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Synthesis and magnetic relaxation properties of new Gd(III) complexes derived from DTPA-bis(amide) conjugates of arylpiperazinyl amines

Abdullah O. Ba-Salem [a], M. Nasiruzzaman Shaikh [b], Nisar Ullah [a,*], Mohamed Faiz [c]

[a] Chemistry Department, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia
[b] Center of Research Excellence in Nanotechnology, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia
[c] Physics Department, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia

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ABSTRACT

Two new Gd(III) complexes **1** and **2** of the type $[Gd(L)H_2O]\cdot nH_2O$ were synthesized from DTPA-bis(amide) conjugates of arylpiperazinyl amines. The relaxivity (R_1) of these complexes was measured in deionized water, which revealed that complex **2** had a higher relaxivity than **1** and Omniscan®, a commercially available MRI contrast agent. The cytotoxicity studies of **1** and **2** indicated that they are non-toxic which warrant their physiological suitability as potential contrast agents for MRI. All the synthesized ligands and complexes were characterized with the aid of analytical and spectroscopic methods including elemental analysis, ^1H NMR, FT-IR, XPS and fast atom bombardment (FAB) mass spectrometry.

Magnetic Resonance Imaging (MRI) is at present one of the safest and efficient non-invasive imaging modality in clinical diagnosis due to no use of harmful high-energy radiation compared to competing radio-diagnostic methods [1–3]. The diagnostic potential of MRI is enormous since, in addition to the assessment of anatomical changes, MRI can also be used for monitoring of organ functions for instance following the functions of the human brain on a real time-scale by functional-MRI (fMRI). In addition, MRI often represents the only reliable diagnostic method for, *e.g.* cranial abnormalities or multiple sclerosis [4,5]. With the advent of clinical MRI scanners, MRI contrast agents are used to study tissue function for instance to track vascular flow for large arteries and veins, vascular perfusion and permeability in capillary networks [6]. MRI contrast agents (CAs) are diagnostic magneto-pharmaceuticals which usually constitute low molecular weight Gd(III) chelates with an acyclic or macrocyclic ligand [7]. The efficacy of the CAs, known as relaxivity, is measured by its ability to transmit the paramagnetic properties into the bulk water proton and thereby shortening the T_1 relaxation times of water proton in the body that in turn provide impressive anatomical information [2]. The prominent position of Gd(III) in most MRI CAs relies on its favorable combination of a large magnetic moment (spin-only $\mu_{\text{eff}} = 1/4$ 7.94 BM, from seven half-filled f orbitals) and long electron spin relaxation time (10^{-8} to 10^{-9} s, from symmetric S electronic state) [8].

Anionic gadolinium complexes, for example $Gd(DTPA)^{2-}$, are generally associated with high osmolality under physiological conditions giving rise to some adverse effects. In addition, they suffer from limitations including limited utility in focal lesion detection. In order to alleviate such liabilities and improve the tissue and/or organ-specificity, preparation of neutral complexes based on the Gd macrocycle, aspiring for highly efficient ("optimized") CAs has been the subject of intensive research in the last 20 years. [9–12]. Based on earlier reports, incorporation of alkyl and aromatic groups in the side arm of the diethylene triamine pentaacetic acid (DTPA) ligand rendered excellent relaxivity and water solubility [13–15]. In light of the above, we have designed and synthesized two novel ligands **13** and **14** from the reaction of diethylene triamine pentaacetic acid dianhydride (DTPAA) with suitably modified arylpiperazines. These ligands were then transformed to their corresponding Gd(III) complexes **1** and **2**. Herein, we wish to disclose the synthesis, characterization, relaxivity (R_1) and cytotoxic studies of these complexes.

Ligands **13** and **14** were synthesized as outlined Scheme 1. Alkylation of commercially available fluoronitrophenol **3** produced **4** and **5**, which were condensed with piperazine **6** to furnish **7** and **8**. The basic hydrolysis of latter under heating generated **9** and **10**, respectively. Initially, we treated **4** and **5** with 1-boc-piperazine to obtain the corresponding condensed products but the acid induced removal of boc group led us to the deprotection of methoxymethyl group. Michael addition of amine **9** with acrylonitrile produced intermediate **11** (for details, see Supplementary information). The reduction of nitro group of **11** by hydrogenation using Pd–C or Ra–Ni as catalysts was problematic, leading to complex

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* Corresponding author.
E-mail address: nullah@kfupm.edu.sa (N. Ullah).

