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WIN 70197: A NOVEL LIVER-TARGETED MAGNETIC RESONANCE IMAGING CONTRAST AGENT

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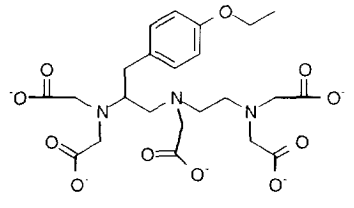
Abstract: WIN 70197 is a novel liver-targeted MRI contrast agent. This report describes the efficient synthesis of the chelator along with preliminary NMRD and toxicity data of the gadolinium complex. X-ray diffraction data was used to confirm the predicted chelate structure.

Magnetic resonance imaging (MRI) provides a potentially powerful method for observing the soft tissue of the body.[3] However, unenhanced MRI has proved seriously limited in detecting abnormal tissues that have T_1 and T_2 relaxation times similar to those of surrounding normal tissues. For example, small metastatic lesions of the central nervous system are frequently missed in unenhanced MRI images.[4] Early in the development of MRI, the ability of paramagnetic agents to increase the conspicuity of certain tissue abnormalities became evident.[5]

The gadolinium chelates of diethylenetriaminepentaacetic acid [Gd(DTPA)$^{2-}$][6] and 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid [Gd(DOTA)$^{-}$][7] are representative of the first generation of MRI contrast agents, the extracellular fluid magnetopharmaceuticals. These small blood-borne paramagnetic chelates share the favorable characteristics of high chemical stability, low toxicity and rapid excretion. Their *in vivo* biological distribution is largely passive and nonspecific, characterized by rapid perfusion into extracellular fluid. As such, these agents enhance conspicuity of lesions that involve significant changes in fluid distribution. Contrast-enhanced MRI has become the method of choice for detection and evaluation of intracranial neoplasms which disrupt the blood-brain barrier.[8]

Considerable research is now being directed towards the generation of paramagnetic chelate complexes which will expand the current scope of utility for MRI. Future generations of magnetopharmaceuticals will possess the ability to target internal organs or diseased tissue directly. Currently detection of cancer and metastatic diseases of the liver by unenhanced MRI is limited in this capacity and contrast enhanced MRI has actually been reported to obscure liver metastases by reducing signal intensity differences between tumor and normal liver.[9] Clearly, agents which enhance conspicuity of liver metastases would expand the scope of MRI.



Gd(EOB-DTPA)$^{2-}$

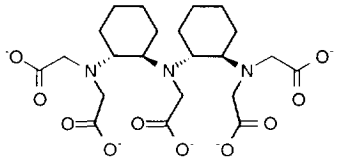
Magnetopharmaceuticals may be directed to the liver by altering the excretory pathway of the gadolinium chelate. For example, substitution of an ethoxybenzyl group to the backbone of the DTPA chelate [Gd(EOB-DTPA)$^{2-}$] enhances hepatobiliary clearance as compared to the parent chelate.[10] Presumably, a degree of lipophilicity is required to enhance hepatobiliary excretion thus directing the gadolinium chelate to the liver.[11]

Due to the toxicity of free lanthanide metals, gadolinium complexes must have high thermodynamic and kinetic stability *in vivo*. Indeed, the acute toxicity of a variety of polyaminocarboxylate gadolinium complexes has been demonstrated to be inversely

proportional to the conditional binding constants of the ligands. Each complex studied was demonstrated to become lethally toxic to mice at the dosage where 13-15 mM of gadolinium was released *in vivo*.[12] A gadolinium complexing agent which shows enhanced *in vivo* stability would be expected to possess a more favorable toxicological profile.

One tact to enhance complex stability is to conformationally constrain the chelating ligand. Preorganization of the chelating functionalities of the ligand to a conformation favorable for metal chelation reduces the entropic barrier to complex formation and thus provides for greater thermodynamic stability of the complex. For example, constraining the ligands of ethylenediaminetetraacetic acid (EDTA) into a cyclohexyl ring has been shown to give a chelate which forms thermodynamically more stable complexes with trivalent metals than EDTA.[13]

In an effort to develop novel liver-directed MRI contrast agents, the gadolinium chelate of dicyclohexenetriaminepentaacetic acid [Gd(DCTPA)$^{2-}$, WIN 70197] was designed and synthesized. It was reasoned that incorporation of two cyclohexane rings in the DTPA backbone would provide sufficient lipophilicity to enhance hepatobiliary clearance of the agent while also improving the conditional stability constant as compared to Gd(DTPA)$^{2-}$. This report describes an efficient synthesis of WIN 70197, as well as preliminary relaxation studies and toxicological evaluations of the complex.



