Synthesis and Relaxivity Studies of a Gadolinium(III) Complex of ATP-Conjugated DO3A as a Contrast Enhancing Agent for MRI

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A gadolinium(III) complex of adenosine 5′-triphosphate (ATP)-appended DO3A has been synthesized in order to attain higher relaxivity and to reduce the in vivo toxicity. DO3A (**2**) was synthesized as the exclusive product by a single step direct trialkylation of cyclen with chloroacetic acid in water (pH 10, –4 °C). ATP was then covalently appended to the DO3A framework through a propyl linker. The 3-bromopropane spacer appended DO3A (**3**) was synthesized by the reaction of DO3A with 1,3-dibromopropane in water/DMF in the presence of triethylamine as proton scavenger. The ATP-appended DO3A (DO3A-Pr-ATP) was synthesized by the reaction of **3** with ATP in water at room temperature. [Gd(DO3A-Pr-ATP)(H_2O)$_2$] (**4**) was synthesized by the reaction of DO3A-Pr-ATP with gadolinium(III) perchlorate hydrate in water. The X- and Q-band EPR spectra of **4** contain a broad band with no hyperfine splitting at both room temperature and liquid nitrogen temperature. The *g*-values are 2.167 and 2.033 at X- and Q-band, respectively. The magnetic moment of **4** is 7.45 BM which is close to the value for free Gd^{III} ion. The longitudinal relaxivity, r_{1p}, of [Gd(DO3A-Pr-ATP)(H_2O)$_2$] is 6.51 $mM^{-1}s^{-1}$ (24 MHz and 35 ± 0.1 °C), which is higher than that of [Gd(DOTA)(H_2O)]$^-$ (r_{1p} = 3.56 $mM^{-1}s^{-1}$, 20 MHz, 39 °C, pH 7.3) and [Gd(DO3A)(H_2O)$_2$] (r_{1p} = 4.8 $mM^{-1}s^{-1}$, 20 MHz, 40 °C). The higher relaxivity of **4** than for other systems with q = 2 is due to the increase in the molecular dimension of the complex by the conjugation of ATP. The relaxivity of **4** at pH 8.4 (TRIS buffer) decreases to 5.64 $mM^{-1}s^{-1}$, probably due to a change in the hydration number by the replacement of the coordinated water molecules by TRIS. The r_{1p} relaxivity of **4** in the presence of β-cyclodextrin is 8.97 $mM^{-1}s^{-1}$ due to the increase in the molecular weight and dimension of the inclusion complex formed by the noncovalent host–guest interaction of the ATP pendant arm with the hydrophobic cavity of β-cyclodextrin. The transverse relaxivity, r_{2p}, of **4** is 7.48 $mM^{-1}s^{-1}$ (24 MHz and 35 ± 0.1 °C). The r_{2p}/r_{1p} ratio of 1.16 indicates that **4** is a T_1-weighted contrast agent. The ATP moiety remains as an extended pendant arm and does not bind with the metal ion, nor does it block the coordination sites for the inner-sphere water molecules.

Introduction

MRI is a state-of-the-art, noninvasive imaging modality in clinical medicine, as exemplified by the award of the 2003 Nobel Prize for medicine to Professor Paul C. Lauterbur (Biomedical Magnetic Resonance Laboratory, University of Illinois) and Professor Sir Peter Mansfield (University of Nottingham) for their seminal discoveries concerning the use of magnetic resonance to visualize different structures leading to the development of modern MRI.[1] An MRI image is a three dimensional signal intensity map of the spatially encoded proton signal of the in vivo water molecules in a given volume element (voxel). The movement of protons from one position to another during the relaxation experiment alters the signal intensity and therefore the moving blood has no detectable signal. It is common to increase the contrast of the image by administering MRI contrast agents (CAs),[2] which enhance the longitudinal and transverse relaxation rates of the water protons which contribute to the intensity of the MRI signal and thus to the contrast of the image. The first example of a paramagnetic substance used for tissue discrimination in MRI was provided by Lauterbur et al.[3] The in vitro and in vivo chemical results reported by Tweedle et al.[4] support the usefulness of strongly chelated Gd^{III} complexes as CAs, and since then Gd^{III} complexes of polyaminocarboxylate ligands have been widely studied.[2a,2e,2f]

Proton relaxivity by Gd^{III}-based CAs is governed by the magnetic coupling interaction of the unpaired electrons of the metal ion and the water proton nuclei by a through-space dipole–dipole mechanism[5] and is interpreted using the Solomon–Bloembergen–Morgan (SBM) theory.[6] Relaxivity is determined by several parameters,[5d,7] and higher relaxivity may be obtained by increasing the correlation time, τ_c, which modulates the dipole–dipole relaxation mechanism. It depends on the exchange lifetime (k_{ex} = $1/\tau_m$) of the coordinated water molecules, the electronic relaxation times ($T_{1e,2e}$), and the rotational correlation time (τ_R) of the complex. One of the practical approaches to gain higher relaxivity by gadolinium-based contrast agents

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is increasing τ_R by increasing the size and molecular weight of Gd^{III} chelates by covalent or noncovalent interaction with macromolecules. Examples of Gd^{III} chelates bound to macromolecules include the HSA adduct of the Gd complex of benzyloxy-α-propionic acid substituted DOTA (r_{1p} = 56 $mM^{-1}s^{-1}$),[8] the Gd complex of a *p*-bromobenzyloxy-substituted pyridine macrocycle (r_{1p} = 8.25 $mM^{-1}s^{-1}$), its HSA adduct (r_{1p} = 48 $mM^{-1}s^{-1}$), and its noncovalent adduct with poly-β-cyclodextrin (r_{1p} = 36 $mM^{-1}s^{-1}$),[9] the squaric ester substituted DO3A linked to polyornithine (r_{1p} = 29 $mM^{-1}s^{-1}$),[10] Gd-DTPA-polylysine[11] (r_{1p} = 13 $mM^{-1}s^{-1}$ and 32 $mM^{-1}s^{-1}$ at pH 8), the Gd dendrimer of the DTPA-functionalized second-generation starburst dendrimer (r_{1p} = 234 $mM^{-1}s^{-1}$ at 25 MHz),[12] the Gd complex of *p*-nitrophenyl-substituted DOTA (r_{1p} = 5.4 $mM^{-1}s^{-1}$, 20 MHz, 25 °C, pH 7.3),[13] the HSA adduct of MS-325 (r_{1p} = 50.8 $mM^{-1}s^{-1}$, 20 MHz),[14] the glucitol-conjugated DO3A (r_{1p} = 5.19 $mM^{-1}s^{-1}$),[15] Gd chelates of *p*-bromobenzyl- and *p*-phosphonatomethylbenzanilido-substituted DO3A (r_{1p} = 7.9 $mM^{-1}s^{-1}$ and 10.7 $mM^{-1}s^{-1}$, respectively), and their HSA adducts (r_{1p} = 24.0 $mM^{-1}s^{-1}$ and 21.0 $mM^{-1}s^{-1}$, respectively),[16] and the multiple Gd-DO3A chelates covalently attached to a polysaccharide backbone (r_{1p} = 14.4 $mM^{-1}s^{-1}$ for each Gd, 20 MHz, 39 °C).[17]

