New Synthetic Routes for 1-Benzyl-1,4,7,10-tetraazacyclododecane and 1,4,7,10-Tetraazacyclododecane-1-acetic Acid Ethyl Ester, Important Starting Materials for Metal-coded DOTA-Based Affinity Tags

Stephan W. Kohl[a], Katharina Kuse[a], Markus Hummert[a], Herbert Schumann[a], Clemens Mügge[b], Katharina Janek[c], and Hardy Weißhoff[b]

[a] Institut für Chemie, Technische Universität Berlin, Straße des 17. Juni 135, D-10623 Berlin, Germany
[b] Institut für Chemie, Humboldt-Universität zu Berlin, Brook-Taylor-Straße 2, D-12489 Berlin, Germany
[c] Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Monbijoustraße 2, D-10098 Berlin, Germany

Reprint requests to Prof. Dr. H. Schumann. Fax +49 30 31422168.
E-mail: schumann@chem.tu-berlin.de

Z. Naturforsch. **2007**, *62b,* 397 – 406; received November 7, 2006

Dedicated to Prof. Helgard G. Raubenheimer on the occasion of his 65th birthday

Two improved routes to synthesize 1-benzyl-1,4,7,10-tetraazacyclododecane (**6**) and 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester (**11**) are described as well as the synthesis of 1-{2-[4-(maleimido-N-propylacetamidobutyl)amino]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (**17**) and its Y, Ho, Tm, and Lu complexes. The ^{1}H and ^{13}C NMR spectra of the new compounds as well as the single crystal X-ray structure analyses of the intermediates 4-benzyl-1,7-bis(*p*-toluenesulfonyl)diethylenetriamine (**3**) and 1,4,7-tris(*p*-toluenesulfonyl)diethylenetriamine (**7**) are reported and discussed. The rare earth complexes of **17** have been characterized by ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry.

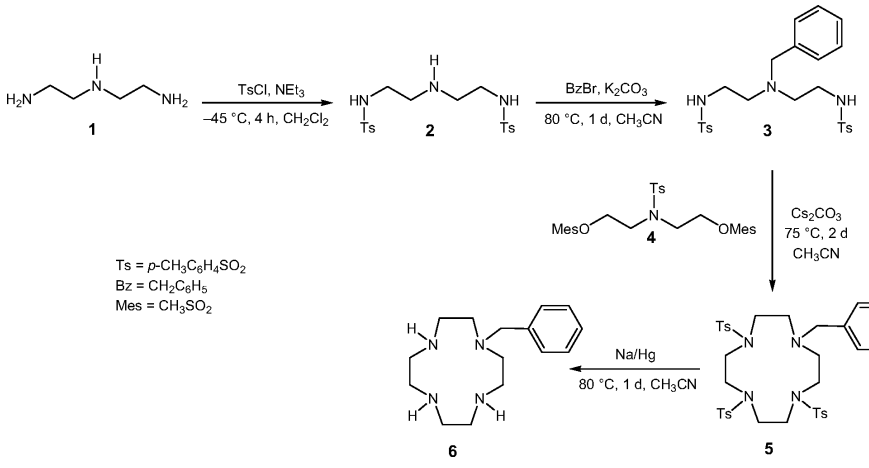
Key words: Tetraazacyclododecane, Macrocycle, DOTA, Affinity Tag, Rare Earth Complexes

Introduction

Macrocyclic polyaminopolycarboxylates have been intensively studied because of their numerous applications, which often require selective functionalization [1, 2]. Metal ion-conjugated peptides with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) or 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) ligands are ideal agents for a spectrum of applications in biomedicine, as therapeutic radiopharmaceuticals, luminescent probes for biochemical analysis, or MRI contrast agents [3 – 6]. Recently a new class of DOTA conjugates was introduced, the so-called element- or metal-coded affinity tags (MECAT) [7, 8]. These reagents can be used in quantitative proteomics, as an additional or alternative method to established 2D-GE and recently developed methods employing isotope-coded affinity tags (ICAT) and isobaric tags for relative and absolute quantitation (ITRAQ) [9 – 13].

Metal-coded affinity tags are reagents composed of a chelating ligand, a monoisotopic metal ion, predominantly rare earth cations, and a reactive group with specificity towards thiol or amino groups. The affinity can be achieved for example by an incorporated group like biotin [11] or by interaction with antibodies [4, 14]. The principle of MECATs is derived from ICAT, but instead of the stable isotope labeling of proteins or peptides a metal ion labeling is applied. The protein mixture of two or more sets of cell states is independently labeled with MECAT reagents containing different metal ions; the samples are combined, and then conventionally cleaved. The MECAT labeled peptides are isolated by affinity chromatography and analyzed by LC-ESI-MS/MS. Peptide sequence information is obtained by tandem mass spectrometry and computer searches of protein data banks. Quantitation of proteins in two cell

0932–0776 / 07 / 0300–0397 $ 06.00



Scheme 1. Route I for the synthesis of 1-benzyl-1,4,7,10-tetraazacyclododecane (**6**).

states is performed by comparing the intensity of the identical peptide peak pair from the samples defined by the mass difference of the complex ions chosen.

Metal ions, and particularly rare earth cations, are suitable for ICP-MS and permit low detection limits of quantitation. Many of the rare earth elements are naturally monoisotopic. Thus, a variety of MECATs with desired mass differences can be synthesized by pairwise integration into ligands. Considering only seven monoisotopic rare earth elements, 19 different mass tags are produceable with mass differences from 2 Da for ^{139}La/^{141}Pr to 86 Da for ^{89}Y/^{175}Lu. Thereby more than two samples can be investigated in parallel, or ambiguous analytical results can be verified in an independent run.

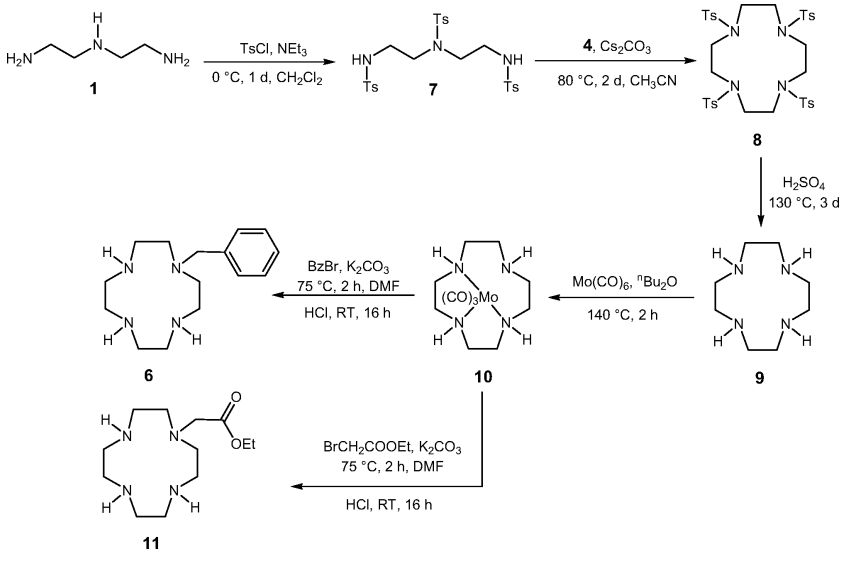
For about 15 years, several research groups have been engaged in the synthesis of mono-functionalized DOTA derivatives [15 – 18]. Meanwhile, N- and C-functionalized DOTA derivatives are commercially available, but still very expensive. To make these important compounds more readily available, we describe in this paper two suitable, cost-efficient synthetic routes to 1-benzyl-1,4,7,10-tetraazacyclododecane (**6**) [19], 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester (**11**) [20] and 1,4,7,10-tetraazacyclododecane-1-acetic acid-4,7,10-tris-(acetic acid *tert*-butyl ester) (tris-^{t}Bu-DOTA) (**15**) [17], the starting materials for the synthesis of N-functionalized DOTA ligands, as well as the synthesis of 1-{2-[4-(maleimido-N-propylacetamidobutyl)amino]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (**17**) and its Y, Ho, Tm, and Lu complexes.

