Gadolinium MRI Contrast Agents Based on Triazine Dendrimers: Relaxivity and In Vivo Pharmacokinetics

Jongdoo Lim,[†] *Baris Turkbey,*[‡] *Marcelino Bernardo,*[§] *L. Henry Bryant Jr.,*[#] Matteo Garzoni,[¶] *Giovanni M. Pavan,*[¶] *Peter L. Choyke,*[‡] *Eric E. Simanek,*[†] *and Hisataka Kobayashi*[‡,*]

[†]Department of Chemistry, Texas Christian University, Fort Worth, TX 76129

[‡]Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

[§]SAIC-Frederick, Inc., NCI-Frederick,, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21702

[#]Laboratory of Diagnostic Radiology Research, Radiology &Imaging Sciences,, Warren Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland 20892

[¶]Department of Innovative Technologies, University of Applied Science of Southern Switzerland, Galleria 2 Manno, SZ

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General procedures

G3 and G5 dendrimers were prepared using methods previously described.[s1] p-NH_2-Bn-DOTA-tetra(t-Bu ester) and p-NH_2-Bn-DTPA-penta(t-Bu ester) were purchased from Macrocyclics. All other chemicals were purchased from Aldrich and Acros. All solvents were ACS grade and used without further purification. Diafiltration purification was performed with Amicon stirred ultrafiltration cell equipment (Model 8050, PLCC membrane, Millipore Corp.) at 35 psi of N_2. NMR spectra were recorded on a Varian Mercury 300 MHz or Inova 500 MHz spectrometer in $CDCl_3$, or $CDCl_3$:MeOH-d_4 (10:1). All mass spectral analyses were carried out by the Laboratory for Biological Mass Spectrometry at Texas A&M.

Synthetic Procedures

Compound 1. A solution of **6** (40 mg, 1.44 μmol), 1-[2-(2-hydroxyethoxy)ethyl]piperazine (60 mg, 0.344 mmol), and DIPEA (0.2 mL, 1.14 mmol) in THF (15 mL) was stirred for 24 h at room temperature. After the temperature was raised to 50 ^oC, the reaction solution was stirred for an additional 24 h and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The resulting product was dissolved in a solution of trifluoroacetic acid (4 mL), dichloromethane (1 mL), and methanol (1 mL). The solution was stirred for 48 h at room temperature and evaporated under vacuum. The crude product was dissolved in dichloromethane and methanol and precipitated by adding hexanes. The precipitation was repeated twice more to afford **1** (37 mg, quantitative) as a white solid. MS (MALDI-TOF) calcd for $C_{1152}H_{1794}N_{396}O_{285}$ 25735.81, found 25500 $(M+H)^+$.

Compound 2. A solution of **7** (0.19 g, 1.68 μmol), 1-[2-(2-hydroxyethoxy)ethyl]piperazine (0.80 g, 4.59 mmol), and DIPEA (0.2 mL, 1.14 mmol) in THF (10 mL) was stirred for 24 h at room temperature. After the temperature was raised to 50 ^oC, the reaction solution was stirred for an additional 24 h and evaporated under

[s1] Lim, J.; Mintzer, M. A.; Perez, L. M.; Simanek, E. E. Synthesis of odd generation triazine dendrimers using a divergent, macromonomer approach. *Org. Lett.* **2010**, *12*, 1148-1151.

vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The resulting product was dissolved in a solution of trifluoroacetic acid (6 mL), dichloromethane (1.5 mL), and methanol (1.5 mL). The solution was stirred for 48 h at room temperature and evaporated under vacuum. The crude product was dissolved in dichloromethane and methanol and precipitated by adding hexanes. The precipitation was repeated twice more to afford **2** (150 mg, 85%) as a white solid. MS (MALDI-TOF) calcd for $C_{4680}H_{7290}N_{1620}O_{1149}$ 104570.18, found 105700 $(M+H)^+$.

Compound 3. A solution of **9** (66 mg, 2.28 μmol), 1-[2-(2-hydroxyethoxy)ethyl]piperazine (0.10 g, 0.574 mmol), and DIPEA (0.2 mL, 1.14 mmol) in THF (15 mL) was stirred for 24 h at room temperature. After the temperature was raised to 50 ^oC, the reaction solution was stirred for an additional 24 h and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The resulting product was dissolved in a solution of trifluoroacetic acid (4 mL), dichloromethane (1 mL), and methanol (1 mL). The solution was stirred for 48 h at room temperature and evaporated under vacuum. The crude product was dissolved in dichloromethane and methanol and precipitated by adding hexanes. The precipitation was repeated twice more to afford **1** (58 mg, quantitative) as a white solid. MS (MALDI-TOF) calcd for $C_{1104}H_{1674}N_{372}O_{333}$ 25470.55, found 24800 $(M+H)^+$.

Compound 4. A solution of **10** (193 mg, 1.65 μmol), 1-[2-(2-hydroxyethoxy)ethyl]piperazine (0.80 g, 4.59 mmol), and DIPEA (0.2 mL, 1.14 mmol) in THF (10 mL) was stirred for 24 h at room temperature. After the temperature was raised to 50 ^oC, the reaction solution was stirred for an additional 24 h and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The resulting product was dissolved in a solution of trifluoroacetic acid (8 mL), dichloromethane (2 mL), and methanol (2 mL). The solution was stirred for 48 h at room temperature and evaporated under vacuum. The crude product was dissolved in dichloromethane and methanol and precipitated by adding

hexanes. The precipitation was repeated twice more to afford **4** (170 mg, quantitative) as a light yellow solid. MS (MALDI-TOF) calcd for $C_{4488}H_{6810}N_{1524}O_{1341}$ 103509.15, found 104700 $(M+H)^+$.

Compound 5. Cyanuric chloride (51 mg, 0.277 mmol) was added to an ice-bath cooled solution of p-NH_2-Bn-DOTA-tetra(t-Bu ester) (0.20 g, 0.272 mmol) and DIPEA (0.20 mL, 1.14 mmol) in THF (20 mL). The solution was stirred for 2 h at 0 ^oC and then concentrated under vacuum. The residue was dissolved in dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by silica gel chromatography (DCM:MeOH = 8:1) to give **5** (0.21 g, 88%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.64 (d, J = 8.1 Hz, 2H), 6.93 (t, J = 9.2 Hz, 2H), 3.35-1.75 (m, 25H), 1.34 (m, 36H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 172.8, 172.7, 172.6 (two lines), 172.5, 170.0, 169.3, 168.7, 167.2, 163.6, 135.5, 135.4, 135.2, 134.8, 128.8, 128.7, 122.2, 122.0, 82.2, 82.1 (two lines), 82.0, 81.9, 81.8, 60.8, 56.3, 55.9, 55.5, 55.3, 52.6, 52.4, 52.3, 52.0, 50.6, 48.6, 48.5, 48.0, 47.8, 42.5, 31.6, 31.0, 27.7; MS (MALDI-TOF) calcd for $C_{42}H_{66}Cl_2N_8O_8$ 880.4381, found 903.4241 $(M+Na)^+$.

Compound 6. A solution of **G3** (40 mg, 5.31 μmol) in THF (1 mL) and H_2O (0.3 mL) was slowly added to a solution of **5** (0.16 g, 0.181 mmol) and DIPEA (0.1 mL, 0.57 mmol) in THF (2 mL) at 0 ^oC. The solution was stirred for 48 h at room temperature and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by precipitation from ethyl acetate to give **6** (0.13 g, 88%) as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.52 (br, 48H), 7.08 (br, 48H), 3.81-1.80 (m, 1140H), 1.42 (m, 864H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 173.0, 172.9, 172.8, 168.9, 167.1, 166.3, 165.2, 164.6, 163.8, 136.7, 134.2, 129.3, 120.7, 82.4, 82.2 (two lines), 82.1, 82.0, 70.6, 70.3, 69.4, 61.4, 55.8, 52.7, 50.9, 48.1, 43.0, 38.3, 31.7, 31.1, 29.7, 28.0; MS (MALDI-TOF) calcd for $C_{1344}H_{2154}Cl_{24}N_{348}O_{237}$ 27801.97, found 27459.14 $(M+H)^+$.

Compound 7. A solution of **G5** (60 mg, 1.89 μmol) in THF (2 mL) and H_2O (0.5 mL) was slowly added to a solution of **5** (0.28 g, 0.317 mmol) and DIPEA (0.2 mL, 1.14 mmol) in THF (3 mL) at 0 ^oC. The solution was stirred for 48 h at room temperature and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by precipitation with hexanes from ethyl acetate to give **7** (0.195 g, 91%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 7.2 Hz, 192H), 7.07 (br, 192H), 3.81-1.81 (m, 4668H), 1.42 (m, 3456H); ^{13}C NMR (75 MHz, $CDCl_3$/MeOH-d_4) δ 172.9, 172.7 (two lines), 172.6, 172.5, 172.4, 168.6, 167.1, 166.0, 164.9, 164.7, 164.2, 136.5, 133.5, 128.9, 120.6, 82.2, 82.1, 82.0, 81.9, 70.3, 69.9, 69.0, 61.0, 55.3, 52.2, 50.6, 48.5, 43.3, 42.3, 37.7, 31.4, 30.9, 29.3, 27.6; MS (MALDI-TOF) calcd for $C_{5448}H_{8730}Cl_{96}N_{1428}O_{957}$ 112834.85, not found.

Compound 8. Cyanuric chloride (48 mg, 0.260 mmol) was added to an ice-bath cooled solution of p-NH_2-Bn-DTPA-penta(t-Bu ester) (0.20 g, 0.257 mmol) and DIPEA (0.20 mL, 1.14 mmol) in THF (20 mL). The solution was stirred for 2 h at 0 ^oC and then concentrated under vacuum. The residue was dissolved in dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by silica gel chromatography (EtOAc:Hex = 2:3) to give **8** (0.21 g, 88%) as a sticky clear oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 3.43-3.38 (m, 10 H), 3.15-2.50 (m, 9H), 1.40 (m, 45H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.2, 170.8, 170.0, 164.0, 138.4, 133.7, 130.1, 121.4, 80.9, 80.7, 63.0, 56.2, 56.0, 55.9, 53.5, 53.0, 52.4, 36.7, 28.2 (two lines); MS (MALDI-TOF) calcd for $C_{44}H_{69}Cl_2N_7O_{10}$ 925.4483, found 926.5064 $(M+H)^+$.