The gadolinium(III) chelates approved by the FDA for human imaging are $(NMG)_2[Gd(DTPA)(H_2O)]$[18] (Magnevist, Schering), $(NMG)[Gd(DOTA)(H_2O)]$[19] (Dotarem, Guerbet), $[Gd(DTPA\text{-}BMA)(H_2O)]$[20] (Omniscan, Nycomed), $[Gd(HP\text{-}DO3A)(H_2O)]$[21] (ProHance, Bracco), $[Gd(DO3A\text{-}Butrol)(H_2O)]$[22] (Gadovist, Schering), and $[Gd(BOPTA)(H_2O)]$[23] (MultiHance, Bracco). New classes of MRI contrast agents[24] such as blood pool agents,[25] targeting CAs,[24] and smart contrast agents such as those that are pH-sensitive,[10,25i,26] oxygen-pressure (pO_2) responsive,[27] enzyme responsive,[28] and metal ion concentration dependent[29] are now emerging.

Clinical MRI applications continue to evolve rapidly. This drives the need for more efficient and pathology-specific contrast agents that will increase the potential of MRI as a diagnostic tool. Increasing the molecular weight of a Gd^{III} chelate with two inner-sphere water molecules by conjugating with a biomacromolecule would lead to a biocompatible CA with high relaxivity. Such chelates with high tissue-specific targeting moieties are able to provide improved image contrasts even at a lower concentration than normally required. DO3A is a well-suited ligand framework for functionalization due to the unique secondary amine nitrogen of the macrocyclic ring. Furthermore, biomacromolecule-appended DO3A is expected to exhibit the same stability as that of the parent DO3A. Herein we report the synthesis and relaxivity studies of gadolinium(III) complex of adenosine 5′-triphosphate appended DO3A.

Results and Discussion

Synthesis of the Ligand

1,4,7-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A) (2)

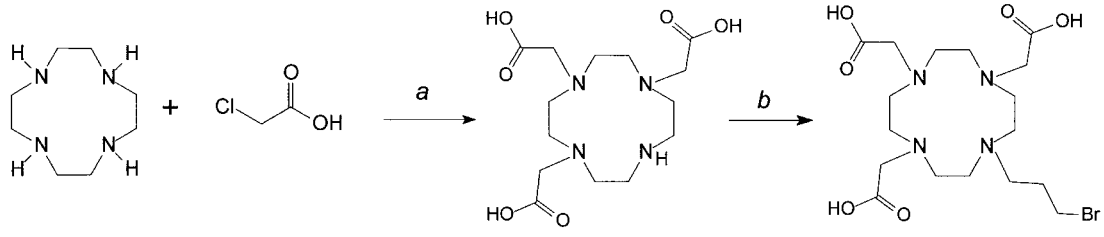
DO3A was synthesized in a single-step direct N-alkylation of cyclen with chloroacetic acid in a 1:3 molar ratio in 69.58% yield. The reaction was carried out at –4 °C and gradually heated to 50 °C over 4 h. The pH of the medium was maintained at 10 throughout the reaction. DO3A was first synthesized by Tweedle et al.[30] by the reaction of cyclen with chloroacetic acid in water at pH 10. The reaction afforded both DO3A and DOTA and the separation was carried out by ion-exchange chromatography. Tweedle et al.[21a] have also reported the synthesis of DO3A by the alkylation of monoprotected cyclen (*p*-toulenesulfonyl or formyl group) with chloroacetic acid followed by deprotection.

Linker-Appended DO3A (3)

DO3A is a heptadentate ligand with three acetate arms and a unique secondary amine nitrogen on the ring. The bromopropyl linker appended DO3A (**3**) was synthesized by the N-alkylation of DO3A with 1,3-dibromopropane in the presence of triethylamine as proton scavenger in DMF/water at room temperature. The use of triethylamine as a base leads to the formation of its hydrobromide salt along with **3**, which is removed by washing with methanol. The reaction is illustrated in Scheme 1.

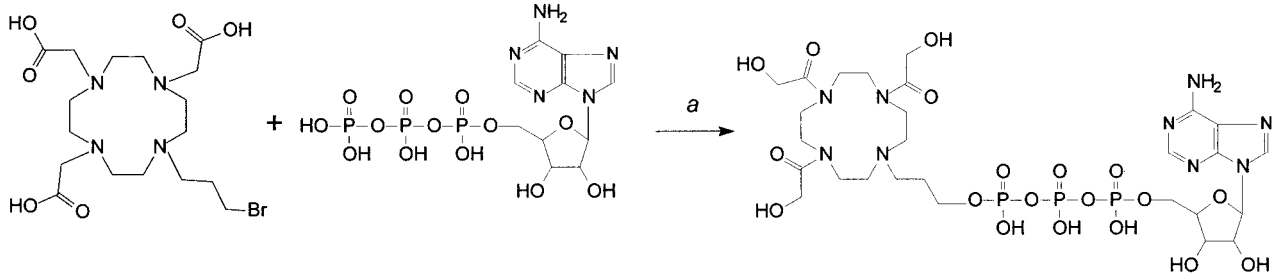
ATP-Appended DO3A (DO3A-Pr-ATP)

ATP was appended onto the DO3A framework through the bromopropyl linker by the reaction of **3** with ATP in a



1 **2** **3**

Scheme 1. Synthesis of linker appended DO3A. Reagents and conditions: (a) –4 °C, H_2O, NaOH, pH 10; (b) 1,3-dibromopropane, DMF/water, triethylamine.



Scheme 2. Synthesis of DO3A-Pr-ATP. Conditions: (a) water, room temp., 36 h.

1:1 molar ratio in water at room temperature (Scheme 2). The reaction of **3** with the commercially available disodium salt of ATP yielded DO3A-Pr-ATP along with sodium bromide as the side product. Since both are soluble in water the isolation of the free ligand becomes difficult. Therefore, the free form of ATP, recovered from its disodium salt by passing an aqueous solution through a cation exchange resin and eluting with water, was reacted with **3**. DO3A-Pr-ATP was isolated as a white solid by adding methanol.

