

Review

N-confused calix[4]pyrroles

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Abstract

This is a first review devoted to N-confused calix[4]pyrroles (NCCPs). NCCPs are a relatively recent arrival to the family of the pyrrole-based anion binding macrocycles, being for the first time identified in 1999. Yet, in a relatively short time these calix[4]pyrrole (CP) isomers attracted attention of the community of research groups interested in anion binding and sensing. This is because they are relatively easy to synthesize, but mainly because they possess anion-binding properties that are different from that of regular calix[4]pyrroles. The difference in anion-binding properties stems from a different binding mode between the NCCP and anion. While the regular CPs adopt in the complex an ideal cone-like conformation where all four pyrroles-NHs engage in hydrogen bonding to the anion, the inverted pyrroles do not allow forming the cone. NCCPs bind anions via a confused cone (CC), by three NH hydrogen bonds with an anion and a CH–anion contact. This different binding mode results also in different anion-binding affinity and selectivity compared to regular CPs. Also, the inverted pyrroles offer a unique possibility for selective chemical modification of the receptor. The corresponding colorimetric sensors were tested for anion binding applications. The results of colorimetric assays for anions are presented and potential applications discussed.

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1. Introduction

Anions play an important role in numerous biological processes and pathological events and they are used as fertilizers

and industrial raw materials, all of which necessitates the development of sensitive anion sensors [1]. Here, materials capable of reversible anion-induced changes in color are particularly attractive [2] as they require little or no instrumentation for practical use. Unfortunately, a few colorimetric anion sensors are able to differentiate selectively between anionic substrates in the presence of competing media such as electrolytes or solvating water molecules [3]. Therefore, significant efforts were mounted

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in the last decade to prepare new receptors and sensors for anions.

Pyrrole-based macrocycles are on the forefront of such efforts since the discovery of anion binding by sapphyrin [4], a pentapyrrolic expanded porphyrin [5] initially discovered by Woodward and co-workers [6]. Since then, numerous pyrrole-based macrocycles were found to bind anions. Alas, few of these materials were easy to synthesize to encourage their further development into potentially applicable anion sensors. This situation was changed dramatically with the discovery of anion-binding properties of calix[4]pyrrole [7–9], a tetrapyrrolic macrocycle with a *meso*-octaalkylporphyrinogen structure (Fig. 1) that can be synthesized in one step in large amounts without chromatographic purification of the product.

2. Calix[4]pyrrole and N-confused calix[4]pyrrole

Interestingly, *meso*-octamethylcalix[4]pyrrole (OMCP) or acetopyrrole was first reported by Baeyer [10], and its structure is shown in Fig. 1, where $R = \text{CH}_3$. The same figure also shows the structure of N-confused calix[4]pyrrole. Both macrocycles are position isomers. While in the regular calix[4]pyrrole the *meso*-carbons are always connected to C2 and C5 of the pyrrole moieties, the N-confused isomer comprises at least one pyrrole moiety connected to the rest of the macrocycle via C2, C4-connection.

Calix[4]pyrroles and porphyrins alike are usually synthesized by acid-catalyzed condensation between a carbonyl compound and pyrrole in the so-called Rothmund condensation [11]. In the case the carbonyl compound used is an aldehyde, a porphyrinogen with alkyl (aryl) and a hydrogen on each of the *meso*-positions is formed. *meso*-Tetraalkyl- or tetraarylporphyrinogen is then oxidized to form a conjugated species, a porphyrin. In contradistinction to the porphyrin synthesis, the products of condensation of ketone with pyrrole cannot be oxidized, and the reaction is arrested at the *meso*-octaalkyl porphyrinogen stage (Fig. 2).

In the early 1990s, first reports emerged describing the identification of porphyrin isomers, in which one or more of the four pyrrole moieties are inverted [12–14]. The inverted pyrrole in these N-confused porphyrins is connected to the rest of the macrocycle via one α -carbon (C2-position) and one β -carbon (C4-position) as opposed to α , α' -carbons (C2, C5-positions) in

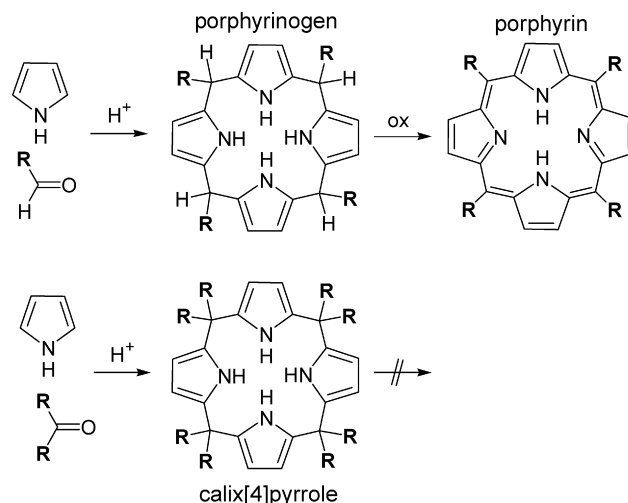


Fig. 2. Synthesis of a porphyrinogen, porphyrin, and calix[4]pyrrole by condensation of pyrrole with a carbonyl compound [11].

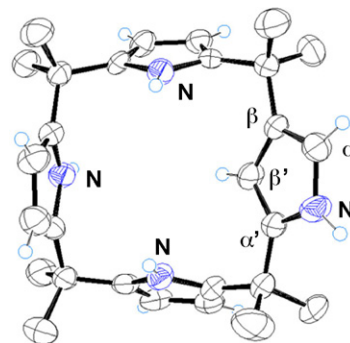


Fig. 3. X-ray structure of N-confused octamethylcalix[4]pyrrole. The hydrogens at *meso*-methyls are omitted for clarity (scaled to 30% probability level) [17].

regular porphyrins. The N-confused porphyrins are formed presumably from a N-confused porphyrinogen by porphyrinogen scrambling.

Similarly to the formation of N-confused porphyrins, the condensation of ketone and pyrrole also produces a certain number of C2, C4-pyrrole connections giving rise to N-confused calix[4]pyrroles. The first N-confused calixpyrrole synthesis was reported in 1975 [15], although the structural assignment may not have been correct, as the NMR spectra were not recorded at the time. Inspired by the earlier report and by further reports on N-confused porphyrins, Dehaen and co-workers mounted the first systematic effort aimed at the preparation of N-confused calix[4]pyrroles [16], which provided the first rational synthesis of this calix[4]pyrrole isomer.

Recently, Anzenbacher and co-workers [17] isolated the simple NC-OMCP and determined its structure by X-ray crystallography (Fig. 3).

3. Synthesis of N-confused calix[4]pyrroles

Although the C2, C5-atoms in pyrrole are significantly more reactive compared to the C3, C4-positions, in most

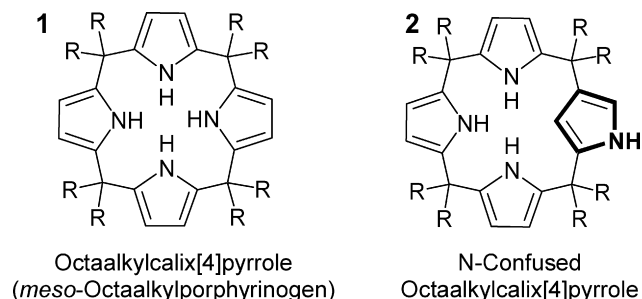


Fig. 1. The structure of octaalkylcalix[4]pyrrole and the corresponding N-confused isomer.

