*antioxidants*

MDPI

# Supplementary Material: Enantioselective Synthesis and Pharmacological Evaluation of Aza-CGP37157–Lipoic Acid Hybrids for the Treatment of Alzheimer's Disease

**Ángel Cores [1,†], Patrycja Michalska [2,†], José Miguel Pérez [1], Enrique Crisman [3,4], Clara Gómez [4], Mercedes Villacampa [1], José Carlos Menéndez [1,*] and Rafael León [4,*]**

[1] Unidad de Química Orgánica y Farmacéutica, Departamento de Química en Ciencias Farmacéuticas, Facultad de Farmacia, Universidad Complutense, 28040 Madrid, Spain; acores@ucm.es (Á.C.); josemigp@ucm.es (J.M.P.); mvsanz@ucm.es (M.V.)
[2] Department of Chemistry, Imperial College London, SW7 2BX London, UK; p.dziama@imperial.ac.uk
[3] Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, 28006 Madrid, Spain; ecrisman@outlook.com
[4] Instituto de Química Médica, Consejo Superior de Investigaciones Científicas (IQM-CSIC), 28006 Madrid, Spain; clara.gomezserrano@estudiante.uam.es
* Correspondence: josecm@ucm.es (J.C.M.); rafael.leon@iqm.csic.es (R.L.)
† These authors contributed equally.

**Table S1**. Nrf2 induction of compounds in the AREc32 cell line expressed as CD values. AREc32 cells were treated with increasing concentrations (0.3, 3, 10 and 30 μM) of the corresponding compound for 24 h and thereafter, luciferase activity was measured. Data are expressed as the concentration required to double the specific luciferase activity (CD).

| Entry | Compound | CD (μM) |
|---|---|---|
| 1 | **CGP37157** | -[2] |
| 2 | ***S*-Lipoic acid** | >600 |
| 3 | ***R*-Lipoic acid** | >600 |
| 4 | ***rac*-3a** | 14.8 ± 1.6[3] |
| 5 | **(*rac*,*R*)-3b** | 9.8 ± 1.6[3] |
| 6 | **(±)-6** | > 30[2] |
| 7 | ***R*-6** | 11.8 ± 0.5 |
| 8 | ***S*-6** | 14.4 ± 0.2 |
| 9 | **(*S*,*S*)-7a** | 26.5 ± 1.3 |
| 10 | **(*S*,*R*)-7b** | 11.9 ± 1.3 |
| 11 | **(*R*,*S*)-7c** | > 30 |
| 12 | **(*R*,*R*)-7d** | > 30 |

Data are means ± SEM of at least five different experiments in duplicate.

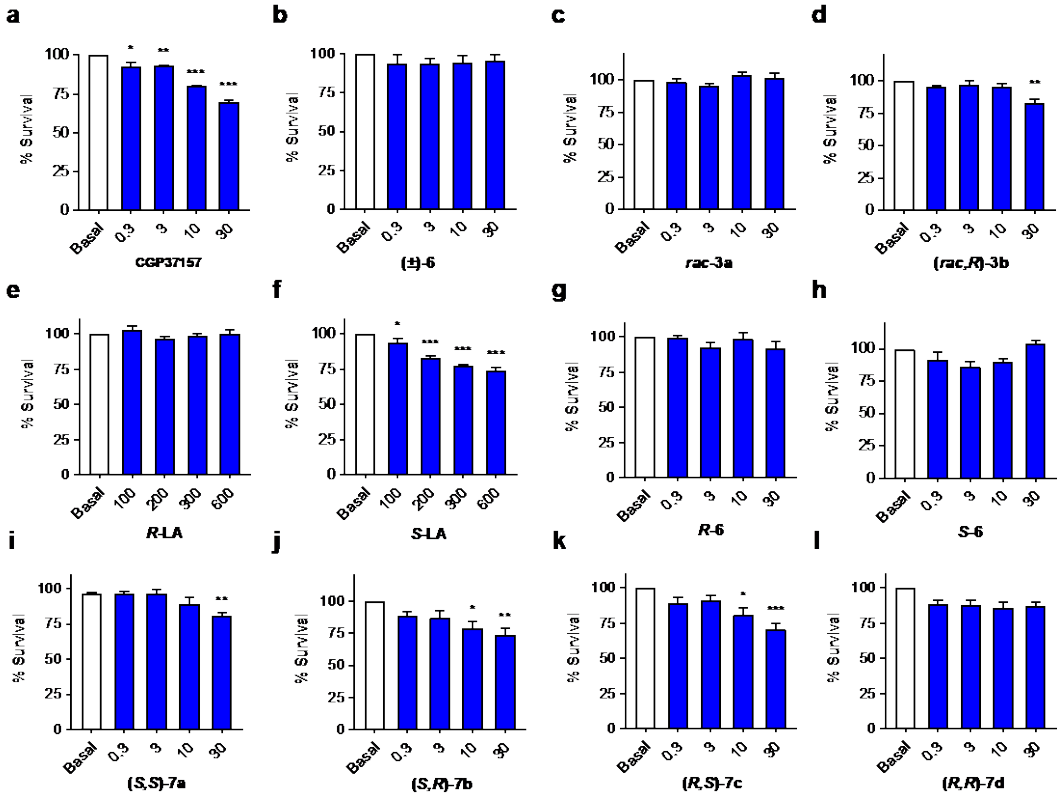



**Figure S1**. Cytotoxicity of CGP37157, **3**, **6**, LA and **7** in the AREc32 cell line. AREc32 cells were treated with each compound at desired concentration (0.3, 3 10 and 30 μM) for 24 h. Thereafter, cell viability was assessed by the MTT method. Data are means of five different cultures in duplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to basal.

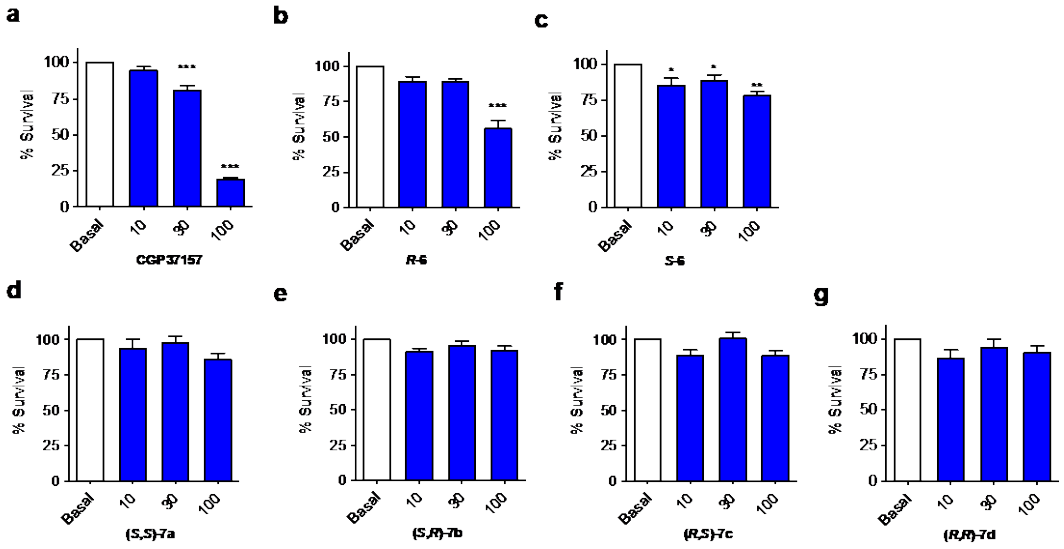
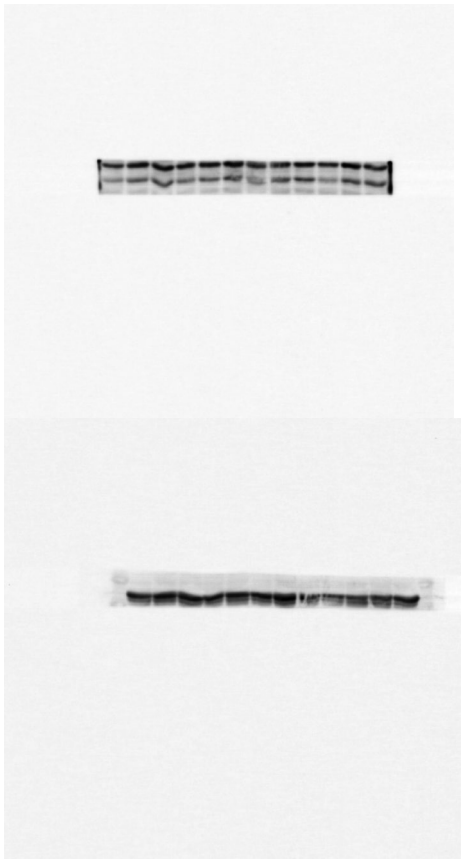



**Figure S2**. Cytotoxicity of CGP37157, **6** and **7** in the SH-SY5Y cell line. SH-SY5Y cells were treated with increasing concentrations of each compound (10, 30 and 100 μM) for 24 h. Thereafter, cell viability was assessed by the MTT method. Data are means of three different cultures in duplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to basal.

**Figure S3: WB original images**

**HO-1**
**β-actin**



**GCLc**
**β-actin**

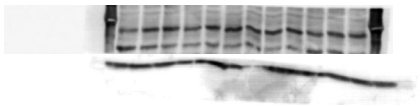


**Table S2**. Neuroprotective effect of compounds in SH-SY5Y cells against the toxic combination R/O. Compounds were pre-incubated (1 μM) for 24 h, then media was replaced for fresh medium containing compounds and R/O (30/10 μM) for another 24 h. Viability was assessed by the MTT method.

| Entry | Compound | % Survival | % Protection |
|---|---|---|---|
| 1 | **Basal** | 100.0 | |
| 2 | **R/O** | 53.4 ± 6.3$^{###}$ | |
| 3 | **Mel** | 68.0 ± 1.5 | 46.1** |

| | | | |
|---|---|---|---|
| 7 | **CGP37157** | 76.2 ± 5.7 | 46.2*** |
| 4 | ***rac*-3a** | 69.0 ± 5.6 | 52.1** |
| 5 | **(*rac*,*R*)-3b** | 72.9 ± 3.5 | 59.3** |
| 6 | **(±)-6** | 74.4 ± 4.1 | 44.1*** |
| 8 | ***R*-6** | 75.1 ± 2.9 | 45.7*** |
| 9 | ***S*-6** | 74.7 ± 2.5 | 51.5*** |
| 10 | **(*S*,*S*)-7a** | 74.8 ± 3.2 | 43.4*** |
| 11 | **(*S*,*R*)-7b** | 81.0 ± 4.7 | 61.1*** |
| 12 | **(*R*,*S*)-7c** | 76.1 ± 2.9 | 47.2*** |
| 13 | **(*R*,*R*)-7d** | 77.6 ± 4.4 | 50.7*** |

Data are means of five different cultures in duplicate. ###$p < 0.001$ compared to basal; **$p < 0.01$, ***$p < 0.001$ compared to R/O.

**Table S3**. Neuroprotective effect of compounds in SH-SY5Y cells against OA. Compounds were pre-incubated (1 μM) for 24 h, then media was replaced for fresh medium containing compounds and OA (30/10 μM) for another 24 h. Viability was assessed by the MTT method.

| Entry | Compound | % Survival | % Protection |
|---|---|---|---|
| 1 | **Basal** | 100.0 | |
| 2 | **OA** | 54.7 ± 4.7### | |
| 3 | **Mel** | 70.3 ± 4.3 | 31.1* |
| 5 | **CGP37157** | 78.8 ± 6.1 | 56.3** |
| 4 | **(±)-6** | 69.1 ± 6.4 | 33.4* |
| 6 | ***R*-6** | 59.3 ± 10.6 | 14.2 |
| 7 | ***S*-6** | 71.5 ± 10.0 | 41.9* |
| 9 | **(*S*,*S*)-7a** | 63.2 ± 7.6 | 21.2 |
| 8 | **(*S*,*R*)-7b** | 70.6 ± 10.3 | 40.2* |
| 11 | **(*R*,*S*)-7c** | 50.7 ± 15.6 | n.p$ |
| 10 | **(*R*,*R*)-7d** | 63.3 ± 8.7 | 21.0 |

Data are means of five different cultures in duplicate. ###$p < 0.001$ compared to basal; **$p < 0.01$, ***$p < 0.001$ compared to R/O. n.p$ compound does not protect cells.

