Electronic Supplementary Information

Reevaluation of the mechanism of cytotoxicity of dialkylated lariat ether compounds

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II. Supplemental figures

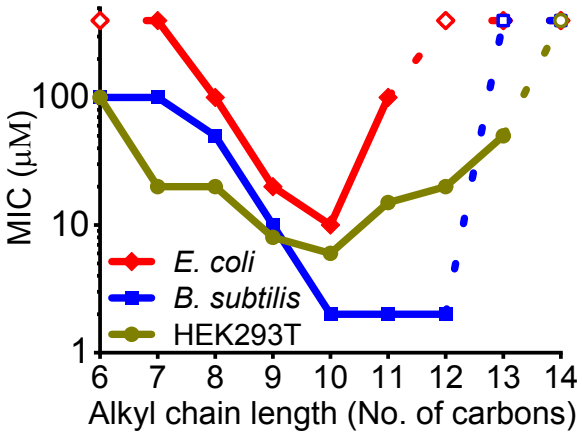


Fig. S1 Toxicity of dialkylated lariat ethers: Minimum inhibitory concentrations (MIC) towards *B. subtilis* (blue), *E. coli* (red), and HEK293T cells (yellow). Open symbols represent non-determined values when the MIC is > 400 μM. Each compound was assayed a minimum of three times at each different concentration.

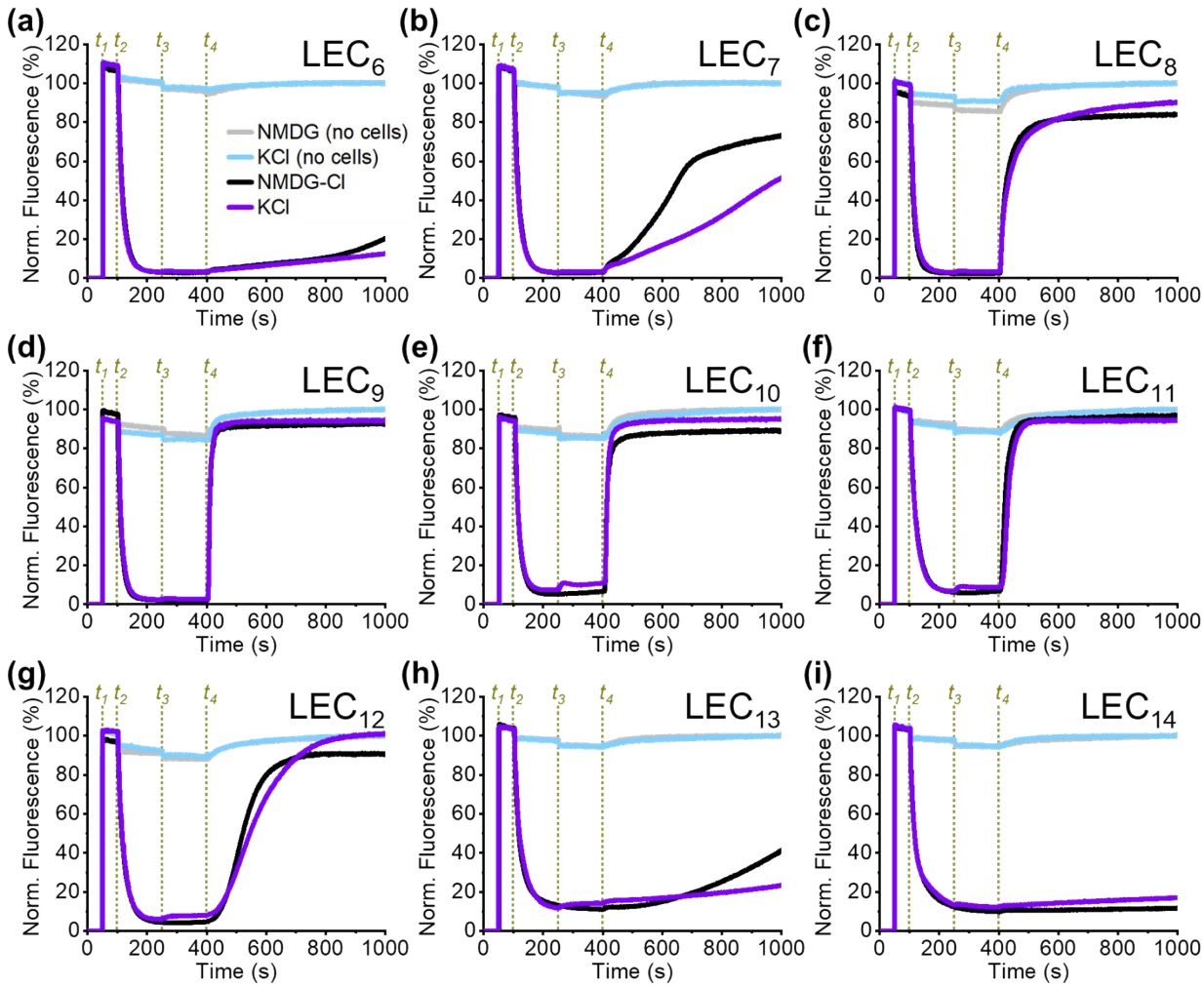


Fig. S2 Depolarizing activity of dialkylated lariat ethers on *B. subtilis*: (a)-(i) Time course of the $DiSC_3(5)$ fluorescence due to the activity of dialkylated lariat ethers with alkyl chains 6 to 14 carbons in length. The experiment was performed in a NMDG-$MeSO_3$ solution (100 mM NMDG, 10 mM HEPES, pH adjusted to 7.4 with methanesulfonic acid). The events are: t_1, addition of dye; t_2, addition of *B. subtilis*; t_3, addition of a 2 M KCl (violet curve) or a 2 M NMDG-Cl (black curve) solution up to a final concentration of 60 mM; t_4, addition of dialkylated lariat ether up to 2 µM). The cyan and gray traces are similar control experiments to the two previously described experiments, with the exception that additional NMDG-$MeSO_3$ solution without cells was added at timepoint t_2. The experiments were normalized relative to the final fluorescence intensity in the control experiments, as described below in the Methods section.

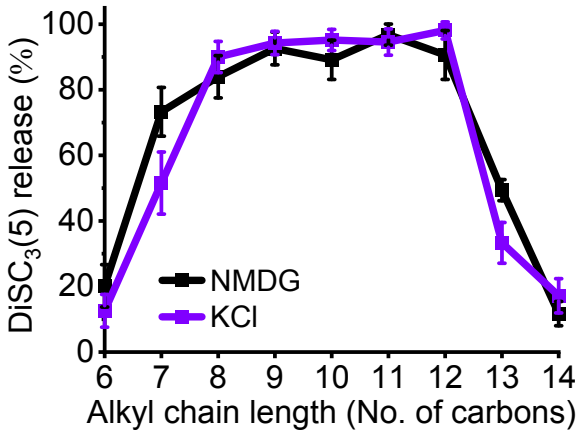


Fig. S3 $DiSC_3(5)$ release elicited by dialkylated lariat ethers: Relative $DiSC_3(5)$ release after 10 min of treatment with 2 µM of various dialkylated lariat ethers in the presence of 60 mM KCl (violet) or 60 mM NMDG-Cl (black). Values are the average ± S.E.M. of values measured at the end of at least three experiments, similar to those described in Supplemental Figure S2.

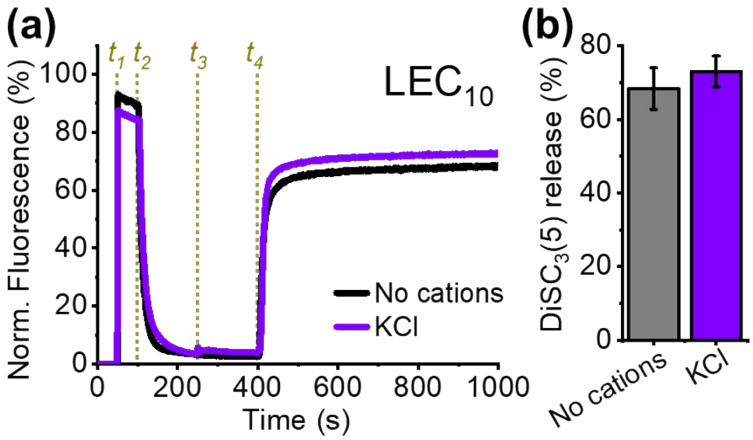


Fig. S4. Depolarizing activity of LEC_{10} in a cation-free solution: (a) Time course of the $DiSC_3(5)$ fluorescence due to the activity of LEC_{10}. This experiment is identical to that described in Supplemental Figure S2e, with the exception that it was performed in a cation-free dextrose solution (200 mM dextrose, 10 mM Tris, pH adjusted to 7.4 with methanesulfonic acid). The events in (a) are: t_1, addition of dye; t_2, addition of *B. subtilis*; t_3, addition of 2 M KCl until a final concentration of 60 mM (violet trace) was reached or the same volume of additional dextrose solution (black trace); t_4, addition of LEC_{10} up to a final concentration of 2 µM. Traces were normalized as described in the Methods section; (b) Relative $DiSC_3(5)$ release after 10 min of treatment with 2 µM LEC_{10} in the presence (violet bar) or the absence (black bar) of 60 mM KCl. The values are averages ± S.E.M. of at least three experiments, similar to those described in (a).

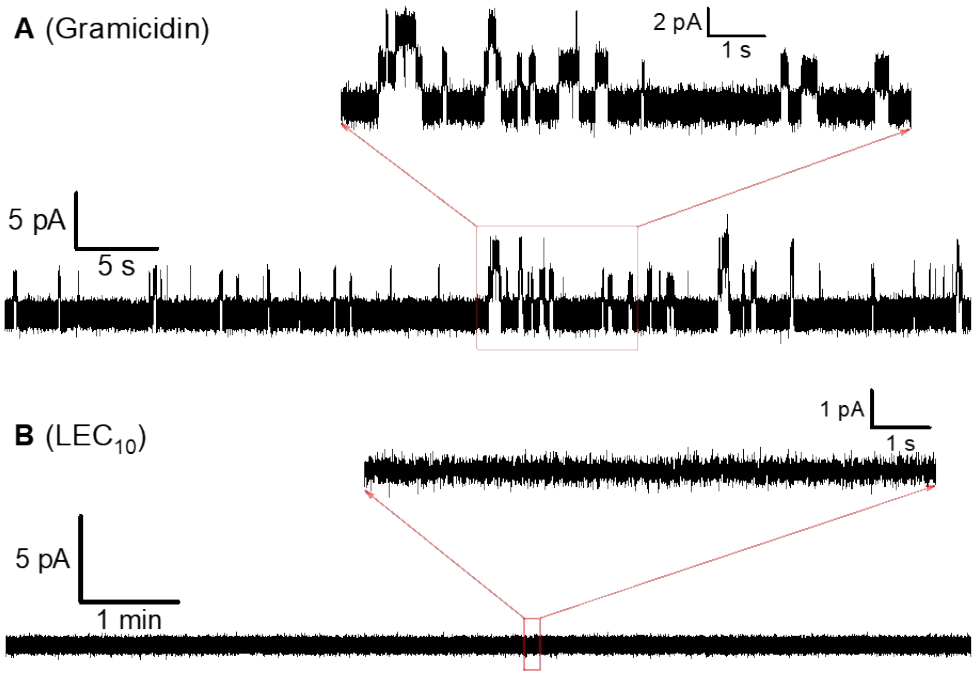


Fig. S5 Lipid bilayer recordings: Current through an asolectin planar lipid bilayer clamped at 100 mV was recorded in the presence of (a) 2 µM Gramicidin and (b) 2 µM LEC_{10}. In both cases, the solution was 150 mM KCl, 10 mM HEPES, pH 7.4.

II. Methods

Chemical synthesis

Symmetrical dialkylated diaza(18-crown-6) ethers with side chains ranging in length between 6 to 14 carbons were synthesized by reductive amination of 4,13-diaza(18-crown-6) with the appropriate aldehyde (as detailed described below in section III). The structures were further confirmed by nuclear magnetic resonance imaging (NMR) and mass spectrometry (MS). Didecyl diaza(18-crown-6) (LEC_{10}) is the only lariat ether that is commercially available (Kryptofix® 22DD; Sigma-Aldrich); the effects of the purchased material were indistinguishable from the synthesized compound.

