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Tris(pyrone) Chelates of Gd(III) as High Solubility MRI-CA

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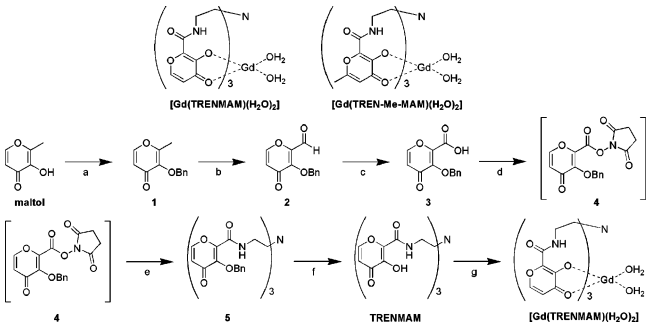
This report describes the synthesis, characterization, and evaluation of a new type of magnetic resonance imaging contrast agent based on pyrone chelators that demonstrate superb aqueous stability, relaxivity, and solubility. The success of magnetic resonance imaging (MRI) as a noninvasive medical imaging technique has been significantly advanced by the development of contrast agents (MRI-CA). These paramagnetic metal chelates or superparamagnetic metal clusters can be used to greatly increase the image quality of MRI by affecting the relaxation of water protons in the immediate vicinity of the MRI-CA.[1]

In 1995, Raymond and co-workers introduced a new class of MRI-CA inspired by the biological coordination chemistry of bacterial siderophores. The ligands used in these MRI-CA are generally comprised of hydroxypyridinones (HOPOs), a class of monoanionic nitrogen heterocycles, which are linked through a common backbone to form hexadentate, tripodal chelators. The archetypical member of this class of compounds, [Gd(TREN-Me-3,2-HOPO)(H_2O)$_2$] (TREN-Me-3,2-HOPO = tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl] amine),[2] shows good stability and selectivity for Gd(III) and high relaxivity (10.5 mM^{-1} s^{-1} at 20 MHz and 37 °C). The high relaxivity of the eight-coordinate [Gd(TREN-Me-3,2-HOPO)(H_2O)$_2$] and its derivatives is attributed to the presence of two inner-sphere water molecules ($q = 2$) and a fast rate of water exchange ($k_{ex} = 1/\tau_M > 25 \times 10^6$ s^{-1}) as a consequence of the associative nature of the exchange mechanism.[3,4] The major liability of [Gd(TREN-Me-3,2-HOPO)(H_2O)$_2$] is poor aqueous solubility.[5] A variety of strategies to solubilize this compound have been investigated.[4,6,7] Derivatives have been prepared with improved solubility, although these generally rely on the introduction of one non-HOPO chelator (resulting in a charged complex)[5] derivatized with a large substituent, such as poly(ethylene)glycols (PEGs)[8] or solubilizing dendrimers.[9]

Pyrones, such as the food additive maltol (3-hydroxy-2-methyl-4-pyrone), are a class of oxygen heterocycles with many parallels to the hydroxypyridinones. They are also monoanionic, hard oxygen donor ligands that form five-membered chelate rings upon metal binding. Notably, chelators, such as maltol, have been found to form metal complexes with good aqueous solubility and biocompatibility; for example, [V=O(maltolato)$_2$] has been widely studied as a soluble therapeutic vanadate source for treating type II diabetes.[10] With this in mind, we turned our efforts to preparing a pyrone analogue of TREN-Me-3,2-HOPO, which we anticipated would form Gd^{3+} complexes with similarly favorable features as an MRI-CA, but with notably improved aqueous solubility. The results of these efforts were two new tripodal chelators, which were used to prepare the MRI-CA, [Gd(TRENMAM)(H_2O)$_2$] and [Gd(TREN-Me-MAM)(H_2O)$_2$] (Scheme 1).

The synthesis of [Gd(TRENMAM)(H_2O)$_2$], beginning with commercially available maltol, is shown in Scheme 1. The

Scheme 1 [a]



[a] Key: (a) BnBr, NaOH(aq), MeOH, 75 °C, 83%; (b) SeO_2, bromobenzene, 155 °C; (c) $NaClO_2$, NH_2SO_3H, H_2O/acetone, 70% (combined yield for steps b and c); (d) NHS, DCC, dry THF; (e) TREN, dry THF, 88% (combined yield for steps d and e); (f) 1:1 HCl:CH_3COOH, 83%; (g) $Gd(NO_3)_3 \cdot 5H_2O$, pyridine, H_2O, 94%.

