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DOTMA-based amides (DOTMAMs) as a platform for the development of PARACEST MRI contrast agents

M. Suchý, A.X. Li, M. Milne, R. Bartha and R.H.E. Hudson

A synthetic methodology leading to previously unknown DOTMA-based secondary amides (DOTMAMs) has been developed. Alkylation of cyclen with l-lactic acid-derived pseudohalides was used as a key step affording the alkyl- and aryl-decorated DOTMAMs. Amino acid-decorated DOTMAMs were obtained via peptide coupling between DOTMA and protected amino acids. Metallation of the DOTMAMs ligand with Tm\(^{3+}\) gave complexes exhibiting proximal amide proton based paramagnetic CEST effects at < -50 ppm relative to water.

Introduction

Nowadays magnetic resonance imaging (MRI) represents an indispensable tool in clinical diagnostics. MRI is primarily based on the detection of water protons in the body and oftentimes is used in combination with an exogenous agent that enhances the image contrast.\(^1\) Such agents are termed ‘contrast agents’ (CAs) and the development of CAs suitable for MRI is an active area of research. Several types of CAs are currently available,\(^2\) among them paramagnetic chemical exchange saturation transfer (PARACEST) MRI CAs\(^3\) represent a relatively new group characterized by exceptional sensitivity to various parameters relevant to clinical diagnostics, such as temperature,\(^4\) pH,\(^5\) redox state,\(^6\) concentration of primary metabolites\(^7\) or enzymatic activity.\(^8\) Despite the potential usefulness of PARACEST CAs, their low in vivo sensitivity requires the administration of high concentrations (20-100 mM) of CAs,\(^9\) thus, it is important to employ ligands that stably bind the potentially toxic lanthanide metal, such as those based on the macrocyclic chelator DOTA, and employ strategies to increase the observable signals.\(^1\)

Upon administration into a biological system PARACEST MRI CAs experience a significant decrease in sensitivity attributable to magnetization transfer (MT) from endogenous macromolecules.\(^11,12\) The MT effect is a competing mechanism capable of saturating the bulk water spins and consequently lowers the contrast efficiency of PARACEST MRI CAs within the MT frequency range (ca. -100 to 100 ppm). Development of PARACEST MRI CAs exhibiting highly shifted CEST signals lying toward or beyond the outer limit of the MT frequency range represents a way to address the low sensitivity associated with these agents. In this regard, we recently demonstrated Tm\(^{3+}\) DOTAM-t-butyl (1, Figure 1),\(^13,14\) possessed an amide proton-based CEST effect clearly lying outside of the MT frequency range. The presence of the highly shifted CEST effect (δ = -102 ppm) was attributed to the predominance of the twisted square antiprism (TSAP) isomer of CA 1 in solution,\(^15\) which was favoured by the sterically demanding t-butylamides.\(^14\)

Unlike the Tm\(^{3+}\) complexes derived from DOTA (2, Figure 1) and unsubstituted DOTAM (3, Figure 1) which exist in solution predominantly (ca. 90%) in the square antiprism isomer (SAP), the Tm\(^{3+}\) complex derived from DOTMA\(^13,15\) (4, Figure 1) favours the TSAP isomer in solution (ca. 90%).\(^11\) This conformational bias is attributed to the steric effect due to the presence of the additional four methyl groups on the side arms of DOTMA (4). We hypothesized tetraamides derived from DOTMA, herein referred to as DOTMAMs,\(^16\) may possess highly shifted CEST signals similar to that observed for 1, and have a greater preference for the TSAP isomer as observed for the carboxylic acid DOTMA, thus potentially resulting in a more intense, highly-shifted CEST effect.

While the syntheses of various ligands based on the DOTA (2) or DOTAM (3) scaffold via peralkylation of cyclen (5, Figure 1) are well documented in the literature,\(^17\) only a small number of DOTMA-based ligands are presently known.\(^16,18\) Furthermore, only the primary amide derived from DOTMA

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Electronic Supplementary Information (ESI) available: Full experimental details, NMR and MS spectra associated with synthetic intermediates and final products. CEST spectra and Ω-plots associated with CAs 6-8, 10 and 11. See DOI: 10.1039/x0xx00000x

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(DOTMAM)\(^{19}\) has been described. This ligand was accessed by the peralkylation of cyclen with racemic 2-propionanamide resulting in a mixture of stereoisomeric ligands of which only the Eu\(^{3+}\) chelate was studied.\(^{19}\)

Our interest in Tm\(^{3+}\) chelates of DOTMAMs (with a secondary amide) required the development of a new synthetic methodology. Herein, we report the preparation of the hitherto unreported Tm\(^{3+}\) complex of known ligand \(^{6}\) and well as the synthesis of five new ligands and the Tm\(^{3+}\) complexes thereof (7-11, Figure 1) as well as evaluation of their CEST properties.

Results and discussion

Our studies began with the synthesis of corresponding electrophiles, which we envisioned using for the peralkylation of cyclen (5). A DCC-mediated peptide coupling between (S)-2-chloropropionic acid (12, Scheme 1) and benzylamine afforded corresponding secondary chloroderivative 13 (Scheme 1) in good yield using a modified literature procedure.\(^{20}\) Despite the use of 2-chloropropionic acid derivatives previously for the preparation of DOTMA,\(^{15}\) the reactivity of electrophile 13 was found to be insufficient to achieve the peralkylation of cyclen. Therefore, we turned our attention to the use of (S)-lactic acid derived pseudohalides as potential electrophiles. Coupling of (S)-lactic acid (14) with a small variety of amines,\(^{21}\) namely benzylamine, t-butylamine, aniline, and (L)-Lys(Boc)-OMe•HCl, proceeded smoothly and afforded the lactamides 15-18 (Scheme 1) in good yields (60-93%).

