Preparation and physicochemical studies of new multiple rings *s*-tetrazines†

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Several new supramolecular *s*-tetrazines have been prepared and studied. Their electrochemical and spectroscopic properties have been investigated, especially in the presence of quenchers. Fluorescence quenching has been shown to occur as expected through a charge transfer mechanism and the cyclophane structure has been shown to lead to an acceleration of the quenching process.

I. Introduction

s-Tetrazine chemistry has been known for more than one century,[1] and the photophysical[2] and electrochemical[3] properties have been recognized; however only recently have these very original properties started to be exploited. With regards to recently developed supramolecular chemistry,[4] the *s*-tetrazine building block appears a very promising and fascinating one. *s*-Tetrazines are highly colored and electroactive heterocycles, displaying the special properties to have a very high electron affinity which make them reducible at high to very high potentials (they are actually the electron poorest class of neutral C–N heterocycles). In addition, they have a low lying π* orbital resulting in a n–π* transition in the visible range. The chemistry of *s*-tetrazines has been recently reviewed,[5] enlightening especially the interest in explosives[6] and coordination chemistry.[7]

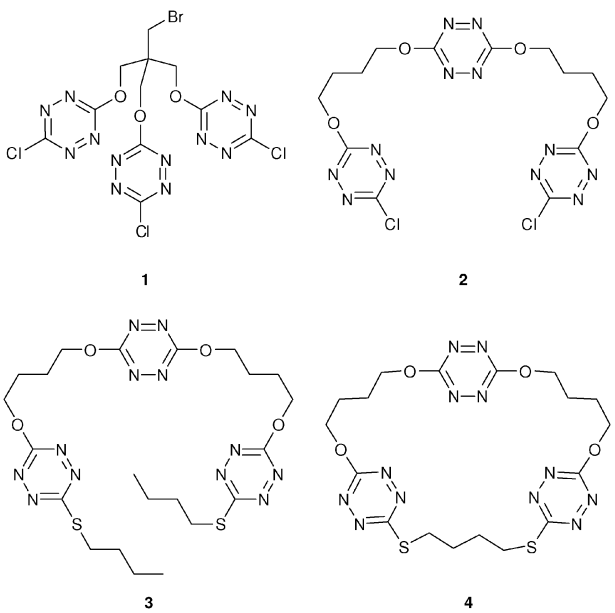
We and others have remarked that *s*-tetrazines substituted with heteroatoms display interesting fluorescence properties.[8–12] Actually, all these compounds are fluorescent on TLC as well as in the crystalline state (unpublished data), which probably place them amongst the smallest organic fluorophores in the visible range ever prepared. This makes them especially attractive in view of sensing applications. Our first studies had shown indeed that chloromethoxy-*s*-tetrazine was among the best compounds, because combination of chlorine and an alkoxy substituent on a *s*-tetrazine appeared to lead to a maximum fluorescence yield (ϕ_F = 0.38 in dichloromethane). An attractive development in this direction was to prepare supermolecules featuring several fluorescent ss-tetrazine moieties, with a controlled spatial arrangement in order to improve either the fluorescence quenching, or the efficiency of the electron transfer in relation to electrochemical applications.

In this article we report the synthesis of several *s*-tetrazines featuring multiple rings in the same molecule, as represented in Scheme 1. We also report their electrochemical and fluorescence properties, including the fluorescence quenching analysis in the presence of several electron donors. The electrochemical behavior of these compounds in the presence of weak acids will also be detailed.

II. Experimental

(1) Synthesis

6,6′-(2-(Bromomethyl)-2-((6-chloro-*s*-tetrazin-3-yloxy)methyl)-propane-1,3-diyl)bis(oxy)bis(3-chloro-*s*-tetrazine) 1. 2-(Bromomethyl)-2-(hydroxymethyl)propane-1,3-diol (0.2 g, 1 mmol), dichloro-*s*-tetrazine (0.45 g, 3 mmol), sodium hydrogenocarbonate (0.34 g, 4 mmol) and 2 drops of 2,4,6-collidine were added under Ar atmosphere into 10 mL of anhydrous dichloromethane and the mixture was stirred at r.t. overnight. The



Scheme 1 Formula of the *s*-tetrazines.

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solids were filtered off and washed with dichloromethane. The filtrate was concentrated by evaporation and passed through a column chromatography (silica, dichloromethane) to give 0.1 g of compound **1** as a red solid (18%) with a "yellow" fluorescence on TLC.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.07(s, 6H), 4.01(s, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.4, 165.5, 68.2, 44.2, 30.9.

MS, *m*/*z*: 560($[M+NH_4]^+$, calc. 560, base peak), 533, 508, 479, 463, 411.

4,4′-(*s*-Tetrazine-3,6-diyl)bis(oxy)dibutan-1-ol 5. NaH (60% dispersion in oil, 265 mg, 6.6 mmol) was added into a solution of 1,4-butanediol (0.59 mL, 6.6 mmol) in 5 mL of anhydrous tetrahydrofuran. The mixture was stirred at r.t. for 0.5 hour, then cooled to −78 °C and transferred slowly with a stainless steel canula into a solution of dichloro-*s*-tetrazine (0.50 g, 3.3 mmol) in 5 mL of tetrahydrofuran at −78 °C. The mixture was allowed to warm slowly to r.t. and stirred for 2 hours. The solids were filtered off, washed with dichloromethane and the filtrate was concentrated by evaporation and passed through a column chromatography (silica, 1:8 methanol/dichloromethane) to give 0.21 g (24%) of 4,4′-(1,2,4,5-*s*-tetrazine-3,6-diyl)bis(oxy)dibutan-1-ol as a red solid, with a "yellow" fluorescence on TLC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60(t, 2H, J = 6.6 Hz), 3.75(t, 2H, J = 6.3 Hz), 2.06–1.97(m, 2H), 1.84–1.75(m, 2H), 1.45 (br., 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 69.8, 62.4, 29.1, 25.3

MS, *m*/*z*: 281.2($[M + Na]^+$ calc. 281.2), 186.3, 142.2.

