Published in final edited form as:

Chemistry. 2016 January 4; 22(1): 264–271. doi:10.1002/chem.201503942.

Chemical Exchange Saturation Transfer (CEST) Agents: Quantum Chemistry and MRI

Jikun Li^[a], Xinxin Feng^[a], Wei Zhu^[a], Nikita Oskolkov^[b], Prof Michael T. McMahon^{*,[b],[c]}, and Prof. Eric Oldfield^{*,[a]}

^[a] Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews, Urbana, IL 61801 (USA)

^[b] The Russell H. Morgan Department of Radiology, The Johns Hopkins University School of Medicine, 991 N. Broadway Baltimore, Maryland 21287 (USA)

^[c] F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, 707 N. Broadway, Baltimore, MD 21287 (USA)

Abstract

Diamagnetic chemical exchange saturation transfer (CEST) contrast agents offer an alternative to Gd³⁺-based contrast agents for MRI. They are characterized by containing protons that can rapidly exchange with water and it is advantageous to have these protons resonate in a spectral window that is far removed from water. Here, we report the first results of DFT calculations of the ¹H nuclear magnetic shieldings in 41 CEST agents, finding that the experimental shifts can be well predicted ($R^2 = 0.882$). We tested a subset of compounds with the best MRI properties for toxicity and for activity as uncouplers, then obtained mice kidney CEST MRI images for three of the most promising leads finding **16** (2,4-dihydroxybenzoic acid) to be one of the most promising CEST MRI contrast agents to date. Overall, the results are of interest since they show that ¹H NMR shifts for CEST agents—charged species—can be well predicted, and that several leads have low toxicity and yield good *in vivo* MR images.

Keywords

CEST MRI; contrast agents; salicylate; density functional theory

Introduction

There is currently considerable interest^[1] in the development of new contrast agents for magnetic resonance imaging (MRI) that function by providing a source of exchangeable protons that resonate far downfield from bulk water. Saturation of these protons results, via chemical exchange, in water signal loss due to saturation transfer. This can be readily measured and used to produce contrast in MR images^[2]. For example, the established CT (X-ray computed tomography) contrast agent iopamidol has exchangeable amide protons

*eoldfield@illinois.edu; mtmcmaho@gmail.com.

Supporting information for this article is given via a link at the end of the document.

with chemical shifts of ~4.3, 5.5 ppm from H₂O—about 9–10 ppm downfield from tetramethylsilane—and has been used in MR imaging [3]. There are two main classes of CEST agents: diamagnetic CEST (diaCEST) agents, organic compounds with labile protons, and paramagnetic CEST (paraCEST) agents, complexes containing paramagnetic metal ions such as some lanthanides^[4], iron^[5] or nickel^[6]. Typical compounds evaluated as diaCEST agents include: iopamidol, glucose^[7], glutamate^[8], creatine^[9], L-arginine^[2a, 10], glycosaminoglycans^[8a, 11], barbituric acid^[12], thymidine derivatives^[13] and human protamine^[14]. In each of these systems the labile protons resonate ~1–6 ppm downfield from water, which can present sensitivity limitations as compared to paraCEST reagents^[1d] when using clinical 3 T scanners, although the lack of metal ions might be advantageous from a toxicity perspective.

We recently reported a series of diaCEST agents having highly deshielded labile protons^[1c-e] including salicylic acids such as **36** (Figure 1; the numbering system from our previous work is used here for clarity) and anthranilic acids such as **38**, with resonances far removed from water—as much as 12 ppm downfield from water in the case of **36**. This is an extremely deshielded value when compared to the established proton chemical shift standard of TMS (tetramethylsilane), corresponding to a chemical shift of ~17 ppm downfield from TMS (i.e. δ~17 ppm, using the IUPAC chemical shift scale). This chemical shift approaches the most highly deshielded ¹H NMR chemical shifts known, which are found in the solid-state with potassium hydrogen malonate^[15] and potassium hydrogen maleate^[16]. In these systems, the acidic protons bridge two carboxylates and form shared-electron, covalent bonds^[17], the experimental (solid-state) NMR chemical shifts being^[18] ~20.5–21 ppm downfield from TMS (i.e. ~15 ppm downfield from water). Here, we sought to determine to what extent it might be possible to compute the chemical shifts seen in a series of diamagnetic CEST agents, in water—expected to be a challenging prospect absent any crystal structures—though if successful this ability could help facilitate the discovery of new CEST agents with improved sensitivity. Also of interest with e.g. **36** (and many other CEST agents) is that they contain carboxylic acids groups, weak acids, as well as phenol groups, both of which might make such compounds uncouplers of oxidative phosphorylation, leading to cytotoxicity. For example, **36** is a nitrophenol and compounds such as 2,4-dinitrophenol are classic uncouplers, collapsing the proton motive force and inhibiting ATP synthesis, so we tested a subset of compounds for activity as uncouplers, as well as for their toxicity to a human cell line. We also explored the ability to predict shifts, exchange rates and contrast using mathematical descriptors and finally, we tested three of the more interesting compounds for *in vivo* CEST MRI activity.

