

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

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General procedures

G3 and G5 dendrimers were prepared using methods previously described.^{s1} p-NH₂-Bn-DOTA-tetra(t-Bu ester) and p-NH₂-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 N₂. NMR spectra were recorded on a Varian Mercury 300 MHz or Inova 500 MHz spectrometer in CDCl₃, or CDCl₃:MeOH-d₄ (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 °C, 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 MgSO₄, 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 C₁₁₅₂H₁₇₉₄N₃₉₆O₂₈₅ 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 °C, 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 MgSO₄, 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 C₄₆₈₀H₇₂₉₀N₁₆₂₀O₁₁₄₉ 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 °C, 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 MgSO₄, 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 C₁₁₀₄H₁₆₇₄N₃₇₂O₃₃₃ 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 °C, 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 MgSO₄, 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-NH₂-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 °C and then concentrated under vacuum. The residue was dissolved in dichloromethane, washed with brine, dried over MgSO₄, 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, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₄₂H₆₆Cl₂N₈O₈ 880.4381, found 903.4241 (M+Na)⁺.

Compound 6. A solution of **G3** (40 mg, 5.31 μmol) in THF (1 mL) and H₂O (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 °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 MgSO₄, 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, CDCl₃) δ 7.52 (br, 48H), 7.08 (br, 48H), 3.81-1.80 (m, 1140H), 1.42 (m, 864H); ¹³C NMR (75 MHz, CDCl₃) 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 C₁₃₄₄H₂₁₅₄Cl₂₄N₃₄₈O₂₃₇ 27801.97, found 27459.14 (M+H)⁺.

Compound 7. A solution of **G5** (60 mg, 1.89 μmol) in THF (2 mL) and H₂O (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 °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 MgSO₄, 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, CDCl₃) δ 7.55 (d, J = 7.2 Hz, 192H), 7.07 (br, 192H), 3.81-1.81 (m, 4668H), 1.42 (m, 3456H); ¹³C NMR (75 MHz, CDCl₃/MeOH-d₄) 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 C₅₄₄₈H₈₇₃₀Cl₉₆N₁₄₂₈O₉₅₇ 112834.85, not found.

Compound 8. Cyanuric chloride (48 mg, 0.260 mmol) was added to an ice-bath cooled solution of p-NH₂-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 °C and then concentrated under vacuum. The residue was dissolved in dichloromethane, washed with brine, dried over MgSO₄, 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, CDCl₃) 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); ¹³C NMR (75 MHz, CDCl₃) 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 C₄₄H₆₉Cl₂N₇O₁₀ 925.4483, found 926.5064 (M+H)⁺.

Compound 9. A solution of **G3** (25 mg, 3.32 μmol) in THF (1 mL) and H₂O (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 °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 MgSO₄, filtered, and evaporated under vacuum. The crude product was purified by silica gel chromatography (from EtOAc:Hex = 2:1 to EtOAc:MeOH:H₂O =

7:2:1) to give **9** (79 mg, 82%) as a white solid. ^1H 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 $\text{C}_{1392}\text{H}_{2226}\text{Cl}_{24}\text{N}_{324}\text{O}_{285}$ 28882.22, found 29324.78 ($\text{M}+\text{H}$) $^+$.

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 $^\circ\text{C}$. 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. ^1H 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 $\text{C}_{5640}\text{H}_{9018}\text{Cl}_{96}\text{N}_{1332}\text{O}_{1149}$ 117155.83, found 117277.83 ($\text{M}+\text{H}$) $^+$.

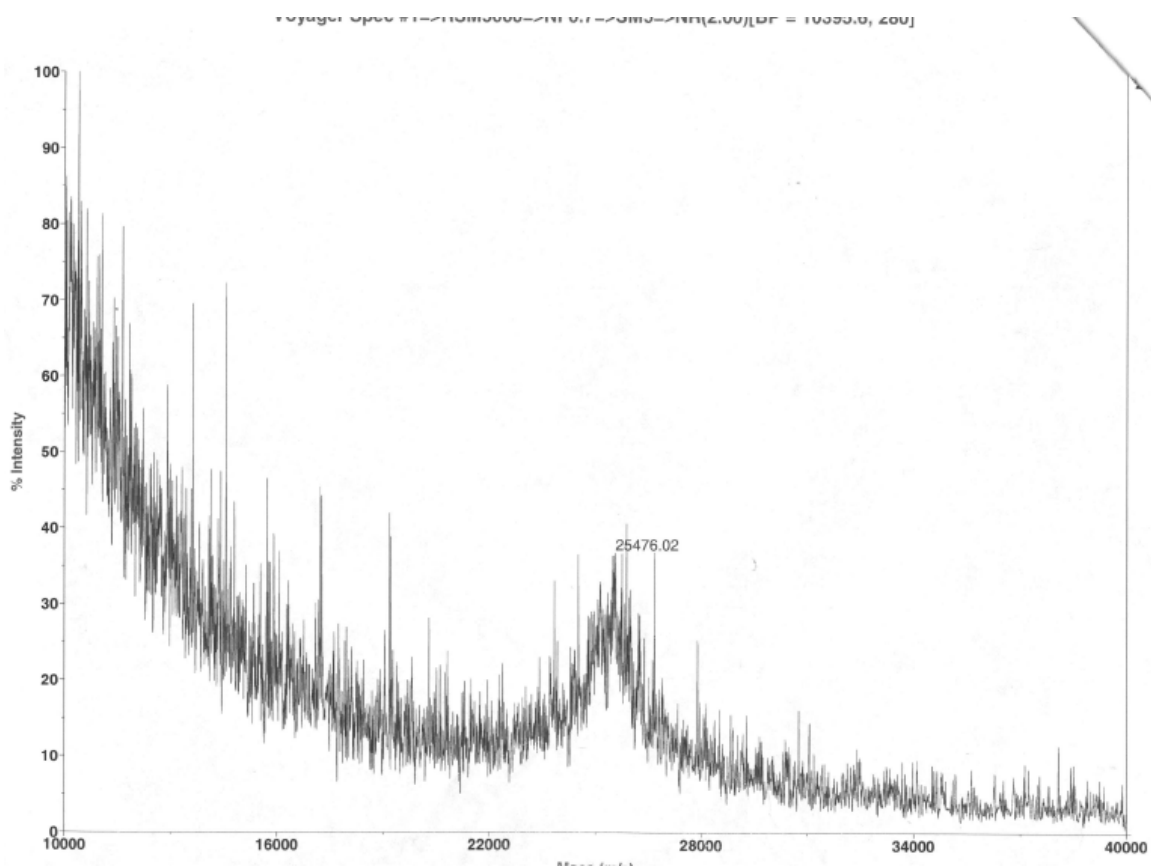


Figure S1. MALDI-TOF mass spectrum of compound **1**.

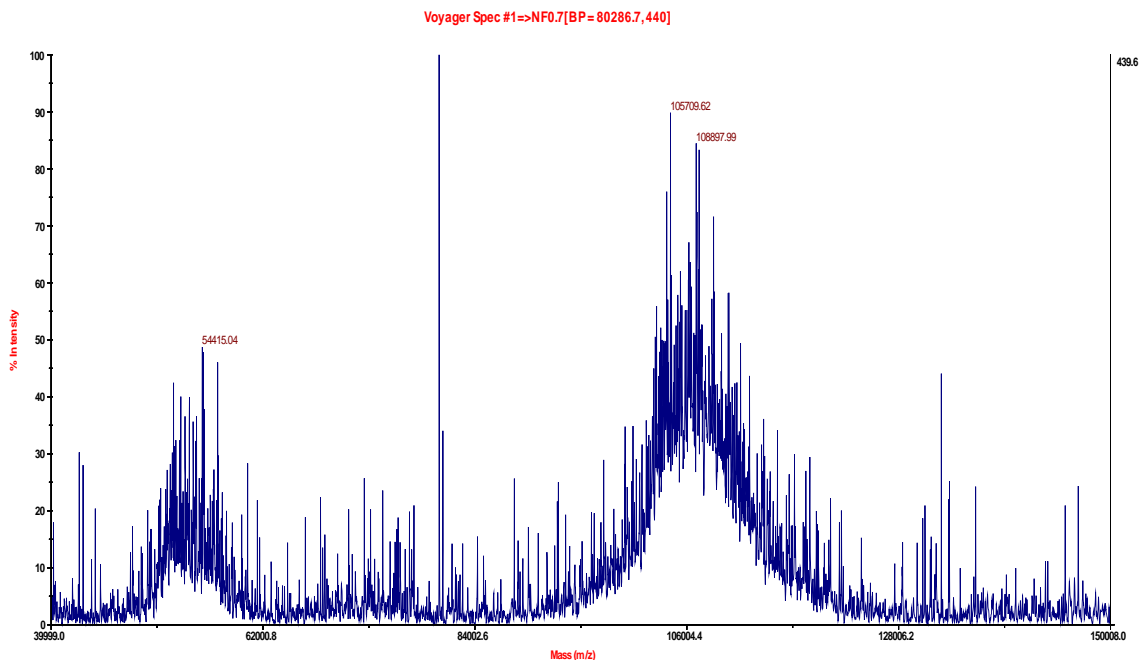


Figure S2. MALDI-TOF mass spectrum of compound **2**.

