

Supporting Information

Self-powered and reagentless electrochemical aptasensor based on DNA walker and tetraferrocene for the detection of aflatoxin B1

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Keywords: DNA walker; DNAzyme; Reagentless; UIO-66 (Zr)-(COOH)₂; AFB1

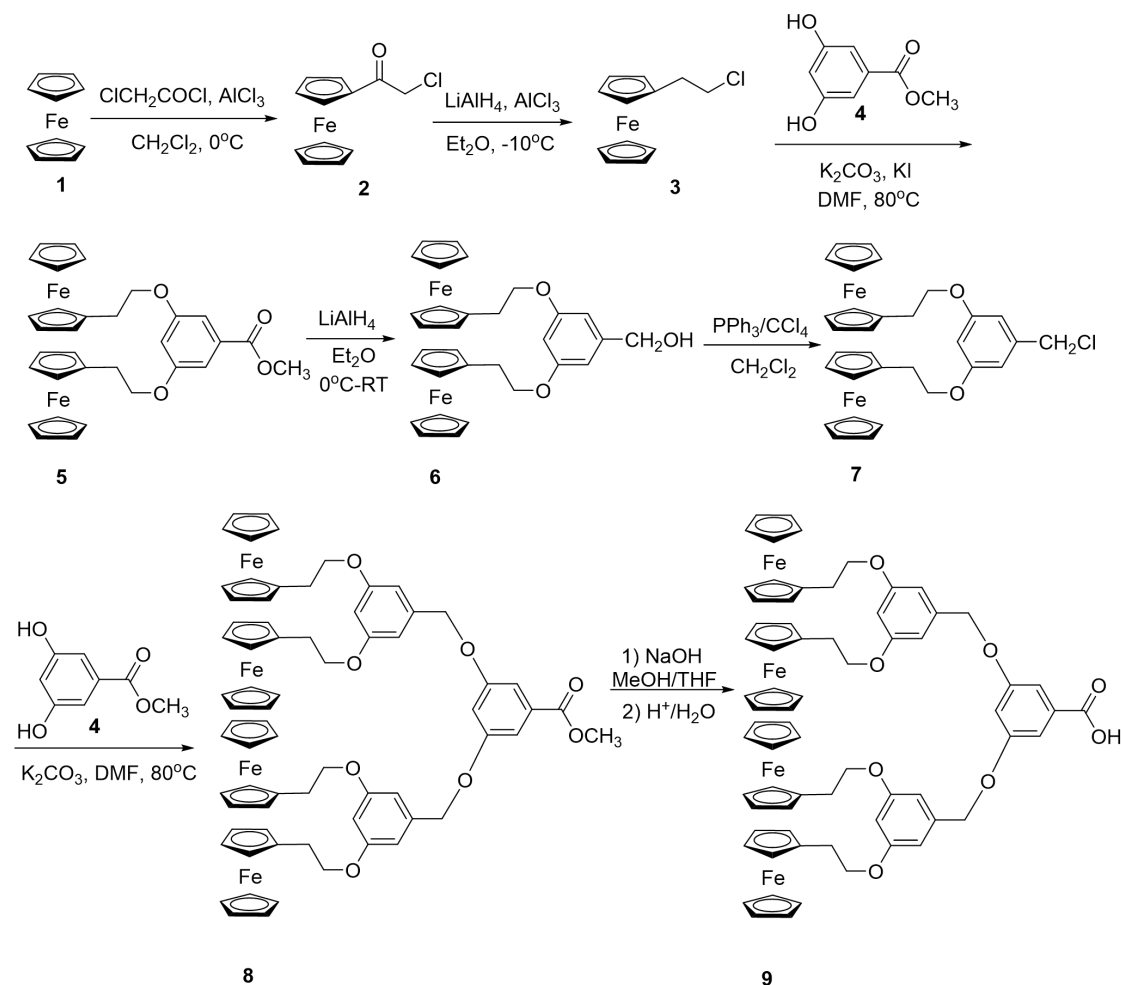


Figure 1 Procedure for the synthesis of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid 9.

