Enantioselective, Ketoreductase-Based Entry into Pharmaceutical Building Blocks: Ethanol as Tunable Nicotinamide Reductant

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SUPPORTING INFORMATION

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I. General Experimental

THF and ether were distilled from sodium benzophenone ketyl. Methanol was distilled over magnesium and iodine. Dichloromethane and diisopropylamine were distilled over CaH_2. Other reagents were obtained from commercial sources and used without further purification. Reaction progress was monitored by TLC or GC-MS (HP model 5890 GC with model 5972 MS). Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). ^{1}H NMR spectra were recorded on Bruker-DRX-Avance-300, 400 or 500 MHz instruments, with chemical shifts reported relative to residual $CHCl_3$ (7.25 ppm). Proton-decoupled ^{13}C NMR spectra were acquired on Bruker-DRX-Avance-300, 400 or 500 MHz instruments, with chemical shifts reported relative to $CDCl_3$ (77.0 ppm). High resolution mass spectra were acquired at the Nebraska Center for Mass Spectrometry (University of Nebraska). Chiral HPLC analysis was performed either on a Chiralcel OD column or an (*S*,*S*)-WHELK O-1 Pirkle column, as specified in the procedures. Elution was carried out with isopropanol/hexane, at the percentages and flow rates given. Yeast aldehyde dehydrogenase (YAlDH) was purchased from Roche, Yeast alcohol dehydrogenase (YADH) and *Lactobacillum kefir* alcohol dehydrogenase (LKADH) were purchased from Sigma. KRED (ketoreductase) enzymes were purchased from Codexis, CPADH and RS1ADH were generously provided by Jülich Chiral Solutions and, unless otherwise specified, S. I. units for all ADH enzymes are defined by the following standard UV assay: the reduction of acetophenone (10 mM) with NAD(P)H (0.2 mM) in 50 mM potassium phosphate buffer, pH 7.0, containing 5% (v/v) DMF, at 22°C.

II. Synthetic Chemistry

Procedure A: Typical Conditions for Ketone Screening

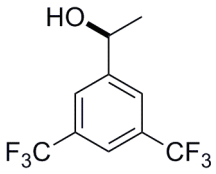
A solution or suspension of ketone bui1ding block (1-5 μmol, 1-5 mM), NAD(P)H cofactor (1-1.1 eq., 1-5.5 μmol, unless otherwise specified), and ADH enzyme (as indicated) in 50 mM KPO_4 buffer pH 6.0 (1 mL final volume) was shaken at 350 rpm at 30°C. Reaction progress was monitored by TLC. Successful combinations of ketone and dehydrogenase were often run for 24 h to provide a point of comparison. The crude reaction mixture was then extracted with ethyl acetate, and the combined extracts dried over Na_2SO_4. Following vacuum filtration and solvent evaporation, in vacuo, the reaction progress was examined by NMR. Conversion was estimated by integration of the characteristic signals of alcohol product and residual starting ketone in the crude ^{1}H NMR spectrum.

Procedure B: Preparation of HPLC Standards (Ketone Reduction with $NaBH_4$)

To a solution of ketone building block (1 mmol) in MeOH (10 mL) at 0°C was added $NaBH_4$ (1 mmol, 37 mg). After completion of the reaction (monitored by TLC), the reaction mixture was quenched by addition of NH_4Cl (aq, sat'd) and extracted with ether. The combined organic layers were dried over Na_2SO_4, filtered and evaporated, in vacuo. Where needed, samples were further purified chromatographically to provide racemic, secondary alcohols as HPLC standards, for the targeted, value-added products.

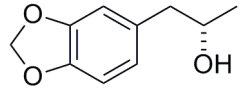
Procedure C: Typical Conditions for Ketone Reductions with EtOH-Based NADH Cofactor Recycling

A solution or suspension of ketone building block (1-5 μmol, 1-5 mM), NAD^+ cofactor (0.001-0.01 eq., 1-50 nmol, 1-50 μM), CPADH stock solution (20-50 μL, 0.13-0.33 acetophenone units), EtOH (5 vol%) and YAlDH (0.5 mg solid) in 50 mM KPO_4 buffer pH 7.5 (1 mL final volume) was shaken at 350 rpm for 1 day at 30°C. The reaction mixture was then extracted with ethyl acetate, dried over Na_2SO_4, and the solvent was evaporated. Conversion was determined by integration of peaks characteristic of product alcohol and residual ketone in the ^1H NMR spectrum.



(*S*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethanol (2).

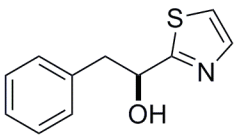
From commercially available 1-[3,5-Bis(trifluoromethyl)phenyl]ethanone following procedure A with CPADH (0.33 acetophenone U), the title compound was obtained with complete conversion and 98% ee for the *S* enantiomer,[1] determined by ^1H NMR spectrum analysis after synthesis of the Mosher ester derivative or chiral HPLC with a Chiralcel OD column, 98% hexane/2% isopropanol, 1 ml/min at 265 nm. NMR spectral data matched those reported earlier.[1]



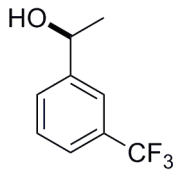
(*S*)-α-Methyl-1,3 benzodioxole-5-ethanol (4).

From 3,4-methylenedioxyphenyl acetone[2] **3** (1 mM), following procedure C (0.002 eq. NAD^+ and 0.33 CPADH acetophenone units), was obtained the title compound in 99% ee (*S* enantiomer) @ 75% conversion (375 cofactor regeneration cycles). Absolute stereochemistry and ee were determined by chiral HPLC (Pirkle (*S*,*S*)-WHELK O-1 column, 1 mL/min flow rate, isopropanol/hexane 1:99, or Chiralcel OD, isopropanol/hexane 1:99, λ = 265 nm).. The assignment of the absolute stereochemistry was made by comparison of the relative retention times to those described by the Eli Lilly Process Chemistry Group.[3] NMR spectral data matched those reported in the literature.[4]

On a larger scale, a solution of 3,4-methylenedioxyphenyl acetone (178 mg, 1 mmol), NAD^+ (2.7 mg, 4 μmol, 0.4 mol %), CPADH (26 acetone U), YADH (4 mg, 1.8 KU), YAlDH (4 mg, 4.4 U, + 4.4 mg after 10h) and BSA (200 mg) in 36 mL KPO_4 buffer pH 7.5 (50 mM) and EtOH (4 mL) was shaken at 350 rpm and 30°C for 20h. The reaction mixture was extracted with Et_2O, the organic phases dried over Na_2SO_4, evaporated and the residue purified by column chromatography on silica gel to give 161 mg (89% yield) of the title compound in 94% ee. $[\alpha]_{365}$ = +120.3° (c 1.1, $CHCl_3$) for 94% ee, {lit[4]: $[\alpha]_D$ = +117.2° (c 1.0, $CHCl_3$) for 99% ee}.

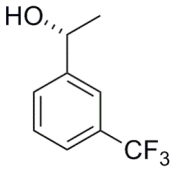


(*S*)-2-Phenyl-1-(thiazol-2-yl)ethanol (6).
From 2-phenyl-1-(thiazol-2-yl)ethanone **5**, following procedure A, with KRED 132 (0.19 acetophenone U), the title compound was obtained in 98% ee *S* enantiomer (@83% conversion), as determined by chiral HPLC (Chiralcel OD column, 1 mL/min flow rate, 2.5% isopropanol/97.5% hexane, *R* alcohol: 29.8 min, *S* alcohol: 33.7 min, λ = 254 nm). NMR spectral data matched those reported previously.[5] Absolute stereochemistry was established by preparing an authentic sample of the *R* alcohol by ketone reduction with (+)-DIPCl, followed by a comparison of HPLC retention times.[6]

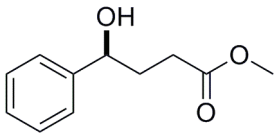


(*S*)-1-(trifluoromethyl)phenylethanol (*S*)-8.
From commercially available 1-(trifluoromethyl)phenylethanone following procedure A (with RS1ADH, 0.51 acetophenone U), the title compound was obtained with complete conversion and 99% ee for the *S* enantiomer, determined by chiral HPLC of the *p*-bromobenzoyl derivative with a Chiralcel OD column, 99% hexane/1% isopropanol, 1 ml/min at 254 nm. NMR spectral data matched those reported earlier.[10]

On a larger scale, a solution of 1-(trifluoromethyl)phenylethanone (188 mg, 1 mmol), NAD^+ (6.8 mg, 10 μmol, 1 mol %), RS1ADH (41 acetophenone U), YADH (4 mg, 1.8 KU), YAlDH (10 mg, 11 U) and BSA (200 mg) in 36 mL KPO_4 buffer pH 7.5 (50 mM) and EtOH (4 mL) was shaken at 350 rpm and 30°C for 12h. The reaction mixture was extracted with Et_2O, the organic phases dried over Na_2SO_4 and evaporated to give 186 mg (98% yield) of (*S*)-1-(trifluoromethyl)phenylethanol in 99% ee. $[\alpha]_D$ = -28.0° (c 1.64, MeOH) for 99% ee, {lit[10]: $[\alpha]_D$ = -27.9° (c 1.64, MeOH) for >99% ee}.



(*R*)-1-(trifluoromethyl)phenylethanol (*R*)-8.
A solution of 1-(trifluoromethyl)phenylethanone (188 mg, 1 mmol), $NADP^+$ (15.2 mg, 20 μmol, 2 mol %, in two portions), LKADH (42 acetophenone U, in 3 portions), YAlDH (40 mg, 44 U) and BSA (200 mg) in 36 mL KPO_4 buffer pH 7.5 (50 mM) and EtOH (4 mL) was shaken at 350 rpm and 30°C for 30h. The reaction mixture was extracted with Et_2O, the organic phases dried over Na_2SO_4 and evaporated, along with residual ketone, to give 122 mg (64% yield) of (*R*)-1-(trifluoromethyl)phenylethanol compound in 86% ee.

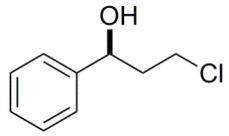


(*S*)-Methyl 4-hydroxy-4-phenylbutyrate (10).
From commercially available methyl 3-benzoylpropionate and following procedure A with KRED 132 (0.19 acetophenone U), the title compound was obtained in 97% ee *S* enantiomer (@ 82% conversion), as determined by

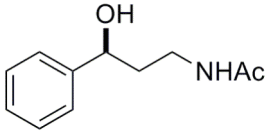
chiral HPLC (Chiralcel OD column, 1 mL/min flow rate, 1.5% isopropanol/98.5 % hexane, λ = 220 nm). NMR spectral data were identical to those reported.[8] Absolute stereochemistry was determined by comparison of HPLC retention time with a known standard. Specifically, methyl 3-benzoylpropionate was asymmetrically reduced using the (*S*)-B-Me-Itsuno-Corey oxazaborolidine catalyst to give the *R* enantiomer.[9]

On a larger scale, a solution of 3 methyl 3-benzoylpropionate (192 mg, 1 mmol), $NADP^+$ (7.6 mg, 10 μmol, 1 mol %), KRED132 (9.5 acetophenone U), LKADH (50 mg, 20 acetophenone U), YAlDH (50 mg, 55 U), BSA (200 mg) and glycerol (5 mL) in 93 mL KPO_4 buffer pH 7.5 (50 mM) and EtOH (2 mL) was shaken at 350 rpm and 30°C for 12h. The reaction mixture was extracted with Et_2O, the organic phases dried over Na_2SO_4, evaporated and the residue purified by column chromatography on silica gel to give 150 mg of a 60:40 mixture of lactone and alcohol (86% yield) in 96% ee.

Some cyclization of the γ-hydroxy ester occurred under the reaction conditions. The resulting lactone can be converted to an intermediate in the synthesis of the targeted drug ((*R*)-Fluoxetine) under the same reaction conditions as for the γ-hydroxy ester.[9]



(*S*)-3-Chloro-1-phenylpropan-1-ol (12).
From commercially available 3-chloro-1-phenylpropan-1-one, following procedure A, with KRED 108 (0.07 acetophenone U), was obtained (*S*)-3-chloro-1-phenylpropan-1-ol in 88% ee (@ 85% conversion) as determined by chiral HPLC (Chiralcel OD column, 1 mL/min flow rate, 10% isopropanol/90% hexane, λ = 220 nm). NMR spectra matched literatue data.[1] $[\alpha]_D$ = -24.1° (c 1.1, $CHCl_3$) for 88% ee, {lit[10]: $[\alpha]_D$ = -23.5° (c 1, $CHCl_3$) for 99% ee}.



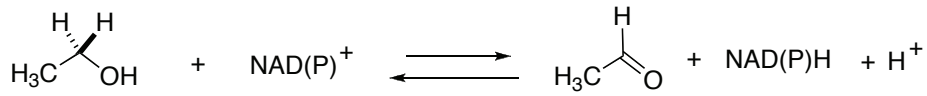
(*S*)-N-(3-hydroxy-3-phenylpropyl)acetamide (14).
From N-(3-oxo-3-phenylpropyl)acetamide **13** following procedure A (5 μmol substrate, with KRED132, 0.38 acetophenone U and 3 eq. NADPH), the title compound was obtained with 67 % conversion and >99% ee for the *S* enantiomer, determined by chiral HPLC with a Chiralcel OD column, 94% hexane/6% isopropanol, 1 ml/min at 210 nm. NMR spectral data matched those reported earlier.[11]
$[\alpha]_D$ = -19.5° (c 0.09, $CHCl_3$) for 99% ee, {lit[11]: $[\alpha]_D$ = -23.6° (c 1.3, $CHCl_3$) for >99% ee}.

III. Thermodynamics/Economics of Nicotinamide Cofactor Reductants

A. Calculation of the ΔG' of the EtOH-mediated NAD(P)H recycling reaction:

From Alberty, R. A. *Arch. Biochem. Biophys.* **1993**, *307*, 8-14:

@ P = 1 Bar, T = 25°C and I (ionic strength) = 0.1:

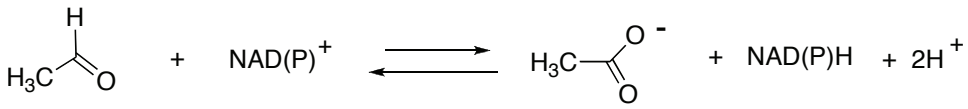


$K = 9.79 \times 10^{-12}$ M

$\Rightarrow$ $K'(pH\ 7) = 9.79 \times 10^{-5}$

$\Rightarrow$ $\Delta G' = -RT\ln K' = -(1.987 \times 10^{-3}\ \text{kcal/mol-K})(298\ \text{K}) \times \ln(9.79 \times 10^{-5})$

$\Rightarrow$ $\Delta G' = +5.47$ kcal/mol



$K = 2.95 \times 10^{-5}\ \text{M}^2$

$\Rightarrow$ $K'(pH\ 7) = 2.95 \times 10^{9}$

$\Rightarrow$ $\Delta G' = -RT\ln K' = -(1.987 \times 10^{-3}\ \text{kcal/mol-K})(298\ \text{K}) \times \ln(2.95 \times 10^{9})$

$\Rightarrow$ $\Delta G' = -12.91$ kcal/mol

Overall: $\Rightarrow$ $\Delta G' = -7.44$ kcal/mol

B. Estimates of ΔG' values for NAD(P)H regeneration with the other reductants were obtained as follows:

Glucose DH reaction (-6.92 kcal/mol from Strecker, H. J. and Korkes, S. *J. Biol. Chem.* **1952**, *196*, 769-784)

Phosphite DH reaction (-15 kcal/mol from van Relyea, H. A.; van der Donk, W. A., *Bioorganic Chemistry* **2005**, *33*, 171-189)

Formate DH reaction @ pH 7, 298 K Alberty lists ΔG' = -19.41 kJ/mol ÷ 4.184 = - 4.6 kcal/mol (Alberty, R. A. *Biophysical Chemistry* **2004**, *111*, 115-122).

