Hyperpolarized Multi-metal ^{13}C-Sensors for MRI

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Supporting Information

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SUPPLEMENTAL RESULTS

Table S1. T_1 relaxation times of metal-coordinating compounds as well as chemical shifts in the presence of calcium. T_1 times (uncertainties of the measurements were ~10^{-2} seconds).

Name	Formula	MW (g/mol)	ppm (δ): ***T_1 (sec)*** **@5.9 Tesla**	ppm (δ): ***T_1 (sec)*** **@11.7 Tesla**	Chemical Shift δ (ppm) to Ca^{2+}
^{13}C-EDTA	$^{12}C_6{}^{13}C_4H_{16}N_2O_8$	296.11	**170.70:** *13.1*	**174.79:** *6.8* 179.98: *5.6 w/$CaCl_2$*	5.2
^{13}C-EGTA	$^{12}C_{10}{}^{13}C_4H_{24}N_2O_{10}$	384.16	**170.24:** *15.0*	**170.34:** *7.6* 180.13: *5.5 w/$CaCl_2$*	9.8
^{13}C-EGTA-d_8	$^{12}C_{10}{}^{13}C_4H_{16}{}^{2}H_8N_2O_{10}$	392.31	-	**170.34**: *10.0*	9.8
^{13}C-Br-Pyro-EGTA	$^{12}C_{14}{}^{13}C_4H_{23}N_2O_{10}$	511.29	**179.40:** *7.8*	**179.97:** *4.5*	0.8
APTRA	$^{12}C_{12}H_{13}NO_7$	283.23	-	**179.60:** *3.9* 177.11: *5.6* 150.74: *1.3*	~0
BAPTA	$^{12}C_{22}H_{24}N_2O_{10}$	476.43	-	**178.86:** *2.9* 149.83: *2.2* 140.06: *5.1* 178.56: *2.0 w/$CaCl_2$*	0.3
HIDA	$^{12}C_6H_{11}NO_5$	177.15	-	**170.31:** *10.0* 170.72: *3.5 w/$CaCl_2$*	0.3
Carboxy Glutamate (CGlu)	$^{12}C_7H_{10}O_6$	191.14	-	**178.38:** *9.7* 177.94: *10.1* 174.59: *10.8*	-
^{13}C-Glycine	$^{12}C_1{}^{13}C_1H_5NO_2$	76.07	**172.39:** *24.8*	-	-
Glycine-1-^{13}C,2,2-d_2	$^{12}C_1{}^{13}C_1H_3{}^{2}H_2NO_2$	78.08	**172.39:** *37.0*	-	-

