Tetrahedron 64 (2008) 6522-6529

Contents lists available at ScienceDirect

# Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Synthesis, characterization and photophysical properties of benzidine-based compounds

Mohamad Hmadeh<sup>a</sup>, Hassan Traboulsi<sup>a</sup>, Mourad Elhabiri<sup>a</sup>, Pierre Braunstein<sup>b,\*</sup>, Anne-Marie Albrecht-Gary<sup>a,\*</sup>, Olivier Siri<sup>c,\*</sup>

<sup>a</sup> Laboratoire de Physico-Chimie Bioinorganique, Institut de Chimie, UMR 7177 CNRS—Université Louis Pasteur, ECPM, 25 rue Becquerel, 67200 Strasbourg, France <sup>b</sup> Laboratoire de Chimie de Coordination, Institut de Chimie, UMR 7177 CNRS—Université Louis Pasteur, 4 rue Blaise Pascal, 67070, Strasbourg Cedex, France <sup>c</sup> Groupe de Chimie Organique et Matériaux Moléculaires, UMR 6114 CNRS, Université de la Méditerranée, Faculté des Sciences de Luminy, case 901, 163 avenue de Luminy, 13288, Marseille Cedex 09, France

# ARTICLE INFO

Article history: Received 6 March 2008 Received in revised form 10 April 2008 Accepted 14 April 2008 Available online 18 April 2008

Keywords: Benzidine N-Alkylation Fluorescent probe Exciplex Protonation constants

1. Introduction

### ABSTRACT

The N-alkylation of the 3,3'-diaminobenzidine with innocent substituents leads to unusual properties. The emission of the benzidine core can be fine-tuned by subtle modifications, and the N-substitution with benzylic groups results in photoinduced exciplexes with distinct and increased emission. This compound constitutes the first example of intramolecular exciplex containing benzidine unit. We also show that these photoinduced processes could be modulated by proton input, and that the diprotonation of the benzidine core disrupted the intramolecular communication in the excited states with a concomitant inhibition of the ligand-centred fluorescence. Furthermore, upon photo-irradiation at 254 nm, semiquinone imine and quinone diimine systems are produced in CH<sub>2</sub>Cl<sub>2</sub> of which the photolysis generates Cl<sup>•</sup> radicals, which rapidly oxidize the tetraamine compounds.

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# 4.4'-Diamino-1.1'-biphenvl (benzidine) **1** is a well known com-

pound, which has been widely used in the past in different areas of science. (i) Molecules of this class were employed for a long time as primary materials in the manufacture of azodyes. Being classified as potentially hazardous and carcinogenic, the importation and industrial use of 1 are now prohibited in many countries except if present in less than 1%.<sup>1</sup>



(ii) Benzidines also play an important role in cell biology and clinical work as valuable staining reagents.<sup>2</sup> Due to its potential risks, research has been carried out to develop analogues for biological and medical purposes. As a result, 3,3',5,5'-tetramethylbenzidine (TMB) was found to be a non-carcinogenic compound, and is now widely used in routine assays.<sup>3,4</sup> (iii) Because of their peculiar redox properties, benzidines have also been

0040-4020/\$ - see front matter © 2008 Elsevier Ltd. All rights reserved. doi:10.1016/j.tet.2008.04.058

successfully employed as key components of proton- or electrondriven molecular machines. Supramolecular shuttles incorporating 4,4'-biphenol and benzidine units display reversible motion of a  $\pi$ electron-deficient tetracationic cyclophane, serving as ring component, which is triggered by oxido-reduction or protonation of the benzidine moiety.<sup>5</sup> (iv) Last, benzidines are relevant examples of simple redox systems, which could find applications as OLEDs<sup>6</sup> or electroactive organic polymeric compounds.<sup>7</sup>

As part of our ongoing investigations on guinone diimines,<sup>8</sup> we describe herein the synthesis and full characterization of Nsubstituted 3,3'-diaminobenzidine systems containing either neopentyl (noted 5) or benzyl (noted 6) groups.



If numerous substituted benzidine derivatives have been reported in the literature, the possibility to introduce electroactive, photoactive or binding units<sup>9</sup> by N-alkylation has received less attention.<sup>10</sup> We show that N-benzvlation of the benzidine backbone confers new properties to these systems.





<sup>\*</sup> Corresponding authors. Tel.: +33 (0)3 90 24 26 38; fax: +33 (0) 3 90 24 26 39. *E-mail address:* amalbre@chimie.u-strasbg.fr (P. Braunstein).

# 2. Results and discussion

# 2.1. Synthesis

Molecules **3** and **5** were prepared as described in the literature.<sup>11</sup> The tetraphenyl 3,3'-diaminobenzidine **6** was synthesized in two steps (Scheme 1) from 3,3'-diaminobenzidine **2**. Its reaction first with 4 equiv of pivaloyl chloride (PhC(O)Cl) afforded the corresponding tetraamido derivative **4** as a grey-white solid, which was then reduced with LiAlH<sub>4</sub> in dry THF to yield the targeted compound **6** as a yellow powder.