Firstly, conversion of the secondary OH group to the corresponding triflate (OTf-lactamide) was attempted. Among the few known preparations of DOTMA-based ligands, peralkylation of cyclen with OTf-(S)-lactic acid esters is used as a key step almost exclusively.\(^{16,18}\) Treatment of lactamide 15 with trifluoroacetic acid anhydride (Tf\(_2\)O)\(^{18a}\) afforded the unstable OTf-lactamide 15b (two signals observed in \(^{19}\)F NMR spectrum). However, treatment of lactamide 16 with Tf\(_2\)O produced the more stable OTf-lactamide 16b (one signal at \(\delta -74.9\) ppm in \(^{19}\)F NMR spectrum after storage for 24 hours at room temperature). Unfortunately, OTf-lactamide 16b was not stable under the forcing conditions required for peralkylation of cyclen (heat, excess of base) resulting in the formation of complex mixture devoid of the desired product of peralkylation.
containing only small amount (< 10%) of the desired product of peralkylation, obtained after careful HPLC purification.

The problem was solved by using OMs-(S)-lactamides 15a-18a (Scheme 1) obtained in excellent yields (> 90%) after treatment of lactamides 15-18 with MsCl under basic conditions (Scheme 1).24 With electrophiles 15a-18a in hand we investigated the alkylation of cyclen. Mixtures of di-, tri- and tetrasubstituted cyclens have been obtained, when electrophiles 15a-17a were used. The desired ligands were purified by HPLC, affording DOTMAM-Bn, DOTMAM-t-Bu and DOTMAM-Ph in low to moderate yields (14-42%) as described in the Supporting Information and shown in Scheme 2.

This methodology also proved successful for the preparation of the DOTMAM ligand with a primary amide group (Scheme 2). Thus 6 was obtained in 51% yield (see the Supporting Information for details) by peralkylation of cyclen with OMs-(S)-lactamide (19a, Scheme 1), followed by purification by flash column chromatography (FCC) on Al₂O₃.† Prolonged heating (48 h at 90 °C) of above mentioned ligands with TmCl₃·H₂O (in dioxane/water mixture, pH ~7) afforded the desired complexes 6-8 (Scheme 3), interestingly no metalation (complex 9) was observed when DOTMAM-Ph was treated with TmCl₃·H₂O under identical conditions (Scheme 2).

Peralkylation of cyclen with OMs-lactamide 18a did not lead to the formation of the desired DOTMAM-Lys(Boc)-OMe; therefore, we explored an alternative synthetic route to obtain the amino acid-decorated DOTMAM derived ligands. An HBTU-mediated coupling between DOTMA tetrasodium salt (4, Scheme 3) prepared according to the literature procedures6,18a and (L)-Lys(Boc)-OMe·HCl afforded the desired DOTMAM-(L)-Lys(Boc)-OMe (in 41% yield after the HPLC purification). Subsequent removal of protecting groups and metalation with TmCl₃·H₂O furnished the complex 10 in 27% overall yield (based on DOTMA tetrasodium salt) as shown in Scheme 3 and described in the Supporting Information. Conjugation of 4 with (L)-Phe-OMe·HCl, followed by removal of ester protecting groups by saponification and metalation with TmCl₃·H₂O gave the complex 11 in 25% overall yield (Scheme 3; see the Supporting Information for experimental details).

With complexes 6-8, 10 and 11 prepared, their potential as PARACEST MRI CAs was investigated. The CEST spectra associated with CAs 6-8, 10 and 11 were acquired as described in the Supporting Information. Temperature and pH sensitivity of the CAs 6-8, 10 and 11 was also investigated. When possible, the results were compared with those obtained previously for related DOTAM-based complexes.13,25 The results of these studies are summarized in Table 1 and discussed below.

**Table 1. CEST properties for CAs 6-8, 10 and 11.**

<table>
<thead>
<tr>
<th>CA</th>
<th>CEST effect (ppm)/signal intensity (%)</th>
<th>Related DOTAM complex; CEST effect (ppm)/signal intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-54 (6); -63 (7)</td>
<td>-46 (32); -51 (33)</td>
</tr>
<tr>
<td>7</td>
<td>-65 (6)</td>
<td>-51 (27)</td>
</tr>
<tr>
<td>8</td>
<td>-115 (3)</td>
<td>-68 (10); -102 (21)</td>
</tr>
<tr>
<td>10</td>
<td>-53 (20); -62 (10)</td>
<td>-50 (15)</td>
</tr>
<tr>
<td>11</td>
<td>-51 (1)</td>
<td>not available</td>
</tr>
</tbody>
</table>

* CEST spectra for 6-8, 10 and 11 were collected on a 9.4 T clinical MRI scanner at 15 mM of complex, 37 °C, pH 7.5 using a saturation pulse of 14 μT for 3.95 s. CEST spectra collected at: 14 T, 10 mM of complex, 37 °C, pH 7.0 using a saturation pulse of 650 Hz for 2 s. CEST spectra collected at: 14 T, 10 mM complex (in 90% D₂O), 37 °C, pH 7.2 using a saturation pulse of 20 μT for 2 s. CEST spectra collected at: 14 T, 10 mM complex, 37 °C, pH 7.0 using a saturation pulse of 20 μT for 10 s.

