# Gd-Complexes of DTPA-bis(amides) Functionalized by Pyridine and Picolinamide: Synthesis, Thermodynamic Stability, and Relaxivity Properties

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A series of DTPA-bis(amides) functionalized by pyridine (**1a-c**) and *N*-phenylpicolinamide) (**1d-e**) and their Gd(III)-complexes of the type [Gd(**1**)($H_2O$)]·x$H_2O$ (**2a-e**) were prepared and characterized by analytical and spectroscopic techniques. Potentiality of **2a-e** as contrast agents for magnetic resonance imaging (MRI CA) was investigated by measuring relevant physicochemical properties and relaxivities and compared with [Gd(DTPA-BMA)($H_2O$)] (DTPA-BMA = N,N''-di(methylcarbamoylmethyl)diethylenetriamine-N,N',N''-triacetate) (Omniscan®). The $R_1$ relaxivities of aqueous solutions of **2a-c** are in the range of 3.33 - 5.02 $m$M$^{-1}$sec$^{-1}$, which are comparable with those of Omniscan® ($r_1$ = 4.58 $m$M$^{-1}$sec$^{-1}$). Complexes **2d-e**, insoluble in water, exhibit relatively higher $R_1$ values (8.1 - 8.3 $m$M$^{-1}$sec$^{-1}$) in HP-β-CD solutions.

**Key Words :** MRI CA, Gd(DTPA-bisamide), Pyridine, Picolinamide, T1 relaxivity

## Introduction

Magnetic resonance imaging (MRI) provides high resolution three-dimensional images of the internal part of the body depending on the difference in the *in vivo* distribution of the water molecules. Noninvasive nature and excellent spatial resolution at the sub-millimeter level render MRI as a powerful diagnostic imaging modality. The relatively low sensitivity of MRI can be overcome by inducing additional contrast in the MR images by the introduction of a contrast agent (CA) prior to the MRI test.[1] These contrast agents catalytically shorten the relaxation time of the nearby water molecules to enhance the contrast with the background tissues in the MR images. The enhanced usage of MRI as the diagnostic imaging modality prompts the development of efficient MRI CAs.[2] The Gd(III) ion is known to possess the highest paramagnetism of all metal ions and the Gd(III) complexes incorporating macrocyclic or acyclic poly(aminocarboxylate) ligands have so far been used widely as MRI CAs. The Gd-based MRI CAs currently available for clinical uses may be classified into two types: (1) an anionic type such as bis-*N*-methylglucamine salt of [Gd(DTPA)($H_2O$)]$^{2-}$ (DTPA = diethylenetriamine-N,N,N',N'',N''-pentaacetic acid) (Magnavist®); (2) a neutral type such as [Gd(DTPA-BMA)($H_2O$)] (DTPA-BMA = N,N''bis-(methylamide) of DTPA) (Omniscan®) and [Gd(HP-DO3A)($H_2O$)] (HP-DO3A = 20-(2-hydroxypropyl) derivative of 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-1,4,7-tetraacetic acid) (Prohance®). Of the two types, the latter is preferred because of relatively low osmotic pressure in the body fluids after intravenous administration.[3,4] Yet, a great number of neutral Gd-complexes incorporating DTPA-bis(amide) ligands are known and some of them are known to exhibit poor water solubility.[5-12] It is worth noting that high relaxivity and non-cytotoxicity in addition to high water solubility are the essential criteria for an efficient MRI CA.

We have recently demonstrated that a slight modification of the ligand such as introduction of polar groups to the alkyl substituents on the amide N-atoms of DTPA-bis(amide) can lead to the formation of a series of highly water soluble Gd-complexes. To our disappointment, however, their use as MRI CAs has been frustrating due to poor relaxivity.[13] In an effort to overcome this problem and at the same time meet the three-fold requirement for an effective MRI CA mentioned above, we have developed a new strategy to further modify DTPA-bis(amide): introduction of polar alkyl substituents with high molecular weight on the amide N-atoms in such a way that the polar groups are directed as far away from the Gd(III) center as possible to minimize any interference with the water exchange equilibrium between the coordinated and the bulk water molecules. In this regard, we chose to prepare novel DTPA-bis(amide) ligands with various pyridines. Herein, we report the syntheses of DTPA-bis(amide) conjugates of pyridine (**1a-c**) and *N*-phenylpicolinamide (**1d-e**) and their Gd(III) complexes (**2a-e**). Also reported are the studies relevant to the potential application of these complexes as practical MRI CAs.

