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Analogs of Eu^{3+} DOTAM-Gly-Phe-OH and Tm^{3+} DOTAM-Gly-Lys-OH: Synthesis and magnetic properties of potential PARACEST MRI contrast agents

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Abstract—Chelated lanthanide ions, especially gadolinium, have found wide use as contrast agents in magnetic resonance imaging. A new paradigm for generating contrast, termed PARACEST, was recently described that requires the slow exchange of water or other exchangeable protons present in the ligand framework. In previous work, we have described a synthetic method for the preparation of dipeptide conjugates of DOTAM for use as PARACEST agents. Two compounds possessed interesting magnetic properties: the Eu^{3+} complex of DOTAM-Gly-Phe-OH and the Tm^{3+} complex of DOTAM-Gly-Lys-OH. To understand the relationship between the structure of these complexes and their magnetic properties, we have expanded our synthetic methodology and prepared several new complexes. Ligands have been prepared in which the terminal phenylalanine moieties have been replaced with tryptophan or tyrosine, the distance to the amino acid residue possessing an α-substituent has been changed, or phenylalanine and lysine have been combined in the peptide sequence. The preparation of lanthanide(III) complexes of these ligands has been achieved and their PARACEST properties have been determined.
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1. Introduction

It is now well established that chelated lanthanide ions can be used as contrast agents in magnetic resonance imaging (MRI). These paramagnetic lanthanide ions are known to induce a large hyperfine shift of the bound water protons and other exchangeable protons that may be present in the surrounding ligand framework.[1] Selective saturation of these exchangeable spins has led to the discovery of a new methodology for generation of MRI contrast: chemical exchange saturation transfer (CEST).[2] In combination with paramagnetic ions (PARA) to generate large chemical shifts, the PARACEST effect has the potential to be a powerful tool for the in vivo measurement of important physiologic parameters, such as temperature[3] and pH[4] that vary between normal physiologic and disease states.[5]

New ligands for PARACEST agents have been designed and synthesized, often derived from DOTA[6] or DOTAM,[7,8] that increase MRI detection sensitivity or provide functional groups. We have recently established a synthetic methodology that allowed the synthesis of a large collection of different lanthanide(III) complexes of DOTAM-derived oligopeptide conjugates.[9] From an initial examination of the PARACEST effects associated with these complexes, two compounds were identified to be of particular interest. Firstly, the Eu^{3+} complex of DOTAM-Gly-Phe-OH (**3**) (Fig. 1) has the potential for in vivo temperature measurement as described recently.[10] Secondly, the Tm^{3+} complex of DOTAM-Gly-Lys-OH (**4**) (Fig. 1) was found to show a PARACEST effect due to exchangeable amide protons and may have applicability for in vivo pH measurement. To understand the relationship between the structure of the aforementioned complexes and their CEST detection sensitivity, we have prepared several new complexes related to **3** and **4**.

Three groups of ligands have been designed as shown in Figure 1. In comparison to compound **3**, Type 1 ligands

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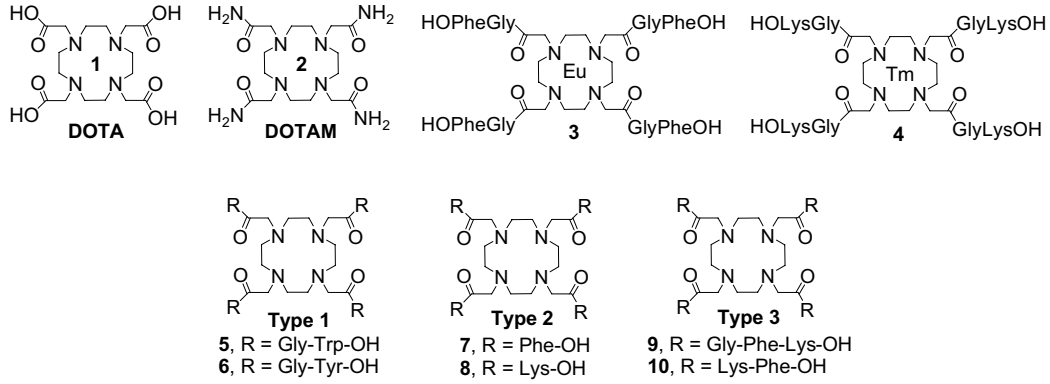


Figure 1. Structure of DOTA (**1**), DOTAM (**2**), complexes **3** and **4** and ligands **5**–**10**.

have had the terminal phenylalanine moieties replaced with other aromatic amino acids, such as tryptophan (**5**) or tyrosine (**6**) (Fig. 1). We also wanted to investigate the effect the number of intervening atoms between the cyclen ring to the amino acid residue possessing α-substitution, such as is present in ligands **3** and **4**, on the CEST sensitivity of the resultant complexes. Consequently, Type 2 ligands (**7** and **8**, Fig. 1), which have had the glycine deleted from the sequence in comparison to **3** and **4** respectively, were prepared. Finally, Type 3 ligands (**9** and **10**, Fig. 1), combine phenylalanine and lysine in their sequence.

A synthetic route allowing for the preparation of the novel lanthanide (III) complexes of ligands **5**–**10** has been developed and their PARACEST properties have been investigated. The results of these studies are described herein.

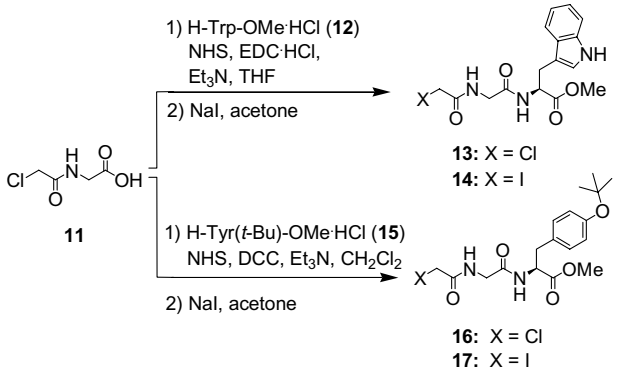
2. Results and discussion

2.1. Synthesis

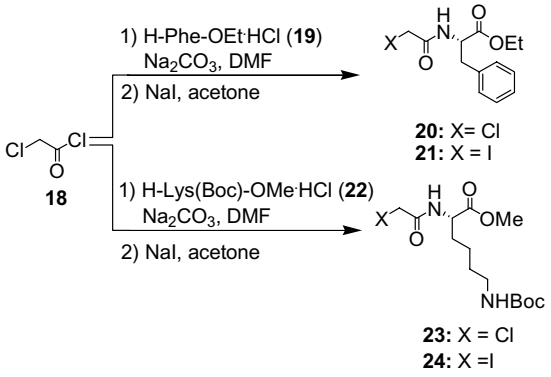
Treatment of *N*-chloroacetylglycine (**11**) with H-Trp-OMe·HCl (**12**) or H-Tyr(*t*-Bu)-OMe·HCl (**15**) under the conditions of NHS/EDC or NHS/DCC-mediated couplings[9] afforded chloroacetyl dipeptides **13** and **16** in good yields (Scheme 1). Halogen exchange under the conditions of Finkelstein reaction (NaI, acetone) proceeded smoothly and iodoacetyl dipeptides **14** and **17** which were intended to be used as electrophiles in the synthesis of Type 1 ligands were obtained in 75% (**14**) and 93% (**17**) yields, respectively (Scheme 1).

Chlorides **20** and **23** have been prepared by treatment of chloroacetyl chloride (**18**) with H-Phe-OEt·HCl (**19**)[11] (78% yield) or H-Lys(Boc)-OMe·HCl (**22**) (96% yield) (Scheme 2). It is worth mentioning that halide **23** has been recently prepared by NHS/EDC-mediated coupling of chloroacetic acid with H-Lys(Boc)-OMe·HCl.[12] Finkelstein reaction of chloroderivatives **20** and **23** afforded electrophiles **21** and **24** (synthesis of Type 2 ligands) in high yields (ca. 80%) (Scheme 2).

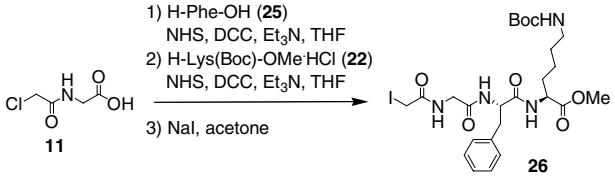
Preparation of iodoacetyl tripeptide **26** as a Type 3 ligand precursor was found to be somewhat troublesome. The main difficulty was the removal of dicyclohexyl urea (DCU) which formed as a side product in two consecutive NHS/DCC-mediated couplings. The use of NHS/EDC·HCl coupling proved to give very low yields. *N*-Chloroacetyl glycine (**11**) was coupled to H-Phe-OH (**25**) (Scheme 3) and the crude dipeptide was extracted together with large quantities of DCU after acidic aqueous work-up. The resulting mixture was subjected to the second NHS/DCC-mediated coupling with H-Lys(Boc)-OMe·HCl (**22**), the corresponding chloroacetyl tripeptide was extracted with $CHCl_3$ and was found to contain approximately 20% of DCU (by ^1H NMR). Purification at this point was not attempted and the mixture was subjected to NaI-promoted halogen exchange. Pure iod-



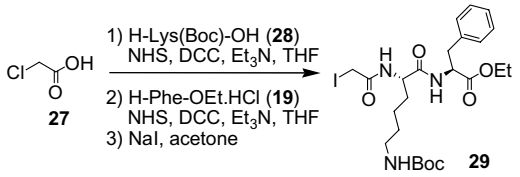
Scheme 1. Synthesis of iodoacetyl dipeptides **14** and **17**.