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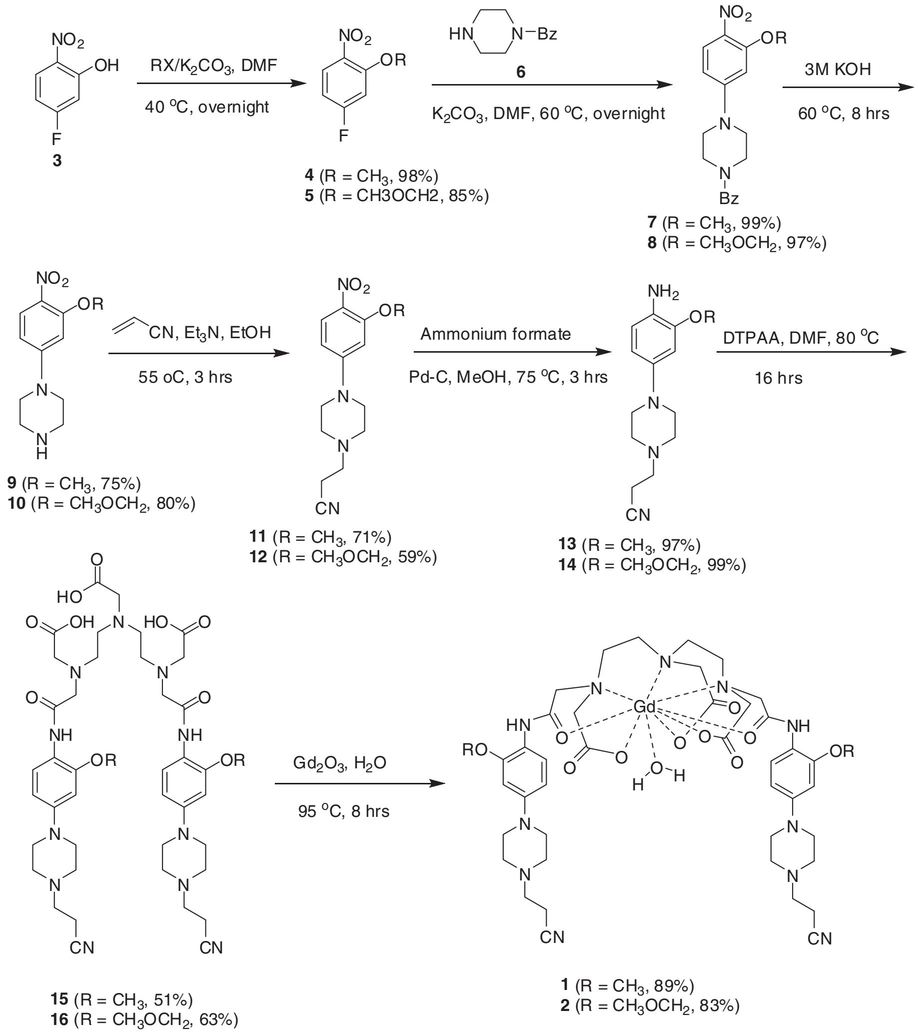

Scheme 1. Synthesis of gadolinium complexes (**1**) and (**2**).

mixture of products, which were difficult to resolve. Therefore, the reduction of nitro group was conceived *via* transfer hydrogenation in methanol with ammonium formate and Pd–C as catalyst to produce aminonitrile **13** in high yield (for details, see Supplementary information). DTPAA [16] was condensed with **13** to construct intermediate **15**, which in turn was heated with Gadolinium(III) oxide to produce complex **1** (Scheme 1). Likewise, complex **2** was synthesized by similar synthetic strategy as employed for **1** (Scheme 1).

