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Gd Complexes of DO3A-(Biphenyl-2,2′-bisamides) Conjugates as MRI Blood-Pool Contrast Agents

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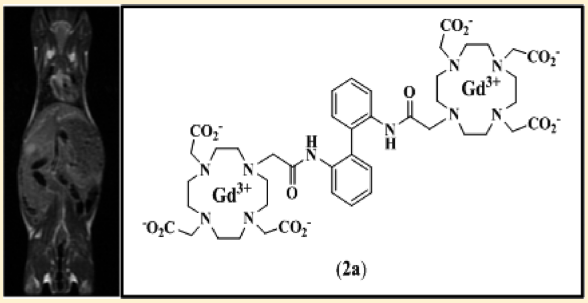
Supporting Information

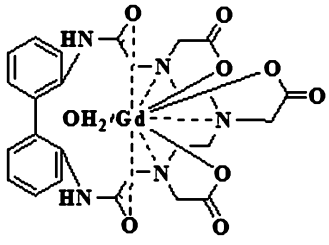
ABSTRACT: We report the synthesis of DO3A derivatives of 2,2′-diaminobiphenyl (**1a**,**b**) and their Gd complexes of the type [Gd(**1**)(H_2O)]·xH_2O (**2a**,**b**) for use as new MRI blood-pool contrast agents (BPCAs) that provide strong and prolonged vascular enhancement. Pharmacokinetic inertness of **2** compares well with that of structurally related Dotarem, a DOTA-based MRI CA currently in use. The R_1 relaxivity in water reaches 7.3 mM^{-1} s^{-1}, which is approximately twice as high as that of Dotarem (R_1 = 3.9 mM^{-1} s^{-1}). They show interaction with HSA to give association constants (K_a) in the order of two ($\sim 10^2$), revealing the existence of the blood-pool effect. The in vivo MR images of mice obtained with **2** are coherent, showing strong signal enhancement in both heart, abdominal aorta, and small vessels. Furthermore, the brain tumor is vividly enhanced for an extended period of time.



KEYWORDS: *Gd chelates, DO3A, biphenyl, MRI BPCA, brain tumor*

Magnetic resonance imaging (MRI) is a powerful technique for noninvasive diagnosis of the human anatomy, physiology, and pathophysiology on the basis of superior spatial resolution and contrast useful in providing anatomical and functional images of the human body.[1] At the clinical level, MRI techniques are mostly performed employing Gd(III) chelates (GdL) to enhance the image contrast by increasing the water proton relaxation rate in the body.[2] Despite their wide and successful applications in clinics, however, conventional Gd(III)-based low-molecular weight CAs are mostly extracellular fluid (ECF) agents exhibiting rapid extravasation from the vascular space. As a result, the time window for imaging is considerably reduced, thus limiting acquisition of high-resolution images.[3−5]

Chart 1



(3)

To overcome such limitations inherent to ECF CAs, the necessity for the development of blood-pool contrast agents (BPCAs) has risen in the expectation that they reside in the blood vessel for an extended period of time and thus are eliminated much more slowly from the circulation than their ECF counterparts. The BPCAs provide strong and prolonged vascular enhancement. The BPCAs thus far developed may be divided into three classes according to their mechanism of action: (i) the noncovalent binding of low-molecular GdL to HSA to prevent immediate leakage into the interstitial space,[6−8] (ii) systems incorporating polymers or liposomes based on an increase in the size of CAs, which slows down leakage through endothelial pores,[9−12] and (iii) systems based on nanoparticles, involving a change in the route of elimination.[13−15] The most successful approach so far is the class (i) represented by MS-325, a Gd−DTPA derivative containing a cholic acid moiety. These complexes bind strongly and reversibly to HSA, leading to not only high R_1 relaxivity at clinical field strengths but also much longer residence times in the blood as compared to ECF agents. The lipophilic component represented by aromatic