3,6-Bis(4-(6-chloro-1,2,4,5-tetrazin-3-yloxy)butoxy)-1,2,4,5-*s*-tetrazine 2. *s*-Tetrazine-diol **5** (0.13 g, 0.5 mmol) and dichloro-*s*-tetrazine (0.151 g, 1 mmol) were dissolved in 15 mL of anhydrous dichloromethane. 2,4,6-Collidine (0.13 mL, 1 mmol) was added dropwise into the solution, and the mixture was stirred overnight under Ar atmosphere. The solvent was evaporated and the residue was passed through a column chromatography (silica, dichloromethane) to give 3,6-bis(4-(6-chloro-1,2,4,5-tetrazin-3-yloxy)butoxy)-1,2,4,5-tetrazine **2** as a red solid (0.22 g, 92%), with a "yellow" fluorescence on TLC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.77(t, 4H, J = 5.7 Hz), 4.67(t, 4H, J = 5.7 Hz), 2.21–2.17(m, 8H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 166.2, 164.6, 70.3, 69.2, 25.3 (2C).

MS, *m*/*z*: 504($[M + NH_4]^+$, (calc. 504), 487(base peak), 408, 391, 357, 325, 295, 247, 171.

3,6-Bis(4-(6-(butylthio)-*s*-tetrazin-3-yloxy)butoxy)-*s*-tetrazine 3. A solution of 1-butanethiol (0.084 mL, 0.78 mmol) and triethylamine (0.11 mL, 0.78 mmol) in 60 mL of acetonitrile was added dropwise through a dropping funnel into a 250 mL round bottom flask containing a solution of *s*-tetrazine **2** (0.19 g, 0.39 mmol) in 60 mL of acetonitrile. The mixture was stirred at r.t. for 2 hours and the solvent was evaporated. After a column chromatography (silica, dichloromethane then 1:34 methanol/dichloromethane) 0.11 g (47%) of **3** was obtained as a red solid which is weakly fluorescent on TLC plate.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.67–4.65(m, 8H), 3.27(t, 4H, J = 7.4 Hz), 2.19–2.15(m, 8H), 1.81–1.71(m, 4H), 1.55–1.45(m, 4H), 0.95(t, 6H, J = 7.4 Hz).

^{13}C NMR (75 MHz, $CDCl_3$) δ 718.0, 166.1, 166.0, 69.3, 69.1, 30.9, 30.7, 25.4, 25.3, 22.0, 13.7.

Cyclophane-*s*-tetrazine 4. In a 500 mL 3-necked round bottom flask containing 200 mL of anhydrous acetonitrile was added simultaneously in 2 hours a solution of 1,4-butanedithiol (0.037 mL, 0.31 mmol) and triethylamine (0.087 mL, 0.62 mmol) in 60 mL of acetonitrile in one dropping funnel and a solution of *s*-tetrazine **2** (0.15 g, 0.31 mmol) in 60 mL of acetonitrile in another dropping funnel. The mixture was stirred for another 0.5 hour and the solvent was evaporated. After column chromatography (silica, 1:2 ethyl acetate/petroleum ether) 0.11 g (67%) of the cyclophane-*s*-tetrazine **4** was obtained as a red solid which is weakly fluorescent on TLC plate.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.77–4.61(m, 8H), 3.32(t, 4H, J = 6.0 Hz), 2.18–2.11(m, 8H), 1.95–1.91(m, 4H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 166.0, 165.9, 68.9, 68.9, 30.3, 28.1, 24.93, 24.9.

MS, *m*/*z*: 537 ($[M + 1]^+$, calc. 537), 351, 334, 310, 261, 243, 211, 190, 156 (base peak), 120.

(2) Electrochemical studies

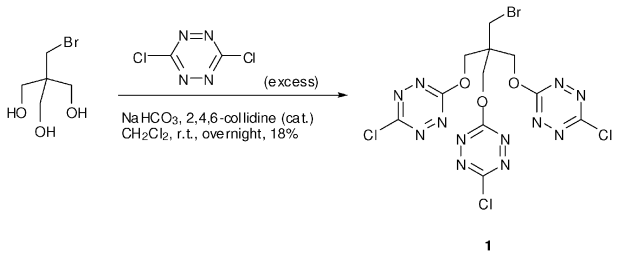
Electrochemical studies were performed using dichloromethane (DM) (SDS, anhydrous for analysis) as a solvent, with *n*-tetrabutylammonium perchlorate (TBAP) (Fluka, puriss.) as the supporting electrolyte. The substrate concentration was *ca.* 5 mM (accurate values are given in the figure captions). A 1 mm diameter Pt electrode was used as the working electrode, along with a Ag^+/Ag (10^{-2} M) reference electrode and a Pt wire counter electrode. The cell was connected to a CH Instruments 600B potentiostat monitored by a PC computer. The reference electrode was checked *vs.* ferrocene as recommended by IUPAC. In our case, $E°(Fc^+/Fc)$ = 0.046 V. All solutions were degassed by argon bubbling prior to each experiment.

