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Synthesis and Physical Chemistry of *s*-Tetrazines: Which Ones are Fluorescent and Why?

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New fluorescent tetrazines have been prepared and their electrochemistry and fluorescence efficiency evaluated. The occurrence of fluorescence as well as the wavelength were found to be strongly dependent on the substituents, which have to be electronegative heteroatoms. This has been rationalized through a computational study that showed that the crucial factor is the nature of the HOMO, which determines the existence or not of fluorescence.

Introduction

s-Tetrazines are relatively old molecules, the discovery of which dates back to the end of the 19th century. The first synthesis was reported by Pinner who performed the reaction of equimolar quantities of hydrazine and benzonitrile and prepared, upon further oxidation of the dihydrotetrazine intermediate, the deep-red 3,6-diphenyl-*s*-tetrazine.[1] All *s*-tetrazines are highly colored, electroactive heterocycles that display a very high electron affinity, which allows them to be reduced at low-to-very-low potentials; they are indeed the least electron-rich class of neutral CN heterocycles.[2] They have a low-lying π* orbital that leads to a n→π* transition in the visible light region, which is responsible for their red color. *s*-Tetrazines are largely used as dienes in inverse Diels–Alder cycloaddition reactions,[3] but are also used in the development of new nitrogen-rich energetic materials.[4] The chemistry of tetrazines has recently been reviewed.[5]

We became interested in *s*-tetrazines to develop new materials having both special optical and electrochemical features. In the course of these studies, we noticed that *s*-tetrazines substituted with heteroatoms can display fluorescence properties.[6,7] Earlier reports of such behavior are scarce.[8,9] Some of these compounds are even fluorescent in the crystalline state, which certainly makes them the smallest organic fluorophores ever prepared and therefore makes them especially attractive for sensing applications. Our first studies showed indeed that 3-chloro-6-methoxy-*s*-tetrazine was among the best of these compounds because the combination of chlorine and an alkoxy substituent in a *s*-tetrazine appeared to lead to a high fluorescence yield. On the other hand, other *s*-tetrazines substituted by nitrogen or sulfur atoms and the diaryl-*s*-tetrazines previously prepared by us appeared to lack any fluorescence. Thus, we became interested in understanding the origin of this behavior and would like eventually to be able to predict it.

We describe herein the preparation of several families of *s*-tetrazines, featuring not only the fluorescent OR- and Cl-substituted tetrazines, but also alkylthio- and dialkylamino-substituted tetrazines, tetrazines unsymmetrically substituted by various heteroatoms, and diaryl-*s*-tetrazines (see Schemes 1 and 2 and Table 1). Many of these compounds are original, and their absorption and fluorescence properties have been investigated. To rationalize the observed properties, quantum calculations were performed on all the *s*-tetrazines prepared and on a few additional examples. The results show in particular that fluorescence occurs when the HOMO orbital has a nonbonding n character, but is absent if the HOMO is a π orbital. The HOMO and the closest occupied HOMO-1 orbitals are very similar in energy in most *s*-tetrazines and their frequent inversion has been found to correlate with the occurrence or not of fluorescence, giving a valuable predictive tool for the fluorescence of this class of compound.

Results and Discussion

All the compounds described were prepared either by starting from the easily accessible dichlorotetrazine by nucleophilic substitution or by a modification of the classical Pinner synthesis[10] (Scheme 3).

