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DO2A-Based Ligands for Gallium-68 Chelation: Synthesis, Radiochemistry and *Ex Vivo* Cardiac Uptake

Supporting Information

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Contents

NMR Spectra

Bis-triphenyl(4-((4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,7-diyl)methyl)benzyl)phosphonium dibromide (**2a**)

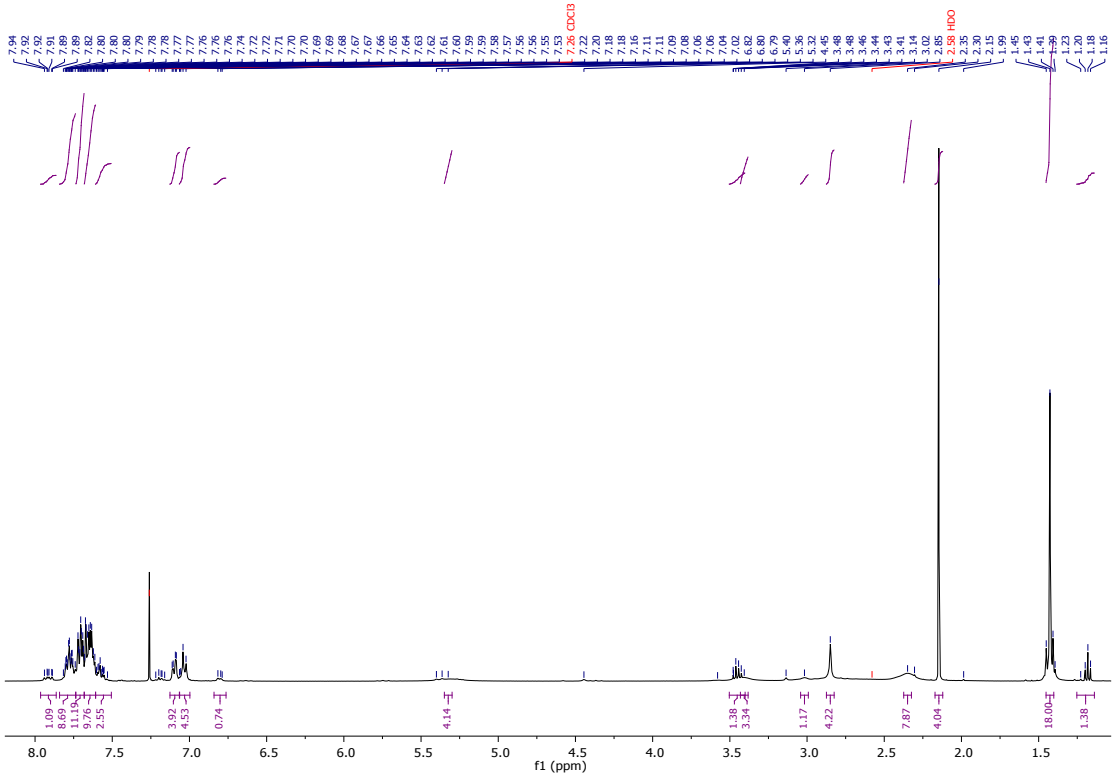


Figure S1: ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, 298 K)

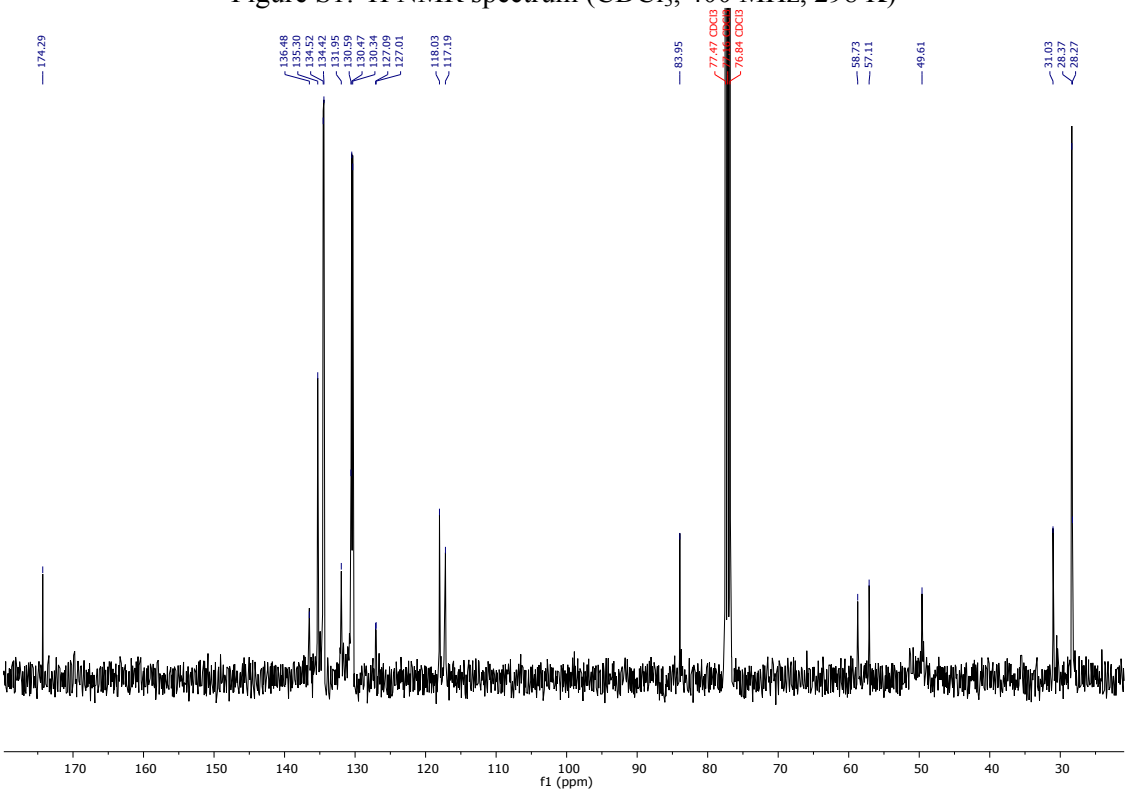


Figure S2: $^{13}C\{^{1}H\}$ NMR spectrum ($CDCl_3$, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

Bis-triphenyl(4-((4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,7-diyl)methyl)4-methylphenyl)phosphonium dibromide (**2b**)

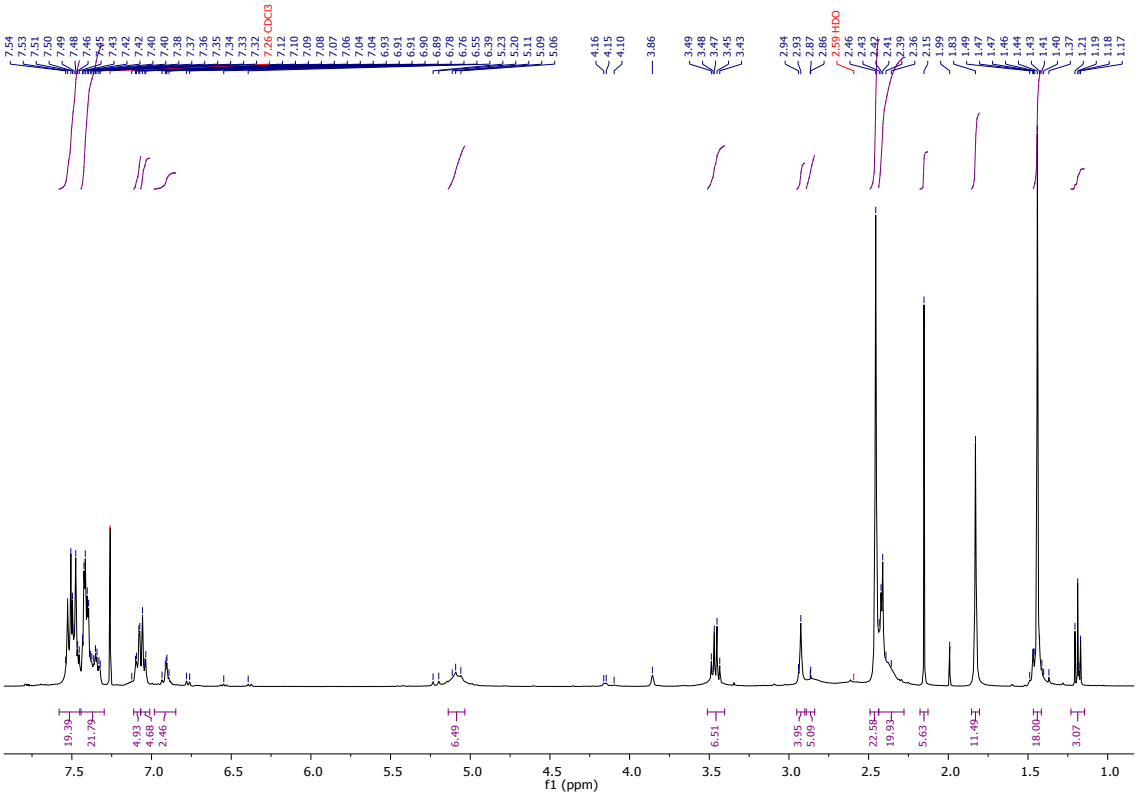


Figure S3: ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, 298 K)

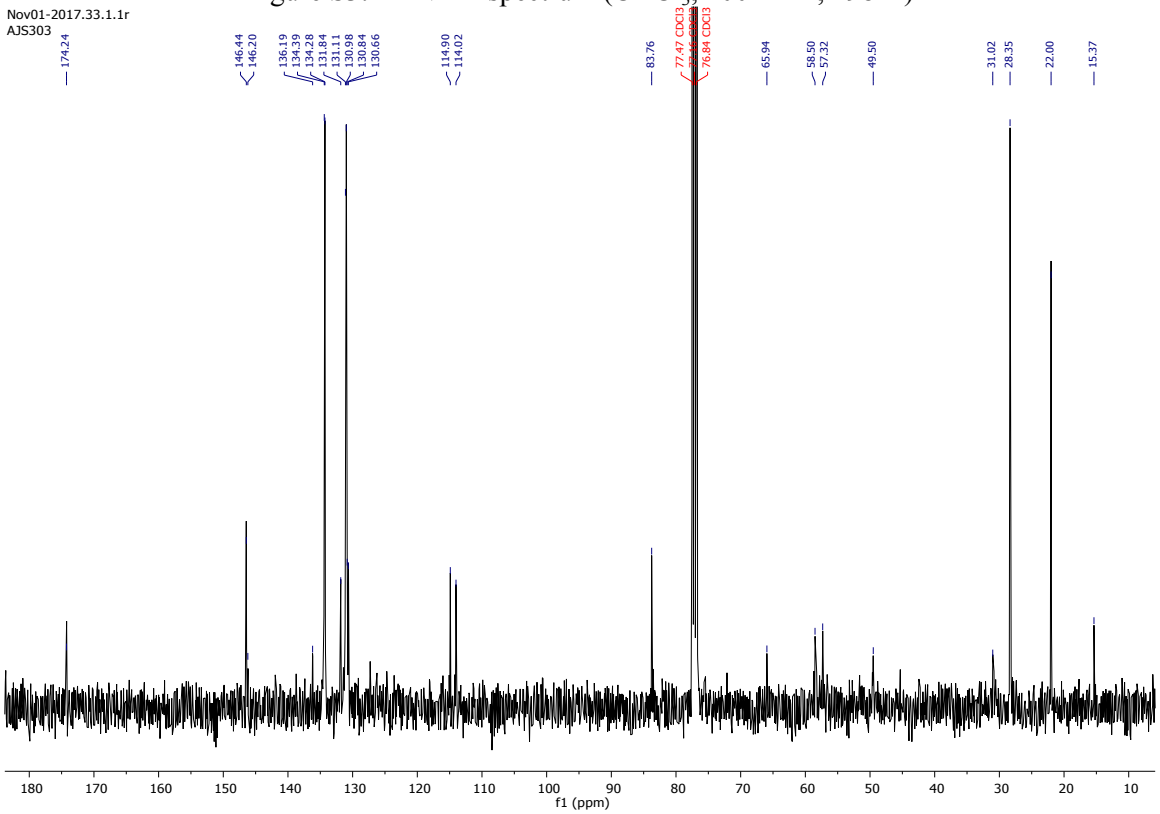


Figure S4: ^{13}C{^{1}H} NMR spectrum ($CDCl_3$, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

Bis-triphenyl(4-((4,10-bis(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1,7-diyl)methyl)3,5-dimethylphenyl)phosphonium dibromide (**2c**)

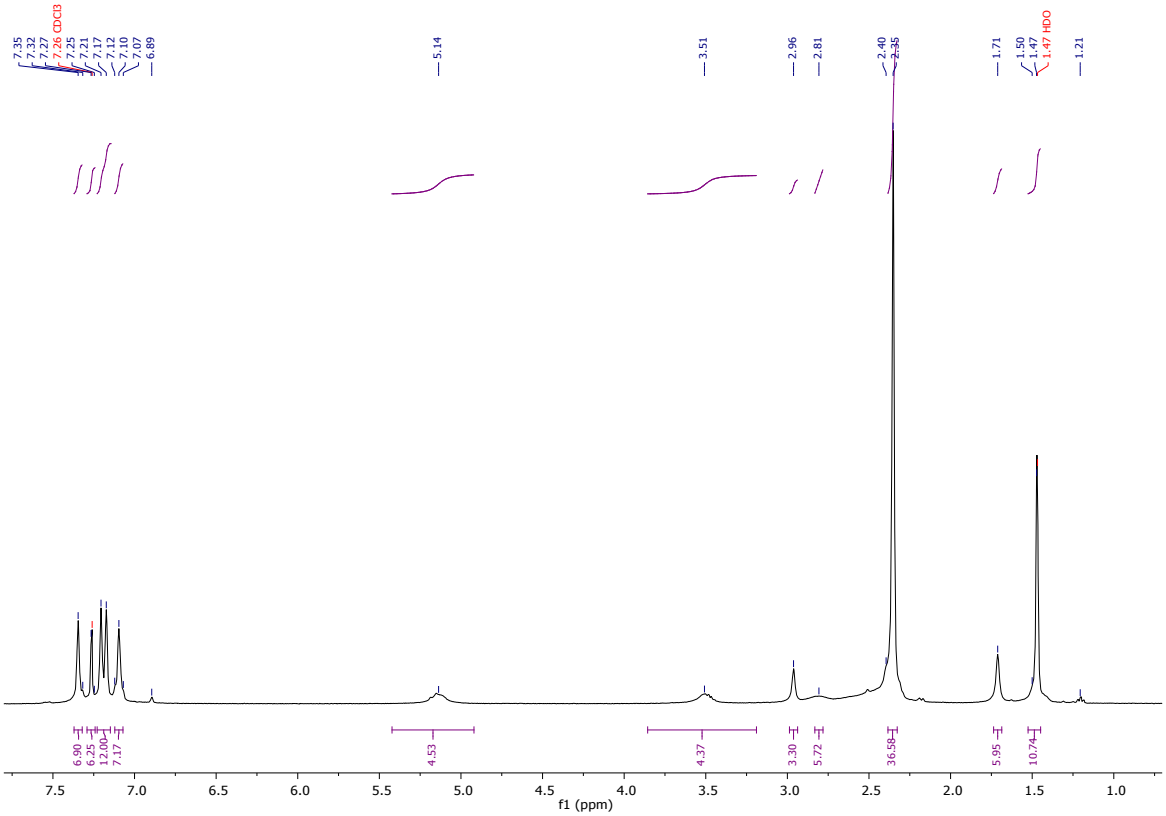


Figure S5: ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, 298 K)