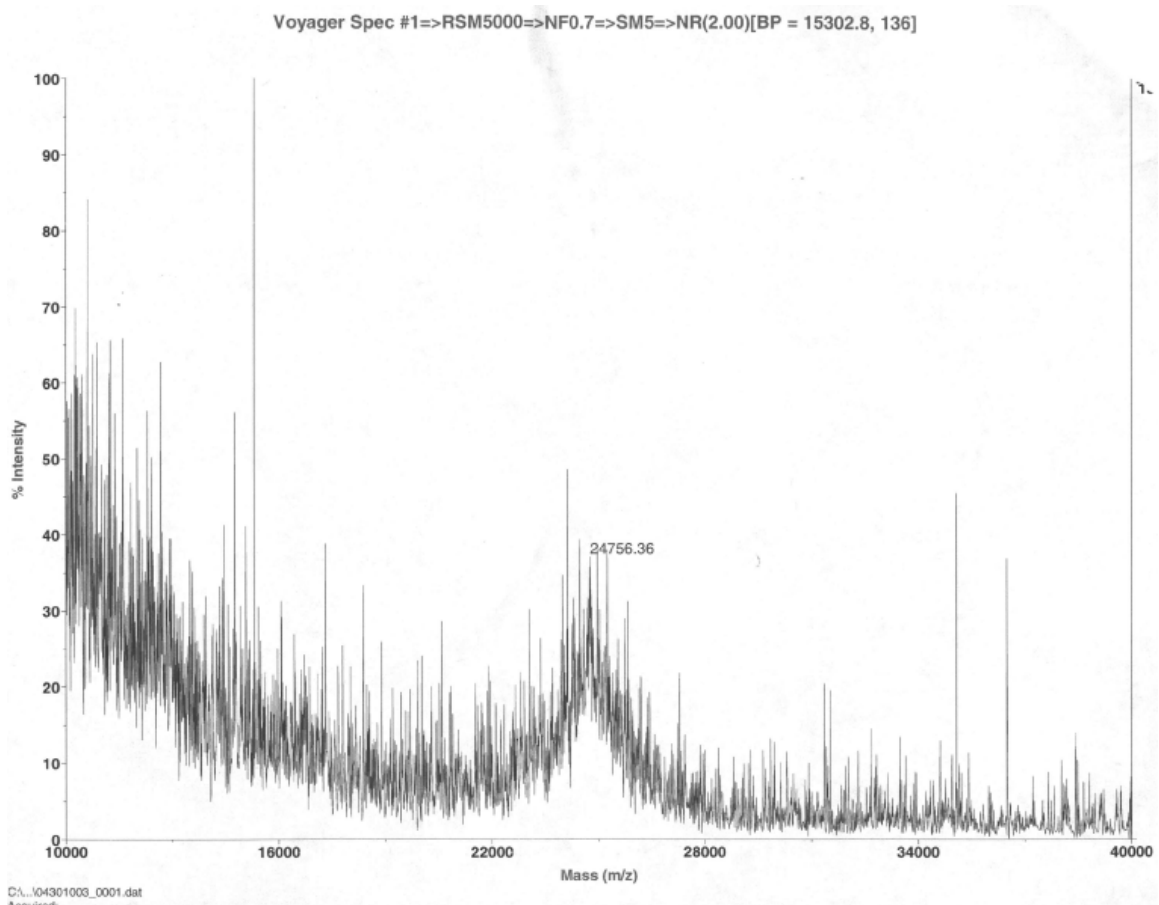


Figure S3. MALDI-TOF mass spectrum of compound **3**.

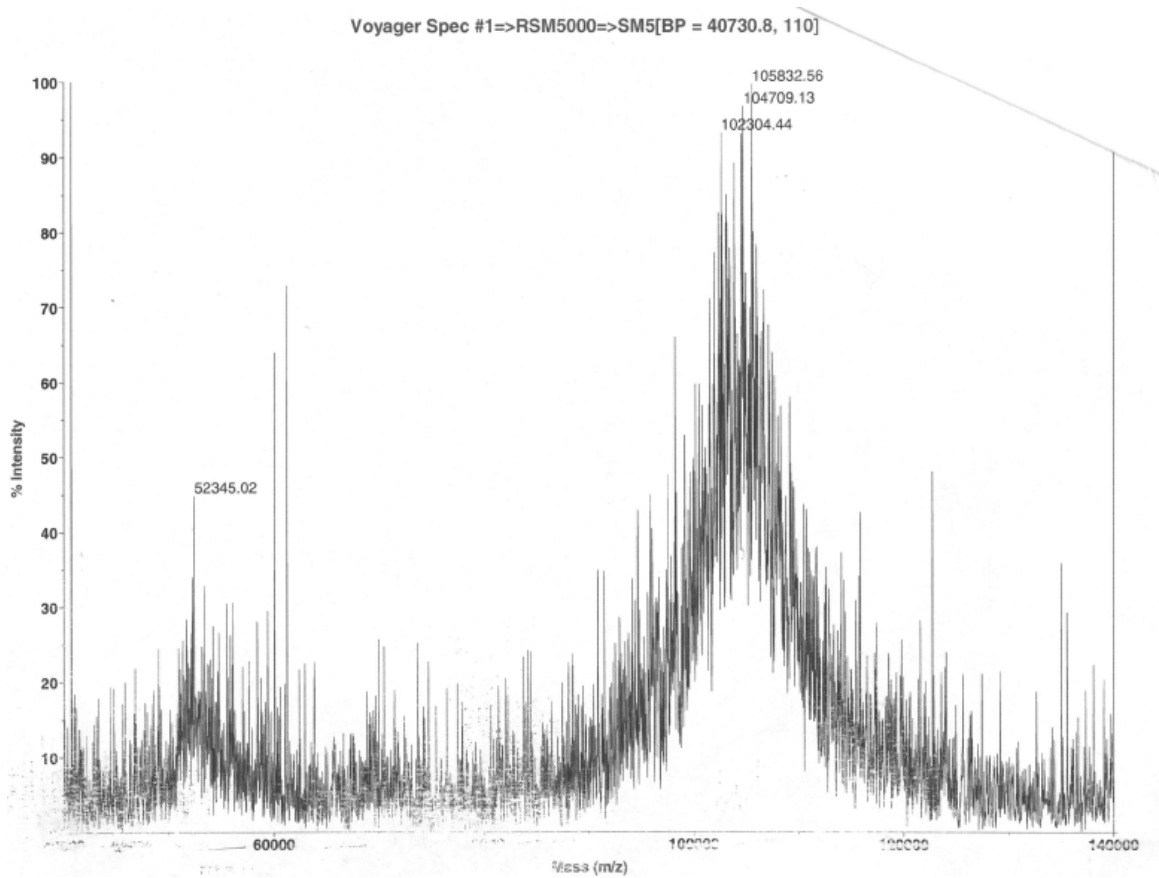


Figure S4. MALDI-TOF mass spectrum of compound **4**.

DOTA-DCT
File: xp
Pulse Sequence: s2pul
Solvent: cdcl3
Ambient temperature
Operator: jdlia
Mercury-300BB "mercury300"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.995 sec
Width 4500.5 Hz
32 repetitions
OBSERVE H1 299.9142799 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 40 sec

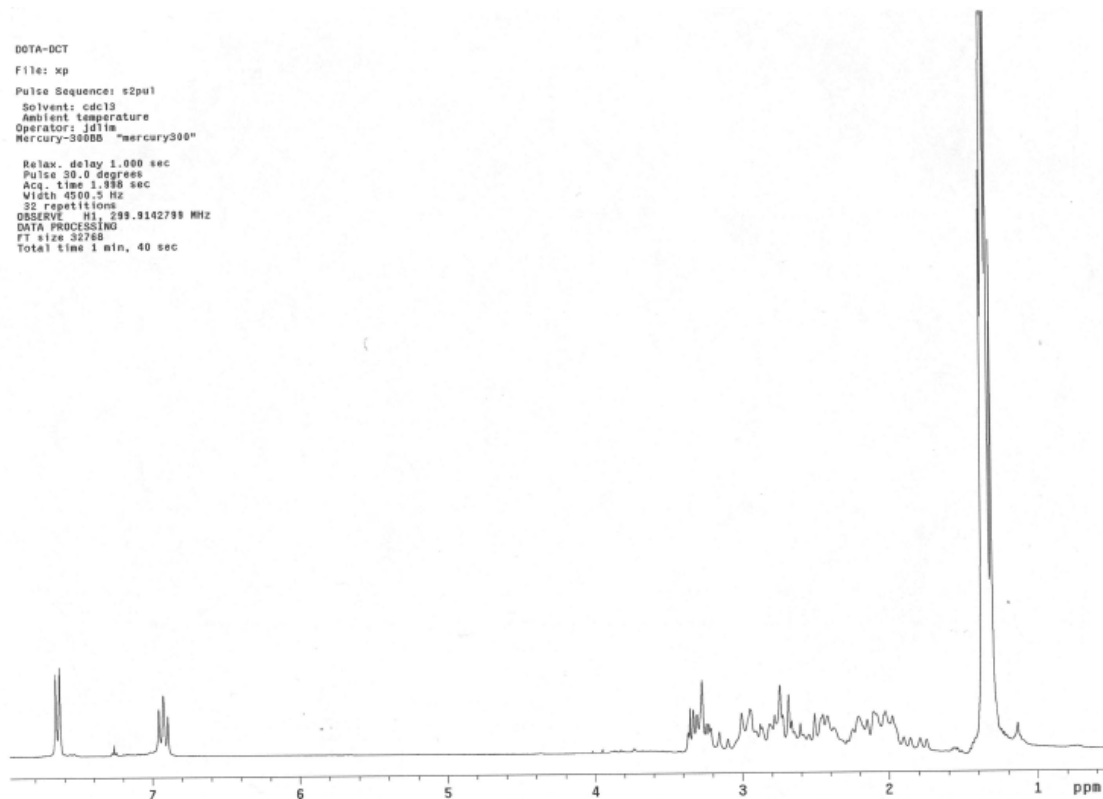


Figure S5. ^1H NMR spectrum of compound **5** (300 MHz, CDCl_3).