1. The Synthetic of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic Acid (9)

1.1 Preparation of α-chloroacetylferrocene (2)

To a solution of ferrocene (11.2 g, 60 mmol) in dichloromethane (60 mL) at 0°C, a solution of chloroacetyl chloride (3.8 mL, 50 mmol) and anhydrous aluminum trichloride (6.6 g, 50 mmol) in dichloromethane (80 mL) was added dropwise. After stirred for 8 h at room temperature, brine (100 mL) was added. The organic phase was separated and washed by brine. The resulting solution was dried over anhydrous Na₂SO₄, filtered and concentrated in vacuum. The residue was purified by flash

column chromatography to give orange crystal in 41.5% yield. ^1H NMR (CDCl_3 , 600MHz): δ 4.88 (Cp-H, t, $J = 2.0$ Hz, 2 H), 4.62 (Cp-H, t, $J = 2.0$ Hz, 2 H), 4.46 (CH_2 , s, 2 H), 4.25 (Cp-H, s, 5 H).

1.2 Preparation of 2-Chloroethylferrocene(3)

A solution of α -chloroacetylferrocene 2 (1.2 g, 4.58 mmol) in Et_2O (120 mL) was added dropwise to a solution of LiAlH_4 (180 mg, 4.7 mmol) and AlCl_3 (609 mg, 4.58 mmol) in Et_2O (90 mL) at -10°C . After 1 h, the reaction was carefully quenched by addition of H_2O (20 mL) and diluted with brine (100 mL). The aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by a silica gel column with ethyl acetate- Petroleum ether (1:15) as the eluent to afford yellow product 3 (0.76 g, 67%). ^1H NMR (CDCl_3 , 600 MHz) δ 4.16 (Cp-H, s, 7H), 4.13 (Cp-H, s, 2H), 3.62 ($\text{CH}_2\text{-O}$, t, $J = 7.2$ Hz, 2H), 2.84 (Fc- CH_2 -, t, $J = 7.2$ Hz, 2H).

1.3 Preparation of Methyl 3,5-bisferrocenethoxybenzoate (5)

2-Chloroethylferrocene 3 (548.1 mg, 2.21 mmol), methyl 3,5-dihydroxybenzoate 4 (154.6 mg, 0.92 mmol), anhydrous potassium carbonate (552 mg, 4 mmol) and potassium iodide (10 mg, 0.06 mmol) in DMF (4 mL) was mixed together and stirred at 80°C until disappearance of starting material was observed by TLC. The mixture was diluted with ethyl acetate (80 mL) and washed with water (3×30 mL) and brine (3×30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave 5 as yellow solid (398 mg, 73%). ^1H NMR (CDCl_3 , 600 MHz) δ 7.19 (Ar-H, s, 1H), 7.11 (Ar-H, d, $J = 2.4$ Hz, 2H), 4.09 (Cp-H, t, $J = 1.5$ Hz, 4H), 4.06 (Cp-H, s, 10H), 4.04-4.02 (Cp-H, - $\text{CH}_2\text{-O}$, m, 8H), 3.82 (O- CH_3 , s, 3H), 2.75 (Fc- CH_2 -, t, $J = 7.2$ Hz, 4H). ^{13}C NMR (CDCl_3 , 150 MHz) δ 166.9, 159.9, 131.9, 107.7, 106.8, 84.6, 68.8, 68.6, 68.6, 67.5, 52.3, 29.5. HRMS (ESI) Calcd. for $\text{C}_{32}\text{H}_{32}\text{Fe}_2\text{O}_4$ $[\text{M}]^+$: 592.0999; found 592.0991.

1.4 Preparation of (3, 5-bisferrocenethoxyphenyl)methanol (6)

To a solution of LiAlH_4 (28.8 mg, 0.76 mmol) in anhydrous ether (10 mL), a solution of methyl 3, 5-bisferrocenethoxy- benzoate 5 (50 mg, 0.084 mmol) in anhydrous ether (15 mL) was added dropwise at 0°C . The mixture was stirred at room temperature until the disappearance of the starting material. And then the reaction was carefully quenched by H_2O (20 mL), the aqueous layer was separated and extracted with diethyl ether (3×25 mL). The combined organic layer was washed with brine (3×50 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography to give orange crystal 6. Yields: 89.0%. ^1H NMR (CDCl_3 , 600 MHz) δ 6.55 (Ar-H, s, 2H), 6.43 (Ar-H, s, 1H), 4.64 (ArCH₂-O, s, 2H), 4.20-4.13 (Cp-H, m, 18H), 4.10 (-CH₂-O, t, $J = 7.2$ Hz, 4H), 2.84 (Fc-CH₂-, t, $J = 7.2$ Hz, 4H). HRMS (ESI) Calcd. for $\text{C}_{31}\text{H}_{32}\text{Fe}_2\text{O}_3$ $[\text{M}]^+$: 564.1050; found 564.1037.

