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Syntheses of Chiroptical Fluorescent Ligands for the Detection of Mercury

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Environmental protection and improvement is a very important issue facing all mankind. Mercury is an extraordinarily malicious pollutant. To curtail and remedy mercury contamination, powerful tools are needed to detect Hg(II). Mercury sensors need to be highly selective to prevent possible interference from other metal ions. They should also be sensitive since even very low concentrations of mercury can do a lot of harm. We intend to fabricate Hg(II) sensors with high selectivity and sensitivity by fusing a sulfur-containing chiral binding motif with a robust fluorophore. Syntheses of two of such ligands and their chiroptical responses to Hg(II) are reported and discussed in this work.

Key Words: Mercury sensors, Methionine, Chiral, Fluorescence, Mercury, Detection.

INTRODUCTION

Environmental protection and improvement is a very important issue facing all mankind. Mercury is an extraordinarily malicious pollutant, which has been of particular concern since the Minamata tragedy[1]. To curtail and remedy mercury contamination, powerful tools are needed to detect Hg^{2+}. Mercury sensors need to be highly selective to prevent possible interference from other metal ions. They should also be sensitive, since even very low concentration of mercury can do much harm. Fluorescent sensors have been pursued intensely due to the high sensitivity of fluorescence spectroscopy and microscopy. A number of switch-on fluorescent sensors for Hg^{2+} have been reported[2-5]. However, there are still needs for improvement in selectivity, sensitivity, photostability and water-solubility.

We intend to fabricate Hg^{2+} sensors with high selectivity through stereochemical control by incorporating a chiral sulfur-containing binding motif, such as methionine, into chiral podand and piperidine scaffolds. Chiral tripodal ligands are widely used in asymmetric synthesis and chiral discrimination[6]. Few have been used previously especially for metal sensing[7] and none has been used for Hg^{2+} sensing. Although it sounds outlandish to modulate achiral metal ion behaviour through chiral organic ligands, it is not without precedence[8,9]. One advantage of using chiral tripodal ligands is that they form complexes with metal ions with defined configuration and chirality can be harnessed to control the stereochemistry of metal complexes[6,10]. An important principle in the rational design of synthetic host molecules is using substitution and stereochemistry to reduce the populations of conformations unfavourable to binding[11,12]. By the same token, substitution and stereochemistry manipulation should be able to reduce the population of conformations favourable to undesirable binding. For example, because of its $5d^{10}6s^0$ configuration, Hg^{2+}, like Zn^{2+} and Cu^{+},[7,13] are not strongly influenced by constraints in its coordination configuration and ligand field. However d^9 metal Cu^{2+} prefers 4-coordinate square planar and 5-coordinate square pyramidal geometries over tetrahedral and trigonal bipyramidal geometries[14-16], while tetrahedral and trigonal stereochemistries are quite common in Hg(II) complexes[17-19]. Therefore, it is necessary to make a ligand that is a mismatch for Cu^{2+} to achieve better Hg(II)/Cu(II) selectivity. Rigidification is a common approach to preorganization[20] and it should also work in promoting preorganization toward mismatch. One can envision that it is possible to construct a ligand whose structure is chirally synchronized and mechanically rigidified so that its trigonal pyramidal configuration cannot be bent to a planar geometry. Hg^{2+}/Cu^{2+} selectivity might be achieved by engineering TPA-based rigidified chiral tripodal receptors[5,7,21]. By incorporating mercury-loving sulfur atoms, Hg^{2+}/Zn^{2+} selectivity might be obtained[2-4]. This stereochemical control approach might offer a new means to address the Hg^{2+}/Cu^{2+} selectivity. The principle behind the proposed approach should also be applicable to some other selectivity problems.

