Supplementary Information for Kelly, et al., "The *Vibrio cholerae* Quorum-Sensing Autoinducer CAI-1: Structural and Mechanistic Analysis of the Biosynthetic Enzyme CqsA"

Supplementary Figures S1-S6, Supplementary Tables S1-S5,
and Supplementary Methods (with Supplementary Figure S7 & Schemes S1-S4)

A. Supplementary Figures

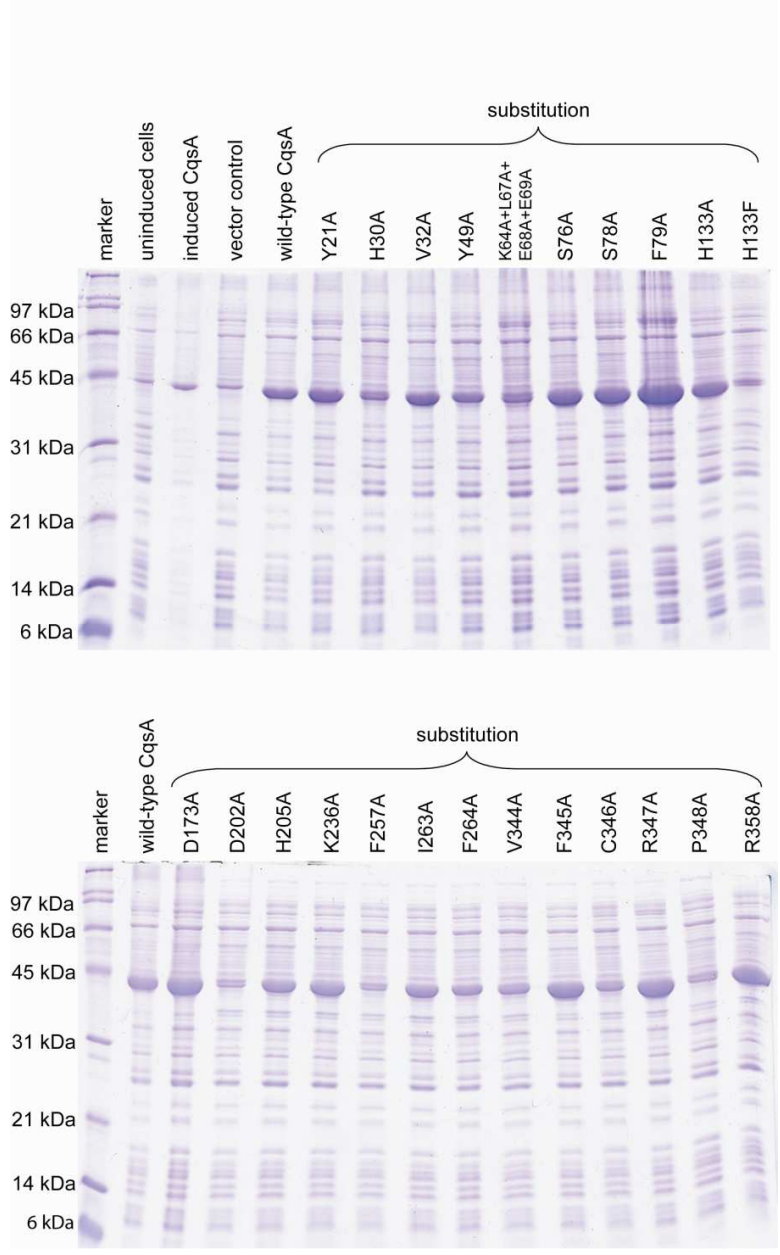


Figure S1 | Expression/Solubility of CqsA Variants. *E. coli* BL21(DE3) cells overexpressing a single CqsA protein (wild-type or mutant) were lysed by sonication and centrifuged to remove insoluble proteins and cell debris. An aliquot of each cleared lysate was analyzed using Coomassie-Blue-stained SDS-PAGE. The prominent band slightly below the 45 kDa marker is CqsA.

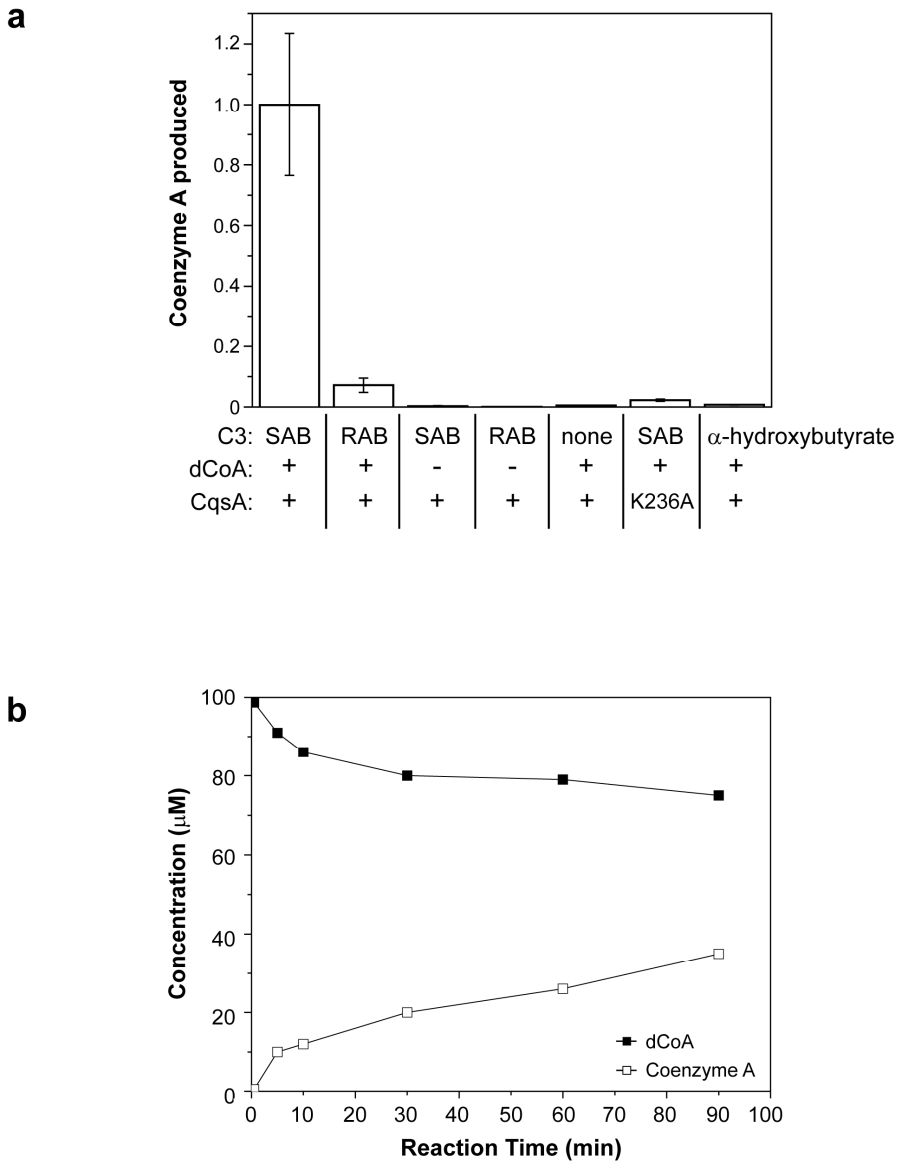


Figure S2 | Coenzyme A Production by CqsA. **a,** CqsA (1 μM) was incubated for two hours with 25 mM SAB (S-**4**) or RAB ((*R*)-2-aminobutyrate, R-**4**) in the presence or absence of 100 μM dCoA (**5**). Evolution of CoA (**7**) was measured using mass spectrometry. The amount of CoA (**7**) produced by the reaction of SAB (S-**4**) and dCoA (**5**) was set to 1.0. **b,** Levels of CoA (**7**, open boxes) and dCoA (**5**, filled boxes), determined using mass spectrometry with labeled standards, upon mixing 1 μM CqsA with 25 mM SAB (S-**4**) and 100 μM dCoA (**5**).

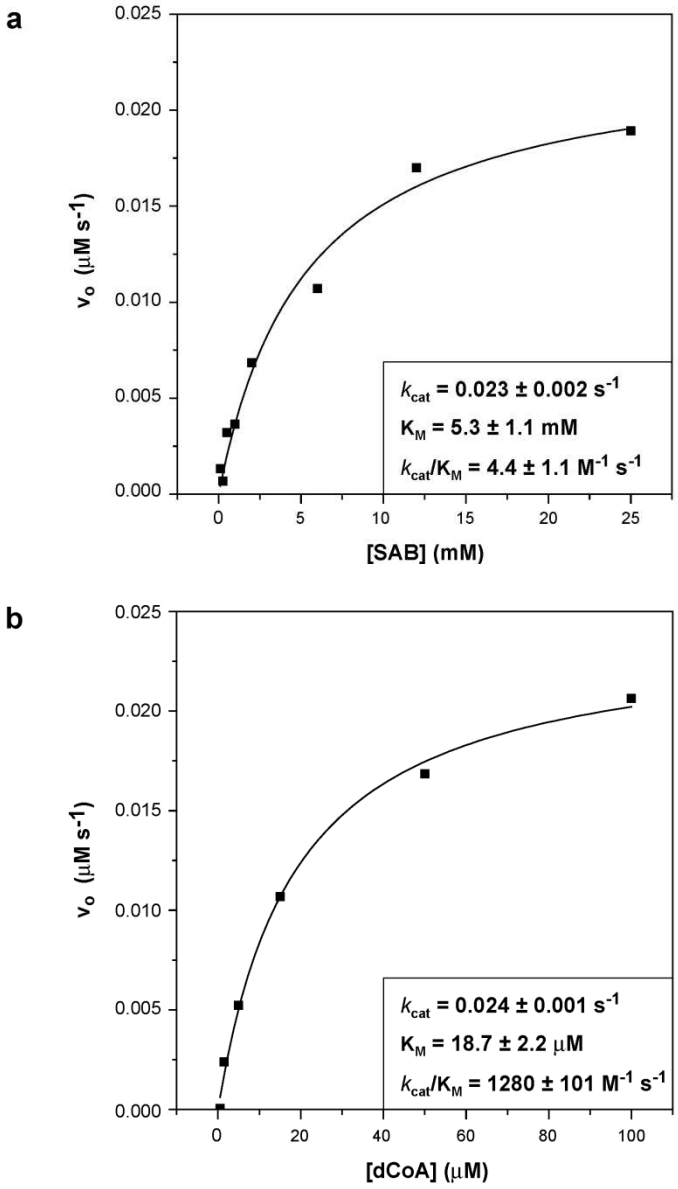


Figure S3 | Kinetic Analysis of CqsA. a, SAB (S-**4**) or **b,** dCoA (**5**) concentrations were varied while the other substrate was held constant at 100 μM (dCoA, **5**) or 25 mM (SAB, S-**4**) in the presence of 1 μM CqsA. In each case, aliquots were placed in acetonitrile (30% (v/v) final concentration) at time points up to 10 min. After removing the precipitated enzyme by centrifugation, amino-CAI-1 (**6**) was quantified by mass spectrometry as in Table S3. Kinetic constants were estimated by fitting initial velocity data to the Michaelis-Menten equation (solid lines).

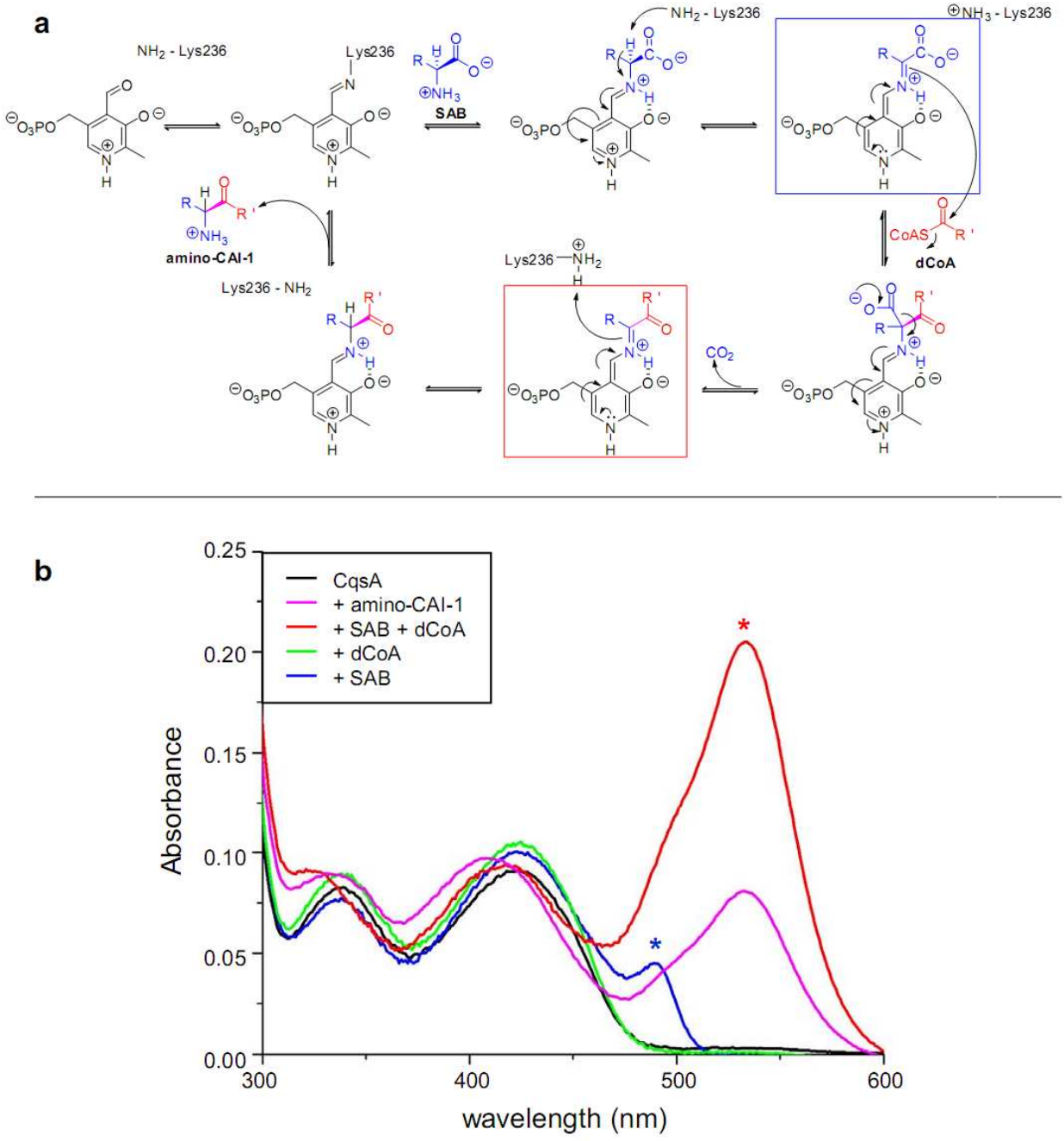


Figure S4: Spectroscopic Analysis of the CqsA Reaction Mechanism. (**a**) Proposed CqsA reaction scheme, inspired by the scheme proposed by Webster and coworkers for AONS[18]. The boxed quinonoid intermediates give rise to the absorbance peaks indicated in panel b by asterisks of the same color. R' = $(CH_2)_8CH_3$. (**b**) The absorbance spectrum of 30 μM holo-CqsA is shown, together with the spectra of CqsA approximately 20 sec after addition of 100 μM amino-CAI-1 (**6**), 25 mM SAB (S-**4**), and/or 100 μM dCoA (**5**). Spectra were recorded at 23°C using a Hewlett-Packard 8452A diode array spectrophotometer.

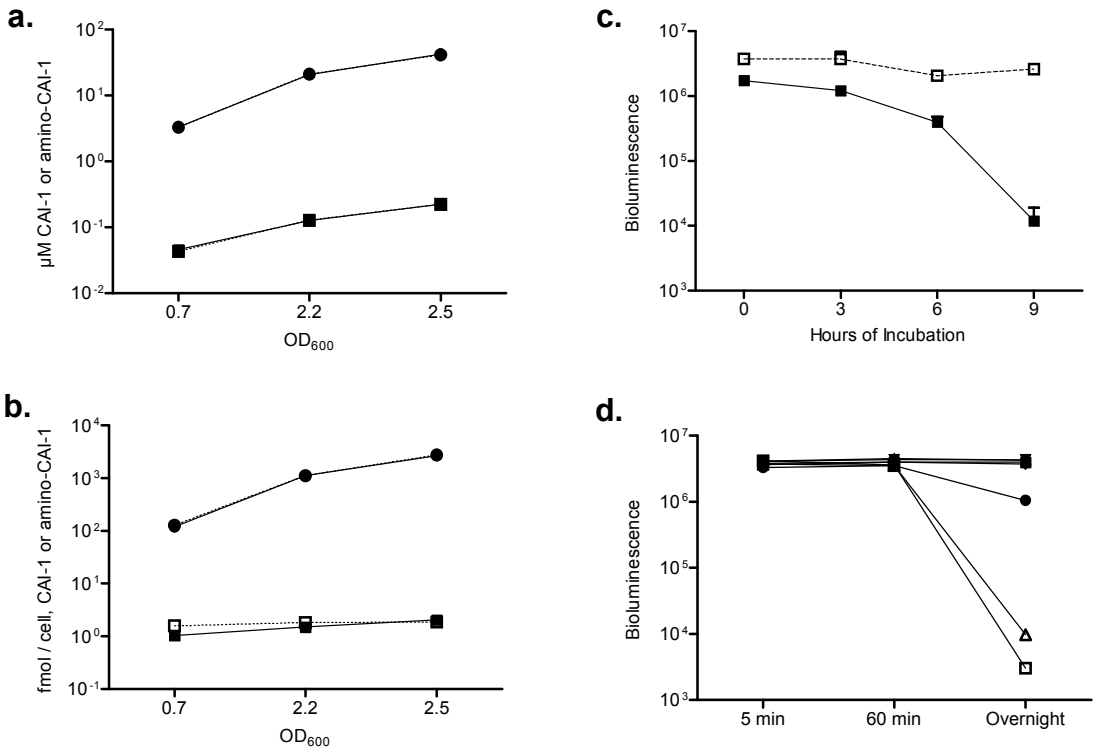


Figure S5 | CAI-1 and Amino-CAI-1: Timecourse and Stability. CAI-1 (**1**, open and filled circles) and amino-CAI-1 (**6**, open and filled squares) levels were determined in duplicate in **a**, cell supernatants and **b**, cell pellets of *E. coli* grown in uniformly ^{13}C-labeled glucose at various stages of growth by spiking with standards at known concentrations. **c,** 4 μM CAI-1 (**1**, filled squares) and amino-CAI-1 (**6**, open squares), both the *S* isomer, were incubated with shaking at 30 ^{o}C in LB medium for the times specified and analyzed for activity in the *V. cholerae* bioluminescence reporter assay. **d,** Amino-CAI-1 (**6**) is stable overnight at acidic and neutral pH values. The autoinducer was incubated at pH 2 (filled squares), 4 (filled triangles), 6 (inverted triangles), 7 (diamonds), 8 (circles), 10 (open squares), and 12 (open triangles) in potassium phosphate buffer for 5 min, 60 min, or overnight before neutralization and bioassay as reported previously.[9]

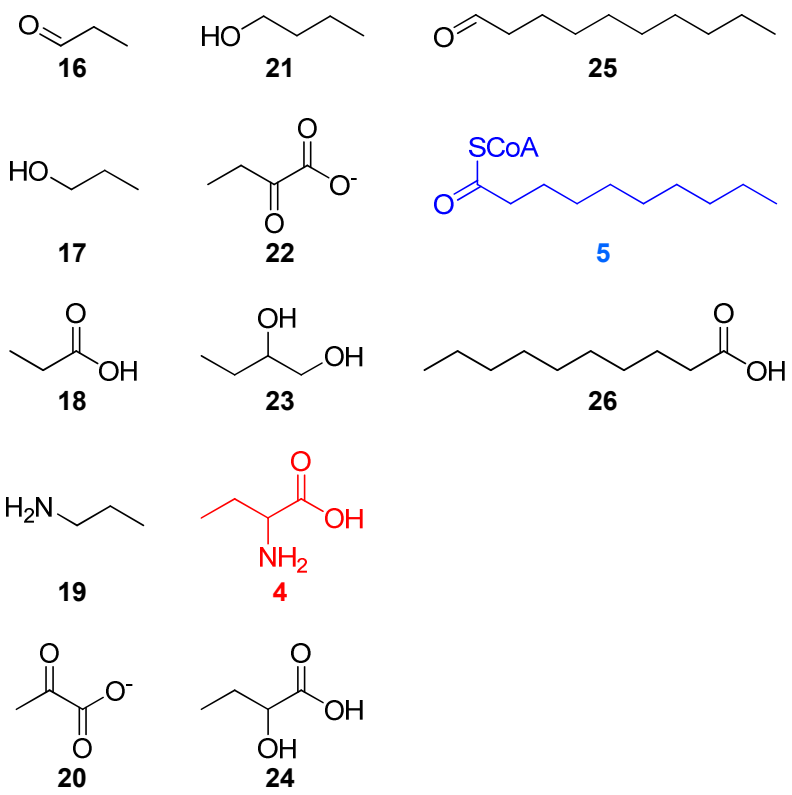


Figure S6 | Structures of Potential Substrates for CqsA. Of the various three-carbon (propionaldehyde (**16**), 1-propanol (**17**), propionic acid (**18**), propylamine (**19**), pyruvic acid (**20**)), four-carbon (1-butanol (**21**), 2-ketobutyric acid (**22**), 1,2-butanediol (**23**), 2-aminobutyric acid (**4**), 2-hydroxybutyric acid (**24**)), and ten-carbon (decanal (**25**), decanoyl-coenzyme A (**5**), decanoic acid (**26**)) compounds tested in pairs for an ability to act as substrates of CqsA, only the combination of **4** and **5** yielded product.

B. Supplementary Tables

Table S1 | Data Collection and Refinement Statistics for CqsA

	Crystal A	Crystal B
Data collection		
Space group	$P2_12_12$	$P2_12_12$
Cell dimensions		
a, *b*, *c* (Å)	71.2, 176.5, 70.5	70.6, 177.4, 70.7
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	50.0-2.70 (2.80-2.70)	50.00-1.80 (1.86-1.80)
R_{sym} or R_{merge} (%)	11.5 (34.4)	5.7 (41.7)
I / σI	11.8 (3.0)	39.6 (4.5)
Completeness (%)	98.1 (86.7)	99.6 (99.2)
Redundancy	5.3 (4.2)	9.4 (8.8)
Refinement		
Resolution (Å)	45.34-2.70	50.00-1.80
No. reflections	23440	78235
R_{work} / R_{free} (%)	18.6/25.0	19.4/23.1
No. atoms		
Protein	6,068	6,077
Ligand/ion	30 (PLP)	32 (PLP) 2 (Mg^{2+}), 10 (SO_4), 16 (dithiothretiol)
Water	95	485
B-factors (Å^2)		
Protein	28.4	29.8
Ligand/ion	23.7	26.3
Water	24.5	36.8
R.m.s. deviations		
Bond lengths (Å)	0.010	0.008
Bond angles (°)	1.228	1.10

*Values in parentheses are for highest-resolution shell.

Table S2 | Activity of Alanine-Substituted CqsA Mutants

Substitution	% wild-type activity
wild-type CqsA	100 ± 0.78
empty vector	<0.01
PLP-coordinating residues	
Y49A	0.01 ± 0.0001
N112A	0.01 ± 0.0002
D173A	0.1 ± 0.03
H205A	<0.01
K236A	<0.01
R358A	<0.01
(H133F)	(0.03 ± 0.02)
(D202A)	(<0.01)
Substrate-binding site: proximal	
S76A'	0.02 ± 0.01
F79A'	<0.01
I263A'	0.03 ± 0.02
F264A'	<0.01
F345A	<0.01
Substrate-binding site: distal	
V32A	10 ± 1
S78A'	81 ± 4
V344A	19 ± 1
C346A	12 ± 3
(H30A)	2 ± 1
(F257A')	3 ± 2
(P348A)	<0.01
Control substitutions	
Y21A	93 ± 36
K64A+L67A+E68A+E69A	94 ± 5
R347A	31 ± 8

Relative autoinducer activity released by *E. coli* cells overexpressing each CqsA protein was measured using the *V. cholerae* CAI-1 bioluminescence assay. Residues contributed by the two CqsA monomers to the active site are distinguished by the presence or absence of a prime (’). Parentheses indicate mutant proteins expressed at levels <20% of wild-type (Fig. S1).

Table S3 | CqsA Produces Amino-CAI-1 and Not CAI-1

Substrates	amino-CAI-1 produced (μM)	CAI-1 produced (μM)
decanoyl-CoA (**5**)	< 0.1	n.d.
(*S*)-2-aminobutyrate (S-**4**)	< 0.1	n.d.
(*S*)-2-aminobutyrate (S-**4**) + decanoyl-CoA (**5**)	37.2±1.9	n.d.
(*R*)-2-aminobutyrate (R-**4**)	< 0.1	n.d.
(*R*)-2-aminobutyrate (R-**4**) + decanoyl-CoA (**5**)	3.1±0.4	n.d.
2-hydroxybutyrate (**24**) + decanoyl-CoA (**5**)	< 0.1	n.d.
(*S*)-2-aminobutyrate (S-**4**) + decanoyl-CoA (**5**)(K236A)	< 0.1	n.d.