Scheme 1. Synthesis of compounds 5 and 6.

#### 2.2. Acido-basic properties

In order to determine the acido-basic properties of the chromophoric benzidine-type units in **5** and **6**, absorption spectrophotometric titrations versus pH were carried out. The spectrophotometric and potentiometric data of **5** between pH 1.5 and 9.5, and of **6** between pH 1.3 and 10.1 are given in Figures S1 and S2 in Supplementary data, respectively. Compounds **5** and **6** possess four protonable amino sites. The statistical treatment<sup>47–50</sup> of the spectrophotometric data versus pH led to the determination of two protonation constants (Eqs. 1 and 2). The reported errors on protonation constants are given as  $3\sigma$  ( $\sigma$ =standard deviation).

$$\mathbf{L}\mathbf{H}_{n-1}^{(n-1)+} + \mathbf{H}^+ \stackrel{K_n^H}{\rightleftharpoons} \mathbf{L}\mathbf{H}_n^{n+} \quad \text{with } 1 \le n \le 4; \ \mathbf{L} = \mathbf{5} \text{ or } \mathbf{6}$$
(1)

$$K_n^{\mathrm{H}} = \frac{[\mathbf{L}\mathbf{H}_n^{n+}]}{[\mathbf{L}\mathbf{H}_{n-1}^{(n-1)+}][\mathbf{H}^+]}$$
(2)

 $K_1^{\rm H}$  and  $K_2^{\rm H}$  values are given in Table 1 together with those determined for closely related compounds benzidine<sup>12</sup> **1**, 3,3'-dimethoxybenzidine<sup>13</sup> **7** and 3,3'-dimethylnaphthidine<sup>13</sup> **8** (Scheme 2). Compounds **1**, **7** and **8** were studied in water at 25 °C by absorption spectrophotometry versus pH.<sup>12b,13</sup> Compound **1** was examined using the same detection method in methanol/water (1:1 v/v).<sup>12a</sup>

Distribution diagrams of the protonated species of **5** and **6** as a function of pH are given in Figures S3 and S4 in Supplementary data. Despite differences in experimental conditions, comparison with benzidine ligands **1**, **7** and **8** allowed us to easily assign  $K_1^{\rm H}$  and  $K_2^{\rm H}$  for **5** and **6** to the amino functions substituted in positions 4 and

#### Table 1

| Protonation constants of <b>5</b> and <b>6</b> and of related compoun |
|---|
|---|

| Equilibrium  | $\log K_n^{\rm H}(\pm$                 | $\log K_n^{\rm H}(\pm 3\sigma)$ |                              |                        |                        |  |
|--|--|---------------------------------|------------------------------|------------------------|------------------------|--|
|  | <b>1</b> <sup>12</sup>                 | 5                               | 6                            | <b>7</b> <sup>13</sup> | <b>8</b> <sup>13</sup> |  |
| $\mathbf{L} + \mathbf{H} \stackrel{K_1^{H}}{\rightleftharpoons} \mathbf{L} \mathbf{H}$   | 4.18 <sup>a</sup><br>4.95 <sup>b</sup> | 3.10(7) <sup>a</sup>            | $2.8(1)^{a}$<br>$3.3(3)^{b}$ | 4.05                   | 3.33                   |  |
| $\mathbf{L}\mathbf{H} + \mathbf{H} \stackrel{K_2^{\mathrm{H}}}{\underset{\kappa^{\mathrm{H}}}{\rightleftharpoons}} \mathbf{L}\mathbf{H}_2$ | 2.92 <sup>a</sup><br>3.84 <sup>b</sup> | 1.6(1) <sup>a</sup>             | $1.2(4)^{a}$<br>$0.9(4)^{b}$ | 2.83                   | 2.62                   |  |
| $LH_2 + H \stackrel{\kappa_3}{\underset{\kappa^{H}}{\rightleftharpoons}} LH_3$   | -                                      | nd                              | nd                           | —                      | _                      |  |
| $LH_3 + H \stackrel{n_4}{\rightleftharpoons} LH_4$   | —                                      | nd                              | nd                           | —                      | -                      |  |

Solvent: CH<sub>3</sub>CN/H<sub>2</sub>O (9:1 v/v); l=0.1 M (NEt<sub>4</sub>ClO<sub>4</sub>) for **5** and **6**; T=25.0(2) °C; nd: not determined under our experimental conditions. For the sake of clarity, charges have been omitted.  $\sigma$ =standard deviation.

<sup>a</sup> Absorption versus pH.

<sup>b</sup> Emission versus pH.



Scheme 2. Chemical structures of benzidine-based compounds 7 and 8.