Compound 9. A solution of **G3** (25 mg, 3.32 μmol) in THF (1 mL) and H_2O (0.3 mL) was slowly added to a solution of **8** (0.10 g, 0.108 mmol) and DIPEA (0.1 mL, 0.57 mmol) in THF (2 mL) at 0 ^oC. The solution was stirred for 48 h at room temperature and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by silica gel chromatography (from EtOAc:Hex = 2:1 to EtOAc:MeOH:H_2O =

7:2:1) to give **9** (79 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, J = 8.1 Hz, 48H), 7.19 (d, J = 7.8 Hz, 48H), 3.80-2.40 (m, 936H), 1.82 (br, 60H), 1.39 (m, 1080H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 171.2, 170.7, 169.1, 166.3, 165.3, 164.6, 163.7, 136.3, 135.6, 129.8, 120.2, 80.8, 80.6, 70.7, 70.3, 69.1, 63.0, 56.0, 55.8, 53.6, 53.0, 52.5, 43.6, 42.8, 38.2, 36.7, 29.7, 28.2; MS (MALDI-TOF) calcd for $C_{1392}H_{2226}Cl_{24}N_{324}O_{285}$ 28882.22, found 29324.78 $(M+H)^+$.

Compound 10. A solution of **G5** (60 mg, 1.89 μmol) in THF (2 mL) and H_2O (0.5 mL) was slowly added to a solution of **8** (0.30 g, 0.324 mmol) and DIPEA (0.2 mL, 1.14 mmol) in THF (3 mL) at 0 ^{o}C. The solution was stirred for 48 h at room temperature and evaporated under vacuum. The residue was dissolved with dichloromethane, washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by precipitation with hexanes from ethyl acetate to give **10** (0.20 g, 90%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, J = 7.5 Hz, 192H), 7.17 (d, J = 7.5 Hz, 192H), 3.81-2.45 (m, 3840H), 1.80 (br m, 252H), 1.39 (m, 4320H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.2, 170.7, 169.1, 166.3, 165.3, 164.6, 163.6, 136.2, 135.6, 129.7, 120.2, 80.8, 80.5, 70.6, 70.2, 69.3, 62.9, 56.0, 55.7, 53.5, 52.9, 52.4, 43.6, 42.9, 42.7, 38.2, 36.6, 29.6, 28.3, 28.2, 28.1, 28.0 (two lines); MS (MALDI-TOF) calcd for $C_{5640}H_{9018}Cl_{96}N_{1332}O_{1149}$ 117155.83, found 117277.83 $(M+H)^+$.

