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ORIGINAL PAPER

HPLC Characterization of *cis* and *trans* Mixtures of Double-Decker Shaped Silsesquioxanes

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Abstract
HPLC was used as the quantitative analysis technique in determining the molar ratio of *cis* and *trans* isomers in the double-decker shaped silsesquioxanes (DDSQ). Different experiments were performed to analyze the effects in the retention times of the polarity of moieties bonded to the DDSQ, as well as other possible adsorption driving forces. As expected, the use of adsorption HPLC was successful in separating *cis* and *trans* DDSQ isomers with the resolution of elution better than 1.5. Interestingly, the molecular size of moiety attached to the DDSQ resulted in significant reduction of the retention time suggesting the sterics constraint plays a critical role in the separation of these cage-like structures along with the strength of hydrogen bonding. In partition HPLC using Si bonded with CN groups as a normal phase resulted in a partial separation for one of the selected systems, which indicates the extent of polarity plays a secondary role in the separation mechanism.

Keywords Double-decker shaped silsesquioxane (DDSQ) · Polarity · Cis and trans isomers · Separation · HPLC

1 Introduction

Functionalized double-decker shaped silsesquioxanes (DDSQ) have been used as the building block in polymerization to obtain inorganic-organic hybrid polymers with enhanced dielectric constants, glass transition temperatures, melting temperatures, among other properties of engineering interest [1–7]. DDSQ are usually synthesized from the condensation reaction between the commercially available DDSQ-$(Ph)_8(OH)_4$ **(1)** and $(R^1)(R^2)$-dichlorosilanes in the presence of triethylamine as seen in Scheme 1. If R^1 is different than R^2, the condensed DDSQ structures have unavoidable *cis* and *trans* isomerism [1, 5, 8–15]. These isomers represent as the product, *cis* and *trans*, show in Scheme 1.

These isomers are different in their physical properties such as crystal structure, melting temperature, recrystallization behavior, solubility, etc. [5]. It was also reported that a polymer made from the all *cis* isomers has a significantly different melting temperature as compared with the same polymer composed of the all *trans* isomer [8]. Quantification of the *cis*-*trans* ratio in a mixture is often based on ^{29}Si-NMR. However, the ^{29}Si-NMR requires a large amount of functionalized DDSQ and requires a long scan time to reduce the signal-to-noise ratio needed for the analysis. To avoid these complications, 2D NMR was used to identify distinctive peaks in the ^{1}H-NMR between *cis* and *trans* isomers in DDSQ samples with aniline and methyl in the R^1 and R^2 positions [9]. The drawback of this procedure is the need to identify characteristic peaks for each moiety, which will be different depending on the R^1 and R^2 used.

Fractional crystallization is the most common method used to obtain nearly-pure *cis*, and nearly-pure *trans* DDSQ isomers [5, 6, 8, 9]. The separation is based on the solubility differences of *cis* and *trans* isomers in a specific solvent [10]. After the fractional crystallization, a liquid chromatography rectification process is often needed to yield a higher isomeric purity [5, 7–11]. Recently, DDSQ-$(Ph)_8(OH)_4$ was condensed with methyltrichlorosilane followed by a

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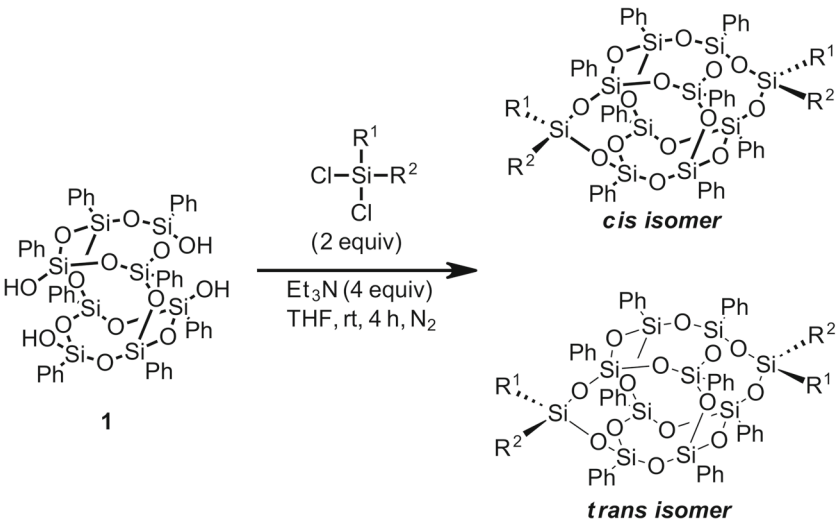

Scheme 1 Condensation reaction of DDSQ-$(Ph)_8(OH)_4$ **(1)** with 2 M equivalents of $R^1R^2SiCl_2$. The resultant product contains *cis* and *trans* isomers DDSQ-$(Ph)_8(R^1)(R^2)$

hydrolysis reaction producing a mixture of DDSQ cages including *cis* and *trans* isomers functionalized with hydroxyl groups. It was found that *cis* and *trans* isomers may be separated by a preparatory silica column and fractions quantified by HPLC [8, 12, 15].

