Supporting Information

Minimalist protein engineering of an aldolase provokes unprecedented substrate promiscuity

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Materials: All reagents and media components were purchased commercially and used without further purification. Hydroxypropanone and propanal were purchased from Sigma-Aldrich. Ketols **2-10** and ether substrates **11-15**,[1] 3-hydroxypropanal (**16**)[2] and D-glyceraldehyde[3] were prepared according to literature procedures. Synthetic oligonucleotides were purchased from Eurofins MWG Operon. Phosphonoglucose isomerase from baker's yeast (*S. cerevisiae*), glucose-6-phosphate dehydrogenase from baker's yeast (*S. cerevisiae*), D,L-glyceraldehyde 3-phosphate (G3P) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were from Sigma-Aldrich. Antibiotics, acrylamide-bisacrylamide and buffer components were from Sigma-Aldrich. Culture media components for bacteria were from Pronadisa. Milli-Q grade water was used for analytical and preparative HPLC and for preparations of buffers and other assay solutions obtained from an Arium® Pro Ultrapure Water Purification System (SartoriusStedim Biotech). All the other solvents used were of analytical grade. Bacterial strains, oligonucleotides and plasmids used in this study are listed in **Table S1**. The plasmid pQE*fsa* containing the gene for expression of FSA (gene code, *fsa*, formerly termed *mipB*)[4] was constructed in the IQAC-CSIC laboratory using routine procedures of molecular biology.

General procedures. Mutagenesis. All the DNA manipulations and bacterial transformation were carried out according to the standard protocols or manufacturers' instructions. D-Fructose-6-phosphate aldolase (FSA) gene mutations were introduced with the QuickChange site-directed mutagenesis kit (QuickChange®, Stratagene), using the plasmid pQE*fsa* as template and performed according to the manufacturer's protocols. The oligonucleotides and the templates used are listed in **Table S1**. *E. coli* Nova Blue competent cells (EMD Millipore) were used for transformation and plasmid preparation. The plasmid DNA was isolated with the High Pure Plasmid Isolation Kit (Roche). DNA sequencing and mass spectra analysis of the expressed proteins (Table S3) confirmed the expected mutations in the gene sequence.

Site directed mutagenesis. The mutants FSA L107A, and FSA L163A were obtained using pQE*fsa* as templates with the mutagenesis primers FSA L107A up and FSA L107A down and FSA L163A up and FSA L163A down, respectively, the double mutants FSA L107A/A129G and FSA A129G/L163A were obtained using pQE*fsa A129G* as template with the mutagenesis primers FSA L107 up and FSA L107 down and FSA L163A up and FSA L163A down, respectively. The double mutants FSA L107A/L163A and FSA A129S/L163A were obtained using pQE*fsa L107A* and pQE*fsa A129S* as templates respectively with the mutagenesis primers FSA L163A up and FSA L163A down. The triple mutants FSA L107A/A129G/L163A and FSA L107A/A129S/L163A were obtained using pQE*fsa A129G/L163A* and pQE*fsa A129S/L163A* as templates respectively with the mutagenesis primers FSA L107A up and FSA L107A down (**Table S1**).

HPLC analyses. HPLC analyses were performed on an X-Bridge™ C18, 5 μm, 4.6×250 mm column from Waters (Milford, USA). Samples (30 μL) were injected and eluted with the following conditions: solvent system (A) aqueous trifluoroacetic acid (TFA) 0.1% (*v/v*) and (B): TFA (0.095% (*v/v*)) in CH_3CN/H_2O (4:1), gradient elution from 10-90% B in 30 min, flow rate 1 mL min^{-1}, detection at 215 nm, column

temperature 30 ºC. The amount of aldol adduct was quantified from the peak areas using and external standard methodology.

Sample derivatization protocol. Reaction samples (10 µl) were mixed with a solution of *O*-benzylhydroxylamine hydrochloride (50 µl 21.1 mg mL^{-1}; 0.14 mmol mL^{-1}) in pyridine:methanol:water 33:15:2. After incubation at 50 ºC for 60 min, samples were diluted in methanol (940 µl) and directly analyzed by HPLC chromatography.

Table S1. Strain, plasmids and oligonucleotides used in this study. Mutagenized codons are highlighted in italic and bold, silent mutations are shown in italic.

Plasmids	Relevant genetic characteristics	Ref./Origin
pQE40	P_{T5}, Amp^r, $ColE1_{ori}$	Quiagen
pQE*fsa*	*fsa* gene (660bp) cloned in pQE40 (*BseRI/HindIII*), the C-terminal His-tag was deleted	[5]
pQE*fsaL107A*	*fsaL107A* gene cloned in pQE40	This study
pQE*fsaA129S*	*fsaA129S* gene cloned in pQE40	[6]
pQE*fsaA129G*	*fsaA129G* gene cloned in pQE40	[7]
pQE*fsaL163A*	*fsaL163A* gene (660bp) cloned in pQE40	This study
pQE*fsaL107A/A129G*	*fsaL107A/A129G* gene (660bp) cloned in pQE40	This study
pQE*fsaL107A/L163A*	*fsaL107A/L163A* gene (660bp) cloned in pQE40	This study
pQE*fsaA129G/L163A*	*fsaA129G/L163A*gene (660bp) cloned in pQE40	This study
pQE*fsaA129S/L163A*	*fsaA129S/L163A* gene (660bp) cloned in pQE40	This study
pQE*fsaL107A/A129G/L163A*	*fsaL107A/A129G/L163A*gene (660bp) cloned in pQE40	This study
pQE*fsaL107A/A129S/L163A*	*fsaL107A/A129S/L163A* gene (660bp) cloned in pQE40	This study
Strains	Relevant genotype	Ref./Origin
E. coli M15[pREP4]	*nal*^{s}, *str*^{s}, *rif*^{s}, *thi*$^-$, *lac*$^-$, *ara*$^+$, *gal*$^+$, *mtl*$^-$, F$^-$, *rec*A$^+$, *uvr*$^+$, *lon*$^+$.	Qiagen
E. coli Nova Blue	*end*A1, *hsd*R17(rB$^+$, mB$^+$), *sup*E44, *thi-1*, *rec*A1, *gyr*A96, *rel*A1 *lac* F´ [*pro*A$^+$B$^+$, *lac*I^q ZΔM15::Tn10] (*Tet*^{R})	EMD Millipore
Oligonucleotides	Oligonucleotide sequences (5´→ 3´)	Ref./Origin
FSAL107A up	CGACG***GCA***GGAACAGCGGTATATGGCG	This study
FSAL107A down	ACCGCTGTTCCTG***CCG***TCGGAATCCCTTCC	This study
FSAL163A up	GAAAGT***GGC***GGCAGCGAGTTTCAAAAC	This study
FSAL163A down	GAAACTCGCTGCC***GCC***ACTTTCGCCTGC	This study

Protein expression and purification. The plasmids were transformed into *E. coli* strain M-15 [pREP-4] (QIAGEN). Cells were grown at 37 ºC in YT medium (4 L) containing ampicillin (100 mg L^{-1}) and kanamycin (25 mg L^{-1}) up to an optical density of 0.6 at 600 nm. For protein expression, temperature was lowered to 30 ºC and IPTG (1 mM final concentration) was added. After additional incubation for 12 h cells were harvested, suspended in starting GlyGly buffer (50 mM, pH 8.5) containing dithiothreithol (DTT) (1 mM) and lysed using a TS 0.75 kW 40K Cell Disrupter (Constant Systems). Cellular debris was removed by centrifugation at 8228 × g for 30 min at 4 ºC. The clear supernatant was treated with heat-shock (70 ºC, 30 min), and centrifuged at 8228×g for 30 min at 4 ºC. The supernatant was dialyzed against GlyGly buffer (5 mM, pH 8.5) containing DTT (0.1 mM) and finally, lyophilized. Protein concentrations were calculated with the Bradford method.

Activity assay: The formation of D-fructose 6-phosphate from dihydroxyacetone (DHA) (300 mM) and D,L-glyceraldehyde 3-phosphate (2.8 mM) was monitored with a coupled assay in GlyGly buffer (50 mM, pH 8.5) containing DTT (1 mM) at 30 ºC using phosphonoglucose isomerase and glucose-6-phosphate dehydrogenase. The reduction of $NADP^+$ (0.5 mM) was monitored at 340 nm for 5 minutes in a Cary Bio UV-Visible Spectrophotometer (Varian, Darmstadt, Germany); the formation of 1 μmol of NADPH

was set equivalent to 1 μmol of D-fructose 6-phosphate formed (one U will produce 1 μmol of D-fructose 6-phosphate (D-F6P) per minute). Table S2 showed the specific activity U mg^{-1} protein Bradford of the mutants used in this work.

Table S2. Specific aldolase activity of the FSA wild-type and mutants used in this work.

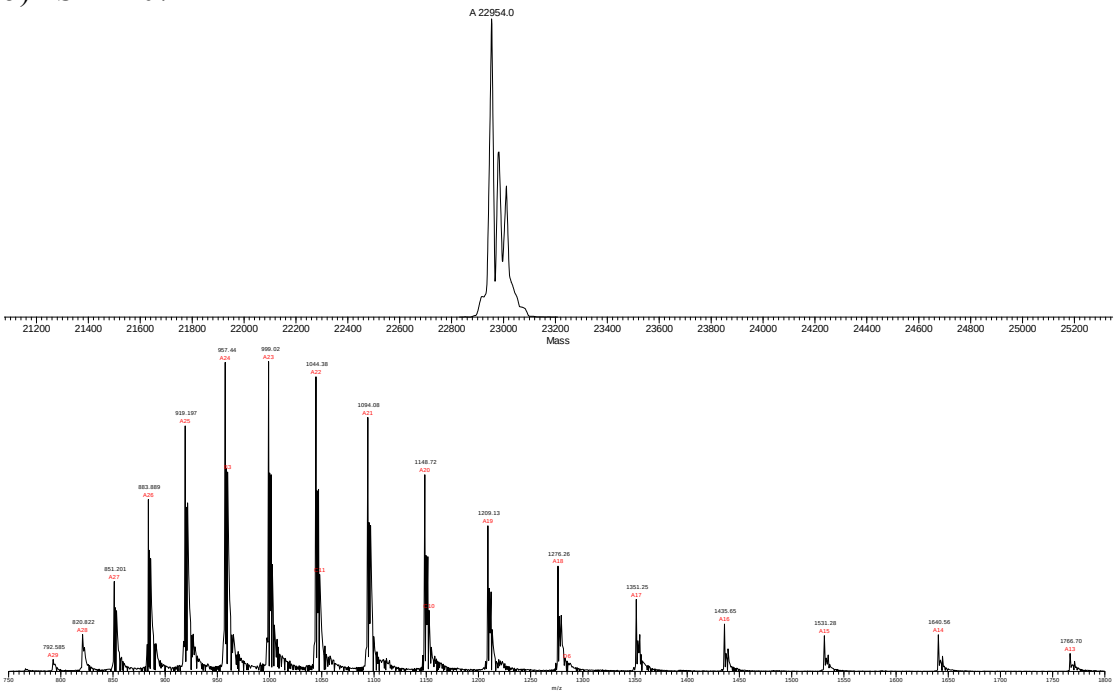
Enzyme	Specific activity U mg^{-1} protein (Bradford)
FSA	0.22±0.01
FSA A129G	0.36±0.1
FSA A129S	9.4±1.3
FSA L107A	0.52±0.03
FSA L163A	0.41±0.10
FSA L107A/129G	0.36±0.04
FSA L107A/L163A	0.50±0.01
FSA A129G/L163A	0.42±0.03
FSA A129S/L163A	0.57±0.04
FSA L107A/A129G/L163A	0.13±0.01
FSA L107A/A129S/L163A	0.56±0.04

Electrospray ionization mass spectrometry of proteins. Sample preparation: each protein (1 mg of lyophilized powder) was resuspended in water (1 mL). To this solution formic acid (150 μL) and acetonitrile (50 μL) was added. Samples (10 μL) were analyzed by UPLC-ESI-MS using an Acquity UPLC™ BEH300 C18 column (1.7 μm, 2.1 × 100 mm), and an ESI-TOF mass spectrometer (LCT Premier Waters, Milford, MA, USA) equipped with a 4 GHz time-to-digital converter (TDC) with a dual ESI source (LockSpray). The second sprayer provided the lock mass calibration with leucine enkephalin (m/z 556.2771). The ESI-TOF was operated in the W-optics mode, thus providing a mass resolution of at least 10 000 full-width at half maximum (FWHM). The acquisition time per spectrum was set to 0.2 s, and the mass range was from 200 to 1800 Da. Data were acquired using a cone voltage of 50 V, capillary voltage of 3000 V, desolvation temperature of 350 ºC, and source temperature of 100 ºC. The desolvation gas flow was set at 400 L h^{-1} and the cone gas flow was set at 30 L h^{-1}. The solvent system used for the elution was: solvent (A): formic acid 0.1% (v/v) in acetonitrile and solvent (B) aqueous formic acid 0.1% (v/v), gradient elution 0% B for 5 minutes, and from 0% to 70% B in 14 min, from 70% to 100% B in 2 min, flow rate 0.3 mL min^{-1}. MassLynx 4.1 (Waters, Milford, MA, USA) was used for data acquisition and processing. MagTran software kindly provided by Dr. Zhongqi Zhang (Amgen, Inc.; Thousand Oaks, CA, USA) was used for molecular weight deconvolution from ESI-MS spectra of proteins. Values are given in Table S3.

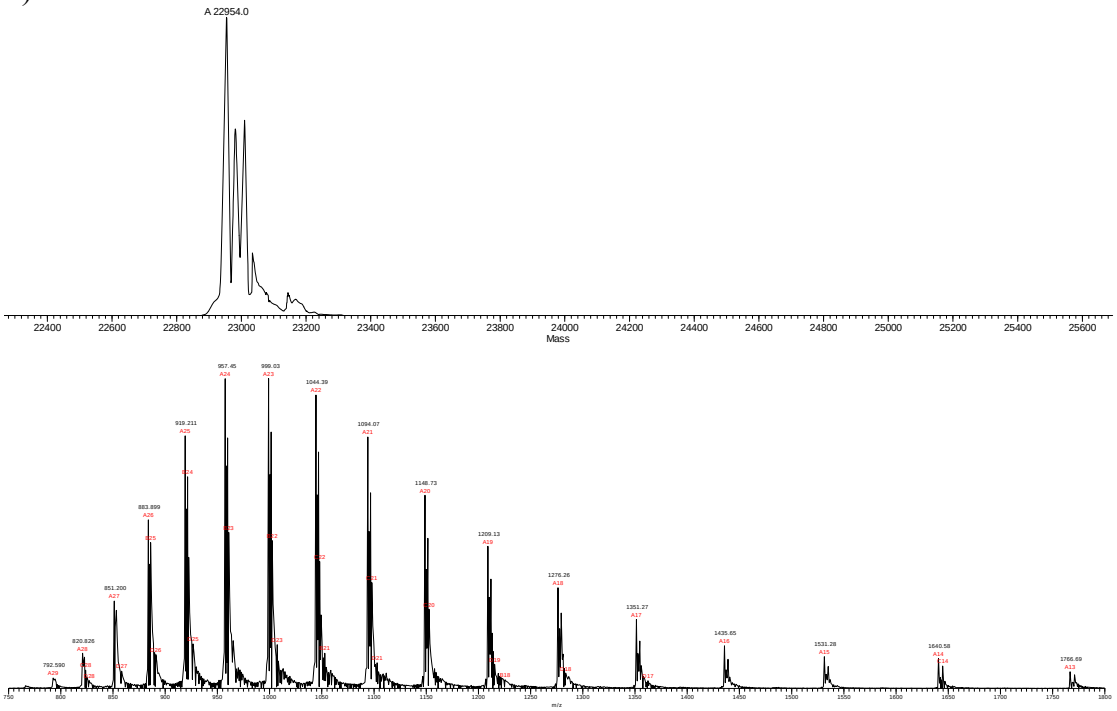
Table S3. Mass spectrometry of the FSA mutants

FSA mutants	**Calculated**	**Observed**	Error abs (Da)
FSA L107A	22953.98	22954.0	0.02
FSA L163A	22953.98	22954.0	0.02
FSA L107A/A129G	22939.95	22940.0	0.05
FSA L107A/L163A	22911.90	22912.0	0.10
FSA A129G/L163A	22939.95	22940.0	0.05
FSA A129S/L163A	22969.97	22969.0	0.97
FSA L107A/A129G/L163A	22897.87	22898.0	0.13
FSA L107A/A129S/L163A	22927.90	22928.0	0.10

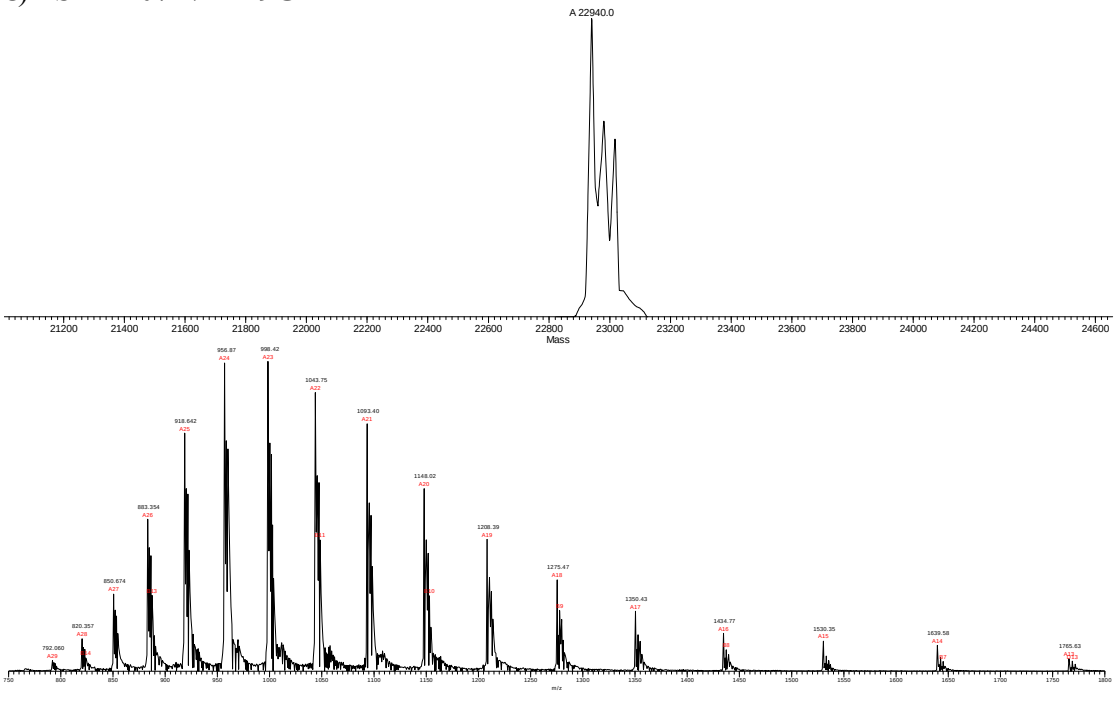
a) FSA L107A



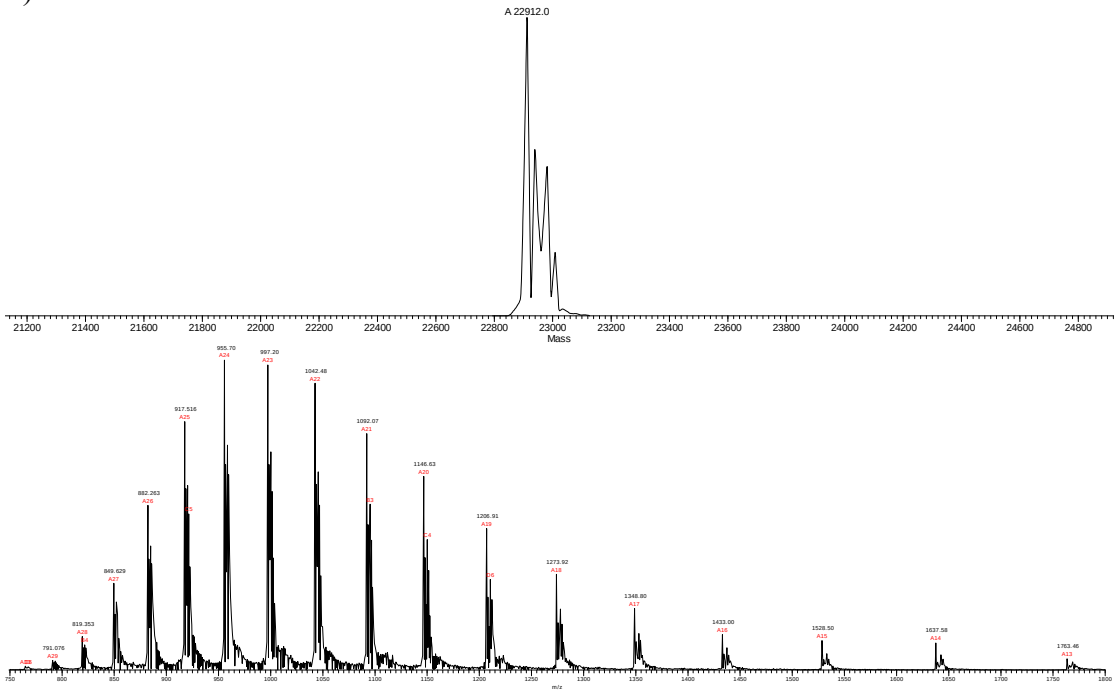
b) FSA L163A



c) FSA L107A/A129G



d) FSA L107A/L163A



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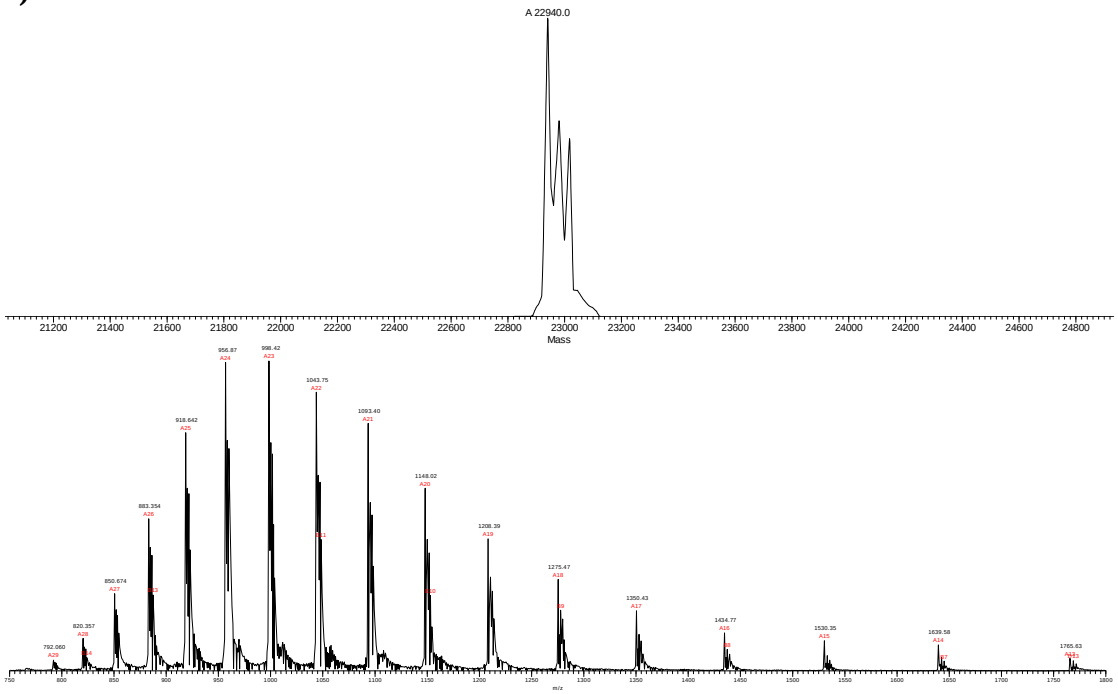
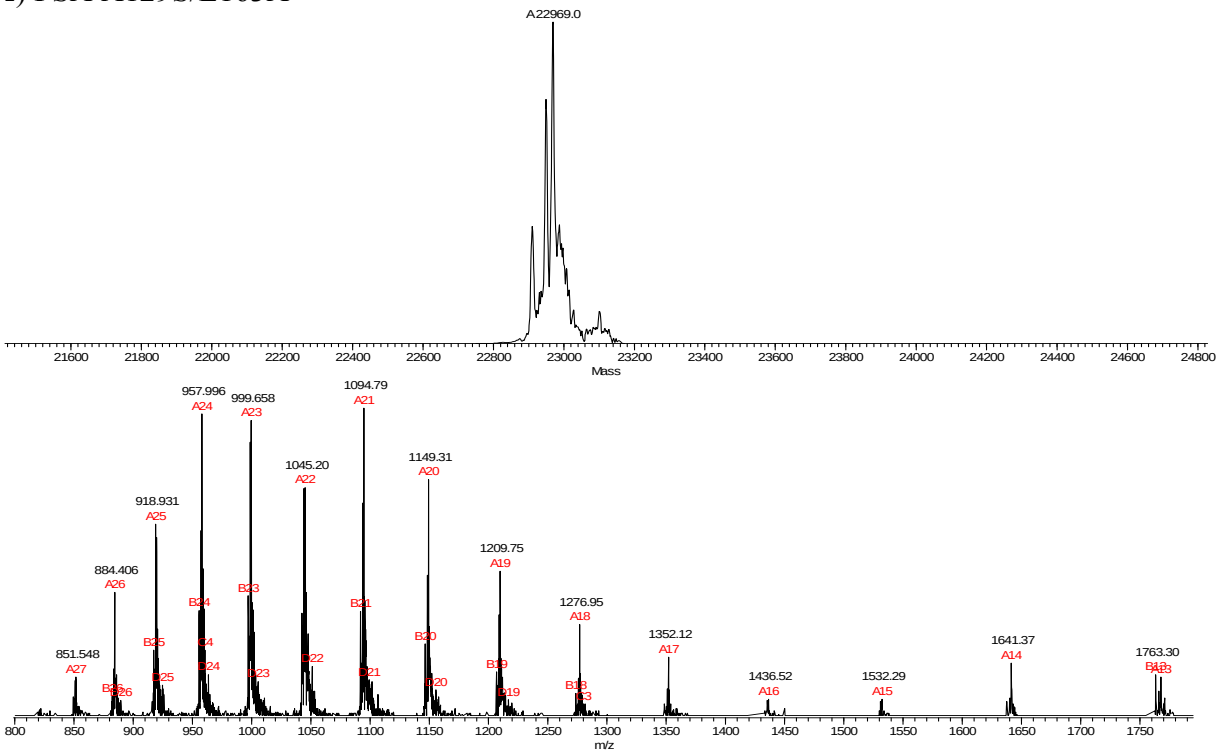
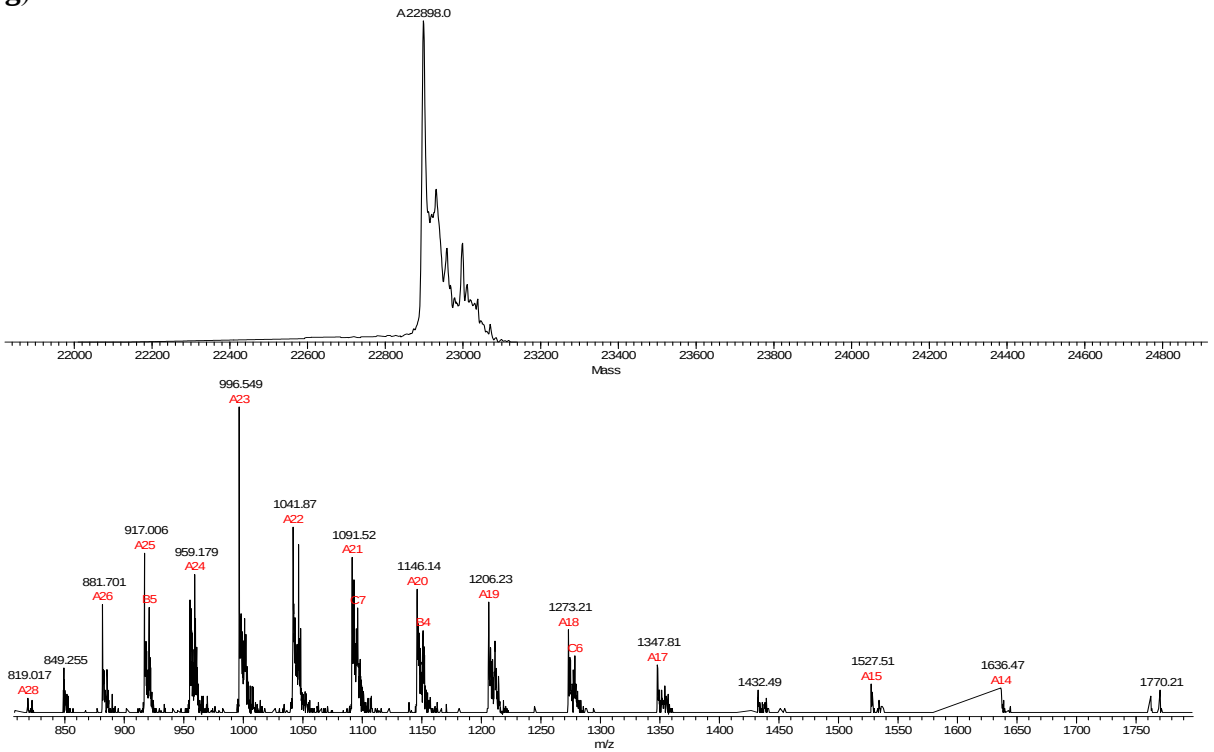