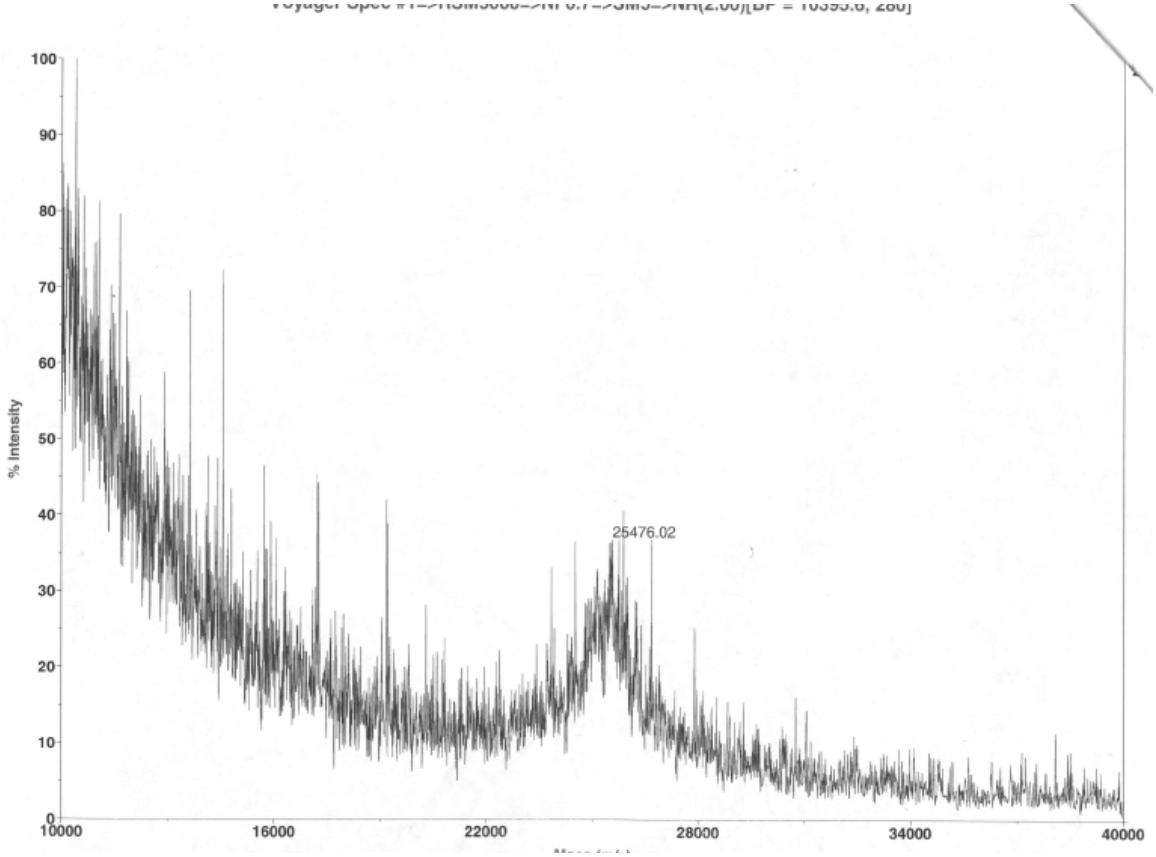

Figure S1. MALDI-TOF mass spectrum of compound **1.**

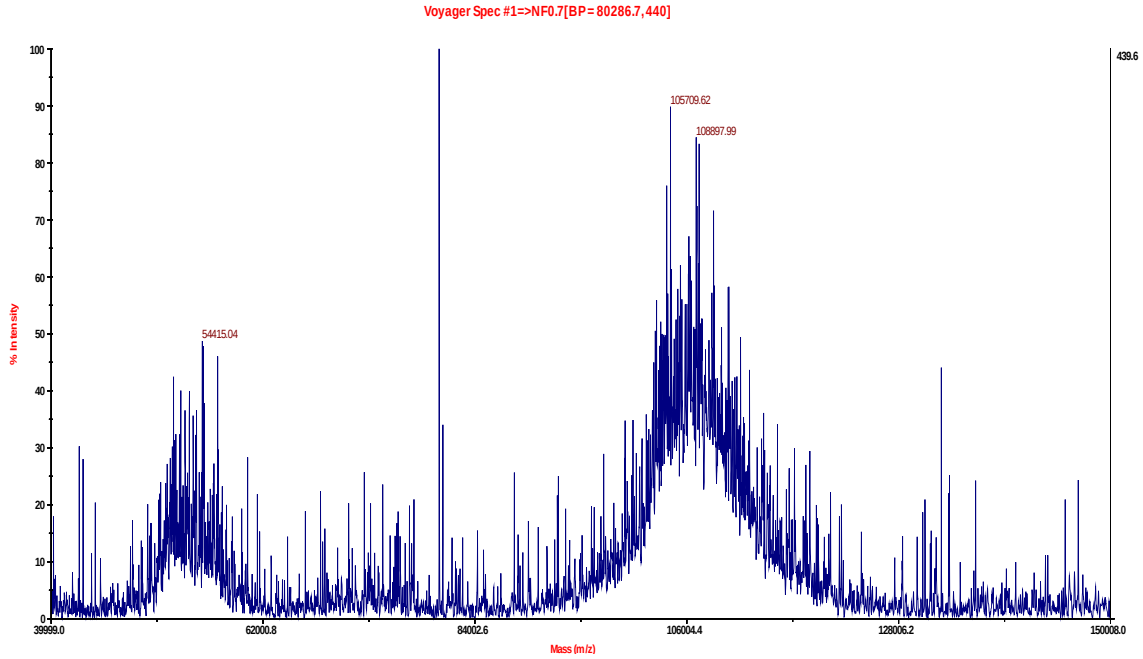

Figure S2. MALDI-TOF mass spectrum of compound **2.**

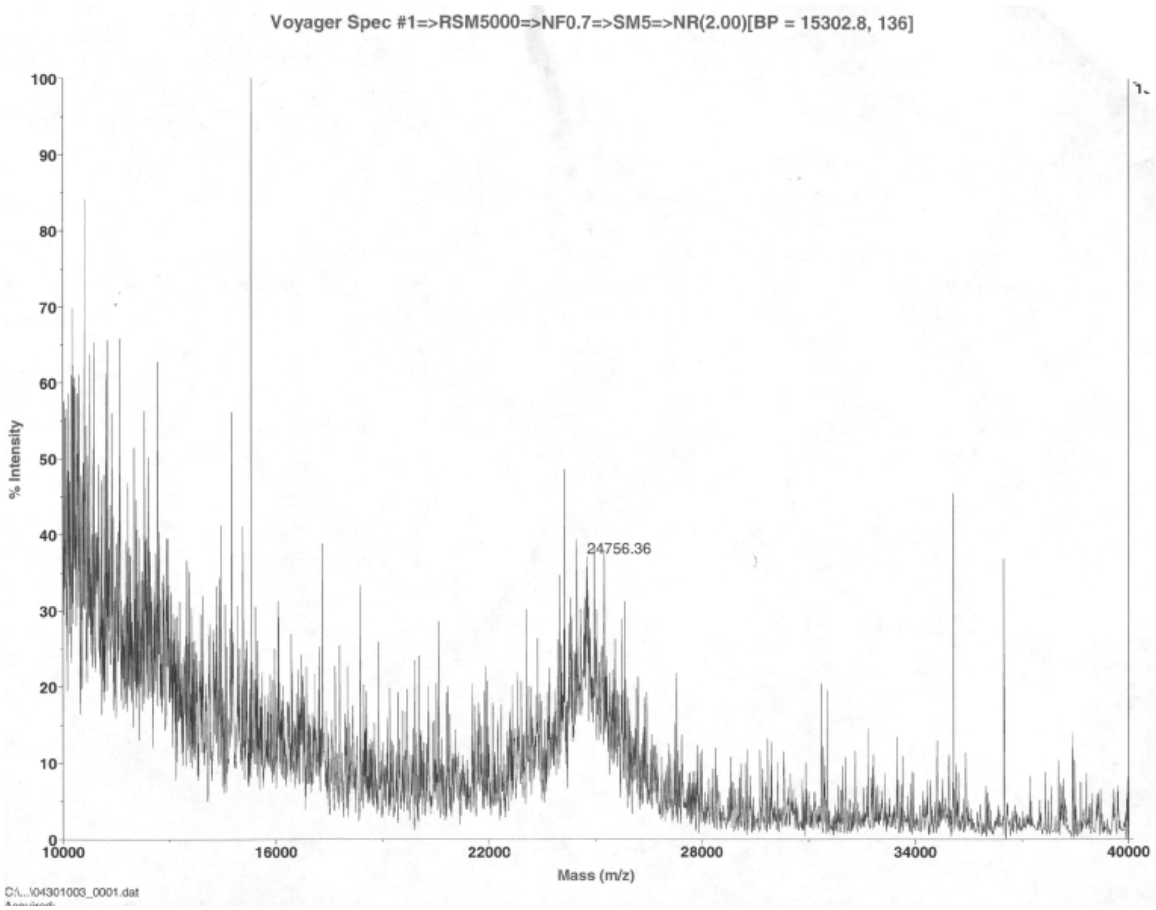

Figure S3. MALDI-TOF mass spectrum of compound **3.**

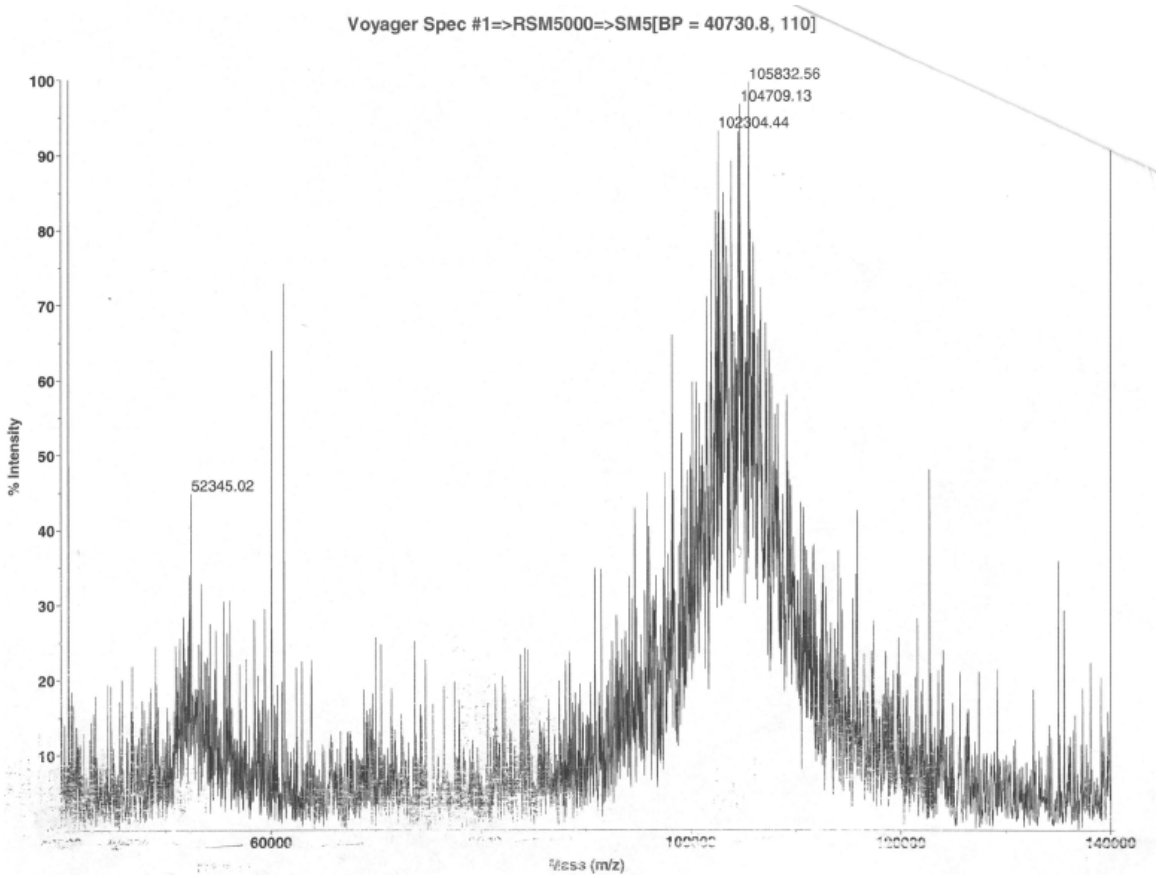

Figure S4. MALDI-TOF mass spectrum of compound **4.**

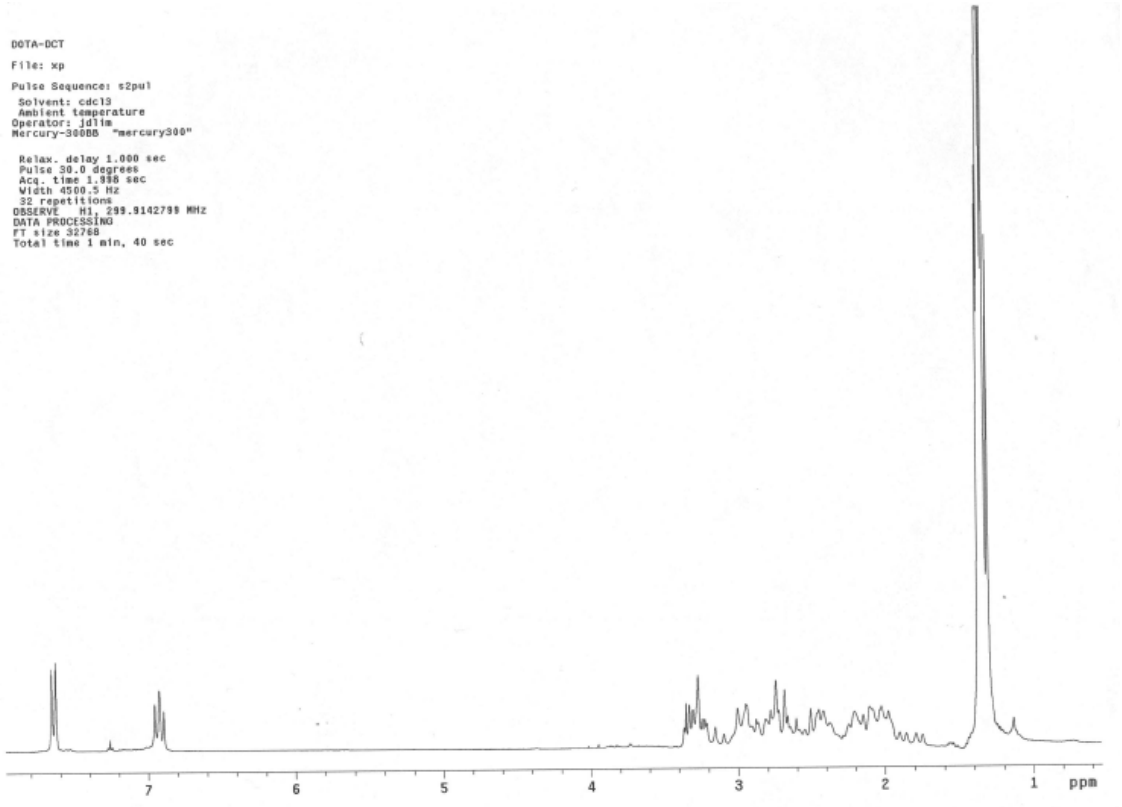

Figure S5. ^{1}H NMR spectrum of compound **5** (300 MHz, $CDCl_3$).

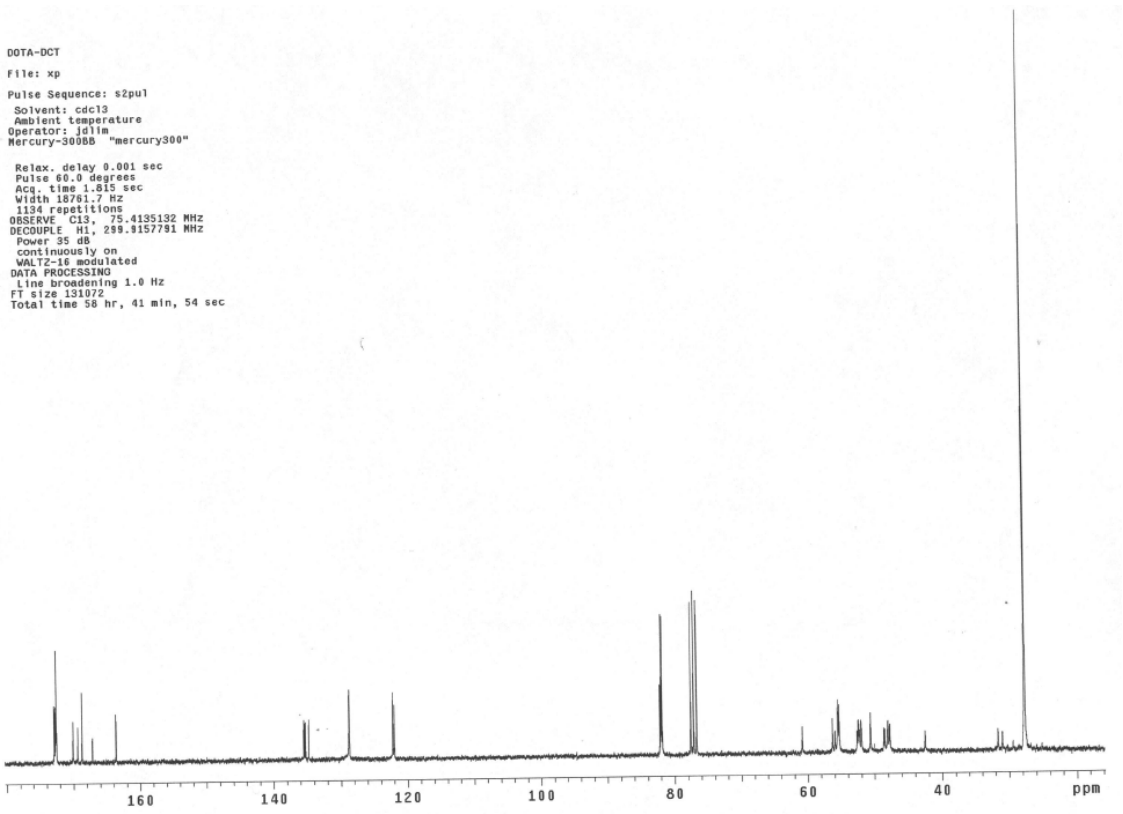

Figure S6. ^{13}C NMR spectrum of compound **5** (75 MHz, $CDCl_3$).

