PET Imaging and Biodistribution of Silicon Quantum Dots in Mice

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Supporting Information

Table of Contents

General: Reagents were obtained from commercial suppliers and used directly, unless otherwise noted. Cupric-64 chloride solution (half life = 12.7 h) (Washington University, St Louse, MO) was used as purchased. Absorption spectra were measured on a Varian Cary 100-Bio UV-vis spectrophotometer at room temperature. Fluorescent spectra were measured on a Jobin Yvon Horiba FluoroMax-P spectrophotometer at room temperature. Mass spectra (ESI^+ MS) were obtained on a Thermo LCQ ion trap (San Jose, CA) operated with an electrospray source in positive ion mode under standard conditions. NMR spectra were measured with a Bruker DRX-500 spectrometer at 296 K, and all chemical shifts and coupling constants are reported in ppm and Hertz, respectively. Elemental analysis was performed by Columbia Analytical Services (previous Desert Analytics Laboratory) of Tucson, Arizona.

3-*t*-Boc-aminopropylbromide (1): Di-*t*-butyl dicarbonate (2 eq, 2.139 g, 9.8 mmol) was added to a solution of 3-bromopropylamine hydrobromide (1 eq, 1.068 g, 4.9 mmol) in methanol (40 mL) and triethylamine (7 mL). The mixture was stirred at 60 °C for 1 hour, and then at room temperature for 16 hours. The mixture was concentrated *in vacuo*, dissolved in dichloromethane, washed successively with hydrochloric acid (1M), brine, saturated sodium bicarbonate aqueous solution, and brine. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with dichloromethane as eluent to give 0.924 grams (79%) of compound **1** as a colorless oil. ESI^+ MS (50% MeOH and 0.1% Formic acid): *m*/*z* 239 (M + H, 7%), 241 (M + 3H, 7%), 138 (100%), 140 (93%). ^1H NMR (500 MHz, $CDCl_3$, 300 K) δ_H 1.45 (9H, s, $C(CH_3)_3$), 2.04-2.07 (2H, m, CH_2), 3.27-3.28 (2H, m, CH_2N), 3.44 (2H, t, *J* 6.5, $BrCH_2$), 4.69 (1H, br s, NH). ^{13}C NMR (126 MHz, $CDCl_3$, 300 K) δ_C 28.64,

31.06, 32.94, 39.24, 67.04, 156.22. Found C, 39.82; H, 6.79; N, 6.18; Br, 33.60; calc. for $C_8H_{16}NO_2Br$: C, 40.35; H, 6.77; N, 5.88, Br, 33.58%.

1-(3-*t*-Boc-aminopropyl)-(1,4,7,10-tetraazacyclododecane) (2). 3-*t*-Boc-aminopropylbromide (**1**) (0.4 eq, 0.787 g, 3.305 mmol) was added to a stirred solution of cyclen (1 eq, 1.48 g, 8.593 mmol) in dry toluene (25 mL). The mixture was refluxed overnight under argon and then extracted with water (3 × 100 mL). The aqueous layers were combined and extracted with dichloromethane (3 × 75 mL). The organic layers were combined and dried over $MgSO_4$. Removal of solvent *in vacuo* gives 0.828 grams (76%) of compound **2** as a white solid. ESI^+ MS (50% MeOH and 0.1% Formic acid): *m*/*z* 330 (M + H, 100%), 230 (21%). ^1H NMR (500 MHz, $CDCl_3$, 300 K) δ_H 1.43 (9H, s, $C(CH_3)_3$), 1.65-1.71 (2H, m, $CH_2CH_2CH_2N$), 2.45-2.65 (16H, m, CH_2 & NH), 2.77-2.79 (4H, m, CH_2), 3.16-3.17 (3H, m, CH_2), 4.93 (1H, br s, NHCO). ^{13}C NMR (126 MHz, $CDCl_3$, 300 K) δ_C 27.98, 28.70, 37.00, 45.46, 46.29, 47.31, 51.88, 52.19, 156.33. Found C, 56.83; H, 10.55; N, 19.85; calc. for $C_{16}H_{35}N_5O_2 \cdot 0.13CH_2Cl_2$: C, 56.37; H, 10.34; N, 21.26%.

1-(3-*t*-Boc-aminopropyl)-4,7,10-(tris-*t*-butylcarboxymethyl)-(1,4,7,10-tetraazacyclododecane) (3). Compound **2** (1 eq, 2.027 g, 6.152 mmol) and potassium carbonate (4.1 eq, 3.492 g, 35.2 mmol) were suspended in dry acetonitrile (30 mL) and stirred for 30 minutes under argon atmosphere. A solution of *tert*-butyl bromoacetate (4 eq, 4.8 g (3.587 mL), 24.608 mmol) in dry acetonitrile (10 mL) was added dropwise to the flask over a period of one hour; then the mixture was stirred for 5 hours. The solids were filtered. The volatile components of the filtrate were evaporated. The crude product was purified by column chromatography on silica gel with dichloromethane/methane (9/1)

as eluent to give 3.349 grams (81%) of compound **3** as a white solid. ESI^+ MS (50% MeOH and 0.1% Formic acid): *m*/*z* 673 (M + H, 100%), 674 (M + 2H, 36%), 573 (7%). ^1H NMR (500 MHz, $CDCl_3$, 300 K) δ_H 1.41-1.46 (36H, m, $C(CH_3)_3$), 1.64 (2H, br s, $CH_2CH_2CH_2N$), 1.99-2.40 (9H, m, CH_2), 2.77 (6H, br s, CH_2), 3.06-3.31 (9H, m, CH_2), 3.42-3.59 (2H, m, CH_2), 4.76 (1H, br s, NHCO). ^{13}C NMR (126 MHz, $CDCl_3$, 300 K) δ_C 28.05, 28.24, 28.39, 28.65, 28.69, 39.03, 48.03, 50.34, 50.57, 50.92, 52.01, 52.90, 53.50, 55.85, 56.01, 56.76, 57.11, 79.38, 82.05, 82.13, 82.67, 83.01, 156.22, 170.28, 172.89, 173.88.

