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Aminoacidic units wired on poly(aryleneethynylene) platforms as highly selective mercury-responsive materials

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ABSTRACT

Novel highly ethynylated materials carrying aminoacidic side arms on poly(aryleneethynylene) (PAE) conjugated backbones have been prepared and fully characterized. The luminescent sensing properties of these materials toward metal ions were investigated. All compounds showed high selectivity toward Hg(II) ions, and a signal amplification in Hg(II) detection was observed for the polymeric compound in comparison with other molecular ligands.

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The widespread role of metal ions in human health ranges from the requirement of daily intake of essential elements, to the toxicity due to metal overload.¹ Because of their large presence in the environment, as a result of human activities such as farming, industry or from contamination during food processing and storage, sources of metal ingestion might be contaminated drinking water, seafood, breast milk, smoking, together with plants and animals used in the diet. In this respect, frequent exposure, although below current admitted levels, may result in accumulative effects and, with the extension of lifespan in the western world, the total accumulation in the body over a lifetime, with special attention to heavy metals, should be taken into serious account.²

Therefore, the development of selective and tunable practical sensors for the detection and quantification of metal ions is the subject of considerable research efforts.^{3–5}

In nature, metal binding is achieved with an unsurpassed degree of selectivity using aminoacids and peptides.^{6–9} In principle, mimicking nature, one could thus succeed in designing and tuning the selectivity of peptides to achieve the desired sensing properties.¹⁰ Despite these premises, only recently sensing systems using aminoacidic and peptide moieties as recognition units for metal sensing have started to appear.^{11–17}

In the past few years we entered this field proposing a new synthetic access to highly conjugated small molecules and polymers characterized by poly(aryleneethynylene)s backbones (PAEs), and their use as sensing material in the construction of efficient chemo- and bio-sensors.^{18–20} In particular²⁰ we reported the preparation of co-polymers made of alternating arylene and phenanthroline or bithiophene moieties spaced by ethynylene units, and their sensing properties toward metal ions.

More recently, we presented our preliminary results obtained with the use of further elaborated structures made of aminoacidic recognition sites covalently linked to conjugated PAEs backbones, and their effectiveness toward metal ion sensing.²¹ The good selectivity and sensitivity obtained for the Hg(II) ion, an environmental heavy metal pollutant of major concern,²² encouraged us to foster the development of the sensing potential of highly ethynylated platforms decorated with aminoacids.

In this Letter we present the synthesis of different PAE structures carrying leucine moieties as side arms, and the investigation of their photophysical behavior upon interaction with metal ions.

Starting from the commercially available terephthalic acid bromides **1** and **2**, formation of derivatives **5** and **6** bearing L-leucine pendants was achieved in two steps by adapting literature procedures (Scheme 1).²³ Subsequently, formation of the conjugated structures **DLM**, **TLM**, and **DLP** was achieved by the use of the Stille



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Scheme 1. Schematic representation of the synthesis of aromatic bromides carrying leucine side arms.

Pd-promoted cross coupling reaction^{19,24} between the tributylethynyltin derivatives **7** and **8** and the aryl halides **5** and **6**, in the presence of tetrakis(triphenylphosphine)palladium(0) catalyst (Scheme 2).

The model compounds N,N'-[2,5-bis(phenylethynyl) terephthaloyl](L,L)di-leucine methyl ester (**DLM**) and N,N',N'',N'''-[(2,2'-ethyne-2,1-diyl)diterephthaloyl](L,L,L,L)tetra-leucine methyl ester (**TLM**), presenting, respectively, two and four leucine units



Scheme 2. Synthetic scheme of the leucine functionalized conjugated materials DLM, TLM, and DLP.

'wired' on different ethynylated platforms were designed with the purpose of investigating the influence of the different number and reciprocal proximity of the aminoacidic units toward metal ion binding efficiency. On the other side, the polymeric material poly[N,N-(2-ethynylterephthaloyl)(L,L)di-leucine methyl ester] (**DLP**), characterized by the regular repetition of leucine units into a long spanning conjugated backbone, represents an example of the so-called molecular wire approach to sensing.^{25,26}

The photophysical characterization of **DLM**, **TLM**, and **DLP** was carried out in acetonitrile. The absorption spectra (Fig. 1a) show, for all compounds, the broad non-structured band profile typical of these species, that can be attributed to delocalized Π - Π * transitions characterized by lower energies as the Π -delocalization increases.^{18,19,27} This is confirmed by the bathochromic shift of the absorption maxima, that goes from 310 nm for TLM to 320 nm for **DLM**. up to 370 nm for the polymer **DLP**. In addition, in the case of TLM and DLM. also the different number of electronwithdrawing imido side substituents enhances this effect.²⁷ The maxima of the fluorescence bands present the same trend (Fig. 1b), but with even more pronounced differences, shifting from 363 nm for TLM, to 392 nm for DLM, up to 505 nm for DLP. The luminescence of these systems has already been attributed to localized states generated by the migration of excitons along the conjugated path with more or less pronounced shoulders due to vibronic transitions.²⁸ Moderate emission quantum yields have been observed: 5×10^{-3} for **TLM** and **DLP** and 1×10^{-2} for **DLM**.

