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Supplemental information

VE607 stabilizes SARS-CoV-2 Spike in the "RBD-up" conformation and inhibits viral entry

Shilei Ding, Irfan Ullah, Shang Yu Gong, Jonathan R. Grover, Mohammadjavad Mohammadi, Yaozong Chen, Dani Vézina, Guillaume Beaudoin-Bussières, Vijay Tailor Verma, Guillaume Goyette, Fleur Gaudette, Jonathan Richard, Derek Yang, Amos B. Smith III, Marzena Pazgier, Marceline Côté, Cameron Abrams, Priti Kumar, Walther Mothes, Pradeep D. Uchil, Andrés Finzi, and Christian Baron

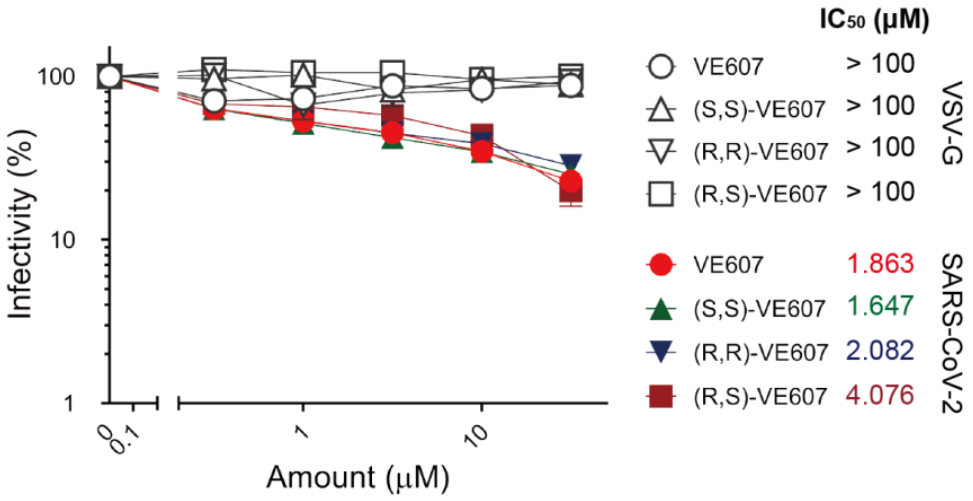


Figure S1. Stereochemical isomers of VE607 have the same capacity as commercially available VE607 to neutralize SARS-CoV-2 pseudovirus particles. Related to Figure 1. All three stereochemical isomers of VE607, (S,S)-VE607, (R,R)-VE607 and (R,S)-VE607 were tested for their inhibition of VSV-G pseudovirus or SARS-CoV-2 D614G pseudovirus. IC_{50} values are shown next to the different isomers. Data represents the average of at least four independent experiments ± SEM.

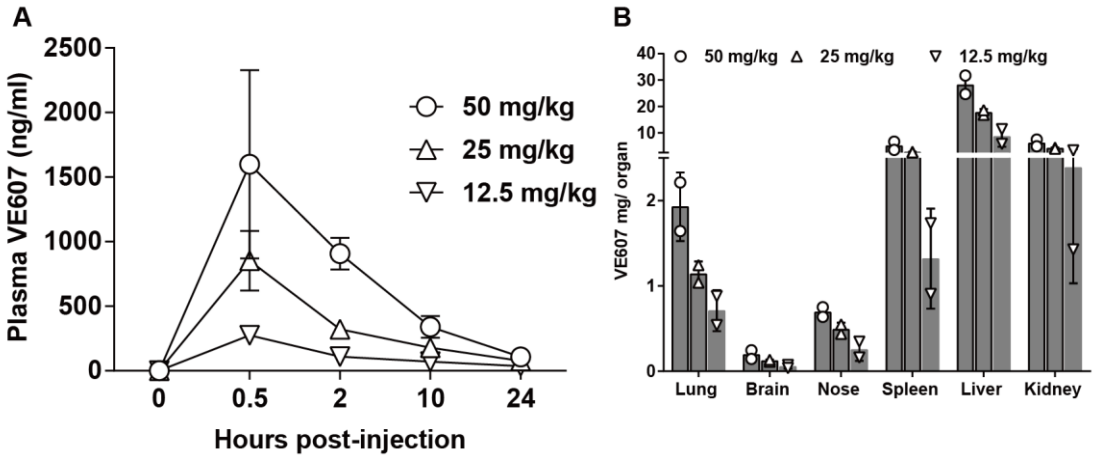
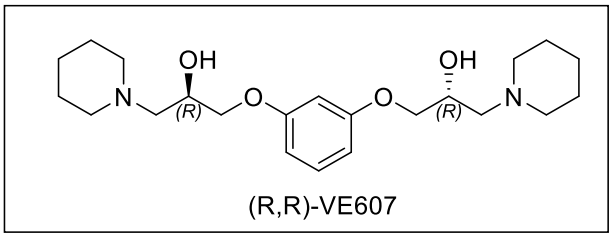


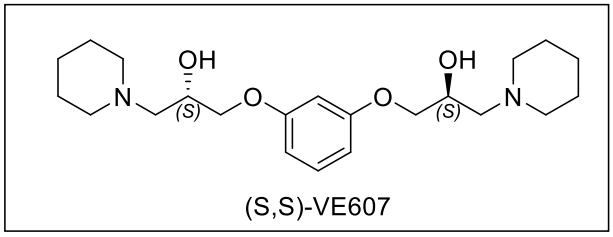
Figure S2. Pharmacokinetics of VE607 in K18-hACE2 Mice. Related to Figure 5.

(**A**) VE607 or vehicle (DMSO) was intraperitoneally injected into K18-hACE2 mice (n=2) at the indicated concentration. Serum samples were collected at 0.5 hour, 2 hour, 10 hour and 24 hour post injection. The VE607 serum concentrations (ng/ml) were determined by liquid chromatography-tandem mass spectrometry using mock-treated samples in parallel as baseline and VE607 in DMSO as standard (see Materials and Methods). (**B**) VE607 concentration (mg/ indicated organ) at 24 hour post-injection.

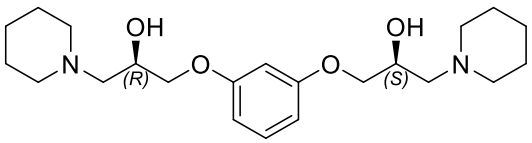
Data S1. Chemical synthesis of the three enantiomers of VE607. Related to figures 2 and 5.



(R,R)-VE607



(S,S)-VE607

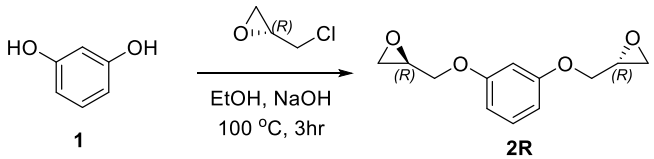


(R,S)-VE607

General Procedures

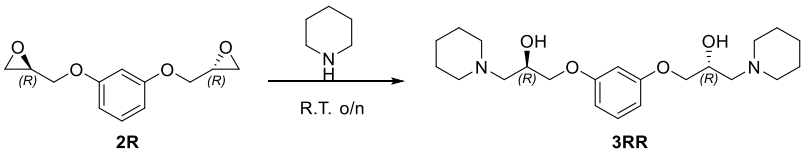
All reactions were conducted in oven-dried glassware under an inert atmosphere of nitrogen, unless otherwise stated. All solvents were reagent or high-performance liquid chromatography (HPLC) grade. Anhydrous THF was obtained from the Pure SolveTM PS-400 system under an argon atmosphere. All reagents were purchased from commercially available sources and used as received. Reactions were magnetically stirred under a nitrogen atmosphere, unless otherwise noted and were monitored by thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F-254 plates (40-55 micron, 230-400 mesh) and visualized by UV light or staining with $KMnO_4$ and heating. Yields refer to chromatographically and spectroscopically pure compounds. Optical rotations were measured on a JASCO P-200 polarimeter. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III 500-MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to chloroform (δ 7.26) or methanol (δ 3.31) for ^{1}H NMR, and chloroform (δ 77.2) or methanol (δ 49.0). High resolution mass spectra (HRMS) were recorded at the University of Pennsylvania Mass Spectroscopy Service Center on either a VG Micromass 70/70H or VG ZAB-E spectrometer. Lyophilization was performed in a Labconco FreeZone 12 Plus lyophilizer (0.148 mbar). SFC analyses were performed with a JASCO system equipped with a PI-280-CO_2 plus CO_2 Delivery System, a BP-2080 plus Automatic Back Pressure Regulator, an MD-2018 plus Photodiode Array Detector (200-648 nm), and PU-2080 plus Intelligent HPLC Pumps. The purity of new compounds were judged by NMR and LCMS (>95%).

Experimental Procedures



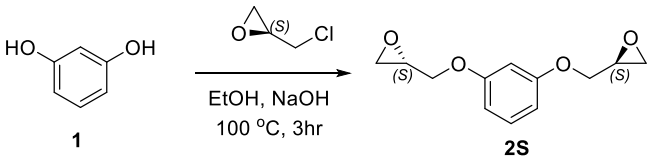
1 **2R**

1,3-bis(((R)-oxiran-2-yl)methoxy)benzene (+)-2R[1]: Resorcinol **(1)** (100 mg, 0.908 mmol, 1 equiv) was dissolved in enantiopure (*R*)-epichlorohydrin (0.569 mL, 7.27 mmol, 8.0 equiv) and heated to 100 °C. A solution of NaOH (72.7 mg, 1.82 mmol, 2.0 equiv) in EtOH (0.75 mL) was then added dropwise. After stirring at 100 °C for three hours, the solution was cooled to r.t., diluted with acetone and filtered through a fritted funnel packed with celite. The filtrate was then concentrated *in vacuo* which was purified via flash column chromatography (5% to 10% EtOAc/hexanes) to yield the title compound as a yellow oil in 97% yield. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.17 (t, J= 8.08 Hz, 1H), 6.55-6.51 (m, 3H), 4.20 (dd, J= 7.84, 3.17 Hz, 2H), 3.95 (dd, J= 5.66, 5.29 Hz, 2H), 3.36 – 3.33 (m, 2H), 2.90 (t, J= 4.77 Hz, 2H). 2.75 (dd, J= 2.63, 2.35 HZ, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 159.76, 130.06, 107.44, 101.94, 68.83, 50.13, 44.87; **HRMS** (ESI) *m/z:* $[M+Na]^+$ calcd 245.0790, found 245.0791; **$[\alpha]_D^{23}$** +17.28 (c 0.74, MeOH).



