Electronic Supporting Information

Chromogenic chemodosimeter for highly selective detection of cyanide in water and
blood plasma based on Si-O cleavage in micellar system

Celso R. Nicoleti, Leandro G. Nandi, and Vanderlei G. Machado*
Departamento de Química, Universidade Federal de Santa Catarina, UFSC, CP 476, Florianópolis,
Santa Catarina, 88040-900, Brazil. Tel.: +55 48 3721 4542.
*vanderlei.machado@ufsc.br

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Experimental Section

1. Reagents

All chemicals used were high-purity commercial reagents. The deionized water used in the measurements was boiled and bubbled with nitrogen and kept in a nitrogen atmosphere to avoid the presence of carbon dioxide. All anions (HSO₄⁻, H₂PO₄⁻, NO₃⁻, CN⁻, CH₃COO⁻, F⁻, Cl⁻, Br⁻ and I⁻) were used as sodium and potassium salts with purity greater than 97-99 %. These salts were purchased from Merck (NaF, NaNO₃, KH₂PO₄, CH₃COONa and NaBr), Vetec (NaI and KHSO₄) and Sigma-Aldrich (Na₂S, NaCl and KCN). Cetyltrimethylammonium bromide (CTABr), p-nitrophenol, imidazole and triisopropylsilyl chloride were purchased from Sigma-Aldrich.

2. Instruments

UV-vis experiments were carried out on an HP 8452A spectrophotometer equipped with a thermostated bath and all measurements were performed at 25 °C, employing a 1 cm quartz cuvette. The maximum wavelength ($\lambda_{\text{max}}$) values of the UV-vis spectra were calculated from the first derivative of the absorption spectrum. Melting points were obtained on a Kofler hot stage and were uncorrected. The nuclear magnetic resonance (NMR) spectra were obtained using a Varian AS-400 spectrometer. Chemical shifts were recorded in ppm with the solvent resonance as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet and m = multiplet), coupling constants (Hz) and integration. Infrared
(IR) spectra were obtained on a Shimadzu spectrophotometer (model Prestige-21), with KBr pellets. High-Resolution Spectra Mass (HRMS) were obtained with an Electro-Spray Ionization (ESI) Quadrupole Time-Of-Flight (QTOF) mass spectrometer.

3. Synthesis and characterization

**2,6-Dibromo-4-nitroph-enol (2).** In a flask containing 5 mL of water at 40 °C, under magnetic stirring, potassium bromide (7.14 g; 60 mmol), p–toluenesulfonic acid (7.61 g; 40 mmol), N–bromosuccinimide (7.12 g; 40 mmol) and p–nitrophenol (3 g; 20 mmol) were mixed. In the course of the reaction (2 h) the formation of a yellow solid was observed. The reaction mixture was cooled to room temperature and the solid was filtered using a Büchner funnel and washed with an aqueous solution of 10 % sodium metabisulfite (3×10 mL). Thereafter, the solid was allowed to dry in a vacuum oven at 100 °C for 4 h. The product was a light yellow solid (5.12 g, 80 %). m.p. obtained: 144-145 °C (m.p. literature: 145 °C); IR (KBr, cm⁻¹): 3366 (O–H), 1688, 1574, 1464 (C=C), 1513, 1339 (N=O), 1232 (C–O); ¹H NMR (DMSO–d₆, 400 MHz) δ/ppm: 8.38 (s, 2H).

**4-Amino-2,6-dibromophenol (3).** The synthesis was carried out using the methodology described in the literature. Compound 2 (0.60 g; 2.0 mmol), tin chloride (SnCl₂.2H₂O) (3.16 g; 14 mmol) and 60 mL of ethanol were added in a round-bottomed flask. The system was maintained under magnetic stirring and reflux in an oil bath for 8 h. After the reflux, the reaction mixture was removed from the heat and left to cool until room temperature. Sodium
hydroxide was then added until pH = 10. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (3×50 mL). The organic layer extracts were dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure. The product obtained was a pale brown solid (0.38 g, 70 % yield). m.p. obtained: 191–192 °C; (m.p. literature:⁴ 191–193 °C). IR (KBr, ν max/cm⁻¹): 3369, 3269 (N–H), 1607, 1481, (C=C), 1218 (C–O); ¹H NMR (DMSO–d₆, 400 MHz) δ/ppm: 7.43 (s, 2H).

