Pergamon

Tetrahedron Letters 43 (2002) 783–786

TETRAHEDRON LETTERS

Synthesis of the Gd(III) complex with a tetrazole-armed macrocyclic ligand as a potential MRI contrast agent

Silvio Aime,[a] Giancarlo Cravotto,[b] Simonetta Geninatti Crich,[a] Giovanni B. Giovenzana,[c] Marinella Ferrari,[d] Giovanni Palmisano[e,*] and Massimo Sisti[e]

[a]*Dipartimento di Chimica I.F.M., Via Giuria 7, 10125 Torino, Italy*
[b]*Dipartimento di Scienza e Tecnologia del Farmaco, Via Giuria 9, 10125 Torino, Italy*
[c]*Dipartimento di Scienze Chimiche Alimentari Farmaceutiche e Farmacologiche, Viale Ferrucci 33, 28100 Novara, Italy*
[d]*Dipartimento di Chimica Organica e Industriale, Via Venezian 21, 20133 Milano, Italy*
[e]*Dipartimento di Scienze Chimiche Fisiche e Matematiche, Via Valleggio 11, 22100 Como, Italy*

Received 12 November 2001; revised 26 November 2001; accepted 29 November 2001

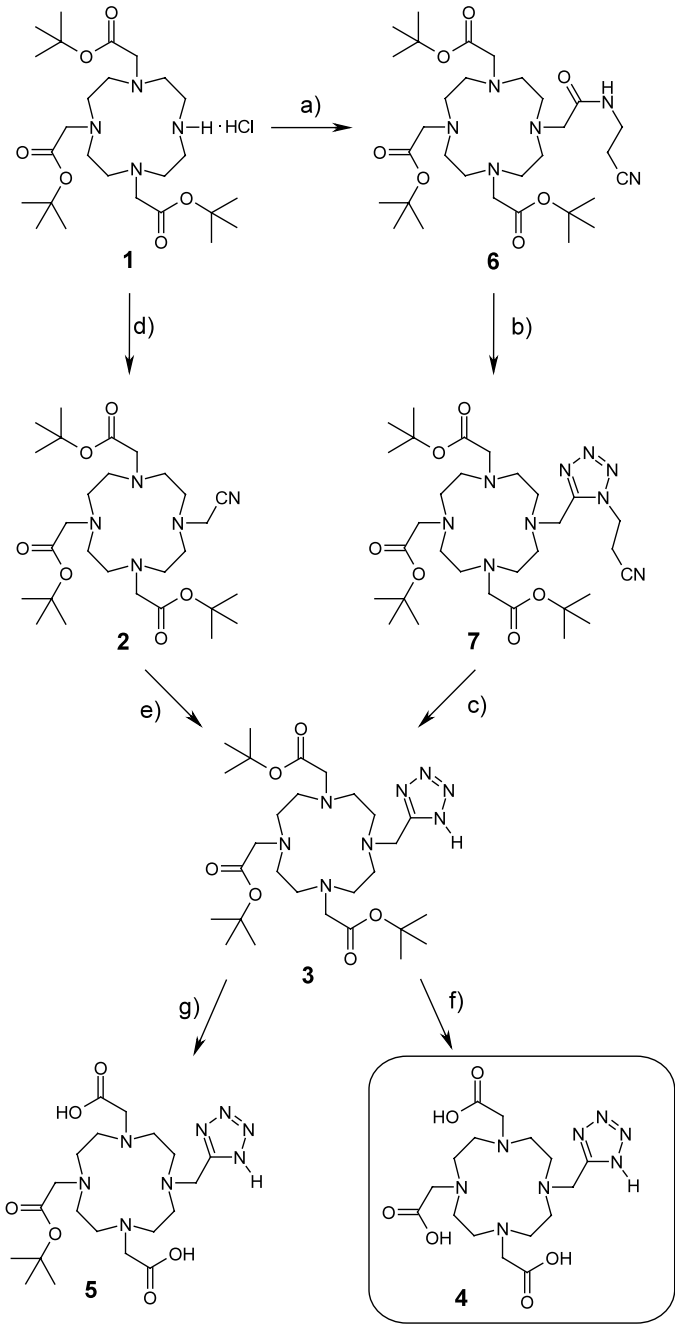
Abstract—We report the synthesis and the properties of the Gd(III) complex with **4** (H_4dotetra), a novel mixed pendant-arm macrocyclic ligand embodying a tetrazole subunit in a N_5O_3 donor set as a potential magnetic resonance imaging (MRI) contrast agent.

