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# Application of RDCs Enhanced NMR Spectroscopy in Structural Analysis of Thiacalix[4]arene Derivatives

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Thiacalix[4]arene spirodienone was rearranged into the corresponding phenoxathiin-based macrocycle. Alkylation of this inherently chiral system to achieve its immobilization led to a mixture of only two (out of four theoretically possible) stereoisomers. As standard NOE and dynamic NMR experiments did not lead to unambiguous determination of the structures we applied the Residual Dipolar Coupling constants (RDCs) method. Poly-γ-ethyl-L-glutamate (PELG) and poly-γ-benzyl-L-glutamate (PBLG) were found to be an easily applicable lyotropic liquid crystalline alignment media for the conformational analysis of thiacalixarene derivatives. Using these media the *1,2-alternate* and the *partial cone* conformations were determined unequivocally.

## Introduction

The introduction of four sulfur atoms instead of the common $CH_2$ bridges imparts to thiacalix[4]arenes[1] considerably different chemical or conformational behaviour when compared with classical calix[n]arenes.[2] As the chemistry of thiacalixarenes has been studied for almost two decades[3] the chemo-, regio- and/or stereoselective oxidation of sulfur atoms to sulfoxides and sulfones,[4] or the alkylation to the corresponding sulfonium salts, has been developed.[5]

Despite these achievements, there are some aspects in the chemistry of thiacalixarenes, which remain virtually unknown or so far unexplored. Among them one can find the synthetic use of the so-called spirodienone derivatives (Figure 1). These compounds were first described in 1992 for classical calixarene series[6] and they can serve as useful intermediates[7] in functionalizing the calixarene skeleton to provide many unusual analogues, including the substitution patterns so far inaccessible by other synthetic methods.[8] Similar reaction in the thiacalixarene series was reported[9] only in 2005, when oxidation of the starting thiacalix[4]arene with chloramine T gave the corresponding spirodienone derivative in high yield (Figure 1). The specific nature of the chemistry of thiacalix[4]arene, compared to that of the classical analogue, can be demonstrated by the unique acidic rearrangement of this spiro-derivative to provide the phenoxathiine-containing macrocycle.[10]

In this paper we report on the first attempt to alkylate the rearranged skeleton to obtain conformationally immobilized derivatives that can be useful building blocks in supramolecular chemistry. The stereochemical outcomes of the products were assigned by a combination of dynamic NMR and residual dipolar coupling (RDC) techniques.

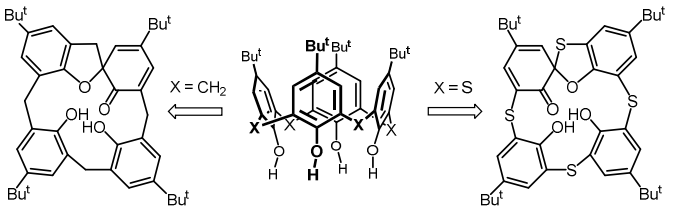
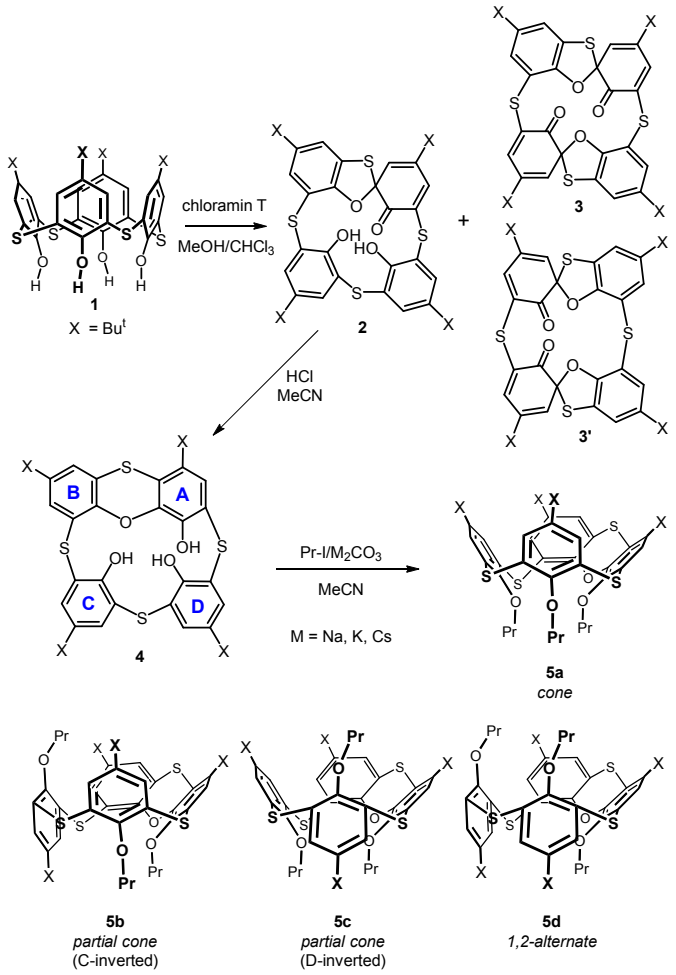



**Figure 1.** The formation of spirodienone derivatives of calix[4]arene and its thia-analogue.

## Results and discussion

The synthesis of the spiro-derivative of thiacalix[4]arene was carried out as reported previously[9] (Scheme 1). The reaction of thiacalixarene **1** (1 g scale) with 1.5 equiv. of chloramine-T·trihydrate in MeOH-$CHCl_3$ at 0 °C for 2 h gave the corresponding orange monospiro compound **2** in 58% yield after column chromatography on silica gel. Closer inspection of the late chromatographic fractions revealed the presence of

another red isomer isolated in 3% yield. Mass spectrometry confirmed the molecular peak (TOF ESI+ *m/z* = 739.32 $[M+Na]^+$) corresponding to the bis(spiro) derivative. Varying the reaction parameters (6 equiv. of chloramine T, room temp., 4 days) increased the yield of the bis(spiro) fraction to 18%. Unfortunately, the $^1$H NMR spectroscopy and HPLC analysis showed that this fraction consisted of at least two different compounds, probably corresponding to regioisomers **3** and **3'**. Moreover, both regioisomers **3**/**3'** could further contain several stereoisomers due to the formation of two new stereogenic centres (spiro-atoms). Not surprisingly, all of our attempts at isolating the individual isomers failed, and as a result, we decided to continue with only monoderivative **2**.




**Scheme 1.** Preparation and alkylation of phenoxathiine-based thiacalix[4]arene.

Spiro derivative **2** was reacted with conc. aqueous HCl in acetonitrile at reflux. The expected phenoxathiin derivative **4**[10] was smoothly obtained in 92% yield after recrystallization of the crude product from $MeCN/CHCl_3$ mixture. Compound **4** represents an interesting inherently chiral derivative with potential applications in supramolecular chemistry as a building block.

