Biocatalytic Deuterium- and Hydrogen-Transfer Using Over-expressed ADH-'A': Enhanced Stereoselectivity and ^{2}H-Labeled Chiral Alcohols.

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Electronic Supporting Information

Contents

General

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^1H) and 90 (^{13}C) MHz or a Bruker DMX Avance 500 at 500 (^1H) and 125 (^{13}C) MHz, respectively. Chemical shifts are reported relative to TMS (δ 0.00) and coupling constants (*J*) are given in Hz. TLC plates were run on silica gel Merck 60 F_{254} and compounds were visualized either by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}*4H_2O$ (100 g l^{-1}), $Ce(SO_4)_2*4H_2O$ (4 g l^{-1}) in H_2SO_4 (10%)] or by UV. Optical rotation values ($[\alpha]_D$) were measured on a Perkin-Elmer polarimeter 341 at 589 nm (Na-line) in a 1dm cuvette.

For anhydrous reactions, flasks were dried and flushed with dry argon just before use. Standard syringe techniques were applied to transfer dry solvents and reagents in an inert atmosphere of dry argon. Anhydrous THF was distilled from potassium. Petroleum ether (bp. 60-90 °C) and EtOAc used for chromatography were distilled prior to use. All other reagents were used as received. d_8-2-Propanol (99+ %D) was obtained from Aldrich.

General protocol for biocatalytic deuterium transfer, analytical scale

Lyophilized cells of *E. coli* Tuner™ (DE3)/pET22b+-ADH-'A' (2 mg) were rehydrated in buffer (Tris-HCl, 500 µl, pH 7.5, 50mM) for 1 h at 30°C with 130 rpm in Eppendorf tubes (1.5 ml). Substrate (20 µl) and d_8-2-propanol (100 µl) were added and the mixture was agitated at 30°C and 130 rpm for 15 minutes. Afterwards the reaction was stopped by addition of ethyl acetate (0.5 ml). The organic layer was separated from the aqueous phase using centrifugation and dried over Na_2SO_4. Conversions and enantioselectivities were determined by using GC analysis.

General protocol for biocatalytic deuterium transfer, preparative scale

Lyophilized cells (50 mg) were rehydrated in buffer (10 ml), substrate (300 mg) and d_8-2-propanol (1 ml) were added and the mixture was agitated for 24 hours as described above. Products were extracted and purified by silica gel chromatography.

Table S1. Results from preparative-scale biocatalytic deuterium transfer for ketones **14a**-**16a**.

Product	e.e. (%)	Isolated yield (mg / mmol)	Yield (%)
(*S*)-1-phenyl-ethanol-1-d_1 **14c**	>99	273 / 2.24	88.9
(*S*)-2-decanol-2-d_1 **15c**	>99	212 / 1.33	69.2
(*S*)-6-methyl-5-hepten-2-ol-2-d_1 **16c**	>99	253 / 1.96	82.3

Determination of the apparent kinetic isotope effect

The apparent KIE was determined by measuring the conversion of the reduction of acetophenone **14a** in the presence of 2-propanol and d_8-2-propanol, respectively employing *E. coli* Tuner™ (DE3)/pET22b+-ADH-'A'after a short reaction time (15 min) to remain in the linear range of the transformation as described in the general protocol for biocatalytic deuterium transfer on an analytical scale.

Table S2. Determination of the apparent KIE for the hydrogen/deuterium transfer reduction of acetophenone **14a** employing *E. coli* Tuner™ (DE3)/pET22b+-ADH-'A'

Substrate	Time	d_8-2-Propanol		2-Propanol		app-KIE[a]
	[min]	conv. [%]	e.e.[%]	conv. [%]	e.e.[%]	
acetophenone **14a**	15	2.4	> 99	7.9	> 99	3.29

[a] apparent kinetic isotope effect, calculated as the ratio between the conversion with d_8-2-propanol and 2-propanol in the linear range.

For NMR spectra of novel compounds see appendix.

1-Phenylethanol-1-d_1 **14c**

^{1}H-NMR ($CDCl_3$) δ 7.28-7.37 (m, 5H), 1.48 (s, 3H);

^{13}C-NMR ($CDCl_3$) δ 145.8, 128.5, 127.4, 125.4, 69.9 (t, J = 22 Hz), 25.0;

2-Decanol-2-d_1 **15c**

^{1}H-NMR ($CDCl_3$) δ 1.26-1.50 (m, 15H), 1.15 (s, 2H), 0.85 (t, 3H);

^{13}C-NMR ($CDCl_3$) δ 67.7 (t, J = 21 Hz), 39.3, 31.9, 29.6, 29.5, 29.3, 25.8, 23.3, 22.6, 14.1;

6-Methyl-5-hepten-2-ol-2-d_1 **16c**

^{1}H-NMR ($CDCl_3$) δ 5.13 (t, 1H), 2.17-2.04 (m, 2H), 1.68 (s, 3H), 1.61 (s, 3H), 1.46 (m, 2H), 1.17 (s, 3H);

^{13}C-NMR ($CDCl_3$) δ 131.9, 124.1, 67.4 (t, J = 22 Hz), 39.0, 25.7, 24.4, 23.2, 17.6;

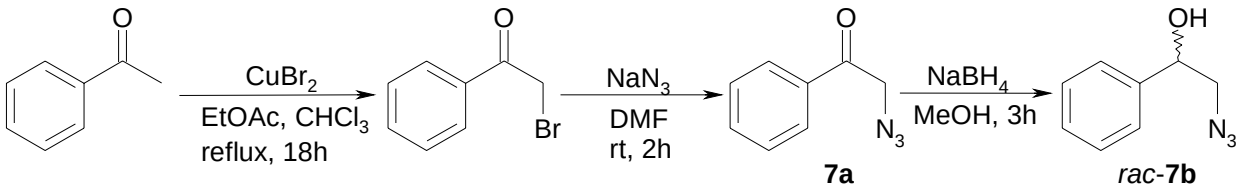
Substrates and reference material

Substrates **1a**-**6a**, **9a**-**16a** and reference compounds *rac*-**1b**-**6b**, *rac*-**9b**-**13b** were either commercially available or synthesized as previously described.[1]

The synthesis of **7a** and *rac*-**7b** was started from acetophenone (Scheme S1). The conversion to the corresponding α-bromo ketone was performed employing the method of King *et al*.[2] using $CuBr_2$. Nucleophilic replacement of bromide by azide was performed under standard conditions

using sodium azide in dimethylformamide. Subsequent sodium borohydride reduction finally yielded in the desired β-azido alcohol.

Scheme S1. Synthesis of substrate **7a** and reference compound *rac*-**7b**.



2-Azido-1-phenylethanone **7a.**

Acetophenone (1.2 g, 10 mmol) was dissolved in EtOAc (17 ml) and $CHCl_3$ (17 ml). $CuBr_2$ (4 g, 17 mmol) was added to the solution and the reaction mixture was refluxed under vigorous stirring for 18 h. The green solution changed to amber whereby the gray Cu(II) complex was reduced to white Cu(I). The mixture was cooled to room temperature and CuBr was filtered off through a pad of Celite. The resulting solution was evaporated under reduced pressure to give 2-bromo-1-phenylethanone as brownish oil (1.7 g, 8.5 mmol, 85 %). The product was identified by comparison of the NMR data with literature.[3] ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.47 (2H, s), 7.48-7.53 (2H, m), 7.60-7.65 (1H, m), 7.96-8.01 (2H, m); ^{13}C NMR (90 MHz, $CDCl_3$) δ 30.9, 128.8, 128.9, 133.1, 134.0, 191.6.

Transformation of the bromo-derivative to the azido ketone **7a** was performed according to literature.[4] A suspension of 2-bromo-1-phenylethanone (1.7 g, 8.5 mmol) in DMF was treated with sodium azide (0.6 g, 9.4 mmol) and the mixture was stirred at 20 °C for 2 h by which time the mixture became homogeneous and red colored. The mixture was diluted with EtOAc and washed with water. The water phase was extracted with EtOAc (3 x). The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Flash chromatography

(p.e./EtOAc = 20:1) gave compound **7a** as a yellow oil (0.7 g, 4.3 mmol, 48 %). Product **7a** was identified by comparison of the NMR data with literature.[10] ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.58 (2H, s), 7.49-7.53 (2H, m), 7.62-7.64 (1H, m) 7.91-7.93 (2H, m); ^{13}C NMR (90 MHz, $CDCl_3$) δ 54.9, 127.9, 128.9, 134.1, 134.4, 193.5.

rac-2-Azido-1-phenyl-1-ethanol *rac*-**7b.**

Adapting a procedure from literature,[5] azidoketone 2-azido-1-phenylethanone **7a** (50 mg, 0.3 mmol) was dissolved in MeOH (4 ml), cooled to 0 °C and $NaBH_4$ (22 mg, 0.6 mmol) was added in portions over 50 min. After stirring for further 2 h at 0 °C the reaction mixture was allowed to warm to room temperature and was quenched with water (1:1). The aqueous phase was extracted with EtOAc (3 x), the organic layers were combined and washed with brine, dried over Na_2SO_4 and evaporated. Flash chromatography (petroleum ether/EtOAc = 5:1) gave **7b** as colorless oil (30 mg, 0.18 mmol, 60 %). The product was identified by comparison of its NMR data with literature values.[6] ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.67 (1H, bs), 3.40-3.51 (2H, m), 4.87 (1H, m), 7.34-7.40 (5H, m); ^{13}C NMR (90 MHz, $CDCl_3$) δ 58.0, 73.4, 125.9, 128.4, 128.7, 140.6.