2R 3RR

(2R,2'R)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (+)-3RR: Epoxide **2R** (25 mg, 0.113 mmol, 1.0 equiv) was dissolved in neat piperdine (0.177 mL, 1.8 mmol, 16 equiv) at 0 °C and stirred overnight at room temperature. Upon completion, solvent was removed *in vacuo*, dissolved in DCM, washed with brine, dried with Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo*. The crude product mixture was then dissolved in water, frozen, and lyophilized to remove excess piperdine. Product was isolated as a yellow oil in 99% yield. **^{1}H NMR** (500 MHz, CD_3OD): δ 7.17-7.13 (m, 1H), 6.55-6.52 (m, 3H), 4.15-4.10 (m, 2H), 3.96 (dd, J= 5.59, 4.31 Hz, 2H), 3.89 (dd, J= 5.93, 3.82, 2H), 2.57-2.46 (m, 12H), 1.64-1.59(m, 8H), 1.51-1.44(m, 4H); **^{13}C NMR** (125 MHz, CD_3OD): δ 161.62, 130.96, 108.15, 102.78, 72.15, 68.36, 63.18, 56.28, 47.47, 27.07, 26.77, 25.55, 25.23; **HRMS** (ESI) *m/z:* $[M+H]^+$ calcd 393.2753, found 393.276; **$[\alpha]_D^{23}$** +3.2 (c 0.33, MeOH).



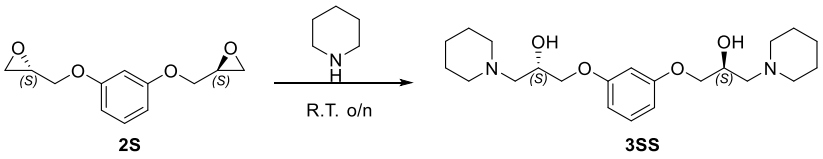
1 2S

1,3-bis(((S)-oxiran-2-yl)methoxy)benzene (-)-2S[2]: Resorcinol **(1)** (100 mg, 0.908 mmol, 1 equiv) was dissolved in enantiopure (*S*)-epichlorohydrin (0.569 mL, 7.27 mmol, 8.0 equiv) and heated to 100 °C. A solution of NaOH (72.7 mg, 1.82 mmol, 2.0 equiv) in EtOH (0.75 mL) was then added dropwise. After stirring at 100 °C for three hours, the solution was cooled to r.t., diluted with acetone and filtered through a fritted funnel packed with celite. The filtrate was then concentrated *in vacuo* which was purified via flash column chromatography (5% to 10% EtOAc/hexanes) to yield the title compound as a yellow oil in 97% yield. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.17 (t, 7.70 Hz, 1H), 6.55-6.51 (m, 3H), 4.20 (dd, J= 7.76, 3.13 Hz, 2H), 3.93 (dd, J= 5.77, 5.34 Hz, 2H), 3.36-3.33 (m, 2H), 2.90 (t, J=

[1] Nouailhas, H., Aouf, C., Le Guernevé, C., Caillol, S., Boutevin, B., & Fulcrand, H. (2011). Synthesis and properties of biobased epoxy resins. Part 1. Glycidylation of flavonoids by epichlorohydrin. *Journal of Polymer Science Part A: Polymer Chemistry*, *49*(10), 2261-2270.

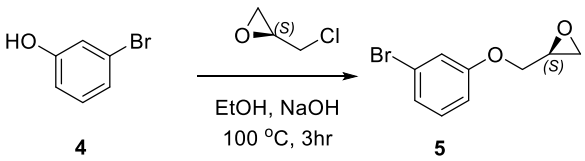
[2] Nouailhas, H., Aouf, C., Le Guernevé, C., Caillol, S., Boutevin, B., & Fulcrand, H. (2011). Synthesis and properties of biobased epoxy resins. Part 1. Glycidylation of flavonoids by epichlorohydrin. *Journal of Polymer Science Part A: Polymer Chemistry*, *49*(10), 2261-2270.

4.87, 2H), 2.75 (dd, 2.68, 2.29, 2H); **^{13}C NMR** (500 MHz, $CDCl_3$): δ 159.74, 130.04, 107.42, 101.92, 68.81, 50.11, 44.74; **HRMS** (ESI) *m/z:* $[M+Na]^+$ calcd 245.0805, found 245.0805; **$[\alpha]_D^{23}$** -17.09 (c 0.74, MeOH).



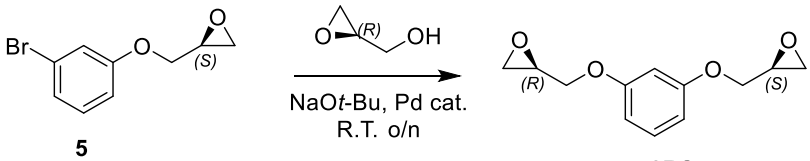
2S **3SS**

(2S,2'S)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (-)-3SS: Epoxide **2** (25 mg, 0.113 mmol, 1.0 equiv) was dissolved in neat piperdine (0.177 mL, 1.8 mmol, 16 equiv) at 0 °C and stirred overnight at room temperature. Upon completion, solvent was removed *in vacuo*, dissolved in DCM, washed with brine, dried with Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo*. The crude product mixture was then dissolved in water, frozen, and lyophilized to remove excess piperdine. Product was isolated as a yellow oil in 99% yield. **^{1}H NMR** (500 MHz, CD_3OD): δ 7.18-7.14 (m, 1H), 6.56-6.52 (m, 3H), 4.18-4.14 (m, 2H), 3.96 (dd, J= 4.51, 4.45 Hz, 2H), 3.89 (dd, J= 5.79, 5.73 Hz), 2.73-2.62 (m, 12H), 1.66-1.57 (m, 8H), 1.56-1.48(m, 4H); **^{13}C NMR** (125 MHz, CD_3OD): δ 121.91, 130.96, 108.14, 102.78, 72.15, 68.33, 63.18, 56.27, 47.59, 27.33, 26.78, 25.74, 25.23; **HRMS** (ESI) *m/z:* $[M+Na]^+$ calcd 415.2567, found 415.2567; **$[\alpha]_D^{23}$** -2.96 (c 0.33, MeOH).



4 **5**

(S)-2-((3-bromophenoxy)methyl)oxirane (+)-5: 3-bromophenol (200 mg, 1.15 mmol, 1.0 equiv) was dissolved in (S)-epichlorohydrin (0.363 mL, 4.624 mmol, 4.0 equiv) and heated to 100 °C. A solution of NaOH (46 mg, 1.15 mmol, 1.0 equiv) was dissolved in EtOH (0.375 mL) and added dropwise. After stirring at 100 °C for three hours, the solution was cooled to r.t., diluted with acetone and filtered through a fritted funnel packed with celite. The filtrate was then concentrated *in vacuo* which was purified via flash column chromatography (5% to 10% EtOAc/hexanes) to yield the title compound as a yellow oil in 95% yield. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.17-7.07 (m, 3H), 6.86 (dq, J= 8.13, 1.35, 0.97 Hz, 1H), 4.22 (dd, 11.03, 3.03 Hz, 1H), 3.93 (dd, J= 5.70, 5.32 Hz, 1H), 3.37-3.32 (m, 1H), 2.91 (t, J=4.47 Hz, 1H), 2.75 (dd, J= 2.63, 2.26 Hz, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 159.27, 130.65, 124.41, 122.86, 118.02, 113.70, 69.03, 49.98, 44.64; **HRMS** (EI) *m/z:* $[M+H]^+$ calcd 227.9786, found 227.9793; **$[\alpha]_D^{23}$** -6.718 (1.08 c, MeOH).

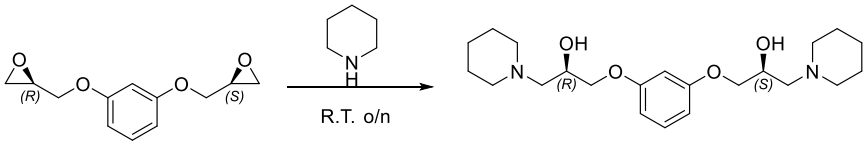


5 **2RS**

1-(((R)-oxiran-2-yl)methoxy)-3-(((S)-oxiran-2-yl)methoxy)benzene (2RS)[3]: Sodium *tert*-butoxide (23.4 mg, 0.2385 mmol, 1.5 equiv) was added to a oven dried flask, sealed with a rubber septa, purged 3x with nitrogen. THF (0.160 mL) was then added to this flask

[3] Zhang, H., Ruiz-Castillo, P., & Buchwald, S. L. (2018). Palladium-catalyzed C–O cross-coupling of primary alcohols. *Organic letters*, *20*(6), 1580-1583.

and sonicated to homogenize the solution. In a separate oven dried 5 mL round tapered flask, *t*-BuBrettPhos Palladacycle Gen. 3 (2.71 mg, 0.00318 mmol, 0.02 equiv), epoxide **(5)** (36.5 mg, 0.159 mmol, 1.0 equiv), and (R)-glycidol (21.27 mg, 0.318 mmol, 2.0 equiv) were added. This flask was sealed with a rubber septa and purged 3x with nitrogen. The sodium *tert*-butoxide in THF solution as then added dropwise to the second flask at room temperature under positive N_2 pressure, and allowed to stir overnight at r.t. Upon completion, the mixture was diluted with EtOAc, and filtered through a fritted funnel packed with celite. The filtrate was then concentrated *in vacuo* which was purified via flash column chromatography (5% to 10% EtOAc/hexanes) as a yellow oil in 35% yield. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.20 (t, J= 8.10, 1Hz), 6.58 - 6.53 (m, 2H), 4.22 (dd, J= 7.85, 3.16 Hz, 2H), 3.96 (dd, J= 5.71, 5.32 Hz, 2H), 3.39 - 3.35 (m, 2H), 2.93 (t, J= 4.50 Hz, 2H), 2.77 (dd, J= 2.64, 2.26 Hz, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 159.76, 130.06, 107.44, 101.95, 68.85, 50.13, 44.78; **HRMS** (ESI) *m/z:* $[M+H]^+$ calcd 223.0970, found 223.0969; **$[\alpha]_D^{23}$** +0.15 (0.936 c, MeOH).