To achieve the immobilization of the 3D-shapes of the cavity in a specific conformation we carried out alkylation of the lower rim (phenolic OH groups) using reaction conditions well-known from the chemistry of calixarenes or thiacalixarenes.[2,3] Interestingly, the reaction of compound **4** with PrI/PrBr in acetone/acetonitrile under reflux using $Na_2CO_3$, $K_2CO_3$ or $Cs_2CO_3$ as a base gave the same two products **5c** and **5d** in various ratios depending on the reaction conditions. Thus, $PrI/K_2CO_3$ in acetone provided **5c** and **5d** in 29% and 20% yield after preparative TLC, respectively. On the other hand, use of $Cs_2CO_3$ led to reversed preferences and **5c** and **5d** were isolated in 18% and 37% yield, respectively.

The assignment of the individual conformers using NMR was not a trivial task as the lack of $CH_2$ bridges, the most indicative part for conformational analysis, made the structure assignment of thiacalixarene derivatives much more difficult when compared with classical calixarenes. Theoretically, four different conformations **5a**-**5d** can be prepared (Scheme 1). Using standard Nuclear Overhauser Effect (NOE) experiments we succeeded to unambiguously determine that compound **5c** adopted the *partial cone* conformation with inversion of ring D (*D-paco*). This conclusion was made based on the NOE contacts found between one aromatic proton of cycle C and one aromatic proton of cycle B, and between the second proton of ring C and the methylene protons (-O-$CH_2$-) attached to ring D (see ESI, Fig. 6). Unfortunately, a similar structural study using NOE could not be performed with compound **5d** due to the severe overlaps in the aromatic region of the $^1$H NMR spectrum. The only clear NOE contact between the aromatic protons of cycles C and D was consistent either with the *cone* (**5a**) or *1,2-alternate* (**5d**) conformations (see ESI, Fig. 12).

### Application of RDCs in structural elucidation of calixarene derivatives

To obtain more structural information about compound **5d** we turned our attention to the method utilizing residual dipolar coupling constants (RDCs).[11] At the same time, this method was also used to confirm the *D-paco* conformation of **5c** obtained by NOE experiment, and thus, to assess the general applicability of this method in the conformational analysis of calixarenes. While the RDC method has recently found a number of applications in the structural elucidation of small organic molecules, to the best of our knowledge, only a handful papers have been reported in calixarene chemistry so far.[12]

The principal of the RDCs method is the measuring of an anisotropic through-space dipole-dipole interaction, that is averaged to zero in solution due to fast molecular tumbling. However, if a sample is partially aligned, the anisotropic interaction becomes observable and contributes to the *J* scalar interaction. As this interaction (*D*) depends on the distance of the coupled nuclei and their orientation within the external magnetic field, the values of the RDCs contain detailed spatial information. Thus, in contrast to data obtained from $^3J$ scalar coupling constants and NOE experiments, the RDCs method can provide "long-range" information. To produce the partial alignment of organic molecules soluble in non-polar solvents, either stretched/compressed polymer gels or lyotropic liquid crystalline (LLC) solutions can be utilized.

### LLC based alignment media

During our continuous study on the conformational analysis of inherently chiral calixarenes we examined a number of recently published LLC based alignment media, such as poly-γ-benzyl-L-glutamate (PBLG),[13] poly-γ-ethyl-L-glutamate (PELG),[14] phenylalanine-derived polyacetylene,[15] valine-derived polyacetylene[16] and polyisocyanide.[17] The applicability of these media has been evaluated mainly with respect to the induced appropriate degree of the alignment, the availability of the alignment media, and the ease of sample preparation. Taking all aspects into consideration the polyglutamate-based homopolypeptides PBLG and PELG were used for the preparation of aligned anisotropic samples of both **5c** and **5d**.

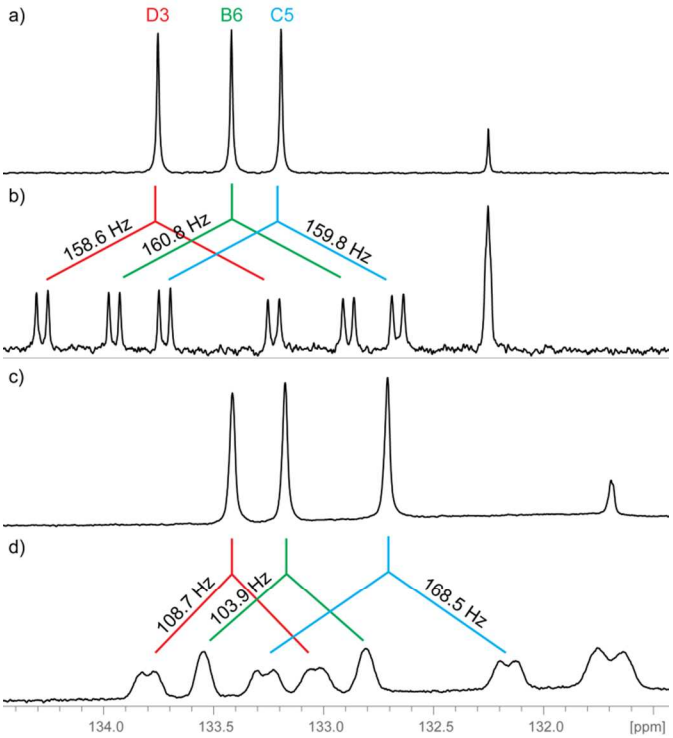



**Figure 1.** Partials of $^{13}$C NMR (150 MHz) spectra of compound **5d** a) $^{13}$C decoupled spectra, isotropic phase in $CDCl_3$, b) $^{13}$C coupled spectra, isotropic phase in $CDCl_3$, c) $^{13}$C decoupled spectra, anisotropic phase in PBLG, $CDCl_3$, d) $^{13}$C coupled spectra, anisotropic phase in PBLG, $CDCl_3$. $^1J_{C\text{-}H}$ and $^1T_{C\text{-}H}$ coupling constant ($J$ = isotropic, $T$ = total) are extracted.