Results and Discussion

We first investigated computationally the chemical shifts of all of the compounds shown in Figure 1, whose saturation offset behavior we reported earlier^[1d], by using density functional theory (DFT). By way of an introduction to the results: in CEST NMR, the experimental “saturation offset” (δ^{expt}) is given in ppm from H₂O (taken as 0 ppm). The actual chemical shift of water is ~5 ppm (depending on temperature and pH) downfield from the IUPAC standard, tetramethylsilane, TMS. In chemical shielding (σ) calculations, the reference is the bare nucleus and TMS is ~5 ppm more shielded than water. The

experimental shielding of pure liquid water (at 34.7°C) is 25.8 ppm^[19]. There are thus 3 sets of numbers: saturation offsets (δ^{expt}); chemical shifts (δ , in ppm from TMS), and computed magnetic shieldings (σ , in ppm from the bare nucleus). Magnetic shieldings are also tensor properties and we report here their principal components, σ_{11} , σ_{22} and σ_{33} . The isotropic shielding is $\sigma_{\text{iso}} = 1/3 \text{ Tr } \sigma$, that is, $1/3 (\sigma_{11} + \sigma_{22} + \sigma_{33})$.

The compounds we investigated are expected to be ionized in aqueous solution at neutral pH values and shielding calculations for charged species in solution are anticipated to be not without difficulty since the (static and dynamic) structures of the species present are not well defined. The CEST agents are expected to ionize, but they will have associated counter-ions as well as complex inter- and intra-molecular hydrogen bonding, and solvent interactions. Nevertheless, we hypothesized that a major contribution to the variations in shielding would be due to intramolecular H-bond interactions and that it might be possible to use density functional theory, incorporating various solvation models, to mimic solvent effects on H-bonding, and thence, chemical shielding. We first performed shielding calculations for 12 representative compounds (**1**, **20-22**, **27**, **36-42**; numbering is the same as in Ref.^[1d]) whose resonance frequencies cover the full δ^{expt} range of 4.8-12 ppm (downfield from water). Results are shown in Table 1. We used the ionized forms shown in Figure 1 as input structures (all carboxyl groups present as carboxylates) and the Gaussian 09 (Rev. D01) program^[20] for geometry optimization, as well as shielding calculations.

In an initial set of calculations we used the GIAO (gauge including atomic orbitals^[21]) approach together with six different structure optimization/solvation approaches: **a**, gas phase optimization/gas phase NMR shielding calculations; **b**, gas phase optimization but with the “SMD”, universal solvation model^[22] in NMR; **c**, SMD optimization but gas phase-NMR; **d**, SMD for optimization and NMR; **e**, PCM (polarizable continuum model^[23]) for both optimization and NMR; **f**, CPCM (polarizable conductor PCM^[24]) for both optimization and NMR; **g**, SMD for optimization and NMR but using IGAIM (individual gauges for atoms in molecules^[25]) and **h**, as **g** but using CSGT (continuous set of gauge transformations^[21]). As can be seen in Table 1, the gas phase results **a** for the shielding are very bad ($R^2 = 0.144$). They improve slightly in **b** ($R^2 = 0.379$), where the SMD solvation model was used during NMR shielding calculations, and significantly with the SMD-optimized structure while retaining the gas phase NMR calculation ($R^2 = 0.835$), **c**. Use of SMD (**d**), PCM (**e**) or CPCM (**f**) during both optimization and shielding calculations all gave good results, the best ($R^2 = 0.881$) being with the SMD model where the average absolute deviation between experimental and calculated shifts was 0.565 ppm, Table 1. We also tested the use of IGAIM (**g**) and CSGT (**h**) shielding calculations: the correlations were both good ($R^2 = 0.87$) as were the average deviations (0.569 ppm). We then evaluated the absolute shieldings (σ_{iso}) for all 41 compounds shown in Figure 1 using (as in the test set) B3LYP/6-311 G (2d,p)/SMD for optimization and B3LYP/TZVPP/SMD for the shielding tensor calculations, with either GIAO, IGAIM or CSGT gauge transformations, all using the Gaussian 09 (Rev. D01) program. Results are shown in Supporting Information Table S1. As expected, there is little difference between the different gauge transformation models and all 3 methods gave good R^2 values, the best being GIAO ($R^2 = 0.882$) which, with this large data set gave a 0.424 ppm average absolute deviation between theory and experiment and a

slope of -0.992 between the computed shielding and experimental saturation offset values, Supporting Information Table S1. The experimental saturation offset (δ^{expt}) versus computed shielding correlation is shown in Figure 2A and has an intercept of ~ 25 ppm. Since the experimental shift offsets are measured from water ($\delta^{\text{expt}} = 0$) this intercept is consistent with the ~ 26 ppm absolute shielding of water reported previously. The principal components of the calculated shielding tensor elements are given in Table 2 and show that the increases in overall shielding arise from the increases in σ_{11} and σ_{22} , as can be seen in Figure 2B.

