

Supporting information

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Dual off-on and on-off Fluorescent Detection of Zn²⁺/Cd²⁺ Ions Based on Carbazolone Substituted 2-Aminobenzamides

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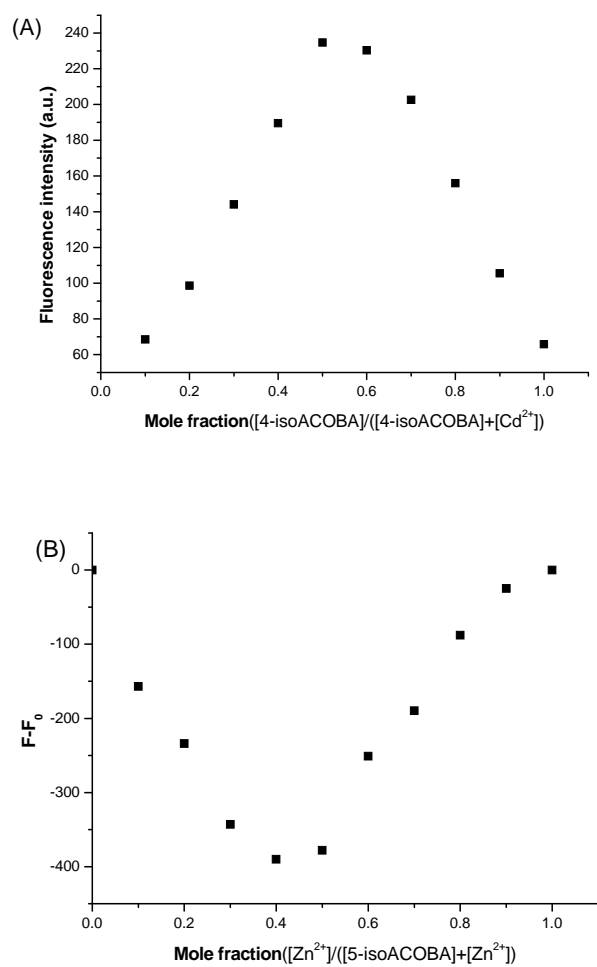


Figure S1 Job's plot for the binding between (a) 4-isoACOBA and Cd²⁺, [4-isoACOBA] + [Cd²⁺] = 0.01 mM; (b) 5-isoACOBA and Zn²⁺, [5-isoACOBA] + [Zn²⁺] = 0.01 mM.