### Measurement of RDCs and structure calculation

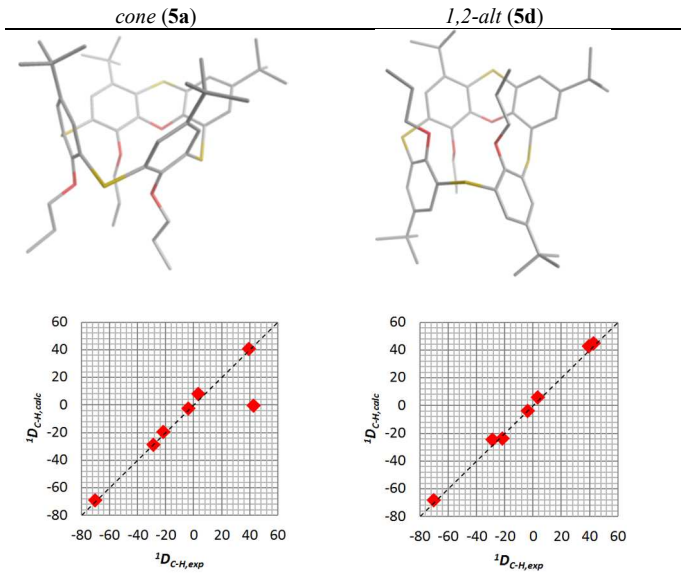
RDCs for the structure determination of both **5c** and **5d** in both PBLG and PELG alignment media were extracted from z-restored $^{13}$C coupled spectra[18] (Fig. 1) or CLIP-HSQC[19] spectra. RDCs were then calculated using the following equation:

$$^1T_{C\text{-}H} = 2\,^1D_{C\text{-}H} + {}^1J_{C\text{-}H},$$

where $^1T_{C\text{-}H}$ is the one-bond heteronuclear coupling constant (total splitting) elucidated from coupled $^{13}$C NMR or CLIP-HSQC spectra of the anisotropic solution, $^1D_{C\text{-}H}$ is the one-bond heteronuclear residual dipolar coupling constant, and $^1J_{C\text{-}H}$ represents the one-bond heteronuclear scalar coupling constant elucidated from coupled $^{13}$C NMR or CLIP-HSQC spectra of the isotropic solution.

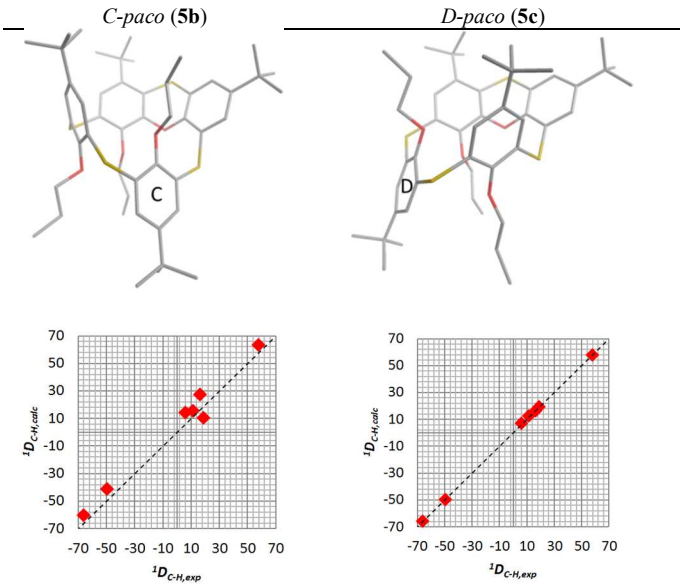
To generate four initial input structures (structural proposals) of **5** (*cone*, *1,2-alt*, *C-paco* and *D-paco*), the *ab initio* calculations (RB3LYP/6-31G* level in Gaussian03[†]) were utilized.[20] Subsequently, the four optimized structure of **5** (Table 1 and 2) together with the experimental one bond RDCs ($^1D_{C\text{-}H}$) were used for the fitting procedure in the program PALES.[21] The comparison of the observed and the back calculated RDCs for *D-paco* of **5c** and *1,2-alt* of **5d** in PBLG resulted in excellent linear correlations (Table 1 and 2). Conversely, the fitting procedures for *C-paco* of **5c** and *cone* of **5d** in PBLG provided large discrepancy between the experimental and the calculated data (Table 1 and 2).

**Table 1** The fitting procedure results for the *cone* (**5a**) and *1,2-alt* (**5d**) – correlations between back-calculated and experimental RDCs.

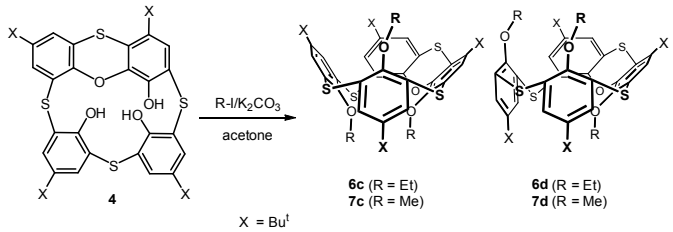



The fitting results for compounds **5c** and **5d** in both alignment media PBLG and PELG for all structural proposals are summarized in Table 3. It is obvious that in both cases PBLG provided slightly better results than PELG due to narrower molecular weight distribution of the alignment medium. Moreover, the fitting results for the spatially different structural proposals of matching **5d** (*1,2-alternate*) and non-matching **5a** (*cone*) differ significantly. In contrast, the fitting results of the structurally very similar **5b** (*C-paco*) and **5c** (*D-paco*) conformations were less distinct.

**Table 2** The fitting procedure results for *partial cone* conformers *C-paco* (**5b**) and *D-paco* (**5c**) – correlations between back-calculated and experimental RDCs.




To provide greater insight into the conformational preferences and to assess the sterical requirements for the immobilization of the phenoxathiin-based skeleton, derivative **4** was alkylated using shorter alkyl groups. As shown in Scheme 2 alkylation with EtI ($K_2CO_3$/acetone) provided again the mixture of only two isomers that were assigned as **6c** and **6d** as per the assignment of isomers **5c** and **5d**. Heating of these samples up to 130 °C did not lead to any change in the $^1$H NMR spectra thus indicating that the ethyl groups were bulky enough for the immobilization of the macrocycle. Interestingly, the same reaction with MeI gave two isomers that were separated using preparative TLC. Standard NMR experiments, including NOE revealed that the structures can be assigned as **7c** (*D-paco)* and **7d** (*1,2-alternate*) – the same conformations as described above (See ESI, Fig. S13-S24). Due to the well resolved $^1$H and $^{13}$C NMR spectra it was not necessary to use the RDC method in this case. Isolation of the two different conformations is very surprising as, to the best of our knowledge, this is the first example of calix[4]arene derivatives where the methyl groups were bulky enough for immobilization of a specific conformers at room temperature.




**Scheme 2.** Alkylation of the phenoxathiin-based thiacalix[4]arene.

The dynamic behaviour of conformations **7c** (*D-paco)* and **7d** (*1,2-alternate*) was studied using temperature dependent $^1$H NMR spectra. Each compound (dissolved in 1,1,2,2-tetrachloroethane-$D_2$) was first gradually heated up to 413 K and then cooled back down to room temperature. Surprisingly, after the cooling down to 298 K, both $^1$H NMR spectra became identical and corresponded to the original spectrum of **7c** (*D-paco*) (Fig. 2). This confirmed that the conformer *D-paco* (**7c)** is thermodynamically much more stable than the *1,2-alternate* (**7d**) conformer. The above finding is in accordance with the results obtained by *ab initio* calculation (RB3LYP/6-31G* level in Gaussian03[†]) for the propyl substituted analogues, where **5c** was favoured over **5d** by 24.07 kJ mol$^{-1}$ (see ESI, Table 7). These results indicated that the formation of conformations 7**c** and 7**d** in almost the same ratio (29% vs 26% yields) was the result of a template effect of the base used ($K^+$ cation) rather than the result of thermodynamic stability of the products. The calculations also showed that the remaining two conformers **5a** (*cone*) and **5b** (*C-paco*), never observed in the reaction mixture, would be even less stable by 29.78 and 30.40 kJ mol$^{-1}$ if compared with **5c**.

