Tetracarboxylic acids on a thiacalixarene scaffold: synthesis and binding of dopamine hydrochloride†


For the first time thiacalix[4]arene derivatives in 1,3-alternate conformation simultaneously containing amide, carboxyl and hydroxyl groups capable of forming 1:1 stoichiometry complexes with dopamine hydrochloride were obtained. The efficiency of dopamine hydrochloride binding was evaluated by a number of spectral methods. Using the methods of fluorescent, UV-Vis and NMR spectroscopy, the mechanism of interaction of the synthesized macrocycles with dopamine has been studied. It was shown that quenching of dopamine fluorescence by the studied macrocycles is carried out through a static mechanism.

Results and discussion


The reaction of cyclic anhydrides (succinic, maleic, citraconic, diglycolic) and L-lactide with tetraamine 1 in 1,3-alternate...
conformation was studied in order to obtain tetrasubstituted thiacalix[4]arene derivatives containing terminal carboxyl and hydroxyl groups. Polar aprotic solvents are generally used for the acylation reaction. Tetraamine 1, selected cyclic anhydrides and l-lactide are readily soluble in two such solvents, pyridine and tetrahydrofuran. However, synthesis in pyridine, according to $^1$H NMR spectroscopy, results in formation of hardly separable mixtures of partially substituted products. Therefore, tetrahydrofuran was chosen for this work. The reaction with all anhydrides studied proceeds for 15 hours at the solvent boiling point, with the exception of succinic anhydride, which reacts more slowly (25 hours) (Scheme 1). Apparently, this is due to the low solubility of succinic anhydride in THF. The reaction of tetraamine 1 with l-lactide also lasts for 25 hours. This can be explained by the lower reactivity of esters compared to carboxylic anhydrides in nucleophilic substitution reactions. As expected,33,34 when the lactide fragment is introduced to the macrocyclic platform, its conformation is kept, which is expressed in the saving of the negative Cotton effect characteristic for l-lactide in the CD spectrum of compound 6 (ESI,† Fig. S29).

Compounds 2–6 were synthesized with high yields of 81–90%. The structures of the obtained p-tert-butylthiacalix[4]arene derivatives were characterized by (1D) $^1$H NMR, $^{13}$C NMR, 2D $^1$H–$^1$H TOCSY, $^1$H–$^{13}$C HSQC, $^1$H–$^{13}$C HMBC spectra, IR spectroscopy, and mass spectrometry, and the composition was confirmed by elemental analysis data.

If the structures of compounds 2 and 4–6 are uniquely determined, for compound 3 the formation of two structures is possible due to the asymmetry of the citraconic anhydride molecule. When ring opening occurs, it is possible to form a mixture of two products with a methyl group in the geminal (product A) or vicinal (product B) positions relative to the amide group (Scheme 2).

It was shown previously35 that the interaction of amines with citraconic anhydride under mild conditions produces a mixture of isomers with the predominance of structure A. However, in some cases, there is further isomerization of product A into the more thermodynamically stable product B.35

The reaction with reflux led to the isolation of the single product with the assumed structure B. The position of the characteristic signal of the proton near the double bond in citraconamic acid can unambiguously assert which carbonyl group is attacked by the nucleophilic nitrogen atom of the amino group.35 According to ref. 35 in the $^1$H NMR spectrum (DMSO-$d_6$) the proton signal is geminal relative to the carboxyl group and appears in the range of 5.7–5.9 ppm, while the signal is geminal relative to the amide group in the 6.0–6.2 ppm region. In the $^1$H NMR spectrum of compound 3 in DMSO-$d_6$, there is a 6.18 ppm signal corresponding to the proton near the double bond. To confirm the structure, homo- and hetero-correlation NMR spectra were recorded: $^1$H–$^1$H TOCSY, $^1$H–$^{13}$C HSQC, $^1$H–$^{13}$C HMBC (Fig. 1 and ESI,† Fig. S16–S18).