3-hydroxyl group of maltol is benzyl protected, followed by oxidation of the 2-methyl group in two steps to give 2-carboxy-3-benzyloxypyran-4(1*H*)-one (**3**).[11] Acid **3** is then converted to the NHS activated ester (**4**), coupled to tris(2-aminoethyl)amine (TREN), and deprotected to give the TRENMAM ligand. The facile and inexpensive synthesis (~$25/g) of the TRENMAM ligand is another attractive feature of this new class of MRI-CA, as these agents generally need to be administered in gram quantities during clinical use. The related ligand TREN-Me-MAM was prepared by a similar route starting from 2-carboxy-3-benzyloxy-6-methylpyran-4(1*H*)-one (see Supporting Information).[12] Combination of either ligand with $Gd(NO_3)_3 \cdot (H_2O)_5$ gives the desired metal complex in ~90% isolated yield. Despite numerous attempts, we were unable to crystallize these Gd^{3+} complexes; however, we did obtain a crystal structure of [Fe(TREN-Me-MAM)] (Figure S1), which illustrated the expected ligand composition, metal coordination, and internal hydrogen bonding typical of these tripodal complexes.[2,5,13]

To determine whether TRENMAM and TREN-Me-MAM formed sufficiently stable Gd^{3+} complexes for use as MRI-CA, the solution thermodynamics of these compounds was evaluated. The ligands and Gd^{3+} complexes were sufficiently soluble for the protonation constants to be measured by potentiometry (Table 1).[5] The pGd values ([Gd] = 10^{-6} M, [L] = 10^{-5} M) were independently determined by competition titrations with DTPA.[14]

The ligands TRENMAM and TREN-Me-MAM are generally more acidic when compared to TREN-Me-3,2-HOPO, with the average of the first three protonation constants (which are attributed to the hydroxyl groups) being 6.0, 6.5, and 7.0, respectively. As expected, the electron-donating methyl substituent on TREN-Me-MAM makes this ligand more basic when compared with TRENMAM. A species distribution plot shows that at physiological pH (7.4) both ligands are essentially fully deprotonated and thereby

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Table 1. Protonation Constants for Pyrone Ligands as Measured by Potentiometric Titrations

constant	TRENMAM	TREN-Me-MAM
log K_1	7.33(1)	7.91(1)
log K_2	5.76(1)	6.30(2)
log K_3	4.97(2)	5.48(2)
log K_4	3.84(2)	4.46(2)

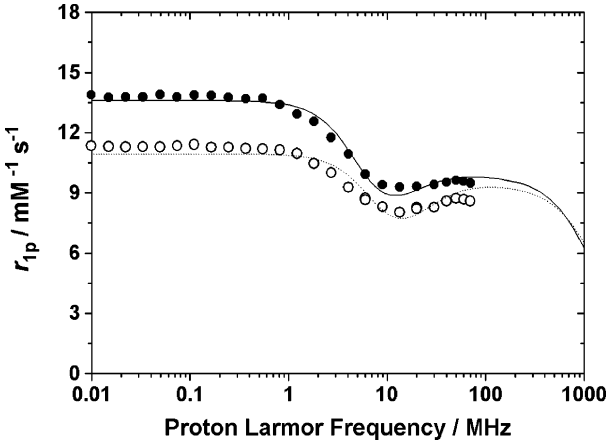

Figure 1. $1/T_1$ NMRD profiles of [Gd(TRENMAM)$(H_2O)_2$] (●) and [Gd(TREN-Me-MAM)$(H_2O)_2$] (○), at 298 K and pH 7.2.

competent to bind metal ions without competition from protons under these conditions (Figure S2). The pGd values of 19.27 ± 0.08 and 19.03 ± 0.04 were determined for TRENMAM and TREN-Me-MAM, respectively, indicating these pyrone ligands form Gd^{3+} complexes with high stability (Figure S3). On the basis of these experiments, the stability of these complexes rivals those of the currently used MRI-CA[1,4] and therefore should be quite adequate for in vivo applications.

