COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Novel coordinating motifs for lanthanide(III) ions based on 5-(2-pyridyl)tetrazole and 5-(2-pyridyl-1-oxide)tetrazole. Potential new contrast agents†

Antonio Facchetti,*[a] Alessandro Abbotto,[b] Luca Beverina,[b] Silvia Bradamante,[c] Palma Mariani,[d] Charlotte L. Stern,[a] Tobin J. Marks,[a] Alberto Vacca[d] and Giorgio A. Pagani*[b]

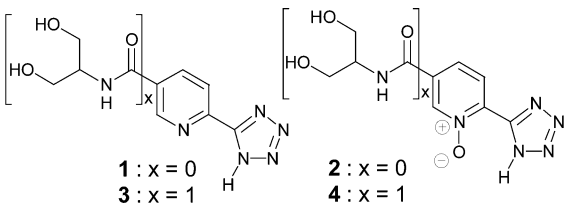
[a] *Department of Chemistry, Northwestern University, 2145 Sheridan Road, 60208, Evanston, IL, USA*
[b] *Department of Materials Science, University of Milano-Bicocca, Via Cozzi 53, I-20125, Milano, Italy*
[c] *CNR-Institute of Molecular Science and Technologies, Via Golgi 19, I-20133, Milano, Italy*
[d] *Department of Chemistry, University of Florence, Via della Lastruccia 3, I 50019, Sesto Fiorentino, Italy*

Received (in Cambridge, UK) 9th February 2004, Accepted 2nd June 2004
First published as an Advance Article on the web 30th June 2004

Water-soluble and neutral Ln(III) and Zn (II) complexes of pyridine- and (pyridine-1-oxide)tetrazole have been synthesized and the Gd derivatives have great potential as high-relaxivity low-osmolarity MRI contrast agents.

The emergence of magnetic resonance imaging (MRI) techniques for medical diagnosis has been accompanied by an intensive growth of interest in the study of paramagnetic metal complexes as contrast agents (CAs) designed to enhance tissue differentiation.[1,2] The majority of the approved CAs are Gd(III) chelates, the efficiency of which is given by the proton relaxivity, r_1, described as the proton relaxation rate enhancement in the presence of the CA compared to a diamagnetic environment. Currently, all Gd(III)-based chelates approved for use in MRI are nine-coordinate complexes in which a ligand occupies eight binding sites at the metal center and the ninth coordination site is occupied by one water molecule. Most of the investigated CAs are derivatives of a limited number of coordinating core structures, such as those of DTPA (diethelenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) which have relaxivity values of ~ 4–5 $mM^{-1}s^{-1}$.[3] Incorporation/functionalization of these ligand motifs into polymeric, dendrimeric, and protein structures increases the rotational correlation time and results in dramatically improved r_1 values.[3,4] However, the maximum values are still about half of the possible theoretical limit (~ 100 $mM^{-1}s^{-1}$) for these cores.[1a,4]

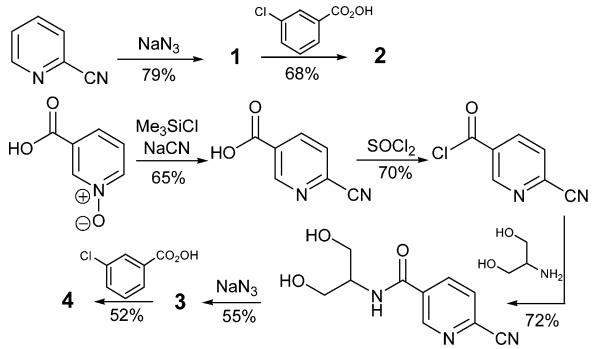
From this intense research effort, *all heteroaromatic*-based ligands are practically absent. This is surprising considering the rich chemistry, stability, and good coordinating properties of many heterocycles.[5] We describe here a new family of ligands (LH = **1–4**) based on the pyridine/pyridine-*N*-oxide–tetrazole skeleton. Ligands **3** and **4** bearing hydrophilic chains were envisioned to improve solubility in water. Thanks to the acidity of tetrazole (pK_a = 4.89 in H_2O)[5] all these systems form stable anions (L^-) at physiological pH range, which act as efficient ligands to transition [Zn(II)] and lanthanide [Gd (III), Eu(III), Dy(III)] metal ions.



