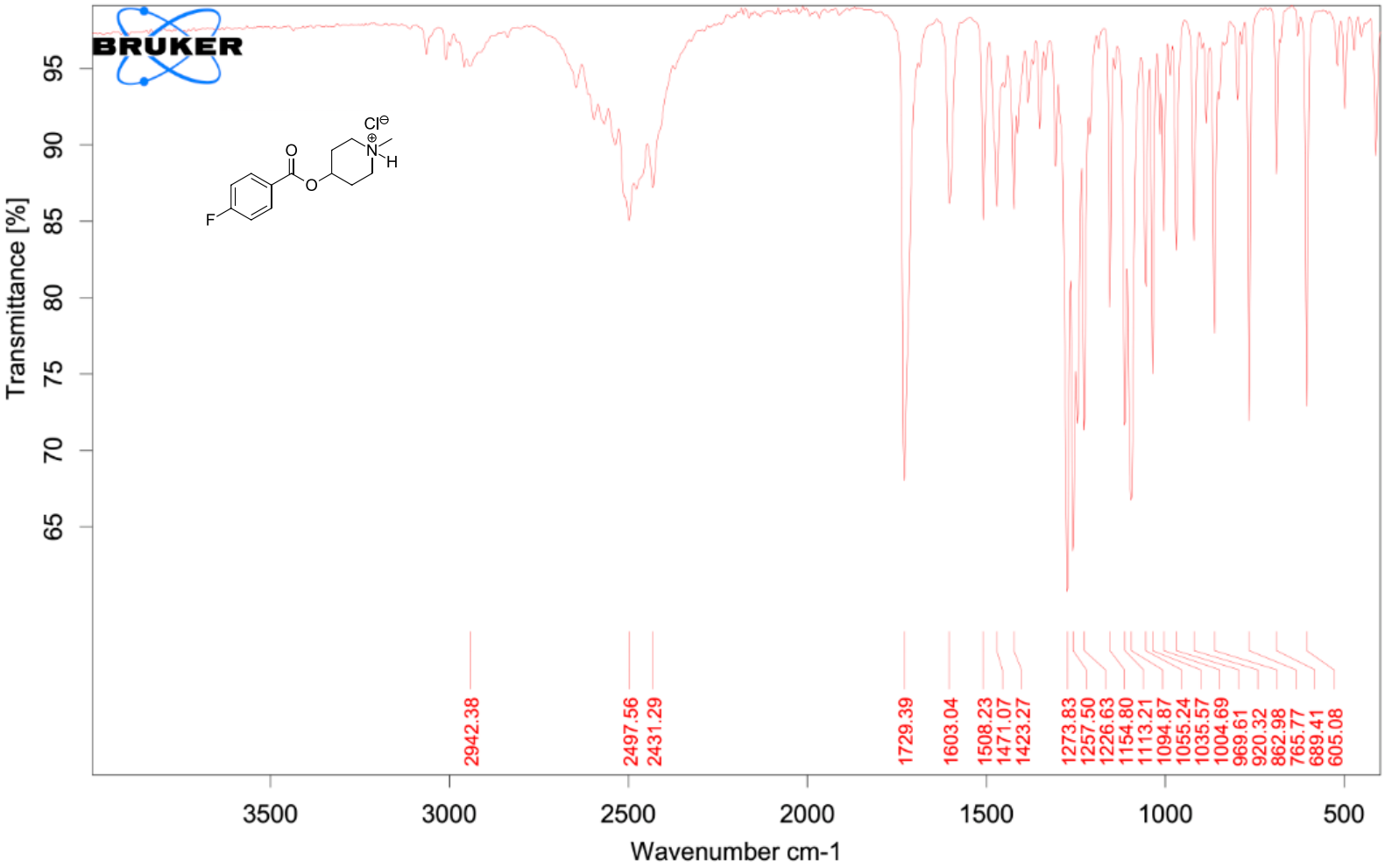


Synthesis: ***N*-Methylpiperidin-4-yl 4-fluorobenzoate**: 4-fluorobenzoyl chloride (0.76 mL, 6.3 mmol), triethylamine (2.2 mL, 15.8 mmol) and DMAP (0.138 g, 1.13 mmol) were dissolved in anhydrous dichloromethane (32 mL) under an argon atmosphere. *N*-Methyl-4-piperidinol (0.979 g, 6.3 mmol) was added to the stirred solution in one portion. The reaction mixture was stirred for 3 hrs at room temperature. After this time, the reaction was quenched with brine (30 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce a clear colourless oil. The crude mixture was purified by silica gel column chromatography (4% MeOH/DCM) to produce a clear colourless oil, *N*-Methylpiperidin-4-yl 4-fluorobenzoate (1.47 g, 98%). The analytical data was similar to previously published data.[1]

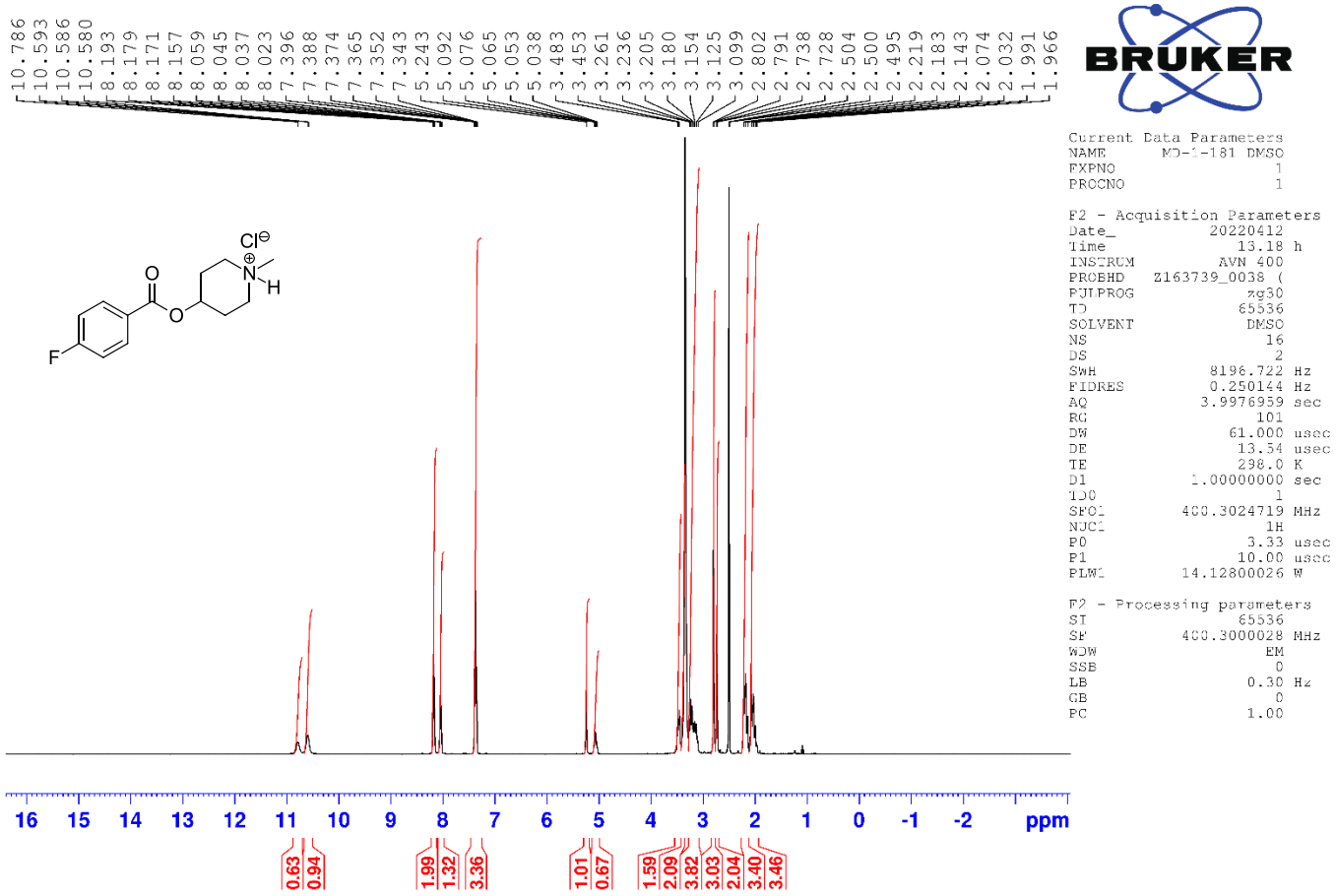
4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium chloride (13): To a flask charged with *N*-methylpiperidin-4-yl 4-fluorobenzoate (0.748 g, 3.15 mmol) anhydrous diethyl ether was added until complete dissolution (63 mL) under an argon atmosphere. HCl in diethyl ether 1N (4.73 mL, 4.73 mmol) was added dropwise and stirred for 1 hr at room temperature. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (20 mL) and solvent was removed with a pipet. This process was repeated two more times. The material was dried *in vacuo* to produce 4-(*p*-fluorobenzoyloxy)-1-methyl-1-piperidinium chloride (**13**, 0.773 g, 90%, 2:3 mixture of isomers) as a white solid.

Spectroscopic data: $MP_{(diethyl\ ether)}$: 219-221°C (Lit $MP_{(acetone/ethanol)}$: 227-228°C); IR (ATR) 2942, 2498, 2431, 1729, 1274, 1257, 766, 605 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ isomer A: 10.78 (br s, 1H), 8.06-8.02 (m, 2H), 7.40-7.34 (m, 2H), 5.10-5.03 (m, 1H), 3.40-3.37 (m, 2H), 3.18-3.10 (m, 2H), 2.73 (d, J = 4.0 Hz, 3H), 2.22-2.14 (m, 2H), 2.07-1.96 (m, 2H); isomer B: 10.59 (br s, 1H), 8.19-8.16 (m, 2H), 7.40-7.34 (m, 2H), 5.26-5.23 (m, 1H), 3.48-3.45 (m, 2H), 3.26-3.18 (m, 2H), 2.80 (d, J = 4.5 Hz, 3H), 2.22-2.14 (m, 2H), 2.07-1.96 (m, 2H); ^{13}C NMR (100.7 MHz, CD_3OD) δ isomer A: 165.3 (d, $^{1}J_{C,F}$ = 246.4 Hz), 163.9, 132.2 (d, $^{3}J_{C,F}$ = 9.7 Hz), 126.0, 115.9 (d, $^{2}J_{C,F}$ = 22.5 Hz), 68.3, 51.2, 41.8, 27.7; isomer B: 165.3 (d, $^{1}J_{C,F}$ = 246.4 Hz), 164.0, 132.5 (d, $^{3}J_{C,F}$ = 9.6 Hz), 126.3, 115.8 (d, $^{2}J_{C,F}$ = 21.9 Hz), 64.6, 48.7, 42.4, 26.7; LRMS (ESI^+): 238.1 (M^+); HRMS (ESI^+): calculated for $C_{13}H_{17}FNO_2^+$: 238.1238 amu; found for $C_{13}H_{17}FNO_2^+$: 238.1244 amu; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.854 mins): 100 %.[6]

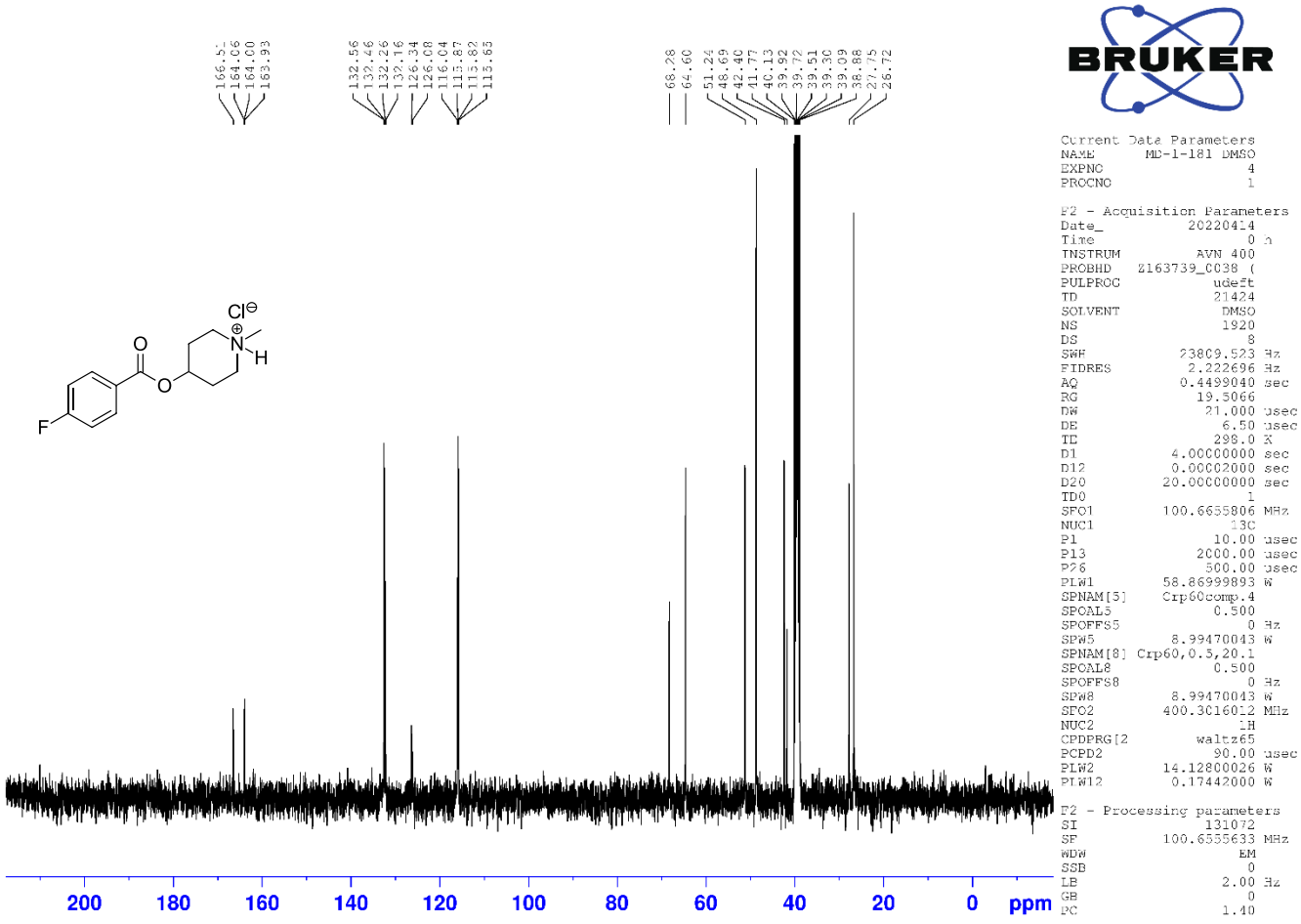
(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride IR(ATR)



(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride ^{1}H NMR 400 MHz (DMSO-*d6*)



(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride ^{13}C NMR 100 MHz (DMSO-*d6*)



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TE                298.0 K
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D12            0.00002000 sec
D20           20.00000000 sec
TD0                    1
SFO1         100.6655806 MHz
NUC1                  13C
P1                 10.00 usec
P13              2000.00 usec
P26               500.00 usec
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SPNAM[8] Crp60,0.5,20.1
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SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2         400.3016012 MHz
NUC2                  1H
CPDPRG[2          waltz65
PCPD2              90.00 usec
PLW2         14.12800026 W
PLW12         0.17442000 W

F2 - Processing parameters
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WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.40
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(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000018.d	Acquisition Date	7/7/2022 10:21:47 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6501 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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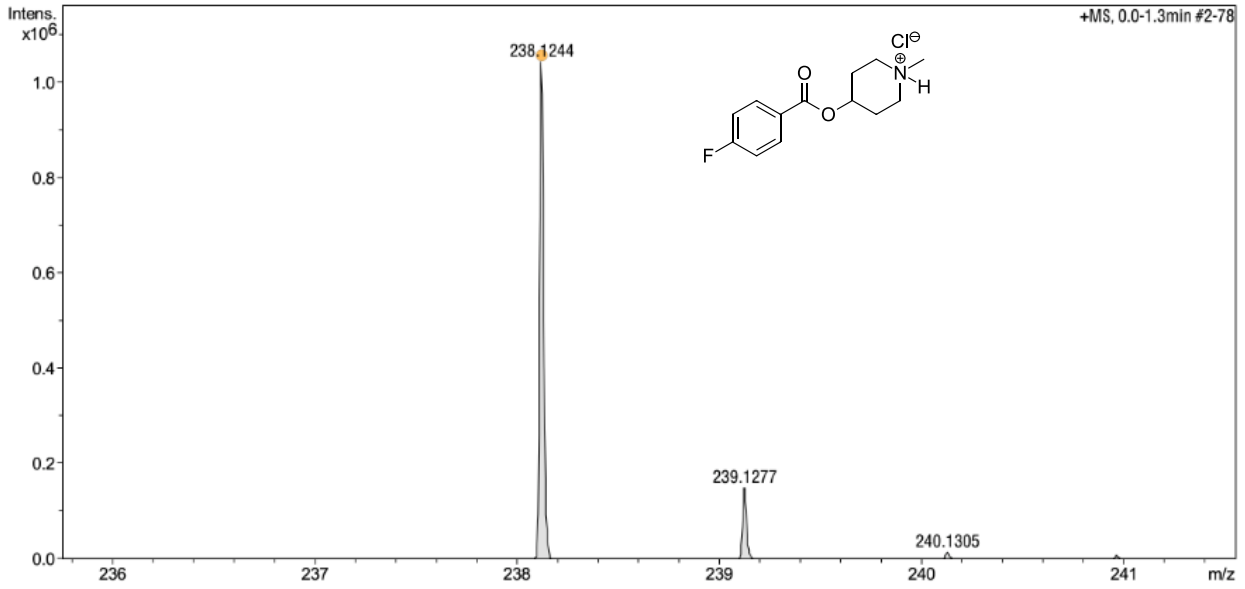

July 07 2022000018.d

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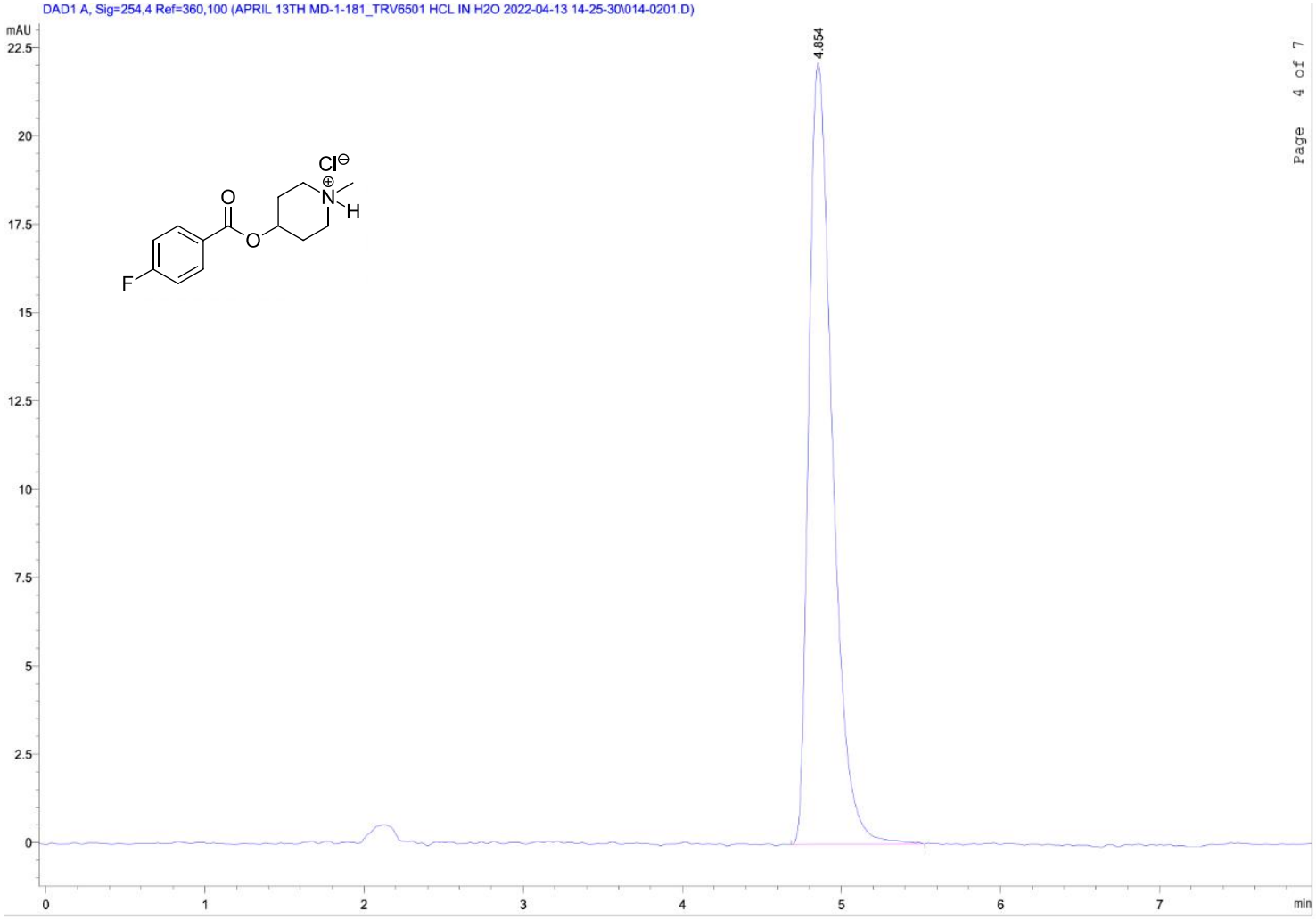
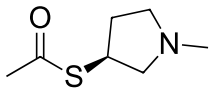
(14) 4-(*p*-Fluorobenzoyloxy)-1-methyl-1-piperidinium Chloride HPLC



Alkyl N-Methylpyrrolidinyl Derivatives

(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate



Synthesis: **(*3R*)-3-(Mesyloxy)-1-methylpyrrolidine**: (3*R*)-(-)-1-Methyl-3-pyrrolidinol (5.00 mL, 45.5 mmol) and triethylamine (9.52 mL, 68.3 mmol) were dissolved in anhydrous dichloromethane (152 mL) and cooled to 0°C with an ice bath. Methanesulfonyl chloride (5.22 mL, 52.4 mmol) was added dropwise at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred overnight. The reaction was quenched with brine (50 mL), extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce a viscous yellow liquid (4.14 g, 51%). Product confirmed by ^{1}H NMR and immediately carried through to next step.

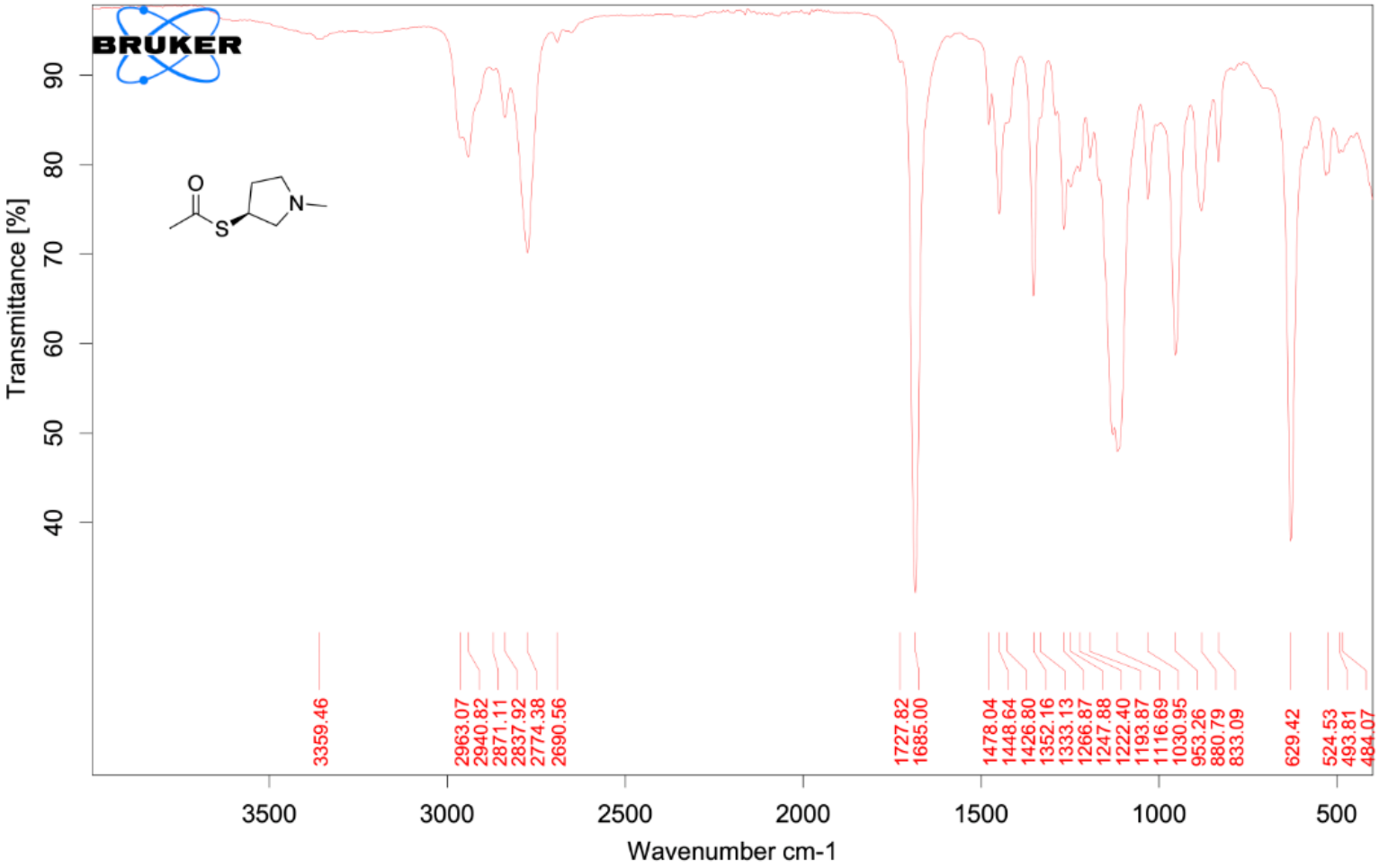
Spectroscopic data: IR(ATR): 3026, 2942, 2784, 1346, 1166, 521 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) d 5.23-5.19 (m, 1H), 3.01 (s, 3H), 2.90 (dd, J = 11.3, 2.2 Hz, 1H), 2.87-2.83 (m, 1H), 2.72 (dd, J = 11.3, 5.7 Hz, 1H), 2.39-2.28 (m, 2H), 2.38 (s, 3H), 2.14-2.06 (m, 1H); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 80.6 (1), 62.4 (2), 54.6(2), 41.9 (3), 38.7 (3), 33.1 (2); HRMS (ESI^+): calculated for $C_6H_{14}NO_3S^+$: 180.0689 amu; found for $C_6H_{14}NO_3S^+$: 180.0689 amu.

(*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3R*)-3-(Mesyloxy)-1-methylpyrrolidine (2.35 g, 13.1 mmol) was dissolved in anhydrous DMF (44 mL) under an argon atmosphere. Potassium thioacetate (4.50 g, 39.4 mmol) was added in 3 portions, allowing the salt to dissolve before adding more. After the potassium thioacetate addition and dissolution, the reaction was heated to

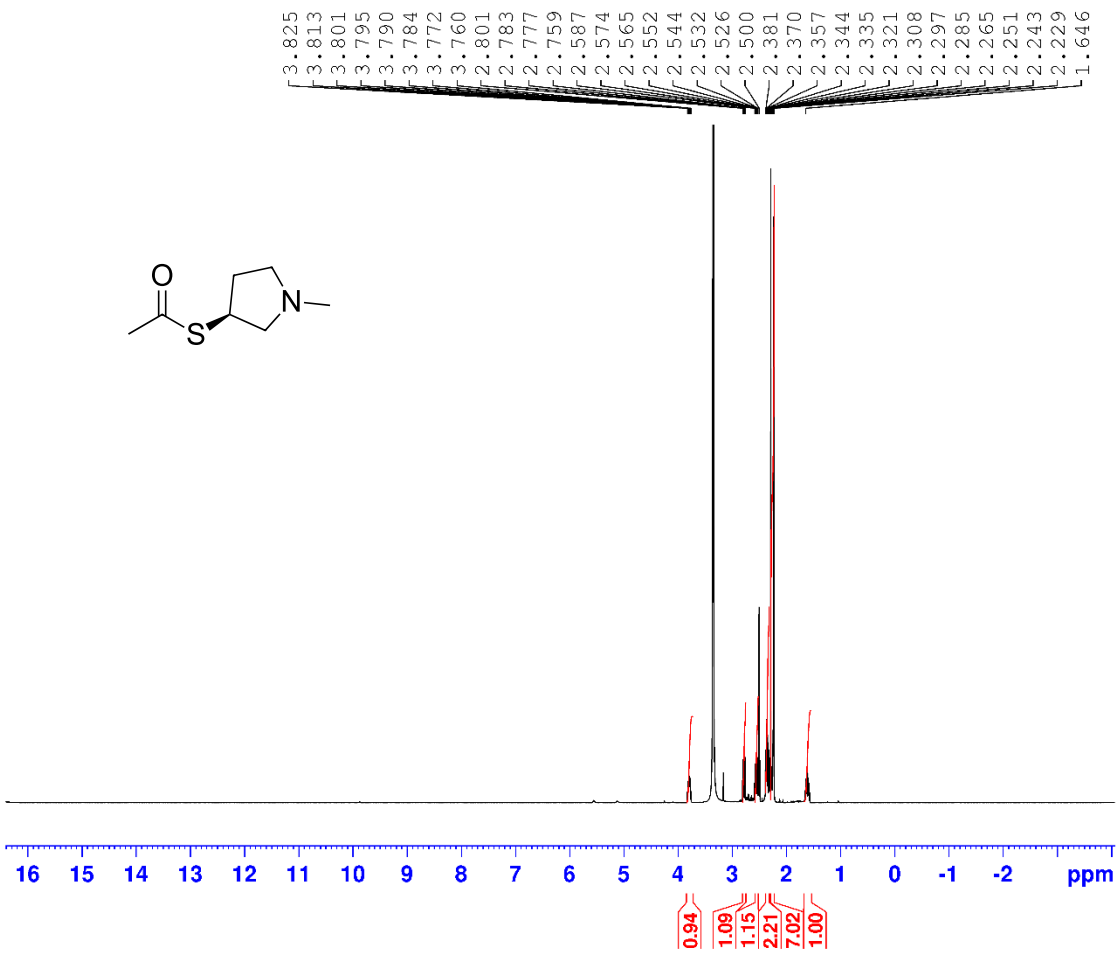
30°C for 1hr. After this time, the reaction was cooled to room temperature, diluted with water (50 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water (5 × 20 mL), dried over Na_2SO_3, and gravity filtered. The filtrate was concentrated *in vacuo* to afford a viscous red liquid (1.88 g). A portion of this material (0.462 g) was purified using flash column chromatography through silica gel (5% MeOH/DCM) to produce a viscous red liquid (0.3183 g, 40% yield).

Spectroscopic Data: IR(ATR): 2963, 2940, 2774, 1685, 1116, 953, 629 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.82-3.76 (m, 1H), 2.78 (dd, J = 9.7, 7.3 Hz, 1H), 2.58-2.53 (m, 1H), 2.38-2.32 (m, 2H), 2.30-2.23 (m, 1H), 2.28 (s, 3H), 2.23 (s, 3H), 1.64-1.57 (m,1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 195.8 (0), 62.2 (2), 54.8 (2), 41.4 (3), 39.8 (1), 31.7 (2), 30.3 (3); HRMS (ESI^+): calculated for $C_7H_{14}NOS^+$: 160.0791 amu; found for $C_7H_{14}NOS^+$: 160.0793 amu; HPLC purity at 230nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 3.330 mins): 91.5 %.

(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate IR(ATR)



(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate ^{1}H NMR 400 MHz (DMSO-*d6*)



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RG                   101
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TE                298.0 K
D1           1.00000000 sec
TD0                    1
SFO1        400.3024719 MHz
NUC1                  1H
P0                 3.33 usec
P1                 9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                 65536
SF          400.3000027 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                  1.00
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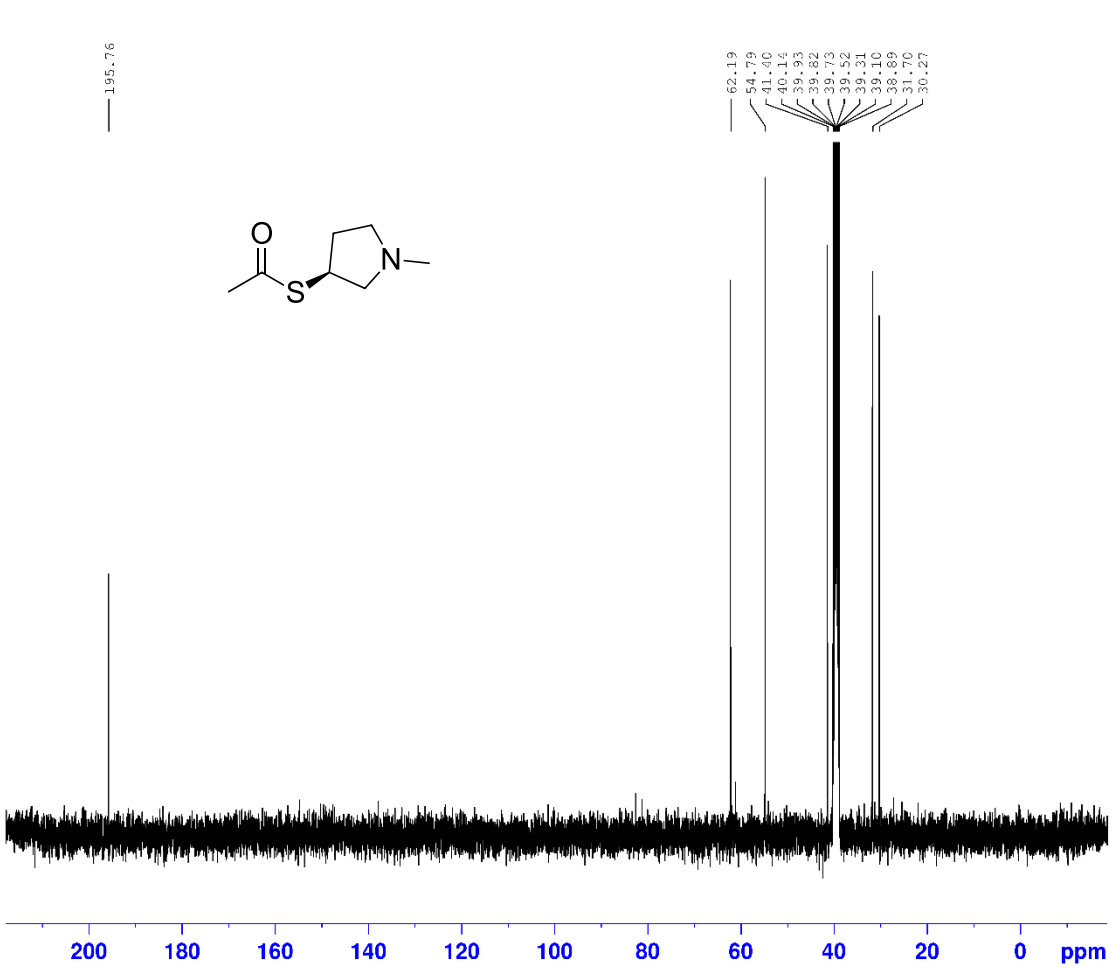
(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate ^{13}C NMR 100 MHz (DMSO-*d6*)



BRUKER

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Current Data Parameters
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PROCNO                 1

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PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 2048
DS                    4
SWH           23809.523 Hz
FIDRES         0.726609 Hz
AQ             1.3762560 sec
RG                14.6672
DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1             3.00000000 sec
D11            0.03000000 sec
TD0                   1
SFO1         100.6655806 MHz
NUC1                13C
P0                 3.33 usec
P1                10.00 usec
PLW1          58.86999893 W
SFO2         400.3016012 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             90.00 usec
PLW2          14.13000011 W
PLW12          0.17375000 W
PLW13          0.08739400 W

F2 - Processing parameters
SI                32768
SF           100.6655604 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
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(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Aug 11 2022000018.d	Acquisition Date	2022-08-11 3:24:25 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV-6019	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	181 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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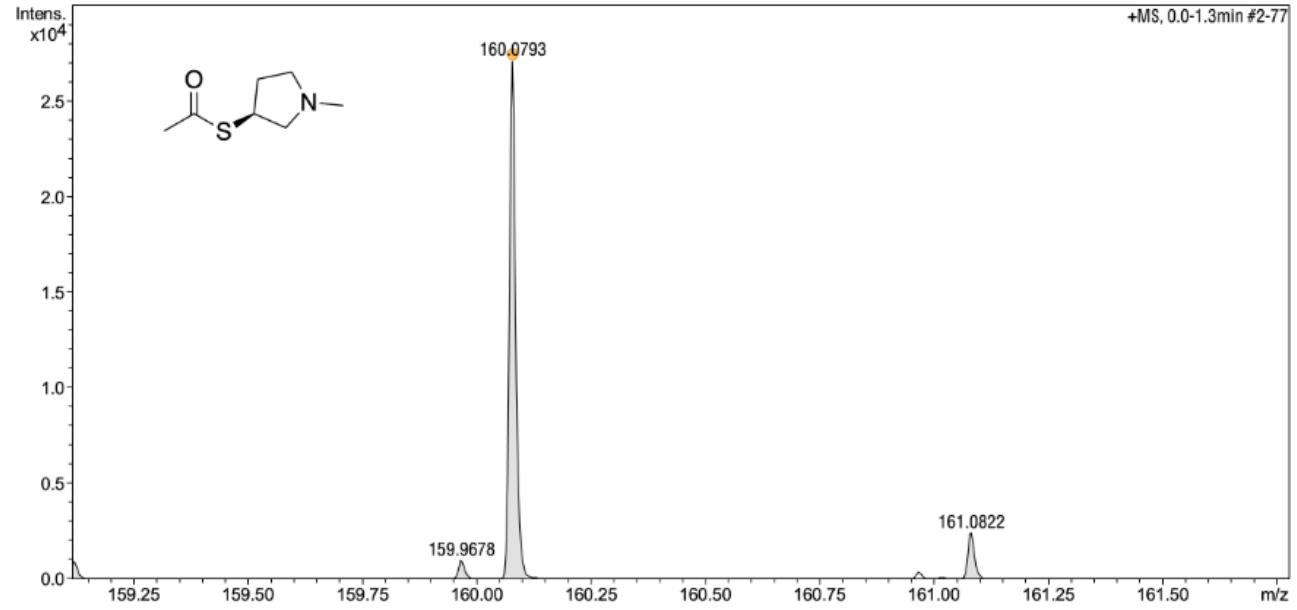

Aug 11 2022000018.d

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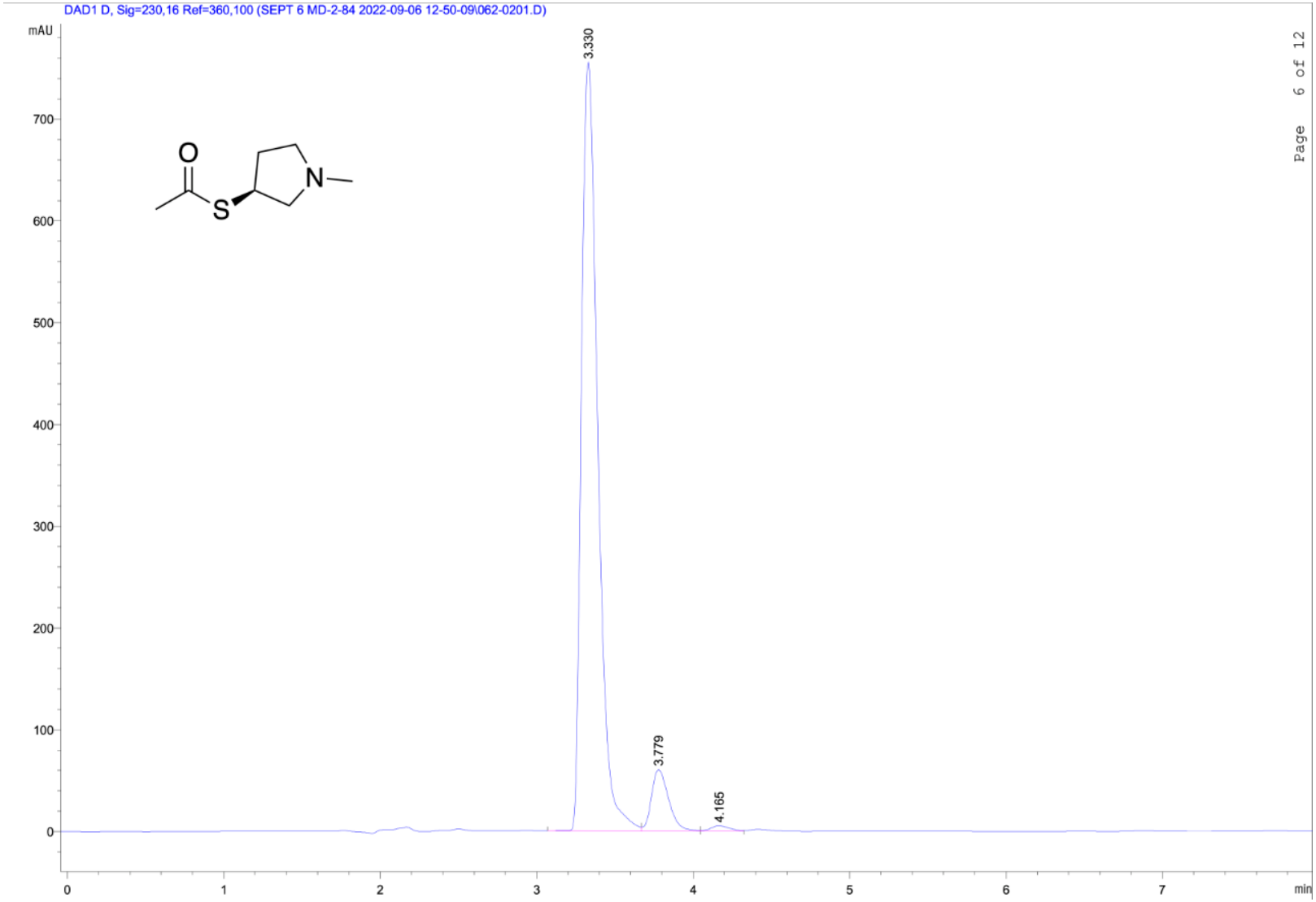
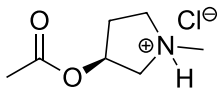
(15) (*3S*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate HPLC



(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride



Synthesis: **(*S*)-1-Methyl-3-pyrrolidinyl acetate**: (*S*)-(+)-1-Methyl-3-pyrrolidinol (0.329 mL, 3 mmol) and triethylamine (1.05 mL, 7.5 mmol) were dissolved in anhydrous dichloromethane (10 mL) and cooled to 0°C with an ice bath. Acetyl chloride (0.213 mL, 3 mmol) was added dropwise at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred for 6 hrs. The reaction was quenched with of brine (10 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was removed *in vacuo* to produce a yellow oil. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to produce (*S*)-1-methyl-3-pyrrolidinyl acetate (0.293 g, 68 %) as a yellow oil.

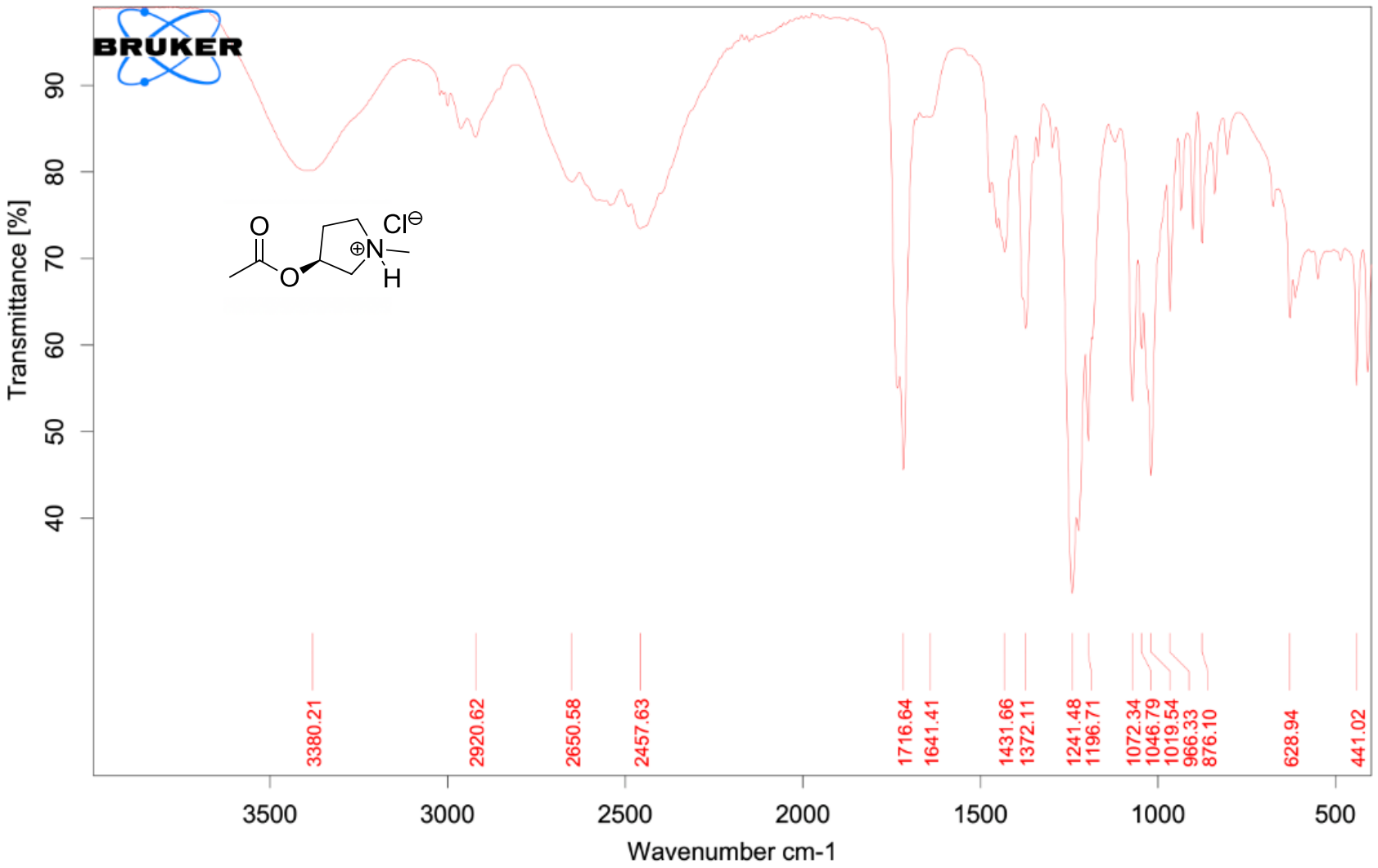
Spectroscopic data: ^{1}H NMR (400 MHz, DMSO-*d6*) δ 5.18-5.14 (m, 1H), 2.85-2.81 (m, 1H), 2.73 (dd, J = 10.9, 1.6 Hz, 1H), 2.57 (dd, J = 10.9, 5.9 Hz, 1H), 2.35 (s, 3H), 2.31-2.21 (m, 2H), 2.04 (s, 3H), 1.89-1.80 (1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 171.1 (0), 74.8 (1), 62.3 (2), 55.1 (2), 42.1 (3), 32.7 (2), 21.4 (3).

