J Incl Phenom Macrocycl Chem (2007) 59:53–63
DOI 10.1007/s10847-007-9294-3

ORIGINAL ARTICLE

Complexing and self-complexing of peptide-cavitands

Christian M. Hülsbusch · Martin Feigel

Received: 24 November 2006 / Accepted: 16 January 2007 / Published online: 10 March 2007

Abstract Resorc[4]arene based peptide-cavitands with four identical chiral amino acids at their upper rim were synthesized and investigated for the complexation of small guest molecules. Competition experiments show, that the tetra amino acid cavitands complex small organic guests in chloroform in the order ethyl acetate <dichloromethane < acetonitrile < ethanol < acetamide < acetic acid. The peptide-cavitands containing aspartic and glutamic acid derivatives enclose parts of their attached amino acids in the cavity, so that these peptide-cavitands are host and guest at the same time. The starting material for the cavitands, the resorc[4]arenetetraamine **3**, is made by a new synthetic route using the Delépine-reaction.

Keywords Amino Acids · Cavitands · Complexation · Host-guest systems · Peptides

Introduction

Methylenedioxy bridged resorc[4]arenes represent molecular vessels being able to enclose small organic guest molecules [1]. Such resorc[4]arene cavitands were prepared with several modifications at their upper and lower rim [2]. For example, cavitands have been used as membrane transporters of anions and a water-soluble resorc[4]arene modified with urotropine ligands complexes anionic aromatic guests [3]. Only a few peptide-modified resorc[4]arenes are known, for example the thioether linked peptide-helices [4]. Amino acids and peptides were mainly attached to the more flexible calixarenes [5].

We have reported that cavitands modified with four identical dipeptides and tripeptides form remarkably stable inclusion complexes with acetonitrile in chloroform solution. A prerequisite to this phenomenon is that a glycine unit is directly bonded to the resorc[4]arene core [6]. Here, we report the synthesis and complexation behaviour of peptide-cavitands with four identical chiral, partially protected amino acids directly attached at the aminomethyl rim of the cavitand. The starting material for these cavitands, the resorc[4]arenetetraamine **3**, is made from the resorc[4]arenetetraurotropinylium tetrabromide **2**.

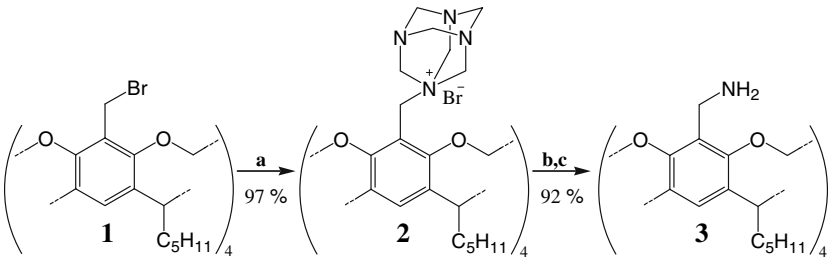
Syntheses

Starting with the resorc[4]arenetetrabromide **1** [7], the resorc[4]arenetetraurotropinylium tetrabromide **2** is prepared with urotropine in chloroform [8]. Compound **2** decomposes to the resorc[4]arenetetraamine **3** with hydrochloric acid in ethanol (Scheme 1). The reasonable yields and the simple isolation without a chromatographic separation are advantages over the common route via the resorc[4]arenetetraphthalimide [9].

The peptide-cavitands are prepared coupling the corresponding protected amino acid or carboxylic acid to the resorc[4]arenetetraamine **3** using standard peptide synthesizing methods. The carboxy component is activated with 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1-Hydroxy-1H-benzotriazole in dichloromethane and a sonicated solution of **3** and triethylamine in dichloromethane is added.

C. M. Hülsbusch · M. Feigel (✉)
Fakultät für Chemie, Ruhr-Universität Bochum,
Universitätsstr. 150, 44780 Bochum, Germany
e-mail: feigel@indi-f.nsc.ruhr-uni-bochum.de

Scheme 1 Synthesis of the resorc[4]arenetetraamine **3**: (**a**) urotopine, $CHCl_3$, RT, 24 h; (**b**) EtOH, HCl, Reflux, 6 h; (**c**) 2 N NaOH, RT, 30 min



After column chromatography on silica reasonable yields (81–89%) are obtained (Scheme 2). The coupled acid derivatives are shown in Table 1.

Table 1 Composition of the amino acid chains, of the peptide cavitands **4–13**

Cavitand	Coupled acid
4	Z-Phe-OH
5	Z-Val-OH
6	Z-Ser-OH
7	Z-Thr-OH
8	Z-Glu(OMe)-OH
9	Z-Asp(OMe)-OH
10	Z-Glu(OH)-OMe
11	Z-Asp(OH)-OMe
12	$MeO_2C(CH_2)_3CO_2H$
13	$MeO_2C(CH_2)_2CO_2H$

Complexation of small organic guest molecules

While there exist many ways to investigate quantitatively complexation, an easy test in the case of the resorcarene derivatives is monitoring the chemical shift of protons of guest molecules in the presence of the resorcarene. A significant chemical shielding of guest signals is observed whenever parts of the guest molecule are included in the cavity and are placed in the influence of the ring currents of the aromatic resorcarene units. Therefore, 5×10^{-3} M solutions of the cavitands **4–7** (with L-phenylalanine, L-valine, L-serine and L-threonine side chains, see Table 1 and Scheme 2) were prepared in chloroform-d at 25°C and treated with approximately equimolar amounts of small guests. After an initial screening, the guests ethyl acetate, dichloromethane, acetonitrile, ethanol, acetamide and acetic acid are added subsequently in that order to the the cavitand solution. Broadening of the guest signals associated with a shift to high field is observed for many guests (Table 2). Usually, the first added guest is replaced by the following guest which can be seen by a resharpening of the NMR signals of the first guest. The results are summarized in Table 2. Here an "+" indicates that signals of the guest molecules are shifted (and in most cases also broadened) in the presence of the amino acid cavitands and a vertical arrow (↑) indicates if the second added guest replaces the first added guest on addition.

For example, the ^{1}H NMR spectra of the cavitands **4** and **5** in chloroform-d solution give no indication for an interaction with the small guest molecules ethyl acetate, dichloromethane or acetonitrile. Merely with ethanol, acetamide and acetic acid, broadening of the cavitand signals occurs and high-field shifts of the signals of guest molecules accompanied with broadening can be noticed. The competition experiments showed that ethanol guest molecules in **4** are displaced immediately by acetamide and subsequently, the acetamide is replaced by acetic acid. The Ser-cavitand **6** and the Thr-cavitand **7** interact stronger with small guest molecules as both cavitands include not only ethanol, acetamide and acetic acid, but also an interaction with dichloromethane and acetonitrile is seen (Table 1).

