

Turn-on Selective Vitamin B6 derivative Fluorescent Probe for Histidine detection in Biological Samples.

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Experimental Section

Physical measurements. Elemental analyses were carried out with Fisons Instruments EA1108 microanalyser at the University of Vigo (CACTI), Spain. Infrared spectra were recorded in KBr windows using a JASCO FT/IR-410 spectrophotometer. ¹H NMR was carried out in a Bruker Avance III 400 at an operating frequency of 400 MHz for ¹H using the solvent peak as internal reference at 25 °C.

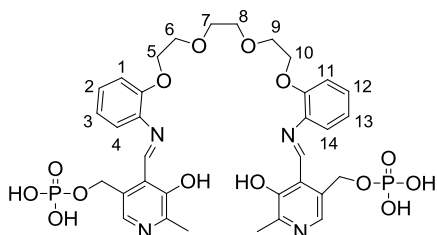
The MALDI-MS analyses were realized in a MALDI-TOF-TOF-MS model Ultraflex II Bruker, Germany, equipped with nitrogen, from the BIOSCOPE group. Each spectrum represents accumulations of 5×50 laser shots. The reflection mode was used. The ion source and flight tube pressure were less than 1.80×10^{-7} and 5.60×10^{-8} Torr, respectively. The MALDI mass spectra of the soluble samples (1 or 2 μg/μL) were recorded using the conventional sample preparation method for MALDI-MS. 1 μL of ligand was put on the sample holder. The sample holder was inserted in the ion source.

Chemicals and starting materials. Na(BF₄).xH₂O, K(BF₄).xH₂O, Ca(CF₃SO₃)₂, Cu(BF₄)₂.6H₂O, Ni(BF₄)₂.6H₂O, Zn(BF₄)₂.xH₂O, Cd(CF₃SO₃)₂, Fe(NO₃)₃.H₂O, Al(NO₃)₃.9H₂O salts, Pyridoxal 5'-phosphate hydrate (vitamin B6), HEPES, histidine, cysteine, tyrosine, dopamine and tryptophan have been purchased from Strem Chemicals, Sigma Aldrich or Solchemar. All compounds were used without previous purification.

Spectrophotometric and spectrofluorimetric measurements. Absorption spectra were recorded by a JASCO V-650 spectrophotometer and a fluorescence emission by a HORIBA Scientific FLUOROMAX-4 spectrofluorimeter. The linearity of the fluorescence emission vs. The concentration was checked out by the concentration used (10^{-4} – 10^{-6} M). A correction of the absorbed light was performed each time that was necessary. The spectrophotometric characterizations and titrations were performed as follows: the stock solutions of the compounds (ca. 10^{-3} M) were prepared by dissolving an appropriated amount of the compounds in a 10 ml

volumetric flask and diluting them to the mark with HEPES buffer at pH 7.7. The solutions were prepared by appropriate dilution of the stock solutions still $10^{-5} - 10^{-6}$ M. Titrations of the ligand **L** was carried out by the addition of microliter amounts of standard solutions of the ions in water. All the measurements were performed at 298 K.

Synthesis and physical characterization of compound L. Compound **1** (0.1 g, 3.01×10^{-4} mol) and vitamin B₆ (compound **2**) (0.148 g, 6.02×10^{-4} mol) were dissolved in absolute ethanol. The



solution was stirred and refluxed at boiling point for 4 hours. The solution was evaporated under reduced pressure yielding an orange oil. The compound was purifying by diethyl ether and cooled ethanol and the final product was obtained as an orange powder.

Colour: orange powder. Yield 0.21 g, (88%), FW = 790.2. Anal. Calc. for C₃₄H₄₀N₄O₁₄P₂: C, 51.70; H, 5.10; N, 7.10. Found: C, 51.80; H, 5.00; N, 6.90 %. IR(cm⁻¹): ν , 1635 (C=N). ¹H-NMR (D₂O/NaOD, 400 MHz): δ_{H} = 2.2 (s, 6H, -CH₃ Vit.B₆), 3.04 (m, 6H, H6-H9), 3.13 (m, 4H, H5, H10), 3.45 (m, 4H, -CH₂ Vit. B₆), 6.20 (m, 8H, H1-H4, H11-H14), 6.4 (s, 2H, pyridine), 7.5 (s, 2H, C=N imine). UV-Vis in HEPES buffer pH 7.7 (λ , nm): Band at 390 nm ($\epsilon = 26429 \text{ cm}^{-1} \cdot \text{M}^{-1}$). Emission spectrum in HEPES buffer pH 7.7 ($\lambda_{\text{exc}} = 390 \text{ nm}$, $\lambda_{\text{emis}} = 470 \text{ nm}$). MALDI-TOF-MS = 790.5 (m/z).

