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Electronic Supplementary Information for

# Dendrimeric Calcium-responsive MRI Contrast Agents with Slow *in vivo* Diffusion

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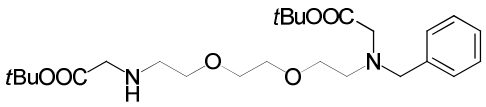
## General remarks

Commercially available reagents and solvents were used without further purification. Compounds **1** and **3** were synthesized according to the published procedure.[1] Purification of the synthesized compounds was performed using silica gel 60 (0.06–0.2 mm) from Carl Roth (Germany). Dendrimers **11** and **12** were purified using Sephadex®LH-20 (lipophilic Sephadex) (bead size: 25-100 μm) and Sephadex®G-15 (bead size: 40-120 μm), both from Sigma-Aldrich (Germany). $^{1}$H, $^{13}$C- NMR spectra and relaxometric experiments were performed on a Bruker Advance III 300 MHz spectrometer, pro-

cessed using TopSpin 2.1 (Bruker GmbH), and analyzed with ACD/SpecManager 9.0 (Advanced Chemistry Development, Inc.). The NMR spectra were obtained either in $CDCl_3$ or $D_2O$, using the deuterium lock frequency. The concentration of $Gd^{3+}$ in analyzed solutions was determined using the bulk magnetic susceptibility shift (BMS).[2] High resolution mass spectra were recorded on a Bruker Daltonics APEX II (FT-ICR-MS) with an electrospray ionization source. ESI-TOF-MS experiments were performed by MAXIS 3G, Bruker Daltonics Inc., Germany. MALDI-TOF-MS analysis was performed by The Scripps Center for Mass Spectrometry, La Jolla, CA. Low resolution mass spectra were recorded on an ion trap SL 1100 system Agilent with an electrospray ionization source.

MRI experiments were performed using a 7 T, 40 cm bore MRI magnet (Kobelco and Jastec, Kobe and Tokyo, Japan) interfaced to a Bruker console (BioSpec Avance-I, Bruker Biospin, Ettlingen, Germany). The volume resonator (72 mm inner diameter, transmission, Bruker Biospin) and the quadrature surface reception coil (rat brain coil, Rapid Biomedical, Rimpar, Germany) were used.

## Synthetic procedures

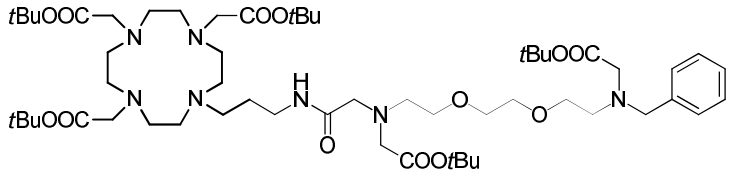



**(2-{2-[2-(Benzyl-*tert*-butoxycarbonylmethyl-amino)-ethoxy]-ethoxy}-ethylamino)-acetic acid *tert*-butyl ester (2).** Bisamine **1** (26.6 mmol, 10.0 g) was dissolved in dimethylformamide (40 mL) and cesium carbonate (21.3 mmol, 6.95 g) was added. Benzyl chloride (21.3 mmol, 2.70 g) was dissolved in dimethylformamide (20 mL) and added dropwise to the mixture. The resulting mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, dichloromethane (50 mL) was added to the residue and the insoluble salts were removed by filtration. Additional dichloromethane (200 mL) was added to the filtrate and the solution was extracted with water (2x200 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (3% methanol/dichloromethane) gave the mono benzyl amine derivative **2** as light yellow oil (5.21 g, 42%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 7.40−7.11 (m, 5H, ArH), 3.82 (br, 2H, NC$H_2$Ar), 3.56 (br., 8H, C$H_2$OC$H_2$C$H_2$OC$H_2$), 3.32-3.22 (m, 4H, $NCH_2COOtBu$), 2.96–2.71 (m, 5H, NHC$H_2$C$H_2$), 1.43 (s, 18H, C(C$H_3$)$_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 171.2, 170.8 (*C*=O), 139.1, 128.8, 128.1, 126.8 (Ar*C*), 80.9, 80.5 (*C*$(CH_3)_3$), 70.4, 70.2, 70.1, 69.9, 58.5, 55.5, 52.8, 51.4, 48.5 (–*C*$H_2$–), 28.0, 27.9 (C(*C*$H_3$)$_3$).

**ESI-HRMS:** $[M+H]^+$ calcd. for $C_{25}H_{43}N_2O_6^+$, 467.3, found 467.3; $[M+Na]^+$ calcd. for $C_{25}H_{42}N_2NaO_6^+$, 489.3, found 489.3.

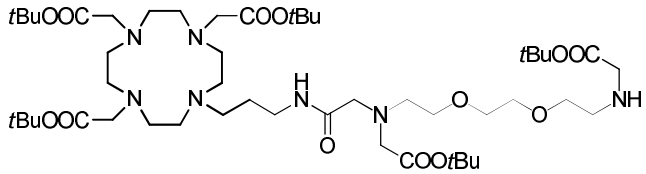


**[Benzyl-(2-{2-[2-(*tert*-butoxycarbonylmethyl-{[3-(4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethyl)-amino]-acetic acid *tert*-butyl ester (4).** The bromide **3** (5.90 g, 6.94 mmol) was slowly added to the suspension of amine **2** (2.70 g, 5.79 mmol) and potassium carbonate (17.37 g, 2.40 mmol) in anhydrous acetonitrile (50 mL). The resulting solution was stirred at 70 °C for 4 h. Upon cooling, the insoluble materials were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in dichloromethane (200 mL) and extracted with water (2x200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the residue by silica gel column chromatography (4% methanol/dichloromethane) gave benzyl amine **4** as a white flocculent powder (4.44 g, 71%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 7.27 (br., 5H, Ar*H*), 3.80 (s, 2H, NC$H_2$Ar) 3.68−1.89 (overlapping m, 44H), 1.85–1.58 (m, 2H, $NCH_2CH_2CH_2$), 1.56−1.31 (m, 45H, $C(CH_3)_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 173.1, 172.1, 171.0, 169.9, 169.5, (*C*=O), 138.6, 128.5, 127.8, 126.8, (ArC), 82.2, 82.0, 81.2, 80.5, ($C(CH_3)_3$), 69.6, 68.5, 58.8, 58.4, 56.1, 55.3, 53.2, 52.4, 51.5, 50.3, 49.7, 36.9, 29.2, (–$CH_2$–), 27.7, 27.6, 27.5, 27.4 ($C(CH_3)_3$).

**ESI-HRMS:** $[M+H]^+$ calcd. for $C_{56}H_{100}N_7O_{13}^+$, 1078.7374, found 1078.7360.

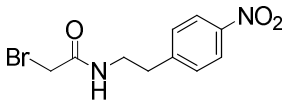


**(2-{2-[2-(*tert*-Butoxycarbonylmethyl-{[3-(4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethylamino)-acetic acid *tert*-butyl ester (5).** The benzyl amine **4** (3.9 g, 0.60 mmol) was dissolved in ethanol (15 mL) and 10% Pd/C (780 mg, 10% w/w) was added. The resulting suspension was shaken for 16 h under a $H_2$ atmosphere (3.0 bar) in a Parr apparatus. The catalyst was removed from the reaction mixture by filtration and solvent evaporated under reduced pressure. The residue was purified by silica gel column chromatography (10 % methanol/dichloromethane) to obtain the pure amine **5** as white fluffy powder (3.4 g, 95%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 8.01 (br. s, 1H, N*H*), 3.74−1.84 (m, 44H), 1.78–1.50 (m, 2H, $NCH_2CH_2CH_2$), 1.47–1.17 (m, 45H, $C(CH_3)_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 173.2, 172.0, 171.7, 171.1, 169.6 (*C*=O), 82.4, 82.1, 81.8, 81.4, 81.3 ($C(CH_3)_3$), 69.3, 69.1, 69.0, 68.7, 68.2, 67.3, 58.9, 58.04, 56.3, 55.3, 53.3, 52.8, 52.5, 51.6, 50.4, 49.8, 48.7, 48.1, 47.5, 37.1, 30.5 (–*C*$H_2$–), 27.7, 27.4 ($C(CH_3)_3$), 24.7 (–*C*$H_2$–).

**ESI-TOF/MS** (m/z): $[M+H]^+$ calcd. for $C_{49}H_{94}N_7O_{13}^+$, 988.6904, found 988.6895.

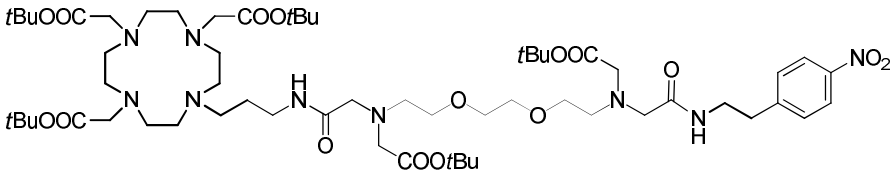



**2-Bromo-*N*-[2-(4-nitro-phenyl)-ethyl]-acetamide (6).** 2-(4-nitrophenyl)ethanamine hydrochloride (5.0 g, 24.67 mmol) and triethylamine (7.57 mL, 54.3 mmol) were dissolved in dichloromethane (100 ml) and cooled to 0 °C. A solution of bromoacetyl bromide (6.0 g, 29.7 mmol) in dichloromethane (20 ml) was added dropwise. Reaction completion was followed by TLC (eluent: ethyl acetate/hexane 7:3 v/v). After completion of the reaction, cold water (200 mL) was added to the reaction mixture and the organic material was extracted with dichloromethane (2x200 mL). The organic phases were combined and dried over sodium sulfate. The suspension was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane, gradient from 1:9 to 7:3 v/v) to obtain the bromide **6** as an off-white solid (3.8 g, 54%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 8.16 (d, *J*=9.0 Hz, 2H; Ar*H*), 7.38 (d, *J*=9.0 Hz, 2H; Ar*H*), 3.85 (s, 2H; Br$CH_2$), 3.59 (q, *J*=7.0 Hz, 2H; Ar$CH_2$), 2.97 (t, *J*=7.0 Hz, 2H; NHC$H_2CH_2$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 165.7 (*C*=O), 146.7, 146.3, 129.6, 123.7 (Ar*C*), 40.7 ($NHCH_2CH_2$), 35.2 (Ar$CH_2$), 28.9 (Br$CH_2$).

**ESI-TOF/MS** (m/z): $[M+Na]^+$ calcd. for $C_{10}H_{11}BrN_2NaO_3^+$, 308.9918, found 308.9840.



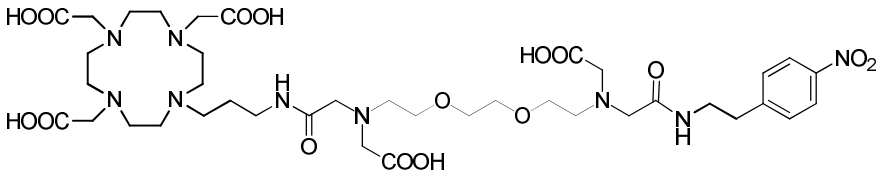

**((2-{2-[2-(*tert*-Butoxycarboxymethyl-{[3-(4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethyl)-{[2-(4-nitro-phenyl)-ethylcarbamoyl]-methyl}-amino)-acetic acid *tert*-butyl ester (7).** The amine **5** (2 g, 2.02 mmol) was dissolved in anhydrous acetonitrile (75 mL) and bromide **6** (0.70 g, 2.44 mmol) and potassium carbonate (0.56g, 4.06 mmol) were added at room temperature. The reaction

mixture was stirred at 70 °C for 24 h. After cooling to room temperature, the insoluble materials were removed by filtration. The residue was dissolved in dichloromethane (200 mL) and washed with water (2x200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, 10% methanol/dichloromethane) to obtain the product **7** as a light brown solid (1.95 g, 81%).

**$^{1}$H NMR** (300 MHz, $CDCl_3$): δ 8.08 (br, 2H; Ar*H*), 7.39 (br, 2H; Ar*H*), 3.64−2.12 (m, 50H), 1.77–1.55 (br, 2H; $NHCH_2CH_2CH_2$), 1.40 (overlapping m, 45H; $C(CH_3)_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 173.0, 172.0, 171.1, 171.0, 170.4, 169.9, 169.5 (*C*=O), 147.0, 146.1, 129.4, 123.1 (Ar*C*), 82.3, 82.0, 81.3, 80.9 ($C(CH_3)_3$), 69.7, 68.7, 58.6, 56.7, 56.3, 56.0, 55.2, 54.0, 52.7, 52.2, 51.4, 50.4, 49.7, 47.4, 39.2, 36.9, 35.1(–$CH_2$–), 27.6, 27.3 ($C(CH_3)_3$), 25.5 (–$CH_2$–).

**ESI-TOF/MS** (m/z): $[M+H]^+$calcd. for $C_{59}H_{104}N_9O_{16}^+$, 1194.7596, found 1194.7583.

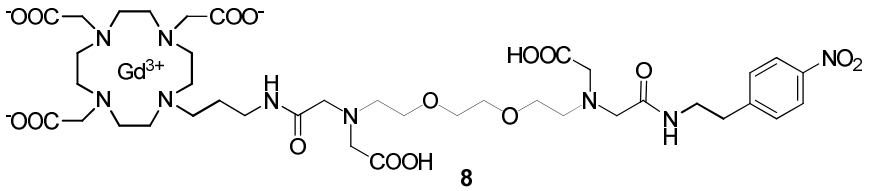



**((2-{2-[2-(Carboxymethyl-{[3-(4,7,10-tris-carboxymethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethyl)-{[2-(4-nitro-phenyl)-ethylcarbamoyl]-methyl}-amino)-acetic acid (8).** The protected monomeric chelator **7** (105 mg, 88 μmol) was dissolved in formic acid (5 mL) and the mixture was stirred at 60 $^{o}$C for 16 h. Formic acid was evaporated under reduced pressure. The residue was dissolved in methanol (1 mL) and added slowly to diethylether (50 mL) with vigorous stirring. The solution was cooled to -20 °C for 16 h. The solvent was decanted and the product was dried by lyophilization to yield the monomeric chelator **8** (65 mg, 81%).

**$^{1}$H NMR** (300 MHz, $D_2O$): δ 8.15 (d, *J*=8.2 Hz, 2H; Ar*H*), 7.45 (d, *J*=8.6 Hz, 2H; Ar*H*), 4.27−2.59 (m, 50H), 1.89 (br, 2H; $NHCH_2CH_2CH_2$).