Azacrowns such as 1,4,7,10-tetraazacyclododecane (cyclen) have a broad spectrum of applications.[1] Their properties have been thoroughly investigated in the design of magnetic resonance imaging (MRI) contrast agents (CAs) based on their paramagnetic ($S=7/2$) Gd(III) complexes[2] as well as diagnostic/therapeutic radiopharmaceuticals carrying radionuclides such as ^{90}Y, $^{64,67}Cu$ or ^{111}In.[3] In order to optimize efficiency in MRI applications, numerous macrocyclic ligands have been synthesized with a wide spectrum of structural features, based on the tetraacetic derivative (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, H_4dota). Isosteric replacement of functional groups (e.g. CO_2H) represents one strategy used in medicinal chemistry for the rational modification of a lead compound to yield safer and more effective agents.[4] Generally, in non-classical isosteres the carboxylate group is replaced by one of the following: (a) point-charge monoprotic acids (e.g. hydroxamic acids and acylsulphonamides), (b) tetrahedral oxyacids (e.g. PO_3H_2, SO_3H and derivatives), (c) charge-distributed monoprotic acids (e.g. tetrazoles and 1,2,4-oxadiazolones). Over the past two decades, several isosteres of H_4dota, including phosphonic, phosphinic and sulphonic acids and carboxamides have been currently evaluated as potential MRI agents.[5] To further clarify the structure–activity relationship (SAR) and to address the role of the CO_2H group in H_4dota-related CAs, we have examined the effect of an isosteric replacement for the CO_2H at a carbon atom of a pendant arm. Accordingly, the tetrazole derivative **4** (H_4dotetra) was selected for synthesis and evaluation (Scheme 1).

This nitrogen-rich five-membered heterocycle (a strong σ-donor and weak π-acceptor ligand) shows a remarkable similarity in size and shape to the carboxyl group and displays acidic properties with pK_a 4.9–5.6 versus 4.2–4.6 for carboxylic acids.[6] Our initial efforts on the preparation of **4** from the known tri(*tert*-butyl)ester hydrochloride **1**[7] proved less straightforward than its structure would suggest. Attempts to directly alkylate **1** with either 5-chloromethyl-1*H*-tetrazole[8] or 2-benzyl-5-chloromethyl-2*H*-tetrazole[9] in refluxing MeCN in the presence of K_2CO_3 yielded intractable mixtures showing little or no evidence of containing the desired tetrazole derivatives. The failure of this approach led us to attempt the alkylation step *prior* to installing the tetrazole moiety. The modification of a single pendant arm of H_4dota to afford the tetrazole derivative **4** was attempted by the method of Duncia[10] as outlined in Scheme 1. Alkylation of **1** with *N*-(2-cyanoethyl)-2-bromoacetamide[11] in MeCN at rt (24 h) in the presence of K_2CO_3 provided cyanoethylamide **6**[12] (69% yield). To our disappointment, treatment of **6** with $TMSN_3$ in the presence of diisopropyl azodicarboxylate (DIAD) and PPh_3 in THF under various reaction conditions failed to effect the cyclization of the putative intermediate imidoyl azide[13] to the protected tetrazole **7**. Then we

* Corresponding author.

0040-4039/02/$ - see front matter
PII: S0040-4039(01)02272-9



Scheme 1. *Reagents and conditions*: (a) $BrCH_2CONHCH_2CH_2CN$, K_2CO_3, CH_3CN, rt, 24 h; (b) $TMSN_3$, DIAD, TPP, THF, rfx; (c) DBU, MeCN, rt; (d) $ClCH_2CN$, KI, K_2CO_3, MeCN, rfx, 3 h; (e) TMS–N_3, Bu_2SnO, PhMe, rfx, 4 h, then 5% aq. HCl, rt, 0.5 h; (f) 12N HCl, rfx, 2 h; (g) TFA, rt, 24 h.