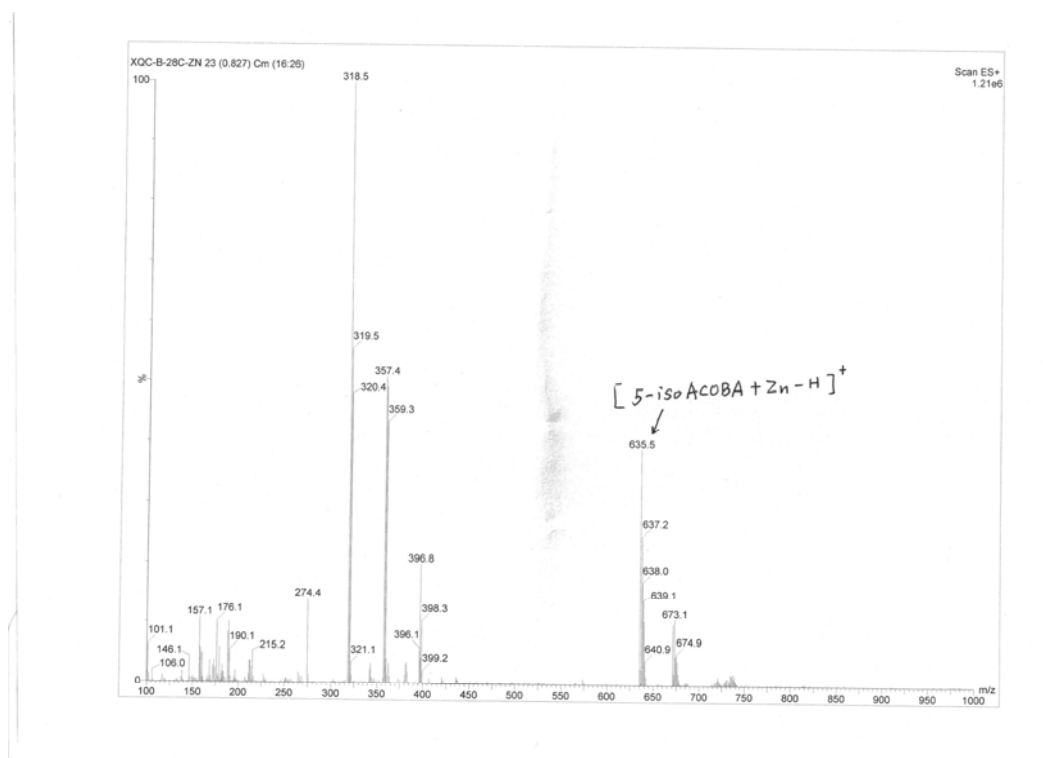
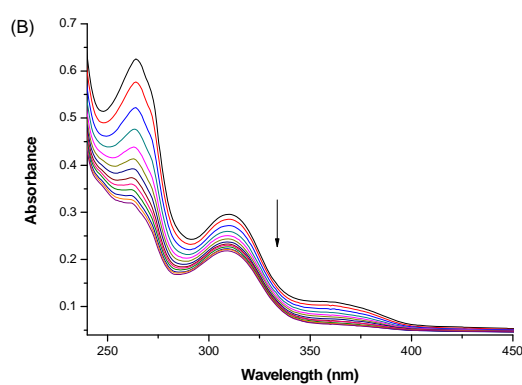
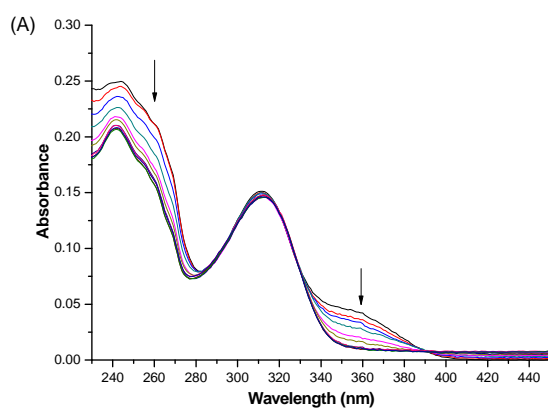
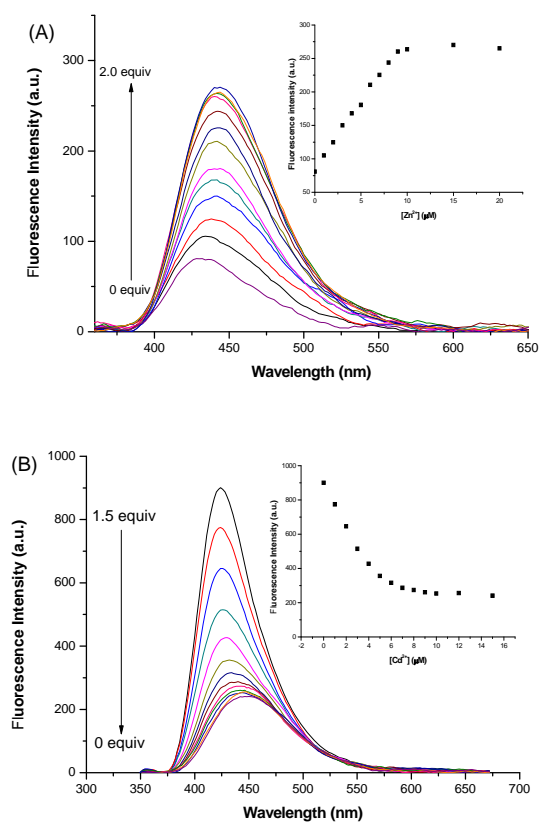