## Experimental Section

**General Remarks.** All reactions were performed under an atmosphere of nitrogen using the standard Schlenk techniques. Solvents were purified and dried using standard procedures. All reagents were purchased from commercial sources and used as received unless otherwise stated. Deionized water was used for all experiments. Hydroxypropyl-$\beta$-cyclodextrin (HP-$\beta$-CD) was obtained from TCI and used without further purification. DTPA-dianhydride was synthesized according to a literature method.[14,15] The $^1$H NMR experiments were performed on a Bruker Advance

400 or 500 Spectrometer by Korea Basic Science Institute (KBSI). Chemical shifts were given as $\delta$ values with reference to tetramethylsilane (TMS) as an internal standard. Coupling constants are in Hz. FAB-mass spectra were obtained by using a JMS-700 model (Jeol, Japan) mass spectrophotometer. Elemental analyses were performed by Center for Instrumental Analysis, KNU. T1 measurements were carried out using an inversion recovery method with a variable inversion time (TI) at 1.5 T (64 MHz). The magnetic resonance (MR) images were acquired at 35 different TI values ranging from 50 to 1750 ms. T1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. For T2 measurements the CPMG (Carr-Purcell-Meiboon-Gill) pulse sequence was adapted for multiple spin-echo measurements. Thirty four images were acquired with 34 different echo time (TE) values ranging from 10 to 1900 ms. T2 relaxation times were obtained from the non-linear least squares fit of the mean pixel values for the multiple spin-echo measurements at each echo time. Relaxivities (R1 and R2) were then calculated as an inverse of relaxation time per mM.

**Potentiometric Measurements.** Potentiometric titrations were carried out with an automatic titrator to determine the protonation constants of the amides and the stability constants of corresponding metal complexes. The auto-titrating system consists of a 798 MPT Titroprocessor, a 728 stirrer and a PT-100 combination pH electrode (Metrohm). The pH electrode was calibrated using standard buffer solutions. All calibrations and titrations were carried out under a $CO_2$-free nitrogen atmosphere in a sealed glass vessel (50 $cm^3$) thermostatted at $25 \pm 0.1^oC$ at an ionic strength of 0.10 mol/$dm^3$ KCl. The concentrations of metal ion and amide solutions were maintained at approximately 0.6 mmol/$dm^3$. A $CO_2$-free KOH solution (0.10 mol/$dm^3$) was used as a titrant to minimize the changes in ionic strength during the titration. Dioxygen and carbon dioxide were excluded from the reaction mixtures by maintaining a positive pressure of purified nitrogen in the titration cell. The electromotive force of the cell is given by $E = E'^0 + Q \log[H^+] + Ej$, and both $E'^0$ and Q were determined by titrating a solution with a known hydrogen-ion concentration at the same ionic strength, using the acid range of the titration. The liquid-junction potential ($Ej$) was found to be negligible under the experimental conditions employed.

**Computational Method.** The protonation constants of DTPA-bis(amides) ligands and the overall stability constants of different metal complexes formed in aqueous solutions were determined from the titration data using the computer program HYPERQUAD.[16] The accuracy of this method was verified by measuring the protonation and the stability constants for Ca(II), Zn(II), Cu(II), and Gd(III) complexes of $[DTPA\text{-}BMA]^{3-}$. These results were compared with literature values.[17]

**Relaxivity of Gd(III) Complexes.** The longitudinal and transverse water proton relaxation rates of *N*-phenylpicolinamide as well as pyridine containing bisamide complexes were measured at 298 K in aqueous HP-$\beta$-CD solutions and aqueous solution respectively. The relaxivity data are collected in Table 4.

***N*-(3-Nitrophenyl)pyridine-2-carboxamide:** 3-Nitroaniline (3.45 g, 25 mmol) was added into a solution of pyridine-2-carboxylic acid (3.08 g, 25 mmol) dissolved in pyridine (10.0 mL). The mixture was stirred under heating at 105 °C for 30 min. Into the hot mixture triphenyl phosphite (6.55 mL, 25 mmol) was added and stirred under heating at 105 °C for 4 h. A pale yellow colored crystalline compound separated from the reaction mixture. The reaction mixture was allowed to attain room temperature and stirred with cold methanol for 30 min. The crystalline product was isolated by filtration, washed with methanol (3×50 mL), and dried under vacuo for 4 h. Yield: 5.35 g (88%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$ = 11.18 (s, 1H), 8.99 (t, $J$ = 2.8 Hz, 1H) 8.77 (d, $J$ = 4.8 Hz, 1H), 8.30 (d, $J$ = 9.2 Hz, 1H), 8.19 (d, $J$ = 9.2 Hz, 1H), 8.09 (t, $J$ = 8.0 Hz, 1H), 7.97 (d, $J$ = 8.0 Hz, 1H), 7.70 (m, 1H), 7.65 (s, 1H) Anal. Calcd. for $C_{12}H_9N_3O_3$: C, 59.26; H, 3.73; N, 17.28. Found: C, 59.13; H, 3.67; N, 17.15.