Results and Discussion

*Synthesis of 1-benzyl-1,4,7,10-tetraazacyclododecane (**6**) and 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester (**11**)*

N-substituted tetraazacyclododecanes are generally synthesized starting with diethylenetriamine (**1**) and diethanolamine as common educts *via* bimolecular cyclization reactions using toluenesulfonyl protecting groups, with subsequent deprotection. To improve our recently published procedure [21], we used a modified way (Scheme 1). The first step, the selective tosylation of the two primary amino groups of **1** is possible at −45 °C in dichloromethane. Thus, the protection and deprotection of the terminal amino groups with phthalic anhydride can be avoided and the yield of 1,7-bis(*p*-toluenesulfonyl)diethylenetriamine (**2**) [22] is increased. The tri-tosylated by-product, 1,4,7-tris(*p*-toluenesulfonyl)diethylenetriamine (**7**) [23], can easily be separated by filtration and used for further preparations. Alkylation of **2** with benzyl bromide and an excess of K_2CO_3 results in the formation of 4-benzyl-1,7-bis(*p*-toluenesulfonyl)diethylenetriamine (**3**) [21], which crystallizes after a few weeks as colorless crystals. Cyclization with 1,5-bis(methylsulfonyloxy)-3-aza-3-(*p*-toluenesulfonylamido)pentane (**4**) [24] according to [21] and elimination of the protecting groups by sodium amalgam yields the monosubstituted cyclen **6** [19] in 80 % yield.

Route II for the synthesis of **6** starts with the complete tosylation of **1** at 0 °C yielding **7** [23] as a white powder which forms colorless single crystals from acetone suitable for X-ray analysis (Scheme 2). Cyclization of **7** with the bis-methylsulf-



Scheme 2. Route II for the synthesis of 1-benzyl-1,4,7,10-tetraazacyclododecane (**6**) and 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester (**11**).

onyloxy compound **4** yields 1,4,7,10-tetrakis(*p*-toluenesulfonyl)-1,4,7,10-tetraazacyclododecane (**8**) [25], which is converted into 1,4,7,10-tetraazacyclododecane (cyclen) (**9**) by heating in concentrated H_2SO_4 for three days [26]. Reaction with $Mo(CO)_6$ following the procedure described by Patinec *et al.* [27, 28] resulted in η^3-1,4,7,10-tetraazacyclododecane molybdenumtricarbonyl (**10**) [26], which was alkylated with benzylbromide and bromoacetic acid ethyl ester in DMF followed by decoordination from the $Mo(CO)_3$ fragment by HCl yielding **6** and 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester (**11**) [20], respectively.

*Molecular structure of 4-benzyl-1,7-bis(p-toluenesulfonyl)diethylenetriamine (**3**) and 1,4,7-tris(p-toluenesulfonyl)diethylenetriamine (**7**)*

The structure of monoclinic crystals of the ditosylated benzylated triamine **3** (Fig. 1) shows non-exceptional averaged bond lengths C–N (1.47 Å) and C–C (1.50 Å) along the chain of the triamine. Similar distances C–N (1.46 Å) and C–C (1.52 Å) were found in the structure of the monoclinic crystals of the tritosylated diethylenetriamine **7** (Fig. 2). The mean N–S distances in the structures of **3** and **7** are 1.61 and 1.62 Å, respectively. The conformation of the dimethylene units in the amine chain is N(1)–C(8)–C(9)–N(2) 66° ((+)-*synclinal*) and N(1)–C(10)–C(11)–N(3) −58° ((–)-*synclinal*) for compound **3** and N(2)–C(3)–C(4)–N(3) 179° (*antiperiplanar*) and N(1)–C(1)–C(2)–N(2) 69° ((+)-*synclinal*) for compound **7**. For **3**

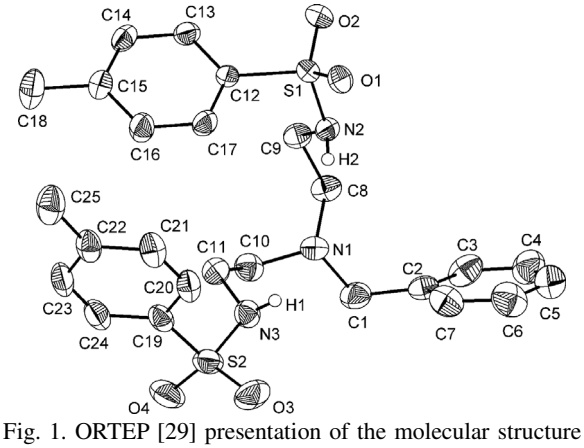


Fig. 1. ORTEP [29] presentation of the molecular structure of **3** (displacement ellipsoids at the 30 % probability level); all hydrogen atoms except H(1) and H(2) have been omitted for clarity; selected bond lengths (Å) and angles (deg): N(1)–C(1) 1.476(3), N(1)–C(8) 1.460(3), N(1)–C(10) 1.476(3), N(2)–C(9) 1.472(3), N(2)–S(1) 1.613(2), N(3)–C(11) 1.458(4), N(3)–S(2) 1.608(3), N(2)–O(1) 3.165(3), N(3)–O(3) 3.090(3); C(9)–N(2)–S(1) 118.11(17), C(11)–N(3)–S(2) 120.4(2).

the distances between the nitrogene atoms N(2) and N(3) and the oxygen atom O(1) of the adjacent molecule are 3.17 and 3.09 Å, respectively. The two molecules are linked *via* intermolecular hydrogen bonds. The bonding of two nitrogen atoms to only one oxygen atom is the reason for the close proximity (3 – 4 Å) of the two tosyl groups in this molecule, compared with **7**, where the terminal tosyl-groups are located far away from each other (9 – 10 Å). Intermolec-

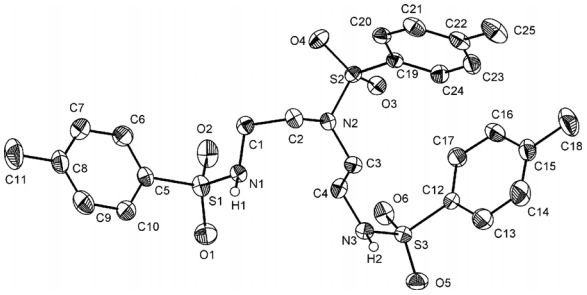


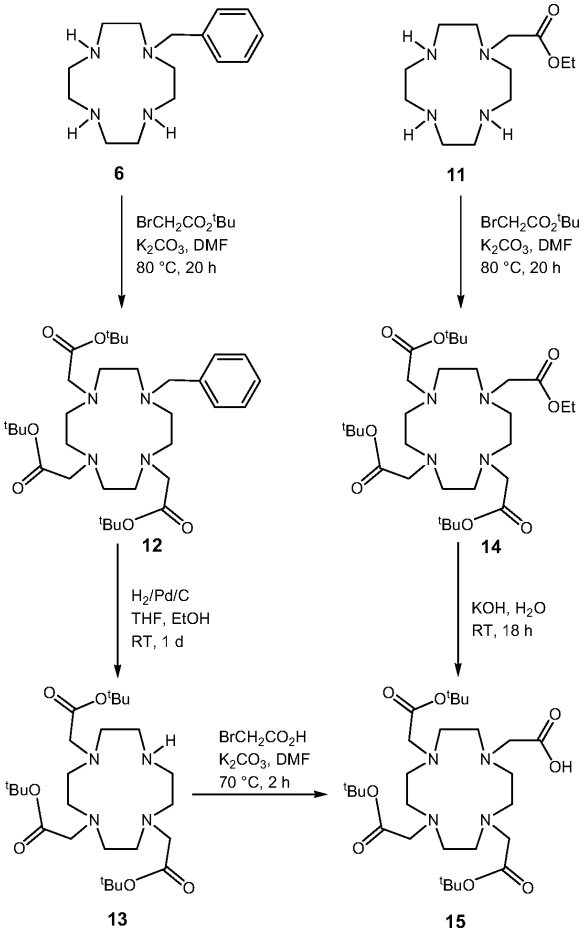
Fig. 2. ORTEP [29] presentation of the molecular structure of **7** (30 % probability ellipsoids); all hydrogen atoms except H(1) and H(2) have been omitted for clarity; selected bond lengths (Å): N(1)–C(1) 1.462(5), N(1)–S(1) 1.620(3), N(2)–C(2) 1.473(4), N(2)–C(3) 1.467(4), N(2)–S(2) 1.473(4), N(3)–C(4) 1.526(5), N(3)–S(3) 1.610(3), N(1)–O(2) 3.008(4), N(3)–O(3) 2.923(4).

ular hydrogen bonds located in **7** between N(1) and O(2) (3.01 Å) and between N(3) and O(6) (2.92 Å) lead to a network in the crystal.

