SUPPORTING INFORMATION

A Highly Selective Fluorescent Sensor for Glucosamine

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Synthesis procedures:

Methyl 3-(2-chloro-5-fluoro-4-methoxyphenyl)-3-oxopropanoate 4:[1] To a solution of NaH 60% (1.96g, 49 mmol) in THF (15 ml) was added dimethyl carbonate (2.836 g, 31.39 mmol) at room temperature. The reaction was refluxed and allowed to stir for 5 minutes, followed by addition of 2'-chloro-4',5'-difluoroacetophenone (2 g, 10.49 ml) in THF (10 ml) dropwise. After 10 h at boiling point, the reaction was cooled to room temperature and slowly added to a mixture of diluted acetic acid (2M) in ice until the evolution of gas ceased. The obtained mixture was extracted with Et_2O (100 ml, 3 times), dried with Na_2SO_4, filtered and concentrated. Purification by flash column chromatography (ethyl acetate:hexane 5:95) followed by recrystallization (hexane: DCM) delivered **4** (2.619 g, 96%) as white crystals. R_f = 0.42 (ethyl acetate:hexane 10:90). The NMR was consistent with a mixture of ketone and enol forms (ketone:enol 3:1). ^{1}H NMR was calibrated for four molecules of **4.**
^{1}H NMR (500 MHz, $CDCl_3$): δ 12.41 (s, 1H), 7.50 (d, 3H, *J* = 10 Hz), 7.35 (d, 1H, *J* = 10 Hz), 6.96 (d, 1H, *J* = 10 Hz), 6.95 (d, 3H, *J* = 10 Hz), 5.63 (s, 1H), 4.02 (s, 6H), 3.91 (s, 9H), 3.88 (s, 3H), 3.77 (s, 3H), 3.71 (s, 9H)
^{13}C NMR (125 MHz, $CDCl_3$): 191.1, 173.0, 168.5, 167.5, 151.5, 151.1, 151.0, 159.5, 149.2, 149.1, 128.7, 128.6, 127.4, 125.2, 118.1, 118.0, 117.1, 117.0, 115.3, 115.2, 92.6, 56.5, 56.4, 52.3, 51.5, 48.6
IR (DCM, cm^{-1}): 2993, 2939, 2844, 1737, 1657, 1603, 1386, 1153, 684
HRMS calculated for $C_{11}H_{10}FClO_4Na^+$ [M + Na^+]: 283.0144, found 283.0143

4-(2-chloro-5-fluoro-4-methoxyphenyl)-7-(diethylamino)-2H-chromen-2-one 5:[2] In a glass tube, 3-diethylaminophenol (0.76 g, 4.60 mmol), β-ketoester **4** (1 g, 3.84 mmol) and of $CoPy_2Cl_2$ (0.333 g, 1.156 mmol) were stirred under nitrogen and solvent free conditions at 110^0C for 24 hours. The reaction was cooled to room temperature and followed by addition of ethyl acetate (100 ml) and stirred for 4 minutes. The mixture was filtered and evaporated solvent. Purification by flash column chromatography (ethyl acetate:DCM 5:95) delivered a crude product. Recrystallization (DCM: n-pentene) afforded pure product (0.64 g, 45%) as light yellow crystals. R_f = 0.63 (ethyl acetate:hexane 25:75).
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.09 (d, 1H, *J* = 10 Hz), 7.03 (d, 1H, *J* = 10 Hz), 6.88 (d, 1H, *J* = 10 Hz), 6.55 (d, 1H, *J* = 2 Hz), 6. 49 (dd, 1H, *J* = 10 Hz, 2 Hz), 5.96 (s, 1H), 3.95 (s, 3H), 3.40 (q, 4H, *J* = 7 Hz), 1.20 (t, 6H, *J* = 7 Hz)
^{13}C NMR (125 MHz, $CDCl_3$): 161.8, 156.5, 152.4, 150.8 (d, J_{F-C} = 246 Hz), 150.7, 148.5 (d, J_{F-C-C} = 11 Hz), 127.6, 127.0 (d, J_{F-C-C}= 6 Hz), 117.6, 117.4, 114.9, 109.9, 108.6, 107.8, 97.7, 56.6, 44.8, 12.4
IR (DCM, cm^{-1}): 2968, 2925, 1705, 1619, 1508, 1411, 830
HRMS calculated for $C_{20}H_{19}FClNO_3Na^+$ [M + Na^+]: 398.0930, found 398.0925

