Supplementary Material (ESI) for Analyst

Fluorescence turn-on detection of protamine based on aggregation-induced emission enhancement characteristics of 4-(6'-carboxyl)hexyloxysalicylaldehyde azine

Xiao-tong Chen,[a] Yu Xiang,[a,b] Na Li,[a] Pan-Shu Song,[a] Ai-jun Tong*[a]

[a] Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, PR China.

[b] Present address: University of Illinois at Urbana-Champaign, #1714 Beckman Institute, Urbana, USA.

* To whom correspondence should be addressed: Fax: +86-10-62782485; Tel: +86-10-62787682; E-mail: tongaj@mail.tsinghua.edu.cn

Electronic Supporting Information

(6 pages)

Characterizations of Compounds

Products were characterized by ^{1}H, ^{13}C NMR, ESI-MS and elemental analyses. ESI mass spectrometry for 4-carboxylmethyloxybenzaldehyde (CMB): m/z 179.16 ($[M - H]^-$); M^- calculated 179.04. ^{1}H-NMR (DMSO-d_6), δ (ppm): 4.83 (s, 2H), 7.11 (d, 2H), 7.86 (d, 2H), 9.87 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 65.1, 115.5, 130.5, 132.2, 163.2, 170.2, 191.6. Elemental Analysis: C 60.09%, H 4.32%, O 35.59%, calculated for $C_9H_8O_4$: C 60.00%, H 4.48%, O 35.52%.

ESI mass spectrometry for 4-(4'-carboxyl)butyloxybenzaldehyde (CBB): *m/z* 221.14 ($[M - H]^-$); M^- calculated 221.09. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.67 (m, 4H), 2.30 (m, 2H), 4.10 (t, 2H), 7.12 (d, 2H), 7.86 (d, 2H), 9.86 (s, 1H), 12.06 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 21.6, 28.4, 33.8, 33.8, 68.26, 115.4, 130.1, 132.3, 164.2, 174.9, 191.8. Elemental Analysis: C 64.97%, H 6.47%, O 28.56%, calculated for $C_{12}H_{14}O_4$: C 64.85%, H 6.35%, O 28.80%.

ESI mass spectrometry for 4-(6'-carboxyl)hexyloxybenzaldehyde (CHB): *m/z* 249.35 ($[M - H]^-$); M^- calculated 249.12. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.38 (m, 6H), 1.74

(m, 2H), 2.22 (t, 2H), 4.08 (t, 2H), 7.11 (d, 2H), 7.86 (d, 2H), 9.87 (s, 1H), 12.00 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 24.9, 25.7, 28.8, 28.9, 34.1, 68.5, 115.4, 130.0, 132.3, 164.2, 175.0, 191.7. Elemental Analysis: C 64.25%, H 7.31%, O 25.44%, calculated for $C_{14}H_{18}O_4$: C 67.18%, H 7.25 %, O 25.57 %.

ESI mass spectrometry for 4-(10'-carboxy)-decyloxybenzaldehyde (DCB): *m/z* 305.13 ($[M - H]^-$); M^- calculated 305.18. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.36 (m, 14H), 1.74 (m, 2H), 2.26 (t, 2H), 4.05 (t, 2H), 7.11 (d, 2H), 7.85 (d, 2H), 9.87 (s, 1H), 11.98 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.3, 25.0, 25.9, 29.0, 29.1, 29.2, 29.3, 29.4, 34.0, 60.1, 68.6, 115.4, 130.0, 132.3, 164.2, 173.4, 191.7. Elemental Analysis: C 70.53%, H 8.41%, O 21.06%, calculated for $C_{18}H_{26}O_4$: C 70.56%, H 8.55 %, O 20.89%.

