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

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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.[10]

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,‡] possessed an amide proton-based CEST effect clearly lying outside of the MT frequency range. The presence of the highly shifted CEST effect ($\delta = -102$ ppm) was attributed to the predominance of the twisted square antiprism (TSAP) isomer of CA **1** in solution[13] 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[15,§] (**4**, Figure 1) favours the TSAP isomer in solution (*ca.* 90%).[16] 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,[§§] 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

(DOTMAM)[19] has been described. This ligand was accessed by the peralkylation of cyclen with racemic 2-propanionamide 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.

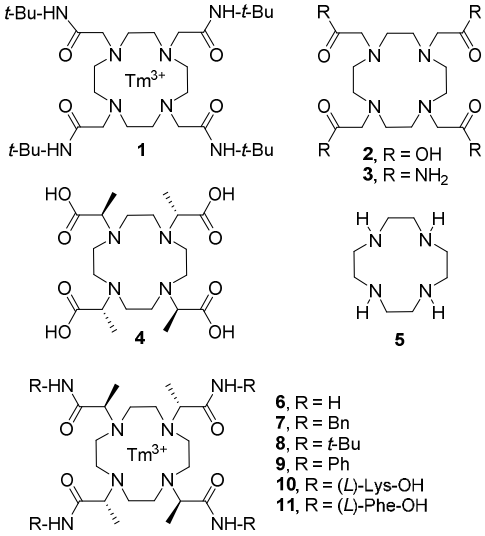


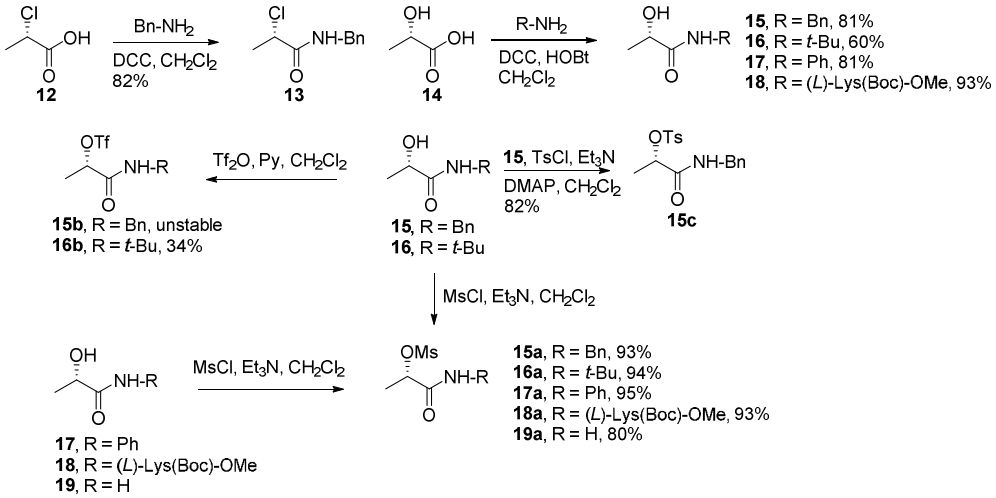
Figure 1. Chemical structures of Tm^{3+} DOTAM-*t*-Bu (**1**), DOTA (**2**), DOTAM (**3**), DOTMA (**4**), cyclen (**5**) and the DOTMA-derived complexes **6-11**.

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 (*O*Tf-lactamide) was attempted. Among the few known preparations of DOTMA-based ligands, peralkylation of cyclen with *O*Tf-(*S*)-lactic acid esters is used as a key step almost exclusively.[16,18] Treatment of lactamide **15** with trifluoroacetic acid anhydride (Tf_2O)[18a] afforded the unstable *O*Tf-lactamide **15b** (two signals observed in ^{19}F NMR spectrum). However, treatment of lactamide **16** with Tf_2O produced the more stable *O*Tf-lactamide **16b** (one signal at δ -74.9 ppm in ^{19}F NMR spectrum after storage for 24 hours at room temperature). Unfortunately, *O*Tf-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.

Scheme 1. Preparation of electrophiles 15a-15c, 16a, 16b, 17a-19a

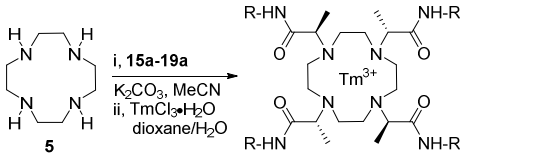


Allen's group has recently reported an alkylation of *N*,*O*-macrocycles related to cyclen by *O*Ts-(*S*)-lactamide.[22] The conversion of lactamide **15** to *O*Ts-(*S*)-benzyl lactamide (**15c**, Scheme 1) proceeded smoothly (82% yield).[23] Unfortunately, the alkylation of cyclen with **15c** resulted in a complex mixture

containing only small amount (< 10%) of the desired product of peralkylation, obtained after careful HPLC purification.