**$^{13}$C{H} NMR** (75 MHz, $D_2O$): δ 171.2, 169.7, 169.5, 167.0, 165.4, 165.0 (*C*=O), 147.6, 146.3, 130.1, 123.7 (Ar*C*), 69.8, 69.4, 64.5, 64.4, 60.8, 57.2, 57.0, 56.1, 55.8, 55.2, 54.9, 51.2, 50.3, 49.4, 49.3, 40.1, 37.0, 34.6, 32.9, 23.9 (–$CH_2$–).

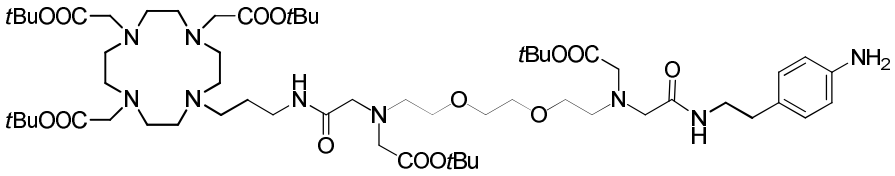
**ESI-TOF/MS** (m/z): $[M-H]^-$ calcd. for $C_{39}H_{62}N_9O_{16}^-$, 912.4320, found 912.4317.



8

**Complex Gd8.** The monomeric chelator **8** (70 mg, 77 µmol) was dissolved in water and pH was adjusted to 7.0 with aqueous sodium hydroxide (0.1 M). A solution of $GdCl_3 \cdot 6H_2O$ (31.3 mg, 84 µmol) in water was added and pH was maintained at 7.0. The mixture was stirred at room temperature for 24 h. Excess $Gd^{3+}$ ions were removed by Chelex®100 and the solvent was evaporated under reduced pressure to obtain **Gd8** as an off-white solid (66 mg, 81%).

**ESI-TOF/MS** (m/z): $[M-H]^-$ calcd. for $[C_{39}H_{59}GdN_9O_{16}]^-$, 1067.3326, found 1067.3352.

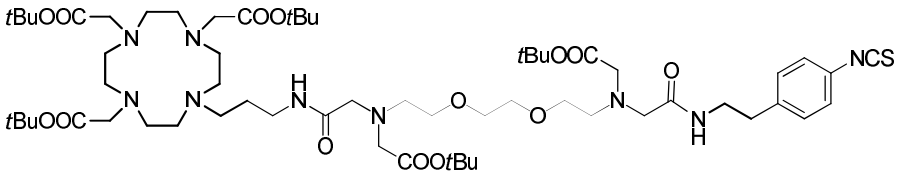


**[{[2-(4-Amino-phenyl)-ethylcarbamoyl]-methyl}-(2-{2-[2-(*tert*-butoxycarbonylmethyl-{[3-(4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethyl)-amino]-acetic acid *tert*-butyl ester (9).** A suspension of the macrocycle **7** (1.85 g, 1.55 mmol) and Pd/C catalyst in ethanol was shaken under a hydrogen atmosphere (3.0 bar) in a Parr hydrogenator. After reaction completion (18 h), the catalyst was removed by filtration through a celite column. The solvent was removed under reduced pressure to obtain **9** as a light brown solid (1.75 g, 97%) which was used in the next step without further purification.

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 6.79 (d, *J*=8.2 Hz, 2H; Ar*H*), 6.43 (d, *J*=8.2 Hz, 2H; Ar*H*), 3.46−1.93 (m, 50H), 1.59−1.45 (br, 2H; $NHCH_2CH_2CH_2$), 1.27 (overlapping m, 45H; $C(CH_3)_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 173.0, 172.0, 170.9, 170.7, 170.5, 170.1, 169.8, 169.4 (*C*=O), 144.8, 128.9, 127.7, 114.7 (Ar*C*), 82.3, 81.9, 81.2, 80.9 ($C(CH_3)_3$), 69.7, 68.7, 58.7, 57.2, 56.7, 56.3, 56.0, 55.2, 53.9, 52.5, 52.1, 51.4, 50.4, 49.6, 48.5, 47.4, 40.1, 36.8, 34.4, 27.6 (–$CH_2$–), 27.5, 27.3 ($C(CH_3)_3$) 25.5 (–$CH_2$–).

**ESI-TOF/MS** (m/z): $[M+H]^+$calcd. for $C_{59}H_{106}N_9O_{14}^+$, 1164.7584, found 1164.7842.

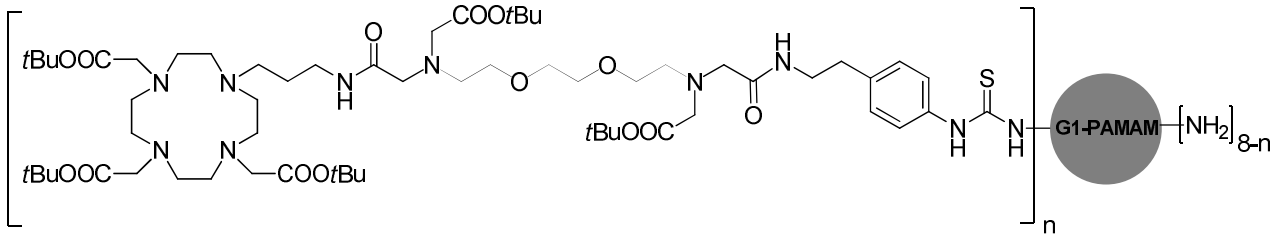


**((2-{2-[2-(*tert*-Butoxycarbonylmethyl-{[3-(4,7,10-tris-*tert*-butoxycarbonylmethyl-1,4,7,10tetraaza-cyclododec-1-yl)-propylcarbamoyl]-methyl}-amino)-ethoxy]-ethoxy}-ethyl)-{[2-(4-isothiocyanato-phenyl)-ethylcarbamoyl]-methyl}-amino)-acetic acid *tert*-butyl ester (10).** The aniline **9** (1.5 g, 1.30 mmol) and triethylamine (0.54 mL, 3.86 mmol) were dissolved in dichloromethane (20 mL) and thiophosgene (0.2 mL, 2.61 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (200 mL) and the solution was washed with water (2x200 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, 10% methanol/dichloromethane) to obtain isothiocyanate **10** as a light brown solid (0.9 g, 58%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 7.09 (dd, *J*=22.8, 8.2 Hz, 4H; Ar*H*), 3.70−1.71 (br, 52H), 1.36 (overlapping m, 45H; C(C$H_3$)$_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 172.3, 171.3, 170.7, 170.5, 170.4 (*C*=O), 138.6, 134.6, 129.8, 128.9, 125.5 (Ar*C*), 82.6, 82.3, 81.2, 81.1 (*C*(CH$_3$)$_3$), 70.0, 69.1, 69.0, 58.9, 56.8, 56.4, 55.5, 54.1, 53.3, 52.1, 51.7, 39.7, 37.1, 35.1 (–*C*H$_2$–), 27.9, 27.7, 27.6 (C(*C*H$_3$)$_3$), 25.7(–*C*H$_2$–).

**ESI-TOF/MS** (m/z): $[M+H]^+$ calcd. for $C_{60}H_{104}N_9O_{14}S$, 1206.7418, found 1206.7404.



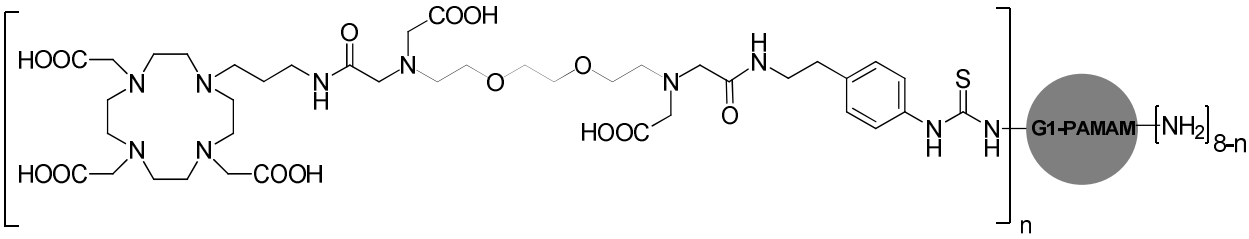
**Dendrimer 11**. G1 PAMAM dendrimer (65 mg, 45 μmol) and isothiocyanate **10** (650 mg, 539 μmol) were dissolved in dimethylformamide and triethylamine (152 μL, 1.09 mmol) was added to the solution. The reaction mixture was stirred at 45 °C for 24 h. The solvent was evaporated and the unreacted ligand was removed using a lipophilic Sephadex (Sephadex®LH-20) column with methanol as the eluent to obtain protected dendrimeric chelator **11** (388 mg, 77%).

**$^1$H NMR** (300 MHz, $CDCl_3$): δ 7.50 (br, Ar*H*), 7.06 (br, Ar*H*), 4.09–1.53 (overlapping m), 1.52–1.26 (overlapping m, C(C$H_3$)$_3$).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 181.4, 173.4, 173.0, 172.4, 172.0, 171.3, 171.3, 170.6, 170.5, 170.4, 170.2, 169.8 (*C*=O), 128.6, 123.6 (Ar*C*), 82.7, 82.3, 81.7, 81.3 (C(*C*H$_3$)$_3$), 70.2, 69.3, 69.0,

59.1, 56.9, 56.6, 56.3, 55.5, 55.3, 54.2, 52.9, 52.4, 51.8, 50.8, 50.3, 50.0, 47.9, 43.9, 40.3, 38.8, 37.2, 36.3, 35.2, 34.2 (–*C*$H_2$–), 28.0, 27.9, 27.7 (*C*($CH_3$)$_3$).

**MALDI-TOF/MS** (m/z): $[M+13Na+K]^+$ calcd. for $C_{542}H_{952}N_{98}Na_{13}KO_{124}S_8^+$, 11412, found 11411; $[M+12Na+2K]^+$ calcd. for $C_{482}H_{849}N_{89}Na_{12}K_2O_{110}S_7^+$, 10223, found 10225; $[M+10Na]^+$ calcd. for $C_{422}H_{746}N_{80}Na_{10}O_{96}S_6^+$, 8893, found 8896.

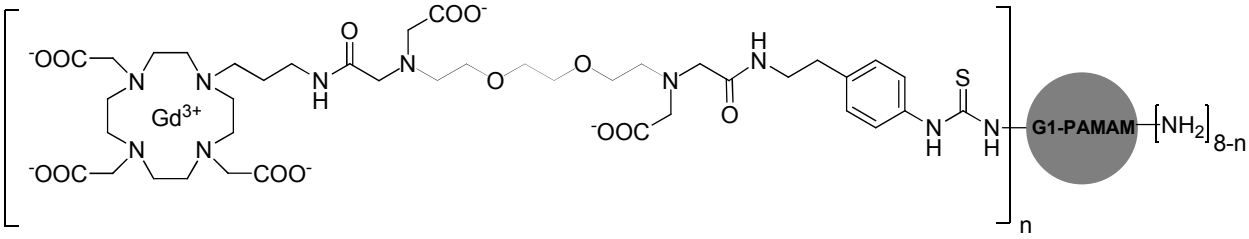



**Dendrimer 12**. The protected dendrimeric chelator **11** (388 mg, 35 μmol) was dissolved in formic acid (5 mL) and the mixture was stirred at 60 °C for 48 h. Formic acid was removed under reduced pressure and the residue was purified by size-exclusion chromatography (G-15 Sephadex column using water as an eluent). The collected fractions were freeze-dried to give dendrimeric chelator **12** as a light brown solid (300 mg, 97%).

**$^1$H NMR** (300 MHz, $D_2O$): δ 7.22 (br, Ar*H*), 4.25−2.50 (overlapping m), 1.83 (br. s).

**$^{13}$C{H} NMR** (75 MHz, $CDCl_3$): δ 180.0, 174.5, 173.6, 173.1, 172.1, 171.9, 170.4, 168.1 (*C*=O), 137.6, 135.4, 129.9, 125.4 (Ar*C*), 69.8, 67.6, 64.8, 63.2, 57.4, 56.6, 56.2, 55.6, 55.3, 55.1, 54.8, 54.2, 50.8, 50.3, 49.7, 49.3, 48.2, 43.6, 40.5, 38.9, 37.0, 36.6, 34.8, 34.2, 31.1, 29.7, 29.1, 23.6 (–*C*$H_2$–).

**MALDI-TOF/MS** (m/z): $[M+13Na+H_2O]^+$ calcd. for $C_{382}H_{632}N_{98}Na_{13}O_{124}S_8(H_2O)^+$, 9149, found 9149; $[M+11Na+3H_2O]^+$ calcd. for $C_{342}H_{569}N_{89}Na_{11}O_{110}S_7(H_2O)_3^+$, 8213, found 8215; $[M+8Na+H_2O]^+$ calcd. for $C_{302}H_{506}N_{80}Na_8O_{96}S_6(H_2O)^+$, 7183, found 7184; $[M+4Na]^+$ calcd. for $C_{262}H_{443}N_{89}Na_{41}O_{82}S_5^+$, 6148, found 6148.




**Dendrimer DSCA**. Dendrimeric chelator **12** (200 mg, 23 μmol) was dissolved in water and pH was adjusted to 7.0 with aqueous sodium hydroxide (0.1 M). A solution of $GdCl_3·6H_2O$ (101 mg, 272 μmol) in water was added and pH was maintained at 7.0. The mixture was stirred at room temperature for 24 h. EDTA (120 mg, 324 μmol) was added into the solution to remove excess $Gd^{3+}$ while maintaining pH at 7.0. Excess of GdEDTA and EDTA were removed by centrifugation using cen-

trifugal filter units with 3 KDa molecular weight cut-off filters to obtain **DSCA** as a light brown solid (220 mg, 97%).

**MALDI-TOF/MS** (m/z): $[M+8Na+8H_2O]^+$ calcd. for $C_{382}H_{608}Gd_8N_{98}Na_8O_{124}S_8(H_2O)_8^+$, 10399, found 10396; $[M+3Na+7H_2O]^+$ calcd. for $C_{342}H_{548}Gd_7N_{89}Na_3O_{110}S_7(H_2O)_7^+$, 9186, found 9185; $[M+3Na+6H_2O]^+$ calcd. for $C_{302}H_{488}Gd_6N_{80}Na_3O_{96}S_7(H_2O)_6^+$, 8087, found 8086.