Many fluorescent sensors suffer from low contrast and therefore low sensitivity because the sensors themselves are quite fluorescent before they interact with the analytes[5,22,23]. Chiral sensors also have the same contrast and sensitivity problem if a chiroptical spectroscopic method such as circular dichroism is used for detection[24]. The sensitivity of chiral fluorescent sensors would be improved through differential circularly polarized fluorescence excitation (CPE) as the detection method since the chiral component's circular dichroism can be detected by the more sensitive fluorescence[25]. Circularly polarized fluorescence excitation is a new fluorescence-detected circular dichroism (FDCD) based approach originally advanced by the Dai *et al*.[24]. This approach can also reduce the high background seen in fluorescence and circular dichroism spectroscopy that originates from biological matrix such as fluorescent proteins and other species. However, the original naphthalene chromophore used in Zn^{2+} sensing was not ideal in that its absorbance and emission are in the UV region, rendering it less desirable. Its low fluorescence quantum yield rendered the sensors not sensitive enough. Furthermore, fluorescence polarization is a significant obstacle for wider application of the approach. Further development by using better fluorophores and newly available FDCD hardware may offer a way to reduce background interference and lay a solid groundwork for developing imaging tools to be used in conjunction with isotropic fluorescence and circular dichroism microscopy[26].

EXPERIMENTAL

All reagents and solvents were purchased from Aldrich or Fisher Scientific/Acros and used as received without further purification unless otherwise noted.

NMR spectra were recorded on a Bruker 400M Hz FT-NMR spectrometer. GC-MS spectra were recorded on an Agilent gas chromatography mass spetrometer (HP 5890-5732 with an electron impact mass spectrometer). LC-MS spectra were acquired on an Agilent LC/MSDTrap XCT system. ESI spectra were taken on the same instrument with direct injection. Fluorescence measurements were performed on a Hitachi F-2500 spectrophotometer. The solvent used in fluorescence studies was spectroscopic grade acetonitrile and $HgCl_2$ was used in Hg-sensing studies. In all measurements, 1 cm quartz cells were used. Excitation wavelength (slit width: 5 nm) was set at 450 nm and emission spectra (slit width: 5 nm) between 460 and 650 nm were recorded.

The detailed synthetic procedure of compound **3**, **4** and **5** were reported in literature and their characterization is reported below[27,28].

Compound 3 (5-bromoacenaphthalene): ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.80 (d, 1H, Ar-H), 7.68 (d, 1H, Ar-H), 7.57 (m, 1H, Ar-H), 7.35 (d, 1H, Ar-H); 7.16 (d, 1H, Ar-H), 3.45 (t, 2H, CH_2), 3.36 (t, 2H, CH_2). MS(EI): m/z M^+ calcd.: 232, found: 232.

Compound 4 (4-bromo-5-nitroacenaphthalene): MS(EI): M^+ calcd.: 277, found: 277.

Compound 5 (4-bromo-5-nitro-1,8-naphthalic anhydride): MS (EI): M^+ calcd.: 321, found: 321.

Compound 1: A solution of 4-bromo-5-nitro-1,8-naphthalic anhydride (compound **5**, 100 mg, 0.31 mmol) methionine methylester (1.63 g, 10 mmol) in 2-methoxylethanol (15 mL) was refluxed for 6 h. After the reaction was completed, the solvent was removed under vacuum. The residue was subjected to silica gel chromatography (CH_2Cl_2/EtOAC 1:1) and then preparative TLC to give the pure compound **1** (70 mg, 0.105 mmol) with a yield of 33 %. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.32 (d, 2H, Ar-H), 6.80 (d, 2H, Ar-H), 5.75 (t, 1H, $(CO)_2$N-CH), 4.55 (m, 2H, Ar-N-CH); 3.75 (s, 6H, COO-CH_3), 3.50 (s, 3H, COO-CH_3), 2.15-2.75 (m, 12H, CH_2), 2.05 (s, 6H, S-CH_3), 2.00 (s, 6H, S-CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm): 175, 172, 151, 133, 131, 121, 122, 107, 56.5, 56, 53, 51.5, 51, 32, 31, 30, 17. MS (ESI): m/z $(M + 1)^+$ calcd.: 666.2, found: 666.4.

Compound 7: MS(EI): m/z M^+ calcd.: 201, found: 201.

