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A Highly Selective and Sensitive Turn-On Fluorescent Chemosensor Based on Rhodamine 6G for Iron(III)**

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Recently, more and more rhodamine derivatives have been used as fluorophores to construct sensors due to their excellent spectroscopic properties. A rhodamine-based fluorescent and colorimetric Fe^{3+} chemosensor 3′,6′-bis(ethylamino)-2-acetoxyl-2′,7′-dimethyl-spiro[1*H*-isoindole-1,9′-[9*H*]xanthen]-3(2*H*)-one (**RAE**) was designed and synthesized. Upon the addition of Fe^{3+}, the dramatic enhancement of both fluorescence and absorbance intensity, as well as the color change of the solution, could be observed. The detection limit of **RAE** for Fe^{3+} was around 7.98 ppb. Common coexistent metal ions showed little or no interference in the detection of Fe^{3+}. Moreover, the addition of CN^- could quench the fluorescence of the acetonitrile solution of **RAE** and Fe^{3+}, indicating the regeneration of the chemosensor **RAE**. The robust nature of the sensor was shown by the detection of Fe^{3+} even after repeated rounds of quenching. As iron is a ubiquitous metal in cells and plays vital roles in many biological processes, this chemosensor could be developed to have applications in biological studies.

Introduction

Fluorescent sensors for detection of transition metal ions, such as Cu^{2+}, Hg^{2+}, and so on, have attracted a great deal of attention in the last decades.[1,2] Some of the more important among them are selective and sensitive fluorescent sensors for Fe^{3+}.[3,4] Iron, which is a ubiquitous metal in cells, plays vital roles in many biological processes.[5] However, deficiencies or excesses in iron are toxic or can lead to disturbances in glucose levels and lipid metabolism.[6] Though the human body can regulate iron to some extent, detection and analysis of bioactive iron remains an important healthcare challenge for chemists. Most literature reports use fluorescence quenching as the readout mechanism for the sensor response,[3] but very few involve a fluorescence "turn-on" response.[4] Moreover, most turn-on fluorescence sensors for Fe^{3+} are not selective over Cr^{3+} and Cu^{2+}.[4a–c] Therefore, new chemosensors that show high selectivity for iron and involve a fluorescence turn-on response appear to be particularly attractive because of the simplicity, high sensitivity, and low detection limit of the fluorescence.

Recently, more and more rhodamine derivatives have been successfully utilized as fluorophores to construct sensors due to their excellent spectroscopic properties: namely, a large molar extinction coefficient, high fluorescence quantum yield, visible light excitation, and long wavelength emission.[2,7] As a result, fluorescent chemosensors for Pb^{2+},[8] Hg^{2+},[9] Cr^{3+},[4a,10] Ag^+,[11] Cu^{2+}[12], and so on, have been developed. Rhodamine derivatives are nonfluorescent and colorless, whereas addition of targeted metal ions leads to ring opening of the corresponding spirolactam, giving rise to a strong fluorescence emission and a color change from colorless to pink. Based on the understanding of the sensing mechanism of rhodamine-based molecular sensors, herein, we report the design and synthesis of a new rhodamine-based chemosensor **RAE** (Scheme 1), which shows a highly selective and sensitive fluorescence enhancement in response to Fe^{3+} in acetonitrile solution. Moreover, the addition of CN^- could quench the fluorescence of the **RAE**–Fe^{3+} complex, indicating the regeneration of the chemosensor **RAE**. The color response allows the rapid and accurate recognition of Fe^{3+} with the naked eye, making this new chemosensor a very promising alternative for the detection of Fe^{3+}.

Results and Discussion

The chemosensor **RAE** was synthesized as shown in Scheme 1. Compounds **2**[13] and **3**[14] were synthesized according to literature methods. The reaction of acetyl chloride **3** and hydroxylamine hydrochloride afforded rhodamine derivative **4** with triethylamine as the base. Subsequently, the chemosensor **RAE** was obtained through the condensation of **4** and acetyl chloride with a yield of 41%. The structure of **RAE** was characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. With **RAE** in hand, we investigated its fluorescence properties by fluorescence measurements. After conducting a preliminary survey with var-