We studied the photophysical behavior of **DLM**, **TLM**, and **DLP** in the presence of different metal ions in order to investigate (i) the influence of different distributions of the leucine groups along the PAE backbones on metal ion coordination, and (ii) the possible occurrence of synergic effects able to enhance the sensing efficiency in more complex structures.

Very interestingly, all compounds presented significant changes in their photophysical properties after the addition of Hg(II) ions, while a hundred-fold excess of Pb(II), Co(II), Cd(II), Ni(II), Cu(II), Fe(II), Zn(II), and Ag(I) did not cause any appreciable modifications either of the absorption or of the emission spectra. The only relevant exception was the significant luminescence variation of the ligand **TLM** in the presence of Cu(II) or Fe(II) (see discussion below and Fig. S3, 4, Supplementary data).²⁹

In the case of **DLM** the complexation of Hg²⁺ induced drastic changes in the absorption and emission spectra. In the absorption spectrum a new band arose above 400 nm, with a concomitant decrease of the intensity maximum at 320 nm (typical band of the non complexed ligand) that shifted to about 350 nm (Fig. S1, Supplementary data), an expected trend since the red shift of the absorption maximum is the most common and well known effect induced by metal complexation. In the emission spectrum



Figure 1. Normalized absorbance spectra: (a) normalized emission spectra; (b) in acetonitrile of TLM (dotted line), DLM (solid line), and DLP (dashed line).



Figure 2. (a) Emission spectra (λ_{exc} 300 nm) of **DLM** 3.0 × 10⁻⁵ M in acetonitrile upon addition of increasing amounts of Hg²⁺. Inset: fluorescence intensity profiles (λ_{exc} 300 nm; λ_{em} 390 nm) vs added equivalents of Hg²⁺; (b) emission spectra (λ_{exc} 300 nm) of **TLM** 5.5 × 10⁻⁵ M in acetonitrile upon addition of increasing amounts of Hg²⁺. Inset: fluorescence intensity profiles (λ_{exc} 300 nm) vs added equivalents of Hg²⁺; (b) emission spectra (λ_{exc} 307 nm (filled circles), λ_{em} 455 nm (open circles); (c) emission spectra (λ_{exc} 375 nm) of **DLP** 8.810⁻⁵ M in acetonitrile upon addition of increasing amounts of Hg²⁺. Inset: fluorescence intensity profile (λ_{exc} 375 nm) vs added equivalents of Hg²⁺. Inset: fluorescence intensity profile (λ_{exc} 375 nm) vs added equivalents of Hg²⁺. Inset: fluorescence intensity profile (λ_{exc} 375 nm) vs added equivalents of Hg²⁺.

(Fig. 2a), a complete quenching of the band centered at 392 nm could be observed.

The plots of both the absorption and emission (Fig. 2a, inset) intensities vs $[Hg^{2+}]$ showed marked variations between 0 and 2 equiv of added metal, to reach then a plateau.

The interpolation of the obtained data with the global analysis software $(SPECFIT)^{30}$ showed evidence of the formation of the two equilibria (1) and (2).

$$\mathbf{L} + \mathbf{H}\mathbf{g}^{2+} \leftrightarrows [\mathbf{L}\mathbf{H}\mathbf{g}]^{2+} \tag{1}$$

$$[L + Hg]^{2+} + Hg^{2+} \Leftrightarrow [LHg_2]^{4+}$$
(2)

Absorption and emission data fitting provided consistent values for the association constants relative to these equilibria: $K_1 = (1.8 \pm 0.3) \times 10^6 \text{ M}^{-1}$ for Eq. (1), and $K_2 = (1.3 \pm 0.3) \times 10^5 \text{ M}^{-1}$ for Eq. (2).

Adding increasing amounts of mercury ions to acetonitrile solutions of **TLM**, we observed absorption variations similar to those of **DLM** (Fig. S2, Supplementary data), while the emission properties presented more drastic changes (Fig. 2b). In this case, the quenching of the emission intensity that centered at 363 nm was accompanied by the formation of a new, non-structured, band with the maximum at 460 nm.