1-(3-Aminopropyl)-4,7,10-(triscarboxymethyl)-(1,4,7,10-tetraazacyclododecane) trifluoroacetic acid salt (4). A mixture of compound **3** (1.0 g, 1.49 mmol) in dichloromethane (8 mL) and trifluoroacetic acid (8 mL) was stirred for 24 hours. The solvent was evaporated in *vacuo* to dryness. The residue was dissolved in methanol, and then the solvent was evaporated to dryness. The process was repeated three times to give 1.244 grams of compound **4** as a white solid. Because the product contains CF_3COOH, see the element analysis result below, the yield after correction is 90%. ESI^+ MS (50% MeOH and 0.1% Formic acid): *m*/*z* 404.6 (M + H, 79%). ^1H NMR (500 MHz, DMSO-d_6, 300 K) δ_H 1.93 (2H, s, $CH_2CH_2CH_2N$),, 2.82-3.01 (10H, m, CH_2), 3.16-3.32 (9H, m, CH_2), 3.48-3.64 (4H, m, CH_2), 3.96 (3H, m, CH_2), 8.05 (3H, br s, $^+NH_3$), 12.76 (2H, br s, COOH). ^{13}C NMR (126 MHz, DMSO-d_6, 300 K) δ_C 21.24, 36.95, 48.33, 48.60, 49.29, 50.09, 50.39, 52.13, 53.71, 54.68, 114.10 (CF_3COOH), 116.48 (CF_3COOH), 118.85 (CF_3COOH), 121.23 (CF_3COOH), 158.65, 158.90, 159.15, 159.40 (COOH), 173.37 (CF_3COOH). ^{19}F NMR (470 MHz, DMSO-d_6, 300 K) δ_F -74.25. Found C, 33.60; H, 4.63; N, 7.88; calc. for $C_{17}H_{33}N_5O_6 \cdot 4.6CF_3COOH$: C, 33.91; H, 4.08; N, 7.55%.

Dextran coated Si_{Mn} QDs (5). DMSO/pyridine (115 mL, 1/1 (v/v)) was added to a flask containing dextran (1.50 g, 27.75 mmol of hydroxyl groups, MW/MN = 6,000 Da) and 4-nitrophenyl chloroformate (0.617 g, 3.05 mmol) at 0-5 °C and argon atmosphere. A 10 mol % (*vs* chloroformate, 0.041 g, 0.3 mmol) of 4-(dimethylamino) pyridine (DMAP) was added as a catalyst. The reaction mixture was stirred under argon for 4 h at 0-5 °C. The reaction product was isolated by precipitation in 400 mL of ether/ethanol (1/1, v/v). The precipitate was isolated by filtration and washed with ethanol/ether (1/1, v/v) and then with ether. The 4-nitrophenyl-activated dextran was dissolved in 205 mL of DMSO/pyridine (2/1, v/v) and then Mn-doped Si QDs prepared by a literature method[1] were added to the flask. After the mixture was stirred under argon for 12 h, compound **4** (53 mg, 0.057 mmol (corrected according to the elemental analysis result of **4**)) was added to the flask and the mixture was stirred for 24 more hours. The reaction product was isolated by precipitation in 500 mL of ether/ethanol (1/1, v/v). The precipitate was isolated by filtration and washed with ethanol/ether (1/1, v/v) and then with ether. The solid was dissolved in water and was dialyzed against deionized water in a dialysis bag with MW cut-off of 12,000-14,000 Da for 72 h (8-10 changes of water) to yield 0.297 grams of white solid.

^{64}Cu labeled dextran Si_{Mn} QDs (6). Dextran Si_{Mn} QDs (**6**) (36.14 mg) was dissolved in 250 µL of 0.2 M pH 5.5 sodium acetate-acetic acid buffer solution in a 1.5 mL Eppendorf vial. After copper 64 (28.64 MBq, specific activity: 94 mCi/ug) was added to the vial the mixture was vortexed for 5 seconds to obtain a uniform solution. The solution was incubated at 55-60 °C for 30 minutes. EDTA aqueous solution (25 µL, 100 mM) was added to the vial. After vortexing for 5 seconds to get the solution uniform, the solution

was incubated at 55-60 ^{o}C for 15 minutes. The crude product was purified by centrifuge filtration with 3K Da nanosep filtration tube (Millipore Inc., Billerica, MA) (30 min @14,000 rpm, then 2 min @ 1,000 rpm) and washed 3 times. The ^{64}Cu labeled dextran Si_{Mn} QDs **6** (~ 15 µL) was diluted to 600 µL with saline (0.9%) that was passed through a sterile 0.22-micron filter before use. The radioactivity was 19.17 MBq. Therefore, the labeling efficiency was 78% after correction with natural decay of ^{64}Cu.

Stability of ^{64}Cu labeled dextran Si_{Mn} QDs (6). Dextran Si_{Mn} QDs (**6**) (12.0 mg) were dissolved in 100 µL of 0.2 M pH 5.5 sodium acetate-acetic acid buffer solution in a 1.5 mL Eppendorf vial. After copper 64 (41.6 MBq) was added to the vial the mixture was incubated at 55-60 $^{\circ}$C for 45 minutes. The solution was centrifuged in 3kDa nanosep filtration tube and washed 3 times. The labeling efficiency was 61%. The purified ^{64}Cu labeled dextran Si_{Mn} QDs (**6**) were diluted with 200 µL of 0.2 M pH 5.5 sodium acetate-acetic acid buffer solution in an Eppendorf vial. EDTA aqueous solution (25 µL, 100 mM) was added to the vial. The solution was incubated at 55-60 $^{\circ}$C for up to 48 h. An aliquot (50 µl) was taken at each time point (1, 4, 24, and 48 h) and measured for initial activity (before purification) using a dose calibrator. Each aliquot was centrifuged in 3kDa nanosep filtration tube and washed 3 times. Final activity (post purification) was measured from the purified Si_{Mn} QDs. Comparisons between values were made using least squares means *t* test. Three parallel solutions of ^{64}Cu labeled dextran Si_{Mn} QDs (**6**) were prepared for the stability test to provide statistical significance.