**Table 3** The comparison of fitting results of possible conformers (**5a**, **5b**, **5c** and **5d**) in PELG and PBLG alignment media.

| | PELG | | PBLG | |
|---|---|---|---|---|
| | RMS [Hz] [a] | $R^2$ [%][a] | RMS [Hz] [a] | $R^2$ [%][a] |
| *Cone* (**5a**) | 21.8 | 68.3 | 16.8 | 81.6 |
| *1,2-alt* (**5d**) | 3.0 | 99.8 | 2.0 | 99.8 |
| *C-paco* (**5b**) | 6.9 | 96.7 | 7.3 | 97.8 |
| *D-paco* (**5c**) | 0.9 | 100.0 | 0.2 | 100.0 |

[a] The lower is the root mean square (RMS) and the higher is the square of correlation coefficient ($R^2$), the better is the fit.

## Conclusions

In conclusion, thiacalix[4]arene spirodienone **3** was rearranged into the corresponding phenoxathiin-based macrocycle **4** under acidic conditions. The alkylation of this inherently chiral system to achieve immobilization led to a mixture of only two (out of four theoretically possible) stereoisomers. As standard NOE and dynamic NMR experiments did not lead to unambiguous determination of the structures we applied the method utilizing the Residual Dipolar Coupling constants. Both PELG (poly-γ-ethyl-L-glutamate) and PBLG (poly-γ-benzyl-L-glutamate) were found to be easily applicable lyotropic liquid crystalline alignment media for the conformational analysis of thiacalixarene derivatives. Using these media the *1,2-alternate* **5d** and the *partial cone* **5c** conformations were determined unequivocally for the propyl substituted derivatives. Dynamic NMR experiments carried out with the corresponding methyl substituted conformers revealed that the *partial cone* conformation **7c** (*D-paco*) represents the most thermodynamically favoured stereoisomer.

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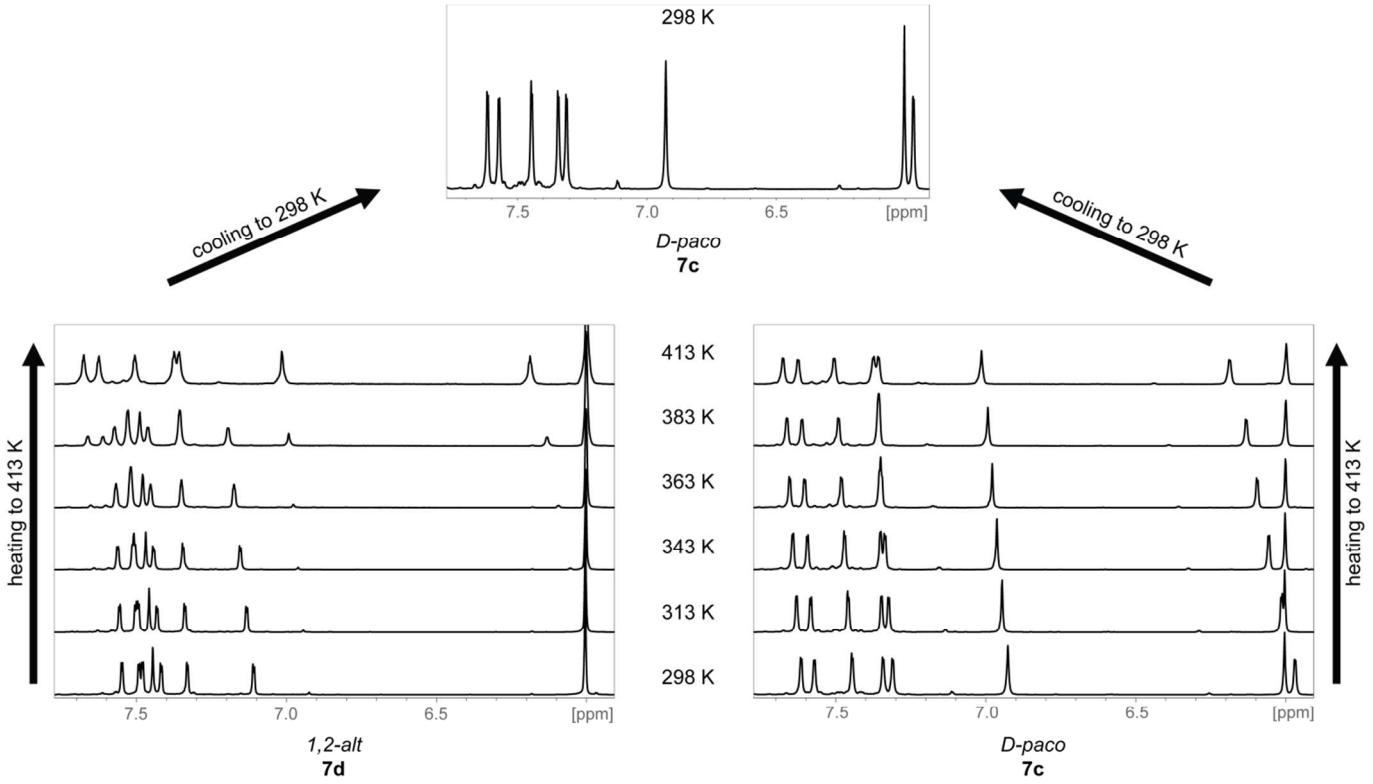



**Figure 2.** An illustration of the heating and cooling process of compounds **7c** and **7d** demonstrated in the aromatic region of 500 MHz $^{1}$H NMR spectra.