Scheme 2 Synthesis of the peptide-cavitands **4–13**: (**c**) EDC · HCl, HOBt, NEt_3, CH_2Cl_2, RT, 18 h; see Table 1 for R

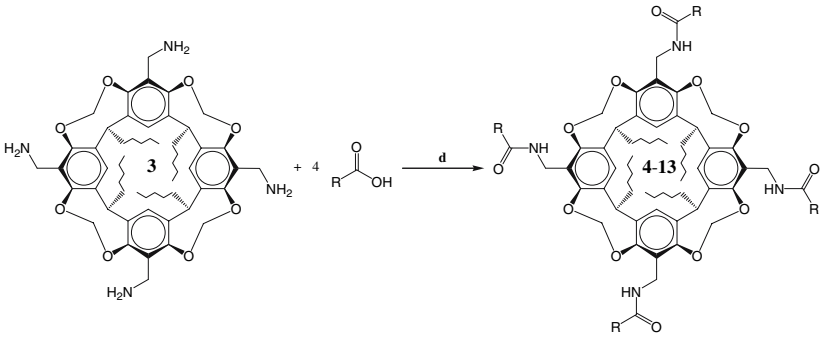


Table 2 Qualitative order of complexation of small guest molecules by the cavitands **4–7** in chloroform-d at 25°C. The "+" sign indicates line broadening and/or chemical shielding of ^{1}H-NMR signals of the guest molecules in the presence of the host. A vertical arrow, followed by a letter denotes that a guest replaces another guest on addition (e.g., acetamide (e) replaces ethanol (d) as guest of the valine cavitand **5**)

Guest	Phe (**4**)	Val (**5**)	Ser (**6**)	Thr (**7**)
a) ethyl acetate	O	O	O	O
b) dichloromethane	O	O	+	O
c) acetonitrile	O	O	+ ↑ **b**	+
d) ethanol	+	+	+ ↑ **b**	+
e) acetamide	+ ↑ **d**	+ ↑ **d**	+ ↑ **b, c, d**	+ ↑ **c, d**
f) acetic acid	+ ↑ **d, e**	+ ↑ **d, e**	+ ↑ **b, c, d, e**	+ ↑ **c, d, e**

The competition experiments give a qualitative ordering of the complexation constants, but do not provide quantitative data without careful integration of broad and overlapping signals. NMR spectra at low temperatures may help but first measurements (see below) show that even at low temperatures the cavitands NMR signals are very complex indicating a disordered cavitand conformation and/or aggregation. We report therefore only the qualitative ordering of complexation constants.

The temperature dependence of the ^{1}H-NMR spectra of the complexes with acetic acid are investigated in more detail: For example, the ^{1}H NMR spectra of **7** with acetic acid in chloroform-d show a very broad signal for the acetic acid methyl group and no signal for the carboxylic acid proton at 303 K (Fig. 1). Two sets of signals are observed at 213 K: Two broad low field signals at $\delta = 12.37$ ppm and $\delta = 10.34$ ppm for the carboxylic acid proton of free and complexed acetic acid and two high field signals at $\delta = 2.10$ ppm and $\delta = -2.54$ ppm for the methyl group protons of free and complexed acetic acid. Clearly the methyl group of the acetic acid is encapsulated. The complexity of the cavitand signals in $CDCl_3$ at low temperature prevents a detailed analysis of the involved conformations and of the kinetics and thermodynamics of complexation. The sharp signals of complexed and free acetic acid give however a clear indication that the rates of complexation and decomplexation are slow on the NMR time scale.

Summarizing the ^{1}H NMR spectroscopic studies leads to the following conclusions: The host-guest-interaction increases with the host-sequence "val-cavitand **5** < phe-cavitand **4** < thr-cavitand **7** < ser-cavitand **6**" and with the guest-sequence "ethyl acetate < dichloromethane < acetonitrile < ethanol < acetamide < acetic acid". More quantitative data are difficult to obtain due to the strong line broadening in the NMR spectra at low temperature.

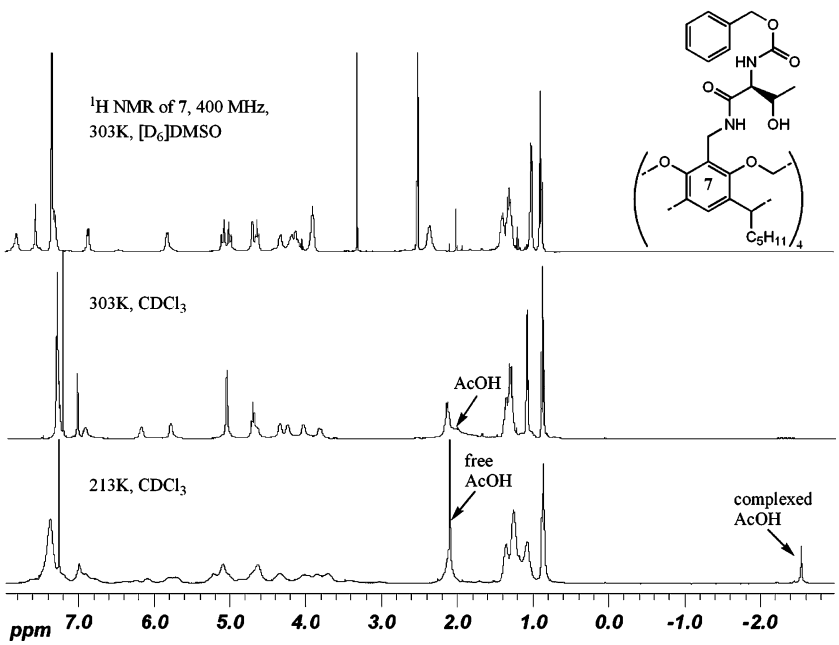


Fig. 1 ^{1}H NMR spectra of **7** in [D$_6$]DMSO and in $CDCl_3$ with added acetic acid

Self-complexing cavitands

Methyl ester groups in the vicinity of the upper rim of the resorc[4]arene cavitands are also welcome guest for the cavitand. This is observed in the ^{1}H NMR spectra of the cavitands **8–13** (Scheme 3). For example, the ^{1}H NMR signal of the methyl group of compound **8** appears in chloroform-d_1 solutions at 303 K as an extremly broad singulett near the baseline between 2.7 and 3.4 ppm. The same signal is a sharp singulett (δ = 3.38 ppm) in dimethyl sulfoxide-d_6 solution at 353 K (Fig. 2).

The signals of the methyl ester split at low temperatures in chloroform and in several other solvents. For example, measurements in acetone-d_6 at 233 K show three singuletts in the range of δ = 3.4–3.6 ppm and one singulett at high field (δ = –0.4 ppm) (Fig. 3). Obviously, one of the four methyl groups of **8** is encapsulated.

As shown in Fig. 4 there are two possible exchange patterns for the methyl groups. One pattern for the random walk, where the methyl ester group to be complexed is picked by chance; the other for peripheral walk, where the already complexed methyl ester group determines the methyl ester which is complexed in the next step. Comparison with the experimental EXSY patterns (Fig. 4) proves that the exchange occurs randomly. An activation barrier of ca. 12.3 ± 0.4 kcal mol^{-1} ($\Delta G^{\neq}_{263}$) for this process can be estimated from the 1D-line shapes.

A Monte carlo search for low energy conformations of **8** by Macromodel [10] produces as global minimum a structure where one of the methyl ester groups is located inside of the cavity as indicated in the NMR spectra (Fig. 5).

Conclusion

A straight synthesis of peptide cavitands is reported based on the easy access to the aminomethyl substituted resorc[4]arene via the Delépine-reaction.