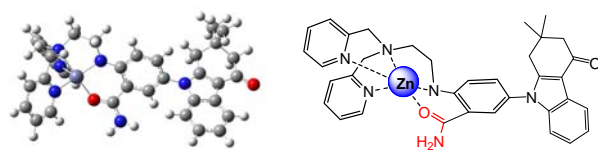
Figure S2 ESI-MS spectrum of 5-isoACOBA-Zn(II) obtained in positive mode.



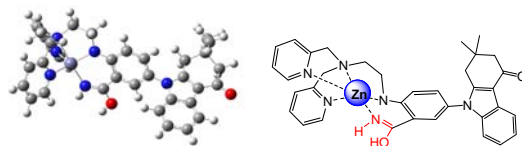
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Figure S3 UV absorption spectra of (a) 4-isoACOBA (10 μM) upon addition of Zn^{2+} (0-1.0 equiv) (b) 5-isoACOBA (10 μM) upon addition of Cd^{2+} (0-1.0 equiv) in 25 mM HEPES buffer containing 15% Ethanol (0.1 M NaClO_4 , pH = 7.4).



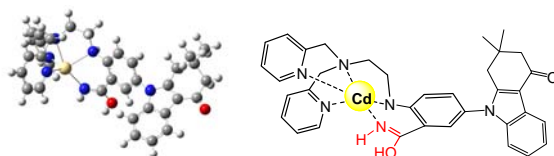
5 **Figure S4** Fluorescence spectra of (a) 4-isoACOBA upon addition of Zn²⁺ (0-2.0 equiv) and (b) 5-isoACOBA upon addition of Cd²⁺ (0-1.5 equiv). *Experimental conditions:* 4- or 5-isoACOBA (10 μM, 25 mM HEPES buffer containing 15% Ethanol, 0.1 M NaClO₄, pH = 7.4), λ_{ex} = 318 nm.



5-isoACOBZA-Zn(II), amide tautomer



5-isoACOBZA-Zn(II), imidic acid tautomer

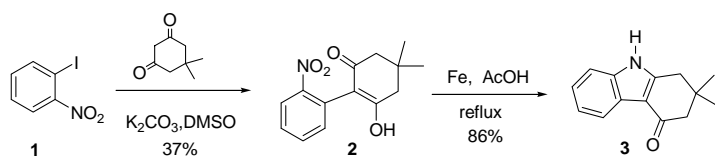


5-isoACOBZA-Cd(II), imidic acid tautomer

Figure S5 The optimized geometry structures of 5-isoACOBZA-Zn(II) and 5-isoACOBZA-Cd(II).

Experimental Section

General. All chemicals were purchased as reagent grade and used without further purification. Buchwald–Hartwig cross-coupling reactions were performed in flame-dried glassware under argon. Toluene was distilled from calcium hydride. The reactions were monitored by analytical thin-layer chromatography (TLC) on silica gel F₂₅₄ glass plates and visualized under UV light (254 and 365 nm) and/or by staining with ninhydrin. Flash column chromatography was performed on silica gel (200–300 mesh). ¹H NMR spectra were recorded with a Bruker Avance III 400 MHz NMR spectrometer at 20 °C. Chemical shifts (in ppm) were determined relative to tetramethylsilane (δ = 0 ppm) in deuteriated solvents. Coupling constants in Hz were measured from the one-dimensional spectra. ¹³C NMR or ¹³C attached-proton test (¹³C-Apt) spectra were recorded with the 400 MHz NMR spectrometer (100 MHz) and calibrated with CDCl₃ (δ = 77.23 ppm). High-resolution mass spectra were recorded with Waters LCT Premier XE or Bruker micrOTOF-QII mass spectrometer. UV absorption and emission spectra were recorded with a GBC Cintra 10e UV/Vis spectrophotometer and a Varian Cary Eclipse spectro-fluorimeter, respectively, in a quartz cell with a 1 cm path length.



