Syntheses of crowned polyamines using isolable succinimidyl esters of N-tritylated linear amino acids and peptides

Nikolaos Tsiakopoulos,a Charilaos Damianakos,a George Karigiannis a
Dimitrios Vahliotis,b Dionissios Papaioannou,a* and Giovanni Sindona c

a Department of Chemistry and b Center of Instrumental Analysis, University of Patras, GR-26500 Patras, Greece
b Center of Instrumental Analysis, University of Patras, GR-26500 Patras, Greece

Abstract
Partial and total syntheses of polyamines incorporating crown ether moieties have been effected using N-tritylated linear amino acids, like β-alanine (βAla) and γ-aminobutyric acid (γAba), to introduce the N–3-C and N–4-C polyamine structural units, respectively. The partial syntheses involve the acylation of commercially available crown bearing amino function(s) or aza-oxa crown ethers with the isolable succinimidyl esters of Trt-βAla-OH or Trt-βAla-γAba-OH, followed by LiAlH₄-mediated reduction of the resulting amides, whereas in the total syntheses the crown and the aza-oxa crown ether moieties are built-up from commercially available starting materials like polyethylene glycols, epichlorohydrin and dibenzylamine.

Keywords: Polyamines, crown ethers, aza-oxa crown ethers, amino acids, active esters, protecting groups

Introduction
Linear polyamines (PAs), like spermidine (SPD) and spermine (SPM) and their conjugates (PACs) with other natural products are widely distributed in the living organisms and are associated with interesting biological functions. For this reason, they have attracted considerable interest, and a variety of PA analogues and PACs have been already synthesized in order to determine structure-activity relationship and to identify possible leads for the development of PA-based pharmaceuticals. On the other hand, the cyclic oligomers of ethylene oxide, collectively known as crown ethers (CEs), and their analogues and derivatives are widely used for complexing and separating metal or organic cations, as well as in the areas of phase transfer.
catalysis, host-guest and supramolecular chemistry. Different types of CEs have been synthesized in order to improve their properties and find suitable applications. In addition, a variety of pharmaceutically interesting compounds have been equipped with crown moieties in order to modify their physiological action. Accordingly, we decided to examine the possibility of introducing CE moieties into PA molecules and study the complexing and biological properties of this new family of organic compounds, which we collectively coin as crowned polyamines (CRPAs). We now wish to report a general method that we have recently developed which facilitates access to CRPAs; CE is commercially available but we have developed alternative methods of preparation. For the introduction of CE moieties into various polyamine skeletons as the purpose of this work, we have chosen the commercially available aza-oxa CEs 1 and 2 (Figure 1), the lariat aminomethyl CE 3 and the diaminodibenzo-CE 4, a mixture of the isomers 4a and 4b.

![Figure 1. Structures of compounds related to this work.](image)

Furthermore, we used simple commercially available building blocks, like polyethylene glycols, epichlorohydrin, and dibenzylamine, in order to build-up various CE skeletons to the appropriate size and content of N atoms, and the ‘isolable’ active esters 5 and 6, readily available from the corresponding linear amino acids β-alanine (βAla) and γ-aminobutyric acid (γAba), as N–3-C and N–3-C–N–4-C synthons for the assembly of various PA skeletons.
Results and Discussion

Linear amino acids and α,ω-diaminoalkanes as building blocks for the assembly of linear polyamine skeletons

In a series of letters, we have shown that the triphenylmethyl (trityl, Trt) N-protected linear amino acids, like βAla and γAba, and their dipeptides, e.g. βAla-γAba, in the form of their corresponding isolable succinimidyl ‘active’ esters (SAEs) 5 and 6 can be successfully employed for the synthesis of a variety of linear and cyclic SPD and SPM analogues and conjugates of the alkaloid kukoamine A type as well as their symmetric and asymmetric dimers, bridged by linear dicarboxylic acids, of the alkaloid tenuilobine type using the ‘amide approach’. The required SAEs are readily obtained as depicted in Scheme 1.

Scheme 1. Syntheses of succinimidy active esters and of linear polyamines (the numbers on the structures serve for the presentation of the NMR data).

(i) Me$_3$SiCl, CH$_2$Cl$_2$/MeCN (5:1), reflux, 30 min. (ii) Et$_3$N/TrtCl, 0 °C, 1 h then 25 °C, 3 h. (iii) MeOH, 97% (7) or 79% (8). (iv) HOSu/DCC, THF/DMF (3:1), 0 °C, 1 h then 25 °C, 12 h, 85% (5) or 88% (6). (v) Me$_3$SiCl, CH$_2$Cl$_2$, 25 °C, 10 min. (vi) 5/Et$_3$N, 0 °C, 45 min then 25 °C, 15 min. (vii) PUT or DAO/Et$_3$N, DMF, 25 °C, 12-24 h, 90% (9a) or 78% (9b) or 82% (11). (viii) LiAlH$_4$, THF, reflux, 2-4 d; 68% (10a) or 61% (10b). (ix) Boc$_2$O/Et$_3$N/cat. DMAP, CHCl$_3$, 0 °C, 30 min then 25 °C, 12 h, 30%. (x) TFA/CH$_2$Cl$_2$ (1:1), 25 °C, 30 min, 75%.

O-Silylation of βAla, followed by N-tritylation and desilylation with MeOH, provided Trt-βAla 7 in 97% yield. SAE 5 was then obtained in 85% yield by the condensation of 7 with of N-
hydroxysuccinimide (HOSu) in the presence of \(N,N^\prime\)-dicyclohexylcarbodiimide (DCC).\(^5\) On the other hand, O-silylation of \(\gamma\)Aba, followed by N-acylation with SAE \(5\) and desilylation with MeOH gave the N-tritylated dipeptide \(8\) in 79% yield. From this compound, SAE \(6\) was easily obtained in 88% yield upon routine activation also with HOSu in the presence of DCC.\(^5\) The applicability of these compounds to build any type of tetra- or hexa-amines of the 3-n-3 or the 3-4-n-4-3 types was readily exemplified by the facile preparation of the tetra-amine derivatives \(10a\) and \(10b\) \((n = 4 \text{ and } 8\), respectively), and the hexa-amine \(14\) \((n = 4)\). Thus, condensation of SAE \(5\) with 1,4-diaminobutane (putrescine, PUT) gave the expected bisamide \(9a\) in 90% yield. This was then reduced with LiAlH\(_4\) to produce \(N^1,N^{12}\)-Trt-SPM \(10a\) in 68% yield.\(^5\) Later, the selective direct ditritylation of SPM to produce \(10a\) was reported.\(^8\) On the other hand, bisacylation of 1,8-diaminooctane (DAO), followed by LiAlH\(_4\) reduction of the bisamide \(9b\) produced the \(N^1,N^{16}\)-ditritylated tetra-amine \(10b\) in 61% yield.\(^4\) Similarly, bisacylation of PUT with SAE \(6\) produced the tetra-amide \(11\) in 82% yield. LiAlH\(_4\) reduction of all four amide functions took place unexceptionally and the thus obtained crude PA derivative \(12\) was per-\(\text{tert}\)-butyloxycarbonylated to facilitate purification by flash column chromatography (FCC). The resulting pure, fully protected, hexa-amine \(13\) \((30\% \text{ overall yield})\) was deprotected with a 50% solution of trifluoroacetic acid (TFA) in CH\(_2\)Cl\(_2\) to produce the 3-4-4-4-3 hexa-amine \(14\) as the hexatrifluoroacetate salt, in 75% yield.\(^4\) In this general methodology, diversion may readily arise from the linear or even branched (e.g. chiral) amino acids or peptides, and the linear or branched or cyclic \(\alpha,\omega\)-diaminoalkanes used to furnish the \(N-C_n-N\) central core of the polyamine.

![Scheme 2](image)

**Scheme 2.** Partial syntheses of crown ethers incorporating polyamine elements (the numbers on the structures serve for the presentation of the NMR data).
Partial syntheses of polyamines incorporating crown and diaza-oxa crown ether moieties

Oxapolyamines, i.e. polyamines incorporating O atom(s) in their skeleton have recently gained considerable interest. Therefore, we decided to extent the above described method to include the cyclic counterparts of poly(ethylene glycols), i.e. CEs. Two possibilities were examined, namely CEs bearing one or two amino-handles and aza-oxo CEs incorporating two N atoms within the ring. Accordingly, condensation of 2-aminomethyl-18-CE-6 with SAE provided the bisamide 15 in 66% yield (Scheme 2). LiAlH₄ reduction of 15 gave the corresponding N-tritylated PA derivative 16 (45% yield); upon TFA detritylation the 8-N-monoalkylated SPD analogue 17 was obtained as its tris(trifluoroacetate) salt (73% yield). Similarly, bisacylation of diaminodibenzo-15-CE-5 with SAE 5 gave bisamide 18 (only isomer 18a is shown in Scheme 2) in 55% yield.

Reduction of 18 with LiAlH₄, followed by detritylation of the resulting CE derivative 19 (82%) gave CE 20 (91%). Compound 20a may be considered as a conformationally restricted dioxa-analogue of the 3-12-3 tetra-amine core. Furthermore, bisacylation of the diaza-oxa-15-CE-5 1 with SAE 5 gave the bisamide 21 in 59% yield (Scheme 3). Upon LiAlH₄ reduction of this compound the fully reduced analogue 22 was obtained in 62% yield.

Scheme 3. Partial syntheses of diaza-oxa crown ethers incorporating polyamine elements (the numbers on the structures serve for the presentation of the NMR data).

Finally, TFA detritylation gave the tetra(trifluoroacetate) salt of CE 23 as in 90% yield. This compound may be considered as a crowned oxa-SPM analogue. When SAE 6 was used to bisacylate diaza-oxa-18-CE-6 2, the expected tetra-amide 24 was obtained in 92% yield. LiAlH₄
reduction of this compound gave a 64% yield of CE derivative 25, from which the hexa(trifluoroacetate) salt of CRPA 26 was obtained in 90% yield.4 This compound may be considered as a dioxo-3-4-8-4-3 PA analogue. It should be noted that the corresponding dioxo-3-8-3 PA analogue has been obtained by bisalkylation of CE 2 with acrylonitrile and subsequent catalytic hydrogenation over Raney Ni.10

**Total syntheses of polyamines incorporating crown ether moieties**

Having established the viability of this method we went on to synthesize more complex CRPAs. Taking into consideration the fact that the CEs so far used are commercially available compounds but very expensive for large scale applications, we decided to develop an alternative method for their preparation. For example, we envisaged that aminomethyl CE 3 could be readily produced using as a key-reaction the nucleophilic displacement of the Cl atom of epichlorohydrin (27) by a synthetically suitable amine. We have used two amines in the past, namely TrtNH\textsubscript{2} \textsuperscript{11} and Bn\textsubscript{2}NH,\textsubscript{5b} to introduce the amino function into the γ-carboxy group of kainic acid, and at position 8 of SPD, respectively. It should be noted that aminomethyl CEs have been obtained so far either from the corresponding hydroxymethyl CEs through phthalimidation, followed by hydrazinolysis\textsuperscript{12} or displacement of the Cl atom of chloromethylated glycol or oligoethylene glycols by ammonia or primary amines followed by CE formation by reaction with the appropriate oligoethylene glycol ditosylates or dichlorides.\textsuperscript{13} In addition, it had been reported that the reaction of hindered amines with 27 leads to either 3-azetidinols in fair yields with primary amines and to a variety of products, some of which coming from EtOH used as the reaction solvent, with secondary amines.\textsuperscript{14} To avoid these solvent-induced side-reactions we performed our exploratory experiments in 27 as the reaction medium. Much cleaner and reproducible results we obtained with Bn\textsubscript{2}NH in place of TrtNH\textsubscript{2}, and therefore, we used Bn\textsubscript{2}NH for the preparation of aminomethyl CEs.