The problem was solved by using *O*Ms-(*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.

Scheme 2. Preparation of CAs **6**-**9**

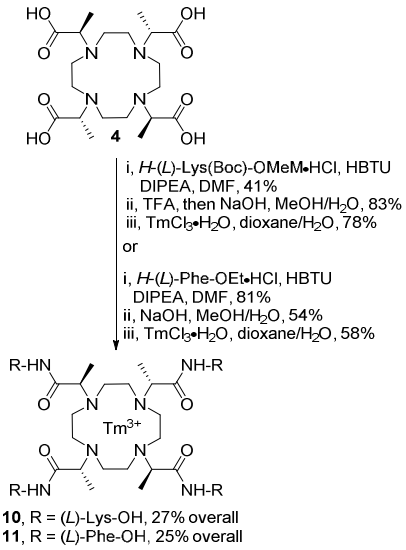


6, R = H, 25% over 2 steps
7, R = Bn, 21% over 2 steps
8, R = *t*-Bu, 10% over 2 steps
9, R = Ph, 14% alkylation, no metallation

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 *O*Ms-(*S*)-lactamide (**19a**, Scheme 1), followed by purification by flash column chromatography (FCC) on Al_2O_3.† Prolonged heating (48 h at 90 °C) of above mentioned ligands with $TmCl_3•H_2O$ (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 $TmCl_3•H_2O$ under identical conditions (Scheme 2).

Peralkylation of cyclen with *O*Ms-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[ʃ,25] between DOTMA tetrasodium salt (**4**, Scheme 3) prepared according to the literature procedures[16,18a] 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 metallation with $TmCl_3•H_2O$ 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-OEt•HCl, followed by removal of ester protecting groups by saponification and metalation with $TmCl_3•H_2O$ gave the complex **11** in 25% overall yield (Scheme 3; see the Supporting Information for experimental details).

Scheme 3. Preparation of CAs **10** and **11**



10, R = (*L*)-Lys-OH, 27% overall
11, R = (*L*)-Phe-OH, 25% overall

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.[13,26] The results of these studies are summarized in Table 1 and discussed below.

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

CA	CEST effect (ppm) signal intensity (%)	Related DOTAM complex; CEST effect (ppm)/signal intensity (%)
6	-54 (6); -63 (7)	-46 (32); -51 (33)[13b$]
7	-65 (6)	-51 (27)[13b$]
8	-115 (3)	-68 (10); -102 (21)[13a†]
10	-53 (20); -62 (10)	-50 (15)[26#]
11	-51 (1)	not available

* 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% D_2O), 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 (Tm^{3+} DOTAM, Tm^{3+} DOTAM-Bn and Tm^{3+} DOTAM-*t*-Bu).[13] 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 Tm^{3+} DOTAM-Bn; 579 μs for **8** *versus* 273 μs for Tm^{3+} DOTAM-*t*-Bu).[27] 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 Eu^{3+} DOTMAM-based complexes (requiring slow exchange rates for bound water)[3] may lead to the formation of PARACEST MRI CAs. These studies are beyond the scope of the present work.

Tm^{3+} DOTMAM-(*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**).[28]

