

Supporting Information

A turn-on near-infrared fluorescent probe for visualization of endogenous
alkaline phosphatase activity in living cells and zebrafish

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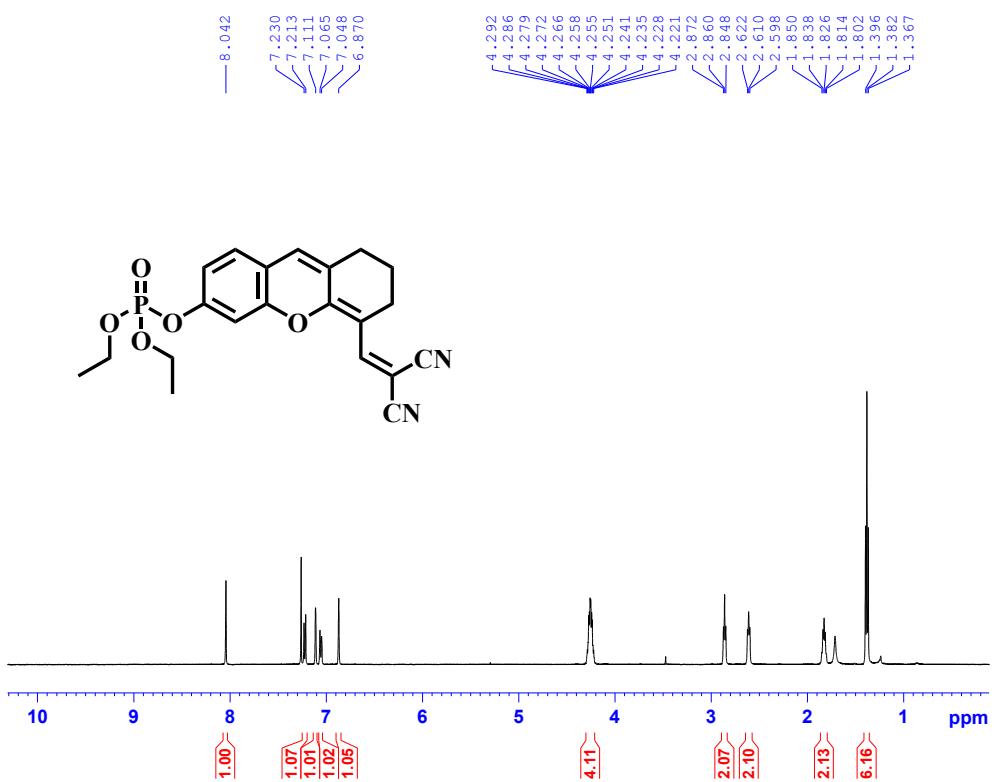


Figure S1. ^1H NMR spectra of compounds 1 in CDCl_3 .

