Salicylic Acid and Analogs: Diamagnetic Chemical Exchange Saturation Transfer (diaCEST) Magnetic Resonance Imaging (MRI) Contrast Agents with Highly Shifted Exchangeable Protons

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1. GENERAL

Phantom Preparation: All compounds were purchased from Sigma Aldrich (St. Louis, MO). Samples were dissolved in 0.01M phosphate-buffered saline (PBS) at the desired concentrations, and titrated using high concentration HCl/NaOH to the desired pH. The solutions were placed into 1 mm glass capillaries and assembled in a holder for CEST MR imaging. The samples were kept in 37°C during imaging. Phantom CEST experiments were taken on a Bruker 11.7 Tesla vertical MR scanner, using a 20 mm birdcage transmit/receive coil. CEST images were acquired using a RARE (RARE = 8) sequence with CW saturation pulse length of 3 seconds and saturation field strength (ω_1) from 1.2 μT to 14.4 μT. The CEST Z-spectra were acquired by incrementing saturation frequency every 0.3 ppm from -15 to 15 ppm for phantoms; TR = 6 s, effective TE = 17 ms, matrix size = 64x48 and slice thickness of 1.2 mm.

Animal Imaging: 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. A 60 μL volume of a 0.25 M salicylic acid solution in PBS (pH 7) was 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 (1.5 mm thick) crossing both renal centre chosen for CEST screening. CEST images were acquired both pre- and post-injection. Image parameters were similar to those for the phantom except for TR/TE = 5s/15 ms, with optimized ω_1 = 7.2 μT.

2. ^{1}H-NMR SPECTRUM OF SALICYLIC ACID IN WATER

Salicylic acid (**1**) was dissolved in 0.01 M PBS with 10% deuterium oxide at the concentration of 25 mM and titrated with HCl/NaOH to pH 7.0. The ^{1}H-NMR was acquired on a 500M Bruker NMR spectrometer at room temperature. The spectra and proton assignment are shown in Figure S1. The C2-OH exchangeable proton was clearly observed at chemical shift 14 ppm from TMS.

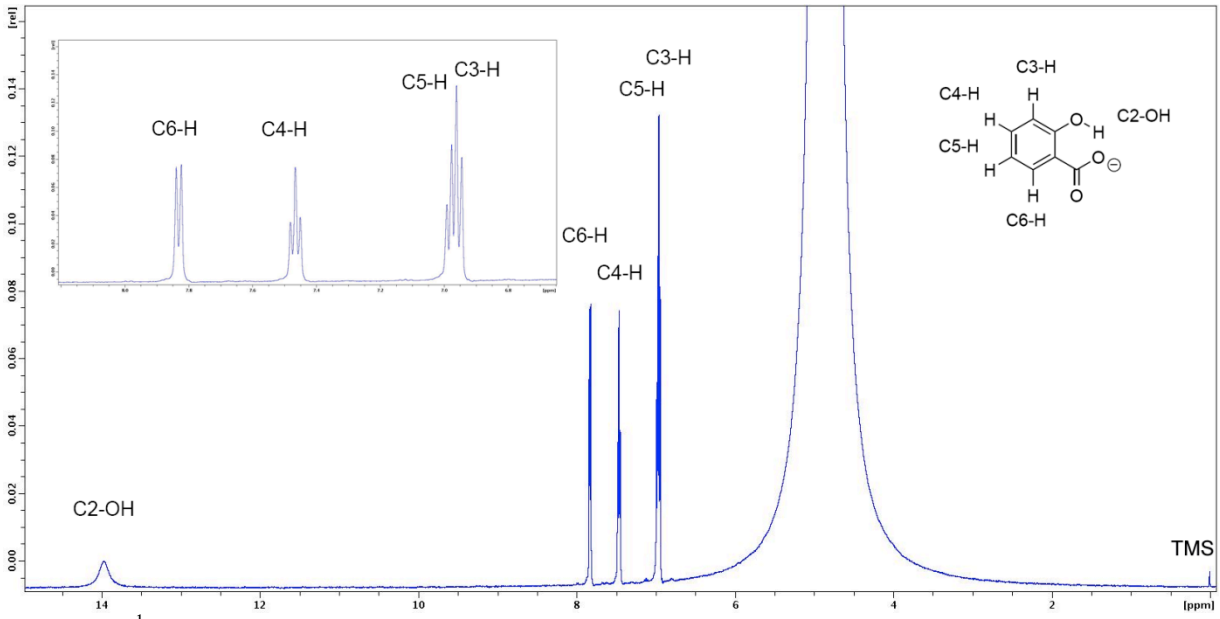


Figure S1. ^{1}H-NMR of salicylic acid (**1**) in water.

3. Z-SPECTRA OF SALICYLIC ACID AT DIFFERENT PH AND CONCENTRATION

A) The effect of pH on the contrast of salicylic acid (**1**) was tested at a concentration of 25 mM, ω_1 = 7.2 μT. The Z-spectra and MTR_{asym} of pH 5.8, 6.5, 6.8, 7.2, 7.6, 8.1, 11.7 were collected and shown in Figure S2. Maximal contrast was observed between pH 6.5 and 7.0.