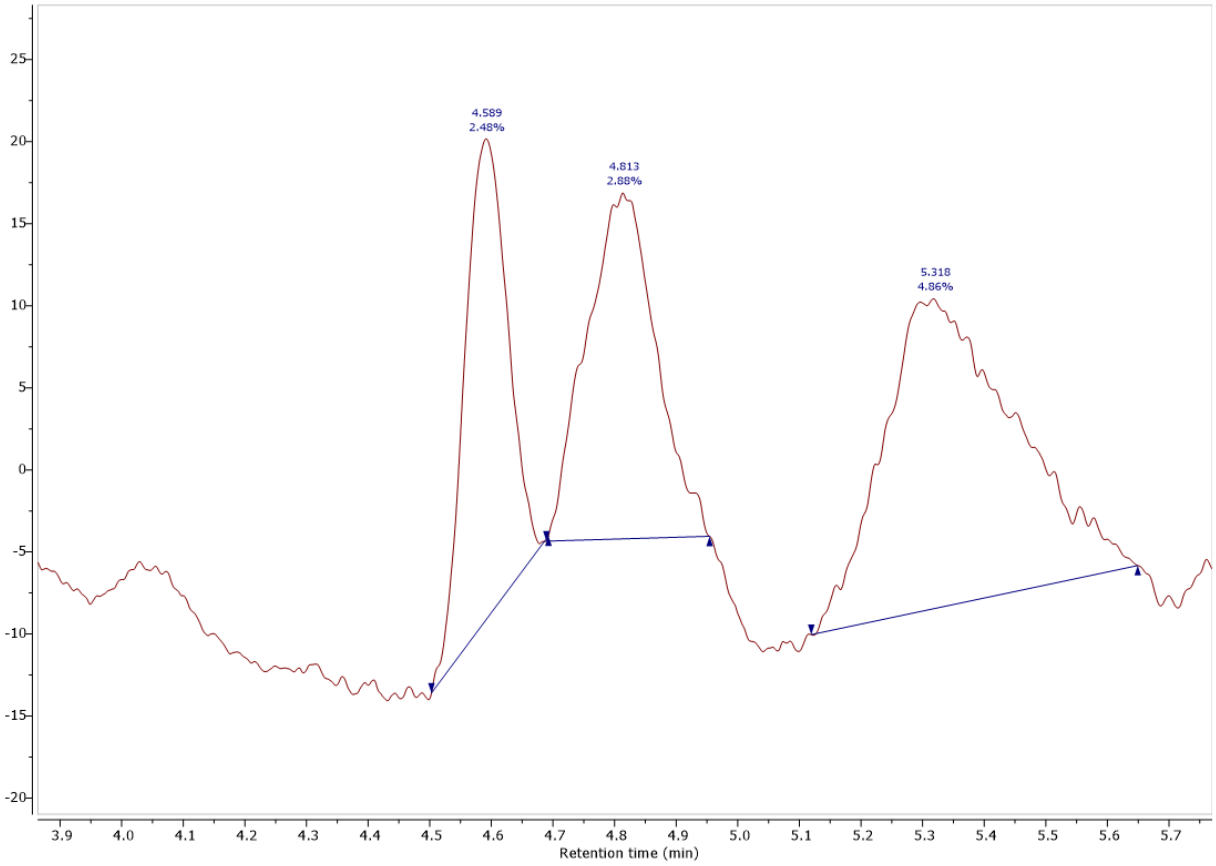
2RS **3RS**

(S)-1-(3-((R)-2-hydroxy-3-(piperidin-1-yl)propoxy)phenoxy)-3-(piperidin-1-yl)propan-2-ol (3RS):

Epoxide **2** (25 mg, 0.113 mmol, 1.0 equiv) was dissolved in neat piperdine (0.177 mL, 1.8 mmol, 16 equiv) at 0 °C and stirred overnight at room temperature. Upon completion, solvent was removed *in vacuo*, dissolved in DCM, washed with brine, dried with Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo*. The crude product mixture was then dissolved in water, frozen, and lyophilized to remove excess piperdine. Product was isolated as a yellow oil in 90% yield. **^{1}H NMR** (500 MHz, CD_3OD): δ 7.17 (t, J= 7.57 Hz, 1H), 6.58-6.53 (m, 3H), 4.23-4.17 (m, 2H), 3.99-3.90 (m, 2H), 2.89-2.70 (m, 12H), 1.78-1.65 (m, 8H), 1.60-1.50 (m, 4H); **^{13}C NMR** (125 MHz, CD_3OD): δ 161.61, 130.96, 108.15, 102.78, 72.14, 68.31, 63.16, 56.26, 26.74, 25.20; **HRMS** (ESI) *m/z:* $[M+H]^+$ calcd 393.2753, found 393.2761; **$[\alpha]_D^{23}$** +0.19 (0.565 c , MeOH).

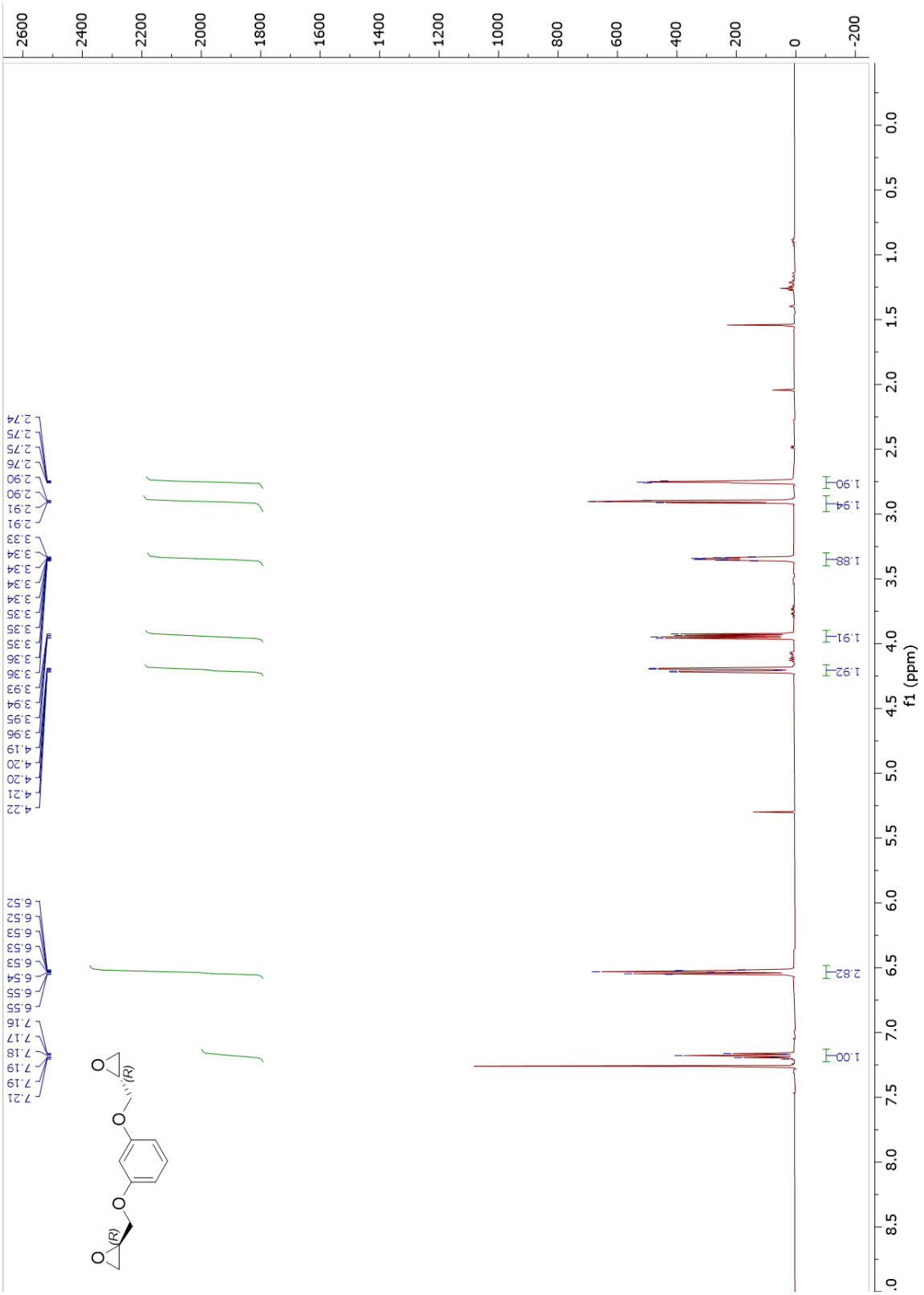
SFC Analysis of commercially available VE607 (see below)

The ratio of the three stereoisomers in the commercial sample of VE607 was determined by SFC to be roughly 1:1:2 of **(S,S)**-VE607: **(R,R)**-VE607: **(R,S)**-VE607. SFC separation was achieved via a Chiralpak® IA-3 column. The elutant gradient was 1% MeOH in supercritical CO_2 to 5% MeOH in supercritical CO_2 over 10 minutes. Flow rate was 2.5 mL/min at 6 MPa. Retention times: **(S,S)-**VE607: 4.6 min, **(R,R)**-VE607: 4.8 min, **(R,S)-**VE607: 5.3 min.