The 41 predicted chemical shieldings are in surprisingly good accord with experiment given that we are investigating solution NMR chemical shifts (or δ^{expt} , from H_2O) and have used geometry optimized anionic structures (without counter-ions) in the shielding calculations, as opposed to the use of e.g. highly accurate neutron structures, periodic boundary conditions and solid-state NMR chemical shifts. However, this may be less surprising given the observation that $\text{O}(\text{N})\text{-H}\cdots\text{O}$ hydrogen bonds in crystals, organic solvents and water have similar lengths and potential energy surfaces^[26]. What the results of Table 1 do clearly show is that the key factor in obtaining a good correlation between experimental shifts and computed shieldings is the incorporation of a solvent model in the geometry optimization, not in the shielding calculations. A comparison of the geometries of the 12 test set structures optimized with or without the SMD solvation model, is shown in Table S2 and indicates that in many cases the carboxylate groups are protonated, as opposed to the phenolic oxygen, and this appears to be the root cause of the failure of the gas phase shielding calculations. For example, with salicylic acid (**1**) the X-H (here, X = O; could be X = N in other systems; the H-bond partner is Y, the carboxyl(ate) oxygen) bond length is $1.01 \text{ \AA}$, about the same as in e.g. **36**, when SMD is used in the geometry optimization calculations. However, in the gas phase optimization calculation, the phenolic O is no longer protonated (O-H distance = $1.49 \text{ \AA}$, Table S2). The H...O H-bond interaction distances all undergo corresponding changes, Table S2. Computed structural and shielding data for all compounds using the SMD approach is given in Table S3. As can be seen in Figure 2C, the computed isotropic shieldings are well correlated with the computed XH...Y distances, that is, the distance between the phenolic OH (or anthranilic acid NH), and the adjacent carboxylate oxygen O^- , the R^2 value being 0.847 . The overall X...Y distance is also well correlated with σ_{iso} , though the X-H distance is not correlated, Figure 2C—because, of course, there is essentially no range in these single bond lengths.

The orientations of the principal components of the shielding tensor are shown in Figure 3 for the salicylate **36** (Figure 3A) and the anthranilate **38** (Figure 3B). As can be seen in Figure 3, in both cases the most shielded component (σ_{33}) is aligned approximately along the XH (i.e. phenol O-H or anthranilate N-H) bond vector. However, as shown in Figure 2B, this highly shielded component does not vary significantly amongst all of the compounds investigated and it is the orthogonal components, σ_{11} and σ_{22} , that vary from one compound to another. The XH...Y distances as well as the XY distances do vary considerably and are well correlated with the overall shielding: shorter distances correlate with less shielding (i.e. downfield shifts). This is what is expected, either from hydrogen bonding or from a purely electrostatic interaction, and similar effects in the fluorobenzene-HF model system are seen

with full *ab initio*, shielding polarizability as well as point charge calculations^[27]. Given the large range of shifts it seems likely that an electrostatic interaction might best describe the more shielded systems (long distances) while the more deshielded systems approach partial covalence, as we reported earlier^[28]. However, the truly covalent (strongly hydrogen bonded) species such as the malonate and maleate acid salts resonate even more downfield, ~20.5 – 21 ppm downfield from TMS (~15 ppm downfield from water) since the acidic protons bridge two carboxylates, forming shared-electron, covalent bonds^[17].

Next, we sought to determine whether our compounds acted as protonophore uncouplers and/or were toxic to human cells. We focused on compounds which we previously reported (see Figure 3 in Ref^[1d]) to be high performance CEST agents^[1d] in aqueous solution. A potential concern with some of the compounds investigated previously^[1c-e], e.g., **27** and **36**, is that in addition to being salicylates, they contain highly electron-withdrawing groups *ortho* or *ortho, para* to the phenolic OH group, so they might have activity as protonophore uncouplers, as does e.g. 2,4-dinitrophenol, so could be rather cytotoxic. We thus first selected a subset of compounds with promising saturation offset (δ^{expt}) and exchange rate (k_{ex}) properties for investigation. Figure 4A shows simulated CEST contrast results at 3T and 5 mM concentrations based on the experimental offset (δ^{expt}) and exchange rates. The most promising compounds are shown by the green circles (compound numbers are given in Supporting Information Table S4). Other compounds are shown by black circles and were not further investigated.

The first assay was inhibition of the growth of the human embryonic kidney cell line HEK293^[29]. The second assay was uncoupling in *E. coli* inverted membrane vesicles in which ATP-powered H⁺-translocation through the ATPase is collapsed in the presence of protonophore uncouplers (as measured by an increase in ACMA fluorescence^[30]). Representative cytotoxicity and uncoupling results are shown in Figures 4B,C and full numerical results are given in Supporting Information Table S4. Only 3 compounds (**27**, **37** and **40**) exhibited cell growth inhibition, and all three were uncouplers. It seems clear that **27** is an uncoupler because it has two strongly electro-withdrawing groups *ortho* and *meta* to the phenol group, just as with 2,4-dinitrophenol, and **37**, fenamic acid, is a known uncoupler^[31]. The origin of the toxicity of **40** is not known.

We next investigated whether it might be possible to carry out shift, contrast as well as exchange rate predictions using a multiple descriptor^[32] approach which, if successful, could facilitate library screening and compound design. For the shift predictions, we found an experimental *versus* prediction R^2 of 0.623 ($p=8.45\times10^{-8}$; Supporting Information Figure S1A), worse than with the DFT calculations although the calculations are far more rapid and this approach could be of future use in screening large libraries of compounds *in silico*. The correlation was worse for the exchange rate ($R^2=0.56$, $p=1.1\times10^{-6}$) and for contrast %, worse still ($R^2=0.48$, $p=2.2\times10^{-4}$, Figures S1B, C). One possibility for the poor agreement with these two latter properties could, however, be that the experimental values are more difficult to determine accurately than are the NMR shifts.