f) FSA A129S/L163A



g) FSA L107A/A129G/L163A



h) FSA L107A/A129S/L163A

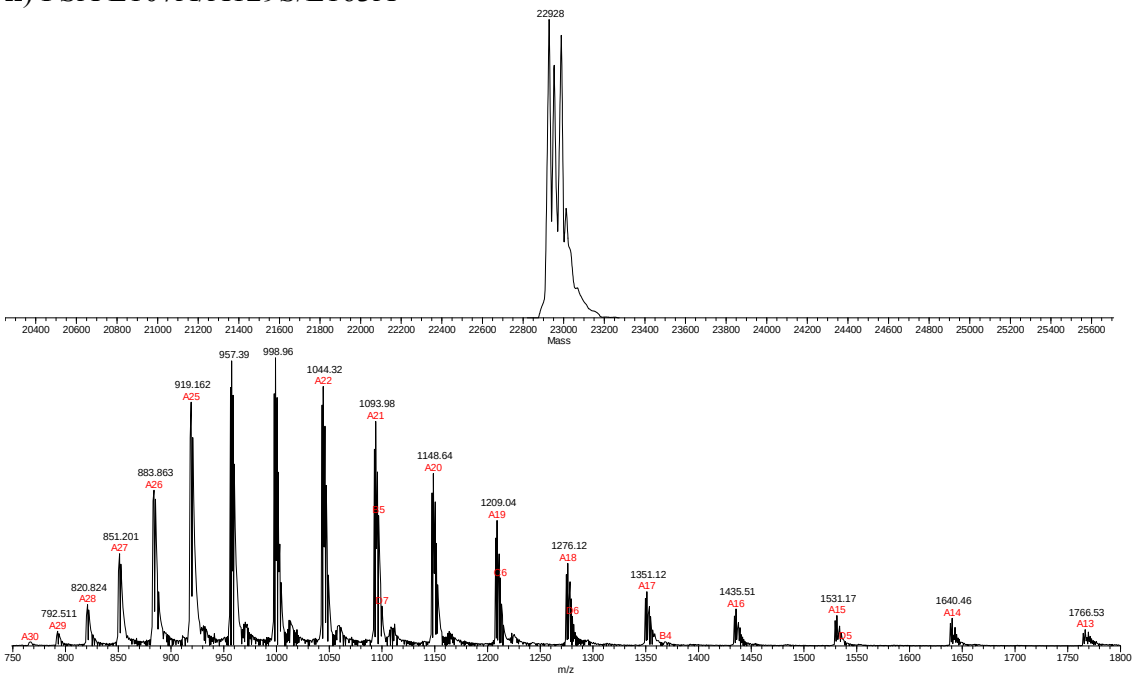


Figure S1. MS ESI/TOF spectra and deconvolution spectra of FSA mutants.

Differential scanning fluorimetry.[8,9] Thermal unfolding of FSA and variants was determined at 1 mg protein mL^{-1} concentration using a 1:500 dilution of SYPRO Orange fluorescent dye (Sigma-Aldrich). The measurements were performed in a total volume of 25 µL in MicroAmp Fast Optical 48-well reaction plates sealed with optical adhesive film using a StepOne Real-Time PCR system (Applied Biosystems). Samples were heated from 25 °C to 95 °C applying a heating ramp of 2 °C min^{-1}. Fluorescence development was monitored with excitation at 480 nm through a 605 nm filter. The melting curves of the individual enzymes were analyzed using the software StepOne 2.0 (Applied Biosystems). The midpoint temperatures obtained are summarized in Table S4 and Figure S2.

Table S4. Midpoint temperatures of the protein-unfolding transition (Tm) for FSA variants.

FSA variant	Tm
wild-type	87.0
L107A	90.4
A129G	86.4
A129S	87.0
L163A	78.4
L107A/L163A	81.4
A129G/L163A	79.4
A129S/L163A	79.5
L107A/A129G	87.6

L107A/A129G/L163A	81.0
L107A/A129S/L163A	84.0

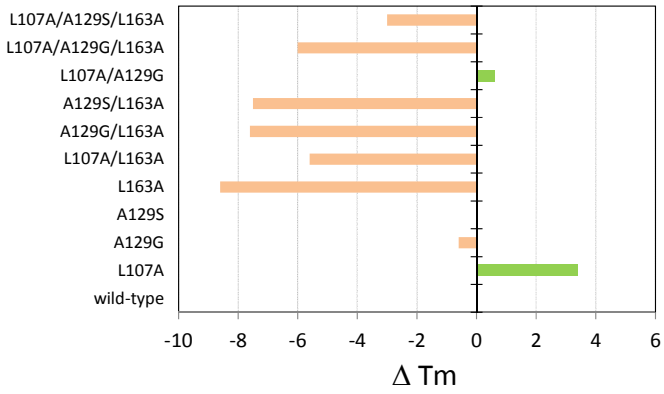


Figure S2. Changes in the unfolding transition temperature (ΔTm) in reference with the FSA wild-type. A negative ΔTm value signifies that the mutation destabilizes the protein, and a positive ΔTm value indicates that the mutation has a stabilizing effect.

Enzymatic aldol reactions with the 3-hydroxy-2-alkanones or ether derivatives and 3-hydroxypropanal. A preliminary set of reactions was conducted to test the obtained mutant proteins with selected nucleophiles. Reactions (200 µL total reaction volume) were performed using 96-deepwell plates stirred with vortex mixer (1200 rpm) at room temperature. To each ketol (0.02 mmol, 100 mM), triethanolamine buffer (50 mM, pH 8, 65 µL) was added. To this solution, 3-hydroxypropanal was added (85 µL, 150 mM final concentration) from a stock solution (350 mM in plain water). The reactions were started by adding the corresponding FSA variant (50 µL, 2 mg mL^{-1} protein, for units see Table S2) from a stock solution (5 mg protein mL^{-1}) in triethanolamine buffer (50 mM, pH 8). Samples (10 µL) were withdrawn at 0, 2, 24 h reaction times and followed the sample derivatization protocol prior to HPLC analysis.

Initial reaction rates of aldol additions of different ketols to 3-hydroxypropanal.**(Table S5).** The most productive enzymes were chosen for a second set of reactions to calculate the initial rates of the aldol reaction (v_o, µmol min^{-1} mg^{-1}). Reactions were carried out on analytical scale in a 96-deepwell plate (1 mL) as follows. To the ketol substrates (0.03 mmol, 100 mM) triethanolamine buffer (50 mM, pH 8, 87-157 µL depending on the amount of enzyme solution used) was added. To this mixture, 3-hydroxypropanal solution (113 µL, 150 mM) was added from a stock solution (350 mM in plain water). The reactions were initiated by the addition of the corresponding FSA mutant in solution (between 30 µL and 100 µL) from stock enzyme preparations (between 3 to 5 mg protein mL^{-1}) in triethanolamine buffer (50 mM, pH 8) to ensure a linear dependence of the product formation versus time at the beginning of the reaction (i.e., reaction conversions <10%) and stirred with the vortex mixer at 1200 rpm and 25 ºC. At different reaction times (0-20 min) samples (10 µL) were withdrawn and followed by the sample derivatization protocol prior to HPLC analysis. Concentrations of the aldol products were calculated with an external standard method from peak areas.

Table S5. Initial rates v_0 (mmol min^{-1} mg^{-1}) for top variants with non-natural substrates.[a]

FSA variant	Ketol					
	1	**2**	**3**	**4**	**8**	
wt	0.31±0.02	0.25±0.03	–[b]	–[b]	–[b]	
L163A	0.98±0.04	0.71	0.11	0.06	–[b]	
L197A/L163A	0.48	1.33	1.25	0.94±0.10	0.32±0.01	
L107A/A129S/L163A	0.47	0.73	0.61±0.08	0.60	0.30±0.01	
FSA variant[a]			**11**	**12**	**13**	**14**
wt			0.12±0.02	–[b]	–[b]	–[b]
L163A			0.76±0.05	0.17±0.02	0.18±0.01	–[b]
L197A/L163A			1.84±0.20	1.28±0.13	0.54±0.08	0.08
L107A/A129S/L163A			1.08±0.19	0.58±0.07	0.62±0.08	0.08

[a] Data presented as the mean of triplicate experiments and for some selected experiments data is the mean of quintuplicate experiments ± standard error of the mean (SE). [b] No reaction.

In situ NMR experiments. Competition reactions were carried out with FSA L107A/L163A to compare the reactivity of 1-hydroxypentan-2-one and 1-hydroxy-3-methoxypropan-2-one in the aldol reactions to 3-hydroxypropanal. The experiments were conducted on a Varian Anova-500 spectrometer. All components were prepared in D_2O. A solution of 1-hydroxypentan-2-one (**3**) and 1-hydroxy-3-methoxypropan-2-one (**11**) (0.075 mmol, 75 mM final concentration each) were prepared in bicarbonate buffer (50 mM, pH 8, 870 µL). To this mixture 3-hydroxypropanal solution was added (113 µL, 150 mM) from a stock solution of (750 mM in plain water). This mixture was transferred into an NMR tube. The initial spectra was taken and the FSA L107A/L163A mutant (1 mg protein, for the activity see Table S2) was then added. The progress of the reaction was monitored by ^{1}H NMR (500 MHz), the water signal was suppressed by presaturation, recording the spectra each 23 min (8 min acquisition time) for a time period of 6 h. A copy of the recorded spectra is shown in Figure S3.

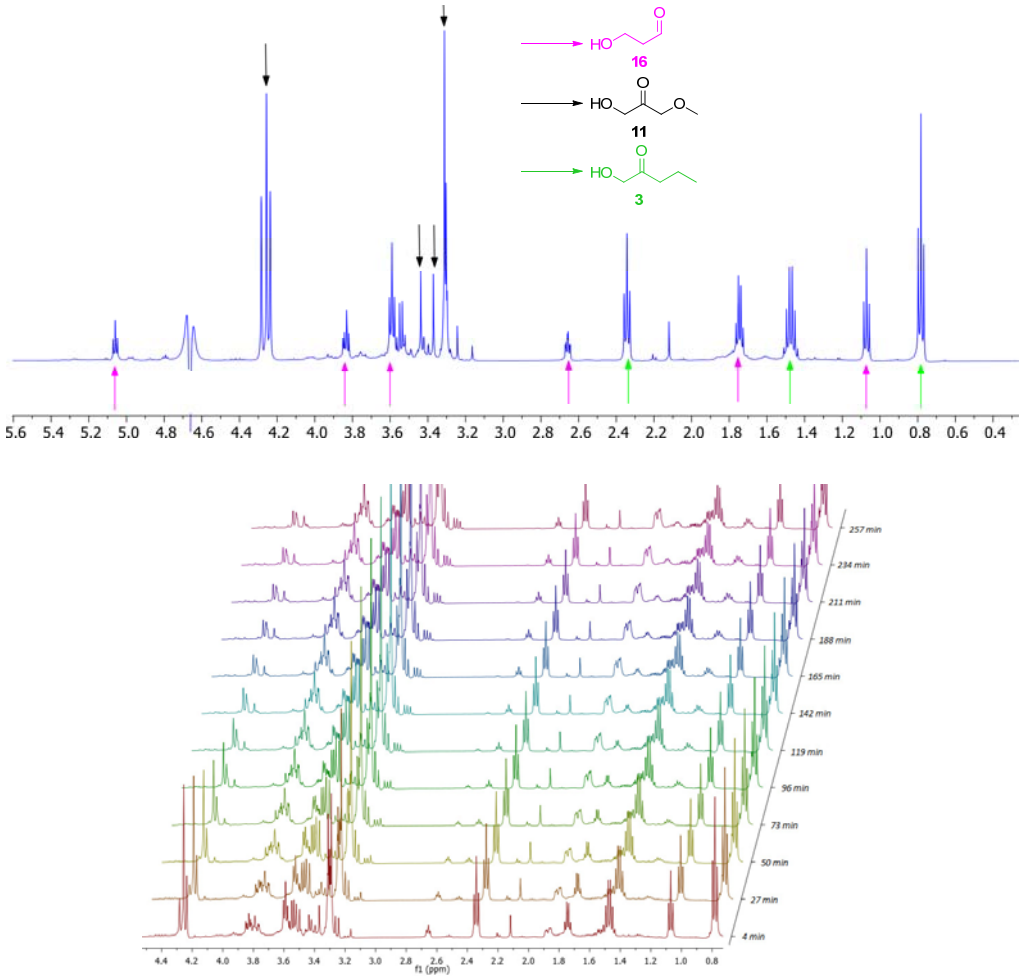


Figure S3. *In situ* NMR monitoring of the competition addition reaction of **3** and **11** to **16** using FSA L107A/L163A as catalyst.

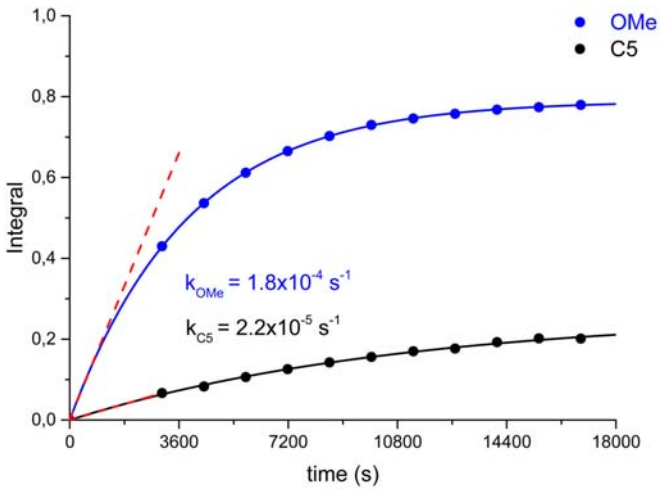
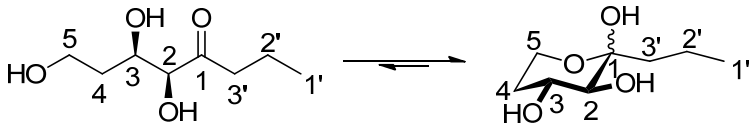


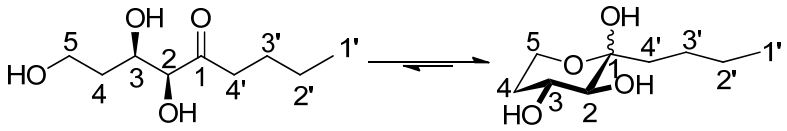
Figure S4. Aldol addition reaction of 1-hydroxypentan-2-one (**3**) and 1-hydroxy-3-methoxypropan-2-one (**11**) to 3-hydroxypropanal (**16**) in competition experiments. Evolution of the aldol adduct concentrations using 1-hydroxypentan-2-one (black squares) and 1-hydroxy-3-methoxypropan-2-one (blue circles) with the reaction time.

General procedure for enzymatic syntheses. Lyophilized FSA L107A/L163A variant (15 mg) was added to a solution (10 mL total reaction volume) containing the respective ketol (150 mM) and electrophile (100 mM) in glycyl-glycine buffer (50 mM, pH 8.5), and the resulting mixture was incubated at room temperature with monitoring at regular intervals by TLC (chloroform-methanol 5:1). Depending on the rate of aldehyde consumption, completed reactions were worked up after 24-48 h by lyophilization of the crude reaction mixture. The residue was purified by silica gel column chromatography using chloroform/methanol (15:1 to 5:1) as eluent to provide the pure aldol products. ^{1}H, ^{13}C NMR and HRMS data for all new compounds is given below. IUPAC nomenclature for carbohydrates as well as for functionalized ketones / ethers are not congruent, and require reversal of numbering with higher carbon chain lengths, although all products share an identical pyranose ring with the same configuration. Thus, for transparent comparison of NMR data, signal assignments are given as an artificial numbering system indicated individually for each structure, in which the underlying sugar skeleton is numbered regularly from the anomeric carbon, to which a "side chain" is added with dashed numbering.

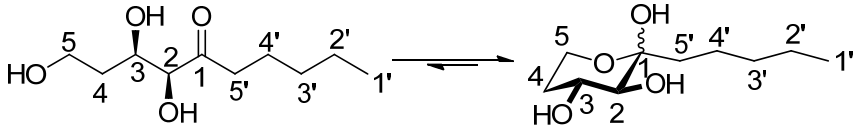
Structural analysis by NMR spectroscopy confirmed that in aqueous solution the products **17-31** participate in a constitutional equilibration in which open-chain isomers reversibly form pyranose hemiacetals as α/β-anomeric forms. Particularly, the well-separated multiplets of the methine protons adjacent to the vicinal diol structure, together with the subterminal methylene unit, prove the relative *trans*-diol structure with equatorially positioned hydroxyl groups. Within both the aliphatic (**17-26**) and ether series (**27-31**), the fraction of α-anomers strongly decreases with increasing bulkiness of the variable constituent, while that of the open-chain isomers increases. For the ketone structures only single C=O resonances were observed, testifying to the high level of stereoselectivity in the enzymatic carboligation, because formation of diastereoisomers would have given rise to additional signal sets. NMR data of compounds 17 and 18 are coincident to those previously described.[10]



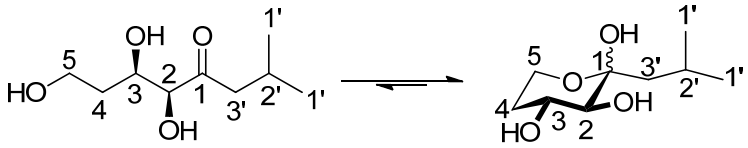
(5*S*,6*R*)-5,6,8-trihydroxyoctan-4-one (19). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (75%). Product ratio: open chain (34%): β-anomer (67%): α-anomer (5%). Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.29 (m, 1H, *H*-2), 4.27 (m, 1H, *H*-3), 3.73 (m, 2H, *H*-5), 2.63 (t, *J* = 7.3 Hz, 2H, *H*-3'), 1.85 (m, 2H, *H*-4), 1.60 (m, 2H, *H*-2'), 0.90 (m, 3H, *H*-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 215.2 (*C*-1), 79.2 (*C*-2), 68.4 (*C*-3), 58.3 (*C*-5), 40.4 (*C*-3'), 35.1 (*C*-4), 16.5 (*C*-2'), 12.9 (*C*-1'). β-Anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.87 (m, 2H, *H*-3, *H*-5$_a$), 3.65 (m, 1H, *H*-5$_{eq}$), 3.30 (d, *J* = 9.5 Hz, 1H, *H*-2), 1.97 (m, 1H, *H*-4$_{eq}$), 1.73 (m, 2H, *H*-3') 1.62 (m, 1H, *H*-4$_a$), 1.38 (m, 2H, H-2'), 0.91 (m, 3H, *H*-1).^{13}C NMR (126 MHz, D_2O) δ (ppm) 99.5 (*C*-1), 74.8 (*C*-2), 68.2 (*C*-3), 58.5 (*C*-5), 39.8 (*C*-3'), 32.8 (*C*-4), 15.6 (*C*-2'), 13.6 (*C*-1'). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.38 (d, *J* = 7.1 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for for $C_8H_{16}O_4Na$ 199.0946, found 199.0940.



(3*R*,4*S*)-1,3,4-trihydroxynonan-5-one (20). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (76%). Product ratio: α-anomer (5%), open chain (41%), β-anomer (54%). Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.36 (m, 1H, *H*-2), 4.33 (m, 1H, *H*-3), 3.81 (dt, *J* = 2.4 Hz, 6.2 Hz, 2H, *H*-5), 2.72 (t, *J* = 7.4 Hz, 2H, *H*-4'), 1.92 (m, 2H, *H*-4), 1.63 (m, 2H, *H*-3'), 1.40 (m, 2H, *H*-2'), 0.96 (m, 3H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 215.4 (*C*-1), 79.3 (*C*-2), 68.6 (*C*-3), 58.4 (*C*-5), 38.3 (*C*-4'), 35.2 (*C*-4), 25.1 (*C*-3'), 21.7 (*C*-2'), 13.1 (*C*-1'). β-Anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.94 (m, 2H, *H*-3, *H*-5_a), 3.72 (m, 1H, *H*-5_{eq}), 3.37 (d, *J* = 9.4 Hz, 1H, *H*-2), 2.04 (m, 1H, *H*-4_{eq}), 1.78 (m, 1H, *H*-4') 1.68 (m, 1H, *H*-4_a), 1.42 (m, 1H, *H*-4'), 1.40 (m, 2H, *H*-3'), 0.96 (m, 3H, H-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 99.60 (*C*-1), 75.0 (*C*-2), 68.4 (*C*-3), 58.6 (*C*-5), 37.3 (*C*-4'), 32.9 (*C*-4), 24.4 (*C*-3'), 22.4 (*C*-2'), 13.3 (*C*-1'). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.46 (d, *J* = 7.0 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for $C_9H_{18}O_4Na$ 213.1103, found 213.1091.

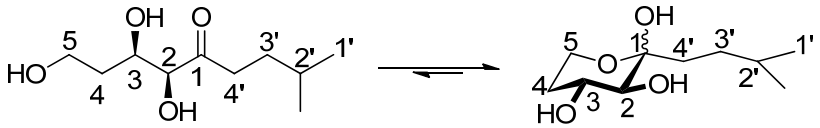


(3*R*,4*S*)-1,3,4-trihydroxydecan-5-one (21). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (50%) as a colorless liquid. Product ratio: α-anomer (4%): open chain (62%), β-anomer (34%). Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.34 (m, 1H, *H*-2), 4.32 (m, 1H, *H*-3), 3.81 (td, *J* = 6.5, 2.3 Hz, 2H, *H*-5), 2.71 (t, *J* = 7.4 Hz, 2H, H-5'), 1.92 (m, 2H, *H*-4),1.66 (m, 2H, *H*-4'), 1.42 (m, 1H, *H*-4'), 1.45 (dt, *J* = 15.3, 7.5 Hz, 4H, *H*-2', *H*-3') 1.40 (m, 2H, *H*-3'), 0.96 (t, *J* = 7.0 Hz, 3H, H-1'), 0.94 (m, 3H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 215.3 (*C*-1), 79.3 (*C*-2), 68.6 (*C*-3), 58.4 (*C*-5), 38.5 (*C*-5'), 35.2 (*C*-4), 30.7 (*C*-4'), 22.7 (*C*-3'), 21.8 (C-2'), 13.3 (*C*-1'). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.94 (m, 2H, *H*-3, *H*-5_a), 3.71 (ddd, *J* = 11.2, 7.0, 4.7 Hz, 1H, *H*-5_{eq}), 3.37 (d, *J* = 9.4 Hz, 1H, *H*-2), 2.04 (m, 1H, *H*-4_{eq}), 1.79 (m, 3H, *H*-5', *H*-4_a), 1.37 (m, 6H, *H*-4', *H*-3', *H*-2'), 1.01 – 0.90 (m, 3H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 99.6 (*C*-1), 75.0 (*C*-2), 68.4 (*C*-3), 58.6 (*C*-5), 37.6 (*C*-5'), 33.0 (*C*-4), 31.4 (*C*-4'), 21.9 (*C*-2'), 13.4 (*C*-1'). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.46 (d, *J* = 7.0 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for $C_{10}H_{20}O_4Na$ 227.1259, found 227.1251.



