Poly(ethylene glycol) modified Mn$^{2+}$ complexes as contrast agents with a prolonged observation window in rat MRA$^\dagger$

Changqiang Wu, $^{a,b}$ Li Yang, $^a$ Zhuzhong Chen, $^e$ Houbing Zhang, $^a$ Danyang Li, $^a$ Bingbing Lin, $^a$ Jiang Zhu, $^{x,c}$ Hua Ai $^{x,d}$ and Xiaoming Zhang $^b$

A novel rigid Mn$^{2+}$ complex (MnL) with pyridine and pyrrolidine rings was designed and synthesized. The complex was further modified with alkyne, and conjugated to azide-terminated PEG by click chemistry. PEGylated MnL shows considerably higher relaxivity (MnL-PEG2k-MnL: $r_1 = 6.38$ mmol$^{-1}$ s$^{-1}$; MnL-PEG4.6k-MnL: $r_1 = 5.63$ mmol$^{-1}$ s$^{-1}$) and much longer blood circulation time than free Mn$^{2+}$ complexes (MnL: $r_1 = 3.73$ mmol$^{-1}$ s$^{-1}$), providing a prolonged time window to acquire longitudinal images for rat contrast enhanced magnetic resonance angiography.

Introduction

Manganese(II) based complexes have been studied as MRI contrast agents for many years, because of their excellent magnetic properties including five unpaired electrons and long electronic relaxation time, which can offer good $T_1$ relaxation enhancement in MRL. $^{1-3}$ Meanwhile, manganese is also a natural cellular constituent, acts as a cofactor for enzymes and receptors in cell biology, and has its own metabolic pathways in vivo. $^{4-6}$ Compared to gadolinium complex based contrast agents, manganese complexes can be good alternatives in terms of biosafety, and possibly having lower chances in developing nephrogenic systemic fibrosis (NSF) in patients with kidney dysfunctions. $^{7-9}$

Chelation ligands are crucial for the stabilization of metal ions and enhancement of relaxivity of manganese complexes. $^{8-10}$ In our previous study, aza-semi-crown pentadentate Mn$^{2+}$ complexes rigidified with pyridine and piperidine rings were developed. $^{11}$ Several other Mn$^{2+}$ complexes based on pentagonal picolinate ligands were also reported recently. $^{12,13}$ The special rigid structure has shown a relatively higher $T_1$ relaxivity (3.6 Mn mmol$^{-1}$ s$^{-1}$, 1.5 T, 20 °C) comparing to commercial agent Teslascan (Mn-DPDP, 2.1 Mn mmol$^{-1}$ s$^{-1}$, 1.5 T, 20 °C). However, the time window for contrast enhanced magnetic resonance angiography (MRA) is usually short because of the fast kidney clearance. In many clinical cases, longer MRI observation window can provide more information to the radiologists for better analysis. In order to overcome the limitation, conjugation of small molecule contrast agents to various frameworks such as micelles, liposomes, dendrimers, proteins, bioactive peptides were chosen. $^{14-18}$ However, complicacy of nano-systems, higher cost of fabrication, and potential toxicity slowed down their clinical translation process.

Polymeric conjugation is a feasible method to prepare macro-molecular contrast agents. As a water-soluble, non-antigenic and biocompatible polymer, poly(ethylene glycol) (PEG) has been approved by the Food and Drug Administration (FDA) for human dermal, oral and intravenous applications. $^{19-22}$ For instance, PEG conjugated interferon (Pegasys®, Roche) provides long circulation time and less side effects than free interferons. $^{24,25}$ PEGylated small molecule contrast agents can slow down kidney clearance rate while have less RES uptake comparing to nanoparticles, presenting a more feasible solution for enhanced MRA imaging. $^{26,27}$

Herein, PEGylated Mn$^{2+}$ complexes containing rigid rings were designed (Fig. 1). We first designed and synthesized a new rigid Mn$^{2+}$ complex (MnL) with pyridine and pyrrolidine rings. Next, the complex was modified with alkynyl group and reacted with azido-terminated PEG. Finally, the relaxivity and rat contrast enhanced MRA of PEGylated MnL and free MnL were studied under clinical MRI scanners.