Scheme 3 Structures of the cavitands **8–13**; methyl ester groups are ''self-complexed''

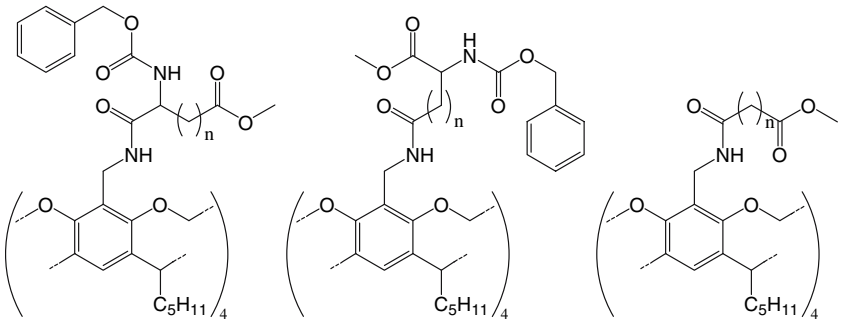
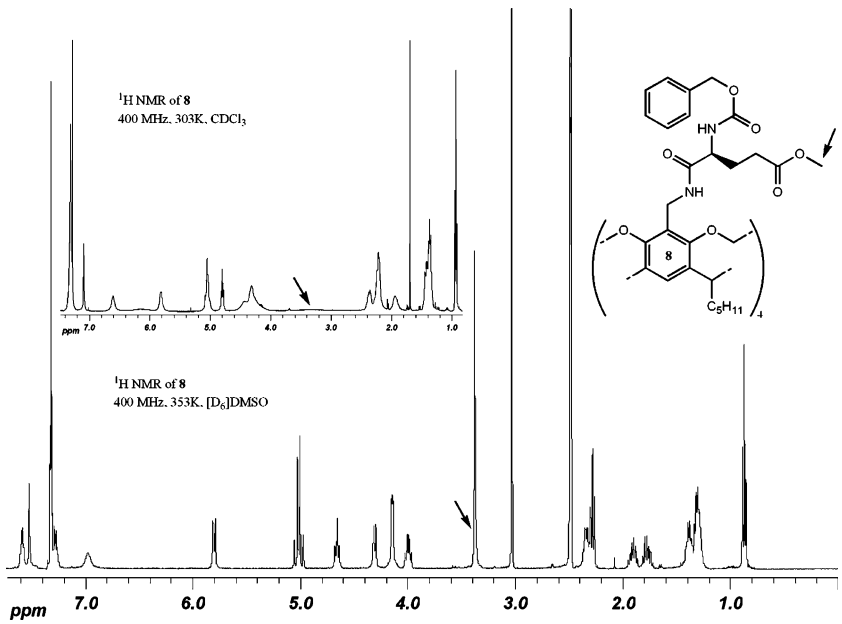


Fig. 2 ^{1}H NMR spectra of **8** in $CDCl_3$ respectively $[D_6]$DMSO



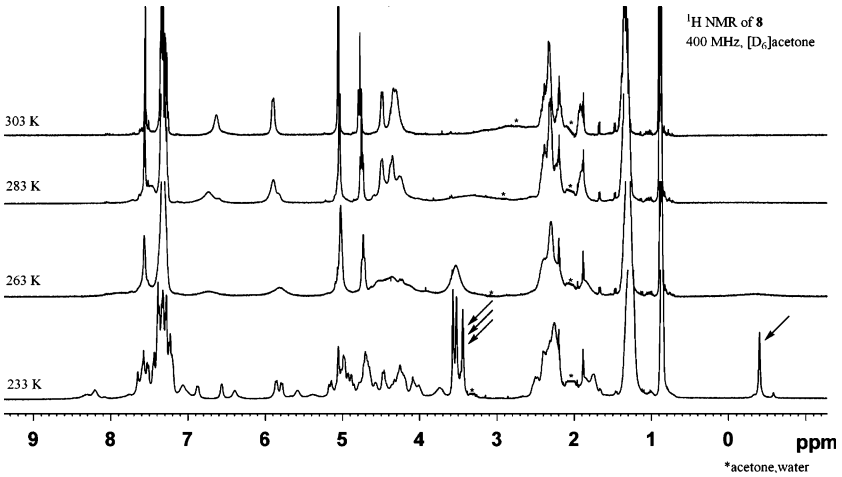


Fig. 3 Temperature dependence of the ^{1}H NMR spectra of **8** in [D$_6$]acetone. Arrows indicate the positions of the signals for the methyl ester groups

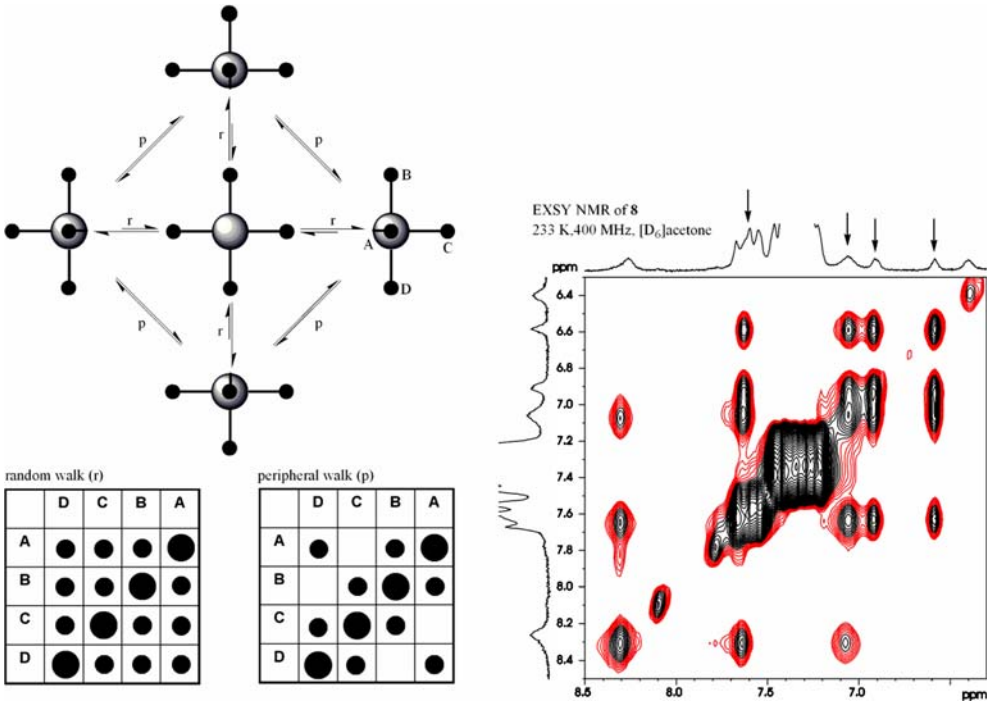


Fig. 4 Possible mechanistic exchange patterns (left) and part of the EXSY NMR spectrum of **8** in [D$_6$]acetone (right). The arrows indicate the four different positions of the Glu(NH)-protons

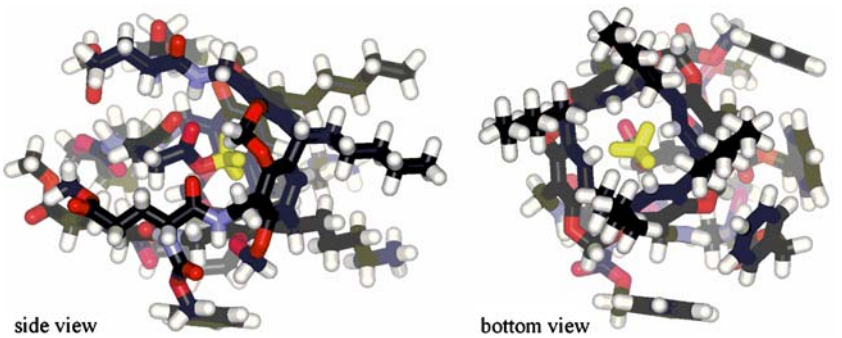


Fig. 5 Structure of **8** obtained by a monte carlo force field computation (Macromodel). The encapsulated methyl group is highlighted in yellow

In contrast to previous studies, where peptides were attached via glycine to the upper rim of resorcarenes, the present study investigates systems in which chiral amino acids are attached directly without any spacer at the rim. The amino acid side chains prevent obviously a well defined pattern of hydrogen bonds between the four attached amino acids.