**Table S4**. Calculated and experimental elemental analysis.

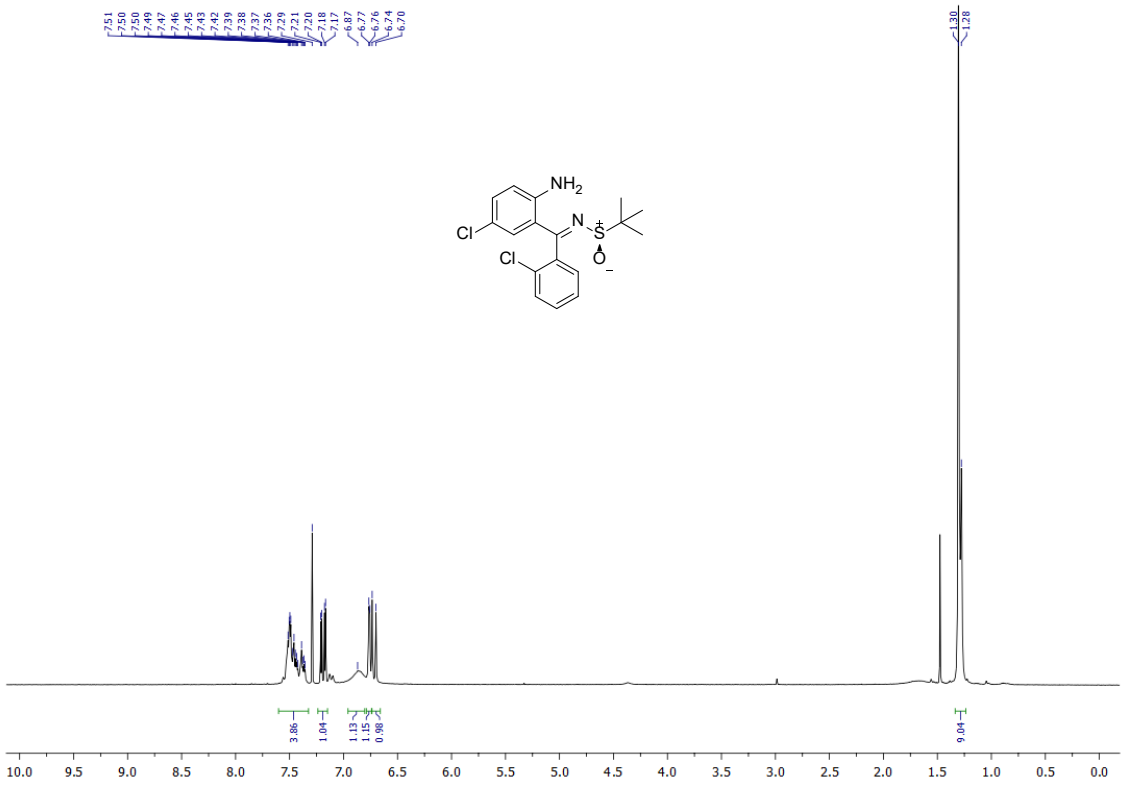
| Compound | Formula | Calculated | | | | Found | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | %C | %H | %N | %S | %C | %H | %N | %S |
| **1** | $C_{17}H_{18}Cl_2N_2OS$ | 55.29 | 4.91 | 7.59 | 8.68 | 55.06 | 4.97 | 7.71 | 8.82 |
| **2** | $C_{17}H_{20}Cl_2N_2OS$ | 54.99 | 5.43 | 7.54 | 8.63 | 55.57 | 5.67 | 7.30 | 8.77 |
| **3** | $C_{13}H_{12}Cl_2N_2$ | 58.45 | 4.53 | 10.49 | -- | 58.28 | 4.39 | 10.79 | -- |
| **4** | $C_{15}H_{16}Cl_2N_2O$ | 57.89 | 5.18 | 9.00 | -- | 55.10 | 4.91 | 8.35 | -- |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **5** | $C_{19}H_{17}BrCl_2N_2O_3$ | 48.33 | 3.63 | 5.93 | -- | 48.18 | 3.52 | 5.82 | -- |
| **6** | $C_{17}H_{16}Cl_2N_2O_2$ | 58.14 | 4.59 | 7.98 | -- | 58.46 | 4.72 | 7.74 | -- |
| **7** | $C_{25}H_{28}Cl_2N_2O_3S_2$ | 55.65 | 5.23 | 5.19 | 11.88 | 55.47 | 5.08 | 5.16 | 11.75 |

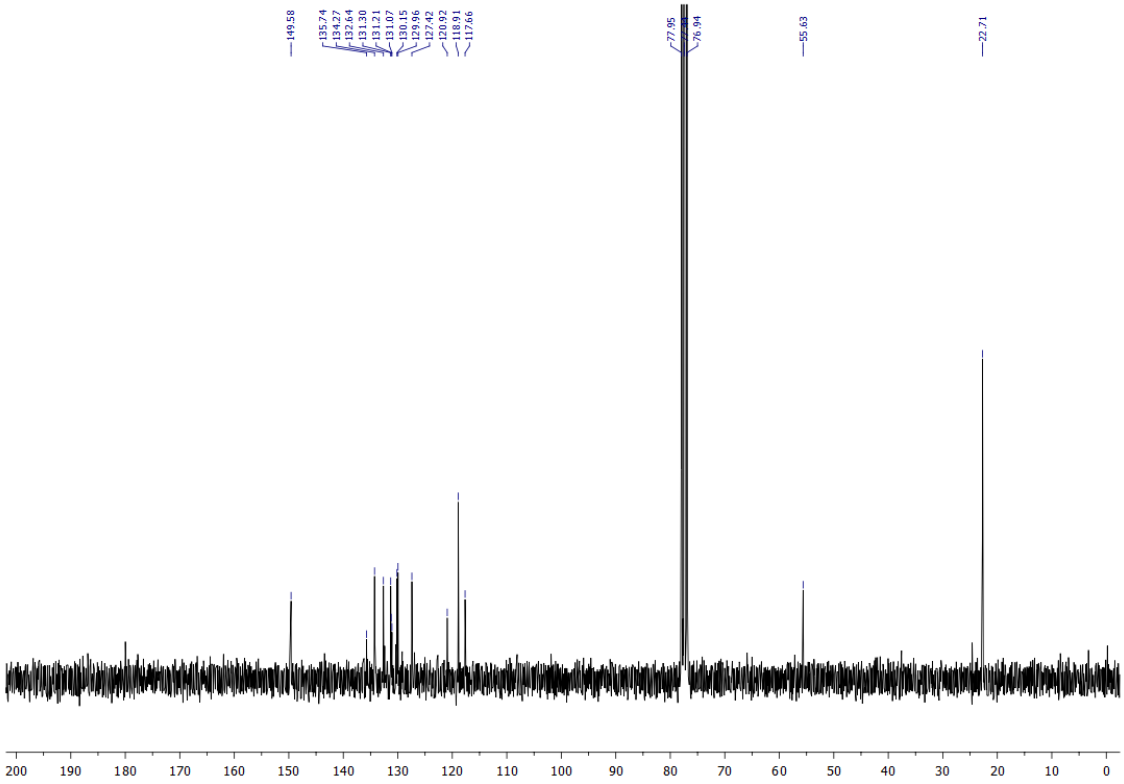
## Copies of NMR spectra

(*R*,*Z*)-*N*-((2-Amino-5-chlorophenyl)(2-chlorophenyl)methylene)-2-methylpropane-2-sulfinamide (***R*-1**)

**$^1$H NMR (250 MHz, $CDCl_3$)**

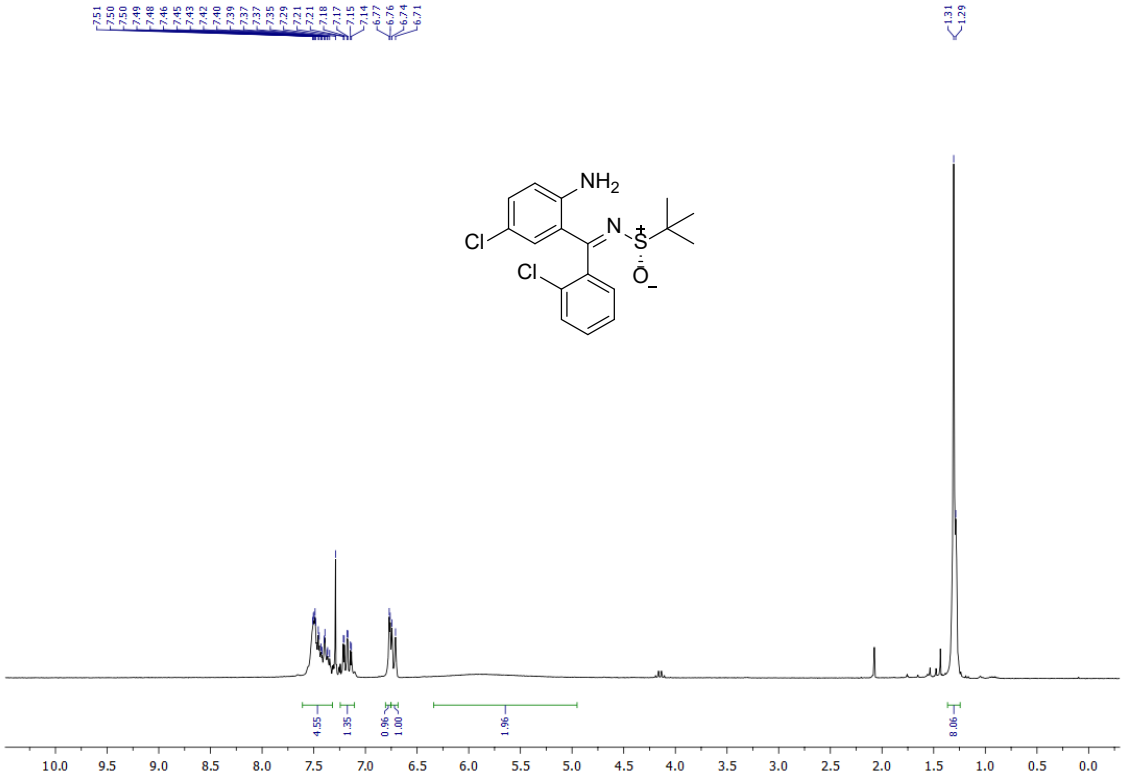



**$^{13}$C NMR (63 MHz, $CDCl_3$)**


149.58
135.74
134.27
132.64
131.30
131.21
131.07
130.15
129.96
127.42
120.92
118.91
117.66
77.95
77.68
76.94
55.63
22.71
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

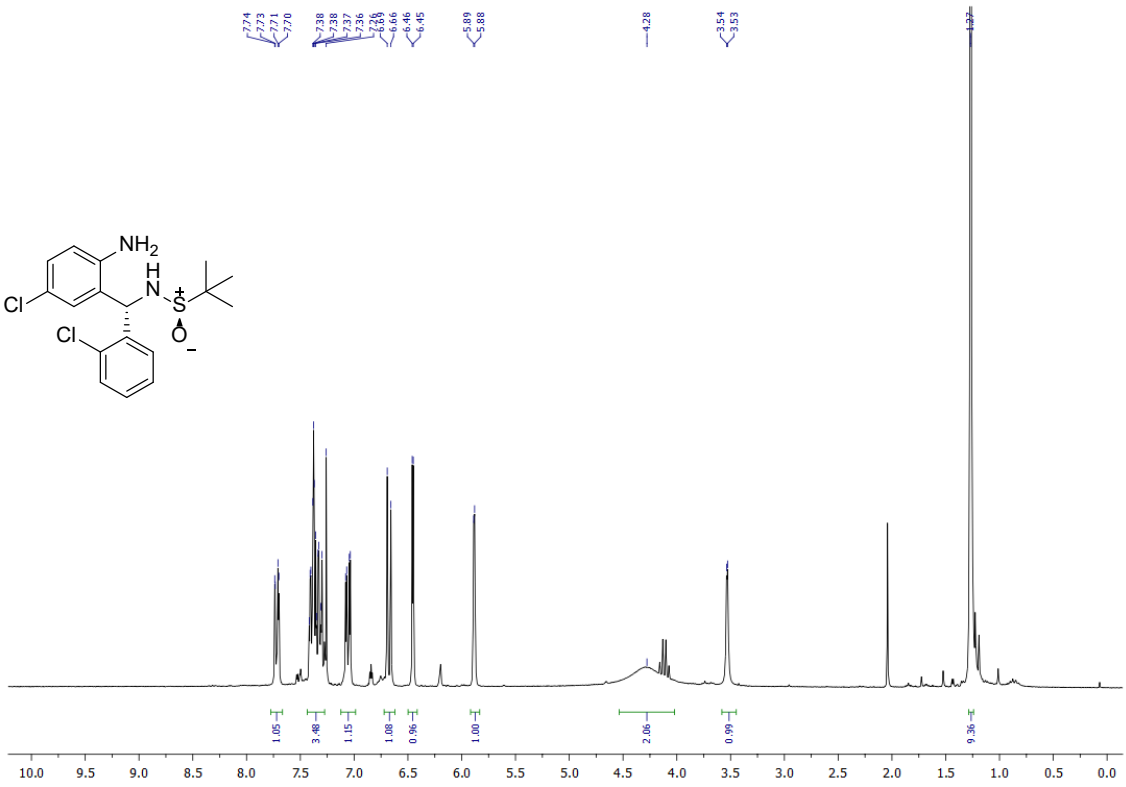
(*S*,*Z*)-*N*-((2-Amino-5-chlorophenyl)(2-chlorophenyl)methylene)-2-methylpropane-2-sulfinamide (***S*-1**)

