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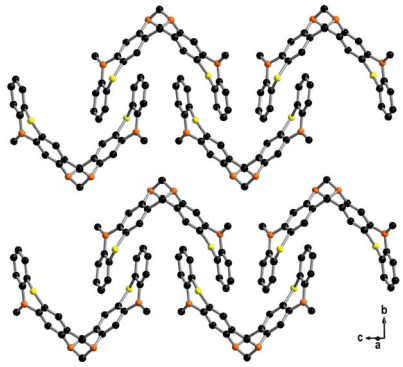
Microwave-assisted catalyzed amination of halogeno-phenothiazines provides precursors for phenothiazine analogues of Tröger's base. DFT and HF computational analysis, electronic properties (fluorescence emission in solution and solid state), and binding to proteins and DNA with effects on prooxidant reactivity were investigated.



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Microwave-Assisted Catalytic Amination of Phenothiazine; Reliable Access to Phenothiazine Analogues of Tröger's Base

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Efficient protocols for microwave-assisted catalyzed amination of halogeno-phenothiazines are described. Phenothiazine analogues of Tröger's base (PTB) were obtained by condensation of amino-phenothiazines with formaldehyde in HCl/EtOH/H_2O and structurally characterized by NMR and XRD analyses. The formation of PTB isomers was predicted by computational analysis based on theoretical methods (DFT and HF). Electronic properties of the parent amino-phenothiazines and PTB were assessed on the basis of cyclic voltammetry and UV/Vis absorption/emission spectroscopy data. The compounds exhibit blue fluorescence emission characterized by extremely large Stokes shifts (8900–10300 cm^{-1}) in both dilute solutions and aggregated states. Interactions with different types of biomolecules show the capacity of binding to proteins and DNA, with effects on prooxidant reactivity including lipid peroxidation.

Introduction

Since 1883, when the first synthesis of phenothiazine was described,[1] derivatives containing this heterocyclic core have constantly attracted scientific interest and, over the years, major applications have been developed in dyes[2] and pharmaceuticals,[3] and as additives for lubricants and polymers.[4] More recently, old and new compounds containing redox-active phenothiazine units[5] were found to exhibit unusual physical properties that were suitable for applications in materials science (electrically conducting charge-transfer composites,[6] materials for photoinduced electron transfer,[7] redox-active fluorophores,[8] or electrode materials[9]). Detailed investigations of the electronic properties of phenothiazine and oligophenothiazine derivatives performed by means of cyclic voltammetry, and absorption and fluorescence spectroscopy, illustrated the possibility of fine-tuning the typical properties associated with this heterocyclic core; shifts of the oxidation potential, bathochromic shifts of the absorption band, varying emission quantum yields, and producing exceedingly large Stokes shifts.[10]

Amino-phenothiazines have been used as versatile key intermediates in the preparation of many synthetic targets containing the phenothiazine core (functional group derivatives, mixed azaheterocyclic structures) and, thus, a specific interest directed towards new synthetic methodologies applicable to the selective preparation of regioisomers has been maintained. Nitro-phenothiazine derivatives have most often been used as starting materials, based on strategies using reducing agents such as Zn/$ZnCl_2$,[11] Sn,[12] or $SnCl_2$,[13] and HCl, Ni and acetic acid or hydrazine hydrate,[14] as well as catalytic hydrogenations with catalysts such as Raney nickel,[15] or palladium on carbon.[16] Other strategies involved the hydrolysis of a pending *N*-acyl-substituent (carbamate,[17] phthalimide[18]) of an aromatic unit of the phenothiazine core previously obtained by ring closure of the correspondingly protected amino-diphenylamine with sulfur,[11] or the rearrangement of an acylazide.[19] Tedious syntheses were developed for chloro-phenothiazine transformations into amino-phenothiazine derivatives by using sodium in liquid ammonia[20] or amines in the presence of alkali.[21] New strategies were developed to complement these traditional methods, which were suggested by the efficient preparation of a wide variety of arylamines by the amination of (hetero)aryl halides based on either Pd-catalyzed,[22] or copper-mediated[23] coupling reactions. These catalyzed C–N bond formation reactions, which typically require long reaction times to reach completion under traditional convective heating conditions (often in inert atmosphere), can be significantly accelerated by microwave heating.[24] Microwave-assisted aminations of bromoaryl derivatives with nitrogen-containing heterocycles in the

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presence of CuI,[25] or copper,[26] as well as palladium-mediated aminations[27] were successfully performed.

A range of heterocyclic analogues of Tröger's base (TB) have been reported.[28] Phenanthroline,[29] porphyrin,[30] and acridine[31] analogues of TB were designed as chiral solvating agents, hydrogen-bond receptors, ligands in metal complexes, and DNA intercalators. The general procedure for the preparation of TB derivatives involves acid-induced condensation of an aromatic amine with formaldehyde or formaldehyde equivalents such as paraformaldehyde,[32] hexamethylene-tetramine[33] or dimethoxymethane.[34] A range of yields of heterocyclic analogues of TB were obtained, depending on the selected reaction conditions and the reactivity of the heterocyclic amine.[35]

As part of our efforts directed to the synthesis and investigation of the chemical, physical and biological properties of new organic compounds containing the phenothiazine core, we here describe a protocol for the convenient microwave-assisted preparation of primary phenothiazinyl-amines and their further use as substrates in the synthesis of phenothiazine analogues of Tröger's base (PTB). The structural features of the new PTB combine the chiral V shaped TB scaffold induced by the pyramidal geometry of the two nitrogen atoms in the methanobenzo-diazocine rigid core, with two folded heterocyclic 1,4-benzothiazine units (which contribute to the molecular complexity with additional stereogenic nitrogen centers and also impart the specific electronic properties of the phenothiazine core). The structural assessments based on computational, analytical, and electrochemical studies presented below, qualify the described phenothiazine derivatives as potential candidates for applications in materials science and molecular biology.