CA 6, derived from the unsubstituted DOTMAM ligand, and CAs 7 and 8, decorated with alkyl groups, all featured amide-proton based CEST effect with intensities that are 4-7 fold smaller (Table 1) than signals produced by the corresponding DOTAM-based counterparts (Tm³⁺ DOTAM, Tm³⁺ DOTAM-Bn and Tm³⁺ DOTAM-t-Bu).25 A CEST...
signal at lower chemical shift was not observed for CA 8 (Table 1), presumably it was too weak to be detected. On the other hand, the CEST signals associated with CAs 6-8 were shifted farther from bulk water signal (by 8-14 ppm, Table 1) as compared to the analogous DOTAM CAs. The signal reduction of DOTMAM-based CAs is caused by slower exchange rates (by ca. 2 fold) compared to related DOTAM-based complexes (244 μs for 7 versus 125 μs for TmDOTAM-Bn; 579 μs for 8 versus 273 μs for TmDOTAM-t-Bu). Should these findings with respect to pendant arm amide proton exchange rates extend to a slowing of water exchange at the metal center, examination of EuDOTMAM-based complexes (requiring slow exchange rates for bound water) may lead to the formation of PARACEST MRI CAs. These studies are beyond the scope of the present work.

TmDOTMAM-(L)-Lys-OH (10) possessed two CEST effects (Table 1), both showed a dependence on pH and temperature (Figure 2). The origin of the two peaks is possibly due to the presence of s-cis and s-trans amide conformations as suggested by the similarity in chemical shifts of the CEST signals to those present in the unsubstituted DOTMAM (6). Curiously, only a very weak CEST effect (Table 1) was observed for TmDOTMAM-(L)-Phe-OH (11). The intensity of the signal was found to increase with increasing pH (see the Supporting Information), implying that the exchange kinetics associated with CA 11 is out of the optimal range (too slow) for the observation of the CEST effect at pH 7; presence of base increases the exchange rate of the side chain amide protons resulting in a somewhat stronger signal at pH 8 (see Supporting Information). Our findings indicate that further variation of the amino acid residues present in DOTMAM-derived CAs might lead to complexes endowed with interesting CEST properties. Examination of the 1H NMR spectra for YbDOTMAM, YbDOTMAM-Bn and YbDOTMAM-t-Bu (supporting information) indicate these complexes exist predominantly as a single isomer. Comparison of the mostly highly shifted signal, the axial cyclen proton designated H4 for Eu3+DOTAM (SAP isomer ~ 40 ppm; TSAP isomer ~ 5 ppm) less highly shifted than the Eu3+DOTAM complex (SAP isomer ~ 44-59 ppm; TSAP isomer ~ 15-20 ppm). The Yb3+DOTAM complex exists as a single isomer (SAP) displaying a chemical shift of H4 = 95 ppm. The Yb3+DOTAM complex prepared during this work shows H4 = 124 ppm, which follows the trend of increasing chemical shift in going from the DOTAM to DOTMAM ligand observed for the Eu3+ complexes. The Yb3+DOTMAM-Bn shows a similar chemical shift for H4 (127 ppm). It is notable that both the Tm3+DOTMAM and Tm3+DOTMAM-Bn complexes both give rise to relatively low-shifted amide-proton CEST signals. This is in contrast to Yb3+DOTMAM-t-Bu which possesses a lower shifted H4 (104 ppm) yet the Tm3+ complex displays the CEST signal with the greatest hyperfine shift. It is tempting to suggest that the lower H4 chemical shift for Tm3+DOTMAM-t-Bu indicates that it exists in a different coordination geometry, possibly TSAP, which is responsible for the high shifting CEST signal and would be consistent with our previous work.13

