Inulin as a Carrier for Contrast Agents in Magnetic Resonance Imaging

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Abstract: Magnetic resonance angiography (MRA) has put forth an impetus for the development of macromolecular Gd^{III} complexes that have a prolonged lifetime in the vascular system. Herein, we report the synthesis and Gd^{III} complexation of a new sugar conjugate based on inulin and the DO3A ligand (DO3A = 1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid). Two API-DO3ASQ conjugates (API = *O*-(aminopropyl)inulin, SQ = squaric acid = 3,4-dihydroxy-3-cyclobutene-1,2-dione) with different degrees of substitution ($ds = 0.7$ and $ds = 1.5$) were prepared from API by using the diethyl ester of squaric acid as a linking agent for the DO3A chelate. The efficacies of the resulting Gd^{III} compounds were evaluated by investigation of their water ^1H longitudinal-relaxation-rate enhancements at variable field (NMRD). A dramatic increase in relaxivity was observed in the more highly substituted conjugate ($ds = 1.5$); this prompted us to do a variable-temperature ^{17}O study in order to further characterize the relaxation parameters involved in this system. [Gd(API-DO3ASQ)] shows promising properties for application as a contrast agent for MRI.

Keywords: chelates · contrast agents · gadolinium complexes · lanthanides · magnetic resonance imaging · polysaccharides

Introduction

One of the most rapidly growing diagnostic techniques used by the medical profession is magnetic resonance imaging (MRI). This method, based on NMR techniques, allows researchers and doctors to image the body in a noninvasive manner. In a similar way to the NMR experiment, the signal measured in MRI is determined by the relaxation of water proton nuclei present in the body. Paralleling the use of MRI as a clinical technique is the development of contrast agents. As their name suggests, this class of pharmaceuticals enhances the image contrast between normal and diseased tissue.

Currently, about one third of all MRI scans are made after the administration of a Gd^{III}-based contrast agent.[1] This serves a dual function: to obtain images with higher resolution and to reduce the time needed to obtain an image. Contrast agents enhance the image contrast by preferentially influencing the relaxation efficiency of the water proton nuclei in the target tissue. The efficiency of a contrast agent is evaluated in terms of the relaxivity, which is defined as the relaxation-rate enhancement of proton nuclei (from water) per mM solution of metal ion. Owing to their high magnetic moment and long electronic relaxation time, which leads to a high relaxation efficiency, Gd^{III}-based contrast agents predominate.[2–4] These complexes contain a Gd^{III}-bound water molecule that rapidly exchanges with the bulk water of the body; this imparts an efficient mechanism for the longitudinal- and transverse-relaxation (T_1 and T_2) enhancement of the water protons. These parameters determine the intensity of the water signal and, ultimately, the contrast of the images. Ironically, the most effective contrast agent would be the Gd^{III}-aquo ion, which contains eight water molecules in the first coordination sphere,[5] but this species is toxic and precipitates as the hydroxide at physiological pH. However, these problems may be overcome by sequestering the Gd^{III} ion with a strong chelator. A delicate balance exists between sufficient binding of the Gd^{III} ion and loss of relaxation-enhancement function. The optimum solution requires an octadentate chelating ligand, which leaves an open site for the binding of one water molecule in the first coordination sphere. Contrast agents currently in use are linear and macrocyclic polyaminocarboxylate complexes of Gd^{III}. Scheme 1 shows some ligands.

Relaxation theory predicts that higher relaxation rates may be obtained upon an increase in the rotational correlation time of the Gd^{III} complexes.[2–4] The application of high-molecular-weight conjugates, obtained by attaching the low-molecular-weight Gd^{III} chelates to polymers, provides a means

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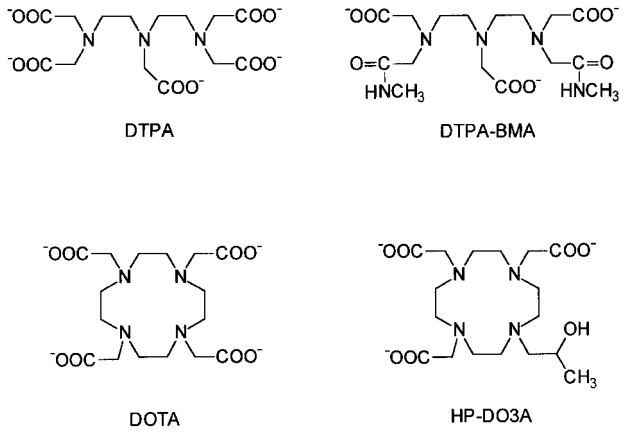
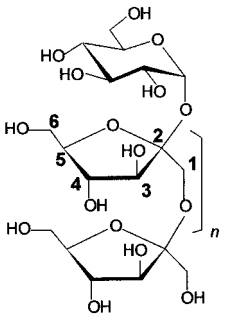


Scheme 1. Ligand structures of linear and cyclic commercially applied contrast agents. HP = 2-hydroxypropyl, BMA = bis(methyl)amide.

to achieving an optimal rotational correlation time. In addition, the nature of these compounds may serve to prolong their residence time in the cardiovascular system, rendering them amenable to applications in magnetic resonance angiography.

Inulin is an oligosaccharide which may serve as an interesting carrier for Gd^{III} chelates. The structure of inulin may be described as a polydisperse mixture of linear β-(2 → 1)-linked α-D-fructofuranosyl chains with a terminal α-D-glucopyranosyl unit at the reducing end (Scheme 2). Depending on the source, the degree of polymerization (*dp*) of inulin



Scheme 2. Representative structure of inulin.

varies from 10 to 30. This leads to an average molecular weight distribution between 1500 and 5000 Da,[6] which is a favorable range for the preparation of macromolecular conjugates. Aqueous solutions of inulin, as well as inulin derivatives, have a low viscosity which is an advantageous property for contrast agents. Furthermore, inulin is not metabolized in blood, and studies have indicated that inulin derivatives behave in the same manner; this makes them pharmacologically inert.[7] The synthesis of an inulin conjugate with diethylenetriaminepentaacetic acid (DTPA) which has a degree of substitution of about 0.2 has been reported.[8] Its efficacy for relaxation enhancement of the water ^{1}H NMR signal was found to be slightly higher than that of commercial contrast agents.