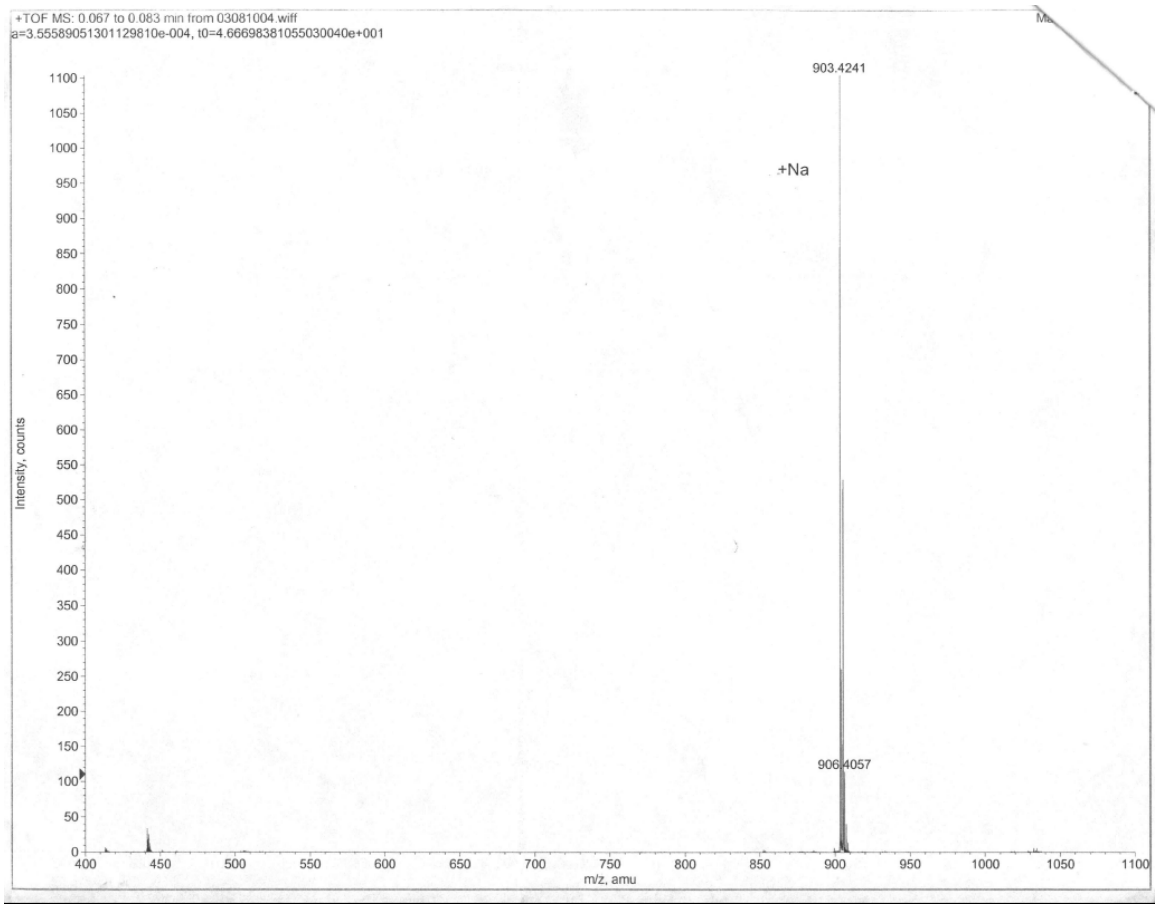

Figure S7. ESI-TOF mass spectrum of compound **5.**

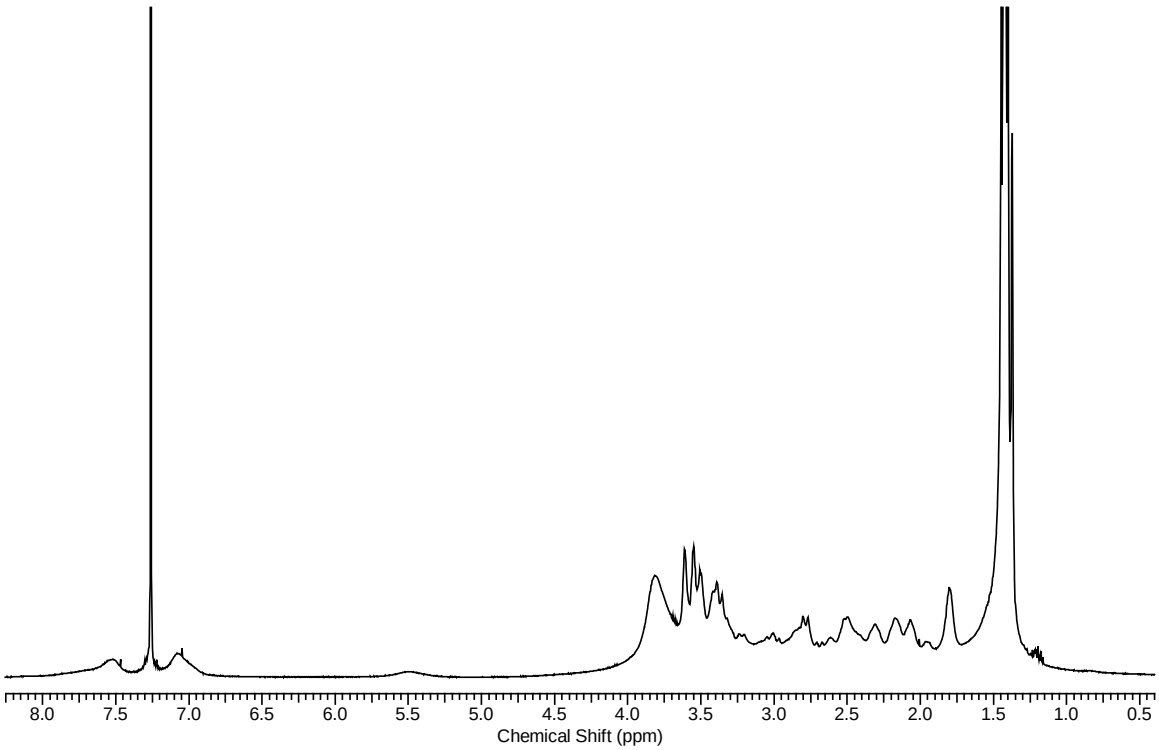

Figure S8. ^{1}H NMR spectrum of compound **6** (500 MHz, $CDCl_3$).

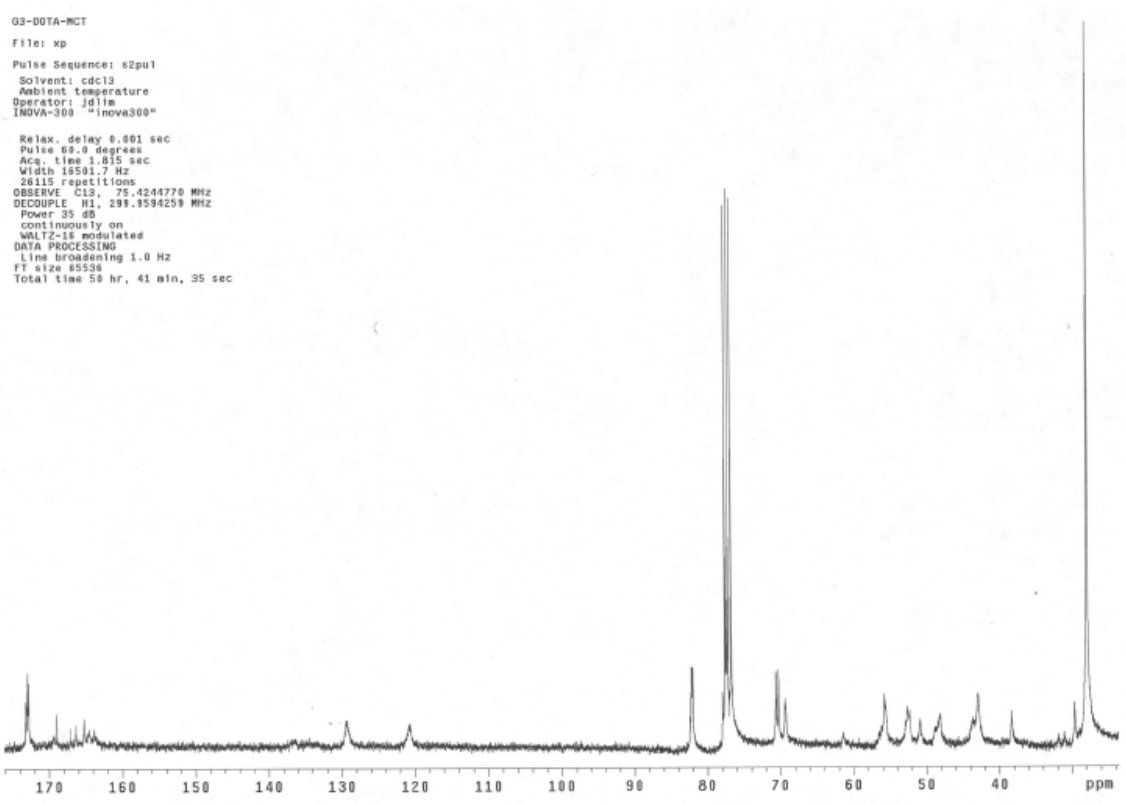

Figure S9. ^{13}C NMR spectrum of compound **6** (75 MHz, $CDCl_3$).

