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Synthesis of new decasubstituted pillar[5]arenes containing glycine fragments and their interactions with Bismarck brown Y

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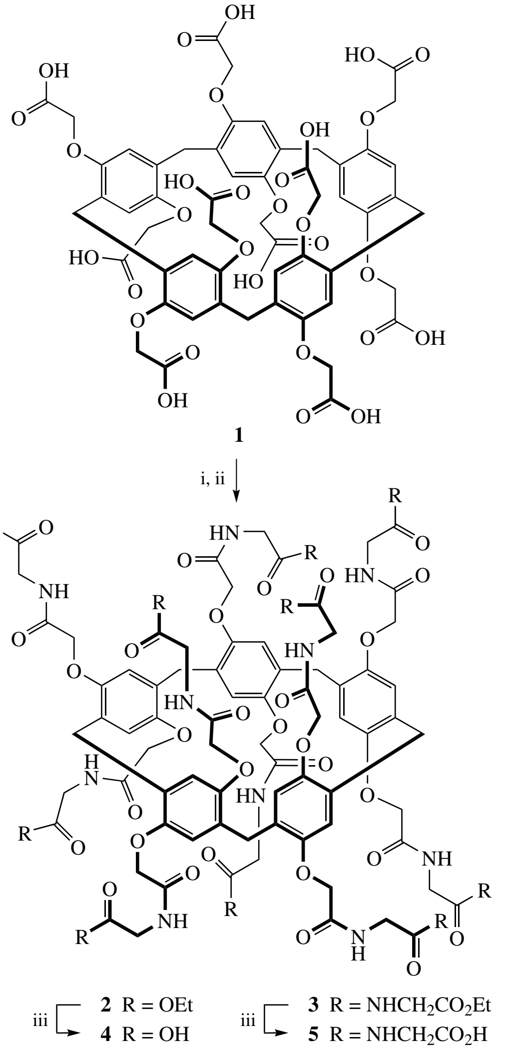
New water-soluble decasubstituted pillar[5]arenes containing glycine and glycylglycine fragments were synthesized and characterized. The UV, DLS and TEM study showed that interaction of self-associates of the glycylglycine derivatives with Bismarck brown Y produced particles of about 59 nm in size.

Azo dyes have found various applications in science, medicine and technology.[1–3] Despite the fact that most water-soluble dyes are weakly reactive and non-toxic at low concentrations, they appear to be main source of water pollution, and pose a serious threat to the environment.[3] Meanwhile, nanoparticles covalently linked or associated with chromophoric or fluorophoric substances have potential use in sensors, biomimetic systems, as catalysts, selective extractants and drug delivery systems.[4–10] We have recently proposed[11] the use of a new class of macrocycles, pillar[5]arenes, as synthetic blocks for constructing water soluble nanoparticles capable of interacting with azo dyes. The *p*-hydroquinone fragments of these macrocycles are linked to each other by methylene bridges.[12] Pillar[*n*]arenes contain an internal hydrophobic cavity with a diameter comparable to the cavity diameter of corresponding cyclodextrins and cucurbit[*n*]urils.[13] Pillar[*n*]arenes similarly to cyclodextrins and cucurbit[*n*]urils tend to form host–guest complexes with organic and inorganic ‘guests’. A distinctive feature of pillar[*n*]arene is the presence of free hydroxyl groups that can be variously functionalized.[14–16]

Previously, self-assembled amphiphilic functionalized pillar[5]arenes[17,18] and supramolecular polymers[19–24] based on inclusion complexes consisting of linear (with carbon number ⩾ 6) molecule fragments in the cavity of the macrocycle were described in the literature. However, there is no data on the possible formation of self-associates by decafunctionalized pillar[5]arenes. On the other hand, structural proteins elastin and collagen are rich in glycine.[25] Collagen fibrils represent a major component in the extracellular matrix and connective tissue providing support for cells.[26] Thus, we hypothesize that attachment of glycine and glycylglycine fragments to pillar[5]arenes can promote self-aggregation and affinity to various biomacromolecules.