*Synthesis of 1-{2-[4-(maleimido-N-propylacetamidobutyl)amino]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (**17**) and its Y, Ho, Tm, and Lu complexes*

Compounds **6** and **11** are the key compounds for the synthesis of 1,4,7,10-tetraazacyclododecane-4,7,10-tris(acetic acid *tert*-butyl ester)-1-acetic acid (tris-^{t}Bu-DOTA) (**15**) [17], which in turn is the starting material for the synthesis of N-functionalized DOTA ligands, which are commercially available, but very expensive. Following published routes [16, 30, 31], **15** is prepared either starting from **6** by alkylation of the unprotected amine functions with $BrCH_2COO^tBu$ to yield 1,4,7,10-tetraazacyclododecane-1-benzyl-4,7,10-tris(acetic acid *tert*-butyl ester) (**12**), followed by removal of the protecting benzyl group with H_2/Pd/C to produce 1,4,7,10-tetraazacyclododecane-4,7,10-tris(acetic acid *tert*-butyl ester) (**13**) [32] and finally by incorporation of an acetate group or from **11** in two steps *via* 1,4,7,10-tetraazacyclododecane-1-acetic acid ethyl ester-4,7,10-tris (acetic acid *tert*-butyl ester) (**14**) (Scheme 3).

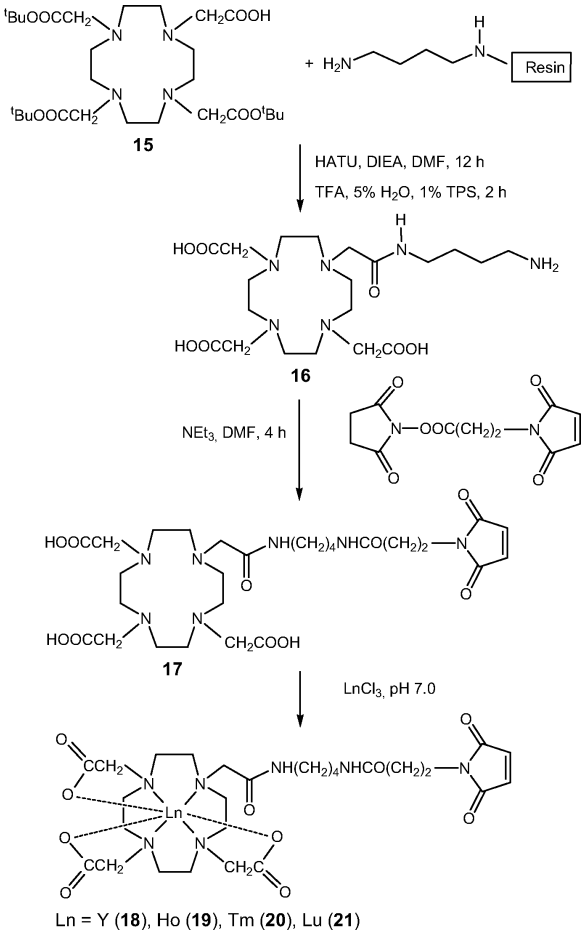
The triply protected DOTA-derivative **15** reacts with 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), Hünig's base (DIEA) and 1,4-diaminobutane trityl resin in DMF with formation of the resin-fixed tris-*tert*-butyl ester of 2-(1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecyl)-acetyl-diaminobutane, which is



Scheme 3. Synthesis of tris-^{t}Bu-DOTA (**15**).

deprotected and cleaved from the resin by trifluoroacetic acid (TFA), water and triisopropylsilane (TPS) yielding **16** in 84 % yield as a white solid (Scheme 4).

Compound **16** reacts with β-maleimidopropionic acid *N*-hydroxysuccinimid ester in the presence of triethylamine in DMF yielding 1-{2-[4-(maleimido-N-propylacetamidobutyl)amino]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (**17**) in 73 % yield as a white solid. Its reaction with lanthanide trichlorides in water at pH 7.0 results in the formation of lanthanide(III) complexes. The yttrium, holmium, thulium, and lutetium complexes **18**, **19**, **20**, and **21** have been characterized by MALDI-TOF mass spectrometry. Fig. 3 shows the MALDI-TOF mass spectrum of **17** chelating Y^{3+}, Ho^{3+}, Tm^{3+} and Lu^{3+} ions. The spectrum demonstrates the utility of rare earths embedded in peptide specific labels as internal standards for quantitative proteomics based



Scheme 4. Synthesis of 1-{2-[4-(maleimido-N-propylacetamidobutyl)amino]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (**17**) and the lanthanide complexes **18** – **21**.

on mass spectrometry. Here, mass tags with differences from 4 Da for ^{165}Ho/^{169}Tm-**17** to 86 Da for ^{89}Y/^{175}Lu-**17** are shown as an example for the variety of different combinations. The use of rare earth elements in addition has the advantage that quantitation can be accomplished by means of ICP MS with very high efficiency and sensitivity. Furthermore, the mass differences between the heavy rare earth-containing tags is useful for the peptide and protein identification in complex mixtures [33].

The lutetium complex **21** was also characterized by elemental analysis and ^{1}H and ^{13}C NMR spectroscopy. These spectra as well as those of **16** and **17** are complicated and very hard to assign because of internal hydrogen bonds which cause very broad signals for the macrocyclic CH_2 protons at low pH values [20, 34].

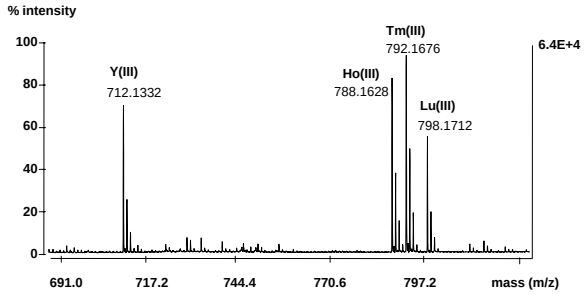


Fig. 3. MALDI-TOF Mass spectrum of the potential thiol-specific MECAT ligand **17** chelating Y, Ho, Tm, and Lu ions.

Further investigations concerning structural analysis of the lanthanide complexes and their application are in progress.

Experimental Section

Unless noted otherwise, all reactions were carried out at r. t. in dried solvents under dry dinitrogen, using standard Schlenk techniques. Chemicals were purchased from Aldrich, Acros, Chempur, and Macrocyclics and used without further purification. *p*-$CH_3C_6H_4SO_2N(CH_2CH_2OSO_2CH_3)_2$ (**4**) was prepared according to the literature [24]. ^{1}H and ^{13}C NMR spectra were recorded with Bruker ARX 200 and Bruker AV 400 spectrometers. Chemical shifts δ were references to TMS or 3-(trimethylsilyl)-propionic acid-D_4 sodium salt (TSP) for measurements in D_2O. Signs of coupling constants were not determined. The MALDI-TOF spectra were recorded with a MALDI-TOF/TOF 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA). Elemental analyses were carried out using a Thermo Finnigan, Flash EA, 1112 Series analyzer.

*$HN(CH_2CH_2NHSO_2C_6H_4CH_3$-p$)_2$ (**2**).* Diethylenetriamine (10.0 g, 0.097 mol) and triethylamine (19.2 g, 0.190 mol) were dissolved in CH_2Cl_2 (300 mL) and cooled to −48 °C. To this solution *p*-toluenesulfonylchloride (36.2 g, 0.190 mol) in CH_2Cl_2 (100 mL) was added over a period of 4 h. The temperature did not exceed −45 °C. After that, the mixture was stirred for 4 h at r. t., and washed three times with water. The organic layer was dried with Na_2SO_4 and the solvents evaporated to give a colorless oil, which was further dried in vacuum. The residue was crystallized from CH_2Cl_2/CH_3OH (1 : 3). Beside the favored ditosylated oily product (**2**), the crystalline tri-tosylated product **7** is formed (m. p. 59 – 61 °C). Yield: 30.0 g (75 %) for **2** and 6.0 g (10 %) for **7**. – ^{1}H NMR (25 °C, 200 MHz, $CDCl_3$): δ = 2.35 (s, 6 H, Ts-CH_3), 2.51 (m, 4 H, Ts-NHCH_2CH_2N), 2.86 (m, 4 H, Ts-NHCH_2CH_2N), 4.56 (br, 3 H, NH), 7.20 (m, 4 H, SO_2CCHC*H*), 7.66 (m, 4 H, SO_2CC*H*CH). – ^{13}C NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 21.0 (Ts-CH_3), 41.9 (NHCH_2CH_2NH-Ts), 47.3 (NH*C*H_2CH_2NH-

Ts), 126.6 (2 × SO_2CCHCH), 129.3 (2 × SO_2CCHCH), 136.3 (2 × SO_2CCH), 142.9 (2 × $SO_2CCHCHCCH_3$). – $C_{18}H_{25}N_3O_4S_2$(411.53): calcd. C 52.53, H 6.12, N 10.21, S 15.58; found C 52.45, H 5.99, N 10.12, S 15.55.