NMR data suggest that the peptide cavitands encapsulate small organic guests in chloroform. Competition experiments show that the guests are taken up in the order ethyl acetate < dichloromethane < acetonitrile < ethanol < acetamide < acetic acid. The complexity and broadness of the signals prevents however a quantitative determination of the free enthalpy of complexation.

Cavitands with attached methyl esters of aspartic and glutamic acid derivatives are found to adopt a special conformation. NMR data suggest, that these amino acids encapsulate one of their own side chain methyl ester groups within the resorcarene bowl, the other three chains are directed outward. The in/out-exchange of the methyl ester groups occurs randomly.

Further investigation should be directed to catalytic functions of the peptide cavitands and to the recognization of protein surfaces. With the given synthesis, the systems are however restricted to resorcarenes having four equivalent peptide or amino acid substituents.

Experimental section

General

The following spectrometers were used: ^{1}H NMR/^{13}C NMR spectra—Bruker DRX 400; FAB-mass spectra—VG AutoSpec; MALDI-mass spectra—Bruker Daltonics autoflex. All solvents were purified according to standard procedure. Chromatography was performed on ICN silica 32–63 μm, 60-Å. Commercial available protected amino acids were obtained from Advanced Chemtech, Aldrich, Fluka and Nova Biochem.

5,11,17,23-Tetrakis[N-urotropinyliummethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene tetrabromide (**2**)

Hexamethylenetetramine (23.83 g, 0.17 mol) was dissolved in chloroform (275 mL) and a solution of tetrakis(bromomethyl)cavitand **1** (5.00 g, 4.2 mmol) in chloroform (25 mL) was added under stirring. The mixture was stirred for 10 min and kept at RT for 24 h. The suspension was filtered and washed with cold chloroform (at least 80 mL). The resulting product was dried in vacuo. Yield: 7.099 g (97%). White solid; m.p. > 180°C (dec.). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30°C): δ = 7.93 (s, 4H, Ar-H), 6.40 (d, 4H, O-CH$_2$-O), 5.15 (s, 24H, Ar-CH$_2$-N-C*H*$_2$), 4.67 (t, 4H, Ar$_2$C*H*-C$_5$H$_{11}$), 4.56 (s, 24H, Ar-CH$_2$-N-CH$_2$-N-C*H*$_2$), 4.11 (d, 4H, O-CH$_2$-O), 3.78 (s, 8H, Ar-CH$_2$), 2.49 (m, 8H, Ar$_2$-CH-C*H*$_2$-(CH$_2$)$_3$-CH$_3$), 1.38 (m, 8H, Ar$_2$-CH-(CH$_2$)$_2$-C*H*$_2$-CH$_2$-CH$_3$), 1.38 (m, 8H, Ar$_2$-CH-CH$_2$-C*H*$_2$-(CH$_2$)$_2$-CH$_3$), 1.32 (m, 8H, Ar$_2$-CH-(CH$_2$)$_3$-C*H*$_2$-CH$_3$), 0.86 (t, 12H, Ar$_2$CH-(CH$_2$)$_4$-C*H*$_3$) ppm. ^{13}C NMR (400 MHz, [D$_6$]DMSO, 30°C): δ = 154.05 (2C, Ar-C2), 138.17 (2C, Ar-C3), 125.44 (Ar-C4), 112.87 (Ar-C1), 99.50 (O-CH$_2$-O), 77.91 (3C, Ar-CH$_2$-N-*C*), 69.53 (3C, Ar-CH$_2$-N-CH$_2$-N-*C*), 49.09 (Ar-CH$_2$), 36.99 (Ar$_2$CH), 31.25 (Ar$_2$-CH-(CH$_2$)$_2$-*C*H$_2$-CH$_2$-CH$_3$), 29.30 (Ar$_2$-CH-*C*H$_2$-(CH$_2$)$_3$-CH$_3$), 26.70 (Ar$_2$-CH-CH$_2$-*C*H$_2$-(CH$_2$)$_2$-CH$_3$), 22.10 (Ar$_2$-CH-(CH$_2$)$_3$-*C*H$_2$-CH$_3$), 13.94 (Ar$_2$CH-(CH$_2$)$_4$-*C*H$_3$) ppm. MS (FAB): m/z = 1670.3 [**2** – Br$^-$]$^+$. C$_{80}$H$_{116}$Br$_4$N$_{16}$O$_8$ · 6H$_2$O (1857.59): calcd. C 51.73, H 6.95, N 12.06; found C 51.97, H 7.25, N 11.82.

5,11,17,23-Tetrakis[aminomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**3**)

Compound **2** (5.00 g, 2.86 mmol) was dissolved in ethanol (95 mL) and hydrochloric acid (37% w/w, 7 mL) was added to the solution. The reaction mixture was refluxed for 6 h and filtered. The precipitate was treated with an aqueous solution of sodium hydroxide (2 N, 100 mL) and stirred for 30 min. The resulting product was filtered and dried in vacuo. Yield: 2.460 g (92%). White solid; m.p. > 175°C (dec.). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 30°C): δ = 7.47 (s, 4H, Ar-H), 5.88 (d, 4H, O-CH$_2$-O), 4.61 (t, 4H, Ar$_2$C*H*-C$_5$H$_{11}$), 4.41 (d, 4H, O-CH$_2$-O), 3.47 (s, 8H, Ar-CH$_2$), 2.70 (br. s, 8H, Ar-CH$_2$-N*H*$_2$), 2.33 (dt, 8H, Ar$_2$-CH-C*H*$_2$-(CH$_2$)$_3$-CH$_3$), 1.37 (m, 8H, Ar$_2$-CH-(CH$_2$)$_2$-C*H*$_2$-CH$_2$-CH$_3$), 1.29 (m, 8H, Ar$_2$-CH-(CH$_2$)$_3$-C*H*$_2$-CH$_3$), 1.27 (m, 8H, Ar$_2$-CH-CH$_2$-C*H*$_2$-(CH$_2$)$_2$-CH$_3$), 0.86 (t, 12H, Ar$_2$CH-(CH$_2$)$_4$-C*H*$_3$) ppm. ^{13}C NMR (400 MHz, [D$_6$]DMSO, 30°C): δ = 152.54 (2C, Ar-C2), 137.75 (2C, Ar-C3), 129.22 (Ar-C1), 120.14 (Ar-C4), 99.51 (O-CH$_2$-O), 36.87 (Ar$_2$CH), 35.20 (Ar-CH$_2$), 31.45 (Ar$_2$-CH-(CH$_2$)$_2$-*C*H$_2$-CH$_2$-CH$_3$), 29.32 (Ar$_2$-CH-*C*H$_2$-(CH$_2$)$_3$-CH$_3$), 27.43 (Ar$_2$-CH-CH$_2$-*C*H$_2$-(CH$_2$)$_2$-CH$_3$), 22.21 (Ar$_2$-CH-(CH$_2$)$_3$-*C*H$_2$-CH$_3$), 13.88 (Ar$_2$CH-(CH$_2$)$_4$-*C*H$_3$) ppm. MS (FAB): m/z = 870.4 [**3** - CH$_2$NH$_2$ - 2NH$_2$]$^+$ (fragmentation peaks show loss of NH$_2$ and CH$_2$NH$_2$ during measurement) [7].