The starting decaacid **1** was synthesized from commercially available reagents according to the published procedures[27] (Scheme 1). It was converted into decachloride by treatment with thionyl chloride in the presence of catalytic amount of DMF.[28] Further acylation of glycine and glycylglycine ethyl ester hydrochlorides with this decachloride gave compounds **2** and **3** in 78 and 70% yields, respectively (see Scheme 1). The acylation proceeded in anhydrous dichloromethane in the presence of triethylamine for 48 h.[†]



1

2 R = OEt
4 R = OH
3 R = $NHCH_2CO_2Et$
5 R = $NHCH_2CO_2H$

Scheme 1 *Reagents and conditions*: i, $SOCl_2$, reflux; ii, glycine/glycylglycine ester hydrochloride, Et_3N/CH_2Cl_2; iii, LiOH, H_2O/THF, room temperature, then HCl.

[†] *General procedure for the synthesis of compounds* **2** *and* **3**. 4,8,14,18,23,26,28,31,32,35-Decakis(methoxycarbonyl)pillar[5]arene **1** (0.30 g, 0.252 mmol) was placed into the round-bottom flask followed by addition of $SOCl_2$ (10 ml, 0.084 mol) and catalytic amount of DMF. The mixture was refluxed for 18 h and then the excess of $SOCl_2$ was removed under reduced pressure. The remainder was dried under reduced pressure for 2 h. The obtained residue was dissolved in 10 ml of dichloromethane. The

To obtain pillar[5]arenes containing carboxyl groups, compounds **2** and **3** were hydrolyzed with lithium hydroxide in aqueous THF (Scheme 1).[‡] This hydrolysis occurred much faster (25 min) than that of decaester **1** previously described.[29] This may probably be attributed to autocatalysis (each subsequent group is hydrolyzed faster than previous one) as well as to high solubility of both esters **2**, **3** and lithium salts of acids **4**, **5** in the THF–water mixture. Prolongation of hydrolysis significantly dropped the yields of products **4**, **5** due to further cleavage of the peptide bonds. Over a 5 h period, compounds **2**, **3** were completely hydrolyzed to decaacid **1**.

Then we examined[§] self-aggregation of the synthesized pillar[5]arenes **4**, **5** and their interaction with azo dye Bismarck brown Y (BBY) which is widely used in biochemical and microbiological studies as well as for cancer treatment.[3] Dynamic light scattering (DLS) study of compounds **4**, **5** in aqueous solution revealed that only macrocycle **5** produced aggregates in the concentrations range of 1×10^{-3}–1×10^{-5} M. For example, monodisperse system (PDI = 0.15) with average hydrodynamic diameter of 195 nm was formed at a molar concentration of 1×10^{-3} M. Lowering the concentration of **5** corresponded to increased hydrodynamic diameter of the particles. In this case, the polydispersity increased simultaneously (0.15–0.31). Compared to literature data,[5,6] it can be assumed that the increased size of the aggregates followed by decrease in pillar[5]arene concentration can be attributed to increased solvation shell as well as to non-spherical form of the aggregates formed.

The interaction of pillar[5]arene **5** with BBY in aqueous solution was also studied by DLS,[§] UV and ^{1}H NMR spectroscopy. It was demonstrated by DLS that combination **5**/BBY in concentration ratio 1:1 formed aggregates with hydrodynamic diameter of 400 nm in a monodisperse system (PDI = 0.17) when the concentration of **5** was 1×10^{-5} M. Raising concentration of the combination **5**/BBY (1:1) to 1×10^{-4} M resulted in decrease in hydrodynamic diameter to 358 nm and increase in polydispersity index (PDI = 0.27). At a concentration of 1×10^{-3} M, this combination gave polydisperse colloid.

Study by UV spectroscopy[¶] revealed that the addition of pillar[5]arene **5** to BBY caused hypochromic shift at 460 nm though no shift of absorption maximum was observed. It is of note that the rise in baseline observed in the UV spectra during the interaction of pillar[5]arenes **5** with BBY can be due to the aggregation of BBY with pillar[4]arene **5**.