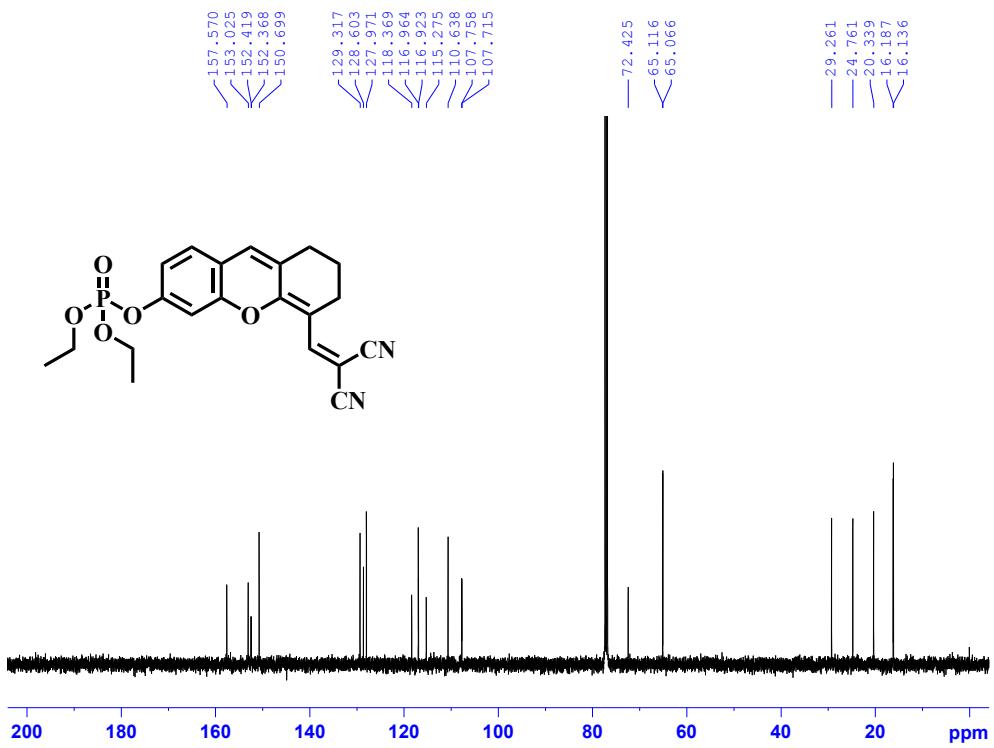


Figure S2. ^{13}C NMR spectra of compounds 1 in CDCl_3 .

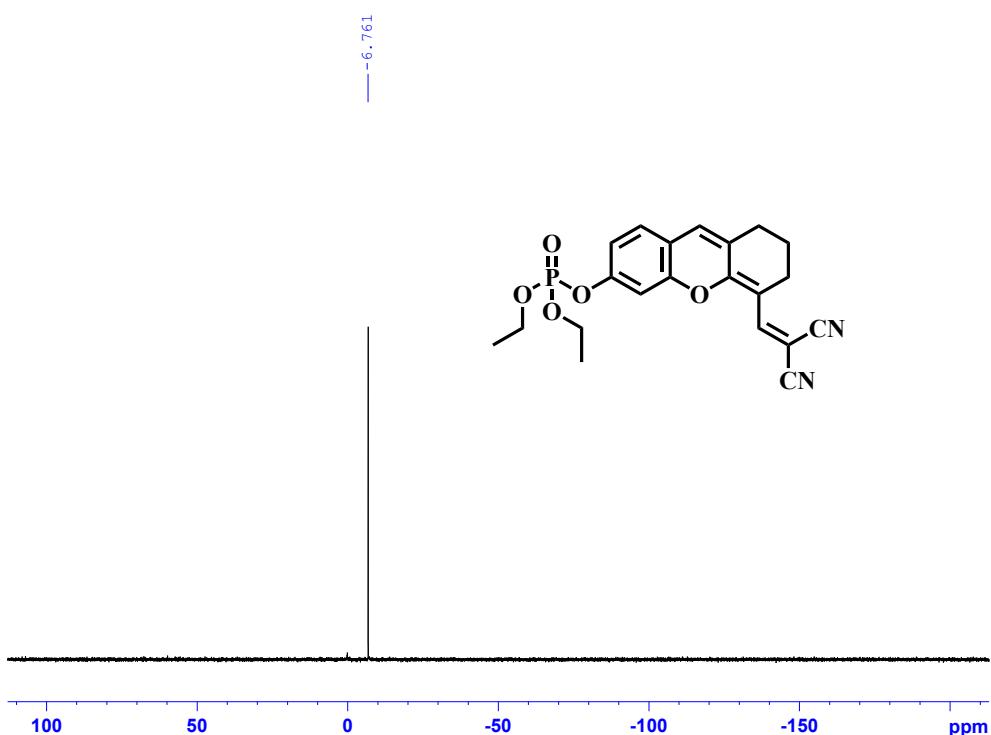


Figure S3. ^{31}P NMR spectra of compounds 1 in CDCl_3 .