Spectroscopic Studies

Infrared Spectrum

The IR spectrum of DO3A-Pr-ATP shows a broad peak at 3420.1 cm^{-1} assignable to the ν_s(OH) vibration. The peaks at 2926.5 and 1476.8 cm^{-1} are assignable to the ν_s(CH) and δ_s(CH) vibrations, respectively. The sharp peaks at 1700 and 1635.4 cm^{-1} are due to the ν_s(C=O) and δ_s(NH) vibrations, respectively. The sharp peak at 1507.1 cm^{-1} is

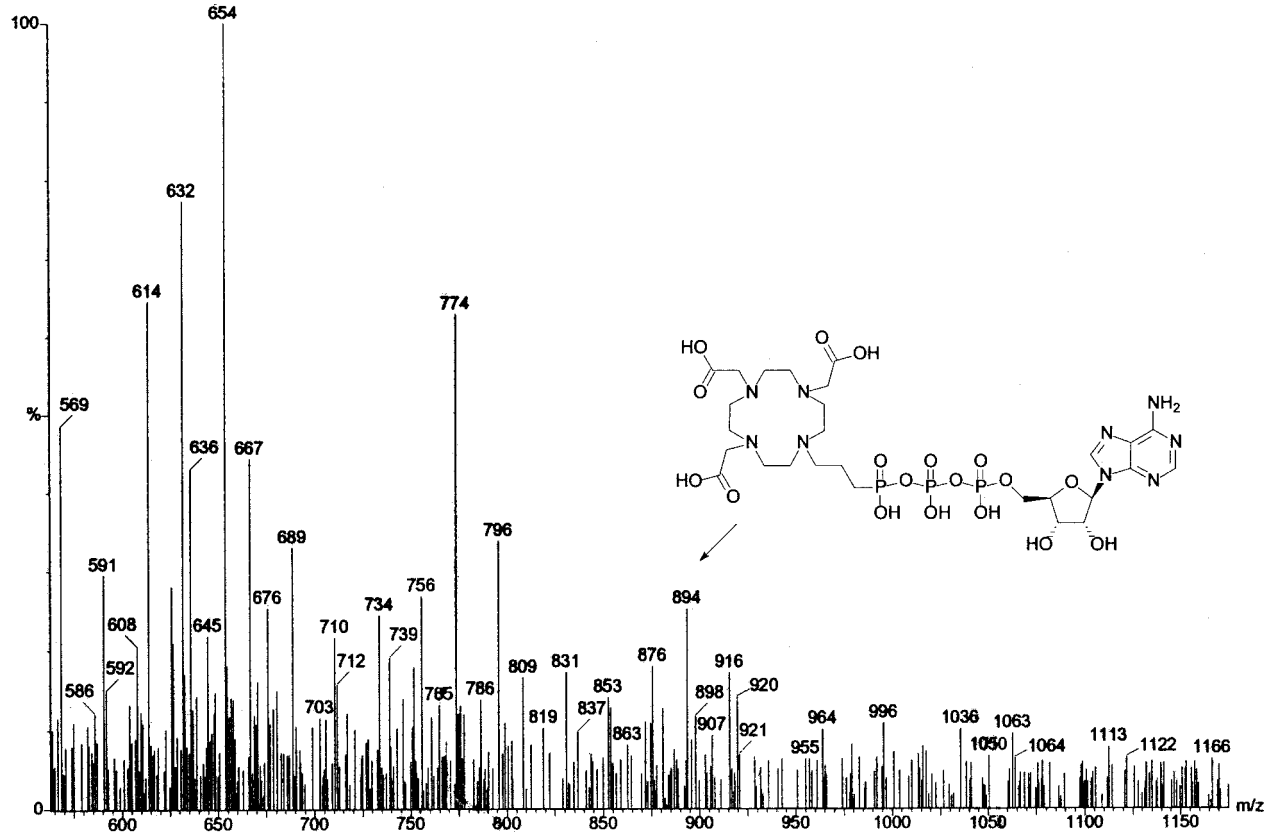


Figure 1. ESI mass spectrum of 10-[3-(adenosine 5′-triphosphate)oxypropyl]-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A-Pr-ATP).

assigned to the ν_s(C–O) vibration of the ether linkage between the DO3A and ATP. The peak at 1087.6 cm^{-1} is assigned to the ν_s(C–N) vibration. The bands at 1228.4 and 900 cm^{-1} are assigned to the ν_s(P=O) and ν_s(P–O) vibrations, respectively.[31] The IR spectrum of the complex contains the characteristic absorption bands of the ligand except for the OH vibrations of the carboxylic acid. The band at 1608.8 cm^{-1} in the complex is typical of the COO functionality.[32]

ESI Mass Spectrum

The ESI mass spectrum of DO3A-Pr-ATP shows a peak at *m*/*z* = 894 for the molecular ion $[M + 1]^+$ ($C_{27}H_{47}N_9O_{19}P_3$). The peak at *m*/*z* = 756 is assignable to the species $[M - C_5H_7N_5]^+$ ($C_{22}H_{39}N_4O_{19}P_3$) formed by the loss of the adenine moiety of ATP. The peak at *m*/*z* = 654 is assignable to the species $[M - C_9H_{13}N_5O_3]^+$ ($C_{18}H_{33}N_4O_{16}P_3$) formed by the loss of the furan moiety of ATP. The peak at *m*/*z* = 348 is assignable to the species $[M - C_{13}H_{19}N_5O_{13}P_3]^+$ ($C_{14}H_{28}N_4O_6$) formed by the loss of the phosphate group of ATP and the propyl linker group. The ESI mass spectrum of DO3A-Pr-ATP is depicted in Figure 1.

^{1}H NMR Spectrum

The ^{1}H NMR spectrum of DO3A-Pr-ATP in D_2O shows resonances for the methylene protons of the cyclen framework as a multiplet at δ = 3.10–3.4 ppm (12 H, NCH_2CH_2N). The triplet at δ = 3.58–3.63 ppm (*J* = 5.5 Hz, 2 H) is assigned to the OCH_2 protons of the linker that is bonded to ATP. The resonance at δ = 4.37 ppm (6 H, CH_2COO) is assigned to the methylene protons of acetate arms. The triplet centered at δ = 4.25 ppm (1 H) is assigned to the proton of the furan ring of the adenine moiety. The doublet at δ = 4.49–4.65 ppm (2 H) is assigned to the OCH_2 protons of the ATP unit. The signals at δ = 2.25 ppm are assigned to the protons of the propyl linker. The resonances at δ = 8.37, 8.54 (m, 2 H), and 6.07 ppm (m, 1 H) are assigned to the aromatic protons of the adenine moiety. The ^{1}H NMR spectrum of DO3A-Pr-ATP is depicted in Figure 2.