7-(diethylamino)-4-(5-fluoro-4-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2H-chromen-2-one 6:[3] In a sealed tube, the mixture of coumarine derivative **5** (100 mg, 0.2563 mmol), bis(pinacolato)diboron (92.3 mg, 0.3848 mmol), [1,1'-bis(diphenyl phosphine)-ferrocene] dichloropalladium (II) in DCM (21.53 mg, 0.0264 mmol), 1,1'-bis(diphenylphosphino)-ferrocene (14.15 mg, 0.0260 mmol) and KOAc (38.5 mg, 0.3923 mmol) were flushed with nitrogen, followed by addition of dry THF (2ml). After being stirred at 105^0C for 48 hours in nitrogen condition, the mixture was evaporated solvent. Purification by flash column chromatography (ethyl acetate:DCM 5:95), followed by recrystallization (hexane:DCM) delivered pure product (55 mg, 46%) as light yellow crystals. R_f = 0.5 (ethyl acetate:hexane 25:75).
^{1}H NMR (500 MHz, CDCl3): δ 7. 73 (d, 1H, *J* = 10 Hz), 7.09 (d, 1H, *J* = 10 Hz), 6.96 (d, 1H, *J* = 10 Hz), 6.57 (d, 1H, *J* = 2 Hz), 6.48 (dd, 1H, *J* = 10 Hz, 2 Hz), 5.96 (s, 1H), 4.01 (s, 3H), 3.42 (q, 4H, *J* = 7 Hz), 1.20 (t, 6H, *J* = 7 Hz), 1.16 (s, 6H), 1.01 (s, 6H)
^{13}C NMR (125 MHz, CDCl3): 162.2, 156.3, 153.6 (J_{F-C} = 250 Hz), 150.4, 147.4, 147.3, 135.2 (d, J_{F-C-C} = 6 Hz) , 127.7, 119.6 (d, J_{F-C-C} = 3 Hz), 116.8, 116.7, 110.0, 109.0, 108.4, 97.7, 84.0, 56.4, 44.8, 24.5, 24.4, 12.4
IR (DCM, cm^{-1}): 2979, 2925, 2358, 1724, 1612, 1522, 1368, 1260, 1142, 859
HRMS calculated for $C_{26}H_{31}FBNO_5Na^+$ [M + Na^+]: 490.2171, found 490.2163

7-(diethylamino)-4-(5-fluoro-4-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-oxo-2H-chromene-3-carbaldehyde 7:[4] A mixture of $POCl_3$ (0.952 ml, 10.2 mmol) and DMF (1.314 ml, 17.04 mmol) was stirred at 0^oC for 45 minutes under nitrogen condition. 0.0453 ml of obtained mixture was added dropwise to a solution of pinacol ester derivative of coumarin **6** (43 mg, 0.075 mmol) in DMF (1ml) at 0^oC. The reaction was allowed to stir for 30 minutes at 0^oC under nitrogen condition before being warmed up to room temperature and stirred under nitrogen condition for 20 hours. The mixture was quickly added to ice water (300 ml) and extracted with hexane. The obtained water layer was extracted with DCM (100 ml, 3 times), dried with Na_2SO_4, filtered and concentrated to give yellow mixture (40 mg). Purification of 2 mg of obtained mixture by HPLC (Zorbax Rx-C8 9.4 mm x 25 cm, MeOH: H_2O 70:30 to 100:0) delivered pure product (1.2 mg, 65%) as a yellow solid.
^{1}H NMR (500 MHz, CDCl3): δ 9.87 (s, 1H), 7. 46 (d, 1H, *J* = 10 Hz), 6.91 (d, 1H, *J* = 10 Hz), 6.75 (d, 1H, *J* = 10 Hz), 6.47 (d, 1H), 6.42 (d, 1H, *J* = 10 Hz), 3.98 (s, 3H), 3.40 (q, 4H), 1.15 (m, 6H), 1.07 (s, 6H), 0.94 (s, 6H)
^{13}C NMR (125 MHz, CDCl3): 188.4, 161.1(J_{F-C} = 158 Hz), 157.4, 154.7, 152.6, 147.3, 147.2, 132.8 (d, J_{F-C-C}= 7 Hz), 130.6, 119.9, 116.6, 116.5, 113.0, 110.6, 109.4, 96.9, 83.9, 56.3, 45.1, 24.6, 24.3, 12.4
IR (DCM, cm^{-1}): 2922, 2843, 1729, 1602, 1566, 1501, 1450, 1370
HRMS calculated for $C_{27}H_{31}BFNO_6Na^+$ [M + Na^+]: 518.2121, found 518.2118

Fluorescence titration

UV-Vis spectra were measured on Cary 100 Bio UV-Vis spectroscopy. Fluorescence emission spectra were obtained using a Shimadzu RF 5301 PC spectrofluorometer. A 10^{-5} M solution of the sensor **2** was prepared in buffer solution (120 mM NaCl, 25 mM HEPES, pH= 7.4). For norepinephrine, a 25 mM HEPES, 50 mM $Na_2S_2O_3$ buffer solution was used due to unstability of norepinephirine. All the titration experiments were carried out slowly at 37 ^{0}C, pH = 7.4 under neutral aqueous conditions. The excited wavelength was 488 nm with slit widths of 3 nm. Binding constants and maximum changes in fluorescence were obtained from GraphPad Prism 5.0 by fitting the fluorescence titration to a single-site binding isotherm.