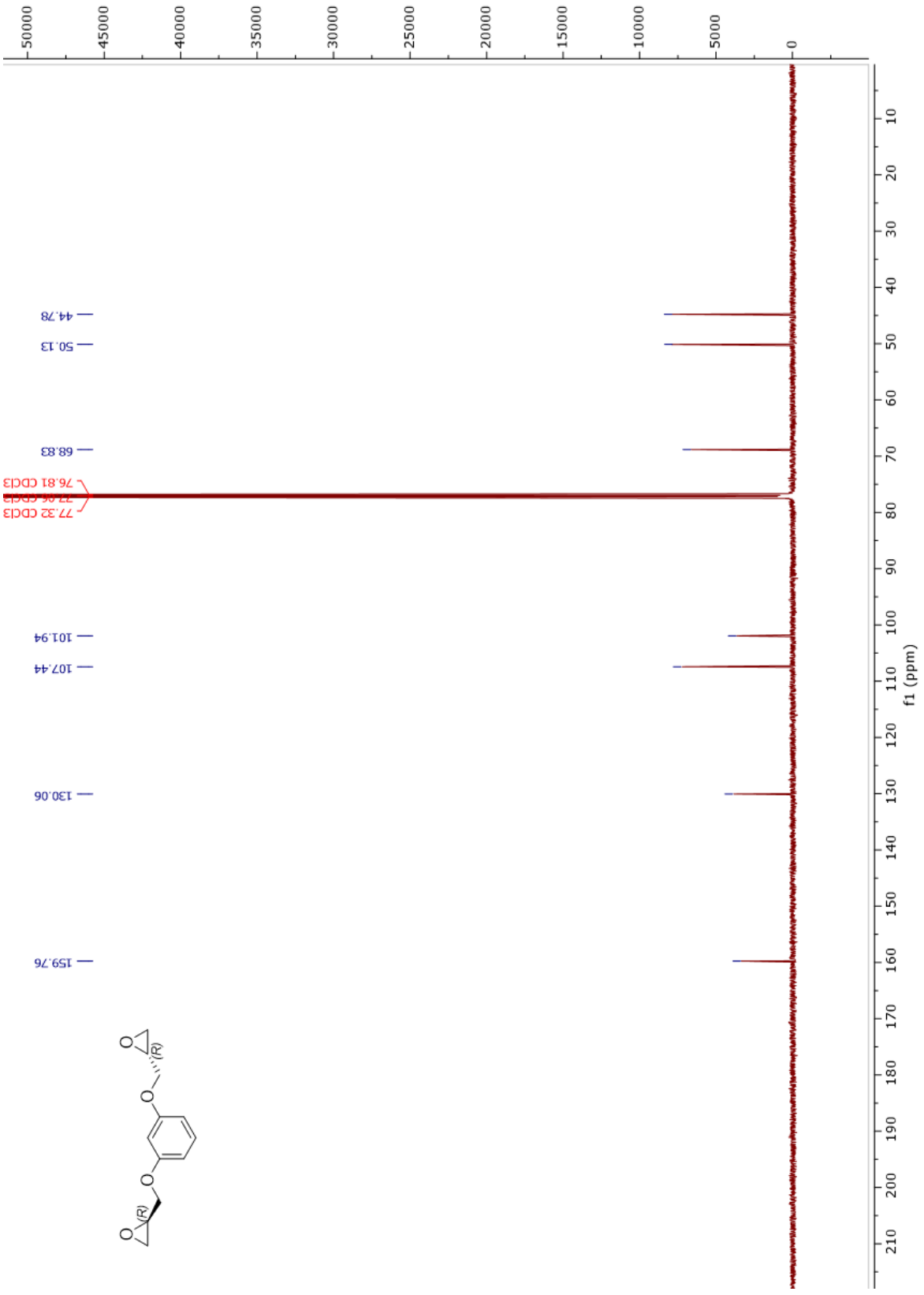


Spectral Data

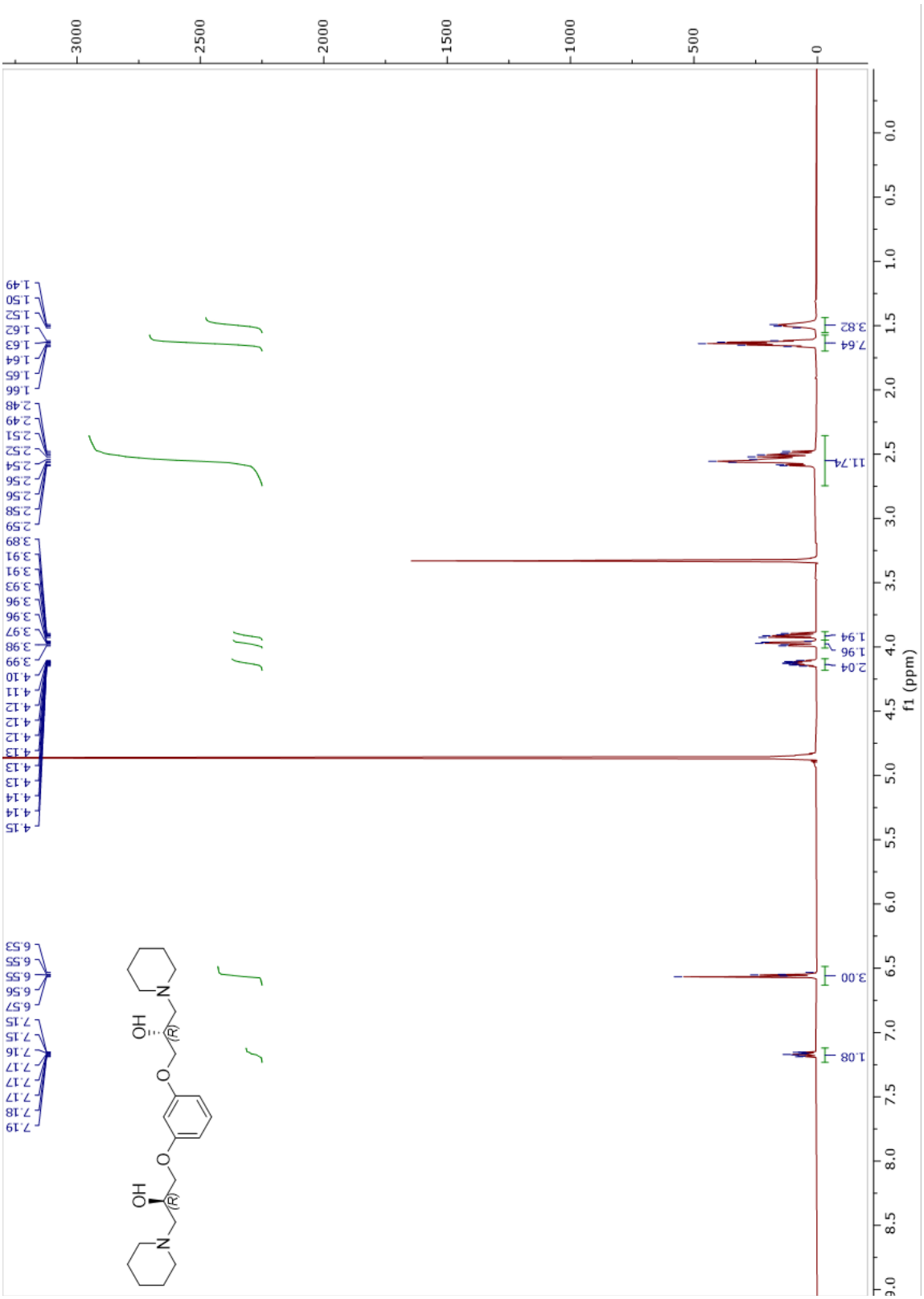
^{1}H NMR for 1,3-bis(((R)-oxiran-2-yl)methoxy)benzene (+)-2R



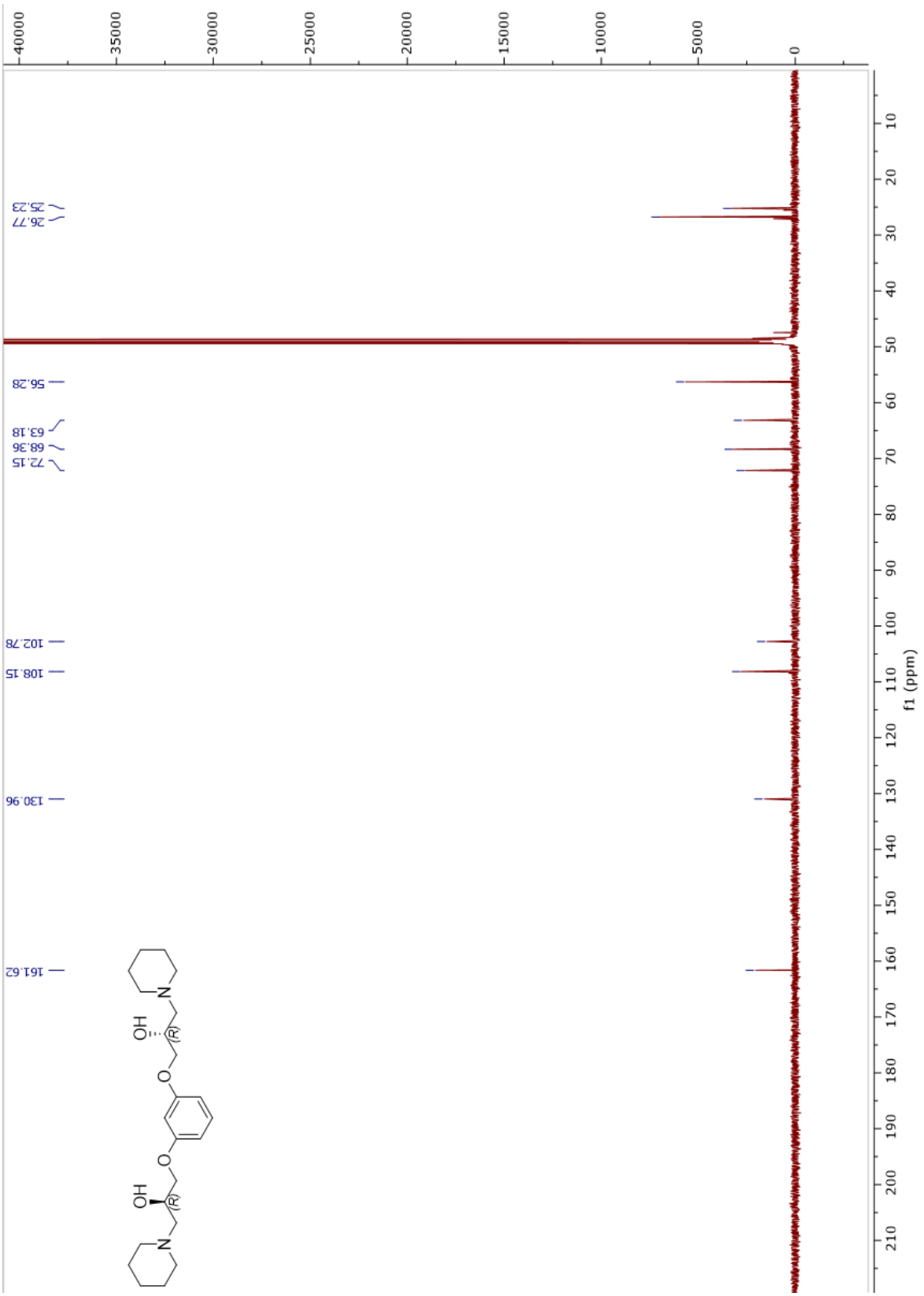
^{13}C NMR for 1,3-bis(((R)-oxiran-2-yl)methoxy)benzene (+)-2R



