SUPPORTING INFORMATION

A Novel Class of Polymeric pH-Responsive MRI CEST Agents

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1) Materials

N,N,N',N",N"-Pentamethyldiethylenetriamine (PMDETA) was purchased from Sigma-Aldrich. 2-(Diisopropyl amino) ethyl methacrylate (DPA) was purchased from Polyscience Company. PEG macroinitiator (MeO-PEG_{114}-Br) were prepared according to the procedure in literature.[1] Other solvents and reagents were used as received from either Sigma-Aldrich or Fisher Scientific Inc.

2) Synthesis of PEG-*b*-PDPA block copolymer

The PEG-*b*-PDPA block copolymer was synthesized by the atom transfer radical polymerization (ATRP) method. First, DPA (2.6 g, 120 mmol), PMDETA (25 μL, 0.12 mmol), and MeO-PEG_{114}-Br (500 mg, 0.1 mmol) were charged into a polymerization tube. Then DMF (1 ml) was added to dissolve the monomer and initiator. After three cycles of freeze-pump-thaw to remove oxygen, CuBr (16 mg, 0.11 mmol) was added into the reaction tube under nitrogen atmosphere, and the tube was sealed *in vacuo*. The polymerization was carried out at 50 ^{o}C for 16 hours. After polymerization, the reaction mixture was diluted with 20 ml THF, and passed through an Al_2O_3 column to remove the catalyst. The THF solvent was removed by rotovap. The residue was dialyzed against the distilled water and lyophilized to provide a white powder. The resulting PEG-*b*-PDPA block copolymers were characterized by ^{1}H 500 MHz NMR, gel permeation chromatography (Viscotek GPCmax, PLgel 5 μm MIXED-D columns by Polymer Labs, THF as eluent at 1 ml/min).

3) Preparation of the micelle solutions for CEST evaluation

Micelles were prepared similar to that of the published procedures.[2] First, 100 mg of the copolymer was dissolved in 5 ml DMF and then added into 40 ml distilled water dropwise under sonication. The mixture was diluted with DI water so that DMF percentage was around 1% (V/V). The DMF was removed through ultrafiltration dialysis with (10 kD) membrane for several times. Then the distilled water was added to adjust the polymer concentration to 15 mg/ml as a stock solution. After micelle formation, the nanoparticles were re-dispersed into 0.1 M MES / MOPS at different pH values and characterized using a 400 MHz NMR spectrometer for CEST efficiencies.

4) pH titration

First, PEG_{114}-*b*-$PDPA_{116}$ copolymer (88 mg) was dissolved in 5 ml 0.1 mol/L HCl and diluted to 20 ml with DI water. The pH titration was carried out by adding the titrant (0.1 - 1 ml increments) of 0.02 M NaOH solution under vigorous magnetic stirring. The pH increase in the range of 2 to 10 was monitored as a function of

the total added volume of NaOH (V_{NaOH}). The pH values were measured using a Mettler Toledo pH meter with a microelectrode.

5) CMC measurements

Critical micelle concentration (CMC) value is the threshold polymer concentration at which micelles would form in solution. CMC of PEG_{114}-*b*-$PDPA_{116}$ copolymer was measured in the 0.2 M sodium phosphate buffer at pH 7.4. First, the stock solution (5 mg/ml) was diluted to different concentrations with the same buffer. In each solution, 5 μL pyrene in THF solution (2×10^{-4} M) was added to 2 ml polymer solution to produce the final pyrene concentration at 5×10^{-7} M. The fluorescence spectra were recorded on a Hitachi fluorometer (F-7500 model) with the excitation wavelength of 339 nm and the excitation and emission slits at 10.0 nm and 1.0 nm, respectively. The I_1 and I_3 values were measured as the maximum emission intensity at *ca*. 372 and 382 nm, respectively. I_1/I_3 ratio was plotted as a function of polymer concentration. I_1/I_3 ratio reflects the polarity of the pyrene environment where partition of pyrene in the hydrophobic micelle core leads to decreased I_1/I_3 values.

