Gd-complexes of DTPA-bis(amide) Conjugates of Phosphonated Tranexamic Esters as MRI Contrast Agents

Mehul A. Patel, Hee-Kyung Kim,[‡] Gang-Ho Lee,[†] Yongmin Chang,[‡,§,*] and Tae-Jeong Kim[*]

Department of Applied Chemistry, Kyungpook National University, Daegu 702-701, Korea. [*]E-mail: tjkim@knu.ac.kr
[†]*Department of Chemistry, Kyungpook National University, Daegu 702-701, Korea*
[‡]*Department of Medical & Biological Engineering, Kyungpook National University, Daegu 702-701, Korea*
[§]*Department of Diagnostic Radiology & Molecular Medicine, Kyungpook National University, Daegu 702-701, Korea*
[*]*E-mail: ychang@knu.ac.kr*

The syntheses of DTPA-bis(amide) conjugates of phosphonated cyclohexane moieties (**5a-d**) and their Gd(III) complexes of the type [Gd(L)(H$_2$O)]·nH$_2$O (**6a-d**; L = **5a-d**) are described. All new compounds have been characterized by microanalysis and spectroscopic techniques. High r_1 relaxivities of aqueous solutions of **6a-d** are observed to be in the range of 10.7-18.3 mM^{-1}sec^{-1}, which compare much better than that of Omniscan[®] (r_1 = 3.90 mM^{-1}sec^{-1}).

Key Words : DTPA-bis(amide), MRI CAs, Phosphonates, Tranexamate, Gd-complexes

Introduction

Magnetic resonance imaging (MRI) possesses an unsurpassed potential for the exploitation of intrinsic tissue and structural differences visualized as image contrast by using a wide variety of pulse sequences. Typically, MRI contrast depends on endogenous differences in water content and relaxation time in the tissue of interest. The specificity and sensitivity of MR imaging can be further enhanced by the utilization of contrast agents (CAs) based on paramagnetic gadolinium chelates (GdL). These low molecular weight compounds distribute rapidly in blood and the extracellular fluid space and enhance image contrast by shortening the longitudinal relaxation time (T_1) and transverse relaxation time (T_2).[1-4] The Gd metal ion has a high magnetic moment and long electronic relaxation times, making it most suitable for this purpose due to strong dipolar interactions with water nuclei protons. However, the introduction of paramagnetic ions directly *in vivo* is limited by inherent toxicity. Therefore, acyclic and macrocyclic polyaminocarboxylates are used as strong ligands to sequester the material and prevent toxicity. These ligands occupy eight of the nine available coordination sites in Gd forming kinetically and thermodynamically stable complexes.[5] The ninth coordination site remains available for fast exchange of coordinated water molecules with those surrounding the complex to transmit the paramagnetic relaxation effect to the bulk solvent. In literature, a number of neutral GdL are reported with DTPA-bis(amide) ligands as a backbone. Yet some of them are known to possess low relaxivity as well as poor water solubility.[6-13]

The mechanism of proton relaxation in GdL is represented by dipolar interaction between the metal ion and proximate water molecules. A second coordination shell may provide an efficient mechanism for paramagnetic relaxation leading to a strong enhancement of the relaxivity of the complexes along with outer sphere water molecules. The selection of group on the ligand capable of forming hydrogen bond may promote the formation of a strong interaction, increase the number of water molecules in the second hydration shell, and also decrease their average distance from the paramagnetic metal center. These possibilities have been explored by considering GdL bearing phosphinates or phosphonates. In fact, it is to be noted that Gd-complexes of DTPA-bis(amide) bearing phosphonate(s) for use as MRI CAs are rare. In one case, an increase in relaxivity has been reported when a phosphonate is attached to the central nitrogen atom of the diethylenetriamine backbone.[14]

Motivated by these findings we have prepared a series of Gd-complexes of DTPA-bis(amide) conjugates of phosphonated tranexamic acid and esters for use as a new class of MRI CAs. In this regard, we have recently found that conjugation of tranexamic moiety with DTPA-bis(amide) significantly enhances r_1-relaxivity of corresponding Gd-complexes.[15] The aim of the present work is therefore twofold: Firstly, to increase solubility of GdL in water due to the presence of hydrophilic phosphonate(s); and secondly to increase r_1-relaxivity of GdL by introduction of the tranexamate- phosphonate conjugate as ligand in GdL.