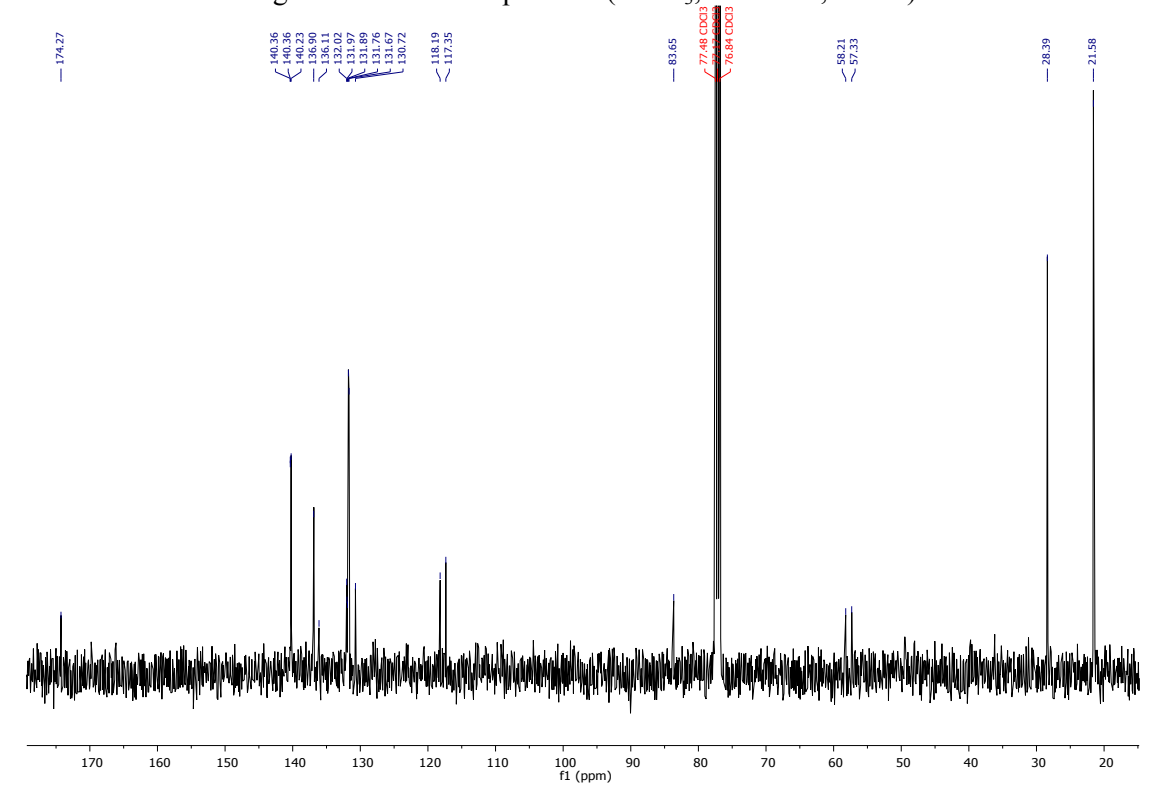


Figure S6: $^{13}C\{^{1}H\}$ NMR spectrum ($CDCl_3$, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

DO2A-(xy-TPP)$_2$ Bistrifluoroacetate (**3a**)

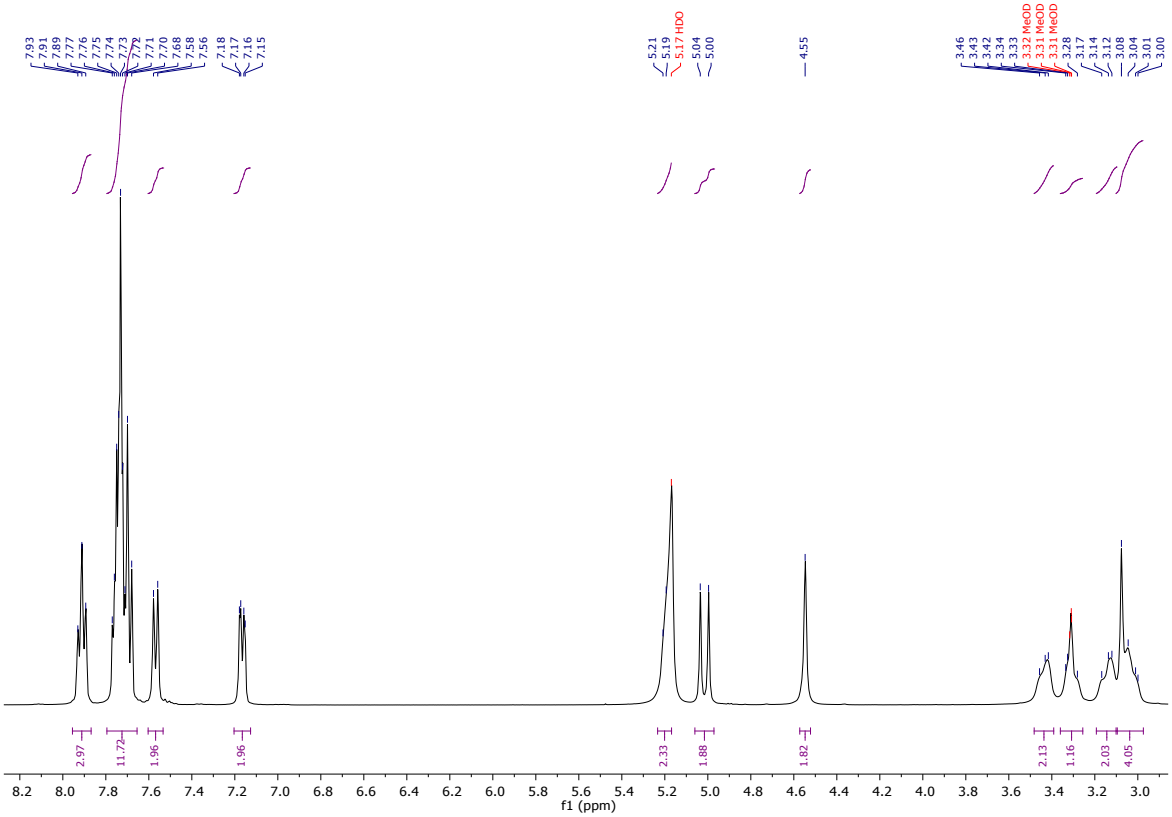


Figure S7: ^{1}H NMR spectrum (MeOD, 400 MHz, 298 K)

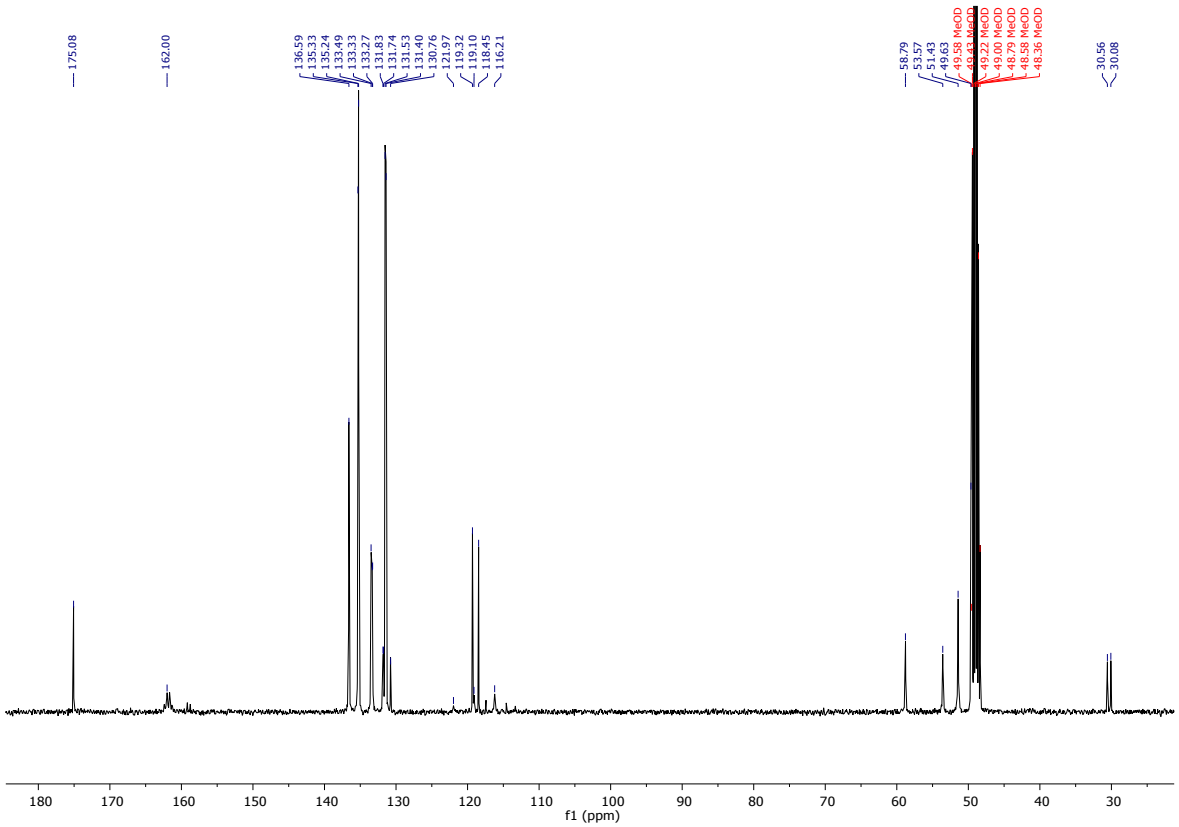


Figure S8: ^{13}C{^{1}H} NMR spectrum (MeOD, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

DO2A-(xy-TTP)$_2$ Bistrifluoroacetate (**3b**)

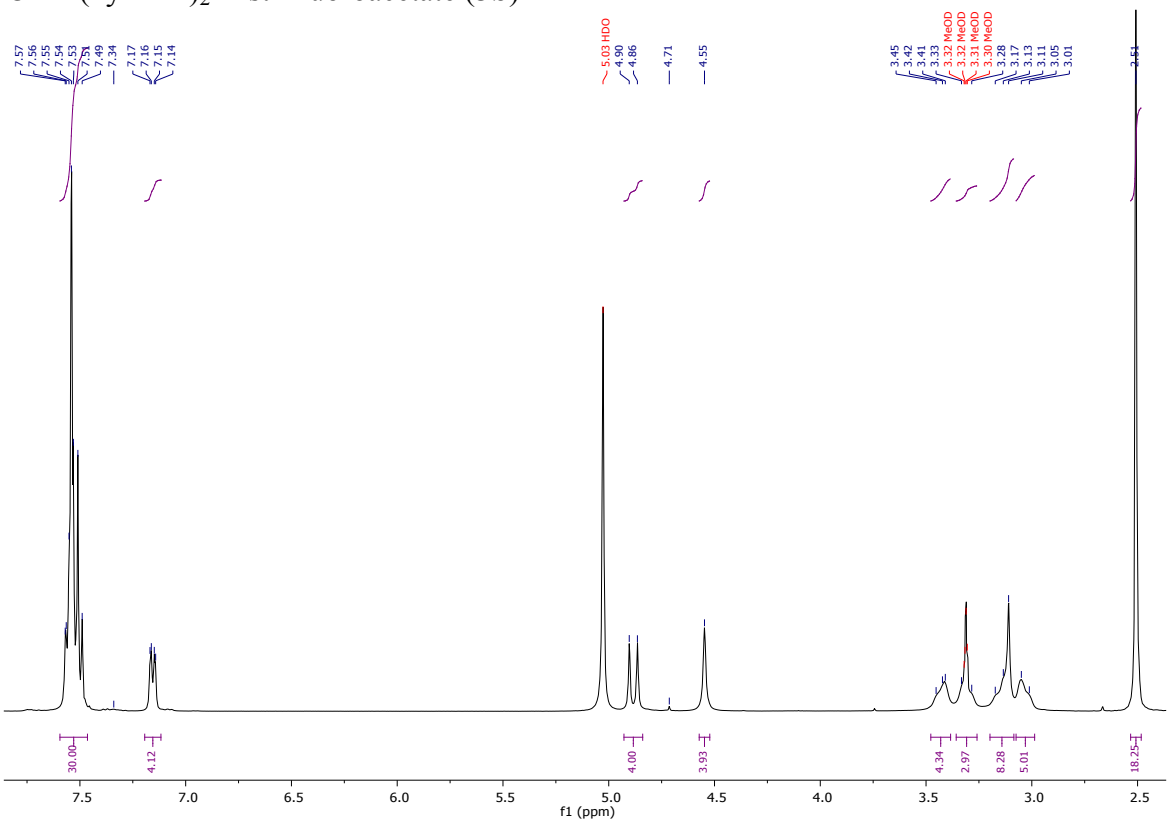


Figure S9: ^{1}H NMR spectrum (MeOD, 400 MHz, 298 K)

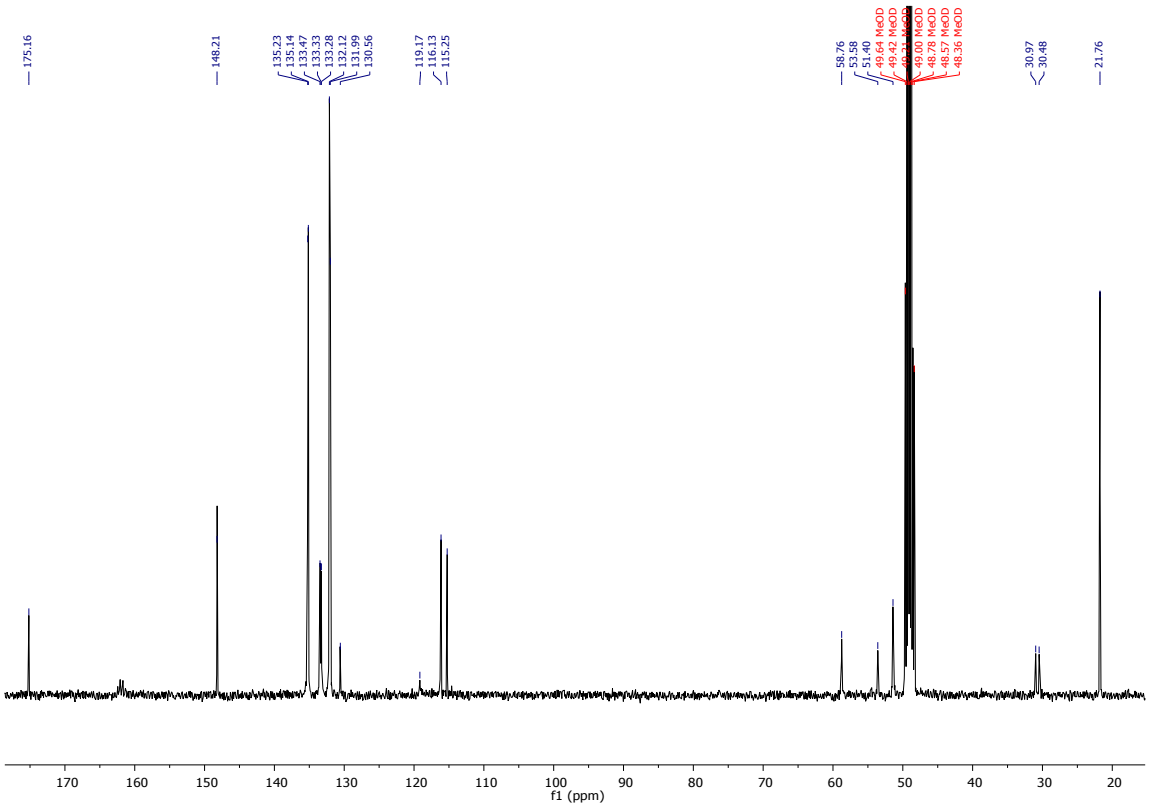


Figure S10: ^{13}C{^{1}H} NMR spectrum (MeOD, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

