Synthesis, Relaxation Properties and In vivo Assessment of Carborane-GdDOTA-Monoamide Conjugate as MRI Blood Pool Contrast Agent

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Scheme 1 Synthesis of CB-GdDOTA-MA. Reagents and conditions: a) Et₂S, Toluene, 85 °C, 2 h. b) Acetonitrile, 65 °C, 12 h, 41%. c) DO3A-t-Bu-ester, N, N-diisopropylethylamine, 85 °C for 12 h, 60%. d) Formic acid, 60 °C, 12 h, 82%. e) GdCl₃·6H₂O, H₂O-MeOH (1:1), pH 6.5, RT, 12 h, 73%. f) DyCl₃·6H₂O, H₂O-MeOH (1:1), pH 6.5, RT, 12 h, 68%.
$^1$H NMR, CDCl$_3$, 500 MHz
$^{13}\text{C NMR, CDCl}_3, 500\text{ MHz}$

$\text{1}$
\[ [M+Na]^+ \]


\[ \text{H NMR, CDCl}_3, 500 \text{ MHz} \]
$^1$H NMR, CD$_3$CN, 160 MHz

2
[M+Br]⁻

[M]⁻

[M+2Br]⁻
$^{1}$H NMR, CDCl$_3$, 400 MHz
$^{13}$CNMR, CDCl$_3$, 100.6 MHz

28.66, 28.77, 30.38, 44.16, 56.38, 56.45, 57.04, 60.79, 76.35, 77.68, 78.00, 78.21, 78.32, 82.76, 82.80, 172.80, 172.97, 173.30
$\text{N}\text{H}\text{O}$

\[\text{CO}_2\text{tBu} \quad \text{CO}_2\text{tBu} \quad \text{tBuO}_2\text{C}\]

$^{11}$B NMR (dc), CDCl$_3$, 128 MHz
[M+Na]^+
$^1$H NMR, CD$_3$OD, 500 MHz
$^{13}$C NMR, CD$_3$OD, 125 MHz
$^1$H NMR, CD$_3$OD, 160 MHz
CB-GdDOTA-MA

Chemical Formula: $\text{C}_{19}\text{H}_{38}\text{B}_{10}\text{GdN}_{5}\text{O}_{7}$
Molecular Weight: 713.8943
Chemical Formula: C₁₉H₃₈B₁₀Dy₅N₇O₇
Molecular Weight: 719.1443
Analytical HPLC analysis of **CB-GdDOTA-MA**
Instrument: Alliance Waters 2695.
Column: Agilent Zorbax SB-C18, 5 μm (4.6 x 250 mm).
Eluent: 1 mL/min; gradient: B 0% to 100% over 45 min, B 100% 45-60 min (A: water, B: acetonitrile).
Detector: Evaporative Scattering Detector (SEDEX 75, operated at 45 °C and 3.5 bars using N₂).

**Fig. S1** IR spectrums of compound 4 and CB-GdDOTA-MA.

**Fig. S2** HPLC trace of CB-GdDOTA-MA.
**Determination of hydration number (q) for CB-DyDOTA-MA**

Varying concentrations of CB-DyDOTA-MA and DyCl₃.6H₂O over the range 10-80 mmol dm⁻³ were prepared in 80% D₂O-H₂O and the pH of the solutions was adjusted to pH 7.0. The ¹⁷O NMR experiments were performed using a Bruker Ascend 400 MHz NMR instrument at RT with the deuterium signal locked. A graph was plotted between the Δδ and the concentration for both CB-DyDOTA-MA and the DyCl₃.6H₂O solutions and slope was obtained. The Δδ value for a complex with the general formula, Dy(ligand)ₙ(H₂O)ᵢ, is given by the following relation:

\[
(\Delta\delta) = q\Delta\text{[Dy(ligand)]}_{n}(\text{H}_2\text{O})_{i}/[\text{H}_2\text{O}]
\]  

The slope of a plot of the Δδ versus the Dy³⁺ concentration is proportional to the q value of the complex (Figure). The q value was obtained by linearly fitting the Δδ value and was found to be 2 for complex CB-DyDOTA-MA.

From the graph, which fit well to a straight line, the slope was calculated. From relation I, the slope of the graph can be equated with the following; Slope = qΔ/[H₂O].

From the graph, the slope was calculated as,

Slope = -381 ppm dm⁻³ mol⁻¹ or,

\[q\Delta/[\text{H}_2\text{O}] = -381 \text{ ppm dm}^{-3} \text{ mol}^{-1}\]

For DyCl₃, the value of q was assumed to be 9, because the coordination number of Dy(III) in such complexes known to be 9. Hence, the value of Δ/[H₂O] is calculated as follows:

\[\Delta/[\text{H}_2\text{O}] = -381 \text{ ppm dm}^{-3} \text{ mol}^{-1} / 9 \]

\[\Delta/[\text{H}_2\text{O}] = -42.3 \text{ ppm dm}^{-3} \text{ mol}^{-1}\]

From this calculation, a value of q for the DOTA-Dy³⁺ complex CB-DyDOTA-MA is calculated as follows:

For complex CB-DyDOTA-MA, Slope = qΔ/[H₂O], or

\[q = \text{slope}/(\Delta/[\text{H}_2\text{O}]) \text{ or } q = -50.4/-42.3, \text{ hence } q = 1.2\]

**Fig. S3** Determination of Hydration number (q) for CB-DyDOTA-MA.
Fig. S4 Representative *in vivo* T1-weighted MRA scans of a mouse at various time points p.i. of Omniscan at a Gd dose of 1.2 mmol/kg.