Tetrahedron 72 (2016) 8479–8485

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Cysteine and homocysteine chemosensor based on photochromic diarylethene with fluorine

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ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form
1 November 2016
Accepted 7 November 2016
Available online 9 November 2016

Keywords:
Photochromic diarylethene
Colorimetric chemosensor
Fluorene group
Cysteine and homocysteine
Recognition of amino acid

ABSTRACT

A new photochromic diarylethene with formyl and fluorene groups, 1-[2-methyl-5-(9,9-diethyl-fluoren-2-yl)-3-thienyl]-2-[(2-methyl-5-formyl)-3-thienyl]perfluorocyclopentene, has been synthesized and characterized with single-crystal X-ray diffraction. Due to the interaction between the formyl group in its ring-closed isomer with cysteine/homocysteine, there was an evident color change in its absorption spectrum from blue to pale yellow and light brick-red upon addition of cysteine and homocysteine, respectively. Moreover, it was revealed that no obvious interference was observed during the titrations with the mixtures of cysteine/homocysteine and other amino acids. These results indicated that the diarylethene could be used as a colorimetric sensor to recognize cysteine/homocysteine with high selectivity.

1. Introduction

It has been well known that amino acids are significantly important to the physiological processes.[1] Among these amino acids in biological systems, the thiol-containing amino acids, such as cysteine (Cys) and homocysteine (Hcy), play crucial roles in maintaining the biological redox homeostasis in reversible redox reactions, and their levels have already been directly linked to some diseases and cancers.[2] For example, in the human blood plasma, Cys deficiency is found in many health problems including retarded growth in children, hair depigmentation, lethargy, liver damage, muscle and fatloss, skinlesions and edema. Abnormal level of Hcy is also related to many diseases, such as slowed growth, Alzheimer's disease and cardiovascular disease.[3] Therefore, it is very important to develop efficient methods to detect and quantify Cys/Hcy in physiological media for both academic studies and clinic applications.[4] Up to date, several analytical techniques have been designed to detect Cys/Hcy, such as high-performance liquid chromatography (HPLC),[5] capillary electrophoresis (CE),[6] electrochemical assay,[7] UV/Vis spectroscopy,[8] mass spectrometry,[9] fluorescence spectroscopy[10] and colorimetric assay.[11] Among these methods, colorimetric indicators are widely studied because of their capability to detect Cys/Hcy through naked-eye.[12] Strongin et al. reported a xanthene dye with a formyl group, which yielded thiazolidines or thiazinanes through the reaction with Cys/Hcy, resulting in a color change.[13] Li et al. also reported many other colorimetric sensors from azo derivatives, which reacted with the Cys or Hcy to afford a five or six-membered ring, resulting in a color change.[14]

Typically, photochromic compounds can suffer reversible change between their open-ring and closed-ring isomers with different spectroscopic properties under the external stimuli of light.[15] Many properties will change in the photoisomerization, such as spectroscopy,[16] refractive index,[17] oxidation/reduction potential,[18] magnetic properties[19] and chiroptical properties.[20] Up to present, the most promising photochromic compounds are piarylethene derivatives with heteroaromatic rings because of their bearing excellent thermally irreversible stability, high photoisomerization quantum yields and remarkable fatigue resistance.[21] For example, some diarylethene chemosensors have already been reported to detect metal ions and fluoride, as well as to image living cells.[22] However, the diarylethene-based colorimetric chemosensor to detect Cys/Hcy has been rarely reported.

In this study, it was found that the synthesized diarylethene derivative, 1-[2-methyl-5-(9,9-diethyl-fluoren-2-yl)-3-thienyl]-2-

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http://dx.doi.org/10.1016/j.tet.2016.11.019
0040-4020/

[(2-methyl-5-formyl)-3-thienyl]perfluorocyclopentene (**1O**), could undergo a reversible photoisomerization reaction in DMF. The ring-closed isomer of compound **1O** could efficiently recognize Cys and Hcy with high selectivity through the significant changes in color and absorption features. The chemical structure and photo-isomerization of the diarylethene are shown in Fig. 1.

2. Experimental

2.1. General methods

All solvents were of spectro-grade and purified through distillation prior to use. NMR spectra were collected on a Bruker AV400 (400 MHz) spectrometer with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as an internal standard. UV–Vis spectra were measured on an Agilent 8453 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. Infrared spectra (IR) were collected on a Bruker Vertex-70 spectrometer. Elemental analysis was carried out on a PE CHN 2400 analyzer. Melting point was measured on a WRS-1B melting point apparatus. Photo-irradiation experiments were performed with an SHG-200 UV lamp, Cx-21 ultraviolet fluorescence analysis cabinet and a BMH-250 visible lamp.

2.2. Synthesis

The synthesis of 1-[2-methyl-5-(9,9-diethyl-fluoren-2-yl)-3-thienyl]-2-[(2-methyl-5-formyl)-3-thienyl]perfluorocyclopentene (**1O**) was shown in Fig. 2.

2.2.1. Synthesis of 2-bromo-9,9-diethyl-fluorene (3)

To a stirred solution of compound **2** (4.90 g, 20.00 mmol) in DMSO (50 mL), NaOH aqueous solution (2 mL, 50% ω/ω) was added. After 5 min of stirring, bromoethane (4.80 g, 44.00 mmol) was added slowly. The reaction mixture was stirred continuously for 10 h at room temperature. Then, 100 mL ethyl acetate was added into the mixture, washed with water to remove DMSO, dried over $MgSO_4$, filtered and the solvent was removed. The residue was purified with chromatography on silica gel (eluting with petroleum ether) to get **3** (4.86 g, 16.20 mmol) as a white solid with 81% yield. M.p. 52–53 °C; ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.31 (t, 6H, J = 8.0 Hz), 1.99–2.04 (m, 4H), 7.33 (s, 3H), 7.46 (d, 2H, J = 4.8 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.66–7.68 (m, 1H).