A TOCSY experiment contains cross-peaks due to protons that can be combined in two spin system. The first spin system is large and contains the resonance signals of the four protons H7–H10. The second spin system is small and combines the signals of the two spectral lines: methyl H14 (δ 1.95 ppm) and methine protons H12 (δ 6.20 ppm) (ESI,† Table S1). Consideration of the correlation signals from the protons H14 and H12 in the HMBC spectrum help to assign carbon atom C13, as well as C11 and C15. In this case, methine carbon atom C13 is assigned unambiguously, as it falls into the characteristic region of 105–145 ppm. The signals of carbon atoms C11 and C15 are close.

![Scheme 1](image1.png)

**Scheme 1** *Reagents and conditions:* (i) maleic anhydride, THF, reflux, 15 hours; (ii) citraconic anhydride, THF, reflux, 15 hours; (iii) succinic anhydride, THF, reflux, 25 hours; (iv) anhydride of diglycolic acid, THF, reflux, 15 hours; (v) (3S)-cis-3,6-dimethyl-1,4-dioxane-2,5-dione, THF, reflux, 25 hours.

![Scheme 2](image2.png)

**Scheme 2** Possible structures of products of interaction of citraconic anhydride with amines.

![Fig. 1](image3.png)

Fig. 1 Overlay of $^1$H–$^{13}$C HSQC (red) and $^1$H–$^{13}$C HMBC (blue) spectra of compound 3 (600 MHz, DMSO-$d_6$, 303 K). The red peaks of the $^1$H–$^{13}$C HSQC spectrum are marked with red text.
However, the signal of the carbon atom of the amide group C11 (δ 164.91 ppm) can be assigned based on the cross-peak from the proton H9 (δ 3.07 ppm). Therefore, the remaining carbon signal is the carboxyl group C15 (δ 167.72 ppm). The correct assignment of C15 is confirmed by the higher intensity of the correlation peak from the H14 protons per carbon atom C15, rather than the carbon atom C11, because of the proximity of the methyl group to the carboxyl fragment.

The proximity of the methine carbon atom C12 to the amide group was verified by the correlation peak from H9 protons (δ 3.07 ppm), which belongs to the first spin group in the TOCSY spectrum. Thus, it can be affirmed that the interaction of citraconic anhydride with tetraamine 1 under reflux in THF produces only one product with a methyl group in the vicinal position relative to the amide fragment (Scheme 1).

Thus, synthesis techniques for new polyfunctional p-tert-butylthiacalix[4]arene derivatives 2-6 in 1,3-alternate conformation simultaneously containing amide, ester, alcohol and carboxyl groups with high yields were developed.

**Binding of dopamine**

One of the most widely used methods for studying dopamine binding is fluorescence spectroscopy. 36–40 It is known that dopamine has significant fluorescence upon excitation at 285 nm. The experiment was carried out in methanol, because in other solvents the compounds proved to be insoluble. Unfortunately, it was not possible to study the interaction of dopamine with compounds 2 and 3 because of their extremely low solubility practically in all organic solvents and water.

It turned out that when all three studied thiacalix[4]arenes 4-6 were added to the solution of dopamine, quenching of its fluorescence with a maximum at 315 nm was observed (ESI,† Fig. S30-S32). However, it remained unclear how the quenching of luminescence was caused: by the formation of the complex or by impingement with the macrocycles molecules. To confirm the complexation, we recorded UV/visible spectra. Dopamine has three absorption maxima with λ<sub>max</sub> at 203, 225, and 285 nm, corresponding to π-π* transitions of the aromatic ring, somewhat shifted to the red region of the spectrum compared to the parent benzene due to substituents effect. 41,42 It turned out that when the dopamine interacts with all thiacalixarenes, the hypochromic effect is observed only at 203 nm, which indicates the complex formation and, accordingly, the static character of fluorescence quenching (Fig. 2 and ESI,† Fig. S33).