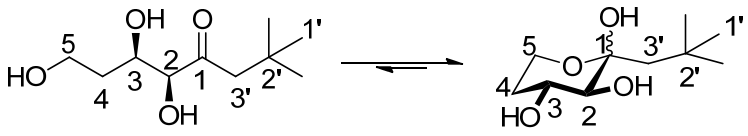
(5*S*,6*R*),5,6,8-trihydroxy-2-methyloctan-4-one (23). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (28%) as a colorless liquid.

Product ratio: α-anomer (<1%), open chain (87%), β-anomer (13%). Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.35 (m, 2H, *H*-2, *H*-3), 3.81 (m, 2H, *H*-5), 2.61 (qd, *J* = 16.6, 7.0 Hz, 2H, *H*-3'), 2.20 (st, *J* = 13.5, 6.8 Hz, 1H, H-2'), 1.92 (m, 2H, *H*-4), 0.99 (dd, *J* = 6.7, 1.6 Hz, 6H). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 214.8 (*C*-1), 79.5 (*C*-2), 68.4 (*C*-3), 58.4 (*C*-5), 47.4 (*C*-3'), 35.3 (*C*-4), 24.2 (*C*-2'), 21.8 (*C*-1'). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.94 (m, 2H, *H*-3, *H*-5_a), 3.72 (m, 1H, *H*-5_{eq}), 3.38 (d, *J* = 9.4 Hz, 1H, *H*-2), 2.04 (m, 1H, *H*-4_{eq}), 1.73 (m, 3H, *H*-3', *H*-4_a), 1.02 (m, 6H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 100.0 (*C*-1), 75.3 (*C*-2), 66.8 (*C*-3), 48.9 (*C*-5), 46.0 (*C*-3'), 32.9 (*C*-4), 23.7 (*C*-2'), 21.7 (*C*-1'). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.37 (d, *J* = 9.3 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for $C_9H_{18}O_4Na$ 213.1103, found 213.1101.



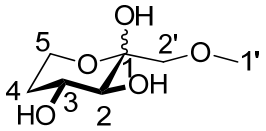
(3*R*,4*S*)-1,3,4-trihydroxy-8-methylnonan-5-one (24). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (25%) as a colorless liquid.

Product ratio: α-anomer (5%), open chain (54%), β-anomer (41%). Some starting ketol substrate also present. Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.27 (m, 2H, *H*-2, *H*-3), 3.73 (m, 2H, *H*-5), 2.65 (t, 2H, *J* = 7.5 Hz, *H*-4'), 1.88 (m, 2H, *H*-4), 1.50 (m, 3H, *H*-2', *H*-3'), 0.88 (t, *J* = 6.5 Hz, 6H, *H*-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 215.6 (*C*-1), 79.3 (*C*-2), 68.6 (*C*-3), 58.4 (*C*-5), 36.7 (*C*-4'), 35.2 (*C*-4), 31.9 (*C*-3'), 27.1 (*C*-2'), 21.6 (*C*-1'). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.86 (m, 2H, *H*-3, *H*-5_a), 3.64 (m, 1H, *H*-5_{eq}), 3.29 (d, *J* = 9.4 Hz, 1H, *H*-2), 1.96 (ddd, *J* = 11.1, 5.1, 2.1 Hz, 1H, *H*-4_{eq}), 1.75 (m, 1H, *H*-4'), 1.61 (m, 1H, *H*-4_a), 1.54 (m, 3H, *H*-2', *H*-3'), 0.88 (t, *J* = 8.1, 4.1 Hz, 6H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 99.7 (*C*-1), 75.0 (*C*-2), 68.4 (*C*-3), 58.6 (*C*-5), 35.5 (*C*-4'), 33.0 (*C*-4), 31.16 (*C*-3'), 27.72 (*C*-2'), 21.89 (*C*-1'). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.38 (d, *J* = 6.8 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for $C_{10}H_{20}O_4Na$ 227.1259, found 227.1254.

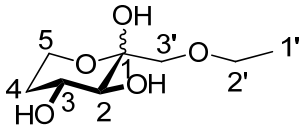


(5*S*,6*R*)-5,6,8-trihydroxy-2,2dimethyloctan-4-one (26). The title compound was prepared according to the general procedure described above. The yellow liquid was

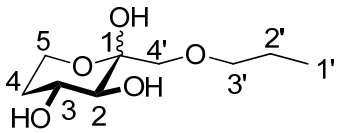
purified by chromatography to give the title compound (25%) as a colorless liquid. The product could not be obtained in pure form due to difficulties in separating undesired side products with similar properties that arise from competing chemical conversions upon the required long reaction times. Product ratio: not determined. Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.13 (m, 2H, *H*-2, *H*-3), 3.89 (m, 2H, *H*-5), 2.31 (s, 2H, *H*-3'), 1.95 (m, 2H, *H*-4), 1.09 (s, 9H, *H*-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 206.1 (*C*-1), 89.7 (*C*-2), 69.6 (*C*-3), 56.9 (*C*-5), 49.0 (*C*-3'), 35.2 (*C*-2'), 30.7 (*C*-4), 22.7 (*C*-1'). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.01 (m, 2H, *H*-3, *H*-5_a), 3.78 (m, 1H, *H*-5_{eq}), 2.09 – 1.98 (m, 5H), 2.04 (m, 1H, *H*-4_{eq}), 1.09 (s, 9H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 97.7 (*C*-1), 76.2 (*C*-2), 69.6 (*C*-3), 57.9 (*C*-5), 49.0 (*C*-3'), 33.0 (*C*-4), 29.21 (*C*-1'), 29.1 (*C*-2'). ESI-MS: m/z $[M + Na]^+$ calculated for $C_{10}H_{20}O_4Na$ 227.1259, found 227.1265.



(3*S*,4*R*)-3,4,6-trihydroxy-1-methoxyhexan-2-one (27). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (89%) as a colorless liquid.
Product ratio: α-anomer (<1%), β-anomer (>99%). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.98 (m, 2H, *H*-3, *H*-5_{eq}), 3.78 (m, 1H, *H*-5_{ax}), 3.70 (d, *J* = 13.1 Hz, 1H, *H*-2'), 3.48 (d, *J* = 13.1 Hz 1H, *H*-2), 3.47 (s, 3H, *H*-1') 3.44 (d, *J* = 13.1 Hz, 1H, *H*-2'), 2.07 (ddt, *J* = 12.7, 4.8, 1.9 Hz 1H, *H*-4_{eq}), 1.72 (tdd, *J* = 13.0, 11.5, 5.3 Hz 1H, *H*-4_{ax}). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 97.8 (*C*-1), 74.0 (*C*-2'), 72.6 (*C*-1'), 68.2 (*C*-3), 59.0 (*C*-5), 58.8 (*C*-2), 32.9 (*C*-4). α-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.38 (d, *J* = 9.3 Hz, 1H, *H*-2). ESI-MS: m/z $[M + Na]^+$ calculated for $C_7H_{14}O_5Na$ 201.1731, found 201.1728.

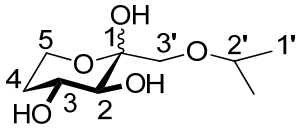


(3*S*,4*R*)-1-ethoxy-3,4,6-trihydroxyhexan-2-one (28). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (82%) as a colorless liquid.
Product ratio: α-anomer (<1%), β-anomer (>99%). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.97 (m, 2H, *H*-3, *H*-5_{eq}), 3.77 (m, 1H, *H*-5_{ax}), 3.62 (m, 3H, *H*-2', *H*-3'), 3.42 (m, 2H, *H*-2, *H*-3'both are d with *J* = 10.0 Hz), 2.05 (m, 1H, *H*-4_{eq}), 1.72 (tdd, *J* = 13.0, 11.5, 5.3 Hz, 1H, *H*-4_{ax}), 1.26 (t, *J* = 7.1 Hz, 3H, *H*-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 97.9 (*C*-1), 72.7 (*C*-2), 71.7 (*C*-2'), 68.2 (*C*-3), 67.4 (*C*-3'), 58.7 (*C*-5), 32.8 (*C*-4), 14.1 (*C*-1'). ESI-MS: m/z $[M + Na]^+$ calculated for $C_8H_{16}O_5Na$ 215.0895, observed 215.0902.



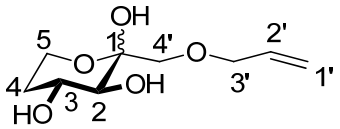
(3*S*,4*R*)-3,4,5-trihydroxy-1-propoxyhexan-2-one (29). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (28%) as a colorless liquid.

Product ratio: α-anomer (<1%), β-anomer (>99%). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.96 (m, 2H, *H*-3, *H*-5_a), 3.76 (ddd, *J* = 11.8, 5.1, 1.6 Hz, 1H, *H*-5_{eq}), 3.70 (d, *J* = 10.4 Hz, 1H, *H*-3'), 3.59 (m, 2H, *H*-4'), 3.50 (two d, 2H, *H*-2, *H*-3' both are d with *J* = 10.0 Hz), 2.04 (m, 1H, *H*-4_{eq}), 1.73 (m, 1H, *H*-4_a), 1.66 (dd, *J* = 14.3, 7.0 Hz, 2H, *H*-2'), 0.96 (t, *J* = 7.4 Hz, 3H, *H*-1').^{13}C NMR (126 MHz, D_2O) δ (ppm) 97.9 (*C*-1), 73.6 (*C*-4'), 72.7 (*C*-2), 72.1 (C-3'), 68.3 (*C*-3), 58.7 (*C*-5), 32.8 (*C*-4), 21.9 (*C*-2'), 9.8 (*C*-1'). ESI-MS: m/z $[M + Na]^+$ calculated for $C_9H_{18}O_5Na$ 229.1052, found 229.1053.



(3*S*,4*R*)-3,4,6-trihydroxy-1-isopropoxyhexan-2-one (30). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (30%) as a colorless liquid.

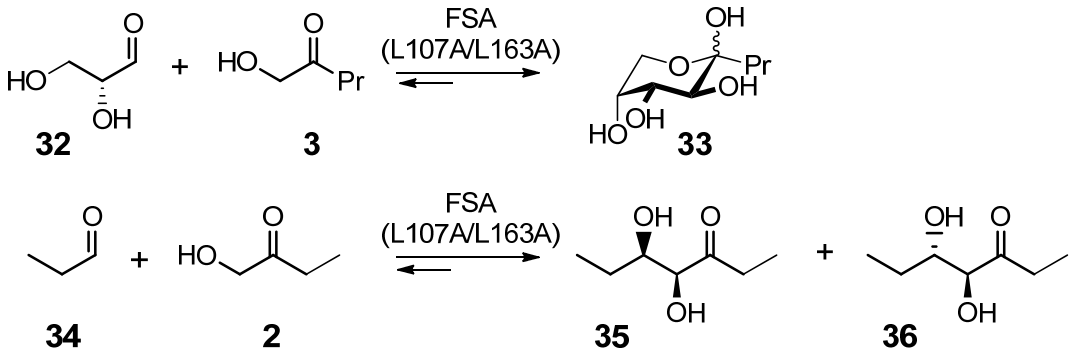
Product ratio: α-anomer (<1%), β-anomer (>99%). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 3.99 (m, 1H, *H*-5_{eq}), 3.96 (m, 1H, *H*-3), 3.82 (m, 1H, *H*-2'), 3.76 (m, 1H, *H*-5_{ax}), 3.69 (d, *J* = 10.3 Hz, 1H, *H*-3'), 3.52 (dd, *J* = 9.9, 4.4 Hz, 2H, *H*-2, *H*-3'), 2.05 (ddt, *J* = 12.9, 4.2, 1.9 Hz, 1H, *H*-4_{eq}), 1.72 (tdd, *J* = 13.1, 11.5, 5.3 Hz, 1H, *H*-4_{ax}), 1.25 (m 6H, *H*-1'). ^{13}C NMR (126 MHz, D_2O) δ (ppm) 98.1 (*C*-1), 73.6 (*C*-2'), 72.7 (*C*-2), 69.8 (*C*-3'), 68.4 (*C*-3), 58.8 (*C*-5), 32.9 (*C*-4), 21.1 (*C*-1'). ESI-MS: m/z $[M + Na]^+$ calculated for $C_9H_{18}O_5Na$ 229.1052, found 229.1053.



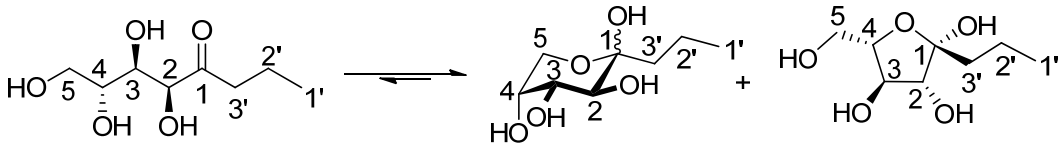
(3*S*,4*R*)-1-(allyloxy)-3,4,5-trihydroxyhexan-2-one (31). The title compound was prepared according to the general procedure described above. The yellow liquid was purified by chromatography to give the title compound (45%) as a colorless liquid.

Product ratio: α-anomer (<1%), β-anomer (>99%). ^{1}H NMR (500 MHz, D_2O) δ (ppm) 6.03 (ddt, *J* = 17.4, 10.4, 5.9 Hz, 1H, *H*-2'), 5.40 (m, 2H, *H*-1'), 4.16 (dd, *J* = 5.9, 1.2 Hz, 2H, *H*-3'), 4.02 (m, 2H, *H*-3, *H*-5_{eq}), 3.78 (ddd, *J* = 11.8, 5.3, 1.6 Hz, 1H, *H*-5_{ax}), 3.73 (d, *J* = 10.3 Hz,1H, *H*-4'), 3.52 (m, 2H, *H*-2, *H*-4' both are d with *J* = 10.0 Hz), 2.05 (m, 1H, *H*-4_{eq}), 1.72 (tdd, *J* = 13.0, 11.5, 5.3 Hz,1H, *H*-4_{ax}).^{13}C NMR (126 MHz, D_2O) δ (ppm) 134.0 (*C*-2'), 118.5 (*C*-1'), 98.0 (*C*-1), 72.8 (*C*-2), 72.5 (*C*-3'), 71.6 (*C*-4'), 68.3 (*C*-3), 58.9 (*C*-5), 32.9 (*C*-4). ESI-MS: m/z $[M + Na]^+$ calculated for $C_9H_{16}O_5Na$ 227.0895, found 227.0899.

Addition of 3 to D-glyceraldehyde and addition of 2 to propanal catalyzed by FSA(L107A/L163A).

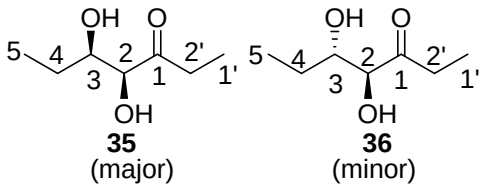


Scheme S1. FSA-catalyzed aldol additions of 1-hydroxypentan-2-one (**3**) and 1-hydroxy-2-butanone (**2**) to D-glyceraldehyde (**32**) and propionaldehyde (**34**), respectively.



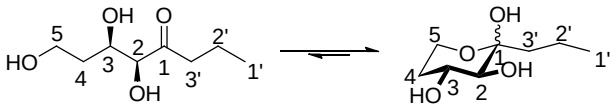
(5*S*,6*R*,7*R*)-5,6,7,8-Tetrahydroxyoctan-4-one (33). The title compound was prepared according to the general procedure described above and purified by chromatography to give the title compound as a colorless liquid. Product ratio: not determined. Open chain: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.15 (dd, *J* = 8.0, 7.3 Hz, 1H, C-3), 4.10 (m, 1H, C-5), 4.01 (d, *J* = 7.9 Hz, 1H, C-2), 3.84 (m, 1H, C-5), 3.73 (m, 1H, C-4), 2.70 (t, *J* = 7.2 Hz, 2H, C-3'), 1.68 (q, *J* = 7.3 Hz, 2H, C-2'), 0.99 (m, 3H, C-1'). ^{13}C NMR (126 MHz, D_2O) δ 215.6 (C-1), 78.4 (C-2), 76.5 (C-3), 74.8 (C-4), 62.9 (C-5), 40.3 (C-3'), 16.7 (C-2'), 12.9 (C-1'). β-anomer: ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.07 (m, 1H, C-5), 4.05 (m, 1H, C-4), 3.93 (dd, *J* = 9.9, 3.4 Hz, 1H, C-3), 3.76 (d, *J* = 10.1 Hz, 1H, C-2), 3.71 (m, 1H, C-5), 4.85-1.79 (m, 2H, C-3'), 1.50 (m, 2H, C-2'), 0.99 (m, 3H, C-1'). ^{13}C NMR (126 MHz, D_2O) δ 99.5 (C-1), 69.9 (C-3), 69.7 (C-2), 69.1 (C-4), 63.3 (C-5), 39.8 (C-3'), 15.8 (C-2'), 13.6 (C-1'). β-furanose. ^{13}C NMR (126 MHz, D_2O) δ 102.9 (C-1), 82.5 (C-4), 81.4 (C-2), 77.6 (C-3), 61.6 (C-5), 36.9 (C-3'), 16.9 (C-2'), 14.8 (C-1').

In an experiment to demonstrate further the scope of the carboligation catalyst and its usefulness for combinatorial variation of both nucleophile and electrophile components, propanal was used as a generic, non-hydroxylated electrophile substrate (Scheme S1). Addition of **2** to **34** catalyzed by FSA(L107A/L163A) gave the expected adduct **35** in high yield (>95%), for which NMR spectroscopic analysis revealed the presence of a second diastereomer **36** in a 10:1 ratio for *threo*/*erythro* configuration, respectively. HPLC monitoring of early stages of conversion confirmed that formation of **36** was of kinetic origin due to incomplete stereoselectivity in the carboligation step and not an artefact from configurational equilibration.



(4*S*,5*R*)- (major) (35) and (4*S*,5*S*)-4,5-dihydroxyheptan-3-one (minor) (36). The title compounds were prepared according to the general procedure described above and purified by chromatography to give the title compounds (**35**:**36** 10:1) as a colorless liquid. ^{1}H NMR (500 MHz, D_2O) (major) δ (ppm) 4.31 (d, *J* = 2.2 Hz, 1H, C-2), 3.99 (ddd, *J* = 7.6, 6.3, 2.2 Hz, 1H, C-3), 2.64 (m, 2H, C-2'), 1.60 (m, 2H, C-4)), 1.03 (t, *J* = 7.3 Hz, 3H, C-1'), 0.95 (t, *J* = 7.5 Hz, 3H, C-5). ^{13}C NMR (126 MHz, D_2O) (major) δ (ppm) 216.29 (C-1), 78.56 (C-2), 73.36 (C-3), 31.96 (C-2'), 25.86 (C-4), 9.60 (C-1'), 6.82 (C-5). ^{1}H NMR (500 MHz, D_2O) (minor) δ 4.27 (d, *J* = 4.8 Hz, 1H, C-2), 3.82 (dt, *J* = 7.6, 5.2 Hz, 1H, C-3), 2.64 (m, 2H, C-2'), 1.46 (m, 2H, C-4), 1.08 (t, *J* = 7.6 Hz, 3H, C-1'), 1.02 (t, *J* = 7.5 Hz, 3H, C-5). ^{13}C NMR (126 MHz, D_2O) (minor) δ 216.63 (C-1), 79.65 (C-2), 73.82 (C-3), 33.27 (C-2'), 24.41 (C-4), 9.45 (C-1'), 6.67 (C-5).

a)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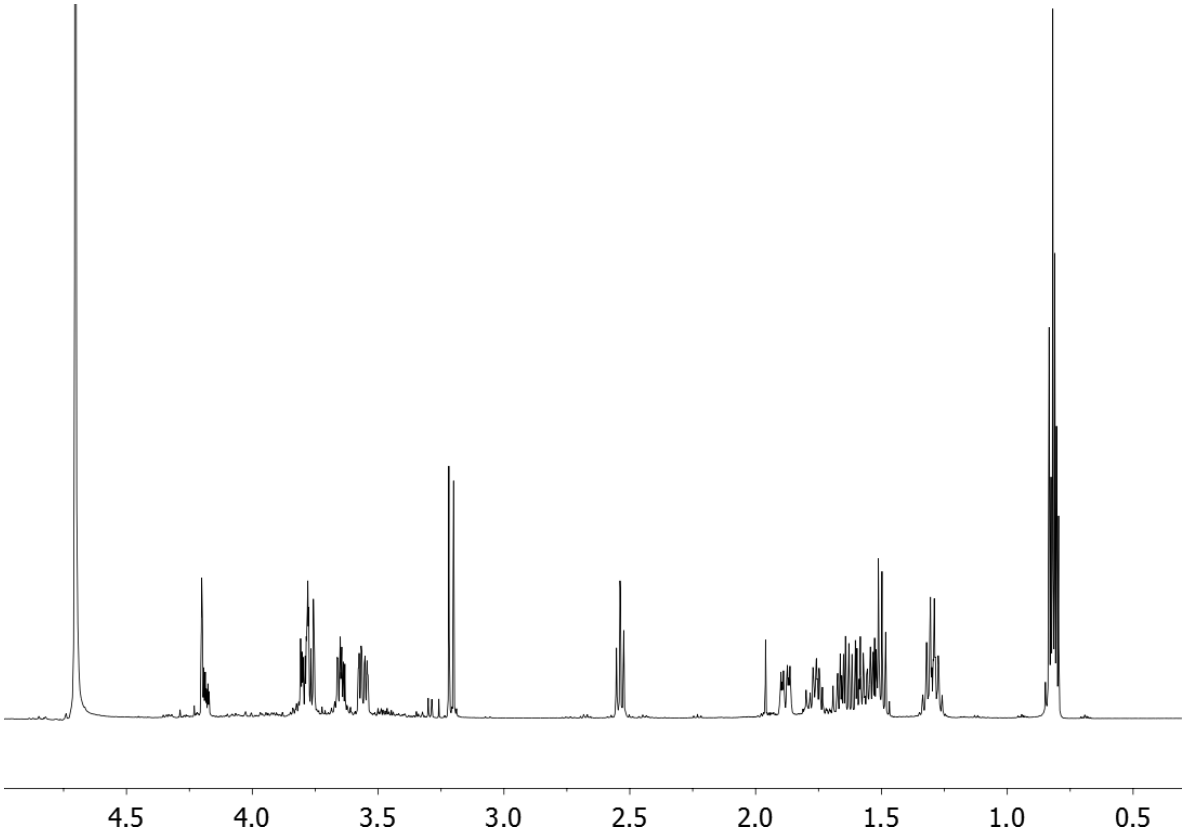
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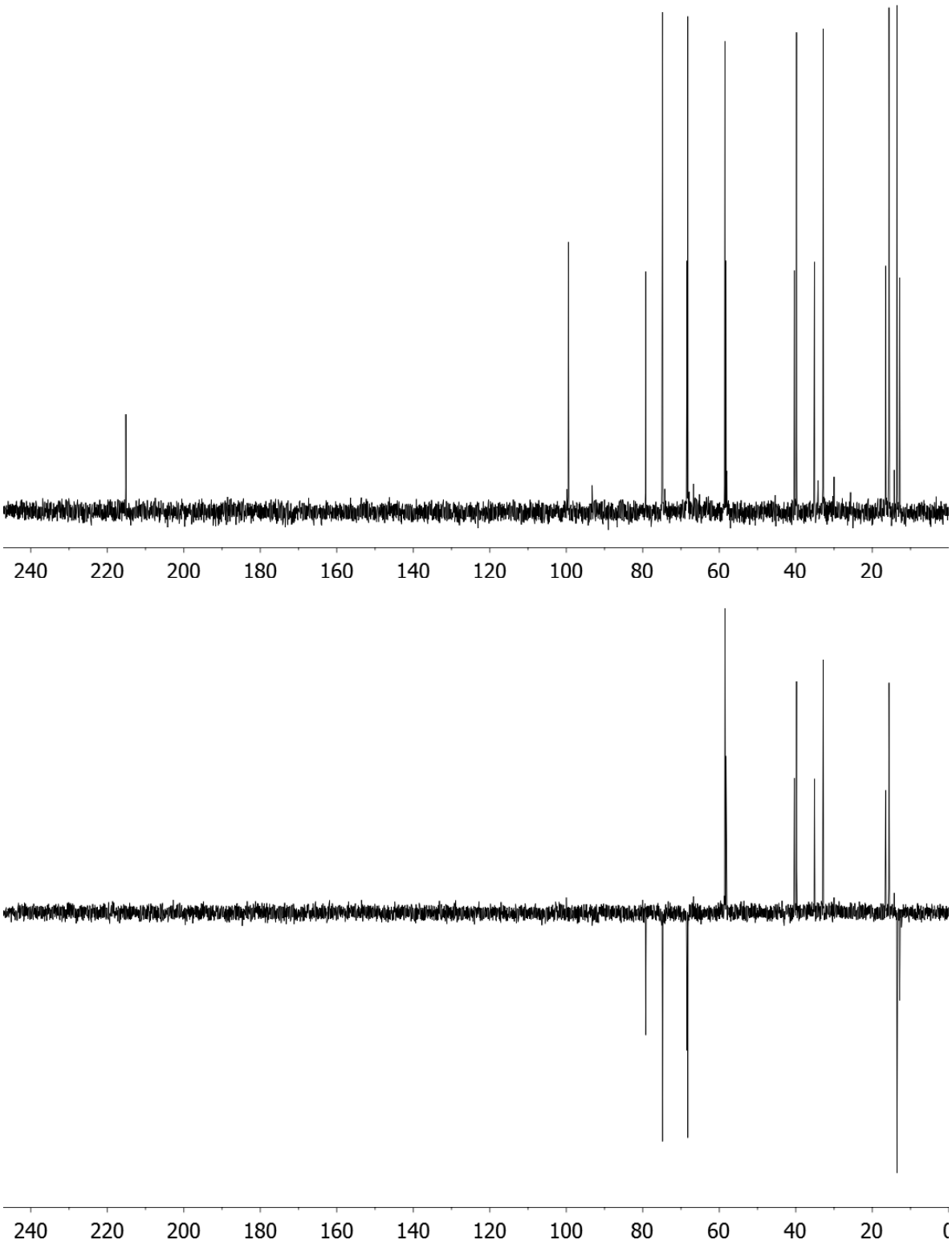
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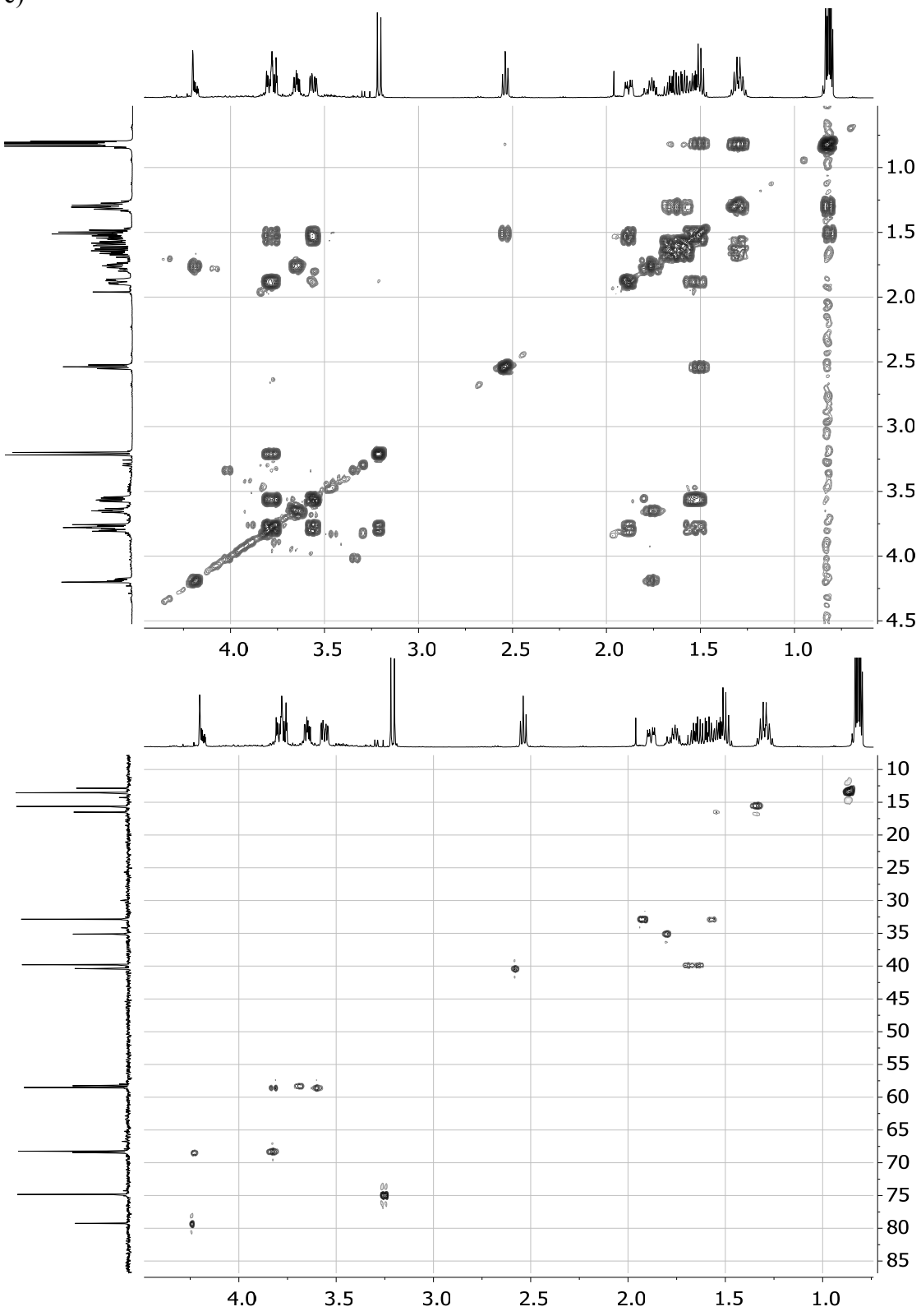
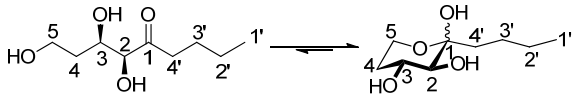