Mass spectrometry measurements of amino-CAI-1 (**6**) and CAI-1 (**1**) production from the specified reactions. Deuterated amino-CAI-1 (d-**6**) and CAI-1 (d-**1**) synthetic standards were used for calibration. n.d.: none detected.

Table S4 | Quantification of Amino-CAI-1 and CAI-1 in Cell-Free Culture Fluids

	CAI-1 (μM)	amino-CAI-1 (μM)
E. coli pcqsA	23 ± 4	0.24 ± 0.03
V. cholerae	0.28 ± 0.05	n.d.

Mass spectrometry measurements of amino-CAI-1 (**6**) and CAI-1 (**1**) production in cell-free culture fluids prepared from wild type *V. cholerae* and *E. coli* overexpressing CqsA. Deuterated amino-CAI-1 (d-**6**) and CAI-1 (d-**1**) synthetic standards were used for calibration.

Table S5 | Inter-Conversion of Amino-CAI-1 and CAI-1

Molecule Supplied	Molecule Detected (μM)	
	amino-CAI-1	***CAI-1***
DMSO	< 0.1	< 0.2
amino-CAI-1	22.6 ± 0.5	5.0 ± 0.4
CAI-1	1.4 ± 0.0	24.1 ± 0.6

Mass spectrometry measurements of amino-CAI-1 (**6**) and CAI-1 (**1**) production following incubation of *E. coli* cells with the specified compound. Deuterated amino-CAI-1 (d-**6**) and CAI-1 (d-**1**) synthetic standards were used for calibration.

C. Supplementary Methods

Quorum Sensing and the *V. cholerae* Life Cycle.

V. cholerae naturally exists in the marine environment, and human infection generally occurs through ingestion of contaminated water. *V. cholerae* expresses numerous virulence factors that are required for colonization of the host intestinal epithelium. Most important are the toxin coregulated pilus, which promotes adherence to the intestinal lining, and cholera toxin, which leads to severe diarrhea and release of the bacterium.[26,27] Critical to the *V. cholerae* infection cycle is its ability to alternate between expression of virulence traits essential for survival inside the host and production of biofilms which are necessary for survival in the marine environment.[26-28] Quorum sensing controls both of these processes. Specifically, *V. cholerae* produces and detects two autoinducers and responds via a shared phosphorelay signaling pathway. The two autoinducer molecules are CAI-1 ((*S*)-3-hydroxytridecan-4-one, **1**), and AI-2 ((2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate, **2**). In the low cell density state (i.e., when autoinducer levels are low), the CAI-1 and AI-2 receptors (CqsS and LuxPQ, respectively) function as kinases, passing phosphate to the LuxO response regulator. LuxO-P activates the expression of four genes encoding the Qrr small regulatory RNAs (sRNAs), which function to destabilize the mRNA encoding the major quorum-sensing regulator, HapR. Under this condition, low cell density specific genes, including genes required for biofilm formation and virulence factor production, are expressed. When the cell density increases, both autoinducers accumulate and bind their respective receptors, causing the receptors to switch to phosphatase activity. As a consequence, LuxO is dephosphorylated and *qrr* expression is terminated. The mRNA encoding HapR is stabilized, and HapR protein is produced. HapR is an activator and a repressor that establishes a program of gene expression appropriate for the high-cell-density state.[29-31] When HapR is produced, biofilm and virulence genes are repressed, potentially promoting dispersal of *V. cholerae* back into the environment.[32]

Spectroscopic Characterization of the CqsA Mechanism.

To determine whether or not CqsA functions by an AONS-type mechanism (Fig. S4a), we monitored spectral changes induced by the interaction of CqsA with SAB (S-**4**), dCoA (**5**), and amino-CAI-1 (**6**; Fig. S4b). First, we examined the PLP (**3**)-bound holoenzyme itself. The absorbance maxima (λ_{max} = 338 and 420 nm; Fig. S4b) are very similar to those reported for AONS (λ_{max} = 334 and 425 nm)[18]. Adding the reaction product, AON (**8**), to AONS yields a new peak at 520 nm[18]. A peak at a similar position, 533 nm, is observed upon adding amino-CAI-1 (**6**) to CqsA (Fig. S4b). The similar λ_{max} values suggest that the product-enzyme complexes formed by CqsA and AONS are likewise similar, and may in both cases represent the quinonoid species represented by the red box in Fig. S4a. The presumptive product quinonoid is also observed when both substrates are added to CqsA (Fig. S4b).

Neither AONS nor CqsA displays spectral changes upon addition of the 'second' (acyl-CoA) substrate only (ref. 18 and Fig. S4b). However, following addition of the 'first' (amino acid) substrate, an interesting difference between AONS and CqsA becomes evident. Specifically,

addition to CqsA of SAB (S-**4**) alone leads to the appearance of a peak at λ_{max} = 488 nm, likely corresponding to the quinonoid intermediate highlighted in blue in Fig. S4a. For AONS, by contrast, although binding of alanine (**9**) alone is apparently unable to stabilize the quinonoid form, a transient peak at a similar position (λ_{max} = 486 nm) is observed following addition of pimeloyl-CoA (**10**) [18]. Taken together, our spectroscopic studies are consistent with the idea that AONS and CqsA utilize similar reaction mechanisms, but with the difference that CqsA is capable of stabilizing the quinonoid intermediate prior to binding the second substrate.

Purification of CqsA.

V. cholerae CqsA was produced in *E. coli* BL21 (DE3) cells grown in LB medium. When the OD_{600} reached 1.0, protein production was induced by addition of 100 μM isopropyl β-D-thiogalactopyranoside. Cells were grown for an additional 6 h at 23°C and harvested by centrifugation. Cell pellets were resuspended in lysis buffer (20 mM HEPES (pH 8.0), 200 mM NaCl, 5 mM β-mercaptoethanol, 100 μM PLP (**3**)) supplemented with 1 mM $MgCl_2$ and 2.5 μg ml^{-1} DNaseI, and then lysed at 15,000 psi. Cleared supernatants were passed over Ni^{2+}-Sepharose 6 Fast Flow affinity resin (GE Healthcare) equilibrated in lysis buffer. After washing the resin with lysis buffer containing 20 mM imidazole, protein was eluted in lysis buffer containing 250 mM imidazole. Further purification was achieved by anion exchange (Source 15Q; GE Healthcare) and size exclusion (Superdex 200; GE Healthcare) chromatography. 100 μM PLP (**3**) was included in all buffers with the exception of the final Superdex 200 buffer. Protein was stored at -80°C in 20 mM HEPES, pH 8.0, 200 mM NaCl, 1 mM dithiothreitol (DTT, **27**).

Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) Analysis.

Samples were analyzed on a Finnigan TSQ Quantum Ultra or DiscoveryMax triple quadrupole mass spectrometer (Thermo Electron Corp., San Jose, CA) operating in selected reaction monitoring (SRM) mode, coupled with a Shimadzu LC-10AD or LC-20AD HPLC system (Shimadzu, Columbia, MD). Samples were divided and different chromatography and ionization methods were used to detect CAI (**1**) and amino-CAI-1 (**6**), with synthetically-produced compounds used as standards (see **Synthetic Details**). Amino-CAI-1 (**6**) was detected in positive ionization mode on a Luna NH2 column (250 mm x 2 mm, 5 μm particle size; Phenomenex, Torrance, CA) at a basic pH with a running time of 40 min.[33] Solvent A was 20 mM ammonium acetate + 20 mM ammonium hydroxide in 95:5 water:acetonitrile, pH 9.45 and solvent B was acetonitrile. The gradient was: *t* = 0, 85% B; *t* = 15 min, 0% B; *t* = 28 min, 0% B; *t* = 30 min, 85% B; *t* = 40 min, 85% B. Other chromatography parameters were as follows: autosampler temperature, 4°C; column temperature, 15°C; injection volume, 5 μl; and solvent flow rate, 150 μl min^{-1}. CAI-1 (**1**) was detected in negative ionization mode on a Synergi Hydro-RP column (150 mm x 2 mm, 4-μm particle size, (Phenomenex, Torrance, CA) at an acidic pH with a running time of 50 min.[34] Solvent A was 10 mM tributylamine plus 15 mM acetic acid in 97:3 water-methanol. Solvent B was methanol. The gradient was as follows: *t* = 0, 0% solvent B; *t* = 5 min, 0% solvent B; *t* = 10 min, 20% solvent B; *t* = 20 min, 20% solvent B; *t* = 35 min, 65% solvent B; *t* = 38 min, 95% solvent B; *t* = 42 min, 95% solvent B; *t* = 43 min, 0% solvent B; and *t*

= 50 min, 0% solvent B. Other liquid chromatography parameters were as follows: autosampler temperature, 4°C; column temperature, 25°C; injection volume, 20 µl; and solvent flow rate, 200 µl/min.

The mass spectrometry parameters were as follows: spray voltage, 3500 V in positive ion mode and -3000 V in negative ion mode; nitrogen as sheath gas at 30 arbitrary units and auxiliary gas at 10 arbitrary units; argon as the collision gas at 1.5 mtorr, and capillary temperature of 325°C. The scan time for each SRM was 0.1 s with a scan width of 1 *m/z*. Unlabeled (**6**) and ^{2}H-labeled amino-CAI-1 (d-**6**) were detected using SRM *m/z* 214→67 at 19 eV and *m/z* 216→69 at 19 eV, respectively, in positive ion mode, with a retention time of 4.5 min on Luna NH2 column. Using this method, the limit of detection was found to be 1 ng ml^{-1}. Unlabeled (**1**) and ^{2}H-labeled CAI-1 (d-**1**) were detected using SRM *m/z* 213→155 at 18 eV and *m/z* 215→155 at 18 eV, respectively, in negative ion mode, with a retention time of 42.8 min on Hydro-RP column. The limit of detection was found to be 1 µg ml^{-1}. Note that the limit of detection of CAI-1 (**1**) is substantially higher than that of amino-CAI-1 (**6**), due to its poor ionization efficiency.

Quantification of Coenzyme A Release.

Evolution of Coenzyme A (**7**) was measured by incubating 1 µM CqsA with 25 mM SAB (S-**4**) or RAB (R-**4**), with or without 100 µM dCoA (**5**) at 23°C. Control reactions include incubation of SAB (S-**4**) and dCoA (**5**) with the catalytic mutant protein K236A and the substitution of SAB (S-**4**) with 2-hydroxybutyric acid (**24**). After 2 h, each reaction was terminated by addition of acetonitrile to 30% (v/v). Following centrifugation to remove the precipitated CqsA, Coenzyme A (**7**) present in the supernatants were measured using mass spectrometry. In addition, at various time points after mixing with CqsA, aliquots of the reaction containing 25 mM SAB (S-**4**) and 100 µM dCoA (**5**) were stopped with acetonitrile and centrifuged as above. Levels of Coenzyme A (**7**) and dCoA (**5**) present in the supernatants were measured using mass spectrometry and plotted to follow reaction progress over time.

Synthetic Details.

The synthesis of 3-position analogs **12 - 15** all proceeded through key racemic intermediate **28** (Scheme S1). Starting material CAI-1 (**1**) was synthesized as reported previously.[9] The conversion of racemic CAI-1 (**1)** to the key tosylate **28** proceeded in high yield using tosyl anhydride; using tosyl chloride resulted in high conversion to α-hydroxychloride **12**. However, the direct conversion of **1** to chloride **12** using tosyl chloride in pyridine produced a significant amount (~25%) of isomer 4-chlorotridecan-3-one, which was inseparable by either column chromatography or HPLC (data not shown). Therefore, analog **12** was instead synthesized from tosylate **28** *via* S_N2 reaction with lithium chloride in DMF. This reaction gave only minor (> 5%) isomer formation. Similarly, the S_N2 reaction of tosylate **28** with lithium bromide gave analog **13** with no detectible isomerization product, whereas the direct conversion of racemic CAI-1 **1** to bromide **13** using *N*-bromosuccinimide and triphenylphosphine in DCM produced a significant amount (~25%) of inseparable isomer 4-bromotridecan-3-one (data not shown).

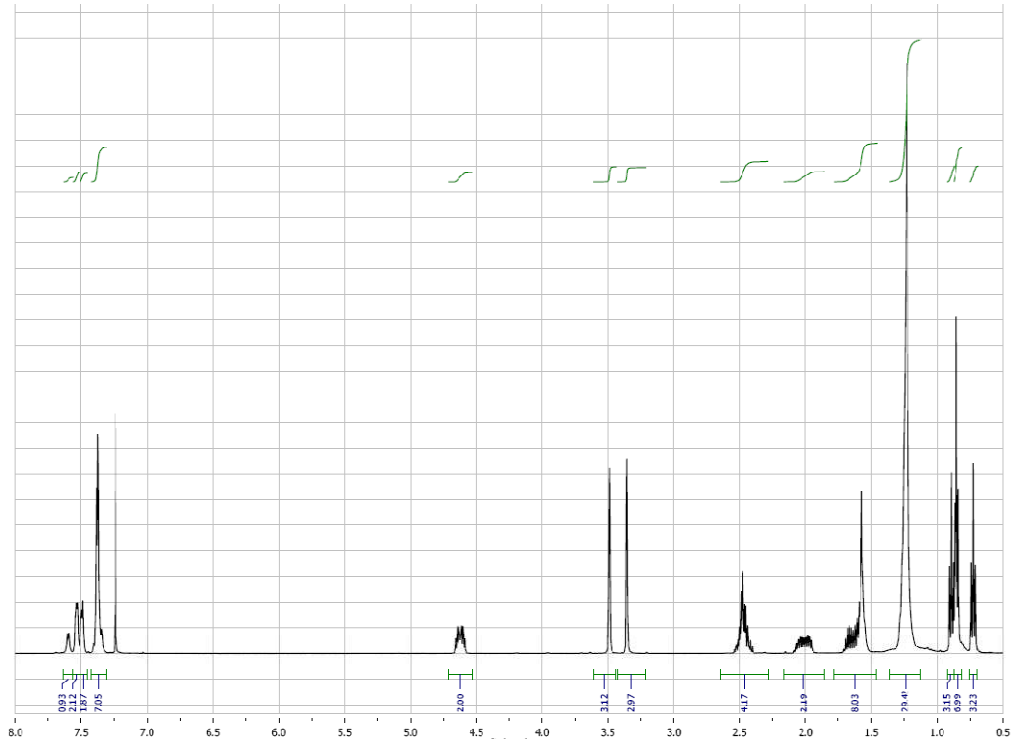
Thiol **14** was also generated from key intermediate tosylate **28** (Scheme S1). Here, tosylate **28** was treated with potassium thioacetate, followed by the removal of the acetate protection with sodium thiomethoxide, to give thiol analog **14**. This thiol was easily oxidized in air to give significant amounts of disulfide compound **14a** in every sample analyzed; a fully reduced sample of thiol **14** could not be maintained. As a result, 25 μM dithiothreitol (DTT, **27**) was added to samples of thiol **14** before assaying.

To synthesize amino analog **6**, the conversion of tosylate **28** to the corresponding azide **29** was followed by hydrogenation with concomitant protection as the *tert*-butoxycarbamate[35] to give compound **30**, as dimerization of the free amine was observed without the use of this protecting strategy (data not shown). Acidic deprotection followed by recrystallization from chloroform gave the hydrochloride salt of 3-amino-CAI-1 **6** as a white crystalline solid (Scheme S1). (3*R*)- and (3*S*)-amino-CAI-1 (R-**6** & S-**6**) were synthesized in homochiral form beginning from enantiopure (*S*)- or (*R*)- CAI-1 (S-**1** or R-**1**). Formation of azide **29** from tosylate **28** proceeds with inversion of configuration; therefore, utilization of (*S*)-CAI-1 (S-**1**) as the starting material gave (*R*)-amino-CAI-1 (R-**6**), and (*R*)-CAI-1 (R-**1**) gave (*S*)-amino-CAI-1 (S-**6**). The enantiopurity of (*R*)-amino-CAI-1 (R-**6**) was determined to be 8:1 by Mosher's method (Scheme S2, Figure S4).

Deuterated CAI-1 (d-**1**) was synthesized by coupling of known dithiane **32**[36] with deuterated propanal and subsequent deprotection[37] (Scheme S3). Conversion of d-**1** to d-**6** followed the synthetic route of Scheme S1.

Oxidation of the secondary hydroxyl group of racemic CAI-1 (**rac-1**) to diketone compound **15** was accomplished by a straightforward PCC oxidation (Scheme S4).

a.



b.

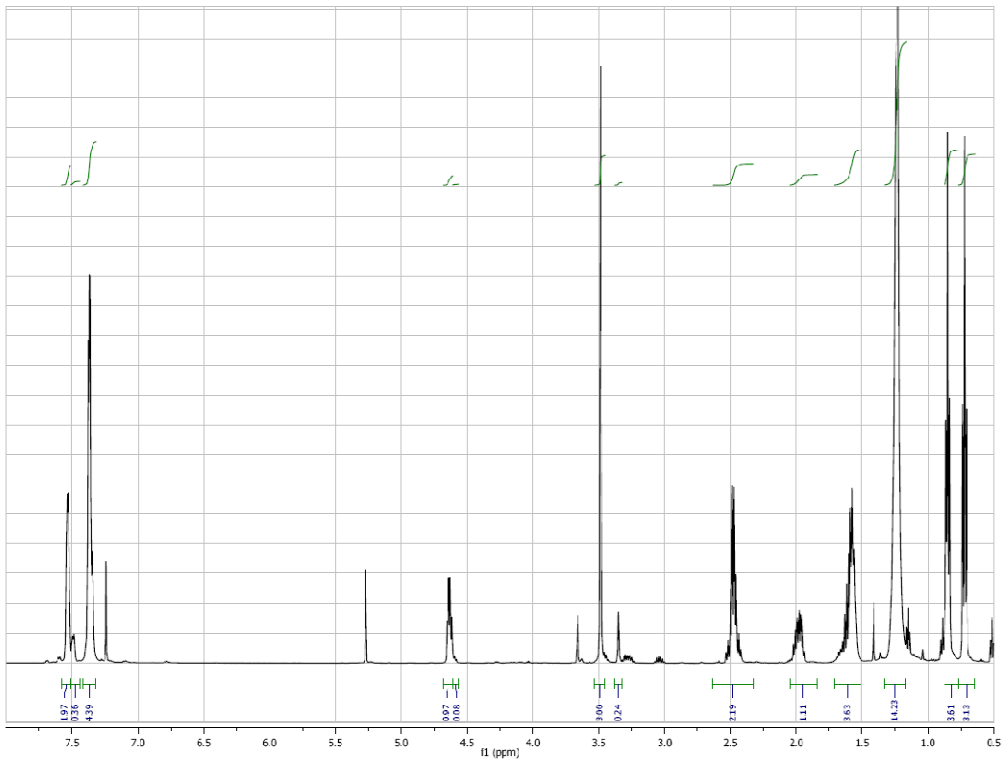
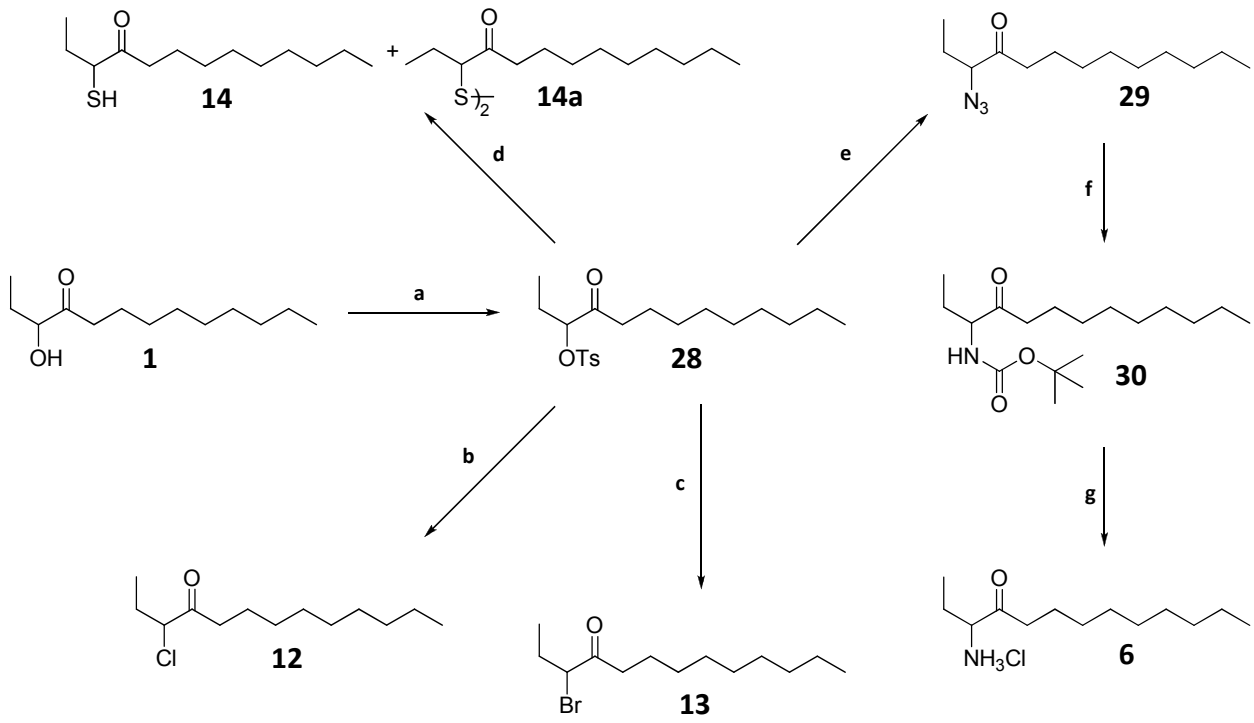
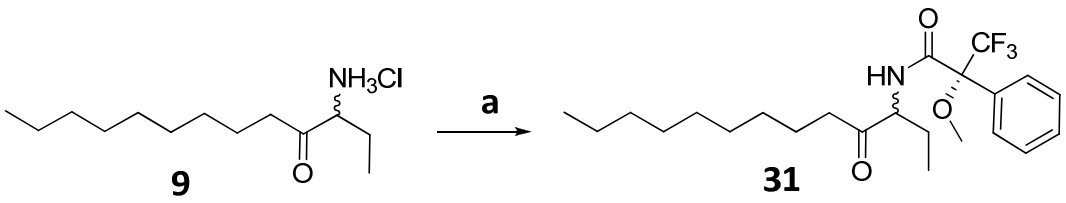


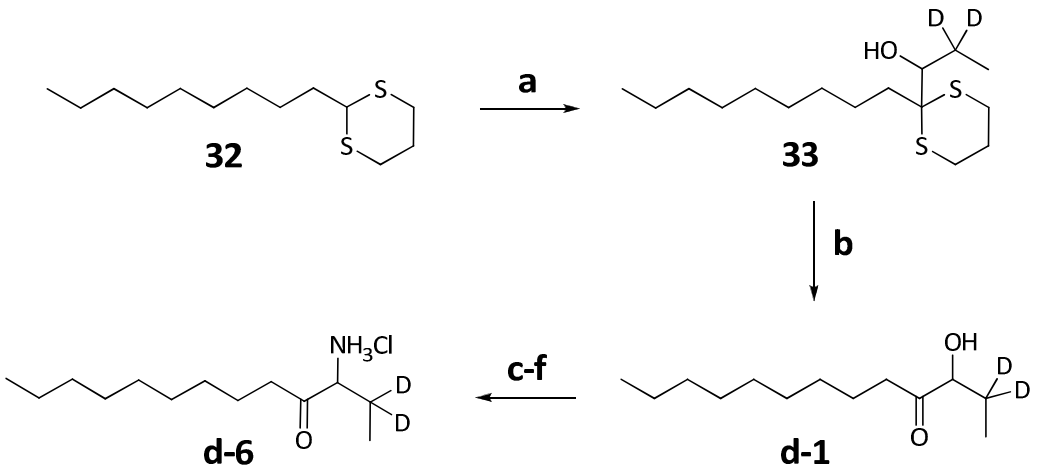
Figure S7 | Determination of Amino-CAI-1 Enantiopurity by Mosher Amide Formation. a, Purified product of Mosher amide formation from racemic CAI-1-NH_3Cl (**6** → **31**, Scheme S2). Integration of methoxy singlets (δ3.49 and δ3.35) shows the expected ~1:1 ratio of diastereomers. **b,** Partially purified product of Mosher amide formation from (*R*)-amino-CAI-1 (R-**6**). Integration of methoxy singlets (δ3.49 and δ3.35) shows an 8:1 mixture of diastereomers.