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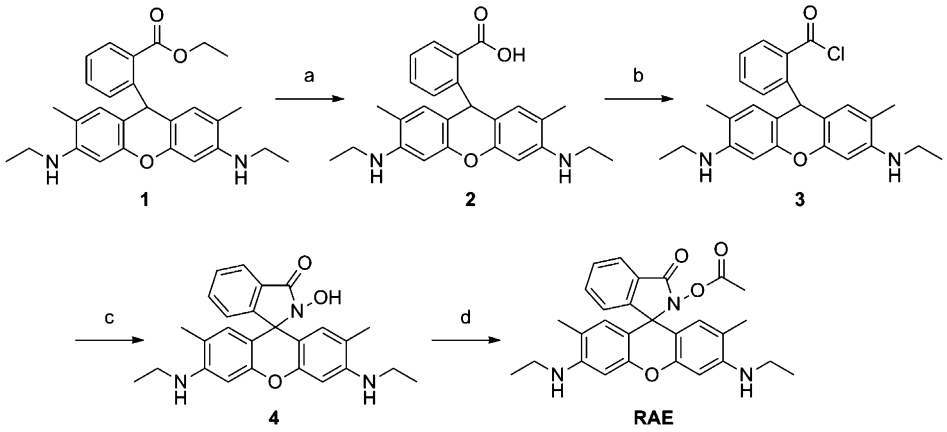

Scheme 1. Synthetic route to **RAE**. *Reagents and conditions*: a) EtOH, NaOH, H_2O, reflux, 2 h, 85%; b) CH_2Cl_2, $POCl_3$, reflux, 3 h, 91%; c) CH_2Cl_2, $NH_2OH{\cdot}HCl$, Et_3N, rt, 6 h, 31%; d) 1. CH_3CN, NaH, CH_3COCl, 0–5 °C, 30 min, 41%.

ious solvent systems, we chose acetonitrile for possible application of the system in metal ion analysis.

Upon the addition of different amounts of Fe^{3+}, the absorbance intensity of **RAE** in acetonitrile became enhanced, and a new absorbance peak at ~520 nm was observed (Figure S2 in the Supporting Information). Therefore, we chose 520 nm as the excitation wavelength in the fluorescence experiments. When the colorless solution containing **RAE** was subjected to fluorescence measurement, the solution (1.0×10^{-6} M) exhibited a very weak emission as shown in Figure 1. At the same time, the pink solution consisting of **RAE** (1.0×10^{-6} M) and Fe^{3+} (10 equiv) (Figure S3 in the Supporting Information) showed relatively strong fluorescence intensity. Similar to some reported rhodamine-based fluorescent sensors for Fe^{3+},[4f,g] the fluorescence enhancement of **RAE** solution in the presence of Fe^{3+} is also attributed to the formation of the spirolactam-ring-opened form of rhodamine induced by Fe^{3+}. Other tested metal ions did not induce any distinct fluorescence enhancement (Figure 1). Thus, sensor **RAE** is capable of fluorescence recognition of Fe^{3+} in acetonitrile.

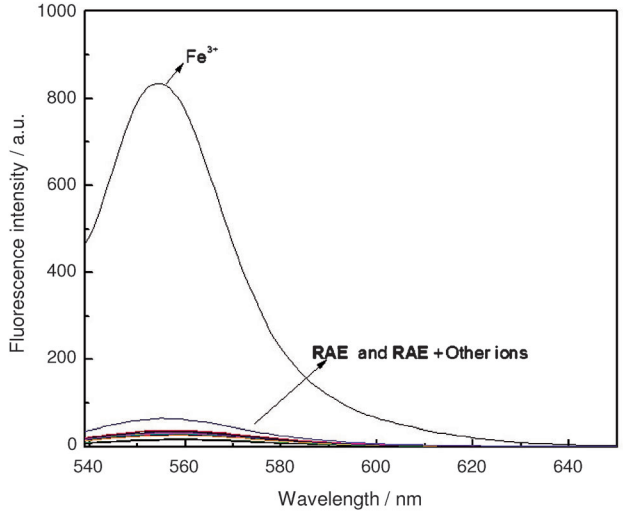

Figure 1. Fluorescence spectra ($\lambda_{ex}=520$ nm) of **RAE** (1 μM) in CH_3CN with different metal ions (10 equiv) (Other ions = Cu^{2+}, Ba^{2+},Ni^{3+}, Mg^{2+}, Na^{+}, Ca^{2+}, Pb^{2+}, Ag^{+}, Co^{3+}, Zn^{2+}, Hg^{2+}, Cr^{3+}, and Al^{3+}).