Study by ^{1}H NMR spectroscopy[¶] showed that the addition of BBY (1×10^{-4} M in D$_2$O) to the solution of pillar[5]arene **5**

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resulting solution was added to a mixture of the amino acid ethyl ester hydrochloride (7.56 mmol) and 5 ml (0.036 mol) of triethylamine in dichloromethane (20 ml) over a 20 min period. The mixture was stirred under argon at room temperature for 48 h, then washed with 2 M HCl (2×30 ml) and water (2×30 ml). The organic layer was separated and concentrated *in vacuo*. The residue was crystallized from the ethanol–acetone mixture. The precipitate obtained was dried under reduced pressure over phosphorus pentoxide.

4,8,14,18,23,26,28,31,32,35-Decakis(5-ethoxy-2,5-dioxo-3-azapentyloxy)pillar[5]arene **2**. Yield 0.40 g (78%), mp 76 °C. ^{1}H NMR (DMSO-d_6) δ: 1.12 (t, 30H, OCH$_2$*Me*, $^3J_{\rm HH}$ 6.9 Hz), 3.86 (br. s, 10H, CH$_2$), 3.96 (d, 20H, NHC*H*$_2$, $^3J_{\rm HH}$ 4.6 Hz), 4.04 (q, 20H, C*H*$_2$Me, $^3J_{\rm HH}$ 6.9 Hz), 4.38 [br. s, 20H, OCH$_2$C(O)], 6.91 (s, 10H, H$_{\rm Ar}$), 8.25 (br. s, 10H, N*H*CH$_2$). ^{13}C NMR (DMSO-d_6) δ: 13.96, 28.73, 40.57, 60.55, 67.60, 114.92, 128.18, 148.90, 168.57, 169.69. ^{1}H–^{1}H NOESY (the most important cross-peaks): H^1/H^3, H^1/H^4, H^2/H^1, H^3/H^4, H^7/H^6, H^7/H^3. IR (ν/cm^{-1}): 3345 (NH), 3069 (NH), 2982 (CH$_2$, CH$_2$Me), 1740 (C=O), 1668 [NHC(O)], 1190 (ArOCH$_2$). MS (MALDI-TOF), *m/z*: 2064.3 [M + Na]$^+$, 2080.2 [M + K]$^+$ (calc. for [M]$^+$, *m/z*: 2041.7). Found (%): C, 55.63; H, 5.44; N, 6.36. Calc. for $C_{95}H_{120}N_{10}O_{40}$ (%): C, 55.88; H, 5.62; N, 6.46.

4,8,14,18,23,26,28,31,32,35-Decakis(8-ethoxy-2,5,8-trioxo-3,6-diazaoctyloxy)pillar[5]arene **3**. Yield 0.46 g (70%), mp 87 °C. ^{1}H NMR (DMSO-d_6) δ: 1.18 (t, 30H, OCH$_2$*Me*, $^3J_{\rm HH}$ 7.1 Hz), 3.80–4.00 (m, 50H, CH$_2$, NHC*H*$_2$), 4.09 (q, 20H, C*H*$_2$Me, $^3J_{\rm HH}$ 7.1 Hz), 4.41 [br. s, 20H, OCH$_2$C(O)], 6.96 (s, 10H, H$_{\rm Ar}$), 8.17 (t, 10H, N*H*CH$_2$, $^3J_{\rm HH}$ 5.3 Hz), 8.37 (t, 10H, N*H*CH$_2$, $^3J_{\rm HH}$ 5.4 Hz). ^{13}C NMR (DMSO-d_6) δ: 14.06, 28.73, 40.70, 41.65, 60.48, 67.43, 114.89, 128.02, 148.81, 168.31, 169.42, 169.79. ^{1}H–^{1}H NOESY (the most important cross-peaks): H^1/H^4, H^1/H^6, H^1/H^5, H^2/H^3, H^4/H^5, H^6/H^7. IR (ν/cm^{-1}): 3300 (NH), 3074 (NH), 2983 (CH$_2$, CH$_2$Me), 1737 (C=O), 1654 [NHC(O)], 1196 (ArOCH$_2$). MS (MALDI-TOF): 2612.8 [M + H]$^+$, 2634.9 [M + Na]$^+$ (calc. for [M]$^+$, *m/z*: 2611.9). Found (%): C, 52.42; H, 5.72; N, 10.52. Calc. for $C_{115}H_{150}N_{20}O_{50}$ (%): C, 52.87; H, 5.79; N, 10.72.