Experimental Section

Generals. All reactions were carried out under an atmosphere of dinitrogen using the standard Schlenk techniques. Solvents were purified and dried using standard procedures. Deionized water was used for all experiments. All reagents were purchased from Aldrich and used as received unless otherwise stated. ^{1}H NMR spectra were recorded on Bruker Advance 400 MHz Spectrometre by Korean Basic Science Institute (KBSI). Elemental analysis was performed by

Centre for Instrumental Analysis, KNU. MALDI-TOF mass spectra were recorded on MALDI-TOF Mass Spectrometer and FAB-Mass spectra on JMS-700 mass spectrophotometer. The Chemical shift (δ) is reported in ppm using chloroform-*d* or DMSO-d_6 as a solvent and calibrated standard solvent signal. DTPA-bis(anhydride) and trans-methyl-4-(aminomethyl) cyclohexane carboxylate hydrochloride (**1**) were prepared according to the literature methods.[15]

Relaxivity Measurements. T_1 measurements were carried out using an inversion recovery method with a variable inversion time (T_1) at 1.5 T (64 MHz). The MR images were acquired at 35 different T_1 values ranging from 50 to 1750 msec. T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each T_1 value. For T_2 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 msec. T_2 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 (r_1 and r_2) were then calculated as an inverse of relaxation time per mM. The determined relaxation times (T_1 and T_2) and relaxivities (r_1 and r_2) were finally image-processed to give the relaxation time map and relaxivity map, respectively.

Synthesis 2: To a suspension of **1** (2.07 g, 10.0 mmol) in chloroform (50 mL) were added successively triethylamine (1.39 mL, 10 mmol) and di-*tert*-butyl dicarbonate (2.18 g, 10 mmol). The mixture was stirred at RT for 18 h. After completion of the reaction, the mixture was poured into water and the aqueous layer extracted with chloroform (3 × 20 mL). The combined organic layer was dried over anhydrous MgSO₄, filtered, concentrated, and purified by column chromatography (silica gel, 20% ethyl acetate in hexane) to give a white solid. Yield: 2.3 g (85%). IR (cm⁻¹) 3410, 2980, 1735, 1685, 1520, 1410, 1250. ¹H-NMR (CDCl₃), δ 0.90-1.01 (2H, m, CH₂ at C₂), 1.39-1.44 (12H, m, CH at C₁, CH₂ at C₆ and 3 x CH₃ merged), 1.79-2.02 (4H, m, CH₂ at C₃ and C₅), 2.23 (1H, m, CH at C₄), 2.97 (2H, m, -C̲H̲₂-NH₂), 3.66 (3H, s, OCH₃), 4.69 (1H, br s, NH). FABMS (*m/z*) Calc. for C₁₄H₂₅NO₄: 271.35 [MH]⁺. Found: 272.40. Anal. Calc. for C₁₄H₂₅NO₄: C, 61.97; H, 9.29; N, 5.16. Found: C, 61.98; H, 9.34; N, 5.13. To a suspension of LiAlH₄ (0.42 g, 11 mmol) in diethyl ether (10 mL) at 0 °C was added a solution of the white solid (2.71 g, 10 mmol) obtained above. The resulting suspension was stirred for 10 h at RT, after which time the reaction mixture was cooled in an ice bath, quenched with cold water, and filtered through Celite. The filtrate was dried over anhydrous MgSO₄, filtered, and the solvent removed under vacuum to give a white solid. This was taken up in hexane (100 mL), and the resulting suspension stirred for 1 h, filtered, washed with hexane, and dried to give **2** as a white solid. Yield: 2.08 g (78%). IR (cm⁻¹) 3400, 2900, 1695, 1510, 1435, 1255. ¹H-NMR (CDCl₃), δ 0.87-1.00 (4H, m, CH₂ at C₂ and C₆), 1.42 (1H,

m, CH at C₁), 1.44 (9H, s, 3 x CH₃), 1.77-1.98 (5H, m, CH₂ at C₃ and C₅ and CH at C₄ merged), 2.97 (2H, m, -C̲H̲₂-NH₂), 3.43 (2H, d, C̲H̲₂-OH, *J* = 4 *Hz*), 4.67 (1H, br s, NH). FABMS (*m/z*) Calc. for C₁₃H₂₅NO₃: 243.34 40 [MH]⁺. Found 244.40. Anal. Calc. for C₁₃H₂₅NO₃: C, 64.16; H, 10.36; N, 5.76. Found: C, 64.39; H, 10.41; N, 5.66.