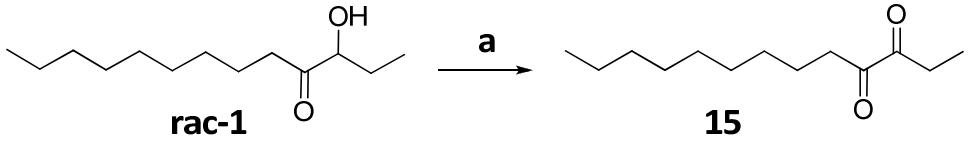
Scheme S1 | Synthesis of Amino-CAI-1 (6) and Chloride (12), Bromide (13), & Thiol (14) Analogs. a.) tosic anhydride, TEA, DCM, 23 °C, 79%; b.) LiCl, DMF, 0 °C, 56%; c.) LiBr, DMF, 0 °C, 93%; d.) KSC(O)Me, EtOH, 0 °C; then NaSMe, MeOH, -15 °C, 64%; e.) NaN_3, DMF, 23 °C, 76%; f.) 5% Pd-C, H_2, Boc_2O, DMF, 23 °C, 46%; g.) 2.0 M HCl in Et_2O, 23 °C; then recrystallization, 45%.



Scheme S2 | Amino-CAI-1 Mosher Amide Formation. a.) TEA, (+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride, DCM, 0 °C, 47%.



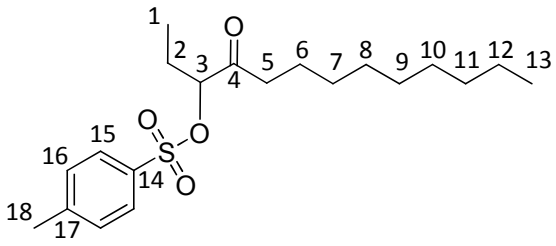
Scheme S3 | Synthesis of Deuterated CAI-1 (d-1) and Deuterated Amino-CAI-1 (d-6). a.) *n*BuLi, -20 ^{o}C, THF; then propionaldehyde-2,2-D_2, -78 ^{o}C, THF, 73%; b.) NCS, $AgNO_3$, 80% aqueous MeCN, 23 ^{o}C, 51%; c.) tosic anhydride, TEA, DCM, 23 ^{o}C, 89%; d.) NaN_3, DMF, 23 ^{o}C, 90%; e.) 5% Pd-C, H_2, Boc_2O, DMF, 23 ^{o}C, 61%; f.) 2.0 M HCl in Et_2O, 23 ^{o}C, then recrystallization, 37%.



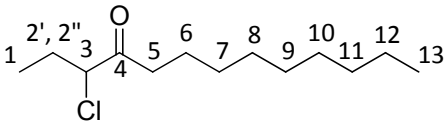
Scheme S4 | Synthesis of Diketone Analog 15. a.) PCC, DCM, reflux, 49%.

Chemical Synthesis.

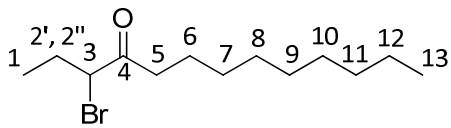
All chemicals were purchased from commercial vendors and used without further purification. Propionaldehyde-2,2-D_2 was purchased from Medical Isotopes, Inc. Unless otherwise noted, all reactions were performed in flame-dried glassware under an atmosphere of argon using dried reagents and solvents. Flash chromatography was performed using standard grade silica gel 60 230-400 mesh from SORBENT Technologies. Thin layer chromatography was carried out using Silica G TLC plates, 200 µm with UV254 (SORBENT Technologies), and visualization was performed by staining (potassium permanganante) and/or by absorbance of UV light. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Avance II (500 MHz) spectrometer. Chemical shifts for ^{1}H and ^{13}C NMR spectra were calibrated according to a $CHCl_3$ internal standard. Infrared spectra of oils dissolved in chloroform and analyzed in an NaCl cell were taken using a Nicolet 730 FT-IR. Infrared spectra of solids were taken using a Nicolet 6700 Smart Orbit IR. Optical rotations were measured in chloroform using a PerkinElmer Model 341 polarimeter at 20 ^{o}C and 589 nm. HRMS analysis was performed using an Aglient Technologies 6220 series Elecrospray/Time-of-Flight spectrometer. EIMS analysis was performed by Dr. John Eng, Princeton University.



Compound 28; 4-oxotridecan-3-yl-4-methyl-benzenesulfonate. Triethylamine (1.5 mL, 11 mmol) was added to a solution of CAI-1 **1** (1.15 g, 5.37 mmol) in DCM (20 mL) at 0 °C. After addition of *p*-toluenesulfonic anhydride (3.49 g, 10.7 mmol), the reaction was warmed to 23 °C and monitored by TLC (20% EtOAc in hexanes). Upon completion, the reaction mixture was diluted to 200 mL DCM and washed with 3 x 50 mL water. The organic solution was then dried over magnesium sulfate, filtered, concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.55) to give **28** (1.56 g, 4.23 mmol, 79%). (*S*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) = -40.9 (*c* = 0.047 g cm^{-3} in $CHCl_3$); (*R*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) = +29.1 (*c* = 0.0019 g cm^{-3} in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.78 (t, *J* = 7.37 Hz, 3H, C13), 0.86 (t, *J* = 6.90 Hz, 3H, C1), 1.26 (m, 12H, C7 – C12), 1.47 (dd, *J* = 14.4, 7.10 Hz, 2H, C6), 1.70 (m, 2H, C2), 2.43 (s, 3H, C18), 2.48 (t, J = 7.31 Hz, 2H, C5), 4.58 (dd, *J* = 7.18, 5.23 Hz, 1H, C3), 7.34 (d, *J* = 8.40 Hz, 2H, C16), 7.78 (d, *J* = 8.21 Hz, 2H, C15); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.3 (C1), 14.4 (C13), 21.9 (C18), 22.89 (C12), 22.94 (C2), 25.4 (C10), 29.2 (C9), 29.5 (C8), 29.60 (C7), 29.63 (C6), 32.1 (C11), 38.5 (C5), 85.8 (C3), 128.2 (C15), 130.2 (C16), 133.2 (C17), 145.5 (C14), 207.7 (C4); IR ($CHCl_3$): 2931, 2855, 1720, 1599, 1369 cm^{-1}. HRMS (*m/z*): $[M]^+$ calcd for $C_{20}H_{32}O_4S$, 368.20213; found: 368.20201.

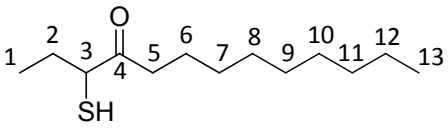


Compound 12; 3-chlorotridecan-4-one. To a solution of tosylate **28** (0.117 g, 0.317 mmol) in DMF (0.5 mL) at 0 °C was added lithium chloride (0.031 g, 0.73 mmol). The reaction mixture monitored by TLC (20% EtOAc in hexanes). Upon completion, the reaction mixture was quenched with water (1 mL) and extracted with DCM (3 x 10 mL). The organic layers were combined and washed with 5 x 10 mL water, then dried over magnesium sulfate, filtered, and concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.79) to give **12** (0.041 g, 0.18 mmol, 56%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.89 Hz, 3H, C13), 1.00 (t, *J* = 7.34 Hz, 3H, C1), 1.25 (m, 12H, C7 – C12), 1.57 (m, 2H, C6), 1.85 (m, 1H, C2'), 1.98 (m, 1H, C2"), 2.62 (t, *J* = 7.37 Hz, 2H, C5), 4.13 (dd, *J* = 8.10 , 5.59 Hz, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.7 (C1), 13.1 (C13), 21.6 (C12), 22.6 (C2), 26.2 (C6), 28.1 (C7), 28.2 (C8), 28.35 (C9), 28.39 (C10), 30.8 (C11), 37.7 (C5), 64.3 (C3), 204.8 (C4); IR ($CHCl_3$): 2961, 2930, 2854, 1717, 1462 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{25}ClO$, 232.15939; found: 232.15931.

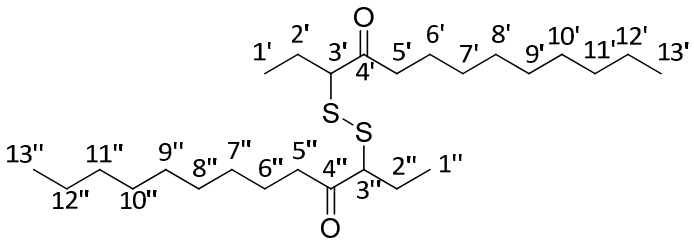


Compound 13; 3-bromotridecan-4-one. To a solution of tosylate **28** (0.111 g, 0.301 mmol) in DMF (0.5 mL) at 0 °C was added lithium bromide (0.054 g, 0.62 mmol). The reaction mixture monitored by TLC (20% EtOAc in hexanes). Upon completion, the reaction mixture was quenched with water (1 mL) and extracted with DCM (3 x 10 mL). The organic layers were combined and washed with 5 x 10 mL water, then dried over magnesium sulfate, filtered, and concentrated, and purified by silica gel chromatography (2.5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.78) to give **13** (0.065 g, 0.28 mmol, 93%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.91 Hz, 3H, C13), 0.98, (t, *J* =

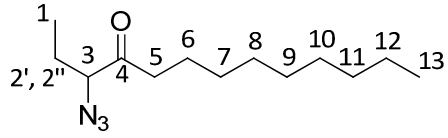
7.31 Hz, 3H, C1), 1.26 (m, 12H, C7 – C12), 1.58 (dq, *J* = 13.96, 7.01, 6.93 Hz, 2H, C6), 1.91 (m, 1H, C2'), 2.02 (m, 1H, C2"), 2.64 (qt, *J* = 17.14, 7.35 Hz, 2H, C5), 4.16 (dd, *J* = 8.00, 6.36 Hz, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 12.2 (C1), 14.3 (C13), 22.9 (C12), 24.1 (C2), 27.1 (C7), 29.3 (C8), 29.47 (C9), 29.58 (C10), 29.62 (C11), 32.1 (C6), 39.3 (C5), 55.7 (C3), 204.7 (C4); IR ($CHCl_3$): 2960, 2929, 2856, 1714, 1364 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{25}BrO$, 276.10888; found: 276.10888.



Compound 14; 3-mercaptotridecan-4-one. To a solution of tosylate **28** (0.208 g, 0.654 mmol) in anhydrous EtOH (5 mL) at 0 °C was added potassium thioacetate (0.330 g, 2.89 mmol). The reaction mixture monitored by TLC (20% EtOAc in hexanes). Upon completion, the reaction mixture was evaporated to dryness and taken up in DCM (25 mL). The organic layer was washed with 3 x 35 mL water, then dried over magnesium sulfate, filtered, and concentrated. The crude acetate was then dissolved in MeOH (5 mL) at -15 °C. Sodium thiomethoxide (0.198 g, 2.82 mmol) was added in one portion. After 30 min, the mixture was poured onto 0.1 M HCl (10 mL) and extracted with DCM (3 x 25 mL). The organic layers were combined, dried over magnesium sulfate, filtered, and concentrated, and purified by silica gel chromatography (25% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.75) to routinely give mixtures of **14** and disulfide **14a** below (0.083 g, 0.36 mmol, 66%). ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 0.85 (t, *J* = 6.93 Hz, 6H), 0.94 (m, 6H), 1.25 (m, 24H), 1.58 (m, 4H), 1.68 (m, 2H), 1.84 (m, 1H), 1.91 (m, 1H), 2.51 (m, 2H), 2.63 (m, 2H), 3.19 (dt, *J* = 10.40, 7.20 Hz, 1H), 3.27 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 12.0 (C1', C1''), 12.1 (C13', C13''), 12.2 (C1), 14.3 (C13), 22.9 (C12), 23.4 (C12'), 23.6 (C12''), 24.00 (C11'), 24.03 (C11''), 24.2 (C11), 27.9 (C10), 29.3 – 29.7 (multiple unresolved signals, C2, C6, C7, C8, C9, C2', C6', C7', C8', C9', C2'', C6'', C7'', C8'', C9''), 40.37 (C5), 40.42 (C5'), 40.6 (C5''), 49.0 (C3), 60.1 (C3'), 61.0 (C3''), 207.0 (C4'), 207.2 (C4''), 208.6 (C4); IR ($CHCl_3$): 2964, 2854, 2570, 1708, 1460 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{26}OS$, 230.17044; found: 230.17074; $[M]^+$ calcd for $C_{26}H_{50}O_2S_2$, 458.32522; found: 458.32569.

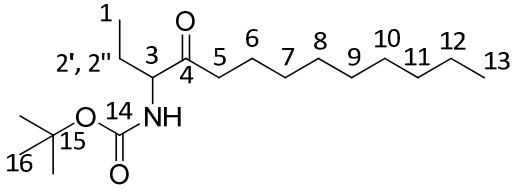


Compound 14a; 3,3'-disulfane-diylditridecan-4-one. Routinely isolated with compound **14**. See above for NMR chemical shift assignments.

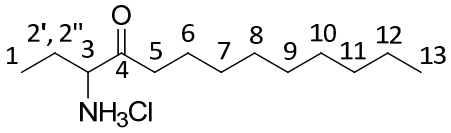


Compound 29; 3-azidotridecan-4-one. To a solution of tosylate **28** (1.5 g, 4.1 mmol) in DMF (7 mL) at 0 °C was added sodium azide (0.59 g, 9.0 mmol). The solution was warmed to 23 °C and monitored by TLC (20% EtOAc in hexanes). Upon completion, the reaction was quenched by addition of 10 mL water, extracted with 3 x 50 mL DCM, and washed five times with 50 mL H_2O. The organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.74) to give **29** (0.745 g, 3.11 mmol, 76%). Compound **29** routinely co-purified with ~10% of isomer 4-azido-3-tridecanone. (*S*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) =

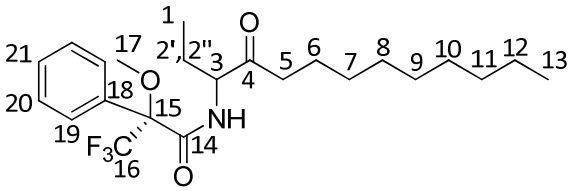
+18.1 (*c* = 0.006 g cm^{-3} in $CHCl_3$); (*R*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) = -25.5 (*c* = 0.012 g cm^{-3} in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 0.84 (t, *J* = 6.95 Hz, 3H, C13), 0.95 (t, *J* = 7.40 Hz, 3H, C1), 1.16 (m, 12H, C7 – C12), 1.55 (m, 2H, C6), 1.69 (tt, *J* = 14.70, 7.42 Hz, 1H, C2'), 1.82 (m, 1H, C2"), 2.47 (m, 2H, C5), 3.72 (dd, *J* = 8.22, 4.99 Hz, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 10.5 (C1), 14.3 (C13), 22.9 (C12), 23.5 (C2), 24.4 (C11), 29.3 (C10), 29.4 (C9), 29.5 (C8), 29.6 (C7), 32.0 (C6), 39.8 (C5), 69.6 (C3), 207.9 (C4); IR ($CHCl_3$): 2927, 2856, 2107, 1719, 1211 cm^{-1}; EIMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{25}N_3O$, 239; found: 155 (C10 acyl fragment), 104, 95, 85, 71.



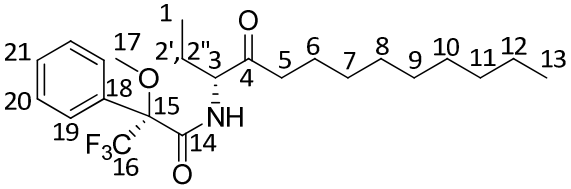
Compound 30; *tert*-butyl-4-oxotridecan-3-yl-carbamate. A solution of azide **29** (0.402 g, 1.68 mmol) and di-*tert*-butyl dicarbonate (1.24 g, 5.66 mmol) in DMF (5 mL) was added to 5% Pd-C suspended in 13 mL DMF under an H_2 atmosphere at 23 ^{o}C and stirred under H_2 for 48 hours. The reaction mixture was then filtered through Celite, diluted with 150 mL hexanes, and washed with 3 x 100 mL brine. The organic layer was then dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.54) to give **30** (0.240 g, 0.767 mmol, 46%). (*S*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) = +12.0 (*c* = 0.047 g cm^{-3} in $CHCl_3$); (*R*)-$[\alpha]_D^{20}$ (deg cm^3 g^{-1} dm^{-1}) = -30.9 (*c* = 0.012 g cm^{-3} in $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 0.85 (t, *J* = 6.83 Hz, 6H, C1 & C13), 1.15 (m, 12H, C7 – C12), 1.36 (s, 9H, C16), 1.50 (m, 3H, C2' & C6), 1.80 (m, 1H, C2"), 2.32 (m, 2H, C5), 4.19 (dd, *J* = 11.98, 6.96, 1H, C3), 5.11 (d, *J* = 7.25 Hz, 1H, -NH); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 9.4 (C1), 14.3 (C13), 22.9 (C12), 23.7 (C2), 25.0 (C10), 28.5 (C16), 29.40 (C9), 29.47 (C8), 29.58 (C7), 29.62 (C6), 32.1 (C11), 40.0 (C5), 60.3 (C3), 78.9 (15), 155.7 (C14), 209.0 (C4); IR ($CHCl_3$): 3431, 2929, 2856, 1703, 1499, 1368 cm^{-1}. HRMS (*m/z*): $[M]^+$ calcd for $C_{14}H_{27}NO_3$ (-^{t}Bu), 257.19909; found: 257.19904.



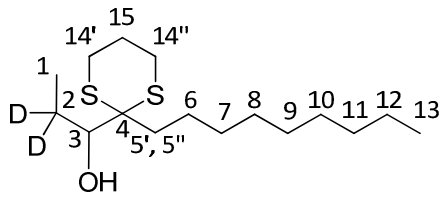
Compound 6; 3-aminotridecan-4-one hydrochloride. 5 mL (1 mmol) 2.0 M HCl in Et_2O was added in one portion to protected α-aminoketone **30** (0.23 g, 0.73 mmol) at 23 ^{o}C and stirred overnight. The reaction mixture was then concentrated to dryness. The white solid was dissolved in minimal warm $CHCl_3$ and recrystallized at 0 ^{o}C. The recovered white solid **6** was dried using aspiration (0.82 g, 3.3 mmol, 45%). Enantiopurity was determined to be 8:1 by Mosher's method (Scheme S.2, Figure S.1, compounds **31** and **(*R, R*)-31**). ^{1}H NMR (500 MHz, D_2O): *δ* 0.79 (t, *J* = 6.77 Hz, 3H, C13), 0.84 (t, *J* = 7.54 Hz, 3H, C1), 1.12 (m, 12H, C7 – C12), 1.43 (m, 2H, C6), 1.87 (dt, *J* = 15.09, 7.47 Hz, 1H, C2'), 1.97 (ddd, *J* = 15.13, 7.57, 4.45 Hz, 1H, C2"), 2.49 (m, 2H, C5), 4.11 (dd, *J* = 7.11, 4.41 Hz, 1H, C3); ^{13}C-NMR (125 MHz, DMSO-d_6): *δ* 8.9 (C1), 14.1 (C13), 22.2 (C12), 22.5 (C2), 22.8 (C10), 28.5 (C9), 28.7 (C8), 28.89 (C7), 28.95 (C6), 31.4 (C11), 38.3 (C5), 58.9 (C3), 207.0 (C4); IR (solid): 2955, 2915, 1725, 1526, 1471 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{27}NO$ (-HCl), 213.20926; found 213.20916.



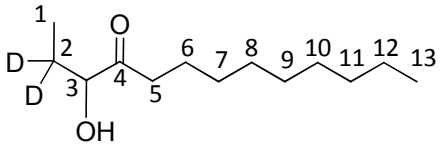
Compound 31; (2*R*)-3,3,3-trifluoro-2-methoxy-*N*-(4-oxotridecan-3-yl)-2-phenylpropanamide. To a solution of compound **6** (0.108 g, 0.431 mmol) in 10 mL DCM was added triethylamine (0.2 g, 0.3 mL, 2 mmol) and (+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (0.16 g, 0.12 mL, 0.65 mmol) each in one portion at 0 °C. The reaction mixture was warmed to 23 °C and stirred overnight. The reaction was then quenched with 10 mL H_2O at 0 °C and extracted with 3 x 25 mL DCM. The organic layer was washed with 3 x 25 mL saturated $NaHCO_3$ (aq), dried over magnesium sulfate, filtered, concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.54) to give Mosher amide **31** (0.016 g, 0.004 mmol, 47%) as an approximately 1:1 mixture of diastereomers (Figure S.1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (t, J = 7.45 Hz, 3H, C13(A)), 0.81 (t, J = 6.92 Hz, 6H, C1(B) & C13(B)), 0.87 (t, J = 7.46 Hz, 3H, C1(A)), 1.23 (m, 24H, C7(A) – C12(A) & C7(B) – C12(B)), 1.58 (m, 5H, C2'(A) & C6(A) and C2'(B) & C2"(B)), 1.65 (m, 2H, C6(B)), 2.01 (m, 2H, C2"(A) & C5(B)), 2.48 (m, 4H, C5(A)), 3.36 (s, 3H, C17(B)), 3.49 (s, 3H, C17(A)), 4.61 (m, 1H, C3(B)), 4.64 (m, 1H, C3(A)), 7.38 (m, 6H, C19(A) & C21(A) and C19(B) & C21(B)), 7.49 (m, 2H, C20(B)), 7.54 (m, 2H, C20(A)); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.35 (C1(A)), 9.43 (C1(B)), 14.3 (C13(A) & C13(B)), 22.9 (C12(A) & C12(B)), 23.63 (C2(A)), 23.64 (C2(B)), 24.51 (C10(A)), 24.54 (C10(B)), 29.35 (C9(A)), 29.36 (C9(B)), 29.45 (C8(A) & C8(B)), 29.56 (C7(A) & C7(B)), 29.59 (C6(A)), 29.60 (C6(B)), 29.9 (C11(B)), 32.1 (C11(A)), 40.0 (C5(A)), 40.1 (C5(B)), 55.1 (C3(B)), 55.5 (C3(A)), 59.0 (C17(A)), 59.3 (C17(B)), 122.6 (C15(A)), 122.9 (C15(B)), 125.0 (C18(A)), 125.2 (C18(B)), 127.5 (C19(A)), 128.1 (C19(B)), 128.7 (C20(A)), 128.8 (C20(B)), 129.69 (C21(A)), 129.70 (C21(B)), 132.1 (C16(B)), 133.2 (C16(A)), 166.3 (C14(B)), 166.4 (C14(A)), 208.6 (C4(A)), 208.7 (C4(B)); IR (solid): 3402, 2930, 2855, 1832, 1689 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{23}H_{34}F_3NO_3$, 429.24908; found 429.24933.



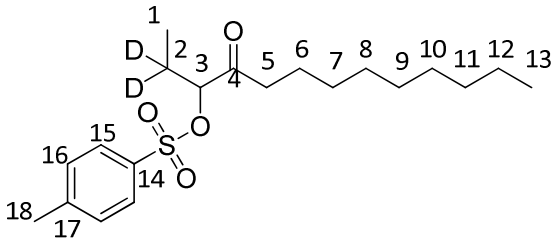
C**ompound (*R,R*)-31; (*R*)-3,3,3-trifluoro-2-methoxy-*N*-((*R*)-4-oxotridecan-3-yl)-2-phenylpropanamide.** Synthesis and purification followed that for compound **31** using (*R*)-CAI-1-NH_3Cl (R-**1**) as the substrate to give **(*R,R*)-31** in 8-fold diastereomeric excess (Figure S.1). Purification by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.54) enabled separation of **(*R,R*)-31** from the minor diastereomer **(*R,S*)-31**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (t, J = 7.46 Hz, 3H, C13), 0.85 (t, J = 6.90 Hz, 3H, C1), 1.23 (m, 12H, C7 – C12), 1.60 (m, 3H, C2' & C6), 1.98 (m, 1H, C2"), 2.48 (m, 2H, C5), 3.49 (s, 3H, C17), 4.64 (dd, J = 11.94, 7.13 Hz, 1H, C3), 7.37 (m, 3H, C19 & C21), 7.53 (m, 2H, C20); ^{19}F-NMR (300 MHz, $CDCl_3$): δ -69.1; ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.4 (C1), 14.3 (C13), 22.9 (C12), 23.6 (C2), 24.5 (C10), 29.35 (C9), 29.49 (C8), 29.56 (C7), 29.59 (C6), 32.1 (C11), 40.0 (C5), 55.5 (C3), 59.0 (C17), 122.6 (C15), 125.0 (C18), 127.5 (C19), 128.7 (C20), 129.7 (C21), 133.2 (C16), 166.4 (C14), 208.6 (C4); IR (solid): 3402, 2930, 2855, 1832, 1689 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{23}H_{34}F_3NO_3$, 429.24908; found 429.24933.