In this work, HPLC was reported as an alternative technique to quantify the *cis-trans* ratio of DDSQ isomers after the capping reaction and/or separations. The wide availability of HPLC as compared to NMR facility makes this quantification method more readily adoptable. Furthermore, the principle of HPLC separation is mainly based on polarity differences between components. Hence, different polar groups may be bonded to DDSQ which increases the number of molecules that can be quantified. In the following, several different mixtures of *cis/trans* compounds are presented and demonstrated a broad applicability of HPLC for the quantification analysis.

2 Experimental

2.1 Materials

All commercially available chemicals were used as received unless otherwise indicated. $(C_6H_5)_8Si_8O_{10}(OH)_4$ 5,11,14,17-Tetra(hydro)octaphenyltetracyclo[7.3.3.(^{3,7})] octasilsesquioxane DDSQ-$(Ph)_8(OH)_4$ was purchased from Hybrid Plastics (Hattiesburg, MS). 3-[Bis(trimethylsilyl)amino] phenylmagnesium chloride $[(CH_3)_3Si]_2NC_6H_4MgCl$ 1 M in THF solution (*m*-PhA$(TMS)_2$-MgCl); vinyltrichlorosilane $(C_2H_3)SiCl_3$; isopropyltrichlorosilane $(C_3H_7)SiCl_3$; isobutyltrichlorosilane $(C_4H_9)SiCl_3$; and 3-cyanopropylmethyldichlorosilane $(C_3H_6CN)(CH_3)SiCl_2$ were purchased from Gelest. Methyltrichlorosilane $(CH_3)SiCl_3$, phenyltrichlorosilane $(C_6H_5)SiCl_3$, deuterated chloroform with 1 vol% tetramethylsilane ($CDCl_3$–1%TMS) were purchased from Sigma-Aldrich. Triethylamine (Et_3N) was purchased from Avantor and distilled over calcium hydride before use. DDSQ bridged with (methyl)(*para*-aniline) dichlorosilane, (methyl)(*meta*-aniline) dichlorosilane, (isobutyl)(*meta*-aniline)dichlorosilane, and (cyclohexyl)(meta-aniline)dichlorosilane moiety were synthesized for our research group and reported in a previous study [5]. Tetrahydrofuran (THF) was refluxed over sodium/benzophenone and distilled. Reagent grade dichloromethane (DCM) and n-hexanes were degassed with helium for HPLC experiments. The previously listed solvents were purchased from Sigma. Si-gel P-60 was obtained from Silicycle. ^{1}H, ^{13}C, and ^{29}Si were recorded on 500 MHz NMR spectrometers.

2.2 Synthesis of (Methyl)(*meta*-Bis(Trimethylsilyl) Amino]Phenyl)Dichlorosilane

A 250 mL round bottom flask containing a magnet stirrer under N_2 was sealed with a rubber septum and submerged in an acetone-dry ice bath. 50 mL of THF and 24.0 mmol (4.0 mL) of isobutyltrichlorosilane were injected respectively to the setup. 20 mmol (20.0 mL) of *m*-PhA$(TMS)_2$-MgCl was added dropwise for 10 min. The ice bath was removed upon completion of the addition, and the solution was stirred overnight until it became a clear yellowish liquid. Volatiles were distilled under N_2 in an oil bath at 90 °C; a second distillation was done under vacuum to collect the expected product as a clear pale-yellow liquid. Spectral information is provided in the supporting document.

2.3 General Synthetic Procedure

DDSQ-$(Ph)_8(R^1)(R^2)$ was synthesized following a previously reported method [9, 14, 15]. In a 250 mL flask purged with dry N_2 for 15 min, DDSQ-$(Ph)_8(OH)_4$ **(1)** (2.00 g, 1.87 mmol, 1 equiv) was dissolved in THF (60 mL) at room temperature. $(R^1)(R^2)SiCl_2$ (3.74 mmol, 2 equiv) was added to the solution

followed by Et_3N (1.04 mL, 7.48 mmol, 4 equiv) under vigorous stirring. The addition of triethylamine took about 5 min in total, a cloudy suspension was formed and stirred continued for 4 additional hours. The solution was then filtered through a fine fritted-funnel-filter to remove the solid triethylamine hydrochloride. The solution was dried in a rotary evaporator and then passed through a silica-gel column using DCM as a solvent. This cleaning step allowed hydrolysis of Si-Cl bond in cages synthesized with trichlorosilanes. The volatiles were removed from the resulting solution and further dried at 0.4 mbar and 50 °C for 12 h to afford DDSQ-$(Ph)_8(R^1)(R^2)$ as a white powder. The structures studied in this work are listed in Fig. 1. NMR spectra for *cis* and *trans* isomer mixtures are provided in the supporting document.