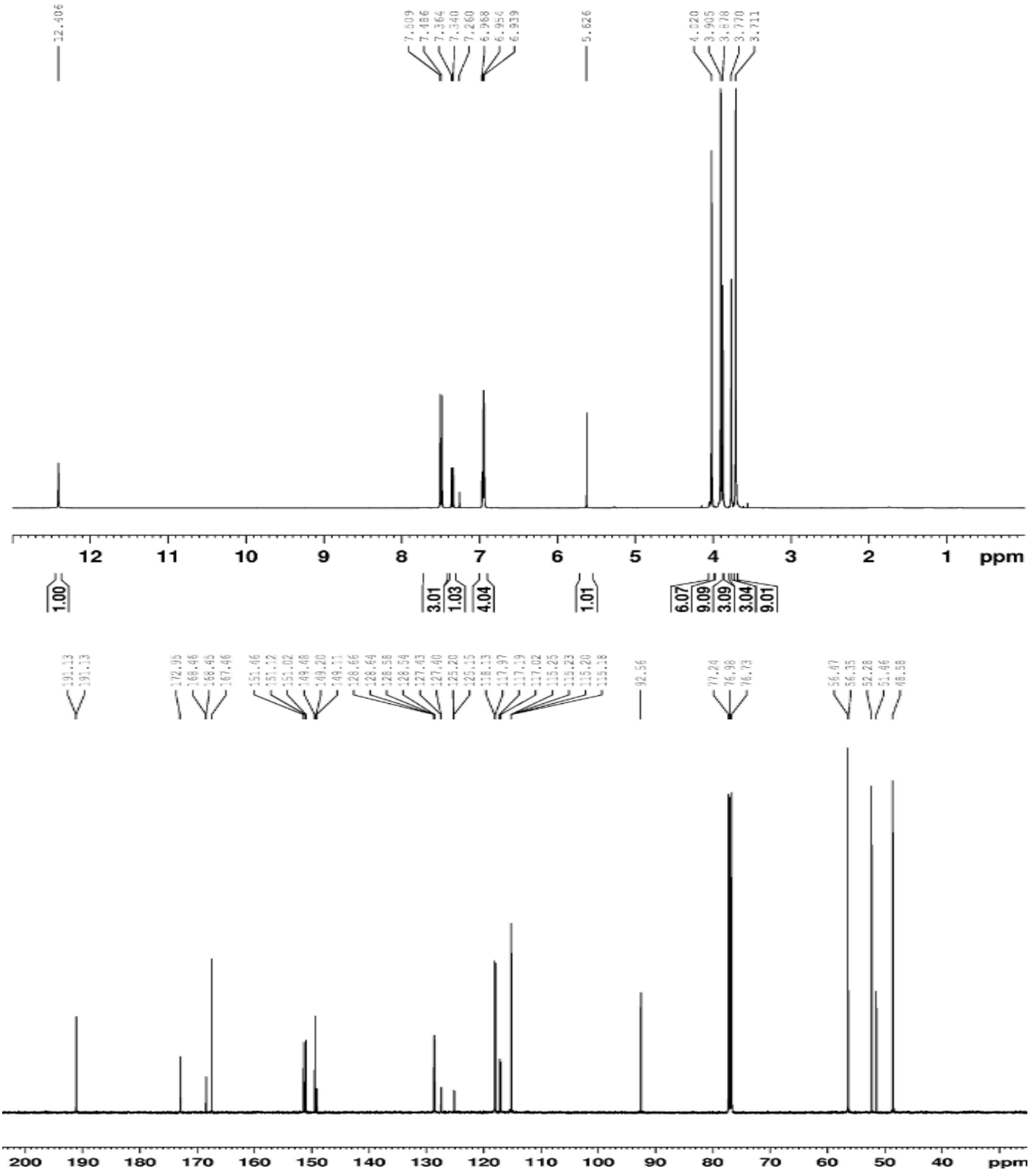
References

(1) Ni, C-L.; Wang, H-Q.; Yan, H. *Youji. Huaxue.,* **2006**, 26(3), 357-359

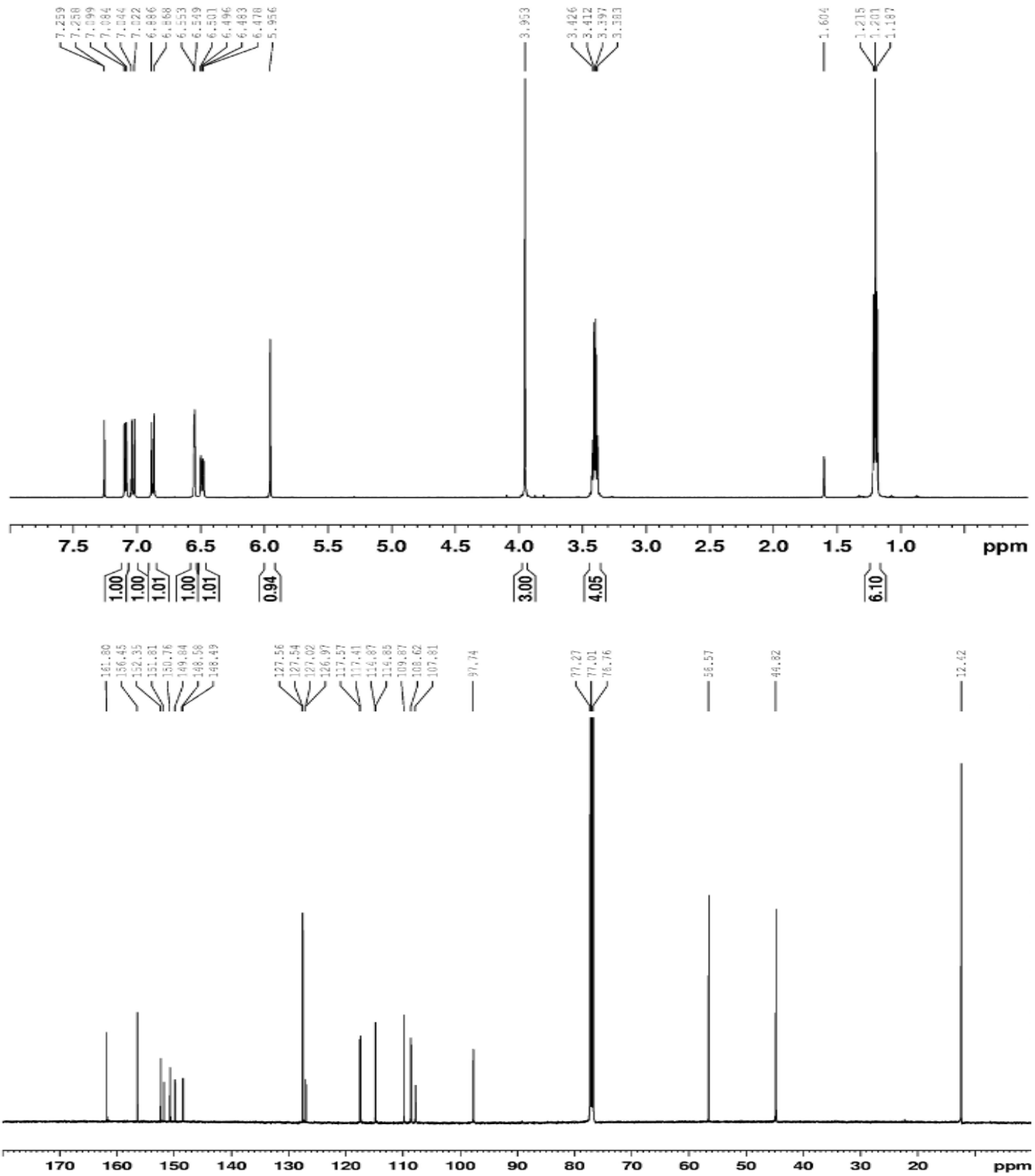
(2) Madhav, J.; Kuarm, B.; Someshwar, P.; Rajitha, B.; Reddy, Y.; Crooks, P. *J. Chem. Res.* **2008**, 4, 232-234