Results and Discussion

Amination of Halogeno-phenothiazine

Two alternative microwave-assisted catalytic amination routes were applied for the regioselective preparation of primary 10*H*-phenothiazinyl-amines starting from the corresponding 1-, 2-, 3- and 4-halogeno-10*H*-phenothiazine (Scheme 1). A modified protocol for Pd-catalyzed amination of aryl halides[22] was applied, using halogeno-10*H*-phenothiazines **1a**–**f** as substrates and $LiN(SiMe_3)_2$ as ammonia equivalent, in the presence of a palladium complex catalyst [Scheme 1, path (i)]. This synthetic protocol gave unsatisfactory yields of amino-10*H*-phenothiazines **2a** and **2b** (explicable by the steric hindrance induced by the *N*-alkyl substituent in **1a**, or the competitive reactivity of the heterocyclic NH group in **1b**, respectively), but afforded various yields of amines **2c**–**f** when starting with regioisomers **1c**–**e**; the experimental results are summarized in Table 1. The regioselective substitution reaction indicates that, at the reaction temperature (140 °C), the elimination–addition mechanism does not appear to be competitive with the catalytic cycle.

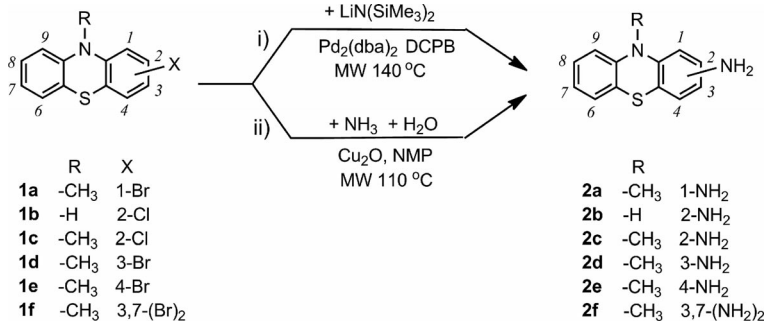
Table 1. Experimental conditions for the microwave-assisted amination of halogeno-phenothiazines; (i) Pd-catalyzed, (ii) copper(I)-mediated.

2	Temp. [°C]		Time [h]		Yield [%]	
	(i)[a]	(ii)[b]	(i)[a]	(ii)[b]	(i)[a]	(ii)[b]
2a	140	110	1	2	–	33
2b	140	110	1	10	87	–
2c	140	110	1	10	90	23
2d	140	110	1	2	28	93
2e	140	110	1	2	–	26
2f	140	110	1	2	43	62

[a] Reaction conditions: $LiN(SiMe_3)_2$ (1 M in THF; 1.2 equiv.), $[Pd_2(dba)_3]$ (0.5 mol-%), DCPB (1.2 mol-%). [b] Reaction conditions: NH_3 (10 mmol), Cu_2O (5 mol-%), H_2O/NMP (1:1).

The copper-catalyzed coupling of aryl halides with aqueous ammonia[36] was alternatively applied for the microwave-assisted amination of halogeno-10*H*-phenothiazines **1a**–**f**, based on a protocol using Cu_2O as catalyst in the presence of *N*-methyl-2-pyrrolidinone [NMP; Scheme 1, path (ii)]. Very good yields of 3-amino-10-methyl-10*H*-phenothiazine (**2d**) were isolated after 2 h irradiation at 110 °C in a sealed vessel, as well as moderate yields of symmetrical 3,7-diamino-10-methyl-10*H*-phenothiazine (**2f**; Table 1). A very long reaction time (10 h) was required for the substitution of 2-chloro-10-methyl-10*H*-phenothiazine **1c** and only small amounts of **2c** were obtained.

Palladium-catalyzed amination appears to be a convenient route for the preparation of 2-amino-10-alkyl-pheno-



Scheme 1. Catalytic amination of halogeno-phenothiazines. *Reagents and conditions:* (i) $LiN(SiMe_3)_2$ (1 M in THF), tris(dibenzylideneacetone)dipalladium $[Pd_2(dba)_3]$ (0.5 mol-%), 2-(dicyclohexylphosphanyl)biphenyl (DCPB; 1.2 mol-%); (ii) 35% aqueous ammonia, Cu_2O (5 mol-%), NMP (5 mol-%).

thiazine derivatives, whereas the Cu-catalyzed pathway is a less expensive alternative method that is suitable for the preparation of 3-amino-10-alkyl-phenothiazine derivatives.

The regioselectivity of the amination reaction appears to be well-correlated with the electron-density distribution in the substituted benzene ring of the phenothiazine core, as suggested by the electrostatic, natural, or Mulliken charges calculated using SPARTAN '06 Semiempirical program[37] and presented in Table 2. In substrate **1c**, lower charge density at C2 indicates an electron-poor aryl chloride (typical substrates for Buchwald–Hartwig reactions), whereas in substrate **1d**, a higher charge density at C3 suggests an electron-rich aryl bromide (with preference for Ullmann–Goldberg reaction). Unfortunately, both synthetic methods provided only small amounts of 1- and 4-amino-substituted regioisomers (Table 1), which may be due to steric hindrance and coordination effects induced by the two heteroatoms in the phenothiazine core. No secondary *N*,*N*-bis(phenothiazinyl)amines were formed during either catalysis conditions.