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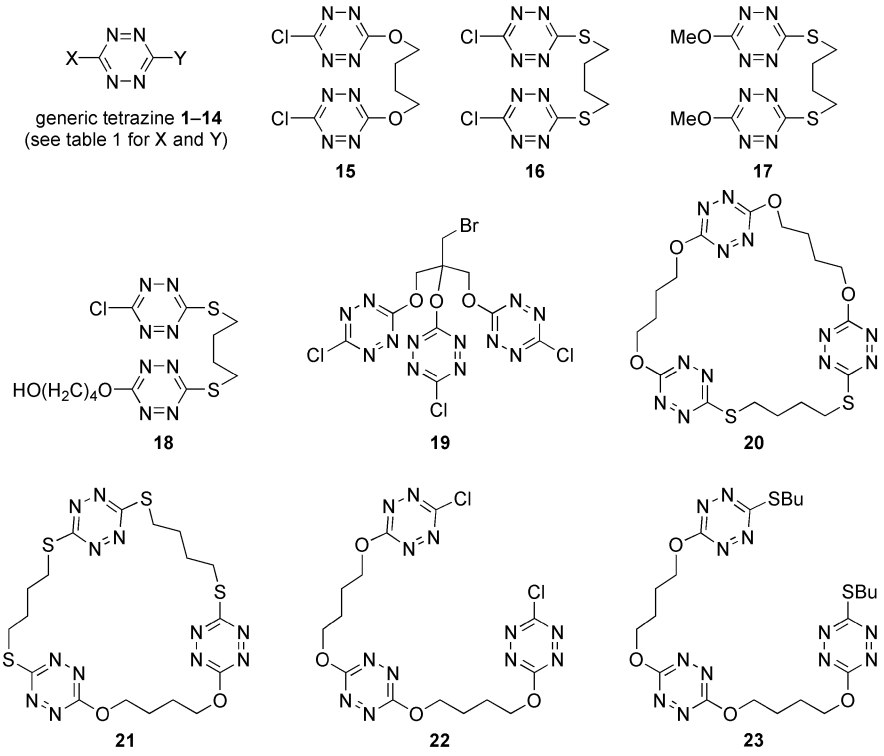
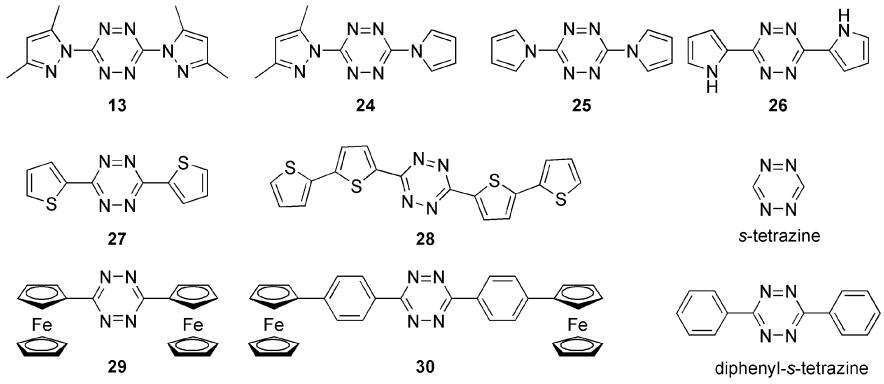


Scheme 1. *s*-Tetrazines substituted by heteroatoms.

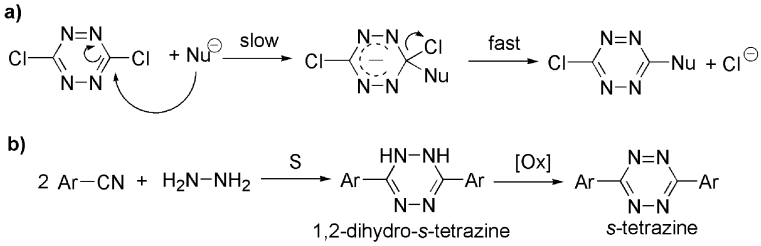


Scheme 2. *s*-Tetrazines substituted by aromatic groups (*s*-tetrazine and diphenyl-*s*-tetrazine have been included for comparison but were not synthesized by us).

Table 1. Substituents in the generic *s*-tetrazines **1**–**14**.

	Substituent X	Substituent Y
1	Cl	Cl
2	Cl	OMe
3	Cl	naphthalen-1-yloxy
4	Cl	pyrrolidin-1-yl
5	Cl	2,3-diphenylaziridin-1-yl
6	OMe	OMe
7	OMe	$O(CH_2)_4OH$
8	$O(CH_2)_4OH$	$O(CH_2)_4OH$
9	OMe	SMe
10	SMe	SMe
11	S-*n*Bu	S-*n*Bu
12	pyrrolidin-1-yl	pyrrolidin-1-yl
13	3,5-dimethyl-1*H*-pyrazol-1-yl	3,5-dimethyl-1*H*-pyrazol-1-yl
14	3,5-dimethyl-1*H*-pyrazol-1-yl	OMe

Most *s*-tetrazines substituted by O, N, or S heteroatoms (Scheme 1 and Table 1) were prepared from the *s*-tetrazine **1**. This compound is easily prepared in five steps from guanidine hydrochloride on a multigram scale. Our need for this valuable starting material led us to optimize some of the steps reported in the literature (see the Supporting Information for details). Nucleophilic substitution of chlorine by amines required the addition of 2 equiv. of reactant to trap the hydrochloric acid formed. Alternatively, triethylamine can be used. Monosubstitution was accomplished at room temperature in high yields in diethyl ether or acetonitrile. Disubstitution was achieved by heating the reaction for a few hours. Thiols proved to be very reactive with **1**, as previously reported.[11] Indeed, it was very difficult to isolate the monosubstituted product of the reaction with



Scheme 3. Synthesis of *s*-tetrazines: a) nucleophilic substitution; b) modified Pinner's synthesis.