## Relaxometric titrations

The $T_1$ determinations were performed at 7.0 T, 25 °C and pH 7.4 (HEPES buffer) using a standard inversion recovery pulse sequence. A solution of $CaCl_2$ of known concentration was added stepwise to the **Gd8** or **DSCA** solution (starting concentration 3.0 mM $Gd^{3+}$) and the $T_1$ was measured after each addition of the analyte. The relaxivity $r_1$ was calculated from Eq. 1: $1/T_{1,obs} = T_{1,d} + r_1 \times [Gd]$, where $T_{1,obs}$ is the measured $T_1$, $T_{1,d}$ is the diamagnetic contribution of the solvent and [Gd] is the actual $Gd^{3+}$ concentration at each point of the titration.

## *In vivo* MRI experiments

Normal male Wistar rats (234.4 ± 21.7 g body weight, 9-11 weeks old, n=3 for **Gd8** and **DSCA**, respectively) were used for MRI experiments. Prior to the MRI scan, all rats were anesthetized with 2.0% isoflurane (Abbott Japan, Japan). Contrast agents (1 μL solution of **Gd8** or **DCSA**, c=10 mM $Gd^{3+}$) were intracerebrally administrated into the cortex (bregma +1 mm frontal, +3 mm right side, and 1.5 mm depth) using a stereotaxic apparatus (Narisige, Tokyo, Japan) and micro-syringe (10 μL, Hamilton Company, Nevada, USA). Rectal temperature was continuously monitored and maintained at 37.0 °C using a heater throughout the experiments. During the MRI scan, the rats were held in place with the help of a handmade ear/bite bar and anesthetized through a facemask with 2.0% isoflurane.

Prior to MRI acquisitions, scout images were acquired to localize the imaging plain. Imaging registration was carefully adjusted to the injected site based on scout scans. MRI acquisitions for $T_1$-weighted imaging ($T_1$WI) were repeated 30 times. The first $T_1$WI scan began typically ~20 minutes after the intracerebral administration.

For the $T_1$WI, a two-dimensional (2D), single-slice image was obtained using a conventional spin-echo sequence with the following parameters: repetition time (TR) = 400 ms, echo time (TE) = 9.6 ms, matrix size = 256 × 256, field of view (FOV) = 32.0 × 32.0 $mm^2$, slice thickness (ST) = 1.0 mm, number of acquisitions (NA) = 4, and slice orientation = trans-axial. For this imaging sequence, the

nominal voxel resolution was 125 × 125 × 1000 μm$^3$. The total acquisition time for the $T_1$WI was 6 min and 42 s. All calculations and analyses were performed using the ParaVision (Bruker Biospin) and MRVision analysis software (MRVision Co., USA).

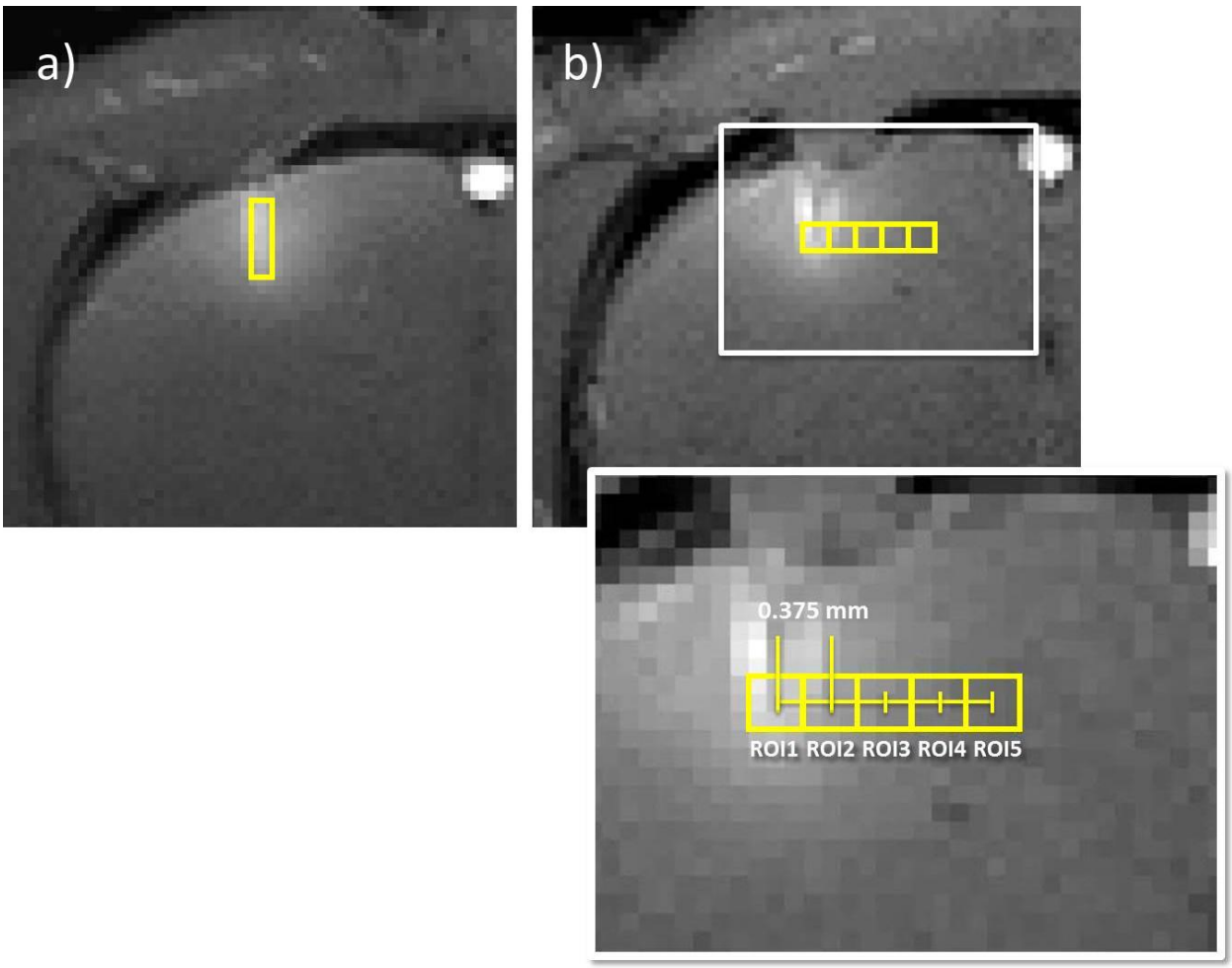



**Figure S1**. Definition of ROI for analysis of MRI data: a) Selection of large ROIs which include 30 voxels at the injected site; b) Selection of small ROIs which were used for longitudinal analysis of SI estimations (only ROI1 were used for both the **Gd8** and **DSCA** in Figure 3c). The expanded framed region is shown on the image below to highlight better the selection of ROI1-ROI5.

**Analysis of MRI data**. Large regions of interest (ROIs, 30 voxels) were selected for all *in vivo* experiments covering enhanced regions on the MR images where the contrast agents were administered (Figure S1a). The obtained signal intensity (SI) was plotted as a function of time (acquisition time from the intracerebral injection) and the values were normalized by the value obtained for the first MR image. For each experiment the data were fitted according to the formula $Y=A\times\ln(X)+C$, where X–acquisition time and Y–normalized SI. The mean of three values obtained for A was calculated for **Gd8** and **DSCA**, respectively. The fitted curves assuming the mean A and C values were plotted together with the single experimental data sets obtained for **Gd8** and **DSCA**, respectively (Figure 2b).

Temporally resolved SI estimations were performed by selecting the smaller ROIs with 9 voxels (Figure S1b). The same type of fitting as explained above was performed for the single *in vivo* MRI session data sets (for **Gd8** and **DSCA**) and the estimated SI differences (in %) after an hypothesized 2 min 'stimulus-on' period were calculated from the fitted values (Figure 2c). The results reported in Figure 2c were obtained only from ROI1, whereas ROI2-ROI5 did not show sufficient signal enhancement (i.e. local contrast agent concentration is low), as depicted in Figure S2.

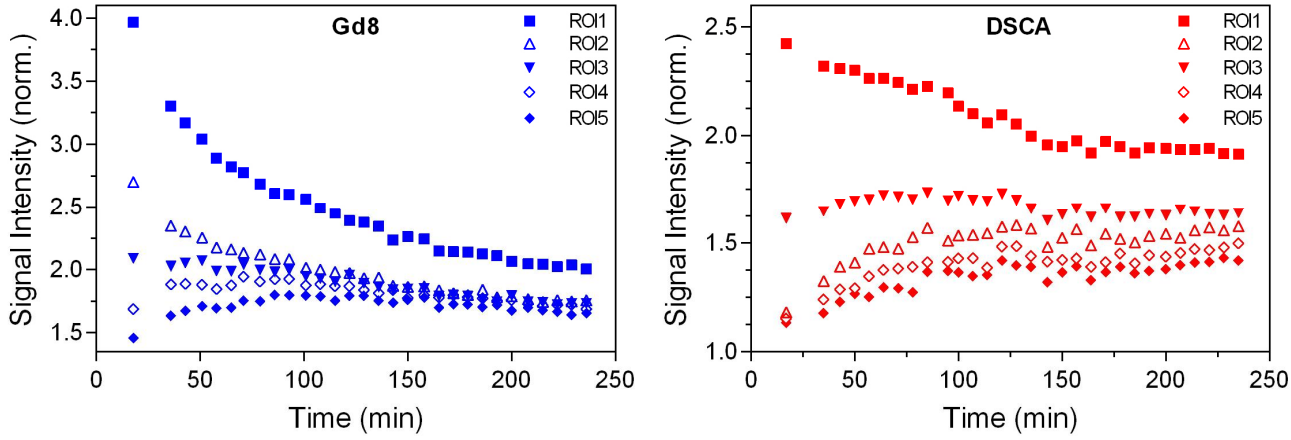
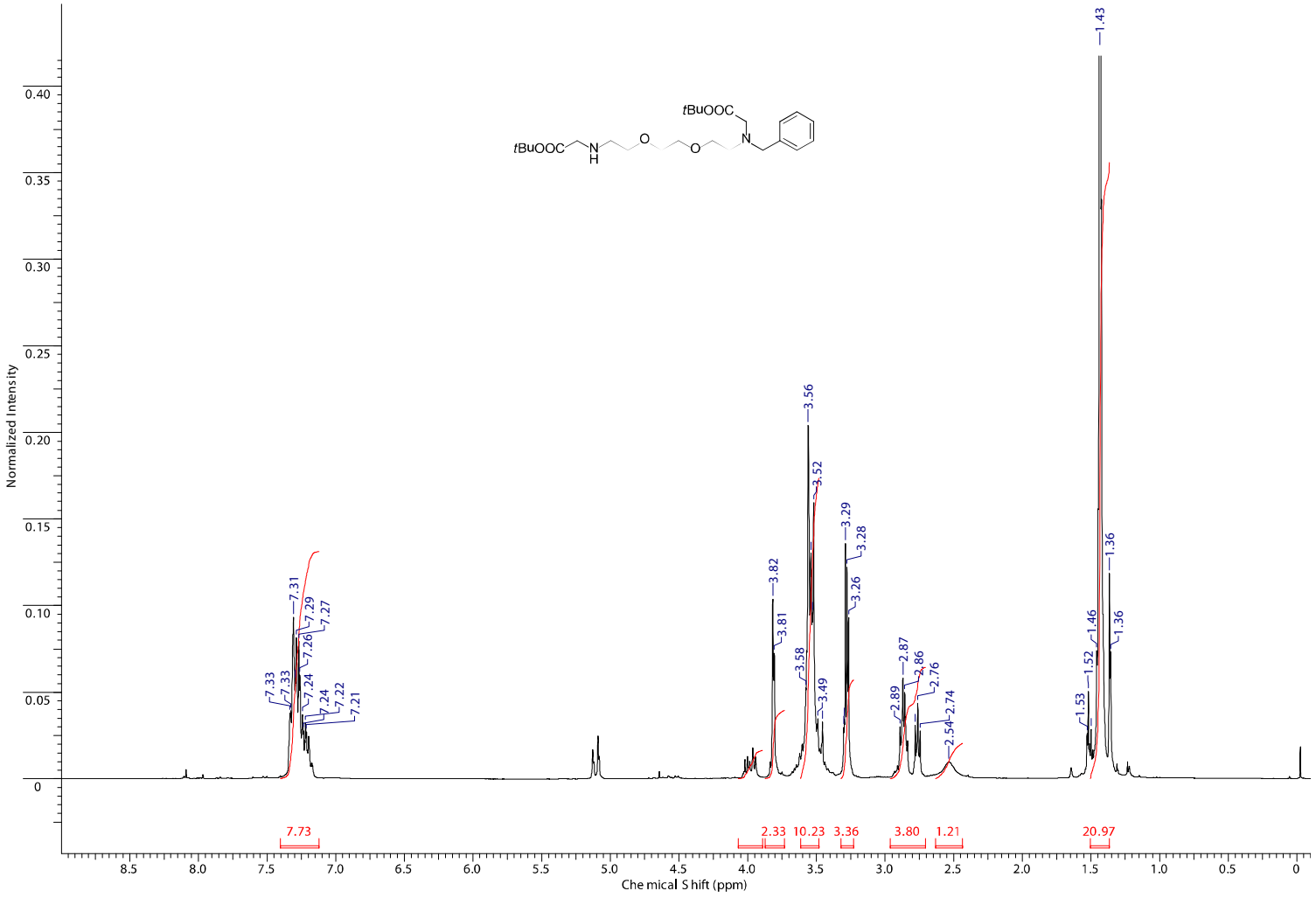



**Figure S2**. Analysis of MRI data from ROI1-ROI5 for **Gd8** (left) and **DSCA** (right). The reported SI values were normalized to the SI of ROI in the muscle where no contrast agent was present, obtained at every time point.

