

Analytical Methods

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4 **A chiroptical chemodosimeter for fast and specific detection of mercury(II) ions**
5
6 **in aqueous media**
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18
19 **Abstract**

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21 A simple salicylamide derivative, *N*-(*o*-propargyloxybenzoyl)-L-phenylglycinol, was
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23 designed and synthesized as a reactive probe for the detection of mercury ions. The
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25 chemodosimeter not only shows a fast and specific CD response to Hg²⁺ ions in
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27 aqueous media but also has well compatibility with some potential organic species
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29 including cysteine and the oxidant *N*-chlorosuccinide (NCS). The proposed
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31 chiroptical method may provide a useful alternative to chemosensors for detecting
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33 heavy-metal ions, which would cause luminescence quenching in chemosensor
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35 systems.
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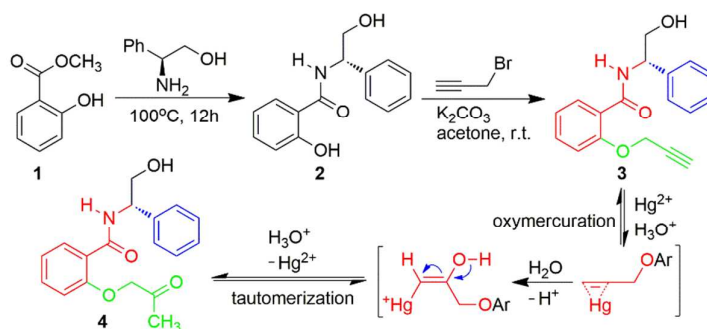
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41 Mercury is one of the most prevalent toxic heavy-metal pollutants in the
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43 environment because it is extensively distributed in water and soil.¹ A major source of
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45 human exposure comes from the contaminated nature water resource, it is therefore
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47 very important to explore powerful techniques for monitoring aqueous Hg²⁺ ions.²
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49 Traditional analytical techniques, including atomic absorption spectrometry,³ cold
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51 vapour atomic fluorescence spectrometry⁴ and inductively coupled plasma mass
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53 spectrometry,⁵ have been used for Hg(II) quantification. However, many of these
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4 methods require complicated, tedious sample preparation and/or sophisticated
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6 instrumentation. In contrast, optical methods are relatively simple, low-cost and easily
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8 operated and thereby more suitable for the on-site detection of mercury. Thus,
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10 numerous Hg(II)-sensors, most of them based on fluorescence and/or UV-vis
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12 spectroscopic techniques, have been developed during the past decades.^{2,6}
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16 On the other hand, the chiroptical property on scales ranging molecules to
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18 nanomaterials has been attracting considerable interest recently in chemical sensing
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20 studies.⁷ In theory, circular dichroism (CD) will occur for a chiral system constituted
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22 with two or more chromophores when their electric transition moments interact in a
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24 stereospecific fashion.⁸ The CD signal could be used to detect chemical which can
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26 disturb the coupling effect between chromophores. There are few reports in the
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28 literature for chiroptical sensing of metal ions in mixed aqueous media. For example,
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30 Yan *et al.* described a CD sensing method for detecting Hg²⁺ using a probe formed
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32 from small chiral organic molecules.⁹ Dai *et al.* reported a kind of chiroptical
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34 fluorescent sensing system, wherein Hg²⁺ could be differentiated from other metal
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36 ions, such as Zn²⁺, Cu²⁺, Ni²⁺, and Pb²⁺, exceptionally well through a combinatorial
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38 fluorescence-exciton-coupled CD recognition.¹⁰ Lately, Habata and co-workers
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40 demonstrated a Hg²⁺-sensing system based on the structures of complexes, using CD
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42 spectroscopy and a chiral bidentate ligand.¹¹ The observed Hg²⁺-specific CD spectral
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44 responses are assigned to the exciton coupling between the chromophoric units of an
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46 *in-situ* formed chiral coordination polymer.
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56 As a proof of concept, herein we designed and synthesized a simple salicylamide
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 4 derivative of L-phenylglycinol (**3**) with the expectation that the propargylated amide
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 6 can serve as a chiroptical probe for Hg(II) detection (Scheme 1). The proposed
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 8 strategy relies on the conformational changes of the dosimeter molecule resulting
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 10 from the oxymercuration of alkynyl ether and subsequent transformations in the
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 12 amide moiety. The results showed that the probe possesses a high selectivity for Hg²⁺
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 14 over other metal ions in aqueous media and a strong resistance to sulfur-containing
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 16 compounds like cysteine as well as to oxidants such as *N*-chlorosuccinide (NCS). To
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 18 the best of our knowledge, no reports on chemodosimetric approaches based on CD
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 20 spectroscopy have been made for mercury detection although quite a number of
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 22 developed fluorescent probes have led to considerable contributions to the metal
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 24 assay.^{6,11,12}
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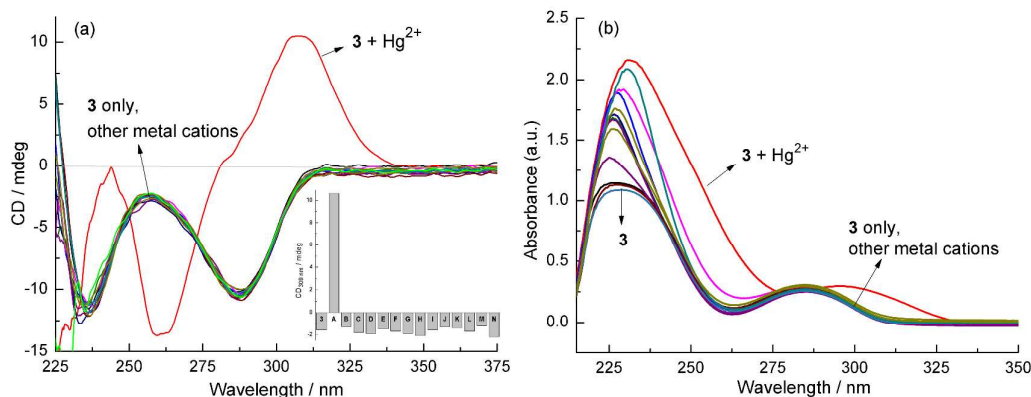