^{13}C NMR Spectrum

The ^{13}C NMR spectrum of DO3A-Pr-ATP in D_2O has a signal at δ = 174.52 ppm assignable to the carboxylic acid carbon. The signals at δ = 152.90–145.57 ppm are assignable to the carbon atoms of the adenine group of ATP. The resonances at δ = 68.43 (OCH_2), 77.38 (OCH_2), and 87.14 ppm are assignable to the C_2, C_3, and C_4 carbons, respectively, of the furan ring. The signal at δ = 34.78 ppm is assignable to the β-CH_2 carbon of the propyl linker. The resonance at δ = 52.99 ppm is due to the methylene carbon of the propyl linker bonded to the nitrogen of the macrocycle. The signal at δ = 68.43 ppm is assigned to the methylene carbon attached to the phosphate group of ATP. The higher chemical shift of this carbon compared to the other two methylene carbons of the linker is indicative of its attachment to the terminal phosphate group of ATP. The signal at δ = 52.99 ppm is assignable to the ethylenic carbon of the macrocyclic framework. The resonance at δ = 57.23 ppm is assignable to the methylene carbon of the acetate arm.

^{31}P NMR Spectrum

The ^{31}P NMR spectrum of DO3A-Pr-ATP in D_2O shows three distinct signals due to the three phosphate groups of ATP in different environments. The signal at δ = 130.20 ppm is assigned to the central phosphate and the signal at δ = 107.35 ppm is assignable to the phosphate attached to the propyl spacer. The signal at δ = 119.30 ppm is assigned to the phosphate that is attached to the furan group of ATP.

Mass Spectrum of [Gd(DO3A-Pr-ATP)(H_2O)$_2$]

The ESI mass spectrum shows a cluster of peaks at *m*/*z* = 1081 assignable to the molecular ion $[M - 2]^+$ $[Gd(C_{27}H_{41}N_9O_{19}P_3)(H_2O)_2]$. The peak at *m*/*z* = 793 is assignable to $[M - C_{11}H_{17}N_5O_5]^+$ $[Gd(C_{17}H_{27}N_4O_{16}P_3)]$, the species formed by the loss of the adenine moiety. The peak at *m*/*z* = 734 is assignable to the species $[M - C_{12}H_{21}N_5O_7]^+$ $[Gd(C_{15}H_{24}N_4O_{14}P_3)]$ formed by the loss of the acetate group. The peak at *m*/*z* = 578 is assignable to the species $[M - Gd(C_{12}H_{20}N_5O_7)]^+$ ($C_{15}H_{25}N_4O_{14}P_3$) formed by the loss of Gd^{III}. The peak at *m*/*z* = 483 is assignable to $[M - Gd(C_{12}H_{20}N_5O_{11}P)]^+$ ($C_{15}H_{25}N_4O_{10}P_2$), the species formed by the loss of one phosphate group. The peak at *m*/*z* = 168 is assignable to $[M - Gd(C_{19}H_{29}N_5O_{21}P_3)]^+$ ($C_8H_{16}N_4$), the species formed by the loss of acetate groups and the propyl linker. The MALDI-TOF mass spectrum shows a peak at *m*/*z* = 1043.80 (100%) assignable to the molecular ion $[M - 2H_2O]^+$ $[Gd(C_{27}H_{41}N_9O_{19}P_3)]$, which further confirms the formation of the complex. The MALDI TOF mass spectrum of [Gd(DO3A-Pr-ATP)(H_2O)$_2$] is given in Figure 3.

EPR Spectra and Magnetic Moment of [Gd(DO3A-Pr-ATP)(H_2O)$_2$]

The X- and Q-band EPR spectra consist of a broad band with no hyperfine splitting at room temperature or at liquid nitrogen temperature. The *g*-value at X-band is 2.167 and at Q-band is 2.0333. The *g*-value at 77 K is 2.103 (X-band). The broad band shows that the dipolar interaction of the metal ion is small. The magnetic moment of [Gd(DO3A-Pr-ATP)(H_2O)$_2$] is 7.5 BM which is close to the value for the free gadolinium(III) ion (7.85 BM).

Relaxivity Studies

Longitudinal Relaxivity (r_{1p})

The ligand DO3A-Pr-ATP has the DO3A framework with an extended ATP pendant arm. It coordinates to the metal ion through four nitrogen donors of the macrocycle and three carboxylate oxygens. Two inner-sphere water molecules complete the coordination geometry. The complex [Gd(DO3A-Pr-ATP)(H_2O)$_2$] (**4**) is expected to have the same coordination geometry as that of [Gd(DO3A)(H_2O)$_2$]. The longitudinal relaxivity of **4** is 6.51 $mM^{-1}s^{-1}$ (normal-

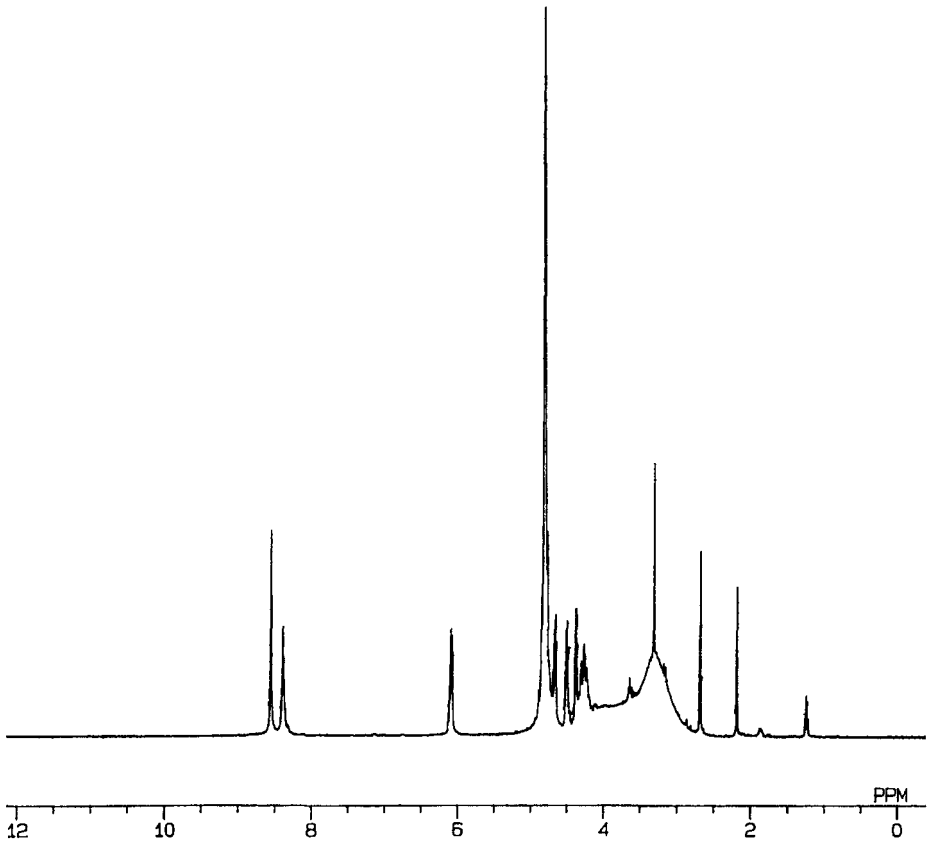


Figure 2. ^{1}H NMR spectrum of 10-[3-(adenosine 5′-triphosphate)oxypropyl]-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A-Pr-ATP).