C. Estimates of Reductant Cost:

Ethanol pricing: $2.20/gallon on 09/20/08 (see http://www.dtnethanolcenter.com/index.cfm?show=10&mid=32)

Where 1 gallon = 3.785 L (0.58/L) and density = 0.789 g/mL and MW = 46.07 g/mol, meaning:

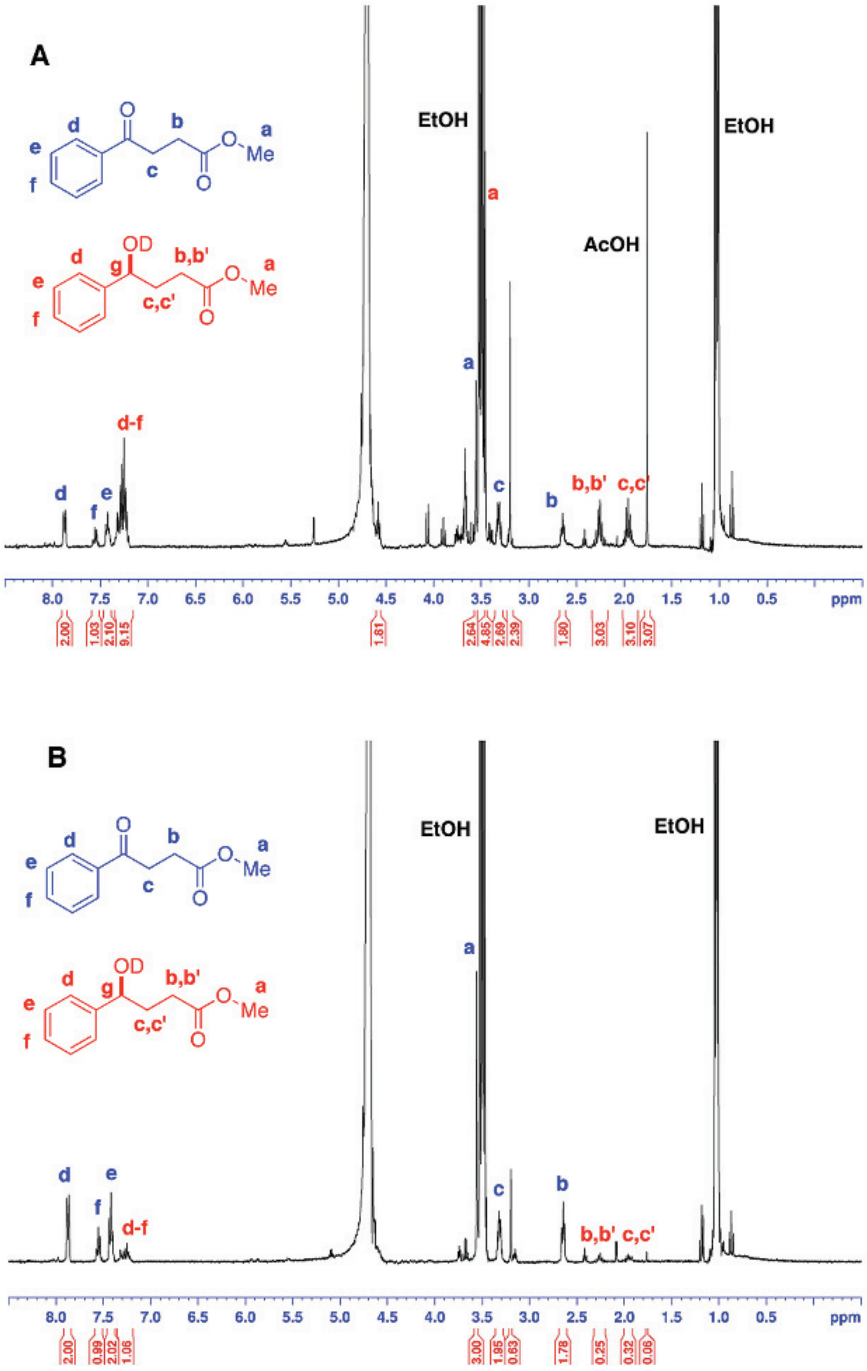
EtOH = (0.58/L ÷ 789 g/L) x 46.07 g/mol = 3.39¢ per mole 25.6 x cheaper than D-Glucose

Glucose (Dextrose) pricing:
$3.99/2 lb on 09/20/08 {see http://wholesalesupplementstore.com/now-dextrose.html (best price) or http://www.bulknutrition.com/p510_Dextrose_Now_Foods.html}
($2.00/lb ÷ 454 g/lb) x 198 g/mol (dextrose-monohydrate) = 87.0 ¢ per mole

Sodium formate pricing:
$60.80/kg (VWR price on 09/20/08) @ 68.01 g/mol
So, we have $0.068/g x 68.01 g/mol = $4.14/mol

Phosphorous acid pricing:
$54.50/500 g (Cole Parmer price on 09/20/08) @ 82.00 g/mol
So, we have $0.109/g x 82 g/mol = $8.94/mol

IV. NMR Model Study – Effect of YAlDH (four electron redox conditions)



Conditions: A solution of methyl 3-benzoylpropionate (0.96 mg, 5 mM), $NADP^+$ (0.1 μmol, 100 μM, 2 mol %), KRED132 (1 mg, 0.19 acetophenone U), LKADH (0.5 mg, 0.2 acetophenone U), EtOH (5.8 μL, 100 mM) with (**A**) or without (**B**) YAlDH (0.5 mg, 0.55 U) in deuterated KPO_4 buffer pH 7.5 (50 mM, 1mL final volume) was shaken at 300 rpm and 30°C for 3h. ^1H NMR spectra were recorded on Bruker-DRX-Avance-400 MHz instrument.

References

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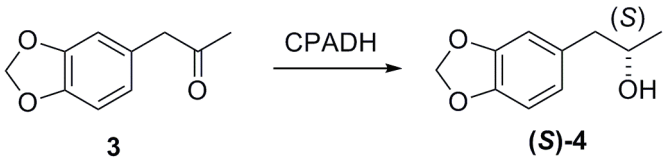
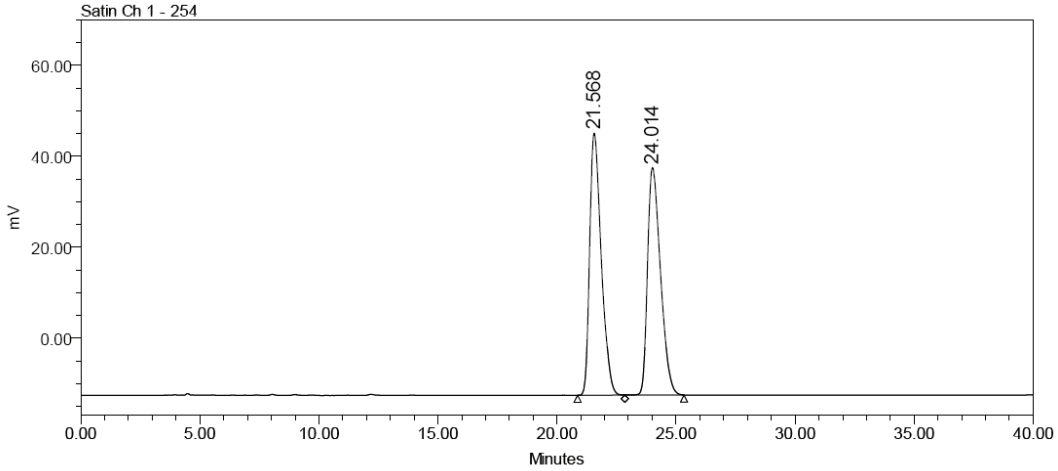
V. HPLC Traces/Mosher Esters

Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 1:99, Flow rate: 1 mL/min, λ = 265 nm.



3 **(*S*)-4**



	RT	Area	% Area	Height
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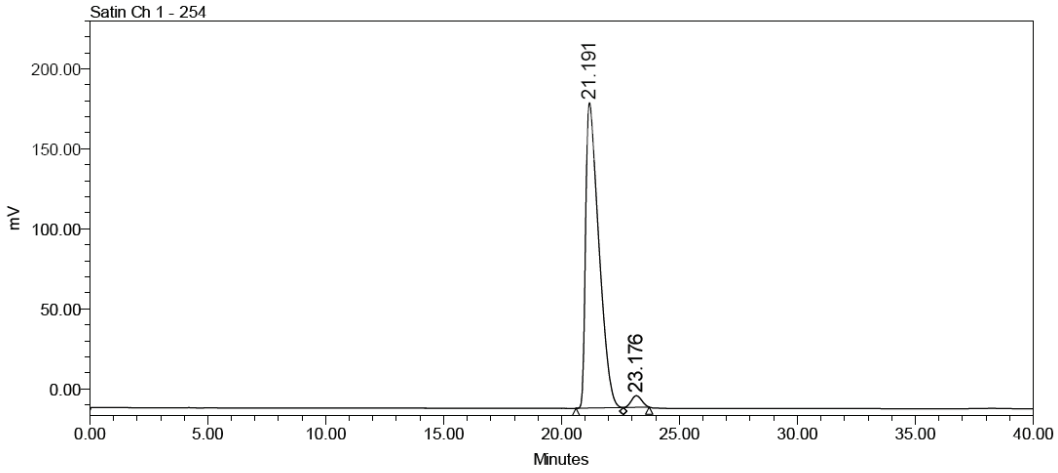
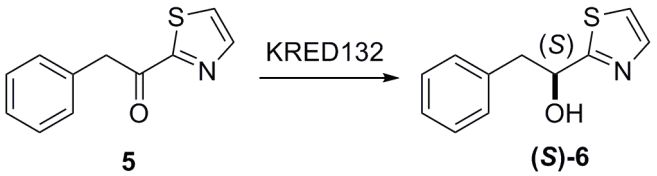
2	24.014	1896009	49.30	50019

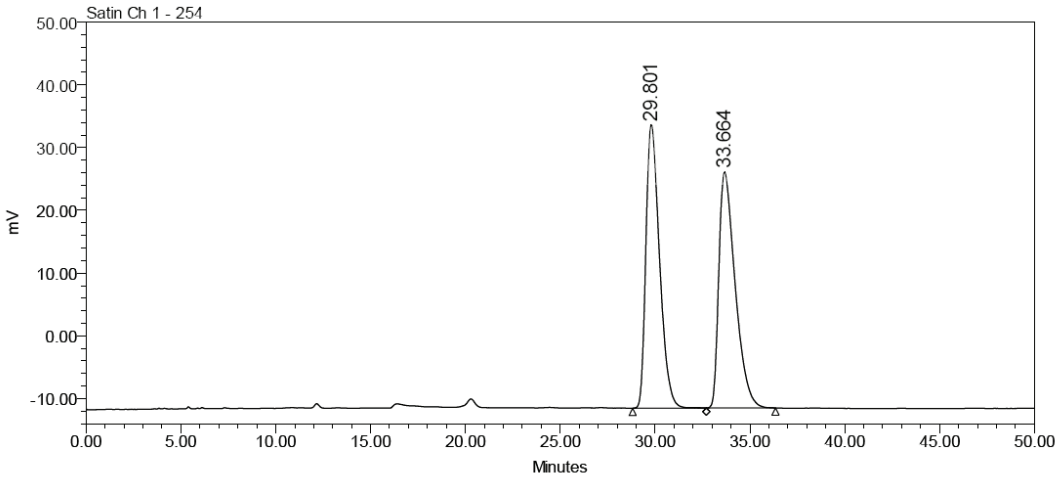


	RT	Area	% Area	Height
1	21.191	7432788	97.12	190995
2	23.176	220706	2.88	7208

Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 2.5:97.5, Flow rate: 1 mL/min, λ = 254 nm.



5 **(*S*)-6**



	Peak Name	RT	Area	% Area	Height
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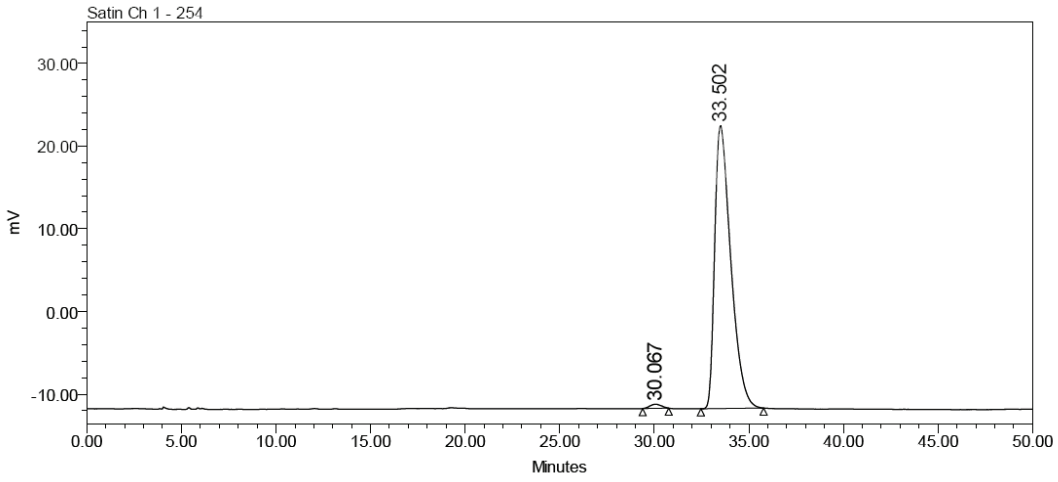
2	Peak2	33.664	2256054	49.91	37621



	Peak Name	RT	Area	% Area	Height
1	Peak1	30.067	21069	1.03	500
2	Peak2	33.502	2023789	98.97	34195

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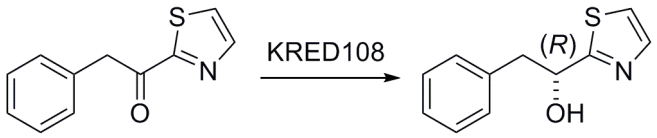
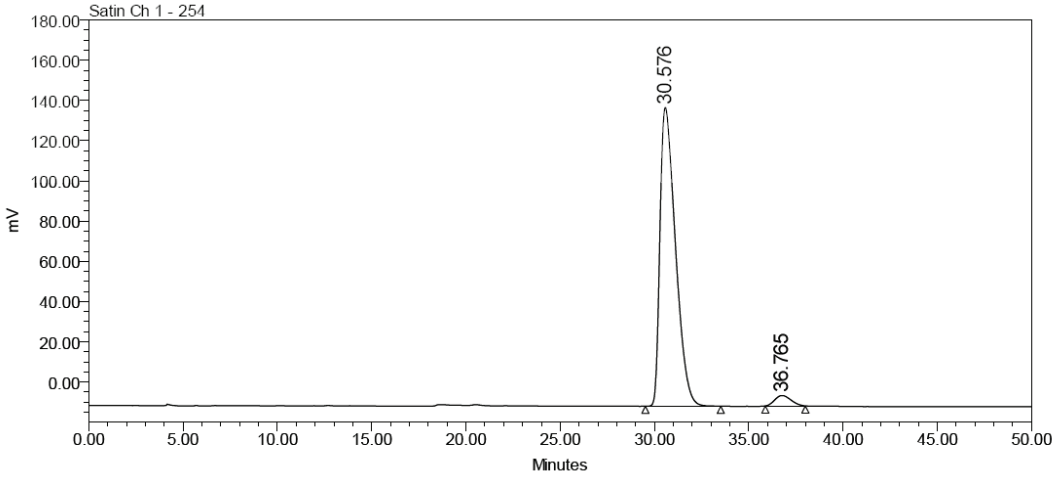
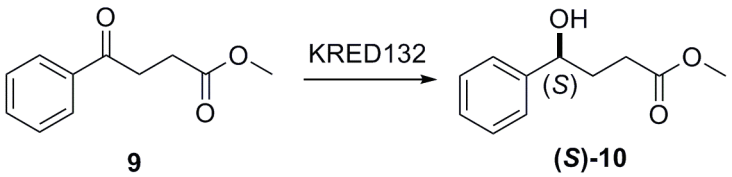


5 **(*R*)-6**

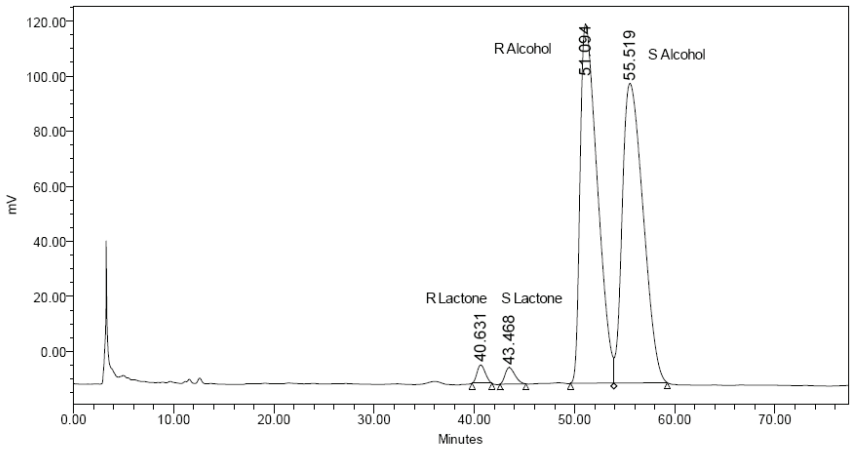


	RT	Area	% Area	Height
1	30.576	8475141	96.51	148536
2	36.765	306239	3.49	5165

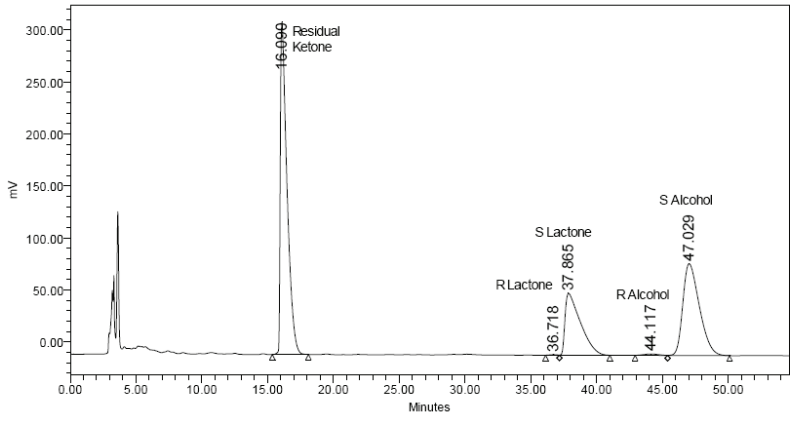
Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 1.5:98.5, Flow rate: 1 mL/min, λ = 220 nm.