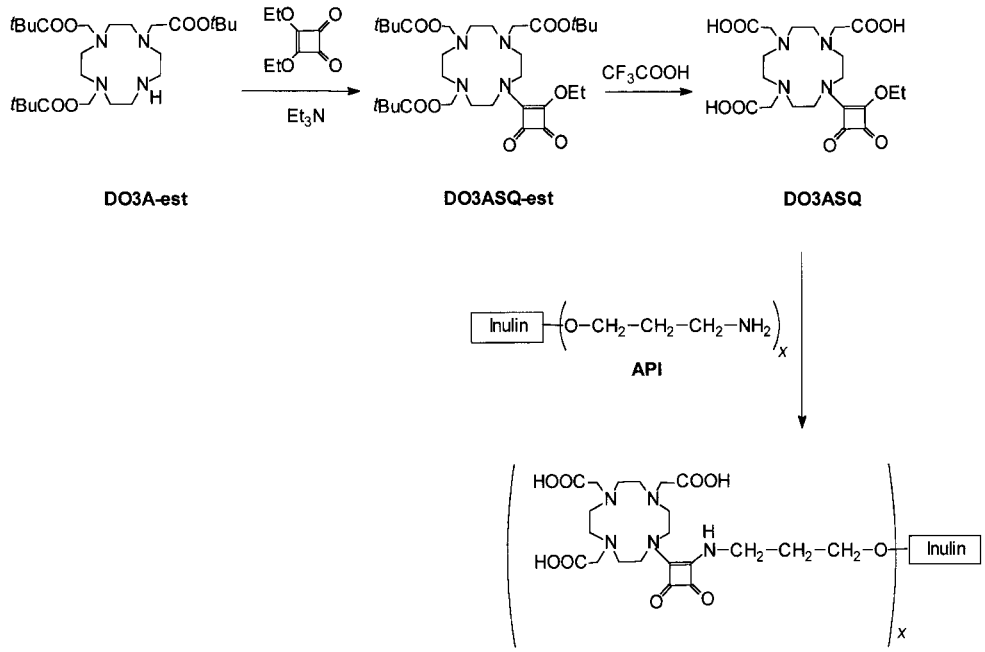
Various other macromolecular systems which incorporate DTPA or functionalized DOTA (1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid) and biopolymers such as dextran and polylysine have also been reported.[9–14] The syntheses involved in these conjugates have several disadvantages that stem from undesirable side-reactions, laborious procedures, and low yields. For these reasons, alternative synthetic routes for obtaining conjugated species that involve activated ligand systems are of particular interest. Recently, Aime et al. reported the synthesis of polylysine and polyornithine conjugates with DO3A by using a squaric acid derivative as a linking agent.[15] This approach proved to be far simpler than previously reported methods and the desired products were isolated in good yield.

Here, we report on the synthesis, purification, and characterization of novel inulin conjugates linked through diethyl squarate to the DO3A ligand. Nuclear magnetic resonance dispersion (NMRD) investigations and variable-temperature ^{17}O NMR measurements of the complexes formed with Gd^{III} were conducted in order to assess their relaxation-enhancing abilities and to gain insight into the parameters which govern these relaxation processes.

Results and Discussion

Synthesis of conjugates and metal complexes: Insulin-DO3ASQ conjugates were obtained by following the reactions outlined in Scheme 3. This route required the addition of a modified inulin derivative with a functionalized chelate ligand (DO3A) that carries the tether for the two species. Inulin was chosen, in part, because of the flexibility of the polymer chain which allows for the preparation of derivatives that have higher degrees of substitution (*ds*: defined as the number of substituents per fructose unit, where the maximum *ds* is three). Modified polymers with high degrees of substitution may then generate more rigid Gd^{III} conjugates, an important factor for increasing the rotational-correlation time (τ_R).[16] *O*-(Aminopropyl)inulin (API) was selected as a starting material as it is easily accessible through the cyanoethylation of inulin.[17] Reduction of the nitrile groups was accomplished with Li metal in liquid ammonia/methanol at low temperature to yield the amino-substituted inulin API.[18] Studies have indicated that the cyanoethyl groups in cyanoethyl inulin (CEI) are distributed uniformly along the inulin chain and that within each fructose unit, the 4-position is the most reactive toward cyanoethylation.[19] The exact distribution of substituents can be determined by following guidelines developed previously.[19] For the CEI derivatives synthesized, the molar fractions of fructose units with substituents at the 3-, 4-, and 6-positions are 0.18, 0.37, and 0.16 (*ds* = 0.7) and 0.40, 0.71, and 0.37 (*ds* = 1.5), respectively. During the reduction of the nitrile groups, essentially no dealkylation occurred and, therefore, it can be assumed that the distribution of the functional groups remained unchanged.

Two APIs that have different degrees of substitution (*ds* = 0.7 and 1.5) were synthesized from inulin (*dp* = 25, long-chain fraction). These materials were coupled to the well-known, strong-chelating macrocyclic ligand DO3A by using diethyl squarate as an activated linking agent (see Scheme 3). DO3ASQ-est, the ester precursor of DO3A, was prepared by treating DO3A, protected as the tri-*tert*-butyl ester, with



Scheme 3. Synthetic route to API-DO3ASQ conjugates.

diethyl squarate; this follows a similar procedure to that reported recently.[15] Attempts to couple the protected DO3ASQ-est with API, followed by treatment with trifluoroacetic acid, failed owing to degradation of the inulin chain. Therefore, DO3ASQ-est was deprotected with trifluoroacetic acid to produce DO3ASQ, which is the starting compound in the synthesis of the conjugates. The DO3ASQ (in excess) was subjected to a second coupling with API under aqueous conditions and in the presence of a base. The resulting conjugate was purified by gel-permeation chromatography in order to remove any unreacted DO3ASQ and residual base. Two API-DO3ASQ conjugates that had degrees of substitution of 0.7 and 1.5 were prepared and purified. The average molecular weights of purified materials were determined to be $\approx$12000 and 22000 Da, respectively, which is in good agreement with the calculated values ($\approx$12470 and 22100 Da for *ds* 0.7 and 1.5, respectively). Formation of the linked conjugates is further supported by inspection of the ^{1}H and ^{13}C NMR spectra which show the disappearance of the ethoxy proton and carbon signals of the parent DO3ASQ compound, as well as slight variations in the signals that correspond to free API and DO3ASQ.