Results and discussion

Characterization of rigid Mn$^{2+}$ complex (MnL)

MnL complex was identified by mass spectrometry. MnL used to obtain single crystals were prepared in MilliQ water directly mixing of ligand and Mn$^{2+}$ at alkaline condition. The product
Three Mn\(^{2+}\) ions are heptadentated at the cavity center, states were both found (coordination number 6 and 7) in one triazole ring. These rigid structures and PEG chain are quite important ligand with rigid pyridine and pyrrolidine rings, and linked PEG by rigid atoms) of one ligand and Mn\(^{2+}\) ion almost locate in the same nitrogen atom, two tertiary amine nitrogen and carboxyl oxygen of ligands in the same way. Five-coordinate atoms (a pyridine nitrogen atom, two tertiary amine nitrogen and carboxyl oxygen atoms) of one ligand and Mn\(^{2+}\) ion almost locate in the same plane, and one coordinate water molecule located on apical positions of this plane to form the pentagonal-bipyramidal coordination sphere. This coordination state is similar with previous reports.\(^{11}\) It’s interesting that one Mn\(^{2+}\) is hexadentated with six carboxyl oxygen atoms of three ligands (two carboxyl oxygen atoms per ligand), forming a standard octahedral coordination sphere. This shows that there are free Mn\(^{2+}\) in our obtained manganese complexes. In the following experiments, MnL was further purified through Sephadex LH-20 column. But unfortunately, the corresponding single crystals were not obtained. So, we think free Mn\(^{2+}\) in MnL might be beneficial to the formation and growth of single crystal. Similar result was also discovered in our previous study.\(^{11}\) The crystals’ structures informed us Mn\(^{2+}\) ions at the cavity center of ligands are heptadentated, and one coordinate water molecule was observed. In the following experiments, MnL was purified through Sephadex LH-20 column to ensure no free Mn\(^{2+}\).

**Synthesis and characterization of L-PEG-L**

**Synthesis of N\(_3\)-PEG-N\(_3\).** Hydroxyl-terminated PEG are commercially available in a variety of molecular weights with narrow dispersity (\(M_n/M_w \leq 1.1\)). For covalent binding with other substrates, terminal hydroxyl group of PEG must be functionalized. In this work, azido-terminated PEG, N\(_3\)-PEG-N\(_3\), was prepared using two steps modification.\(^{28}\) Hydroxyl-terminated PEG with different molecular weights (2 kDa and 4.6 kDa) were used. Tosylation of PEG diols is considered the most critical step because the conversion yield are involved in final replacement rate of azide. In order to achieve high conversion rate, \(p\)-toluenesulfonyl chloride with five equivalents to hydroxyl was added, and a long reaction time of 24 hours was kept. Results show that the degree of substitution were approximately 92% (PEG2k) and 91% (PEG4.6k) respectively, as calculated from the integral values of PEG (\(\delta\ 3.9\text{--}3.4\)) and the methylene protons adjacent to the tosyl group (\(\delta\ 4.15\)). Then, azido reaction carried out in DMF at 60 °C, and identified by disappearing of characteristic peaks of the tosyl group in \(^1\)H NMR spectrum (Fig. 3).

**Synthesis of L-PEG-L.** The click chemistry of L-Alkynyl and N\(_3\)-PEG-N\(_3\) was carried out in water. The resulted solution was dialyzed 2 days against water (MWCO 1 kDa membrane) to remove free ligands and then lyophilized. Fig. 4 shows the \(^1\)H NMR spectra of N\(_3\)-PEG-N\(_3\), L-Alkynyl and L-PEG-L, simultaneously. The characteristic peaks of PEG (a), ligand (d–j) and triazole ring linkage (k) appeared together in \(^1\)H NMR spectrum of L-PEG-L, and used to calculate grafting efficiency respectively (list in Table 1). The grafting ratio was calculated as the amount of ligand per PEG molecule. As a result, relatively higher ligand grafting rates are shown in NMR. However, lower molecular weight PEG2k has higher grafting rate (an average grafting ratio of 1.33) than that of PEG4.6k (an average grafting ratio of 1.10), depicting that the grafting efficiency was reduced with the hindering effect of longer PEG chains.