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Chart 2

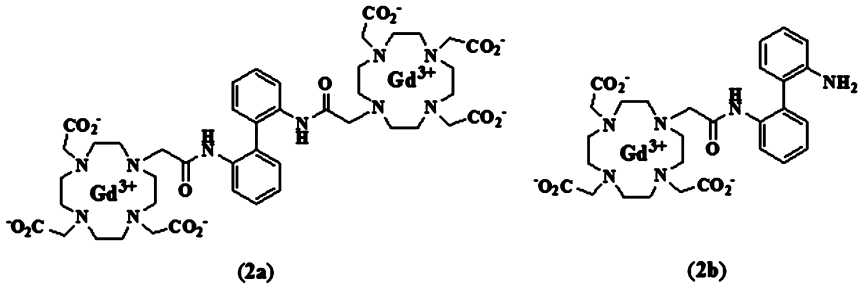

Table 1. Relaxivity Data of 2a, 2b, Dotarem, and MS-325 (64 MHz, 293 K)[a]

	R_1 (mM^{-1} s^{-1})			R_2 (mM^{-1} s^{-1})		
	water	PBS[b]	HSA[c]	water	PBS[b]	HSA[c]
2a	7.3	4.3	8.1	7.6	5.3	15.3
2b	7.0	3.4	6.8	7.6	4.4	11.3
Dotarem	3.9	3.6	5.3	4.1		7.2
MS-325[22]		5.2	19.0		5.9	37.0

[a]Concentrations are given in [Gd]. [b]PBS: pH 7.4. [c][HSA] = 0.67 mM in PBS.

moieties in the chelate is responsible for the generation of noncovalent interactions between the corresponding GdL and HSA.[16,17] The anionic phosphodiester moiety in MS-325 is also known to contribute in a synergistic manner to the noncovalent binding interaction with HSA.

We have also been involved for some time in the design and synthesis of a new series of Gd-based MRI BPCAs.[18−21] More recent work includes the synthesis of a series of macrocyclic DTPA-(biphenyl-2,2′-bisamides) (**3**) (Chart 1).

They form neutral Gd(III) complexes belonging to class (i) of BPCAs and exhibit very high blood-pool enhancement due to the presence of highly lipophilic biphenyl in the macrocyclic chelate backbone.[21] One disadvantage of these imaging probes, however, is much lower thermodynamic and kinetic stabilities

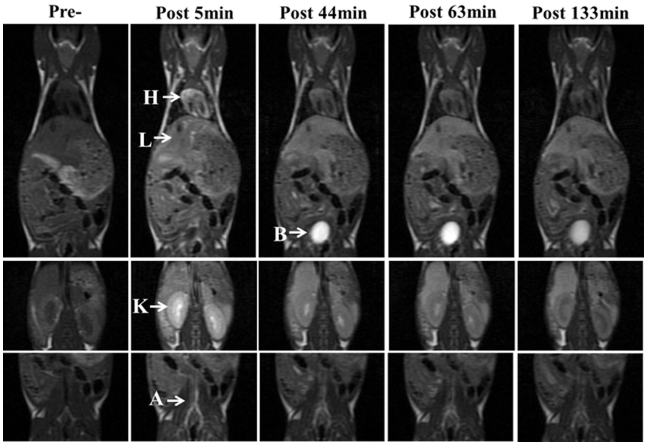

Figure 2. In vivo MR T_1 weighted spin echo (SE) coronal images of mice obtained by tail vein injection with **2a** (0.1 mmol/kg): H, heart; L, liver; K, kidney; B, bladder; and A, abdominal aorta (64 MHz).

as compared with their DO3A analogues, although those stabilities are slightly higher than those of their DTPA counterparts.[21] Motivated by these findings and in an effort to increase thermodynamic and kinetic stabilities, we have developed further two new DO3A-(biphenyl-2,2′-bisamide) chelates (**1**) and their Gd complexes (**2**) (Chart 2).