DOTA-DCT
File: xp
Pulse Sequence: s2pu1
Solvent: cdcl3
Ambient temperature
Operator: jdlm
Mercury-3000B "mercury300"

Relax. delay 9.001 sec
Pulse 60.0 degrees
Acq. time 1.515 sec
Width 15761.7 Hz
1134 repetitions
OBSERVE C13, 75.4135132 MHz
DECOUPLE H1, 299.9157791 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 58 hr, 41 min, 54 sec

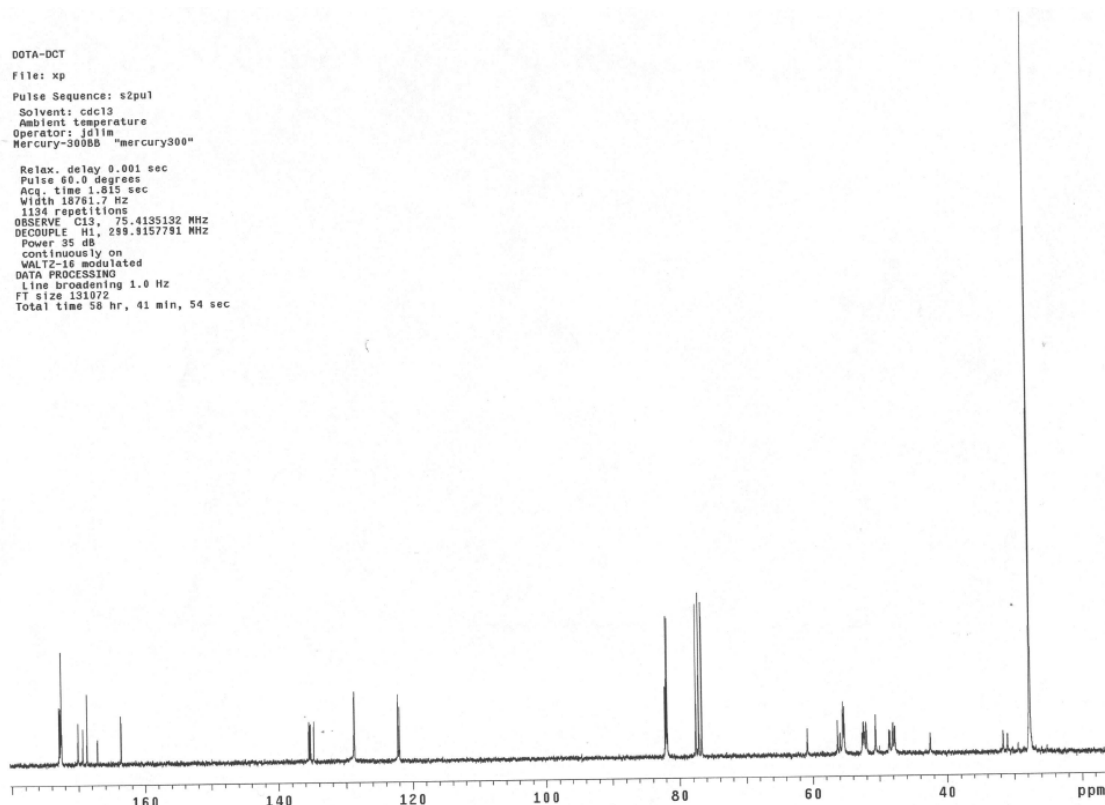


Figure S6. ^{13}C NMR spectrum of compound **5** (75 MHz, CDCl_3).

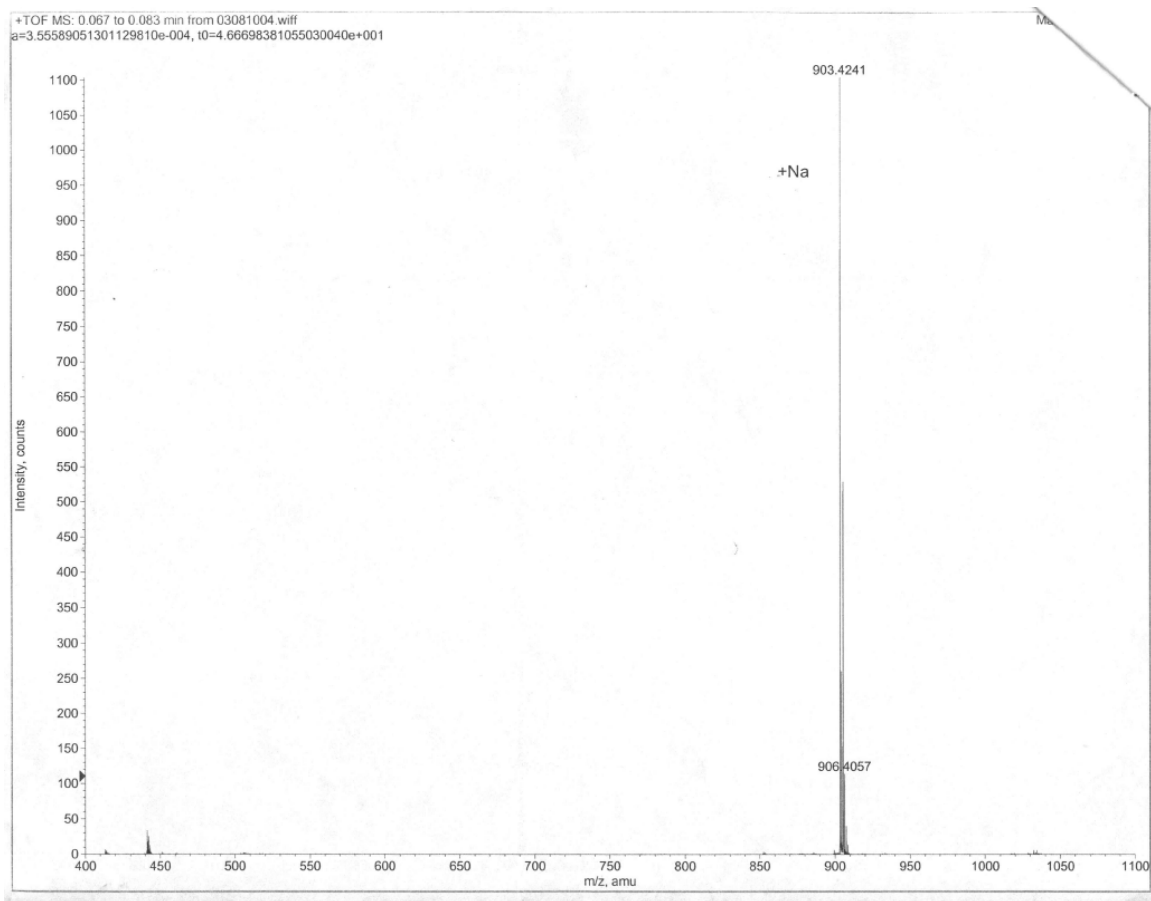


Figure S7. ESI-TOF mass spectrum of compound **5**.

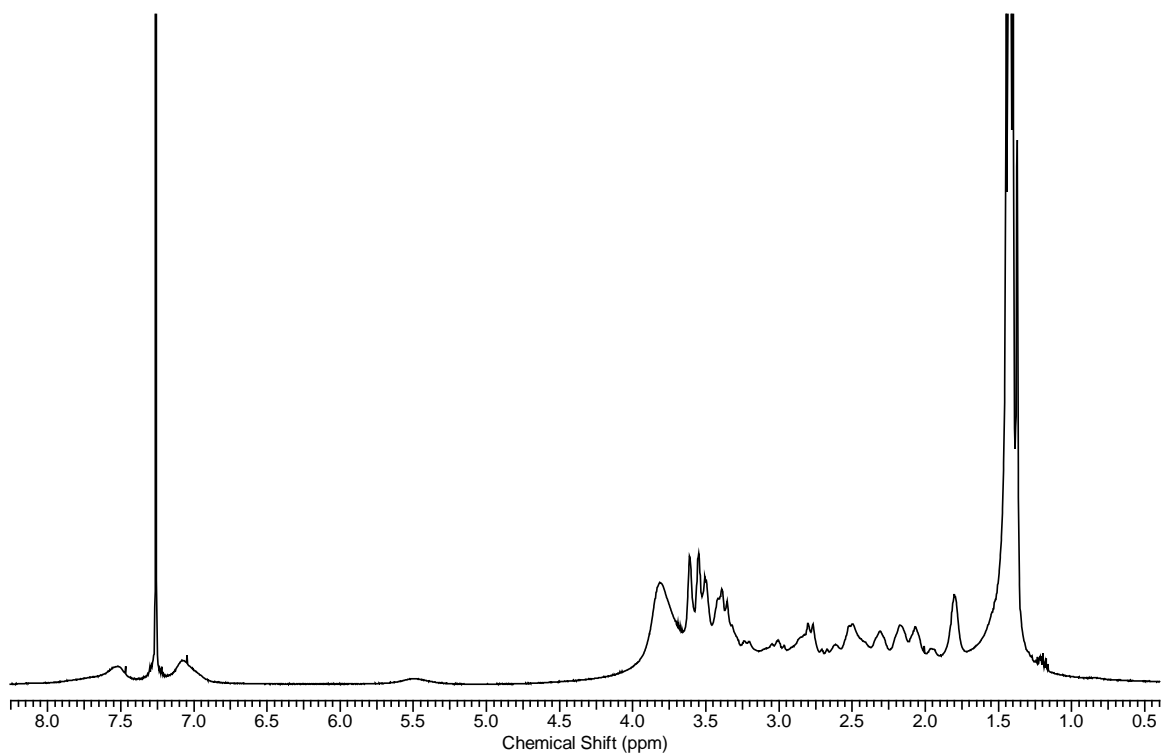


Figure S8. ¹H NMR spectrum of compound **6** (500 MHz, CDCl₃).