2-Azido-1-(4-hydroxyphenyl)ethanone **8a.**

The synthesis of **8a** was performed in analogy as described for **7a** starting from 2-bromo-1-(4-hydroxyphenyl)ethanone (4 g, 19 mmol), NaN_3 (1.3 g, 20 mmol) in DMF (30 ml). Flash chromatography (petroleum ether/EtOAc = 5:1) gave compound **8a** as white powder (1.6 g, 8.9 mmol, 47 %). For NMR spectra see appendix. ^{1}H NMR (360 MHz, d_6-acetone) δ 4.46 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.77 (2H, d, J = 8.7 Hz), 8.72 (1H, bs); ^{13}C NMR (90 MHz, d_6-acetone) δ 54.4, 115.3, 115.8, 130.5, 162.3, 191.6.

2-Azido-1-(4-hydroxyphenyl)-1-ethanol **8b**.

The synthesis of **8b** was performed as described for **7b** starting from 2-azido-1-(4-hydroxyphenyl)-1-ethanone **8a** (1 g, 5.6 mmol), MeOH (10 ml) and $NaBH_4$ (0.5 g, 13 mmol). Flash chromatography (petroleum ether/EtOAc = 5:1) gave compound **8b** as colorless oil (0.5 g, 2.8 mmol, 50 %). For NMR spectra see appendix. ^{1}H NMR (360 MHz, d_6-acetone) δ 3.28 (1H, dd, J_1 = |12.6| Hz, J_2 = 3.8 Hz), 3.40 (1H, dd, J_1 = |12.6| Hz, J_2 = 8 Hz), 4.82-4.84 (1H, m), 6.82 (2H, d, J = 8.5 Hz), 7.26 (2H, d, J = 8.5 Hz), 8.35 (1H, bs); ^{13}C NMR (90 MHz, d_6-acetone) δ 57.8, 73.0, 115.0, 127.3, 133.3, 156.9.

Sequencing of ADH-'A' and construction of the vector pET-22b+-ADH-'A'

Rhodococcus ruber was cultivated in a complex medium (10 g l^{-1} yeast extract, Oxoid L21; 10 g l^{-1} bacteriological peptone, Oxoid L37; 10 g l^{-1} glucose, Fluka 49150; 2 g l^{-1} NaCl, Roth 9265.1; 0.15g l^{-1} $MgSO_4x7H_2O$, Fluka 63140; 1.3 g l^{-1} NaH_2PO_4, Fluka 71496; 4.4 g l^{-1} K_2HPO4, Merck 5101) in baffled shake flasks (250 ml medium in a one liter shake flask) for 48 hours at 30°C and 130 rpm.

After enzyme purification of "ADH-A" as previously reported[7] MS/MS de novo-sequencing after tryptic digestion was performed.[8] Four different tryptic peptides were identified (EVGADAA, SGAGAADA[L/I]R, V[L/I]AVD[L/I]DDDR, [L/I]MEVVA[L/I]AR, whereby [L/I]) is leucine or isoleucine).

For the identification of an initial partial sequence of the ADH-'A' gene, a PCR-screening strategy using degenerated primer was applied. On the basis of known internal peptide sequences

described above in addition with conserved regions of published sequences from public databases, a set of oligonucleotides was designed. These oligonucleotides were used for a PCR screening with chromosomal DNA of *Rhodococcus ruber* as a template. Chromosomal DNA was prepared after lysozyme/proteinaseK/RNase A incubation of the cell material and standard phenolysation/precipitation procedure. PCR was performed using the HotStar-Taq Master-Mix (Qiagen, Hilden, Germany) according with the instruction provided by the manufacturer. By using an unusual annealing time of 2 min at 50°C a fragment of approximately 350 bp was generated which could be characterized as a part of an ADH-gene by sequence database comparison.

For establishing a DNA bank, the Expand I-cosmid vector with an insert capacity of 9-16 kb (Roche Diagnostics GmbH, Mannheim, Germany) was used. Chromosomal DNA from *Rhodococcus ruber* was partially digested enzymatically and ligated into the vector. In vitro packaging and transformation of the cosmid library into *E. coli* DH5α was performed according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). After insert analysis a total of 2500 clones was generated and archived in 384-MTP-format.

For identification of the MTP coordinates of the clone with the complete ADH-'A' sequence, a grid PCR screening was performed using primers derived from the internal sequences mentioned above. For this purpose the cosmid DNA of each MTP row was prepared and used as a template for a screening PCR with specific internal primers. In the following the clones of the positive row were tested separately for identification of the ADH harboring candidate. An open reading frame of 1038 bp was identified by inside-out sequencing of the cosmid DNA using the initial identified partial sequence.

The complete amino acid sequence is as follows (underlined sequences represent the internal sequences identified by MS/MS):

MKAVQYTEIGSEPVVVDIPTPTPGPGEILLKVTAAGLCHSDIFVMDMPAAQYAYGLPLTL
GHEGVGTVAELGEGVTGFGVGDAVAVYGPWGCGACHACARGRENYCTRAADLGITPP
GLGSPGSMAEYMIVDSARHLVPIGDLDPVAAAPLTDAGLTPYHAISRVLPLLGPGSTAVV
IGVGGLGHVGIQILRAVSAARVIAVDLDDDRLALAREVGADAAVKSGAGAADAIRELTG
GQGATAVFDFVGAQSTIDTAQQVVAVDGHISVVGIHAGAHAKVGFFMIPFGASVVTPY
WGTRSELMEVVALARAGRLDIHTETFTLDEGPAAYRRLREGSIRGRGVVVP.

An expression plasmid was constructed on the basis of the T7-promoter system pET22b+ (Novagen, Merck KGaA, Darmstadt, Germany). The ADH gene was amplified using high fidelity PCR with oligonucleotides coding for the entire gene and adjacent restriction sites for cloning into the MCS of pET22b+ (NdeI-BamHI). After transformation into *E. coli* DH5α, insert-harboring clones were sequence-checked and an error free plasmid was used for transformation into various T7-RNA polymerase hosts (see below).

Testing of various hosts

For the isolation of plasmids Promega Plasmid DNA Purification System was used. Transformation were performed according to Novagen® pET System Manual page 25ff. The following hosts were tested: BL21(DE3), BL21 Star (DE3), Tuner™ (DE3), Rosetta™2 (DE3), BLR (DE3), NovaBlue (DE3), Origami™ (DE3), Origami B (DE3).

For each host the growth conditions (temperature before and after induction, time until induction and until harvest, concentration of IPTG) were optimized with respect to get highest cell density and highest activity per mg lyophilized cells. Reaction conditions for hydrogen transfer were optimized with respect to co-substrate concentration.

Optimized batch procedure for preparation of lyophilized cells of *E. coli* Tuner™ (DE3)/pET22b+-ADH-'A'

Medium LB-amp: Luria broth (25 g l^{-1}, Sigma L-3522), ampicillin sodium salt (100 mg l^{-1}, Sigma A9518-5G), KH_2PO_4 (1.4 g l^{-1}, Fluka 60220), K_2HPO_4 (4.4 g l^{-1},Fluka 60355).