4′ of the biphenyl backbone. Values of  $\log K_3^H$  and  $\log K_4^H$  were estimated to be significantly lower than 0.9, probably owing to electrostatic interactions and electronic properties. Large  $\Delta \log K = \log K_1^H - \log K_2^H$  values<sup>14</sup> of about 1.3(2) were calculated for compounds **1** and **5–8** (Table 1). This result reflects strong electrostatic repulsions between the two molecular subunits.<sup>14</sup> It is noteworthy that the diimines derived from benzidine, which were obtained by oxidation with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, displayed two significantly higher pK values of ≈ 8.95 and 5.05.<sup>15</sup> The increase of the pK values with respect to benzidine (4.95 and 3.84, Table 1) could be mainly explained by inductive and mesomeric effects occurring in the conjugated quinone diimine systems.<sup>8</sup>

Moreover, we report here the spectrophotometric properties of 5 and 6 under various acido-basic conditions. The electronic spectra of the tetramino species 5 and 6 (Fig. 1) are characterized by the presence of two main absorption bands (322 and 236 nm for 5; 324 and 240 nm for 6) in the UV region. These absorptions are attributed to short-axis  $(\pi_2)$  and long-axis  $(\pi_1)$  polarized  $\pi$ - $\pi$ \* transitions of the biphenyl backbone, respectively.<sup>16</sup> Interestingly, the absorption spectrum of biphenyl in cyclohexane is constituted of a broad and structureless band centred at 247 nm  $(\varepsilon = 1.55 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1})$ .<sup>17</sup> The substitution of the 4,4'-positions of the biphenyl chromophore by two amino functions (1) induces a large bathochromic shift ( $\lambda$ =278 nm;  $\epsilon^{278}$ =2.26×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> in water) of the long-axis-polarized transitions.<sup>12b</sup> Furthermore, introduction of two additional amino groups in positions 3 and 3' (5 and 6) resulted in a bathochromic shift of the latter transitions  $(\lambda=322 \text{ nm}; \epsilon^{322}=1.89\times10^4 \text{ M}^{-1} \text{ cm}^{-1} \text{ for } \mathbf{5}; \lambda=324 \text{ nm}; \epsilon^{324}=1.57\times10^4 \text{ M}^{-1} \text{ cm}^{-1} \text{ for } \mathbf{6}, \text{ Fig. 1}).$  The comparison of the electronic spectrum of **6** with that of **5** evidenced a hyperchromic effect at  $\approx$  250–300 nm due to the presence of the four benzylic arms  $(\lambda_{\text{max}}=259 \text{ nm}, \varepsilon^{259}=1.86 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1} \text{ per benzyl unit}).^{18,19}$ 

The first protonation of compounds **5** and **6** induces a hypochromic effect and a concomitant broadening of the absorption bands lying at lower energies (Fig. 1). Besides, hypochromic (20–30% of decrease) and bathochromic shifts ( $\Delta\lambda$ =10 nm for **5** and  $\Delta\lambda$ =6 nm for **6**) of the short-axis-polarized transitions could be also observed. These spectrophotometric data are in agreement with those reported for the monoprotonated benzidine **1**, for which similar



**Figure 1.** Electronic spectra of the protonated species of **5** (a) and **6** (b). Solid line: neutral species; dash line: monoprotonated species; dotted line: diprotonated species. Solvent: CH<sub>3</sub>CN/H<sub>2</sub>O (9:1 v/v); *I*=0.1 M (NEt<sub>4</sub>ClO<sub>4</sub>); *T*=25.0(2) °C.

trends ( $\lambda$ =278 nm;  $\varepsilon^{278}$ =2.26×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> for **1**;  $\lambda$ =273 nm;  $\varepsilon^{273}$ =1.76×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> for **1**H<sup>+</sup>) were observed.<sup>12b</sup> The second protonation leads to significant blue shifts of the long-axis-polarized transitions ( $\Delta\lambda$ =24 nm for **5** and  $\Delta\lambda$ =14 nm for **6**) simultaneously with a hypochromic shift of the short-axis-polarized transitions. These data also agree with benzidine 1 spectrophotometric data ( $\lambda$ =247 nm;  $\varepsilon^{247}$ =1.63×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> for **1**H<sub>2</sub><sup>2+</sup>).<sup>12b</sup> As it was pointed out for biphenyl, the blurring of the absorption bands in the ground state strongly indicates a distortion away from planarity. In solution, the preferred twist angle for biphenyl was indeed estimated to be  $\approx 42^{\circ}$ ,<sup>20</sup> while various X-ray diffraction studies pointed to a planar structure for crystalline biphenyl.<sup>21</sup> However, a recent X-ray study<sup>22</sup> revealed that benzidinium dichloride possesses polymorphic properties (triclinic and orthorhombic structures). Interestingly, the angle between the two benzene rings within the two polymorphs is equal to  $18.12(5)^{\circ}$  and  $45.60(5)^{\circ}$ , respectively. Density functional calculations supported these observations and showed that the most stable conformation corresponds to  $\theta$ =30° rotated conformation about the C-C biaryl bond.<sup>23</sup>