the disubstituted product always predominating. In those cases in which we did obtain alkylthio(chloro)-*s*-tetrazines, the second chlorine could be replaced by oxygen by reaction with alcohols at reflux or under pressure. Compounds **17** and **18** were prepared in this way. In contrast, alcohols reacted with **1** with more difficulty. We tried various methods to accomplish monosubstitution in high yields. The use of alcoholate proved not to be very satisfactory (20% yield on average) as a considerable degradation took place. A low temperature (–78 °C) was required in this case. We attempted the reaction with a variety of inorganic bases but with little improvement. The use of pressure led to better results (50% yields). Only when we used 2,4,6-collidine in dichloromethane did we obtain monoalkoxy-*s*-tetrazine in high yields (90% or more) at room temperature. Addition of a drying agent such as molecular sieves to keep moisture off the reaction mixture allows a high yield to be maintained when the reaction time is prolonged (>1 h). The second substitution was still a problem. Indeed, only alcoholates at a low temperature (–78 °C) in THF gave acceptable results with either s-tetrazine **1** (symmetrical product) or chloro(alkoxy)-*s*-tetrazines (unsymmetrical product). Other *s*-tetrazines can be used for nucleophilic substitution. Bis(methylthio)-*s*-tetrazine (**10**) was for a long time the intermediate of choice in this type of chemistry[12] until the discovery of an easy access to **1**. Even this derivative was at first prepared from **10**.[13] The sole derivative prepared by nucleophilic substitution from **10** in this work is **9**. Disubstitution in this case was sometimes possible. 3,5-Dimethylpyrazolyl can also act as a leaving group, but less efficiently than chlorine.[11] There are recent examples of substitution with amines.[14] We found that **13** reacted readily with methanol under pressure to give both mono- and disubstituted derivatives.

All the diaryl-*s*-tetrazines (Scheme 2) were prepared by our modified version of the Pinner synthesis followed by oxidation with isoamyl nitrite, which is a mild oxidant and gives *s*-tetrazines cleanly and in high yields (Scheme 3).

The redox potentials for the first electrochemical reduction of *s*-tetrazines were measured in dichloromethane (Table 2) and are correlated to the LUMO, with discrepancies related to solvation variations. However, it should be pointed out that the *s*-tetrazine core in which the electron is located (Figure 1) undergoes only small structural changes. Besides, the first reduction is located on the *s*-tetrazine core, as evidenced by the fully reversible behavior observed for most compounds. The redox potentials highlight the role played by the substituents on the position of the LUMO level: Strong electron-withdrawing groups like chlorine or 3,5-dimethylpyrazolyl shift the standard potential towards more positive values, whereas electron-rich substituents like ferrocene or pyrrole shift it in the opposite direction. Substitution of oxygen by sulfur, for example, replacing alkoxy with a methylthio group, has little influence on the redox potential of the *s*-tetrazine. Very interesting is the comparison between *N*-substituted *s*-tetrazines like pyrrolidine and pyrrole: In the former case, the reduction occurs at a very negative potential due to the π-donor effect of the lone pair on the nitrogen that destabilizes the anion radical, whereas in the latter, this effect is strongly alleviated by the aromatic character of the substituent.

Table 2. Half-wave reduction potential of *s*-tetrazines in dichloromethane vs. ferrocene.

	Substituent X	Substituent Y	$E°_{red}$ [V]
1	Cl	Cl	–0.68[a]
2	Cl	OMe	–0.99[a]
3	Cl	naphthalen-1-yloxy	–0.97[a]
4	Cl	pyrrolidin-1-yl	–1.35
5	Cl	2,3-diphenylaziridin-1-yl	–1.04
6	OMe	OMe	–1.25[a]
9	OMe	SMe	–1.23
10	SMe	SMe	–1.20
12	pyrrolidin-1-yl	pyrrolidin-1-yl	–1.73
13	3,5-dimethyl-1*H*-pyrazol-1-yl	3,5-dimethyl-1*H*-pyrazol-1-yl	–0.96[c,d]
14	3,5-dimethyl-1*H*-pyrazol-1-yl	OMe	–0.89
15			–0.99[a]
16			–0.90
19			–0.94[b]
20			–1.24
21			–1.21[b]
24	3,5-dimethyl-1*H*-pyrazol-1-yl	pyrrol-1-yl	–0.97[c]
25	pyrrol-1-yl	pyrrol-1-yl	–1.07[c]
26	pyrrol-2-yl	pyrrol-2-yl	–1.31[c]
27	2-thienyl	2-thienyl	–1.24[c]
28	2,2′-bithienyl-5-yl	2,2′-bithienyl-5-yl	–1.25[e,f]
29	ferrocenyl	ferrocenyl	–1.28[d,g]
30	4-ferrocenylphenyl	4-ferrocenylphenyl	–1.32[d,g]
s-Tetrazine			–1.16[h,i]
Diphenyl-*s*-tetrazine			–1.21[h,i]

[a] From ref.[3] [b] From ref.[5] [c] From ref.[14] [d] Not fully reversible. [e] From ref.[15] [f] As a polymer. [g] From ref.[16] [h] From ref.[15] [i] Recorded in acetonitrile.