***(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium chloride**: To a flask charged with (*S*)-1-Methyl-3-pyrrolidinyl acetate (0.124 g, 0.867 mmol) anhydrous diethyl ether was added until complete dissolution (17 mL) under an argon atmosphere. HCl in diethyl ether (1 N solution, 1.3 mL, 1.3 mmol) was added dropwise and stirred for 1 hr. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated three times with diethyl ether (20 mL), solvent was

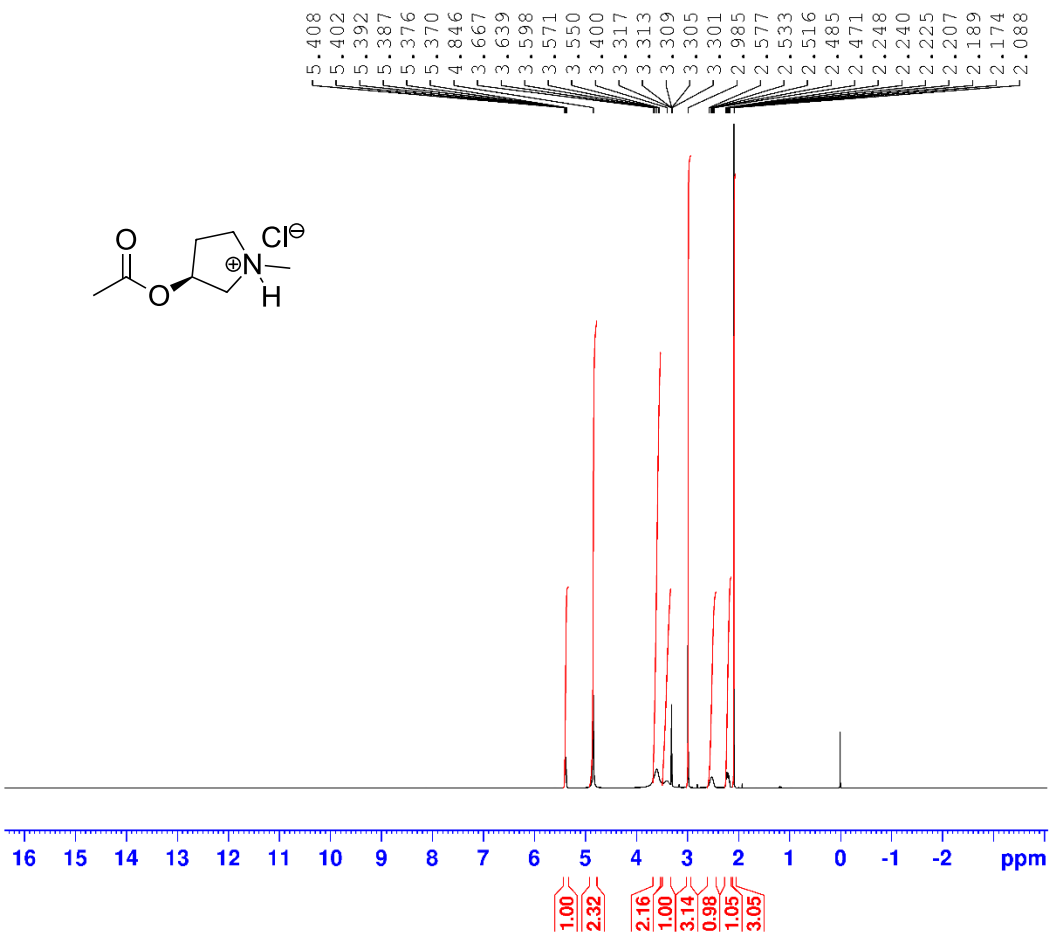
removed with a pipet and compound placed under vacuum (1 mm Hg) to produce *(3S)*-3-acetoxy-1-methyl-1-pyrrolidinium chloride (0.141 g, 90%) as a white solid.

Spectroscopic data: IR(ATR): 3380, 2921, 2650, 2458, 1717, 1241, 1047 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 5.41-5.37 (m, 1H), 4.85 (br s, 2H), 3.67-3.55 (m, 2H), 3.42-3.38 (m, 1H), 2.98 (s, 3H), 2.58-2.47 (m, 1H), 2.25-2.17 (m, 1H), 2.09 (s, 3H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 171.7 (0), 73.8 (1), 61.7 (2), 55.5 (2), 41.9 (3), 31.7 (2), 20.8 (3); LRMS (ESI^+): 144.1 (M^+); HRMS (ESI^+): calculated for $C_7H_{14}NO_2^+$: 144.1019 amu; found for $C_7H_{14}INO_2^+$: 144.1022 amu; HPLC purity at 210nm (75% CH3CN : 10% CH3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 3.066 mins): >99 %.

(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (CD_3OD-*d4*)



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Current Data Parameters
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EXPNO                        8
PROCNO                       1

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Time                     16.14 h
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PROBHD    Z163739_0038 (
PULPROG                  zg30
TD                      65536
SOLVENT                  MeOD
NS                         16
DS                          2
SWH                   8196.722 Hz
FIDRES                0.250144 Hz
AQ                    3.9976959 sec
RG                         101
DW                      61.000 usec
DE                      13.63 usec
TE                      298.0 K
D1                    1.00000000 sec
TD0                          1
SFO1                400.3024719 MHz
NUC1                        1H
P0                       3.16 usec
P1                       9.49 usec
PLW1                14.13000011 W

F2 - Processing parameters
SI                       65536
SF                  400.3000081 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
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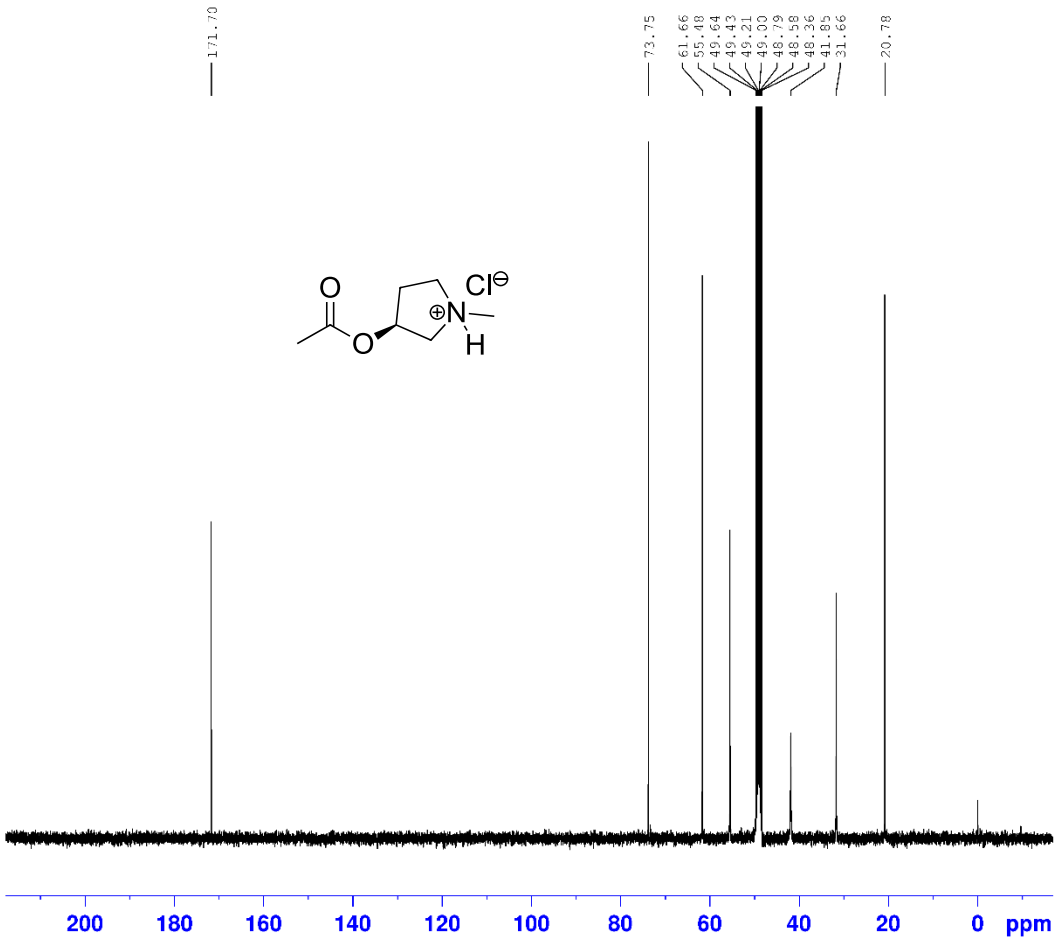
(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride ^{13}C NMR 100 MHz (CD_3OD-*d4*)



BRUKER

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Current Data Parameters
NAME                  SB-1-132
EXPNO                        9
PROCNO                       1

F2 - Acquisition Parameters
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Time                     22.00 h
INSTRUM                AVN 400
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PULPROG                zgpg30
TD                        65536
SOLVENT                   MeOD
NS                        3072
DS                           4
SWH                  23809.523 Hz
FIDRES                0.726609 Hz
AQ                    1.3762560 sec
RG                      37.4408
DW                      21.000 usec
DE                       6.50 usec
TE                      298.0 K
D1                    3.00000000 sec
D11                   0.03000000 sec
TD0                          1
SFO1                100.6655806 MHz
NUC1                        13C
P0                       3.33 usec
P1                      10.00 usec
PLW1                58.86999893 W
SFO2                400.3016012 MHz
NUC2                        1H
CPDPRG[2                waltz65
PCPD2                    90.00 usec
PLW2                14.13000011 W
PLW12                0.15710001 W
PLW13                0.07902300 W

F2 - Processing parameters
SI                        32768
SF                  100.6553746 MHz
WDW                          EM
SSB                          0
LB                       1.00 Hz
GB                           0
PC                       1.40
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(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

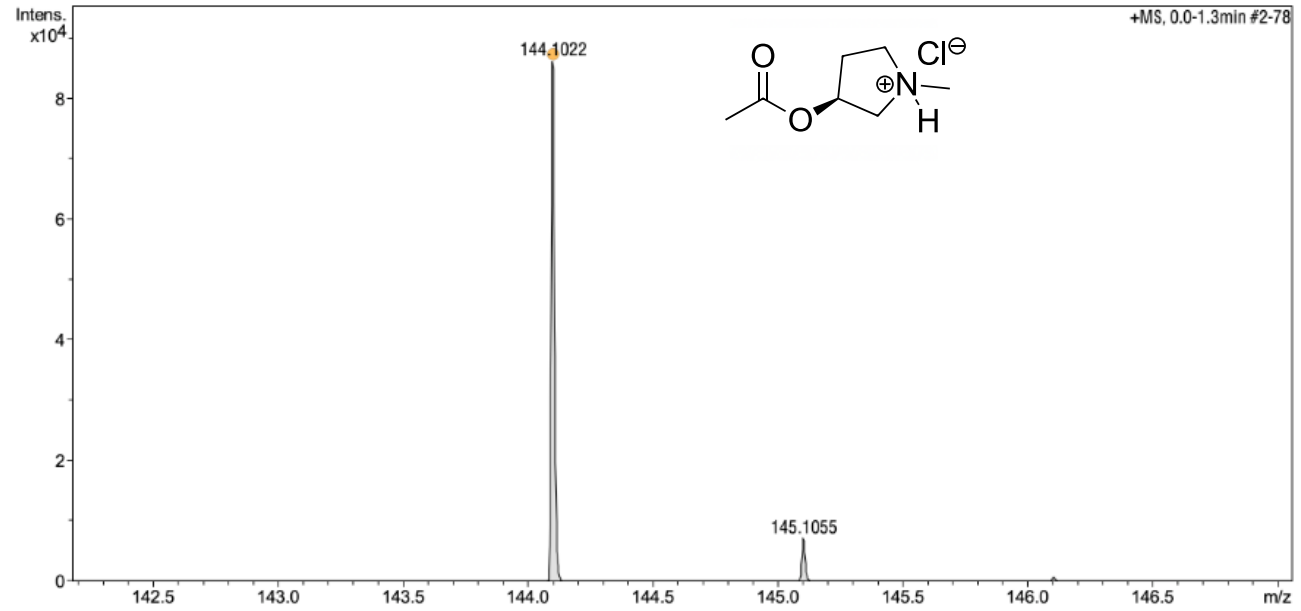
Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000014.d	Acquisition Date	7/7/2022 10:04:14 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6017 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
144.1022	C7H14NO2	144.1019	-2.0



July 07 2022000014.d

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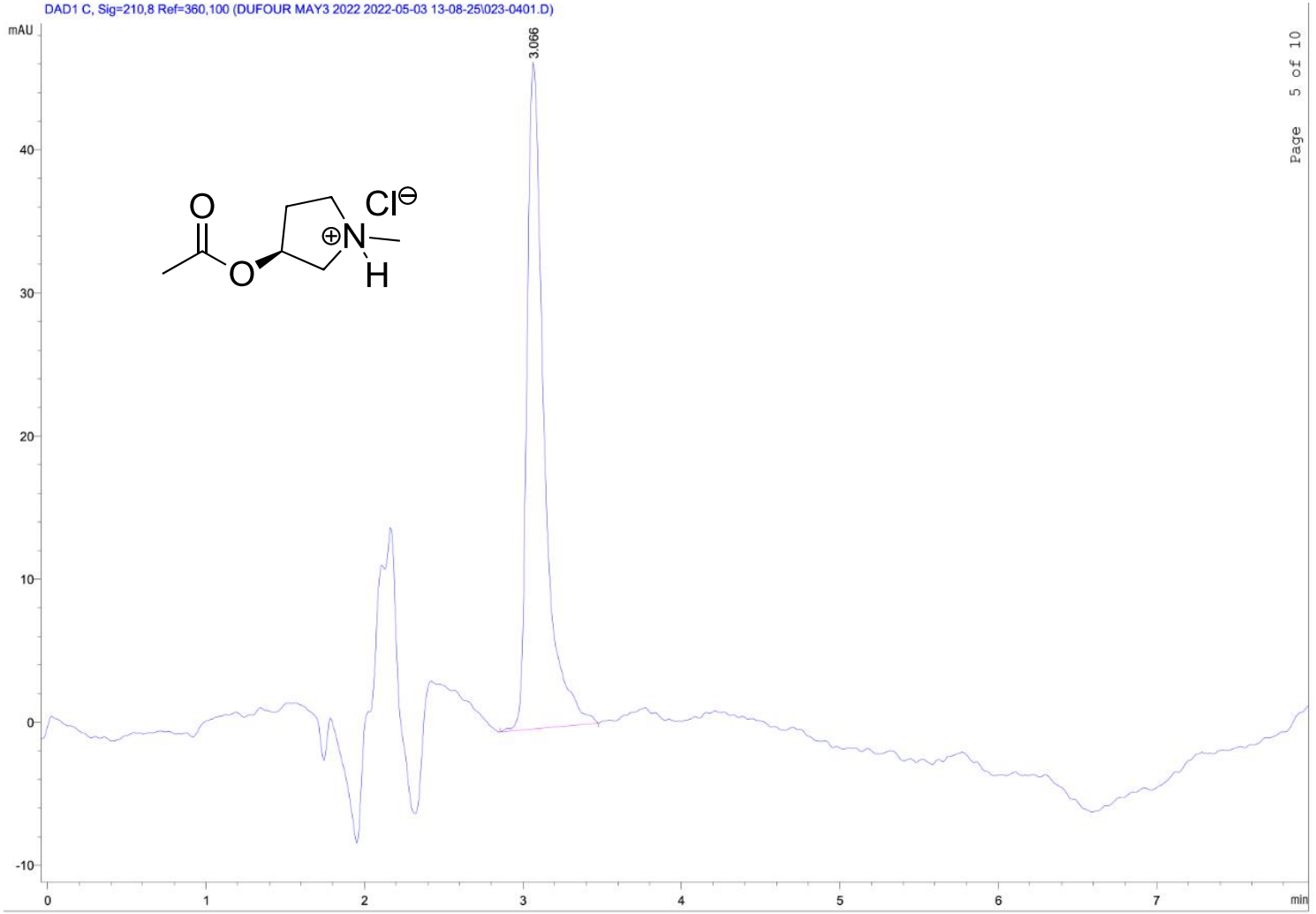
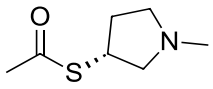
(16) *(3S)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 3.066 min.



(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate

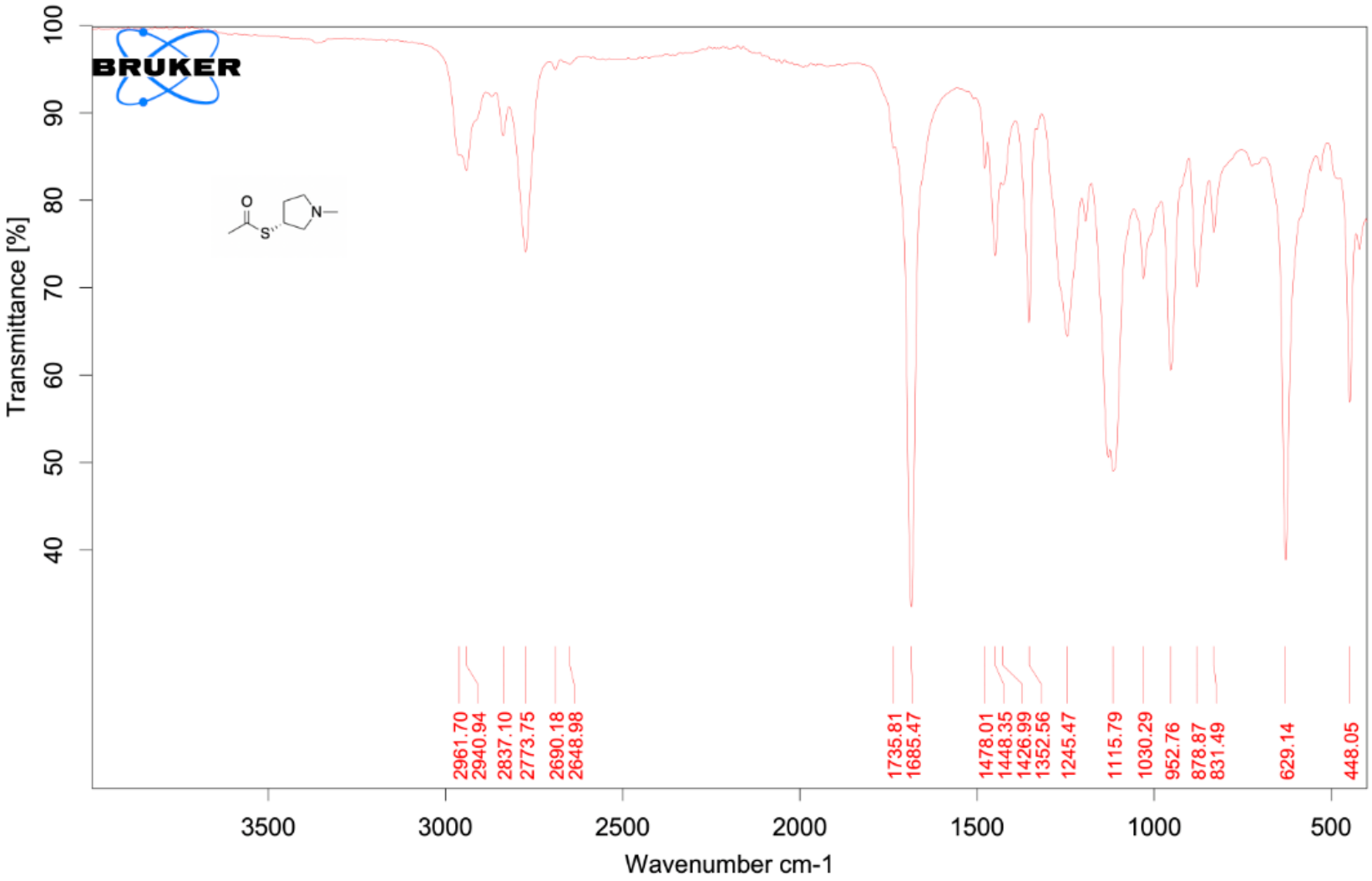


Synthesis: **(*3R*)-*S*-(1-methylpyrrolidin-3-yl) ethanethioate**: (*3S*)-(+)-1-Methyl-3-pyrrolidinol (6.00 mL, 54.6 mmol) was dissolved in dry dichloromethane (180 mL) and cooled in an ice bath. Triethylamine (11.4 mL, 81.9 mmol) was added dropwise. The mixture was stirred for 5 mins before the dropwise addition of methanesulfonyl chloride (4.90 mL, 62.6 mmol). The mixture was warmed slowly to room temperature and stirred for 16 hrs. The reaction was then quenched with the addition of brine (50 mL). The layers were separated, and the aqueous layer was back-extracted with dichloromethane (2 × 30 mL). The combined organics were washed with saturated $NaHCO_{3(aq)}$, dried over Na_2SO_4, gravity filtered, and the filtrate was concentrated to afford 8.01 g (82% yield) of the labile mesylate as an oil, which was used immediately without purification. The crude mesylate (6.80 g, 38.0 mmol) was charged to a round-bottom flask. To this was added 18-crown-6 ether (15.1 g, 57.0 mmol), THF (125 mL), and then potassium thioacetate (6.50 g, 57.0 mmol) in one portion. The mixture was heated to reflux and stirred overnight for 16 hrs. After cooling to room temperature, the reaction was diluted with H_2O (60 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic layers were washed with saturated $NH_4Cl_{(aq)}$, H_2O (3 × 60 mL), brine (60 mL), and dried with Na_2SO_4. The mixture was gravity filtered and the filtrate was concentrated to give 3.59 g of a purple oil. Vacuum distillation (heating at 115^{o}C at 1 mmHg and collecting the distillate at 74-76^{o}C) afforded 2.43 g of an orange oil. This material was then dissolved in ethyl acetate and extracted with 1N HCl (2 x 15 mL). The combined aqueous extracts were basified by the dropwise addition of 2N Na_2CO_3(aq) and then quickly extracted with dichloromethane (3 x 30 mL). The combined dichloromethane

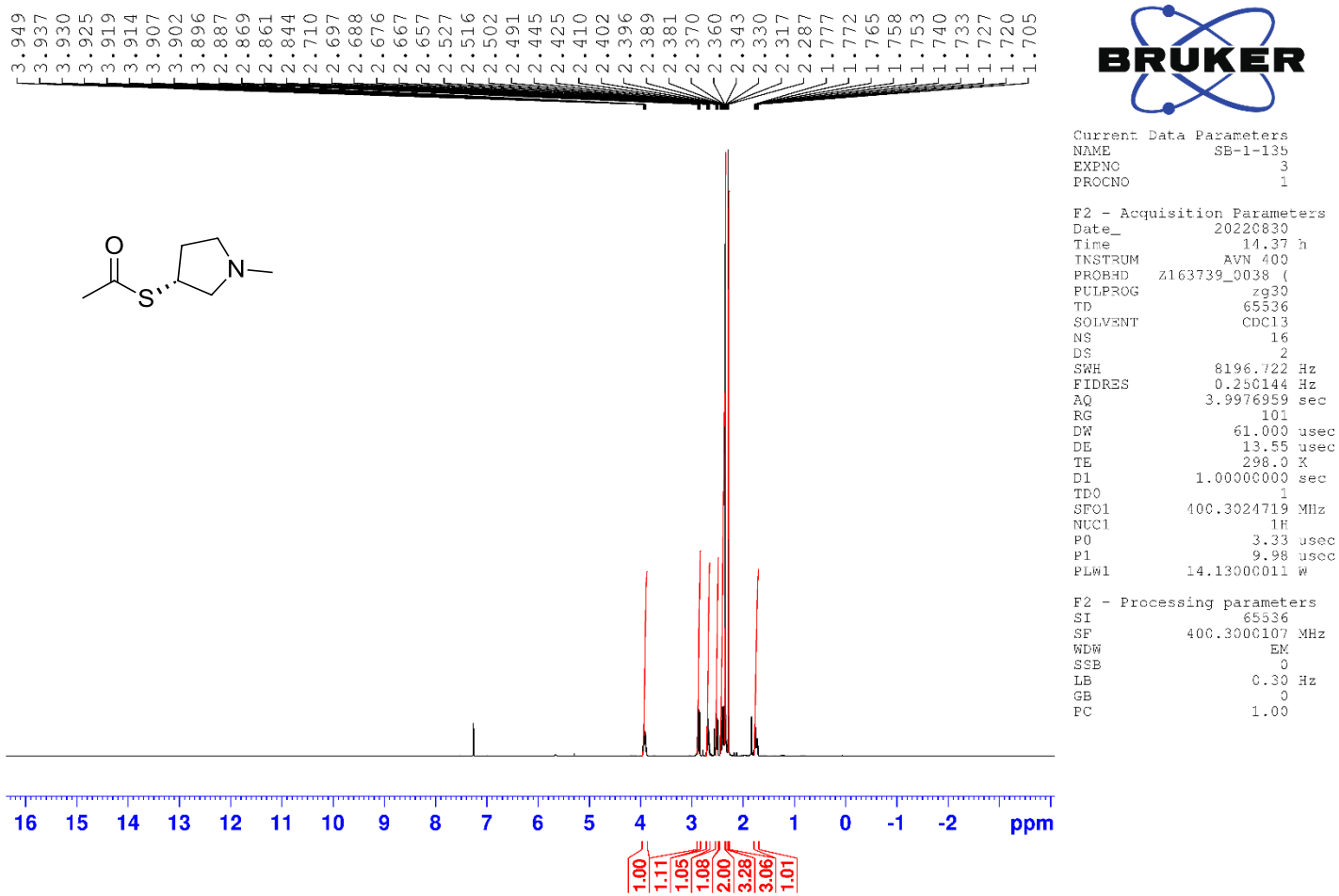
extracts were washed with brine, dried (Na_2SO_4), gravity filtered, and the filtrate was concentrated *in vacuo* to give 1.57 g of a yellow oil. This material was dissolved in *i*-PrOH (100 mL) and treated with oxalic acid (0.875 g, 9.72 mmol). After stirring for 2 hrs, the thick white precipitate was collected by filtration, washing with *i*-PrOH (50 mL). The crude solid was then recrystallized from *i*-PrOH to give 1.85 g of a yellowish solid, which was immediately treated with 50 mL of saturated $NaHCO_3$(aq) and then extracted with DCM (3 x 60 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated to give (*3R*)-*S*-(1-methylpyrrolidin-3-yl) ethanethioate (0.985 g,16% yield) as a yellow viscous liquid.

Spectroscopic data: IR(ATR) 2962, 2941, 2774, 1685, 1116, 629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.95-3.88 (m, 1H), 2.87 (dd, J = 10.1, 7.1 Hz, 1H), 2.71-2.64 (m, 1H), 2.51 (dd, J = 10.1, 4.6 Hz, 1H), 2.44-2.36 (m, 2H), 2.34 (s, 3H), 2.29 (s, 3H), 1.78-1.71 (m, 1H); ^{13}C NMR (100.7 MHz, CD_3OD-*d4*) δ 196.5 (0), 63.0 (2), 55.6 (2), 42.0 (3), 40.5(1), 32.1 (2), 30.5 (3); LRMS (ESI^+): 160.1 (M^+); HRMS (ESI^+): calculated for $C_7H_{14}NOS^+$: 160.0791 amu; found for $C_7H_{14}NOS^+$: 160.0788 amu; HPLC purity at 230 nm (25% CH_3CN : 75% CH_3OH, retention time: 3.323 mins): 95%.

(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate IR(ATR)



(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate ^{1}H NMR 400 MHz ($CDCl_3$)



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PROCNO                 1

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TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             8196.722 Hz
FIDRES          0.250144 Hz
AQ              3.9976959 sec
RG                   101
DW                61.000 usec
DE                13.55 usec
TE                298.0 K
D1             1.00000000 sec
TD0                    1
SFO1          400.3024719 MHz
NUC1                  1H
P0                  3.33 usec
P1                  9.98 usec
PLW1          14.13000011 W

F2 - Processing parameters
SI                 65536
SF            400.3000107 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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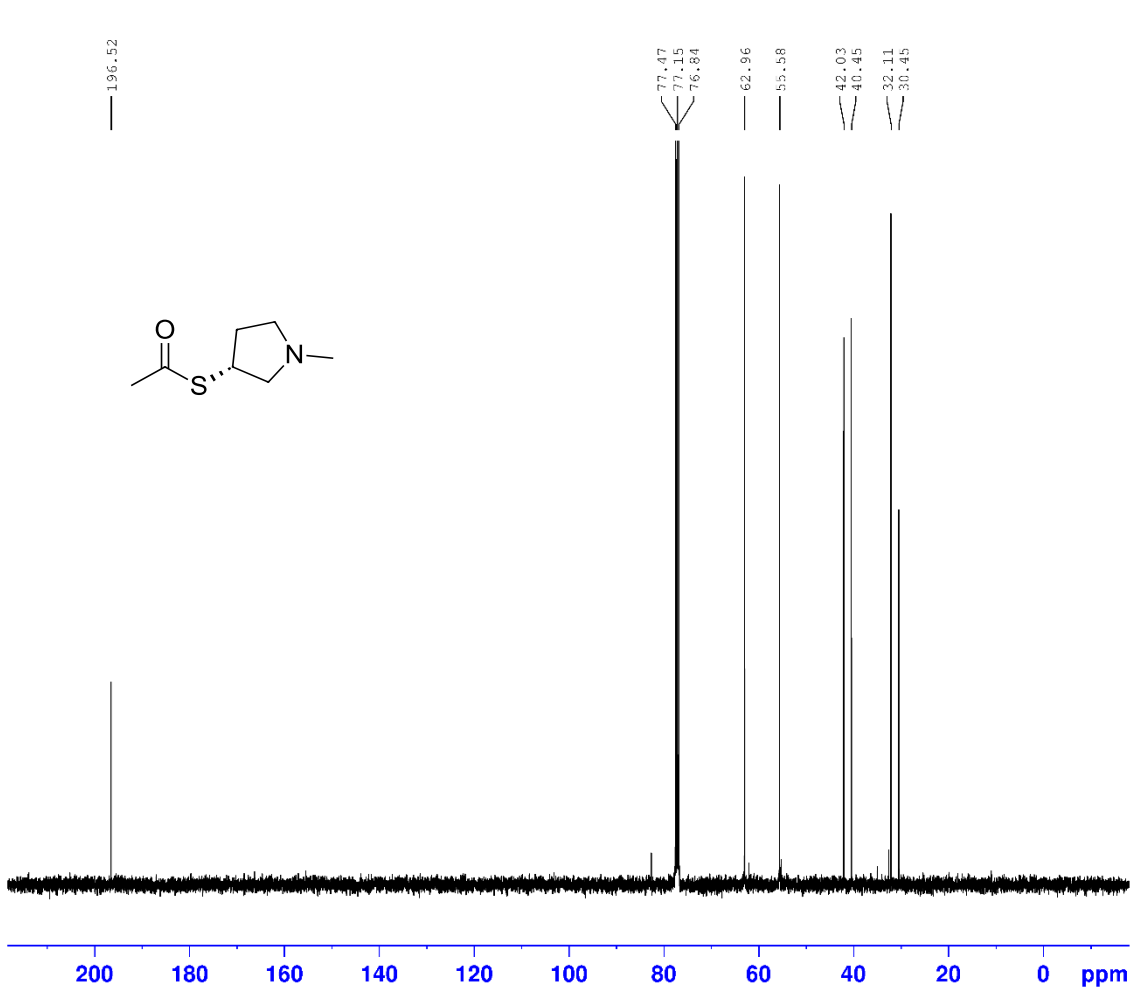
(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate ^{13}C NMR 100 MHz ($CDCl_3$)

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Current Data Parameters
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EXPNO                    4
PROCNO                   1

F2 - Acquisition Parameters
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Time                22.10 h
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PULPROG             zgpg30
TD                   65536
SOLVENT              CDCl3
NS                    4096
DS                       4
SWH             23809.523 Hz
FIDRES           0.726609 Hz
AQ               1.3762560 sec
RG                9.25165
DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                      1
SFO1         100.6655806 MHz
NUC1                  13C
P0                  3.33 usec
P1                 10.00 usec
PLW1         58.86999893 W
SFO2         400.3016012 MHz
NUC2                  1H
CPDPRG[2          waltz65
PCPD2              90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W
PLW13         0.08739400 W

F2 - Processing parameters
SI                   32768
SF          100.6555027 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                   1.40
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(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Oct 14 2022000001.d	Acquisition Date	2022-10-14 10:19:36 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6020	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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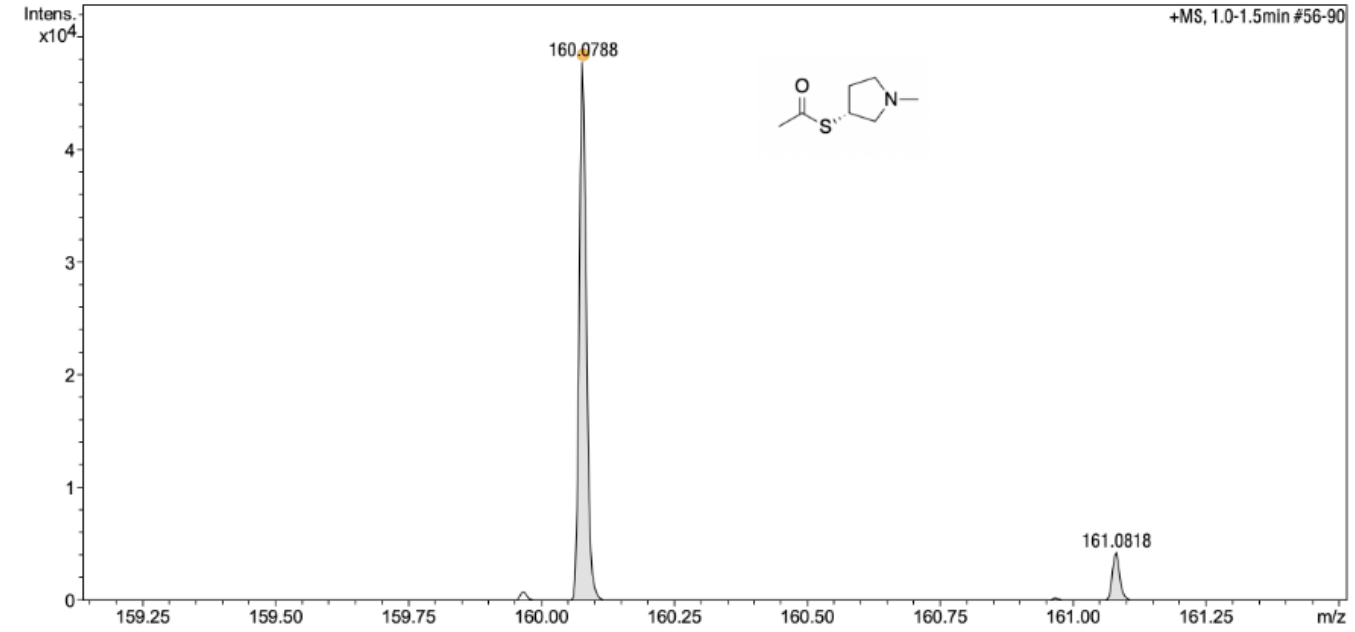

Oct 14 2022000001.d

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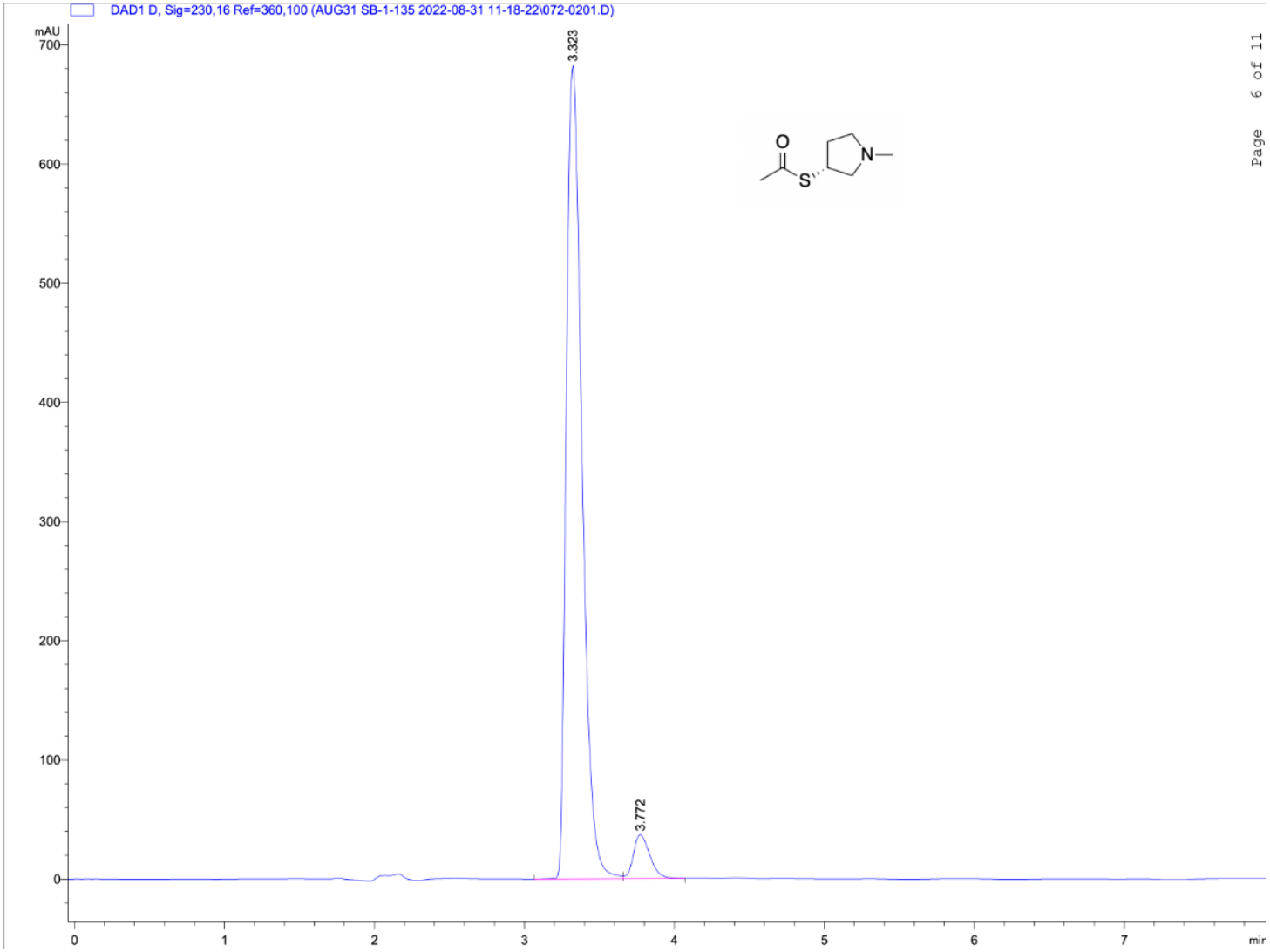
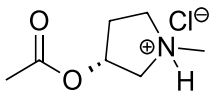
(17) (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) Ethanethioate HPLC



(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride



Synthesis: **(*3R*)-1-Methyl-3-pyrrolidinyl acetate**: (*3R*)-(-)-1-Methyl-3-pyrrolidinol (0.549 mL, 5.00 mmol) and triethylamine (1.74 mL, 12.5 mmol) were dissolved in anhydrous dichloromethane (17 mL) and cooled to 0°C with an ice bath. Acetyl chloride (0.356 mL, 5.00 mmol) was added dropwise at 0°C. The ice bath was removed after 5 min, and the reaction was stirred for 3 hrs. The reaction was quenched with 20 mL of brine, extracted with DCM (3×15 mL) and dried over Na_2SO_3. After gravity filtration of the drying agent, the solvent was removed *in vacuo* to produce a yellow oil. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to produce (*3R*)-1-methyl-3-pyrrolidinyl acetate (0.227 g, 32%) as a yellow oil.

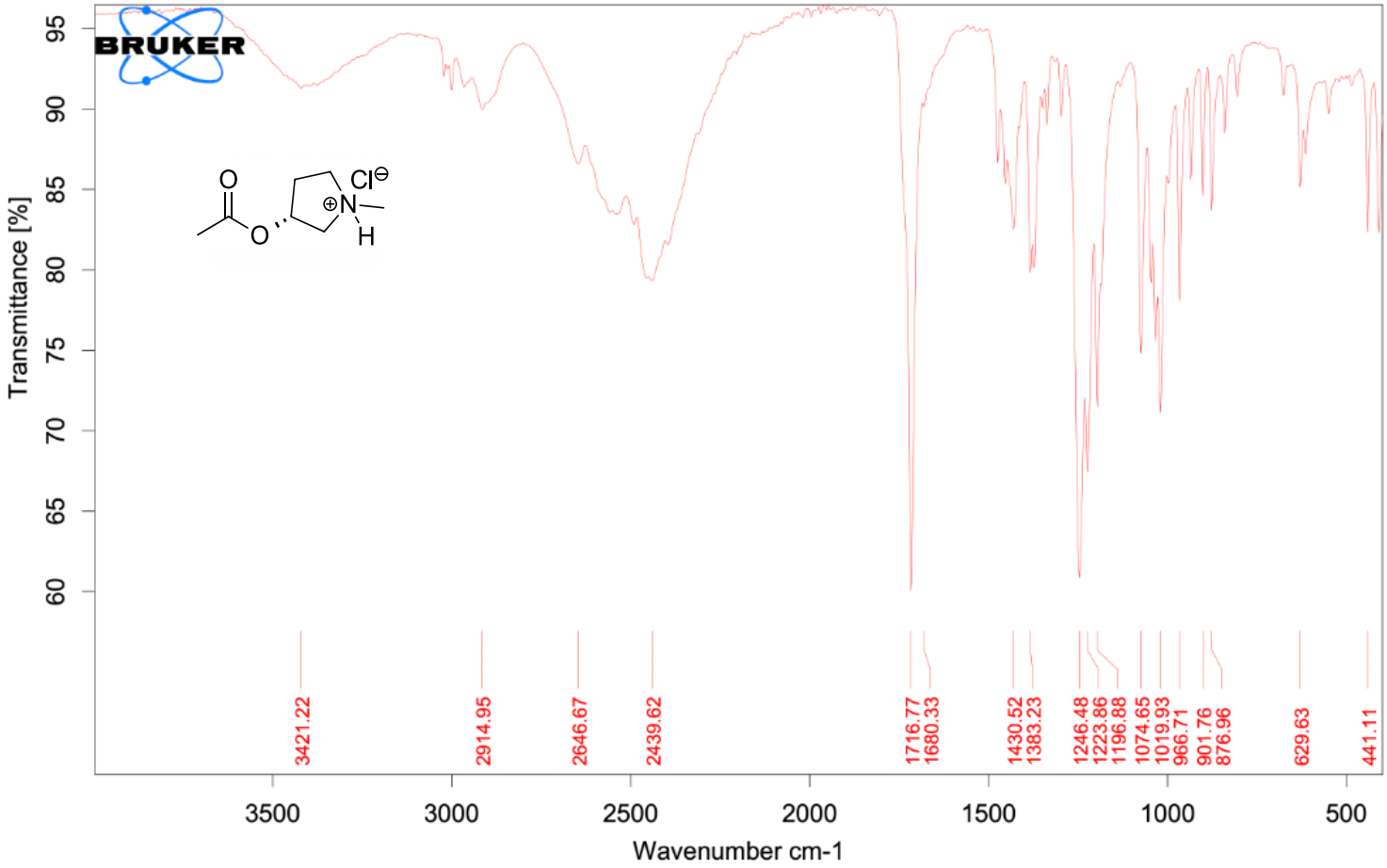
Spectroscopic data: IR(ATR) 2944, 2839, 2780, 1735, 1236, 887cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21-5.16 (m, 1H), 2.89-2.84 (m, 1H), 2.73 (d, J = 10.6 Hz, 1H), 2.58 (dd, J = 10.9, 5.9 Hz, 1H), 2.36 (s, 3H), 2.30-2.23 (m, 2H), 2.04 (s, 3H), 1.89-1.83 (m, 1H); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 171.2 (0), 74.8 (1), 62.3 (2), 55.1 (2), 42.1 (3), 32.7 (2), 21.4 (3); HRMS (ESI^+): calculated for $C_7H_{14}NO_2^+$: 144.1019 amu; found for $C_7H_{14}NO_2^+$: 144.1019 amu; HPLC purity at 200 nm (25% CH_3CN : 75% CH_3OH, retention time: 3.09 mins): >99%.

***(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride**: To a flask charged with (*3R*)-1-methyl-3-pyrrolidinyl acetate (0.122 g, 0.85 mmol) anhydrous diethyl ether was added until complete dissolution (17 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 1.28 mL, 1.28 mmol) was added dropwise and stirred for 1hr. The solvent was removed *in vacuo* resulting in a white

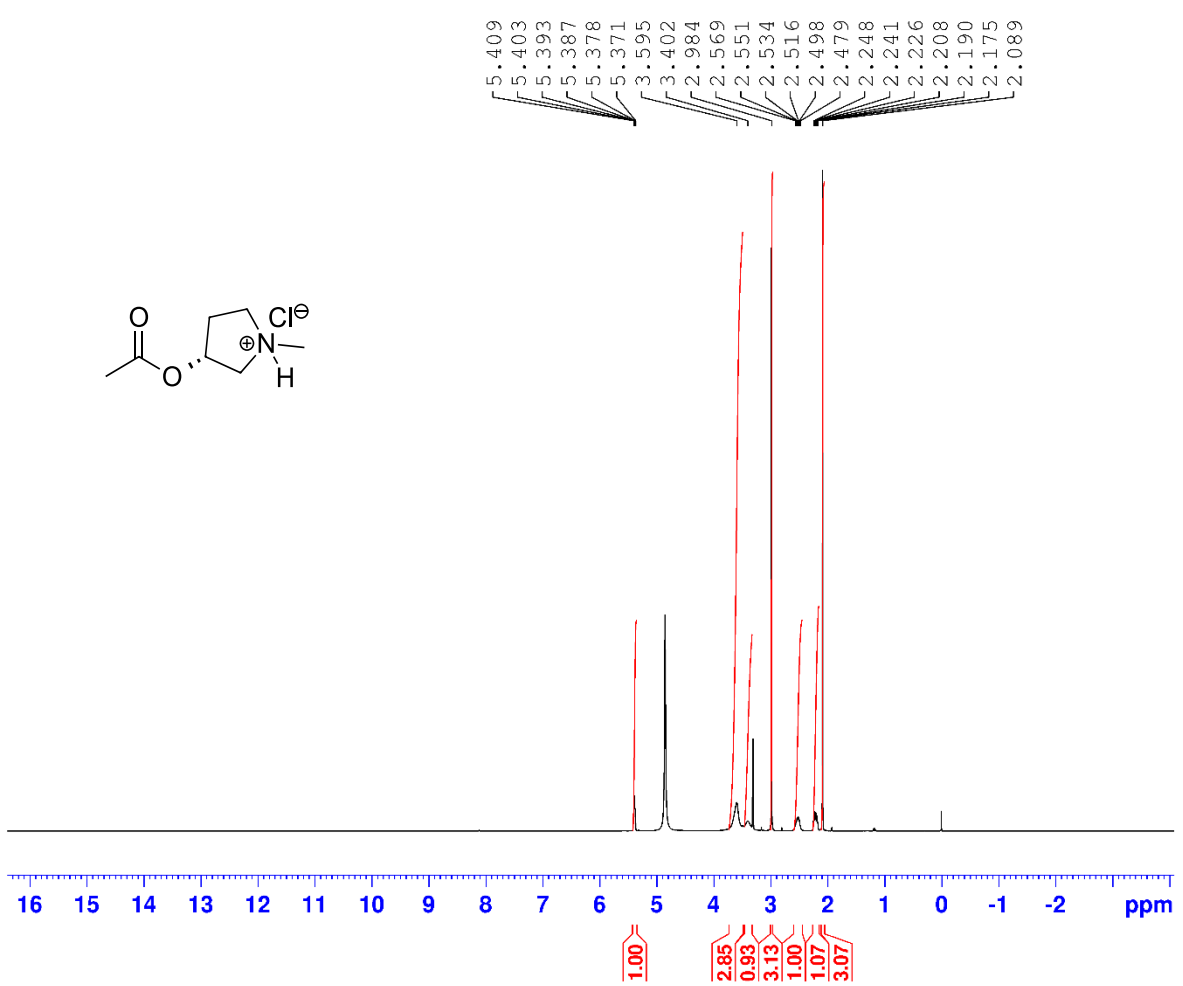
solid. The compound was triturated with diethyl ether (20 mL), and the solvent was removed with a pipet. This process was repeated two more times. The compound was dried *in vacuo* to produce *(3R)*-3-acetoxy-1-methyl-1-pyrrolidinium chloride (0.146 g, 96%).

Spectroscopic data: $MP_{(diethyl\ ether)}$: 90-93^{o}C; IR(ATR) 2915, 2647, 2440, 1717, 1246, 1224, 967 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD-*d4*) δ 5.41-5.37 (m, 1H), 3.60 (app br s, 3H), 3.40 (app br s, 1H), 2.98 (s, 3H), 2.57-2.48 (m, 1H), 2.25-2.17 (m, 1H), 2.09 (s, 3H); ^{13}C NMR (100.7 MHz, CD_3OD-*d4*) δ 171.7 (0), 73.8 (1), 61.7 (2), 55.5 (2), 41.9 (3), 31.7 (2), 20.8 (3); LRMS (ESI^+): 144.1 (M^+); HRMS (ESI^+): calculated for $C_7H_{14}NO_2^+$: 144.1019 amu; found for $C_7H_{14}NO_2^+$: 144.1018 amu; HPLC purity at 230 nm (25% CH_3CN : 75% CH_3OH, retention time: 3.097 mins): >99%.