1.5 Preparation of 3, 5-bisferrocenethoxybenzyl chloride (7)

To a solution of PPh_3 (0.4313 g, 1.65 mmol) in CCl_4 (10 mL), a solution of (3, 5-bisferrocenethoxyphenyl) methanol 6 (0.7739 g, 1.37 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The mixture solution was refluxed for 30 min and observed by TLC. The mixture was diluted with dichloromethane (40 mL) and washed with water (3×20 mL) and brine (3×20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to gave 7 as dark orange solid. ^1H NMR (CDCl_3 , 600 MHz) δ 6.55 (Ar-H, d, $J = 1.8$ Hz, 2H), 6.43 (Ar-H, t, $J = 2.4$ Hz, 1H), 4.53 (ArCH₂-O, s, 2H), 4.18 (Cp-H, t, $J = 1.8$ Hz, 4H), 4.15 (Cp-H, s, 10H), 4.11 (Cp-H, t, $J = 1.8$ Hz, 4H), 4.09 (-CH₂-O, t, $J = 7.2$ Hz, 4H), 2.83 (Fc-CH₂-, t, $J = 7.2$ Hz, 4H). ^{13}C NMR (CDCl_3 , 150 MHz) δ 160.3, 139.5, 107.1, 101.3, 84.6, 68.7, 68.6, 68.6, 67.5, 46.2, 29.2. HRMS (ESI) Calcd. for $\text{C}_{31}\text{H}_{31}\text{ClFe}_2\text{O}_2$ $[\text{M}]^+$: 582.0711; found 582.0731.

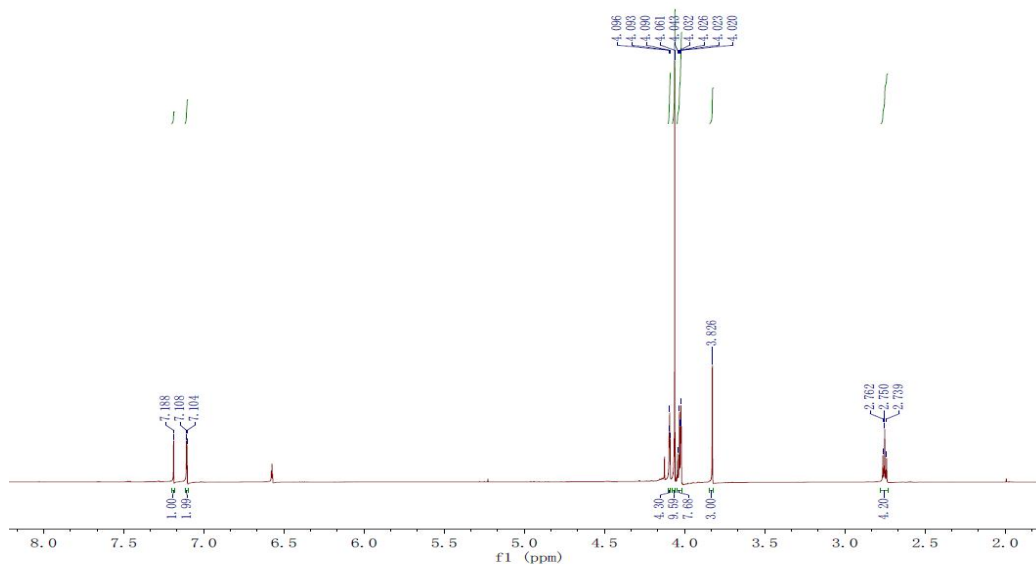
1.6 Preparation of Methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate (8)