*$C_6H_5CH_2N(CH_2CH_2NHSO_2C_6H_4CH_3$-p)$_2$ (**3**).* The tosylated trisamine **2** (5.8 g, 14 mmol) was dissolved in CH_3CN (200 mL) and 5.5 g (39 mmol) of dried K_2CO_3 were added. The mixture was heated to 80 °C and after rapid dropwise addition of $C_6H_5CH_2Br$ (1.7 g, 14 mmol) refluxed for 24 h. The resulting precipitate was filtered and washed 3 times with CH_2Cl_2 (30 mL). The organic layers were washed with water (4 × 40 mL) and dried with Na_2SO_4. The solvent was removed by rotary evaporation to leave **3** as a pale yellow oil, which crystallizes after several days. M. p. 52 °C. Yield: 6.9 g (98 %). – ^{1}H NMR (25 °C, 400 MHz, $CDCl_3$): δ = 2.30 (s, 6 H, CH_3), 2.35 – 2.40 (m, 4 H, NCH_2CH_2NH), 2.85 (br, 4 H, NCH_2CH_2NH), 3.50 (s, 2 H, benzyl-CH_2), 5.64 (br; 2 H, NH), 7.13 – 7.26 (m, 5 H, benzyl-H), 7.27 – 7.29 (m, 4 H, SO_2CCHCH), 7.63 – 7.75 (m, 4 H, SO_2CCHCH). – ^{13}C NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 21.3 (Ts-CH_3), 44.9 (NH-CH_2CH_2NH-Ts), 51.3 ($NHCH_2CH_2$NH-Ts), 59.3 (PhCH_2-), 126.8 (2 × SO_2CCHCH), 127.35 ($CH_2CCHCHCH$), 128.34 (CH_2CCHCH), 129.92 (CH_2CCHCH), 130.1 (2 × SO_2C-$CHCH$), 136.1 (2 × SO_2CCH), 136.31 (CH_2CCH), 142.7 (2 × $SO_2CCHCHC$). – $C_{25}H_{31}N_3O_4S_2$ (501.66): calcd. C 59.86, H 6.23, N 8.38, S 12.78; found C 59.55, H 6.13, N 8.20, S 12.71.

*$C_6H_5CH_2N[CH_2CH_2N(SO_2C_6H_4CH_3$-p)$]_3CH_2CH_2$ (**5**)* was prepared according to [21] from 10.7 g (21 mmol) of **3** and 8.9 g (21 mmol) of **4**. M. p. 161 – 164 °C. Yield: 9.9 g (65 %). – ^{1}H NMR (25 °C, 400 MHz, $CDCl_3$): δ = 2.39 (s, 6 H, Ts-CH_3), 2.44 (s, 3 H, Ts-CH_3), 2.73 (dd, 4 H, benzyl-NCH_2), 3.08 (dd, 4 H, benzyl-NCH_2CH_2), 3.34 (dd, 4 H, benzyl-$NCH_2CH_2NCH_2$), 3.46 (dd, 4 H, benzyl-NCH_2CH_2-NCH_2CH_2), 3.61 (s, 2 H, PhCH_2-), 7.14 – 7.20 (m, 5 H, Ph), 7.26 (m, 4 H, SO_2CCHCH), 7.33 (m, 2 H, SO_2CCHCH), 7.57 (m, 4 H, SO_2CCHCH), 7.72 (m, 2 H, SO_2CCHCH). – ^{13}C NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 21.48 (Ts-CH_3), 21.53 (2 × Ts-CH_3), 48.62 (benzyl-NCH_2CH_2), 50.93 (benzyl-$NCH_2CH_2NCH_2CH_2$), 51.68 (benzyl-NCH_2-CH_2NCH_2), 55.12 (benzyl-NCH_2CH_2), 59.52 (PhCH_2-), 127.42 (2 × SO_2CCHCH), 127.57 ($CH_2CCHCHCH$), 128.25 (CH_2CCHCH), 129.70 (2 × SO_2CCHCH), 129.92 (CH_2CCHCH), 134.68 (2 × SO_2CCH), 135.62 (SO_2CCH), 136.31 (CH_2CCH), 143.45 ($SO_2CCHCHC$), 143.58 (2 × $SO_2CCHCHC$). – $C_{36}H_{44}N_4O_6S_3$ (724.95): calcd. C 59.65, H 6.12, N 7.73, S 13.27; found C 59.08, H 6.14, N 7.94, S 13.22.

*$C_6H_5CH_2N(CH_2CH_2NH)_3CH_2CH_2$ (**6**).* Route I: 5.8 g (8.0 mmol) of **5**, 6.8 g (48 mmol) of anhydrous Na_2HPO_4, and sodium amalgam (2 %, 9.5 g, 48 mmol) were stirred in CH_3CN (250 mL) at 80 °C for one day. The colourless mixture changed to white, and mercury precipitated which was separated. The solvent was removed on a rotary evaporator and the grey residue was dissolved in $CHCl_3$ (80 mL) and washed three times with water (55 mL). The organic phases were combined and dried with Na_2SO_4. The solvent was removed and the crude product was dried under vacuum. Recrystallization from CH_2Cl_2/CH_3OH (10 : 1) yielded **6** as a bright yellow solid. M. p. 83 – 85 °C. Yield: 1.6 g (80 %). Route II: 5.1 g (14 mmol) of **10** and 6.8 g (49 mmol) of K_2CO_3 were suspended in DMF (150 mL) and stirred for 30 min at 75 °C. Afterwards $C_6H_5CH_2Br$ (1.7 mL, 14 mmol) was added dropwise and the mixture was refluxed for 2 h precipitating a white solid. After cooling to r. t., filtering and evaporating the solvent, the yellow residue was treated with HCl (35 mL, 10 %) and stirred at r. t. on air for further 16 h. After raising the pH to 8 a brown solid was formed and removed by centrifugation. The resulting clear blue solution was extracted with $CHCl_3$ (4 × 35 mL). The combined organic layers were dried with Na_2SO_4. A yellow solid was obtained after evaporation of all volatiles and drying under vacuum. Yield: 2.49 g (68 %). – ^{1}H NMR (25 °C, 400 MHz, $CDCl_3$): δ = 2.44 – 2.77 (m, 16 H, macrocyclic CH_2), 3.52 (s, 2 H, PhCH_2), 7.12 – 7.25 (m, 5 H, benzyl-H). – ^{13}C-NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 44.54 (benzyl-N$(CH_2)_2NCH_2CH_2$), 45.92 (benzyl-N$(CH_2)_2NCH_2$), 46.70 (benzyl-NCH_2CH_2), 50.90 (benzyl-NCH_2), 58.91 (PhCH_2-), 126.82 ($CCHCHCH$), 128.06 ($CCHCHCH$), 129.06 ($CCHCHCH$), 138.78 ($CCHCHCH$). – $C_{15}H_{26}N_4$ (262.40): calcd. C 68.66, H 9.99, N 21.35; found C 68.28, H 9.80, N 21.28.