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Scheme 1 Synthetic route of the chiral chemodosimeter molecule **3** and the proposed
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 44 mechanism for the hydrolysis of **3** in the presence of Hg(II) ions.
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 48 The probe **3** was readily synthesized by two-step functional transformations in
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 50 moderate yield. The structures of the intermediate and goal product were confirmed
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 52 by NMR, FTIR, and mass spectral analysis (see ESI Fig. S1–S2). In this probe
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 54 molecule, a propargyl group as a selective reaction site was connected to the
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 56 salicylamide motif through an ether linkage, because the approach of oxymercuration
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of alkynyl ether has been successfully used to develop fluorescent chemodosimeters for Hg^{2+} .^{13,14} Meanwhile, the hydroxyl group would endow it with a good water compatibility.

As expected, the probe itself displays an evident CD spectrum with two negative Cotton effects (CE) associated with the absorption near 232 nm and 285 nm, respectively, which may be ascribed to the electric dipole-dipole coupling interaction of the two chromophoric units at the chiral center (Fig. 1a). The UV-vis and CD patterns are quite similar to that seen in the case of salicylamide of (*S*)- α -phenylethylamine.¹⁵ Upon titration of **3** with Hg^{2+} in a pH 4 buffer solution, the absorption band at 232 nm was remarkably increased and slightly red-shifted, while the band at 285 nm shifted to a higher wavelength position (~300 nm, Fig. 1b). Although there are only small variations in the UV-vis spectrum of **3** when it is titrated with Hg^{2+} , this metal ion produced a drastically different CD profile, featuring a positive CE at 309 nm and a negative CE at 260 nm. In contrast, other metal cations tested caused little effects on the probe's CD pattern. Also, under the same conditions a sharp depression in fluorescence was observed when mixing **3** with Hg^{2+} , while a small enhancement happened with most other ions (Fig. S3).