Compound 33; 1-(2-nonyl-1,3-dithian-2-yl)propan-1-ol-2,2-D_2. To a solution of known dithiane **32** (5.0 g, 20 mmol) in THF (150 mL) at -20 °C was added *n*-butyllithium (2.5 M in hexanes, 10 mL, 25 mmol) dropwise. The anion was stirred at -20 °C for 4 hours. After cooling to -78 °C, a solution of propionaldehyde-2,2-D_2 (1.0 g, 1.25 mL, 17.2 mmol) in THF (10 mL) was added dropwise. The reaction was allowed to slowly reach 23 °C stirring overnight and was then poured onto 500 mL ice water, extracted with 3 x 250 mL DCM, washed once each with 100 mL cold 2 N KOH and brine. The organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (2.5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.59) to give **33** (3.84 g, 12.6 mmol, 73%) and 1.4 g unreacted dithiane **11**. ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 0.83 (t, *J* = 6.97 Hz, 3H, C13), 1.04 (s, 3H, C1), 1.13 (m, 12H, C7 – C12), 1.45 (m, 3H, C5' & C6), 1.79 (m, 2H, C15), 2.02 (dqd, *J* = 10.46, 5.21, 2.86 Hz, 1H, C5"), 2.58 (m, 2H, C14'), 2.69 (d, J = 1.67 Hz, 1H, -OH), 2.95 (m, 2H, C14"), 3.84 (s, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 12.4 (C1), 14.3 (C13), 22.9 (C12), 24.69 (C15), 24.73 (C14'), 25.2 (C14"), 26.2 (C11), 29.5 (C10), 29.6 (C9), 29.7 (C8), 30.3 (C7), 32.0 (C6), 34.6 (C5), 59.4 (C4), 73.4 (C3); IR ($CHCl_3$): 3471, 2905, 2853, 2124 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{16}H_{30}D_2OS_2$, 306.20201; found: 306.20193.

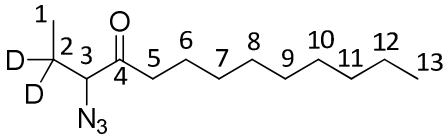


Compound d-1; 3-hydroxytridecan-4-one-2,2-D_2. To a solution of *N*-chlorosuccinimide (7.69 g, 57.6 mmol) and silver(I) nitrate (10.9 g, 64.1 mmol) in 300 mL 80% aqueous MeCN at 0 °C was added a solution of protected α-hydroxy-ketone **33** (3.84 g, 12.6 mmol) in 20 mL MeCN in one portion. The reaction was stirred for 30 minutes and then quenched sequentially with 40 mL each of saturated Na_2SO_3 (aq), saturated $NaHCO_3$ (aq), and saturated NaCl (aq), stirring ~1 min in between each. The reaction mixture was then poured onto a solution of 1:1 DCM: hexanes (800 mL) and filtered through silica gel. The recovered organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography (5% EtOAc in hexanes; R_f (20% EtOAc in hexanes) = 0.47) to give **d-1** (1.4 g, 6.4 mmol, 51%). ^{1}H-NMR (500 MHz, $CDCl_3$): *δ* 0.84 (t, *J* = 6.84 Hz, 3H, C13), 0.87 (s, 3H, C1), 1.23 (m, 12 H, C7 – C12), 1.59 (m, 2H, C6), 2.42 (m, 2H, C5), 3.41 (d, *J* = 4.67 Hz, 1H, -OH), 4.05 (d, *J* = 4.28 Hz, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): *δ* 8.8 (C1), 14.3 (C13), 22.8 (C12), 23.8 (C11), 29.40 (C10), 29.42 (C9), 29.53 (C8), 29.57 (C7), 32.0 (C6), 38.0 (C5), 77.2 (C3), 212.7 (C4); IR ($CHCl_3$): 3440, 2928, 2856, 1708, 1215 cm^{-1}. HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{24}D_2O_2$, 216.20583; found: 216.20595.

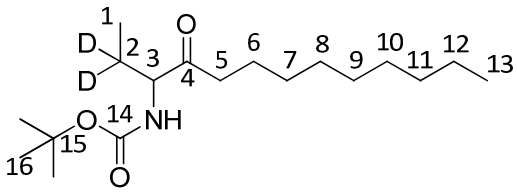


Compound d-28; 4-oxotridecan-3-yl-4-methyl-benzenesulfonate-2,2-D_2. Synthesis and purification followed that described for compound **28** to give deuterated tosylate **d-28** (1.65 g, 4.46 mmol, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ* 0.76 (s, 3H, C1), 0.82 (t, *J* = 6.95 Hz, 3H, C13), 1.11 (m, 12H, C7 – C12), 1.40 (m, 2H, C6), 2.38 (s, 3H, C18), 2.46 (t, *J* = 7.44 Hz, 2H, C5), 4.52 (s, 1H, C3), 7.29 (d, *J* = 8.12 Hz, 2H, C16), 7.68 (*J* =

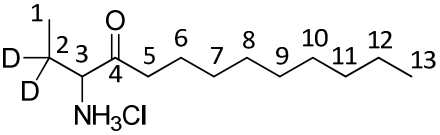
8.28 Hz, 2H, C15); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.1 (C1), 14.4 (C13), 21.9 (C18), 22.89 (C12), 22.93 (C10), 29.2 (C9), 29.5 (C8), 29.60 (C7), 29.63 (C6), 32.1 (C11), 38.5 (C5), 85.7 (C3), 128.2 (C15), 130.2 (C16), 133.2 (C17), 145.5 (C14), 207.7 (C4); IR ($CHCl_3$): 2853, 1720, 1599, 1460, 1365 cm^{-1}. HRMS (*m/z*): $[M]^+$ calcd for $C_{20}H_{30}D_2O_4S$, 370.21468; found: 370.21505.



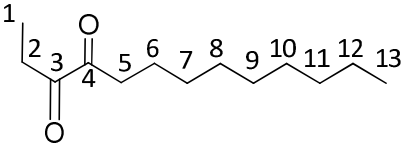
Compound d-29; 3-azidotridecan-4-one-2,2-D$_2$. Synthesis and purification followed that described for compound **29** to give deuterated azide **d-29** (0.926 g, 3.83 mmol, 90%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.83 Hz, 3H, C13), 0.91 (s, 3H, C1), 1.15 (m, 12H, C7 – C12), 1.57 (dd, *J* = 14.74, 8.54 Hz, 2H, C6), 2.34 (m, 2H, C5), 3.66 (s, 1H, C3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 10.3 (C1), 14.3 (C13), 22.9 (C12), 23.5 (C11), 29.3 (C10), 29.45 (C9), 29.58 (C8), 29.60 (C7), 32.1 (C6), 39.9 (C5), 69.5 (C3), 208.0 (C4); IR ($CHCl_3$): 2924, 2855, 2111, 1719 cm^{-1}; EIMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{23}D_2NO$ (-N_2), 215; found: 215, 155, 129, 95, 85, 71.



Compound d-30; *tert*-butyl-4-oxotridecan-3-yl-carbamate-2,2-D$_2$. Synthesis and purification followed that for compound **30** to give **d-30** (0.721 g, 2.28 mmol, 61%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.83 (s, 3H, C1), 0.85 (t, *J* = 7.01 Hz, 3H, C13), 1.21 (m, 12H, C7 – C12), 1.34 (s, 9H, C16), 1.47 (m, 2H, C6), 2.33 (m, 2H, C5), 4.16 (d, *J* = 7.57 Hz, 1H, C3), 5.15 (d, *J* = 7.34 Hz, 1H, -NH); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 9.2 (C1), 14.3 (C13), 22.9 (C12), 23.7 (C10), 28.5 (C16), 29.41 (C9), 29.47 (C8), 29.59 (C7), 29.62 (C6), 32.1 (C11), 40.0 (C5), 60.2 (C3), 79.8 (C15), 155.6 (C14), 209.8 (C4); IR ($CHCl_3$): 3432, 2929, 2857, 1703, 1497 cm^{-1}. HRMS (*m/z*): $[M]^+$ calcd for $C_{14}H_{25}D_2NO_3$ (-^{t}Bu), 259.21165; found: 259.21195.



Compound d-6; 3-aminotridecan-4-one-2,2-D$_2$ hydro-chloride. Synthesis and purification followed that for compound **6** to give deuterated CAI-1-NH_3Cl **d-6**. (0.082 g, 0.330 mmol, 45%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.85 (m, 6H, C1 & C13), 1.24 (m, 12H, C7 – C12), 1.48 (m, 2H, C6), 2.57 (td, *J* = 7.10, 2.57 Hz, 2H, C5), 4.09 (s, 1H, C3), 8.17 (s, 3H, -NH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 8.9 (C1), 14.1 (C13), 22.2 (C12), 22.5 (C10), 22.8 (C9), 28.5 (C8), 28.7 (C7), 28.9 (C6), 29.0 (C11), 31.4 (C5), 38.3 (C3), 58.9 (C4); IR (solid): 2914, 2847, 1724, 1525, 1470 cm^{-1}; HRMS (*m/z*): $[M]^+$ calcd for $C_{13}H_{25}D_2NO$ (-HCl), 215.22182; found 215.22187.



Compound 15; tridecan-3,4-dione. To a solution of pyridinium chlorochromate (0.82 g, 3.8 mmol) in DCM (3 mL) at 0 ^{o}C was added a solution of racemic CAI-1 **1** (0.16 g, 0.75 mmol) dropwise over 5 min. The solution was then heated to reflux and monitored by TLC (20% EtOAc in hexanes). Upon completion, 20 mL Et_2O was added and the supernatant was recovered. The residual black gum was rinsed with Et_2O. The organic layers were then combined and filtered through a plug of silica gel, which was

thoroughly rinsed with ether. Diketone **15** was routinely used without further purification (0.078 g, 0.37 mmol, 49%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.85 (t, J = 6.9 Hz, 3H, C13), 1.05 (t, J = 7.3 Hz, 3H, C1), 1.24 (m, 12H, C7 – C12), 1.54 (m, 2H, C6), 2.72 (dt, J = 20.0, 7.3 Hz, 4H, C2 & C5); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 7.1 (C1), 14.3 (C13), 22.9 (C12), 23.2 (C11), 29.3 (C10), 29.5 (C9), 29.6 (C8), 29.8 (C7), 29.9 (C2), 32.1 (C6), 36.3 (C5), 200.3 (C4), 200.6 (C3); IR ($CHCl_3$): 2960, 2855, 1713, 1466, 667 cm^{-1}; HRMS (m/z): $[M]^+$ calcd for $C_{13}H_{24}O_2$, 212.17763; found: 212.17768.

D. Supplementary References

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27. Faruque, S.M. & Nair, G.B. Molecular ecology of toxigenic Vibrio cholerae. *Microbiology and Immunology* **46**, 59-66 (2002).
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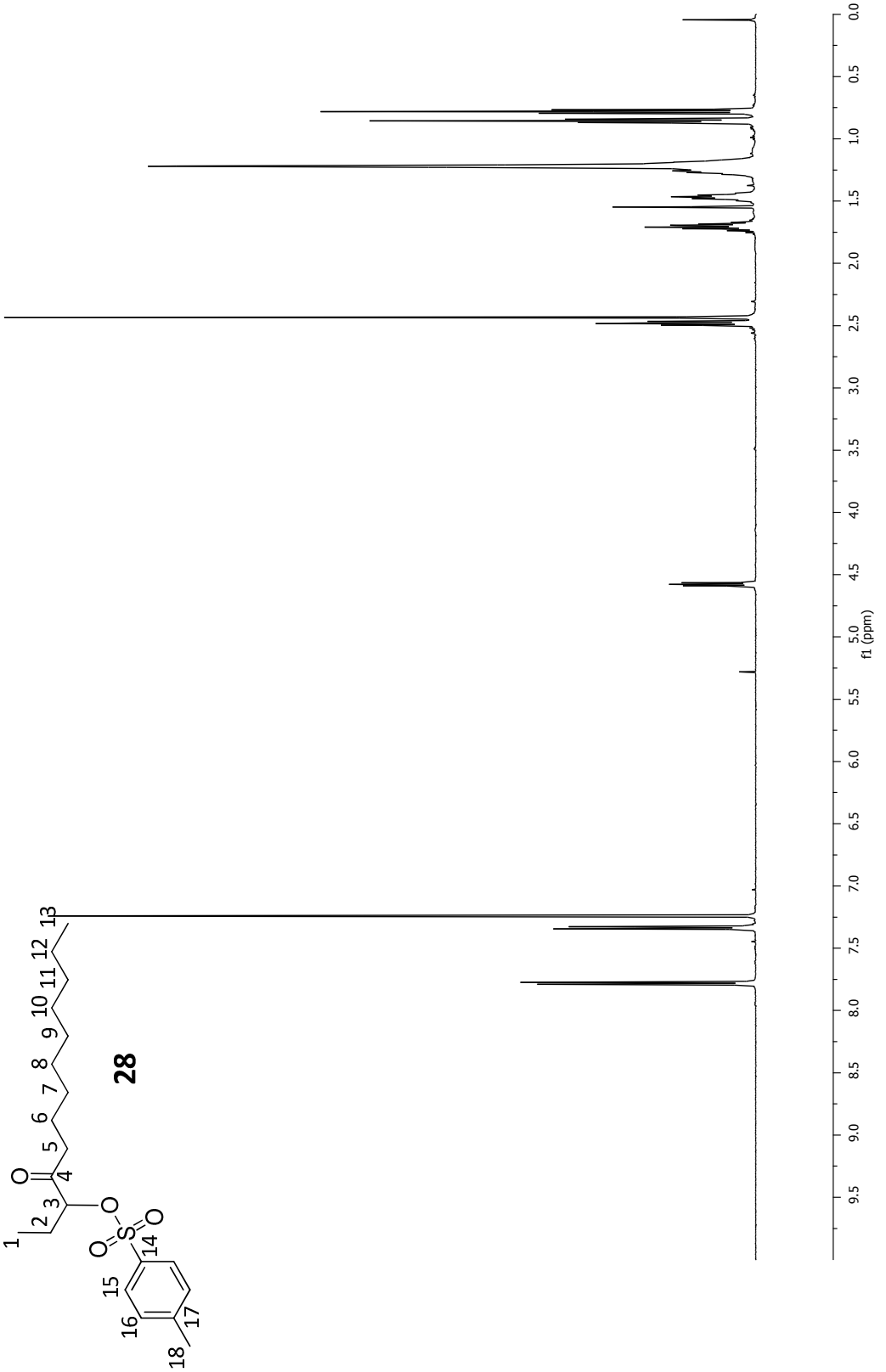
E. Supplementary Spectra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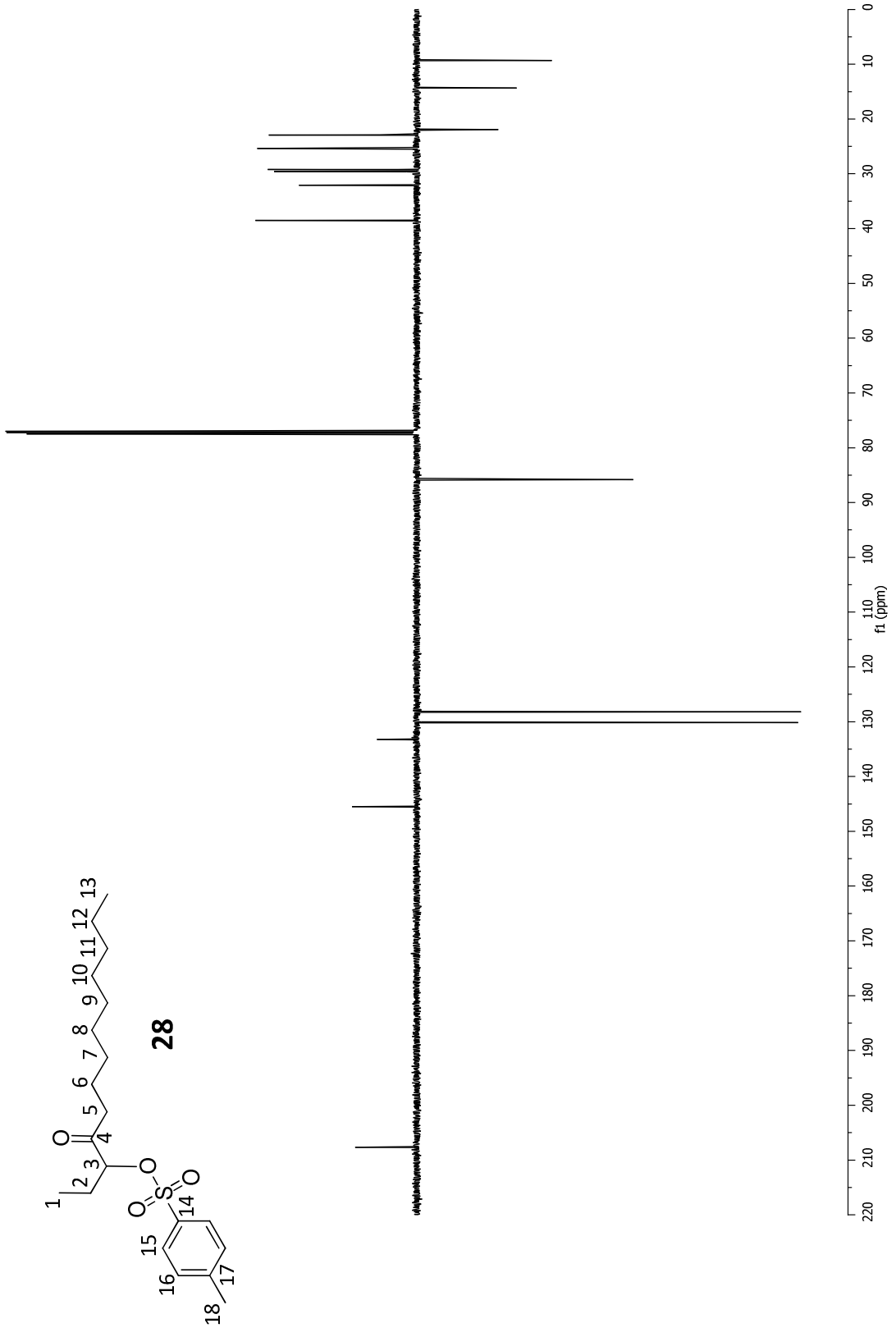
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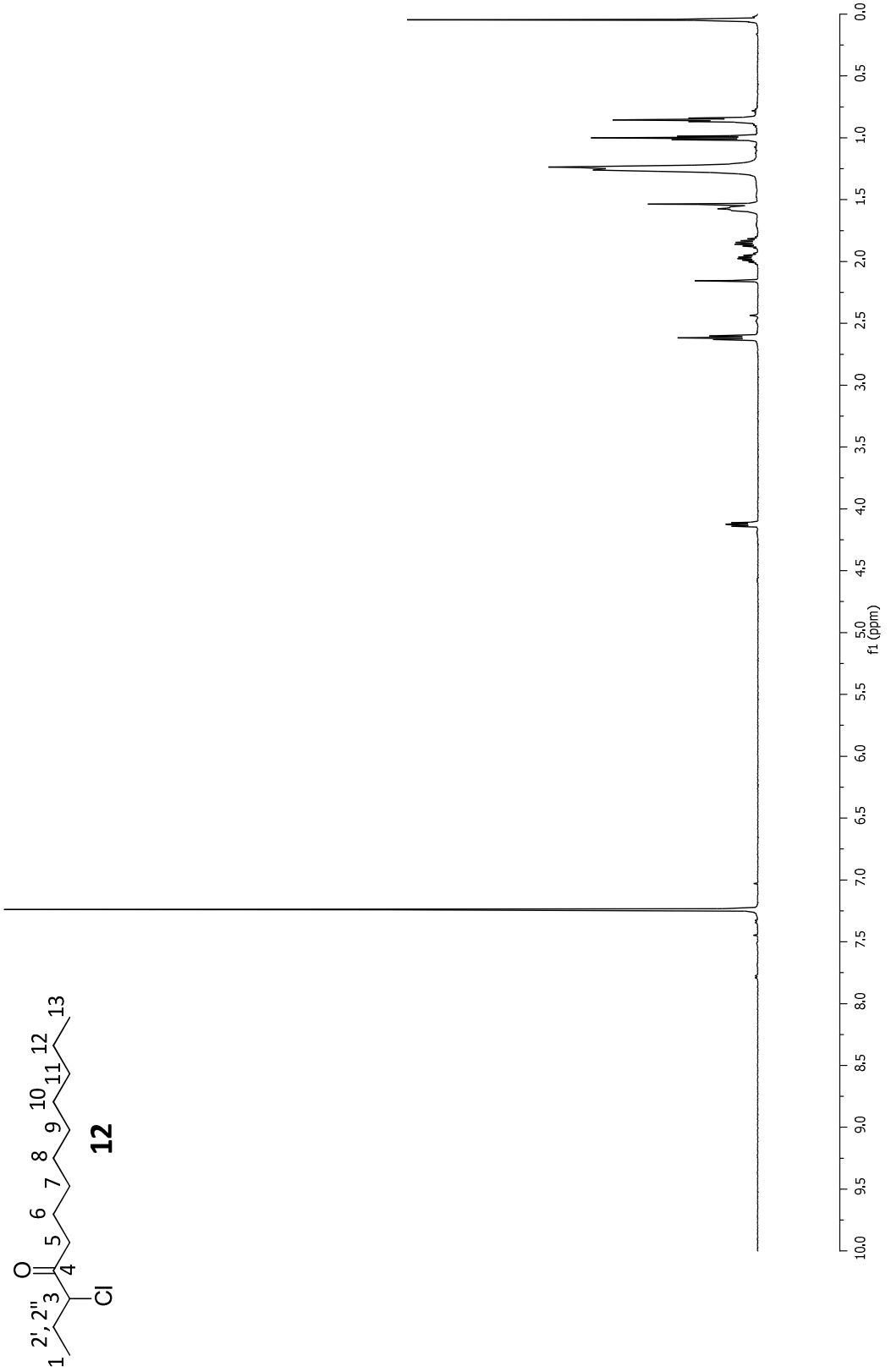
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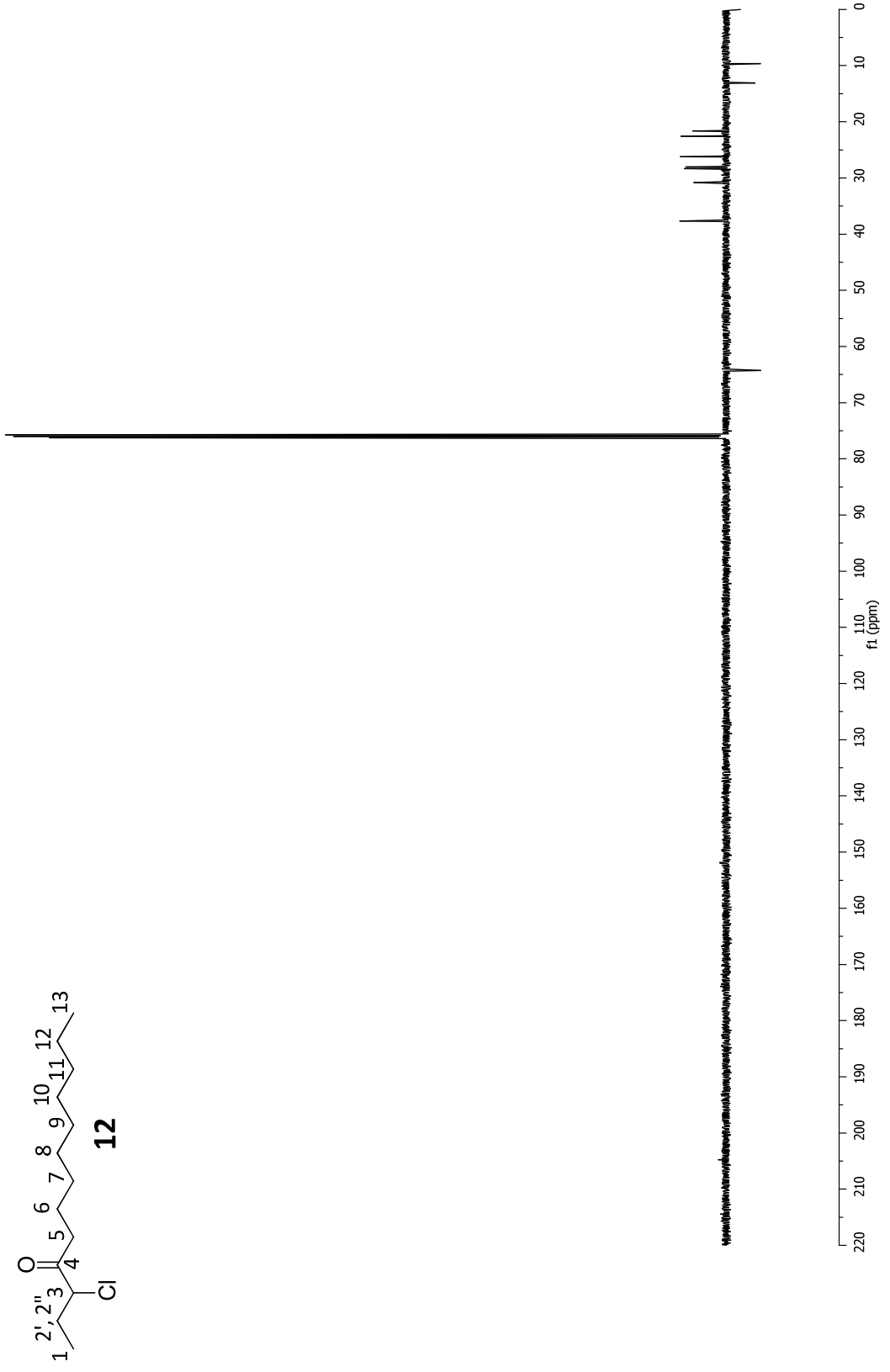