Toxicity

On bacteria. XL1 blue strain *Escherichia coli* cells transformed with a pET28a plasmid (which contains a kanamycin resistance gene) were grown at 37 °C in 2 mL of LB medium (Miller's LB broth; Research Products International) supplemented with 50 µg/mL of kanamycin until the optical density (600 nm) reached 0.600. The starting density of bacteria in the remainder of the experiments was a 1/100 dilution of this bacterial density, which was split into 4 mL cultures. A series of 400, 100, 10 and 5 mM stock solutions of each dialkylated diaza(18-crown-6) compounds in trifluoroethanol (TFE) were diluted by adding to the 4 mL cultures until final concentrations of 400, 200, 100, 50, 20, 15, 10, 5, 4, 3, 2 and 1 µM were reached, with the final TFE concentration never > 0.1% v/v. Negative controls in the presence of TFE 0.1% v/v or the absence of any treatment were done for each batch. The cultures were incubated at 37 °C with agitation for 12 h. *Bacillus subtilis* (168 WT) toxicity tests were carried out using an identical protocol, except for the addition of the antibiotic. For both bacterial species, the minimum inhibitory concentrations (MIC) were determined as the lowest compound concentration that inhibited growth after 12 h as judged by visual turbidity. Each compound was assayed a minimum of three times at each tested concentration.

On HEK293T cells. Human embryonic kidneys (HEK293T) cells (Thermofisher Scientific) were grown attached in cell culture dishes, detached by trypsin treatment and diluted in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with phenol red indicator (Thermofisher scientific) until the density reached 100,000 viable cells/mL. An aliquot of 1 mL of this cell suspension was added to each well of a 24-wells culture plate. After 2 h, when the cells were already attached, a custom amount of 400, 100, 10 or 5 mM TFE solution of the dialkylated diaza(18-crown-6) ether compounds were added until final concentration of 400, 200, 100, 50, 20, 15, 10, 8, 6, 5, 4, 3, 2 or 1 µM were reached. The final TFE concentration never was over 0.1% v/v. Negative controls in the presence of TFE 0.1% v/v or the absence of any treatment were done for each plate. The plates were cultured at 37 °C. The MIC was determined as the lowest compound concentration that killed all the cells after 48 h, as judged by trypan blue dye. The change in the pH of the medium, as judged by the change in the color (red to yellow) of the medium after 72 h, was also considered; this only occurred in those wells where the cells were still alive and multiplying. Each compound was assayed a minimum of three times at each reported concentration.

$DiSC_3(5)$ depolarization assays

A 2-mL liquid culture of *B. subtilis* cells was grown at 37 °C in LB media until OD_{600} = 0.600, and then collected by centrifugation at 2000 rpm during 3 min. The bacteria were washed once in NMDG-$MeSO_3$ solution (100 mM NMDG, 10 mM HEPES, pH adjusted to 7.4 with methanesulfonic acid) or dextrose solution (200 mM dextrose, 10 mM Tris, pH adjusted to 7.4 with HCl) depending on the experiment. The centrifugation step was repeated and the bacteria were resuspended until OD_{600} = 1.0 in the same solution. The working dye solution was a 200 µM solution of $DiSC_3(5)$ (3,3'-dipropylthiadicarbocyanine iodide; Tokyo Chemical Industry) in DMSO. 2M solutions of NMDG-Cl, NaCl and KCl were prepared, they contained 10 mM HEPES and the pH was adjusted to pH 7.4 with NMDG, NaOH or KOH, respectively, to avoid any pH changes after their addition.
The experiment was initiated with 3 mL of NMDG-$MeSO_3$ or dextrose solution in a quartz cuvette, then 5 µL of the dye solution was added (timepoint t_1, 50 sec; Fig. S2a) for a 0.3 µM final concentration by the end of experiment. This was followed by the addition of 100 µL of the *B. subtilis* suspension (timepoint t_2, 100 sec; Fig. S2a). After 150 sec (timepoint t_3, 250 sec; see Fig. S2a) the fluorescence stabilized to a minimum intensity and 100 µL of 2M NMDG-Cl, 2M NaCl or 2 M KCl solution was added to reach a about 60 mM final concentration of the salt. The final step was the addition 0.64 µL of a 10 mM stock solution of the desired dialkylated lariat ether compound in TFE (timepoint t_4, 400 sec; Fig. S2a), for a final concentration of 2 µM. Negative controls were performed by adding the same volume of TFE without any dialkylated lariat ether. Fluorescence intensity was recorded each second using a Horiba Fluoromax 4 spectrometer (excitation wavelength = 640 nm; emission wavelength = 670 nm). The solution inside the cuvette was vigorously mixed throughout the experiment by using a magnetic stirrer and the temperature was kept constant at 25 °C. To normalize the fluorescence, experiments in the same conditions but without adding bacteria were performed, normalization was done by dividing the fluorescence by the final fluorescence level of these experiments.

Planar lipid bilayer experiments

Experiments in planar lipid bilayers were performed via a Nanion Orbit-mini planar bilayer system. The planar membranes were formed by painting a 25 mg/mL solution of lipids in n-nonane over the four 50 µm apertures on the chip. The lipids used were asolectin from soybean (Sigma-Aldrich) or a 3:1 w/w mix of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) (Avanti Polar lipids). The solutions tested were KCl solution (150 mM KCl, 10 mM HEPES, pH adjusted to 7.4 with KOH), NaCl solution (150 mM NaCl, 10 mM HEPES, pH adjusted to 7.4 with NaOH), and a concentrated KCl solution (500 mM KCl, 10 mM HEPES, pH adjusted to 7.4 with KOH). When the appropriate membrane formed, the voltage was clamped at 100 mV or -100 mV, and a stock solution of gramicidin D (Sigma-Aldrich) in DMSO or LEC_{10} in TFE were added up to the desired concentration. The tested concentration of gramicidin D was 1 nM and 2, 10, 100 and 200 µM were tested for LEC_{10}. Two different temperatures were tested for each condition: 25 and 37 °C. The current was recorded over 10 min for the gramicidin test and up to 1 h for the LEC_{10} test. The data was analysed with Clampfit 10.7 software (Axon Instruments).

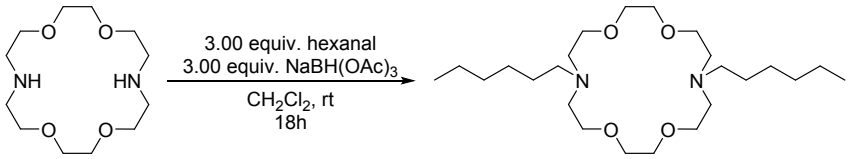
Lactate dehydrogenase activity determination

A 2-mL liquid culture of *B*. *subtilis* cells was grown at 37 °C in media until OD_{600} = 0.600, and then collected by centrifugation at 2000 rpm during 3 min. Bacteria were washed once in lactate/NAD^+ solution (95 mM lithium lactate, 10 mM NAD^+, 10 mM HEPES, pH adjusted to 7.4 with LiOH). The centrifugation step was repeated and the bacteria were resuspended until OD_{600} = 1.0 and then diluted 100X in lactate/NAD^+ solution. The working fluorophore solution was a 1 mM solution of resazurin sodium salt (Alfa Cesar) in water. The experiment was started with 3 mL of lactate/NAD^+ solution in a quartz cuvette, then 3.1 µL of resazurin solution were added (timepoint t_1, 50 sec; see Figure 5B) for a 1 µM final concentration, followed by the addition of 100 µL of the diluted *B*. *subtilis* suspension (timepoint t_2, 100 sec; Fig. 3). After 150 sec (timepoint t_3, 250 sec; Fig. 3) 10 µL of the diaphorase (Worthintong) solution was added. The final step involved the addition of 0.62 µL of a 10 mM stock solution of LEC_{10} in TFE (timepoint t_4, 400 sec; Fig. 3), for a final LEC_{10} concentration of 2 µM. Fluorescence intensity was recorded each second using a Horiba Fluoromax 4 spectrometer (excitation wavelength = 550 nm; emission wavelength = 583 nm). The solution inside the cuvette was vigorously mixed throughout the experiment by using a magnetic stirrer and the temperature was kept constant at 37 °C. As a control, one experiment was performed under the same conditions, except the same amount of TFE was added without the dialkylated lariat ether, up to a final concentration of 0.02%. To normalize, the fluorescence was divided by the fluorescence level just before timepoint t_4.

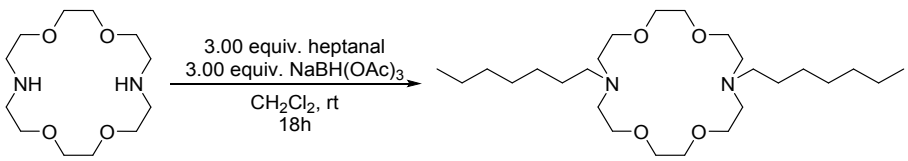
III. Synthesis of Dialkylated Diaza(18-Crown-6) Lariat Ethers

All glassware was either oven dried at 140°C or flame dried under vacuum and purged with nitrogen immediately prior to use. Hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanol, and pyridinium chlorochromate (PCC) were obtained from Millipore Sigma. Unless otherwise specified, reagents were used as obtained from the supplier without further purification. Acetonitrile (MeCN), toluene, and dichloromethane (CH_2Cl_2) were freshly distilled from calcium hydride or passed through an alumina column immediately prior to use. Other solvents were purified using accepted procedures from the sixth edition of "Purification of Laboratory Chemicals".[1] Air- and moisture- sensitive reactions were performed using standard Schlenk techniques under an inert nitrogen atmosphere, unless otherwise specified. Analytical thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F24 plates containing a fluorescent indicator. Reaction products were visualized using 254 nm UV light and ceric ammonium molybdate (CAM), $KMnO_4$, and I_2 stains unless otherwise specified. Preparative chromatography using a gradient method with mixtures of MeOH and CH_2Cl_2 or EtOAc and hexanes, unless otherwise specified, was performed using SilicaFlash P60 silica gel (230-400 mesh) via Still's method.[2]

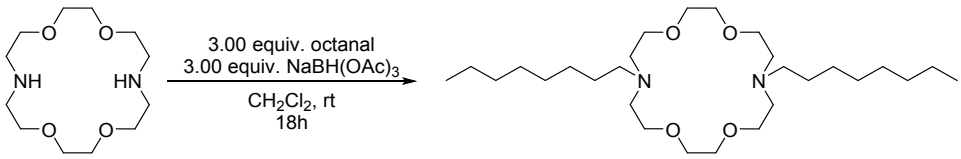
^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker Avance-500 spectrometers. Chemical shifts are reported relative to the tetramethylsilane peak (δ 0.00 ppm). Accurate mass measurements were acquired at the University of Wisconsin, Madison, using a Micromass LCT (electrospray ionization or electron impact methods).



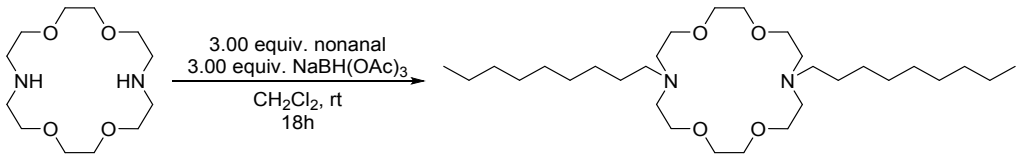
Dihexyl diaza(18-crown-6) (LEC$_6$). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.14 mL (1.14 mmol, 3.00 equiv.) hexanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC$_6$**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 95.0 mg (0.22 mmol, 58%) of **LEC$_6$**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.71 – 3.51 (m, 16H), 2.85 – 2.69 (m, 8H), 2.58 – 2.41 (m, 4H), 1.51 – 1.37 (m, 4H), 1.35 – 1.20 (m, 12H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.1, 56.1, 53.9, 31.8, 27.2, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{24}H_{50}N_2O_4$ $[M + H]^+$ 431.3843, found 431.3843.



Diheptyl diaza(18-crown-6) (LEC_7). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.16 mL (1.14 mmol, 3.00 equiv.) heptanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_7**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 49.0 mg (0.11 mmol, 29%) of **LEC_7**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.64 – 3.56 (m, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.51 – 2.44 (m, 4H), 1.49 – 1.39 (m, 4H), 1.33 – 1.21 (m, 16H), 0.91 – 0.84 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.8, 70.1, 70.1, 56.1, 54.0, 31.9, 29.3, 27.5, 27.3, 22.6, 14.1. HRMS (ESI) *m/z* calculated for $C_{26}H_{54}N_2O_4$ $[M + H]^+$ 459.4156, found 459.4153.