3a: To a stirring solution of **2** (1.21 gm, 5 mmol) and triethylamine (0.84 mL, 6 mmol) in diethyl ether at −40 °C was added drop wise phosphorous oxychloride (0.72 mL, 5 mmol). Stirring was continued for 4 h, and the temperature was raised to 0 °C to stir additional 3 h. Water and triethylamine were added drop wise successively, and the reaction mixture left for another 10 h at 0 °C. The Et₃N·HCl salt was removed by filtration and the organic filtrate dried over anhydrous MgSO₄. The product was isolated as a white solid after removal of the solvent under vacuum. Yield: 0.97 g (60%). IR (cm⁻¹) 3450, 2985, 1650, 1415, 1230, 1115. ¹H-NMR (CDCl₃), δ 0.90-1.03 (4H, m, CH₂ at C₂ and C₆), 1.41-1.43 (1H, m, CH at C₁), 1.45 (9H, s, 3 x CH₃), 1.60-1.62 (1H, m, CH at C₄), 1.77-1.84 (4H, m, CH₂ at C₃ and C₅), 2.97 (2H, m, -C̲H̲₂-NH₂), 3.81-3.85 (2H, m, -C̲H̲₂O-), 4.72 (1H, br s, NH), 9.66 (2H, br s, OH). FABMS (*m/z*) Calc. for C₁₃H₂₆NO₆P: 323.32 [MH]⁺. Found: 324.30. Anal. Calc. for C₁₃H₂₆NO₆P: C, 48.29; H, 8.11; N, 4.33. Found: C, 48.30; H, 8.09; N, 4.35.

3b: The title compound was prepared according to the procedure described for the synthesis of **3a** by simply replacing phosphorus oxychloride with dimethyl chlorophosphate. The product was obtained as colorless oil. Yield: 85%. IR (cm⁻¹) 3445, 2960, 1665, 1410, 1250, 1105. ¹H-NMR (CDCl₃) δ 0.91-1.02 (4H, m, CH₂ at C₂ and C₆), 1.40-1.41 (1H, m, CH at C₁), 1.43 (9H, s, 3 x CH₃), 1.61-1.64 (1H, m, CH at C₄), 1.78-1.85 (4H, m, CH₂ at C₃ and C₅), 2.97 (2H, m, -C̲H̲₂-NH₂), 3.75 and 3.78 (6H, two s, 2 x OCH₃), 3.82-3.87 (2H, m, -C̲H̲₂O-), 4.28 (1H, br s, NH). FABMS (*m/z*) Calc. for C₁₅H₃₀NO₆P: 351.28 [MH]⁺. Found 352.50. Anal. Calc. for C₁₅H₃₀NO₆P: C, 51.27; H, 8.61; N, 3.99. Found: C, 51.30; H, 8.59; N, 4.01.

3c: The title compound was prepared according to the procedure described for the synthesis of **3a** by simply replacing phosphorus oxychloride with dieethyl chlorophosphate. The product was obtained as colorless oil. Yield: 85%. IR (cm⁻¹) 3440, 2895, 1615, 1420, 1225, 1110. ¹H-NMR (CDCl₃) δ 0.90-1.05 (4H, m, CH₂ at C₂ and C₆), 1.30-1.52 (16H, m, 3 x -OCH₂C̲H̲₃, CH at C₁, 3 x CH₃ merged), 1.61-1.63 (1H, m, CH at C₄), 1.76-1.84 (4H, m, CH₂ at C₃ and C₅), 2.96 (2H, m, -C̲H̲₂-NH₂), 3.81-3.86 (2H, m, -C̲H̲₂O-), 4.08 (4H, q, -OC̲H̲₂CH₃), 4.81 (1H, br s, NH). FABMS (*m/z*) Calc. for C₁₇H₃₄NO₆P: 379.43 [MH]⁺. Found: 379.30. Anal. Calc. for C₁₇H₃₄NO₆P: C, 53.81; H, 9.03; N, 3.69. Found: C, 53.83; H, 9.00; N, 3.72.

3d: To a stirred solution of **2** (1.22 g, 5 mmol), 1,2,4-triazole (2.76 g, 40 mmol), and triethylamine (5.57 mL, 40 mmol) in pyridine (25 mL) at 0 °C was added drop wise phenyl dichlorophosphate (2.97 mL, 20 mmol). After the addition was complete, the reaction mixture was allowed to warm to RT. Stirring was continued for 3 h, and the volume

1024 *Bull. Korean Chem. Soc.* **2011**, Vol. 32, No. 3

Mehul A. Patel et al.