*p-$CH_3C_6H_4SO_2N(CH_2CH_2NHSO_2C_6H_4CH_3$-p)$_2$ (**7**).* 2.0 g (19 mmol) of **1** and NEt_3 (7.9 mL, 57 mmol) were dissolved in CH_2Cl_2 (100 mL) and the mixture cooled to −2 °C. A solution of *p*-toluenesulfonylchloride (10.9 g, 57 mmol) in CH_2Cl_2 (50 mL) was added dropwise keeping the temperature at 0 °C. The mixture was stirred at this temperature for 24 h and then washed with water (3 × 55 mL). The organic layer was dried with Na_2SO_4. Evaporation and drying under vacuum resulted in **7** as a white solid. M. p. 59 – 61 °C. Yield: 10.2 g (95 %). – ^{1}H NMR (25 °C, 200 MHz, [D_6]DMSO): δ = 2.37 (s, 9 H, Ts-CH_3), 2.81 – 3.02 (m, 8 H, $NHCH_2CH_2N$), 5.37 (br, 2 H, NH), 7.33 – 7.40 (m, 6 H, SO_2CCHCH), 7.54 (d, ^3J = 8.2 Hz, 2 H, SO_2CCH), 7.66 (m, 6 H, (d, ^3J = 8.2 Hz, 4 H, $NHSO_2CCH$). – ^{13}C NMR (25 °C, 50.32 MHz, [D_6]DMSO): δ = 20.97 (Ts-CH_3), 41.59 (2× $NHCH_2$), 48.40 (2× $NHCH_2CH_2$), 126.54 (2× $NHSO_2CCHCH$), 126.83 (NSO_2CCHCH), 129.68 (2× $NHSO_2CCHCH$), 129.88 (NSO_2CCHCH), 135.31 (NSO_2CCH), 137.36 (2× $NHSO_2CCH$), 142.76 (2× $NHSO_2CCHCHCCH_3$), 143.46 ($NSO_2CCHCHCCH_3$). – $C_{25}H_{31}N_3O_6S_3$ (565.73): calcd. C 53.08, H 5.52, N 7.43, S 17.00; found C 53.15, H 5.45,

N 17.29, S 17.29.

*(p-$CH_3C_6H_4SO_2NCH_2CH_2)_4$ (**8**)*. Cs_2CO_3 (50.6 g, 0.150 mol) was suspended in a solution of **7** (29.3 g, 0.052 mol) in CH_3CN (300 mL) and heated to 80 °C. Afterwards 21.5 g (0.052 mol) of **4**, dissolved in CH_3CN (250 mL), were added over a period of 1 h and the mixture stirred at this temperature for 2 d. After cooling to r. t. 500 mL of water were added in order to separate the partially precipitated product from the excess carbonate by filtration. The remaining solution was extracted with CH_2Cl_2 (5 × 80 mL), the organic layers were combined, the solvent was reduced in volume to about one third and CH_3OH (100 mL) was added. After storing the solution for 3 d at 5 °C, **8** was obtained as a white solid. Decomposition > 260 °C. Yield: 27.9 g (68 %). – ^{1}H NMR (25 °C, 200 MHz, $CDCl_3$): δ = 2.45 (s, 12 H, Ts-CH_3), 3.43 (s, 16 H, macrocyclic C*H*$_2$), 7.28 – 7.35 (m, 8 H, SO_2CCHCH), 7.61 – 7.71 (m, 8 H, SO_2CCHCH). – ^{13}C NMR (25 °C, 50.32 MHz, $CDCl_3$): δ = 21.38 (Ts-CH_3), 44.41 (CH_2), 126.43 (SO_2CCHCH), 129.68 (SO_2CCHCH), 135.41 (SO_2CCH), 142.75 (SO_2CCHCH). – $C_{36}H_{44}N_4O_8S_4$ (789.01): calcd. C 54.80, H 5.62, N 7.10, S 16.25; found C 54.45, H 5.44, N 7.29, S 16.00.

*($HNCH_2CH_2)_4$ (**9**)*. 65.3 g (0.083 mol) of **8** was stirred with 100 mL of concentrated sulphuric acid for 3 d at 130 °C. The initially colorless solution changed to brown after a few h and a black precipitate occurred. The mixture was cooled to 0 °C, diluted with 150 mL of water and then the pH was adjusted to > 13 by addition of solid KOH (130 g, 2.32 mol). The filtered precipitate was washed with EtOH (2 × 90 mL) and the aqueous and the organic phases were combined and evaporated. The brownish residue was dissolved in 80 mL of 0.1 M HCl and washed with CH_2Cl_2 (4 × 30 mL). The pH of the aqueous phase was adjusted again to > 13 and the solution extracted with $CHCl_3$ (4 × 30 mL). After combining and drying of the organic phases with K_2CO_3 the solvent was removed and the white solid of **9** was dried in a vacuum. M. p. 113 – 114 °C. Yield: 8.6 g (60 %). – ^{1}H NMR (25 °C, 400 MHz, $CDCl_3$): δ = 2.17 (s, 4 H, NH), 2.68 (s, 16 H, CH_2). – ^{13}C NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 46.12 (CH_2). – $C_8H_{20}N_4$ (127.27): calcd. C 55.78, H 11.70, N 32.52; found C 55.13, H 11.99, N 32.50.

*($CO)_3Mo(HNCH_2CH_2)_4$ (**10**)*. 2.7. g (16 mmol) of **9** and 4.6 g (16 mmol) of $Mo(CO)_6$ were suspended in *n*-dibutylether (80 mL) and heated to 140 °C for 2 h. The yellow precipitate was filtered off and washed with diethyl ether (3 × 15 mL) to yield 5.2 g (92 %) of **10**. – $C_{11}H_{20}N_4O_3Mo$ (352.24): calcd. C 37.51, H 5.72, N 15.91; found C 36.93, H 5.83, N 15.61.

*$C_2H_5OC(O)CH_2N(CH_2CH_2NH)_3CH_2CH_2$ (**11**)*. **11** was prepared in analogy to the synthesis of **6** following route II using 1.1 g (3.0 mmol) of **10**, 1.4 g (49 mmol) of K_2CO_3, 0.33 mL (3.0 mmol) of $BrCH_2COOEt$, and 80 mL of DMF. Yield: 0.40 g (55 %) of **11** as a light yellow solid. M. p. 89 – 91 °C. – ^{1}H NMR (25 °C, 400 MHz, $CDCl_3$): δ = 1.20 (t, ^3J = 8.9 Hz, 3 H, CH_2CH_3), 2.48 – 2.84 (m, 16 H, macrocyclic CH_2), 3.30 (s, 2 H, NCH_2CO), 4.10 (q, ^3J = 8.9 Hz, 2 H, CH_2CH_3). – ^{13}C NMR (25 °C, 100.64 MHz, $CDCl_3$): δ = 13.95 (CH_2CH_3), 51.05 (NCH_2CO), 46.30 (ester-$N(CH_2)_2NCH_2CH_2$), 47.03 (ester-$N(CH_2)_2NCH_2$), 50.34 (ester-NCH_2CH_2), 55.70 (ester-NCH_2), 60.65 (CH_2CH_3), 172.34 (NCH_2CO). – $C_{12}H_{26}N_4O_2$ (258,36): calcd. C 55.79, H 10.14, N 21.69; found C 55.95, H 10.30, N 21.80.

*$C_6H_5CH_2NCH_2CH_2[N(CH_2COO^tBu)CH_2CH_2]_3$ (**12**)*. 1.1 g (4 mmol) of **6** and 1.7 g (12 mmol) of dried K_2CO_3 were suspended in DMF (180 mL) and heated to 80 °C for 30 min. Afterwards $BrCH_2COO^tBu$ (2.34 mL, 12 mmol) was added dropwise and the mixture refluxed for 20 h, precipitating KBr as a white solid. The solvent was evaporated, the residue dissolved in CH_2Cl_2 (50 mL) and filtered. The colorless solution was then washed with water (3 × 45 mL) and the organic layer was dried with Na_2SO_4. A yellow solid was obtained after evaporation of the volatiles and drying in vacuum. Yield: (1.81 g, 75 %). M. p. 95 – 98 °C. – ^{1}H NMR (25 °C, 200 MHz, $CDCl_3$): δ = 1.39 (s, 18 H, ^{t}Bu), 1.43 (s, 9 H, ^{t}Bu), 2.57 – 2.81 (m, 16 H, macrocyclic C*H*$_2$), 3.17 (s, 4 H, $CH_2CO_2^tBu$), 3.29 (s, 2 H, $CH_2CO_2^tBu$), 3.49 (s, 2 H, benzyl-C*H*$_2$), 7.20 – 7.25 (m, 5 H, benzyl-*H*). – ^{13}C NMR (25 °C, 50.32 MHz, $CDCl_3$): δ = 28.72 ($C(CH_3)_3$), 52.41 (benzyl-$N(CH_2)_2NCH_2CH_2$), 52.60 (benzyl-NCH_2CH_2), 52.72 (benzyl-NCH_2CH_2), 56.68 (NCH_2CO), 60.11 (NCH_2-benzyl), 81.75 ($C(CH_3)_3$), 127.32 (benzyl-C_{para}), 128.51 (benzyl-C_{meta}), 128.97 (benzyl-C_{ortho}), 136.01 (benzyl-C_{quart}), 169.68 ($NCH_2CO_2{}^tBu$). – $C_{33}H_{56}N_4O_6$ (604.82): calcd. C 65.53, H 9.33, N 9.26; found C 65.83, H 9.51, N 9.40.