2.4 Analytical Methods

2.4.1 Preparatory Separation of Cis and Trans Isomers

Liquid chromatography was performed to separate *cis* and *trans* isomers in **2**, **3**, and **11**, and those hydrolyzed structures synthesized with trichlorosilanes in **6** to **10** with a procedure previously described [10, 12, 15]. A glass preparatory chromatography column, 60 cm in length and 4 cm internal diameter, with 500 mL round top reservoir was packed with 60 g of Si-gel resulting with packing height of about 40 cm. DCM was then flushed through the packed bed under pressure generated by a dry N_2 stream. Wetting of the packed bed was complete until no air bubbles, or dry space was observed. A

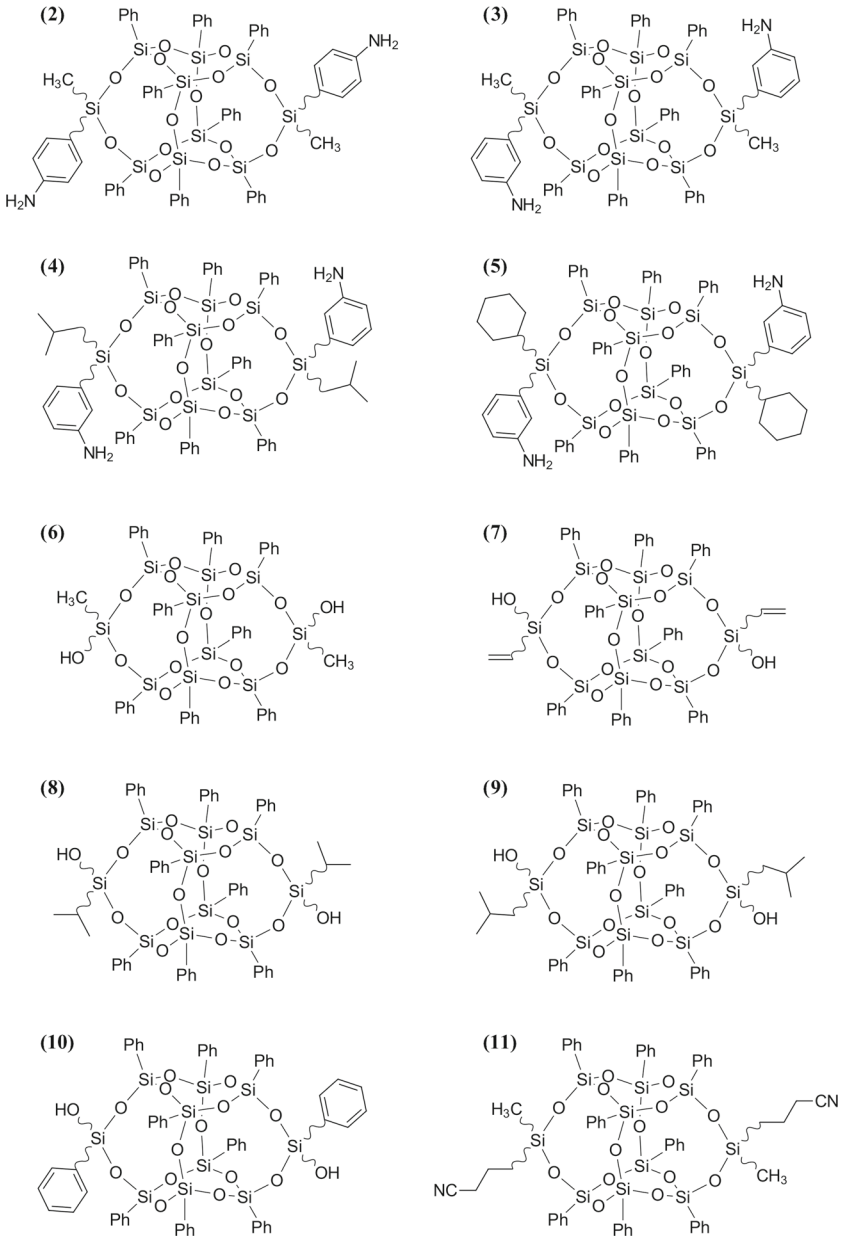

Fig. 1 DDSQ-$(Ph)_8(R^1)(R^2)$ isomers studied in this work. **(2)** DDSQ-$(Ph)_8$(para-aniline)(methyl), **(3)** DDSQ-$(Ph)_8$(meta-aniline)(methyl), **(4)** DDSQ-$(Ph)_8$(meta-aniline)(isobutyl), **(5)** DDSQ-$(Ph)_8$(meta-aniline)(cyclohexyl), **(6)** DDSQ-$(Ph)_8$(hydroxyl)(methyl), **(7)** DDSQ-$(Ph)_8$(hydroxyl)(vinyl), **(8)** DDSQ-$(Ph)_8$(hydroxyl)(isopropyl), **(9)** DDSQ-$(Ph)_8$(hydroxyl)(isobutyl), **(10)** DDSQ-$(Ph)_8$(hydroxyl)(phenyl), **(11)** DDSQ-$(Ph)_8$(cyanopropyl)(methyl)

concentrated solution of DDSQ isomeric mixture in DCM (5 mL, 0.2 g/mL) was gently injected from the top of the wet Si-gel bed and moved into the packed bed until no solution was observed above the packed bed. The column was then gently charged with an additional 500 mL of DCM and flushed using the N_2 stream with an average flow rate of 10 mL/min. Fractions of 5 mL were collected at the bottom of the column until the DCM reached the top of the bed. Each fraction was injected in 5 cm TLC plates of Si-gel supported in aluminum. TLC was evaluated with DCM and then analyzed under a 245 nm UV-lamp. Similar fractions were combined and dried for further experiments. The retardation factor (R_f) in Eq. 1 was used as a measure of the separation efficiency after the preparatory LC experiments.