The IR spectrum shows a vibrational band at 1635 cm⁻¹ assigned to ν (C=N) linkage. Otherwise the absence of other band attributed to the free amine, confirms the imine formation¹. In the ¹H-NMR evidence the characteristic signals of the proton in the imine linkage at 7.5 ppm. The MALDI-TOF-MS spectrum reveals a peak at 790.5 m/z corresponded to the compound **L**. Other fragments of **L** were founded, as can be seen in figure 1A. The photophysical characterization of **L** was carried out in HEPES buffer at pH 7.7 (see figure 1B).

Urine samples. The urine samples were collected from healthy volunteers, and diluted 100 times before analysis. No other pre-treatments were needed in the experiment for detection of histidine in the real samples.

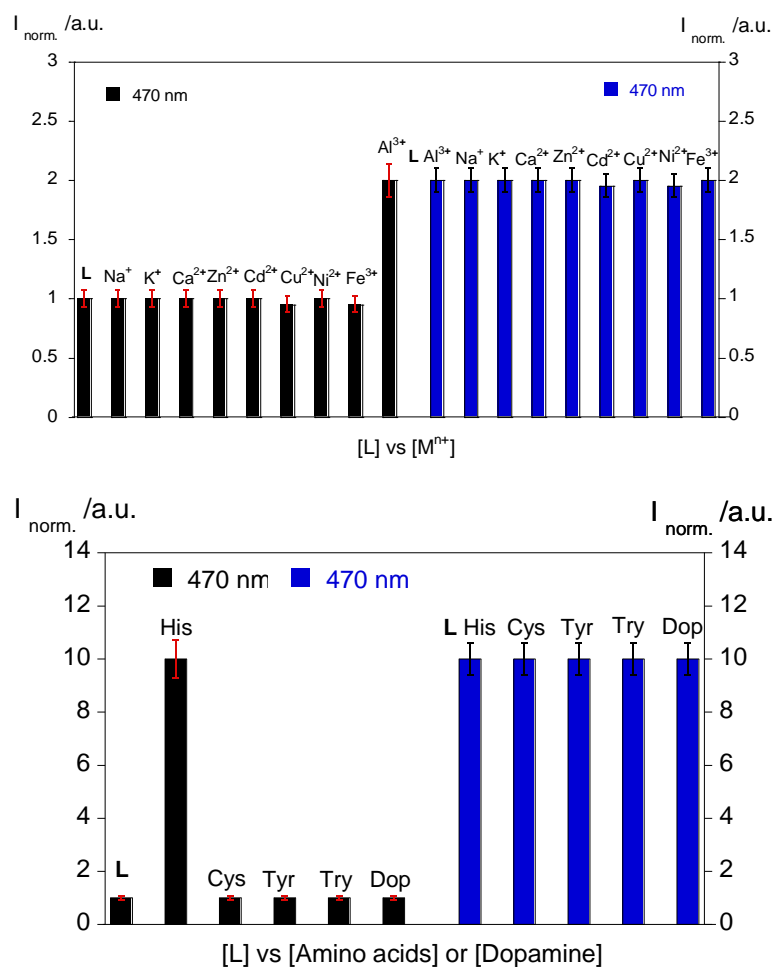


Figure S11. - Above: Normalized emission intensity at 470 nm, of compound **L** upon addition of 10 equivalents of Mⁿ⁺ metal ions (black bar); and normalized emission intensity of system LAI³⁺ upon addition of Mⁿ⁺ (blue bar) ([L] = 1×10⁻⁵ M, solvent: Hepes buffer pH 7.7, λ_{exc} = 390 nm, Mⁿ⁺ = Na⁺, K⁺, Ca²⁺, Zn²⁺, Cd²⁺, Cu²⁺, Ni²⁺, Fe³⁺). Below: Normalized emission intensity at 470 nm, of compound **L** upon addition of 10 equivalents of amino acids (black bar) or dopamine; and normalized emission intensity of system LHis upon addition of 100 equivalents of amino acids. ([L] = 1×10⁻⁵ M, solvent: Hepes buffer pH 7.7, λ_{exc} = 390 nm, amino acids = Histidine (His), cysteine (Cys), Tyrosine (Tyr), Tryptophan (Try), and Dopamine (Dop)).

¹ R. M. Silverstein, G. C. Bassler, T. C. Morrill, *Spectrometric Identification of Organic Compounds*, 4th Edition, John Wiley & Sons, 1980.