9 **(*S*)-10**

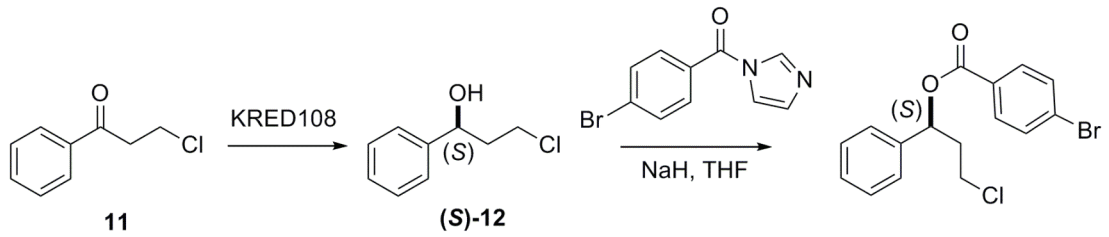


	RT	Area	% Area	Height
1	40.631	382296	1.19	6663
2	43.468	405163	1.26	5978
3	51.094	15594875	48.53	130391
4	55.519	15752099	49.02	108803

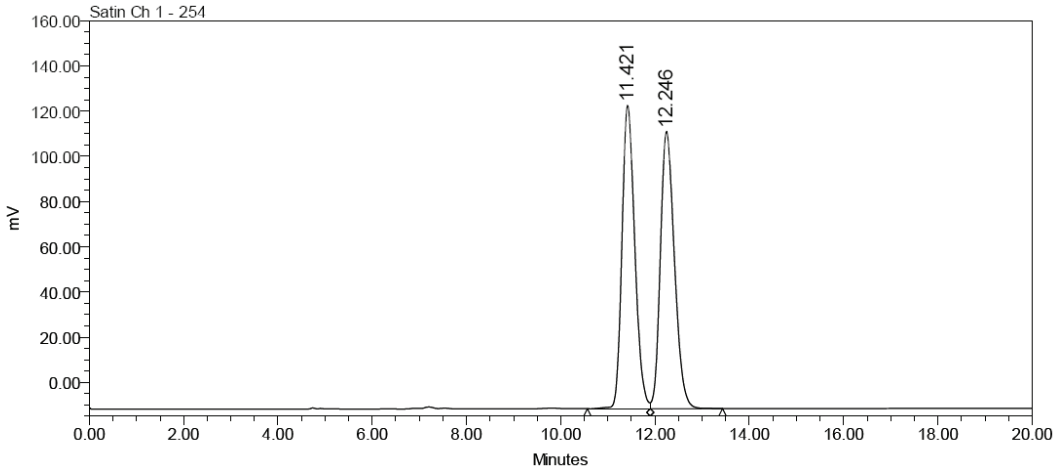


	RT	Area	% Area	Height
1	16.090	11292184	47.08	320007
2	36.718	18317	0.08	478
3	37.865	4827002	20.13	59391
4	44.117	101210	0.42	1338
5	47.029	7745808	32.30	88228

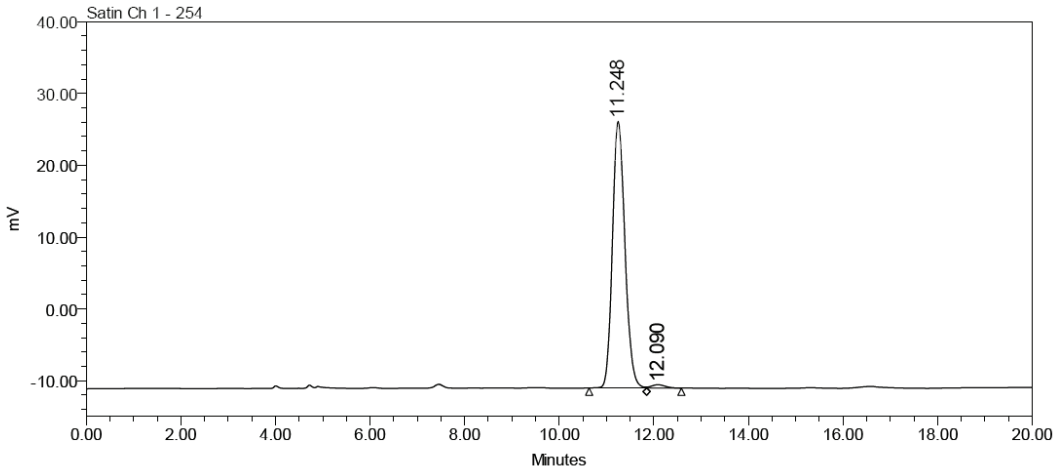
Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 1:99, Flow rate: 1 mL/min, λ = 254 nm.



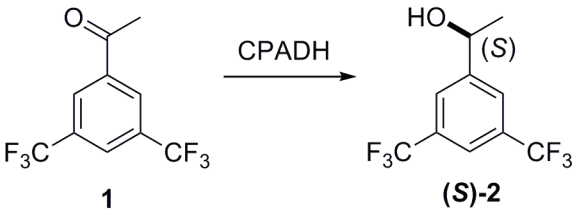
11 **(*S*)-12**



	Peak Name	RT	Area	% Area	Height
1	Peak1	11.421	2542025	49.94	134343
2	Peak2	12.246	2547637	50.06	122780

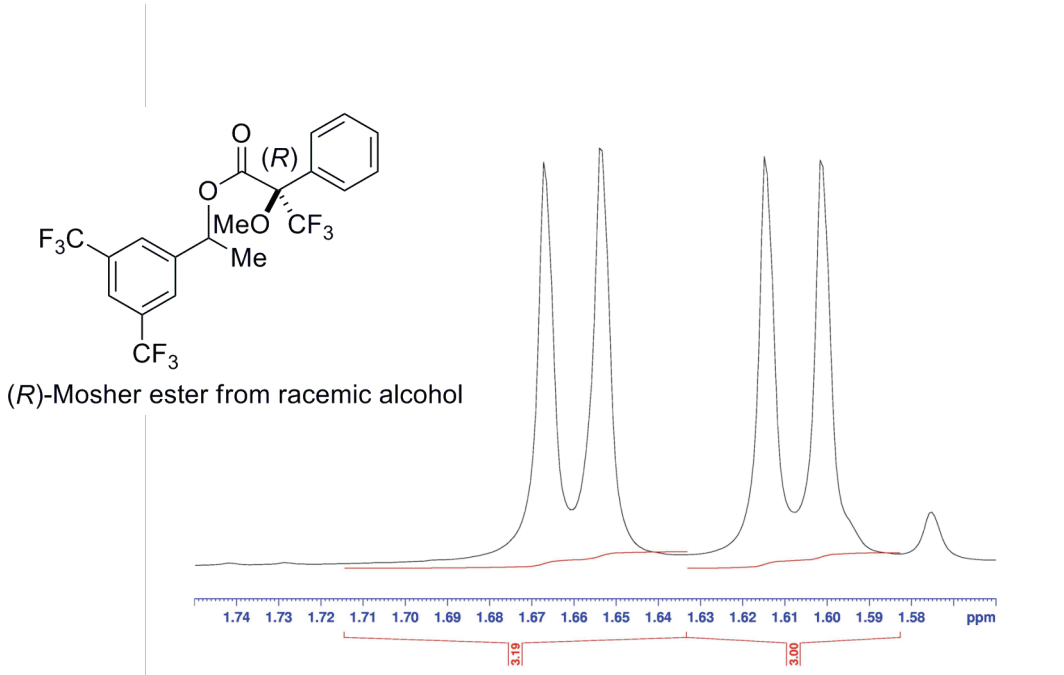


	RT	Area	% Area	Height
1	11.248	683397	98.63	37167
2	12.090	9495	1.37	468

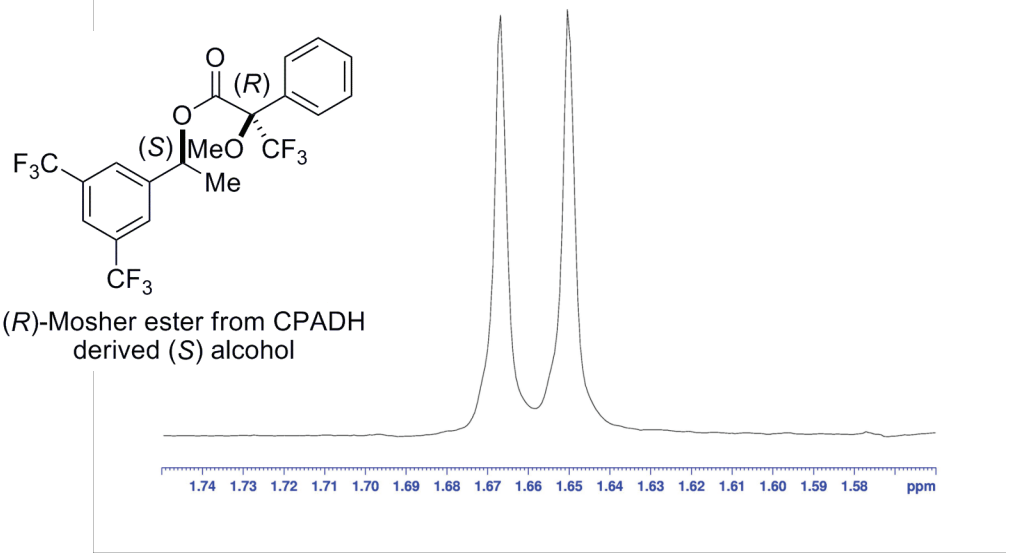


1 **(*S*)-2**

^{1}H NMR section ($CDCl_3$, 400 MHz) of the Mosher ester derivatives.

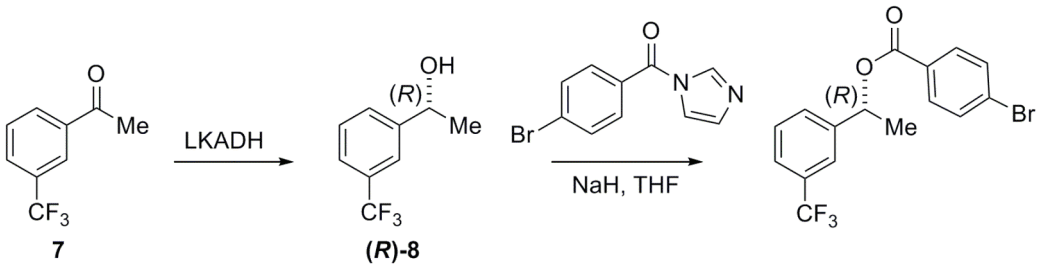


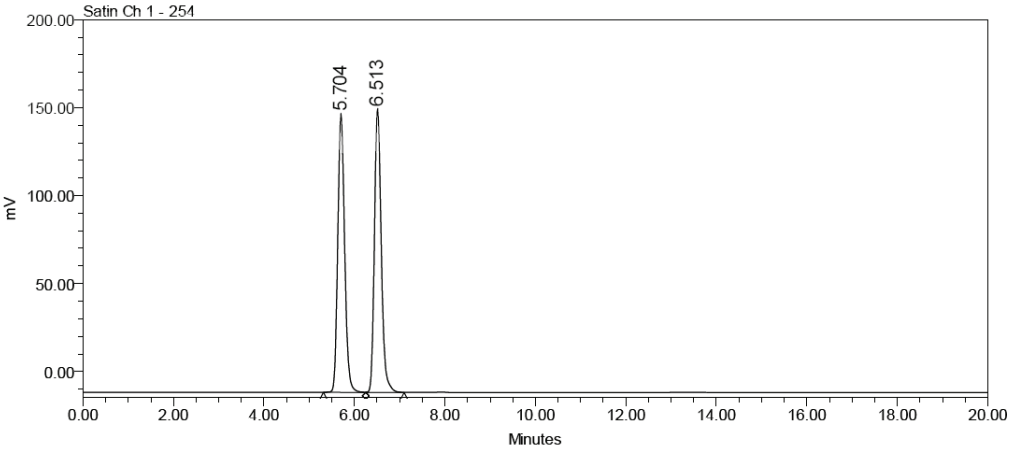
(*R*)-Mosher ester from racemic alcohol



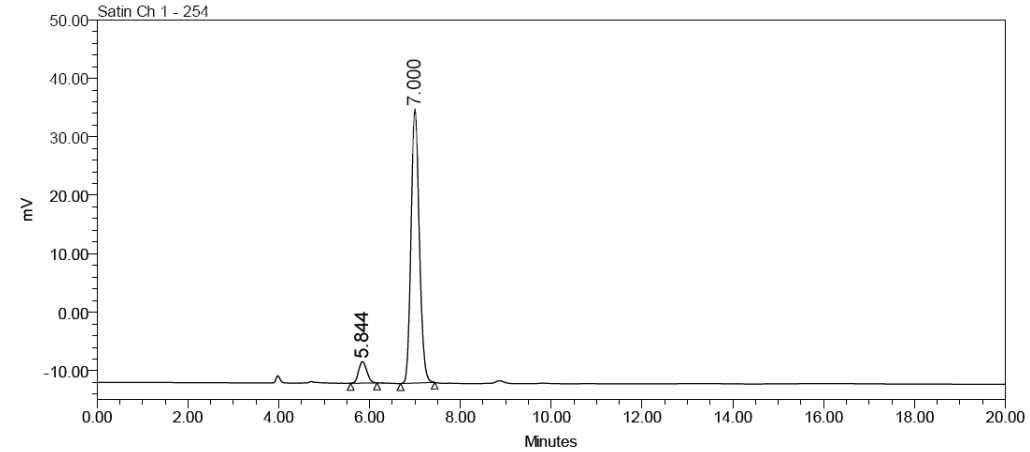
(*R*)-Mosher ester from CPADH derived (*S*) alcohol

Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 1:99, Flow rate: 1 mL/min, λ = 254 nm.