E. coli Tuner™ (DE3)/pET22b+-ADH-'A' was stored at –86°C in a glycerol/LB-amp 15:85 solution. Prior to use it was plated on LB-amp, a single colony was plated again on LB-amp (16 h, 37°C), before a loop of cells were used to inoculate 250 ml of LB-amp medium in one liter baffled shake flasks with additional Zn^{2+} ($ZnCl_2$, 100 mg l^{-1} final concentration, Aldrich 20,808-6), which was added from a stock solution (1 ml of 25 mg $ZnCl_2$ per ml water) by sterile filtration. After incubation for 24 hours at 30°C at 130 rpm an OD of ~5 was reached and the expression of ADH-'A' was induced by the addition of IPTG (450 mg l^{-1}, 2 mM final concentration, preqlab Biotechnologie GmbH 37-2020). Again, additionally ampicillin sodium salt (50 mg l^{-1}) was added. The incubation was performed at 20°C to avoid the formation of inclusion bodies for 24 hours at 130 rpm. The cells were harvested by centrifugation (8000 rpm, 3000 g, 20 min, 4°C), the medium was decanted and the cells were resuspended in water, shock-frozen (liquid nitrogen) and lyophilized.

Analytics

GC Analyses for determination of conversion

The following columns were used: column A: Hewlett Packard HP 1301 (30 m x 0.25 mm x 0.25µm, 1.0 bar N_2); column B: J&W Scientific Agilent Technologies HP-1 (30 m x 0.25 mm x 0.25 µm, 1.0 bar N_2); column C: Chrompack Chirasil Dex (25 m x 0.32 mm x 0.25 µm, 1.0 bar H_2); column D: J&W Scientific Agilent Technologies CP 1301 (30 m x 0.25 mm x 0.25 µm, 1.0 bar N_2).

Table S3. Determination of conversion.

Compound	Program[a]	Column	Retention time [min]	
			Ketone **a**	Alcohol **b**
1	120/0/20/250/0	B	4.6	4.4
2	120/10/20/160/0	C	4.7	7.9, 8.4[c]
3	90/0/5/120/0/30/270/2	D	7.7	7.6
4	100/0/15/270/2	B	10.1	11.1
5	55/7/5/80/0/10/160/0	A	6.8	7.1
6	50/8/10/150/2	C	8.2	10.2, 10.8[b,c]
7	120/0/5/170/25	C	8.6	21.9, 23.5[c]
9	100/0/5/145/0/30/270/0	D	5.9	6.2
10	90/0/5/120/0/30/270/0	D	7.9	6.4
11	50/8/10/150/2	C	18.0	10.1, 12.1[b,c]
12	90/9/15/130/0/20/250	B	8.8	10.0
13	90/0/5/120/0/30/270/2	D	9.0	9.2
14	100/5/12/160/10	C	2.1	5.5, 6.0[c]
15	100/9/30/240/0	A	10.4	10.6
16	50/5/4/90/0/12/150	C	7.4	11.7

[a] Program: initial temp. [°C]/time [min]/slope [°C/min]/ temp. [°C]/ time [min]/slope [°C/min]/ temp. [°C]. [b] measured as acetate. [c] retention times of enantiomers using chiral column.

GC Analyses on chiral columns

The following chiral columns were used: column C: Chrompack Chirasil Dex (25 m x 0.32 mm x 0.25 μm, 10 psi H_2); column E: Astec Chiraldex G-PN (30 m x 0.32 mm, 1.0 bar H_2).

Table S4. Determination of ee values

Compound	Program[a]	Column	Retention time [min]
1b[b]	80/6.5/10/160/10	C	13.3 (*S*), 13.5 (*R*)
3b[b]	112/0/0.5/116/0/15/170/1	C	7.8 (*R*), 8.6 (*S*)
4b[b]	112/0/0.5/116/0/15/170/1	C	9.8 (*R*), 10.0 (*S*)
5b[b]	55/7/5/80/10/160	E	10.4 (*S*), 10.6 (*R*)
6b[b]	50/8/10/150/2	C	10.2 (*S*), 10.8 (*R*)
7b	120/0/5/170/25	C	21.9 (*S*), 23.5 (*R*)
9b[b]	112/0/0.5/116/0/15/170/1	C	4.8 (*R*), 5.6 (*S*)
10b	112/0/0.5/116/0/15/170/1	C	5.3 (*R*), 5.5 (*S*)
11b[b]	50/8/10/150/2	C	10.1 (*S*), 12.1 (*R*)
12b	65/5/2/150/10	C	11.8 (*S*), 12.1 (*R*)
13b[b]	112/0/0.5/116/0/15/170/1	C	11.4 (*R*), 11.6 (*S*)
14c	100/5/12/160/10	C	5.5 (*R*), 6.0 (*S*)
15c[b]	80/7/10/160	E	10.3 (*S*), 10.4 (*R*)
16c	50/5/4/90/12/150	C	11.7 (*S*), 12.2 (*R*)

[a] Program: initial temp. [°C]/time [min]/slope [°C/min]/ temp. [°C]/ time [min]/slope [°C/min]/ temp. [°C]. [b] Enantiomeric excess determined of the corresponding acetate derivative.

HPLC Analyses on chiral column

Daicel Chemical Ind. Ltd. Chiralpak OD-H (0.46 cm x 25 cm); *n*-heptane/*i*-propanol/trifluoroacetic acid (90/10/0.1), 18°C, flow 0.8 ml min^{-1};

Retention time: **8a** :17.7 min, **8b**: 21.0 (*S*), 23.5 (*R*).

Determination of absolute configuration

Absolute configurations were assigned by (i) comparison of elution order on chiral GC with published data or by (ii) optical rotations as previously described or by (iii) co-injection with commercial available material or independent synthesized chiral reference material as described before.[1]

For determination of the absolute configuration of **8b** by comparison of the optical rotation with literature data the *p*-hydroxy moiety had to be methylated. 2-Azido-1-(4-hydroxy)-phenyl-1-ethanol **8b** was methylated using freshly prepared diazomethane: To a solution of aqueous KOH (3 ml, 40 %), 2-ethoxy-ethanol (2 ml), and 10 mL *iso*-propyl ether, diazalde (4 g, 18.7 mmol) was added and the mixture was stirred vigorously. Through a steam of argon the resulting diazomethane was bubbled into an ice-cold ether solution. The reaction was finished as the ether phase was colored dark yellow. Alcohol **8b** (45 mg) was dissolved in MeOH (500 µl), diazomethane in ether was added until reaction was finished as followed by TLC. A few drops of 2 N NaOH were added, the ether phase was dried over Na_2SO_4, the solvent evaporated and the methylated azido alcohol was obtained as colorless oil (13 mg). $[\alpha]_D^{20}$ –17.7 (c = 0.7 g, $CHCl_3$, >97 % e.e.); Lit.[9]: $[\alpha]_D^{25}$ = –56.9 (c = 1.1, $CHCl_3$, 55 % e.e.)

Optical Rotation

Optical rotation values for **1b**, **3b-5b** and **9b-13b** are published in precedent papers.[1]

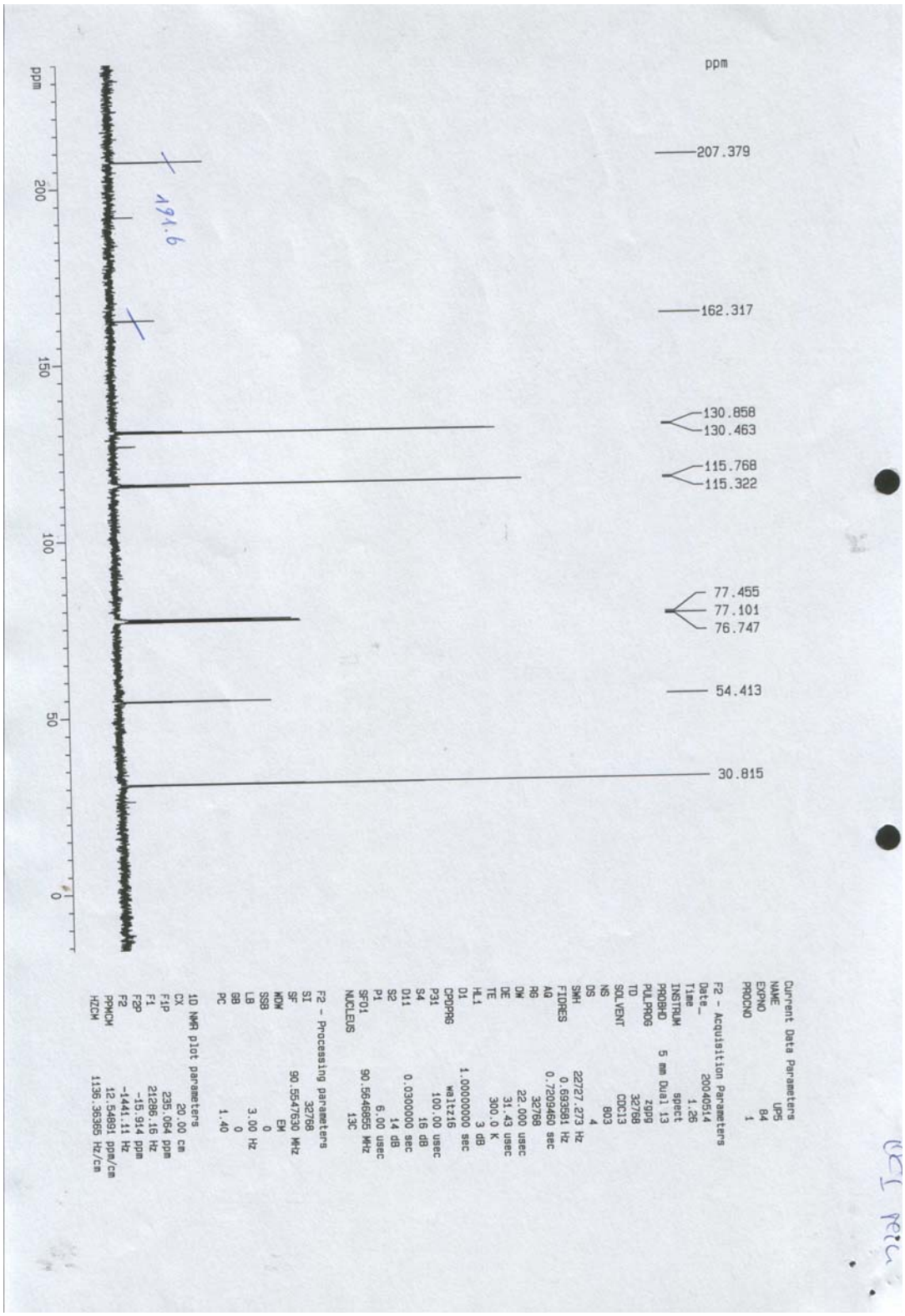
Table S5. Optical rotations of alcohols.