## Experimental

### General experimental procedures

All chemicals were purchased from commercial sources and used without further purification. $Hg(ClO_4)_2 \cdot 3H_2O$ was stored in a desiccator filled with $P_2O_5$. Solvents were dried and distilled using conventional methods. Melting points were measured on Heiztisch Mikroskop - Polytherm A (Wagner & Munz, Germany) and are not corrected. IR spectra were measured on an FT-IR spectrometer Nicolet 740 in $CHCl_3$ and/or in KBr at a spectral resolution of 4 $cm^{-1}$. NMR spectra were performed on Varian Gemini 300 ($^{1}$H: 300 MHz, $^{13}$C: 75 MHz), Bruker Avance III$^{TM}$ ($^{1}$H: 500 MHz, $^{13}$C: 125 MHz) and on Bruker 600 Avance$^{III}$ ($^{1}$H: 600.1 MHz, $^{13}$C: 150.9 MHz) spectrometers. Deuterated solvents used are indicated in each case. Chemical shifts (δ) are expressed in ppm and are referenced to the residual peak of solvent or TMS as an internal standard; coupling constants (*J*) are in Hz. $^{199}$Hg chemical shifts were referenced to diphenylmercury(II) as an external standard in $CD_2Cl_2$ or $CDCl_3$ (0.5 M solutions). Signal assignment was supported by $^{1}$H-$^{1}$H COSY, $^{1}$H-$^{13}$C HMQC or $^{1}$H-$^{13}$C HMBC 2D NMR and 1D $^{1}$H-DPFGSE NOE experiments using the standard pulse sequences provided by Bruker. For RDCs extractions either z-restored (zrsegp) or F2-coupled clean inphase HSQC (CLIP-HSQC) were utilized. Mass analyses were performed using ESI technique on a Q-TOF (Micromass) spectrometer. The purity of the substances and the courses of reactions were monitored by TLC using TLC aluminum sheets with Silica gel 60 $F_{254}$ (Merck) and analyzed at 254 or 365 nm. Column chromatography was performed using Silica gel Geduram 60 $F_{254}$ (Merck, particle size 0,063-0,200 mm). Preparative TLC chromatography was carried out on 20 x 20 cm glass plates covered by Silica gel 60 $GF_{254}$ (Merck).

### Preparation of NMR samples for RDCs measurements

A solution of each compound in the alignment medium was prepared directly in an NMR tube due to the high viscosity of liquid crystalline solution. A weighted amount of alignment medium PELG (Sigma-Aldrich, mol wt >100,000) or PBLG

(Sigma-Aldrich, mol wt 150,000-350,000), calixarene derivative, and $CDCl_3$ (ARMAR Chemicals, 99.8 Atom%D) were added to an NMR tube. To obtain well resolved spectra it was necessary to homogenize the sample. After a few hours, the NMR tube was centrifuged upside down to mix up the viscous content. Then a capillary with DMSO-$d_6$ (ARMAR Chemicals, 99.8 Atom%D) was added as an external standard. Homogenization of the anisotropic sample was repeated until the lines of split $CDCl_3$ and non-split DMSO-$d_6$ in the $^2$H NMR spectrum were narrow and no signal of residual non-aligned solvent was observed.

**Table 3** Preparation of anisotropic solutions.

| | PELG | | PBLG | |
|---|---|---|---|---|
| | **5c** (*D-paco*) | **5d** (*1,2-alt*) | **5c** (*D-paco*) | **5d** (*1,2-alt*) |
| $m_{MED}$[a] *[mg]* | 72.9 | 73.4 | 70.9 | 71.0 |
| $m_{CMPD}$[b] *[mg]* | 20.0 | 20.0 | 24.6 | 24.8 |
| $m_{SOLV}$[c] *[mg]* | 964.5 | 865.0 | 840.0 | 825.0 |
| $w_{MED}$ *[%]* | 6.9 | 7.7 | 7.6 | 7.7 |
| $\nu_Q$[d] *[Hz]* | 440 | 449 | 236 | 242 |

[a] weight of alignment medium (PELG or PBLG); [b] weight of calixarene derivative; [c] weight of solvent ($CDCl_3$); [d] quadrupolar splitting of DMSO-$d_6$ in the capillary

**Synthetic procedures**

**Mono(spirodienon)-*p*-tert-butylthiacalix[4]arene (2) and Bis(spirodienone)-*p*-tert-butylthiacalix[4]arenes (3 and 3').** Compound **1** (1.00 g, 1.40 mmol) was dissolved in a mixture of chloroform (90 ml) and methanol (30 ml). The solution was cooled to 0 °C in an ice bath, chloramine T trihydrate was added (0.587 g, 2.1 mmol, 1.5 equiv) and the reaction mixture was stirred for 2 h. The mixture was quenched with brine, concentrated *in vacuo*, and extracted with $CH_2Cl_2$. The combined organic layers were washed with water, dried over $MgSO_4$, and evaporated *in vacuo* to dryness. The crude product was purified by column chromatography on $SiO_2$ ($CH_2Cl_2$:hexane = 7:10 v/v) to yield 580 mg (58%) of **2** as an orange solid. Further elution with $CH_2Cl_2$ yielded 28 mg (3%) of bis(spirodienone) **3/3'** fraction as a red solid.

**2:** $^1$H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): 8.25 (s, 1H, -OH); 7.79 (s, 1H, -OH); 7.62 (d, 1H, Ar-H, *J* = 2.6 Hz); 7.54 (d, 1H, Ar-H, *J* = 2.5 Hz); 7.53 (d, 1H, Ar-H, *J* = 2.3 Hz); 7.44 (d, 1H, Ar-H, *J* = 2.6 Hz); 7.33 (d, 1H, Ar-H, *J* = 2 Hz); 7.27 (d, 1H, Ar-H, *J* = 2.5 Hz); 7.03 (d, 1H, Ar-H, *J* = 2 Hz); 6.44 (d, 1H, Ar-H, *J* = 2.3 Hz); 1.25 (s, 9H, *t*-Bu); 1.24 (s, 9H, *t*-Bu); 1.24 (s, 9H, *t*-Bu); 1.22 (s, 9H, *t*-Bu). All characteristics are in agreement with those previously described.[9]

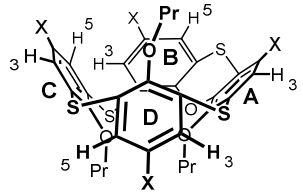
**3/3':** M.p. 300-305 °C. IR (KBr) ν ($cm^{-1}$): 1701. MS-ESI ($C_{40}H_{44}O_4S_4$) *m/z* calcd.: 739.18 $[M+Na]^+$, found: 739.31 $[M+Na]^+$. $^1$H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): (major isomer) 7.41 (d, 2H, Ar-H, *J* = 2.2 Hz), 6.81 (s, 4H, C=CH), 6.24 (d, Ar-H, *J* = 2.2 Hz), 1.15 (s, 18H, *t*-Bu), 1.09 (s, 18H, *t*-Bu). $^{13}$C NMR ($CDCl_3$, 75 MHz, 298 K) δ (ppm): 186.57 (**C**=O), 149.67, 145.90, 141.30 (Ar, **C**-H), 129.95, 127.80 (Ar, **C**-H), 127.32, 121.09 (C=**C**-H), 117.22 (C=**C**-H), 116.40, 90.17, 34.91 (dienone-**C**$(CH_3)_3$), 34.48(Ar-**C**$(CH_3)_3$), 31.36 (Ar-C(**C**$H_3)_3$), 28.37 (dienone-C(**C**$H_3)_3$). $^1$H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): (minor isomer) 7.51 (d, 2H, Ar-**H**, *J* = 2.3 Hz), 7.11 (d, 2H, C=C**H**, *J* = 1.9 Hz), 7.01 (d, 2H, C=C**H**, *J* = 2.0 Hz), 6.35 (d, 2H, Ar-**H**, *J* = 2.4 Hz), 1.21 (s, 18H, *t*-**Bu**), 1.18 (s, 18H, *t*-**Bu**), 1.15 (s, 18H, *t*-**Bu**), 1.05 (s, 18H, *t*-**Bu**).