(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (CD_3OD-*d4*)



BRUKER

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PROCNO                  1

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SOLVENT             MeOD
NS                    16
DS                     2
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AQ               3.9976959 sec
RG                   101
DW                61.000 usec
DE                13.54 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1
SFO1         400.3024719 MHz
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P1                10.00 usec
PLW1         14.12800026 W

F2 - Processing parameters
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SF           400.3000077 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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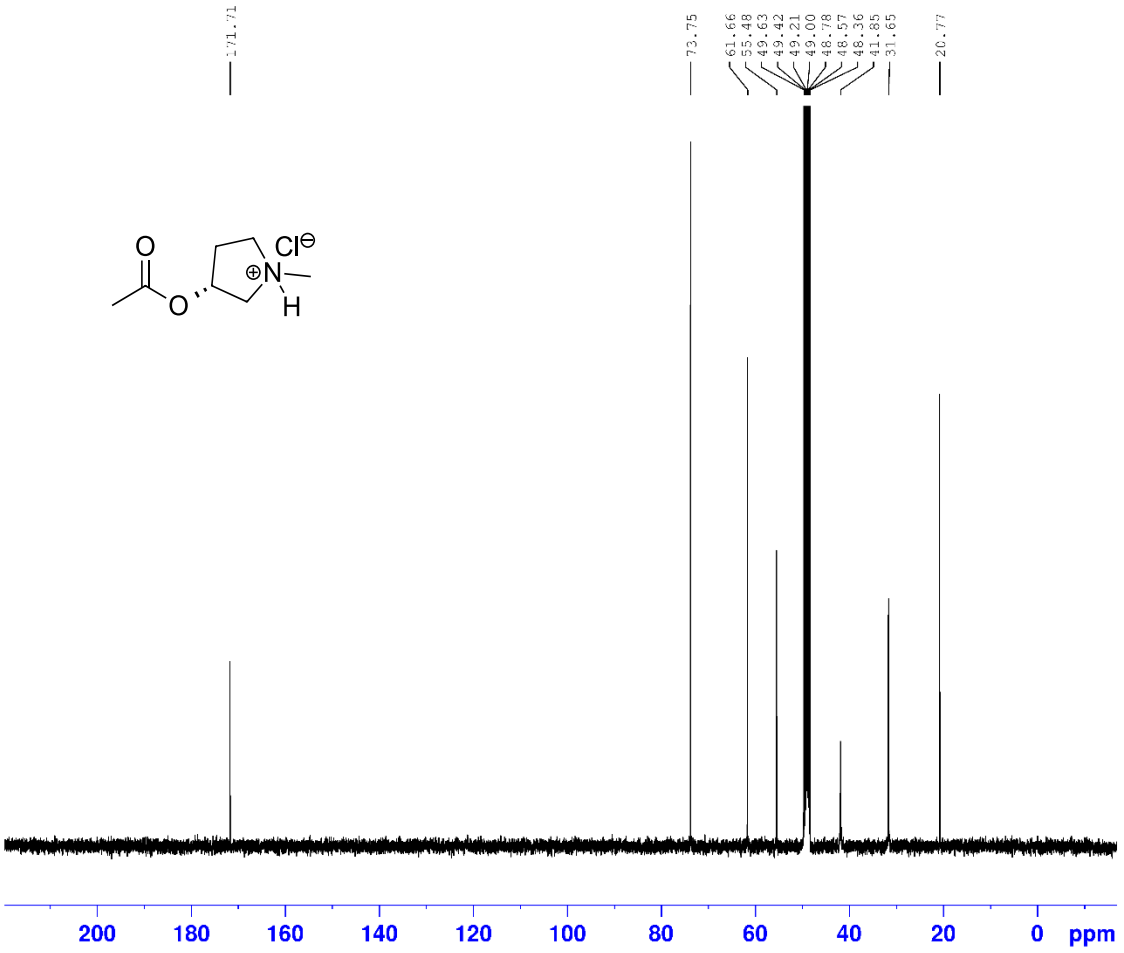
(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride ^{13}C NMR 100 MHz (CD_3OD-*d4*)



BRUKER

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PROCNO                 1

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DW               21.000 usec
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TE                298.0 K
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D11          0.03000000 sec
TD0                    1
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NUC1                13C
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PLW1        58.86999893 W
SFO2        400.3016012 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             90.00 usec
PLW2        14.12800026 W
PLW12        0.17442000 W
PLW13        0.08773200 W

F2 - Processing parameters
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SF          100.6553754 MHz
WDW                   EM
SSB                    0
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GB                     0
PC                 1.40
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(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 06 2022000010.d	Acquisition Date	7/6/2022 2:53:34 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6018 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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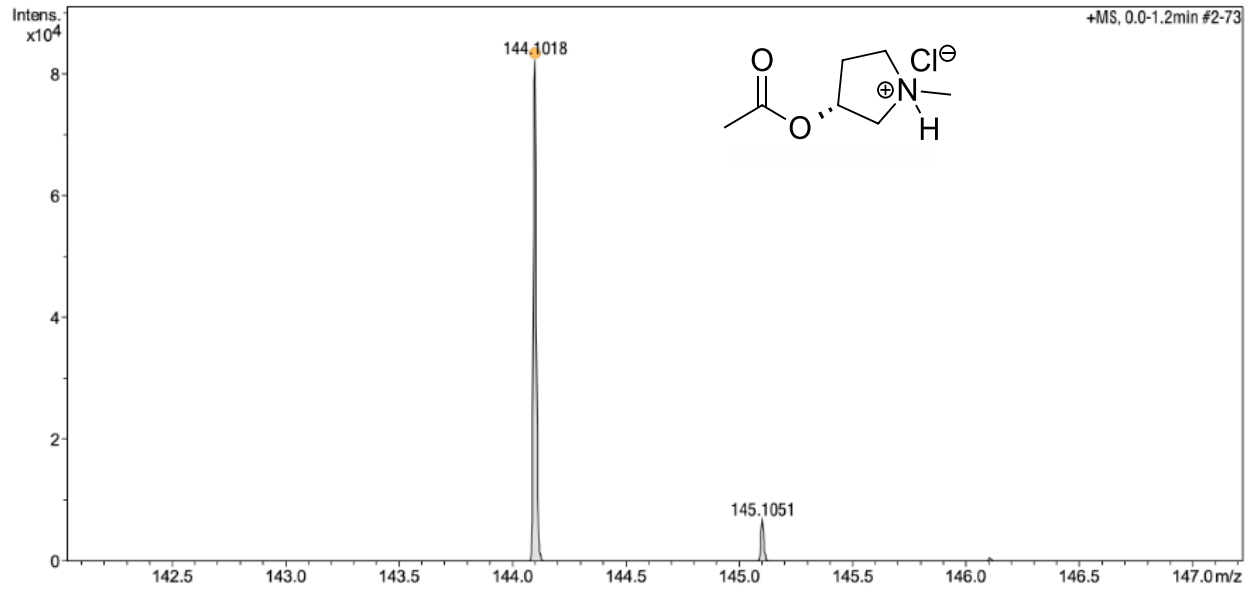

July 06 2022000010.d

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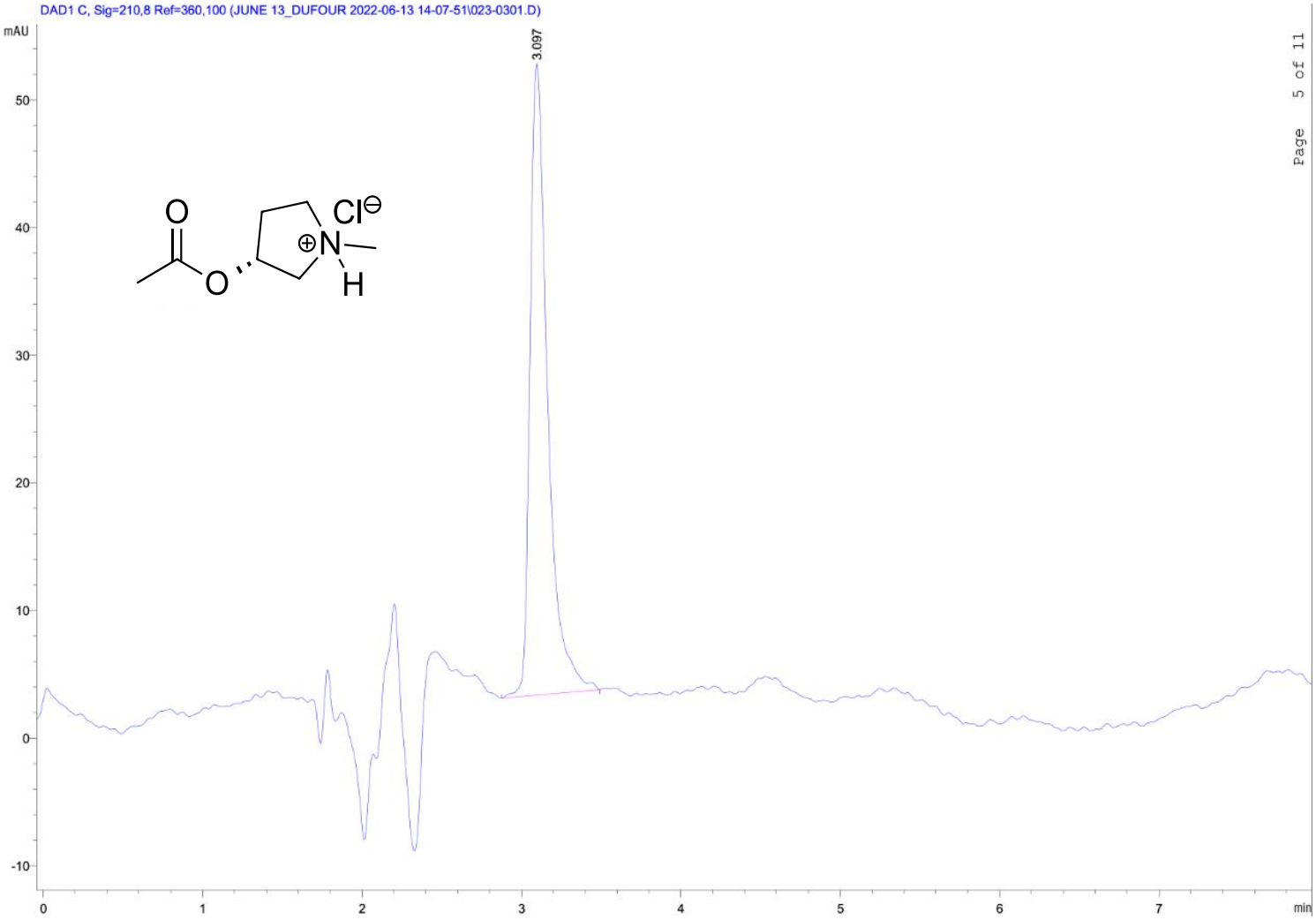
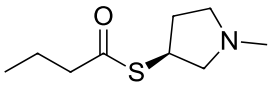
(18) *(3R)*-3-Acetoxy-1-methyl-1-pyrrolidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 3.097 min.



(19) ((*3S)*-1-Methylpyrrolidin-3-yl) Butanethioate



Synthesis: **(*3R*)- 3-(Mesyloxy)-1-methylpyrrolidine**: (*3R*)-(-)-1-Methyl-3-pyrrolidinol (6.0 mL, 54.6 mmol) was dissolved in anhydrous dichloromethane (180 mL) and cooled to 0°C with an ice bath. Triethylamine (11.4 mL, 81.9 mmol) and methanesulfonyl chloride (4.9 mL, 62.8 mmol) were added dropwise at 0°C. The ice bath was removed after 5 min, and the reaction was stirred overnight (16 hrs). The reaction was quenched with brine (50 mL), extracted with dichloromethane (3 × 15 mL) and dried over Na_2SO_3. The solvent was removed *in vacuo* to produce (*3R*)-3-(mesyloxy)-1-methylpyrrolidine as a yellow oil (6.11 g, 54%). Product confirmed by ^{1}H NMR and immediately carried through to next step.

Spectroscopic data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.23-5.19 (m, 1H), 3.01 (s, 3H), 2.90 (dd, J = 11.6 Hz, 2.0 Hz, 1H), 2.87-2.83 (m, 1H), 2.71 (dd, J = 11.3, 5.7 Hz, 1H), 2.37 (s, 3H), 2.35-2.28 (m, 2H), 2.14-2.05 (m, 1H).

(*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3R*)-3-(Mesyloxy)-1-methylpyrrolidine (6.11g, 34.11 mmol), was dissolved in anhydrous THF (114 mL) under an argon atmosphere. 18-Crown-6-ether (9.91 g, 37.5 mmol) was added followed by potassium thioacetate (4.29 g, 37.5 mmol), added in 3 portions. Once all potassium thioacetate had gone into solution, the reaction was heated to 30°C and stirred overnight. The reaction was gravity filtered and the filtrate was diluted with water (50 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo*

to produce a red oil. This crude material was distilled under vacuum (1 mm Hg at 95 °C) to give (*3S*)-S-(1-methylpyrrolidin-3-yl) ethanethioate as a yellow oil (2.33 g, 37%). Product confirmed by ^{1}H NMR and immediately carried through to next step.

Spectroscopic data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95-3.89 (m, 1H), 2.94 (dd, J = 10.2 Hz, 7.3 Hz, 1H), 2.76-2.71 (m, 1H), 2.56 (dd, J = 10.4, 4.8 Hz, 1H), 2.52-2.46 (m, 1H), 2.40-2.35 (m, 1H), 2.38 (s, 3H).

(*3S*)-1-Methyl-3-pyrrolidinethiol: (*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate (0.952 g, 5.98 mmol) was dissolved in MeOH (30 mL) and sodium methoxide (0.333 g, 5.98 mmol) was added to the stirring solution. After (*3S*)-S-(1-methylpyrrolidin-3-yl) ethanethioate was no longer visible by thin layer chromatography developed by a $KMnO_4$ stain, the reaction was concentrated *in vacuo*. The resulting material was taken up in dichloromethane and concentrated *in vacuo*, and this process was repeated twice.

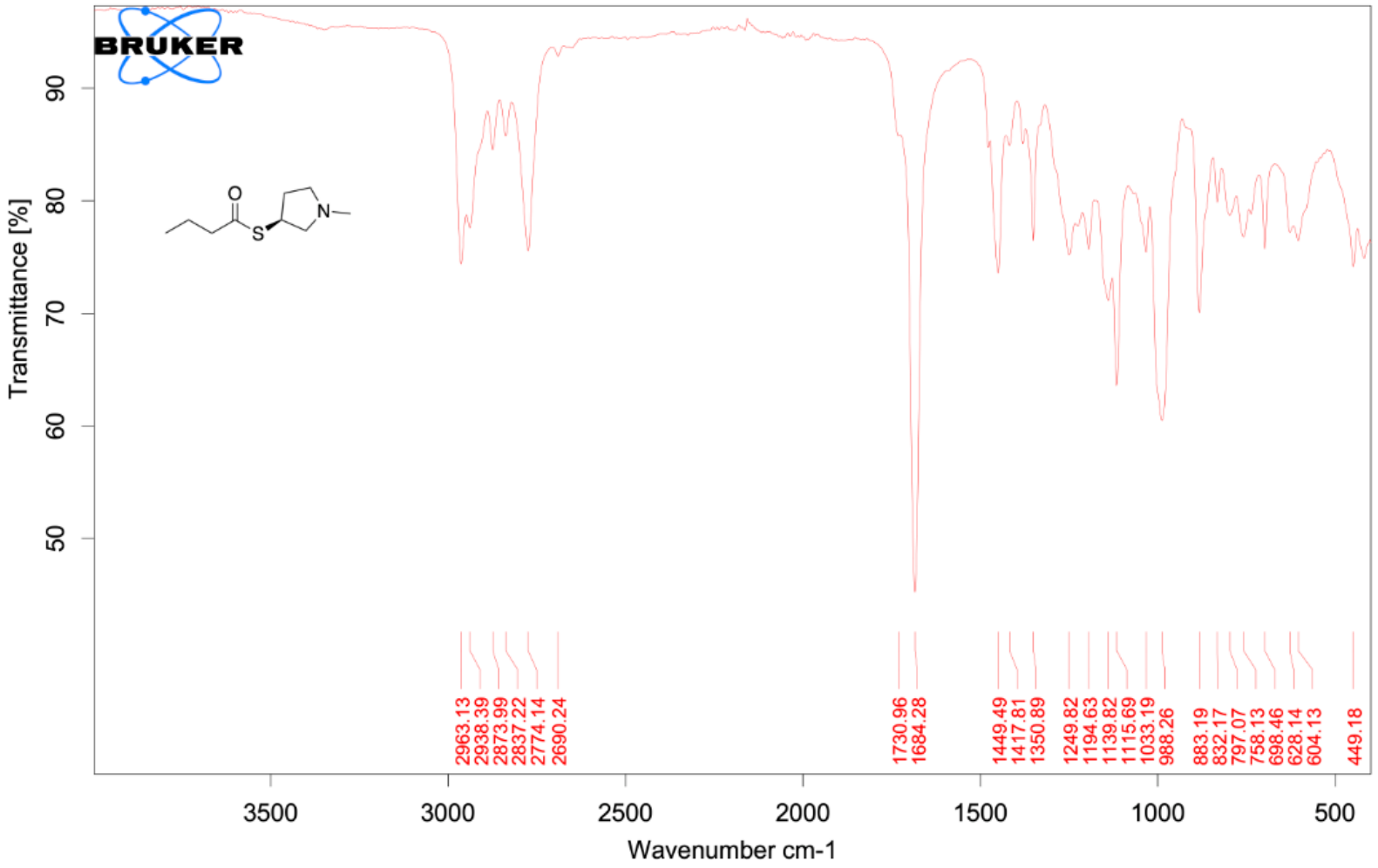
Product confirmed by ^{1}H NMR and immediately carried through to next step.

Spectroscopic data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40-3.35 (m, 1H), 2.79-2.71 (m, 2H), 2.52-2.48 (m, 1H), 2.33-2.36 (m, 3H), 2.31 (s, 3H), 1.72-1.63 (m, 1H).

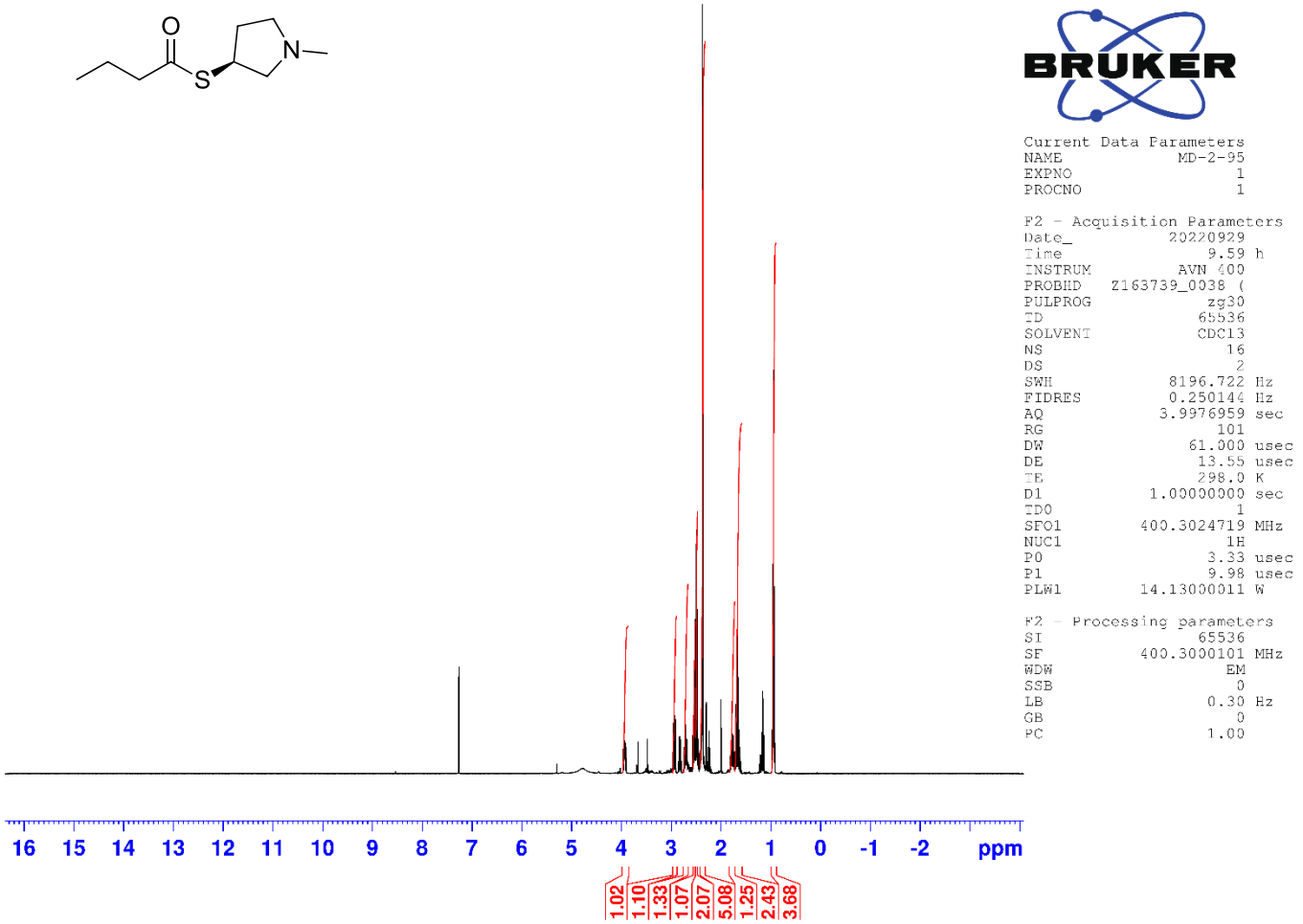
(*(3S)*-1-Methylpyrrolidin-3-yl) butanethioate: The entire material of (*3S*)-1-methyl-3-pyrrolidinethiol from the previous step was dissolved in dichloromethane (20 mL) and the resulting solution was cooled to 0°C in an ice bath. Butyryl chloride (0.621 mL, 5.98 mmol) was added followed by triethylamine (1.67 mL, 12.0 mmol). The ice bath was removed after 30 mins and the reaction was stirred overnight for 16 hrs. The reaction was concentrated *in vacuo* to afford a white material. This crude material was placed in diethyl ether (20 mL), gravity filtered to remove the insoluble material, and the filtrate was concentrated *in vacuo* to give (*(3S)*-1-methylpyrrolidin-3-yl) butanethioate as a clear oil (0.786 g, 70%).

Spectroscopic data: IR(ATR) 2963, 2938, 2774, 1684, 1116, 988, 883 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.96-3.90 (m, 1H), 2.94 (dd, J = 10.2, 7.3 Hz, 1H), 2.74-2.68 (m, 1H), 2.54 (dd, J = 10.12, 5.2 Hz, 1H), 2.47 (t, J = 7.5 Hz, 2H), 2.38-2.36 (m, 2H), 2.36 (s, 3H), 1.80-1.72 (m, 1H), 1.65 (sextet, J = 7.4 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 200.1 (0), 62.8 (2), 55.5 (2), 45.8 (2), 42.0 (2), 40.0 (1), 32.2 (2), 19.2 (2), 13.6 (3); HRMS (ESI^+): calculated for $C_9H_{18}NOS^+$: 188.1104 amu; found for $C_9H_{18}NOS^+$: 188.1100 amu; HPLC purity at 230 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.506 mins): 89%.

(19) ((*3S)*-1-Methylpyrrolidin-3-yl) Butanethioate IR(ATR)



(19) ((*3S)*-1-Methylpyrrolidin-3-yl) Butanethioate ^{1}H NMR 400 MHz ($CDCl_3$)



BRUKER

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PROCNO 1

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SOLVENT CDCl3
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DS 2
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FIDRES 0.250144 Hz
AQ 3.9976959 sec
RG 101
DW 61.000 usec
DE 13.55 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
SFO1 400.3024719 MHz
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P0 3.33 usec
P1 9.98 usec
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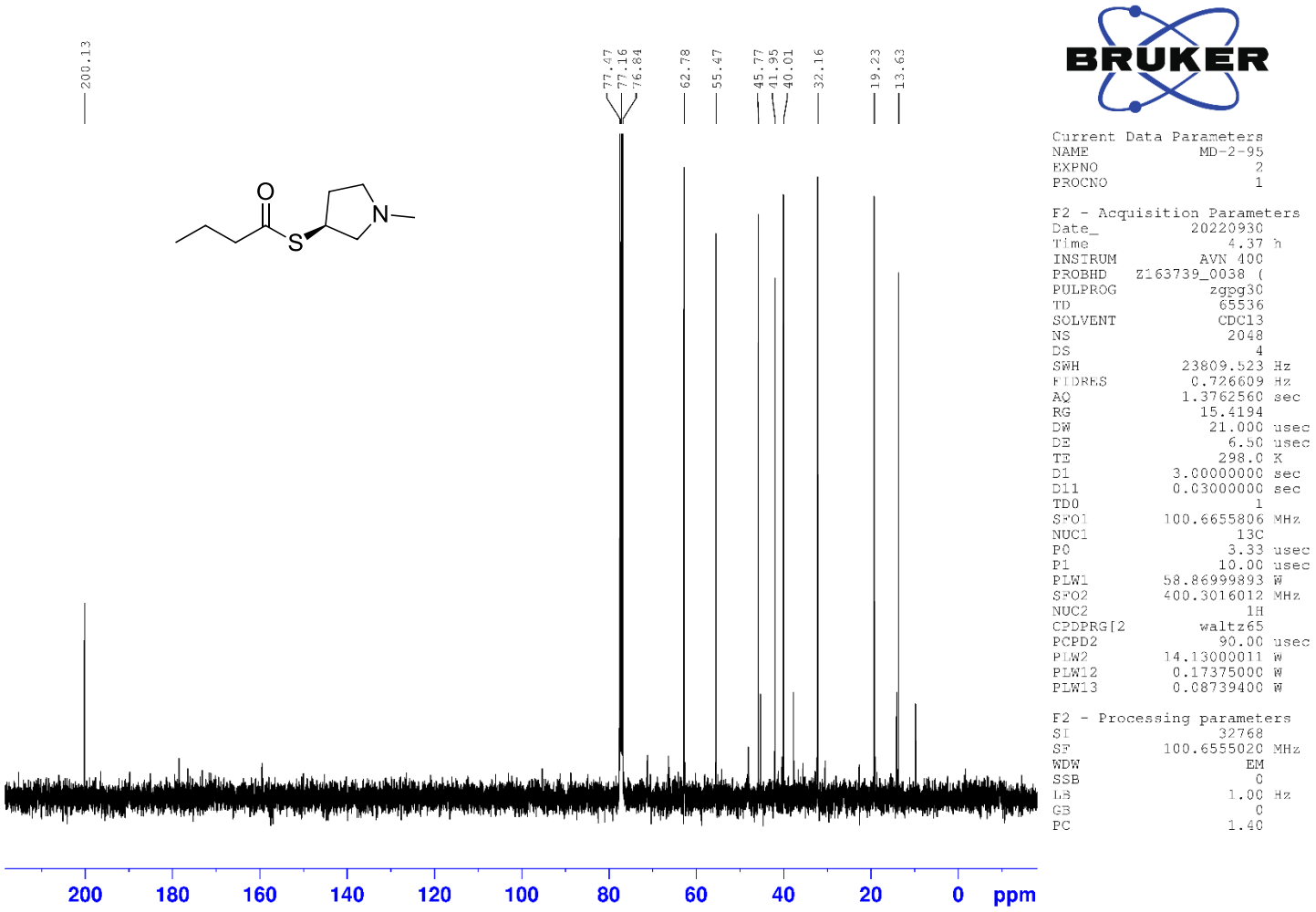
F2 - Processing parameters
SI 65536
SF 400.3000101 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

(19) (*(3S)*-1-Methylpyrrolidin-3-yl) Butanethioate ^{13}C NMR 100 MHz ($CDCl_3$)



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PROCNO                      1

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DS                        4
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FIDRES             0.726609 Hz
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RG                   15.4194
DW                   21.000 usec
DE                    6.50 usec
TE                   298.0 K
D1                3.00000000 sec
D11               0.03000000 sec
TD0                       1
SFO1            100.6655806 MHz
NUC1                    13C
P0                     3.33 usec
P1                    10.00 usec
PLW1           58.86999893 W
SFO2            400.3016012 MHz
NUC2                    1H
CPDPRG[2            waltz65
PCPD2                 90.00 usec
PLW2           14.13000011 W
PLW12           0.17375000 W
PLW13           0.08739400 W

F2 - Processing parameters
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WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
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(19) ((*3S)*-1-Methylpyrrolidin-3-yl) Butanethioate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Oct 14 2022000006.d	Acquisition Date	2022-10-14 11:10:14 AM
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Sample Name	TRV 6023	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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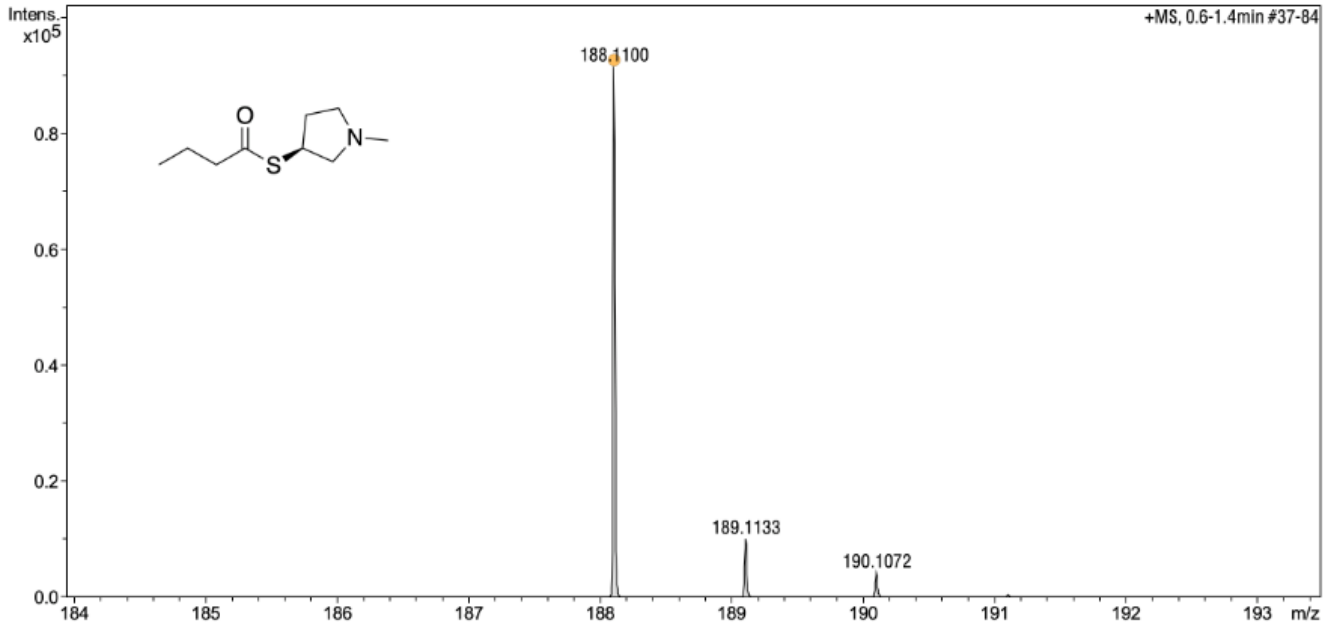

Oct 14 2022000006.d

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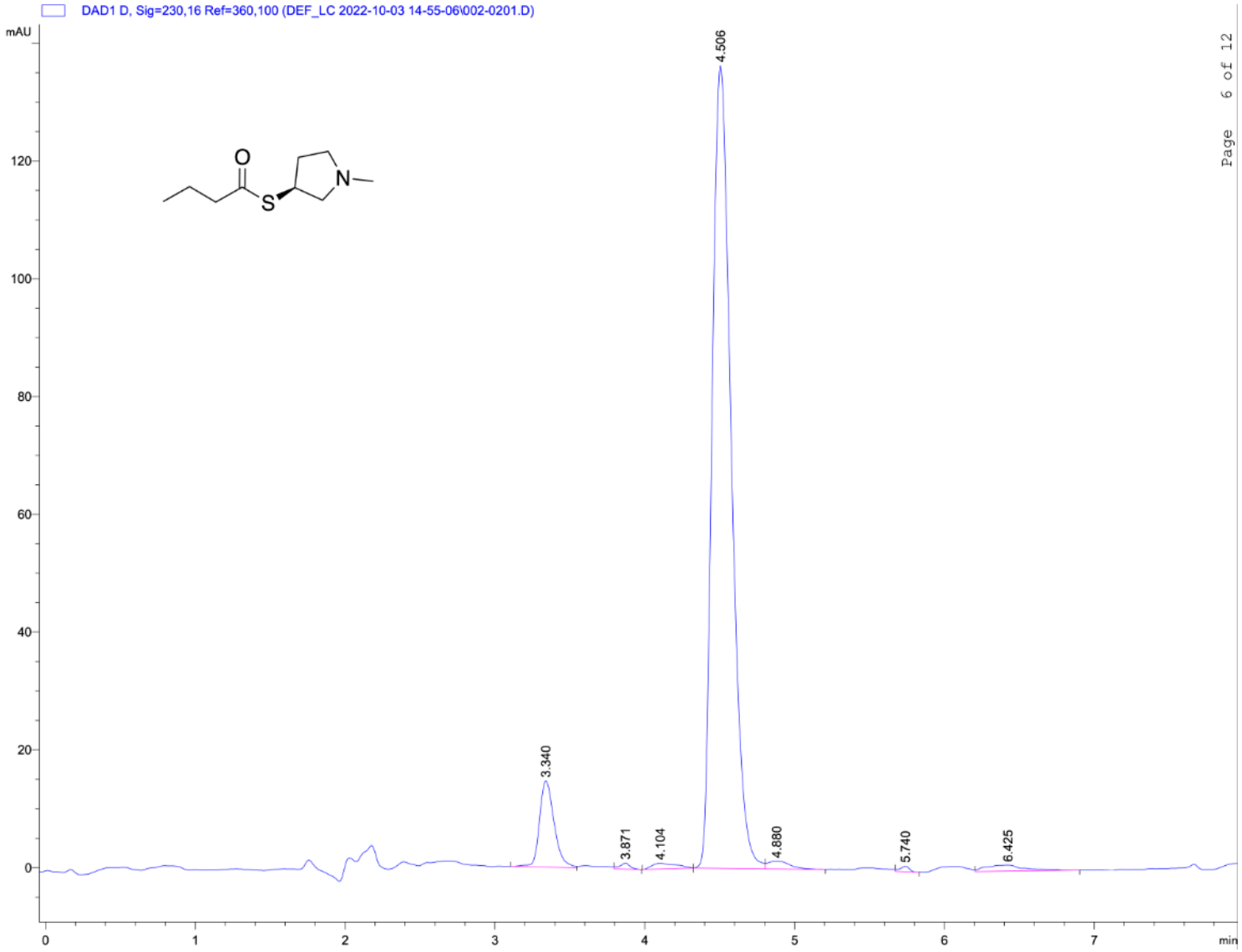
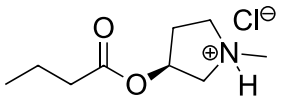
(19) ((*3S)*-1-Methylpyrrolidin-3-yl) Butanethioate HPLC



(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride



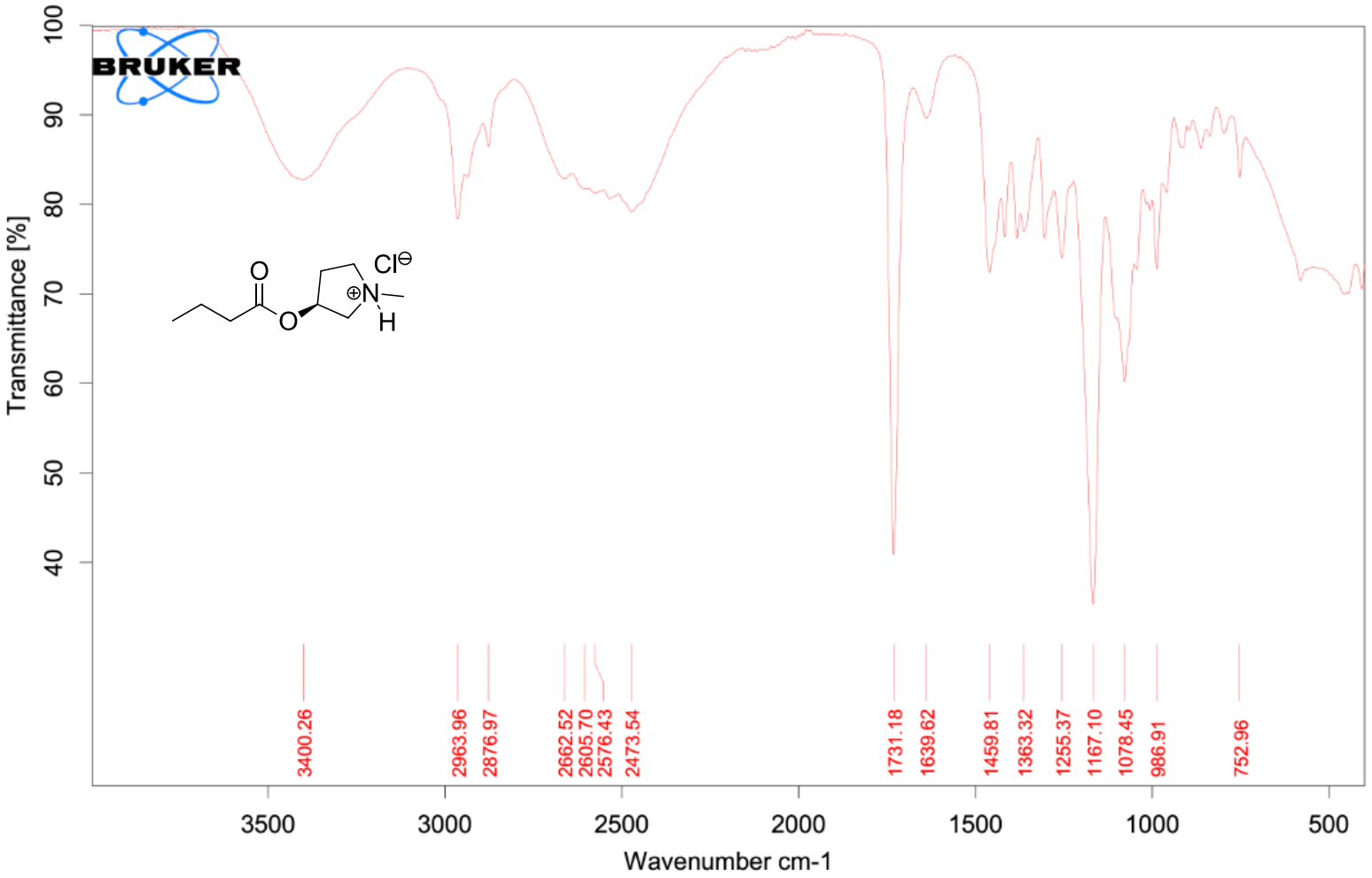
Synthesis: **(*3S*)-1-Methyl-3-pyrrolidinyl butyrate**: (*3S*)-(+)-1-Methyl-3-pyrrolidinol (0.329 mL, 3 mmol) and triethylamine (1.05 mL, 7.5 mmol) were dissolved in anhydrous dichloromethane (10 mL) and cooled to 0°C with an ice bath. Butyryl chloride (0.312 mL, 3.00 mmol) was added dropwise at 0°C. The ice bath was removed after 5 min, and the reaction was stirred for 3hrs. The reaction was quenched with brine (10 mL), extracted with dichloromethane (3 × 15 mL) and dried over Na_2SO_3. The solvent was removed *in vacuo* to produce a yellow oil. The crude mixture was purified using flash chromatography through silica gel column (5% MeOH/DCM) to afford (*3S*)-1-methyl-3-pyrrolidinyl butyrate as a yellow viscous liquid (0.367 g, 71%). The analytical data was similar to previously published data.[4]

***(3S)*-3-Butyroxy-1-methyl-1- pyrrolidinium chloride**: To a flask charged with (*3S*)-1-methyl-3-pyrrolidinyl butyrate (0.367 g, 1.77 mmol) anhydrous diethyl ether was added until complete dissolution (35 mL) under an argon atmosphere. HCl in diethyl ether 1 N (2.65 mL, 2.65 mmol) was added dropwise and stirred for 1hr. The solvent was removed in vacuo resulting in a white solid. The compound was triturated three times with diethyl ether (20 mL), solvent was removed with a pipet. This process was repeated two more times. The resulting white solid was dried *in vacuo* to produce *(3S)*-3-butyroxy-1-methyl-1- pyrrolidinium chloride (0.303 g, 82%).

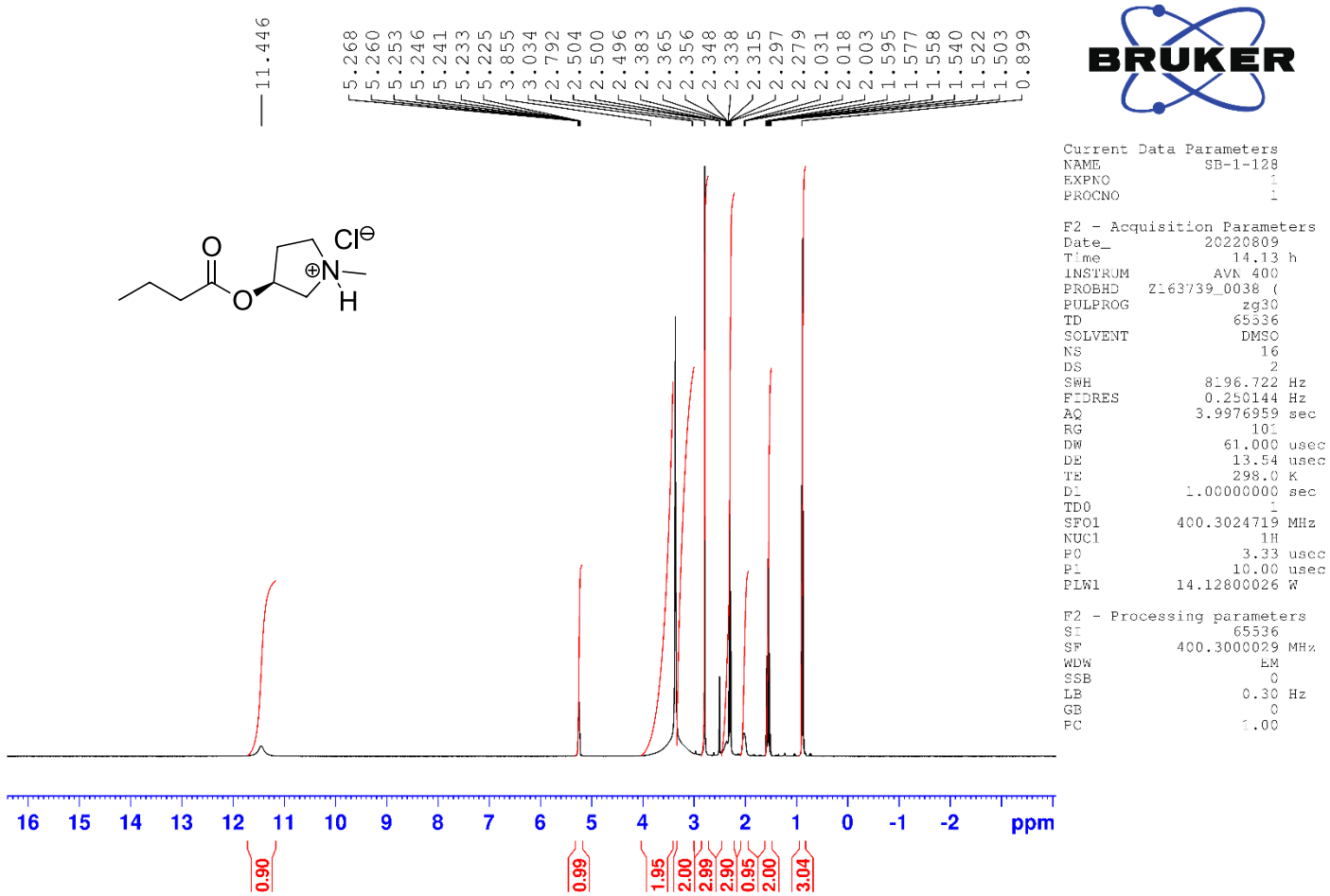
Spectroscopic data: IR(ATR) 3400, 2964, 2877, 2663, 2606, 2576, 2473, 1731, 1167, 1078 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 11.45 (br s, 1H), 5.27-5.23 (m, 1H), 3.85-3.03 (br app m, 4H), 2.79 (s, 3H), 2.38-2.34 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.03-2.00 (m, 1H), 1.55 (sext, J = 7.4

Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 172.4 (0), 72.1 (1), 59.0 (2), 53.1 (2), 40.6 (3), 35.2 (2), 30.3 (2), 17.7 (2), 13.4 (3); LRMS (ESI^+): 158.1 (M^+); HRMS (ESI^+): calculated for $C_9H_{18}NO_2^+$: 172.1332 amu; found for $C_9H_{18}NO_2^+$: 172.1336 amu; HPLC purity at 210 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 3.786 mins): >99%.