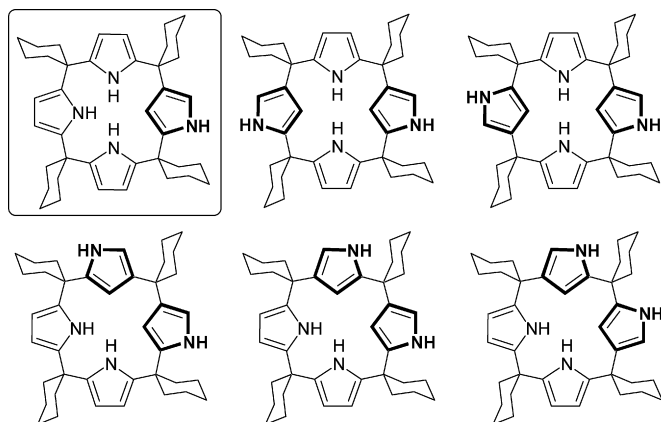


Fig. 4. Typical products of the cyclohexanone–pyrrole condensation: a mixture of regular calix[4]pyrrole (not shown), N-confused and doubly N-confused calix[4]pyrroles.

calix[4]pyrrole syntheses 10–30% of the “faulty connections” are established. Because the electrophilic substitution of pyrrole by the tertiary benzylic carbocation is reversible (largely due to the high stability of the tertiary benzylic cation) and because of high thermodynamic stability of the regular calix[4]pyrrole, the N-confused isomer is usually found in the crude condensation product in a significantly lower amount (6–22%). However, Dehaen and co-workers demonstrated that careful tuning of the reaction conditions (solvent, acid catalyst) can result in up to 30% yield of the N-confused isomer or up to 35% of a mixture of doubly N-confused isomers (Fig. 4) [16]. Interestingly, when the conditions were set up to obtain the maximum amount of the doubly confused calixpyrrole isomers (Fig. 4), the relative amount of the one-time N-confused calix[4]pyrrole was found to be dramatically decreased. Thus, it appears that the doubly confused isomers are formed preferentially from the N-confused isomer. This is, presumably, due to the lower stability of the N-confused calix[4]pyrroles. The increasing amount of the doubly N-confused isomers in the mixture, which one would expect might be even less stable than single-confused calix[4]pyrroles, is likely due to their low solubility resulting in their precipitation from the reaction mixture.

The fact is that NCCP-type materials are formed during every calixpyrrole synthesis. Most of the time such syntheses are carried out in alcohol [7,16], from which the calixpyrroles products precipitate when formed. Collecting the solid precipitate followed by simple recrystallization of the calixpyrrole crude product, usually from a polar solvent such as acetone, yields pure calix[4]pyrroles as the first fractions. Because of lower symmetry and non-zero dipole moment, N-confused calix[4]pyrroles are in general more polar. Hence, the mother liquors from the crystallization carried out from a polar solvent are enriched with N-confused calixpyrroles. Likewise, the original alcohol-filtrate (after separation of the calixpyrrole crude product) comprises a mixture of N-confused and expanded calixpyrroles as well as open-chain oligomers and polymers. It is therefore only a matter of patience and effort to obtain new N-confused calixpyrroles. After all, they are patiently waiting to be discovered.

4. Structure and anion binding

At this time, the only information available regarding the structure and anion-binding properties of N-confused calix[4]pyrroles are derived from studies of N-confused octamethylcalix[4]pyrrole (NC-OMCP). Limited availability of the doubly confused isomers due to low solubility and the complexity of the isomeric mixtures so far effectively precluded binding studies using these materials.

4.1. Binding mode

The regular calix[4]pyrroles form with competent anions such as F^- , Cl^- , $H_2PO_4^-$, CN^- , and AcO^- an ideally symmetrical hydrogen-bonded complex with a cone-like conformation. The high degree of symmetry is easy to observe in the NMR spectra and X-ray structures (Figs. 5 and 6, respectively).

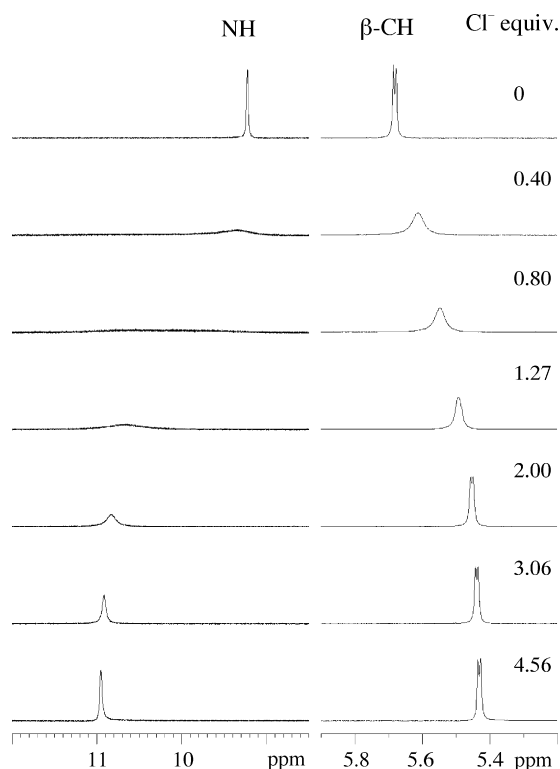


Fig. 5. OMCP titrated with tetrabutylammonium (TBA) chloride in $DMSO-d_6$.

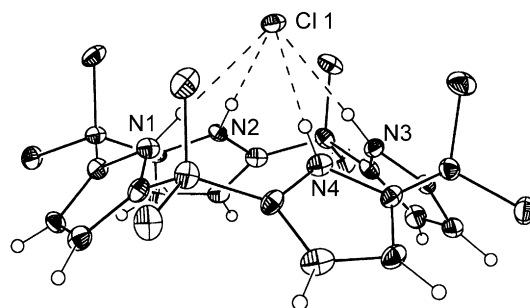


Fig. 6. OMCP–chloride complex displays a typical cone-like conformation with all four pyrroles NHs pointing toward the anion [7].