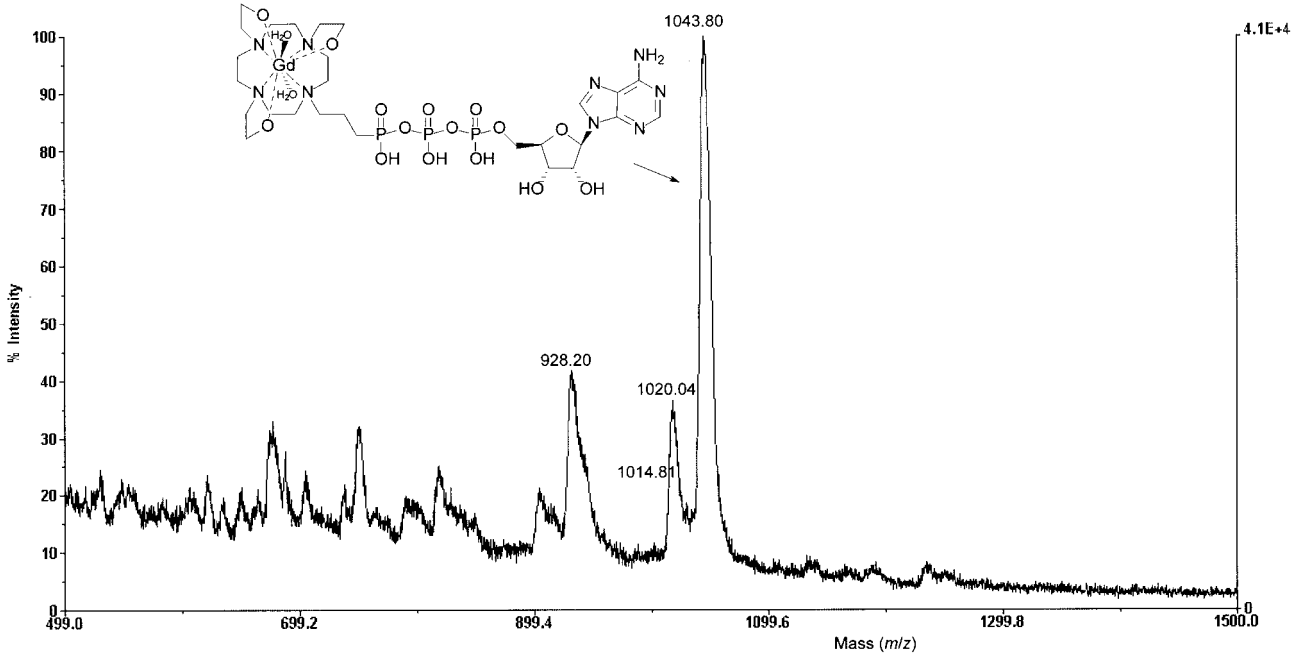


Figure 3. MALDI TOF mass spectrum of [Gd(DO3A-Pr-ATP)(H_2O)$_2$] (**4**).

ized to 1 mM). The contribution by the inner-sphere mechanism to the overall relaxivity is approximately 1.7 mM^{-1} s^{-1} per coordinated water molecule.[2a,2d] The higher longitudinal relaxivity of **4** than that of [Gd(DOTA)(H_2O)]$^-$ (r_{1p} = 3.56 mM^{-1} s^{-1}, 20 MHz, 39 °C, pH 7.3),[15] a q = 1 system, and [Gd(DO3A)(H_2O)$_2$] (r_{1p} = 4.8 mM^{-1} s^{-1} at 20 MHz, 40 °C),[33] a q = 2 system, is attributed not only to the presence of two inner-sphere water molecules bound to the me-

tal ion, but also to the longer rotational correlation time, τ_R, due to the increase in the molecular dimensions of the complex by the conjugation of ATP. As the molecular tumbling rate is reduced by increasing the molecular dimension, the dipole–dipole interaction between the paramagnetic metal ion and the water proton is efficient and, as a result, a greater paramagnetic effect is transferred to the bulk solvent (water). Tweedle et al.[34] have shown that the relaxivity of Gd^{III} complexes increases with increasing molecular weight. The outer-sphere contribution to overall relaxivity is approximately 2.3–2.5 $mM^{-1}s^{-1}$ deduced on the basis of the relaxivity of Gd^{III} complexes with no inner-sphere water molecules ($q = 0$).[35]

Longitudinal Relaxivity in the Presence of β-Cyclodextrin

The longitudinal relaxivity of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ increases to 8.97 $mM^{-1}s^{-1}$ in the presence of β-cyclodextrin. This is attributed to the formation of an inclusion complex through a nonbonding interaction between the ATP pendant arm and β-cyclodextrin with a concomitant increase in the molecular dimension and τ_R of the complex, which leads to an enhancement in relaxivity. An aqueous solution of β-cyclodextrin (50 mM) under the same experimental conditions does not show enhancement of water proton relaxivity. The concentration of β-cyclodextrin was maintained at 50-times the concentration of the complex to ensure the formation of the inclusion complex. Aime and co-workers[36] have reported that the formation of ternary inclusion complexes between the hydrophobic cavity of β-cyclodextrin and Gd^{III} chelates leads to a 10-fold increase of r_{1p}. Many complexes form inclusion complexes with β-cyclodextrin through noncovalent interactions between the substituent and β-cyclodextrin cavity.[9] The nature of the substituent also plays an important role. Aime et al.[13] have reported a large K_D (dissociation constant) value as a consequence of the poor fit of the substituent into the β-cyclodextrin cavity compared to complexes with no substituent. The presence of the substituent in the *para* position of the phenyl ring is expected to strengthen the binding and favor the formation of the inclusion complex.[37] Thus, the enhancement of the relaxivity of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ in the presence of β-cyclodextrin is attributed to the formation of the inclusion complex by a noncovalent host–guest interaction between the free amine group in the *para* position of the phenyl ring of ATP and the hydrophobic cavity of β-cyclodextrin. A plot of the concentration of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ vs. $1/T_1$ in the presence and absence of β-cyclodextrin is given in Figure 4.

pH Dependence of the Relaxivity

The relaxivity of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ at pH 8.4 (TRIS buffer) is 5.64 $mM^{-1}s^{-1}$, which is one unit lower than the relaxivity of the complex without pH adjustment (pH 5.4). The hydration number of the metal plays an important role on relaxivity. $[Gd(H_2O)_8]^{3+}$ has a q value of 8 or 9 and exhibits a relaxivity of 11.8 $mM^{-1}s^{-1}$. As the q value decreases, the relaxivity also decreases. Sherry et al.[38] have observed that the decrease in relaxivity at higher pH is due to the competition of the hydroxide ion for coordination to the Gd^{III} ion. The decrease in the relaxivity of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ at higher pH may be due to the replacement of bound water molecules by TRIS.