To a solution of methyl 3,5-dihydroxybenzoate 4 (6.6 mg, 0.039 mmol) and anhydrous K_2CO_3 (21.5 mg, 0.156 mmol) in 10 mL of DMF, s3,

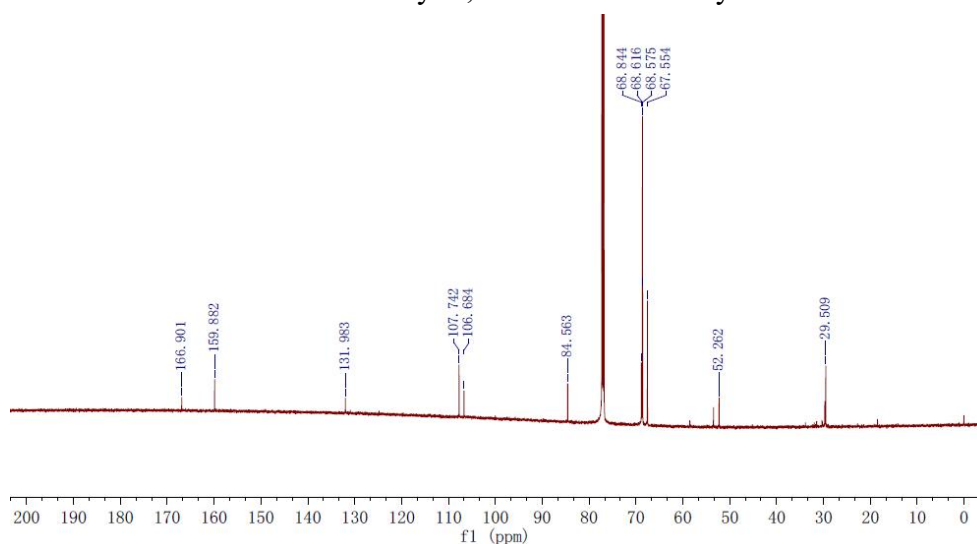
5-bisferrocenethoxybenzyl chloride **7** (50 mg, 0.085 mmol) was added. The reaction was stirred at 80 °C under nitrogen atmosphere and observed by TLC. The mixture was diluted with ethyl acetate (30 mL) and washed with brine (3×15 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent was removed in vacuum. The residue was purified by a silica gel column with ethyl acetate-petroleum ether as the eluent to give **8** as dark yellow solid. ¹H NMR (CDCl₃, 600 MHz) δ 7.30 (Ar-H, d, J = 2.4 Hz, 2H), 6.82 (Ar-H, t, J = 2.4 Hz, 1H), 6.59 (Ar-H, d, J = 2.4 Hz, 4H), 6.45 (Ar-H, t, J = 2.4 Hz, 2H), 5.01 (ArCH₂-O, s, 4H), 4.18 (Cp-H, t, J = 1.2 Hz, 8H), 4.15-4.09 (Cp-H and -CH₂-O, m, 36H), 3.91 (-COOCH₃, s, 3H), 2.83 (Fc-CH₂-, t, J = 7.2 Hz, 8H).