**\(T_1\) relaxivity studies in vitro**

In order to study the relaxation property changes of manganese complexes before and after PEGylation, we simultaneously...
tested relaxivities of MnL, MnL-PEG2k-MnL and MnL-PEG4.6k-MnL under a 1.5 T clinical MRI scanner. Fig. 5A displays the $T_1$ relaxation rates at different Mn concentrations. Three curve slopes represent the longitudinal relaxivities ($r_1$) of MnL, MnL-

PEG2k-MnL and MnL-PEG4.6k-MnL respectively. The relaxivity of free MnL complexes is 3.73 mmol$^{-1}$s$^{-1}$. As expected, PEGylated manganese complexes show a higher relaxivities (MnL-PEG2k-MnL: $r_1 = 6.38$ mmol$^{-1}$s$^{-1}$; MnL-PEG4.6k-MnL: $r_1 = 5.63$ mmol$^{-1}$s$^{-1}$) than free MnL complexes. One contributing factor is the rigid rings and PEG chain, which usually results in a longer correlation time ($\tau_R$). However, longer PEG chain with faster local motion does not lead to higher relaxivity, and it might be on account of the molecular structure of PEG, which is a flexible thread-like molecule. The similar result was also reported in the studies on PEGylated GdDOTA chelates. T$_1$-weighted images of water at different concentration of MnL, MnL-PEG2k-MnL and MnL-PEG4.6k-MnL (from 0.5 to 0.1 mmol) were studied in Fig. 5B, water solutions with PEGylated MnL shown obviously higher signal than water solutions with free MnL contrast group in the same manganese concentrations.

**In vivo MRA studies**

Contrast enhanced MRA is an important clinical tool to detect blood diseases, such as myocardial infarction, atherosclerotic plaque, and tumor angiogenesis. It is quite challenging to obtain sequential high-resolution blood vessel imaging in MRA using small molecule contrast agents due to low relaxivity and short plasma half-life. In this work, contrast enhanced MRA study of SD rats was carried out on a clinical 3.0 T MR scanner to evaluate vascular contrast enhancement effect of free MnL and PEGylated MnL. As shown in Fig. 6, blood vessel images of the chest and neck regions of rats were obtained after intravenous
injection of free MnL and MnL-PEG4.6k-MnL at a dosage of 0.25 Mn mmol per kg body weight. The vascular imaging window of PEGylated MnL group reached 10 minutes, was obviously longer than that of the free MnL group (3 minutes of vascular imaging window). The results stated that PEGylation of small molecule agents are beneficial for prolonged plasma half-life, and probably due to lower kidney clearance rates. In Fig. 7, MRI signal changes in jugular vein, heart and liver at different time points (in 30 minutes) were quantitatively studied. A $J_{\text{hearts liver}}$ for 4 h, and then its pH was readjusted to 7 under nitrogen protection. The resulting solution was stirred at 45 °C for 12 h. Filtered inorganic salts from solution, acetonitrile was evaporated off. The product was purified by silica gel column chromatography (methylene dichloride/methanol = 20/1). Yield: 2.2 g (76%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.62 (t, $J= 7.7$ Hz, 1H), 7.34 (d, $J= 7.6$ Hz, 2H), 4.02 (d, $J= 13.7$ Hz, 2H), 3.78 (d, $J= 13.7$ Hz, 2H), 3.66 (s,6H), 3.43 (t, $J= 13.5$ Hz, 2H), 3.10 (d, $J= 7.0$ Hz, 2H), 2.62–2.45 (m, 2H), 2.22–2.07 (m, 2H), 2.03–1.87 (m, 4H), 1.87–1.74 (m, 2H). $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 174.61 (s), 158.02 (s), 136.80 (s), 121.54 (s), 65.24 (s), 60.11 (s), 53.39 (s), 51.69 (s), 29.37 (s), 23.31 (s). HRMS (ESI): $m/z = 362.1823$ (M + H$^+$), 384.1761 (M + Na$^+$).