(3) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, 60, 7508-7510

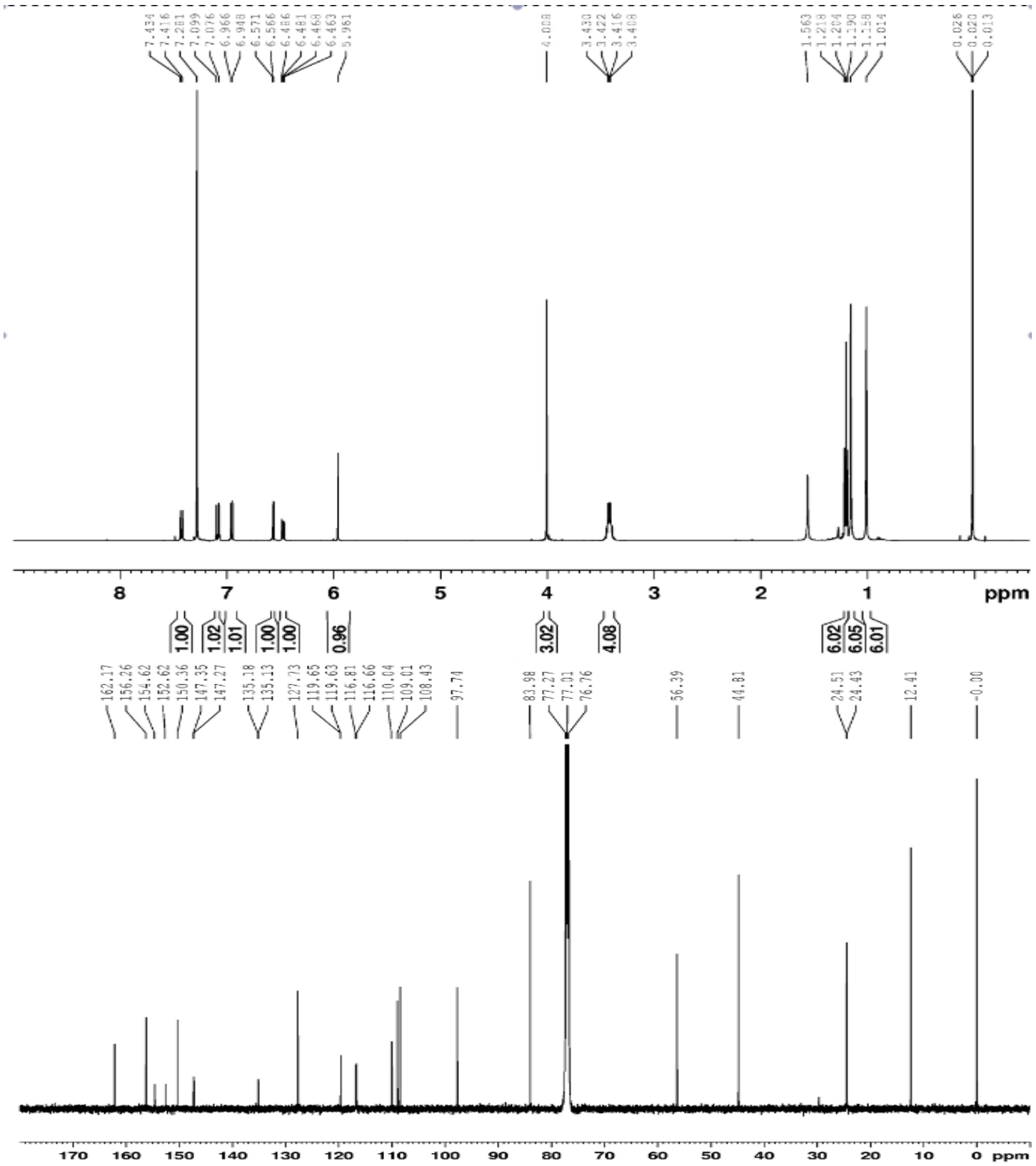
(4) Rajanna, K.; Solomon, F.; Ali, M. *Int. J. Chem. Kinet.* **1996**, 28 (12), 865-872