Titration of **RAE** solution (1 μM) in acetonitrile by addition of 0–110 equiv Fe^{3+} was subsequently carried out. Upon incremental addition of Fe^{3+}, the fluorescence intensity of **RAE** solution at 548 nm increases gradually and reaches the saturation point when 110 equiv of Fe^{3+} is added (Figure 2). The inset picture shows relative intensity (I/I_0) versus the concentration of Fe^{3+} in the low concentration region up to 7.98 ppb.

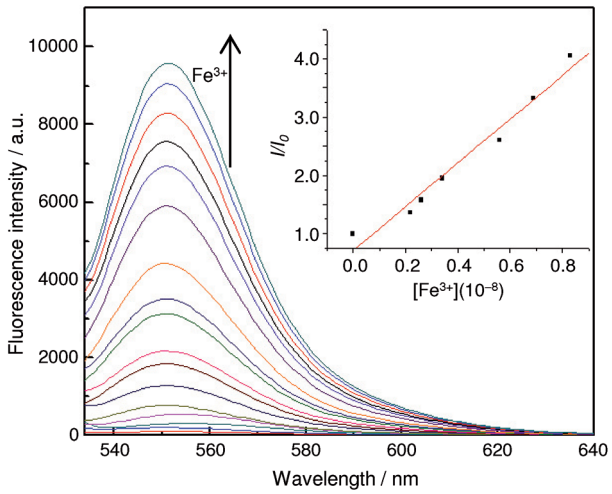

Figure 2. Fluorescence titration of **RAE** (1 μM) in CH_3CN with increasing Fe^{3+} concentration ($\lambda_{ex}=520$ nm). Inset: the fluorescence of **RAE** (1 μM) ($\lambda_{em}=548$ nm) as a function of the Fe^{3+} concentration ($0–8\times10^{-9}$ M).

Achieving a highly selective response to the target analyte over a complex background of potentially competitive species is an important requirement for a chemosensor. Thus, the competition experiments in the presence of potentially competitive metal ions were conducted, and the results are shown in Figure 3. The results of the competitive-metal-ion binding studies clearly suggest a lack of interference by the other metal ions (100 equiv) on the selective detection of Fe^{3+} (10 equiv) by **RAE**.

We also found that **RAE** could complex with Fe^{3+} in a 1:1 ratio, confirmed by the Job plot. A maximum emission intensity is seen when the molecular fraction of Fe^{3+} is ~0.50, which indicates the formation of a 1:1 complex between **RAE** and Fe^{3+} with a total concentration of 10 μM (Figure 4). The bind-

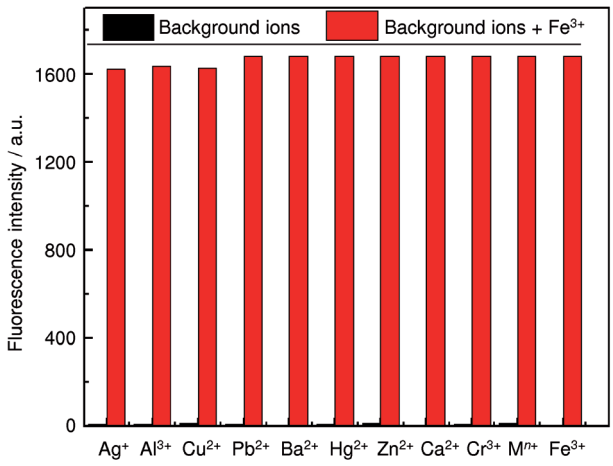

Figure 3. Metal-ion selectivity of **RAE** in CH_3CN. Dark bars represent the fluorescence intensity of a solution of **RAE** (1 μM) and 100 equiv of other metal ions (M^{n+} = Na^+, K^+, Mg^{2+}, Ce^{3+}). Red bars show the fluorescence intensity after addition of Fe^{3+} (10 equiv) to the solution of **RAE** (1 μM) and different metal ions (100 equiv).