Scheme 2. Synthesis of electrophiles **21** and **24**.



Scheme 3. Synthesis of iodoacetyl tripeptide **26**.



Scheme 4. Synthesis of iodoacetyl dipeptide **29**.

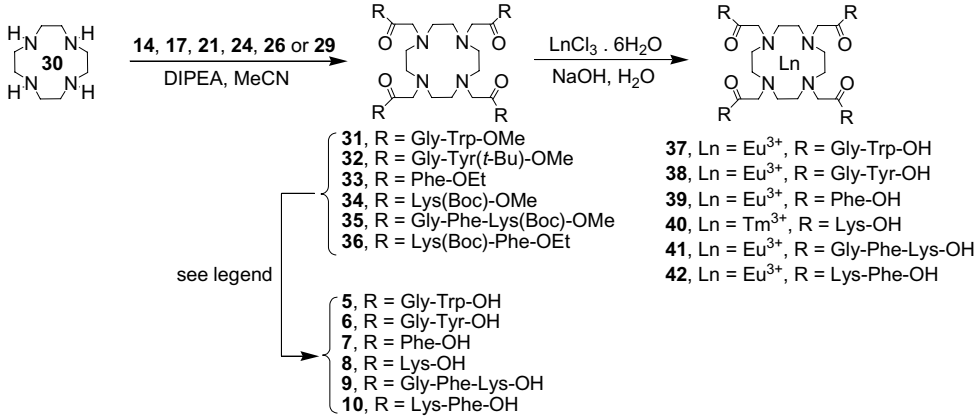
oacetyl tripeptide **26** was obtained in 52% yield, based on *N*-chloroacetyl glycine (**11**) (Scheme 3), after chromatography and recrystallization.

A similar one-pot strategy has been used for the synthesis of another Type 3 ligand precursor, iodoacetyl dipeptide **29** (Scheme 4). Chloroacetic acid (**27**) was subjected to consecutive NHS/DCC-mediated couplings, first with H-Lys(Boc)-OH (**28**), followed by H-Phe-OEt·HCl (**19**, Scheme 4), to yield the chloroacetyl dipeptide that was contaminated with DCU (10%, by ^{1}H NMR) despite having undergone column chromatography. This material was treated with NaI in acetone to give the pure electrophile **29** (Scheme 4) in 40% yield, based on chloroacetic acid (**27**).

Peralkylation of cyclen (**30**) with iodine containing electrophiles **14**, **17**, **21**, **24**, **26** and **29** was carried out using a recently established protocol.[9] Tetraalkylated cyclens **31**–**35** were obtained in very good yield (ca. 90%), and the yield of tetraalkylated cyclen **36** was somewhat lower (64%) (Scheme 5). Compounds **31**–**36** were purified by trituration in hexanes. The protecting groups were removed using standard protocols: ester groups in conjugates **31** and **33** were removed by saponification (NaOH in THF/H_2O) and Boc or *t*-Bu protecting groups in **32** and **34**–**36** were removed by treatment with TFA. Reaction mixtures containing TFA were concentrated and crude products obtained were saponified (NaOH, THF, or MeOH/H_2O) to afford ligands **5**–**10** (Scheme 5). Ligands **5**, **6**, **8**, **9**, and **10** represent new structures, while ligand **7** and its Cd^{2+}, Cu^{2+}, and Zn^{2+} complexes have been recently described.[13] The metalation of ligands **6**–**10** proceeded smoothly (Table 1) resulting in complexes **38**–**42** which were purified by size exclusion chromatography (SEC) as previously described.[9] Identification of fractions containing complexes **37**–**39** was made by UV visualization on TLC or staining by I_2 vapors (**37**–**39**). The ninhydrin test was used to identify the fractions containing complexes **40**–**42**. Complexes **37**–**42** were obtained in moderate to very good yields (Table 1). The absence of free Eu^{3+} or Tm^{3+} after size exclusion chromatography was confirmed for all complexes by employing the xylenol orange test.[14] HPLC chromatograms (Method B, general experimental procedures) of fractions obtained after size exclusion chromatography (complexes **38** and **39**) indicated that <5% (based on peak area) of free ligands **6** and **7** were present. The complexes were characterized by ^{1}H NMR spectroscopy and mass spectrometry (ESI-TOF) (Table 1, experimental procedures). The metalation of Trp-OH containing ligand **5** was found to be difficult and was not completed even using 5 equivalents of $EuCl_3·6H_2O$ and elevated temperature. After SEC, the Eu^{3+} complex **37** obtained was found to contain ca. 15% of free ligand **5** (HPLC analysis, Method B, general experimental procedures). Since the impure complex **37** did not possess particularly striking magnetic properties (Fig. 2), no studies on the optimization of the metalation reaction were carried out. It is worth mentioning that a similar problem occurred when attempts to introduce Eu^{3+} to DOTAM-Gly-Arg(NO_2)-OMe were made.[9]

2.2. PARACEST properties associated with complexes 37–42

Complexes **37**–**42** have been designed based on the structures of Eu^{3+} complex **3** and Tm^{3+} complex **4** and



Scheme 5. Synthesis of ligands **5**–**10** and their lanthanide(III) complexes **37**–**42**; **31** → **5**. Reagents: NaOH, THF/H_2O; **32** → **6**: (i) TFA, CH_2Cl_2; (ii) NaOH, THF/H_2O; **33** → **7**: NaOH, THF/H_2O; **34** → **8**: (i) TFA; (ii) NaOH, MeOH/H_2O; **35** → **9**: (i) TFA; (ii) NaOH, MeOH/H_2O; **36** → **10**: (i) TFA, CH_2Cl_2; (ii) NaOH, THF/H_2O.

Table 1. Selected data for the synthesis and characterization of complexes **37**–**42**

	Complex	Yield[a] (mass, percent)	HRMS (ESI-TOF) *m/z*
37	Eu^{3+} DOTAM-Gly-Trp-OH	28 mg, 44%	1528.5098 $[M-2H]^+$ (1528.5072 calcd for $C_{68}H_{79}N_{16}O_{16}Eu$)
38	Eu^{3+} DOTAM-Gly-Tyr-OH	28 mg, 36%	1433.4343 $[M-2H]^+$ (1433.4341 calcd for $C_{60}H_{74}N_{12}O_{20}Eu$)
39	Eu^{3+} DOTAM-Phe-OH	22 mg, 32%	1142.3730 $[M-2H]^+$ (1142.3764 calcd for $C_{52}H_{63}N_8O_{12}Eu$)
40	Tm^{3+} DOTAM-Lys-OH	84 mg, 77%	1083.4867 $[M-2H]^+$ (1083.4891 calcd for $C_{40}H_{74}N_{12}O_{12}Tm$)
41	Eu^{3+} DOTAM-Gly-Phe-Lys-OH	127 mg, 98%	1884.8392 $[M-2H]^+$ (1884.8435 calcd for $C_{84}H_{123}N_{20}O_{20}Eu$)
42	Eu^{3+} DOTAM-Lys-Phe-OH	34 mg, 34%	1653.7559 $[M-2H]^+$ (1653.7484 calcd for $C_{76}H_{110}N_{16}O_{16}Eu$)

[a] Complex **37** contained ca. 15% of free ligand **5**.