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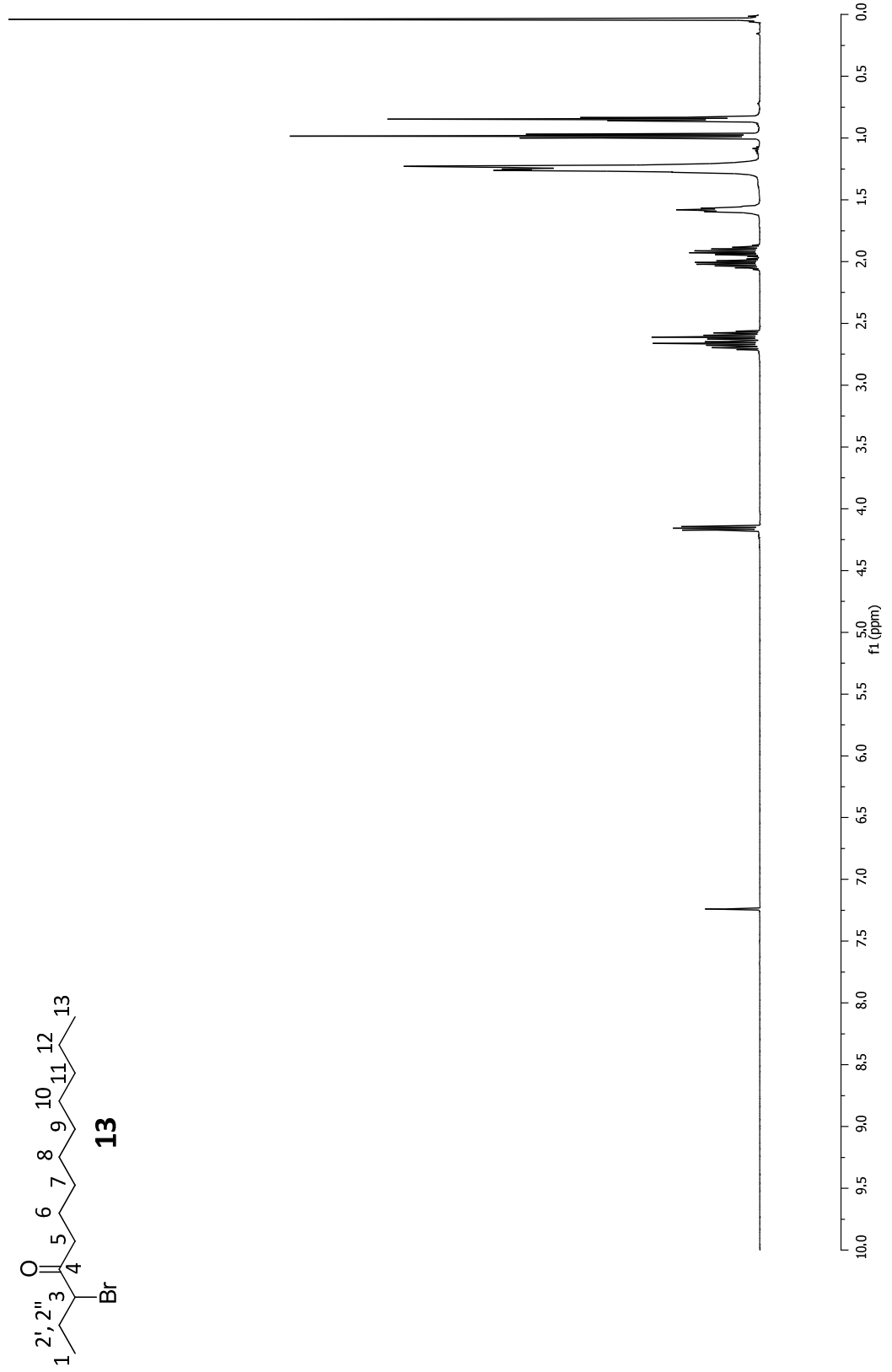
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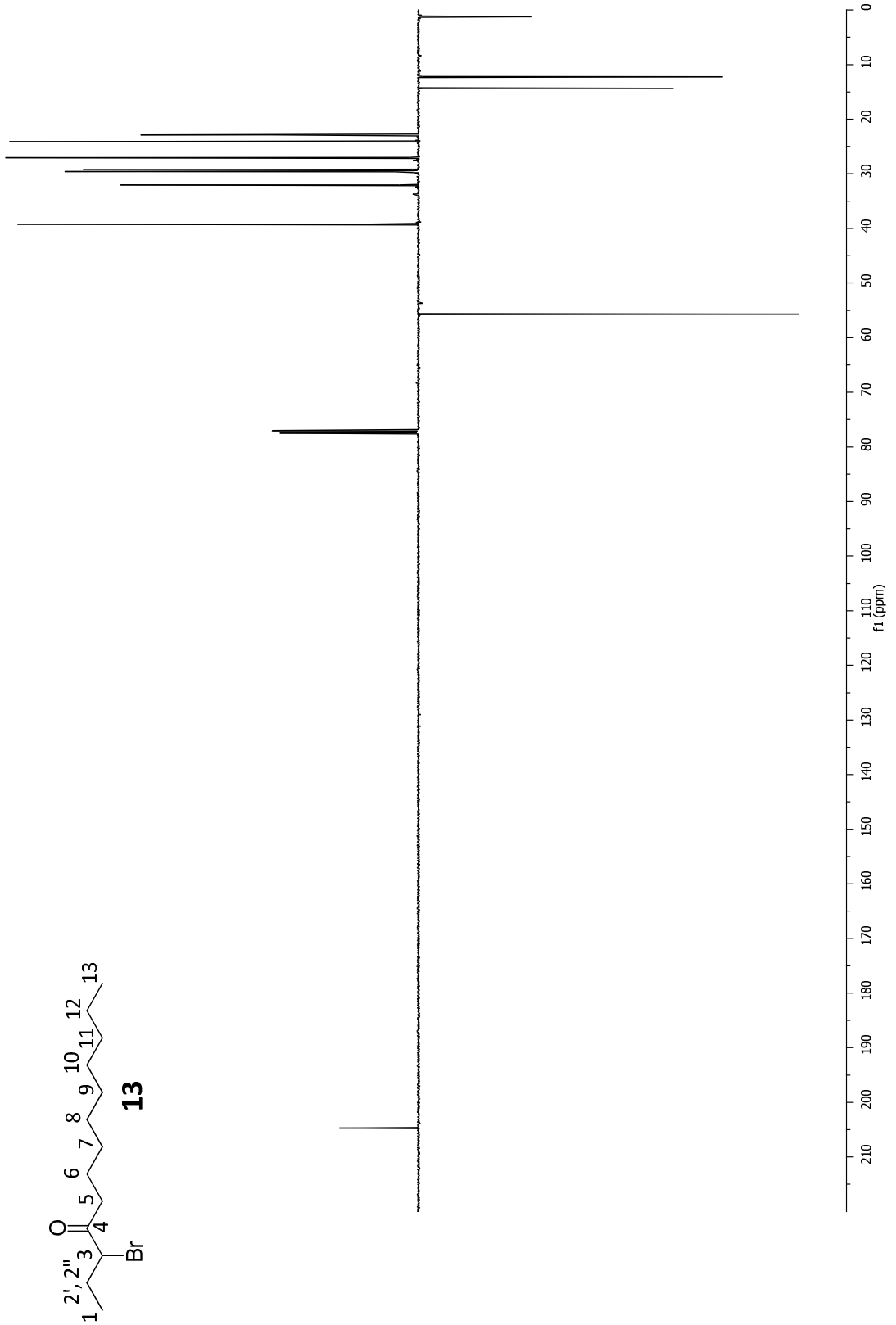
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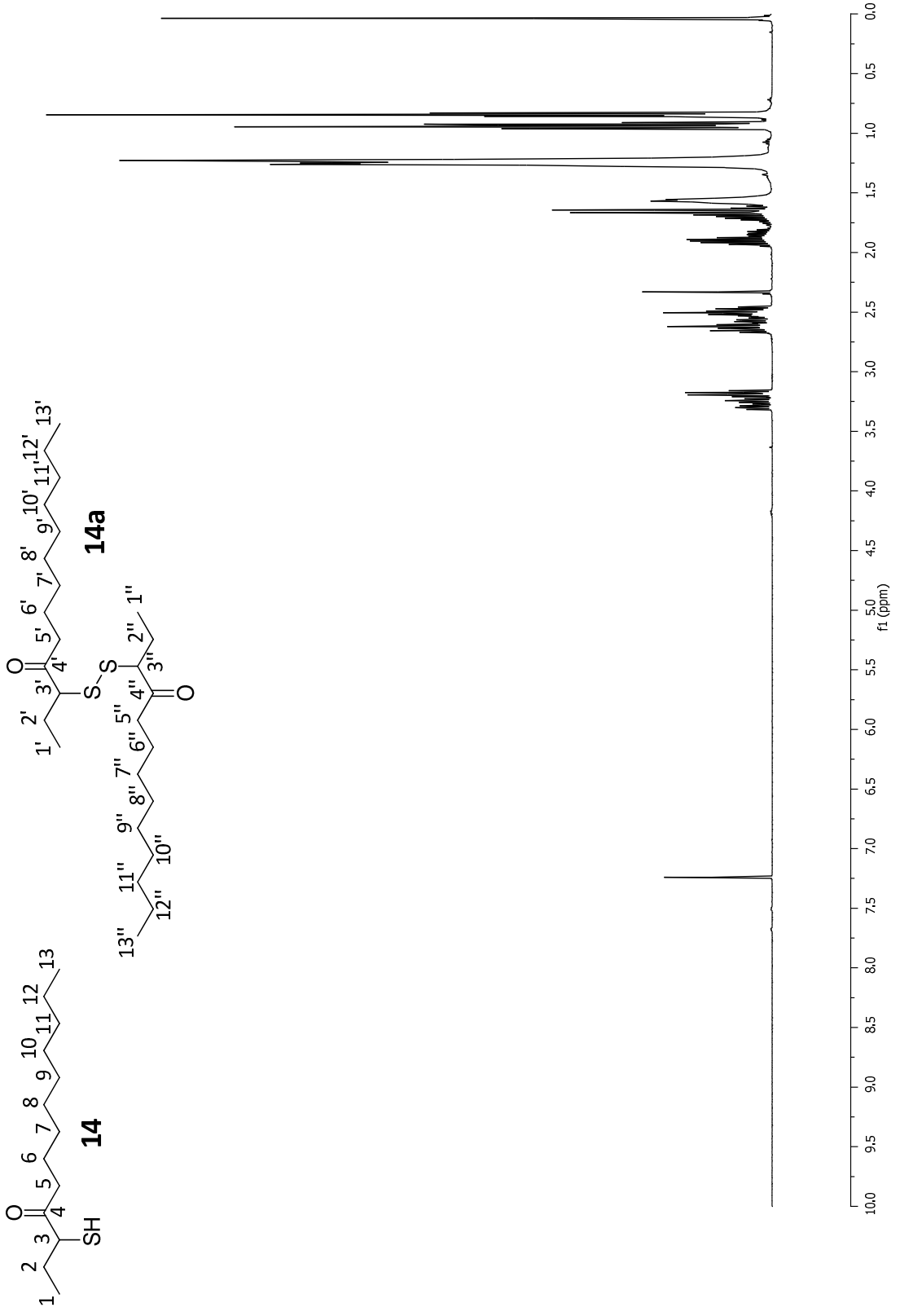
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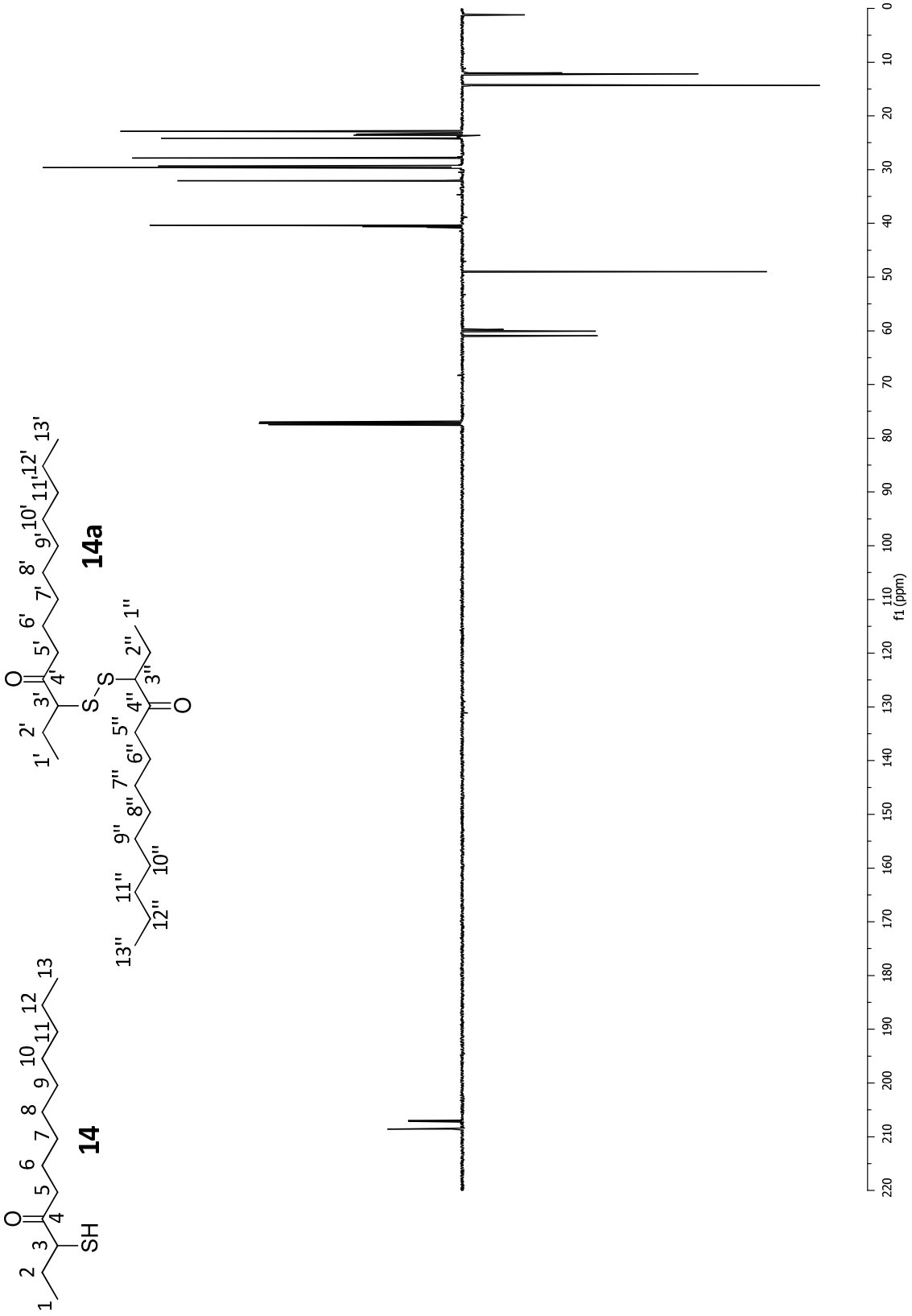
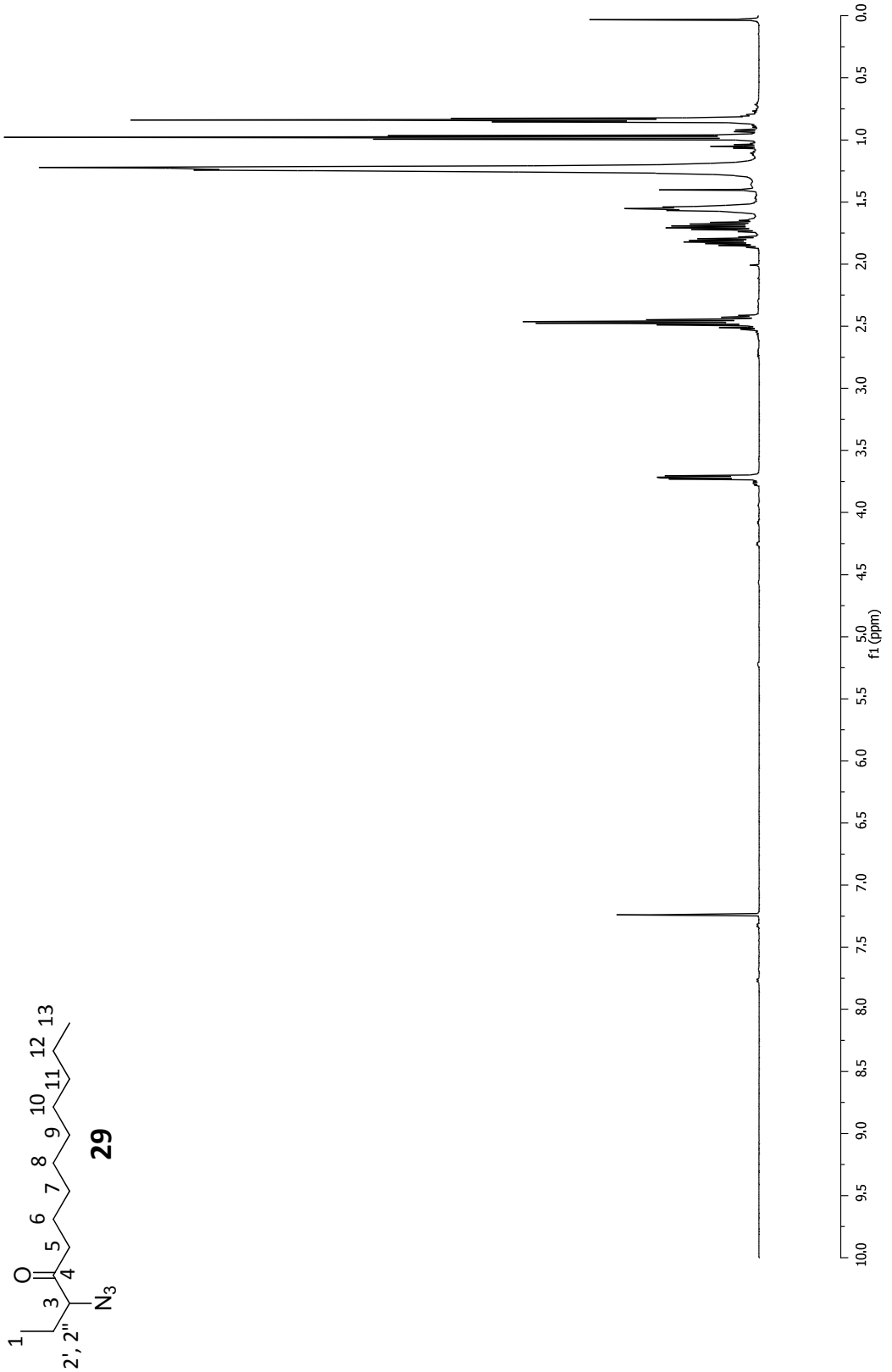
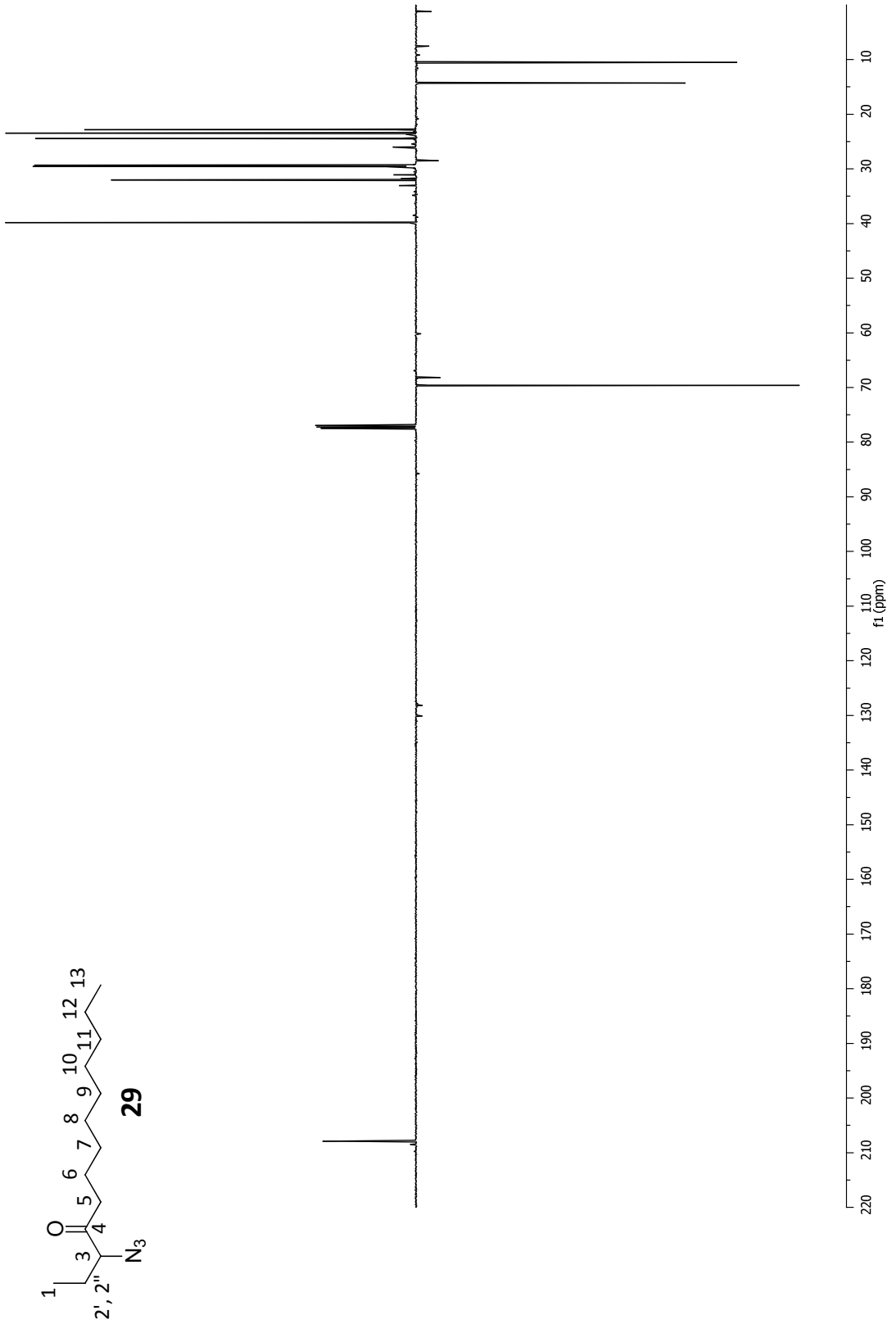
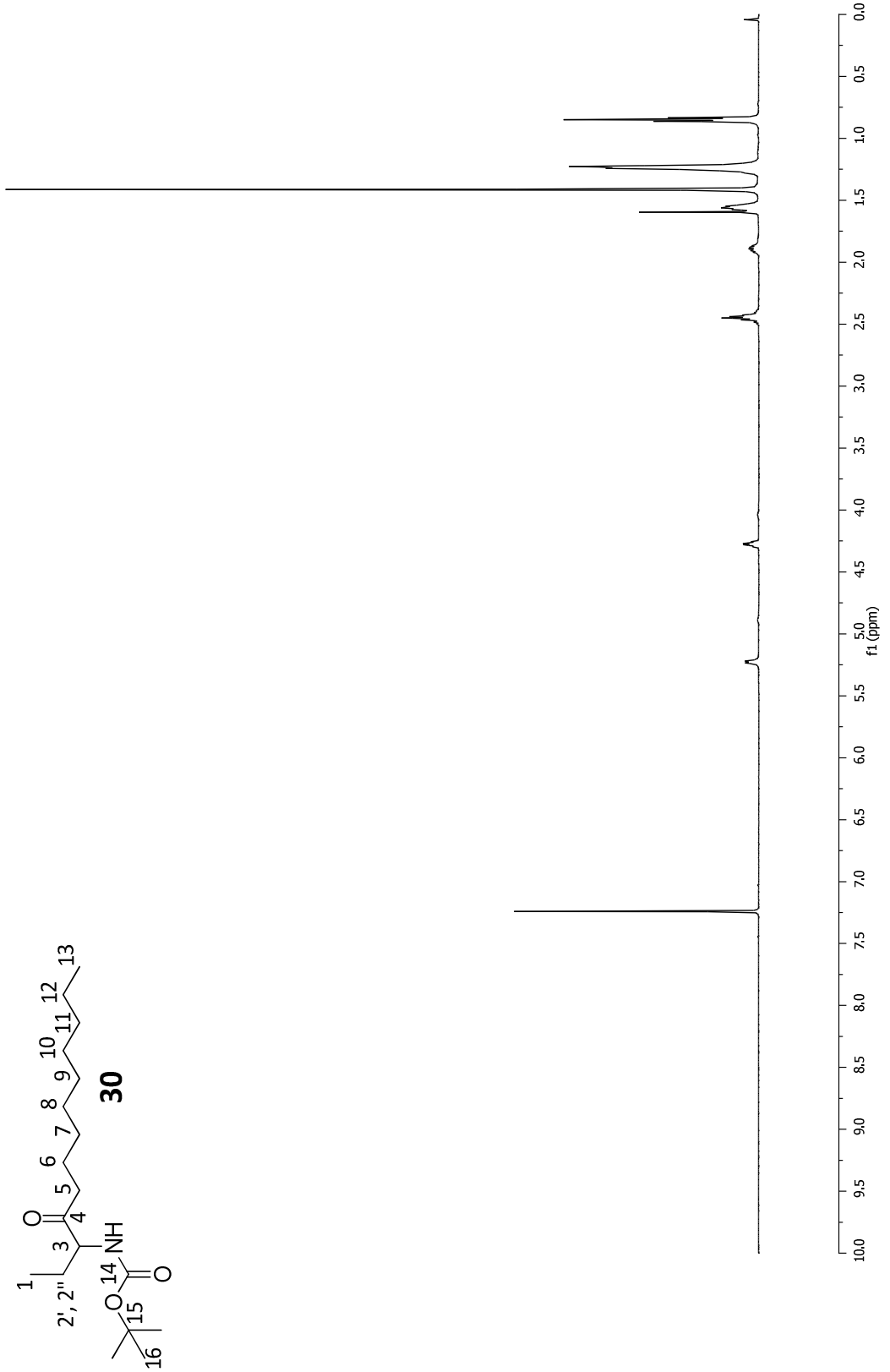
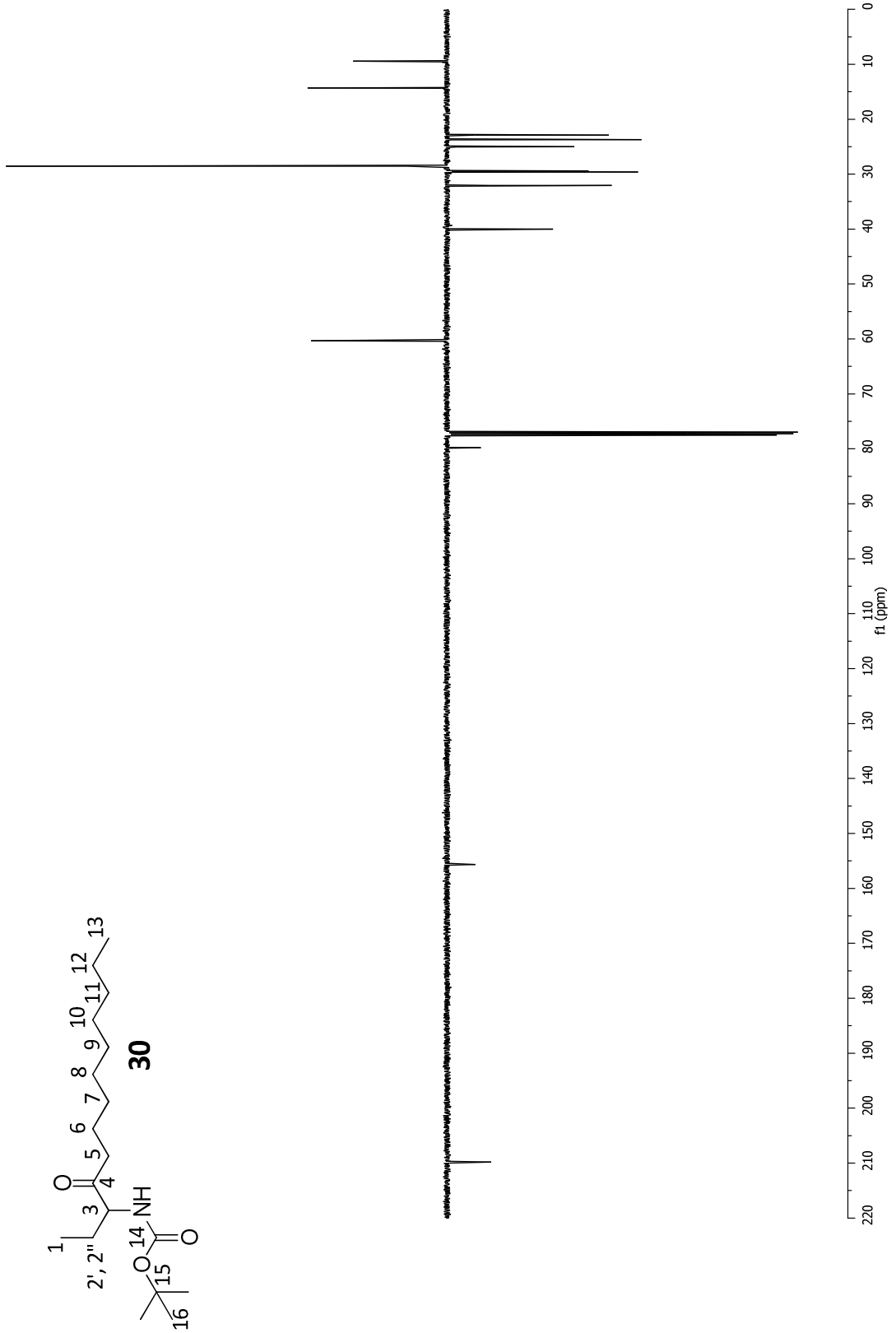
f1 (ppm)