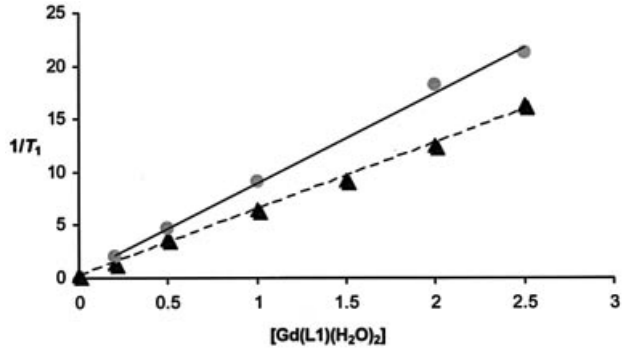


Figure 4. A plot of concentration of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ vs. T_1^{-1} in the presence (●) and in the absence (▲) of β-cyclodextrin.

Transverse Relaxivity (r_{2p})

The transverse relaxivity of $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ is 7.48 $mM^{-1}s^{-1}$ (24 MHz and 35 ± 0.1 °C), which is higher than that of $[Gd(DOTA)(H_2O)]^-$ (r_{2p} = 4.57 $mM^{-1}s^{-1}$, 20 MHz). The r_{2p}/r_{1p} ratio for $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ is 1.16, showing that the complex is a T_1-weighted contrast agent. The maximum signal intensity attained is determined by the r_{2p}/r_{1p} ratio, with the highest signal intensity occurring when the ratio is unity.

Conclusions

In this work, the synthesis and relaxivity studies of a Gd^{III} complex of adenosine 5′-triphosphate conjugated DO3A has been reported. The increase in the molecular weight of the chelate leads to an increase in the r_{1p} and r_{2p} relaxivities due to the slow tumbling rate of the complex in solution. The covalent attachment of ATP causes the complex to remain intact under different experimental conditions and it is expected to be biocompatible and nontoxic. $[Gd(DO3A\text{-}Pr\text{-}ATP)(H_2O)_2]$ is expected to reduce the in vivo clearance rate due to its noncovalent interaction with blood serum albumin and could be used as an intravascular contrast agent as the persistence of the complex in blood would be higher. Thus, there is a wide scope for studying the interaction and relaxivity of this complex in the presence of blood serum albumin to evaluate its suitability as a blood pool agent. Furthermore, the ATP-conjugated derivative is particularly interesting since the NH_2 group provides a site for further modification of the ligand or covalent attachment of a substituent. The appended ATP does not bind with the central metal ion or block the coordination site of the inner-sphere water molecules and remains as an extended pendant arm. It is worthwhile to study the behavior of this complex in response to biological events such as enzyme action. The advent of attaching metal complexes to larger biological molecules has created a new

realm of research in the field of bioconjugates. Conjugates of chelates with biological macromolecules that have affinity for specific tissues can be employed to study specific biological processes and physiological function. Design of MRI contrast agents that remain in the body for longer time and target specific tissues is a need for the future.

Experimental Section

Chemicals: Triethylenetetraamine (99%), *p*-toluenesulfonyl chloride, 1,2-dibromoethane, 1,3-dibromopropane, chloroacetic acid, xylenol orange, β-cyclodextrin, Celite, anhydrous potassium carbonate, and TRIS (Fluka) were used as such. Adenosine 5′-triphosphate disodium salt (Aldrich) was converted into the free form by passing through Amberlite IR120 (H^+, strongly acidic) (Aldrich) cation exchange resin and eluting with triply distilled water. The resin was washed with distilled water five times before use. Triethylamine, perchloric acid (Merck, India), sodium hydroxide and potassium hydroxide pellets (Rankem, India), and gadolinium(III) carbonate (Indian Rare Earths Ltd.) were used as such. $Gd(ClO_4)_3 \cdot nH_2O$ was prepared from gadolinium(III) carbonate and perchloric acid in water. The perchlorate salt was recrystallized from triply distilled water prior to use. Cyclen was synthesized by the method of Chavez and Sherry.[39] The solvents were purified by the standard procedures.[40]

Physical Measurements: Infrared spectra were recorded on a Perkin–Elmer Spectrum RX-I FT-IR spectrometer in the range of 4000–400 cm^{-1} using KBr pellets. Potassium bromide (FT IR grade, Aldrich) was used to make pellets. FAB mass spectra were recorded on a Jeol SX-102/DA 6000 mass spectrometer/data system using argon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzyl alcohol as the matrix. Electrospray ionization mass spectra were recorded with a Micromass Quattro-II Triple Quatrapole mass spectrometer. The sample was dissolved in water and introduced into the ESI capillary using a 5-μL syringe pump. The ESI capillary was set at 3.5 kV with a cone voltage of 40 V. CHN microanalyses were carried out using Perkin–Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin–Elmer AD 6 Autobalance. Helium (analytical grade) was used as the carrier gas. Analytical and preparative HPLC analysis were carried out using Varian PrepStar 218 (Varian Instruments Inc., USA) binary gradient solvent delivery module with an inline three channel degasser Model 2000 for solvent delivery. A Rheodyne injector valve (20 μL) was used for the sample injection. HPLC column (250 × 4.6 mm × 1/4 in Valco, Microsorb-MV 100-5 C18) (analytical) and DYNAMAX HPLC column (250 × 10.0 mm, Microsorb 300–10 C18) (preparative) were used. UV/Visible detector Model 345 operating in the range 190–1100 nm was used. The fractions were collected using a Model 704 fraction collector. EPR spectra were recorded on a Jeol instrument at Q-band (34.5 MHz) and X-band (9.4 MHz) with a scan range of 8000 g and the field was set at 12500 T. Magnetic susceptibility measurements were carried out on EG&G PAR Model 155 vibrating sample magnetometer at 25 °C. ^1H NMR spectra were recorded in D_2O and $CDCl_3$ (9.995 atom% D, Aldrich) on a Jeol GSX-400 multinuclear NMR spectrometer working at 400 MHz at 25 °C. The ^{13}C NMR spectra were recorded at 100 MHz using a Jeol GSX-400 instrument. The ^{31}P NMR spectra were recorded at 162 MHz.