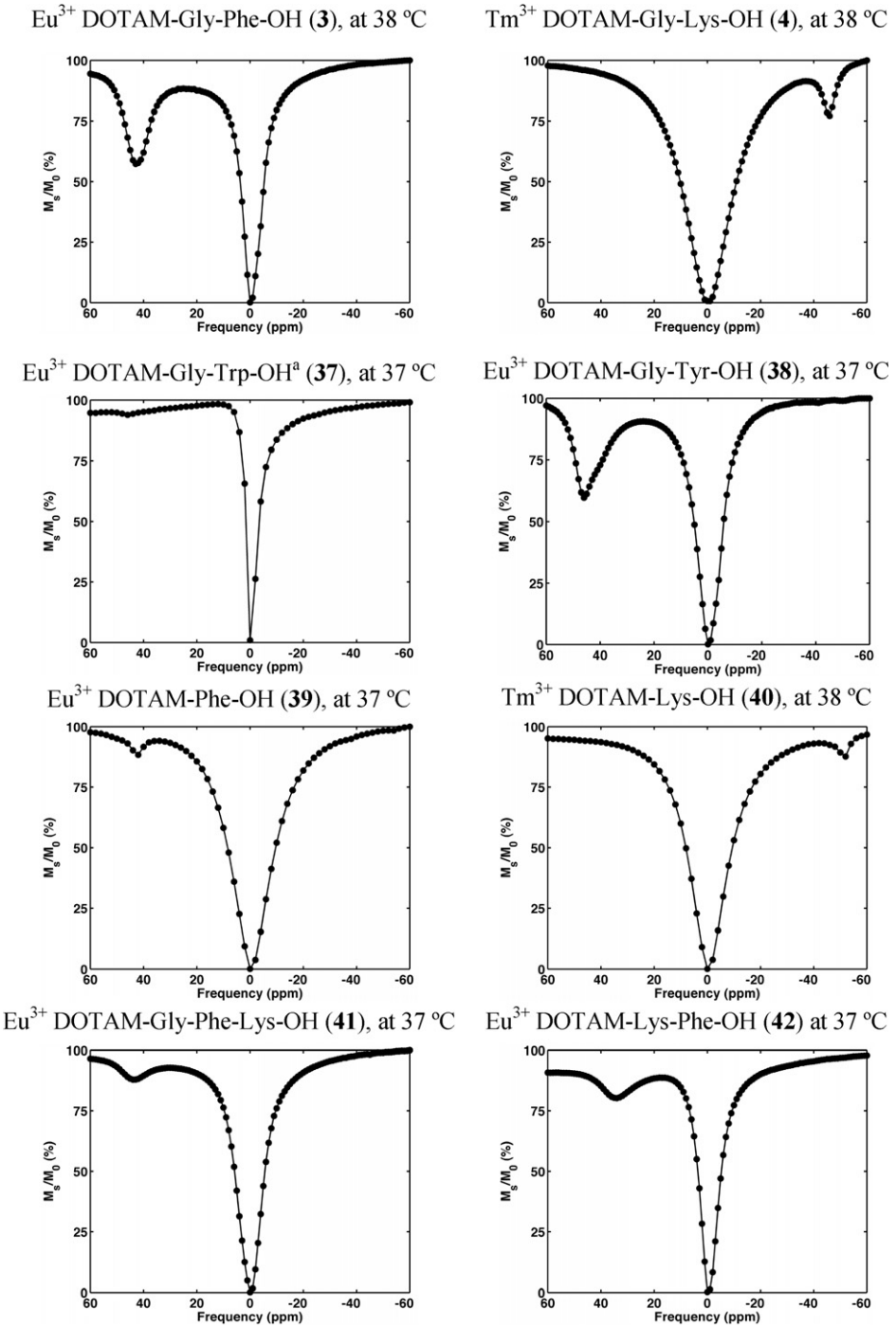


Figure 2. PARACEST spectra of compounds **3**, **4**, **37**–**42**, determined at 10 mM concentration, pH 7.0. All spectra were collected using a 9.4 T NMR spectrometer with saturation power of 14 μT (saturation time = 10 s) at the temperature indicated. A single repetition was used at each saturation frequency and there was no additional delay without saturation [a]The PARACEST spectrum of Eu^{3+} DOTAM-Gly-Trp-OH (**37**) was obtained using the complex containing ca.15% (HPLC analysis) of free ligand **5**.

synthesized as potentially new MRI PARACEST agents. With the complexes **37**–**42** in hand, the survey of their CEST efficiency under the conditions of near physiological temperature and pH was performed. The results were compared with those for Eu^{3+} DOTAM-Gly-Phe-OH (**3**) and Tm^{3+} DOTAM-Gly-Lys-OH (**4**) as shown in Figure 2. Weak to moderate PARACEST effects (<20%) due to bound water protons are observed with Eu^{3+} DOTAM-Gly-Trp-OH (**37**, ca. 45–50 ppm), Eu^{3+} DOTAM-Phe-OH (**39**, ca. 45–50 ppm), Eu^{3+} DOTAM-Gly-Phe-Lys-OH (**41**, ca. 45–50 ppm), and Eu^{3+} DOTAM-Lys-Phe-OH (**42**, ca. 35 ppm) designed as analogs of Eu^{3+} DOTAM-Gly-Phe-OH (**3**) (Fig. 2). The PARACEST effect (at ca. −50 ppm) associated with Tm^{3+} DOTAM-Lys-OH (**40**) (due to amide protons) is slightly weaker (15%) compared to the PARACEST effect of the glycine-containing analog Tm^{3+} DOTAM-Gly-Lys-OH (**4**) (25%, ca. −50 ppm). The PARACEST spectrum of Eu^{3+} DOTAM-Gly-Tyr-OH (**38**) exhibits the presence of a strong PARACEST effect due to bound water protons (40%, ca. 50 ppm) similar to parent compound **3**.

The results show that replacement of the terminal phenylalanine (Phe) subunit in **3** with other natural aromatic amino acids has dramatically different effects. While the structurally modest change from Phe (**3**) to tyrosine (Tyr, **38**) preserves the PARACEST properties, the change to tryptophan (Trp, **37**) results in significant loss of the PARACEST effect. Similarly, a substantial loss in the PARACEST effect (due to bound water protons) was also observed with complexes bearing shorter (Eu^{3+} DOTAM-Phe-OH, **39**), longer (Eu^{3+} DOTAM-Gly-Phe-Lys-OH, **41**) or modified (Eu^{3+} DOTAM-Lys-Phe-OH, **42**) side chains. The exact reasons for the exceptionally large PARACEST effect associated with Eu^{3+} DOTAM-Gly-Phe-OH[10] (**3**) are not fully understood at the moment; however, it is reasonable to ascribe this effect to favorable bound-bulk water exchange rate. The rate of exchange of the pool of bound water with bulk water influences the magnitude of the PARACEST effect and the exchange rate in turn is affected by the nature of the ligand, the identity of the metal, and environmental factors such as pH and temperature. Recent studies have elucidated some electronic[15,16] and steric[17] factors affecting water exchange rates within closely related series of DOTA and DOTAM analogs. The electronic effect is observed when the basicity of a chelating atom, either the nitrogen of the cyclen ring or the oxygen of the carbonyl, is modulated by substituent effects. Due to the structural similarity of the ligands described herein, and all other factors (pH, temperature, and metal ion) being constant, the changes in the observed PARACEST effect is likely caused by changes in the water exchange rate due to varying the steric environment around the metal.

The PARACEST effect due to amide protons was also found to be dependent on the changes in the structure of the complex, as evidenced by comparison of the data for Tm^{3+} DOTAM-Lys-OH (**40**) and Tm^{3+} DOTAM-Gly-Lys-OH (**4**). Although the PARACEST effect is diminished, it is not obliterated and this observation indicates that analogous complexes bearing similar PARACEST properties might be synthesized without significant loss in the PARACEST effect associated with amide protons.

3. Conclusions

We have shown that our recently developed synthetic methodology for the synthesis and purification of lanthanide(III) complexes of cyclen oligopeptide conjugates[9] may be easily modified such that complexes with peptidic appendages that are shorter (glycine deletion), longer (e.g., tripeptides), or vary the amino acid (Trp, Tyr) can be prepared in reasonable yields. The PARACEST spectra associated with new complexes were acquired under standard conditions[9] and were compared to those associated with potentially useful MRI contrast agents Eu^{3+} DOTAM-Gly-Phe-OH (**3**) (for temperature measurement) and Tm^{3+} DOTAM-Gly-Lys-OH (**4**) (for pH measurement). Most notably, it was found that only the conservative replacement of the terminal phenylalanine (Phe) subunit in **3** by tyrosine (Tyr, **38**) was tolerated and resulted in a compound with a strong PARACEST effect due to bound water protons. Conversely, replacement of Phe by tryptophan (Trp, **37**) resulted in a compound with very poor PARACEST properties under the conditions of measurement. Other changes to the length or composition of the peptide arms (**39**, **41**, and **42**) were all found to be detrimental to the PARACEST effect due to bound water protons. To aid the rational design of subsequent contrast agents, insight in to the origin of the PARACEST effect is needed, especially with respect to structural studies on the complexes and the effect of structure on the rate of exchange of water with the metal center.

The PARACEST effect due to amide protons was found to be slightly less dependent on the changes in the structure of the complex, as evidenced by comparison of the data for Tm^{3+} DOTAM-Lys-OH (**40**) and Tm^{3+} DOTAM-Gly-Lys-OH (**4**). This observation indicates that more analogous complexes bearing similar PARACEST properties could be synthesized without significant loss in the PARACEST effect associated with amide protons.