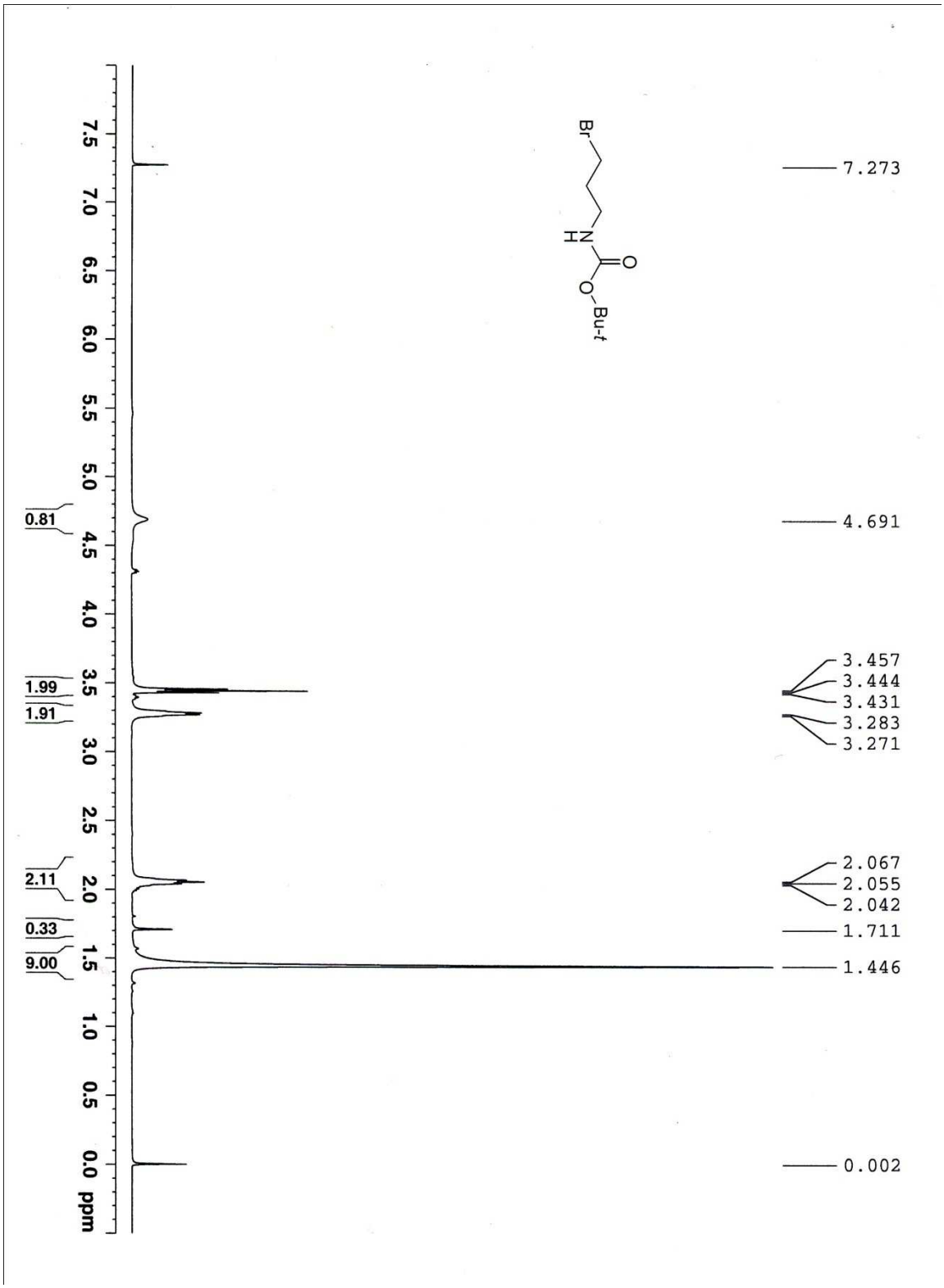
Blood clearance. All animal experiments were conducted under a protocol approved by the University of California, Davis, International Animal Care and Use Committee. Under isoflurane (2 – 3.0%) anaesthesia three C57BL/6 mice (Charles River, Wilmington,

MA), ~25 grams each, were injected via the tail vein with 4.92, 4.96, and 4.14 MBq of the Si_{Mn} QDs **6** (~5 mg Si per kg mouse) in 0.15 mL of 0.9% saline followed by a 0.2 mL of saline flush. Blood samples were collected at 2, 5, 10, 30, 60, and 120 min postinjection via tail nicking and the activities were counted by a Perkin-Elmer Wizard 1470 Automatic Gamma Counter.

***In vivo* PET imaging and *ex vivo* well gamma counting.** Under isoflurane (2 – 3.0%) anaesthesia four C57BL/6 mice (Charles River, Wilmington, MA), ~25 grams each, were injected via the tail vein with 8.44, 7.25, 7.55, and 8.14 MBq of the Si_{Mn} QDs **6** (~5 mg Si per kg mouse) in 0.15 mL of 0.9% saline followed by a 0.2 mL of saline flush. At 5 min, 1, 4, 24, and 48 h postinjection, the mice were scanned on a microPET-Focus 120 (Siemens Medical Solutions, Malvern, PA). The Focus 120 has a transaxial field of view of 10 cm and an axial field of view of 7.6 cm, so is adequate for the whole body imaging of 2 mice at the same time. Images were reconstructed using a maximum a posteriori algorithm (MAP). Static scans yield a single image that represents the average intensities for a given acquisition time.[2] The mice were sacrificed after 48-h PET scan. Major organs were harvested and the activities were counted by a Perkin-Elmer Wizard 1470 Automatic Gamma Counter.

1. Tu, C. Q.; Ma, X. C.; Pantazis, P.; Kauzlarich, S. M.; Louie, A. Y., Paramagnetic, Silicon Quantum Dots for Magnetic Resonance and Two-Photon Imaging of Macrophages. *J. Am. Chem. Soc.* **2010,** *132* (6), 2016-2023.