**3,5-Dibromo-4-[(triisopropylsilyl)oxy]aniline (4).** For the synthesis of 4, the methodology described by Ischay, Lu and Yoon⁵ was adapted. Compound 3 (0.70 g; 2.6 mmol), imidazole (0.53 g; 7.80 mmol) and 5 mL of dry DMF were mixed in a 100 mL round-bottomed flask under stirring. Triisopropylsilyl chloride (0.67 mL, 3.12 mmol) was then added and the reaction mixture was stirred in a nitrogen atmosphere for 8 h at 36 °C. The reaction was monitored by thin layer chromatography (TLC) using hexane:ethyl acetate (3:1; v/v) as the eluent. When the reaction was completed, water was added, the aqueous phase was extracted twice with n-hexane and the combined organic extracts were dried over anhydrous MgSO₄ and filtered. The solvent was removed by rotary evaporation and the purification of 4 was performed by column chromatography using silica gel and hexane:ethyl acetate (3:1; v/v) as the eluent, to give 4 an amorphous pale brown solid (0.70 g, 63 % yield). m.p. obtained: 77 °C. IR (KBr, ν max/cm⁻¹): 3429, 3353 (N-H), 2943, 2864 (C-H), 1601, 1468 (C=C), 1242, 883 Si(CH₃)₃; ¹H NMR (400 MHz, CDCl₃) δ/ppm: 6.81 (2H, s, Ar–H), 3.44 (2H, s, -NH₂), 1.55-1.47 (3H, m, -CH), 1.13 (18H, d, J = 7.82 Hz, -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ/ppm:
143.85, 141.28, 119.25, 115.87, 18.08, 14.36; HRMS (ESI, TOF): m/z calcd for C_{13}H_{25}Br_{2}NOSi [M + H]^+ 424.0125, found 424.0127.

3,5-Dibromo-N-(4-nitrobenzylidene)-4-[(triisopropylsilyl)oxy]aniline (1). 4-Nitrobenzaldehyde (0.50 g; 2.5 mmol) and compound 4 (0.66 g, 2.5 mmol) and absolute ethanol (5 mL) were added to a round-bottomed flask. The reactants were heated slowly with stirring until the solubilization of the reagents. One drop of acetic acid (AcOH) was then added and the reaction mixture was stirred for 4 h. The reaction was monitored by thin layer chromatography (TLC) eluting with hexane:ethyl acetate (3:1; v/v). The precipitate in the reaction medium was filtered off under vacuum, washed with iced ethanol and recrystallized two times from ethanol:acetone (2:1; v/v). After drying, 0.52 g (60 % yield) of a solid product comprised of yellow needles were obtained. m.p. obtained: 162-163 °C. IR (KBr, $\bar{\nu}_{\text{max}}$/cm$^{-1}$): 2943, 2866 (C–H), 1601 (C=N), 1634, 1456 (C=C), 1522, 1342 (N=O), 1295, 885 Si(CH$_3$)$_3$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$/ppm: 8.52 (1H, s, C=N) 8.33-8.31 (2H, d, $J$ = 9.0 Hz, Ar–H), 8.04-8.02 (2H, d, $J$ = 8.6 Hz, Ar–H), 7.48 (2H, s, Ar–H), 1.61-1.52 (3H, m, -CH(CH$_3$)$_2$), 1.15 (18H, d, $J$ = 7.4 Hz, -CH(CH$_3$)$_2$; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$/ppm: 157.44, 151.01, 149.7, 145.04, 141.42, 129.71, 125.88, 124.37, 116.42, 18.36, 14.77; HRMS (ESI, TOF): m/z calcd for C$_{22}$H$_{28}$Br$_2$N$_2$O$_3$Si [M + H]$^+$ 557.0290, found 557.0284.