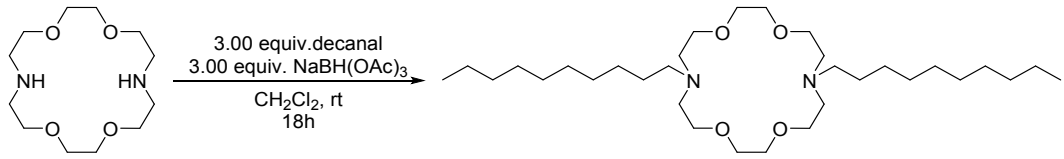


Dioctyl diaza(18-crown-6) (LEC_8). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.18 mL (1.14 mmol, 3.00 equiv.) octanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_8**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 49.0 mg (0.10 mmol, 26%) of **LEC_8**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.64 – 3.56 (m, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.53 – 2.44 (m, 4H), 1.48 – 1.38 (m, 4H), 1.32 – 1.18 (m, 20H), 0.91 – 0.86 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.7, 69.8, 55.9, 53.8, 31.8, 29.7, 29.5, 29.4, 29.3, 27.5, 22.7, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{28}H_{58}N_2O_4$ $[M + H]^+$ 487.4469, found 487.4471.

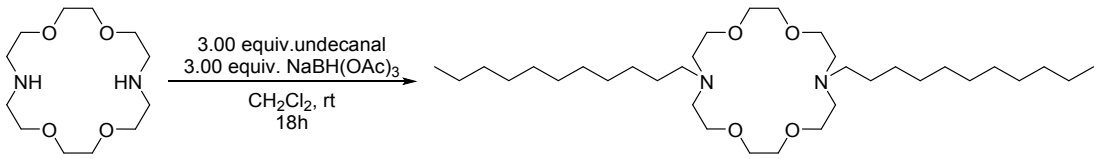


Dinonyl diaza(18-crown-6) (LEC_9). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.20 mL (1.14 mmol, 3.00 equiv.) nonanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_9**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 66.0 mg (0.13 mmol, 34%) of **LEC_9**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (d, *J* = 7.4 Hz, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.52 – 2.43 (m, 4H), 1.48 – 1.38 (m, 4H), 1.26 (s, 24H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.0, 56.1,

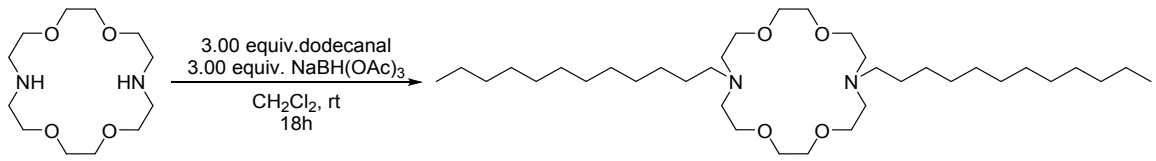
53.9, 31.9, 29.7, 29.6, 29.6, 29.3, 27.5, 27.3, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{30}H_{62}N_2O_4$ $[M + H]^+$ 515.4782, found 515.4787.



Didecyl diaza(18-crown-6) (LEC_{10}). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.21 mL (1.14 mmol, 3.00 equiv.) decanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_{10}**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 19.0 mg (0.03 mmol, 8%) of **LEC_{10}**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (d, *J* = 8.6 Hz, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.52 – 2.43 (m, 4H), 1.43 (dq, *J* = 13.3, 6.7, 6.0 Hz, 4H), 1.26 (s, 28H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.1, 56.1, 54.0, 31.9, 29.7, 29.7, 29.6, 29.6, 29.3, 27.5, 27.3, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{32}H_{66}N_2O_4$ $[M + H]^+$ 543.5095, found 543.5097.

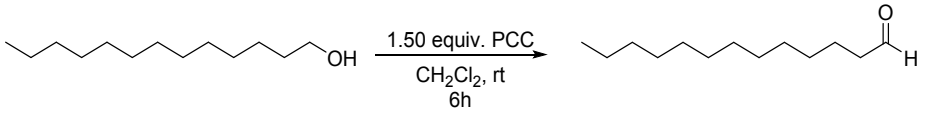


Diundecyl diaza(18-crown-6) (LEC_{11}). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.24 mL (1.14 mmol, 3.00 equiv.) undecanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_{11}**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 119 mg (0.21 mmol, 55%) of **LEC_{11}**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.76 – 3.47 (m, 16H), 2.90 – 2.64 (m, 8H), 2.57 – 2.41 (m, 4H), 1.51 – 1.37 (m, 4H), 1.34 – 1.18 (m, 32H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.7, 70.0, 63.1, 56.0, 53.9, 32.8, 31.9, 31.9, 29.6, 29.6, 29.4, 29.4, 27.5, 27.2, 25.7, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{34}H_{70}N_2O_4$ $[M + H]^+$ 571.5408, found 571.5405.

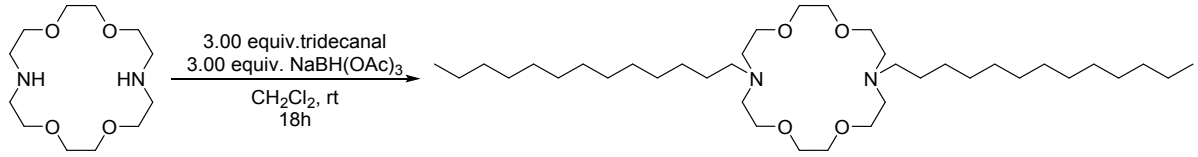


Didodecyl diaza(18-crown-6) (LEC_{12}). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.25 mL (1.14 mmol,

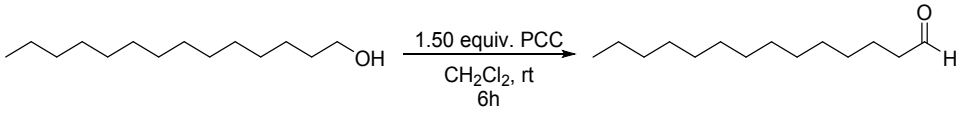
3.00 equiv.) dodecanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_{12}**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 118 mg (0.20 mmol, 53%) of **LEC_{12}**as a white solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.61 (d, *J* = 8.7 Hz, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.51 – 2.44 (m, 4H), 1.76 – 1.69 (m, 2H), 1.43 (dq, *J* = 12.9, 6.9, 6.5 Hz, 4H), 1.26 (s, 34H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.1, 56.1, 54.0, 31.9, 29.7, 29.7, 29.6, 29.4, 27.5, 27.3, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{36}H_{74}N_2O_4$ $[M + H]^+$ 599.5721, found 599.5716.



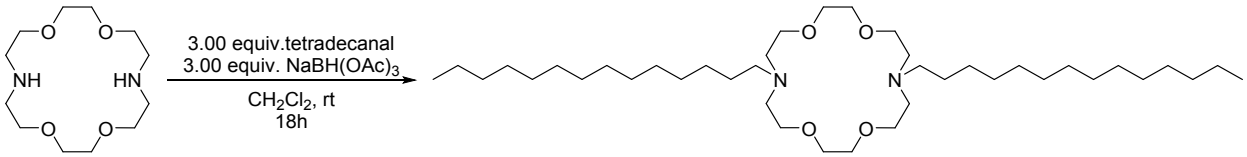
Tridecanal. To a stirred solution of 1.00 g (4.99 mmol, 1.00 equiv.) tridecanol in 50 mL CH_2Cl_2 was added 1.61 g (7.49 mmol, 1.50 equiv.) PCC. The resulting black solution was stirred at room temperature for 6h, then 1.61 g celite was added and the light brown suspension stirred at room temperature for an additional 30 minutes. The crude reaction mixture was then filtered through a plug of silica gel using CH_2Cl_2 as eluent to afford 732 mg (3.69 mmol; 74%) tridecanal as a white solid which was used without any additional purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.9 Hz, 1H), 2.41 (td, *J* = 7.3, 1.9 Hz, 2H), 1.63 (p, *J* = 7.4 Hz, 2H), 1.37 – 1.20 (m, 18H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.9, 43.9, 31.9, 29.6, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 22.7, 22.1, 14.1. HRMS (ESI) *m/z* calculated for $C_{13}H_{26}O$ $[M + H]^+$ 199.2056, found 199.2055.



Ditridecyl diaza(18-crown-6) (LEC_{13}). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 0.27 mL (1.14 mmol, 3.00 equiv.) tridecanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_{13}**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 58.0 mg (0.09 mmol, 24%) of **LEC_{13}**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.66 – 3.55 (m, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.51 – 2.45 (m, 4H), 1.78 (s, 2H), 1.43 (p, *J* = 7.1 Hz, 4H), 1.25 (s, 38H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.1, 56.1, 54.0, 31.9, 29.7, 29.7, 29.7, 29.6, 29.4, 27.5, 27.3, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{38}H_{78}N_2O_4$ $[M + H]^+$ 627.6034, found 627.6021.

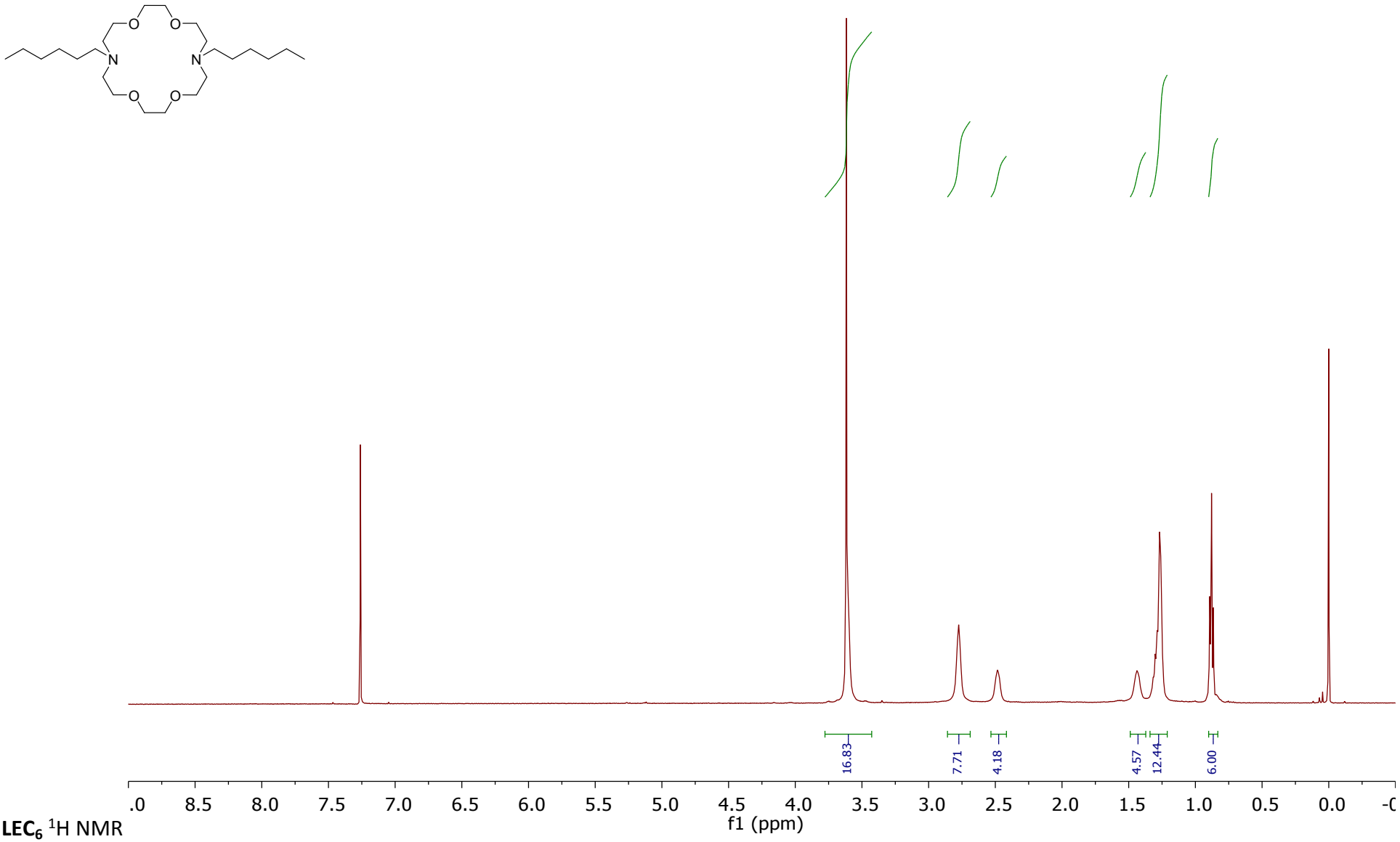


Tetradecanal. To a stirred solution of 1.00 g (4.66 mmol, 1.00 equiv.) tetradecanol in 47 mL CH_2Cl_2 was added 1.51 g (6.99 mmol, 1.50 equiv.) PCC. The resulting black solution was stirred at room temperature for 6h, then 1.51 g celite was added and the light brown suspension stirred at room temperature for an additional 30 minutes. The crude reaction mixture was then filtered through a plug of silica gel using CH_2Cl_2 as eluent to afford 801 mg (3.77 mmol; 81%) tetradecanal as a white solid which was used without any additional purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (t, *J* = 1.9 Hz, 1H), 2.42 (td, *J* = 7.3, 1.9 Hz, 2H), 1.63 (p, *J* = 7.4 Hz, 2H), 1.37 – 1.19 (m, 20H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.9, 43.9, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.2, 22.7, 22.1, 14.1. HRMS (ESI) *m/z* calculated for $C_{14}H_{28}O$ $[M + H]^+$ 213.2213, found 213.2210.