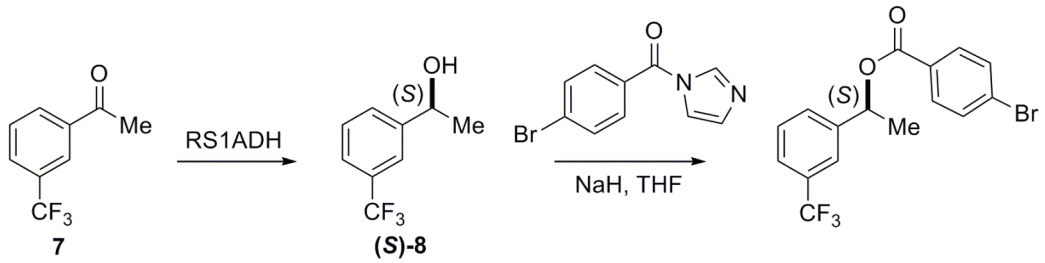


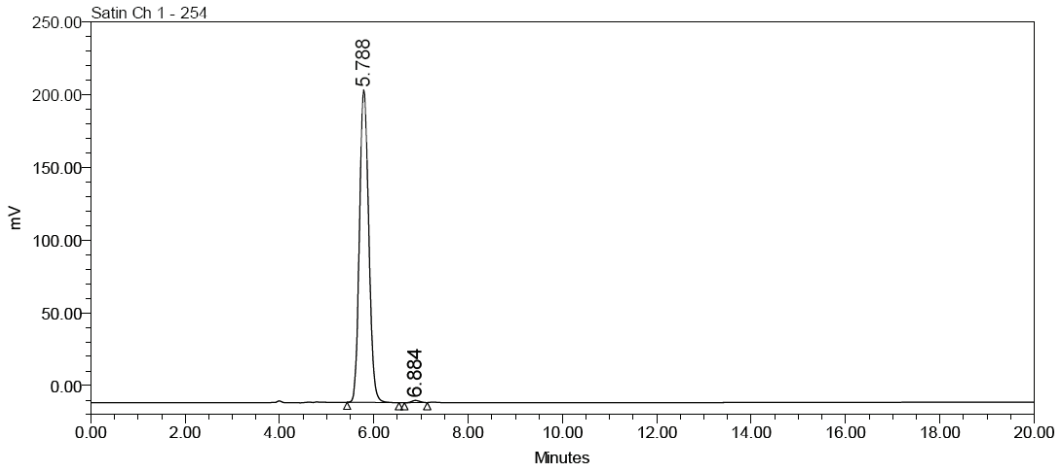
	RT	Area	% Area	Height
1	5.704	1671003	49.61	158699
2	6.513	1697065	50.39	161583



	Peak Name	RT	Area	% Area	Height
1	Peak1	5.844	46140	7.20	3682
2	Peak2	7.000	594773	92.80	46799

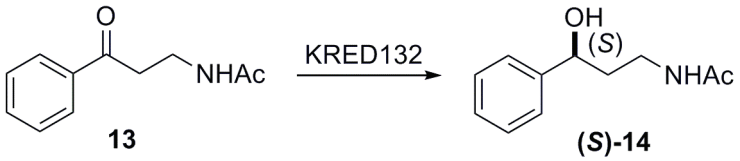
Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 1:99, Flow rate: 1 mL/min, λ = 254 nm.



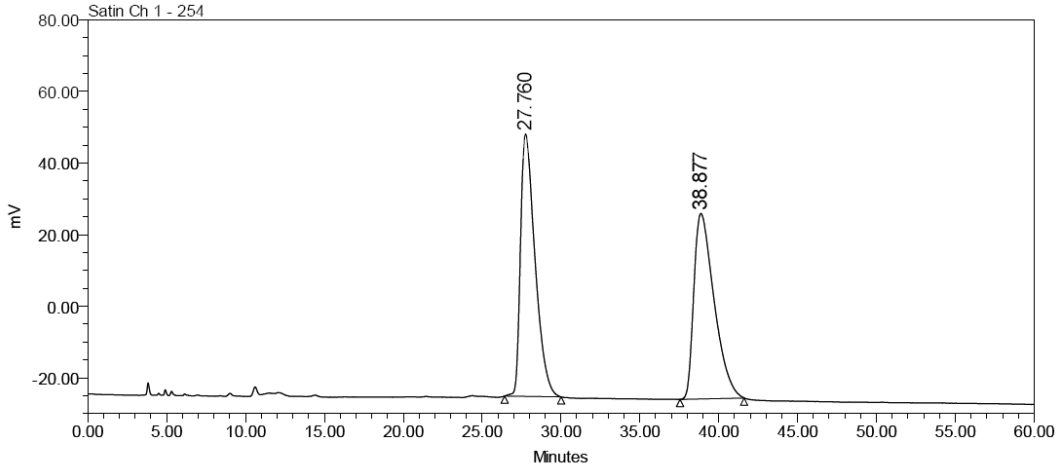


	Peak Name	RT	Area	% Area	Height
1	Peak1	5.788	2944227	99.42	214980
2	Peak2	6.884	17078	0.58	1524

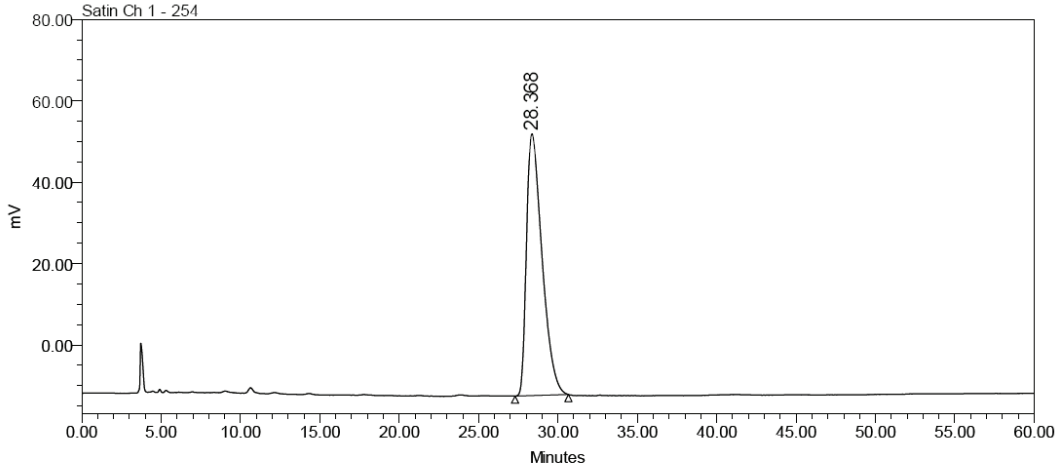
Conditions: Column Chiralcel OD, Eluent: isopropanol/hexane 6:94, Flow rate: 1 mL/min, λ = 210 nm.



13 **(*S*)-14**

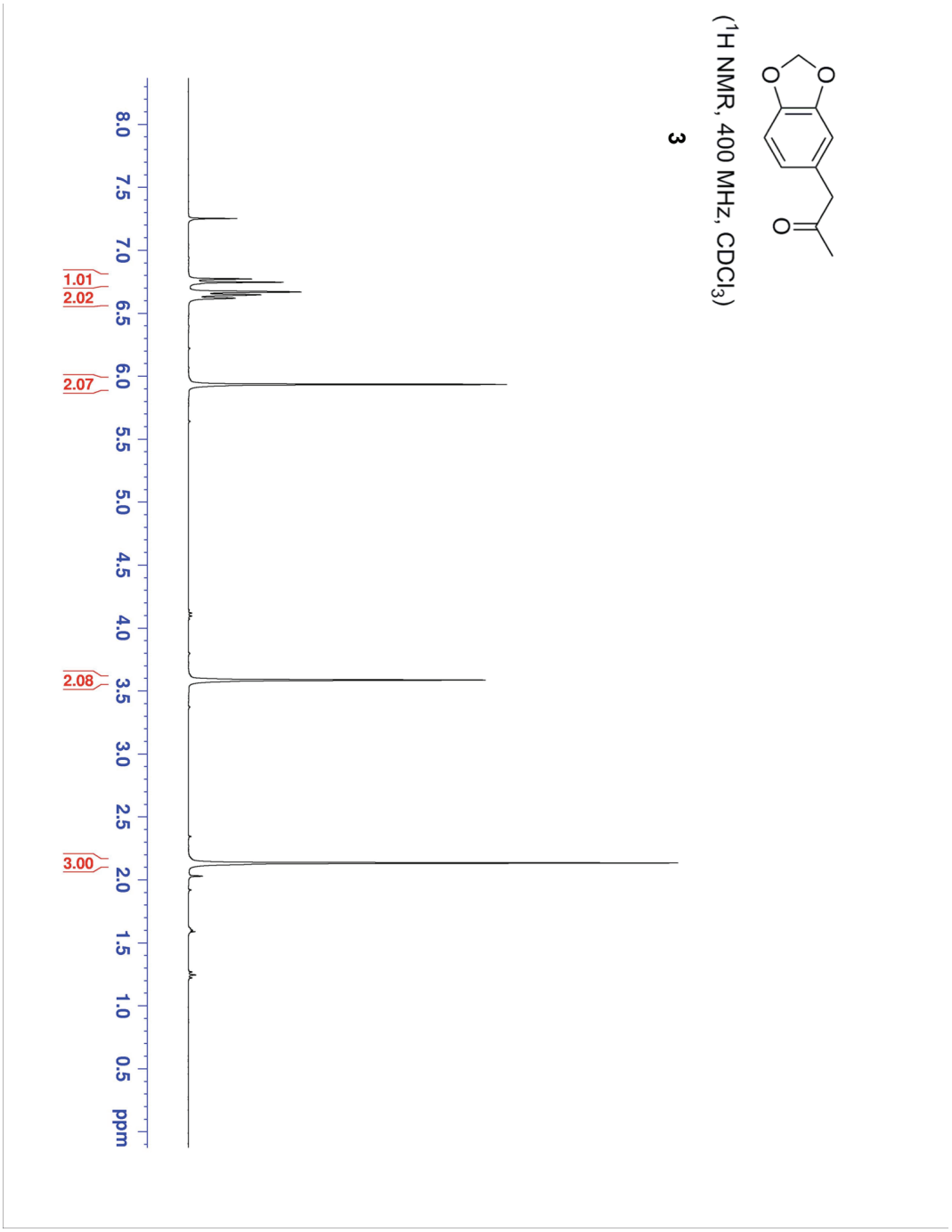


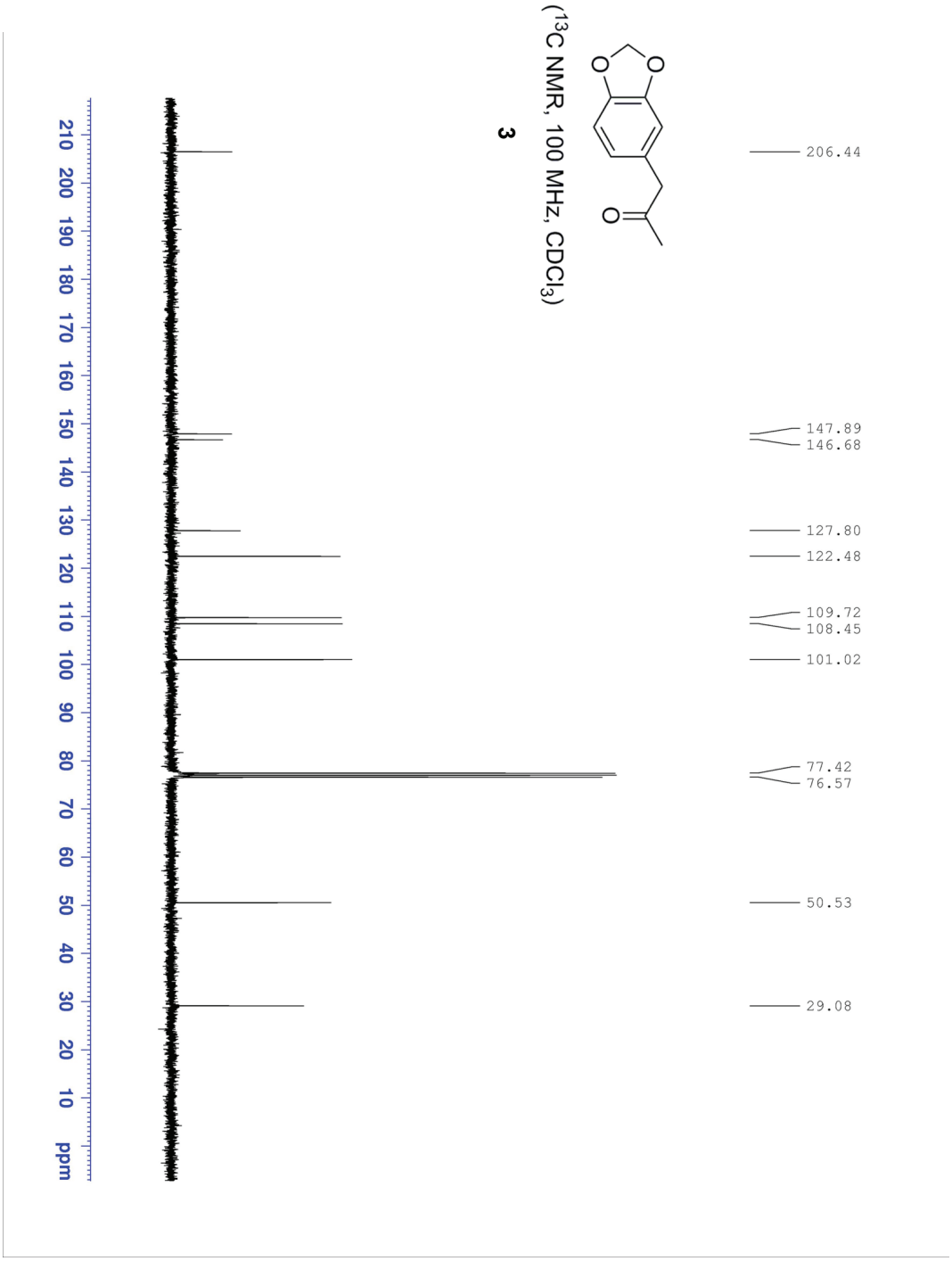
	Peak Name	RT	Area	% Area	Height
1	Peak1	27.760	4682637	50.27	73256
2	Peak2	38.877	4631813	49.73	51762