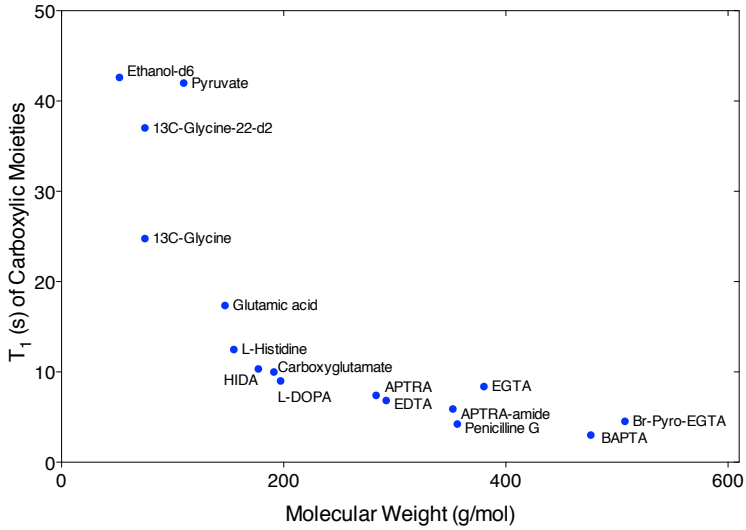
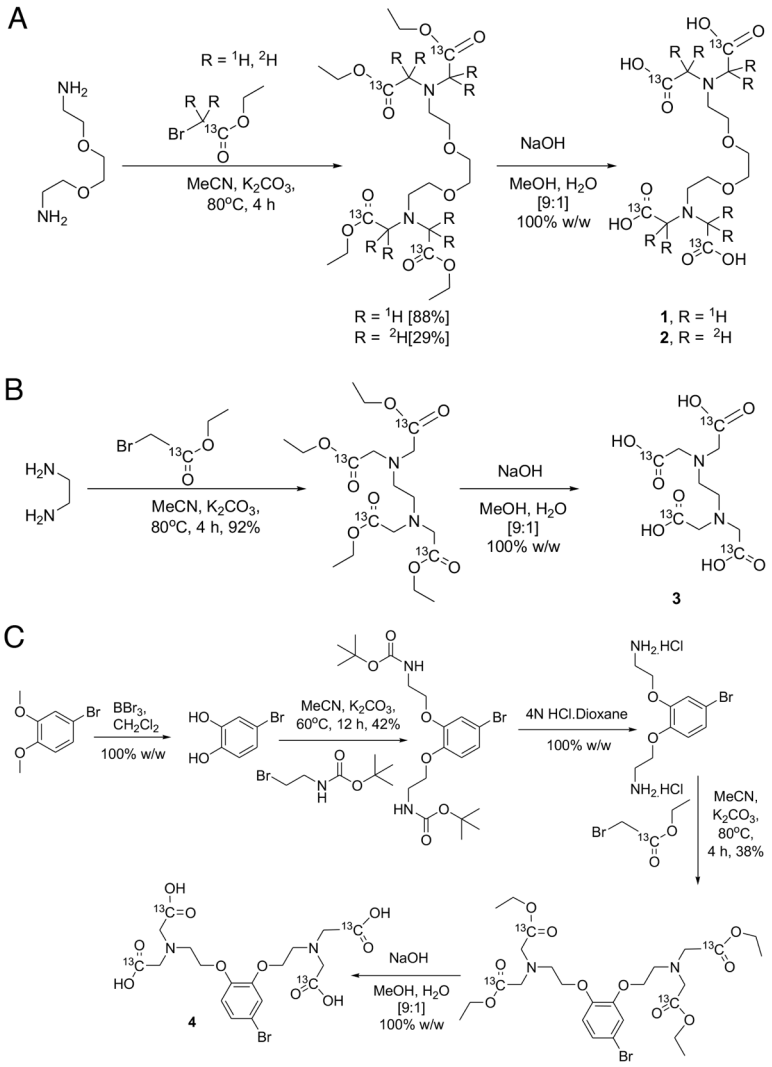


Figure S1. T_1 relaxation (11.7 T) times as a function of molecular weight.

Table S2. T_1 relaxation times obtained from hyperpolarized ^{13}C-sensors at 1 Tesla tabulated for each NMR signal peak shown in Figure 3.

	sans Ca^{2+}	**subsat. Ca^{2+}**	**sat. Ca^{2+}**
^{13}C-EGTA T_1 (sec) @ 171 / 181 ppm	15.1 / -	12.6 / 11.6	- / 8.7
^{13}C-EDTA T_1 (sec) @ 174 / 181 ppm	13.5 / -	11.9 / 11.5	- / 10.4
^{13}C-EGTA-d_8 T_1 (sec) @ 171 / 181 ppm	-	25.6 / 26.2	-



Scheme S1. Synthetic scheme. A) ^{13}C-EGTA and ^{13}C-EGTA-d_8; B) ^{13}C-EDTA; C) ^{13}C-Br-Pyro-EGTA.