In fact, reaction of Bn\textsubscript{2}NH with 27 in the presence of \textsuperscript{1}Pr\textsubscript{2}NEt as HCl scavenger, at 110 °C for 2 h produced a mixture of two compounds which were readily separated in a small scale experiment by FCC and identified as the chlorohydrin 28 and the epoxide 29 (Scheme 4). Although this reaction mixture would have been used as such for the following transformation, namely the reaction with an oligoethylene glycol (OEG) in the presence of NaH, much better results were obtained when this mixture (ratio \textsuperscript{28}:\textsuperscript{29} = 3:2; or 2:3) by NMR, was first cleanly converted to the epoxide 29 (67% overall yield based on Bn\textsubscript{2}NH) by treatment with NaH in THF at ambient temperature. NaH-catalyzed nucleophilic ring-opening of epoxides by OEGs have been used for the production of higher homologues of OEGs, suitable for CEs preparation.\textsuperscript{15} Thus, treatment of epoxide 29 with diethylene glycol (DEG) in the presence of a catalytic quantity of NaH at refluxing THF for 2 days produced the anticipated triethylene glycol (TEG) derivative 30 in 75% yield. Preparation of the desired aminomethyl CE derivative 31 required as the final step the condensation of diol 30 with triethylene glycol ditosylate (TGT). Although several OEG ditosylates are commercially available, we used for the large scale preparation of DGT the method prescribed in ref. 2d, p. 84, an adaptation of the method by Ouchi et al.,\textsuperscript{16} TGT
and tetraethylene glycol ditosylate (TEGT; for its application cf. the following discussion). This method involves treatment of OEGs with tosyl chloride in the presence of NaOH in a mixture of THF/H$_2$O (2:1) as the solvent. On the other hand, an improved method has been recently published for the preparation of CEs exploiting the significant enhancement of the alkylation efficiency of alkoxides by ditosylates by using an unwashed 60% NaH dispersion in anhydrous DMSO.$^{17}$

![Scheme 4](image)

**Scheme 4.** Total synthesis of 2-aminomethyl-16-CE-6 and of related crowned polyamines (the numbers on the structures serve for the presentation of the NMR data). (i) Bn$_2$NH/iPr$_2$NEt, 110 ºC, 2 h, 40% 28 and 27% 29. (ii) NaH/cat. imidazole, THF, 25 ºC, 1 h, 100%. (iii) NaH, THF, reflux, 2 d, 75%. (iv) 60% NaH dispersion, DMSO, 25 ºC, 2 d, 57%. (v) H$_2$(3 atm)/Pd(OH)$_2$ on C, MeOH, 25 ºC, 3 d, 96%. (vi) 5/Et$_3$N, DMF, 25 ºC, 12 h, 93%. (vii) TFA/CH$_2$Cl$_2$ (1:1), 25 ºC, 30 min, 95% 32b or 85% 35. (viii) 6/Et$_3$N, DMF, 0 ºC, 1 h then 25 ºC, 1 d, 79%. (ix) LiAlH$_4$, THF, reflux, 4 d. (x) ZCl/iPr$_2$NEt, CHCl$_3$, 0 ºC, 30 min then 25 ºC, 12 h, 60%.
(xi) MeS-C(=NZ)-NHZ/iPr₂NEt, CHCl₃, 0 ºC, 30 min then 25 ºC, 12 h, 82%. (xii) H₂(1 atm)/10% Pd-C, AcOH/MeOH/H₂O (4:1:0.1), 25 ºC, 6 h, then HCl/MeOH, 70%.

Indeed, using this method, the anticipated compound 31 was obtained in 57% yield. From this compound, the amino function was unmasked by catalytic hydrogenolysis at 3 atm and ambient temperature, using Pearlman’s catalyst [Pd(OH)₂ on C] to remove both benzyl groups.⁵b,⁶b

The aminomethyl CE 3 (obtained in 96% yield) was acylated without any further purification by SAE 5 to introduce the first N–3-C–N unit of SPM into CE. FCC purification afforded amide 32a in 93% yield. This compound was detritylated with a solution of TFA in CH₂Cl₂, and the amino function was acylated with SAE 6 in the presence of Et₃N in order to introduce the remaining 4-C–N–3-C–N of SPM into CE. The expected trisamide 33 was obtained in 75% overall yield. Reduction of this amide with LiAlH₄ followed by complete benzyloxy carbonylation with benzyloxy carbonyl chloride (ZCl) produced the fully protected crowned SPM (CRSPM) 34 in 60% overall yield.⁴ Complete benzyloxy carbonylation serves two purposes. It facilitates purification by FCC of the crude product produced by LiAlH₄ reduction of the amide functions and allows selective removal of the Trt group in the presence of the Z groups by mild acidolysis for further primary amino function modifications. For example, detritylation of CRSPM derivative 34 followed by guanidylation of the free primary amino group with the commercially available reagent 1,3-bis(benzyloxy carbonyl)-2-methyl-2-thiopseudourea (BZMTU) produced the fully protected guanidylated CRSPM derivative 36 in 70% yield. Complete deprotection is then readily effected by catalytic hydrogenolysis to produce the final product, the guanidylated CRSPM 37 in 70% yield. Natural and synthetic guanidylated polyamines¹a and other types of organic compounds incorporating the guanidinium function¹⁸ show very interesting biological properties, due to the fact that this function interacts strongly through hydrogen bonds and electrostatic interactions with other functional groups present in enzymes or receptors.

We then decided to synthesize diaza-oxa CEs incorporating PA elements in their skeleton, such as the CRPAs 23, 43, 48 and 51 (Scheme 5). We envisaged that CEs of this type might be readily available from a common precursor, i.e. the ditritylated oxo-SPM analogue 41, by alkylation with various OEG diiodides (OEGIs). We based our reasoning on a well-established methodology (the Dale reaction) to produce aza-oxa CEs through the alkylation of primary or secondary diamines by OEGIs in the presence of anhydrous Na₂CO₃.¹⁹ OEGIs are readily available from the corresponding ditosylates OEGTs through the classical displacement with NaI in acetone (see experimental section) whereas oxo-SPM derivative would be assembled from the diamine 39 and SAE 5. Although several OEG α,ω-diamines (OEGAs) are commercially available, we obtained the required OEGA 39 from DGT through the classical two-steps sequence involving displacement with potassium phthalimide, followed by hydrazinolysis. For the purpose of this work, we isolated the bisphthalylhydrazide salt of OEGA 39 and used it as such for the coupling with SAE 5 in the presence of Et₃N. The expected bisamide 40 was
obtained in 77% yield. LiAlH₄ reduction of bisamide 40 proceeded unexceptionally to give the key-intermediate 41 in 85% yield. Bisalkylation of this intermediate with TGI in dry MeCN in the presence of anhydrous Na₂CO₃ produced ditritylated CRPA 22 in very good yield (73%), whereas its bisalkylation by TEGI in the presence of anhydrous K₂CO₃ gave access to the next higher CE homologue 42 in 66% yield. These CEs incorporate a symmetric oxa-3-5-3 tetra-amine skeleton and can be considered as oxa-SPM analogues.

Scheme 5. Total syntheses of diaza-oxa crowned polyamines. (the numbers on the structures serve for the presentation of the NMR data).

(i) PhthNK, DMF, 100-120 ºC, 12 h, 50%. (ii) H₂NNH₂.H₂O, MeOH, reflux, 30 min, 69%. (iii) SAE 5/Et₃N, DMF, 0 ºC, 30 min then 25 ºC, 12 h, 77%. (iv) LiAlH₄, THF, reflux, 2 d, 85%. (v) Na₂CO₃ (TGI) or K₂CO₃ (TEGI), MeCN, reflux, 2 d, 73% (22) or 66% (42). (vi) TFA/CH₂Cl₂ (1:3), 1 h, 25 ºC, 90-100%. (vii) SAE 6/Et₃N, DMF, 0 ºC, 30 min then 25 ºC, 1 d, 68% (44) or 55%
Detritylation of these compounds provided the corresponding CRPAs 23 and 43, which upon coupling with the SAE 6, also in the presence of Et₃N, gave the tetra-amides 44 and 45 in 68 and 55% yield, respectively. These tetra-amides were subsequently reduced with LiAlH₄ at refluxing THF to produce the corresponding crude ditritylated CRPAs 46 and 49. For purification by FCC, these compounds were per-tert-butoxycarbonylated with bis(tert-butyl)dicarbonate (Boc₂O) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) to give the fully protected CRPAs 47 and 50 in 35% and 26% overall yields, respectively, based on the starting tetra-amides. Finally, TFA-mediated detritylation of these intermediates gave the desired CRPAs 48 and 51 in 88% and 83% yield, respectively. These CEs incorporate a symmetric oxa-3-4-3-5-3-4-3 octa-amine skeleton.

Conclusions

Linear and crowned polyamines of variable length or ring-sizes and number of nitrogen functions in their skeleton can be readily assembled using the isolable succinimidyl active esters of the N-tritylated amino acid βAla and peptide βAla-γAba to couple to α,ω-diamino-alkanes and -dibenzocrown ethers, diaza-oxa crown ethers and aminomethyl crown ethers, followed by LiAlH₄-mediated reduction of the thus obtained polyamides. The required amino components were either commercially available or were readily synthesized in our laboratory employing, also commercially available, building blocks like oligoethylene glycols, epichlorohydrin and dibenzylamine and the alkylation of either oligoethylene alkoxides by oligoethylene tosylates or oligoethylene diamines by oligoethylene diiodides as key-reactions for the assembly of the crown or diaza-oxa crown ether skeleton, respectively. Further applications of this general approach to the synthesis of other types of crowned polyamines and conjugates and the evaluation of the metal-complexing and biological properties of the novel crowned polyamines described in this work are currently under investigation.