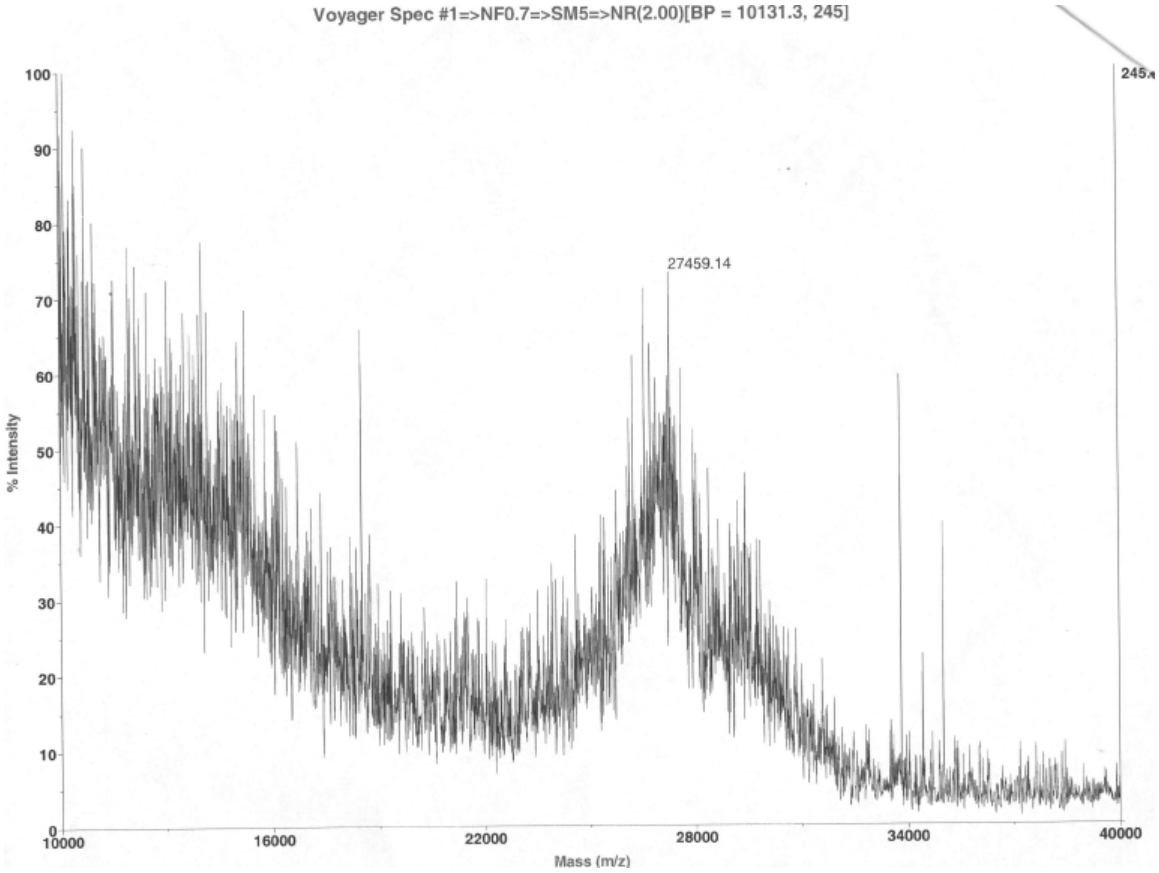

Figure S10. MALDI-TOF mass spectrum of compound **6.**

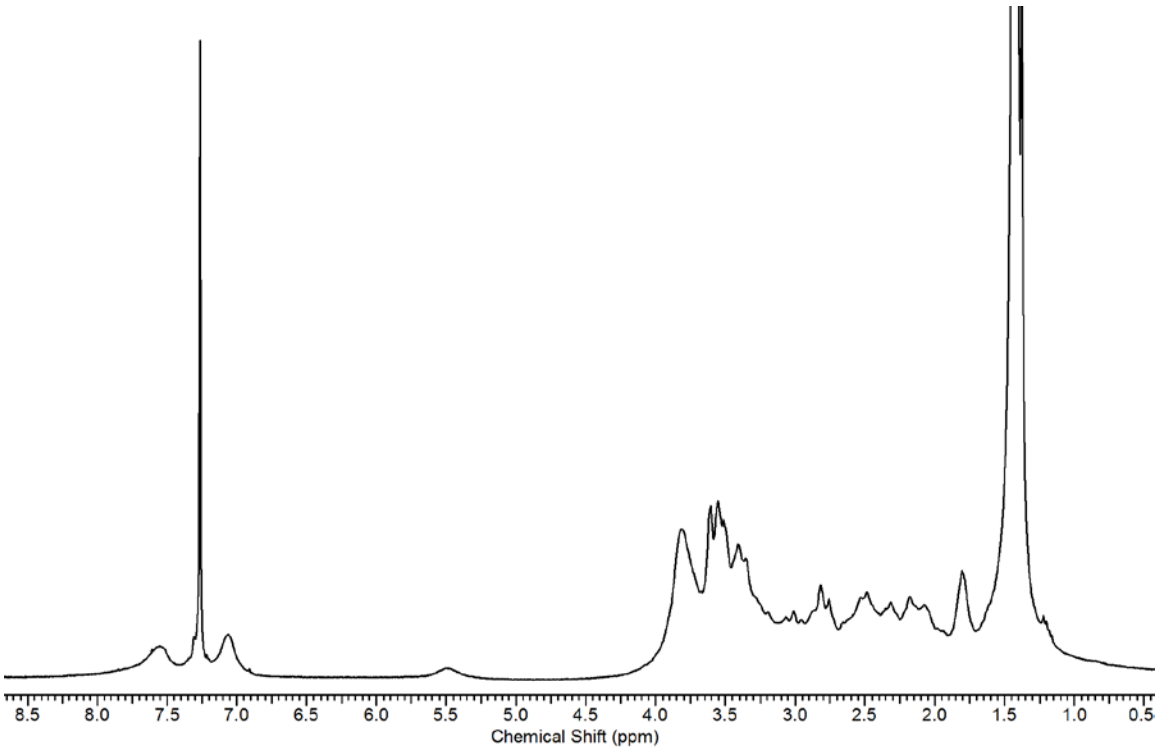

Figure S11. ^{1}H NMR spectrum of compound **7** (300 MHz, $CDCl_3$).

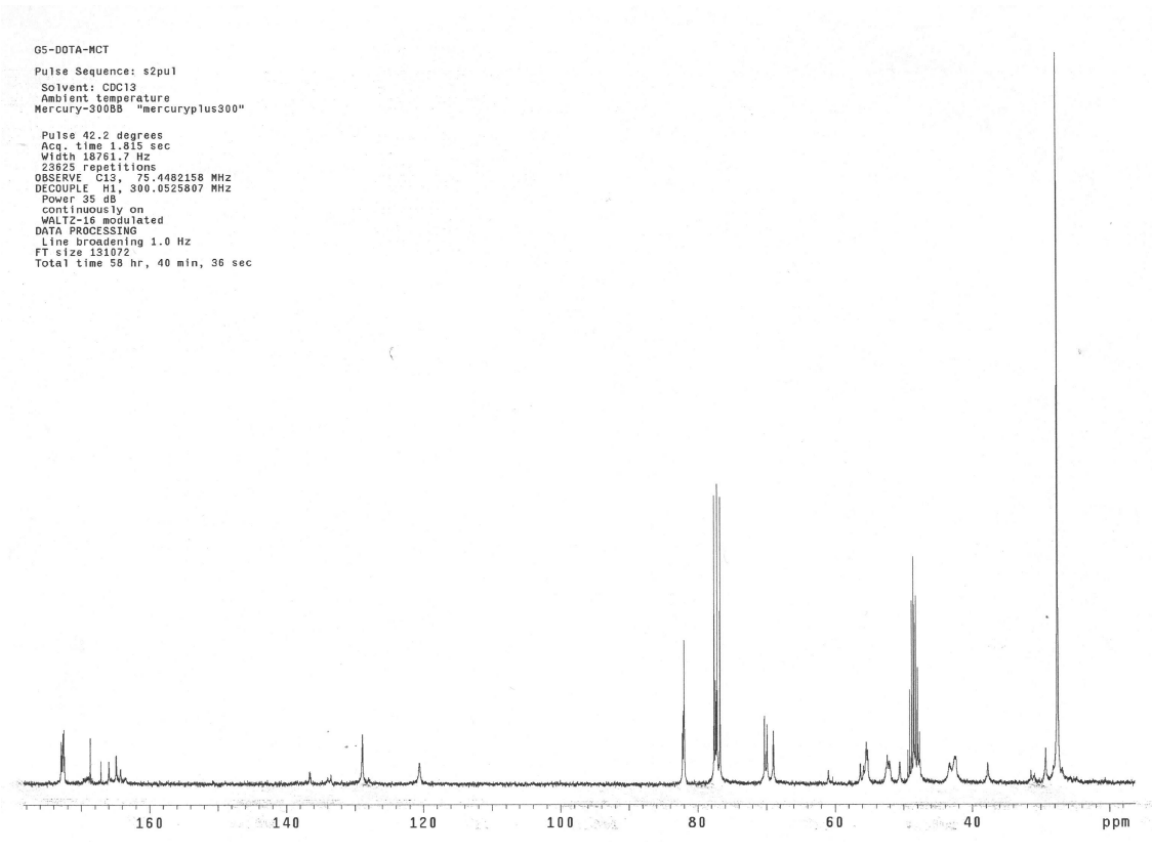

Figure S12. ^{13}C NMR spectrum of compound **7** (75 MHz, $CDCl_3$/MeOH-d_4).

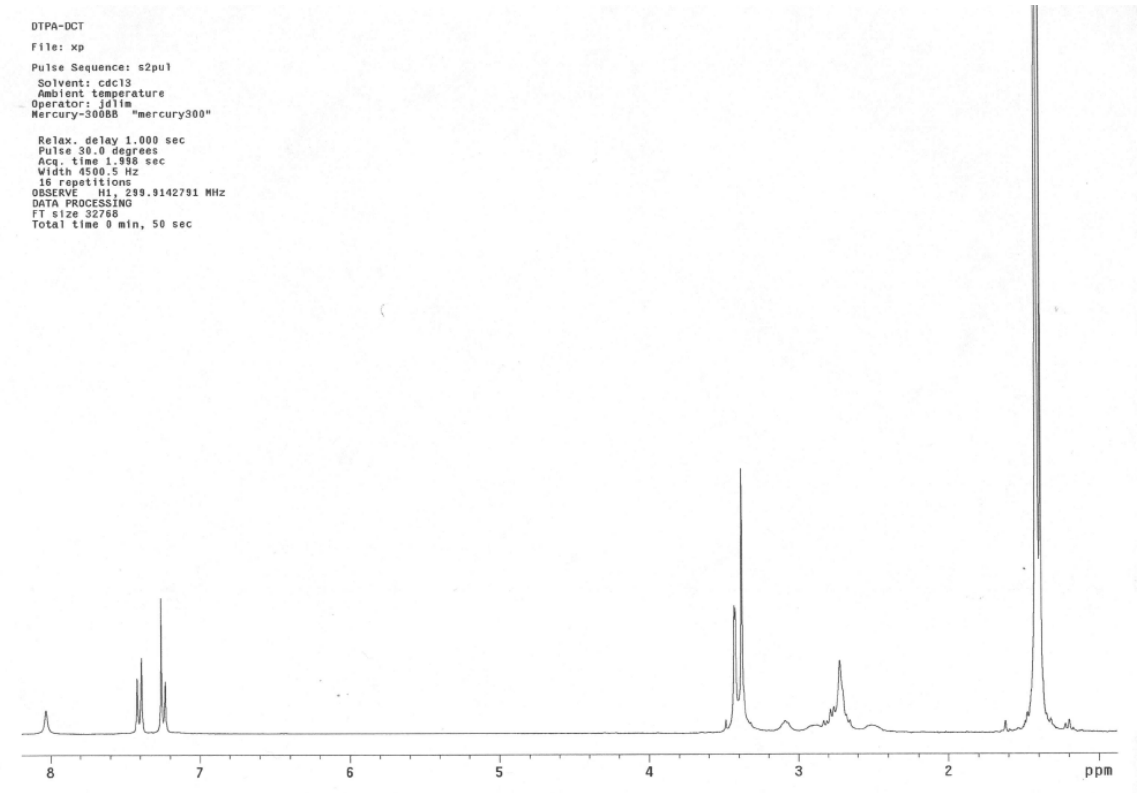

Figure S13. ^{1}H NMR spectrum of compound **8** (300 MHz, $CDCl_3$).