6) Preparation of samples for DLS and pH measurements

PEG_{114}-*b*-$PDPA_{116}$ (125 mg) was dissolved in 50 ml DI water by adding 2 ml 1M HCl. After fully dissolved, the total volume was adjusted to 100 ml by adding DI water. The initial pH is measured, $pH_{(initial)}$ = 1.4. This solution was titrated by adding the titrant (0.1 – 1 ml incremnets) of 0.02 M NaOH solution under vigorous magnetic stirring. At pH values of interest, the 1.5 ml mixtures were taken and filtrated through 0.45 μm syringe filters. These samples were used to measure the hydrodynamic diameters and Zeta potentials.

7) DLS protocols

Dynamic light scattering (DLS) was measured on a Zetasizer μV model (with He-Ne laser, λ= 632 nm) (Malvern Instruments Ltd, Worcestershire, UK). For hydrodynamic diameter measurement, Zen0040 disposable micro cuvettes were used as the sample cell, with 173^{o} backscatter (NIBS default), number of runs = 5, duration time = 10 s, six measurements per data point, and automatic attenuation. For the Zeta potential measurement, DTS1060C Clear Disposable zeta cell was used as the sample cell, with automatic measurement duration (minimum 30 and maximum 100), automatic attenuation, and six measurements per sample.

8) NMR CEST characterization

^{1}H NMR CEST evaluation was performed with an Agilent Technologies 9.4 T vertical bore NMR spectrometer (formerly Varian, Inc.) at the room temperature (around 20 ^{o}C inside the bore). A presat pulse sequence was used to collect CEST spectra with a 5 s square-shape hard pre-saturation pulse at a power level of 25 db

(equivalent to B_1 = 9.4 μT) and the arrayed saturation frequency offset from +8,000 Hz to -8,000 Hz, and a step of -100 Hz (namely, 161 spectra in total were acquired in order to obtain a CEST spectrum).

For CEST dependence on concentration (Fig. 2d), a much shorter saturation duration time of 3 s was used. Other parameters were same as stated above.

9) MRI protocols

MRI CEST images were recorded with an Agilent Technologies 9.4 T / 31 cm bore-hole small animal MRI system (formerly Varian, Inc.) with a 38 mm quad coil (Doty Scientific, Inc.) as both excitation and receiver device. The temperature inside the magnet bore was around 20 ^{o}C. A customer modified fast spin echo (fsems) pulse sequence was used to acquire MRI raw images. The key modification was that a 3 s hard pre-saturation pulse at power level of 36 db (equivalent to B_1 = 8.2 μT) was applied at different saturation frequency offsets, varying from +10 ppm to -10 ppm with a decreased step of 0.2 ppm. Other major parameters are: the repetition time TR = 3.15 s, ETL = 8, ESP = 4, kzero = 4, the effective echo time TE_{eff} = 48 ms, 1 average, 2 dummy scans, the matrix size of 128x128, the field of view (FOV) = 30 x 30 mm and the slice thickness of 1 mm.

10) CEST spectral fitting method

The CEST spectra were fitted to a 2-pool or 3-pool chemical exchange model of Block Equations using the Matlab platform (version 7.0, Mathworks, Natick, MA) by following the published procedures.[3]

Tables S1. The parameters for fitting the CEST spectrum of PEG_{114}-*b*-$PDPA_{116}$ at pH 5.0 presented in **Fig. 2a** of the main text.[a)]

τ_{ex}**NH** (μs)	B_1 (Hz)	**Δδ** (ppm)	T_1**Water** (s)	T_2**Water** (s)	T_1**NH** (s)	T_2**NH** (s)
890	400	3.90	3.00	1.31	1.11	0.002

a) The parameters listed in this Table:

τ_{ex}NH: the lifetime of proton on the tertiary amines of block copolymer (in unit of micro-second)

B_1: the power of the pre-saturation pulse (in unit of Hz)

Δδ: the chemical shift difference between the proton of tertiary amines of block copolymer and the bulk water (in unit of ppm)

T_1 or T_2 values: the corresponding longitudinal or transversal relaxation times (in unit of second)