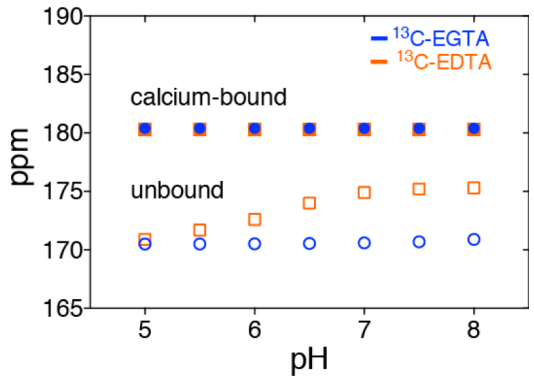


Figure S2. Response of ^{13}C-sensors to pH. Chemical shifts are plotted for the calcium-bound and free chelators (1 mM ^{13}C-EGTA and ^{13}C-EDTA) dissolved in 15 mM MOPS buffer adjusted to 5-8 pH without (open symbols) or with (filled symbols) equimolar concentrations of calcium. 0.5 µL ^{13}C-methanol was used as an internal reference. Measurements were taken at 310 K, 500 MHz (^{13}C: 125.83 MHz)

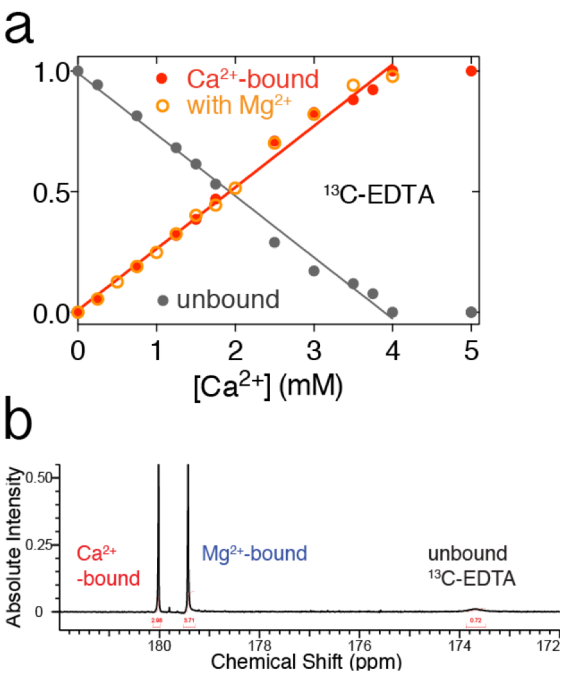


Figure S3. Binding curves and multiplexed metal detection. (**a**) The Area under the Curve (AUC) was plotted for ^{13}C-EDTA (4mM) with increasing amounts of Ca^{2+} in the absence of Mg^{2+} (filled red circles, slope 0.257) or in the presence of 1.4 mM Mg^{2+} (open yellow circles, slope 0.261) or unbound chelator (gray circles, slope -0.257**).** (**b**) Exemplary spectrum showing multiplexed detection of Ca^{2+} and Mg^{2+}.

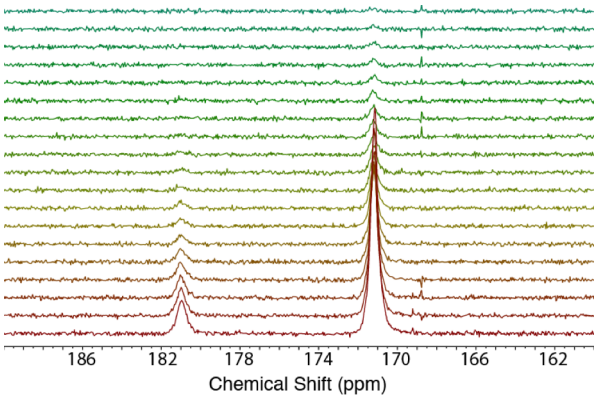


Figure S4. Calcium detection in human serum by hyperpolarized ^{13}C-EGTA. The sensor was subjected to DNP for one hour (1.3 K and 3.35 Tesla), dissolved, mixed with human serum, (1:1 in 0.1 M MOPS, 7.5 mM final concentration of ^{13}C-EGTA) and measured at 1 Tesla. The two peaks correspond to the unbound carboxylic moieties (171 ppm, T_1 = 12.9 s) and the Ca^{2+}-bound species (181 ppm, T_1 = 11.6 s).