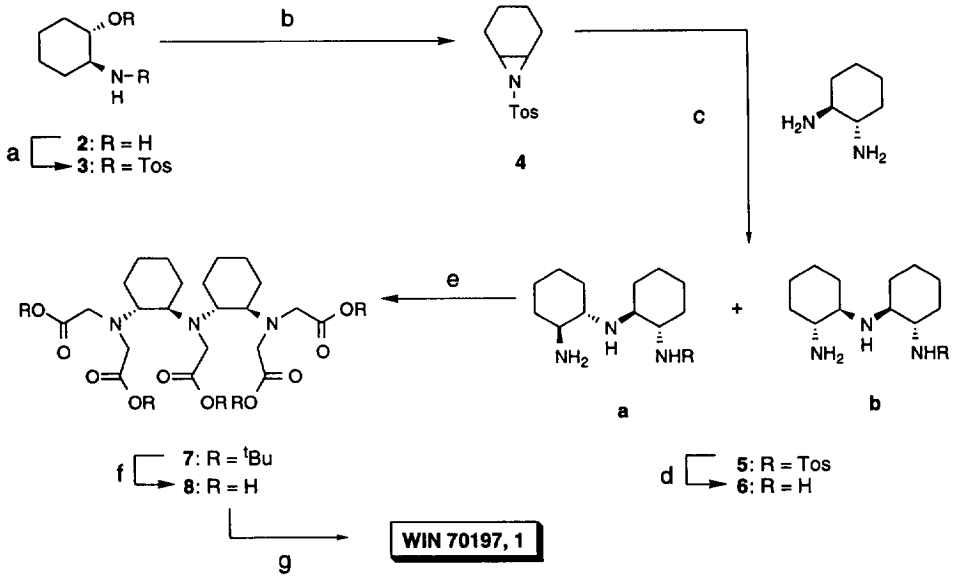
WIN 70197, 1

The designed dicyclohexyl backbone which has four stereochemical centers could exist as a number of diastereomers capable of complexing gadolinium. Intuitively, one would expect that the set of isomers consisting of two trans-1,2-diaminocyclohexyl units to be better disposed for chelation than the set of isomers consisting of one cis- and one trans-, or, two cis-1,2,-diaminocyclohexane units since this should result in a chelate with the least number of nonbonded interactions. This prediction was corroborated by modeling studies which show the all-trans (R,R,R,R) isomer to form a stable complex with the Gd^{3+} ion.[14]

The synthesis of racemic all-trans-DCTPA and its gadolinium chelate was achieved as depicted in **Scheme 1**. Treatment of trans-2-aminocyclohexanol hydrochloride with p-toluenesulfonyl chloride in pyridine gave ditosylate **3** which was converted to N-tosylaziridine **4** by portionwise addition of **3** to a suspension of sodium hydride in THF. The aziridine was opened by heating **4** in the presence of trans-1,2-diaminocyclohexane to give diastereomers **5a** and **5b** as an inseparable 4:1 mixture, respectively. Treating the mixture with concentrated sulfuric acid for 20 h cleaved the tosylamide to give triamines **6a** and **6b**. After separation by flash column chromatography, the desired all-trans isomer (**6a**) was isolated in 31% yield from **4**. Exhaustive alkylation of triamine **6a** with *tert*-butyl bromoacetate gave **7** in 46% yield after recrystallization from 2-propanol. The *tert*-butyl esters were cleaved upon treatment with trifluoroacetic acid (TFA) to give pentaacid **8** as the tris(TFA) salt in 94% yield after trituration from hexanes.[15] Prior to complexation, DCTPA (**8**) was converted to the ammonium salt by treatment with a saturated solution of ammonia in 2-propanol. Complexation of DCTPA was achieved by titration of an aqueous solution of gadolinium nitrate into an aqueous solution of the ammonium salt of **8**. Gd(III)DCTPA^{2-} was isolated as the diammonium salt in 71% yield after trituration with acetone.[16]