4. Experimental

4.1. General experimental procedures

All amino acids (naturally occurring *L* isomers) and reagents were commercially available, unless otherwise stated. All solvents were of HPLC grade and used as such, except for CH_2Cl_2 and THF (dried over Al_2O_3, in a solvent purification system) and deionized water (18.2 MΩ/cm). Organic extracts were dried over Na_2SO_4 and solvents were removed under reduced pressure in a rotary evaporator. Flash column chromatography (FCC) was carried out using silica gel, mesh size 230–400 Å. Size exclusion chromatography was carried out on BIO-GEL P2, 45–90 μm mesh resin (20 g, column size 15 × 2 cm per 0.1 mmol of compound). Ten fractions

(10 mL each) were collected. Fractions were identified by 5% solution of ninhydrin in AcOH/EtOH (tetraacetyl-Lys-OH, Gly-Phe-Lys-OH, and Lys-Phe-OH derivatives) or by UV/I_2 vapors (tetraacetyl-Gly-Trp-OH, Gly-Tyr-OH, and Phe-OH derivatives). Thin-layer chromatography (TLC) was carried out on Al backed silica gel plates, and compounds were visualized by UV light or I_2 vapors. Melting points were obtained on Fisher–Johns apparatus and are uncorrected. Specific rotations $[\alpha]_D$ were determined by Atago Polax-2L polarimeter at ambient temperature using a 5-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. HPLC analysis (Waters 600E) was carried out using a Microsorb-CN column (particle size 5 μm; 100 Å pore; 4.6 id × 250 mm). Mobile phase: Method A: 75% H_2O/25% MeCN–25% H_2O/75% MeCN over 35 min, linear gradient, at a flow rate 1 mL/min; Method B: 25% H_2O/75% MeCN–100% MeCN over 30 min, linear gradient, at a flow rate 1 mL/min. NMR spectra were recorded on Varian-Mercury 400 MHz spectrometer at room temperature (~21 °C); for ^{1}H (400.1 MHz), δ values were referenced as follows $CDCl_3$ (7.26 ppm); DMSO-d_6 (2.49 ppm); D_2O (4.75 ppm) for ^{13}C (100.6 MHz) $CDCl_3$ (77.0 ppm); DMSO-d_6 (39.5 ppm). Mass spectra (MS) were obtained on mass spectrometers, using electron impact (EI, Finnigan MAT 8200) and electron spray (ESI, Micromass LCT) for ionization.

4.1.1. NHS/EDC·HCl-mediated coupling of *N*-chloroacetylglycine (11) with H-Trp-OMe·HCl (12). To a stirred solution of *N*-chloroacetylglycine (**11**, 303 mg, 2 mmol) in dry THF (6 mL) were added NHS (231 mg, 2 mmol) and EDC·HCl (767 mg, 4 mmol). The mixture was stirred for an additional 18 h at rt, after which it was concentrated. The residue was dissolved in water (30 mL) and was extracted against EtOAc (30 mL +;3× 20 mL). The combined organic extract was dried and concentrated to afford 353 mg (71%) of *O*-hydroxysuccinimidyl *N*-chloroacetylglycinate. The crude product was dissolved in dry THF (12 mL), the solution was cooled to 0 °C, and H-Trp-OMe·HCl (**12**, 360 mg, 1.41 mmol) and Et_3N (590 μL, 4.24 mmol) were added. The resulting mixture was stirred for 2 h at 0 °C and for another 2 h at rt. The solvent was evaporated; the residue was dissolved in EtOAc (30 mL) and was washed with water (30 mL). The aqueous phase was extracted with EtOAc (20 mL), the combined organic extract was dried, concentrated, and the residue was subjected to FCC on 50 g SiO_2 (CH_2Cl_2/MeOH, 9:1). From chromatography 375 mg of *N*-chloroacetyl-Gly-Trp-OMe (**13**) was isolated (74%, based on *O*-hydroxysuccinimidyl *N*-chloroacetylglycinate; 52%, based on *N*-chloroacetylglycine). Colorless solid; $[\alpha]_D^{25}$ +26 (*c* 0.38, MeOH). HPLC: t_R 7.3 min; ^{1}H NMR (DMSO-d_6) δ 10.88 (s, D_2O exch., 1H); 8.39 (m, D_2O exch., 1H); 7.47 (d, J = 8 Hz, 1H); 7.33 (d, J = 8 Hz, 1H); 7.15 (d, J = 2 Hz, 1H); 7.06 (dd, J = 8, 8 Hz, 1H); 6.98 (dd, J = 8, 8 Hz, 1H); 4.53 (m, 1H); 4.11 (s, 2H); 3.80 (dd, J = 16.5, 5.5 Hz, 1H); 3.73 (dd, J = 16.5, 5.5 Hz, 1H); 3.57 (s, 3H); 3.16 (dd, J = 14.5, 5 Hz, 1H); 3.05 (dd, J = 14.5, 8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 172.1, 168.3, 166.1, 136.1, 127.0, 123.8, 121.0, 118.4, 117.9, 111.4, 109.2, 53.1, 51.9, 42.5, 41.9, 27.1. HRMS (EI) *m*/*z*: found 351.0982 (351.0986 calcd for $C_{16}H_{18}ClN_3O_4$). LRMS (EI) *m*/*z* (rel abundance): 351 $[M+H]^+$ (10), 201 (23), 130 (100).

4.1.2. NHS/DCC-mediated coupling of *N*-chloroacetylglycine (11) with H-Tyr(*t*-Bu)-OMe·HCl (15). To a stirred suspension of *N*-chloroacetylglycine (**11**, 758 mg, 5 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C were added NHS (575 mg, 5 mmol) and DCC (1.341 g, 6.5 mmol). The stirring was continued for 30 min at 0 °C and then H-Tyr(*t*-Bu)-OMe·HCl (**15**, 1.275 g, 5 mmol) and Et_3N (1.4 mL, 6 mmol) were added and the resultant mixture was stirred for 18 h at rt and then set aside for 6 h at −20 °C. The precipitate (*N*,*N*′-dicyclohexylurea (DCU) and Et_3N·HCl) was filtered off and the filtrate was washed with 1 M HCl, was dried, concentrated, and then subjected to FCC on 60 g SiO_2 (CH_2Cl_2/MeOH, 9:1). The eluate was concentrated, the residue was dissolved in a small amount of acetone (ca. 5 mL), and the solution was set aside for 2 h at −20 °C. An additional amount of DCU was filtered off and the filtrate was concentrated to give *N*-chloroacetyl-Gly-Tyr(*t*-Bu)-OMe (**16**) (1.18 g, 61%). Colorless oil; $[\alpha]_D^{25}$ +85 (*c* 0.47, CH_2Cl_2). HPLC: t_R 7.2 min; ^{1}H NMR ($CDCl_3$) δ 7.27 (s, D_2O exch., 1H); 6.97 (d, J = 8 Hz, 2H); 6.89 (d, J = 8 Hz, 2H); 6.48 (d, D_2O exch., J = 7.5 Hz, 1H); 4.82 (dd, J = 6, 6 Hz, 1H); 4.04 (s, 2H); 3.95 (d, J = 5 Hz, 1H); 3.69 (s, 3H); 3.08 (dd, J = 14, 6.5 Hz, 1H); 3.03 (dd, J = 14, 6.5 Hz, 1H); 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.8, 167.8, 166.6, 154.4, 130.3, 129.6, 124.1, 78.4, 53.4, 52.3, 42.9, 42.2, 37.1, 28.7. HRMS (EI) *m*/*z*: found 384.1448 (384.1452 calcd for $C_{18}H_{25}ClN_2O_5$). LRMS (EI) *m*/*z* (rel abundance): 384 $[M+H]^+$ (10), 178 (100), 151 (10), 107 (48).

4.1.3. Acylation of H-Phe-OEt·HCl (19) and H-Lys(-Boc)-OMe·HCl (22) with chloroacetyl chloride (18). To separate suspensions of H-Phe-OH·HCl (**19**, 574 mg, 2.5 mmol) and H-Lys(Boc)-OMe·HCl (**22**, 742 mg, 2.5 mmol) in dry DMF (2 mL each) was added Na_2CO_3 (530 mg, 5 mmol). The individual mixtures were stirred for 30 min at rt followed by dropwise addition (over a 1 min period) of chloroacetyl chloride (**18**, 200 μL, 2.5 mmol). The mixtures were then stirred for 1 h at rt after which time they were diluted with brine (50 mL) and extracted with EtOAc (2× 30 mL). The combined organic extracts were washed with brine (2× 50 mL), dried, and concentrated to give *N*-chloroacetyl-Phe-OEt (**20**) and *N*-chloroacetyl-Lys(Boc)-OMe (**23**), respectively. Compound **20** was purified by crystallization from CH_2Cl_2/hexane solution (524 mg, 78%), compound **23** (809 mg, 96%) was used for the next step without further purification.

N-Chloroacetyl-Phe-OEt (**20**), white crystals, mp 55–57 °C (61–64 °C, lit.[11]); $[\alpha]_D^{25}$ +55 (*c* 0.64, CH_2Cl_2). HPLC: t_R 9.1 min; For ^{1}H NMR ($CDCl_3$) and HRMS (EI) data see reference[11]; ^{13}C NMR ($CDCl_3$) δ 170.6, 165.4, 135.3, 129.0, 128.3, 127.0, 61.4, 53.2, 42.1, 37.5, 13.8.

N-Chloroacetyl-Lys(Boc)-OMe (**23**), colorless oil. HPLC: t_R 10.3 min; For $[\alpha]_D^{20}$, ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$), and HRMS (FAB) data see Ref. 12.

