SUPPORTING INFORMATION

Protein Expression and Purification. *E. coli* XL-1 Blue was used as a bacterial host for manipulation of plasmid DNA and standard molecular cloning techniques were employed throughout the construction of the DEBS overexpression clones. DNA sequence analysis of all clones generated in this study was performed at the University of Michigan DNA sequencing core facility. PCR amplifications were performed using pFU Turbo DNA Polymerase (Stratagene) or LA-Taq DNA polymerase (Takara Bio). The DEBS module 5 was amplified from cosmid pDHS1178, previously identified to contain the DEBS PKS gene cluster, by PCR using the following primer pair: 5'-gggaggagcatatgagcggtgacaacggc-3' and 5'-gagggatccttagagccgctccaggtagtg-3'. Following restriction digestion with *Nde*I and *Bam*HI, the purified PCR product was ligated into similarly digested pET28b to give the Ery5 overexpression plasmid pDHS1177. Similarly, the DEBS module 6 was PCR amplified from the following pair of oligonucleotides: 5'-gaggctagcgacccgatcgcgatcg-3' and 5'-ctcctcctcgagtcatgaattccctccgcccag-3'. Upon restriction digest with *Nhe*I and *Hind*III, the PCR product was cloned into pET21b to give the Ery6 expression plasmid pDHS1174. The unnatural fusion protein, Ery5-TE, DEBS module 5 was constructed in two steps. First, module 5 was PCR amplified as above using primers designed with engineered *Nde*I and *Bam*HI restriction sites; however, in this case, the *Bam*HI containing reverse primer was slightly redesigned to eliminate inclusion of the stop codon. The resulting PCR fragment was ligated into pET21b following restriction digestion with *Nde*I and *Bam*HI to give plasmid pJKDEBS5. In a second PCR reaction, DNA encoding the DEBS TE was amplified from the *Bam*HI restriction site containing primer 5'-

gagggatcccagcagctcgacagcgggact-3' and the *Hind*III restriction site containing primer 5'-ctcctcctcgagtcatgaattccctccgcccag -3'. The doubly digested PCR product was subsequently cloned into similary-digested pJKDEBS5 to yield the Ery5-TE overexpression plasmid pDHS1172. Cloning of PikAIII, PikAIII-TE, PikAIV, Pik TE and DEBS TE have been previously described.[1-3]

Protein Expression and Purification. The expression and purification of all proteins has been previously reported[1-4] and similar protocols were followed in this study. The expression of each module containing polypeptide (PikAIII, PikAIV, PikAIII-TE, Ery5, Ery6, Ery5-TE) was accomplished in the *E. coli* (BAP1) expression strain, a bacterial strain that has been genetically engineered to express type I modular PKS proteins.[5] This *E. coli* strain encodes the phosphopanteitheinyl transferase (*pptase*) gene from *B. subtilis* on its chromosome. Expression of the heterologous Pptase ensures the efficient post-translational modification of ACP domains. *E. coli* (BAP1) was generously provided to the Sherman laboratory by Stanford University Professor Chaitain Khosla. BL21(DE3) *E. coli* was used to express Pik TE and DEBS TE. Cultures were grown at 37 °C with shaking to an OD_{600} of 0.8-1.0 in TB media. Protein expression was induced with the addition of IPTG to a final concentration of 0.4 mM. The cultures continued to incubate, with shaking, at 18° C for 16-18 hours (overnight).

Each polyhistidine-tagged protein was subsequently purified by nickel-affinity chromatography. Cells from overexpression cultures (typically 1 L) were harvested by centrifugation (5000g, 12 min, 4 ºC) and resuspended in approximately 25 mL lysis buffer (50 mM Tris, pH 7.5, 300 mM NaCl, 10% glycerol, 20 mM imidazole). Following membrane disruption via sonication (6 x 20 s with 30 s intervening between pulses), the

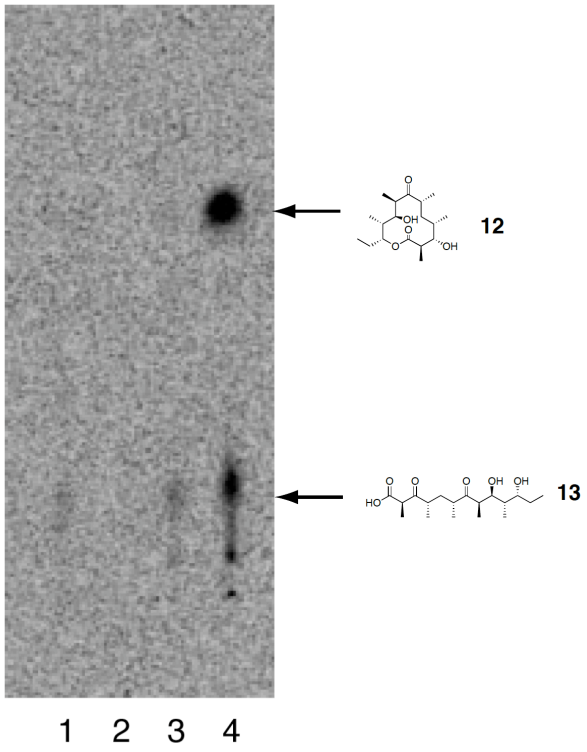
cellular debris was pelleted by centrifugation (40,000g, 30 minutes, 4 ºC). The cell free lysate was sterile filtered with a 0.45 mm syringe filter after which it was directly loaded onto a 5 mL his-trap column using an AKTA FPLC (GE Healthcare). Polyhistidine-tagged proteins were eluted with a linear gradient of lysis buffer with 400 mM imidazole and fractionated into 4 mL fractions. Protein containing fractions, as identified by A280 intensities and confirmed by SDS-PAGE, were pooled, concentrated and exchanged into storage buffer (100 mM NaH_2PO_4, pH 7.2, 1 mM EDTA, 1 mM TCEP, 20% glycerol) using PD-10 desalting columns (GE Healthcare). Protein purity was confirmed to be greater than 95% by SDS-PAGE analysis. Purified proteins were dispensed into 100 μL aliquots, flash frozen in liquid nitrogen and stored at -80 °C. Final protein concentrations were determined A280 measurements, from theoretical extinction coefficients.

Biochemical Assay of DEBS and Pik monomodules. Reactivity of PikAIII, PikAIV, Ery5, Ery5-TE and Ery6 with native pentaketide substrates was assessed by incorporation of 2-[^{14}C]-methylmalonyl CoA to form extended polyketide products. 2-[^{14}C]-methylmalonyl CoA (55.5 mCi/mmol) was purchased from American Radiolabeled Chemicals and diluted to give a stock solution with specific activity of 1 mCi/mmol and a final concentration of 4 mM. All other chemicals were purchased from Sigma. Enzymatic reactions were run at a final volume of 50 μL in a buffer containing 400 mM sodium phosphate (pH 7.4), 5 mM NaCl, 1 mM EDTA, 1 mM TCEP, 4 mM NADPH (when used), 20% glycerol and 5% DMSO. For radioassay experiments, enzymes and substrates were added to a final concentration of 5 μM and 1 mM, respectively. The reactions were incubated for 18 h at 30°C, then quenched by addition of 250 μL EtOAc and extracted with an additional 2 × 250 μL EtOAc. The organic extracts were concentrated under a

stream of N_2, re-suspended in 30 μL CH_2Cl_2, and spotted on a Merck silica gel TLC plate. The TLC plate was developed in 5% MeOH/CH_2Cl_2, exposed to a phophorimager screen for 36 h and analyzed using a Typhoon phosphorimager (Molecular Dynamics). For LC-MS/MS experiments, 100 μL reactions were used with 5 μM enzyme and 1 mM substrate, and the reactions again were incubated for 18 h at 30°C. The reactions were stopped by filtering through a Microcon YM-10 centrifugal filter device (Millipore) to remove the enzyme. 100 μL of 20% CH_3CN/H_2O was run through the filter to ensure elution of all polyketide products. LC-MS/MS analysis was performed on a ThermoElectron Finnigan LTQ Linear Ion Trap. Chromatographic separation was achieved on a Phenomenex Luna C18(2) column (3 μm, 2.0 × 250 mm) using a CH_3CN/H_2O gradient (20%, 20 min; 20% to 100%, 60 min, 0.1 mL/min).

Confirmation of Macrolactone 12. A large scale (2 mL) enzymatic reaction of Ery5-TE with DEBS pentaketide and cold methymalonyl-CoA was incubated for 24h followed by extraction with 3 × 4 mL EtOAc. The crude organic extracts were concentrated and dissolved in 100 μL MeOH and purified by semi-preparative HPLC on a Phenomenex Luna C18(2) column (5 μm, 4.6 × 250 mm) using a gradient of CH_3CN/10 mM NH_4OAc buffer, pH 6.0 (20 to 100%, 30 min, 1 mL/min). Fractions containing the desired compound, as determined by ESI(+)-MS, were pooled and concentrated to give < 1 mg of **12**. ^1H NMR of macrolactone **12** was obtained on a Varian MR600 spectrometer fitted with a capillary flow probe, and the spectrum was correlated to that previously described.[6] HRMS ESI^+ (*m/z*): 351.2138 (Predicted $[M+Na]^+$ for $C_{19}H_{32}O_5$ is 351.2147).

Figure S1. Radio-TLC Analysis of Ery5 and Ery6 with DEBS pentaketide SNAC. Lane 1: Ery5 + Ery6; Lane 2: Ery5; Lane 3: Ery6; Lane 4: Ery5-TE (positive control)



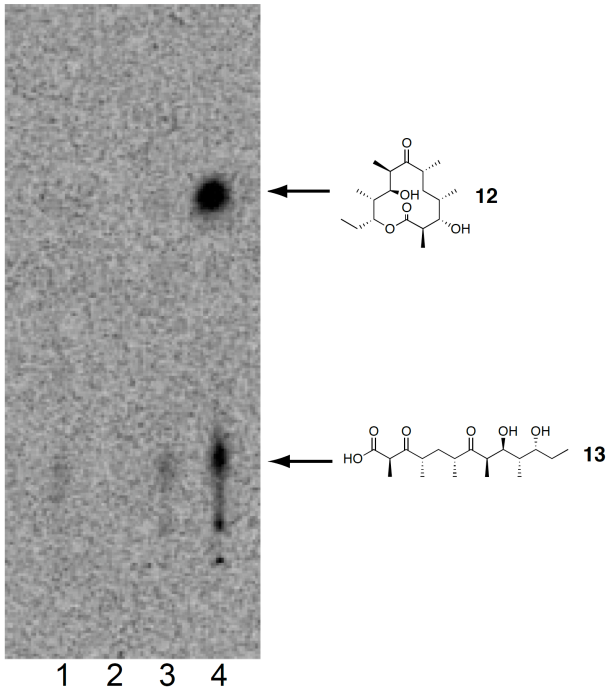
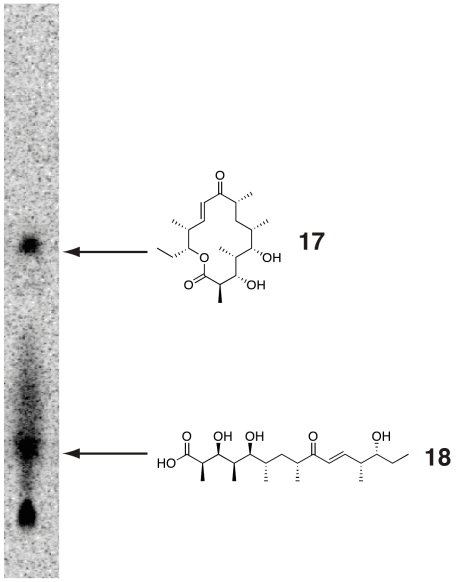


Figure S2. Radio-TLC of Ery6 incubated with Pik hexaketide SNAC.