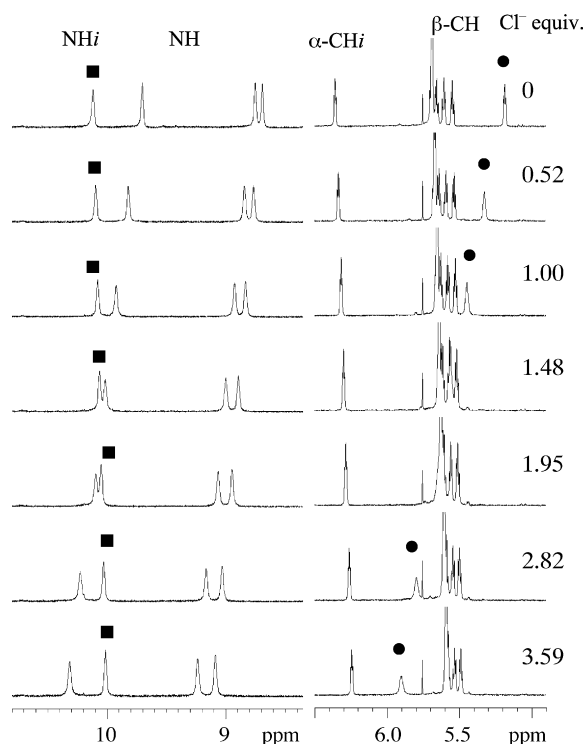


Fig. 7. NC-OMCP titrated with tetrabutylammonium (TBA) chloride in DMSO- d_6 . Labeling: inverted pyrrole β -CHi (●); inverted pyrrole NHi (■) [17].

The symmetrical nature of the OMCP–chloride complex is clearly visible in the X-ray structure of the complex (Fig. 6) [7]. The nitrogen-to-chloride anion distances are in the range of 3.264(7)–3.331(7) Å.

Interestingly, ^1H NMR titrations of NC-OMCP with anions revealed a very different pattern: the three non-inverted pyrroles show the usual behavior (a down-field shift of NHs and up-field shift of the pyrrole β -H signals) while the inverted pyrrole shows a concerted up-field shift of the inverted pyrrole NHi signal as well as a strong down-field shift of the β -pyrrole proton (● in Fig. 7).

The concerted up-field shift of the inverted pyrrole NHi suggests that this NH moiety does not participate in anion binding. Conversely, the down-field shift of the inverted β -CHi suggests its close contact with the anion or even participation in the NC-OMCP–Cl complex formation.

These results imply that the NC-OMCP receptor binds anions via a different binding mode compared to OMCP. Further insight into the binding mode was obtained from the density functional theory (DFT) study [17]. From the combination of NMR data and DFT energy-minimized structures it appears that this binding mode, new in calixpyrrole-anion binding, involves the β -pyrrole CHi-anion contact or bond to participate in the receptor–anion complex alongside the three non-inverted pyrroles. Density functional theory calculations were carried out for both OMCP and NC-OMCP and their respective complexes with tetramethylammonium fluoride (TMAF). The energy-optimized [18] structures of complexes OMCP·TMAF and NC-OMCP·TMAF are shown on computer-generated models in Fig. 8.

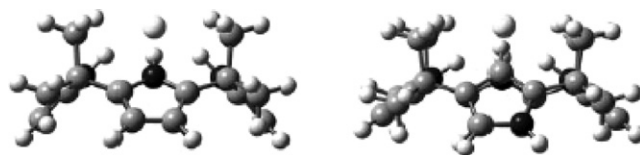


Fig. 8. Calculated structures of OMCP·TMAF (OMCP·F-tetramethylammonium) and NC-OMCP·TMAF complexes. Optimization was performed using Gaussian'03 [18] at B3LYP/6-31G* level of theory. The TMA is omitted for clarity.

The DFT study aimed at interpretation of shifts in NMR spectra corresponding to the energy-minimized model for NC-OMCP in the resting state and in the complex with anions was performed. The DFT-GIAO magnetic shields were calculated for NC-OMCP and NC-OMCP·F-tetramethylammonium in the energy minimum (a cone-like conformation involving the inverted β -pyrrole hydrogen). The calculation showed the same trends (shifts of ^1H -resonances) upon complexation of NC-OMCP with tetramethylammonium fluoride observed in the ^1H NMR titration experiments for NC-OMCP with tetrabutylammonium fluoride in DMSO- d_6 .

The final proof of the NC-OMCP-anion binding mode came from the single crystal X-ray structure studies by Gale and co-workers [19] (Fig. 9). The NC-OMCP·Cl crystal was obtained using ethyl- and butyl-methylimidazolium as a cation. The chloride anion is bound by three N–H···Cl hydrogen bonds with Cl···H distances in the range 2.712(6)–2.358(5) Å [2.604(7)–2.337(5) Å] and one C–H···Cl contact of 2.605(4) Å [2.770(9) Å]. The chloride is slightly displaced from the center of the macrocycle towards the three NH groups. The X-ray structure is in remarkable agreement with the results of the DFT calculations.

Thus the N-confused calix[4]pyrrole structure seems to prefer to form complexes with anions via a more symmetrical cone-like conformation that involves β -pyrrole hydrogen of the inverted pyrrole rather than a less symmetrical conformation that could involve all four pyrrole NHs. This behavior was observed in all materials utilizing the NC-OMCP receptor with various anions.

4.2. Anion-binding affinities

In general, NMR binding studies performed using OMCP and NC-OMCP in DMSO- d_6 show that both receptors bind

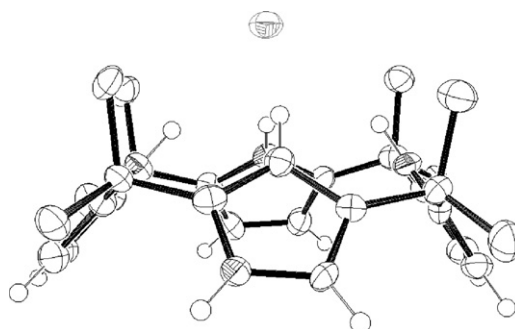


Fig. 9. NC-OMCP·Cl X-ray structure by Gale and co-workers (scaled to 35% probability level; methyl hydrogen atoms were omitted for clarity) [19].

similar anions, F^- , Cl^- , $H_2PO_4^-$, CN^- , AcO^- , albeit with different relative affinity. Thus, for example, the OMCP binds chloride with $K_{asoc} = 1000\text{ M}^{-1}$, while the N-confused isomer shows $K_{asoc} = 20\text{ M}^{-1}$. Similarly, the OMCP binds fluoride with $K_{asoc} > 5 \times 10^5\text{ M}^{-1}$, while the N-confused isomer shows $K_{asoc} \leq 10^3\text{ M}^{-1}$ (the uncertainty in the determination of the K_{asoc} for fluoride is due to the slow exchange, where the constants are derived from integration of broad signals in the 1H NMR spectra) [17].

It appears that the N-confused calixpyrroles show less affinity to spherical anions, presumably because these anions require formation of a high symmetry receptor–anion complex. Also, it appears that the four *versus* three NH-hydrogen bonds in OMCP and NC-OMCP, respectively, may contribute to lower anion affinity observed for NC-OMCPs. It is conceivable that the $CH \cdots anion$ interaction is just a close contact rather than a hydrogen bond.

5. Anion sensors utilizing N-confused calix[4]pyrroles

In order to achieve construction of practical and reliable schemes for sensing of anions, a relatively large number of sensors are required. This is because few molecular sensors have a wide dynamic range, where one could obtain clearly observable output signal changes over two or three orders of a magnitude in the analyte concentration. The development of reliable anion sensor devices would most likely require combinations of several molecular sensors to cover the wide range of analytes and their concentrations. Calix[4]pyrroles and their N-confused congeners could, however, help bridging these voids as they provide a platform for generating molecular sensors with similar, yet different anion-binding properties.