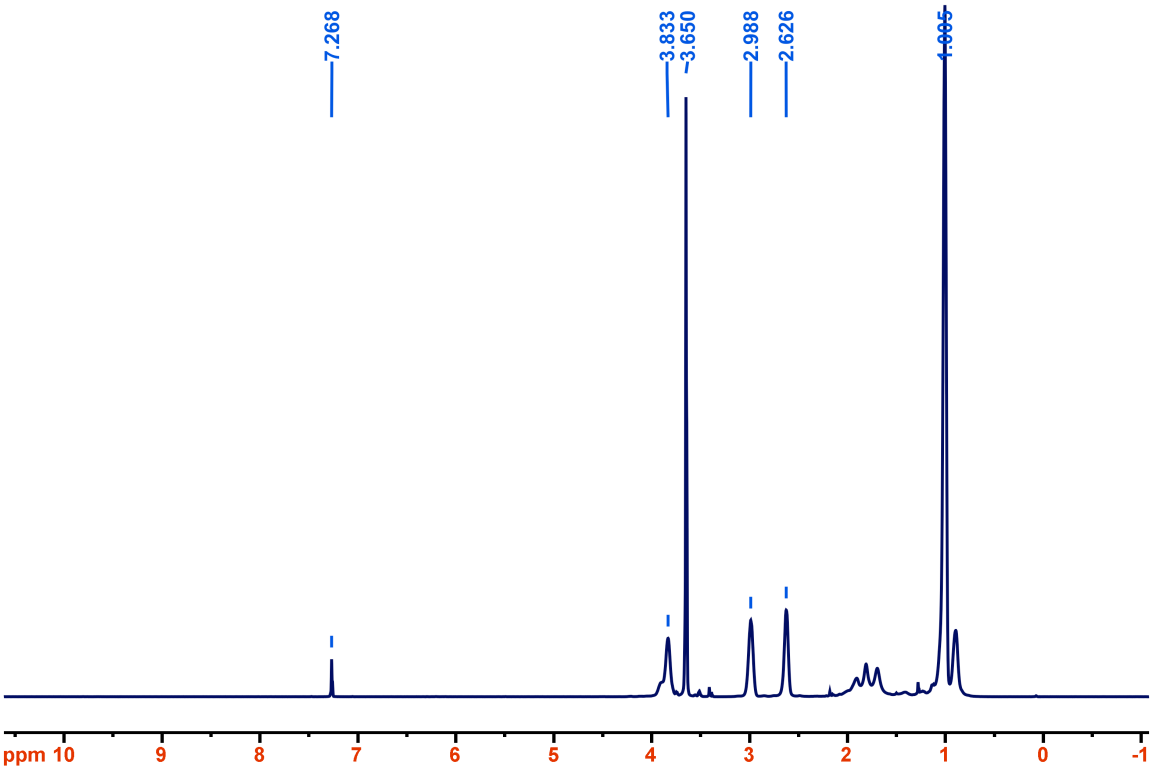

Figure S1. ^{1}H NMR spectrum of PEG_{114}-*b*-$PDPA_{116}$ in $CDCl_3$. The spectrum was recorded using a Varian 500 MHz NMR spectrometer.