The coordination geometry of [Gd(DO3ASQ)] is similar to that of other DOTA-like complexes in which the Gd^{III} ion is bound by the four N atoms of the macrocycle. In this case, it is also bound by the three carboxylate oxygen atoms of the arms. Studies reported by Aime et al. indicated that the squaric acid moiety participates in the chelation of the Gd^{III} ion.[15] The coordination cage is completed by one bound water molecule.[15] For the API-DO3ASQ conjugates, the formation of Gd^{III} complexes was achieved by using an NMR titration procedure in which complete complexation is detected by monitoring the ^{17}O NMR signal of the solvent water.[3] In a typical experiment, a solution of the ligand in D_2O is placed in a 10 mm NMR tube and titrated with solid portions of $GdCl_3 \cdot 6H_2O$ at 75 °C while maintaining neutral pH. Complexation occurs immediately, and free Gd^{III} ions are easily detected by a dramatic increase in the ^{17}O water chemical shift. The amount of API-DO3ASQ required for back titration of the excess Gd^{III} may then be determined by evaluation of the chemical shift data (see Figure 1 for plot). From the titration data, and from the known degrees of substitution in the ligand conjugates, we were able to determine the average relative composition of Gd^{III} chelate/inulin chain as 17 [Gd(DO3ASQ)]/inulin ($W_M \approx 15000$, $ds = 0.7$) and 37 [Gd(DO3ASQ)]/inulin ($W_M \approx 28000$, $ds = 1.5$).

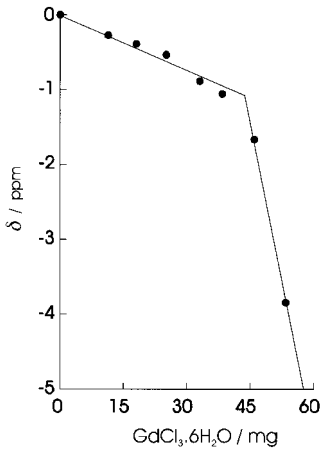


Figure 1. Plot of Gd^{III} induced chemical ^{17}O shift of water (δ) versus the amount of $GdCl_3 \cdot 6H_2O$ for Gd^{III} complexation titration of 114.3 mg API-DO3ASQ ($ds = 1.5$) in 2.4 mL D_2O at 75 °C and 7.05 T.

Variable-temperature ^{17}O NMR and NMRD measurements: The conjugate with a high degree of substitution, [Gd_{37}{API-$(DO3ASQ)_{37}$}], was investigated by water ^{1}H longitudinal-

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relaxation-time measurements at several temperatures (5, 15, 25, 37, and 45 °C), at magnetic-field strengths varying between 2.5×10^{-4} and 1.2 T (NMRD), and by variable-temperature ^{17}O NMR shift and relaxation measurements in order to assess the relaxation processes in this system. For comparison, NMRD measurements on the nonconjugated [Gd(API-DO3ASQ)] complex were included in this study. The ^{17}O NMR relaxation studies on the nonconjugated complex have been reported previously by Aime et al.[15] The equations applied for the analysis of these data are given in the Appendix.

The shape of the NMRD curves (Figure 2), particularly the local maximum at a resonance frequency of about 20 MHz, is characteristic for high-molecular-weight compounds with slow molecular tumbling.[2] A dramatic increase in the relaxivity is observed in going from $[Gd_{17}\{API\text{-}(DO3ASQ)_{17}\}]$ ($ds = 0.7$) to $[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$ ($ds = 1.5$). This suggests a large increase in the rotational-correlation time with increasing degree of substitution which may be ascribed to a substantial reduction in local motions owing to the increasing steric hindrance. Apparently, these local motions contribute significantly to the over-all rotational-correlation time at a low *ds* but to a much lesser extent at a higher *ds*.

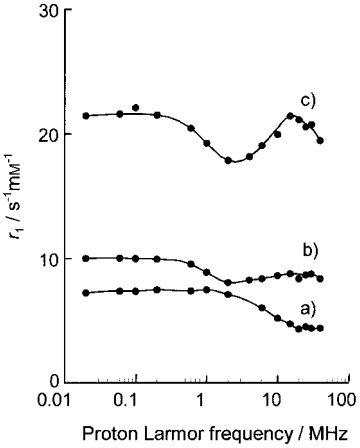

Figure 2. NMRD profiles at 37 °C of a) [Gd(DO3ASQ)], b) $[Gd_{17}\{API\text{-}(DO3ASQ)_{17}\}]$, and c) $[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$. The lines are guides to the eye.

A reasonable fit of the NMRD and ^{17}O NMR data of $[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$ with Equations (1) – (17) (see Appendix) was obtained with the parameters compiled in Table 1 (see Figure 3). For comparison, previously published data for the "nonconjugated chelate" [Gd(DO3ASQ)] have been included in Table 1.[15] A constraint was introduced during the fitting procedure by fixing the hydration number, *q*, at "1". The correctness of this assumption is confirmed by the value obtained for the scalar coupling constant ($A/\hbar$), which is similar to values reported for other Gd^{III} polyaminocarboxylate complexes that have one inner-sphere water molecule.[20, 21] The shift induced by a Gd^{III} complex to the water ^{17}O resonance is proportional to *q*, if the exchange between Gd^{III} bound water and the bulk is rapid on the NMR timescale.[3] If *q* had had a value other than one, this would have been reflected in the ^{17}O chemical shifts at high temperature. In that case, the incorrect assumption of $q = 1$, would have been reflected in the value of $A/\hbar$ obtained in the fitting procedure [see Appendix, Eq. (4)).