Table 2. Charge-density distribution in the substituted benzene unit of the lowest energy conformer of halogeno-phenothiazines **1c** and **1d** and amino-phenothiazines **2c** and **2d** obtained after molecular mechanics optimization of the conformers generated within the Confanal module of Spartan'06, followed by semiempirical (PM3) and 6-31G(d) B3LYP DFT calculations.

Charge[a]	Position	1c	1d	2c	2d
E	C1	–0.29438	–0.24010	–0.43922	–0.18504
	C2	0.16595	–0.08281	0.44925	–0.33753
	C3	–0.24929	–0.05143	–0.38109	0.39218
	C4	–0.08339	–0.07407	–0.09123	–0.32270
N	C1	–0.28744	–0.25868	–0.31747	–0.25019
	C2	–0.03163	–0.23732	0.18107	–0.27337
	C3	–0.26250	–0.12553	–0.29652	0.16102
	C4	–0.21712	–0.24259	–0.21116	–0.27518
M	C1	–0.17148	–0.16555	–0.22874	–0.17684
	C2	–0.08225	–0.16314	0.30870	–0.19030
	C3	–0.12032	0.02192	–0.16680	0.32558
	C4	–0.17747	–0.19694	–0.18249	–0.22616

[a] E: electrostatic, N: natural, M: Mulliken.

The successful microwave-assisted palladium-catalyzed amination protocol was further applied to the preparation of several new 2-amino-10-alkyl-10*H*-phenothiazines **4** (Scheme 2), containing variable alkyl chain lengths. Comparable results were obtained in the case of *N*-methyl- (**1c**) and *N*-ethyl- (**3a**) substituted substrates (above 90% yields), whereas bulkier substituents such as *n*-hexyl or *n*-octyl in **3b** and **3c**, respectively, induced increasing steric hindrance and consequently slightly lower yields. Similar reactivity was observed in the bis-amination of symmetrical 1,6-bis(2-chloro-phenothiazin-10-yl)hexane (**3d**), which afforded good yields of the new 1,6-bis(2-amino-phenothiazin-10-yl)hexane (**4d**). The structures of amines **2** and **4** were confirmed by spectroscopic methods (FTIR, ^{1}H and ^{13}C NMR spectroscopic and MS analyses).

Phenothiazine Analogues of Tröger's Base

The formation of the methano[1,5]diazocine unit during the condensation of 2-amino-10-alkyl-phenothiazine with formaldehyde under acidic conditions is based on electrophilic aromatic substitution involving either C1 or C3 (atom labeling in Scheme 1) and thus, three possible regioisomers of the phenothiazine analogues of Tröger's base (PTB) can be formed: one asymmetric (1,3′) and two symmetric (1,1′ and 3,3′) (Figure 1).

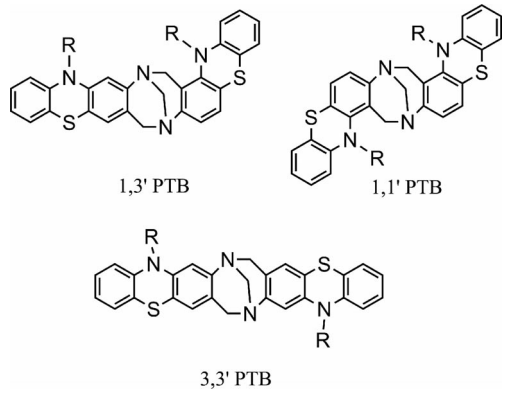
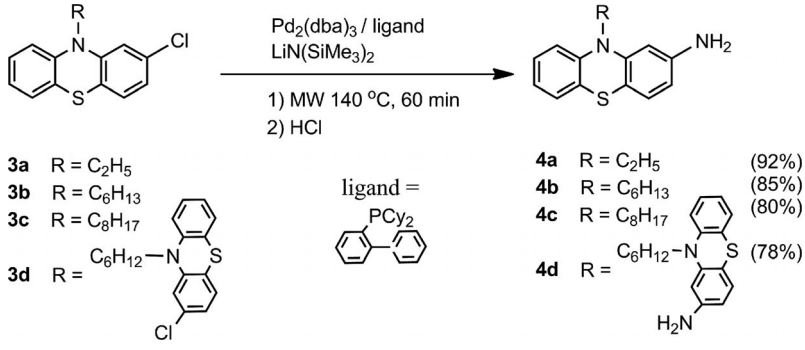
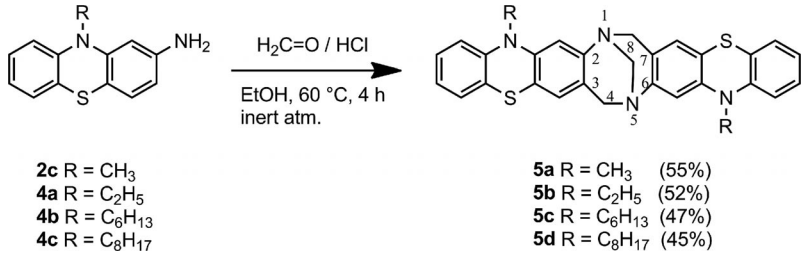


Figure 1. PTB regioisomers.