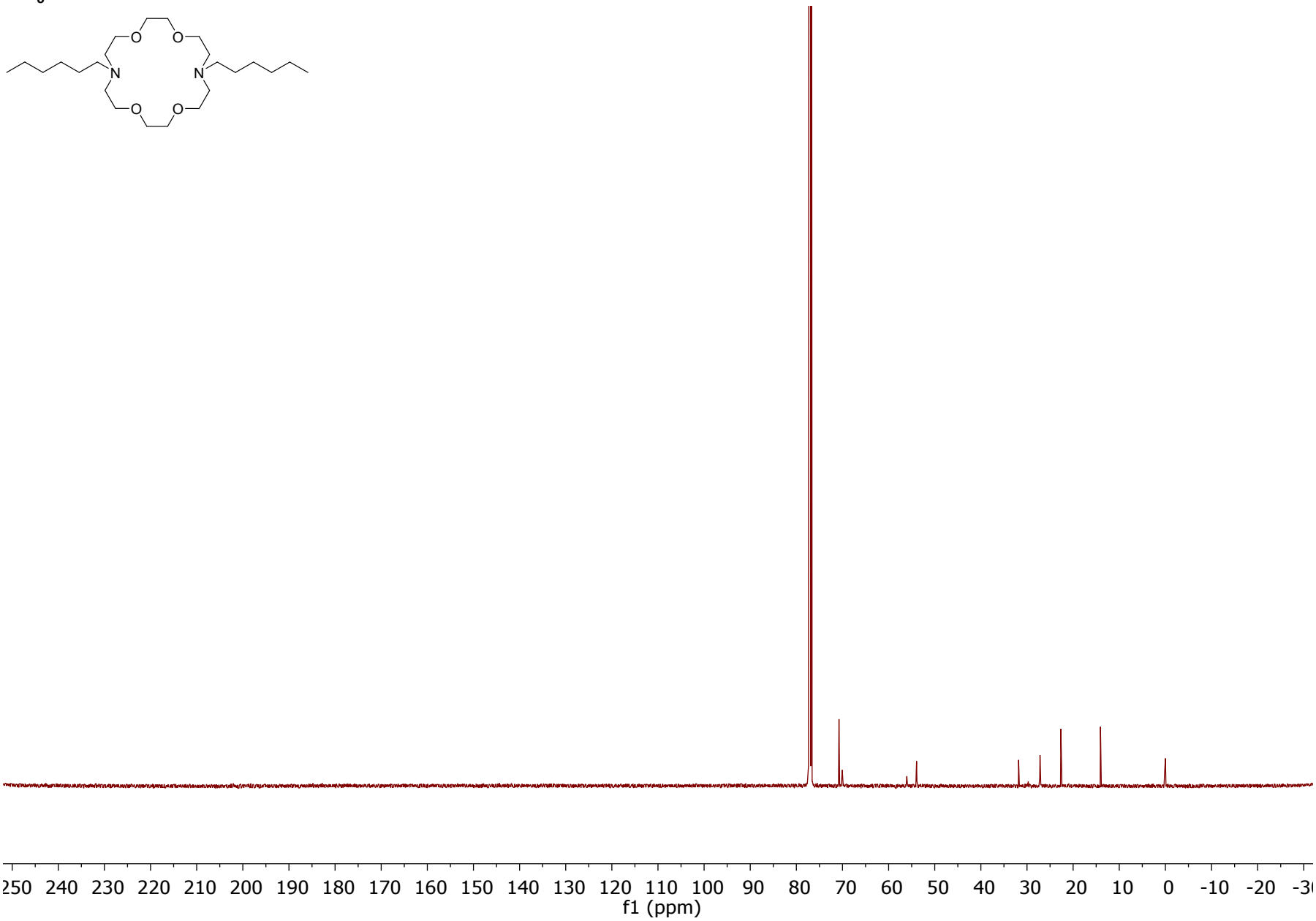
Ditetradecyl diaza(18-crown-6) (LEC_{14}). To a stirred solution of 100.0 mg (0.38 mmol, 1.00 equiv.) 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane in 3.80 mL CH_2Cl_2 was added 242 mg (1.14 mmol, 3.00 equiv.) tetradecanal, followed by 242 mg (1.14 mmol, 3.00 equiv.) $NaBH(OAc)_3$. The white suspension was stirred at room temperature for 18 h, then filtered through a pad of celite. The filter pad was washed with additional CH_2Cl_2, and the volatiles removed in vacuo to afford crude **LEC_{14}**. The crude material was purified via flash column chromatography on alumina using a 0-25% gradient of EtOAc in hexanes to afford 78.0 mg (0.12 mmol, 32%) of **LEC_{14}**as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 – 3.53 (m, 16H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.52 – 2.42 (m, 4H), 1.58 (s, 8H), 1.43 (s, 4H), 1.25 (s, 36H), 0.88 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 70.8, 70.1, 56.1, 54.0, 31.9, 29.7, 29.7, 29.7, 29.4, 27.5, 27.3, 22.7, 14.1. HRMS (ESI) *m/z* calculated for $C_{40}H_{82}N_2O_4$ $[M + H]^+$ 655.6347, found 655.6339.

IV. NMR Spectra

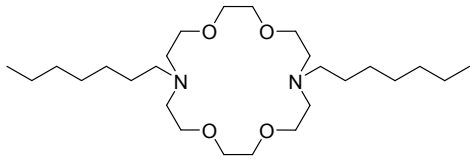


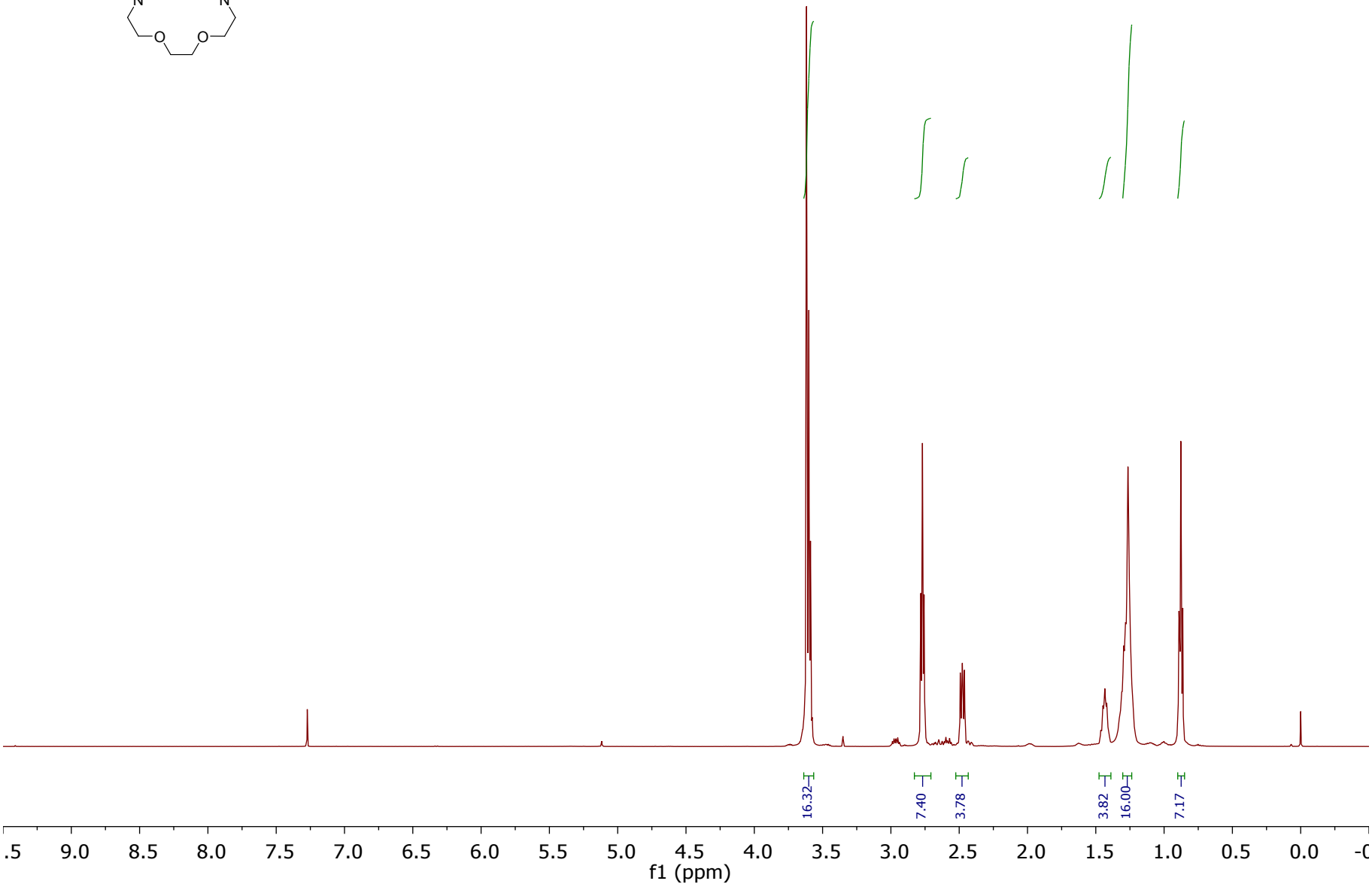
LEC$_6$ ^{1}H NMR

LEC$_6$ ^{13}C NMR

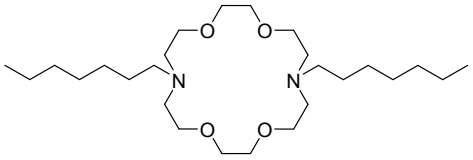

O
O
N
N
O
O
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

LEC$_7$ ^{1}H NMR


O
O
N
N
O
O

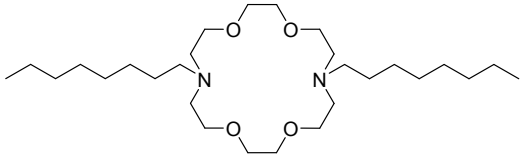

16.32
7.40
3.78
3.82
16.00
7.17
.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -(
f1 (ppm)