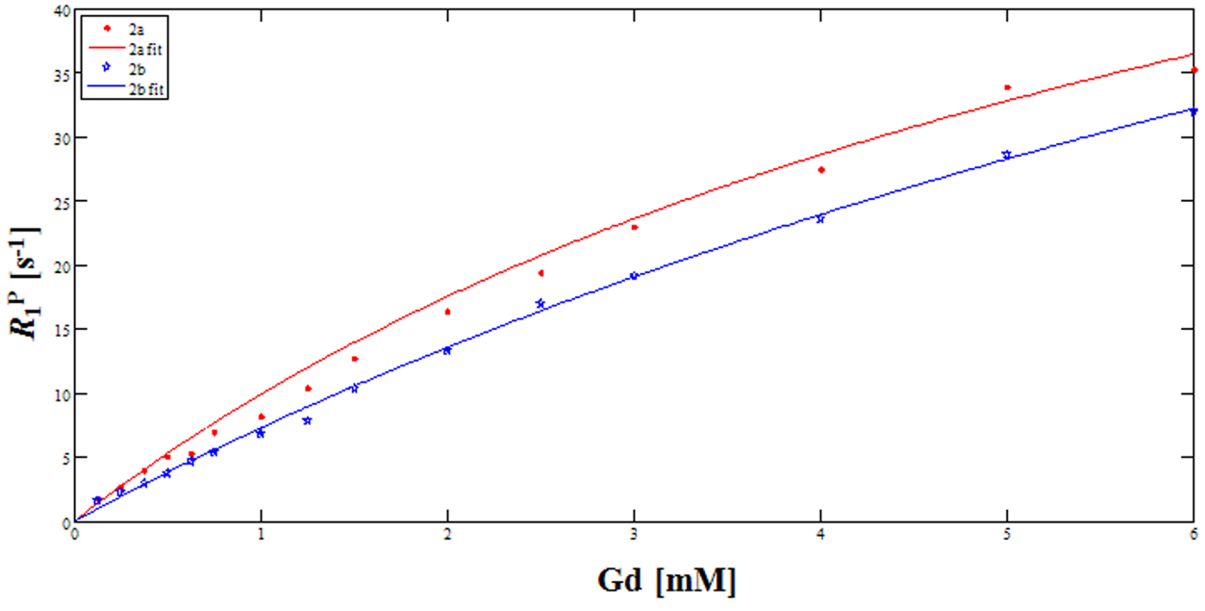

Figure 1. Proton longitudinal paramagnetic relaxation rates of **2a** and **2b** as a function of [Gd] in PBS (pH 7.4) solutions of HSA (0.67 mM) at 64 MHz and 293 K.

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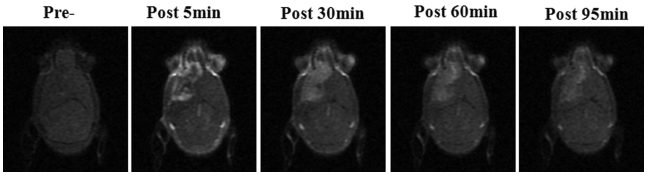

Figure 3. In vivo MR T_1 weighted SE coronal images of brain tumor (C6-glyoma cell) obtained with **2a** (0.1 mmol/kg) (64 MHz).

The synthesis of **1** and **2** is straightforward as described in Scheme S1 in the Supporting Information. The synthesis of **2** proceeds in two steps: That is, initial formation of the DO3A-diaminobiphenyl conjugate (**1**) followed by complexation with $GdCl_3$. For the preparation of the mono-Gd analogue (**2b**), it is initially required to protect one amino group in 2,2′-bis(amino)biphenyl with di-*tert*-butyl dicarbonate. The formation of **1** and **2** was confirmed by microanalytical, spectroscopic (NMR and MS), and HPLC methods (cf. ESI).

Table 1 shows the proton relaxivities, R_1 and R_2, of **2a** and **2b** along with Gd-DOTA (Dotarem) and MS-325 for comparative purposes. Measurements were made in three different media: water, PBS (pH 7.4), and a PBS solution of HSA. R_1 relaxivities of **2a** and **2b** in water are almost twice as high as that of structurally analogous Dotarem. In PBS, however, the same relaxivities drop to almost half the original values. The observations such as these may be expected when invoking possible displacement of the inner-sphere water molecule in **2** by the phosphate anion of PBS. Related observations such as a R_1 drop in the PBS solution of HSA have already been noted and rationalized in terms of the inner-sphere water molecule displaced by a donor atom from a carboxylate on the protein or the phosphate anion of PBS.[23] R_1 relaxivities of **2a** and **2b** are almost doubled in the PBS solution of HSA, demonstrating the existence of interaction with HSA, although the interactions are not as strong as the one observed with MS-325.

