Introduction

The fabrication and design of new chemosensors capable of selective and sensitive detection of toxic heavy metal ions have attracted considerable attention because of their wide use in biological, analytical, and environmental fields. Among various heavy metal ions, Hg$^{2+}$ has been intensively studied owing to its toxic nature and threat to human life and ecosystems. Mercury pollution has sparked interest in the development of new tactics to monitor Hg$^{2+}$ in biological and environmental samples. The Environmental Protection Agency (EPA) standard for the maximum allowable level of inorganic Hg$^{2+}$ in drinking water is 2 ppb. Thus, chemosensors for the sensitive detection of Hg$^{2+}$ by effective analytical methods are of high interest. Various techniques are reported for the recognition of Hg$^{2+}$; however, fluorescence spectroscopy has gained importance over other techniques because of its simplicity, quick response, and high sensitivity.

Abstract

A novel fluorescent chemosensor containing a coumarin–thiourea unit and a β-N-glycosyl moiety is designed, synthesized, and characterized by $^1$H NMR, $^{13}$C NMR, and elemental analysis. The chemosensor can selectively and efficiently detect Hg$^{2+}$ in CH$_3$CN with a detection limit of 2.6 μM.

Keywords

coumarin, fluorescent sensor, N-glycosyl, selectively, thiourea

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as high hydrophilicity, flexible stereo-structures, low toxicity
and biological compatibility,11,12 and are usually called fluo-
rescent glycoconjugates. Many fluorophores—such as rhoda-
mine,13,14 fluorescein,15 dicyanomethylene 4H-pyran,16
tetraphenylethylene,17 1,8-naphthalimide,18 tetraphenyleth-
ylene (TPE),19 and boron-dipyromethene (BODIPY)20—
have been conjugated with carbohydrates, producing
structurally and functionally diverse fluorescent glycocon-
ters, which can recognize different ions or proteins.

Recently, we reported sugar–rhodamine21 and sugar–cou-
marin22 fluorescent probes for detecting Hg2+ and bovine
serum albumin (BSA), respectively. Investigations revealed
that introducing a carbohydrate could significantly improve
the hydrophilicity and selectivity. Inspired by these results,
we report herein the design of a novel coumarin-based β-N-
glycoside (GC1), which is connected by a thiourea group. As
the sulfur atom in thiourea showed very good affinity toward
mercury recognition23,24 the sensor was used to detect Hg2+.
The UV-Vis and fluorescence spectra were recorded to eval-
uate the detecting abilities of the chemosensor.

Results and discussion

The strategy undertaken for the synthesis of the target sensor
GC1 is shown in Scheme 1 and involves the preparation of
precursors 2 and 5. 2,3,4,6-β-O-acetyl-α-L-glucopyranosyl
isothiocyanate (2) was synthesized from glucopyranosyl
bromide (1).25 3-Amino-7-diethylaminochromene-2-one
(5) was synthesized in two steps according to the litera-
ture.26 GC2 was obtained in 87% yield by the condensation
of 2 and 5, finally, cleavage of the acetyl groups in GC2
afforded sensor GC1.

To our delight, the sensor demonstrated comparatively
good water solubility due to the polyhydroxy carbohydrate
moiety, and it was fully soluble in 100% HEPES buffer
solution. We initially examined the fluorescence spectral
response of GC1 (50 μM) with and without addition of 10
equiv. of Hg2+ ions (500 μM) in 100% HEPES buffer solu-
tion and CH3CN, respectively. No discernible changes in
the emission intensities were observed at 648 nm in 100%
HEPES buffer (Figure 1(a)). However, a significant

Scheme 1. Synthesis of GC1: (a) KSCN, TBAB, MeCN; (b) CH3(NO2)COOEt, n-BuOH; (c) SnCl2, H2O, HCl; (d) THF, RT;
(e) CH3OH, CH3ONa.

Figure 1. Fluorescence spectra of GC1 (50 μM) in the absence and presence of Hg2+ (500 μM) in 100% HEPES buffer solution (a)
and CH3CN (b). λex: 385 nm and slit width (5, 5).
increase in the fluorescence at 670 nm occurred in CH₃CN with Hg²⁺ ions, along with a bathochromic shift (625–670 nm) (Figure 1(b)). The good water solubility resulted in a higher background fluorescence intensity in 100% HEPES buffer, and this did not allow detection ion of Hg²⁺. The changes after adding Hg²⁺ in CH₃CN were accompanied by a striking color change from green to bright yellow, while the color of the solution of GC1 on addition of other ions did not change (see inset, Figure 1(b)).