**Rearranged calix[4]arene derivative (4).** Spirodienone **2** (500 mg, 0.70 mmol) was suspended in a mixture of acetonitrile (600 ml) and conc. aqueous HCl (15 ml), and the reaction mixture was heated at reflux for 90 min. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. Trituration with $CHCl_3$ gave a white powder (product of acetonitrile self-condensation) which was removed by filtration. The remaining clear solution from the filtration was evaporated to dryness, and the crude product was recrystallized from MeCN:$CHCl_3$ (3:1 v/v) to give 463 mg (92%) of compound **4** as white crystals. $^1$H NMR ($CDCl_3$, 300 MHz, 298 K) δ (ppm): 8.38 (s, 1H, -OH), 8.37 (s, 1H, -OH), 7.82 (s, 1H, -OH), 7.77 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.49 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.45 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.37 (d, 2H, Ar-H, *J* = 2.6 Hz), 7.34 (s, 1H, Ar-H), 7.27 (d, 1H, Ar-H, *J* = 2.3 Hz), 1.43 (s, 9H, *t*-Bu), 1.31 (s, 9H, *t*-Bu), 1.15 (s, 9H, *t*-Bu), 1.14 (s, 9H, *t*-Bu). All characteristics are in agreement with those previously described.[10].

**Tripropoxy derivative (*partial cone* – D inverted) (5c) and tripropoxy derivative (*1,2-alternate*) (5d).** Spirodienone **2** (100 mg, 0.140 mmol) was dissolved in 20 ml of dry acetone and $K_2CO_3$ (0.084 g, 6 equivs., 0.84 mmol) was added. The reaction mixture was stirred for 30 min at room temp. Propyl iodide (284 mg, 12 equivs.) was then added and the mixture was heated to reflux for one week. The reaction mixture was then cooled to rt and evaporated to dryness *in vacuo*. The residue was diluted with water and extracted with $CH_2Cl_2$ (3 x 20 ml). The collected organic fractions were washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by preparative TLC on $SiO_2$ (EtOAc:hexane = 1:8 v/v) to yield 35 mg (29%) of **5c** and 23 mg (20%) of **5d**.

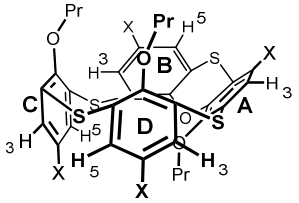
**5c**: White solid, m.p. 205-208 °C (DCM/MeOH). $^1$H NMR ($CDCl_3$, 600 MHz, 298 K) δ (ppm): 7.64 (d, 1H, H-3-B, *J* = 2.3 Hz), 7.46 (d, 1H, H-5-D, *J* = 2.5 Hz), 7.35 (d, 1H, H-3-D, *J* = 2.5 Hz), 7.26 (d, 1H, H-5-B, *J* = 2.3 Hz), 7.17 (d, 1H, H-3-C, *J* = 2.4 Hz), 6.98 (s, 1H, H-3-A), 6.36 (d, 1H, H-5-C, *J* = 2.4 Hz), 4.42 (m, 1H, -$OCH_2$´-A), 4.07 (m, 1H, -$OCH_2$´-C, 3.67 (m, 1H, -$OCH_2$´´-C), 3.66 (m, 1H, -$OCH_2$´-D), 3.58 (m, 1H, -$OCH_2$´´-D); 3.55 (m, 1H, -$OCH_2$´´-A), 1.75 (m, 1H, -$CH_2$´-D), 1.72 (m, 2H, -$CH_2$-C), 1.42 (m, 1H, -$CH_2$´-A), 1.38 (s, 9H, *t*-Bu-A), 1.38 (s, 9H, *t*-Bu-B), 1.35 (s, 9H, *t*-Bu-D), 1.21 (m, 1H, -$CH_2$´´-A), 1.02 (t, 3H, -$CH_3$-C, *J* = 7.6 Hz), 0.93 (s, 9H, *t*-Bu-C), 0.85 (m, 1H, -$CH_2$´´-D), 0.79 (t, 3H, -$CH_3$-A, *J* = 7.6 Hz), 0.42 (t, 3H, -$CH_3$-D, *J* = 7.6 Hz). $^{13}$C NMR ($CDCl_3$, 150.9 MHz, 298 K, $CDCl_3$) δ (ppm): 156.2 (1-D), 154.8 (1-C), 150.0 (1-B), 147.4 (4-B), 146.5 (1-A), 144.9 (4-D), 144.6 (4-C), 139.3 (4-A), 135.7 (3-B), 129.2 (3-C), 128.7 (5-D), 128.0 (3-D), 126.8 (5-C), 124.8 (5-B), 121.9 (3-A), 75.5 (-$OCH_2$-A), 75.0 (-$OCH_2$-C), 71.5 (-$OCH_2$-D), 35.8 (q. *t*-Bu-A), 34.6 (q. *t*-Bu-B), 34.3 (q. *t*-Bu-D), 33.9 (q. *t*-Bu-C), 31.5 (*t*-Bu-B), 31.4 (*t*-Bu-D), 30.9 (*t*-Bu-C), 30.2 (*t*-Bu-A), 23.5 (-$CH_2$-C), 22.9 (-$CH_2$-A), 22.3 (-$CH_2$-D), 10.9 (-$CH_3$-C), 10.4 (-$CH_3$-A), 10.0 (-$CH_3$-D), 147.5,

132.2, 128.3, 127.7, 126.8, 125.3, 124.2, 123.8 and 117.1 (q. 2-A, 2-C, 2-D, 6-D, 6-C, 5-A, 2-B, 6-B and 6-A). HR MS-ESI ($C_{49}H_{64}O_4S_4$) *m/z* (% int.) calcd.: 867.35796 $[M+Na]^+$, found: 867.35825 $[M+Na]^+$ (100).