1.7 Preparation of **3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid (9)**

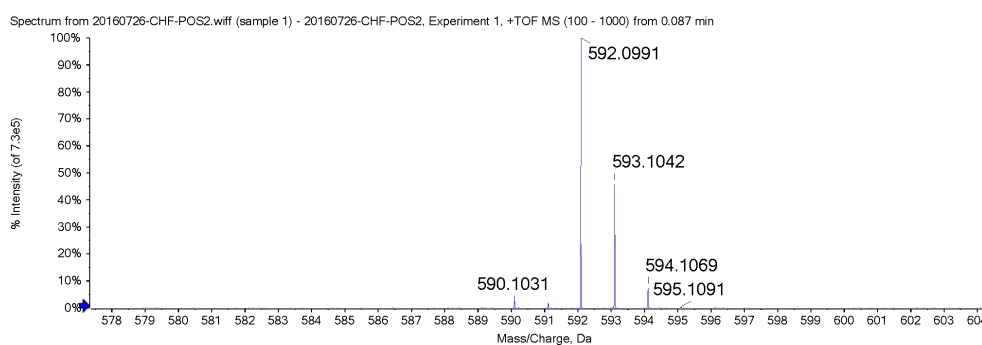
To a solution of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate **8** (50 mg, 0.04 mmol) in THF/MeOH (10mL, v/v = 1: 1) was added 10% aqueous sodium hydroxide (2 mL) at room temperature. The mixture was kept stirring overnight, the pH was adjusted to about 5 with dilute hydrochloric acid. The resulting orange crystals were collected by filtration, washed with water, and dried under vacuum. A pure sample was obtained by recrystallization from dichloromethane/ petroleum ether to give **9** (32 mg, 65% yield, purity > 95%) as dark yellow solid. ¹H NMR (CDCl₃, 600 MHz) δ 7.32 (Ar-H, s, 2H), 6.85 (Ar-H, s, 1H), 6.59 (Ar-H, s, 4H), 6.44 (Ar-H, s, 2H), 5.01 (ArCH₂-O, s, 4H), 4.20-4.11 (Cp-H, m, 36H), 4.09 (-CH₂-O, t, J = 7.2 Hz, 8H), 2.83 (Fc-CH₂-, t, J = 6.6 Hz, 8H). HRMS (ESI) Calcd. for C₆₉H₆₆Fe₄O₈ [M]⁺: 1246.2155; found 1246.2091



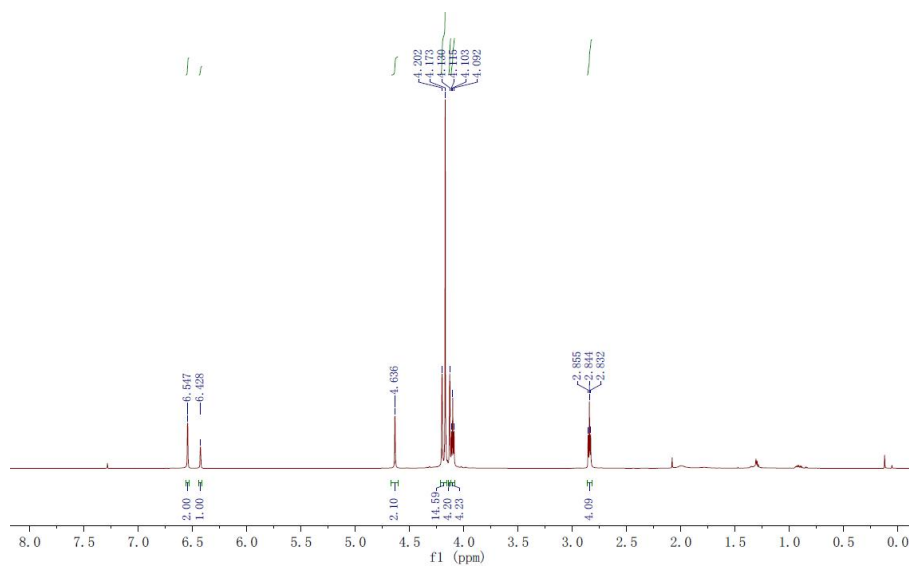
¹H NMR of methyl 3,5-bisferrocenethoxybenzoate **5**