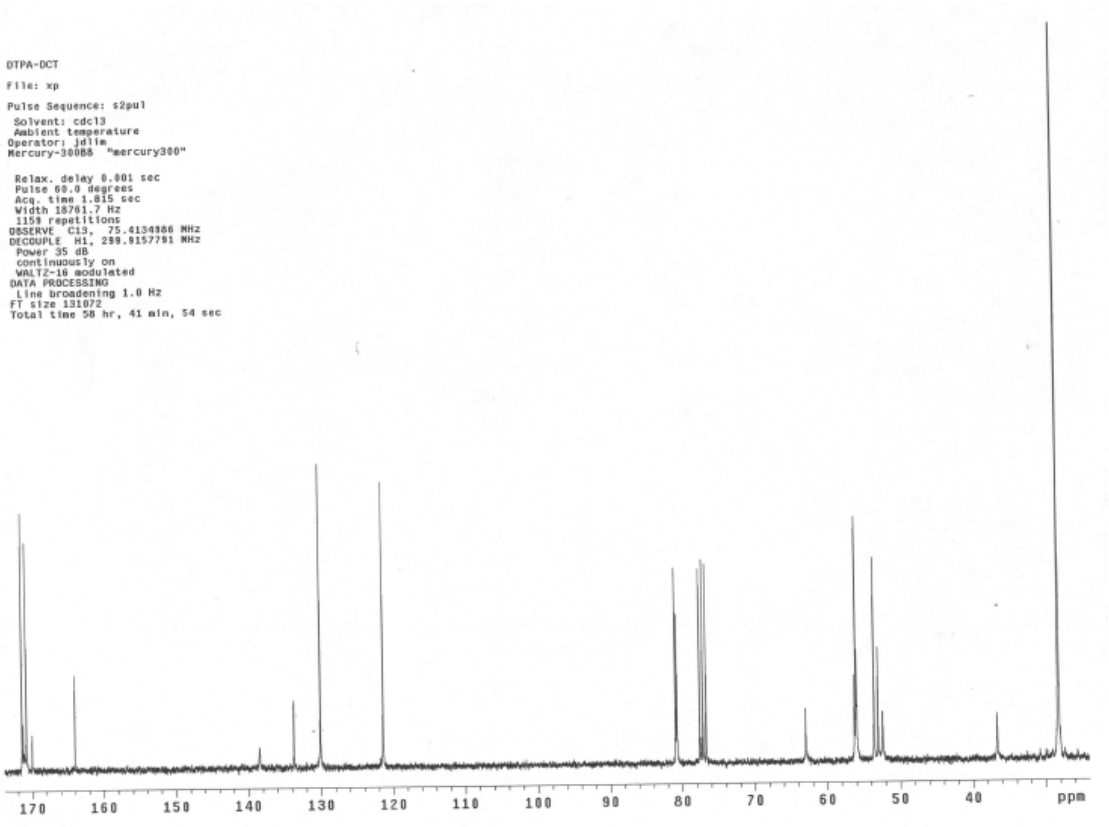

Figure S14. ^{13}C NMR spectrum of compound **8** (75 MHz, $CDCl_3$).