03-DOTA-MGT
File: xp
Pulse Sequence: s2pu1
Solvent: cdcl3
Ambient temperature
Operator: jdlie
INOVA-300 "inova300"

Relax. delay 9.001 sec
Pulse 63.0 degrees
Acq. time 1.835 sec
Width 16501.7 Hz
26115 repetitions
OBSERVE C13, 75.4244770 MHz
DECOUPLE H1, 299.3594259 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 59 hr, 41 min, 35 sec

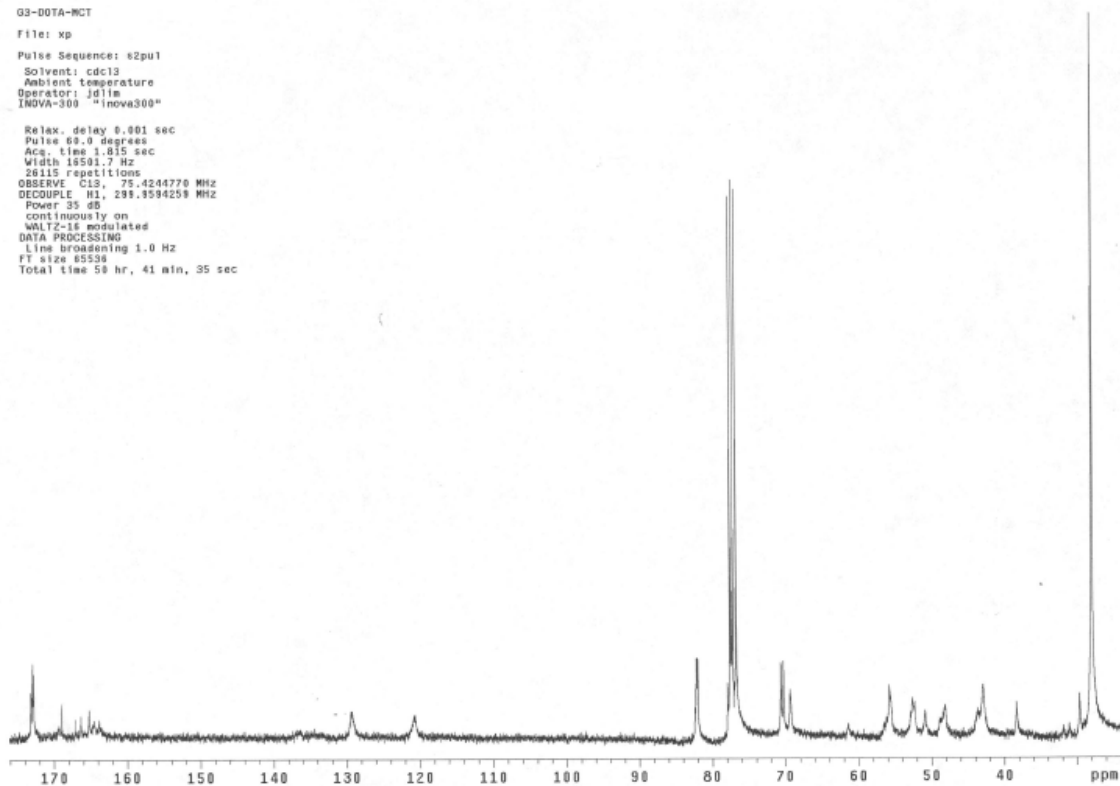


Figure S9. ^{13}C NMR spectrum of compound **6** (75 MHz, CDCl_3).

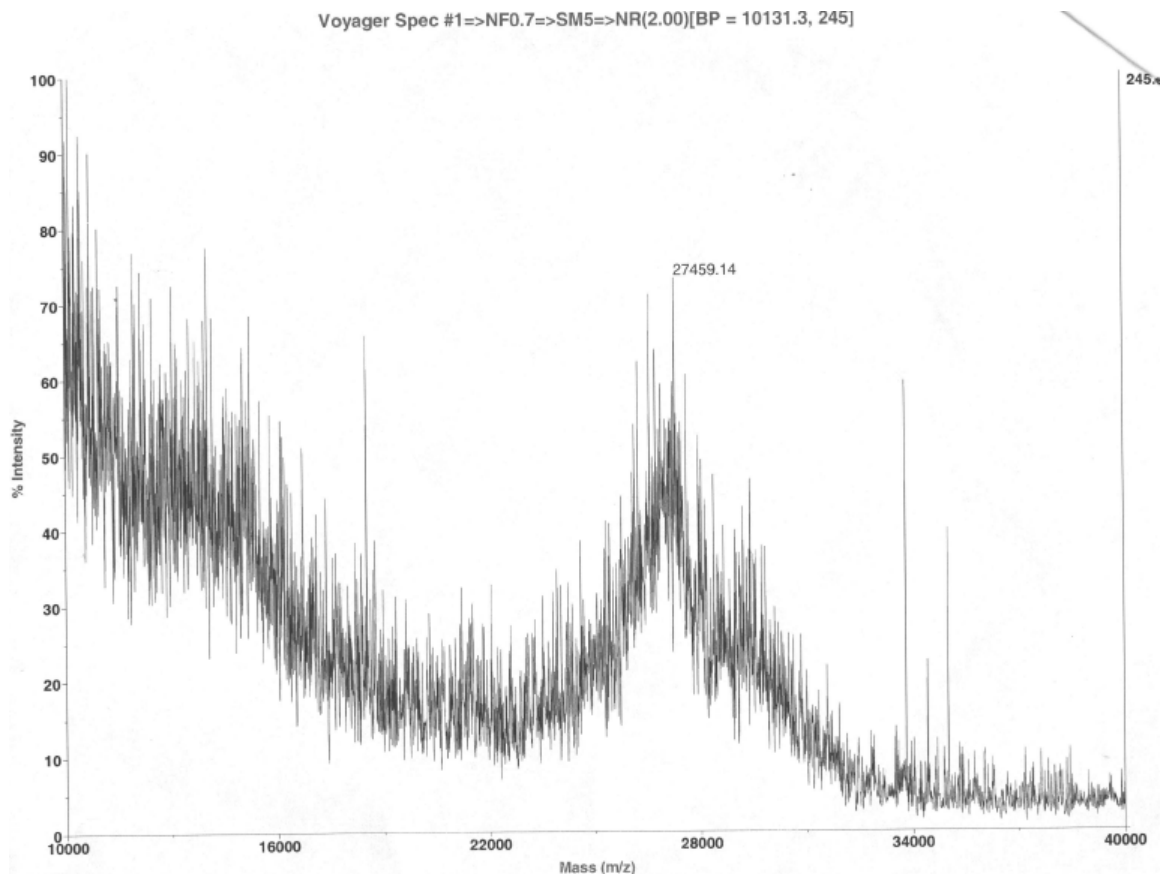


Figure S10. MALDI-TOF mass spectrum of compound **6**.

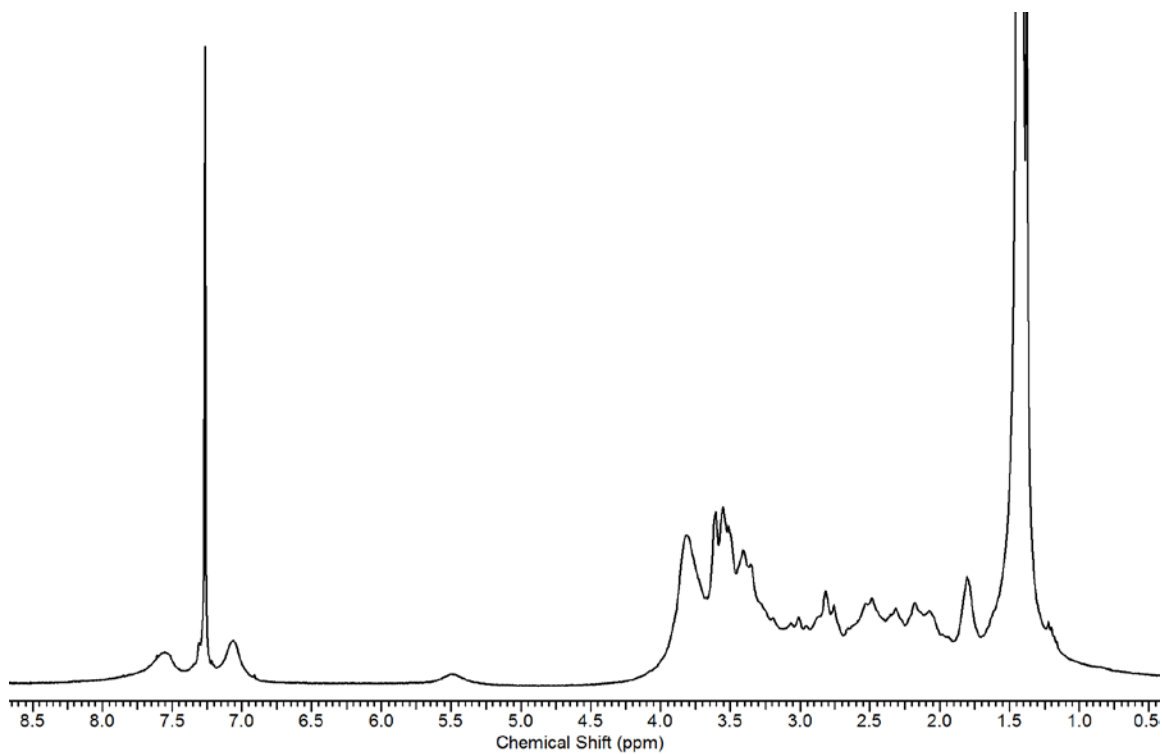


Figure S11. ^1H NMR spectrum of compound **7** (300 MHz, CDCl_3).