***N*-(4-Nitrophenyl)pyridine-2-carboxamide.** Yield: 4.8 g (79%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$ = 11.22 (s, 1H), 8.77 (d, $J$ = 6.4 Hz, 1H), 8.23 (m, 5H), 8.09 (t, $J$ = 10.0 Hz, 1H), 7.71 (m, 1H). Anal. Calcd. for $C_{12}H_9N_3O_3$: C, 59.26; H, 3.73; N, 17.28. Found: C, 58.97; H, 3.59; N, 16.93.

***N*-(3-Aminophenyl)pyridine-2-carboxamide.** A suspension of *N*-(3-nitrophenyl)pyridine-2-carboxamide (2.43 g, 10 mmol) in 25 mL dry methanol was treated with Pd-C (10%) (0.2 g) and ammonium formate (3.15 g, 50 mmol) under a continuous flow of nitrogen. The resulting mixture was stirred at room temperature under nitrogen for 2 h. The progress of the reaction was monitored by TLC. The reaction mixture was filtered through celite to remove Pd-C, washed with methanol and the solvent was distilled out from the filtrate under reduced pressure. The residue was taken in dichloromethane (50 mL), washed twice with 30 mL portions of water and then with 30 mL brine, dried over anhydrous sodium sulphate. Distillation of the solvent under reduced pressure yielded *N*-(3-aminophenyl)pyridine-2-carboxamide. Yield: 1.65 g (77%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$ = 9.94 (s, 1H), 8.61 (d, $J$ = 6.0 Hz, 1H), 8.30 (d, $J$ = 10.4 Hz, 1H), 7.90 (t, $J$ = 6.0 Hz, 1H), 7.47 (m, 2H), 7.17 (t, $J$ = 10.8 Hz), 6.96 (d, $J$ = 8.8 Hz, 1H) 6.48 (d, $J$ = 8.8 Hz, 1H), Anal. Calcd. for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.34; H, 5.03; N, 19.55.

***N*-(4-Aminophenyl)pyridine-2-carboxamide.** Yield: 2.07 g (97%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$ = 9.84 (s, 1H), 8.60 (d, $J$ = 5.6 Hz, 1H), 8.28 (d, $J$ = 7.6 Hz, 1H), 7.89 (t, $J$ = 8.0 Hz, 1H), 7.56 (d, $J$ = 6.4 Hz , 2H), 7.45 (t, $J$ = 8.0 Hz, 1H) 7.66 (d, $J$ = 6.4 Hz, 2H). Anal. Calcd. for $C_{12}H_{11}N_3O$: C, 67.59; H, 5.20; N, 19.71. Found: C, 67.44; H, 5.20; N, 19.46.

**1a·$H_2O$.** To a suspension of DTPA-bis(anhydride) (0.71 g, 2 mmol) in DMF (15 mL) was added 2-aminopyridine (0.37 g, 4 mmol). The mixture was stirred at 65 °C for 6 h, after which the solvent was removed under reduced pressure, and the residue was taken up in methanol (10 mL). The solution was passed through a short silica gel column with methanol as an eluent. An off-white solid was obtained after

removal of the solvent under vacuo at 50 °C for 8 h. Yield 0.87 g (81%). $^1$H [DMSO-$d_6$, 400 MHz], $\delta$ = 10.32 (s, 2H), 8.27 (d, $J$ = 4.4 Hz, 2H), 8.07 (d, $J$ = 8.28 Hz, 2H), 7.76 (m, 2H), 7.07 (m, 2H), 3.46 (s, 2H) 3.43 (s, 8H), 2.87 (t, $J$ = 5.02 Hz, 4H), 2.85 (t, $J$ = 5.02, 4H). FABMS (m/z): Calc. for $C_{24}H_{31}N_7O_8$: 546.21 $[MH]^+$. Found: 546.07 $[MH]^+$. Anal. Calc. for $C_{24}H_{31}N_7O_8 \cdot H_2O$: C, 51.15; H, 5.90; N, 17.40. Found: C, 50.77; H, 5.93; N, 17.23.

**1b·2H$_2$O.** This compound was obtained by following the same procedure as that for **1a** by replacing 2-aminopyridine with 3-aminopyridine. Yield 0.92 g (85%). $^1$H [DMSO-$d_6$, 400 MHz], $\delta$= 10.33 (s, 2H), 8.82 (d, $J$ = 2.48 Hz, 2H), 8.22 (d, $J$ = 5.04 Hz, 2H), 8.07 (t, $J$ = 8.52 Hz, 2H), 7.27 (d, $J$ = 5.0 Hz, 2H), 3.50 (s, 2H) 3.45 (s, 8H), 3.09 (t, $J$ = 6.8 Hz, 4H), 2.98 (t, $J$ = 6.8 Hz, 4H). FABMS (m/z): Calc. for $C_{24}H_{31}N_7O_8$: 546.21 $[MH]^+$. Found: 546.25 $[MH]^+$. Anal. Calc. for $C_{24}H_{31}N_7O_8 \cdot 2H_2O$: C, 49.56; H, 6.07; N, 16.86. Found: C, 49.69; H, 6.00; N, 16.80.