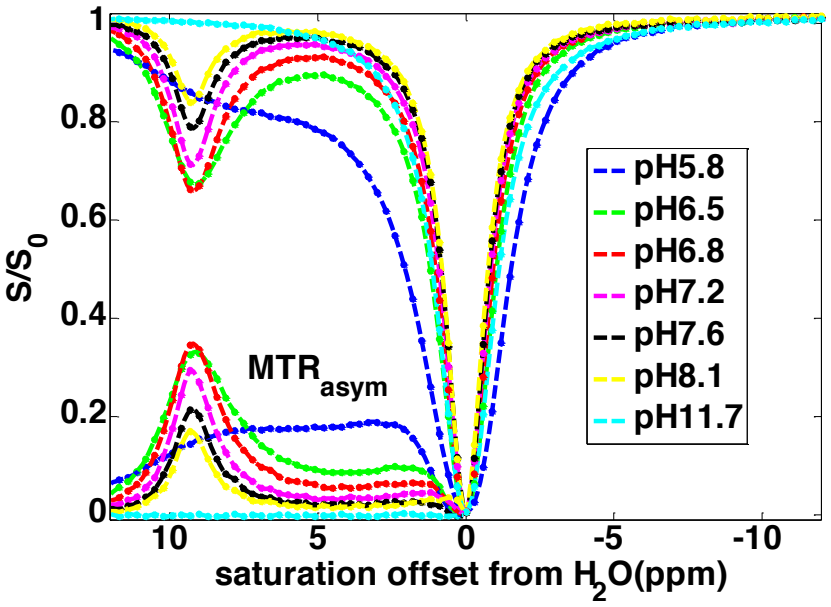


Figure S2. pH effect on the contrast of salicylic acid (**1**)

B) The concentration dependence of the contrast of salicylic acid (**1**) at pH 7.3-7.4 was measured at a saturation field strength (ω_1) = 7.2 μT. The Z-spectra and MTR_{asym} spectra at concentrations 1.5 mM, 3.1 mM, 6.3 mM, 12.5 mM, 25.0 mM and 50.0 mM were collected and are shown below. 4% contrast was obtained at 1.50 mM.

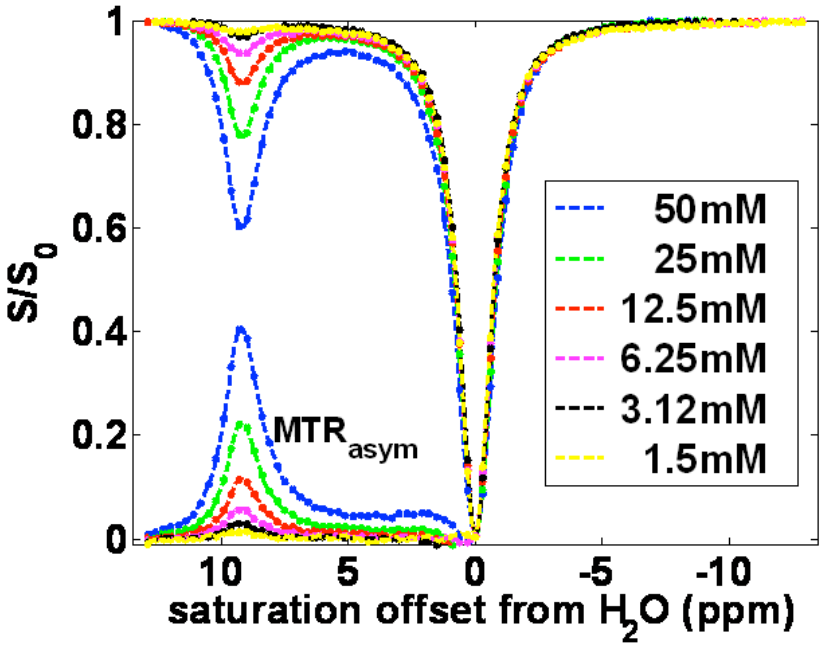
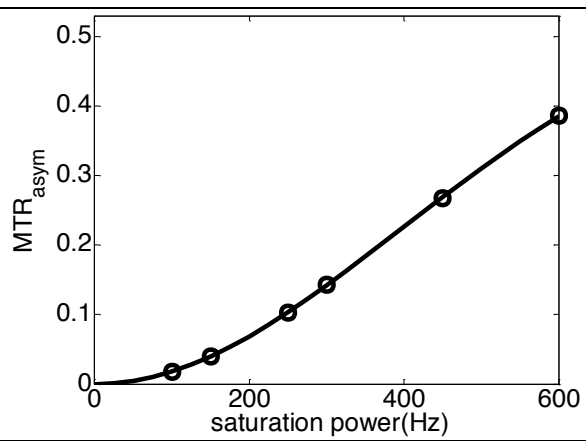