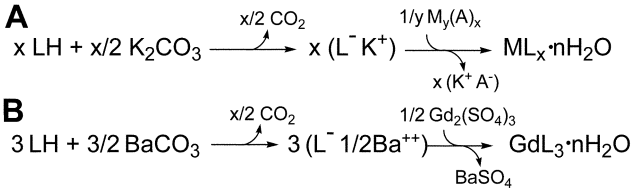
Compound **1**[6] and new ligands **2–4** were prepared according to Scheme 1.‡ The tetrazole ring in **1** and **3** was synthesized by reaction of the corresponding cyanopyridine with HN_3 (NaN_3 + NH_4Cl) in DMF at 130 °C. Regioselective oxidation of the pyridine nitrogen of **1** and **3** to afford **2** and **4**, respectively was achieved employing *m*-chloroperbenzoic acid in MeOH. This result is remarkable considering the large number of azine nitrogens present in **1** and **3**. Indeed, other oxidizing agents such as H_2O_2 and CH_3CO_3H afford inseparable mixture of products. The protonation constant of **1** (pK_a = 4.11) and **2** (pK_a = 3.55) were obtained *via* potentiometric titration. The pK_a values are lower than in tetrazole due to conjugation with the electron-poor pyridine ring. To fully explore the ligand capacities of both –N=C–C–N⁻– and O⁻–N⁺=C–C–N⁻– moieties present in this new class of ligands, anions $\mathbf{1}^-$ and $\mathbf{2}^-$ were reacted with both a transition metal ion [Zn(II), to allow chelate ^{1}H NMR analysis] and a set of lanthanide [Gd(III), Eu(III), and Dy(III)] ions, whereas ligands $\mathbf{3}^-$ and $\mathbf{4}^-$ were investigated targeting both Zn(II) and Gd(III) ions.

The synthesis of neutral chelates **1–4** was planned depending on their expected solubility properties. Poorly or relatively soluble chelates were prepared by reacting LH with the stoichiometric amount of K_2CO_3 in H_2O (Scheme 2A). The corresponding anion L^- was then reacted with concentrated aqueous solutions of the corresponding metal salts (acetate or chloride) and the resulting complexes isolated by filtration. For highly soluble Gd chelates, the ligand anion was prepared by reacting LH with $BaCO_3$ (Scheme 2B). A solution of $Gd_2(SO_4)_4$ was then added to the barium salt, and the insoluble $BaSO_4$ filtered off. Evaporation of water afforded the chelate. All the chelates (Table S1, Supporting Information†) were purified by crystallization from either H_2O or H_2O–MeOH.

Single crystals of Gd($\mathbf{1}^-$)$_3$, Gd($\mathbf{2}^-$)$_3$, and Zn($\mathbf{2}^-$)$_2$ were obtained either by cooling of a saturated solution or slow evaporation of an aqueous solution of the complex.§ The Gd(III) ion in Gd($\mathbf{1}^-$)$_3$ and Gd($\mathbf{2}^-$)$_3$ (Fig. 1) is nona- and octacoordinate by three bidentate



Scheme 1 Synthetic routes to ligands **1–4**.



Scheme 2 Synthetic routes to the neutral chelates.

† Electronic supplementary information (ESI) available: chelate analytical data, crystal structure of Zn($\mathbf{2}^-$)$_2$, potentiometric titrations, and relaxivity plots. See http://www.rsc.org/suppdata/cc/b4/b401919a/

DOI: 10.1039/b401919a

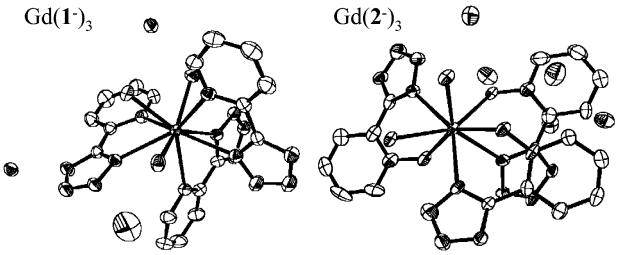


Fig. 1 Crystal structure of Gd(**1**$^{-}$)$_3$ and Gd(**2**$^{-}$)$_3$.

ligands and three/two H_2O molecules, respectively. The different coordination number probably reflects the dissimilar steric requirements[3] of a five- *versus* a six-membered chelation motif for **1** and **2**, respectively. Interestingly, three/five additional H_2O molecules are present in the second coordination sphere, suggesting large relaxivity values. The geometry of Zn(**2**$^{-}$)$_2$ (Figure S2†) is octahedral with two equatorial ligands and two H_2O molecules in the axial positions.