$$R_f = \frac{Total\ TLC\ length}{Distance\ traveled\ by\ fraction\ n} \quad (1)$$

2.4.2 UV-VIS Spectroscopy

Wavelength sweep readings were developed for isolated isomers **2** to **5** with DCM as the solvent. Individual isomers were solubilized forming master batches of 0.2 mg/mL. Then, the solutions were diluted reaching lower concentrations in progressive steps until a value close to 0.02 mg/mL. All readings were contrasted against a DCM blank.

2.4.3 Analysis and Quantification of Isomers by HPLC

All HPLC experiments were performed using an Agilent 1100 HPLC equipped with a UV detector. The columns selected for this work were Supelco LiChrospher® Si-60 for adsorption chromatography and ZORBAX® CN column for partition normal phase chromatography (pHPLC). DCM was used as the mobile phase for the adsorption chromatography. Different ratios of DCM:hexanes ranging from pure dichloromethane to 70% hexane for pHPLC. Solvents were degassed using helium for a minimum of 15 min prior to all HPLC experiments. A flow rate of 1 mL/min at a giving a constant pressure of 32 bars was used. The temperature was set in 25 °C, and the injection volume was 5 μL. The UV detector was emitting at 254 nm. Once the elution was finished, a blank sample containing mobile phase was injected and flushed trough the column for verification of the baseline and to confirm complete elution of the previous injection.

Standard plate theory of chromatography was used for quantitative analysis of separation [16]. Two different methods were used to evaluate the separation resolution based on peak width (W_n) and full width at half height of the peak ($W_{n@0.5}$) show in Eqs. 2 and 3, respectively. The retention time (t_r) is the elution time at the peak maximum. Values of t_r, W_n, and $W_{n@0.5}$ were calculated using the Agilent chemstation software. The theoretical plate number (N) or column efficiency based on Gaussian distribution was calculated using Eq. 4.

$$R_S = \frac{(t_{r2} - t_{r1})}{0.5(W_1 + W_2)} \quad (2)$$

$$R_{S\,0.5} = \frac{1.18(t_{r2} - t_{r1})}{0.5(W_{1@0.5} + W_{2@0.5})} \quad (3)$$

$$N = 16\left(\frac{t_r}{W}\right)^2 \quad (4)$$

3 Results and Discussion

3.1 Separation of *cis* and *trans* Isomers

Two spots were observed by TLC for structures **2** to **5** and for **11**. These two spots indicate the presence of *cis* and *trans* isomers; their corresponding retardation factors are listed in Table 1. The *cis* and *trans* isomers were separated by a preparatory LC, and the isolated fractions were evaluated using ^{29}Si-NMR as shown in Fig. 2. The spectra obtained for the first fraction was assigned to *trans* isomers; the spectra obtained for the second fraction was assigned to *cis* isomers. A previous report from our research group describes the peak assignments in detail [10]. Mass balance for the material injected in the column resulted in 75% recovery in two main fractions after elution. This result is comparable with a previous report for separation of DDSQ mixtures by LC [15].

The difference in elution times between *cis* and *trans* isomers is related with the orientation of the polar moieties. For *cis* isomers, both polar groups are pointing at the same direction. This configuration slows the elution rate due to possible stronger adsorption. For *trans* isomers, polar groups are pointing at the opposite direction. Here, only one of the polar groups is attracted to the stationary phase surface increasing the elution rate. A similar relation between positional isomers was reported previously for small molecules [17].

Table 1 TLC retardation factors, R_f, with dichloromethane as mobile phase for **2** DDSQ-$(Ph)_8$(*p*-aniline)(methyl), **3** DDSQ-$(Ph)_8$(*m*-aniline)(methyl), **4** DDSQ-$(Ph)_8$(*m*-aniline)(isobutyl), **5** DDSQ-$(Ph)_8$(*m*-aniline)(cyclohexyl), and **11** DDSQ-$(Ph)_8$(cyanopropyl)(methyl)

Compounds	*trans*	*Cis*
2	0.28	0.14
3	0.43	0.28
4	0.66	0.44
5	0.77	0.51
11	0.86	0.73