(3) Fluorescence measurements

All solvents were of spectroscopic grade.

Steady-state spectroscopy. A UV-Vis. Varian CARY 500 spectrophotometer was used. Excitation and emission spectra were measured on a SPEX Fluorolog-3 (Jobin-Yvon). A right-angle configuration was used. Optical density of the samples was checked to be less than 0.1 to avoid reabsorption artifacts.

Time-resolved spectroscopy. The fluorescence decay curves were obtained with a time-correlated single-photon-counting method using a titanium-sapphire laser (82 MHz, repetition rate lowered to 4 MHz thanks to a pulse-peaker, 1 ps pulse width, a doubling crystal is used to reach 495 nm excitation) pumped by an argon ion laser. The Levenberg-Marquardt algorithm was used for non-linear least square fit. In order to estimate the quality of the fit, the weighted residuals were calculated. In the case of single photon counting, they are defined as the residuals, *i.e.* the difference between the measured value and the fit, divided by the square root of the fit.



Scheme 2 Synthesis of the tripodal *s*-tetrazine **1**.

χ^2 is equal to the variance of the weighted residuals. A fit was said to be good for χ^2 values between 0.8 and 1.2.

3. Results and discussion

(1) Synthesis

The following synthetic route (Scheme 2) was used for the preparation of the tripodal *s*-tetrazine **1**.

The choice of the base was not obvious, since when only $NaHCO_3$ or $KHCO_3$ were used, a high temperature and long reaction times were required, and even so a considerable quantity of partially substituted triol was obtained. Considering as well that the use of polyalkoxyl anions is too harsh and leads to the decomposition of *s*-tetrazines in most cases, we turned to 2,4,6-collidine which is an often-used organic base with no nucleophilicity. We added a catalytic amount of it and the reaction went smoothly to give the expected trisubstituted product.

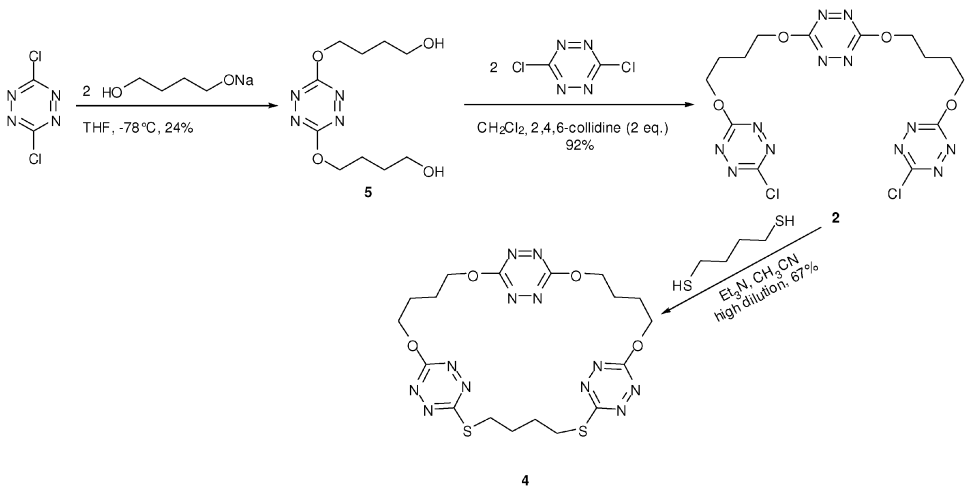
The synthetic scheme for the synthesis of the cyclophane **4** is displayed below (Scheme 3). We had already prepared a cyclophane containing several *s*-tetrazine rings,[11] but the aim of this more tricky synthesis was to prepare a cyclophane containing a dialkoxy-*s*-tetrazine subunit, which is much more fluorescent than sulfur substituted ones. The main synthetic difficulty occurred at the beginning, with the formation of the dialkoxyl *s*-tetrazine which requires the use of the mono anion of butane-1,4-diol leading to the desired molecule **5** but with a modest yield (24%) along with numerous side products, while the addition of two equivalents of 2,4,6-collidine allowed us to prepare **2** in the second step. Indeed, if the mono functionalisation of chloro-*s*-tetrazine with an alcohol is efficient using 2,4,6-collidine, its disubstitution is only possible with stronger nucleophiles (alkoxide in this case), albeit with poor yields. The ring closure was not a problem since sulfur nucleophiles are known to be much more reactive than oxygen ones.

(2) Electrochemistry

Like all *s*-tetrazine compounds investigated so far, the tripod **1** exhibits a fully reversible voltammogram, corresponding to the reduction of the three *s*-tetrazine moieties into stable anion radicals, as evidenced by the single peak in the CV (Fig. 1). Comparison with chloromethoxy-*s*-tetrazine (CMTZ) gives valuable information on the number of electroactive sites in the tripod: both compounds are reduced at approximately the same potential (−0.85 V for **1** and −0.90 V for CMTZ *vs.* Ag^+/Ag) but with a significantly higher peaks separation for the tripod (160 mV *vs.* 85 mV at 10 mV/s). This is expected since all the *s*-tetrazines in the tripod are not strictly reduced at the same potential, because of the separation of the standard potentials due to the presence of more than one redox center on the same molecule (this arises out of entropy variations, the theoretical value for this difference is [2RT/F]*ln*3, that is 57 mV[13]). Since the measured difference is slightly higher, it is also likely that repulsive interactions exist between the injected negative charges. The comparison between CVs for both compounds also allowed us to check the overall number of exchanged electrons for the tripod $n_{\text{tripod}\mathbf{1}}$, compared to CMTZ n_{CMTZ}. Peak currents *vs.* square root of the scan rate give linear variations in both cases, with the slope ratio equal to:

$$\frac{n_{\text{tripod}\mathbf{1}}}{n_{\text{CMTZ}}}\sqrt{\frac{D_{\text{tripod}\mathbf{1}}}{D_{\text{CMTZ}}}} = 1.75$$

according to the Randles-Sevčik equation. The expected value for the electron number ratio leads to: $\frac{D_{\text{tripod}\mathbf{1}}}{D_{\text{CMTZ}}} = 0.35$, which is in rather good agreement with the theoretical value deduced from the estimated size of both compounds, taking into account that CMTZ has a rather more ellipsoidal shape than the tripod.[14] This evidences the fact that all the *s*-tetrazine cores in the tripod compound are actually electroactive.



Scheme 3 Synthetic scheme of cyclophane-*s*-tetrazine **4**.

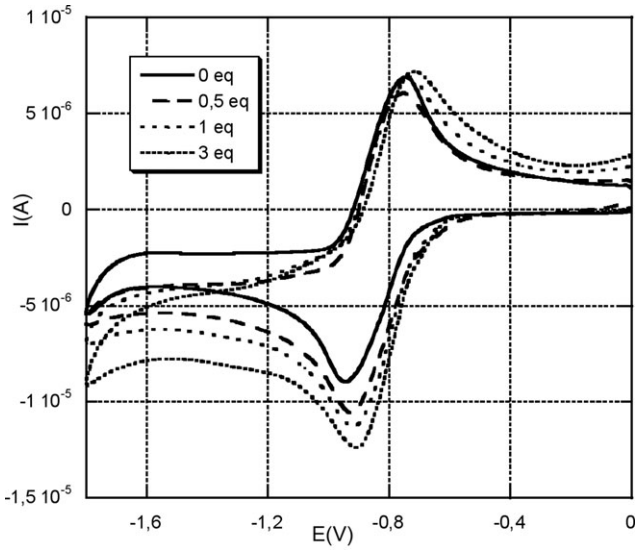


Fig. 1 Electrochemical behaviour of tripod **1** *ca.* 1 mM in DM + TBAP in the presence of increasing amounts of resorcinol (scan rate: 100 mV/s).

An interesting feature is the sensitivity of the anion radical to weak acids. The Fig. 1 also shows the evolution of the CVs upon successive additions of resorcinol. It is clear that the peaks featuring the *s*-tetrazine moieties reduction shift toward positive value and also that the ratio of backward *vs.* forward current decreases, indicative of a protonation of the anion-radical by resorcinol. We already had noticed this effect in the case of *s*-tetrazine **5**.[11]

We focused then on the electrochemical features of cyclophane **4**. Fig. 2 displays the CVs of the cyclophane compared to those of dimethoxy-*s*-tetrazine (DMTZ): it is clear that the redox behaviour is similar in particular concerning the standard potentials which are very close as expected (−1.20 and −1.21 V *vs.* Ag^+/Ag, respectively). Similarly to the tripod case, the peak potentials are more separated in the case of the cyclophane compound than for the DMTZ standard (*ca.* 145 mV *vs.* 90 mV at 10 mV/s), but here the potential widening is equal to the theoretical prediction[13] which tends to demonstrate that this time, the *s*-tetrazine moieties in **4** are strictly reduced at the same potential. The comparison between CVs for both compounds also allowed us to check the overall number of exchanged electrons for the cyclophane, DMTZ acting as a standard. In that case, peak currents vary linearly *vs.* the square root of the scan rate in both cases (see suppl. mat.), with the slope ratio equal to:

$$\frac{n_{\text{cyclo}\,\mathbf{4}}}{n_{\text{DMTZ}}}\sqrt{\frac{D_{\text{cyclo}\,\mathbf{4}}}{D_{\text{DMTZ}}}} = 1.49$$

according to the Randles-Sevčik equation. The expected value for the electron number ratio leads to: $\frac{D_{\text{cyclo}\,\mathbf{4}}}{D_{\text{DMTZ}}} = 0.25$, which is as expected less than the value found for the tripod, in agreement with the compared estimated diameters (*ca.* 11 Å for the cyclophane **4** *vs. ca.* 8 Å for the tripod **1**). Assuming the same diffusion coefficient for DMTZ and CMTZ, the ratio