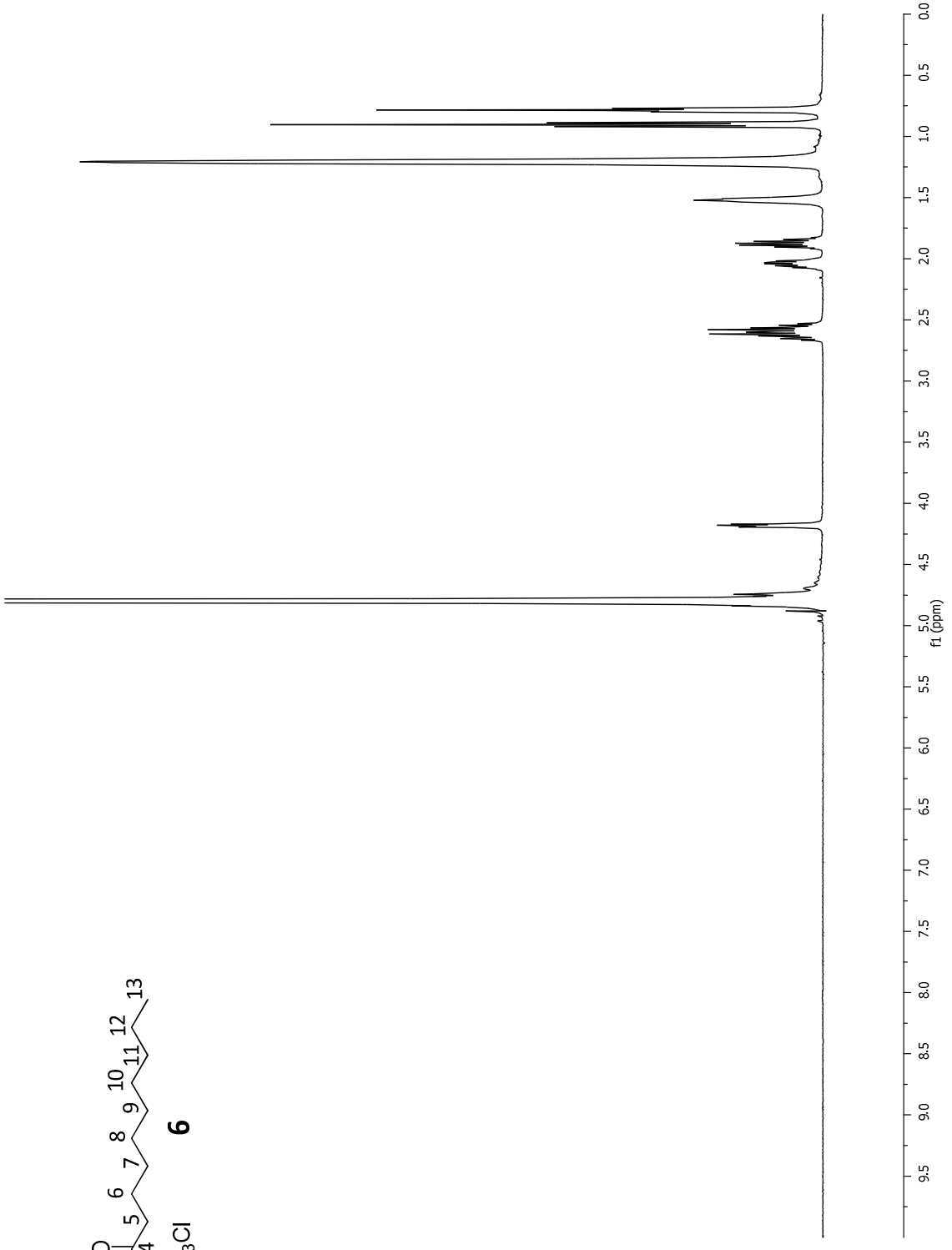

14
14a
f1 (ppm)

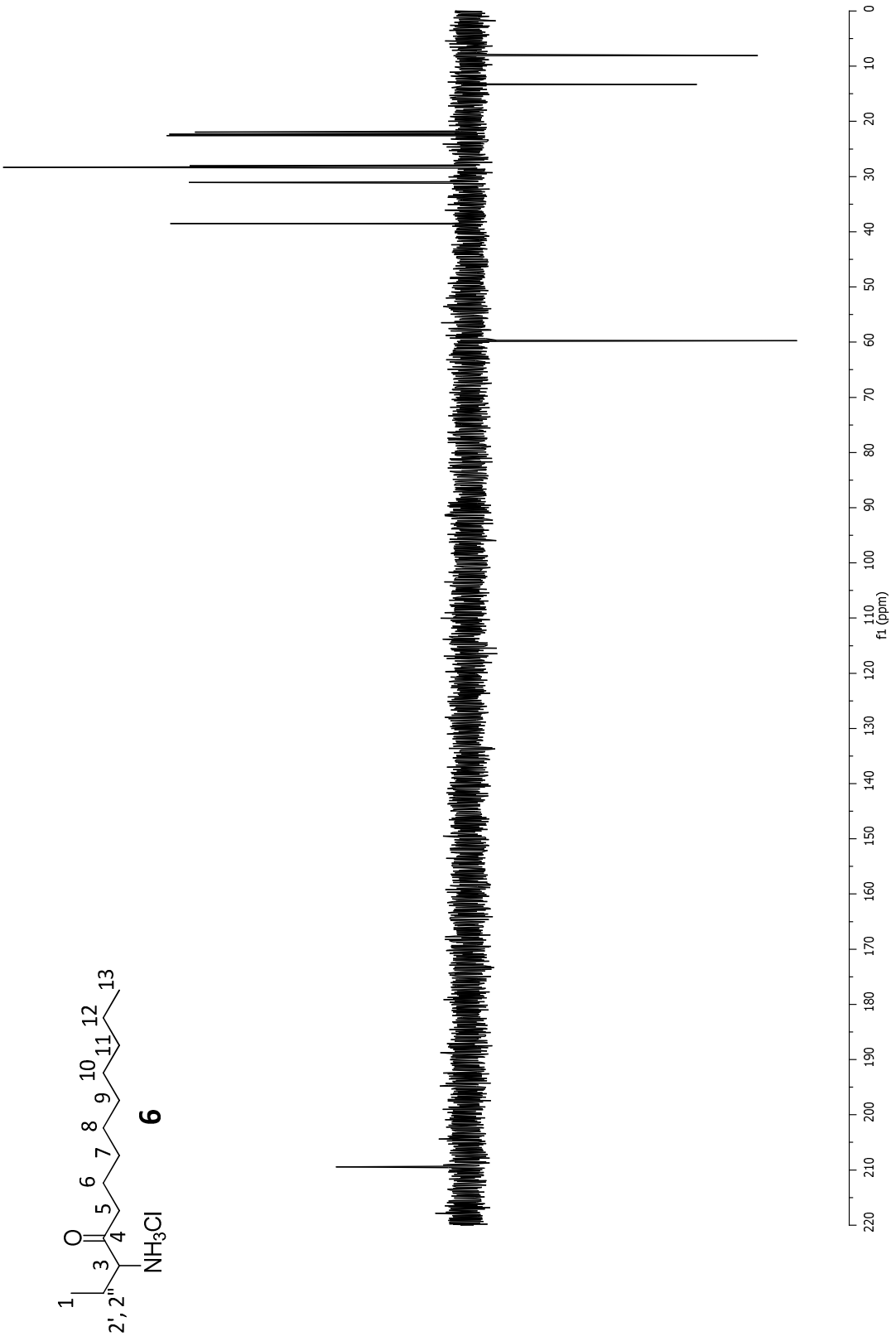

O
N3
1
2', 2"
3
4
5
6
7
8
9
10
11
12
13
29
f1 (ppm)

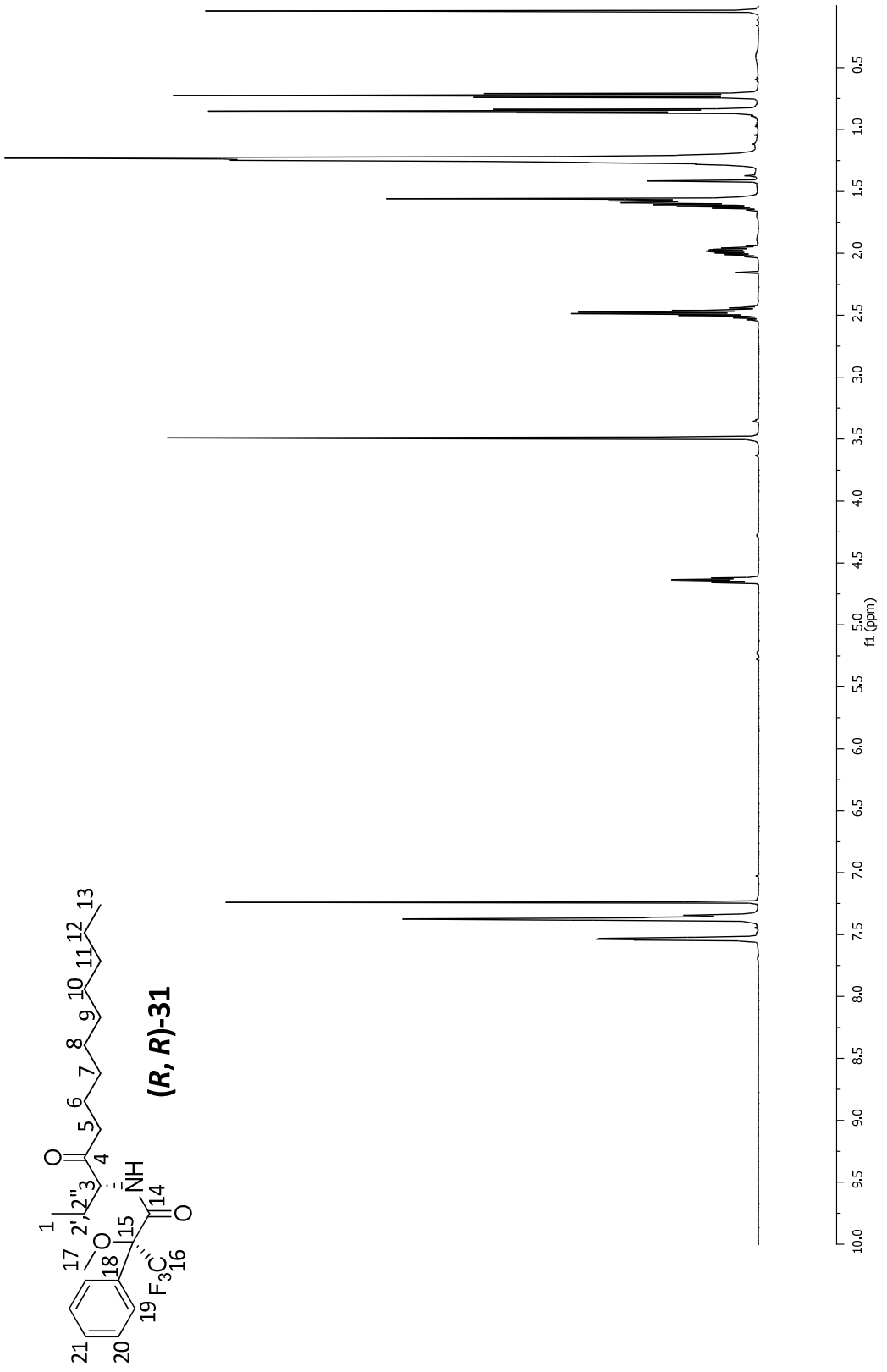

f1 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
O
N3
1
2', 2"
3
4
5
6
7
8
9
10
11
12
13
29

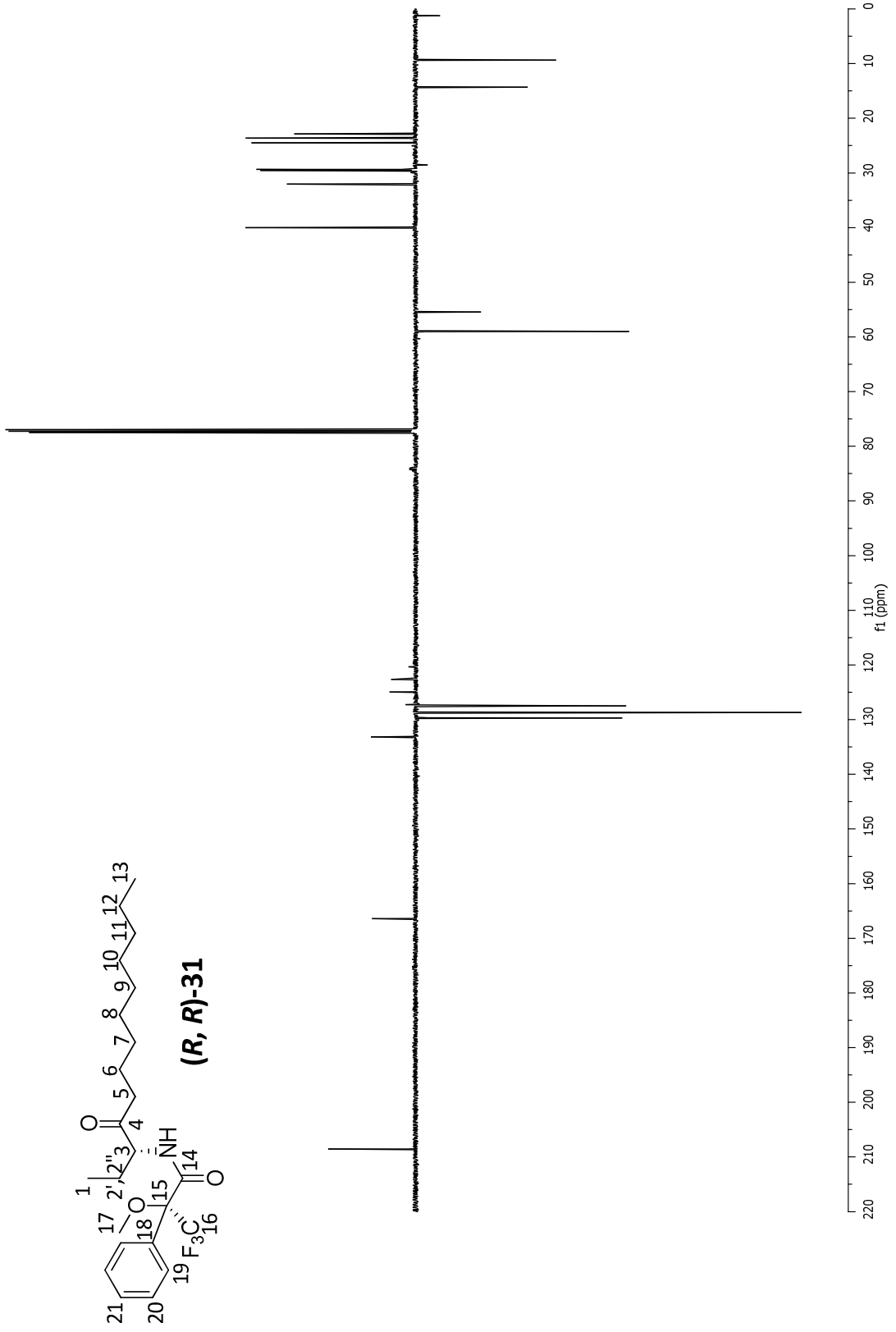

30
1
2', 2"
3
4
O
5
6
7
8
9
10
11
12
13
NH
14
O
O
15
16
f1 (ppm)

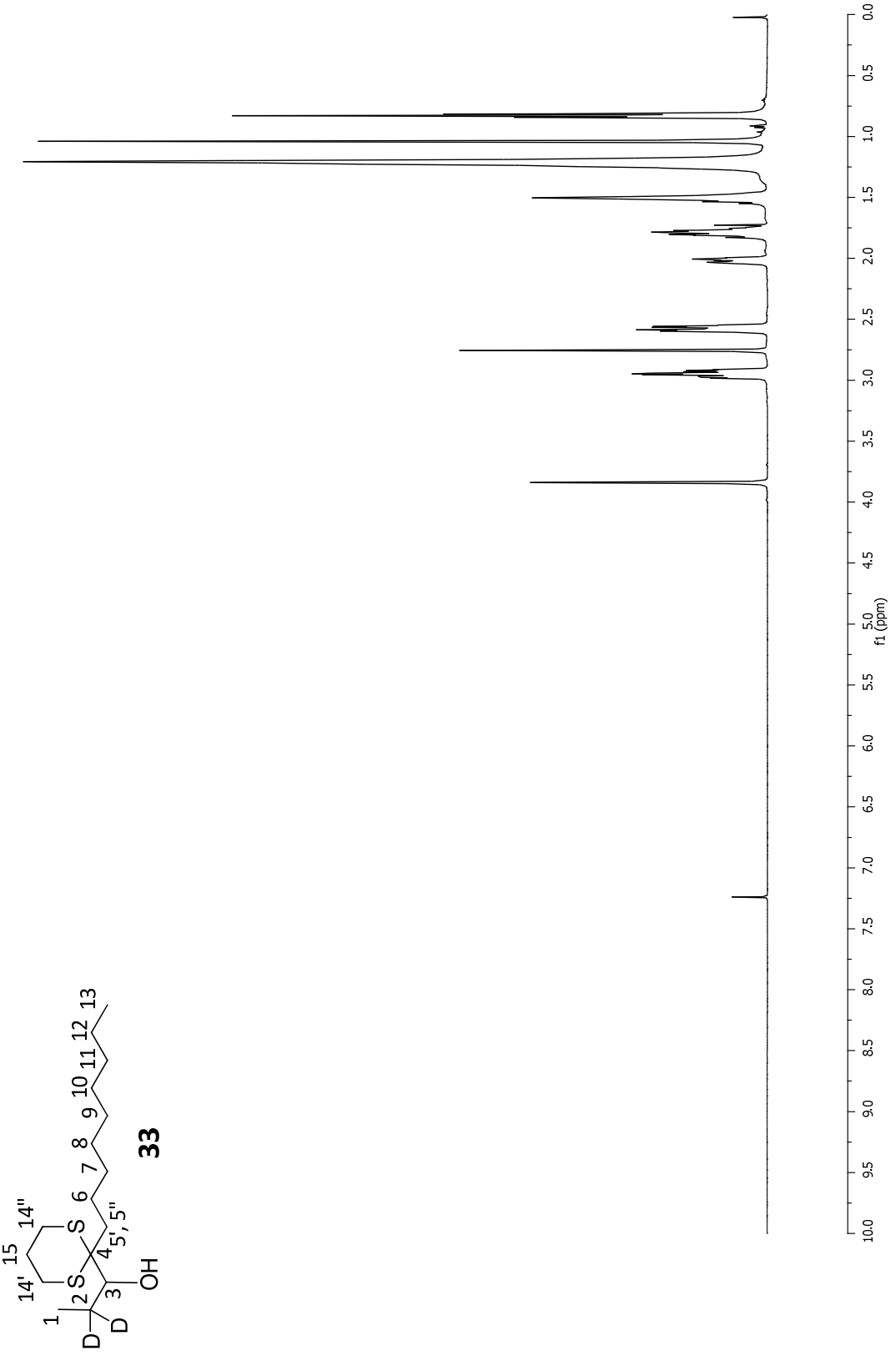

30
f1 (ppm)