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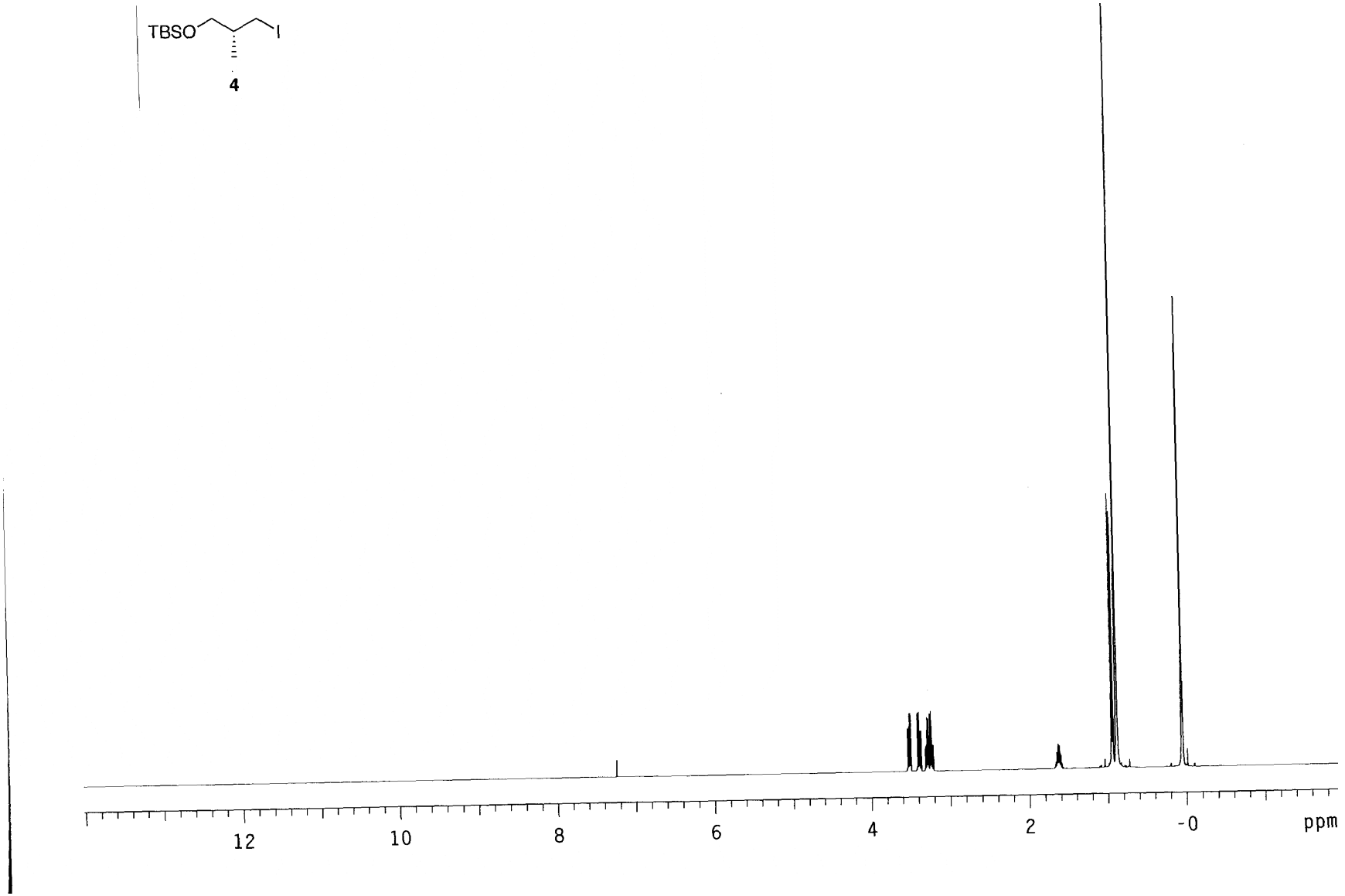
2. Lu, H. X.; Tsai, S. C.; Khosla, C.; Cane, D. E. *Biochemistry* **2002**, *41*, 12590-12597.
3. Gokhale, R. S.; Hunziker, D.; Cane, D. E.; Khosla, C. *Chem. Biol.* **1999**, *6*, 117-125.
4. Gokhale, R. S.; Tsuji, S. Y.; Cane, D. E.; Khosla, C. *Science* **1999**, *284*, 482-5.