ESI mass spectrometry for CMSA: *m/z* 297.07 ($[M - H]^-$); M^- calculated 297.10. ^{1}H-NMR (DMSO-d_6), δ (ppm): 4.78 (s, 2H), 6.99 (m, 4H), 7.39 (m, 1H), 7.68 (m, 1H), 8.69 (d, 1H), 8.97 (d, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 115.4, 117.0, 117.1, 118.7, 119.9, 130.4, 130.7, 131.6, 133.4, 159.2, 161.1, 162.4, 163.1, 163.3, 170.4. Elemental Analysis: C 64.30%, H 4.85%, N 9.25%, O 21.60%, calculated for $C_{16}H_{14}N_2O_4$: C 64.42%, H 4.73%, N 9.40%, O 21.45%.

ESI mass spectrometry for CBSA: m/z 339.10 ($[M - H]^-$); M^- calculated 339.14. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.70 (m, 4H), 2.30 (t, 2H), 4.06 (t, 2H), 6.97 (d, 2H), 7.06 (d, 2H), 7.39 (t, 1H), 7.67 (t, 1H), 7.82 (d, 2H), 8.73 (s, 1H), 8.92 (s, 1H), 11.41 (s, 1H), 12.05 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 21.7, 28.5, 33.8, 68.0, 115.5, 117.0, 118.8, 120.0, 126.5, 130.9, 131.8, 133.3, 159.2, 161.2, 162.0, 163.0, 174.9. Elemental Analysis: C 67.22%, H 5.96%, N 8.12%, O 18.70%, calculated for $C_{19}H_{20}N_2O_4$: C 67.05%, H 5.92%, N 8.23%, O 18.80%.

ESI mass spectrometry for CHSA: m/z 367.22 ($[M - H]^-$); M^- calculated 367.17. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.39 (m, 4H), 1.51 (m, 2H), 1.72 (m, 2H), 4.02 (t, 2H), 7.04 (m, 4H), 7.38 (t, 1H), 7.64 (d, 1H), 7.82 (2H), 8.72 (s, 1H), 8.92 (s, 1H), 11.85 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 25.2, 25.8, 28.9, 29.0, 34.9, 68.2, 115.4, 117.0, 118.8, 119.9, 126.5, 130.8, 131.7, 133.3, 159.3, 162.0, 162.4, 162.9, 176.0. Elemental Analysis: C 68.61%, H 6.67%, N 7.47%, O 17.25%, calculated for $C_{21}H_{24}N_2O_4$: C 68.46%, H 6.57%, N 7.60%, O 17.37%.

ESI mass spectrometry for CDSA: m/z 423.31 ([M – H]$^-$); M$^-$ calculated 423.24. ^{1}H-NMR (DMSO-d_6), δ (ppm): 1.18 (m, 14H), 1.73 (t, 2H), 2.21 (t, 2H), 4.03 (t, 2H), 7.01 (m, 4H), 7.38 (t, 1H), 7.65 (d, 1H), 7.81 (d, 2H), 8.66 (d, 1H), 8.95 (d, 1H), 11.12 (s, 1H), 11.40 (s, 1H), 11.92 (s, 1H). ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.3, 25.0, 25.9, 29.0, 29.1, 29.2, 29.3, 29.4, 34.0, 60.1, 68.6, 115.4, 130.0, 132.3, 164.2, 173.4, 191.7. Elemental Analysis: C 70.55%, H 7.74%, N 6.47%, O 15.24%, calculated for $C_{25}H_{32}N_2O_4$: C 70.73%, H 7.60%, N 6.60%, O 15.07%.