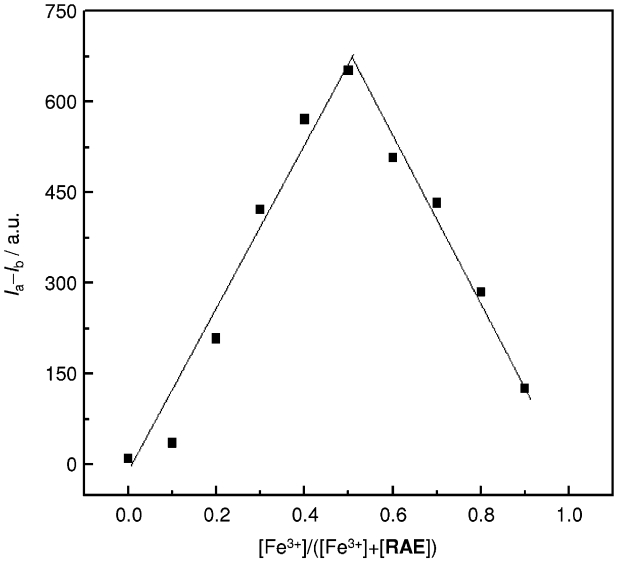

Figure 4. Job plot according to the method of continuous variations, indicating the 1:1 stoichiometry for **RAE**–Fe^{3+} (the total concentration of **RAE** and Fe^{3+} is 10.0 μM).

ing ratio of **RAE** and Fe^{3+} was also confirmed by using the Benesi–Hidebrand method (Figure 5).

Regeneration of the probe is a prerequisite in developing novel chemosensors for practical applications. The regeneration of the receptor **RAE** was performed by the addition of the Fe^{3+}-binding agent CN^-. As shown in Figure 6, addition of CN^- to the solution of receptor **RAE** and Fe^{3+} results in diminution of the fluorescence intensity at 548 nm, which indicates the regeneration of the free receptor **RAE**. Furthermore, the fluorescence of the solution of **RAE** and Fe^{3+} can be recovered even after four cycles of Fe^{3+} addition followed by CN^--induced quenching (Figure 7). Such a regeneration process is important for the fabrication of Fe^{3+} sensors.

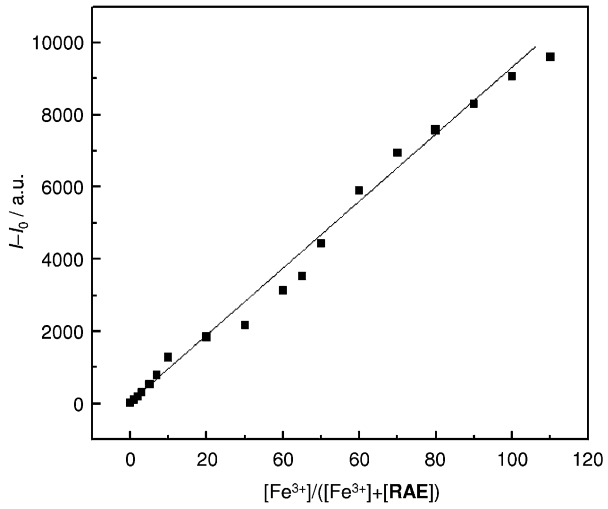

Figure 5. Benesi–Hildebrand plot of **RAE** (1 μM in CH_3CN, 548 nm) assuming 1:1 stoichiometry between **RAE** and Fe^{3+}.

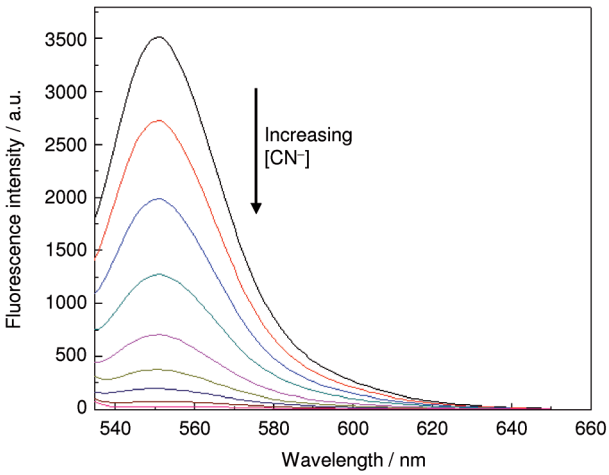

Figure 6. Fluorescence spectra of the solution of **RAE** (1 μM, CH_3CN) and Fe^{3+} (10 equiv) with addition of different amount of CN^- (increasing concentrations of CN^- from 0 to 46 equiv) in CH_3CN, λ_{ex} = 520 nm.