5. Pfeifer, B. A.; Admiraal, S. J.; Gramajo, H.; Cane, D. E.; Khosla, C. *Science* **2001**, *291*, 1790-2.
6. Kao, C. M.; Luo, G. L.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1995**, *117*, 9105-9106.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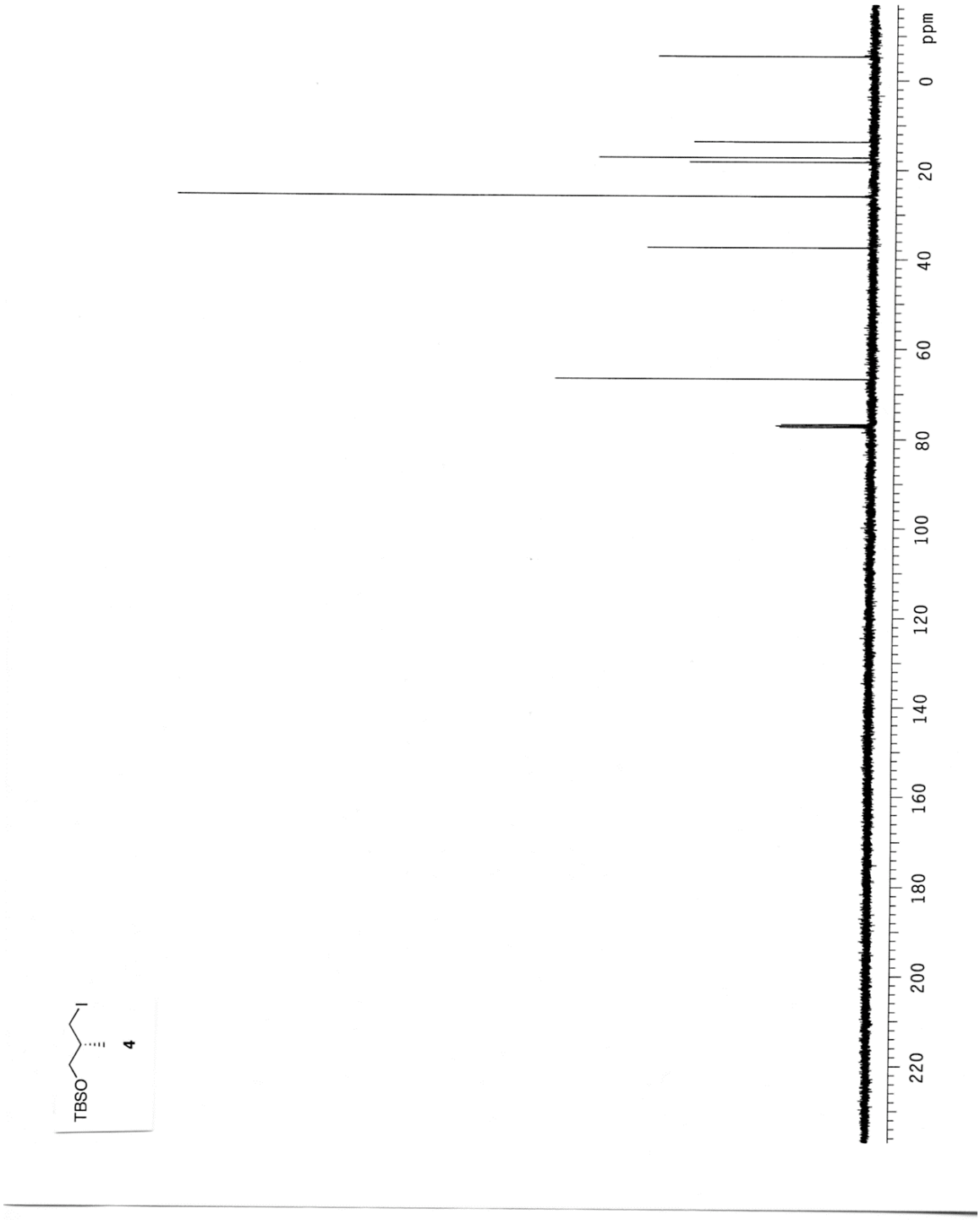