X-ray-quality crystals of WIN 70197 were grown by vapor-phase diffusion of acetone into an aqueous solution of the complex. As illustrated by the stereo diagram in **Figure 1,** X-ray diffraction of these crystals

Scheme 1: Synthesis of WIN 70197



Reagents and conditions: a) 2.1 eq p-toluenesulfonyl chloride, pyridine, 4 °C, 92%; b) 1.6 eq NaH, THF, 97%; c) 6 eq (+/-)-*trans*-1,2-diaminocyclohexane, CH_3CN, reflux; d) H_2SO_4, 100-110 °C, **6a**: 31% from **4**; e) 7 eq *tert*-butyl bromoacetate, 7 eq K_2CO_3, 4Å molecular sieves, CH_3CN, 50 °C, 46%; f) TFA, 25 °C, 94% as $(TFA)_3$ salt, g) NH_3 in 2-propanol, then gadolinium nitrate in water, 71%.

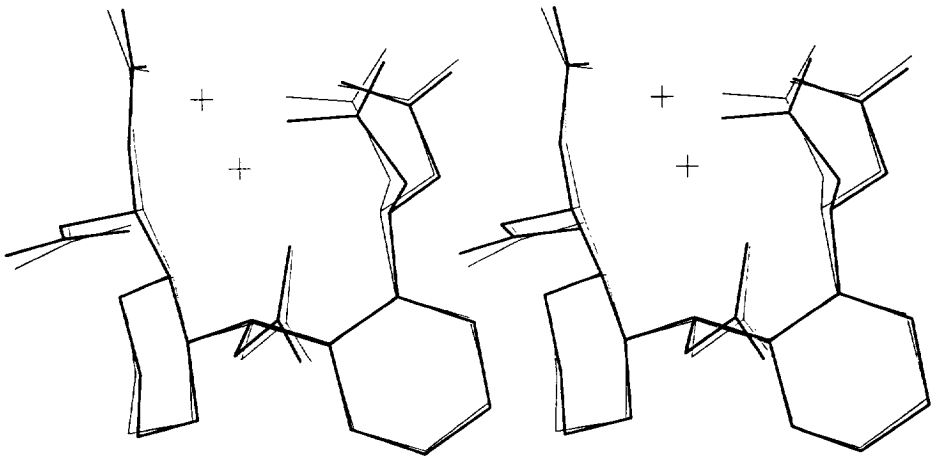
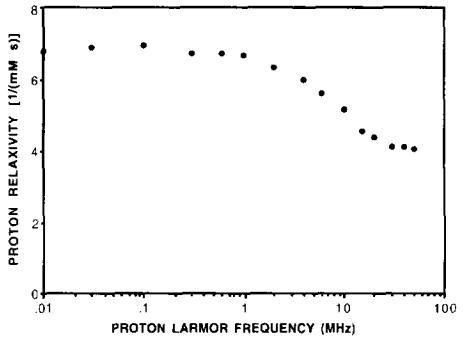


Figure 1 Stereo views of the modeled chelator structure (bold lines) and the X-ray structure of WIN 70197 (thin lines). The crosses are the bound water (top) and Gd ion in the X-ray structure. Hydrogens omitted for clarity.

confirmed the all-trans nature of the ligand. As expected, the DCTPA ligand occupied eight of the nine coordination sites of the gadolinium ion. The remaining coordination site of the metal ion is occupied by a water oxygen (i.e., q=1 system). The gadolinium to oxygen distance was found to be 2.42 Å, a value similar to the 2.49 Å reported for $Gd(DTPA)^{2-}$.[17]

FIGURE 2. WIN 70197 IN WATER AT 25 C



Because the molecular dimensions and the gadolinium to proton on bound water molecular distance (approximated by the Gd to O distance) are similar for $Gd(DTPA)^{2-}$ and WIN 70197, and because both complexes are q=1 systems, it is expected that the high-field relaxivities are similar for both complexes. **Figure 2** shows the nuclear magnetic relaxation dispersion (NMRD) profile of WIN 70197 in water at 25 °C, where the longitudinal relaxivity is plotted as function of magnetic field strength. Indeed, at high field strengths (greater than about 10 MHz in units of the proton Larmor frequency), the relaxivities are similar to those reported for $Gd(DTPA)^{2-}$.[18]