2,6-Dibromo-4-[(4-nitrobenzylidene)amino]phenol (5a). 4-Nitrobenzaldehyde (0.28 g; 1.87 mmol) and compound 3 (0.50 g, 1.87 mmol) and absolute ethanol (5 mL) were added to a round-bottomed flask. The reactants were heated slowly with stirring until the solubilization
of the reagents. One drop of acetic acid (AcOH) was then added and the reaction mixture was stirred for 4 h. The reaction was monitored by thin layer chromatography (TLC) eluting with hexane:ethyl acetate (3:1; v/v). The precipitate in the reaction medium was filtered off under vacuum, washed with iced ethanol and recrystallized three times from ethanol:ethyl acetate (1:1; v/v). After drying, 0.37 g (50 % yield) of a brown solid product were obtained. m.p. obtained: 179-180 °C. IR (KBr, $\tilde{\nu}_{\text{max}}$/cm$^{-1}$): 3451 (O-H), 3112, 3088 (C-H), 1595 (C=N), 1617, 1464 (C=C), 1511, 1342 (N=O), 1238 (C-O); $^1$H NMR (400 MHz, C$_3$D$_6$O) $\delta$/ppm: 8.86 (1H, s, C=N), 8.73 (1H, s, OH), 8.39-8.36 (2H, d, $J = 8.99$ Hz, Ar–H), 8.23-8.21 (2H, d, $J = 8.99$ Hz, Ar–H), 7.65 (2H, s, Ar–H); $^{13}$C NMR (100 MHz, C$_3$D$_6$O) $\delta$/ppm: 159.21, 150.69, 150.21, 145.45, 142.49, 130.40, 126.39, 124.68, 111.72; HRMS (ESI, TOF): $m/z$ calcd for C$_{13}$H$_8$Br$_2$N$_2$O$_3$ [M + H]$^+$ 400.8955, found 400.8952.

4. UV-vis study of 1 with the anions

A solution of 1 was prepared in propanone in a concentration of 1.0×10$^{-2}$ mol L$^{-1}$. An aliquot of the solution, sufficient to give a concentration of the compound of 4.0×10$^{-5}$ mol L$^{-1}$, was collected with a microsyringe and placed in a volumetric flask. After evaporation of the solvent, 5 mL of an aqueous stock solution of CTABr at a concentration of 2.0×10$^{-3}$ mol L$^{-1}$ with the pH adjusted to 8.0 were added. This solution was then used to prepare the solution of each anion in a concentration of 1.0×10$^{-3}$ mol L$^{-1}$, using 5 mL volumetric flasks. Subsequently, these solutions were transferred to cuvettes hermetically closed with rubber
stoppers and UV-vis spectra were obtained. The absorbance values were collected for the $\lambda_{\text{max}}$ verified for each mixture.

5. Titration of 1 and determination of the detection and quantification limits

The titration was performed in the aqueous CTABr micellar system at pH 8.0 with the preparation of the solution of 1 as described previously. This solution was used to prepare the stock anion solution, using a vial closed with rubber stoppers and the titrations were carried out by adding small amounts of the salt solution with a microsyringe to closed quartz cuvettes containing the solution of 1. The UV–vis spectra were taken after each addition and the absorbance values were collected at 474.0 nm [Figure 4A].

The linear segment of the titration curve was used to calculate the detection (DL) and quantification (QL) limits. After the linear fitting of the experimental data, the DL and QL were obtained according to the procedure reported in the literature, using Eqs. (1) and (2):6-8

\[
\text{DL} = 3 \times \text{Sb}_1 / S \quad \text{Eq. (1)}
\]

\[
\text{QL} = 10 \times \text{Sb}_1 / S \quad \text{Eq. (2)}
\]

where $\text{Sb}_1$ is the standard deviation of the blank solution and $S$ is the slope of the calibration curve.
**Figure S-1.** Titration curve of 1 in aqueous CTABr micellar system (pH = 8.0) with increasing amounts of CN⁻. The linear segment of the curve was used to calculate the DL and QL.