GS-DOTA-MCT
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300BB "mercuryplus300"

Pulse 42.2 degree
Acq. time 1.815 sec
Width 18761.7 Hz
23825 repetitions
OBSERVE C13, 75.4482158 MHz
DECOUPLE H1, 300.0525807 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 58 hr, 40 min, 36 sec

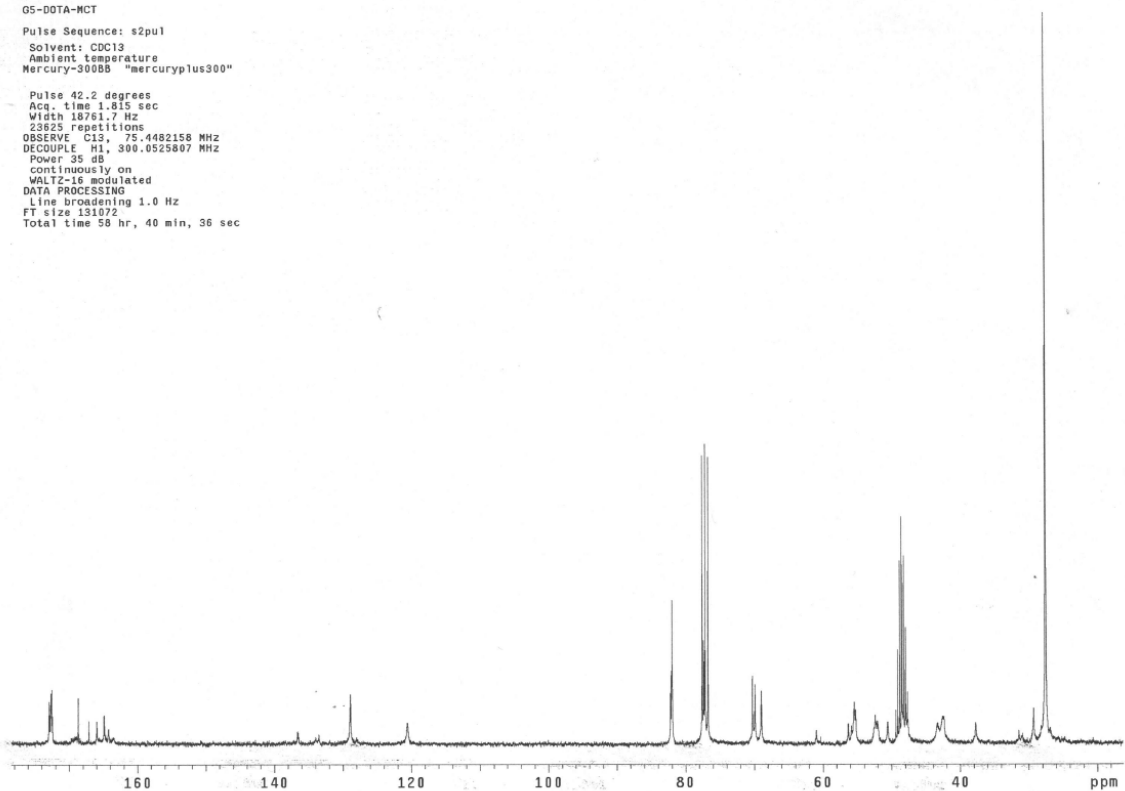


Figure S12. ^{13}C NMR spectrum of compound **7** (75 MHz, $\text{CDCl}_3/\text{MeOH-d}_4$).

DTPA-DCT
File: xp
Pulse Sequence: s2pul
Solvent: cdcl3
Ambient temperature
Operator: jdlm
Mercury-30000 "mercury300"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.938 sec
Width 4500.5 Hz
18 repetitions
OBSERVE H1, 299.9142791 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 50 sec

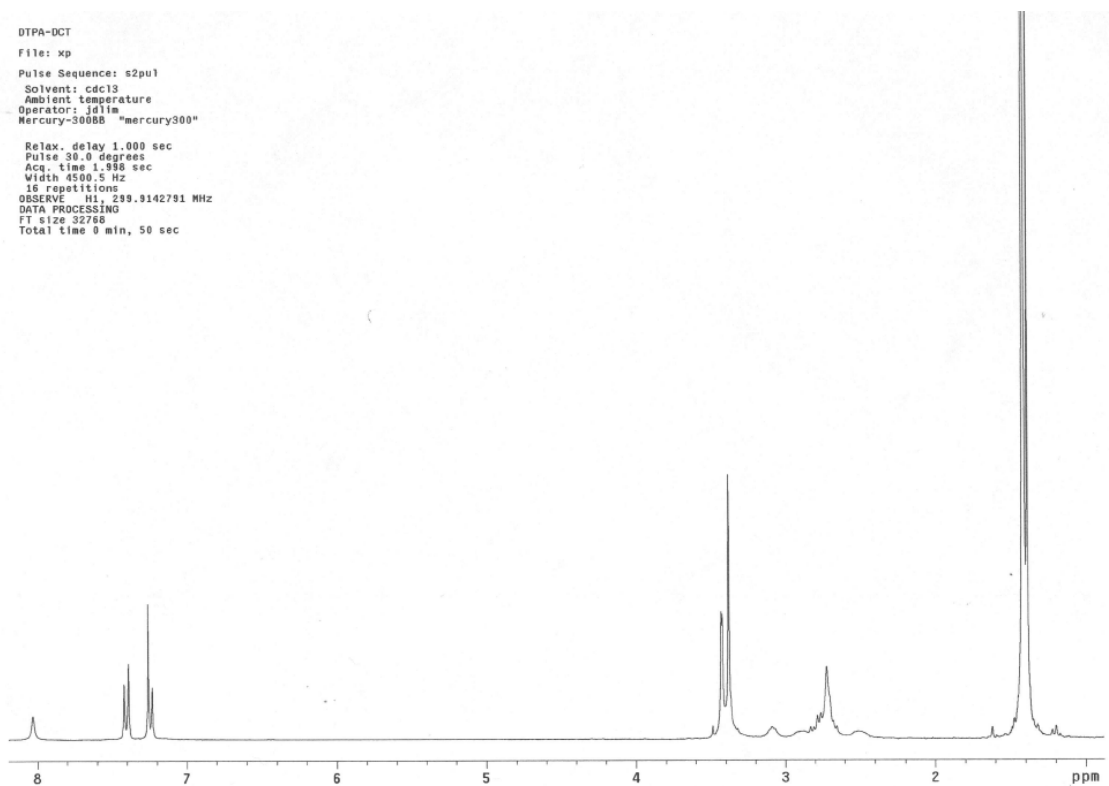


Figure S13. ^1H NMR spectrum of compound **8** (300 MHz, CDCl_3).

DTPA-DCT
File: xp
Pulse Sequence: s2pu1
Solvent: cdcl3
Ambient temperature
Operator: jdlie
Mercury-30000 "mercury300"

Relax. delay 0.901 sec
Pulse 00.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1153 repetitions
OBSERVE C13, 75.4134386 MHz
DECOUPLE H1, 299.8157791 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 56 hr, 41 min, 54 sec

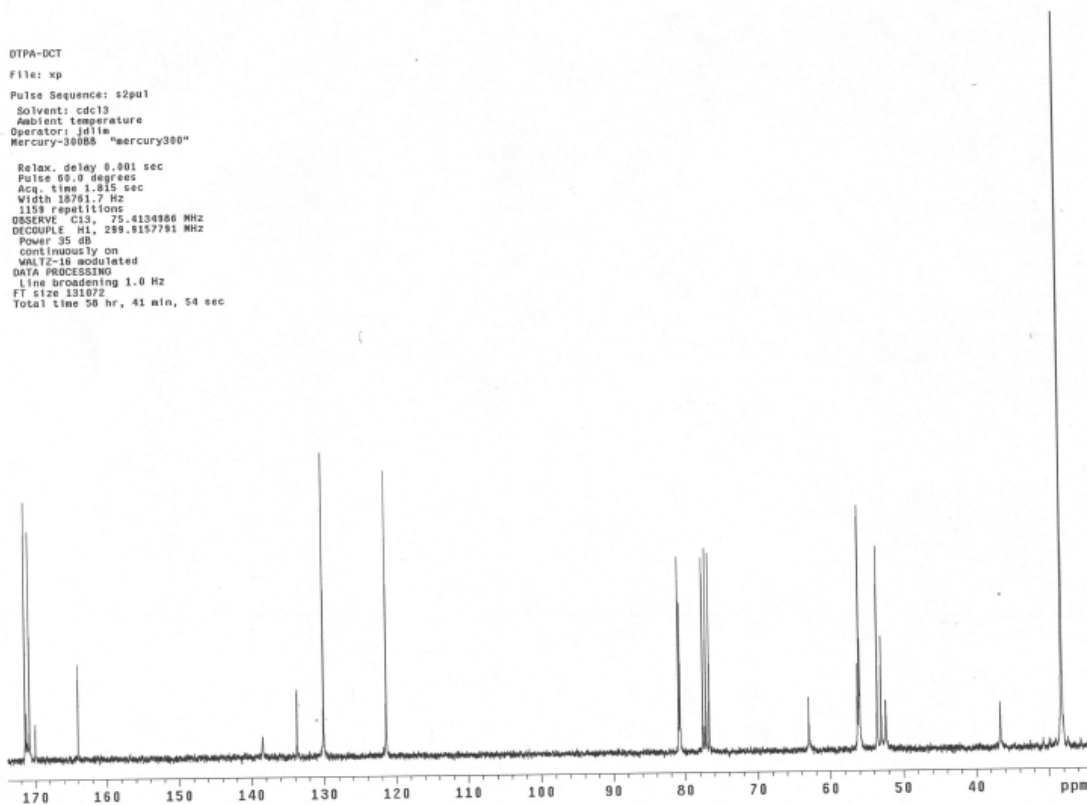


Figure S14. ^{13}C NMR spectrum of compound **8** (75 MHz, CDCl_3).