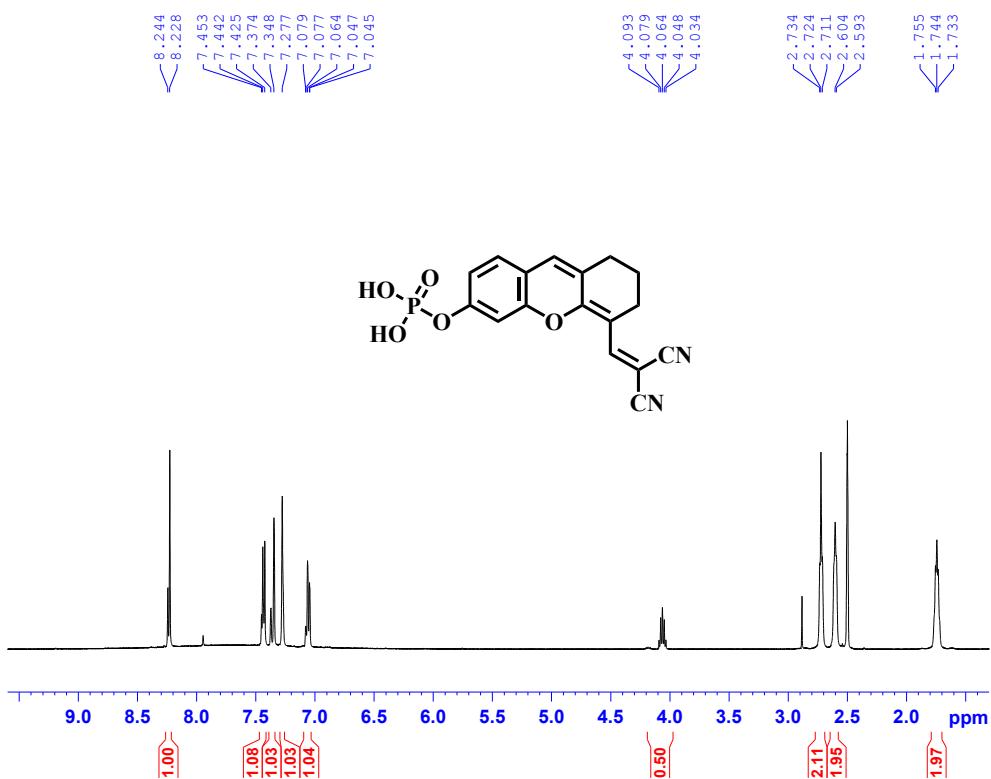


Figure S4. ^1H NMR spectra of DXMP in $\text{DMSO}-d_6$.

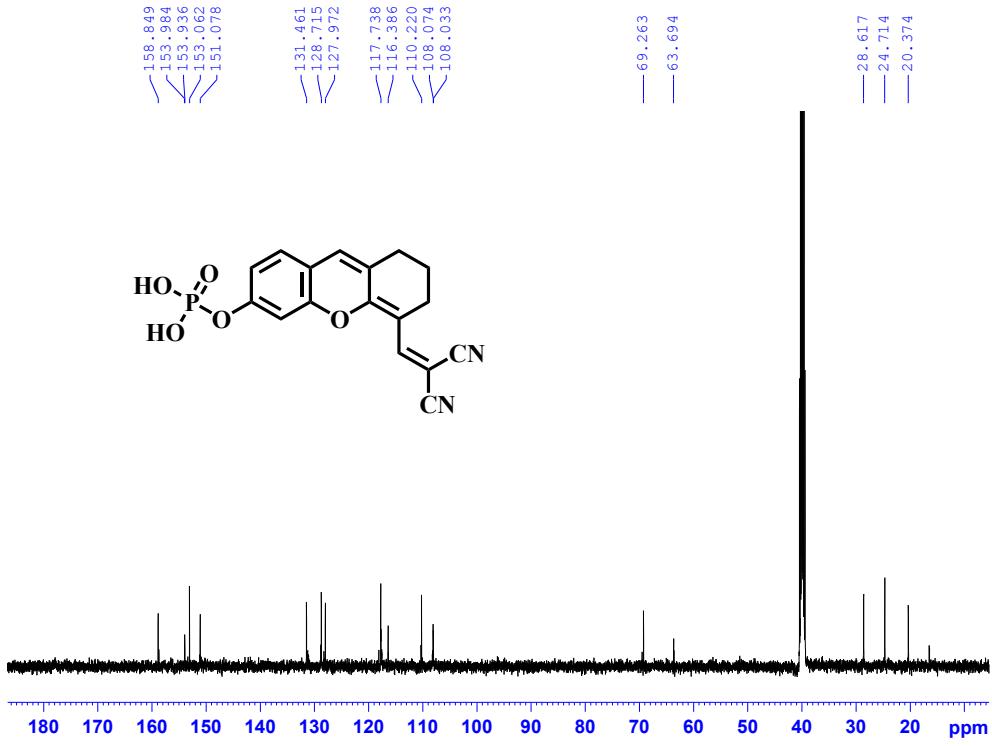


Figure S5. ^{13}C NMR spectra of DXMP in $\text{DMSO}-d_6$.