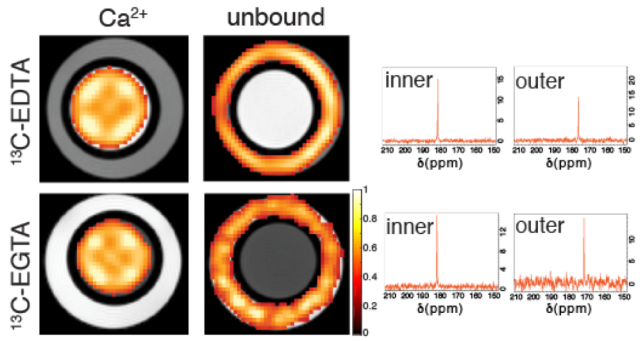


Figure S5. **Chemical Shift Imaging of metal distributions with non-hyperpolarized sensors**. Two NMR tubes filled with ^{13}C-EDTA or ^{13}C-EGTA at thermal equilibrium (200 mM) were concentrically positioned inside the bore of a MR microimaging system with saturating Ca^{2+} concentrations (300 mM) added to the inner tube. NMR spectra obtained from chemical shift images are overlaid on a proton MSME (Multi Slice Multi Echo) image and areas under the curve (AUC) of the Ca^{2+}-bound (left column) or free chelator (right column) are overlaid on a hot color scale. The corresponding spectra are shown on the right.

EXPERIMENTAL SECTION

General

The chemicals and anhydrous solvents were purchased from commercial suppliers (Aldrich, Fluka, and VWR) and were used without further purification unless otherwise stated. All reactions were carried out under an inert (nitrogen) atmosphere. All glassware was washed with a mixed acid solution, thoroughly rinsed with deionized, distilled water and predried with a heat gun under vacuum. Ultra-pure deionized water (18 MΩ cm^{-1}) was used throughout.

Chromatography

Flash column chromatography was performed by using flash silica gel 60 (70–230 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel plates with 0.2 mm thick silica gel 60 F_{254} (E. Merck) using different mobile phases. The compounds were visualized by UV irradiation (254 nm), iodine and Dragondorff staining reagent.

Spectroscopy

Each synthetic step was characterized by NMR and Mass spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV250 and Bruker AV500 spectrometer equipped with a cryoprobe (^{1}H; internal reference $CDCl_3$ at 7.27 ppm or D_2O at 4.75 ppm; ^{13}C; internal reference $CDCl_3$ at 77.0 ppm). All experiments were performed at 23°C. Electrospray mass spectra (ESI-MS) were recorded on SL 1100 system (Agilent, Germany) with ion-trap detection in positive and negative ion mode. HRMS were measured on a Thermo Finnigan LQT.

Synthesis of chemical shift metal sensors.
(1) ^{13}C-labeled EGTA:
[3,12-bis[carboxy(^{13}C)methyl](1,14-$^{13}C_2$)-6,9-dioxa-3,12-diazatetradecanedioic acid].
^{13}C-Labeled EGTA was synthesized in two steps as described in the literature on non-^{13}C labelled EGTA. A solution of 2,2'-(ethane-1,2-diylbis(oxy))diethanamine (0.1 g, 0.68 mmol) and K_2CO_3 (0.56 g, 4.06 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl bromoacetate-1^{13}C (0.34 mL, 3.04 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.4) to give *dimethyl 3,12-bis(2-methoxy(¹³C)-2-oxoethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-dioate(¹³C)* (0.295 g, 88%) as a yellow gum.

^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.24 (t, *J*=6 Hz, 12H), 2.94 (t, *J*=6 Hz, 4H), 3.42 - 3.70 (m, 16H), 4.13 (q, *J*=7 Hz, 8H).^{13}C NMR ($CDCl_3$, 62.9 MHz) δ ppm: 14.1, 53.6, 55.3, 56.2, 60.4, 70.1, 171.4. HR-MS (ES^+) *m/z* $^{12}C_{18}{}^{13}C_4H_{41}N_2O_{10}$ requires 497.2895 $[M+H]^+$; found 497.2880 $[M+H]^+$.