DO2A-(xy-TXP)$_2$ Bistrifluoroacetate (**3c**)

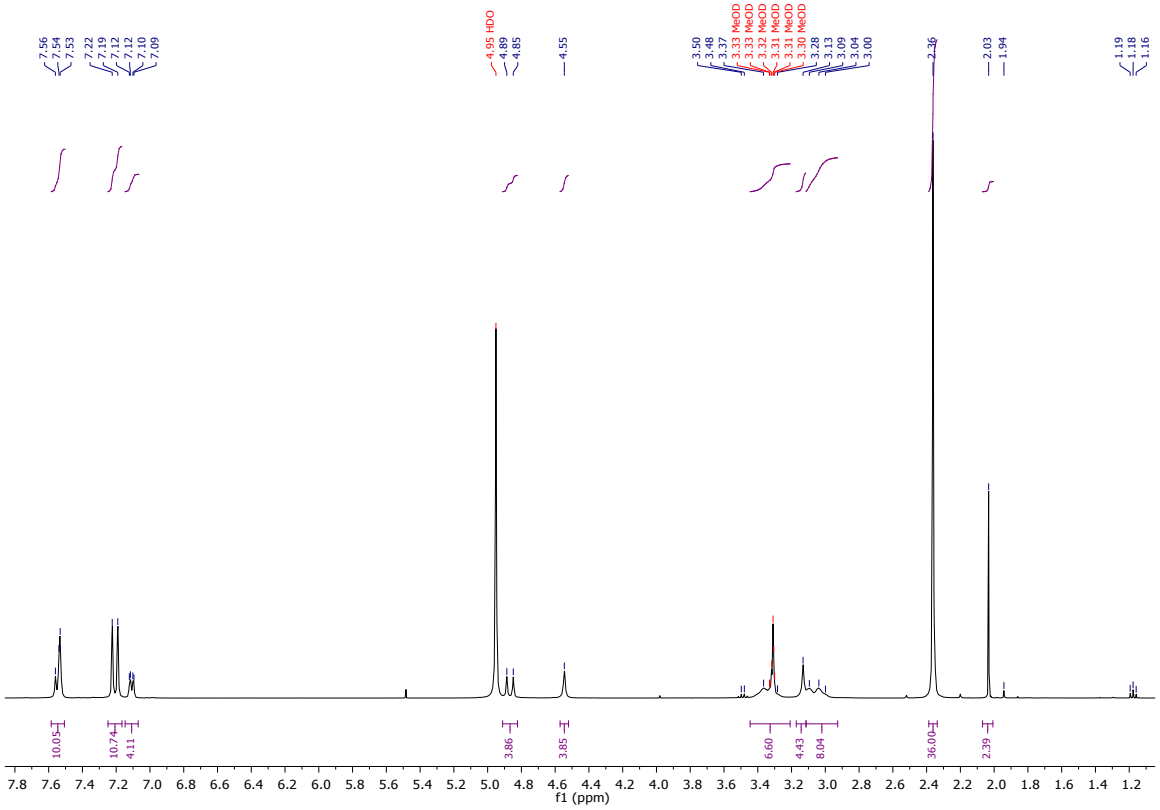


Figure S11: ^{1}H NMR spectrum (MeOD, 400 MHz, 298 K)

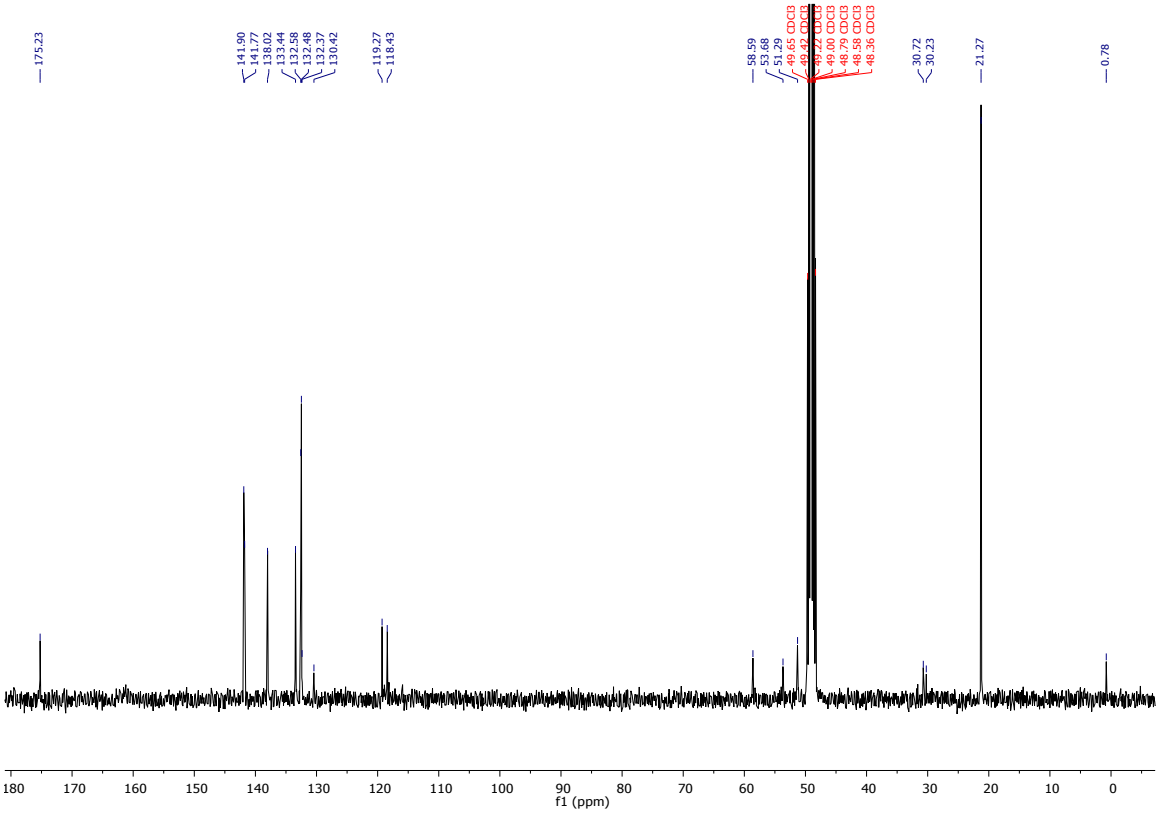


Figure S12: ^{13}C{^{1}H} NMR spectrum (MeOD, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

Di-*tert*-butyl 2,2′-(4,10-dibenzyl-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (**5a**)

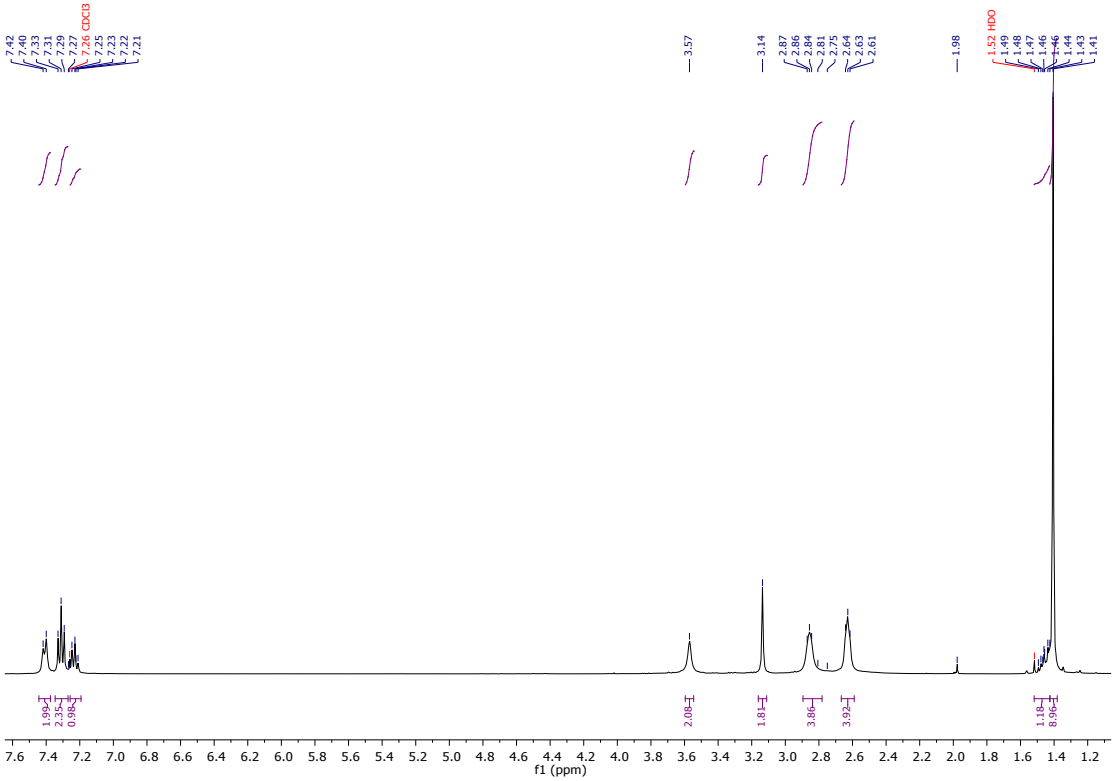


Figure S13: ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, 298 K)

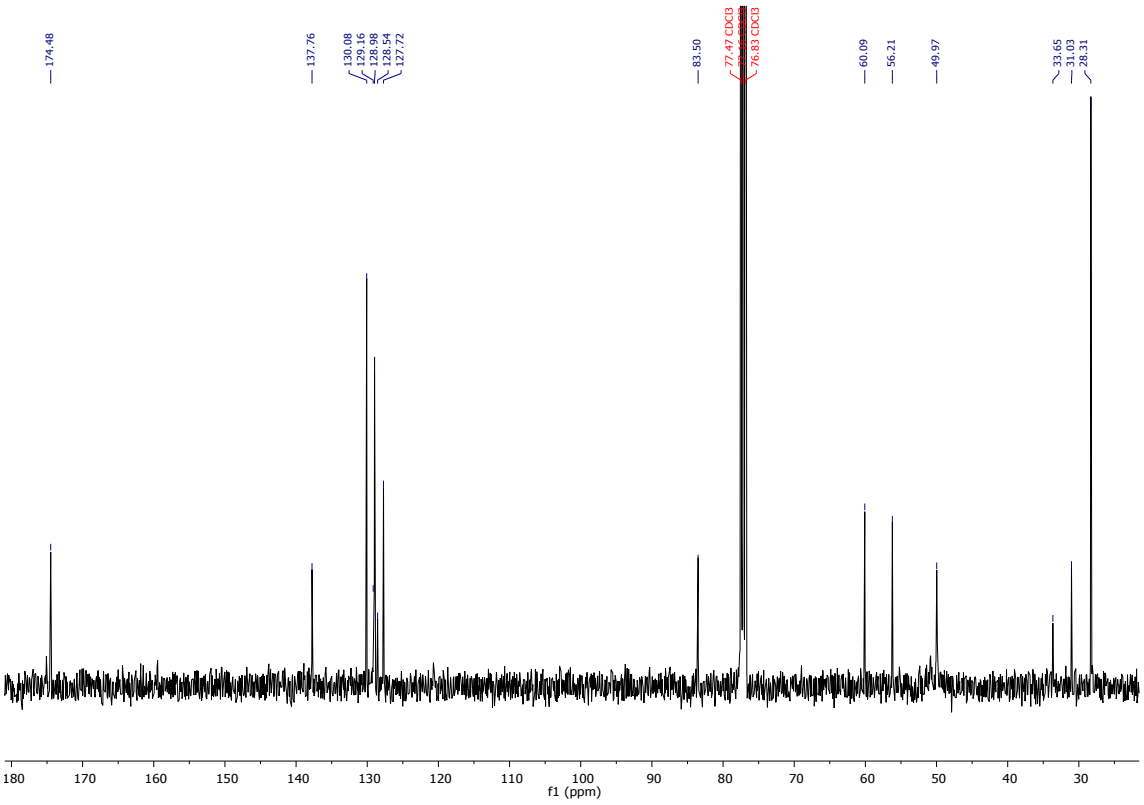


Figure S14: ^{13}C{^{1}H} NMR spectrum ($CDCl_3$, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

DO2A-Bn_2 (**6a**)