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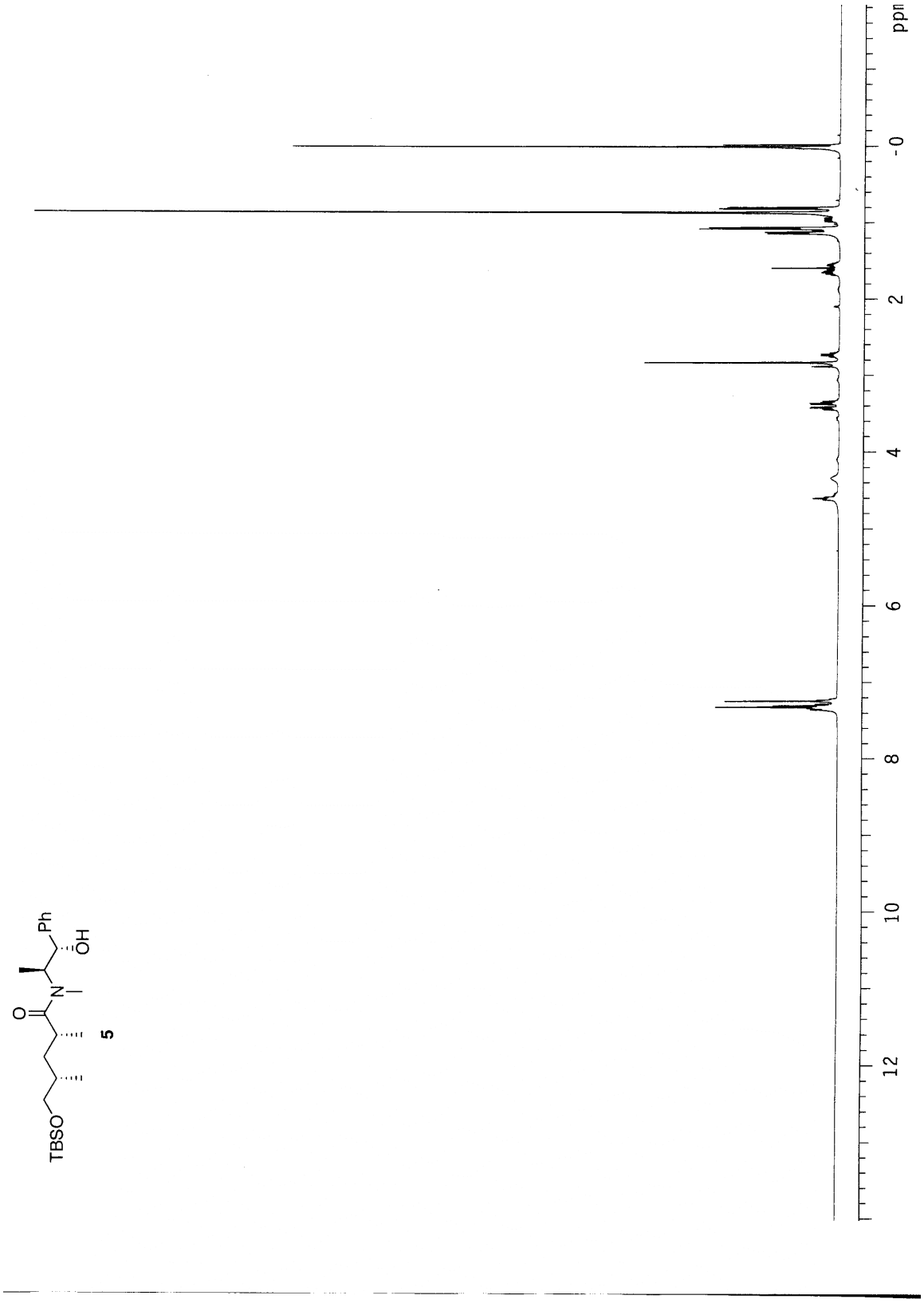
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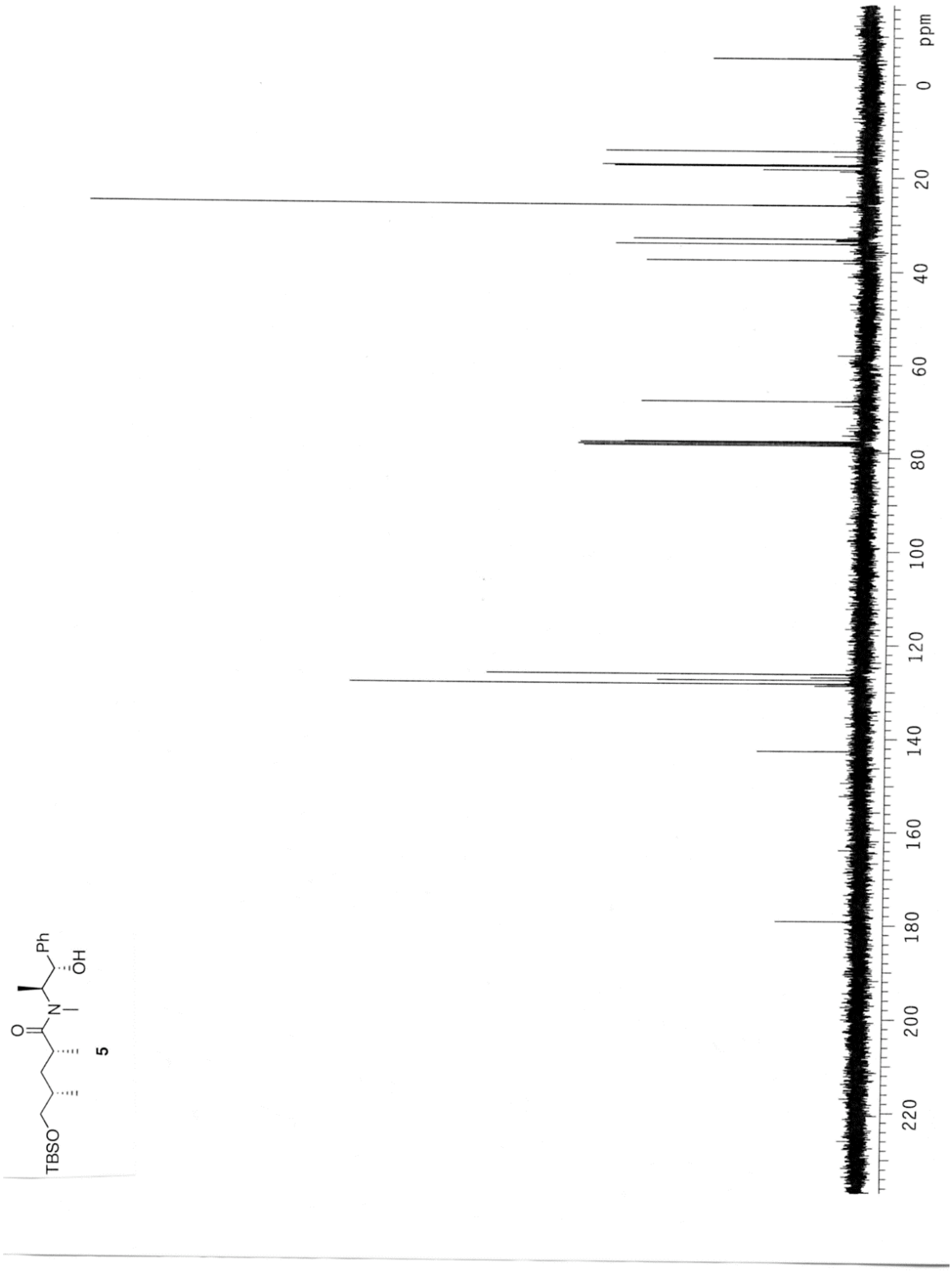
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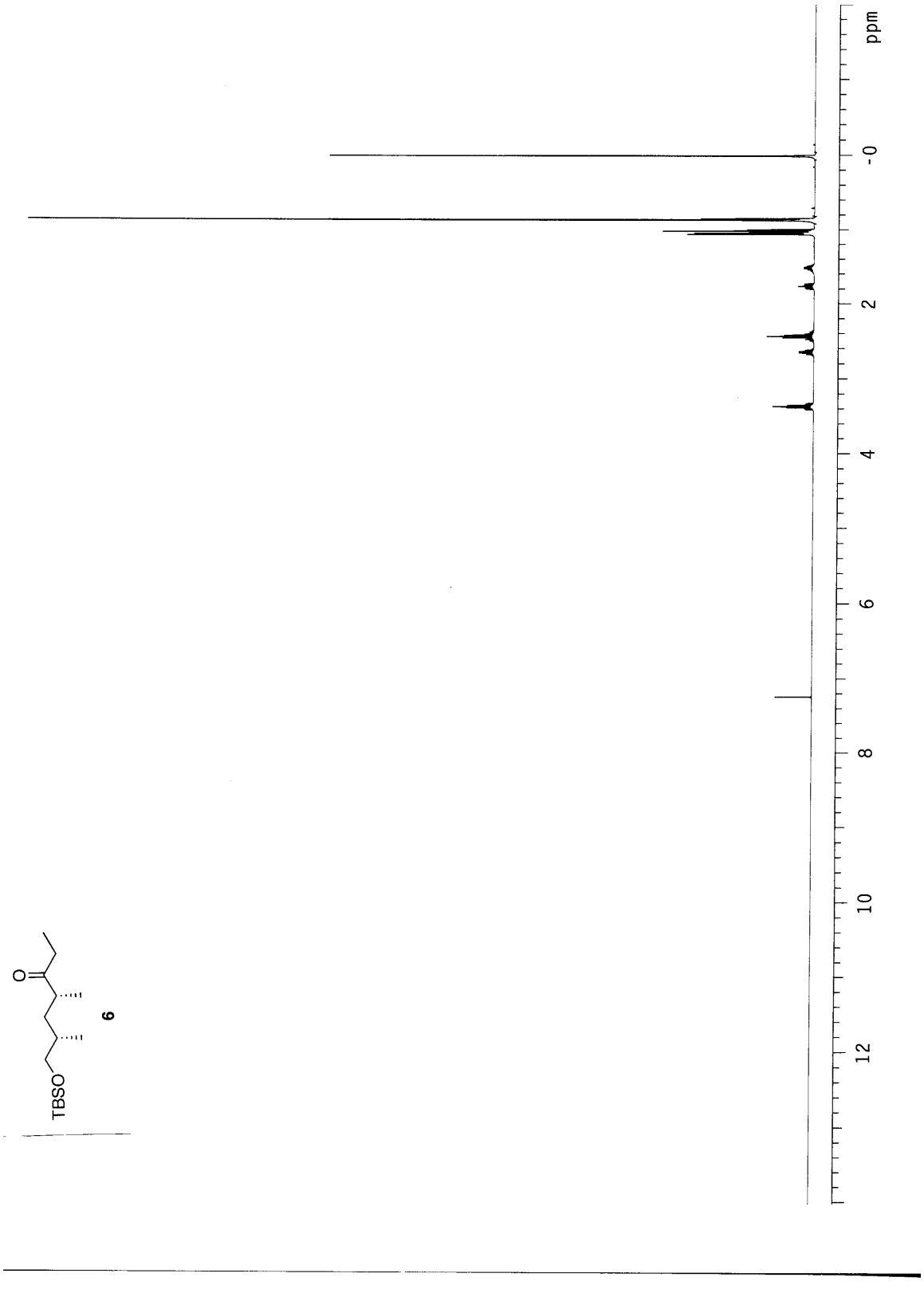
