Inorganic Chemistry

ARTICLE

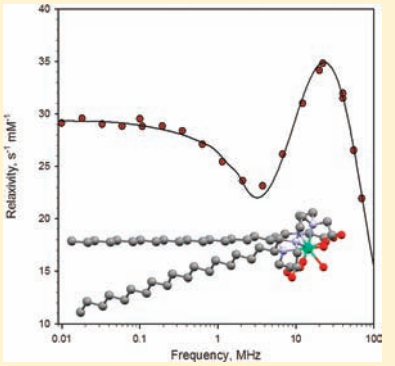
pubs.acs.org/IC

Gadolinium DOTA Chelates Featuring Alkyne Groups Directly Grafted on the Tetraaza Macrocyclic Ring: Synthesis, Relaxation Properties, "Click" Reaction, and High-Relaxivity Micelles

Christian Vanasschen, Nouri Bouslimani, David Thonon, and Jean F. Desreux*

Coordination and Radiochemistry, University of Liège, Sart Tilman B16, B4000 Liège, Belgium

ABSTRACT: This paper reports on the synthesis and relaxivity properties of tetraacetic DOTA-type chelating agents featuring one or two alkyne groups directly grafted on the tetraaza macrocyclic ring and available for "click" reactions with azide-bearing substrates. The racemic DOTAma ligand bearing one alkyne group was obtained by a bisaminal template route. The same approach was used to prepare ligand DOTAda substituted by two alkyne groups located on two adjacent carbon atoms. The *S,S* enantiomer of DOTAda was also prepared by a "crab-like" condensation. This ligand is the first example of a DOTA derivative featuring two reactive functions adjacent to each other on the macrocyclic ring. A triacetic monoalkyne ligand (DO3ma) was also synthesized for comparison purposes. NMR studies indicate that the Yb(III) chelates of DOTAma and DOTAda adopt two conformations in solutions in which the tetraaza ring is rigidified. The hydration state of the Eu(III) chelates was determined by luminescence spectroscopy, and the water exchange time of the Gd(III) complexes was measured by ^{17}O NMR. Ring substitution accelerates the water exchange. These data were used to interpret nuclear magnetic relaxation dispersion curves of the Gd(III) chelates. Two long aliphatic chains have been added to DOTAda by a "click" procedure to form the $(C18)_2$DOTAda ligand. The corresponding Gd(III) complex forms micelles of unusually high relaxivity presumably because of the close proximity of the aliphatic chains on the macrocyclic ring that ensures a rigid double anchoring into the micelles.



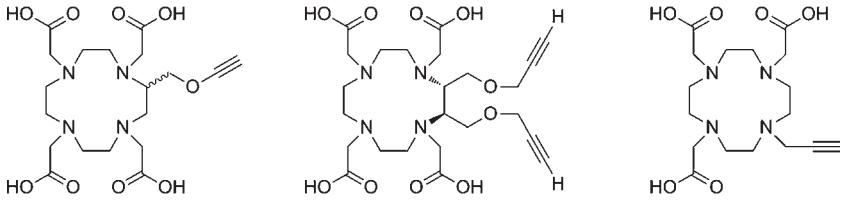
1. INTRODUCTION

Magnetic resonance imaging (MRI) using gadolinium-containing contrast agents is now a commonly used high-resolution clinical imaging modality.[1] Gadolinium complexes drastically increase the longitudinal relaxation rate $1/T_1$ of the water protons, thus generating bright spots in T_1-weighted MRI images. The efficacy of a contrast agent depends on several parameters, most importantly the water exchange time τ_m, the electronic relaxation time τ_s, and the rotational correlation time τ_r. High increases in relaxation rate per millimolar of metal ion (or relaxivity) can be obtained by slowing down the tumbling rate of a contrast agent thanks to a coupling with a macromolecule or nanoparticle. Moreover, attachment of a large number of paramagnetic complexes to one macromolecular substrate increases the local Gd(III) concentration and leads to a better sensitivity. The chelates should be rigidly bonded to a high molecular weight entity and also exhibit fast water exchange rates. A variety of macromolecular contrast agents have been obtained by grafting paramagnetic DTPA- or DOTA-type chelates through amide or thiourea bonds to polymers[2−4] and dendrimers[5−9] or through noncovalent interaction of lipophilic Gd(III) complexes with proteins such as albumin.[10−12]

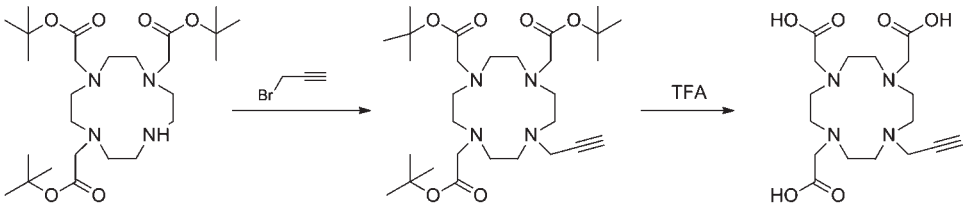
The excellent yield reported for the copper-catalyzed 1,3-dipolar Huisgen cycloaddition ("click" reaction) between an azide and a terminal alkyne[13,14] as well as its fast rate, robustness, and bio-orthogonality[15] make this transformation an excellent candidate for coupling high payloads of Gd(III) complexes to a particular macromolecule. "Click" chemistry has already been used for grafting Gd-DOTA monoamide chelates to viral particles[16] or β-cyclodextrins.[17] However, it is well known that complexes bearing amide chelating units are less stable and exhibit slower water exchange rates that are detrimental to the relaxivity.[18,19] Gd(III) diethylenetriaminetetraacetate (GdDTTA) has also been coupled to β-cyclodextrin, but this chelate is again less stable than the clinically approved Gd−DOTA and Gd−DTPA.[20] In the present work, we report on the synthesis of two novel alkyne-bearing macrocyclic ligands as well as on the physical properties of their lanthanide chelates that are of interest for a relaxometric evaluation. Even if the synthesis of macrocyclic chelates is more difficult and time consuming than the preparation of their noncyclic analogues, we preferred to prepare DOTA derivatives because of their well-recognized kinetic inertness and thermodynamic stability.[21] Only one "clickable" tetraacetic DOTA ligand has been reported so far.[22] This ligand features an alkyne group on one of its acetate arms, and it was clicked to an azide-bearing somatostatin analogue. We chose to graft one or two alkyne moieties directly on the tetraaza ring of DOTA (compounds **1** and **2**) to avoid steric crowding, Scheme 1. We also investigated the Gd(III) chelate of the less strongly complexing triacetic derivative **3** that has already been prepared recently by Lowe et al.[23,24] and which is included here for comparison purposes. The GdDO3ma complex is an easily obtainable metal complex that can be used for testing various reaction conditions in a "click" condensation.

Received: May 24, 2011
Published: August 22, 2011

Scheme 1



Scheme 2. Synthesis of DO3ma, 3



Despite the added difficulties entailed by the synthesis of DOTAda, **2**, we wanted to obtain this ligand because it features two alkyne substituents on the same ethylenediamine unit. These closely spaced reacting groups could be particularly interesting for firmly anchoring a Gd(III) complex on a macromolecule or grafting on chemically modified proteins[25] thanks to two attachment points. In the present paper, ligand **2** is converted into an amphiphilic gadolinium complex that self-assembles into paramagnetic micelles of very high relaxivities.

2. EXPERIMENTAL SECTION

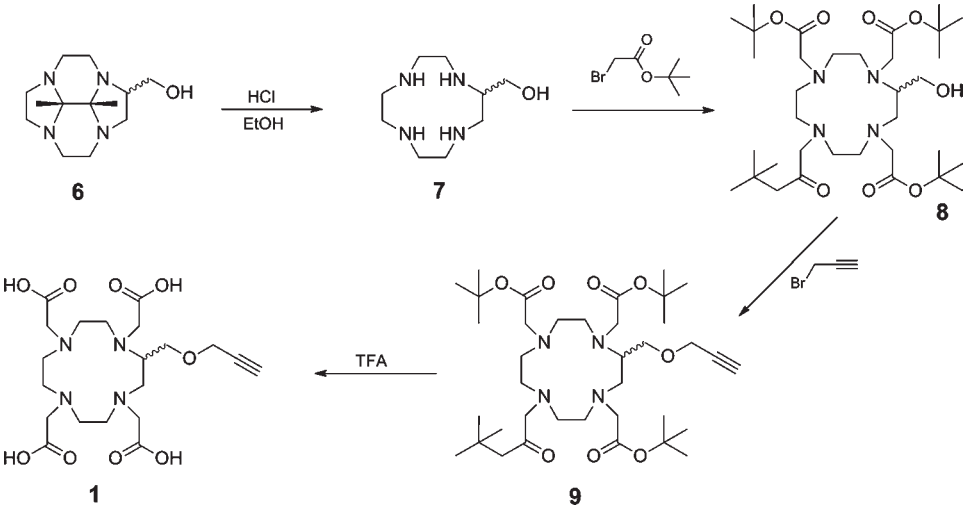
^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer, and nuclear magnetic relaxation dispersion (NMRD) data were collected from 0.01 to 70 MHz on an upgraded Stelar Spinmaster FFC2000 relaxometer (Stelar, Mede, PV, Italy) to which a Bruker 2T permanent magnet had been connected. All experimental T_1 relaxation times were corrected for the T_1 value of pure water. ^{17}O NMR measurements were carried out on 4.6−7.6 mM (pH = 5.7) solutions of Gd(III) complexes. ^{17}O-Enriched water (10% $H_2{}^{17}O$, Cortecnet, Paris) was added to solutions of metal complexes in order to reach a 2% ^{17}O enrichment. The ^{17}O transverse relaxation times were measured using the standard Carr−Purcell−Meiboom−Gill spin−echo pulse sequence on a Bruker Avance AM250 spectrometer. Luminescence lifetimes were measured on a Photon Technology International (PTI, Birmingham, NJ) spectrometer equipped with a GL-3300 nitrogen laser using a PBBO dye to excite the Eu^{3+} ion at 394 nm. The recorded luminescence decay curves (emission monitored at 592 nm) were fitted to a monoexponential function using the PTI Time Master software. Quantitative interpretations of the ^{17}O, NMRD, and luminescence data were performed with the Scientist software program (Micromath, USA). Electrospray mass spectra were obtained on Fisons VG platform (ESI-MS) and Bruker Daltonics MicrOTOF (TOF-ESI-MS) spectrometers. Melting or decomposition points were measured on an Electrothermal IA 9100 apparatus.

Synthesis of 1-(Prop-2-ynyl)-4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 5 (Scheme 2). (*t*-Bu)$_3$DO3A, **4** (496 mg, 0.96 mmol, CheMatech, USA), was dissolved in acetonitrile (20 mL) and placed in a 100 mL round-bottomed flask under argon atmosphere. Potassium carbonate (293 mg, 2.1 mmol) and propargyl bromide (126 μL of an 80% solution in toluene, 1.16 mmol) were added to the solution. After 4 h at room temperature, potassium carbonate was filtered off and washed with dichloromethane (100 mL). The filtrate was washed with water (20 mL) and brine (20 mL) and dried over $MgSO_4$. After solvent evaporation under reduced pressure, a yellowish oil remained (504 mg, yield 95%). MS (ESI+): m/z 553 $(M + H)^+$, 575 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{29}H_{52}N_4NaO_6$ $(M + Na)^+$ 575.3779, found 575.3788. ^{1}H NMR ($CDCl_3$) δ: 3.31 (CH≡CCH_2, s, 2H), 3.16 (m, 6H), 2.8−2.65 (m, 12H), 2.58 (m, 4H), 2.05 (CH≡CCH$_2$, s, 1H), 1.33 and 1.34 (2s, 27H) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.5 and 172.5 (C=O), 82.0, 81.8, 79.1 (CH≡CCH$_2$), 72.5 (CH≡CCH$_2$), 56.5, 55.7, 52.1 (2C), 51.9, 51.7, 43.0 (CH≡CCH$_2$), 27.7, 27.8 ppm.