of the reaction mixture was reduced to dryness under reduced pressure. The oily residue was taken up in a mixture of chloroform (50 mL) and water (60 mL), transferred into a separatory funnel, and the organic layer extracted. The volume of the extract was reduced to a minimum amount to be chromatographed on silica-gel (eluent:ethyl acetate containing growing amounts of methanol (from 1 to 10%). The product was obtained as amorphous powder after usual workups. Yield: 1.41 g (71%). IR (cm^{-1}) 3455, 2910, 1645, 1420, 1235, 1125. ^{1}H-NMR (CDCl$_3$) δ 0.84-1.00 (4H, m, CH$_2$ at C$_2$ and C$_6$), 1.41-1.42 (1H, m, CH at C$_1$), 1.44 (9H, s, 3 x CH$_3$), 1.58-1.60 (1H, m, CH at C$_4$), 1.72-1.79 (4H, m, CH$_2$ at C$_3$ and C$_5$), 2.95 (2H, m, -CH$_2$-NH$_2$), 3.99-4.03 (2H, m, -CH$_2$O-), 4.67 (1H, br s, NH), 7.15-7.36 (5H, m, Ar-H). FABMS (*m/z*) Calc. for C$_{19}$H$_{30}$NO$_6$P: 399.42 [M]$^+$. Found: 399.40. Anal. Calc. for C$_{19}$H$_{30}$NO$_6$P: C, 57.13; H, 7.57; N, 3.51. Found: C, 57.15; H, 7.55; N, 3.50.

Typical Procedure for the Synthesis of 4. To a solution of **3** (1.50 g) in ethyl acetate (20 mL) was added hydrogen chloride in ethyl acetate (4 M, 20 mL). The stirring was continued at RT for 6 h by which time the reaction was complete as confirmed by a TLC spot test. All volatiles were removed with gentle heating under high vacuum, and the residue re-dissolved in ethyl acetate (30 mL) to be transferred to a separatory funnel, where it was washed successively with potassium carbonate (saturated solution) and brine. The organic phase was separated, dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*, leaving pure amine, which was used directly without further purification.

4a: Colorless oil. Yield: 78%. IR (cm^{-1}) 3450, 2990, 1655, 1420, 1250, 1115. ^{1}H-NMR (DMSO-d_6), δ = 0.83-0.96 (4H, m, CH$_2$ at C$_2$ and C$_6$), 1.47-1.50 (2H, m, CH at C$_1$ and C$_4$), 1.64-1.72 (4H, m, CH$_2$ at C$_3$ and C$_5$), 2.70 (2H, t, -CH$_2$-NH$_2$, *J* = 8 *Hz*), 3.60-3.66 (2H, m, -CH$_2$O-), 8.15 (2H, br s, NH$_2$). FABMS (*m/z*) Calc. for C$_8$H$_{18}$NO$_4$P: 223.21. Found: 224.30 [MH]$^+$. Anal. Calc. for C$_8$H$_{18}$NO$_4$P: C, 43.05; H, 8.13; N, 6.28. Found: C, 43.08; H, 8.11; N, 6.30.

4b: Colorless oil. Yield: 80%. IR (cm^{-1}) 3460, 2975, 1650, 1415, 1230, 1105. ^{1}H-NMR (DMSO-d_6), δ 0.91-0.98 (4H, m, CH$_2$ at C$_2$ and C$_6$), 1.51-1.53 (2H, m, CH at C$_1$ and C$_4$), 1.73-1.82 (4H, m, CH$_2$ at C$_3$ and C$_5$), 2.63 (2H, t, -CH$_2$-NH$_2$, *J* = 8 *Hz*), 3.64 and 3.67 (6H, two s, 2 x OCH$_3$), 3.77-3.80 (2H, m, -CH$_2$O-), 8.01 (2H, br s, NH$_2$). FABMS (*m/z*) Calc. for C$_{10}$H$_{22}$NO$_4$P: 251.26. Found: 252.40 [MH]$^+$. Anal. Calc. for C$_{10}$H$_{22}$NO$_4$P: C, 47.80; H, 8.83; N, 5.57. Found: C, 47.83; H, 8.80; N, 5.56.

4c: Colorless oil. Yield: 75%. IR (cm^{-1}) 3445, 2960, 1640, 1415, 1235, 1120. ^{1}H-NMR (DMSO-d_6), δ 1.00-1.32 (4H, m, CH$_2$ at C$_2$ and C$_6$), 1.34 (6H, t, -OCH$_2$CH$_3$, *J* = 4 *Hz*), 1.64-1.76 (2H, m, CH at C$_1$ and C$_4$), 1.83-1.98 (4H, m, CH$_2$ at C$_3$ and C$_5$), 2.86 (2H, t, -CH$_2$-NH$_2$, *J* = 8 *Hz*), 3.81-3.86 (2H, m, -CH$_2$O-), 4.11 (4H, q, -OCH$_2$CH$_3$, *J* = 4 *Hz*), 8.24 (2H, br s, NH$_2$). FABMS (*m/z*) Calc. for C$_{12}$H$_{26}$NO$_3$P: 279.31. Found: 280.34 [M+H]$^+$. Anal. Calc. for C$_{12}$H$_{26}$NO$_3$P: C, 51.60; H, 9.38; N, 5.01. Found: C, 51.64; H, 9.36; N, 4.99.