turned our attention to nitriles as dipolarophiles in the intermolecular [3+2] cycloaddition for the assembly of a tetrazole ring in **4**. Thus, **1** was reacted with freshly distilled $ClCH_2CN$ under heterogeneous conditions (K_2CO_3, cat. KI, MeCN, reflux, 3 h) to afford the required nitrile **2**[12] in 76% isolated yield. Subsequent thermal [3+2] cycloaddition of **2** with HN_3 (or equivalents, i.e. NaN_3–NH_4Cl–LiCl in DMF,[14] NaN_3–$AlCl_3$ in THF,[15] or Me_3SnN_3 in *o*-xylene[16]) under various conditions gave erratic results. Gratifyingly, reaction of **2** with $TMSN_3$ in refluxing toluene (4 h) in the presence of 1.0 equiv. of Bu_2SnO[10,17] resulted in the tetrazole triester **3**[12] in 56% isolated yield after acidic workup (5% aq. HCl, rt, 0.5 h) to protodestannylate the 5-stannyltetrazole product. Full deprotection of *tert*-butyl esters in **3** to reach the target **4**[12] (91%) was cleanly achieved with 12N HCl at reflux (2 h), whereas selective deprotection took place in neat TFA (rt, 24 h) yielding the symmetrical diacid **5**[12] in 85% yield.

The protonation constants of H_4dotetra **4** were determined by conventional pH potentiometry [pK_{a1} 11.2(4), pK_{a2} 9.1(8), pK_{a3} 4.7(2), pK_{a4} 3.3(8)]. The Gd(III) complex of **4** Na^+[Gd-**4**]$^-$ was synthesized by mixing a stoichiometric amount of Gd(III) chloride hexahydrate with an aqueous solution of **4**, while maintaining the pH at 7.5 by the addition of 1N NaOH. Excess free Gd(III) ion was easily removed by precipitation of $Gd(OH)_3$ at basic pH, followed by centrifugation of the precipitate. The key requirements for a potential Gd-containing contrast agent are: (i) high thermodynamic and kinetic stability (vide infra), (ii) good water solubility, (iii) low osmolality and (iv) marked ability to enhance the relaxation rate of solvent water. The magnitude of this effect is generally expressed as relaxivity (R^{1p}), the latter being function of many parameters (i.e. the denticity and the nature of donor atom set). Relaxivity measurements (pH 7.4, 298 K, 0.47 T) with Na^+[Gd-**4**]$^-$ gave values of $R^{1p}=4.8$ mM^{-1} s^{-1}. Interestingly, this value is higher than those reported for octadentate ligands such as $Gd(dota)^{-}$[18] and $Gd(dtpa)^{2-}$ (dtpa is diethylenetriamine–pentaacetic acid),[19] both used nowadays in clinical applications on a routine basis.[20] A careful analysis of R^{1p} at various frequencies strongly suggests a close similarity of [Gd-**4**]$^-$ with $Gd(dota)^-$: H_4dotetra should then be coordinated to Gd(III) ion in an octadentate fashion via five N atoms (four of the macrocycle rim and one of the tetrazole ring) and three carboxylate oxygens (N_5O_3 donor set) with one water molecule in the inner sphere. The kinetic stability of Na^+[Gd-**4**]$^-$ was assessed by measuring the pH dependence of relaxivity in a closed cell under dinitrogen with CO_2-free KOH. Under these conditions R^{1p} remains constant in the range 2<pH< 11.5 (Fig. 1).

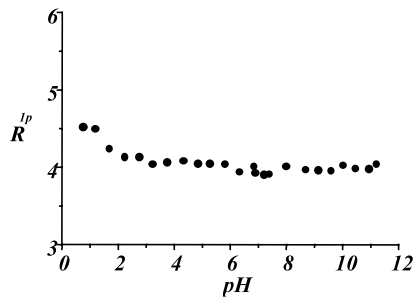


Figure 1. Relaxivity as a function of pH for [Gd-**4**]$^-$ (25°C, 0.47 T).

A high in vivo stability of the complex under normal metabolic conditions is required as free Gd(III) is highly toxic and is retained in the liver, spleen and bone. Potentiometric determination of the Na^+[Gd-**4**]$^-$ thermodynamic stability constant K_{GdL} gave a log K_{GdL} 19.0, indicating a reasonable stability compared to the lowest value (16.85) for Gd(dtpa-bma), a commercially used MRI agent (Omniscan™, Nycomed). The tetrazole, like other surrogates of the CO_2H function, would be expected to have a pronounced effect on the metabolic stability[16] of the ligand; consequently, it was thought that it was poorly adsorbed, thus exhibiting an even better safety profile. Furthermore, we can envisage for the tetrazole group the occurrence in vivo of non-covalent interactions with slowly moving biomolecules such as proteins. This could lead to CAs with longer correlation times and consequently higher relaxivity. Specific tissue affinity could also be improved. In summary, we have shown that H_4dotetra **4**, arising from isosteric substitution of a single CO_2H pendant arm by a tetrazole moiety, represents a good framework to produce an octadentate N_5O_3 ligand system for Gd(III) ion. The resulting complex Na^+[Gd-**4**]$^-$ exhibits a satisfactory stability and interesting relaxometric properties that make it suitable for MRI applications.