*$HNCH_2CH_2[N(CH_2COO^tBu)CH_2CH_2]_3$ (**13**)*. Hydrogen gas was bubbled through a suspension of **12** (1.2 g, 2.0 mmol) and the catalyst Pd/C (10 % Pd, 200 mg) in a mixture of CH_3OH and THF (1 : 1, 300 mL) at r. t. over night. After removing the catalyst by filtration over celite, the solvent was evaporated and the brownish residue dried in vacuum. Crystallization from a mixture of acetone/diisopropylether (2 : 1) yielded a light yellow solid (0.41 g, 40 %). M. p. 47 – 50 °C. – IR (KBr): ν = 1733 (s, C=O, ester), 1158 (s, C–O, ester), 1059 (m, C–N) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, r. t.): δ = 1.39 (s, 27 H, ^{t}Bu), 2.52 (m, 4 H, $NHCH_2CH_2$), 2.68 (s, 8 H, $NRCH_2CH_2NR$), 2.77 (m, 4 H, $NHCH_2$), 3.26 (s, 6 H, $CH_2CO_2{}^tBu$). – ^{13}C{^{1}H} NMR (100.64 MHz, $CDCl_3$, r. t.): δ = 28.24 ($C(CH_3)_3$), 47.56 ($NHCH_2CH_2$), 50.70 ($NH(CH_2)_2NRCH_2$), 52.16 ($NHCH_2$), 52.24 ($NH(CH_2)_2NRCH_2CH_2$), 52.80 ($NH(CH_2)_2N$-$R(CH_2)_2NCH_2CO$), 57.23 (s, $NH(CH_2)_2NCH_2CO$), 81.78 ($C(CH_3)_3$), 171.14 (NCH_2CO). – MS (EI, 70 eV): *m/z* (%) = 514 (24.10) $[M]^+$, 413 (100) $[M - CO_2 - {}^tBu]^+$. – $C_{26}H_{50}N_4O_6$ (514.70): calcd. C 60.67, H 9.79, N 10.89;

found C 60.38, H 9.62, N 10.39.

*$EtOC(O)CH_2NCH_2CH_2[N(CH_2COO^tBu)CH_2CH_2]_3$ **(14)*** was prepared as described for **12** from **11** (0.25 g, 1.0 mmol), K_2CO_3 (0.41 g, 3.0 mmol), $BrCH_2COO^tBu$ (0.44 mL, 3.0 mmol), and 25 mL of DMF as a white solid. Yield: 0.44 g (74 %). Decomposition > 140 °C. – ^{1}H NMR (200 MHz, $CDCl_3$, r. t.): δ = 1.21 (t, ^3J = 8.85 Hz, 3 H, OCH_2CH_3), 1.39 (s, 18 H, ^{t}Bu), 1.41 (s, 9 H, ^{t}Bu), 2.50 – 2.86 (m, 16 H, macrocyclic CH_2), 3.20 – 3.31 (m, 6 H, $CH_2CO_2{}^t$Bu), 3.34 (s, 2 H, NCH_2CO_2Et), 4.10 (q, ^3J = 8.85 Hz, 2 H, OCH_2CH_3). – $^{13}C\{^1H\}$ NMR (50.32 MHz, $CDCl_3$, r. t.): δ = 14.03 (OCH_2CH_3), 27.72 ($C(CH_3)_3$), 51.59 (NCH_2CO), 55.91 ($EtO_2CCH_2N(CH_2)_2NCH_2CH_2$), 56.25 ($EtO_2CCH_2N(CH_2)_2NCH_2$), 56.41 ($EtO_2CCH_2NCH_2CH_2$), 56.78 ($EtO_2CCH_2NCH_2$), 60.85 ($OCH_2CH_3$), 81.75 ($C(CH_3)_3$), 171.98 ($NCH_2CO_2{}^t$Bu), 172.64 ($NCH_2CO_2Et$). – $C_{30}H_{56}N_4O_8$ (600.80): calcd. C 59.98, H 9.39, N 9.33; found C 59.83, H 9.31, N 9.20.

*$HOC(O)CH_2NCH_2CH_2[N(CH_2COO^tBu)CH_2CH_2]_3$ **(15)***. a) **15** was prepared as described above for **12** from **13** (0.1 g, 0.2 mmol), K_2CO_3 (28 mg, 0.2 mmol), $BrCH_2COOH$ (0.014 mL, 0.2 mmol) and 10 mL of DMF at 70 °C (2 h), as a white solid. Yield: 74 mg (65 %). M. p. 127 – 130 °C. b) **14** (0.3 g, 0.5 mmol) was suspended in aqueous KOH solution (1 M, 5 mL) and stirred for one day at 30 °C. The mixture was brought to dryness, the residue suspended in C_2H_5OH (10 mL) and then filtered. This process was repeated five times. The combined organic layers were dried with Na_2SO_4. A white microcrystalline solid was obtained after evaporation of the solvents and drying in vacuum. Yield: 0.17 g (59 %). M. p. 129 – 131 °C. – IR (KBr): ν = 1738 (s, C=O, ester), 1644 (s, C=O, acid), 1161 (s, C–O, ester), 1120 (m, C–N) cm^{-1}. – ^{1}H NMR (400 MHz, $CDCl_3$, r. t.): δ = 1.42 (s, 27 H, ^{t}Bu), 2.76 (s, 8 H, acid-$N(CH_2)_2NCH_2CH_2$), 3.04 (m, 4 H, acid-NCH_2CH_2), 3.29 (s, 4 H, acid-$N(CH_2)_2NCH_2CO$), 3.37 (s, 2 H, acid-$N\{(CH_2)_2N\}_2CH_2CO$), 3.60 (m, 4 H, acid-NCH_2), 3.69 (s, 2 H, CH_2COOH). – $^{13}C\{^1H\}$ NMR (100.64 MHz, $CDCl_3$, r. t.): δ = 28.13 ($C(CH_3)_3$), 48.47 (acid-NCH_2CH_2), 50.28 (acid-$N(CH_2)_2NCH_2$), 53.51 (acid-$NCH_2CH_2NCH_2CH_2$), 55.75 (NCH_2COOH), 56.07 (acid-$N\{(CH_2)_2N\}_2CH_2CO$), 56.75 (acid-$N(CH_2)_2NCH_2CO$), 81.80 ($C(CH_3)_3$), 166.95 ($COOH$), 169.93 (acid-$N(CH_2)_2NCH_2CO$), 170.69 (acid-$N\{(CH_2)_2N\}_2CH_2CO$). – MS (EI, 70 eV): *m/z* (%) = 572 (3.10) $[M]^+$, 471 (100) $[M - CO_2 - {}^tBu]^+$. – $C_{28}H_{52}N_4O_8$ (572.74): calcd. C 58.72, H 9.15, N 9.78; found C 58.61, H 9.07, N 9.51.