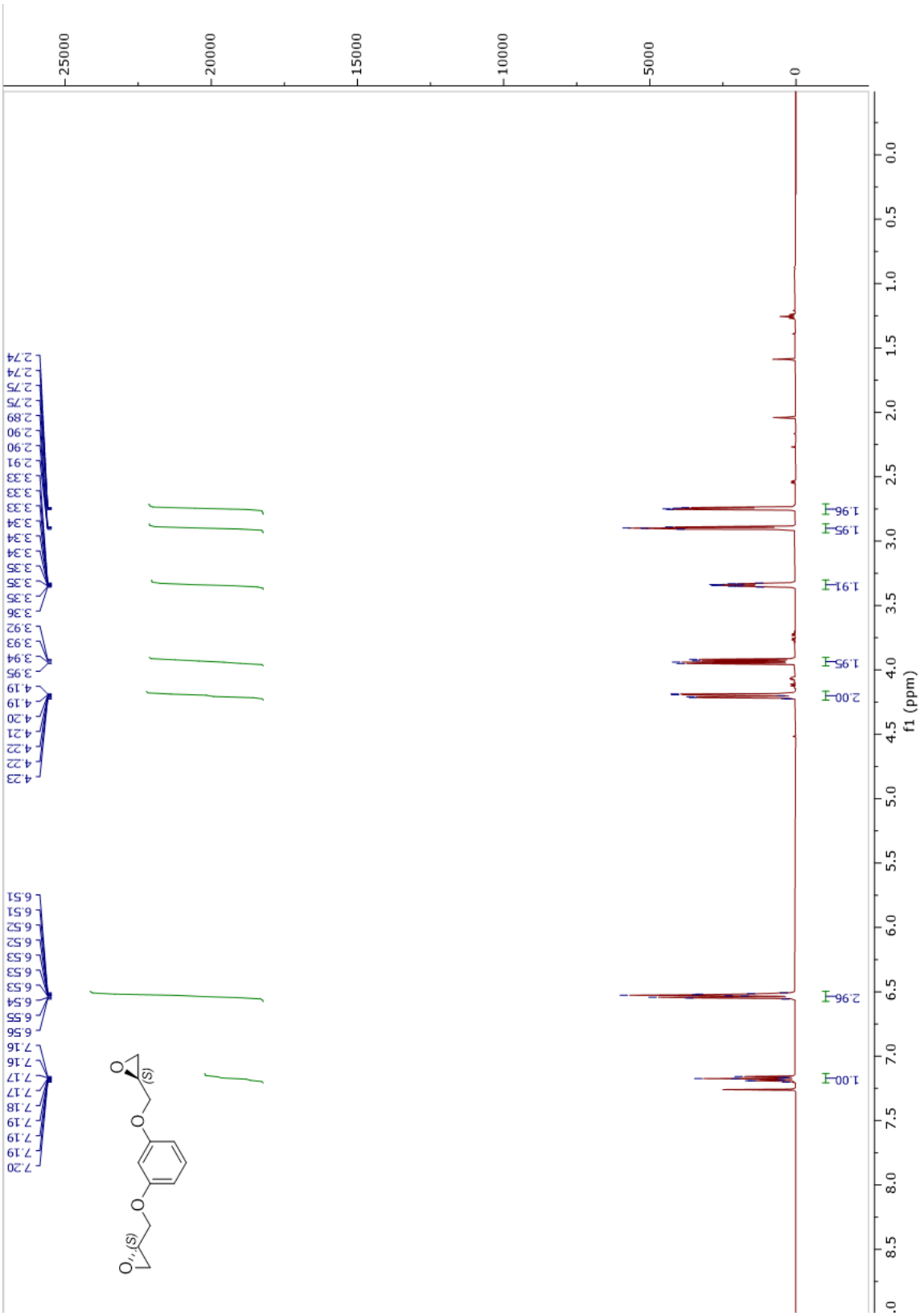
^{1}H NMR for (2R,2'R)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (+)-3RR



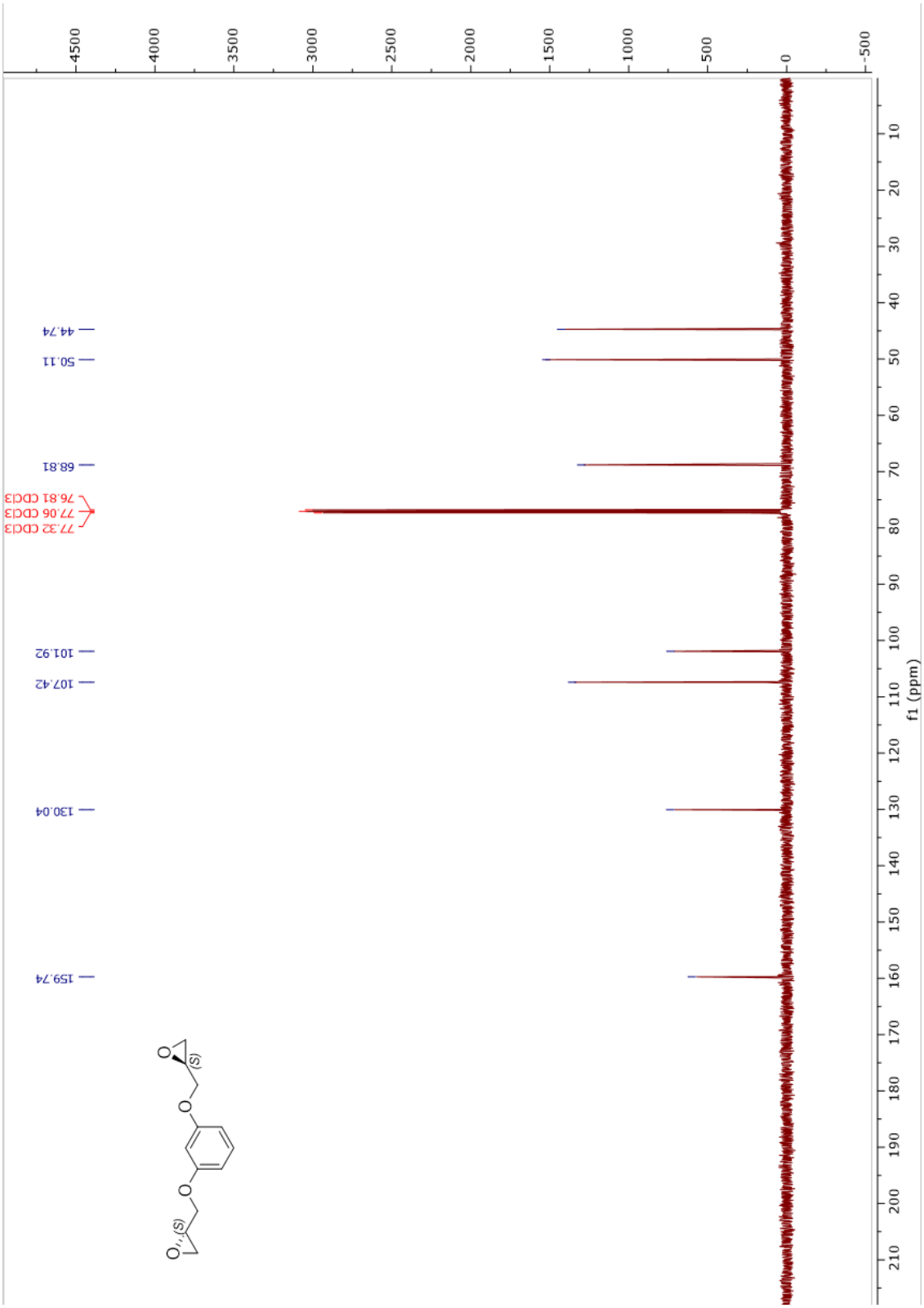
^{13}C NMR for (2R,2'R)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (+)-3RR