Condensation of several 2-amino-10-alkyl-10*H*-phenothiazines with formaldehyde and hydrochloric acid in ethanol afforded PTB **5a**–**d** (Scheme 3) in moderate yields (45–55%). Experimental results show a preference for substitution at C3 (3,3′-PTB). In the case of amine **2c**, a comparison of the computed charge densities at C1 and C3 (shown in Table 2) indicates higher values at C1, supporting the as-



Scheme 2. Palladium-catalyzed amination of 2-chloro-10-alkyl-10*H*-phenothiazines.



2c R = CH_3
4a R = C_2H_5
4b R = C_6H_{13}
4c R = C_8H_{17}

5a R = CH_3 (55%)
5b R = C_2H_5 (52%)
5c R = C_6H_{13} (47%)
5d R = C_8H_{17} (45%)

Scheme 3. PTB formation by condensation of 2-amino-10-alkyl-phenothiazine with formaldehyde.

sumption of steric control of the electrophilic attack at the less sterically crowded C3. 2-Amino-10*H*-phenothiazine **2b**, which does not contain a protective *N*-alkyl group, gave a complex mixture containing only small amounts of PTB. Other methods involving formaldehyde equivalents such as paraformaldehyde or hexamethylenetetramine in strong acids[38] were not successful for the preparation of PTB.

Long alkyl chains attached to the heterocyclic nitrogen atom dramatically lowered the melting points of the PTB [cf. m.p. 312 (**5a**) and 68 (**5d**) °C].

The core structures of PTB **5a**–**d** were identical, as established by ^1H, ^{13}C, 1D NOEDIFF, HMQC, HMBC, and COSY NMR experiments. For example, in the ^1H NMR spectrum of **5b**, the protons in the diazocine unit appear as a singlet (δ = 4.3 ppm for the methylene bridge) and a well-separated AB spin system for the *endo* (δ = 4.1 ppm, d) and *exo* (δ = 4.6 ppm, d) methylene protons in the equivalent positions 4 and 8 (atom labeling in Scheme 3). The equivalence of the two alkyl-phenothiazine units generates a reduced number of well-separated signals that are located in the aromatic (δ = 6.6–7.2 ppm) and aliphatic (δ = 1.5 ppm, 3 H, t, and 4 ppm, 2 H, q) region, respectively. The recorded NOEDIFF spectra of **5b** show spatial interactions between aromatic protons in the 4-position of the phenothiazine (δ = 6.64 ppm, s) and the *endo* CH_2 protons of the diazocine unit, thus providing evidence for the regioselectivity of the cyclization.

The stereoisomers of PTB **5a**, induced by the chirality of the methano[1,5]diazocine unit and the pyramidal geometry of the nitrogen atoms in the folded phenothiazine units, were analyzed by using computational tools. DFT optimizations (M062x/6-31G**) reveal that three pairs of diastereoisomers are feasible (Figure 2). The conformational analysis of the phenothiazine unit (folded along an axis passing through the heteroatoms N and S) indicates a preference for a quasi-equatorial orientation of the methyl substituent, but minima with one or both methyl groups in quasi-axial orientation were also identified (Figure 3), which are higher in energy by 5–12 kcal/mol, depending on the method employed. On the methodological side, we note that minima identifiable by classical HF theory [as well as by semiempirical AM1 (data not shown)] could not be identified as proper local minima when using a DFT method particularly adapted for dealing with weak supramolecular

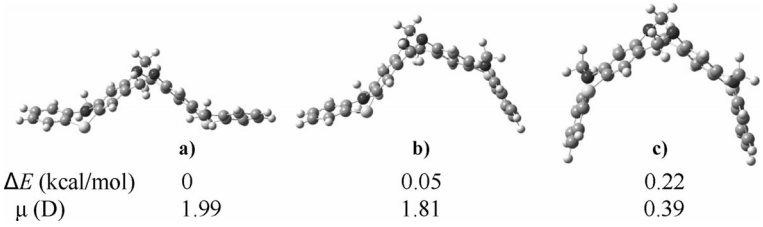


Figure 2. Structural models, computed relative energies, and dipole moments for diastereoisomers of **5a** characterized by *S*,*S* configuration at the nitrogen atoms in the diazocine bridge, quasi-equatorial positions of the methyl groups, and variable configurations at the stereogenic nitrogen atoms in the folded phenothiazine units: (a) *R*,*R*; (b) *R*,*S*; (c) *S*,*S*.

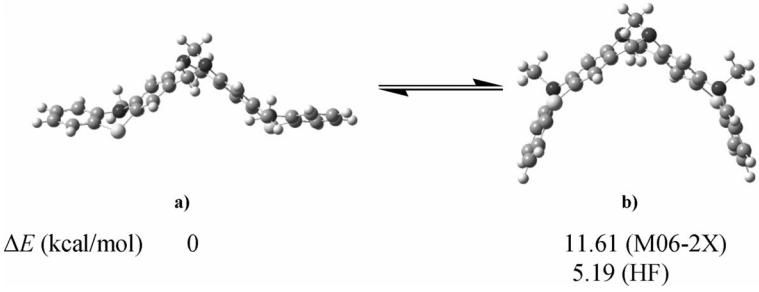


Figure 3. Structural models and computed relative energies for conformers of **5a** with *S*,*S* configuration at nitrogen atoms in the diazocine bridge and *R*,*R* configurations in the phenothiazine units: (a) quasi-equatorial orientation of the *N*-methyl groups; (b) quasi-axial orientation of the *N*-methyl groups.