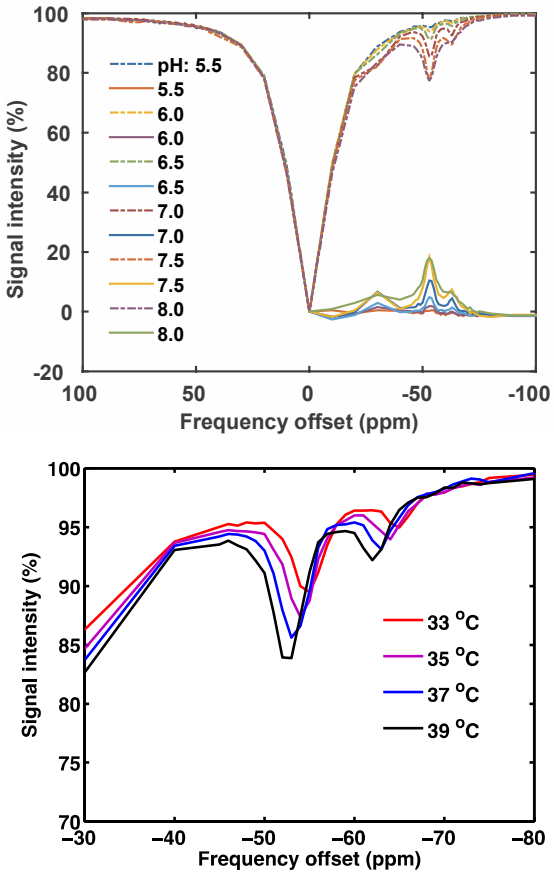


Figure 2. CEST spectra associated with Tm^{3+} DOTMAM-(*L*)-Lys-OH (**10**), measured at 9.4 T, 15 mM of complex, using a saturation pulse of 14 μT for 3.95 s. The CEST effects are modulated by both pH (top) and temperature (bottom).

Curiously, only a very weak CEST effect (Table 1) was observed for Tm^{3+} DOTMAM-(*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)[R] 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).[29] Our findings indicate that further variation of the amino acid residues[30] present in DOTMAM-derived CAs might lead to complexes endowed with interesting CEST properties.

Examination of the ^1H NMR spectra for Yb^{3+} DOTMAM, Yb^{3+} DOTMAM-Bn and Yb^{3+} DOTMAM-*t*-Bu (supporting information) indicate these complexes exist predominantly in as a single isomer. Comparison of the mostly highly shifted signal, the axial cyclen proton designated H_4, is commonly done discriminate coordination complex geometry (i.e. SAP versus TSAP),[31] and estimate population distributions of the two isomers, but is unenlightening in the current case. Comparison of Eu^{3+} to Yb^{3+} metallated ligands show the following trends. The chemical shift of H_4 for Eu^{3+}DOTAM (SAP isomer ~40 ppm; TSAP isomer ~ 5 ppm)[19] less highly shifted than the Eu^{3+}DOTMAM complex (SAP isomer ~44-59 ppm; TSAP isomer ~ 15-20 ppm). The Yb^{3+}DOTAM complex exists as a single isomer (SAP) displaying a chemical shift of H_4 = 95 ppm. The Yb^{3+}DOTMAM complex prepared during this work shows H_4 = 124 ppm, which follows the trend of increasing chemical shift in going from the DOTAM to DOTMAM ligand observed for the Eu^{3+} complexes. The Yb^{3+}DOTMAM-Bn shows a similar chemical shift for H_4 (127 ppm). It is notable that both the Tm^{3+}DOTMAM and Tm^{3+}DOTMAM-Bn complexes both give rise to relatively low-shifted amide-proton CEST signals. This is in contrast to Yb^{3+}DOTMAM-*t*-Bu which possesses a lower shifted H_4 (104 ppm) yet the Tm^{3+} complex displays the CEST signal with the greatest hyperfine shift. It is tempting to suggest that the lower H_4 chemical shift for Tm^{3+}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 MΩ cm millipore water), CH_2Cl_2 and DMF (dried over Al_2O_3, 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 Na_2SO_4. Flash column chromatography (FCC) was carried out using silica gel (SiO_2), mesh size 230 - 400 Å and basic alumina (Al_2O_3), 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 I_2 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 $[\alpha]_D$ were determined by polarimeter at ambient temperature using a 1 ml, 10 cm path length cell; the units are 10^{-1} deg cm^2 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 HR-ESI-MS detector. Mobile phase: Method A: 100% H_2O – 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 C_{18} 300 Å column (particle size 15 µm; 8 × 100 mm Radial-Pak cartridge). Mobile phase for Method B (DOTMAM-Bn) was 0 min, 90% H_2O – 10% MeCN (for each method both solvents containing 0.1% TFA) to 13 min, 20% H_2O – 80% MeCN,; Method C (DOTMAM-*t*-Bu, **7**): 0 min, 90% H_2O – 10% MeCN to 11 min, 13% H_2O – 87% MeCN; Method D (DOTMAM-Ph): 0 min, 90% H_2O – 10% MeCN to 13 min, 100% MeCN; Method E [DOTMAM-(*L*)-Lys(Boc)-OMe, DOTMAM-(*L*)-Phe-OEt]: 0 min, 90% H_2O – 10% MeCN to 10 min, 100% MeCN; Method F (**8**): 0 min, 90% H_2O – 10% MeCN to 11 min, 20% H_2O – 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 I_2 vapours and UV light. Absence of free Tm^{3+} was verified by xylenol orange test.[32] NMR spectra were recorded on a 400 MHz spectrometer for ^{1}H NMR spectra δ values were recorded as follows: $CDCl_3$ (7.27 ppm); CD_3OD (3.31; 4.87 ppm), D_2O (4.75 ppm) for ^{13}C (125 MHz) δ $CDCl_3$ (77.0 ppm); CD_3OD (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 mm^2, matrix: 32 × 32, TR = 4000 ms, 4 echoes, and TE = 10 ms), preceded by a frequency selective saturation pulse (B_1 = 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 Ω-plot method as described previously.[33]