# 2.3. Emission spectrophotometry

Figure 2 shows the normalized absorption and emission spectra of **5** in CH<sub>2</sub>Cl<sub>2</sub>. The corresponding electronic spectrum  $(\lambda_{max}=320 \text{ nm}; \epsilon^{320}=1.84 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1})$  is similar to that previously determined for **5** in acetonitrile/water (9:1 v/v) mixture (Fig. 1). Upon excitation into the transitions at 276 nm, compound **5** displays a broad and structureless band centred at 400 nm, and is

characterized by a large Stokes shift of  $\approx 80$  nm. The absolute fluorescence quantum yield of **5** is equal to 0.54(5)%. The fluorescence emission spectrum of biphenyl in cyclohexane displays a fine vibrational structure ( $\lambda_{max}^{em} = 326 \text{ nm with } \Delta \lambda = 1050 \text{ cm}^{-1}$ ). These data are characteristic of a chromophore that is non-planar in the ground state and more planar and linear in its excited singlet and triplet states.<sup>16,24</sup> By contrast, the absence of a structured profile for the absorption and emission bands demonstrates that 5 possesses a non-planar arrangement either in the ground state or in the excited state. Introduction of neo-pentylamino units in positions 3, 3', 4 and 4' induces a red shift of 74 nm of the emission band with respect to biphenyl. Moreover, our spectrophotometric data are in good agreement with those obtained for benzidine 1, for which an emission signal centred at  $\approx$  380 nm, and attributed to the biphenyl core was observed. A Stokes shift of  $\approx 100 \text{ nm}$  and a fluorescence quantum yield of 0.39<sup>43</sup> were measured for **1**.



**Figure 2.** Normalized absorption (solid line) and emission (dashed line) spectra of compound **5**. Solvent: CH<sub>2</sub>Cl<sub>2</sub>; T=25.0(2) °C. Compound **5**:  $\lambda_{exc}=276$  nm; emission and excitation slits=7 nm. Compound **6**:  $\lambda_{exc}=269$  nm; emission and excitation slits=8 nm.

The N-alkylation with benzyl groups significantly altered the absorption and emission properties of the 3,3',4,4'-tetraaminobiphenyl moiety. Upon excitation at 269 nm, compound **6** indeed exhibits an intense, broad and non-structured fluorescence emission band at 486 nm together with a shoulder at  $\approx$  386 nm (Fig. 3).



**Figure 3.** Normalized emission spectra of **6.** Solvent: CH<sub>2</sub>Cl<sub>2</sub>; T=25.0(2) °C. Solid line:  $\lambda_{exc}=269$  nm; dashed line:  $\lambda_{exc}=318$  nm; dotted line:  $\lambda_{exc}=362$  nm; emission and excitation slits=10 nm.

The fluorescence emission centred at 386 nm most likely originates from the 3,3'-diaminobenzidine backbone as shown by the similar shape of the excitation ( $\lambda_{ana}$ =386 nm) and absorption spectra (Fig. S5 in Supplementary data). By contrast, the excitation spectrum recorded with  $\lambda_{ana}$ =482 nm is strikingly different from the absorption spectrum of **6**, and is slightly red shifted ( $\lambda_{max}$ =332 nm) as expected from a ground state association. These spectrophotometric features suggest the formation of a photoinduced exciplex. This exciplex type emission $^{25,26}$  can thus be ascribed to intramolecular  $\pi$ -stacking interactions in the excited state between the benzylic units and the 3,3'-diaminobenzidine moiety. This intramolecular process, which follows the excitation of one of the chromophores, leads to an excited species for which the two aromatic residues are brought into proper superposition by  $\pi$ -stacking.<sup>27</sup> Such properties were already reported for various non-symmetric bichromophoric compounds such as fluorescent chemosensors,<sup>28</sup> switches,<sup>29</sup> antibodies,<sup>30</sup> dyads<sup>31</sup> or chiral recog-nition markers.<sup>32</sup> Amine intramolecular exciplexes<sup>33</sup> are unlikely to be generated, since no red-shifted exciplex emission was observed with compound **5**. It should also be mentioned that dibenzylamine, dibenzylether and other dibenzyl derivatives<sup>34</sup> may undergo intramolecular excimer formation with the appearance of a fluorescence emission band with a maximum at about 335 nm in addition to the normal emission at 285 nm. Intramolecular excimer solely involving benzyl units within compound 6 could be excluded since no emission at  $\approx$  335 nm was observed.

At  $\lambda_{exc}$ =368 nm, the absolute fluorescence quantum yield of **6** (3.9(4)%) was found to be more than seven times higher than that of **5** under the same experimental conditions.<sup>35</sup> This result clearly shows that the emission properties of the 3,3'-diaminobenzidine moiety can be tuned by subtle modification (e.g., N-alkylation). Introduction of aromatic residues led to unexpected photoinduced exciplex species with increased fluorescence efficiency. Importantly, compound **6** reveals to be the first example of intramolecular exciplex containing benzidine unit.