4.1.4. Reaction of *N*-chloroacetyl-Gly-Trp-OMe (13), *N*-chloroacetyl-Gly-Tyr(*t*-Bu)-OMe (16), *N*-chloroacetyl-Phe-OEt (20) and *N*-chloroacetyl-Lys(Boc)-OMe (23) with NaI. To separate solutions of *N*-chloroacetyl-Gly-Trp-OMe (**13**, 718 mg, 2 mmol), *N*-chloroacetyl-Gly-Tyr(*t*-Bu)-OMe (**16**, 770 mg, 2 mmol), *N*-chloroacetyl-Phe-OEt (**20**, 539 mg, 2 mmol) and *N*-chloroacetyl-Lys(-Boc)-OMe (**23**, 674 mg, 2 mmol) in acetone (12 mL) was added NaI (899 mg, 6 mmol). The individual mixtures were stirred for 18 h at rt and then were concentrated to approximately 1/3 of their original volumes. The mixtures were each diluted with EtOAc (30 mL) and then washed with 10% Na_2SO_3 solution (20 mL). The aqueous phases were extracted with EtOAc (20 mL) and the combined organic extracts were dried and concentrated to give *N*-iodoacetyl compounds **14**, **17**, **21**, and **24**. These were purified as follows: *N*-iodoacetyl-Gly-Trp-OMe (**14**), crystallization from acetone/hexane solution; *N*-iodoacetyl-Gly-Tyr(*t*-Bu)-OMe (**17**), FCC on 40 g SiO_2 (CH_2Cl_2/MeOH, 19:1); *N*-iodoacetyl-Phe-OEt (**21**), crystallization from dichloromethane/hexane solution; *N*-iodoacetyl-Lys(Boc)-OMe (**24**), FCC on 40 g SiO_2 (CH_2Cl_2/MeOH, 19:1) followed by crystallization from dichloromethane/hexane solution.

N-Iodoacetyl-Gly-Trp-OMe (**14**, 664 mg, 75%), colorless crystals; mp 175–177 °C; $[\alpha]_D^{25}$ +21 (*c* 0.48, MeOH). HPLC: t_R 7.9 min; ^{1}H NMR (DMSO-d_6) δ 10.87 (s, D_2O exch., 1H); 8.45 (t, D_2O exch., J = 5.5 Hz, 1H); 8.35 (d, D_2O exch., J = 7.5 Hz, 1H); 7.46 (d, J = 8 Hz, 1H); 7.32 (d, J = 8 Hz, 1H); 7.15 (d, J = 2 Hz, 1H); 7.05 (dd, J = 8, 8 Hz, 1H); 6.97 (dd, J = 8, 8 Hz, 1H); 4.52 (m, 1H); 3.75 (dd, J = 16.5, 5.5 Hz, 1H); 3.69 (m, 3H); 3.15 (dd, J = 14.5, 6 Hz, 1H); 3.04 (dd, J = 14.5, 8 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 172.1, 168.4, 167.8, 136.1, 127.0, 123.8, 121.0, 118.4, 117.9, 111.4, 109.1, 53.1, 51.8, 42.1, 30.7, 27.1. HRMS (EI) *m*/*z*: found 443.0347 (443.0342 calcd for $C_{16}H_{18}IN_3O_4$). LRMS (EI) *m*/*z* (rel abundance): 443 $[M+H]^+$ (10), 201 (42), 130 (100).

N-Iodoacetyl-Gly-Tyr(*t*-Bu)-OMe (**17**, 884 mg, 93%), colorless solid; $[\alpha]_D^{25}$ +50 (*c* 0.60, CH_2Cl_2). HPLC: t_R 7.8 min; ^{1}H NMR ($CDCl_3$) δ 7.11 (t, D_2O exch., J = 5 Hz, 1H); 7.00 (d, J = 8 Hz, 2H); 6.90 (d, J = 8 Hz, 2H); 6.75 (d, D_2O exch., J = 8 Hz, 1H); 4.81 (dd, J = 6, 6 Hz, 1H); 3.95 (dd, J = 16.5, 5 Hz, 1H); 3.88 (dd, J = 16.5, 5 Hz, 1H); 3.71 (s, 2H); 3.68 (s, 3H); 3.08 (dd, J = 14, 6 Hz, 1H); 3.03 (dd, J = 14, 6.5 Hz, 1H); 1.31 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.7, 168.4, 168.3, 154.2, 130.5, 129.6, 124.1, 78.4, 53.6, 52.3, 43.4, 37.1, 28.7. HRMS (EI) *m*/*z*: found 476.0801 (476.0808 calcd for $C_{18}H_{25}IN_2O_5$). LRMS (EI) *m*/*z* (rel abundance): 476 $[M+H]^+$ (10), 420 (10), 243 (37), 178 (100), 107 (42).

N-Iodoacetyl-Phe-OEt (**21**, 568 mg, 82%), colorless crystals; mp 60–61 °C; $[\alpha]_D^{25}$ +68 (*c* 0.44, CH_2Cl_2). HPLC: t_R 10.3 min; ^{1}H NMR ($CDCl_3$) δ 7.27 (m, 3H); 7.14 (m, 2H), 6.48 (d, D_2O exch., 6.5 Hz, 1H); 4.83 (dd, J = 6, 5.5 Hz, 1H); 4.19 (q, J = 7 Hz, 2H); 3.69 (d, J = 11.5 Hz, 1H); 3.65 (d, J = 11.5 Hz, 1H); 3.17 (dd, J = 14, 6 Hz, 1H); 3.12 (dd, J = 14, 6 Hz, 1H); 1.26 (t, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.0, 166.4, 135.4, 129.4, 128.6, 127.2, 61.7, 53.8, 37.7, 14.1. HRMS (EI) *m*/*z*: found 361.0173 (361.0175 calcd for $C_{13}H_{16}INO_3$). LRMS (EI) *m*/*z* (rel abundance): 361 $[M+H]^+$ (10), 288 (10), 176 (100), 131 (19), 120 (24).

N-Iodoacetyl-Lys(Boc)-OMe (**24**, 655 mg, 77%), colorless crystals; mp 68–70 °C; $[\alpha]_D^{25}$ +23 (*c* 0.64, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 6.64 (m, D_2O exch., 1H); 4.55 (m, 1H); 3.75 (s, 3H); 3.74 (d, J = 11.5 Hz, 1H); 3.70 (d, J = 11.5 Hz, 1H); 3.11 (d, J = 6 Hz, 1H); 3.08 (d, J = 6 Hz, 1H); 1.89 (m, 1H); 1.72 (m, 1H); 1.42 (s, 9H); 1.40 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 172.4, 167.4, 156.0, 79.0, 52.4, 39.9, 31.5, 29.4, 28.3, 22.2. HRMS (EI) *m*/*z*: found 429.0873 (429.0842 calcd for $C_{14}H_{25}IN_2O_5$). LRMS (EI) *m*/*z* (rel abundance): 429 $[M+H]^+$ (10), 355 (23), 245 (45), 184 (33), 142 (100), 84 (67).

4.1.5. Synthesis of *N*-iodoacetyl-Gly-Phe-Lys(Boc)-OMe (26). To a stirred suspension of *N*-chloroacetylglycine (**11**, 303 mg, 2 mmol) and NHS (230 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added DCC (454 mg, 2.2 mmol). The cooling bath was removed and the mixture was stirred for 18 h at rt. The solvent was evaporated, the residue was suspended in dry THF (8 mL), and then H-Phe-OH (**25**, 330 mg, 2 mmol) and Et_3N (560 μL, 4 mmol) were added. The mixture was stirred for 2 h at rt after which time it was diluted with 1 M HCl (50 mL) and was extracted with EtOAc (40 + 3× 20 mL). The combined organic extract was dried, concentrated, and the residue was dissolved in dry THF (20 mL). The solution thus obtained was cooled to 0 °C, NHS (230 mg, 2 mmol) and DCC (495 mg, 2.4 mmol) were added, and the mixture was stirred for 30 min at 0 °C, followed by the addition of H-Lys(-Boc)-OMe·HCl (**22**, 594 mg, 2 mmol) and Et_3N (560 μL, 4 mmol). The cooling bath was removed and the mixture was stirred for 18 h at rt. The mixture was concentrated to about 1/3 of its original volume, diluted with brine (50 mL), and extracted with $CHCl_3$ (40 + 2× 20 mL). The combined organic extracts were dried, concentrated, and the residue obtained [*N*-chloroacetyl-Gly-Phe-Lys(Boc)-OMe containing ca. 20% of DCU (by ^{1}H NMR)] was dissolved in acetone (15 mL). To this mixture was added NaI (899 mg, 6 mmol) and the resultant suspension was stirred for 18 h at rt. The mixture was then concentrated to ca. 1/3 of its original volume, diluted with EtOAc (30 mL), and washed with 10% Na_2SO_3 solution (20 mL). The aqueous phase was extracted with EtOAc (20 mL), and the combined organic extract was dried and concentrated to give crude *N*-iodoacetyl compound **26**. Compound **26** was purified by FCC on 50 g SiO_2 (CH_2Cl_2/MeOH, 19:1) followed by crystallization from dichloromethane/hexane solution.