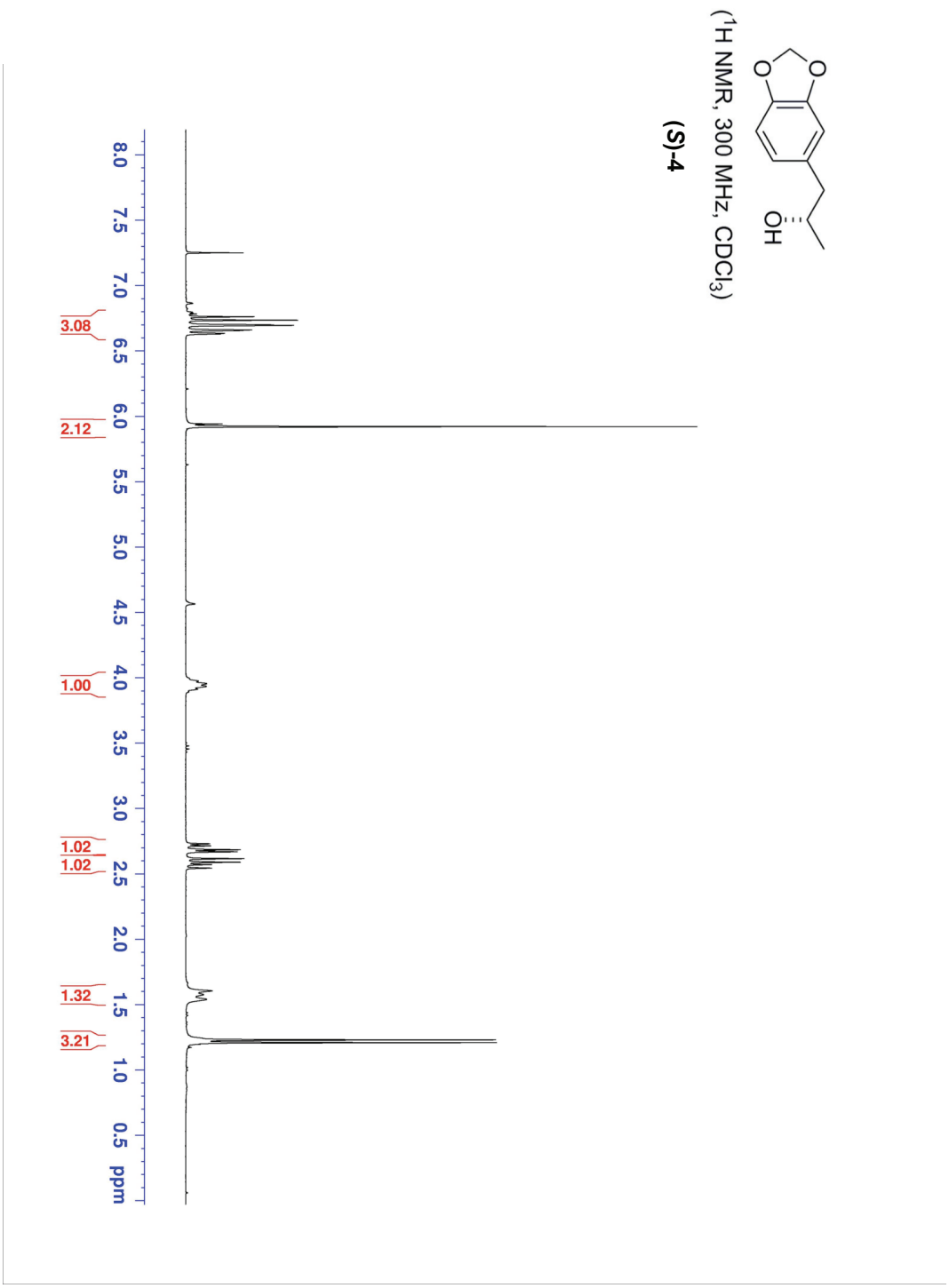


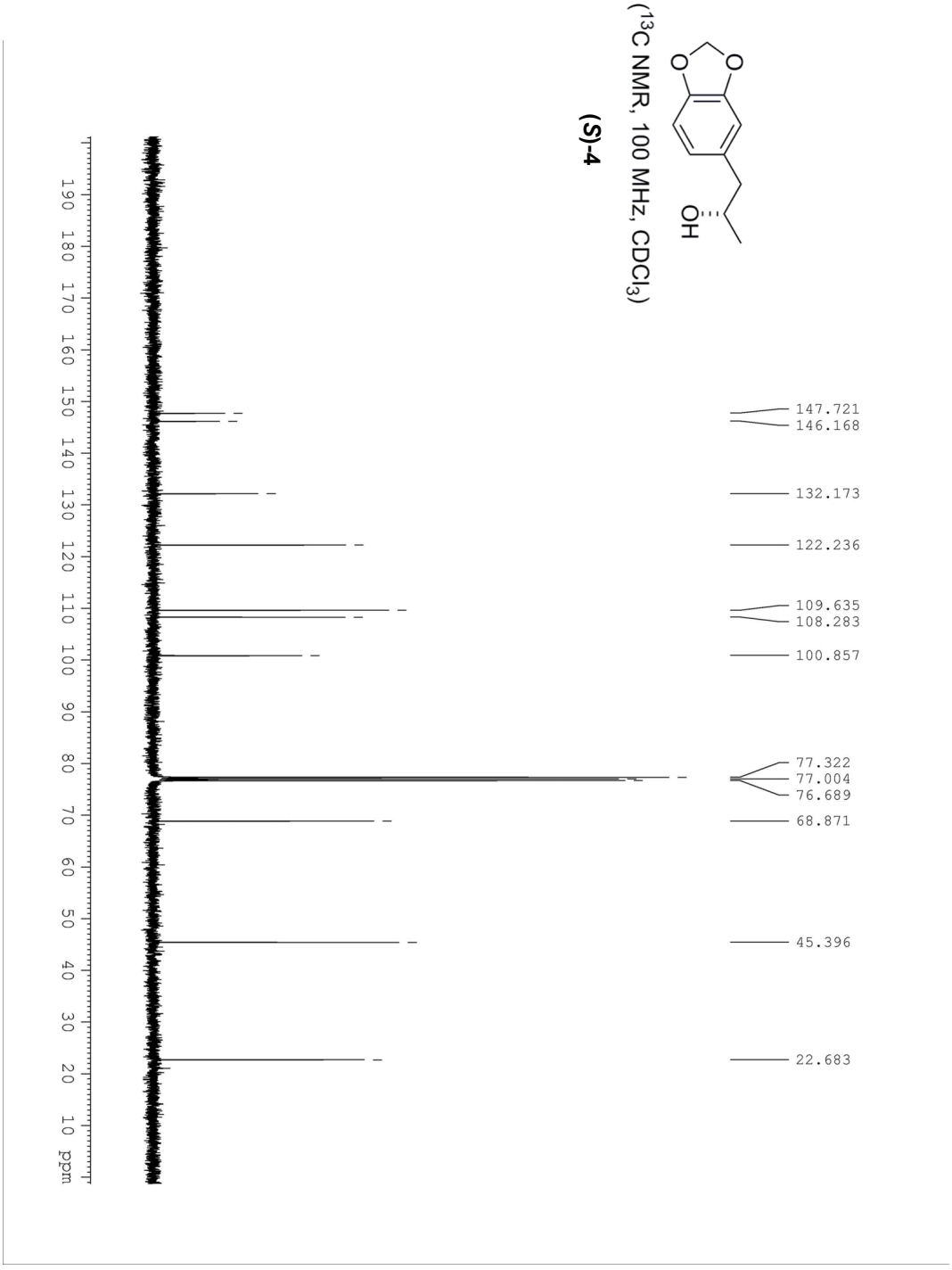
	Peak Name	RT	Area	% Area	Height
1	Peak1	28.368	4387085	100.00	64357

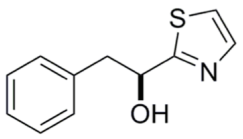
VI. ^{1}H and ^{13}C NMR Spectra




(13C NMR, 100 MHz, CDCl3)
3
206.44
147.89
146.68
127.80
122.48
109.72
108.45
101.02
77.42
76.57
50.53
29.08
ppm

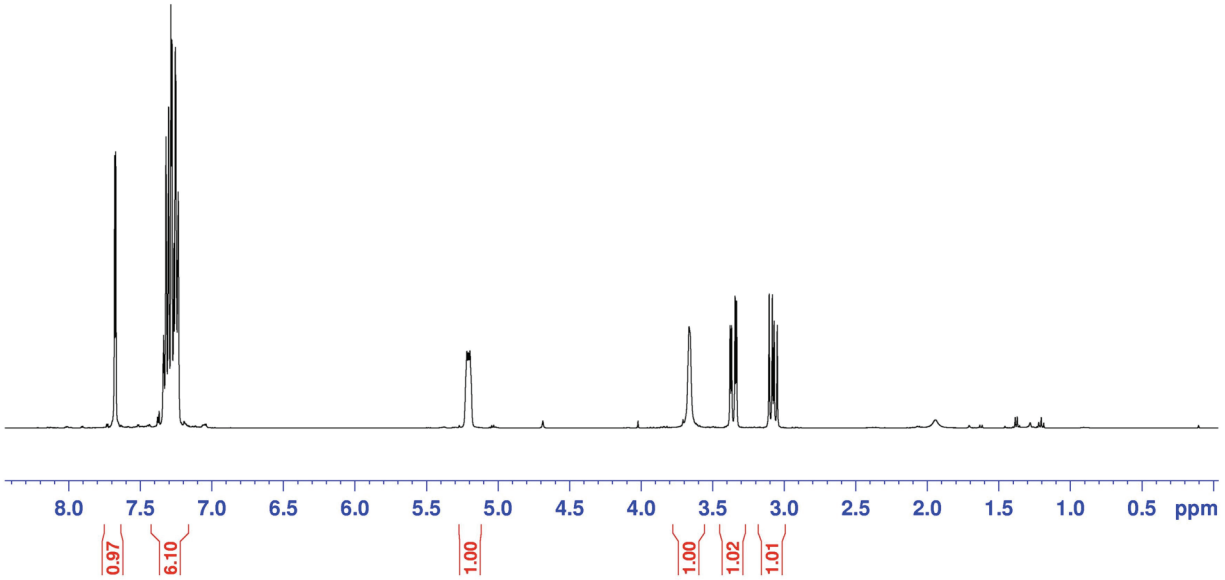

(S)-4
(^{1}H NMR, 300 MHz, $CDCl_3$)
OH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
3.08
2.12
1.00
1.02
1.02
1.32
3.21

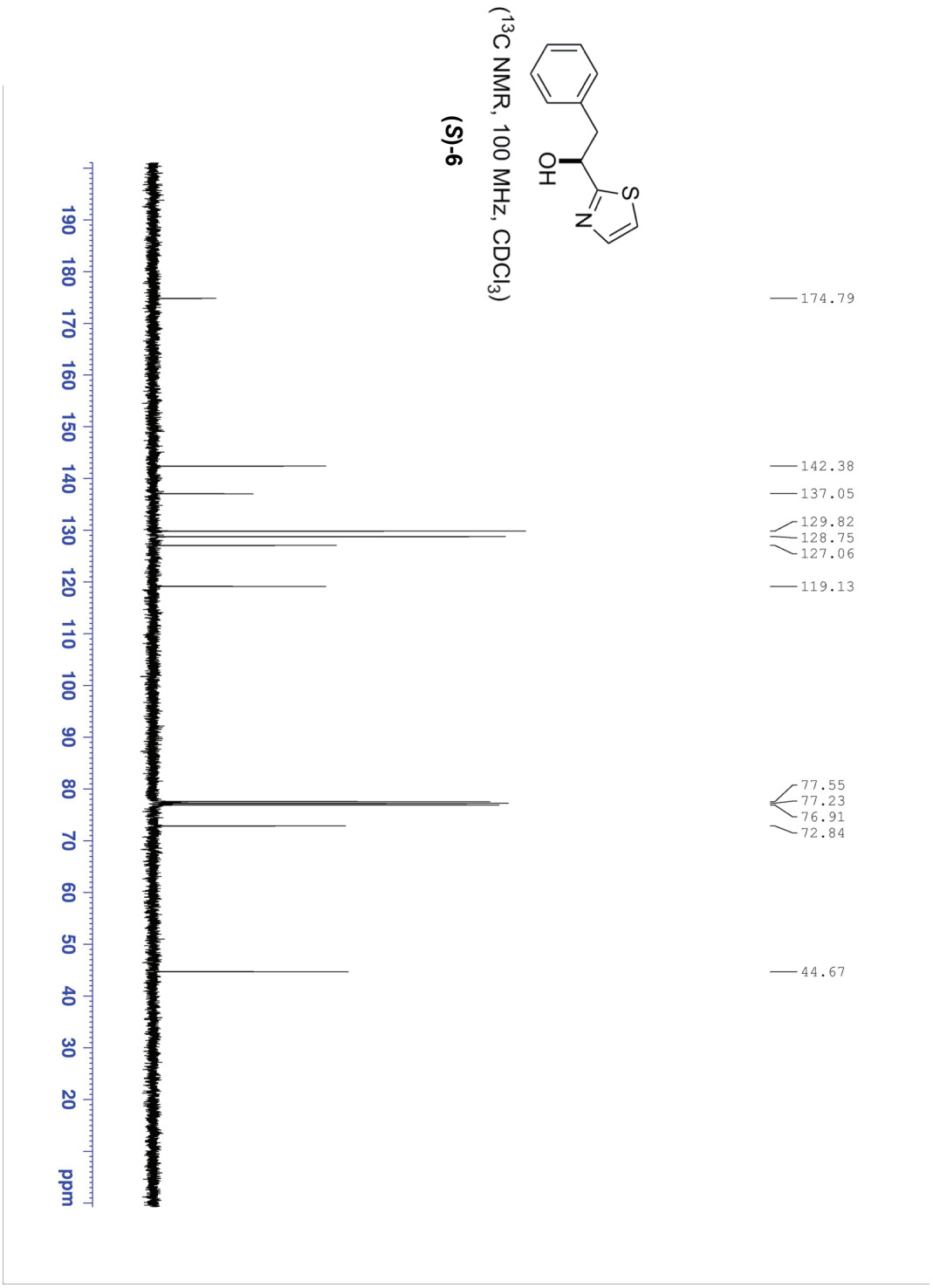

(S)-4
(^{13}C NMR, 100 MHz, CDCl$_3$)
OH
147.721
146.168
132.173
122.236
109.635
108.283
100.857
77.322
77.004
76.689
68.871
45.396
22.683
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

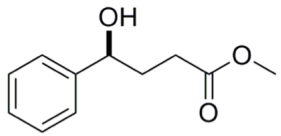


(^{1}H NMR, 400 MHz, $CDCl_3$)

(*S*)-6

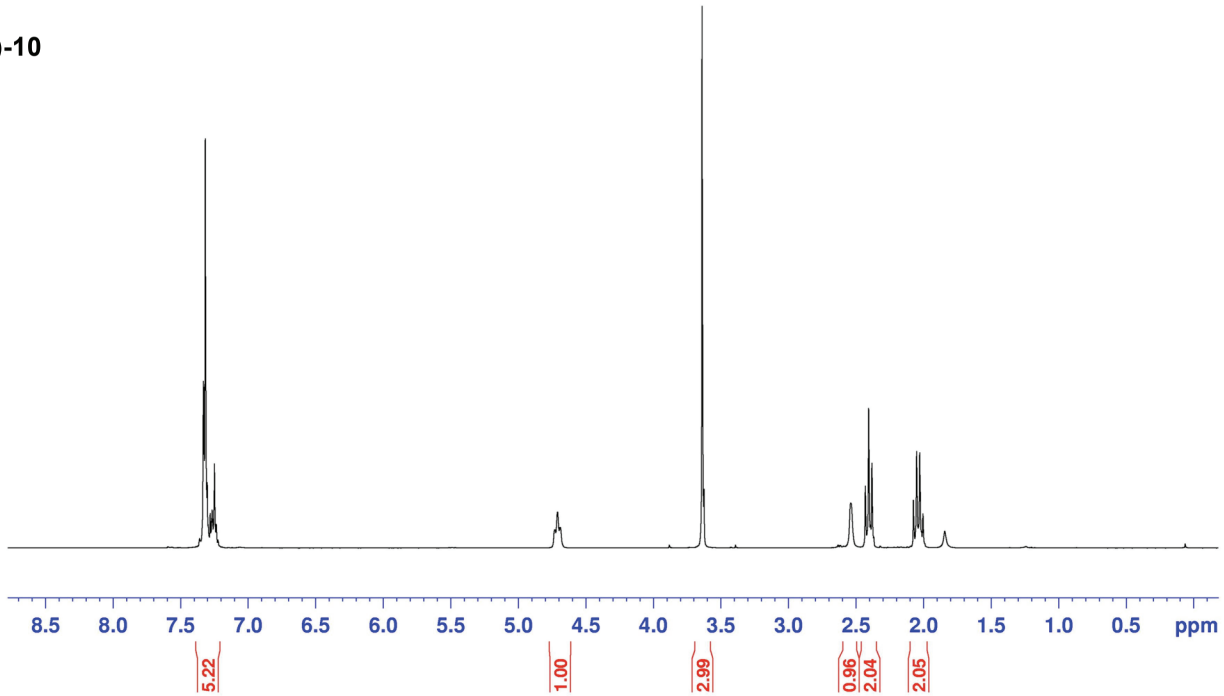


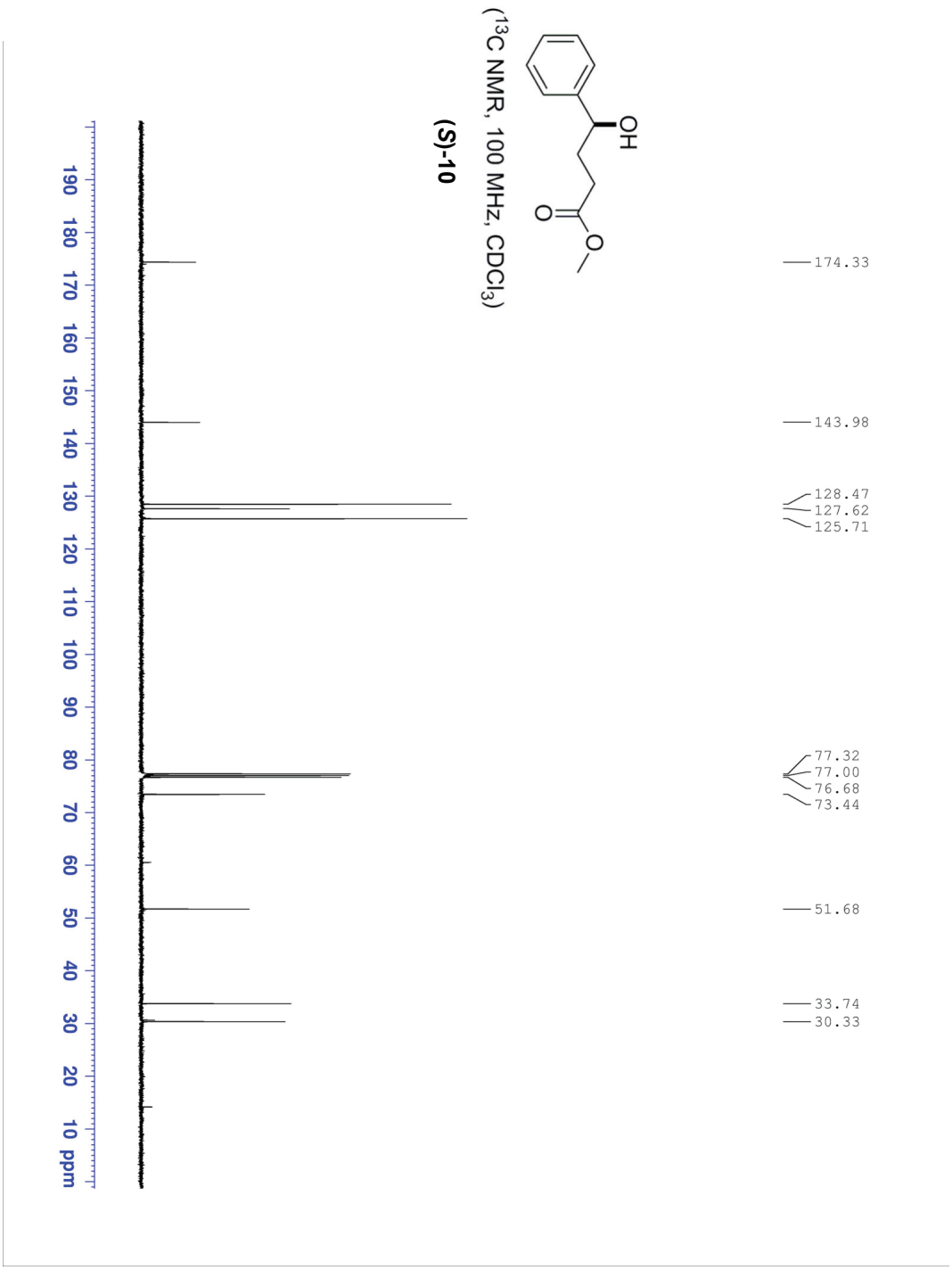

(S)-6
(^{13}C NMR, 100 MHz, CDCl$_3$)
OH
S
N
174.79
142.38
137.05
129.82
128.75
127.06
119.13
77.55
77.23
76.91
72.84
44.67
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

