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Synthesis of Gd-DTPA-cholesterol: a new lipophilic gadolinium complex as a potential MRI contrast agent

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Abstract—A straightforward and efficient synthesis of Gd-DTPA-cholesterol is reported. This new lipophilic gadolinium complex can be easily incorporated into mixed micelles in order to achieve MRI contrast agents with increased relaxivity. These supramolecular systems can find potential application in magnetic resonance angiography (MRA). © 2003 Elsevier Science Ltd. All rights reserved.

Magnetic resonance imaging (MRI) is a powerful and non-invasive diagnostic technique useful in providing images of the inside of the human body.[1] Nowadays, around 30% of all the MRI scans are performed employing a contrast agent,[2,3] which is an exogenous compound able to enhance the relaxation rates of water protons. In this way the quality of the MRI images obtained is greatly improved.

Polyaminopolycarboxylic complexes of gadolinium ion are the most widely used contrast agents in MRI.[2,3] Since 1988, when the use of Gd-DTPA (Fig. 1) was approved for humans, several other gadolinium complexes have reached the market (e.g. Gd-DOTA, Gd-HPDO3A)[4,5] (Fig. 1) but there is still a great effort in synthesizing new products with improved properties and for specific applications.

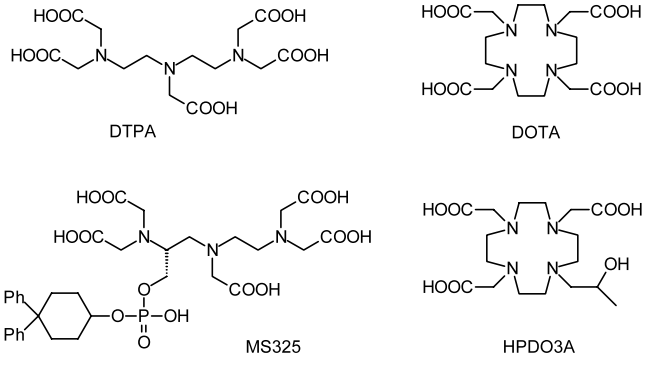

Figure 1. Chemical structure of some polyaminopolycarboxylic ligands used in MRI.

A very urgent and specific medical need is represented by the imaging of the cardiovascular system.[6,7] To perform a magnetic resonance angiography (MRA) the administered contrast agent must stay in the blood stream for a long time and three main different strategies have been proposed.

Serum protein binders, like MS325[8] (Fig. 1), are currently in development while polymeric[9] or dendrimeric[10] gadolinium complexes, which stay confined into the blood vessels because of their large dimension, are examples of a different approach. Finally, supramolecular systems such as liposomes[11] or micelles[12] have also been used. In particular, we focused our attention on mixed micelles which are three component aggregates of: (i) a non-ionic, biocompatible surfactant (e.g. Synperonic® F108); (ii) a phospholipid; (iii) a lipophilic gadolinium complex (Fig. 2). Mixed micelles containing lipophilic gadolinium complexes bearing one or two aliphatic chains have been already studied and have given promising results as MRA contrast agents.[13]

Here we report the synthesis of the new gadolinium complex **5** containing a cholesterol unit as lipophilic moiety. Cholesterol is an important component of natural membranes and in literature there are several examples of molecules (e.g. polyamines,[14] oligonucle-