Table 1. Parameters obtained from the analysis of ^{17}O NMR and NMRD data.

Parameter	$[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$	[Gd(DO3ASQ)][a]
τ_M^{298} [ns]	260 ± 10	134
ΔH_M [kJ mol^{-1}]	46 ± 2	41
τ_R^{298} [ps]	735 ± 25	78 ± 2
E_R [kJ mol^{-1}]	10.4 ± 13	–
τ_v^{298} [ps]	66 ± 5	19 ± 3
E_v [kJ mol^{-1}]	1[b]	–
$A/\hbar$ [$\times 10^6$ rad s^{-1}]	-3.6 ± 0.2	–
Δ^2 [$\times 10^{19}$ s^{-2}]	0.56 ± 0.06	6.3 ± 0.4
δg_L^2	0.19 ± 0.03	–
D_{GdH}^{298} [$\times 10^{-9}$ m^2 s^{-1}]	1.6 ± 0.2	–
E_{DGdH} [kJ mol^{-1}]	24.7 ± 2.6	–

[a] The data listed for [Gd(DO3ASQ)] have been reported previously by Aime, et al. in ref. [15] and are provided here for comparison. [b] It was necessary to fix this parameter while fitting the data, since the calculation failed if it was left as variable.

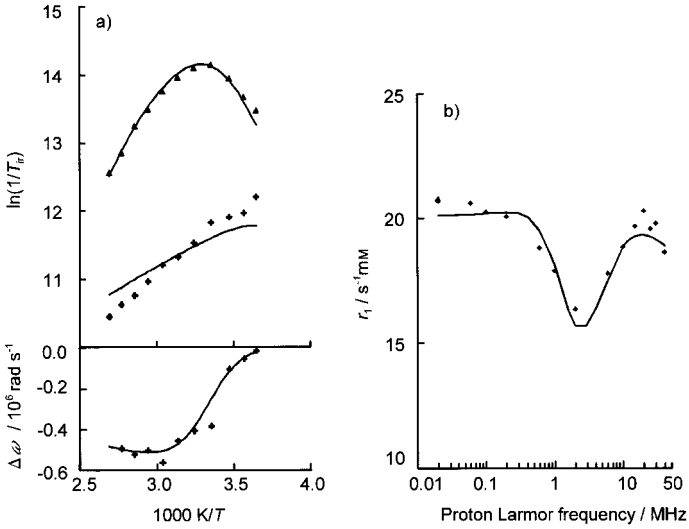

Figure 3. Temperature dependence of a) the reduced ^{17}O transverse (▲) and longitudinal (+) relaxation rates of $[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$, expressed as the $\ln(1/T_{ir})$, and the reduced chemical shifts, $\Delta\omega_r$, and b) NMRD profile of $[Gd_{37}\{API\text{-}(DO3ASQ)_{37}\}]$ at 37 °C. The lines represent simultaneous least-squares fits (see text).

The residence time of water in the first coordination sphere of Gd^{III} in the conjugate at 298 K (τ_M^{298}) was determined to be 260 ns, a value which is typical for DTPA and DOTA-type compounds, yet an order of magnitude smaller than those found for the bis(amide)-DTPA ligands.[20, 22] This number is somewhat higher than that reported for the free [Gd(DO3ASQ)] of 134 ns.[15] The relaxivity as a function of temperature shows a maximum at about 30 °C (see Figure 4). This indicates that, below this temperature, τ_M is limiting the relaxivity: $\tau_M > T_{1M}$ (see Appendix, Eq. (13)). Increasing the temperature results in a decrease of τ_M and, consequently, an increase in r_1. Above 30 °C, T_{1M} becomes the limiting factor. This parameter increases with temperature and, therefore, r_1 decreases upon increasing the temperature (for $T > 30$ °C). However, it is clear that at 37 °C the water exchange rate does

not pose any limitations in achieving relaxation enhancements for the macromolecular [Gd(API-DO3ASQ)] conjugates.

In order to assess the relevance of the rotational correlation time (τ_R^{298}) obtained from the fit, it was necessary to rule out possible problems arising from the distribution of molecular weights of the inulin conjugates. Simulations to investigate the effect of molecular-weight distribution were carried out by constructing a dataset of 20 calculated r_1 values for a range of τ_R values (100 to 190 ps) and by using fixed typical values for the other parameters. The resulting r_1 values were averaged, by assuming a Lorentzian distribution of the τ_R values, and then entered into a normal fitting procedure. The parameters obtained from this fit were found to be in good agreement with the input (within error) and the resulting τ_R value corresponded well with the average value; this indicates that fitting the experimental data should, in principle, be possible and not be problematic owing to a distribution of molecular weights.

It should be noted that the τ_R value is much smaller than expected for such a macromolecular compound, based solely on its molecular weight. From the Debye–Stokes–Einstein equation, τ_R is estimated as 10^{-8} s for compounds in this molecular weight-range.[23] This discrepancy in τ_R value may be accounted for by local rotations of the tether that connects the Gd^{III} chelate to the polymer; these may be more rapid compared with the overall rotation of the conjugate. Similar observations have been reported for macromolecular compounds with Gd^{III} chelates attached to other polymeric carriers, including polylysine,[15, 24] polyornithine,[15] dextrans,[16] and dendrimers.[25] As already stated above, the mobility of the tether will decrease upon increase of the *ds* due to an increase of the steric crowding. This may explain the disproportional increase in relaxivity upon increase of the *ds* of the conjugate.