Synthesis of 1-(Prop-2-ynyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, 3, DO3ma (Scheme 2). Triester **5** (647 mg, 1.2 mmol) was dissolved in trifluoroacetic acid (10 mL), and the solution was stirred at room temperature overnight. Evaporation of the solvent under reduced pressure yielded a viscous brown oil. This residue was dissolved in water (3 mL, pH ≈ 2) and loaded onto a Dowex 50x2-200 (H^+ form) cation-exchange resin. The column was washed with water (500 mL), and the sought product was recovered by elution with 0.5 M aqueous NH_3. The eluate was evaporated under reduced pressure, and the remaining light brown oil was dissolved in decarbonated water (3 mL). The pH was brought to 10 with aqueous NH_3, and the solution was transferred onto a Dowex (OH^- form) 1x2-200 anion-exchange resin. The column was washed with decarbonated water (500 mL), and the sought product was eluted using a formic acid gradient (0.005, 0.04, 0.08, 0.15, 0.3, 0.5, 1 M). The fractions containing the desired product (HCOOH 0.08 and 0.15 M) were combined and evaporated to dryness in vacuo. Repetitive evaporations after addition of water were performed in order to remove formic acid. Finally, 293 mg of compound **3** was obtained as a golden oil. Yield 65%. MS (ESI+): m/z 385 $(M + H)^+$. ESI-TOF-MS: calcd for $C_{17}H_{29}N_4O_6$ $(M + H)^+$ 385.2082, found 385.2078. ^{1}H NMR (D_2O) δ: 3.27 (CH≡CCH_2, s, 2H), 3.23 (m, 6H), 2.9−2.6 (m, 12H), 2.56 (m, 4H), 2.12 (CH≡CCH$_2$, s, 1H) ppm. ^{13}C NMR (D_2O) δ: 177.7 and 170.3 (C=O), 77.8

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

Scheme 3. Synthesis of DOTAma, 1



($CH{\equiv}CCH_2$), 75.5 ($CH{\equiv}CCH_2$), 57.2, 55.9, 51.9, 51.0, 48.1, 47.5, 42.3 ($CH{\equiv}CCH_2$) ppm.

Synthesis of 2-Hydroxymethyl-1,4,7,10-tetraazacyclododecane, 7 (Scheme 3). The synthesis of **7** was performed according to the experimental procedure described by Boschetti et al.[26] with some modifications. Triethylenetetramine (6 g, 41 mmol) was dissolved in acetonitrile (180 mL) and cooled in an ice bath. A solution of butanedione (3.53 g, 41 mmol in 18 mL of acetonitrile) was added dropwise over 30 min, and the ice bath was removed after 4 h of vigorous stirring. The yellow reaction mixture was heated to 60 °C, and potassium carbonate (28.4 g, 205 mmol) and a solution of 2,3-dibromo-1-propanol (9.02 g, 41 mmol in 18 mL of acetonitrile) were added. After 48 h at this temperature, potassium carbonate was removed by filtration over Celite, and the filtrate was evaporated to dryness in vacuo. The residue was transferred onto an alumina column and eluted with a dichloromethane−methanol 97:3 mixture. After removal of the solvents under reduced pressure, the red oil was dissolved in acetonitrile (20 mL) and left at −20 °C for 24 h. The formed crystals were collected by filtration and washed with cold acetonitrile, hexane, and diethyl ether. This afforded a purple solid, which was found to contain a mixture of the *R,S,R* and *R,S,S* isomers of compound **6**, which could not be separated. The two diastereoisomers were then dissolved in a mixture of ethanol (540 mL) and concentrated HCl (180 mL). After heating this solution at 60 °C for 48 h, the precipitate was filtered off and washed with cold ethanol and diethyl ether to obtain 1.83 g of compound **7** as a beige powder. Yield 22.3%. MS (ESI+): m/z 203 $(M + H)^+$. ESI-TOF-MS: calcd for $C_9H_{23}N_4O$ $(M + H)^+$ 203.1866, found 203.1870. ^{1}H NMR (D_2O) δ: 3.78−3.05 (m, 17H) ppm. ^{13}C NMR (D_2O) δ: 59.0 (CH_2OH), 56.2 ($CHCH_2OH$), 46.7, 44.6, 44.0, 43.6, 43.5, 43.0, 42.1 ppm.

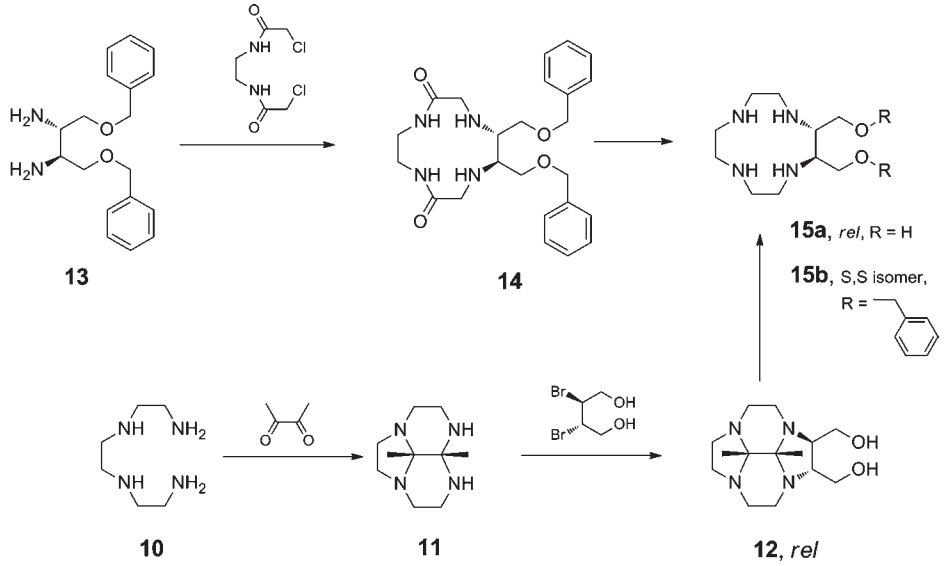
Synthesis of 2-Hydroxymethyl-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 8 (Scheme 3). Potassium carbonate (1.136 g, 8.22 mmol) and *tert*-butyl bromoacetate (1.604 g, 8.22 mmol) were successively added to a solution of **7** (416 mg, 2.06 mmol) in 20 mL of dimethylformamide at 50 °C. After 3 h, ethyl acetate (50 mL) was added to the reaction mixture and potassium carbonate was filtered off. The filtrate was washed three times with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Compound **8** was partially purified by eluting the crude mixture through a silica gel column first with ethyl acetate and finally with methanol in order to recover the sought product **8** (979 mg of an orange oil, yield ≈ 72%). MS (ESI+): m/z 681.4 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{33}H_{62}N_4NaO_9$ $(M + Na)^+$ 681.4409, found 681.4398. ^{1}H NMR ($CDCl_3$) δ: 3.85−1.8 (m, 25H), 1.30 (s, 36H), 1.18 (O*H*, m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.2, 172.9, 172.4, and 172.2 (C═O), 82.1 (4C), 58.8, 58.2, 55.9, 55.4, 55.2, 55.0, 54.5, 53.7, 52.3, 52.1, 50.4, 49.9, 48.7, 47.5, and 27.4 (4C) ppm.

Synthesis of 2-(Prop-2-ynyloxymethyl)-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 9 (Scheme 3). Tetraester **8** (598 mg, ∼0.91 mmol) was dissolved in dichloromethane (20 mL), and this solution was cooled to 0 °C. Propargylbromide in toluene (80% solution, 292 μL, 2.71 mmol, Aldrich), tetrabutylammonium iodide (catalytic amount), and cesium hydroxide monohydrate (457 mg, 2.72 mmol) were successively added to the reaction mixture. After 30 min of vigorous stirring, the ice bath was removed and the reaction mixture was left for 1 h at room temperature. After allowing the cesium base to settle at the bottom of the flask, the supernatant was taken up and washed with 15 mL of water. The organic phase was then dried over $MgSO_4$ and evaporated to dryness, and the remaining residue was chromatographed over a silica gel column (elution gradient: CH_2Cl_2−EtOAc 50:50 to EtOAc−MeOH 85:15) to yield 443 mg of a yellowish oil after evaporation of the solvents (yield ≈ 70%) . MS (ESI+): m/z 719 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{36}H_{64}N_4NaO_9$ $(M + Na)^+$ 719.4566, found 719.4568. ^{1}H NMR ($CDCl_3$) δ: 4.11 ($CH{\equiv}CCH_2$, m, 2H), 4.06−1.61 (m, 26H), 1.30 (s, 36H) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.4, 173.2, 172.5, and 172.4 (C═O), 81.5 (4C), 79.1 and 79.3 ($CH{\equiv}CCH_2$), 74.9 and 74.8 ($CH{\equiv}CCH_2$), 67.4 and 66.3 ($CHCH_2OCH_2$), 58.5 and 58.0 ($CH{\equiv}CCH_2$), 55.9, 54.8, 53.9, 53.4, 51.8, 51.2, 50.8, 50.4, 49.4, 49.0, 47.3, 47.1, 27.4 ppm (*t*-But) ppm.

Synthesis of 2-(Prop-2-ynyloxymethyl)-1,4,7,10-tetra(carboxymethyl)-1,4,7,10-tetraazacyclododecane, 1, DOTAma (Scheme 3). A solution of tetraester monoalkyne **9** (774 mg, ∼1.11 mmol) in trifluoroacetic acid (15 mL) was stirred at room temperature overnight. Evaporation of the solvent under reduced pressure yielded a viscous brownish oil. This residue was dissolved in water (3 mL, pH ≈ 2) and loaded onto a Dowex 50x2-200 (H^+ form) cation-exchange resin. The column was washed with water (500 mL), and the sought product as an ammonium salt was recovered by elution with 0.5 M aqueous NH_3. The eluate was evaporated under reduced pressure, and the light brown oil was dissolved in decarbonated water (3 mL). The pH was brought to 10 with aqueous NH_3, and the solution was transferred onto a Dowex (OH^- form) 1x2-200 anion-exchange resin. The column was washed with decarbonated water (500 mL), and the sought product was eluted using a formic acid gradient (0.005, 0.04, 0.08, 0.15, 0.3, 0.5, 1 M). The fractions containing the desired product were combined and evaporated to dryness in vacuo. Formic acid was eliminated by repetitive evaporations after addition of water. Compound

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

Scheme 4. Synthesis of 1,4,7,10-Tetraazacyclododecane-2,3-diyl)dimethanol 15a and Its Dibenzylated Derivative 15b



1 (331 mg) was obtained as a yellowish oil (yield ≈ 63%). MS (ESI+): m/z 473 $(M + H)^+$, 495 $(M + Na)^+$, 511 $(M + K)^+$. ESI-TOF-MS: calcd for $C_{20}H_{32}N_4NaO_9$ $(M + Na)^+$ 495.2061, found 495.2075. ^{1}H NMR (D_2O) δ: 4.16 ($CH{\equiv}CCH_2$, m, 2H), 4.10−1.56 (m, 26H) ppm. ^{13}C NMR (D_2O) δ: 173.0 (2C) and 170.6 (2C) (C═O), 79.6 ($CH{\equiv}CCH_2$), 76.5 ($CH{\equiv}CCH_2$), 65.4 ($CHCH_2OCH_2$), 58.2 ($CH{\equiv}CCH_2$), 57.5−46.4 (br) ppm.