Alcohol	$[\alpha]_D^{20}$	literature
(*S*)-**6b**	+10.2 (c 1.2, $CHCl_3$)	+10.1 (*S*) (c 0.67, $CHCl_3$)[c]
(*R*)-**7b**	-113.1 (c 1.7, $CHCl_3$)	–89.3 (*R*) (c 0.8, $CHCl_3$)[b]
(*R*)-**8b**	-137.6 (c 1.6, $CHCl_3$)	-
(*S*)-**14c**	-169 (c 3.1, $CHCl_3$)	+ 30 (*R*)[a]
(*S*)-**15c**	+35 (c 5.0, $CHCl_3$)	-
(*S*)-**16c**	+25.4 (c 3.0, $CHCl_3$)	-

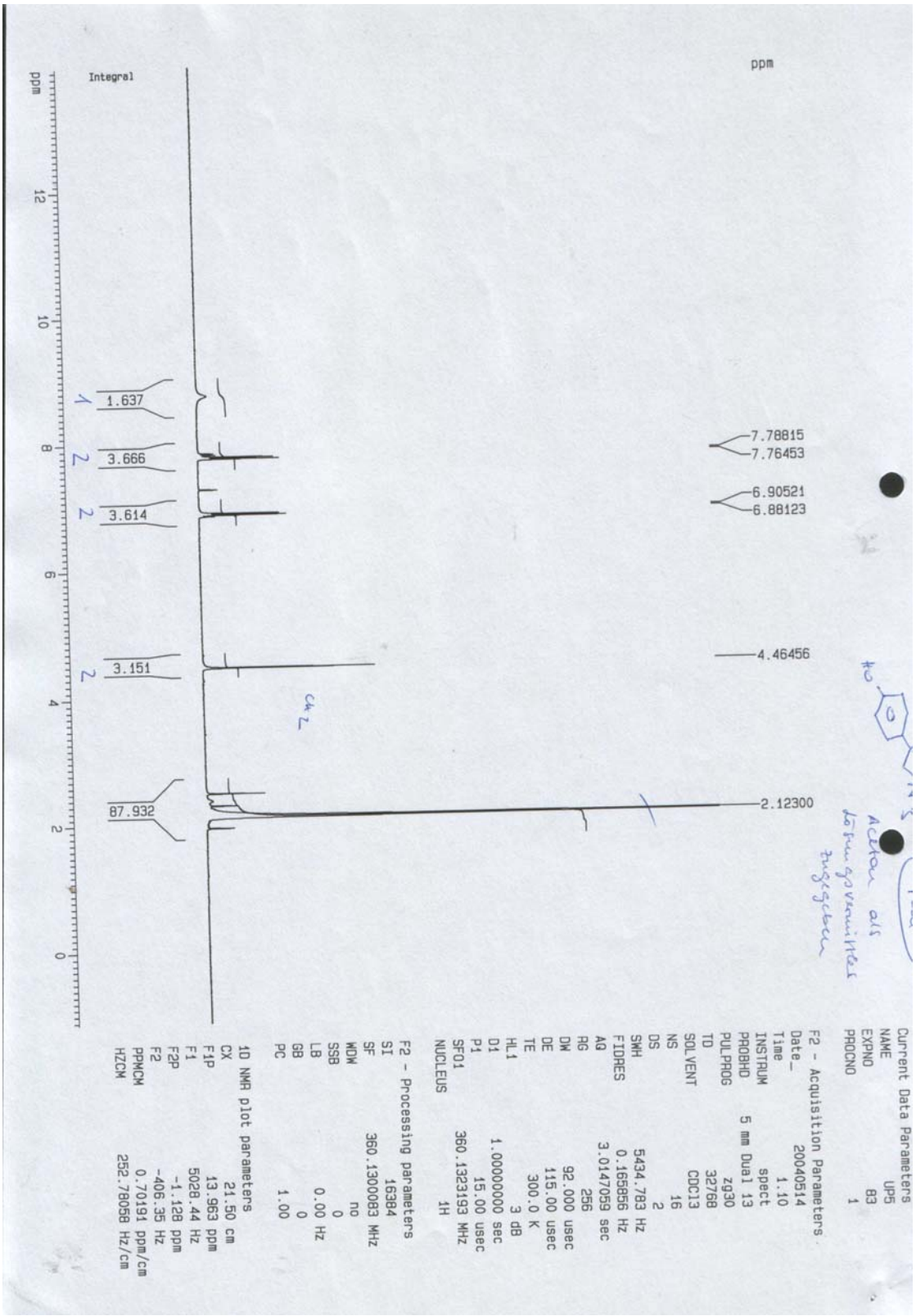
[a] Ref. 10. [b] Ref. 11. [c] Ref. 12.

NMR Spectra

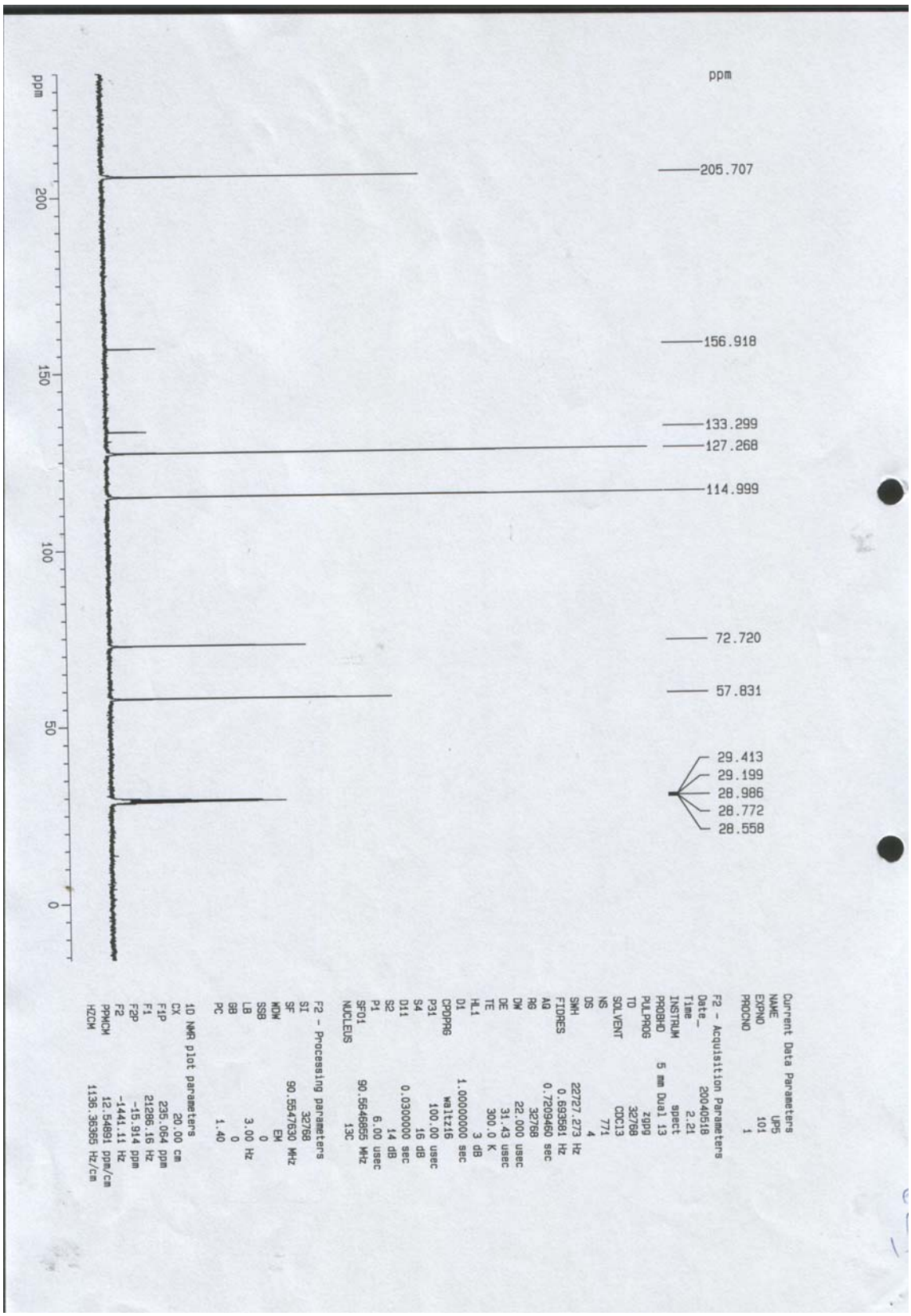
^{13}C NMR of 2-Azido-1-(4-hydroxyphenyl)ethanone **8a.**