Experimental Section

General Procedures. Melting points were determined with a Buchi SMP-20 apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a Perkin Elmer 16PC FT-IR spectrophotometer. ¹H-NMR spectra were obtained at 400.13 MHz and ¹³C-NMR spectra at 100.62 MHz on a Bruker DPX spectrometer. CDCl₃ and tetramethylsilane (TMS) were used as the solvent and internal standard, respectively, unless otherwise stated. Chemical shifts are reported in δ units, parts per million () downfield from TMS. The assignments of the ¹H spectra
are based on chemical shift arguments, analysis of coupling patterns and signal intensities whereas the $^{13}$C spectra were assigned taking into consideration chemical shift arguments. Data for the aromatic region (trityl group) are omitted for $^1$H and $^{13}$C NMR spectra of the N-tritylated compounds for the sake of brevity. The values observed for compound 7 are typical: $^1$H NMR: $\delta$ 7.417 (6H, d, $J = 8.0$ Hz, $o$-H), 7.268 (6H, t, $J = 7.6$ Hz, $m$-H), 7.191 (3H, t, $J = 7.2$ Hz, $p$-H). $^{13}$C NMR: $\delta$ 146.321 (ipso-C), 128.957 (o-C), 128.460 (m-C), 126.630 (p-C).

Electron-impact ionization mass spectra (EI-MS) were obtained on a Fisons VG 7070E mass spectrometer with electron beam energy of 70 eV. Fast atom bombardment (FAB) mass spectra were recorded on a Fisons VG ZAB 2F operating at an accelerating potential of 8 kV and neutral xenon beam of 9 eV and using m-nitrobenzyl alcohol (NBA) as the matrix. Liquid secondary ion ionization (LSI) mass spectra in the positive mode were obtained on a Fisons VG ZAB-T four-sector instrument. The compounds were ionized using a caesium ion gun operated at an acceleration voltage of 30 kV. NBA was also used as the matrix solution which was carefully mixed with a 1 µL of sample solution in CHCl$_3$ on the tip of the probe prior to analysis. Electron-spray ionization (ESI) mass spectra were recorded on a Micromass-Platform LC spectrometer using MeOH or MeCN as solvents. The ESI high-resolution MS (HR-MS) experiments were carried out in a hybrid QqTOF mass spectrometer equipped with an ion spray ionization source. Compounds were dissolved in a solution containing 0.1% acetic acid in methanol/water 50/50 and analyzed by direct infusion (5 L/min) at the optimum ion spray voltage of 4800V. The nitrogen gas flow was set at 30 psi, whereas the orifice, the focusing ring and the skimmer voltages were kept at 30, 50 and 25V, respectively. MS/MS experiments were performed in the collision cell $q$ on the isotopically pure ($^{12}$C) peak of the selected precursor ions by keeping the Q1 at 20 or 30V and unit resolution, and scanning the TOF analyser. All the acquisitions were averaged over 50 scans at 8000 TOF resolution with a standard deviation of 0.01 cps. Microanalyses were performed on a Carlo Erba EA 1108 CHNS elemental analyzer in the Center of Instrumental Analysis of the University of Patras.

Flash column chromatography (FCC) was performed on Merck silica gel 60 (230-400 mesh) and TLC on Merck 60F$_{254}$ films (0.2 mm) precoated on aluminium foil. The solvent or solvent systems used for elution were: (A) PhMe/EtOAc (98:2), (B) PhMe/EtOAc (8:2), (C) PhMe/EtOAc (1:1), (D) PhMe/EtOAc (4:6), (E) EtOAc, (F) CHCl$_3$/MeOH (95:5), (G) CHCl$_3$/MeOH (9:1), (H) CHCl$_3$/MeOH/conc. NH$_3$ (95:5:0.5), (I) CHCl$_3$/MeOH/conc. NH$_3$ (9:1:0.05), (J) CHCl$_3$/MeOH/conc. NH$_3$ (9:1:0.1), (K) CHCl$_3$/MeOH/conc. NH$_3$ (85:15:1.5), (L) CHCl$_3$/MeOH/conc. NH$_3$ (8:2:0.2), (M) CHCl$_3$/MeOH/conc. NH$_3$ (9:2:5:0.2), (N) CHCl$_3$/MeOH/conc. NH$_3$ (7:3:0.3), (O) CHCl$_3$/MeOH/conc. NH$_3$ (6:4:0.4). Spots were visualized with UV light at 254 nm and the ninhydrin agent. All solvents (Merck) were dried and/or purified according to standard procedures prior to use. Anhydrous Na$_2$SO$_4$ was used for drying organic solvents, unless otherwise indicated, and subsequently solvents were routinely removed at ca. 40 °C under reduced pressure (water aspirator). All reagents employed in the present work were purchased from either Aldrich or Fluka and used without further purification. NaH was purchased as a 60% dispersion in mineral oil. It was routinely washed off oil by
repeated washings with hexane prior to use, unless otherwise stated. With the exception of TFA-mediated deprotections, all other reactions were routinely carried out in an atmosphere of Ar.

**N-Trityl-β-alanine (7).** To a magnetically stirred suspension of βAla (17.82 g, 0.2 mol) in anhydrous CH₂Cl₂ (250 mL) and MeCN (50 mL) was added Me₃SiCl (27.9 mL, 0.22 mol), and the reaction mixture was refluxed with exclusion of moisture for 30 min. To the ice-cooled (0 °C) resulting solution anhydrous Et₃N (61 mL, 0.44 mol) was added dropwise followed by the addition of TrtCl (58.5 g, 0.21 mol) in three equal portions within 15 min. Stirring was continued at 0 °C for 1 h, and at ambient temperature for 3 h. MeOH (20 mL) was then introduced into the reaction mixture and the solvents were evaporated to dryness. To the resulting residue, aqueous NaOH solution (1N, 700 mL) was added and then extracted twice with Et₂O. The aqueous phase was then ice-cooled and brought to pH 5 by the dropwise addition of glacial AcOH. A 5% aqueous citric acid solution was then added to further acidify the reaction mixture until no more precipitate was formed. The precipitate was filtered and washed on the filter twice with H₂O and then with ice-cold Et₂O and finally dried under vacuo at 40 °C overnight to give pure product 7 (64.3 g, 97%), mp 166–69 °C; Rₕ (G) 0.33. FT-IR: 3455-2500 (CO-OH), 3298 (NH-Trt), 1708 (CO-OH), 1594 (Ph C=C) cm⁻¹. EI-MS (m/z): 331 (M⁺), 243 (Trt), 165 (Trt-PhH). ¹H NMR: δ 4.900 (2H, br. s, NH, CO₂H), 2.506 (2H, t, J = 5.2 Hz, NC₄H₂), 2.451 (2H, t, J = 5.2 Hz, CH₂CO). ¹³C NMR: δ 176.736 (C-O₂H), 71.866 (Ph 3C), 39.785 (NCH₂), 35.061 (CH₂CO₂H). Anal. Calcd for C₂₂H₂₁NO₂ (331.42): C, 79.73; H, 6.39; N, 4.23. Found: C, 79.93; H, 6.11; N, 4.05.

**2,5-Dioxopyrrolidin-1-yl N-trityl-β-alaninate (5).** To an ice-cold suspension of Trt-βAla (24.4 g, 73.6 mmol) in THF (150 mL) and DMF (50 mL) was added HOSu (10.2 g, 88.3 mmol) and subsequently after 10 min DCC (16.7 g, 81 mmol). The resulting reaction mixture was stirred at 0 °C for 1 h and then at ambient temperature overnight. The precipitated N,N'-dicyclohexylurea (DCU) was filtered off, and the residue on the filter was washed with hot THF. The filtrate was evaporated under reduced pressure, and the residue was taken up in EtOAc (300 mL) and washed twice with an ice-cold saturated aqueous NaCl solution (brine). Drying, evaporation to one third of the volume and refrigeration overnight gave crystalline product SAE 5 (26.8 g, 85%), mp 178–80 °C; Rₕ (E) 0.63. FT-IR: 3236 (NH-Trt), 1818 and 1782 (CO-OSu), 1746 (N-CO)) cm⁻¹. LSI-MS (m/z): 451 (MNa), 429 (MH), 351 (MH-PhH), 243 (Trt), 165 (Trt-PhH). ¹H NMR: δ 2.788 (6H, m, H-2 and CO(CH₂)₂CO), 2.518 (2H, t, J = 6.4 Hz, H-3), 2.083 (1H, br. s, NH). ¹³C NMR: δ 169.452 (COCH₂), 168.302 (C-1), 71.367 (Ph₃C), 39.496 (C-3), 32.947 (C-2), 25.982 (COCH₂). Anal. Calcd for C₂₆H₂₄N₂O₄ (428.49): C, 72.88; H, 5.65; N, 6.54. Found: C, 73.03; H, 5.75; N, 6.24.

**N-Trityl-β-alanyl-γ-aminobutyric acid (8).** To a magnetically stirred suspension of γAba (6.6 g, 64 mmol) in anhydrous CH₂Cl₂ (90 mL) was added Me₃SiCl (8.9 mL, 70.4 mmol), and the reaction mixture was stirred at ambient temperature for 10 min. The resulting solution was ice-cooled and then treated with SAE 5 (24.9 g, 58 mmol) followed by the dropwise addition of anhydrous Et₃N (16 mL, 115 mmol) within 30 min. Stirring was continued at 0 °C for 15 min and at ambient temperature for 15 min. MeOH (5 mL) was introduced, the resulting reaction mixture...
was diluted with CH₂Cl₂ (300 mL) and then washed once with an ice-cold 5% aqueous citric acid solution and twice with H₂O. Drying and evaporation to dryness left a residue which, upon dissolution in a minimum volume of hot EtOAc and overnight refrigeration gave crystalline dipeptide acid 8 (19.1 g, 79%), mp 157–59 ºC; R₆(G) 0.16. FT-IR: 3300-2500 (OH), 3298 (NH), 1712 (CO-OH), 1634 (NH-CO) cm⁻¹. EI-MS (m/z): 417 (M⁺H), 339 (M⁺H-PhH), 243 (Trt), 165 (Trt-PhH). ¹H NMR (DMSO-d₆): δ 7.848 (1H, unresolved t, H-5), 3.024 (2H, q, J = 6.0 Hz, H-4), 2.247 (2H, t, J = 6.4 Hz, H-8), 2.200 (2H, t, J = 7.2 Hz, H-2), 2.145 (2H, t, J = 6.4 Hz, H-7), 1.600 (2H, quintet, J = 7.2 Hz, H-3). ¹³C NMR: δ 175.082 (C-1), 172.263 (C-6), 71.127 (Ph₃C), 40.342 (C-4), 38.664 (C-8), 36.995 (C-2), 31.944 (C-7), 24.869 (C-3). Anal. Calcd for C₂₆H₂₈N₂O₃ (416.52): C, 74.98; H, 6.78; N, 6.72. Found: C, 75.14; H, 6.50; N, 6.44.