## References


1. G. Angelovski, P. Fouskova, I. Mamedov, S. Canals, E. Toth and N. K. Logothetis, *ChemBioChem*, 2008, **9**, 1729-1734.
2. D. M. Corsi, C. Platas-Iglesias, H. van Bekkum and J. A. Peters, *Magn. Reson. Chem.*, 2001, **39**, 723-726.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H
O
O
N
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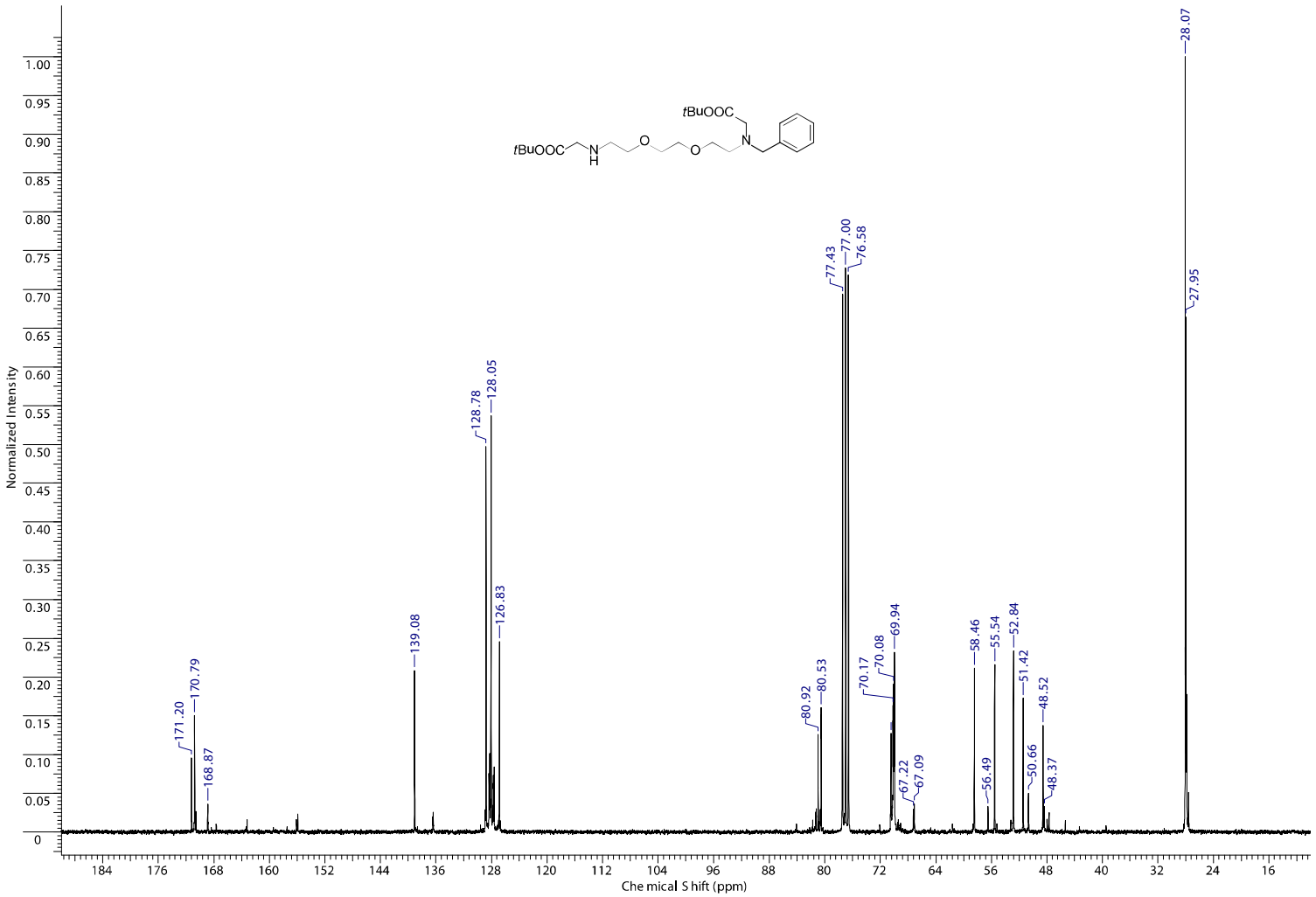
3.80
1.21
20.97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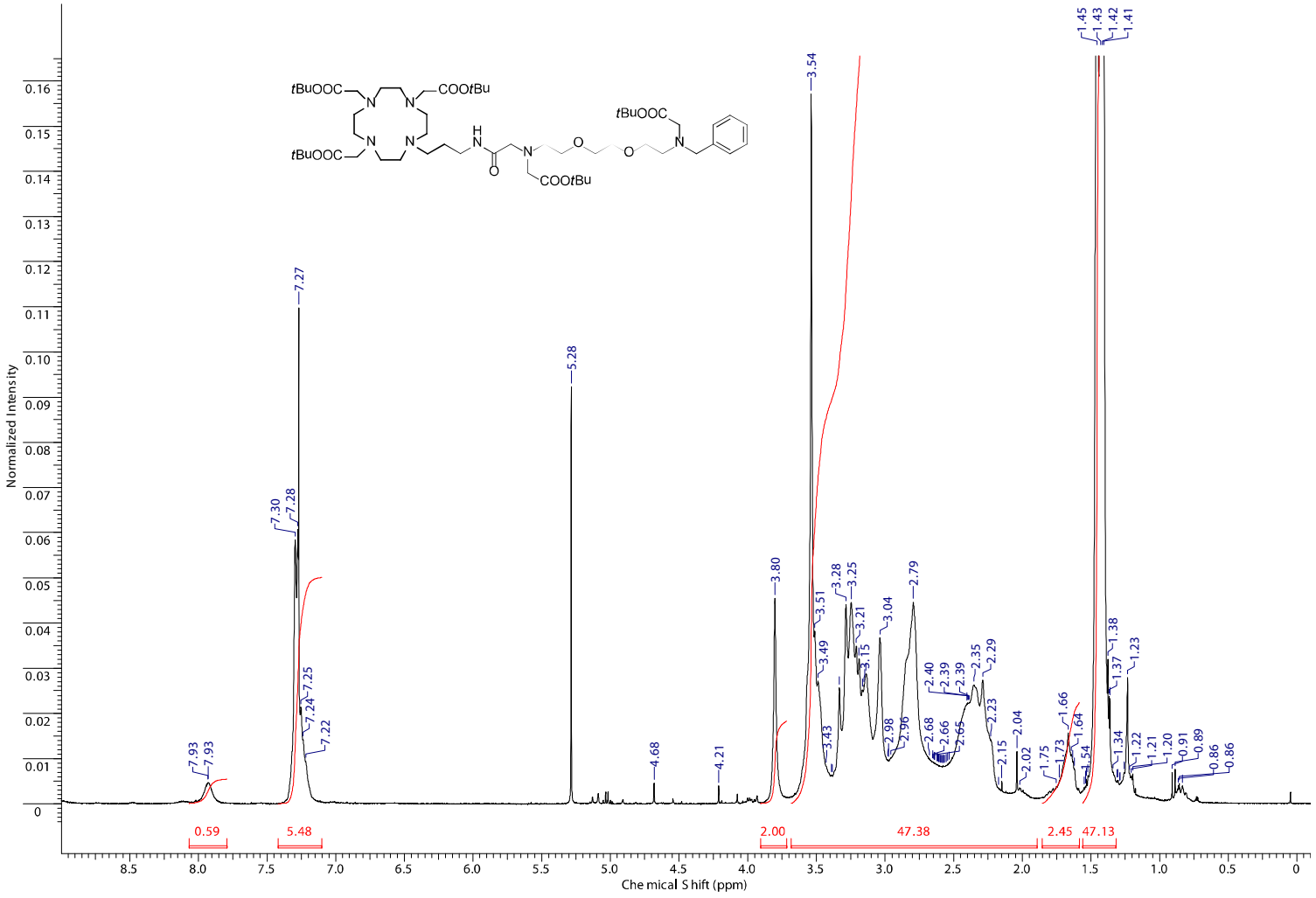
N
H
O
O
N
tBuOOC
Normalized Intensity
Che mical S hift (ppm)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tBuOOC
COOtBu
tBuOOC
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Normalized Intensity
Chemical Shift (ppm)
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7.27