The proton relaxation enhancement (PRE) was measured to obtain more detailed information about such an interaction with protein. The extent of relaxation enhancement, an ε* titration, is as expected in that the relaxation rate increase is much larger in the presence of HSA, and the same rate does not increase linearly with concentration, indicating the presence of protein binding (cf. Figure S1 in the Supporting Information).[24] The association constants (K_a) characterizing the interaction between HSA and **2a** and **2b** are 2.11×10^2 and 1.07×10^2 M^{-1}, respectively. These values can be derived from the so-called M-titration, proton longitudinal relaxation rate as a function of the concentration of the paramagnetic substrate at the fixed concentration of HSA (Figure 1). Fitting of the data was carried out according to the literature method.[25] Here, it is worth noting that the MS-325, a well-known BPCA, shows a K_a value of 6.1×10^3 M^{-1}, greater by almost 1 order of magnitude than those of the **2a** and **2b**.[25] As is the case with MS-325, the role of two aromatic units via a lipophilic interaction may be invoked to rationalize the increase in the relaxivity in a PBS solution, although such increases are not so dramatic. Observations such as these in connection with the biphenyl moiety have already been noted in our previous work with the DTPA-biphenyl-2,2′-bis(amide) analogues (**3**).[21] In short, **2a** may serve as a novel BPCA in that it carries macrocyclic DOTA units, which would provide further thermodynamic and kinetic stabilities than acyclic DTPA analogues. To the best of our knowledge, **2a** is the first example of BPCA possessing DOTA as a chelate.

Figure 2 shows in vivo coronal images of mice obtained by tail vein injection with **2a**. The figure reveals clearly blood-pool enhancement in heart and abdominal aorta, and the image lasts as long as 1 h to be eventually excreted through kidney. Supportive with these observations is the biodistribution data of **2a** and the CNR profiles provided in Figures S3 and S4 in the Supporting Information, respectively. Such slow renal clearance as well as high stabilities (both thermodynamic and kinetic) may compensate for the potential risk of nephrogenic systemic fibrosis (NSF), which has often been reported with acyclic DTPA series such as Omniscan.

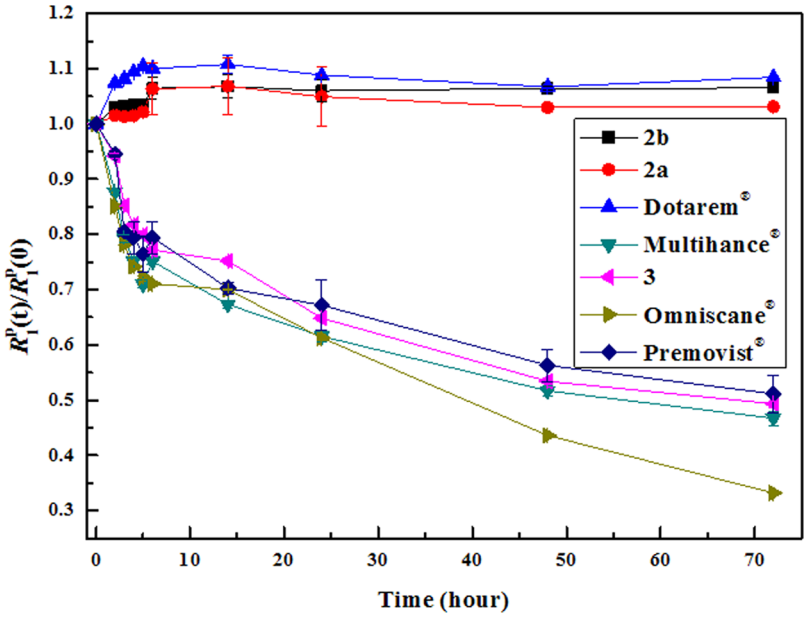

Figure 4. Evolution of longitudinal relaxation rates $R_1^P(t)/R_1^P(0)$ as a function of time for various MRI CAs {$[Gd]_0$ and $[ZnCl_2]_0$ = 2.5 mM in PBS (pH 7.4) at 128 MHz and 293 K}.