**$^1$H NMR (250 MHz, $CDCl_3$)**

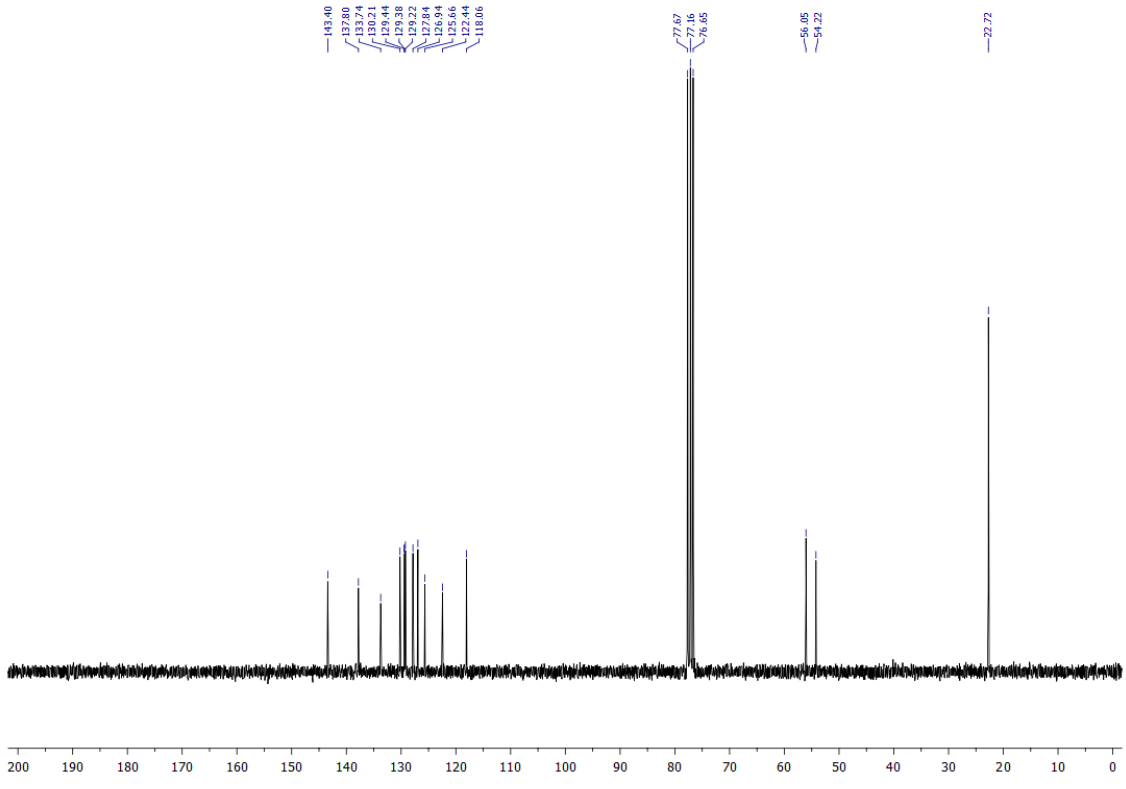


(*R*)-*N*-[(*S*)-(2-Amino-5-chlorophenyl)(2-chlorophenyl)methyl]-2-methylpropane-2-sulfinamide (***R,S*-2**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

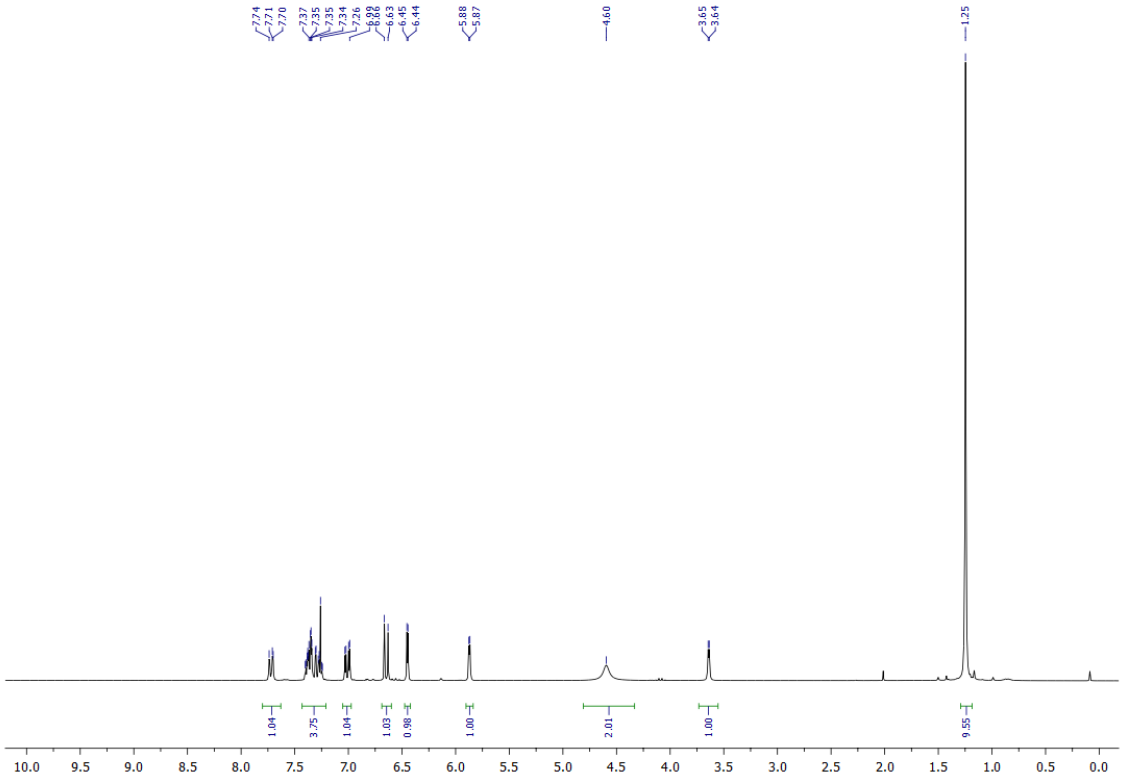



**$^{13}$C NMR (63 MHz, $CDCl_3$)**



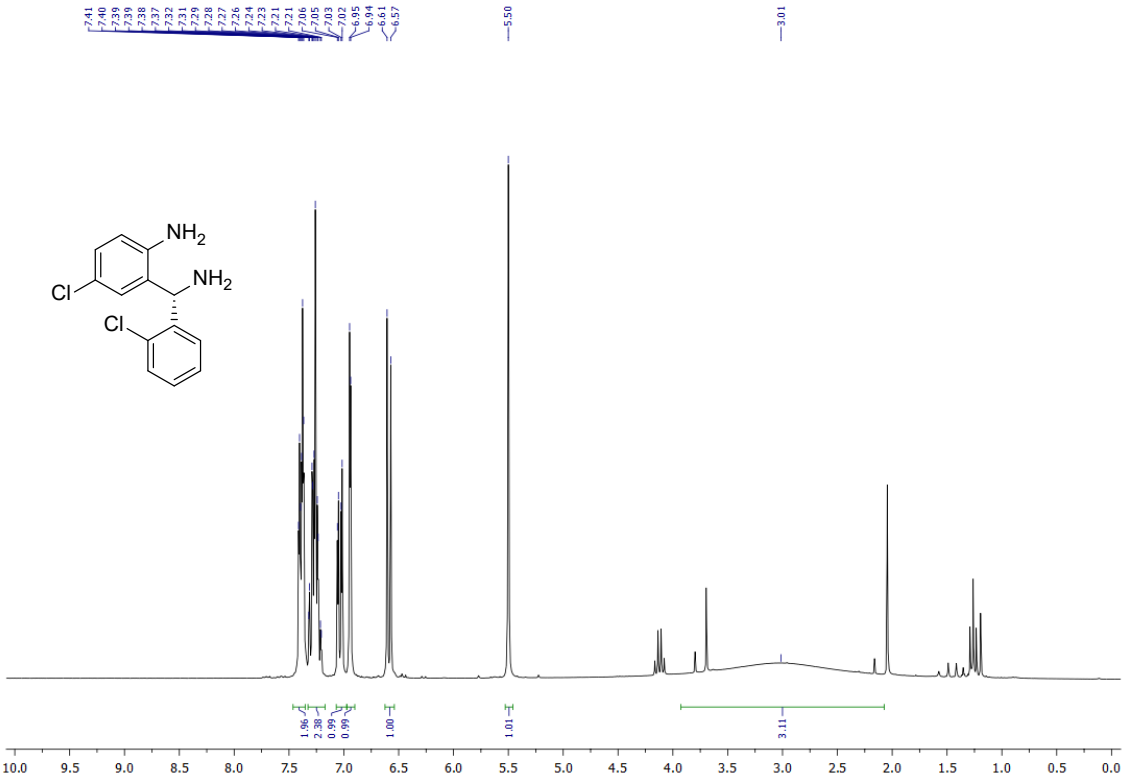
(*S*)-*N*-[(*R*)-(2-Amino-5-chlorophenyl)(2-chlorophenyl)methyl]-2-methylpropane-2-sulfinamide(***S,R*-2**)

## $^{1}$H NMR (250 MHz, $CDCl_3$)

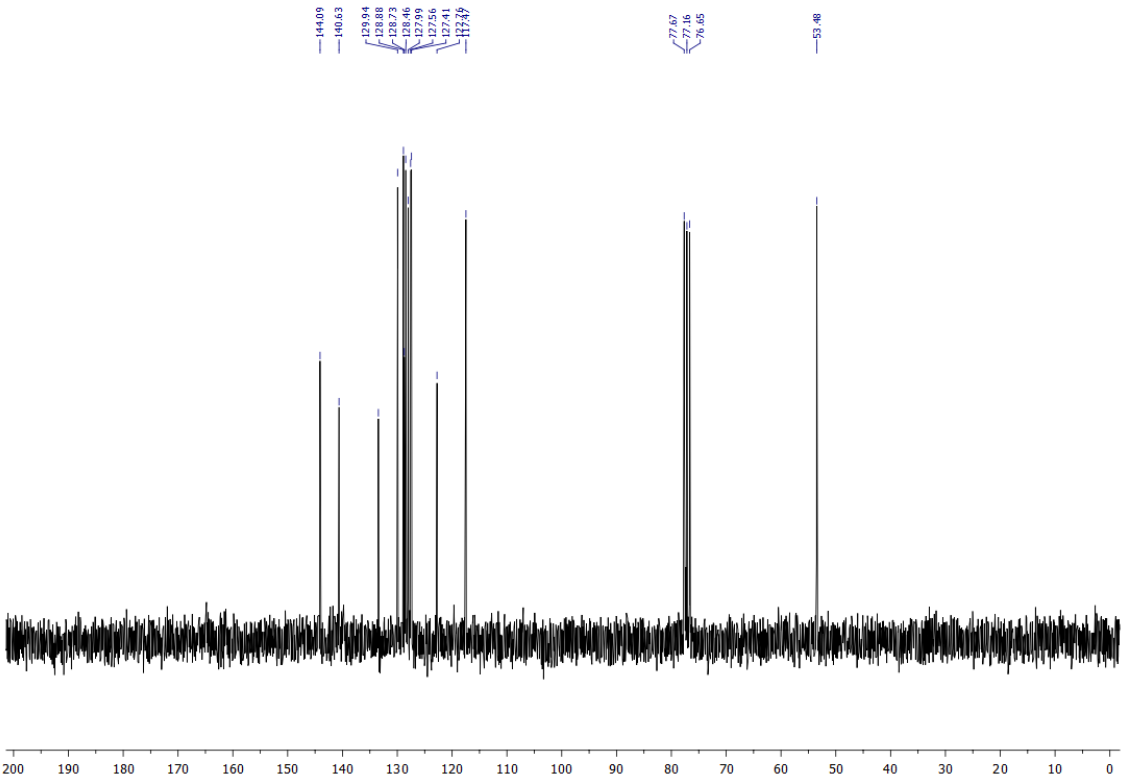


(*S*)-2-[Amino(2-chlorophenyl)methyl]-4-chloroaniline (***S*-3**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