¹³C NMR of methyl 3,5-bisferrocenethoxybenzoate **5**

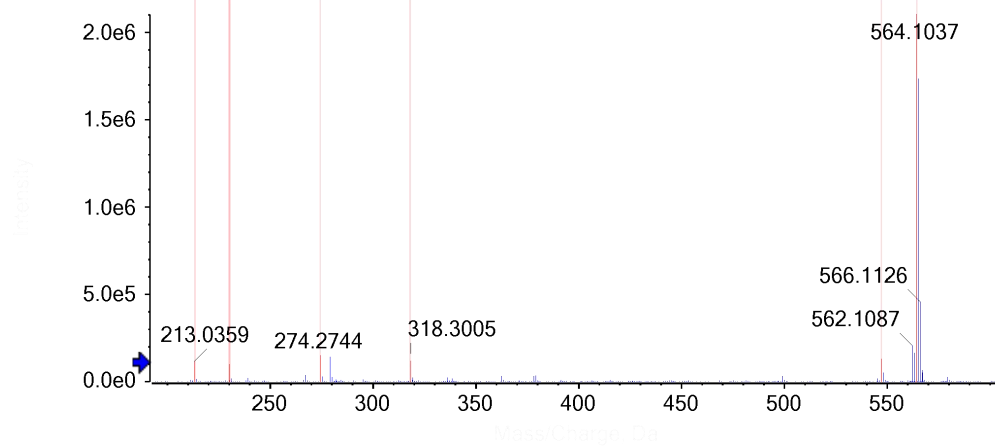


HRMS of methyl 3,5-bisferrocenethoxybenzoate **5**

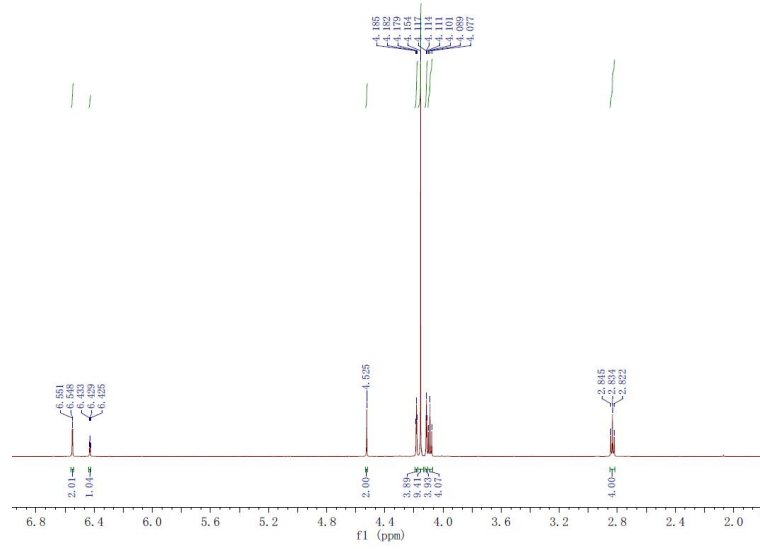


¹H NMR of (3, 5-bisferrocenethoxyphenyl)methanol **6**

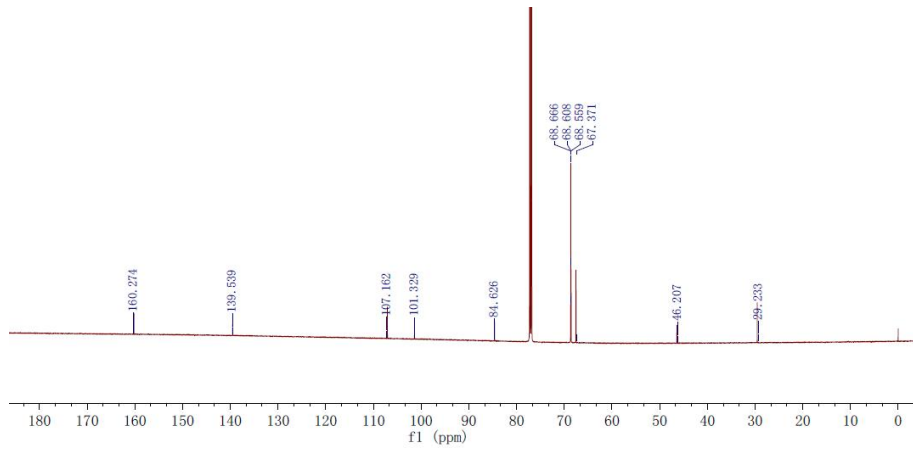
Spectrum from 20161215-POS-H161209-1.wiff (samp...m 0.104 min, noise filtered, Gaussian smoothed)