Compound 8: Na_2CO_3 (4.24 g, 0.04 mol) was added to a DMF solution (50 mL) of methionine methyl ester hydrochloride (2.00 g, 0.01 mol) and compound **7** (4.02 g, 0.02 mol). The mixture was stirred vigorously for 5 days. Then the solid was removed from the mixture by filtration. Solvent was removed from the filtrate under vacuum, resulting in a brown oil of compound **8** (4.05 g, 0.01 mol), yield 100 %. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65 (t, 2H, Py-H), 7.25 (d, 2H, Py-H), 7.05 (d, 2H, Py-H), 4.62 (s, 4H, Py-CH_2-O), 4.08 (dd, 4H, Py-CH_2-N-C (chiral)); 3.75 (s, 3H, COO-CH_3), 3.60 (t, 1H, N-CH), 2.60 (m, 2H, CH_2), 2.05 (t, 2H, S-CH_2), 2.00 (s, 3, S-CH_3). MS (ESI): m/z $(M + Na)^+$ calcd.: 428.2, found: 428.3.

Compound 10: The compound methanesulfonic chloride MsCl (2.0 mL, 3.0 g, 26 mmol) was dissolved in 25 mL of dry CH_2Cl_2 at -20 ºC. To this solution were added compound **8** (2.03 g, 5 mmol) and TEA (4.0 mL, 29 mmol) in 25 mL of dry CH_2Cl_2. The solution was stirred for 3 h before water was added. The mixture was extracted with three 30 mL portions of CH_2Cl_2. The organic layers were combined and dried over sodium sulfate. The solvent was removed under reduced pressure to give the desired compound **9** as light yellowish solid, which was used without further purification in the following procedure: (Caution!: Sodium azide and alkyl azides are explosive and toxic!) sodium azide (1.42 g, 21.8 mmol) and 18-crown-6 (0.23 g, 0.86 mmol) were dissolved in acetonitrile (10 mL) and stirred vigorously for 20 min at room temperature before all the compound **9** obtained earlier in 10 mL acetonitrile was added. The reaction mixture was thoroughly stirred for another 1 h. Filtration of the mixture and evapouration of the solvent yielded the crude product, which was subjected to silica gel chromatography (eluent $CHCl_3$) to afford the pure compound **10** (2.15 g, 4.73 mmol). Yield: 94.5 %. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65 (t, 2H, Py-H), 7.45 (d, 2H, Py-H), 7.15 (d, 2H, Py-H), 4.45 (s, 4H, Py-CH_2-N_3), 4.05 (s, 4H, Py-CH_2-N-C (chiral)); 3.75 (s, 3H, COO-CH_3), 3.65 (t, 1H, N-CH), 2.65 (m, 2H, CH_2), 2.05 (t, 2H, S-CH_2), 2.00 (s, 3, S-CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm): 173.2, 159.7, 155.9, 137.0, 122.2, 120.3, 61.9, 57.2, 55.5, 51.7, 46.7, 30.8, 29.1, 15.2. MS (ESI): m/z $(M + 1)^+$ calcd.: 456.2, found: 456.3.

Compound 11: To a solution of compound **10** (2.15 g, 4.73 mmol) in THF (9 mL) was added triphenylphosphine