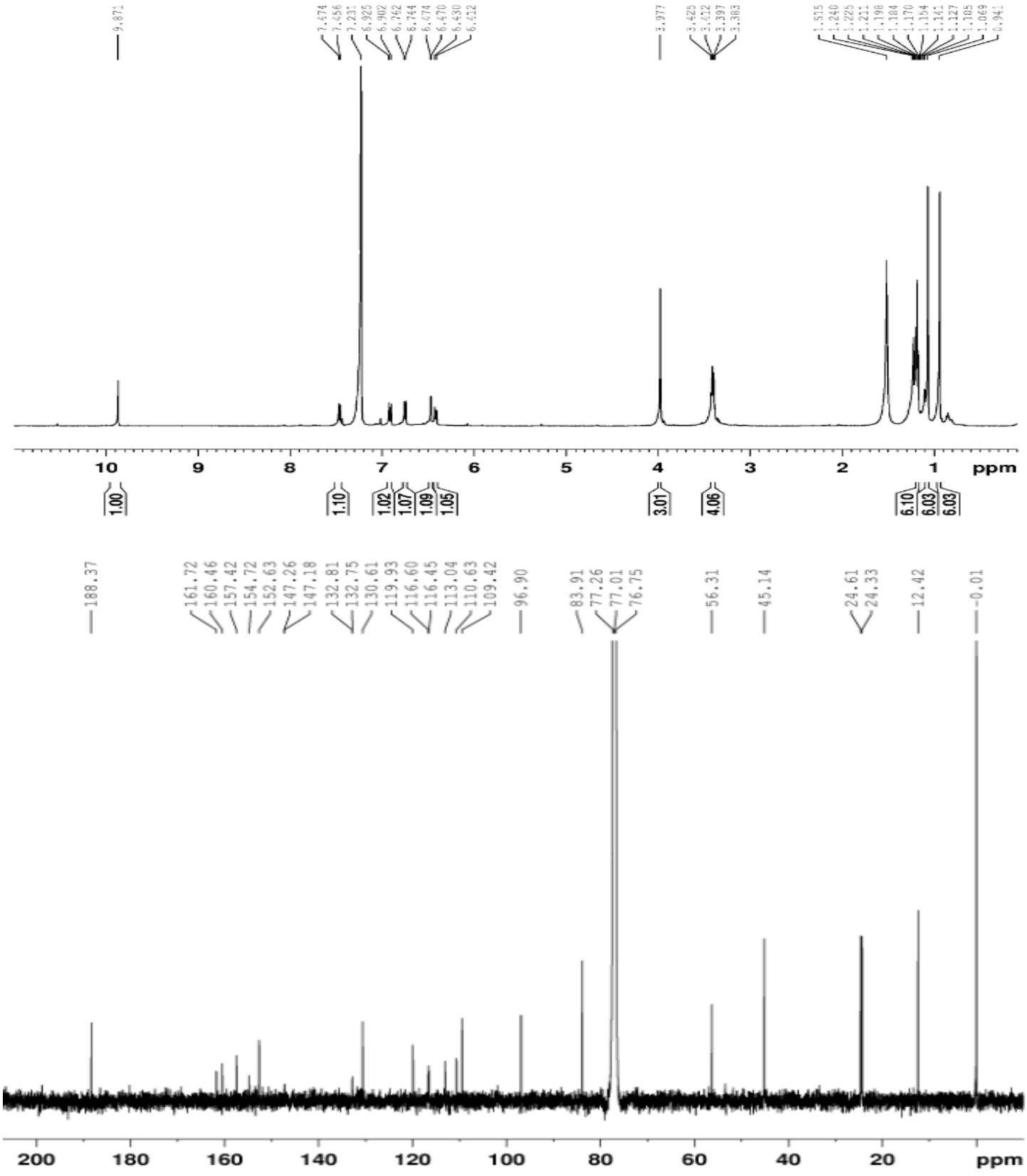
Supporting Figure SF1. ^{1}H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 125 MHz) spectra of methyl 3-(2-chloro-5-fluoro-4-methoxyphenyl)-3-oxopropanoate **4** in $CDCl_3$