The tetra-acid (3,12-bis[carboxy(¹³C)methyl](1,14-$^{13}C_2$)-6,9-dioxa-3,12-diazatetradecanedioic acid) was obtained by deprotection of the ethyl group on *dimethyl 3,12-bis(2-methoxy(¹³C)-2-oxoethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-dioate(¹³C)* (0.25 g, 0.65 mmol) with NaOH (0.25 g, 3 mmol) in 10 mL MeOH: H_2O (9:1) for 2 h at room temperature. After completion of the reaction, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ^{13}C-labeled EGTA (0.19 g, 100% w/w) as an off-white solid.

^{1}H NMR (D_2O, 250 MHz) δ ppm: 3.23 (t, *J*=7 Hz, 4H), 3.54 - 3.76 (m, 12H), 3.80 (t, *J*=7 Hz, 4H). ^{13}C NMR (D_2O, 62.9 MHz) δ ppm: 54.5, 58.7, 65.9, 69.6, 170.4. HR-MS (ES^+) *m/z* $^{12}C_{10}{}^{13}C_4H_{25}N_2O_{10}$ requires 385.1643 $[M+H]^+$; found 385.1634 $[M+H]^+$.

(2) ^{13}C-labeled EGTA-d_8:
[1,14-diethyl 3,12-bis[2-ethoxy-2-oxo($^2H_2$)ethyl](2,2,13,13-2H4)-6,9-dioxa-3, 12-diazatetradecanedioate($^{13}C_4$)].

^{13}C-d_8-Labeled EGTA was synthesized in similar way as compound 1 (^{13}C-Labeled EGTA). A solution of 2,2'-(ethane-1,2-diylbis(oxy))diethanamine (0.1 g, 0.68 mmol) and K_2CO_3 (0.56 g, 4.06 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl 2-bromo($^2H_2$)acetate-1^{13}C (0.52, 3.06 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.4) to give 1,14-diethyl 3,12-bis[2-ethoxy-2-oxo($^2H_2$)ethyl](2,2,13,13-$^2H_4$(¹³C))-6,9-dioxa-3, 12-diazatetradecanedioate (0.10 g, 29%) as a yellow gum.

^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 1.30 (t, *J*=7 Hz, 12H), 3.21 (t, *J*=4.5 Hz, 4H), 3.66 (t, *J*=5 Hz, 4H), 3.79 (s, 4 H), 4.22 (qd, *J*=7, 3 Hz, 8H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ ppm: 14.0, 29.7, 53.4, 61.8, 68.1, 69.6, 173.5. LR-MS (ES^+) *m/z* $^{12}C_{18}{}^{13}C_4H_{36}{}^{2}H_8N_2O_{10}$ requires 505.3 $[M+H]^+$; found 505.5 $[M+H]^+$.

The tetra-acid (3,12-bis[carboxy(^{13}C,$^{2}H_2$)methyl](2,2,13,13-$^{2}H_4$($^{13}C_4$))-6,9-dioxa-3,12-diazatetradecanedioic acid) was obtained by deprotection of the ethyl group on 1,14-diethyl 3,12-bis[2-ethoxy-2-oxo($^{2}H_2$)ethyl](2,2,13,13-$^{2}H_4$)-6,9-dioxa-3, 12-diazatetradecanedioate($^{13}C_4$) (0.1 g, 0.65 mmol) with NaOH (0.25 g, 3 mmol) in 10 mL MeOH: H_2O (9:1) for 2 h at room temperature. After completion of the reaction, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ^{13}C-$^{2}H_8$-Labeled EGTA (0.078 g, 100% w/w) as an off-white solid.

^{1}H NMR (D_2O, 500 MHz) δ ppm: 3.40 (t, *J*=5 Hz, 4H), 3.62 (s, 4 H), 3.80 (t, *J*=5 Hz, 4H).^{13}C NMR (D_2O, 126 MHz) δ ppm: 38.8, 54.7, 64.7, 69.9, 170.4. LR-MS (ES^-) *m/z* $^{12}C_{10}{}^{13}C_4H_{16}{}^{2}H_8N_2O_{10}$ requires 391.2 $[M-H]^-$; found 391.6 $[M+-H]^-$.

(3) ^{13}C-labeled EDTA:
[2-[(2-{bis[carboxy(^{13}C)methyl]amino}ethyl)[carboxy(^{13}C)methyl]amino](1-^{13}C)acetic acid].