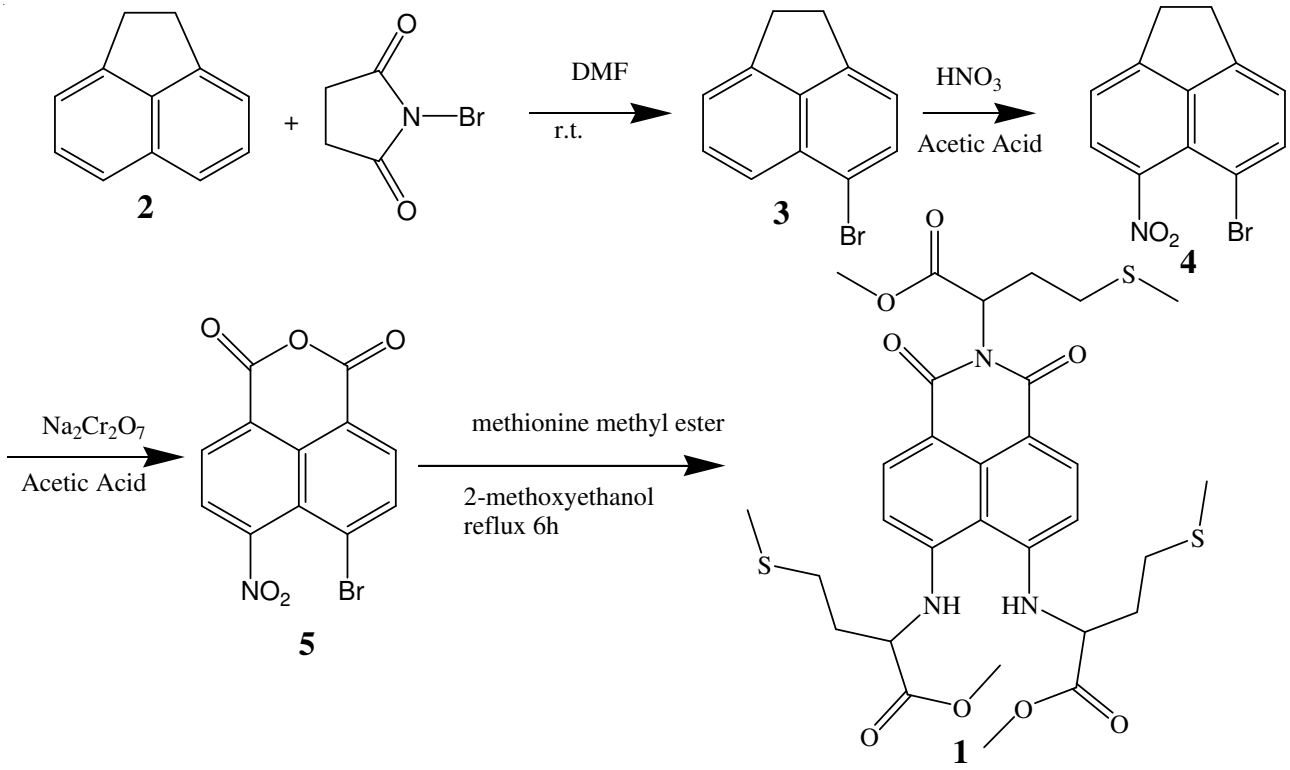
(2.88 g, 11.0 mmol) slowly. The reaction mixture was stirred at room temperature for 2 h and then H_2O (0.40 mL, 22 mmol) was added. The stirring was continued for another 16 h. The solvent was removed under vacuum and the residue was partitioned between ether (3 × 30 mL) and 2 % HCl (50 mL). The organic portions were discarded and the aqueous layer was basified carefully with saturated NaOH solution until the pH reached 10. The basified aqueous layer was then extracted with methylene chloride (3 × 30 mL). The organic portions were combined and dried over Na_2SO_4 before the solvent was evapourated to yield the crude product, which was subsequently purified by silica gel chromatography using EtOAc and 20 % methanol in EtOAc as gradient eluent to give pure compound **11** (1.55 g, 3.85 mmol) with a yield of 81.4 %. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.52 (t, 2H, Py-H), 7.25 (d, 2H, Py-H), 6.95 (d, 2H, Py-H), 3.93 (s, 4H, Py-CH_2-N(primary amine), 3.85 (s, 4H, Py-CH_2-N-C (chiral)); 3.62 (s, 3H, COO-CH_3), 3.55 (t, 1H, N-CH), 2.90 (b, 4H, NH_2), 2.50 (m, 2H, CH_2), 2.00 (t, 2H, S-CH_2), 1.90 (s, 3, S-CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm): 173.2, 160.1, 158.8, 136.8, 121.7, 119.5, 61.8, 57.3, 52.0, 47.0, 30.8, 29.1, 15.2. MS (ESI): m/z (M + 1)$^+$ calcd.: 404.2, found: 404.3.