Synthesis of (5*S*,6*S*)-5,6-Bis(benzyloxymethyl)-1,4,7,10-tetraazacyclododecane-2,9-dione, 14 (Scheme 4). Sodium carbonate (3.76 g, 35.5 mmol) and *N,N′*-bis(chloroacetyl)ethylenediamine (418 mg, 1.97 mmol) were added to a solution of (2*S*, 3*S*)-1,4-bis-(benzyloxy)butane-2,3-diamine[27] and **13** (590 mg, 1.97 mmol) in acetonitrile (62 mL), and this mixture was heated to reflux for 24 h under vigorous stirring. After cooling to room temperature, the reaction mixture was filtered and the filtrate was evaporated to dryness in vacuo. Recrystallization of the remaining residue in boiling ethanol afforded 174 mg of cycle **14** as a white solid (mp 164 °C, yield 20%). MS (ESI+): m/z 441 $(M + H)^+$. ESI-TOF-MS: calcd for $C_{24}H_{33}N_4O_4$ $(M + H)^+$ 441.2496, found 441.2511. ^{1}H NMR (CD_3OD) δ: 7.45−7.30 (Ar, m, 10H), 4.48 (benzylic CH_2, s, 4H), 3.50−3.02 (m, 14H) ppm. ^{13}C NMR (CD_3OD) δ: 175.2 (C═O), 139.9, 129.7, 129.4, and 129.0 (Ar), 74.6 (benzylic CH_2), 70.5 ($CHCH_2O$), 58.7 (CHNH), 51.8 ($CH_2C{=}O$), 39.0 ($CH_2NHC{=}O$) ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(benzyloxymethyl)-1,4,7,10-tetraazacyclododecane, 15b (Scheme 4). Cyclic compound **14** (920 mg, 2.10 mmol) was dissolved in dry toluene (20 mL), placed under argon atmosphere, and cooled to 0 °C. Red-Al (5 mL of a 3.5 M solution in toluene, Aldrich) was added to this solution, and the resulting viscous mixture was maintained at 0 °C for 30 min and then heated at 80 °C for 3 h. After dropwise addition of water (5 mL) at 0 °C, the reaction mixture was stirred for 16 h at room temperature. The solvents were evaporated in vacuo; the residue was dissolved in methanol and purified by dry flash chromatography using a large silica gel column (40 cm length, 5 cm diameter). Elution with methanol followed by methanol saturated with NH_3 allowed recovering tetraamine **15b** as a colorless oil (680 mg, yield 78%). MS (ESI+): m/z 413 $(M + H)^+$. ESI-TOF-MS: calcd for $C_{24}H_{37}N_4O_2$ $(M + H)^+$ 413.2911, found 413.2906. ^{1}H NMR (CD_3OD) δ: 7.45−7.20 (Ar, m, 10H), 4.43 (benzylic CH_2, m, 4H), 3.60−3.30 (CH_2CHNH and $CHCH_2O$, m, 6H), 2.80−2.55 (m, 12H) ppm. ^{13}C NMR (CD_3OD) δ: 139.9, 129.7, 129.4, and 129.0 (Ar), 74.6 (benzylic CH_2), 70.4 ($CHCH_2O$), 58.0 (CHNH), 48.0, 47.6, 44.5 ppm.

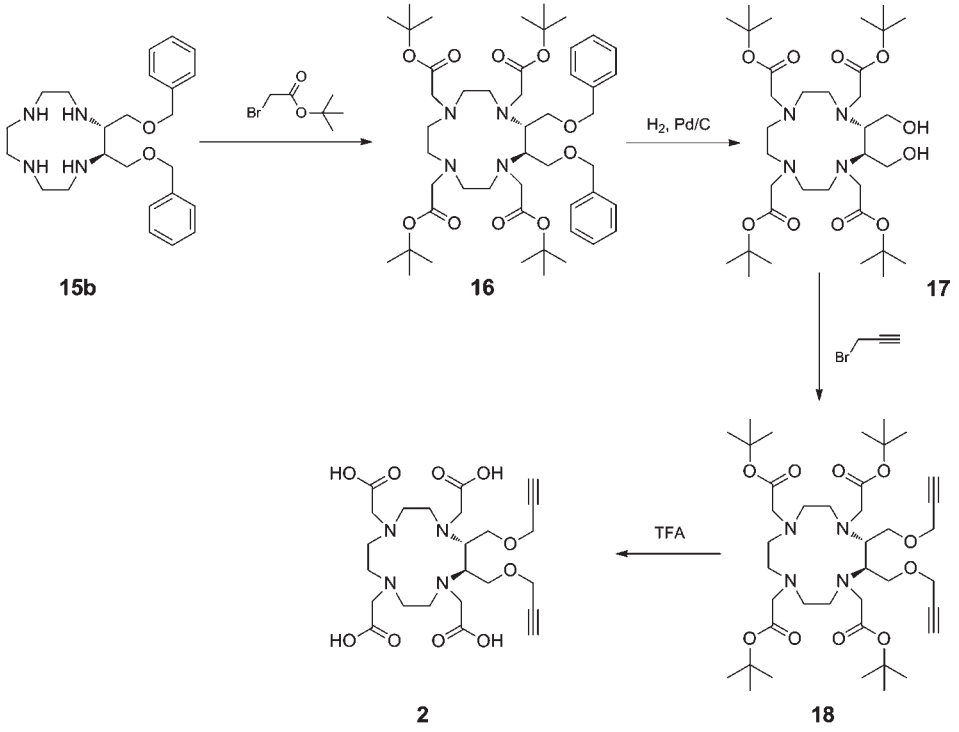
Synthesis of *rel*-((2*S*,3*S*)-1,4,7,10-Tetraazacyclododecane-2,3-diyl)dimethanol, 15a (Scheme 4). Although **15a** could be obtained by a one-pot procedure, we opted for a step by step approach in which each intermediate was isolated. Preparation of **11** has been reported[28] without experimental details.

An ethanolic solution of butanedione (1.77 g, 20.5 mmol in 30 mL) was added dropwise to a solution of triethylenetetraamine, **10**, in ethanol (3 g, 20.5 mmol in 100 mL) at 0 °C. After 4 h of stirring, the solvent was removed under reduced pressure without heating. The remaining yellow solid was taken up in hexane (80 mL) to obtain a yellow solution with an insoluble residue. The latter is highly soluble in methanol and was eliminated by treating the mother solution of hexane with small amounts of methanol (3 × 5 mL). The pale yellow hexane fraction was separated, and the solvent was removed under reduced pressure to yield a white solid (1.2 g, 21%). Note: In our hands, the ethanol/hexane procedure reported above was more convenient than synthesis in acetonitrile because bisaminal **11** crystallized more readily. However, solid **11** undergoes a relatively rapid alteration in contact with air at ambient temperature; its color changes from white to yellow in less than 5 h. Colorless solutions ($CHCl_3$, MeOH) change to orange within 1 day even in anhydrous conditions, indicating complete degradation of **11** as evidenced by mass spectrometry. ^{1}H NMR (CD_3OD, 298 K) δ: 2.5−3.05 (m, 12H, N−CH_2), 1.16 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), ^{13}C NMR (CD_3OD, 298 K) δ: 77.02, 68.05 (NCN), 51.26, 48.37, 40.53 (NCH_2), 22.41, 10.91 ($NCCH_3$). MS (ESI+): $[M + Na]^+$ (m/z) found, 219.1616; calcd, 219.1586.

A solution of *rel*-(2*R*,3*R*)-2,3-dibromo-1,4-butanediol (1.51 g, 6.1 mmol) in 10 mL of acetonitrile was added to a mixture of the bisaminal intermediate **11** (1.2 g, 6.1 mmol) and Cs_2CO_3 (10 g, 30.5 mmol) in acetonitrile (40 mL), and the resulting mixture was refluxed for 48 h under argon. The reaction mixture was filtered through a pad of Celite to obtain a colorless to pale yellow solution, and the solvent was removed to yield the protected macrocycle **12** as a yellowish oil (1.4 g, 81%). The product can be used as such in the next synthetic step or better be recrystallized from cold acetonitrile. ^{1}H NMR (CD_3OD, 233 K) δ: 4.1 (m, 1 H, NC*H*CH_2OH), 3.56−3.76 (m, 2 H, NCHCH_2OH), 3.5 (m, 1 H, NC*H*CH_2OH), 3.07−3.27 (m, 2 H, NCHCH_2OH), 2.35−3.07 (m, 12 H, NCH_2), ^{13}C NMR (CD_3OD, 233K) δ: 79.84, 74.97 (NCN),

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

Scheme 5. Synthesis of DOTAda, 2



78.20, 74.07 ($NCHCH_2OH$), 56.87, 52,81 ($NCHCH_2OH$), 50.52, 49.65, 49.50, 47.30, 45.87, 45.44 (NCH_2). MS (ESI^+): $[M+H]^+$ (m/z) found, 283.2129; calcd, 283.2129.

The bridged macrocycle **12** obtained in the preceding step was dissolved in ethanol (50 mL), and 10 mL of concentrated HCl (37%) was added carefully. The solution was refluxed for 48 h under argon. The solid hydrochloride was collected by filtration and rinsed with small amounts of cold ethanol and ether. Attempts to recrystallize the remaining beige solid were unsuccessful. After dissolution in 30 mL of water, the product was purified by adjusting the pH to 12 with a 4 M NaOH solution followed by evaporating the solvent under vacuum. Acetonitrile (150 mL) was added to the residual solid, and the resulting suspension was stirred overnight at 45 °C. The insoluble material was filtered off, and the solvent was evaporated to yield **15a** as an orange oil (0.7 g, 61%). The NMR spectrum of **15a** is very sensitive to pH, particularly the $NCHCH_2OH$ resonances. The best-resolved ^1H and ^{13}C NMR spectra were obtained between pH 3 and 4. ESI-TOF-MS: $[M + H]^+$ (m/z) found, 233.1969; calcd, 233.1972. ^1H NMR (D_2O, 298 K) δ: 4.57 (m, 2 H, $NCHCH_2OH$, assigned checked with the HSQC spectrum), 3.28−3.81 (m, 16 H, NCH_2 and CH_2OH). ^{13}C NMR (D_2O, 298 K) δ: 68.43 ($NCHCH_2OH$), 48.72 (CH_2OH), 45.97, 45.48, 43.30 (NCH_2).

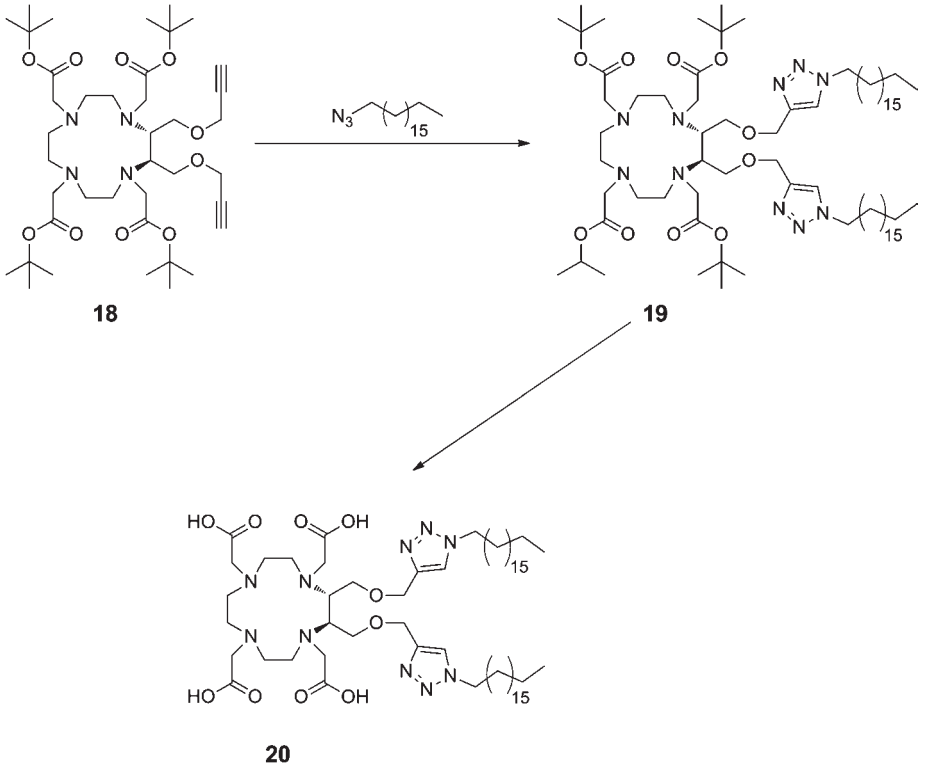
Synthesis of (2*S*,3*S*)-2,3-Bis(benzyloxymethyl)-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 16 (Scheme 5). Potassium carbonate (280 mg, 2.04 mmol) and *tert*-butyl bromoacetate (395 mg, 2.04 mmol) were added to tetraamine **15b** (210 mg, 0.51 mmol) dissolved in DMF (10 mL). After 3 h of stirring at 50 °C, the reaction mixture was diluted with ethyl acetate (50 mL) and washed three times with brine. The organic layer was dried over $MgSO_4$, and the solvents were removed by evaporation under reduced pressure. The remaining residue was purified by silica gel column chromatography, eluting first with EtOAc and finally with a EtOAc−MeOH 3:1 mixture. The fractions containing the sought product were evaporated in vacuo to give 408 mg of **16** as an orange oil; yield 91%. MS (ESI+): m/z 869 $(M + H)^+$. ESI-TOF-MS: calcd for $C_{48}H_{77}N_4O_{10}$ $(M + H)^+$ 869.5634, found 869.5649. ^1H NMR ($CDCl_3$) δ: 7.10−7.00 (Ar, m, 10 H), 4.33 (benzylic CH_2, m, 4 H), 4.15−1.78 (m, 26 H), 1.22 (s, 36 H) ppm. ^{13}C NMR ($CDCl_3$) δ: 174.6, 174.2, 173.7, and 173.4 (C═O), 138.5 (2 C), 129.1 (2 C), 128.8 (2 C), and 128.5 (2 C) (Ar), 82.8, 82.6, 82.5, 82.3, 74.0, and 73.9 (benzylic CH_2), 67.4, 65.8, 59.7, 56.5 (2 C), 55.6, 54.4, 54.0, 53.4, 53.2, 50.2, 49.0, 48.9, 45.6, 28.7 (4 C) ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(hydroxymethyl)-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 17 (Scheme 5). Compound **16** (200 mg, 0.23 mmol) was dissolved in ethanol (15 mL) and placed in a reactor (Miniclave, Büchi). Concentrated HCl (192 μL) and Pd/C 10% catalyst (57.5 mg, Degussa, E101 NE/W, Aldrich) were added, and the reaction mixture was hydrogenated for 72 h (5 bar, RT). After filtration and abundant washing of the catalyst with methanol, the combined organic fractions were eluted on a column of Amberlyst A-21 resin (free base form, ACROS) to eliminate HCl. Evaporation of the solvents under reduced pressure afforded diol **17** as a green-yellow oil (95 mg, yield 60%). MS (ESI+): m/z 690 $(M + H)^+$, 711.4 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{34}H_{65}N_4O_{10}$ $(M + H)^+$ 689.4695, found 689.4683. ^1H NMR ($CDCl_3$) δ: 3.83−1.85 (m, 26H), 1.33 (s, 36H), 1.16 (O*H*, m, 2 H) ppm. ^{13}C NMR ($CDCl_3$) δ: 174.7, 174.1, 173.7, and 173.5 (C═O), 82.9, 82.8, 82.7, 82.6, 62.8, 58.2, 58.0, 56.6 (2C), 56.3, 53.6, 53.5 (2C), 53.4, 49.9, 49.1, 48.9, 45.7, 28.8 (4C) ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(prop-2-ynyloxymethyl)-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 18 (Scheme 5). A solution of propargyl bromide in toluene (385 μL of a 80% solution, 3.58 mmol), a catalytic amount of tetrabutylammonium iodide, and cesium hydroxide monohydrate (600 mg, 3.58 mmol) were added successively to a solution of diol **17** (410 mg, 0.595 mmol) in dichloromethane (20 mL) at 0 °C. After 30 min of vigorous stirring, the ice bath was removed and the reaction mixture was left for 1 h at room temperature. After allowing the cesium base to settle at the bottom of the flask, the supernatant was collected and washed with 15 mL of water. The organic phase was dried over $MgSO_4$ and evaporated to dryness, and the obtained residue was purified by elution