Finally, we investigated whether any of the compounds that were predicted to have good NMR properties *in vitro* and no/low toxicity in the human cell growth/uncoupler assays gave

good CEST MRI results in mice (and had no or low toxicity) by following their kidney uptake at 11.7 Tesla. Results for **16** (2,4-dihydroxybenzoic acid) are shown in Figure 5. A T_2 weighted image is shown in Figure 5A and a pre-injection CEST overlay image is shown in Figure 5B. As is shown in the post-injection image results in Figures 5C (20 minutes) and 5D (50 minutes), the calyces of the kidneys are clearly visualized (regions outside the kidneys are masked due to visceral movements). The time courses of the asymmetric magnetization transfer ratios (MTR_{asym}) for **1** (salicylic acid), **16**, and **43** are shown in Figures 6A-C and superimposed, in Figure 6D. Clearly, the most promising results are found with **16**.

Compound **16** is an analog of salicylic acid **1**, and we were interested in several similar analogs that also had good NMR properties, such as **42** and **43**. However, **42** was very toxic to cells due, perhaps, to its ability to be oxidized to the quinone **42a**, Figure 7, setting up a redox shuttle. Both **42** and **43** have two sets of the OH/CO₂H substituents found in salicylic acid but in **43**, the OH groups occupy *meta* positions, so the ring cannot be metabolized to a quinone. Unfortunately, **43** had relatively low CEST activity *in vivo*. Removal of one carboxyl group to give **16**, a hydroxy-analog of salicylic acid, gave the best results, Figure 6B, but incorporation of a nitro-group (**36**, with excellent NMR properties) resulted in a compound with relatively low solubility, and addition of a 3-MeO gave a species, **32**, that was toxic to mice. So, addition of a single OH group to **1** to yield the relatively slowly metabolizing compound, **16**, a.k.a. β -resorcylic acid, found in human plasma after cranberry juice administration^[33], resulted in the most promising lead, with good *in vivo* activity and no apparent toxicity.

Conclusions

In summary, the results we have described above are of interest for two distinct reasons. First, they represent the first accurate calculations of CEST NMR shifts, achieved by using density functional theory methods with several different solvation models. Since the structures are not obtained from crystallography and the species of interest are charged, the correlations between theory and experiment are very encouraging. A more empirical descriptor-based method gave less good accord with experiment, although exchange rate predictions were significant. Second, we screened a series of 19 compounds for toxicity in a human cell growth inhibition assay, as well as for activity as uncouplers, finding several compounds that had good CEST MRI properties that also appeared non-toxic. We then tested a subset of 4 such compounds *in vivo* in mice finding that the cranberry juice metabolite **16** displayed excellent *in vivo* contrast, as well as no apparent toxicity.

Experimental Section

DFT calculation of magnetic shielding tensors

All calculations were performed with Gaussian 09 Rev. D01^[20]. Structures of compounds were converted to 3D coordinates with Avogadro (<http://avogadro.cc>). In the first step, geometry optimization was performed with the B3LYP functional^[34] and a 6-311G+(2d,p)^[35] basis set, with (6-311(d,p) for the iodines in compound **28**^[36]). Calculations were performed using either gas phase or solvation models (in water): SMD^[22], PCM^[23] and

CPCM [24]. The optimized geometries were then used to calculate the NMR shielding tensors using the B3LYP functional and a TZVPP [37] basis set, with one of the following methods: GIAO [21], IGAIM [25] and CGST [21], once again with or without solvation. After an initial run with 12 compounds, a production run for all 41 compounds was performed using SMD and GIAO.

HEK293 Cell Toxicity

HEK293 cell growth inhibition assays were carried out as described previously [29]. A broth microdilution method was used to determine the growth inhibition IC₅₀ values. Briefly, $\sim 5 \times 10^4$ cells suspended in 100 μ L of DMEM supplemented with 10% fetal bovine serum (FBS), 4.5 g/L glucose and L-glutamine and preserved with 1% penicillin–streptomycin were seeded in 96-well plates (Corning Inc., Corning, NY) and incubated at 37 °C in a 5% CO₂ atmosphere. The cells were cultured for 36 h before the assays. The cells were then incubated with 400 μ M, 200 μ M, 100 μ M, 25 μ M, 12.5 μ M and 6.25 μ M of compounds and DMSO as a control for 2 h [29]. Then, an MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay (ATCC, Manassas, VA) was performed to obtain dose–response curves.

Uncoupling Activity

E. coli inverted membrane vesicles (IMVs) were prepared by three passages through a pre-cooled French pressure cell at 20,000 psi. The lysate was centrifuged at 14,000g at 4 °C for 20 min to remove unbroken cells. The supernatant was centrifuged at 370,000g at 4 °C for 1 h, and the pellet, consisting of the IMVs, was washed with 50 mM MOPS–KOH (pH 7.5), 2 mM MgCl₂. After the second centrifugation step, membranes were resuspended in 50 mM MOPS–KOH (pH 7.5), 2 mM MgCl₂, 10% glycerol, and stored at –80 °C. Proton translocation into IMVs was measured by the decrease of ACMA fluorescence. The excitation and emission wavelengths were 410 and 480 nm, respectively. IMVs (0.1 mg/mL membrane protein) were pre-incubated at 37 °C in 10 mM HEPES–KOH (pH 7.5), 100 mM KCl, 5 mM MgCl₂ containing 2 μ M ACMA, and the baseline was monitored for 5 min. The reaction was then initiated by adding 1 mM ATP. When the signal had stabilized, compounds were added and proton translocation was measured, fluorimetrically.