Experimental

General Experimental Procedures

Reagents were commercially available and all solvents were HPLC grade except for water (18.2 MQ cm millipore water), CH2Cl2 and DMF (dried over Al2O3, in a solvent purification system). Solvents were removed under reduced pressure in a rotary evaporator, aqueous solutions were lyophilized and organic extracts were dried over Na2SO4. Flash column chromatography (FCC) was carried out using silica gel (SiO2), mesh size 230 - 400 Å and basic alumina (Al2O3), pH 9.5-10.5, mesh size 10 – 100 Å. Thin-layer chromatography (TLC) was carried out on Al backed silica gel or alumina plates with compounds visualised by I2 vapours, anisaldehyde stain, 5% ninhydrin stain, phosphomolybdic acid stain, and UV light. Melting points (mp) were obtained on Fisher–Johns apparatus.
and are uncorrected. Specific rotations [α]_D were determined by polarimeter at ambient temperature using a 1 ml, 10 cm path length cell; the units are 10⁻² deg cm² g⁻¹ and the concentrations are reported in g/100 mL. Ultra performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7μm; 1.0 id × 100 mm) and H R-ESI-MS detector. Mobile phase: Method A: 100% H₂O – 100% MeCN (both solvents containing 0.1% HCOOH) over 5 min, then 100% MeCN for 2 min, linear gradient, flow rate 0.1 mL/min. HPLC purification was performed using a Delta-Pak C18 300 Å column (particle size 15 μm; 8 × 100 mm Radial-Pak cartridge). Mobile phase for Method B (DOTMAM-Bn) was 0 min, 90% H₂O – 10% MeCN (for each method both solvents containing 0.1% TFA) to 13 min, 20% H₂O – 80% MeCN.; Method C (DOTMAM-t-Bu, 7): 0 min, 90% H₂O – 10% MeCN to 11 min, 13% H₂O – 87% MeCN; Method D (DOTMAM-Ph): 0 min, 90% H₂O – 10% MeCN to 13 min, 100% MeCN; Method E [DOTMAM-(L)-Lys(Boc)-OMe, DOTMAM-(L)-Phe-OEt]: 0 min, 90% H₂O – 10% MeCN to 10 min, 100% MeCN; Method F (Bz t-Bu O): 0 min, 90% H₂O – 10% MeCN to 11 min, 20% H₂O – 80% MeCN; linear gradient and 3 mL/min flowrate were used for each method. Size exclusion chromatography (SEC) was carried out on Bio-Gel P2, 45-90 μm mesh resin (8 g, per 0.1 mmol of compound). Ten fractions (10 ml each) were collected and identified with i3 vapours and UV light. Absence of free Tm³⁺ was verified by xylanol orange test. NMR spectra were recorded on a 400 MHz spectrometer for ¹H NMR spectra δ values were recorded as follows: CDCl₃ (7.27 ppm); CD₂OD (3.31; 4.87 ppm), D₂O (4.75 ppm) for ¹³C (125 MHz) δ CDCl₃ (77.0 ppm); CD₂OD (49.2 ppm). Mass spectra (MS) were obtained using electron impact (EI) or electrospray ionisation (ESI). Chemical exchange saturation transfer (CEST) spectra were acquired using a 9.4 T small animal clinical MRI scanner as follows: NMR tubes with solutions of the complexes (15 mM, pH 7.5) were imaged at 37 °C [the temperature was monitored and controlled by blowing hot air using a Model 1025 Small Animal Monitoring and Gating System (SA Instruments, Inc., Stony Brook, NY)] using a fast spin echo pulse sequence (FOV: 12.8 × 12.8 mm², matrix: 32 × 32, TR = 4000 ms, 4 echoes, and TE = 10 ms), preceded by a frequency selective saturation pulse (B1 = 14 μT, saturation range = -150 to 50 ppm in steps of 1 ppm, saturation time = 3.95 s). CEST spectra were generated using the average signal intensity from each tube. Similar methodology was used to evaluate the temperature (15 mM, pH 7.5, temperature range 33-39 °C) and pH (15 mM, 37 °C, pH range 6.0-8.0) sensitivity of selected agents. The exchange rates of the amide protons with bulk water were measured by 0-plot method as described previously. 

Preparation of (S)-N-benzyl-2-chloropropanamide

The reaction was carried out as described in the literature, starting from 430 μL (5 mmol) of S-chloropropionic acid (12). The product was isolated by FCC on 70 g SiO₂, eluting with hexanes/EtOAc (3:1), obtained (S)-N-benzyl-2-chloropropanamide (13, 813 mg, 82%), colorless solid. [α]_20D -1.3 (c 1, MeOH); lit. [α]_20D -3.8 (c 1, MeOH). ¹H NMR (CDCl₃) δ 7.37 (m, 2H); 7.31 (m, 3H); 6.88 (br s, D₂O exch, 1H); 4.49 (m, 3H); 1.79 (d, J = 7.0 Hz, 3H). ¹³C NMR (CDCl₃) δ 169.4, 137.4, 128.6, 127.5 (2 × C), 55.6, 43.7, 22.5. HRMS (EI) m/z; found 197.0602 [M⁺]⁺ (calcd 197.0607 for C₈H₁₀NO); LRMS (EI) m/z (rel. abundance): 197 [M⁺] (23), 162 (85), 106 (14).

Coupling of (S)-lactic acid with aniline, benzylamine and t-butylamine

Separate mixtures containing (S)-lactic acid (14, 450 mg, 5 mmol for benzylamine or 180 mg, 2 mmol), HOBT (1.35 g, 10 mmol for benzylamine or 540 mg, 4 mmol), DCC (1.55 g, 7.5 mmol for benzylamine or 619 mg, 3 mmol) and benzylamine (660 μL, 6 mmol), aniline (220 μL, 2.4 mmol) or t-butylamine (220 μL, 2.4 mmol) in dry CH₂Cl₂ (15 mL for benzylamine or 6 mL) were stirred for 24 h at room temperature (rt). The solids were filtered off, the filters were washed with ice cold CH₂Cl₂. Resulting solutions were washed with saturated NaHCO₃ solution (30 mL for benzylamine or 20 mL), the aqueous layers were extracted with CH₂Cl₂ (2 × 30 mL for benzylamine or 2 × 20 mL). Combined organic extracts were dried, were concentrated, the residues were dissolved in small amount of cold acetone (ca. 5 – 10 mL) and precipitated dicyclohexylurea was filtered off using a Pasteur pipette with a cotton plug. The volatiles were evaporated and the residues were subjected to FCC on 40 g SiO₂, eluting with hexanes/EtOAc (3:1, reaction with benzylamine); 30 g SiO₂, eluting with CH₂Cl₂/MeOH (98:2, reaction with aniline) or 25 g SiO₂, eluting with CH₂Cl₂/MeOH (95:5). Evaporation of the eluates afforded (S)-N-benzyl lactamide (15, 727 mg, 81%), (S)-N-t-butyl lactamide (16, 175 mg, 60%) or (S)-N-phenyl lactamide (17, 266 mg, 81%).

(S)-N-benzyl lactamide (15), colorless oil. [α]_20D -6.4 (c 1, MeOH); lit. [α]_20D -6.8 (c 6.9, CHCl₃). ¹H NMR (CDCl₃) δ 7.29 (m, 5H); 7.11 (br s, D₂O exch, 1H); 4.41 (d, J = 6.0 Hz, 2H); 4.24 (q, J = 7.0 Hz, 1H); 1.42 (d, J = 7.0 Hz, 3H). ¹³C NMR (CDCl₃) δ 174.7, 137.8, 128.7, 127.6, 127.5, 68.3, 43.0, 21.2. HRMS (EI) m/z; found 179.0952 [M⁺]⁺ (calcd 179.0946 for C₈H₁₁NO₂); LRMS (EI) m/z (rel. abundance): 179 [M⁺] (33), 161 (15), 134 (10), 91 (100), 65 (12).