In order to verify the selectivity of compound GC1 for Hg²⁺, the UV-Vis spectral responses of GC1 (50 μM) were examined in CH₃CN and with various nitrate salts of metal cations, such as Ag⁺, Bi³⁺, Cu²⁺, Co²⁺, Ca²⁺, Cd²⁺, Pb²⁺, Fe³⁺, Na⁺, and Hg²⁺ in CH₃CN.

At the same time, working on the fluorescence spectra titration data from Figure 4(a), a plot of the fluorescence intensity of GC1 against the concentration of Hg²⁺ indicated a good linear relationship in the range of 0–400 μM (Figure 4(b)). The linear equation was \( y = 1.93971E^6 + 29,245.5x \) \( R² = 0.99202 \); the detection limit was calculated according to the literature²⁷ from this titration experiment, and it was found to be 2.6 μM, which represents a moderate level. Moreover, the fluorescence titration experiment clearly revealed that there is a linear relationship between the emission intensity and the concentration of Hg²⁺ (up to 4 M), which is advantageous for quantitative analysis of [Hg²⁺].

As shown in Figure 5, when 10 equiv. of Hg²⁺ were added, the fluorescence intensity of GC1 in CH₃CN was enhanced and reached a maximum within 8 min. Furthermore, the fluorescence intensity of the GC1 solution with Hg²⁺ ions remained steady over the following 30 min. The rapid signaling of Hg²⁺ ions by GC1 could well satisfy the requirement of real-time and quick detection.

The proposed mechanism for detecting Hg²⁺ ions requires that the S atom in thiourea binds with thiophilic Hg²⁺ and promotes an Hg²⁺-induced desulfurization of the thiocarbonyl moiety, leading to an increase in the intramolecular charge transfer character of the excited-state coumarin moiety, which results in the fluorescent enhancement (Scheme 2). The hydrolytic conversion of thioureas into ureas catalyzed by certain metal ions is known to be very efficient. In the present case, the conversion was efficient exclusively with Hg²⁺ ions in acetonitrile.

The proposed transformation was confirmed by ¹³C NMR measurements. On comparing the ¹³C NMR data of GC1 with that of GC1 in the presence of Hg²⁺ (Figure 6), we found that the C=S group at 180 ppm had vanished and...
Figure 4. (a) The fluorescence spectra of GC1 (50 μM) in the presence of various concentrations of Hg$^{2+}$ (0–1.2 mM) in CH$_3$CN, λ$_{ex}$: 385 nm, slit width (5, 5). The inset shows the fluorescent intensity enhancement as a function of [Hg$^{2+}$]. (b) The linear fit plot of the GC1–Hg$^{2+}$ complex as a function of Hg$^{2+}$ concentration showing a linear progression coefficient of 0.9987 in the range of 0–400 μM.

Figure 5. Time response of GC1 toward 10 equiv. of Hg$^{2+}$ in CH$_3$CN. [GC1] = 50 μM.

that the C=O group at 160 ppm was present during the desulfurization process from GC1 to 6.

**Conclusion**

In summary, we have designed and prepared a novel $N$-glycosyl coumarin-based fluorescent sensor GC1, which exhibited high selectivity toward Hg$^{2+}$ over other metal ions. The sensor was based on the thiourea moiety as a binding site and a coumarin moiety as a signaling group. Upon addition of 10 equiv. of Hg$^{2+}$, the color changed from green fluorescence to bright yellow. The sensor showed a lower detection limit of 2.6 μM, which indicated that it could potentially be useful as a probe for monitoring Hg$^{2+}$ levels in physiological and environmental systems. This work may contribute to the development of more efficient and more useful Hg$^{2+}$ probes.
Experimental section

General

All chemicals were of analytical grade and were purchased from commercial sources and used without further purification. Solvents were dried before use over 3 or 4 Å molecular sieves according to standard procedures. Reactions were monitored using thin-layer chromatography on silica gel–coated thin layer chromatography (TLC) plates, and the detection was performed by UV absorption (254 nm) where applicable, and by spraying with 50% sulfuric acid in ethanol followed by charring at 150 °C. Column chromatography was performed on silica gel (200–300 mesh) using petroleum ether (60–80 °C) or petroleum ether–ethyl acetate mixtures as eluents. 1H and 13C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Elemental analyses were obtained using a Vario EL III elemental analyzer. The UV-Vis absorption and fluorescence spectra were recorded with a TU-1901 spectrophotometer and a Fluoromax-4 spectrofluorometer, respectively.