3 T CEST Simulations

The Bloch-McConnell equations were numerically solved for a two-pool system for a long continuous wave saturation pulse over a range of solute proton k_{ex} and δ^{expt} to determine which compounds are the most promising CEST contrast agents for use on 3 T scanners. The simulation parameters were: $t_{\text{sat}} = 3$ s, $X_{\text{CA}} = 10$ mM, $T_{1w} = 3$ s, $T_{2w} = 0.1$ s, $T_{1s} = 3$ s, $T_{2s} = 0.1$ s, $\omega_1 = 4$ μ T.

Animal Imaging

All experiments conducted with mice were performed in accordance with protocols approved by the Johns Hopkins University Institutional Animal Care and Use Committee (IACUC), animal protocol #MO13M251 CEST imaging of mice.

BALB/c mice weighing 20–25 g (Charles River Laboratories, Wilmington, MA) were maintained under specific pathogen free conditions in the animal facility of Johns Hopkins University. For MRI, mice were anesthetized by using 0.5–2% isoflurane and placed in a 23 mm transmit/receive mouse coil. Breath rate was monitored throughout *in vivo* MRI experiments using a respiratory probe. 100 μ L volumes of 0.25 M salicylate solutions in PBS (pH 7) were slowly injected *via* a catheter into the tail vein. *In vivo* images were acquired on a Bruker Biospec 11.7 T horizontal MR scanner, with one axial slice (1.0 mm thick) crossing both renal centres chosen for the kidney uptake study. CEST images were acquired both pre- and post-injection. The CEST Z-spectra were acquired by incrementing saturation frequency every 0.3 ppm from –10.1 to –9.2 and +9.2 to +10.1 ppm; TR = 5.5 s, effective TE = 4 ms, matrix size = 64 $\times$ 48, slice thickness of 1.0 mm, ω_1 = 5.9 μ T, repeatedly.

Image Post-processing

MR images were processed using custom-written Matlab scripts with the CEST contrast quantified by calculating the asymmetry in the magnetization transfer ratio (MTR_{asym}) using $MTR_{asym} = (S_{-\omega} - S_{+\omega}) / S_0$ for OH protons at the frequency offset from water (ω) for the compound. S_0 is the signal of water without saturation, S with saturation and therefore frequency dependent. The relative MTR_{asym} was calculated by subtracting the pre-contrast values from that of the post-contrast values.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the United States Public Health Service (National Institutes of Health grants GM065307, CA158191, and EB015031), by a Harriet A. Harlin Professorship, and by the University of Illinois Foundation/Oldfield Research Fund.

References

- [1]. a) Longo DL, Dastru W, Digilio G, Keupp J, Langereis S, Lanzardo S, Prestigio S, Steinbach O, Terreno E, Uggeri F, Aime S. *Magnet. Reson. Med.* 2011; 65:202. b) Aime S, Calabi L, Biondi L, De Miranda M, Ghelli S, Paleari L, Rebaudengo C, Terreno E. *Magnet. Reson. Med.* 2005; 53:830. c) Yang X, Song X, Li Y, Liu G, Ray Banerjee S, Pomper MG, McMahon MT. *Angew. Chem. Int. Ed. Engl.* 2013; 52:8116. [PubMed: 23794432] d) Yang X, Yadav NN, Song X, Ray Banerjee S, Edelman H, Minn I, van Zijl PC, Pomper MG, McMahon MT. *Chemistry.* 2014; 20:15824. [PubMed: 25302635] e) Song X, Yang X, Ray Banerjee S, Pomper MG, McMahon MT. *Contrast Media Mol. Imaging.* 2015; 10:74. [PubMed: 24771546]
- [2]. a) Liu GS, Song XL, Chan KWY, McMahon MT. *NMR Biomed.* 2013; 26:810. [PubMed: 23303716] b) Hancu I, Dixon WT, Woods M, Vinogradov E, Sherry AD, Lenkinski RE. *Acta Radiol.* 2010; 51:910. [PubMed: 20828299] c) Terreno E, Castelli DD, Aime S. *Contrast Media Mol. Imaging.* 2010; 5:78. [PubMed: 20419761] d) van Zijl PCM, Yadav NN. *Magnet. Reson. Med.* 2011; 65:927.
- [3]. Sun PZ, Longo DL, Aime S. *Proc. Intl. Soc. Mag. Reson. Med.* 2011; 19:4496.
- [4]. a) Zhang S, Winter P, Wu K, Sherry AD. *J. Am. Chem. Soc.* 2001; 123:1517. [PubMed: 11456734] b) Ferrauto G, Castelli DD, Terreno E, Aime S. *Magnet. Reson. Med.* 2013;

69:1703.c) Ali MM, Liu GS, Shah T, Flask CA, Pagel MD. *Acc. Chem. Res.* 2009; 42:915. [PubMed: 19514717]