Longitudinal Relaxivity (r_{1p}) Measurements: The longitudinal relaxivity of the Gd^{III} complex was determined from the spin-lattice relaxation time, T_1. The T_1 measurements were carried out on a Maran wide line NMR (Resonance Instruments Ltd., UK) operating at 24 MHz and 35 ± 0.1 °C. The temperature was controlled using a temperature console. The complex solutions in triply distilled water were placed in a 10-mm stoppered quartz tube and the instrument parameters were optimized for each T_1 measurement. The T_1 measurements were made using the standard inversion recovery pulse sequence (180°–τ–90°) with phase-sensitive detection[41] with varying τ values ranging from 50 μs to 6 s for each concentration of the complex. The computer program Winfit was used to plot the time vs. signal intensity to get an exponential plot and the T_1 values were calculated from the plot. A delay of at least 5 T_1 was maintained in between successive pulses to allow the complete return of the spin system to equilibrium. The T_1 values for five different concentrations of the complex were measured. A plot of $1/T_1$ vs. concentration of the complex gave a straight line and the slope was taken as the longitudinal relaxivity r_{1p}. The longitudinal relaxivity at pH 8.5 was measured by maintaining the pH by adding TRIS buffer. The longitudinal relaxivity in the presence of β-cyclodextrin was determined by adding aqueous solution of a known amount of β-cyclodextrin and keeping the solution overnight at ambient temperature to attain equilibrium. Six different concentrations (0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) of the complex in water were used for relaxivity measurements.

Transverse Relaxivity (r_{2p}) Measurements: The transverse relaxivity, r_{2p}, was determined from the spin–spin relaxation time T_2. A standard CPMG (Carl–Purcel–Meiboon–Gill) pulse sequence (90°–τ–180°)[42] with a τ value of 50 μs was used to determine T_2. The computer program Winfit was used to plot the time vs. signal intensity to get an exponential curve and T_2 was calculated from the graph. The T_2 values for six different concentrations were measured. The transverse relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex vs. $1/T_2$ by a least-squares fitting method. Six different concentrations (0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) of the complex were prepared in triply distilled water.

1,4,7-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A) (2): Chloroacetic acid (2.820 g, 30 mmol) in 20 mL of water was placed in a double-walled, round-bottomed flask, connected to a cryostat maintained at –4 °C, and aqueous sodium hydroxide (5 N) was added dropwise whilst stirring until the pH reached 5. Cyclen sulfate (3.684 g, 10 mmol) in water (50 mL) was neutralized with 5 N sodium hydroxide and added to the chloroacetic acid solution in the flask whilst stirring at –4 °C. The pH of the mixture was increased to 10 by the addition of 5 N aqueous sodium hydroxide. The solution was maintained at –4 °C with constant stirring whilst maintaining pH 10 by the frequent addition of 5 N aqueous sodium hydroxide for 3 h. The contents of the flask were transferred to another round-bottomed flask, fitted with a double surface condenser, and slowly heated to room temperature for 5 h while maintaining a pH of 10. The solution was further heated to 50 °C over a period of 4 h and the temperature was maintained for 8 h at pH 10. The solution was cooled to room temperature and loaded onto a column containing Amberlite IR 120 (H^+, strongly acidic) cation exchange resin and eluted with deionized water. The solvent was removed under reduced pressure in a rotary evaporator and the resulting white solid was washed with dry acetone and methanol, filtered, and dried in a vacuum desiccator. The purity of DO3A was checked by HPLC (R_f = 3.244, H_2O/CH_3CN 9:1 *v*/*v*); yield 69.58%; m.p. 260 °C. IR (KBr): $\tilde{\nu}$ = 3406.5 cm^{-1} ν_s(OH), 2964.0 ν_s(CH), 1723.0 ν_s(C=O), 1623.6 δ(NH), 1459.8 ν(CH), 1391.6 δ(OH), 1212.3 ν_s(CO), 1087.0 ν_s(CN), 985 δ(CH), 720 δ_r(CH). MS (70 eV, EI): *m*/*z* (%) = 344 (80) $[M - 2]^+$ ($C_{14}H_{24}N_4O_6$),

329 (50) $[M - NH]^+$ ($C_{14}H_{23}N_3O_6$), 302 $[M - C_2NH_3]^+$ ($C_{12}H_{20}N_3O_6$), 243 (20) $[M - C_4H_7NO_2]^+$ ($C_{10}H_{17}N_3O_4$), 185 (50) $[M - C_6H_9NO_4]^+$ ($C_8H_{15}N_3O_2$), 57 (100) ($C_2H_2O_2$). ^{1}H NMR (400 MHz, D_2O, TMS): δ = 3.21 (m, 12 H, CH_2), 3.39 (m, 4 H, N–CH_2), 3.60 (s, 6 H, CH_2COOH), 3.9 (d, 1 H, J = 5.3 Hz, NH) ppm. ^{13}C NMR (100.34 MHz, D_2O): δ = 42.5 (CH_2), 47.8 (N–CH_2), 52.06 (CH_2COOH), 174.5 (COOH) ppm. $C_{14}H_{26}N_4O_6$ (346.08): calcd. C 48.58, H 7.51, N 16.18; found C 48.14, H 7.43, N 15.94.

10-Bromopropyl-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (3): DMF (30 mL) and 5 drops of triethylamine were added, whilst stirring, to a solution of DO3A (3.45 g, 10 mmol) in 60 mL of distilled water in a round-bottomed flask, kept over a rotamantle, and the mixture was heated to 80 °C. The temperature was maintained at 80 °C for 1 h with stirring. 1,3-Dibromopropane (2.01 g, 10 mmol) in 10 mL of DMF was then added slowly and the reaction mixture was stirred for 36 h at 80 °C. The mixture was filtered and the filtrate was evaporated in a rotary evaporator to dryness. The solid compound that separated out was washed with dry methanol to remove triethylamine hydrobromide salt and then with acetone. The white solid was dried in a vacuum desiccator and recrystallized from water. The purity of **3** was checked by HPLC (R_f = 4.20, H_2O/CH_3CN, 3:2 *v*/*v*); yield 67%; m.p. 290 °C (dec). IR (KBr): $\tilde{\nu}$ = 3432.2 cm^{-1} ν_s(OH), 2954.0 ν_s(CH),1692.0 ν_s(C=O), 1460.3 δ(CH), 1385.4 δ(OH), 1214.4 ν_s(CO), 1087.3 ν_s(CN), 980.7 δ(CH), 699.4 δ_r(CH). MS (FAB): *m*/*z* = 466 $[M - 1]^+$ ($C_{17}H_{30}N_4O_6Br$), 341 $[M - C_3H_9Br]^+$ ($C_{14}H_{21}N_4O_6$), 284 $[M - C_5H_{11}O_2Br]^+$ ($C_{12}H_{20}N_4O_4$), 255 $[M - C_7H_{16}O_2Br]^+$ ($C_{10}H_{15}N_4O_4$). ^{1}H NMR (400 MHz, D_2O, TMS): δ = 2.63 (m, 2 H, CH_2), 3.23 (m, 12 H, CH_2), 3.41 (s, 6 H, N–CH_2), 3.84 (CH_2COOH) ppm. ^{13}C NMR (100.34 MHz, D_2O): δ = 36.84 (N-CH$_2$-*C*H$_2$), 52.73 (*C*H$_2$), 58.28 (*C*H$_2$COOH) 174.31 (*C*OOH) ppm. $C_{17}H_{31}BrN_4O_6$ (467.01): calcd. C 43.71, H 6.63, N 11.99; found C 43.18, H 6.33, N 11.53.