# 2.4. Effect of pH on emission

It was of interest to examine the effect of pH on the emission properties of **6** in acetonitrile/water. It is noteworthy that when 3,3'-diaminobenzidine is N-substituted by an alkyl group (R=neopentyl, **5**), no emission signal from the biphenyl skeleton was observed in acetonitrile/water (9:1 v/v). In contrast, **6** with benzylic *N*-substituents exhibited interesting emission properties. A spectrofluorimetric titration of **6** versus pH was therefore undertaken. Upon excitation at 250 nm (Fig. 1 and Figs. S1 and S2 in Supplementary data), an emission maximum was observed at 392 nm at pH $\approx$ 5 (Fig. 4a). Since exciplexes involve different fluorophores, some degree of charge separation could be expected.

According to classical effects of solvent polarity.<sup>26,29</sup> red-shifted emission could be expected upon going from acetonitrile/water to dichloromethane. In contrast, a blue shift was observed for 6 under these conditions. Compound 6 is indeed an intricate system with one to four benzyl units being able to interact with the benzidine core in the excited state. Moreover, it has been suggested that photoinduced folded structures could be favoured in less polar or non-polar solvents, more open arrangements appearing in polar solvents.<sup>36</sup> The first protonation of one of the amine functions induces a significant bathochromic shift of  $\approx 40$  nm of the ligandcentred fluorescence signal, suggesting that the positive charge borne by the 3,3'-diaminobenzidine core may favour folded arrangements within the corresponding excited species. The second protonation process induces a quenching of the ligand-centred fluorescence emission (Fig. 4b). Electrostatic repulsions most likely disrupted the interactions between the two aromatic chromophores in the excited state and prevent the formation of the



**Figure 4.** (a) Fluorescence spectra of **6** ( $5.25 \times 10^{-6}$  M) versus pH. Solvent: acetonitrile/ water (9:1 v/v); T=25.0(2) °C; l=0.1 M (NEt<sub>4</sub>ClO<sub>4</sub>); l=1 cm,  $\lambda_{exc}=250$  nm. Spectra: (1) pH=0.85; (2) pH=2.35; (3) pH=4.97. Excitation and emission slits: 5 nm. (b) Fluorescence intensities versus pH at  $\lambda$ =395 nm and  $\lambda$ =430 nm.

exciplex species under acidic conditions.<sup>37</sup> Therefore, the excited unfolded diprotonated species  $6H_2^{2+}$  behaves as compound **5** with no emission properties. Interestingly, under these experimental conditions, **6** possesses valuable fluorescent on/off switch properties, which can be easily modulated by proton input.

The processing of the spectrofluorimetric data versus pH led to the determination of two protonation constants close to those determined in the ground state by absorption spectrophotometry (Table 1). The recalculated and normalized emission spectra of **6** and **6**H<sup>+</sup> are given in Figure 5.

#### 2.5. Photo-irradiation of 5 and 6 in CH<sub>2</sub>Cl<sub>2</sub>

Benzidines are relevant examples of simple redox systems, which in non-aqueous media, undergo stepwise electrochemical oxidation<sup>6,7</sup> to form monoradical cations and diradical (diimine) dications.<sup>38,39</sup> These oxidation processes and the resulting products were also observed from closely related compounds using free radical initiators,<sup>40</sup> oxidizing reagents<sup>41</sup> and peroxidase/H<sub>2</sub>O<sub>2</sub> system.<sup>42</sup> It was reported that photolysis of halomethanes, such as CH<sub>2</sub>Cl<sub>2</sub>, leads to the formation of free radicals able to abstract electrons from benzidine **1**.<sup>43</sup> Benzidine **1** was consequently transformed to coloured mono- and diradical cations. Similarly, photo-irradiation ( $\lambda_{exc}$ =254 nm) of **5** (Fig. 6a and Fig. S6 in



**Figure 5.** Relative recalculated fluorescence spectra of **6** protonated species. Solvent: acetonitrile/water (9:1 v/v); *T*=25.0(2) °C; *I*=0.1 M (NEt<sub>4</sub>ClO<sub>4</sub>); **6**H<sub>2</sub><sup>2+</sup> was assumed to be a non-emitting species in the statistical processing.

Supplementary data) and **6** (Fig. 6b and Fig. S7 in Supplementary data) in CH<sub>2</sub>Cl<sub>2</sub> is associated with significant spectral changes.

After photo-irradiation ( $\approx 20 \text{ s}$ ), the intensity of the absorption bands centred at  $\approx 320 \text{ nm}$  strongly decreases with concomitant formation of weak absorption bands in the visible region ( $\lambda_{abs}$ =547 nm for **5** and  $\lambda_{abs}$ =553 nm for **6**). Prolonged irradiation induces red shifts of these transitions ( $\lambda_{abs}$ =593 nm for **5** and  $\lambda_{abs}$ =584 nm for **6**). These spectrophotometric variations are assigned to the formation of coloured monoradical and diradicals (Figs. S6 and S7 in Supplementary data). These radical cations are produced in solution according to Scheme 3 in which methylene chloride is excited ( $\lambda_{exc}$ =254 nm) and photolyzed to yield halogen radicals.