An attempt to confirm or confute this type of quenching mechanism was also undertaken by using the fluorescence method. It is known that in a single type of quenching (dynamic or static) in the Stern-Volmer coordinates, the quenching value is linearly related to the quencher concentration, and for a combination of types, linearity is disturbed. 43 The recording of emission spectra at different temperatures (283 and 303 K) showed the linear dependence in the whole range of concentrations studied. However, it turned out unexpectedly that the slope of the curves at different temperatures is constant (ESI,† Fig. S34). The absence of the growth in the quenching constant with temperature increasing unambiguously testifies against the dynamic mechanism of quenching.

The binding constants were determined from analysis of binding isotherms (obtained by fluorescence spectroscopy in the absence of dynamic quenching) and fitted to a 1 : 1 binding model. 44–46 The Bindfit application, which was developed for supramolecular systems, 47 was used to process the results. To confirm the proposed stoichiometry, the titration data were also processed by the binding model at the ratio “host : guest” = 1 : 2. However, in this case the constants are determined with much greater uncertainty (ESI,† Fig. S30–S32).

The determination of the association constants showed that all compounds bind well enough to dopamine hydrochloride and still have similar values of association constants. Compound 6 most strongly binds dopamine-HCl (K<sub>a</sub> = 1.91 x 10<sup>4</sup> M<sup>-1</sup>), while for others the bonding is weaker: (K<sub>a</sub> = 1.50 x 10<sup>4</sup> M<sup>-1</sup> for 5 and 0.95 x 10<sup>4</sup> M<sup>-1</sup> for 4).

It is known that amide fragments are often used as binding sites for anionic substrates. 48–50 The presence of amide moieties in the structures of compounds 4–6 may lead to the fact that binding of the anion is carried out (Cl<sup>-</sup>). However, the study of the UV spectra of the obtained thiacalixarenes in the presence of tetrabutylammonium chloride indicates the absence of this kind of interaction, as evidenced by the absence of both hypo- and hyper-chromic effects in the electronic spectra of these systems (ESI,† Fig. S35). Thus, catecholamine binding, rather than its anionic fragment, is observed, which makes it possible to extend the proposed mechanism of interaction not only to the salt, but also to the free amine.

To further confirm the complexation and establish the structure of the complex, we also employed the 1H NMR spectroscopy method (ESI,† Fig. S36). In the spectrum of free dopamine hydrochloride, signals of aromatic protons are observed as a multiplet (6.56–6.74 ppm), as well as two triplets of methylene protons (3.41 and 2.98 ppm). When thiacalixarene 5 is added, a significant upfield shift of the proton signals of the methylene groups of amine into the region of 3.11 and 2.79 ppm is observed, and a small shift of the singlet in the weak fields (6.69 ppm) indicates dopamine binding with a macrocycle.

The formation of the complex between macrocycle 6 and dopamine hydrochloride and its spatial structure were also...
confirmed by 2D $^1$H–$^1$H NOESY NMR spectroscopy (Fig. 3). The presence of cross-peaks between the protons of the pyro-
catechol and ethylene fragments of dopamine, as well as
aromatic protons and methylene protons bound with amide
groups of 6 indicates the formation of an endohedral complex
between the compounds studied.

The two-dimensional DOSY spectroscopy method also testifies in
favor of complex formation of 6 with dopamine-HCl. The diffusion coefficients of 6, dopamine-HCl and mixture of dopamine-HCl–6 at
298 K (1 mM) were determined (Table 1). The DOSY spectrum for
the mixture of catecholamine with 6 in 1:1 ratio (1 mM) indicates the presence of only one type of particles with a diffusion coefficient
lower than for the individual compounds. The greatest decrease in
the diffusion rate was shown by dopamine-HCl in the presence of
6 (Table 1). The electronic absorption spectra were recorded using
a Shimadzu UV-3600 spectrometer in methanol in quartz
cuvettes with the thickness of the transmissive layer at 10 mm.