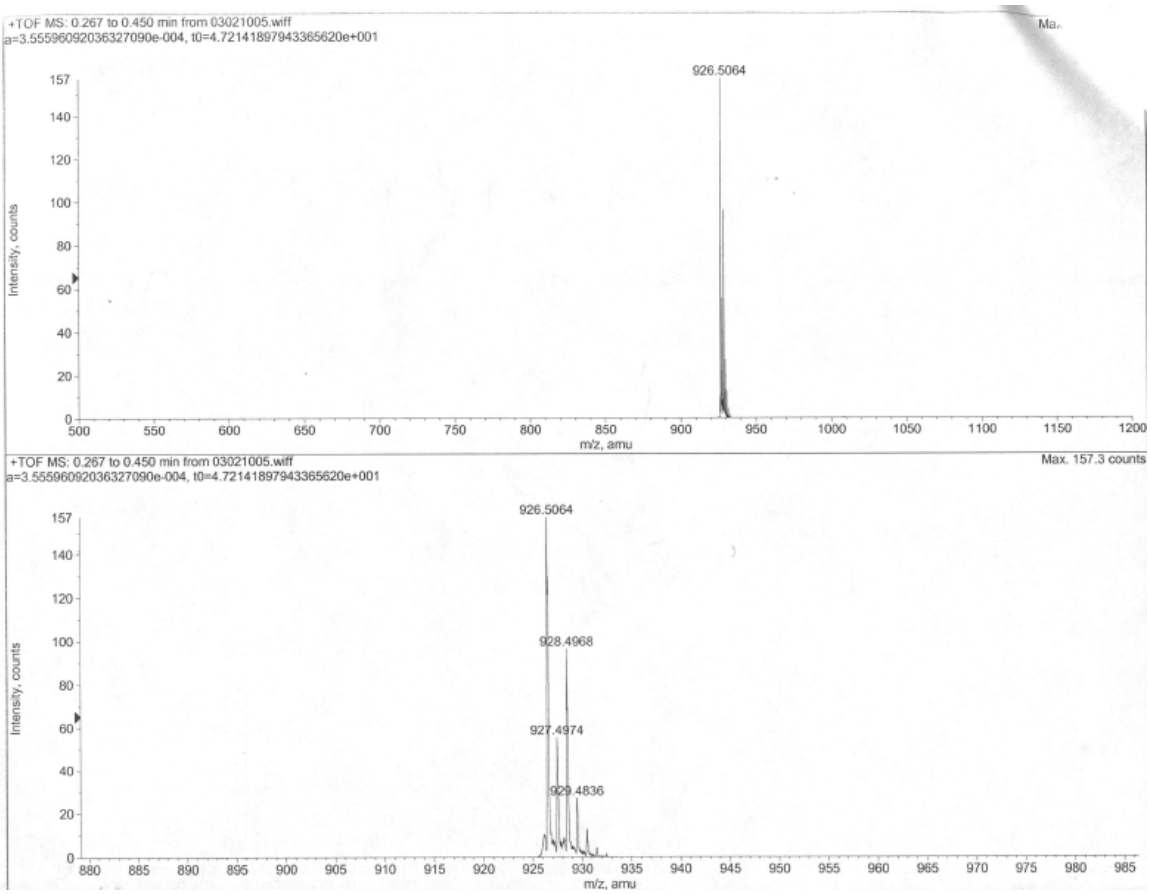

Figure S15. ESI-TOF mass spectrum of compound **8.**

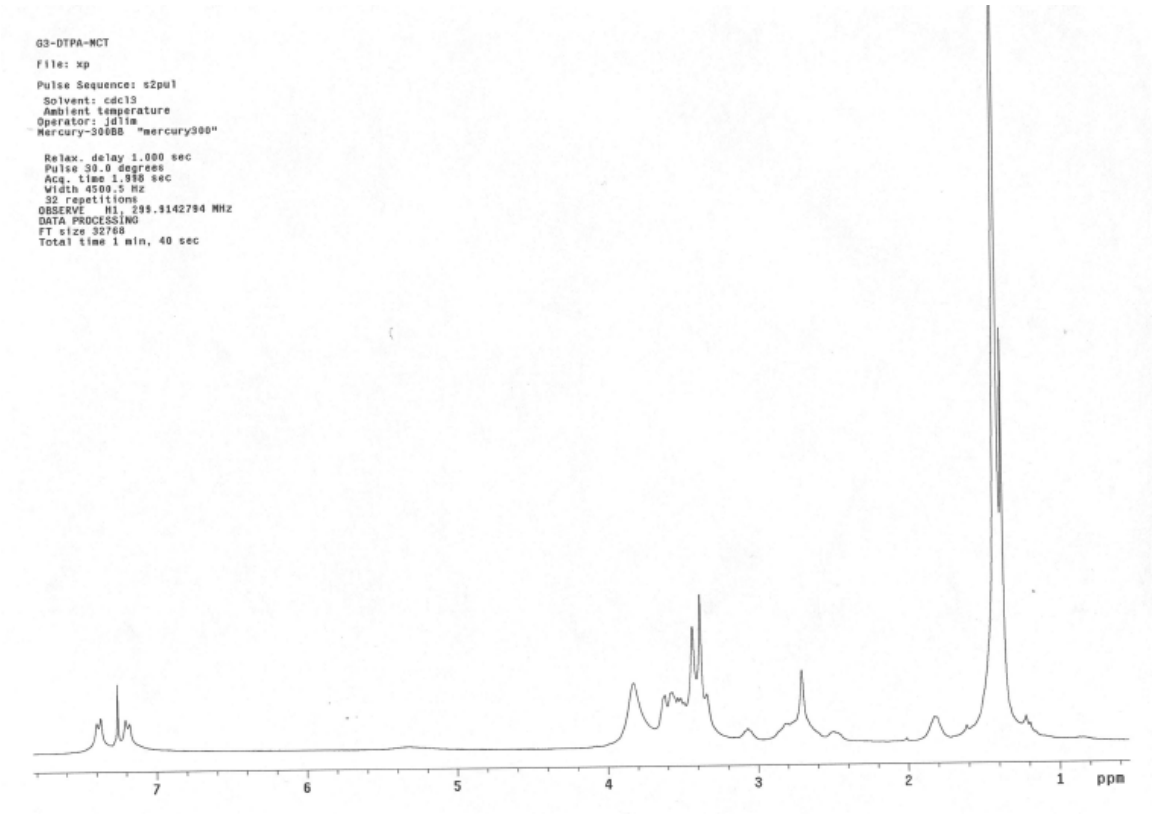

Figure S16. ^{1}H NMR spectrum of compound **9** (300 MHz, $CDCl_3$**).**

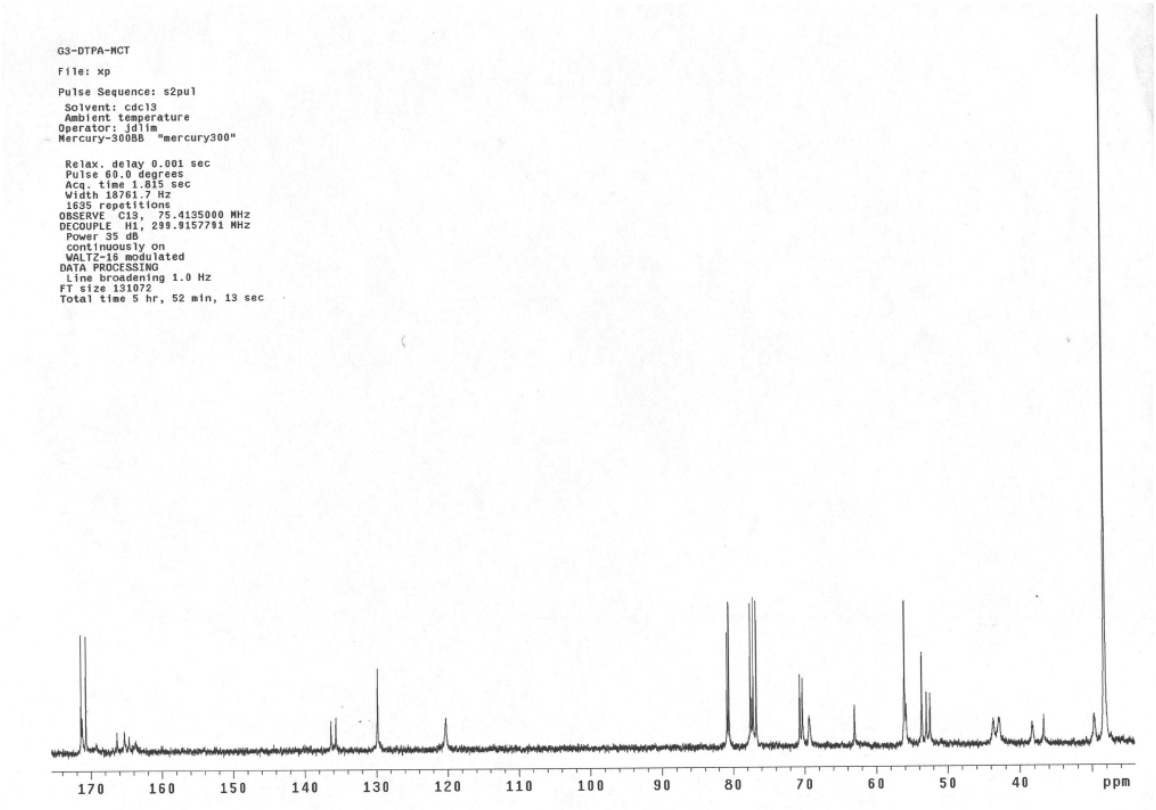

Figure S17. ^{13}C NMR spectrum of compound **9** (75 MHz, $CDCl_3$**).**

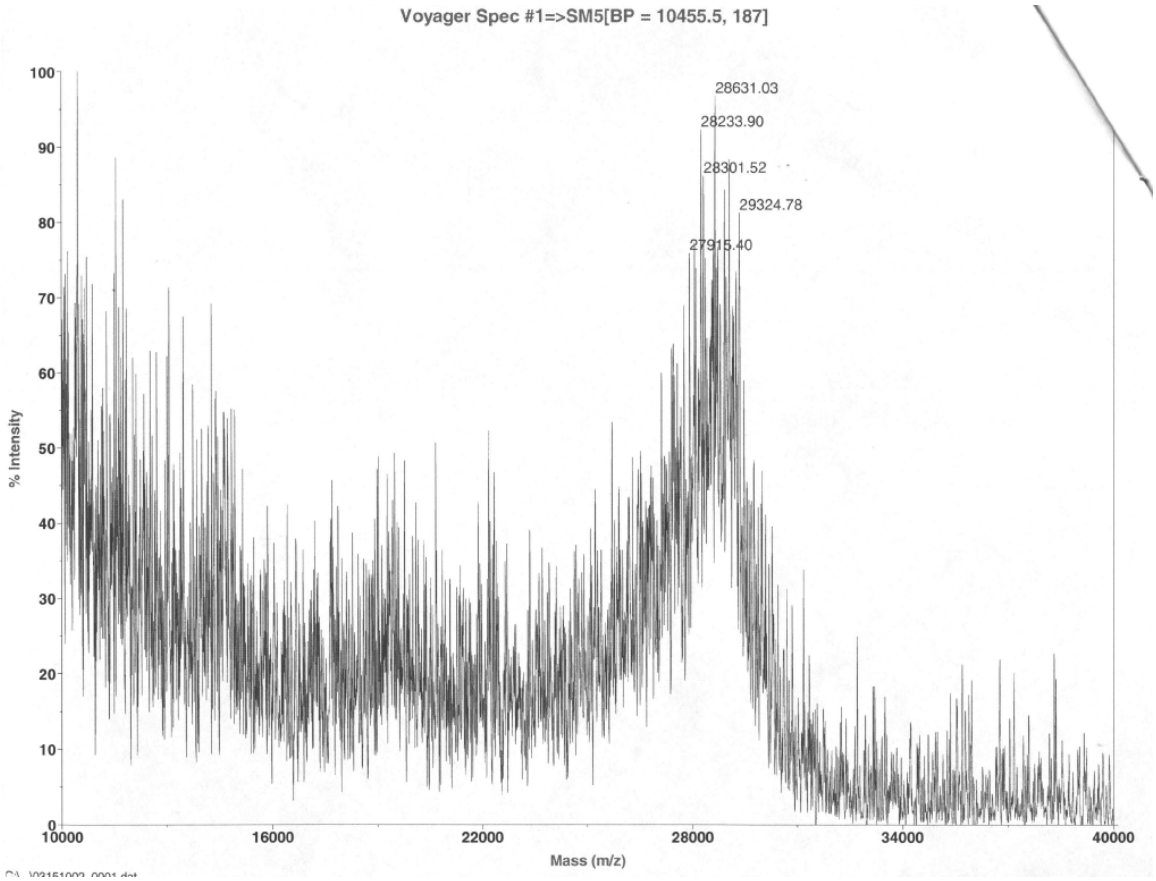

Figure S18. MALDI-TOF mass spectrum of compound **9.**

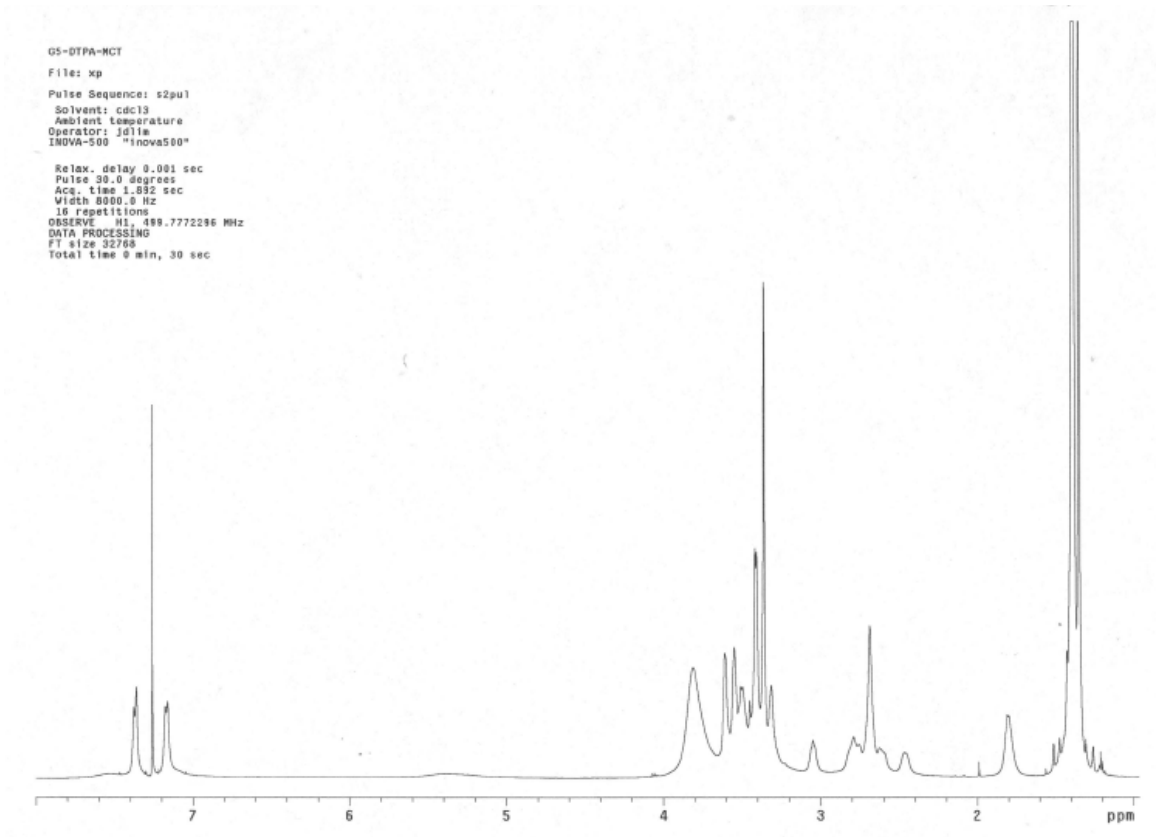