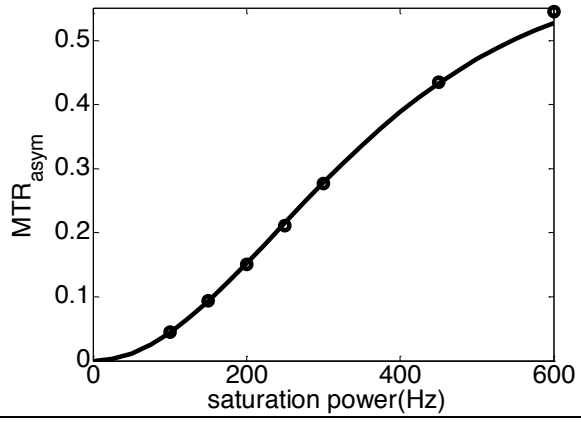
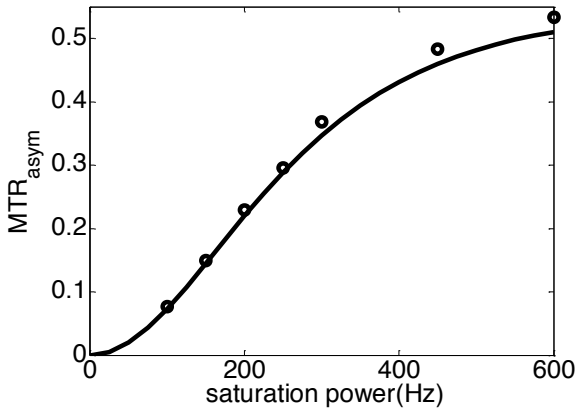
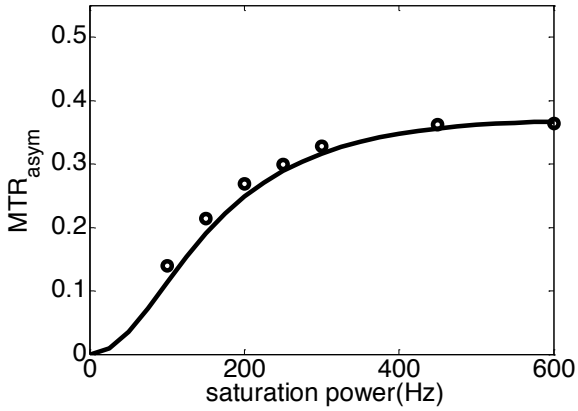
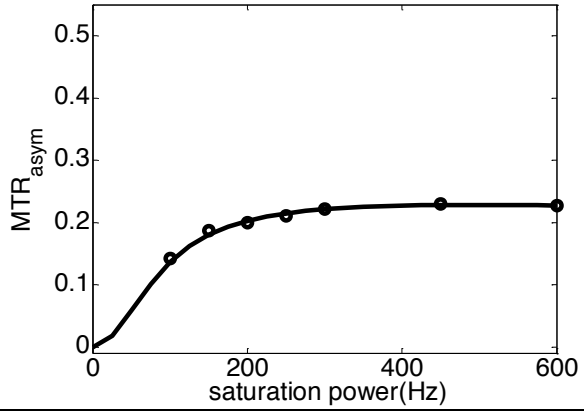
Figure S2. Concentration effect on the contrast of salicylic acid (**1**)

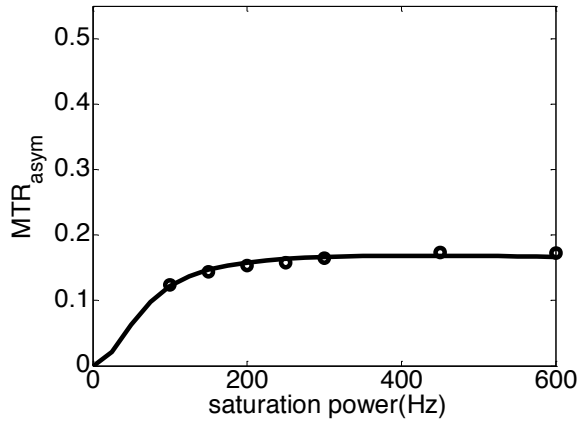
4. PROTON EXCHANGE OF SALICYLIC ACID AT DIFFERENT PH

QUESP datasets for salicylic acid (**1**) at 9.3 ppm were collected as a function of pH using ω_1 = 1.2 μT, 2.4 μT, 3.6 μT, 5.4 μT, 7.2 μT, 10.8 μT and 11.4 μT. The solvent to water exchange rate (k_{sw}) was calculated according to fitting to a 2-pool Bloch equation model.[1] The parameters for all fitting were: R_{1w} = 0.3, R_{2w} = 0.6, R_{1s} = 0.71, R_{2s} = 39, T_{sat} = 3s. The results at pH 5.8, 6.2, 6.5, 7.0, 7.4 and 7.8 were summarized in Table S1.

Table S1. The calculated proton exchange rate of salicylic acid (**1**) at different pH.

entry	pH	QUESP fitting[1]	Exchange rate (k/sec.)
1	5.8		9.7

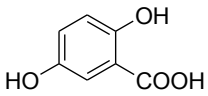
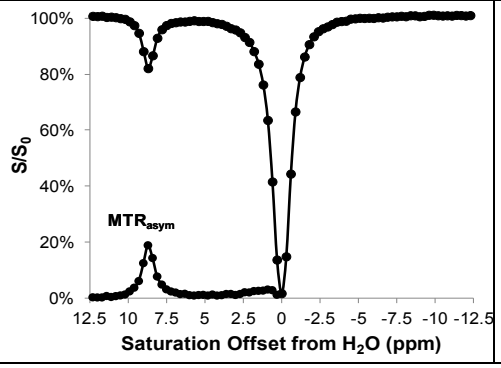
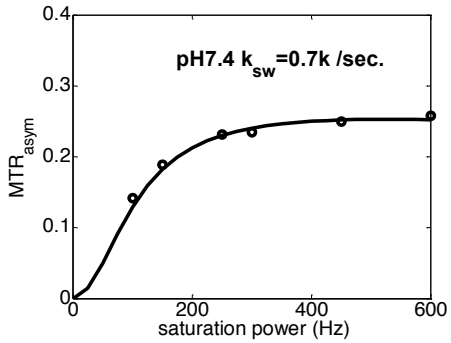
2	6.2		4.2
3	6.5		2.4
4	7.0		1.2
5	7.4		0.6

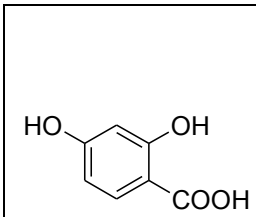
6	7.8		0.4

5. Z-SPECTRA OF SALICYLIC ACID ANALOGUES

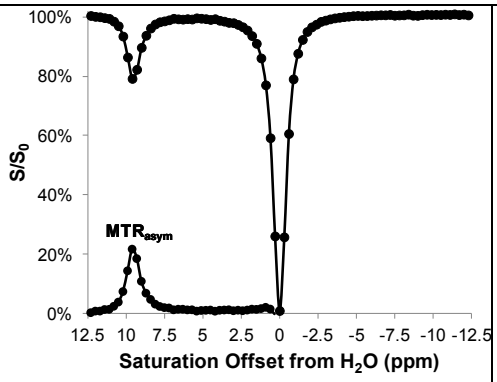
25 mM salicylic acid analogs (**4** – **11**) were made at pH 7.1-7.4 and tested with the phantom condition as mentioned in general section. The Z-spectra were obtained by using T_{sat} = 3 sec, ω_1 = 3.6 μT at 37 ºC. The Z-spectra, MTR_{asym} and QUESP curve are listed in the Table S2.