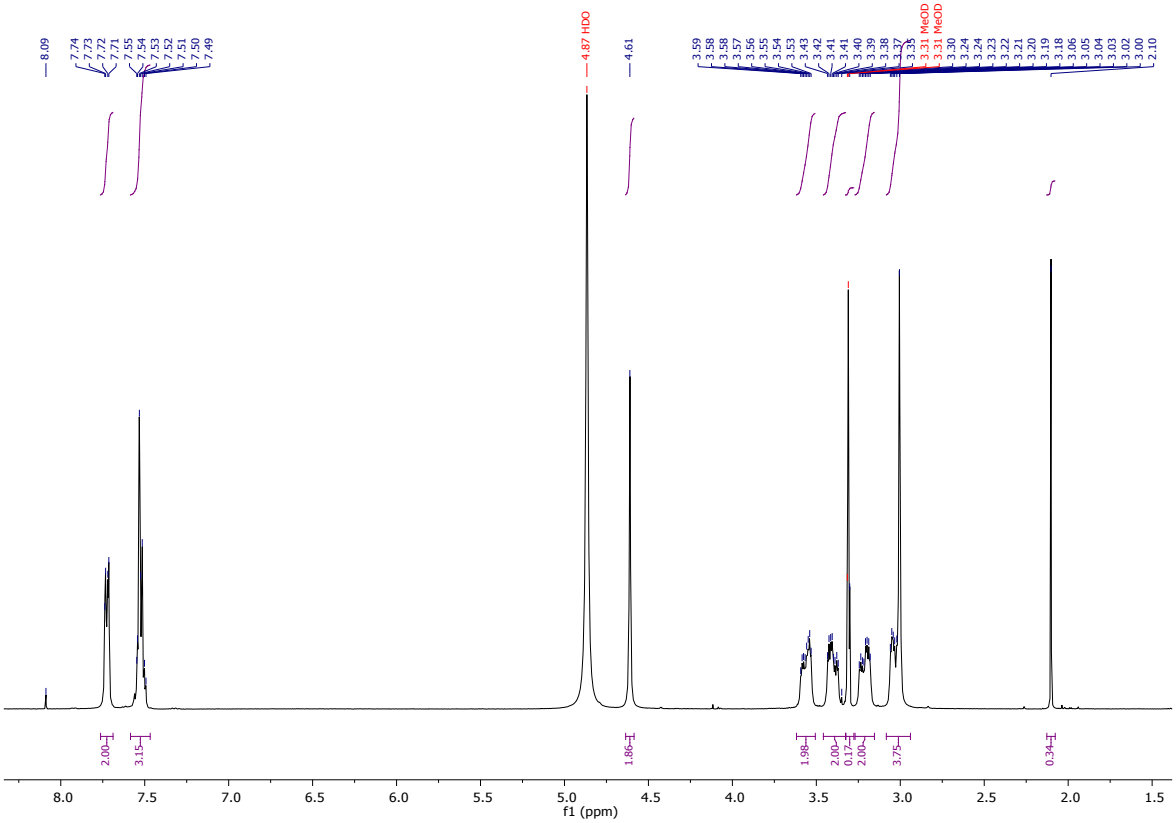


Figure S15: ^{1}H NMR spectrum (MeOD, 400 MHz, 298 K)

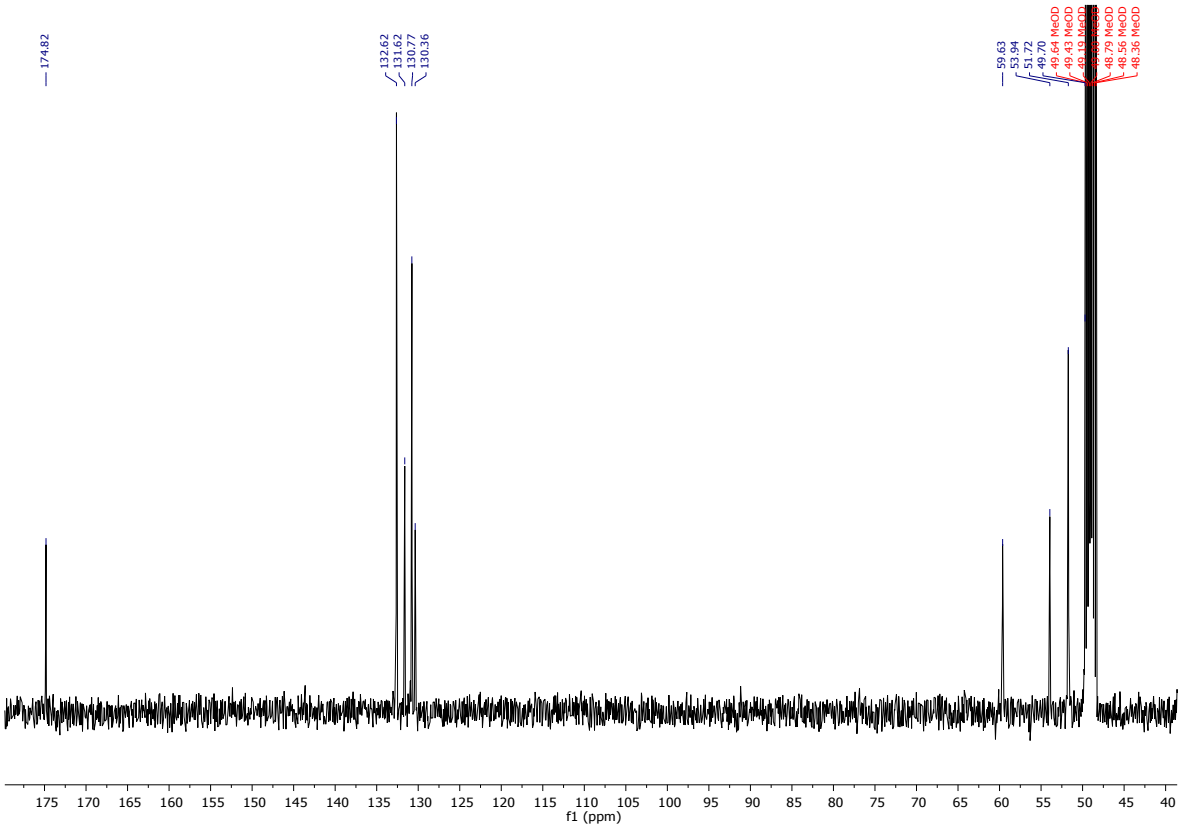


Figure S16: $^{13}C\{^{1}H\}$ NMR spectrum (MeOD, 100 MHz, 298 K). The residual solvent peak has been truncated for clarity.

DO2A-Xy_2 (**6b**)

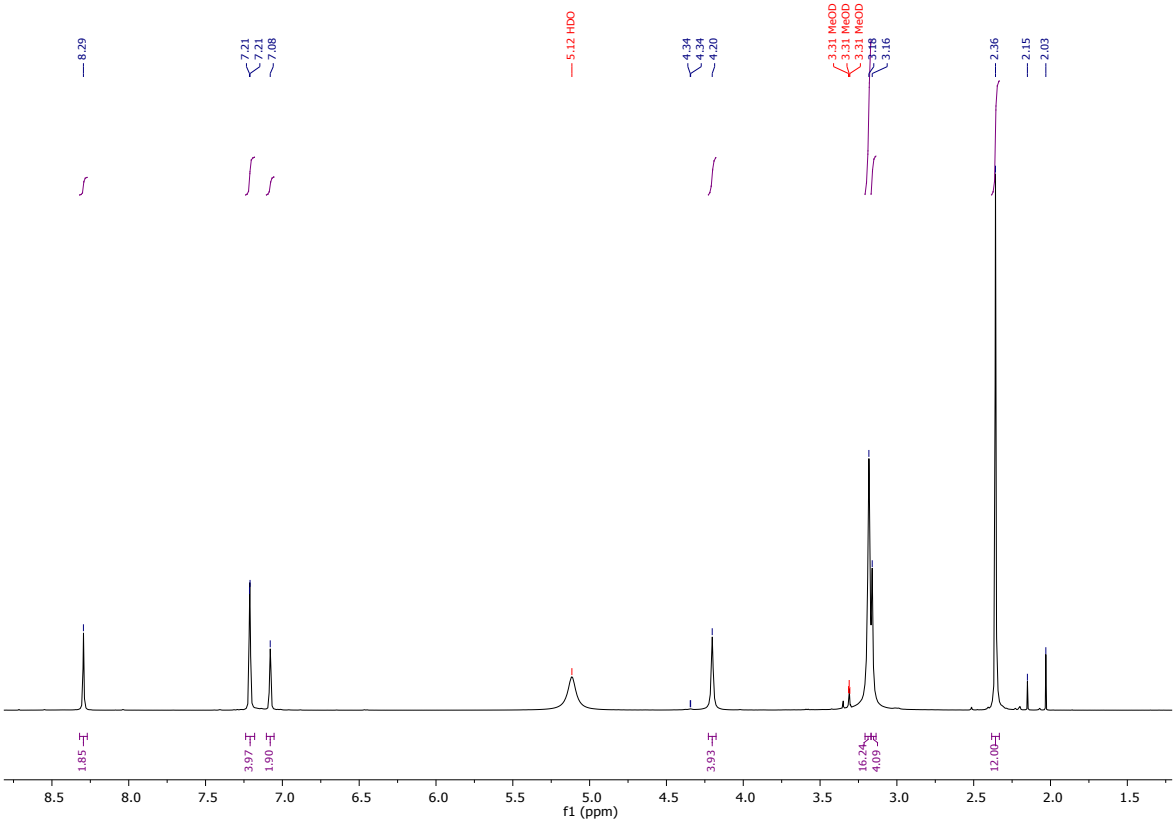


Figure S17: ^{1}H NMR spectrum (MeOD, 400 MHz, 298 K)

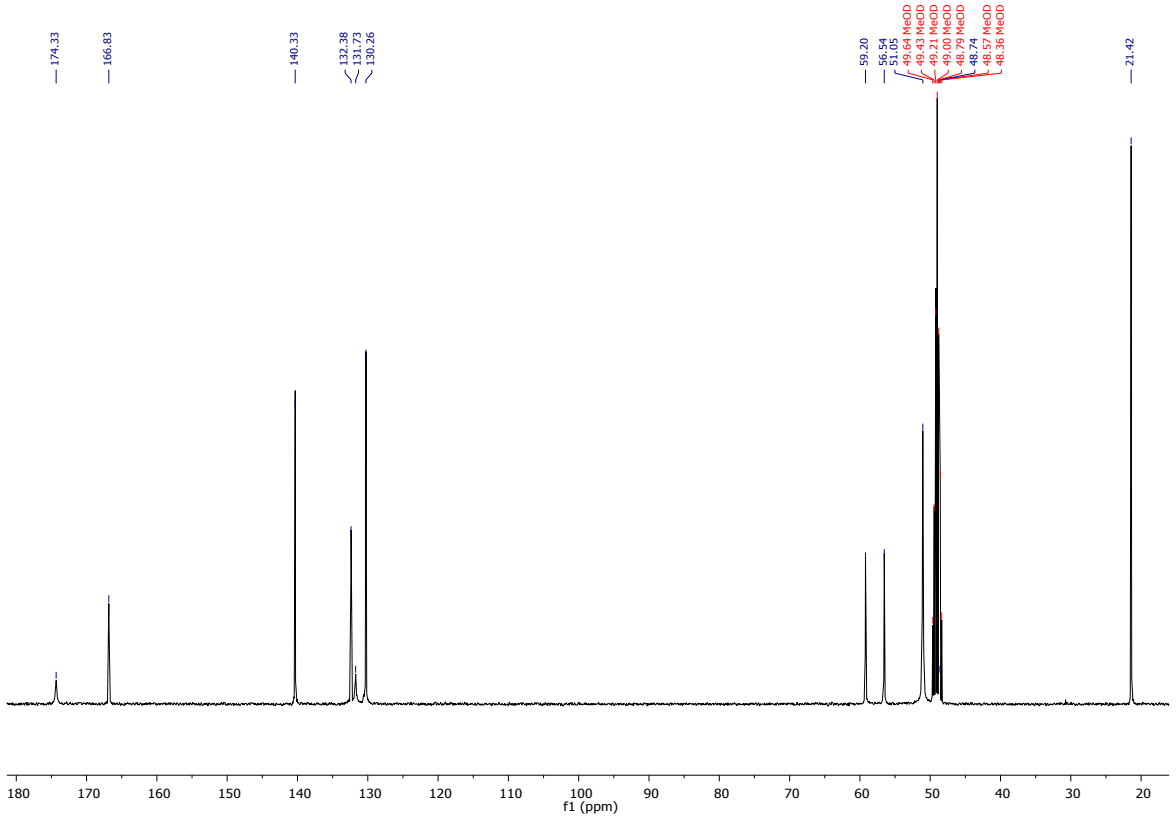


Figure S18: $^{13}C\{^{1}H\}$ NMR spectrum (MeOD, 100 MHz, 298 K)

RadioHPLC Analysis

[^{68}Ga]Ga3a

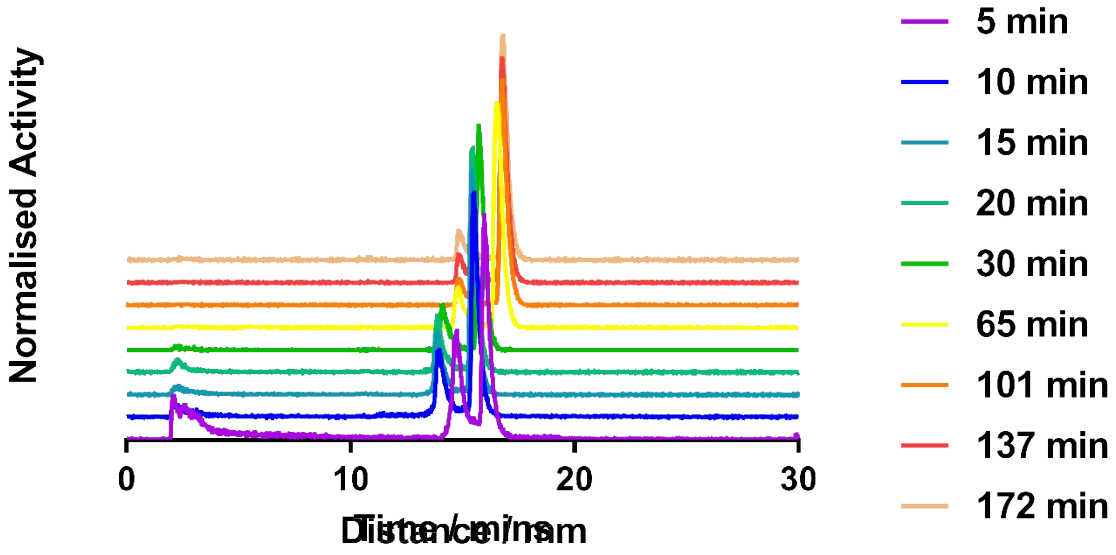


Figure S19: RadioHPLC traces of the reaction time alteration experiments on **[^{68}Ga]Ga3a**. Reaction conditions: 100 °C, 0.2 M NaOAc. Eluent gradient: 100 % A for 5 min, 0-100 % B in A for 20 min, 100 % B for 5 min; flow rate 1 mL min^{-1}. Traces offset for clarity.

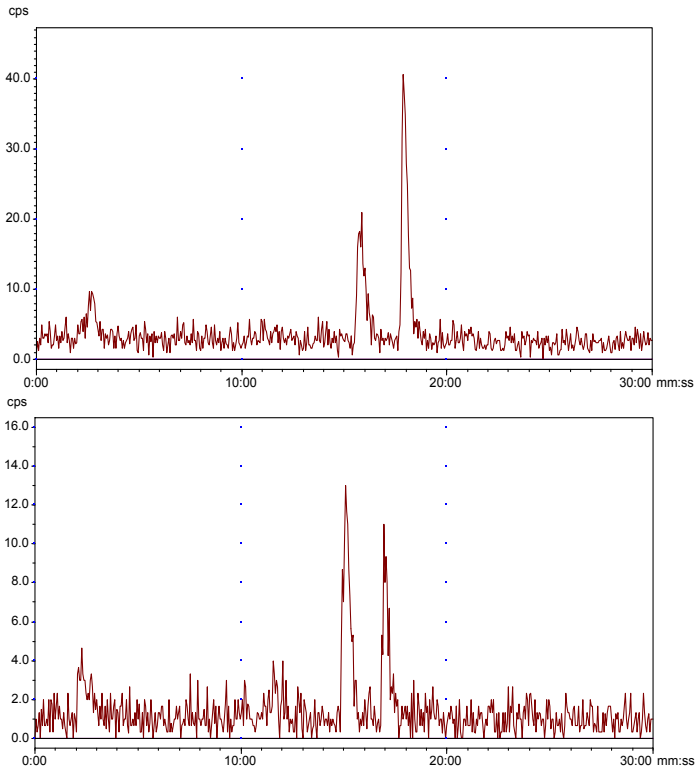


Figure S20: RadioHPLC traces of the isolated kinetic peak experiments on **[^{68}Ga]Ga3a**. Reaction conditions: (Top) 65 min, 100°C, 0.2 M NaOAc; (Bottom) 100 min, 25°C, 0.2 M NaOAc. Eluent gradient as described for Figure S19.