Cl' radicals are hence able to abstract electrons from benzidine with subsequent formation of the two benzidine radicals. It is noteworthy that compounds **5** and **6** are photostable in tetrahydrofuran and acetonitrile/water. It is a good indication that the oxidized products result from reactions with methylene chloride. Emission spectra of **5** and **6** are also notably altered with large blue shift and strong increase of the ligand-centred emission bands (Fig. 6a and b). Prolonged irradiation in CH<sub>2</sub>Cl<sub>2</sub> induces a photobleaching of the samples, which results in intricate photo-degraded products as described for benzidine **1** by mass spectrometric methods (ESMS).<sup>44</sup> These results agree well with the spectral



**Figure 6.** Effect of photo-irradiation ( $\lambda_{exc}$ =254 nm) on emission spectra of (a) **5** and (**6**) in CH<sub>2</sub>Cl<sub>2</sub>. *T*=25.0(2) °C; (a) [**5**]<sub>tot</sub>=5.53×10<sup>-6</sup> M;  $\lambda_{exc}$ =276 nm; excitation and emission slits: 7 nm; the irradiation times are: 0 (1), 10, 20, 30, 40, 50, 60, 80, 110, 160, 220 (2) s; (b) [**6**]<sub>tot</sub>=3.5×10<sup>-6</sup> M;  $\lambda_{exc}$ =269 nm; excitation and emission slits: 8 nm; the irradiation times are: 0 (1), 10, 20, 30, 40, 50, 60, 80 (2) s.

changes monitored for benzidine **1** in various halomethanes (CHCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, CCl<sub>4</sub>).<sup>43</sup> Moreover, oxidation in acetonitrile/water by *tert*-butyl-hydroperoxide under basic conditions results in slow conversion of diamine to diimine as shown by the appearance of an



Scheme 3. Proposed oxidation scheme of benzidine-type compounds 5 and 6 following photo-irradiation of CH<sub>2</sub>Cl<sub>2</sub> at 254 nm.

absorption band centred at  $\approx$  598 nm for 5 (Fig. S8 in Supplementary data). Under these experimental conditions, no intermediate could be evidenced. It was reported<sup>42</sup> that the horseradish peroxidase (HRP) catalyzed oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H<sub>2</sub>O<sub>2</sub>, and led to the formation of the two-electron oxidation product (diimine), which was characterized by an intense absorption band at 450 nm  $(\varepsilon^{450}=5.9\times10^4 \text{ M}^{-1} \text{ cm}^{-1})$ . Prior to the formation of this species, a blue charge transfer complex of the parent diamine and the diimine oxidation product was evidenced ( $\lambda_{max}$ =370 and 652 nm;  $\epsilon^{652}$ =3.9×10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>). This charge transfer complex resulted from dimerization of two 3,3',5,5'-tetramethylbenzidine semiquinone imine cation free radicals (TMB+•) and subsequent disproportionation within these species, but is, itself, in equilibrium with the diamine and the diimine  $(\log K=5.45)$ .<sup>42</sup> With compounds **5** and **6**, no charge transfer complexes could be evidenced either by photo-irradiation in CH<sub>2</sub>Cl<sub>2</sub> or by oxidation with H<sub>2</sub>O<sub>2</sub> in tetrahydrofuran/water (9:1 v/v), probably due to the bulkiness of the N-substituents.

# 3. Conclusion

Our results show that N-alkylation of 3,3'-diaminobenzidine with innocent substituents leads to unusual properties. Indeed, the emission properties of the benzidine core can be fine-tuned by subtle modifications, and the introduction of benzylic residues led to unexpected photoinduced exciplexes with distinct emission properties and improved fluorescence efficiency. Importantly, compound 6 constitutes the first example of intramolecular exciplex containing benzidine unit. We could show that these photoinduced properties could be modulated by proton input, and the diprotonation of the benzidine unit disrupted the intramolecular communication in the excited states with a concomitant inhibition of the ligand-centred fluorescence emission. The semiquinone imine and quinone diimine systems were generated in CH<sub>2</sub>Cl<sub>2</sub> upon photo-irradiation at 254 nm. Photolysis of halomethane indeed generates Cl' radicals, which were able to rapidly oxidize the tetraamine compounds. It will be of particular interest to examine the dynamics of the exciplex formation by time-resolved fluorescence spectroscopy. The potential of these new systems as chromogenic substrates for measuring HRP activity using absorption and emission techniques will be also examined. Furthermore, with two bischelating sites and owing to their photophysical properties, compounds 5 and 6 will be also used as ligands for the coordination of metal centres.<sup>45</sup>

# 4. Experimental section

# 4.1. General

<sup>1</sup>H NMR (300 MHz), <sup>13</sup>C NMR (75 MHz) spectra were recorded on a Bruker AC-300 instrument. FAB mass spectral analyses were recorded on an autospec HF mass spectrometer and MALDI-TOF mass spectral analyses were recorded on a Finnigan TSQ 700. Elemental analyses were performed by the Spectropole center (Marseille, France). Solvents were freshly distilled under nitrogen prior to use. Molecule **5** was prepared according to the literature.<sup>11</sup> All reactions of air- or water-sensitive compounds were performed using standard Schlenk techniques under dry argon atmosphere.