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

Scheme 6. Synthesis of $(C18)_2$DOTAda, 20



on a silica gel column (elution gradient CH_2Cl_2–EtOAc 50:50 to EtOAc–MeOH 85:15). After evaporation of the fractions containing the desired compound (ESI-MS), dialkyne **18** was recovered as a yellowish oil (409 mg, yield 90%). MS (ESI+): *m/z* 765 $(M + H)^+$, 787 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{40}H_{68}N_4NaO_{10}$ $(M + Na)^+$ 787.4828, found 787.4832. ^{1}H NMR ($CDCl_3$) δ: 3.98–1.58 (m, 28H), 4.03 ($CH{\equiv}CCH_2$, m, 4 H), 1.28 (s, 36H) ppm. ^{13}C NMR ($CDCl_3$) δ: 173.7, 173.1, 172.7, and 172.4 (C=O), 81.8, 81.7, 81.6, 81.5, 79.1, and 79.2 ($CH{\equiv}CCH_2$), 75.1 and 74.9 ($CH{\equiv}CCH_2$), 66.3 and 64.6 ($CHCH_2O$) 58.0 (2C, $CH{\equiv}CCH_2$), 55.6, 55.5, 54.3, 53.6, 53.4, 52.9, 52.5, 52.3, 49.2, 48.0, 47.9, 44.7, 27.8 (4C) ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(prop-2-ynyloxymethyl)-1,4,7,10-tetra(carboxymethyl)-1,4,7,10-tetraazacyclododecane, 2, DOTAda (Scheme 5). Dialkyne **18** (660 mg, 0.86 mmol) was dissolved in trifluoroacetic acid (15 mL), and the obtained solution was stirred at room temperature overnight. Evaporation of the solvent under reduced pressure yielded a viscous brownish oil. This residue was dissolved in water (3 mL, pH ≈ 2) and loaded onto a Dowex 50x2-200 (H^+ form) cation-exchange resin. The column was washed with water (500 mL), and the ammonium salt of DOTAda was recovered by elution with 0.5 M aqueous NH_3. The eluate was evaporated under reduced pressure, and the light brown oil was dissolved in decarbonated water (3 mL). The pH was brought to 10 with aqueous NH_3, and the solution was transferred onto a Dowex (OH^- form) 1x2-200 anion-exchange resin. The column was washed with decarbonated water (500 mL), and the sought product was eluted using a formic acid gradient (0.005, 0.04, 0.08, 0.15, 0.3, 0.5, 1 M). The fractions containing the desired product (HCOOH 0.08 and 0.15 M) were combined and evaporated to dryness in vacuo. Repetitive evaporations after addition of water were performed in order to remove formic acid. Finally, 313 mg of ligand **2** was obtained as a yellowish oil in 67% yield. MS (ESI+): *m/z* 541 $(M + H)^+$, 563 $(M + Na)^+$, 579 $(M + K)^+$. ESI-TOF-MS: calcd for $C_{24}H_{36}N_4NaO_{10}$ $(M + Na)^+$ 563.2324, found 563.2341. ^{1}H NMR (D_2O) δ: 4.07 ($CH{\equiv}CCH_2$, m, 4 H), 3.97–1.60 (m, 28 H) ppm. ^{13}C NMR (D_2O) δ: 176.5, 176.4, 171.6, and 171.2 (C=O), 79.5 and 79.2 ($CH{\equiv}CCH_2$), 76.5 and 76.4 ($CH{\equiv}CCH_2$), 65.1 and 63.7 ($CHCH_2O$), 62.4, 58.0 (2 C, $CH{\equiv}CCH_2$), 56.5, 56.2, 55.9 (2 C), 52.8, 51.0 (2 C), 49.8, 49.3, 48.7, 46.0 ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(1-octadecyl-1*H*-[1,2,3]triazol-4-ylmethoxymethyl)-1,4,7,10-tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, 19 (Scheme 6). 1-Azidooctadecane[29] (69.7 mg, 0.236 mmol), 27 μL of 2,6-lutidine (0.236 mmol), 41 μL of diisopropylethylamine (0.236 mmol), and 25 mg of CuI (0.13 mmol) were added successively to a solution of tetraester dialkyne **18** (90 mg, 0.118 mmol) in dry dichloromethane (10 mL) under argon atmosphere. After 3 h, a 1 M aqueous DTPA solution was added (10 mL), and the resulting mixture was stirred vigorously overnight. The blue aqueous phase was discarded, and the organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The remaining residue was purified by chromatography on a silica gel column, eluting with dichloromethane. Product **19** was recovered as a yellowish oil (109 mg, yield, 68%). MS (ESI+): *m/z* 1378 $(M + Na)^+$. ESI-TOF-MS: calcd for $C_{76}H_{142}N_{10}NaO_{10}$ $(M + Na)^+$ 1378.0803, found 1378.0817. ^{1}H NMR ($CDCl_3$) δ: 8.25 and 8.13 (ss, triazole ring protons), 4.43 (m, 4 H, $OCH_2C{=}C$), 3.8–1.75 (m, 30 H), 1.58 (m, 4 H, CH_2CH_2N), 1.42 (s, 36 H), 1.54–1.03 (m, 60 H, aliphatic CH_2), 0.85 (t, 6 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$) δ: 174.0, 173.3, 172.7, and 172.5 (C=O), 143.5 and 143.4 ($OCH_2C{=}C$), 124.5 and 124.3 ($OCH_2C{=}C$), 81.8, 81.7 (2 C), 81.5, 63.9, and 63.7 ($OCH_2C{=}C$), 66.3, 64.9, 58.8, 55.7, 55.6, 54.4, 53.4, 53.0, 52.6, 52.3, 49.2, 48.3, 48.1, 44.8, 50.4 (2 C, $CH_2N{-}N{=}N$), 31.8 (2 C, $CH_2CH_2CH_3$), 30.4, 30.3, 30–28.5, 26.6, and 26.5 (28 C, aliphatic CH_2), 27.8 (4 C, *tert*-butylic CH_3), 22.6 (2 C, CH_2CH_3), 14.0 (2 C, CH_2CH_3) ppm.

Synthesis of (2*S*,3*S*)-2,3-Bis(1-octadecyl-1*H*-[1,2,3]triazol-4-ylmethoxymethyl)-1,4,7,10-tetra(carboxymethyl)-1,4,7,10-tetraazacyclododecane, 20, $(C18)_2$DOTAda (Scheme 6). Compound **19** (94 mg, 0.07 mmol) was dissolved in a 1:1 mixture of

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

trifluoroacetic acid and dichloromethane and vigorously stirred overnight at room temperature. After evaporation of the solvents in vacuo, the remaining brownish oil was dissolved in dichloromethane (5 mL) and diethyl ether (30 mL) was added to this solution. The formed precipitate was isolated by centrifugation, washed with diethyl ether, and dried under reduced pressure to obtain ligand **20** as a pale yellow oil in 67% yield (52 mg). MS (ESI+): m/z 1132 $(M + H)^+$. ESI-TOF-MS: calcd for $C_{60}H_{111}N_{10}O_{10}$ $(M + H)^+$ 1131.8479, found 1131.8468. ^{1}H NMR ($CDCl_3$) δ: 7.77 (b, triazole ring protons), 5.0 − 0.8 (br, cycle and aliphatic chains) ppm. ^{13}C NMR ($CDCl_3$) δ: 171.4 (2 C) and 171.2 (2 C) (C═O), 143.0 and 142.8 (OCH_2C═C), 123.8 and 123.6 (OCH_2C═C), 67−44 (18 C, br), 31.9 (2 C, $CH_2CH_2CH_3$), 29.7, 29.4, 30−28.5, 26.6, and 26.5 (28 C, aliphatic CH_2), 22.7 (2 C, CH_2CH_3), 14.0 (2 C, CH_2CH_3) ppm.

Preparation of Metal Complexes. Typically, 1 equiv of DOTAma, **1**, DOTAda, **2**, or DO3ma, **3**, was dissolved in water (5 mL), and a slight stoichiometric excess of a lanthanide trichloride solution (1.1 equiv) was added dropwise. The solution was stirred at 50 °C while the pH was continuously maintained at pH 5.5 by adding 1 M KOH until no more pH variation was observed. After stirring 24 h at this temperature, the solution was passed through a column of Chelex 100 resin (Aldrich) that was eluted with water to remove the excess of metal ions. Evaporation under reduced pressure yielded the complexes as pale yellow solids. The absence of free metal ions was checked with xylenol orange.[30]

Micelles of $(C18)_2$DOTAda, **20,** were obtained by adding very slowly (portions of 20 μL) a THF solution of the ligand (10 mg in 400 μL THF) to 5 mL of water under vigorous stirring. The stirring was maintained overnight in a recipient that was not covered to let the organic solvent evaporate. The remaining solution was sonicated for 5 min to obtain a stable micellar suspension to which a $GdCl_3$ solution (1.1 equiv) was added dropwise. The reaction mixture was stirred at 50 °C, while the pH was continuously maintained at 5.5 by adding 1 M KOH until no more pH variation was observed. After stirring 24 h at 50 °C, 500 mg of Chelex 100 resin was added and the suspension was stirred for 3 h at room temperature. The supernatant was collected and lyophilized to afford Gd$(C18)_2$DOTAda as a colorless material. The Gd content was checked by ICP atomic emissions spectroscopy and/or measuring the relaxation after mineralization in concentrated acid. Ligand solutions were prepared by weight and successive dilutions. Three samples of Gd$(C18)_2$DOTAda micelles yielded the same relaxivity curves within the errors limits.

3. RESULTS AND DISCUSSION

Syntheses of Alkyne-Substitued Ligands. Chelating agent DO3ma, **3**, was readily synthesized starting from commercially available DO3A$(t\text{-Bu})_3$, **4**, and following the procedure recently reported by Lowe et al.[23] with minor modifications (Scheme 2). Alkylation of the free amino group using propargyl bromide afforded DO3ma$(t\text{-Bu})_3$, **5**, in 95% yield. A purified sample of the DO3ma ligand, **3**, was obtained with a 62% global yield after deprotection of the triester with TFA followed by cationic and anionic ion-exchange chromatography.