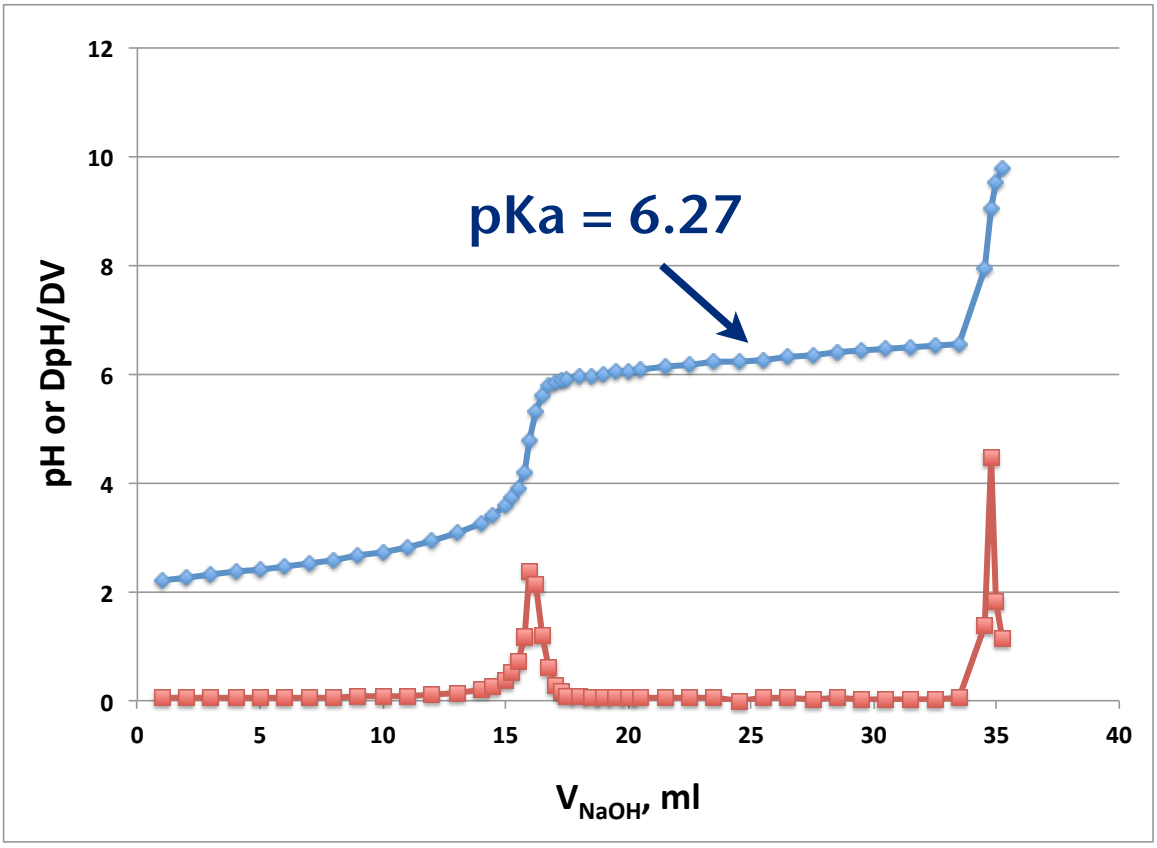

Figure S2. pH titration curve and the corresponding differential curve of 88 mg PEG_{114}-*b*-$PDPA_{116}$ with 0.02 M NaOH aqueous solution.

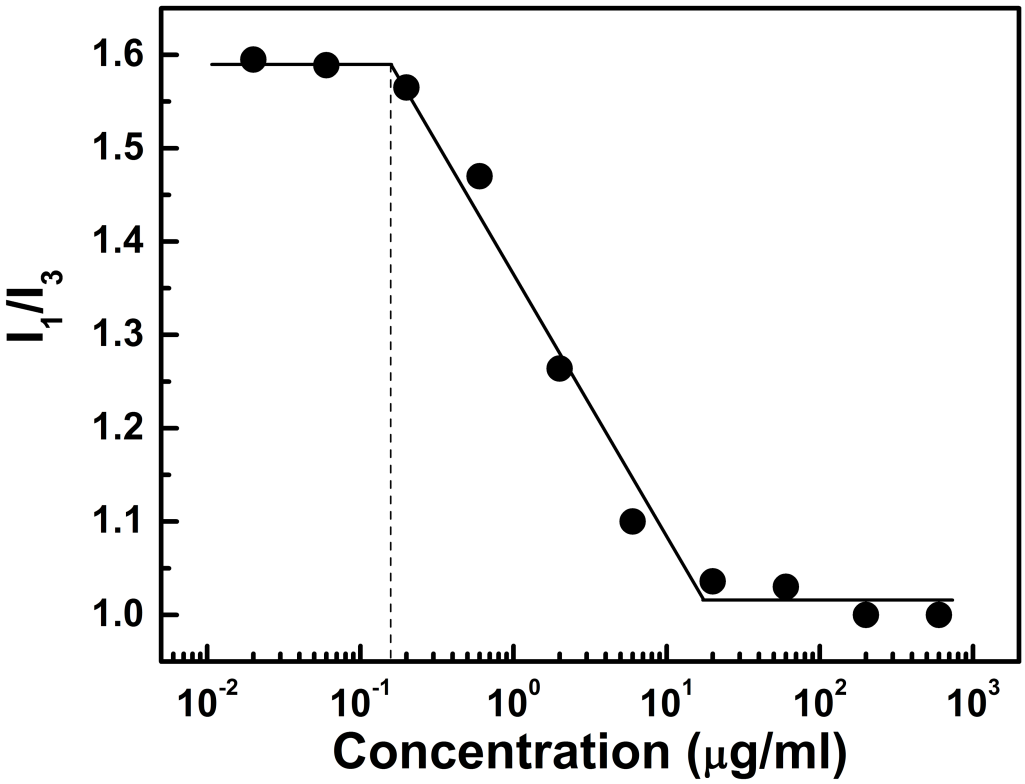

Figure S3. A plot of I_1/I_3 values *vs.* the concentrations for PEG_{114}-*b*-$PDPA_{116}$, which yielded a CMC of 0.15 μg/ml.