10-[3-(Adenosine5′-triphosphate)oxypropyl]-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DO3A-Pr-ATP): Adenosine 5′-triphosphate disodium salt (0.551 g, 1 mmol) was dissolved in 5 mL of water and passed through a column packed with Amberlite IR-120 (H^+, strongly acidic) cation exchange resin and eluted with distilled water. The linker-appended DO3A (**3**; 0.466 g, 1 mmol) in 25 mL of water was added to the eluent, which contains free ATP, and stirred for 24 h at room temperature. The solvent was removed under reduced pressure in a rotary evaporator whilst maintaining the bath temperature below 40 °C. The vitreous solid that separated out became white upon the addition of methanol and was washed with acetone, dried in a vacuum desiccator, and stored in a refrigerator. The purity of DO3A-Pr-ATP was checked by HPLC (R_f = 6.11, H_2O/CH_3CN, 9:5 *v*/*v*); yield 53.4%; m.p. 153 °C (dec). IR (KBr): $\tilde{\nu}$ = 3420.1 cm^{-1} ν_s(OH), 2926.5 ν_s(CH), 1476.8 δ_s(CH), 1700 ν_s(C=O), 1635.4 δ_s(NH), 1507.1 ν_s(C–O), 1087.6 ν_s(C–N), 995 δ(C–H), 719.8 δ_r(C–H), 1228.4 ν_s(P=O), 900 ν_s(P–O). MS (ESI): *m*/*z* = 894 $[M + 1]^+$ ($C_{27}H_{47}N_9O_{19}P_3$), 756 $[M - C_5H_7N_5]^+$ ($C_{22}H_{39}N_4O_{19}P_3$), 654 $[M - C_9H_{13}N_5O_3]^+$ ($C_{18}H_{33}N_4O_{16}P_3$), 348 $[M - C_{13}H_{19}N_5O_{13}P_3]^+$ ($C_{14}H_{28}N_4O_6$). ^{1}H NMR (400 MHz, D_2O, TMS): δ = 2.25 (t, 2 H, N-CH$_2$-CH_2), 3.10–3.4 (m, 12 H, NCH_2CH_2N), 3.58–3.63 (t, J = 5.5 Hz, 2 H; OCH$_2$), 4.25 (t, 1 H), 4.37 (6 H, CH_2COO), 4.49–4.65 (d, J = 7.08 Hz, 2 H), 6.073 (m, 1 H), 8.37, 8.54 (m, 2 H) ppm. ^{13}C NMR (100.34 MHz, D_2O): δ = 34.78 (*C*H$_2$), 52.99 (*C*H$_2$), 57.23 (*C*H$_2$COOH), 68.43 (O*C*H$_2$), 77.38 (O*C*H$_2$), 87.14 (C_2, C_3, and C_4 of furan ring), 174.52 (*C*OOH), 152.90–145.57 (aromatic ring) ppm. ^{31}P NMR (162 MHz, D_2O): δ = 107.35 (*P*–P–P), 119.30 (P–P–*P*), 130.20 (P–*P*–P) ppm. $C_{27}H_{46}N_9O_{19}P_3$ (893.05): calcd. C 36.31, H 5.15, N 14.10; found C 36.14, H 4.96, N 13.94.

[Gd(DO3A-Pr-ATP)(H$_2$O)$_2$] (4): Gadolinium(III) perchlorate hydrate (0.11 g, 0.25 mmol) was added to a solution of DO3A-Pr-ATP (0.22 g, 0.25 mmol) in 30 mL of water and stirred at room temperature for 36 h. Dowex cation exchange resin (10 g) was added to the reaction mixture and stirred for 15 min at room temperature and the absence of uncomplexed Gd^{III} ions was confirmed by testing the solution with xylenol orange indicator.[43] The solution was filtered to remove solid particles and the filtrate was distilled off under reduced pressure in a rotary evaporator to dryness. The white solid was recrystallized from triply distilled water. The purity of the complex was checked by HPLC (single peak, retention time 4.420 (H_2O/CH_3CN, 9:1, *v*/*v*); yield 59.09%; m.p. 175 °C (dec). IR (KBr): $\tilde{\nu}$ = 3303.4 cm^{-1} ν_s(OH), 1608.8 (COO), 1410.6 δ_s(OH), 1238.0 ν_s(P=O), 909 ν_s(P–O). MS (ESI): *m*/*z* = 1081 $[M - 2]^+$ $[Gd(C_{27}H_{41}N_9O_{19}P_3)(H_2O)_2]$, 793 $[M - C_{11}H_{17}N_5O_5]^+$ $[Gd(C_{17}H_{27}N_4O_{16}P_3)]$, 734 $[M - C_{12}H_{21}N_5O_7]^+$ $[Gd(C_{15}H_{24}N_4O_{14}P_3)]$, 578 $[M - Gd(C_{12}H_{20}N_5O_7)]^+$ ($C_{15}H_{25}N_4O_{14}P_3$), 483 $[M - Gd(C_{12}H_{20}N_5O_{11}P)]^+$ ($C_{15}H_{25}N_4O_{10}P_2$), 168 $[M - Gd(C_{19}H_{29}N_5O_{21}P_3)]^+$ ($C_8H_{16}N_4$). $Gd(C_{27}H_{43}N_9O_{19}P_3)(H_2O)_2$ (1083.05): calcd. C 29.94, H 4.33, N 11.63; found C 29.68, H 4.25, N 11.46.

Supporting Information Available (see footnote on the first page of this article): Chart S1. Gadolinium(III) chelates approved by FDA for human imaging. FAB mass spectroscopic data of 10-(3-bromopropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Figure S1), T_1 values for different concentrations of $[Gd(L1)(H_2O)_2]$ (Table S1), the plot of concentration of $[Gd(L1)(H_2O)_2]$ vs. $1/T_1$ (r_{1p} relaxivity measurements) (Figure S2), T_1 values for different concentrations of $[Gd(L1)(H_2O)_2]$ in the presence of β-cyclodextrin (Table S2), the plot of concentration of $[Gd(L1)(H_2O)_2]$ vs. $1/T_1$ in the presence of β-cyclodextrin (Figure S3), T_1 values for different concentrations of $[Gd(L1)(H_2O)_2]$ at pH 8.4 (Table S3), and a plot of concentration of $[Gd(L1)(H_2O)_2]$ vs. $1/T_1$ (r_{1p} relaxivity measurements at pH 8.4; Figure S4).

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