(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (DMSO-*d6*)

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PROCNO                       1

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FIDRES                0.250144 Hz
AQ                    3.9976959 sec
RG                         101
DW                      61.000 usec
DE                      13.54 usec
TE                      298.0 K
D1                    1.00000000 sec
TD0                          1
SFO1                400.3024719 MHz
NUC1                        1H
P0                       3.33 usec
P1                      10.00 usec
PLW1                14.12800026 W

F2 - Processing parameters
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WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
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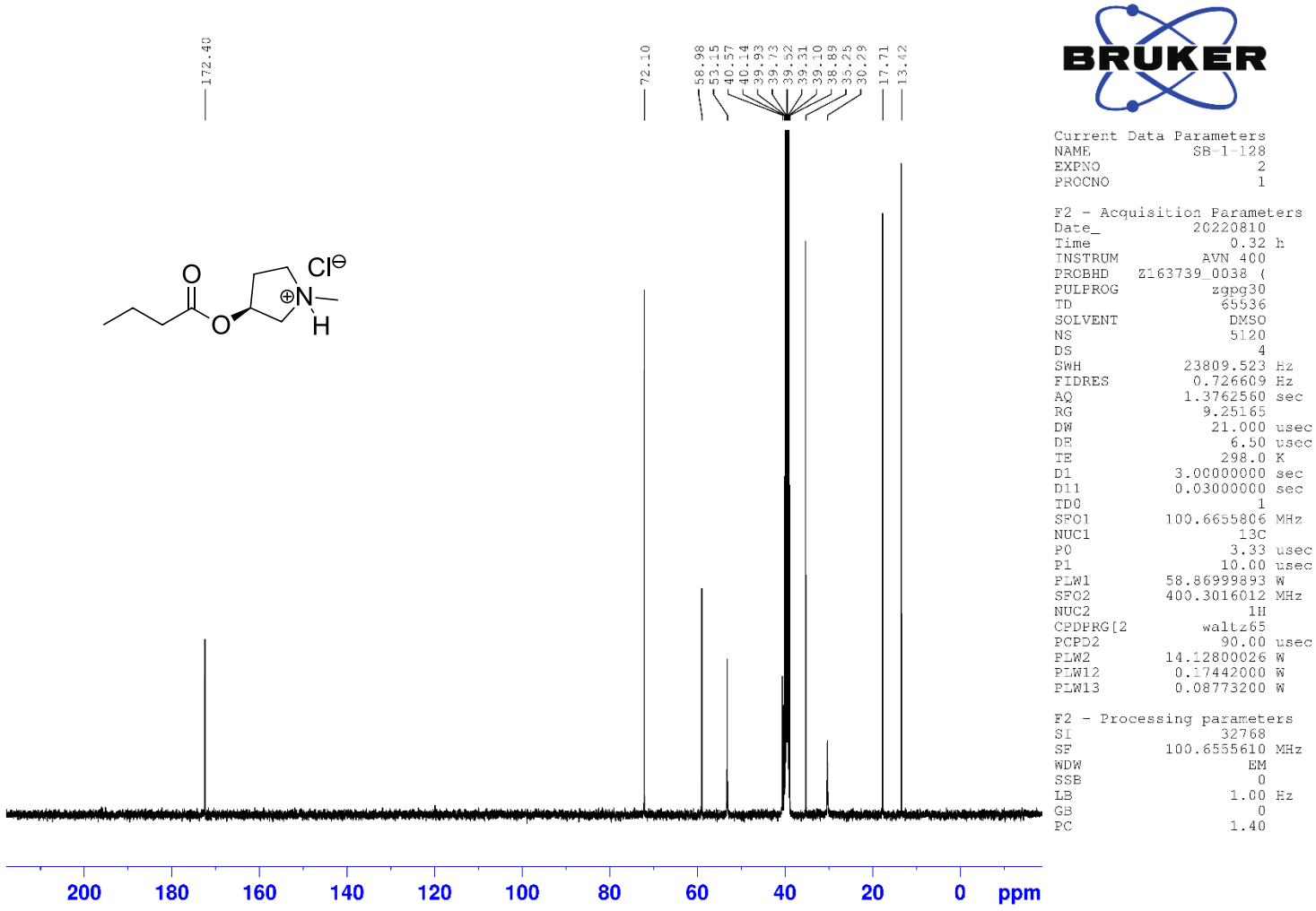
(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride ^{13}C NMR 100 MHz (DMSO-*d6*)



BRUKER

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Current Data Parameters
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EXPNO                        2
PROCNO                       1

F2 - Acquisition Parameters
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PULPROG              zgpg30
TD                    65536
SOLVENT                DMSO
NS                      5120
DS                         4
SWH               23809.523 Hz
FIDRES             0.726609 Hz
AQ                1.3762560 sec
RG                   9.25165
DW                  21.000 usec
DE                   6.50 usec
TE                  298.0 K
D1              3.00000000 sec
D11             0.03000000 sec
TD0                        1
SFO1           100.6655806 MHz
NUC1                    13C
P0                    3.33 usec
P1                   10.00 usec
PLW1           58.86999893 W
SFO2           400.3016012 MHz
NUC2                    1H
CPDPRG[2             waltz65
PCPD2                90.00 usec
PLW2           14.12800026 W
PLW12           0.17442000 W
PLW13           0.08773200 W

F2 - Processing parameters
SI                     32768
SF             100.6555610 MHz
WDW                       EM
SSB                        0
LB                      1.00 Hz
GB                         0
PC                      1.40
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(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

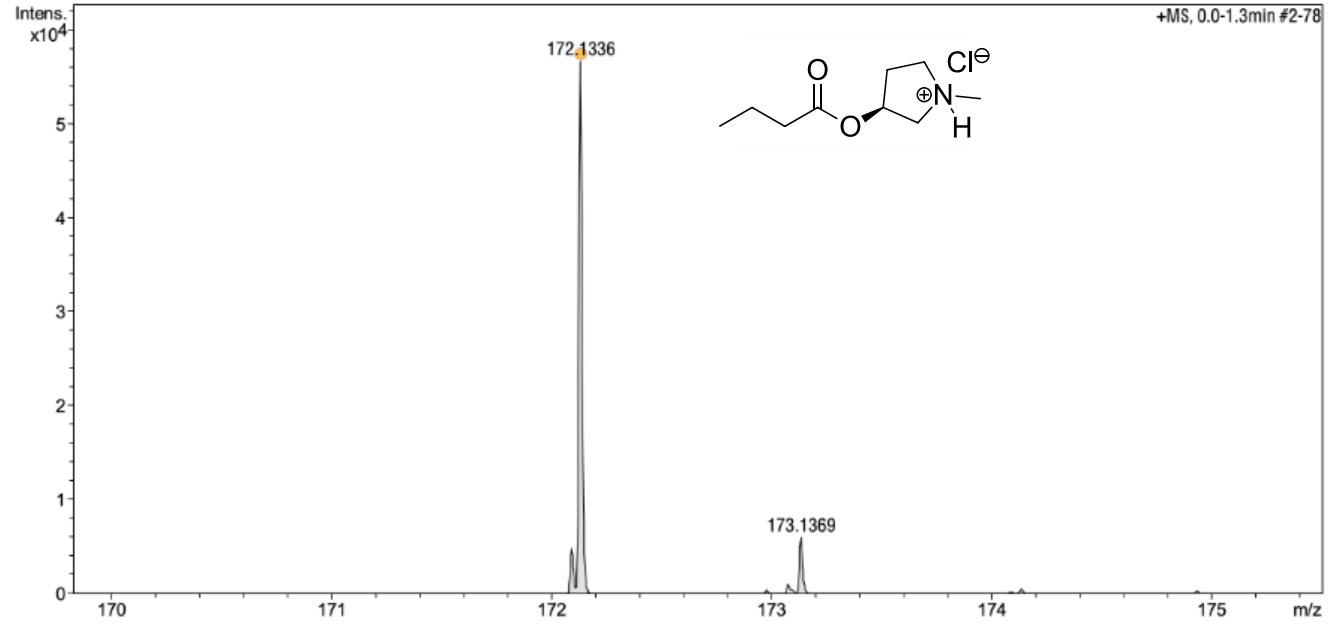
Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000010.d	Acquisition Date	7/7/2022 9:46:34 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6015 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

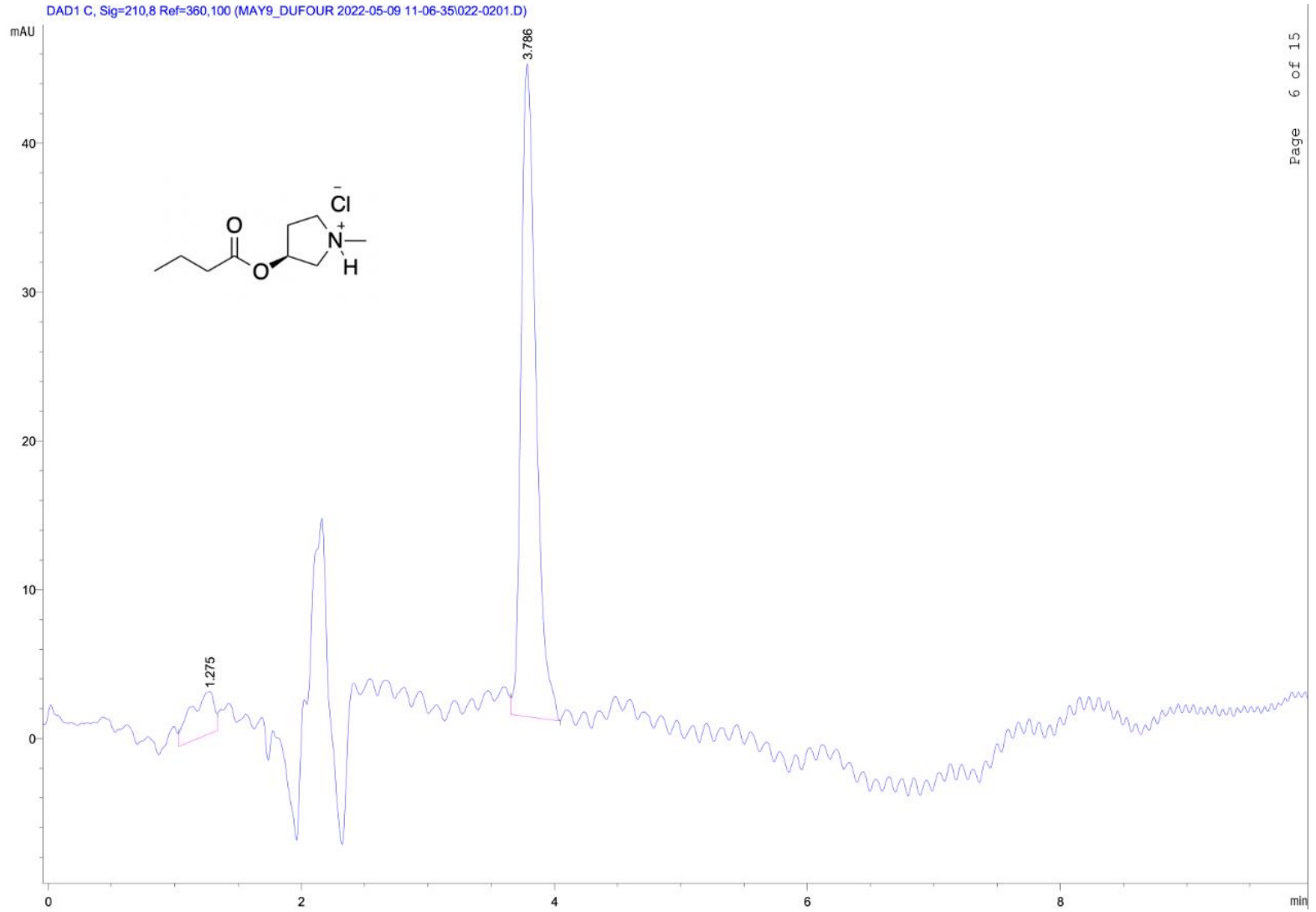
Meas. m/z	Ion Formula	m/z	err [ppm]
172.1336	C9H18NO2	172.1332	-2.2



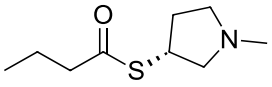
July 07 2022000010.d

Bruker Compass DataAnalysis 4.3 printed: 7/7/2022 9:49:58 AM by: x Page 1 of 1

(20) *(3S)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 3.786 mins.



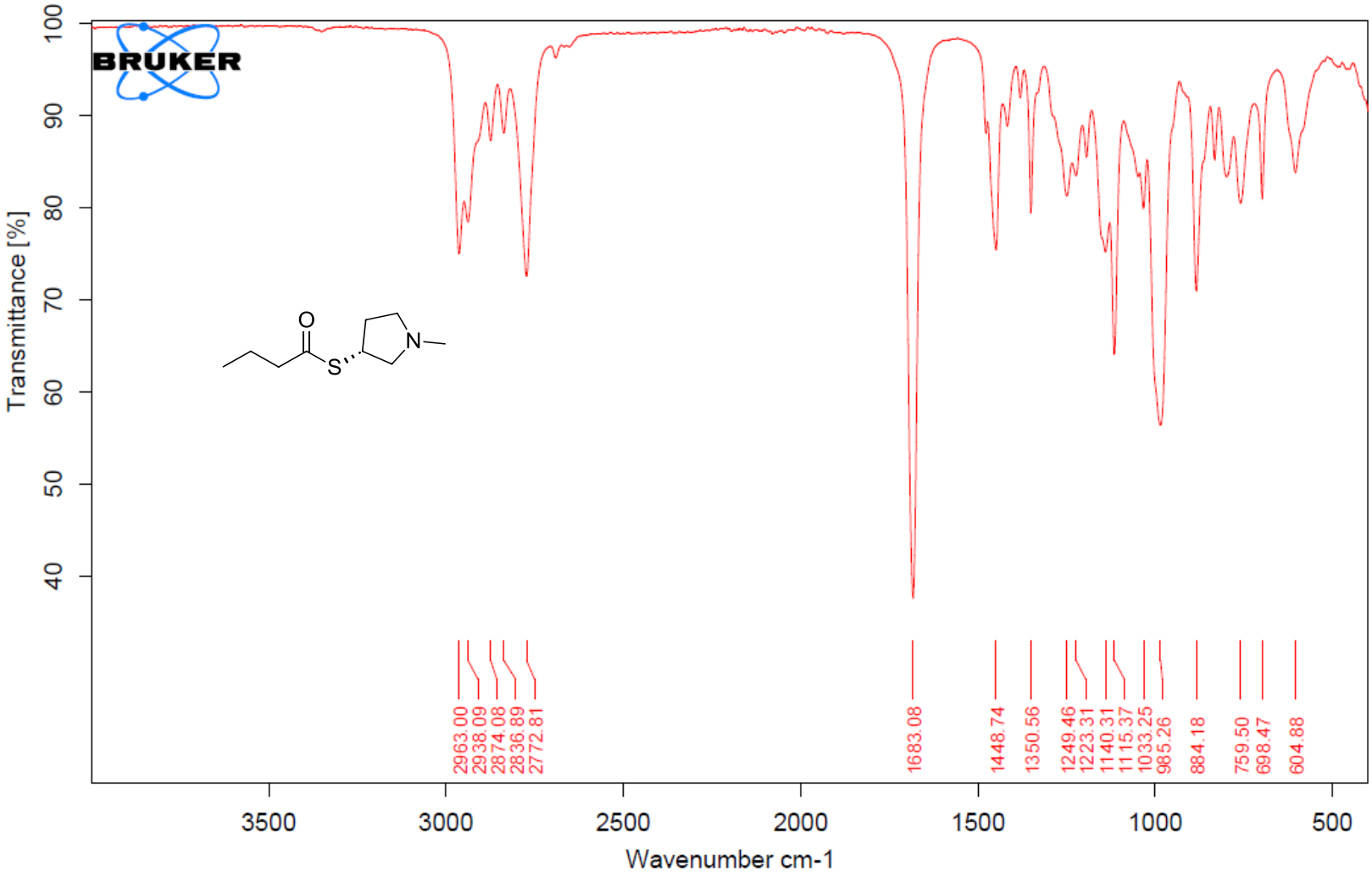
(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate



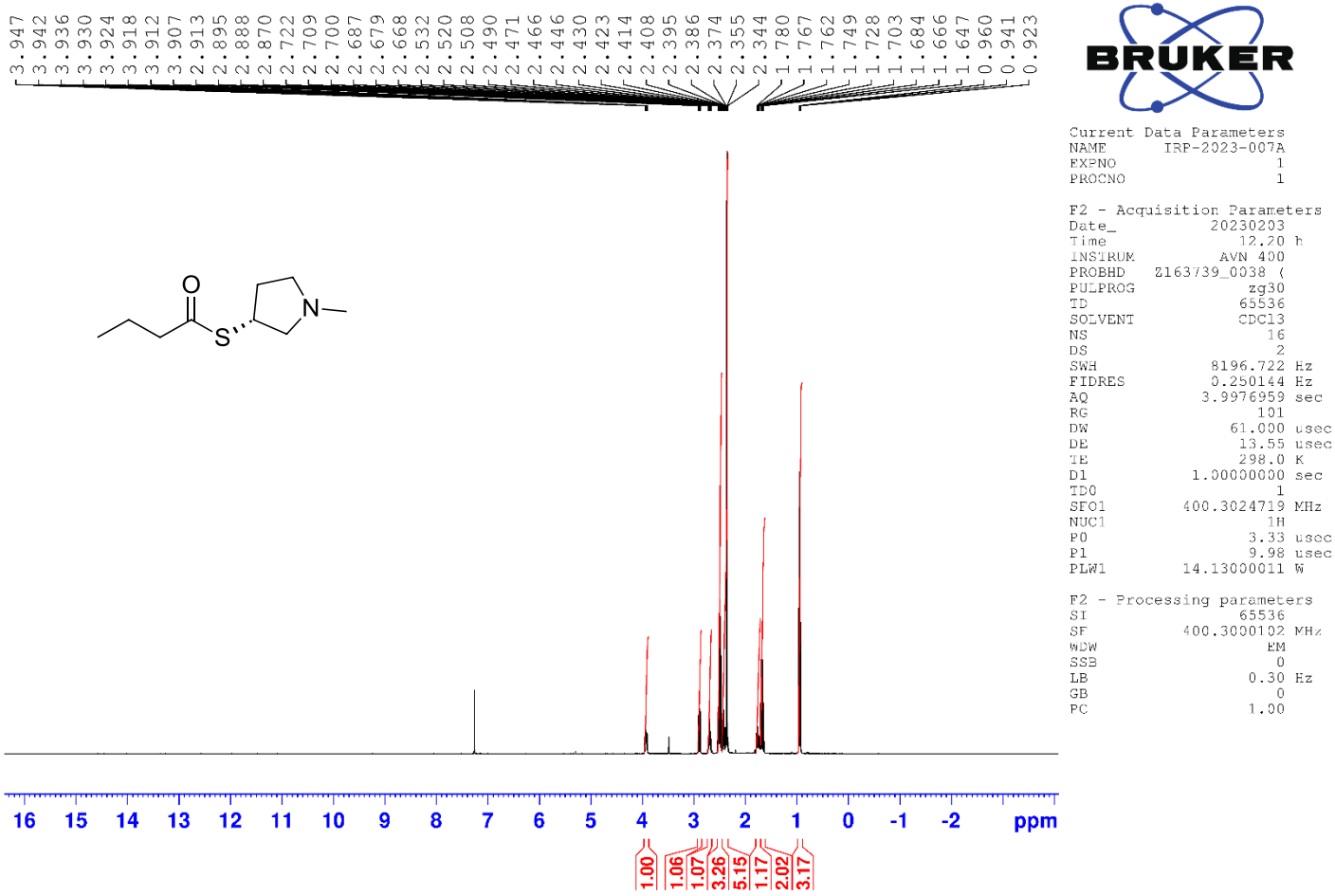
Synthesis: **((*3R*)-1-Methylpyrrolidin-3-yl) butanethioate**: (*3R*)-*S*-(1-Methylpyrrolidin-3-yl) ethanethioate (1.08 g, 6.76 mmol) was dissolved in MeOH (30 mL) and cooled in an ice bath. Sodium methoxide (0.438 g, 8.11 mmol) was added in one portion and the reaction was stirred for 3 hrs. The reaction was concentrated *in vacuo* to ensure all methanol was removed. Anhydrous THF (25 mL) and Et_3N (2.80 mL, 20.3 mmol) was added to this mixture. The stirring solution was cooled to 0^oC using an ice bath. Butyryl chloride (1.05 mL, 10.1 mmol) was added dropwise, and a white precipitate formed immediately. The reaction was warmed to room temperature and stirred for 1.5 hrs. The reaction was diluted with diethyl ether (30 mL) washed with water (2 x 30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, gravity filtered, and the filtrate was concentrated *in vacuo*. The crude material was subjected to flash chromatography through silica gel (9% methanol in dichloromethane) to afford ((*3R*)-1-methylpyrrolidin-3-yl) butanethioate (69%, 0.861 g, 4.59 mmol) of as a clear light red liquid.

Spectroscopic data: IR(ATR) 2963, 2938, 2773, 1683, 1449, 1116, 985, 884 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.96-3.89 (m, 1H), 2.89 (dd, J = 10.1, 7.2 Hz, 1H), 2.72-2.67 (m, 1H), 2.53-2.47 (m, 1H), 2.49 (t, J = 7.4 Hz, 2H), 2.45-2.37 (m, 2H), 2.36 (s, 3H), 1.79-1.72 (m, 1H), 1.68 (sext, J = 7.4 Hz, 2H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 200.2 (0), 63.1 (2), 55.6 (2), 45.8 (2), 42.1 (3), 40.1 (1), 32.2 (2), 19.2 (2), 13.6 (3); LRMS (ESI^+): XXX (M^+); HRMS (ESI^+): calculated for $C_9H_{18}NOS^+$: 188.1104 amu; found for $C_9H_{18}NOS^+$: 188.1106 amu; HPLC purity at 230 nm (25% CH_3CN : 75% CH_3OH, retention time: 3.323 mins): 98%.

(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate IR(ATR)



(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate ^{1}H NMR 400 MHz ($CDCl_3$)



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Current Data Parameters
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
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Time              12.20 h
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PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8196.722 Hz
FIDRES         0.250144 Hz
AQ             3.9976959 sec
RG                  101
DW                61.000 usec
DE                13.55 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1
SFO1         400.3024719 MHz
NUC1                 1H
P0                 3.33 usec
P1                 9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                65536
SF         400.3000102 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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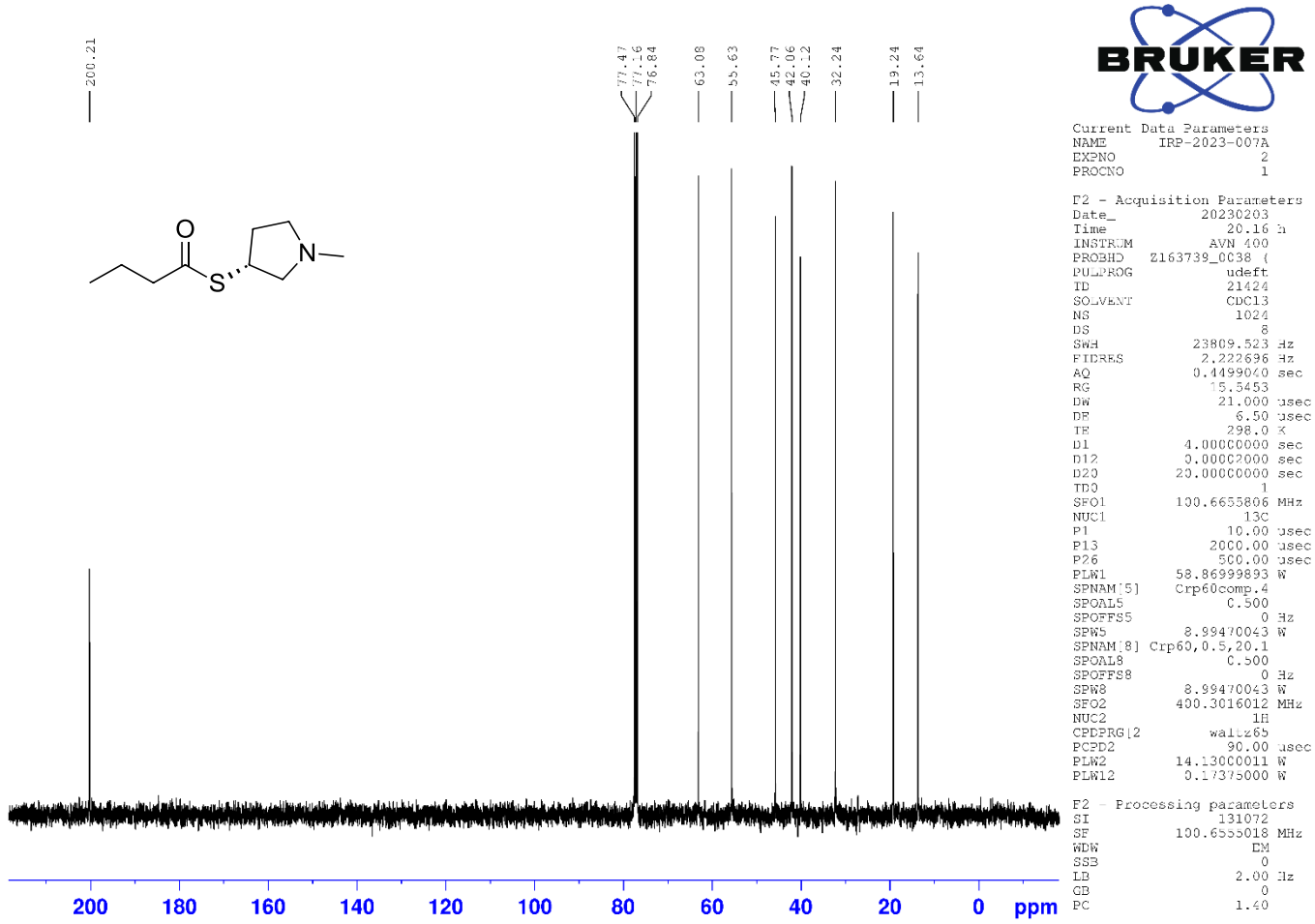
(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate ^{13}C NMR 100MHz ($CDCl_3$)



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Current Data Parameters
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EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
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Time              20.16 h
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PULPROG           udeft
TD                21424
SOLVENT           CDCl3
NS                 1024
DS                    8
SWH           23809.523 Hz
FIDRES         2.222696 Hz
AQ            0.4499040 sec
RG               15.5453
DW               21.000 usec
DE                6.50 usec
TE                298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                   1
SFO1        100.6655806 MHz
NUC1                13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         58.86999893 W
SPNAM[5]    Crp60comp.4
SPOAL5             0.500
SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2        400.3016012 MHz
NUC2                1H
CPDPRG[2          waltz65
PCPD2             90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W

F2 - Processing parameters
SI                131072
SF          100.6555018 MHz
WDW                 EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.40
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(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

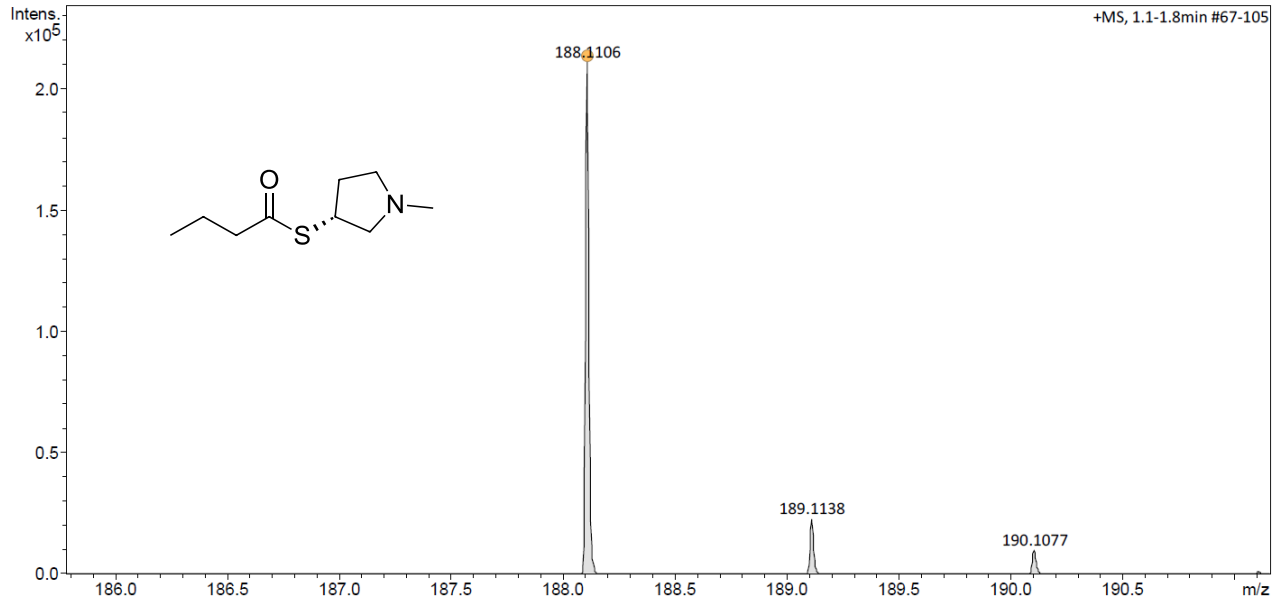
Analysis Info

Analysis Name	D:\Data\Xiao\Feb 17 2023000007.d	Acquisition Date	2023-02-17 9:28:19 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV6024	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
188.1106	C9H18NOS	188.1104	-1.3



Feb 17 2023000007.d

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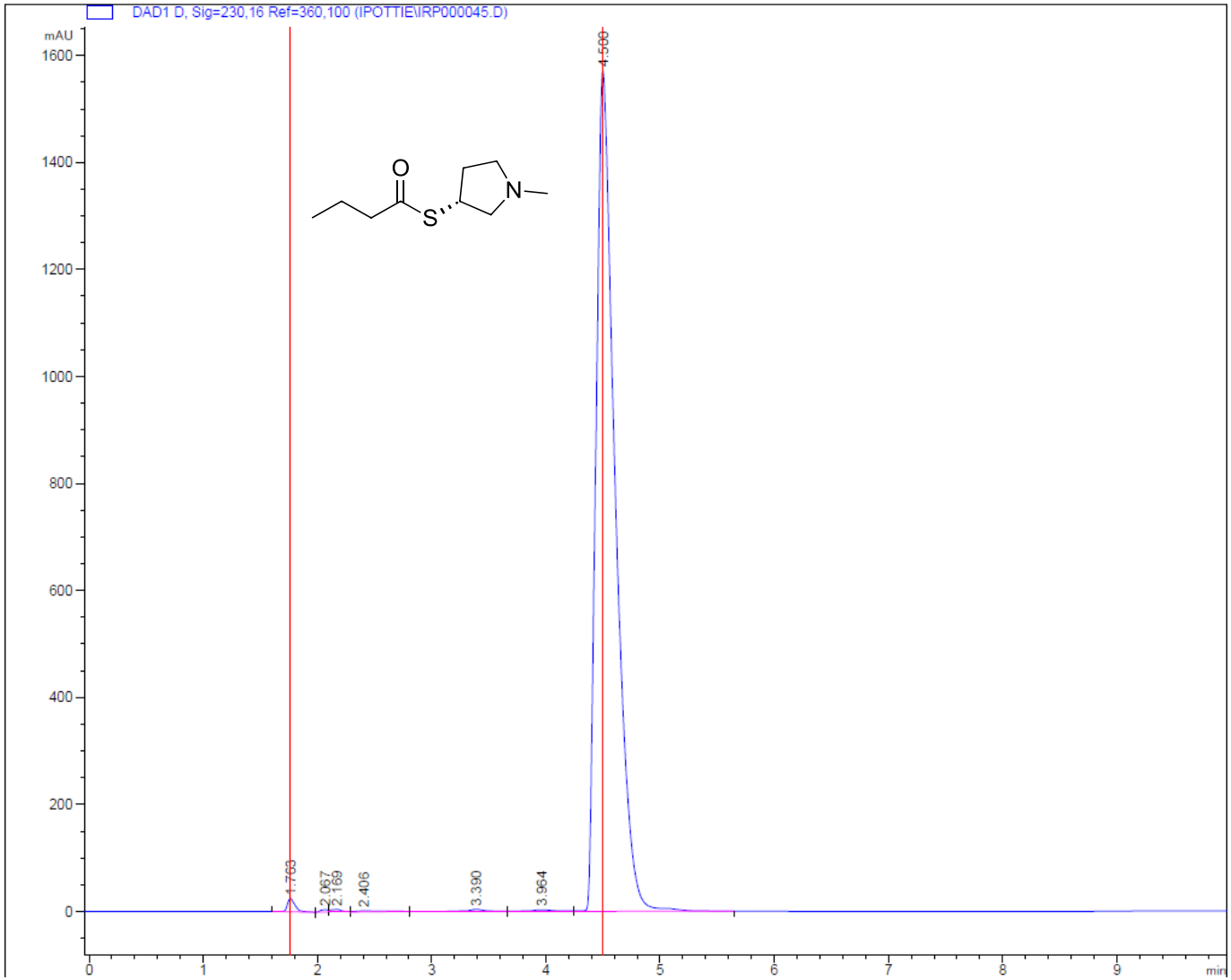
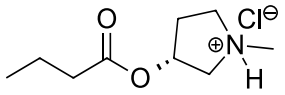
(21) ((*3R*)-1-Methylpyrrolidin-3-yl) Butanethioate HPLC



(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride

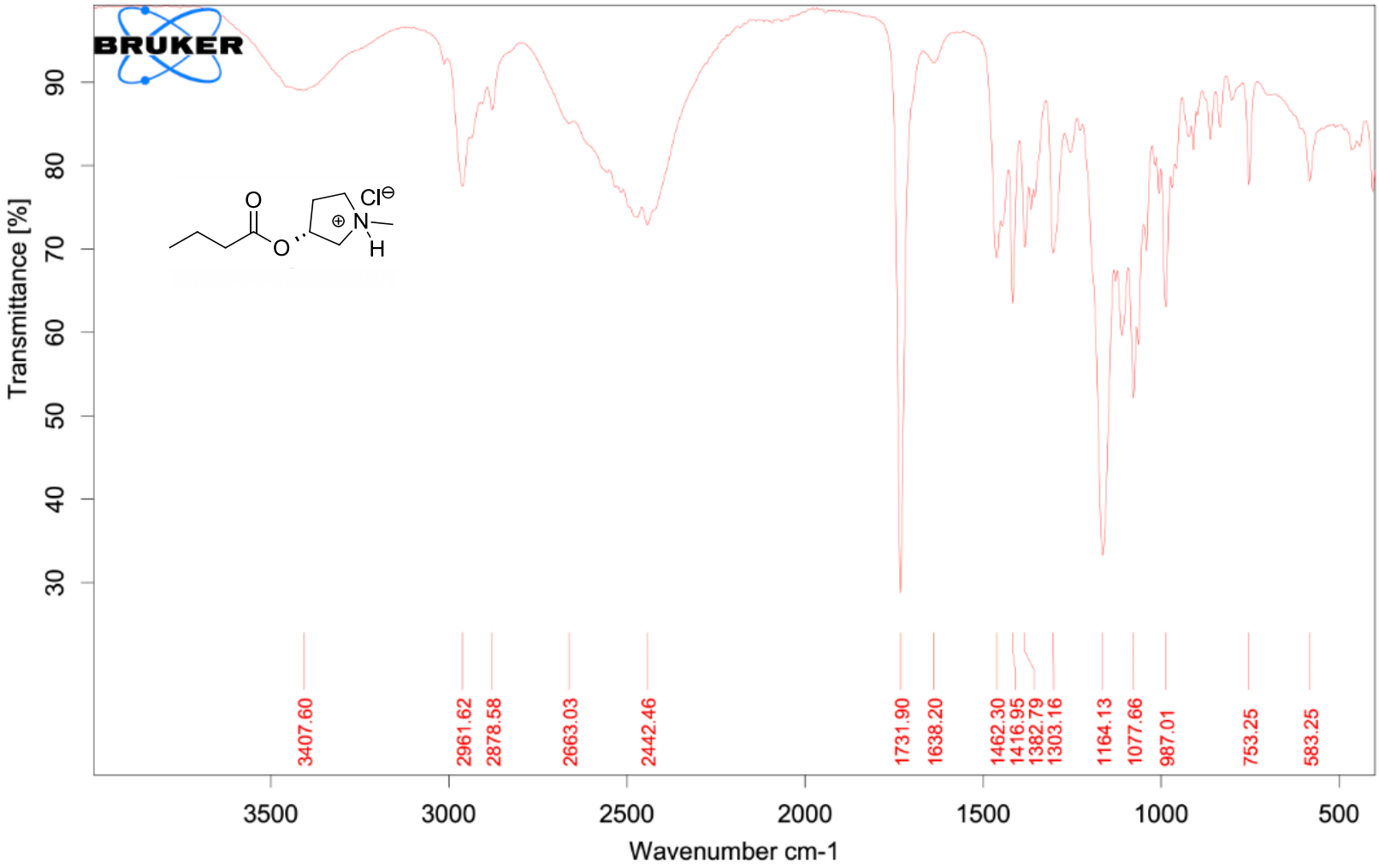


Synthesis: **(*3R*)-1-methyl-3-pyrrolidinyl butyrate**: (*3R*)-(-)-1-Methyl-3-pyrrolidinol (0.549 mL, 5.00 mmol) and triethylamine (1.74 mL, 12.5 mmol) were dissolved in anhydrous dichloromethane (17 mL) and cooled to 0°C with an ice bath. Butyryl chloride (0.519 mL, 5.00 mmol) was added dropwise at 0 °C. The ice bath was removed after 5 mins, and the reaction was stirred for 3hrs. The reaction was quenched with brine (20 mL), extracted with dichloromethane (3 × 15 mL) and dried over Na_2SO_3. The drying agent was removed was removed *via* gravity filtration and the solvent was removed *in vacuo* to produce a yellow oil. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to produce (*3R*)-1-methyl-3-pyrrolidinyl butyrate (0.729 g, 85%) as a yellow viscous liquid.

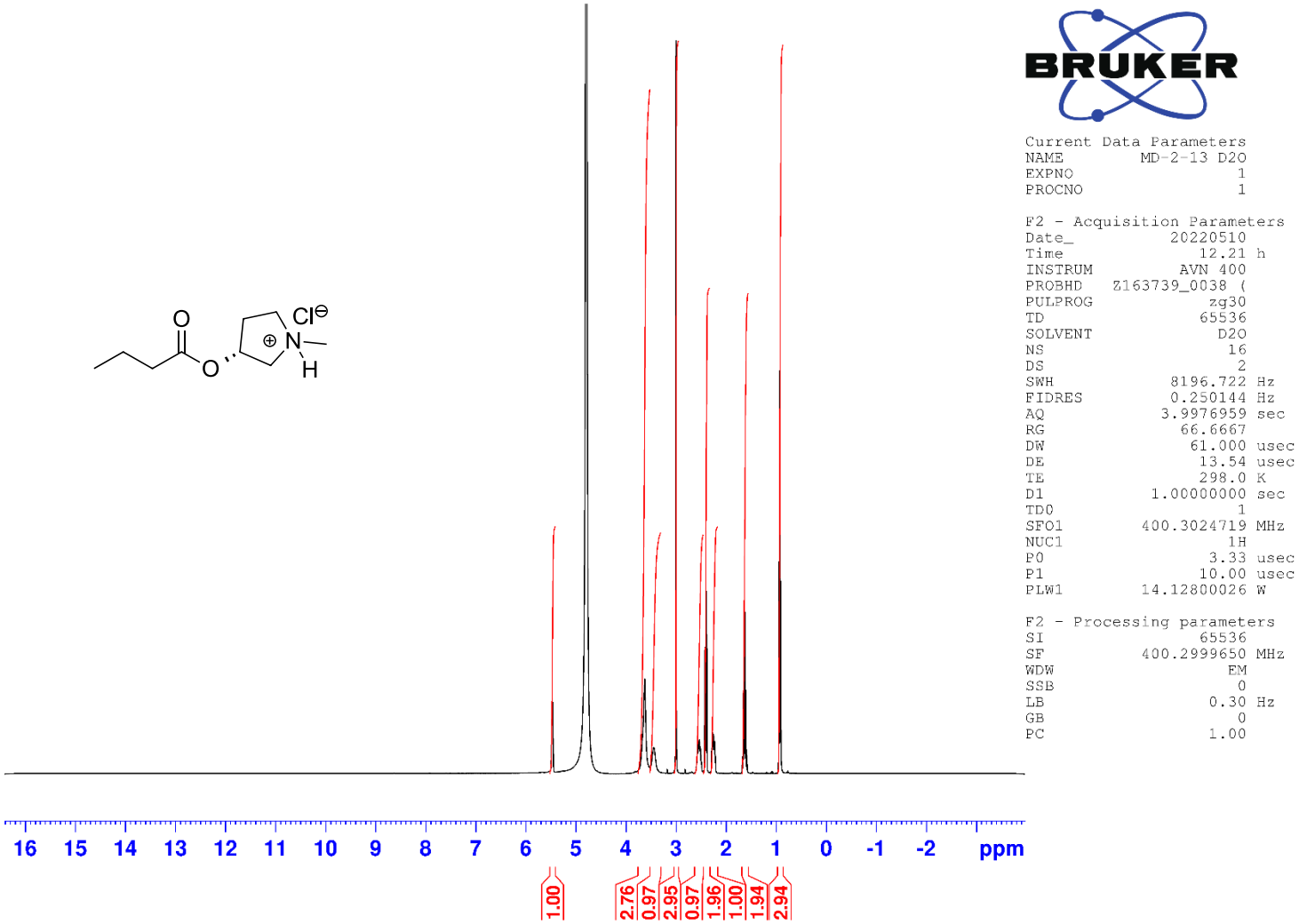
Spectroscopic data: IR(ATR) 2964, 2939, 2777, 1730, 1251, 1175 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.20-5.15 (m, 1H), 2.84-2.79 (m, 1H), 2.69 (dd, J = 10.9, 2.2 Hz, 1H), 2.61 (dd, J = 10.9, 5.9 Hz, 1H), 2.35 (s, 3H), 2.28-2.25 (m, 4H), 1.88-1.79 (m, 1H), 1.63 (sext, J = 7.4 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, CD_3OD-*d4*) δ 173.8 (0), 74.5 (1), 62.4 (2), 55.1 (2), 42.32 (3), 36.4 (2), 32.8 (2), 18.6 (2), 13.8 (3); LRMS (ESI^+): 172.1 (M^+); HRMS (ESI^+): calculated for $C_9H_{18}NO_2^+$: 172.1332 amu; found for $C_9H_{18}NO_2^+$: 172.1334 amu; HPLC purity at 210 nm (75% CH_3CN : 10% CH_3OH, 15 % aqueous triethylamine (0.1 % triethylamine in water) retention time: 3.803 mins): 99%.

***(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium chloride**: To a flask charged with (*3R*)-1-methyl-3-pyrrolidinyl butyrate (0.364 g, 1.75 mmol) anhydrous diethyl ether was added until complete dissolution (35 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 2.63 mL, 2.63 mmol) was added dropwise and stirred for 1 hr. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated with diethyl ether (20 mL), solvent was removed with a pipet. The process was repeated two more times. The material was dried *in vacuo* to produce *(3R)*-3-butyroxy-1-methyl-1- pyrrolidinium chloride (0.350 g, 96%) as a white solid. *Spectroscopic data:* IR(ATR) 3407, 2962, 2879, 2663, 2442, 1732, 1164, 1078 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 5.47 (app br s, 1H), 3.62 (app br s, 3H), 3.44 (app br s, 1H), 3.00 (s, 3H), 2.59-2.49 (m, 1H), 2.40 (t, J = 7.4 Hz, 2H), 2.28-2.22 (m, 1H), 1.63 (sext, J = 7.4 Hz, 2H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100.7 MHz, D_2O) δ 175.8 (0), 72.9 (1), 60.4 (2), 54.3 (2), 41.2 (3), 35.6 (2), 30.4 (2), 17.7 (2), 12.8 (3); LRMS (ESI^+): 172.1 (M^+); $^+$); HRMS (ESI^+): calculated for $C_9H_{18}NO_2^+$: 172.1332 amu; found for $C_9H_{18}NO_2^+$: 172.1331 amu; HPLC purity at 210 nm (75% CH_3CN : 10% CH_3OH, 15 % aqueous triethylamine (0.1 % triethylamine in water) retention time: 3.805 mins): >99%.

(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (D_2O)



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EXPNO                 1
PROCNO                1

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PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    16
DS                     2
SWH             8196.722 Hz
FIDRES          0.250144 Hz
AQ             3.9976959 sec
RG                66.6667
DW                61.000 usec
DE                 13.54 usec
TE                 298.0 K
D1            1.00000000 sec
TD0                    1
SFO1         400.3024719 MHz
NUC1                  1H
P0                  3.33 usec
P1                 10.00 usec
PLW1         14.12800026 W

F2 - Processing parameters
SI                 65536
SF           400.2999650 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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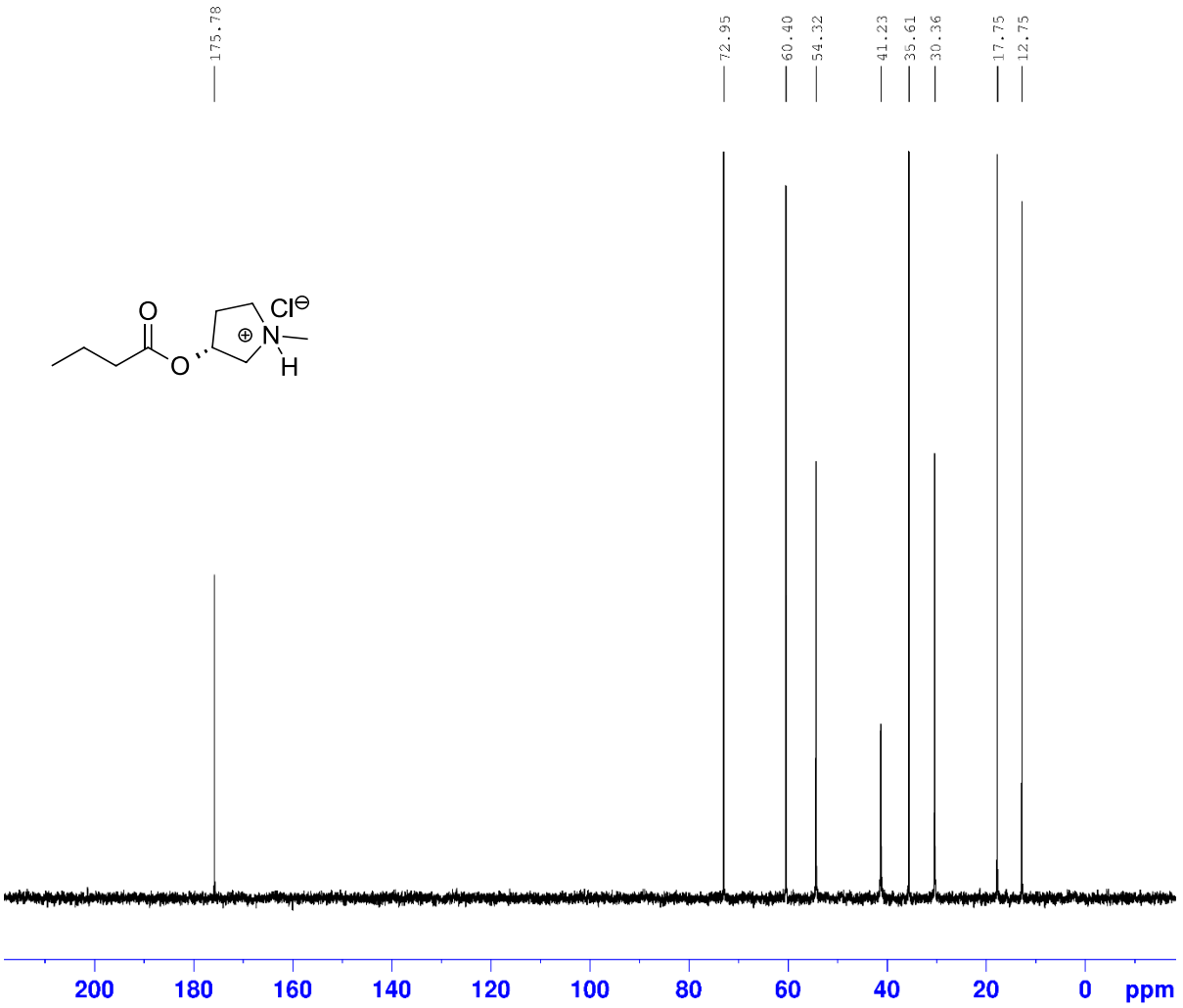
(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride ^{13}C NMR 100 MHz (D_2O)



BRUKER

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Current Data Parameters
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EXPNO                  2
PROCNO                 1

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Time                0.43 h
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PULPROG             udeft
TD                  21424
SOLVENT               D2O
NS                   1920
DS                      8
SWH             23809.523 Hz
FIDRES          2.222696 Hz
AQ              0.4499040 sec
RG                26.3136
DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                    1
SFO1        100.6655806 MHz
NUC1                 13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         58.86999893 W
SPNAM[5]     Crp60comp.4
SPOAL5             0.500
SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2        400.3016012 MHz
NUC2                 1H
CPDPRG[2         waltz65
PCPD2             90.00 usec
PLW2         14.12800026 W
PLW12         0.17442000 W

F2 - Processing parameters
SI                131072
SF          100.6555151 MHz
WDW                 EM
SSB                    0
LB                2.00 Hz
GB                     0
PC                1.40
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(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

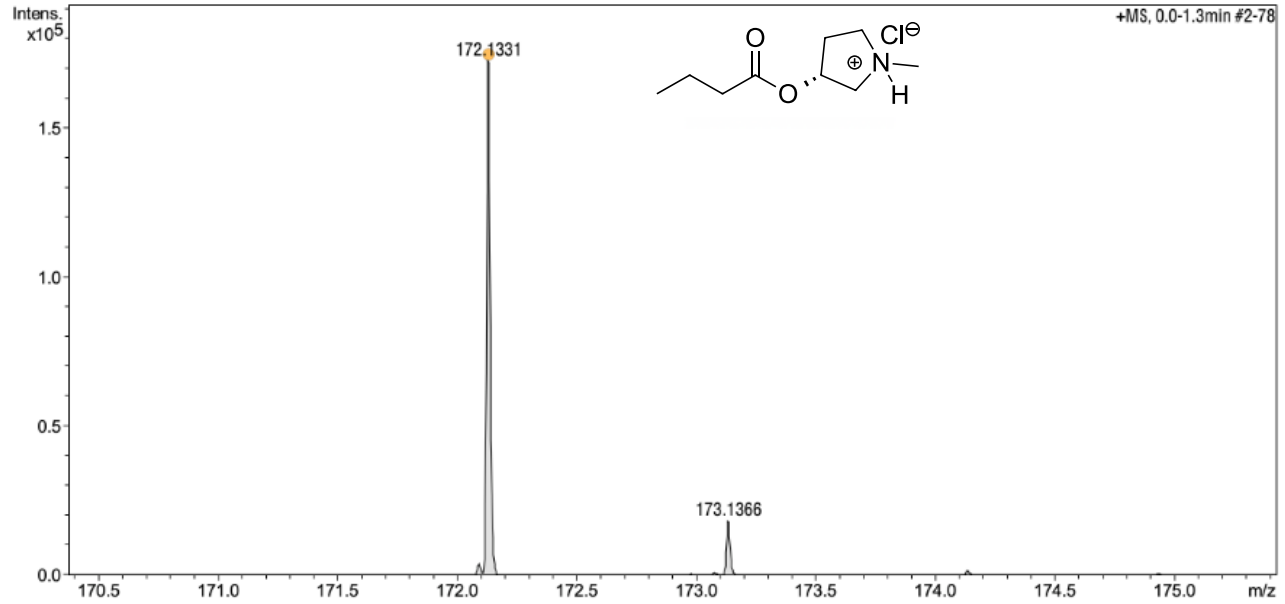
Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000012.d	Acquisition Date	7/7/2022 9:55:17 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6016 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
172.1331	C9H18NO2	172.1332	0.5



July 07 2022000012.d

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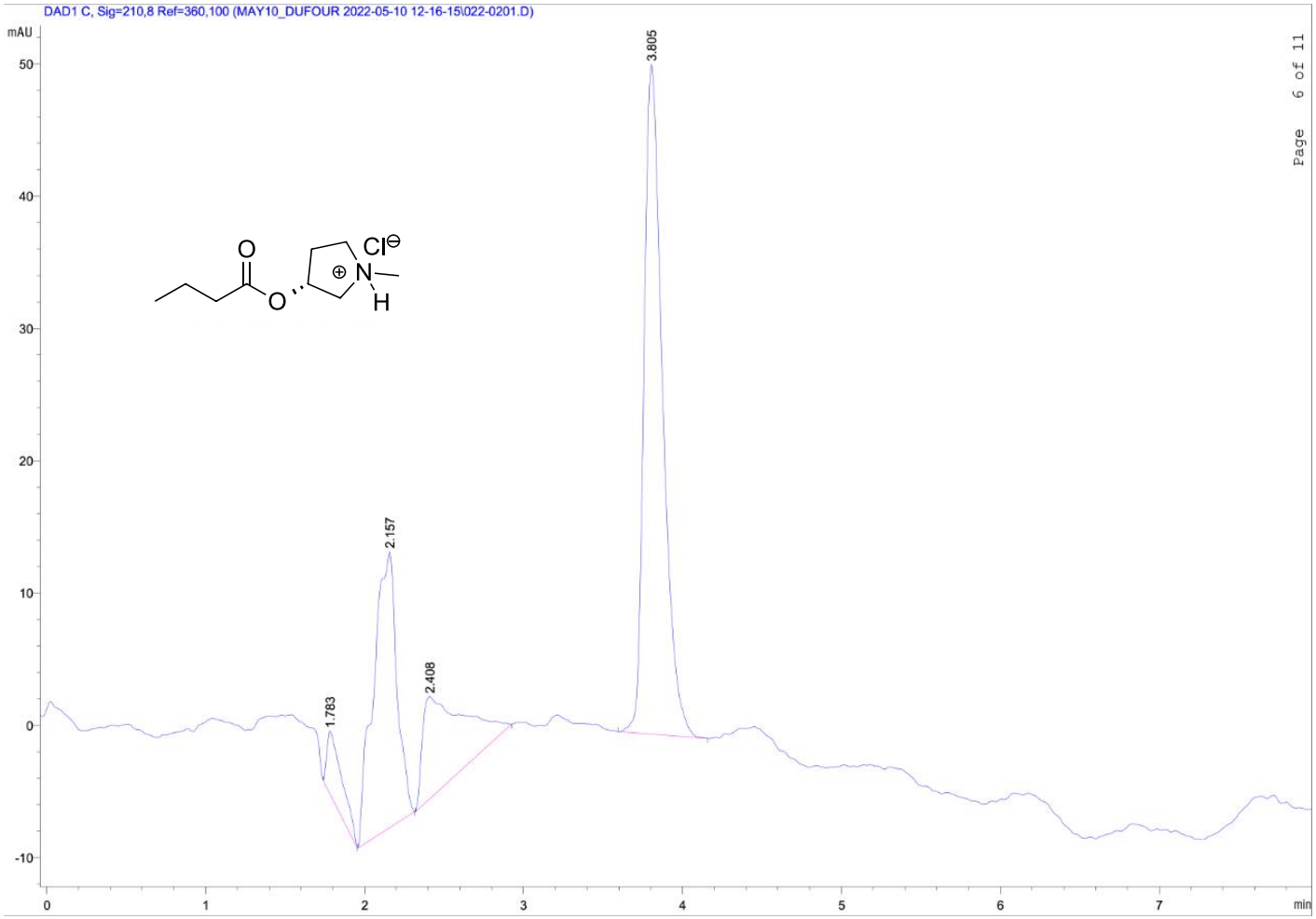
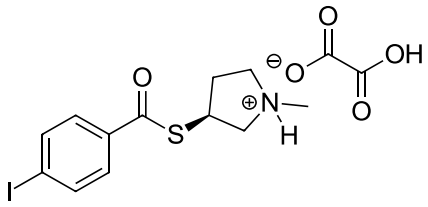
(22) *(3R)*-3-Butyroxy-1-methyl-1-pyrrolidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 3.805 min.