**1c·H$_2$O.** This compound was obtained by following the same procedure as that for **1a** by replacing 2-aminopyridine with 4-aminopyridine. Yield 0.8 g (74%). $^1$H [DMSO-$d_6$, 400 MHz], $\delta$= 10.08 (s, 2H), 7.57 (d, $J$ = 8.52 Hz, 4H), 7.27 (d, $J$ = 8.52 Hz, 4H), 3.52 (s, 2H) 3.45 (s, 8H), 3.13 (t, $J$ = 6.8 Hz, 4H), 2.98 (t, $J$ = 6.8 Hz, 4H). FABMS (m/z): Calcd. for $C_{24}H_{31}N_7O_8$: 546.21$[MH]^+$; $C_{24}H_{31}N_{7Na}O_8$: Found: 546.22 $[MH]^+$. Anal. Calc. for $C_{24}H_{31}N_7O_8 \cdot H_2O$: C, 51.15; H, 5.90; N, 17.40. Found: C, 51.39; H, 5.97; N, 17.14.

**1d·H$_2$O.** A mixture of DTPA-bisanhydride (0.36 g, 1.0 mmol) and N-(3-aminophenyl)pyridine-2-carboxamide (0.42 g, 2.0 mmol) was suspended in 10 mL pyridine under nitrogen. The mixture was heated at 60°C for 6 h. The reaction mixture was allowed to attain the room temperature and the solvent was removed from the reaction mixture under reduced pressure. The residue was heated with 20 mL methanol. The solid product was isolated from the reaction mixture by filtration, washed thoroughly with cold methanol (3×25 mL), dried under vacuo at room temperature for 6 h. Yield: 0.74 g (98%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$= 10.51 (s, 2H), 10.39 (s, 2H), 8.73 (d, $J$ = 3.4 Hz, 2H), 8.25 (s, 2H), 8.15 (d, $J$ = 7.9 Hz, 2H), 8.05 (t, $J$ = 7.7 Hz, 2H), 7.66 (m, 2H), 7.52 (d, $J$ = 9.2 Hz, 2H), 7.45 (d, $J$ = 8.1 Hz , 2H) 7.26 (t, $J$ = 8.1 Hz, 2H ), 3.52 (s, 2H) 3.49 (s, 4H), 3.46 (s, 4H), 3.00 (t, $J$ = 6.8 Hz, 4H), 2.93 (t, $J$ = 6.8 Hz,4H). FABMS (m/z): Calcd. for $C_{38}H_{41}N_9O_{10}$: 784.30 $[MH]^+$. Found: 784.34 $(MH)^+$. Anal. Calcd. for $C_{38}H_{41}N_9O_{10} \cdot H_2O$: C, 56.92; H, 5.41; N, 15.72. Found: C, 57.19; H, 5.39; N, 15.97.

**1e·H$_2$O.** This compound was obtained by following the same procedure as that for **1e** by replacing *N*-(3-aminophenyl)pyridine-2-carboxamide with *N*-(4-aminophenyl)-pyridine-2-carboxamide. Yield: 0.62 g (80%) $^1$H [DMSO-$d_6$, 400 MHz], $\delta$= 10.53 (s, 2H), 10.12 (s, 2H), 8.72 (d, $J$ = 5.0 Hz, 2H), 8.14 (d, $J$ = 7.8 Hz, 2H), 8.05 (t, $J$ = 7.6 Hz, 2H), 7.80 (d, $J$ = 7.8 Hz, 4H), 7.66 (m, 6H), 3.49 (s, 2H) 3.41 (s, 8H), 3.03 (t, $J$ = 6.8 Hz), 2.94 (t, $J$ = 6.8, 4H). FABMS (m/z): Calcd. for $C_{38}H_{41}N_9O_{10}$: 784.30 $[MH]^+$. Found: 783.38 $[MH]^+$. Anal. Calcd. for $C_{38}H_{41}N_9O_{10} \cdot H_2O$: C, 56.92; H, 5.41; N, 15.72. Found: C, 57.16; H, 5.38; N, 15.94.