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4 **Fig. 1** (a) Circular dichroism and (b) UV-vis spectral changes observed at 25°C after
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6 10 min for a 1:6 mixture of **3** (10^{-4} M) and each of metal ions as their nitrate salts
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8 except for $\text{Hg}(\text{ClO}_4)_2$ in NaOAc-HOAc buffer (pH = 4) containing 1% methanol.
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10 Inset: a bar graph showing the relative CD intensity of the various ions in
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12 comparison with probe **3** (the peak height at 309 nm); alphabets from A to N denote
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14 Hg^{2+} , Ag^+ , Al^{3+} , Ca^{2+} , Zn^{2+} , Cd^{2+} , Cr^{3+} , Cu^{2+} , K^+ , Na^+ , Mg^{2+} , Mn^{2+} , Ni^{2+} , and Pb^{2+} ,
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16 respectively.
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21 The above-observed CD spectral changes for probe **3** are most likely due to the fact
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23 that Hg^{2+} promoted the hydration of its terminal alkyne group, thereby disturbing the
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25 spatial arrangement of chromophores at the stereogenic center. According to the
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27 well-established chemistry of Kucherov reaction,¹⁶ the alkyne hydrolysis proceeds
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29 through a three-step sequence involving oxymercuration, protonation, and
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31 tautomerization, as shown in Scheme 1. In a controlled hydrolysis experiment, ¹H
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33 NMR spectroscopy monitored the formation of the expected keto product **4** as judged
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35 from the negligible acetylenic proton signals at 2.50 ppm along with the appearance
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37 of distinctive peaks of the acetyl group around 2.15 ppm (Fig. S4). The observation is
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39 in agreement with that reported by the Choi's group.¹⁴
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47 Next, we optimized conditions for the Hg(II)-catalyzed hydrolysis of **3** in various
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49 buffers (for details, see Table S1 in the Supporting Information) at ambient
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51 temperature. It was found that when the pH varies from 3.5 to 8, the Cotton effect at
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53 309 nm in the induced CD spectra initially rises, reaches a maximum at pH 4 and then
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55 decreases with further increasing pH values (Fig. S5). However, no obvious spectral
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changes were observed for free **3** during the pH adjustment. Therefore, the subsequent CD signaling experiments were carried out in a pH 4 NaOAc-HOAc buffer solution containing 1% methanol as co-solvent.

As can be seen in Fig. 2, there is a significant time dependence of the hydrolysis of **3** in the presence of Hg^{2+} , which is a typical feature for the dosimetric detecting systems.^{12a-d,13} With the reaction, the CD amplitudes at both 309 nm and 260 nm increased and reached a platform rapidly (inset of Fig. 2). The observation along with the ^1H NMR analysis results (Fig. S3) let us to conclude that the hydrolysis was nearly complete within about 10 min at room temperature. It is noteworthy that the propargyl ether-type probe is much more reactive than its butynyl analog reported by Koide et al., for the latter a higher temperature (90°C) and longer time (1 h) are required to effect the oxycurmeration reaction.¹³

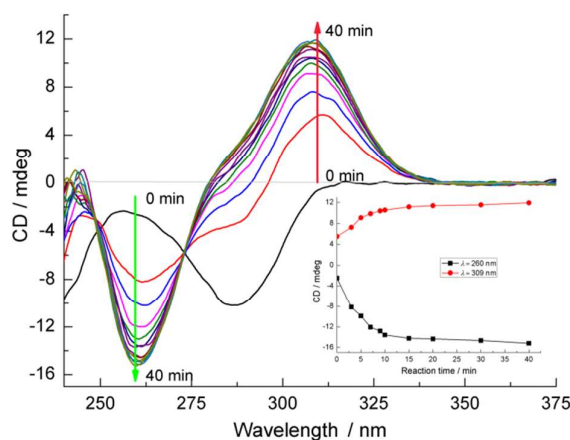


Fig. 2 CD spectral evolution of **3** (10^{-4} M) upon addition of Hg^{2+} (6 equiv.) in NaOAc-HOAc buffer (pH = 4, containing 1% CH_3OH) at 25°C. The inset shows the time-dependent CD intensities at 260 nm and 309 nm, respectively.

To examine the selectivity of probe **3** toward Hg^{2+} over environmentally relevant metal ions, we undertook competitive experiments. The selective experiments were

conducted in the presence of Hg^{2+} (6 equiv.) mixed with 10 equivalents of different metal species at room temperature. Overall, all the tested ions did not interfere substantially the detection of Hg^{2+} even at a higher concentration, as shown in Fig. 3,. Of the thirteen metal ions screened, high oxidation state metal ions such as Al^{3+} and Cr^{3+} have somewhat greater effects on the spectral response, which leading to a CD signal decrease by approximate 32% and 45%, respectively. One possible reason for this phenomenon could be related to the complexation of these co-existing ions with the hydrolyzed product of **3**.