These trends in the redox potentials are confirmed by the positions of the corresponding LUMO levels. In particular, the differences between the reduction potentials in comparison with **1** (in V) are very similar to the corresponding differences in the LUMO energy levels (in eV), as shown in

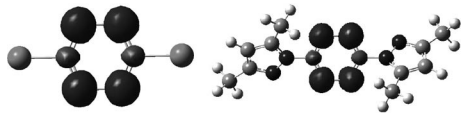


Figure 1. Spin density (isodensity 0.004 a.u.) from B3LYP calculations on the radical anions of **1** (left) and **13** (right).

Table 3 and Figure 2. These results confirm that the reduction of these compounds is centered on the four nitrogen atoms of the aromatic core with very little reorganization in addition to the electron transfer.

Table 3. Values of the differences between standard potentials and the differences between LUMO levels compared with tetrazine **1** as the standard.

Compound	Measured $\Delta E°$ [V]	Calculated ΔE_{LUMO} [eV]
2	0.31	0.28
4	0.67	0.70
6	0.57	0.55
12	1.05	1.27

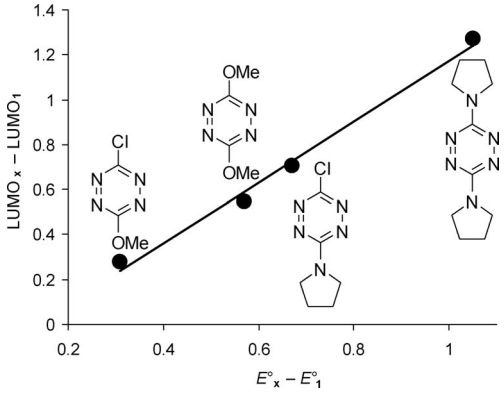


Figure 2. Correlation between the differences in the LUMO energies of *s*-tetrazines **2**, **4**, **6**, and **12** in comparison with **1** and the differences in their standard potentials (R^2 = 0.9904).

The absorption and fluorescence (when relevant) wavelengths for the *s*-tetrazines are reported in Table 4. The absorption spectra exhibit two main bands (λ_1 and λ_2) and in some cases a smaller one (λ_3). The first transition is attributed to the n→π* transition of the *s*-tetrazine. This forbidden transition has a small molar extinction coefficient ε that ranges from 400 to 2000 $L\,mol^{-1}\,cm^{-1}$. Furthermore, this band is weakly sensitive to the substitution pattern on the ring, as expected for this type of transition.[16] The second band is attributed to a π→π* transition and is more intense (the ε values are usually higher by a factor of 10). Its position is strongly correlated to the electronegativity of the substituents present on the ring. As expected electron-withdrawing groups shift this band to higher energies (hypsochromic shift). An intermediate situation is reached when two different heteroatoms are present when compared to their symmetrical counterparts (for example, see **1**, **2**, and **6** or **1**, **4**, and **12**). The nature of the alkyl chain has little influence, as evidenced by the very similar behavior of **6**, **7**, and **8**. Finally, note that in the case of multi-*s*-tetrazine derivatives, the spectral features are very similar to their monomeric equivalents (compare **15** and **19** with **2** or **17** and **9**). For differently substituted *s*-tetrazines, a more complicated situation is found, but in some cases two bands can be seen to arise from the second transition (compounds **20** and **23**).

The fluorescence spectra of all the compounds were recorded. Only the *s*-tetrazines substituted by heteroatoms display fluorescence, and only in the case of oxygen and chlorine substituents are relatively high fluorescence quantum yields observed. Most *s*-tetrazines substituted by aromatic rings were found not to be fluorescent. Nevertheless diphenyl-*s*-tetrazine is reported to be weakly fluorescent.[17] The position of the emission band is also correlated to the electron-donating strength of the substituents. The most efficient derivatives are those containing one chlorine and one alkoxy. This is true for monomer **2**, but also, and very interestingly, for the multichromophoric **15** and **19**. In these molecules, the fluorescence parameters (quantum yields and lifetimes) are retained, which indicates that the *s*-tetrazines do not interact. The presence of one sulfur atom, as in **16**, induces a marked decrease in the fluorescence. This loss is even more dramatic when sulfur is combined with oxygen in **17**. The presence of two sulfur atoms leads to a nearly complete loss of fluorescence as demonstrated for **11**. Unfortunately, the multichromophoric systems with different *s*-tetrazines **18** and **20**–**23** exhibit the characteristics of the less fluorescent subunit are retained.