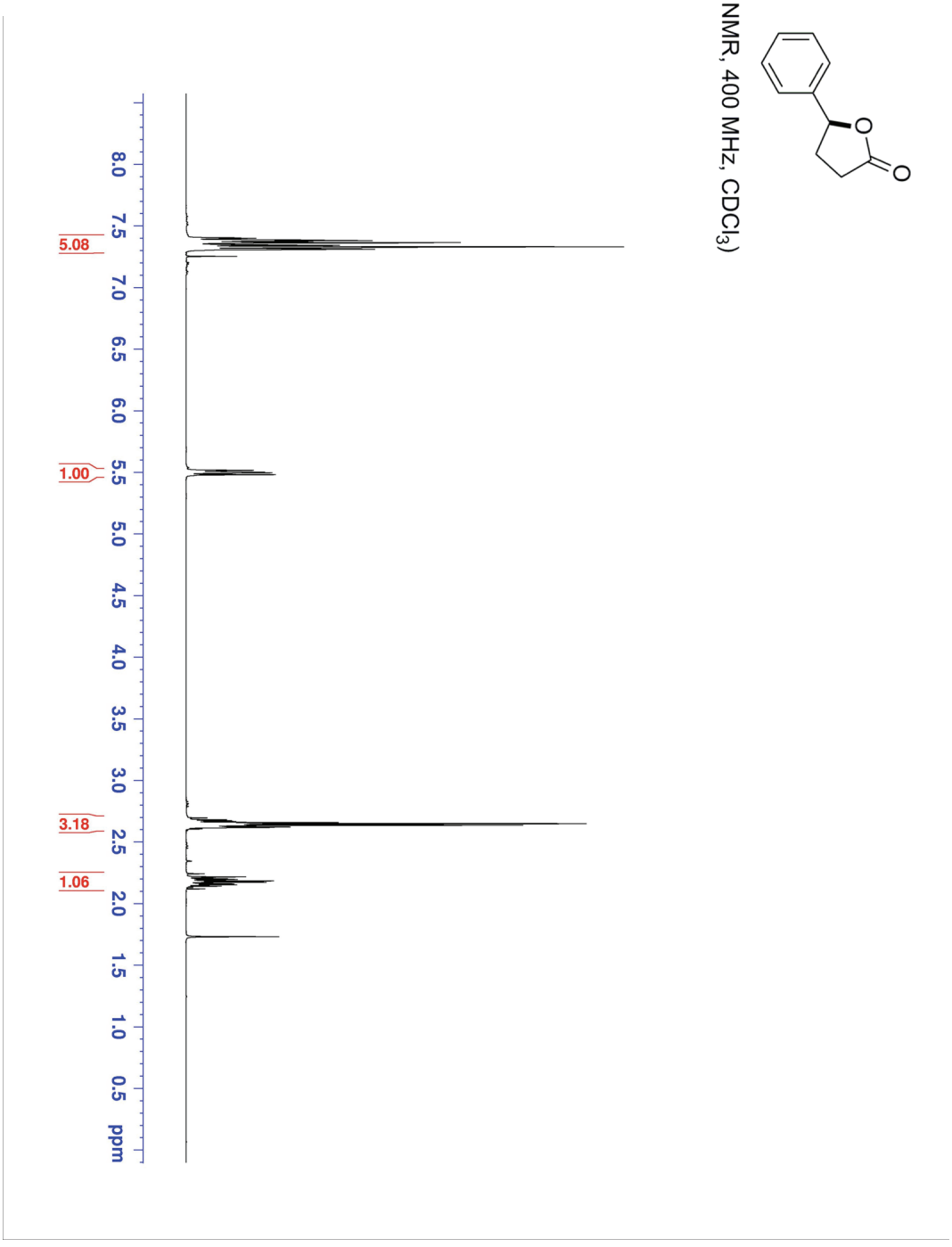


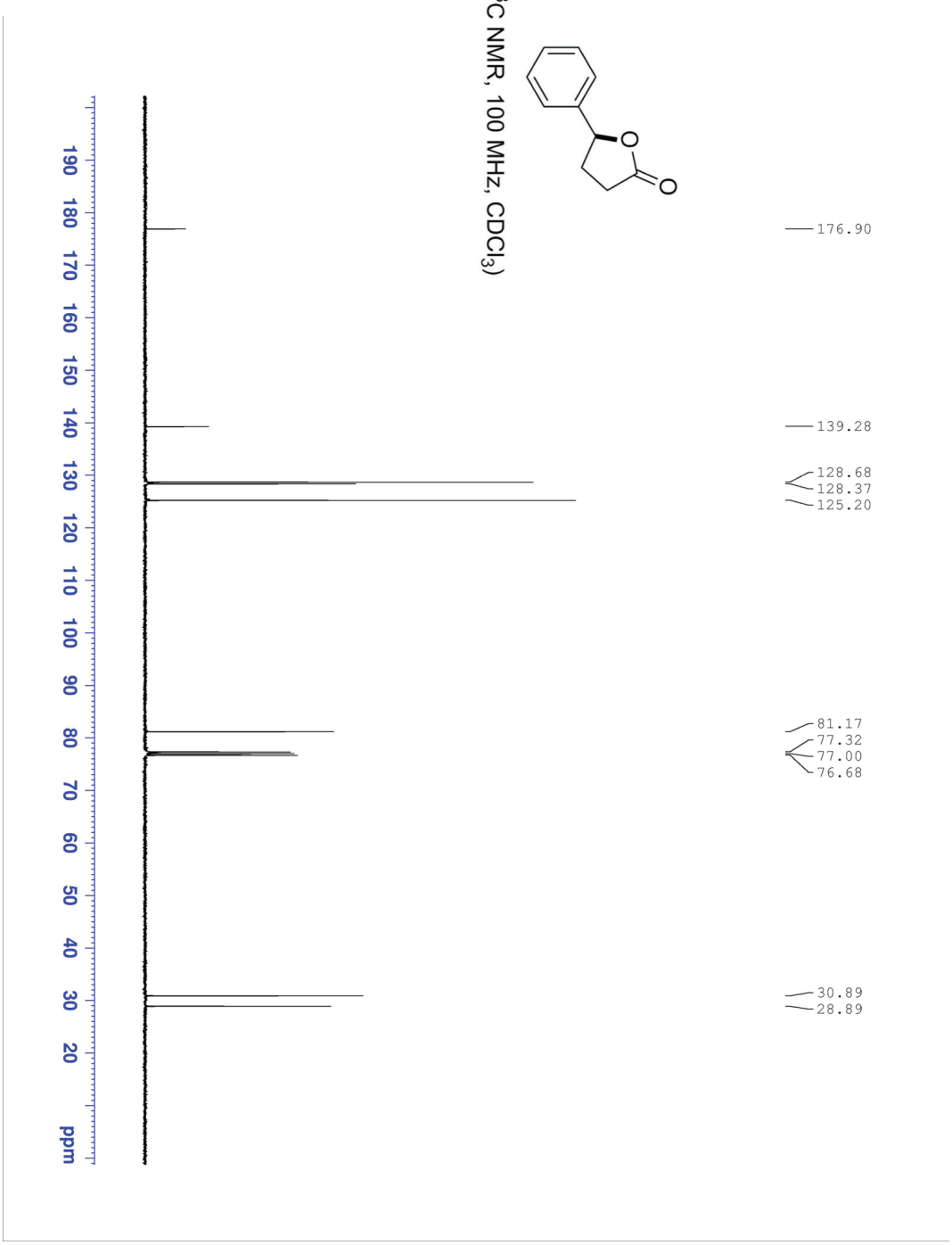
(^{1}H NMR, 300 MHz, $CDCl_3$)