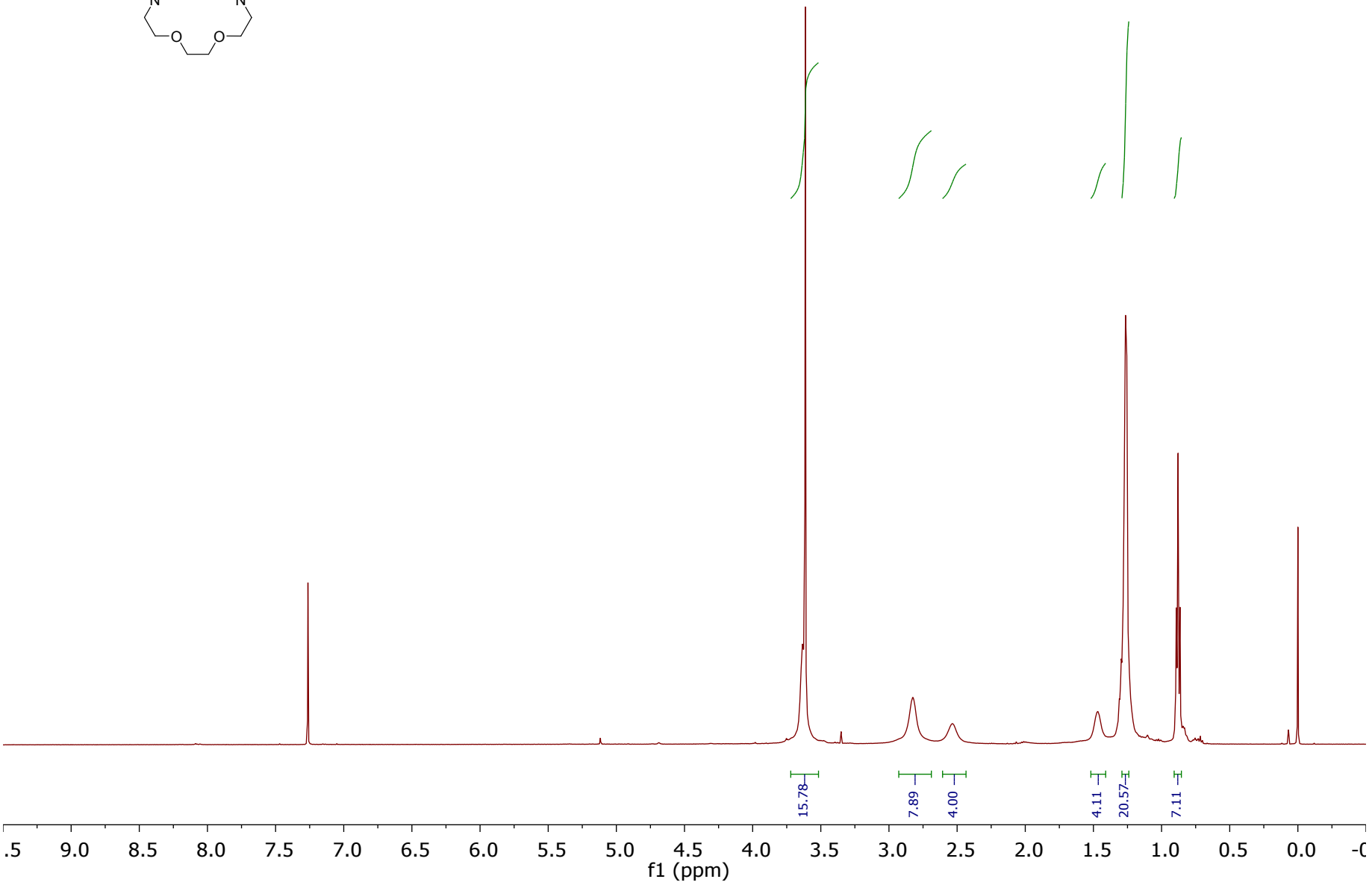
LEC$_7$ ^{13}C NMR


O
O
N
N
O
O

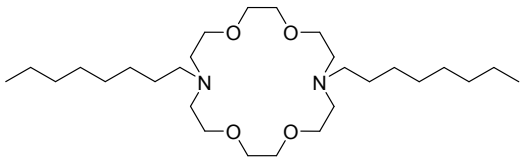
70.8
70.8
70.1
70.1
56.1
54.0
31.9
29.3
27.5
27.3
22.6
14.1
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

LEC_8 ^{1}H NMR


O
O
N
N
O
O

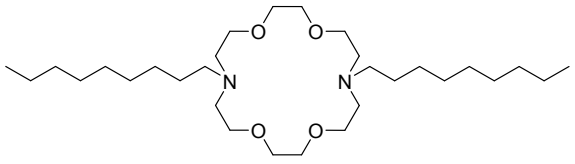

15.78
7.89
4.00
4.11
20.57
7.11
.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

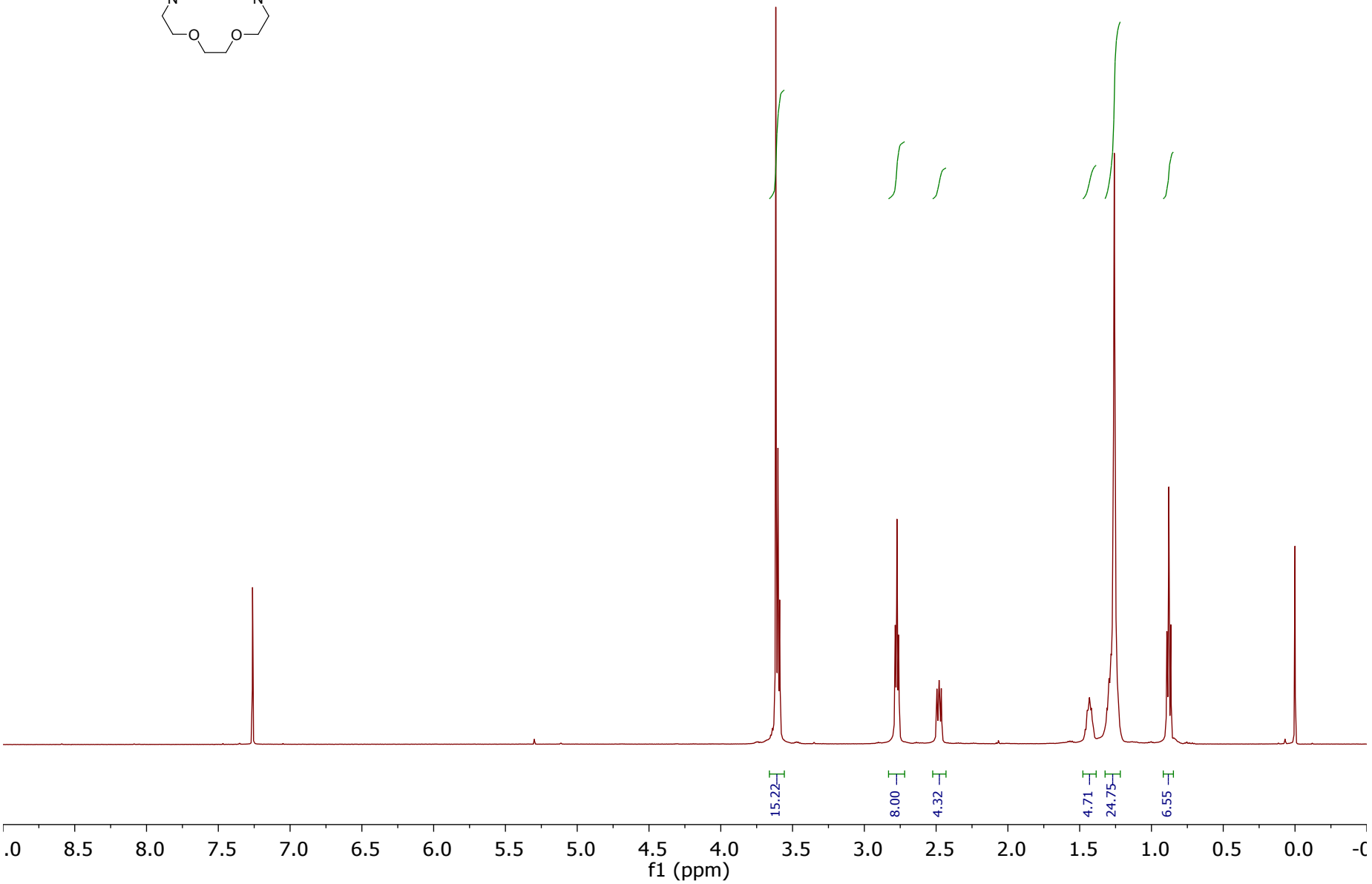
LEC$_8$ ^{13}C NMR


O
O
N
N
O
O

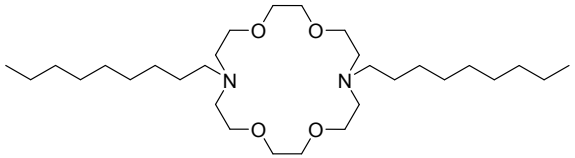
70.7
69.8
55.9
53.8
31.8
29.7
29.5
29.4
29.3
27.5
22.7
22.7
14.1
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -3
f1 (ppm)

LEC$_9$ ^{1}H NMR


O
O
N
N
O
O

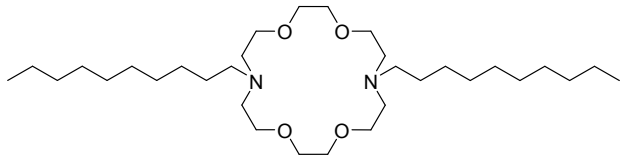

15.22
8.00
4.32
4.71
24.75
6.55
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

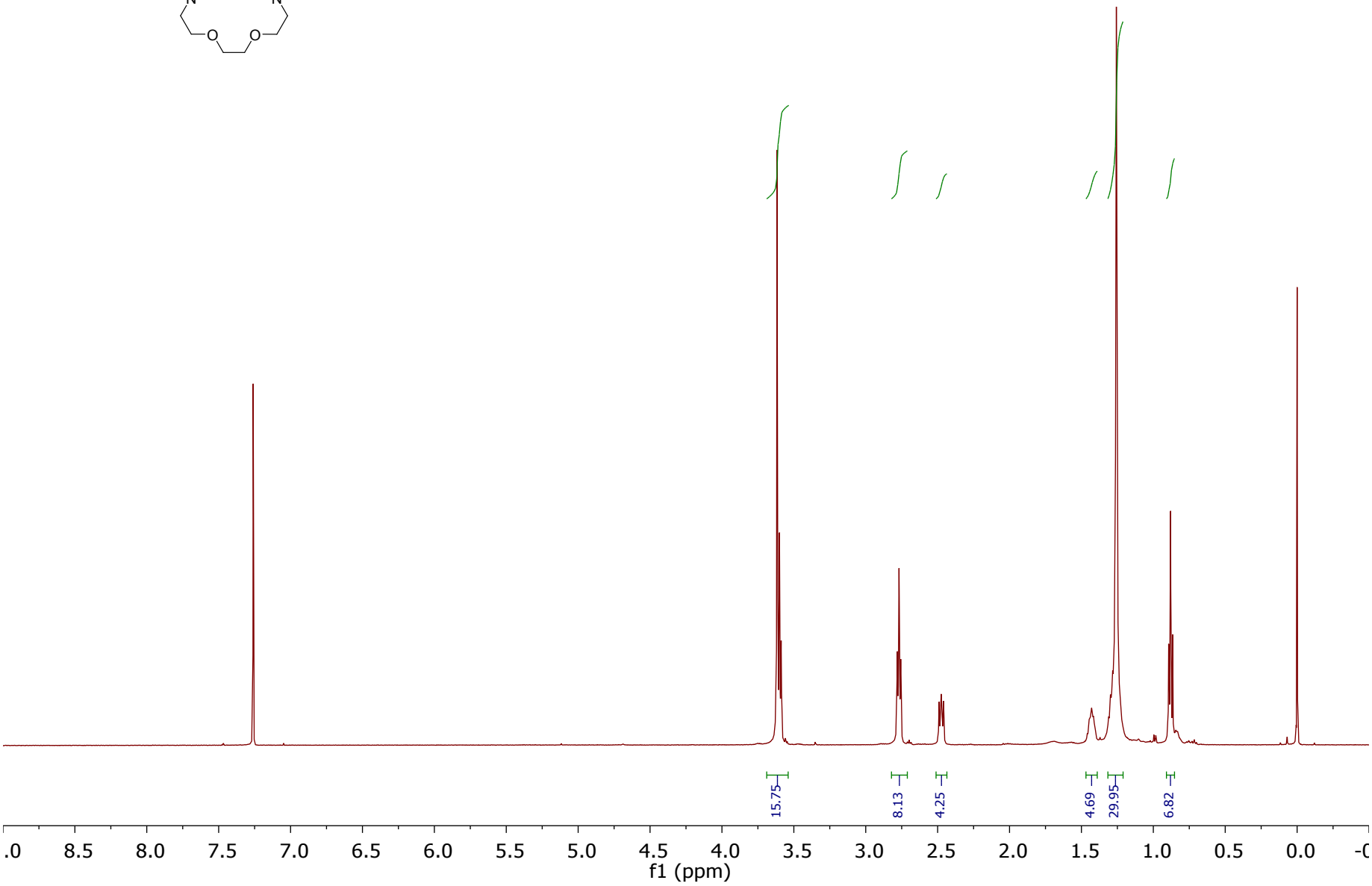
LEC$_9$ ^{13}C NMR


O
O
N
N
O
O

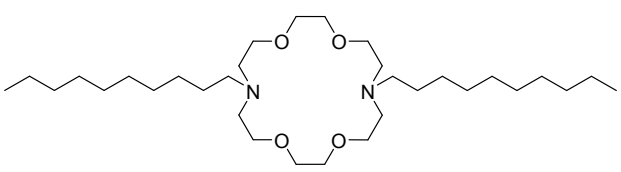
70.8
70.0
56.1
53.9
31.9
29.7
29.6
29.6
29.3
27.5
27.3
22.7
14.1
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