[‡] *General procedure for the synthesis of compounds* **4** *and* **5**. Pillar[5]arene **3** or **4** (0.1146 mmol) was placed into a round-bottom flask and 6 ml of THF, 4 ml of water and LiOH (0.22 g, 9.10 mmol) were added. The mixture was stirred under argon at room temperature for 25 min. THF was then evaporated *in vacuo* and 20 ml of 2 M HCl were added. The precipitate formed was centrifuged, washed with water, crystallized from the ethanol–acetone and dried under reduced pressure over phosphorus pentoxide.

4,8,14,18,23,26,28,31,32,35-Decakis(5-hydroxy-2,5-dioxo-3-azapentyloxy)pillar[5]arene **4**. Yield 0.18 g (90%), mp 135 °C. ^{1}H NMR (DMSO-d_6) δ: 3.75–4.00 (m, 30H, CH$_2$, NHC*H*$_2$), 4.40 [br. s, 20H, OCH$_2$C(O)], 6.92 (s, 10H, H$_{\rm Ar}$), 8.18 (br. s, 10H, N*H*CH$_2$), 12.69 (br. s, 10H, OH). ^{13}C NMR (DMSO-d$_6$) δ: 28.69, 40.61, 67.50, 114.96, 128.06, 148.98, 168.47, 171.24. IR (ν/cm^{-1}): 3378 (NH), 2927 (CH$_2$, CH$_2$Me), 1726 (C=O), 1653 [NHC(O)], 1196 (ArOCH$_2$). MS (MALDI-TOF), *m/z*: 1767.3 [M + Li]$^+$ (calc. for [M]$^+$, *m/z*: 1760.4). Found (%): C, 51.03; H, 4.29; N, 7.82. Calc. for $C_{75}H_{80}N_{10}O_{40}$ (%): C, 51.14; H, 4.58; N, 7.95.

4,8,14,18,23,26,28,31,32,35-Decakis(8-hydroxy-2,5,8-trioxo-3,6-diazaoctyloxy)pillar[5]arene **5**. Yield 0.20 g (77%) mp 128 °C. ^{1}H NMR (DMSO-d_6) δ: 3.70–4.00 (m, 60H, CH$_2$, NHC*H*$_2$), 4.41 [br. s, 20H, OCH$_2$C(O)], 6.96 (s, 10H, H$_{\rm Ar}$), 8.17 (t, 10H, N*H*CH$_2$, $^3J_{\rm HH}$ 4.6 Hz), 8.28 (t, 10H, N*H*CH$_2$, $^3J_{\rm HH}$ 5.1 Hz), 12.61 (br. s, 10H, OH). ^{13}C NMR (DMSO-d_6): 28.70, 40.65, 41.63, 67.42, 114.86, 128.01, 148.79, 168.26, 169.20, 171.18. ^{1}H-^{1}H NOESY (the most important cross-peaks): H^1/H^4, H^1/H^2, H^1/H^3, H^3/H^2, H^3/H^4. IR (ν/cm^{-1}): 3299 (NH), 2933 (CH$_2$, CH$_2$Me), 1731 (C=O), 1647 [NHC(O)], 1197 (ArOCH$_2$). MS (MALDI-TOF), *m/z*: 2332.0 [M + H]$^+$, 2339.0 [M + Li]$^+$ (calc. for [M]$^+$, *m/z*: 2331.6). Found (%): C, 48.72; H, 4.53; N, 11.95. Calc. for $C_{95}H_{110}N_{20}O_{50}$ (%): C, 48.93; H, 4.75; N, 12.01.

[§] *Determination of shape and size of the particles by TEM.* Imaging was carried out with a Carl Zeiss Merlin scanning electron microscope. Images were processed with the STEM detector on the 300 mesh copper grid coated with Formvar. Probe preparation was performed using negative staining protocol with 2% uranyl acetate solution. The concentrations of BBY and pillar[5]arene **5** were equal to 10^{-5} M. Images of the mixtures were recorded in 1 h after mixing the solutions at 20 °C.