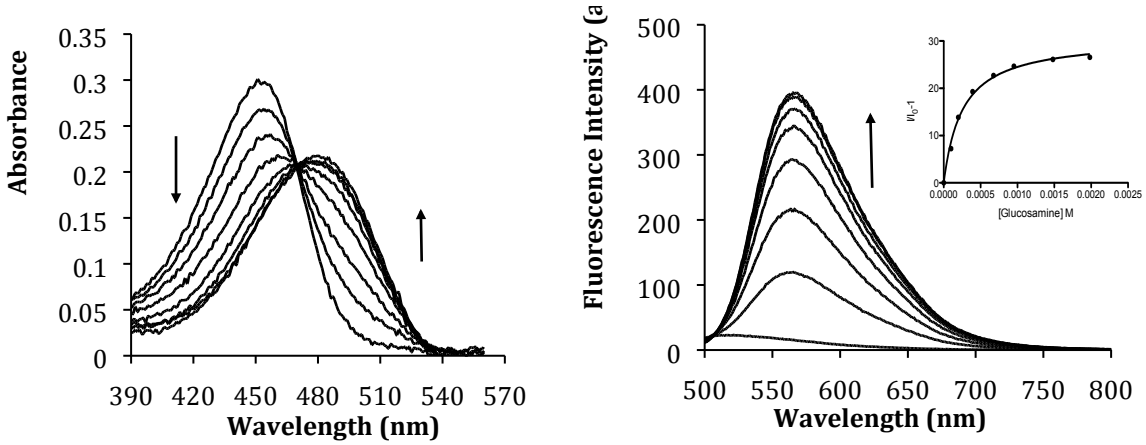
Supporting Figure SF2. ^{1}H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 125 MHz) spectra of 4-(2-chloro-5-fluoro-4-methoxyphenyl)-7-(diethylamino)-2H-chromen-2-one **5** in $CDCl_3$



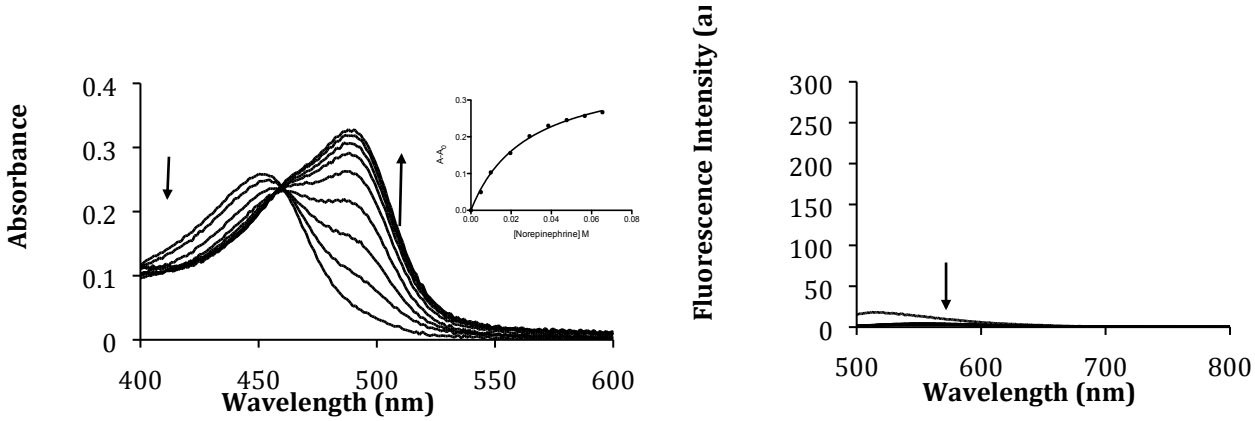
Supporting Figure SF3. ^{1}H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 125 MHz) spectra of 7-(diethylamino)-4-(5-fluoro-4-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2H-chromen-2-one **6** in $CDCl_3$



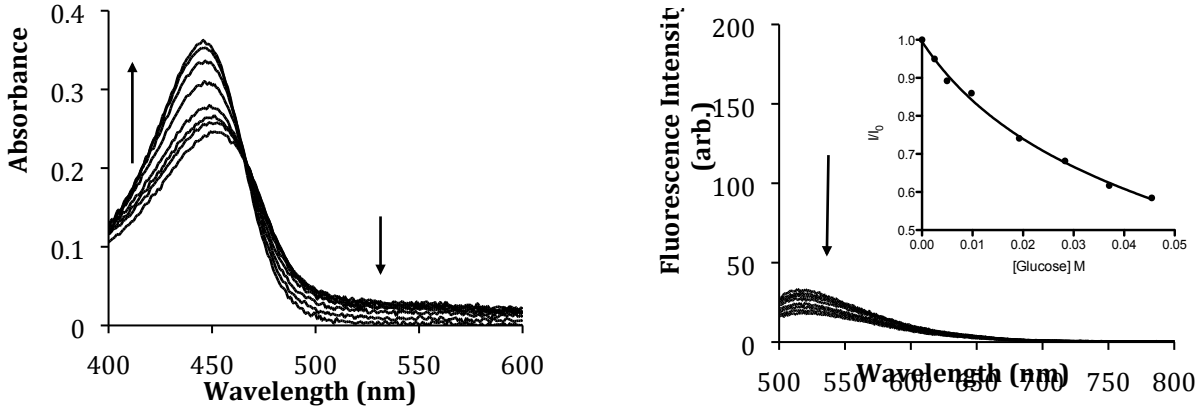
Supporting Figure SF4. ^{1}H NMR (top, 500 MHz) and ^{13}C NMR (bottom, 125 MHz) spectra of 7-(diethylamino)-4-(5-fluoro-4-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-oxo-2H-chromene-3-carbaldehyde **7** in $CDCl_3$