Evidence that WIN 70197 is liver specific and will significantly enhance liver contrast in MR imaging is provided by relaxation studies. WIN 70197 was injected into a mouse at a dose of 100 μmol/kg. The liver was excised five minutes post-injection and the NMRD profile was measured. A control liver was also excised from a mouse that was not injected with contrast agent and its NMRD profile also measured. The effect of the WIN 70197 is evident when the NMRD profile of the control liver is subtracted from the NMRD of the liver from the treated mouse (**Figure 3**). The NMRD profile for WIN 70197 in liver is clearly different from that in water, with the most pertinent difference being the appearance of a peak in the relaxation rate at 30 MHz in liver. The appearance of such a peak in relaxation rates results from an increase in rotational correlation time of the paramagnetic complex.[8] In this sample, this increase is most likely caused by one of two possibilities. Either WIN 70197 becomes bound to a macromolecule or cell membrane in the liver, or, gadolinium is released from WIN 70197 and becomes bound to a macromolecule or cell membrane in the liver. The NMRD profile cannot distinguish between these two scenarios but the absence of toxic effects in the preliminary toxicological studies (vide infra) provides an indication that gadolinium is not released from the chelate, at least not in quantities necessary to account for the NMRD results.

FIGURE 3. WIN 70197 IN WHOLE MOUSE LIVER AT 35 C

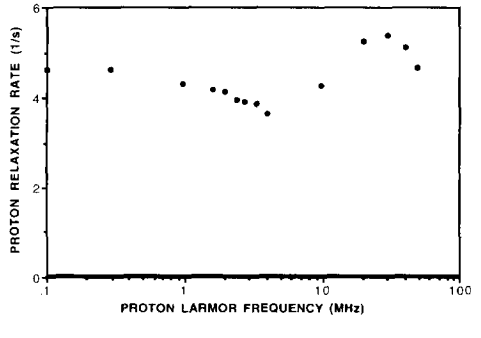
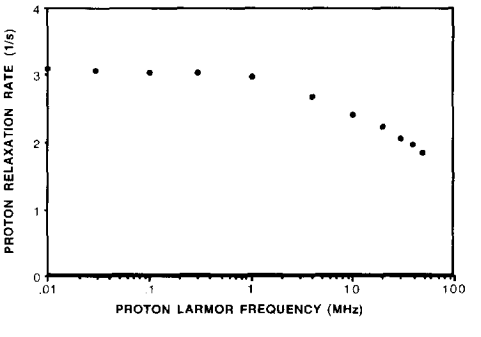


FIGURE 4. WIN 70197 IN WHOLE MOUSE PLASMA AT 35 C



The magnitude of the increase in relaxation rate over background observed in the liver shows that WIN 70197 will enhance liver MR images at this dose and time point. Measurements of the relaxation rate at 0.47 T, or 20 MHz in units of the proton Larmor frequency, show that the relaxation rate returns to the control rate in approximately 60 minutes.

To demonstrate liver specificity, a similar experiment was conducted in mouse plasma (except that blood was removed about one minute post-injection of WIN 70197); no peak was present in the NMRD profile (**Figure 4**). The NMRD profile in plasma is qualitatively similar to that in water indicating that WIN 70197 does not interact with proteins or other macromolecules present in the plasma as it does in the liver.

Initial evidence for the *in vivo* stability of WIN 70197 came from acute tolerance studies performed in mice. A saline solution of WIN 70197 (100 mM) was administered in doses of 1000 and 2000 mmol/kg to groups of three mice. No adverse clinical signs were observed in either group. Upon necropsy, no gross visible lesions were observed in any mice. These observations are consistent with the complex being stable *in vivo* as free gadolinium is extremely toxic even in small doses.