- [5]. Dorazio SJ, Tsitovich PB, Sifers KE, Sperry JA, Morrow JR. *J. Am. Chem. Soc.* 2011; 133:14154. [PubMed: 21838276]
- [6]. Olatunde AO, Dorazio SJ, Sperry JA, Morrow JR. *J. Am. Chem. Soc.* 2012; 134:18503. [PubMed: 23102112]
- [7]. a) Chan KW, McMahon MT, Kato Y, Liu G, Bulte JW, Bhujwala ZM, Artemov D, van Zijl PC. *Magnet. Reson. Med.* 2012; 68:1764.b) Yadav NN, Xu J, Bar-Shir A, Qin Q, Chan KW, Grgac K, Li W, McMahon MT, van Zijl PC. *Magnet. Reson. Med.* 2014; 72:823.c) Walker-Samuel S, Ramasawmy R, Torrealdea F, Rega M, Rajkumar V, Johnson SP, Richardson S, Goncalves M, Parkes HG, Arstad E, Thomas DL, Pedley RB, Lythgoe MF, Golay X. *Nat. Med.* 2013; 19:1067. [PubMed: 23832090] d) Nasrallah FA, Pages G, Kuchel PW, Golay X, Chuang KH. *J. Cerebr. Blood F. Met.* 2013; 33:1270.
- [8]. a) Singh A, Haris M, Cai K, Kassey VB, Kogan F, Reddy D, Hariharan H, Reddy R. *Magnet. Reson. Med.* 2012; 68:588.b) Kogan F, Singh A, Debrosse C, Haris M, Cai K, Nanga RP, Elliott M, Hariharan H, Reddy R. *Neuroimage.* 2013; 77:262. [PubMed: 23583425] c) Cai K, Singh A, Roalf DR, Nanga RP, Haris M, Hariharan H, Gur R, Reddy R. *NMR Biomed.* 2013; 26:1278. [PubMed: 23553932]
- [9]. a) Kogan F, Haris M, Debrosse C, Singh A, Nanga RP, Cai K, Hariharan H, Reddy R. *J. Magn. Reson. Imaging.* 2014; 40:596. [PubMed: 24925857] b) Kogan F, Haris M, Singh A, Cai K, Debrosse C, Nanga RP, Hariharan H, Reddy R. *Magnet. Reson. Med.* 2014; 71:164.
- [10]. Liu GS, Moake M, Har-el YE, Long CM, Chan K W Y, Cardona A, Jamil M, Walczak P, Gilad AA, Sgouros G, van Zijl PCM, Bulte JWM, McMahon MT. *Magnet. Reson. Med.* 2012; 67:1106.
- [11]. a) Ling W, Regatte RR, Navon G, Jerschow A. *Proc. Natl. Acad. Sci. USA.* 2008; 105:2266. [PubMed: 18268341] b) Kim M, Chan Q, Anthony MP, Cheung KM, Samartzis D, Khong PL. *NMR Biomed.* 2011; 24:1137. [PubMed: 21387446] c) Saar G, Zhang B, Ling W, Regatte RR, Navon G, Jerschow A. *NMR Biomed.* 2012; 25:255. [PubMed: 22253087]
- [12]. Chan K W Y, Yu T, Qiao Y, Liu Q, Yang M, Patel H, Liu GS, Kinzler KW, Vogelstein B, Bulte JWM, van Zijl PCM, Hanes J, Zhou SB, McMahon MT. *J. Control. Release.* 2014; 180:51. [PubMed: 24548481]
- [13]. Bar-Shir A, Liu GS, Liang YJ, Yadav NN, McMahon MT, Walczak P, Nimmagadda S, Pomper MG, Tallman KA, Greenberg MM, van Zijl PCM, Bulte JWM, Gilad AA. *J. Am. Chem. Soc.* 2013; 135:1617. [PubMed: 23289583]
- [14]. Oskolkov N, Bar-Shir A, Chan K W Y, Song XL, van Zijl PCM, Bulte JWM, Gilad AA, McMahon MT. *ACS Macro Lett.* 2015; 4:34. [PubMed: 25642384]
- [15]. Soler MJ, Riera M, Battle D. *Exp. Diabetes Res.* 2012; 2012:616313. [PubMed: 22461787]
- [16]. Li J, Shi M, Zhang H, Yan L, Xie M, Zhuang L, Zhu Y, Chen J. *Clin. Nephrol.* 2012; 77:305. [PubMed: 22445474]
- [17]. Currie M, Speakman JC. *J. Chem. Soc. A.* 1970:1923.
- [18]. Harris RK, Jackson P, Merwin LH, Say BJ, Hägele G. *J. Chem. Soc., Faraday Trans. 1.* 1988; 84:3649.
- [19]. a) Winkler PF, Kleppner D, Myint T, Walther FG. *Phys. Rev. A.* 1972; 5:83.b) Phillips WD, Cooke WE, Kleppner D. *Phys. Rev. Lett.* 1975; 35:1619.
- [20]. Frisch, MJ.; Trucks, GW.; Schlegel, HB. Gaussian, Inc.; Wallingford, CT, USA: 2009.
- [21]. Cheeseman JR, Trucks GW, Keith TA, Frisch MJ. *J. Chem. Phys.* 1996; 104:5497.
- [22]. Marenich AV, Cramer CJ, Truhlar DG. *J. Phys. Chem. B.* 2009; 113:6378. [PubMed: 19366259]
- [23]. Tomasi J, Mennucci B, Cammi R. *Chem. Rev.* 2005; 105:2999. [PubMed: 16092826]
- [24]. Cossi M, Rega N, Scalmani G, Barone V. *J. Comput. Chem.* 2003; 24:669. [PubMed: 12666158]
- [25]. Keith TA, Bader RFW. *Chem. Phys. Lett.* 1993; 210:223.
- [26]. Sigala PA, Ruben EA, Liu CW, Piccoli PM, Hohenstein EG, Martinez TJ, Schultz AJ, Herschlag D. *J. Am. Chem. Soc.* 2015; 137:5730. [PubMed: 25871450]