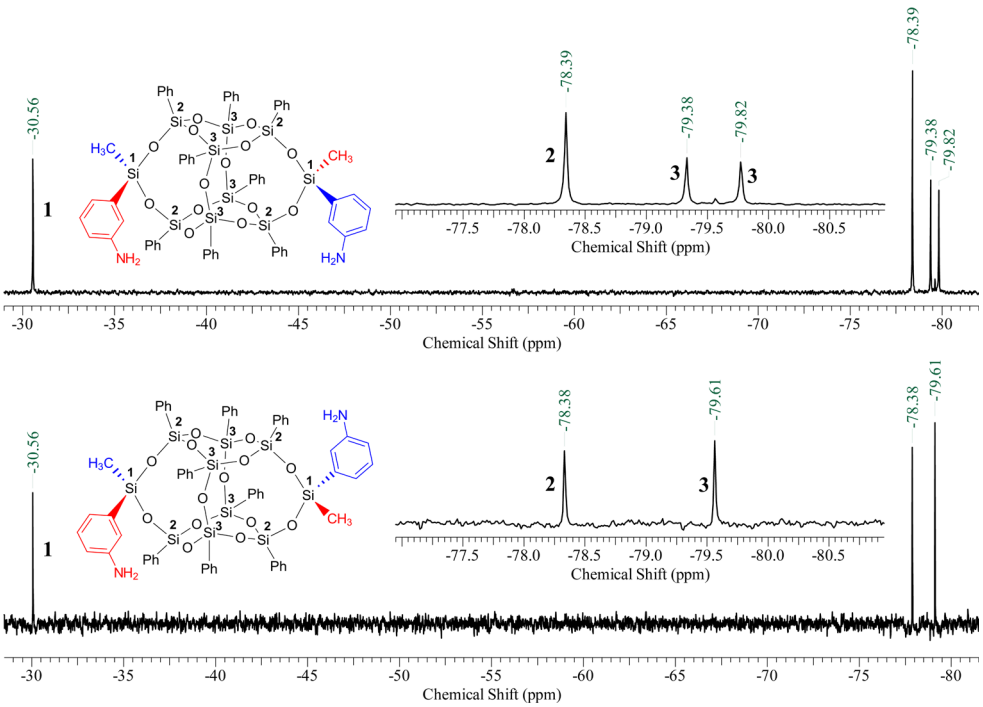

Fig. 2 ^{29}Si NMR for *cis* and *trans* isomers after separation of **3**. (**a**) *trans* isomer, corresponding to the first spot in TLC; (**b**) *cis* isomer, corresponding to the second spot in TLC

3.2 UV Absorbance Intensity of Positional Isomers of Phenylamine

UV detectors are highly employed in organic chemistry due to its lower concentration limit compare with other class of detection devices. In this work HPLC detection and quantification was developed by UV. However, other class of detectors with less definition such as refractive index (RI), or with higher resolution like mass spectroscopy can also be used to detect and quantify ratios between *cis* and *trans* DDSQ molecules. *Cis* and *trans* isomers for molecules **2** to **5** show a maximum absorbance at 254 nm which is associated with the chromophores in the phenyl rings [18]. However, at the

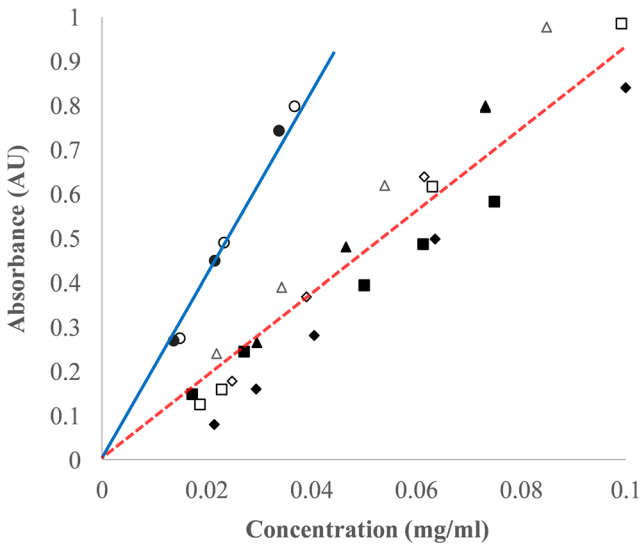

Fig. 3 UV absorbance results at λ = 254 nm. Open symbols are for *trans* isomers, and filled symbols are for *cis* isomers. ● DDSQ-$(Ph)_8$(*p*-aniline)(methyl) **2**; ▲ DDSQ-$(Ph)_8$(*m*-aniline)(methyl) **3**; ▪ DDSQ-$(Ph)_8$(*m*-aniline)(isobutyl) **4**; ♦ DDSQ-$(Ph)_8$(*m*-aniline)(cyclohexyl) **5**. The red dashed line represents the linear trend of all the *para* samples, and the straight blue line is the linear trend for the *meta* samples. The slope of *para* was determined to be two times the slope for *meta*