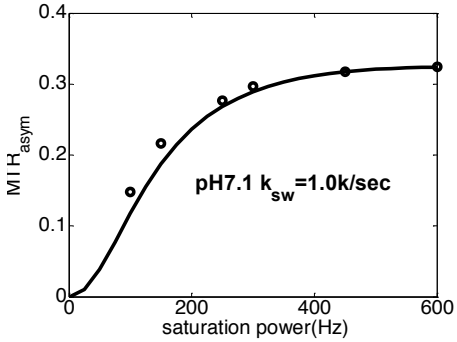
Table S2. The Z-spectra and MTR_{asym} of salicylic acid analogs

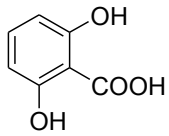
Compound	Z-spectra and MTR_{asym}	Exchange rate fitting
 **4**		


HO
OH
COOH

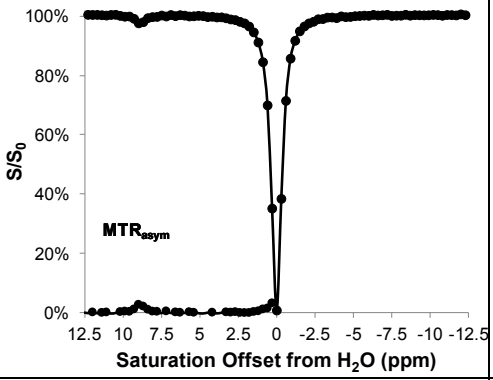
5

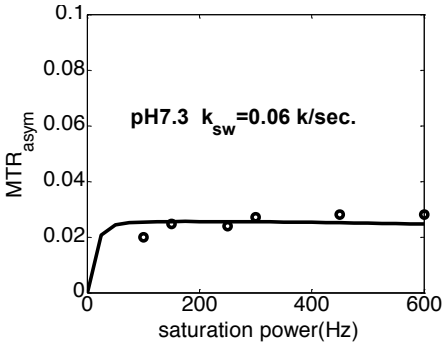

S/S0
MTRasym
Saturation Offset from H2O (ppm)

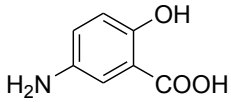

MTRasym
pH7.1 ksw=1.0k/sec
saturation power(Hz)


OH
COOH
OH

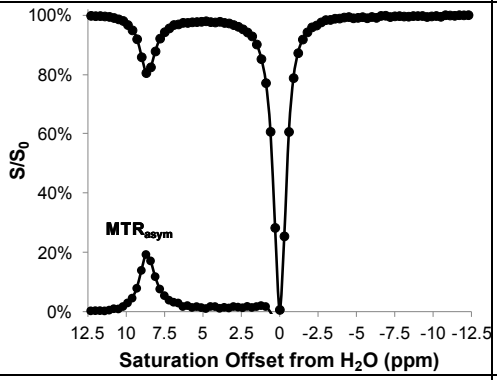
6

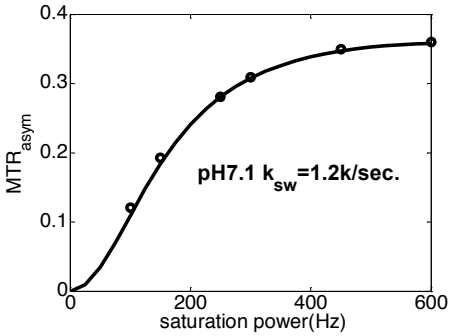

S/S0
MTRasym
Saturation Offset from H2O (ppm)

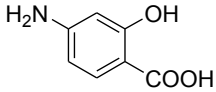

MTRasym
pH7.3 ksw=0.06 k/sec.
saturation power(Hz)


OH
H2N
COOH

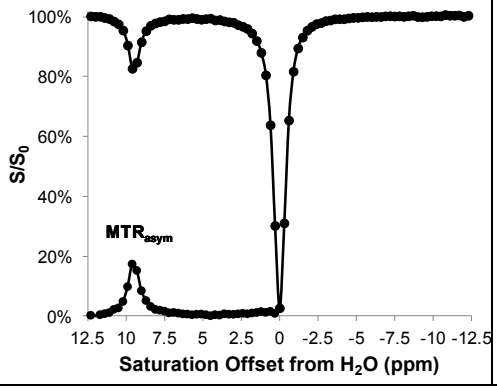
7

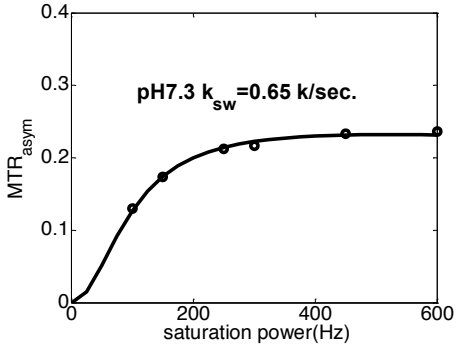

S/S0
MTRasym
Saturation Offset from H2O (ppm)