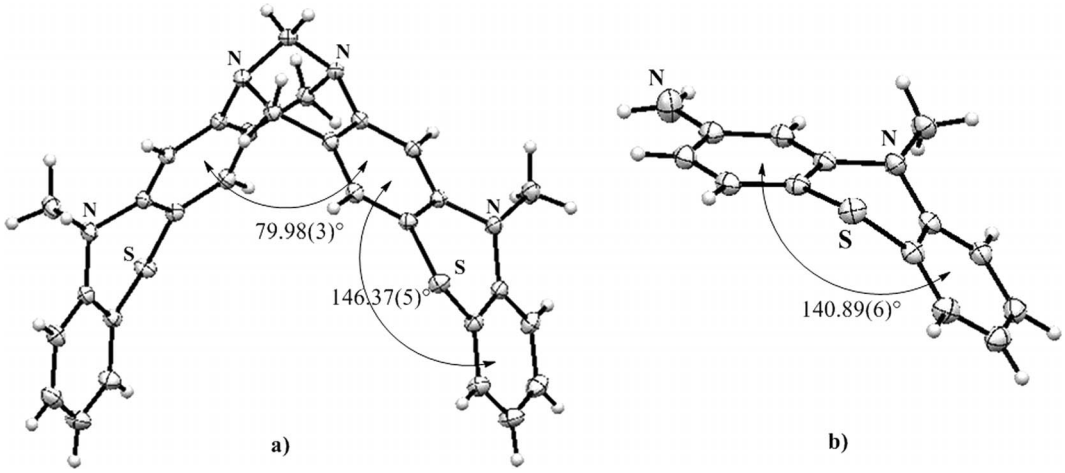


Figure 4. ORTEP representations of the molecular structures of PTB (a) **5a** and (b) **2c**. Selected angles between the planes of aromatic rings are presented.

interactions, which gives particularly good results in conformational and supramolecular issues.[39]

To elucidate the preferred molecular structures in the solid state, single crystals of PTB **5a** and **5b** that were suitable for X-ray analysis were obtained from ethanol. The molecular structures of **5a** and its amino-substituted precursor **2c** are given in Figure 4. In **5a**, a central TB scaffold and a pair of adjacent 1,4-benzothiazine rings is present. The rigid central methylene bridge in the diazocine moiety imposes a twisted V shape with C_2 symmetry on the molecule. The dihedral angle between the two adjoining benzene rings is 80.0° (close to the values reported for TB analogues), whereas the phenothiazine units show folding angles of 146.4° and quasi-equatorial orientations of the methyl substituents (typical for 10-methyl-phenothiazine derivatives).[40] A quasi-equatorial orientation of the methyl substituents was also observed in the structure of the amino-substituted precursor **2c** (Figure 4, b), which is characterized by a folding angle of 140.9°, a value slightly smaller than that reported for 10-methyl-phenothiazine (143.7°).[40] Neighboring molecules of **2c** are associated by NH···N hydrogen bonds [2.70(3) Å], NH···S hydrogen bonds [2.75(3) Å] and offset parallel π–π interactions (3.39 Å) between aromatic units.

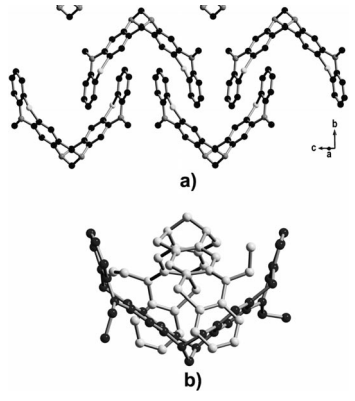


Figure 5. Crystal packing diagram of PTB (a) **5a** and (b) **5b**.

A different packing is observed for orthorhombic **5a** and monoclinic **5b** (Figure 5). In both compounds, a pair of enantiomers with similar configurations at the four stereogenic nitrogen atoms is present; *S*,*S*,*S*,*S* and *R*,*R*,*R*,*R* enantiomer, with *S*,*S* or *R*,*R* configuration for the two nitrogen atoms located in the diazocine unit and *S*,*S* or *R*,*R* for the two nitrogen atoms in the adjacent phenothiazine units.

Electronic Properties

The electronic properties of the new PTB compounds **5a**–**d** and their amino-10*H*-phenothiazines precursors **2b**, **2c**, and **4a**–**d**, determined by UV/Vis absorption spectroscopy, fluorescence spectroscopy, and cyclic voltammetry, are summarized in Table 3.

Table 3. Electronic properties of PTB and amino-10*H*-phenothiazines (UV/Vis absorption/emission data and cyclic voltammetry parameters).