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

The reaction was carried out as described in the literature,[34] starting from 430 µL (5 mmol) of *S*-chloropropionic acid (**12**). The product was isolated by FCC on 70 g SiO_2, eluting with hexanes/EtOAc (3:1), obtained (*S*)-*N*-benzyl-2-chloropropanamide (**13**, 813 mg, 82%), colorless solid. $[\alpha]_D$ -1.3 (c 1, MeOH); lit.[34] $[\alpha]_D$ -3.8 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 2H); 7.31 (m, 3H); 6.88 (br s, D_2O exch, 1H); 4.49 (m, 3H); 1.79 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) 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 $C_{10}H_{12}ClNO$); 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 CH_2Cl_2 (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 CH_2Cl_2. Resulting solutions were washed with saturated $NaHCO_3$ solution (30 mL for benzylamine or 20 mL), the aqueous layers were extracted with CH_2Cl_2 (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 SiO_2, eluting with hexanes/EtOAc (3:1, reaction with benzylamine); 30 g SiO_2, eluting with CH_2Cl_2/MeOH (98:2, reaction with aniline) or 25 g SiO_2, eluting with CH_2Cl_2/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. $[\alpha]_D$ -6.4 (c 1, MeOH); lit.[35] $[\alpha]_D$ -6.8 (c 6.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 5H); 7.11 (br s, D_2O 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). ^{13}C NMR ($CDCl_3$) 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 $C_{10}H_{13}NO_2$); 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. $[\alpha]_D$ -28.0 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.47 (br s, D_2O exch, 1H); 4.09 (q, *J* = 6.5 Hz, 1H); 3.52 (br s, D_2O exch., 1H); 1.38 (d, *J* = 6.5 Hz, 3H); 1.36 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 174.5, 68.3, 50.7, 28.6, 21.1. HRMS (EI) m/z; found 145.1107 $[M]^+$ (calcd 145.1103 for $C_7H_{15}NO_2$); LRMS (EI) m/z (rel. abundance): 145 $[M^+]$ (95), 130 (100), 101 (67).

(*S*)-*N*-phenyl lactamide (**17**), colorless oil. $[\alpha]_D$ -41.0 (c 0.5, MeOH); lit.[36] $[\alpha]_D$ -23.5 (c 0.48, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 8.67 (br s, D_2O 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, D_2O exch, 1H); 1.48 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$) 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 $C_9H_{11}NO_2$); 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 (*S*)-lactic acid (**14**, 180 mg, 2 mmol), *H*-(*L*)-Lys(Boc)-OMe · HCl (594 mg, 2 mmol), HOBt (270 mg, 2 mmol) and Et_3N (280 µL, 2 mmol) in dry CH_2Cl_2 (15 mL) was cooled to 0 ºC, 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 CH_2Cl_2. Resulting solution was washed with saturated $NaHCO_3$ solution (30 mL), the aqueous layer was extracted with CH_2Cl_2 (30 mL). Combined organic extract was dried, 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 SiO_2, eluting with CH_2Cl_2/MeOH (95:5). Evaporation of the eluate afforded (*S*)-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18**, 621 mg, 93%) as colorless oil. $[\alpha]_D$ -11.9 (c 1.4, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.20 (d, D_2O exch., *J* = 8.5 Hz, 1H); 4.71 (br s, D_2O 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). ^{13}C NMR ($CDCl_3$) 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 $[M + H]^+$ (calcd. 333.2026 for $C_{15}H_{29}N_2O_6$).