Figure S5. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **19**.

a)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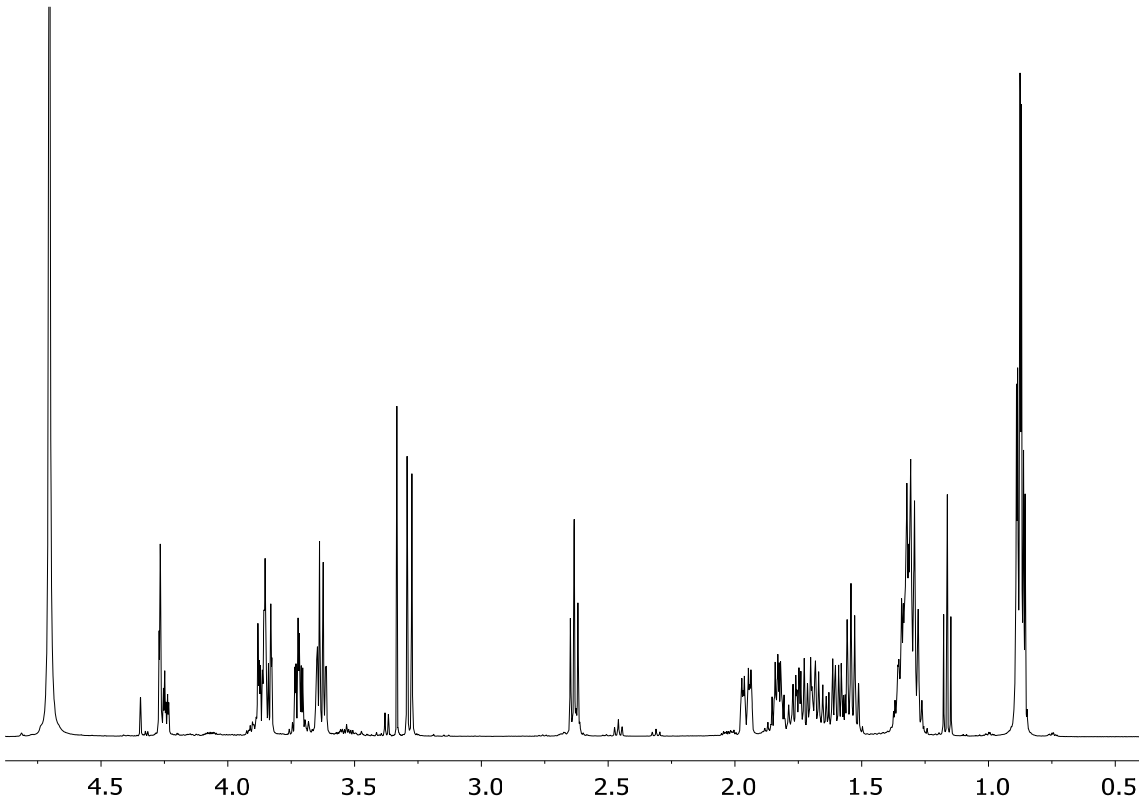
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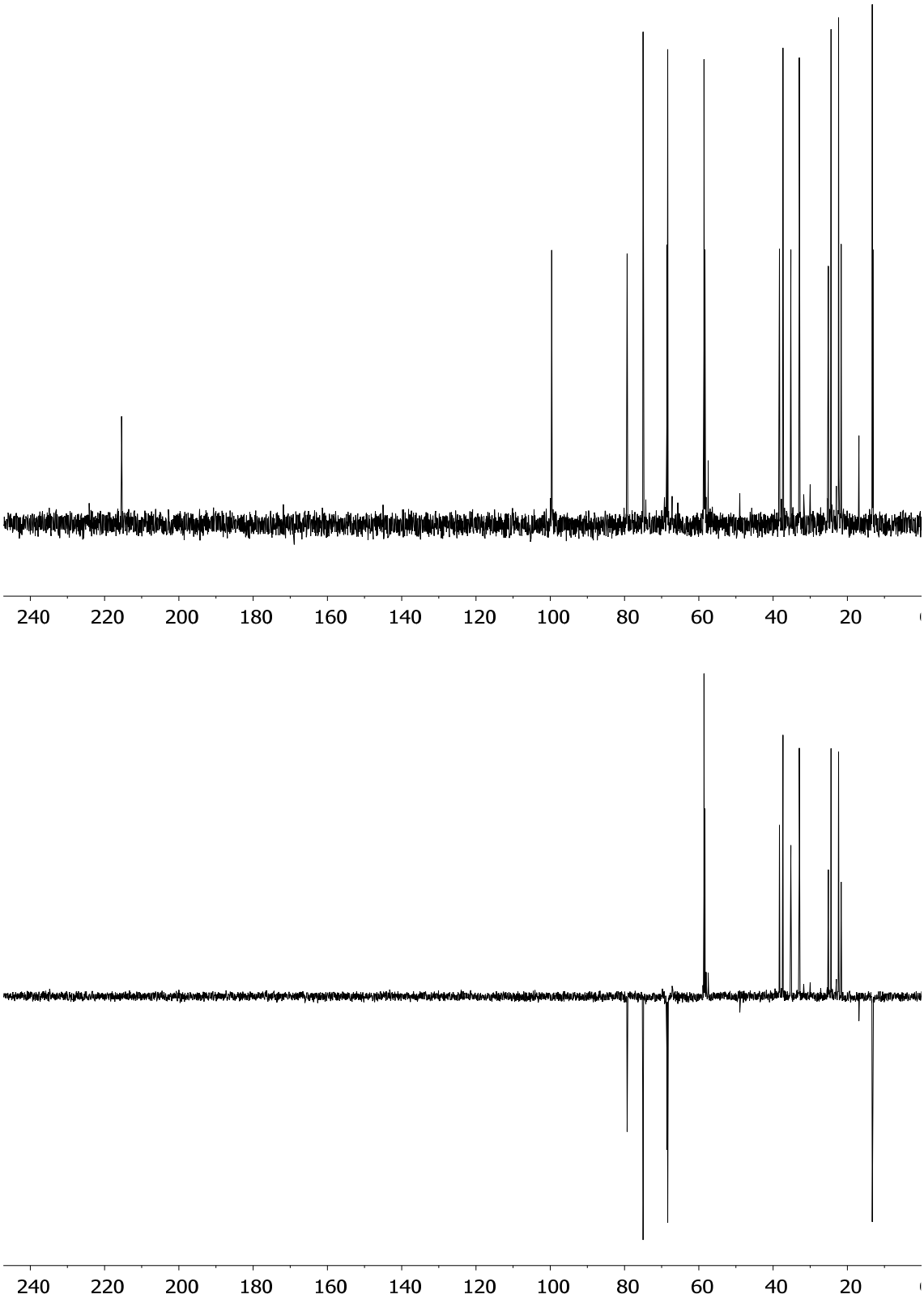
b)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c)

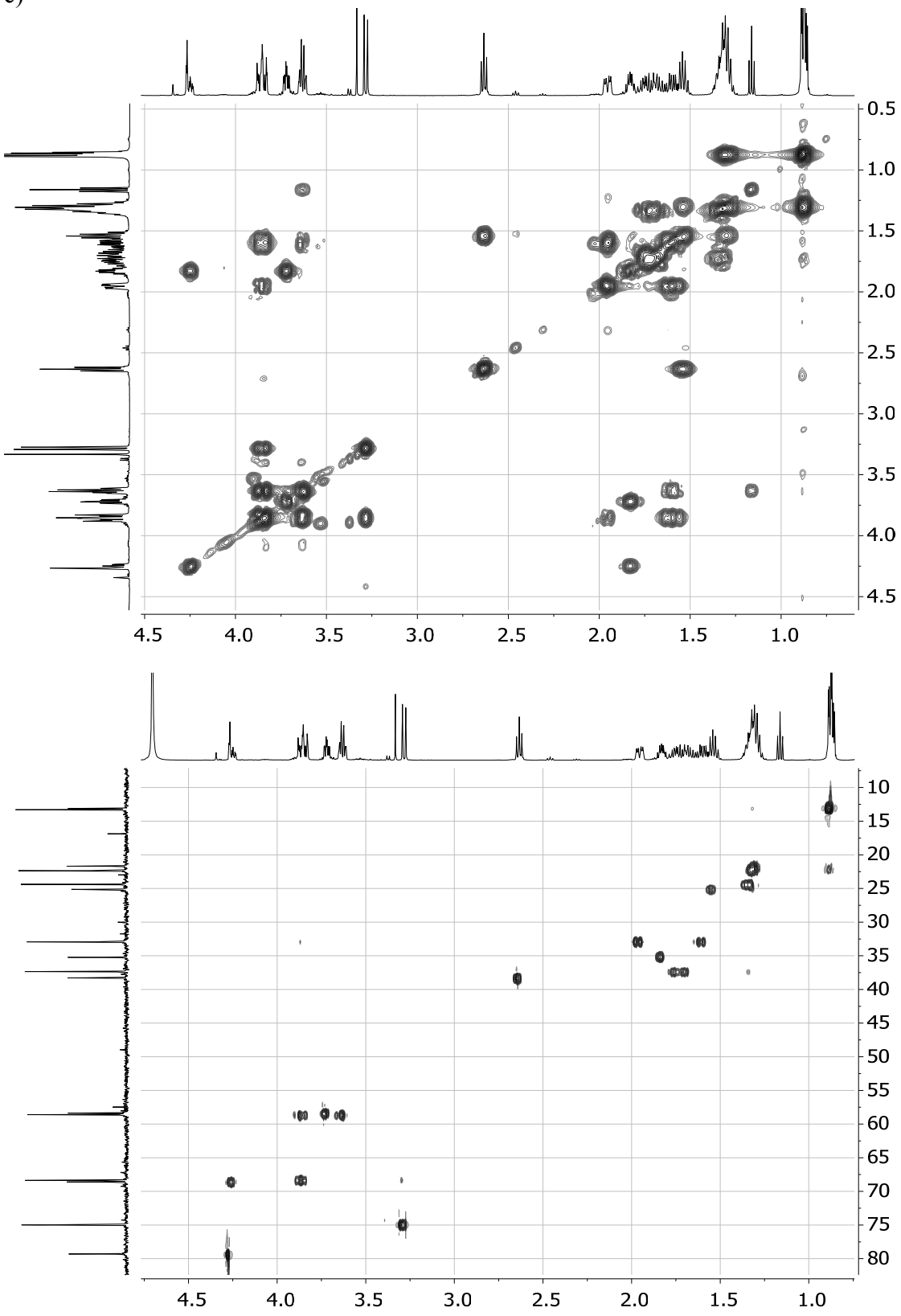
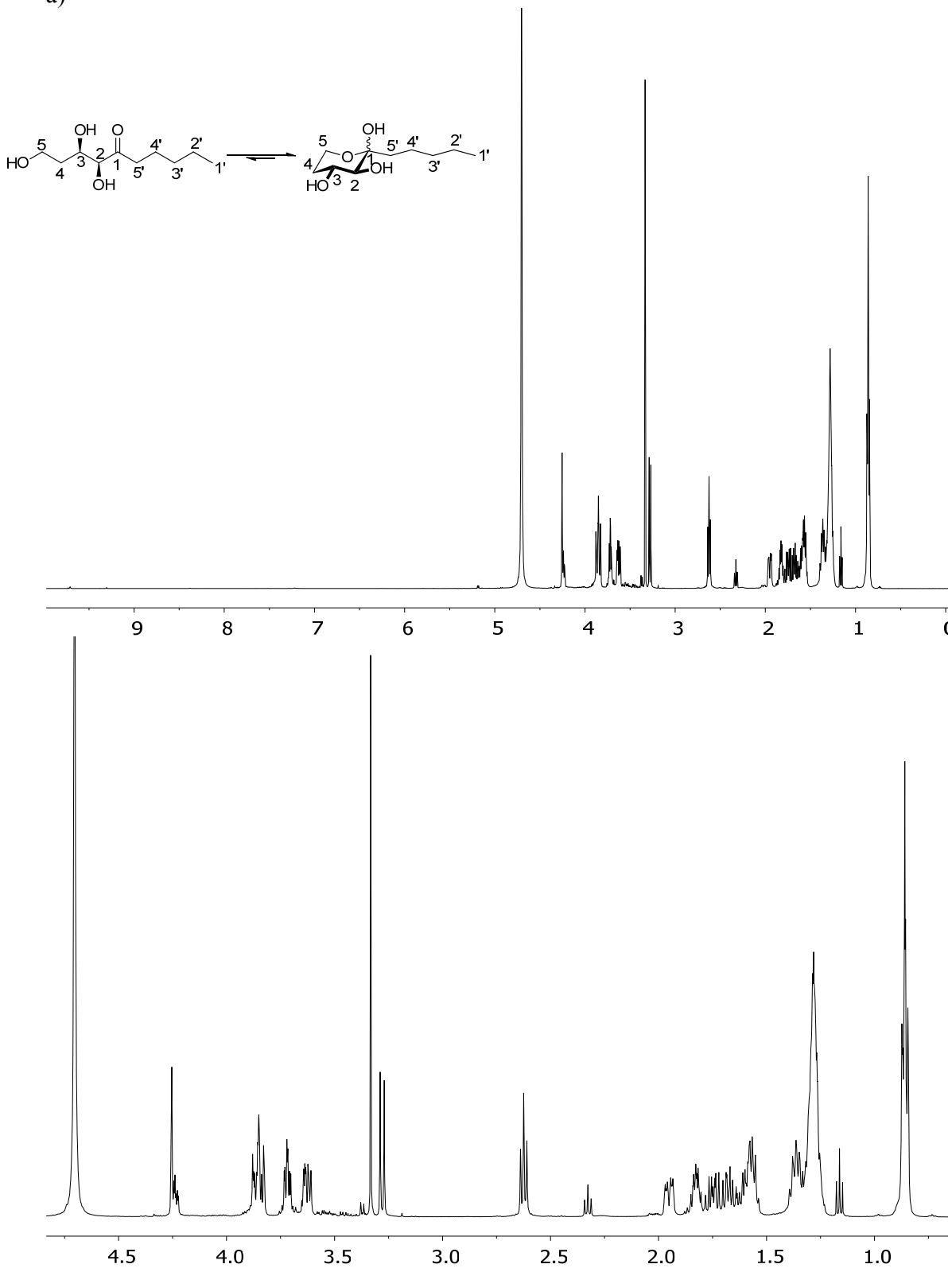


Figure S6. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **20**.

a)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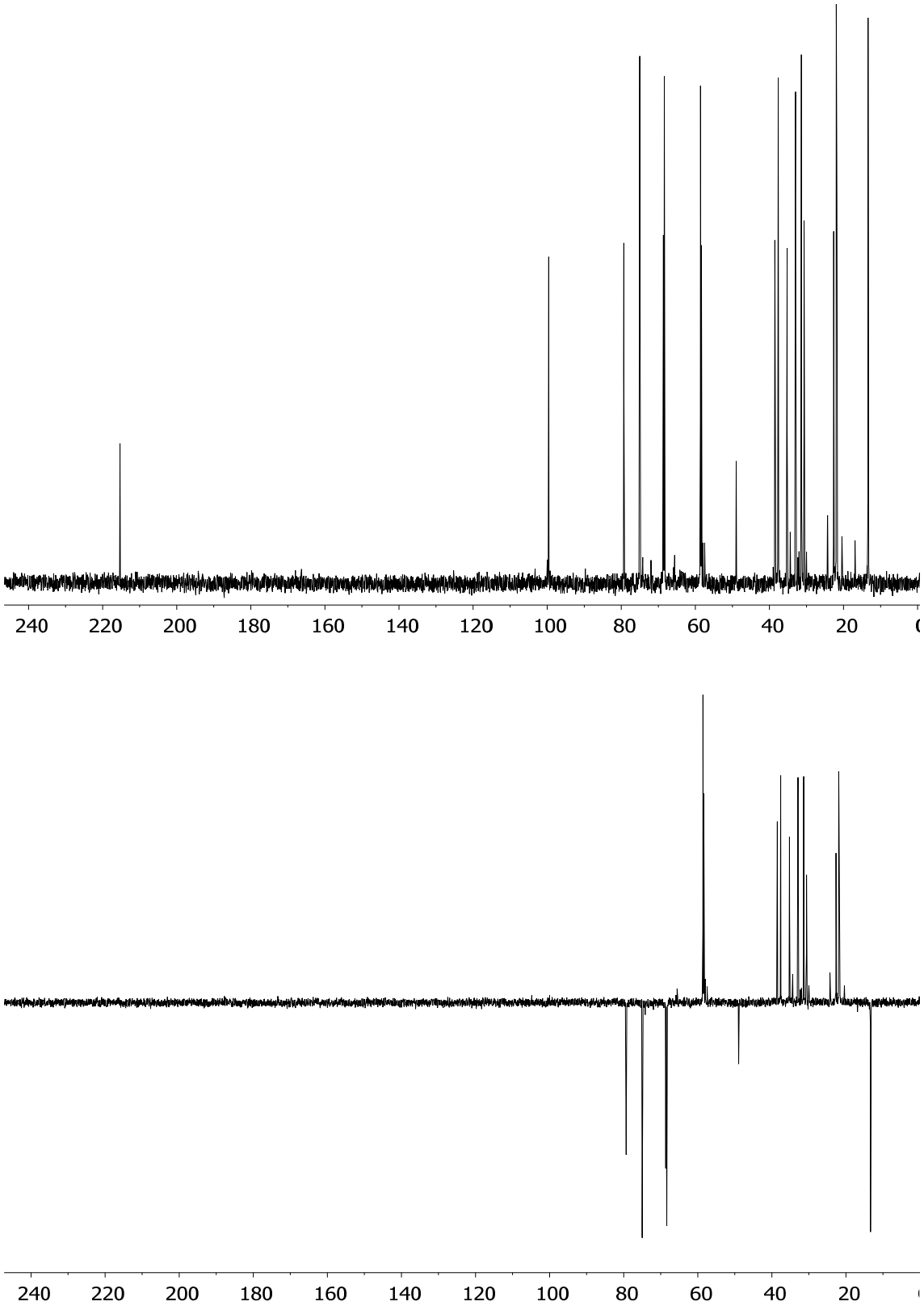
b)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c)

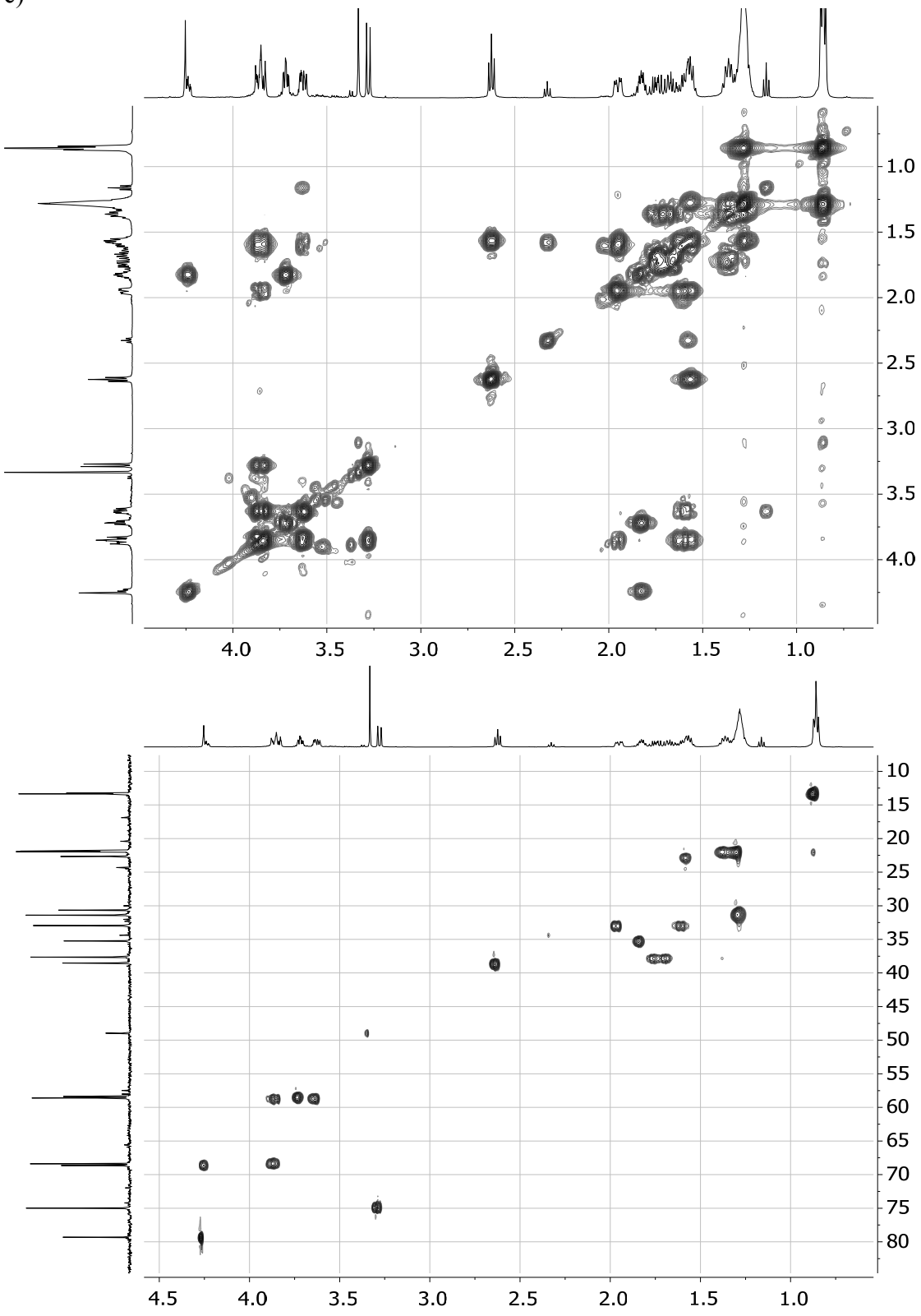
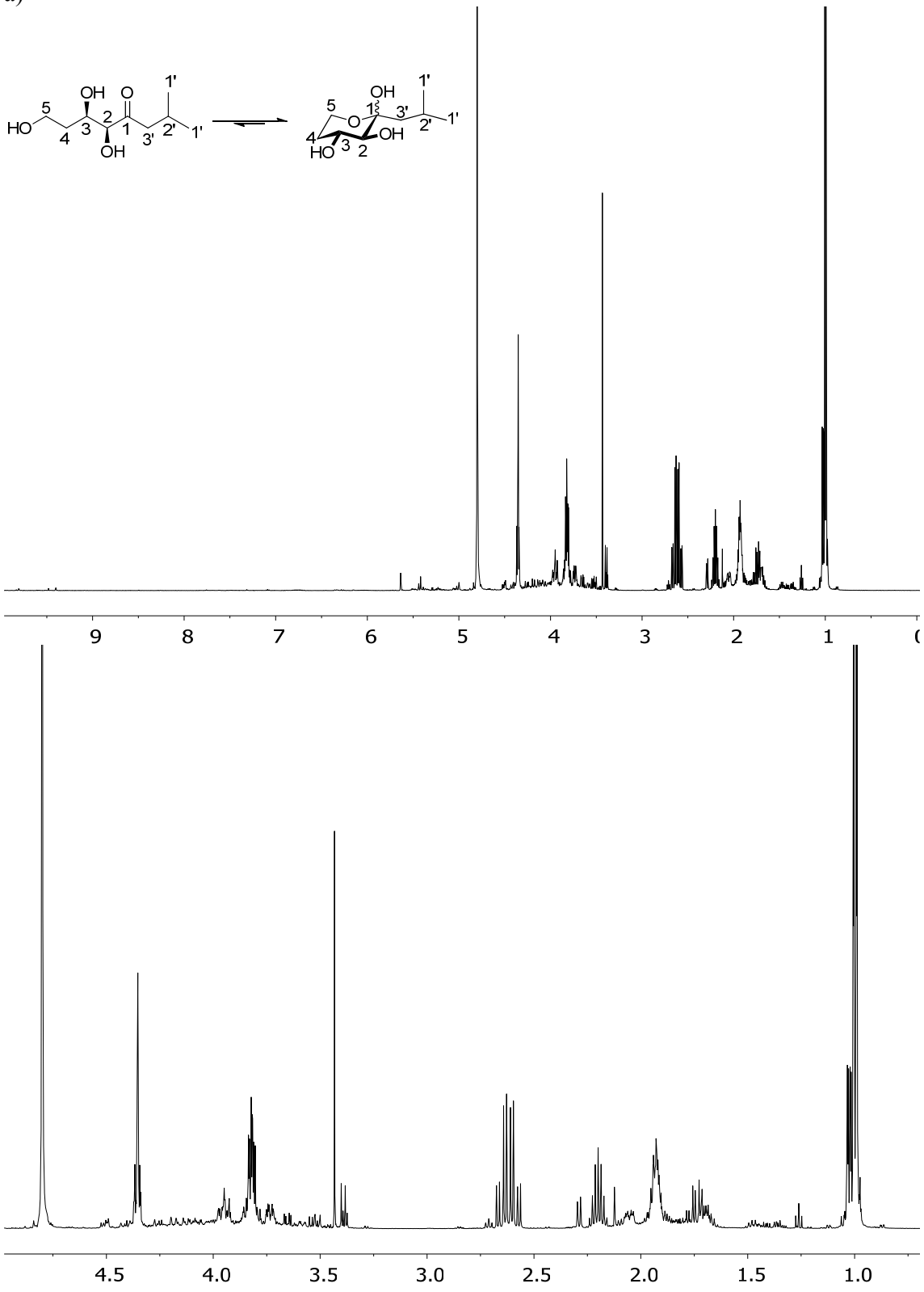