	$\lambda_{max,abs}$ [nm]	$\lambda_{max,em}$ [nm]	Stokes shift [cm^{-1}]	Quantum yield Φ_f [%][a]	$E_0^{0/+1}$ [mV][b]	$E_0^{+1/+2}$ [mV][b]
2b	272, 324	487	10300	9.50	755	1085
2c	272, 315	457	9800	6.20	897	1211
2d	275, 317	444	8900	5.43	524	883
2f	275, 315	449	9400	9.60	–	–
4a	275, 315	463	10000	6.18	781	1180
4b	272, 315	457	9800	4.55	692	1109
4c	271, 315	457	9800	4.85	841	1255
4d	271, 317	465	10200	12.30	–	–
5a	274, 321	451	8900	6.90	902	1449[b]
5b	267, 321	455	9100	6.23	938	1716[b]
5c	276, 321	447	8600	5.12	992	1772[b]
5d	276, 321	457	9100	6.00	978	1912[b]
6	268, 324	458, 520 (sh)	9000	7.75	–	–

[a] The fluorescence quantum yield Φ_f was determined by using perylene as standard (Φ_f = 94%). [b] In CH_2Cl_2, 20 °C, v = 100 mV/s, electrolyte: nBu$_4$N$^+$PF$_6^-$, Pt working electrode, Pt counter electrode, Ag/AgCl reference electrode.

In the UV/Vis absorption spectra of PTB compounds **5a**–**d** and amino-10*H*-phenothiazines **2b**–**d**, **2f**, and **4a**–**d**, two absorption bands with maxima at 268–275 and 315–324 nm are observed due to electronic transitions in the phenothiazine unit. Upon UV excitation by irradiation with the corresponding longest wavelength, all compounds emit blue fluorescence due to extremely large Stokes shifts (8600–10300 cm^{-1}), with 4–12% fluorescence quantum yields. The fluorescence emissions observed in diluted solutions are also present in the solid states of PTB **5a**–**d**, which is supported by the lack of strong face-to-face π-π stacking interaction between the aromatic units of the phenothiazine fluorophore (which are usually responsible for fluorescence quenching in the solid state). Figure 6 shows the UV/Vis absorption and fluorescence emission spectra of dichloromethane solutions of PTB **5b** and amine precursor **2c** as well as the emission properties of the crystalline solids; the latter show a small hypsochromic shift.

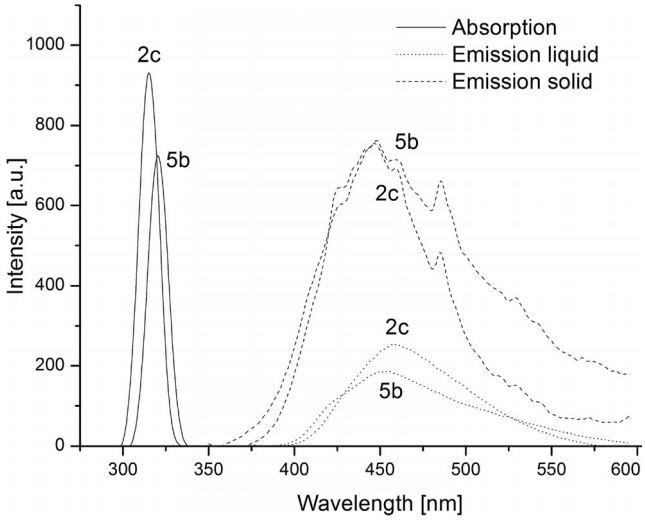


Figure 6. UV/Vis absorption and emission spectra in dilute dichloromethane solution (10^{-5} M) and the crystalline state for PBT **5b** and amino-substituted precursor **2c**.

Electrochemical data were obtained by cyclic voltammetry studies on PTB **5a**–**d** and amino-substituted precursors **2b**–**d** and **4a**–**c** in dichloromethane using ferrocene/ferrocenium (Fc/Fc^+) as internal standard, with scanning in the anodic (up to 2 V) and cathodic (up to –0.2 V) region.

In comparison with the parent 10*H*-phenothiazine, which was characterized by the first one-electron oxidation potential $E_0^{0/+1}$ = 624 mV, alkylation of the heterocyclic nitrogen atom results in an anodical shift (10-Me-10*H*-phenothiazine $E_0^{0/+1}$ = 767 mV), which increases in the case of the synthesized amines [e.g., 2-amino-10-Me-10*H*-phenothiazine (**2c**) $E_0^{0/+1}$ = 897 mV]. A second irreversible oxidation peak appears at higher anodic potential (ca. 1500 mV).

A comparison between the CV patterns recorded for PTB and the corresponding amino-substituted phenothiazine precursors revealed not only an anodic shift of both oxidation potentials of PTB, pointing towards a higher stability, but also an independent participation of the two equivalent phenothiazine units, as indicated by the increased current intensities (see for example the CV of PTB **5c** in Figure 7).