In this study, to highlight the high sensitivity of the emissive properties of the *s*-tetrazine to the nature of the substituent, one case is of particular importance. When one considers compounds **4** and **5**, it appears, very surprisingly at first sight, that a very small change in the nature of the amine (aziridine to pyrrolidine) can completely switch off the fluorescence of the *s*-tetrazine. This result led us to look at the electronic structure of these derivatives in more depth; we looked at a possible relation between the structure of the *s*-tetrazines and their photophysical properties. A survey of the literature attracted our attention to the special crystal structure of an analogue of compound **4**, bis(aziridin-1-yl)-*s*-tetrazine,[18] in which the rigidity of the aziridine ring forces it out of the plane of the *s*-tetrazine. As a comparison, in piperazine, a larger cyclic amine, the nitrogen atom attached to the tetrazine ring was found to be conjugated with the aromatic ring.[14a] Our DFT calculations were able to account for this structure and revealed one interesting consequence of this structural difference. We found that the nature of the HOMO and HOMO-1 orbitals in compounds **4** and **5** are reversed (Figure 3), which reflects the difference in the donating ability of the nitrogen atoms in the two cycles. For instance, studies on the influence of the nature of the cyclic amine on the color of azo dyes have shown that the electron-donating power of the nitrogen atom decreases in the following order: Pyrrolidinyl > piperidinyl > azetidinyl >> aziridinyl.[19]

We then systematically performed calculations on all the *s*-tetrazine derivatives that we had prepared and on some others of potential interest and plotted the four most important orbitals in relation to fluorescence processes, that

Table 4. Absorption and fluorescence maxima recorded in dichloromethane.

	Substituent X	Substituent Y	UV/Vis absorption λ_1^{abs} [nm]	λ_2^{abs} [nm]	λ_3^{abs} [nm]	Fluorescence $\lambda^{em[a]}$ [nm]	ϕ_f[b]	τ [ns][c]
1	Cl	Cl	515	307	–	551; 567	0.14	58
2	Cl	OMe	520	327	269	567	0.38	160
3	Cl	naphthalen-1-yloxy	523	–	281	567	0.004	120; 6
4	Cl	pyrrolidin-1-yl	514	436	–	–	–	–
5	Cl	2,3-diphenylaziridin-1-yl	522	365	259	576	0.045	–
6	OMe	OMe	524	345	275	575	0.11	49
7	OMe	$O(CH_2)_4OH$	526	347	–	572	0.10	59
8	$O(CH_2)_4OH$	$O(CH_2)_4OH$	528	348	–	575	0.09	–
9	OMe	SMe	528	394	–	–	–	–
10	SMe	SMe	528	422	287	–	–	–
11	S-*n*Bu	S-*n*Bu	528	428	290	590	0.0009	<1>[d]
12	pyrrolidin-1-yl	pyrrolidin-1-yl	525	496	274	–	–	–
13	3,5-dimethyl-1*H*-pyrazol-1-yl	3,5-dimethyl-1*H*-pyrazol-1-yl	524	383	278	–	–	–
14	3,5-dimethyl-1*H*-pyrazol-1-yl	OMe	529	367	–	578	–	–
15			521	328	270	572	0.36	144
16			522	391	273	576	0.025	13; 6
17			529	396	258	580	0.005	7; 2
18			524	394	263	577	0.005	<5>[d]
19			518	323	269	565	0.29	150
20			528	397; 349	257	585	0.006	–
21			529	403	288	585	0.002	–
22			522	330	269	570	0.13	59
23			529	398; 348	259	575	0.006	10
s-Tetrazine[e]			542	320	252	575	0.0006	1.5
Diphenyl-*s*-tetrazine[f]			542	298	–	602	–	20

[a] $\lambda^{ex} = \lambda_1^{abs}$. [b] $\phi_f \pm 8\%$. [c] $\tau \pm 2\%$. [d] Average of a multiexponential decline. [e] From ref.[2] [f] From ref.[17]

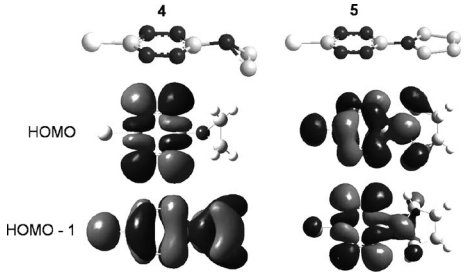


Figure 3. Calculated HOMO and HOMO-1 orbitals of compounds **4** and **5**.

is, the HOMO, the HOMO-1, the LUMO, and the LUMO+1 (see Figure 4 and Supporting Information). It appears that for substituents that are good electron donors (in the case of amines, aromatics, etc.), the HOMO orbital is of π type and the fluorescent character vanishes. This inversion of orbitals has already been proposed in an earlier report by Gleiter et al.,[20] who recorded and rationalized the photoelectron spectra of *s*-tetrazines. Neugebauer and co-workers also studied the radical cations of *s*-tetrazines[21] and observed that, depending on the substituents, the radical was delocalized only on the central ring (n type *s*-tetrazines) or on the overall molecule (π type *s*-tetrazines). In contrast, even though a lot of calculations (semiempirical, HF, or DFT) have already been conducted on *s*-tetrazine[22] and its derivatives, these trends have never been highlighted before.