The values obtained for the parameters that determine the electronic relaxation rates (τ_v^{298} and Δ^2) are outside the range of values usually observed. Attempts to fit the experimental data of the conjugates with these parameters fixed at more common values failed, particularly in the region between 1 and 5 MHz, which appears to be flatter than expected. Similar phenomena in the low-field region have been observed for [Gd(DO3ASQ)]-linked polylysine and polyornithine conjugates[15] and for interactions of noncovalent, paramagnetic adducts with human serum albumin.[26, 27] It is known, however, that the generally applied equations are inappropriate to describe the low-field range of the NMRD profile for slow-rotating systems, especially as regards the electron-spin system.[28] Aime et al. have suggested that higher relaxivities observed in this region may be due to contributions from water molecules bound to the surface of the Gd^{III} macromolecule and within the vicinity of the paramagnet.[15]

Clinically, MRI exams are performed at a magnetic field between 0.5 and 2 T (corresponding with a ^1H resonance frequency of 20–85 MHz). The relaxivity of the newly developed Gd_{37}[(API-(DO3ASQ)$_{37}$] at 20 MHz and 37 °C (20.3 $mM^{-1}s^{-1}$, see Figure 2) is about five times higher than that of the currently commercially available contrast agents. It is clear that the inulin conjugates, particularly those with a high degree of substitution, possess very efficient relaxation-rate-enhancing properties and, therefore, are promising potential MRI contrast agents.

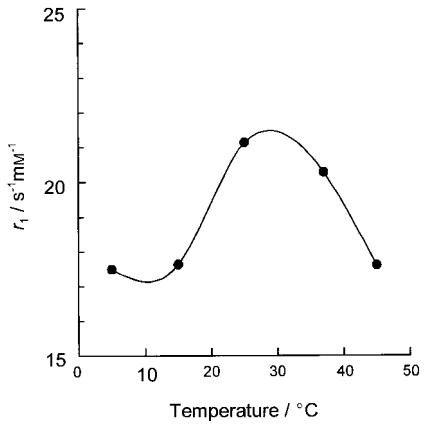

Figure 4. Temperature dependence of the longitudinal proton relaxivity at 20 MHz for Gd_{37}[API-(DO3ASQ)$_{37}$]. The line is a guide to the eye.

Conclusion

This report has highlighted the synthesis and relaxivity properties of an interesting new conjugate, [Gd(API-DO3ASQ)]. NMRD studies of two conjugates that have different degrees of substitution show a dramatic increase in relaxivity from *ds* = 0.7 to 1. 5. Apparently, the local motion decreases upon increase of *ds*. A molecular model has shown this to be a reasonable explanation. Analysis of the variable-temperature ^{17}O NMR and NMRD data suggests that both the rotational correlation time (τ_R) and the electronic relaxation time (T_{1e}) appear to increase without a corresponding decrease in the water exchange rate. To our knowledge, such a high relaxivity (20.3 $mM^{-1}s^{-1}$ at 37 °C) has never been measured for a sugar conjugate; this makes [Gd(API-DO3ASQ)] a promising new potential contrast agent.

Experimental Section

Materials and methods: Diethyl squarate and gadolinium chloride ($GdCl_3 \cdot 6H_2O$) were obtained from Aldrich and used without further purification. ^{17}O-enriched water (10% labeling) was purchased from Cortec (Paris, France). All other reagent-grade chemicals were purchased from commercial sources and used without further purification. Inulin (*dp* 25) was donated by Sensus Coöperatie Cosun U.A. (Roosendaal, The Netherlands). This "long-chain" fraction was obtained by recrystallization of inulin (*dp* 10), thereby selectively removing the low-molecular-weight components. *O*-(Aminopropyl)inulin samples with degrees of substitution (*ds*) of 0.7 and 1.5 were synthesized from inulin (*dp* 25) by following a procedure described previously.[18] The tris(*tert*-butyl ester) of DO3A was a gift from Prof. J. Klaveness (University of Oslo, Norway).

Residual salts formed in the preparation of API and API-DO3ASQ were removed by ultrafiltration of aqueous solutions at neutral pH with a UTC-60 membrane filter (Toray Industries, Tokyo, Japan) under high pressure (20 bar N_2). Solid samples of [Gd(API-DO3ASQ)] were isolated after lyophilization.

Tris(*tert*-butyl ester) of 10-(2-ethoxy-3,4-dioxo-1-cyclobutenyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid (DO3ASQ-est): This compound

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was prepared in a similar manner to that recently reported by Aime et al.[15] One equivalent of triethylamine (0.51 g) was added to a solution of the DO3A-tris(ester)·HBr (3.0 g, 5.0 mmol) in ethanol (45 mL). After 5 min, diethyl squarate (1.1 g, 6.4 mmol) in ethanol (20 mL) was added to the stirred mixture in one portion and stirring was continued at room temperature for four days. The reaction progress was followed by TLC (SiO_2, $CHCl_3$/acetone, 1:1) by noting the consumption of diethyl squarate ($R_f = 0.60$). The solvents were evaporated to leave a crude residue which was purified by column chromatography on SiO_2 with a gradient of $CHCl_3$/acetone (4:1–1:9); this yielded 3.1 g (96%) of a pale yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.72$ (q, $J = 7.1$ Hz, 2H), 3.93 (t, $J = 6.0$ Hz, 2H), 3.78 (t, $J = 6.0$ Hz, 2H), 3.26 (s, 2H), 3.25 (s, 4H), 2.98 (m, 4H), 2.72 (m, 8H), 1.42 (s, 27H), 1.30 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 189.0$, 182.0, 176.1, 172.0, 170.6, 80.9, 69.2, 58.0, 57.6, 55.4, 54.9, 54.5, 53.1, 52.9, 52.2, 51.9, 49.6, 48.8, 28.1, 15.8; IR (neat): $\tilde{\nu} = 2978$, 2933, 2843, 1797, 1731, 1714, 1605 cm^{-1}; $R_f = 0.50$ (SiO_2, $CHCl_3$/acetone, 1:1).