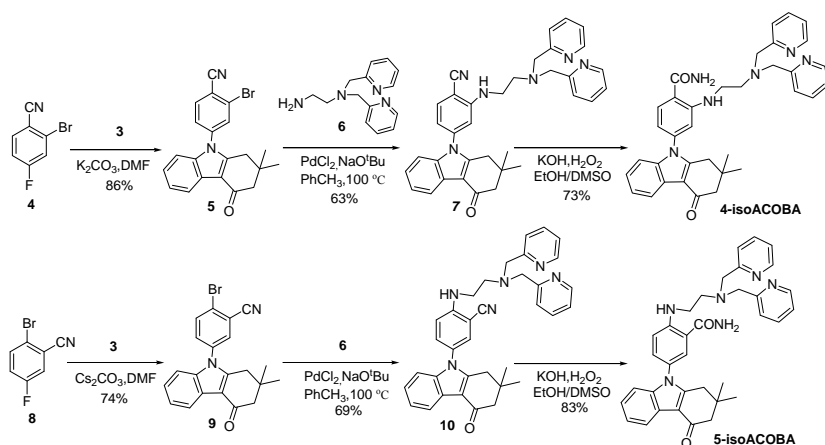
5,5-dimethyl-2-(2-nitrophenyl)-1,3-cyclohexanedione (2)

5,5-dimethyl-1,3-cyclohexanedione (341 mg, 2.43 mmol, 1.20 equiv.) was dissolved in DMSO (10 ml), and 2-iodonitrobenzene (498 mg, 2.00 mmol, 1.00 equiv.) and K₂CO₃ (994 mg, 7.20 mmol, 3.60 equiv.) were added. The reaction mixture was stirred at 85–90 °C for 5 h. After cooling to room temperature, the mixture was poured into water. The resulting solution was acidified with concentrated HCl and extracted with CH₂Cl₂. The combined organic phase was washed with saturated NaCl solution, and dried with MgSO₄. After removal of the solvent, the mixture was purified by column chromatography (CH₂Cl₂/MeOH = 200/1) to give **2** (195 mg, 0.75 mmol, 37%).

2,3-dihydro-2,2-dimethyl-1H-carbazol-4(9H)-one (3)

5,5-dimethyl-2-(2-nitrophenyl)-1,3-cyclohexanedione **2** (195 mg, 0.75 mmol, 1.00 equiv.) was taken in acetic acid (3.75 mL) and heated until the solution became homogeneous. Fe powder (250.2 mg, 4.48 mmol, 6.00 equiv.) was

added and the mixture was heated to reflux temperature until the starting material had been completely consumed as detected by TLC. After cooling to room temperature and acetic acid was removed in vacuo. The resulting mixture was diluted with EtOAc, and filtered through Celite to remove any iron impurities. The filtrate was washed with saturated NaCl solution, dried with MgSO₄, and the resulting crude product was purified by column chromatography to give **3** (138 mg, 0.647 mmol, 86%).



2-bromo-4-(2,2-dimethyl-4-oxo-1,2,3,4-tetrahydrocarbazol-9-yl)benzonitrile (**5**):

2,3-dihydro-2,2-dimethyl-1H-carbazol-4(9H)-one **3** (69 mg, 0.32 mmol, 1.00 equiv.) was dissolved in DMF (4 ml), and 2-bromo-4-fluorobenzonitrile (96 mg, 0.48 mmol, 1.50 equiv.) and K₂CO₃ (132 mg, 3.00 mmol, 3.00 equiv.) were added. The reaction mixture was stirred in a 60 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution, and dried with MgSO₄. After removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc = 4/1) to give **5** (108 mg, 0.92 mmol, 86%) as a colorless solid. Mp. 241–242 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.31 (d, *J* = 7.8 Hz, 1 H), 7.90 (d, *J* = 8.2 Hz, 1 H), 7.79 (d, *J* = 1.9, 1 H), 7.50 (dd, *J* = 1.9, 8.3 Hz, 1 H), 7.35 (td, *J* = 0.8, 7.9 Hz, 1 H), 7.28 (td, *J* = 1.2, 8.2 Hz, 1 H), 7.18–7.20 (m, 1 H), 2.70 (s, 2 H), 2.51 (s, 2 H), 1.16 (s, 6 H). ¹³C-Apt (100 MHz, CDCl₃): δ 193.7, 149.5, 137.5, 135.5, 131.7, 126.6, 126.4, 124.9, 124.1, 123.8, 122.0, 116.3, 115.8, 113.8, 109.9, 52.1, 37.1, 35.7, 28.6. HRMS (ESI-TOF): *m/z* Calcd for C₂₁H₁₈BrN₂O [M+H]⁺ 393.0602, found: 393.0597.

2-((2-[bis(pyridin-2-ylmethyl)amino]ethyl)amino)-4-(2,2-dimethyl-4-oxo-1,2,3,4-tetrahydrocarbazol-9-yl)benzamide (4-isoACOBA):

A Schlenk flask was charged with compound **5** (94 mg, 0.24 mmol, 1.00 equiv.), BPEA **6** (58 mg, 0.24 mmol, 1.00 equiv.), sodium *tert*-butoxide (58 mg, 0.60 mmol, 2.50 equiv.), palladium chloride (8.5 mg, 0.048 mmol, 0.20 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (DPPF; 26.6 mg, 0.048 mmol, 0.20 equiv.), and toluene (10 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH = 30/1) on silica gel to give **7** (83 mg, 0.15 mmol, 63%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 8.56 (d, *J* = 4.8 Hz, 2 H), 8.28 (d, *J* = 7.8, 1 H), 7.70 (t, *J* = 7.7, 2 H), 7.58-7.61 (m, 3 H), 7.30 (t, *J* = 7.2 Hz, 1 H), 7.22 (t, *J* = 8.1 Hz, 1 H), 7.15 (t, *J* = 6.2 Hz, 3 H), 6.62 (dd, *J* = 1.6, 8.1 Hz, 1 H), 6.53 (d, *J* = 1.4 Hz, 1 H), 6.27 (bs, 1 H), 3.93 (s, 4 H), 3.22-3.23 (m, 2 H), 2.93 (t, *J* = 5.1 Hz, 2 H), 2.66 (s, 2 H), 2.45-2.46 (m, 2 H), 1.12 (s, 6 H). Then, KOH (50 mg, 0.89 mmol, 8.12 equiv.) and compound **7** (60 mg, 0.11 mmol, 1.00 equiv.) were added to a solution of EtOH and DMSO (4/1, 2.5 mL). The reaction mixture was stirred in a 40 °C oil bath, and H₂O₂ (30%, 0.3 mL) was slowly added dropwise through a syringe over 0.5 h. Then the resulting solution was stirred for another 2.0 h until the disappearance of benzonitrile as shown by TLC. After removal of the solvent by rotary evaporation, the mixture was diluted with DCM (150 mL), washed with saturated NaCl solution, dried with MgSO₄, filtered, and concentrated. The mixture was purified by column chromatography (CH₂Cl₂/MeOH = 20/1) to give 4-isoACOBA (44 mg, 0.08 mmol, 73%) as a pale yellow powder. Mp. 199–200 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.44 (d, *J* = 4.3 Hz, 2 H), 8.30 (d, *J* = 7.8 Hz, 1 H), 7.74 (d, *J* = 7.8 Hz, 2 H), 7.65 (t, *J* = 1.4, 2 H), 7.59 (d, *J* = 8.6 Hz, 1 H), 7.31 (t, *J* = 7.0 Hz, 1 H), 7.21 (t, *J* = 7.2 Hz, 1 H), 7.15 (s, 1 H), 7.11 (t, *J* = 6.2 Hz, 2 H), 7.52-6.54 (m, 2 H), 5.99 (bs, 2 H), 3.98 (s, 4 H), 3.32 (s, 2 H), 2.67 (s, 2 H), 2.93 (s, 2 H), 2.49 (s, 2 H). ¹³C-Apt (100 MHz, CDCl₃): δ 193.8, 171.1, 158.9, 150.9, 150.5, 148.8, 140.6, 138.1, 136.7, 130.0, 124.7, 123.4, 123.3, 123.0, 122.2, 121.6, 113.9, 112.8, 112.7, 110.6, 110.1, 60.5, 52.4, 52.3, 40.5, 37.1, 35.6, 29.7, 28.6. HRMS (ESI-TOF): *m/z* Calcd for C₃₅H₃₇N₆O₂ [M+H]⁺ 573.2978, found: 573.2969.

2-bromo-5-(2,2-dimethyl-4-oxo-1,2,3,4-tetrahydrocarbazol-9-yl)benzotrile (9):

2,3-dihydro-2,2-dimethyl-1H-carbazol-4(9H)-one **3** (292 mg, 1.37 mmol, 1.00 equiv.) was dissolved in DMF (10 ml), and 2-bromo-5-fluorobenzotrile (548 mg, 2.74 mmol, 2.00 equiv.) and Cs₂CO₃ (1.33 g, 4.11 mmol, 3.00 equiv.) were added. The reaction mixture was stirred at room temperature for 30 min and then heated in an 80 °C oil bath until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum to leave a yellowish oil. The crude oil was then diluted with DCM (150 mL), washed with saturated NaCl solution, and dried with MgSO₄. After removal of the solvent, the mixture was purified by column chromatography (hexanes/EtOAc = 4/1) to give **9** (400 mg, 1.02 mmol, 74%) as a colorless solid. Mp. 209–210 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.30 (d, *J* = 7.6 Hz, 1 H), 7.95 (d, *J* = 8.5 Hz, 1 H), 7.71 (d, *J* = 1.6 Hz, 1 H), 7.53–7.56 (m, 1 H), 7.31 (t, *J* = 7.2 Hz, 1 H), 7.25 (t, *J* = 7.6 Hz, 1 H), 7.11–7.13 (m, 1 H), 2.62 (s, 2 H), 2.39 (s, 2 H), 1.12 (s, 6 H). ¹³C-Apt (100 MHz, CDCl₃): δ 193.6, 149.9, 137.8, 136.1, 135.0, 132.9, 132.6, 125.4, 124.7, 124.0, 123.6, 121.8, 117.7, 116.0, 113.4, 109.8, 52.0, 36.8, 35.6, 28.6. MS (ESI): *m/z* [M+H]⁺ 393.06.

2-((2-[bis(pyridin-2-ylmethyl)amino]ethyl)amino)-5-(2,2-dimethyl-4-oxo-1,2,3,4-tetrahydrocarbazol-9-yl)benzamide (5-isoACOBA):

A Schlenk flask was charged with compound **9** (114 mg, 0.29 mmol, 1.05 equiv.), BPEA **6** (78 mg, 0.32 mmol, 1.11 equiv.), sodium *tert*-butoxide (70 mg, 0.73 mmol, 2.50 equiv.), palladium chloride (10.3 mg, 0.58 mmol, 0.20 equiv.), 1,1'-bis(diphenylphosphanyl)ferrocene (DPPF; 32.2 mg, 0.58 mmol, 0.20 equiv.), and toluene (10 mL) under argon. The flask was immersed in an oil bath at 100 °C with stirring until the starting material had completely disappeared as judged by TLC analysis. The solution was then allowed to cool to room temperature, diluted with DCM (100 mL), filtered through Celite, and concentrated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH = 30/1) on silica gel to give **10** (111 mg, 0.20 mmol, 69%) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 8.61 (d, *J* = 4.3 Hz, 2 H), 8.27 (d, *J* = 7.8 Hz, 1 H), 7.73 (td, *J* = 1.6, 7.7 Hz, 2 H), 7.61–7.63 (m, 2 H), 7.40 (d, *J* = 2.4 Hz, 1 H), 7.28–7.33 (m, 2 H), 7.19–7.23 (m, 3 H), 7.06 (d, *J* = 8.1, 1 H), 6.72 (d, *J* = 9.0, 1 H), 6.29 (s, 1 H), 3.95 (s, 4 H), 3.34–3.35 (m, 2 H), 2.98 (t, *J* = 5.7 Hz, 2 H), 2.61 (s, 2 H), 2.47 (s, 2 H), 1.13 (s, 6 H).

Next, KOH (100 mg, 1.79 mmol, 8.95 equiv.) and compound **10** (111 mg, 0.20 mmol, 1.00 equiv.) were added to a solution of EtOH and DMSO (4/1, 2.0 mL). The reaction mixture was stirred in a 40 °C oil bath, and H₂O₂ (30%, 0.5 mL) was slowly added dropwise through a syringe over 0.5 h. Then the resulting solution was stirred for another 2.0 h until the disappearance of benzonitrile as shown by TLC. After removal of the solvent by rotary evaporation, the mixture was diluted with DCM (150mL), washed with saturated NaCl solution, dried with MgSO₄, filtered, and concentrated. The mixture was purified by column chromatography (CH₂Cl₂/MeOH = 20/1) to give 5-isoACOBA (95 mg, 0.166 mmol, 83%) as a colorless solid. Mp. >250 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.69 (bs, 1 H), 8.46 (d, *J* = 4.0 Hz, 2 H), 8.03 (d, *J* = 7.2 Hz, 1 H), 7.92 (bs, 1 H), 7.78-7.80 (m, 2 H), 7.71-7.73 (m, 3 H), 7.43 (bs, 1 H), 7.30 (d, *J* = 8.7 Hz, 1 H), 7.16-7.24 (m, 4H), 7.09 (d, *J* = 7.5 Hz, 1H), 6.76 (d, *J* = 8.9 Hz, 1 H), 3.81 (s, 4 H), 3.15 (s, 2 H), 2.70 (d, *J* = 13.9 Hz, 4 H), 2.37 (s, 2 H), 1.05 (s, 6 H). ¹³C-Apt (100 MHz, DMSO-*d*₆): δ 192.9, 171.2, 159.6, 152.3, 150.1, 149.1, 138.9, 136.9, 131.7, 128.2, 124.3, 123.3, 123.2, 122.8, 122.6, 121.9, 120.7, 114.8, 112.6, 111.2, 60.1, 52.4, 52.3, 36.4, 35.4, 28.6. HRMS (ESI-TOF): *m/z* Calcd for C₃₅H₃₇N₆O₂ [M+H]⁺ 573.2978, found: 573.2963.

Spectroscopic Materials and Methods: Stock solutions (0.5 mM) of zinc perchlorate and cadmium sulphate were prepared in deionized water respectively. Stock solutions (1.0 mM) of 4-isoACOBA was prepared in Ethanol and 5-isoACOBA was prepared in DMSO. All the fluorescence spectra of probes were measured in HEPES buffer (25 mM HEPES, 0.1 M NaClO₄, pH = 7.4, 15% (v/v) ethanol) and the excitation wavelength was 318 nm with excitation and emission slit widths of 5 nm at room temperature.

DFT Calculation: The ground state structures of sensors were optimized using density functional theory (DFT) with B3LYP functional and 6-31G (d) basis set. No imaginary frequencies were found in frequency analysis of all calculated structures. All calculations were performed using the Gaussian 09.¹

Reference:

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Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09; Revisions A.02 ed., Gaussian, Inc.: Wallingford CT, 2009.