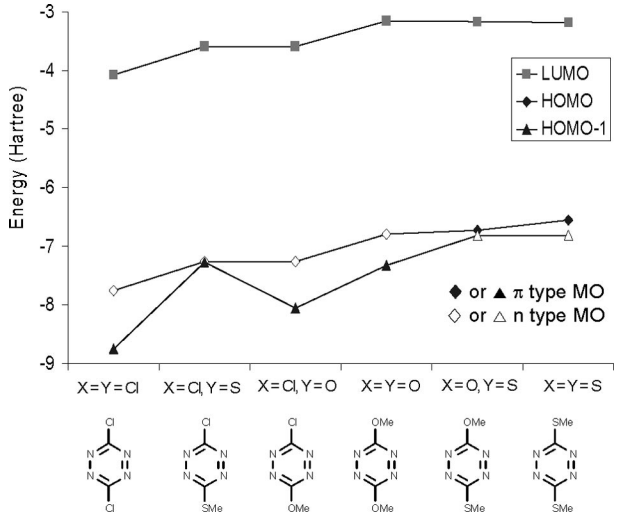


Figure 4. Positions of the HOMO (♦), HOMO-1 (▲), and LUMO (■) orbitals of **1**, **2**, **6**, **9** **10**, and 3-chloro-6-methylthio-*s*-tetrazine. Black denotes π-type orbitals and white n-type orbitals.

It was thus clear that the HOMO orbital plays a crucial role because of the mixing or not of the heteroatom lone pair or the π system of the aromatic substituent. It is also clear that the LUMO and LUMO+1 orbitals are only weakly affected by the substituents, as could be expected, especially in the case of the LUMO. This orbital corresponds to the π* orbital of the tetrazine ring and is located entirely on the four-nitrogen-atom system and its position depends mainly on the inductive effect of the substituents,

as suspected on the basis of the electrochemistry and UV/Vis absorption spectra.

On the other hand, the HOMO and HOMO-1 orbitals are strongly affected by the substituent. A very interesting situation of orbital-crossing may happen depending on the substituent. If the substituent is a weak mesomer donor, or exerts only inductive effects without orbital-mixing (in the case of chlorine and alkoxy substituents), insufficient orbital-mixing occurs and the tetrazine π system is the HOMO-1 orbital, whereas the lone pairs of the tetrazine ring mix and form the HOMO. On the other hand, when the substituents are good mesomer donors and mix sufficiently with the accepting tetrazine π system (in the case of all aromatics, amino, and alkylthio type substituents), this latter orbital energy rises above the energy of the n orbital (which is more or less constant) and forms the HOMO (whereas the n system is the HOMO-1 orbital). The case of **9** is a special one in which both orbitals are almost at the same level, which explains the very weak fluorescence observed. In these limiting cases, a better representation of the orbital spacing can be obtained by running a further minimization in dichloromethane. For example, compound **4** has two orbitals that are very similar in energy (n: –0.24529 hartree; π: –0.24588 hartree) in the gas phase, whereas in dichloromethane they are reversed and more widely spaced (π: –0.24329 hartree; n: –0.25496 hartree), which reflects better its nonfluorescent nature. Note that some *s*-tetrazine derivatives, for example, dimethyl- and diphenyl-*s*-tetrazines, previously reported as weakly fluorescent fit the observed trend. On the other hand, known compounds like the bis(trifluoromethyl)-, dicyano-, or bis(*x*-pyridyl)-*s*-tetrazine (x = 2, 3, or 4) have never been described as fluorescent but will be tested in due course.

We can also understand from these results the behavior of the multi-*s*-tetrazine compounds prepared. Indeed, the relative positions of the orbitals of the simple *s*-tetrazines shown in Figure 4 show that the HOMO of the least fluorescent one is always of higher energy. Hence, the emissive properties of compounds **15**–**23** are always governed by the less emissive nucleus (thio oxygen for **20** or dithiol for **21**). Hopefully, calculations will provide a reliable predictive tool for the design of future fluorescent multi-*s*-tetrazine compounds.

Conclusions

From the results above it is clear that tetrazines, already recognized for their applications in energetic materials and coordination chemistry, also have good potential for the elaboration of new performance molecular materials. Owing to the efficient synthesis of a fluorescent *s*-tetrazine comprising one alkoxy substituent and a chlorine atom, we now have access to a large variety of derivatives. The factors determining whether fluorescence occurs or not has been thoroughly investigated and now opens the way to the elaboration of more fluorescent molecules. The synthesis and study of additional new *s*-tetrazines and their use in the development of sensors as well as electrofluorescent switches is currently underway.