#### 4.2. Syntheses

### 4.2.1. Synthesis of 4

To a solution of 3,3'-diaminobenzidine (0.50 g, 2.33 mmol) in Et<sub>2</sub>O (500 mL) and NEt<sub>3</sub> (5 mL) was added PhC(O)Cl (9.33 mmol) and the mixture was stirred for 18 h at room temperature. After evaporation of Et<sub>2</sub>O, the crude product was taken up in water. The

insoluble grey-white solid **4** in suspension was then isolated by filtration, washed with water and dried under air (88% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 7.04 (d, <sup>3</sup>*J*<sub>HH</sub>=9 Hz, 2H, aromatic C–H), 7.35–7.60 (m, 10H, aromatic C–H), 8.03 (d, <sup>3</sup>*J*<sub>HH</sub>=9 Hz, 4H, aromatic C–H), 8.29 (m, 6H, aromatic C–H), 10.44 (s, 2H, N–H), 10.52 (s, 2H, N–H). Anal. Calcd for C<sub>40</sub>H<sub>30</sub>N<sub>4</sub>O<sub>4</sub>·H<sub>2</sub>O: C, 74.06; H, 4.97; N, 8.64. Found: C, 74.10; H, 5.09; N, 8.52. MS (MALDI-TOF): *m/z*: 631.2 [M+1]<sup>+</sup>.

# 4.2.2. Synthesis of 6

The tetraamide **4** was dissolved in dry THF and an excess of LiAlH<sub>4</sub> (10 equiv) was added to the solution. The mixture was then refluxed for 12 h and the excess of LiAlH<sub>4</sub> was quenched by addition of water. After filtration of aluminium salts and evaporation to dryness, the crude product was taken up in CH<sub>2</sub>Cl<sub>2</sub> and then extracted with water. The organic phase was dried with MgSO<sub>4</sub> and evaporated to dryness after filtration. Yellow product **4** was collected by filtration after precipitation from a mixture of ether and petroleum ether (85% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ : 3.65 (br s, 4H, N–H), 4.24 (s, 4H, CH<sub>2</sub>), 4.27 (s, 4H, CH<sub>2</sub>), 6.66 (d, <sup>3</sup>*J*<sub>HH</sub>=9 Hz, 2H, aromatic C–H), 6.75 (s, 2H, aromatic C–H), 6.84 (d, <sup>3</sup>*J*<sub>HH</sub>=9 Hz, 2H, aromatic C–H), 7.30 (m, 20H, aromatic C–H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 49.00 (CH<sub>2</sub>), 49.03 (CH<sub>2</sub>), 110.93–112.24–117.58–127.28–127.86–127.95–128.64–128.68–133.55–135.89–137.32–139.50 (aryl C). Anal. Calcd for C<sub>40</sub>H<sub>38</sub>N<sub>4</sub>·1/2H<sub>2</sub>O: C, 82.30; H, 6.73; N, 9.60. Found: C, 82.52; H, 6.67; N, 9.45. MS (MALDI-TOF): *m/z*: 575.3 [M+1]<sup>+</sup>.

# 4.3. Physico-chemistry

#### 4.3.1. Starting materials and solvents

For solubility reasons, the acido-basic properties of ligands **5** and **6** were examined in a mixture of acetonitrile/water (9:1 v/v). Distilled water was further purified by passing through a mixed bed of ion-exchanger (BIOBLOCK SCIENTIFIC R3-83002, M3-83006) and activated carbon (BIOBLOCK SCIENTIFIC ORC-83005). The distilled water, spectroscopic grade acetonitrile and  $CH_2Cl_2$  (MERCK, Uvasol<sup>®</sup>, p.a.) were de-oxygenated using CO<sub>2</sub>- and O<sub>2</sub>-free argon prior to use (SIGMA Oxiclear cartridge). All the stock solutions were prepared by weighting solid products using an AG 245 METTLER TOLEDO analytical balance (precision 0.01 mg). The complete dissolution of the ligands was achieved with the help of ultrasonic bath. The ionic strength was adjusted to 0.1 M with tetra-ethylammonium perchlorate (NEt<sub>4</sub>ClO<sub>4</sub>, FLUKA, puriss).