7.25
7.24
7.22
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4.21
3.80
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1.37
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1.22
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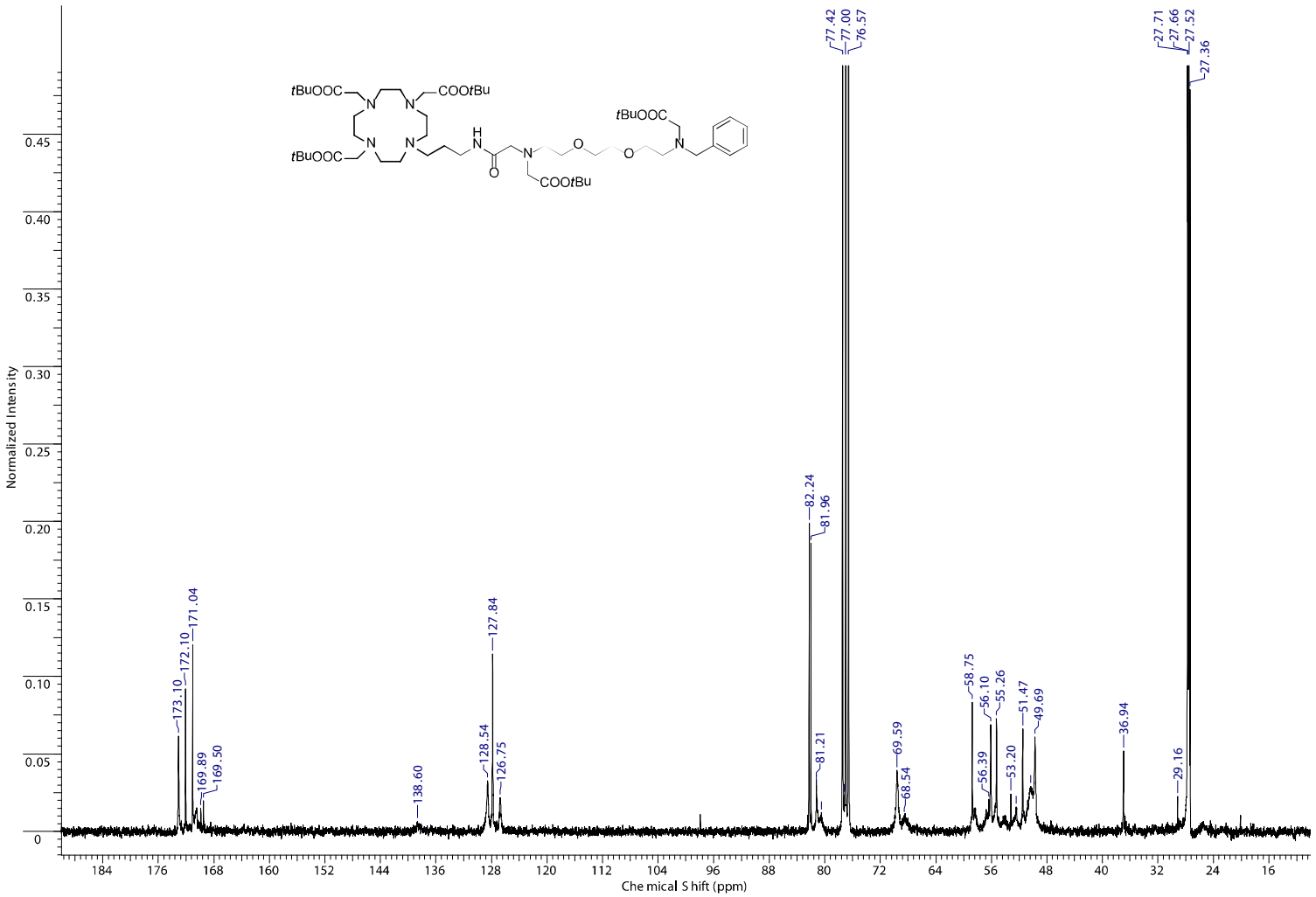
47.13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COOtBu
tBuOOC
COOtBu
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169.50
138.60
128.54
127.84
126.75
82.24
81.96
81.21
77.42
77.00
76.57
69.59
68.54
58.75
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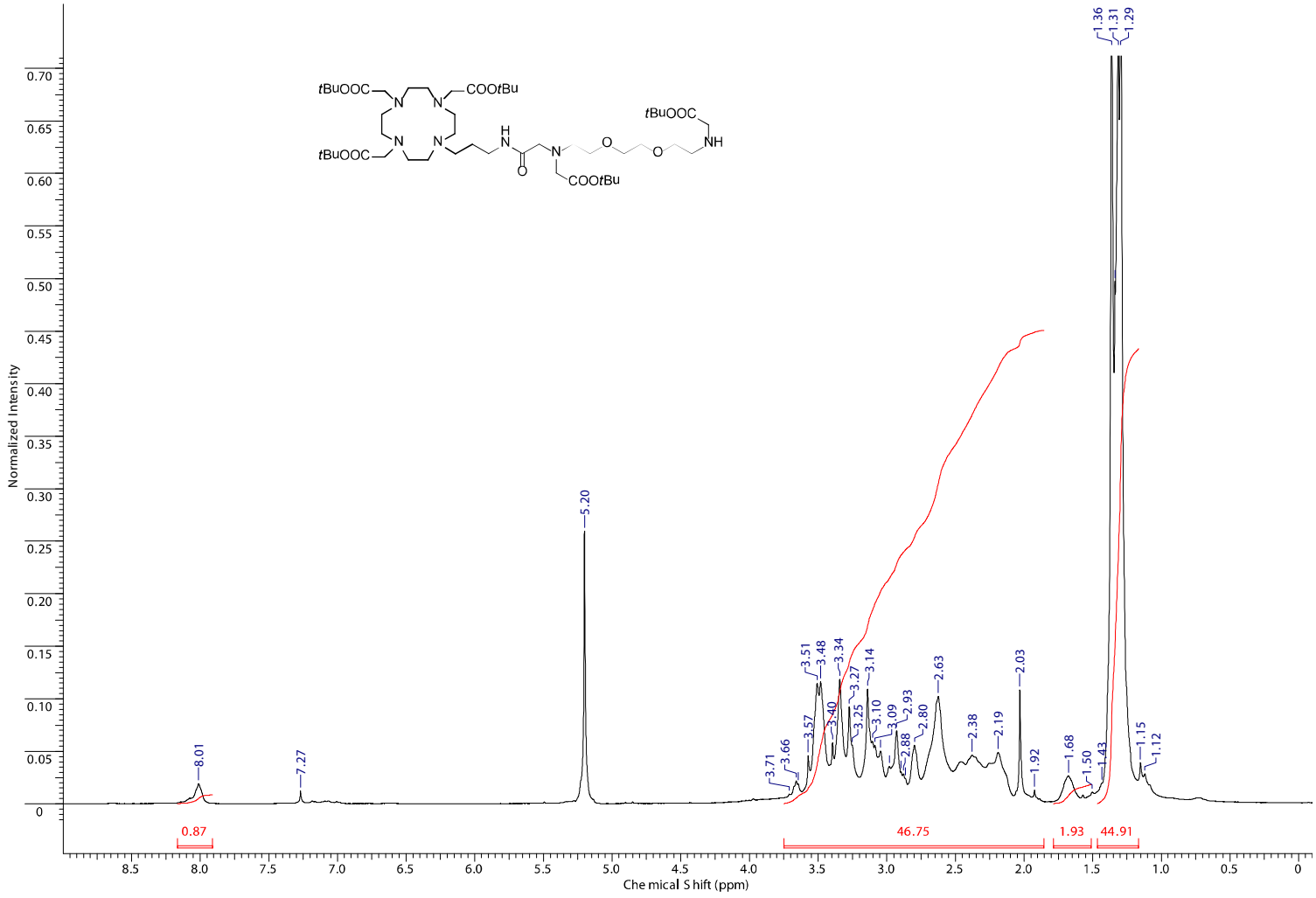
27.36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NH
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5.20
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3.66