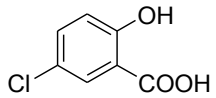
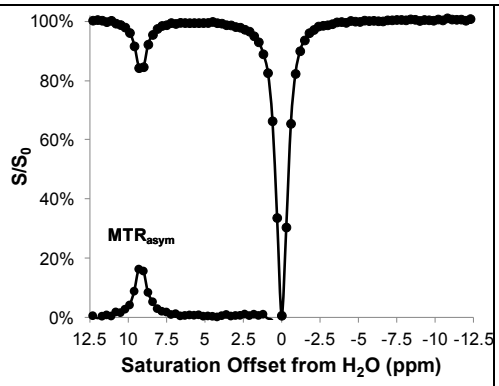
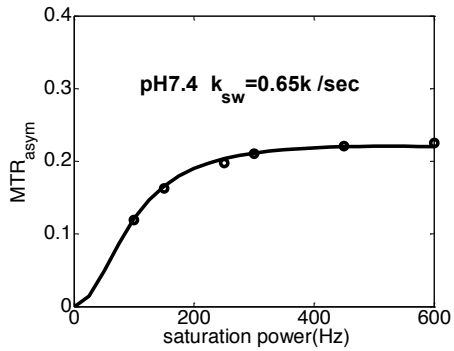
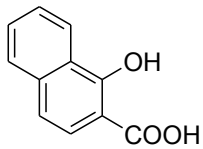
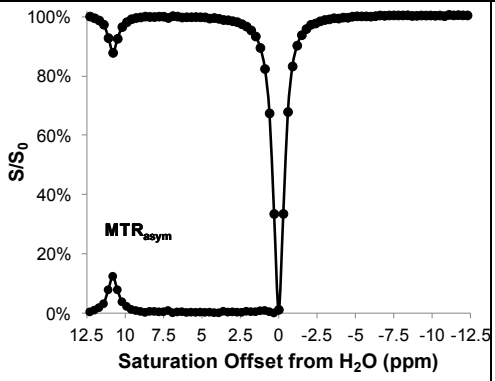
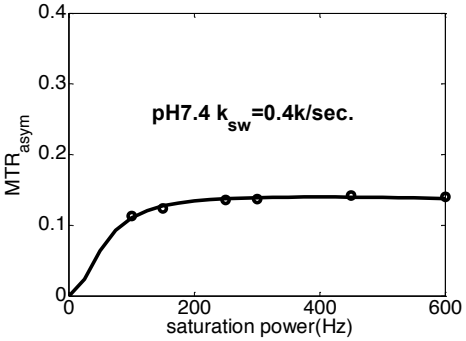
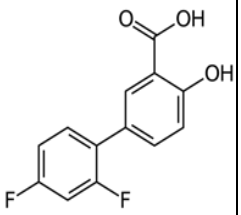
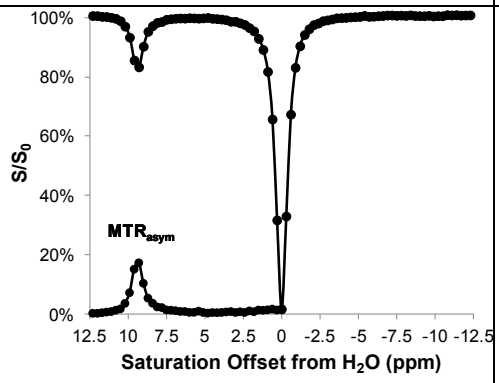
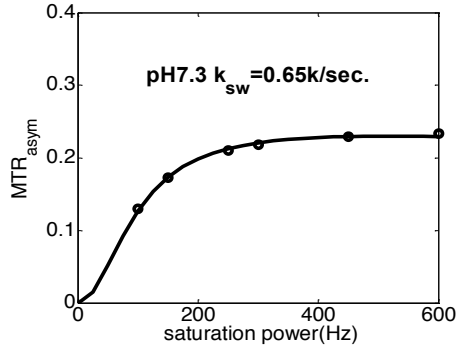
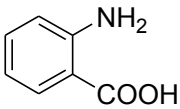
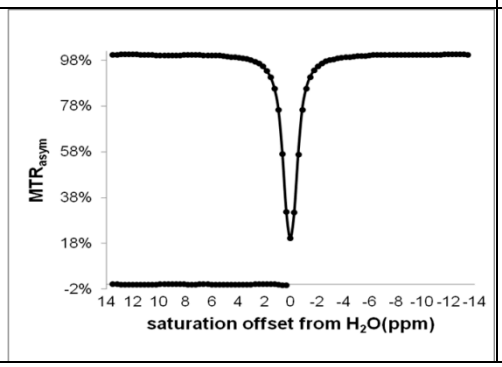

MTRasym
pH7.1 ksw=1.2k/sec.
saturation power(Hz)


H2N
OH
COOH

8


S/S0
MTRasym
Saturation Offset from H2O (ppm)


MTRasym
pH7.3 ksw=0.65 k/sec.
saturation power(Hz)

 **9**		
 **10**		
 **12**		
 **11**		NA

6. IN VIVO DATA

A) *In vivo* data collection scheme 1

For the initial test, we started with an injection of 100 μl of 250 mM compound **1** solution into the mouse tail vein (i.v.), and acquired CEST images using a saturation field strength of 5.9 μT. A Z-spectrum was acquired before injection. For the dynamic CEST contrast measurements, we used a 6-offset scheme (± 9.6 ppm, ± 9.3 ppm, ± 9.0 ppm, 5 min temporal resolution) after i.v. injection to ensure robustness to B_0 inhomogeneity and high contrast-noise-ratio. The CEST contrast map was calculated with averaging over the 3 offsets (9.6 ppm, 9.3 ppm and 9.0 ppm). The kidney reached maximum CEST contrast at around 5 min, and then the contrast started decaying at 10 min. The Z-spectra are plotted in Figure S3, and the pre- and post- injection maps are shown in Table S3.