2. Palko, H. A.; Fung, J. Y.; Louie, A. Y., Positron emission tomography: A novel technique for investigating the biodistribution and transport of nanoparticles. *Inhalation Toxicology* **2010,** *22* (8), 657-668.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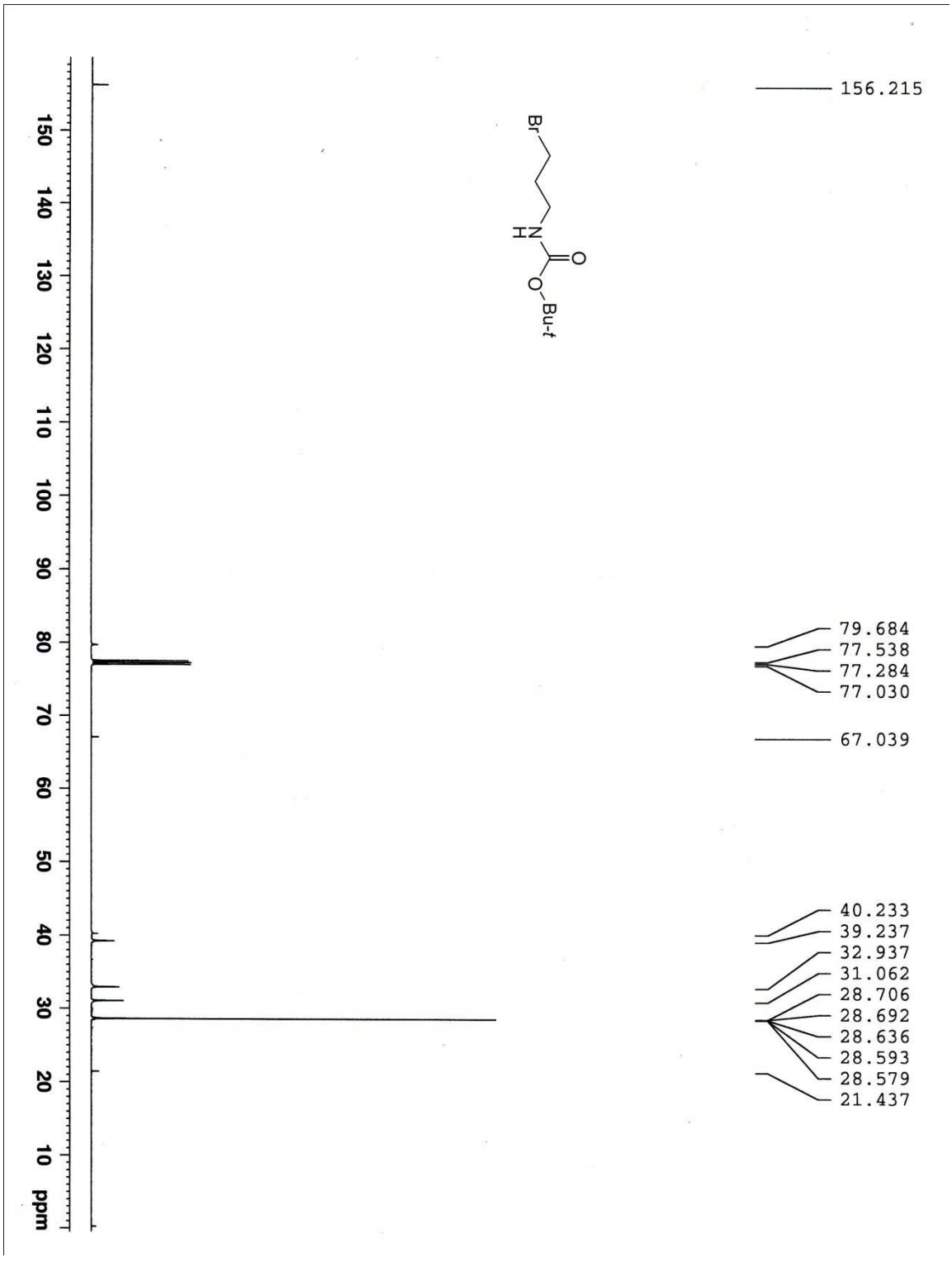
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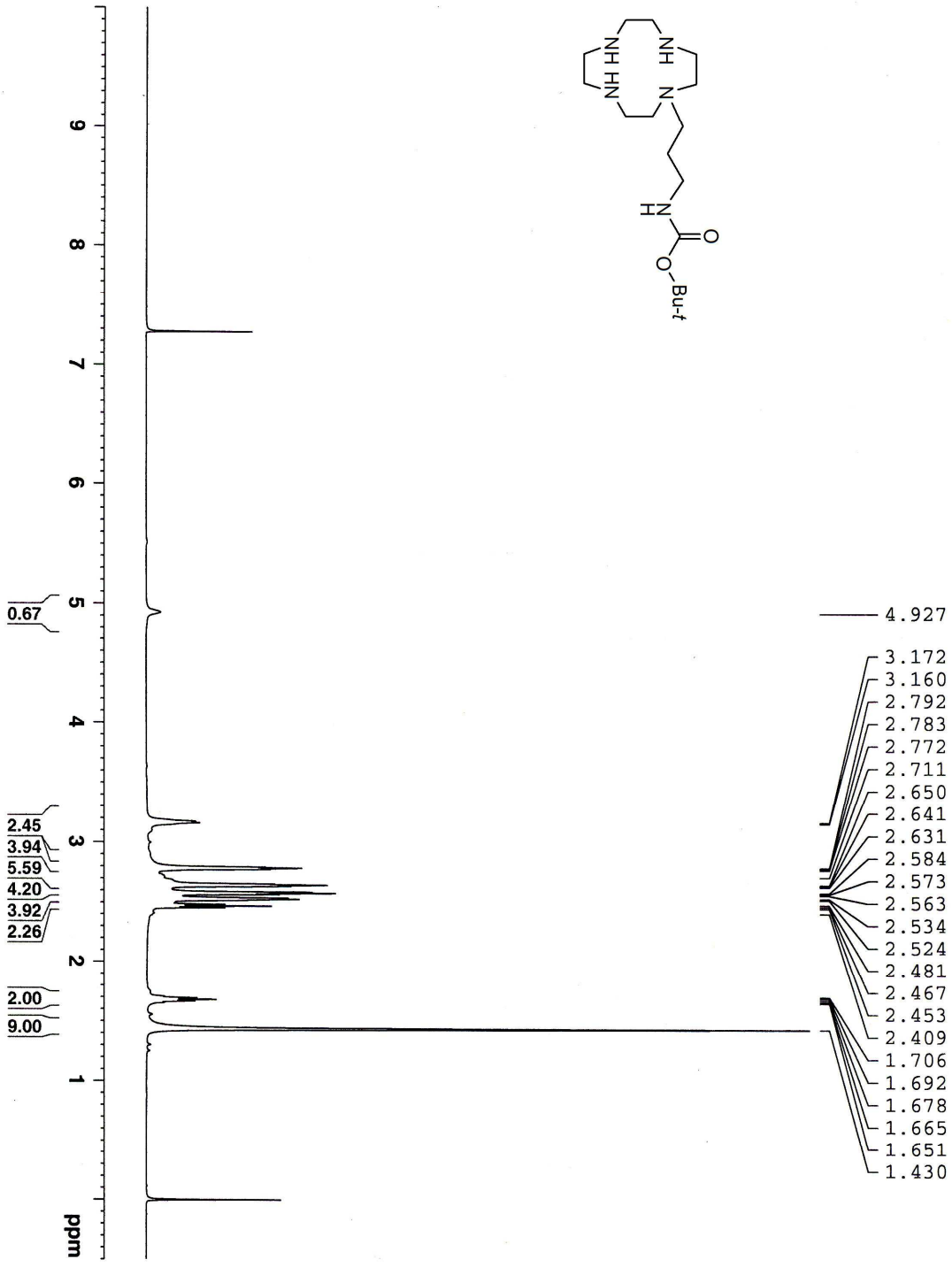
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