A solution of ethane-1,2-diamine (0.1 g, 1.67 mmol) and K_2CO_3 (1.38 g, 10 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. ^{13}C -labeled ethyl bromoacetate-1^{13}C (0.84 mL, 7.5 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.5) to give *tetraethyl-2-[(2-{bis[carboxy(¹³C)methyl]amino}ethyl)[carboxy(¹³C)methyl]amino](1-¹³C) acetate* (0.625 g, 92%) as a yellow gum.

^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.28 (t, *J*=7 Hz, 12H), 2.92 (t, *J*=6 Hz, 4H), 3.61 (s, 8H), 4.17 (q, *J*=7 Hz, 8H).^{13}C NMR ($CDCl_3$, 62.9 MHz) δ ppm: 14.1, 51.4, 55.3, 60.4, 171.3. HR-MS (ES^+) *m/z* $^{12}C_{14}{}^{13}C_4H_{33}N_2O_8$ requires 409.2371 $[M+H]^+$; found 409.2365 $[M+H]^+$.

The tetra-acid (2-[(2-{bis[carboxy(¹³C)methyl]amino}ethyl)[carboxy(¹³C)methyl]amino](1-¹³C)acetic acid) was obtained by deprotection of the ethyl group on *tetraethyl-2-[(2-{bis[carboxy(¹³C)methyl]amino}ethyl)[carboxy(¹³C)methyl]amino](1-¹³C) acetate* (0.6 g, 2.05 mmol) with NaOH (0.36 g, 9 mmol) in 10 mL MeOH: H_2O (9:1) for 2 h at room temperature. After the reaction was completed, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ^{13}C-labeled EDTA (0.435 g, 100% w/w) as an off-white solid.

^{1}H NMR (D_2O, 250 MHz)) δ ppm: 3.71 (s, 4H), 3.94 (d, *J*=4.5, 8H).^{13}C NMR (D_2O, 62.9 MHz) δ ppm: 51.7, 58.5, 170.8. HR-MS (ES^+) *m/z* $^{12}C_6{}^{13}C_4H_{17}N_2O_8$ requires 297.1119 $[M+H]^+$; found 297.1118 $[M+H]^+$.

(4) Bromo derivative of ^{13}C-labeled Pyro-EGTA
[2-({2-[2-(2-{bis[carboxy(¹³C)methyl]amino}ethoxy)-5-bromophenoxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetic acid].

The bromo-derivative of ^{13}C-labeled Pyro-EGTA was synthesized in 5 steps following a similar synthesis from the literature (Mishra *et al.*, 2011). The methyl groups were removed by using successive addition of borontribromide in the solution of *4-bromo-1,2-dimethoxybenzene* in anhydrous CH_2Cl_2 at -4°C to room temperature for 2 h. The reaction was quenched by MeOH and solvent was evaporated under reduced pressure to afford *4-bromobenzene-1,2-diol*. Stepwise alkylation of *4-bromobenzene-1,2-diol* with *tert*-butyl 2-bromoethylcarbamate in anhydrous MeCN (6 h at 80°C) afforded compound di-*tert*-butyl 2,2'-(4-bromo-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate, which was de-Boc by using 4N HCl dioxane solution (30 min at room temperature) to give 2,2'-(4-bromo-1,2-phenylene)bis(oxy)diethanamine in good yield.

A solution of 2,2'-(4-bromo-1,2-phenylene)bis(oxy)diethanamine (0.1 g, 0.37 mmol) and K_2CO_3 (0.40 g, 2.91 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl 2-bromoacetate-1^{13}C (0.185 mL, 1.64 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.55) to give tetramethyl 2-({2-[2-(2-{bis[carboxy(¹³C)methyl]amino}ethoxy)-5-bromophenoxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetate (0.06 g, 38%) as a yellow gum.