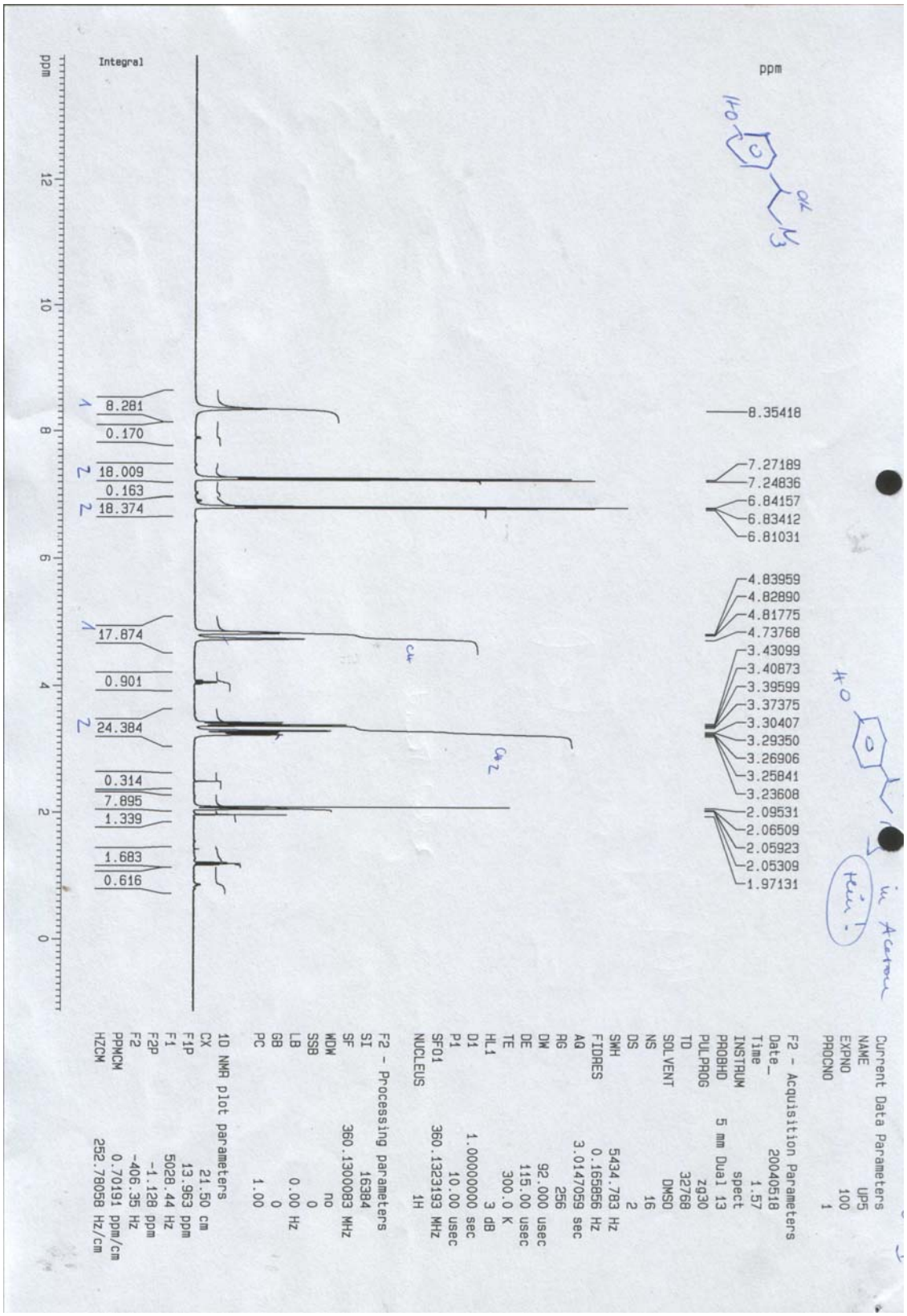
^{1}H NMR of 2-Azido-1-(4-hydroxyphenyl)ethanone **8a.**



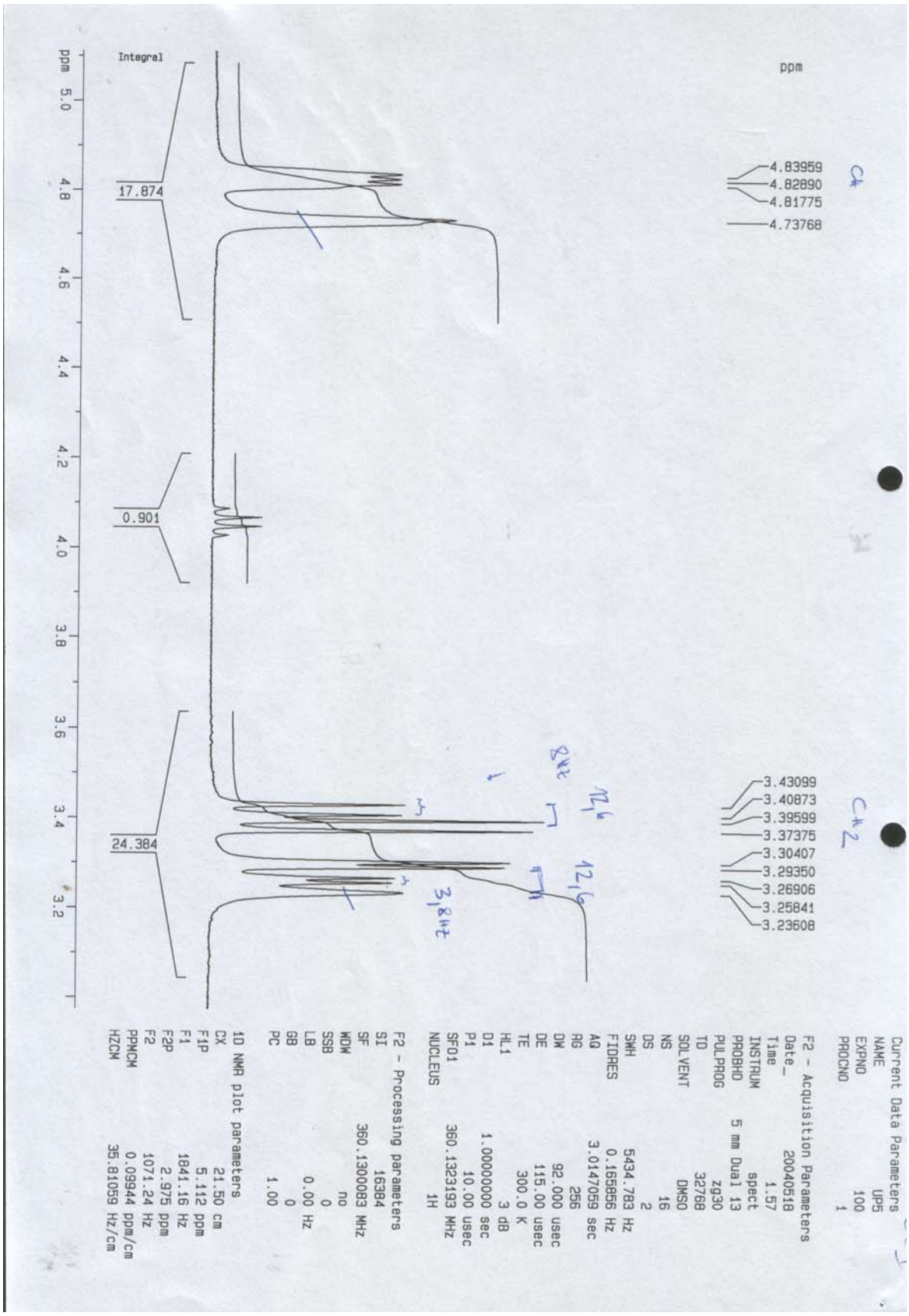
^{13}C NMR of 2-Azido-1-(4-hydroxy)-phenyl-1-ethanol **8b**.