Determination of the hydrodynamic size of the particles by DLS. The particle size was determined by a Zetasizer Nano ZS instrument at 20 °C containing a 4 mW He–Ne laser operating at a wavelength of 633 nm and incorporating noninvasive backscatter optics (NIBS). The measurements were performed at a detection angle of 173°, and the measurement position within the quartz cuvette was automatically determined by the software. The results were processed with the DTS (Dispersion Technology Software 4.20) software package. Deionized water (obtained using a Millipore-Q purification system) with resistivity > 18.0 MΩ cm was used for the preparation of solutions. During the experiments, the concentration ratio [pillar[5]arene]/[BBY] was 1:1, the concentration of compounds was equal to 1×10^{-5}, 1×10^{-4} and 1×10^{-3} M. The particle size was determined in 1 h after the sample preparation. To determine the kinetic stability of the systems, the measurements were also carried out under similar conditions after 3 and 5 h (see Online Supplementary Materials).

[¶] *The study of the interaction of pillar[5]arenes* **1**, **4**, **5** *with BBY by UV spectroscopy.* UV-VIS spectra were recorded using a Shimadzu UV-3600 spectrometer; the cell thickness was 1 cm, slit width 1 nm. Deionized water (obtained using a Millipore-Q purification system) with resistivity > 18.0 MΩ cm was used for the preparation of solutions. The concentration ratio [pillar[5]arene]/[BBY] was 1:1, the concentration of compounds was 1×10^{-5}, 1×10^{-4} and 1×10^{-3} M. The recording of the absorption spectra of the mixtures of BBY with pillar[5]arenes was carried out in 1 h after mixing the solutions at 20 °C (see Online Supplementary Materials).

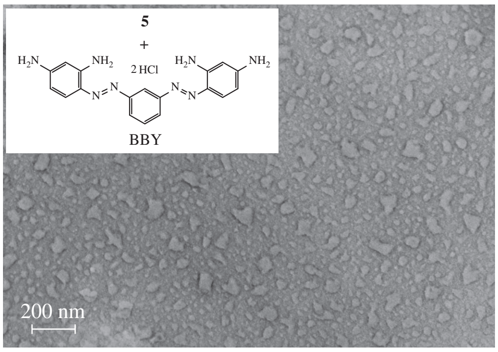

Figure 1 TEM images of aggregates of pillar[5]arene **5** at 1×10^{-5} M and BBY at 1×10^{-5} M concentration.

(1×10^{-4} M in D_2O) caused the following changes in chemical shifts: macrocycle protons shifted upfield by 0.1–0.2 ppm and the BBY protons shifted downfield by 0.1–0.4 ppm, which confirms the interaction between the pillar[5]arene and BBY. Changes less than 1 ppm in the chemical shifts of the dye protons indicate that the BBY molecule does not enter into the hydrophobic cavity of the macrocycle but rather interacts with the functional groups of the pillar[5]arene substituents.

The obtained aggregates based on macrocycle **5** and BBY were also investigated by transmission electron microscopy (TEM).[§] Figure 1 shows TEM image of the nanoparticles formed by aggregates of pillar[5]arene **5** and BBY. According to the TEM data, the average diameter of aggregates was 59 nm. As a rule of thumb, the differences in the size of the aggregates as determined by DLS and TEM can be explained by dynamic nature of aggregation, the influence of solvent and the absence of hydration shells in TEM images.

In conclusion, new water-soluble decasubstituted pillar[5]-arenes containing glycine and glycylglycine fragments were synthesized and characterized. It was shown by DLS that the pillar[5]arene containing glycylglycine fragments is able to form aggregates of about 195–617 nm. The interaction of the macrocycles with BBY was studied by UV spectroscopy, DLS and TEM. It was demonstrated that the interaction of self-aggregates based on decasubstituted pillar[5]arenes containing glycylglycine fragments with BBY resulted in formation of the particles of about 59 nm in size. These results offer new opportunities for the design of supramolecular systems for controlled release and targeted drug delivery.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.11.011.

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[§] *The study of the interaction of pillar[5]arene* **5** *with BBY by NMR spectroscopy*. The ^1H NMR spectra of pillar[5]arene **5** (1×10^{-4} M in D_2O), BBY (1×10^{-4} M in D_2O) and complexes of compound **5** (1×10^{-4} M in D_2O) with BBY (1×10^{-4} M in D_2O) were recorded on a Bruker Avance 400 spectrometer (400 MHz) using D_2O as internal standard (see Online Supplementary Materials).