Figure S7. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **21**.

a)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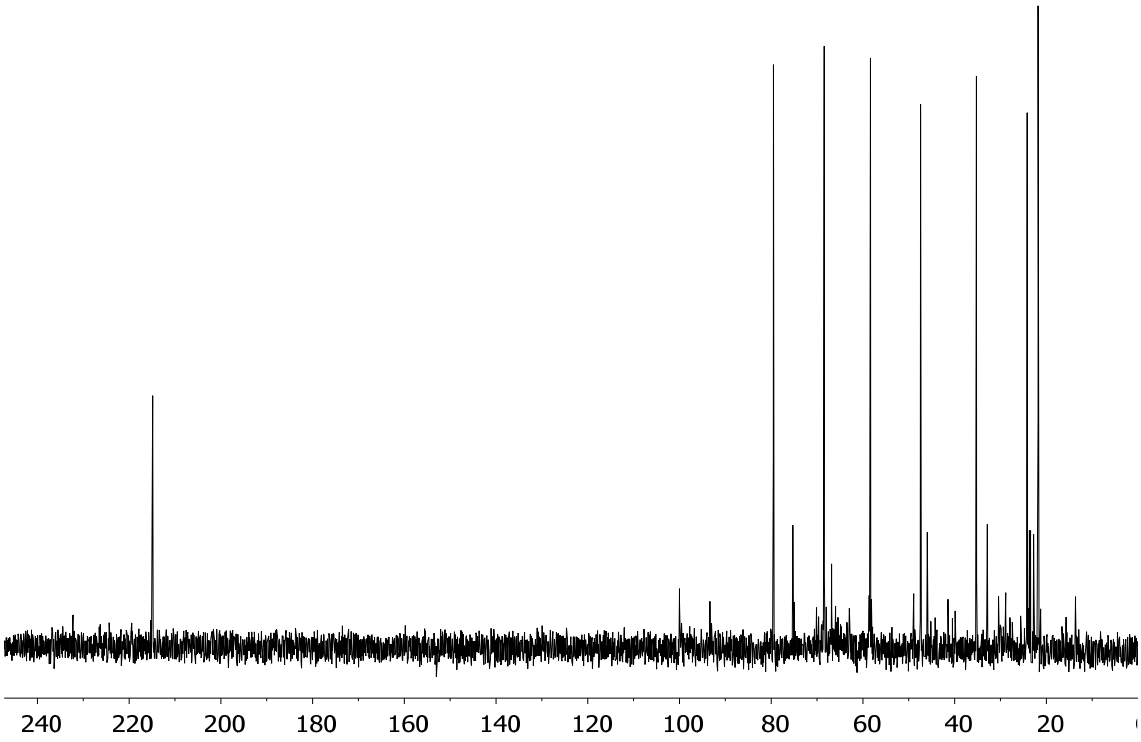
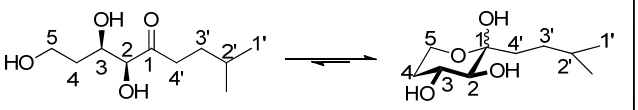


Figure S8. a) ^{1}H and b) ^{13}C NMR spectra of compound **23**.

a)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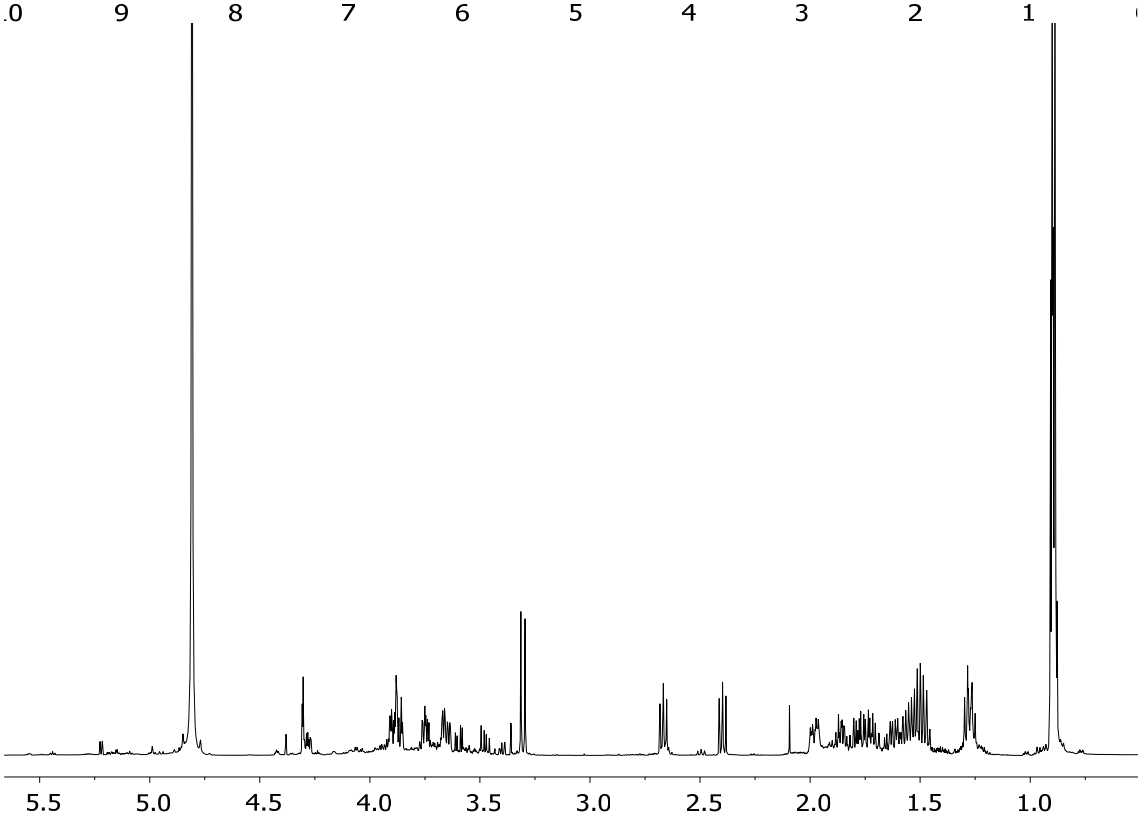
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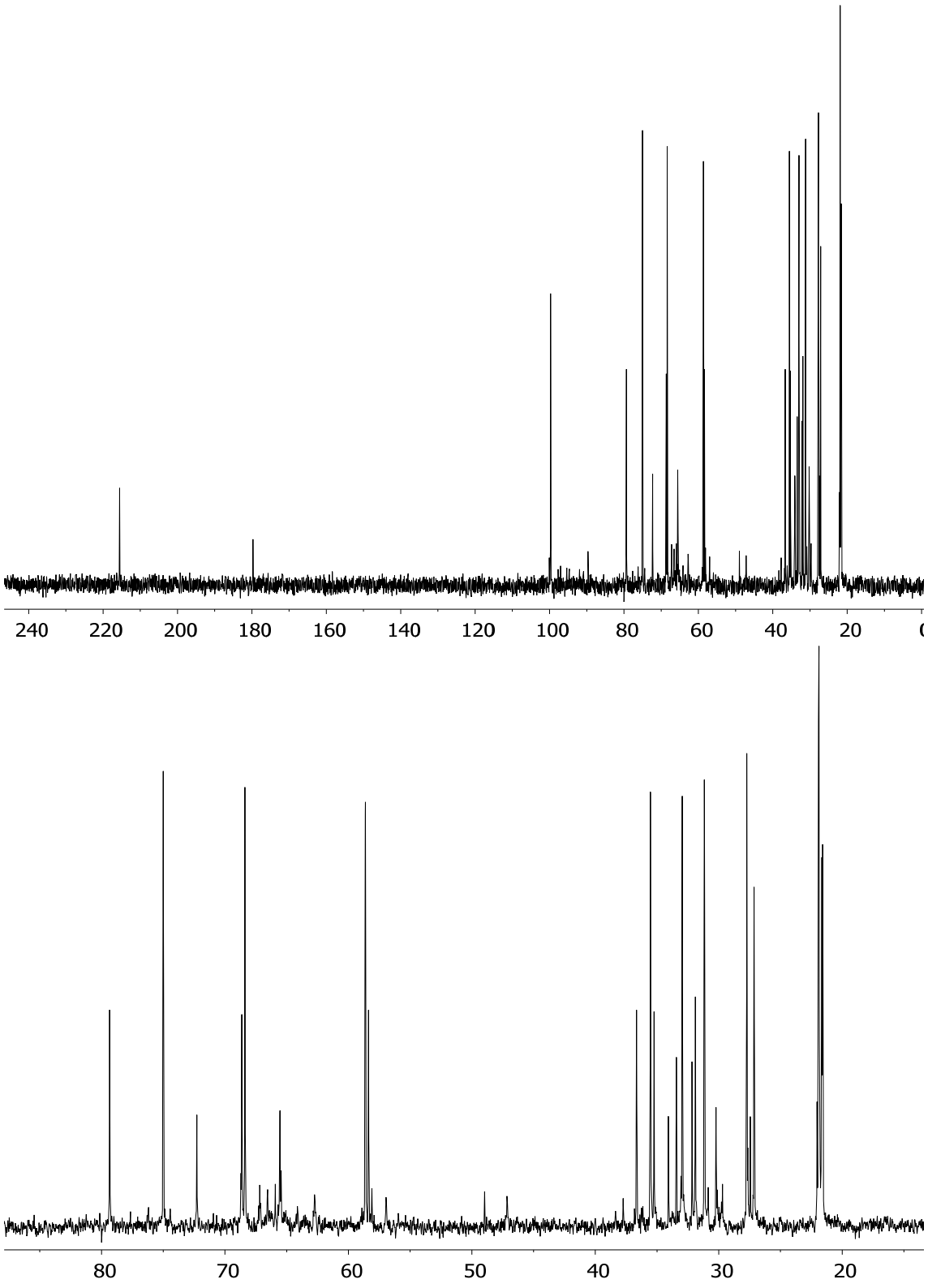

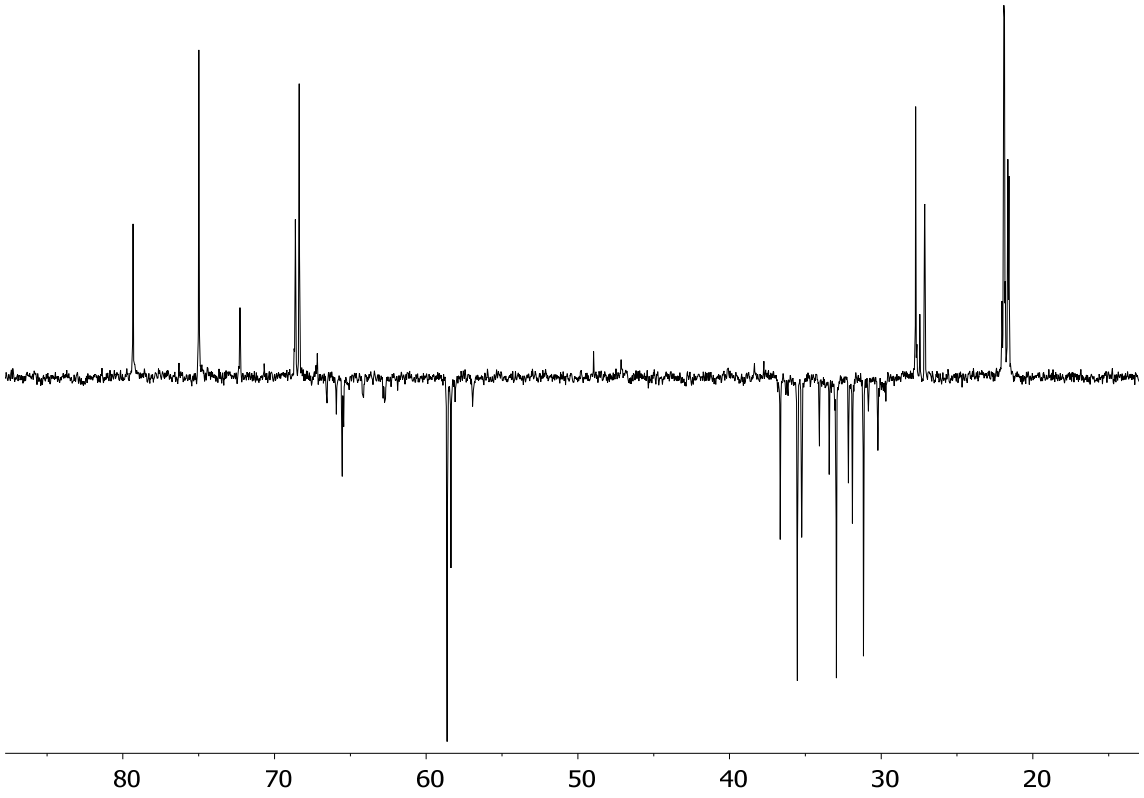

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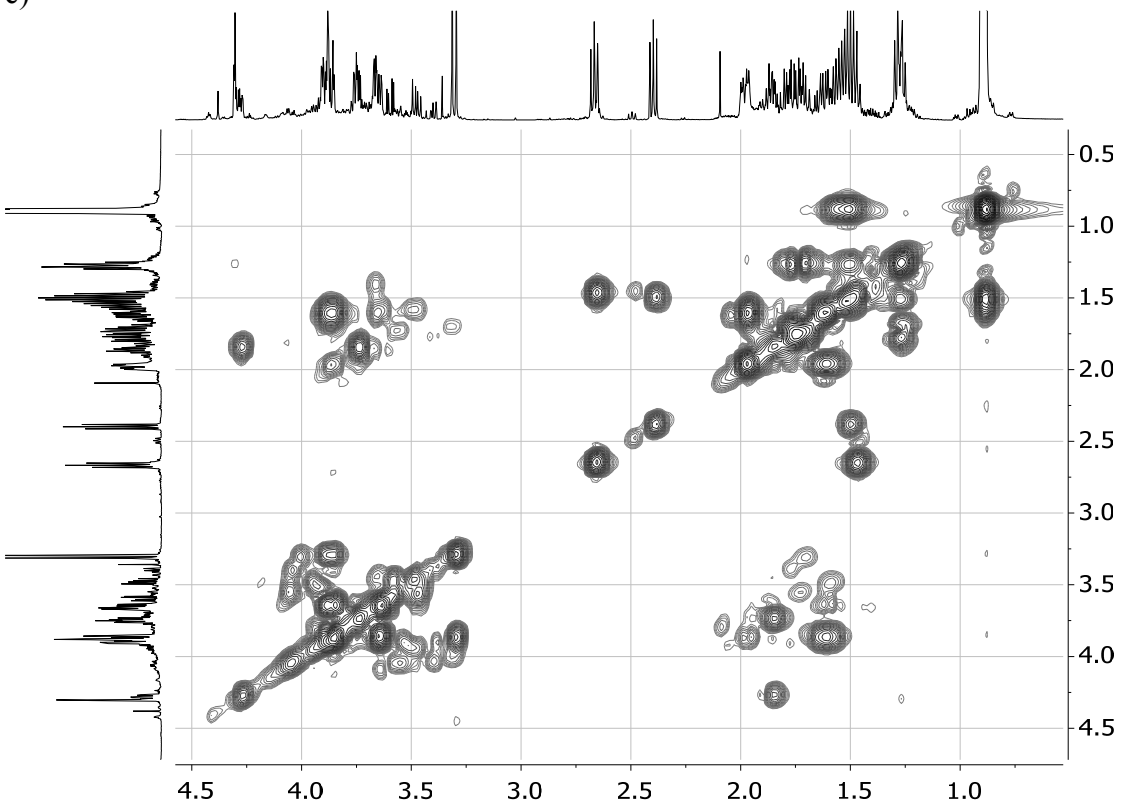

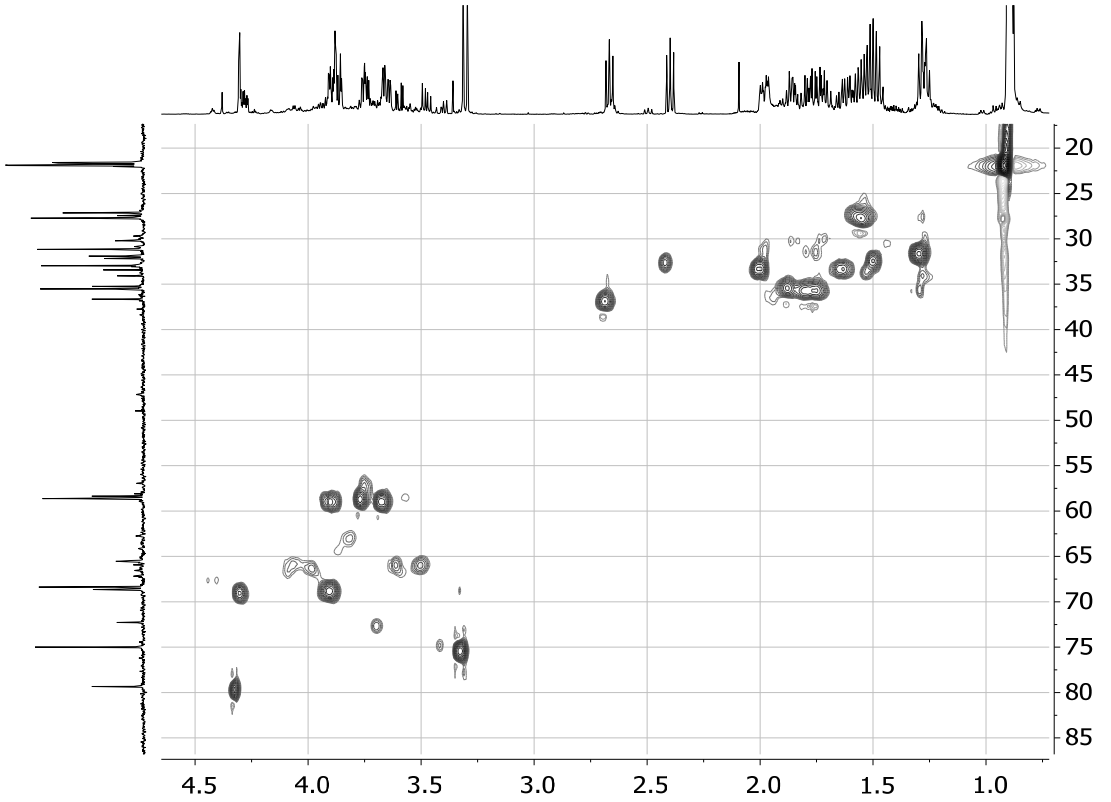

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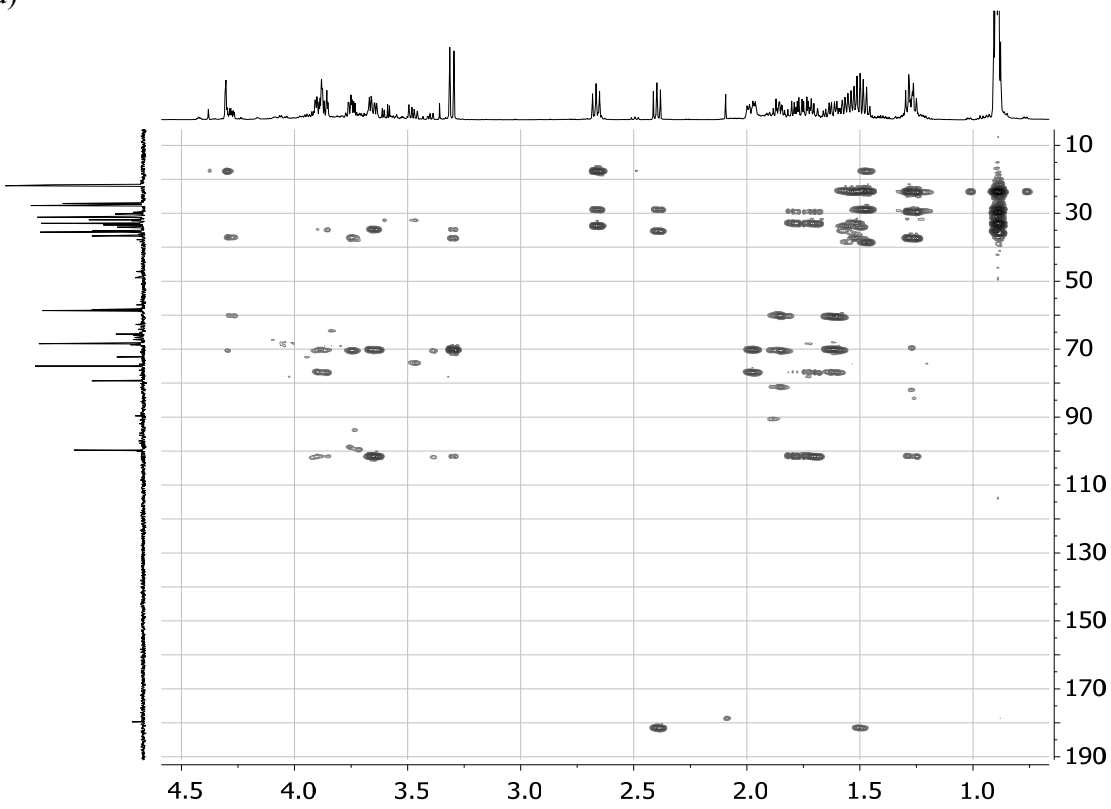
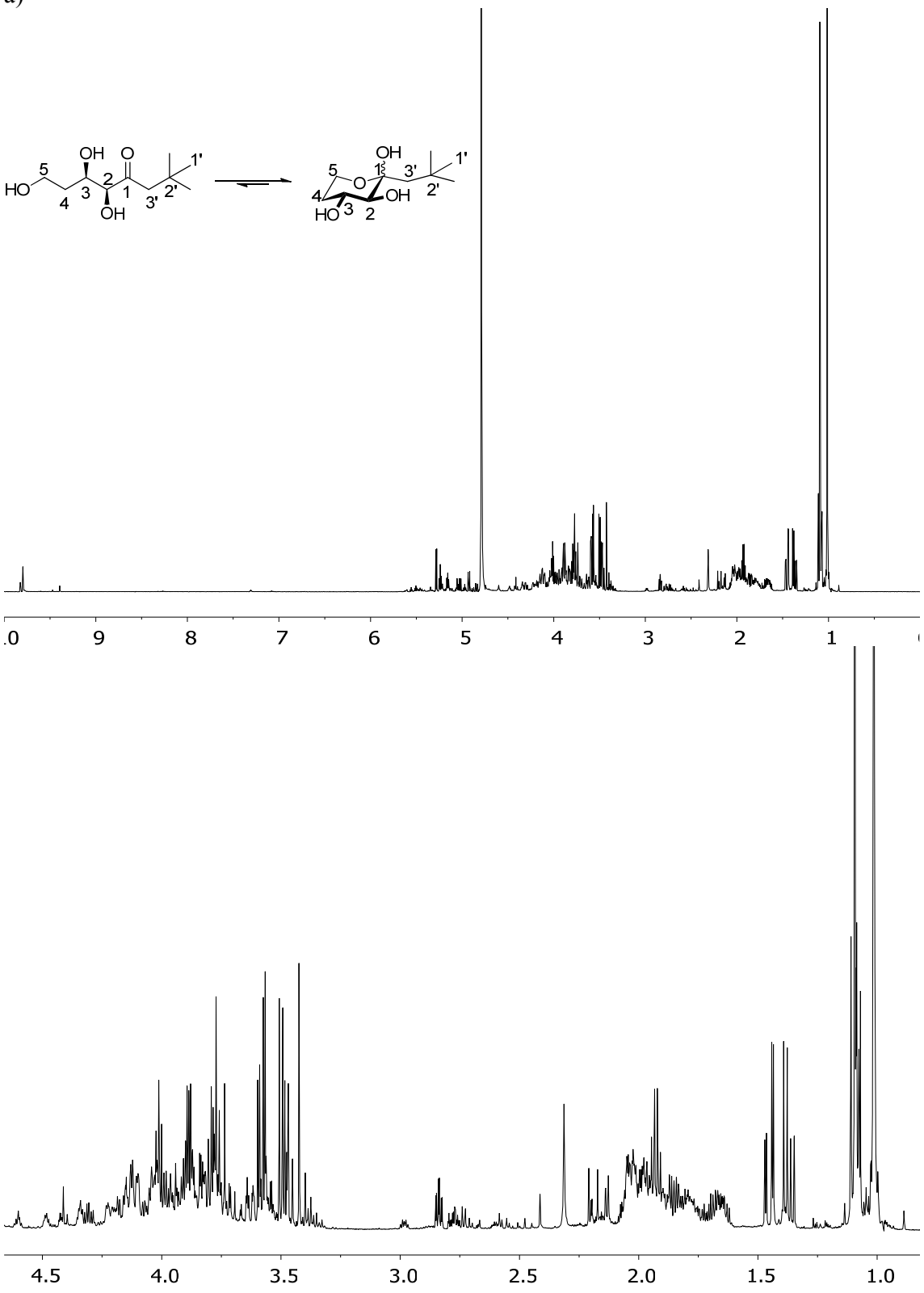