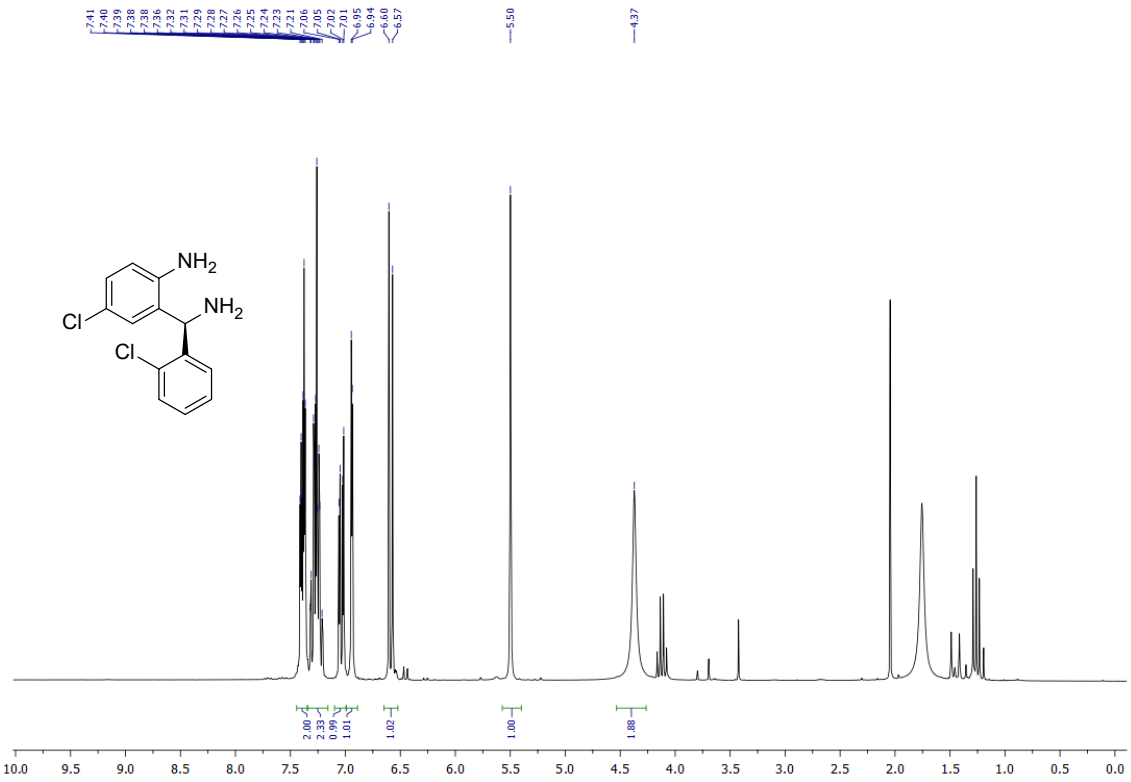



**$^{13}$C NMR (63 MHz, $CDCl_3$)**



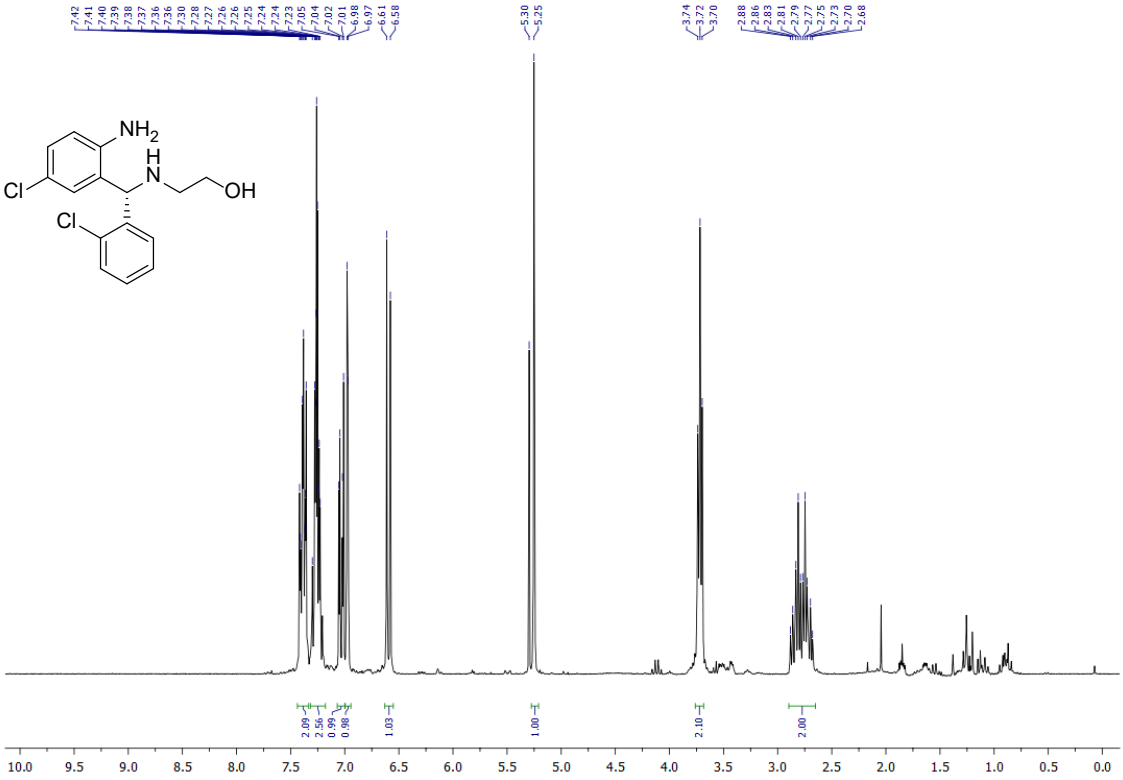

(*R*)-2-(Amino(2-chlorophenyl)methyl)-4-chloroaniline (***R*-3**)

## $^{1}$H NMR (250 MHz, $CDCl_3$)

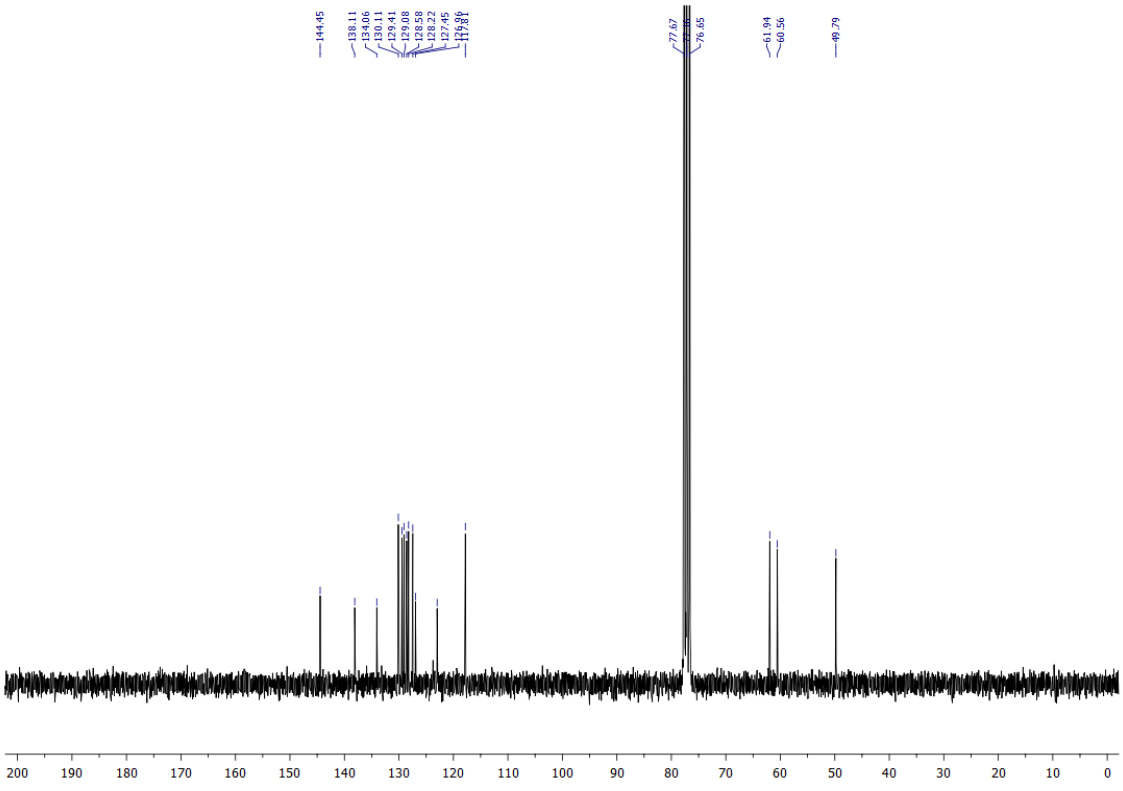


(*S*)-2-(((2-Amino-5-chlorophenyl)(2-chlorophenyl)methyl)amino)ethan-1-ol (***S*-4**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

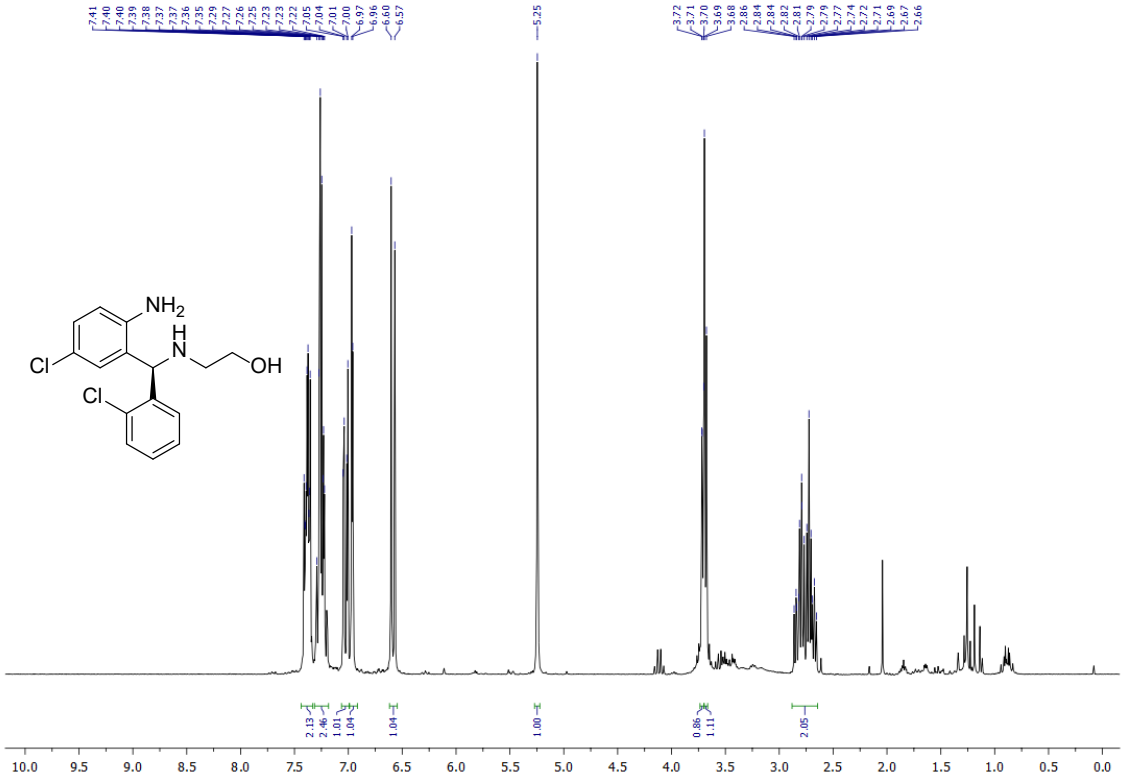



**$^{13}$C NMR (63 MHz, $CDCl_3$)**



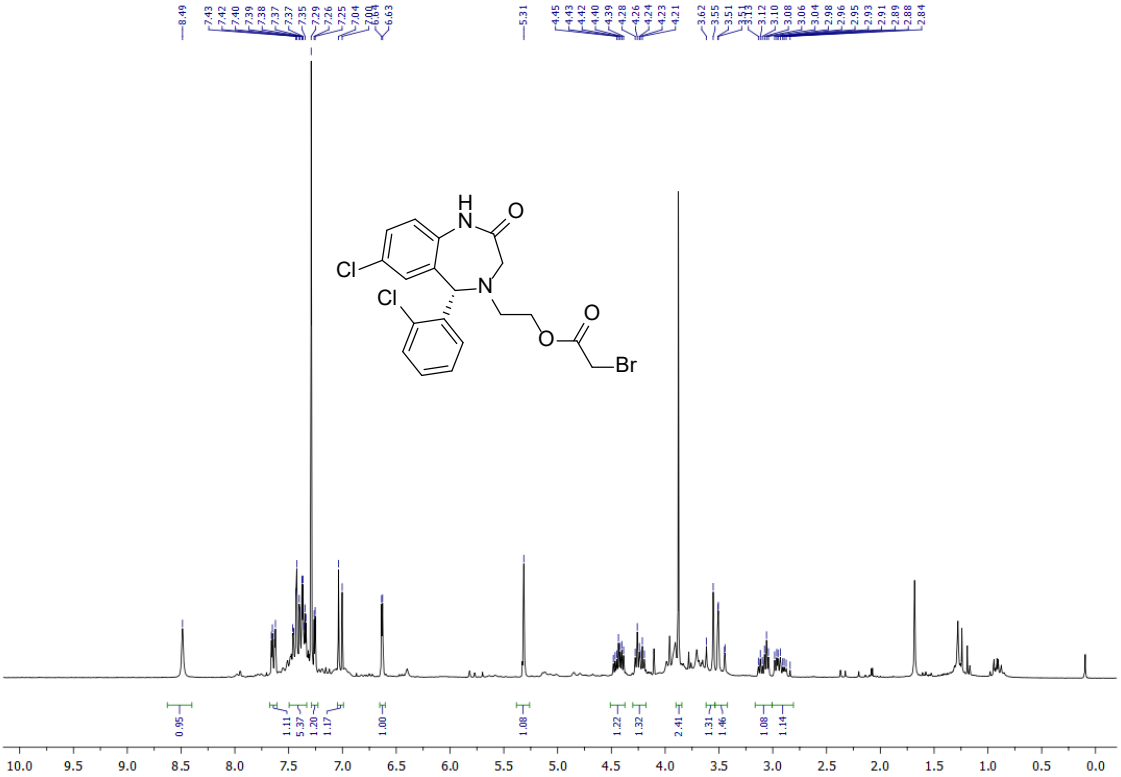
(*R*)-2-(((2-amino-5-chlorophenyl)(2-chlorophenyl)methyl)amino)ethan-1-ol (***R*-4**)

**$^1$H NMR (250 MHz, $CDCl_3$)**

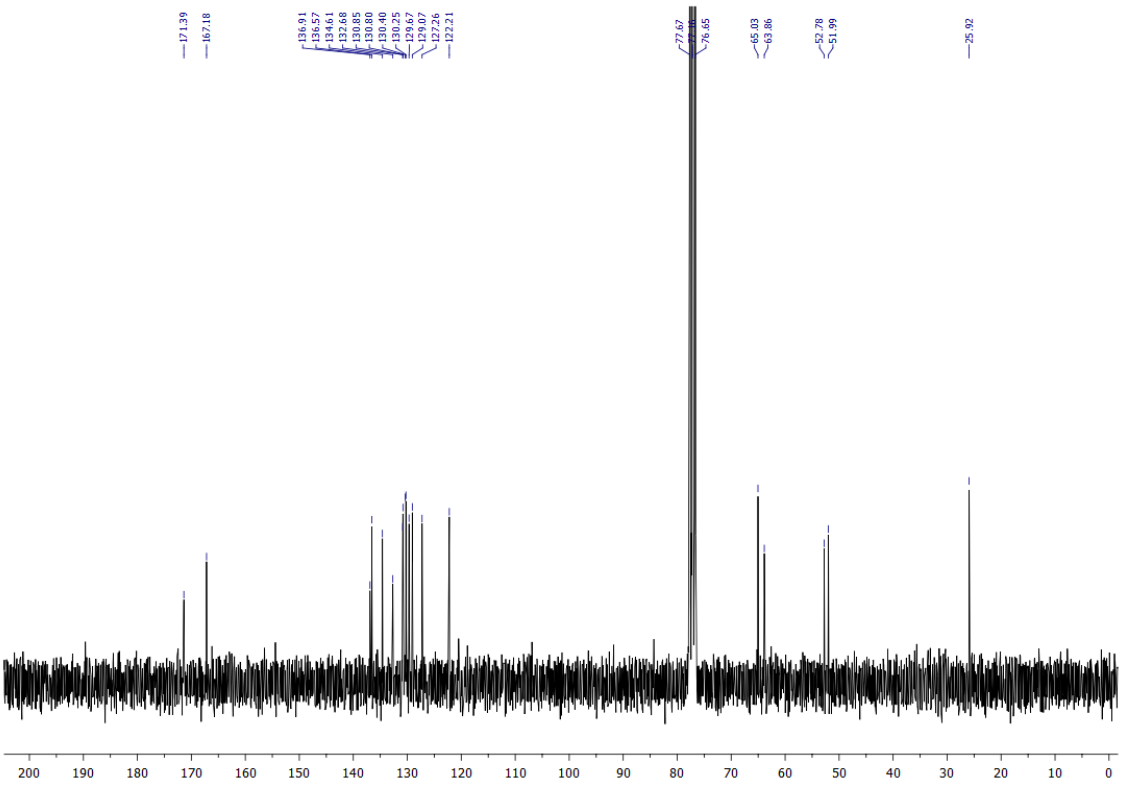


(*S*)-2-(7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 2-bromoacetate (***S*-5**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