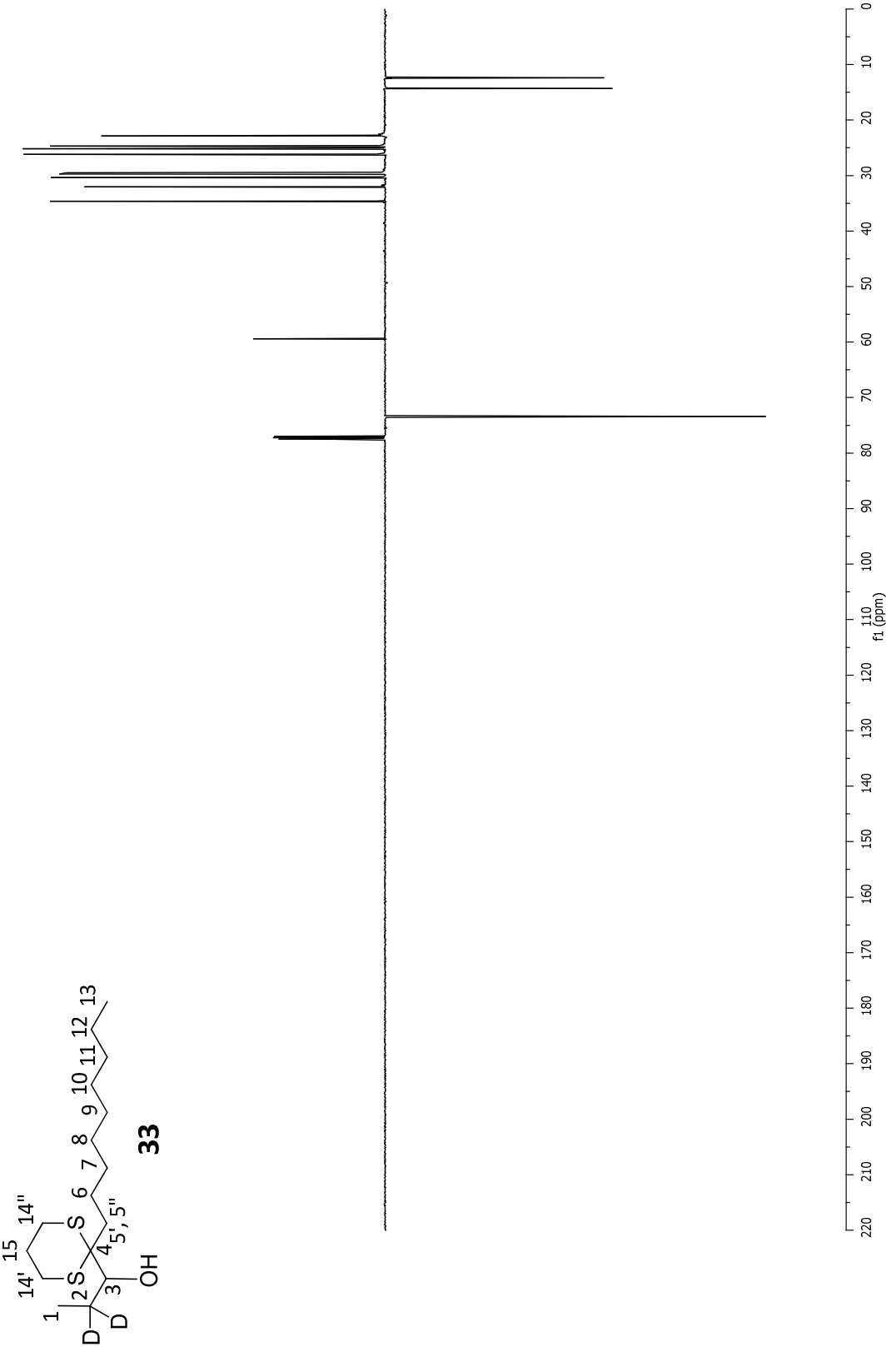

1
2', 2"
3
4
O
5
6
7
8
9
10
11
12
13
NH3Cl
6
f1 (ppm)


1
2', 2"
3
4
O
5
6
7
8
9
10
11
12
13
NH3Cl
6
f1 (ppm)


(R, R)-31
f1 (ppm)

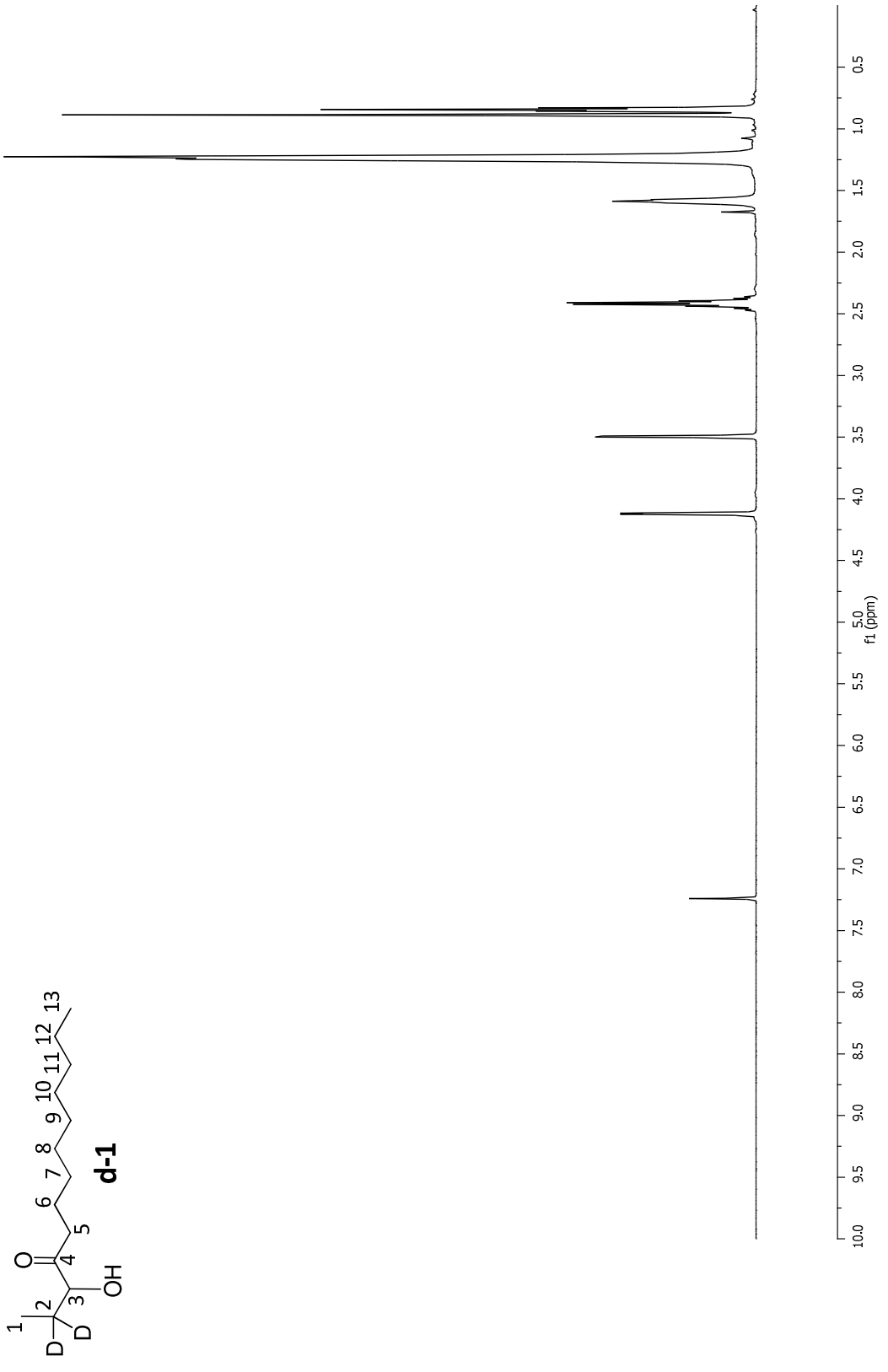

(R, R)-31
f1 (ppm)

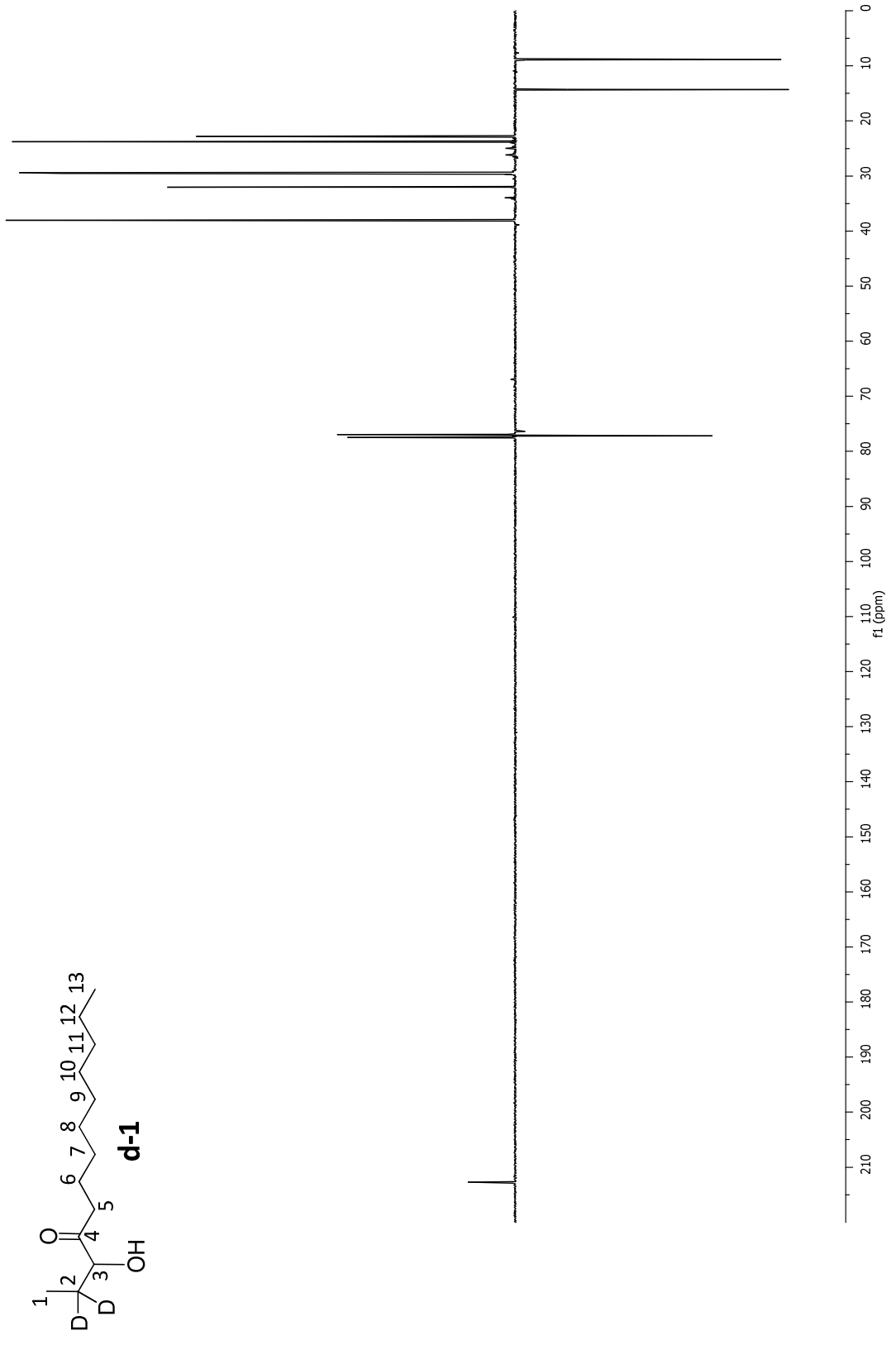

f1 (ppm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
33
OH
D
D
S
S
1
2
3
4', 5"
6
7
8
9
10
11
12
13
14'
14"
15

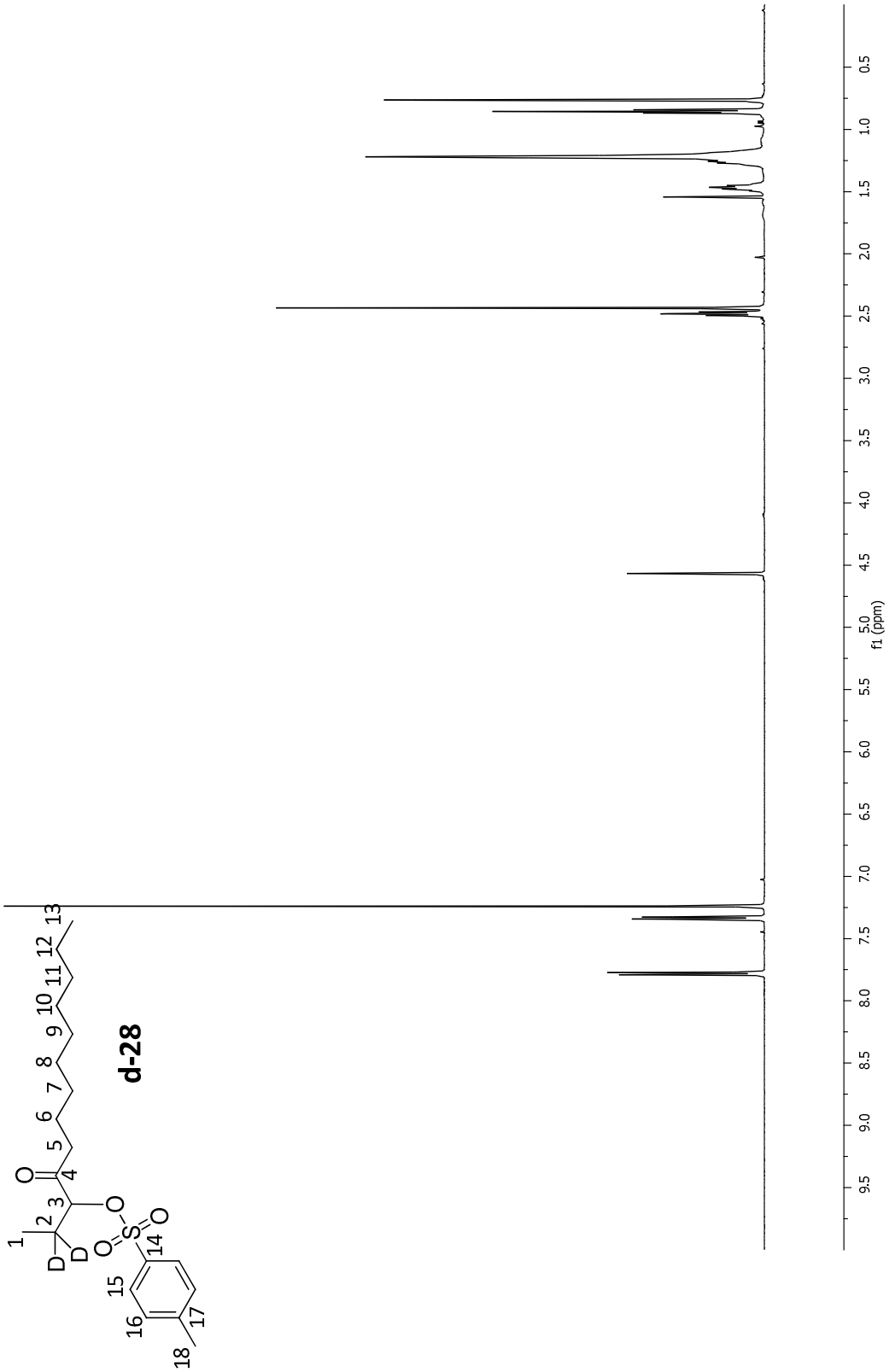

f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

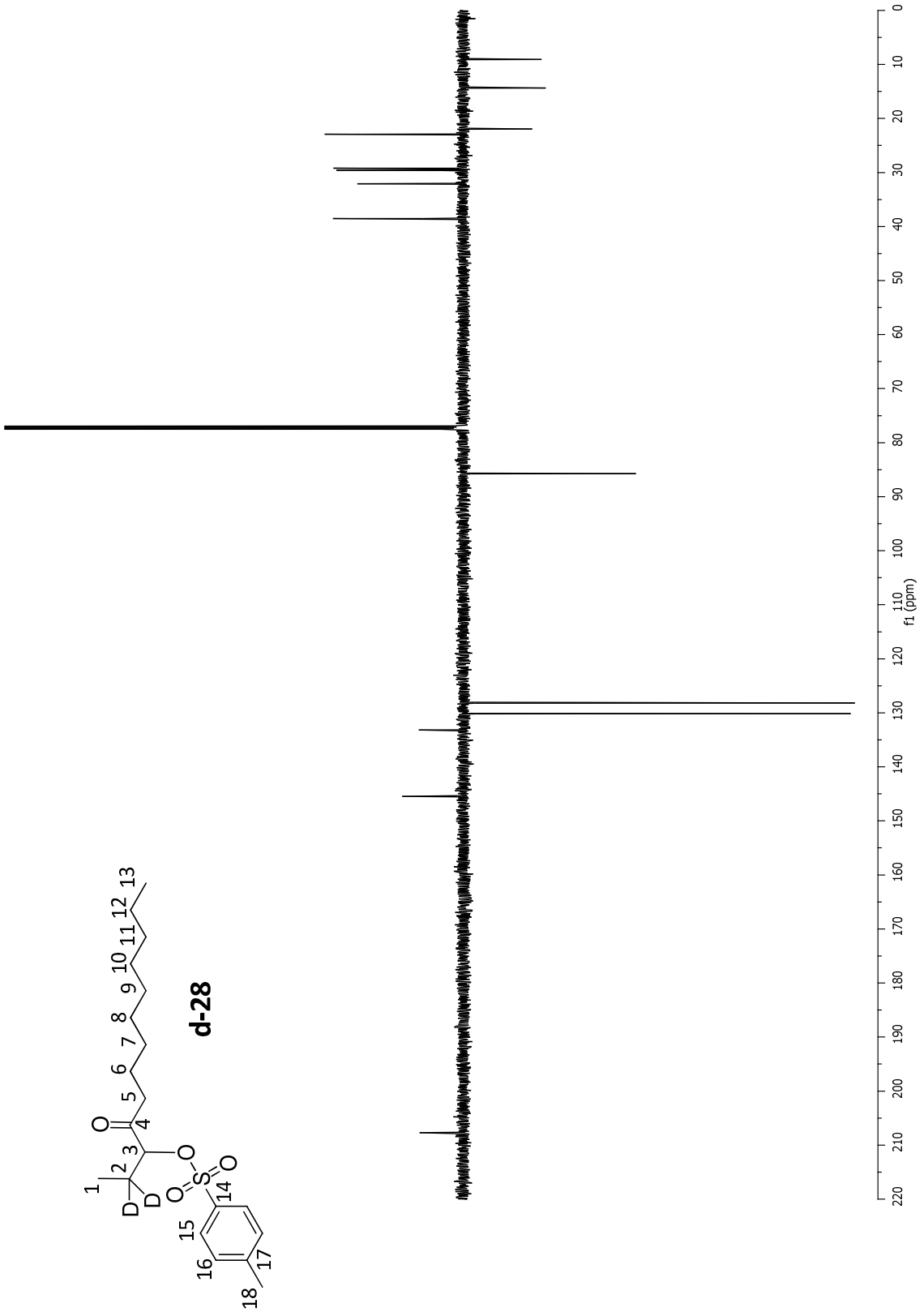
1
D
D
2
S
3
OH
4', 5"
S
14'
15
14"
6
7
8
9
10
11
12
13