(S)-N-t-butyl lactamide (16), colorless solid. [α]_20D -28.0 (c 1, MeOH). ¹H NMR (CDCl₃) δ 6.67 (s, D₂O exch, 1H); 4.09 (q, J = 6.5 Hz, 1H); 3.52 (br s, D₂O exch, 1H); 1.38 (d, J = 6.5 Hz, 3H); 1.36 (s, 9H). ¹³C NMR (CDCl₃) δ 174.5, 68.3, 50.7, 28.6, 21.1. HRMS (EI) m/z; found 145.1107 [M⁺]⁺ (calcd 145.1103 for C₈H₁₃NO); LRMS (EI) m/z (rel. abundance): 145 [M⁺] (95), 130 (100), 101 (67).

(S)-N-phenyl lactamide (17), colorless oil. [α]_20D -41.0 (c 0.5, MeOH); lit. [α]_20D -23.5 (c 0.48, CHCl₃). ¹H NMR (CDCl₃) δ 6.87 (br s, D₂O exch, 1H); 7.52 (m, 2H); 7.30 (m, 2H); 7.12 (m, 1H); 4.31 (q, J = 6.5 Hz, 1H); 4.14 (br s, D₂O exch, 1H); 1.48 (d, J = 6.5 Hz, 3H). ¹³C NMR (CDCl₃) δ 173.2, 137.0, 129.0, 124.6, 119.9, 68.7, 21.0. HRMS (EI) m/z; found 165.0797 [M⁺]⁺ (calcd 165.0790 for C₈H₁₂NO₂); LRMS (EI) m/z (rel. abundance): 165 [M⁺] (50), 121 (21), 93 (100), 65 (13).

Coupling of (S)-lactic acid with H-(L)-Lys(Boc)-OMe•HCl
A stirred mixture containing (5)-lactic acid (14, 180 mg, 2 mmol), H-Lys(Boc)-OMe·HO (594 mg, 2 mmol), HOBt (270 mg, 2 mmol) and Et3N (280 μL, 2 mmol) in dry CH2Cl2 (15 mL) was cooled to 0 °C, followed by an addition of DCC (825 mg, 4 mmol). The stirring continued for further 30 min at 0 °C, the cooling bath was removed and the stirring was continued for further 48 h at rt. The solids were filtered off, the filter was washed with ice cold CH2Cl2. Resulting solution was washed with saturated NaHCO3 solution (30 mL), the aqueous layer was extracted with CH2Cl2 (30 mL). Combined organic extracts was diluted, was concentrated, the residue was dissolved in small amount of cold acetone (ca. 5 – 10 mL) and precipitated dicyclohexylurea was filtered off using a Pasteur pipette with a cotton plug. The volatiles were evaporated and the residue was subjected to FCC on 50 g SiO2, eluting with CH2Cl2/MeOH (95:5). Evaporation of the eluate afforded (S)-(S)-(Lys(Boc)-OMe) lactamide (18, 621 mg, 93%) as colorless oil. [α]D -11.9 (c 1.4, MeOH).1H NMR (CDCl3) δ 7.20 (d, D2O exch., J = 8.5 Hz, 1H); 4.71 (br s, D2O exch, 1H); 4.57 (m, 1H); 4.24 (q, J = 6.5 Hz, 1H); 3.73 (s, 3H); 3.08 (m, 2H); 1.86 (m, 1H); 1.71 (m, 1H); 1.49 (m, 2H); 1.43 (m, 12H); 1.35 (m, 2H).13C NMR (CDCl3) δ 174.8, 172.8, 156.1, 79.2, 68.3, 52.4, 51.5, 40.1, 31.9, 29.4, 28.4, 22.4, 21.0. HRMS (ESI) m/z: found 333.2025 [M + H]+ (calcld. 333.2026 for C19H20N2O4). Reaction of (S)-N-t-butyl lactamide with TfO

A round bottom flask containing (S)-(S)-t-butyl lactamide (16, 202 mg, 1.39 mmol) was flushed with N2, followed by the addition of dry CH2Cl2 (2 mL) and dry pyridine (120 μL, 1.46 mmol). The solution was cooled to 0 °C, followed by a dropwise addition (over ca. 1 min) of TfO (230 μL, 1.39 mmol). The stirring continued for further 1 h at 0 °C, the solvent was removed and the residue was subjected to FCC on 25 g SiO2, eluting with hexanes/EtOAc (1:1). Evaporation of the eluate afforded (S)-(O)-Tf-N-t-butyl lactamide (16b, 223 mg, 34%) as colorless solid. [α]D -16.8 (c 1.4, MeOH).1H NMR (CDCl3) δ 5.94 (br s, D2O exch, 1H); 5.14 (q, J = 7.0 Hz, 1H); 1.70 (d, J = 7.0 Hz, 3H); 1.38 (s, 9H).13C NMR (CDCl3) δ 166.0, 123.1, 120.0, 116.8, 113.6, 83.4, 52.1, 28.4, 19.1.19F NMR (CDCl3) δ -74.9. HRMS (EI) m/z: found 277.0591 [M]+ (calcld. 277.0596 for C4H14F2O2N5S); LRMS (EI) m/z (rel. abundance): 373 [M]+ (18), 262 (100), 222 (44), 177 (20), 112 (42), 69 (90). Reaction of (S)-N-benzyl lactamide with TsCl