2.2.2. Synthesis of 3-bromo-5-(9,9-diethyl-fluoren-2-yl)-2-methylthiophene (5)

Compound **5** was prepared from 3-bromo-2-methyl-5-thienylboronic acid (**4**) (3.00 g, 13.50 mmol) and compound **3** (3.68 g, 12.25 mmol) in the presence of $Pd(PPh_3)_4$ (0.40 g, 0.35 mmol) and Na_2CO_3 (6.50 g, 61.25 mmol) in THF (80 mL). After 20 h of refluxing, the product was extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, filtrated and evaporated. The crude product was purified on silica gel column with petroleum ether as the eluent. 3.54 g of compound **5** was obtained as a white solid in 73% yield. M.p. 130–131 °C; ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.26 (t, 6H, J = 8.0 Hz), 1.94–2.00 (m, 4H), 2.36 (s, 3H), 7.10 (s, 1H), 7.24–7.28 (m, 3H), 7.38–7.43 (m, 2H), 7.60–7.63 (m, 2H).

2.2.3. Synthesis of [2-methyl-5-(9,9-diethyl-fluoren-2-yl)-3-thienyl]perfluorocyclopentene (6)

Into a stirred solution of compound **5** (2.32 g, 5.80 mmol) in anhydrous THF, n-BuLi (2.55 mL, 2.4 mol L^{-1}) was injected at −78 °C under an argon atmosphere. The solution was continuously stirred for another 30 min. Excess octafluorocyclopentene (0.94 mL) was then added and stirred for 1 h at −78 °C. The mixture was warmed to room temperature and extracted with ether. The organic layer was collected and washed with saturated salt water (30 mL) and then water (50 mL). The organic layer was dried over $MgSO_4$, filtrated and evaporated. The crude product was purified on silica gel column with petroleum ether as the eluent to give **6** (2.12 g, 4.16 mmol) as a light yellow solid in 72% yield. M.p. 74–76 °C; ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.16 (t, 6H, J = 8.0 Hz), 1.86–1.92 (m, 4H), 2.33 (s, 3H), 7.12 (s, 1H), 7.16 (s, 3H), 7.30 (s, 1H), 7.36 (d, 1H, J = 8.0 Hz), 7.53 (d, 2H, J = 8.0 Hz).

2.2.4. Synthesis of 3-bromo-5-[(1,3-dioxolane)-yl]-2-methylthiophene (8)

Compound **7** (1.51 g, 7.40 mmol), glycol (2.1 mL, 37.00 mmol) and p-toluenesulfonic acid (0.03 g, 0.15 mmol) were dissolved in benzene (100 mL). Under the Dean–Stark condition, the reaction mixtures were refluxed overnight and then washed sequentially three times with saturated salt water (30 mL) and water (50 mL). The combined benzene layers were dried, filtered and evaporated under vacuum to give 1.74 g of compound **8** as yellow oil in 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 2.45 (s, 3H), 4.09–4.10 (m, 4H), 6.03 (s, 1H), 7.29 (s, 1H).

2.2.5. Synthesis of 1-[2-methyl-5-(9,9-diethyl-fluoren-2-yl)-3-thienyl]-2-[(2-methyl-5-formyl)-3-thienyl]perfluorocyclopentene (***1O***)

Compound **8** (0.80 g, 3.23 mmol) in anhydrous THF was injected into n-BuLi (1.48 mL, 2.4 mol L^{-1}) at −78 °C under an argon atmosphere. The solution was continuously stirred for 30 min. Compound **6** (1.79 g, 3.55 mmol) dissolved in anhydrous THF (5 mL) was then slowly added to the reaction mixture at −78 °C and stirred for another 1 h. The reaction was quenched with water (20 mL). The mixture was warmed to room temperature and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtrated and evaporated. The crude product was purified on silica gel column with petroleum ether/ethyl acetate (8: 1) as the eluent to get purple solid intermediates.

The purple solid intermediates and p-toluenesulfonic acid (0.54 g) were dissolved in a mixture of water (20 mL) and acetone (80 mL). Pyridine (0.9 mL) was added into the mixture and then refluxed for 24 h. After the reaction, the mixture was washed sequentially with aqueous $NaHCO_3$ and water. The organic layer

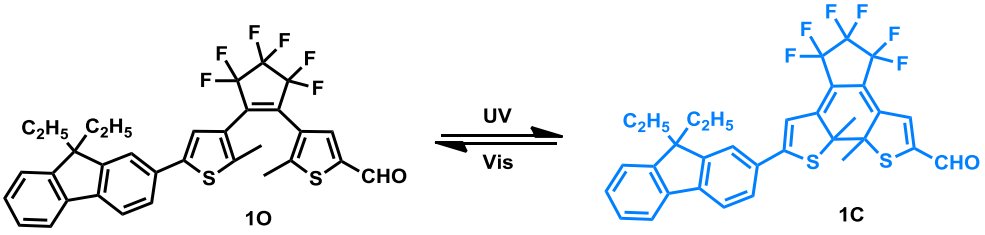

Fig. 1. Photochromism of diarylethene **O**.