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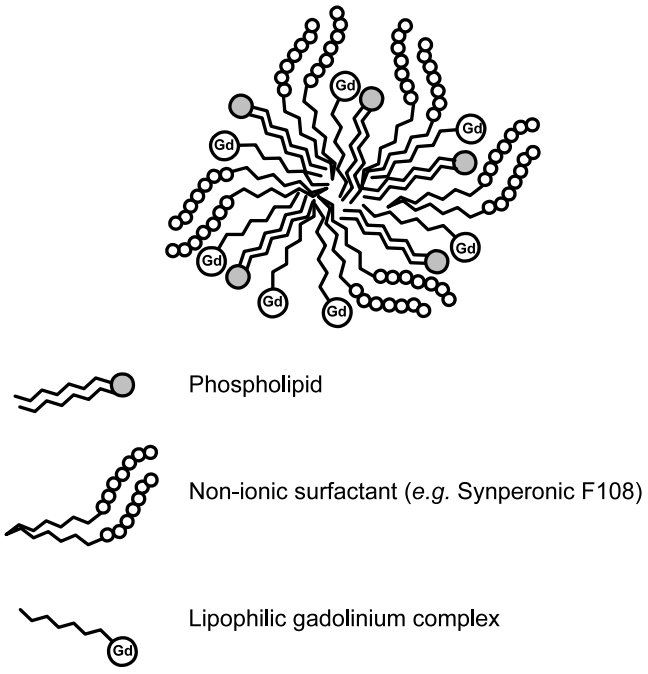
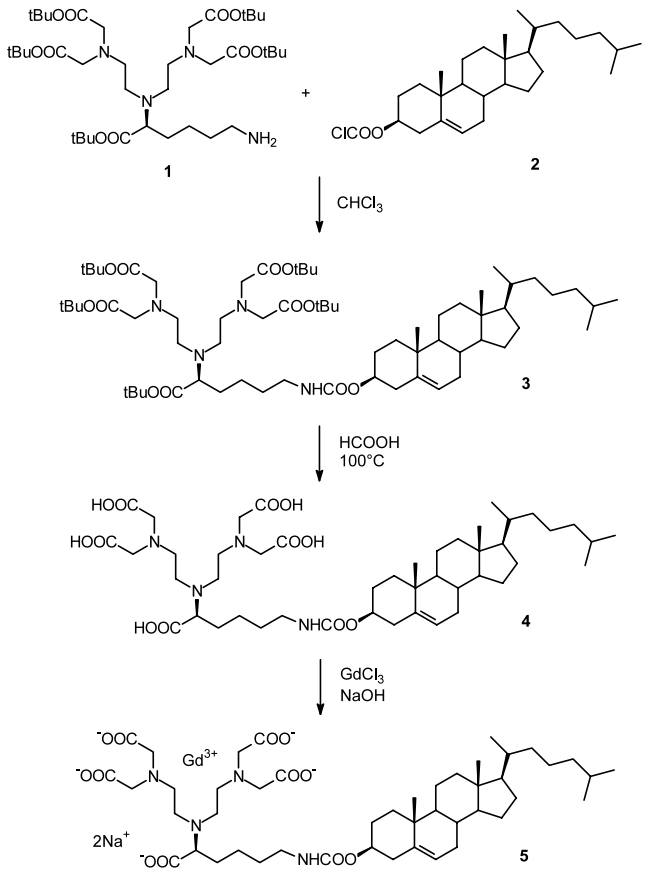


Figure 2. Schematic representation of a mixed micelle.



Scheme 1.

otides,[15] PEGs,[16,17] polysaccharides[18]) that have been conjugated to cholesterol in order to increase their lipophilicity, allowing their incorporation into liposomes, micelles or biomembranes.

On these bases we thought to apply this strategy to DTPA and to the best of our knowledge **5** is the first example of a Gd-DTPA complex attached to cholesterol.[19]

The synthesis of **5** is easy and straightforward (Scheme 1) and takes advantage of the very useful bifunctional pro-chelating agent **1**,[20] which was coupled to cholesteryl chloroformate **2** to give the pentaester **3**.† Deprotection of **3** with formic acid at 100°C gave the DTPA-cholesterol ligand **4**,‡ which was then reacted with gadolinium chloride to obtain the corresponding complex **5**§ in 50% overall yield.

Complex **5** is soluble in water up to 0.5 g mL^{-1} and shows a high relaxivity ($r_1 = 27.2$ mM^{-1} s^{-1} in buffer of Tris/glycerol, pH 7.8). This value of relaxivity is extremely high if compared to the relaxivity of Gd-DTPA ($r_1 = 3.7$ mM^{-1} s^{-1}),[21] and it is an indirect evidence of a micellar self-organization of **5** in water. In fact, such high relaxivity is only achieved with multimeric gadolinium complexes like dendrimers or poly-