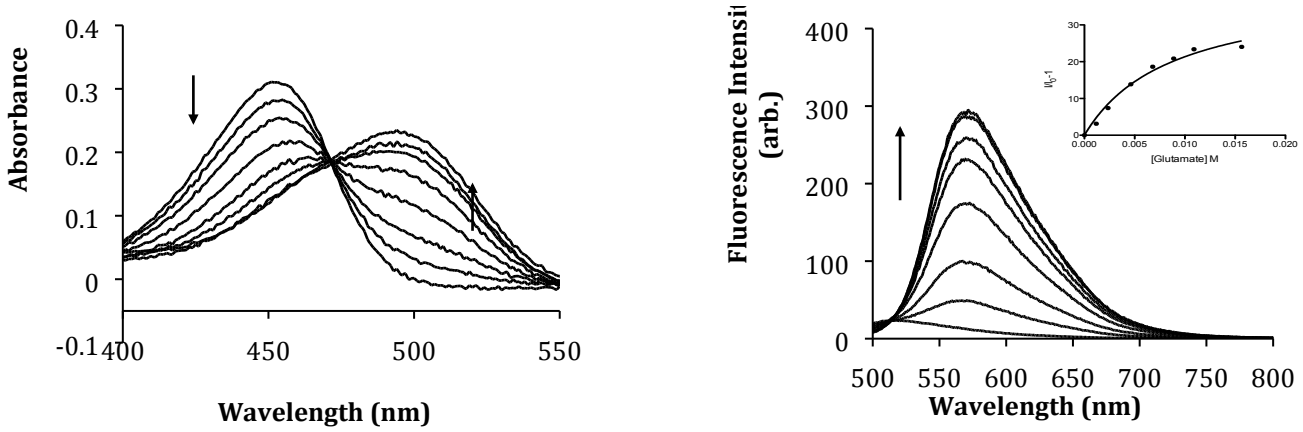
Supporting Figure SF5. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of D-glucosamine (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 568 nm.



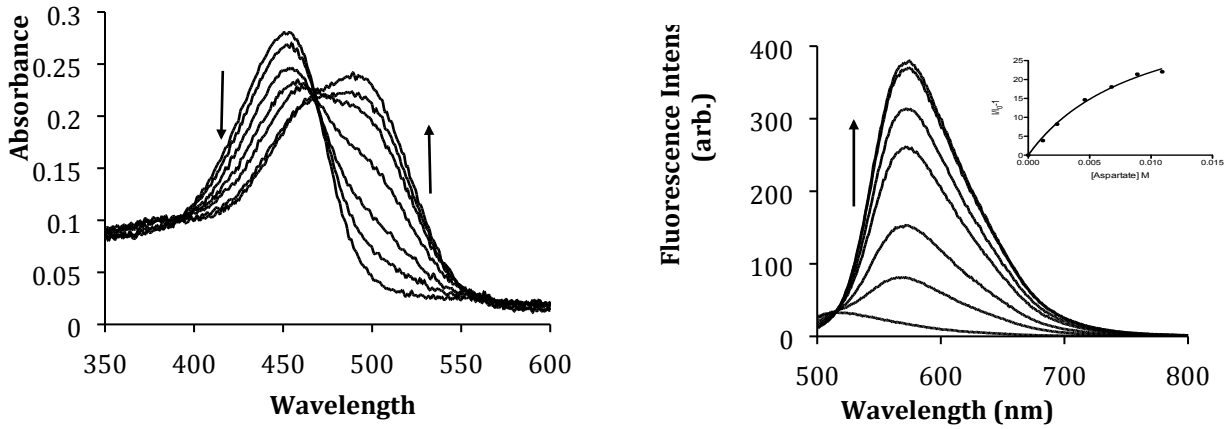
Supporting Figure SF6. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of norepinephrine (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, by UV changes at 488 nm.



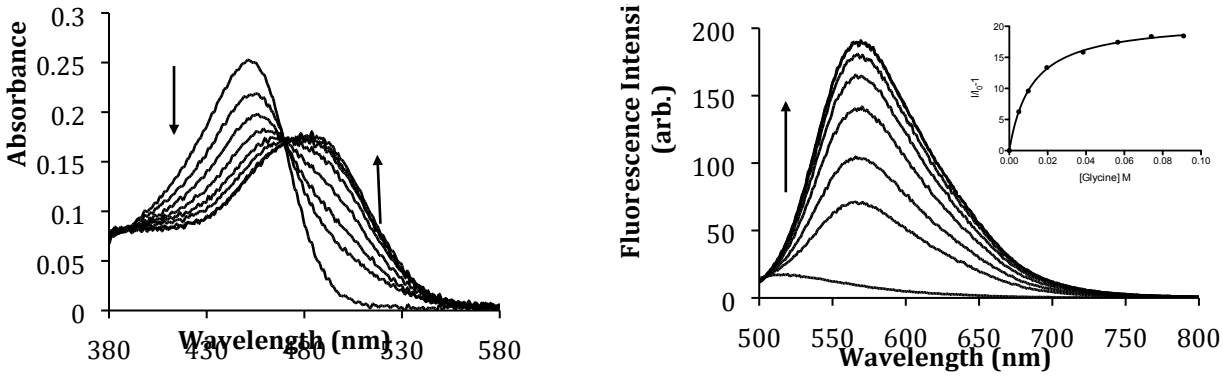
Supporting Figure SF7. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of glucose (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 519 nm.