Figure S9. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC and d) HMBC spectra of compound **24**.

a)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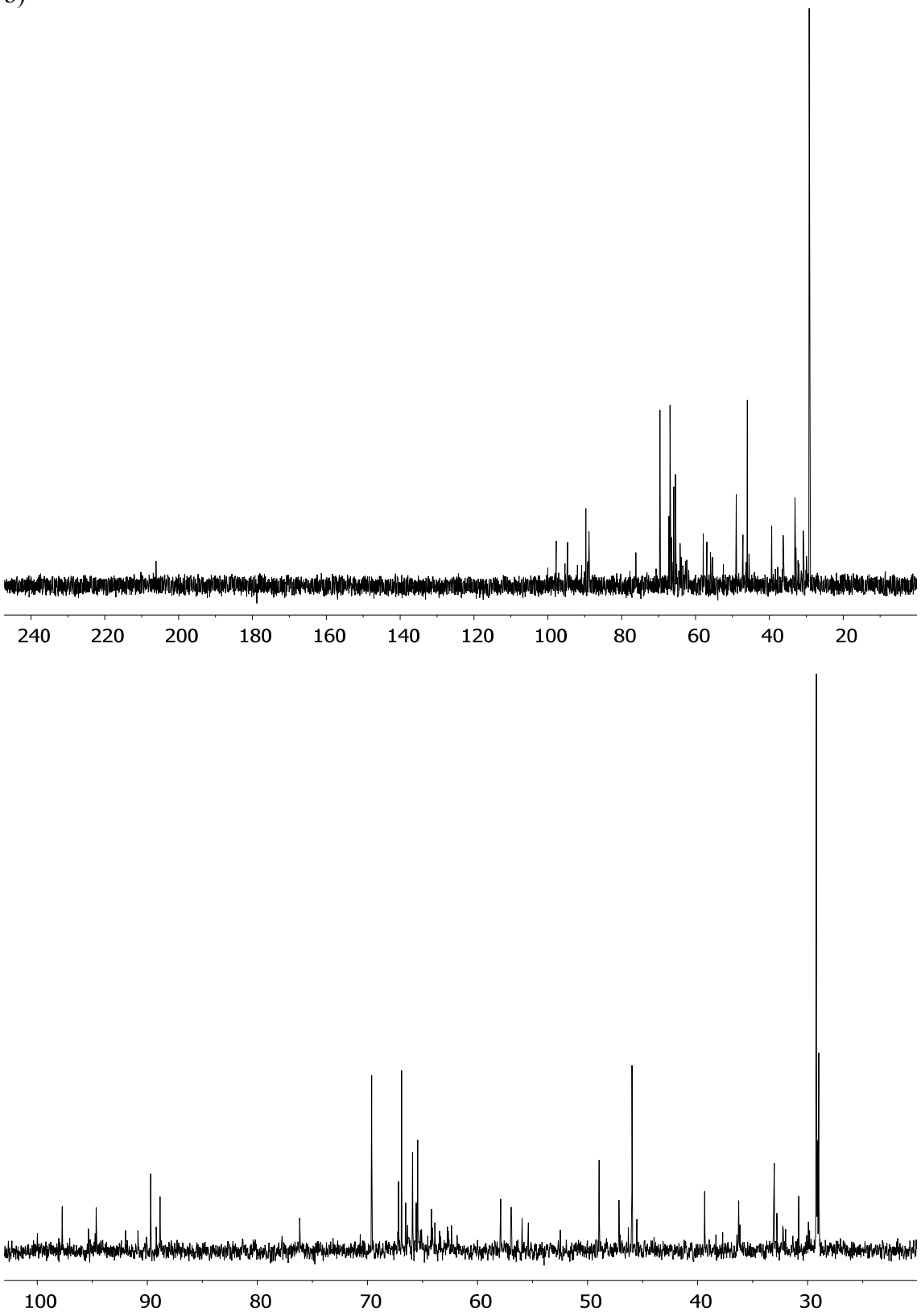

Figure S10. a) ^{1}H and b) ^{13}C NMR spectra of compound **26**. See remark on page S16 regarding compound purification.

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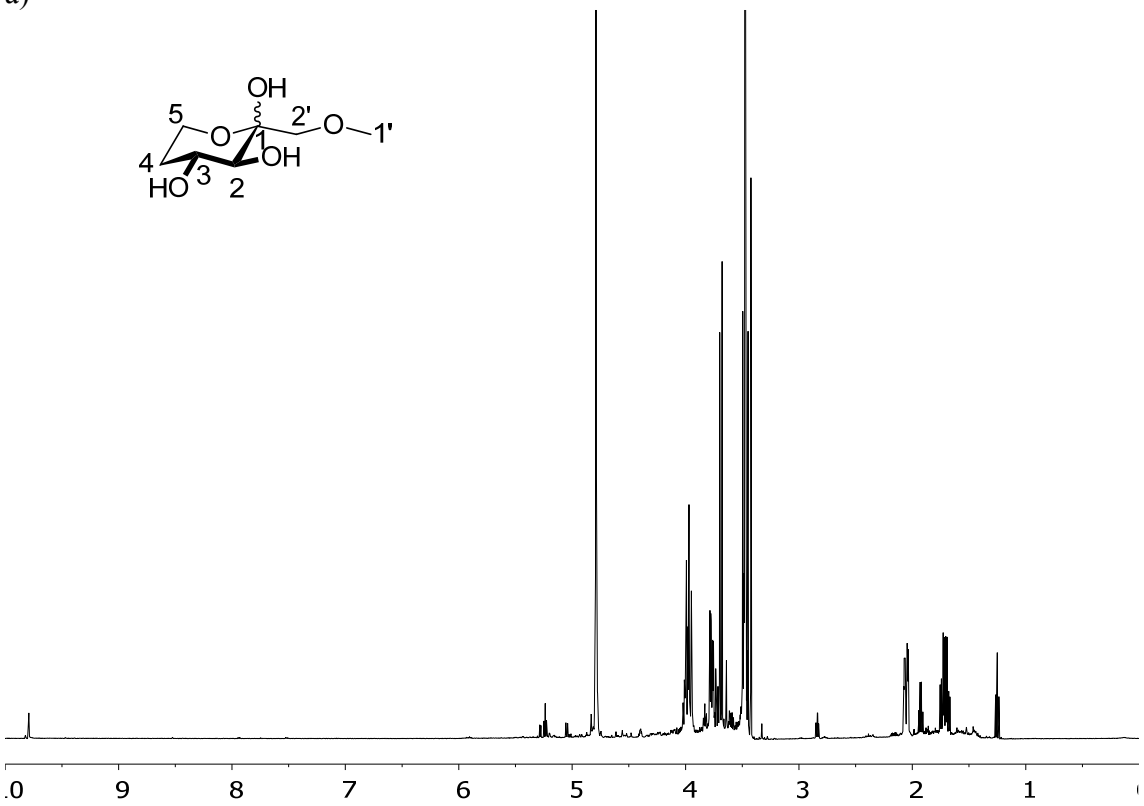

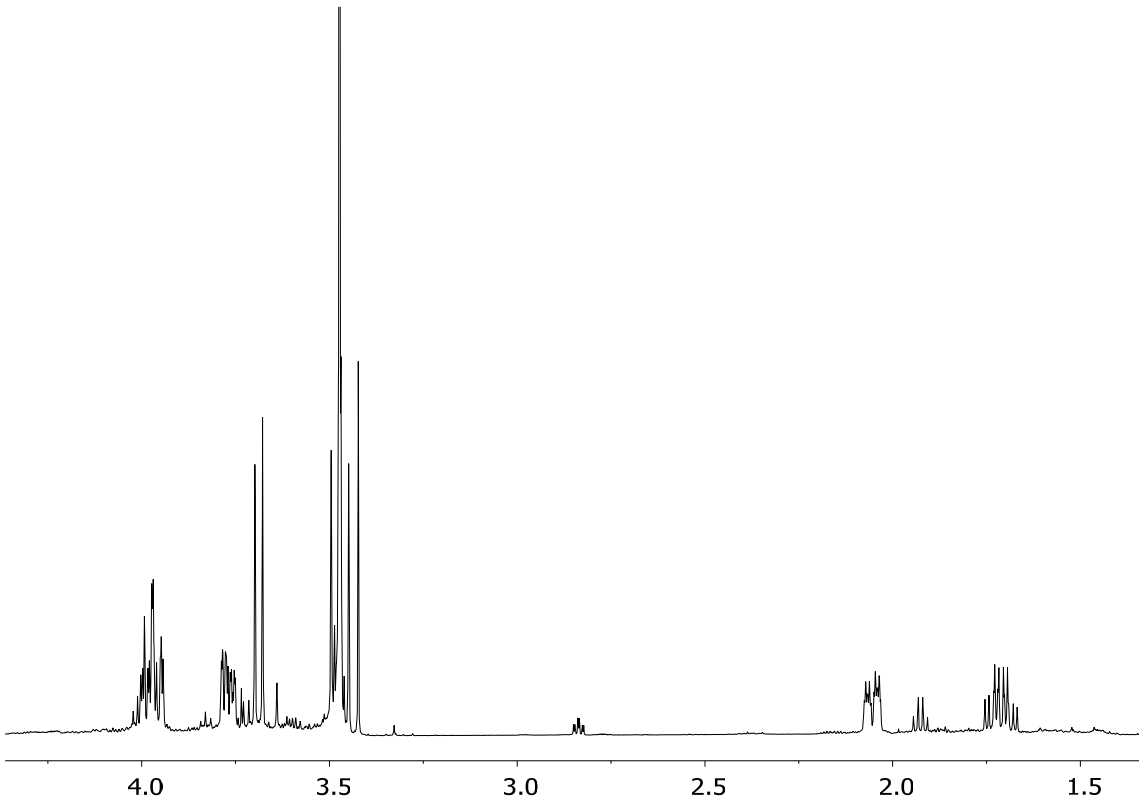

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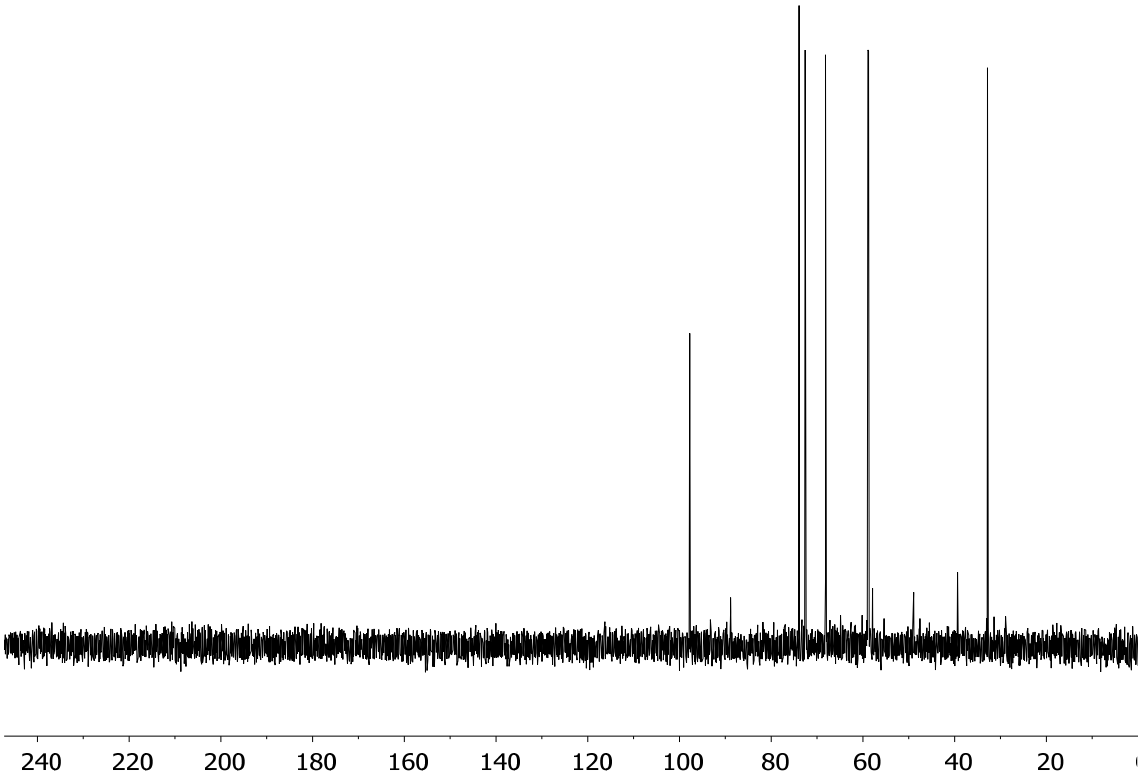
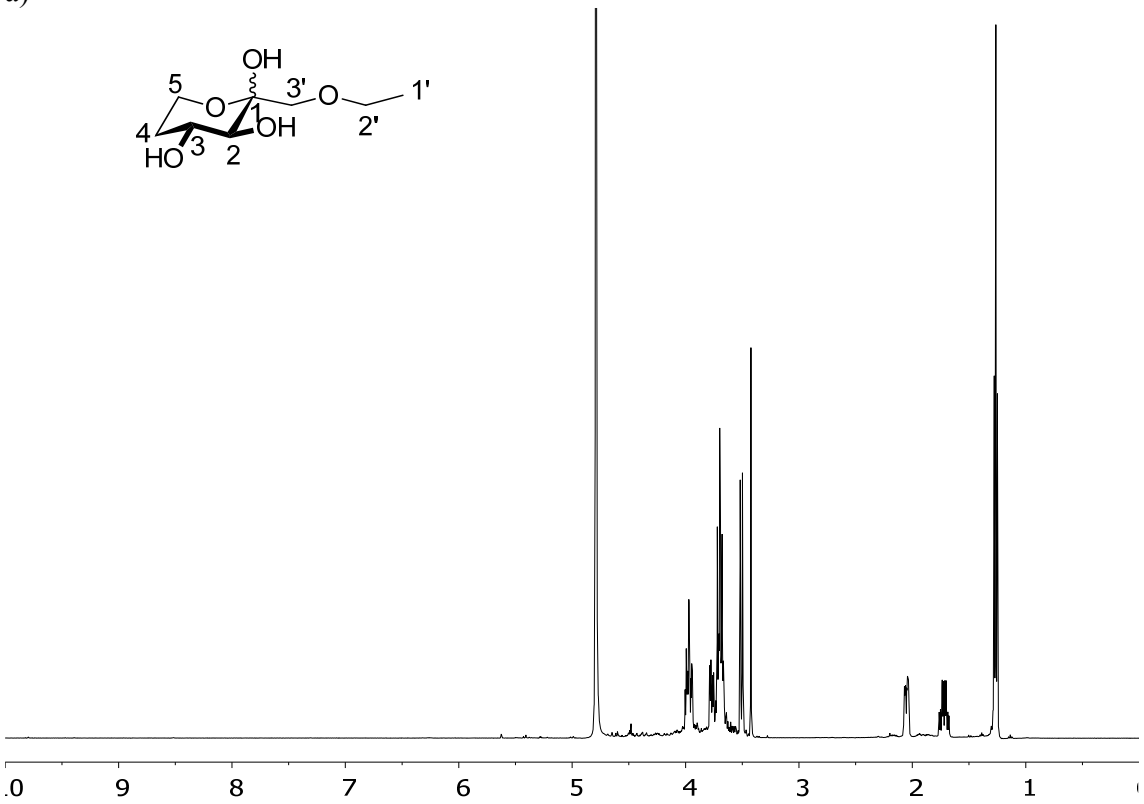
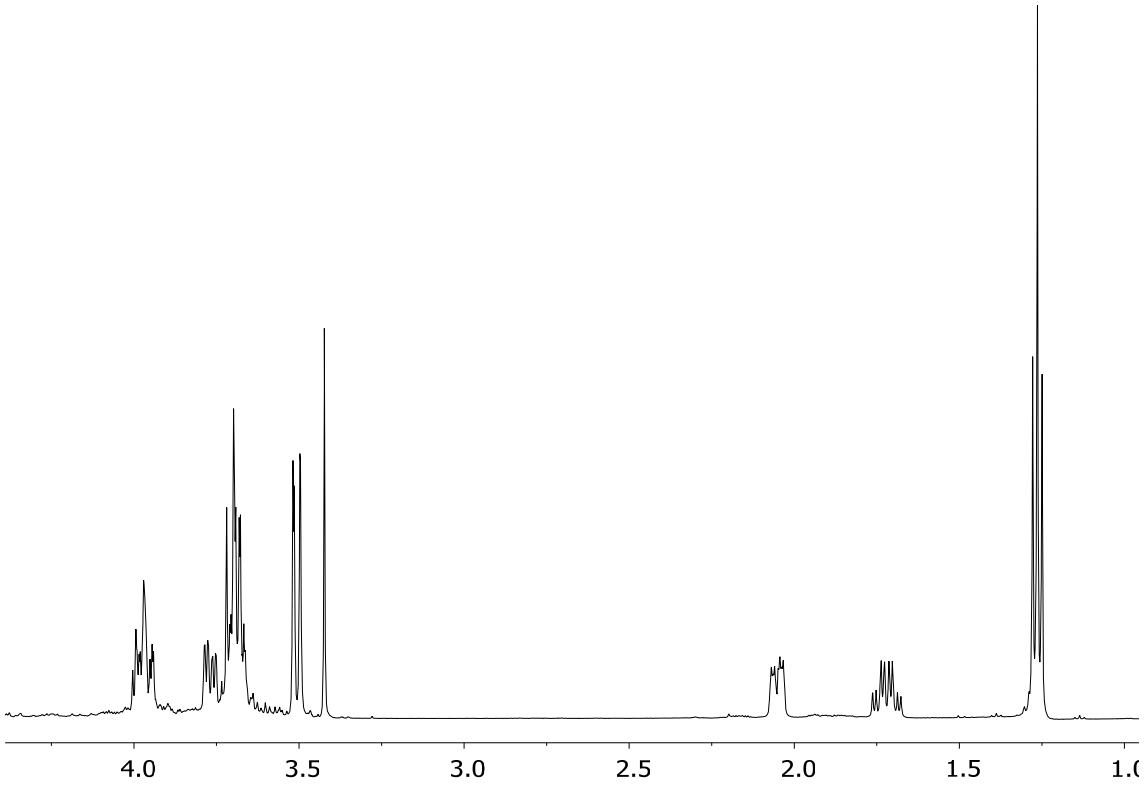


Figure S11. a) ^{1}H and b) ^{13}C NMR and DEPT, spectra of compound **27**.

a)





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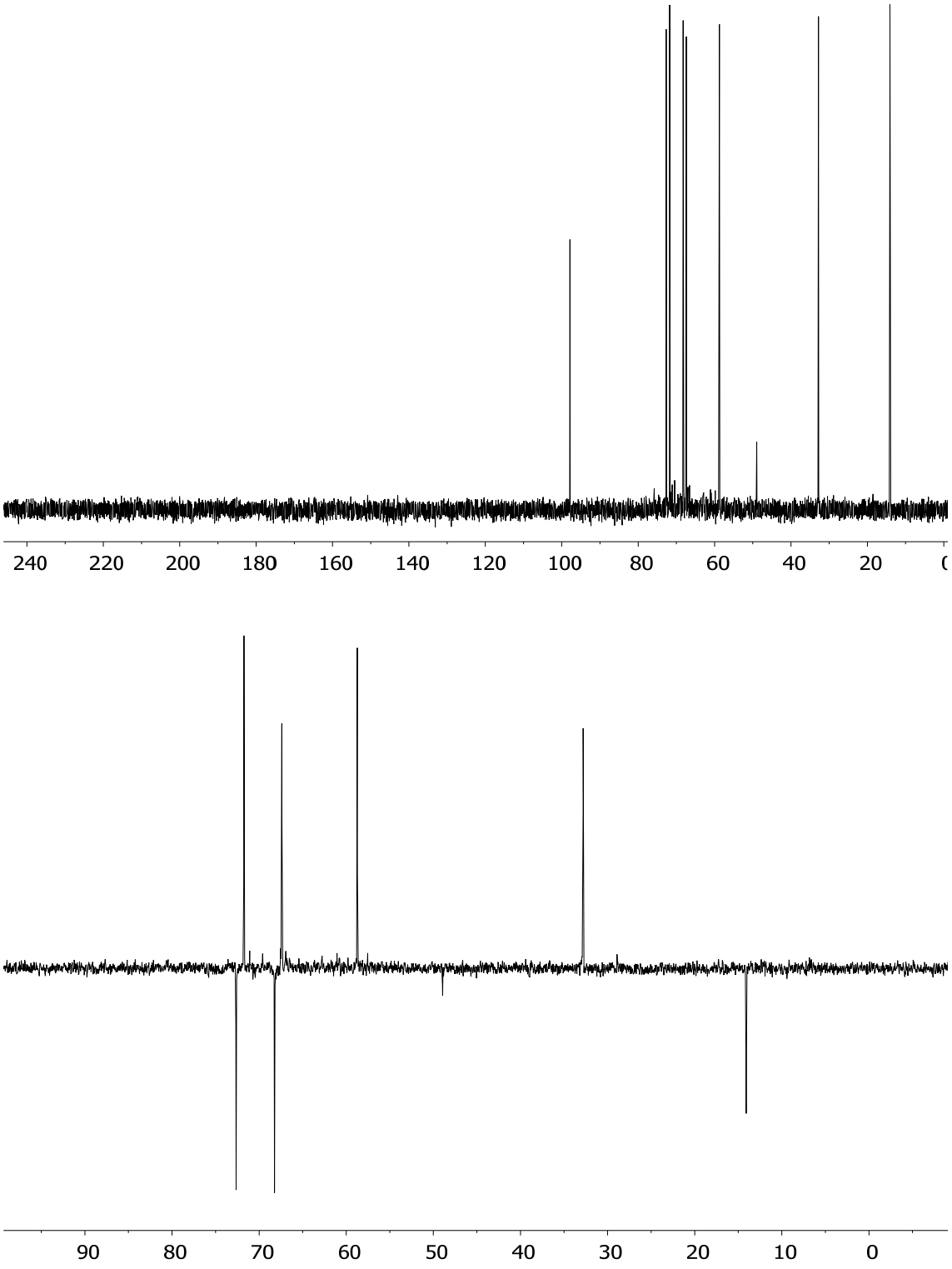
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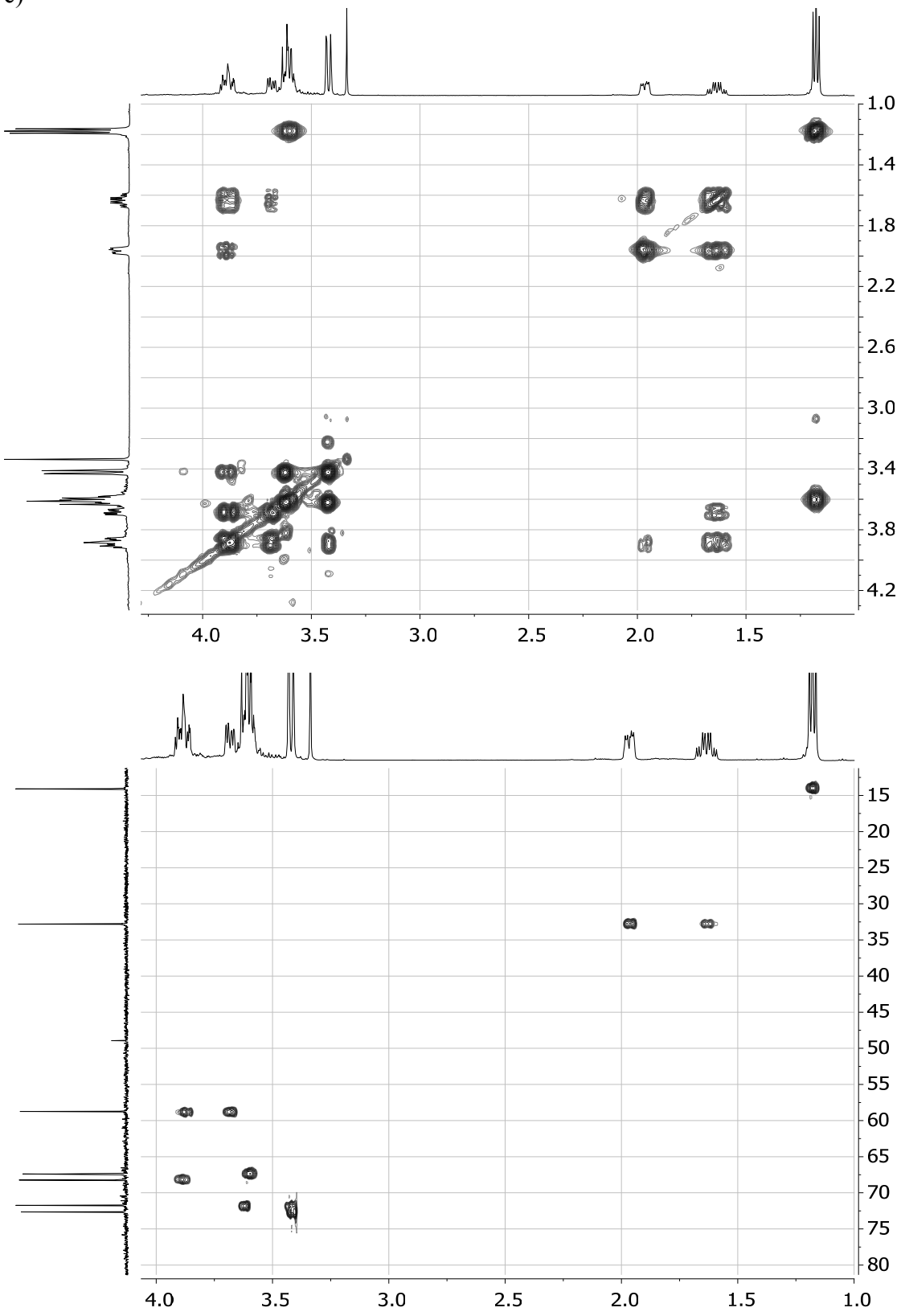

Figure S12. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **28**.