This is a very interesting result, since the possibility of monitoring two distinct signals, the emission quenching at 363 nm and the emission enhancement at 460 nm, is very valuable from an applicative point of view, allowing ratiometric sensing.^{5b,31} In the case of **TLM**, this can be particularly useful for the selective detection of mercury ions in samples containing also Cu(II) and/or Fe(II), that, as we have already mentioned, have a lower but not negligible affinity for this ligand. Despite the fact that Cu(II) and Fe(II) ions, similarly to Hg(II), were both provoking the rising of the band at 460 nm, the intensity decrease of the band at 363 nm was exclusively related to the presence of Hg²⁺ ions (Fig. S3–6, Supplementary data).

Plotting the values of the **TLM** absorption and emission (Fig. 2b, inset) intensities vs [Hg²⁺], the trends were very similar to that observed for **DLM** with marked variations between 0 and 2 equiv of added metal, to reach then a plateau. The treatment of both the absorption and emission titration data provided consistent values for the association constants of equilibria (1 and 2): $K_1 = (1.7 \pm 0.5) \times 10^7 \text{ M}^{-1}$ and $K_2 = (2.9 \pm 0.7) \times 10^6 \text{ M}^{-1}$, respectively.

It is interesting to note that these values are both ten times higher with respect to those obtained for **DLM**, clearly evidencing the effectiveness of the different ligand architecture toward metal complexation.

Markedly different absorption and emission outputs were observed upon exposure of **DLP** to the mercury ions. For clarity, in order to facilitate the direct comparison with **DLM** and **TLM**, concentration of **DLP** is expressed in terms of concentration of monomeric repeating units. In the absorption spectrum (Fig. S7, Supplementary data), no appreciable variations were observed up to the addition of 0.15 equiv of Hg²⁺ to an acetonitrile solution of the ligand. Only for higher amounts of the metal ions a gradual decrease of the absorbance could be detected without, however, reaching a plateau, even in the presence of a 20-fold excess of mercury ions. On the contrary, the trend in the emission plot was dramatically different (Fig. 2c). A drastic but not complete quenching of the emission intensity was observed up to 0.2 equiv of added mercury, and then no further changes of the residual luminescence could be evidenced even in the presence of a 20-fold excess of the metal ion (Fig. 2c, inset).

These results clearly indicated that an adduct is formed in which, on average, each Hg(II) ion affected the photophysical properties of 5 surrounding repeating units of the polymer chain.

Further additions of metal ions yield complex structures displaying different absorption spectra, but without affecting the efficiency of the quenching process. Comparing the emission intensity changes occurring for **TLM** and **DLP** in the presence of Hg(II), while for the first one each single complexation event induces the quenching of up to monomeric units, in **DLP** one binding event quenches up to 5 repeating units. This is a very interesting result that indicates the occurrence of positive cooperation effects inducing an amplification of the response variations in species presenting a higher number of monomeric repeating units.

For all compounds detection limits (LOD) were calculated by 3σ and literature³² methods. The obtained values, coincident by the two methods, were 0.42 ppm, 0.52 ppm, and 0.34 ppm for **DLM**, **TLM**, and **DLP**, respectively. Interestingly, the detection limit obtained for **DLP** is significatively lower than that of **TLM**, an amplification effect in line with the molecular wire effect.^{25,26} In this respect, although the LOD values we obtained are in line with Hg²⁺ detection procedures based on the use of other conjugated sensing materials,^{3–5} the unprecedented use of aminoacidic functionalized PAE platform might disclose new opportunities in the sensoristic field.

Moreover it is worth noting that also the 'small molecules' **DLM** and **TLM** present very interesting sensing properties toward Hg²⁺ ions, in contrast to what was previously observed for other monomeric units of similar aminoacidic-functionalized conjugated systems.¹³

In conclusion, by the use of the Stille Pd-catalyzed synthetic protocol, we have prepared oligomeric and polymeric systems, characterized by poly(aryleneethynylene) (PAE) conjugated backbones carrying aminoacidic side arms. These systems resulted highly selective toward Hg²⁺ ions, a species of particular interest due to its dramatic toxicity and environmental impact.

To the best of our knowledge, the compounds **DLM**, **TLM**, and **DLP** described in this study represent the first example of sensing materials made of aminoacidic groups directly linked to PAE structures. Although more studies are necessary to understand the specific selectivity of these systems for mercury,³³ and, at this stage only studies in acetonitrile solvent are reported, while for practical applications aqueous environments are preferable, the enormous range of possibilities offered by the proper choice and assembly of aminoacidic and/or peptidic recognition sites with the PAE backbone confers to these systems a great potential for the development of innovative materials for applications in the analytical field.

The study of other PAE systems carrying different aminoacids and oligopeptides as side arms is ongoing.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.034.

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