\[
DL = 1.48 \times 10^{-5} \text{ mol L}^{-1} \quad QL = 4.93 \times 10^{-5} \text{ mol L}^{-1}
\]

6. **Kinetics of the reaction of 1 with CN⁻ in aqueous CTABr micellar system at different pH values**

All kinetics runs were carried out under pseudo-first-order conditions using an excess of potassium cyanide \((1.0 \times 10^{-3} \text{ mol L}^{-1})\). A stock solution of 1 \((1.0 \times 10^{-2} \text{ mol L}^{-1})\) was prepared as described in section 4. All reactions were initiated by mixing a small volume of the stock solution of 1 in a hermetically closed 3 cm³ quartz-stoppered cuvette (10 mm path length) containing 2 mL of CTABr at the concentration of \(2.0 \times 10^{-3} \text{ mol L}^{-1}\) with the pH adjusted to 8.0. The final concentration of compound 1 was \(4.0 \times 10^{-5} \text{ mol L}^{-1}\) in all kinetic experiments. After the thermal equilibrium at 25 °C (using a thermostated bath) was reached, a volume of 40 µL of a stock solution of \(5.0 \times 10^{-2} \text{ mol L}^{-1}\) of CN⁻ in CTABr \((2.0 \times 10^{-3} \text{ mol L}^{-1})\), with the pH adjusted to 8.0, was added to the cuvette and the kinetic data were collected after an
interval of 60 s. The reaction was monitored through the change in absorbance \( (A_t) \) at 474 nm as a function of the reaction time \( t \). The \( k_{obs} \) values were calculated from Eq. (3) through nonlinear least-squares regression curve-fitting (Figure S-2) using the ORIGIN 6.1 program.

\[
A_t = [\exp(-k_{obs} \cdot t)] + A_\infty \quad \text{Eq. (3)}
\]

where \( A_t \) represents the absorbance of the reaction mixture at any reaction time \( t \), \( A_\infty \) the absorbance of the reaction mixture at reaction time \( t = \infty \) and \( k_{obs} \) the pseudo-first-order rate constant. The half-life times \( (t_{1/2}) \) were calculated by means of the following expression: \( t_{1/2} = \ln 2 / k_{obs} \). The \( k_{obs} \) and \( t_{1/2} \) values are shown in Table S-1.

\[\begin{array}{c|c|c|c|c|c|c|c|c}
\hline
\text{Time / s} & 0 & 330 & 660 & 990 & 1320 & 1650 & 1980 & 2310 & 2640 \\
\hline
\text{Absorbance} & \quad & \quad & \quad & \quad & \quad & \quad & \quad & \quad & \quad \\
\hline
\end{array}\]
Table S-1. Values of $k_{obs}$ obtained for the reaction of 1 with CN$^-$ in aqueous micellar medium.

<table>
<thead>
<tr>
<th>pH</th>
<th>$10^{-3}k_{obs}$ (s$^{-1}$)</th>
<th>$t_{1/2}$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>1.78 ± 0.03</td>
<td>389</td>
</tr>
<tr>
<td>8.8</td>
<td>1.35 ± 0.032</td>
<td>513</td>
</tr>
<tr>
<td>10.0</td>
<td>1.21 ± 0.034</td>
<td>573</td>
</tr>
<tr>
<td>11.0</td>
<td>0.81 ± 0.176</td>
<td>856</td>
</tr>
</tbody>
</table>