- [27]. a) de Dios AC, Pearson JG, Oldfield E. Science. 1993; 260:1491. [PubMed: 8502992] b) de Dios AC, Oldfield E. J. Am. Chem. Soc. 1994; 116:7453.
- [28]. Arnold WD, Oldfield E. J. Am. Chem. Soc. 2000; 122:12835.
- [29]. Lv W, Banerjee B, Molland KL, Seleem MN, Ghafoor A, Hamed MI, Wan B, Franzblau SG, Mesecar AD, Cushman M. Bioorgan. Med. Chem. 2014; 22:406.
- [30]. Li K, Schurig-Briccio LA, Feng X, Upadhyay A, Pujari V, Lechartier B, Fontes FL, Yang H, Rao G, Zhu W, Gulati A, No JH, Cintra G, Bogue S, Liu YL, Molohon K, Orlean P, Mitchell DA, Freitas-Junior L, Ren F, Sun H, Jiang T, Li Y, Guo RT, Cole ST, Gennis RB, Crick DC, Oldfield E. J. Med. Chem. 2014; 57:3126. [PubMed: 24568559]
- [31]. Masubuchi Y, Yamada S, Horie T. Biochem. Pharmacol. 1999; 58:861. [PubMed: 10449197]
- [32]. Mukkamala D, No JH, Cass LM, Chang TK, Oldfield E. J. Med. Chem. 2008; 51:7827. [PubMed: 19053772]
- [33]. Kai Z, Yuegang Z. J. Agr. Food Chem. 2004; 52:222. [PubMed: 14733499]
- [34]. Becke AD. J. Chem. Phys. 1993; 98:5648.
- [35]. a) Krishnan R, Binkley JS, Seeger R, Pople JA. J. Chem. Phys. 1980; 72:650. b) McLean AD, Chandler GS. J. Chem. Phys. 1980; 72:5639.
- [36]. Glukhovtsev MN, Pross A, McGrath MP, Radom L. J. Chem. Phys. 1995; 103:1878.
- [37]. Weigend F, Ahlrichs R. Phys. Chem. Chem. Phys. 2005; 7:3297. [PubMed: 16240044]

Figure 1.
Structures of compounds studied. The compound numbers are those used in Refs 4 and 5.

Figure 2.

Computed magnetic shieldings of the 41 compounds. (A) The experimental saturation offset (δ^{expt}) versus computed absolute shielding correlation. (B) The calculated magnetic shielding (σ_{iso}) versus tensor eigenvalues correlation. (C) The calculated magnetic shielding (σ_{iso}) versus distance correlation.

Figure 3.
The orientation of the shielding tensor elements for (A) salicylate **36** and (B) anthranilate **38**.

Figure 4.

Selection criteria and toxicity assays for diaCEST agents. (A) Simulated CEST contrast at 3T at 5 mM concentration according to δ^{expt} and k_{ex} . The green circles represent compounds selected with ($2000 \text{ s}^{-1} > k_{\text{ex}} > 350 \text{ s}^{-1}$) and to $\delta^{\text{expt}} > 4.5 \text{ ppm}$; black circles represent the remaining salicylates. (B) Effects of various compounds on viability of HEK293 (human embryonic kidney) cells. (C) Effects of selected compounds on collapsing the pH gradient in *E. coli* inverted membrane vesicles.

Figure 5.
In vivo contrast for **16**. (A) T_{2w} image. (B) Overlay MTR_{asy} (9.6 ppm) map pre-injection. (C) Overlay MTR_{asy} (9.6 ppm) map at 20 min post-injection. (D) Overlay MTR_{asy} (9.6 ppm) map at 50 min post-injection.

Figure 6. Dynamic time course of the MTR_{asym} (9.6 ppm) for regions of interest enclosing the whole left and right kidney calyces. $\omega_1 = 5.9 \mu T$ ($n = 2$) for (A) **1**, (B) **16**, and (C) **43**. (D) Superimposition of A to C.

Figure 7. Effects of ring substituents on efficacy of various salicylic acid (**1**) CEST MRI contrast agents. **42** and **43** had good NMR properties in vitro but were either toxic in cells (**42**) or had poor pharmacokinetics (**43**) *in vivo*. Adding an OH improved *in vivo* efficacy (**16**) but the methoxy **32** was toxic (*in vivo*) and the nitro **36** had poor solubility.