Succinimidyl N-trityl-β-alanyl-γ-aminobutyrate (6). To an ice-cold suspension of Trt-βAla-γAba (19.6 g, 47 mmol) in THF (110 mL) and DMF (40 mL) was added HOSu (6.1 g, 53 mmol) followed after 10 min by DCC (10.5 g, 51 mmol). The resulting reaction mixture was stirred at 0 ºC for 1 h and then at ambient temperature overnight. The precipitated DCU was filtered off and washed on the filter with hot THF. The filtrate was evaporated under reduced pressure, and the residue was taken up in CH₂Cl₂, overnight refrigeration and filtration removed an additional amount of DCU. Evaporation of CH₂Cl₂ and addition of Et₂O followed by overnight refrigeration gave the crystalline product SAE 6 (21.2 g, 88%), mp 101–04 ºC; R₆(E) 0.57. FT-IR: 3326 and 3296 (NH), 1816 and 1788 (CO-OSu), 1740 (N-CO) and 1630 (NH-CO) cm⁻¹. LSI-MS (m/z): 536 (M⁺Na), 514 (M⁺H), 436 (M⁺H-PhH), 339 [M⁺H-N(COCH₂)₂-Ph], 243 (Trt), 165 (Trt-PhH). ¹H NMR: δ 6.720 (1H, unresolved t, H-5), 3.363 (2H, q, J = 6.4 Hz, H-4), 2.790 (4H, br. s, CO(CH₂)₂CO), 2.638 (2H, t, J = 6.8 Hz, H-2), 2.473 (2H, t, J = 5.6 Hz, H-8), 2.372 (2H, t, J = 5.6 Hz, H-7), 1.980 (2H, quintet, J = 6.8 Hz, H-8). ¹³C NMR: δ 172.268 (C-6), 169.627 (CO(CH₂)₂CO), 168.767 (C-1), 71.558 (Ph₃C), 46.342 (C-4), 38.646 (C-8), 37.057 (C-2), 29.062 (C-7), 26.448 (CO(CH₂)₂CO), 25.927 (C-3). Anal. Calcd for C₃₀H₃₁N₃O₅ (513.59): C, 70.16; H, 6.08; N, 8.18. Found: C, 69.93; H, 6.30; N, 8.29.

1N,12N-Bis(tritylamino)-4,9-diazadodecane-3,10-dione (9a). To a magnetically stirred solution of SAE 5 (25.71 g, 60 mmol) in DMF (230 mL) was added PUT (2.64 g, 30 mmol). After 15 min at ambient temperature, the pH of the reaction mixture was adjusted to 8 by the addition of Et₃N, followed by stirring at for 24 h. The reaction mixture was then diluted with H₂O (600 mL), and the precipitated product was extracted with CH₂Cl₂. The organic phase was washed sequentially once with H₂O, twice with a 5% aqueous NaHCO₃ solution and finally twice with H₂O, dried and evaporated to a volume that induced crystallization. Refrigeration, filtration of the precipitated solid and washing on the filter initially with ice-cold CH₂Cl₂ followed by Et₂O and hexane gave pure bisamide 9a (19.3 g, 90%), mp 182–85 ºC; R₆(G) 0.68. FT-IR: 3328 and 3277 (NH), 1634 (NH-CO) cm⁻¹. FAB-MS (m/z): 716 (M⁺H), 473 (M⁺Trt), 395 (M⁺Trt-PhH), 243 (Trt). ¹H NMR: δ 6.503 (2H, unresolved t, H-4 and H-9), 3.371 (4H, q, J = 5.6 Hz, H-5 and H-8), 2.434 (4H, t, J = 6.0 Hz, H-1 and H-12), 2.295 (4H, t, J = 6.0 Hz, H-2 and H-11), 1.546 (4H, m, H-6, H-7). ¹³C NMR: δ 172.782 (C-3 and C-10), 71.381 (Ph₃C), 40.334 (C-5...
and C-8), 39.377 (C-1 and C-12), 37.526 (C-2 and C-11), 27.417 (C-6 and C-7). Anal. Calcd for C₄₈H₅₀N₄O₂ (714.96): C, 80.64; H, 7.05; N, 7.84. Found: C, 80.84; H, 6.87; N, 7.60.

1N,12N-Bis[3-(tritylamino)propyl]butane-1,4-diamine (10a). To a magnetically stirred suspension of LiAlH₄ (6.4 g, 168 mmol) in anhydrous THF (220 mL) at reflux temperature was added bisamide 9a (20 g, 28 mmol) in ca. equal portions within 30 min, and refluxing was continued for 4 d. The resulting reaction mixture was ice-cooled, and excess LiAlH₄ was carefully quenched by dropwise addition of a saturated aqueous Na₂SO₄ solution. The resulting salts were filtered off by vacuum filtration and washed on the filter with distilled THF. The filtrate was concentrated to dryness, and the resulting oily residue was dissolved in EtOAc and washed twice with brine. Drying and evaporation gave an oily residue which upon trituration with Et₂O and refrigeration gave SPM derivative 10a (13 g, 68%). This product was recrystallized from MeOH (700 mL), mp 116–17 ºC; Rf(M) 0.78. FT-IR: 3268 (NH) cm⁻¹. FAB-MS (m/z): 688 (M⁺H), 609 (M-PhH), 445 (M-Trt), 367 (M-Trt-PhH), 243 (Trt). HR-MS: Found 687.4441 (M++1), C₄₈H₅₅N₄ requires M++1 = 687.4427. 1H NMR: δ 2.656 (4H, t, J = 7.2 Hz, H-1 and H-12), 2.580 (4H, unresolved t, H-3 and H-10), 2.184 (4H, unresolved t, H-5 and H-8), 1.752 (2H, br. s, H-4 and H-9), 1.650 (4H, quintet, J = 6.8 Hz, H-2 and H-11), 1.475 (4H, unresolved quintet, H-6 and H-7). 13C NMR: δ 71.320 (Ph 3C), 50.440 (C-1 and C-12), 48.966 (C-3 and C-10), 42.528 (C-5 and C-8), 31.498 (C-2 and C-11), 28.499 (C-6 and C-7). Anal. Calcd for C₄₈H₅₄N₄ (686.99): C, 83.92; H, 7.92; N, 8.16. Found: C, 83.71; H, 8.05; N, 8.30.

1N,16N-Bis(tritylamino)-4,13-diazahexadecane-3,14-dione (9b). To a magnetically stirred ice-cold solution of DAO (0.72 g, 5 mmol) in dry DMF (8 mL) was added anhydrous Et₃N (1.4 mL, 10 mmol), followed by SAE 5 (4.46 g, 10.4 mmol). The reaction mixture was stirred at 0 ºC for 30 min and then at ambient temperature overnight. It was then diluted with CHCl₃ and washed sequentially twice with a 5% aqueous NaHCO₃ solution and three times with H₂O, dried, evaporated, and the residue was triturated with Et₂O and refrigeration gave pure bisamide 9b (3 g, 78%), mp 158–59 ºC; R₆(E) 0.43. FT-IR: 3290 (NH), 1629 (NHCO) cm⁻¹. ESI-MS (m/z): 772.13 (MH). 1H NMR: δ 6.332 (2H, t, J = 5.20 Hz, H-4 and H-9), 3.210 (4H, t, J = 6.4 Hz, H-5 and H-8), 2.446 (4H, t, J = 6.0 Hz, H-1 and H-12), 2.311 (4H, t, J = 6.0 Hz, H-2 and H-11), 1.487 (4H, quintet, J = 6.8 Hz, H-6 and H-7). 13C NMR: δ 172.454 (C-3 and C-10), 71.437 (Ph₃C), 40.362 (C-5 and C-8), 39.789 (C-1 and C-12), 37.638 (C-2 and C-11), 30.046 (C-6 and C-7), 29.520 (C-6 and C-7). Anal. Calcd for C₅₂H₅₈N₄O₂ (876.99): C, 81.00; H, 7.58; N, 7.27. Found: C, 81.20; H, 7.32; N, 7.15.

1N,16N-Bis[3-(tritylamino)propyl]octane-1,8-diamine (10b). Bisamide 9b (1.55 g, 2 mmol) was treated with LiAlH₄ (0.46 g, 12 mmol) in anhydrous THF (25 mL) for 2 d as described for 9a. Following identical work-up procedure followed by FCC purification using the solvent system I as eluant., pure tetra-amine 10b was obtained as an oil (0.9 g, 67%), R₆(I) 0.5. ESI-MS (m/z): 743.05 (MH), 501.39 (M-H-Trt), 243 (Trt). HR-MS (m/z): Found 743.5053 (M⁺+1), C₅₂H₅₃N₄ requires M⁺+1 = 743.5056. 1H NMR: δ 2.741 (4H, t, J = 7.2 Hz, H-1 and H-12), 2.204 (4H, t, J = 6.8 Hz, H-5 and H-8), 1.734 (4H, quintet, J =
6.0 Hz, H-2 and H-11), 1.517 (4H, quintet, J = 6.8 Hz, H-6 and H-7), 1.299 (8H, m, (C-6′,7′,6″,7″)). 13C NMR: δ 71.338 (Ph3C), 50.287 (C-3 and C-10), 48.898 (C-1 and C-12), 42.449 (C-5 and C-8), 30.584 (C-2 and C-11), 29.815 (C-6 and C-7), 29.449 (C-6′,7′), 27.388 (C-6′′,7′′). Anal. Calcd for C52H62N4 (743.09): C, 84.05; H, 8.41; N, 7.54. Found: C, 84.20; H, 8.15; N, 7.41.

1N,22N-Bis(tritylamino)-1,4,9,14,19,22-hexazadocosane-3,8,15,20-tetraone (11). To a magnetically stirred ice-cold solution of PUT (0.32 g, 3.6 mmol) in dry DMF (10 mL) was added anhydrous Et3N (1 mL, 7.2 mmol), followed by SAE 6 (3.7 g, 7.2 mmol). The reaction mixture was stirred at 0 ºC for 30 min and then at ambient temperature overnight. It was then diluted with CHCl3 and washed sequentially with a 5% aqueous NaHCO3 solution (twice) and with H2O (three times), dried, evaporated to a volume that induced crystallization. Overnight refrigeration gave pure tetra-amide 11 (2.61 g, 82 %), mp 185–86 ºC; Rf (E) 0.32. FT-IR: 3290 (NH), 1635 (NH CO) cm⁻¹. ESI-MS (m/z): 886.41 (MH), 243.12 (Trt). Anal. Calcd for C56H64N6O4 (885.17): C, 75.99; H, 7.29; N, 9.49. Found: C, 75.80; H, 7.49; N, 9.67.