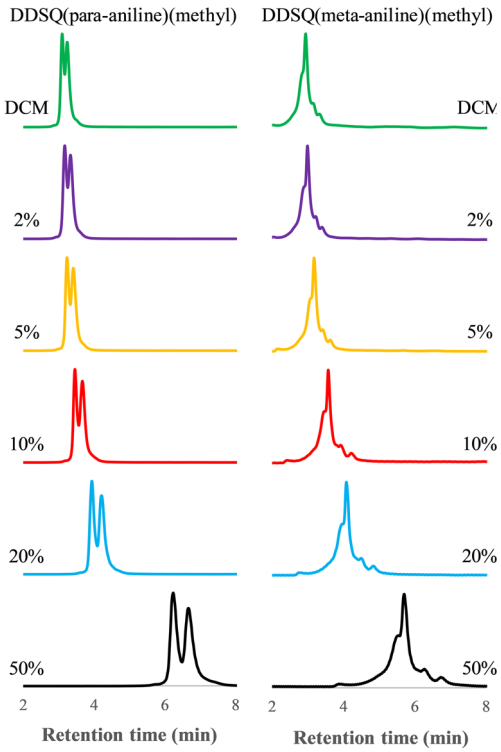

Fig. 4 Effect of dichloromethane/hexane mobile phase ratios in normal phase pHPLC for *cis-trans* of 2 and 3. The percentual value indicated represents the volume percentage of hexanes in the mobile phase

Table 2 Comparison of the resolution obtained using eqs. 2 and 3 in *cis-trans* of 2

Hexane % *v*/v in DCM:Hexane solution	0	2	5	10	20	30	50	70
R_s	0.77	1.73	1.63	1.64	1.58	1.68	1.81	1.93
$R_{s0.5}$	0.36	0.99	0.90	1.03	1.02	1.09	1.04	1.04

same concentrations, absorbance values for *meta* molecules are lower when compare to the absorbance values for *para* isomers. It was determined that the slope of absorbance versus concentration for all *para* isomers is twice as that of *meta* isomers show in Fig. 3. This result agrees with previous reports for isomers of positional phenylamine [17]. However, there are no differences in the slope of the absorbance versus concentration curve between *cis* and *trans* isomer. This result implies for the case of separation by HPLC with a UV detector, the UV absorbance for *cis* and *trans* isomers can be directly correlated to their ratio without concentration correction. In addition, there is no effect on the absorbance values when different R groups were evaluated, as the main contribution to the UV intensity is the eight phenyl groups surrounding the DDSQ, and not the anilines attached to the DDSQ-$(Ph)_8$ core.

3.3 Analysis by HPLC of Isomeric Mixtures and Individual Isomers

3.3.1 pHPLC of 2 and 3

HPLC evaluation by pHPLC in silica column bonded with cyano (CN) moieties show a single broad peak for the *cis-trans* mixture of **3** while two peaks were clearly observed for the *cis-trans* mixture of **2** using DCM as the mobile phase. By modifying the mobile phase with the addition of hexanes to DCM, the polarity of the mobile phase was reduced; this change increased the retention time in each peak. The observed effect can be interpreted as a preference of the molecules to be adsorbed in the polar stagnant layer, causing a reduction in the mass transfer between the stagnant layer and the mobile phase. An additional effect of addition of hexanes is the enhancement of R_S as seen in Fig. 4. The R_S value is based on peak analogy to normal distribution and it is an indicative of the separation efficiency. Less than 1% overlapping between two normal distribution has been defined as 6σ between two normal distributions or $R_S = 1.5$. R_S calculated with Eq. 2 was improved from 0.77 when only DCM was flushed as the mobile phase to 1.93 when the mobile phase was modified to a 7:3 volumetric ratio of DCM:Hexanes. For mobile phase containing less than 10% of hexane, evaluation of peak widths (W) was difficult due to a pronounced overlap. This resulted in a significant error on the calculated value of R_S. Alternatively, resultion of elution based on Eq. 3 may be used for these highly overlapped peaks. Results of the resolution were tabulated in Table 2.

3.3.2 pHPLC Accuracy Verification

HPLC experiments were performed for quantification of ratios between *cis* **2** and *trans* **2** isomers mixed from isolated samples. Five Mixtures with different *cis* and *trans* ratios described in Table 3 were diluted in DCM. The solutions were injected to pHPLC and eluted with a mobile phase with 1:1 volumetric ratio of DCM:hexanes to achieve optimal R_s. The mixtures with known ratios of isomers were evaluated with the use of a UV detector attached to the HPLC. Eluted chromatograms observed in Fig. 5 match with the known ratio of isomers in the mixtures. This result indicates that quantification by HPCL-UV is possible and the standard deviations are lower than 5% as refer in Table 3.

3.3.3 Separation by Adsorption Chromatography

For adsorption chromatography, pure DCM was chosen to activate the Si-OH surface in the stationary phase of the column [17]. Different than pHPLC, adsorption chromatography allowed the separation of *meta*-aniline isomers. Resolution of elution between *cis* **2** and *trans* **2** isomers and between *cis* **3** and *trans* **3** isomers are much higher in adsorption chromatography than pHPLC. Using DCM as the mobile phase,

Table 3 Comparison between *cis* and *trans* percentages of **2** calculated by weighting nearly-pure *cis* and nearly-pure *trans* and calculated from the area under the peaks in the chromatograms presented in Fig. 5. The standard deviation was calculated based on the known percentage and the area percentage for each isomer