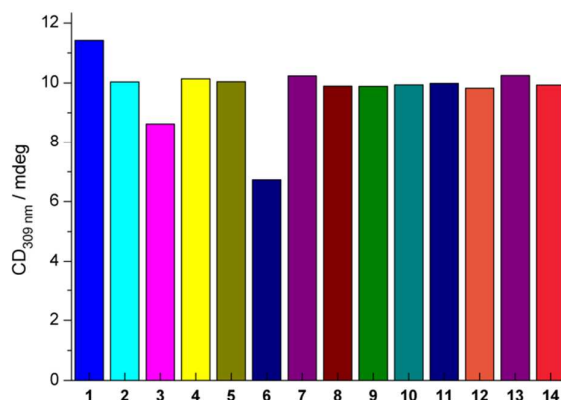


Fig. 3 CD intensity at 309 nm of **3** (10^{-4} M) upon addition of 6 equiv Hg^{2+} in the presence of various metal cations (10 equiv.) in NaOAc-HOAc buffer (pH = 4, containing 1% methanol). 1: none; 2: Ag^{+} ; 3: Al^{3+} ; 4: Ca^{2+} ; 5: Cd^{2+} ; 6: Cr^{3+} ; 7: Cu^{2+} ; 8: Mg^{2+} ; 9: Mn^{2+} ; 10: Na^{+} ; 11: Ni^{2+} ; 12: Pb^{2+} ; 13: Zn^{2+} , 14: K^{+} .

Fig. 4 shows the CD titration spectra of **3** with Hg^{2+} ions. In the titration experiments employing **3** of 100 μM , the negative CE amplitude at 285 nm gradually became smaller with the incremental addition of Hg^{2+} (0–10 equiv.), and eventually the original spectrum turned into a positive exciton-coupled circular dichroism spectrum. The CD signal intensity at 309 nm increased linearly in proportion to

concentration in the range of $0 \sim 6 \times 10^{-4}$ M. However, the probe displayed a higher detection limit (~ 0.12 mM), which may arise in part from the lower detection sensitivity of the equipment employed.

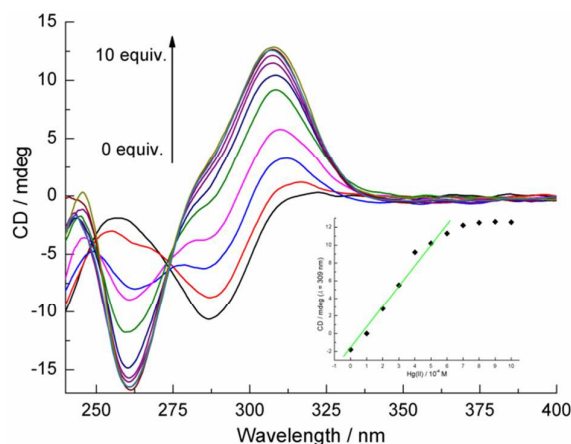


Fig. 4 CD spectra of probe **3** (10^{-4} M) in NaOAc-HOAc buffer solution (pH 4) containing 1% methanol upon the incremental addition of Hg^{2+} (0–10 equiv) at 25°C . Inset shows plots of the CD intensity at 309 nm as a function of Hg^{2+} concentration ($0 \sim 10 \times 10^{-4}$ M).

As the protocol of the US EPA indicates,¹⁷ the oxidative pretreatment of mercury-containing environmental samples is to transform various forms of organic and sulfur-bound mercury species to sulfur-free inorganic mercury(II). Therefore, anti-oxidation should be a highly desirable property for a Hg(II)-probe. In addition, Koide et al.¹³ found that the Hg–S bond could be oxidatively disrupted by using *N*-chlorosuccinimide (NCS) instead of Br–Cl as an oxidant. Thus, we exploited the spectral response of propargyl ether **3** to Hg^{2+} in the presence of NCS as those previously described.^{12b,13} From Fig. 5 we can see that the Hg(II)-induced CD signal intensities at 309 nm decreased to a certain extent when 10 equivalents

of NCS relative to **3** were added. However, this probe is basically stable against the organic oxidant while remaining its detectable CD response to the envisaged analyte at higher concentrations.