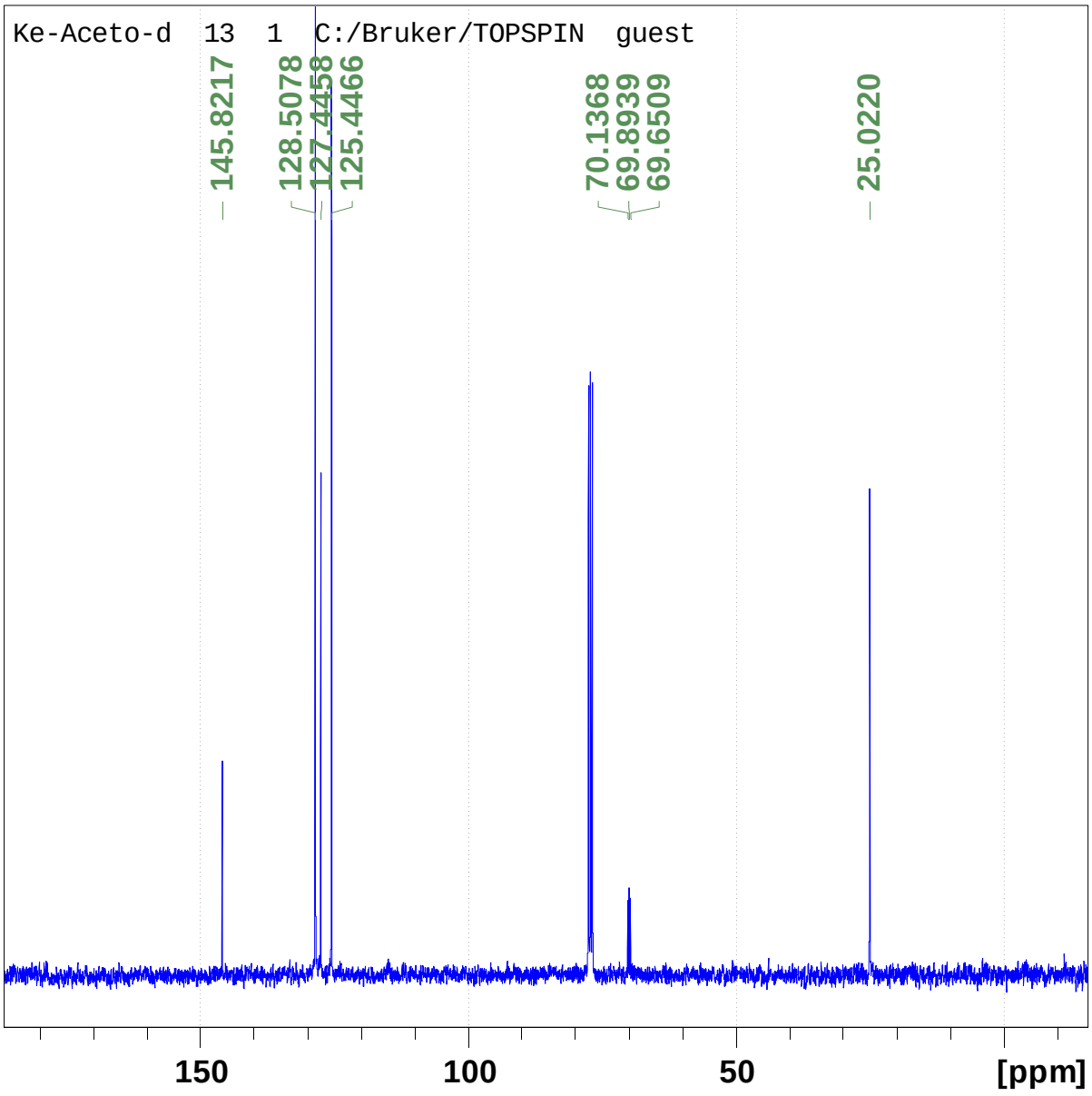
^{1}H NMR of 2-Azido-1-(4-hydroxy)-phenyl-1-ethanol **8b**.



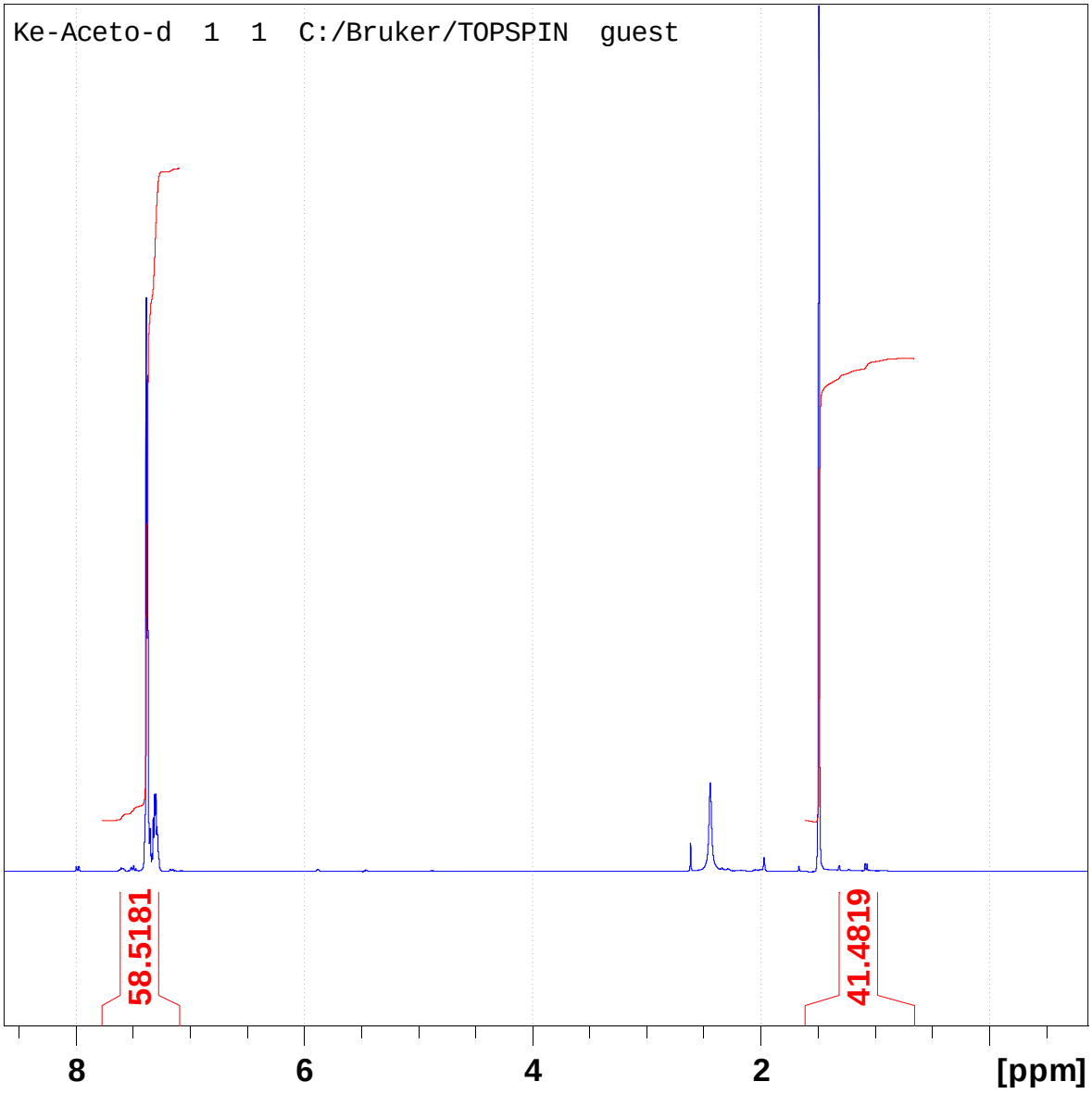
Part of ^{1}H NMR of 2-Azido-1-(4-hydroxy)-phenyl-1-ethanol **8b**.