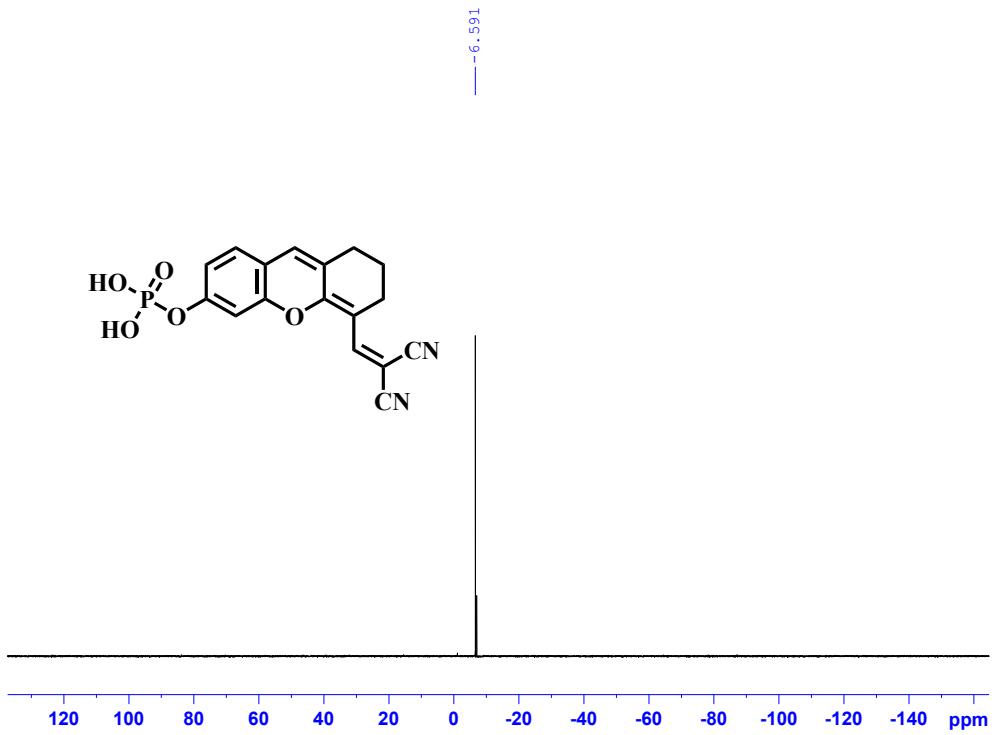


Figure S6. ^{31}P NMR spectra of DXMP in $\text{DMSO}-d_6$.