7. $pK_a$ values for 5a in aqueous and micellar solutions

A solution of 5a was prepared at a concentration of 1.0×10$^{-2}$ mol L$^{-1}$ in propanone, and stored in glass flasks closed with rubber stoppers to avoid the evaporation of the solvent. Aliquots of the solution, corresponding to the concentration of 5a of 4.0×10$^{-5}$ mol L$^{-1}$, were collected with a microsyringe and placed in 10 mL vials containing distilled water or aqueous CTABr (2.0×10$^{-3}$ mol L$^{-1}$). Subsequently, the pH was adjusted through the addition of small aliquots of HCl (0.1 mol L$^{-1}$) or KOH (0.1 mol L$^{-1}$). The UV-vis spectrum of the compound was recorded at 25 °C. Each absorbance value at 428.0 nm (in distilled water) or at 474.0 nm (in aqueous CTABr 2.0×10$^{-3}$ mol L$^{-1}$) was collected and plotted as a function of the pH (Figure S-3 A and B). Data was fitted using a sigmoid equation to give the $pK_a$ value for 5a of 7.37±0.04 in water and 4.03±0.04 in the aqueous CTABr micellar medium.
**Figure S-3.** Absorbance values as a function of pH for 5a (A) at 428.0 nm in water and (B) at 474.0 nm in aqueous CTABr (2.0×10⁻³ mol L⁻¹). The theoretical curves were traced using a sigmoid equation.

8. Stability tests of 1

**Figure S-4.** Stability test of 1 (4.0×10⁻⁵ mol L⁻¹) in pH 8.0 at 25 °C (A) in aqueous CTABr (2.0×10⁻³ mol L⁻¹) micellar system and (B) in water.
**Figure S-5.** UV-vis spectra of 1 in an aqueous CTABr micellar system for 1 in the absence (a) and in the presence of F⁻ (b) and CN⁻ (c) after 20 min (A), 1h (B), 2 h (C), and 19 h (D) of reaction at 25 °C.

9. Detection of cyanide in human blood plasma

A blood sample was collected with EDTA from a non-smoking volunteer. The blood (1 mL) was spiked with potassium cyanide \( c(\text{CN}^-) = 10 \text{ mmol L}^{-1} \) and 80 mmol L\(^{-1} \), and was subsequently centrifuged at 1500 rpm for 10 min at room temperature to remove cellular components. After centrifugation, three plasma aliquots (10, 20 and 60 μL) were placed in a hermetically closed 3 cm\(^3\) quartz-stoppered cuvette (10 mm path length) containing the solution of 1 (4.0×10\(^{-5}\) mol L\(^{-1}\)) in 2 mL of CTABr (2.0×10\(^{-3}\) mol L\(^{-1}\)) with the pH adjusted to 8.0. Fifteen minutes later, the UV-vis spectra were obtained and the absorbance at 474 nm was read (**Figure 5**). The concentrations of CN⁻ were calculated using the titration curve (**Figure 4B**).
10. IR, NMR and mass spectra

Figure S-6. IR spectrum for 2 (KBr pellet).
Figure S-7. $^1$H NMR spectrum for 2 in DMSO-d$_6$ (400 MHz).

Figure S-8. IR spectrum for 3 (KBr pellet).
Figure S-9. $^1$H NMR spectrum for 3 in DMSO-d$_6$ (400 MHz).

Figure S-10. IR spectrum for 4 (KBr pellet).
Figure S-11. $^1$H NMR spectrum for 4 in CDCl$_3$ (400 MHz).

Figure S-12. $^{13}$C NMR spectrum for 4 in CDCl$_3$ (100 MHz).
Figure S-13. HRMS for compound 4.

Figure S-14. IR spectrum for 1 (KBr pellet).
**Figure S-15.** $^1$H NMR spectrum for 1 in CDCl$_3$ (400 MHz).

**Figure S-16.** $^{13}$C NMR spectrum for 1 in CDCl$_3$ (100 MHz).
**Figure S-17.** HRMS for compound 1.

**Figure S-18.** IR spectrum for 5a (KBr pellet).
Figure S-19. \( ^1\)H NMR spectrum for 5a in C\(_3\)D\(_6\)O (400 MHz).

Figure S-20. \( ^{13}\)C NMR spectrum for 5a in C\(_3\)D\(_6\)O (100 MHz).
Figure S-21. HRMS for compound 5a.

11. References

(8) Isaad, J.; El Achari, A.; Malek, F. Dyes Pigm. 2013, 97, 134-140.