Table 1Results for benchmark shielding calculations^a.

		a	b	c	d	e	f	g	h
Compound #	Experimental Saturation Offset δ^{expt} (ppm)	Gas-opt, Gas-NMR	Gas-opt SMD-NMR	SMD-Opt gas-NMR	SMD	PCM	CPCM	SMD	SMD
		GIAO	GIAO	GIAO	GIAO	GIAO	GIAO	IGAIM	CSGT
01	9.3	12.3159	12.8430	15.4695	16.2038	15.0980	15.1017	16.9685	16.9670
20	9.5	13.1733	12.9859	13.5874	14.2329	12.6162	12.6142	14.9531	14.9510
21	9.0	13.0725	12.8555	14.3598	15.1533	13.2651	13.2681	15.8663	15.8646
22	9.3	13.2107	12.9734	13.9493	14.7672	13.4961	13.5120	15.5309	15.5293
27	10.5	13.8677	13.5975	14.6455	15.3640	13.8806	13.8855	16.2196	16.2209
36	12.0	13.8124	13.6974	12.2175	12.7743	11.4320	11.4499	13.5945	13.5939
37	4.8	14.8852	15.9267	18.5236	19.5947	18.5256	18.5296	20.1616	20.1637
38	6.3	15.1161	15.9722	18.3400	19.2678	17.0927	17.0963	19.7993	19.8026
39	7.3	13.5305	14.3524	16.3247	17.3947	15.9970	16.0021	18.0436	18.0472
40	7.8	12.9689	13.7155	16.2177	17.2646	15.9837	16.9727	17.8678	17.8708
41	9.3	10.2870	10.9643	14.0071	14.9339	13.8448	13.8555	15.9189	15.9243
42	8.3	15.7021	15.8651	17.0511	17.2294	16.3716	16.3791	18.0259	18.0247
Statistics									
R ²		0.144	0.379	0.835	0.881	0.847	0.824	0.870	0.870
Slope		−0.511	−0.775	−0.891	−0.877	−0.851	−0.813	−0.911	−0.911
Maximum Absolute Deviation		3.55	3.29	1.22	1.17	1.10	1.07	1.25	1.25
Average Absolute Deviation		1.32	1.09	0.667	0.565	0.627	0.714	0.569	0.568

^aB3LYP/6-311+G(2d,p)/SMD for geometry optimization and B3LYP/TZVPP/SMD for magnetic shielding calculations.

Table 2

DFT-calculated magnetic shielding tensor eigenvalues^a.

Comp ound #	Experi mental g_{expt} (ppm)	Calcu lated σ_{iso} (ppm)	Calculated Eigenvalues of σ Tensor (ppm)			Comp ound #	Experi mental g_{expt} (ppm)	Calcu lated σ_{iso} (ppm)	Calculated Eigenvalues of σ Tensor (ppm)		
			σ_{11}	σ_{22}	σ_{33}				σ_{11}	σ_{22}	σ_{33}
1	9.3	16.2038	0.4808	12.1384	35.9921	29	10.5	14.7437	-2.2778	10.6398	35.8693
5	9.5	16.0227	-1.2043	13.6568	35.6157	30	10.5	14.6886	-2.2636	10.5792	35.7502
8	7.8	17.1329	2.2819	13.9533	35.1636	31	9	15.997	-0.2876	11.4971	36.7814
10	8.5	16.9089	1.323	13.0403	36.3633	32	9.3	15.9252	-0.5822	11.4293	36.9286
11	8.5	17.1395	1.5375	13.252	36.6291	33	9	16.0212	-0.3169	11.4669	36.9138
12	9	16.2287	0.5605	12.3987	35.727	34	10.5	14.5844	-1.5549	10.0415	35.2665
13	9.5	15.9459	0.1462	12.1827	35.5087	35	9.5	15.7453	-0.3596	11.0767	36.5187
14	10.3	14.5067	-1.2327	10.6195	34.1272	36	12	12.7743	-4.0108	8.1399	34.1938
16	9.5	16.0617	0.4413	11.8401	35.9038	37	4.8	19.5947	5.5884	19.8844	33.3112
17	9.5	16.2315	0.5923	11.9141	36.1882	38	6.3	19.2678	8.6763	16.5069	32.6201
18	9.3	16.3844	0.6166	12.3844	36.1522	39	7.3	17.3947	4.7723	14.0667	33.3452
19	9.5	15.8398	0.1995	11.7274	35.5964	40	7.8	17.2646	5.9718	13.1947	32.6273
20	9.5	14.2329	-2.586	9.6492	35.6356	41	9.3	14.9339	0.453	16.1734	28.1751
21	9	15.1533	-1.1004	10.5572	36.0031	42	8.3	17.2294	1.583	13.3957	36.7262
22	9.3	14.7672	-1.5376	10.0058	35.8334	43	9.8	15.7588	0.0198	11.6624	35.5943
23	9.5	15.9625	-0.2561	11.4693	36.6743	44	9.5	15.0626	-1.6707	9.5312	37.3272
24	9.3	15.9855	-1.0153	10.9574	38.0145	A1	4.8	19.2877	5.1759	19.3325	33.3546
25	9.5	15.7426	-0.5874	11.2319	36.5834	A2	4.8	20.1646	7.8186	18.7743	33.9011
26	10.3	15.3213	-1.2752	10.823	36.4161	A3	7.3	17.7833	5.6766	14.7227	32.9506
27	10.5	15.364	-1.3911	10.8233	36.6599	A4	7	17.6648	5.0759	14.5602	33.3584
28	10.8	15.3306	-1.7147	10.5758	37.1307						

^aB3LYP/TZVPP/SMD.