Synthesis of ligands substituted with one or two alkyne groups grafted on the tetraaza cyclododecane ring requires full construction of the macrocyclic tetraaza unit. Essentially two approaches can be followed to achieve this goal: (a) a reaction between triethylenetetramine and butanedione to form a *geminal-cis* bisaminal possessing the requisite configuration to undergo a (1 + 1) cyclocondensation with a 1,2-disubstituted ethylene derivative featuring two leaving groups[26,28,31] or (b) a "crab-like" synthesis between a bis-chloroacetamide and a diamine.[32] The bisaminal route is more direct, but in our hands, the yields were not always reproducible.[28] This is the route selected by Boschetti et al.[26] to obtain 1,4,7,10-tetraazacyclododecan-2-yl)methanol, **7**, in a one-pot procedure starting from triethylenetetramine and butanedione to form a bisaminal that is directly reacted with 2,3-dibromopropan-1-ol or 1,3-ditosylpropan-1-ol. In our hands, simple filtration of crude **6** through an alumina column (97:3 CH_2Cl_2−MeOH) as suggested by these authors[26] led to only partial purification, and we had to isolate the bisaminal **6** by recrystallization in ice-cold acetonitrile in order to obtain pure macrocyclic derivative **7** as a mixture of two diastereoisomers (*R,S,R* and *R,S,S*) displaying 26 carbon NMR resonances as also observed for its amino analogue[33] (yield from triethylenetetramine 22.3%).

tert-Butyl ester arms were added to **7** in basic medium, and a Williamson-type *O*-alkylation using propargyl bromide and cesium hydroxide in dichloromethane led to the monoalkyne **9** in less than 2 h (yield 95%). It is interesting to note that all carbon atoms of the prop-2-ynyloxymethyl substituent give rise to two resonances in the ^{13}C NMR spectrum of **9**. Partial differentiation of the *R* and *S* enantiomers appears to be due to a rigidification effect by the *tert*-butyl ester groups that forces the alkyne substituents to be located in two slightly different environments. Cleavage of the esters by trifluoroacetic acid followed by ion-exchange chromatography afforded pure DOTAma ligand, **1** (yield 65%). Since this molecule no longer experiences the rigidifying effect of the *tert*-butyl ester arms, the carbon atoms of the prop-2-ynyloxymethyl substituent now give rise to single NMR peaks. Monoalkyne ligand **1** could be obtained in 6 steps with an overall yield of 7% starting from triethylenetetraamine.

The tetraaza cycle featuring two alcohol groups was prepared by the bisaminal template route as a racemic mixture and by the "crab-like" technique as the *S,S* isomer. Caution was needed to ensure that the two alcohol substituents would not prevent the tetraazacyclododecane cycle from adopting its preferred [3.3.3.3] square conformation[34] in which the lone pairs of all nitrogen atoms are pointing toward the same side of the macrocyclic unit and are thus available for binding a metal ion. This implies that the substituents in the tetraaza cycle be located in the two equatorial positions rather than in the axial positions where the steric crowding would become so large that complexing could not take place. The diol unit in the macrocyclic ring thus has to be in the *S,S* or *R,R* conformation.

In the template approach to preparation of the basic unit of DOTAda (Scheme 4), bisaminal **11** obtained from triethylenetetramine and 2,3-butanedione[28] was reacted with *dl*-2,3-dibromobutane-1,4-diol[35] in the presence of Cs_2CO_3. Removal of the central bridging unit of **12** with concentrated HCl led to diol **15a**. The latter could not be obtained in a pure state without isolating intermediate **12**. Moreover, we could not obtain this compound by reacting **11** with a ditosylated reagent rather than a dibromide. Unsuccessful attempts at cyclizing tricyclic bisaminal have already been reported and assigned to an improper orientation of the secondary nitrogen atoms for a cyclocondensation.[28] In the present case, it is not clear why steric requirements could prevent a reaction between a tricyclic bisaminal and 1,4-bis-(benzyloxy)butane-2,3-diol dimethanesulfonate.

The "crab-like" approach to preparation of the optically pure *S,S* isomer of diol **15b** with the alcohol groups protected by benzyl functions is also illustrated in Scheme 4. It should be noted here that an enantiomer of **15b** could not be prepared by the template pathway reported above because optically pure enantiomers of 2,3-dibromobutane-1,4-diol have not been prepared so far. Our

synthesis started from (2*S*,3*S*)-1,4-bis(benzyloxy)-2,3-diaminobutane, **13**, which was obtained in three steps as reported by Scheurer et al.[27] from commercially available 1,4-di-*O*-benzyl-L-threitol, which was submitted to the classical transformation into a dimesylate, a diazide, and finally a diamine by hydrogenation in dry THF rather than in methanol as reported in a more recent publication by the same authors.[36] The "crab-like" cyclization with *N*,*N*′-(ethane-1,2-diyl)bis(2-chloroacetamide)[37] followed by recrystallization in boiling ethanol afforded pure 5,6-bis-(benzyloxymethyl)-1,4,7,10-tetraazacyclododecane-2,9-dione, **14**. The poor yield obtained for this reaction (20%) is in agreement with previous works[38] and with the results obtained for similar cyclizations undertaken in our laboratory.[39] The reduction of amide groups in a macrocycle is usually carried out with a BH_3-THF complex, but as expected, the drastic conditions needed to convert the intermediate amine−borane complexes into amines (6 M HCl) caused partial deprotection of the alcohol groups. The mild reducing agent Red-Al was thus preferred as it is known to leave the benzyl ether groups untouched.[40] After flash chromatography purification using silica gel, tetraamine **15b** was obtained in 78% yield. The latter was reacted with *tert*-butyl bromoacetate to yield tetraester **16** (Scheme 5), which must adopt a preferred conformation as the 2-fold symmetry observed in the ^{13}C NMR spectra of compounds **14** and **15b** is no longer observed. Debenzylation of **16** proved to be a challenging task. Use of common reaction conditions[41] (3 atm of H_2, 25 °C, Pd/C 10%) in different organic solvents failed even in the presence of large amounts of catalyst. The lack of reaction in the presence of amines has already been reported[42] and could be avoided by addition of a small amount of acid. With large amounts of Pearlman's catalyst ($Pd(OH)_2/C$) and acetic acid, diol **17** was observed in mass spectrometry but the reaction yield remained lower than 30%. The use of acids such as TFA and CCl_3COOH, of Raney nickel as catalyst, or of several deprotection methods not based on catalytic hydrogenation[41] led to hydrolysis of the *tert*-butyl ester to slow kinetics or to formation of large amounts of side products. Finally, addition of a small amount of concentrated HCl[43] to an ethanol solution of **16** followed by catalytic hydrogenation for 3 days afforded the target diol **17** in 60% yield while preserving the integrity of the ester functions. Two alkyne units were then introduced by a Williamson-type *O*-alkylation. In the case of aliphatic alcohols, this reaction is usually carried out in the presence of NaH and propargyl bromide.[44,45] Compound **17** proved to be unstable when sodium hydride was added to a toluene or THF solution: mass spectrometry indicated that the diol underwent a double intramolecular transesterification. Different conditions of phase transfer catalysis (PTC) were tested as the synthesis of propargylic ethers from allylic,[46] benzylic,[47] and propargylic[48] alcohols have already been reported by this method. In our hands, *O*-alkylation of **17** using NaOH or KOH (as 50% aqueous solution or as pellets), tetrabutylammonium iodide in dichloromethane and propargyl bromide (80% solution in toluene) went to completion only after 14 days. The reaction was even slower in pure propargyl bromide. Dueno et al.[49] reported that cesium bases generate highly nucleophilic cesium alkoxides able to accelerate *O*-alkylation reactions of aliphatic alcohols. This was confirmed by the observation of a mass peak for dialkyne **18** after 10 h when a 50% aqueous solution of cesium hydroxide was used. The accelerating effect of cesium was even more impressive when solid $CsOH\cdot H_2O$ was directly added to the reaction mixture in dichloromethane: **18** could be isolated after 90 min. This corresponds to a 200-fold increase in rate compared to sodium hydroxide. Dialkyne **18** was then converted to DOTAda, **2**, by cleaving the *tert*-butyl esters using TFA followed by purification by ion-exchange chromatography. It is worthwhile to note that the two alkyne groups of **2** give rise to separate ^{13}C peaks, although they are far removed from the macrocyclic unit.

The template procedure illustrated in Scheme 4 is obviously much more effective than the "crab-like" approach (three steps instead of five, higher overall yield). A well-defined isomer can only be synthesized thanks to a cyclization with a diamide dichloride as in Scheme 4, but three steps are required for obtaining the starting derivative **13**.

Solution Structures. Solutions of the lanthanide complexes with ligands **1**−**3** were prepared by adding a 10% excess of metal ion to a solution of ligand and adjusting the pH to 5.5 at 50 °C. Once the pH was constant, the reaction mixtures were eluted on a Chelex 100 resin to eliminate the metal ions in excess.

It is now well established[34,50,51] that the tetraaza cycle of the DOTA-like complexes adopts either a $\delta\delta\delta\delta$ or a $\lambda\lambda\lambda\lambda$ arrangement depending on the N−C−C−N torsion angles in the ethylenediamine units that all have either the same positive (δ) or negative (λ) values. Moreover, the pendant acetate arms form either a clockwise (Λ) or a counterclockwise (Δ) four-bladed propeller. This leads to four stereoisomers, two of which are in a square antiprismatic (SAP) arrangement (Λ $\delta\delta\delta\delta$ and Δ $\lambda\lambda\lambda\lambda$) while the other two adopt a twisted square antiprism (TSAP) geometry (Δ $\delta\delta\delta\delta$ and Λ $\lambda\lambda\lambda\lambda$). These four stereoisomers are related as two pairs of enantiomers that give rise to separate peaks because of a slow intermolecular exchange on the NMR time scale. The resonances most shifted to low fields of the Yb^{3+} chelates are known[34,50,51] to correspond to the axial ring protons located on the carbon atom on the side of the [3.3.3.3] square conformation in the SAP geometry (also called $H_{ax\ up}$,[52] see Figure 1). In the TSAP geometry, these resonances are also found at low fields; they are less shifted and easily distinguishable from the SAP peaks. Figure 2 presents the low-field part of NMR spectra of the Yb^{3+} complexes with DOTAda and DOTAma.

A chiral carbon atom in the tetraaza ring transforms the four potential isomers of a DOTA complex into diastereoisomers whose number is increased to eight if this complex is a racemic mixture as in the case of YbDOTAma. Instead of observing the expected eight sets of four resonances for the axial proton shifted to low field ($H_{ax,up}$ between 150 and 60 ppm), only four series of four peaks are visible (four resonances are overlapping to form the resonance at 148 ppm). Molecular modeling calculations confirmed by analyses of the dipolar NMR shifts indicate that ring substituents cannot occupy axial positions because of steric crowding and that the "eq up" orientation is preferred to the "eq down" position.[52−54] This preference is sufficiently strong to prevent the inversion of the ethylenediamine groups in the tetraaza ring as already found in the EXSY spectra of an Yb^{3+} complex with a DOTA ligand substituted with one methyl group (MDOTA).[52] With a methyl[52] or benzyl[55] ring substituent in an *S* enantiomer, this necessarily leads to a $\delta\delta\delta\delta$ arrangement that will also be found for the *R* enantiomer of DOTAma. Conversely, the *S* enantiomer will adopt a $\lambda\lambda\lambda\lambda$ conformation. Both conformations are found in the spectrum of racemic YbDOTAma with a "frozen" cycle and either Δ or Λ arrangements of the acetate arms. Only four sets of four peaks are thus observed in the NMR spectrum of YbDOTAma, and the SAP form is the major species in solution (TSAP/SAP = 1:2 for YbDOTAma).

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

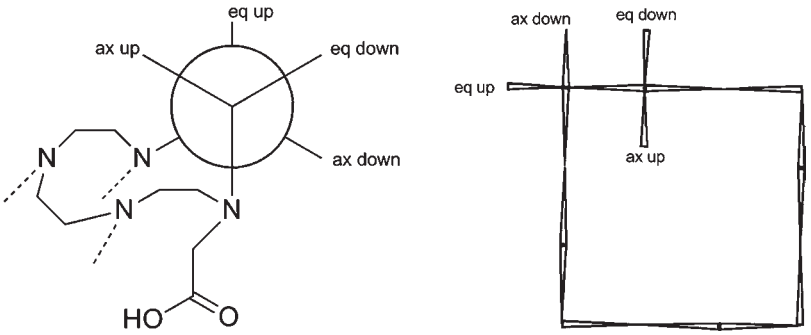


Figure 1. (Left) Conformation of an ethylenediamine group in a lanthanide DOTA complex. (Right) [3.3.3.3] Conformation of the tetraaza ring in a lanthanide DOTA complex with the proton nomenclature.