Reaction of (*S*)-*N*-*t*-butyl lactamide with Tf_2O

A round bottom flask containing (*S*)-*N*-*t*-butyl lactamide (**16**, 202 mg, 1.39 mmol) was flushed with N_2, followed by the addition of dry CH_2Cl_2 (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 Tf_2O (230 µL, 1.39 mmol). The stirring continued for further 1 h at 0 °C, the solvent was evaporated and the residue was subjected to FCC on 25 g SiO_2, 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. $[\alpha]_D$ -16.8 (c 0.5, MeOH). ^{1}H NMR ($CDCl_3$) δ 5.94 (br s, D_2O exch, 1H); 5.14 (q, *J* = 7.0 Hz, 1H); 1.70 (d, *J* = 7.0 Hz, 3H); 1.38 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 166.0, 123.1, 120.0, 116.8, 113.6, 83.4, 52.1, 28.4, 19.1. ^{19}F NMR ($CDCl_3$) δ -74.9. HRMS (EI) m/z; found 277.0591 $[M]^+$ (calcd 277.0596 for $C_8H_{14}F_3NO_4S$); LRMS (EI) m/z (rel. abundance): 277 $[M^+]$ (16), 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 CH_2Cl_2 (6 mL) was cooled to 0 °C, followed by the addition of Et_3N (460 µL, 3.29 mmol), DMAP (24 mg, 0.2 mmol) and TsCl (471 mg, 2.47 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 SiO_2, eluting with hexanes/EtOAc (3:1). Evaporation of the eluate afforded (*S*)-*O*-Ts-*N*-benzyl lactamide (**15c**, 450 mg, 82%) as colorless solid. $[\alpha]_D$ -53.9 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) 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, D_2O 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). ^{13}C NMR ($CDCl_3$) 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 $[M]^+$ (calcd 179.0946 for $C_{10}H_{13}NO_2$); LRMS (EI) m/z (rel. abundance): 179 $[M^+]$ (33), 161 (15), 134 (10), 91 (100), 65 (12). HRMS (ESI) *m/z*: found 334.1124 $[M + H]^+$ (calcd. 334.1113 for $C_{17}H_{20}NO_4S$).

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*)-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18**, 279 mg, 0.84 mmol) or (*S*)-lactamide (**19**, 891 mg, 10 mmol) in dry CH_2Cl_2 [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*)-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18**); 15 mL in the case of (*S*)-lactamide (**19**)] and Et_3N [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*)-*N*-(*L*)-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*)-*N*-(*L*)-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*)-*N*-(*L*)-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 SiO_2, eluting with hexanes/EtOAc (1:1) in the case of (*S*)-*N*-benzyl lactamide (**15**) and (*S*)-*N*-phenyl lactamide (**17**); 30 g SiO_2, eluting with hexanes/EtOAc (1:1) in the case of (*S*)-*N*-*t*-butyl lactamide (**16**); 30 g SiO_2, eluting with CH_2Cl_2/MeOH (95:5) in the case of (*S*)-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18**); 60 g SiO_2, eluting with CH_2Cl_2/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-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18a**, 322 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, was washed with hexanes and was dried to afford (*S*)-*O*-Ms-lactamide (**19a**, 1.34 g, 80%).

(*S*)-*O*-Ms-*N*-benzyl lactamide (**15a**), colorless solid. $[\alpha]_D$ -39.8 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5H); 6.64 (br s, D_2O exch, 1H); 5.14 (q, *J* = 7.0 Hz, 1H); 4.53 (dd, *J* = 15.0, 6.0 Hz, 1H); 4.47 (dd, *J* = 15.0, 6.0 Hz, 1H); 3.08 (s, 3H); 1.67 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 168.7, 137.4, 128.6, 127.5, 127.4, 76.5, 43.2, 38.4, 18.9. HRMS (EI) m/z; found 258.0806 $[M + H]^+$ (calcd 258.0800 for $C_{11}H_{16}NO_4S$); LRMS (EI) m/z (rel.

abundance): 258 [M^+] (10), 161 (62), 133 (23), 106 (44), 91 (100).

(*S*)-*O*-Ms-*N*-*t*-butyl lactamide (**16a**), colorless solid. $[\alpha]_D$ -68.8 (c 0.5, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.09 (br s, D_2O 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 ($CDCl_3$) δ 167.7, 51.6, 38.8, 28.5, 18.8. HRMS (EI) m/z; found 223.0874 $[M]^+$ (calcd 223.0878 for $C_8H_{17}NO_4S$); LRMS (EI) m/z (rel. abundance): 223 [M^+] (29), 208 (100), 180 (16), 136 (15), 123 (50), 84 (79).

(*S*)-*O*-Ms-*N*-phenyl lactamide (**17a**), colorless solid. $[\alpha]_D$ -64.6 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.13 (br s, D_2O exch., 1H); 7.55 (m, 2H); 7.35 (m, 2H); 7.17 (m, 1H); 5.21 (q, *J* = 7.0 Hz, 1H); 3.16 (s, 3H); 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 166.6, 136.6, 129.1, 125.2, 120.2, 76.6, 38.9, 18.8. HRMS (EI) m/z; found 243.0562 $[M]^+$ (calcd 243.0565 for $C_{10}H_{13}NO_4S$); LRMS (EI) m/z (rel. abundance): 243 [M^+] (83), 120 (100), 92 (43).