5.1. Synthesis of optical sensors

Investigation of electrophilic aromatic substitution of OMCP resulted in one step modification of the calix[4]pyrrole skeleton. Recently, Anzenbacher and co-workers utilized electrophiles such as tricyanoethene (TCE) [20] and various diazonium salts [21] to generate chromogenic calixpyrrole derivatives **TCE-1** and **Azo-1** (Fig. 10). Independently of each other Dehaen and co-workers [22] investigated the electrophilic substitution of NC-OMCP and generated several materials with the electrophile attached to the most reactive α -CH, among them also sensors **TCE-2** and **Azo-2** (Fig. 10).

The sensors were characterized by both NMR and X-ray crystallography. While the **TCE-1** showed behavior typical for calixpyrroles [3], **TCE-2** displays propensity for intramolecular cyclization to yield a cyclic form **TCE-2C** depicted in Fig. 11.

The structures of all four sensors: **TCE-1**, **TCE-2C**, **Azo-1**, and **Azo-2** were also characterized by X-ray crystallography (Fig. 12) [17].

Finally, the free α -carbon in N-confused calix[4]pyrrole is much easier to modify than in regular calix[4]pyrroles that have all α -carbons bonded to *meso*-carbons. Not only that the free α -carbon is more reactive than the one in β -positions, it is also

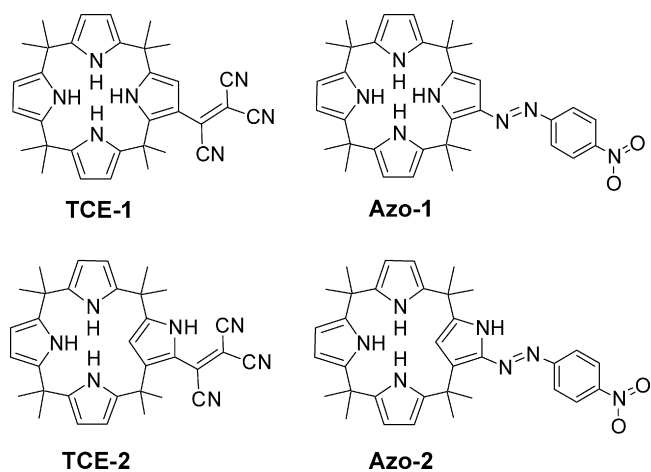


Fig. 10. The structure of chromogenic sensors utilizing OMCP and NC-OMCP receptors.

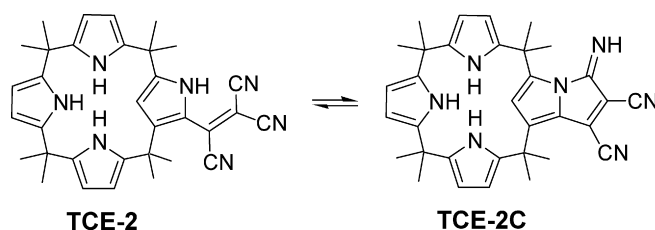


Fig. 11. Intramolecular cyclization of **TCE-2** to **TCE-2C**.

the only one unsubstituted α -carbon, which dramatically simplifies the resulting product mixtures. In fact, modifications of regular calix[4]pyrroles always result in a complex mixture of products. This aspect might be a reason for increased interest in N-confused calix[4]pyrroles in the future.

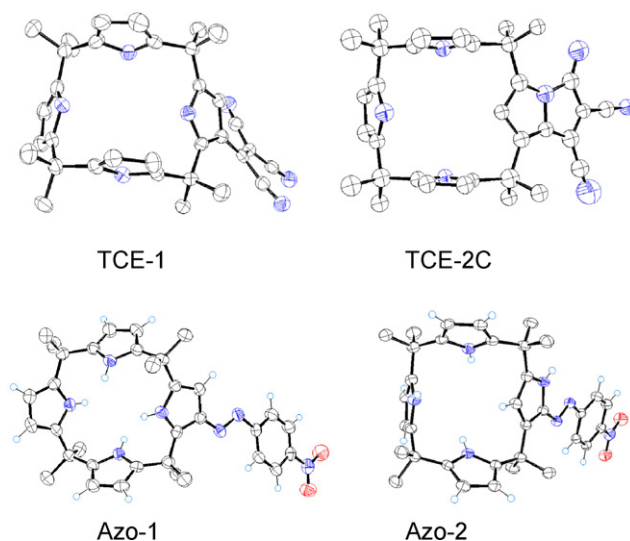


Fig. 12. Top: X-ray structures of **TCE-1** and **TCE-2C** (displacement ellipsoids are scaled to the 50% probability level; the hydrogen atoms have been removed for clarity). Bottom: X-ray structures of **Azo-1** and **Azo-2** (the displacement ellipsoids are scaled to the 50% probability level; the methyl hydrogen atoms have been omitted for clarity) [17].

5.2. Colorimetric sensors

In general, the attachment of dye precursors to NC-OMCP converts the originally colorless species into chromogenic materials, which show changes in color upon binding of an anion. Both the naked-eye observation and absorption spectroscopy may be used to study different anion binding behavior of chromogenic OMCPs and NC-OMCPs.

For example, the isomeric pairs of sensors (**TCE-1** and **TCE-2C**, **Azo-1** and **Azo-2**) show different relative affinity toward anionic substrates. **TCE-1**, which yields a pink DMSO solution, turns to orange in the presence of fluoride but shows only a negligible change in color and minute changes in UV–vis spectra in the presence of chloride (Fig. 13, top). At the same concentration, the solution of **TCE-2C** shows a vivid pink color and the corresponding absorption is also three times stronger. The addition of fluoride to **TCE-2C** induced a large hypsochromic shift corresponding to a pink-to-orange color change. On the other hand, the addition of chloride into a **TCE-2C** solution resulted in a bathochromic shift in the absorption spectrum and an easily discernible pink-to-purple color change (Fig. 13, bottom). The black dot (●) in the graph shows the region of spectrum