† **Synthesis of 3**: A solution of cholesteryl chloroformate **2** (22 mmol) in $CHCl_3$ (60 mL) was added in 1 h to a stirred solution of compound **1** (20 mmol) in $CHCl_3$ (150 mL) at room temperature. After 18 h the reaction mixture was washed with 5% aq. $NaHCO_3$ (2×150 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (EtOAc/*n*-hexane, 15:85) to give **3** as a yellowish oil (18.2 g; 78%). ^{13}C NMR (200 MHz, $CDCl_3$) δ 11.72 (CH_3), 18.58 (CH_3), 19.21 (CH_3), 20.91 (CH_2), 22.43 (CH_3), 22.70 (CH_3), 23.24 (CH_2), 23.68 (CH_2), 24.15 (CH_2), 28.06 (broad, several signals), 29.39 (CH_2), 31.75 (CH_2), 35.65 (CH), 36.04 (CH_2), 36.41 (C), 36.88 (CH_2), 38.49 (CH_2), 39.38 (CH_2), 39.60 (CH_2), 40.70 (CH_2), 42.17 (C), 49.87 (CH), 50.11 (CH_2), 53.47 (CH_2), 55.85 (CH_2), 55.98 (CH), 56.55 (CH), 63.83 (CH), 73.79 (CH), 80.50 (C), 80.63 (C), 122.21 (CH), 139.77 (C), 156.04 (CO), 170.52 (CO), 172.61 (CO). ESI MS (*m*/*z*): 1180 [M+Na^+]. Anal. calcd for $C_{66}H_{116}N_4O_{12}$: C, 68.48; H, 10.10; N, 4.84; Found C, 68.89; H, 10.08; N, 4.75%.

‡ **Synthesis of 4**: A solution of pentaester **3** (10.9 mmol) in formic acid (250 mL) was heated at 100°C for 1.5 h then the reaction mixture was evaporated under reduced pressure. The residue was taken up with H_2O, stirred for 30 min, filtered and dried (P_2O_5) to give ligand **4** as a pale yellow solid (7.8 g; 81%). Mp: 200°C (dec.). ^{13}C NMR (200 MHz, d_6-DMSO, 80°C) δ 11.65 (CH_3), 18.60 (CH_3), 18.93 (CH_3), 20.68 (CH_2), 22.27 (CH_3), 22.45 (CH_3), 23.37 (CH_2), 23.52 (CH_2), 23.85 (CH_2), 27.33 (CH), 27.66 (CH_2), 27.96 (CH_2), 28.57 (CH_2), 29.38 (CH_2), 31.42 (CH_2), 31.59 (CH), 35.14 (CH), 35.83 (CH_2), 36.21 (C), 36.74 (CH_2), 38.39 (CH_2), 39.04 (CH_2), 39.40 (CH_2), 40.24 (CH_2), 42.02 (C), 49.81 (CH), 50.00 (CH_2), 53.02 (CH_2), 55.46 (CH_2), 55.94 (CH), 56.32 (CH), 64.08 (CH), 73.03 (CH), 121.56 (CH), 140.05 (C), 155.68 (CO), 172.11 (CO), 173. 21 (CO). ESI MS (*m*/*z*): 876 [M^+]. Anal. calcd for $C_{46}H_{76}N_4O_{12}$: C, 62.99; H, 8.73; N, 6.39; Found C, 62.70; H, 8.89; N, 6.31%.

§ **Synthesis of 5**: A solution of $GdCl_3$ (5.8 mmol) in H_2O (10 mL) was added dropwise to a solution of **4** (5.8 mmol) in H_2O (300 mL) and 2N NaOH (5.8 mL), maintaining the reaction mixture at pH 6.8 by constant addition of 2N NaOH. After 1 h the solution was concentrated to 50 mL and MeCN (300 mL) was added. The precipitate was filtered and dried (P_2O_5) to obtain the complex **5** as a white solid (4.9 g; 79%). Mp: >250°C. ESI MS (*m*/*z*): 1052 [M–Na^+], 514 [M–2Na^+]. Anal. calcd for $C_{46}H_{71}GdN_4Na_2O_{12}$: C, 51.38; H, 6.66; N, 5.21; Gd, 14.62; Na, 4.28; Found C, 51.37; H, 6.83; N, 5.17; Gd, 14.51; Na, 4.02%.

mers.[2,3] Moreover, it is worth noticing that mixed micelles formulated with complex **5** show the highest relaxivity ($r_1 = 25$ mM^{-1} s^{-1}) we have ever obtained so far with these types of aggregates.