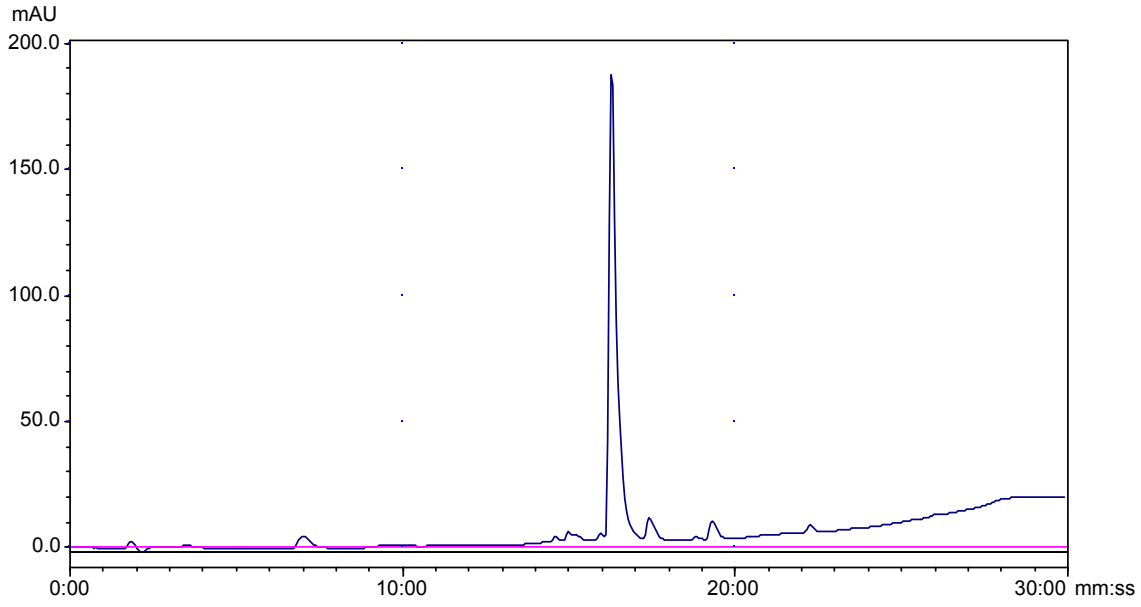


Figure S21: Radio HPLC of the isolated thermodynamic peak of **[^{68}Ga]Ga3a**.
Reaction conditions: 65 min, 100°C, 0.2 M NaOAc. Eluent gradient as described for Figure S19.

Figure S22: RadioHPLC traces of the reaction temperature alteration experiments on **[^{68}Ga]Ga3a**.
Reaction conditions: 30 min, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.

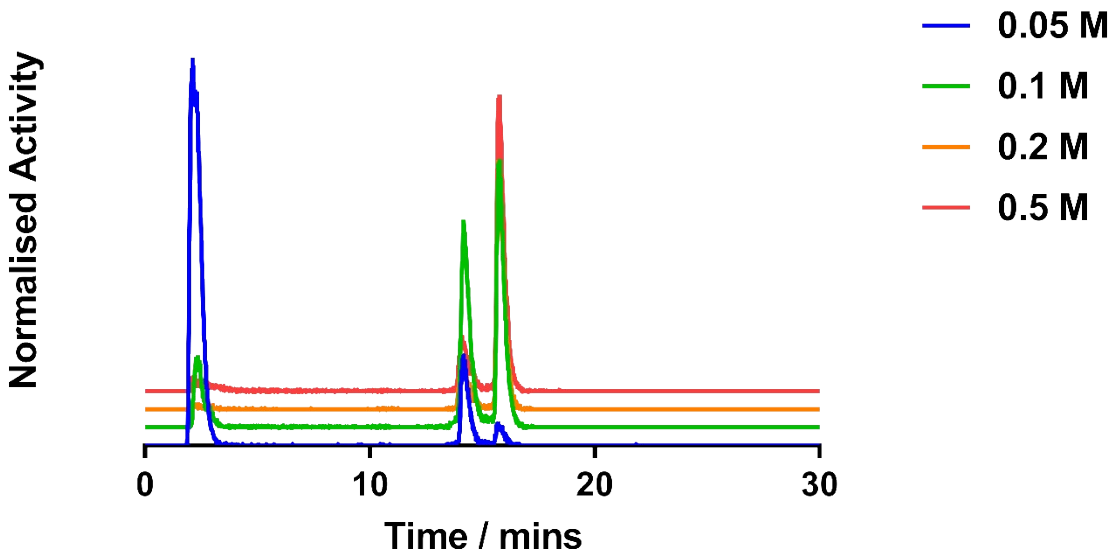


Figure S23: RadioHPLC traces of the sodium acetate concentration alteration experiments on **[^{68}Ga]Ga3a**. Reaction conditions: 100 °C, 30 mins. Eluent gradient as described for Figure S19. Traces offset for clarity.

[^{68}Ga]Ga3b

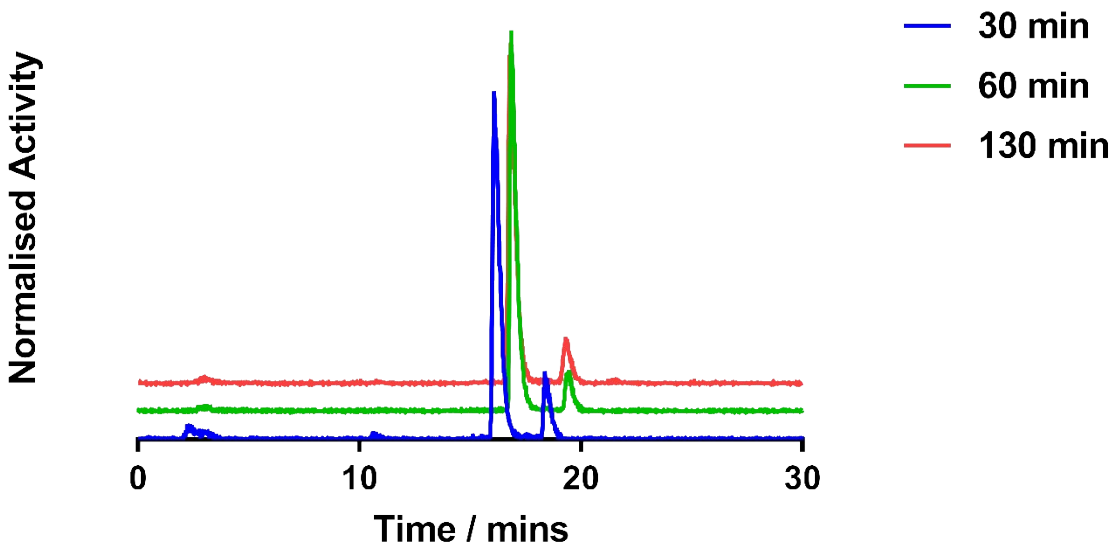


Figure S24: RadioHPLC traces of the reaction time alteration experiments on **[^{68}Ga]Ga3b**. Reaction conditions: 100 °C, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.

[^{68}Ga]Ga3c

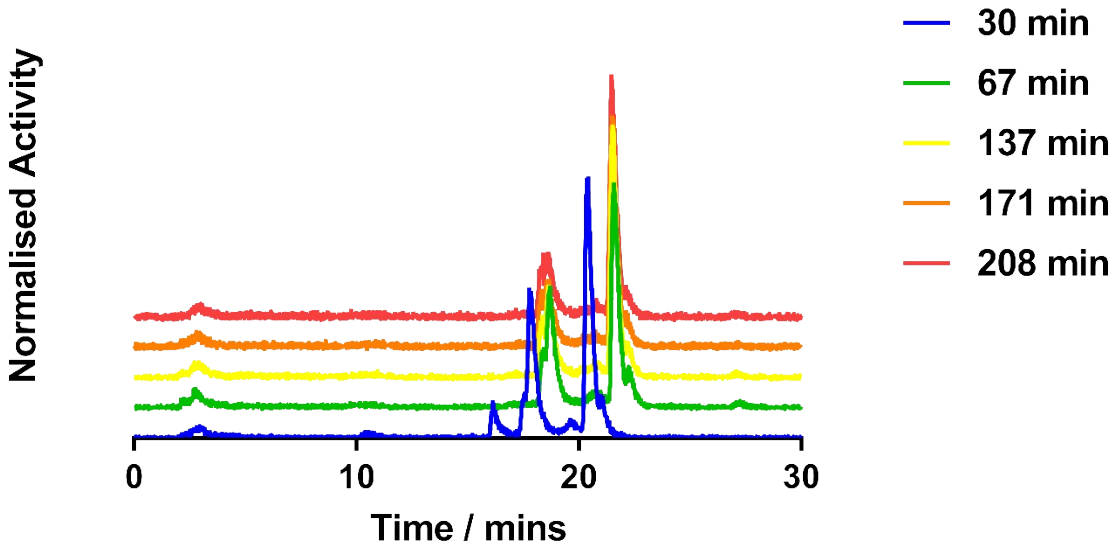


Figure S25: RadioHPLC traces of the reaction time alteration experiments on **[^{68}Ga]Ga3c.** Reaction conditions: 100 °C, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.

[^{68}Ga]Ga6b

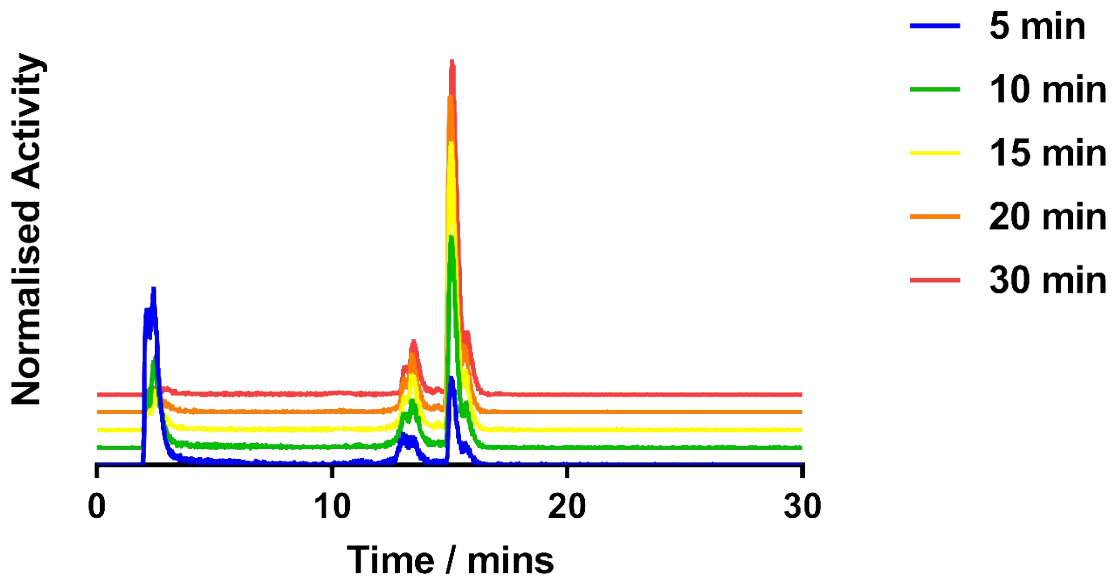


Figure S26: RadioHPLC traces of the reaction time alteration experiments on **[^{68}Ga]Ga6b**. Eluent gradient as described for Figure S19. Reaction conditions: 100 °C, 0.2 M NaOAc. Traces offset for clarity.

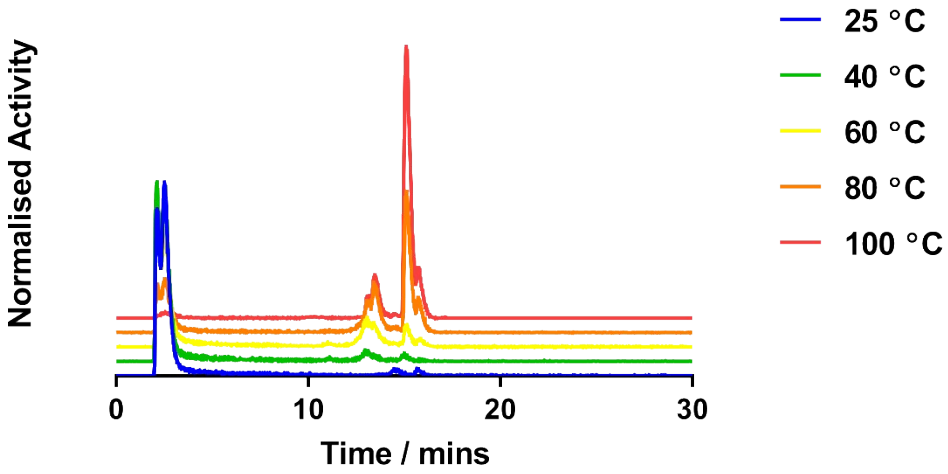


Figure S27: RadioHPLC traces of the reaction temperature alteration experiments on **[^{68}Ga]Ga6b**. Reaction conditions: 30 min, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.