(*S*)-*O*-Ms-*N*-(*L*)-Lys(Boc)-OMe lactamide (**18a**), pale yellow oil. $[\alpha]_D$ -36.5 (c 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 6.89 (d, D_2O exch., *J* = 8.0 Hz, 1H); 5.08 (q, *J* = 7.0 Hz, 1H); 4.63 (br s, D_2O exch., 1H) 4.58 (m, 1H); 3.74 (s, 3H); 3.14 (s, 3H); 3.10 (m, 2H); 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 ($CDCl_3$) δ 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 $[M + H]^+$ (calcd. 411.1801 for $C_{16}H_{31}N_2O_8S$).

(*S*)-*O*-Ms-lactamide (**19a**), colorless crystals; mp 110-112 ºC. $[\alpha]_D$ -50.9 (c 1, MeOH). ^{1}H NMR (CD_3OD) δ 5.02 (q, *J* = 7.0 Hz, 1H); 3.16 (s, 3H); 1.57 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (CD_3OD) δ 174.6, 77.0, 38.6, 19.6. HRMS (EI) m/z; found 167.0247 $[M]^+$ (calcd 167.0252 for $C_4H_9NO_4S$); LRMS (EI) m/z (rel. abundance): 167 [M^+] (10), 123 (56), 109 (12), 65 (29).

Alkylation of cyclen with (*S*)-*O*-Ms-lactamides 15a-19a

K_2CO_3 [320 mg, 2.31 mmol or 767 mg, 5.55 mmol in the case of (*S*)-*O*-Ms-lactamide (**19a**)] was added to separate solutions of cyclen [**5**, 43 mg, 0.25 mmol or 103 mg, 0.6 mmol in the case of (*S*)-*O*-Ms-lactamide (**19a**)] and (*S*)-*O*-Ms-*N*-benzyl lactamide (**15a**, 257 mg, 1 mmol), (*S*)-*O*-Ms-*N*-*t*-butyl lactamide (**16a**, 223 mg, 1 mmol), (*S*)-*O*-Ms-*N*-phenyl lactamide (**17a**, 243 mg, 1 mmol) or (*S*)-*O*-Ms-lactamide (**19a**, 401 mg, 2.4 mmol) in MeCN [1.5 mL or 5 mL in the case of (*S*)-*O*-Ms-lactamide (**19a**)]. The mixtures were stirred for 48 h at 60 °C, were cooled to rt, were diluted with brine (40 mL) and were extracted with EtOAc (2 × 20 mL). In the case of (*S*)-*O*-Ms-lactamide (**19a**) the solids were filtered off with suction and the filtrate was washed with MeCN. Combined organic extracts were dried and were concentrated along with the filtrate obtained in the case of (*S*)-*O*-Ms-lactamide (**19**). The residues obtained after extraction 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 DOTMAM-Bn × $4CF_3COO^-$ (115 mg, 42%), DOTMAM-*t*-Bu × $4CF_3COO^-$ (67 mg, 24%) or DOTMAM-Ph × $4CF_3COO^-$ (43 mg, 14%) The residue obtained in the case of (*S*)-*O*-Ms-lactamide (**19a**) was subjected to FCC on 25 g Al_2O_3, eluting first with CH_2Cl_2/MeOH/NH_4OH (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 DOTMAM (141 mg, 51%).

DOTMAM-Bn × $4CF_3COO^-$, colorless hygroscopic solid. HPLC, Method B, t_R 9.1 min. ^{1}H NMR (D_2O) δ 6.82-6.71 (br m, 20H); 4.17-2.47 (br m, 28H); 1.48 (br s, 6H); 0.92 (br s, 6H). HRMS (ESI) *m/z*: found 817.5093 $[M + H]^+$ (calcd. 817.5129 for $C_{48}H_{65}N_8O_4$).

DOTMAM-*t*-Bu × $4CF_3COO^-$, colorless hygroscopic solid. HPLC, Method C, t_R 7.9 min. ^{1}H NMR (D_2O) δ 4.12-2.60 (br m, 20H); 1.49 (br s, 6H); 1.22 (br s, 36H); 1.08 (br s, 6H). HRMS (ESI) *m/z*: found 681.5723 $[M + H]^+$ (calcd. 681.5755 for $C_{36}H_{73}N_8O_4$).

DOTMAM-Ph × $4CF_3COO^-$, colorless hygroscopic solid. HPLC, Method D, t_R 7.8 min. ^{1}H NMR (D_2O) δ 7.32-6.88 (br m, 20H); 4.74-2.66 (br m, 20H); 1.68-1.17 (br m, 12H). HRMS (ESI) *m/z*: found 761.4512 $[M + H]^+$ (calcd. 761.4503 for $C_{44}H_{57}N_8O_4$).