N-Iodoacetyl-Gly-Phe-Lys(Boc)-OMe [**26**, 670 mg, 52% based on *N*-chloroacetylglycine (**11**)], colorless crystals; mp 110–112 °C; $[\alpha]_D^{25}$ +36 (*c* 0.42, CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 8.42 (m, D_2O exch., 2H); 8.28 (m, D_2O exch., 1H); 8.13 (m, D_2O exch., 1H); 7.21 (m, 4H); 6.79 (m, 1H); 4.56 (m, 1H); 4.18 (m, 1H); 3.67 (s, 2H); 3.65 (m, 2H); 3.60 (s, 3H); 2.99 (m, 1H); 2.86 (m, 2H);

2.71 (m, 1H); 1.61 (m, 3H); 1.34 (s, 9H); 1.29 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 172.3, 171.2, 168.5, 168.1, 167.8, 155.5, 137.6, 129.2, 128.0, 126.2, 77.3, 53.5, 52.1, 51.8, 42.2, 42.1, 37.6, 30.7, 30.5, 29.1, 28.3, 22.7. HRMS (ESI) *m/z*: found 655.1596 $[M+Na]^+$ (655.1605 calcd for $C_{25}H_{37}IN_4O_7Na$).

4.1.6. Synthesis of *N*-iodoacetyl-Lys(Boc)-Phe-OEt (29). To a stirred suspension of chloroacetic acid (**27**, 190 mg, 2 mmol) and NHS (230 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added DCC (495 mg, 2.4 mmol). The cooling bath was removed and the mixture was stirred for 18 h at rt. The solvent was evaporated, the residue was suspended in dry THF (10 mL), and H-Lys(Boc)-OH (**28**, 493 mg, 2 mmol) and Et_3N (560 μL, 4 mmol) were added. The resultant mixture was stirred for 4 h at rt after which it was diluted with 1 M HCl (30 mL) and was extracted with EtOAc (2× 20 + 3× 10 mL). The combined organic extract was dried, concentrated, and the residue was dissolved in dry THF (10 mL). The solution thus obtained was cooled to 0 °C, NHS (230 mg, 2 mmol) and DCC (495 mg, 2.4 mmol) were added, and the mixture was stirred for 30 min at 0 °C and for 18 h at rt. The mixture was diluted with brine (30 mL) and then extracted with EtOAc (30 + 2× 20 mL). The organic extract was dried and concentrated and the residue was dissolved in dry THF (10 mL) followed by the addition of H-Phe-OEt·HCl (**19**, 459 mg, 2 mmol) and Et_3N (1.12 mL, 8 mmol). The mixture was stirred for 4 h at rt, was diluted with saturated $NaHCO_3$ solution (30 mL) and was extracted with EtOAc (30 + 2× 20 mL). The combined organic extract was dried, concentrated and the residue was subjected to FCC on 30 g SiO_2 (hexane/acetone, 2:1). The product obtained [*N*-chloroacetyl-Lys(Boc)-Phe-OEt containing *ca.* 10% of DCU (by ^{1}H NMR)] was dissolved in acetone (7 mL) and NaI (513 mg, 3.42 mmol) was added. The mixture was stirred for 18 h at rt and was diluted with EtOAc (30 mL). The organic phase was washed with 10% Na_2SO_3 solution (20 mL) and aqueous phase was extracted with EtOAc (20 mL). The combined organic extract was dried and was concentrated to give crude *N*-iodoacetyl compound **29**, which was subsequently purified by FCC on 30 g SiO_2 (hexane/acetone, 2:1).

N-Iodoacetyl-Lys(Boc)-Phe-OEt [**29**, 467 mg, 40% based on chloroacetic acid (**27**)], colorless solid; $[\alpha]_D^{25}$ +36 (*c* 0.56, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 3H); 7.11 (m, 2H); 6.78 (d, D_2O exch., J = 7.5 Hz, 1H); 6.47 (d, D_2O exch., J = 7.5 Hz, 1H); 4.81 (m, 1H); 4.68 (br s, D_2O exch., 1H); 4.37 (m, 1H); 4.17 (q, J = 7 Hz, 2H); 3.66 (s, 2H); 3.16–3.01 (m, 4H); 1.82 (m, 1H); 1.61 (m, 1H); 1.46 (m, 2H); 1.42 (s, 9H); 1.33 (m, 2H); 1.23 (t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 171.3, 171.0, 167.9, 156.0, 135.7, 129.2, 128.4, 127.0, 78.8, 61.4, 53.5, 53.2, 39.9, 37.7, 32.1, 29.4, 28.4, 22.5, 22.2, 14.0. HRMS (ESI) *m/z*: found 612.1537 $[M+Na]^+$ (612.1547 calcd for $C_{24}H_{36}IN_3O_6Na$).

4.1.7. Peralkylation of cyclen (30) with *N*-iodoacetyl compounds 14, 17, 21, 24, 26, and 29. To separate solutions of cyclen **30** (43 mg, 0.25 mmol for electrophiles **14**, **17**, **21**, **24**, **26** or 17 mg, 0.1 mmol for electrophile **29**) and DIPEA (**14**, **17**, **21**, **24**, and **26**, 175 μL, 1 mmol; **29**, 70 μL, 0.4 mmol) in MeCN (**14**, **17**, **21**, **24**, and **26**, 5 mL; **29**, 3 mL) were added four equivalents of the *N*-iodoacetyl compounds **14**, **17**, **21**, **24, 26** (1 mmol), and **29** (0.4 mmol). The reaction mixtures were stirred at elevated temperature as follows: *N*-iodoacetyl-Gly-Trp-OMe (**14**), 72 h at 50 °C; *N*-iodoacetyl-Gly-Tyr(*t*-Bu)-OMe (**17**) and *N*-iodoacetyl-Gly-Phe-Lys(Boc)-OMe (**26**), 24 h at 70 °C; *N*-iodoacetyl-Phe-OEt (**21**) and *N*-iodoacetyl-Lys(Boc)-OMe (**24**) 18 h at 50 °C; *N*-iodoacetyl-Lys(Boc)-Phe-OEt (**29**), 48 h at 70 °C. The reaction mixture obtained after alkylation of cyclen with *N*-iodoacetyl-Gly-Trp-OMe (**14**) was cooled to rt, poured on crushed ice (100 mL), and the resulting precipitate was isolated by vacuum filtration. After washing the precipitate with water, tetraacetyl-Gly-Trp-OMe cyclen (**31**) of satisfactory purity was obtained. The reaction mixtures obtained from alkylation of cyclen with *N*-iodoacetyl compounds **17**, **21**, **24**, and **26** were cooled to rt, diluted with EtOAc (30 mL), and washed with water (30 mL). The aqueous phases were extracted with EtOAc (20 mL) and combined organic extracts were dried and concentrated. The peralkylated cyclens **32**–**35** were purified by repeated trituration (twice) in hexanes. The reaction mixture obtained after alkylation of cyclen with *N*-iodoacetyl-Lys(Boc)-Phe-OEt (**29**) was cooled to rt and the precipitate was isolated by filtration. The precipitate was washed with cold acetonitrile to give tetraacetyl-Lys(Boc)-Phe-OEt cyclen (**36**) of satisfactory purity.

Tetraacetyl-Gly-Trp-OMe cyclen (**31**, 335 mg, 93%); colorless solid; $[\alpha]_D^{25}$ +26 (*c* 0.39, MeOH). HPLC: t_R 24.7 min; ^{1}H NMR (DMSO-d_6) δ 10.86 (s, D_2O exch., 4H); 8.38 (m, D_2O exch., 2H); 8.24 (m, D_2O exch., 4H); 8.05 (m, D_2O exch., 2H); 7.44 (d, J = 7.5 Hz, 4H); 7.30 (m, 4H); 7.12 (s, 4H); 7.04 (dd, J = 7.5, 7.5 Hz, 4H); 6.95 (dd, J = 7.5, 7.5 Hz, 4H); 4.51 (m, 4H); 3.73 (m, 8H); 3.54 (m, 20H); 3.15–2.84 (br m, 24H); ^{13}C NMR (DMSO-d_6) δ 172.2, 171.0, 169.3, 169.0, 168.8, 136.1, 127.1, 123.8, 121.1, 118.6, 118.0, 111.5, 109.2, 53.2, 53.1, 51.9, 50.9, 41.6, 27.2. HRMS (ESI) *m/z*: found 1433.6708 $[M+H]^+$ (1433.6642 calcd for $C_{72}H_{89}N_{16}O_{16}$).

Tetraacetyl-Gly-Tyr(*t*-Bu)-OMe cyclen (**32**, 381 mg, 99%); colorless solid; $[\alpha]_D^{25}$ +38 (*c* 0.66, CH_2Cl_2). HPLC: t_R 25.8 min; ^{1}H NMR ($CDCl_3$) δ 8.03 (br s, D_2O exch., 4H); 7.77 (br s, D_2O exch., 4H); 7.04 (m, 8H); 6.87 (d, J = 8 Hz, 8H); 4.73 (m, 4H); 3.84 (m, 8H); 3.63 (s, 12H); 3.62–3.64 (m, 8H); 3.05–2.60 (br m, 24H); 1.29 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 172.2, 169.1, 154.1, 129.6, 124.1, 114.9, 78.3, 53.6, 52.2, 46.3, 42.4, 36.9, 28.7. HRMS (ESI) *m/z*: found 1565.8562 $[M+H]^+$ (1565.8507 calcd for $C_{80}H_{117}N_{12}O_{20}$).