General procedure for coupling of carboxylic acids or Z-protected amino acids to **3**

The corresponding carboxylic acid or Z-protected amino acid (2.5 mmol), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.575 g, 3 mmol) and 1-Hydroxy-1H-benzotriazole (0.406 g, 3 mmol) were dissolved in dichloromethane (50 mL) and stirred at room temperature for 1 h. A mixture of compound **3** (0.466 g, 0.5 mmol) and triethylamine (0.5 mL, 3.6 mmol) in dichloromethane (10 mL) was sonicated, added to the activated amino acid and stirred at room temperature for 18 h. The solvent was evaporated in vacuo and the residue taken up in a mixture of ethyl acetate (60 mL) and aqueous sodium hydrogen sulphate (10% w/w, 50 mL). The phases were separated and the organic layer was extracted with aqueous sodium hydrogen sulphate (10% w/w, 2 × 50 mL), saturated sodium hydrogen carbonate in water (3 × 50 mL) and brine (2 × 50 mL). The organic phase was dried over anhydrous sodium sulphate and filtered. The solvent was removed in vacuo and the resulting product was separated by column chromatography on silica (2.5 × 25 cm^2).

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-phenylalanylamidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**4**)

Z-Phe-OH (0.748 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate / *n*-hexane 1/2, 2. ethyl acetate). Yield: 0.873 g (85%). White solid; m.p. 112–115°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30°C): δ = 7.80 (br. t, 4H, Ar-CH_2-N*H*), 7.55 (s, 4H, Ar-H), 7.43 (d, 4H, Phe-NH), 7.26 (m, 20H, Z-Ar-H), 7.20 (m, 20H, Phe-Ar-H), 5.71 (d, 4H, O-CH_2-O), 4.93 (dd, 8H, Z-CH_2-O), 4.63 (t, 4H, Ar_2CH-C_5H_{11}), 4.29 (d, 4H, O-CH_2-O), 4.20 (m, 4H, Phe-αH), 4.20 (m, 4H, Ar-C*H*$_2$-NH), 4.07 (m, 4H, Ar-CH_2-NH), 2.88 (m, 4H, Phe-βH), 2.71 (m, 4H, Phe-βH), 2.34 (m, 8H, Ar_2-CH-C*H*$_2$-$(CH_2)_3$-CH_3), 1.37 (m, 8H, Ar_2-CH-$(CH_2)_2$-C*H*$_2$-CH_2-CH_3), 1.29 (m, 8H, Ar_2-CH-$(CH_2)_3$-C*H*$_2$-CH_3), 1.27 (m, 8H, Ar_2-CH-CH_2-C*H*$_2$-$(CH_2)_2$-CH_3), 0.85 (t, 12H, Ar_2CH-$(CH_2)_4$-C*H*$_3$) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 171.00 (Phe-*C*O), 155.66 (Z-CO), 153.21 (Ar-C2a), 152.95 (Ar-C2b), 137.95 (2C, Ar-C3), 137.74 (PheAr-C1), 136.99 (Z-C1), 129.08 (2C, PheAr-C3),128.14 (2C, Z-C3), 127.90 (2C, PheAr-C2), 127.48 (Z-C4), 127.25 (2C, Z-C2), 126.14 (PheAr-C4), 123.86 (Ar-C1), 121.34 (Ar-C4), 99.36 (O-CH_2-O), 65.15 (Z-CH_2), 55.95 (Phe-αC), 37.65 (Phe-βC), 36.81 (Ar_2CH), 32.82 (Ar-CH_2-NH), 31.39 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 29.20 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.33 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.18 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 13.86 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (MALDI-TOF): m/z = 2080.63 $[4 + Na]^+$, 2096.87 $[\mathbf{4}+K]^+$. $C_{124}H_{136}N_8O_{20} \cdot 3H_2O$ (2112.49): calcd. C 70.50, H 6.78, N 5.30; found C 70.74, H 6.92, N 5.31.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-valinylamidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**5**)

Z-Val-OH (0.628 g, 2.5 mmol) was used in procedure A (Chromatography: ethyl acetate / *n*-hexane 1/1). Yield: 0.776 g (83%). White solid; m.p. 106–109°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30°C): δ = 7.77 (br. t, 4H, Ar-CH_2-N*H*), 7.55 (s, 4H, Ar-H), 7.31 (m, 20H, Z-Ar-H), 7.16 (d, 4H, Val-NH), 5.76 (d, 4H, O-CH_2-O), 5.00 (dd, 8H, Z-CH_2-O), 4.62 (t, 4H, Ar_2CH-C_5H_{11}), 4.36 (d, 4H, O-CH_2-O), 4.17 (m, 4H, Ar-C*H*$_2$-NH), 4.07 (m, 4H, Ar-CH_2-NH), 3.76 (m, 4H, Val-αH), 2.34 (m, 8H, Ar_2-CH-C*H*$_2$-$(CH_2)_3$-CH_3), 1.86 (m, 4H, Val-βH), 1.37 (m, 8H, Ar_2-CH-$(CH_2)_2$-C*H*$_2$-CH_2-CH_3), 1.29 (m, 8H, Ar_2-CH-$(CH_2)_3$-C*H*$_2$-CH_3), 1.28 (m, 8H, Ar_2-CH-CH_2-C*H*$_2$-$(CH_2)_2$-CH_3), 0.86 (t, 12H, Ar_2CH-$(CH_2)_4$-C*H*$_3$), 0.79 (s, 12H, Val-CH_3), 0.78 (s, 12H, Val-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 170.78 (Val-CO), 155.94 (Z-CO), 153.19 (Ar-C2a), 153.03 (Ar-C2b), 137.89 (2C, Ar-C3), 137.08 (Z-C1), 128.17 (2C, Z-C3), 127.55 (Z-C4), 127.45 (2C, Z-C2), 123.93 (Ar-C1), 121.31 (Ar-C4), 99.25 (O-CH_2-O), 65.28 (Z-CH_2), 60.09 (Val-αC), 36.75 (Ar_2CH), 32.66 (Ar-CH_2-NH), 31.34 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 30.30 (Val-βC), 29.18 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.28 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.18 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 18.96 (Val-CH_3), 18.14 (Val-CH_3), 13.86 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (MALDI-TOF): m/z = 1888.30 $[\mathbf{5}+Na]^+$, 1904.25 $[\mathbf{5}+K]^+$. $C_{108}H_{136}N_8O_{20} \cdot 5H_2O$ (1956.35): calcd. C 66.30, H 7.52, N 5.73; found C 66.25, H 7.46, N 5.69.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-serinylamidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**6**)

Z-Ser-OH (0.598 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate, 2. ethyl acetate / ethanol 9/1). Yield: 0.800 g (88 %). White solid; m.p. 109–112°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30°C): δ = 7.85 (br. t, 4H, Ar-CH_2-N*H*), 7.55 (s, 4H, Ar-H), 7.33 (m, 20H, Z-Ar-H), 7.11 (d, 4H, Ser-NH), 5.78 (d, 4H, O-CH_2-O), 5.01 (dd, 8H, Z-CH_2-O), 4.77