Aryl N-Methylpyrrolidinyl Derivatives

(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate



Synthesis: **(*3R*)-3-(Mesyloxy)-1-methylpyrrolidine**: (*R*)-(-)-1-Methyl-3-pyrrolidinol (6.00 mL, 54.6 mmol) was dissolved in anhydrous dichloromethane (180 mL) and cooled to 0°C with an ice bath. Triethylamine (11.4 mL, 81.9 mmol) and methanesulfonyl chloride (4.90 mL, 62.8 mmol) were added dropwise at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred overnight. Brine (50 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce a yellow viscous liquid (6.11 g, 54%). Product confirmed by ^{1}H NMR and the crude material was immediately carried through to next step without further purification.

(*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3R*)-3-(Mesyloxy)-1-methylpyrrolidine (6.11 g, 34.1 mmol), was dissolved in anhydrous THF (114 mL) under an argon atmosphere. 18-crown-6-ether (9.91 g, 37.5 mmol) was added followed by potassium thioacetate (4.29 g, 37.5 mmol), added in 3 portions. Once all potassium thioacetate had gone into solution, the reaction was heated to 30°C and left overnight. The reaction was gravity filtered, the filtrate diluted with water (50 mL) and it was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_3, gravity filtered, and concentrated *in vacuo* to produce a red oil. The crude material was then distilled to give (*3S*)-S-(1-methylpyrrolidin-3-yl) ethanethioate as a yellow oil (2.33 g, 37%). The product was confirmed by ^{1}H NMR.

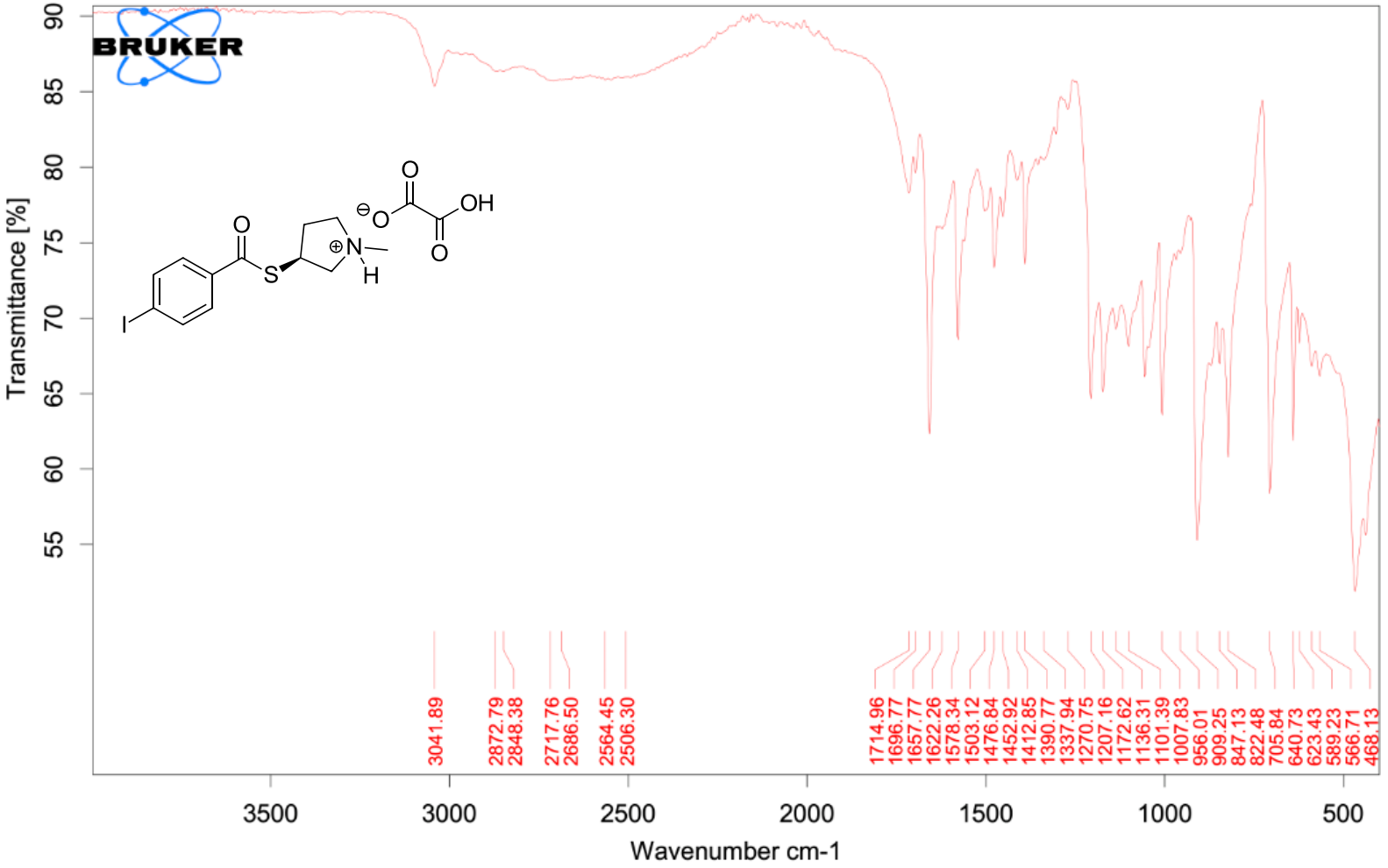
(*S*)-1-Methyl-3-pyrrolidinethiol: (*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate (0.442g, 2.77 mmol) was dissolved in MeOH (10 mL) and sodium methoxide (0.155 g, 2.78 mmol) was added to the stirring solution. After all starting material was shown to be consumed by TLC

experiment, the MeOH was removed *in vacuo* and the resulting material was washed with DCM and concentrated twice. This material was used immediately in the next step.

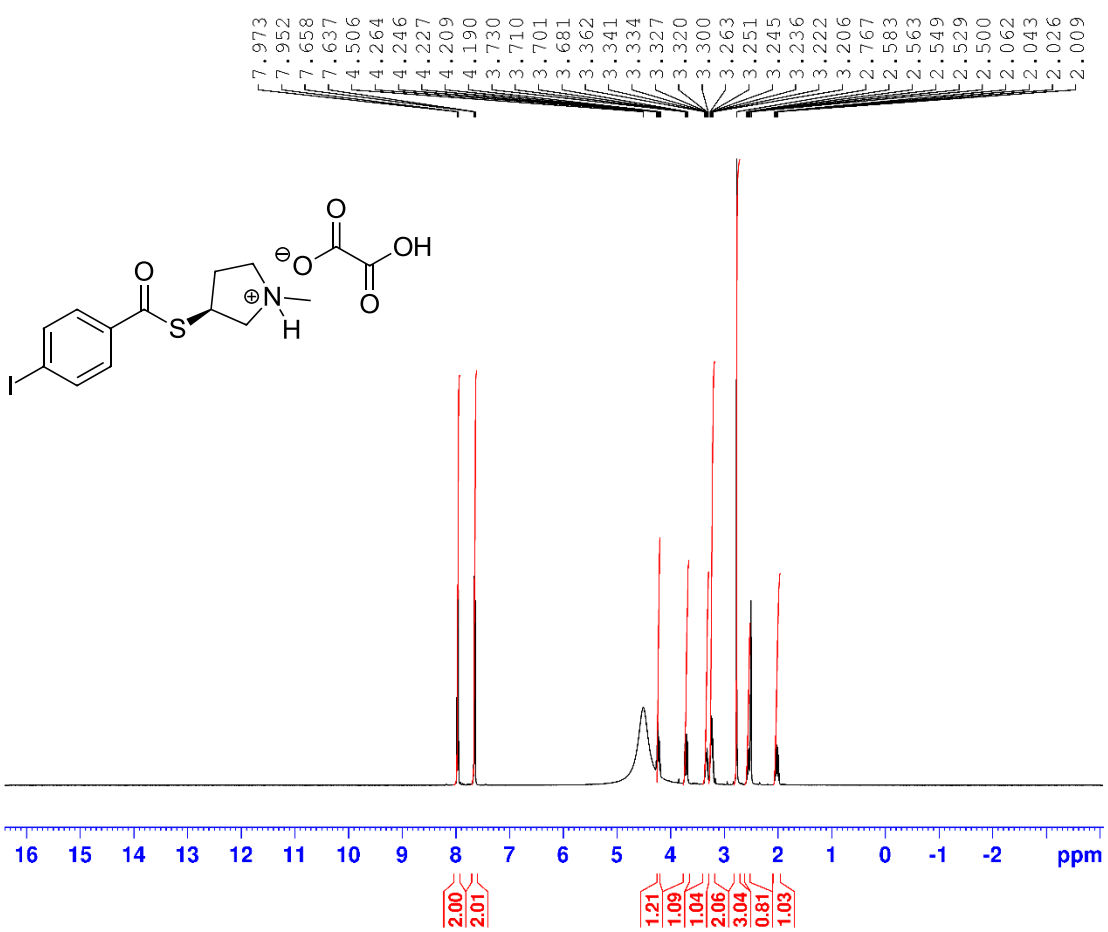
((*3S*)-1-Methylpyrrolidin-3-yl) *p*-iodobenzenethioate: (*S*)-1-Methyl-3-pyrrolidinethiol from the previous step was dissolved in dichloromethane (10 mL) and cooled to 0°C using an ice bath. 4-Iodobenzoyl chloride (0.740 g, 2.78 mmol) was added followed by triethylamine (0.774 mL, 5.55 mmol). The ice bath was removed after 30 mins and the reaction was left overnight. The next morning the solvent was removed *in vacuo.* The crude material was washed with diethyl ether, gravity filtered to remove all precipitate, and the filtrate was concentrated *in vacuo* to give a white solid. This material recrystallized with methanol and isopropanol to give a white solid (0.727 g).

((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate: ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-iodobenzenethioate (0.600 g, 1.73 mmol) was completely dissolved in isopropanol (32.5 mL). With stirring, oxalic acid (0.156 g, 1.73 mmol) was added and stirred for 3 hrs at room temperature. The solution was concentrated *in vacuo* to give a white solid and immediately recrystallized with isopropanol. For further purification, this material was recrystallized from methanol to give ((*3S*)-1-methylpyrrolidin-3-yl) *p*-iodobenzenecarbothioate oxylate as a white solid (0.357 g, 47%).
Spectroscopic Data: IR(ATR): 3041, 2718, 2564, 1714, 1697, 1578, 909 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.97-7.95 (m, 2H), 7.66-7.64 (m, 2H), 4.51 (br s, 2H, overlapping with H_2O in DMSO), 4.26-4.19 (m, 1H), 3.71 (dd, J = 11.7, 8.1 Hz, 1H), 3.36-3.30 (m, 1H), 3.26-3.21 (m, 2H), 2.77 (s, 3H), 2.58-2.52 (m, 1H), 2.06-1.97 (m, 1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 190.3 (0), 164.3 (0), 138.9 (1), 135.1 (0), 128.5 (1), 103.0 (0), 59.7 (2), 54.1 (2), 40.4 (3), 38.6 (1), 30.0 (2); HRMS (ESI^+): calculated for $C_{12}H_{15}INOS^+$: 347.9913; found for $C_{12}H_{15}INOS^+$: 347.9912; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 8.245 mins): 97.2 %.

(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate IR(ATR)



(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate ^{1}H NMR 400 MHz (DMSO-*d6*)



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PROCNO                1

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TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8196.722 Hz
FIDRES        0.250144 Hz
AQ            3.9976959 sec
RG                  101
DW               61.000 usec
DE               13.55 usec
TE                298.0 K
D1            1.00000000 sec
TD0                   1
SFO1        400.3024719 MHz
NUC1                 1H
P0                3.33 usec
P1                9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                65536
SF          400.3000000 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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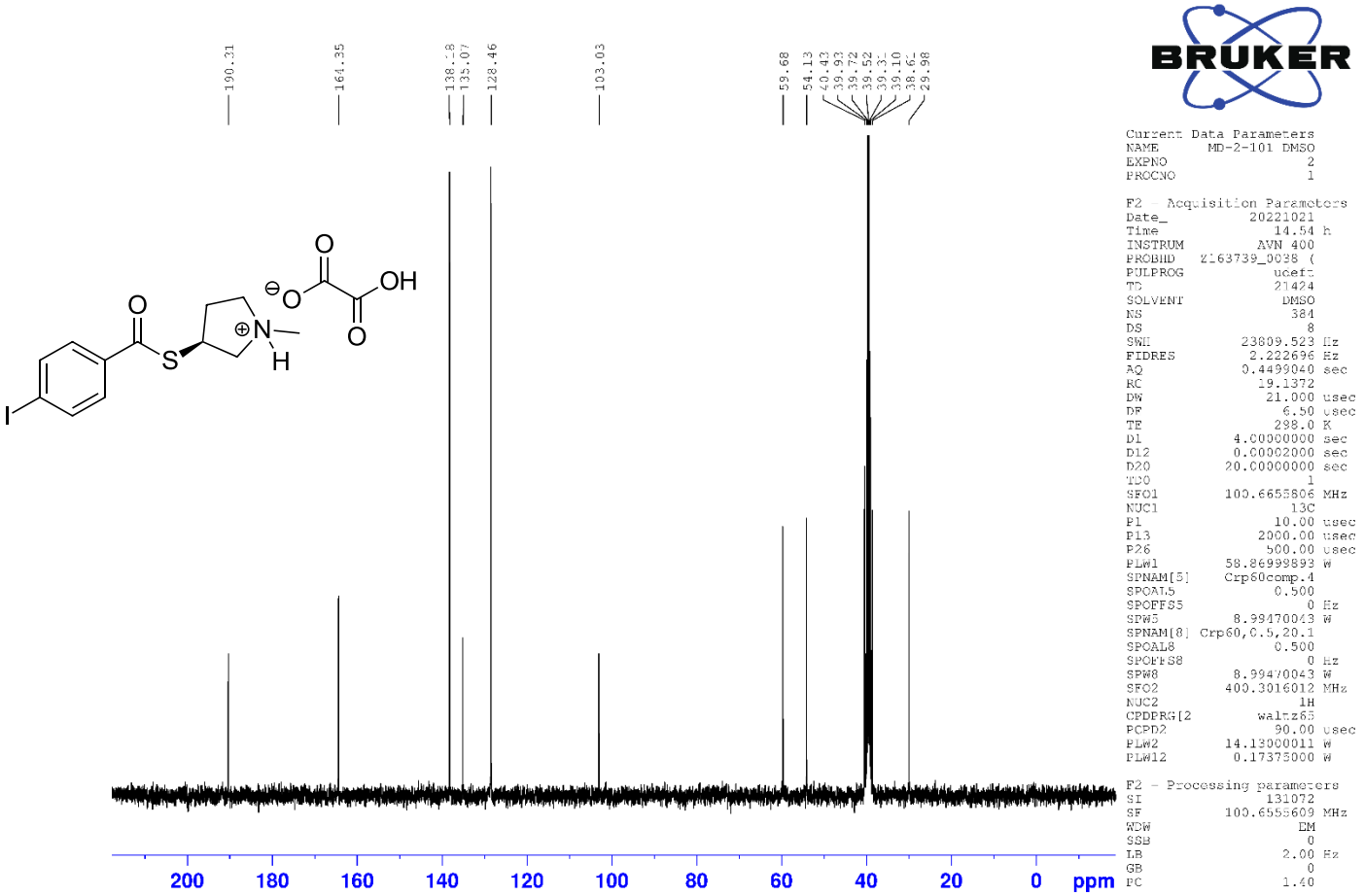
(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate ^{13}C NMR 100 MHz (DMSO-*d6*)

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PROCNO                1

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PULPROG           ucefp
TD                21424
SOLVENT            DMSO
NS                  384
DS                    8
SWH           23809.523 Hz
FIDRES         2.222696 Hz
AQ            0.4499040 sec
RG               19.1372
DW              21.000 usec
DE               6.50 usec
TE              298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                   1
SFO1        100.6655806 MHz
NUC1                13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1        58.86999893 W
SPNAM[5]    Crp60comp.4
SPOAL5            0.500
SPOFFS5               0 Hz
SPW5         8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8            0.500
SPOFFS8               0 Hz
SPW8         8.99470043 W
SFO2        400.3016012 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             90.00 usec
PLW2        14.13000011 W
PLW12        0.17375000 W

F2 - Processing parameters
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SF          100.6655609 MHz
WDW                 EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.40
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(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Nov 01 2022000004.d	Acquisition Date	2022-11-01 2:53:21 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6025	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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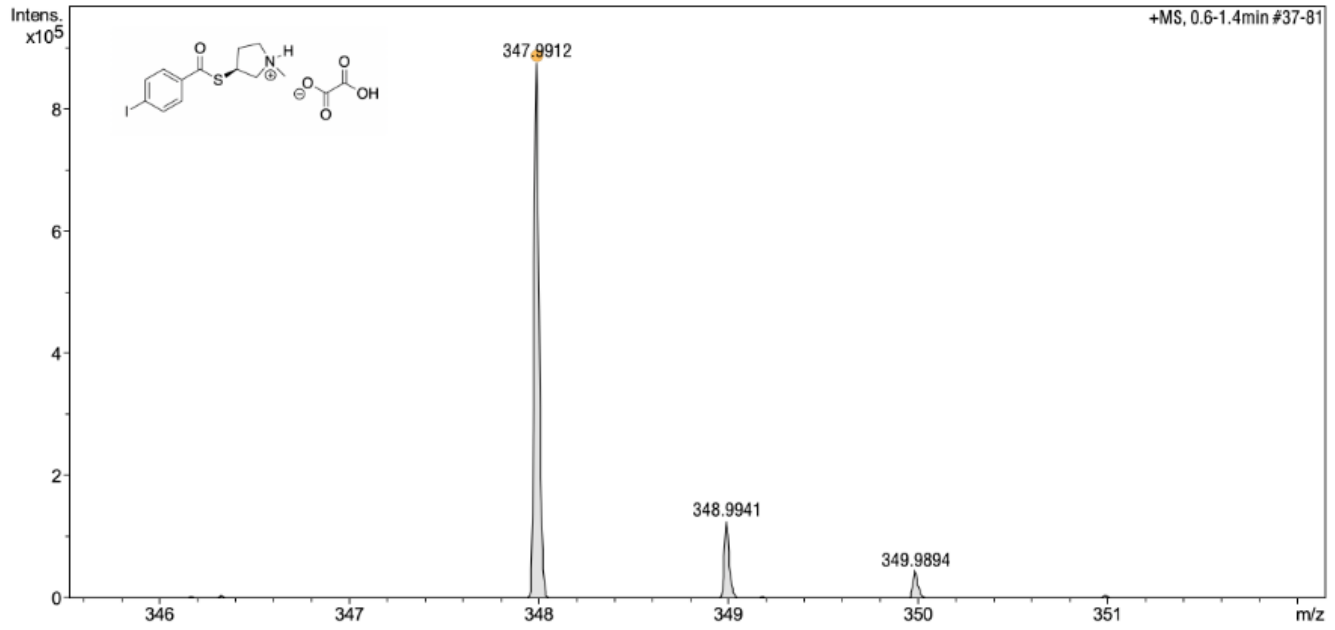
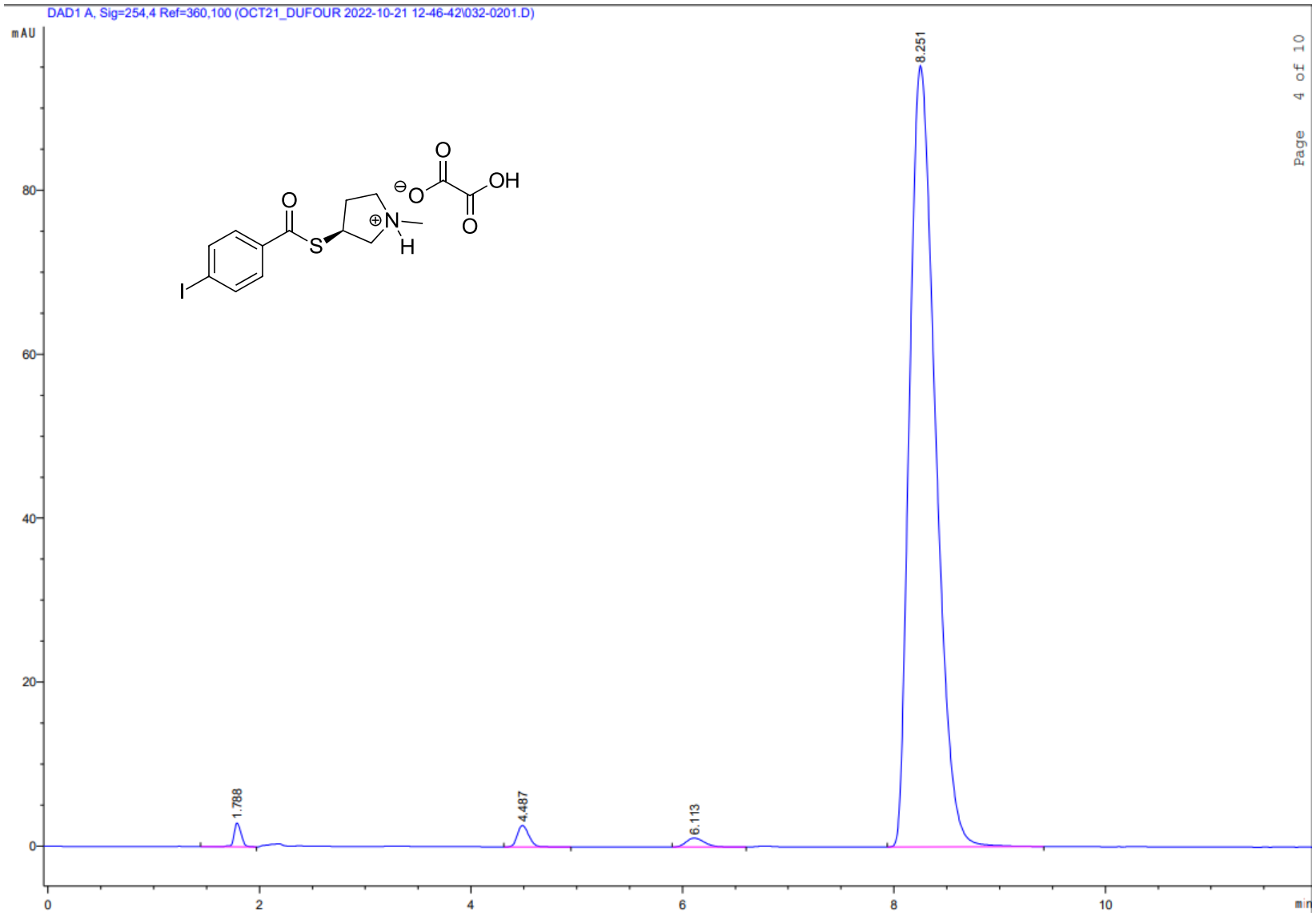


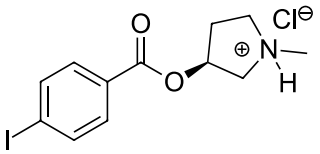
Nov 01 2022000004.d

Bruker Compass DataAnalysis 4.3 printed: 2022-11-08 10:41:42 AM by: x Page 1 of 1

(23) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate



(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride



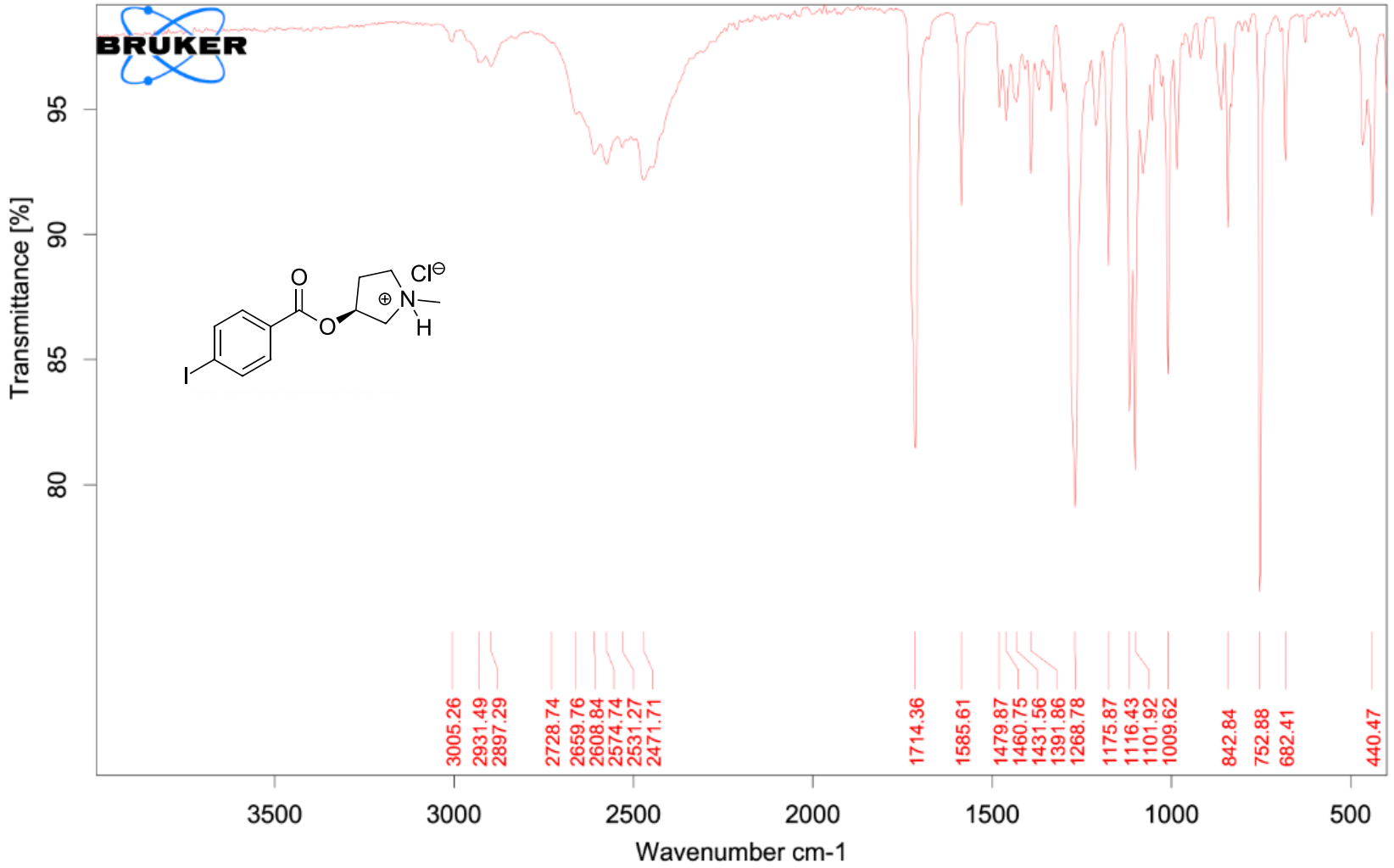
Synthesis: **(*3S*)-1-Methyl-3-pyrrolidinyl *p*-iodobenzoate**: 4-Iodobenzoyl chloride (0.799 g, 3.00 mmol), triethylamine (1.05 mL, 7.50 mmol) and 4-dimethylaminopyridine (DMAP) (0.0659 g, 0.54 mmol) were dissolved in anhydrous dichloromethane (20 mL) under an argon atmosphere. (*S*)-(+)-1-Methyl-3-pyrrolidinol (0.329 mL, 3.00 mmol) was added at 0°C. The ice bath was removed after 5 min, and the reaction was stirred for 3h at room temperature. The reaction was quenched with brine (20 mL), extracted with dichloromethane (3 x 15 mL) and the combined organic layers were dried over Na_2SO_3. After the drying agent was removed *via* filtration the solvent was removed *in vacuo* to produce a white solid. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to afford (*3S*)-1-methyl-3-pyrrolidinyl *p*-iodobenzoate (0.274 g, 28%) as a white solid. The analytical data was similar to previously published data.[7]

***(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium chloride**: (*3S*)-1-Methyl-3-pyrrolidinyl *p*-iodobenzoate (0.136 g, 0.410 mmol) was dissolved in anhydrous diethyl ether (9 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 0.62 mL, 0.620 mmol) was added dropwise resulting in the immediate formation of a white precipitate. The reaction was stirred for an additional 60 mins. The precipitate was collected *via* suction filtration, washed with Et_2O (2 x 10 mL), to afford *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium chloride, (0.100 mg, 66% yield) as a white solid.

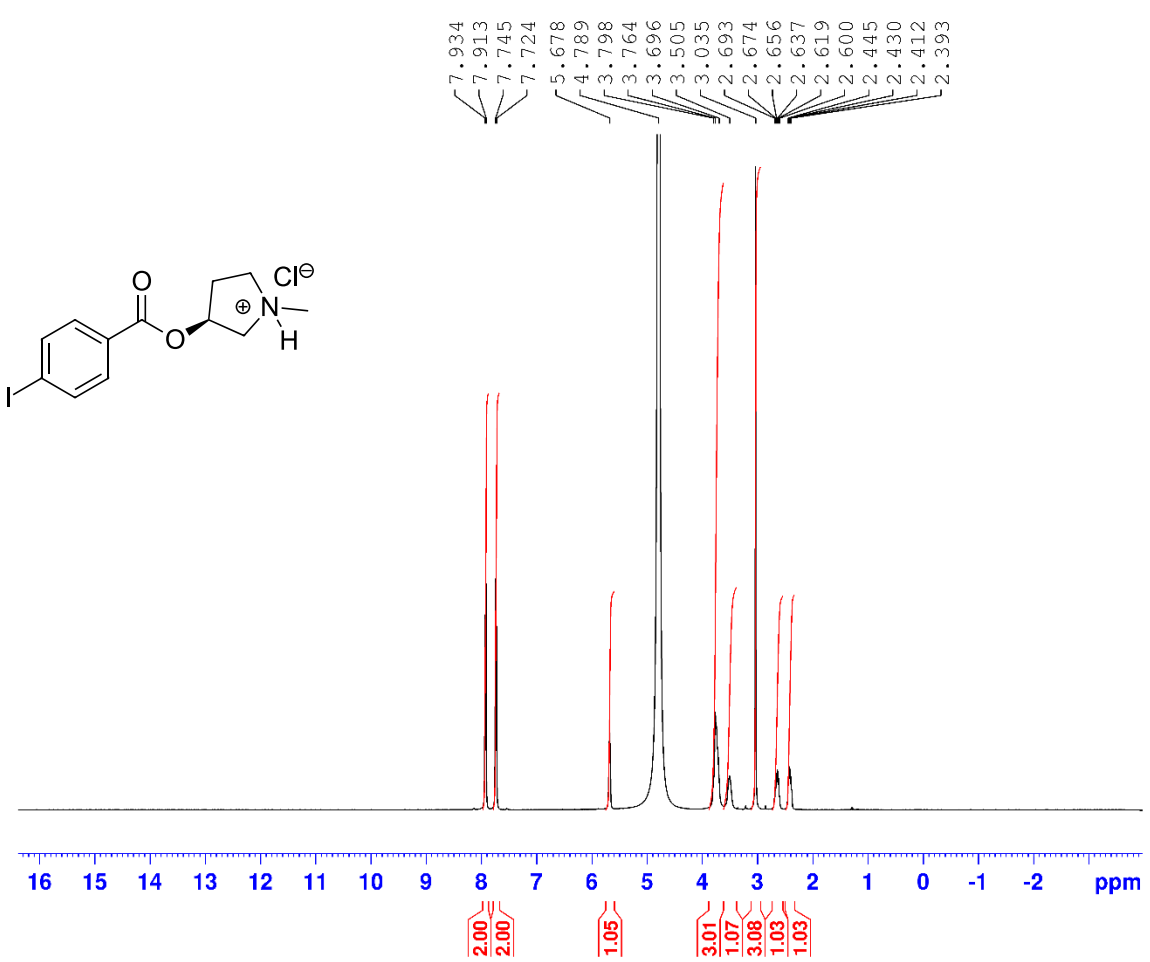
Spectroscopic data: $MP_{(diethyl\ ether)}$: 243-245ºC; IR (ATR) 3005, 2931, 2897, 2574, 2472, 1714, 1586, 1269, 1102, 753; ^1H NMR (400 MHz, D_2O) δ 7.93-7.91 (m, 2H), 7.75-7.72 (m, 2H), 5.68 (br app s, 1H), 3.80-3.70 (m, 3H), 3.50 (br app s, 1H), 3.03 (s, 3H), 2.69-2.60 (m, 1H), 2.45-2.39 (m, 1H); ^{13}C NMR (100.7 MHz, D_2O) δ 167.7 (0), 138.7 (1), 131.5 (1), 128.9 (0), 102.3 (0), 74.7 (1), 61.1 (2), 55.1 (2), 42.0 (2), 31.1 (2); LRMS (ESI^+): 332.0 (M+); HRMS (ESI^+): calculated for $C_{12}H_{15}INO_2^+$: 332.0142; found for $C_{12}H_{15}INO_2^+$: 332.0133; HPLC purity at 254 nm (75%

CH_3CN : 10% CH_3OH, 15 % aqueous triethylamine (0.1 % triethylamine in water) retention time: 5.723 mins): >99%.

(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (D_2O)

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PROCNO                       1

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TD                       65536
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FIDRES                0.250144 Hz
AQ                    3.9976959 sec
RG                      66.6667
DW                       61.000 usec
DE                       13.54 usec
TE                       298.0 K
D1                  1.00000000 sec
TD0                          1
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NUC1                        1H
P0                       3.33 usec
P1                      10.00 usec
PLW1               14.12800026 W

F2 - Processing parameters
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SF                 400.2999656 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
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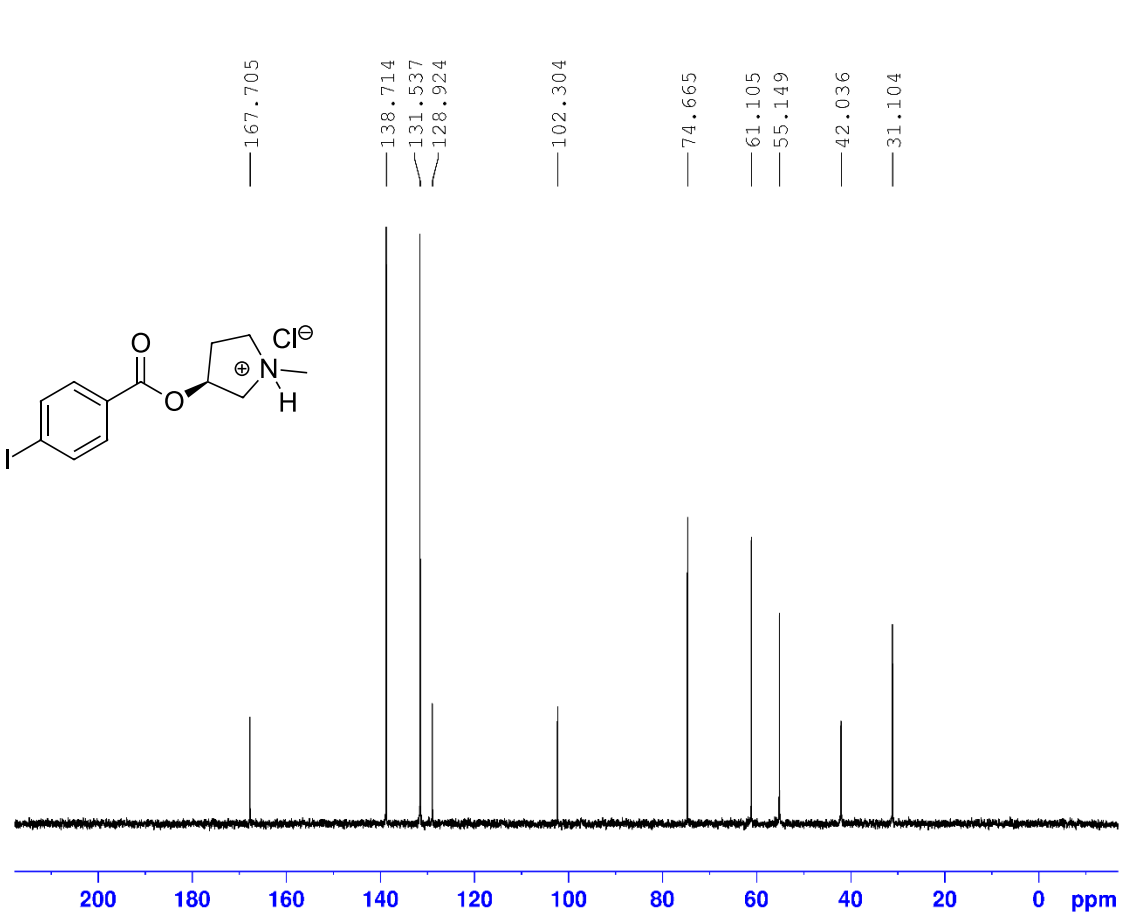
(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{13}C UDEFT NMR 100 MHz (D_2O)



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Current Data Parameters
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EXPNO                    9
PROCNO                   1

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PULPROG              udeft
TD                   21424
SOLVENT                D2O
NS                    2304
DS                       8
SWH             23809.523 Hz
FIDRES           2.222696 Hz
AQ              0.4499040 sec
RG                 22.6702
DW                 21.000 usec
DE                  6.50 usec
TE                 298.0 K
D1              4.00000000 sec
D12             0.00002000 sec
D20            20.00000000 sec
TD0                      1
SFO1           100.6655806 MHz
NUC1                   13C
P1                   10.00 usec
P13                2000.00 usec
P26                 500.00 usec
PLW1           58.86999893 W
SPNAM[5]     Crp60comp.4
SPOAL5               0.500
SPOFFS5                  0 Hz
SPW5            8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8               0.500
SPOFFS8                  0 Hz
SPW8            8.99470043 W
SFO2           400.3016012 MHz
NUC2                    1H
CPDPRG[2           waltz65
PCPD2                90.00 usec
PLW2           14.12800026 W
PLW12           0.17442000 W

F2 - Processing parameters
SI                  131072
SF             100.6554448 MHz
WDW                    EM
SSB                     0
LB                   2.00 Hz
GB                      0
PC                   1.40
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(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 07 2022000021.d	Acquisition Date	7/7/2022 11:30:06 AM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6002 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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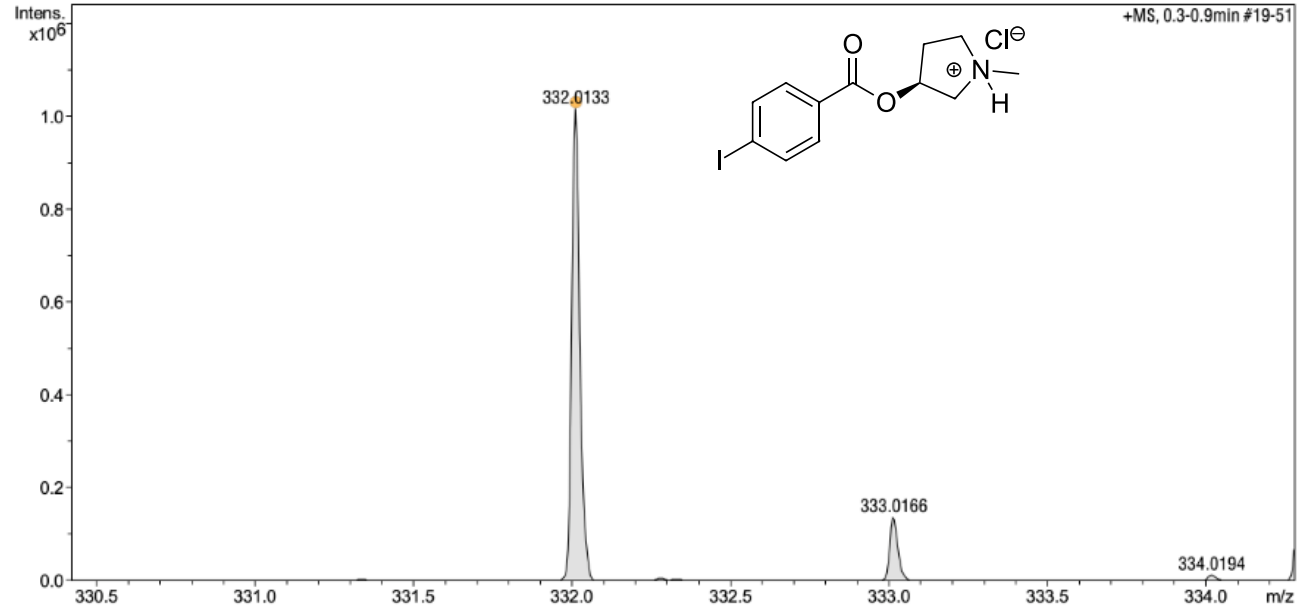
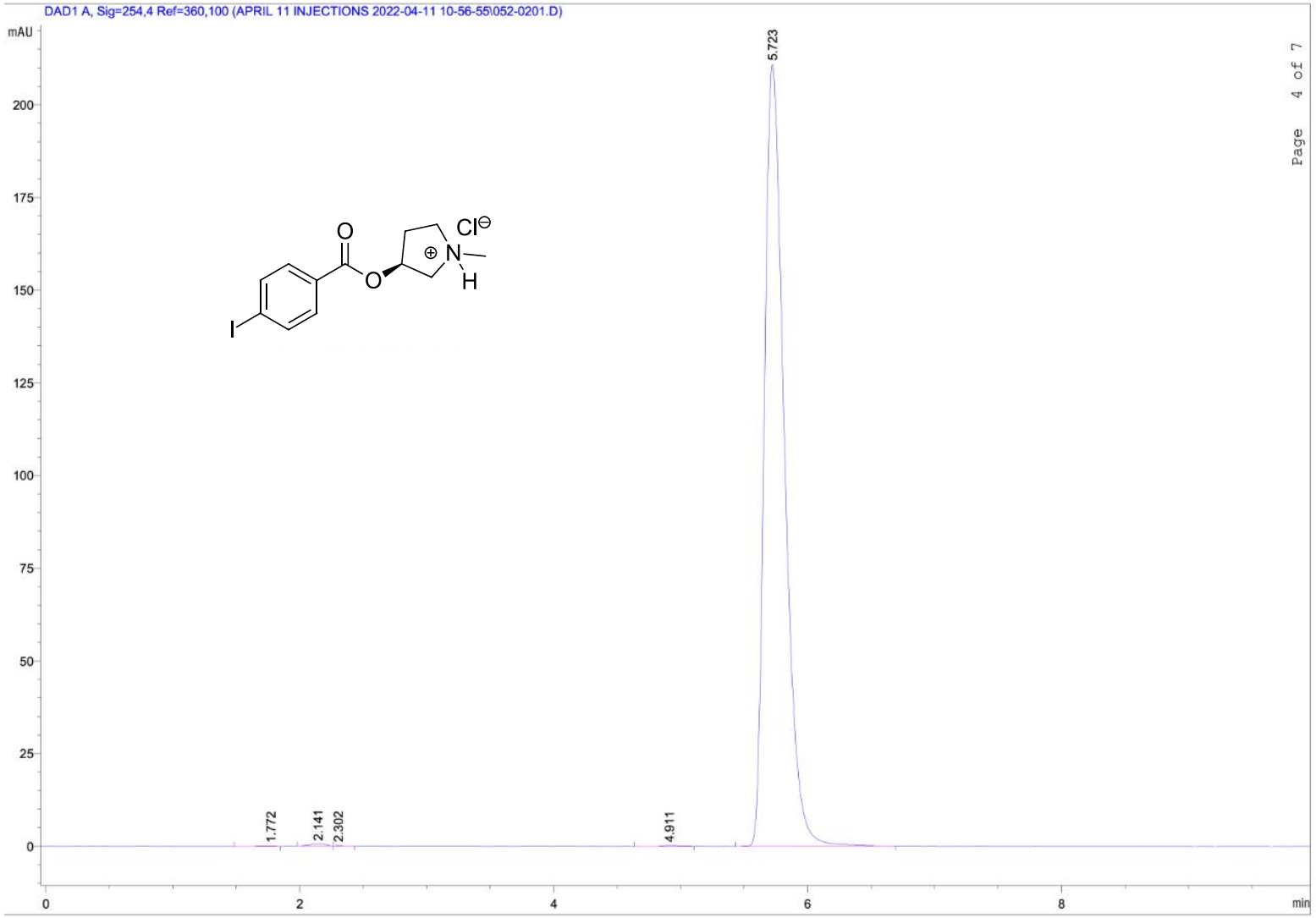
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
332.0133	C12H15INO2	332.0142	2.7

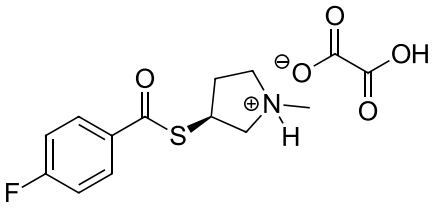


July 07 2022000021.d

(24) *(3S)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride HPLC



(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate



Synthesis: **(*3R*)-3-(Mesyloxy)-1-methylpyrrolidine**: (*R*)-(-)-1-Methyl-3-pyrrolidinol (6.00 mL, 54.6 mmol) was dissolved in anhydrous dichloromethane (180 mL) and cooled to 0°C with an ice bath. Triethylamine (11.4 mL, 81.9 mmol) and methanesulfonyl chloride (4.90 mL, 62.8 mmol) were added dropwise at 0°C. The ice bath was removed after 5 min, and the reaction was stirred overnight. Brine (50 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 x 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce a yellow viscous liquid (6.11 g, 54%). Product was confirmed by ^{1}H NMR and the crude material was immediately carried through to next step without further purification.

(*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3R*)-3-(Mesyloxy)-1-methylpyrrolidine (6.11 g, 34.1 mmol) was dissolved in anhydrous THF (114 mL) under an argon atmosphere. 18-crown-6-ether (9.91 g, 37.5 mmol) was added followed by potassium thioacetate (4.29 g, 37.5 mmol), added in 3 portions. Once all potassium thioacetate had gone into solution, the reaction was heated to 30°C and left overnight. The reaction was gravity filtered, the filtrate diluted with water (50 mL) and it was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_3, gravity filtered, and concentrated *in vacuo* to produce a red oil. The crude material was then distilled to give (*3S*)-S-(1-methylpyrrolidin-3-yl) ethanethioate as a yellow oil (2.33 g, 37 %). The product was confirmed by ^{1}H NMR and the material was immediately carried through to next step without further purification.