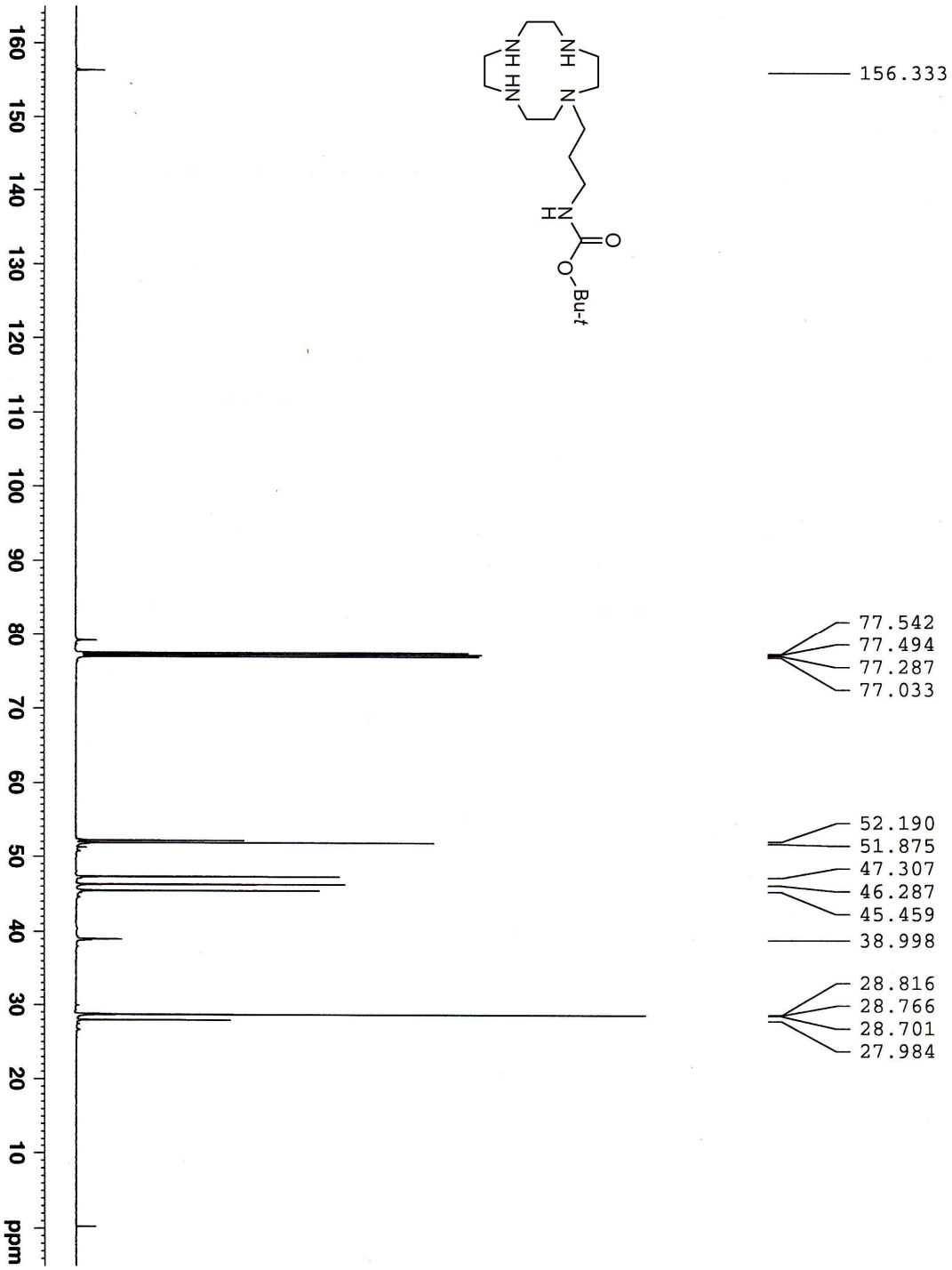
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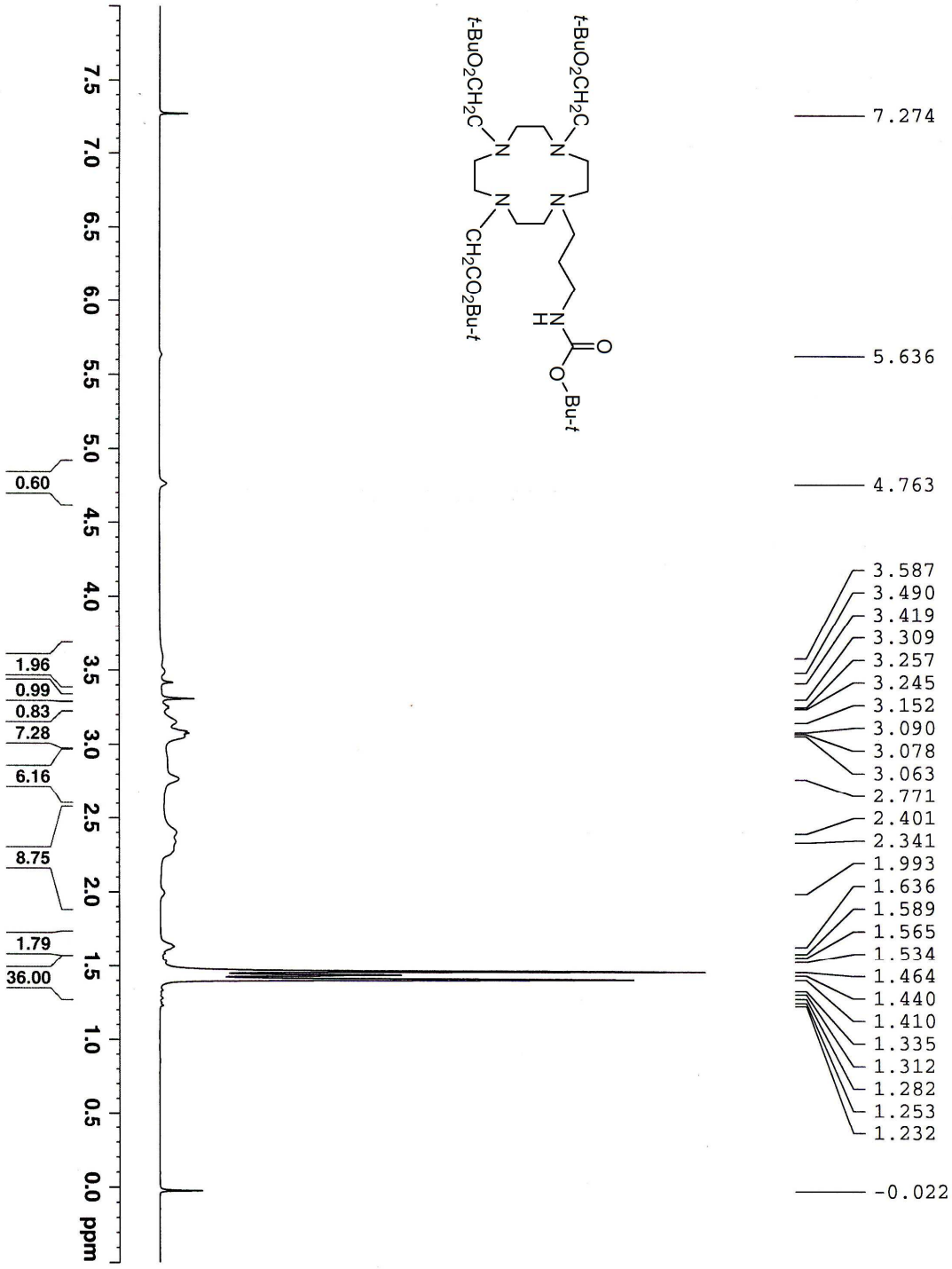
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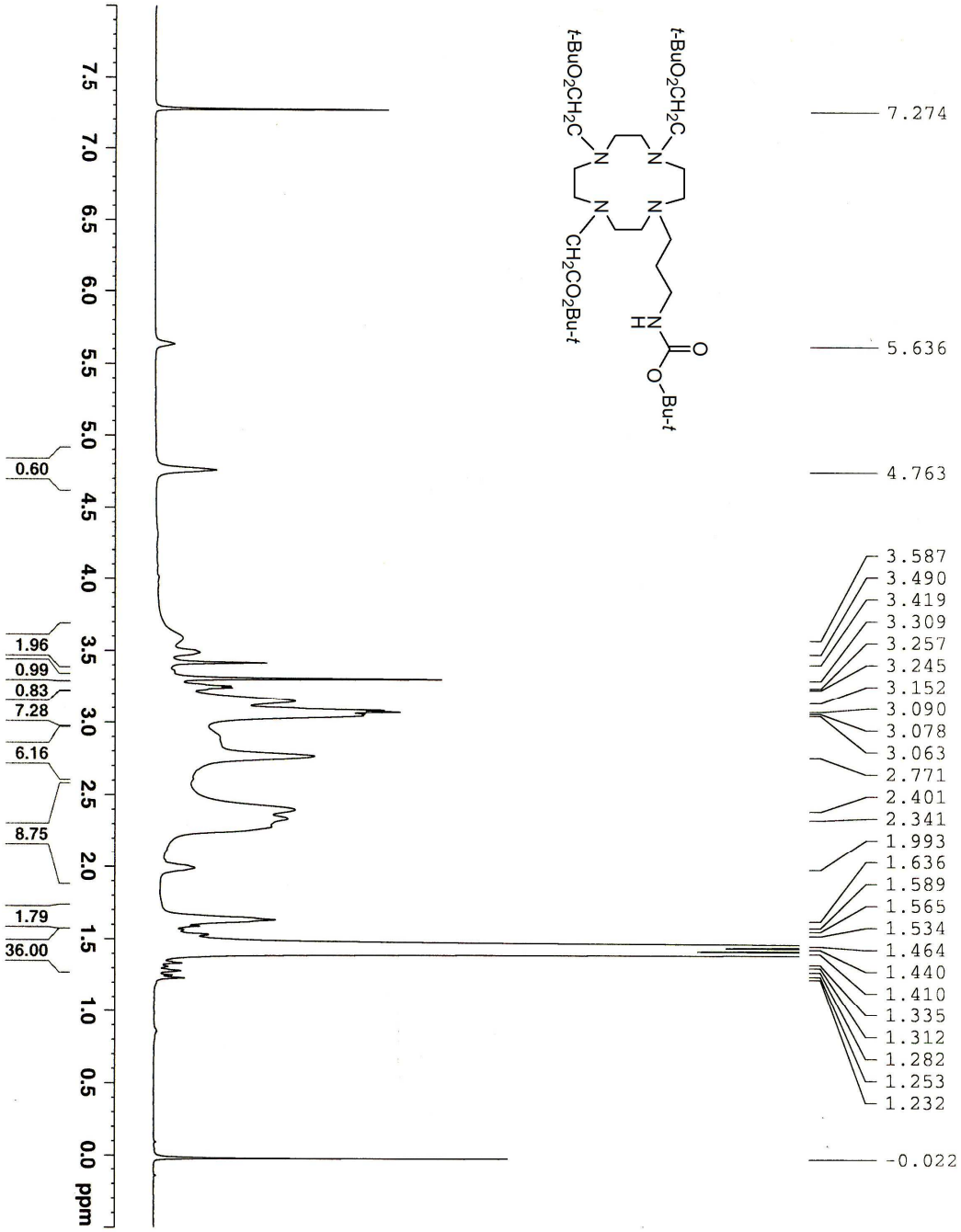