Langendorff Isolated Heart Perfusion

Triple γ-Detector System Raw Data for **[^{68}Ga]Ga3c**

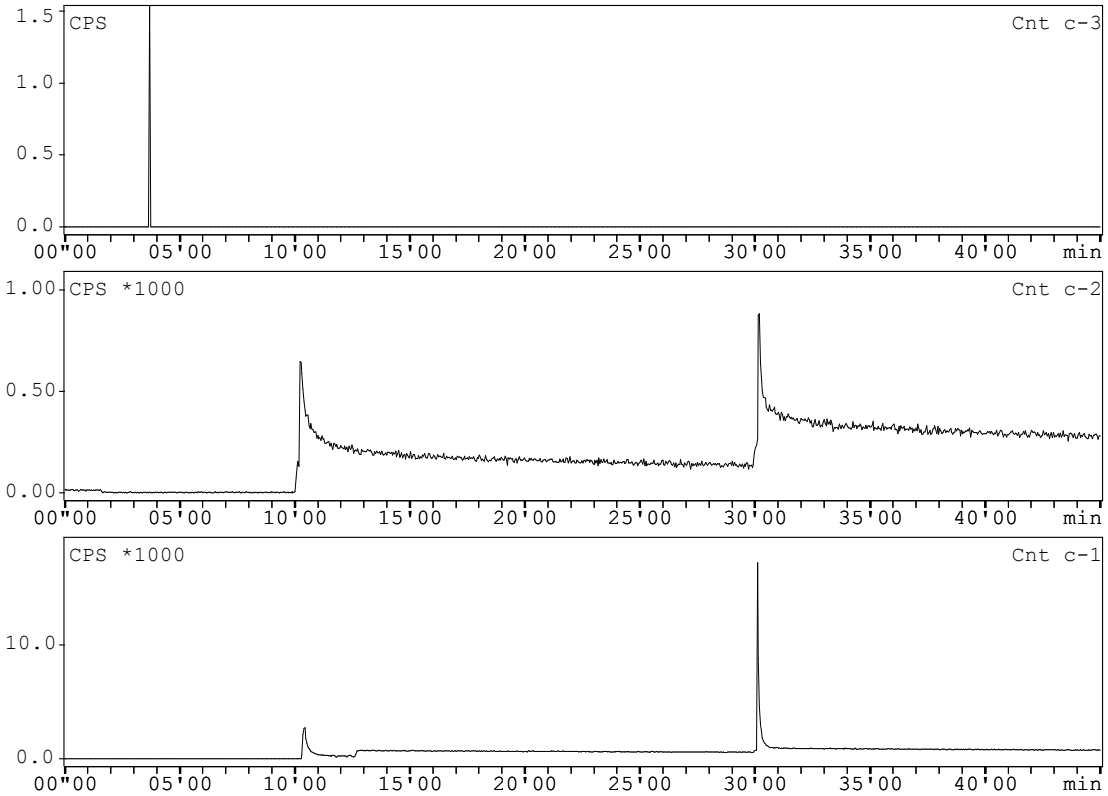


Figure S28: Experiment 1. Cnt c-1 refers to arterial activity, Cnt c-2 refers to heart activity, Cnt c-3 refers to venous activity, however the detector was damaged and as such no trace could be obtained.

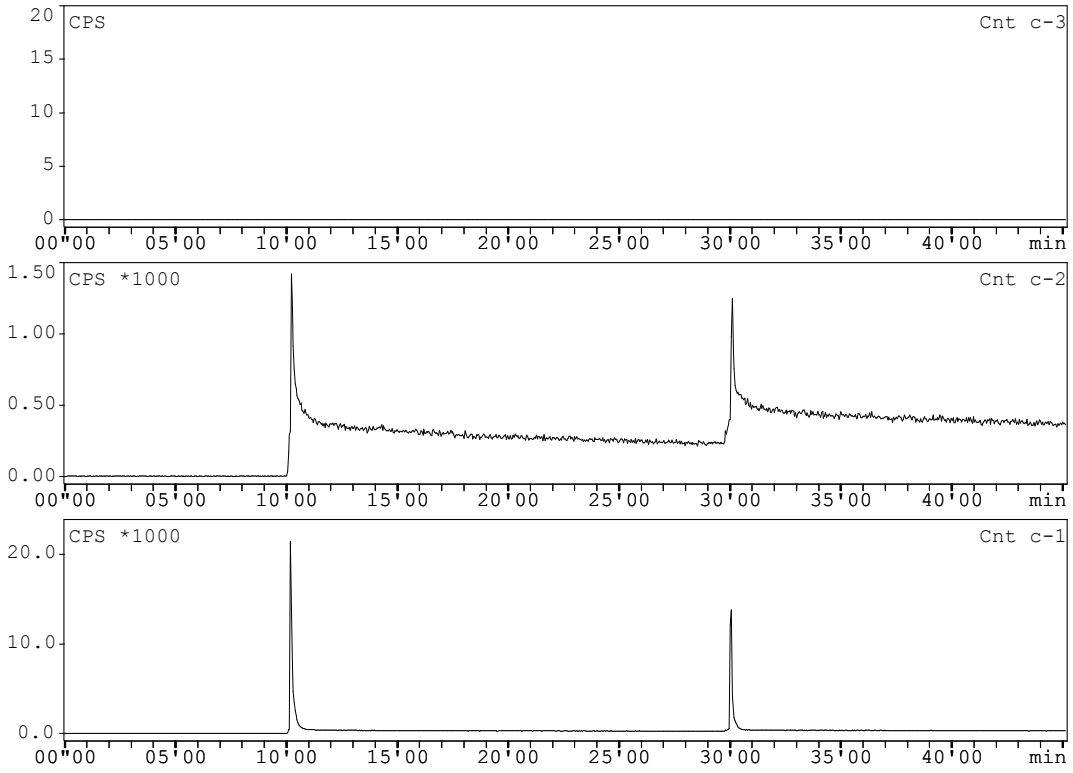


Figure S29: Experiment 2.

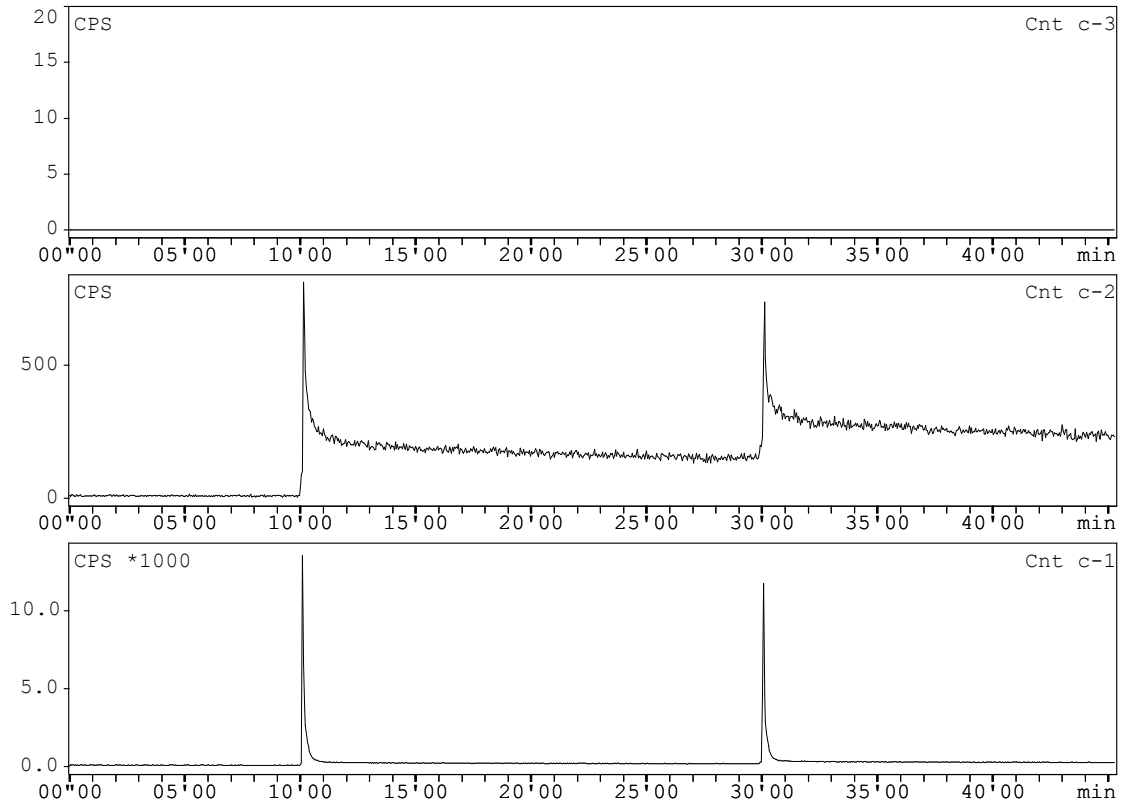


Figure S30: Experiment 3.

Triple γ-Detector System Raw Data for **[^{68}Ga]Ga6b**

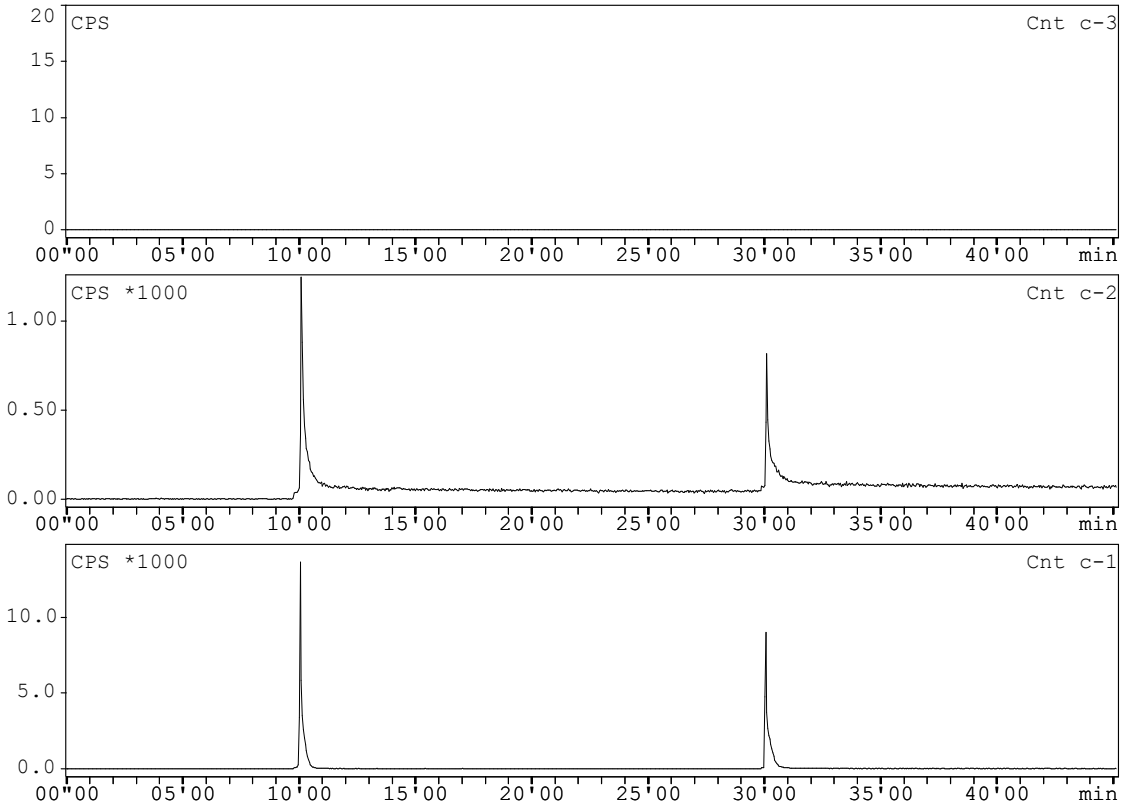


Figure S31 Experiment 1.

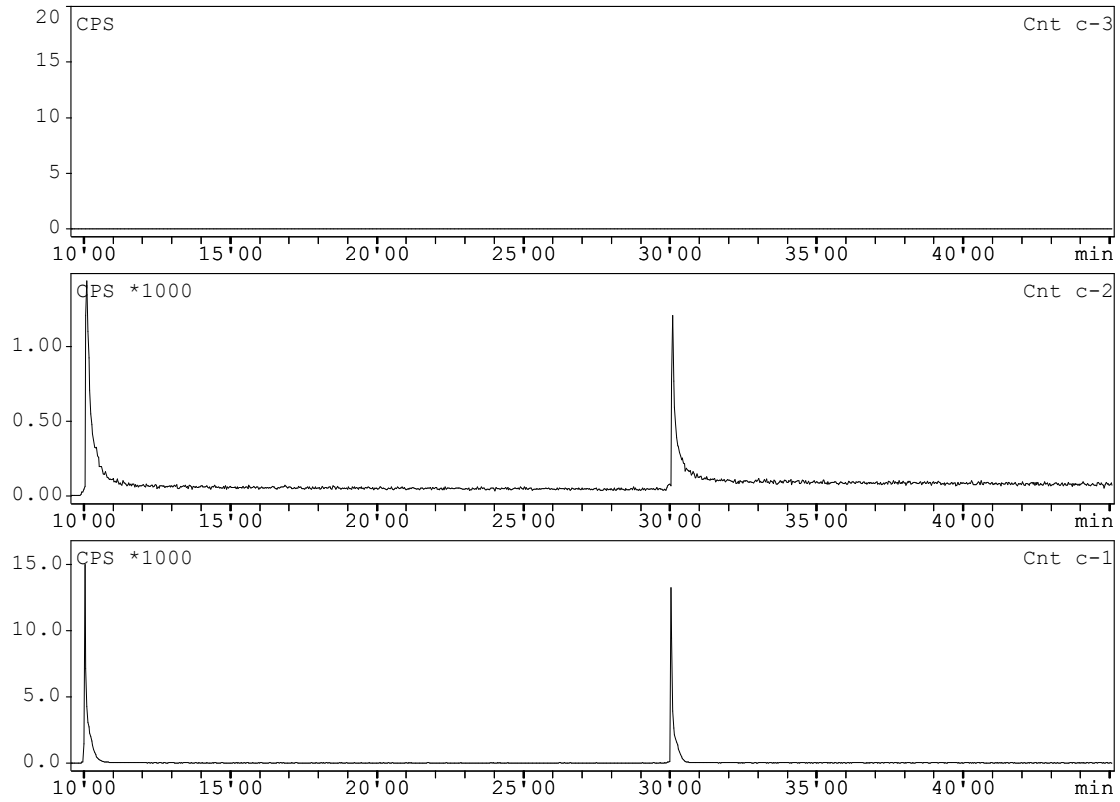


Figure S32: Experiment 2.