**Synthesis of the ligand (5).** Compound 4 (700 mg, 2.0 mmol) was dissolved in methanol (20 mL), NaOH (320 mg, 8 mmol) and 2 mL MilliQ water was added. The solution was heated to 45 °C and kept for 2 h. Evaporated off all the solvents to obtain light-yellow solid, which was dissolved in water and then acidified with concentrated HCl (37%, 1.0 mL). The water was evaporated off, ethanol added to dissolve the crude product. Filtered the salts, ethanol was evaporated off to get the product. Yield: 390 mg (99%). $^1$H NMR (400 MHz, DMSO) $\delta$ 7.81 (t, $J= 7.7$ Hz, 1H), 7.40 (d, $J= 7.7$ Hz, 2H), 4.17 (d, $J= 14.0$ Hz, 2H), 3.95 (d, $J= 14.0$ Hz, 2H), 3.50 (m, $J= 9.0$, 5.2 Hz, 2H), 3.23–3.12 (m, 2H), 2.74–2.60 (m, 2H), 2.11 (dq, $J= 12.3$, 8.5 Hz, 2H), 1.88 (tt, $J= 12.8$, 4.1 Hz, 2H), 1.83–1.65 (m, 4H), 1.03 NMR (400 MHz, DMSO) $\delta$ 171.84 (s), 155.24–153.24 (m), 137.93 (s), 122.94 (s), 66.26 (s), 58.15 (s), 53.75 (s), 28.50 (s), 22.84 (s). MS-ESI: $m/z = 332.11$ (M – H$^+$), 368.09 (M + Cl$^-$) (Fig. 8).

**Preparation of Mn$^{2+}$ complexes (MnL).** Ligand (5) (180 mg, 0.55 mmol) was dissolved in MilliQ water (12 mL) and the pH of the solution was adjusted to 6 by adding NaOH solution (0.2 M). Manganese chloride tetrahydrate (100 mg, 0.55 mmol) was added under nitrogen protection. The resulting solution was stirred at room temperature for 4 h, and then its pH was readjusted to 7 by addition of NaOH (0.2 M aq.). The water was removed by rotary evaporation and the resulting solid was suspended in ethanol. The insoluble salts were removed by filtration and the filtrate was evaporated. The product was obtained as a colorless solid. HRMS-ESI: $m/z = 385.0835$ (M – H$^+$), 421.0371 (M + Cl$^-$).

**Experimental**

**Synthesis and characterization of rigid Mn$^{2+}$ complexes (MnL)**

The results showed that Mn concentration in blood vessel is gradually reducing, and there are certain uptake of agents in heart and liver. This phenomenon follows the metabolic pathway of manganese, uptake by mitochondria rich organs.$^{2,3}$

**Fig. 6** Contrast enhancement MRA study of SD rat at a clinical 3.0 T scanner. Dosage: 0.25 Mn mmol kg$^{-1}$ Bw of MnL (A) and MnL-PEG4.6k-MnL (B).

**Fig. 7** Quantification of MRI signal changes in jugular vein, heart and liver at different time points after administration with MnL-PEG4.6k-MnL (dosage: 0.25 Mn mmol kg$^{-1}$ Bw).
solution (MnL concentration: 10 mg mL⁻¹). MnL complexes in the following experiments were further purified through Sephadex LH-20 column before use.