N4,N8,N14,N19-Tetra(tert-butoxycarbonyl)-N1,N22-ditrityl-1,22-diamino-4,9,14,19-tetraaza-docosane (13). Tetra-amide 11 (1.28 g, 1.45 mmol) was treated with LiAlH4 (0.55 g, 14.5 mmol) in anhydrous THF (10 mL) for 4 d as described for 9a. Identical work-up afforded the crude product 12 as an oil, Rf (O) 0.16. To a solution of this product in anhydrous CHCl3 (4 mL) were added dry Et3N (1 mL, 7.25 mmol) and DMAP (0.1 g, 0.8 mmol), the mixture was cooled to 0 ºC and treated with Boc2O (1.58 g, 7.25 mmol) for 30 min at this temperature and at ambient temperature overnight. The resulting solution was diluted with more CHCl3 and washed sequentially with a 5% aqueous NaHCO3 solution and with H2O (twice), dried and evaporated to leave a residue. From this residue upon FCC purification using solvent system B as eluant pure product 13 (0.54 g, 30%) was obtained as a white foam, Rf(B) 0.37. ESI-MS (m/z): 1,229.82 (M), 987.81 (M-Trt), 243 (Trt). C76H104N6O8 requires M⁺ = 1,229.70.

4,9,14,19-Tetraazadocosane-1,22-diamine (14). A solution of the protected hexa-amine 13 (0.39 g, 0.32 mmol) in CH2Cl2/TFA (1:1, 4 mL) was kept at ambient temperature for 30 min. Evaporation to dryness, trituration with Et2O and overnight refrigeration gave the hexa(trifluoroacetate) salt of hexa-amine 14 (0.25 g, 75%) as an oil. HR-MS: Found 345.3715 (M++1), C18H45N6 requires M++1 = 345.3706.

2-(10′-Tritylamino-3′,8′-dioxo-2′,7′-diazadecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (15). To a magnetically stirred solution of commercial crown ether 5 (1 g, 3.41 mmol) in anhydrous DMF (4 mL) at ambient temperature were added sequentially dry Et3N (0.5 mL, 3.6 mmol) and SAE 6 (1.75 g, 3.41 mmol). After an additional 1 h the reaction mixture was diluted with CHCl3 and washed sequentially with a 5% aqueous NaHCO3 solution and with brine (twice). Drying and evaporation to dryness furnished a residue from which upon FCC purification with solvent system G as eluant bisamide 15 (1.56 g, 66%) was obtained as an oil, Rf(G) 0.08. ESI-MS (m/z): 731,35 (M⁺Trt), 715.08 (M⁺Na), 693.02 (M⁺H), 243.11 (Trt). C30H53N3O8 requires M⁺ = 691.83.
2-(10'-Tritylamino-2',7'-diazadecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (16). A solution of bisamide 15 (1.56 g, 2.25 mmol) in anhydrous THF (13 mL) was added dropwise within 45 min to a magnetically stirred suspension of LiAlH₄ (0.44 g, 13.5 mmol) in refluxing THF (5 mL). The reaction mixture was stirred at this temperature overnight and was worked-up as described for the preparation of 10a. From the resulting crude reaction product upon FCC purification using the solvent system L as eluant pure product 16 (0.66 g, 45%) was obtained as an oil, Rᶠ(N) 0.21. ESI-MS (m/z): 687 (M⁺Na), 665 (M⁺H), 422 (M⁺H-Trt), 243 (Trt). C₃₀H₅₇N₃O₆ requires M⁺ = 663.90. ¹H NMR: δ 3.907 (1H, ddd, J = 6.6, 4.4 and 3.6 Hz, CH₂-O), 3.678 (22H, m, CH₂-O), 2.695 (4H, m, H-1’ and H-3’), 2.614 (4H, m, H-6’ and H-8’), 2.179 (2H, t, J = 6.8 Hz, H-10’), 2.054 (3H, br. s, NH), 1.687 (2H, m, H-9’), 1.520 (4H, m, H-4’ and H-5’). ¹³C NMR: δ 78.880 (C=O), 71.337 (Ph₃C), 73.395, 71.251-70.959 and 70.187 (11C, CH₂-O), 51.466 (C-1’), 50.298 and 50.255 (C-6’ and C-8’), 48.786 (C-3’), 42.490 (C-10’), 31.287 (C-9’), 28.307 (C-4’ and C-5’).

2-(10'-Amino-2',7'-diazadecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (17). A solution of tritylated triamine 16 (0.45 g, 0.68 mmol) in 6 mL of CH₂Cl₂/TFA (1:1) was kept at ambient temperature for 1 h. Evaporation to dryness, trituration with Et₂O and refrigeration for several days gave pure triamine 17 as tristrifluoroacetate salt (0.38 g, 73%), mp 136–40 ºC; Rᶠ(N) 0.06. HR-MS (m/z): Found 422.2647 (M⁺+1), C₂₀H₄₄N₃O₆ requires M⁺+1 = 422.2656.

Ν⁴,Ν⁴(S‘)-Bis(3-tritylamino propanoyl)-4’,4’(5’)-diaminodibenzo-15-crown ether-5 (18). To a magnetically stirred suspension of crown ether 6 (1 g, 2.89 mmol) in anhydrous DMF (6 mL) iPr₂NEt (1 mL, 6 mmol) and SAE 5 (2.49 g, 5.8 mmol) were added sequentially, and the resulting reaction mixture was kept at 60 ºC for 2 h. It was then treated with an additional quantity of SAE 5 (0.43 g, 1 mmol) for a further 1 h at that temperature and for overnight at ambient temperature. The reaction mixture was diluted with CHCl₃ and washed sequentially with a 5% aqueous K₂CO₃ solution (twice) and with H₂O, dried (MgSO₄) and evaporated to leave a residue. Upon FCC purification using EtOAc as eluant pure 18 (1.55 g, 55%) was obtained as a beige foam, Rᶠ(E) 0.27. ESI-MS (m/z): 996 (M⁺H), 258(TrtNH(CH₂)₂CHO+H), 243 (Trt). C₆₂H₆₄N₄O₇ requires M⁺ = 973.18. Ν⁴,Ν⁴(S’)-Bis(3-tritylaminopropyl)-4’,4’(5’)-diaminodibenzo-15-crown ether-5 (19). Bisamide 18 (1.5 g, 1.54 mmol) was added portionwise within 30 min to a magnetically stirred suspension of LiAlH₄ (0.3 g, 9 mmol) in refluxing THF (10 mL). The reaction mixture was stirred at this temperature for 2 d. Excess hydride was destroyed at –10 ºC by the dropwise addition of a saturated aqueous K₂SO₄ solution. Filtration, washing with distilled THF, and finally evaporation of the filtrates left a residue. This was taken-up in EtOAc and washed twice with the above mentioned K₂SO₄ solution (twice) and with H₂O, dried (MgSO₄) and evaporated to dryness. FCC purification using initially solvent system C followed by solvent system D gave pure product 19 (1.19 g, 82%) as an oil, Rᶠ(C) 0.25. ESI-MS (m/z): 946 (MH), 258(TrtNH), 243 (Trt). C₆₂H₆₄N₄O₇ requires M⁺ = 945.11. ¹H NMR: δ 6.819 (2H, d, J = 8.8 Hz, H-4”), 6.219 (2H, unresolved d, H-1”), 6.143 (2H, dd, J = 8.8 and 2.4 Hz, H-3”), 4.26-4.25 (4H, m, H-5 and H-6), 4.128 (4H, t, J = 4.0 Hz, H-1 and H-4), 3.919 (4H, t, J = 4.4 Hz, H-2 and H-3), 3.163 (4H, t, J =
6.4 Hz, H-1'), 2.270 (4H, t, J = 6.0 Hz, H-3'), 1.755 (4H, quintet, J = 6.0 Hz, H-2'). $^{13}$C NMR: $\delta$ 151.311 (C-6''), 144.896 (C-5''), 142.095 (C-2''), 102.564 (C-1''), 105.542 (C-3''), 119.113 (C-4''), 71.511 (Ph3C), 70.873 (C-5 and C-6), 70.298 and 70.255 (4C, C-1/2/3/4), 43.648 (C-1'), 42.280 (C-3'), 30.869 (C-2').

$^{N, N'}$:Bis(3-aminopropyl)-4',4'-'(5'')-diaminodibenzo-15-crown ether-5 (20). A solution of tritylated tetra-amine 19 (1.1 g, 1.16 mmol) in CH$_2$Cl$_2$/TFA (3:1, 10 mL) was kept at ambient temperature for 1 h. Evaporation to dryness, trituration with Et$_2$O and refrigeration for 1 d, followed by decantation of the supernatant liquor and repetition of this procedure gave pure tetra-amine 20 (0.97 g, 91%) as the tetra(trifluoroacetate) salt in the form of a beige foam, R$_d$(G) 0.02. HR-MS (m/z): Found 461.2044 (M$^{++1}$), C$_{24}$H$_{37}$N$_4$O$_5$ requires M$^{++1}$ = 461.2039.

$^{N, N'}$:Bis(3-tritylamino)propanoyl)-1,4,10-trioxa-7,13-diazacyclopentadecane (21). To a magnetically stirred solution of aza-oxy crown ether 1 (1 g, 4.58 mmol) in anhydrous DMF (5 mL) and CHCl$_3$ (5 mL) were added dry Et$_3$N (1.4 mL, 10 mmol), SAE 5 (4.28 g, 10 mmol) and a catalytic amount of HOBt (54 mg, 0.4 mmol), and the resulting reaction mixture was stirred at ambient temperature overnight. Dilution with CHCl$_3$, washing with H$_2$O (twice), drying and evaporation left a residue from which upon FCC purification using CHCl$_3$ as eluant and followed by solvent system G pure bisamide 21 (2.28 g, 59%) was obtained as an oil, R$_f$(G) 0.85. ESI-MS (m/z): 846.27 (MH), 243.11 (Trt). C$_{54}$H$_{60}$N$_4$O$_5$ requires M$^+$ = 845.09. From the same column, monoacylated 1 (0.34 g, 14%) was also eluted; ESI-MS (m/z): 532.44 (MH), 243.11 (Trt). C$_{32}$H$_{41}$N$_3$O$_4$ requires M$^+$ = 531.62.

$^{N, N'}$:Bis(3-tritylamino)propanoyl)-1,4,10-trioxa-7,13-diazacyclopentadecane (22). A solution of bisamide 21 (2.28 g, 2.7 mmol) in anhydrous THF (10 mL) was added dropwise within 1 h to a magnetically stirred suspension of LiAlH$_4$ (0.46 g, 14 mmol) in refluxing THF (5 mL). The reaction mixture was stirred at this temperature for 1 d and was then worked-up under an identical manner to that for product 19. Upon FCC purification using solvent system J for elution pure product 22 (1.37 g, 62%) was obtained as an oil, R$_d$(J) 0.57. ESI-MS (m/z): 840.18 (M$^{++1}$), 817.96 (MH), 243 (Trt). HR-MS (m/z): Found 817.5049 (M$^{++1}$), C$_{54}$H$_{64}$N$_4$O$_3$ requires M$^{++1}$ = 817.5057.

