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

Supporting Information

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NMR Spectra
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Figure S1: $^1$H NMR spectrum (CDCl$_3$, 400 MHz, 298 K)

Figure S2: $^{13}$C\{\text{\textsuperscript{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-diy1)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 (3e)**

**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-Xy₂ (6b)

Figure S17: "H NMR spectrum (MeOD, 400 MHz, 298 K)

Figure S18: "C{"H} NMR spectrum (MeOD, 100 MHz, 298 K)
RadioHPLC Analysis

$[^{68}\text{Ga}]\text{Ga3a}$

Figure S19: RadioHPLC traces of the reaction time alteration experiments on $[^{68}\text{Ga}]\text{Ga3a}$. 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 $[^{68}\text{Ga}]\text{Ga3a}$. 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 $[^{68}\text{Ga}]{\text{Ga}3a}$.
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 $[^{68}\text{Ga}]{\text{Ga}3a}$.
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 [68Ga]Ga3a. Reaction conditions: 100 °C, 30 mins. Eluent gradient as described for Figure S19. Traces offset for clarity.

[68Ga]Ga3b

Figure S24: RadioHPLC traces of the reaction time alteration experiments on [68Ga]Ga3b. Reaction conditions: 100 °C, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.
Figure S25: RadioHPLC traces of the reaction time alteration experiments on $[^{68}\text{Ga}]\text{Ga3c}$. Reaction conditions: 100 °C, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.

Figure S26: RadioHPLC traces of the reaction time alteration experiments on $[^{68}\text{Ga}]\text{Ga6b}$. 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 $^{68}$GaGa6b.
Reaction conditions: 30 min, 0.2 M NaOAc. Eluent gradient as described for Figure S19. Traces offset for clarity.
Langendorff Isolated Heart Perfusion

Tripple γ-Detector System Raw Data for [68Ga]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}\text{Ga}]\text{Ga-DO2A-(xy-TPP)} \text{2 Trisnitrate}

Compound 3a (0.08 g, 0.07 mmol) and Ga(NO\(_3\))\(_3\)\(\cdot\)H\(_2\)O (0.02 g, 0.07 mmol) were suspended in NH\(_4\)OAc (0.5 M, 0.15 mL), and heated at 100 °C 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) \(\delta\) (ppm): 7.90 (6H, td, \(^3\)J\(_{HH}\) = 7.3, \(^4\)J\(_{HH}\) = 1.9 Hz, p-Ph), 7.77 – 7.63 (24H, m, o/m-Ph), 7.41 (4H, d, \(^3\)J\(_{HH}\) = 8.1 Hz, C\(_6\)H\(_4\)), 7.08 (4H, dd, \(^3\)J\(_{HH}\) = 8.3, \(^4\)J\(_{HP}\) = 2.6 Hz, C\(_6\)H\(_4\)), 4.99 (4H, d, \(^2\)J\(_{HP}\) = 15.3, CH\(_2\)), 4.01 (4H, s, CH\(_2\)), 3.94 (4H, s, CH\(_2\)), 3.58 – 3.34 (12H, m, macrocycle H), 3.02 – 2.90 (4H, m, macrocycle H).

\(^1^3\)C\({}^{1}\)H-NMR (100 MHz, MeOD) \(\delta\) (ppm): 173.7 (C=O), 136.5 (p-Ph), 135.4 (d, \(^3\)J\(_{CP}\) = 9.5 Hz, m-Ph), 133.3 (C\(_6\)H\(_4\)), 132.7 (d, \(^3\)J\(_{CP}\) = 5.3 Hz, C\(_6\)H\(_4\)), 132.4 (C\(_6\)H\(_4\)), 131.4 (d, \(^2\)J\(_{CP}\) = 12.7 Hz, \(\text{o-Ph}\)), 130.7 (C\(_6\)H\(_4\)), 119.0 (d, \(^1\)J\(_{CP}\) = 85.7 Hz, i-Ph), 65.6 (CH\(_2\)), 61.1 (CH\(_2\)), 58.4 (macrocycle C), 55.6 (macrocycle C), 51.9 (macrocycle C), 30.4 (d, \(^1\)J\(_{CP}\) = 48.5 Hz, CH\(_3\)P). \(^{31}\)P\({}^{1}\)H-NMR (162 MHz, MeOD) \(\delta\) (ppm): 22.9. HRMS (ES-TOF+) m/z calcd for C\(_{64}\)H\(_{66}\)N\(_4\)O\(_4\)P\(_2\)Ga ([M]+) 361.7938. found: 361.7928.

ES-TOF+ MS of \[^{nat}\text{Ga}]\text{Ga-DO2A-(xy-TPP)} \text{2 Trisnitrate}

C\(_{64}\)H\(_{66}\)N\(_4\)O\(_2\)P\(_2\)Ga +3 ION = 361.7938
FOUND MASS = 361.7928
Figure S34: $^1$H NMR of [natGa]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate (MeOD, 400 MHz, 298 K)

Figure S35: $^{13}$C-$^1$H NMR of [natGa]Ga-DO2A-(xy-TPP)$_2$ Trisnitrate (MeOD, 101 MHz, 298 K)
Figure S36: $^{31}\text{P}$-{$^1\text{H}$} NMR of $[^{nat}\text{Ga}]\text{Ga-DO2A-(xy-TPP)}_2$ Trisnitrate (MeOD, 162 MHz, 298 K)

Figure S37: LCMS spectra of $[^{nat}\text{Ga}]\text{Ga-DO2A-(xy-TPP)}_2$ Trisnitrate.
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Figure S39: VT $^1$H NMR spectra of $[^{nat}]$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\textit{Ga}]Ga-DO2A-Bn\textsubscript{2} Nitrate

Compound 6a (0.10 g, 0.21 mmol) and Ga(NO\textsubscript{3})\textsubscript{3}·H\textsubscript{2}O (0.06 g, 0.21 mmol) were suspended in NH\textsubscript{4}OAc (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\textsubscript{2}, 0-100 % B in A) to yield the desired product (0.01g, 0.02 mmol, 11 %). \textsuperscript{1}H-NMR (400 MHz, MeOD) \(\delta_H\) (ppm): 7.59 – 7.50 (4H, m, \textit{m}-Ph), 7.45 (6H, m, \textit{o/p}-Ph), 4.12 (4H, s, CH\textsubscript{2}), 4.00 (4H, s, CH\textsubscript{2}), 3.60 (4H, td, \(J_{HH} = 13.8, J_{HH} = 4.9\) Hz, macrocycle H), 3.41 (8H, m, macrocycle H), 3.02 – 2.93 (4H, m, macrocycle H). \textsuperscript{13}C-NMR (101 MHz, MeOD) \(\delta_C\) (ppm): 173.77 (C=O), 132.66 (\textit{m}-Ph), 132.15 (\textit{o}-Ph), 130.63 (\textit{p}-Ph), 129.99 (\textit{i}-Ph), 66.55, 61.11 (CH\textsubscript{2}), 58.33, 55.74 (macrocycle, C). HRMS (ES-TOF+): \textit{m}/\textit{z} calcld for C\textsubscript{26}H\textsubscript{34}N\textsubscript{4}O\textsubscript{4}Ga ([M]+) 535.1830. found: 535.1836.

Figure S40: \textsuperscript{1}H NMR of [nat\textit{Ga}]Ga-DO2A-Bn\textsubscript{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.