^{1}H NMR ($CDCl_3$, 250 MHz) δ ppm: 1.25 (t, J=7.0, 12H), 3.05 - 3.33 (m, 4 H), 3.68 (d, J=5.0 Hz, 8H), 3.99 - 4.29 (m, 12H), 6.73 (d, J=9.0 Hz, 1H), 6.92 - 7.13 (m, 2H).^{13}C NMR ($CDCl_3$, 62.9 MHz) δ ppm: 14.2, 53.4, 55.5, 56.4, 60.5, 112.9, 116.5, 119.3, 123.7, 149.2. 151.4, 171.4. HR-MS (ES^+) *m/z* $^{12}C_{22}{}^{13}C_4H_{40}BrN_2O_{10}$ requires 623.2001 $[M+H]^+$; found 623.1996 $[M+H]^+$.

The tetra-acid [2-({2-[2-(2-{bis[carboxy(¹³C)methyl]amino}ethoxy)-5-bromophenoxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetic acid] was obtained by deprotection of the ethyl group on tetramethyl 2-({2-[2-(2-{bis[carboxy(¹³C)methyl]amino}ethoxy)-5-bromophenoxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetate (0.06 g, 0.096 mmol) with NaOH (0.03 g, 0.75 mmol) in 10 mL MeOH: H_2O (9:1) for 2 h at room temperature. After reaction completion, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ^{13}C-labeled *m*-Br-PyroEGTA (0.05g, 100% w/w) as an off-white solid.

^{1}H NMR (D_2O, 250 MHz)) δ ppm: 3.02 (t, *J*=5 Hz, 4H), 3.18 - 3.45 (m, 8H), 4.08 (t, *J*=5 Hz, 4H), 6.92 (d, J=8.5 Hz, 1H), 7.07 - 7.26 (m, 2H).^{13}C NMR (D_2O, 62.9 MHz) δ ppm: 53.5, 58.3, 65.0, 112.4, 113.3, 115.2, 123.7, 146.6, 147.9, 179.4. HR-MS (ES^+) *m/z* $^{12}C_{14}{}^{13}C_4H_{24}BrN_2O_{10}$ requires 511.0749 $[M+H]^+$; found 511.0746 $[M+H]^+$.

NMR-based metal detection of thermally polarized compounds

NMR experiments were performed at 23°C at 500 MHz (^{13}C 125 MHz) or 250 MHz (^{13}C 62.5 MHz) and as indicated. pH values were measured with a standard pH electrode. All solutions were prepared in (3-(N-morpholino)propanesulfonic acid) MOPS buffer (concentrations as indicated in the respective experiment) and deuterated water in a ratio of 1:1, at pH 7.4 and were measured at 300K. ^{13}C-methanol was used as reference. A thermal spectrum was acquired (number of scans, ns, 1000, number of points, np, 16) using a 30° pulse and 90 degree pulse for decoupling. ^{13}C T_1 values were calculated from a fit to a series of spectra acquired with varying repetition times (from 0.01 s to 50 s). Compounds were dissolved in 15 mM MOPS with H_20/D_20 (1:1), 1 μL ^{13}C-methanol was used as an internal reference. For NMR-based determination of calcium concentrations, human serum was diluted to 50% v/v in 0.1 M MOPS buffer. The reference binding curve for calcium and ^{13}C-EGTA was obtained from 0.1 M MOPS buffer containing 0.45 mM Mg^{2+} and 4 g/dL Bovine Serum Albumin (BSA). In parallel, metal concentrations in the serum sample were also obtained from the routine colorimetric test conducted at the clinical chemistry department of the Hospital of the Technical University of Munich.