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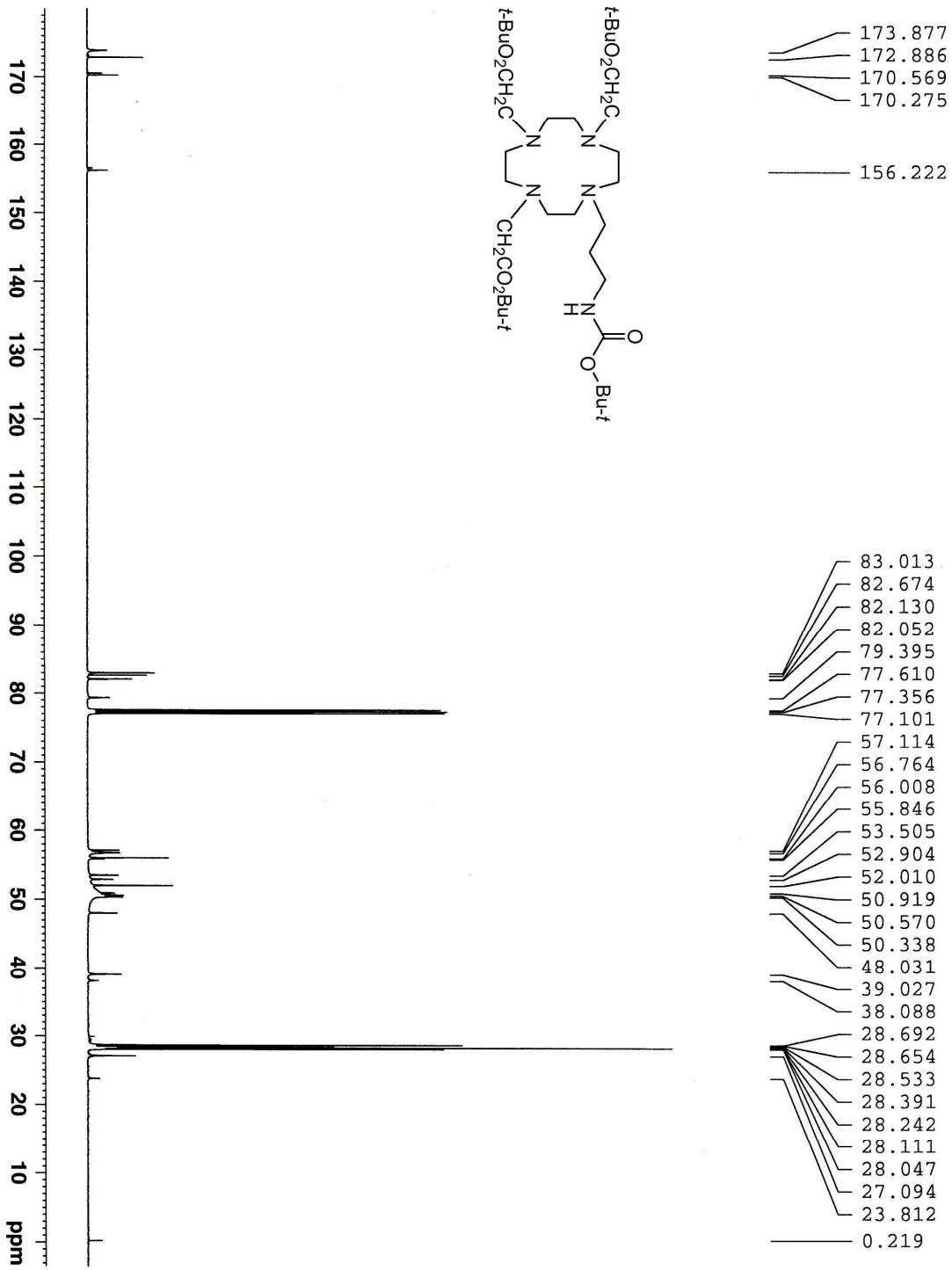
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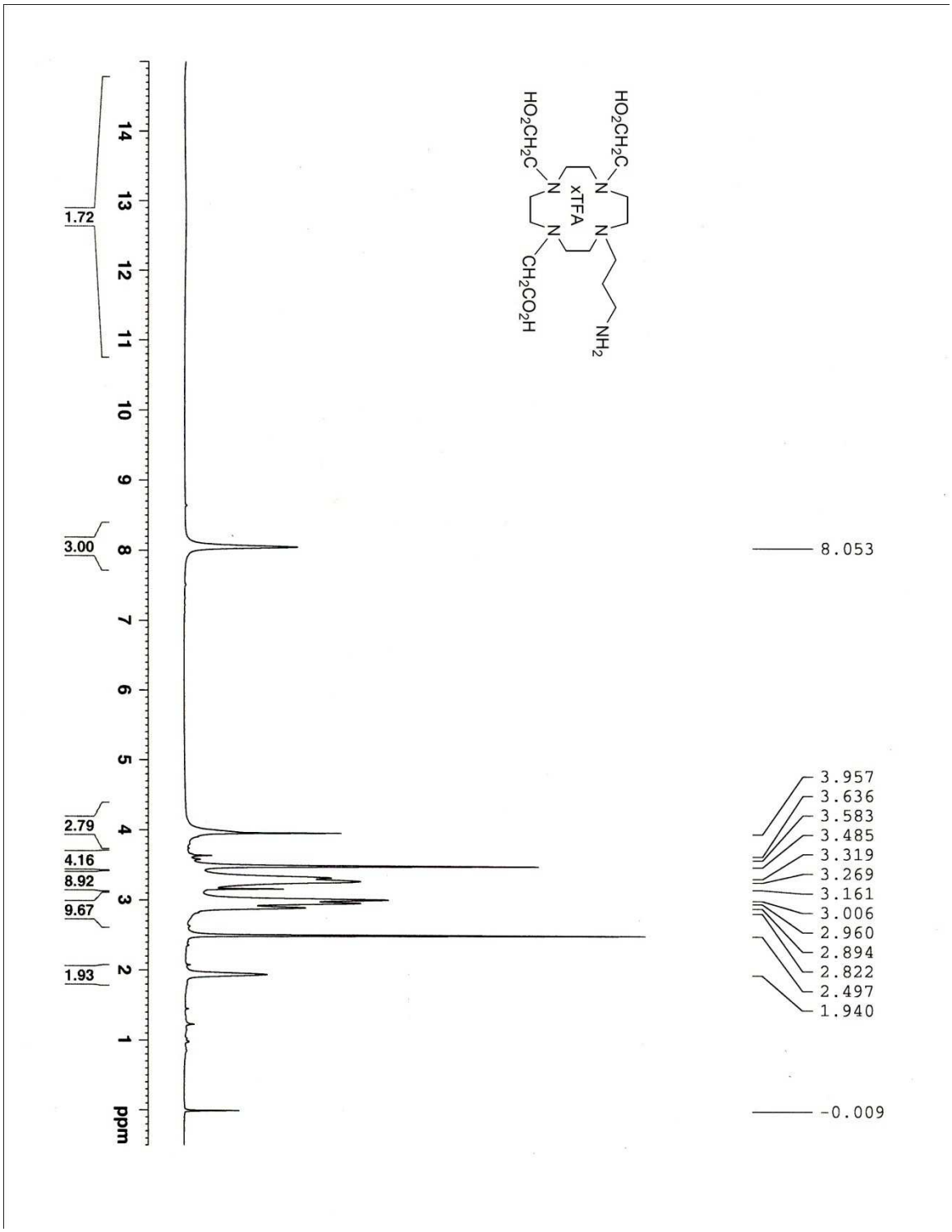