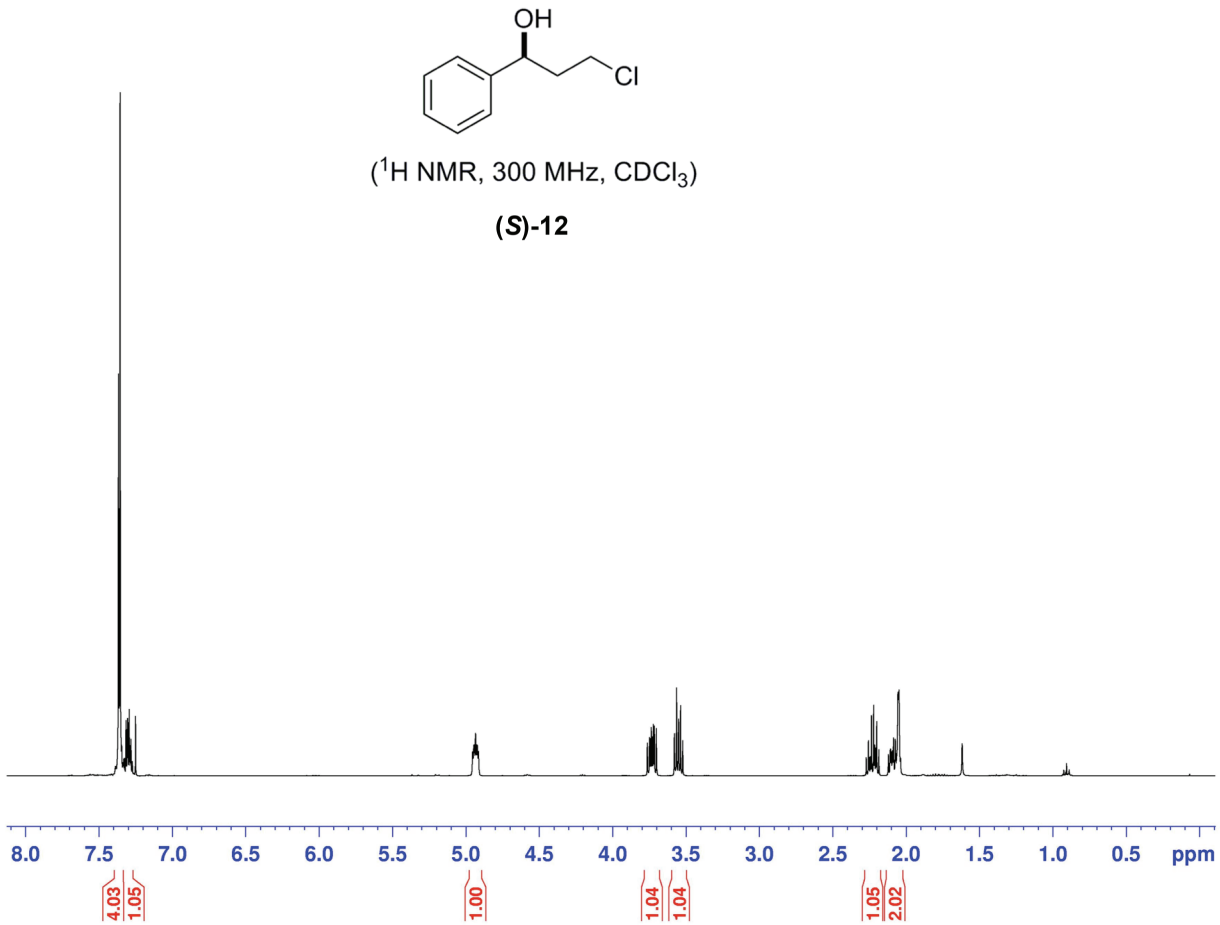
(*S*)-10

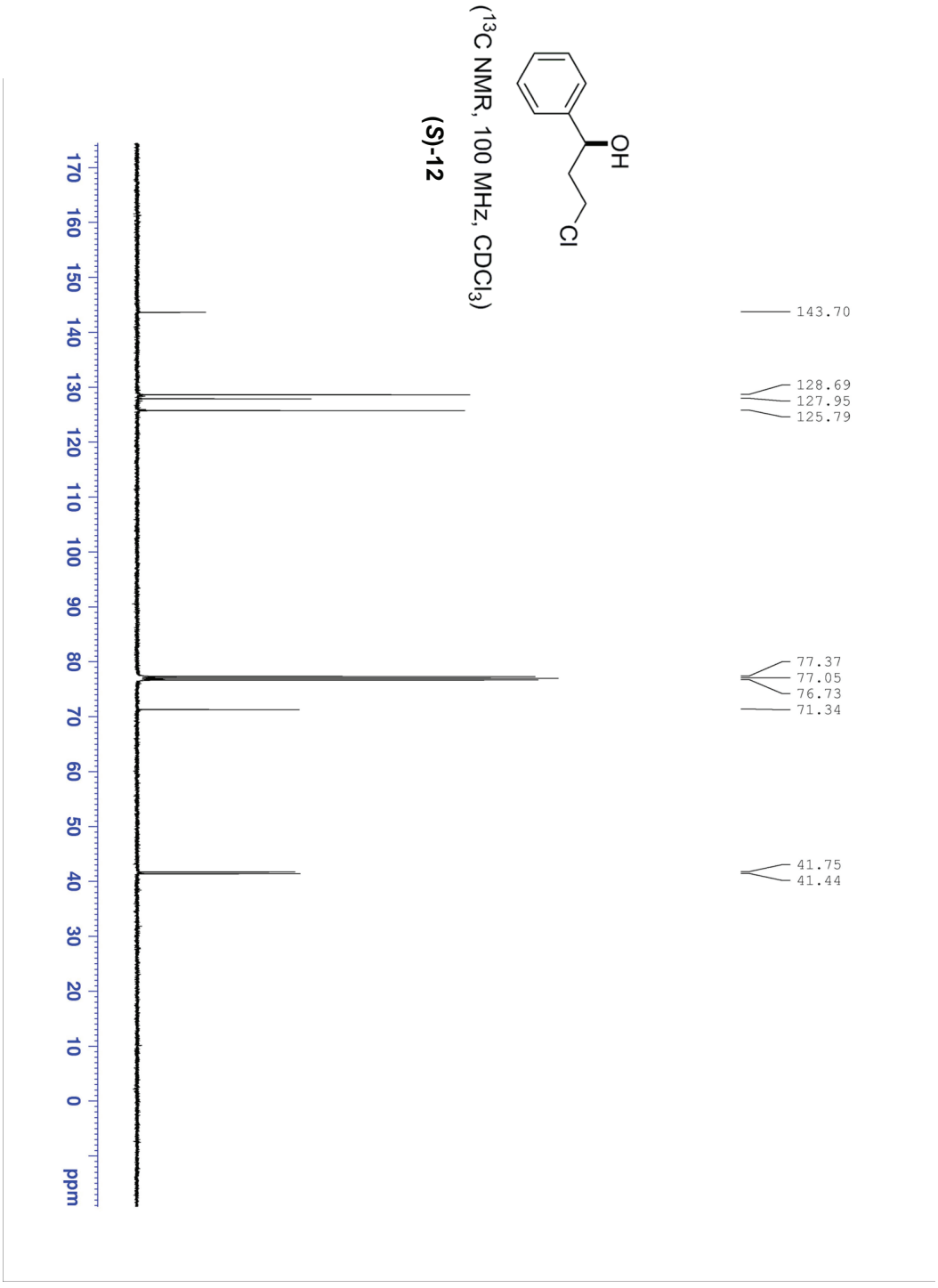


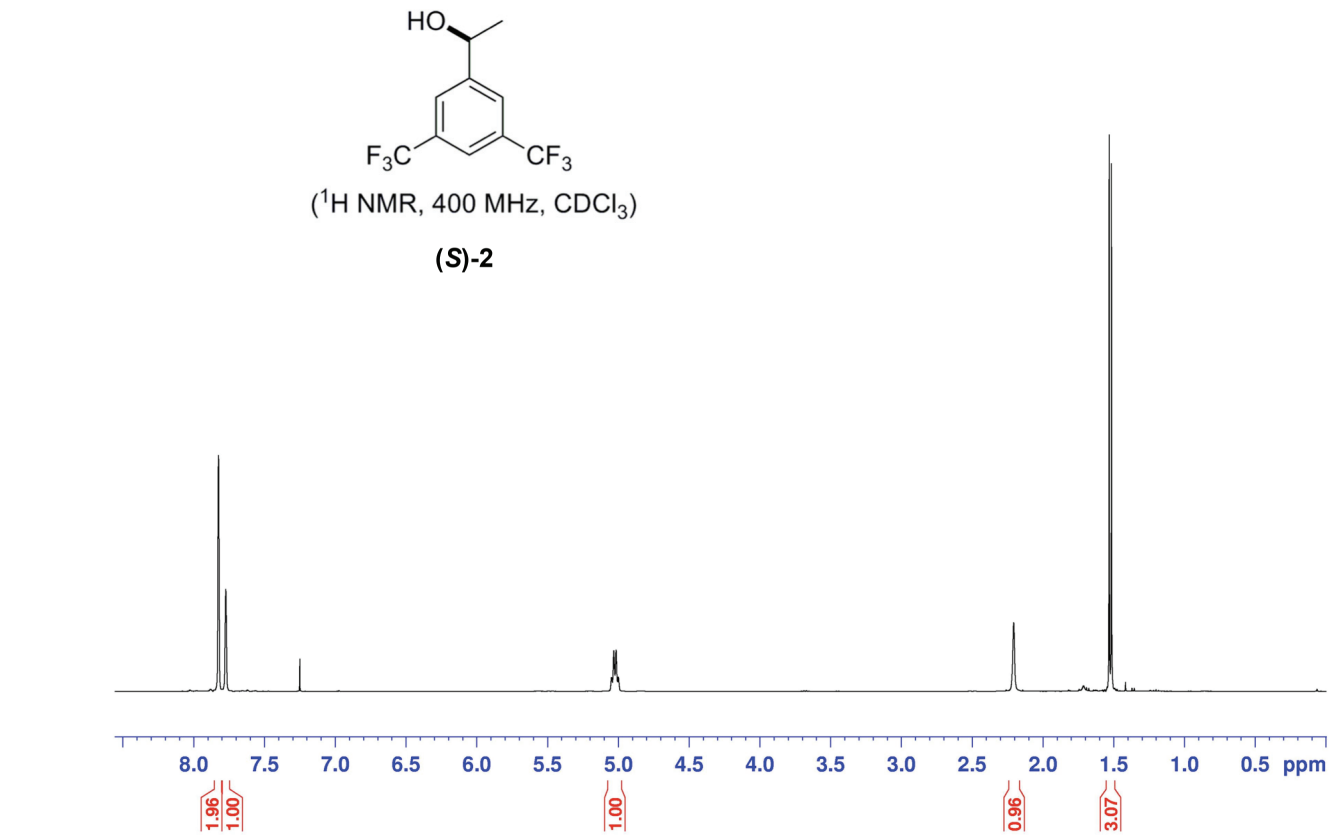

(S)-10
(^{13}C NMR, 100 MHz, CDCl$_3$)
OH
O
O
174.33
143.98
128.47
127.62
125.71
77.32
77.00
76.68
73.44
51.68
33.74
30.33
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

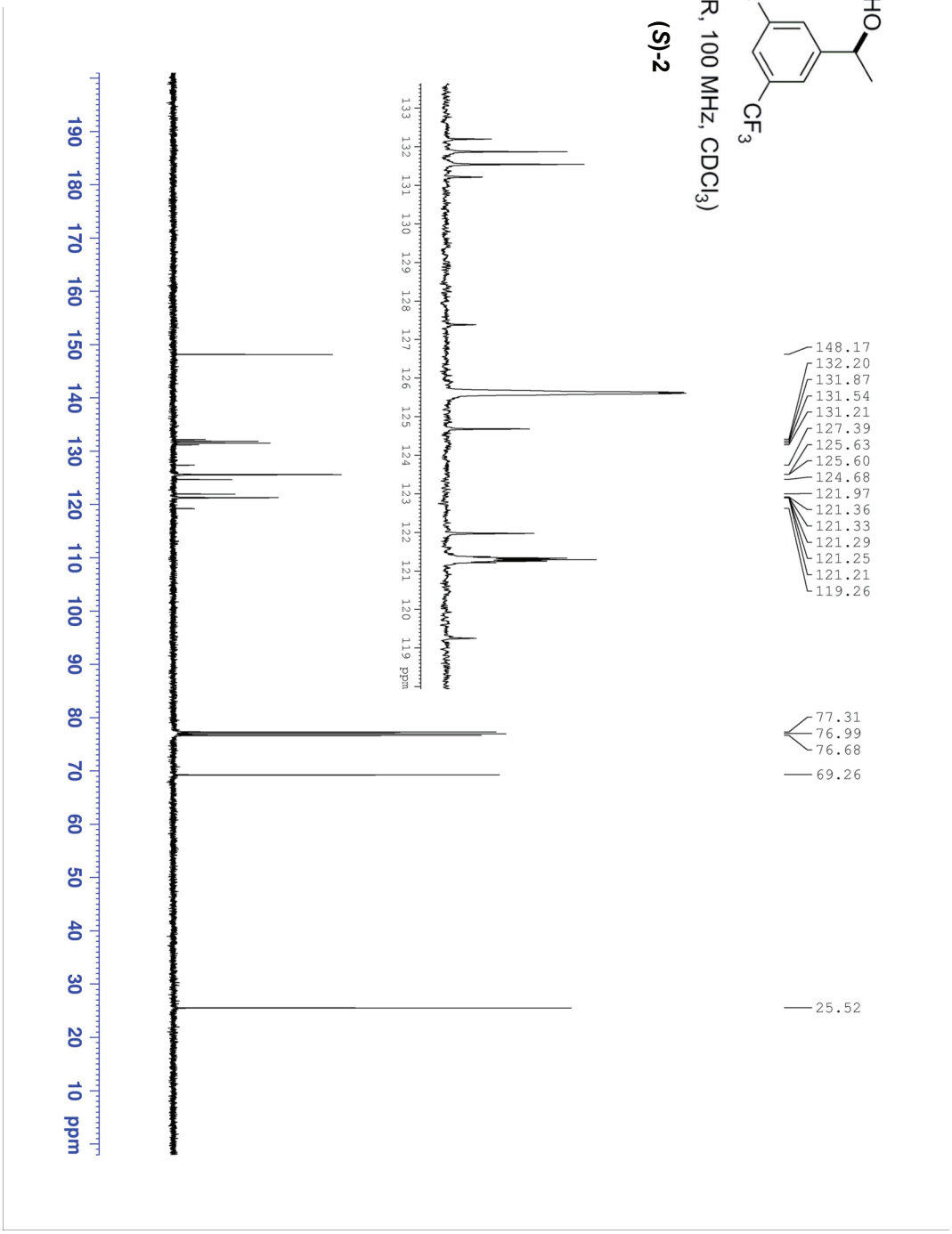

(1H NMR, 400 MHz, CDCl3)
O
O
5.08
1.00
3.18
1.06
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

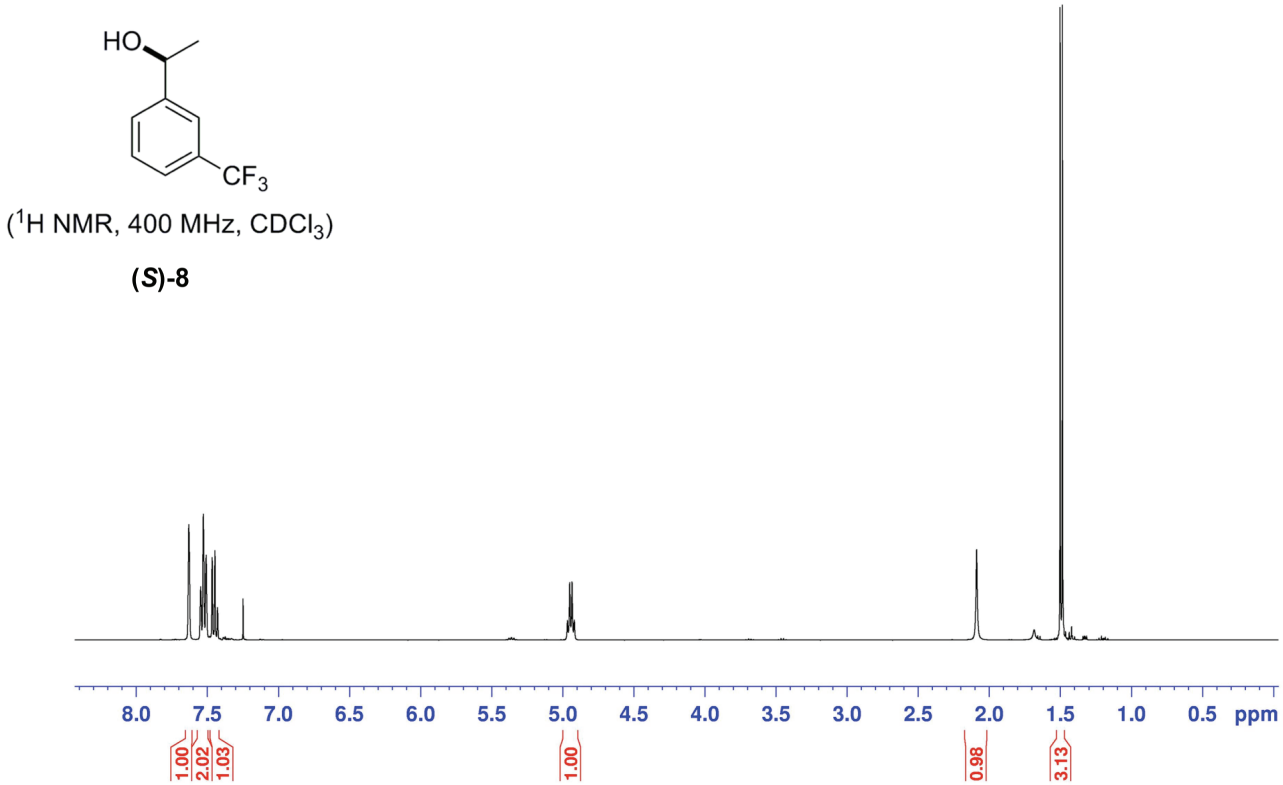

(^{13}C NMR, 100 MHz, CDCl$_3$)
176.90
139.28
128.68
128.37
125.20
81.17
77.32
77.00
76.68
30.89
28.89
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

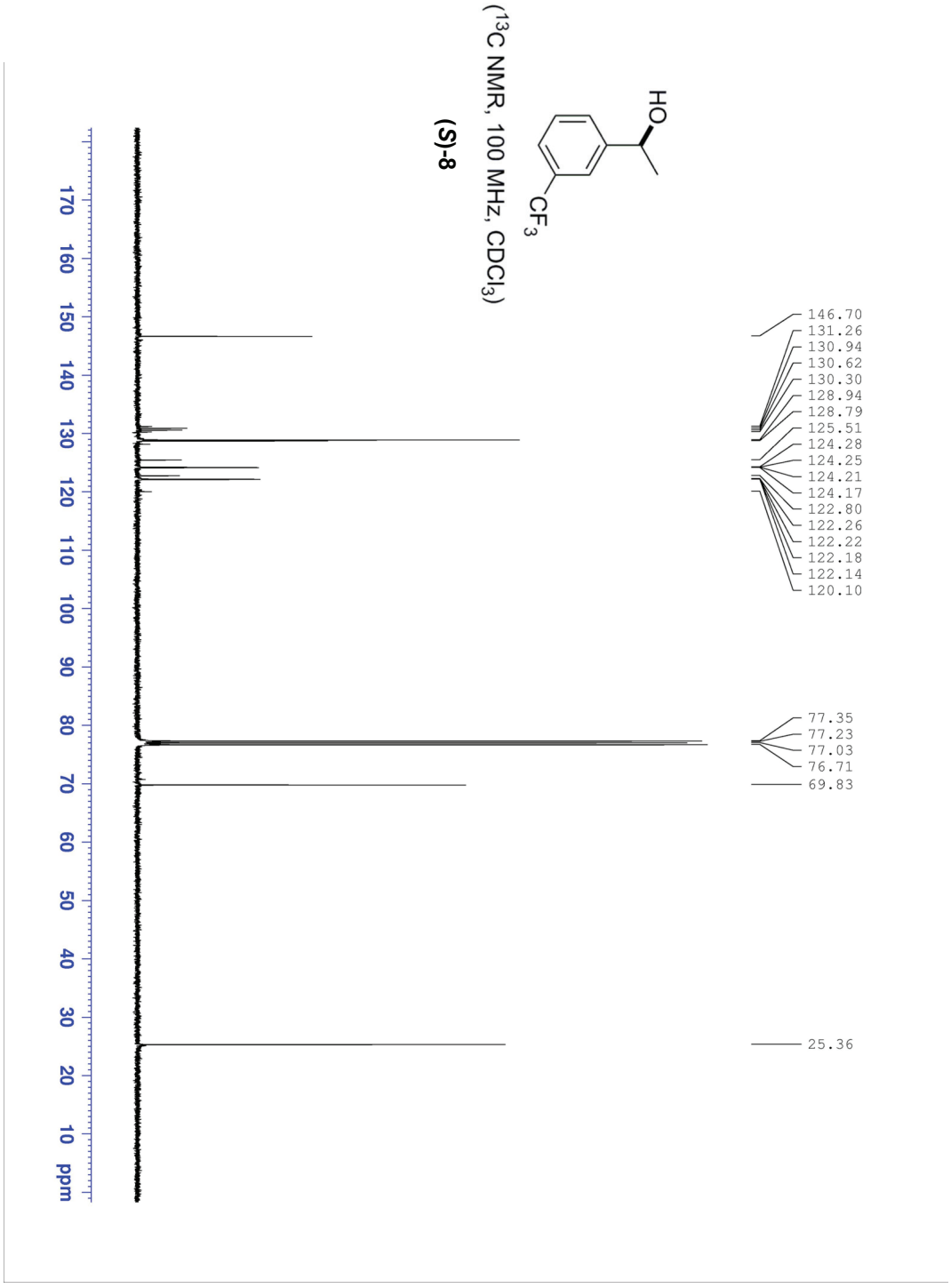

OH
Cl
(1H NMR, 300 MHz, CDCl3)
(S)-12
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
4.03
1.05
1.00
1.04
1.04
1.05
2.02