Tetraacetyl-Phe-OEt cyclen (**33**, 246 mg, 89%); colorless solid; $[\alpha]_D^{25}$ +46 (*c* 0.44, CH_2Cl_2). HPLC: t_R 27.0 min; ^{1}H NMR ($CDCl_3$) δ 7.57 (br s, D_2O exch., 4H); 7.23 (m, 16H); 7.10 (m, 4H); 4.83 (m, 4H); 4.13 (q, J = 7 Hz, 8H); 3.18–2.95 (m, 12H); 2.57–2.33 (m, 12H); 1.21 (t, J = 7 Hz, 12H); ^{13}C NMR ($CDCl_3$) δ 171.4, 171.1,

170.3, 136.4, 136.0, 129.2, 128.8, 128.0, 126.5, 61.0, 56.3, 52.7, 51.8, 37.0, 13.7. HRMS (ESI) *m/z*: found 1127.5836 $[M+Na]^+$ (1127.5793 calcd for $C_{60}H_{80}N_8O_{12}Na$).

Tetraacetyl-Lys(Boc)-OMe cyclen (**34**, 340 mg, 99%); colorless solid; $[\alpha]_D^{25}$ +12 (*c* 0.41, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 7.72 (br s, D_2O exch., 4H); 4.91 (m, D_2O exch., 4H); 4.44 (m, 4H); 3.85–3.65 (br m, 8H); 3.72 (s, 12H); 3.22–2.67 (br m, 24H); 1.83 (m, 12H); 1.56–1.26 (m, 12H); 1.43 (s, 36H); ^{13}C NMR ($CDCl_3$) δ 172.5, 156.0, 78.8, 56.5, 53.3, 52.3, 51.6, 39.9, 30.7, 29.3, 28.4, 28.3, 22.8. HRMS (ESI) *m/z*: found 1373.8469 $[M+H]^+$ (1373.8507 calcd for $C_{64}H_{117}N_{12}O_{20}$).

Tetraacetyl-Gly-Phe-Lys(Boc)-OMe cyclen (**35**, 466 mg, 94%); colorless solid; $[\alpha]_D^{25}$ +10 (*c* 0.49, CH_2Cl_2). ^1H NMR (DMSO-d_6) δ 8.41–8.20 (br m, D_2O exch., 12H); 7.19 (m, 16H); 6.74 (m, 4H); 4.58 (br s, D_2O exch., 4H); 4.19 (m, 4H); 3.81–3.54 (br m, 16H); 3.59 (s, 12H); 3.21–2.49 (br m, 32H); 1.77–1.57 (br m, 12H); 1.49–1.12 (br m, 12H); 1.34 (s, 36H); ^{13}C NMR (DMSO-d_6) δ 172.5, 172.3, 170.1, 169.8, 169.5, 155.6, 129.2, 128.0, 115.8, 77.4, 52.0, 51.8, 41.4, 37.6, 34.3, 33.0, 31.1, 30.7, 29.2, 28.3, 22.3. HRMS (ESI) *m/z*: found 2212.1941 $[M+Na]^+$ (2212.1922 calcd for $C_{108}H_{164}N_{20}O_{28}Na$).

Tetraacetyl-Lys(Boc)-Phe-OEt cyclen (**36**, 129 mg, 64%); colorless solid; $[\alpha]_D^{25}$ −8 (*c* 0.65, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 7.70 (br s, D_2O exch., 8H); 7.28–7.12 (m, 20H); 4.93 (m, 4H); 4.75 (br s, D_2O exch., 4H); 4.43 (m, 4H); 4.12 (m, 8H); 3.11–3.00 (br m, 28H); 2.62 (br s, 12H); 1.76–1.65 (br m, 8H); 1.41 (s, 36H); 1.35–1.13 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.5, 171.3, 155.9, 135.9, 129.1, 128.4, 126.9, 78.8, 61.3, 58.7, 53.4, 53.2, 53.1, 40.0, 37.7, 31.5, 29.3, 28.4, 22.8, 13.9. HRMS (ESI) *m/z*: found 2018.1862 $[M+H]^+$ (2018.1870 calcd for $C_{104}H_{161}N_{16}O_{24}$).

4.1.8. Tetraacetyl-Gly-Trp-OH cyclen (5). A solution of NaOH (1 M, 1.58 mL) was added to a solution of tetraacetyl-Gly-Trp-OMe cyclen (**31**, 113 mg, 0.079 mmol) in THF (2.5 mL). The mixture was vigorously stirred for 1 h at rt and then the THF was evaporated leaving an aqueous residue that was cooled to 0 °C. The pH was adjusted to 2 and the mixture was set aside for 1 h at 0 °C after which a precipitate was isolated by filtration, washed with water, and dried to afford tetraacetyl-Gly-Trp-OH cyclen (**5**, 81 mg, 74%) of sufficient purity. Colorless solid; $[\alpha]_D^{25}$ +31 (*c* 0.32, DMSO). HPLC: Method A: t_R 19.7 min; Method B: t_R 2.6 min; ^1H NMR (DMSO-d_6) δ 10.84 (s, D_2O exch., 4H); 8.28 (m, D_2O exch., 4H); 8.21 (m, D_2O exch., 4H); 7.49 (d, J = 8 Hz, 4H); 7.30 (d, J = 8 Hz, 4H); 7.12 (s, 4H); 7.03 (dd, J = 8, 8 Hz, 4H); 6.94 (dd, J = 8, 8 Hz, 4H); 4.48 (m, 4H); 3.74 (m, 8H); 3.46 (m, 8H); 3.17–2.98 (br m, 12H); 2.82 (m, 12H); ^{13}C NMR (DMSO-d_6) δ 173.2, 170.8, 168.6, 136.1, 127.3, 123.8, 121.0, 118.5, 118.2, 111.5, 109.6, 53.1, 51.1, 51.0, 41.7, 27.2. HRMS (ESI) *m/z*: found 1377.6010 $[M+H]^+$ (1377.6016 calcd for $C_{68}H_{81}N_{16}O_{16}$).

4.1.9. Tetraacetyl-Gly-Tyr-OH cyclen (6). To a solution of tetraacetyl-Gly-Tyr(*t*-Bu)-OMe cyclen (**32**, 157 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL). The mixture was stirred for 3 h at rt, then the solvent was evaporated, and the residue was dissolved in THF (2 mL). To this solution was added NaOH (1 M, 2 mL) and the mixture was vigorously stirred for 1 h at rt. The THF was evaporated and the aqueous residue was cooled to 0 °C. The pH was adjusted to 6 (1 M HCl) and the mixture was set aside for 1 h at 0 °C. After the aqueous phase was decanted, an oily precipitate that had deposited on the flask walls was triturated with hexanes to afford tetraacetyl-Gly-Trp-OH cyclen (**6**, 70 mg, 51%). Colorless solid; $[\alpha]_D^{25}$ +65 (*c* 0.31, DMSO). HPLC: Method A: t_R 5.7 min; Method B: t_R 3.7 min; ^1H NMR (DMSO-d_6) δ 8.25 (br s, D_2O exch., 4H); 8.27 (br s, D_2O exch., 4H); 8.19 (m, D_2O exch., 4H); 6.98 (d, J = 8.5 Hz, 8H); 6.63 (d, J = 8.5 Hz, 8H); 4.35 (m, 4H); 3.74 (m, 8H); 3.49–3.23 (m, 8H); 3.15–2.71 (br m, 24H); ^{13}C NMR (DMSO-d_6) δ 172.9, 171.8, 168.8, 156.1, 130.1, 127.3, 115.2, 57.0, 54.0, 46.3, 41.7, 36.2. HRMS (ESI) *m/z*: found 1285.5415 $[M+H]^+$ (1285.5377 calcd for $C_{60}H_{77}N_{12}O_{20}$).

4.1.10. Tetraacetyl-Phe-OH cyclen (7). A solution of NaOH (2.5 M, 1.6 mL) was added to a solution of tetraacetyl-Phe-OEt cyclen (**33**, 180 mg, 0.16 mmol) in THF (1.6 mL). The mixture was vigorously stirred for 2 h at rt, the THF was evaporated and the aqueous residue was cooled to 0 °C. The pH was adjusted to 5 (1 M HCl) and the mixture was transferred to a centrifuge tube and was centrifuged for 5 min at 1000 rpm. The supernatant was decanted and the pellet was triturated with hexanes to afford tetraacetyl-Phe-OH cyclen (**7**, 110 mg, 69%). Colorless solid; $[\alpha]_D^{25}$ −37 (*c* 0.81, CH_2Cl_2). HPLC: Method A: t_R 17.1 min; Method B: t_R 4.1 min; ^1H NMR (DMSO-d_6) δ 8.36 (br s, D_2O exch., 4H); 7.16 (m, 20H); 4.43 (m, 4H); 3.74–2.56 (br m, 32H); ^{13}C NMR (DMSO-d_6) δ 173.0, 169.8, 137.7, 129.2, 128.2, 126.1, 56.2, 52.4, 50.6, 48.2, 37.4. HRMS (ESI) *m/z*: found 993.4673 $[M+H]^+$ (993.4722 calcd for $C_{52}H_{65}N_8O_{12}$).