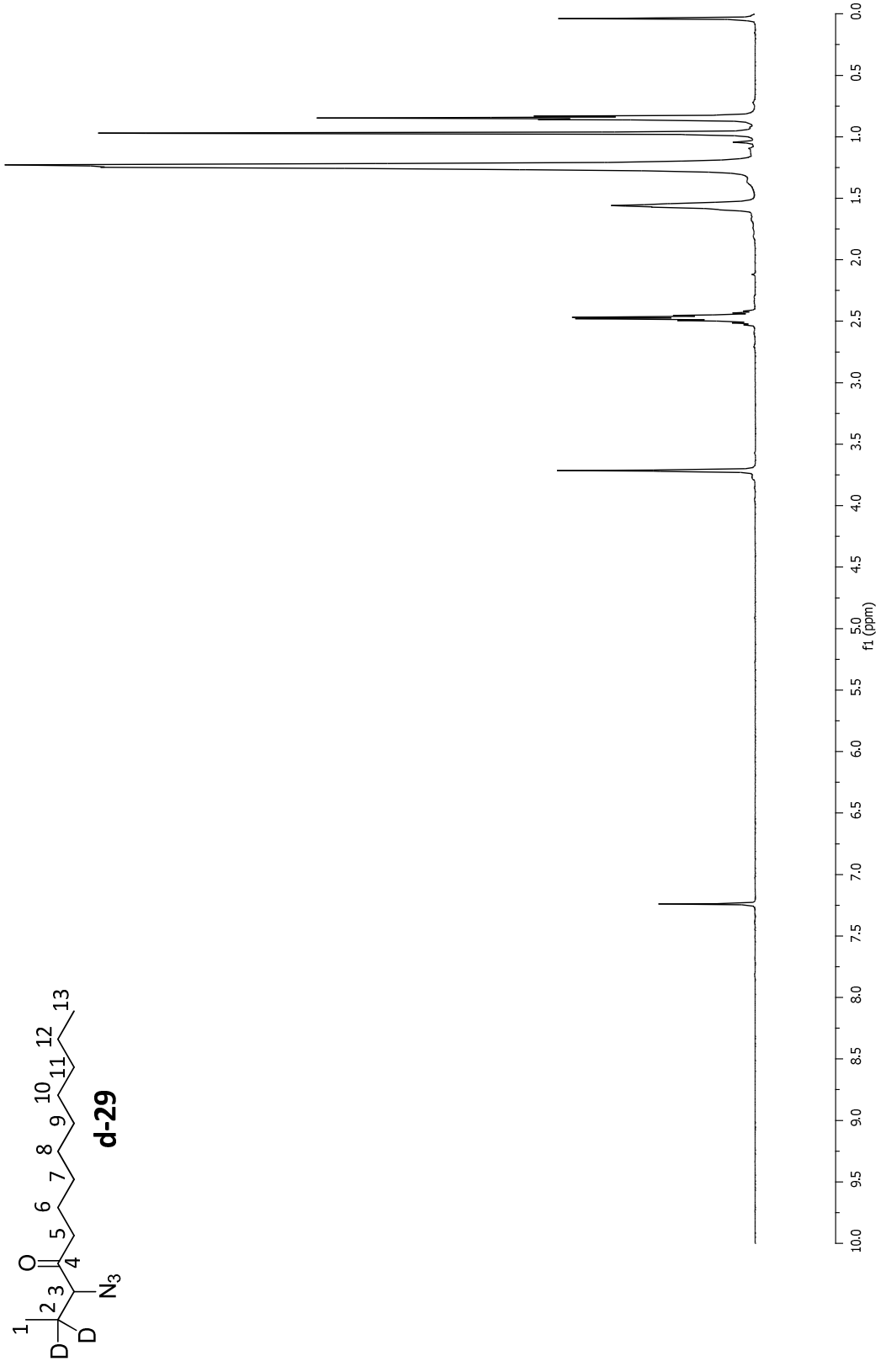
33

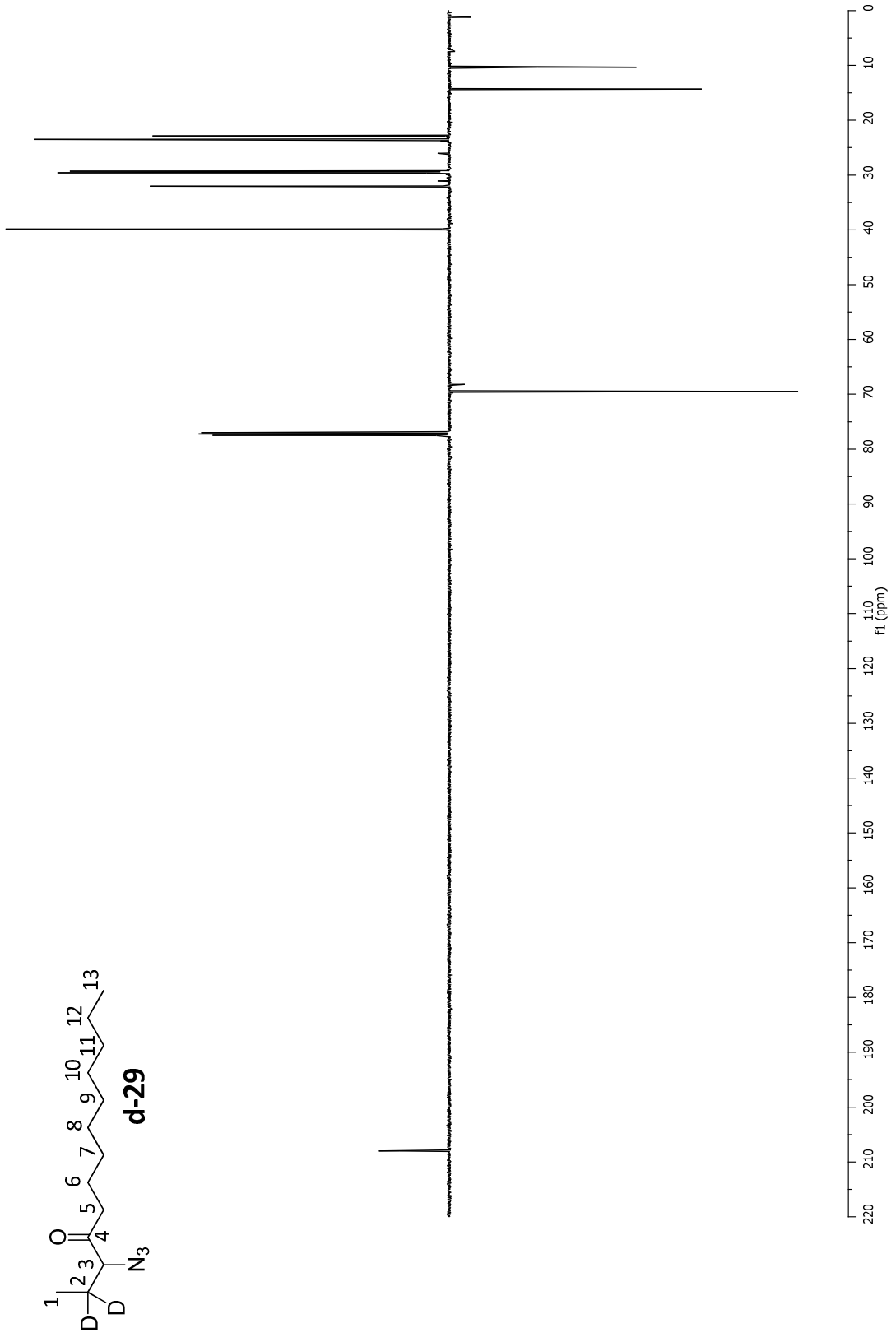

1
D
D
2
3
OH
4
O
5
6
7
8
9
10
11
12
13
d-1
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

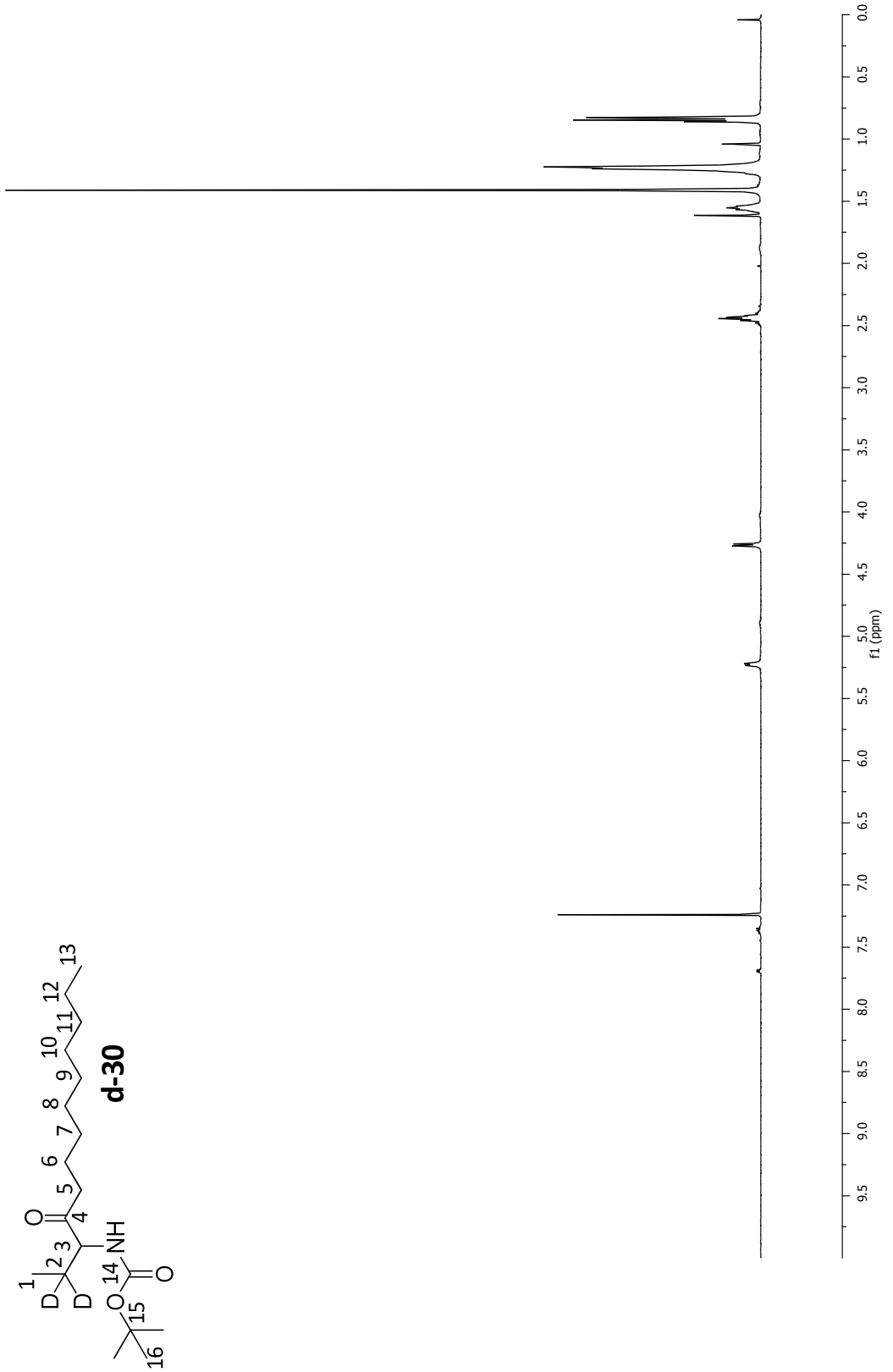

f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
1
2
3
4
5
6
7
8
9
10
11
12
13
D
D
O
OH
d-1

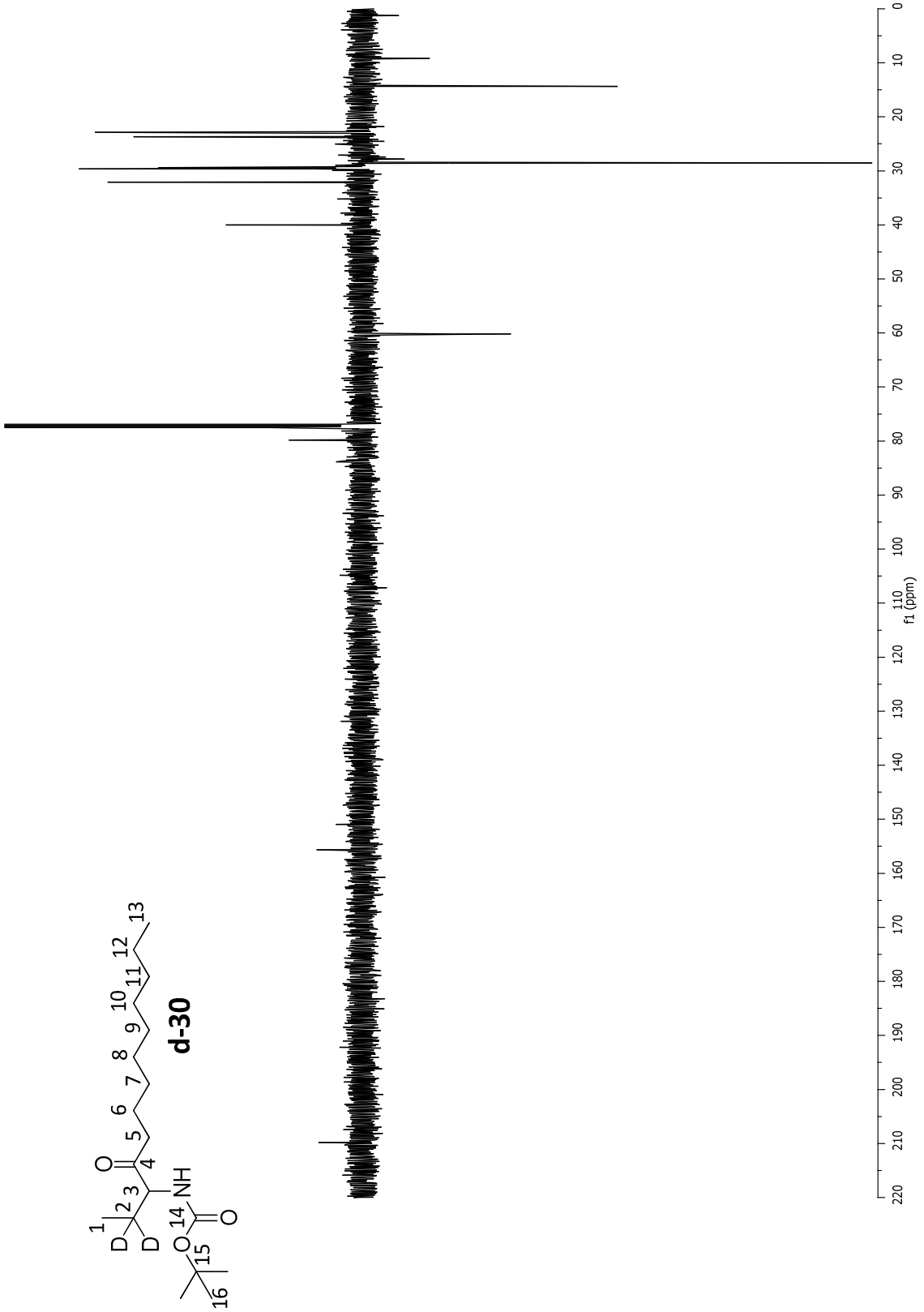

d-28
f1 (ppm)

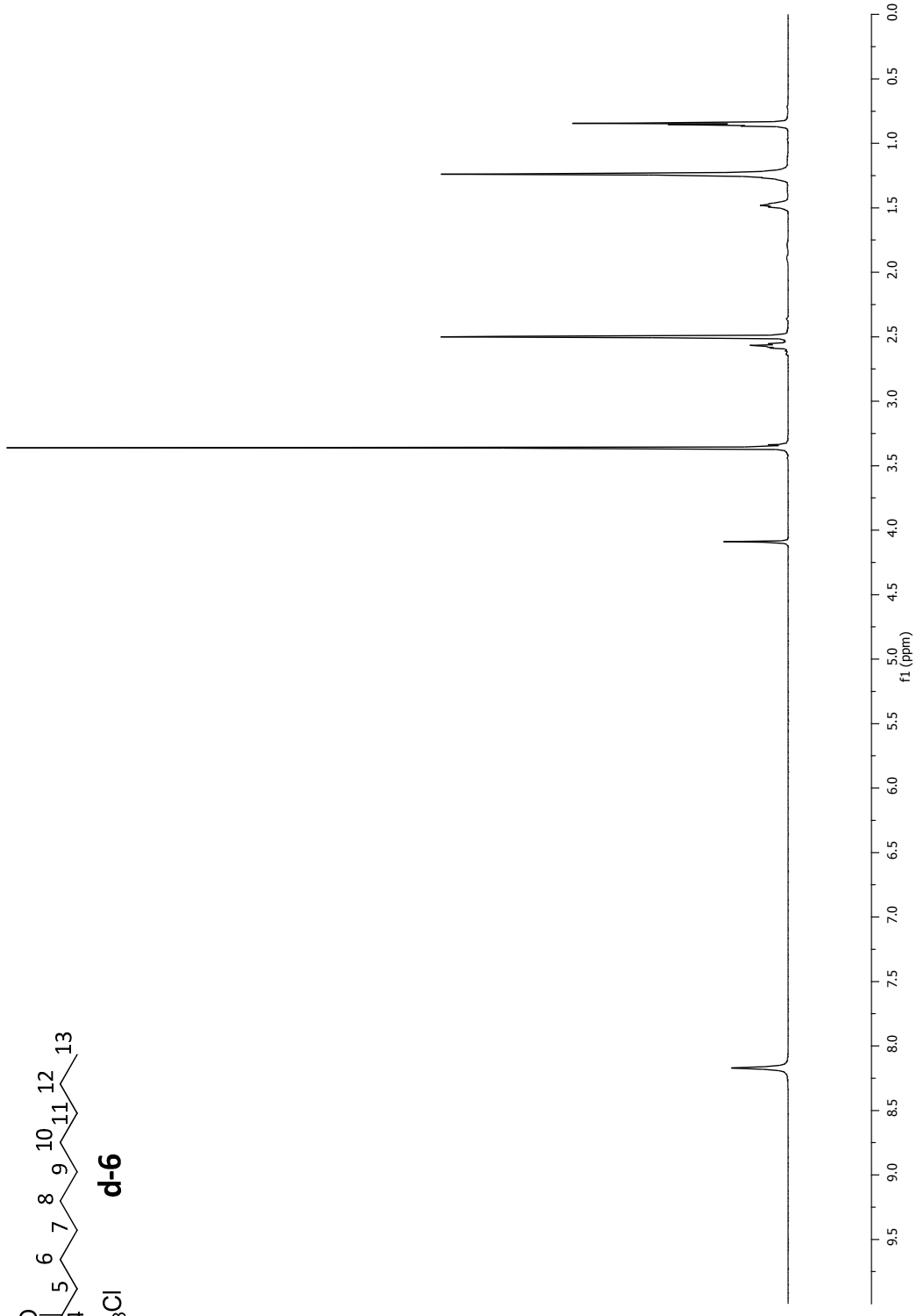

d-28
f1 (ppm)

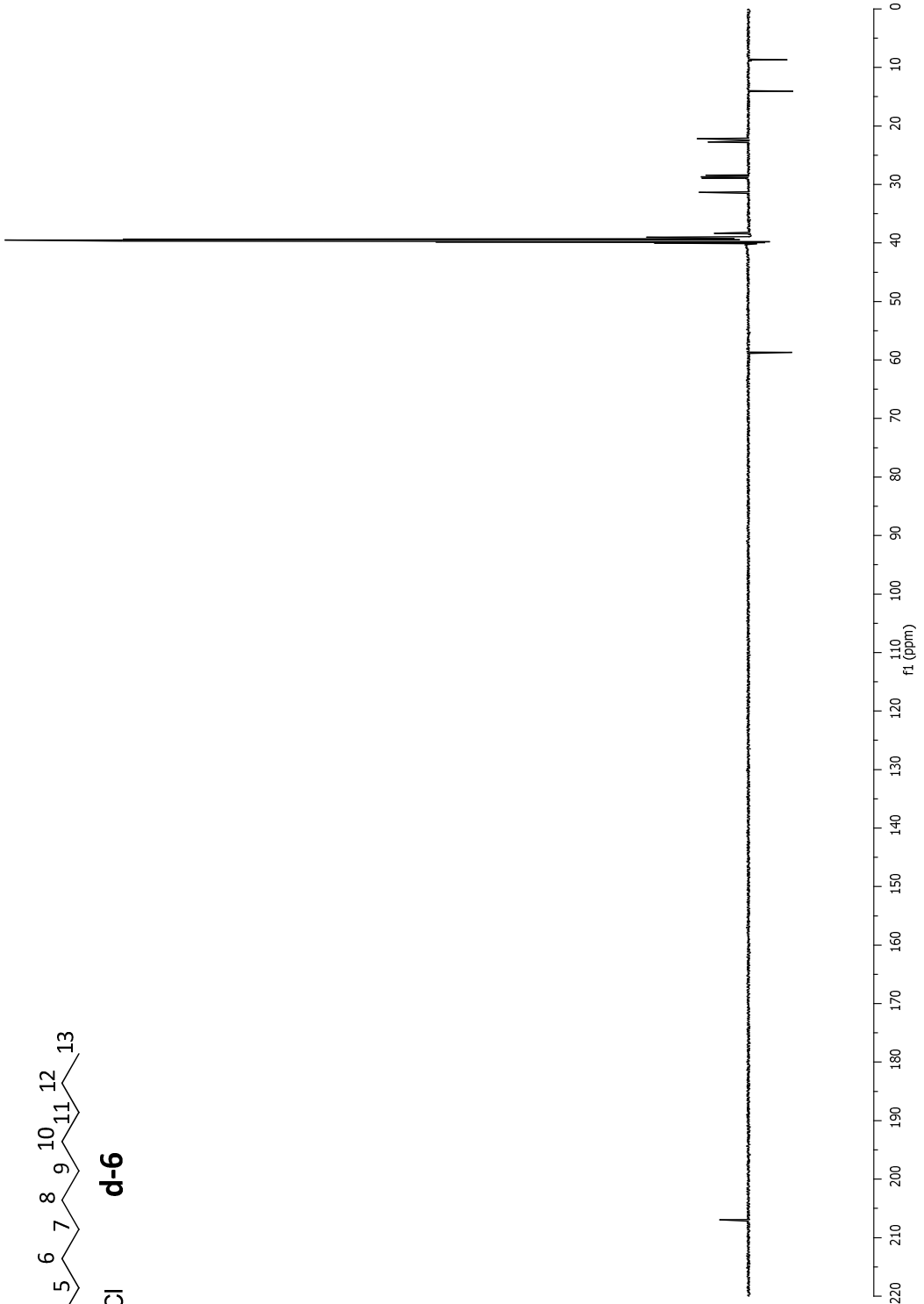

d-29
f1 (ppm)

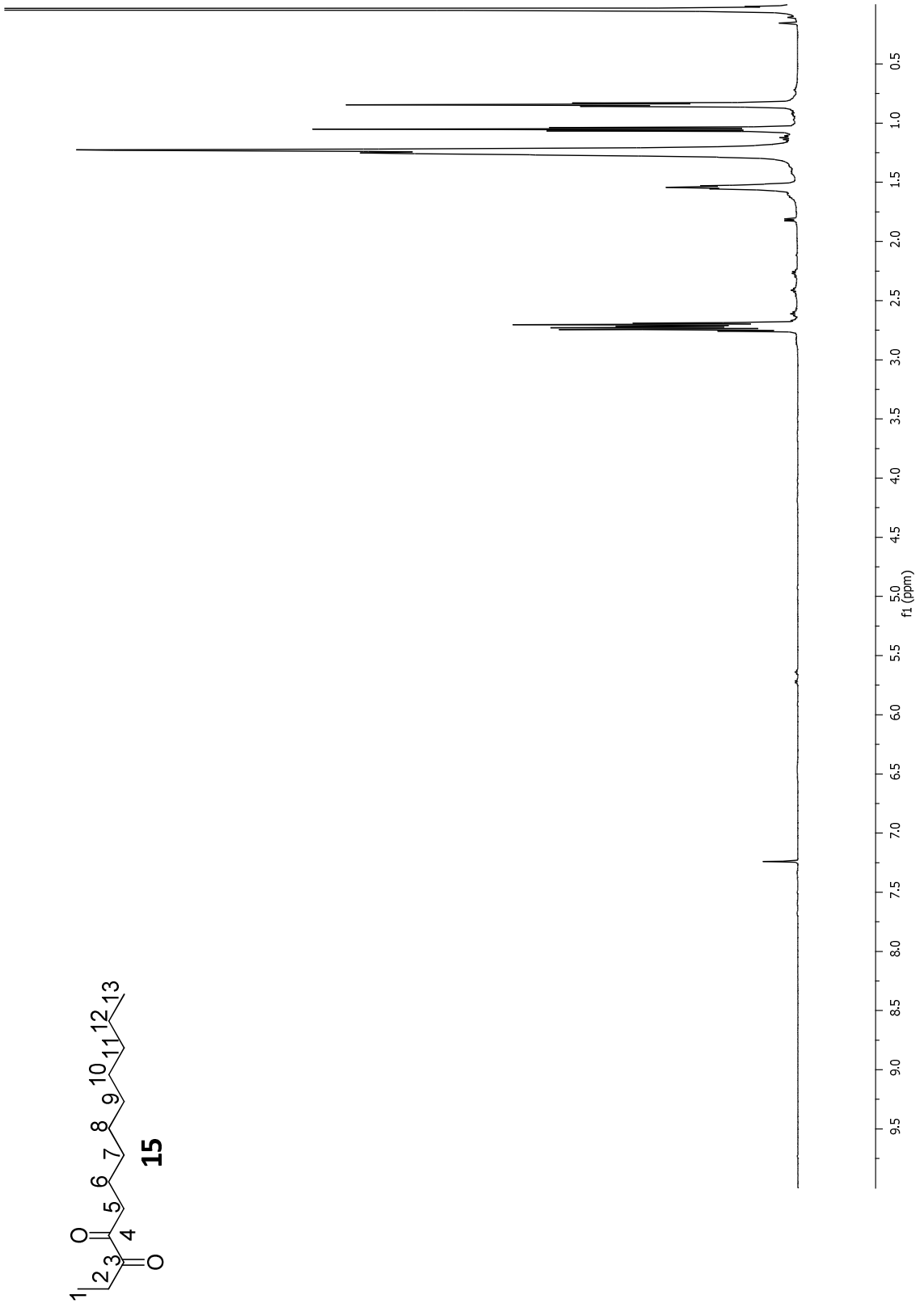

d-29
f1 (ppm)


d-30
f1 (ppm)

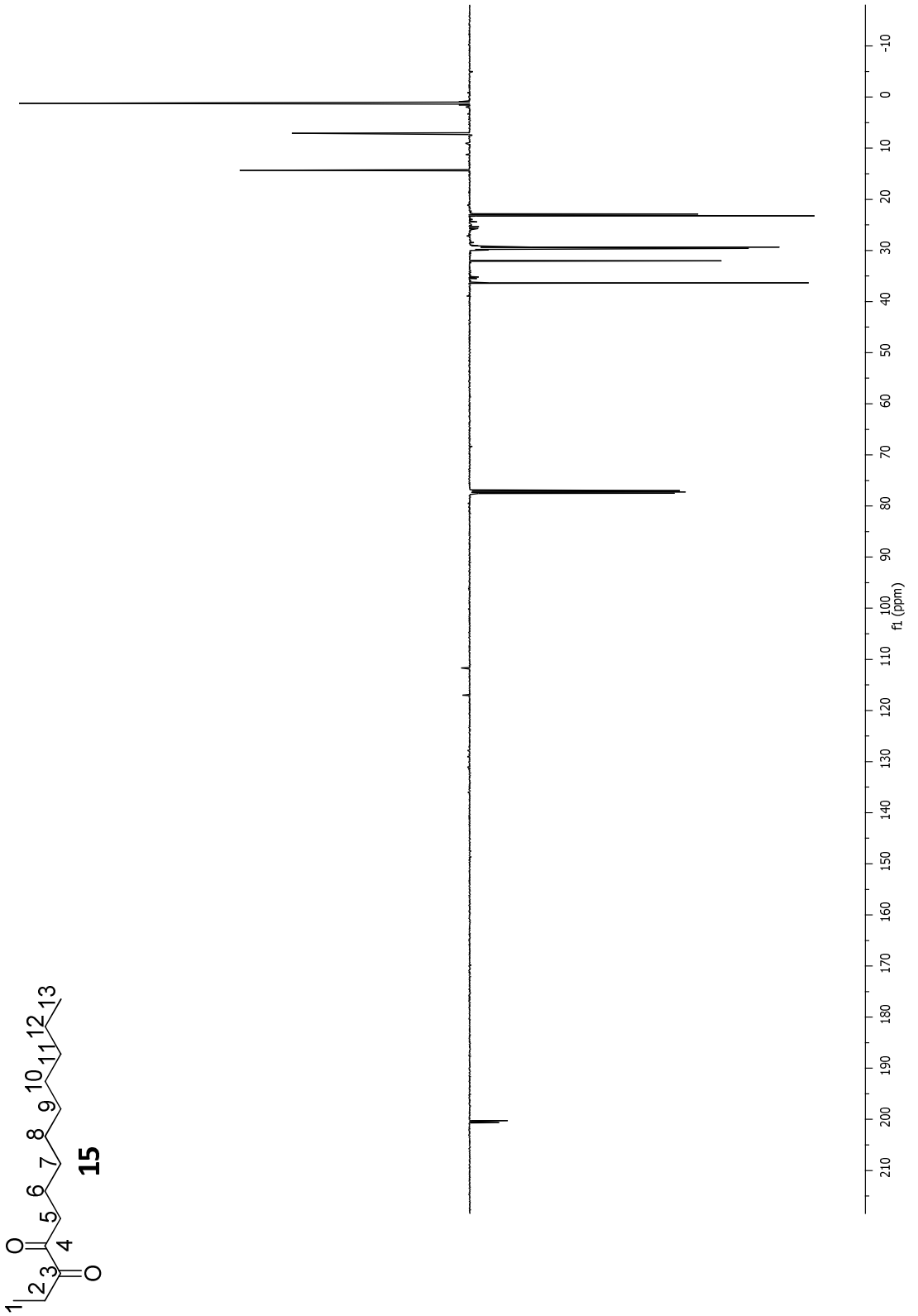

d-30
f1 (ppm)


1
2
3
D
D
4
O
5
6
7
8
9
10
11
12
13
NH3Cl
d-6
f1 (ppm)


1
2
3
4
5
6
7
8
9
10
11
12
13
D
D
O
NH3Cl
d-6
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
f1 (ppm)


f1 (ppm)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
O
O
1
2
3
4
5
6
7
8
9
10
11
12
13

15


f1 (ppm)

15