Conclusions

Thus, new $p$-tert-butylthiacalix[4]arene derivatives 2–6 in 1,3-
alternate conformation simultaneously containing amide,
carboxyl and hydroxyl groups were synthesized. The ability of
thiacalix[4]arenes 4–6 to bind dopamine hydrochloride was established in methanol by spectral methods (UV, fluorescence,
NMR spectroscopy). By fluorescence spectroscopy, it was shown that quenching of dopamine fluorescence by the studied
macrocycles is carried out through a static mechanism. All
the complexes formed have 1:1 stoichiometry, while for all
studied compounds the association constants were close (in the
order of $(1-1.9) \times 10^{-1}$ M$^{-1}$). Unfortunately, at this stage, the
possibility of using the synthesized compounds to determine
dopamine is not revealed, but the obtained results can be useful
for the development of new synthetic dopamine receptors.

Experimental

General

The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance
400 spectrometer (400.17 MHz for H-atoms) in DMSO-$d_6$, CDCl$_3$, and methanol-$d_4$. The residual solvent peaks
were used as an internal standard. Homo- and hetero-
correlation NMR spectra were recorded at 30 °C on a Bruker
Avance III 600 MHz NMR spectrometer equipped with a 5 mm
TXI Probe. Assignments were done using 2D $^1$H–$^1$H TOCSY,
$^1$H–$^{13}$C HSQC and $^1$H–$^{13}$C HMBC experiments. Repetition delay
d$_1$ was set to 2 s for all 2D correlation spectra. The mixing time
for $^1$H–$^1$H TOSCY experiment was 80 ms. All NMR data were
processed and analyzed using Topspin 3.5 software.

The IR spectra were recorded using a Spectrum 400
(PerkinElmer) IR spectrometer.

Elemental analysis was performed on a PerkinElmer 2400
Series II instrument.

Electrospray ionization mass spectra (ESI) were obtained
on an AmazonX mass spectrometer (Bruker Daltonik GmbH,
Bremen, Germany). The measurements were carried out in the
positive ions registration regime in the $m/z$ range from 100 to
2800. The voltage on the capillary was $-4500$ V. Nitrogen was
used as the drying gas with a temperature of 300 °C and a
flow rate of 10 L min$^{-1}$. The compounds were dissolved in
acetonitrile to a concentration of $10^{-6}$ g L$^{-1}$. Data were pro-
cessed using DataAnalysis 4.0 (Bruker Daltonik GmbH, Bremen,
Germany).

The MALDI mass spectra were recorded on an Ultraflex III
mass spectrometer. $p$-Nitroaniline was used as the matrix.

Melting points were determined using Boetius Block apparatus.

The electronic absorption spectra were recorded using
a Shimadzu UV-3600 spectrometer in methanol in quartz
cuves with the thickness of the transmissive layer at 10 mm.
The mixtures of thiacalixarenes ($10 \mu$L) and dopamine-HCl
($100 \mu$L) in methanol were incubated for 5 minutes. The experiment was carried out at 293 K.

The CD spectra were recorded using a Jasco-1500 spectrophotometer in quartz cuvettes with the thickness of the trans-
misse layer at 1 mm. The spectra were measured with a scan
rate of 15 nm min$^{-1}$, spectral range of 210–260 nm, slit width of
1 nm, sampling step of 1 nm and 5 scans co-addition.

Fluorescence spectra were recorded on a Fluorolog 3
luminescent spectrometer (Horiba Jobin Yvon). The excitation
wavelength was selected as 285 nm. The emission scan range
was 300–540 nm. Excitation and emission slits were 3 nm. Quartz cuvettes with optical path length of 10 mm were used. Emission spectra were automatically corrected by the Fluorescence program. The fluorescence spectra were recorded in methanol solutions with a dopamine concentration of 10 μM. The concentrations of thiacalix[4]arenes ranged from 0 to 90 μM. The experiment was carried out at 293 K. The temperature dependences of fluorescence for all compounds were determined at 283 and 303 K on a LS-55 Fluorescence Spectrometer (PerkinElmer) using a Peltier RT/1 cuvette thermostatic holder.