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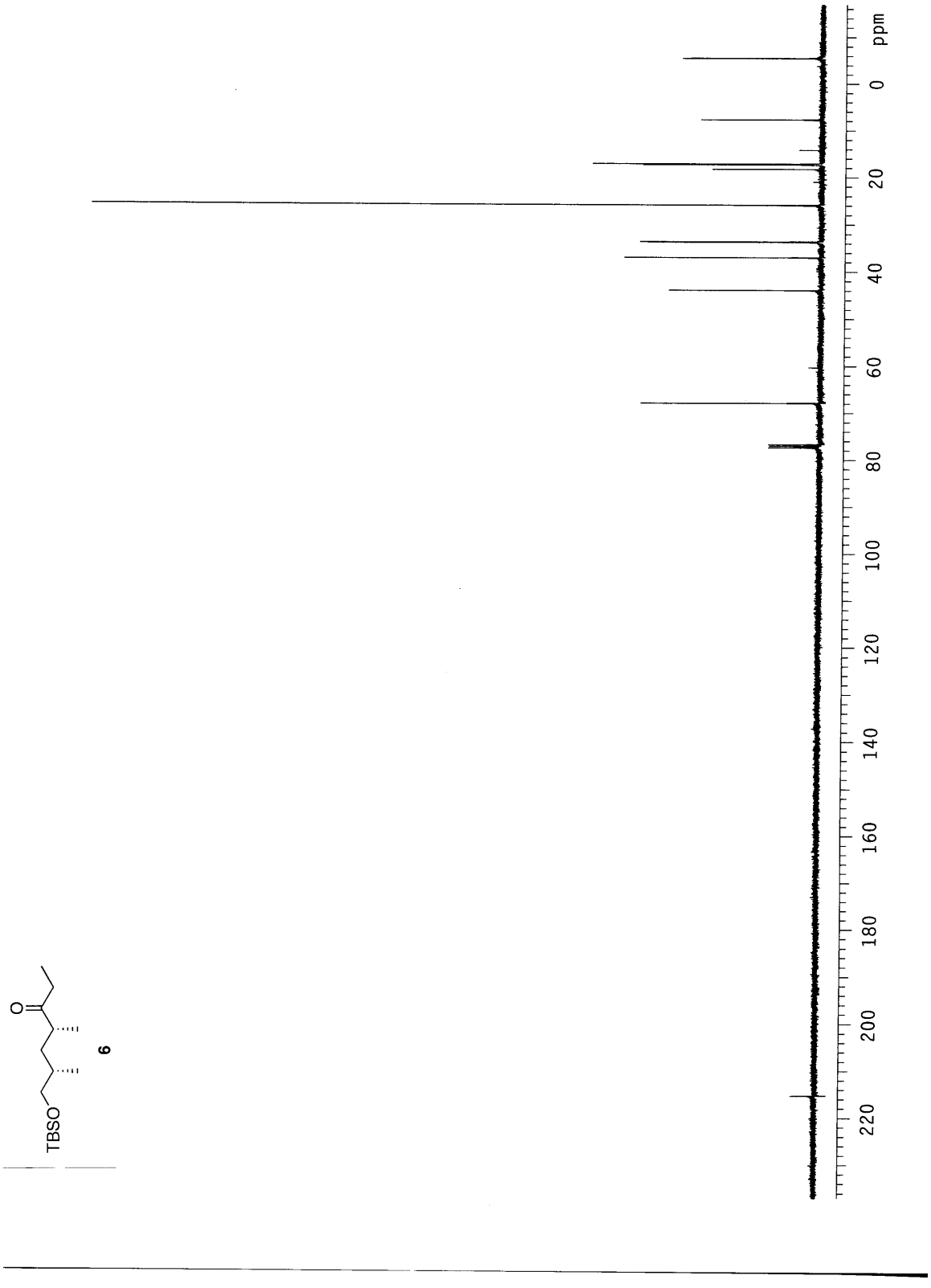
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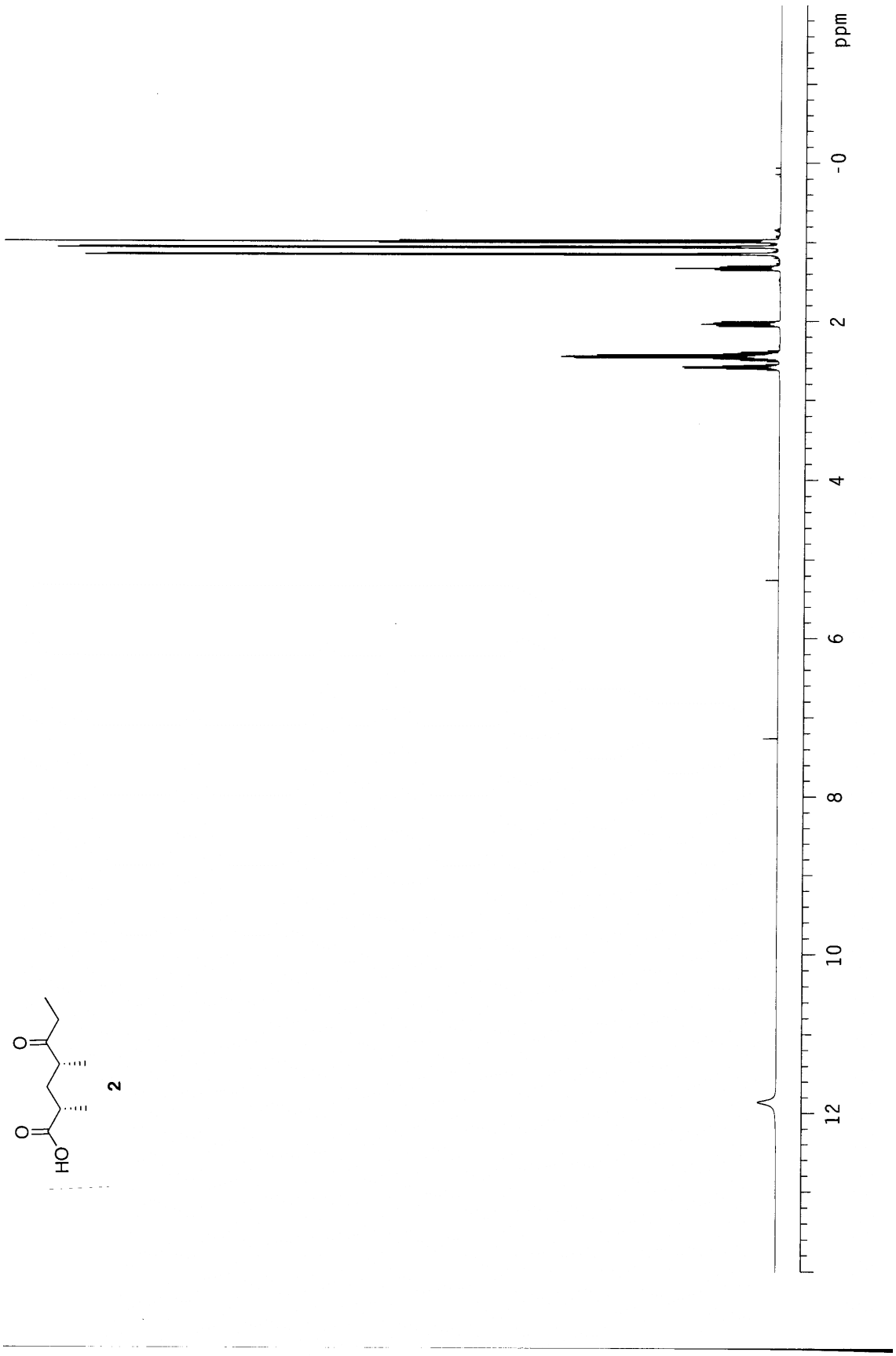
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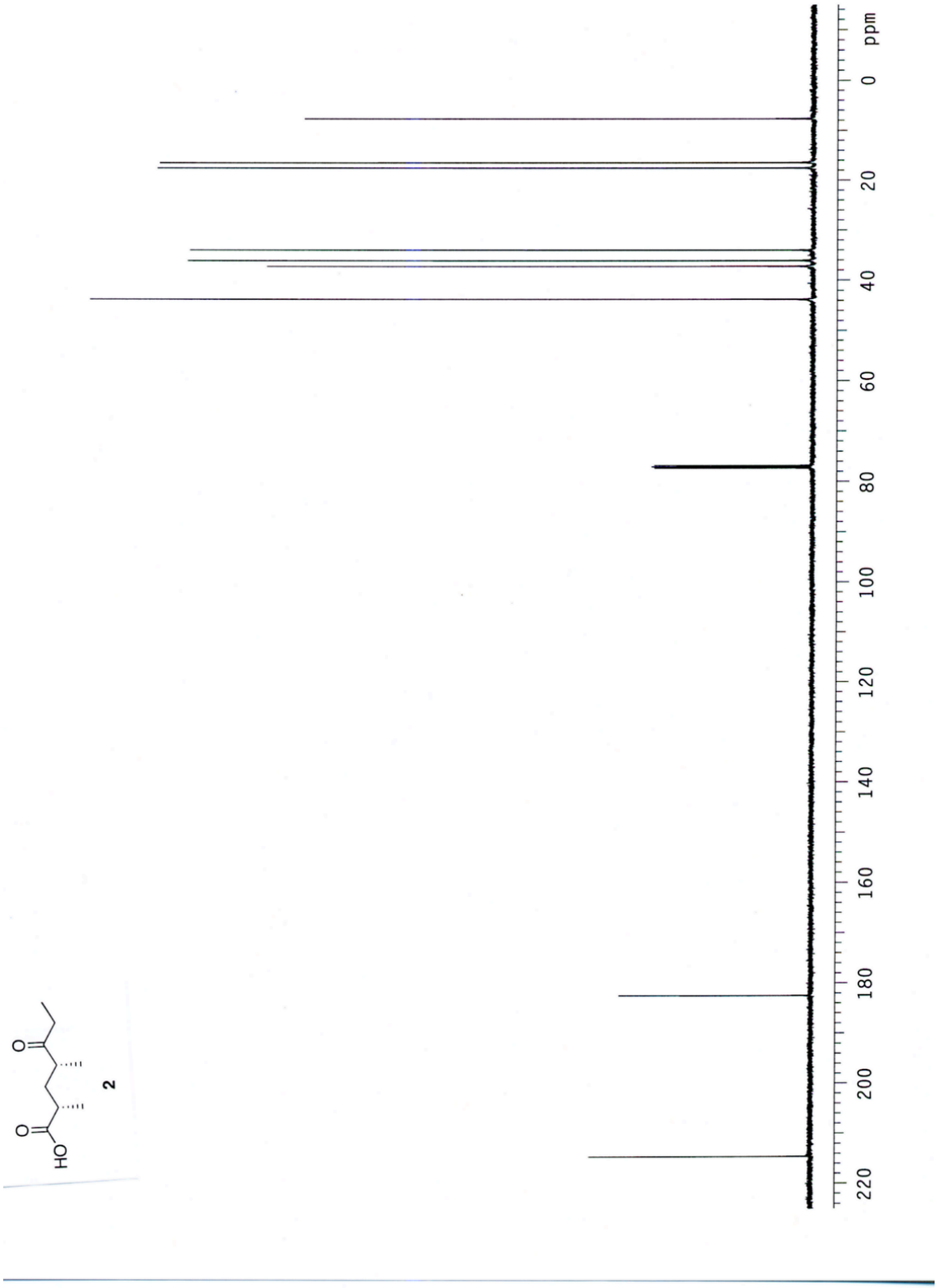
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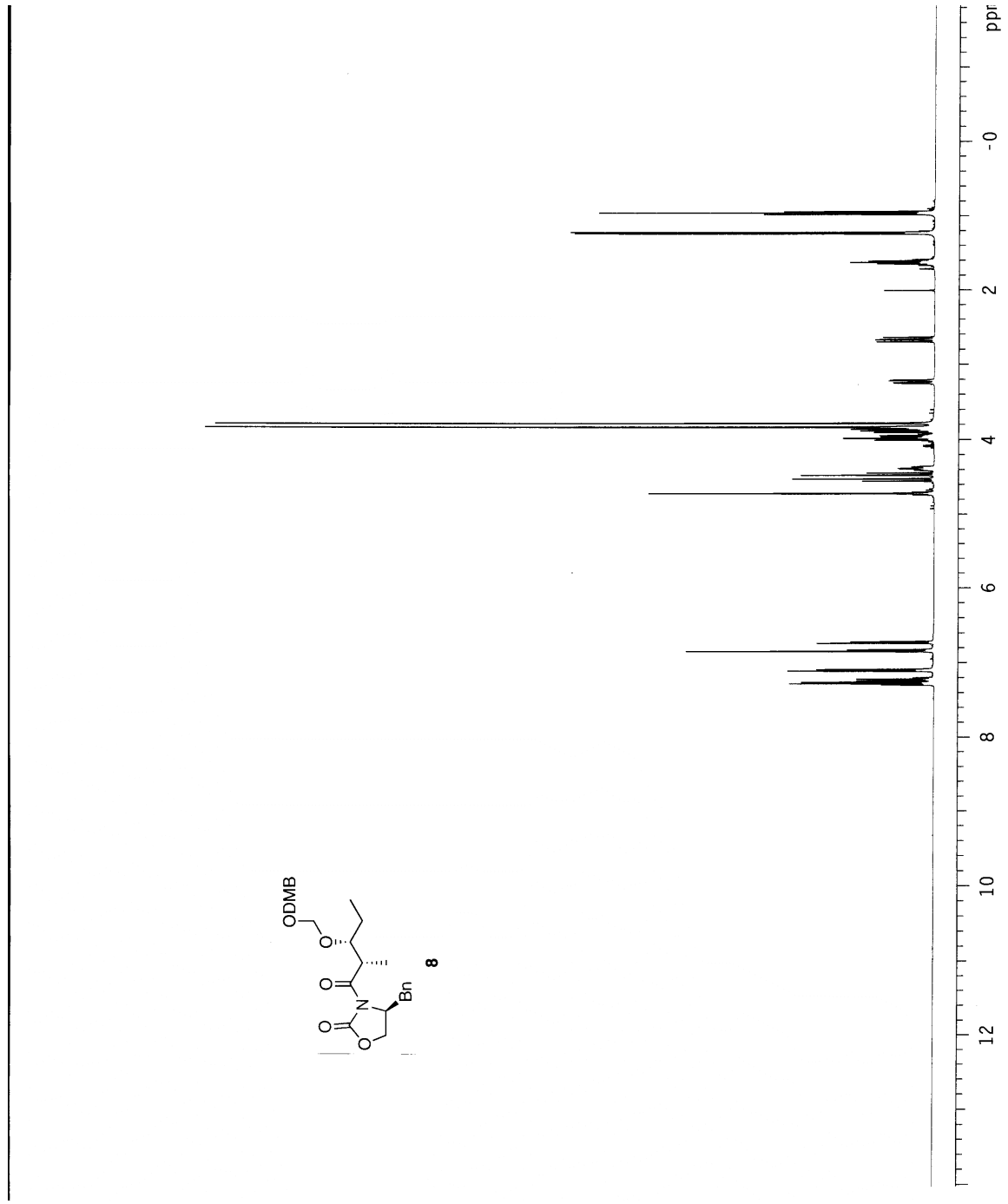