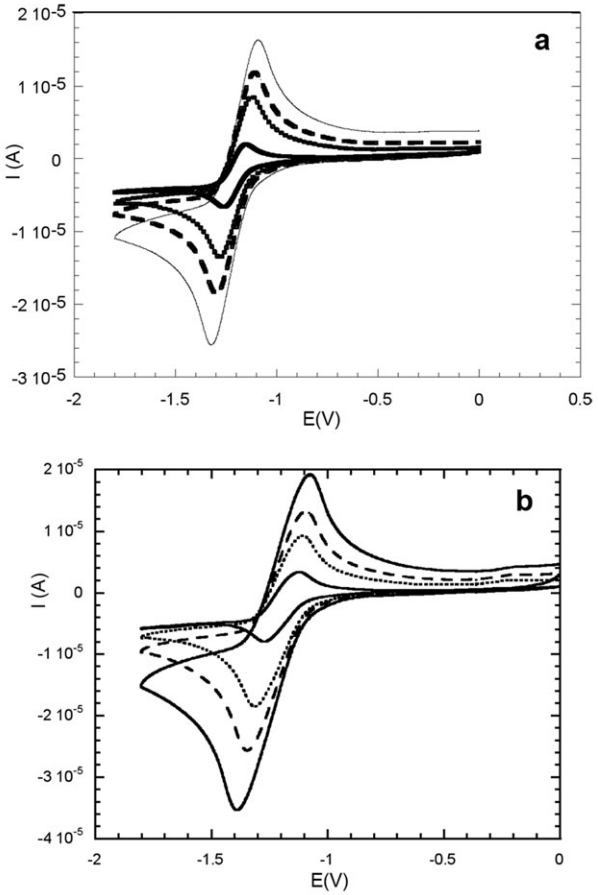


Fig. 2 CVs of (a) dimethoxy-*s*-tetrazine and (b) cyclophane **4**, each 5 mM in DM + TBAP on Pt at various scan rates (10; 50; 100; 200 mV/s).

$D_{\text{cyclo}\,\mathbf{4}}/D_{\text{tripod}\,\mathbf{1}}$ agrees very well with the ratio of diameters estimated by molecular modeling (Chem. 3D, see supp. Mater.†). This corroborates our former assumption about the number of involved electrons in the reduction process, and thus demonstrates that all the *s*-tetrazine cores in the cyclophane compound are actually electroactive.

In order to check the sensitivity of cyclophane-*s*-tetrazine **4** to three-fold symmetrical donor compounds, several equivalents of either 1,3,5-trimethoxybenzene or triethanolamine were added to the solution of **4**. The results are displayed in Fig. 3 and clearly evidence a redox potential shift and decrease of backward current only in the case of triethanolamine. Therefore it is likely that no interaction takes place with the electron donor 1,3,5-trimethoxybenzene and the neutral tetrazine. Correlatively it is clear that the presence of an acidic proton is required to observe a modification of the electrochemical behaviour. Actually the main interaction probably takes place between the protic donor and the reduced form of the tetrazine cyclophane (acid base interaction) since the redox potential is shifted toward more positive values upon additions of triethanolamine.[13] However it is not fully clear why the effect becomes discernable only above 2 eq. of triethanolamine, the role of the cyclophane symmetry is here unclear.

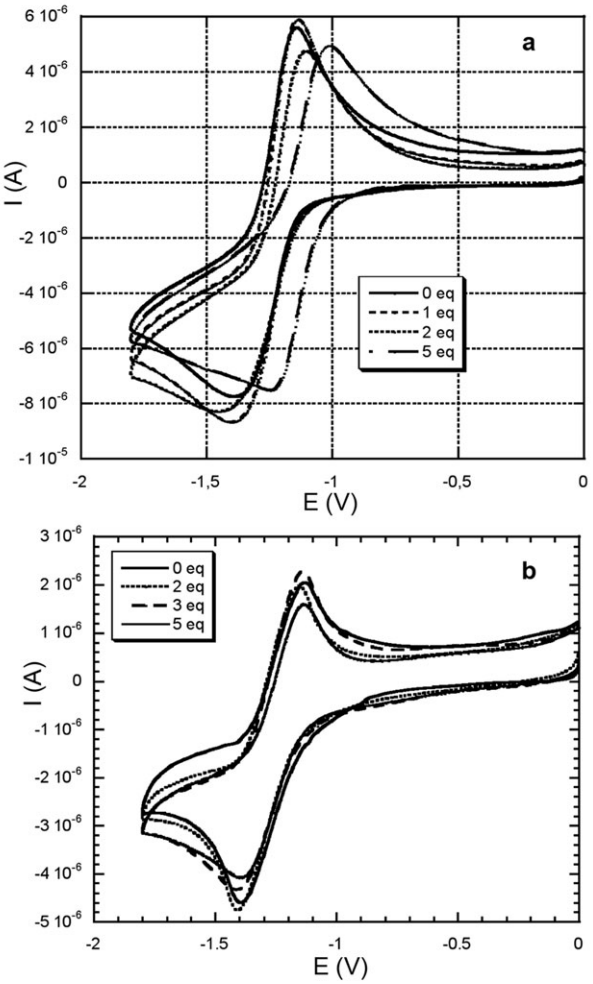


Fig. 3 CVs of cyclophane-tetrazine **4**, 5 mM in DM + TBAP on Pt at 100 mV/s, in presence of: (a) triethanolamine and (b) 1,3,5-trimethoxybenzene.

(3) Fluorescence study

All the new *s*-tetrazines are fluorescent in DM. Table 1 below gathers the main photophysical characteristics of the four molecules in solution, while the fluorescence spectra of the three most relevant compounds are represented in Fig. 4. The excitation wavelength was chosen at 495 nm for all compounds.

It seems that in each molecule containing *s*-tetrazine rings of different types, the general behaviour in fluorescence corresponds unfortunately to the one of the less fluorescent ring. For example, the open structure **2** has one dialkoxy-*s*-tetrazine moiety, and two 1-chloro-4-alkoxy-*s*-tetrazine moieties. The values found for fluorescence lifetimes and quantum yield (τ_0 = 59 ns, Φ_F = 0.13) are in the same range as those of DMTZ (τ_0 = 49 ns, Φ_F = 0.11).[10] Correlatively, the fluorescence lifetime and quantum yield are markedly lower compared to CMTZ (τ_0 = 160 ns, Φ_F = 0.38). Therefore, this compound displays the fluorescence characteristics of a dialkoxy-*s*-tetrazine rather than a chloroalkoxy-*s*-tetrazine. Similarly, cyclophane **4** displays the characteristics of a 1-alkoxy-4-thioalkoxy-*s*-tetrazine, with unfortunately a relatively low fluorescence yield.