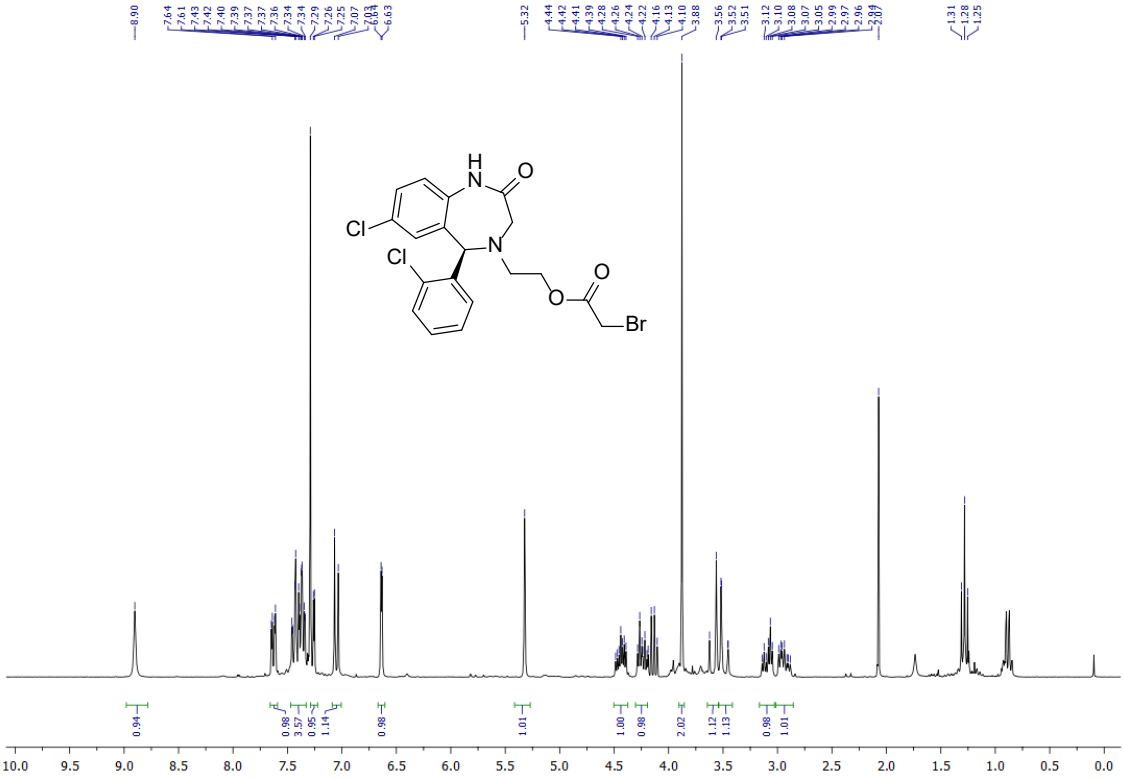



**$^{13}$C NMR (63 MHz, $CDCl_3$)**



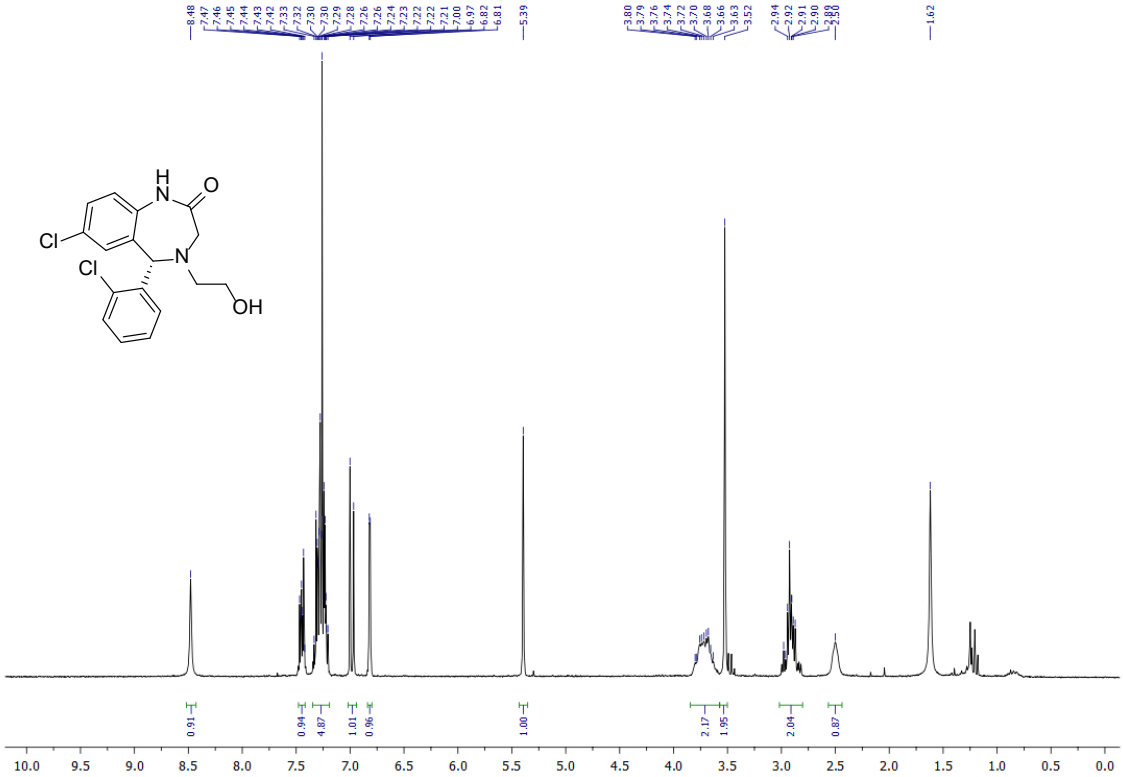
(*R*)-2-(7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 2-bromoacetate (***R*-5**)

**$^1$H NMR (250 MHz, $CDCl_3$)**

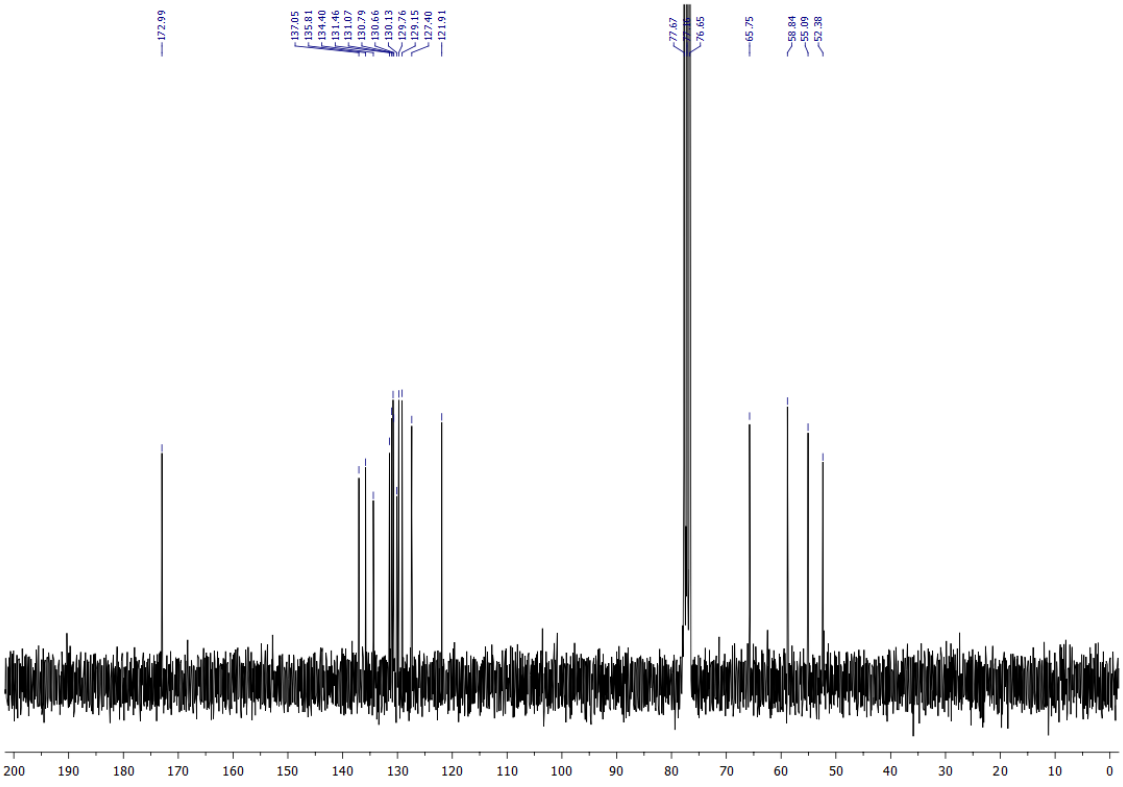


(5*S*)-7-Chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (***S*-6**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