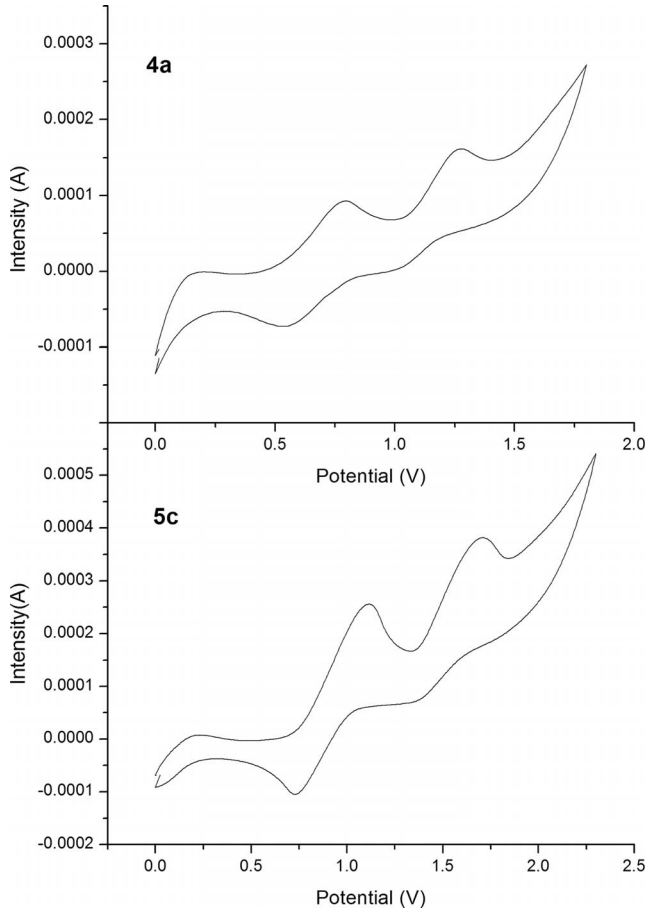


Figure 7. Cyclic voltammograms of **4a** and **5c** (recorded in CH_2Cl_2, 20 °C, v = 100 mV/s, electrolyte: nBu$_4$N$^+$PF$_6^-$, Pt working electrode, Pt counter electrode, Ag/AgCl reference electrode) using ferrocene/ferrocenium (Fc/Fc^+) as internal standard.

Interaction with Biomolecules

Several experiments were performed to test the capacity of the synthesized amino-substituted phenothiazines and PTBs to interact with proteins and DNA. Autoxidation of the physiologically-useful oxy form of hemoglobin and myoglobin into the toxic ferric ("met") forms, monitored by UV/Vis absorption spectroscopy, indicated that amino-substituted phenothiazines have a strong effect on the autoxidation rates of hemoglobin (Hb) (Figure 8) and myoglobin (Mb), accelerating this process in a qualitatively similar manner for both proteins. On the other hand, the corresponding PTB showed much smaller or even negligible effects (Table 4).

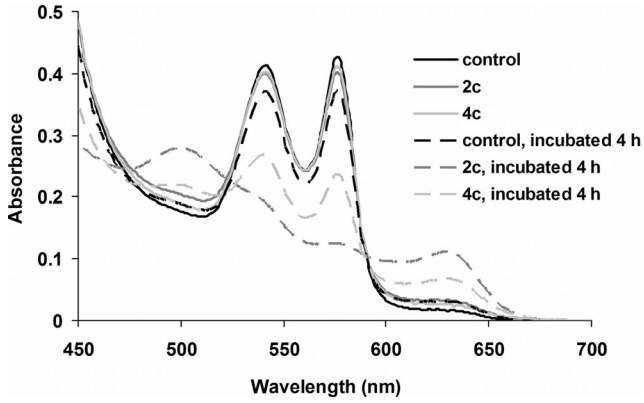


Figure 8. UV/Vis spectra of hemoglobin, collected at the initial and final time points during the autooxidation experiments in the presence of **2c** and **4c**.

Table 4. Increases in autooxidation rates induced in hemoglobin and myoglobin, and inhibition of lipid peroxidation by amino-substituted phenothiazines and PTBs.

	Hb [%][a]	Mb [%][a]	Lipid inhibition[b]
2d	+362.1	+71.9	–
2c	+358.4	+76.9	+
4a	+358.9	+73.4	+
4b	+118.3	+72.1	+
4c	+170.4	+70.0	+
5a	+8.3	+15.6	+
5b	–1.9	+29.0	+
5c	+10.7	n.d.	–
5d	–35.2	n.d.	–

[a] Percentage of increase relative to the native form of Hb/Mb under the same conditions. [b] Compounds marked with "–" showed no difference from the control over a 10 h reaction time; compounds marked with "+" showed significant delays in the absorbance increase associated with lipid peroxidation in this experiment.

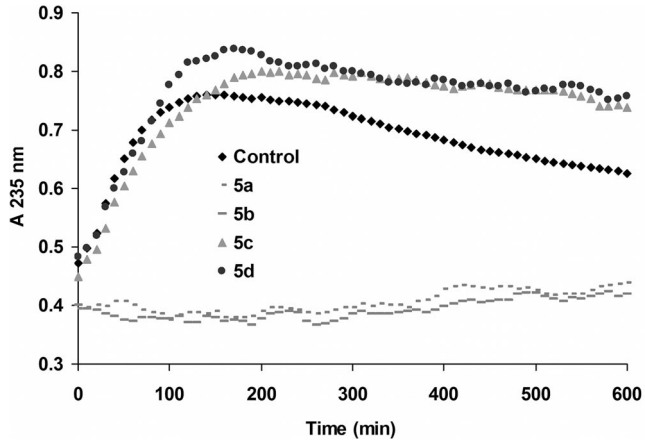


Figure 9. Lipid peroxidation induced by cytochrome *c* (1.5 μM) in liposomes (5 μg/mL) in the presence/absence of PTB (10 μM), in phosphate buffer (20 mM), pH 7.4.

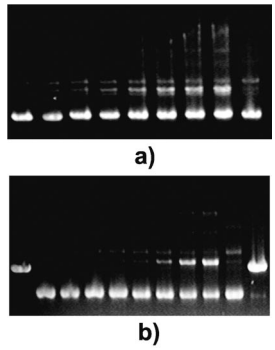


Figure 10. Electrophoresis data for (a) **2c** and (b) **5a**. The lanes contain 250 ng plDNA with 0, 0.5, 1, 2, 4, 6, 8, 10, and 0 μL of each compound. In the case of compound **5a**, the first and last lanes show the linear-DNA control.