Conclusions

A novel fluorescent sensor **RAE** was designed and synthesized. In acetonitrile, **RAE** exhibits highly selective and sensitive detection of Fe^{3+} over other metal ions with a fluorescence turn-on effect, and the detection limit is 7.98 ppb. Moreover, the addition of CN^- could quench the fluorescence of the **RAE**–Fe^{3+} complex, indicating the regeneration of chemosensor **RAE**. Further efforts will be focused on the structure modification of the sensor so that it could also be operated in aqueous solution for possible biological applications.

Experimental Section

Instruments and materials: The fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorometer. A 1.0 cm quartz cuvette with a volume of 3.0 mL was used for all spectra collection. Thin-

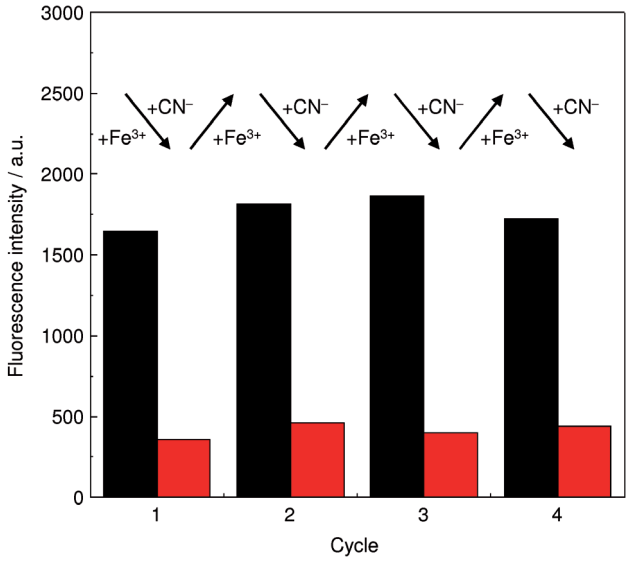

Figure 7. Regeneration of **RAE** (1 μM, CH_3CN) upon repeated addition of Fe^{3+} (10 equiv) followed by CN^- (25 equiv), $\lambda_{ex}=520$ nm. Four cycles of Fe^{3+} and CN^- addition are shown. The bars show the fluorescence intensity after addition of Fe^{3+} (black) and after the addition of CN^- (red).

layer chromatography (TLC) was performed on glass plates coated with SiO_2 GF254. The plates were inspected by UV light or in I_2 vapor. Column chromatography was performed on silica gel (200–300 mesh). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 500 NMR (500 MHz) using tetramethylsilane (TMS) as an internal standard. Matrix-assisted laser desorption/ionization mass spectrometry (MS-MALDI) was performed on a Bruker Daltonics Biflex III. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard procedures prior to use. CH_3CN in chromatographic grade was used throughout the experiments as solvent. For the CH_3CN stock solutions of the various metal ions (0.01 mM), the perchlorate salts of Fe^{3+}, Cu^{2+}, Ba^{2+}, Ni^{3+}, Mg^{2+}, Na^+, and Ca^{2+}, the nitrate salts of Pb^{2+} and Ag^+, and the chloride salts of Co^{3+}, Zn^{2+}, and Hg^{2+} were used. The stock solution of compound **RAE** (0.01 M) was prepared by dissolving accurately weighed **RAE** in CH_3CN.

General procedure: Typically, 3.0 mL of the solution of **RAE** (1 μM) was placed in a quartz cell (1.0 cm width), and the appropriate aliquot of Fe^{3+} solution was added. The resulting solution was stirred thoroughly and allowed to stand at rt for 2 min, and then the fluorescence spectrum was recorded. For fluorescence intensity measurements, the excitation and emission wavelengths were at 520 nm and 548 nm, respectively. The slit width was 5 nm/5 nm. The synthetic route is shown in Scheme 1.

2-[3′,6′-Bis(ethylamino)-2′,7′-dimethyl-9*H*-xanthen-9-yl]benzoic acid (2):[13] To a solution of rhodamine 6G **1** (540 mg, 1.13 mmol) in EtOH (14 mL) was added NaOH (135 mg, 3.39 mmol) in H_2O (2 mL), and the reaction mixture was stirred for 2 h at reflux. After addition of distilled H_2O (15 mL), the solution was cooled to rt. The resulting precipitate was isolated by filtration and dried at 70 °C for 30 min to give compound **2** (400 mg, 85%). No further purification was conducted.