2,3,4,6-β-O-acetyl-α-d-glucopyranosyl isothiocyanate (2)

To a solution of 4 Å molecular sieves in anhydrous CH3CN (50 mL) was added tetrabutylammonium bromide (0.4 g, 1.2 mmol) and KSCN (0.2 g, 2.4 mmol). The mixture was stirred at room temperature (RT) for 2 h. Next, 2,3,4,6-penta-O-acetyl-α-d-glucopyranosyl bromide (0.5 g, 1.2 mmol) was added to the reaction mixture, which was stirred at RT for another 2 h. After completion, the reaction mixture was filtered, the solvent was removed under reduced pressure and the product was purified by silica gel column chromatography to afford 2,3,4,6-penta-O-acetyl-α-d-glucopyranosyl isothiocyanate (2) as a white powder (0.3 g, 60%), m.p. = 111.7–112.5 °C (lit.28 m.p. = 112–113 °C). 1H NMR (400 MHz, CDCl3): δ 6.64 (d, J = 4.0 Hz, 1H), 5.59 (t, J = 9.7 Hz, 1H), 5.19 (s, 1H), 4.87 (dd, J = 10.0, 4.0 Hz, 1H), 4.35 (d, J = 10.8 Hz, 2H), 4.16 (d, J = 10.6 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H). 13C NMR (100 MHz, CDCl3): δ 170.50, 170.04, 169.17, 168.99, 144.25, 83.49, 77.20, 74.06, 72.49, 71.89, 67.68, 61.50, 20.64, 20.47. Anal. calcd for C15H19NO9S: C, 46.27; H, 4.92; N, 3.60; S, 8.23; found: C, 46.54; H, 4.89; N, 3.56; S, 8.32.

7-Diethylamino-3-nitrocholene-2-one (4)

A mixture of 4-diethylaminosalicylaldehyde (0.4 g, 2.1 mmol), ethyl nitroacetate (0.3 mL, 3.0 mmol), piperidine (0.1 mL) and glacial acetic acid (0.2 mL) in n-BuOH (20 mL) was refluxed for 9 h. An orange solid formed while cooling which was filtered and washed with n-BuOH (2 × 10 mL) and finally dried under vacuum to give an
orange solid (0.5 g, 90%), m.p. = 169.2–169.8 °C (lit. 29 m.p. = 170 °C). 1H NMR (400 MHz, CDCl3): δ 8.70 (s, 1H), 7.42 (d, J = 7.1 Hz), 6.37 (d, J = 2.3 Hz, 1H), 6.24 (d, J = 2.2 Hz, 1H), 5.77 (s, 1H), 4.62 (d, J = 6.6 Hz, 1H), 3.99–3.85 (m, 1H), 3.74 (d, J = 12.2, 5.1 Hz, 1H), 3.55–3.41 (m, 9H), 1.52–1.20 (m, 8H). 13C NMR (100 MHz, MeOD): δ 181.49, 161.57, 157.49, 151.57, 134.93, 119.52, 114.32, 108.04, 104.89, 97.30, 79.65, 77.94, 69.91, 68.33, 61.41, 44.15, 29.33, 11.60. Anal. calcd for C13H14N2O4: C, 59.54; H, 5.38; N, 10.68; found: C, 59.36; H, 5.50; N, 10.58.

3-Amino-7-diethylamino-chromene-2-one (5)

The compound 5 was synthesized according to the literature.30 To a 100-mL round-bottomed flask were added SnCl2 (1.5 g, 7.9 mmol) and 15% HCl (5 mL). 7-Diethylamino-3-nitrochromene-2-one (4) (0.5 g, 1.9 mmol) was added portion-wise, and the solution was stirred at RT for 6 h. A 5-M solution of NaOH was added to neutralize the excess acid. The aqueous phase was extracted with ethyl acetate (3 × 15 mL). The combined organic layer was dried over anhydrous Na2SO4, filtered and evaporated to dryness. Purification by column chromatography gave a yellow solid, which was directly used for the next step without characterization.

**Synthesis of GC2**

To a solution of 3-amino-7-diethylaminochromene-2-one (5) (0.10 g, 0.43 mmol) in THF (10 mL) was added 3-Amino-7-diethylamino-chromene-2-one (3) (0.05 g, 63%), orange powder, m.p. = 105.2–105.7 °C. 1H NMR (400 MHz, CDCl3): δ 7.30 (d, J = 8.9 Hz, 1H), 6.75 (s,

**Detection limit**

The detection limit was calculated on the basis of the fluorescence titration. The fluorescence emission spectrum of GC1 was measured 10 times. To measure the slope, the fluorescence emission was plotted as a function of the concentration of Hg2+ from the titration experiment. The detection limit was then calculated using the following equation: Detection limit = 3σ/k, where σ is the standard deviation of the blank measurement, and k is the slope between the fluorescence emission intensity versus [Hg2+].

**Declaration of conflict interests**

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