$^{N, N'}$:Bis(3'-aminopropyl)-11,7,10-trioxa-4,13-diazacyclopentadecane (23). A solution of crown ether derivative 22 (1.47 g, 1.8 mmol) in CH$_2$Cl$_2$/TFA (3:1; 8 mL) was kept at ambient temperature for 1 h and was then concentrated to dryness. The residue was triturated with Et$_2$O and refrigerated whereby the product separated as an oil. The supernatant liquor was decanted and this procedure was repeated once more. Thus, the tetra(trifluoroacetate) salt of 23 (1.38 g, 90%) was obtained in the form of a yellowish powder, R$_d$(J) 0.22. ESI-MS (m/z): 356.11 (M$^{++1}$), C$_{16}$H$_{37}$N$_4$O$_3$ requires M$^{++1}$ = 333.2866.
**N^7,N^{16}-Bis(8-tritylamino-6-oxo-5-azaoctanoyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (24).** To a magnetically stirred solution of aza-oxy crown ether 2 (0.52 g, 2 mmol) in anhydrous DMF (3 mL) and CHCl₃ (1 mL) were added dry Et₃N (0.56 mL, 4 mmol) and SAE 6 (2.05 g, 4 mmol). The resulting reaction mixture was stirred at ambient temperature for 2 d. Dilution with CHCl₃, washing with a 5% aqueous NaHCO₃ solution (twice) and with H₂O (once), drying and evaporation left a residue from which upon FCC purification using the solvent system F as eluant pure bisamide 24 (1.95 g, 92%) was obtained as a viscous oil, Rᵡ(F) 0.23. ESI-MS (m/z): 1,060.31 (M+), 817.47 (M+Trt), 243.09 (Trt). C₆₄H₇₈N₆O₈ requires M⁺ = 1,059.36.

**N^7,N^{16}-Bis(8-tritylamino-5-azaoctyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (25).** A solution of tetra-amide 24 (1.91 g, 1.8 mmol) in anhydrous THF (15 mL) was added dropwise within 20 min to a magnetically stirred suspension of LiAlH₄ (0.72 g, 22 mmol) in refluxing THF (5 mL). The reaction mixture was stirred at this temperature for 1 d, and then excess LiAlH₄ was carefully destroyed at −10 °C by the dropwise addition of a saturated aqueous Na₂SO₄ solution. Filtration and washing with more distilled THF followed by evaporation of the filtrates gave a residue which was taken up in EtOAc. The organic layer was washed twice with brine, dried and evaporated to leave crude hexa-amine derivative 25. Upon FCC purification using solvent system J, then system L and finally system N for elution pure product 25 (1.15 g, 64%) was obtained as a viscous oil, Rᵡ(N) 0.47. ESI-MS (m/z): 1026 (M⁺Na), 1004 (M⁺H), 656 [M⁺Na-TrtNH(CH₂)₃NH(CH₂)₂CH=CH₂], 633 656 [M⁺H-TrtNH(CH₂)₃NH(CH₂)₂CH=CH₂], 243 (Trt). C₆₄H₈₆N₆O₄ requires M⁺ = 1003.42. ¹H-NMR: δ 3.594 (16H, m, H-2-5 and H-8-11), 2.753 (8H, t, J = 5.6 Hz, H-1, H-6, H-7 and H-12), 2.674 (4H, t, J = 6.8 Hz, H-1’), 2.589 (4H, m, H-4’), 2.489 (4H, m, H-6’), 2.183 (4H, t, J = 6.4 Hz, H-8’), 1.761 (4H, quintet, J = 6.4 Hz, H-7’), 1.464 (8H, m, H-2’ and H-3’). ¹³C NMR: δ 71.364 (Ph₃C), 71.148 (C-3, C-4, C-9 and C-10), 70.420 (C-2, C-5, C-8 and C-11), 56.250 (C-1’), 54.410 (C-1, C-6, C-7 and C-12), 50.335 (C-4’), 48.824 (C-6’), 42.512 (C-8’), 31.323 (C-2’), 28.446 (C-3’), 25.698 (C-7’).

**N^7,N^{16}-Bis(8-amino-5-azaoctyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (26).** A solution of bistritylated hexa-amine 25 (1.1 g, 1.1 mmol) in CH₂Cl₂/TFA (3:1, 10 m) was kept at ambient temperature for 1 h and was then concentrated to dryness. The residue was triturated with Et₂O and refrigerated whereby the product separated as an oil. The supernatant liquor was decanted and this procedure was repeated once more to furnish 26 (1.19 g, 90%) as the hexa(trifluoroacetate) salt in the form of a brownish powder, Rᵡ(N) 0.07. HR-MS (m/z): Found 519.4589 (M⁺+1), C₂₆H₅₉N₆O₄ requires M⁺+1 = 519.4598.

**Reaction of epichlohydrin (27) with dibenzylamine, Formation of a mixture of 1-dibenzylamino-3-chloro-2-propanol (28) and dibenzylaminomethyloxirane (29).** A solution consisting of 27 (30 mL, 0.38 mol), Bn₂NH (20 mL, 0.1 mol) and anhydrous iPr₂NEt (20 mL, 0.11 mol) was kept at 110 °C (bath temperature) for 2 h. It was then diluted with Et₂O and washed sequentially with a 5% aqueous NaHCO₃ solution, with H₂O (twice), and with brine (once), dried and evaporated to dryness to leave an oily residue. From this oil, upon purification by FCC using PhMe as the eluant mixture consisted of chlorohydrin 28 and epoxide 29 (18.34 g; ratio 3:2 by 1H NMR) was obtained. This mixture was used as such for the next experiment.
However, in a small scale experiment (2 mL from each of Bn2NH and iPr2NEt and 3 mL of 27), the resulting mixture was separated by FCC using solvent system A as eluant to give pure alcohol 28 and epoxide 29, both as oils.

Chlorohydrin 28: Rf(PhMe/EtOAc =98:2) 0.24. $^1$H NMR: $\delta$ 7.35–7.23 (10H, m, Ph-H), 3.751 (1H, dq, $J = 8.6$ and 5.2 Hz, $CH$-OH), 3.753 and 3.503 (4H, two d-ABq, $J = 13.4$ Hz, NCH$_2$Ph), 3.450 (2H, dd, $J = 5.0$ and 3.1 Hz, CH$_2$Cl), 3.225 (1H, br. s, OH), 2.631 (1H, dd, $J = 11.9$ and 4.8 Hz, CHCH$_2$N) and 2.587 (1H, dd, $J = 11.9$ and 8.5 Hz, CHCH$_2$N). $^{13}$C NMR: $\delta$ 138.754 (ipso C), 129.470 (o-C), 128.938 (m-C), 127.835 (p-C), 68.211 (C$_6$H-OH), 59.298 (NCH$_2$Ph), 57.178 (CH$_2$Cl), 45.451 (NCH$_2$CH). Anal. Calcd for C$_{17}$H$_{20}$ClNO (289.81): C, 70.46; H, 6.96; N, 4.83. Found: C, 70.26; H, 7.10; N, 4.97.

Epoxide 29: Rf(PhMe/EtOAc =98:2) 0.32. $^1$H NMR: $\delta$ 7.37–7.20 (10H, m, Ph-H), 3.796 and 3.557 (4H, two d-ABq, $J = 13.7$ Hz, NCH$_2$Ph), 3.067 (1H, dq, $J = 6.4$ and 3.7 Hz, $CH$-O), 2.760 (1H, dd, $J = 13.8$ and 6.3 Hz, CH$_2$O), 2.391 (1H, dd, $J = 5.0$ and 2.8 Hz, NCH$_2$CH). $^{13}$C NMR: $\delta$ 139.802 (ipso C), 129.265 (o-C), 128.701 (m-C), 127.427 (p-C), 59.410 (NCH$_2$Ph), 56.315 (CH-O), 51.520 (CH$_2$O), 45.443 (NCH$_2$CH). Anal. Calcd for C$_{17}$H$_{19}$NO (253.34): C, 80.60; H, 7.56; N, 5.53. Found: C, 80.38; H, 7.70; N, 5.81.

Conversion of the mixture of chlorohydrin 28 and epoxide 29 to epoxide 29 and reaction of the latter with DEG. Preparation of 9-dibenzylamino-3,6-dioxanonane-1,8-diol (30). A solution consisted of chlorohydrin 28 (3.5 g, 12 mmol) and epoxide 29 (2 g, 8 mmol) in anhydrous THF (15 mL) was added dropwise within 30 min to two-necked round bottom flask equipped with a side-armed dropping funnel containing NaH (0.29 g, 12 mmol) and a catalytic amount of imidazole. The resulting reaction mixture was stirred at ambient temperature for a further 1 h and then filtered. The precipitate was washed with THF (10 mL), and the filtrate containing pure (by TLC) epoxide 29 was used as such to the following experiment.

DEG (19 mL, 0.2 mol) was added dropwise within 15 min to a magnetically stirred suspension of NaH (0.1 g, 4 mmol) in anhydrous THF (10 mL) placed in a two-necked round bottom flask equipped with a side-armed dropping funnel and a reflux condenser. It was then heated to reflux, and the above described solution of 29 was added dropwise from the dropping funnel within 2.5 h. Reflux was kept on for additional 2 d. THF was then evaporated, and the residue was dissolved in EtOAc and washed three times with brine. Drying and evaporation left an oily residue from which after FCC using the solvent system F as eluant pure TEG derivative 30 (5.4 g, 75%) was obtained as an oil, R$_f$(F) 0.38. ESI-MS (m/z): 741.65 (2M+Na), 719.69 (2M+H), 382.34 (MNa), 360.42 (MH). $^1$H NMR: $\delta$ 7.33–7.23 (10H, m, Ph-H), 3.912 (1H, m, H-6), 3.741 (2H, d, $J = 13.4$ Hz, H-8), 3.680 (2H, t, $J = 4.6$ Hz, H-1), 3.609-3.551 (6H, m, H-2-4), 3.520 (2H, d, $J = 13.5$ Hz, H-8$'$), 3.483 (1H, dd, $J = 10.2$ and 3.7 Hz, H-5), 3.533 (1H, dd, $J = 10.2$ and 6.4 Hz, H-5$'$), 2.557 (1H, dd, $J = 12.8$ and 8.4 Hz, H-7), 2.504 (1H, dd, $J = 12.9$ and 5.1 Hz, H-7$'$). $^{13}$C NMR: $\delta$ 139.095 (ipso C), 129.429 (o-C), 128.793 (m-C), 127.611 (p-C), 74.847 (C-6), 72.915 (C-1), 71.182 (C-2), 70.711 (C-5), 67.569 (C-4), 62.111 (C-3), 59.028 (C-8),
56.413 (C-7). Anal. Calcd for C_{21}H_{29}NO_{4} (359.46): C, 70.17; H, 8.13; N, 3.90. Found: C, 70.47; H, 7.94; N, 3.70.