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3.25
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2.38
2.19
2.03
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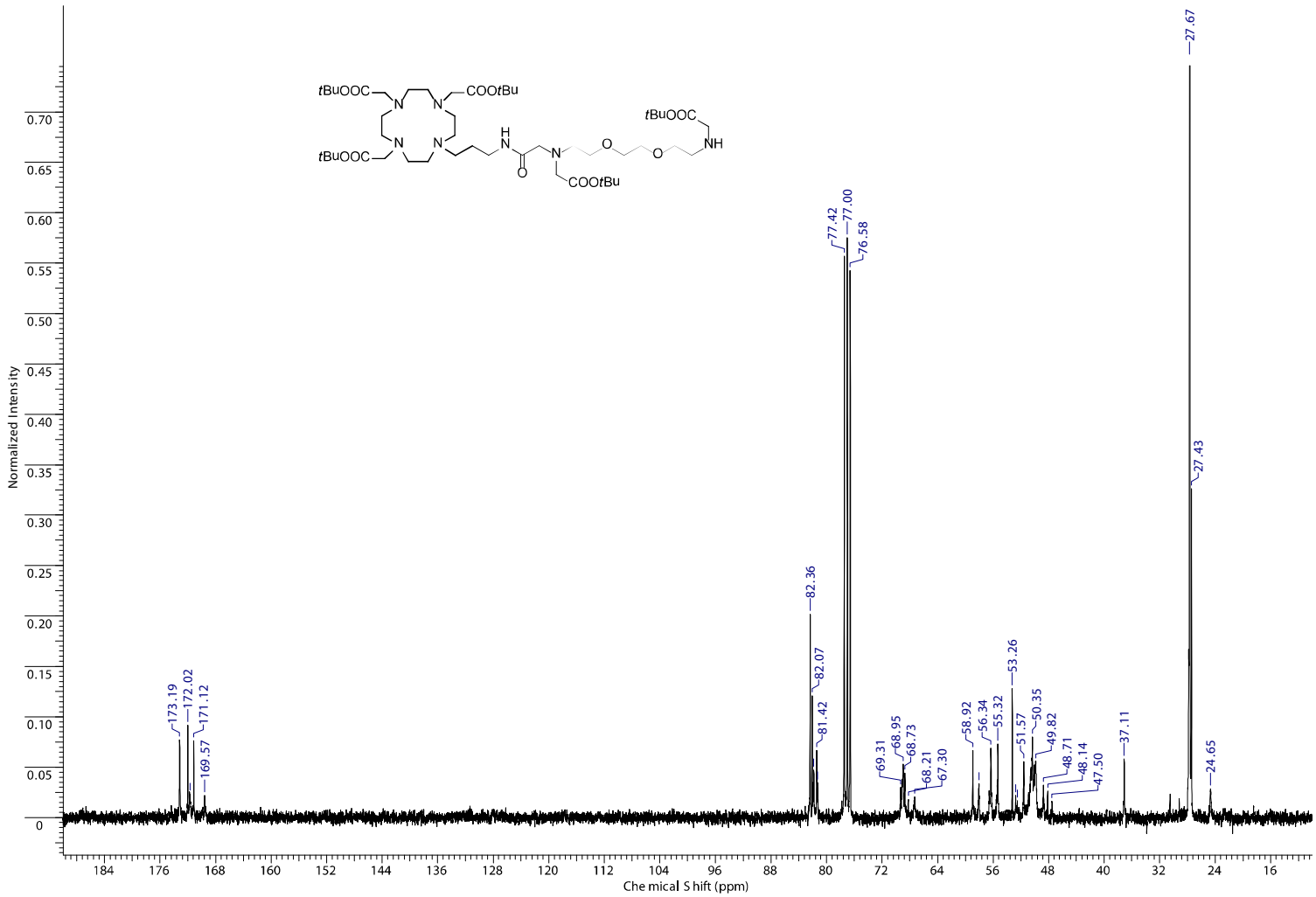
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COOtBu
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COOtBu
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77.00
76.58
69.31
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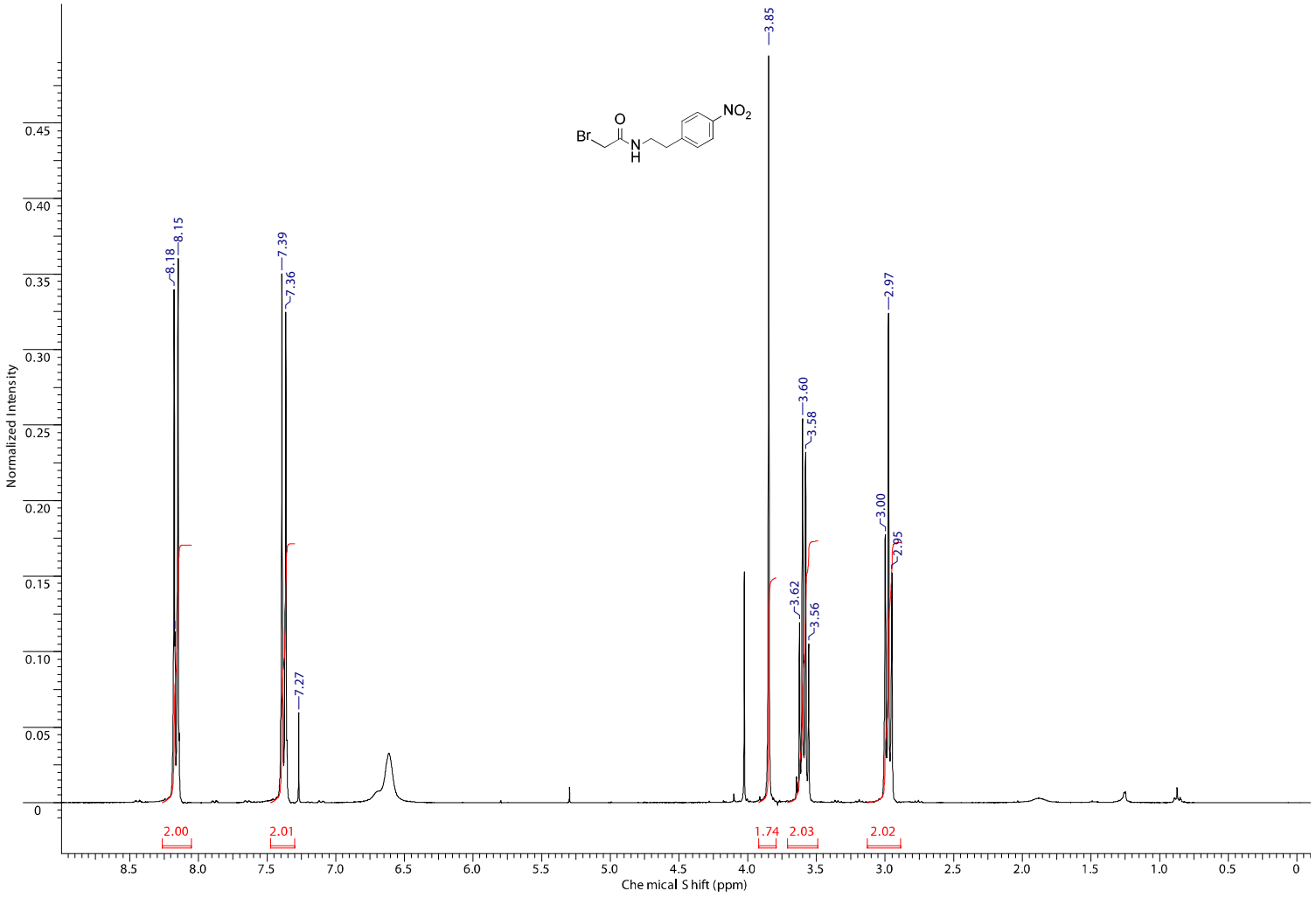
24.65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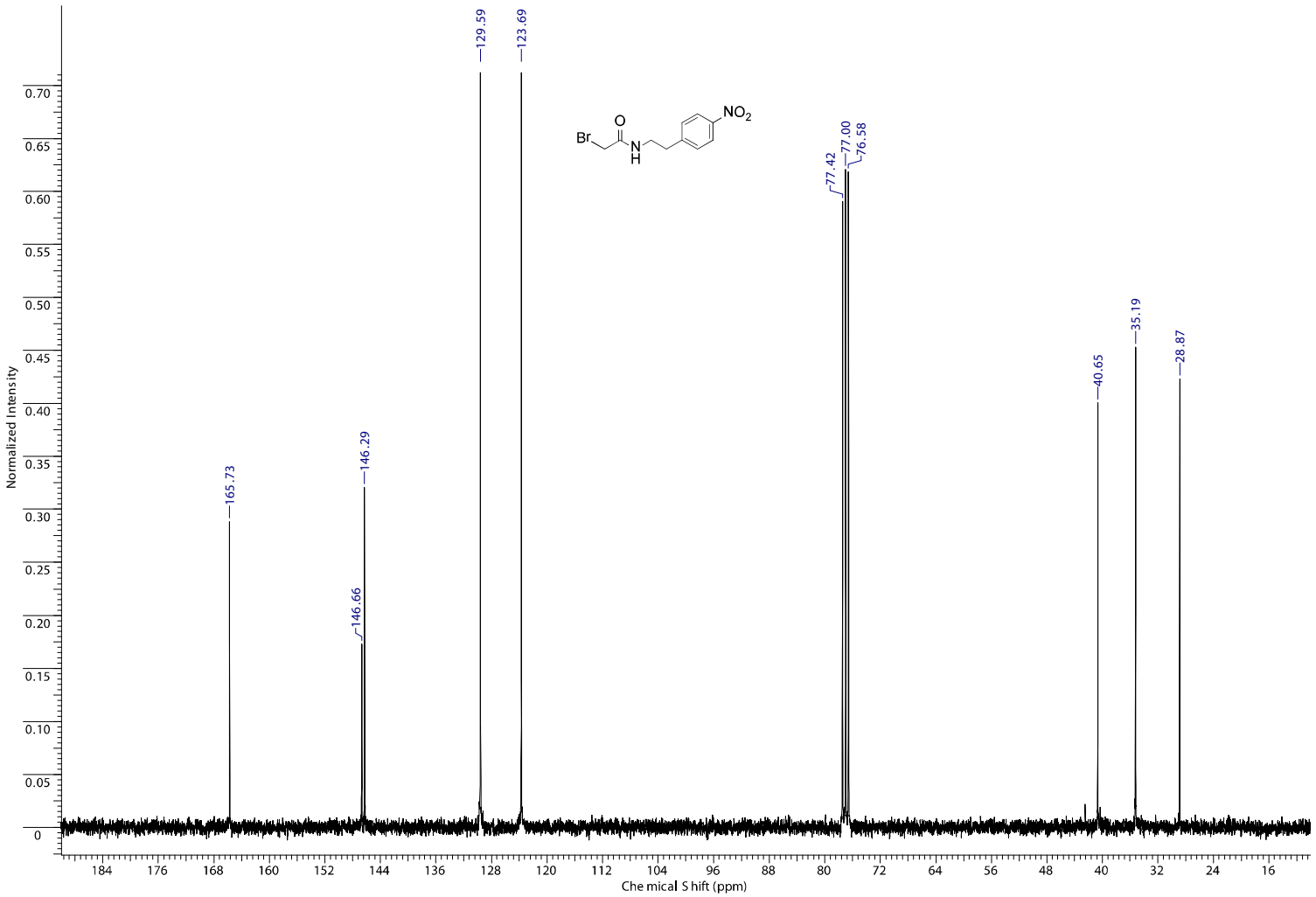
Normalized Intensity
Chemical Shift (ppm)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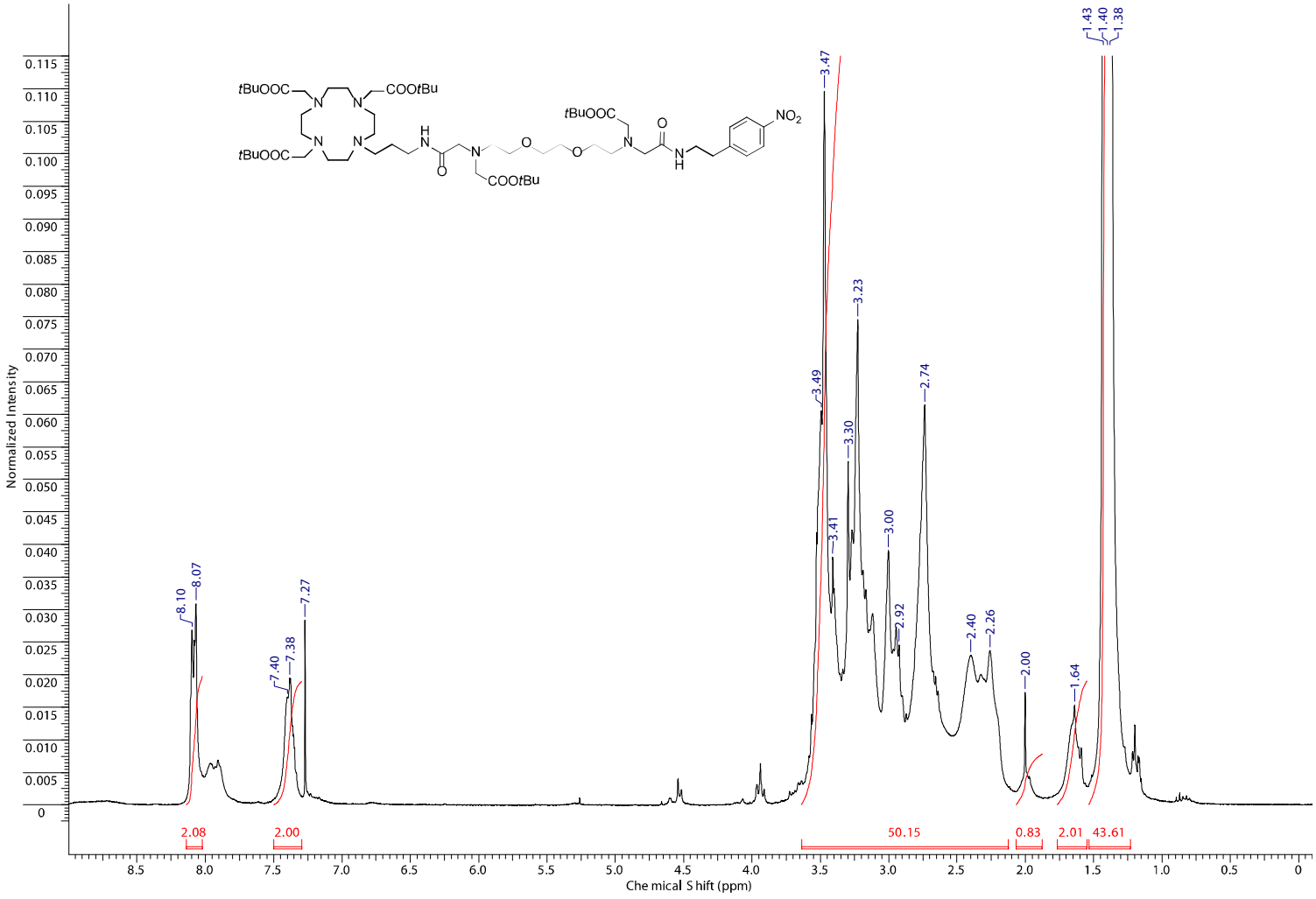
Normalized Intensity