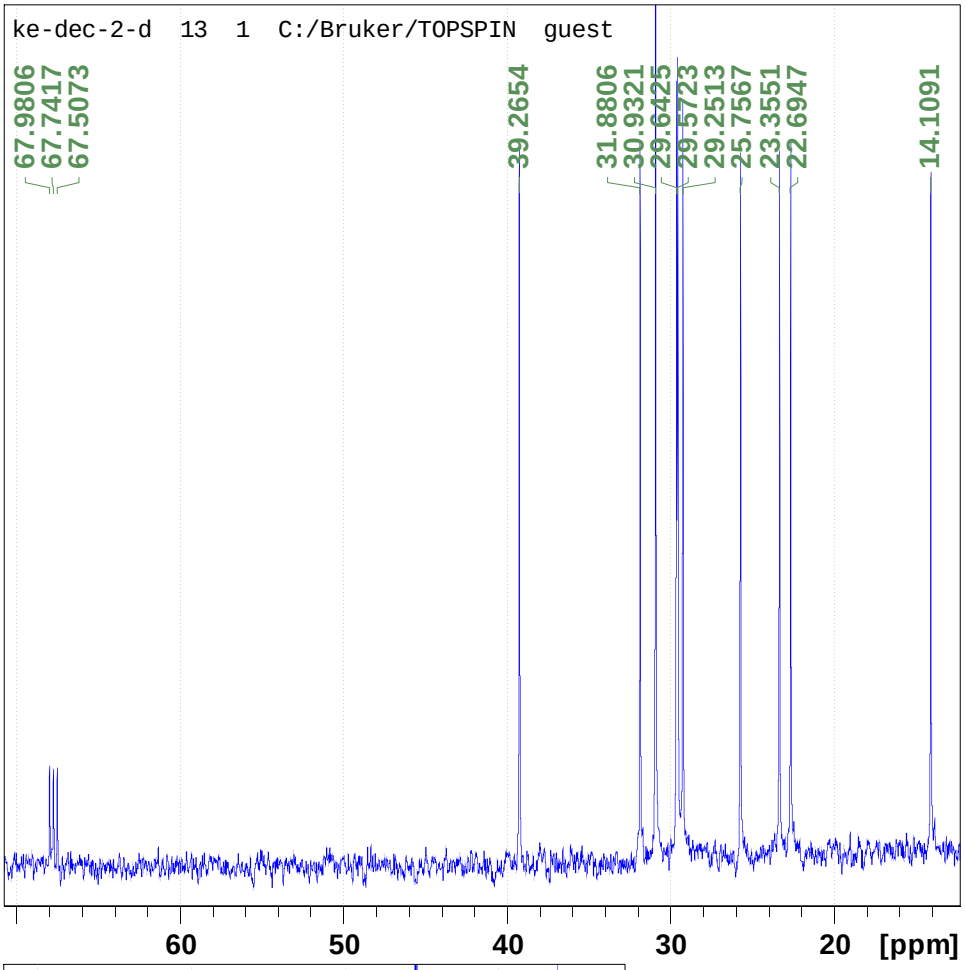
^{13}C NMR of (*S*)-1-phenyl-ethanol-1-d_1 **14c**

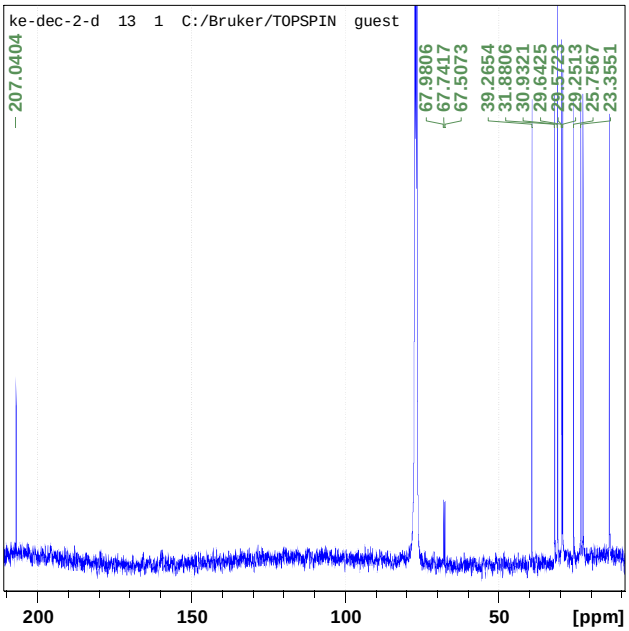


^{1}H NMR of (*S*)-1-phenyl-ethanol-1-d_1 **14c**



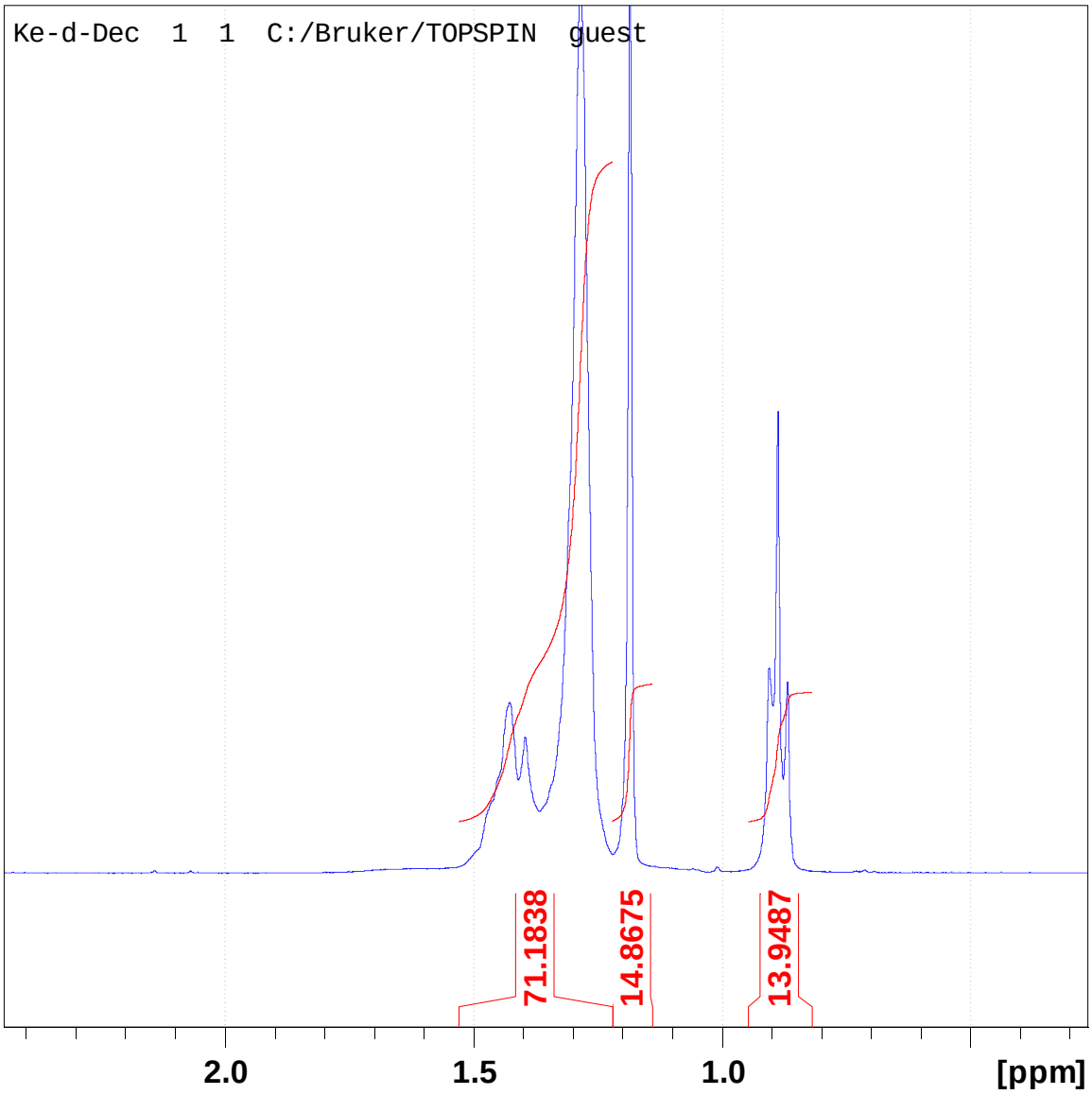
^{13}C NMR of (*S*)- 2-decanol-2-d_1 **15c**