Having demonstrated that pyrone-based complexes were sufficiently stable and soluble for use as MRI-CA, we sought to evaluate their physical properties as contrast agents. Several measurements were performed, including nuclear magnetic resonance dispersion (NMRD) profiles, pH-dependent relaxometry, and variable temperature ^{17}O NMR relaxometry. The relaxivity, r_{1p}, of the complexes, as measured at 20 MHz and 298 K, is 9.3 and 8.2 mM^{-1} s^{-1} for [Gd(TRENMAM)$(H_2O)_2$] and [Gd(TREN-Me-MAM)$(H_2O)_2$], respectively, and these values are constant in the pH range of 4−9. These values are very similar to those found for [Gd(TREN-Me-3,2-HOPO)$(H_2O)_2$] and related chelates, suggesting that in the pyrone complexes the Gd^{3+} ion retains two water molecules in its inner coordination sphere. This was confirmed by a detailed NMRD (at 298 and 310 K, Figure S4) and variable temperature ^{17}O NMR (at 2.12 T, Figure S5) study. Figure 1 shows the $1/T_1$ NMRD profiles of the two complexes recorded at 298 K over the frequency range of 0.01−70 MHz. A simultaneous fitting of both the NMRD and ^{17}O NMR data provided the parameters listed in Table 2.

The complex [Gd(TRENMAM)$(H_2O)_2$] shows a slightly higher relaxivity than that of [Gd(TREN-Me-MAM)$(H_2O)_2$] over the entire range of magnetic field strength that is explained by a shorter value of Δ^2 and a slightly longer rotational correlation time τ_R. On the other hand, both complexes are endowed with a fast rate of water exchange ($^{298}k_{ex} \approx 8 \times 10^8$ s^{-1}), similar to that measured for the Gd^{3+} aqua ion.[4] The very rapid water exchange kinetics[15] may represent an advantage for MRI-CA applications at high fields (80−100 MHz), where the optimal τ_M values for achieving high relaxivities are close to 1 ns.[8] Finally, we measured the relaxation rate of the complexes versus concentration in the range of 0.5−100 mM (at 0.1 MHz and 298 K). From this relaxometric approach, 0.1 M represents the lower limit of the solubility of the complexes, a value that is almost an order of magnitude higher than that for similar tripodal chelates.[4]

Table 2. Parameters Obtained from the Simultaneous Fitting of ^1H NMRD and ^{17}O NMR Data

	[Gd(TRENMAM)]	[Gd(TREN-Me-MAM)]
$\Delta^2/10^{19}$ s^{-2}	5.6 ± 0.3	10.8 ± 0.2
$^{298}\tau_v$/ps	19.0 ± 0.8	15.2 ± 1.2
$^{298}\tau_R$/ps	145 ± 6	120 ± 9
$^{298}\tau_M$/ns	1.1 ± 0.3	1.0 ± 0.4
ΔH_M/kJ mol^{-1}	27.6 ± 0.9	22.4 ± 1.8
E_v/kJ[a]	1	1
r_{GdH}/Å	3.09 ± 0.2	3.04 ± 0.3
r_{GdO}/Å[a]	2.48	2.48
$A/\hbar/10^6$ rad·s^{-1}	−3.6 ± 0.2	−3.8 ± 0.1
q[a]	2	2
a/Å[a]	4.0	4.0
$D/10^{-5}$ cm^2 s^{-1}	2.27 ± 0.3	2.30 ± 0.2
E_D/kJ mol^{-1}[a]	22	22

[a] Values were fixed in the fitting procedure.

In conclusion, we have described a new class of pyrone-derived tripodal chelators that form stable and soluble Gd^{3+} complexes with high relaxivity for use as MRI-CA. The compounds are among the most soluble with a $q = 2$ value, and one of them can be readily and economically synthesized from the food additive maltol. Continuing studies with derivatives of TRENMAM and TREN-Me-MAM are ongoing and will be reported in due course.

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Supporting Information Available: Details for syntheses, structures, titration experiments, and complete ref 15. X-ray crystallographic files in CIF format are available at http://www.ccdc.cam.ac.uk (CCDC ref 289563). This material is available free of charge via the Internet at http://pubs.acs.org.

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