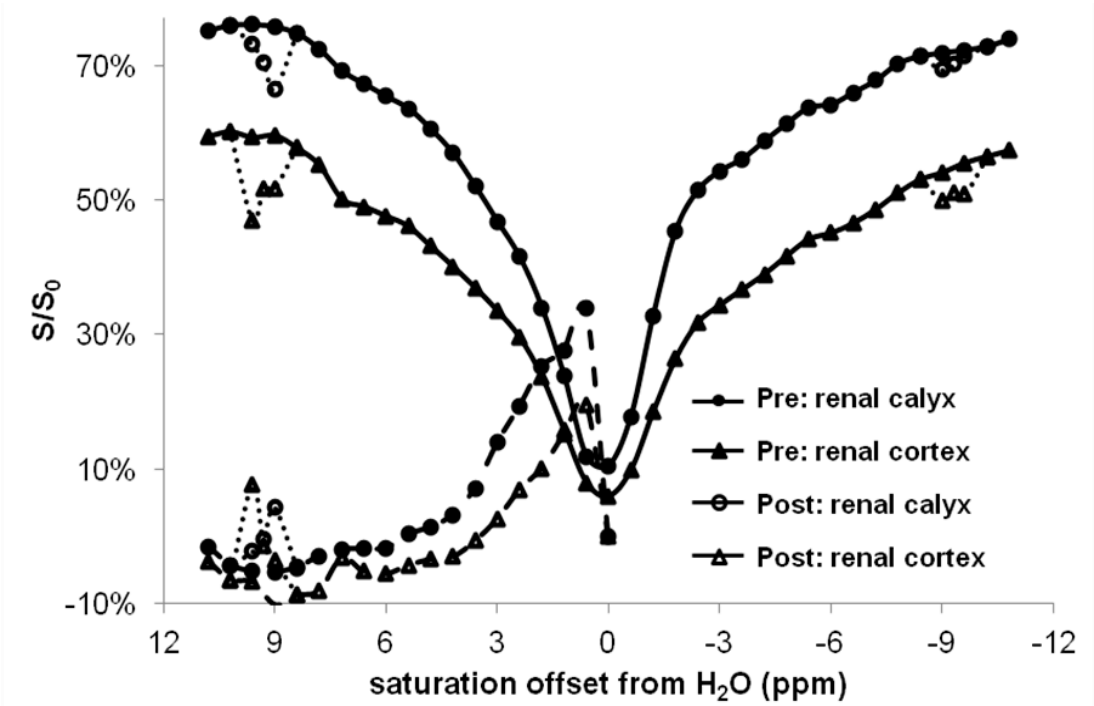
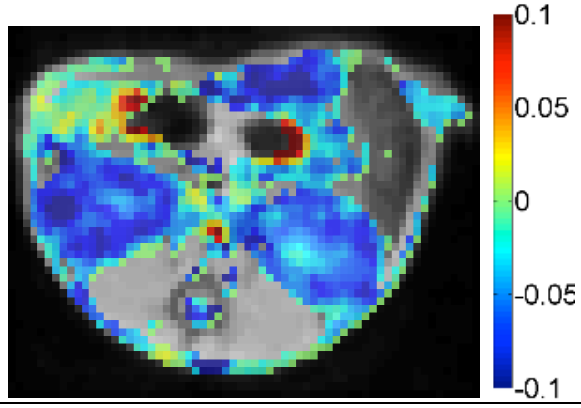
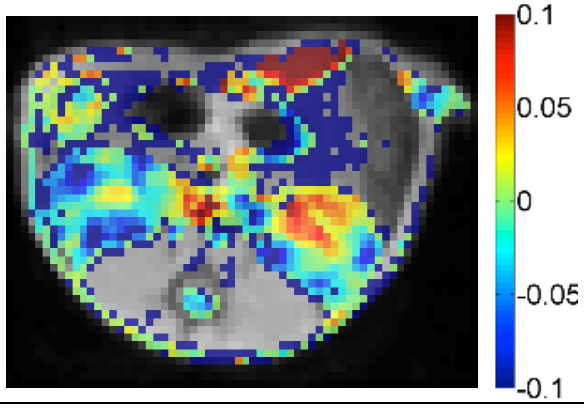
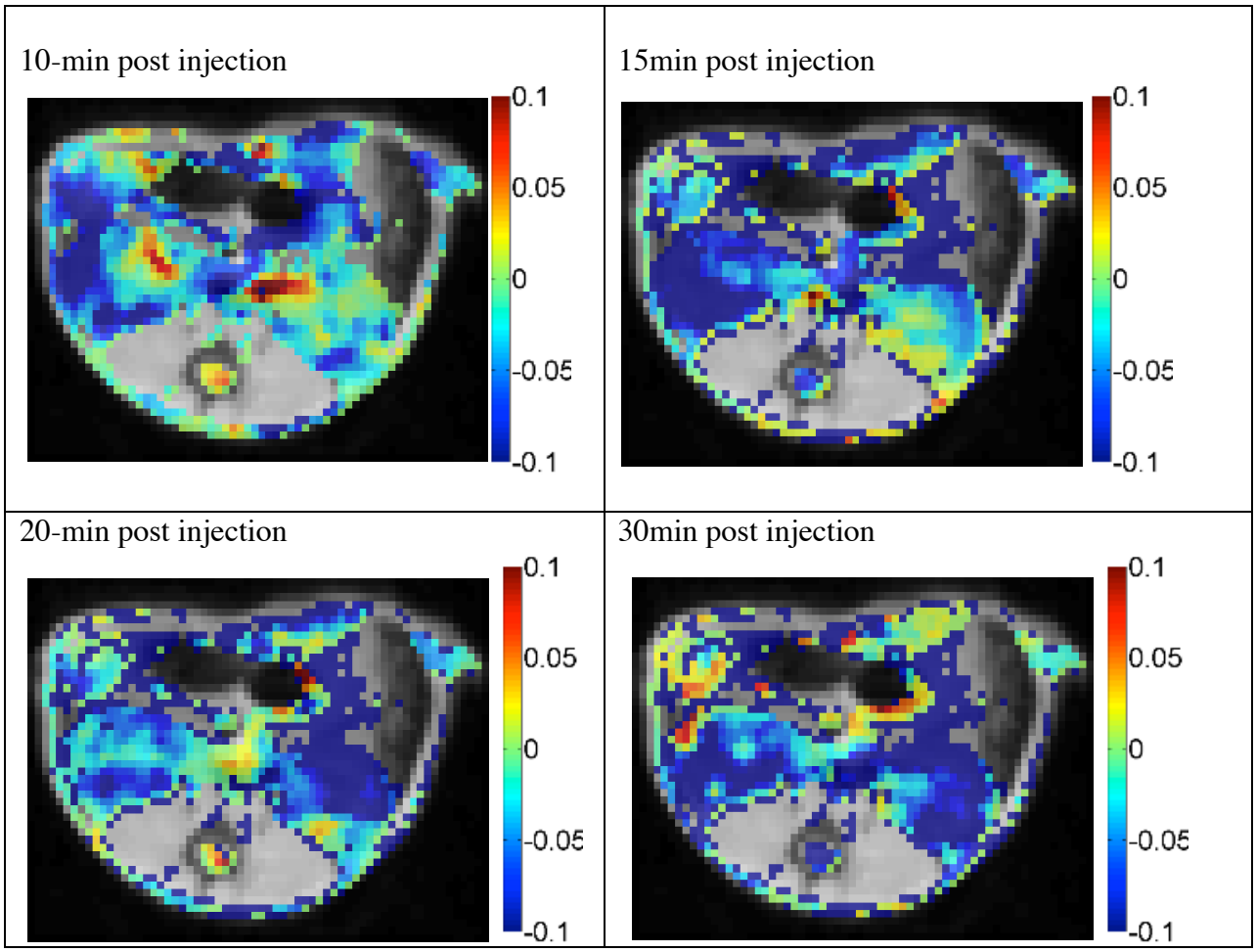