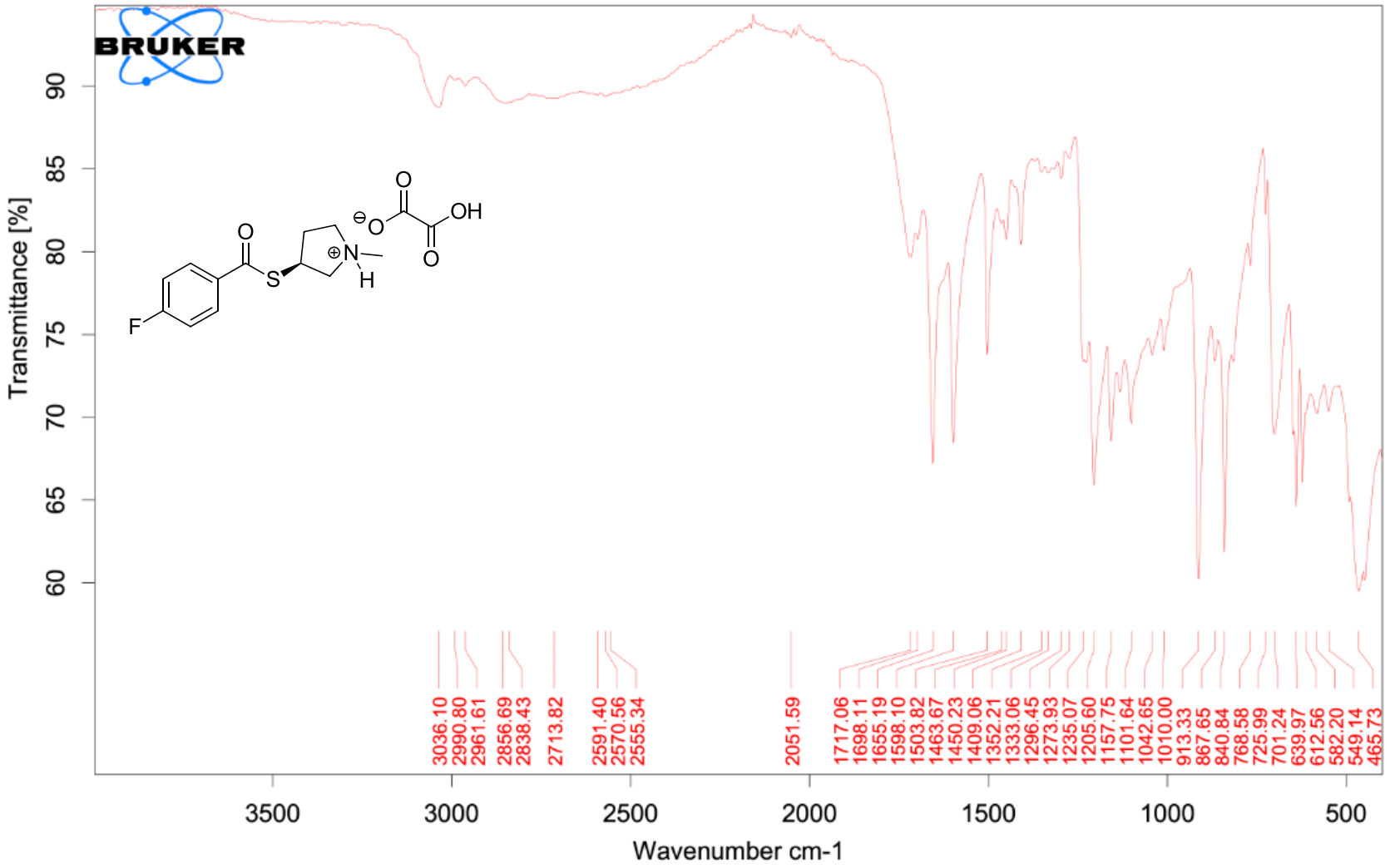
(*S*)-1-Methyl-3-pyrrolidinethiol: (*3S*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate (0.344 g, 2.16 mmol) was dissolved in MeOH (11 mL) and sodium methoxide (0.120 g, 2.16 mmol) was added to the stirring solution. After all starting material was shown to be consumed by TLC

experiment, the MeOH was removed *in vacuo*. The resulting material was placed dichloromethane (20 mL) and concentrated *in vacuo*. This process was repeated, and the material was immediately used in the next step.

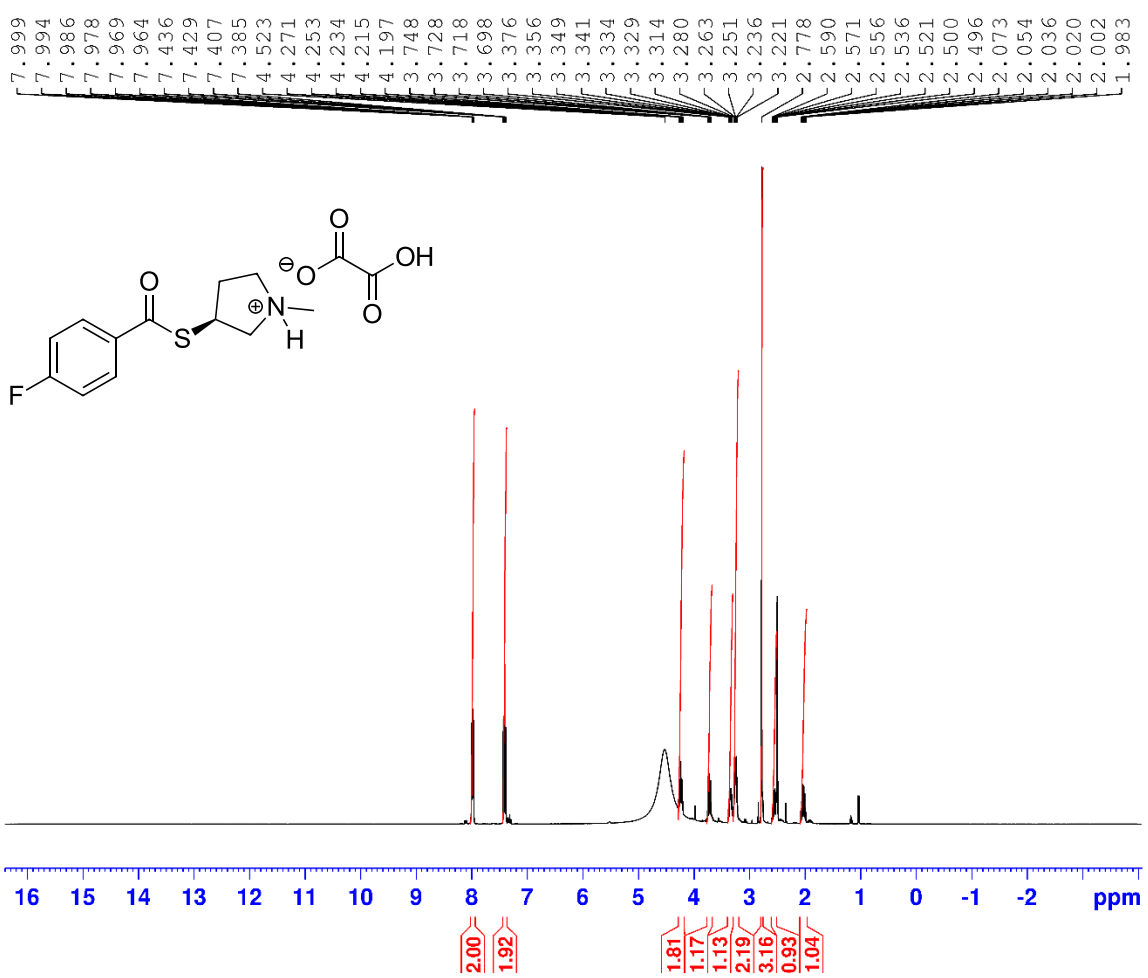
((*3S*)-1-Methylpyrrolidin-3-yl) *p*-fluorobenzenethioate: (*S*)-1-Methyl-3-pyrrolidinethiol was dissolved in dichloromethane (7 mL) and cooled to 0°C using an ice bath. 4-Fluorobenzoyl chloride (0.602 g, 4.32 mmol) was added followed by triethylamine (0.774 mL, 5.55 mmol). The ice bath was removed after 30 mins and warmed to room temperature. After all starting material was shown to be consumed by TLC experiment (developed using $KMnO_4$ stain), the reaction was concentrated *in vacuo.* The crude material was washed with diethyl ether, gravity filtered to remove all precipitate, and the filtrate was concentrated *in vacuo* to give a yellow oil (0.406 g).

((*3S*)-1-Methylpyrrolidin-3-yl) *p*-fluorobenzenecarbothioate oxylate: A portion of ((*3S*)-1-methylpyrrolidin-3-yl) *p*-fluorobenzenethioate (0.0.324 g, 1.36 mmol) was completely dissolved in isopropanol (14 mL). With stirring, oxalic acid (0.220 g, 1.36 mmol) was added and stirred for 3 hrs at room temperature. The solution was concentrated *in vacuo* to give a white solid and immediately recrystallized from isopropanol to give ((*3S*)-1-methylpyrrolidin-3-yl) *p*-fluorobenzenecarbothioate oxylate as a white solid (0.289 g, 64%).
Spectroscopic Data: $MP_{(isopropanol)}$: 130-134°C; IR(ATR): 3036, 2961, 2713, 2591, 1717, 1698, 1598, 913 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 8.00-7.96 (m, 2H), 7.44-7.38 (m, 2H), 4.52 (br s, 2H, overlapping with H_2O in DMSO), 4.27-4.20 (m, 1H), 3.72 (dd, J = 11.8, 8.1 Hz, 1H), 3.37-3.31 (m, 1H), 3.28-3.22 (m, 2H), 2.78 (s, 3H), 2.59-2.52 (m, 1H), 2.07-1.98 (m, 1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 189.4 (0), 166.8 (d, $^1J_{C,F}$ = 252.7 Hz, 0), 164.3 (1), 132.5 (d, $^4J_{C,F}$ = 2.8 Hz, 0), 129.9 (d, $^3J_{C,F}$ = 9.6 Hz, 1), 116.4 (d, $^2J_{C,F}$ = 22.4 Hz, 1), 59.7 (2), 54.1 (2), 40.4 (3), 38.6 (1), 30.0(2); ^{19}F NMR (376.6 MHz, DMSO-*d6*) δ -104.1; HRMS (ESI^+): calculated for $C_{12}H_{15}FNOS^+$: 240.0853; found for $C_{12}H_{15}FNOS^+$: 240.0853; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.817 mins): 98.2 %.

(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate IR(ATR)



(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate ^{1}H NMR 400 MHz (DMSO-*d6*)

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PROCNO                1

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PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH             8196.722 Hz
FIDRES          0.250144 Hz
AQ              3.9976959 sec
RG                   101
DW                61.000 usec
DE                13.55 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1
SFO1        400.3024719 MHz
NUC1                  1H
P0                  3.33 usec
P1                  9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                 65536
SF          400.3000028 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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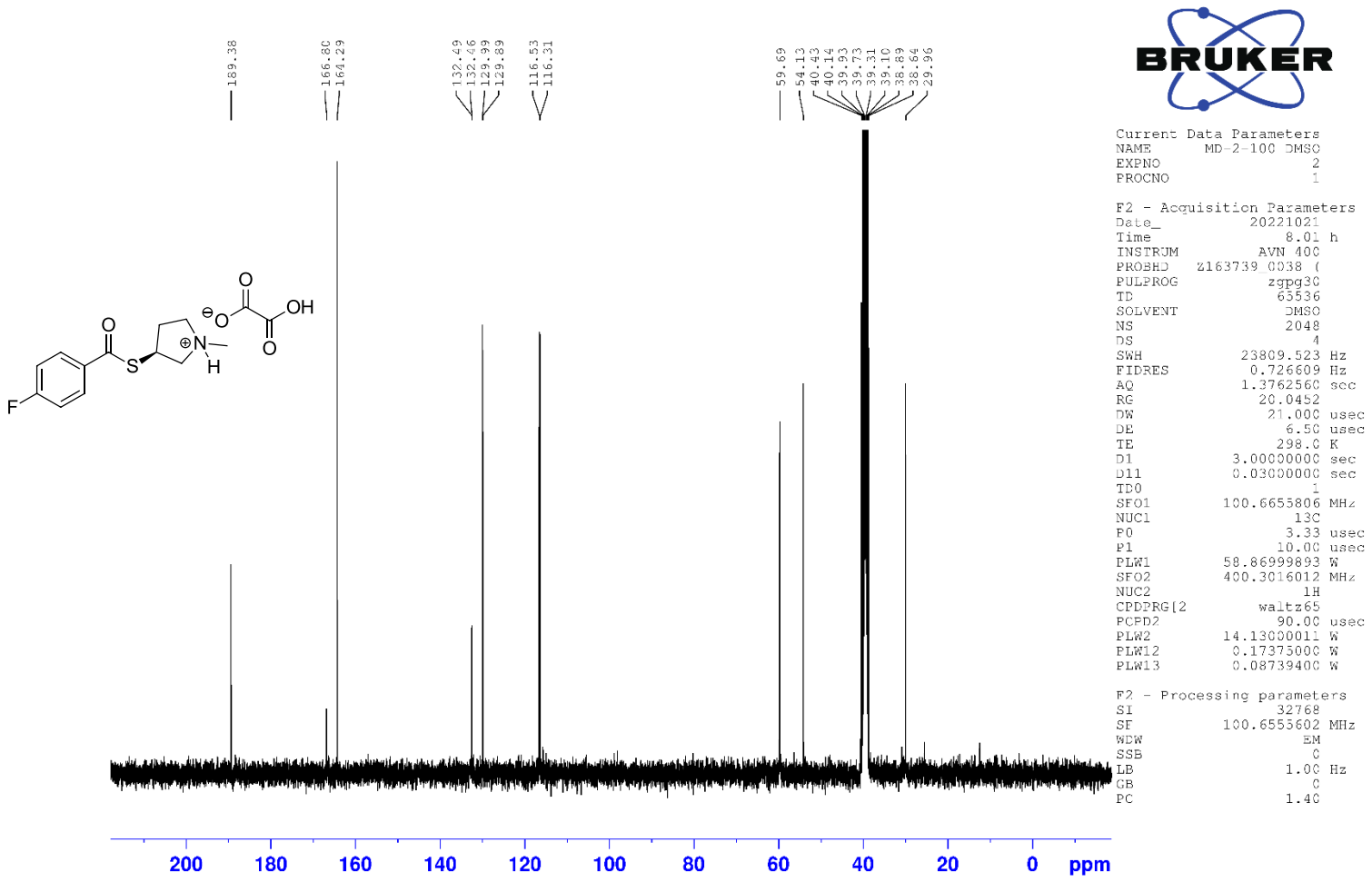
(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate ^{13}C NMR 100 MHz (DMSO-*d6*)

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Current Data Parameters
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PROCNO                1

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PROBHD   Z163739 0038 (
PULPROG         zgpg30
TD               65536
SOLVENT           DMSO
NS                2048
DS                   4
SWH          23809.523 Hz
FIDRES        0.726609 Hz
AQ            1.3762560 sec
RG              20.0452
DW               21.000 usec
DE                6.50 usec
TE               298.0 K
D1           3.00000000 sec
D11          0.03000000 sec
TD0                  1
SFO1       100.6655806 MHz
NUC1               13C
P0                3.33 usec
P1               10.00 usec
PLW1       58.86999893 W
SFO2       400.3016012 MHz
NUC2               1H
CPDPRG[2        waltz65
PCPD2            90.00 usec
PLW2       14.13000011 W
PLW12       0.17375000 W
PLW13       0.08739400 W

F2 - Processing parameters
SI                32768
SF         100.6555602 MHz
WDW                EM
SSB                 0
LB                1.00 Hz
GB                  0
PC                1.40
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(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Nov 01 2022000002.d	Acquisition Date	2022-11-01 2:43:55 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6026	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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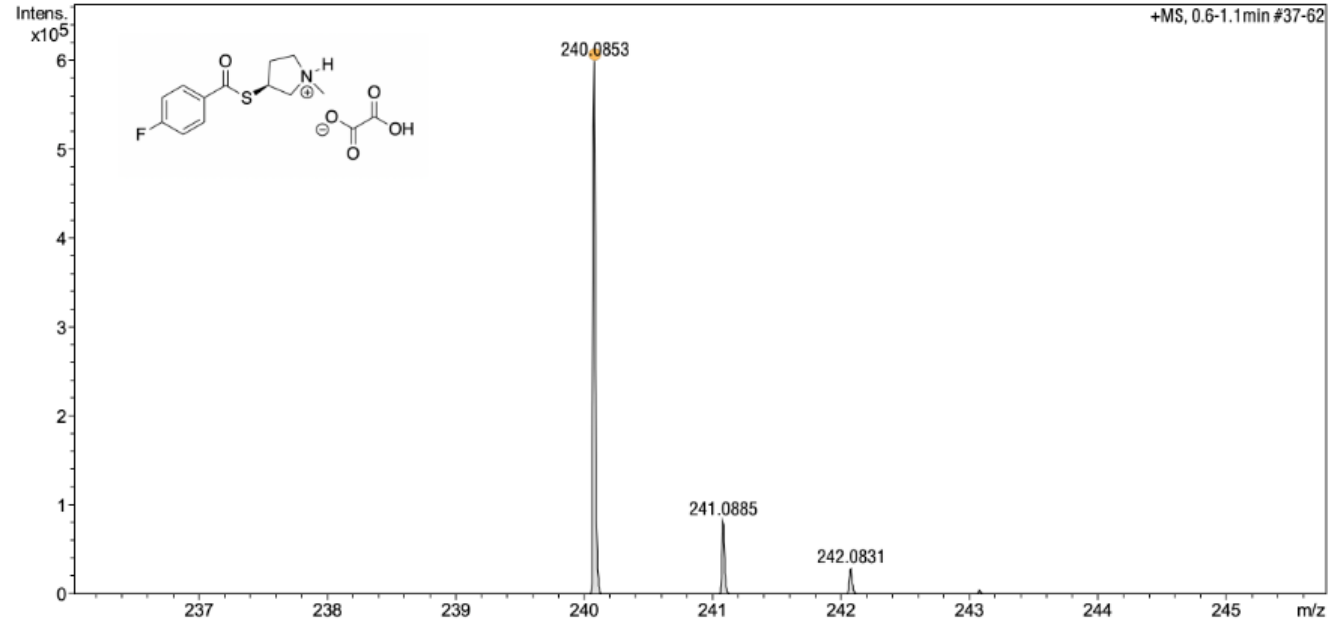
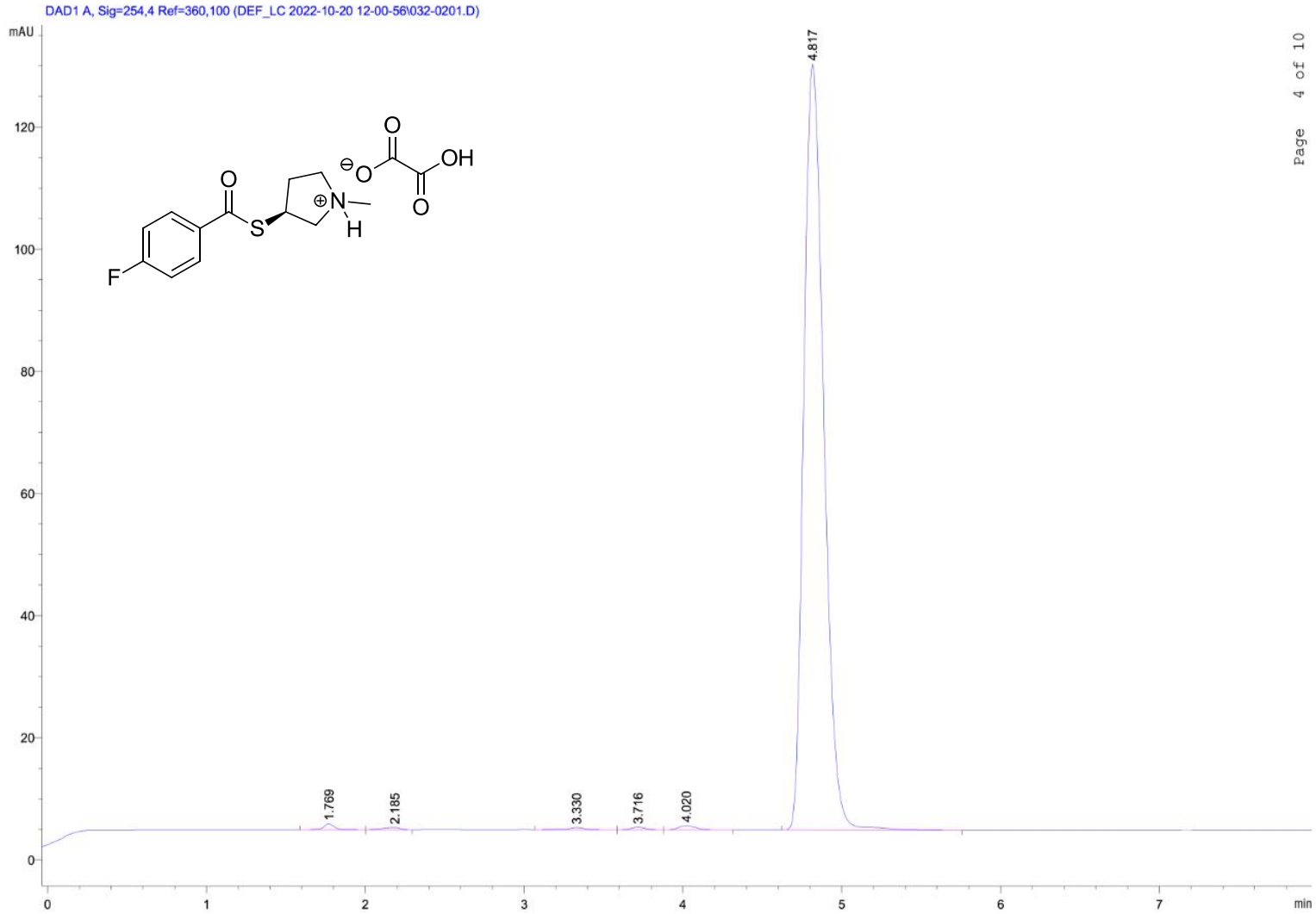


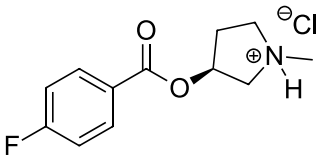
Nov 01 2022000002.d

Bruker Compass DataAnalysis 4.3 printed: 2022-11-08 10:39:33 AM by: x Page 1 of 1

(25) ((*3S*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate HPLC



(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride



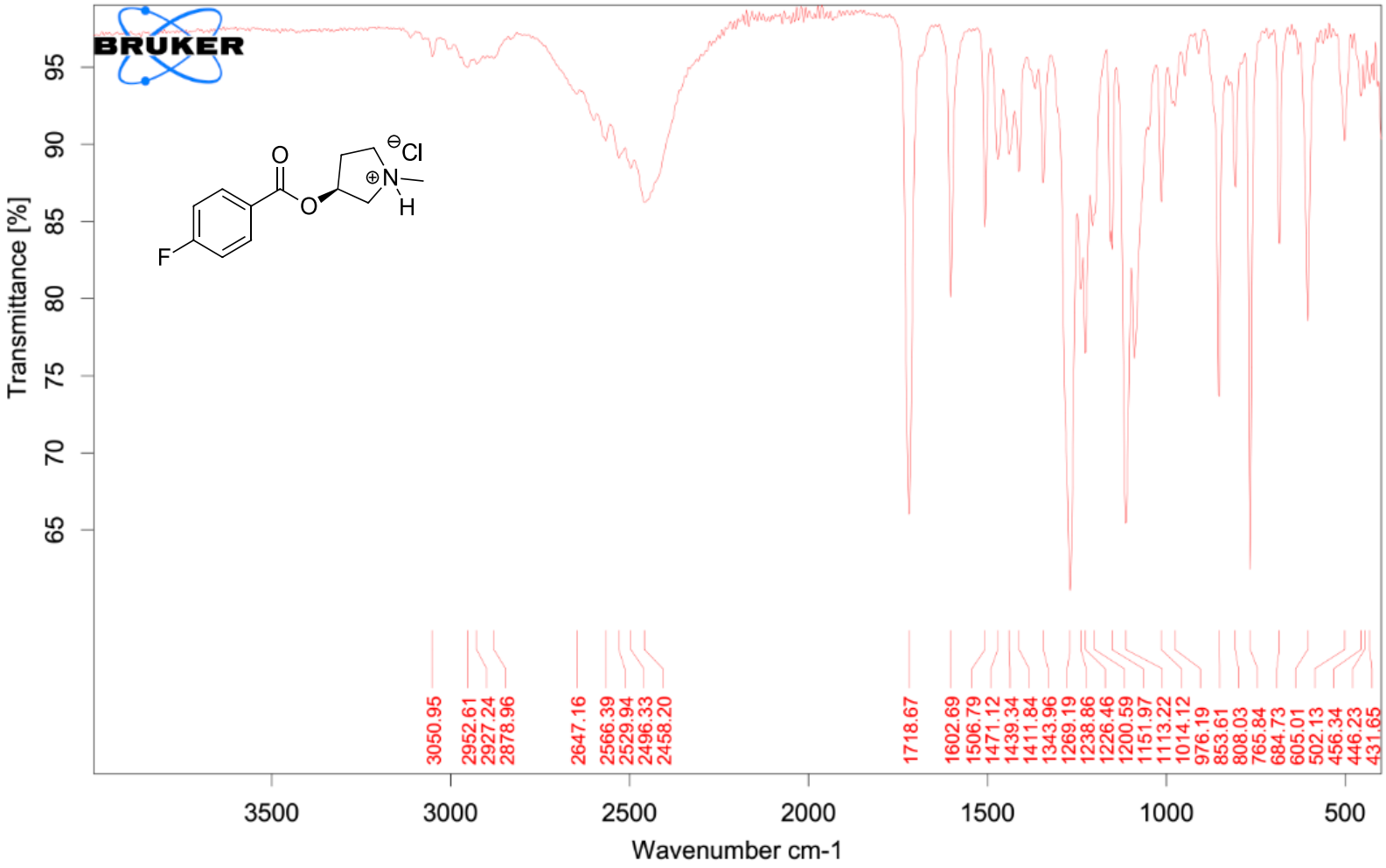
Synthesis: **(*3S*)-1-Methyl-3-pyrrolidinyl *p*-fluorobenzoate**: (*3S*)-(+)-1-Methyl-3-pyrrolidinol (0.20 mL, 1.82 mmol) was dissolved in anhydrous dichloromethane (6.00 mL) under an argon atmosphere and cooled to 0°C. To this solution, triethylamine (0.501 mL, 3.64 mmol) and 4-fluorobenzoyl chloride (0.218 g, 1.82 mmol). The ice bath was removed after 5 min, and the reaction was stirred for 24 hrs. At this time water (20 mL) was added, layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce an orange oil. This crude material was purified by flash chromatography through silica gel (5% MeOH/DCM/1%TEA) to afford (*3S*)-1-methyl-3-pyrrolidinyl *p*-fluorobenzoate as an orange oil (0.338 g, 83%).

Spectroscopic data: IR(ATR): 2965, 2942, 2777, 1712, 1603, 1507, 1268, 1113, 688 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.03-7.99 (m, 2H), 7.37-7.33 (m, 2H), 5.31-5.27 (m, 1H), 2.75-2.69 (m, 3H), 2.33-2.25 (m, 2H), 2.26 (s, 3H), 1.90-1.81 (m, 1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 165.1 (d, $^{1}J_{C,F}$ = 251.3 Hz, 0), 164.7 (0), 132.1 (d, , $^{3}J_{C,F}$ = 9.7 Hz, 1), 126.4 (d, $^{4}J_{C,F}$ = 2.7 Hz, 0), 115.9 (d, $^{2}J_{C,F}$ = 22.1 Hz, 1), 75.3 (1), 61.6 (2), 54.4 (2), 41.6 (3), 32.2 (2); HPLC purity at 254 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.106 mins): 98.7 %.

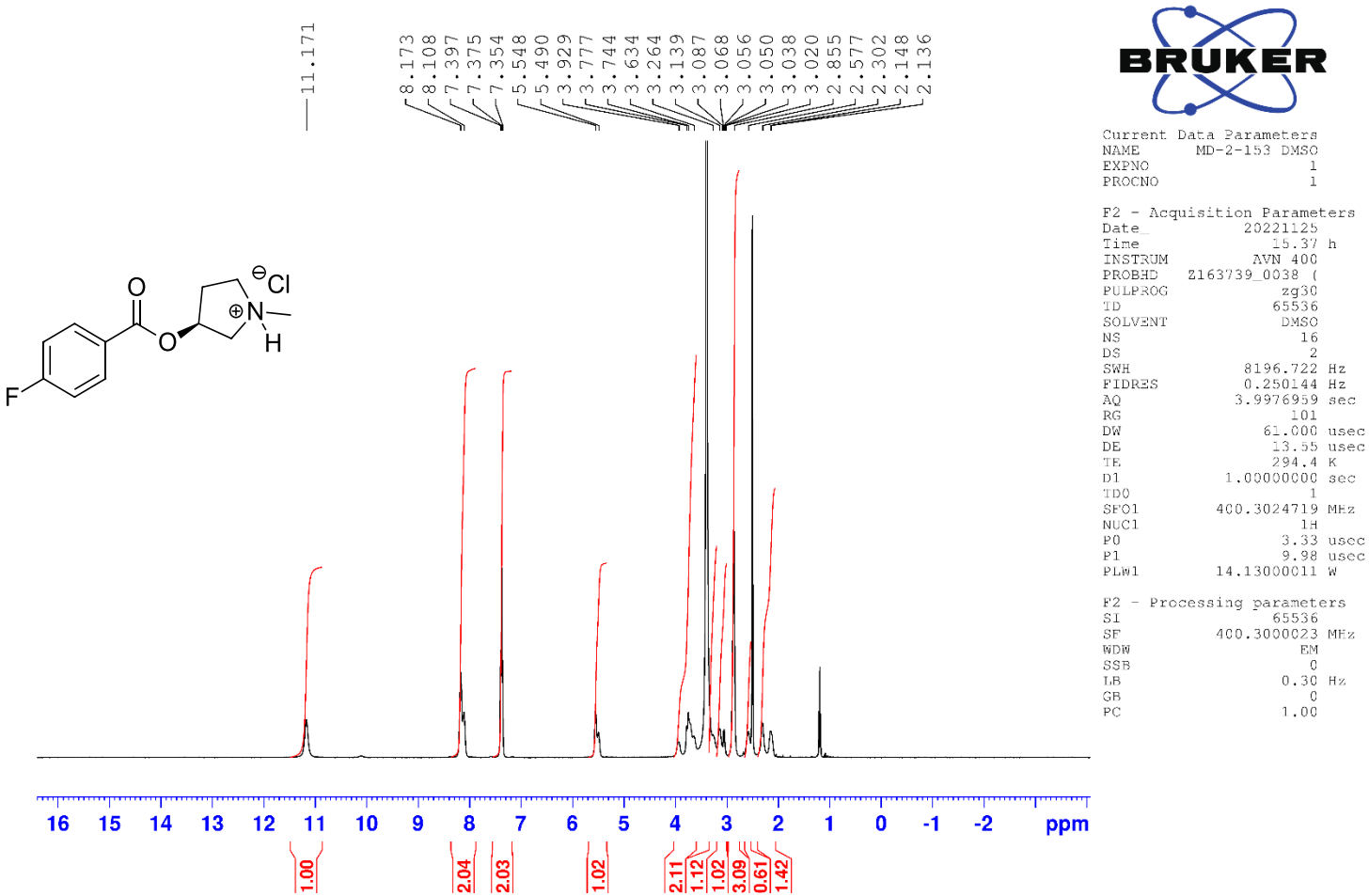
(*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride: S-((*3S*)1-Methylpyrolidin-3-yl) *p*-fluorobenzenecarbothioate (0.2484 g, 1.11 mmol) was dissolved in anhydrous diethyl ether (22mL) under an argon atmosphere. HCl in diethyl ether 1 N (1.7 mL, 1.67 mmol) was added dropwise and stirred for 3 hrs. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated three times with diethyl ether (20mL), solvent was removed with a pipet and compound was dried to produce (*3S*)-3-(*p*-fluorobenzoyloxy)-1-methyl-1-pyrrolidinium chloride (0.148 g, 51%) as a mixture of isomers.

Spectroscopic data: $MP_{(diethyl\ ether)}$: 143-145 °C; IR(ATR): 3051, 2953, 2927, 2647, 2566, 2530, 1719, 1603, 1269, 1113, 766 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 11.17 (br s, 1H), 8.17-8.11 (m, 2H), 7.40-7.35 (m, 2H), 5.55-5.49 (m, 1H), 3.93-3.63 (m, 2H), 3.30-3.24 (m, 1H), 3.14-3.02 (m, 1H), 2.85 (s, 3H), 2.58-2.14 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 165.4 (d, $^{1}J_{C,F}$ = 251.8 Hz, 0), 164.2 (0), 132.6 (1), 125.9 (0), 115.8 (d, $^{2}J_{C,F}$ = 22.5 Hz, 1), 73.3 (1), 59.5 (2), 53.2 (2), 40.4 (3), 30.3 (2); HRMS (ESI^{+}): calculated for $C_{12}H_{15}FNO_2^{+}$: 224.1081 amu; found for $C_{12}H_{15}FNO_2^{+}$: 224.1083 amu; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.118 mins): 98.8 %.

(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{1}HNMR 400 MHz (DMSO-*d6*)



BRUKER

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PROCNO                1

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PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8196.722 Hz
FIDRES         0.250144 Hz
AQ             3.9976959 sec
RG                  101
DW                61.000 usec
DE                13.55 usec
TE                294.4 K
D1           1.00000000 sec
TD0                   1
SFO1         400.3024719 MHz
NUC1                  1H
P0                 3.33 usec
P1                 9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                65536
SF         400.3000023 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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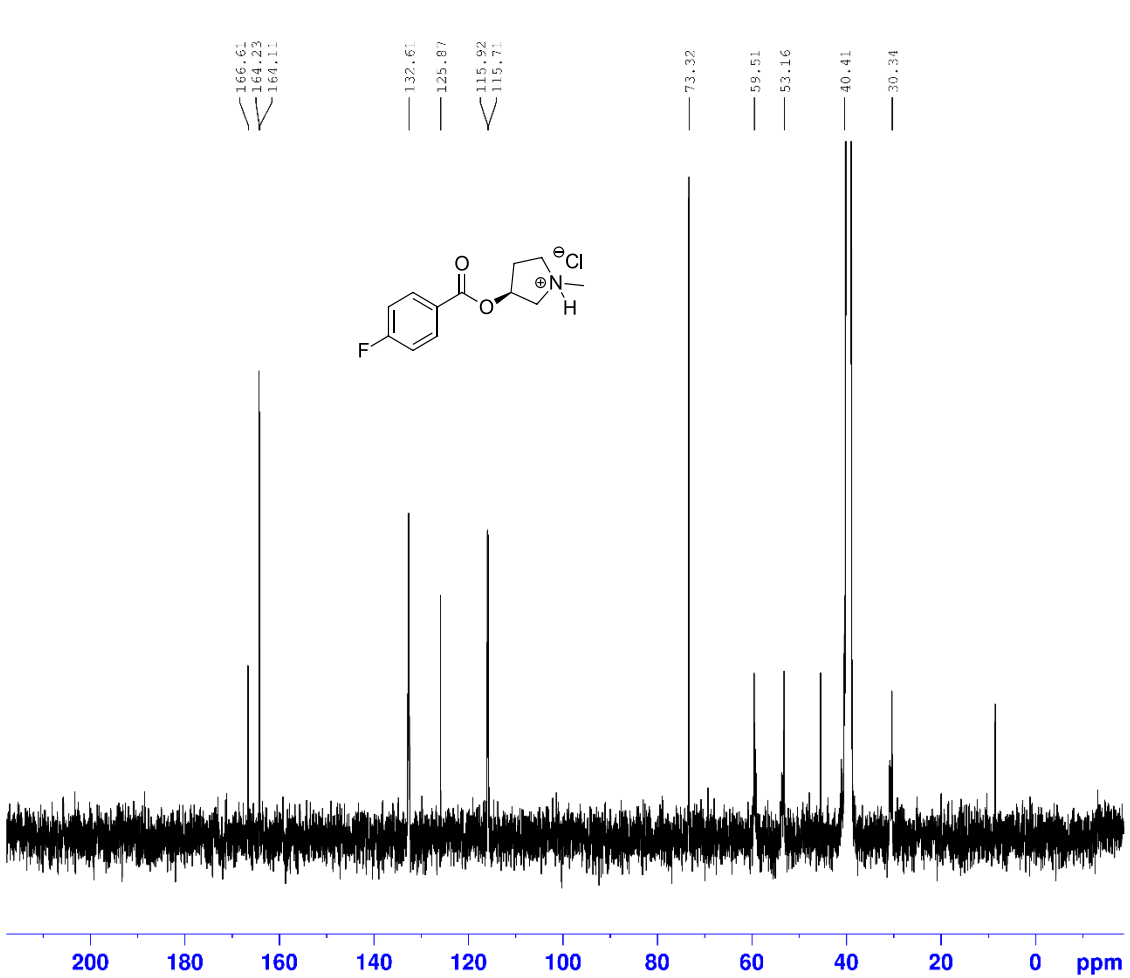
(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{13}CNMR 100 MHz (DMSO-*d6*)

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Current Data Parameters
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PROCNO                1

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Time               1.47 h
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PULPROG            udeft
TD                 21424
SOLVENT             DMSO
NS                    768
DS                      8
SWH            23809.523 Hz
FIDRES          2.222696 Hz
AQ              0.4499040 sec
RG                20.5375
DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1             4.00000000 sec
D12            0.00002000 sec
D20           20.00000000 sec
TD0                    1
SFO1         100.6655806 MHz
NUC1                 13C
P1                 10.00 usec
P13              2000.00 usec
P26               500.00 usec
PLW1         58.86999893 W
SPNAM[5]     Crp60comp.4
SPOAL5             0.500
SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2         400.3016012 MHz
NUC2                 1H
CPDPRG[2          waltz65
PCPD2              90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W

F2 - Processing parameters
SI                131072
SF           100.6555602 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.40
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(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Dec 13 2022000004.d	Acquisition Date	2022-12-13 2:18:41 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV6027 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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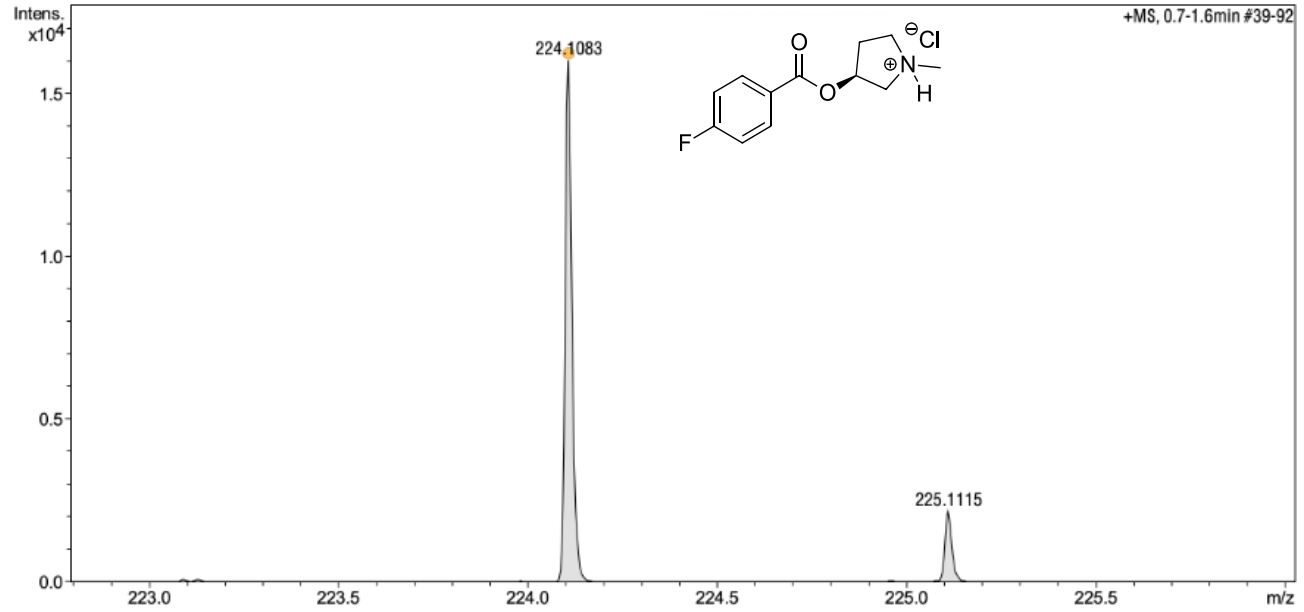
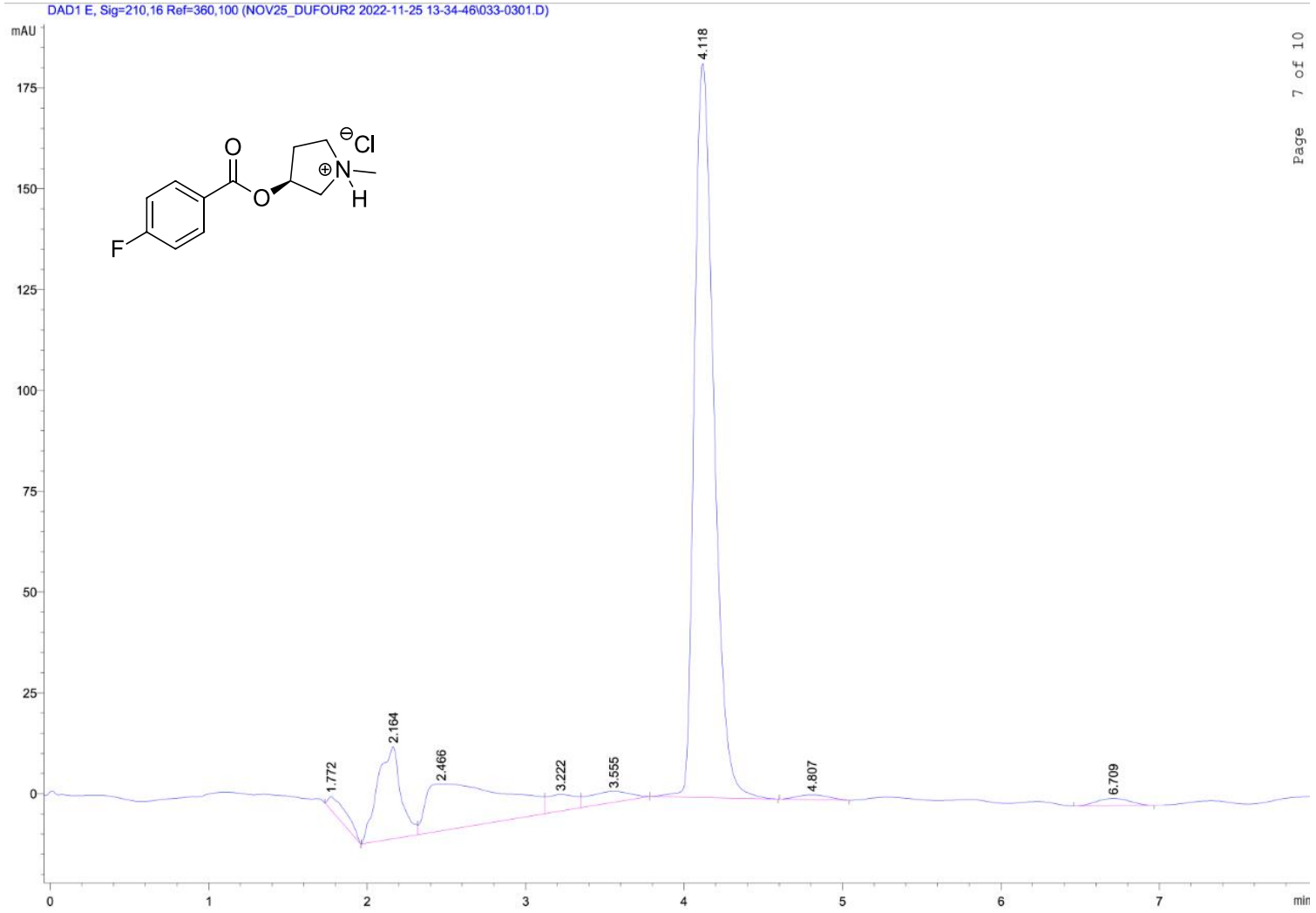
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

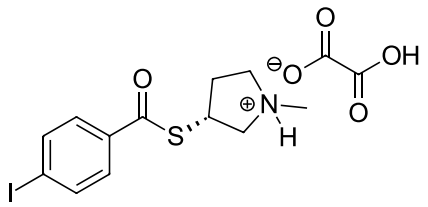
Meas. m/z	Ion Formula	m/z	err [ppm]
224.1083	C12H15FNO2	224.1081	-0.6



(26) (*3S*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 4.118 min.



(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate



Synthesis: **(*3S*)-3-(Mesyloxy)-1-methylpyrrolidine**: (*S*)-(-)-1-Methyl-3-pyrrolidinol (6.00 mL, 54.6 mmol) was dissolved in anhydrous DCM (130 mL) and cooled to 0°C with an ice bath. Triethylamine (11.4 mL, 81.9 mmol) and methanesulfonyl chloride (4.90 mL, 62.8 mmol) were added dropwise at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred overnight. Brine (50 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3. Gravity filtered, and the filtrate was concentrated in vacuo to produce a yellow oil (8.87 g, 91%). Product confirmed by ^{1}H NMR and the crude material was immediately carried through to next step without further purification.

(*3R*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3S*)-3-(Mesyloxy)-1-methylpyrrolidine (6.99 g, 39.0 mmol), was dissolved in anhydrous THF (130 mL) under an argon atmosphere. 18-crown-6-ether (15.5 g, 58.5 mmol) was added followed by potassium thioacetate (6.68 g, 58.5 mmol), added in 3 portions. Once all potassium thioacetate had gone into solution, the reaction was heated to 30°C and left overnight. The reaction was filtered then diluted with water (50 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_3 and concentrated *in vacuo* to produce a red oil. The crude material was then distilled under vacuum (1 mm Hg) at 100 °C to give a yellow oil (4.45 g, 72%). Product confirmed by ^{1}H NMR and the crude material was immediately carried through to next step without further purification.

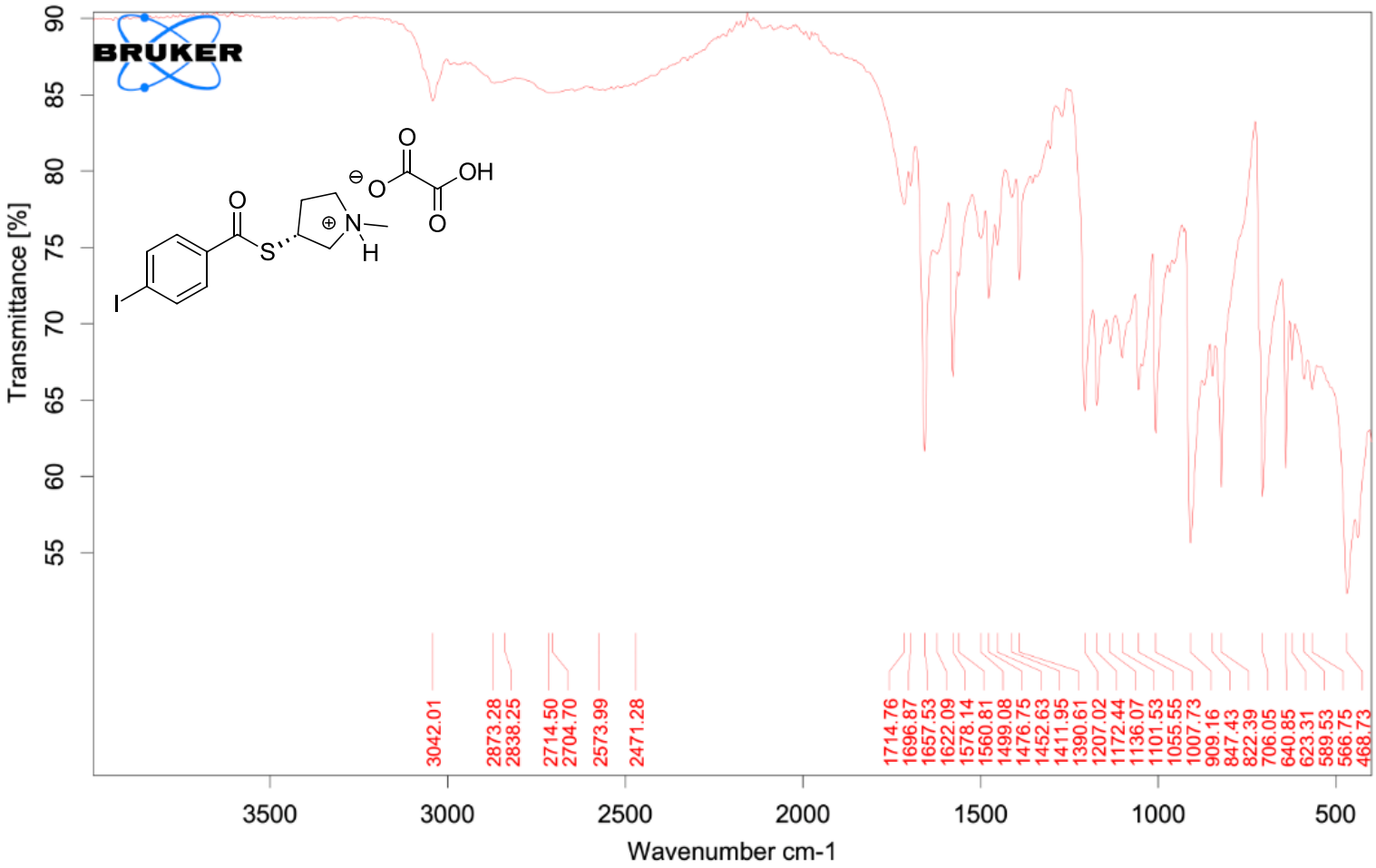
(*R*)-1-Methyl-3-pyrrolidinethiol: (*3R*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate (0.951 g, 5.97 mmol) was dissolved in MeOH (30 mL) and sodium methoxide (0.333 g, 5.97 mmol) was added to the stirring solution. After all starting material was consumed by TLC using a $KMnO_4$ stain, MeOH was removed *in vacuo* and a solvent swap with DCM was done and used in the next step immediately.