**Synthesis of the ligand with alkynyl group**

**Synthesis of dimethyl 4-hydroxypyridine-2,6-dicarboxylate (7).** Chelidamic acid 6 (10.0 g, 54.6 mmol) was suspended in 150 mL absolute methanol (MeOH). Then, 2,2-dimethoxypropene (DMP, 60 mL, 75 mmol) and concentrated HCl (7.5 mL, 20 mmol) were slowly added in with magnetic stirring. The solution was stirred for 12 h and heated to reflux for 5 h. The crude mixture was removed solvent under vacuum and 100 mL of aqueous solution of sodium bicarbonate was added in. The precipitate was isolated by filtration and washed with light-yellow solid was dissolved in 5 mL water, added concentrated HCl to adjust pH to 6.5, and water was evaporated off. Ethanol added to dissolve the product, filtered and stirred for another 5 h. The crude mixture was collected (11.9 g, yield 88%). ¹H NMR (400 MHz, CDCl₃) δ 7.65 (s, 2H), 3.97 (t, J = 12.4 Hz, 2H), 3.82 (m, 2H), 3.04 (m, 2H), 2.54 (q, J = 6.9 Hz, 2H), 1.70 (m, 8H). Then, the product (830 mg, 2 mmol) was dissolved in 20 mL methanol, NaN₃ (320 mg, 8 mmol) was dissolved in 2 mL MilliQ water and added in. Stirring at 40 °C for 5 h, methanol and water was evaporated off. The light-yellow solid was dissolved in 5 mL water, added concentrated HCl to adjust pH to 6.5, and water was evaporated off. The precipitate was filtered and ethanol added to dissolve the product, filtered and stirred for another 5 h. The crude mixture was collected (2.5 g, 13 mmol) was slowly added in 20 mL of SOCl₂ at 0 °C. The reaction mixture was stirred at room temperature for 1 h and heated to reflux for another 2 h. The crude mixture was removed solvent under vacuum and 20 mL of H₂O was added. The solution was filtered and saturated aqueous solution of sodium bicarbonate was added in drops into the filtrate. The precipitate was isolated by filtration to afford compound 10: 2.4 g, (80%). ¹H NMR (400 MHz, CDCl₃) δ 7.05 (s, 2H), 4.81 (d, J = 2.4 Hz, 2H), 4.48 (s, 4H), 3.66 (t, J = 2.4 Hz, 1H).

**Synthesis of 2,6-bis (chloromethyl)-4-(prop-2-yn-1-yloxy) pyridine (10).** Compound 9 (2.5 g, 13 mmol) was slowly added in 20 mL of SOCl₂ at 0 °C. The reaction mixture was stirred at room temperature for 1 h and heated to reflux for another 2 h. The crude mixture was removed solvent under vacuum and 20 mL of H₂O was added. The solution was filtered and saturated aqueous solution of sodium bicarbonate was added in drops into the filtrate. The precipitate was isolated by filtration to afford compound 10: 2.4 g, (80%). ¹H NMR (400 MHz, D₂O) δ 7.91 (s, 2H), 4.52 (d, J = 13.8 Hz, 2H), 4.40 (d, J = 12.4 Hz, 2H), 3.94 (m, 2H), 3.82–3.63 (m, 3H, 2H), 3.21 (m, 2H), 2.58–2.39 (m, 2H), 2.26–1.96 (m, 7H). ¹³C NMR (101 MHz, D₂O) δ 166.49 (s), 157.20 (s), 109.51 (s), 77.59 (s), 77.23 (s), 56.34 (s), 44.87 (s). ESI-MS: m/z = 230.01 (M + H⁺).