Figure S3. *In vivo* Z-spectra and MTR_{asym} spectra for renal calyx and cortex, acquired both pre-injection and at 5 min post injection.

Table S3. Dynamic CEST contrast maps pre- and post- injection at 5.9 μT.

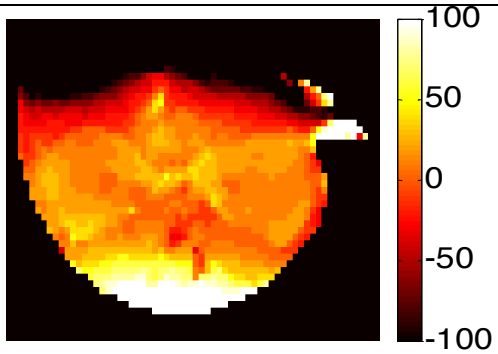
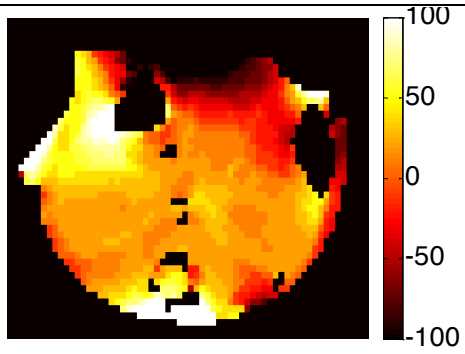
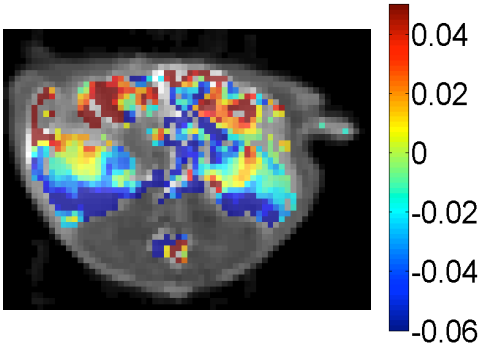
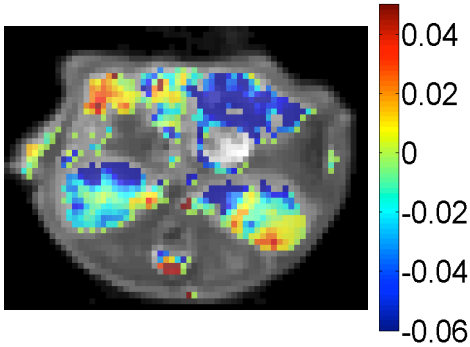
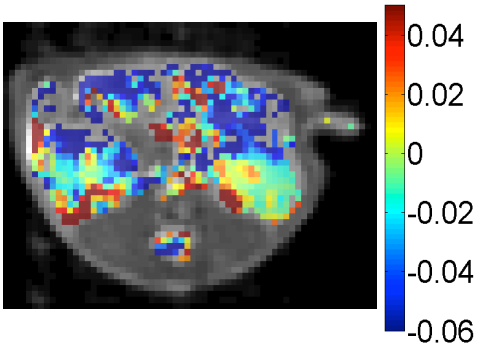
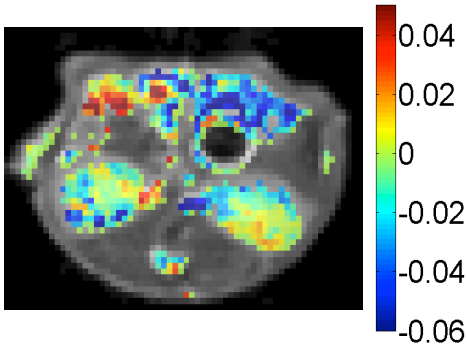
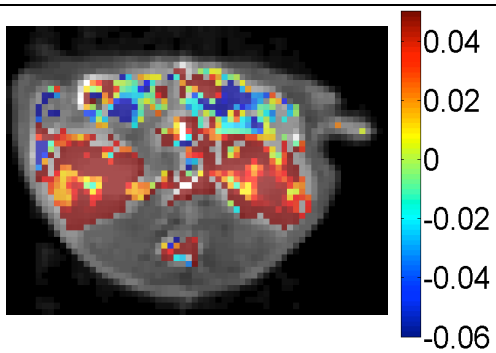
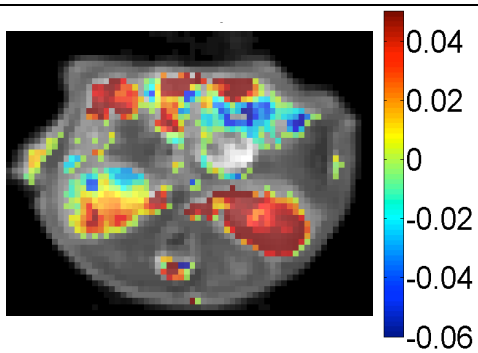
Pre-injection	5 min post injection
	