The fluorescence of the open structure **3** (which differs from the cyclophane only by the fact that the ring is not closed) displays exactly the same characteristics as the cyclophane **4**. Therefore, the fluorescence loss can not be ascribed to the conformation of the molecule. It is also noteworthy that the close proximity of the same subunit in a molecule, as it is the case with tripod **1** does not lead to a marked alteration of the fluorescence properties of the *s*-tetrazines compared to their monomeric model.

(4) Fluorescence quenching experiments

We had already demonstrated the possibility of performing efficient fluorescence quenching with chloroalkoxy-*s*-tetrazines.[10] Quenching experiments were performed with three of the *s*-tetrazine presented, tripod **1**, the open *s*-tetrazine **2** and the cyclophane **4**, in order to check if the particular conformation of these *s*-tetrazines could lead to an improvement of the quenching efficiency.

(a) Tripod behavior. The fluorescence quenching has been tested using various electron donors, since we had previously demonstrated that electron transfer in the excited state is the quenching mechanism in the case of another related

Table 1 Main spectral data of compounds **1–4** in dichloromethane

Compound	λ_{max}^{abs}/nm	ε_{abs}/L mol^{-1} cm^{-1} (± 5%)	λ_{max}^{em}/nm	ϕ_F[a] (± 5%)	Lifetime/ns (± 5%)
Tripod **1**	518	1800			
	323	7960	565	0.29	150
	269	1250			
Open structure **2**	522	2100			
	330	8510	570	0.13	59
	269	3890			
Open structure **3**	529	1650			
	399	2100			
	348		575	0.006	10
	259				
Cyclophane **4**	528	1650			
	397	2100	585	0.006	2.7 (99.6%)
	350				38.3 (0.4%)
	257				4.6[b]

[a] Quantum yields measured by comparison with a Rhodamine 6G standard in ethanol.[15] [b] Average lifetime.

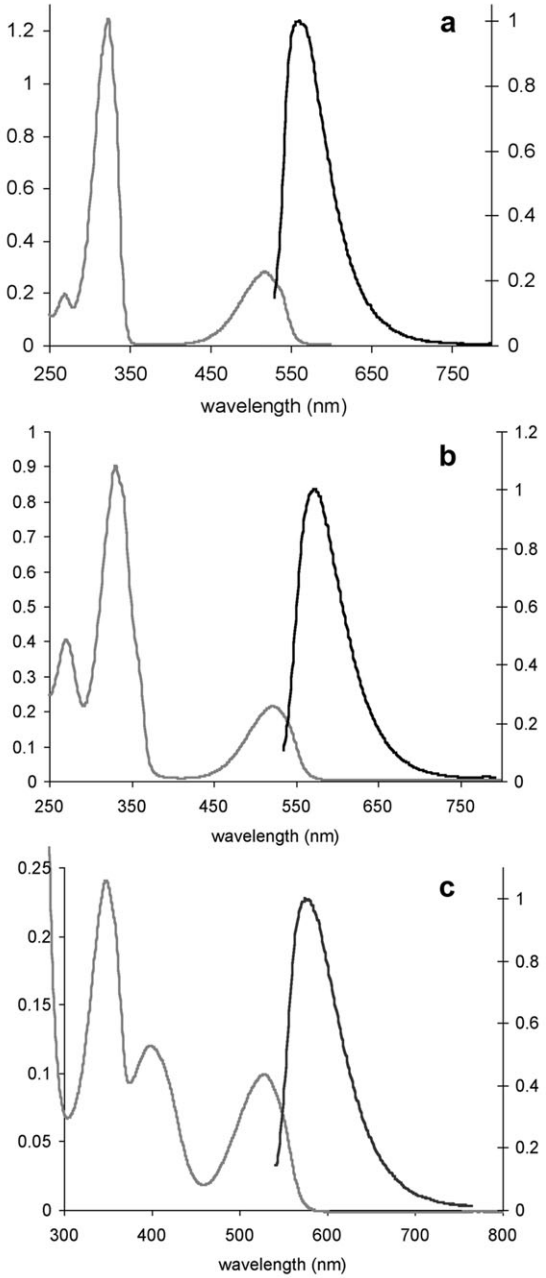


Fig. 4 Absorption (grey line) and normalized fluorescence (black line) spectra of (a) compound **1**, (b) compound **2** and (c) compound **4** (λ_{exc} = 495 nm).

s-tetrazine, because of the oxidative character of its excited state. The Fig. 5 below displays (a) the changes observed in the fluorescence spectrum upon addition of increasing amounts of triphenylamine, and (b) the concentration-quenching dependence for different donors using the Stern–Volmer equation:

$$\left(\frac{I_0}{I}\right) - 1 = K_{SV} \cdot [Q]$$

For all donors, a linear correlation is obtained which can be attributed either to a static or dynamic quenching. The best electron donors like tetrathiafulvalene give as expected the most efficient quenching.