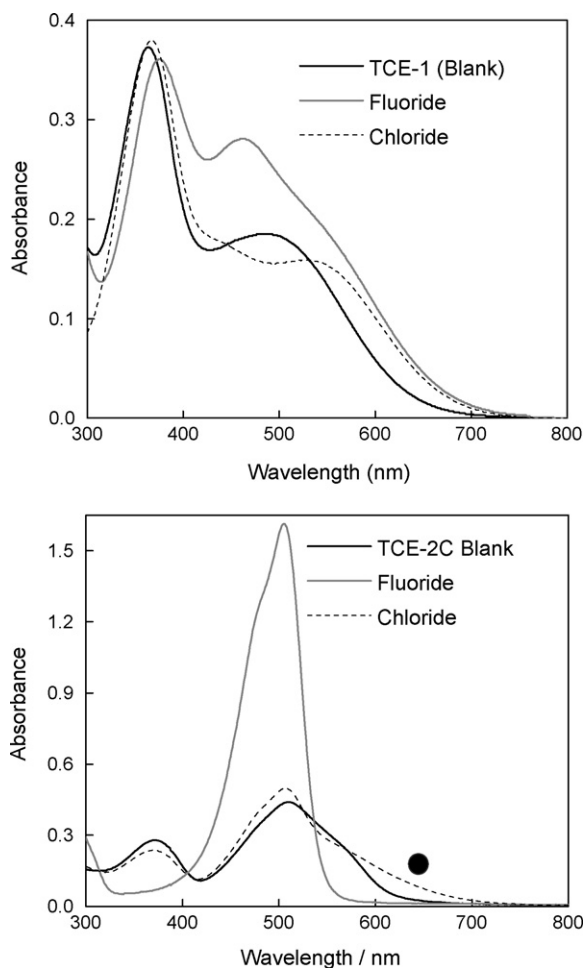


Fig. 13. Top: solution of **TCE-1** before and after the addition of fluoride and chloride anions. Bottom: solution of **TCE-2C** before and after the addition of fluoride and chloride anions. Both solutions were 5.0×10^{-5} M in DMSO.

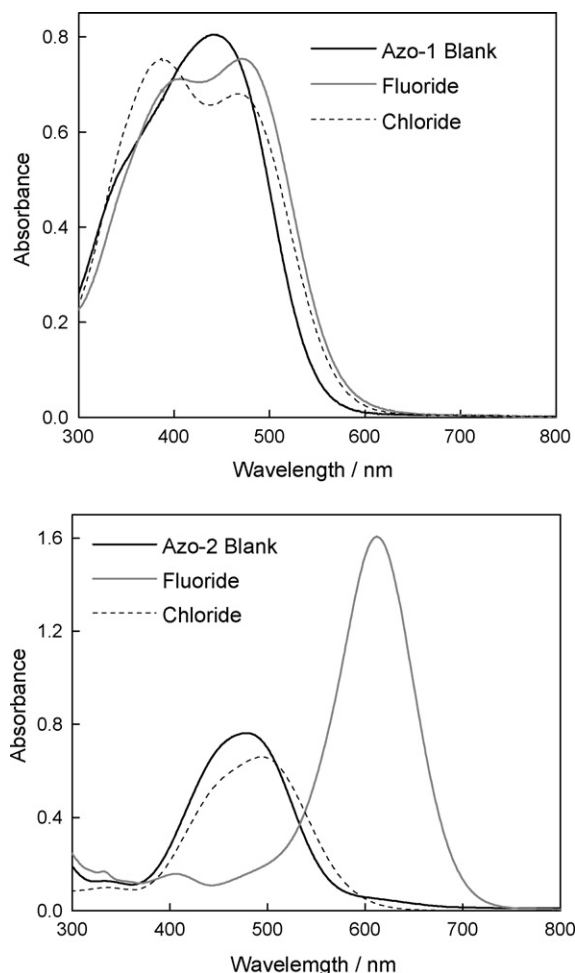


Fig. 14. Top: solution of **Azo-1** before and after addition of fluoride and chloride anions. Bottom: solution of **Azo-2** before and after addition of fluoride and chloride anions. Both solutions were 5.0×10^{-5} M in DMSO.

(600–700 nm) responsible for the purple component of the color [17].

The addition of anions to a DMSO solution of **Azo-1** and **Azo-2** sensors also resulted in changes in color. Thus, **Azo-1** forms a yellow solution, the color of which changes to orange upon addition of fluoride with a corresponding small bathochromic shift. The color of the solution upon addition of a chloride anion remains virtually unchanged (Fig. 14, top). Dehaen and co-workers [22] reported titrations of **Azo-2** with fluoride, which showed a large bathochromic shift with a dramatic orange-to-blue change in color, possibly due to the deprotonation of the inverted pyrrole NH. Upon the addition of chloride **Azo-2** showed an orange-to-red color change (Fig. 14, bottom) [17].

The comparison of binding constants calculated for **TCE-1** and **TCE-2C** shows that both compounds strongly bind fluoride and acetate, and both bind chloride weakly. **TCE-1**, like other regular calix[4]pyrroles, does not bind dihydrogenphosphate and hydrogenpyrophosphate strongly. By comparison, **TCE-2C** binds dihydrogenphosphate as well as acetate with high affinity. Similarly, both **Azo-1** and **Azo-2** show affinity for fluoride, acetate, and hydrogenpyrophosphate, albeit weaker compared to the **TCE**-sensors. This is rationalized by stronger electron-

withdrawing effect of the three-cyano groups of the TCE moiety.

Interestingly, both N-confused sensors (**TCE-2C** and **Azo-2**) show dramatic increase in selectivity toward the acetate anion over chloride and phosphate compared to their counterparts (**TCE-1** and **Azo-1**). This is expressed as ratios of binding constants $K_{\text{AcO}^-}/K_{\text{Cl}^-}$ and $K_{\text{AcO}^-}/K_{\text{H}_2\text{PO}_4^-}$. For example, the affinity for acetate over chloride increased ca. 50-times for **TCE-2C** versus regular **TCE-1**, and 28-times for **Azo-2** versus the regular **Azo-1**. Thus it appears, that the N-confused calix[4]pyrroles accommodate easier non-spherical anions.

The UV–vis spectra indicate that the relative strength in the respective color transitions observed for the sensors and their complexes with anions does not necessarily correlate with the receptor–anion affinities (K_{as}). That is most likely because the magnitude of the change in the transition dipole moment does not generally correlate with the thermodynamic association constants but rather with the degree of difference in the frontier orbital density between the resting state and the respective receptor–anion complex. The fact that the changes in color (both wavelength and intensity) are not the same for all of the sensor-anion combinations adds yet another dimension to the potential application of these sensors for qualitative substrate determination in a naked-eye regime or serve as complementary sensor moieties in sensor arrays.

5.3. Sensor arrays toward practical anion sensing in water

Numerous efforts were focused on the rational design and preparation of anion sensors that display clearly distinguishable colorimetric signaling and may be used in optical sensor arrays utilizing pattern recognition [23,24]. An important feature of sensor arrays is that individual receptors are usually not highly selective toward specific analytes and that the specificity comes from the recognition of the response pattern. A pattern specific for a given analyte is based on optical signal response [25,26] from a large number of sensor units that do not exhibit a highly selective interaction with the analyte, but rather small perturbation arising from the non-specific nature of the interactions between the analyte and the sensor units. In order to develop colorimetric sensor array for anions, one has to generate sufficiently broad dataset of optical changes from a relatively large number of sensors. Combinations of regular and N-confused calix[4]pyrroles-based colorimetric sensors appears to be an interesting avenue for such an endeavor.