LEC_{10} ^{1}H NMR

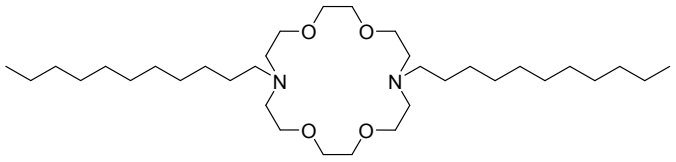

O
O
N
N
O
O

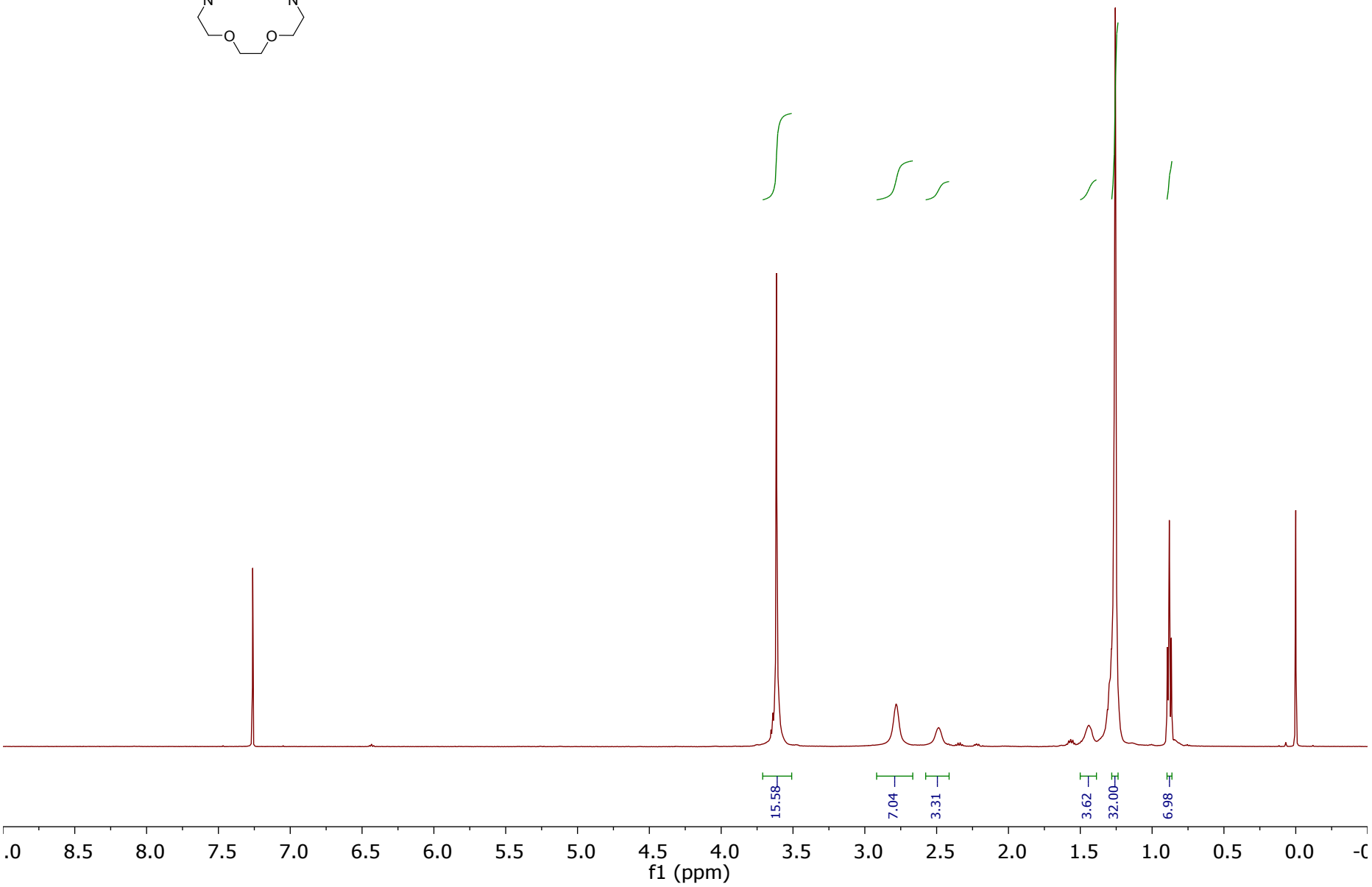

15.75
8.13
4.25
4.69
29.95
6.82
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

LEC_{10} ^{13}C NMR

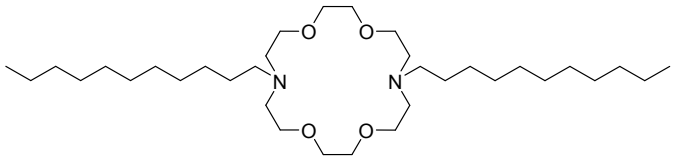


LEC_{11} ^{1}H NMR

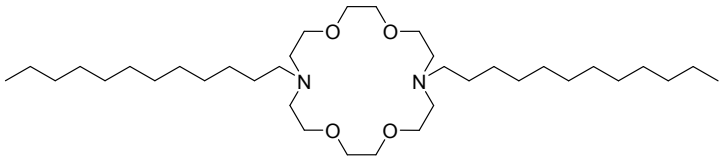

O
O
N
N
O
O

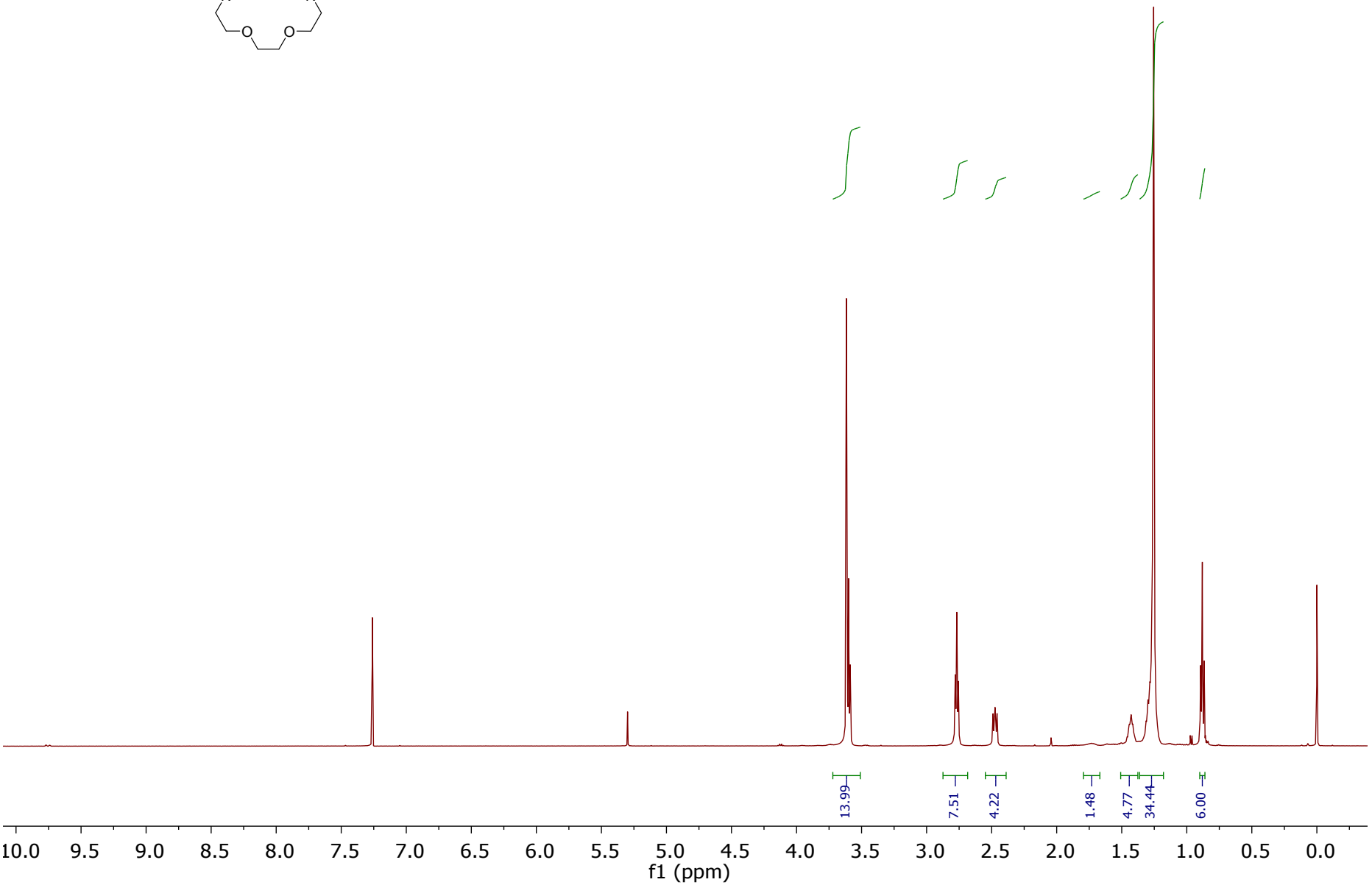

15.58
7.04
3.31
3.62
32.00
6.98
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