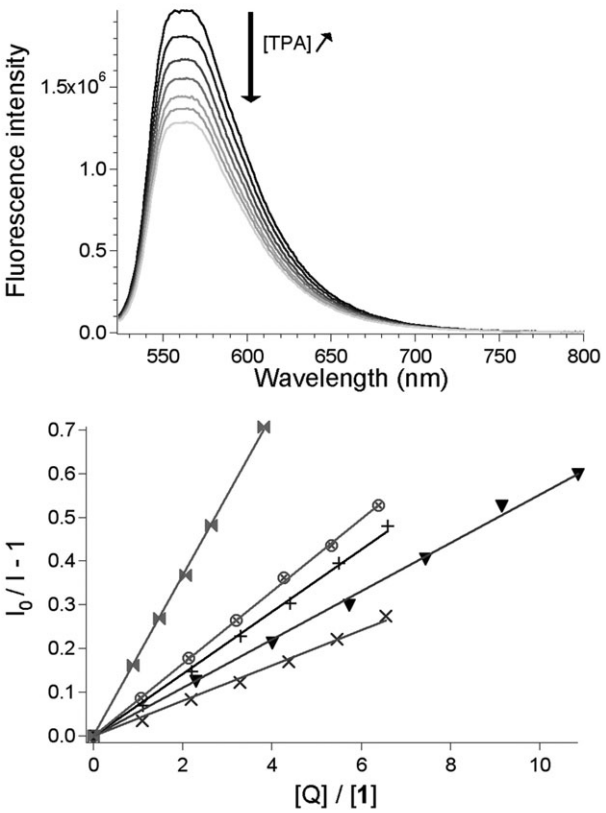


Fig. 5 (a) Modification of fluorescence spectra of **1** upon addition of triphenylamine (TPA) from 0.06 mM (1 eq.) to 0.36 mM (6 eq.). Quenching of compound **1**'s fluorescence is observed (λ_{exc} = 495 nm); (b) Stern–Volmer plots ($\frac{I_0}{I} - 1$ as a function of the concentration ratio of quencher Q over **1**); I_0 is the fluorescence intensity without quencher, I is the fluorescence intensity with quencher) for the quenching of compound **1** with five different quenchers (Q), namely tetrathiafulvalene (⋈), triphenylamine (⊗), tri(4-bromophenyl)amine (+), pyrrole (♥) and 1,3,5-trimethoxybenzene (×).

Time-dependent experiments were then carried out with tetrathiafulvalene (see supp. material†), and have shown that the quenching was purely dynamic. The initial fluorescence intensity of the fluorescence decay keeps nearly constant upon addition of increasing amounts of quencher, while the lifetime becomes shorter and shorter but still monoexponential (*i.e.* unique): this indicates that the mechanism of quenching is dynamic and not static. Unfortunately, this result rules out the formation of a complex between the tripod in its ground state and the quencher.

Nevertheless thanks to this observation, one can write $K_{SV} = k_q \cdot \tau_0$ and it is therefore possible to correlate more accurately the donor strength with the quenching efficiency (k_q), through the Rehm–Veller relationship, in the case of a quenching occurring through a charge transfer process:

$$\Delta G^0 = E(D/D^+) - E(A/A^-) - E_{0-0} - \frac{e^2}{\varepsilon_r \cdot r}$$

For a specified acceptor, the $E(A/A^-)$ and E_{0-0} are fixed, and $e^2/(\varepsilon_r \cdot r)$ is constant, therefore ΔG^0 can be expressed as a linear function of the redox potential of donors ($E^0_{ox}(D)$), which

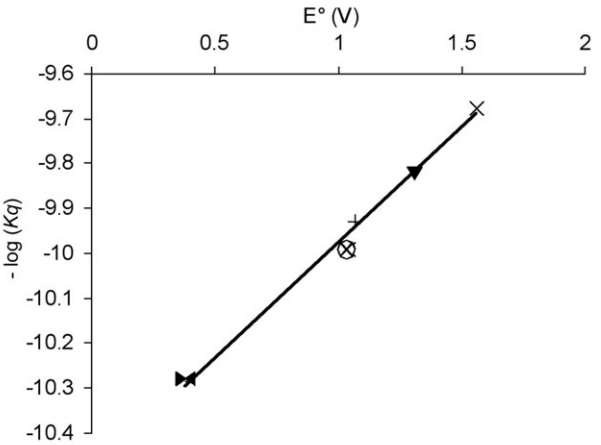


Fig. 6 Rehm–Weller plot for **1** (R = 0.992). Potentials are literature data expressed *vs.* SCE.

constitutes a simplified Rehm–Weller equation:

$$\frac{\Delta G^0}{R \cdot T} = -\mathrm{Log}(k_q) = a \cdot E^0_{ox}(D) + b$$

As shown in Fig. 6 below, there is an excellent correlation between the donor standard potential and the quenching rate constant, and therefore confirms that the quenching occurs effectively through a charge transfer process.

According to theoretical treatments,[16] $+\Delta E_{0-0}$ can be calculated from the spectroscopic data:

$$+\Delta E_{0-0} = \frac{E_{abs} + E_{em}}{2e} = \frac{hc}{2e}\left(\frac{1}{\lambda_{abs}} + \frac{1}{\lambda_{em}}\right)$$

where E_{abs} and E_{em} represent, respectively the absorption and emission energy, and $+\Delta E_{0-0}$ is expressed in eV (and therefore can be directly correlated to the electrochemical potential). (The other symbols e, h, c have their usual meanings.)