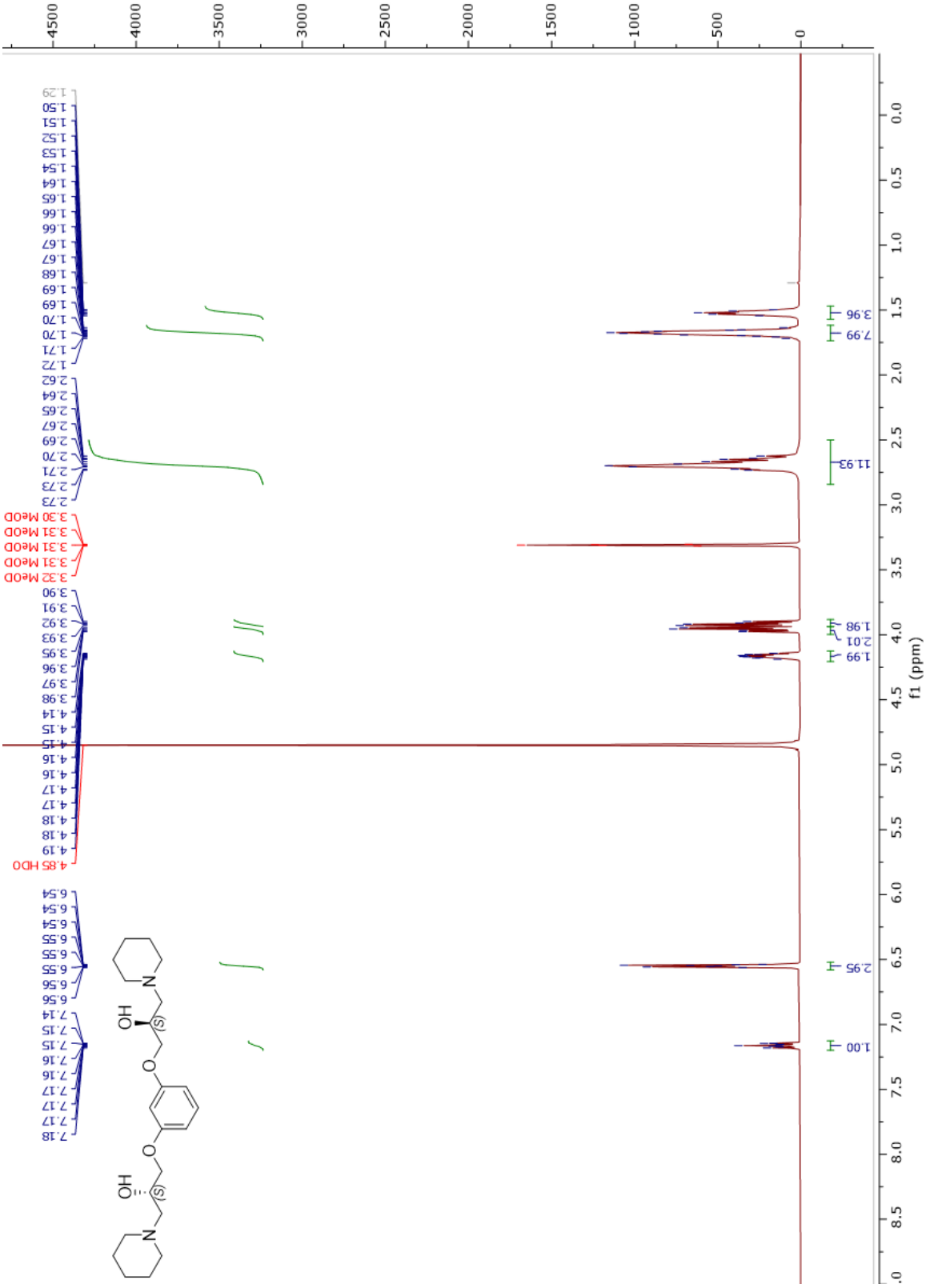
1H NMR for 1,3-bis(((S)-oxiran-2-yl)methoxy)benzene (-)-2S



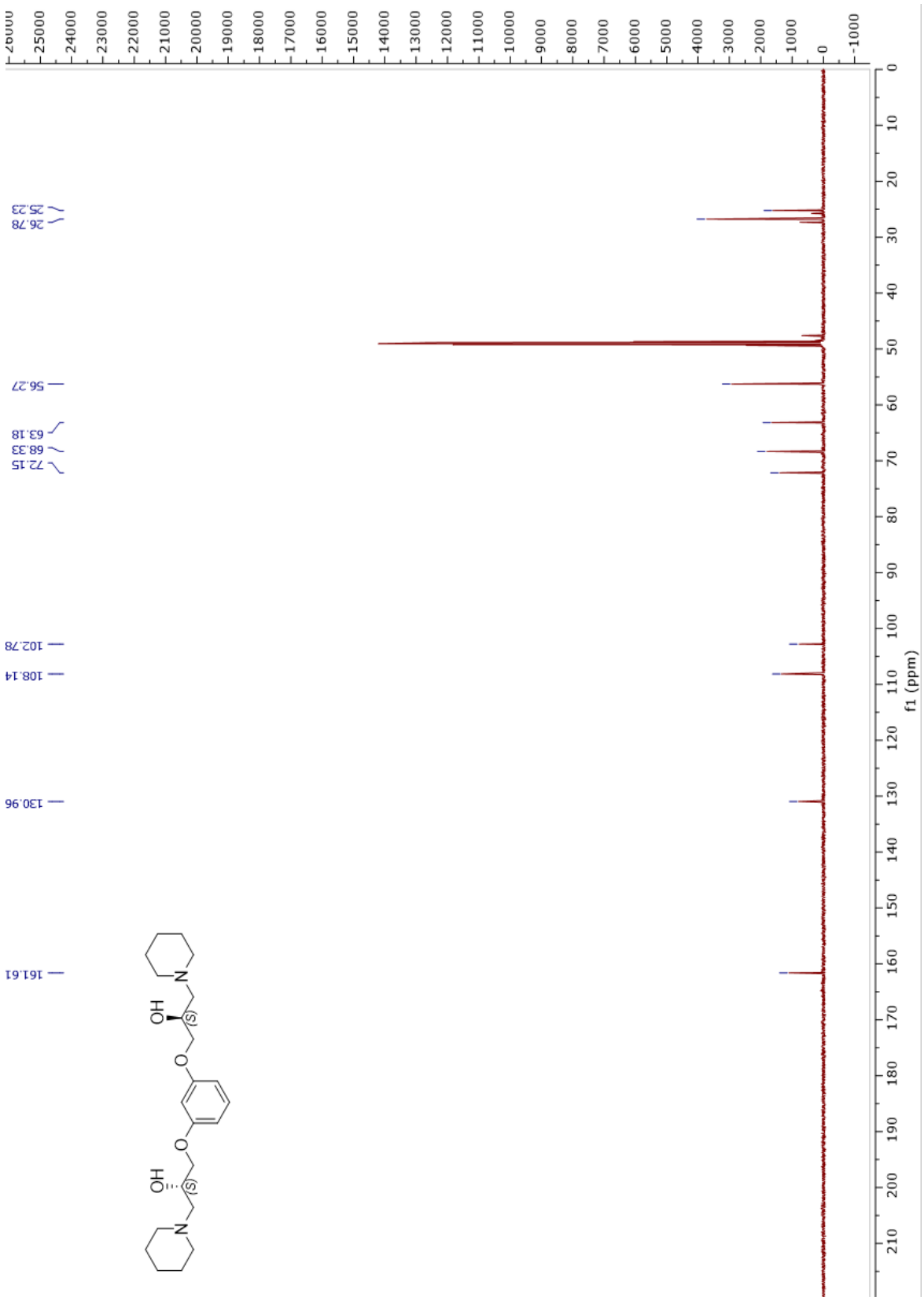
^{13}C NMR for 1,3-bis(((S)-oxiran-2-yl)methoxy)benzene (-)-2S



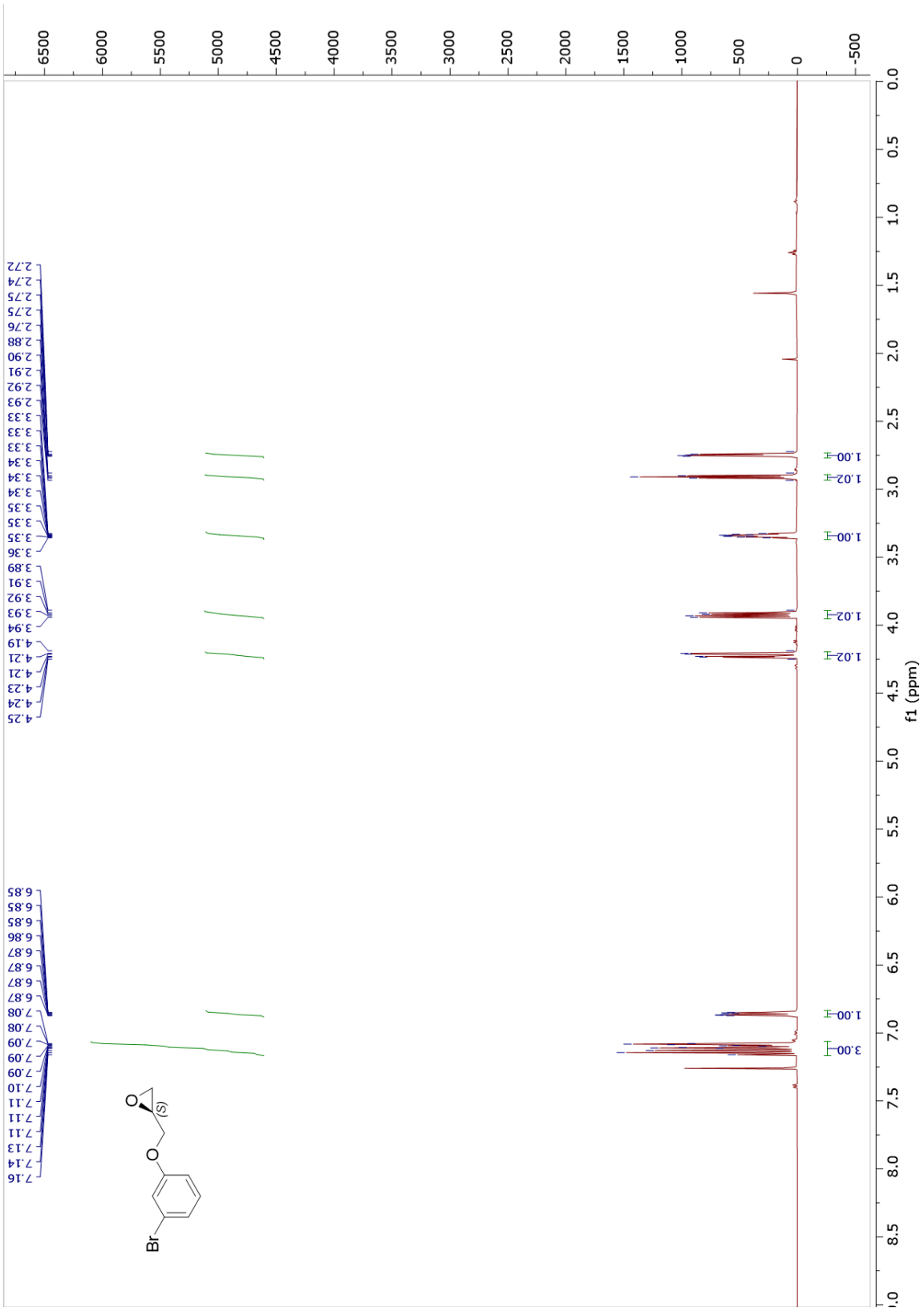
[1]H NMR for (2S,2'S)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (-)-3SS