% Weighted *cis*	% Weighted *trans*	% Area *cis*	% Area *trans*	Standard deviation *cis* (%)	Standard deviation *trans* (%)
27	73	25	75	1.5	1.5
41	59	44	56	2.3	2.3
55	45	58	41	2.1	2.1
69	31	74	26	3.3	3.4
83	17	86	14	1.9	1.9

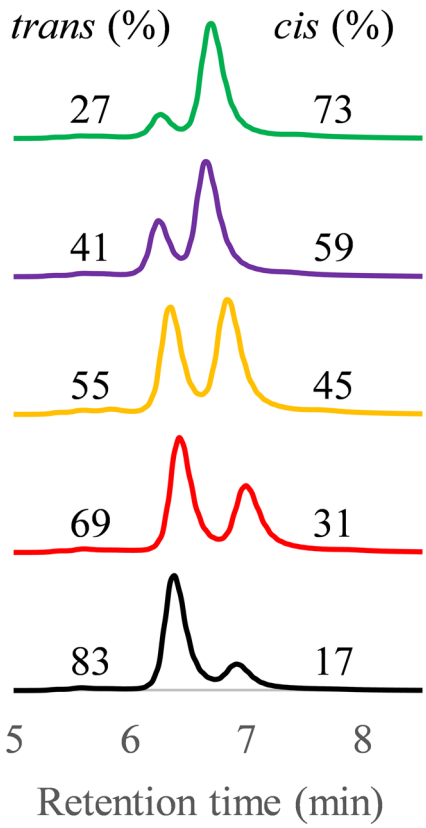

Fig. 5 Quantitative analysis of *cis-trans* mixtures of DDSQ-$(Ph)_8$(*p*-aniline)(methyl) by pHPLC. The individual isomers were first isolated and then mixed to a known ratio for comparison against the area under each peak. The weighted precent of isolated isomers in mixtures was indicated next to each curve

resolution of elution has a value of 6 or higher. In addition, as shown in Table 4, the retention time is high. To reduce t_r, 2% acetonitrile was added to the mobile phase. This change also decreases R_s to an optimal value of 1.5. Like pHPLC, it was observed that in adsorption chromatography *trans* isomers migrate faster than *cis* isomers as could be seen from the retention times presented in Table 4.

Table 4 Retention time (t_r), peak width (W), and plate number (N) after separation by adsorption HPLC with DCM as the mobile phase; Retention time (t_{rAcN}) after separation by adsorption HPLC with a mobile phase composed by DCM:Acetonitrile in the volumetric ratio 98:2

Compound	t_r (min)	W (min)	N	t_{rAcN} (min)
trans **2**	32.1	3.0	1831.8	7.6
cis **2**	60.5	5.0	2342.5	11.4
trans **3**	43.7	4.4	1578.2	8.5
cis **3**	66.4	5.6	2249.4	14.7
trans **4**	23.3	2.3	1642.0	–
cis **4**	47.5	4.7	1634.2	–
trans **5**	20.0	3.0	711.1	–
cis **5**	41.9	6.3	707.7	–

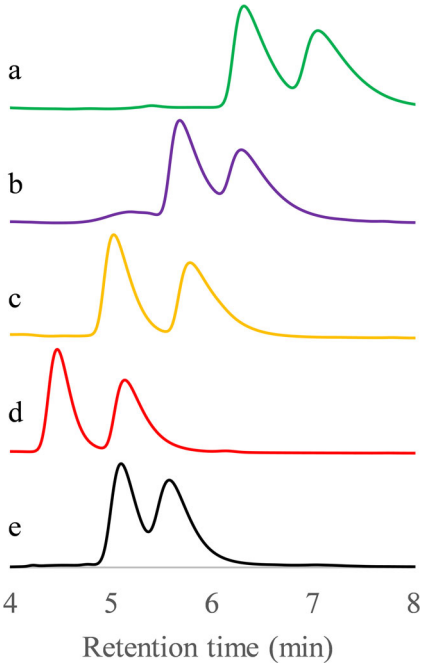

Fig. 6 Retention times for *cis* and *trans* DDSQ-$(Ph)_8$(hydroxyl)(R^2). (**a**) R^2: methyl **6**; (**b**) R^2: vinyl **7**; (**c**) R^2: isopropyl **8**; (**d**) R^2: isobutyl **9**; and (**e**) R^2: phenyl **10**

DDSQ with *meta*-aniline moieties has higher retention times compared with *para*-aniline. The reason for this retention time difference is not apparent. However, from crystallographic data, amine groups in DDSQ with *meta*-aniline are pointing to the same direction, and it is possible the amine moiety is more exposed than in the *para* position [5, 19].