(br. t, 4H, Ser-OH), 4.62 (t, 4H, Ar_2CH-C_5H_{11}), 4.33 (d, 4H, O-CH_2-O), 4.17 (m, 4H, Ar-CH_2-NH), 4.12 (m, 4H, Ar-CH_2-NH), 4.01 (m, 4H, Ser-αH), 3.51 (m, 8H, Ser-βH), 2.33 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 1.38 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.30 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.27 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.87 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 169.77 (Ser-*C*O), 155.78 (Z-CO), 153.20 (Ar-C2a), 152.95 (Ar-C2b), 137.84 (2C, Ar-C3), 136.90 (Z-C1), 128.21 (2C, Z-C3), 127.62 (Z-C4), 127.61 (2C, Z-C2), 123.89 (Ar-C1), 121.24 (Ar-C4), 99.45 (O-CH_2-O), 65.44 (Z-CH_2), 61.71 (Ser-βC), 57.08 (Ser-αC), 36.85 (Ar_2CH), 33.00 (Ar-CH_2-NH), 31.42 (Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 29.27 (Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 27.36 (Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 22.18 (Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 13.87 (Ar_2CH-$(CH_2)_4$-CH_3) ppm. MS (MALDI-TOF): m/z = 1839.98 [**6**+Na]$^+$, 1855.95 [**6**+K]$^+$. $C_{100}H_{120}N_8O_{24} \cdot 3H_2O$ (1872.11): calcd. C 64.16, H 6.78, N 5.99; found C 64.02, H 6.93, N 5.96.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-threoninylamidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**7**)

Z-Thr-OH (0.633 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate, 2. ethyl acetate / ethanol 4/1). Yield: 0.754 g (81%). White solid; m.p. 122–125°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30°C): δ = 7.80 (br. t, 4H, Ar-CH_2-N*H*), 7.55 (s, 4H, Ar-H), 7.33 (m, 20H, Z-Ar-H), 6.85 (d, 4H, Thr-NH), 5.80 (d, 4H, O-CH_2-O), 5.02 (dd, 8H, Z-CH_2-O), 4.67 (d, 4H, Thr-OH), 4.62 (t, 4H, Ar_2CH-C_5H_{11}), 4.30 (d, 4H, O-CH_2-O), 4.15 (m, 4H, Ar-CH_2-NH), 4.10 (m, 4H, Ar-CH_2-NH), 3.88 (m, 4H, Thr-βH), 3.87 (m, 4H, Thr-αH), 2.33 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 1.37 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.30 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.26 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.99 (d, 12H, Thr-CH_3), 0.86 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 169.92 (Thr-*C*O), 155.97 (Z-CO), 153.21 (Ar-C2a), 152.96 (Ar-C2b), 137.86 (2C, Ar-C3), 136.96 (Z-C1), 128.20 (2C, Z-C3), 127.61 (Z-C4), 127.51 (2C, Z-C2), 123.87 (Ar-C1), 121.29 (Ar-C4), 99.35 (O-CH_2-O), 66.64 (Thr-βC), 65.47 (Z-CH_2), 60.44 (Thr-αC), 36.81 (Ar_2CH), 32.94 (Ar-CH_2-NH), 31.39 (Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 29.23 (Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 27.33 (Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 22.18 (Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 19.87 (Thr-CH_3), 13.87 (Ar_2CH-$(CH_2)_4$-CH_3) ppm. MS (MALDI-TOF): m/z = 1896.43 [**7**+Na]$^+$, 1912.40 [**7**+K]$^+$. $C_{104}H_{128}N_8O_{24} \cdot 3H_2O$ (1928.21): calc. C 64.78, H 7.00, N 5.81; found C 64.69, H 7.02, N 5.76.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-glutamic acidyl γ-methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**8**)

Z-Glu(OMe)-OH (0.738 g, 2.5 mmol) was used in procedure A (Chromatography: ethyl acetate / *n*-hexane 5/1). Yield: 0.839 g (82%). White solid; m.p. 114–117°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 80°C): δ = 7.59 (t, 4H, Ar-CH_2-N*H*), 7.52 (s, 4H, Ar-H), 7.30 (m, 20H, Z-Ar-H), 6.98 (br. d, 4H, Glu-NH), 5.80 (d, 4H, O-CH_2-O), 5.02 (dd, 8H, Z-CH_2-O), 4.66 (t, 4H, Ar_2CH-C_5H_{11}), 4.31 (d, 4H, O-CH_2-O), 4.15 (m, 8H, Ar-CH_2-NH), 4.00 (m, 4H, Glu-αH), 3.38 (s, 12H, Glu-OCH_3), 2.34 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 2.28 (m, 8H, Glu-γH), 1.91 (m, 4H, Glu-βH), 1.77 (m, 4H, Glu-βH), 1.39 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.32 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.30 (m, 8H, Ar_2-CH- CH_2-CH_2-$(CH_2)_2$-CH_3), 0.87 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 80°C): δ = 172.15 (*C*OOCH_3), 170.26 (*C*O$CH(CH_2)_2COOCH_3$), 155.26 (Z-CO), 152.91 (Ar-C2a), 152.73 (Ar-C2b), 137.57 (2C, Ar-C3), 136.59 (Z-C1), 127.76 (2C, Z-C3), 127.16 (Z-C4), 127.03 (2C, Z-C2), 123.57 (Ar-C1), 120.90 (Ar-C4), 99.05 (O-CH_2-O), 65.20 (Z-CH_2), 53.69 (Glu-αC), 50.44 (Glu-OCH_3), 36.58 (Ar_2CH), 32.64 (Ar-CH_2-NH), 30.94 (Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 29.46 (Glu-γC), 28.99 (Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 26.98 (Glu-βC), 26.89 (Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 21.64 (Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 13.25 (Ar_2CH-$(CH_2)_4$-CH_3) ppm. MS (MALDI-TOF): m/z = 2064.38 [**8**+Na]$^+$, 2080.44 [**8**+K]$^+$. $C_{112}H_{136}N_8O_{28} \cdot 2H_2O$ (2078.35): calc. C 64.72, H 6.79, N 5.39; found C 64.50, H 6.68, N 5.37.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-aspartic acidyl β-methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**9**)

Z-Asp(OMe)-OH (0.703 g, 2.5 mmol) was used in procedure A (Chromatography: ethyl acetate / *n*-hexane 2/1). Yield: 0.844 g (85%). White solid; m.p. 113–116°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 100°C): δ = 7.52 (s, 4H, Ar-H), 7.46 (br. t, 4H, Ar-CH_2-N*H*), 7.31 (m, 20H, Z-Ar-H), 7.01 (d, 4H, Asp-NH), 5.81 (d, 4H, O-CH_2-O), 5.04 (dd, 8H, Z-CH_2-O), 4.68 (t, 4H, Ar_2CH-C_5H_{11}), 4.33 (m, 4H, Asp-αH), 4.30 (d, 4H, O-CH_2-O), 4.25 (m, 8H, Ar-CH_2-NH), 3.28

(s, 12H, Asp-OCH_3), 2.69 (m, 4H, Asp-βH), 2.57 (m, 4H, Asp-βH), 2.35 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 1.40 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.33 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.31 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.88 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 170.48 (*C*$OOCH_3$), 169.97 (*C*$OCHCH_2COO$ CH_3), 155.70 (Z-CO), 153.19 (Ar-C2a), 152.91 (Ar-C2b), 137.85 (2C, Ar-C3), 136.82 (Z-C1), 128.22 (2C, Z-C3), 127.68 (Z-C4), 127.58 (2C, Z-C2), 123.96 (Ar-C1), 121.28 (Ar-C4), 99.47 (O-CH_2-O), 65.53 (Z-CH_2), 51.13 (Asp-αC), 51.13 (Asp-OCH_3), 36.83 (Ar_2CH), 36.06 (Asp-βC), 32.91 (Ar-CH_2-NH), 31.41 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 29.24 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.35 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.19 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 13.87 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (MALDI-TOF): m/z = 2008.59 [**9**+Na]$^+$, 2024.54 [**9**+K]$^+$. $C_{108}H_{128}N_8O_{28} \cdot 3H_2O$ (2040.25): calc. C 63.58, H 6.62, N 5.49; found C 63.38, H 6.63, N 5.51.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-glutamic acidyl α-methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**10**)