Synthesis of **1**: To a solution of compound **15** (0.100 g, 0.114 mmol) in deionized water (10 mL) was added Gd_2O_3 (0.021 g, 0.057 mmol) and the reaction was stirred at 90 °C for 8 h. The mixture was filtered through a pad of celite while hot and the filtrate was concentrated

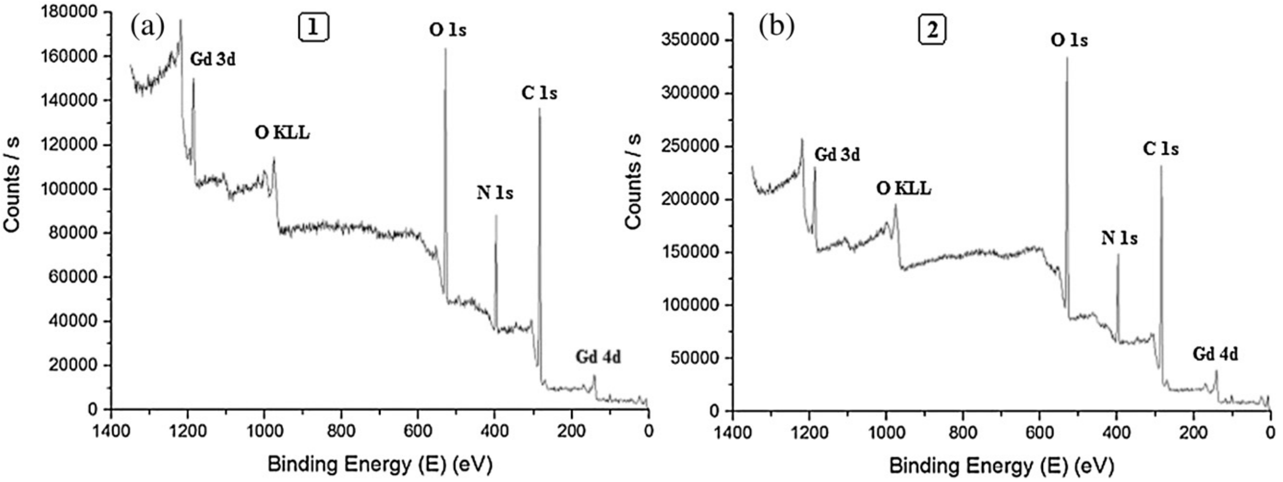

Fig. 1. Survey XPS spectrum of (**1**) and (**2**), showing all elements except hydrogen.

Table 1
Relaxivity and cytotoxicity data of complexes **1** and **2** compared with Omniscan[a].

CA	T_1 (ms)	R_1 (mM^{-1} s^{-1})	*In vitro* cytotoxicity (IC_{50} µg/mL)	
1	293.51 ± 1.21	3.40	**1**	>50
2	235.67 ± 1.01	4.24	**2**	>50
Omniscan®[b]		3.2	Cyclohexamide	0.13 ± 0.02

[a] Data measured under same condition.

[b] Data obtained from reference [20].

under reduced pressure. The residue was then added drop wise to cold acetone (25 ml) and the precipitated product was filtered, dried under reduced pressure to afford compound **1** as a light blue solid. Yield: 89%. Anal. Calcd for $C_{42}H_{56}GdN_{11}O_{10}\cdot 4H_2O$: C, 45.68; H, 5.84; and N, 13.95. Found: C, 45.62; H, 5.90; and N, 13.89. FAB-MS (*m*/*z*): calcd for $C_{42}H_{56}GdN_{11}O_{10}$, 1032.35 ($[MH]^+$). Found: 1032.35 ($[MH]^+$). Melting point: >300 °C. IR (KBr): 3444, 3030, 2927, 2250, 1611, 1456, 1399, 1323, 1250, 1204, 1135, 1093 cm^{-1}.

Synthesis of **2**: Following the same procedure adopted for the synthesis of **1**, condensation of ligand **16** with Gd_2O_3 afforded compound **2** as light purple solid. Yield: 83%. Anal. Calcd for $C_{44}H_{60}GdN_{11}O_{12}\cdot 4H_2O$: C, 45.39; H, 5.89; and N, 13.23. Found: C, 45.32; H, 5.95; and N, 13.26. FAB-MS (*m*/*z*): calcd for $C_{44}H_{60}GdN_{11}O_{12}$, 1092.37 ($[MH]^+$). Found: 1092.37 ($[MH]^+$). Melting point: >300 °C. IR (KBr): 3486, 3012, 2845, 2253, 1616, 1403, 1322, 1246, 1123, 1090, 990 cm^{-1}.