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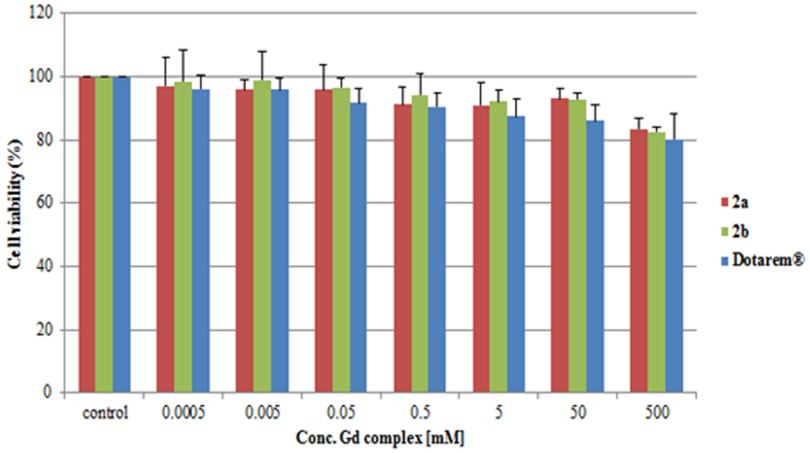

Figure 5. IMR-90 cell viability for **2** and Dotarem at various concentrations.

An additional feature of **2a** is that it is capable of enhancing the brain tumor for as long as an hour, which is rarely observed with an ordinary ECF agent such as Dotarem (cf. Figure 3).

An additional advantage of **2a**, when compared with existing MS-325, lies in the fact that the former incorporates DOTA as a chelate backbone rather than DTPA. Thus, greater enhancement in kinetic stabilities would be expected with **2** than their DTPA-based counterparts without a significant loss of the blood-pool effect. Indeed, such an expectation is demonstrated in Figure 4, which shows the plots of $R_1^P(t)/R_1^P(0)$ as a function of time for various MRI CAs. The relative value of R_1^P at any time t, $R_1^P(t)/R_1^P(0)$, is therefore a good estimator of the extent of transmetalation by zinc. Among endogenous ions, only Zn^{2+} can displace a significant amount of Gd because the concentration of the former in blood is relatively high.[26] Its evolution over time gives relevant information about the kinetics of the reaction. Quantitative transmetalation kinetic data are provided (Figure S2 and Table S1 in the Supporting Information). A dramatic increase in kinetic stability is observed with **2** as compared with the plots exhibited by DTPA-based CAs including **3**, a new type of BPCA recently developed by us.[21] In addition, the kinetic stability of **2** compares well with that of Dotarem. Little change in R_1 observed in the pH range 1−7 demonstrates that **2** is stable enough under highly acidic conditions (Figure S7 in the Supporting Information).

The cytotoxicity of the present series compares well with that of Dotarem (Figure 5). The comparison was made by employing IMR-90 human embryonic lung fibroblasts, a normal cell line that is typically used in the nontoxicity test.

In conclusion, we have put into a new entry two new Gd complexes (**2a** and **2b**) as novel MRI BPCAs. One of the most characteristic features of **2a** is that they not only reveal great signal enhancement in R_1 relaxivity in water but also exhibit a certain degree of interaction with HSA solutions with a dramatic increase in kinetic stability as well. The structural uniqueness of **2** lies in that it is neutral in charge and thus makes no resort to electrostatic interaction, supposedly one of the essential factors for the blood-pool effect.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of **2** and other detailed procedures: NMR and HPLC spectra, relaxivity measurements, relaxation enhancement of **2** with HSA, kinetic constants, biodistribution, and CNR profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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