In conclusion, we have shown a simple and efficient entry to the new lipophilic gadolinium complex **5**. This derivative can easily be incorporated into supramolecular systems like mixed micelles, a new and very promising class of blood pool MRI/MRA contrast agents.

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References

1. Rinck, P. A. *Magnetic Resonance in Medicine*; Blackwell Scientific Publications: Oxford, UK, 1993.
2. Merbach, A. E.; Tóth, É. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; John Wiley & Sons Ltd: Chichester, UK, 2001.
3. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293–2352.
4. Reichert, D. E.; Lewis, J. S.; Anderson, C. J. *Coord. Chem. Rev.* **1999**, *184*, 3–66.
5. Thunus, L.; Lejeune, R. *Coord. Chem. Rev.* **1999**, *184*, 125–155.
6. Kroft, L. J. M.; de Roos, A. *J. Magn. Reson. Imaging* **1999**, *10*, 395–403.
7. Bogdanov, A. A., Jr.; Lewin, M.; Weissleder, R. *Adv. Drug Del. Rev.* **1999**, *37*, 279–293.
8. Caravan, P.; Cloutier, N. J.; Greenfield, M. T.; McDermid, S. A.; Dunham, S. U.; Bulte, J. W. M.; Amedio, J. C., Jr.; Looby, R. J.; Supkowski, R. M.; Horrocks, W. DeW., Jr.; McMurry, T. J.; Lauffer, R. B. *J. Am. Chem. Soc.* **2002**, *124*, 3152–3162.
9. Ladd, D. L.; Hollister, R.; Peng, X.; Wei, D.; Wu, G.; Delecki, D.; Snow, R. A.; Toner, J. L.; Kellar, K.; Eck, J.; Desai, V. C.; Raymond, G.; Kinter, L. B.; Desser, T. S.; Rubin, D. L. *Bioconjugate Chem.* **1999**, *10*, 361–370.
10. Krause, W.; Hackmann-Schlichter, N.; Maier, F. K.; Müller, R. *Top. Curr. Chem.* **2000**, *210*, 261–308.
11. Unger, E. C.; Shen, D.-K.; Fritz, T. A. *J. Magn. Reson. Imaging* **1993**, *3*, 195–198.
12. Tournier, H.; Hyacinthe, R.; Schneider, M. *Acad. Radiol.* **2002**, *9* (*suppl 1*), S20–S28.
13. Anelli, P. L.; Lattuada, L.; Lorusso, V.; Schneider, M.; Tournier, H.; Uggeri, F. *MAGMA* **2001**, *12*, 114–120.
14. Cooper, R. G.; Etheridge, C. J.; Stewart, L.; Marshall, J.; Rudginsky, S.; Cheng, S. H.; Miller, A. D. *Chem. Eur. J.* **1998**, *4*, 137–151.
15. Vu, H.; Schmaltz Hill, T.; Jayaraman, K. *Bioconjugate Chem.* **1994**, *5*, 666–668.
16. Ishiwata, H.; Vertut-Doï, A.; Hirose, T.; Miyajima, K. *Chem. Pharm. Bull.* **1995**, *43*, 1005–1011.
17. Boden, N.; Bushby, R. J.; Clarckson, S.; Evans, S. D.; Knowles, P. F.; Marsh, A. *Tetrahedron* **1997**, *53*, 10939–10952.
18. Akiyoshi, K.; Takanabe, H.; Sato, T.; Sato, T.; Kondo, H.; Sumanoto, J. *Chem. Lett.* **1990**, *3*, 473–476.
19. A Gd-DO3A-cholesterol conjugate has been reported for adrenal imaging: Mühler, A.; Platzek, J.; Radüchel, B.; Frenzel, T.; Weinmann, H.-J. *J. Magn. Reson. Imaging* **1995**, *5*, 7–10.
20. Anelli, P. L.; Fedeli, F.; Gazzotti, O.; Lattuada, L.; Lux, G.; Rebasti, F. *Bioconjugate Chem.* **1999**, *10*, 137–140.
21. Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901–927.