Ligands **1–4** and the corresponding Gd(III) chelates are highly soluble in H_2O. Chelate solubility increases going from the unsubstituted **1** (2.1 g L^{-1}) and **2** (4.7 g L^{-1})) to the hydroxyalkyl-substituted **3** (25.6 g L^{-1}) and **4** (21.2 g L^{-1}) systems. An additional requirement for practical application of Gd(III) complexes is thermodynamic stability, to avoid toxicity of the metal. Potentiometric data obtained by titration of a Gd:LH (LH = **1** and **2**, Figs. S3 and S4†) mixture in the pH range of 2–5 (T = 298 K, I = 0.1 M) can be fitted assuming the stepwise addition of up to three L^{-} to the Gd ion,[7] leading to a cumulative stability constant (logβ) values of 6.7(2) and 8.66(7) for Gd(**1**$^{-}$)$_3$ and Gd(**2**$^{-}$)$_3$, respectively. As expected, these values are lower than those of approved CAs based on macrocycle octadentate ligands (> 15.8).[3]

Finally, the relaxivity r_1 is the key property of a potential contrast agent. Measurements were performed at pH = 5.7–7.2 and r_1 values (mM^{-1} s^{-1}) were obtained from the slope of a plot of $1/T_1$ *vs.* [GdL$_3$] (concentration range 0.2–1 mM, Fig. S5†) and were found to be: Gd(**1**$^{-}$)$_3$ (9.98); Gd(**2**$^{-}$)$_3$ (9.25); Gd(**3**$^{-}$)$_3$ (10.79); Gd(**4**$^{-}$)$_3$ (17.72). These very high values cannot be explained solely on the basis of a large number of water molecules probably coordinating the metal ion in aqueous solution. Rather, very fast exchange between inner/outer sphere water molecules with the bulk H_2O might be involved, as suggested for complexes of heptadentate ligands.[8]

In summary, we described here new bidentate ligand motifs based solely on heteroaromatic units and leading to water-soluble neutral chelates. The Gd(III) complexes exhibit a large number of coordinated H_2O molecules and Gd(**4**$^{-}$)$_3$ exhibits the highest r_1 values reported to date for a low molecular weight molecule. Thanks to the facile functionalization of the pyridine ring, incorporation of these chelating motifs into a single tripodal hexadentate structure should greatly improve stability (necessary for application) and retain the intrinsic high relaxivity. Starting from these large values, r_1 can be further improved by ligand incorporation into slower rotational motion substrates such as proteins, dendrimers, and polysaccharides,[1,2] as demonstrated successfully for functionalized DTPA-protein bound (r_1 ~ 50 mM^{-1}s^{-1})[4a] and DOTA-dendrimer (r_1 ~ 15–19 mM^{-1}s^{-1}) systems.[4d] Efforts in this direction are in progress.

This work was supported by grants MURST II (0103-97) and NSF CHE-0078998.

Notes and references

‡ *Synthetic details.* Ligands **1** and **3**. A mixture of parent cyanopyridine (5.3 mmol), NaN_3 (8.0 mmol), NH_4Cl (8.0 mmol) in anhydrous DMF (27 mL) was reacted at 130 °C for 2–4 h. After cooling, the inorganic salts were discarded by filtration and the solvent removed under reduced pressure. The residue was taken up with dilute HCl (0.1 M, 20 mL) and the formed solid collected and recrystallized from H_2O. **1** (78%): mp 213 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.79 (dd, J = 5.0, 1.7, H-6); 8.22 (d, J = 8.0, H-3); 8.08 (td, H-4); 7.63 (dd, J = 8.0, H-5). **3** (76%): mp > 240 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.18 (d, J = 2.0, H-2); 8.47 (dd, J = 8.3, 2.0, H-4); 8.44 (d, J = 8.0, –CONH–); 8.32 (d, J = 8.0, H-5); 4.70 (s, OH); 4.01 (dtt, J = 8.0, 5.8, 5.8, CH); 3.60–3.48 (m, CH_2). Ligands **2** and **4**. A mixture of parent pyridyltetrazole (6.8 mmol) and *m*-chloroperbenzoic acid (11.6 mmol) in MeOH (200 mL) was reacted overnight at room temperature without stirring. **2** (66%) was isolated by filtration and recrystallized from H_2O. mp > 240 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.56 (d, J = 6.4, H-6); 8.41 (dd, J = 7.8, 2.1, H-3); 7.66 (td, H-4); 7.63 (dd, J = 7.7, H-5). **4** (80%) was isolated by evaporating the solvent and, after washing the solid with CH_2Cl_2 (300 mL), recrystallized from EtOH/H_2O. mp > 240 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.97 (s, H-2); 9.49 (d, J = 8.5, H-4); 8.53 (d, J = 8.5, – CONH–); 7.92 (d, J = 8.5, H-5); 4.70 (s, OH); 4.01 (m, CH); 3.60–3.40 (m, CH2).