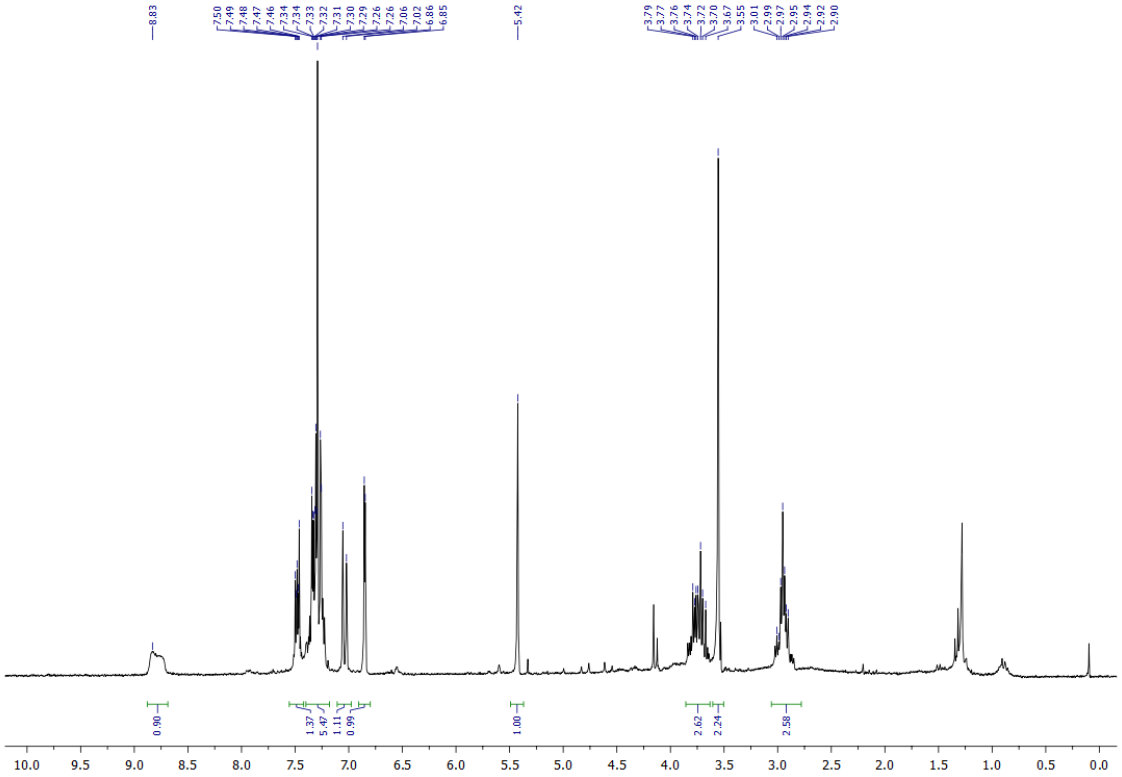



**$^{13}$C NMR (63 MHz, $CDCl_3$)**



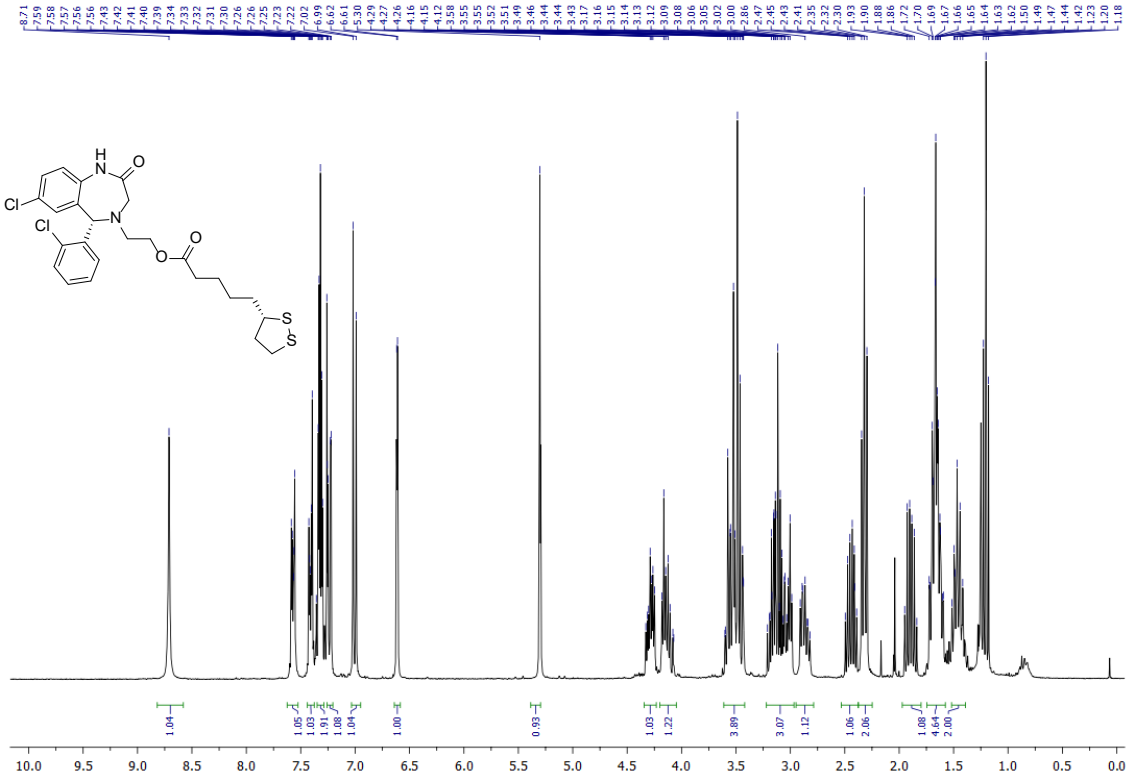
(5*R*)-7-Chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (***R*-6**)

**$^1$H NMR (250 MHz, $CDCl_3$)**

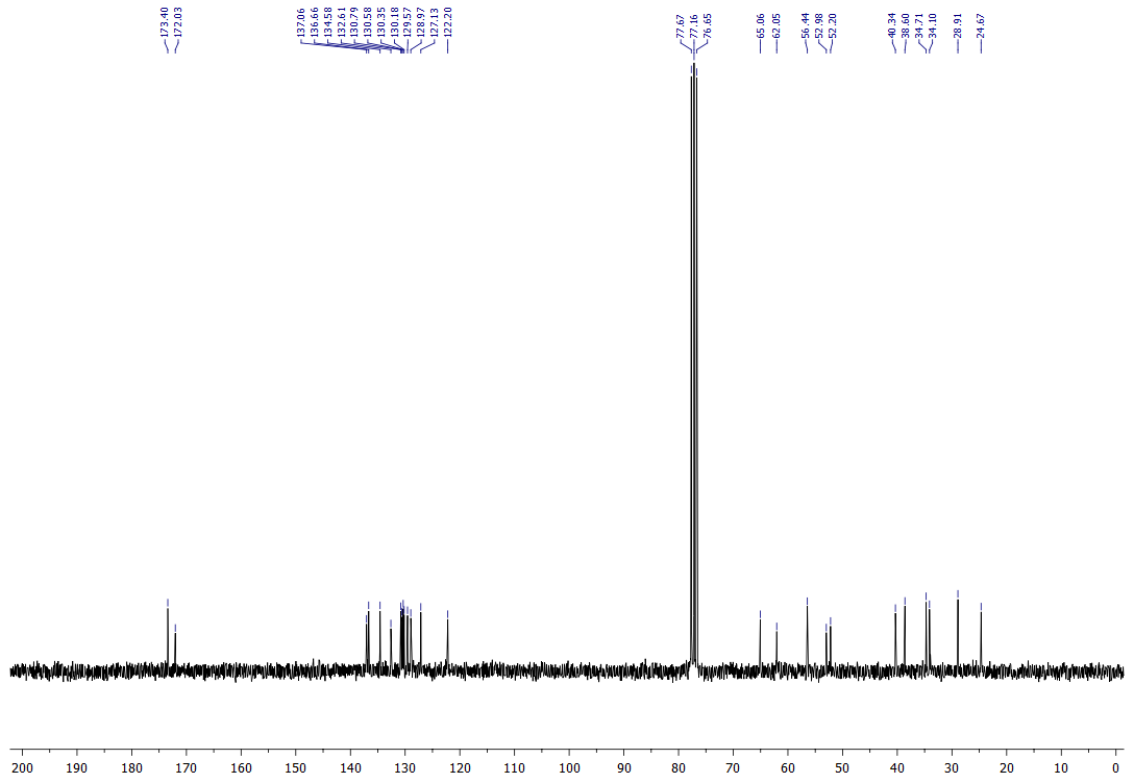


2-((*S*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*S*)-1,2-dithiolan-3-yl)pentanoate (**7a**)

**$^{1}$H NMR (250 MHz, $CDCl_3$)**

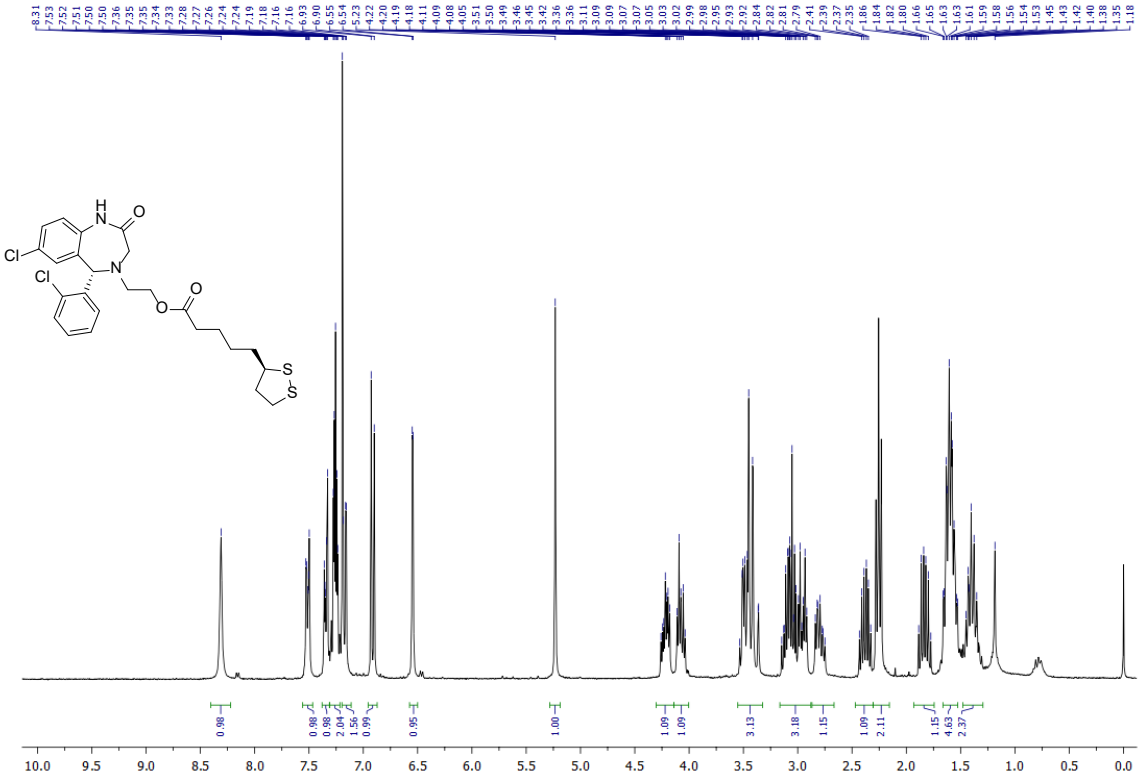


**$^{13}$C NMR (63 MHz, $CDCl_3$)**



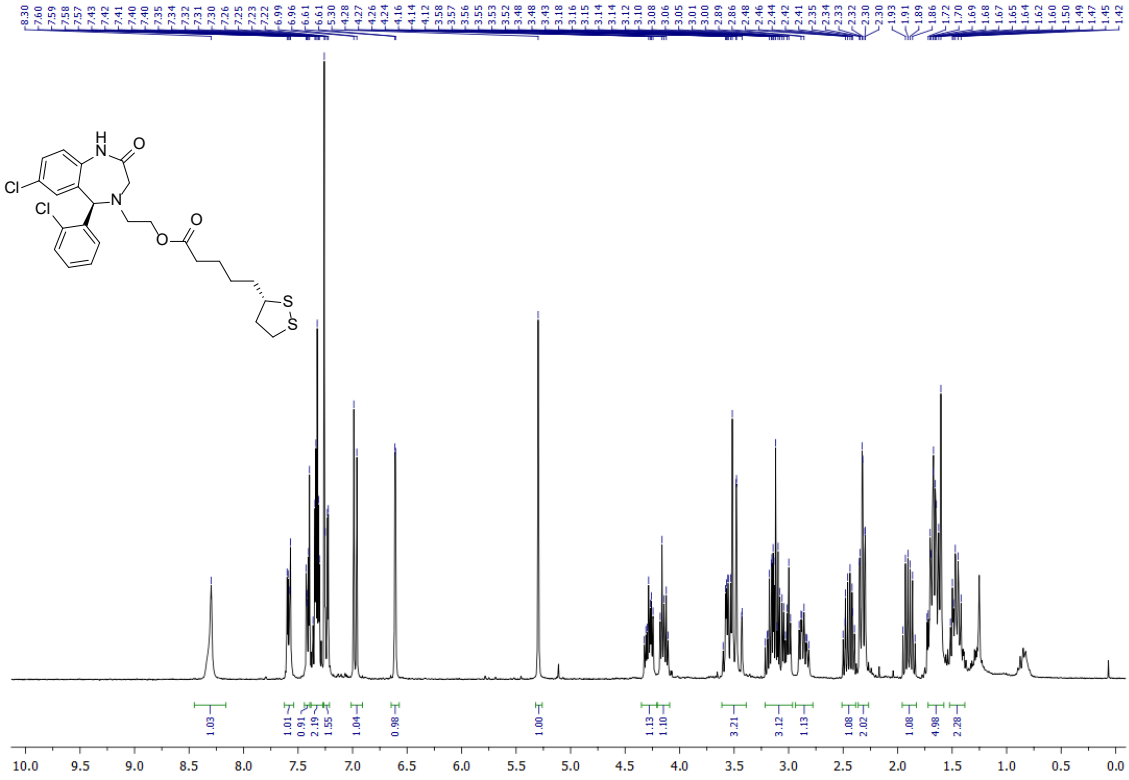
2-((*R*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*S*)-1,2-dithiolan-3-yl)pentanoate (**7b**)

**$^1$H NMR (250 MHz, $CDCl_3$)**



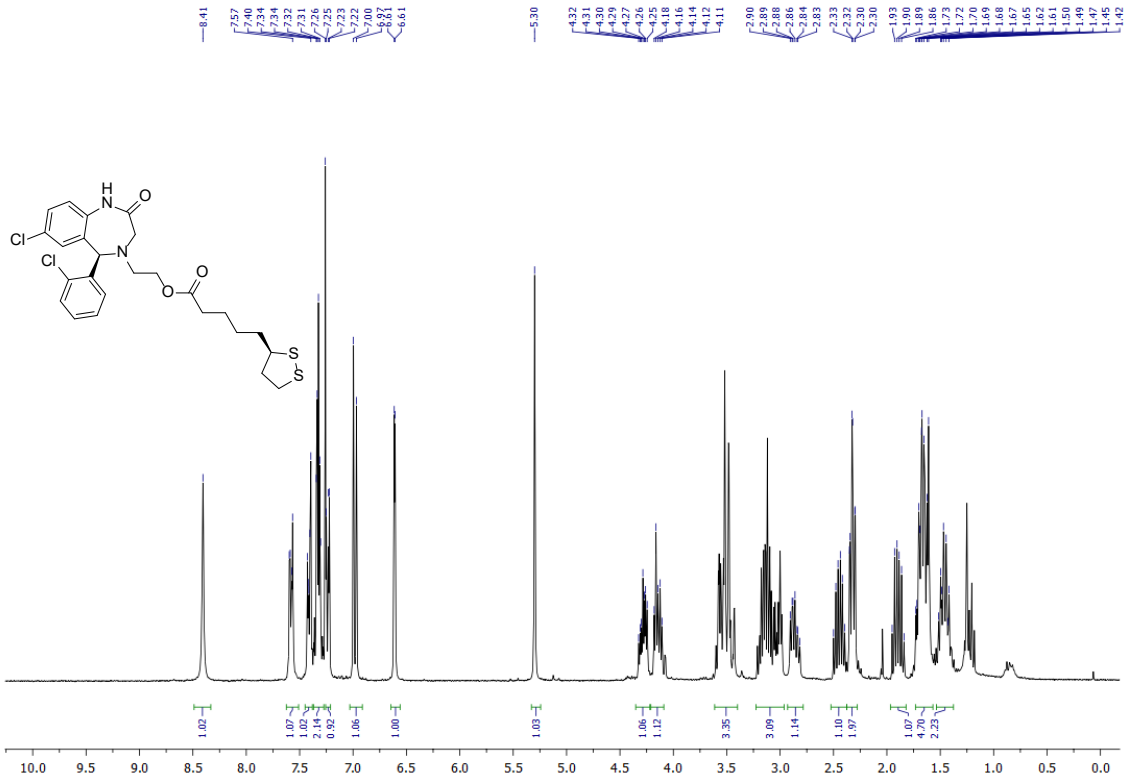
2-((*S*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*R*)-1,2-dithiolan-3-yl)pentanoate (**7c**)

**$^1$H NMR (250 MHz, $CDCl_3$)**



2-((*R*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*R*)-1,2-dithiolan-3-yl)pentanoate (**7d**)

**$^1$H NMR (250 MHz, $CDCl_3$)**



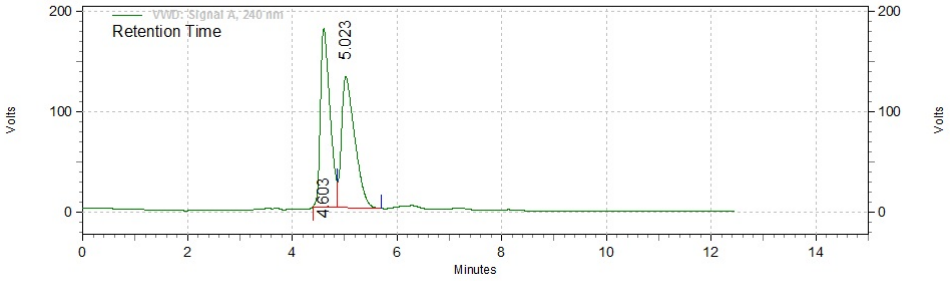
## Copies of chromatograms

(rac)-2-(((2-amino-5-chlorophenyl)(2-chlorophenyl)methyl)amino)ethan-1-ol (***rac*-4**)

HPLC (ULTRON ES OVM Chiral Analytical Reverse Phase, 5µm, 4.6 mm x 150 L, buffer HNaPO4 20 mM pH 5.9/ $CH_3CN$ 80:20, flow 1.0 mL/min.