Chemical Shift (ppm)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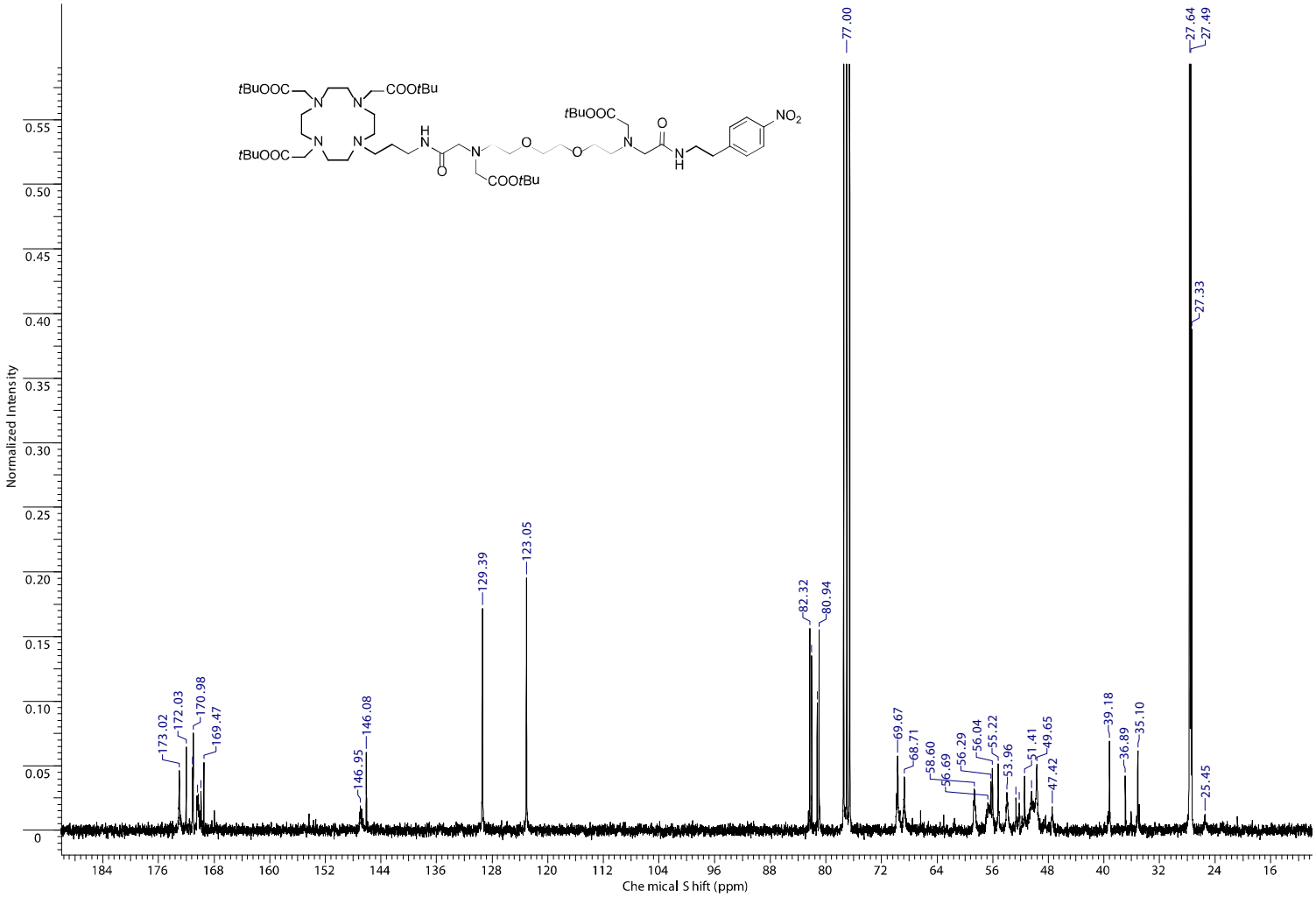
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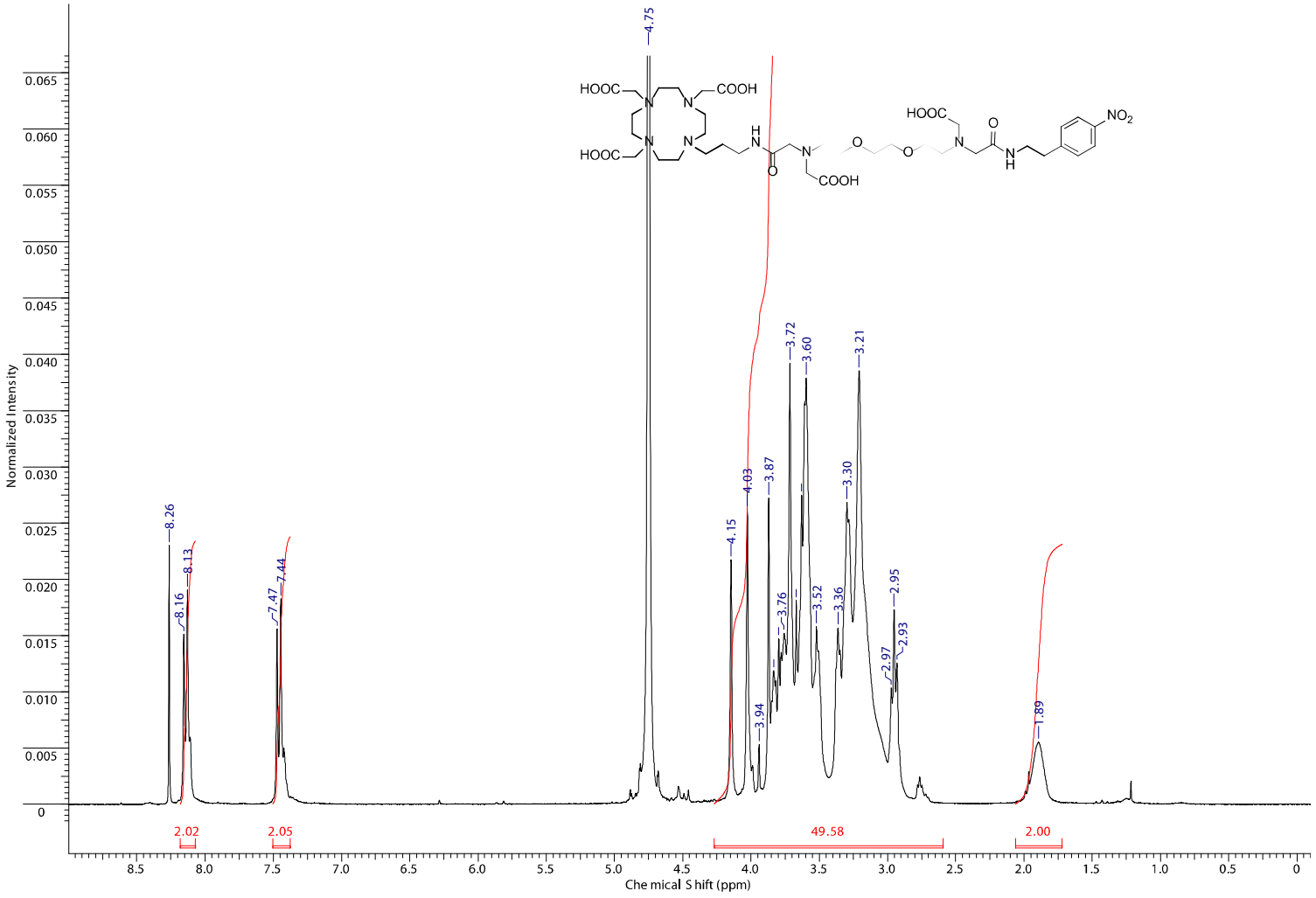
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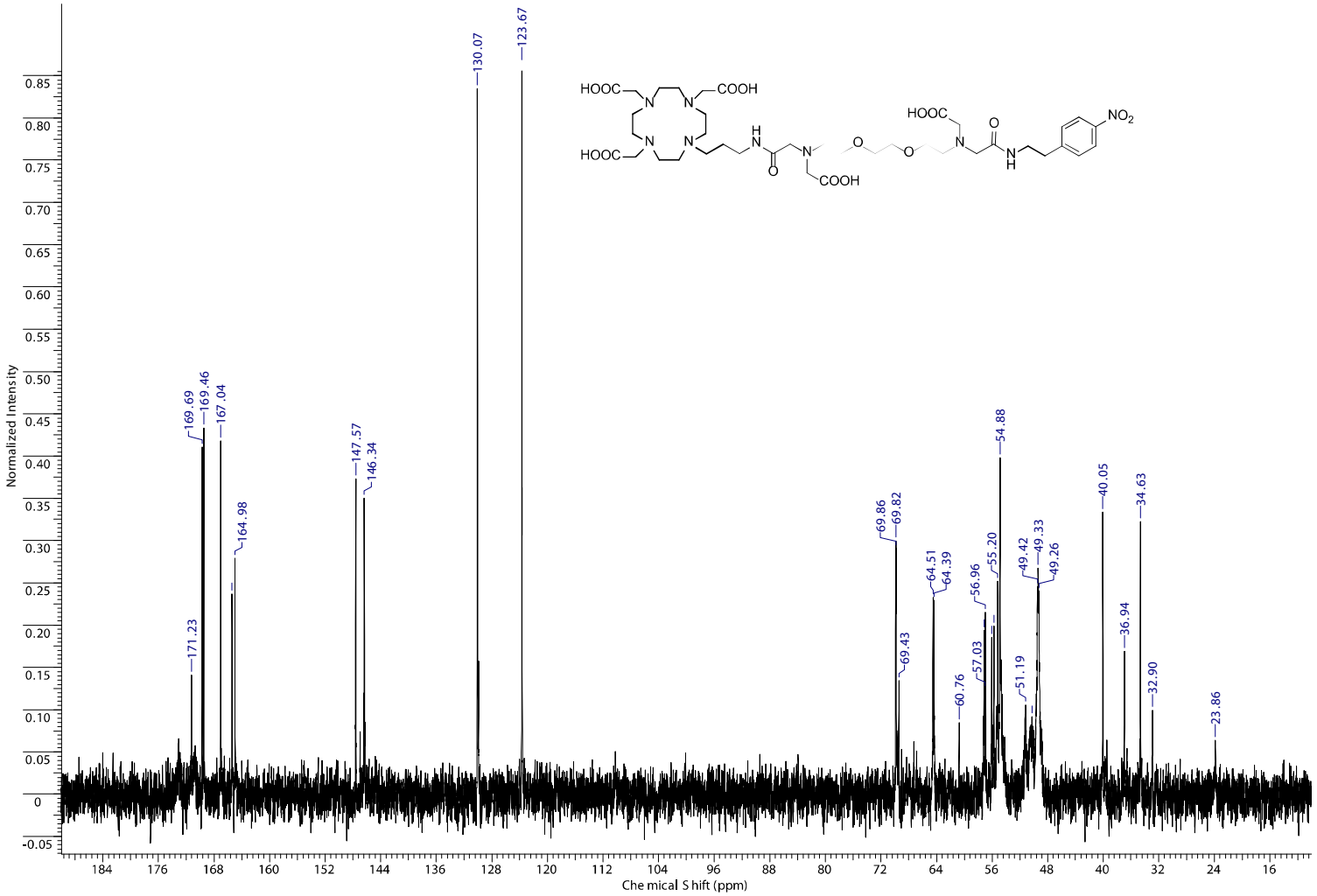
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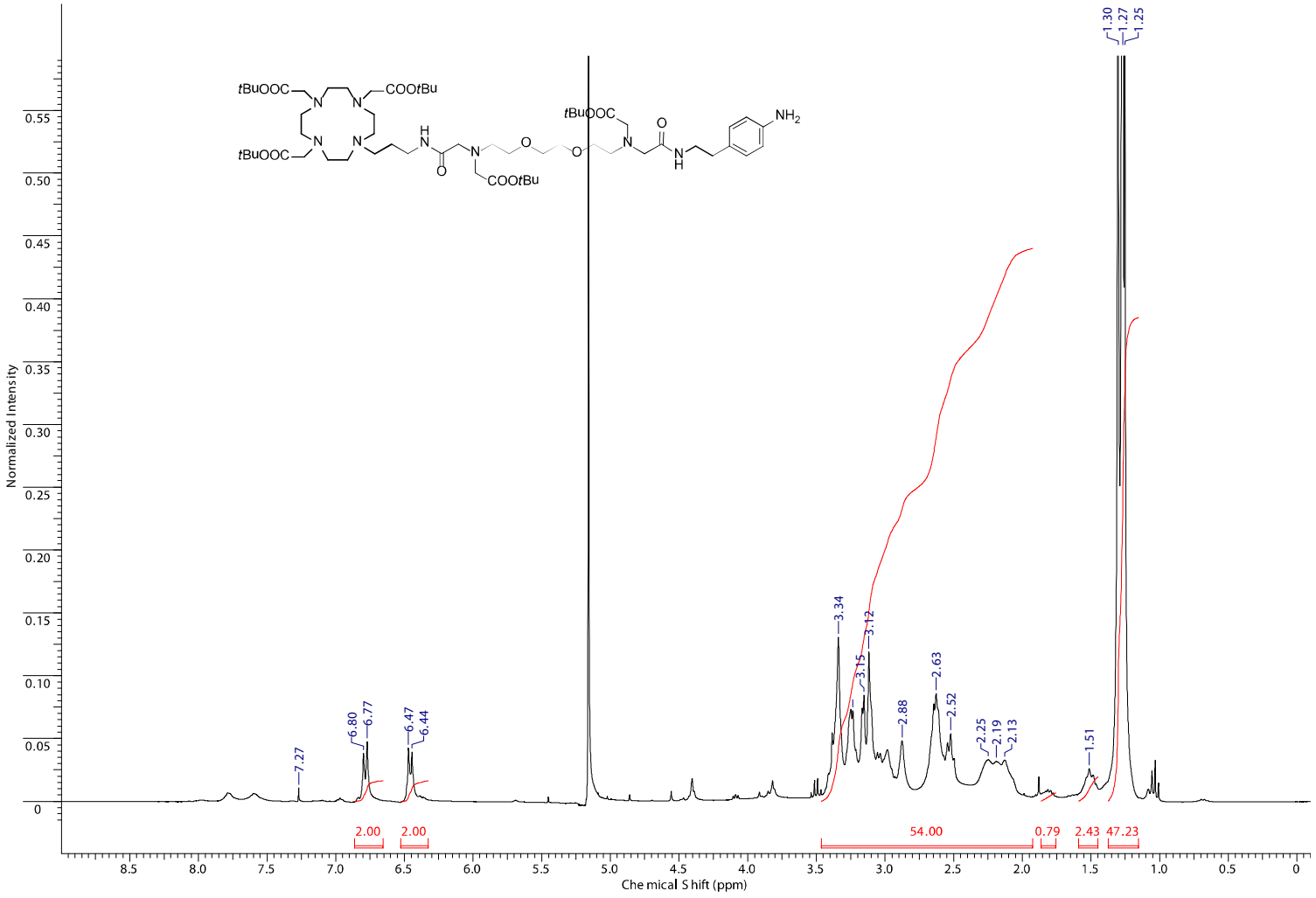
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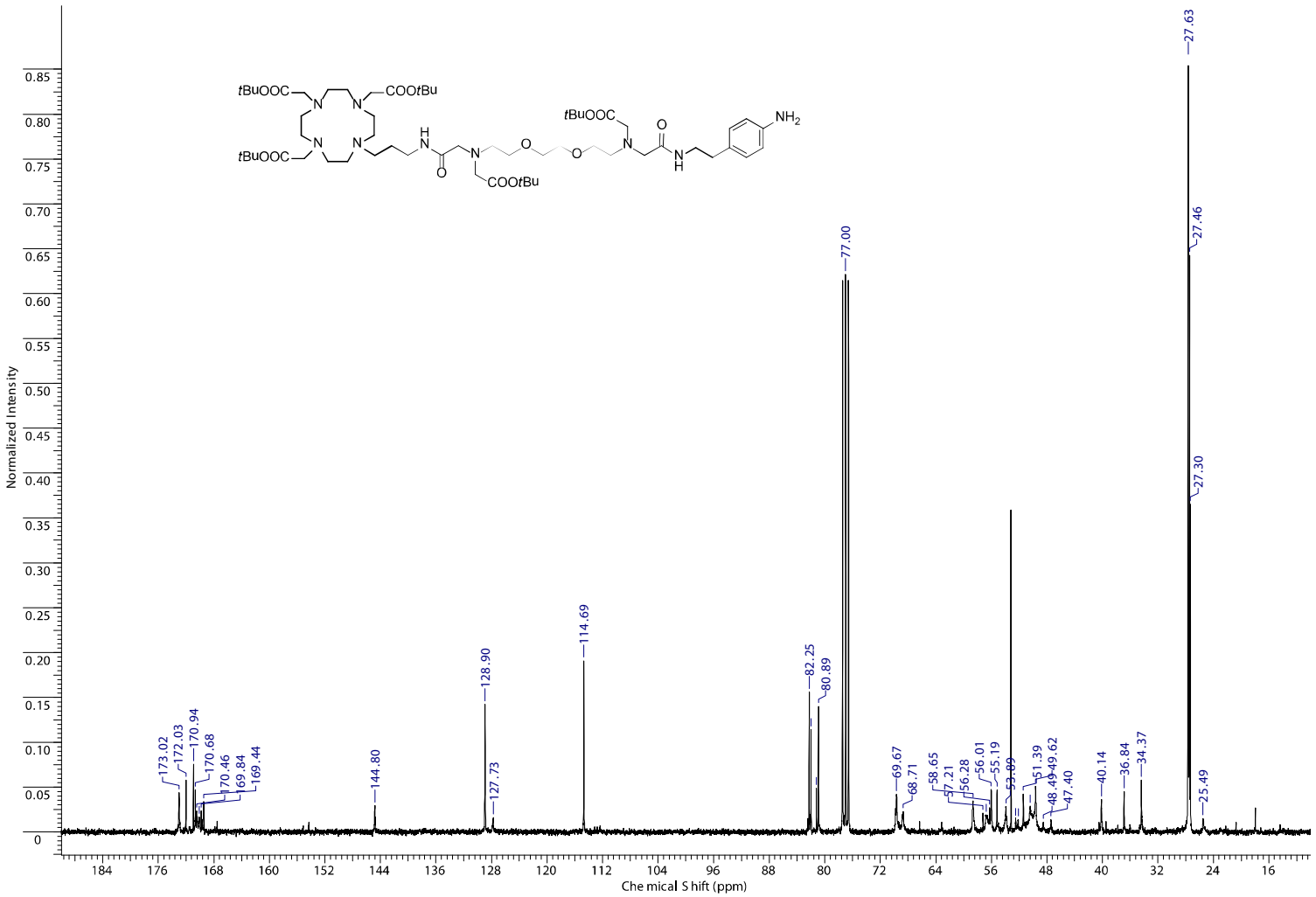
Che mical S hift (ppm)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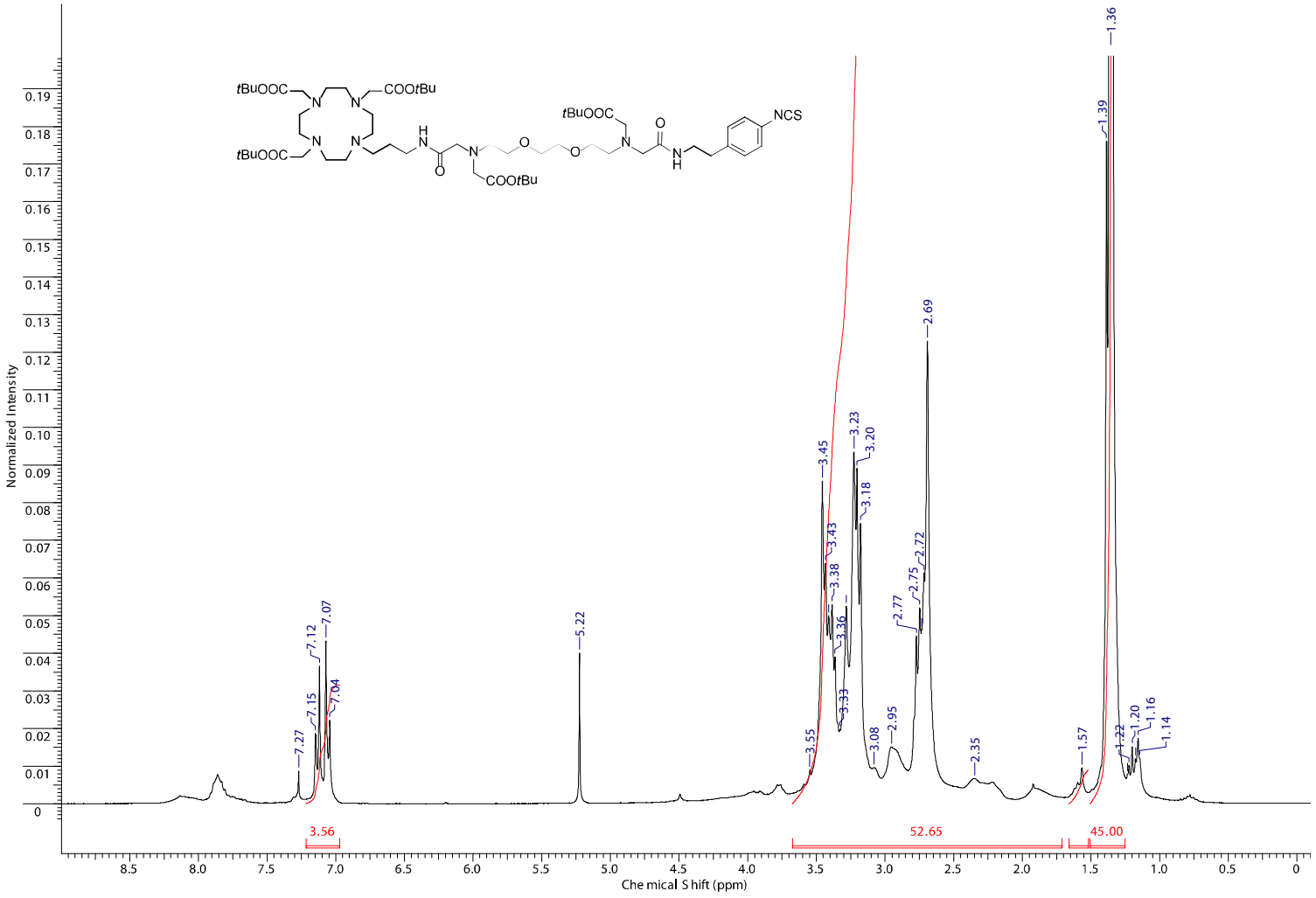
Normalized Intensity
Che mical S hift (ppm)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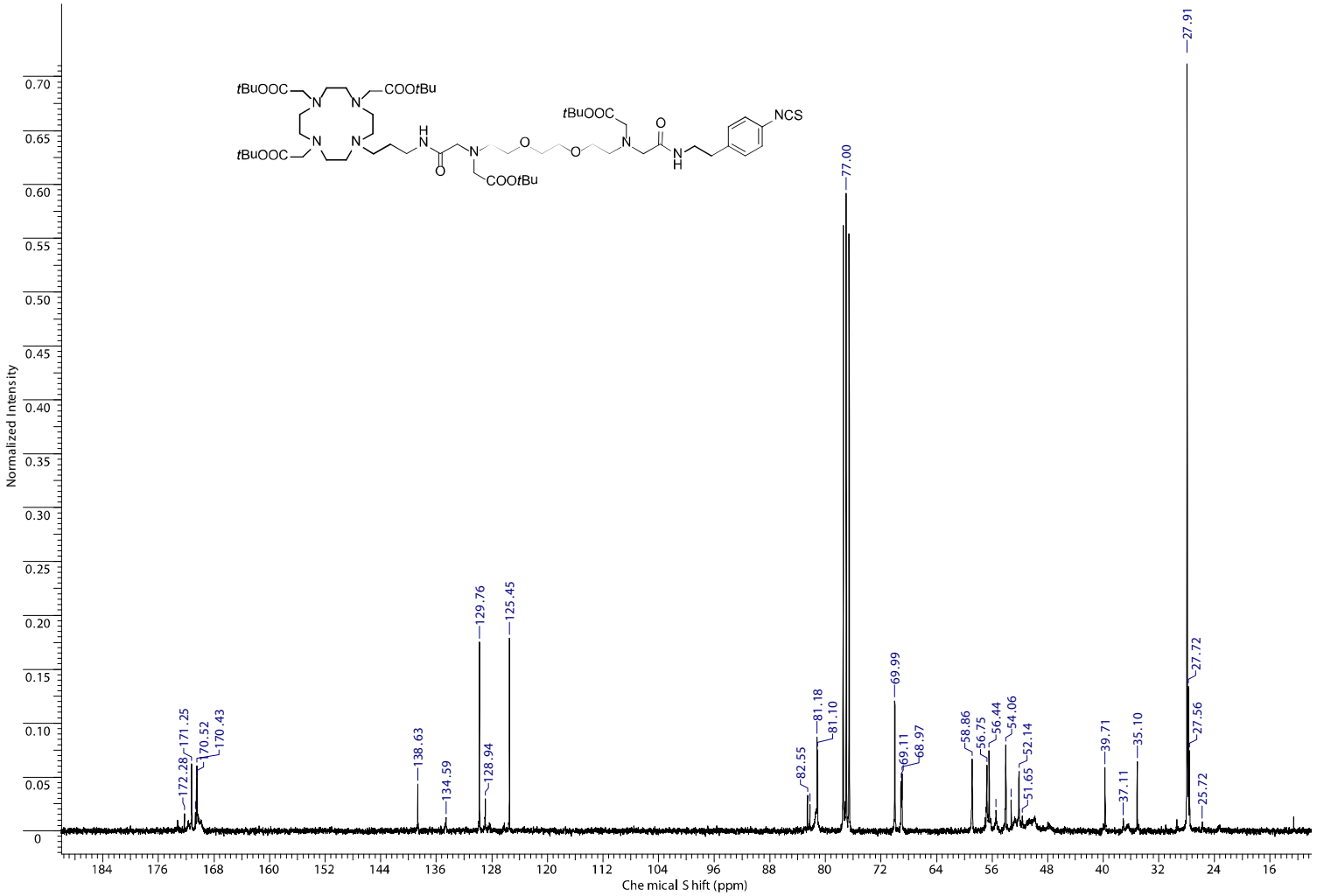
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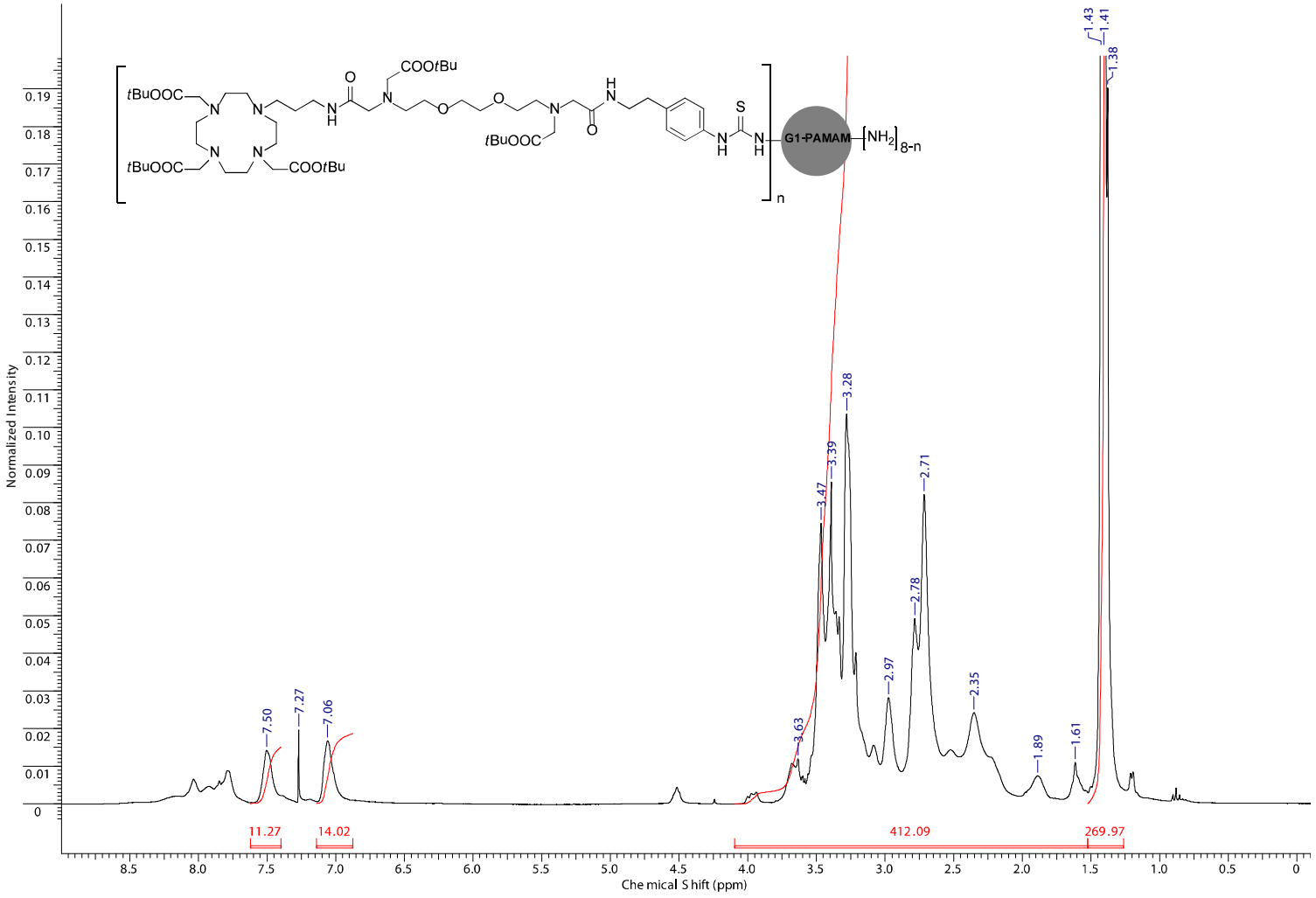