Among the most important practical issues in anion sensing is the fact that anions occur mostly as solutes in water or aqueous media. While a number of anion sensors that are soluble in water-miscible solvents such as DMSO or MeCN tolerate a small percentage of water in the medium, the fact remains that most simple receptors and sensors that utilize hydrogen bonding to establish the anion–receptor complex do not operate successfully in water. This is because only a few of such materials are actually water-soluble, but mainly because the anion hydration strongly competes with the receptor for hydrogen bonding to the anion [2b,27]. Both these factors, however, render the reliable

Table 1

Association constants (M^{-1}) for **TCE-1**, **TCE-2C**, **Azo-1**, **Azo-2** and anions in DMSO at 22 °C determined by absorption spectroscopy titrations^a [17]

Anion	TCE-1	TCE-2C	Azo-1	Azo-2
F^-	$>10^6$	$>10^6$	$>10^6$	7240
Cl^-	1370	319	741	<50
AcO^-	242,000	$>10^6$	8540	16,600
H_2PO_4^-	5230	810,000	3330	430
$\text{HP}_2\text{O}_7^{3-}$	584,000	n.d.	92,200	5650
$K_{\text{AcO}^-}/K_{\text{Cl}^-}$	176	$>10,000$	12	330
$K_{\text{AcO}^-}/K_{\text{H}_2\text{PO}_4^-}$	46	25	3	39

^a Anions were used in the form of their Bu_4N^+ (TBA) salts. The errors in all fits are $\pm 15\%$.

sensing of anions by synthetic receptors such as calix[4]pyrroles somewhat difficult.

The utility of polymeric matrices in the sensor development has been demonstrated on many occasions [28,29]. It has been shown that this problem with anion hydration may, in part, be circumvented by carrying out the anion sensing at a solution–polymer interface where the degree of hydration is more favorable than the one in bulk water [30]. Polymers, if properly engineered, may be capable of attracting or even extracting anions from the bulk aqueous medium [3]. Anzenbacher and co-workers [3,30] previously demonstrated that anion sensors utilizing hydrogen bonding to establish receptor–anion complexes may be successfully used in conjunction with polymers capable of internally displacing the bulk water, thus giving the receptor better chance to establish the anion–receptor complex. The degree of polymer carrier ability to internally displace water

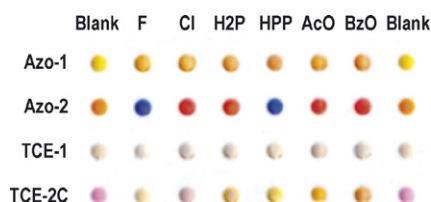


Fig. 15. Micro-assay utilizing **Azo-1**, **Azo-2**, **TCE-1**, and **TCE-2C** doped polyurethane. The anions were administered in pure water (200 nL, 10 mM). The anions are abbreviated as follows: F (fluoride), Cl (chloride), H_2P (dihydrogen phosphate), HPP (hydrogen pyrophosphate), AcO (acetate), BzO (benzoate) [17].

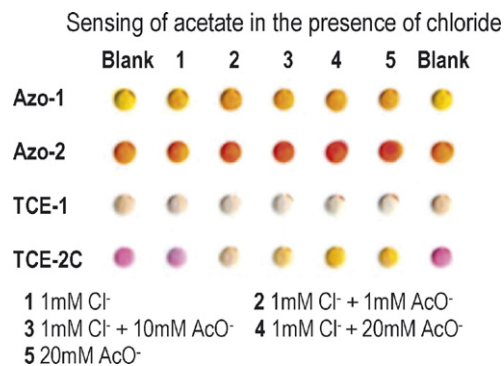


Fig. 16. Competitive micro-assay utilizing **Azo-1**, **Azo-2**, **TCE-1**, and **TCE-2C** doped polyurethane. Sensing of acetate (0–20 mM) administered as an aqueous solution containing 1 mM Cl^- [17].

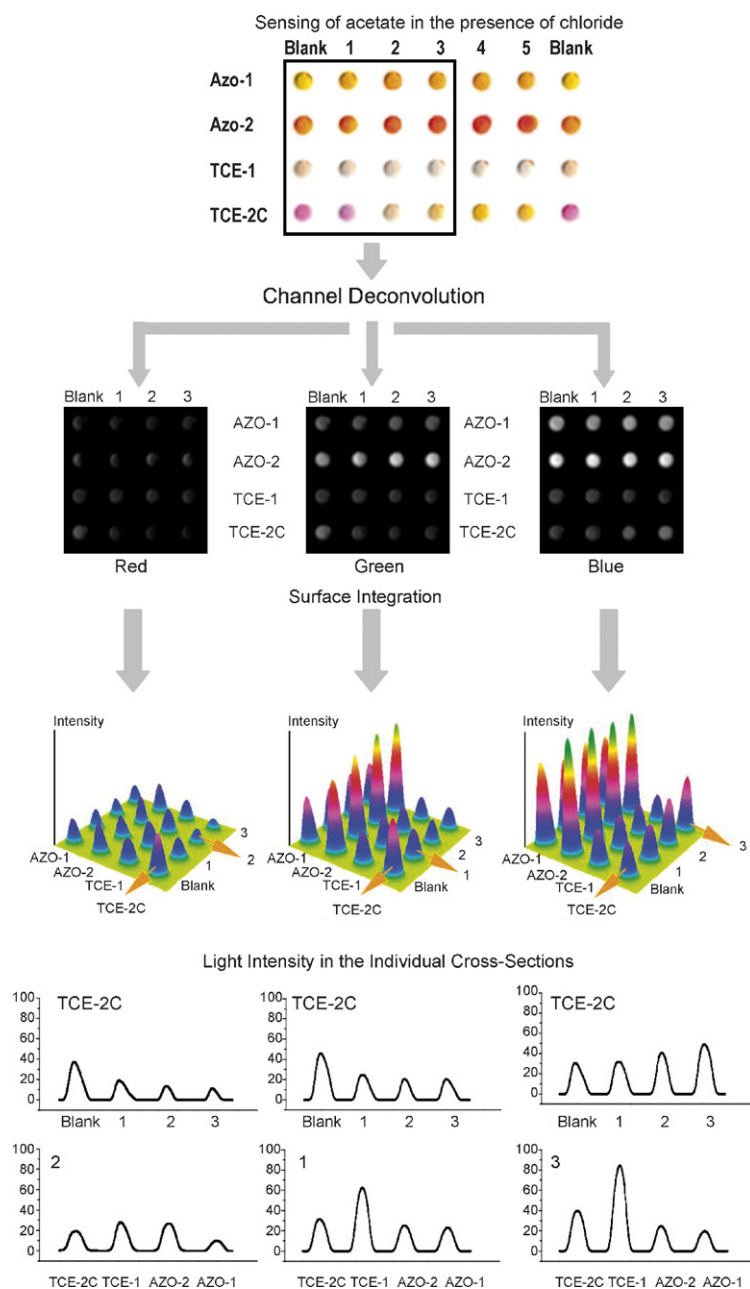


Fig. 17. Sensors **Azo-1**, **Azo-2**, **TCE-1**, **TCE-2C** (horizontal lines) were solution-cast in a polyurethane solution into a 80-well microplate to form a 50–100 μm thick film in each well. Each well then received a 200 nL sample of an anion (10.0 mM in water). The full-color image was deconvoluted into RGB channels and light intensity in each of the wells was integrated to create X – Y –intensity quasi-3D graphs. The individual horizontal/vertical cross-section of these quasi-3D graphs provide information regarding the specific response of each of the sensor to an individual anion and *vice versa*. The X cross-section shows a response of a sensor to the increasing acetate concentration while the Y cross-section shows how different sensors react to the presence of one anion.

is therefore a key variable in the design of polymer-embedded colorimetric sensors. For example, polyurethanes offer a good balance of useful properties as hosts for anion sensors: they are (i) colorless and optically transparent, (ii) partially hydrophilic, and (iii) comprise amide moieties that aid in the anion extraction from the water and anion binding [3,30].