§ *Crystal Structure.* All measurements were made on a Bruker SMART CCD diffractometer with graphite monochromated MoKα (0.71073 Å) radiation. The data were collected at a temperature of 153(2) K and the structures were solved by direct methods and expanded using Fourier techniques using SHELXTL. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in idealized positions and not refined. Intensities were corrected for absorption. Gd(**1**$^{-}$)$_3$. Monoclinic, $P2(1)/n$, Z = 4. Cell dimensions: a = 9.0273(9) Å; b = 17.714(3) Å; c = 16.7480(19) Å; α = 90°; β = 99.819(9)°; γ = 90.00°. V = 2639.0(6) Å^3, ρ_{calcd} = 1.794 g cm^{-3}, total/independent reflections = 24502/6489, R(int) = 0.0415, R = 0.0280. Zn(**2**$^{-}$)$_2$. CCDC 232483.Monoclinic, $C2/c$, Z = 8. Cell dimensions: a = 31.01(2) Å; b = 8.561(4) Å; c = 7.546(3) Å; α = 90°; β = 100.65(5)°; γ = 90°. V = 1968.8(18) Å^3, ρ_{calcd} = 1.875 g cm^{-3}, total/independent reflections = 8819/2401, R(int) = 0.0564, R = 0.0469. Gd(**2**$^{-}$)$_3$. CCDC 232481. Monoclinic, $P2(1)/n$, Z = 4. Cell dimensions: a = 7.847(3) Å; b = 39.783(7) Å; c = 9.092(3) Å; α = 90°; β = 96.50(3)°; γ = 90.00°. V = 2820.0(2) Å^3, ρ_{calcd} = 1.780 g cm^{-3}, total/independent reflections = 18878/5168, R(int) = 0.0393, R = 0.0762. CCDC 232482. See http://www.rsc.org/suppdata/cc/b4/b401919a/ for crystallographic data in .cif or other electronic format.

1 (*a*) *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, eds. A. Merbach and E. Toth, John Wiley and Sons, New York, 2001; (*b*) S. Aime, M. Botta, M. Fasano and E. Terreno, *Acc. Chem. Res.*, 1999, **32**, 941; (*c*) P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293; (*d*) S. H. Koenig and R. D. Brown III, *Prog. NMR Spectrosc.*, 1990, **22**, 487; (*e*) B. L. Engelstad and G. L. Wolf, in *Magnetic Resonance Imaging*, eds. D. D. Stark and W. G. Bradley, Jr., The C. V. Mosby Company, St. Louis, 1988.

2 (*a*) L. Luttuada and G. Lux, *Tetrahedron Lett.*, 2003, **44**, 3893; (*b*) L. Vander Elst, M. Port, I. Raynal, C. Simonot and R. N. Muller, *Eur. J. Inorg. Chem.*, 2003, 2495; (*c*) K. Kimpe, T. N. Parac-Vogt, S. Laurent, C. Pierart, L. Vander Elst, R. N. Muller and K. Binnemans, *Eur. J. Inorg. Chem.*, 2003, 3021; (*d*) R. D. Bolskar, A. F. Benedetto, L. O. Husebo, R. E. Price, E. F. Jackson, S. Wallace, L. J. Wilson and J. M. Alford, *J. Am. Chem. Soc.*, 2003, **125**, 5471; (*e*) M. K. Thompson, M. Botta, G. Nicolle, L. Helm, S. Aime, A. E. Merbach and K. N. Raymond, *J. Am. Chem. Soc.*, 2003, **125**, 14274; (*f*) A. Borel, L. Helm and A. E. Merbach, *Chem. Eur. J.*, 2001, **7**, 600.

3 *Contrast Agents I/Magnetic Resonance Imaging.*, Ed. W. Krause, *Topics in Current Chemistry*, Springer, Berlin, 2002, **Vol 221**.

4 (*a*) R. N. Muller, B. Raduchel, H. R. Maecke and A. E. Merbach, *Eur. J. Inorg. Chem.*, 1999, 1949; (*b*) É. Tóth, F. Connac, L. Helm, K. Adzamli and A. E. Merbach, *J. Biol. Inorg. Chem.*, 1998, **3**, 606; (*c*) S. Aime, M. Botta, S. G. Crich, G. Giovenzana, M. Sisti and E. Terreno, *J. Biol. Inorg. Chem.*, 1997, **2**, 470; (*d*) É. Tóth, D. Pubanz, S. Vauthey, L. Helm and A. E. Merbach, *Chem. Eur. J.*, 1996, **2**, 1607; (*e*) V. S. Vexler, O. Clement, H. Schmitt-Willich and R. C. Brasch, *J. Magn. Res. Imaging*, 1994, **4**, 381.

5 A. R. Katritzky in, *Handbook of Heterocyclic Chemistry*, Pergamon Press, Oxford, 1985.

6 J. M. Mc Manus and R. M. Herbst, *J. Org. Chem.*, 1959, **24**, 1462.

7 Potentiometric data were analysed using HYPERQUAD program . P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739.

8 Y. Bretonniere, M. Mazzanti, J. Pecaut, F. A. Dunand and A. E. Merbach, *Chem. Commun.*, 2001, 621.