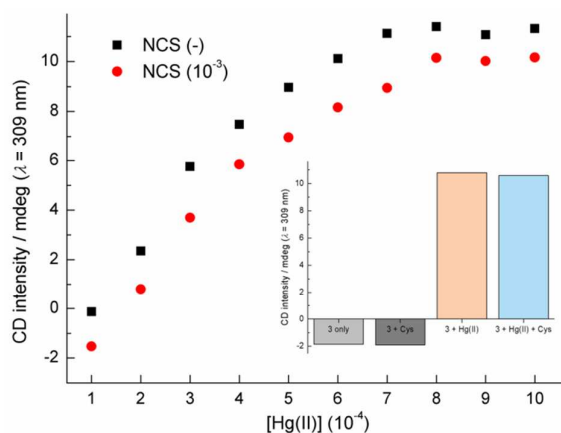


Fig. 5 Correlation between CD intensities at 309 nm and $[\text{Hg}^{2+}]$ in the absence and presence of NCS (10^{-3} M). Inset: Hg(II) detection in the presence of 6 equiv. cysteine relative to **3**. All measurements were performed in NaOAc-HOAc buffer (pH = 4, containing 1% CH_3OH) at 25°C after reaction for 10 min, $[\mathbf{3}] = 10^{-4}$ M, $[\text{Hg}^{2+}] = 6 \times 10^{-4}$ M.

It is well known that the compounds containing soft donor atoms possess high affinity for soft metal ions such as $\text{Hg}(\text{II})$,¹⁸ which would bring adverse effects to the metal detection in the case of probe **3**. With this in mind, we chose cysteine as a model pollutant to assess preliminarily whether the dosimetric detection method could be compatible with organic components in environmental samples. As inferred from Fig. 5 inset, only a slight effect to the CD spectra was observed upon addition of cysteine, suggesting that the oxymercuration reaction of **3** is specific toward mercury(II) ions, which allows the CD probe can recognize this ion even if the reaction solution was contaminated with sulfhydryl-containing species.

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4 In summary, we have developed a chiroptical method for the detection of Hg²⁺ ions
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6 in aqueous media using a simple chiral dosimeter molecule and CD spectroscopy.
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8 This water-compatible CD probe processes a rapid response for Hg²⁺ at ambient
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10 temperature with specific selectivity over other tested metal ions and a stronger
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12 tolerance to some organic species including NCS and cysteine. The proposed
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14 chiroptical procedure may provide a useful alternative to chemosensors for detecting
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16 heavy-metal ions, which would cause luminescence quenching in chemosensor
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18 systems. Further improvements of this method, specifically in regard to the detection
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20 sensitivity, are underway in our laboratory.
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26 **Acknowledgements:** The authors are grateful for the financial support provided by
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28 the National Natural Science Foundation of China (Grant No. 21474089).
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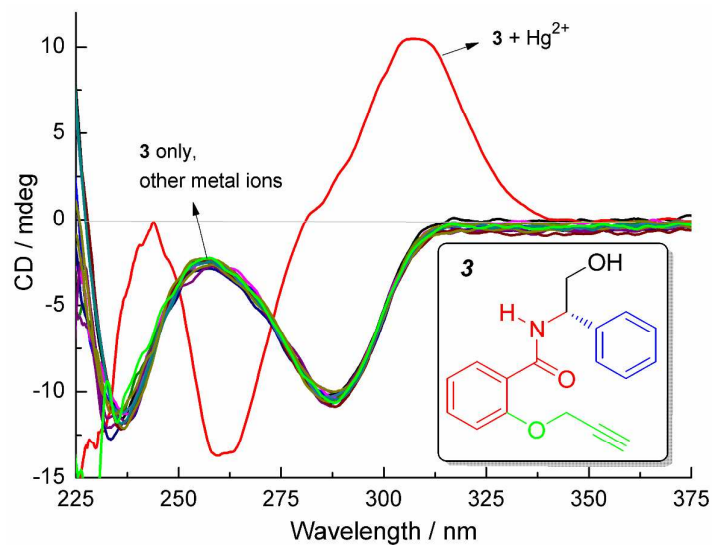
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49 18 Taking advantage of the high thiophilicity of the Hg²⁺ ion, the introduction of soft
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51 sulfur donors into a molecular scaffold is useful strategy of designing mercury
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53 sensors. See: Ref. 6a–c and 12f.
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Graphic abstract



A simple Hg^{2+} -specific CD probe has been developed taking advantage of an alkynyl ether oxymercuration mechanism for detection.