4d: Colorless oil. Yield: 79%. IR (cm^{-1}) 3435, 2980, 1665, 1405, 1230, 1125. ^{1}H-NMR (DMSO-d_6), δ 0.91-1.02 (4H, m, CH$_2$ at C$_2$ and C$_6$), 1.56-1.58 (2H, m, CH at C$_1$ and C$_4$), 1.75-1.86 (4H, m, CH$_2$ at C$_3$ and C$_5$), 2.67 (2H, t, -CH$_2$-NH$_2$, *J* = 8 *Hz*), 3.85 (2H, m, -CH$_2$O-), 7.20-7.45 (5H, m, Ar-H) 8.15 (2H, br s, NH$_2$). FABMS (*m/z*) Calc. for C$_{14}$H$_{22}$NO$_2$P: 299.30. Found: 300.60 [MH]$^+$. Anal. Calc. for C$_{14}$H$_{22}$NO$_4$P: C, 56.18; H, 7.41; N, 4.68. Found: C, 56.20; H, 7.40; N, 4.66.

Typical Procedure for the Synthesis of 5. To a stirred suspension of **4** (20 mmol) in DMF (20 mL) was added DTPA-bis(anhydride) (10 mmol). The mixture was stirred at 80 °C for 6 h, after which the solvent was removed, and the residue dissolved in methanol (2 mL). The solution was passed through a short column of silica gel (60 meshes) with methanol as an eluent. The residue obtained after removal of the solvent from the eluate was triturated with a mixture of acetone and diethyl ether (30:70 v/v, 150 mL). The solid product was isolated by filtration, washed with acetone (3 x 30 mL), and dried *in vacuo*.

5a: Yield 85%. IR (cm^{-1}) 3410, 2955, 1745, 1685, 1400, 1250, 1100. ^{1}H-NMR (DMSO-d_6), δ 0.71-1.01 (8H, m, H11), 1.35 (2H, m, H13), 1.49 (2H, m, H10), 1.65-1.85 (8H, m, H12), 2.98-3.30 (8H, m, H9 and H14 merged), 3.35-3.42 (8H, m, H5 and H7 merged), 3.45-3.65 (12H, m, H2, H3/H4 and 2 x -COOH merged), 8.01 (2H, br s, -NH-). FABMS (*m/z*) Calc. for C$_{30}$H$_{55}$N$_5$O$_{16}$P$_2$: 803.73. Found: 804.30 [MH]$^+$. Anal. Calc. for C$_{30}$H$_{55}$N$_5$O$_{16}$P$_2$·8H$_2$O: C, 38.01; H, 7.55; N, 7.39. Found: C, 38.05; H, 7.59; N, 7.35.

5b: Yield 78%. IR (cm^{-1}) 3450, 2900, 1735, 1675, 1410, 1235, 1110. ^{1}H-NMR (DMSO-d_6), δ 0.78-1.05 (8H, m, H11), 1.29-1.53 (4H, m, H13 and H10 merged), 1.67-1.83 (8H, m, H12), 2.93 (4H, m, H9), 3.04 (4H, m, H14), 3.27 (4H, s, H5), 3.47-3.75 (18H, m, H7, 4 x -OCH$_3$ and H2 merged), 3.72 (8H, m, H3/H4), 8.10 (2H, br s, -NH-). FABMS (*m/z*) Calc. for C$_{34}$H$_{63}$N$_5$O$_{16}$P$_2$: 859.84. Found: 859.50 [M]$^+$. Anal. Calc. for C$_{34}$H$_{63}$N$_5$O$_{16}$P$_2$·4H$_2$O: C, 43.82; H, 7.68; N, 7.52. Found: C, 43.85; H, 7.71; N, 7.56.

5c: Yield 70%. IR (cm^{-1}) 3420, 2905, 1750, 1680, 1405, 1240, 1110. ^{1}H-NMR (DMSO-d_6), δ 0.79-0.99 (8H, m, H11), 1.23 (12H, t, -OCH$_2$CH$_3$, *J* = 8 *Hz*), 1.35 (2H, m, H13), 1.46 (2H, m, H10), 1.59-1.85 (8H, m, H12), 2.94 (4H, m, H9), 3.22 (4H, m, H14), 3.38-3.58 (10H, m, H5, H7 and H2 merged), 3.81 (8H, m, H3/H4), 3.99 (8H, q, -OCH$_2$CH$_3$, *J* = 8 *Hz*), 7.95 (1H, S, -COOH), 8.07 (2H, br s, NH). FABMS (*m/z*) Calc. for C$_{38}$H$_{71}$N$_5$O$_{16}$P$_2$: 915.94. Found: 916.70 [MH]$^+$. Anal. Calc. for C$_{38}$H$_{71}$N$_5$O$_{16}$P$_2$·5H$_2$O: C, 45.37; H, 8.12; N, 6.96. Found: C, 45.39; H, 8.09; N, 6.99.