DOTMAM, colorless solid. ^{1}H NMR (CD_3OD) δ 3.63 (q, *J* = 7.0 Hz, 4H); 3.00 (t, *J* = 13.5 Hz, 4H); 2.82 (t, *J* = 12.5 Hz, 4H); 2.38 (d, *J* = 14.0 Hz, 4H); 2.15 (d, *J* = 13.5 Hz, 4H); 1.19 (d, *J* = 7.0 Hz, 12H). ^{13}C NMR (CD_3OD) δ 179.5, 57.3, 47.7, 46.2, 7.6. HRMS (ESI) *m/z*: found 457.3230 $[M + H]^+$ (calcd. 457.3251 for $C_{20}H_{41}N_8O_4$).

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[37,38] and *H*-(*L*)-Lys(Boc)-OMe•HCl (178 mg, 0.6 mmol) or *H*-(*L*)-Phe-OEt•HCl (138 mg, 0.6 mmol) in DIPEA (210 µL, 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 considerations. The fractions containing the desired product were combined and concentrated to leave DOTMAM-(*L*)-Lys(Boc)-OMe × $4CF_3COO^-$ (117 mg, 41%) or DOTMAM-(*L*)-Phe-OEt × $4CF_3COO^-$ (195 mg, 81%).

DOTMAM-(*L*)-Lys(Boc)-OMe × $4CF_3COO^-$, colorless solid. HPLC, Method E, t_R 7.5 min. ^{1}H NMR (CD_3OD) δ 4.78-3.94 (br m, 4H); 3.76 (br s, 12H); 3.47-2.55 (br m, 24H); 1.86-1.34 (br m, 72H). HRMS (ESI) *m/z*: found 1429.9065 $[M + H]^+$ (calcd. 1429.9133 for $C_{68}H_{125}N_{12}O_{20}$).

DOTMAM-(*L*)-Phe-OEt × $4CF_3COO^-$, pale brown solid. HPLC, Method E, t_R 7.8 min. ^{1}H NMR (CD_3OD) δ 7.31-7.02 (br m, 20 H); 5.06 (br m, 4H); 4.46-4.12 (br m, 12H); 3.41-2.31 (br m, 24H); 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 $C_{64}H_{89}N_8O_{12}$).

Deprotection of DOTMAM-(*L*)-Lys(Boc)-OMe

A solution of DOTMAM-(*L*)-Lys(Boc)-OMe × $4CF_3COO^-$ (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 ºC, MeOH was evaporated, the mixture was cooled to 0 °C and the pH was adjusted to ca. 7 (1 M HCl). Resulting aqueous solution was subjected to SEC as described in General experimental procedures. The fractions containing the product were combined and concentrated to leave DOTMAM-(*L*)-Lys-OH (50 mg, 83%) as colorless solid. ^{1}H NMR (D_2O) δ 4.09 (m, 4H); 3.78 (m, 4H); 3.09-2.88 (br m, 24H); 1.76-1.30 (br m, 36H). HRMS (ESI) *m/z*: found 973.6386 $[M + H]^+$ (calcd. 973.6410 for $C_{44}H_{85}N_{12}O_{12}$).

Saponification of DOTMAM-(*L*)-Phe-OEt

A solution of NaOH (64 mg, 1.6 mmol) in H_2O (1 mL) was added to a solution of DOTMAM-(*L*)-Phe-OEt × $4CF_3COO^-$ (117 mg, 0.1 mmol) in THF (1 mL). The mixture was vigorously stirred for 24 h at 60 °C, was diluted with small amount of H_2O (ca. 3 mL) and was cooled to 0 °C. The pH was adjusted to ca. 4-5 (1 M HCl), the mixture was set aside for 4 h at 3 °C, the precipitate was filtered off with suction, was washed with water and was dried to leave DOTMAM-(*L*)-Phe-OH (57 mg, 54%) as pale brown solid. ^{1}H NMR (CD_3OD) δ 7.19 (m, 20 H); 4.71 (br m, 4H); 3.58-2.82 (br m, 28H); 1.14 (br m, 12H). HRMS (ESI) *m/z*: found 1049.5399 $[M + H]^+$ (calcd. 1049.5348 for $C_{56}H_{73}N_8O_{12}$).