In summary, the efficient synthesis of the conformationally restrained, lipophilic gadolinium chelate, WIN 70197, is described. Relaxation studies show that WIN 70197 is an effective liver imaging agent, presumably because it binds to macromolecules or cell membranes in the liver, and clears from the liver rapidly. Furthermore, studies have indicated that WIN 70197 is well tolerated in mice at up to twenty times the projected imaging dose of 100 mmol/kg. Future reports will describe binding constants and imaging studies of this promising liver-targeted MRI contrast agent.

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14. Of the possible four racemic pairs and two meso compounds, only four isomers were of sufficiently low strain energy to form a reasonable chelate of gadolinium with all eight ligand atoms. Based on

coordinates obtained from the crystallographic structure of Nd(III)DTPA^{2-} complex (Cambridge CDB), atomic models for the four diastereomeric complexes (S,R,R,S; S,R,S,R; R,R,R,S and R,R,R,R) of Gd(III)DCTPA^{2-} were built in Quanta and subjected to restrained energy minimizations using the CHARMm force field. The atomic positions of all nitrogens and carboxylate oxygens were fixed based on the reference crystal structure. The final energies were then compared to give a qualitative prediction.

15. Pure ligand, free from TFA, could be obtained by the following procedure: a solution of the TFA salt of **8** (1.5 g) in deionized water (3 ml) was adjusted to pH 8 by the addition of a saturated solution of $Ba(OH)_2$ and heated for 30 min. The resulting slurry was filtered and washed with water. The solid was suspended in water (5 ml) and the pH of the solution was adjusted to 2 with 20% sulfuric acid solution. The suspension was stirred at 80 °C and the pH readjusted to 2 with aqueous $Ba(OH)_2$ and dilute sulfuric acid. The precipitate was filtered and the filtrate evaporated and dried to provide DCTPA monohydrate as a white solid (0.75 g).

16. Data for selected compounds. **7**: white crystals; mp 106-108 °C; ^{1}H NMR (300 MHz, $CDCl_3$)) d 3.61-3.50 (m, 10 H), 2.71 (m, 2 H, C<u>H</u>-N), 2.54 (m, 2 H, C<u>H</u>-N), 2.04 (m, 4 H), 1.61 (m, 4 H), 1.46 (s, 9 H, C<u>H</u>$_3$), 1.42 (s, 36 H, C<u>H</u>$_3$), 1.19-1.05 (m, 8 H); ^{13}C NMR (75 MHz, $CDCl_3$) d 173.96, 172.47, 80.59, 80.17, 65.73, 64.31, 54.05, 47.58, 31.98, 29.81, 28.75, 26.47; HRMS (LSIMS, CH_2Cl_2-CH_3OH) calcd for $C_{42}H_{76}N_3O_{10}$ $(M+H)^+$ 782.55307; found $(M+H)^+$ 782.55664. **8 (DCTPA hydrate)**: white solid; mp 170°C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) d 3.78 (d, 1 H, J=48.6 Hz, N-C<u>H</u>-CO), 3.73 (d, 1 H, J=48.6 Hz, N-C<u>H</u>-CO), 3.62 (d, 4 H, J=41.8 Hz, N-C<u>H</u>-CO), 3.56 (d, 4 H, J=41.8 Hz, N-C<u>H</u>-CO), 3.17 (m, 2 H), 2.87 (m, 2 H), 2.33 (m, 2 H), 1.61 (m, 4 H), 1.31-1,12 (m, 8 H); ^{13}C NMR (75 MHz, DMSO-d_6) d 178.46, 176.17, 71.40, 69.03, 53.32, 34.66, 32.61, 32.55, 30.51, 30.35. HRMS (LSIMS, CH_3OH-DMSO) calcd for $C_{22}H_{35}N_3O_{10}$ $(M+H)^+$ 502.24007; found 502.24077. Analysis calcd for $C_{22}H_{35}N_3O_{10}\cdot H_2O$: C, 50.86; H, 7.18; N, 8.09. Found: C, 51.29; H, 7.12; N, 8.12. **1 (WIN 70197)**: white solid; mp 220°C (dec). Analysis calcd for $C_{22}H_{38}N_3O_{10}\cdot 2NH_3\cdot 3\,H_2O$: C, 35.52; H, 5.96, N, 9.41; Gd, 21.14. Found: C, 35.41; H, 6.04; N, 9.16; Gd, 20.98.

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