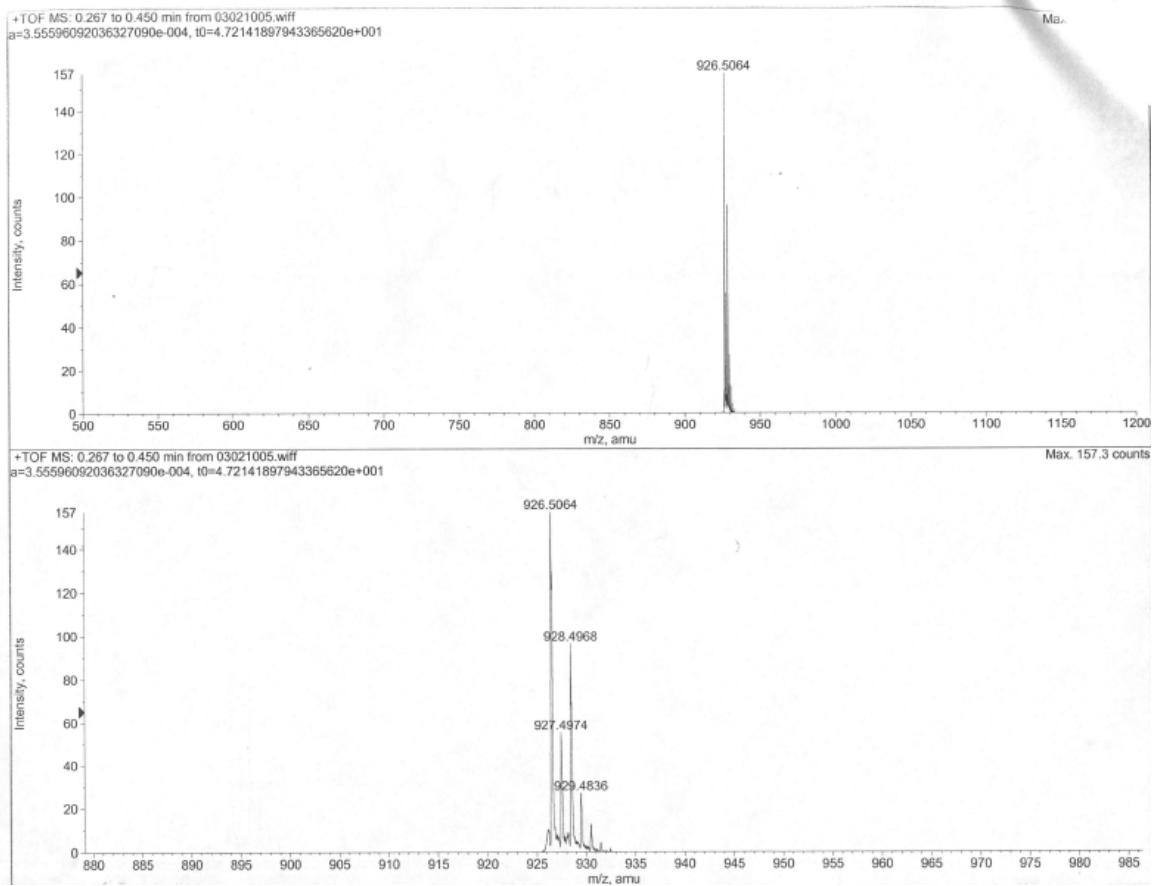


Figure S15. ESI-TOF mass spectrum of compound **8**.

G3-DTPA-MCT
File: xp
Pulse Sequence: s2pul
Solvent: cdcl3
Ambient temperature
Operator: jdlia
Mercury-3000B "mercury300"
Relax. delay 1.000 sec
Pulse 30.0 degree
Acq. time 1.916 sec
Width 4500.5 Hz
32 repetitions
OBSERVE H1, 299.9142794 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 40 sec

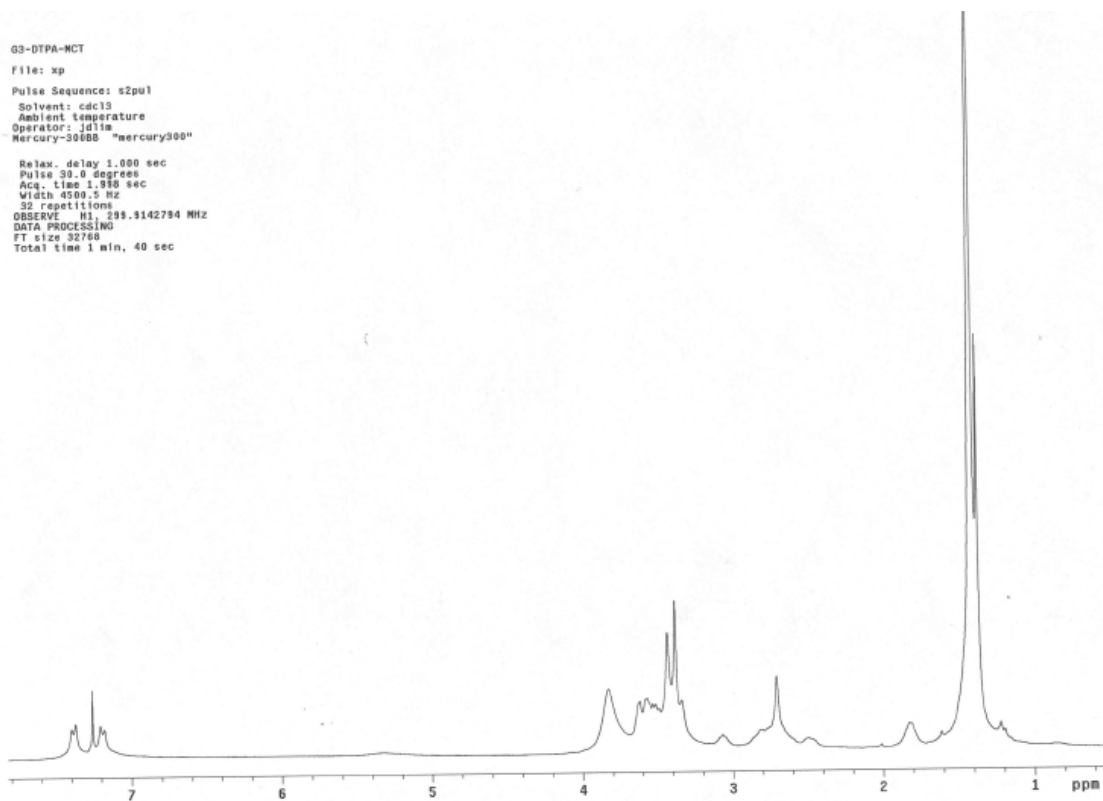


Figure S16. ¹H NMR spectrum of compound **9** (300 MHz, CDCl₃).

G3-DTPA-NCT
File: xp
Pulse Sequence: s2pu1
Solvent: cdcl3
Ambient temperature
Operator: jdlm
Mercury-300B "mercury300"
Relax. delay 0.001 sec
Pulse 60.0 degrees
Acq. time 1.815 sec
Width 18761.7 Hz
1635 repetitions
OBSERVE C13, 75.4135000 MHz
DECOUPLE H1, 298.9157791 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F1 size 131072
Total time 5 hr, 52 min, 13 sec

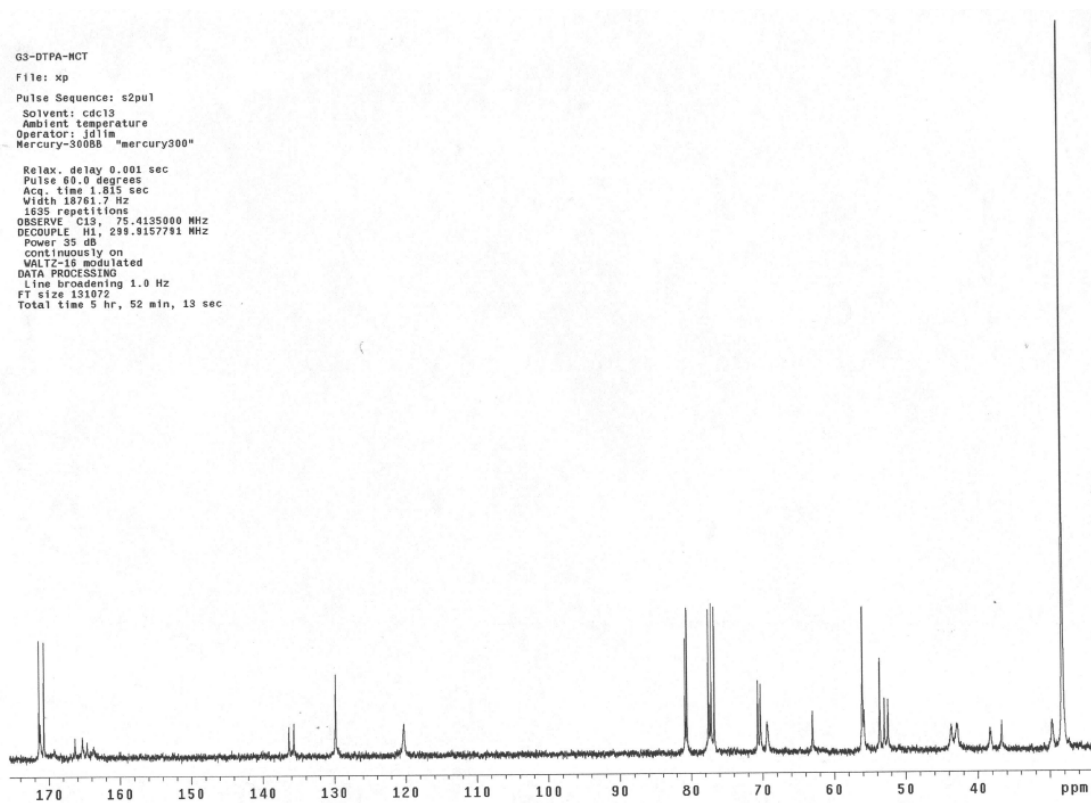


Figure S17. ^{13}C NMR spectrum of compound **9** (75 MHz, CDCl_3).