The number of theoretical plates (N) is a measure of the peak broadening in HPLC. in the column for *cis* **3** and *trans* **3** isomers, resulted in higher values compared with the N value for *cis* **4** and *trans* **4** and for *cis* **5** and *trans* **5** as seen in Table 3. It is remarkable that the non-polar group attached to the D-Si affects the column efficiency. From the data collected in Table 3, the column was more efficient resolving **2** and **3** containing methyl group as R^2; followed by **4** with isobutyl as R^2 moiety; and lastly, the lower efficiency was attributed to **5** which has the bulkier cyclohexane group in the R^2 position. Comparison of N between *cis* and *trans* in evaluated molecules does not show a recognizable trend. N for *cis* of **2** and **3**

Table 5 Retention time for DDSQ functionalized with methyl and different polar groups. R stands for *para*-aniline **2**, hydroxyl **6**, and cyanopropyl **11**

DDSQ-$(Ph)_8$(R)(methyl)	t_r *trans* (min)	t_r *cis* (min)
R: *para*-aniline (**2**)	32.19	61.08
R: hydroxyl (**6**)	6.31	7.03
R: cyanopropyl (**11**)	5.31	6.23

is larger than N for *trans* of **2** and **3**. In this case, N of *cis* was favored by t_r and not by W. However, when t_r was lower for **4** and **5**, W had a bigger effect in the calculation of N ending with slightly better N values for *trans* compared against *cis*.

3.3.4 Effect of R^2 Groups in Retention Times

It was observed that the size of organic groups attached to the DDSQ core influences the retention times. For R^1 is *meta*-aniline, the retention time increases as the size of R^2 decreases, $t_r(R^2 = \text{methyl}) > t_r(R^2 = \text{isobutyl}) > t_r(R^2 = \text{cyclohexyl})$. When the polar group was changed from *meta*-aniline to hydroxyl, the retention time was also observed to be affected by the size of R^2 group. Similar to *meta*-anilines, the bulkier group was related to a lower retention time show in Fig. 6. However, for R^2 was phenyl, the retention time was higher as compared to R^2 was isobutyl. This result suggests the isobutyl moiety has a higher steric effect than phenyl, which reduces the overall adsorption between the adsorption site and the hydroxyl group attached to the DDSQ-$(Ph)_8$ core. Integration of DDSQ-Ph_8(OH)(R) after synthesis by ^{29}Si-NMR results in a mixture with approximately 50% *cis* and 50% *trans* isomers. After separation by HPLC the calculation of the area of each peak in the chromatogram resulted in 50% ± 1.6% of the total area verifying the results obtained previously for DDSQ bonded to para-aniline. Integrations can be seen in the supplemental information file.

3.3.5 Effect of Polar Groups (R^1) in t_r

DDSQ-$(Ph)_8(OH)_4$ was functionalized with different polar groups, *para*-aniline; hydroxyl; or cyanopropryl, and its separation characteristics evaluated using adsorption chromatography. It was found that the retention time for *trans* **2** (*p*-aniline) was five times longer than the retention times for *trans* **6** (hydroxyl) and *trans* **11** (cyanopropyl). The retention time was ten times higher for *cis* moeity of *p*-aniline than hydroxyl (*cis* **6**) and cyanopropyl (*cis* **11**) as seen in Table 5.

The retention time differences between **6** and **2** could be related to the location of the polar groups. For DDSQ-$(Ph)_8$(*para*-aniline)(methyl), the amine moiety is extended away from the core of the DDSQ by a phenyl ring to avoid the steric hindrance from the eight phenyl groups surrounding the core. The steric effect is expected to be higher for DDSQ-$(Ph)_8$(hydroxyl)(methyl) as the hydroxyl moiety is directly attached to the cage. The steric effect weakens the absorption to the stationary phase, which resulted in a significant decrease in retention time. Even it is generally recognized OH has stronger adsorption than NH_2.

For both **2** and **11**, the polar group is not directly attached to the core. However, a difference in the retention time was still observed. This result can be justified based on the hydrogen bonding. For **2**, the oxygen atom from the silanol at the stationary phase and the nitrogen atom from **2** can act as hydrogen acceptors; and both have hydrogen donors. For **11**, the cyano group can act only as the acceptor and forms a weaker hydrogen bonding with the stationary phase as compared with **2**.

4 Conclusions

This work confirmed that separation of DDSQ *cis/trans* isomer mixtures by liquid chromatography and quantification by HPLC is possible as the primary separation technique. Evaluation of isomers by NMR and HPLC showed that the isomer expected after separation was present in percentages better than 90% purity. It was observed that the position of the amine group in the aniline affected the UV absorbance values at the same concentration. However, no concentration effect was found in absorbances between *cis* and *trans* isomers. This enables one to determine the *cis/trans* ratio in a mixture directly from the UV detector signal of an HPLC experiment.

Separation by normal phase partition chromatography was distinguished as a technique for identification and quantification of *cis* and *trans* DDSQ-$(Ph)_8$(*p*-aniline)(methyl). This chromatography mode does not permit separation of the *meta* isomers. Adsorption chromatography using silica as stationary phase was a better separation technique allowing optimal resolution of the elution for every case studied in this work.

The moiety next to the polar group bonded to the D-Si has a steric effect that affects the adsorption. Bulkier groups reduced the adsorption and in consequence the retention time. However, the elution order is not changed by the adjacent groups it means that for DDSQ functionalized cages, the *trans* isomers will always elute first. Polarity in the DDSQ is a crucial factor for allowance the separation process. In addition, the strength of hydrogen bonding affected by the sterics of the surrounding groups significantly influnced the elution time.

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