2-[3′,6′-Bis(ethylamino)-2′,7′-dimethyl-9*H*-xanthen-9-yl]benzoyl chloride (3):[14] To a solution of **2** (400 mg, 0.96 mmol) in CH_2Cl_2 (10 mL) was added $POCl_3$ (0.26 mL, 2.88 mmol) dropwise over 2 min. The solution was heated at reflux for 3 h. The reaction mixture was cooled to rt and evaporated in vacuo to give compound **3** (380 mg, 91%), which was used in the next step without purification.

3′,6′-Bis(ethylamino)-2-hydroxy-2′,7′-dimethyl-spiro{1*H*-isoindole-1,9′-[9*H*]xanthen}-3(2*H*)-one (4): To the crude acid chloride **3** dissolved in CH_2Cl_2 (10 mL), Et_3N (0.3 mL, 2.15 mmol) was added dropwise after addition of $NH_2OH{\cdot}HCl$ (150 mg, 2.16 mmol). The reaction mixture was stirred for 6 h at rt, then extracted with CH_2Cl_2 (3×20 mL), and the combined organic layers were dried over anhydrous Na_2SO_4. The solution was filtered, concentrated in vacuo, and the crude product was purified by column chromatography (hexanes/EtOAc, 2:1→1:1) to give compound **4** as a pink solid (150 mg, 0.35 mmol, 36% from rhodamine 6G). The pink colored product was recrystallized from CH_2Cl_2/hexanes (1:1, *v/v*) to give compound **4** as a white solid (117 mg, 31%); ^1H NMR (400 MHz, $CDCl_3$): $\delta=7.87$–7.84 (m, 1H), 7.47–7.42 (m, 2H), 7.07–7.04 (m, 2H), 6.40 (s, 2H), 6.36 (s, 2H), 3.53 (br s, 2H), 3.25–3.20 (m, 4H), 2.13 (s, 6H), 1.24 ppm (t, $J=7.2$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 163.6, 152.3, 150.9, 147.7, 132.8, 128.6, 128.3, 127.9, 123.8, 123.0, 117.9, 104.7, 97.1, 65.9, 38.5, 16.9, 14.9 ppm; IR (film): $\tilde{\nu}=3390$, 2963, 2924, 1683, 1645, 1623, 1519, 1467, 1416, 1377, 1342, 1277, 1208, 1156, 1091, 1044, 1014 cm^{-1}.

3′,6′-Bis(ethylamino)-2-acetoxyl-2′,7′-dimethyl-spiro[1*H*-isoindole-1,9′-[9*H*]xanthen]-3(2*H*)-one (RAE): To the solution of **4** (1.5 g, 3.73 mmol) in anhydrous CH_3CN (45 mL), NaH (0.11 g, 4.5 mmol) was added at 0–5 °C. The mixture was stirred in an ice bath for 30 min. CH_3COCl (0.39 g, 4.11 mmol) dissolved in CH_3CN (10 mL) was added at 0–5 °C dropwise over 20 min. The mixture was stirred in an ice bath for more than 30 min. Impurities were removed by filtration, and the filtrate was concentrated in vacuo. Purification by flash column chromatography (hexanes/EtOAc, 5:1) gave **RAE** as an ivory-white solid (0.62 g, 41%); ^1H NMR (500 MHz, CD_3COCD_3): $\delta=10.11$ (s, 1H), 7.87 (d, $J=7.0$ Hz, 1H), 7.62–7.56 (m, 2H), 7.05 (d, $J=7.5$ Hz, 1H), 6.42 (s, 2H), 6.32 (s, 2H), 4.55 (s, 2H), 3.25 (t, $J=6.0$ Hz, 4H), 1.95 (s, 9H), 1.29 ppm (q, $J=5.5$ Hz, 6H); ^{13}C NMR (125 MHz, CD_3COCD_3): $\delta=167.5$, 163.4, 153.2, 152.3, 149.1, 134.4, 129.6, 129.0, 124.9, 123.7, 118.9, 105.2, 97.0, 66.6, 39.0, 18.0, 17.3, 14.9 ppm; MS-MALDI: $m/z=472.1$ $[M+H]^+$.

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