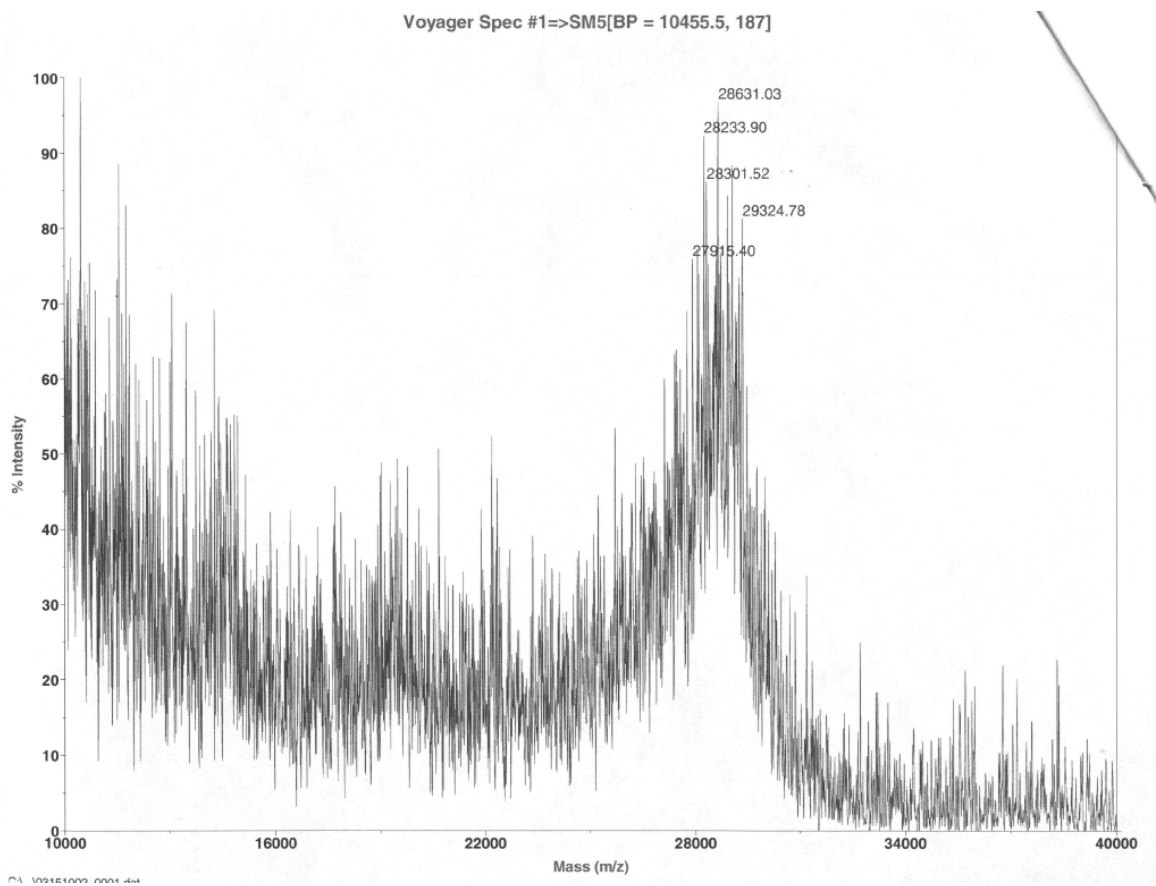


Figure S18. MALDI-TOF mass spectrum of compound **9**.

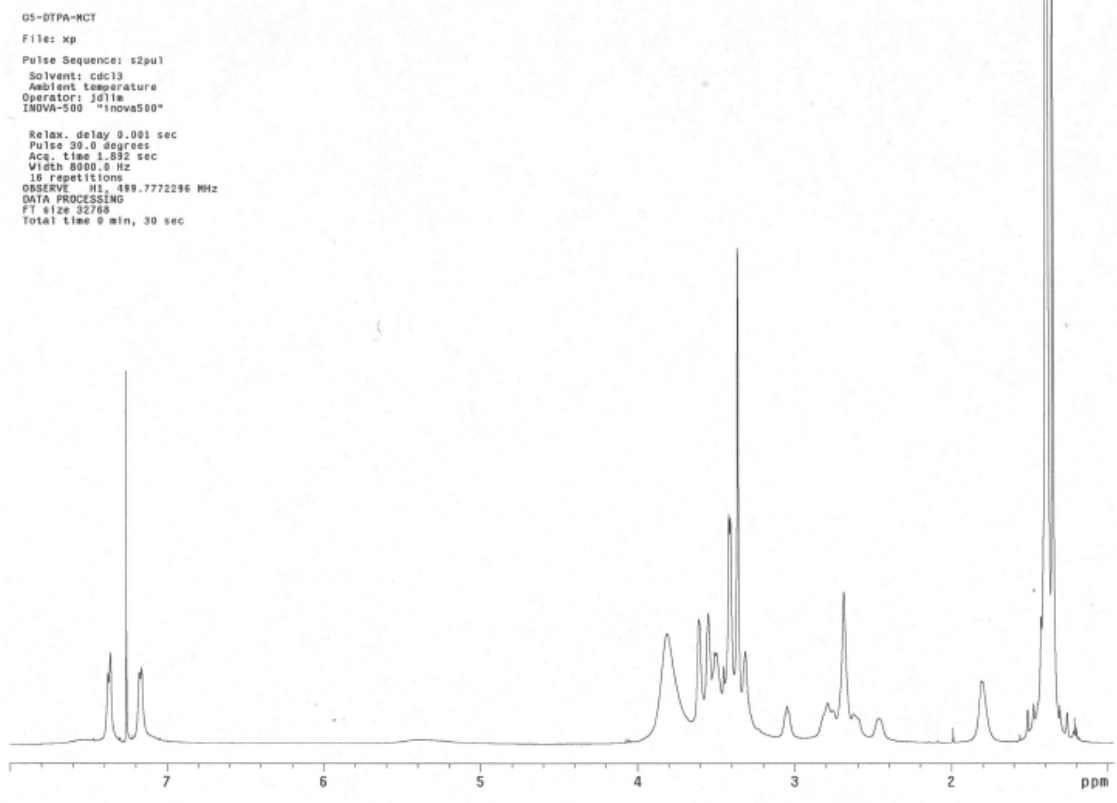


Figure S19. ^1H NMR spectrum of compound **10** (500 MHz, CDCl_3).

05-DTPA-NCT

File: xp

Pulse Sequence: s2pu1

Solvent: cdcl3

Ambient Temperature

Operator: jdl1m

INOVA-500 "inova500"

Relax. delay 0.001 sec

Pulse 60.0 degrees

Acq. time 1.300 sec

Width 25000.0 Hz

2313 repetitions

OBSERVE C13, 125.6699807 MHz

DECOUPLE H1, 499.7797227 MHz

Power 43 dB

continuously on

WALTZ-16 modulated

DATA PROCESSING

Line broadening 0.5 Hz

FT size 65536

Total time 36 hr, 23 min, 59 sec

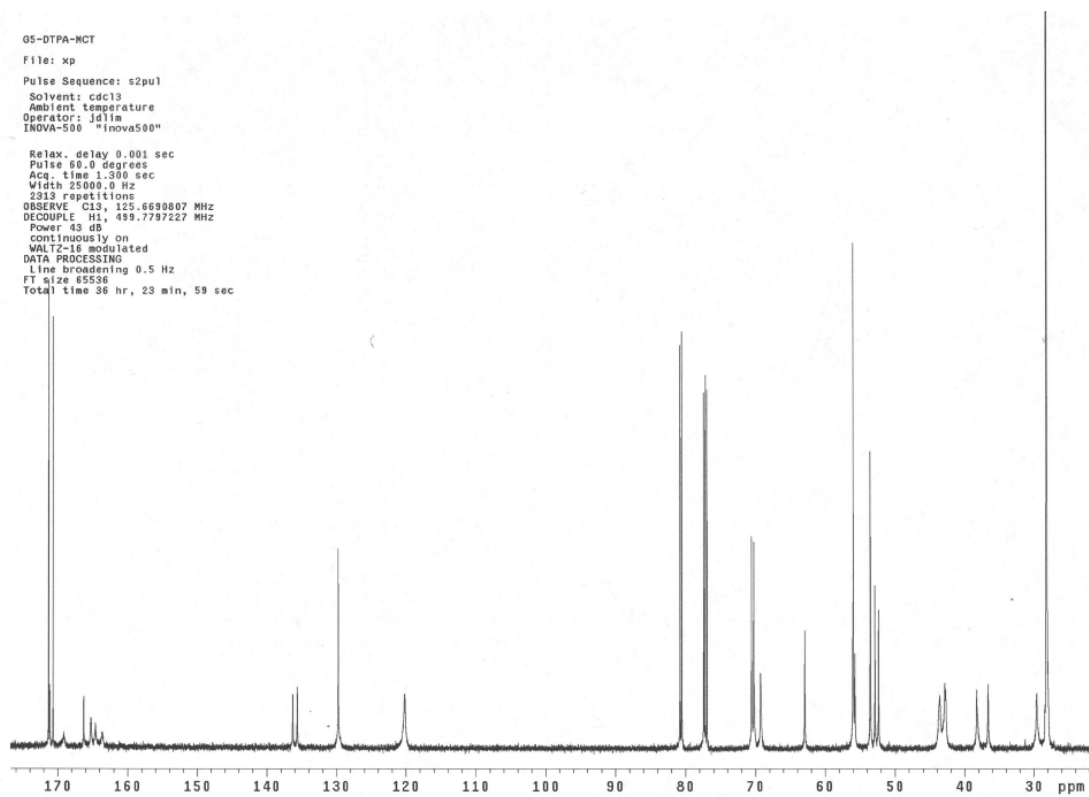


Figure S20. ^{13}C NMR spectrum of compound **10** (125 MHz, CDCl_3).