Compound 13: To a stirring solution of compound **11** (100 mg, 0.25 mmol) in 5 mL of methanol was added fluorescamine (compound **12**, 200 mg, 0.72 mmol) in four portions over 2 h. Then the solvent was removed and the residue was subjected to silica gel chromatography using EtOAc and 20 % methanol in EtOAc as gradient eluent to give pure compound **13** (160 mg, 0.17 mmol) with a yield of 67 %. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.75 (s, 2H, Ar-H), 7.55 (d, 2H, Ar-H), 7.70 (d, 4H, Py-H), 7.45 (m, 2H, Ar-H), 7.39 (m, 4H, Ar-H), 7.25 (m, 6H, Ar-H), 7.20 (d, 2H, Ar-H), 7.10 (d, 2H, Ar-H), 6.75 (d, 2H, =C-H), 4.30 (s, 4H, Py-CH_2-N-Ar), 3.95 (s, 4H, Py-CH_2-N-C(chiral)), 3.75 (s, 3H, COO-CH_3), 3.74 (t, 1H, N-CH), 2.60 (m, 2H, CH_2), 2.02 (s, 3, S-CH_3), 1.95 (t, 2H, S-CH_2), ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm): 191.7, 173.1, 168.0, 165.6, 159.5, 154.1, 137.4, 134.6, 131.0, 128.9, 128.3, 127.1, 126.2, 124.5, 122.2, 120.7, 109.7, 94.4, 60.3, 57.1, 51.5, 50.1, 30.7, 29.1, 15.2. MS (ESI, negative mode): m/z (M - 1)$^+$ calcd.: 958.3, found: 958.4.

RESULTS AND DISCUSSION

To make selective fluorescent sensors for Hg^{2+}, our first strategy is to incorporate Hg-loving sulfur atom(s) into the binding moieties of the sensor(s). To achieve this, lignad **1** was constructed with several methionine moieties, which contains Hg-loving sulfur atoms[2,23]. As is shown in **Scheme-I**, acenaphthalene (compound **2**) was reacted with N-succinimide bromide to afford[27] compound **3**, which was subsequently treated with a mixture of fuming nitric acid and glacial acetic acid to give[28] compound **4**. Oxidizing compound **4** with $Na_2Cr_2O_7$ in glacial acetic acid afforded compound **5**, which was reacted with methionine methyl ester to give the target ligand **1**. Although compounds **2** through **5** were reported previously[27,28], their NMR and mass spectroscopy data is reported for the first time in this paper (see the experimental section).

As shown in Fig. 1, the new ligand **1** emitted green fluorescence (λ_{em} = 507 nm) when excited at 450 nm. The visible region excitation wavelength is long enough for this ligand to be more desirable than those sensors that have to be excited by the harmful UV light. Its green fluorescence emission is also desirable because green light is most-sensitive to human eyes. Upon addition of Hg^{2+}, there is a response in the form of