1H diffusion ordered spectroscopy (DOSY). The spectra were recorded on a Bruker Avance 400 spectrometer, at 9.4 Tesla, at the resonating frequency of 400.17 MHz for 1H, using a BBO Bruker 5 mm gradient probe. The temperature was regulated at 298 K and no spinning was applied to the NMR tube. DOSY experiments were performed using the STE bipolar gradient pulse pair (stepgpgps) pulse sequence. 16 scans of 16 data points were collected. The maximum gradient strength produced in the z direction was 5.35 G mm⁻¹. The duration of the magnetic field pulse gradients (δ) was optimized for each diffusion time (Δ) in order to obtain a 2% residual signal with the maximum gradient strength. The values of δ and Δ were 1.800 μs and 100 ms, respectively. The pulse gradients were incremented from 2 to 95% of the maximum gradient strength in a linear ramp.⁵¹,⁵²

### General procedure for the synthesis of compounds 2–4

A mixture of 5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrasubstituted(3-aminopropano)Substituted-8,14,20-tetra[1]calix[4]arene (1)¬⁴ (0.50 g, 0.33 mmol) and the corresponding anhydride (4.20 mmol) in 7 ml of dry tetrahydrofuran was refluxed for 15 hours (25 hours in the case of compound 4). After cooling the reaction mixture, the solvent was removed under reduced pressure. 20 ml of chloroform was added to the residue and then stirred for 1.5 hours. It was then heated to the boiling point of the solvent and the insoluble precipitate was filtered hot. The resulting precipitate was washed with 30 ml of hot methanol and dried in a vacuum desiccator over P₂O₅.


1H NMR (DMSO-d₆, δ, ppm, J/Hz): 1.19 (s, 36H, (CH₃)₂C), 1.39 (m, 8H, –CH₃–CH₂–CH₃), 3.12 (dt, 8H, –CH₂–NH–, 3JHH = 5.6 Hz, 3JHN = 6.6 Hz), 3.86 (t, 8H, O–CH₂–, 3JHH = 7.4 Hz), 6.27 (d of AB system, 4H, –CH–CH₃, 3JHH = 12.8 Hz, 6.43 (d of AB system, 4H, –CH–CH₃, 3JHH = 12.8 Hz), 7.37 (s, 8H, Ar-H), 9.11 (br.t, 4H, NH), 15.13 (br.s, 4H, –OH).

13C NMR (DMSO-d₆, δ, ppm): 28.36, 30.86, 33.85, 36.35, 67.48, 127.64, 128.62, 131.64, 133.26, 145.57, 156.87, 165.30, 165.50.


IR (υ/cm⁻¹): 3272 (NH); 3092 (–CH₃); 2962, 1008 (OH); 2909, 2870, 1708, 1443, 1241 (COOH); 1632, 1570, 1264 (CO–NH). MS (MALDI-TOF): calculated [M⁺] m/z = 1341.7, found [M + H⁺] m/z = 1342.5, [M + Na⁺] m/z = 1363.5, [M + K⁺] m/z = 1380.5. El. anal. calc'd for C₆₈H₃₇N₃O₇S₄ (％): C, 60.88; H, 6.31; N, 4.18; S, 9.56. Found (％): C, 60.46; H, 5.95; N, 3.95; S, 9.23.

### 5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrasubstituted(3-3-carboxyethylamino)Substituted-8,14,20-tetra[1]calix[4]arene (1,3-alternate-4). Yield: 0.64 g (89%). M.p.: 243 °C.

1H NMR (DMSO-d₆, δ, ppm, J/Hz): 1.21 (s, 36H, (CH₃)₂C), 1.28 (tt, 8H, –CH₂–CH₂–CH₂–, 3JHH = 7.0 Hz, 3JHN = 7.2 Hz), 2.31 (AB part of AA’BB’ system, 8H, –CH₂–CH₂–C(O), 3JHABH = 6.8 Hz, 3JHAB = 13.6 Hz), 2.41 (A’B’ part of AA’BB’ system, 8H, –CH₂–CH₂–C(O), 3JHABH = 6.8 Hz, 3JHABx = 13.6 Hz), 2.95 (dt, 8H, –CH₂–CH₂–NH–, 3JHH = 6.0 Hz, 3JHN = 6.2 Hz), 3.79 (t, 8H, O–CH₂–, 3JHH = 7.2 Hz), 7.35 (s, 8H, Ar-H), 7.77 (t, 4H, NH, 3JHN = 5.2 Hz), 12.03 (br.s, 4H, –OH).