A solution of (S)-N-benzyl lactamide (15, 295 mg, 1.65 mmol) in dry CH2Cl2 (6 mL) was cooled to 0 °C, followed by the addition of TsCl (471 mg, 3.29 mmol), DMAP (24 mg, 0.2 mmol) and TsCl (471 mg, 2.46 mmol). The mixture was stirred for 1 h at 0 °C, then for 24 h at rt. The solvent was evaporated and the residue was subjected to FCC on 30 g SiO2, eluting with hexanes/EtOAc (3:1). Evaporation of the eluate afforded (S)-(O)-Ts-N-benzyl lactamide (15c, 450 mg, 82%) as colorless solid. [α]D -53.9 (c 1, MeOH).1H NMR (CDCl3) δ 7.77 (d, J = 8.0 Hz, 2H); 7.33 (m, 5H); 7.20 (d, J = 6.5 Hz, 2H); 6.62 (br s, D2O exch, 1H); 4.92 (q, J = 7.0 Hz, 1H); 4.41 (d, J = 6.0 Hz, 2H); 2.46 (s, 3H); 1.47 (d, J = 7.0 Hz, 3H).13C NMR (CDCl3) δ 168.6, 145.4, 137.3, 132.6, 130.0, 128.6, 127.7, 127.4, 77.3, 43.1, 21.6, 18.7. HRMS (EI) m/z: found 179.0952 [M]+ (calcld. 179.0946 for C12H12N2O3S); LRMS (EI) m/z (rel. abundance): 179 [M]+ (33), 161 (15), 134 (10), 91 (100), 65 (12). HRMS (ESI) m/z: found 334.1124 [M + H]+ (calcld. 334.1113 for C12H12N2O3S). Reaction of (S)-lactamides 15-19 with MsCl

Separate suspensions or solutions of (S)-N-benzyl lactamide (15, 788 mg, 4.4 mmol), (S)-N-t-butyl lactamide (16, 175 mg, 1.21 mmol), (S)-N-phenyl lactamide (17, 266 mg, 1.61 mmol), (S)-(S)-(Lys(Boc)-OMe) lactamide (18, 279 mg, 0.84 mmol) or (S)-(S)-lactamide (19, 891 mg, 10 mmol) in dry CH2Cl2 (20 mL in the case of (S)-N-benzyl lactamide (15); 6 mL in the case of (S)-N-t-butyl lactamide (16); 8 mL in the case of (S)-N-phenyl lactamide (17); 4 mL in the case of (S)-(S)-(Lys(Boc)-OMe) lactamide (18); 15 mL in the case of (S)-(S)-lactamide (19) and Et3N (1.12 mL, 8 mmol in the case of (S)-N-benzyl lactamide (15); 310 μL, 2.19 mmol in the case of (S)-N-t-butyl lactamide (16); 410 μL, 2.93 mmol in the case of (S)-N-phenyl lactamide (17); 580 μL, 4.2 mmol in the case of (S)-(S)-(Lys(Boc)-OMe) lactamide (18); 2.54 mL, 18.2 mmol in the case of (S)-lactamide (19)) were cooled to 0 °C, followed by a dropwise addition (over ca. 1 min) of MsCl (430 μL, 5.58 mmol in the case of (S)-N-benzyl lactamide (15); 120 μl, 1.53 mmol in the case of (S)-N-t-butyl lactamide (16); 160 μL, 2.04 mmol in the case of (S)-N-phenyl lactamide (17); 90 μL, 1.07 mmol in the case of (S)-(S)-(Lys(Boc)-OMe) lactamide (18); 980 μL, 12.7 mmol in the case of (S)-lactamide (19)). The cooling baths were removed and the mixtures were stirred at rt as follows: 2 h in the case of (S)-N-benzyl lactamide (15) and (S)-(S)-(Lys(Boc)-OMe) lactamide (18); 4 h in the case of (S)-N-t-butyl lactamide (16) and (S)-N-phenyl lactamide (17); 24 h in the case of (S)-lactamide (19). The volatiles were evaporated and the residues were subjected to FCC as follows: 40 g SiO2, eluting with hexanes/EtOAc (1:1) in the case of (S)-N-benzyl lactamide (15) and (S)-N-phenyl lactamide (17); 30 g SiO2, eluting with hexanes/EtOAc (1:1) in the case of (S)-N-t-butyl lactamide (16); 30 g SiO2, eluting with CH2Cl2/MeOH (95:5) in the case of (S)-(S)-(Lys(Boc)-OMe) lactamide (18); 60 g SiO2, eluting with CH2Cl2/MeOH (95:5) in the case of (S)-lactamide (19). Evaporation of the eluates afforded (S)-(O)-Ms-N-benzyl lactamide (15a, 1.05 g, 93%), (S)-(O)-Ms-N-t-butyl lactamide (16a, 253 mg, 94%), (S)-(O)-Ms-N-phenyl lactamide (17a, 373 mg, 95%) and (S)-(O)-Ms-(S)-(Lys(Boc)-OMe) lactamide (18a, 422 mg, 93%). In the case of (S)-lactamide (19) was the eluate concentrated to ca. one quarter of its original volume (a precipitate started to form), hexanes were added and the mixture was set aside for 2 h at -10 °C. Separated precipitate was filtered off, washed with hexanes and dried to afford (S)-(O)-Ms-lactamide (19a, 1.34 g, 80%).
(S)-O-Ms-N-t-butyl lactamide (16a), colorless solid. $\delta_{1}^1$H NMR (CDCl$_3$) $\delta$ 6.09 (br s, D$_2$O exch, 1H); 4.95 (q, $J$ = 7.0 Hz, 1H); 3.10 (s, 3H); 1.60 (d, $J$ = 7.0 Hz, 3H); 1.38 (s, 9H). $^{13}$C NMR (CDCl$_3$) $\delta$ 167.7, 51.6, 38.8, 28.5, 18.8. HRMS (EI) m/z: found 223.0874 $[M]$ (calcld. 223.0878 for C$_{14}$H$_{21}$NO$_3$); LRMS (EI) m/z (rel. abundance): 223 $[M]^+$ (29), 208 (100), 180 (16), 135 (12), 84 (79).