((*3R*)-1-Methylpyrrolidin-3-yl) *p*-iodobenzenethioate: (*R*)-1-Methyl-3-pyrrolidinethiol was dissolved in DCM (20mL) and cooled to 0 °C in an ice bath. 4-Iodobenzoyl chloride (1.59 g, 5.97 mmol) was added to the reaction flask followed by triethylamine (1.66 mL, 11.9 mmol). The ice bath was removed after 30 mins, reaction was warmed to room temperature and stirred for 16 hrs. Once the reaction was complete, the solvent was removed *in vacuo.* The crude material was washed with diethyl ether, filtered to remove all precipitate, and the filtrate was concentrated *in vacuo* to give an orange solid, (1.63 g, 79%). This material was used carried through immediately without further purification.

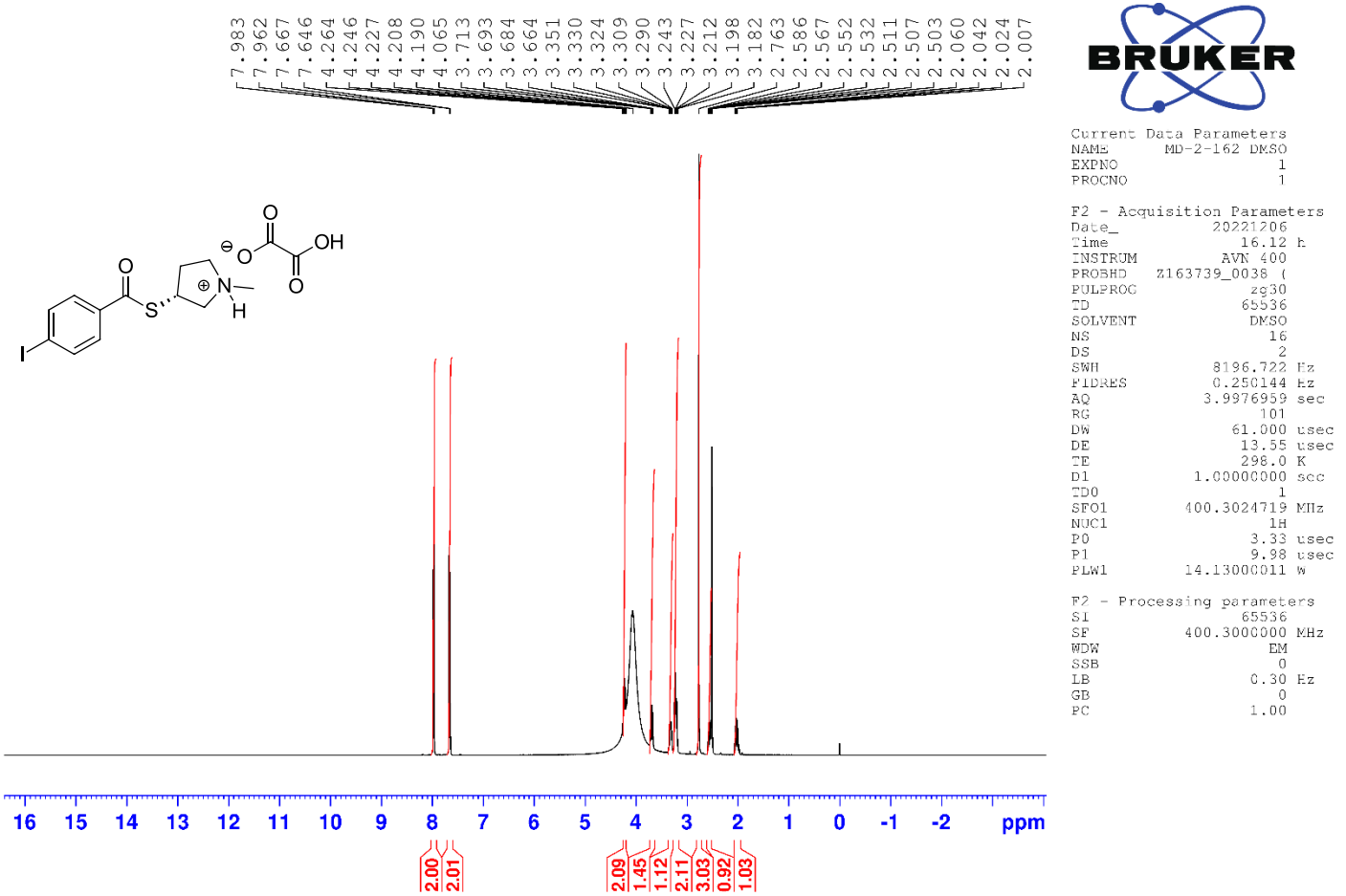
((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate: ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-iodobenzenethioate (1.14 g, 3.28 mmol) was completely dissolved in isopropanol (63 mL). With stirring, oxalic acid (0.296 g, 3.28 mmol) was added and left to react for 3 hrs. The solution was concentrated *in vacuo* to give a white solid and immediately recrystallized with isopropanol. For further purification, this material was recrystallized with methanol twice to give ((*3R*)-1-methylpyrrolidin-3-yl) *p*-iodobenzenecarbothioate Oxylate as a white solid (0.610 g, 42%)

Spectroscopic Data: $MP_{(isopropanol)}$: 197–200 °C; IR(ATR): 3042, 2838, 2705, 2574, 1658, 1561, 909, 706 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.98-7.96 (m, 2H), 7.67-7.65 (m, 2H), 4.26-4.19 (m, 1H), 4.06 (br s, 2H, overlapping with H_2O in DMSO); 3.69 (dd, J = 11.7, 8.0 Hz, 1H), 3.35-3.29 (m, 1H), 3.24-3.18 (m, 2H), 2.76 (s, 3H), 2.59-2.52 (m, 1H), 2.06-1.97 (m, 1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 190.3 (0), 164.2 (0), 138.2 (1), 135.1 (0), 128.5 (1), 103.0 (0), 59.8 (2), 54.2 (2), 40.5 (3), 38.6 (1), 30.0 (0); HRMS (ESI^+): calculated for $C_{12}H_{15}INOS^+$: 347.9913 amu; found for $C_{12}H_{15}INOS^+$: 347.9910 amu; HPLC purity at 230nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 8.331 mins): 99.5 %.

(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate IR(ATR)



(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate ^{1}HNMR 400 MHz (DMSO-*d6*)

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PROCNO                1

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Time              16.12 h
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PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH            8196.722 Hz
FIDRES        0.250144 Hz
AQ            3.9976959 sec
RG                  101
DW               61.000 usec
DE               13.55 usec
TE               298.0 K
D1            1.00000000 sec
TD0                   1
SFO1         400.3024719 MHz
NUC1                 1H
P0                3.33 usec
P1                9.98 usec
PLW1         14.13000011 W

F2 - Processing parameters
SI                65536
SF           400.3000000 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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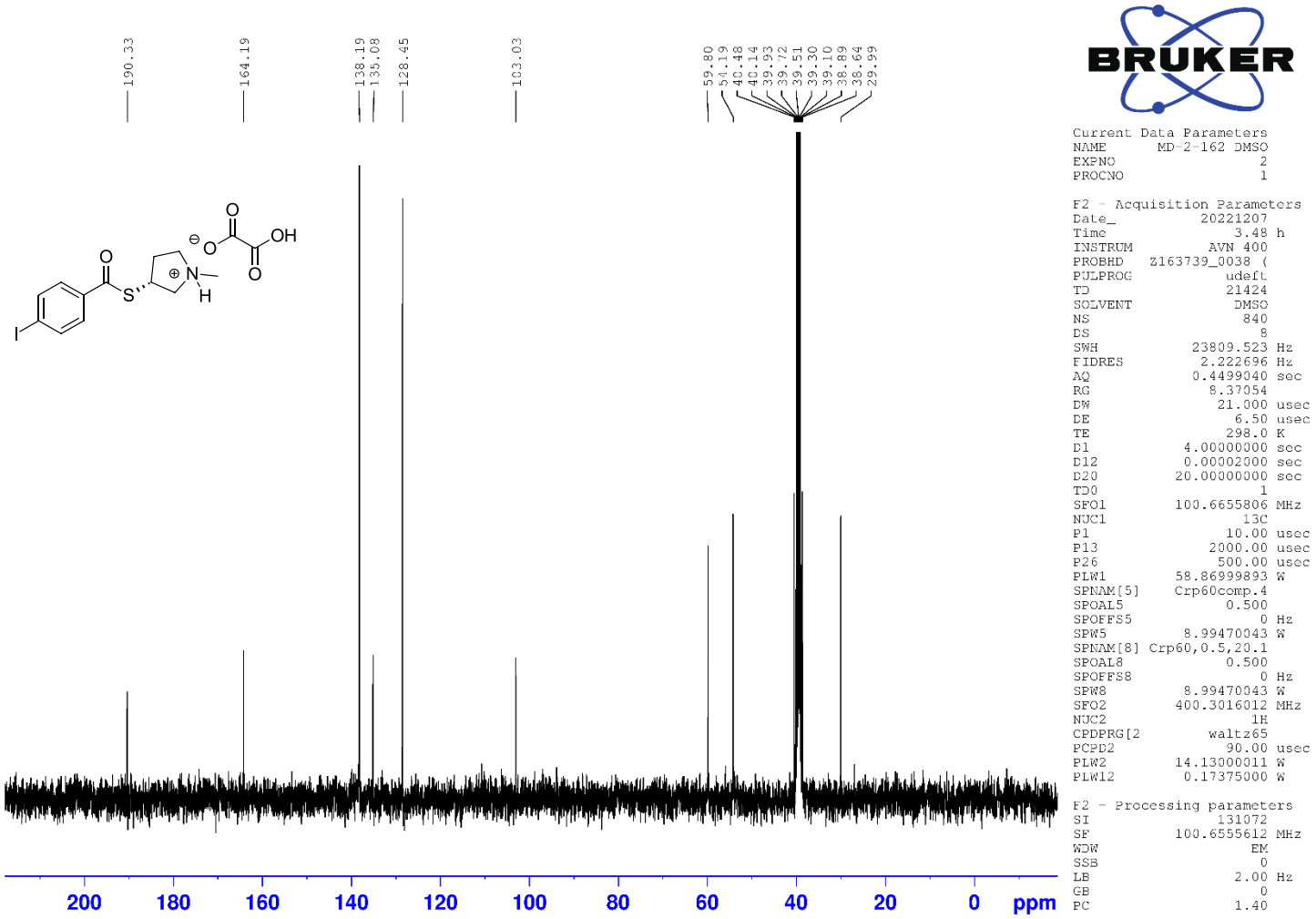
(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate ^{13}CNMR 100 MHz (DMSO-*d6*)



BRUKER

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Current Data Parameters
NAME       MD-2-162 DMSO
EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
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Time               3.48 h
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PULPROG            udeft
TD                 21424
SOLVENT             DMSO
NS                   840
DS                     8
SWH            23809.523 Hz
FIDRES          2.222696 Hz
AQ             0.4499040 sec
RG              8.37054
DW               21.000 usec
DE                6.50 usec
TE                298.0 K
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D12          0.00002000 sec
D20         20.00000000 sec
TD0                    1
SFO1         100.6655806 MHz
NUC1                 13C
P1                 10.00 usec
P13              2000.00 usec
P26               500.00 usec
PLW1         58.86999893 W
SPNAM[5]    Crp60comp.4
SPOAL5             0.500
SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2         400.3016012 MHz
NUC2                 1H
CPDPRG[2         waltz65
PCPD2              90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W

F2 - Processing parameters
SI                131072
SF           100.6555612 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.40
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(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Dec 13 2022000002.d	Acquisition Date	2022-12-13 2:01:53 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV6029 Oxalate	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active	Set Capillary	3500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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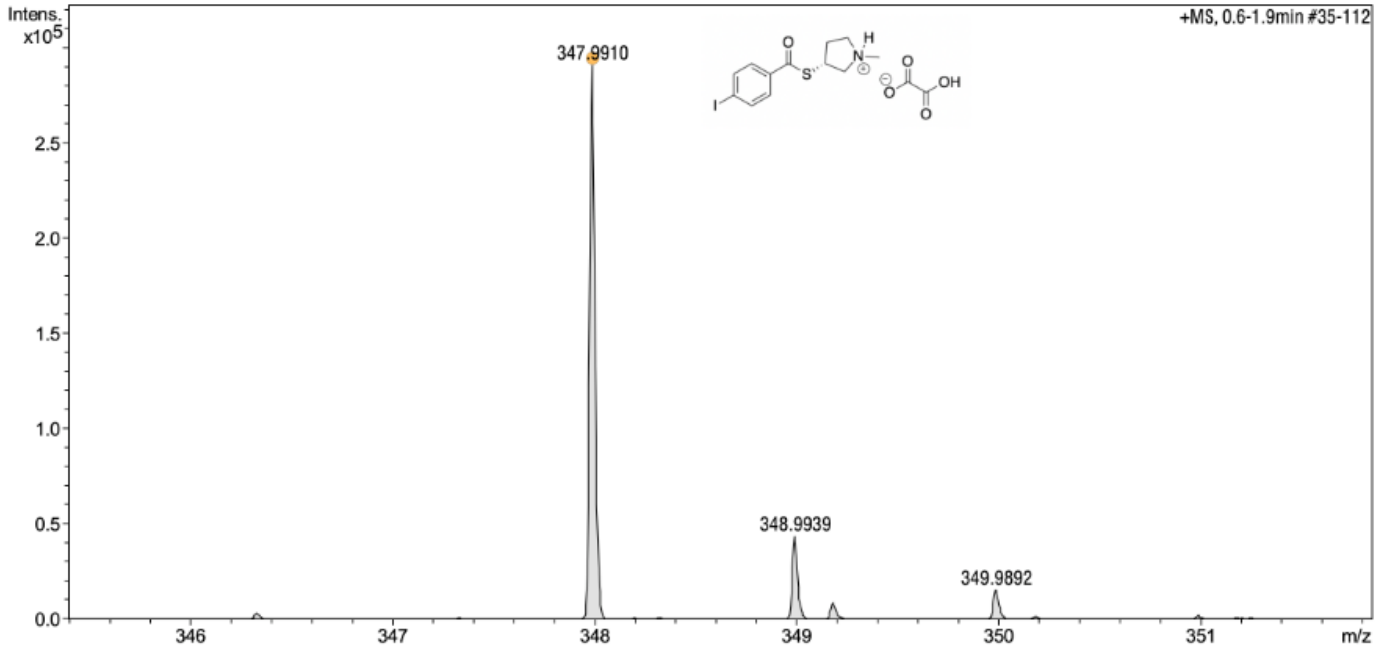
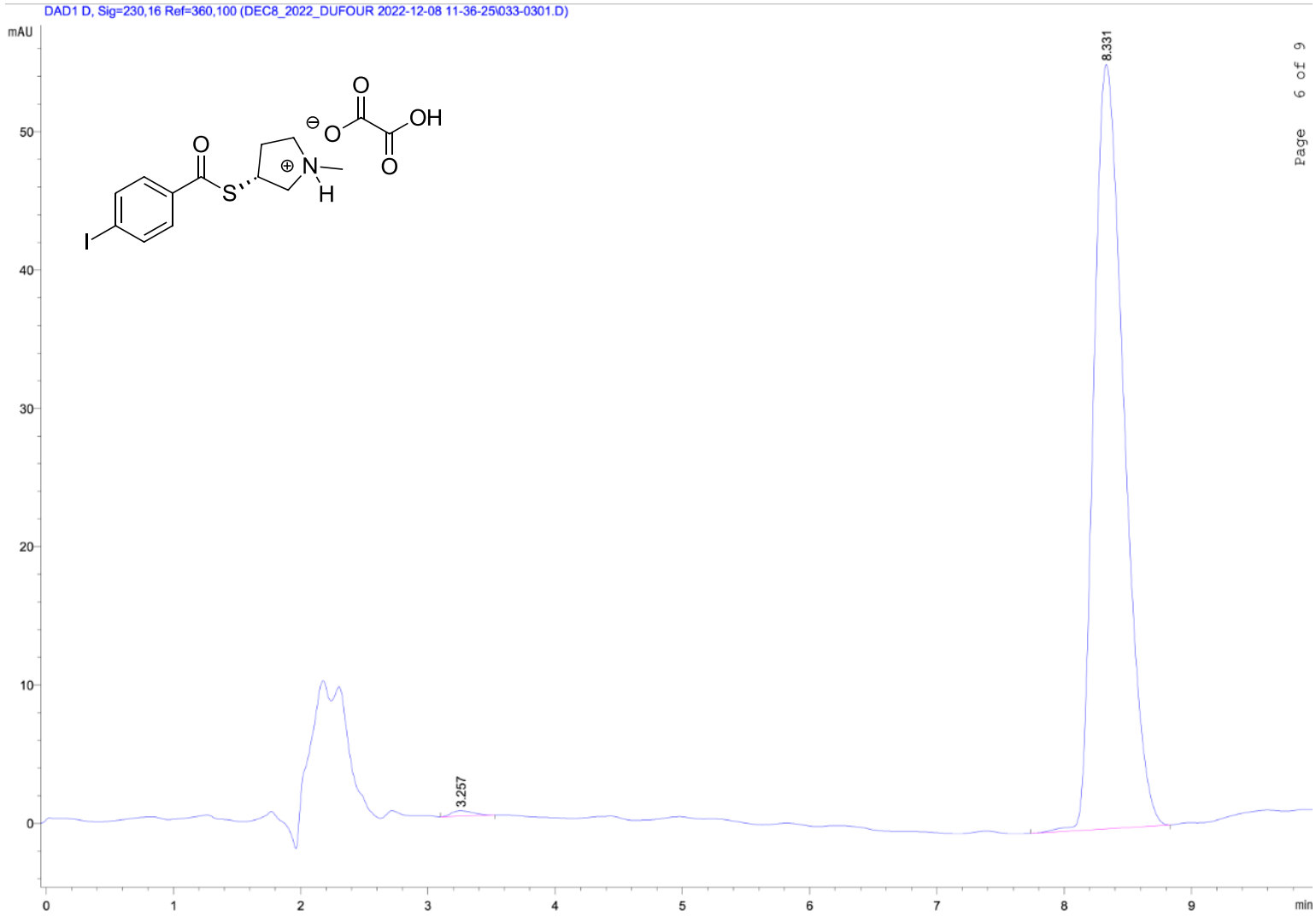
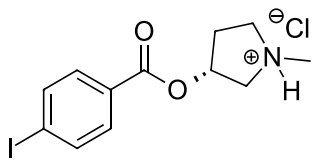


(27) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Iodobenzenecarbothioate Oxylate HPLC. Note: Noise from the injection has a retention time from 1.6 min until 2.5 min. The compound of interest has a retention time of 8.331 min.



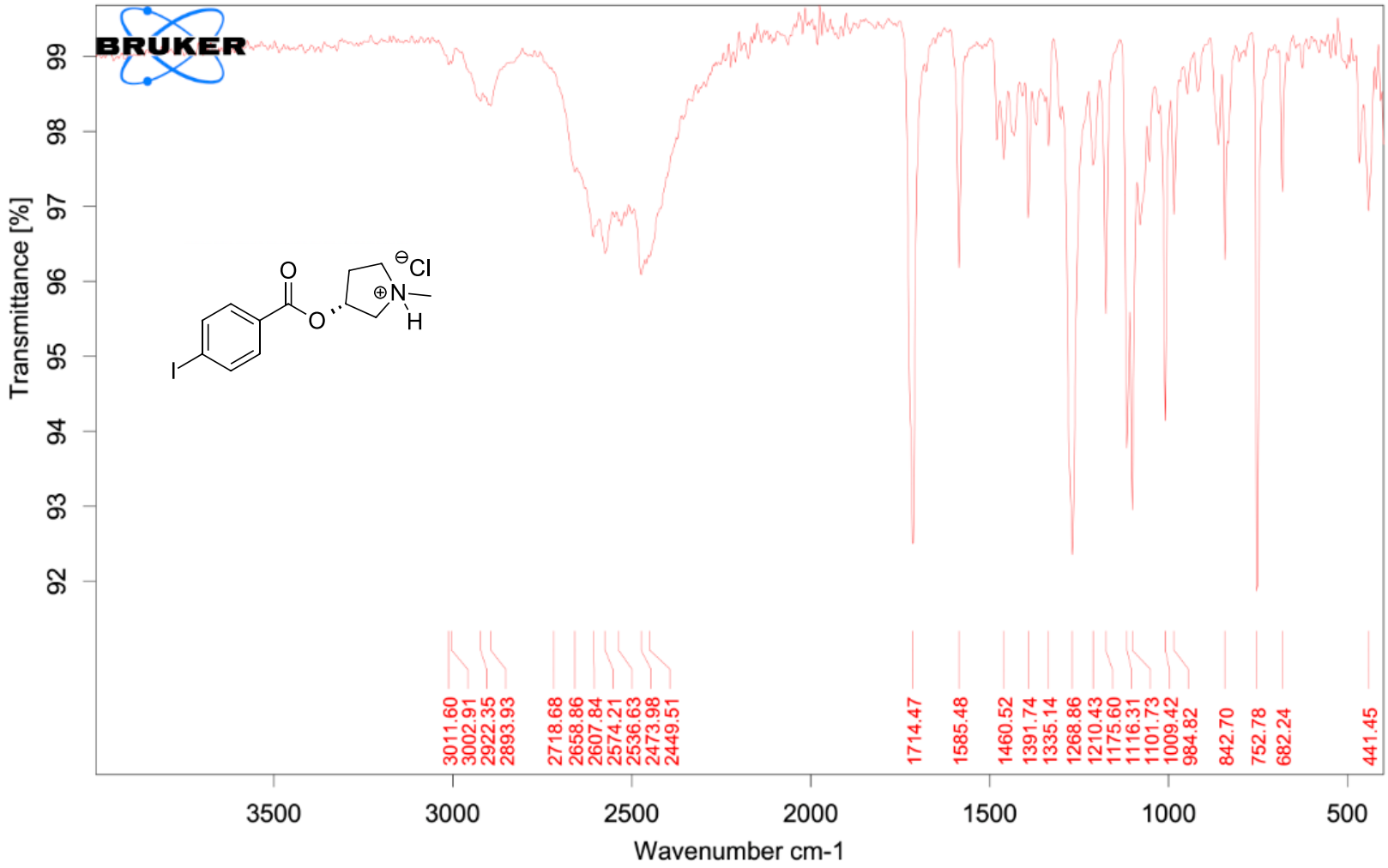
(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium chloride



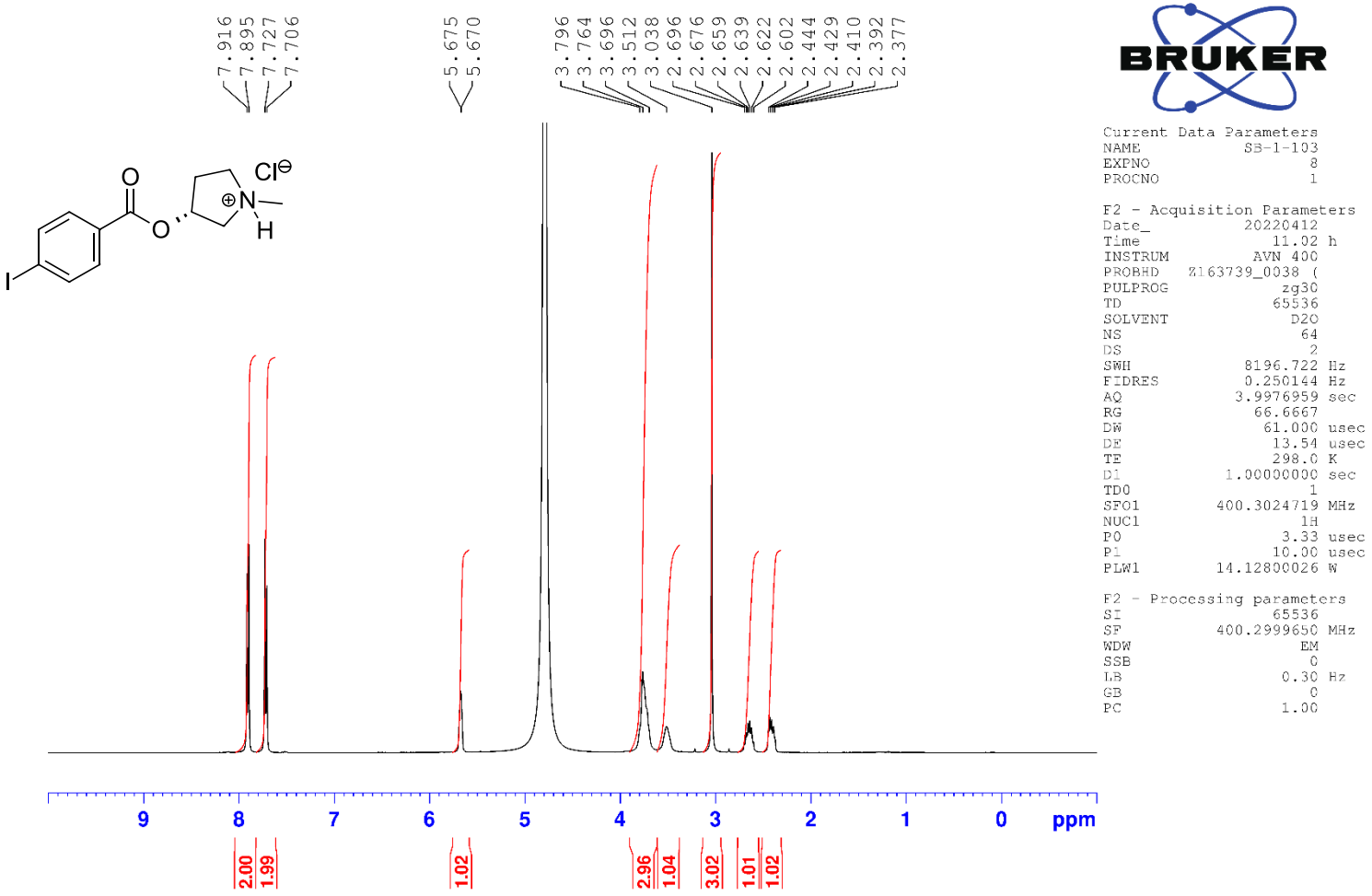
Synthesis: **(*3R*)-1-Methyl-3-pyrrolidinyl *p*-iodobenzoate**: 4-Iodobenzoyl chloride (0.799 g, 3.00 mmol), triethylamine (1.05 mL, 7.50 mmol) and 4-dimethylaminopyridine (DMAP) (0.0659 g, 0.540 mmol) were dissolved in anhydrous dichloromethane (20.0 mL) under an argon atmosphere. (R)-(-)-1-Methyl-3-pyrrolidinol (0.329 mL, 3.00 mmol) was added at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred for 3hrs. The reaction was quenched with brine (20mL), extracted with DCM (3×15 mL) and the combined organic layers were dried over Na_2SO_3. The solvent was removed was *in vacuo* to produce a pink solid. The crude mixture was purified by silica gel column chromatography (5% MeOH/DCM) to produce a pink solid, (*3R*)-1-methyl-3-pyrrolidinyl *p*-iodobenzoate (0.409 g, 41%). The analytical data was similar to previously published data.[7]

***(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium chloride**: (*3R*)-1-Methyl-3-pyrrolidinyl *p*-iodobenzoate (0.257 g, 0.776 mmol) was dissolved in anhydrous diethyl ether (16 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 1.2 mL, 1.16 mmol) was added dropwise resulting in the immediate formation of a white precipitate. The reaction was stirred for an additional 60 mins at room temperature. The precipitate was collected via suction filtration, washing with Et_2O (2 x 10 mL), to afford *(3R)*-3-(*p*-iodobenzoyloxy)-1-methyl-1-pyrrolidinium chloride (0.150 mg, 53 % yield) as a white solid. Spectroscopic data: $MP_{(diethyl\ ether)}$: 243-245 ^oC; IR (ATR) 3012, 3003, 2922, 2894, 2574, 2474, 1714, 1585, 1269, 1102, 753; ^1H NMR (400 MHz, D_2O) δ 7.92-7.90 (m, 2H), 7.73-7.71 (m, 2H), 5.68-5.66 (m, 1H), 3.80-3.70 (m, 3H), 3.51 (br app s, 1H), 3.04 (s, 3H), 2.70-2.60 (m, 1H), 2.44-2.38 (m, 1H); ^{13}C NMR (100.7 MHz, D_2O) δ 167.7, 138.7, 131.5, 128.9, 102.3, 74.7, 61.1, 55.2, 42.0, 31.1; LRMS (ESI^+): 332.0 (M^+); HRMS (ESI^+): calculated for $C_{12}H_{15}INO_2^+$: 332.0142; found for $C_{12}H_{15}INO_2^+$: 332.0133; HPLC purity at 254 nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 5.755 mins): >99%.

(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{1}H NMR 400 MHz (D_2O)



BRUKER

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EXPNO                        8
PROCNO                       1

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Time                     11.02 h
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PULPROG                   zg30
TD                       65536
SOLVENT                    D2O
NS                           64
DS                            2
SWH                   8196.722 Hz
FIDRES                0.250144 Hz
AQ                    3.9976959 sec
RG                      66.6667
DW                       61.000 usec
DE                       13.54 usec
TE                       298.0 K
D1                    1.00000000 sec
TD0                          1
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NUC1                        1H
P0                       3.33 usec
P1                      10.00 usec
PLW1                14.12800026 W

F2 - Processing parameters
SI                       65536
SF                  400.2999650 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
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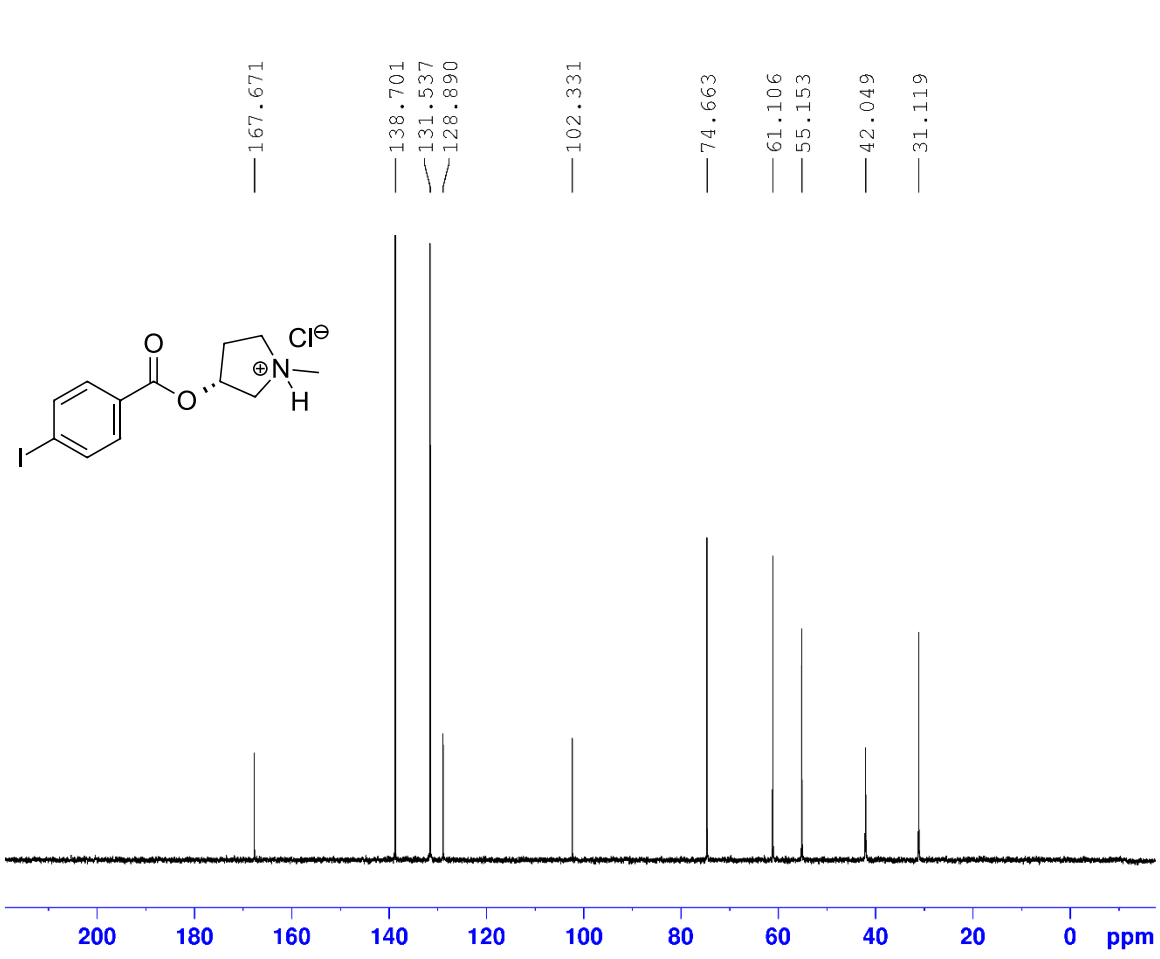
(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{13}C NMR 100 MHz (D_2O)



BRUKER

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EXPNO                  9
PROCNO                 1

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PULPROG           udeft
TD                21424
SOLVENT             D2O
NS                 2304
DS                    8
SWH           23809.523 Hz
FIDRES         2.222696 Hz
AQ            0.4499040 sec
RG               30.6195
DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                    1
SFO1        100.6655806 MHz
NUC1                13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         58.86999893 W
SPNAM[5]    Crp60comp.4
SPOAL5            0.500
SPOFFS5               0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8            0.500
SPOFFS8               0 Hz
SPW8          8.99470043 W
SFO2        400.3016012 MHz
NUC2                1H
CPDPRG[2         waltz65
PCPD2             90.00 usec
PLW2         14.12800026 W
PLW12         0.17442000 W

F2 - Processing parameters
SI                131072
SF          100.6554448 MHz
WDW                 EM
SSB                  0
LB                2.00 Hz
GB                   0
PC                1.40
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(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\July 06 2022000014.d	Acquisition Date	7/6/2022 3:12:40 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV 6003 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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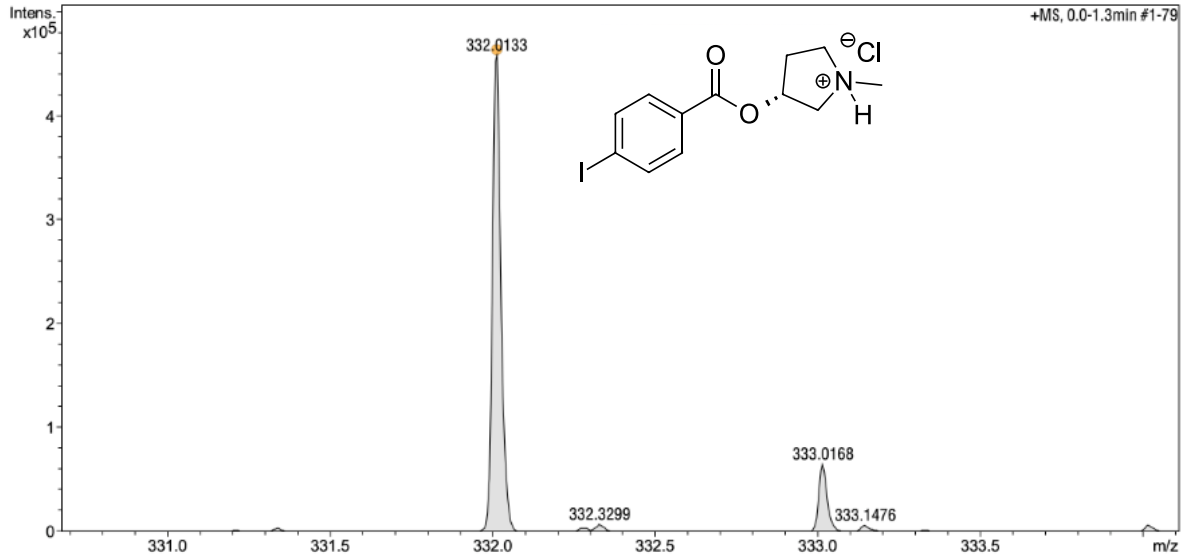
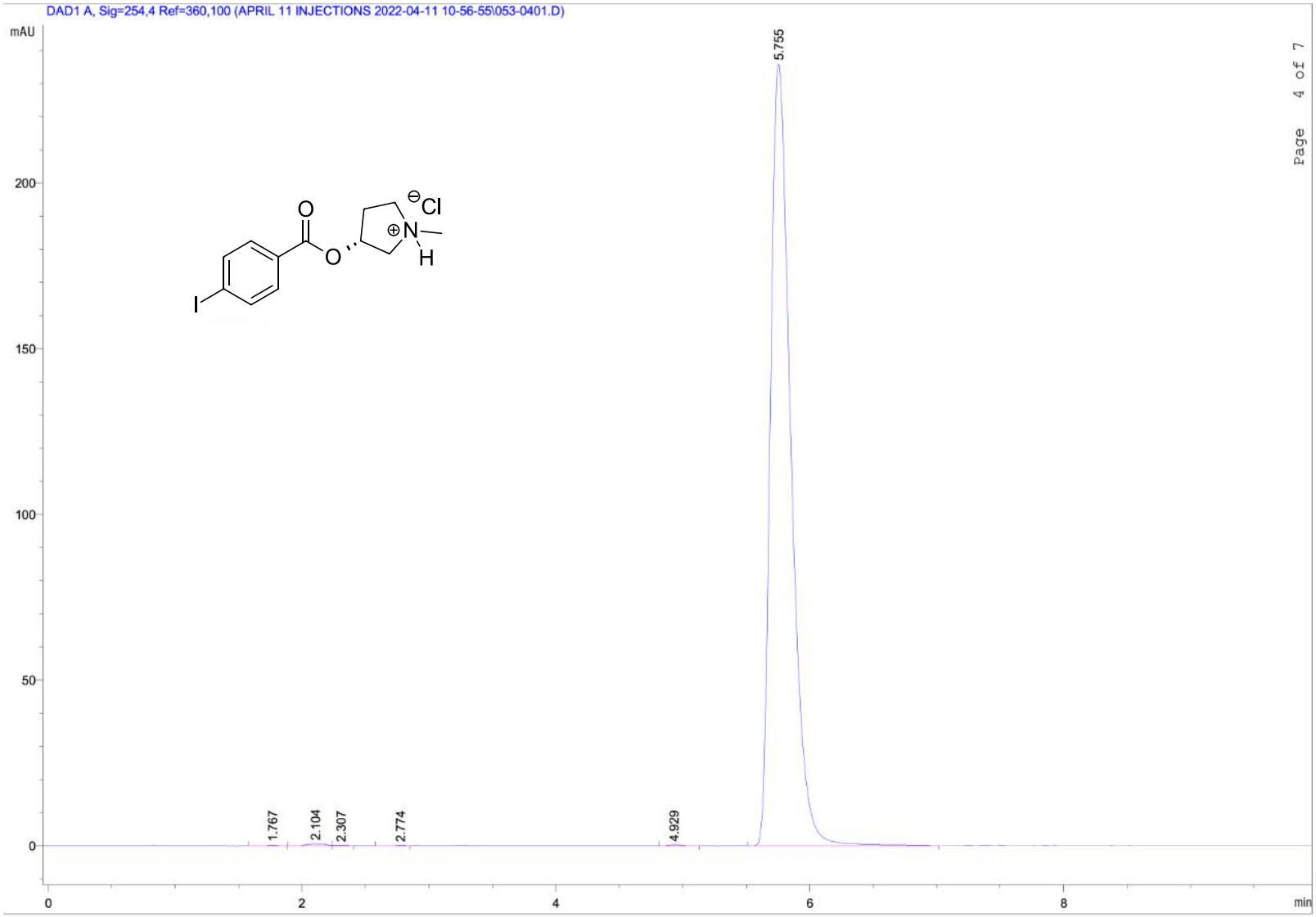


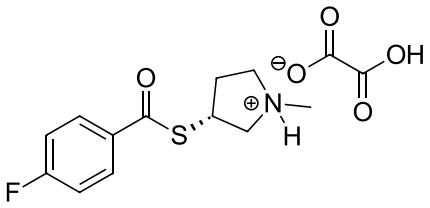
July 06 2022000014.d

Bruker Compass DataAnalysis 4.3 printed: 7/6/2022 3:16:03 PM by: x Page 1 of 1

(28) *(3R)*-3-(*p*-Iodobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride HPLC



(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate



Synthesis: **(*3S*)-3-(Mesyloxy)-1-methylpyrrolidine:** (*3S*)-(-)-1-Methyl-3-pyrrolidinol (6.0 mL, 54.6 mmol) was dissolved in anhydrous dichloromethane (130 mL) and cooled to 0°C with an ice bath. Triethylamine (11.4 mL, 81.9 mmol) and methanesulfonyl chloride (4.9 mL, 62.8 mmol) were added dropwise at 0°C. The ice bath was removed after 5 mins, and the reaction was stirred overnight. Brine (50 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce (*3S*)-3-(mesyloxy)-1-methylpyrrolidine as a yellow oil (8.87 g, 91%). Product was confirmed by ^{1}H NMR and the crude material was immediately carried through to next step without further purification.

(*3R*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate: (*3S*)-3-(Mesyloxy)-1-methylpyrrolidine (6.99 g, 39.0 mmol) was dissolved in anhydrous THF (130 mL) under an argon atmosphere. 18-crown-6-ether (15.5 g, 58.5 mmol) was added followed by potassium thioacetate (6.68 g, 58.5 mmol), added in 3 portions. Once all potassium thioacetate had gone into solution, the reaction was heated to 30°C and left overnight. The reaction was filtered, the filtrate diluted with water (50 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water (3 × 20 mL) and brine (3 × 20 mL), dried over Na_2SO_3, gravity filtered, and concentrated *in vacuo* to produce (*3R*)-S-(1-methylpyrrolidin-3-yl) ethanethioate as a red oil. The crude material was then distilled under vacuum (1 mm Hg) at 100-101°C to give a yellow oil (4.45 g, 72%). Product was confirmed by ^{1}H NMR and the material was immediately carried through to next step without further purification.

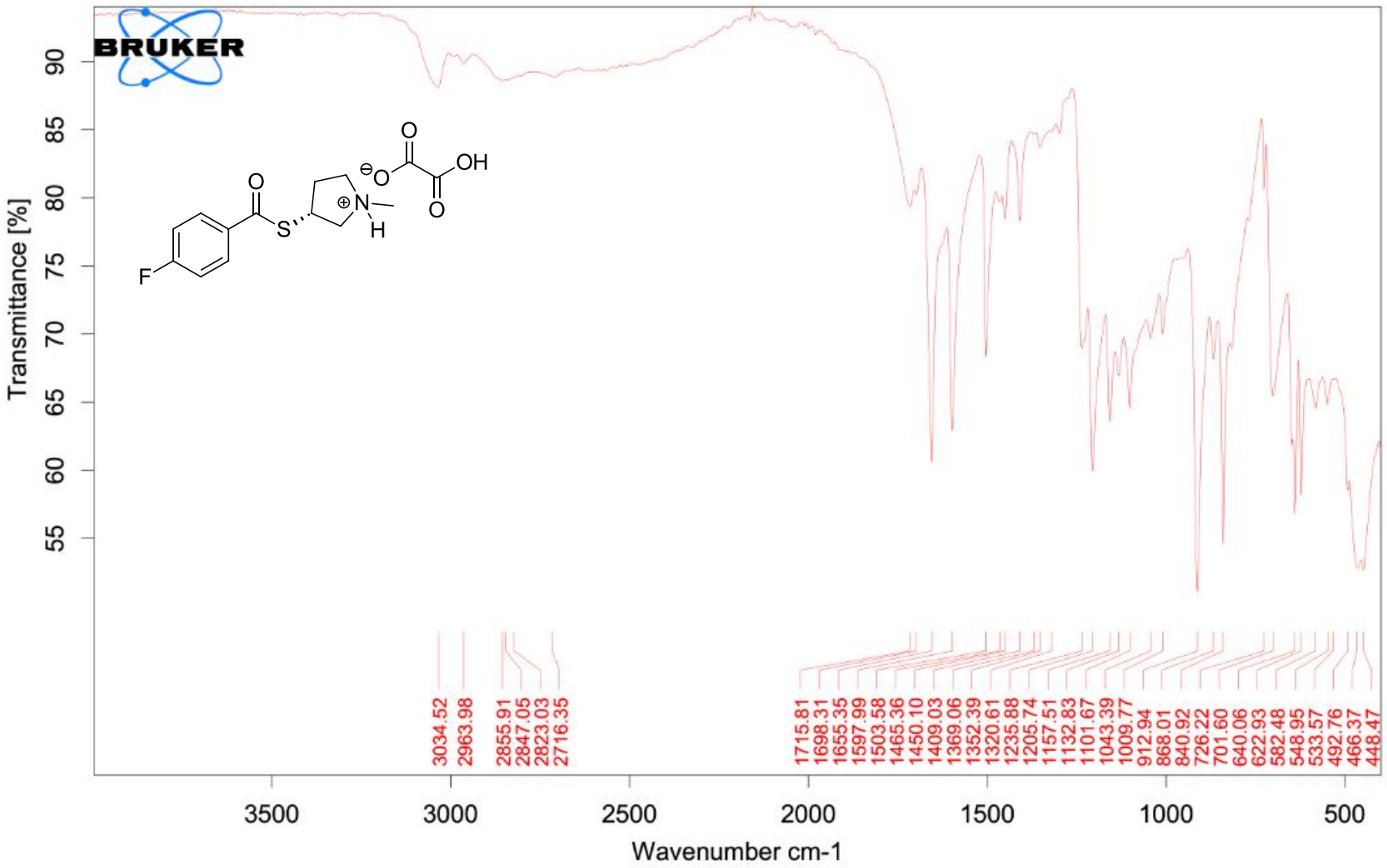
(*S*)-1-Methyl-3-pyrrolidinethiol: (*3R*)-S-(1-Methylpyrrolidin-3-yl) ethanethioate (0.950 g, 5.97 mmol) was dissolved in MeOH (30 mL) and sodium methoxide (0.332 g, 5.97 mmol) was added to the stirring solution. After all starting material was shown to be consumed by TLC experiment

the reaction was concentrated *in vacuo.* The resulting material was placed dichloromethane (20 mL) and concentrated *in vacuo*. This process was repeated and the material was immediately used in the next step.