**Synthesis and characterization of ligand with alkynyl group**

(L-Alkynyl, 11). Compound 2 (2.65 g, 16 mmol) and compound 10 (2.1 g, 8 mmol) were dissolved in 30 mL dry acetonitrile, K₂CO₃ (11 g, 80 mmol) and KI (30 mg) were added. The mixture was stirred at 45 °C for 12 h. Filtered inorganic salt from solution, solvent was evaporated off. The product was purified by silica gel column chromatography (methylene dichloride/ethyl acetate = 2/1). ¹H NMR (400 MHz, D₂O) δ 6.98 (s, 2H), 4.75 (d, J = 1.6 Hz, 2H), 3.97 (t, J = 15.7 Hz, 2H), 3.76 (d, J = 14.2 Hz, 2H), 3.67 (s, 6H), 3.49–3.37 (m, 2H), 3.18–3.04 (m, 2H), 2.54 (q, J = 8.2 Hz, 3H), 2.25–1.70 (m, 8H). Then, the product (830 mg, 2 mmol) was dissolved in 20 mL methanol, NaOH (320 mg, 8 mmol) was dissolved in 2 mL MilliQ water and added in. Stirring at 40 °C for 5 h, methanol and water was evaporated off. The light-yellow solid was dissolved in 5 mL water, added concentrated HCl to adjust pH to 6.5, and water was evaporated off. Ethanol added to dissolve the product, filtered and stirred for another 5 h. The crude mixture was collected (2.4 g, 80%). ¹H NMR (400 MHz, D₂O) δ 7.16 (s, 2H), 4.93 (s, 2H, 4.52 (d, J = 13.8 Hz, 2H), 4.40 (d, J = 12.4 Hz, 2H), 3.94 (m, 2H, 3.82–3.63 (m, 3H, 2H), 3.21 (m, 2H), 2.58–2.39 (m, 2H), 2.26–1.96 (m, 7H). ¹³C NMR (101 MHz, D₂O) δ 165.21 (s), 157.19 (s), 110.63 (s), 77.70 (s), 76.77 (s), 69.07 (s), 58.89 (s), 57.38 (s), 56.13 (s), 28.96 (s), 22.61 (s). HRMS (ESI): m/z = 410.1647 (M + Na⁺) (Fig. 9).

**Synthesis of PEGylated Mn³⁺ complex (MnL-PEG-MnL)**

**Synthesis of PEG with terminal azide group (N₃-PEG-N₃).** Methylene chloride layer was dried over anhydrous magnesium sulfate. Filtered and saturated aqueous solution of sodium bicarbonate was added in. The solution was stirred at room temperature for 24 h. The mixture was washed three times with 50 mL water in a separatory funnel. Methylene chloride layer was dried with anhydrous magnesium sulfate. Filtered and evaporated off chloroform to get the product 8 (4.5 g, 90%). ¹H NMR (400 MHz, CDCl₃) δ 7.91 (s, 2H), 4.88 (d, J = 1.4 Hz, 2H), 4.02 (s, 6H), 2.63 (s, 1H).

**Synthesis of (4-(prop-2-yn-1-yl)oxy)pyridine-2,6-diyldimethanol (9).** Compound 8 (4.5 g, 18 mmol) was dissolved in 80 mL ethanol, NaBH₄ (2.7 g, 72 mmol) was added in slowly at 0 °C. The reaction mixture was stirred at room temperature for 2 h and heated to reflux for another 5 h. The crude mixture was concentrated under vacuum and 100 mL saturated aqueous solution of potassium carbonate was added. After stirred at 60 °C for 2 h, products were extracted out with chloroform (100 mL, three times). The organic phase dried over sodium sulfate and concentrated. Compound 9 was obtained as a white solid; 2.8 g (80%). ¹H NMR (400 MHz, DMSO) δ 6.92 (s, 2H), 5.41 (s, 2H), 4.90 (d, J = 2.4 Hz, 2H), 4.48 (s, 4H), 3.66 (t, J = 2.4 Hz, 1H).

**Synthesis of 2,6-bis (chloromethyl)-4-(prop-2-yn-1-olox) pyridine (10).** Compound 9 (2.5 g, 13 mmol) was slowly added in 20 mL of SOCl₂ at 0 °C. The reaction mixture was stirred at room temperature for 1 h and heated to reflux for another 2 h. The crude mixture was removed solvent under vacuum and 20 mL of H₂O was added. The solution was filtered and saturated aqueous solution of sodium bicarbonate was added in drops into the filtrate. The precipitate was isolated by filtration to afford compound 10: 2.4 g, (80%). ¹H NMR (400 MHz, CDCl₃) δ 7.05 (s, 2H), 4.81 (d, J = 2.4 Hz, 2H), 4.48 (s, 4H), 3.66 (t, J = 2.4 Hz, 1H).
vacuum distillation. The product was dissolved in 30 mL chloroform, and filtrated. Filtrate dropped into cold diethyl ether to obtain precipitate. Dried in vacuo to give a white powder product.