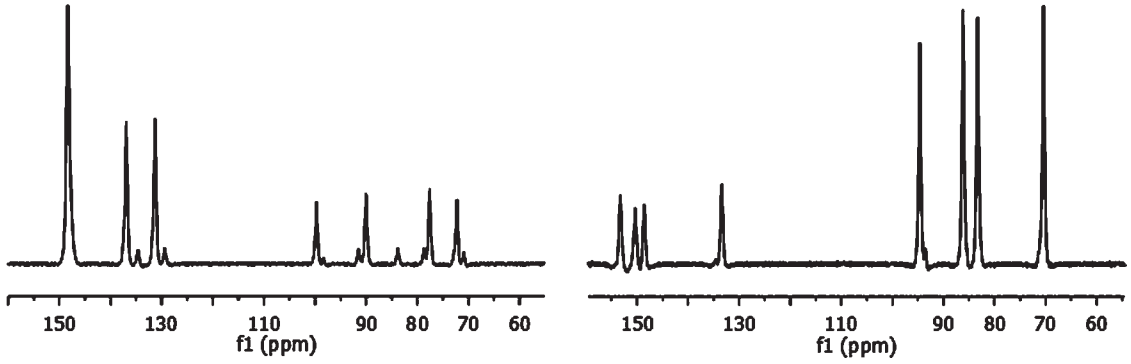


Figure 2. Downfield part of the NMR spectra of the Yb(III) complexes with DOTAma, **1** (left), and DOTada, **2** (right).

The Yb^{3+} complex with the enantiopure DOTAda ligand, **2,** is the first known example of a lanthanide chelate with two adjacent asymmetric carbon atoms in the tetraaza cycle. The two C substituents have no choice but to occupy the two equatorial positions that have been shown to be the least crowded by molecular modeling.[52] As DOTAda is an *S,S*-enantiomer, the tetraaza cycle must thus again be in a $\delta\delta\delta\delta$ arrangement but the TSAP geometry is now dominant (TSAP/SAP ratio close to 2:1) presumably because of the higher steric crowding. A 9:1 SAP/TSAP ratio was found in the case of the Yb(III) (*S*-2-nitrobenzyl)-DOTA complex.[53]

Relaxivity Properties. The paramagnetic lanthanide ion Gd(III) induces a remarkable increase in the experimental relaxation rate r_{1exp} of the water protons because of its long electronic relaxation time T_{1e}. The dipolar interaction taking place in the first coordination sphere is accounted for by the Solomon–Bloembergen–Morgan (SBM) equations[56,57] that are summarized below for a complex GdL

$$r_{1exp} = \frac{q_{H2O}*[GdL]}{55.56*(T_{1M} + \tau_m)} + R_{1outer} \quad (1)$$

$$\frac{1}{T_{1M}} = \text{function}(r^{-6}, \tau_c, \Delta^2, B_0) \quad (2)$$

$$\frac{1}{\tau_c} = \frac{1}{\tau_r} + \frac{1}{\tau_m} + \frac{1}{T_{1e}} \quad (3)$$

where τ_r and τ_m are the rotational and water exchange correlation times and where the relaxivity r_{1exp} is expressed in s^{-1} mM^{-1}. The other parameters are the hydration number of the encapsulated metal ion q_{H2O}, the metal–proton distance r, the correlation time of the modulation of the zero-field splitting τ_v, and the mean-square zero-field splitting energy Δ^2. The solvent molecules outside the first coordination sphere also make a contribution to the relaxivity that depends on the metal–proton distance r_{outer} and the diffusion coefficient D_{diff}.[56,57] Small complexes such as GdDO3ma, GdDOTAma, and GdDOTAda are tumbling rapidly in solution, and one can safely assume that τ_r is the predominant factor in the above equations at most fields B_0 because it is the smallest correlation time in eq 3. For such chelates, the nuclear magnetic relaxation dispersion (NMRD) curve between 0.01 and 80 MHz is an S-shaped curve as found in Figure 3.

The main difficulty when using the SBM eqs 1–3 is that they depend on a large number of unknown parameters. A reliable and quantitative interpretation of the NMRD curves in Figure 3 requires determination of as many parameters as possible by independent methods. The hydration number q_{H2O} was deduced from the measurements of the fluorescence lifetimes τ_{H2O} and τ_{D2O} of the Eu(III) complexes in water and D_2O using the modified Horrocks equation[58]

$$q_{H2O} = 1.2\left[\left(\frac{1}{\tau_{H2O}} - \frac{1}{\tau_{D2O}}\right) - 0.25\right] \quad (4)$$

The q_{H2O} value determined for EuDOTAma and EuDOTAda was 1.1 ± 0.3, and both species are assumed to be monohydrated as are all tetraacetic DOTA-type chelates.[59] The measurements carried out on EuDO3ma indicate that this species is bishydrated ($q_{H2O} = 2.1 \pm 0.3$), in keeping with the hydration

dx.doi.org/10.1021/ic2010997 |*Inorg. Chem.* 2011, 50, 8946–8958

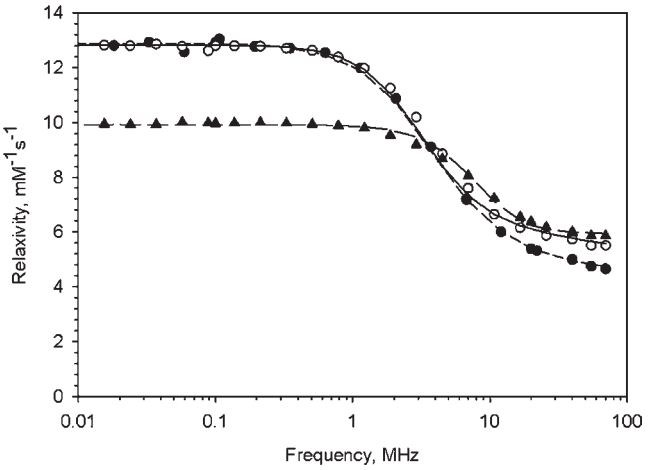


Figure 3. Nuclear magnetic relaxation curves (NMRD) of GdDOTAma (●), GdDOTAda (○), and GdDO3ma (▲) at 25 °C.

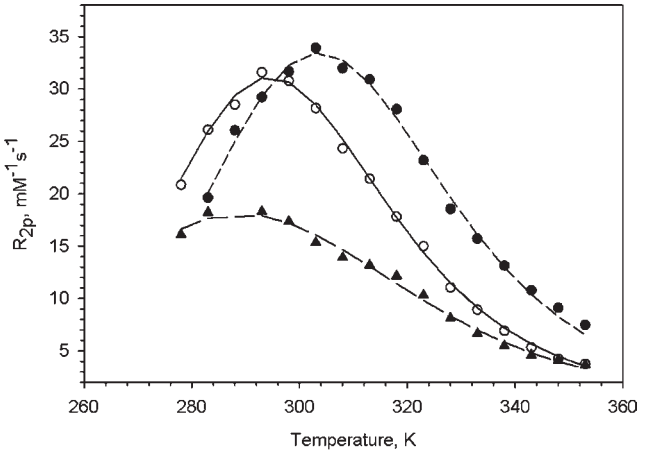


Figure 4. Temperature dependence of the paramagnetic contribution R_{2p} to the transverse ^{17}O water relaxation rate of GdDO3ma (▲), GdDOTAma (●), and GdDOTAda (○) at 5.87 T, pH 5.7, [Gd] = 4.65, 7.04, and 7.61 mM, respectively. Experimental data and calculated curves.

numbers reported for DO3A-type complexes bearing only three acetate arms.[60,61]

The rate of water exchange $1/\tau_m$ of GdDO3ma, GdDOTAma, and GdDOTAda is another parameter in the SBM equations that can be determined independently. The $1/\tau_m$ values were deduced from the paramagnetic contribution of the Gd^{3+} complexes to the ^{17}O transverse relaxation rate of water (R_{2p}) between 278 and 353 K using the Swift and Connick equations[62] (see Figure 4).

The hyperfine coupling constant ($A/\hbar$) was fixed at -3.5×10^6 rad s^{-1},[57] the activation energy of the modulation of the zero field splitting was fixed at 1 kJ/mol, and we also assumed an Eyring temperature dependence of the water exchange time.[57,63] The parameters giving the best agreement between the experimental and the computed transverse relaxation times of the three Gd(III) complexes are given in Table 1.

It has been shown that the water exchange time is about 10 times smaller in the more open TSAP than in the SAP form in the case of the optically pure isomers of GdDOTA chelates bearing

Table 1. Relaxivity Parameters Obtained by Best Fittings of the ^{17}O Data

complex	$\Delta H^{\ddagger}$ (kJ·mol^{-1})	Δ^2 (10^{19} s^{-2})	τ_v^{298K} (ps)	τ_m^{298K} (ns)
GdDO3ma	43	7.9	12	89
GdDOTAma	52	5.8	12.5	183
GdDOTAda	48	3.6	14	122

Table 2. Relaxivity Parameters Obtained by Best Fittings of the NMRD Data

complex	Δ^2 (10^{19} s^{-2})	τ_v^{298K} (ps)	τ_m^{298K} (ns)	τ_r^{298K} (ps)
Gd-DO3ma	9.9	13.7	89[a]	60
Gd-DOTAma	3.6	14.8	183[a]	80
Gd-DOTAda	2.0	14.4	122[a]	107
Gd-(C18)$_2$DOTA[b]	0.52[c]	26[c]	323[c]	3950[c]
	0.81[d]	36[d]	333[d]	135[d,e]
				5206[d,e]

[a] Deduced from the ^{17}O measurements. [b] Above cmc. [c] Bertini–Kowalewski–Luchinat (BKL) approach[66,67] with an axial component of the zero-field splitting D = 0.017 cm^{-1}. [d] SBM and Lipari–Szabo approach[68] with second sphere.[69] [e] Rotational correlation times for fast and slow motion with rigidity factor S^2 = 0.78.

one 4-nitrobenzyl group in the tetraaza ring and one methyl substituent on each acetate arm.[55] The experimental values reported in Table 1 follow this trend even if it should be remembered that GdDOTAma is a racemic mixture. The water exchange is faster for GdDOTAma than in the case of the unsubstituted GdDOTA (τ_m = 243 ns), a chelate which adopts essentially an SAP geometry, and it is still faster for GdDOTAda for which the TSAP geometry is the major contributor. In the latter case, the exchange water time τ_m becomes closer to the theoretically "ideal" value of about 20 ns at 0.47 T for slowly rotating Gd(III) complexes. This value could probably be reached if the acetate arms of the alkyne chelates were substituted by methyl groups so as to increase the steric crowding of the complex. As expected, the water exchange is much faster in the case of the bishydrated electrically neutral triacetate complex GdDO3ma and is very close to the value measured for DO3A, its analogue with an unsubstituted secondary amine (τ_m = 80 ns).[64]

The dispersion of the relaxivity of Gd-DO3ma, Gd-DOTAma, and Gd-DOTAda with the frequency at 298 K (Figure 3) was fitted using the SBM equations and experimental values of the water exchange time τ_m. Several parameters were fixed prior to the calculations: the metal–water proton distances in the first coordination sphere and in the outer sphere were set at 3.1 and 3.6 Å,[10] whereas the relative diffusion constant of the complexes was fixed at 2.5×10^{-5} cm^2 s^{-1}.[65] The calculated parameters are reported in Table 2. The low values of the rotational correlation times τ_r are in good agreement with those reported for similar rapidly rotating chelates.[18,57,60] Variations between the Δ^2 values in Tables 1 and 2 are probably not physically significant. Indeed, the parameters deduced from the NMRD curves always have to be considered with some caution since several factors, especially τ_v and Δ^2, are strongly correlated. Nonetheless, τ_m and τ_r are considered to be reliable, as the influence of the other parameters on these factors is more limited.

dx.doi.org/10.1021/ic2010997 |Inorg. Chem. 2011, 50, 8946–8958

Micellar Assembly Based on the GdDOTAda Complex. As a proof of the versatility of the click reaction with alkyne DOTA derivatives and also of the interest of having two closely spaced alkyne moieties, we prepared a gadolinium complex able to self-assemble into micelles in aqueous media. Forming micelles is a well-known procedure for slowing down the tumbling rate τ_r of contrast agents and hence to obtain higher relaxivities. Amphiphilic chelates have been obtained by appending aliphatic substituents to DOTA or DTPA-type ligands[70−74] and to other polyaminopolyacetic derivatives.[64,75] The ligand $(C18)_2$DOTAda is a new addition to this series of amphiphilic Gd(III) chelates from which it differs by having two long aliphatic chains directly grafted onto the tetraaza macrocycle on two adjacent carbon atoms. Accardo et al.[74] reported another rare example of this approach in the case of a DTPA chelate that also features two aliphatic chains, but they are far removed from the chelating unit.