*$H_2N(CH_2)_4NHC(O)CH_2NCH_2CH_2[N(CH_2COOH)CH_2CH_2]_3$ **(16)***. To a solution of **15** (1.34 g, 2.35 mmol) in DMF (40 mL), 0.983 g (2.585 mmol) of HATU and 0.5 mL of Hünig's base were added. The mixture was stirred for 5 min and added to 5 g of 1,4-diaminobutane trityl resin (loading 0.47 mmol/g, 2.35 mmol) in DMF. The reaction mixture was agitated at r. t. overnight and the solvent removed in vacuum. Afterwards the cleavage from the resin was carried out with 50 mL of TFA, 5 % water and 1 % tri-*iso*-propylsilane for 2 h. The mixture was filtered and the filtrate was evaporated in vacuum. The residue was washed with ether yielding **16** (935 mg, 84 %). Further purification was achieved by preparative HPLC (Agilent-Prep-C18 column; solvent A: 0.1 % TFA in water; solvent B: 10 % of aq. 0.1 % TFA, 90 % aq. CH_3CN). Removal of the mobile phase gave the product as a lyophilized solid. M. p. 168 – 170 °C. – ^{1}H NMR (400 MHz, D_2O): δ = 1.40 (m, 2 H, $NH_2CH_2CH_2$), 1.48 (m, 2 H, $NHCH_2CH_2$), 2.89 (t, 2 H, NH_2CH_2), 3.08 (m, 2 H, $CONHCH_2$), 2.70 – 3.50 (broad, 16 H, NCH_2CH_2N), 3.60 – 4.20 (broad, 8 H, $NHCH_2CO$). – $^{13}C\{^1H\}$ NMR (100.64 MHz, D_2O): δ = 24.2 ($NHCH_2CH_2$), 25.4 ($NH_2CH_2CH_2$), 38.6 ($CONHCH_2$), 39.1 (NH_2CH_2), 47.0 – 53.5 (broad, NCH_2CH_2N), 53.5 – 57.0 (broad, $NHCH_2CO$), 174.0 – 175.0 (broad, CO) – MALDI-TOF MS: *m/z* = 457 $[M+H]^+$. – $C_{20}H_{38}N_6O_7$ (474.56): calcd. C 50.62, H 8.07, N 17.71; found C 50.53, H 8.01, N 17.81.

*$C_4H_2O_2N(CH_2)_2C(O)NH(CH_2)_4NHC(O)CH_2NCH_2$-$CH_2[N(CH_2COOH)CH_2CH_2]_3$ **(17)***. To a solution of **16** (500 mg, 1.05 mmol) in 25 mL of DMF, 0.75 mL of NEt_3 and a solution of 560 mg (2.1 mmol) of β-maleimidopropionic acid N-hydroxysuccinimide ester in 10 mL of DMF were added. The mixture was allowed to stand for 4 h at r. t. with occasional stirring. The precipitate was filtered and the filtrate was evaporated to dryness. Impurities were removed by washing with $CHCl_3$ and CH_3OH. Yield: 480 mg (73 %) of **17**. Further purification was achieved by preparative HPLC (Agilent-Prep-C18 column; solvent A: 0.1 % TFA in water; solvent B: 10 % of aq. 0.1 % TFA, 90 % aq. CH_3CN). M. p. 183 – 185 °C. – ^{1}H NMR (400 MHz, D_2O): δ = 1.42 (m, 2 H, $NHCH_2CH_2CH_{22}$), 1.46 (m, 2 H, $NHCH_2CH_2CH_2$), 2.38 (t, 2 H, NCH_2CH_2CO), 3.05 (t, 2 H, $CONHCH_2$), 3.10 (m, 2 H, $CONHCH_2$), 3.12 – 3.54 (broad, 16 H, NCH_2CH_2N), 3.69 (t, 2 H, NCH_2CH_2CO), 3.64 – 4.23 (broad, 8 H, $NHCH_2CO$), 6.88 (s, 2 H, CH=CH). – $^{13}C\{^1H\}$ NMR (100.64 MHz, D_2O): δ = 26.4 ($NHCH_2CH_2CH_2$), 27.1 ($NHCH_2CH_2CH_2$), 34.0 (NCH_2CH_2CO), 37.9 (NCH_2CH_2CO), 39.6 (CH_2NHCO), 40.2 ($CONHCH_2$), 47.0 – 54.0 (broad, NCH_2CH_2N), 54.0 – 57.0 (broad, $NHCH_2CO$), 135.6 (CH=CH), 167.0 – 174.0 (broad, CO) – MALDI-TOF MS: *m/z* = 626 $[M+H]^+$. – $C_{27}H_{43}N_7O_{10}$ (625.68): calcd. C 51.83, H 6.93, N 15.67; found C 51.23, H 7.12, N 15.71.

*$LnC_4H_2O_2N(CH_2)_2C(O)NH(CH_2)_4NHC(O)CH_2NCH_2$-$CH_2[N(CH_2COO)CH_2CH_2]_3$ **(18)** – **(21)***. Using a 0.1 M Na_2CO_3/HCl solution (pH = 7.5 buffer), 0.15 mmol of YCl_3, $HoCl_3$, $TmCl_3$ or $LuCl_3$ were dissolved and combined with 60 mg (0.096 mmol) of **17**. The pH was adjusted to 7.0 and the samples were kept at r. t. over night. Analytical HPLC was carried out to verify the

Table 1. Parameters of the single crystals, data collection and structure refinement of **3** and **7**.

Compound	**3**	**7**
Empirical formula	$C_{25}H_{31}N_3O_4S_2$	$C_{25}H_{31}N_3O_6S_3$
Formula weight [g mol^{-1}]	501.65	565.71
Crystal size [mm^3]	$0.75 \times 0.52 \times 0.35$	$0.52 \times 0.24 \times 0.15$
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/n$
Z	4	4
a [Å]	11.9855(4)	5.1910 (1)
b [Å]	10.9267(4)	27.4576(5)
c [Å]	20.0058(7)	18.9445(4)
β [deg]	97.530(1)	93.168(1)
V [Å^3]	2597.40(16)	2696.08(9)
D_{calcd} [g cm^{-3}]	1.283	1.394
Absorption coefficient [mm^{-1}]	0.240	0.320
Min./max. transmission	0.9207 / 0.8404	0.8880 / 0.4807
$F(000)$ [e]	1064	1192
2θ Range for data collection [deg]	$1.71 \leq \theta \leq 26.00$	$1.48 \leq \theta \leq 27.50$
Data set	$-14 \leq h \leq 12$	$-6 \leq h \leq 6$
	$-13 \leq k \leq 13$	$-30 \leq k \leq 35$
	$-22 \leq l \leq 24$	$-24 \leq l \leq 21$
Reflections, collected	17564	20467
Reflections, unique	5095 (R_{int} = 0.0773)	6177 (R_{int} = 0.0859)
Data / restraints / parameter	5095 / 0 / 317	6177 / 1 / 345
Goodness-of-Fit (F^2)	0.988	1.081
Final R indices ($I \geq 2\sigma(I)$)	$R1$ = 0.0516	$R1$ = 0.0798
	$wR2$ = 0.1180	$wR2$ = 0.1410
R indices (all data)	$R1$ = 0.0971	$R1$ = 0.1404
	$wR2$ = 0.1394	$wR2$ = 0.1625
Largest diff. peak and hole [e Å^{-3}]	0.234 / −0.382	0.334 / −0.338

coordination to the *Ln*(III) ions. The complexes were purified on an Agilent-Prep-C18 column (solvent A: 0.1 % TFA in water; solvent B: 10 % of 0.1 % TFA, 90 % aq. CH_3CN). Yield ∼ 30 mg. – MALDI-TOF MS: *m/z* = 712 ($C_{27}H_{40}N_7O_{10}Y$, $[M+H]^+$); *m/z* = 788 ($C_{27}H_{40}N_7O_{10}Ho$, $[M+H]^+$); *m/z* = 792 ($C_{27}H_{40}N_7O_{10}Tm$, $[M+H]^+$); *m/z* = 798 ($C_{27}H_{40}N_7O_{10}Lu$, $[M+H]^+$). The analytical HPLC method for the metal-DOTA-conjugates used an Agilent 1100 HPLC system and was performed on a Zorbax 300SB-C18 4.6 × 150 mm column (Agilent) with a flow rate of 1 mL/min and a linear gradient of 100 % solution A to 60 % solution B in 30 min (A: 0.1 % TFA in water; solvent B: 0.1 % TFA, 90 % aq. CH_3CN) with spectrophotometric monitoring at λ = 220 nm. The retention time was the same (9.05 min) for all complexes. **18**, **19**, **20** and **21**: decomposition > 220 °C. **21**: ^{1}H NMR (400 MHz, D_2O): δ = 1.40 (m, 2 H, $NHCH_2CH_2CH_2$), 1.45 (m, 2 H, $NHCH_2CH_2CH_2$), 2.23 – 2.82 (broad, 12 H, NCH_2CH_2N; 2 H, NCH_2CH_2CO), 3.09 (t, 2 H, $CONHCH_2$), 3.12 (m, 2 H, $CONHCH_2$), 3.12 – 3.73 (broad, 8 H, $NHCH_2CO$; broad 4 H, NCH_2CH_2N), 3.75 (broad, 2 H, NCH_2CH_2CO), 6.74 (s, 2 H, CH=CH). – ^{13}C NMR (100.64 MHz, D_2O): δ = 26.6 ($NHCH_2CH_2CH_2$), 26.7 ($NHCH_2CH_2CH_2$), 34.3 (NCH_2CH_2CO), 38.2 (NCH_2CH_2CO), 39.8 (CH_2NHCO), 40.0 ($CONHCH_2$), 54.3 – 56.8 (8 × CH_2, NCH_2CH_2N), 63.4 (1 × CH_2, $NHCH_2CO$), 65.7 (3 × CH_2, $NHCH_2CO$), 134.8 (CH=CH), 171.4 (NCH_2CH_2CO), 174.2 (=CHCO), 180.9 (1 × CO, CO), 181.2 (3 × CO, CO). – $C_{27}H_{40}N_7O_{10}Lu$ (797.65): calcd. C 40.66, H 5.05, N 12.29; found C 40.39, H 5.12, N 12.12.