Metallation of DOTMAM, DOTMAM-Bn, DOTMAM-*t*-Bu, DOTMAM-(*L*)-Lys-OH and DOTMAM-(*L*)-Phe-OH with $TmCl_3•H_2O$

Separate solutions of $TmCl_3•H_2O$ [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 × $4CF_3COO^-$ (54 mg, 0.043 mmol), DOTMAM-*t*-Bu × $4CF_3COO^-$ (22 mg, 0.019 mmol), DOTMAM-(*L*)-Lys-OH × $4CF_3COO^-$ (54 mg, 0.055 mmol) and DOTMAM-(*L*)-Phe-OH × $4CF_3COO^-$ (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 HaOH solution). Resulting solutions were subjected to SEC as described in General experimental procedures. The fractions containing the desired product were combined and concentrated to leave Tm^{3+} DOTMAM-Bn (**7**, 21 mg, 50%) and Tm^{3+} DOTMAM-*t*-Bu (**8**, 7 mg, 40%). The residues obtained from DOTMAM, DOTMAM-(*L*)-Lys-OH and DOTMAM-(*L*)-Phe-OH were dissolved in water (2 mL) and were subjected to SEC as described in General experimental procedures. The fractions containing the desired product were combined and concentrated to leave Tm^{3+} DOTMAM (**6**, 32 mg, 49%), Tm^{3+} DOTMAM-(*L*)-Lys-OH (**10**, 49 mg, 78%) and Tm^{3+} DOTMAM-(*L*)-Phe-OH (**11**, 38 mg, 58%).

Tm^{3+} DOTMAM (**6**), colorless solid. HRMS (ESI) *m/z*: found 623.2344 $[M - 2H]^+$ (calcd. 623.2358 for $C_{20}H_{38}N_8O_4Tm$).

Tm^{3+} DOTMAM-Bn (**7**), colorless solid. HPLC, Method C, t_R 6.1 min. HRMS (ESI) *m/z*: found 983.4254 $[M - 2H]^+$ (calcd. 983.4236 for $C_{48}H_{62}N_8O_4Tm$).

Tm^{3+} DOTMAM-*t*-Bu (**8**), colorless solid. HPLC, Method F, t_R 6.6 min. HRMS (ESI) *m/z*: found 847.4824 $[M - 2H]^+$ (calcd. 847.4862 for $C_{36}H_{70}N_8O_4Tm$).

Tm^{3+} DOTMAM-(*L*)-Lys-OH (**10**), colorless solid. HRMS (ESI) *m/z*: found 1139.5552 $[M - 2H]^+$ (calcd. 1139.5523 for $C_{44}H_{82}N_{12}O_{12}Tm$).

Tm^{3+} DOTMAM-(*L*)-Phe-OH (**11**), colorless solid. HRMS (ESI) *m/z*: found 1237.4275 $[M - 3H + Na]^+$ (calcd. 1237.4239 for $C_{56}H_{69}N_8O_{12}NaTm$).

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 *O*Ms-lactamides as a key step for the preparation of simple alkyl- and aryl-decorated ligands, while peptide coupling of DOTMA tetrasodium salt with protected amino acids furnished amino acid-decorated DOTMAM ligands. Furthermore, the Tm^{3+} complexes of these ligands were prepared and their CEST properties were investigated. Although the CEST effects were more highly shifted than comparable Tm^{3+}DOTAM-based 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 **10** displayed temperature- and pH-dependent CEST effects in the physiological range. Based on similarity to the analogous DOTAM-based agent, it is expected to have reasonable biocompatibility[39] and may be suitable for future *in vivo*

studies.[9,39] Future work should investigate Eu^{3+} DOTMAM-alkyl decorated complexes as well as a wider selection of Tm^{3+} DOTMAM-amino acid decorated complexes for useful CEST properties.

Examination of the Yb^{3+} complexes for DOTMAM, DOTMAM-Bn and DOTMAM-*t*-Bu indicated an overwhelming predominance of a single coordination isomer in solution although the *t*-butyl amide appeared different than the other two. The Tm^{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_4 proton chemical shift we speculate that the TSAP isomer is most favoured in this series by the combination of the *t*-butyl amide substituent and DOTMAM ligand, although further studies are needed to elucidate the geometry unambiguously.

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Notes and references

‡ DOTAM refers to the tetraamide 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 α, α', α'', α '''-tetramethyl-1,4,7,10-tetraazacyclododecane-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 Merbach 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_2O_3, the best results were obtained with 10-100 mesh, pH 9.5-10.5.
∫ The coupling between DOTMA•$4Na^+$ and benzylamine (7% yield) was found to be inferior to that obtained by tetraalkylation of cyclen with *O*Ms-(*S*)-benzyl lactamide.
¤ The CEST effect associated with CA **11** was too weak to measure the exchange rate.

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