Recently, Anzenbacher and co-workers [17] developed a sensing array using micro-well plates quartz slides to generate 80 (8×10) wells ($230 \pm 10 \mu\text{m}$ diameter, $230 \pm 10 \mu\text{m}$ deep, Fig. 15). Polyurethanes doped with sensors were solution-cast to create ca. 50–100 μm coating in each respective well. Fig. 15

shows a 4×8 section of the assay that accommodates sensors **Azo-1**, **Azo-2**, **TCE-1**, **TCE-2C** (horizontal lines) and blank, (water) and six different aqueous anionic analytes in the vertical lines. The anions used in this assay were added as aqueous solutions (200 nL, 10 mM). While the changes in color in the assay may be clearly observed by a naked eye, a variety of absorption spectroscopy and imaging techniques can be used to record the response generated by the array. Also, the methods of evaluating the response in the non-specific sensor arrays [31,32] utilizing pattern recognition [32,33] may be used for analysis of anionic analytes.

While the micro-assay in Fig. 15 suggests that **Azo-1**, **Azo-2**, **TCE-1**, and **TCE-2C** can act as colorimetric sensors for aqueous anions, for practical purposes it is also necessary to demonstrate the possibility of sensing the anions in the presence of competing electrolytes. The selectivity coefficients (see Table 1) suggest that it should be possible to sense carboxylates in the presence of chloride. The competitive assay confirmed that acetate may be sensed in water and in the presence of a competing chloride anion. Fig. 16 shows that changes in color start to be observable at an equimolar acetate–chloride concentration. Further increase in the acetate (analyte) compared to the competitor concentration shows gradual increase in the color change confirming that the change in color is due to the presence of the acetate analyte. This result is also significant for potential application of anion sensing in biological media [34].

The utility of the isomeric calix[4]pyrroles **Azo-1**, **Azo-2**, **TCE-1**, and **TCE-2C** in colorimetric arrays was demonstrated in the latter assay (shown in Fig. 16). First, the color changes in the assay were recorded by a CCD-based imager, and the full-color color image was deconvoluted into red, green, and blue channels, and the light intensity in each channel was integrated. This process (shown in Fig. 17) yields quasi-3D representation of the sensor array where *X* and *Y* correspond to the *XY* dimensions of the sensor chip and the *Z*-scale corresponds to the integrated light density (integration of the non-zero pixels).

The analysis of the data obtained from the cross from the array and the individual profiles confirms that sensors **Azo-1**, **Azo-2**, **TCE-1**, **TCE-2C** may be used to obtain data regarding the presence of anions in an aqueous analyte, and construct sensor arrays capable of providing output that can be used to identify unknown anion samples. The preliminary results also show such arrays will provide quantitative information regarding the concentration of the anionic analyte. Multisensor arrays utilizing pattern recognition [35] by harnessing the information from small changes in multiple sensors are currently being developed for practical applications [32,36–38]. The color changes in the array utilizing calixpyrrole-based sensors **Azo-1**, **Azo-2**, **TCE-1**, and **TCE-2C** suggest that these simple materials may be successfully used for sensing of anions administered as purely aqueous solutions.

5.4. Potentiometric sensors

Regular cyclohexylcalix[4]pyrrole, its N-confused congener, and cyclohexyldipyrromethane were applied as sensory elements in a liquid membrane of potentiometric sensors aimed at recognition of neutral forms of nitrophenol isomers [39,40]. The potentiometric responses obtained from the three electrodes indicated an affinity for protons. Not surprisingly, the potential of liquid membranes, containing pyrrole derivatives, strongly depended on the pH of the aqueous phase. The response to the presence of nitrophenol derivatives were then investigated at three different pHs: 4.0, 6.0 and 8.0 [40]. The three electrodes utilizing each of the cyclohexylcalix[4]pyrrole, its N-confused congener, and cyclohexyldipyrromethane, showed affinity to neutral nitrophenols and significantly lower affinity to the cor-

responding nitrophenolate anion. All three sensors emembranes displayed higher selectivity towards 4-nitrophenol. Also, the sensors derived from the two calix[4]pyrrole macrocycles displayed higher affinity compared to the dipyrromethane-based electrode. The symmetry of the calix[4]pyrrole receptor did not seem to play crucial parameter in nitrophenol binding. Rather, the number of pyrrole rings in the ligand structure affected the sensitivity of the sensors.

6. Conclusions

In this short review the authors took upon themselves to summarize the field of supramolecular chemistry of N-confused calix[4]pyrroles. Arguably, with only a few papers published on the topic and only 7 years since the first spectroscopic characterization of a N-confused calix[4]pyrrole derivative, this was an easy job to do. The reason for doing so was to demonstrate how a narrow yet very lively field took off and made a giant leap from N-confused calix[4]pyrrole being discovered as a trace impurity to the synthesis of materials, which may help its older and more frequently studied isomer, the regular calix[4]pyrrole, to enter the realm of practical application in sensor arrays.

In summary, the structure of N-confused calix[4]pyrroles has been determined and their binding mode with anionic analytes has been established both in solution as well as in the solid state. N-confused calix[4]pyrroles can today be produced on a multi-gram scale, can be selectively derivatized and converted into optical sensors for anions. Colorimetric assays utilizing N-confused calix[4]pyrrole sensors were fabricated. It was demonstrated how these sensors may be useful as a simple yet powerful tool for sensing of anions in water and in the presence of competing electrolytes. The authors felt that this success story deserved to be encapsulated and shared with the community of supramolecular chemists to enjoy.

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References

- [1] (a) R.F. Schmidt, G. Thews, Human Physiology, 2nd ed., Springer-Verlag, Berlin, 1989;
(b) W.J. Marshall, S.K. Bangert, Clinical Chemistry, 5th ed., Elsevier, Edinburgh, 2004;
(c) Ullmann's Encyclopedia of Industrial Chemistry, 6th ed., Wiley-VCH, New York, 1999.
- [2] (a) J.L. Sessler, P.A. Gale, W.-S. Cho, Anion Receptor Chemistry. Monographs in Supramolecular Chemistry, RSC Publishing, Cambridge, 2006;
(b) C. Suksai, T. Tuntulani, Top. Curr. Chem. 255 (2005) 163;
(c) R. Martínez-Máñez, F. Sancenón, Chem. Rev. 103 (2003) 4419;
(d) P.D. Beer, P.A. Gale, Angew. Chem. Int. Ed. 40 (2001) 486.
- [3] R. Nishiyabu, P. Anzenbacher Jr., J. Am. Chem. Soc. 127 (2005) 8270.
- [4] J.L. Sessler, S. Camiolo, P.A. Gale, Coord. Chem. Rev. 240 (2003) 17.