10-(2-Ethoxy-3,4-dioxo-1-cyclobutenyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid (DO3ASQ): DO3ASQ-est (0.5 g) was dissolved in neat trifluoroacetic acid (5 mL) and stirred for 18 h at room temperature. The resulting solution was concentrated in vacuo; this left a golden residue which was taken up in a minimal amount of methanol (2 mL). Addition of diethyl ether afforded a white solid which was collected, washed with diethyl ether, and dried in vacuo to yield 0.35g (95%) of the DO3ASQ. FAB MS: *m/z*: 471 $[M+H]^+$; ^{1}H NMR (300 MHz, D_2O, pD 5.0): $\delta = 4.71$ (q, $J = 7.2$ Hz, 2H), 4.10–3.70 (m, 10H), 3.51–3.30 (m, 12H), 1.39 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75.5 MHz, D_2O, pD 5.0): $\delta = 189.9$, 185.9, 179.4, 177.3, 174.7, 172.7, 73.3, 56.3, 56.1, 55.0, 53.0, 51.2, 51.1, 50.1, 49.7, 16.4.

***O*-(Aminopropyl)inulin-DO3ASQ (*ds* = 0.7) (API-DO3ASQ)**: *O*-(Aminopropyl)inulin (*ds* = 0.7, 0.18 g, 0.5 mmol amino groups) was dissolved in H_2O (2 mL), after which, triethylamine (2 mL, 27 mmol) was added to the stirred solution. After 5 min, a solution of DO3ASQ (0.33 g, 0.6 mmol) in H_2O (3 mL) was added in one portion, and the solution was stirred at room temperature for ten days. The reaction was monitored by GPC-HPLC (TSK-Gel 3000 PW_{XL}, 0.2 M NaCl, 20 °C, flow rate = 0.5 mL min^{-1}) for the disappearance of DO3ASQ. The resulting mixture was concentrated in vacuo to leave a yellow residue. This material was redissolved in ethanol and addition of diethyl ether precipitated a pale yellow powder (0.4 g). Unreacted DO3ASQ and residual base were removed by size exclusion chromatography (Sephadex G-25, H_2O eluent) and the fractions were analyzed by GPC-HPLC. Pure fractions ($R_t = 14.3$ min) were collected and freeze-dried (yield = 0.19 g). ^{1}H NMR (300 MHz, D_2O, pD 5.0): $\delta = 4.40$–3.60, 3.36, 3.07, 1.89; ^{13}C NMR (75.5 MHz, D_2O, pD 5.0): $\delta = 184.1$, 183.5, 176.4, 174.5, 170.3, 170.0, 104.9, 82.7, 82.1, 78.3, 75.8, 69.9, 63.6, 62.5, 58.2, 55.0, 53.0, 52.2, 50.9, 43.2, 32.1, 28.5.

***O*-(Aminopropyl)inulin-DO3ASQ (*ds* = 1.5) (API-DO3ASQ)**: *O*-(Aminopropyl)inulin (*ds* = 1.5, 0.20 g, 1.0 mmol amino groups) was dissolved in H_2O (2 mL), after which, triethylamine (5 mL, 35 mmol) was added to the stirred solution. After 5 min, a solution of DO3ASQ (0.61 g, 1.2 mmol) in H_2O (3 mL) was added in one portion, and the solution was stirred at room temperature for ten days. The reaction was monitored by GPC-HPLC (TSK-Gel 3000 PW_{XL}, 0.2 M NaCl, 20 °C, flow rate = 0.5 mL min^{-1}) for the disappearance of DO3ASQ. The resulting mixture was concentrated in vacuo to leave a yellow residue. This material was redissolved in ethanol and addition of diethyl ether precipitated a pale yellow powder (0.4 g). Unreacted DO3ASQ and residual base were removed by SEC (Sephadex G-25, H_2O eluent) and fractions were analyzed by GPC-HPLC. Pure fractions ($R_t = 14.3$ min) were collected and freeze-dried (yield = 0.36 g). ^{1}H NMR (300 MHz, D_2O, pD 5.0): $\delta = 4.40$–3.60, 3.31, 3.04, 2.98, 1.98 (shoulder), 1.89; ^{13}C NMR (75.5 MHz, D_2O, pD 5.0): $\delta = 183.4$, 183.2, 179.1, 173.5, 170.1, 169.8, 105.5, 84.5, 82.4, 80.5, 78.2, 76.1, 72.7, 69.9, 63.0, 62.3, 59.1, 58.1, 55.2, 52.6, 43.2, 39.1, 32.2, 28.8, 28.4.

Preparation of Gd^{III} complexes: The complexes were formed by titration of aqueous solutions (D_2O) of the ligand with solid aliquots of $GdCl_3$ while maintaining neutral pH by addition of NaOH(aq). The formation of the complex was monitored by measuring the ^{17}O NMR signal shift of water at 75 °C.[3] The presence of free Gd^{III} was readily detected by a dramatic increase in the chemical shift and width of the signal, whereupon evaluation of the titration data allowed the amount of ligand required for complete complexation of free Gd^{III} to be determined. The solutions were then adjusted to pH 9 to precipitate any remaining Gd^{III} as the hydroxide, which was then removed by centrifugation. Finally, the solutions were neutralized and freeze-dried to yield solid samples.

Physical methods: ^{1}H (300 MHz), ^{13}C (75.5 MHz) and ^{17}O (40.7 MHz) NMR spectra were recorded on a Varian INOVA-300 spectrometer with 5 mm (^{1}H and ^{13}C NMR) or 10 mm sample tubes (^{17}O NMR). Chemical shifts are reported as δ values. For measurements in D_2O, *tert*-butyl alcohol was used as an internal standard with the methyl signal calibrated at $\delta = 1.2$ (^{1}H) or 31.2 (^{13}C). D_2O (100%) was used as an external chemical shift reference for ^{17}O resonances. The pH of samples was measured at ambient temperature with a Corning 125 pH meter with a calibrated micro-combination probe purchased from Aldrich. The pH values reported are direct meter readings without correction for D-isotope effects. HPLC (SEC) analysis was carried out with a set-up consisting of two consecutive UltraHydrogel Shodex columns (OHpak SB-802.5 HQ and OHpak SB-803 HQ) and a NaCl (0.9%)/MeOH (70:30 v/v) mobile phase at a flow rate of 0.8 mL min^{-1}. Detection was performed with a refractive index detector (Waters) and a diode array (Waters). The columns were calibrated with standard dextrans.