**2a·4H$_2$O.** To a solution of **1a** (0.54 g, 1 mmol) in deionized water (10 mL) was added $Gd_2O_3$ (0.18 g 0.5 mmol). The suspension was stirred for 6 h at 90°C during which time a pale yellow solution resulted. The reaction mixture was cooled to RT and passed through a Celite to remove any solid impurities. The solvent was removed and the residue was taken up in methanol (5 mL). Acetone (100 mL) was added to precipitate the product as a white solid. Yield 0.56 g (78%). FABMS (m/z): calcd for $C_{30}H_{46}GdN_5O_{12}$ 701.42 $[M-H_2O]^+$, found 701.40. Anal. Calcd for $C_{24}H_{30}GdN_7O_9 \cdot 4H_2O$: C, 36.50; H, 4.85; N, 12.41. Found: C, 36.57; H, 4.59; N, 12.28.

**2b·4H$_2$O.** This was synthesized by following the same procedure as that for **2a** by replacing **1a** with **1b**. Yield 0.52 g (72%). FABMS (m/z): calcd for $C_{30}H_{46}GdN_5O_{12}$ 701.39 $[M-H_2O]^+$, found 701.35. Anal. Calcd for $C_{24}H_{30}GdN_7O_9 \cdot 4H_2O$: C, 36.50; H, 4.85; N, 12.41. Found: C, 36.73; H, 4.64; N, 12.49.

**2c·3.5H$_2$O.** This was synthesized by following the same procedure as that for **2a** by replacing **1a** with **1c**. Yield 0.54 g (75%). FABMS (m/z): calcd for $C_{30}H_{46}GdN_5O_{12}$ 701.46 $[M-H_2O]^+$, found 701.41. Anal. Calcd for $C_{24}H_{30}GdN_7O_9 \cdot 3.5H_2O$: C, 36.92; H, 4.78; N, 12.56. Found: C, 36.46; H,4.68; N, 12.29.

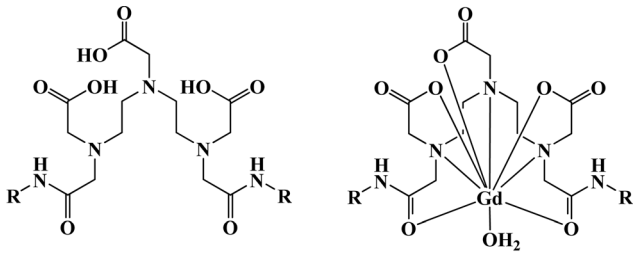
**2d·3H$_2$O.** To a solution of $GdCl_3 \cdot 6H_2O$ (0.37 g, 1 mmol) in 2.0 mL was added **1e** (0.78 g, 1 mmol) followed by 25 mL pyridine. The resulting suspension was heated for 6 h at 70°C. The solvents were evaporated from the reaction mixture and the residue was heated with 25 mL water at 70 °C for 1 h. After cooling to the room temperature the precipitated product was isolated by filtration, washed with 20 mL water, dried in vacuo for 12 h. Yield: 0.88 g (92%). FABMS (m/z): Calcd. for $C_{38}H_{40}GdN_9O_{11}$: 956.21. Found: 938.23 $[M-H_2O]^+$. Anal. Calcd. for $C_{38}H_{38}GdN_9O_{10} \cdot 3H_2O$: C, 46.01; H, 4.47; N, 12.71. Found: C, 45.95; H, 4.64; N, 12.67.

**2e·3H$_2$O.** This was synthesized by following the same procedure as that for **2e** by replacing **1d** with **1e**. Yield: 0.524 g (55%). FABMS (m/z): Calcd. for $C_{38}H_{40}GdN_9O_{11}$: 956.21. Found: 938.27 $[M-H_2O]^+$. Anal. Calcd. for $C_{38}H_{38}GdN_9O_{10} \cdot 3H_2O$: C, 46.01; H, 4.47; N, 12.71. Found: C, 45.92; H, 4.28; N, 12.71.

## Results and Discussion

**Synthesis.** Chart 1 shows a series of novel pyridine-based DTPA-bis(amides) (**1a-e**) and their Gd(III) complexes of the type $[Gd(L)(H_2O)] \cdot nH_2O$ (**2a-e**). Simple condensation of DTPA-bis-anhydride with two equivalents of aromatic amine in DMF results in the corresponding DTPA-bis-amides (**1a-e**) in almost quantitative yields.

These ligands form Gd(III) complexes of the type $[Gd(L)(H_2O)] \cdot nH_2O$ (L = **1a-e**) by simple complexation with an equimolar amount of gadolinium oxide or gadolinium chloride. All complexes were isolated as a white solid by precipitating in cold acetone from the reaction mixture. While the complexes **2a-c** are hygroscopic and were highly



| R = | $H_3$[DTPA-BMA] | [Gd(DTPA-BMA)$H_2O$] |
|---|---|---|
| $CH_3$ | **1a** | **2a** |
| 2-py | **1b** | **2b** |
| 3-py | **1c** | **2c** |
| 4-py | **1d** | **2d** |
| $C_6H_4$-3-(NHCOpy) | **1e** | **2e** |

**Chart 1**

soluble in water, **2d-e** were insoluble, limiting their study of thermodynamic properties. All the compounds were characterized by various analytical and spectroscopic techniques.