Dynamic Nuclear Polarization of ^{13}C-EGTA and ^{13}C-EDTA and NMR detection

Samples were prepared dissolving 4.2 mg of trityl radical (OX063, GE-Healthcare, Amersham UK) in 100 μL of 0.5 M of ^{13}C-EGTA (or ^{13}C-EDTA) solution (DMSO/D2O) and a trace amount of Dotarem (Guerbet, Birmingham UK). The samples were polarized via dynamic nuclear polarization on a HyperSense DNP polarizer system (Oxford Instruments Molecular Biotools, Oxford, UK) using 60-100 min of 94.1 GHz microwave irradiation at 1.3 K and 3.35 T. The polarized samples were dissolved in a heated and pressurized solution of 100 mM MOPS buffer prepared in D_2O/DMSO-d6 (9:1) with or without saturating concentrations of $CaCl_2$, leading to a ~13 mM solution of the hyperpolarized substrate with a pH of 7.4 +/- 0.2. Polarization levels were on average 1-2% for ^{13}C-EDTA and ^{13}C-EGTA and 4-5% for ^{13}C-EGTA-d_8. Build-up time constant was ~800 s. Hyperpolarized samples were measured at 1T (42.5 MHz proton and 10.8 Carbon) with NMR benchtop spectrometer (Spinsolve, Magritek) and NMR spectra were analyzed with Mestrenova 10 (Mestrelab Research). Final calcium concentrations were confirmed by ICP-MS. The average time interval between the end of polarization and start of the NMR measurement was 12.0 +/- 0.6 seconds.

Chemical Shift Imaging of ^{13}C-EGTA and ^{13}C-EDTA at equilibrium polarization

200 mM of either ^{13}C-EDTA or ^{13}C-EGTA (in 200 mM MOPS in D_2O buffer pH 7.4) were filled in an NMR tube with 5 mm diameter. The same solution with addition of 300 mM calcium was filled into a 3 mm diameter NMR tube that was positioned concentrically inside the 5 mm NMR tube. Microimaging was performed on a Bruker WideBore UltraShield 500WB PLUS with Avance3HD Console Great60 - 60A Gradient Amplifiers. The instrument was equipped with Micro5 gradient system (5G/cm/A -> 300G/cm = 3T/M), BLAX500 - 500W rf power amplifier, and 5 mm double tuned ^{1}H/^{13}C coil. The anatomical proton images were acquired with Multi-Slice Multi-Echo (MSME) pulse sequence (TR=5000ms, TE=2.8ms, FA=90°, FOV=8mm, and MTX=128). The CSI sequence was run with TR=8000ms, TE=0.865ms, FOV=8mm, and MTX=128. The data were pre-analyzed by CSI tool software [1] and analyzed with ParaVision 6.0 and TopSpin 3.2.

Chemical Shift Imaging of hyperpolarized ^{13}C-EGTA and ^{13}C-EDTA

CSI experiments with hyperpolarized chelators (10 mM) and saturating metal concentrations (15 mM) were performed on a 7T small animal scanner (GE/Agilent MRI 901 7T). A fast Spin Echo se-

quence was used to acquire the proton images with a dual-tuned ^{1}H-^{13}C-volume coil for radiofrequency transmission and reception. The Fast Spin Echo was acquired using 256 steps of frequency and phase encoding, TE=20 ms, TR=3000 ms, FA=90°, echo train length=4, bandwidth=15.63, FOV=6 cm and slice thickness=1 mm. The ^{13}C scans were acquired using a Flex Surface Coil $^{13}C/^{1}H$ (RAPID Biomedical GmbH). A spectro-spatial (SPSP) IDEAL spiral [2] sequence was used for fast Chemical Shift Imaging with TR=125 ms, FA=8°, FOV=6 cm, slice thickness=8 mm. Chemical shifts were referenced against ^{13}C-Urea measured before each experiment. Data processing was performed using custom-written routines [3] in MATLAB (R2015a, Mathworks).

SUPPLEMENTAL REFERENCES

(1) Flögel, U.; Jacoby, C.; Gödecke, A.; Schrader, J. *Magn Reson Med* **2007**, *57* (1), 50.

(2) Wiesinger, F.; Weidl, E.; Menzel, M. I.; Janich, M. A.; Khegai, O.; Glaser, S. J.; Haase, A.; Schwaiger, M.; Schulte, R. F. *Magnetic Resonance Medicine* **2012**, *68* (1), 8.

(3) Durst, M.; Koellisch, U.; Frank, A.; Rancan, G.; Gringeri, C. V.; Karas, V.; Wiesinger, F.; Menzel, M. I.; Schwaiger, M.; Haase, A.; Schulte, R. F. *NMR Biomed.* **2015**, *28* (6), 715.