HRMS of (3, 5-bisferrocenethoxyphenyl)methanol **6**

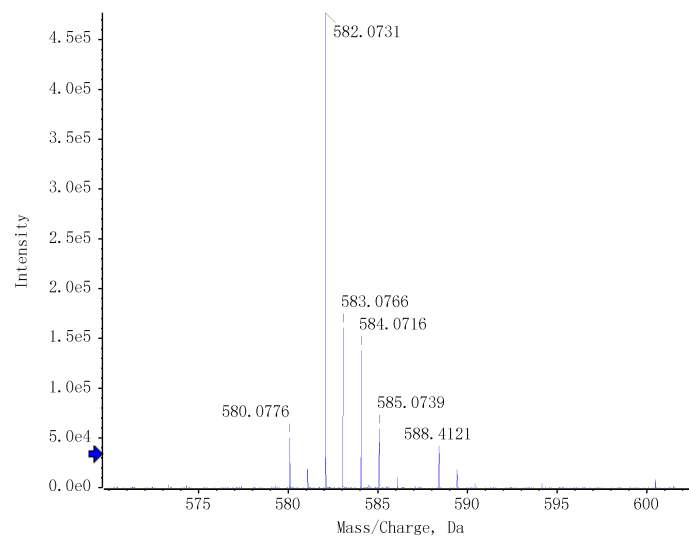


¹H NMR of 3, 5-bisferrocenethoxybenzyl chloride 7

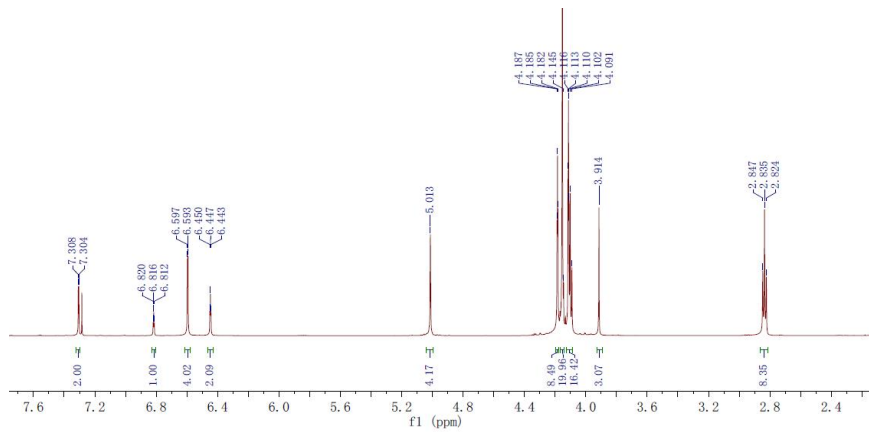


¹³C NMR of 3, 5-bisferrocenethoxybenzyl chloride 7

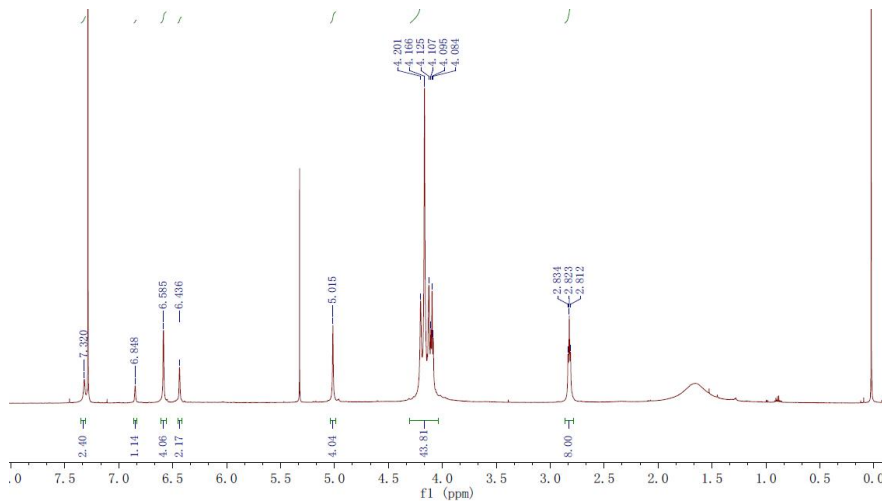
Spectrum from 20170418-POS-H170414.wif..., +TOF MS (100 - 3000) from 0.101 min