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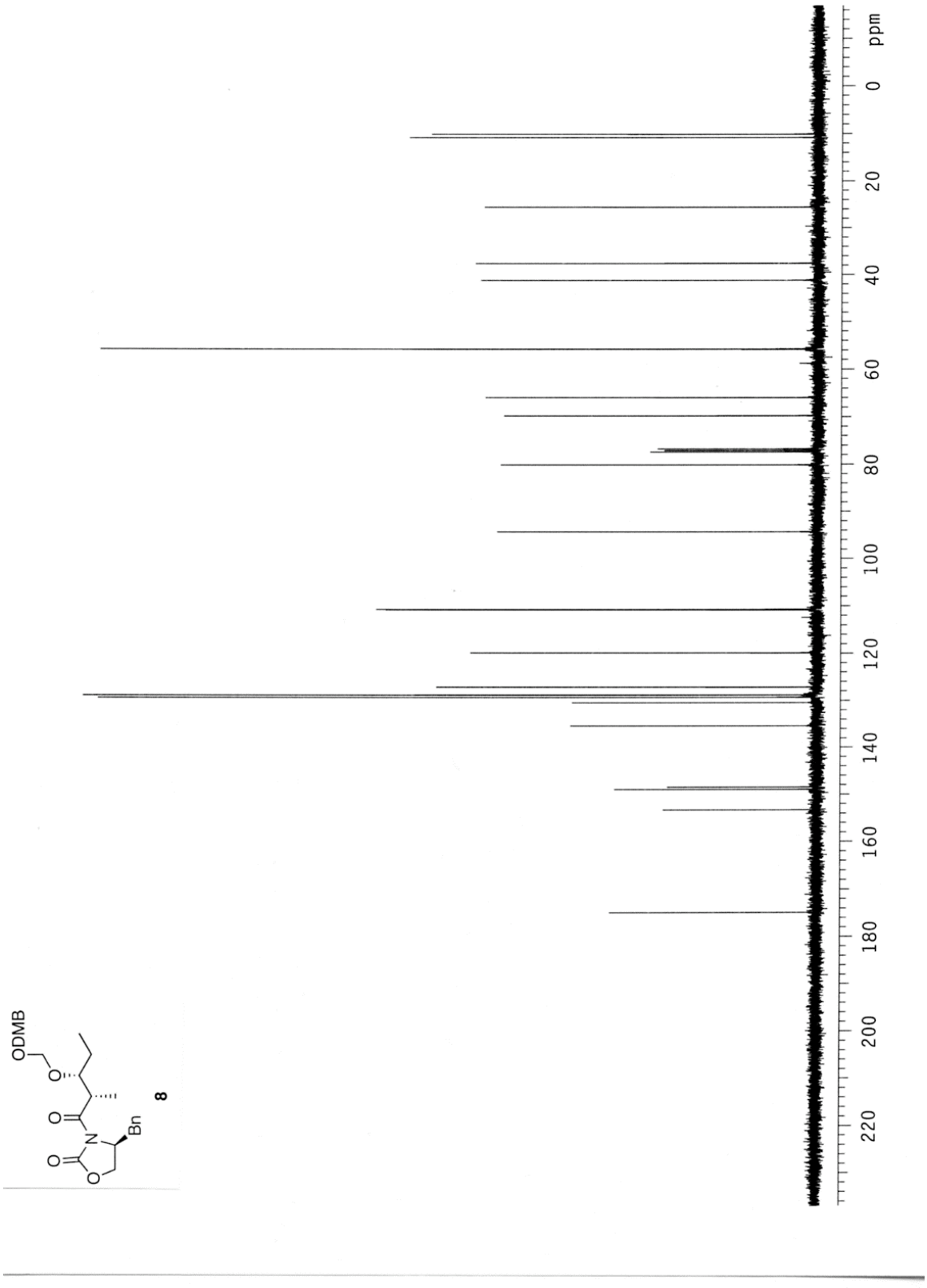
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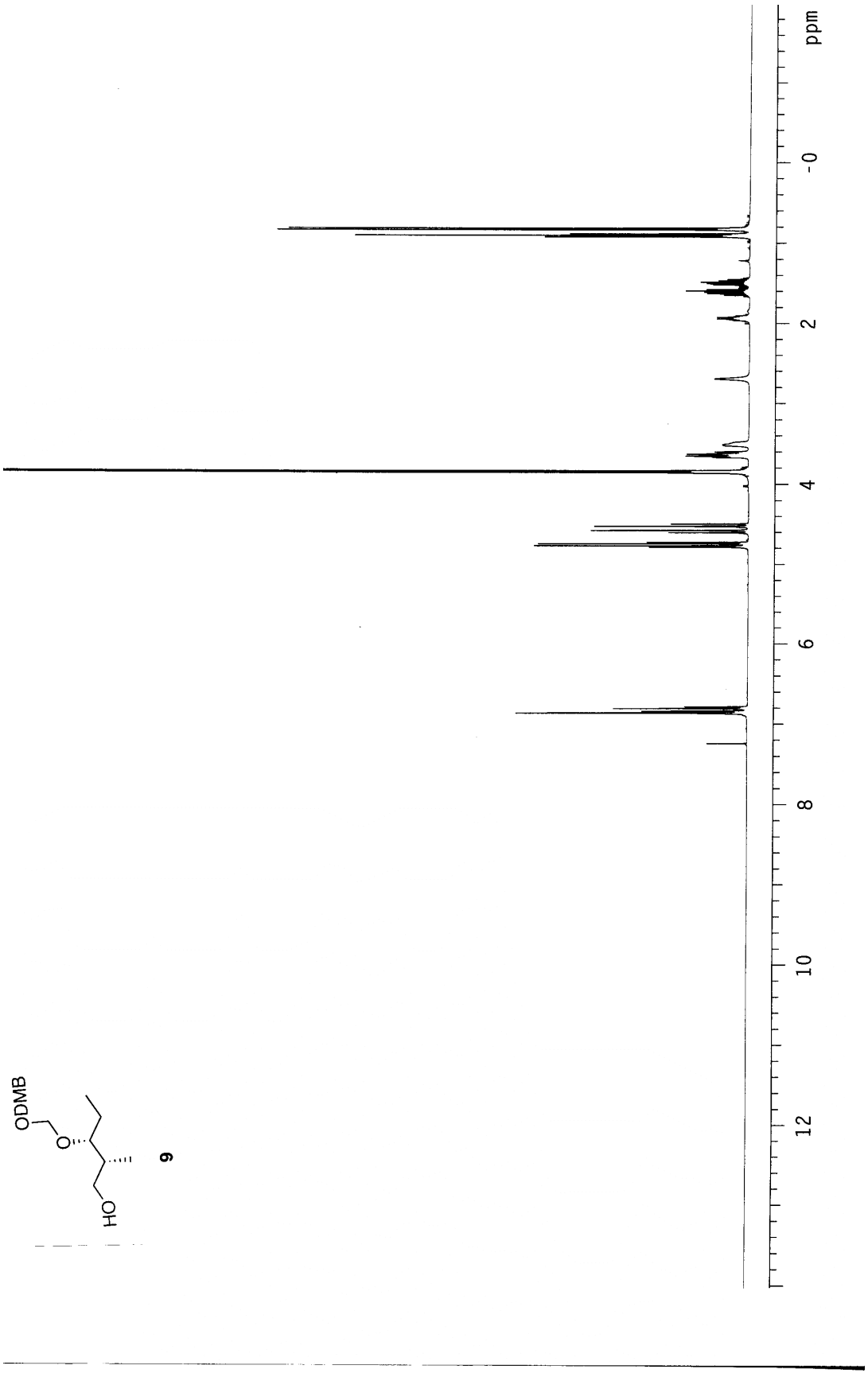
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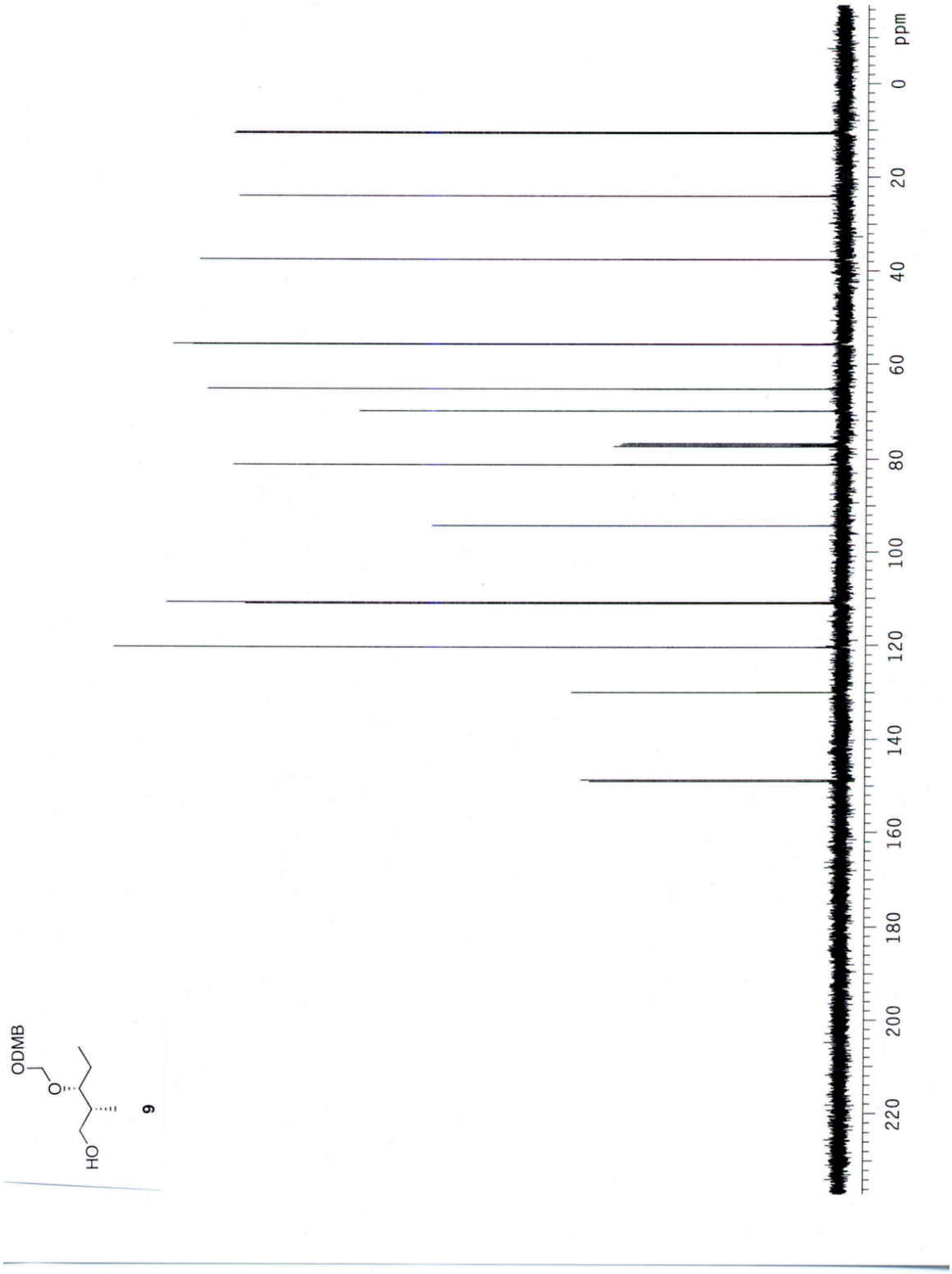