Scheme-I: Synthesis of ligand **1**

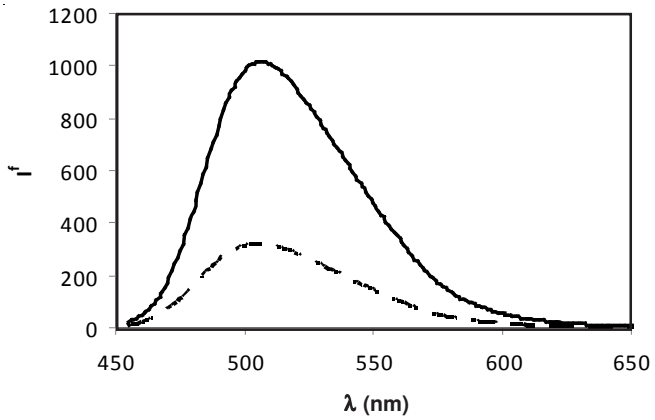
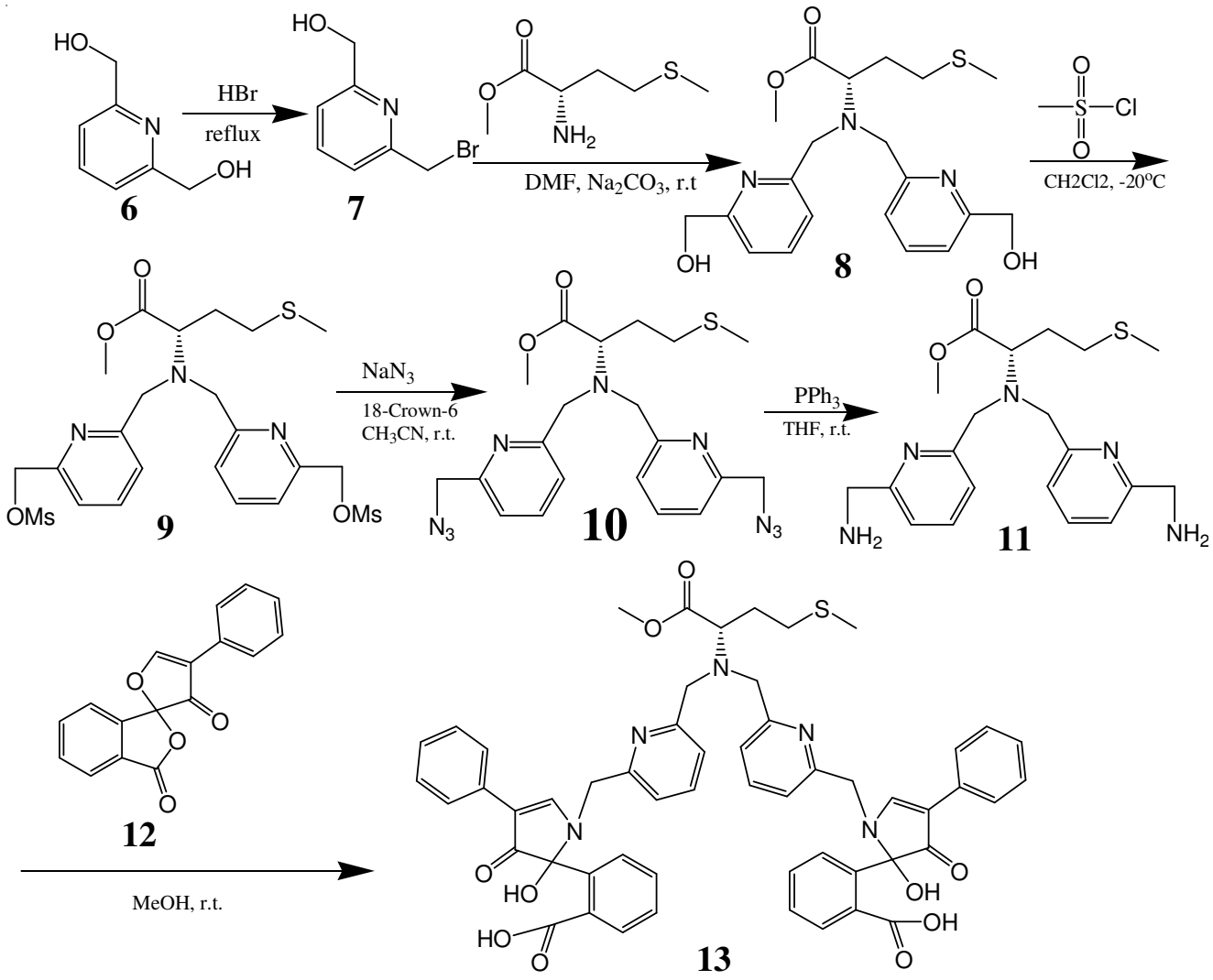


Fig. 1. Fluorescence of ligand **1** (solid line) and its response to Hg^{2+} (broken line). Ex: 450 nm; solvent: acetonitrile; concentration: 1 μM

a decrease in fluorescence signal, which means this ligand can be used as a fluorescent sensor for Hg^{2+}.