**Synthesis of PEGylated ligand (L-PEG-L) by click chemistry reaction.** L-Alkynyl (1.2 g, 3 mmol), N3-PEG2k-N3 or N3-PEG4.6k-N3 (1 mmol), anhydrous cupric sulfate and sodium ascorbate were added in 100 mL radius flask. 40 mL deoxygenated water was added to dissolve compounds. The mixture was stirred under inert gas protection and heated to 60 °C for 48 h. The solution was filtrated and dialyzed 3 days against water (MWCO 1000 Da membrane and then lyophilized to obtain PEGylated ligand (L-PEG2k-L or L-PEG4.6k-L).

**Preparation of PEGylated Mn**

2+ complexes solution. PEGylated ligand (L-PEG2k-L or L-PEG4.6k-L) and manganese chloride (5–10 times molar excess with regard to the L) were dissolved in 10 mL MilliQ water. After stirring for 1 hour, the solution was dialyzed 2 days against water (MWCO 1000 Da membrane) to ensure no free Mn2+. Mn concentration of PEGylated Mn2+ complexes solution was measured by elemental analyses using atomic absorption spectroscopy.

### T1 relaxation and MRI phantom studies

T1 relaxivities of free MnL, PEGylated Mn2+ complexes (MnL-PEG2k-MnL or MnL-PEG4.6k-MnL) were measured at 1.5 T on a clinical MR scanners (Siemens, Sonata) at 25 °C as previous reports.9 In brief, samples were diluted to different concentrations (0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, and 0.5 mM of Mn). The T1-weighted MRI phantom were acquired with a conventional spin-echo (SE) acquisition with TR values ranging from 20 to 1000 ms (TE = 5.3 ms, slice thickness = 3 mm, 292 × 146 mm, flip angle = 90°). Signal intensities at different TR were collected and used to fitting signal decay curve by OriginPro 8, and therefore obtained T1 time parameters. Relaxivity values (r1) were calculated through the linear fitting of 1/T1 time (s−1) versus the Mn concentration (mM) in Microsoft excel 2013.

### Conclusions

In summary, a rigid Mn2+ complex (MnL) was designed and synthesized. Then, further PEG (PEG2k and 4.6k) modification of MnL complexes was achieved by click chemistry with high ligand grafting rates (above one ligand per PEG molecule). PEGylated MnL present higher T1 relaxivities (MnL-PEG2k-MnL: r1 = 6.38 mmol−1 s−1; MnL-PEG4.6k-MnL: r1 = 5.63 mmol−1 s−1) than free MnL complexes. In vivo SD rat MRA studies show that PEGylated MnL have a longer vascular imaging window (up to 10 minutes) than that of free MnL at the same manganese dosage (0.25 Mn mmol kg−1 Bw).

### Conflicts of interest

There are no conflicts to declare.

### Acknowledgements

This work was supported by grants from the Chinese Ministry of Sciences 973 Program (2013CB933903), National Key Technology R&D Program (2012BAI23B08), and National Natural Science Foundation of China (81620103, 20974065, 51173117, 50830107, 81671675 and 81601490). Science and Technology Project of Sichuan, China (2016YJ0172), the Scientific Research Start-up Fund of North Sichuan Medical College (CBY15-QD02).