CAUTION: perchlorate salts combined with organic ligands are potentially explosive and should be handled in small quantity and with the necessary precautions.<sup>46</sup>

# 4.3.2. Potentiometry

The free hydrogen ion concentrations were measured with an Ag/AgCl combined glass electrode (METROHM 6.0234.500, Long Life) filled with 0.1 M NaCl (FLUKA, p.a.) in water. Potential differences were given by a TACUSSEL Isis 20.000 millivoltmeter. Standardization of the millivoltmeter and the verification of the linearity of the electrode response were performed using a set of commercial MERCK buffered solutions (pH=4.00, 7.00 and 9.00). The experiments were carried out at 25.0(2) °C maintained with the help of HAAKE FJ thermostats.

# 4.3.3. Protonated species of 5 and 6

Solutions of **5**  $(5.46 \times 10^{-5} \text{ M})$  and **6**  $(5.25 \times 10^{-5} \text{ M})$  were prepared by quantitative dissolution of a solid sample in acetonitrile/ water (9:1 v/v). An aliquot of 40 mL was introduced into a jacketed cell (METROHM 6.1414.150) maintained at 25.0(2) °C by the flow of a HAAKE FJ thermostat. The solution was continuously deoxygenated by bubbling with oxygen-free argon. The titration of **5** (1.47 < pH < 9.46) or **6** (1.34 < pH < 10.06) was carried out by addition of known volumes of a 0.1 M standardized perchloric acid solution. Special care was taken to ensure that complete equilibration was attained. Absorption spectra versus pH were recorded using a Varian CARY 50 spectrophotometer fitted with HELLMA optical fibers (HELLMA, 041.002-UV) and an immersion probe made of quartz suprazil (HELLMA, 661.500-QX).

#### 4.4. Fluorescence titration

An aliquot of 40 mL of **6**  $(5.25 \times 10^{-6} \text{ M})$  was introduced into a jacketed cell (METROHM 6.1414.150) maintained at 25.0(2) °C by the flow of a HAAKE FI thermostat. The solution was continuously de-oxygenated by bubbling with oxygen-free argon. The initial pH was adjusted at 0.85 with concentrated perchloric acid, and the titration of 6 was carried out by addition of known volumes of a tetrametylammonium hydroxide solution (0.1 M). An aliquot (2 mL) was taken after each addition of base, and a fluorescence emission spectrum (250-700 nm) was recorded versus pH with 1 cm quartz optical cell (HELLMA, 110-QS) on a PERKIN-ELMER LS-50B spectrofluorimeter maintained at 25.0(2) °C by the flow of a HAAKE FJ thermostat. The excitation wavelength was 250±1 nm, and the excitation and emission slit widths were set at 5 nm. The absorbance at the excitation wavelength ( $\lambda_{exc} \ge 250 \text{ nm}$ ) was kept lower than 0.1 to minimize reabsorption processes and to keep it constant throughout the fluorescence titration. The light source was a pulsed xenon flash lamp with a pulse width at half peak height  $< 10 \ \mu s$  and power equivalent to 20 kW.

# 4.5. Refinement of the data

The spectrophotometric and potentiometric data were processed with the SPECFIT program, which adjusts the stability constants and the corresponding molar extinction coefficients ( $M^{-1}$  cm<sup>-1</sup>) of the species at equilibrium. SPECFIT<sup>47–50</sup> uses factor analysis to reduce the absorbance matrix and to extract the eigenvalues prior to the multiwavelength fit of the reduced data set according to the Marquardt algorithm.<sup>51,52</sup> Distribution curves of the various species were calculated using the Haltafall program.<sup>53</sup> Origin 5.0 was used to process the analytical results.<sup>54</sup> For the sake of simplicity, charges are omitted in all the chemical equilibria. The errors are given as  $3\sigma$  with  $\sigma$ =standard deviation.

#### 4.6. Photophysics

Emission spectra were collected on a PERKIN-ELMER LS-50B luminescence spectrophotometer with solutions of absorbance smaller than 0.1 at wavelength  $>\lambda_{exc}$  in order to avoid errors due to the inner filter effect. Absorption spectra were also measured along the fluorescence titration by using a Varian CARY 300 spectrophotometer. Fluorescence quantum yields were determined relative to fluorescent standard quinine sulfate ( $\Phi_{abs}$ =0.546, 0.5 M H<sub>2</sub>SO<sub>4</sub>) with possibility of correcting for differences between the refractive index of the reference  $n_r$ , and the sample solutions  $n_s$  using the expression:

$$\varphi_{\rm f}({\rm s}) = \varphi_{\rm f}({\rm r}) \frac{\int I_{\rm s}(\lambda) \times D_{\rm r} \times n_{\rm s}^2}{\int I_{\rm r}(\lambda) \times D_{\rm s} \times n_{\rm r}^2}$$

The indices s and r denote sample and reference, respectively. The integrals over *I* represent areas of the corrected emission spectra, *D* is the optical density at the wavelength of excitation.

#### Acknowledgements

This work has been supported by the Centre National de la Recherche Scientifique (UMR 7177 and UMR 6114). M.H. and H.T.

thank the French Ministry of Research and Education for granting them a Ph.D. fellowship.

# Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.04.058.

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