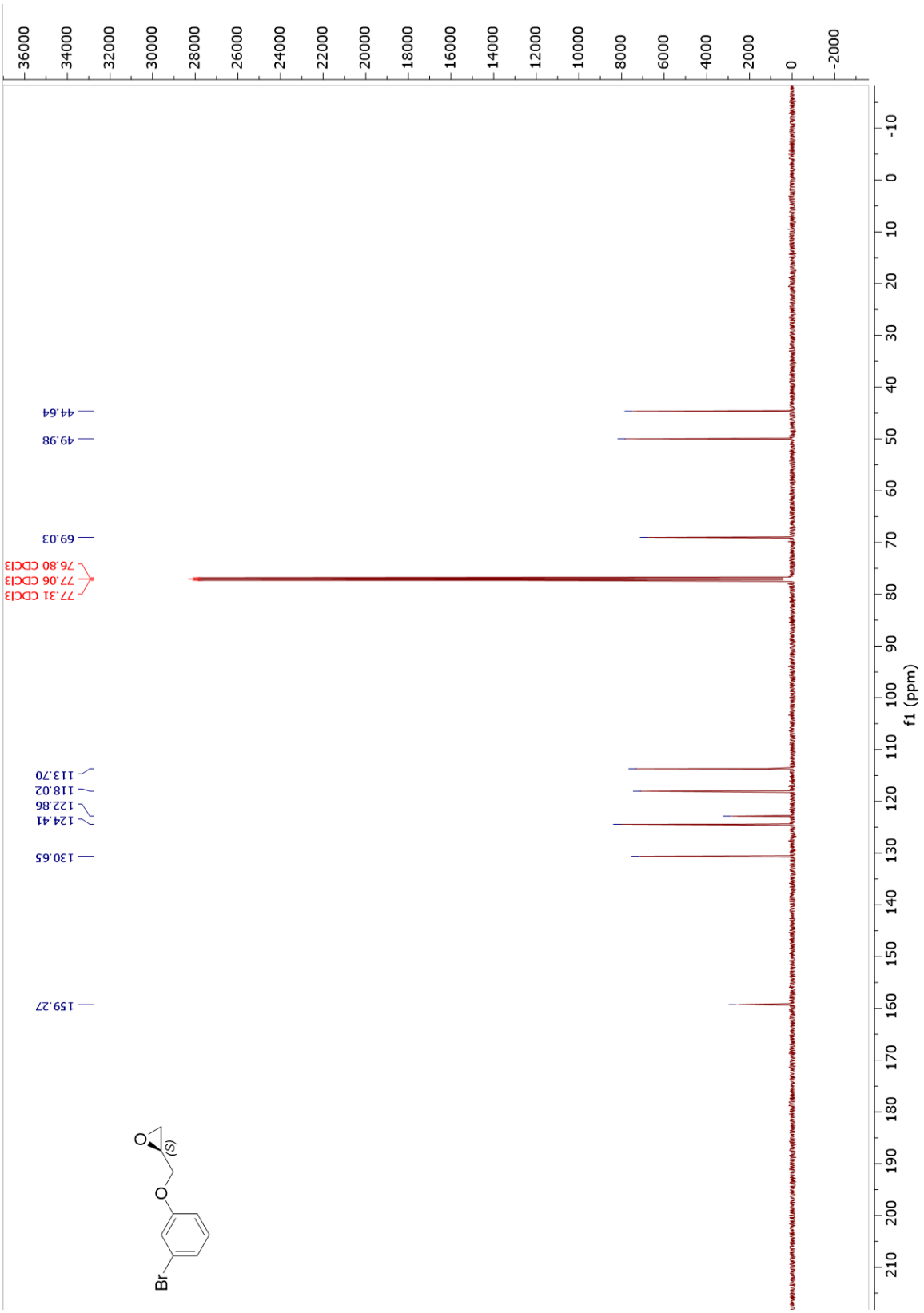
^{13}C NMR for (2S,2'S)-3,3'-(1,3-phenylenebis(oxy))bis(1-(piperidin-1-yl)propan-2-ol) (-)-3SS



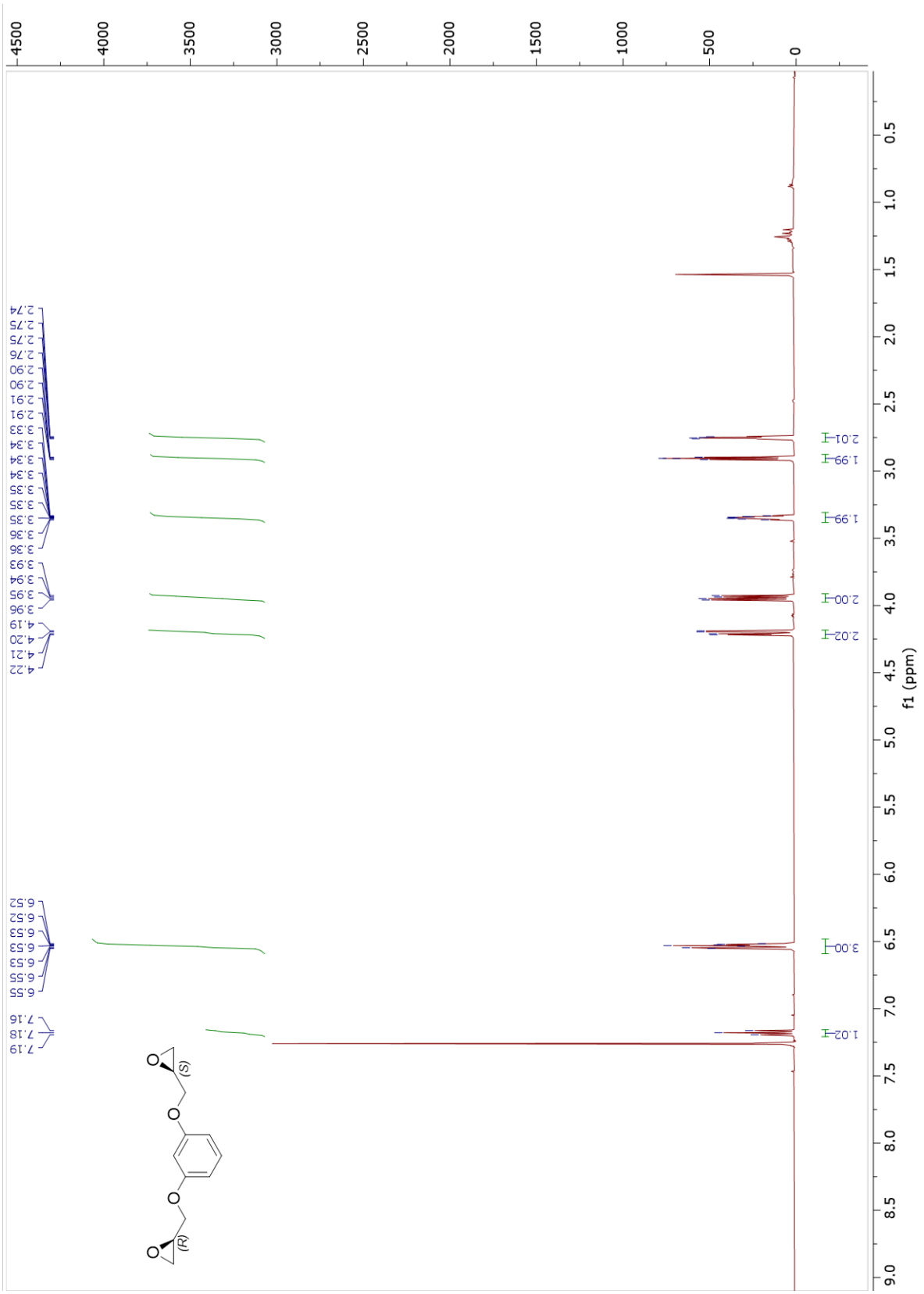
^{1}H NMR for (S)-2-((3-bromophenoxy)methyl)oxirane (+)-5



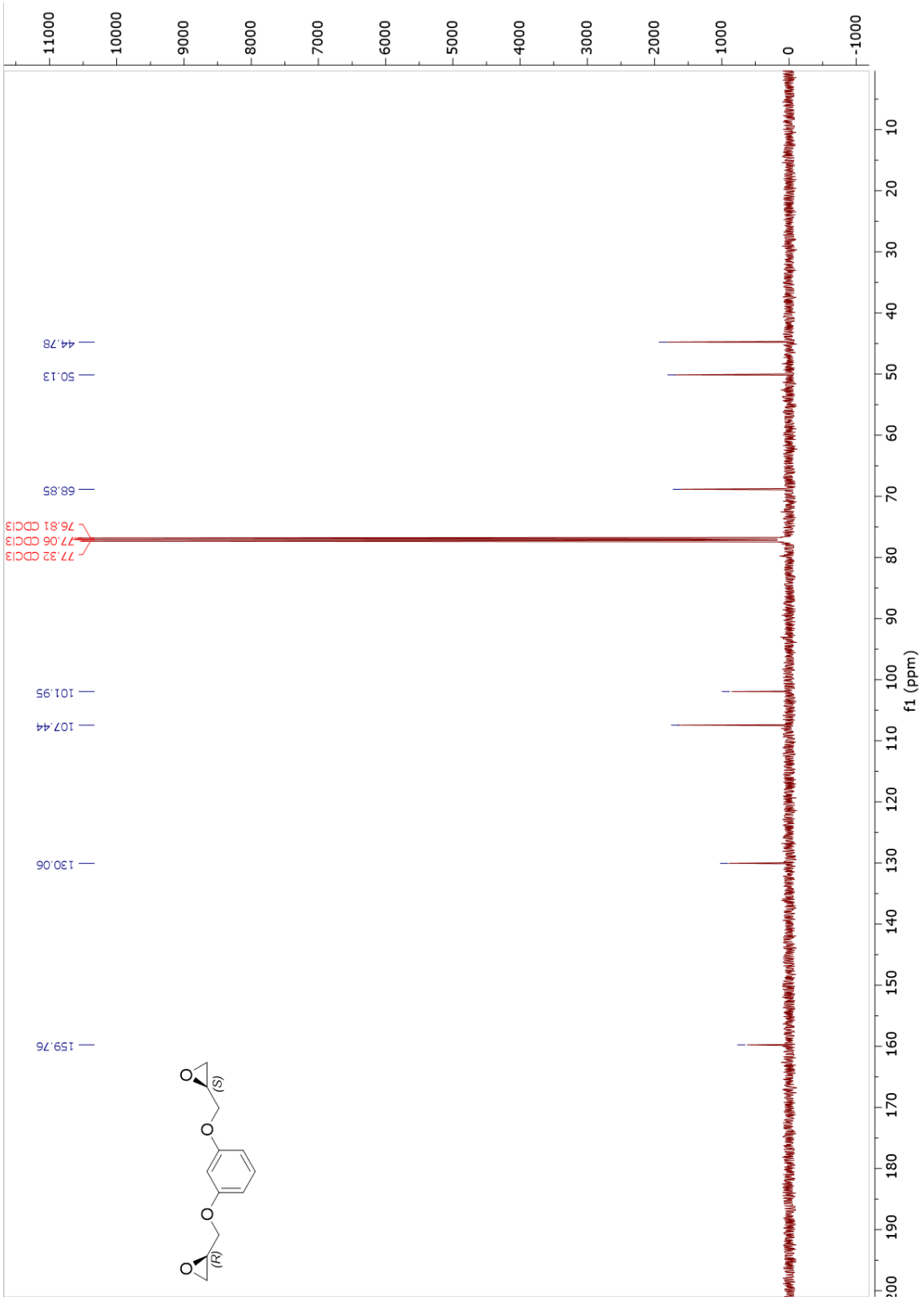
^{13}C NMR for (S)-2-((3-bromophenoxy)methyl)oxirane (+)-5