To make more selective "turn-on" fluorescent sensors for Hg^{2+} and sensors more suitables for CPE studies, sulfur atom(s) needs to be into the binding moieties which can impose a trigonal bipyramidal coordination geometry. To this end, tripodal podand ligand **13** was constructed with a methionine moiety, which contains Hg-loving sulfur and two arms containing 2-picolylamine moieties (**Scheme-II**). First, 2,6-*bis*-(hydroxymethyl)pyridine (compound **6**) was treated with 47 % HBr to give compound **7** in 50 % yield, which was higher than published yield[29]. Compound **7** was coupled with methionine methyl ester under very mild conditions to afford the dialkylated tripodal compound **8**, whose two alcohol functional groups were activated with mesyl chloride to give compound **9**. Reacting compound **9** with sodium azide afforded the azidyl compound **10**, which was subsequently reduced by triphenylphosphine to yield compound **11**. Fluorescamine (compound **12**) reacted easily with the primary amines in compound **11** to afford the targeted tripodal fluorescent chiral ligand **13**.

In this ligand **13**, the tripodal scaffold was tagged with two moieties of the fluorophore fluorescamine. Fluorescamine was chosen because it can react easily with primary amines to yield compounds with bright green fluorescence, which is most sensitive to human eyes. Fluorescamine has been attached to peptides to give products which showed very interesting fluorescence[30] and circular dichroism properties[31].



Scheme-II: Synthesis of the fluorescent chiral tripodal ligand **13**

Model studies using analogs of this ligand showed that in the presence of Hg(II), the fluorescence of these sensor would be turned on because Hg(II) coordination has proven to be able to disrupt the photoinduced electron transfer quenching of the emission from several fluorophores by the lone pair of the amino nitrogen and sulfur atoms[3,7]. It was also expected that Hg(II) complexes with this methionine-containing podand ligand would give a positive couplet in ECCD. If a metal, such as Cu(II) or Zn(II) does change the CD of the ligands containing the methionine moiety, it poses the risk of false-positive even when the fluorescence enhancement is substantially lower than that induced by Hg(II). However, if the methyl ester in ligand **13** is hydrolyzed, it is predicted that Cu(II) or Zn(II) would give opposite ECCD (unbroken curve) signal to that of Hg(II) complex because Cu(II) or Zn(II) has higher preference for -COO- than S-atom of the methionine (Fig. 2)[32], the false-positive from the fluorescence approach can be excluded. Fluorescence and circular dichroism studies of ligand **13**, however, this does not show the expected behaviour. There was little change in fluorescence of circular dichroism upon exposing the compound to Hg(II). We are investigating the mechanism and will improve our design accordingly.

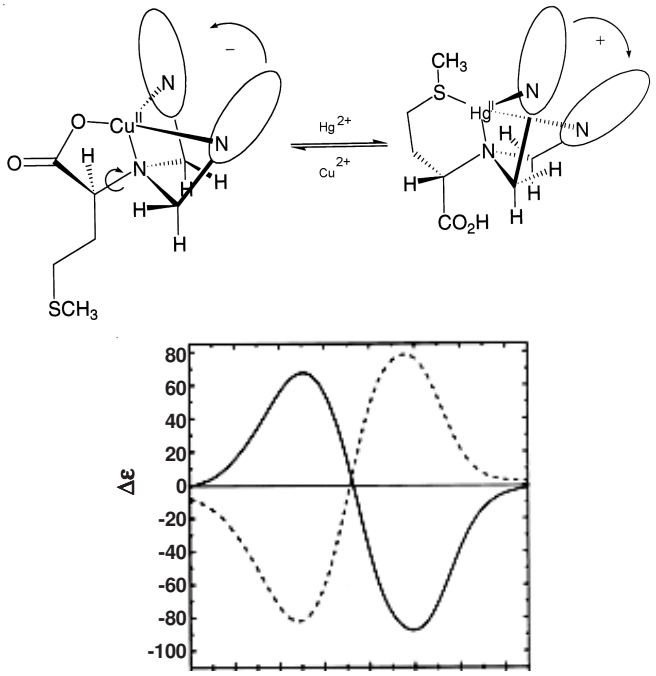


Fig. 2. Expected chiroptical responses of **13** to different metal ions

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