The $1/T_1$ NMRD profiles were measured at 5, 15, 25, 37, and 45 °C with an IBM Research Relaxometer by using the field-cycling method and covering a continuum of magnetic fields from 2.5×10^{-4} to 1.2 T. This corresponds to a proton Larmor frequency range of 0.01–50 MHz. The variable-temperature ^{17}O measurements were performed at a magnetic field of 7.05 T with a Varian INOVA-300 spectrometer equipped with a 5 mm probe. An aqueous solution of the Gd^{III} complex of [Gd_{37}{API-(DO3ASQ)$_{37}$}] with a Gd^{III} concentration of 0.027 M at pH 5.0 was prepared for this study. In addition, an acidified water sample (pH 5.0) was used as a reference for these measurements. Solutions were prepared with ^{17}O-enriched water (5%) and the samples were sealed under an argon atmosphere. For each temperature, spectral parameters were collected for both the Gd^{III} complex and the acidified water sample. The measurements were conducted without a frequency lock and no sample spinning. Longitudinal relaxation rates ($1/T_1$) were determined with the inversion-recovery method,[29] and the transverse relaxation rates ($1/T_2$) were obtained by the Carr–Purcell–Meiboom–Gill spin-echo technique.[30]

Calculations: Experimental variable-temperature ^{17}O NMR and NMRD data were fit with a computer program written by É. Tóth and L. Helm (University of Lausanne, Switzerland) using Micromath Scientist version 2.0 (Salt Lake City, UT, USA).

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Appendix

^{17}O NMR spectroscopy: From the measured ^{17}O NMR relaxation rates ($1/T_1$ and $1/T_2$) and the angular frequencies (ω) of the [Gd(API-DO3ASQ)] solution and of the acidified water reference ($1/T_{1A}$, $1/T_{2A}$ and ω_A) it is possible to calculate the reduced relaxation rates and chemical shifts ($1/T_{1r}$, $1/T_{2r}$ and ω_r) by using Equations (1)–(3).[31–34]

$$\frac{1}{T_{1r}} = \frac{1}{P_M}\left[\frac{1}{T_1} - \frac{1}{T_{1A}}\right] = \frac{1}{T_{1M} + \tau_M} \quad (1)$$

$$\frac{1}{T_{2r}} = \frac{1}{P_M}\left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right] = \frac{1}{\tau_M}\frac{T_{2M}^{-2} + \tau_M^{-1}T_{2M}^{-1} + \Delta\omega_M^2}{(\tau_M^{-1} + T_{2M}^{-1})^2 + \Delta\omega_M^2} \quad (2)$$

$$\Delta\omega_r = \frac{1}{P_M}(\omega - \omega_A) = \frac{\Delta\omega_M}{(1 + \tau_M T_{2M}^{-1})^2 + \tau_M^2\Delta\omega_M^2} + \Delta\omega_{os} \quad (3)$$

in which $1/T_{1M}$ and $1/T_{2M}$ represent the relaxation rates for bound water molecules, τ_M is the residence lifetime of a bound water molecule, $\Delta\omega_M$ is

the chemical shift difference between the bound water molecules and the bulk water (in the absence of a paramagnetic interaction) and P_M is the mole fraction of bound water. The overall outer-sphere contribution to the chemical shift is given by $\Delta\omega_{os}$. Previous investigations have shown that the outer-sphere contributions to the relaxation rates in Equations (1) and (2) may be omitted.[21, 35]

The value of $\Delta\omega_M$ is determined by the chemical shift of the Gd^{III}-bound water molecules which is governed by the hyperfine interaction between the Gd^{III} electron spin and the ^{17}O nucleus [Eq. (4)].

$$\Delta\omega_M = \frac{g_L \mu_B S(S+1) B}{3 k_B T} \frac{A}{\hbar} \tag{4}$$

Here, g_L is the isotropic Landé g-factor ($g_L = 2.0$ for Gd^{III}), S is the electron spin ($S = 7/2$ for Gd^{III}), B is the magnetic field, k_B is the Boltzmann constant, and $A/\hbar$ is the hyperfine or scalar coupling constant. The outer-sphere contribution to $\Delta\omega_r$ may be assumed to have a temperature dependence similar to $\Delta\omega_M$, which is given by Equation (5), in which C_{os} is an empirical constant.

$$\Delta\omega_{os} = C_{os}\Delta\omega_M \tag{5}$$

The ^{17}O longitudinal relaxation rates for bound water molecules in Gd^{III} solutions are dominated by dipole–dipole and quadrupolar interactions[21, 35] and are given by Equation (6)

$$\frac{1}{T_{1m}} = \left[\frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma_I^2\gamma_s^2}{r_{GdO}^6} S(S+1)\right]\left[6\tau_{d1} + 14\left(\frac{\tau_{d2}}{1 + \omega_s^2\tau_{d2}^2}\right)\right] + \frac{3\pi^2}{10}\frac{2I+3}{I^2(2I-1)}\chi^2\left(1 + \frac{\eta^2}{3}\right)\tau_R \tag{6}$$