### Notes and references

1. D. Pan, S. D. Caruthers, A. Senpan, A. H. Schmieder, S. A. Wickline and G. M. Lanza, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2011, 3, 162–173.
2. E. Chabanova, V. B. Logayer, J. M. Moller and H. S. Thomsen, *Open Drug Saf. J.*, 2011, 2, 29–38.
3. D. Pan, A. H. Schmieder, S. A. Wickline and G. M. Lanza, *Tetrahedron*, 2011, 67, 8431–8444.
4. B. Drahos, J. Kotek, I. Cisarova, P. Hermann, L. Helm, I. Lukes and E. Toth, *Inorg. Chem.*, 2011, 50, 12785–12801.
5 M. A. Sieber, T. Steger-Hartmann, P. Lengsfeld and H. Pietsch, J. Magn. Reson. Imag., 2009, 30, 1268–1276.
6 T. S. Henrik, Radiol. Clin., 2009, 47, 827–831.
7 R. Jin, B. Lin, D. Li and H. Ai, Curr. Opin. Pharmacol., 2014, 18, 18–27.
8 E. J. Werner, A. Datta, C. J. Jocher and K. N. Raymond, Angew. Chem., Int. Ed., 2008, 47, 8568–8580.
9 B. Drahos, J. Kotek, I. Cisarova, P. Hermann, L. Helm, I. Lukes and E. Toth, Inorg. Chem., 2011, 50, 12785–12801.
10 S. Wang and T. D. Westmoreland, Inorg. Chem., 2008, 48, 719–727.
11 H. Su, C. Wu, J. Zhu, T. Miao, D. Wang, C. Xia, X. Zhao, Q. Gong, B. Song and H. Ai, Dalton Trans., 2012, 41, 14480–14483.
12 A. Forgács, R. Pujales-Paradelo, M. Regueiro-Figueroa, L. Valencia, D. Esteban-Gómez, M. Botta and C. Platas-Iglesias, Dalton Trans., 2017, 46, 1546–1558.
13 A. Forgács, M. Regueiro-Figueroa, J. L. Barriada, D. Esteban-Gómez, A. de Blas, T. Rodriguez-Blas, M. Botta and C. Platas-Iglesias, Inorg. Chem., 2015, 54, 9576–9587.
14 H. Ai, Adv. Drug Delivery Rev., 2011, 63, 772–788.
15 F. Kielar, L. Tei, E. Terreno and M. Botta, J. Am. Chem. Soc., 2010, 132, 7836–7837.
16 Y. Song, E. K. Kohlmeir and T. J. Meade, J. Am. Chem. Soc., 2008, 130, 6662–6663.
17 C. Wu, D. Li, L. Yang, B. Lin, H. Zhang, Y. Xu, Z. Cheng, C. Xia, Q. Gong, B. Song and H. Ai, J. Mater. Chem. B, 2015, 3, 1470–1473.
18 L. O. Liepold, M. J. Abedin, E. D. Buckhouse, J. A. Frank, M. J. Young and T. Douglas, Nano Lett., 2009, 9, 4520–4526.
19 C. Wu, Y. Xu, L. Yang, J. Wu, W. Zhu, D. Li, Z. Cheng, C. Xia, Y. Guo, Q. Gong, B. Song and H. Ai, Adv. Funct. Mater., 2015, 25, 3581–3591.
20 Y. Xu, C. Wu, W. Zhu, C. Xia, D. Wang, H. Zhang, J. Wu, G. Lin, B. Wu, Q. Gong, B. Song and H. Ai, Biomaterials, 2015, 58, 63–71.
21 H. Y. Su, C. Q. Wu, D. Y. Li and H. Ai, Chin. Phys. B, 2015, 24, 127506.
22 D. Wang, B. Lin, T. Shen, J. Wu, C. Xia, B. Song and H. Ai, Sci. Bull., 2016, 61, 1023–1030.
23 J. Xie, G. Liu, H. S. Eden, H. Ai and X. Chen, Acc. Chem. Res., 2011, 44, 883–892.
24 B. Podobnik, B. Helk, V. Smilović, Š. Škrajnar, K. Fidler, S. Jevšvar, A. Godwin and P. Williams, Bioconjugate Chem., 2015, 26, 452–459.
25 C. Dhalluin, A. Ross, L.-A. Leuthold, S. Foser, B. Gsell, F. Müller and H. Senn, Bioconjugate Chem., 2005, 16, 504–517.
26 A. M. Mohs, X. Wang, K. C. Goodrich, Y. Zong, D. L. Parker and Z. R. Lu, Bioconjugate Chem., 2004, 15, 1424–1430.