LEC$_{11}$ ^{13}C NMR

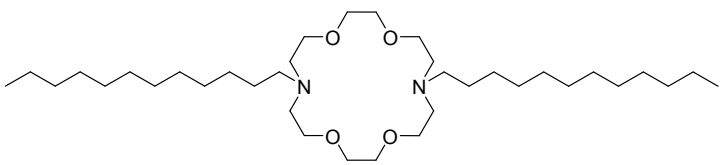


LEC_{12} ^{1}H NMR

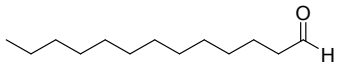

O
O
N
N
O
O

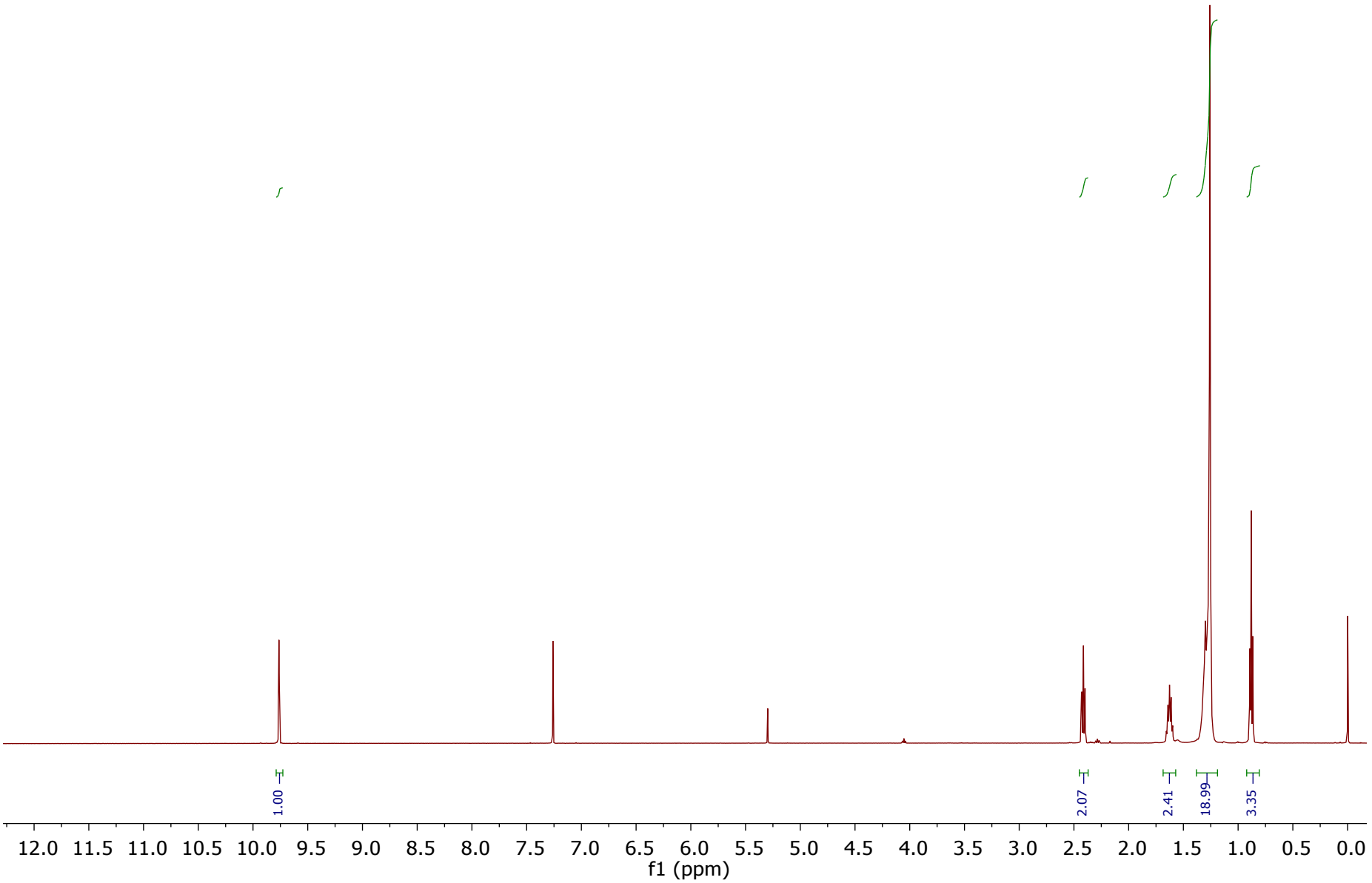

13.99
7.51
4.22
1.48
4.77
34.44
6.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