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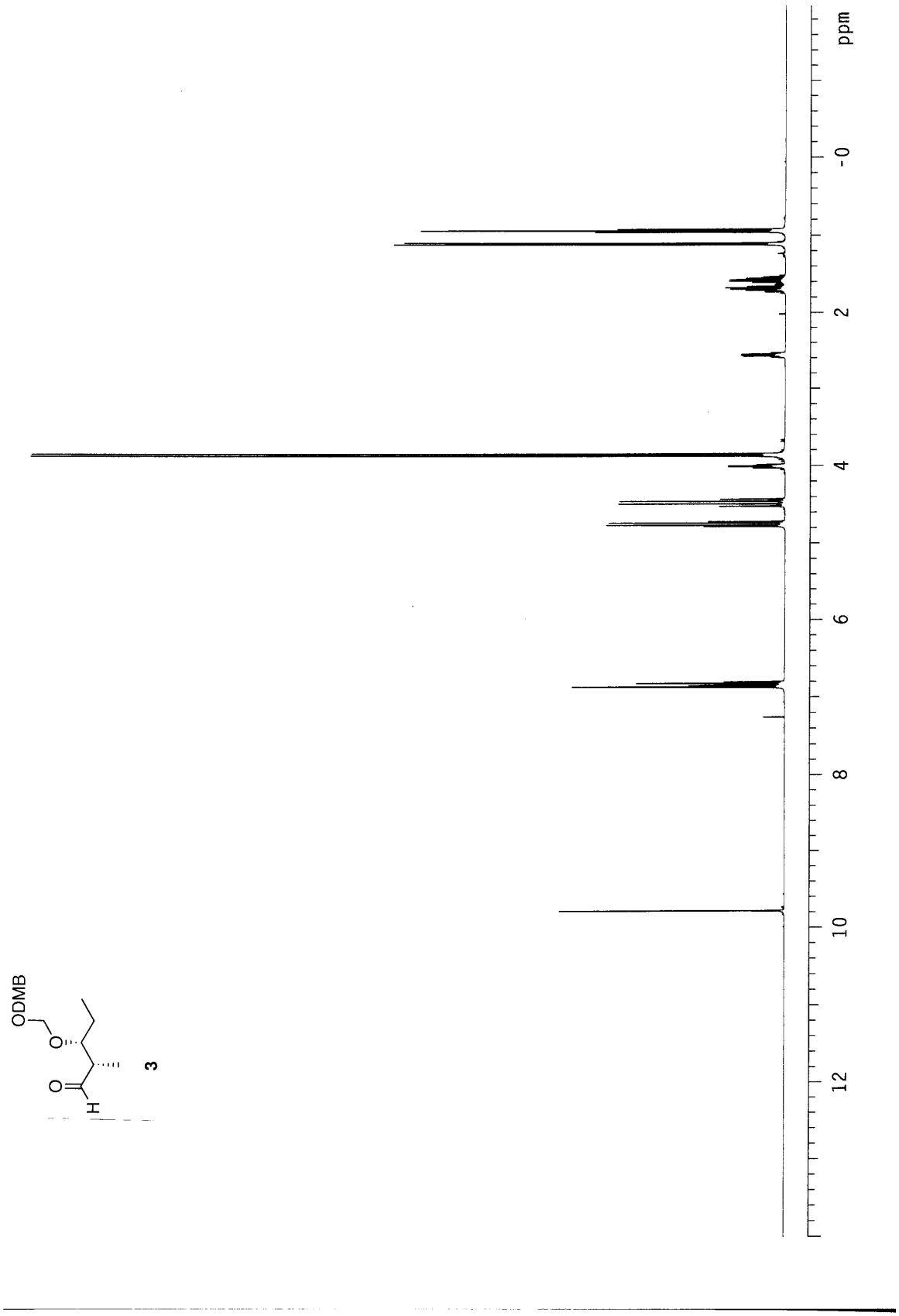
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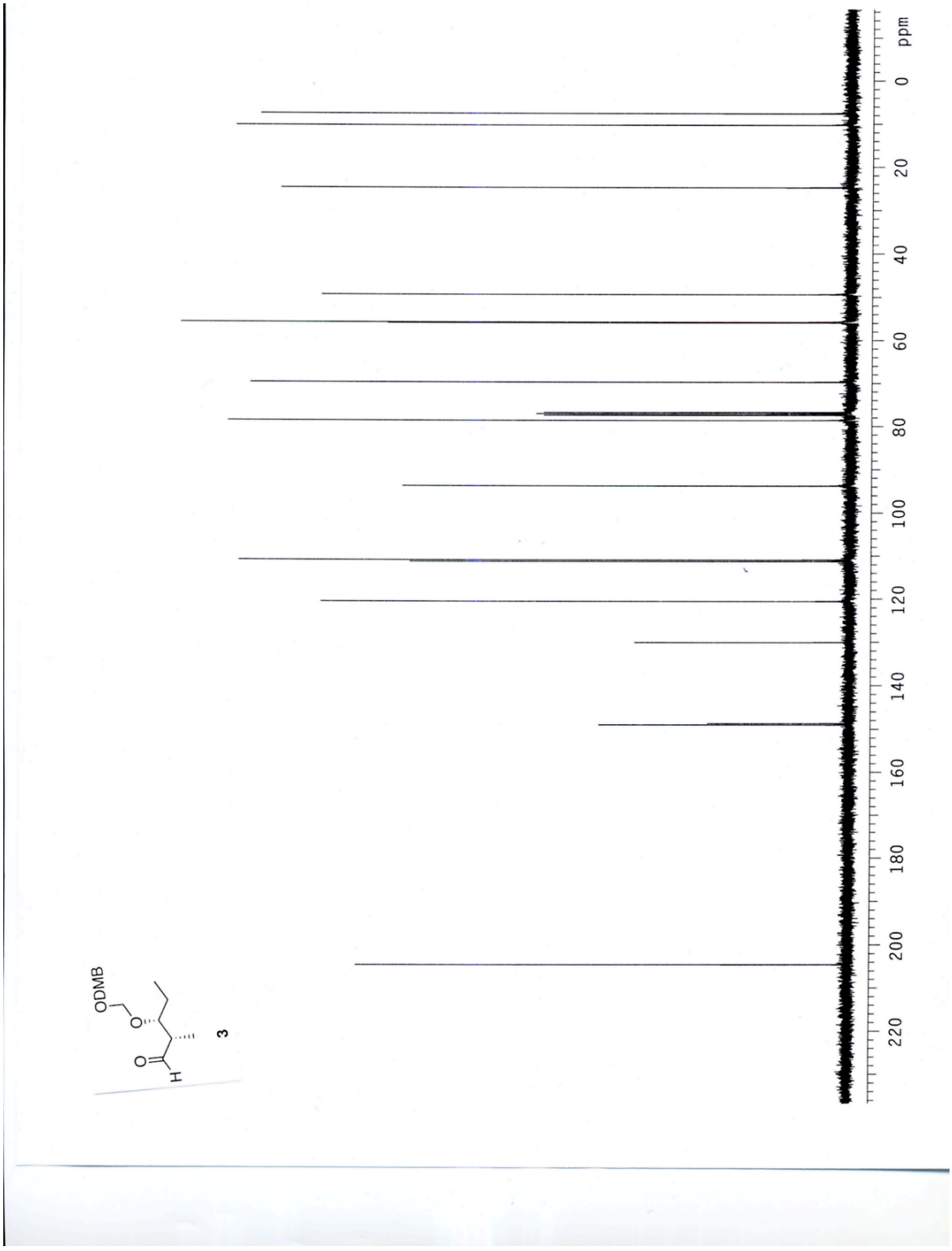
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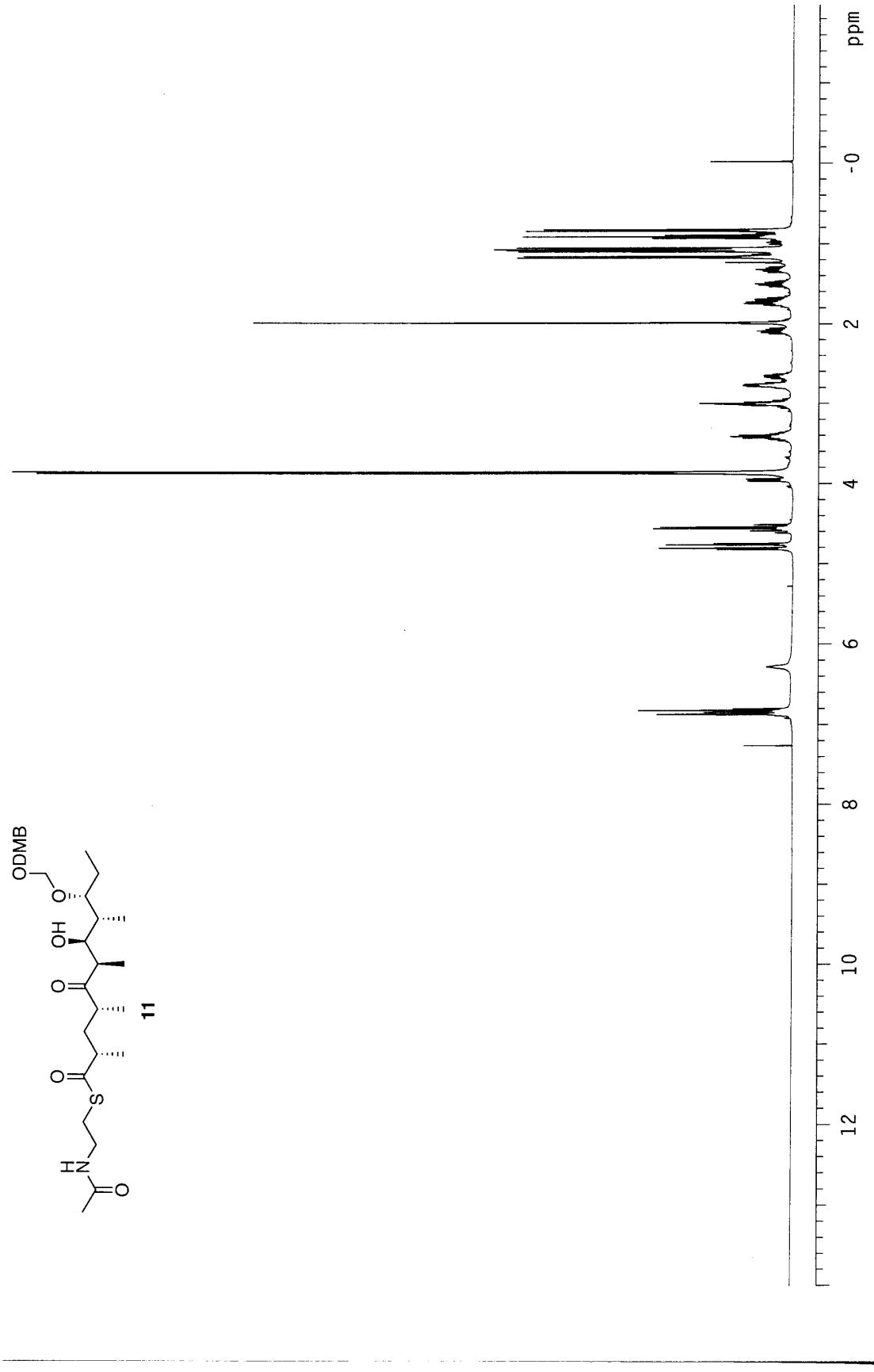
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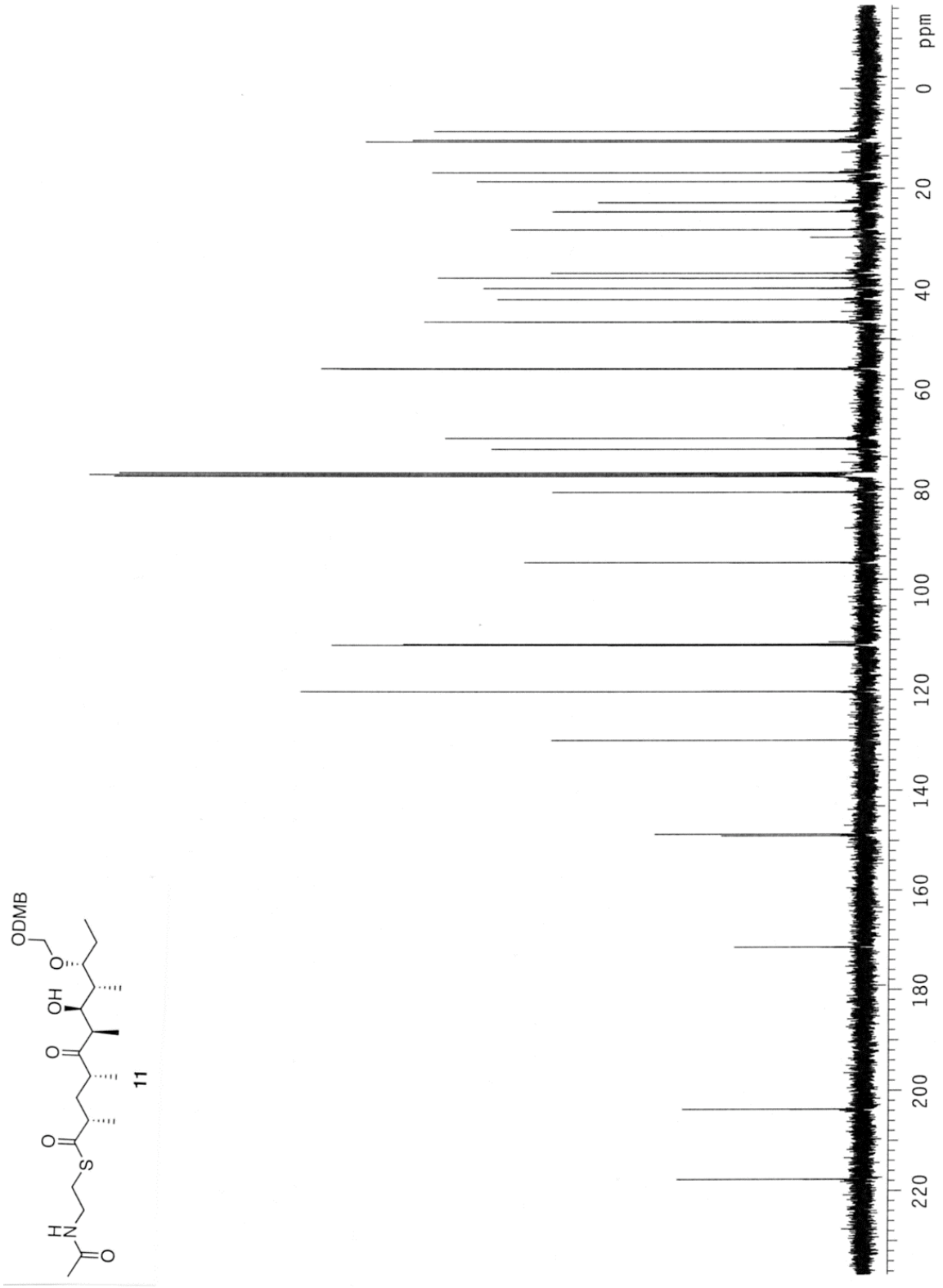