**Potonation Constants and Thermodynamic Stability Constants.** The protonation constants ($K_i^H$) of the ligands (**1a-c**), and the stability constants of their metal complexes (**2a-c**) are defined in equations (1) and (2), respectively, where $H_iL$ ($i = 1, 2,..$) is the protonated ligand, L totally deprotonated free ligand, M unhydrolyzed aqua metal ion, and ML the non-protonated and unhydrolyzed complex.

$$K_i^H = [H_iL]/[H_{i-1}L][H^+] \quad (1)$$

$$K_{ML(therm)} = [ML]/[M][L] \quad (2)$$

The protonation constants and the stability constants of their Gd(III), Ca(II), Zn(II), and Cu(II) complexes were determined by potentiometric titration, and relevant data are collected in Tables 1 and 2 along with those for DTPA and DTPA-BMA for comparative purposes. It is known that for the DTPA-bis(amide) ligands the first protonation constant ($K_1^H$) takes place at the central nitrogen atom, while the second ($K_2^H$) and the third ($K_3^H$) at the terminal amine nitrogen atoms.[12] Table 1 shows that all ligands exhibit higher protonation constants ($\log K_i^H$) and $\Sigma pK_a$ values than DTPA-BMA. It is probable that the presence of pyridine nitrogen (**1a-c**) in the amide side-arms seems to render the protonation of the amine nitrogen(s) facile by some cooperative interaction to exhibit higher protonation constants. When the comparison is made among the present series, **1b** shows the highest values and even higher values than the parent DTPA. In general, substitution of the acetate groups on the terminal amine nitrogen atoms of DTPA reduces its basicity, as reflected in the lower $\log K_i^H$ and $\Sigma pK_a$ values of corresponding DTPA-bis-amide ligands. High basicity of **1a-c** will surely lead to high thermodynamic stability of their metal complexes.

Table 2 shows the thermodynamic stability constants for the complexes of Ca(II), Zn(II), and Cu(II) complexes. A direct potentiometric method can not be applied for the measurement of the stability constants of **2a-c** since they are formed at low pH. Instead, they were determined by employing the method of ligand-ligand competition potentiometric titration between EDTA and **1a-c** for Gd(III) ion.[18-20] Thermodynamic stability of Gd(III) complexes measures the tendency of dissociation of the complexes in solution to generate free Gd(III) ion, which shows acute cytotoxicity in the physiological system. The table shows quite expectedly that high basicity of **1a-c** leads to high thermodynamic stability of their metal complexes as compared with that of corresponding metal complexes of DTPA-BMA, with **1b** exhibiting the highest thermodynamic stability for the same reason described above.

**Table 1**. Protonation constants ($\log K_i^H$) of **1a-c** ($I = 0.10$ mol/dm$^3$)

| Equilibrium | log $K$ (25°C, $\mu = 0.10$ M (KCl)) | | | | |
|---|---|---|---|---|---|
| | **1a** | **1b** | **1c** | **DTPABMA**[a] | **DTPA**[b] |
| [HL]/[L][H] | 9.55 | 10.93 | 9.71 | 9.37 | 10.49 |
| [$H_2$L]/[HL][H] | 6.08 | 6.07 | 6.53 | 4.38 | 8.60 |
| [$H_3$L]/[$H_2$L][H] | 4.24 | 4.52 | 4.73 | 3.31 | 4.28 |
| [$H_4$L]/[$H_3$L][H] | 3.92 | 4.39 | 4.02 | – | 2.64 |
| $\Sigma pK_a$ | 23.79 | 25.91 | 24.99 | 17.06 | 26.01 |

[a]Data obtained from ref. 17. [b]Data obtained from ref. 25.