Crystal structure determination

Crystals suitable for X-ray diffraction were obtained by crystallization of **3** from the pure oil and of **7** from acetone. The data were collected on a Siemens SMART CCD diffractometer (graphite monochromated MoK_α radiation, λ = 0.71070 Å) by use of ω scans at 293 K (**3**) and 173 K (**7**). The structures were solved by Direct Methods using SHELXS-97 [35] and refined on F^2 using all reflections with SHELXL-97 [36]. All non-hydrogen atoms were refined anisotropically and the carbon-bound hydrogen atoms were placed in calculated positions and assigned to an isotropic displacement parameter of U_{iso} = 0.08 Å^2. Hydrogen atoms bonded to nitrogen were found. SADABS [37] was used to perform area-detector scaling and absorption corrections. Important parameters of the single crystals, data collection and the refinement of the structure are listed in Table 1. Further crystallographic data were deposited as supplementary publication no. CCDC 608991 (**7**) und CCDC 608992 (**3**) and can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SPP "Lanthanoidspezifische Funktionalitäten in Molekül und Material") and the Fonds der Chemischen Industrie. We thank Barbara Brecht-Jachan, Prisca Kunert and Dr. Peter Henklein, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, for the purification of compounds **16** – **21**.

[1] S. Liu, D. S. Edwards, *Bioconjugate Chem.* **2001**, *12*, 7.
[2] V. Jacques, J. Desreux in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging* (Eds.: A. E. Merbach, E. Tóth), Wiley, Chichester, **2001**, p. 157.
[3] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[4] R. R. Edelman, J. R. Hesselink, M. B. Zlatkin in *MRI: Clinical Magnetic Resonance Imaging*, Saunders, Philadelphia, **1996**.
[5] D. C. Onthank, S. Liu, P. J. Silva, J. A. Barrett, T. D. Harris, S. P. Robinson, D. S. Edwards, *Bioconjugate Chem.* **2004**, *15*, 235.
[6] M. Woods, A. D. Sherry, *Inorg. Chem.* **2003**, *42*, 4401.
[7] M. Krause, C. Scheler, U. Boettger, H. Weisshoff, M. Linscheid, DE10227599A1 (Proteome Factory AG, Humboldt University Berlin) **2002**.
[8] P. A. Whetstone, N. G. Butlin, T. M. Corneillie, C. F. Meares, *Bioconjugate Chem.* **2004**, *15*, 3.
[9] A. J. Link, *Electrophoresis* **1997**, *18*, 1314.
[10] A. Shevchenko, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1440.
[11] S. P. Gygi, B. Rist, T. J. Griffin, J. Eng, R. Aebersold, *J. Proteome Res.* **2002**, *1*, 47.
[12] R. Aebersold, M. Mann, *Nature* **2003**, *422*, 198.
[13] S. P. Gygi, B. Rist, S. A. Gerber, F. Turecek, M. H. Gelb, R. Aebersold, *Nat. Biotechnol.* **1999**, *17*, 994.
[14] T. M. Corneillie, A. J. Fisher, C. F. Meares, *J. Am. Chem. Soc.* **2003**, *125*, 15039.
[15] J. P. L. Cox, A. S. Craig, I. M. Helps, K. J. Jankowski, D. Parker, M. A. W. Eaton, A. T. Millican, K. Millar, N. R. A. Beeley, B. A. Boyce, *J. Chem. Soc., Perkin Trans. 1* **1990**, 2567.
[16] D. D. Dischino, E. J. Delaney, J. E. Emswiler, G. T. Gaughan, J. S. Prasad, S. K. Srivastava, M. F. Tweedle, *Inorg. Chem.* **1991**, *30*, 1265.
[17] A. Heppeler, S. Froidevaux, H. R. Mäcke, E. Jermann, M. Béhé, P. Powell, M. Hennig, *Chem. Eur. J.* **1999**, *5*, 1974.
[18] N. V. Gerbeleu, V. B. Arion, J. Burgess, *Template Synthesis of Macrocyclic Compounds*, Wiley-VCH, Weinheim, **1999**.
[19] H. Bernard, J. J. Yaouanc, J. C. Clement, H. des Abbayes, H. Handel, *Tetrahedron Lett.* **1991**, *32*, 639.
[20] J. P. André, C. F. G. C. Geraldes, J. A. Martins, A. E. Merbach, M. I. M. Prata, A. C. Santos, J. J. P. de Lima, E. Tóth, *Chem. Eur. J.* **2004**, *10*, 5804.
[21] H. Schumann, K. Kuse, S. Dechert, *Z. Naturforsch.* **2004**, *59b*, 1415.
[22] A. E. Martin, T. M. Ford, J. E. Bulkowski, *J. Org. Chem.* **1982**, *47*, 412.
[23] T. J. Atkins, J. E. Richman, W. F. Oettle, *Org. Synth.* **1978**, *58*, 86.
[24] F. P. Schmidtchen, *Chem. Ber.* **1980**, *113*, 2175.
[25] J. E. Richman, T. J. Atkins, *J. Am. Chem. Soc.* **1974**, *96*, 2268.
[26] D. Parker, *Macrocycle Synthesis. A Practical Approach.* Oxford University Press, **1996**.
[27] V. Patinec, J. J. Yaouanc, J. C. Clément, H. Handel, H. des Abbayes, M. M. Kubicki, *J. Organomet. Chem.* **1995**, *494*, 215.
[28] V. Patinec, I. Gardinier, J. J. Yaouanc, J. C. Clément, H. Handel, H. des Abbayes, *Inorg. Chim. Acta* **1996**, *244*, 105.
[29] Diamond, Crystal and Molecular Structure Visualization, Crystal Impact – K. Brandenburg & H. Putz GbR, Bonn (Germany) **2004**.
[30] M. S. Ali, S. M. Quadri, *Bioconjugate Chem.* **1996**, *7*, 576.
[31] L. A. Carpino, H. Imazumi, A. El-Faham, F. J. Ferrer, C. Zhang, Y. Lee, B. M. Foxman, P. Henklein, C. Hanay, C. Mügge, H. Wenschuh, J. Klose, M. Beyermann, M. Bienert, *Angew. Chem.* **2002**, *114*, 458.
[32] C. Li, W. T. Wong, *J. Org. Chem.* **2003**, *68*, 2956.
[33] M. P. Hall, S. Ashrafi, I. Obegi, R. Petesch, J. N. Peterson, L. V. Schneider, *J. Mass. Spectrom.* **2003**, *38*, 809.
[34] C. F. G. C. Geraldes, A. D. Sherry, M. P. M. Marques, M. C. Alpoim, S. Cortes, *J. Chem. Soc., Perkin Trans.* **1991**, 137.
[35] G. M. Sheldrick, SHELXS-97, Program for the Solution of Crystal Structures, University of Göttingen, Göttingen (Germany) **1990**.
[36] G. M. Sheldrick, SHELXL-97, Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen (Germany) **1997**.
[37] G. M. Sheldrick, SADABS, Program for Empirical Absorption Correction of Area Detector Data, University of Göttingen, Göttingen (Germany) **1996**.