Acknowledgements

Financial support from Bracco Imaging (Milan) and Italian CNR (Target Project on Biotechnology) are gratefully acknowledged.

References

1. (a) Dietrich, B.; Viout, P.; Lehn, J.-M. *Macrocyclic Chemistry*; VCH: Weinheim, 1993; (b) Gokel, G. *Monographs in Supramolecular Chemistry*: *Crown Ethers and Cryptands*; The Royal Society of Chemistry: Cambridge, 1991; (c) Alexander, V. *Chem. Rev*. **1995**, *95*, 273.
2. (a) Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev*. **1995**, *95*, 2529; (b) Lauffer, R. B. *Chem. Rev*. **1987**, *87*, 901; (c) Hohenschuh, E.; Watson, A. D. *Magnetic Resonance Imaging of the Body*: *Contrast Media-Theory and Mechanisms of Contrast-Enhancing Agents*, 3rd ed.; Lippincott-Raven Press: New York, 1997; pp. 1439–1464; (d) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev*. **1999**, *99*, 2393; (e) Lauffer, R. B.; Parmelee, D. J.; Dunham, S. U.; Ouellet, H. S.; Dolan, R. P.; Witte, S.; McMurry, T. J.; Walovitch, R. C. *Radiology* **1998**, *207*, 529; (f) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev*. **1987**, *87*, 901.
3. (a) Volkert, W. A.; Hoffmann, T. J. *Chem. Rev*. **1999**, *99*, 2269; (b) Anderson, C. J.; Welch, M. J. *Chem. Rev*. **1999**, *99*, 2219.
4. (a) Wermuth, C. G. *The Practice of Medicinal Chemistry*: *Molecular Variations Based on Isosteric Replacement*; Academic Press: Oxford, 1996; pp. 204–232; (b) Patani, G. A.; LaVoie, E. J. *Chem. Rev*. **1996**, *96*, 3147.
5. Broan, C. J.; Cole, E.; Jankowski, K. J.; Parker, D.; Pulukkody, K.; Boyce, B. A.; Beeley, N. R. A.; Millar, K.; Millican, A. T. *Synthesis* **1992**, 63.
6. Singh, H.; Chawla, A. S.; Kapoor, V. K.; Paul, D.; Malhotra, R. K. *Progress in Medicinal Chemistry*: *Medicinal Chemistry of Tetrazoles*; Elsevier: Amsterdam, 1980; pp. 151–183.
7. Heppeler, A.; Froidevaux, S.; Maecke, H. R.; Jermann, E.; Behe, M.; Powell, P.; Hennig, M. *Chem. Eur. J*. **1999**, *5*, 1974–1981.
8. (a) Behringer, H.; Kohl, K. *Chem. Ber*. **1956**, *89*, 2648; (b) Yaouanc, J. J.; Sturtz, G.; Kraus, J. L.; Chastel, C.; Colin, J. *Tetrahedron Lett*. **1980**, *21*, 2689.
9. Moderhack, D. *Chem. Ber*. **1975**, *108*, 887.
10. (a) Duncia, J. V.; Pierce, M. E.; Santella, J. B. *J. Org. Chem*. **1991**, *56*, 2395; (b) Wallace, E. M.; Moliterni, J. A.; Moskal, M. A.; Neubert, A. D.; Marcopulos, N.; Stamford, L. B.; Trapani, A. J.; Savage, P.; Chou, M.; Jeng, A. Y. *J. Med. Chem*. **1998**, *41*, 1513.
11. Kano, S.; Komoto, K.; Uchiyama, Y.; Noguchi, T. GB Patent 1,090,608 (1967) (to Japan Soda Co. Ltd); (*Chem. Abstr*. 68:21179).