Liposome oxidation experiments, monitored by recording the typical absorbance band situated at 235 nm for 10 h, indicated that some of the synthesized amino-phenothiazines and PTB inhibit the lipid peroxidation induced by cytochrome *c* (Figure 9). Both types of protein-related reactivity reported in Table 4 can be related to free radical stress, as free radicals (superoxide, peroxides) are known to be involved in accelerating protein autooxidation and lipid peroxidation.[41]

DNA electrophoresis experiments using plasmid pTZ57R treated with dimethyl sulfoxide (DMSO) solutions of amino-phenothiazines and PTBs respectively at different ratios, indicated that several of the compounds examined can not only bind to DNA, but also cause detectable changes in its physical properties. Thus, the amino-substituted phenothiazine **2c** and the corresponding PTB **5a** induce relaxation of the circular plasmid, as illustrated in Figure 10.

The experiments shown here, illustrating binding to proteins with effects on prooxidant reactivity including lipid peroxidation and binding to DNA, suggest that the family of phenothiazine derivatives described here is amenable to applications involving biological activity with respect to more than one class of biomolecules – proteins, lipids, and nucleic acids.

Conclusions

Microwave-assisted catalyzed C–N bond formation reactions can be successfully applied in the preparation of primary amino-10-alkyl-10*H*-phenothiazines. The Pd-catalyzed amination is suitable for the preparation of 2-amino-phenothiazine, whereas the Cu-catalyzed amination conveniently generates the 3-amino-phenothiazine regioisomer.

New PTB compounds, combining the chiral V shaped TB scaffold with the folded 1,4-benzothiazine unit, are formed by regioselective condensation of 2-amino-10-alkyl-10*H*-phenothiazines with formaldehyde. Computational analysis of the stereoisomers of PTB revealed three possible pairs of diastereoisomers. A pair of enantiomers each containing similar configurations at the four stereogenic nitrogen atoms was evidenced by XRD.

The UV/Vis absorption/emission and redox processes involving PTBs appear to be rather insensitive to the effects

of the rigid methano-diazocine bridge, which electronically decouples the two phenothiazine units in the PTB. Compounds **5a**–**d** exhibit blue fluorescence emission in dilute solution and the solid state, and are characterized by extremely large Stokes shifts (8900–10300 cm^{-1}) and moderate quantum yields (4.5–12.3%).

The experiments shown here, illustrating the supramolecular assemblies, chirality, redox, and fluorescence properties of amino-substituted phenothiazines and their related PTB, qualify this family of phenothiazine derivatives for further studies in materials science; their capacity to bind to proteins or nucleic acids, and additional prooxidant reactivity (including lipid peroxidation) point towards applications involving biological activity with respect to more than one class of biomolecules.

Experimental Section

General Procedure for Microwave-Assisted Palladium-Catalyzed Synthesis of Amino-Phenothiazines: A microwave reaction tube was charged with 10-alkyl-chloro-10*H*-phenothiazine (1 mmol), $[Pd_2(dba)_3]$ (4.6 mg, 5 μmol, 0.5 mol-%), and 2-(dicyclohexylphosphanyl)biphenyl (4.2 mg, 12 μmol, 1.2%), the air was replaced by argon, and $LiN(SiMe_3)_2$ (1 M in THF, 1.2 mL, 1.2 mmol) was carefully added. The reaction tube was sealed and placed in the microwave reactor cavity. The reaction mixture was stirred under irradiation at 140 °C for 1 h and, after cooling to room temperature, diethyl ether (150 mL) was added and the mixture was acidified to pH 3 with aqueous hydrochloric acid (5 mL, 15%) under vigorous stirring. The precipitate was isolated by filtration and washed with diethyl ether. The amine can be further purified by column chromatography on basified silica gel.

General Procedure for Microwave-Assisted Copper-Catalyzed Synthesis of Amino-Phenothiazines: A microwave reaction tube was charged with 10-alkyl-bromo-10*H*-phenothiazine (1 mmol), aqueous ammonia (1 mL, 35%), Cu_2O (0.01 g, 0.7 mmol), and *N*-methyl-pyrrolidone (0.1 mL, 1 mmol), then sealed and subjected to irradiation in the microwave reactor cavity. The reaction mixture was stirred under irradiation at 110 °C for 2 h. After cooling to room temperature, the reaction mixture was poured in water (150 mL) and the precipitate was isolated by filtration and further purified by column chromatography over silica gel with toluene as eluent.

General Procedure for the Preparation of PTB: A solution of concentrated aqueous HCl (0.5 mL, 32%), formalin (0.7 mL, 37%), and ethanol (20 mL, 95%) was cooled in an ice bath for 2 h. The amino-10-alkyl-phenothiazine (1 mmol) was added and the mixture was progressively heated to 60 °C and then the temperature was maintained for 4 h. The reaction mixture was cooled, neutralized with NaOH (1 M) and the solid was collected by vacuum filtration. Purification was performed by column chromatography on silica gel with toluene/dichloromethane as eluent.

Supporting Information (see footnote on the first page of this article): Detailed experimental synthetic protocols, structural assignments based on spectroscopic methods (NMR, FT IR, UV/Vis, HRMS, XRD) and experimental protocols applied in the study of interaction with biomolecules.

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