**Table 2**. Stability Constants and selectivity constants of Gd(III), Ca(II), Zn(II), and Cu(II) complexes of **1a-c**

| Equilibrium | log $K$ (25 °C, $\mu = 0.10$ M (KCl)) | | | | |
|---|---|---|---|---|---|
| | **1a** | **1b** | **1c** | **DTPABMA**[a] | **DTPA**[b] |
| [GdL]/[Gd][L] | 18.85 | 19.92 | 19.59 | 16.85 | 22.46 |
| [GdHL]/[GdL][H] | 4.75 | 4.99 | 8.47 | – | 2.39 |
| {log$K_{GdL}$(pH 7.4)} | 16.67 | 16.37 | 17.23 | 14.84 | 18.14 |
| [CaL]/[Ca][L] | 7.55 | 8.91 | 8.13 | 7.17 | 10.75 |
| [CaHL]/[CaL][H] | 4.87 | 4.25 | 7.24 | 4.45 | 6.11 |
| {log$K_{CaL}$(pH 7.4)} | 5.37 | 5.36 | 5.77 | 5.11 | 6.43 |
| [ZnL]/[Zn][L] | 11.25 | 11.46 | 11.40 | 12.04 | 18.70 |
| [ZnHL]/[ZnL][H] | 4.85 | 5.37 | 7.11 | 4.04 | 5.60 |
| {log$K_{ZnL}$(pH 7.4)} | 9.07 | 7.91 | 9.04 | 10.02 | 14.38 |
| [CuL]/[Cu][L] | 11.47 | 12.07 | 11.95 | 13.03 | 21.38 |
| [CuHL]/[CuL][H] | 6.55 | 6.87 | 7.42 | 3.36 | 4.81 |
| {log$K_{CuL}$(pH 7.4)} | 9.29 | 8.52 | 9.59 | 11.06 | 17.06 |
| [log$K_{sel}$(Gd/Ca)] | 11.30 | 11.01 | 11.46 | 9.68 | 11.71 |
| [log$K_{sel}$(Gd/Zn)] | 7.60 | 8.46 | 8.19 | 4.81 | 3.76 |
| [log$K_{sel}$(Gd/Cu)] | 7.38 | 7.85 | 9.82 | 3.82 | 1.08 |
| log$K'_{sel}$ | 11.89 | 12.73 | 12.46 | 9.03 | 7.04 |

[a]Data obtained from ref. 17. [b]Data obtained from ref. 25.

**Conditional Stability Constants and Selectivity Constants.** The conditional stability constant ($K'_{Sel}$) can be calculated by considering all equilibria present at physiological pH.[17] The $K'_{Sel}$ have been shown to correlate with the experimental $LD_{50}$ value.[21] The thermodynamic stability constant reveals the extent of complexation at the given pH. The stability constants of the complexes have been evaluated using equation (3), where $K_n^H$ ($n = 1, 2, 3$, etc) are the stepwise protonation constants of the ligands.

$$K_{ML(cond)} = K_{ML(therm)}(1 + K_1^H[H] + K_1^H K_2^H[H]^2 + K_1^H K_2^H K_3^H[H]^3 + ...)^{-1}. \quad (3)$$

**Table 3**. The pM[a] values of the complexes of Gd(III), Ca(II), Zn(II), and Cu(II) of **1a-c** at pH 7.4

| Equilibrium | log *K* (25 °C, $\mu$ = 0.10 M (KCl)) | | | | |
|---|---|---|---|---|---|
| | **1a** | **1b** | **1c** | **DTPABMA**[b] | **DTPA**[c] |
| pGd | 15.67 | 15.37 | 16.23 | 13.88 | 17.14 |
| pCa | 4.37 | 4.36 | 4.77 | 4.19 | 5.45 |
| pZn | 8.08 | 6.91 | 10.04 | 9.06 | 13.39 |
| pCu | 8.30 | 7.52 | 8.59 | 10.05 | 16.06 |

[a]pM = $-\log[M^{n+}]_{free}$ at pH 7.4; $[M^{n+}]_{total}$ = 1 $\mu$mol/dm$^3$; $[L]_{total}$ = 1.1 $\mu$mol/dm$^3$. [b]Data obtained from ref. 17. [c]Data obtained from ref. 25.

**Table 4**. Relaxivity data for Gd(III) complexes, **2a-e**

| CA | T1 (*msec*) | R1 ($m$M$^{-1}$ sec$^{-1}$) | T2 (*msec*) | R2 ($m$M$^{-1}$ sec$^{-1}$) |
|---|---|---|---|---|
| **2a**[a] | 198.64 ± 1.46 | 5.02 ± 0.37 | 184.48 ± 1.95 | 5.40 ± 0.57 |
| **2b**[a] | 249.43 ± 1.93 | 4.06 ± 0.31 | 224.66 ± 1.98 | 4.50 ± 0.32 |
| **2c**[a] | 300.80 ± 2.82 | 3.33 ± 0.23 | 272.31 ± 8.06 | 3.70 ± 0.41 |
| **2d**[b] | 123.61 ± 1.64 | 8.10 ± 0.03 | 141.33 ± 6.31 | 7.30 ± 0.08 |
| **2e**[b] | 130.15 ± 4.44 | 8.30 ± 0.03 | 121.59 ± 9.8 | 8.28 ± 0.06 |
| Omniscan®[a] | 204.08 ± 1.38 | 4.90 ± 0.16 | 294.12 ± 1.54 | 3.40 ± 0.52 |