Experimental Section

Synthesis: Solvents were dried by conventional methods, distilled from nitrogen, and deoxygenated prior to use. Starting compounds were purchased and used without further purification. *s*-Tetrazines **1**,[23] **2**,[6] **3**,[6] **4**,[11] **6**,[6] **7**,[7] **8**,[7] **9**,[24] **10**,[25] **11**,[11] **13**,[26] **15**,[7] **19**,[27] **20**,[27] **21**,[7] **22**,[27] **23**,[27] **24**,[10] **25**,[10] **26**,[10] **27**,[10] **28**,[28] **29**,[29] and **30**[29] were synthesized according to reported procedures. The NMR spectra were recorded with a Bruker AC 300 spectrometer with a 5 mm probehead. The ^{1}H and ^{13}C NMR chemical shifts are referenced relative to the residual proton signal and the central peak of the carbon triplet of the deuteriated solvent ($CDCl_3$), respectively.

3-Chloro-6-(2,3-diphenylaziridin-1-yl)-*s*-tetrazine (5): In an Erlenmeyer flask, *s*-tetrazine **1** (0.1 g, 0.7 mmol) was dissolved in dry diethyl ether (25 mL). 2,3-Diphenylaziridine[30] (0.3 g, 0.15 mmol) was added dropwise at room temperature. The reaction was stirred for 10 min and water (20 mL) was added. The organic layer was separated and the aqueous phase was extracted with diethyl ether (20 mL). The organic layers were combined, dried with $MgSO_4$, and concentrated under vacuo. Compound **5** was isolated by column chromatography (SiO_2, CH_2Cl_2) in 66% yield (0.135 g). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.13 (s, 2 H), 7.32 (m, 10 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 50.3, 127.1, 128.7, 128.83, 133.5, 164.0, 167.0 ppm. HRMS (ESI$^+$): calcd. for $C_{16}H_{13}{}^{35}ClN_5$ [M + H]$^+$ 310.0854; found 310.0866.

3,6-Bis(pyrrolidin-1-yl)-*s*-tetrazine (12): In a 250 mL three-necked round-bottomed flask, *s*-tetrazine **1** (2.02 g, 13.3 mmol) was dissolved in dry diethyl ether (130 mL). Pyrrolidine (4.40 mL, 52.7 mmol) was added dropwise at room temperature. The reaction was heated at reflux for 2 h and quenched with water (100 mL). The organic layer was separated and the aqueous phase was extracted with diethyl ether (100 mL). The organic layers were combined, dried with $MgSO_4$, and concentrated in vacuo. Compound **4** was isolated by column chromatography (SiO_2, CH_2Cl_2) in the first fraction and compound **12** was then recovered by using a 1:10 mixture of ethyl acetate/CH_2Cl_2; yield 29% (0.85 g). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.01 (m, 4 H), 3.62 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.5, 46.5, 158.3 ppm. HRMS (ESI$^+$): calcd. for $C_{10}H_{16}N_6Na$ [M + Na]$^+$ 243.1329; found 243.1326.

3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-methoxy-*s*-tetrazine (14): *s*-Tetrazine **13** (1.0 g, 3.7 mmol), methanol (3 mL), and a small amount of sodium sulfate were placed in a pressure-resistant reactor. The reaction vessel was sealed and warmed at 100 °C for 2 h. After cooling, the mixture was dissolved in CH_2Cl_2 and extracted twice with water. The combined organic layers were dried with $MgSO_4$ and concentrated in vacuo. Compound **6** was isolated by column chromatography (SiO_2, CH_2Cl_2) in the first fraction and compound **14** was then recovered by using a 1:10 mixture of ethyl acetate/CH_2Cl_2; yield 67% (0.51 g). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.31 (s, 3 H), 2.58 (s, 3 H), 4.28 (s, 3 H), 6.10 (s, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 13.7, 14.2, 56.9, 111.1, 143.0, 153.5, 159.4, 166.4 ppm. HRMS (ESI$^+$): calcd. for $C_8H_{10}N_6ONa$ [M + Na]$^+$ 229.0808; found 229.0814.

1,4-Bis(6-chloro-*s*-tetrazin-3-ylthio)butane (16): Butane-1,4-dithiol (0.4 g, 3.3 mmol) and triethylamine (0.93 mL, 6.6 mmol) were mixed in acetonitrile (30 mL) in a dropping funnel and the mixture

was added slowly to a solution of *s*-tetrazine **1** (1.0 g,6.6 mmol) in acetonitrile (10 mL) at room temperature. After the addition the mixture was stirred for another 2 h until TLC indicated the reaction was over (1:5 ethyl acetate/petroleum ether). The solids were filtered, washed with diethyl ether, and the filtrate evaporated. After column chromatography (SiO_2, 1:6 ethyl acetate/petroleum ether), 0.18 g (15%) of the product **16** was obtained. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.02 (m, 4 H), 3.37 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 27.5, 30.0, 165.6, 175.7 ppm. HRMS (ESI$^+$): calcd. for $C_8H_8{}^{35}Cl_2N_8S_2Na$ [M + Na]$^+$ 372.9583; found 372.9587.