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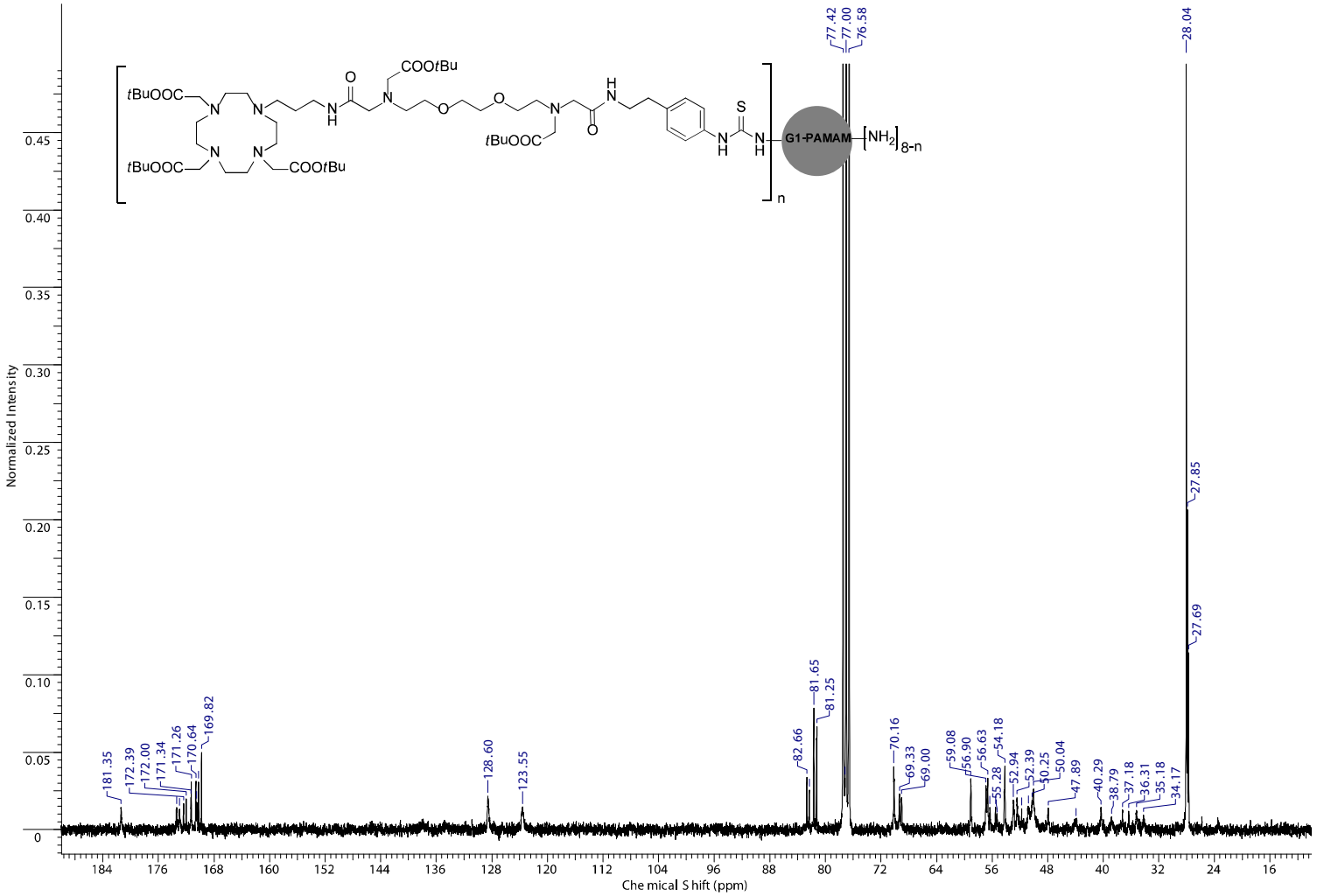
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50.04
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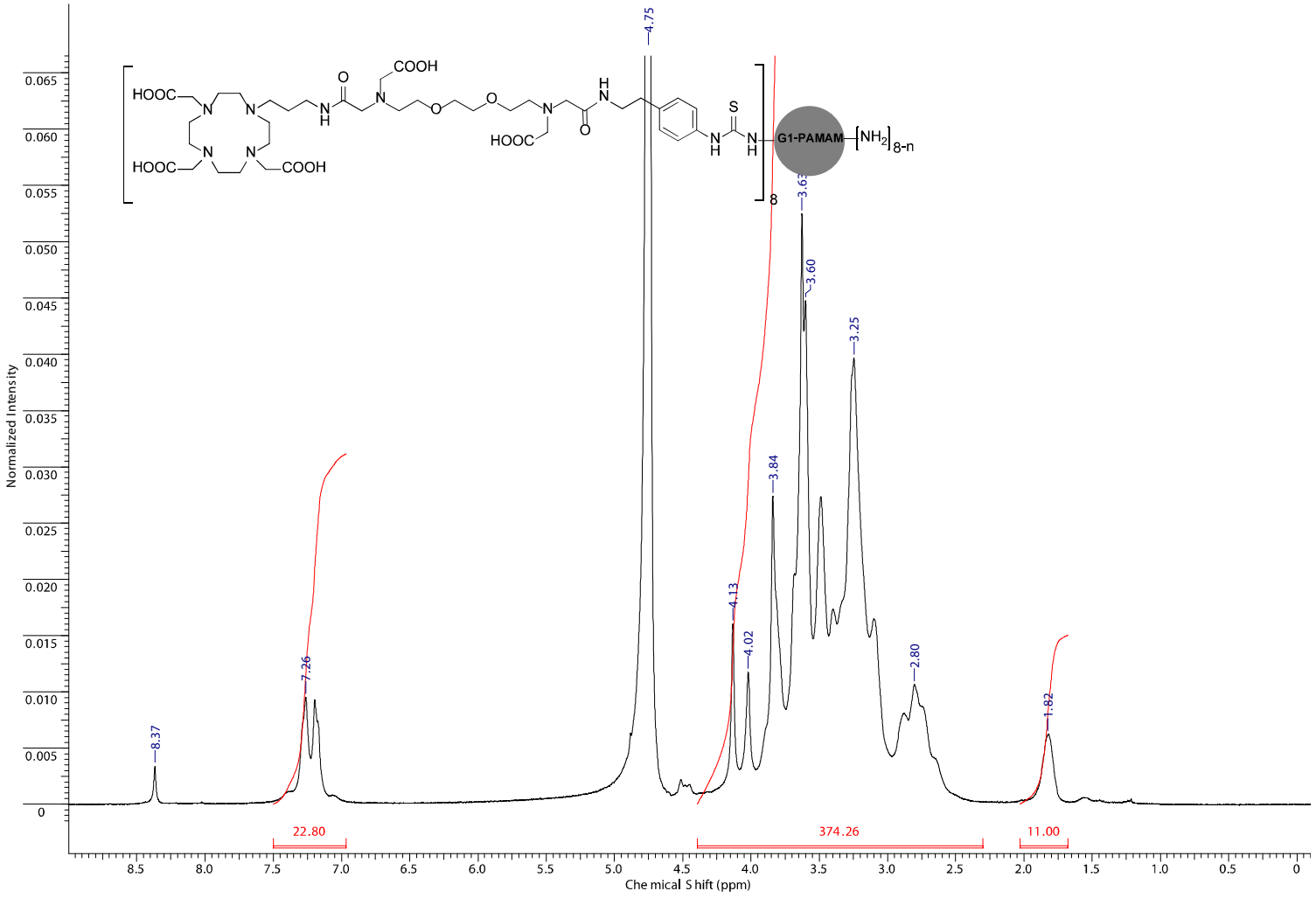
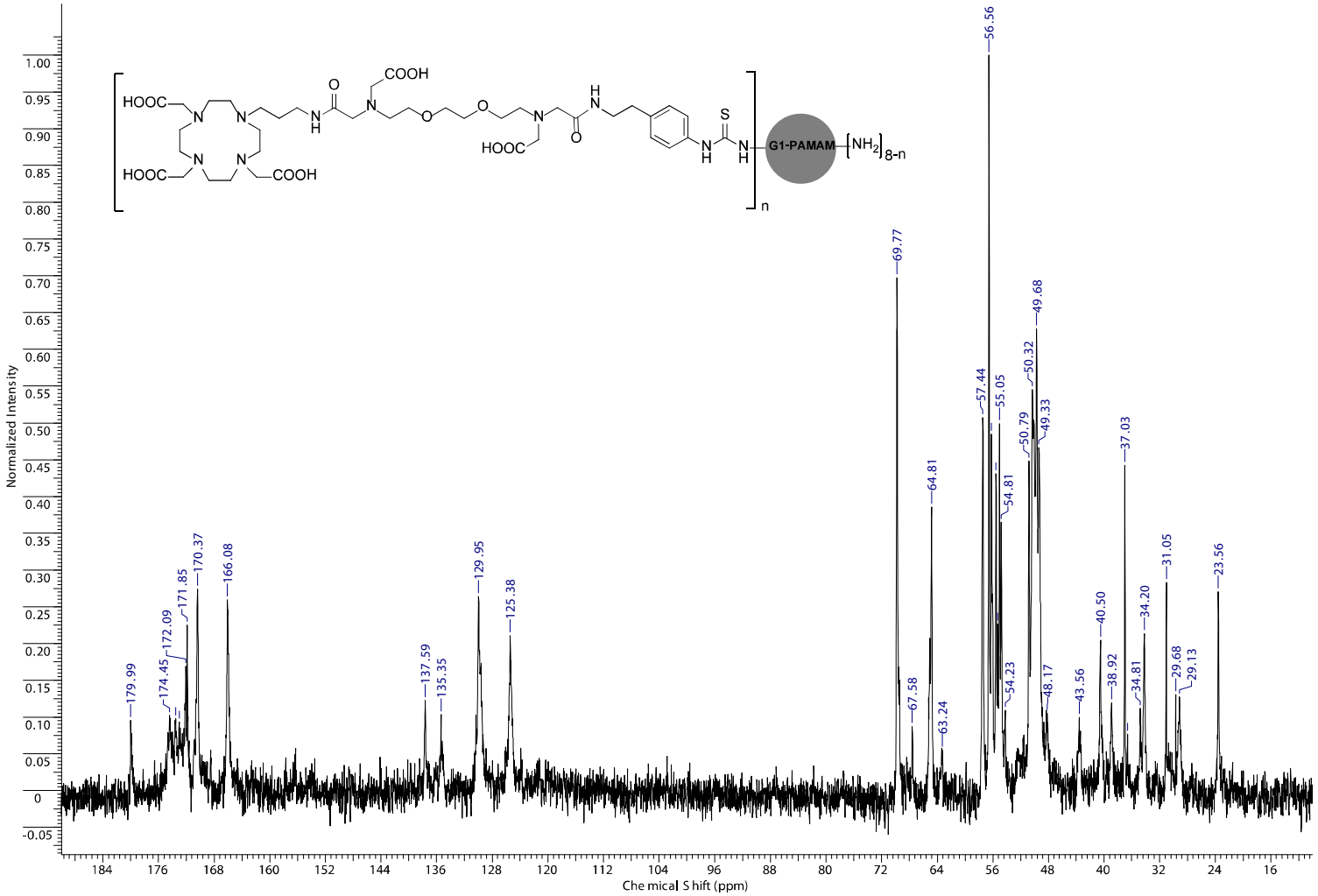
40.29
38.79
37.18
36.31
35.18
34.17


HOOC
COOH
HOOC
COOH
HOOC
COOH
HOOC
G1-PAMAM
[NH2]8-n
8
Normalized Intensity
Chemical Shift (ppm)
8.37
7.26
4.75
4.13
4.02
3.84
3.63
3.60
3.25
2.80
1.82
22.80
374.26
11.00


HOOC
COOH
HOOC
COOH
HOOC
G1-PAMAM
[NH2]8-n
n
Normalized Intensity
Chemical Shift (ppm)
179.99
174.45
172.09
171.85
170.37
166.08
137.59
135.35
129.95
125.38
69.77
67.58
64.81
63.24
56.56
57.44
55.05
54.81
54.23
50.79
50.32
49.68
49.33
48.17
43.56
40.50
38.92
37.03
34.81
34.20
31.05
29.68
29.13
23.56