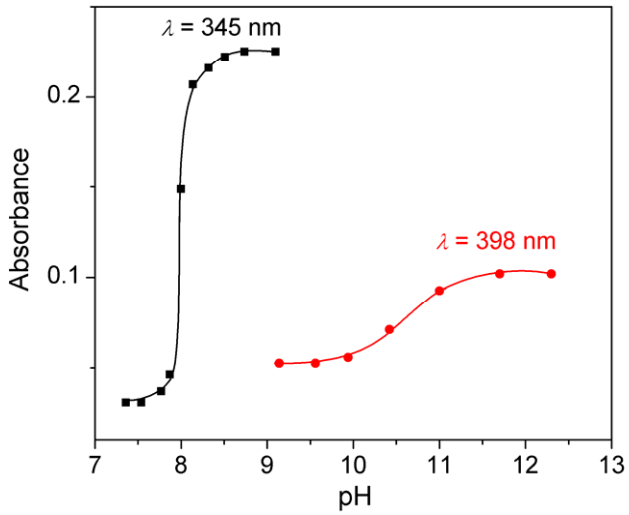

Fig. S1 pH effect on the absorbance of CHSA at 345 and 398 nm measured with 0.25 cm cuvette. *c*(Tris-HCl) = 10 mM, *c*(CHSA) = 45 μM.

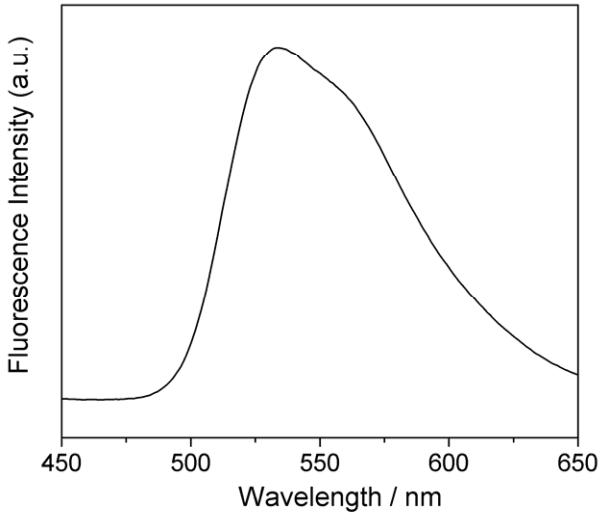

Fig. S2 Solid state fluorescence emission spectra of CHSA, which was similar to its AIEE fluorescence spectra in aggregate state. The solid fluorescence quantum yield was 0.09. Excitation was performed at 340 nm.

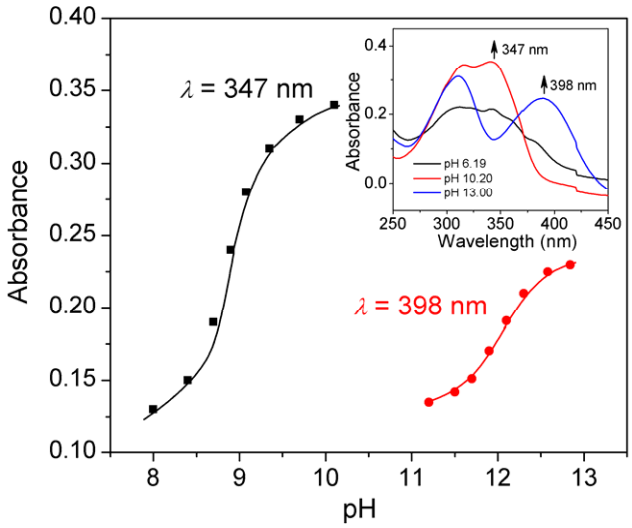

Fig. S3 pH effect on absorbance of CDSA at 347 and 398 nm, respectively. *c*(Tris-HCl) = 10 mM, *c*(CDSA) = 20 μM. Inset was absorbance spectra of CHSA at pH 6.19, 10.20 and 13.00.