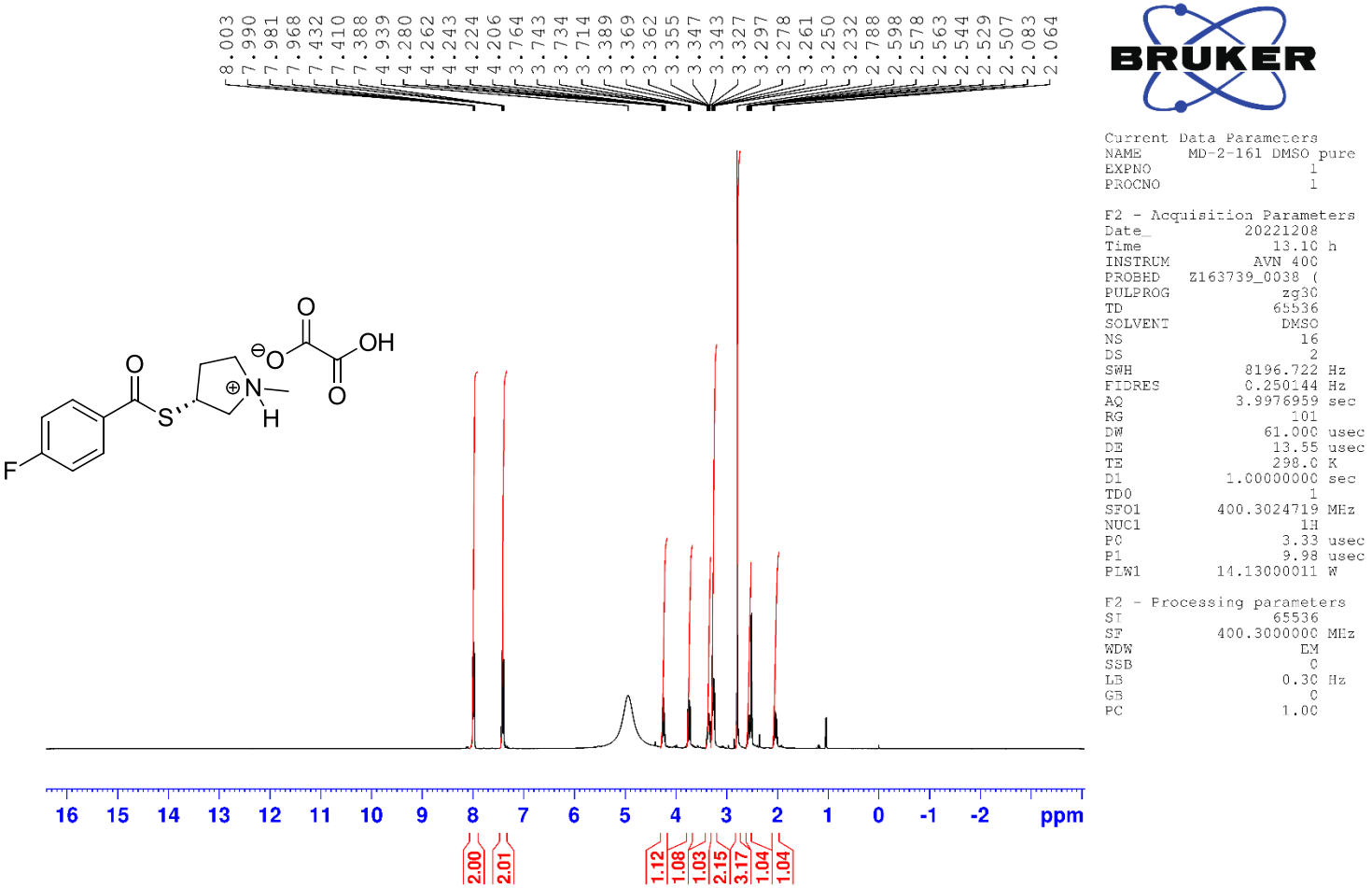
((*3R*)-1-Methylpyrrolidin-3-yl) *p*-fluorobenzenecarbothioate oxylate: The (*S*)-1-methyl-3-pyrrolidinethiol was dissolved in dichloromethane (20 mL) and cooled to 0°C using an ice bath. 4-fluorobenzoyl chloride (0.716 mL, 5.97 mmol) was added followed by triethylamine (1.66 mL, 11.9 mmol). The ice bath was removed after 30 mins and warmed to room temperature. After all starting material was shown to be consumed by TLC experiment (developed using $KMnO_4$ stain), the solvent was removed *in vacuo.* The crude material was washed with diethyl ether, filtered to remove all precipitate, and the filtrate was concentrated *in vacuo* to give an orange oil (1.52 g). A portion of this orange oil (1.03 g, 4.30 mmol) was completely dissolved in isopropanol (43 mL). With stirring, oxalic acid (0.387 g, 4.30 mmol) was added and the mixture stirred for 3 hrs. The solution was concentrated *in vacuo* to give a white solid and immediately recrystallized with isopropanol four times to give ((*3R*)-1-methylpyrrolidin-3-yl) *p*-fluorobenzenecarbothioate oxylate as a white solid (0.539 g, 38%).

Spectroscopic Data: $MP_{(isopropanol)}$: 134–137 °C; IR(ATR): 3035, 2964, 2847, 2716, 1655, 1598, 1206, 913, 841 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.00-7.97 (m, 2H), 7.43-7.39 (m, 2H), 4.24 (pent, J = 7.5 Hz, 1H), 3.74 (dd, J = 12.0, 8.3 Hz, 1H), 3.39-3.34 (m, 1H), 3.30-3.23 (m, 2H), 2.79 (s, 3H), 2.60-2.53 (m, 1H), 2.08-1.99 (m, 1H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 189.4 (0), 165.5 (d, $^{1}J_{CF}$ = 253.3 Hz, 0), 164.5 (1), 132.47 (d, $^{4}J_{CF}$ = 2.6 Hz, 0), 129.9 (d, $^{3}J_{CF}$ = 9.8 Hz, 1), 116.4 (d, $^{2}J_{CF}$ = 22.4 Hz, 1), 59.6 (2), 54.1 (2), 40.4 (3), 38.6 (1), 30.0 (2); ^{19}F NMR (376.6 MHz, DMSO-*d6*) δ 104.1; HRMS (ESI^{+}): calculated for $C_{12}H_{15}FNOS^{+}$: 240.0853 amu; found for $C_{12}H_{15}FNOS^{+}$: 240.0853 amu; HPLC purity at 254nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 5.031 mins): 98.2%.

(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate IR(ATR)



(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate ^{1}HNMR 400 MHz (DMSO-*d6*)

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PROCNO                     1

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PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
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FIDRES          0.250144 Hz
AQ              3.9976959 sec
RG                   101
DW                61.000 usec
DE                13.55 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1
SFO1         400.3024719 MHz
NUC1                  1H
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P1                 9.98 usec
PLW1        14.13000011 W

F2 - Processing parameters
SI                 65536
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WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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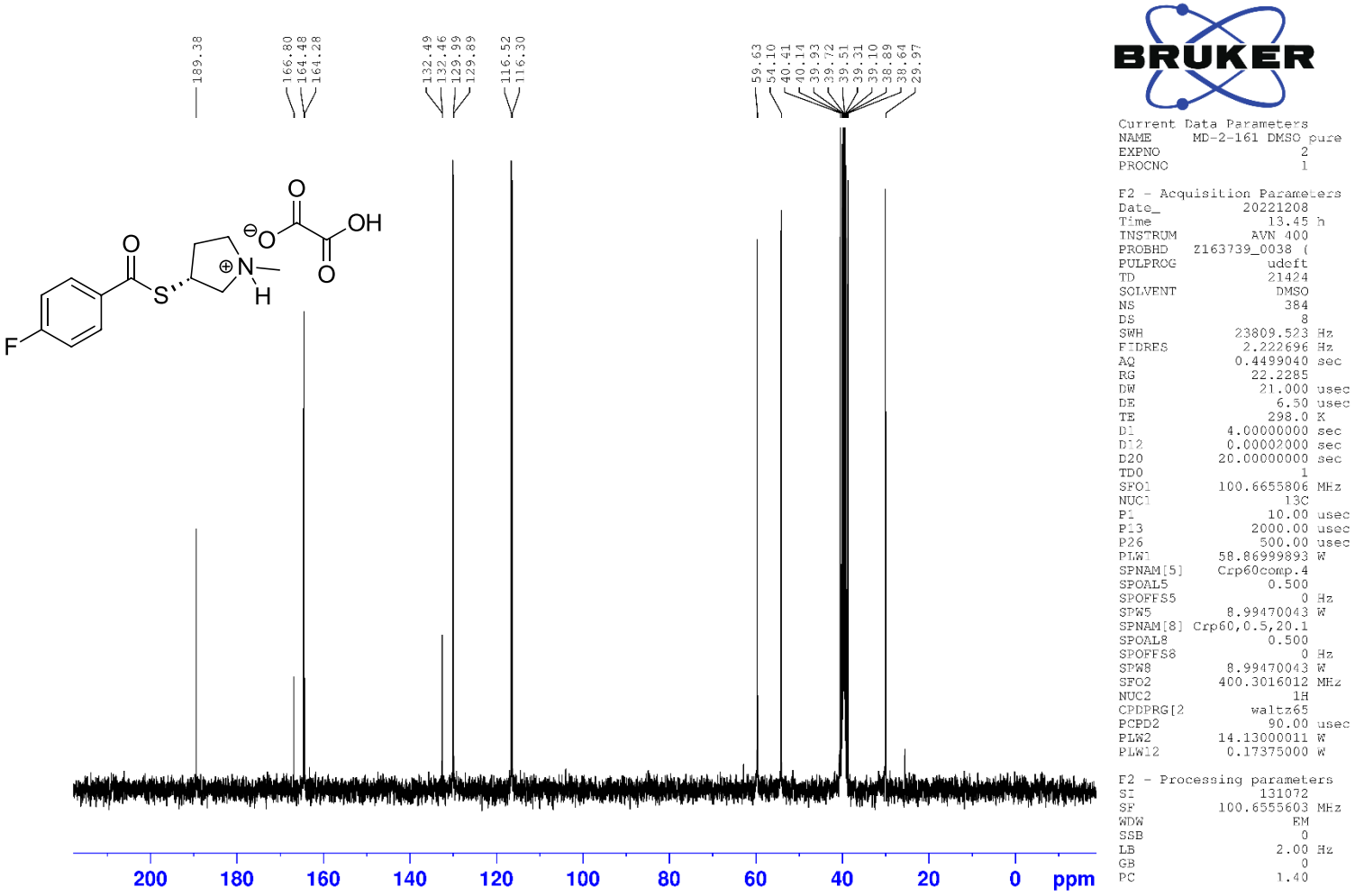
(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate ^{13}CNMR 100 MHz (DMSO-*d6*)

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Current Data Parameters
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PROCNO                     1

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PULPROG           udeft
TD                21424
SOLVENT             DMSO
NS                    384
DS                      8
SWH           23809.523 Hz
FIDRES         2.222696 Hz
AQ            0.4499040 sec
RG              22.2285
DW               21.000 usec
DE                6.50 usec
TE               298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                    1
SFO1        100.6655806 MHz
NUC1                  13C
P1                10.00 usec
P13             2000.00 usec
P26              500.00 usec
PLW1         58.86999893 W
SPNAM[5]     Crp60comp.4
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SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2        400.3016012 MHz
NUC2                  1H
CPDPRG[2          waltz65
PCPD2             90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W

F2 - Processing parameters
SI                131072
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LB                2.00 Hz
GB                   0
PC                1.40
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(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Dec 13 2022000001.d	Acquisition Date	2022-12-13 1:52:45 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV6030 Oxalate	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

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Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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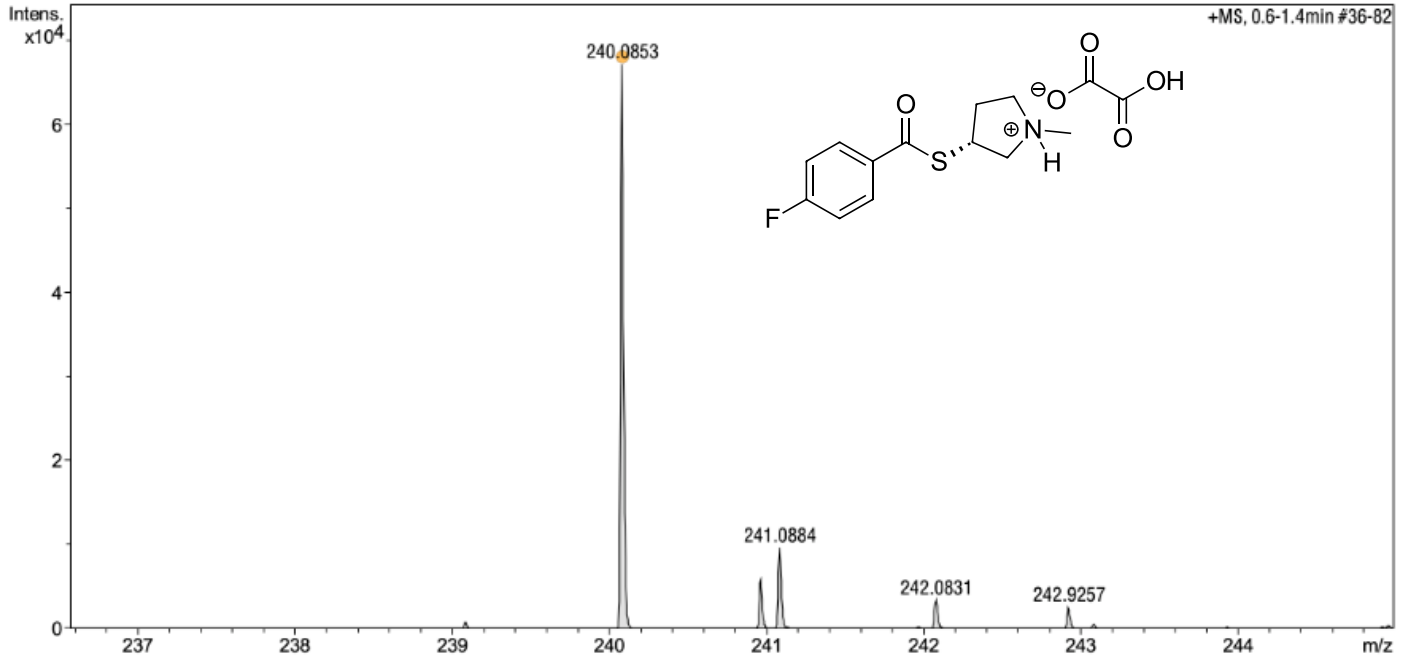
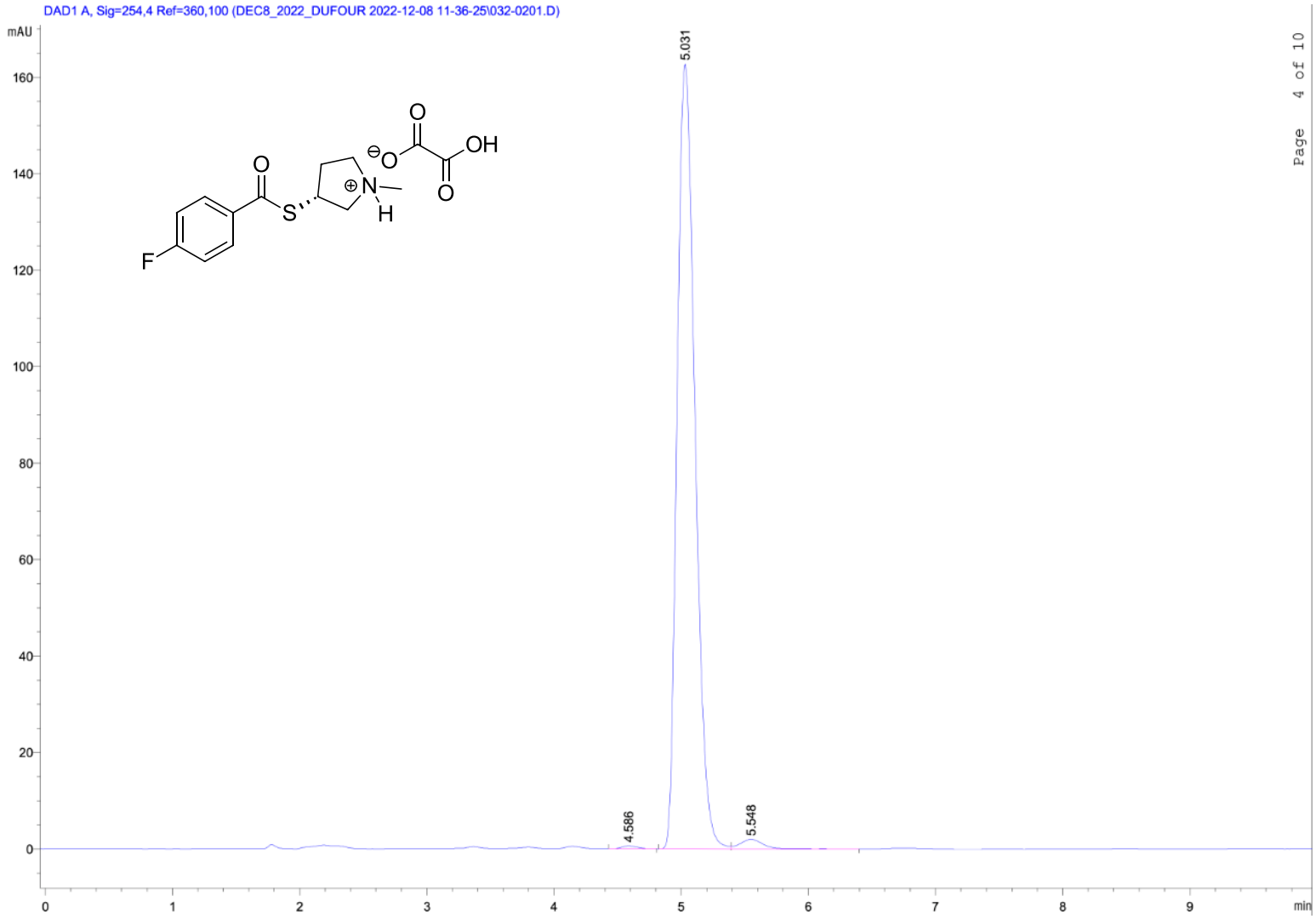
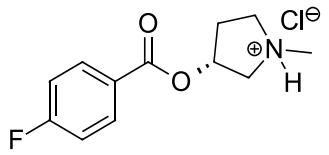


(29) ((*3R*)-1-Methylpyrrolidin-3-yl) *p*-Fluorobenzenecarbothioate Oxylate HPLC



(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride



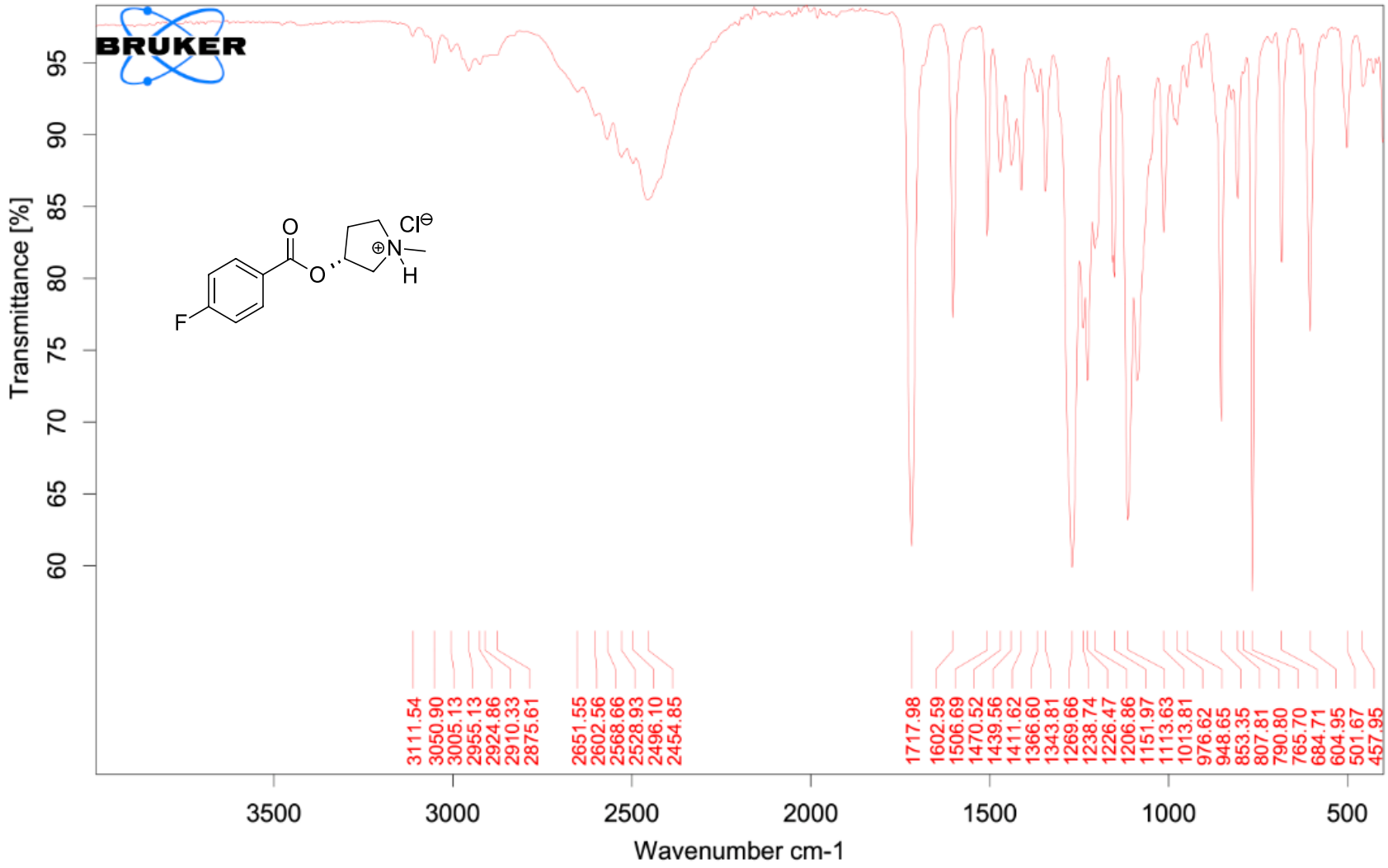
Synthesis: **(*3R*)-1-Methyl-3-pyrrolidinyl *p*-fluorobenzoate**: (*3R*)-(+)-1-Methyl-3-pyrrolidinol (0.659 mL, 6.00 mmol) was dissolved in anhydrous dichloromethane (20 mL) under an argon atmosphere and cooled to 0°C. To this solution, triethylamine (1.67 mL, 12.0 mmol) and 4-fluorobenzoyl chloride (0.720 g, 6.00 mmol). The ice bath was removed after 5 min, and the reaction was stirred for 24hrs. At this time water (20 mL) was added, layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were dried over Na_2SO_3, gravity filtered, and the filtrate was concentrated *in vacuo* to produce an orange oil. This crude material was purified by flash chromatography through silica gel (5% MeOH/DCM/1%TEA) to afford (*3R*)-1-methyl-3-pyrrolidinyl *p*-fluorobenzoate as an orange oil (1.09 g, 82%).

(*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride: (*3R*)-1-Methyl-3-pyrrolidinyl *p*-fluorobenzoate (0.248 g, 1.11 mmol) was dissolved in anhydrous diethyl ether (22 mL) under an argon atmosphere. HCl in diethyl ether (1 N - 1.70 mL, 1.67 mmol) was added dropwise and stirred for 3 hrs. The solvent was removed *in vacuo* resulting in a white solid. The compound was triturated three times with diethyl ether (20 mL), solvent was removed with a pipet and compound was dried to produce (*3R*)-3-(*p*-fluorobenzoyloxy)-1-methyl-1-pyrrolidinium chloride (0.148 g, 51%).

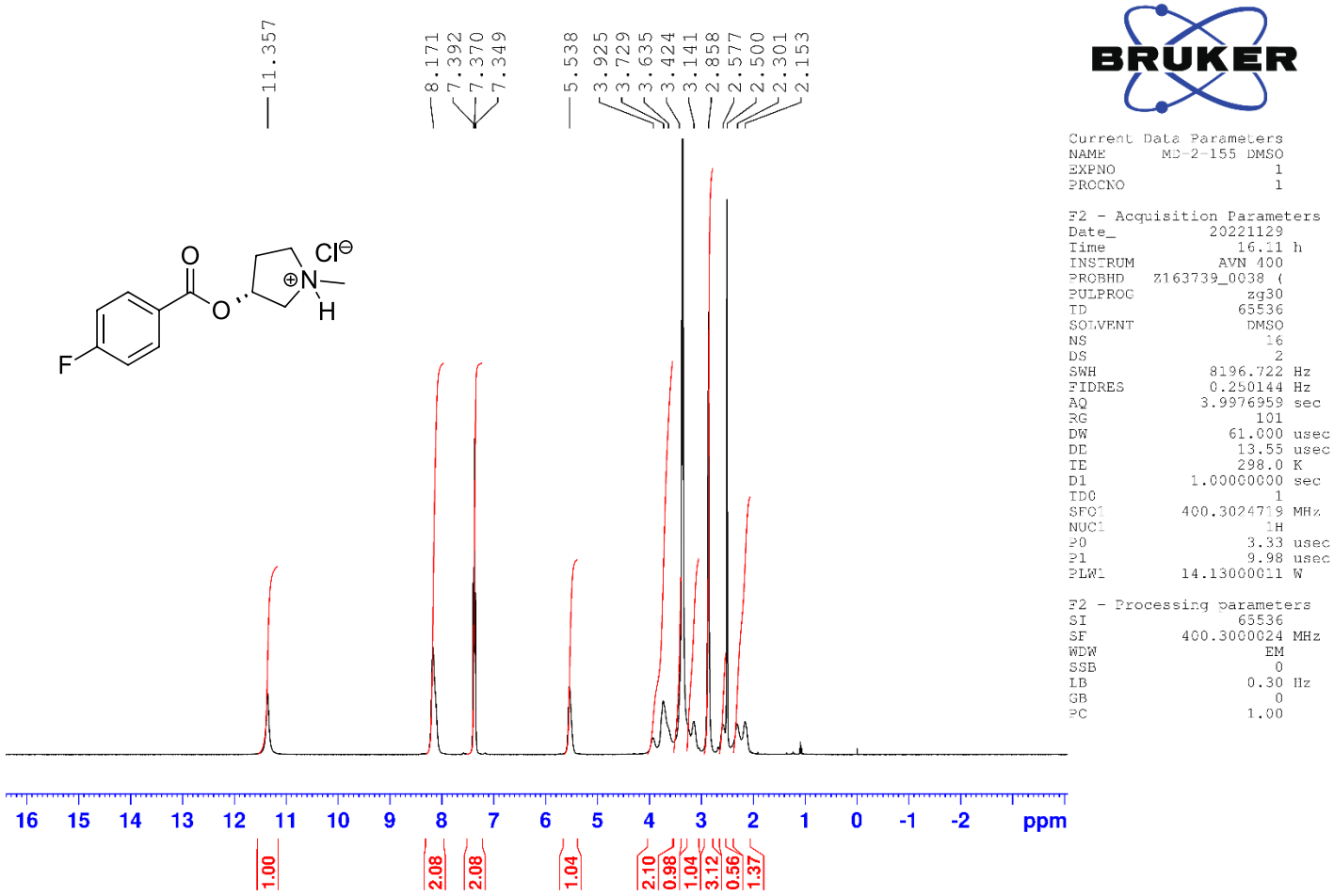
Spectroscopic Data: $MP_{(diethyl\ ether)}$: 144–149 °C; IR(ATR): 3112, 3051, 2955, 2603, 2569, 2455, 1718, 1602, 1270, 1114, 853, 685 cm^{-1}; ^1H NMR (400 MHz, DMSO-*d6*) δ 11.36 (br s, 1H),

8.16-8.15 (m, 2H), 7.39-7.35 (m, 2H), 5.54 (br s, 1H), 3.92-3.63 (m, 2H), 3.42 (br s, 1H), 3.14 (br s, 1H), 2.86 (s, 3H), 2.58-2.15 (m, 2H); ^{13}C NMR (100.7 MHz, DMSO-*d6*) δ 165.3 (d, $^{1}J_{C,F}$ = 251.8 Hz, 0), 164.2 (0), 132.6 (1), 125.8 (d, $^{4}J_{C,F}$ = 2.4 Hz, 0), 115.8 (d, $^{2}J_{C,F}$ = 22.2 Hz, 1), 73.3 (1), 59.4 (2), 53.1 (2), 40.4 (3), 30.4 (2); HRMS (ESI^{+}): calculated for $C_{12}H_{15}FNO_2^{+}$: 224.1081 amu; found for $C_{12}H_{15}FNO_2^{+}$: 224.1086 amu; HPLC purity at 230nm (75% CH_3CN : 10% CH_3OH : 15% aqueous triethylamine [0.1% triethylamine in H_2O], retention time 4.052 mins): 98.4%.

(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride IR(ATR)



(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{1}HNMR 400 MHz (DMSO-*d6*)

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AQ            3.9976959 sec
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DE               13.55 usec
TE               298.0 K
D1            1.00000000 sec
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NUC1                 1H
P0                3.33 usec
P1                9.98 usec
PLW1         14.13000011 W

F2 - Processing parameters
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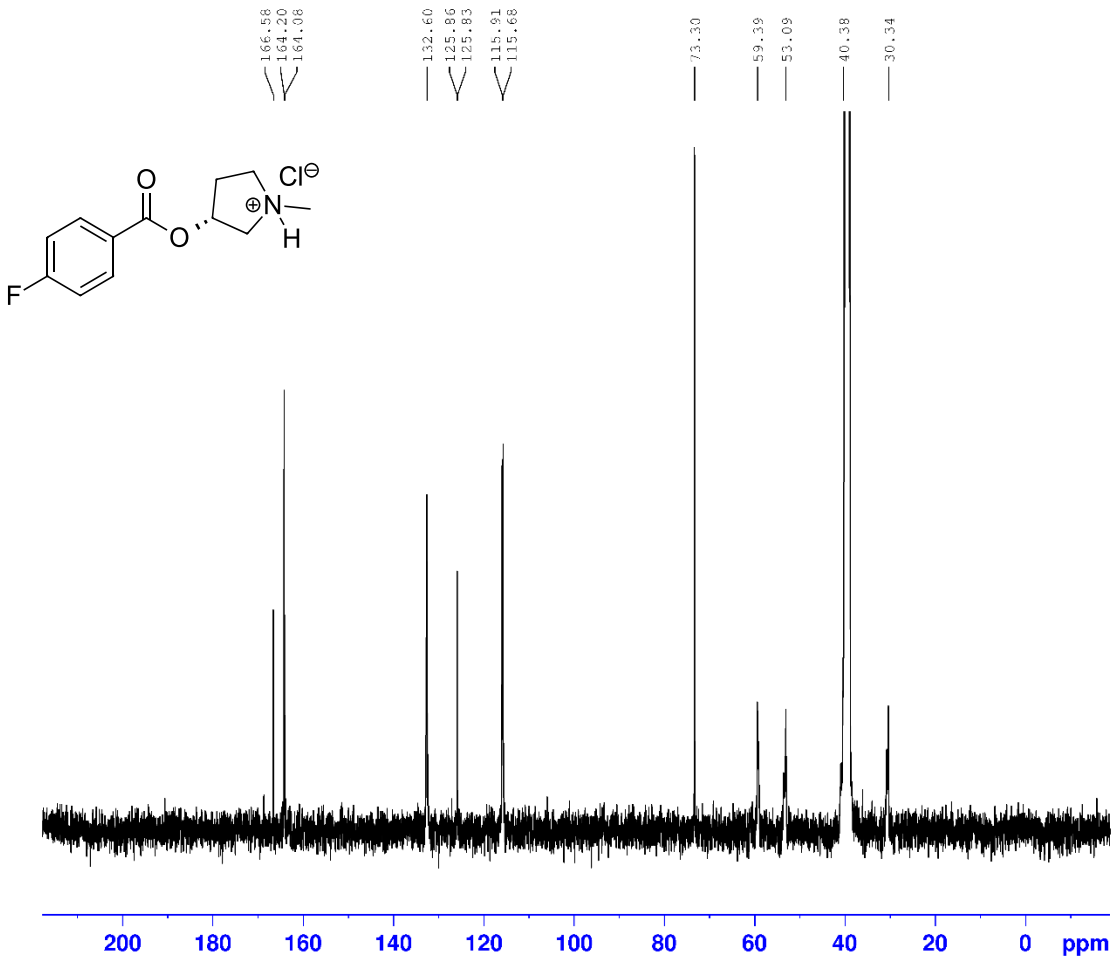
(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride ^{13}CNMR 100 MHz (DMSO-*d6*)

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DW                21.000 usec
DE                 6.50 usec
TE                298.0 K
D1           4.00000000 sec
D12          0.00002000 sec
D20         20.00000000 sec
TD0                    1
SFO1         100.6655806 MHz
NUC1                 13C
P1                 10.00 usec
P13              2000.00 usec
P26               500.00 usec
PLW1         58.86999893 W
SPNAM[5]    Crp60comp.4
SPOAL5             0.500
SPOFFS5                0 Hz
SPW5          8.99470043 W
SPNAM[8] Crp60,0.5,20.1
SPOAL8             0.500
SPOFFS8                0 Hz
SPW8          8.99470043 W
SFO2         400.3016012 MHz
NUC2                 1H
CPDPRG[2         waltz65
PCPD2              90.00 usec
PLW2         14.13000011 W
PLW12         0.17375000 W

F2 - Processing parameters
SI                131072
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WDW                  EM
SSB                   0
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GB                    0
PC                 1.40
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(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride High Resolution Mass Spectrum (ESI, positive mode)

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Xiao\Dec 13 2022000003.d	Acquisition Date	2022-12-13 2:10:47 PM
Method	Xiao all 1.m	Operator	x
Sample Name	TRV6028 HCl	Instrument	compact 8255754.20059
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
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Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

Meas. m/z	Ion Formula	m/z	err [ppm]
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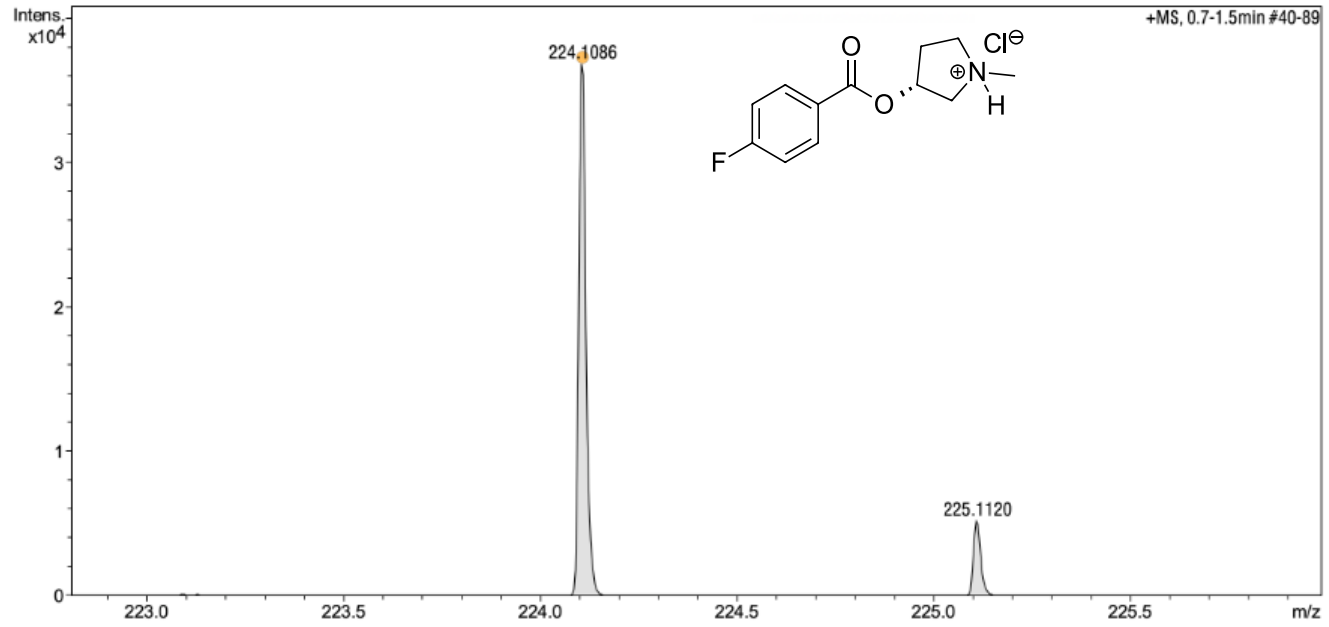
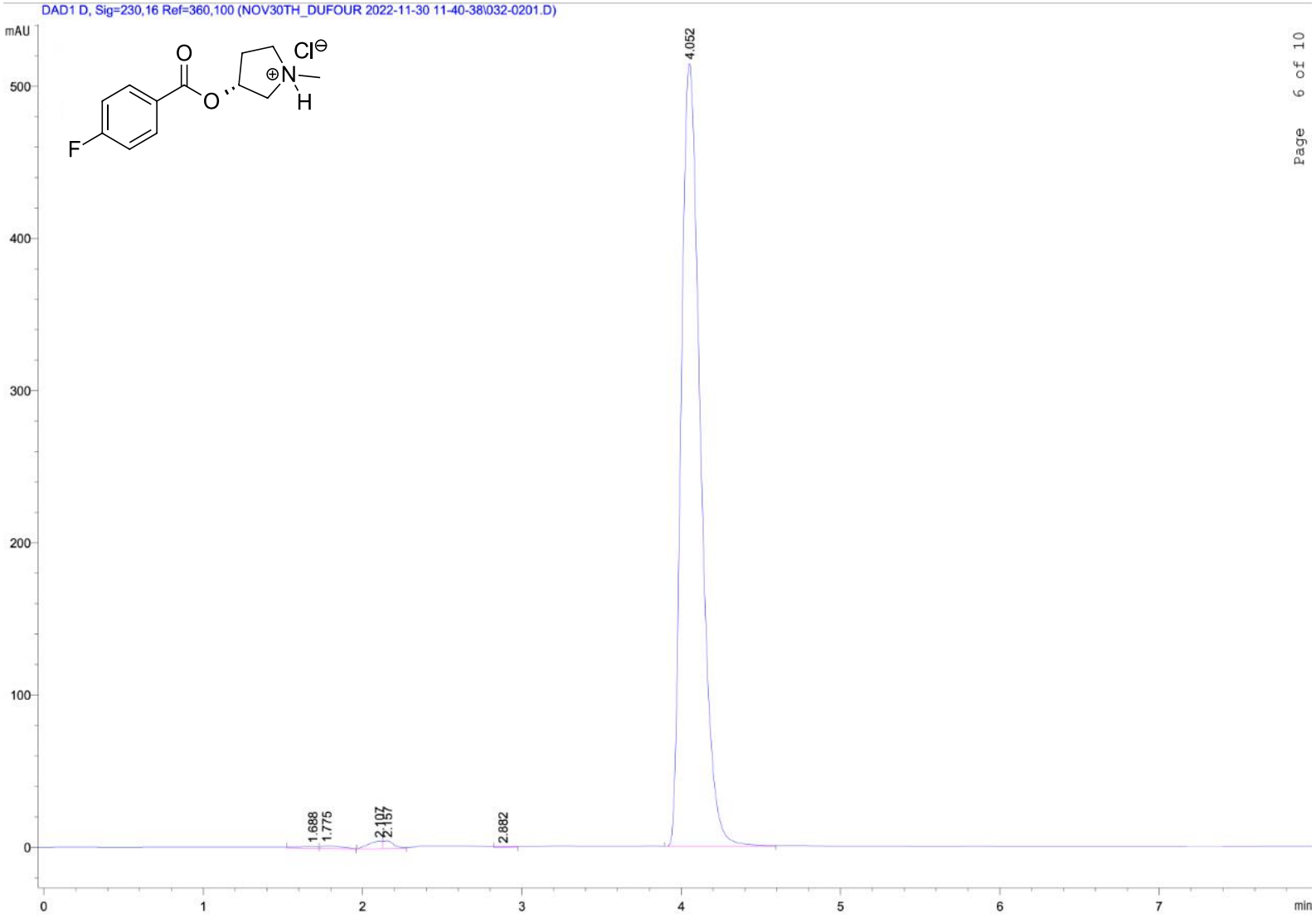


Dec 13 2022000003.d

Bruker Compass DataAnalysis 4.3 printed: 2022-12-13 2:14:03 PM by: x Page 1 of 1

(30) (*3R*)-3-(*p*-Fluorobenzoyloxy)-1-methyl-1-pyrrolidinium Chloride HPLC



References:

[1] Macdonald IR, Jollymore CT, Reid GA, Pottie IR, Martin E, Darvesh S. Thioesters for the in vitro evaluation of agents to image brain cholinesterases. J Enzyme Inhib Med Chem. 2013;28(3):447-455. DOI: 10.3109/14756366.2011.647008

[2] Bormans G, Sherman P, Snyder SE, Kilbourn MR. Synthesis of carbon-11- and fluorine-18-labeled 1-methyl-4-piperidyl-4'-fluorobenzoate and their biodistribution in mice. Nucl Med Biol. 1996;28(4):513-517. DOI 10.1016/0969-8051(96)00033-9.

[3] Hoeltje HD, Jensen B, Lambrecht G. Cyclic acetyl choline analogs part 4 4 acetoxy piperidines and 4 acetoxythia cyclo hexanes. Eur J Med Chem. 1978;13(5):453-464.

[4] Kikuchi T, Fukushi K, Ikota N, Ueda T, Nagatsuka S-I, Arano Y, Irie T. Synthesis of piperidinyl and pyrrolidinyl butyrates for potential in vivo measurement of cerebral butyrylcholinesterase activity. J Labelled Compd Radiopharm. 2001;44(1):31-41. DOI: 10.1002/jlcr.429.

[5] Cheng CY, Brochmann-Hanssen E, Waters JA. Quantitative structure-activity relationships of aromatic esters of 1-methyl-4-piperidinol as analgesic. J Med Chem. 1982;25(2):145-152. DOI: 10.1021/jm00344a011.

[6] Waters JA. Aromatic esters of nonquaternary carbon-4 piperidinols as analgesics. J Med Chem. 1978;21(7):628-633. DOI: 10.1021/jm00205a007.

[7] Macdonald IR, Reid GA, Joy EE, Pottie IR, Matte G, Burrell S, Mawko G, Martin E, Darvesh S. Synthesis and preliminary evaluation of piperidinyl and pyrrolidinyl iodobenzoates as imaging agents for butyrylcholinesterase. Mol Imaging Biol. 2011;13(6):1250-1261. DOI: 10.1007/s11307-010-0448-0.

B. Figures for histochemistry

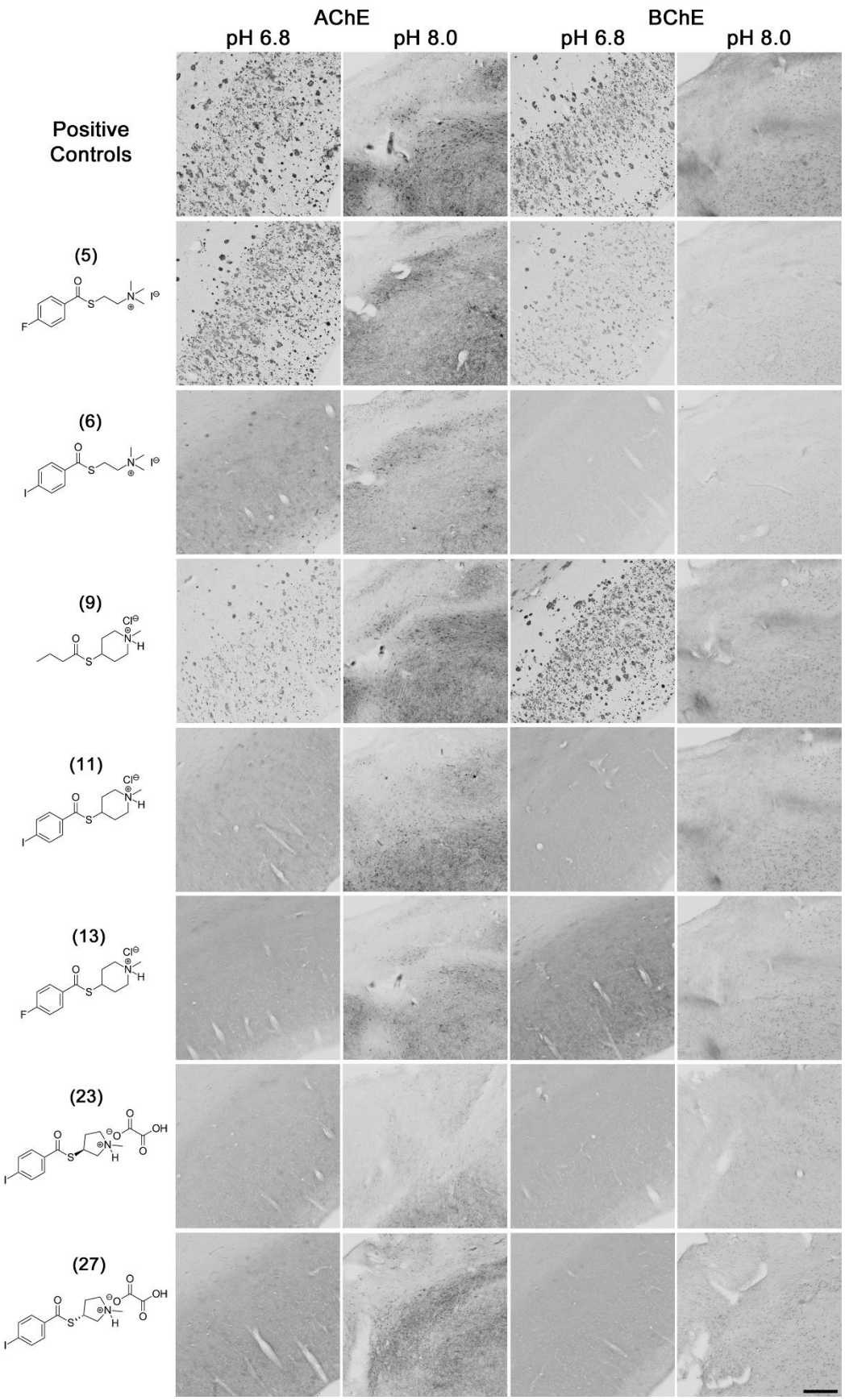

AChE
BChE
pH 6.8
pH 8.0
pH 6.8
pH 8.0
Positive Controls
(5)
(6)
(9)
(11)
(13)
(23)
(27)

Figure S1. Representative photomicrographs of histochemical staining of AChE (columns 1 and 2) and BChE (columns 3 and 4) at pH 6.8 and 8.0 in human brain tissues demonstrating that, when used as inhibitors, some compounds engaged with cholinesterases. Optimal staining for plaques at pH 6.8 is shown in the orbitofrontal cortex, optimal staining of normal neural elements at pH 8.0 is shown in the thalamus. Scale bar =400 μm.

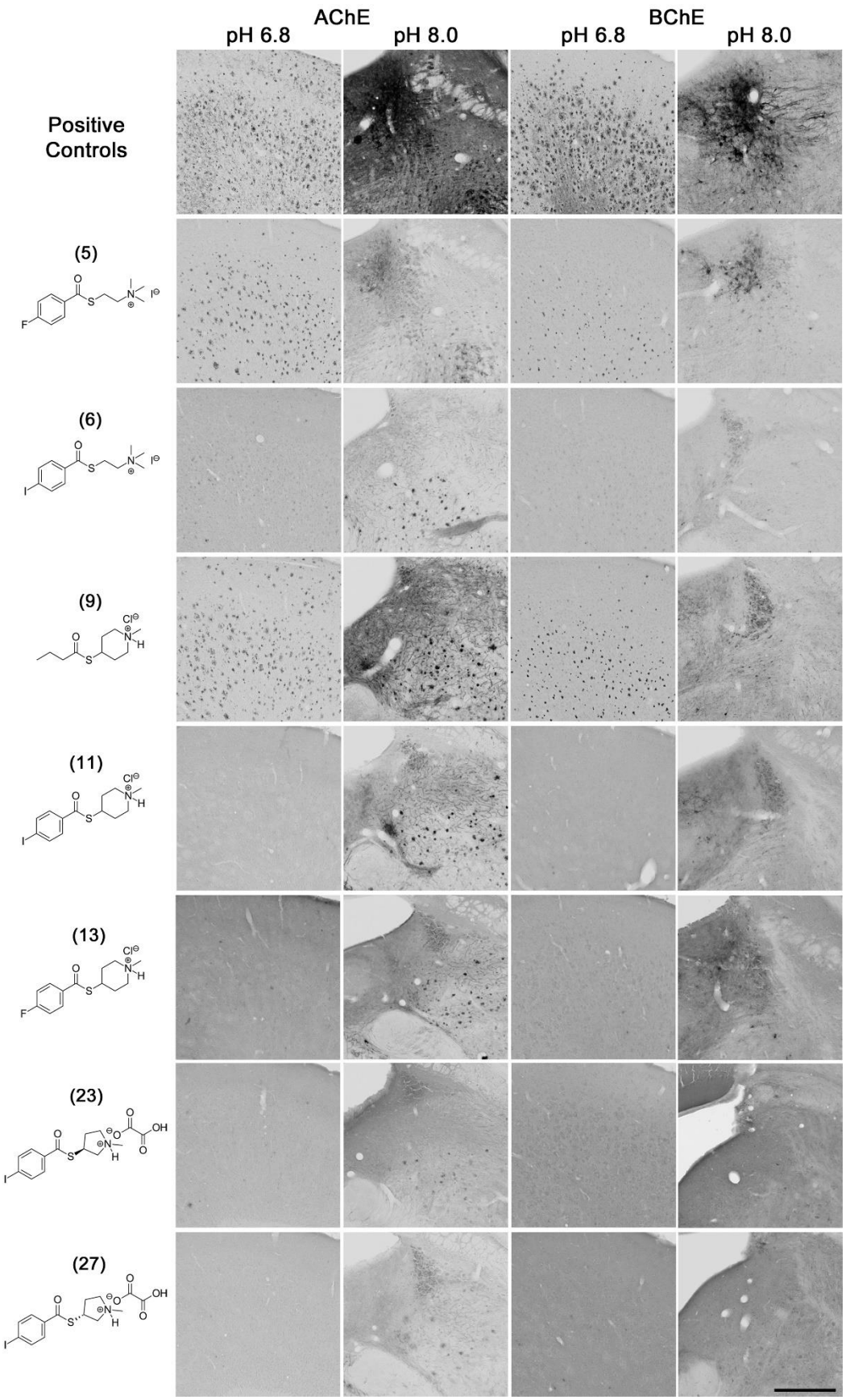

AChE
BChE
pH 6.8
pH 8.0
pH 6.8
pH 8.0
Positive Controls
(5)
(6)
(9)
(11)
(13)
(23)
(27)

Figure S2. Representative photomicrographs of histochemical staining of AChE (columns 1 and 2) and BChE (columns 3 and 4) at pH 6.8 and 8.0 in mouse brain tissues demonstrating that, when used as inhibitors, some compounds engaged with cholinesterases. Optimal staining for plaques at pH 6.8 is shown in the orbitofrontal cortex, optimal staining of normal neural elements at pH 8.0 is shown in the thalamus. Scale bar = 200 µm.