(S)-O-Ms-N-phenyl lactamide (17a), colorless solid. $\delta_{1}^1$H NMR (CDCl$_3$) $\delta$ 8.13 (br s, D$_2$O exch, 1H); 7.55 (m, 2H); 7.35 (m, 2H); 7.17 (m, 1H); 1.90 (m, 1H); 1.72 (m, 1H); 1.63 (d, $J$ = 7.0 Hz, 3H); 1.49 (m, 2H); 1.42 (s, 9H); 1.35 (m, 2H). $^{13}$C NMR (CDCl$_3$) $\delta$ 172.2, 168.6, 156.0, 79.1, 76.3, 52.5, 51.8, 40.0, 38.7, 31.8, 29.4, 28.4, 22.3, 19.2. HRMS (ESI) m/z: found 411.1786 $[M + H]^+$ (calcld. 411.1801 for C$_{14}$H$_{21}$N$_2$O$_5$).

(SO-Ms-4-(L)-Lys-(Boc)-OMe lactamide (18a), pale yellow oil. $\delta_{1}^1$H NMR (CDCl$_3$) $\delta$ 7.00 (s, D$_2$O exch, J = 8.0 Hz, 1H); 5.08 (q, $J$ = 7.0 Hz, 1H); 4.63 (br s, D$_2$O exch, 1H) 4.58 (m, 1H); 3.74 (s, 3H); 3.14 (s, 3H); 1.50 (m, 1H); 1.51 (m, 1H); 1.48 (m, 2H); 1.45 (m, 2H); 1.38 (s, 9H); 1.35 (m, 2H). $^{13}$C NMR (CDCl$_3$) $\delta$ 167.7, 66.6, 36.8, 18.8. HRMS (ESI) m/z: found 817.5093 $[M + H]^+$ (calcld. 817.5129 for C$_{46}$H$_{51}$N$_2$O$_8$).

Coupling of DOTMA × 4Na$^+$ with H-(L)-Lys(Boc)-OMe • HCl and H-(L)-Phe-OEt • HCl

A stirred separate suspensions of DOTMA×4Na$^+$ (4, 82 mg, 0.15 mmol), prepared according to previously described procedures$^{57,58}$ and H-(L)-Lys(Boc)-OMe • HCl (178 mg, 0.16 mmol) or H-(L)-Phe-OEt • HCl (138 mg, 0.6 mmol) in DIPA (210 $\mu$L, 1.2 mmol) and dry DMF (1 mL) were cooled to 0°C, followed by the addition of HBTU (228 mg, 0.6 mmol). The mixtures were stirred for 10 min at 0°C, then for 24 h at 60°C, then they were cooled to rt and were diluted with brine (50 mL), followed by the extraction with EtOAc (2 × 25 mL). Combined organic extracts were washed with brine (3 × 50 mL), were dried and were concentrated. The residues were dissolved in MeOH (3 mL) and were subjected to semi-preparative HPLC purification as described in General experimental procedures. The fractions containing the desired product were combined and concentrated to leave DOTMA-Bn × 4CF$_3$COO$^-$ (115 mg, 42%), DOTMA-t-Bu × 4CF$_3$COO$^-$ (67 mg, 24%) or DOTMA-Ph × 4CF$_3$COO$^-$ (43 mg, 14%). The residue obtained in the case of (S)-O-Ms-lactamide (16a) was subjected to FCC on 25 g Al$_2$O$_3$, eluting first with CH$_2$Cl$_2$/MeOH/NH$_4$OH (80:19:1, 3 fractions ca. 30 mL each) later with MeOH (8-10 fractions, ca. 30 mL each). The later fractions were concentrated to give DOTMA (141 mg, 51%).
DOTMAM-(L)-Phe-OEt × 4CF₃COO, pale brown solid. HPLC, Method E, tₑ₉ 7.8 min. ¹H NMR (CD₂OD) δ 7.31-7.02 (br m, 20 H); 5.06 (br m, 4H); 4.46-4.12 (br m, 2H); 3.41-2.31 (br m, 2H); 1.48 (br s, 6H); 1.35 (m, 12H); 1.08 (br s, 6H). HRMS (ESI) m/z: found 1161.6594 [M + H]+ (calcd. 1161.6600 for C₄₅H₆₀N₈O₁₂).

Deprotection of DOTMAM-(L)-Lys(Ob)c-OMe
A solution of DOTMAM-(L)-Lys(Ob)c-OMe × 4CF₃COO (117 mg, 0.062 mmol) in TFA (1 mL) was stirred for 20 min at rt. The volatiles were evaporated, the residue was dissolved in MeOH (600 µL), followed by the addition of NaOH solution (2.5 M, 1 mL, 2.5 mmol). Resulting mixture was stirred for 2 h at 60 °C, the mixture was cooled to 0 °C and the pH was adjusted to ca. 7.0 (1 M HCl). Resulting aqueous solution containing the desired product was concentrated, were dissolved in water (1 mL) and were neutralized (pH ca. 7.0, 1 M NaOH solution).