- [5] J.L. Sessler, S.J. Weghorn, Expanded, Contracted and Isomeric Porphyrins. Tetrahedron Organic Chemistry Series, vol. 15, Pergamon–Elsevier Science, Oxford, 1997.
- [6] V.J. Bauer, D.J.L. Clive, D. Dolphin, J.B. Paine III, F.L. Harris, M.M. King, J. Loder, S.C. Wang, R.B. Woodward, *J. Am. Chem. Soc.* 105 (1983) 6429.
- [7] P.A. Gale, J.L. Sessler, V. Král, V. Lynch, *J. Am. Chem. Soc.* 118 (1996) 5140.
- [8] P.A. Gale, J.L. Sessler, V. Král, *Chem. Commun.* (1998) 1.
- [9] P.A. Gale, P. Anzenbacher Jr., J.L. Sessler, *Coord. Chem. Rev.* 222 (2001) 57.
- [10] A. Baeyer, *Ber. Deut. Chem. Ges.* 19 (1886) 2184.
- [11] P. Rothmund, C.L. Gage, *J. Am. Chem. Soc.* 77 (1955) 3340.
- [12] P.J. Chmielewski, L. Latos-Grazynski, K. Rachlewicz, T. Glowiak, *Angew. Chem. Int. Ed.* 33 (1994) 805.
- [13] P.J. Chmielewski, L. Latos-Grazynski, K. Rachlewicz, T. Glowiak, *Angew. Chem. Int. Ed.* 33 (1994) 779.
- [14] H. Furuta, T. Asano, T. Ogawa, *J. Am. Chem. Soc.* 116 (1994) 779.
- [15] O. Tsuge, M. Tashiro, Y. Kiryu, *Org. Prep. Proc. Int.* 7 (1975) 39.
- [16] S. Depraetere, M. Smet, W. Dehaen, *Angew. Chem. Int. Ed.* 38 (1999) 10780.
- [17] R. Nishiyabu, M.A. Palacios, W. Dehaen, P. Anzenbacher Jr., *J. Am. Chem. Soc.* 128 (2006) 11496–11504.
- [18] M.J. Frisch, G.W. Trucks, H.B. Schlegel, P.M.W. Gill, B.G. Johnson, M.A. Robb, J.R. Cheeseman, T.A. Keith, G.A. Peterson, J.A. Montgomery, K. Raghavachari, M.A. Al-Laham, V.G. Zakrzewski, J.V. Ortiz, J.B. Foresman, J. Cioslowski, B.B. Stefanov, A. Nanayakkara, M. Challacombe, C.Y. Peng, P.Y. Ayala, W. Chen, M.W. Wong, J.L. Andres, E.S. Replogle, R. Gomperts, R.L. Martin, D.J. Fox, J.S. Binkley, D.J. Defrees, J. Baker, J.P. Stewart, M. Head-Gordon, C. Gonzalez, J.A. Pople, *Gaussian'03* (Revision B.04), Gaussian Inc., Pittsburgh, PA, 2003.
- [19] G.W. Bates, M. Kostermans, W. Dehaen, P.A. Gale, M.E. Light, *Cryst. Eng. Commun.* 8 (2006) 444.
- [20] R. Nishiyabu, P. Anzenbacher Jr., *J. Am. Chem. Soc.* 127 (2005) 8270.
- [21] B. Wex, P. Anzenbacher Jr., Proceedings of the 223rd National Meeting of the American Chemical Society, Orland, FL, April 7–11, American Chemical Society, Washington, DC, 2002 (ORGN-245).
- [22] R. Gu, S. Depraetere, J. Kotek, J. Budka, E. Wagner-Wysiecka, J.F. Biernat, W. Dehaen, *Org. Biomol. Chem.* 3 (2005) 2921.
- [23] D.L. Eastwood, O.O. Soyemi, J. Karunamuni, L. Zhang, H. Li, M.L. Myrick, *Proc. SPIE: Int. Soc. Opt. Eng.* 4199 (2001) 105.
- [24] D.-S. Lee, J.-K. Jung, J.-W. Lim, J.-S. Huh, D.-D. Lee, *Sens. Actuators B* 77 (2001) 228.
- [25] J.J. Lavigne, S. Savoy, M.B. Clevenge, J.E. Ritchie, B. McDoniel, S.-J. Yoo, E.V. Anslyn, J.T. McDevitt, J.B. Shear, D. Neikirk, *J. Am. Chem. Soc.* 120 (1998) 6429.
- [26] A. Goodey, J.J. Lavigne, S.M. Savoy, M.D. Rodriguez, T. Curey, A. Tsao, G. Simmons, J. Wright, S.-J. Yoo, Y. Sohn, E.V. Anslyn, J.B. Shear, D.P. Neikirk, J.T. McDevitt, *J. Am. Chem. Soc.* 123 (2001) 2559.
- [27] J.W. Steed, J.L. Atwood, *Supramolecular Chemistry*, Wiley, Chichester, 2000.
- [28] (a) Y. Osada, D.E. DeRossi (Eds.), *Polymer Sensors and Actuators*, Springer, Berlin, 2000;
(b) D.T. McQuade, A.E. Pullen, T.M. Swager, *Chem. Rev.* 100 (2000) 2537.
- [29] D. Aldakov, P. Anzenbacher Jr., *J. Am. Chem. Soc.* 126 (2004) 4752.
- [30] D. Aldakov, M.A. Palacios, P. Anzenbacher Jr., *Chem. Mater.* 17 (2005) 5238.
- [31] M. Schena, *Microarray Analysis*, Wiley–Liss, Hoboken, 2003.
- [32] Y. Vlasov, A. Legin, A. Rudnitskaya, C. Di Natale, A. D'Amico, *Pure Appl. Chem.* 77 (2005) 1965.
- [33] J.J. Lavigne, E.V. Anslyn, *Angew. Chem. Int. Ed.* 40 (2001) 3118.
- [34] R.F. Schmidt, G. Thews, *Human Physiology*, 2nd ed., Springer-Verlag, Berlin, 1989.
- [35] J.J. Lavigne, E.V. Anslyn, *Angew. Chem. Int. Ed.* 40 (2001) 3118.
- [36] C. Krantz-Rulcker, M. Stenberg, F. Winquist, I. Lundstrom, *Anal. Chim. Acta* 426 (2001) 217.
- [37] K.J. Albert, N.S. Lewis, C.L. Schauer, G.A. Sotzing, S.E. Stitzel, T.P. Vaid, D.R. Walt, *Chem. Rev.* 100 (2000) 2595.
- [38] D. James, S.M. Scott, Z. Ali, W.T. O'Hare, *Microchim. Acta* 149 (2005) 1.
- [39] T. Piotrowski, H. Radecka, J. Radecki, S. Depraetere, W. Dehaen, *Electroanalysis* 13 (2001) 342.
- [40] T. Piotrowski, H. Radecka, J. Radecki, S. Depraetere, W. Dehaen, *Mater. Sci. Eng. C* 18 (2001) 223.