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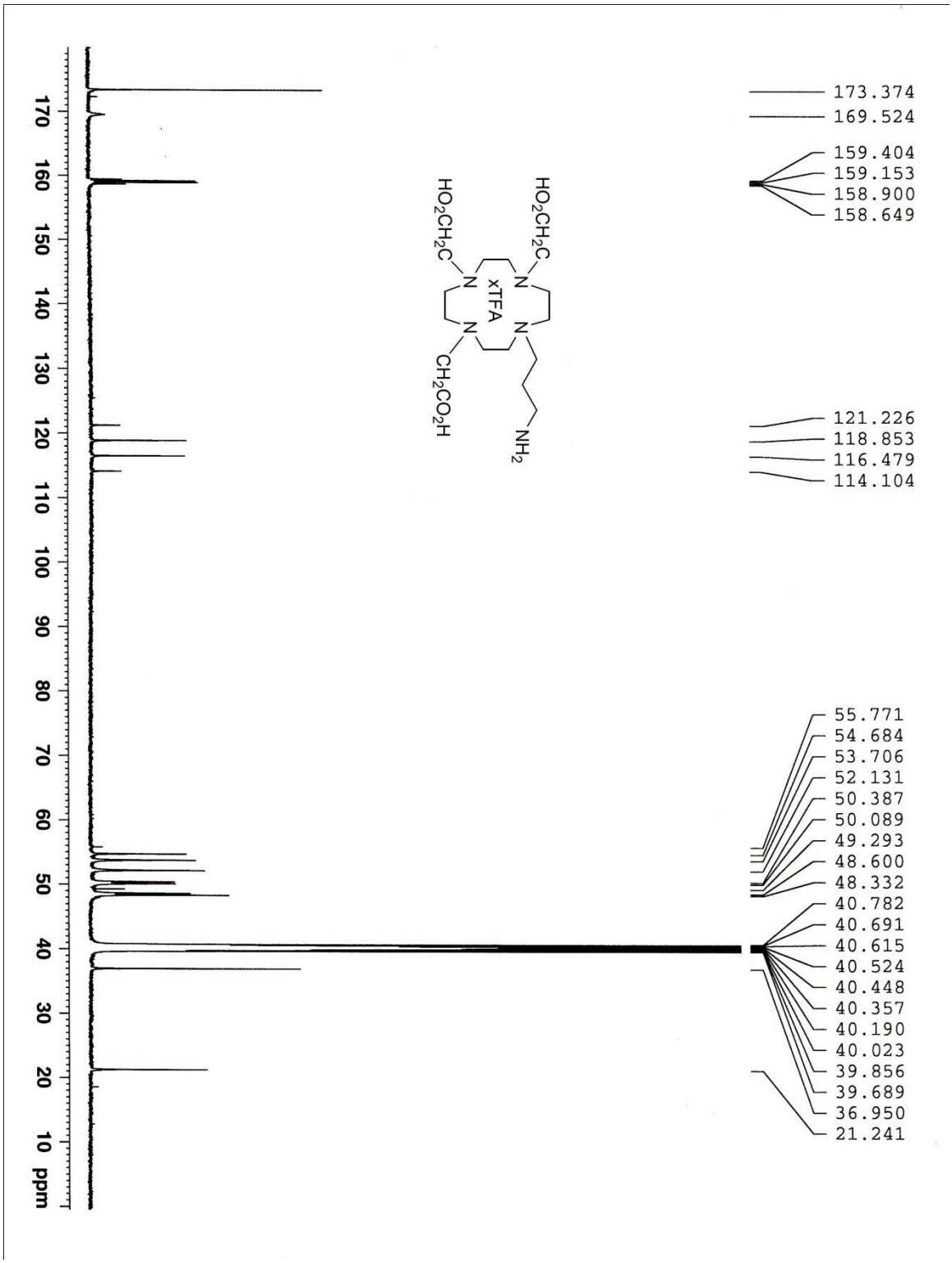
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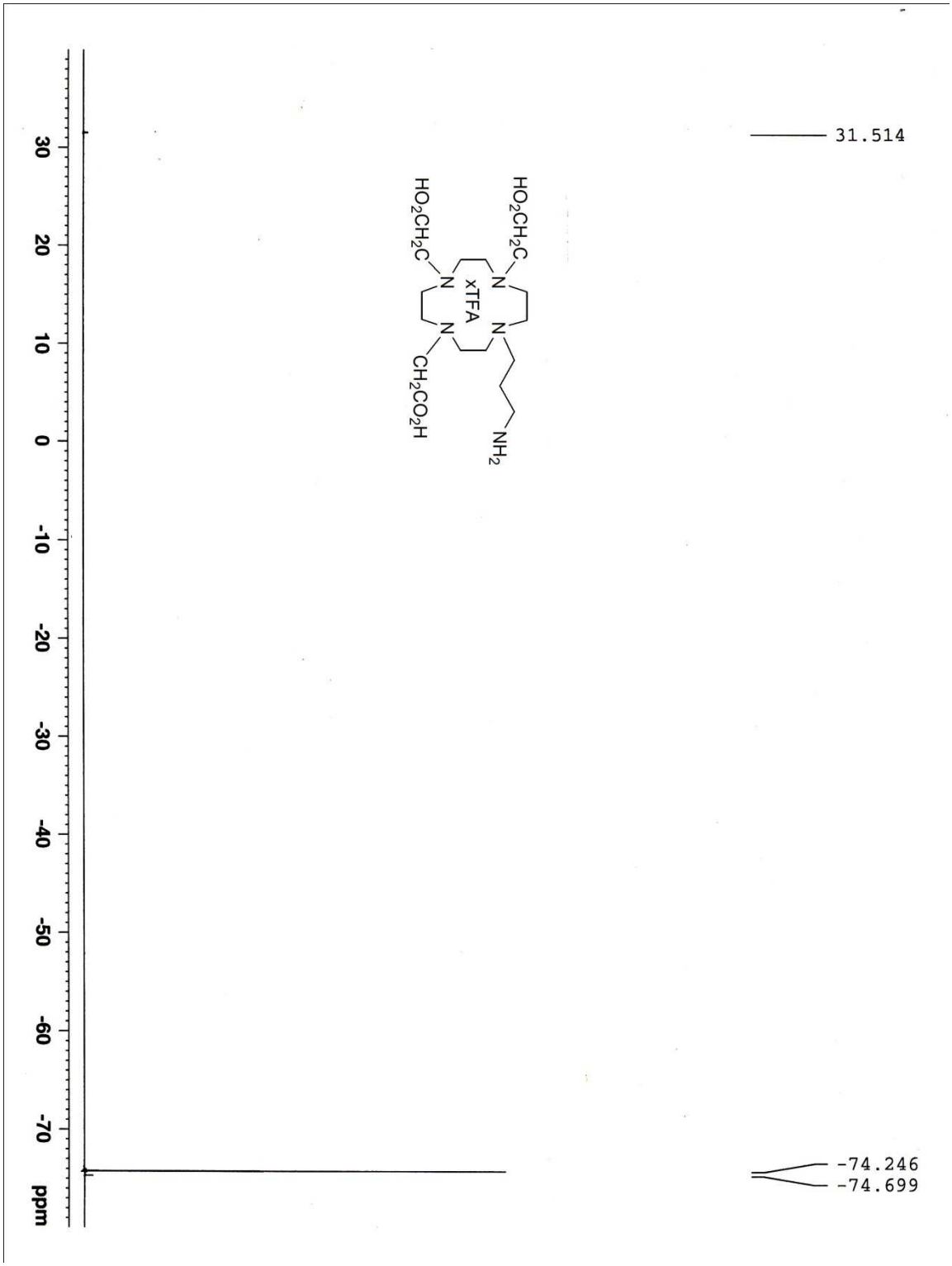
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