2-Dibenzylaminomethyl-18-crown ether-6 (31). To a solution of diol 30 (4.8 g, 13.4 mmol) in anhydrous DMSO (50 mL) was first added NaH (2.68 g of a 60% dispersion in oil, 67 mmol) and then TGT (6.15 g, 13.4 mmol). The resulting reaction mixture was stirred at ambient temperature for 2 d and then diluted with H_{2}O (150 mL) and brine (50 mL). The resulting mixture was extracted three times with Et_{2}O, the organic layers were combined, washed three times with brine and finally dried (anhydrous MgSO_{4}) and evaporated to dryness to leave an oily residue. This residue was subjected to FCC using solvent system G as eluant to give pure product 31 (3.62 g, 57%) as an oil, R_{f}(G) 0.20. ESI-MS (m/z): 513.46 (M^{+}K), 496.49 (M^{+}Na), 474.46 (M^{+}H). \text{H NMR: } \delta 7.34–7.20 (10H, m, Ph-H), 3.81–3.32 (24H, m, CHO, CH_{2}O and NCH_{2}Ph), 2.563 (1H, dd, J = 12.6 and 5.7 Hz, OCHC_{2}H_{2}N), 2.506 (1H, dd, J = 12.6 and 4.8 Hz, OCHC_{2}H_{2}N). Anal. Calcd for C_{27}H_{39}NO_{6} (473.60): C, 68.47; H, 8.30; N, 2.96. Found: C, 68.70; H, 8.05; N, 2.69.

2-Aminomethyl-18-crown ether-6 (3). To a solution of crown ether 31 (2.2 g, 4.6 mmol) in MeOH (30 mL) was added of Pearlman's catalyst (0.5 g), and the resulting mixture was subjected to catalytic hydrogenation at 3 atm pressure at ambient temperature for 3 d. Filtration of the catalyst and evaporation of the MeOH filtrate to dryness left the oily crown ether 3 (1.3 g, 96%), R_{f} (G) 0.11. This product 3 was identical in all respects with a commercially available sample and was used without any further purification in the preparation of amide 32a.

2-(5′-Tritylamino-3′-oxo-2′-azapentyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (32a). To a solution of aminomethyl crown 3 (1.3 g, 4.43 mmol) and dry Et_{3}N (0.62 mL, 4.43 mmol) in anhydrous DMF (5 mL) was added in three equal portions within 30 min SAE 5 (1.9 g, 4.43 mmol). The resulting solution was kept at ambient temperature overnight and was then diluted with CHCl_{3} and washed sequentially with a 5% aqueous NaHCO_{3} solution (twice) and with brine (once), dried (MgSO_{4}) and evaporated to dryness to leave an oily residue. Following FCC purification with solvent system J as eluant pure amide 32a (2.5 g, 93%) was obtained as a foam, R_{f}(J) 0.19. FT-IR: 3322 (NH), 1652 (NHCO) cm^{-1}. ESI-MS (m/z): 1,214.08 (2M^{+}H), 646.48 (M^{+}Na), 629.71 (M^{+}H), 243 (Trt). C_{35}H_{46}N_{2}O_{7} requires M^{+} = 606.76.

2-(14′-Tritylamino-3′,7′,12′-trioxo-2′,6′,11′-triazatetradecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (33). A solution of amide 32a (1.2 g, 2 mmol) in CH_{2}Cl_{2}/TFA (1:1; 8 mL) was kept at ambient temperature for 30 min. It was then evaporated to dryness, triturated with Et_{2}O and refrigerated overnight. The supernatant liquor was decanted and the residue was triturated with Et_{2}O followed by refrigeration; finally decantation was repeated to leave the trifluoroacetate salt of 32b (0.91 g, 95%) as a thick oil. HR-MS (m/z): Found 365.2231 (M^{+}+1), C_{16}H_{33}N_{2}O_{7} requires M^{+}+1 = 365.2228. This oil was dissolved in anhydrous DMF (3 mL) and treated at 0 ºC in turn with Et_{3}N (0.5 mL, 3.8 mmol) and SAE 6 (0.98 g, 1.9 mmol) for 1 h and then at ambient temperature for 1 d. Identical work-up as for 32a and FCC purification using solvent system G for elution gave pure trisamide 33 (1.13 g, 79%) as a foam, R_{f}(G) 0.26. FT-IR: 3284 (NH), 1652 (NHCO) cm^{-1}.
ESI-MS (m/z): 801.62 (M+) 785.60 (MNa), 763.75 (MH), 243 (Trt). HR-MS (m/z): Found 763.4292 (M++1), C42H59N4O9 requires M++1 = 763.4282.

2-(N2,N6,N11′-Tris(benzylloxycarbonyl)-14′-tritylamino-2′,6′,11′-triazatetradecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (34). A solution of trisamide 33 (0.7 g, 0.92 mmol) in anhydrous THF (8 mL) was added dropwise within 30 min to a magnetically stirred suspension of LiAlH4 (0.31 g, 8.3 mmol) in refluxing THF (3 mL). The reaction mixture was stirred at this temperature for 4 d and was then worked-up as described for the preparation of 10a to give crude tetra-amine 34 (0.45 g, 0.63 mmol). This was dissolved in dry CHCl3 (5 mL) and treated sequentially twice with a 5% aqueous NaHCO3 solution and once with H2O, dried (MgSO4) and evaporated to leave an oily residue. Upon FCC purification using solvent system F for elution pure product 34 (0.60 g, 60% based on trisamide 33) was obtained as an oil, Rf(F) 0.36. HR-MS (m/z): Found 1,123.6032 (M++1), C66H83N4O12 requires M++1 = 1,123.6008.

2-(N2,N6,N11′-Tris(benzylloxycarbonyl)-14′-[bis(benzylloxycarbonyl)guanidyl]-2′,6′,11′-triazatetradecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (36). Intermediate 34 (0.16 g, 0.14 mmol) was detritylated under identical way to that for amide 32a to give the trifluoroacetate salt of 35 (0.12 g, 85%) as an oil. ESI-MS (m/z): 882.50 (M+H), 881.51 (M+Na). C47H68N4O12 requires M+ = 881.08. This oil was dissolved in anhydrous CHCl3 (0.5 mL) and treated at 0 °C in turn with iPr2NEt (0.044 mL, 0.25 mmol) and the reagent BZMTU (0.043 g, 0.12 mmol) for 30 min and at ambient temperature overnight. Identical work-up as for 34 and final FCC purification with solvent system F gave pure, fully protected, intermediate 36 (0.13 g, 82%) as a foam, Rf(F) 0.11. ESI-MS (m/z): 1,230.64 (M+K), 1,214.63 (M+Na), 1,191.67 (M+H), 608.02 [(M+H+Na)/2]. C64H82N6O16 requires M+ = 1,191.39.

2-(14′-guanidyl-2′,6′,11′-triazatetradecyl)-1,4,7,10,13,16-hexaoxacyclohexadecane (37). To a solution of fully protected polyamine 36 (0.12 g, 0.1 mmol) in glacial AcOH (4 mL), MeOH (1 mL) and H2O (0.1 mL) was added Pd on C (10%; 0.03 g), and the resulting reaction mixture was hydrogenated at ambient temperature for 6 h. Filtration and evaporation gave pure product 37, Rf(L) 0.27. This product was dissolved in a solution of HCl in anhydrous MeOH (1.8 M, 1 mL). Trituration with Et2O and refrigeration gave the tetrahydrochloride of polyamine 37 (0.054 g, 70%) as a powder. HR-MS (m/z): Found 521.4038 (M++1), C24H53N6O6 requires M++1 = 521.4027.

1,8-Diido-3,6-dioxaoctane (TGI) and 1,11-Diido-3,6,9-trioxaoctadecane (TEGI). To a magnetically stirred solution of either TGT (4.59 g, 10 mmol) or TEGT (5.03 g, 10 mmol) in acetone (60 mL), NaI (6 g, 40 mmol) was added, and the resulting reaction mixture was stirred at ambient temperature for 1 d. Filtration and evaporation of the solvent left a residue which was triturated with Et2O and refrigerated for overnight. Filtration and evaporation of the solvent left the pure diiodides in quantitative yield as pale red liquids which were used as such without any further purification for the preparation of theaza-oxa crown ethers.
Preparation of 3-oxapentane-1,5-diamine (39) as bisphtalhydrazide salt. A solution of DGT (11.03 g, 26.6 mmol) and potassium phthalimide (9.85 g, 53.2 mmol) in DMF (25 mL) was heated at 100-20 ºC (bath temperature) overnight. It was then diluted with CHCl₃ and washed in turn with a aqueous NaOH (2N) and with brine (twice) and H₂O (twice). Drying and evaporation left a residue which upon trituration with Et₂O and overnight refrigeration gave pure bisphtalimide 38 (4.85 g, 50%), R₆(C) 0.36.

Bisphtalimide 38 (4.8 g, 13.2 mmol) and hydrazine monohydrate (1.28 mL, 26.4 mmol) were heated under reflux in MeOH (15 mL) for 30 min. The reaction mixture was then refrigerated overnight and the precipitated product the bisphtalyl-hydrazide salt of 39 (3.9 g, 69%) was obtained by filtration and washing with ice-cold MeOH. This salt was used as such for the preparation of bisamide 40.

N¹,N¹³-Ditritylamino-3,11-dioxo-7-oxa-4,10-diazatridecane (40). To a magnetically stirred, ice-cold solution of salt 39 (3.43 g, 8 mmol) in dry DMF (30 mL) was added anhydrous Et₃N (4.5 mL, 32 mmol), followed by SAE 5 (6.86 g, 16 mmol). The reaction mixture was stirred at this temperature for 30 min and then at ambient temperature overnight. It was then diluted with CHCl₃, washed in turn with aqueous NaHCO₃ solution (5%, twice) and with H₂O (three times), dried, evaporated, and the residue was triturated with EtOAc. Overnight refrigeration gave pure bisamide 40 (4.5 g, 77%), mp 164–65 ºC; R₆(G) 0.71. FT-IR: 3328 and 3254 (NH), 1646 (NH- CO) cm⁻¹. ESI-MS (m/z): 731.87 (M+H), 489.64 (M-Trt), 243 (Trt). ¹H NMR: δ 6.678 (2H, t, J = 5.2 Hz, H-4 and H-10), 3.544 (4H, t, J = 5.2 Hz, H-6 and H-8), 3.395 (4H, q, J = 5.2 Hz, H-5 and H-9), 2.364 (4H, t, J = 6.0 Hz, H-1 and H-13), 2.131 (4H, t, J = 6.0 Hz, H-2 and H-12). ¹³C NMR: δ 172.796 (C-3 and C-11), 71.452 (Ph ₃C), 70.540 (C-6 and C-8), 40.226 (C-5 and C-9), 39.512 (C-1 and C-13), 37.259 (C-2 and C-12). Anal. Calcd for C₄₈H₅₀N₄O₃ (730.96): C, 78.87; H, 6.90; N, 7.76. Found: C, 78.59; H, 7.05; N, 7.99.