5d: Yield 80%. IR (cm^{-1}) 3415, 2950, 1745, 1675, 1450, 1255, 1120. ^{1}H-NMR (DMSO-d_6), δ 0.82-1.22 (8H, m, H11), 1.39 (2H, m, H13), 1.55 (2H, m, H10), 1.70-1.91 (8H, m, H12), 2.98 (4H, m, H9), 3.11 (4H, m, H14), 3.41 (4H, s, H5), 3.45-3.69 (7H, s, H7, H2 and –COOH merged), 3.82 (8H, m, H3/H4), 4.38 (2H, br s, 2 x -COOH), 7.15-7.52 (10H, m, Ar-H), 8.06 (2H, br s, -NH-), 8.22 and 8.40 (2H, s, -OH). FABMS (*m/z*) Calc. for C$_{42}$H$_{63}$N$_5$O$_{16}$P$_2$: 955.92. Found: 956.90 [MH]$^+$. Anal. Calc. for C$_{42}$H$_{63}$N$_5$O$_{16}$P$_2$·7H$_2$O:

C, 46.62; H, 7.17; N, 6.47. Found: C, 46.64; H, 7.20; N, 6.45.

Typical Procedure for the Synthesis of 6. To a solution of **5** (0.73 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 100 °C during which time the color of the solution turn into pale yellow. The reaction mixture was cooled RT and passed through a Celite to remove any solid impurities. The solvent was removed and the residue taken up in methanol (5 mL). Acetone (100 mL) was added to precipitate the product as a white solid.

6a: Yield 88%. IR (cm^{-1}) 3400, 2925, 1645, 1555, 1410, 1220, 1095. FABMS (*m/z*) Calc. for $C_{30}H_{54}GdN_5O_{17}P_2$: 975.97. Found: 959.22 [M+H–H_2O]$^+$. Anal. Calc. for $C_{30}H_{54}GdN_5O_{17}P_2 \cdot 10H_2O$: C, 31.17; H, 6.45; N, 6.06. Found: C, 31.20; H, 6.41; N, 6.10.

6b: Yield 89%. IR (cm^{-1}) 3435, 2910, 1640, 1575, 1420, 1225, 1100. FABMS (*m/z*) Calc. for $C_{34}H_{62}GdN_5O_{17}P_2$: 1032.08. Found: 1014.20 [M–H_2O]$^+$. Anal. Calc. for $C_{34}H_{62}GdN_5O_{17}P_2 \cdot 7H_2O$: C, 35.26; H, 6.61; N, 6.05. Found: C, 35.28; H, 6.60; N, 6.08.

6c: Yield 92%. IR (cm^{-1}) 3430, 2915, 1660, 1565, 1400, 1250, 1115. FABMS (*m/z*) Calc. for $C_{38}H_{70}GdN_5O_{17}P_2$: 1088.18. Found: 1071.00 [M+H–H_2O]$^+$. Anal. Calc. for $C_{38}H_{70}GdN_5O_{17}P_2 \cdot 3H_2O$: C, 39.96; H, 6.71; N, 6.13. Found: C, 39.94; H, 6.75; N, 6.08.

6d: Yield 82%. IR (cm^{-1}) 3420, 2960, 1650, 1580, 1400, 1245, 1115. FABMS (*m/z*) Calc. for $C_{42}H_{62}GdN_5O_{17}P_2$: 1128.16. Found: 1110.30 [M–H_2O]$^+$. Anal. Calc. for $C_{42}H_{62}GdN_5O_{17}P_2 \cdot 4H_2O$: C, 42.03; H, 5.88; N, 5.84. Found: C, 42.07; H, 5.91; N, 5.86.