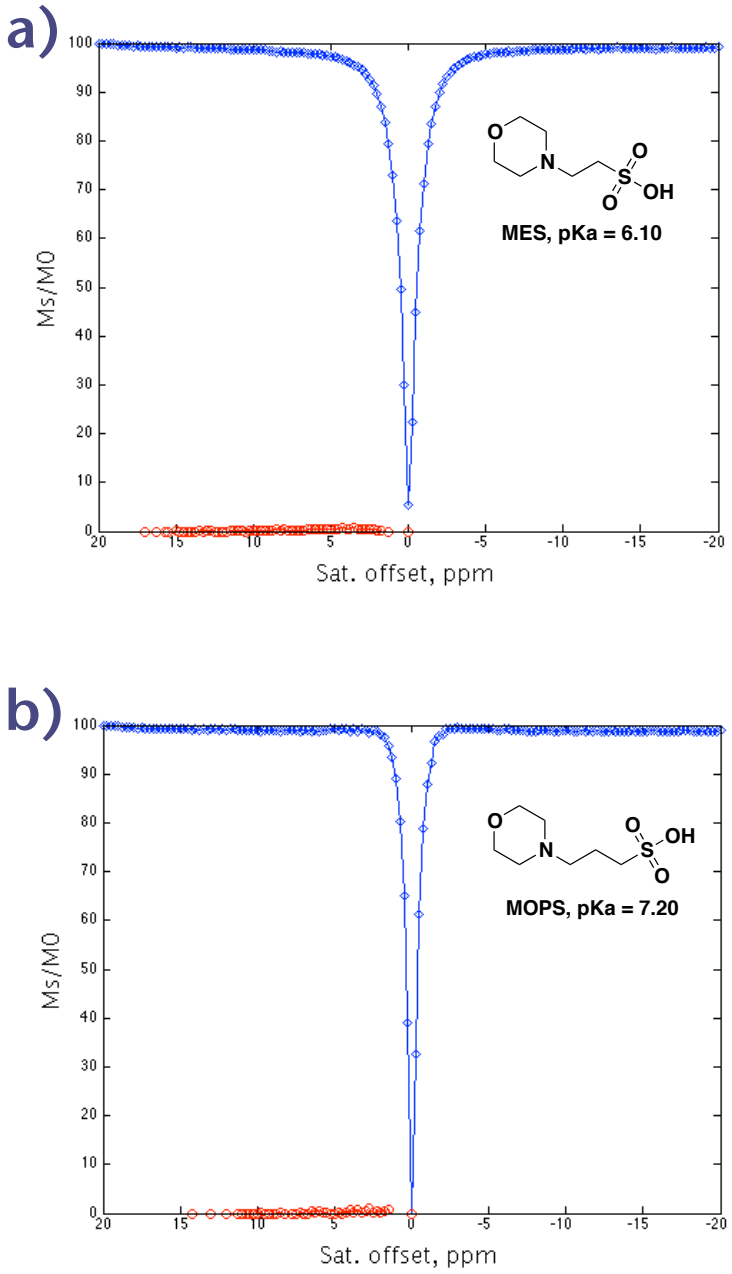

Figure S4. The CEST spectra of pure buffers: a) 0.1 M MES at pH 5.3 and b) 0.1 M MOPS at pH 7.5, respectively. The CEST asymmetries were also presented to better visualize the CEST effects (namely, $CEST_{asym} = [M_s/M_0]_{downfield} - [M_s/M_0]_{upfield}$).