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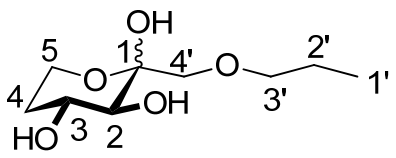

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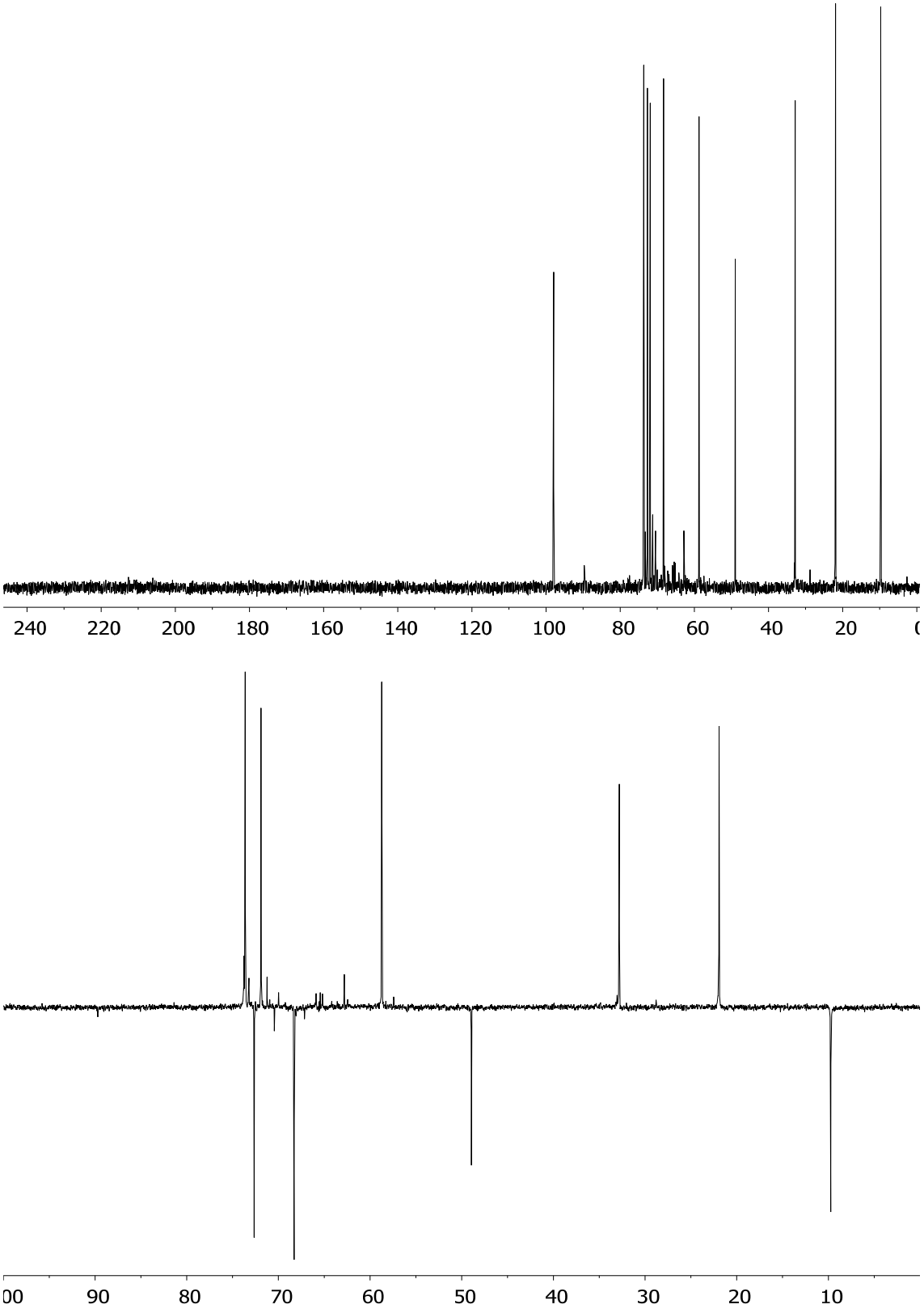
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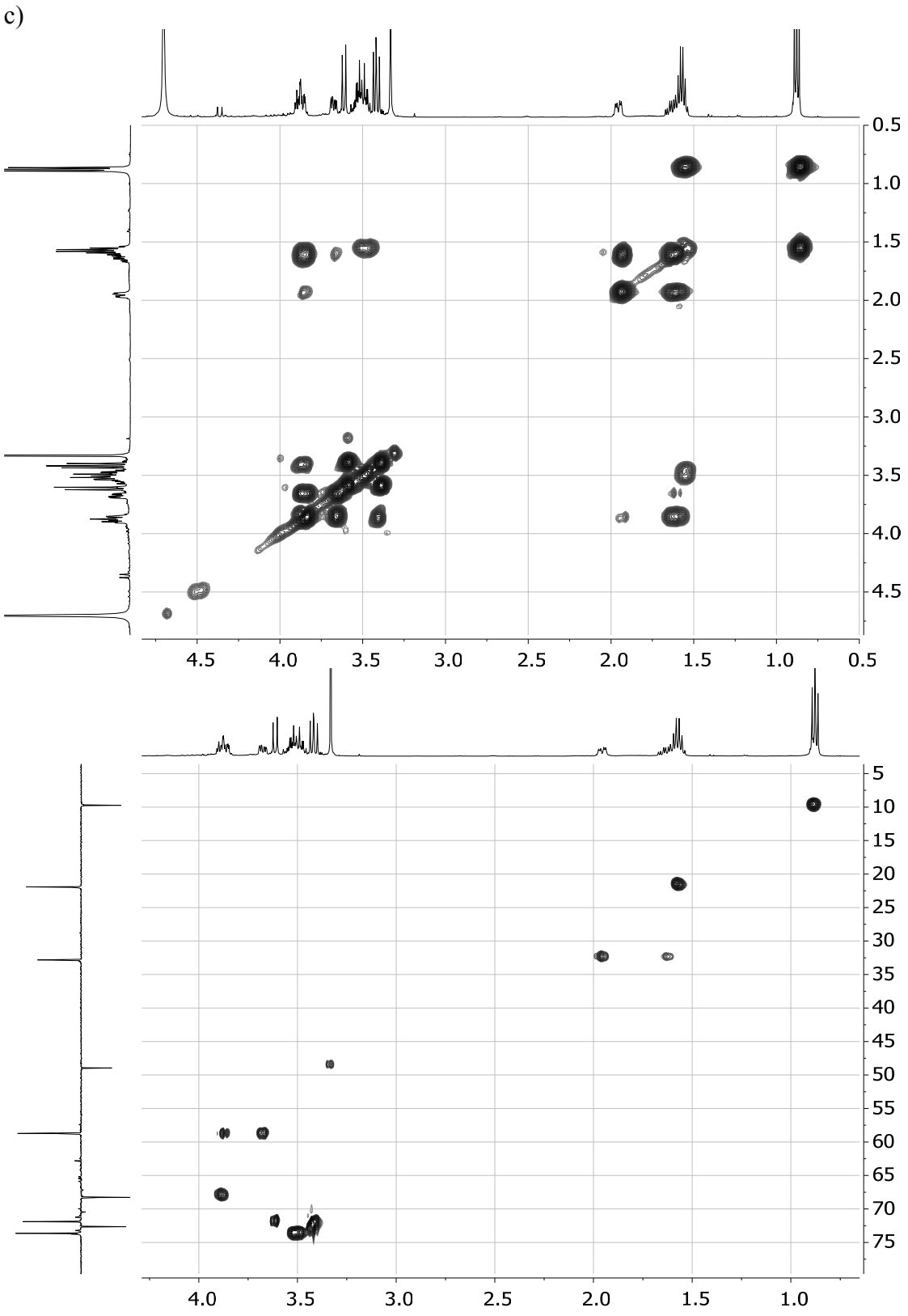
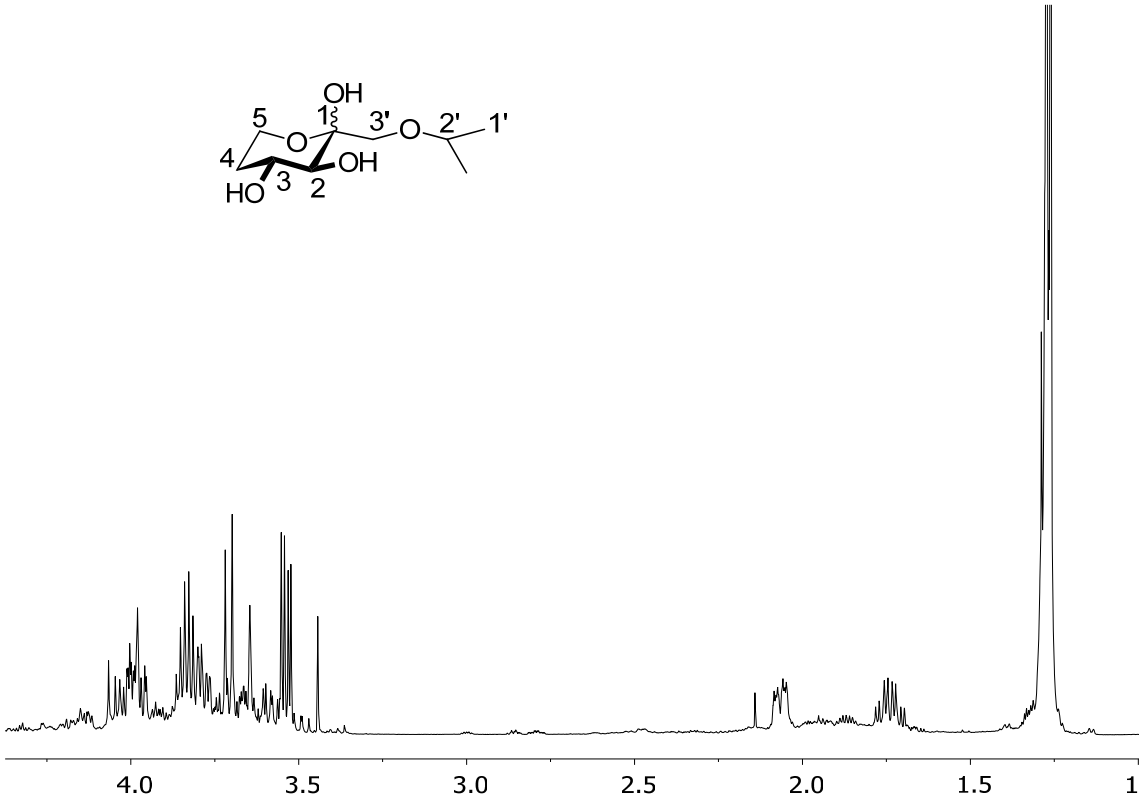
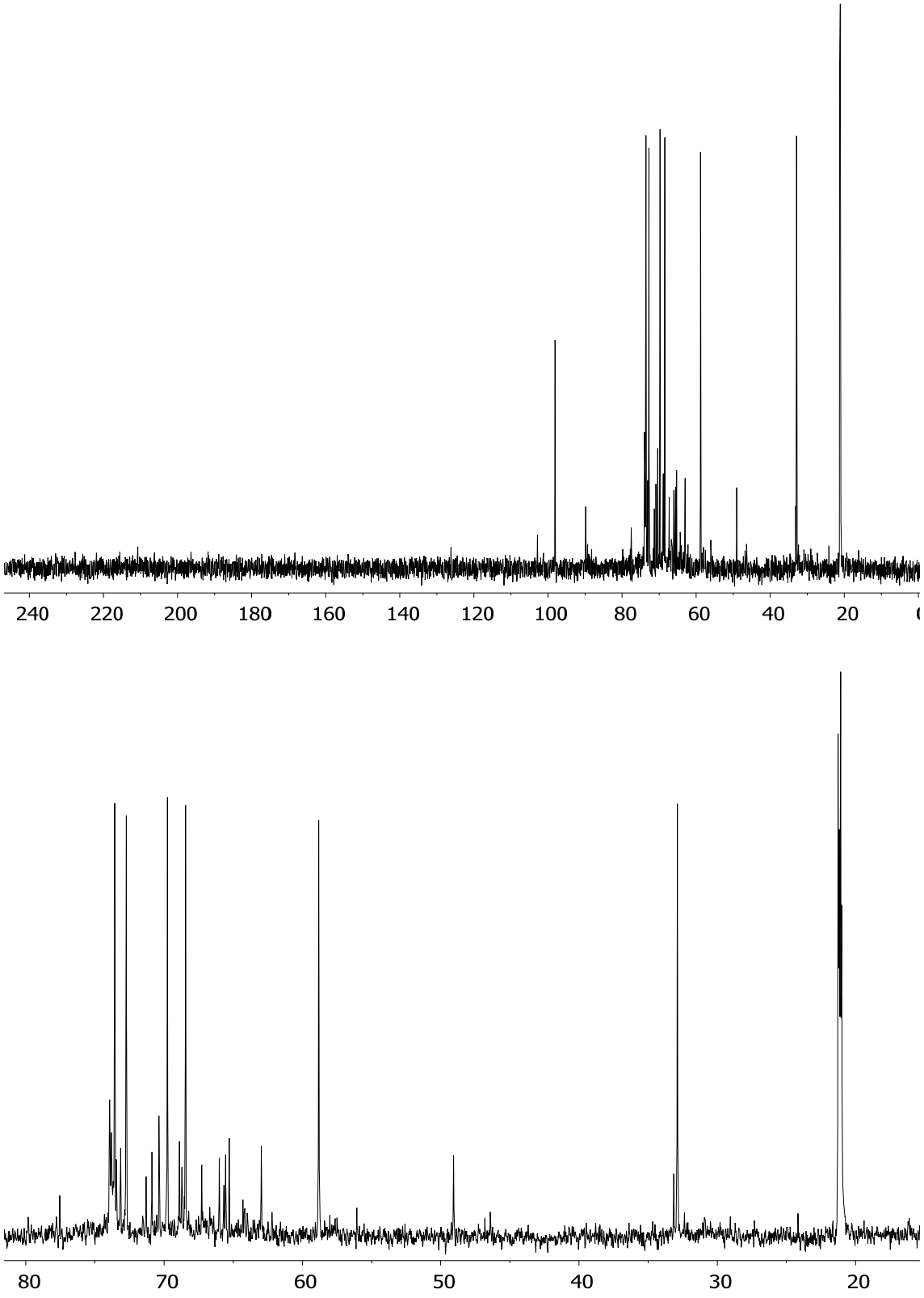


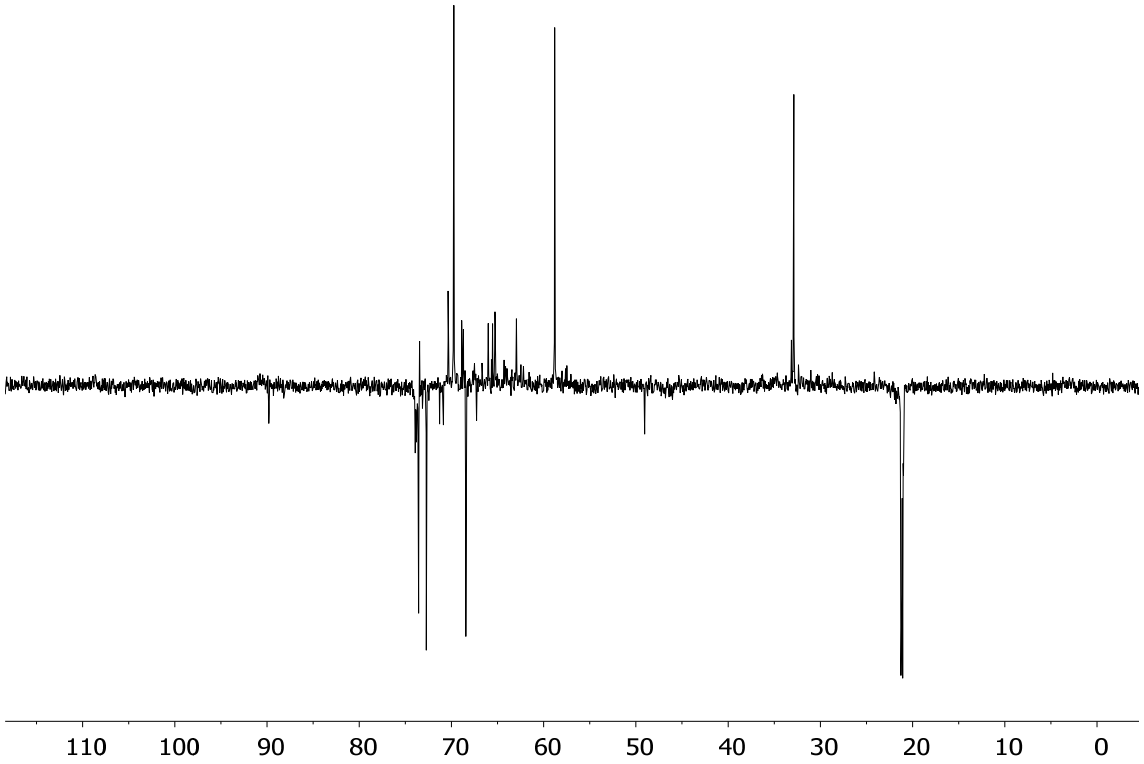
Figure S13. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **29**.

a)



b)

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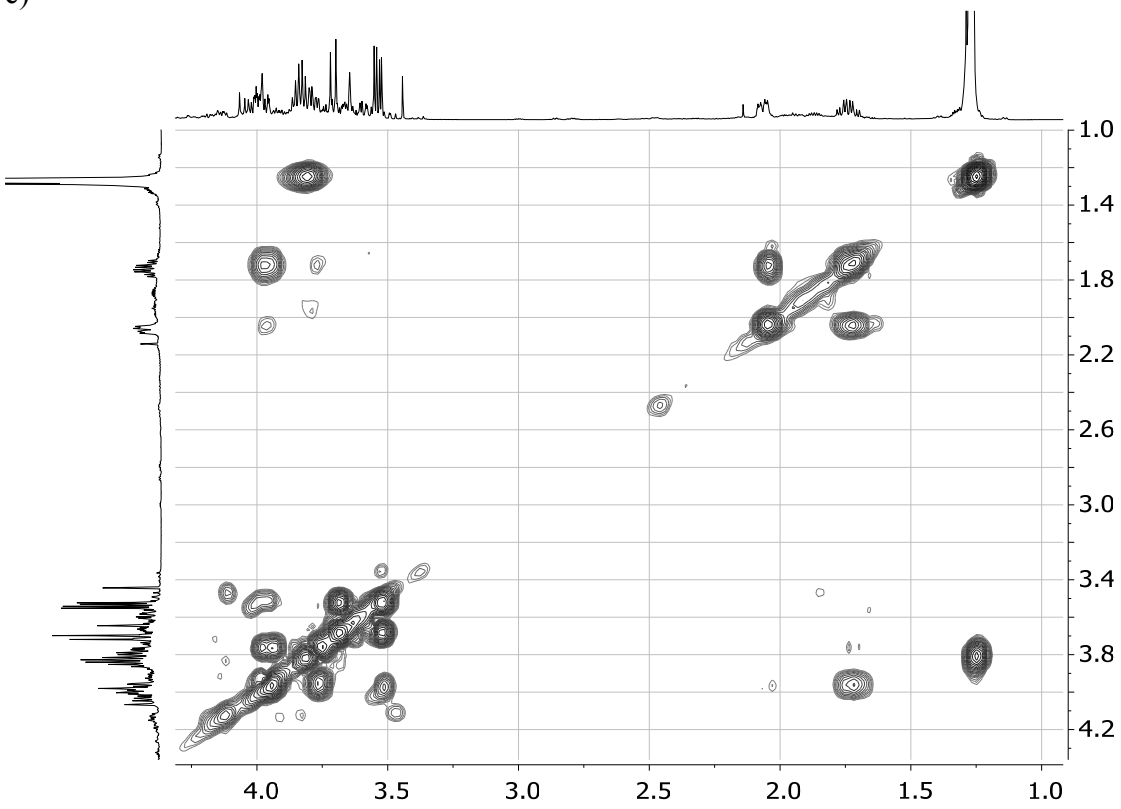
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1.4
1.8
2.2
2.6
3.0
3.4
3.8
4.2
4.0
3.5
3.0
2.5
2.0
1.5
1.0