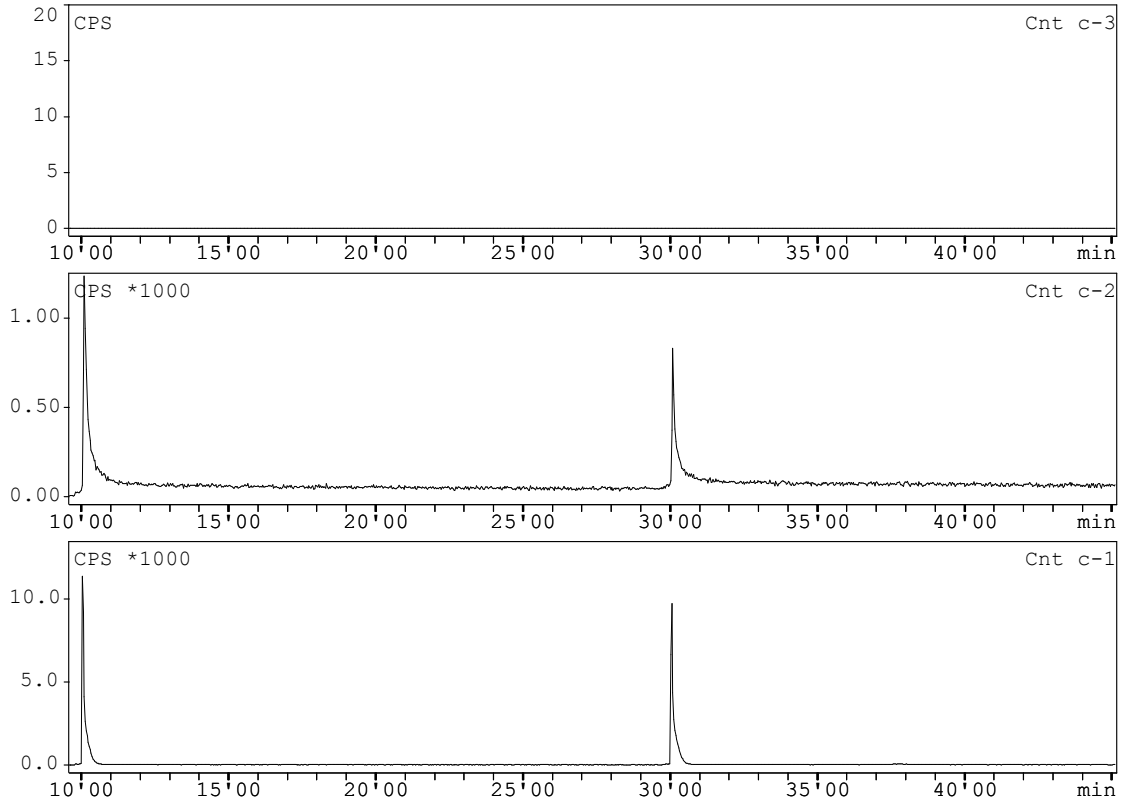
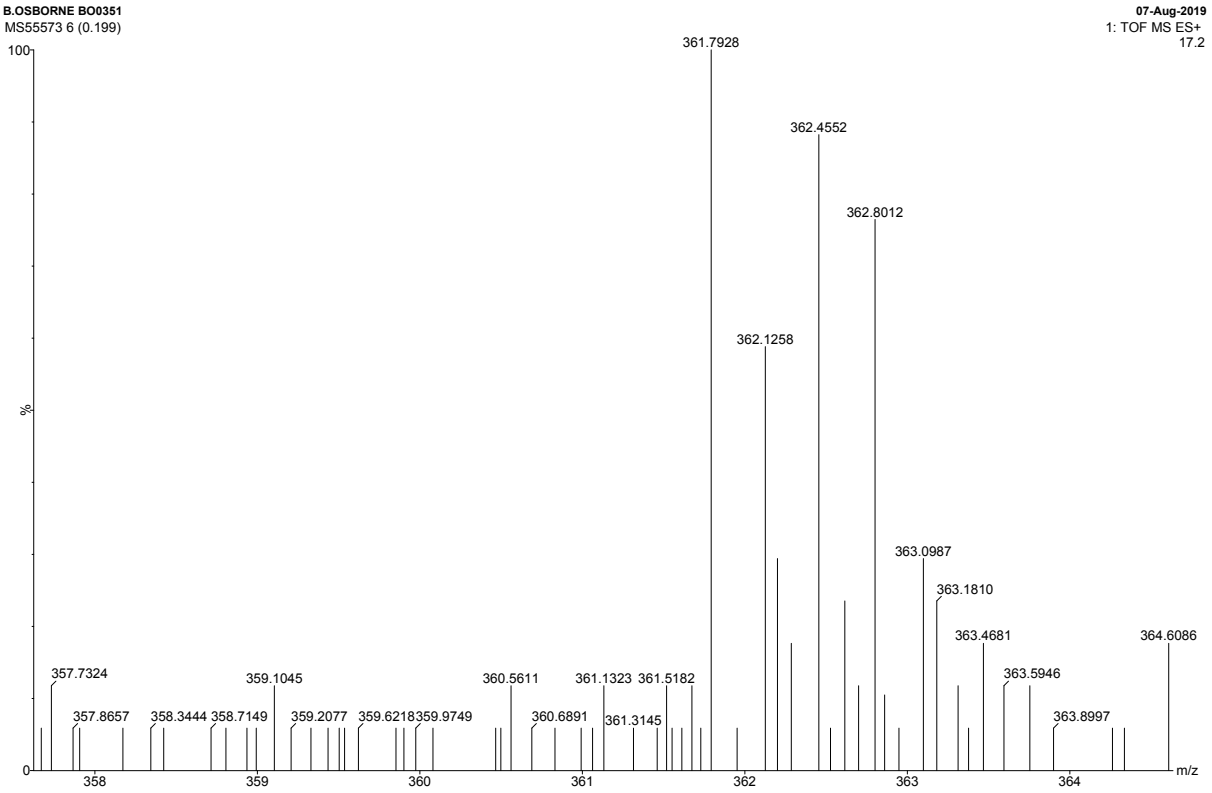


Figure S33: Experiment 3.

Synthesis of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate**

Compound **3a** (0.08 g, 0.07 mmol) and $Ga(NO_3)_3.H_2O$ (0.02 g, 0.07 mmol) were suspended in NH_4OAc (0.5 M, 0.15 mL), and heated at 100 ^oC for 30 min. The filtrate was isolated and the solvent was removed under reduced pressure, before the residue was purified by reverse-phase flash chromatography (C-18 SiO_2, 0-100 % B in A) to yield the desired product (0.05 g, 0.04 mmol, 58 %). ^{1}H-NMR (400 MHz, MeOD) δ_H (ppm): 7.90 (6H, td, $^3J_{HH}$ = 7.3, $^4J_{HH}$ = 1.9 Hz, *p*-Ph), 7.77 – 7.63 (24H, m, *o*/*m*-Ph), 7.41 (4H, d, $^3J_{HH}$ = 8.1 Hz, $C_6\underline{H}_4$), 7.08 (4H, dd, $^3J_{HH}$ = 8.3, $^4J_{HP}$ = 2.6 Hz, $C_6\underline{H}_4$), 4.99 (4H, d, $^2J_{HP}$ = 15.3, $C\underline{H}_2$), 4.01 (4H, s, $C\underline{H}_2$), 3.94 (4H, s, $C\underline{H}_2$), 3.58 – 3.34 (12H, m, macrocycle $\underline{H}$), 3.02 – 2.90 (4H, m, macrocycle $\underline{H}$). ^{13}C{^{1}H}-NMR (100 MHz, MeOD) δ_C (ppm): 173.7 ($\underline{C}$=O), 136.5 (*p*-Ph), 135.4 (d, $^3J_{CP}$ = 9.5 Hz, *m*-Ph), 133.3 ($\underline{C}_6H_4$), 132.7 (d, $^3J_{CP}$ = 5.3 Hz, $\underline{C}_6H_4$), 132.4 ($\underline{C}_6H_4$), 131.4 (d, $^2J_{CP}$ = 12.7 Hz, *o*-Ph), 130.7 ($\underline{C}_6H_4$), 119.0 (d, $^1J_{CP}$ = 85.7 Hz, *i*-Ph), 65.6 ($\underline{C}H_2$), 61.1 ($\underline{C}H_2$), 58.4 (macrocycle $\underline{C}$), 55.6 (macrocycle $\underline{C}$), 51.9 (macrocycle $\underline{C}$), 30.4 (d, $^1J_{CP}$ = 48.5 Hz, $\underline{C}H_2P$). ^{31}P{^{1}H}-NMR (162 MHz, MeOD) δ_P (ppm): 22.9. HRMS (ES-TOF+): *m/z* calcd for $C_{64}H_{66}N_4O_4P_2Ga$ ($[M]^{3+}$) 361.7938. found: 361.7928.

ES-TOF+ MS of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate**



C64H66N4O2P2Ga +3 ION = 361.7938

FOUND MASS = 361.7928

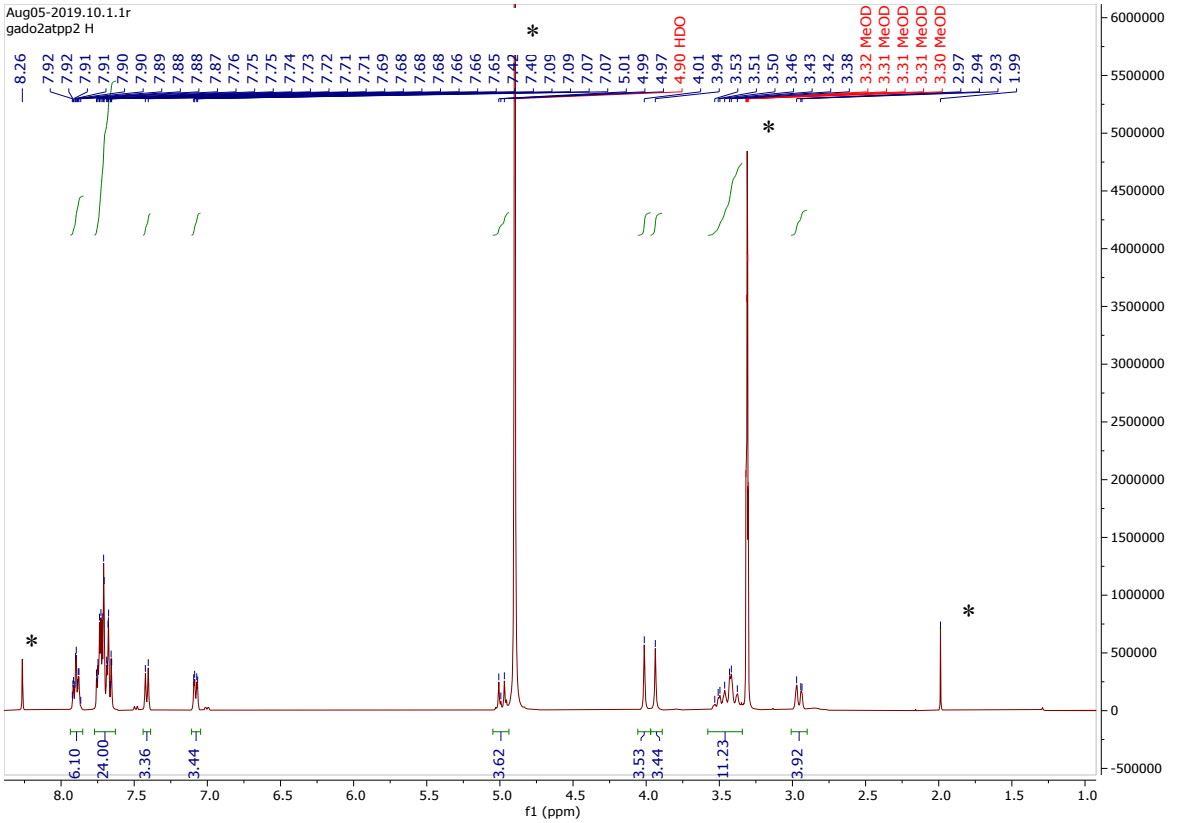


Figure S34: ^{1}H NMR of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate** (MeOD, 400 MHz, 298 K)

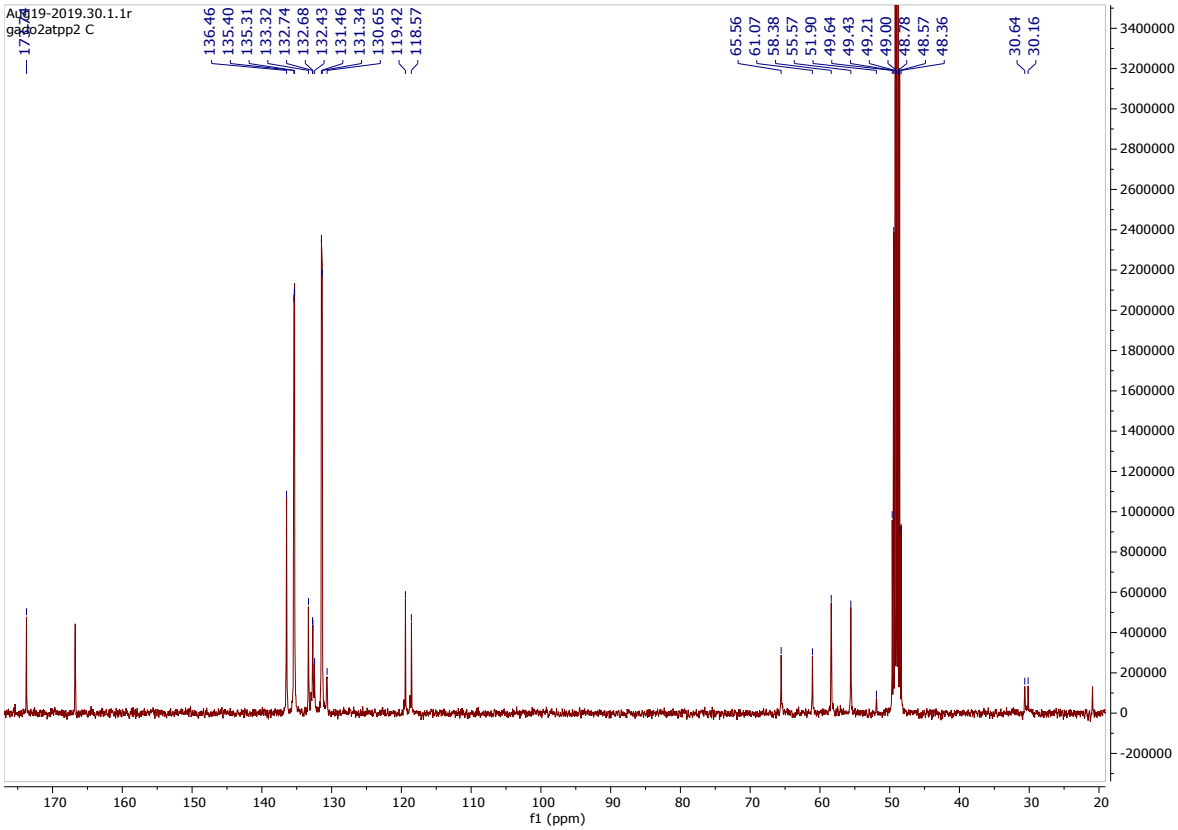


Figure S35: ^{13}C-{^{1}H} NMR of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate** (MeOD, 101 MHz, 298 K)