4.1.11. Boc-deprotection and hydrolysis of peralkylated cyclens 34–36. Tetraacetyl-Lys(Boc)-OMe (**34**, 137 mg, 0.1 mmol), tetraacetyl-Gly-Lys(Boc)-Phe-OEt (**35**, 150 mg, 0.068 mmol) and tetraacetyl-Lys(Boc)-Phe-OEt (**36**, 151 mg, 0.075 mmol) were dissolved in TFA (1.5 mL, compounds **34** and **35**) or in CH_2Cl_2 (2 mL) and TFA (1 mL, compound **36**) and the reaction mixture was stirred for 15 min at rt. The solvents were evaporated, the residues were dissolved in MeOH (**34**, 1 mL; **35**, 700 μL) or THF (**36**, 1.5 mL), and NaOH solutions (2.5 M; **34**, 1 mL; **35**, 700 μL or 1 M; **36**, 1.5 mL) were added. The mixtures were stirred for 2 h at 60 °C (compounds **34** and **35**) or for 1 h at rt (compound **36**). The hydrolysis mixtures were then cooled to 0 °C and the pH was adjusted to 6 (1 M HCl). The resultant aqueous solutions were subjected for size exclusion chromatography; fractions containing the products (identified by ninhydrin test) were combined and were lyophilized to afford ligands **8**–**10**.

Tetraacetyl-Lys-OH cyclen (**8**, 78 mg, 85%); colorless oil; $[\alpha]_D^{25}$ −9 (*c* 0.55, H_2O). ^1H NMR (D_2O) δ 4.00 (m,

4H); 3.83–2.72 (br m, 24H); 1.72–1.59 (m, 16H); 1.37–1.24 (m, 8H); ^{13}C NMR (D_2O) δ 163.1, 54.0, 38.1, 37.3, 30.0, 25.2, 22.6, 21.2, 13.4; HRMS (ESI) *m/z*: found 917.5826 $[M+H]^+$ (917.5784 calcd for $C_{40}H_{77}N_{12}O_{12}$).

Tetraacetyl-Gly-Phe-Lys-OH cyclen (**9**, 78 mg, 66%); colorless oil; $[\alpha]_D^{25}$ +5 (*c* 0.92, H_2O). ^{1}H NMR (D_2O) δ 7.13 (m, 20H); 3.98–3.69 (m, 8H); 3.40–2.63 (br m, 48H); 1.61–1.44 (m, 16H); 1.26–1.12 (m, 8H); ^{13}C NMR (D_2O) δ 173.2, 173.1, 163.6, 163.2, 162.9, 162.5, 129.5, 128.9, 121.0, 118.1, 115.2, 112.3, 55.2, 55.1, 53.2, 42.5, 39.5, 34.5, 31.4, 31.2, 26.6, 22.6, 22.4, 22.3, 22.1; HRMS (ESI) *m/z*: found 1734.9384 $[M+H]^+$ (1734.9457 calcd for $C_{84}H_{126}N_{20}O_{20}$).

Tetraacetyl-Lys-Phe-OH cyclen (**10**, 100 mg, 88%); colorless solid; $[\alpha]_D^{25}$ −17 (*c* 0.60, H_2O). ^{1}H NMR (D_2O) δ 7.45–7.23 (m, 20H); 4.48–4.45 (m, 4H); 3.92–3.88 (m, 4H); 3.60–2.64 (br m, 40H); 1.81–1.50 (m, 12H); 1.28–1.07 (m, 12H); ^{13}C NMR (D_2O) δ 163.7, 163.4, 163.0, 162.7, 130.0, 128.9, 118.1, 115.2, 55.2, 39.5, 39.3, 38.3, 30.6, 26.8, 26.5, 22.4, 21.6; HRMS (ESI) *m/z*: found 1506.8600 $[M+2H]^+$ (1506.8599 calcd for $C_{76}H_{114}N_{16}O_{16}$).

4.1.12. Metalation of ligands 5–10. Separate solutions of tetraacetyl-Gly-Trp-OH cyclen (**5**, 58 mg, 0.042 mmol), tetraacetyl-Gly-Tyr-OH cyclen (**6**, 70 mg, 0.054 mmol), tetraacetyl-Phe-OH cyclen (**7**, 60 mg, 0.06 mmol), tetraacetyl-Lys-OH cyclen (**8**, 92 mg, 0.1 mmol), tetraacetyl-Gly-Phe-Lys-OH cyclen (**9**, 119 mg, 0.068 mmol) and tetraacetyl-Lys-Phe-OH cyclen (**10**, 90 mg, 0.06 mmol) in water (**5** and **6**, 5 mL; **7**, 4 mL; **8**and **10**, 3 mL; **9**, 2 mL) were treated with lanthanide(III) chloride hexahydrates as follows: **5**, $EuCl_3{\cdot}6H_2O$, 77 mg, 0.21 mmol; **6**, $EuCl_3{\cdot}6H_2O$, 24 mg, 0.065 mmol; **7**, $EuCl_3{\cdot}6H_2O$, 44 mg, 0.12 mmol; **8**, $TmCl_3{\cdot}6H_2O$, 58 mg, 0.15 mmol; **9**, $EuCl_3{\cdot}6H_2O$, 25 mg, 0.068 mmol; **10**, $EuCl_3{\cdot}6H_2O$, 22 mg, 0.06 mmol. The reaction mixtures were treated with NaOH (2.5 M solution) to adjust the pH to 9 and were stirred for 18 h at 50 °C (ligand **5**) or at rt (ligands **6**–**10**) followed by size exclusion chromatography. The fractions containing the lanthanide(III) complexes (identified by UV/I_2 vapors, complexes **37**–**39**; ninhydrin test, complexes **40**–**42**) were combined and were freeze-dried to afford complexes **37**–**42**. Selected data are reported in Table 1.

Eu^{3+} DOTAM-Gly-Trp-OH (**37**). Colorless solid. HPLC (Method B): t_R 4.0 min. HPLC analysis indicated the presence of ca. 15% of free ligand **5**. The ^{1}H NMR has not been acquired.

Eu^{3+} DOTAM-Gly-Tyr-OH (**38**). Colorless solid. HPLC (Method B): t_R 4.1 min. ^{1}H NMR (D_2O) δ 44.88 (s); 34.15 (s); 24.42 (s); 22.41 (s); 8.24 (s); 7.24 (s); 6.70–6.61 (m); 3.25–2.82 (m); 2.39–2.03 (m); 1.73–1.02 (m); −3.01 (s); −3.52 (s); −3.94 (s); −4.95 (s); −8.14 (s); −8.84 (s); −9.03 (s); −9.72 (s); −12.03 (s); −12.92 (s); −81.04 (s).

Eu^{3+} DOTAM-Phe-OH (**39**). Colorless solid. HPLC (Method B): t_R 6.2 min. ^{1}H NMR (D_2O) δ 27.11 (s); 24.61 (s); 22.45 (s); 17.77 (s); 17.55 (s); 10.04 (s); 7.88 (s); 7.21–6.90 (m); 6.42–6.29 (m); 5.97 (s); 4.04–0.68 (m); −1.73 (s); −2.60 (s); −2.82 (s); −3.98 (s); −5.81 (s); −8.59 (s); −9.22 (s); −9.64 (s); −10.32 (s); −11.45 (s); −12.01 (s); −13.03 (s); −13.94 (s); −19.91 (s).

Tm^{3+} DOTAM-Lys-OH (**40**). Colorless oil. ^{1}H NMR (D_2O) δ 45.27 (s); 34.30 (s); 3.78–1.92 (m); 1.43–1.01 (m); −0.08 (s); −1.19 (s); −4.67 (s); −8.50 (s); −10.13 (s); −14.23 (s); −81.56 (s); −94.42 (s).

Eu^{3+} DOTAM-Gly-Phe-Lys-OH (**41**). Colorless oil. ^{1}H NMR (D_2O) δ 8.17 (s); 7.21–6.81 (m); 3.94–2.30 (m); 1.86 (s); 1.70–0.72 (m); −3.05 to −3.57 (m); −8.82 to −9.08 (m); −12.13 to −12.69 (m).

Eu^{3+} DOTAM-Lys-Phe-OH (**42**). Colorless solid. ^{1}H NMR (D_2O) δ 19.04 (s); 8.39 (s); 7.67 (s); 7.27–6.98 (m); 3.18 (s); 2.85–2.44 (m); 2.03 (s); 1.58 (s); 1.20–0.95 (m); 0.22–0.09 (m); −1.63 (s); −3.09 (s); −4.45 (s); −6.36 (s); −9.80 (s); −10.28 (s); −11.33 (s); −12.20 (s).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.04.038.

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