Ligand $(C18)_2$DOTAda, **20**, was synthesized as shown in Scheme 6. The reaction conditions of Scheurer et al.[27] were used to convert 1-bromooctadecane into 1-azidooctadecane **16** that was "click" coupled with tetraester dialkyne **18** using copper iodide as catalyst in dichloromethane. Complicating side reactions were minimized by using two equivalents of two nitrogen bases, diisopropylethylamine and 2,6-lutidine,[76] per macrocycle **18** and by performing the reaction in the absence of oxygen. In our hands, 1.1 equiv of CuI was needed for obtaining **19**, presumably because the DOTA unit of ester **18** forms a stable Cu(II) complex and totally consumes one equivalent of this reagent. Compound **18** had completely reacted after 3 h as shown by the ES-MS. After removal of the copper ions by extracting the reaction mixture with a 1 M DTPA solution overnight followed by silica gel purification, bisaliphatic compound **19** was recovered in 68% yield. This compound features two ^{1}H NMR resonances at 8.13 and 8.25 ppm that correspond to the two triazole substituents that are nonequivalent as are the two alkyne groups of ligand **2** (vide supra). Cleavage of the *tert*-butyl esters of **19** with TFA led to the target ligand $(C18)_2$DOTAda, **20**. The Gd$(C18)_2$DOTAda complex was obtained by adding gadolinium trichloride to an aqueous micellar suspension of ligand **20** (see Experimental Section). Preparation of Gd(III)-containing micelles was usually carried out by preparing first the metal complex and then proceeding to formation of micelles. In our hands, several variations of this procedure (synthesis in pyridine[73b] or in water[74] with or without sonication) led to nonreproducible results including sedimentation and degradation. The compactness of $(C18)_2$DOTAda, the substitution of its macrocyclic ring by two long aliphatic chains rather than one, and the low cmc of its Gd complex could be at the origin of these difficulties.

As reported by different authors,[72,77] the critical micellar concentration (cmc) of amphiphilic chelates can be determined by measuring the ^{1}H relaxation rates as a function of the Gd(III) complex concentration. A relaxivity vs complex concentration plot exhibits a marked break when a surfactant changes from its monomeric state to a slowly tumbling micellar form. As shown in Figure 5, Gd$(C18)_2$DOTA possesses a very low cmc of about 4×10^{-3} mM. An even lower cmc value has been determined for a DTPA amphiphilic complex with two long aliphatic chains (cmc = 9.6×10^{-4} mM).[74]

At concentrations above the cmc (0.158 mM), the NMRD profile of a solution of Gd$(C18)_2$DOTAda micelles displays a pronounced maximum at 20−40 MHz and high relaxivities at low fields, i.e., two features that are characteristic of slowly

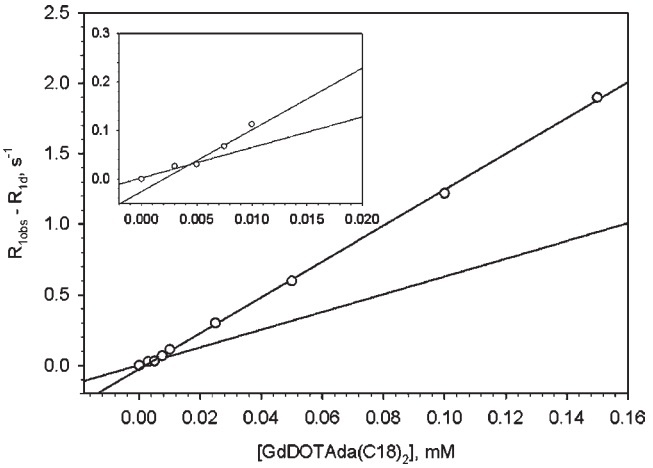


Figure 5. Plot of the variation of the water ^{1}H longitudinal relaxation rate $1/T_1$ versus the concentration of $(C18)_2$GdDOTAda at 20 MHz and 25 °C.

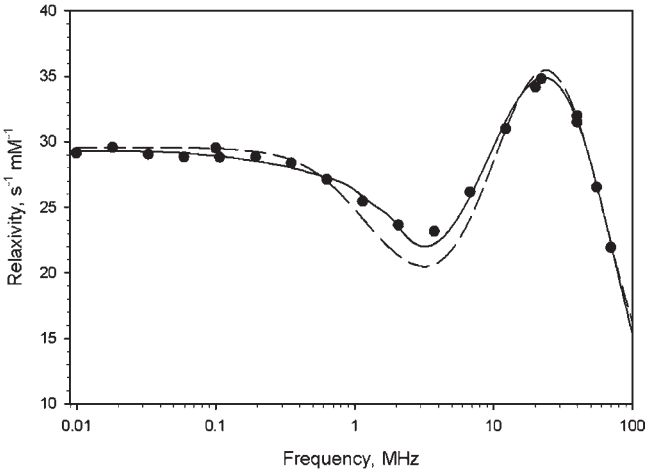


Figure 6. Nuclear magnetic relaxation curve (NMRD) of a micellar suspension of Gd$(C18)_2$DOTAda at a concentration of 0.158 mM and 25 °C. Calculated NMRD curves by the Bertini−Kowalewski−Luchinat (BKL) approach[66,67] (solid line) and the SBM and Lipari−Szabo approach[68,69] (dashed line).

rotating species (see Figure 6). The 20 MHz relaxivity is close to 35 s^{-1} mM^{-1}, which is approximately six times higher than the value obtained for the parent Gd−DOTAda complex. The 20 MHz relaxivity is also higher than or comparable to the maxima reported for high-generation Gd^{3+}-containing dendrimers[78] and for the paramagnetic micellar systems that exhibit the highest efficacy reported so far.[77] We ascribe the high relaxivity at 20−40 MHz to the double anchoring of the Gd(III) complex into the micellar structure and to the close spacing between the two octadecyl chains that both prevent independent rotations of the metal chelates and the micelles.

There are several approaches for interpreting relaxivity curves of slowly rotating Gd(III) complexes. In the Bertini−Kowalewski−Luchinat (BKL) approach,[66,67] the SBM equations have been modified to take into account a static zero-field splitting (ZFS) in addition to the transient ZFS that causes the electron spin relaxation. In another approach, the SBM equations have been adapted to better represent the partially independent motions of

a chelate and of the macromolecule to which it is linked. The Lipari−Szabo theory that is applied for this purpose yields different rotational correlation times for the slow and fast motions as well as a rigidity factor that describes the internal flexibility. Mathematical fits with the BKL computer program[66,67] and by the BMS−Lipari−Szabo approach led to the relaxivity parameters listed in Table 2. Caution must again be advised in accepting the values collected in this table because the number of parameters involved in the fit procedures is even larger than in the simple BMS treatment. In addition, several parameter sets can give equally good fits, and the parameters τ_v and Δ^2 in eq 2 are strongly correlated. We also neglected the relaxivity of the monomeric complex as its contribution to relaxivity must be very weak compared to the micelles because of its fast rotational time and as its concentration is very small because of its low cmc.[72] These problems have already been mentioned several times,[79] and ranges of values are probably more reliable. This being taken into account, there is a sizable increase in rotational correlation times upon micelle formation and the high value of the rigidity factor (S^2 = 0.78) supports the assumption that the Gd-$(C18)_2$DOTAda complex has little mobility in the micelles thanks to their two closely spaced aliphatic substituents. It should also be noted that the computed water exchange time τ_m of the micelles is two to three times higher than the one found for the parent unsubstituted chelates complex and is thus detrimental to a high relaxivity maximum at high fields. This does not prevent the Gd$(C18)_2$DOTAda micelles from exhibiting very high relaxivities. Finally, it should be noted that the changes undergone by the water exchange time τ_m upon formation of micelles remains poorly understood. The τ_m factors of Gd(III)-containing micelles have been reported to be identical,[72] larger,[73] or smaller[74] than that of the corresponding monomers.

4. CONCLUSIONS

Obtaining the very high relaxivities that could be reached according to the SBM equations remains an elusive goal, although much progress has been made. The easiest procedure consists in slowing down the rotation of a Gd^{3+} chelate by linking it covalently or noncovalently to a macromolecule whether synthetic or biological. However, relaxivities remain lower than expected because of a water exchange time that is too long and because of the flexibility of the chelate−macromolecule link. We report here an attempt at solving this problem by a double anchoring of the metal chelate. A DOTA ligand featuring two alkyne moieties grafted on two adjacent carbon atoms in the tetraaza ring was synthesized for this purpose and reacted by a "click" approach to add long aliphatic chains. The relaxivity of resulting micelles is particularly high presumably due to the double anchoring. In a future extension of the present research work, GdDOTAda will be reacted with polyazido ester polymers.[80] The monoalkyne ligands DOTAma and DO3ma were synthesized for comparison purposes, but they are interesting in their own right as reacting them with polyazido polymers is more straightforward.[81] Finally, the bisalkyne ligand DOTAda can be substituted with a large variety of chemical functions thanks to a "click" reaction with azide-featuring substrates. The rigid association of DOTAda lanthanide chelates with proteins for structure determination from the dipolar shifts and from the residual dipolar couplings is one application of potential interest.[25]

AUTHOR INFORMATION

Corresponding Author
*E-mail: jf.desreux@ulg.ac.be.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Fonds de la Recherche Scientifique (FNRS) and the Institut Interuniversitaire des Sciences Nucléaires of Belgium for their financial support.