in which $\mu_0/4\pi$ is the magnetic permeability in a vacuum, $\hbar$ is the Dirac constant, γ_I is the nuclear gyromagnetic ratio, $\gamma_S = g_L\mu_B/\hbar$ is the electron gyromagnetic ratio ($\gamma_s = 1.76 \times 10^{11}\ rad\,s^{-1}\,T^{-1}$ for $g_L = 2.0$), r_{GdO} is the distance between the electron charge and the ^{17}O nucleus, $\tau_{di}^{-1} = \tau_M^{-1} + T_{ie}^{-1} + \tau_R^{-1}$. T_{ie} is the electronic relaxation time, τ_R is the rotational correlation time for the Gd^{III}-O vector, I is the nuclear spin ($I = 5/2$ for ^{17}O), χ is the quadrupolar coupling constant and η is the asymmetry parameter. The ^{17}O transverse relaxation rates of bound water molecules in Gd^{III} chelates are governed by electron–nucleus scalar mechanisms and may be expressed in terms of Equation (7) in which $\tau_{is}^{-1} = \tau_M^{-1} + T_{ie}^{-1}$.[21, 36]

$$\frac{1}{T_{2M}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left[\tau_{1s} + \frac{\tau_{2s}}{1 + \omega_s^2\tau_{2s}^2}\right] \tag{7}$$

For Gd^{III} complexes, the electronic relaxation rates may be interpreted in terms of the zero field splitting (ZFS) interaction,[37] which results from transient distortions of the complex, and a spin rotation (SR) mechanism [Eq. (8)].[38–40]

$$\frac{1}{T_{ie}} = \left(\frac{1}{T_{ie}}\right)^{ZFS} + \left(\frac{1}{T_{ie}}\right)^{SR} \tag{8}$$

The ZFS contribution to the longitudinal and transverse electronic relaxation rates may be accurately described by Equations (9) and (10).[41]

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_v[4S(S+1) - 3]\left(\frac{1}{1 + \omega_s^2\tau_v^2} + \frac{1}{1 + 4\omega_s^2\tau_v^2}\right) \tag{9}$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2\tau_v\left(\frac{5.26}{1 + 0.372\omega_s^2\tau_v^2} + \frac{7.18}{1 + 1.24\omega_s\tau_v}\right) \tag{10}$$

Here, Δ^2 represents the mean-square ZFS energy and τ_v is the correlation time, which describes the modulation of the electronic-spin-state splitting. The spin rotation (SR) contribution is a magnetic-field-independent mechanism which may be approximated with Equation (11) in which $\delta g_L^2 = \Sigma_i \delta g_{Li}^2$, which refers to the deviations from the free-electron value of g_L.

$$\left(\frac{1}{T_{1e}}\right)^{SR} = \frac{\delta g_L^2}{9\tau_R} \tag{11}$$

It should be noted that the transverse electronic-relaxation rates have a negligible influence on the ^1H and ^{17}O relaxation rates.

Nuclear magnetic resonance dispersion (NMRD): In NMRD studies, relaxivity, r_1 ($s^{-1}\,mM^{-1}$), is the usual notation for the longitudinal proton-relaxation-rate enhancements, with the overall relaxivity resulting from inner-sphere and outer-sphere contributions [Equation (12)].

$$r_1 = r_{1is} + r_{1os} \tag{12}$$

While the inner-sphere contribution is a short-range interaction arising from the chemical exchange of water molecules in the first coordination sphere of the Gd^{III} ion, the outer-sphere effects result from long-range interactions with the bulk water which diffuses in the vicinity of the paramagnetic center. The inner-sphere contribution to the observed relaxivity is given by Equation (13).

$$r_{1is} = \left(\frac{q}{1000 \times 55.5}\right)\frac{1}{T_{1m} + \tau_m} \tag{13}$$

Here q is the number of inner-sphere water molecules in the Gd^{III} chelate. The ^1H longitudinal relaxation rate of inner-sphere water molecules is dominated by dipolar interactions and may be expressed by the Solomon–Bloembergen equation[42, 43] [Eq. (14)])

$$\frac{1}{T_{1M}} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\hbar^2\gamma_s^2\gamma_I^2}{r_{GdH}^6} S(S+1)\left[\frac{3\tau_{d1}}{1 + \omega_I^2\tau_{d1}^2} + \frac{7\tau_{d2}}{1 + \omega_s^2\tau_{d2}^2}\right] \tag{14}$$

in which ω_I and ω_S are the proton and electron Larmor frequencies, respectively, and r_{GdH} is the effective distance between the gadolinium electron spin and the water protons (fixed at 3.1×10^{-10} m).

The outer-sphere contribution to the observed relaxivity can be described by Equations (15) and (16).[44]

$$r_{1os} = \frac{32\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2\gamma_s^2\hbar^2 S(S+1)\frac{N_A}{a_{GdH}D_{GdH}}[3J_{os}(\omega_1, T_{1e}) + 7J_{os}(\omega_2, T_{2e})] \tag{15}$$

$$J_{os}(\omega, T_{je}) = \mathrm{Re}\left\{\frac{1 + \frac{1}{4}\left[i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right]^{1/2}}{1 + \left[i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right]^{1/2} + \frac{4}{9}\left[i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right] + \frac{1}{9}\left[i\omega\tau_{GdH} + \frac{\tau_{GdH}}{T_{je}}\right]^{3/2}}\right\} \tag{16}$$

Here Re is the real part of the complex number, N_A is Avogadro's number, a_{GdH} is the distance of closest approach of an outer-sphere water molecule to the Gd^{III} ion, D_{GdH} is the diffusion coefficient, and τ_{GdH} is the correlation time which corresponds to a_{GdH}^2/D_{GdH}. The electronic-relaxation dependence is expressed by the spectral density functions, J_{os} (ω_i, T_{je}) ($j = 1, 2$) in Equation (16).

All correlation times and D_{GdH}, are assumed to obey an exponential temperature dependence [Eq. (17)] in which τ_x^T and τ_x^{298} are the values of the concerned parameter at temperature T and 298.15 K, respectively, E_x is the associated activation energy and R is the gas constant.

$$\tau_x^T = \tau_x^{298}\exp\left[\frac{E_x}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \tag{17}$$

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