1,4-Bis(6-methoxy-*s*-tetrazin-3-ylthio)butane (17): *s*-Tetrazine **16** (94 mg, 0.27 mmol) and $MgSO_4$ (0.5 g) were added to methanol (50 mL) and the mixture was heated at reflux under Ar for 20 h. After filtration of the solids the filtrate was evaporated and the residue was purified by column chromatography (SiO_2, 3:10 ethyl acetate/petroleum ether). After evaporation of the solvents 46 mg (50%) of **17** was obtained. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.03–1.96 (m, 4 H), 3.35–3.30 (m, 4 H), 4.24 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 28.0, 30.2, 56.5, 166.3, 171.4 ppm. HRMS (ESI$^+$): calcd. for $C_{10}H_{14}N_8O_2S_2Na$ [M + Na]$^+$ 365.0573; found 365.0593.

4-{6-[4-(6-Chloro-*s*-tetrazin-3-ylthio)butylthio]-*s*-tetrazin-3-yloxy}-butan-1-ol (18): *s*-Tetrazine **17** (143 mg, 0.41 mmol), butane-1,4-diol (0.365 mL, 4.1 mmol), $NaHCO_3$ (34 mg, 0.4 mmol), $MgSO_4$ (0.3 g), and dichloromethane (6 mL) were added to a 20 mL pressure tube. The mixture was stirred and heated at 120 °C for 2 h and then cooled to room temperature. After column chromatography (SiO_2, ethyl acetate) 36 mg (22%) of the product **18** was obtained. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.60 (br., 1 H), 1.80 (m, 4 H), 2.02 (m, 4 H), 3.32 (m, 4 H), 3.73 (t, J = 6.26 Hz, 2 H), 4.61 (t, J = 6.44 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 25.2, 27.7, 28.0, 28.9, 30.2, 30.3, 62.3, 69.7, 165.8, 166.1, 171.2, 175.9 ppm. HRMS (ESI$^+$): calcd. for $C_{12}H_{17}{}^{35}ClN_8O_2S_2Na$ [M + Na]$^+$ 427.0497; found 427.0505.

Cyclic Voltammetry: The electrochemical studies were performed using an EG&G PAR 273 potentiostat interfaced to a PC computer. The reference electrode used was an Ag^+/Ag electrode filled with 0.01 M $AgNO_3$. This reference electrode was checked versus ferrocene, as recommended by IUPAC. In our case, $E°(Fc^+/Fc)$ = 0.045 V in acetonitrile or dichloromethane with 0.1 M tetrabutylammonium perchlorate (TBAP). TBAP was purchased from Fluka (puriss). Acetonitrile (Aldrich, 99.8%), dichloromethane (SDS, 99.9%), and toluene (Aldrich, 99.5%) were used as received. All solutions were deaerated by bubbling with argon for a few minutes prior to electrochemical measurements.

Photophysical Studies

Steady-State Spectroscopy: A UV/Vis Varian CARY 500 spectrophotometer was used. Excitation and emission spectra were measured with a SPEX Fluorolog-3 (Jobin–Yvon) instrument. A right-angled configuration was used. The optical densities of the samples were checked to be less than 0.1 to avoid reabsorption artifacts. The relative fluorescence quantum yields of the *s*-tetrazines were measured relative to that of Rhodamine 6G in ethanol (ϕ_f = 0.95). The solutions were of equal absorbance at the excitation wavelength (λ_{exc}=529 nm).

Time-Resolved Spectroscopy: The fluorescence decay curves were obtained by a time-correlated single-photon-counting method using a titanium–sapphire laser (82 MHz, repetition rate lowered to 4 or 0.8 MHz depending on the lifetime measured by a pulse-peaker, 1 ps pulse width, a doubling crystal was used to reach 495 nm excitation) pumped by an argon ion laser. The Levenberg–Marquardt algorithm was used for nonlinear least-squares fits.

Theoretical Modeling: All the geometry optimizations were performed in vacuo with a Nec TX7 with 32 Itanium 2 processors at ENS Cachan. The hybrid density functional B3LYP potential with the 6-31(d)G* basis set was used as implemented in Gaussian 03.[31] Harmonic vibrations were also calculated for all the obtained structures to verify that a true minimum was observed. In some cases (see text) a solvent model was included (IEFPCM) to account for the effects of solvation on the electronic properties. Orbitals and spin densities were generated using the cubgen module of Gaussian and visualized with GaussView 3.0 of Gaussian Inc.

Supporting Information (see also the footnote on the first page of this article): Detailed preparations of tetrazine **1**, UV/Vis and fluorescence spectra of **5**, **9**, **11**, **14** **16**, **17** and **18** and plots of the energy levels of calculated orbitals for all compounds.

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