Z-Glu(OH)-OMe (0.738 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate, 2. ethyl acetate / ethanol 10/1). Yield: 0.899 g (88 %). White solid; m.p. 118–121°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 100°C): δ = 7.48 (s, 4H, Ar-H), 7.43 (br. t, 4H, Ar-CH_2-N*H*), 7.33 (m, 20H, Z-Ar-H), 7.17 (br. d, 4H, Glu-NH), 5.84 (d, 4H, O-CH_2-O), 5.04 (br. dd, 8H, Z-CH_2-O), 4.67 (t, 4H, Ar_2C*H*-C_5H_{11}), 4.35 (d, 4H, O-CH_2-O), 4.11 (m, 8H, Ar-CH_2-NH), 4.02 (m, 4H, Glu-αH), 3.16 (br. s, 12H, Glu-OCH_3), 2.34 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 2.18 (m, 8H, Glu-γH), 1.96 (m, 4H, Glu-βH), 1.82 (m, 4H, Glu-βH), 1.39 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.34 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.32 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.88 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 172.73 (*C*$OOCH_3$), 170.60 (*C*$O(CH_2)_2CHCOOCH_3$), 155.89 (Z-CO), 153.22 (2C, Ar-C2), 137.61 (2C, Ar-C3), 136.80 (Z-C1), 128.23 (2C, Z-C3), 127.69 (3C, Z-C2/C4), 124.10 (Ar-C1), 120.93 (Ar-C4), 99.60 (O-CH_2-O), 65.50 (Z-CH_2), 53.72 (Glu-αC), 51.67 (Glu-OCH_3), 36.82 (Ar_2CH), 32.86 (Ar-CH_2-NH), 31.45 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 31.16 (Glu-γC), 29.29 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.42 (Ar_2-CH-CH_2-*C*H_2-(C $H_2)_2$-CH_3), 26.18 (Glu-βC), 22.20 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 13.88 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (MALDI-TOF): m/z = 2064.51 [**10**+Na]$^+$, 2080.49 [**10**+K]$^+$. $C_{112}H_{136}N_8O_{28} \cdot 4H_2O$ (2114.38): calc. C 63.62, H 6.86, N 5.30; found C 63.42, H 6.75, N 5.30.

5,11,17,23-Tetrakis[benzyloxycarbonyl-L-aspartic acidyl α-methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**11**)

Z-Asp(OH)-OMe (0.703 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate / *n*-hexane 2/1, 2. ethyl acetate). Yield: 0.818 g (82%). White solid; m.p. 116–119°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 100°C): δ = 7.50 (s, 4H, Ar-H), 7.49 (br. t, 4H, Ar-CH_2-N*H*), 7.33 (m, 20H, Z-Ar-H), 7.00 (d, 4H, Asp-NH), 5.85 (d, 4H, O-CH_2-O), 5.05 (s, 8H, Z-CH_2-O), 4.68 (t, 4H, Ar_2C*H*-C_5H_{11}), 4.41 (m, 4H, Asp-αH), 4.31 (d, 4H, O-CH_2-O), 4.12 (m, 8H, Ar-CH_2-NH), 3.27 (s, 12H, Asp-OCH_3), 2.54 (m, 8H, Asp-βH), 2.34 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 1.40 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.33 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.32 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.88 (t, 12H, Ar_2 CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 172.00 (*C*$OOCH_3$), 168.40 (*C*$OCH_2CHCOOOCH_3$), 155.76 (Z-CO), 153.20 (Ar-C2a), 153.12 (Ar-C2b), 137.85 (2C, Ar-C3), 136.83 (Z-C1), 128.32 (2C, Z-C3), 127.80 (Z-C4), 127.66 (2C, Z-C2), 124.07 (Ar-C1), 121.25 (Ar-C4), 99.61 (O-CH_2-O), 65.58 (Z-CH_2), 51.66 (Asp-OCH_3), 50.69 (Asp-αC), 36.90 (Ar_2CH), 36.76 (Asp-βC), 32.86 (Ar-CH_2-NH), 31.52 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 29.33 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.48 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.29 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 13.98 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (MALDI-TOF): m/z = 2008.36 [**11**+Na]$^+$, 2024.40 [**11**+K]$^+$. $C_{108}H_{128}N_8O_{28} \cdot 4H_2O$ (2058.27): calc. C 63.02, H 6.66, N 5.44; found C 62.74, H 6.51, N 5.39.

5,11,17,23-Tetrakis[glutaric acidyl methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**12**)

Glutaric acid monomethyl ester (0.365 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate, 2. ethyl acetate / ethanol 10/1). Yield: 0.638 g (88%). White solid; m.p. 103–106°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 100°C): δ = 7.50 (s, 4H, Ar-H), 7.36 (br. t, 4H, Ar-CH_2-N*H*), 5.86 (d, 4H, O-CH_2-O), 4.68 (t, 4H, Ar_2C*H*-C_5H_{11}), 4.33 (d, 4H, O-CH_2-O), 4.11 (d, 8H, Ar-CH_2-NH), 3.32 (s, 12H,

Glu-OCH_3), 2.34 (dt, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 2.26 (t, 8H, R-$(CH_2)_2$-CH_2-$COOCH_3$), 2.09 (t, 8H, R-CH_2-$(CH_2)_2$-$COOCH_3$), 1.75 (tt, 8H, R-CH_2-CH_2-CH_2-$COOCH_3$), 1.39 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.34 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.32 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.88 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 173.01 (*C*$OOCH_3$), 171.19 (*C*O$(CH_2)_3COOCH_3$), 153.21 (2C, Ar-C2), 137.72 (2C, Ar-C3), 124.19 (Ar-C1), 121.15 (Ar-C4), 99.84 (O-CH_2-O), 50.89 (Glu-OCH_3), 36.85 (Ar_2CH), 33.93 (R-*C*H_2-$(CH_2)_2$-$COOCH_3$), 32.90 (Ar-CH_2-NH), 32.73 (R-$(CH_2)_2$-*C*H_2-$COOCH_3$), 31.49 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 29.36 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 27.44 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.28 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 20.37 (R-CH_2-*C*H_2-CH_2-$COOCH_3$), 13.97 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (FAB): m/z = 1446.2 [**12**+H]$^+$, 1468.1 [**12**+Na]$^+$. $C_{80}H_{108}N_4O_{20} \cdot 4H_2O$ (1517.79): calc. C 63.31, H 7.70, N 3.69; found C 63.53, H 7.71, N 4.07.