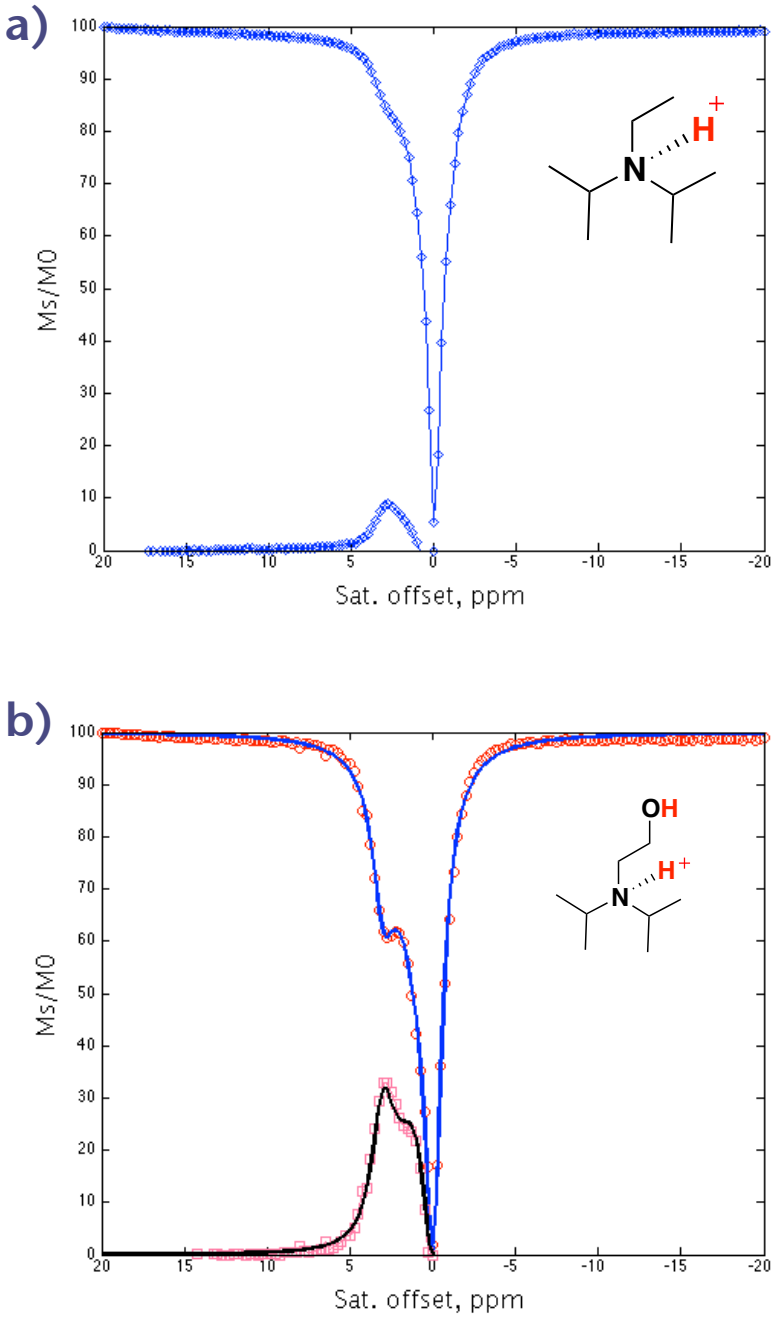

Figure S5. The CEST spectra of small analog molecules: a) 116 mM N,N-diisopropylethylamine (DIPEA) in 0.1 M MES at pH 5.3 and b) 116 mM *N*,*N*-diisopropylaminoethanol (DIPAE) in 0.1 M MOPS at pH 5.8, respectively. The CEST asymmetries were also presented to better visualize the CEST effects (namely, $CEST_{asym} = [M_s/M_0]_{\text{downfield}} - [M_s/M_0]_{\text{upfield}}$).