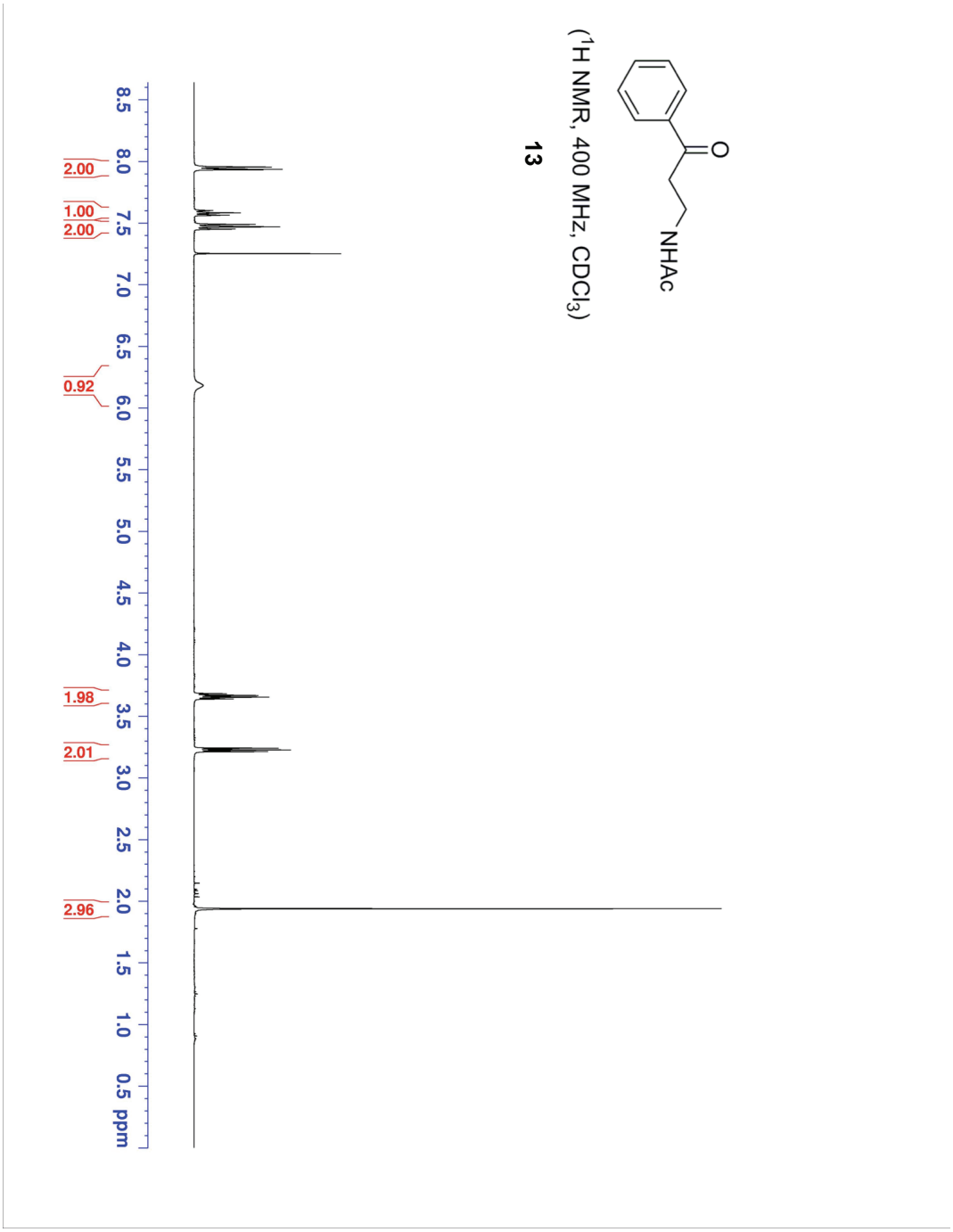

(13C NMR, 100 MHz, CDCl3)
(S)-12
OH
Cl
143.70
128.69
127.95
125.79
77.37
77.05
76.73
71.34
41.75
41.44
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

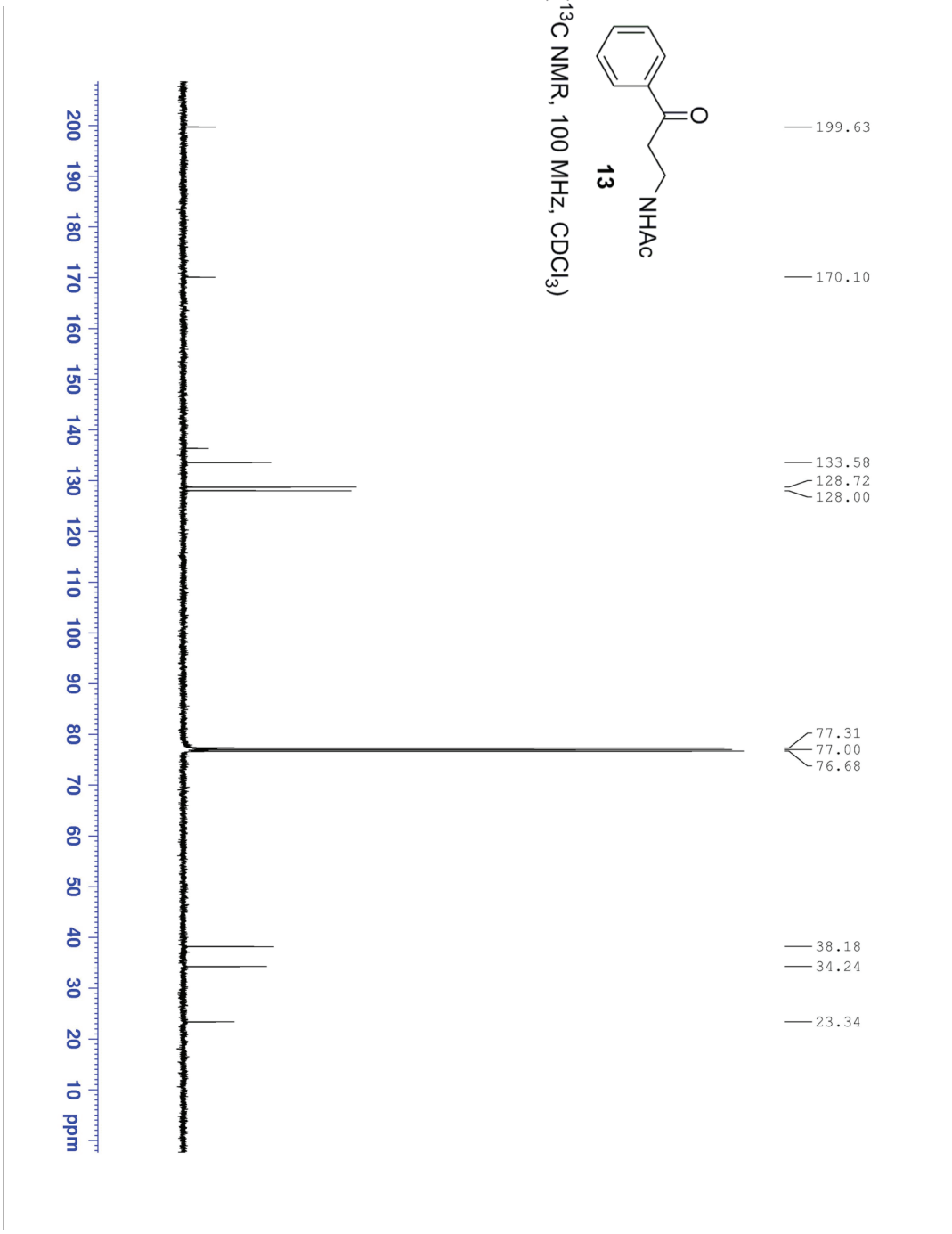

HO
F3C
CF3
(1H NMR, 400 MHz, CDCl3)
(S)-2
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.96
1.00
1.00
0.96
3.07

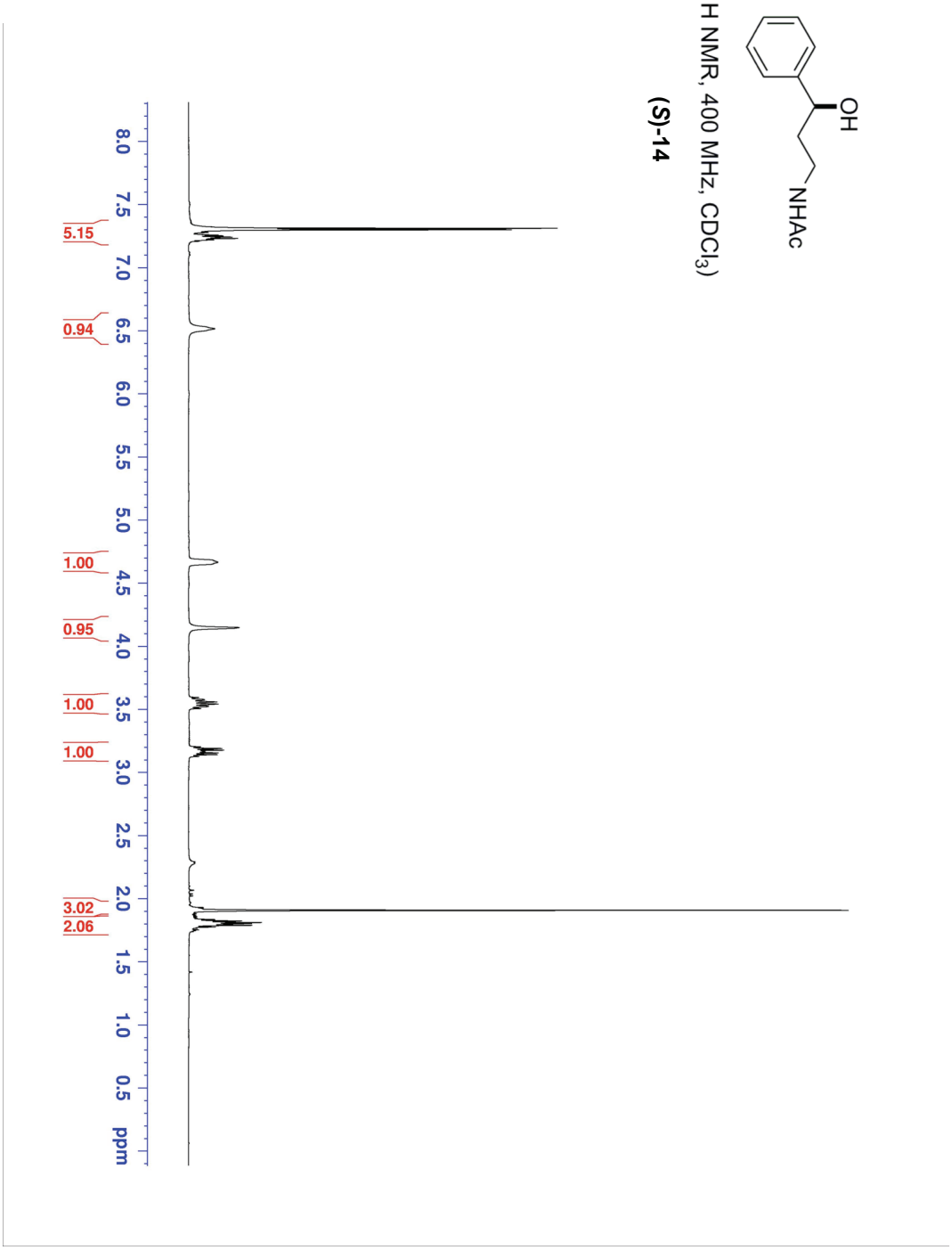

(S)-2
(^{13}C NMR, 100 MHz, CDCl$_3$)
F$_3$C
CF$_3$
HO
148.17
132.20
131.87
131.54
131.21
127.39
125.63
125.60
124.68
121.97
121.36
121.33
121.29
121.25
121.21
119.26
77.31
76.99
76.68
69.26
25.52
ppm

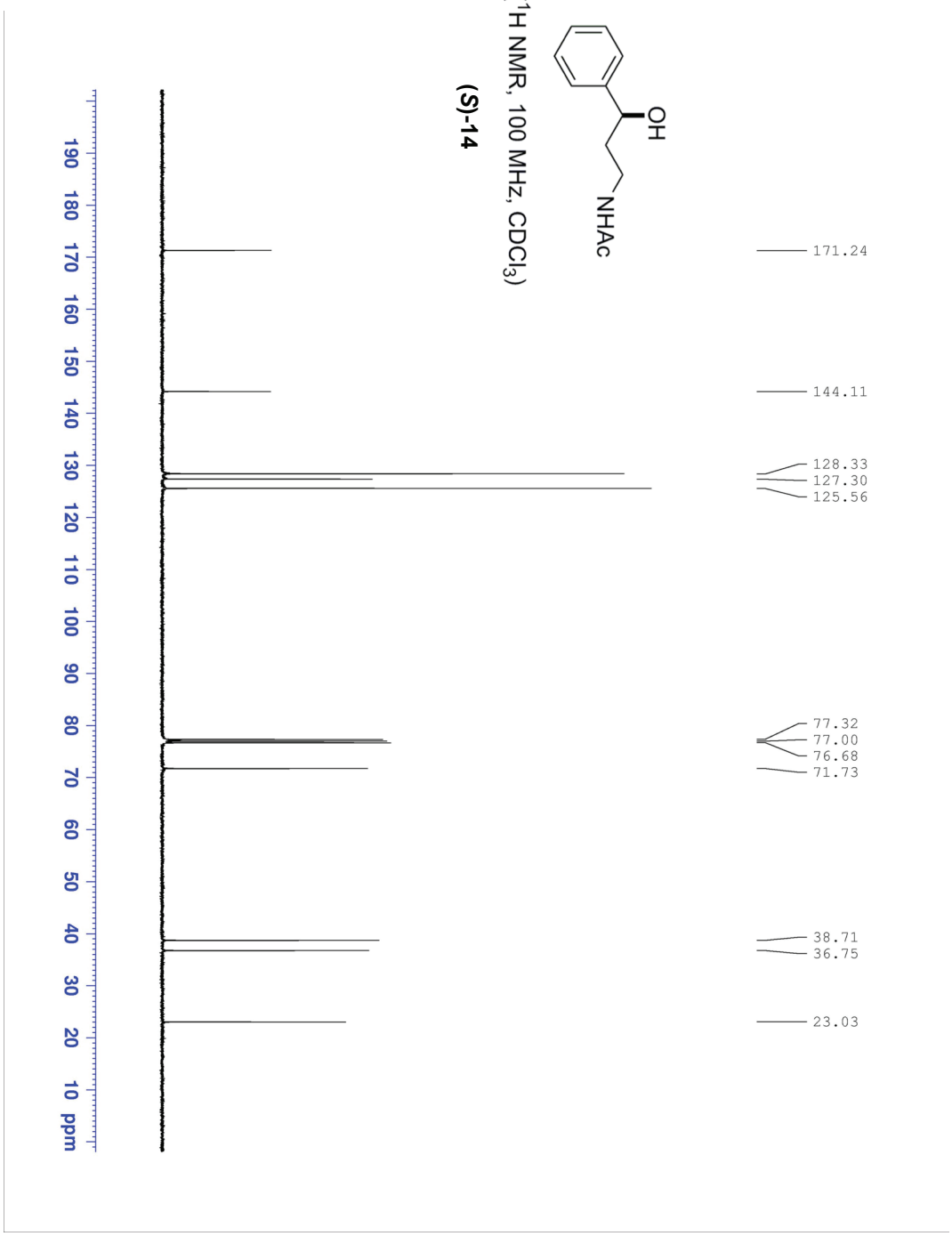

HO
CF3
(1H NMR, 400 MHz, CDCl3)
(S)-8
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
2.02
1.03
1.00
0.98
3.13


(S)-8
(^{13}C NMR, 100 MHz, CDCl$_3$)
HO
CF$_3$
146.70
131.26
130.94
130.62
130.30
128.94
128.79
125.51
124.28
124.25
124.21
124.17
122.80
122.26
122.22
122.18
122.14
120.10
77.35
77.23
77.03
76.71
69.83
25.36
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


O
NHAc
(1H NMR, 400 MHz, CDCl3)
13
2.00
1.00
2.00
0.92
1.98
2.01
2.96
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5 ppm


(13C NMR, 100 MHz, CDCl3)
O
NHAc
13
199.63
170.10
133.58
128.72
128.00
77.31
77.00
76.68
38.18
34.24
23.34
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


OH
NHAc
(1H NMR, 400 MHz, CDCl3)
(S)-14
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
5.15
0.94
1.00
0.95
1.00
1.00
3.02
2.06


(S)-14
(^{1}H NMR, 100 MHz, CDCl$_3$)
OH
NHAc
171.24
144.11
128.33
127.30
125.56
77.32
77.00
76.68
71.73
38.71
36.75
23.03
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm