*Electronic Supplementary Information*

# Squaramide-based Metal-organic Framework as Luminescent Sensor for the Detection of Lactose in Aqueous Solution and in Milk

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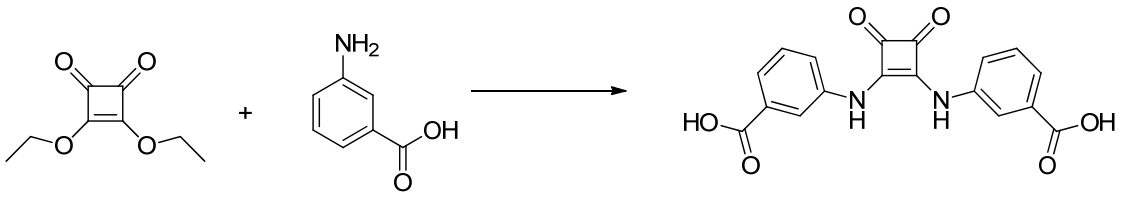
## 1. Materials and Methods.

**Reagents and chemicals:** All reagents and solvents were of AR grade and used without further purification unless otherwise noted. $Co(NO_3)_2 \cdot 6H_2O$, Lactose and other sugars were purchased from Alfa Aesar. Stock solution ($2\times10^{-2}$ M) of the sugar molecules of Tre (Trehalose), Mal (Maltose), Cel (Cellobiose), Suc (Sucrose), Mel (Melibiose), Xyl (Xylose), Man (Mannose), Rib (Ribose), Glu(Glucose) and Lac(lactose) were prepared in water for further experiments.

**Instruments and spectroscopic measurements:** The elemental analyses of C, H and N were performed on a Vario EL III elemental analyzer. $^1$H NMR spectra were measured on a Bruker-400 spectrometer with $Me_4Si$ as an internal standard, the samples Co−DBPY after treated with Lac were first decomposed by DCl (deuterated hydrochloric acid) and then DMSO-$d_6$ was added to perform $^1$H-NMR measurement. X-Ray powder diffraction (XRD) patterns of the Co–DBPY was recorded on a Rigaku D/max-2400 X−ray powder diffractometer (Japan) using Cu-*K*α (λ = 1.5405 Å) radiation. FT−IR spectra were recorded as KBr pellets on JASCO FT/IR−430. Thermogravimetric analysis (TGA) was carried out at a ramp rate of 5 °C/min in a nitrogen flow with a Mettler-Toledo TGA/SDTA851 instrument. Fluorescence spectra of the solution were obtained using the F−4600 spectrometer (Hitachi). The fluorescent quantum yields were measured using an absolute photoluminescence quantum yield measurement system (Hamamatsu, C9920-02). The radiative deactivation curves for the fluorescence were recorded on Edinburgh Instruments, model FL 920. The data were recorded at the emission maximum of each sample. The samples were measured in a suspension of dispersed powder before and after treated with Lac in HEPES.

**Solution Fluorescent Spectra Detection:** For sugar sensing, the high concentration stock solutions of related sugar-analysts ($2.0 \times 10^{-2}$ M) were prepared directly in water, and the Co−DBPY emulsion was prepared by introducing 1 mg of Co−DBPY powder into 3.00 mL of HEPES buffer (pH=7.4). For the Lac sensing in milk, it was performed through direct addition of the milk to 3.00 mL of HEPES buffer (pH=7.4) containing 1 mg of Co−DBPY. The excitation wavelength was 320 nm. Both excitation and emission slit widths were 10 nm. Fluorescence measurements were carried out in a 1 cm quartzcuvette with stirring the suspension of Co−DBPY.

**Synthesis of the $H_2$dbda (3,3'-((3,4-dioxocyclobut-1-ene-1,2-diyl)bis(azanediyl))dibenzoic acid) ligand**.




3-Aminobenzoic acid (576 mg, 4.2 mmol), $Zn(CF_3SO_3)_2$ (145 mg, 0.4 mmol), and 3,4-diethoxy-3-cyclobutene-1,2-dione (295 uL, 2 mmol) was added into 19.0 mL toluene and 1.0 mL NMP. After heating to reflux at 100 ºC for 24 h under a $N_2$ atmosphere, a yellow precipitate was harvested by filtration and washed with MeOH (10 mL). To further purify the product, the yellow solid was stirred in boiling MeOH (20 mL) for 5 min and then isolated by vacuum filtration, and washed with MeOH (3×5 mL). This purification procedure was repeated two more times, and the product was dried at 80 ºC for 12 h. Yield: 0.6 g (85%) based on 3,4-diethoxy-3-cyclobutene-1,2-dione. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.36 (s, 2H), 8.13 (s, 2H), 7.99 (d, *J*

= 8.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.50 (t, *J* = 8.0 Hz, 2H). ESI-MS (*m/z*): $[M]^-$ calculated for $[C_{18}H_{11}N_2O_6]^-$ 351.3, found 350.9.

**Synthesis of** Co−DBPY**:** A mixture of 3,3'-((3,4-dioxocyclobut-1-ene-1,2-diyl)bis(azanediyl))dibenzoic acid ($H_2$dbda) (1.8 mg, 2.5 mM), 4,4'-bipyridine (bpy) (0.9 mg, 2.9 mM) and $Co(NO_3)_2 \cdot 6H_2O$ (1.6 mg, 2.7 mM) were dissolved in N,N-Dimethylacetamide/Ethanol/water (1/1/0.5, 2.5 mL) in a screw capped vial. The resulting mixture was placed in an oven at 80 ºC for 3 days. After cooling, red block crystals were collected by filtration. Yield: 73%. Anal calc. for $C_{28}H_{18}CoN_4O_7$: C 57.84, H 3.12, N 9.64%; Found: C 57.82, H 3.14, N 9.69%.

## 2. X-ray Crystallography (Single-crystal diffraction) and Characterizations of Co−DBPY.

### 2.1 Crystal data of Co−DBPY:

| | After squeeze | Before squeeze |
|---|---|---|
| **formula** | $C_{28}H_{18}CoN_4O_7$ | $C_{28}H_{18}CoN_4O_7$ |
| **formula weight** | 581.39 | 581.39 |
| **crystal system** | Monoclinic | Monoclinic |
| **space group** | *P*2(1)/*c* | *P*2(1)/*c* |
| ***a* /Å** | 11.386(2) | 11.386(2) |
| ***b* /Å** | 23.961(5) | 23.961(5) |
| ***c* /Å** | 14.850(3) | 14.850(3) |
| ***α* /°** | 90.00 | 90.00 |
| ***β* /°** | 104.161(15) | 104.161(15) |
| ***γ* /°** | 90.00 | 90.00 |
| ***V* /Å$^3$** | 3928.5(14) | 3928.5(14) |
| **Z** | 4 | 4 |
| **$\rho_{calcd}$/g cm$^{-3}$** | 0.983 | 0.983 |
| **$\mu$ /mm$^{-1}$** | 0.473 | 0.473 |
| ***T* /K** | 296(2) | 296(2) |
| **Collected reflections** | 6896 | 6896 |
| **unique reflections** | 2618 | 2618 |
| **$R_{int}$** | 0.1162 | 0.1162 |
| **$R_1$ [$I > 2\sigma(I)$]** | 0.0704 | 0.1110 |
| **$wR_2$ (all data)** | 0.1735 | 0.3419 |
| **GOOF** | 1.002 | 1.007 |
| **CCDC** | 1843727 | |

**2.2 Crystallography**:

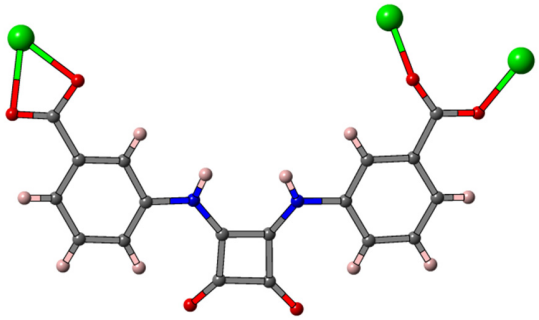
Intensities were collected on a Bruker SMART APEX CCD diffractometer with graphitemonochromated Mo-Kα ($\lambda$ = 0.71073 Å) using the SMART and SAINT programs. The structure was solved by direct methods and refined on $F^2$ by full-matrix least-squares methods with SHELXTL *version* 5.1. Non-hydrogen atoms of the ligand backbones were refined anisotropically. Hydrogen atoms within the ligand backbones were fixed geometrically at calculated positions and allowed to ride on the parent non-hydrogen atoms, and the data were treated with the SQUEEZE routine within PLATON.

**2.3 Table S1** Selective bond distance (Å) and angle (°) in Co−DBPY.

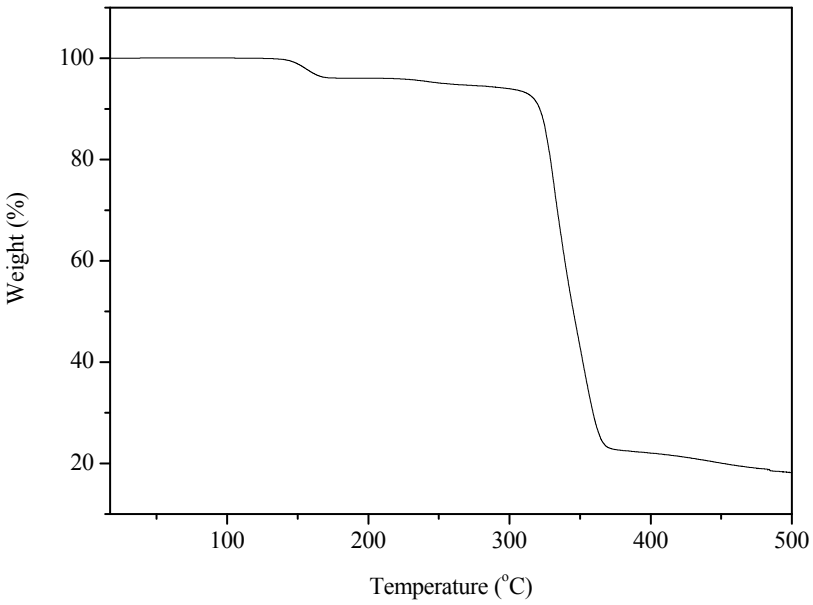
| | | | |
|---|---|---|---|
| Co(1)–O(1A) | 1.982(4) | Co(1)–O(4B) | 1.997(4) |
| Co(1)–O(3) | 2.119(4) | Co(1)–N(2) | 2.153(4) |
| Co(1)–N(1C) | 2.168(4) | Co(1)–O(2) | 2.264(4) |
| O(1A)–Co(1)–O(4B) | 118.76(15) | O(1A)–Co(1)–O(3) | 150.61(17) |
| O(4B)–Co(1)–O(3) | 90.63(17) | O(1A)–Co(1)–N(2) | 87.35(16) |
| O(4B)–Co(1)–N(2) | 88.52(16) | O(3)–Co(1)–N(2) | 93.46(15) |
| O(1A)–Co(1)–N(1C) | 91.28(16) | O(4B)–Co(1)–N(1C) | 92.43(16) |
| O(3)–Co(1)–N(1C) | 87.57(15) | N(2)–Co(1)–N(1C) | 178.6(2) |
| O(1A)–Co(1)–O(2) | 90.93(16) | O(4B)–Co(1)–O(2) | 150.28(16) |
| O(3)–Co(1)–O(2) | 59.68(15) | N(2)–Co(1)–O(2) | 91.82(15) |
| N(1C)–Co(1)–O(2) | 87.86(15) | | |

Symmetry code A: -x, 0.5+y, 0.5-z; B: -x, -y, -z; C: x, -0.5-y, -0.5+z.

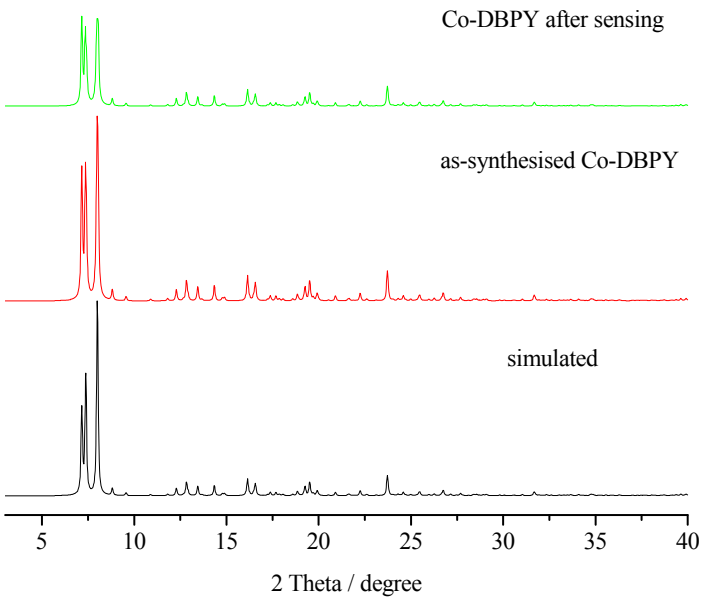
**2.4 Figure S1** The coordination mode of **dbda**$^{2-}$ ligands in Co−DBPY.



**2.5 Figure S2** TGA traces of Co−DBPY ranging from room temperature to 500 °C.

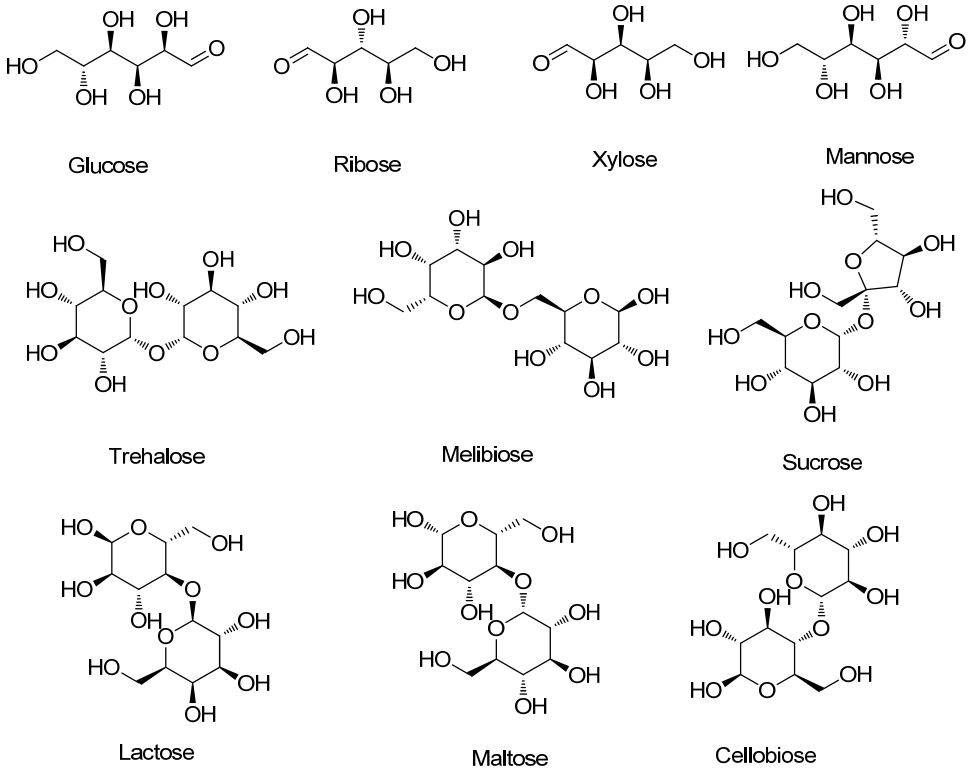


**2.6 Figure S3** Powder XRD patterns of Co−DBPY simulated from single-crystal X-ray diffraction results and the as-synthesized Co−DBPY.

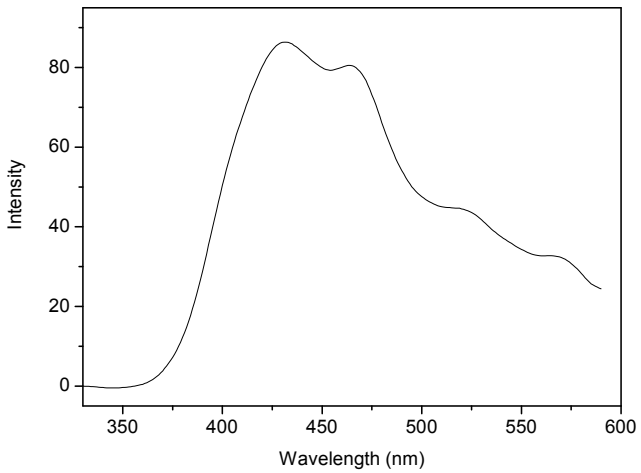


## 3. Studies on the sugar sensing based on Co−DBPY and related ligands.

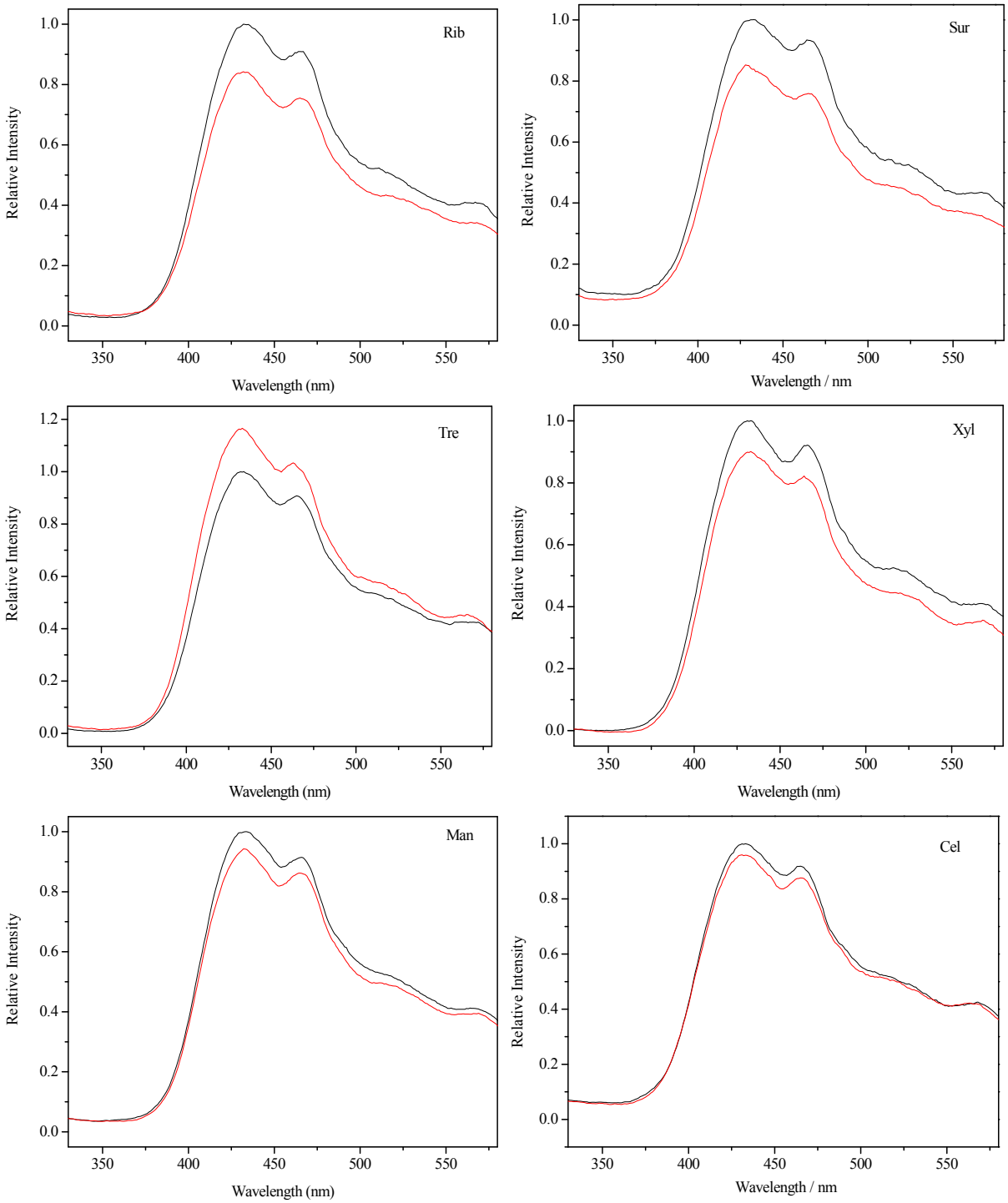
**3.1** Molecular structure of selected sugars.

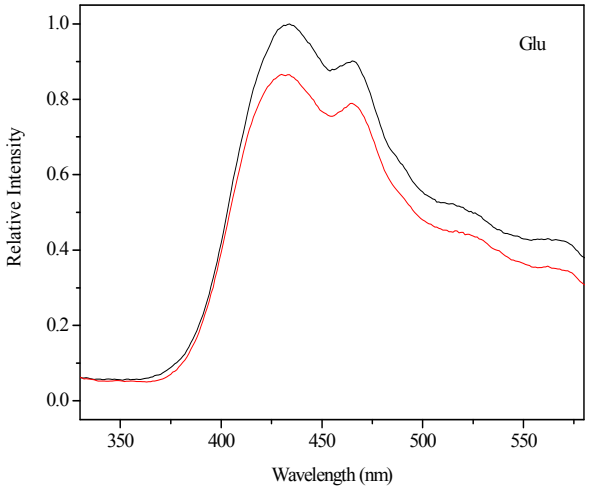


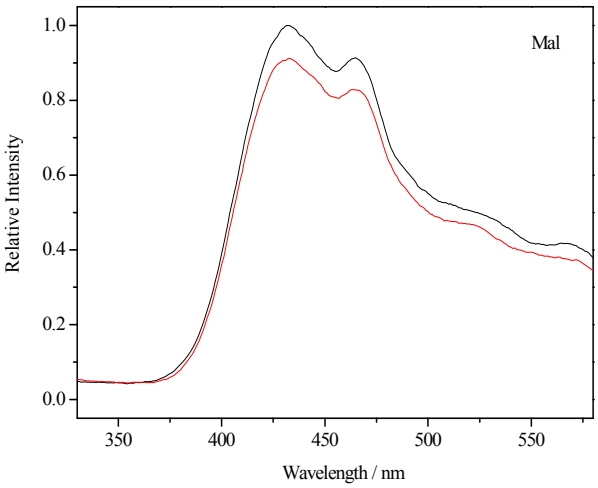

**3.2 Figure S4** The emission spectrum of Co−DBPY in HEPES buffer (pH=7.4), recorded at room temperature with the excitation at 320 nm.

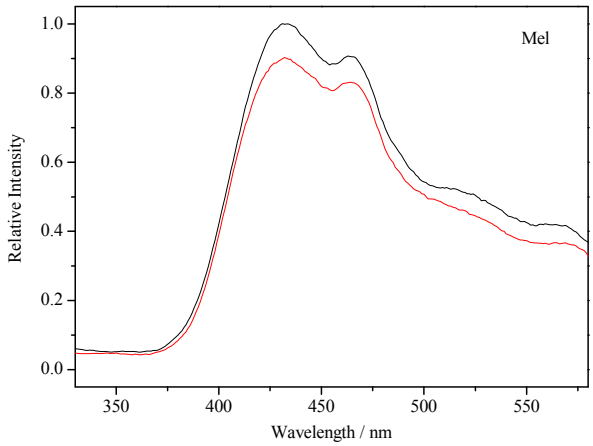


**3.3 Figure S5** Families of various fluorescence spectra of 0.33 g/L Co−DBPY in HEPES buffer solution upon the addition of 5.07 mM of different selected sugars.

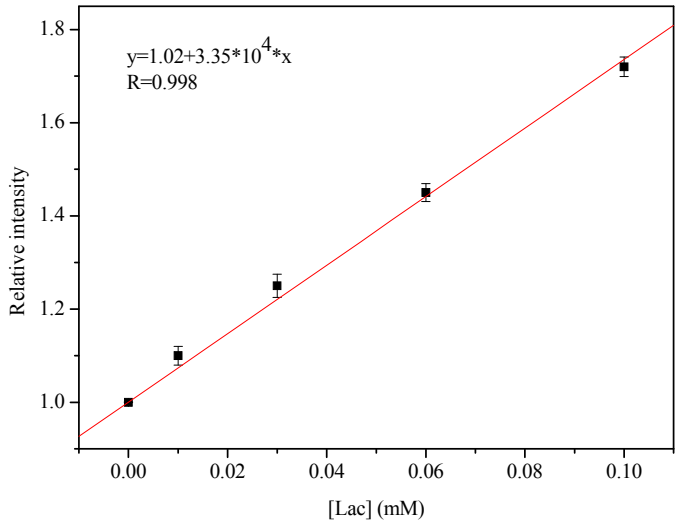



Glu
Relative Intensity
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550


Mal
Relative Intensity
Wavelength / nm
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550


Mel
Relative Intensity
Wavelength / nm
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550

**3.4 Figure S6** Plot of the relative fluorescence intensity of Co−DBPY as the concentration of Lac in the range of 0-0.1 mM.




To determine the S/N ratio, the emission intensity of Co−DBPY without Lac was measured for 10 times and the standard deviation of blank measurements was determined. Three independent duplication measurements of emission intensity were performed in the presence of Lac and each average value of the intensities was plotted as a concentration of Lac for determining the slope, in which each error bar represented the data range. The detection limit is then calculated with the following equation.

Linear Equation: $y = 3.35 \times 10^4 \times x + 1.02$; $R = 0.998$

$$S = 3.35 \times 10^4;\ \delta = \sqrt{\frac{\sum(F_0 - F_1)^2}{N-1}} = 0.335\ (N = 10);\ K = 3$$

$LOD = K \times \delta / S = 3.0 \times 10^{-5}\ M \approx 30\ \mu M$ ($F_0$ is the fluorescence intensity of Co−DBPY; $F_1$ is the average of the $F_0$.)

### 3.5 Nonlinear fitting of fluorescence intensity against Lac concentration

The following considerations apply for nonlinear fitting of fluorescence intensity against Lac concentration defined as

$$K = \frac{[HG]}{[H][G]} \qquad (1)$$

The measurements are performed under conditions where the fluorescence intensity of the free MOFs $F_0$ is proportional to the concentration $c_0$:

$$F_0 = ac_0 \qquad (2)$$

After addition of a given amount of Lac at a concentration $c_M$, the fluorescence intensity becomes

$$F = a[G] + b[HG] \qquad (3)$$

In addition to this relation, we have

$$c_0 = [H] + [HG] \qquad (4)$$

$$c_M = [G] + [HG] \qquad (5)$$

In the presence of an excess of Lac so that is fully formation the host-guest complexes, $F$ reaches the limiting value $F_{lim}$:

$$F_{lim} = bc_0 \qquad (6)$$

From eqs 1-6, it was deriving the usual relation:

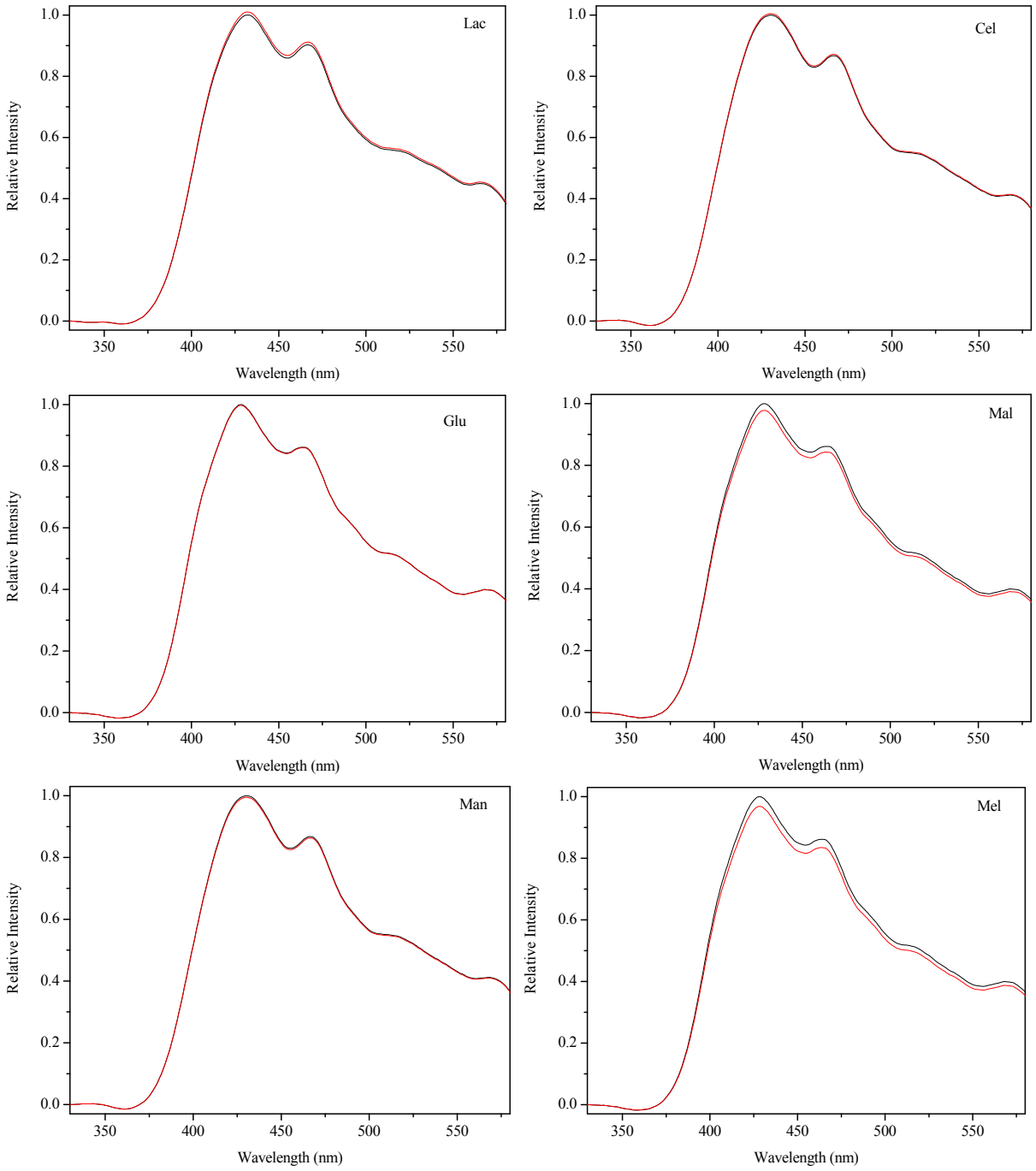
$$\frac{F_0 - F}{F - F_{lim}} = K[G] \qquad (7)$$

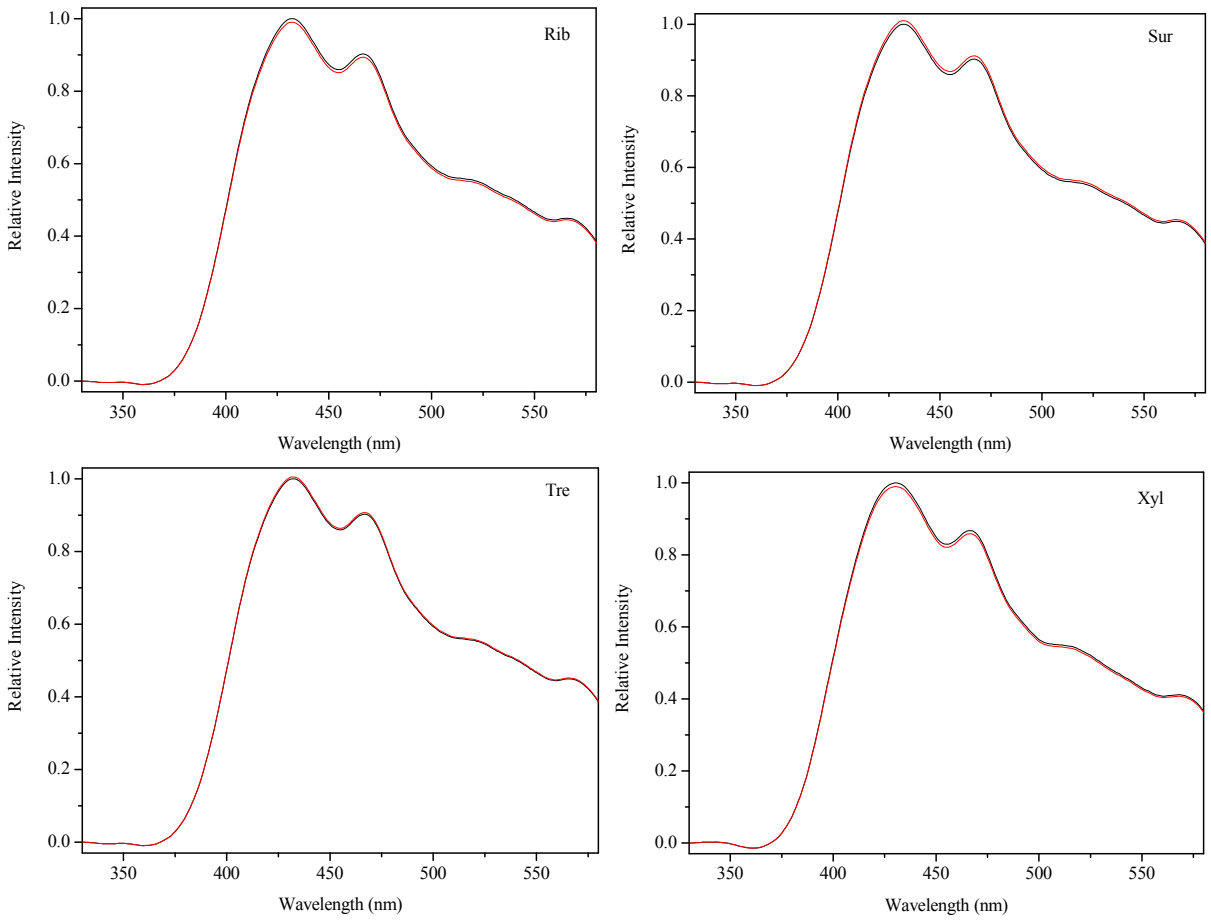
Since [G] cannot be approximated to $c_M$, then the following relation derived from the above equations are used:

$$F = F_0 + \frac{F_{lim} - F_0}{2c_0}\left[c_0 + c_M + \frac{1}{K} - \left[\left(c_0 + c_M + \frac{1}{K}\right)^2 - 4c_0c_M\right]^{1/2}\right]$$

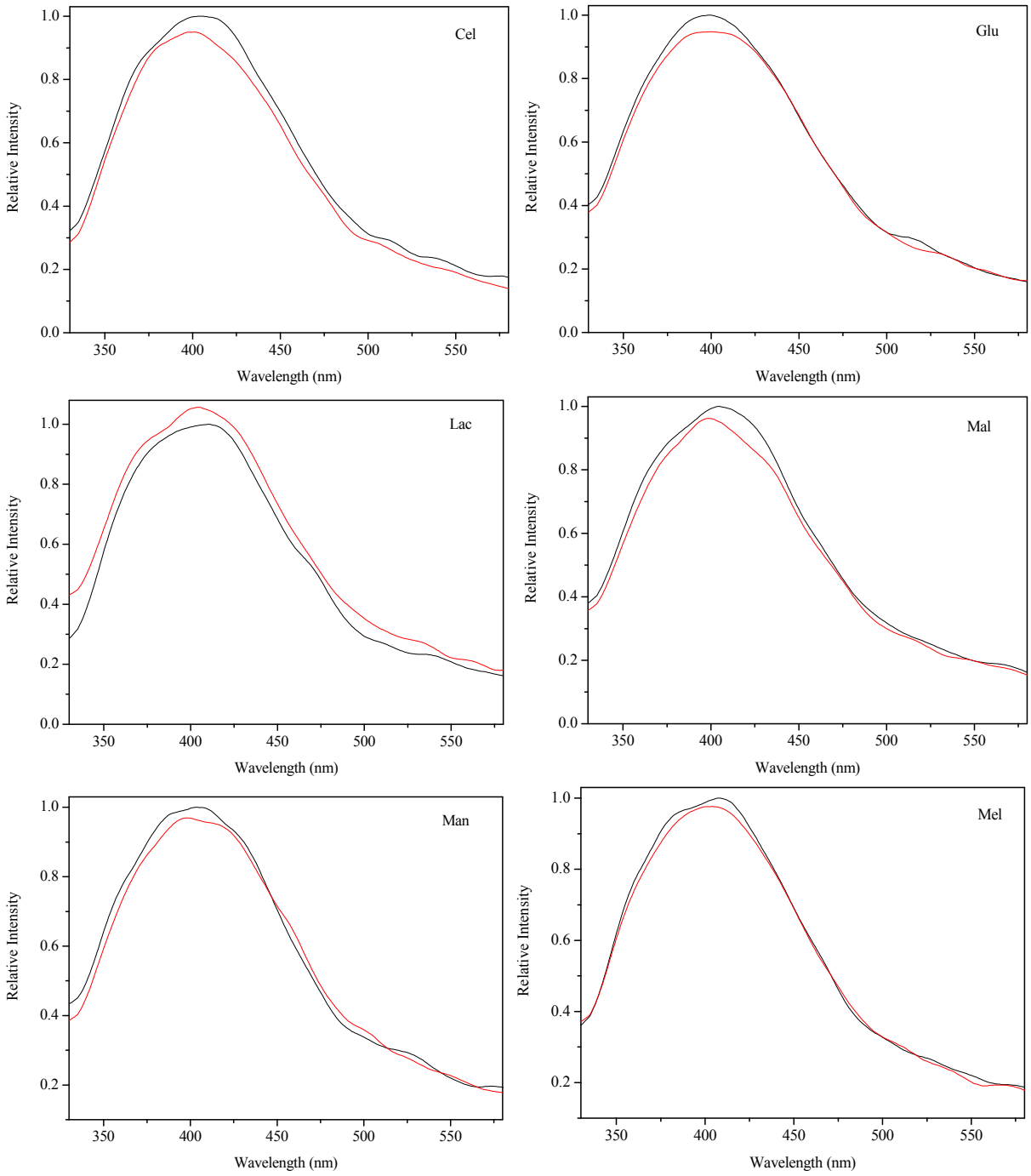
The nonlinear least-squares by analysis of $F$ versus $c_M$.

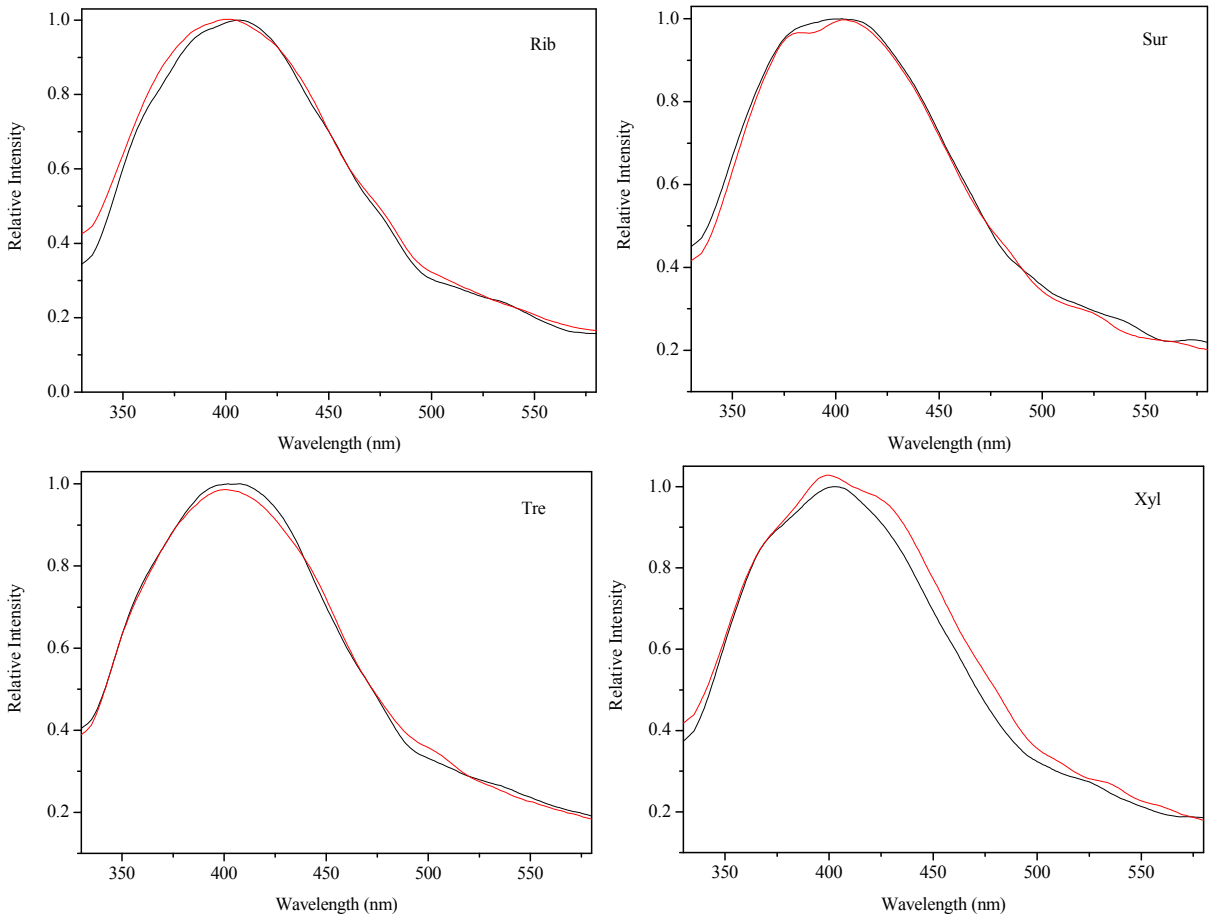
**3.6 Figure S7** Families of various fluorescence spectra of 0.33 g/L $H_2$dbda in HEPES buffer solution upon the addition of 5.07 mM of different selected sugars.




Rib
Sur
Tre
Xyl
Relative Intensity
Wavelength (nm)
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550

**3.7 Figure S8** Families of various fluorescence spectra of 0.33 g/L bpy in HEPES buffer solution upon the addition of 5.07 mM of different selected sugars.




Rib
Sur
Tre
Xyl
Relative Intensity
Wavelength (nm)
0.0
0.2
0.4
0.6
0.8
1.0
350
400
450
500
550

**3.8 Table S2** Comparison with different methods for Lac sensing.

| Entry | Methods | LOD | Ref. |
|---|---|---|---|
| 1 | Hydrophilic interaction chromatography (HILIC) method | 29 μM | [S1] |
| 2 | Electrochemical method | 0.29 μM | [S2] |
| 3 | Biostrip technology | 58 mM | [S3] |
| 4 | Biosensor method | 1.13 mM | [S4] |
| 5 | Alternative chromatographic method | 78.3 μM | [S5] |
| 6 | Electrochemical method | 10 μM | [S6] |
| 7 | Fluorescent method | 0.2 mM | [S7] |
| 8 | Fluorescent method | 30 μM | This work |

**Reference:**

S1. B. Beilmann, P. Langguth, H. Hausler and P. Grass, *J. Chromatogr. A*, 2006, **1107**, 204.

S2. X. Zhang, Y. Cao and J. Ye, *Food Chem.*, 2001, **72**, 385.

S3. S. K. Sharma, N. Sehgal and A. Kumar, *Biotechnol. Lett.*, 2002, **24**, 1737.

S4. P. P. Campos, M. L. Moraes, D. Volpati, P. B. Miranda, O. N. Oliveira, Jr. and M. Ferreira, *ACS Appl. Mater. Interfaces*, 2014, **6**, 11657.

S5. L. N. de O. Neves, R. G. de Carvalho, J. A. K. de Aguiar and P. H. F. da Silva, *Anal. Methods*, 2017, **9**, 4657.

S6. F. Lopez, S. Ma, R. Ludwig, W. Schuhmann and A. Ruff, *Electroanal.*, 2017, 29, 154.

S7. X. Zhang, S. Wang and G. Xing, *Chem. Asian J.*, 2015, **10**, 2594.