13C NMR (DEPT) (DMSO-d₆, δ, ppm): 25.05, 29.28, 30.07, 30.91, 33.89, 35.86, 67.80, 127.68, 128.54, 145.43, 156.88, 170.89, 173.82. 1H-1H NOESY NMR spectrum (most important cross-peaks are presented): H₄⁵/H₄⁶, H₄⁷/H₄⁸, H₅⁵/H₅⁶, H₅⁷/H₅₈, H₉⁴/H₁₀⁴, H₉⁵/H₁₀⁵, H₉⁶/H₁₀₆, H₉⁷/H₁₀₇, H₉⁸/H₁₀₈, H₁₀⁴/H₁₀⁵, H₁₀⁵/H₁₀⁶, H₁₀⁶/H₁₀₇, H₁₀⁷/H₁₀₈, H₁₀⁸/H₁₀₉.

IR (υ/cm⁻¹): 3350, 3206 (NH); 2960, 954 (OH); 2903, 2867, 1718, 1708, 1407, 1245, 1239 (COOH); 1652, 1537, 1267 (CO–OH). MS (MALDI-TOF): calculated [M⁺] m/z = 1349.7, found [M + H⁺] m/z = 1351.0, [M + Na⁺] m/z = 1372.6, [M + K⁺] m/z = 1388.6. El. anal. calc'd for C₆₀H₃₈N₃O₇S₄ (％): C, 60.51; H, 6.67; N, 4.15; S, 9.50. Found (％): C, 59.97; H, 6.65; N, 3.86; S, 8.99.

### 5,11,17,23-Tetra-tert-butyl-25,26,27,28-tetrasubstituted(3-3-carboxymethoxy)Substituted-8,14,20-tetra[1]calix[4]arene (1,3-alternate-5). A mixture of 5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrasubstituted(3-3-aminopropano)Substituted-8,14,20-tetra[1]calix[4]arene (1)¬⁴ (0.50 g, 0.33 mmol) and anhydride of diglyceic acid (0.49 g, 4.20 mmol) in 7 ml of dry tetrahydrofuran was refluxed for 15 hours. After cooling the reaction mixture, the solvent was removed under reduced pressure. 20 ml of methylene chloride was added to the residue and then stirred for 30 minutes. It was then heated to the boiling point of the solvent and the insoluble precipitate was filtered hot. 20 ml of distilled water was added to the resulting precipitate and stirred for 1 hour. Then the insoluble precipitate was filtered off in a warm form and dried in a vacuum desiccator over P₂O₅.
Yield: 0.62 g (83%). M.p.: 241 °C. 1H NMR (DMSO-d6, δ, ppm, J/Hz): 1.21 (s, 36H, (CH3)2C), 1.25 (tt, 8H, −CH2−CH2−CH2−, JHH = 6.4 Hz, JHH = 7.2 Hz), 3.00 (dt, 8H, −CH2−NH−, JHH = 6.0 Hz, JHH = 6.0 Hz), 3.80 (t, 8H, O−CH2−, JHH = 7.2 Hz), 3.95 (s, 8H, (−CH2−C(O))2), 4.12 (s, 8H, (−CH2−C(O))), 7.35 (s, 8H, Ar−H), 7.60 (brt, 4H, NH), 12.81 (brs, 4H, OH). 13C NMR (DMSO-d6, δ, ppm): 29.00, 30.87, 33.90, 35.20, 67.17, 67.78, 70.01, 127.60, 128.12, 132.30, 140.00, 146.97, 153.97, 166.56, 168.62, 171.38.

Notes and references


47 Bindfit v0.5 (Open Data Fit, 2016); http://supramolecular.org/bindfit/.