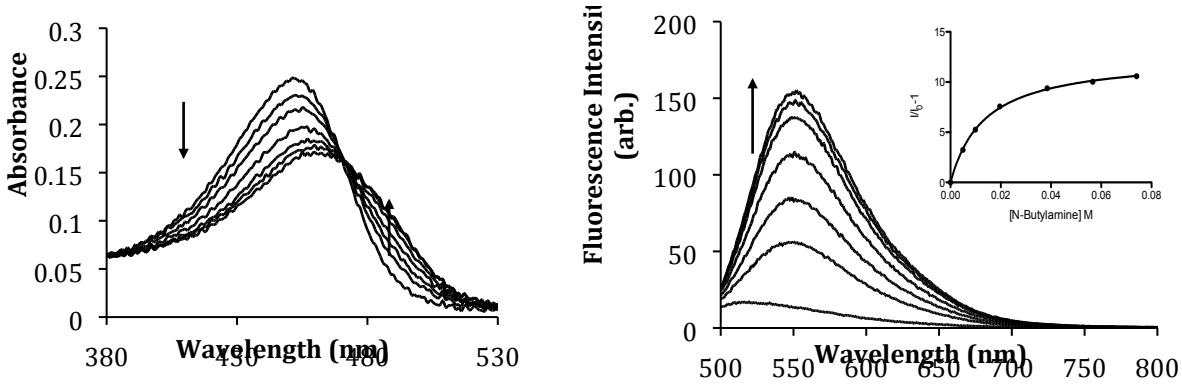
Supporting Figure SF8. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of L-glutamic acid (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 572 nm.



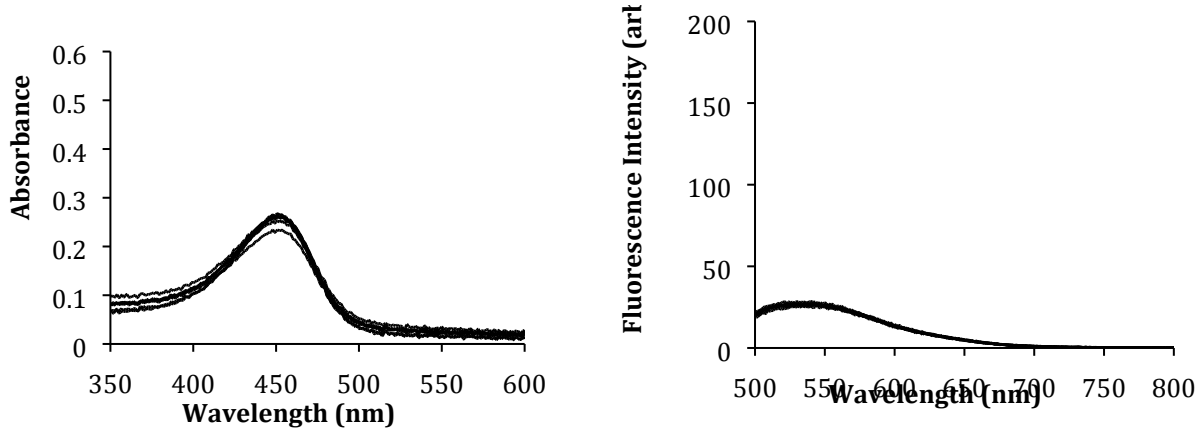
Supporting Figure SF9. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of L-aspartic acid (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 575 nm.



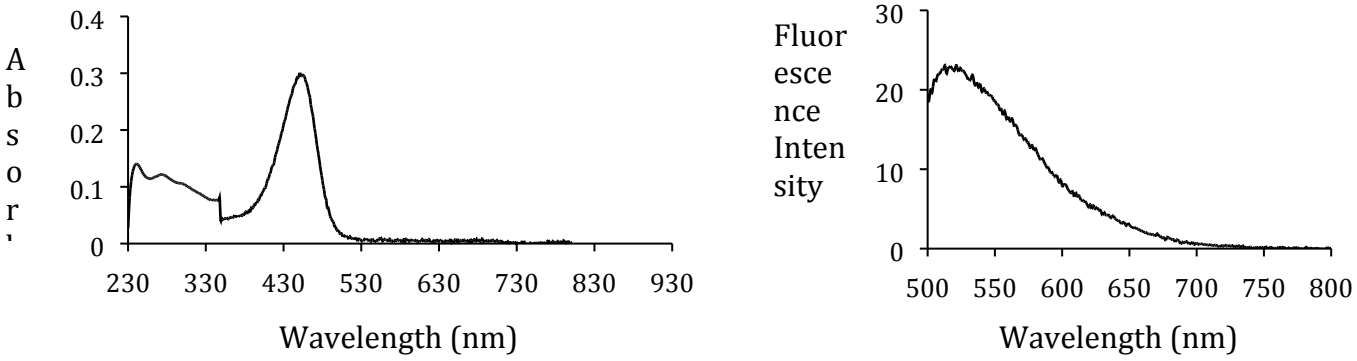
Supporting Figure SF10. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of glycine (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 565 nm.



Supporting Figure SF11. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of N-butylamine (λ_{ex} = 488 nm). Inset is the fit to a one-site binding isotherm, at 550 nm.



Supporting Figure SF12. UV-Vis absorbance spectra (left) and fluorescent spectra changes (right) of sensor 2 upon addition of diethylamine (λ_{ex} = 488 nm).



Supporting Figure SF13. UV-Vis absorbance spectra (left) and fluorescent spectra (right) of sensor 2 (λ_{ex} = 488 nm).