The structures of all the intermediates and final complexes **1** and **2** were established by spectroscopic techniques such as elemental analysis, ^1H and ^{13}C NMR, FAB mass spectrometry. Complexes **1** and **2** were highly hygroscopic and isolated as a hydrated solid. The appearance of the ν (OH) band from the water of crystallization at 3444 and 3486 cm^{-1} supported this observation [13]. Likewise, the disappearance of carbonyl stretching bands of free ligands **15** and **16** at 1706 and 1729 cm^{-1} in both **1** and **2** complexes indicated the participation of the carbonyl groups in coordination [17].

The metal contents and chemical composition of **1** and **2** were determined by X-ray photoelectron spectroscopy (XPS) (Figs. 1, 2 in Supplementary information). In compound **1**, C 1 s region fitted with three peaks (eV) at 284.8 (C–C), 286.0 (C–N, C–O) and 288.2 (C═C, C–O) [18]. Whereas Gd 4d region was fitted with four peaks, showing multiplet structure, N 1 s region was fitted with one peak at 399.7 eV. Likewise, O 1 s region was fitted with peaks (eV) at 531.4 (C–O) and 532.8 (C═O) (Fig. 2) [18]. The XPS measurement of compound **2** showed similar peaks pattern like in the case of **1** (Fig. 3, Supplementary information).

Relaxivities (R_1) were calculated as an inverse of relaxation times per mM based on the earlier report [19]. The R_1 data (Table 1) revealed that **2** had a higher relaxivity than **1** and Omniscan®, a commercially available MRI contrast agent. This higher relaxivity of **2** could be attributed to its relatively higher molecular weight along with the dangling alkylnitrile moiety, attached to the piperazine functionality, which may render stability through hydrogen bonding to the water molecule that is coordinated to the Gd(III).

Toxicity of compounds **1** and **2** was analyzed on adherent 3T3 mouse embryonic fibroblastic cells by MTT assay following earlier procedures with some modification [21]. The cytotoxicity assay indicated that compounds **1** and **2** were non-toxic, which warrant their physiological suitability as potential contrast agents for MRI.

In conclusion, two new non-ionic complexes of type $[Gd(L)H_2O]\cdot nH_2O$ have been synthesized and evaluated them for relaxivity and cytotoxicity. The relaxivity of these complexes turned out to be higher or comparable to Omniscan®. The cytotoxicity assay revealed that these complexes were non-toxic and hence make them prospective candidate as potential contrast agents for MRI.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2015.03.042.

References

[1] P.C. Lauterbur, Image formation by induced local interactions: examples employing nuclear magnetic resonance, Nature 242 (1973) 190–191.

[2] P. Caravan, J.J. Ellison, T.J. McMurry, R.B. Lauffer, Gadolinium (III) chelates as MRI contrast agents: structure, dynamics, and applications, Chem. Rev. 99 (1999) 2293–2352.

[3] K. Aneta, Z. Thomas, Bioorganic synthesis of some recent tactical application of bioisosteres in drug design, Med. Chem. 54 (8) (2011) 2529–2591.

[4] S. Ogawa, T.-M. Lee, A.S. Nayak, P. Glynn, Oxygenation-sensitive contrasts in magnetic resonance image of rodent brain at high magnetic fields, Magn. Reson. Med. 14 (1990) 68–78.

[5] J. Belliveau, B. Rosen, H. Kantor, R. Rzedzian, D. Kennedy, R. McKinstry, J. Vevea, M. Cohen, I. Pykett, T. Brady, Functional cerebral imaging by susceptibility-contrast NMR, Magn. Reson. Med. 14 (1990) 538–546.

[6] D.V. Hingorania, A.S. Bernsteinb, M.D. Pagela, A review of responsive MRI contrast agents: 2005–2014, Contrast Media Mol. Imaging (2014)http://dx.doi.org/10.1002/cmmi.1629.

[7] K.W.-Y. Chan, W.-T. Wong, Small molecular gadolinium(III) complexes as MRI contrast agents for diagnostic imaging, Coord. Chem. Rev. 251 (2007) 2428–2451.