**Area % Report**

Data File: D:\Enterprise\Projects\Default\Result\Diamina pH5.9_AcCN20%.rslt\Diamina pH5.9_AcCN20%.rslt.dat
Method: D:\Enterprise\Projects\Default\Method\Angel ph59-CH3CN20% 1ml.met
Acquired: 4/11/2018 2:01:30 PM (GMT +02:00)
Printed: 4/17/2018 4:23:56 PM (GMT +02:00)




**VWD: Signal A, 240 nm Results**

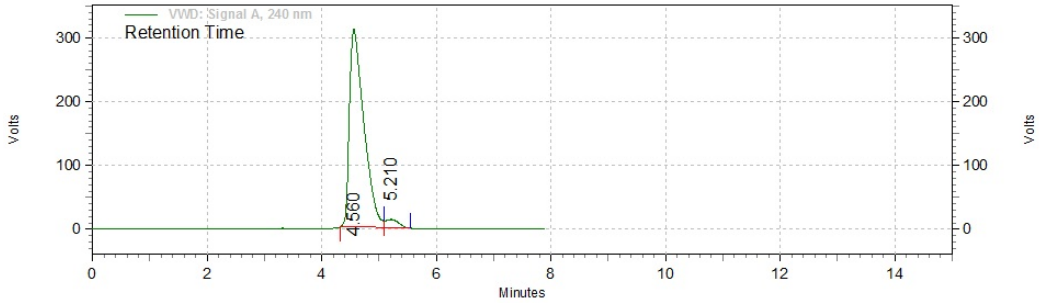
| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 4.603 | 37797369 | 49.52 | 2979248 | 57.70 |
| 5.023 | 38527866 | 50.48 | 2183790 | 42.30 |
| Totals | 76325235 | 100.00 | 5163038 | 100.00 |

(*S*)-2-(((2-Amino-5-chlorophenyl)(2-chlorophenyl)methyl)amino)ethan-1-ol (***S*-4**)

*ee* 93% The enantiomers were separated by HPLC (ULTRON ES OVM Chiral Analytical Reverse Phase, 5µm, 4.6 mm x 150 L, buffer $NaH_2PO_4$ 20 mM pH 5.9/ $CH_3CN$ 80:20, flow 1.0 mL/min.

## Area % Report

Data File: D:\Enterprise\Projects\Default\Result\diasteroA_Diamina pH5.9_AcCN20%b.rslt\diasteroA_Diamina pH5.9_AcCN20%b.rslt.dat
Method: D:\Enterprise\Projects\Default\Result\diasteroA_Diamina pH5.9_AcCN20%b.rslt\Angel ph59-CH3CN20% 1ml.met
Acquired: 4/11/2018 2:37:07 PM (GMT +02:00)
Printed: 4/17/2018 4:26:54 PM (GMT +02:00)



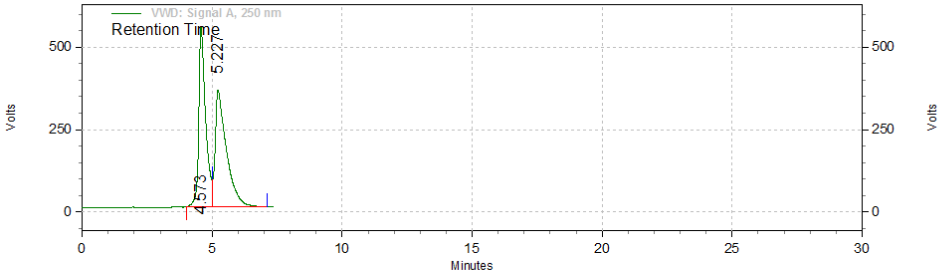

**VWD: Signal A, 240 nm Results**

| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 4.560 | 91315109 | 96.35 | 5194146 | 95.97 |
| 5.210 | 3463364 | 3.65 | 217952 | 4.03 |
| Totals | 94778473 | 100.00 | 5412098 | 100.00 |

(*rac*)-7-Chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (***rac*-6**)

**Area % Report**

Data File: D:\Enterprise\Projects\Default\Result\bzdrac3.rslt\bzdrac3.rslt.dat
Method: D:\Enterprise\Projects\Default\Result\bzdrac3.rslt\Angel ph59-iPrOH20% 1ml.met
Acquired: 3/7/2019 7:14:50 PM (GMT +02:00)
Printed: 7/31/2019 2:59:15 PM (GMT +02:00)




**VWD: Signal A, 250 nm Results**

| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 4.573 | 167514860 | 48.44 | 9151422 | 60.66 |
| 5.227 | 178334005 | 51.56 | 5934470 | 39.34 |

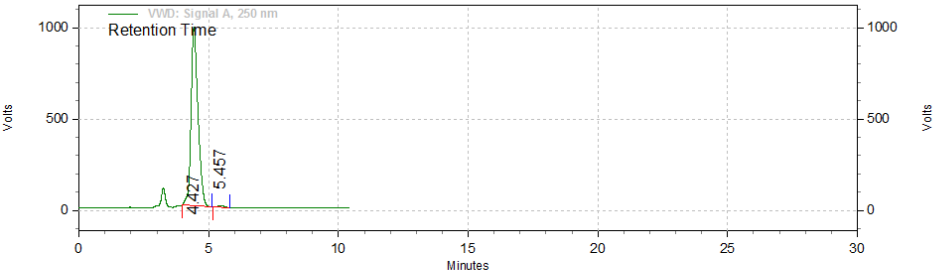
The enantiomers were separated by HPLC (ULTRON ES OVM Chiral Analytical Reverse Phase, 5µm, 4.6 mm x 150 L, buffer $NaH_2PO_4$ 20 mM pH 5.9/ *i*-PrOH 80:20, flow 1.0 mL/min.

(5*S*)-7-Chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (***S*-6**)

*ee* 93% The enantiomers were separated by HPLC (ULTRON ES OVM Chiral Analytical Reverse Phase, 5µm, 4.6 mm x 150 L, buffer $NaH_2PO_4$ 20 mM pH 5.9/ *i*-PrOH 80:20, flow 1.0 mL/min.

**Area % Report**

Data File: D:\Enterprise\Projects\Default\Result\ac102a.rslt\ac102a.rslt.dat
Method: D:\Enterprise\Projects\Default\Result\ac102a.rslt\Angel ph59-iPrOH20% 1ml.met
Acquired: 3/7/2019 8:23:51 PM (GMT +02:00)
Printed: 7/31/2019 3:13:07 PM (GMT +02:00)



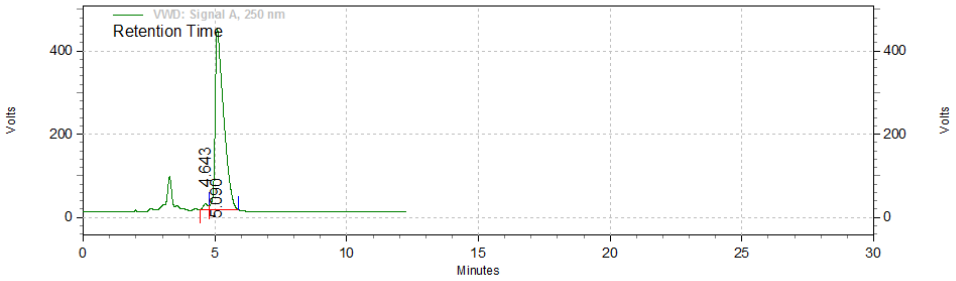

**VWD: Signal A, 250 nm Results**

| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 4.427 | 306622680 | 99.04 | 16399498 | 98.92 |
| 5.457 | 2973713 | 0.96 | 178412 | 1.08 |
| Totals | 309596393 | 100.00 | 16577910 | 100.00 |

(5*S*)-7-Chloro-5-(2-chlorophenyl)-4-(2-hydroxyethyl)-4,5-dihydro-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one (***R*-6**)
HPLC (ULTRON ES OVM Chiral Analytical Reverse Phase, 5µm, 4.6 mm x 150 L, buffer $NaH_2PO_4$ 20 mM pH 5.9/ *i*-PrOH 80:20, flow 1.0 mL/min.

**Area % Report**

Data File: D:\Enterprise\Projects\Default\Result\ac101a.rslt\ac101a.rslt.dat
Method: D:\Enterprise\Projects\Default\Result\ac101a.rslt\Angel ph59-iPrOH20% 1ml.met
Acquired: 3/7/2019 8:08:44 PM (GMT +02:00)
Printed: 7/31/2019 3:07:30 PM (GMT +02:00)

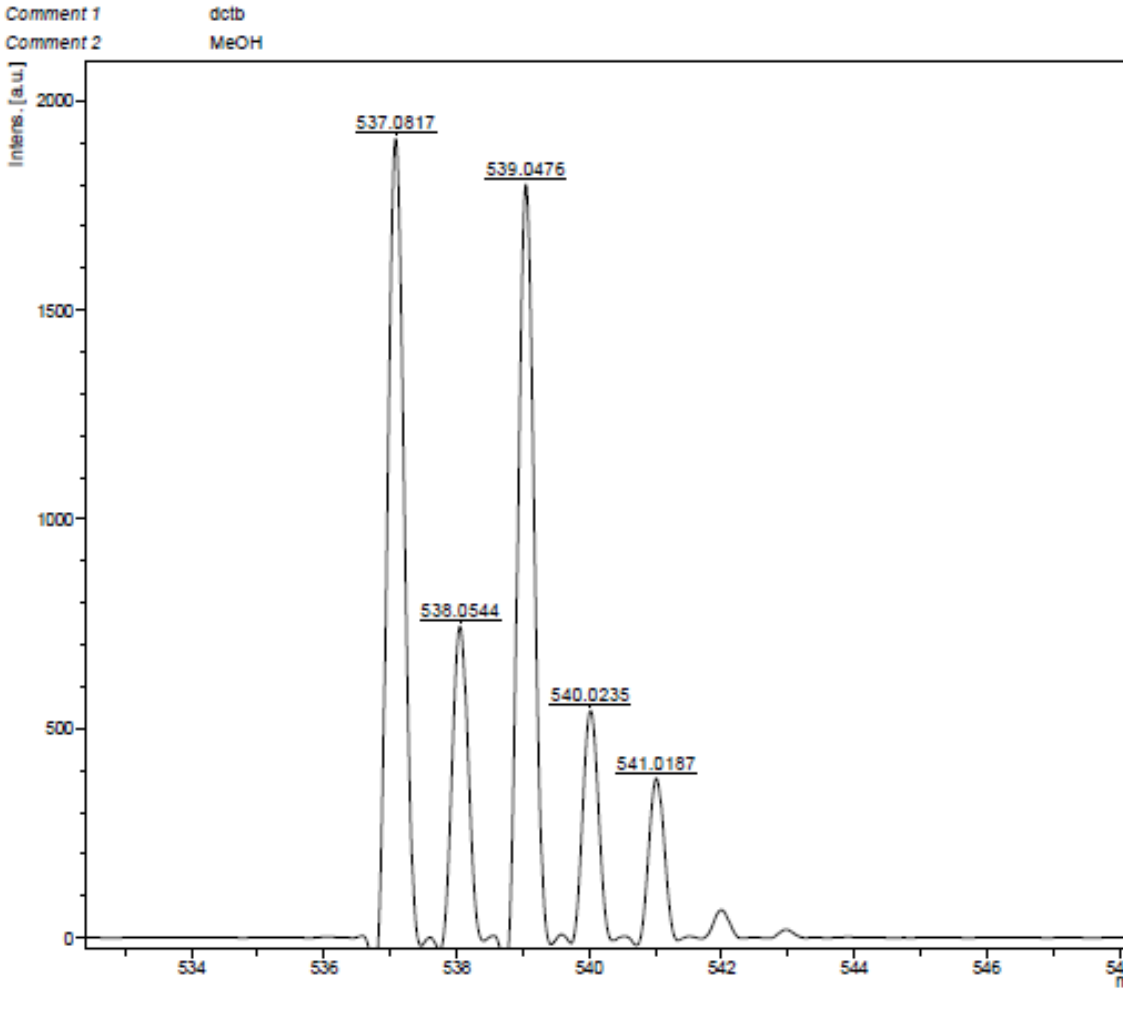



**VWD: Signal A, 250 nm Results**

| Retention Time | Area | Area % | Height | Height % |
|---|---|---|---|---|
| 4.643 | 3077942 | 1.84 | 249477 | 3.29 |
| 5.090 | 163830168 | 98.16 | 7326067 | 96.71 |
| Totals | 166908110 | 100.00 | 7575544 | 100.00 |