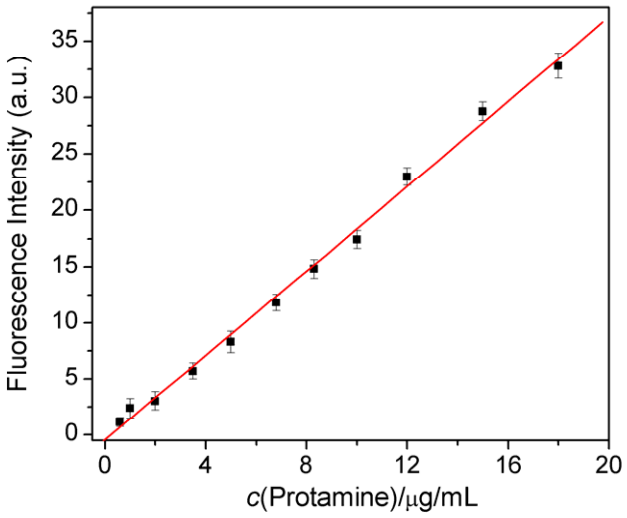

Fig. S4 The fluorescence intensity of $CHSA^-$-protamine *vs.* the concentration of protamine (0 to 18 μM). *c*(Tris-HCl) = 10 mM, *c*(CHSA) = 45 μM. λ_{ex} = 340 nm, λ_{em} = 538 nm.

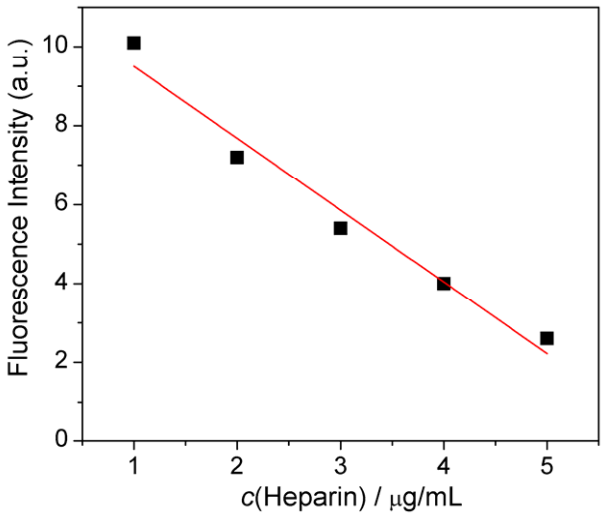

Figure S5. The fluorescence intensity of $CHSA^-$-protaime-heparin *vs.* the concentration of heparin (1 to 5 μM). The linear range was 1 – 5 μg/mL with a relative correlation coefficient of *R* = 0.963. *c*(Tris-HCl) = 10 mM, *c*(CHSA) = 45 μM, *c*(protamine) = 20 μg / mL. λ_{ex} = 340 nm, λ_{em} = 538 nm.

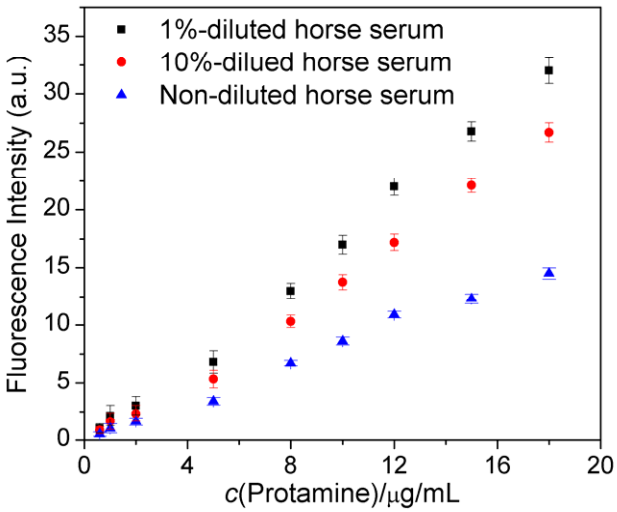

Fig. S6 The fluorescence intensity of CHSA$^-$-protamine *vs.* the concentration of protamine (0 to 18 μM) in 1%-diluted (squares), 10%-diluted (dot) and non-diluted horse serum (triangles). λ_{ex} = 340 nm, λ_{em} = 538 nm.