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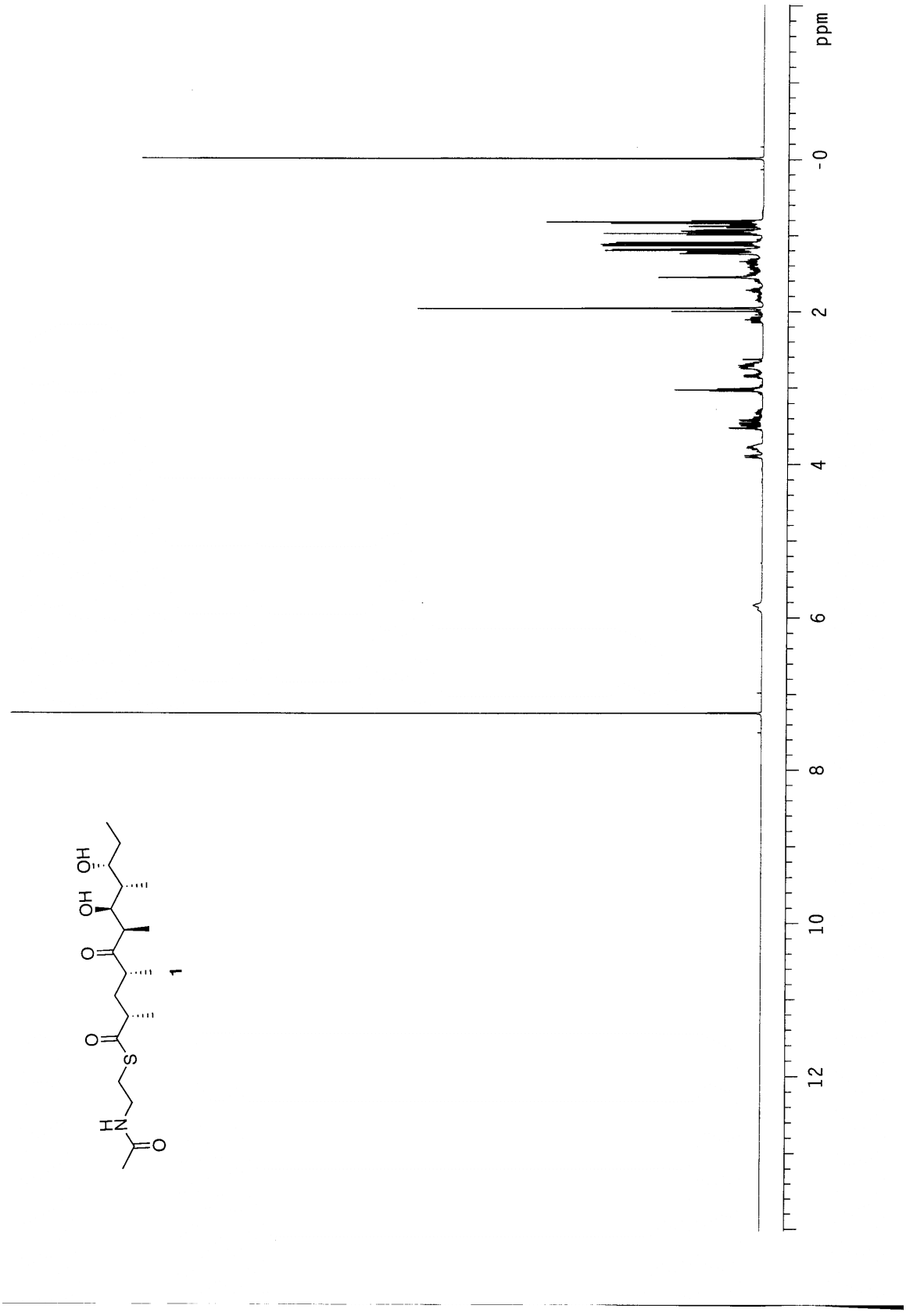
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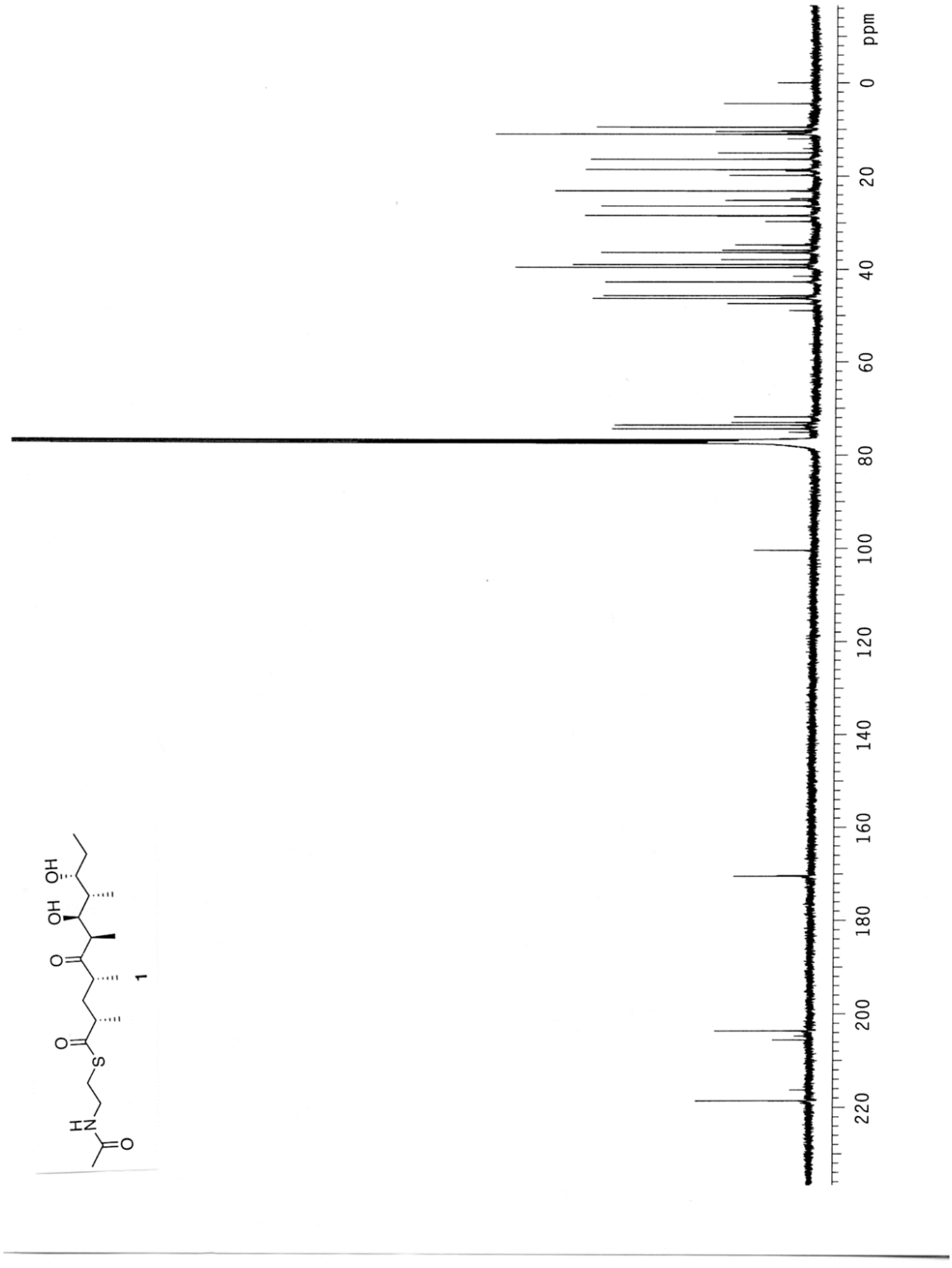
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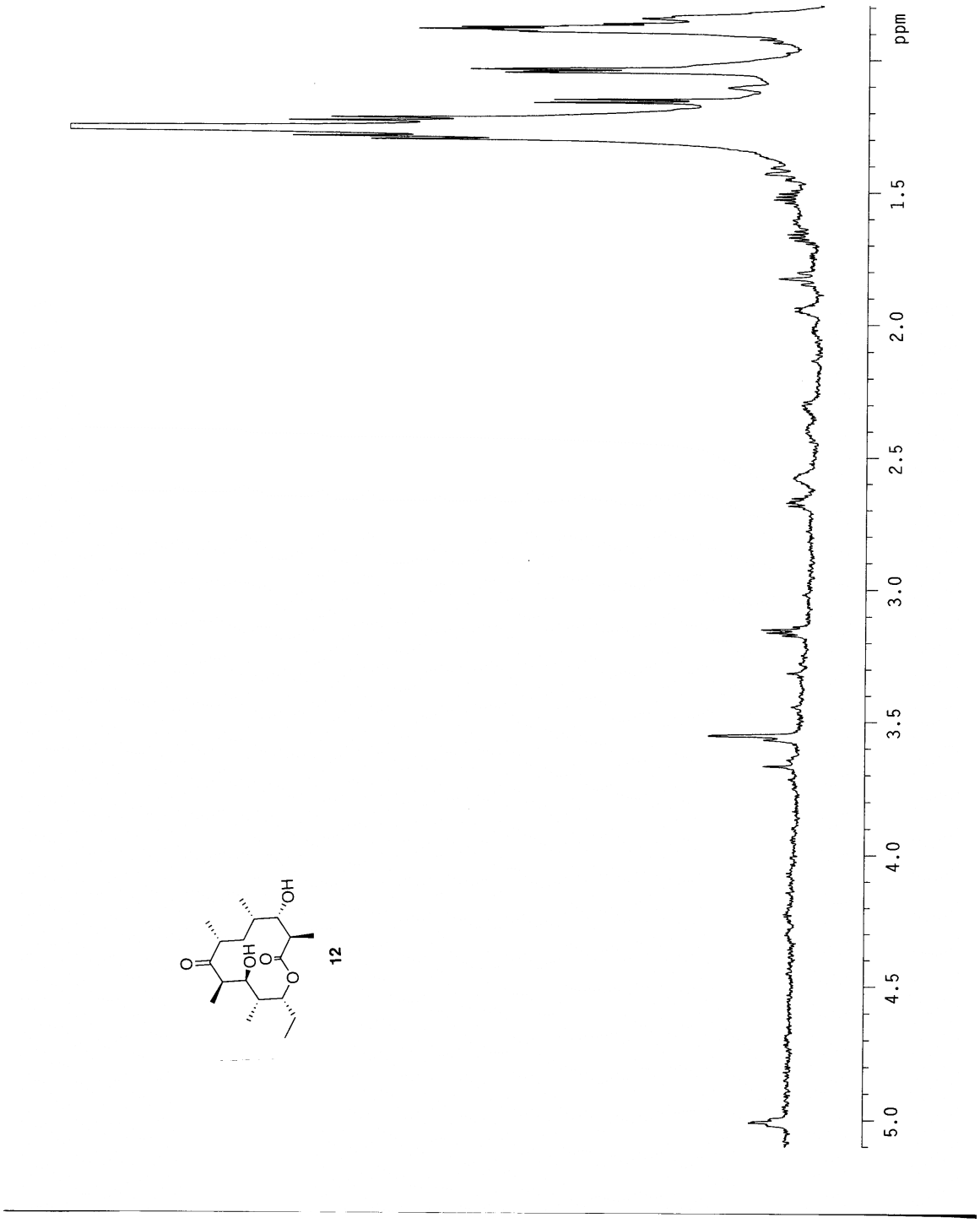
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