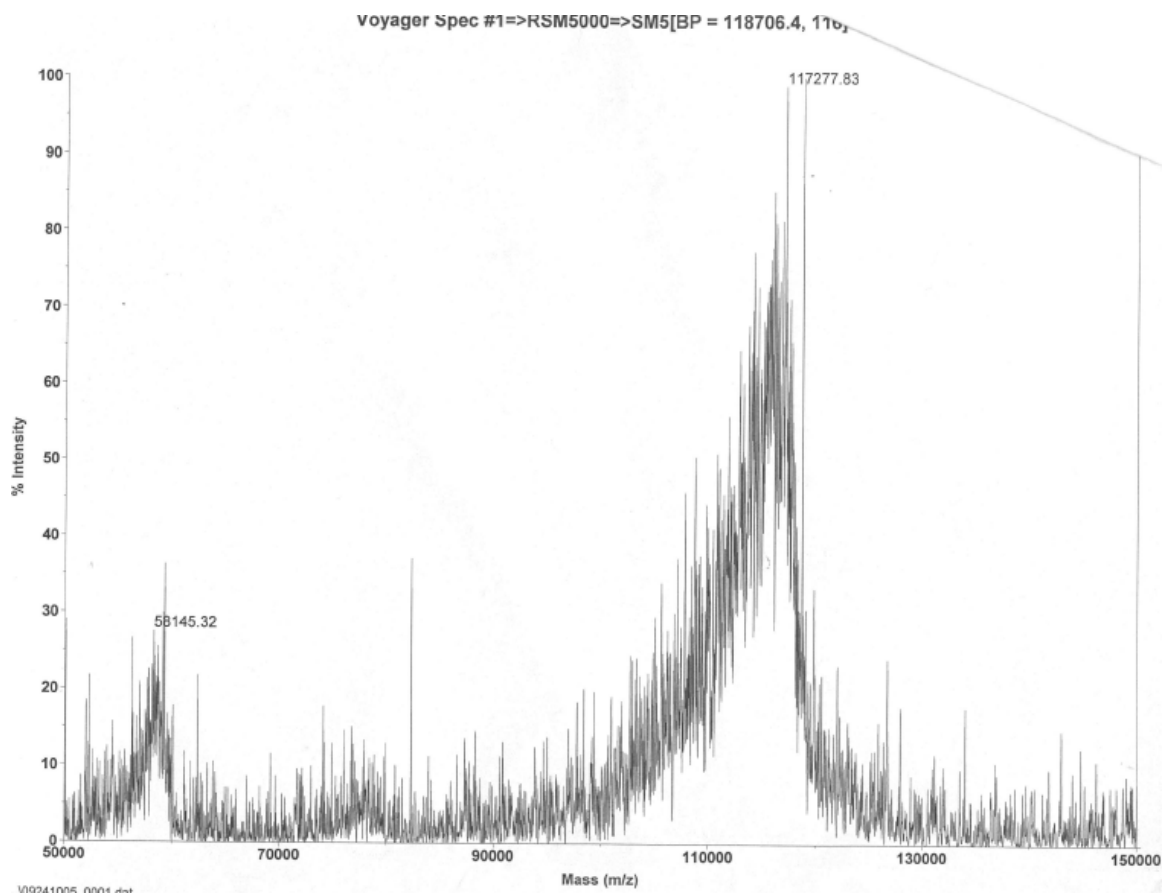
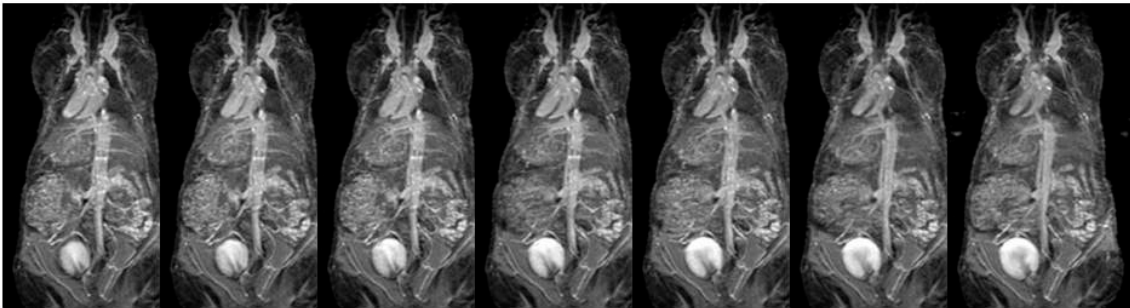
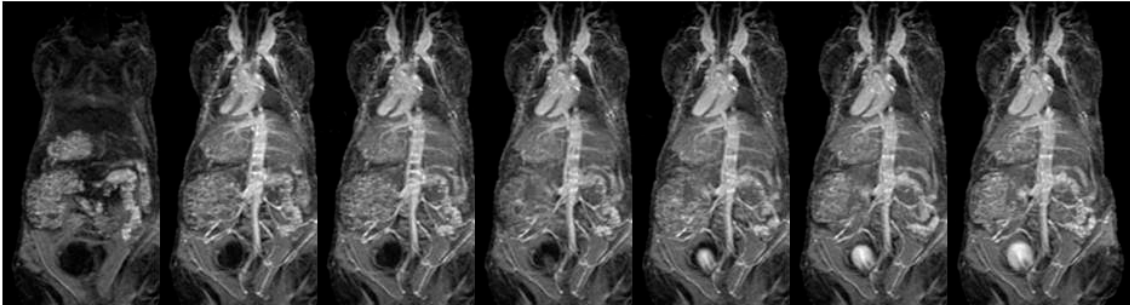


Figure S21. MALDI-TOF mass spectrum of compound **10**.

PAMAM-G5-DPTA (2min/frame)



PAMAM-G5-DOTA (2min/frame)

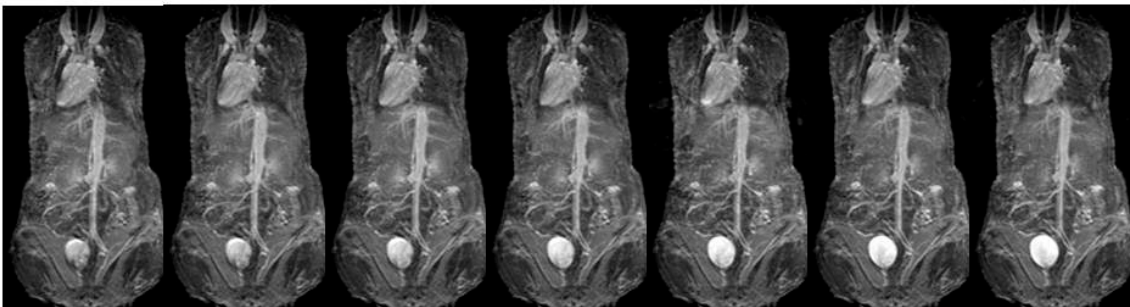
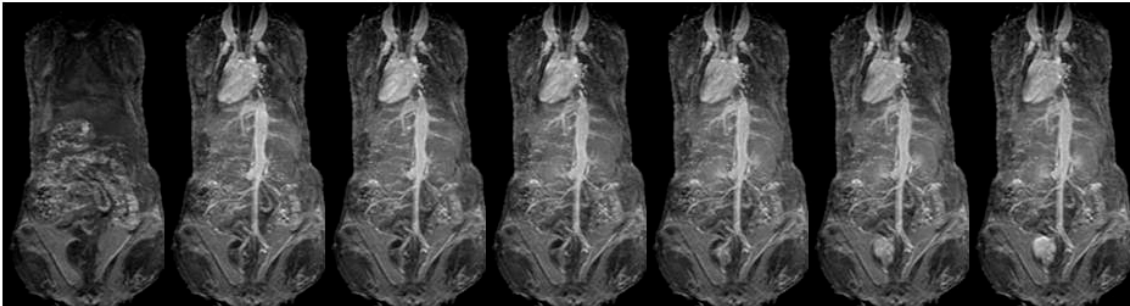


Figure S22. Dynamic MRI of mice using 0.03mmolGd/kg of PAMAM-G5-DPTA and PAMAM-G5-DOTA. No clear difference on relaxivity is shown between DPTA and DOTA conjugates.