Electronic Supplementary Information

A highly selective and light-up red emissive fluorescent probe for imaging of penicillin G amidase in Bacillus cereus

Jianguo Wang,‡a Qingqing Chen,‡a Jie Wu,a Wenping Zhu,a Yongquan Wu,a Xiaolin Fan,a Guanxin Zhang,b Yibao Li*a and Guoyu Jiang*a

aKey Laboratory of Organo-Pharmaceutical Chemistry, Gannan Normal University, Ganzhou 341000, P. R. China

bOrganic Solids Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China.
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**Fig. S2.** $^{13}$C NMR of 6-formylnaphthalen-2-yl acetate (compound 2) in $d_6$-DMSO.
**Fig. S3.** $^1$H NMR of HCyN in $d_6$-DMSO.

**Fig. S4.** $^{13}$C NMR of HCyN in $d_6$-DMSO.
Fig. S5. HRMS spectrum of HCyN.

HCyN

Chemical Formula: C_{21}H_{29}NO^+

Exact Mass: 328.1696

Fig. S6. ^1H NMR of HCyNB in d_6-DMSO.
Fig. S7. $^{13}$C NMR of HCyNB in $d_6$-DMSO.

Fig. S8. HRMS spectrum of HCyNB.
Fig. S9. HRMS spectrum of HCyNB after incubation with PGA (0.1 U/mL) for 15 min at 37 °C.

Fig. S10. The fluorescence intensity at 593 nm of HCyNB (10 μM) incubated with different concentrations of PGA as a function of time. ($E_{ex} = 443$ nm).

Fig. S11. Stability of HCyNB. $I/I_0$ is the fluorescence intensity ratio at 570 nm after and before t-minute incubation at 37 °C.
**Fig. S12.** Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: \( V = V_{\text{max}} [S] / (K_{m} + [S]) \), where \( V \) is the reaction rate, \([S]\) is the concentration of the probe HCyNB, and \( K_{m} \) is the Michaelis constant. Points were fitted using a linear regression model (correlation coefficient \( R^2 = 0.992 \)). \( Y = 162.73X + 15.56 \).

**Fig. S13.** Confocal fluorescence microscope images of HCyNB in penicillinase-producing *Bacillus cereus* strain CMCCB 63301 in the presence of different concentrations (0, 5 and 20 mM) of PGA inhibitor (penicillin G sodium salt).
Fig. S14. Average PL intensity of probe HCyNB in penicillinase-producing *Bacillus cereus* strain CMCCB 63301 vs the concentration of PGA inhibitor.