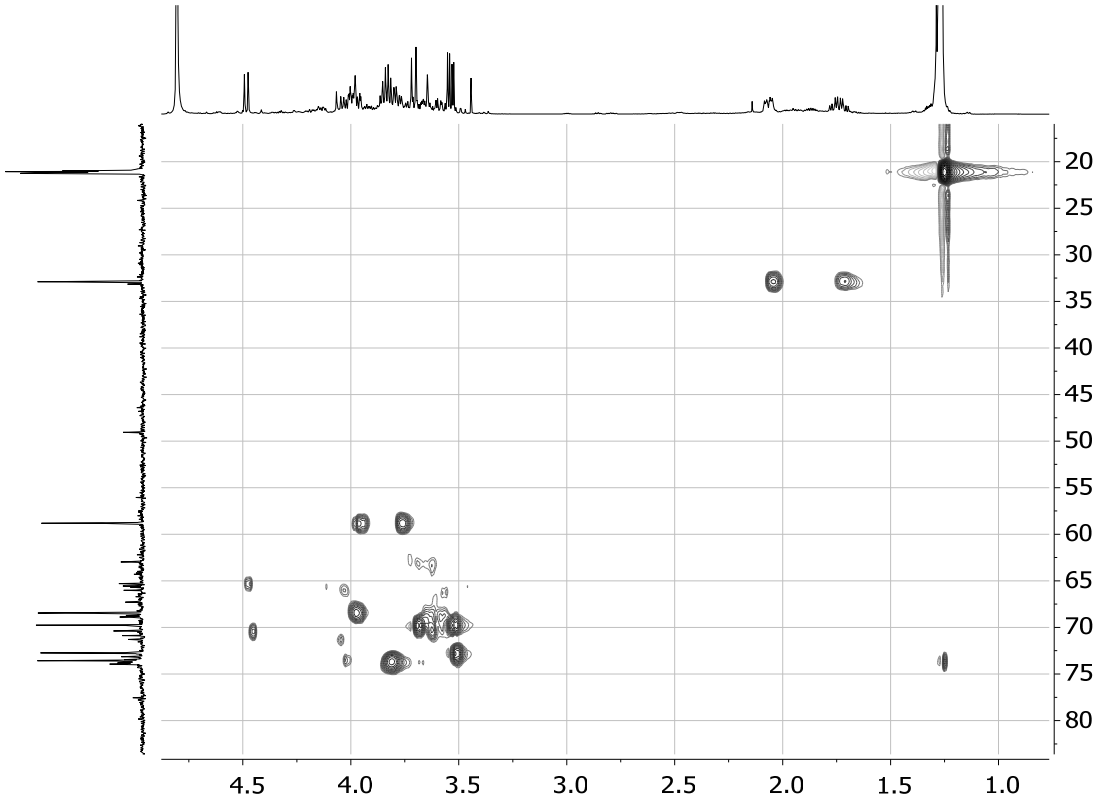
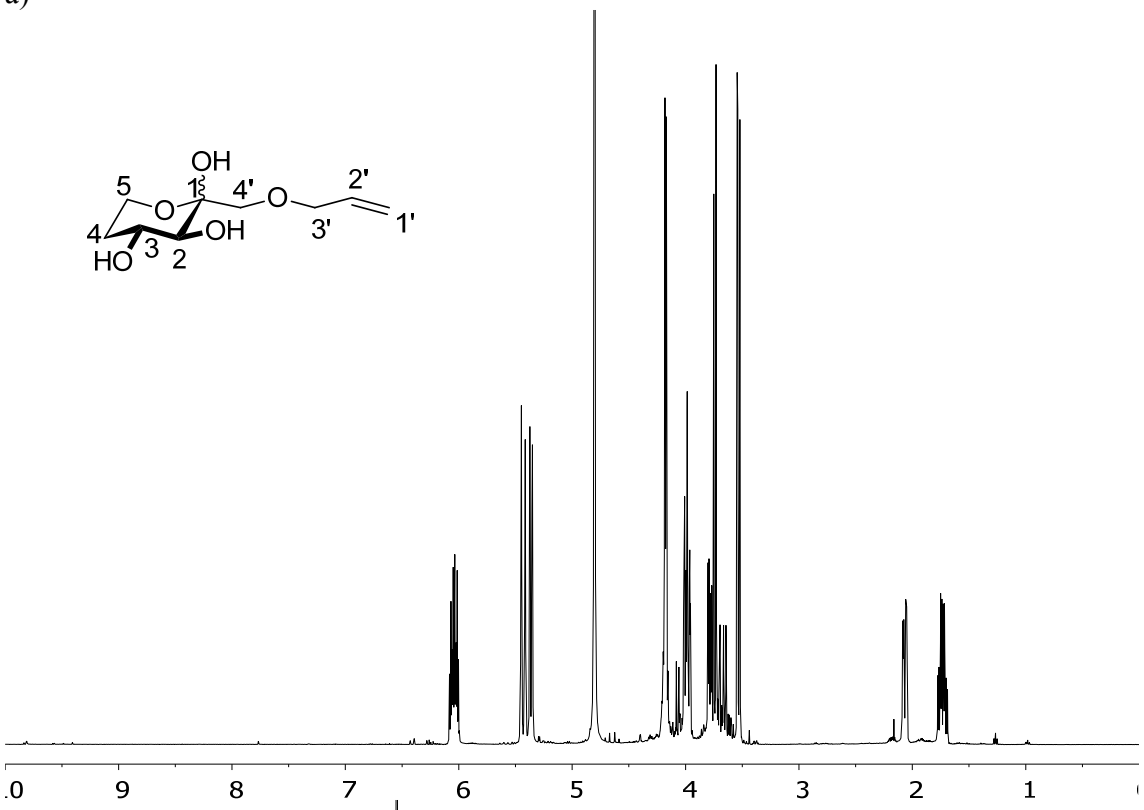
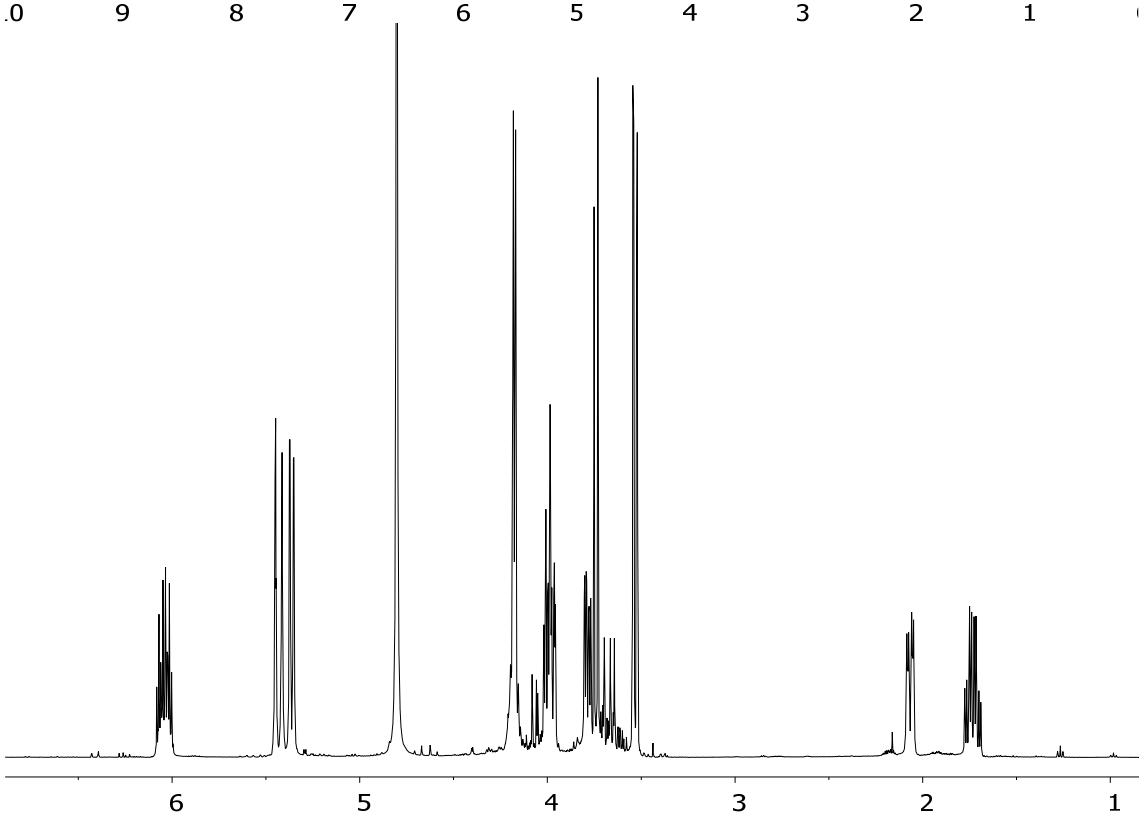


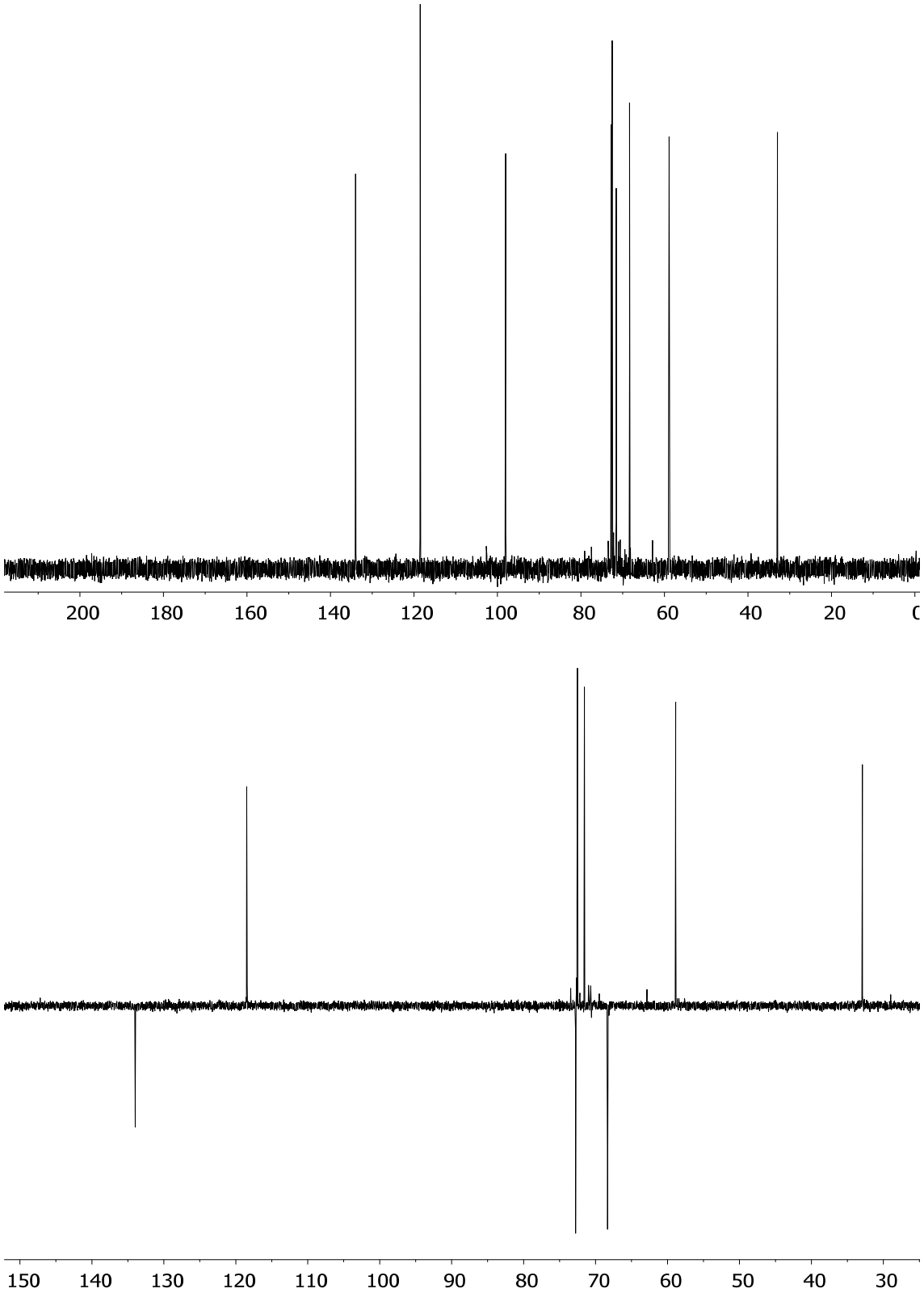
Figure S14. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **30**.

a)





b)


200
180
160
140
120
100
80
60
40
20
150
140
130
120
110
100
90
80
70
60
50
40
30

c)

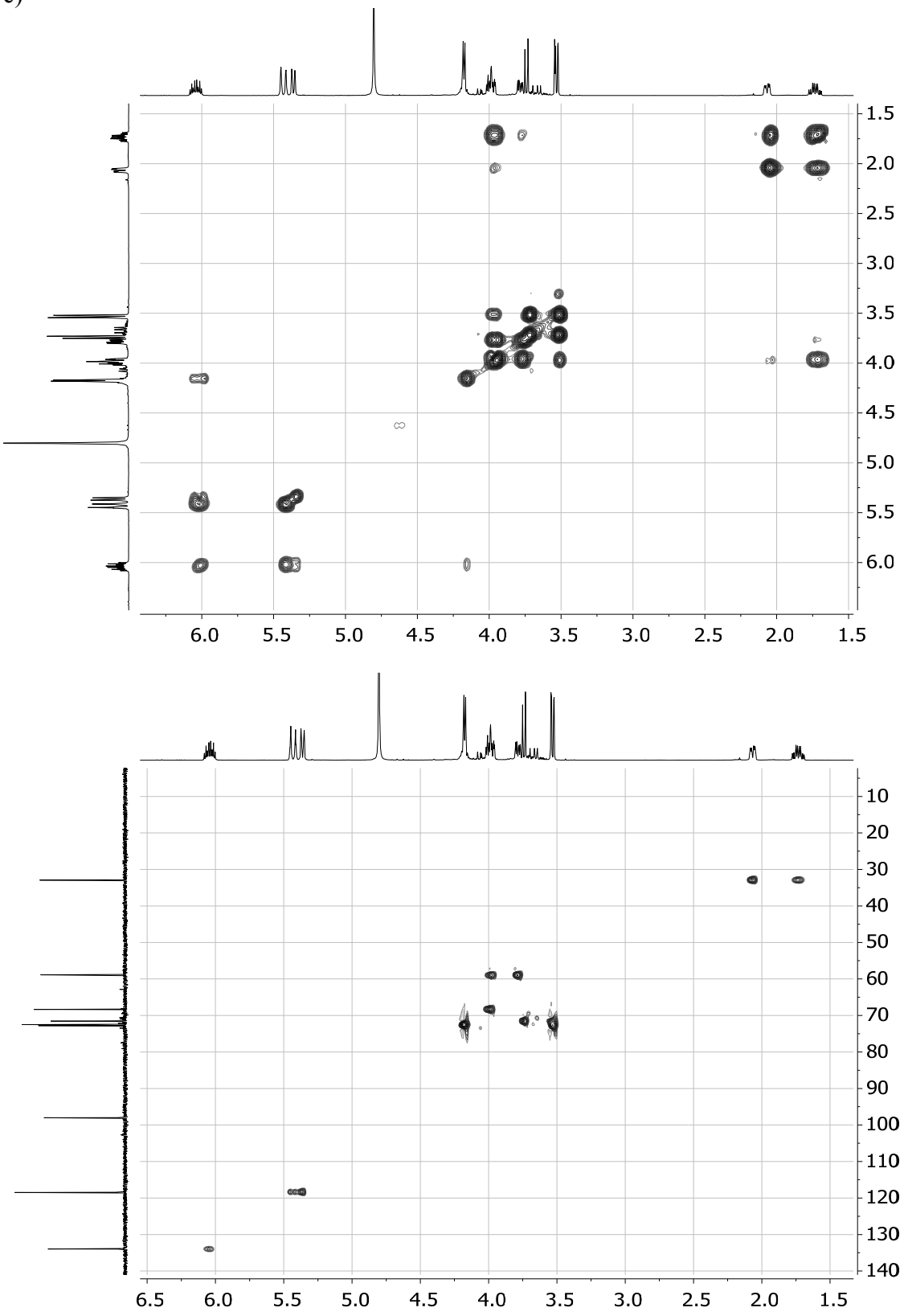
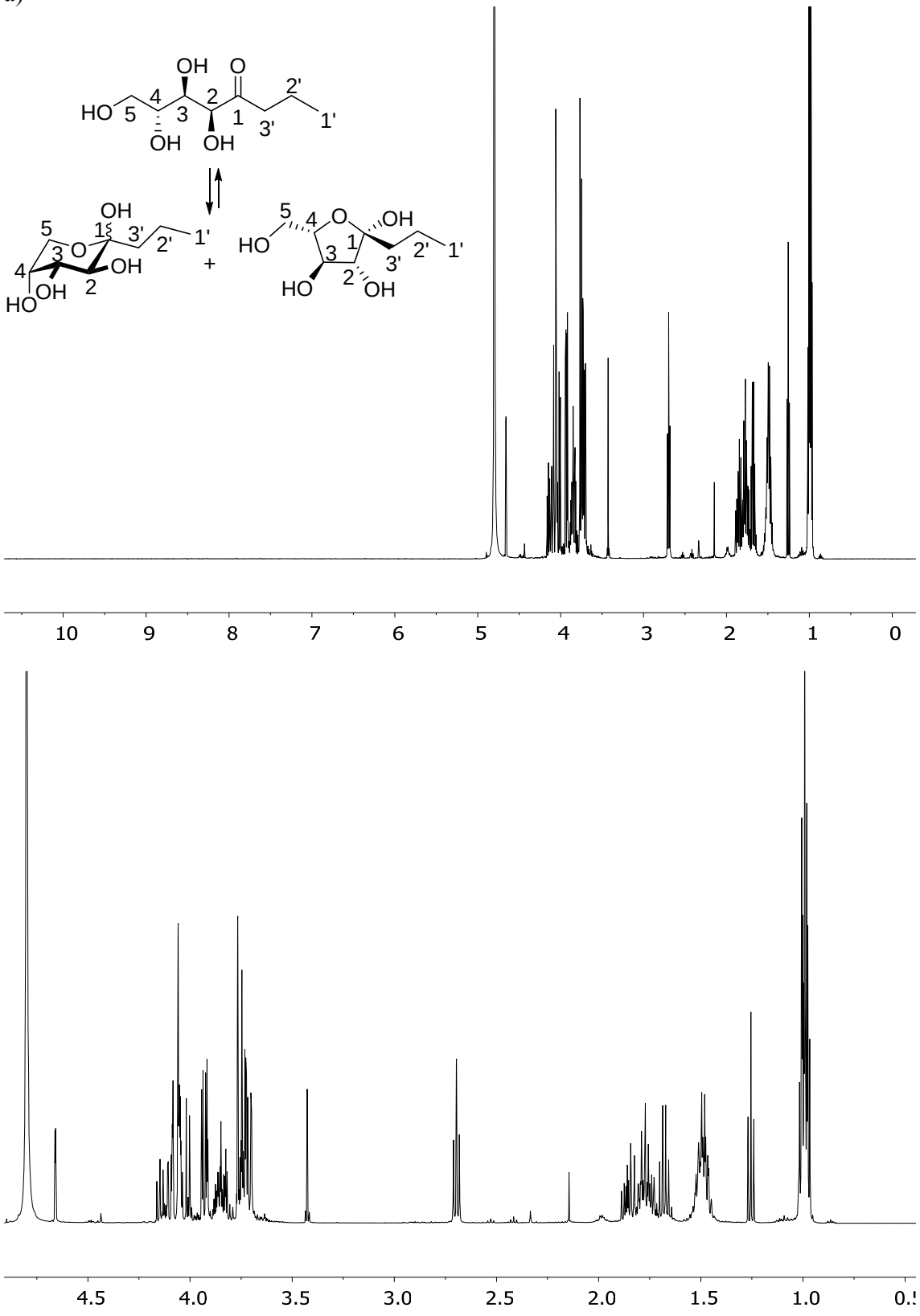
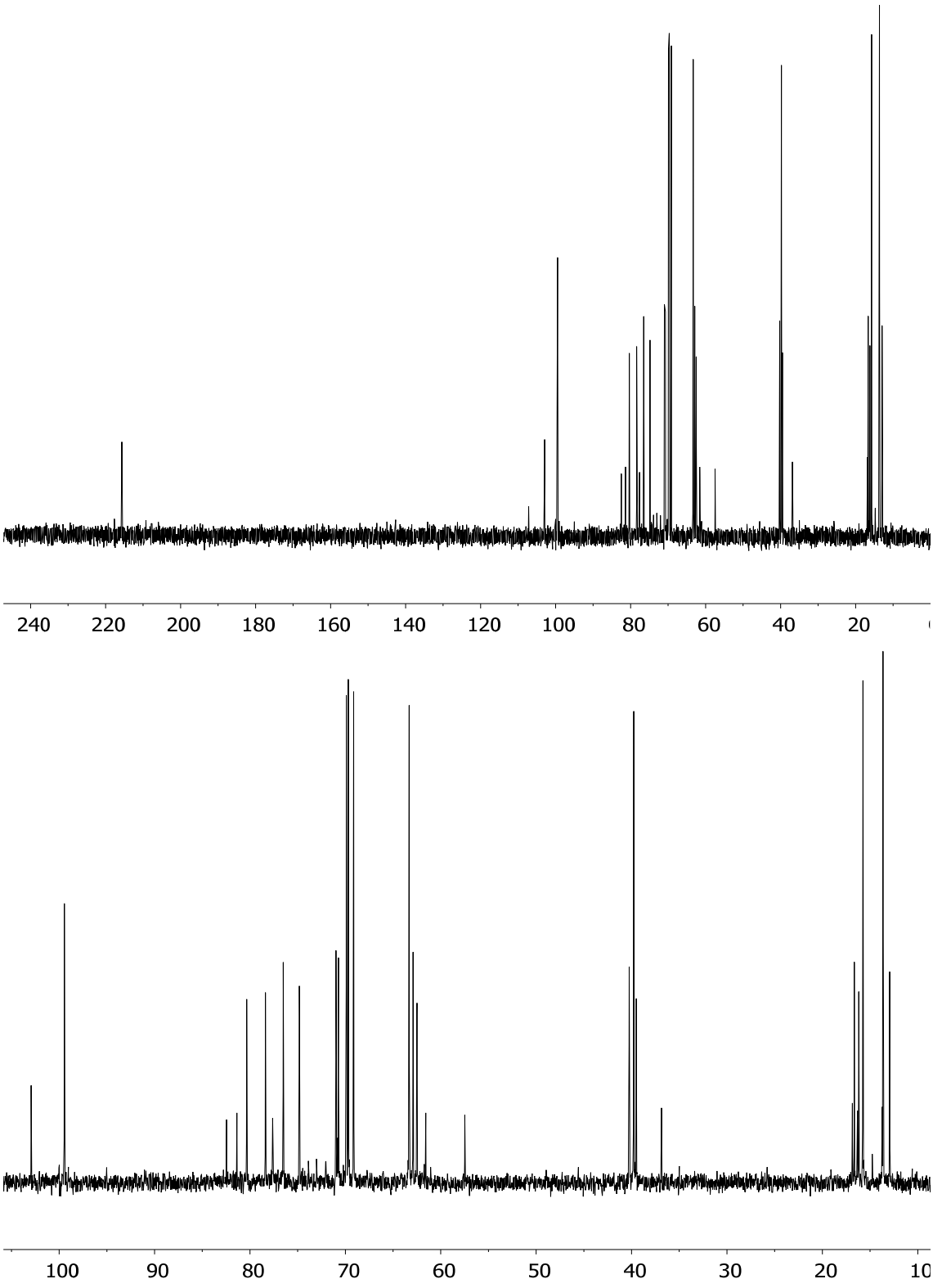


Figure S15. a) ^{1}H and b) ^{13}C NMR and DEPT, c) COSY and HSQC spectra of compound **31**.

a)


OH
O
HO
OH
OH
OH
OH
OH
HO
OH
OH
HO
OH
10
9
8
7
6
5
4
3
2
1
0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

b)


240
220
200
180
160
140
120
100
80
60
40
20
100
90
80
70
60
50
40
30
20
10

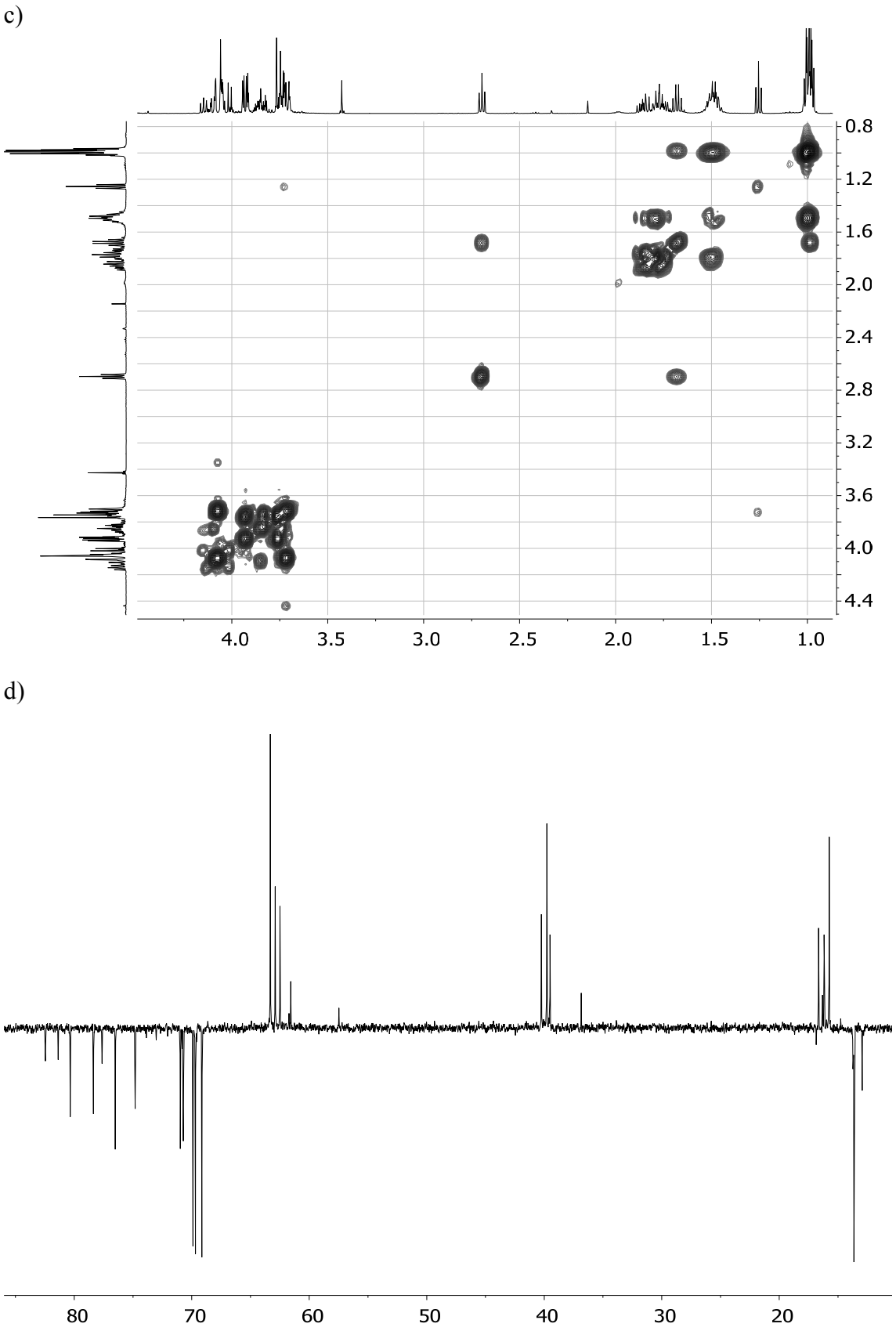
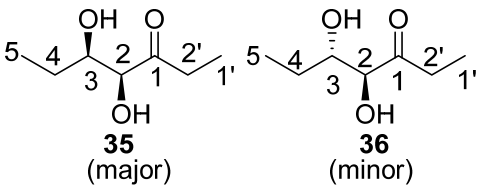


Figure S16. a) ^{1}H and b) ^{13}C NMR, c), COSY and d) DEPT spectra of compound **33**.

a)



35
(major)

36
(minor)

b)

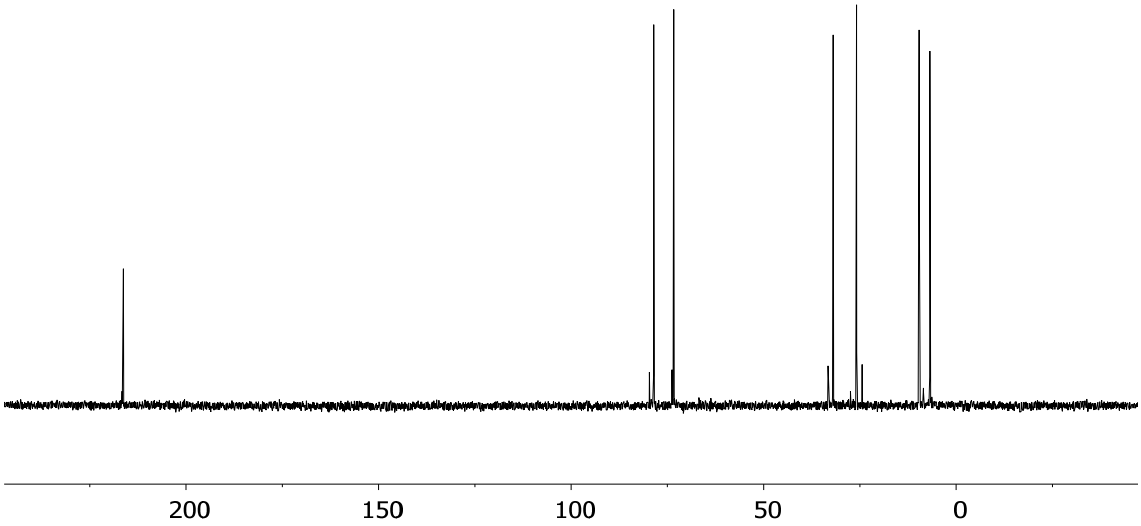


Figure S17. a) ^{1}H and b) ^{13}C NMR spectra of compound **35** (major) and **36** (minor).

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