Results and Discussion

Synthesis and Characterization. Scheme 1 illustrates a synthetic route leading to the formation of a series of DTPA-bis(amide) conjugates of phosphorylated tranexamates (**5a-d**) and their Gd(III)-complexes of the type [Gd(L)(H_2O)]·xH_2O (**6a-d**; L = **5a-d**). The synthesis initially requires the protection of amine in **1** by di-*tert*-butyl dicarbonate. This was followed by reduction of the ester group with lithium aluminum hydride. The resulting alcohol **2** was then converted to various phosphorylated derivatives **3a-d**. Subsequent deprotection of the amine group in **3** with hydrochloric acid resulted in **4**. The reaction of **4** with DTPA-bis(anhydride) yielded corresponding condensation products, DTPA-bis(amides) (**5**). They form Gd(III) complexes of the type [Gd(**5**)(H_2O)]·xH_2O (**6**) by simple complexation with an equimolar amount of Gd_2O_3 in water. All complexes were isolated as white, hygroscopic solids by repeated precipitation with cold diethyl ether from the reaction mixture.

The formation of **5** and **6** was confirmed by microanalysis and spectroscopic techniques such as ^{1}H and ^{13}C NMR, HRFAB- and MALDI-TOF mass spectrometry. IR spectro-
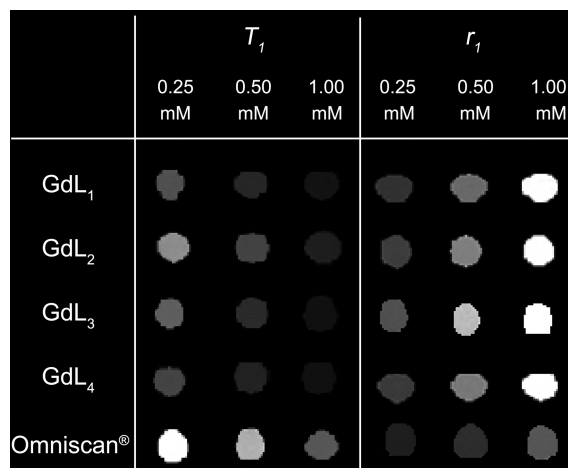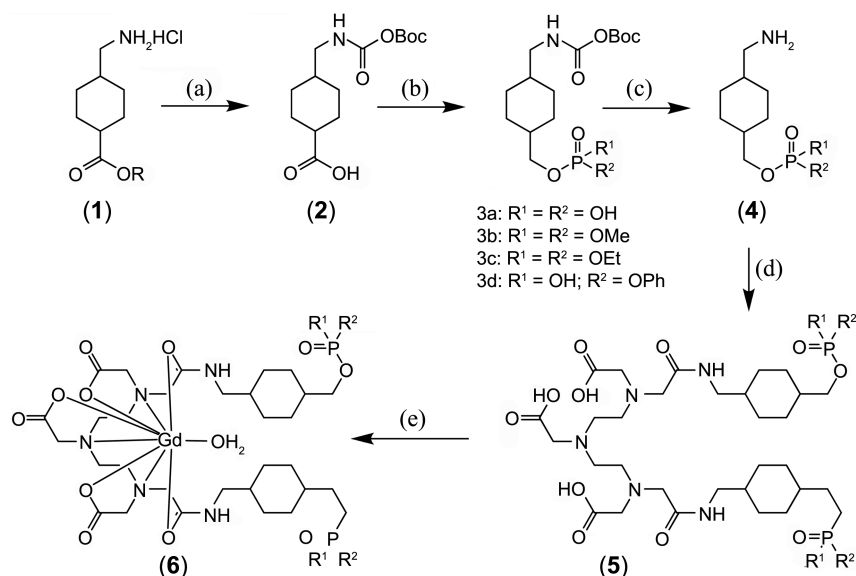

Figure 1. The T_1 and r_1 maps for **6a-d** and Omniscan®.

Scheme 1. ^{a}Key: (a) (i) (Boc)$_2$O, TEA, CHCl$_3$, RT; (ii) LAH, Et$_2$O, 0 °C to RT; (b) **3a**: POCl$_3$ TEA, −40 to 0 °C; **3b-d**: PO(OR)$_2$Cl, TEA, 0 °C to RT; (c) HCl (4 M), EtOAc, RT; (d) DTPA-bis(anhydride), DMF, 90 °C; (e) Gd$_2$O$_3$, water, 100 °C.

Table 1. T_1, r_1, T_2 and r_2 values for **6** and Omniscan®

Sample	T_1 ms	r_1 mM^{-1}s^{-1}	T_2 ms	r_2 mM^{-1}s^{-1}
6a	93.7 ± 3.1	10.7 ± 0.3	85.9 ± 2.4	11.6 ± 0.3
6b	80.5 ± 0.6	12.4 ± 0.1	75.2 ± 1.1	13.3 ± 0.1
6c	54.7 ± 1.7	18.3 ± 0.5	53.3 ± 1.0	19.1 ± 0.3
6d	80.5 ± 2.0	12.4 ± 0.3	72.2 ± 1.4	13.8 ± 0.2
Omniscan®	258.7 ± 1.7	3.9 ± 0.2	225.4 ± 9.7	4.4 ± 0.1

scopy is also informative in that the presence of carbonyl groups can be confirmed by a pair of intense carbonyl stretching bands assignable to the amide carbonyl (NHC=O) and carboxylic carbonyl (C=O) groups in the range 1675-1685 cm^{-1} and 1735-1750 cm^{-1}, respectively. The stretching frequency for the amide carbonyl, due to its participation in coordination with Gd(III), appears at longer wavelengths.