Figure S19. ^{1}H NMR spectrum of compound **10** (500 MHz, $CDCl_3$**).**

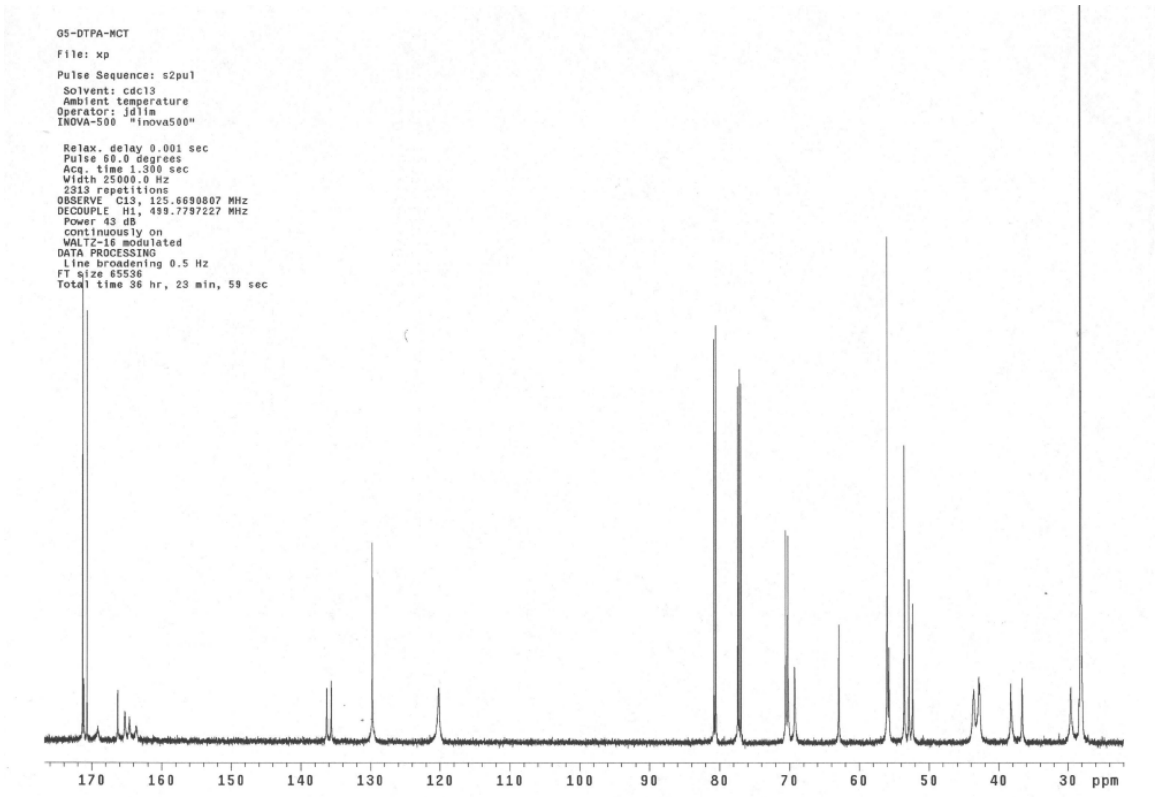

Figure S20. ^{13}C NMR spectrum of compound **10** (125 MHz, $CDCl_3$**).**

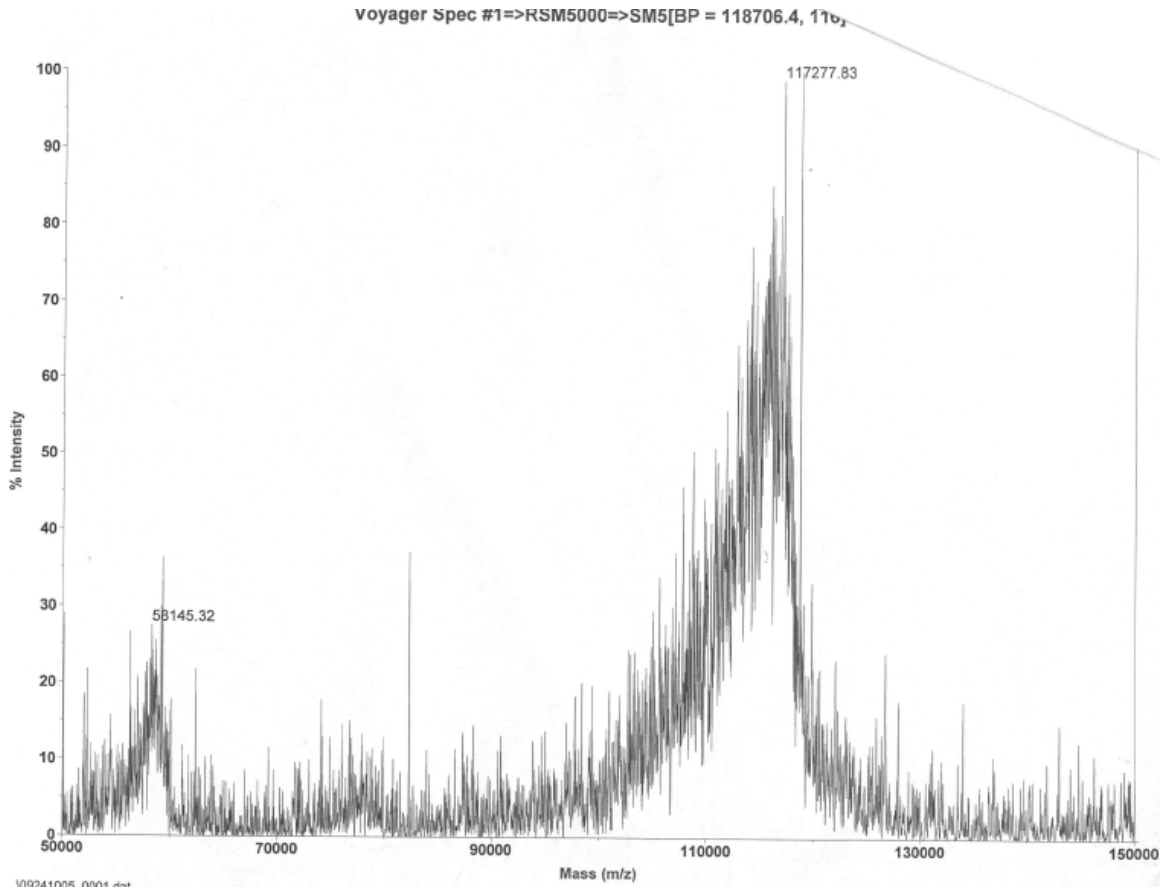

Figure S21. MALDI-TOF mass spectrum of compound **10**.

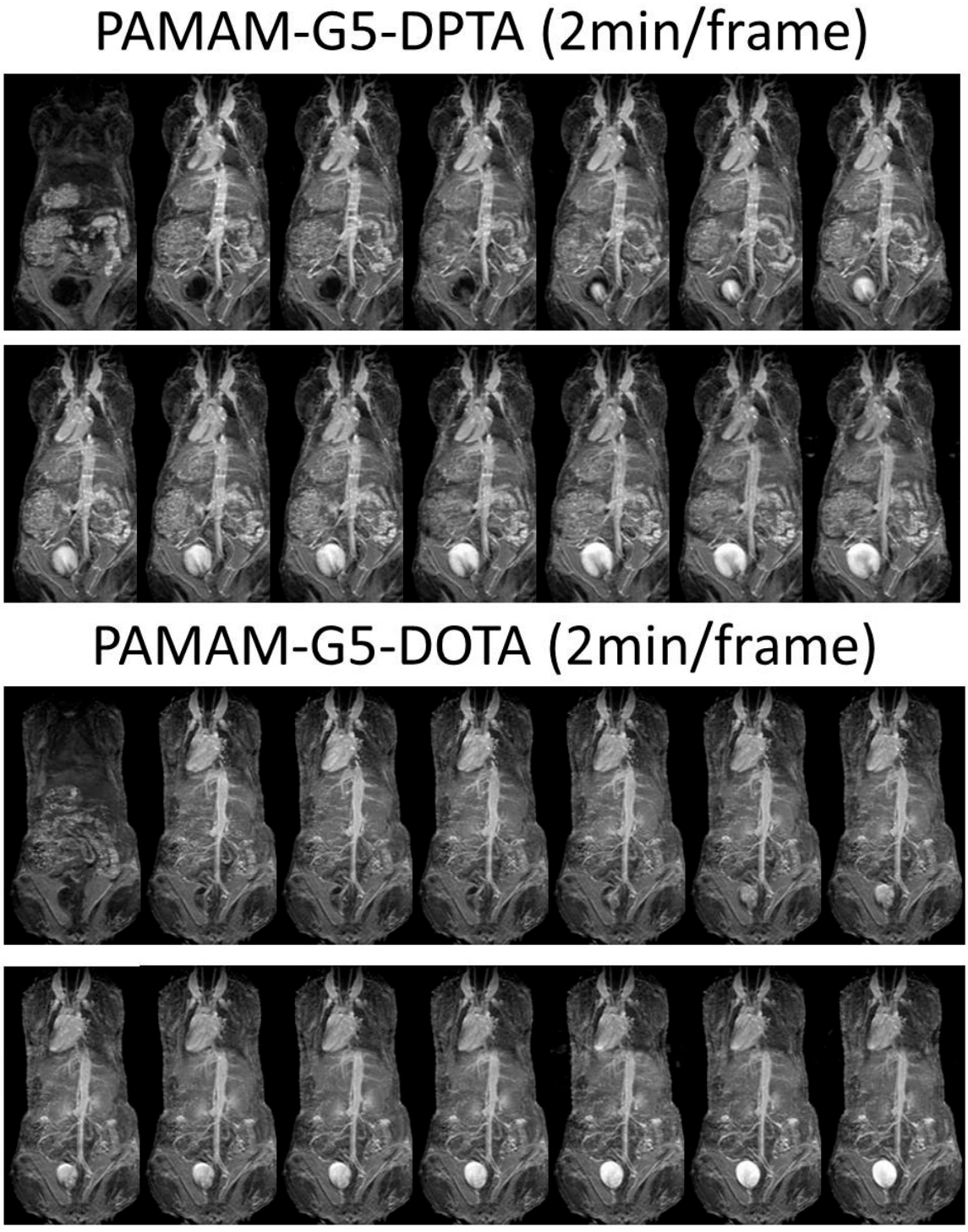

Figure S22. Dynamic MRI of mice using 0.03mmolGd/kg of PAMAM-G5-DPTA and PAMAM-G5-DOTA. No clear difference on relaxivity is shown between DPTA and DOTA conjugates.