**5c** (X = Bu$^t$)
*D-paco*

**5d**: White solid, m.p.: 195-198 °C (DCM). $^1$H NMR ($CDCl_3$, 600 MHz, 298 K) δ (ppm): 7.55 (d, 1H, H-3-B, *J* = 2.3 Hz), 7.52 (d, 1H, H-3-C, *J* = 2.5 Hz), 7.37 (d, 1H, H-3-D, *J* = 2.5 Hz), 7.34 (d, 1H, H-5-B, *J* = 2.5 Hz), 7.33 (s, 1H, H-3-A), 7.31 (d, 1H, H-5-B, *J* = 2.3 Hz), 7.24 (d, 1H, H-5-C, *J* = 2.5 Hz), 4.18 (m, 1H, -$OCH_2$´-A), 3.75 (m, 1H, -$OCH_2$´-D, 3.66 (m, 1H, -$OCH_2$´´-D), 3.58 (m, 1H, -$OCH_2$´´-A), 3.47 (m, 1H, -$OCH_2$´-C); 2.59 (m, 1H, -$OCH_2$´´-C), 1.53 (s, 9H, *t*-Bu-A), 1.46 (m, 1H, -$CH_2$-D), 1.30 (s, 9H, *t*-Bu-C), 1.28 (s, 9H, *t*-Bu-B), 1.20 (s, 9H, *t*-Bu-D), 1.06 (m, 2H, -$CH_2$-A), 0.90 (m, 1H, -$CH_2$´-C), 0.85 (t, 3H, -$CH_3$-D, *J* = 7.6 Hz), 0.37 (t, 3H, -$CH_3$-A, *J* = 7.6 Hz), 0.29 (t, 3H, -$CH_3$-C, *J* = 7.6 Hz). $^{13}$C NMR ($CDCl_3$, 150.9 MHz, 298 K, $CDCl_3$) δ (ppm): 157.6 (1-D), 157.2 (1-C), 154.3 (1-B), 148.8 (1-A), 147.3 (4-B), 145.8 (4-C), 145.4 (4-D), 140.4 (4-A), 133.8 (5-D), 133.4 (3-B), 133.2 (3-C), 129.9 (3-D), 126.5 (5-C), 125.0 (5-B), 124.8 (3-A), 77.0 (-$OCH_2$-C), 76.2 (-$OCH_2$-D), 74.2 (-$OCH_2$-A), 35.9 (q. *t*-Bu-A), 34.9 (q. *t*-Bu-B), 34.3 (q. *t*-Bu-C), 34.1 (q. *t*-Bu-D), 31.4 (*t*-Bu-B), 31.4 (*t*-Bu-C), 31.2 (*t*-Bu-D), 30.3 (*t*-Bu-A), 22.9 (-$CH_2$-D), 22.8 (-$CH_2$-A), 20.9 (-$CH_2$-C), 10.7 ($CH_3$-D), 10.1 ($CH_3$-A), 9.2 ($CH_3$-C), 147.8, 132.3, 130.0, 129.1, 127.7, 126.9, 124.1, 122.8 and 122.2 (q. 2-A, 6-C, 2-C, 6-C, 2-D, 6-A, 6-B, 2-B and 5-A). MS-ESI ($C_{49}H_{64}O_4S_4$) *m/z* (% int.) calcd.: 867.35796 $[M+Na]^+$, found: 867.35748 $[M+Na]^+$ (100).




**5d** (X = Bu$^t$)
*1,2-alt*

The same reaction conditions as described above using $Cs_2CO_3$ as a base provided **5d** in 37% and **5c** in 18% yield, respectively, after the preparative TLC.

**Triethoxy derivative (*partial cone*) (6c) and triethoxy derivative (*1,2-alternate*) (6d).** The same procedure as described for the preparation of compounds **5c**/**5d** starting from spirodienone **2** (100 mg, 0.140 mmol), 20 ml of dry acetone, $K_2CO_3$ (0.084 g, 6 equivs., 0.84 mmol), and ethyl iodide (260 mg, 12 equivs.). The crude product was purified by preparative TLC on $SiO_2$ (EtOAc:hexane = 1:6 v/v) to yield 32 mg (29%) of **6c** and 27 mg (25%) of **6d**.

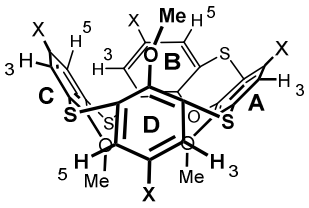
**6c**: White solid, m.p. 221-227 °C (DCM/MeOH). $^1$H NMR ($CDCl_3$, 300 MHz, 298 K, $CDCl_3$) δ (ppm): 7.65 (d, 1H, Ar-H, *J* = 2 Hz), 7.47 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.36 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.27 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.19 (d, 1H, Ar-H, *J* = 2.3 H), 6.99 (s, 1H, Ar-H), 6.28 (d, 1H, Ar-H, *J* = 2.3 Hz), 4.43 (m, 1H, -CH(H)), 4.12 (m, 1H, -CH(H)), 3.81 (m, 2H, -$OCH_2$-), 3.68 (m, 2H, -$OCH_2$-), 1.72 (m, 3H, -$CH_3$), 1.55 (m, 3H, -$CH_3$), 1.35 (s, 18H, *t*-Bu), 1.28 (s, 9H, *t*-Bu), 0.96 (s, 9H, *t*-Bu), 0.78 (t, 3H, -$CH_3$, *J* = 7 Hz). $^{13}$C NMR ($CDCl_3$, 75.4 MHz, 298 K, $CDCl_3$) δ (ppm): 156.20, 154.67, 150.14, 148.14, 147.63, 146.58, 145.32, 144.88, 139.91, 135.89, 132.97, 129.51, 128.83, 128.78, 128.17, 127.99, 127.23, 127.18, 125.99, 125.03, 124.58, 124.23, 121.96, 117.13, 68.84, 68.96, 41.69, 36.32, 36.08, 34.90, 34.77, 34.55, 24.04, 31.84, 31.09, 29.95, 29.61, 27.97, 27.14, 25.50, 22.89, 22.82, 20.89, 18.98, 15.89, 15.711, 14.66, 14.51, 14.31. MS-ESI ($C_{46}H_{58}O_4S_4$) *m/z* (% int.) calcd.: 825.31 $[M+Na]^+$, found: 825.12 $[M+Na]^+$ (100).

**6d**: Yellowish solid, m.p. 205-210 °C (DCM/MeOH). $^1$H NMR ($CDCl_3$, 300 MHz, 298 K, $CDCl_3$) δ (ppm): 7.55 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.51 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.36 (d, 1H, Ar-H, *J* = 2.6 Hz), 7.33 (d, 2H, Ar-H, *J* = 2.3 Hz), 7.32 (s, 1H, Ar-H), 7.31 (d, 1H, Ar-H, *J* = 2.3 Hz), 7.24 (d, 1H, Ar-H, *J* = 2.6 Hz), 4.43 (m, 1H, -$OCH_2$-), 4.12 (m, 1H, -$OCH_2$-), 3.81 (m, 2H, -$OCH_2$-), 3.68 (m, 2H, -$OCH_2$-), 1.55 (m, 3H, -$CH_3$) 1.48 (s, 9H, *t*-Bu), 1.46 (s, 9H, *t*-Bu), 1.22 (t, 3H, -$CH_3$, *J* = 7.2 Hz), 0.99 (s, 9H, *t*-Bu), 0.85 (s, 9H, *t*-Bu), 0.63 (t, 3H, -$CH_3$, *J* = 7.2 Hz). $^{13}$C NMR ($CDCl_3$, 75.4 MHz, 298 K, $CDCl_3$) δ (ppm): 158.31, 156.12, 151.89, 149.21, 147.61, 146.01, 144.95, 144.88, 140.91, 137.12, 134.27, 130.43, 129.13, 128.52, 128.04, 127.75, 126.91, 126.18, 125.91, 125.43, 123.27, 124.11, 122.37, 118.08, 70.42, 69.24, 43.58, 38.37, 37.91, 35.47, 34.00, 33.61, 32.81, 31.11, 30.22, 29.45, 29.02, 28.25, 26.12, 25.58, 23.79, 21.62, 20.51, 19.99, 16.42, 15.11, 14.72, 13.21, 12.85. MS-ESI ($C_{46}H_{58}O_4S_4$) *m/z* (% int.) calcd.: 825.31 $[M+Na]^+$, found: 825.12 $[M+Na]^+$ (100).