Relaxivity Studies. Figure 1 shows the relaxation time (T_1) and relaxivity (r_1) maps on **6** along with those for Omniscan® for comparative purposes. The phantom images were obtained with 1 mM solution of complexes. Table 1 summarizes the relaxation times (T_1 and T_2) and relaxivities (r_1 and r_2) for **6a-d** and Omniscan®. The most significant feature of Table 1 is that all new complexes exhibit significantly higher r_1 values than that of Omniscan®, the highest value reaching with **6c** up to almost five times as high as that of Omniscan®. Parallel observations in the r_1 increase have also been made earlier by us with non-phosphorylated analogues such as Gd-complexes of DTPA-(bisamide) conjugates of tranexamates.[15] When the comparison is made between the present series (**6**) and their non-phosphorylated counterparts, the presence of the phosphonate in the ligand seems conspicuous as the former series exhibit even higher r_1 value than the latter. In this connection, it is worth noting a report that an additional increase in the relaxivity is obtained by virtue of the presence of two water molecules in the second coordination sphere and which are probably bound to the phosphonate or phosphinate moiety through hydrogen bonds.[14]

References

1. Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901.
2. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
3. Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19.
4. Toth, E.; Helm, L.; Merbach, A. E. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; Wiley: 2001; p 45.
5. Moreau, J.; Guillon, E.; Pierrard, J.-C.; Rimbault, P. M.; Aplincourt, M. *Chem. Eur. J.* **2004**, *10*, 5218.
6. Bligh, S. W. A.; Chowdhury, A. H. M. S.; McPartlin, M.; Scowen, I. J.; Bulman, R. A. *Polyhedron* **1995**, *14*, 567.
7. Lammers, H.; Maton, F.; Pubanz, D.; van Laren, M. W.; van Bekkum, H. A.; Merbach, E. R.; Muller, N.; Peters, J. A. *Inorg. Chem.* **1997**, *36*, 2527.
8. Aime, S.; Benetollo, F.; Bombieri, G.; Colla, S.; Fasano, M.; Paoletti, S. *Inorg. Chim. Acta* **1997**, *254*, 63.
9. Zhao, X.; Zhuo, R.; Lu, Z.; Liu, W. *Polyhedron* **1997**, *16*, 2755.
10. Lammers, H.; van der Heijden, A. M.; van Bekkum, H.; Geraldes, C. F. G. C.; Peters, J. A. *Inorg. Chim. Acta* **1998**, *268*, 249.
11. Bligh, S. W. A.; Chowdhury, A. H. M. S.; Kennedy, D.; Luchinat, C.; Parigi, G. *Mag. Reson. Med.* **1999**, *41*, 767.
12. Wang, Y.-M.; Wang, Y.-J.; Sheu, R.-S.; Liu, G.-C.; Lin, W.-C.; Liao, J.-H. *Polyhedron* **1999**, *18*, 1147.
13. Geraldes, C. F. G. C.; Urbano, A. M.; Alpoim, M. C.; Sherry, A. D.; Kuan, K.-T.; Rajagopalan, R.; Maton, F.; Muller, R. N. *Magn. Reson. Imaging* **1995**, *13*, 401.
14. Kotek, J.; Lebdskova, P.; Hermann, P.; van der Elst, L.; Muller, R.N.; Geraldes, C. F. G. C.; Maschmeyer, T.; Lukes, I.; Peters, J. A. *Chem. Eur. J.* **2003**, *9*, 5899.
15. (a) Dutta, S.; Park, J.-A.; Jung, J.-C.; Chang, Y.; Kim, T.-J. *Dalton Trans* **2008**, 2199. (b) Dutta, S.; Kim, S.-K.; Patel, D. B.; Kim, T.-J.; Chang, Y. *Polyhedron* **2007**, *26*, 3799. (c) Dutta, S.; Kim, S.-K.; Lee, E. J.; Kim, T.-J.; Kang, D.-S.; Chang, Y.; Kang, S. O.; Han, W.-S. *Bull. Korean Chem. Soc.* **2006**, *27*, 1038.
16. Botta, M. *Eur. J. Inorg. Chem.* **2000**, 399.