Considering the following equation, we can estimate the excited-state oxidation potential of the acceptor (Tripod **1**) as $E^{0*} = E^0 + \Delta E_{0-0}$. Then $E^{0*} \approx 1.44\ \mathrm{V} \pm 0.1$ (*vs.* Ag^+/Ag). It is therefore clear that the linear behaviour of Rehm–Weller was likely to be expected since the reduction potential of each donor investigated lies much below 1.44 V ± 0.1 (about 1.76 *vs.* SCE).

(b) Open structure 2 and cyclophane 4. We have performed parallel quenching experiments in order to check any possible different behavior between cyclophane **4** and the analogous open structure **3**, which is very similar but for the ring closure.

Like in the case of **1**, the fluorescence quenching of **2** was carried out in the presence of the same electron-rich quenchers: triphenylamine (TPA), tris(4-bromophenyl)amine, pyrrole and 1,3,5-trimethoxybenzene. Fig. 7a shows the modification of fluorescence spectra of **2** upon sequential addition of TPA. The data for all quenchers were summarized to obtain the Stern–Volmer plots (Fig. 7b).

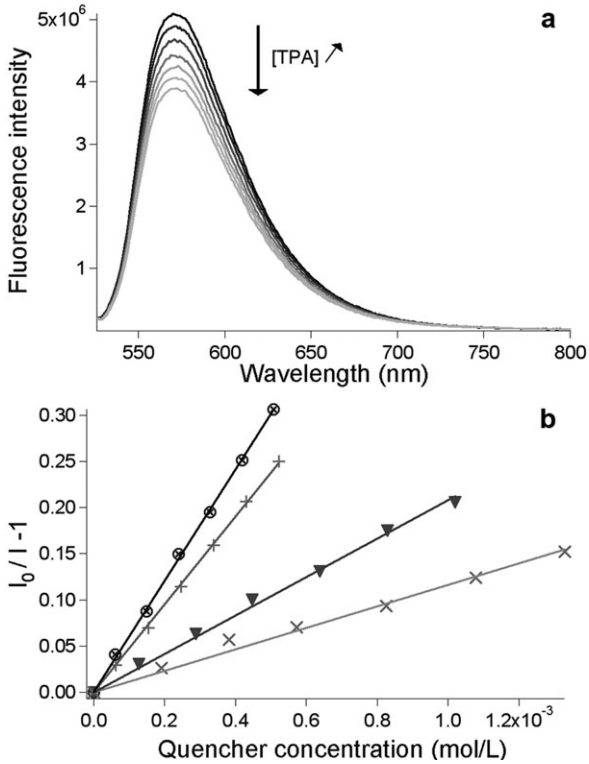


Fig. 7 (a) Modification of fluorescence spectra (λ_{exc} = 495 nm) of **2** upon addition of triphenylamine (TPA); (b) Stern–Volmer plots for the quenching of **2** with triphenylamine (⊗), tri(4-bromophenyl)-amine (+), pyrrole (♥) and 1,3,5-trimethoxybenzene (×).

The Rehm–Veller equation is again verified, indicating the occurrence of a quenching through electron transfer, as it could be expected.

Despite the weak fluorescence of cyclophane **4**, it has been possible to perform fluorescence quenching in the case of the good donor triphenylamine. The results are summarized in Table 2.

It comes from the table that the quenching rate constant is more than two times higher in the case of the cyclophane than in the case of the other *s*-tetrazine compounds. Therefore, it is likely that the threefold symmetry structure of **1** and the open structure of **2** do not play any accelerating role; on the other hand the closed cyclophane structure leads to an increase of the quenching rate. It is however not likely, especially in view

Table 2 Stern–Volmer constants, quenching rate and lifetimes in presence of triphenylamine

Compound	$k_{SV} = k_q \cdot \tau_0$/L mol^{-1}	$k_q/10^9$ L mol^{-1} s^{-1}	τ_0/ns
Tripod **1**	1486	9.8	150
Open structure **2**	603	10.2	59
Cyclophane **4**			2.7 ± 0.1 (99.6%)
	102	22.2	38.3 ± 0.8 (0.4%)
			$\overline{\tau_0} = 4.6$

of the electrochemistry results that this comes from the formation of a pre-inclusion complex, in which case the acceleration of the quenching would probably have been even faster.

In order to take a closer look at this, we have performed NMR experiments of cyclophane **4**, upon addition of various amounts of triphenylamine, to check if formation of a complex in the neutral state occurred to some extent. We chose one of the main O–CH_2 protons signals which gives an accurate enough peak (with a complex concentration of 5.4 10^{-3} M). Actually a very small shift of the δ values is observable upon triphenylamine addition, probably accounting for the formation of a complex in the neutral state (see supp. material†). However, the determination of the association constant by a standard fitting method[17] gives a low value of 2.6 for the association constant. This therefore excludes any appreciable complex formation at the 10^{-4}–10^{-5} M concentration range used for the fluorescence study.

IV. Conclusion

We have prepared several new multi-ring (multi-functional) *s*-tetrazines and examined their spectroscopic and electrochemical properties, as well as their fluorescence quenching. All the *s*-tetrazines examined have their fluorescence quenched by electron donors according to a charge transfer process, thus confirming their potential in the sensing of aromatic electron rich compounds. Except in the case of a closed cyclophane, the spatial arrangement of the *s*-tetrazine does not seem to influence noticeably the rate of the fluorescence quenching. In addition, electrochemistry experiments have shown that the electrogenerated anion-radical of the *s*-tetrazines, was sensitive to weak acids, especially in the case of triacids displaying a symmetry fitting the one of the *s* arrangement in the supermolecule.

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