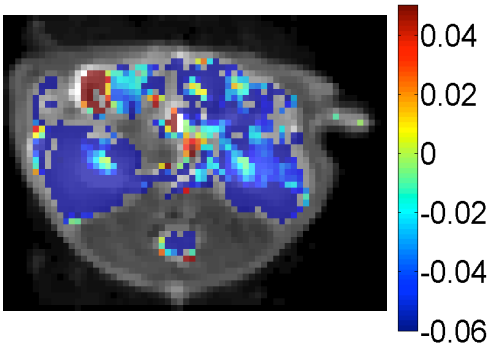
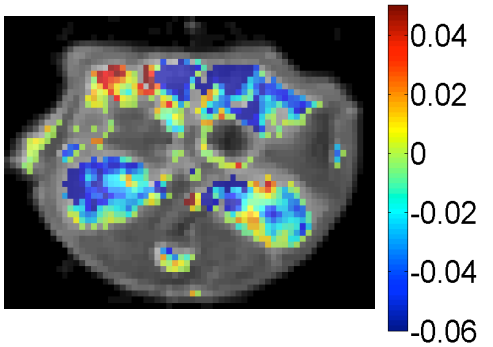
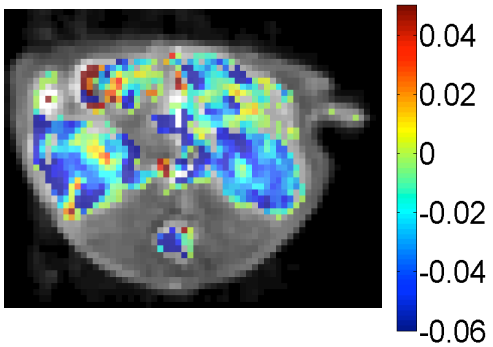
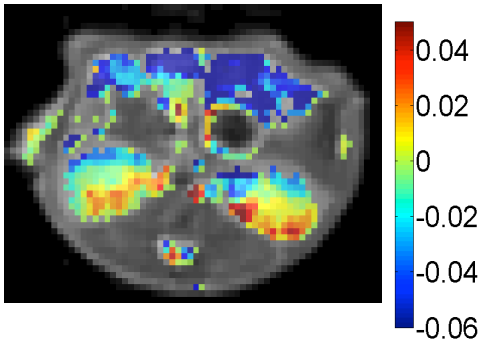
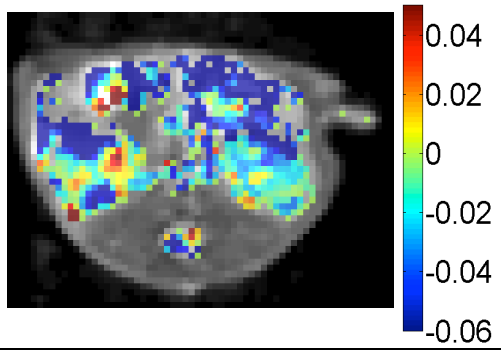
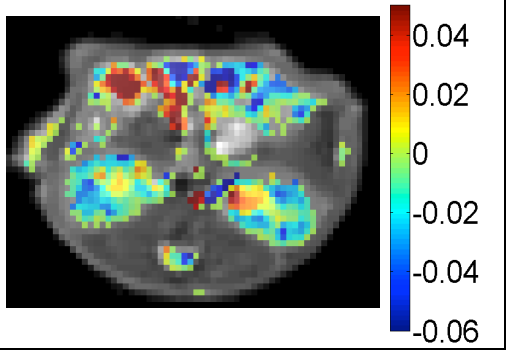


B) Scheme 2: Improved CEST imaging of kinetics:

According to the initial results in Figure S3 and Table S3, we modified the *in vivo* data collection scheme, in order to improve the kinetic data for compound **1** in the kidney. In addition, we further reduce the dose of agent to 60 μl of 250 mM solution. Instead of time consuming 5.9 μT 6-offset, we used 7.2 μT 2 offset at 9.3 ppm and -9.3 ppm for the dynamic CEST image acquisition. For the image post-processing, the CEST contrast maps at 9.3 ppm was smoothed by adding a 2x2 medium filter and overlapped to the saturation weighted image at -9.3 ppm. Images at every two adjacent time points were also averaged to increase the contrast-noise-ratio, with a temporal resolution of 3 min. For 2 mice, the dynamic contrast maps are shown in Table S4.

Table S4. The dynamic contrast maps for two mice

Entry	Time (min)	Mouse 1 (0309M2)	Mouse 2 (0311M3)
1	B0 Map		
2	0 Pre-inject		
3	3		
4	7		

5	11		
6	15		
7	30		

7. References

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