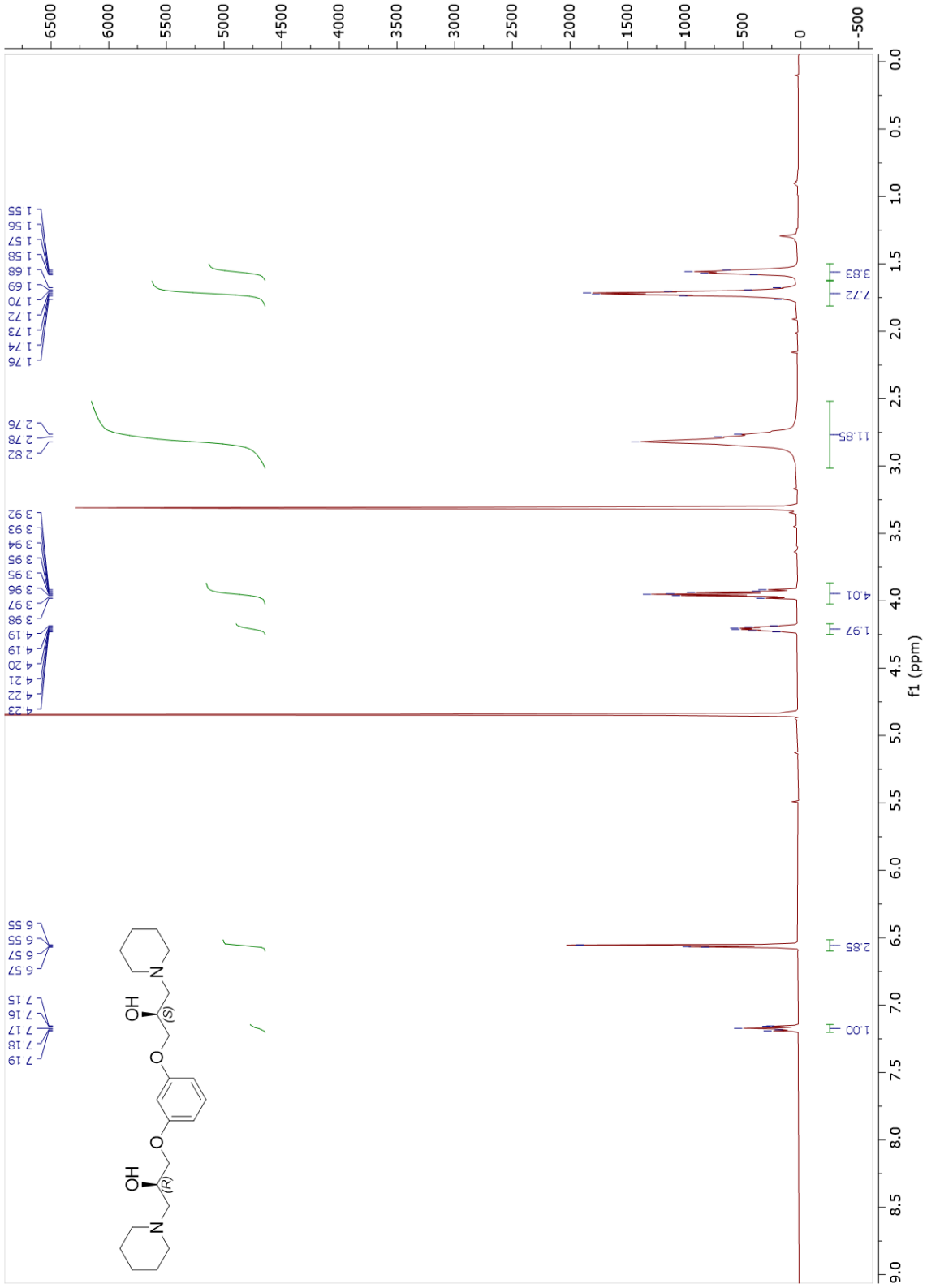
^{1}H NMR for 1-(((R)-oxiran-2-yl)methoxy)-3-(((S)-oxiran-2-yl)methoxy)benzene 2RS



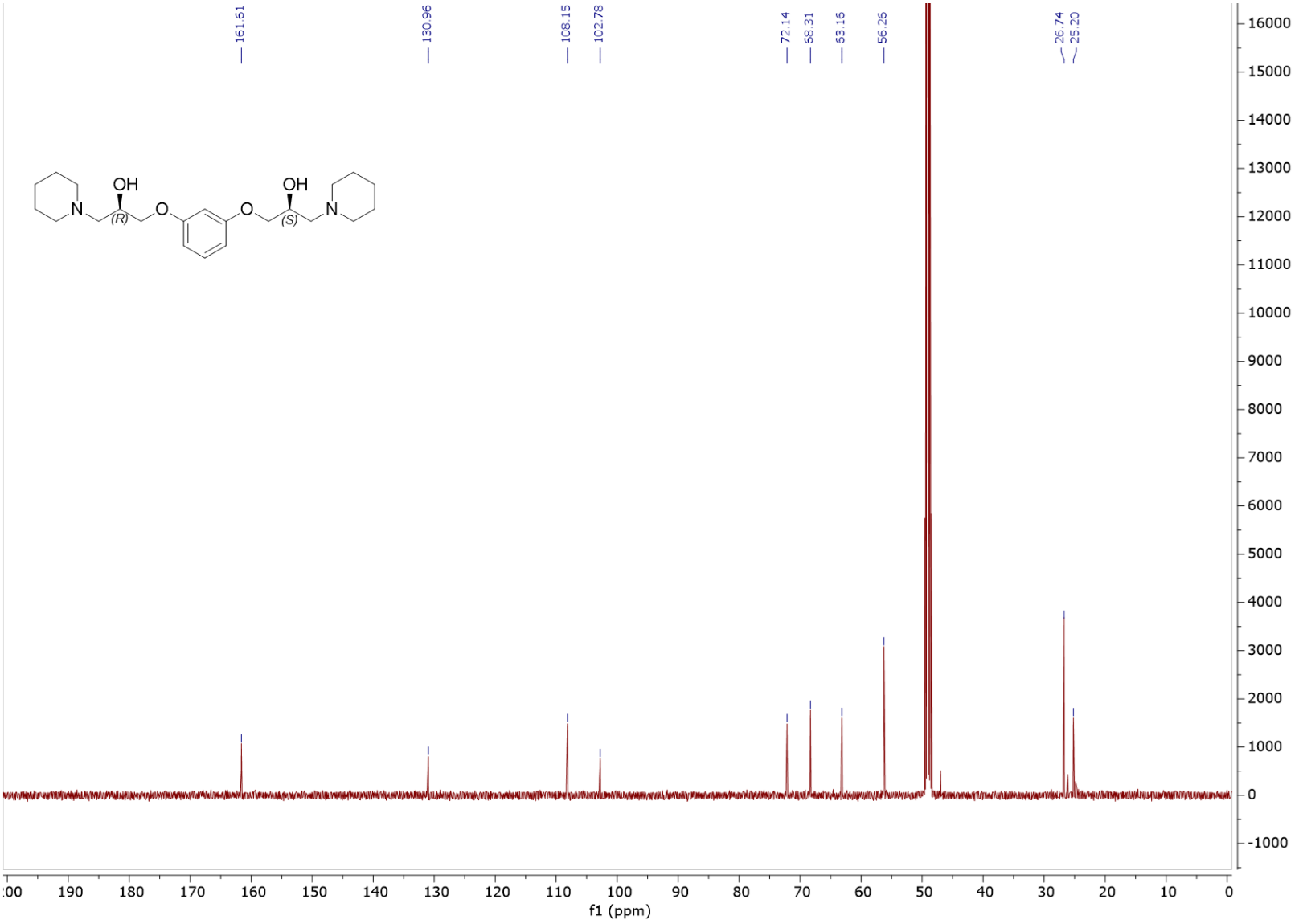
^{13}C NMR for 1-(((R)-oxiran-2-yl)methoxy)-3-(((S)-oxiran-2-yl)methoxy)benzene 2RS



^{1}H NMR for (S)-1-(3-((R)-2-hydroxy-3-(piperidin-1-yl)propoxy)phenoxy)-3-(piperidin-1-yl)propan-2-ol 3RS



^{13}C NMR for (S)-1-(3-((R)-2-hydroxy-3-(piperidin-1-yl)propoxy)phenoxy)-3-(piperidin-1-yl)propan-2-ol 3RS


161.61
130.96
108.15
102.78
72.14
68.31
63.16
56.26
26.74
25.20
OH
OH
N
O
O
N
(R)
(S)
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)