12. Analytical data. Compound **2**: mp 176°C (dec.). ^{1}H NMR ($CDCl_3$) 3.63 s[2H], 3.13 s[4H], 3.00 s[2H], 2.91–2.12 m[16H], 1.39 s[18H], 1.34 s[9H]. ^{13}C NMR ($CDCl_3$) 173.6 (s), 173.5 (s), 115.0 (s), 82.9 (s), 82.6 (s), 56.7 (t), 55.7 (t), 50.7 broad (4t), 42.7 (t), 27.8 (q), 27.7 (q). MS(CI) 554 (MH^+). Anal. calcd for $C_{28}H_{51}N_5O_6$ (553.7) C, 60.73; H, 9.28; N, 12.65. Found C, 60.55; H, 9.38; N, 12.50. Compound **3**: mp 98–100°C. ^{1}H NMR ($CDCl_3$) 3.09 s[2H], 2.95 s[4H], 2.91 [4H], 3.10–2.20 m[16H], 1.47 s[9H], 1.44 s[18H]. ^{13}C NMR ($CDCl_3$) 172.0 (s), 171.4 (s), 159.9 (s), 82.7 (s), 81.8 (s), 56.0 (t), 55.7 (t), 50.0 (t), 49.6 bs(4t), 27.9 (q), 27.8 (q). MS(CI) 597 (MH^+). Anal. calcd for $C_{28}H_{52}N_8O_6$ (596.8) C, 56.35; H, 8.78; N, 18.78. Found C, 56.29; H, 8.89; N, 18.59. Compound **4**: mp 255–260°C (dec.). ^{1}H NMR (D_2O) 3.79 s[2H], 3.75 s[4H], 3.51 s[2H], 3.10–2.90 m[16H]. MS(FAB$^+$) 451 (MNa^+), 429 (MH^+), 411. Anal. calcd for $C_{16}H_{28}N_8O_6$ (428.4) C, 44.85; H, 6.59; N, 26.15. Found C, 44.69; H, 6.72; N, 26.09. Compound **5**: mp 189–192°C (dec.). ^{1}H NMR (D_2O) 3.09 s[4H], 2.92 s[4H], 2.91 s[2H], 1.58 s[9H]. MS(FAB$^+$) 507 (MNa^+), 485 (MH^+), 467. Anal. calcd for $C_{20}H_{36}N_8O_6$ (484.6) C, 49.58; H, 7.49; N, 23.13. Found C, 49.51; H, 7.47; N, 22.89. Compound **6**: mp 125–127°C (dec.). ^{1}H NMR ($CDCl_3$) 8.97 t[1H] (J=5.6 Hz), 3.51 q[2H] (J=6.6 Hz), 2.66 t[2H] (J=7.1 Hz), 3.35–2.24 m[28H], 1.41 s[18H], 1.39 s[9H]. ^{13}C NMR ($CDCl_3$) 172.3 (2·s), 172.2 (2·s), 118.6 (s), 82.4 (2·s), 58.3 (t), 57.2 (t), 56.9 (t), 54–52 broad (4·t) 35.3 (t), 28.4 (q), 28.3 (q), 18.6 (t). MS(CI) 625 (MH^+). Anal. calcd for $C_{31}H_{56}N_6O_7$ (624.8) C, 59.59; H, 9.03; N, 13.45. Found C, 59.61; H, 9.10; N, 13.32.
13. Harvill, E. K.; Herbst, R. M.; Schreiner, E. C.; Roberts, C. W. *J. Org. Chem*. **1950**, *15*, 662.
14. Butler, R. N.; Quinn, K. F.; Welke, B. *J. Chem. Soc., Chem. Commun*. **1992**, 1481.
15. Arnold, C.; Thatcher, D. N. *J. Org. Chem*. **1969**, *34*, 1141.
16. Ashton, W. T.; Cantone, C. L.; Chang, L. L.; Hutchins, S. M.; Strelitz, R.; MacCross, M.; Chang, R. S. L.; Lotti, V. J.; Faust, K. A.; Chen, T.-B.; Bunting, P.; Schorn, T. W.; Sivlighn, S. D.; Siegl, P. K. S. *J. Med. Chem*. **1993**, *36*, 591.
17. Luitjen, J. G.; Janssen, M. J.; Van der Kirk, G. J. M. *Recl. Trav. Chim. Pays Bas* **1963**, *81*, 286.

18. Aime, S.; Anelli, P. L.; Botta, M.; Fedeli, F.; Grandi, M.; Paoli, P.; Uggeri, F. *Inorg. Chem.* **1992**, *31*, 2422–2428.
19. Aime, S.; Botta, M.; Panero, M.; Grandi, M.; Uggeri, F. *Magn. Reson. Chem.* **1991**, *29*, 923–927.
20. The current MRI contrast agents in clinical use based on polyamino carboxylate complexes of Gd(III) are Magnevist™ (Gd-dtpa, Schering), ProHance™ (Gd-hpdo$_3$a, Bracco), Dotarem™ (Gd-dota, Guerbet) and MultiHance™ (Gd-bopta, Bracco).