Saponification of DOTMAM-(L)-Lys-OH
A solution of DOTMAM-(L)-Lys-OH × 4CF₃COO (117 mg, 0.055 mmol) in dioxane [2 mL] and was subjected to SEC as described in General experimental procedures. The fractions containing the desired product were combined and concentrated to leave DOTMAM-(L)-Lys-OH (50 mg, 83%) as colorless solid.

Metallation of DOTMAM, DOTMAM-Bn, DOTMAM-t-Bu, DOTMAM-(L)-Lys-OH and DOTMAM-(L)-Phe-OH with TmCl₄•H₂O
Separate solutions of TmCl₄•H₂O [34 mg, 0.13 mmol in the case of DOTMAM; 13 mg, 0.047 mmol in the case of DOTMAM-Bn; 6 mg, 0.021 mmol in the case of DOTMAM-t-Bu; 18 mg, 0.066 mmol in the case of DOTMAM-(L)-Lys-OH and DOTMAM-(L)-Phe-OH] in water [2 mL in the case of DOTMAM, DOTMAM-(L)-Lys-OH and DOTMAM-(L)-Phe-OH; 2.5 mL in the case of DOTMAM-Bn; 1 mL in the case of DOTMAM-t-Bu] were added to separate solutions of DOTMAM (46 mg, 0.1 mmol), DOTMAM-Bn × 4CF₃COO (54 mg, 0.043 mmol), DOTMAM-t-Bu × 4CF₃COO (22 mg, 0.019 mmol), DOTMAM-(L)-Lys-OH × 4CF₃COO (54 mg, 0.055 mmol) and DOTMAM-(L)-Phe-OH × 4CF₃COO (57 mg, 0.055 mmol) in dioxane [2 mL in the case of DOTMAM, DOTMAM-(L)-Lys-OH and DOTMAM-(L)-Phe-OH; 2.5 mL in the case of DOTMAM-Bn; 1 mL in the case of DOTMAM-t-Bu]. The mixtures were stirred for 48 h at 90 °C, while the pH was maintained at ca. 6.5-7.0 (1 M NaOH solution). Reaction mixtures were transferred into centrifuge tubes and were lyophilized. The residues obtained from DOTMAM-Bn and DOTMAM-t-Bu were dissolved in MeOH/water (1.5 mL each) and were subjected to semipreparative HPLC purification as described in General experimental procedures. The fractions containing the desired product were combined, were concentrated, were dissolved in water (1 mL) and were neutralized (pH ca. 7.0, 1 M NaOH solution).

Conclusions
In summary, we have developed a new synthetic methodology and prepared the first examples of DOTMAM ligands possessing secondary amides including amino acids. This was achieved by tetraalkylation of cyclen with Tm⁺ ions and prepared the first examples of DOTMAM ligands possessing secondary amides including amino acids. This was achieved by tetraalkylation of cyclen with Tm⁺ ions. The complexes, the intensity of the CEST effects were observed to be weaker. This has been ascribed to less favourable (slower) amide proton exchange rates. The L-lysine conjugated agent displayed temperature- and pH-dependent CEST effects in the physiological range. Based on similarity to the analogous DOTMAM-based agent, it is expected to have reasonable biocompatibility and may be suitable for future in vivo applications.
studies.\textsuperscript{9,29} Future work should investigate Eu\textsuperscript{3+} DOTMAM-alkyl decorated complexes as well as a wider selection of Tm\textsuperscript{3+} DOTMAM-amino acid decorated complexes for useful CEST properties.

Examination of the Yb\textsuperscript{3+} complexes for DOTMAM, DOTMAM-Bn and DOTMAM-\textit{t}-Bu indicated an overwhelming predominance of a single coordination isomer in solution although the \textit{t}-butyl amide appeared different than the other two. The Tm\textsuperscript{3+} complexes of these ligands also show the greatest difference in the chemical shift of the amide-proton CEST signal. On the basis of the cyclen H\textsubscript{4} proton chemical shift we speculate that the TSAP isomer is most favoured in this series by the combination of the \textit{t}-butyl amide substituent and DOTMAM ligand, although further studies are needed to elucidate the geometry unambiguously.

Acknowledgements

Financial support from the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged. We thank Prof. J. M. Chong (University of Waterloo) for the assistance with optical rotation measurements.

Notes and references

\# DOTAM refers to the tetramide derivative of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid).

\$ The term DOTMA is used to refer to the RRRR-enantiomer of \(\alpha,\alpha',\alpha'',\alpha'''\) -tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetate in this manuscript.

\% The term DOTMAM is used to denote DOTMA-based amides in analogy to DOTAM which is used to denote DOTA-based amides. This abbreviation was first used by Merchbach and coworkers with respect to the primary amide derivative of DOTMA. See reference 19.

\* The outcome of FCC purification depended strongly on quality of Al\textsubscript{2}O\textsubscript{3}, the best results were obtained with 10-100 mesh, pH 9.5-10.5.

\( The coupling between DOTMA+4Na\textsuperscript{+} and benzylamine (7\% yield) was found to be inferior to that obtained by tetraalkylation of cyclen with OMs-(S)-benzyl lactamide.

\( The CEST effect associated with CA 11 was too weak to measure the exchange rate.

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R = H, alkyl, aryl, amino acid