LEC_{12} ^{13}C NMR

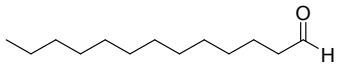


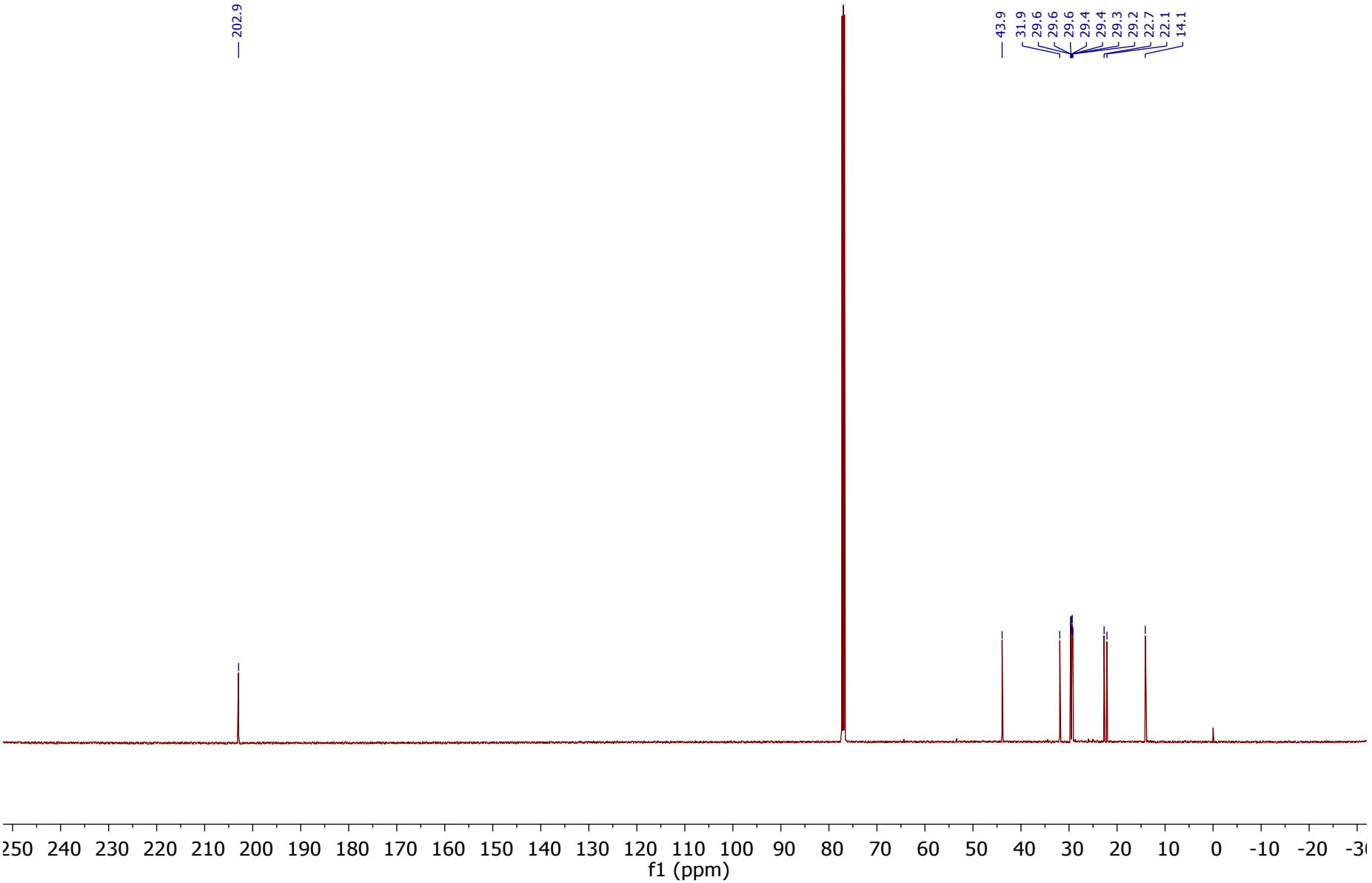
Tridecanal ^{1}H NMR



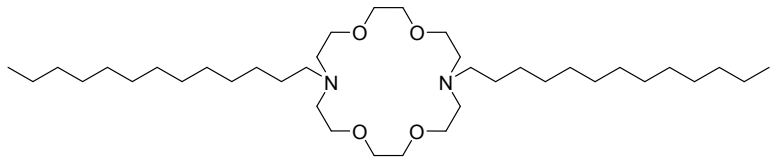


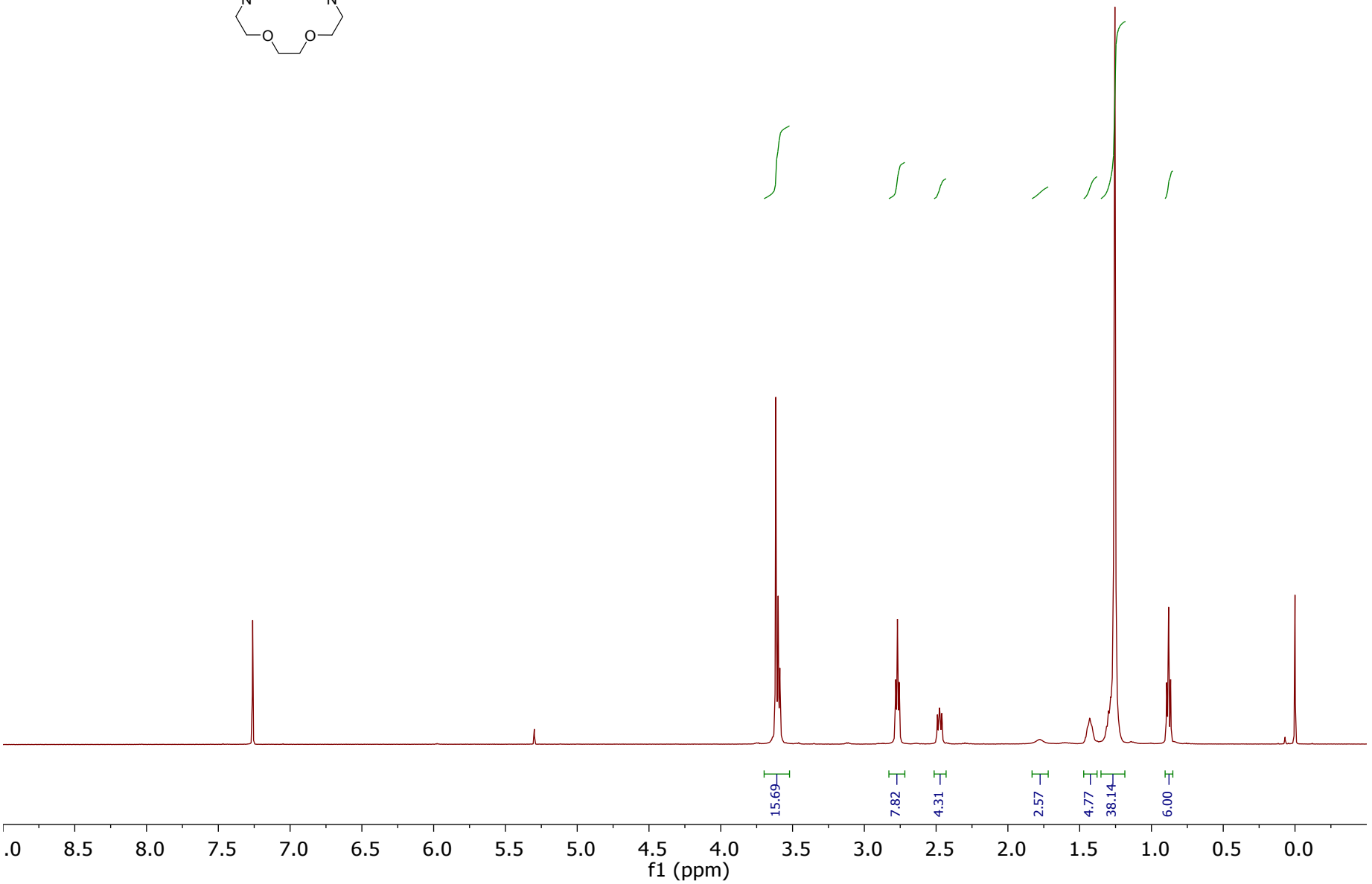
Tridecanal ^{13}C NMR



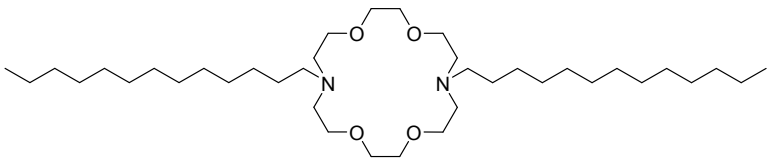


LEC_{13} ^{1}H NMR

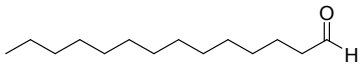

O
O
N
N
O
O

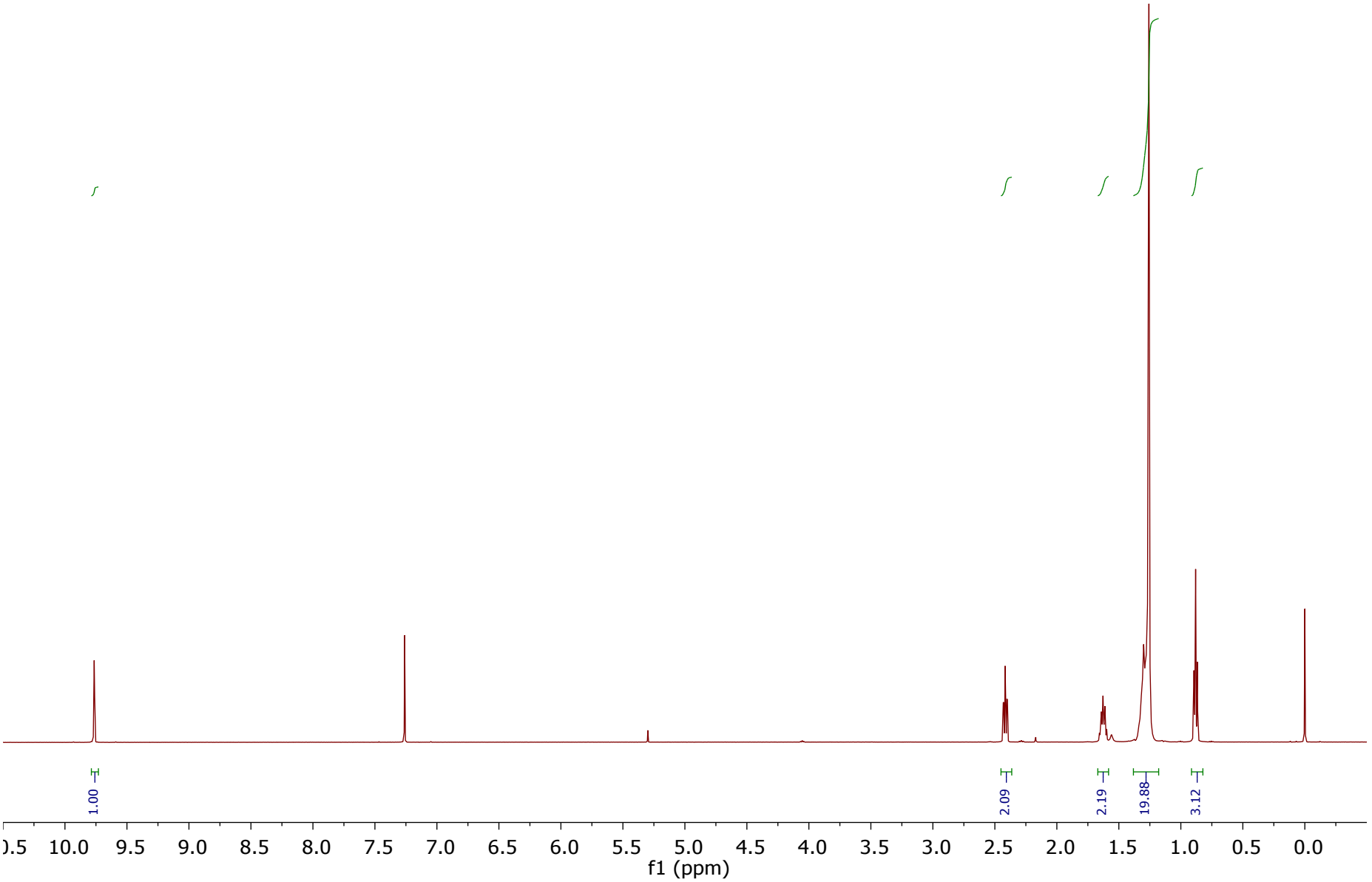

15.69
7.82
4.31
2.57
4.77
38.14
6.00
.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

LEC$_{13}$ ^{13}C NMR

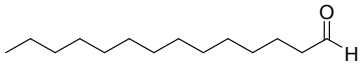


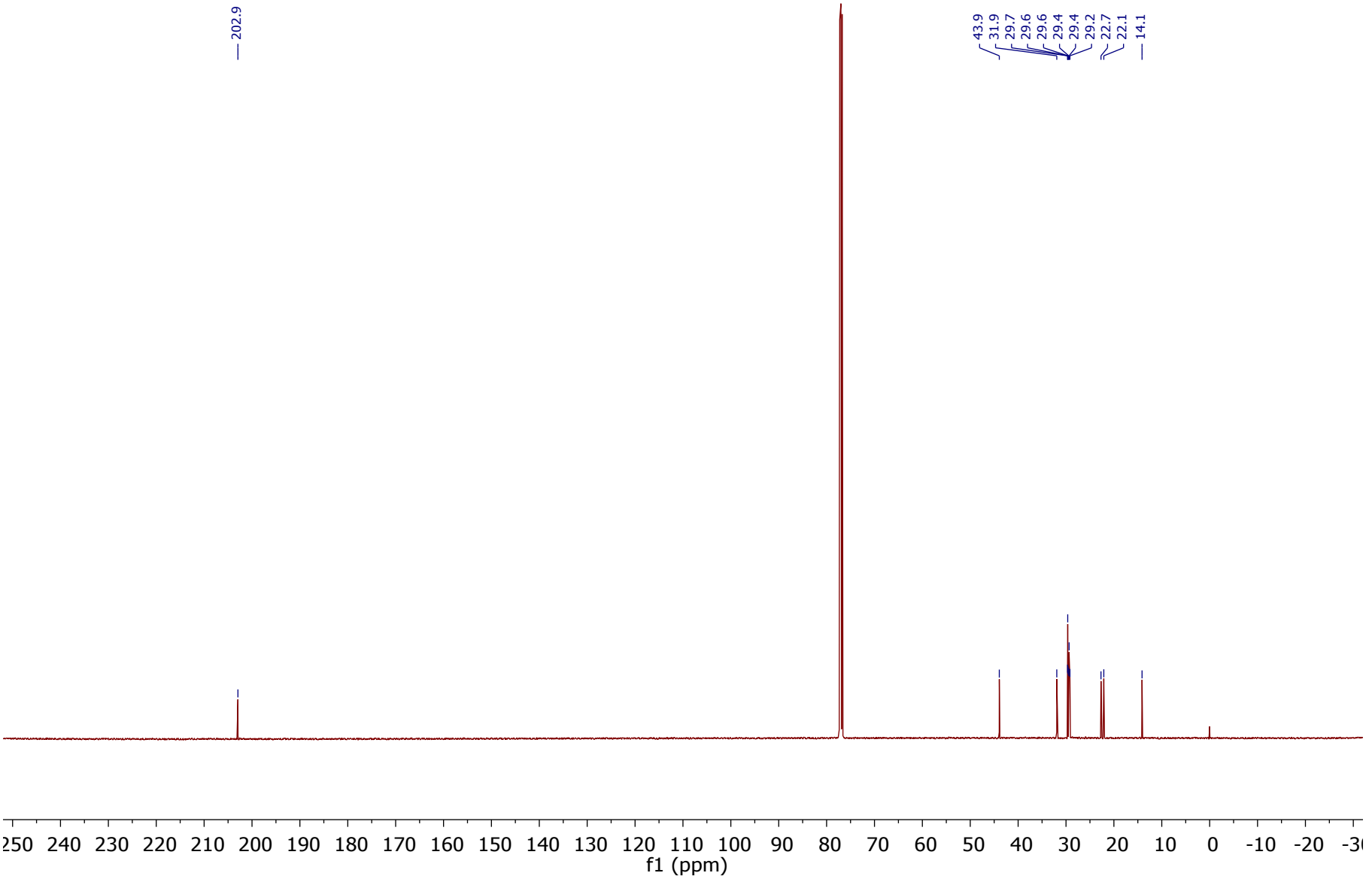
Tetradecanal ^{1}H NMR



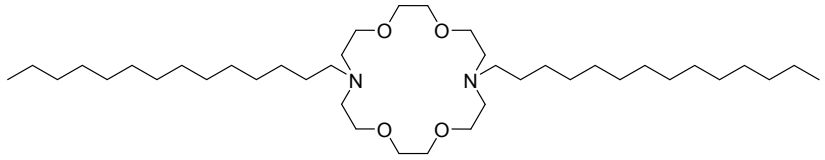


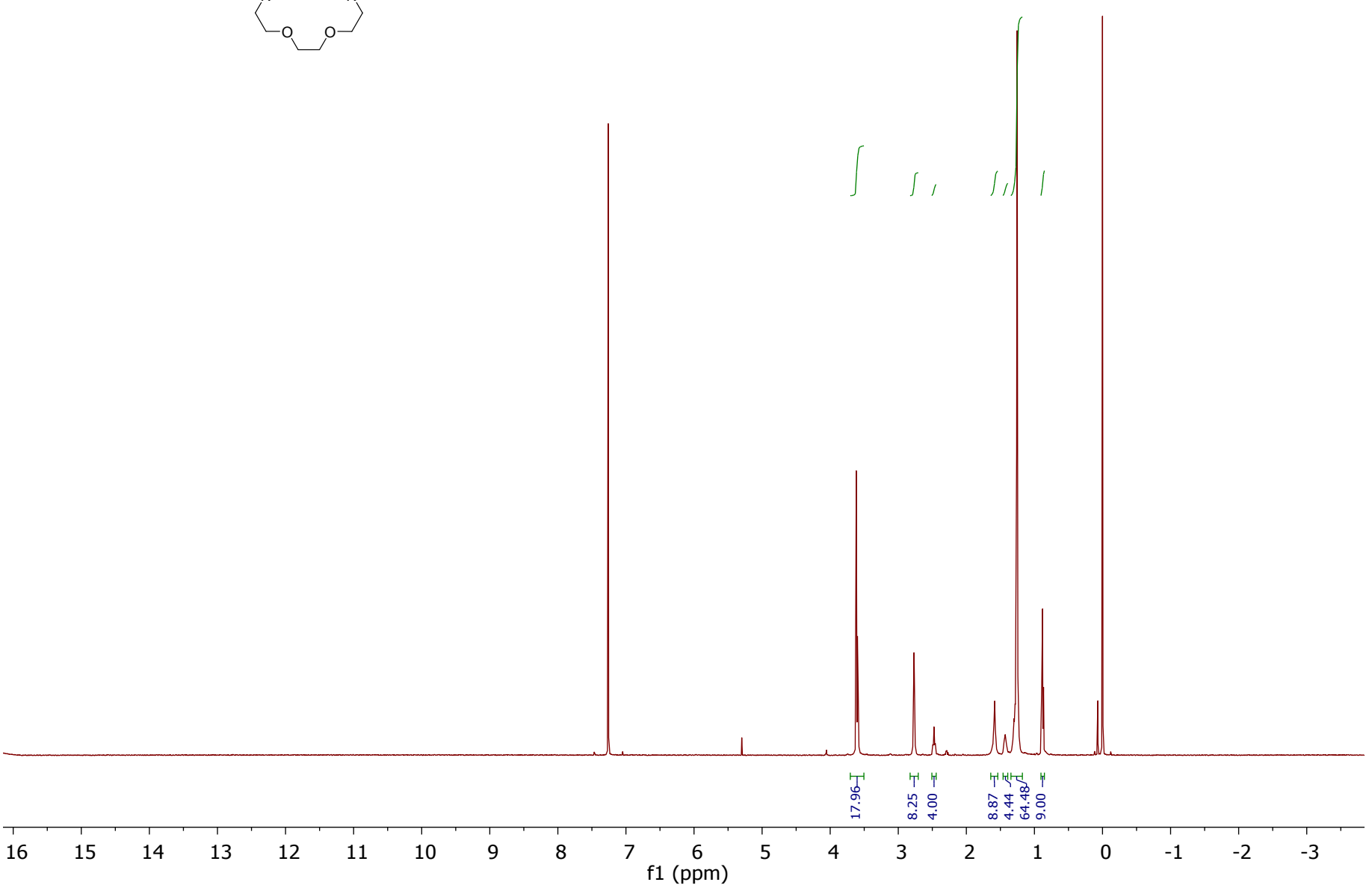
Tetradecanal ^{13}C NMR



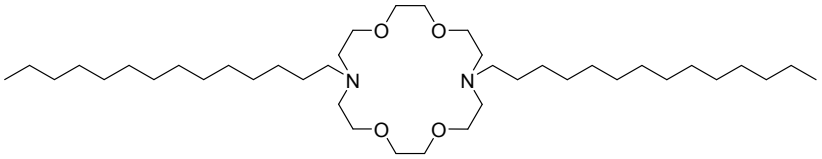


LEC_{14} ^{1}H NMR


O
O
N
N
O
O


17.96
8.25
4.00
8.87
4.44
64.48
9.00
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)

LEC_{14} ^{13}C NMR



IV. References

1. Armarego, W. L. F.; Chai, C. L. L. Chapter 4 – Purification of Organic Chemicals. In *Purification of Laboratory Chemicals (Sixth Edition)*, Butterworth-Heinemann: Oxford, 2009; pp 88-444.
2. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, 43, 2923-2925.