N¹,N¹³-Ditrityl-1,13-diamino-7-oxa-4,10-diazatridecane (41). Bisamide 40 (4.53 g, 6.2 mmol) was treated with LiAlH₄ (1.4 g, 37 mmol) in anhydrous THF (50 mL) for 2 d, and the resulting reaction mixture was worked-up in an identical way to that described for 9a to give pure product 41 (3.7 g, 85%), 111–12 ºC, R₆(G) 0.42. ESI-MS (m/z): 703.80 (M+H). ¹H NMR: δ 3.530 (4H, t, J = 5.2 Hz, H-6 and H-8), 2.733 (4H, t, J = 5.2 Hz, H-5 and H-9), 2.643 (4H, t, J = 6.8 Hz, H-1 and H-13), 2.148 (4H, unresolved t, H-3 and H-11), 1.696 (2H, br. s, H-4 and H-10), 1.621 (4H, quintet, J = 6.8 Hz, H-2 and H-12). ¹³C NMR: δ 71.310 (Ph₃C), 70.997 (C-6 and C-8), 49.869 (C-5 and C-9), 48.974 (C-1 and C-13), 42.552 (C-3 and C-11), 31.480 (C-2 and C-12). Anal. Calcd for C₄₈H₅₄N₄O (702.99): C, 82.01; H, 7.74; N, 7.97. Found: C, 82.21; H, 7.35; N, 7.77.

N⁴,N¹³-Di(3′-tritylaminopropyl)-1,7,10-trioxa-4,13-diazacyclopentadecane (22) from tetra-amine derivative 41. To a solution of tetra-amine derivative 41 (1.2 g, 1.7 mmol) and diiodide TGI (0.63 g, 1.7 mmol) in anhydrous MeCN (30 mL) was added Na₂CO₃ (1.04 g, 10 mmol), and the resulting mixture was refluxed in an atmosphere of Ar for 2 d. It was then refrigerated, filtered, and the residue on the filter was washed with ice-cold MeCN. The filtrate was evaporated, and the residue was dissolved in EtOAc and washed with H₂O (twice). Drying
(MgSO₄), filtration and evaporation left a residue from which after FCC purification and using solvent system J as eluant the pure product 22 (1.01 g, 73%) was obtained as a foam.

**N⁴,N¹⁶-Di(3′-trityliminopropyl)-1,7,10,13-tetraoxa-4,16-diazacyclooctadecane (42).** Under identical reaction conditions and work-up, compound 41 (0.98 g, 1.4 mmol), diiodide TEGI (0.58 g, 1.4 mmol) and K₂CO₃ (1.11 g, 8 mmol) in anhydrous MeCN (30 mL) gave after FCC purification with solvent system H as eluant the pure product 42 (0.80 g, 66%) as a foam, Rₜ(H) 0.33. ESI-MS (m/z): 862.04 (MΗ). HR-MS (m/z): Found 861.5332 (M++1), C₅₆H₆₉N₄O₄ requires M++1 = 861.5319. ¹H NMR: δ 3.586 and 3.509 (16 H, two m, CH₂O), 2.776 (8H, m, C-1, C-6, C-7 and C-10), 2.596 (4H, m, C-1″), 2.118 (4H, t, J = 6.8 Hz, H-3″), 1.654 (4H, quintet, J = 6.4 Hz, H-2″).

**N⁴,N¹⁴-Di(3′-aminopropyl)-1,7,10,13-tetraoxa-4,16-diazacyclooctadecane (43).** Under identical reaction conditions and work-up procedure as for 23, aza-crown derivative 42 (0.8 g, 0.94 mmol) in CH₂Cl₂/TFA (3:1; 4 mL) 43 was obtained as the tetra(trifluoroacetate) salt (0.74 g, ca. 100%), Rₜ(J) 0.21. This salt was used as such for the next step. ESI-MS (m/z): 377.54 (MΗ). HR-MS (m/z): Found 377.3142 (M++1), C₁₈H₄₁N₄O₄ requires M++1 = 377.3128.

**N⁴,N¹⁶-Di(12′-tritylamino-5′,10′-dioxo-4′,9′-diazadodecyl)-1,7,10-trioxa-4,13-diaza-cyclopentadecane (44).** To an ice-cold solution of the tetratrifluoroacetate salt 23 (0.70 g, 0.89 mmol) in anhydrous DMF (5 mL) Et₃N (0.77 mL, 5.5 mmol) was added followed by SAE 6 (0.92 g, 1.8 mmol). The resulting reaction mixture was stirred at this temperature for 30 min and then overnight at ambient temperature. Additional SAE 6 (0.10 g, 0.2 mmol) was then added, and stirring was continued for 1 d. Dilution with EtOAc, washing sequentially with an aqueous K₂CO₃ solution (5%) and with a saturated aqueous MgSO₄ solution (twice), drying (MgSO₄) and evaporation left a residue. This residue was subjected to FCC purification and using solvent system J as eluant to furnish pure tetra-amide 44 (1 g, 68%) as a white foam, Rₜ(L) 0.55. FT-IR: 3307 (NH), 1657 (NHCO) cm⁻¹. ESI-MS (m/z): 1,169.16 (MK), 1,153.22 (MNa), 1,130.27 (MΗ), 565.80 [(M+2H)/2]. C₆₈H₈₈N₈O₇ requires M+ = 1,129.50.

**N⁴,N¹³-Di(12′-tritylamino-5′,10′-dioxo-4′,9′-diazadodecyl)-1,7,10-trioxa-4,13-diaza-cyclopentadecane (45).** Identical reaction conditions and work-up procedure as for 44 were employed with the exception of using an aqueous NaHCO₃ solution (5%) instead of K₂CO₃. Thus, from the tetratrifluoroacetate salt 43 (0.74 g, 0.89 mmol) in anhydrous DMF (5 mL) Et₃N (0.77 mL, 5.5 mmol) was added followed by SAE 6 (0.92 g, 1.8 mmol). The resulting reaction mixture was stirred at this temperature for 30 min and then overnight at ambient temperature. Additional SAE 6 (0.10 g, 0.2 mmol) was then added, and stirring was continued for 1 d. Dilution with EtOAc, washing sequentially with a aqueous K₂CO₃ solution (5%) and with a saturated aqueous MgSO₄ solution (twice), drying (MgSO₄) and evaporation left a residue. This residue was subjected to FCC purification and using solvent system K as eluant to furnish pure tetra-amide 45 (1 g, 68%) as a white foam, Rₜ(L) 0.55. FT-IR: 3307 (NH), 1657 (NHCO) cm⁻¹. ESI-MS (m/z): 1,213.10 (MK), 1,153.22 (MNa), 1,130.27 (MΗ), 565.80 [(M+2H)/2]. C₇₀H₉₂N₈O₈ requires M+ = 1,173.55. ¹H NMR: δ 3.59–3.54 and 3.276 (16H, two m, CH₂O), 2.643 (8H, m, H-3″ and H-8″), 2.499, 2.409 and 2.347 (12H, three m, H-1, H-6, H-7, H-10 and H-1″), 2.237 and 2.128 (10H, two m, NH, H-8″ and H-11″), 1.633 (4H, m, H-2″), 1.811 (4H, quintet, J = 6.8 Hz, H-7″).

**N⁴,N¹⁶-Di(12′-tritylamino-5′,10′-dioxo-4′,9′-diazadodecyl)-1,7,10,13-tetraoxa-4,16-diaza-cyclooctadecane (47).** To a magnetically stirred suspension of LiAlH₄ (0.36 g, 9.6 mmol) in refluxing anhydrous THF (4 mL) a solution of tetra-amide 44 (0.9 g, 0.8 mmol) in THF (6 mL) was added dropwise within 30 min. Refluxing was continued for 4 d and then the
resulting reaction mixture was ice-cooled. Excess LiAlH₄ was destroyed carefully by the dropwise addition of a saturated aqueous K₂SO₄ solution, the precipitated salts were filtered off and washed on the filter with distilled THF. The filtrate was evaporated under reduced pressure and the residue was taken-up in EtOAc and was washed twice with a saturated aqueous KCl solution. Drying (MgSO₄), filtration and evaporation left the crude product 46 (0.73 g, 0.68 mmol). ESI-MS (m/z): 1,074.36 (M+H). This product was dissolved in anhydrous CHCl₃ (3 mL) and treated sequentially at 0 °C with Et₃N (0.57 mL, 4.08 mmol), a catalytic amount of DMAP and Boc₂O (0.74 g, 3.4 mmol) and was then left at ambient temperature overnight. Dilution with CHCl₃, sequential washing once with a 5% aqueous K₂CO₃ solution and with H₂O (twice), drying (MgSO₄), filtration and evaporation left an oily residue. From this residue, after FCC purification with solvent system I as eluant pure product 47 (0.41 g, 35% overall yield based on tetra-amide 44) was obtained as a foam, R₆(L) 0.34. ESI-MS (m/z): 1,475.62 (M+H). HR-MS (m/z): Found 1,473.9772 (M⁺+1), C₈₈H₁₂₉N₈O₁₁ requires M⁺+1 = 1,473.9781.

**N⁴,N¹³-Di(12'-amino-4',9'-diazadodecyl)-11,7,10-trioxa-4,13-diazacyclopentadecane (48).** A solution of fully protected octa-amine derivative 47 (0.41 g, 0.28 mmol) in CH₂Cl₂/TFA (1:1; 4 mL) was kept at ambient temperature for 20 min. Evaporation to dryness, trituration with Et₂O and overnight refrigeration gave the octa(trifluoroacetate) salt of octa-amine 48 (0.37 g, 88%) as a foam, R₆(L) 0.19. HR-MS (m/z): Found 589.5498 (M⁺+1), C₃₀H₆₉N₈O₃ requires M⁺+1 = 589.5493.

**N⁴,N¹⁶-Di[12'-tritylamino-4',9'-diazadodecyl]-1,7,10,13-tetraoxa-4,16-diazacyclooctadecane (50).** Identical reaction conditions and work-up procedure, with the exception that Na₂SO₄ was used instead of both K₂SO₄ and KCl, as for the preparation of 46 were used for the preparation of product 49. Thus, from of tetra-amide 45 (0.9 g, 0.77 mmol) the crude product 49 (0.4 g) was obtained. ESI-MS (m/z): 1,119.49 (M⁺), 1,118.48 (M⁻), 560.55 [(M+2H)/2]. This product was directly converted into the corresponding per(tert-butoxycarbonylated) oily product 50 (0.3 g, 26% overall yield based on the tetra-amide 45b) after FCC purification with solvent system J as eluant. R₆(L) 0.22. ESI-MS (m/z): 1,519.96 (M⁺). HR-MS (m/z): Found 1,518.0031 (M⁺+1), C₉₀H₁₃₉N₈O₁₂ requires M⁺+1 = 1,518.0043.

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4. Part of this work has been presented in the following review article: Tsiakopoulos, N.; Vassis, S.; Militisopoulou, M.; Damianakos, C.; Gatos, P.; Voyatzis, K.; Pantazaka, E.; Papaioannou, D. Drug Discovery and Design: Medical Aspects In Biomedical and Health Research, Matsoukas, J.; Mavromoustakos, T. Eds; IOS Press: Amsterdam, 2002; Vol. 55, p 53.