[8] M.T. Vlaardingerbroek, J.A. den Boer, Magnetic Resonance Imaging. Theory and practice, Springer Verlag, Germany, 1996.

[9] H. Schmitt-Willich, M. Brehm, C.L.J. Evers, G. Michl, A. Muller-Fahrnow, O. Petrov, J. Platzek, B. Raduchel, D. Sulzle, Synthesis and physicochemical characterization of a new gadolinium chelate: the liver-specific magnetic resonance imaging contrast agent Gd-EOB-DTPA, Inorg. Chem. 38 (1999) 1134–1144.

[10] H.J. Weinmann, R.C. Brash, W.R. Press, Characteristics of gadolinium-DTPA complex: a potential NMR contrast agent, AJR Am. J. Roentgenol. 142 (1984) 619–622.

[11] C.A. Chang, Magnetic resonance imaging contrast agents. Design and physicochemical properties of gadodiamide, Investig. Radiol. 28 (Suppl. 1) (1993) S21–S27.

[12] S.K. Mark, C.D. William, B.L. David, N.R. Kenneth, C.Q. Steven, M.R. Scott, Gadolinium complexation by a new DPTA-amide ligand. Amide oxygen coordination, J. Inorg. Chem. 29 (1990) 1488–1491.

[13] S. Dutta, S.-K. Kim, E.J. Lee, T.-J. Kim, D.-S. Kang, Y. Chang, S.O. Kang, W.-S. Han, Synthesis and magnetic relaxation properties of paramagnetic Gd-complexes of new DTPA-bis-amides. The X-ray crystal structure of $[Gd(L)(H_2O)]\cdot 3H_2O$ (L = DTPA-bis(4-carboxylicphenyl)amide), Bull. Korean Chem. Soc. 27 (2006) 1038–1042.

[14] Y.-M. Wang, T.-H. Cheng, G.-C. Liu, R.-S. Sheu, Synthesis of some N,N″-bis(amide) derivatives of diethylenetriaminepentaacetic acid and the stabilities of their complexes with Gd3 +, Ca2 +, Cu2 + and Zn2 +, J. Chem. Soc. Dalton Trans. 5 (1997) 833–837.

[15] S. Dutta, J.-A. Park, J.-C. Jung, Y. Chang, T.-J. Kim, Gd-complexes of DTPA-bis(amide) conjugates of and its with high relaxivity and stability for magnetic resonance imaging, Dalton Trans. (2008) 2199–2206.

[16] D.J. Hnatowich, W.W. Layne, R.L. Childs, The preparation and labeling of DTPA-coupled albumin, Appl. Radiat. Isot. 33 (1982) 327–332.

[17] D. Zhang, X. Zhang, Z. Linb, H. Zhang, Y. Chen, S. He, Synthesis and relaxation properties of two non-ion complexes of gadolinium(III) and manganese(II) with derivatives from diethylene triamine pentaacetic acid and isoniazid, Inorg. Chem. Commun. 40 (2014) 66–68.

[18] D. Briggs, M.P. Seah, Practical surface analysis, second ed.vol. 1 Wiley, New York, 1990.

[19] K.-H. Jung, H.-K. Kim, G.H. Lee, D.-S. Kang, J.-A. Park, K.M. Kim, Y. Chang, T.-J. Kim, Gd Complexes of macrocyclic diethylenetriaminepentaacetic acid (DTPA) biphenyl-2,20-bisamides as strong blood-pool magnetic resonance imaging contrast agents, J. Med. Chem. 54 (2011) 5385–5394.

[20] M. Rohrer, H. Bauer, J. Mintorovitch, M. Requardt, H.-J. Weinmann, Comparison of magnetic properties of MRI contrast media solutions at different magnetic field strengths, Investig. Radiol. 40 (2005) 715–724.

[21] M.A. Mesaik, Z. Haq, S. Murad, Z. Ismail, N.R. Abdullah, H.K. Gill, A. Rahman, M. Yousaf, R.A. Siddiqui, A. Ahmad, M.I. Choudhary, Biological and molecular docking studies on coagulin-H: human IL-2 novel natural inhibitor, Mol. Immunol. 43 (2006) 1855–1863.