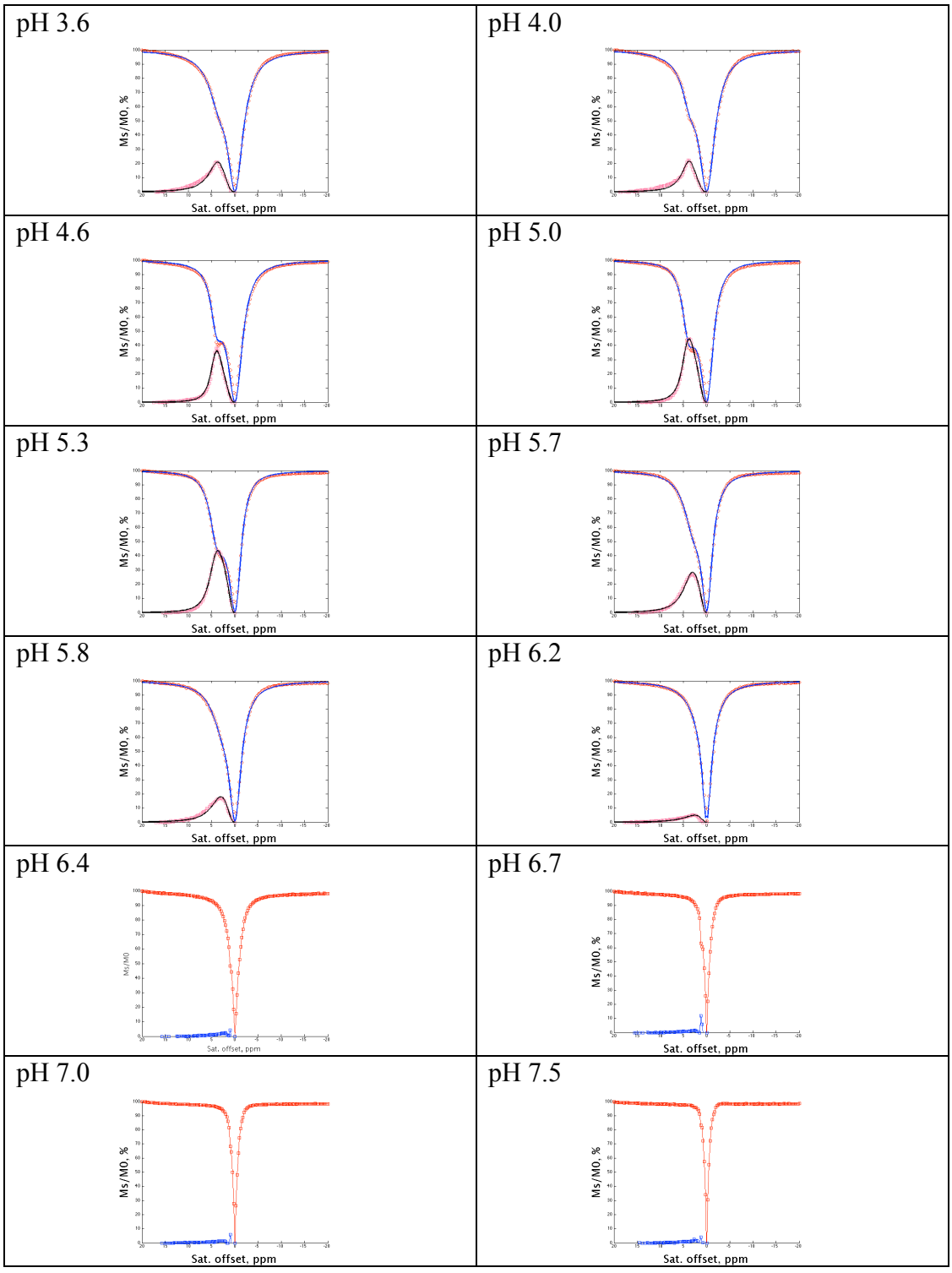

Figure S6. The CEST spectra of 0.5 mM PEG_{114}-*b*-$PDPA_{116}$ in 0.1 M MES and/or MOPS at the different pH values as labeled in the figures. The CEST asymmetries were also presented to better visualize the CEST effects (namely, $CEST_{asym} = [M_s/M_0]_{\text{downfield}} - [M_s/M_0]_{\text{upfield}}$).

11) References:

1. K. Zhou, Y. Wang, X. Huang, K. Luby-Phelps, B. D. Sumer and J. Gao, *Angewandte Chemie International Edition*, 2011, **50**, 6109-6114.
2. N. Nasongkla, E. Bey, J. Ren, H. Ai, C. Khemtong, J. S. Guthi, S.-F. Chin, A. D. Sherry, D. A. Boothman and J. Gao, *Nano Letters*, 2006, **6**, 2427-2430.
3. D. E. Woessner, S. Zhang, M. E. Merritt and A. D. Sherry, *Magnetic Resonance in Medicine*, 2005, **53**, 790-799.