## Copies of high-resolution mass spectra

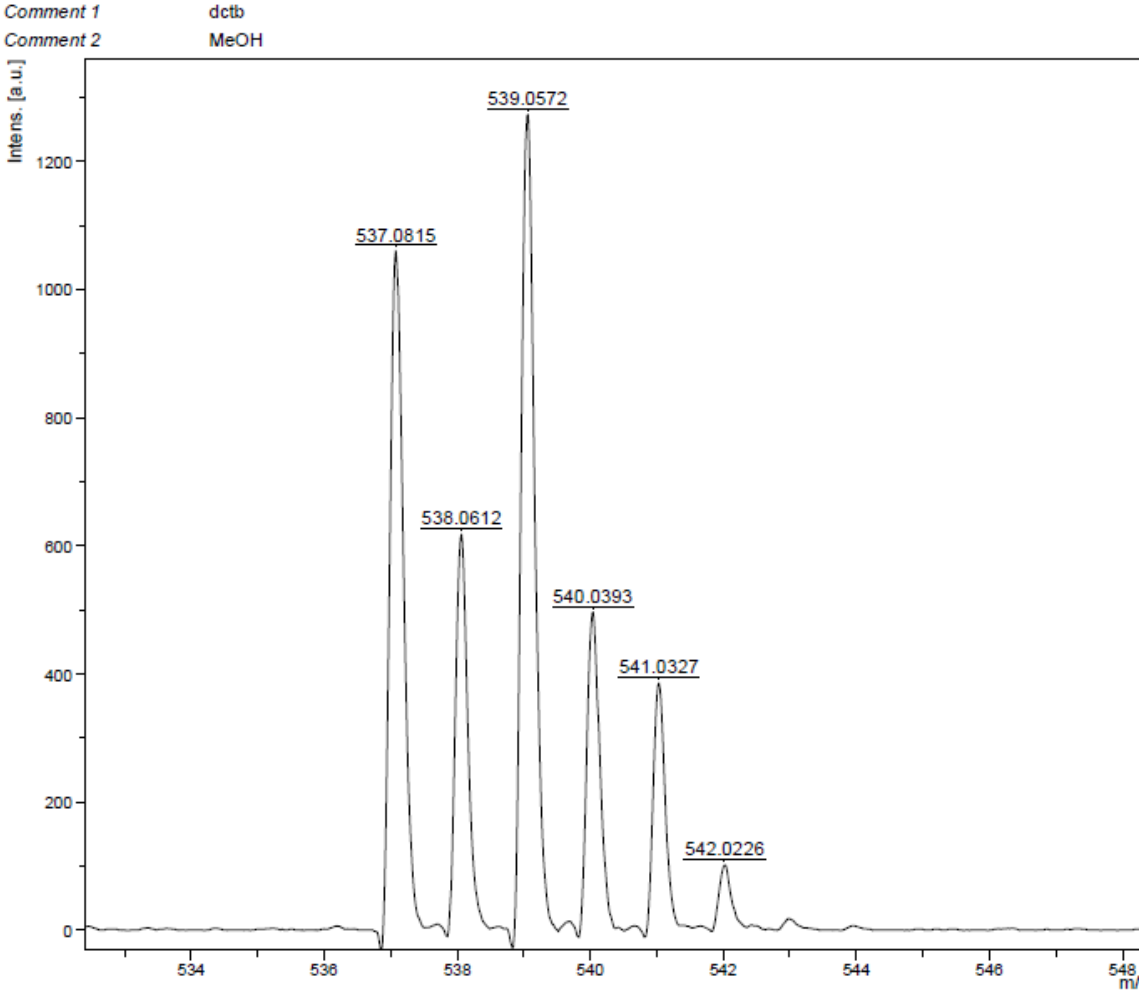
2-((*S*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*S*)-1,2-dithiolan-3-yl)pentanoate (**7a**)




**Acquisition Parameter**

| | |
|---|---|
| *Date of acquisition* | 2021-12-22 12:54:17 |
| *Acquisition method name* | D:\Methods\flexControlMethods\RP_Proteomics_HPC_20211222.par |
| *Aquisition operation mode* | Reflector |
| *Voltage polarity* | POS |
| *Number of shots* | 200 |
| *Name of spectrum used for calibration* | 09-00-calpep\0_M4\2 |
| *Calibration reference list used* | PeptideCalibStandard mono |

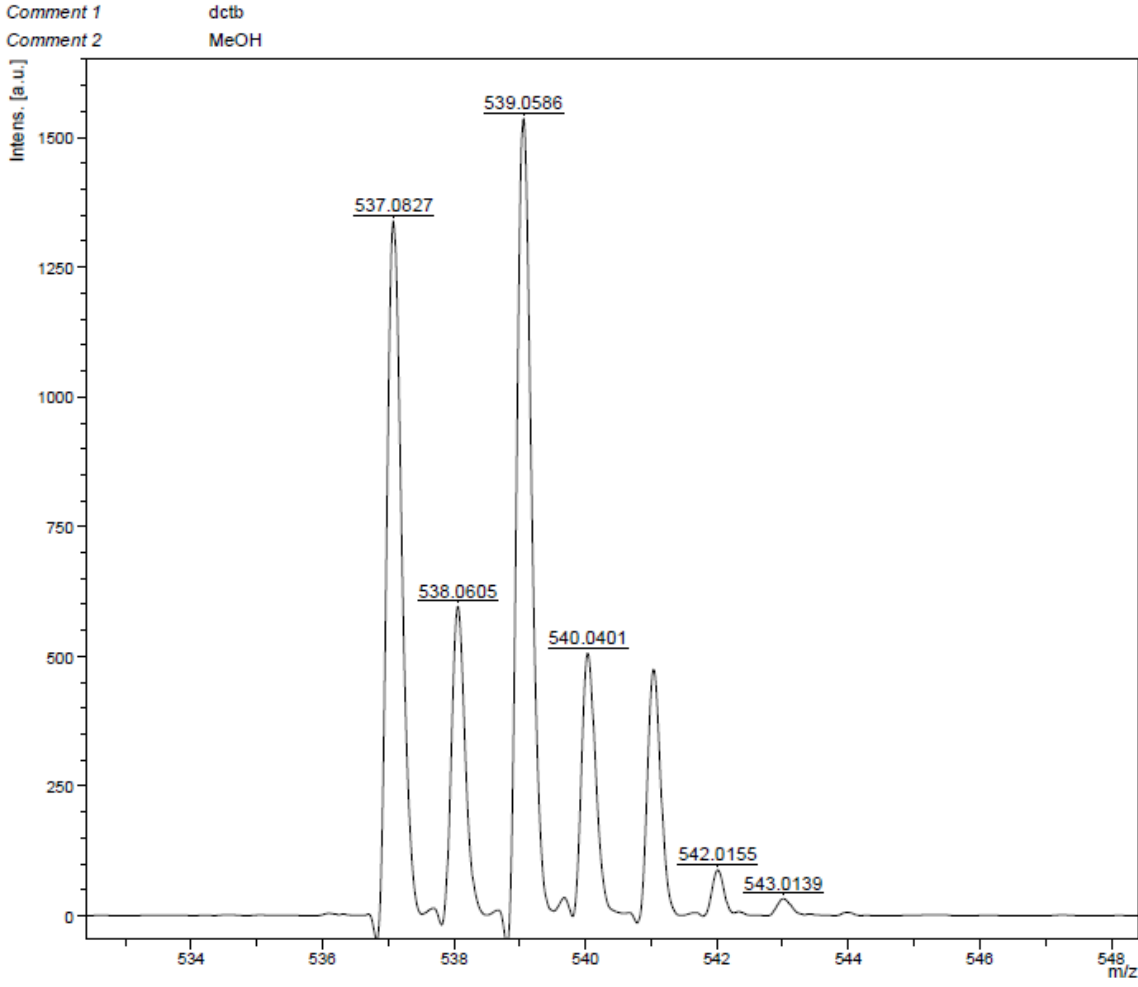
2-((*R*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*S*)-1,2-dithiolan-3-yl)pentanoate (**7b**)




**Acquisition Parameter**

| | |
|---|---|
| *Date of acquisition* | 2021-12-22 12:54:39 |
| *Acquisition method name* | D:\Methods\flexControlMethods\RP_Proteomics_HPC_20211222.par |
| *Aquisition operation mode* | Reflector |
| *Voltage polarity* | POS |
| *Number of shots* | 200 |
| *Name of spectrum used for calibration* | 09-00-calpep\0_M4\2 |
| *Calibration reference list used* | PeptideCalibStandard mono |

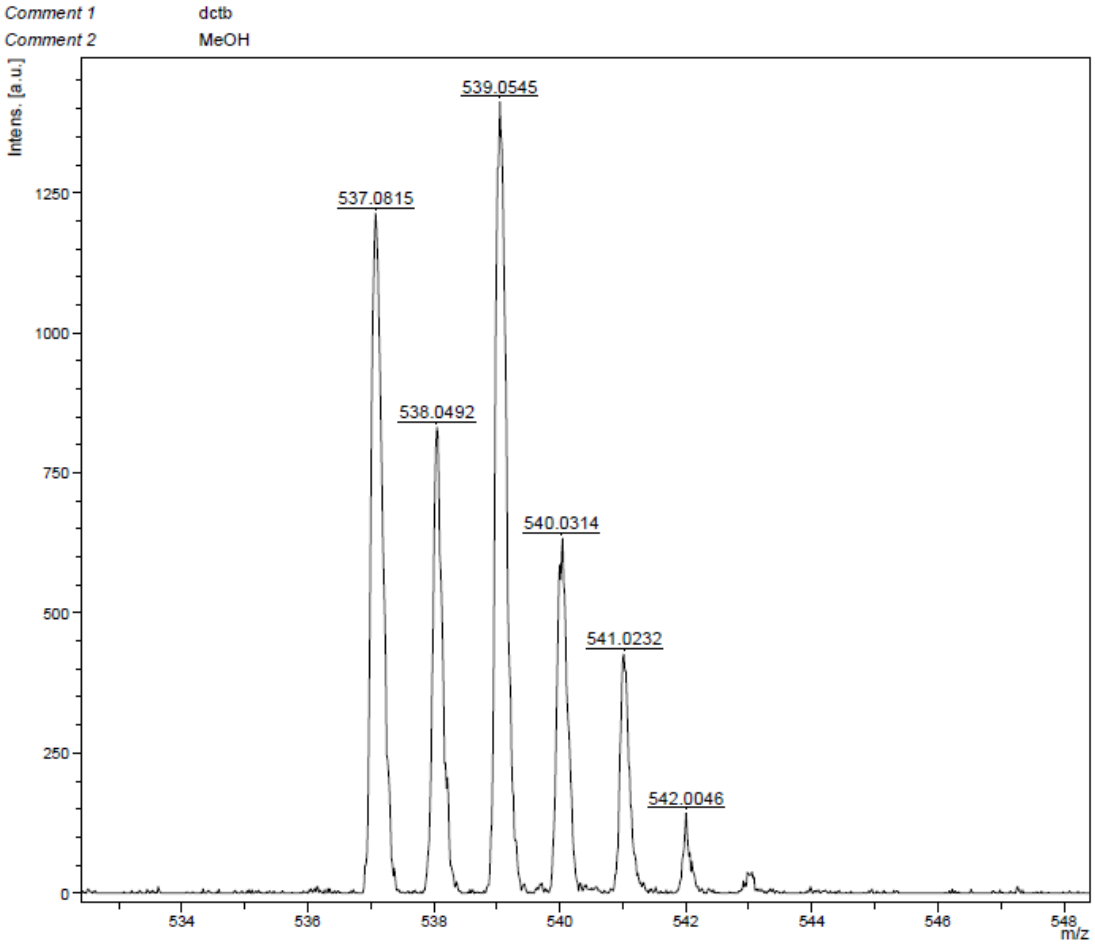
2-((*S*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*R*)-1,2-dithiolan-3-yl)pentanoate (**7c**)




**Acquisition Parameter**

| | |
|---|---|
| *Date of acquisition* | 2021-12-22 12:54:53 |
| *Acquisition method name* | D:\Methods\flexControlMethods\RP_Proteomics_HPC_20211222.par |
| *Aquisition operation mode* | Reflector |
| *Voltage polarity* | POS |
| *Number of shots* | 200 |
| *Name of spectrum used for calibration* | 09-00-calpep\0_M4\2 |
| *Calibration reference list used* | PeptideCalibStandard mono |

2-((*R*)-7-Chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4*H*-benzo[*e*][1,4]diazepin-4-yl)ethyl 5-((*R*)-1,2-dithiolan-3-yl)pentanoate (**7d**)




**Acquisition Parameter**

| | |
|---|---|
| *Date of acquisition* | 2021-12-22 12:55:09 |
| *Acquisition method name* | D:\Methods\flexControlMethods\RP_Proteomics_HPC_20211222.par |
| *Aquisition operation mode* | Reflector |
| *Voltage polarity* | POS |
| *Number of shots* | 200 |
| *Name of spectrum used for calibration* | 09-00-calpep\0_M4\2 |
| *Calibration reference list used* | PeptideCalibStandard mono |