[a]Relaxivity in water. [b]Relaxivity in 50 mM HP-$\beta$-CD solution

Table 2 demonstrates that **1a-c** reveal higher log$K'_{Sel}$ values than DTPA-BMA and DTPA suggesting that their Gd(III) complexes are to exhibit little cytotoxicity. Larger the pM value, the higher the affinity of the ligand for the metal ion under the given condition.[22-24] Table 3 shows that **1a-c** exhibit higher pM values with Gd(III) than with Ca(II), Zn(II), or Cu(II); indication is that the Gd(III) complexes of 1a-c are stable enough to avoid any interference by other endogenous metal ions. In addition to the pM value, the conditional stability constant ($K'_{Sel}$) has also to be taken into consideration under the physiological condition. This is because a Gd(III) complex injected as an MRI CA into the physiological system through the blood pool competes not only with endogenous metal ions such as Ca(II), Zn(II), and Cu(II) but also with proton at the physiological pH.[21]

**Relaxivity of Gd(III) Complexes.** The relaxivities, $R_1$ and $R_2$, for the complexes **2a-c** in water and **2d-e** in HP-$\beta$-CD solution measured at 298 K and 1.5 T were collected in Table 4. Figure 1 represents the relaxation time (T1) maps and the corresponding relaxivity (R1) maps on **2a-e** at different concentrations.The relaxivities of **2a-c** are in the order **2a** > **2b** > **2c** which are comparable with Omniscan. The position of the pyridine nitrogen seems to have a profound effect on the rate of water exchange although the exact rationalization has yet to be found out. On the other hand, the aqueous HP-$\beta$-CD solutions of **2d-e** show much enhanced relaxivities. For the complex **2d**, the $R_1$ and the $R_2$ values were found to be 8.1 and 7.3 mM$^{-1}$sec$^{-1}$, respectively, while for the complex **2e**, the $R_1$ and the $R_2$ values were found to be 8.3 and 8.28 mM$^{-1}$sec$^{-1}$, respectively. It is well established that in the presence of $\beta$-CD and HP-$\beta$-CD, the noncovalent interaction of the hydrophobic substituents on the ligand of a Gd-based contrast agent with the hydrophobic cavity of the $\beta$-CD rings leads to an enhancement of the water proton relaxivity. The enhanced relaxivity has been attributed to the slowing down of the tumbling motion of the Gd(III) complex due to the aforementioned noncovalent interaction, which leads to the formation of "host-guest" inclusion complex.[19]

Also, the substitution on the benzene ring in **2d-e** seems to influence the relaxivity. In **2e**, the presence of the 1,4-disubstituted benzene rings between the pyridyl ring and DTPA moiety in the ligand, directs the pyridyl nitrogen far away from the Gd(III) centre and thereby avoiding any kind of interference with the water exchange equilibrium between the coordinated water molecule and the bulk water.[15] However, in **2d**, the presence of the 1,3-disubstituted benzene rings between the pyridyl ring and DTPA moiety in the ligand, directs the pyridyl nitrogen towards the Gd(III) centre leading to interference with the water exchange equilibrium causing a slight decrease in the water proton relaxivity.[20]

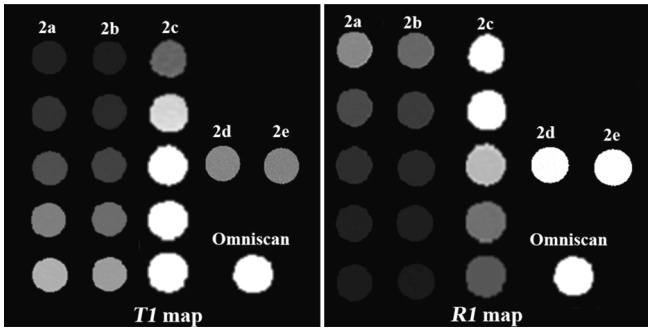



**Figure 1**. The relaxation time (T1) maps and the corresponding relaxivity (R1) maps on **2a-e** at different concentrations, 4, 2, 1, 0.5 and 0.25 mmol (from top to bottom) and Omniscan is at 1 mmol concentration level.

## Conclusions

The synthesis and characterization of DTPA-bis(amide) conjugates of pyridine (**1a-c**) and N-phenylpicolinamide (**1d-e**), and their Gd(III) complexes (**2a-e**) are described. The potentiality of these gadolinium complexes as practical MRI CAs was investigated by measuring their $R_1$ and $R_2$ relaxivities and their relevant physicochemical properties. Complexes **2a-c** show comparable relaxivities with Omniscan® in aqueous solution. But, on the other hand, the Gd-complexes **2d-e** exhibit much higher $R_1$ relaxivity than Omniscan®, reaching upto 8.30 mM$^{-1}$s$^{-1}$, in HP-$\beta$-CD solution. Thermodynamic stability constants, conditional stability constants, and the pM values for **2a-c** demonstrate higher stability of these complexes under physiological conditions. Further studies are directed towards establishing these complexes as practical MRI CAs.

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