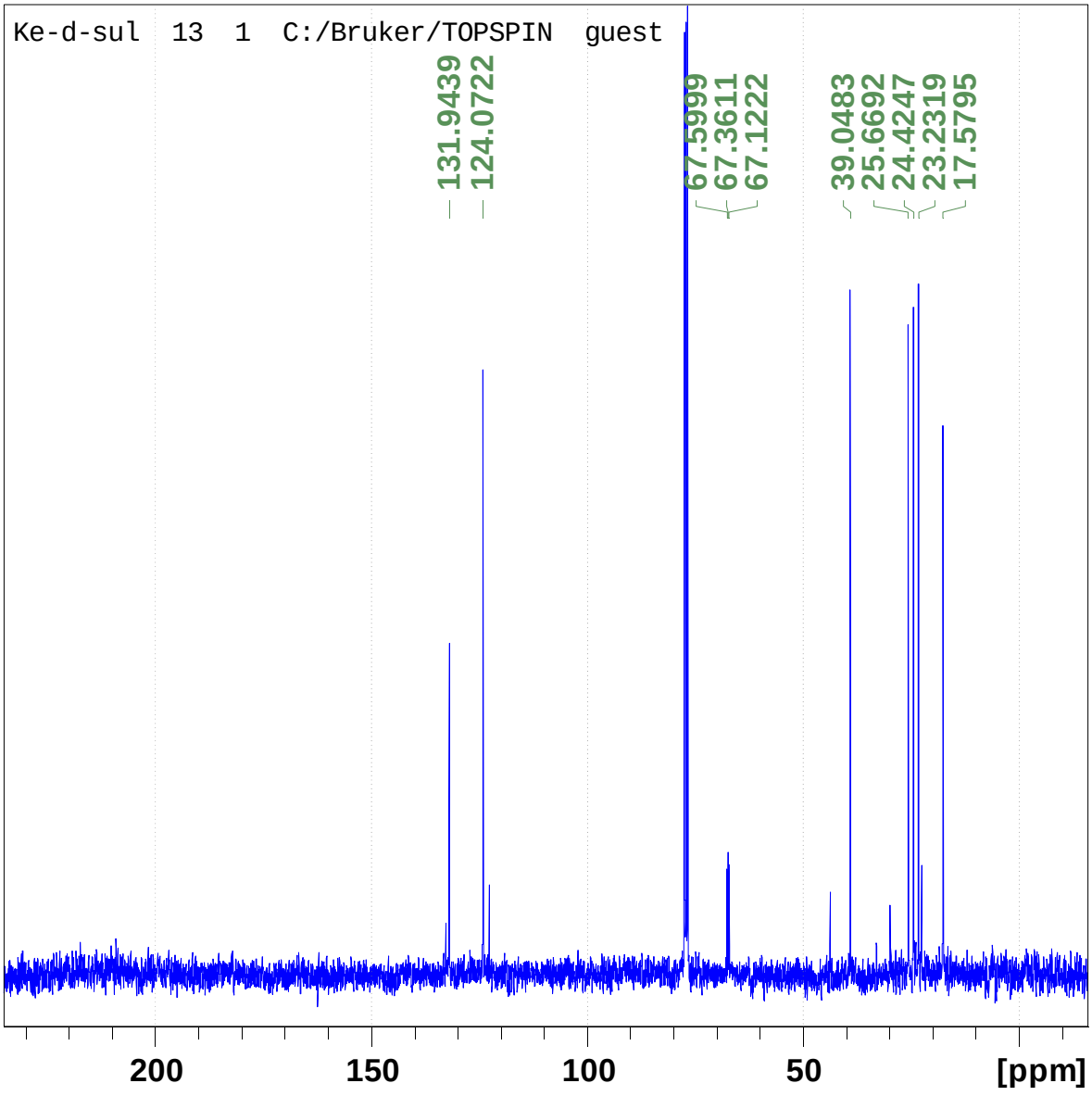


(δ 207 and 30.9 originate from acetone)

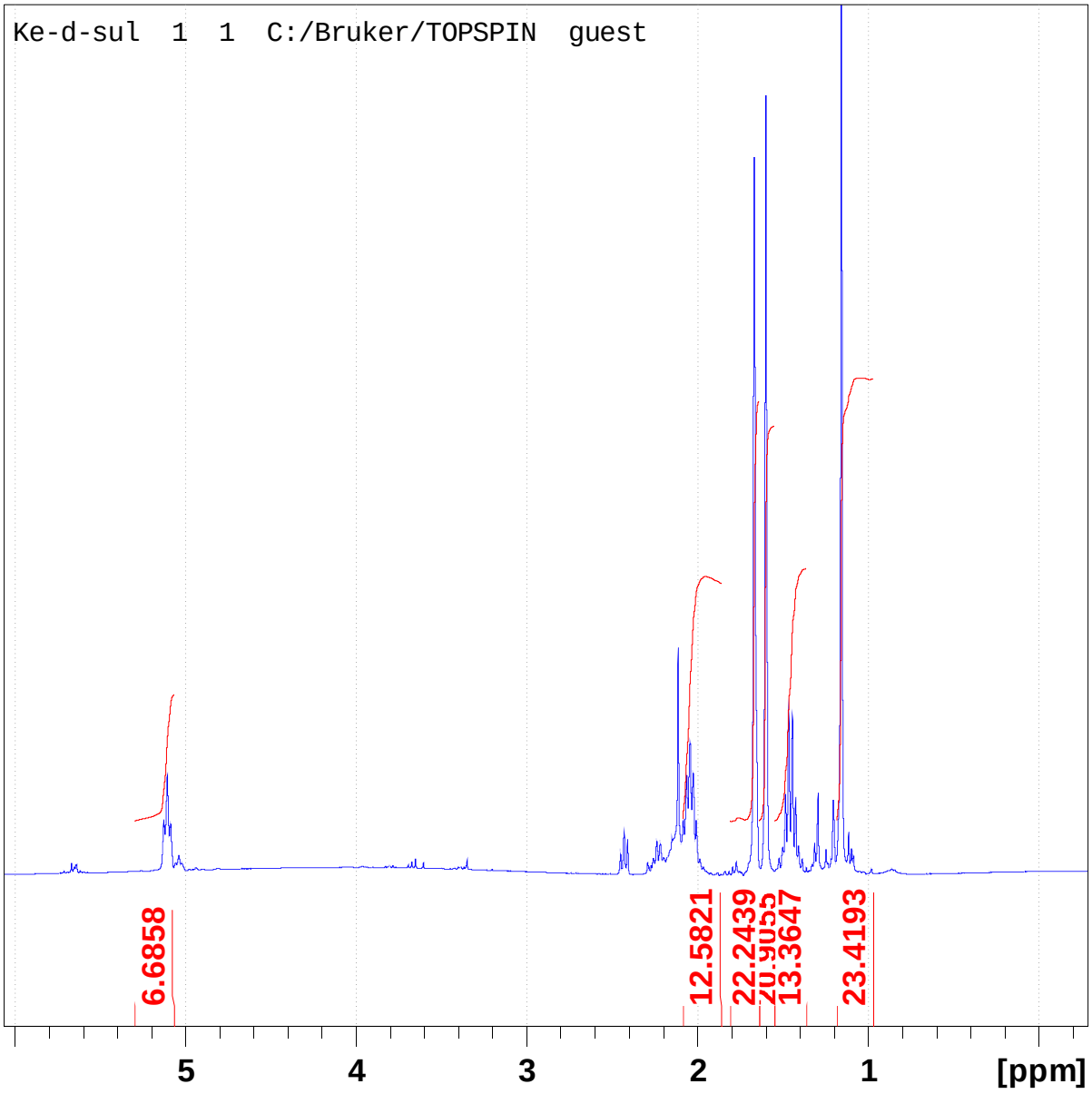
^{1}H NMR of (*S*)-2-decanol-2-d_1 **15c**



^{13}C NMR of (*S*)-6-methyl-5-hepten-2-ol-2d_1 **16c**



^{1}H NMR of (*S*)-6-methyl-5-hepten-2-ol-2d_1 **16c**



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