REFERENCES

(1) (a) Merbach, A. E.; Toth, E. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Wiley: Chichester, 2001. (b) Caravan, P.; Ellison, J. J.; Mc.Murry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293–2352.
(2) Rebizak, R.; Schaefer, M.; Dellacherie, E. *Eur. J. Pharm. Sci.* **1999**, *7*, 243–248.
(3) Corsi, D. M.; Vander Elst, L.; Muller, R. N.; van Bekkum, H.; Peters, J. A. *Chem.—Eur. J.* **2001**, *7*, 1383–1389.
(4) Lebduskova, P.; Kotek, J.; Hermann, P.; Vander Elst, L.; Muller, R. N.; Likes, I.; Peeters, J. A. *Bioconjugate Chem.* **2004**, *15*, 881–889.
(5) Jaszberenyi, Z.; Moriggi, L.; Schmidt, P.; Weidensteiner, C.; Kneuer, R.; Merbach, A. E.; Helm, L.; Toth, E. *J. Biol. Inorg. Chem.* **2007**, *12*, 406–420.
(6) Langereis, S.; Dirksen, A.; Hackeng, T. M.; van Genderen, M. H. P.; Meijer, E. W. *New J. Chem.* **2007**, *31*, 1152–1160.
(7) Nicolle, G. M.; Toth, E.; Schmitt-Willich, H.; Raduchel, B. E.; Merbach, A. E. *Chem.—Eur. J.* **2002**, *8*, 1040–1048.
(8) Xu, H.; Regino, C. A. S.; Bernardo, M.; Koyama, Y.; Kobayashi, H.; Choyke, P. L.; Brechbiel, M. W. *J. Med. Chem.* **2007**, *50*, 3185–3193.
(9) Rudovsky, J.; Hermann, P.; Botta, M.; Aime, S.; Lukes, I. *Chem. Commun.* **2005**, 2390–2392.
(10) Caravan, P.; Cloutier, N. J.; Greenfield, M. T.; McDermid, S. A.; Dunham, S. U.; Bulte, J. W. M.; Amedio, J. C. J.; Looby, R. J.; Supkowski, R. M.; Horrocks, W. D., Jr.; McMurry, T. J.; Lauffer, R. B. *J. Am. Chem. Soc.* **2002**, *124*, 3152–3162.
(11) Caravan, P.; Parigi, G.; Chasse, J. M.; Cloutier, N. J.; Ellison, J. J.; Lauffer, R. B.; Luchinat, C.; McDermid, S. A.; Spiller, M.; McMurry, T. J. *Inorg. Chem.* **2007**, *46*, 6632–6639.
(12) Henoumont, C.; Henrotte, V.; Laurent, S.; Vander Elst, L.; Muller, R. N. *J. Bioinorg. Biochem.* **2008**, *102*, 721–730.
(13) Rostovsev, V. C.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 2596–2599.
(14) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *8*, 1040–1048.
(15) Wu, P.; Fokin, V. V. *Aldrichim. Acta* **2007**, *40*, 7–17.
(16) Prasuhn, D. E.; Yeh, R. M.; Obenaus, A.; Manchester, M.; Finn, M. G. *Chem. Commun.* **2007**, 1269–1271.
(17) Song, Y.; Kohlmeir, E. K.; Meade, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 6662–6663.
(18) Powell, D. H.; Ni Dhubhghaill, O. N.; Pubanz, D.; Helm, L.; Lebedev, Y. S.; Schlaepfer, W.; Merbach, A. E. *J. Am. Chem. Soc.* **1996**, *118*, 9333–9346.
(19) Toth, E.; Burai, L.; Brücher, E.; Merbach, A. E.; Paight, E. S. *J. Chem. Soc., Dalton Trans.* **1997**, 1587–1594.
(20) Bryson, J. M.; Chu, W. J.; Lee, J. H.; Reineke, T. M. *Bioconjugate Chem.* **2008**, *19*, 1505–1509.
(21) Morcos, S. K. *Eur. Radiol.* **2011**, 496–500.
(22) Knör, S.; Modlinger, A.; Poethko, T.; Schottelius, M.; Wester, H. J. *Chem.—Eur. J.* **2007**, *13*, 6082–6090.
(23) Stasiuk, G. J.; Lowe, M. P. *Dalton Trans.* **2010**, 9725–9727.
(24) Jauregui, M.; Perry, W. S.; Allain, C.; Vidler, L. R.; Willis, M. C.; Kenwright, A. M.; Snaith, J. S.; Stasiuk, G. J.; Lowe, M. P.; Faulkner, S. *Dalton Trans.* **2009**, 6283–6285.
(25) Keizers, P. H. J.; Desreux, J. F.; Overhand, M.; Ubbink, M. *J. Am. Chem. Soc.* **2007**, *129*, 9292–9293.

(26) Boschetti, F.; Denat, F.; Espinosa, E.; Lagrange, J.-M.; Guilard, R. *Chem. Commun.* **2004**, 588–589.
(27) Scheurer, A.; Mosset, P.; Saalfrank, R. W. *Tetrahedron: Asymmetry* **1997**, *8*, 1243–1251.
(28) Herve, G.; Bernard, H.; Le Bris, N.; Yaouanc, J.-J.; Handel, H.; Toupet, L. *Tetrahedron Lett.* **1998**, *39*, 6861–6864.
(29) Marti, M. J.; Rico, I.; Ader, J. C.; De Savignac, A.; Lattes, A. *Tetrahedron Lett.* **1989**, *30*, 1245–1248.
(30) Barge, A.; Cravotto, G.; Gianolio, E.; Fedeli, F. *Contrast Media Mol. Imaging* **2006**, *1*, 184–188.
(31) Chuburu, F.; Tripier, R.; Le Baccon, M.; Handel, H. *Eur. J. Org. Chem.* **2003**, 1050–1055.
(32) Uggeri, F.; Aime, S.; Anelli, P. L.; Botta, M.; Brochetta, M.; de Haën, C.; Ermondi, G.; Grandi, M.; Paoli, P. *Inorg. Chem.* **1995**, *34*, 633–642.
(33) Rousselin, Y.; Sok, N.; Boschetti, F.; Guilard, R.; Denat, F. *Eur. J. Org. Chem.* **2010**, 1688–1693.
(34) Desreux, J. F. *Inorg. Chem.* **1980**, *19*, 1319–1324.
(35) Chickos, J. S.; Bausch, M.; Alul, R. *J. Org. Chem.* **1981**, *46*, 3559–3562.
(36) Scheurer, A.; Mossett, P.; Saalfrank, R. W. *Tetrahedron: Asymmetry* **1999**, *10*, 3559–3570.
(37) Lin, W. O.; Figueira, J. A. A.; Alt, H. G. *Monatsh. Chem.* **1985**, *116*, 217–221.
(38) Krakowiak, K. E.; Bradshaw, J. S.; Izatt, R. M. *J. Heterocycl. Chem.* **1990**, *27*, 1585–1589.
(39) Desreux, J. F.; Tweedle, M. F.; Ratsep, P. C.; Wagler, T. R.; Marinelli, E. R. U.S. Patent US 5358704, 1994; pp 1−22.
(40) Anelli, L.; Beltrami, A.; Franzini, M.; Paoli, P.; Rossi, P.; Uggeri, F.; Virtuani, M. *Inorg. Chim. Acta* **2001**, *317*, 218–229.
(41) Greene, T. W.; Wuts, P. G. M. *Protective groups in organic synthesis*, 3rd ed.; Wiley: New York, 1999.
(42) Czech, B. P.; Bartsch, R. A. *J. Org. Chem.* **1984**, *49*, 4076–4078.
(43) Sato, S.; Nemoto, M.; Kumazawa, T.; Matsuba, S.; Onodera, J. I.; Aoyama, M.; Obarab, H.; Kamadab, H. *Carbohydr. Res.* **2004**, *339*, 2425–2432.
(44) Labadie, G. R.; Viswanathan, R.; Poulter, C. D. *J. Org. Chem.* **2007**, *72*, 9291–9297.
(45) Lucas, R.; Neto, V.; Bouazza, H.; Zerrouki, R.; Granet, R.; Ktausz, P.; Champavier, Y. *Tetrahedron Lett.* **2008**, *49*, 1004–1007.
(46) Mikami, K.; Kataoka, S.; Yusa, Y.; Aikawa, K. *Org. Lett.* **2004**, *6*, 3699–3701.
(47) Lamberth, C.; Jeanguenat, A.; Cederbaum, F.; De Mesmaeker, A.; Zeller, M.; Kempf, H. J.; Zeun, R. *Bioorg. Med. Chem.* **1990**, *27*, 1585–1589.
(48) Jones, J. B.; Wright, J. M.; Hybd, G.; Wyatt, J. K.; Warner, P. M.; Huber, R. S.; Li, A.; Kilgore, M. W.; Sticca, R. P.; Pollenz, R. S. *J. Org. Chem.* **2002**, *67*, 5727–5733.
(49) Dueno, E. E.; Chu, F.; Kim, S. I.; Jung, K. W. *Tetrahedron Lett.* **1999**, *40*, 1843–1846.
(50) Aime, S.; Botta, M.; Fasano, M.; Marques, M. P. M.; Geraldes, C. F. G. C.; Pubanz, D.; Merbach, A. E. *Inorg. Chem.* **1997**, *36*, 2059–2068.
(51) Howard, J. A. K.; Kenwright, A. M.; Moloney, J. M.; Parker, D.; Port, M.; Navet, M.; Rousseau, O.; Woods, M. *J. Chem. Soc., Chem. Commun.* **1998**, 1381–1382.
(52) Ranganathan, R. S.; Raju, N.; Fan, H.; Zhang, X.; Tweedle, M. F.; Desreux, J. F.; Jacques, V. *Inorg. Chem.* **2002**, *41*, 6856–6866.
(53) Woods, M.; Kovacs, Z.; Kiraly, R.; Brücher, E.; Zhang, S.; Sherry, A. D. *Inorg. Chem.* **2004**, *43*, 2845–2851.
(54) Di Bari, L.; Pescitelli, G.; Sherry, A. D.; Woods, M. *Inorg. Chem.* **2005**, *44*, 8391–8398.
(55) Woods, M.; Kovacs, Z.; Zhang, S.; Sherry, A. D. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 5889–5892.
(56) Banci, L.; Bertini, I.; Luchinat, C. *Nuclear and electron relaxation*; VCH: Weinheim, 1991.
(57) Bruce, J. I.; Lowe, M. P.; Parker, D. Photophysical aspects of lanthanide(III) complexes. In *The chemistry of contrast agents in MRI*; Merbach, A. E., Toth, E., Ed.; Wiley: New York, 2001; p 1.
(58) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493–503.
(59) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293–2352.
(60) Aime, S.; Botta, M.; Geninatti Crich, S.; Giovenzana, G.; Pagliarin, R.; Sisti, M.; Terreno, E. *Magn. Reson. Chem.* **1998**, *36*, 200–208.
(61) Botta, M.; Quici, S.; Pozzi, G.; Marzanni, G.; Pagliarin, R.; Barra, S.; Crich, S. G. *Org. Biol. Chem.* **2004**, *2*, 570–577.
(62) Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962**, *37*, 307–320.
(63) Paris, J.; Gameiro, C.; Humblet, V.; Mohapatra, P. K.; Jacques, V.; Desreux, J. F. *Inorg. Chem.* **2006**, *45*, 5092–5102.
(64) Aime, S.; Botta, M.; Bruce, J. I.; Mainero, V.; Parker, D.; Terreno, E. *Chem. Commun.* **2001**, *1*, 115–116.
(65) Vander Elst, L.; Sessoye, A.; Laurent, S.; Muller, R. N. *Helv. Chim. Acta* **2005**, *88*, 574–587.
(66) Bertini, I.; Galas, O.; Luchinat, C.; Parigi, G. *J. Magn. Reson. A* **1995**, *113*, 151–158.
(67) Bertini, I.; Kowalewski, J.; Luchinat, C.; Nilsson, T.; Parigi, G. *J. Chem. Phys.* **1999**, *111*, 5795–5807.
(68) Dunand, F. A.; Toth, E.; Hollister, R.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2001**, *6*, 247–255.
(69) Rudovsky, J.; Botta, M.; Hermann, P.; Hardcastle, K. I.; Lukes, I.; Aime, S. *Bioconjugate Chem.* **2006**, *17*, 975–987.
(70) Torres, S.; Martins, J. A.; André, J. P.; Geraldes, C. F. G. C.; Merbach, A. E.; Toth, E. *Chem.—Eur. J.* **2006**, *12*, 940–948.
(71) André, J. P.; Toth, E.; Fischer, H.; Seelig, A.; Mäcke, H. R.; Merbach, A. E. *Chem.—Eur. J.* **1999**, *5*, 2977–2983.
(72) Nicolle, G. M.; Toth, E.; Eisenwiener, K. P.; Mäcke, H. R.; Merbach, A. E. *J. Biol. Inorg. Chem.* **2002**, *7*, 757–769.
(73) (a) Parac-Vogt, T. N.; Kimpe, K.; Laurent, S.; Piérart, C.; Vander Elst, L.; Muller, R. N.; Binnemans, K. *Eur. J. Inorg. Chem.* **2004**, 3538–3543. (b) Kimpe, K.; Parac-Vogt, T. N.; Laurent, S.; Piérart, C.; Vander Elst, L.; Muller, R. N.; Binnemans, K. *Eur. J. Inorg. Chem.* **2003**, 3021–3027.
(74) Accardo, A.; Tesauro, D.; Morelli, G.; Gianolio, E.; Aime, S.; Vaccaro, M.; Mangiapia, G.; Paduano, L.; Schillen, K. *J. Biol. Inorg. Chem.* **2007**, *12*, 267–276.
(75) Gianolio, E.; Giovenzana, G.; Ciampa, A.; Lanzardo, S.; Imperio, D.; Aime, S. *ChemMedChem* **2008**, *3*, 60–62.
(76) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 9372–9376.
(77) Gianolio, E.; Giovenzana, G. B.; Longo, D.; Longo, I.; Menegotto, I.; Aime, S. *Chem.—Eur. J.* **2007**, *13*, 5785–5797.
(78) Laus, S.; Sour, A.; Ruloff, R.; Toth, E.; Merbach, A. E. *Chem.—Eur. J.* **2005**, *11*, 3064–3076.
(79) Kruk, D.; Nilsson, T.; Kowalewski, J. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4907–4917.
(80) Riva, R.; Schmeits, S.; Jérôme, C.; Jérôme, R.; Lecomte, P. *Macromolecules* **2007**, *40*, 796–803.
(81) Kimura, K.; Matsuba, T.; Tsujimura, Y.; Yokoyama, M. *Anal. Chem.* **1992**, *64*, 2508–2511.