5,11,17,23-Tetrakis[succinic acidyl methyl ester amidomethyl]-4(24),6(10),12(16),18(22)-tetramethylenedioxy-2,8,14,20-tetrapentylresorc[4]arene (**13**)

Succinic acid monomethyl ester (0.330 g, 2.5 mmol) was used in procedure A (Chromatography: 1. ethyl acetate, 2. ethyl acetate / ethanol 10/1). Yield: 0.621 g (89%). White solid; m.p. 118–121°C. ^{1}H NMR (400 MHz, [D_6]DMSO, 30°C): δ = 7.81 (br. t, 4H, Ar-CH_2-N*H*), 7.53 (s, 4H, Ar-H), 5.86 (d, 4H, O-CH_2-O), 4.60 (t, 4H, Ar_2CH-C_5H_{11}), 4.28 (d, 4H, O-CH_2-O), 4.08 (d, 8H, Ar-CH_2-NH), 3.45 (s, 12H, Suc-OCH_3), 2.43 (t, 8H, R-CH_2-CH_2-$COOCH_3$), 2.29 (t, 8H, R-CH_2-CH_2-$COOCH_3$), 2.29 (m, 8H, Ar_2-CH-CH_2-$(CH_2)_3$-CH_3), 1.38 (m, 8H, Ar_2-CH-$(CH_2)_2$-CH_2-CH_2-CH_3), 1.30 (m, 8H, Ar_2-CH-$(CH_2)_3$-CH_2-CH_3), 1.28 (m, 8H, Ar_2-CH-CH_2-CH_2-$(CH_2)_2$-CH_3), 0.87 (t, 12H, Ar_2CH-$(CH_2)_4$-CH_3) ppm. ^{13}C NMR (400 MHz, [D_6]DMSO, 30°C): δ = 172.75 (*C*$OOCH_3$), 170.35 (*C*O(CH_2)COO CH_3), 153.14 (2C, Ar-C2), 137.78 (2C, Ar-C3), 124.12 (Ar-C1), 121.15 (Ar-C4), 99.81 (O-CH_2-O), 51.06 (Suc-OCH_3), 36.82 (Ar_2CH), 32.84 (Ar-CH_2-NH), 31.40 (Ar_2-CH-$(CH_2)_2$-*C*H_2-CH_2-CH_3), 29.46 (R-*C*H_2-CH_2-$COOCH_3$), 29.31 (Ar_2-CH-*C*H_2-$(CH_2)_3$-CH_3), 28.60 (R-CH_2-*C*H_2-$COOCH_3$), 27.35 (Ar_2-CH-CH_2-*C*H_2-$(CH_2)_2$-CH_3), 22.19 (Ar_2-CH-$(CH_2)_3$-*C*H_2-CH_3), 13.87 (Ar_2CH-$(CH_2)_4$-*C*H_3) ppm. MS (FAB): m/z = 1389.4 [**13**+H]$^+$, 1411.5 [**13**+Na]$^+$. $C_{76}H_{100}N_4O_{20} \cdot 4H_2O$ (1461.68): calc. C 62.45, H 7.45, N 3.83; found C 62.06, H 7.41, N 4.04.

References

1. (a) Moran, J.R., Ericson, J.L., Dalcanale, E., Bryant, J.A., Knobler, C.B., Cram, D.J.: Vases and kites as cavitands. J. Am. Chem. Soc. **113**, 5707 (1991); (b) Cram, D.J., Karbach, S., Kim, H.-E., Knobler, C.B., Maverick, E.F., Ericson, J.L., Helgeson, R.: Host-guest complexation. 46. Cavitands as open molecular vessels form solvates. J. Am. Chem. Soc. **110**, 2229 (1988); Tunstad, L.M., Tucker, J.A., Dalcanale, E., Weiser, J., Bryant, J.A., Sherman, J.C., Helgeson, R.C., Knobler, C.B., Cram, D.J.: Host-guest complexation. 48. Building blocks for cavitands and carcerands. J. Org. Chem. **54**, 1305 (1989)
2. (a) Timmerman, P., Verboom, W., Reinhoudt, D.N.: Resorcinarenes. Tetrahedron **52**, 2663 (1996); (b) Jasat, A., Sherman, J.C.: Carceplexes and hemicarceplexes. Chem. Rev. **99**, 931 (1999); (c) Verboom, W.: Cavitands. In: Asfari, Z., Böhmer, V., Harrowfield, J.McB., Vicens, J. (eds.), Calixarenes 2001, Kluwer, Dordrecht (2001)
3. (a) Boerrigter, H., Grave, L., Nissink, J.W.M., Chrisstoffels, L.A.J., van der Maas, J.H., Verboom, W., de Jong, F., Reinhoudt, D.N.: (Thio)urea resorcinarene cavitands. Complexation and membrane transport of halide anions. J. Org. Chem. **63**, 4174 (1998); (b) Ahn, D.-R., Kim, T.W., Hong, J.-L.: Water-soluble resorcin[4]arene: Complexation of anionic aromatic Guests by cooperativity of electrostatic and hydrophobic interactions. Tetrahedron Lett. **40**, 6045 (1999)
4. (a) Mezo, A.R., Sherman, J.C.: Cavitands are effective templates for inducing stability and native like structure in De Novo four-helix bundles. J. Am. Chem. Soc. **121**, 8983 (1999); (b) Gibb, B.C., Mezo, A.R., Causton, A.S., Fraser, J.R., Tsai, F.C.S., Sherman, J.C.: Efficient coupling of amino acid derivatives to rigid organic scaffolds: Model syntheses for De Novo proteins. Tetrahedron **51**, 8719 (1995)
5. (a) Hamuro, Y., Calama, M.C., Park, H.S., Hamilton, A.D.: Ein Calixaren mit vier Peptidschleifen: ein Antikörper-Mimeticum zur Erkennung von Proteinoberflächen. Angew. Chem. **109**, 2797 (1997); (b) Hamuro, Y., Calama, M.C., Park, H.S., Hamilton, A.D.: A calixarene with four peptide loops: An antibody mimic for recognition of protein surfaces. Angew. Chem. Int. Ed. Engl. **36**, 2680 (1997); (c) Lin, A., Hamilton, A.D.: Design and synthesis of multiple-loop receptors based on a calix[4]arene scaffold for protein surface recognition. Comptes Rendus **5**, 441 (2002); (d) Sansone, F., Barboso, S., Casnati, A.,Fabbi, M., Pochini, A., Ugozzoli, F., Ungaro, R.: Synthesis and Structure of Chiral *Cone* Calix[4]arenas Functionalized at the Upper Rim with L-Alanine Units. Eur. J. Org. Chem. 897 (1998); (e) Bauer, W., Soi, A., Hirsch, A.: Application of DPFGSE-ROE to calixarene derivatives under conditions near NOE zero-crossing. Magn. Reson. Chem. **38**, 500 (2000); (f) Lazzarotto, M., Sansone, F., Baldini, L., Casnati, A., Cozzini, P., Ungaro, R.: Synthesis and properties of *upper rim C*-linked peptidocalix[4]arenes. Eur. J. Org. Chem. 595 (2001)
6. Berghaus, C., Feigel, M.: Peptide-Cavitands based on resorc[4]arenes – synthesis and structure. Eur. J. Org. Chem. 3200 (2003)
7. Boerrigter , H., Verboom, W., Reinhoudt, D.N.: Novel resorcinarene cavitand-based CMP(O) cation ligands: synthesis and extraction properties. J. Org. Chem. **62**, 7148 (1997)
8. The reaction type is known as the Delépine reaction: Delépine, M.: Sur lhexaméthyléne-amine (suite). Solubilités, hydrate, bromure, sulfate, phosphate. Bull. Soc. Chim. Fr. **13**, 352 (1895)

9. Boerrigter, H., Verboom, W., Reinhoudt, D.N.: Ligands for EuIII, FeIII, SrII, and UO2II based on CMPO-functionalized resorcinarene cavitands; Synthesis and Extraction. Liebigs Ann./Recueil, 2247 (1997)
10. MacroModel V4.0: Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T., Still, W.C.: MacroModel – An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. J. Comput. Chem. **11**, 440 (1990)