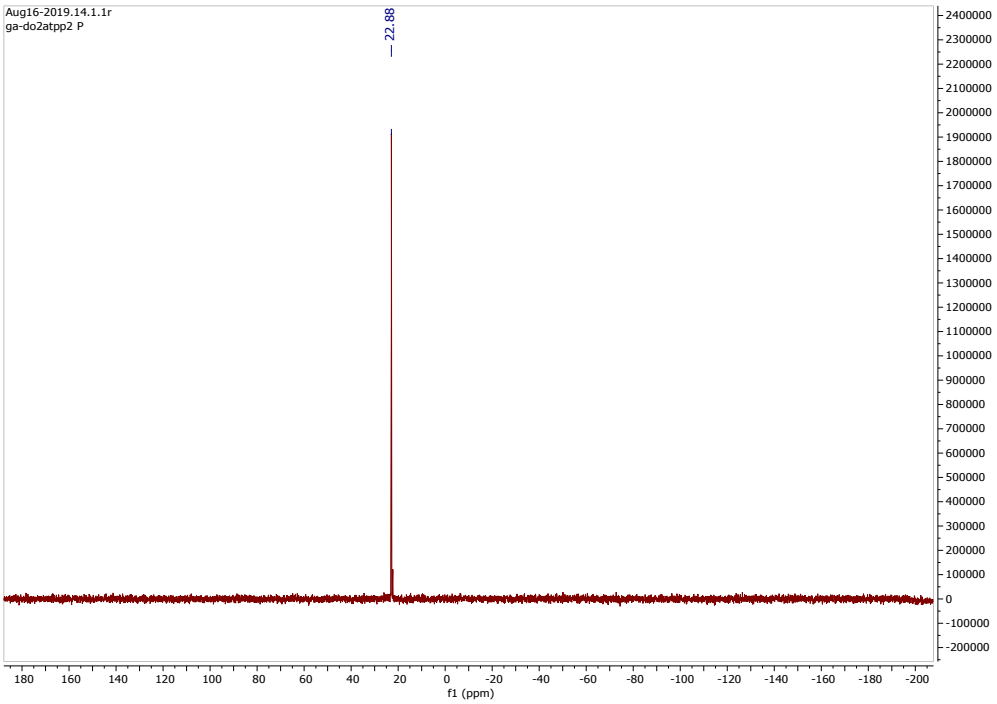


Figure S36: ^{31}P-{^{1}H} NMR of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate** (MeOD, 162 MHz, 298 K)

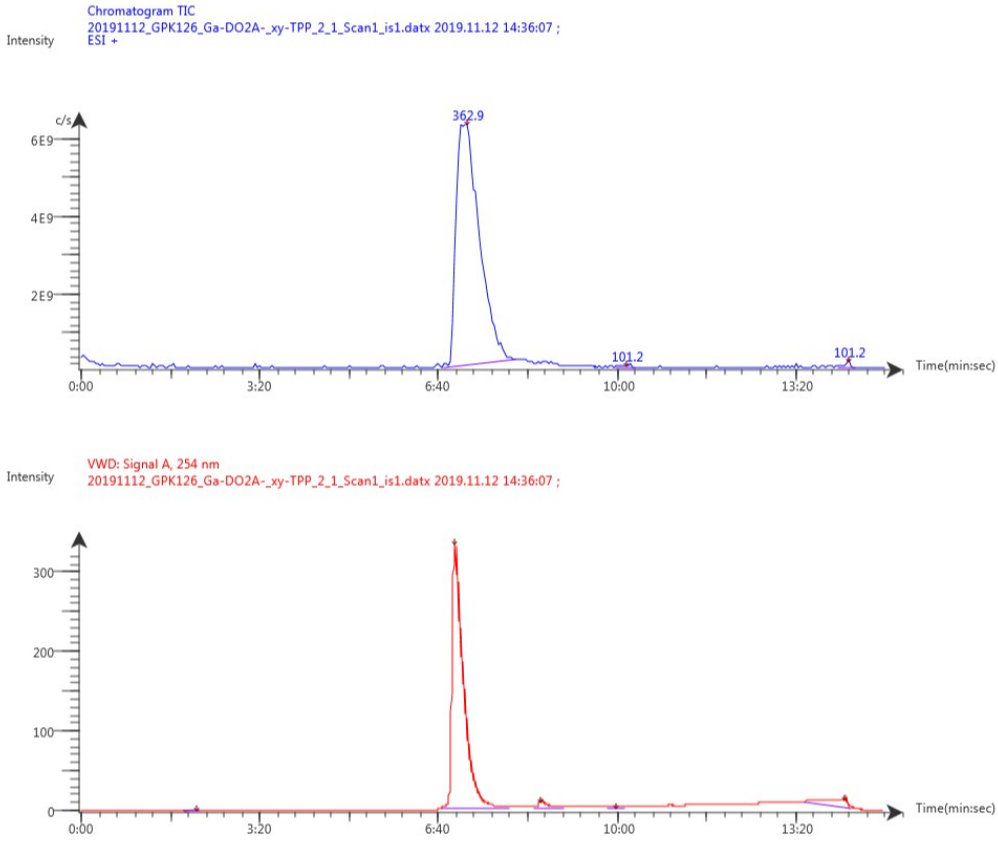


Figure S37: LCMS spectra of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate**.

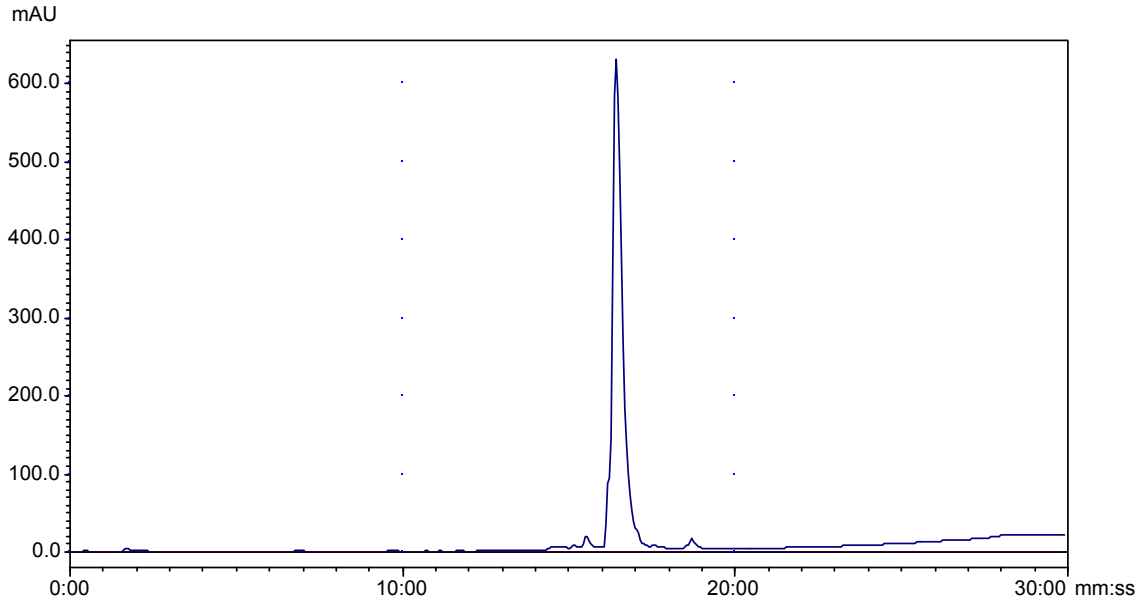


Figure S38: HPLC trace of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate**. Eluent gradient as described for Figure S19.

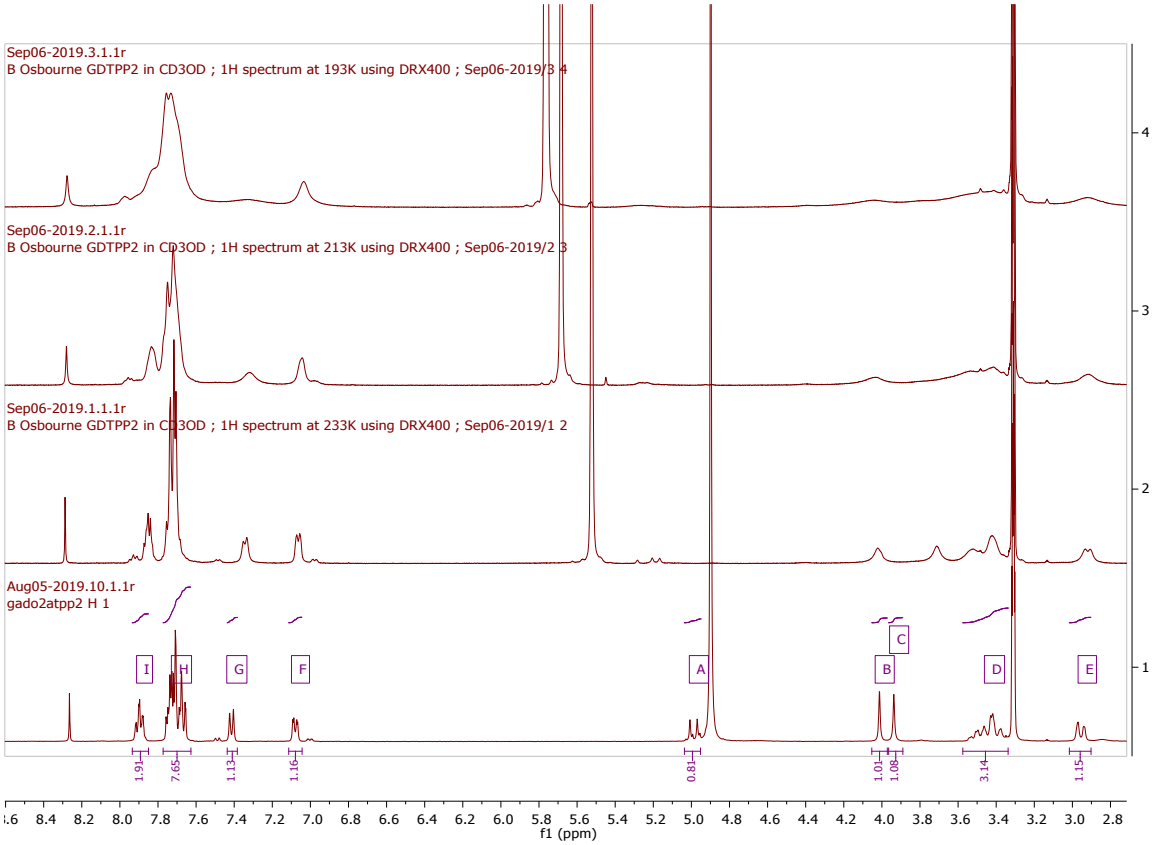


Figure S39: VT ^{1}H NMR spectra of **[^{nat}Ga]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate** (MeOD, 400 MHz) at different temperatures: 1 = 298 K, 2 = 233 K, 3 = 213 K, 4 = 193 K.

Synthesis of **[^{nat}Ga]Ga-DO2A-Bn$_2$ Nitrate**

Compound **6a** (0.10 g, 0.21 mmol) and $Ga(NO_3)_3.H_2O$ (0.06 g, 0.21 mmol) were suspended in NH_4OAc (0.5 M, 1.0 mL), and heated overnight at 100 °C. The filtrate was isolated and the solvent was removed under reduced pressure, before the residue was purified by reverse-phase flash chromatography (C-18 SiO_2, 0-100 % B in A) to yield the desired product (0.01g, 0.02 mmol, 11 %). ^{1}H-NMR (400 MHz, MeOD) δ_H (ppm): 7.59 – 7.50 (4H, m, *m*-Ph), 7.45 (6H, m, *o*/*p*-Ph), 4.12 (4H, s, C$\underline{H}_2$), 4.00 (4H, s, C$\underline{H}_2$), 3.60 (4H, td, $^3J_{HH}$ = 13.8, $^4J_{HH}$ = 4.9 Hz, macrocycle $\underline{H}$), 3.41 (8H, m, macrocycle $\underline{H}$), 3.02 – 2.93 (4H, m, macrocycle $\underline{H}$). ^{13}C-NMR (101 MHz, MeOD) δ_C (ppm): 173.77 (C=O), 132.66 (*m*-Ph), 132.15 (*o*-Ph), 130.63 (*p*-Ph), 129.99 (*i*-Ph), 66.55, 61.11 (C$\underline{H}_2$), 58.33, 55.74 (macrocycle, $\underline{C}$). HRMS (ES-TOF+): *m/z* calcd for $C_{26}H_{34}N_4O_4Ga$ ([M]$^+$) 535.1830. found: 535.1836.

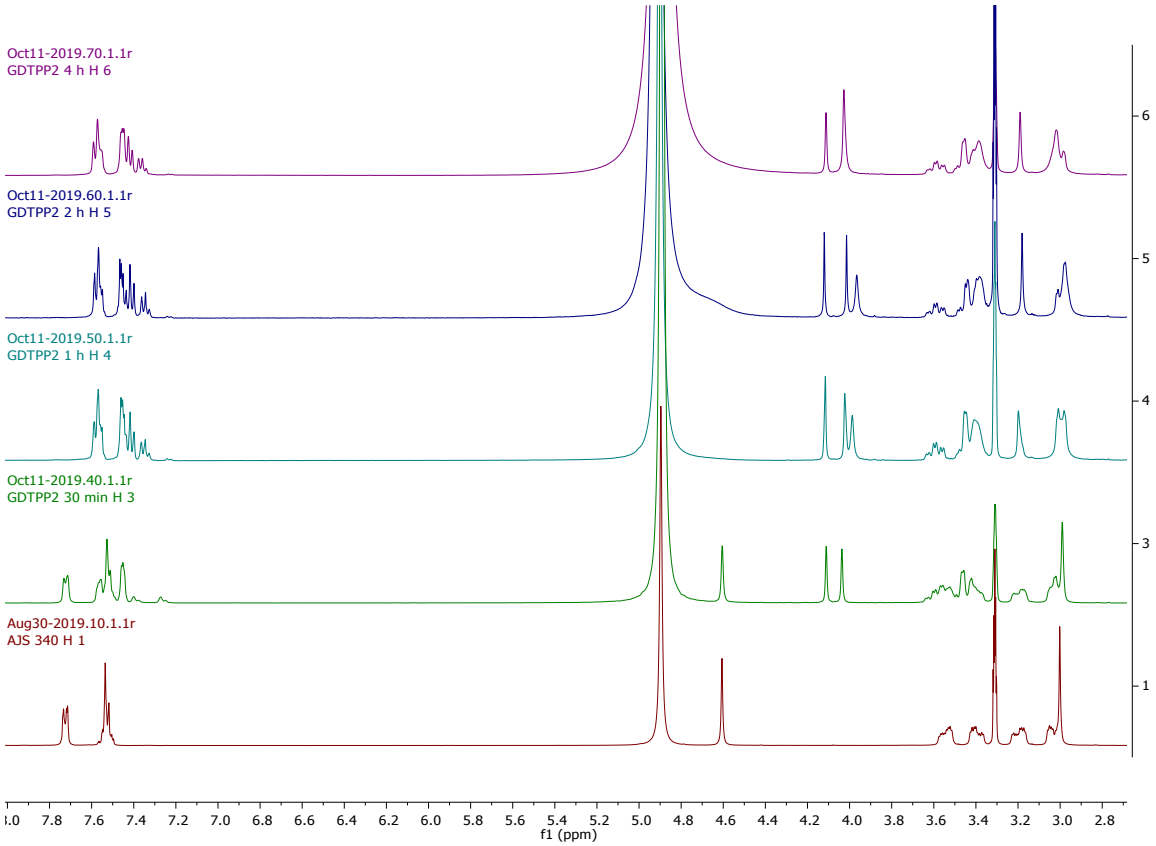


Figure S40: ^{1}H NMR of **[^{nat}Ga]Ga-DO2A-Bn$_2$ Trisnitrate** (MeOD, 400 MHz, 298 K) at different time points: 1 = 0 min, 3 = 30 min, 4 = 1 h, 5 = 2 h, 6 = 4 h.