HRMS of 3, 5-bisferrocenethoxybenzyl chloride 7



¹H NMR of methyl 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoate 8



¹H NMR of 3, 5-bis(3, 5-bisferrocenethoxybenzyloxy)benzoic acid 9

2. Synthesis of Mn²⁺@UiO-66(Zr)-(COOH)₂

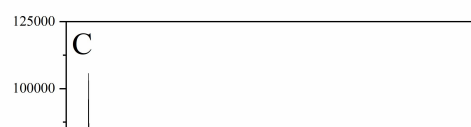
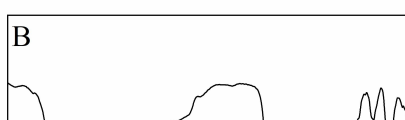
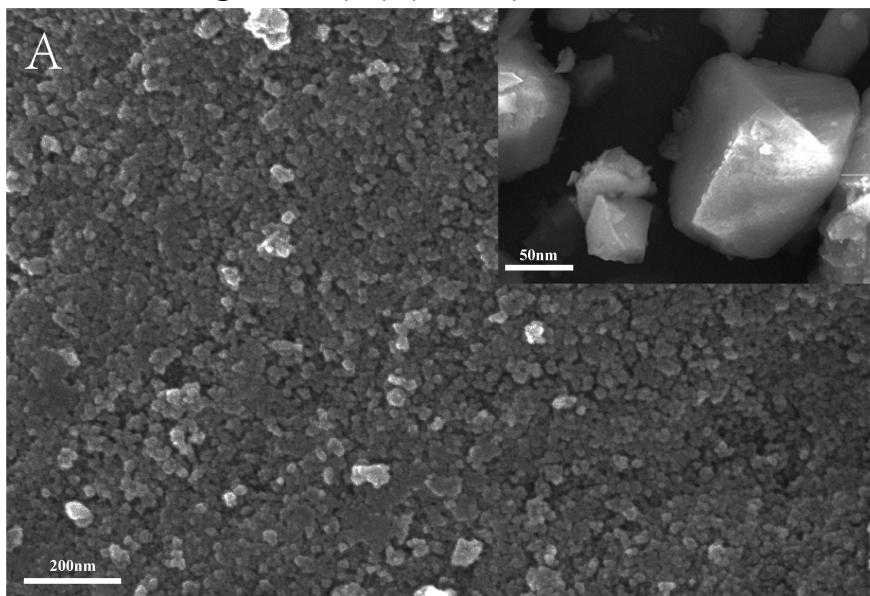


Fig.S1. (A) SEM images of as synthesized a UiO-66(Zr)-(COOH)₂. (B) FTIR spectrum of UiO-66(Zr)-(COOH)₂. (C) EDS characterization of Mn²⁺@UiO-66(Zr)-(COOH)₂.

3. Analytical performance of the electrochemical aptasensor for AFB1

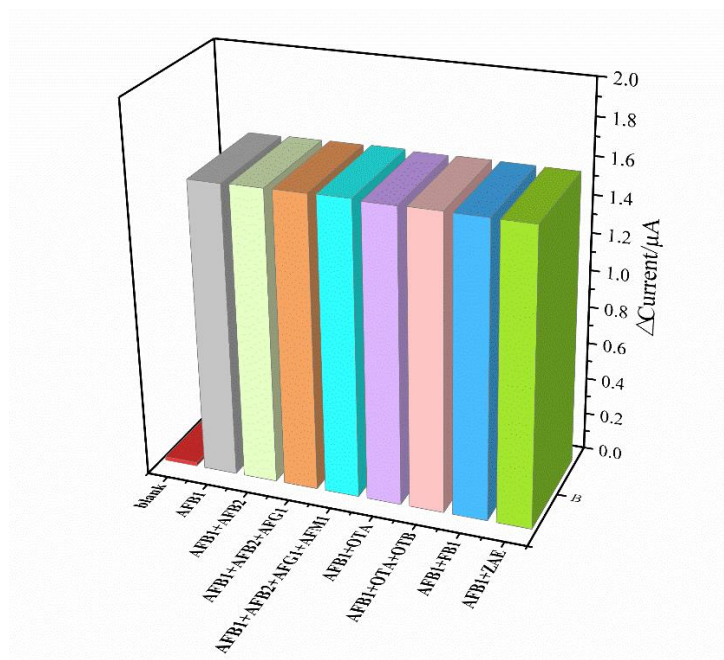


Fig.S2. Specificity experiments of E-AB sensor for AFB1 detection. Interfering substances include AFB1+AFB2, AFB1+AFB2+AFG1, AFB1+AFB2+AFG1+AFM1, AFB1+OTA, AFB1+OTA+OTB, AFB1+FB1 and AFB1+ZAE