**Trimethoxy derivative (*partial cone*– D inverted) (7c) and trimethoxy derivative (*1,2-alternate*) (7d).** The same procedure as described for the preparation of compounds **5c**/**5d** starting from spirodienone **2** (100 mg, 0.140 mmol), 20 ml of dry acetone, $K_2CO_3$ (0.084 g, 6 equiv., 0.84 mmol), and methyl iodide (260 mg, 12 equivs.). The crude product was purified by preparative TLC on $SiO_2$ (EtOAc:hexane = 1:6 v/v) to yield 32 mg (29%) of **7c** and 27 mg (25%) of **7d**.

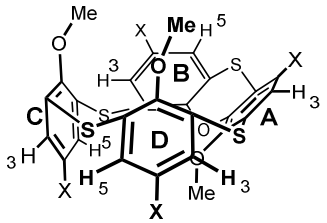
**7c**: White solid, m.p. 152-156 °C (MeOH). $^1$H NMR ($C_2D_2Cl_4$, 600.13 MHz, 298 K), δ (ppm): 7.64 (d, 1H, H-3-*B*, *J* = 1.7 Hz); 7.60 (d, 1H, H-5-*D*, *J* = 2.0 Hz); 7.47 (d, 1H, H-3-*D*, *J* = 1.9 Hz); 7.37 (d, 1H, H-5-*B*, *J* = 1.7 Hz); 7.34 (d, 1H, H-3-*C*, *J* = 1.5 Hz); 6.95 (s, 1H, H-3-*A*); 6.00 (d, 1H, H-5-*C*, *J* = 1.9 Hz); 3.89 (s, 3H, $OCH_3$-*C*); 3.54 (s, 3H, $OCH_3$-*D*); 3.39 (s, 3H, $OCH_3$-*A*); 1.59 (s, 9H, *t*-Bu-*A*); 1.42, 1.40 and 1.40 (3 x s, 27H, 3 x *t*-Bu-*A, B, D*); 0.88 (s, 9H, *t*-Bu-*C*). $^{13}$C NMR ($C_2D_2Cl_4$, 150.92 MHz, 298 K), δ (ppm): 157.8 (C-1-*D*); 155.1 (C-1-*C*); 150.1 (C-1-*B*); 148.25 and 148.20 (C-5-*A* and C-4-*B*); 146.1 (C-1-*A*); 145.5 (C-4-*A*); 144.7 (C-4-*C*); 140.7 (C-4-*D*); 135.5 (C-3-*B*); 132.5, 128.3, 126.9, 126.2, 126.1, 124.4, 123.5 and 116.9 (8 x C-2,6); 131.9 (C-3-*C*); 130.3 (C-5-*D*); 129.3 (C-3-*D*); 125.6 (C-5-*A*); 125.2 (C-5-*B*); 120.6 (C-3-*A*); 60.3 ($OCH_3$-*C*); 59.9 ($OCH_3$-*A*); 57.7 ($OCH_3$-*D*); 36.0, 34.6, 34.3 and 33.7 (4 x quater. *t*-Bu-); 31.5, 31.4, 30.8 and 30.3 (4 x *t*-Bu-). MS-ESI ($C_{43}H_{52}O_4S_4$)

*m/z* (% int.) calcd.: 783.26406 $[M+Na]^+$, found: 783.26446 $[M+Na]^+$ (100).



**7c** (X = Bu$^t$)
*D-paco*

**7d**: White solid, m.p. 233-236 °C (MeOH). $^1$H NMR ($C_2D_2Cl_4$, 600.13 MHz, 298 K), δ (ppm): 7.57 (d, 1H, H-3-*C*, *J* = 2.5 Hz); 7.52 (d, 1H, H-3-*D*, *J* = 2.5 Hz); 7.57 (d, 1H, H-3-*C*, *J* = 2.5 Hz); 7.47 (s, 1H, H-3-*A*); 7.44 (d, 1H, H-5-*C*, *J* = 2.5 Hz); 7.36 (d, 1H, H-5-*B*, *J* = 2.3 Hz); 7.14 (d, 1H, H-5-*D*, *J* = 2.5 Hz); 3.76 (s, 3H, $OCH_3$-*A*); 3.64 (s, 3H, $OCH_3$-*D*); 2.34 (s, 3H, $OCH_3$-*C*); 1.59 (s, 9H, *t*-Bu-*A*); 1.37 (s, 9H, *t*-Bu-*C*); 1.27 (s, 9H, *t*-Bu-*B*); 1.18 (s, 9H, *t*-Bu-*D*). $^{13}$C NMR ($C_2D_2Cl_4$, 150.92 MHz, 298 K), δ (ppm): 158.9 (C-1-*D*); 158.6 (C-1-*C*); 154.0 (C-1-*B*); 149.2 (C-5-*A*); 148.20 and 148.26 (C-1-*A* and C-4-*B*); 146.4 (C-4-*D*); 145.9 (C-4-*C*); 140.7 (C-4-*A*); 133.9 (C-5-*D*); 133.5 (C-3-*B*); 133.2 (C-3-*C*); 132.3, 130.6, 129.0, 128.0, 126.8, 125.0, 124.4 and 123.1 (8 x C-2,6); 131.7 (C-3-*D*); 128.8 (C-5-*C*); 125.7 (C-3-*A*); 125.0 (C-5-*B*); 60.9 ($OCH_3$-*D*); 60.2 ($OCH_3$-*A*); 58.9 ($OCH_3$-*C*); 36.0 (quater. *t*-Bu-*A*); 34.6 (quater. *t*-Bu-*B*); 34.3 (quater. *t*-Bu-*C*); 34.1 (quater. *t*-Bu-*D*); 31.5 (*t*-Bu-*C*); 31.4 (*t*-Bu-*B*); 31.3 (*t*-Bu-*D*); 30.7 (*t*-Bu-*A*). MS-ESI ($C_{46}H_{58}O_4S_4$) *m/z* (% int.) calcd.:



**7d** (X = Bu$^t$)
*1,2-alt*

783.26406 $[M+Na]^+$, found: 783.26444 $[M+Na]^+$ (100).

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## Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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