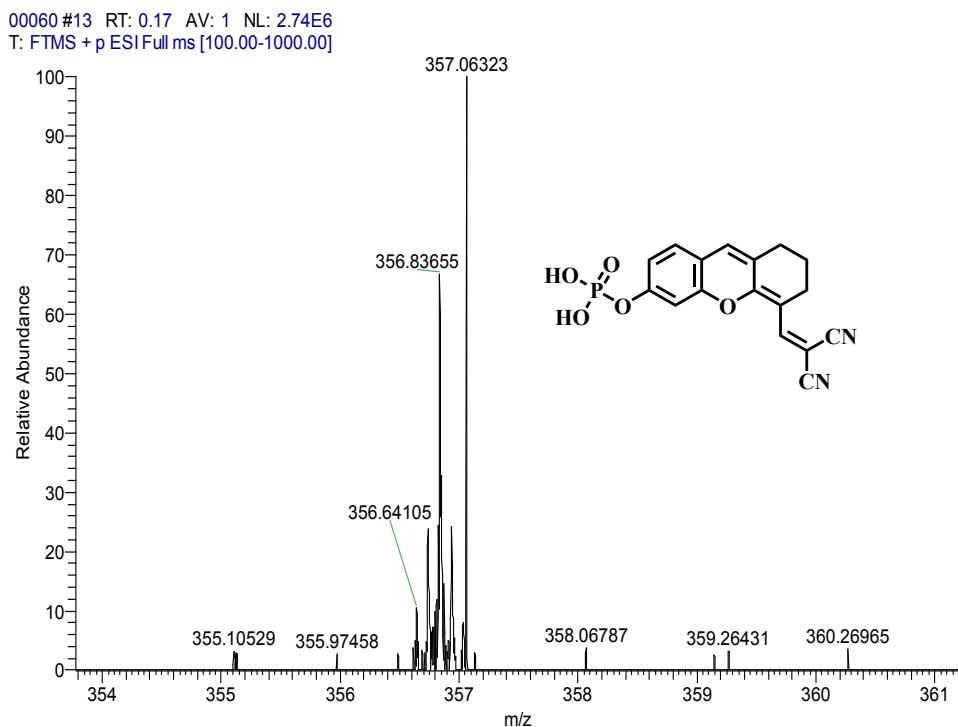


Figure S7. HRMS spectra of DXMP.

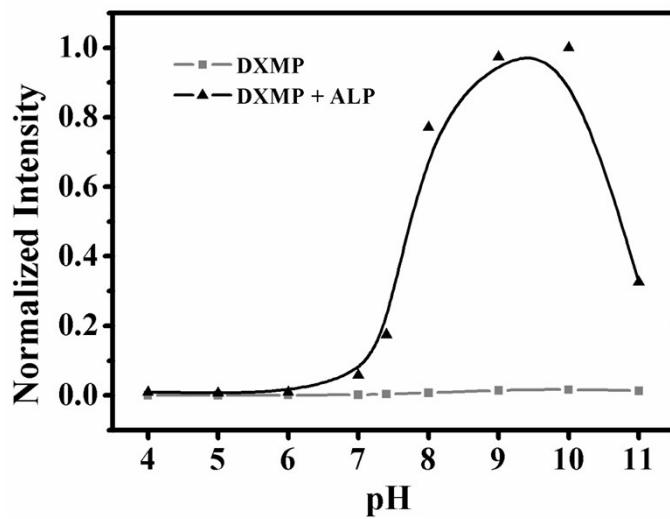


Figure S8. pH effect on fluorescence intensity of DXMP (10 μ M) and DXMP (10 μ M) incubated with ALP (200 U/L) in buffered DMSO/Tris-HCl (v/v = 2:3) solution. ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 600/640$ nm, slits: 5 nm/5 nm).

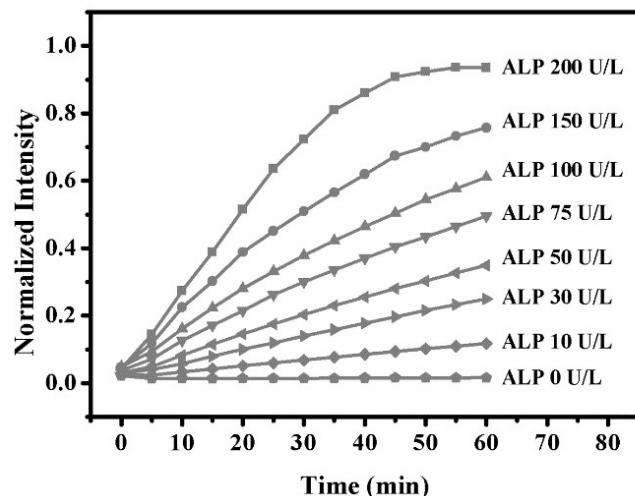


Figure S9. Time-dependent fluorescence intensity increment at 640 nm using DXMP (10 μ M) with different amounts of ALP (0, 10, 30, 50, 75, 100, 150, 200 U/L) within 60 min in buffered DMSO/Tris-HCl (v/v = 2:3, pH = 8.0). ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 600/640$ nm, slits: 5 nm/5 nm).

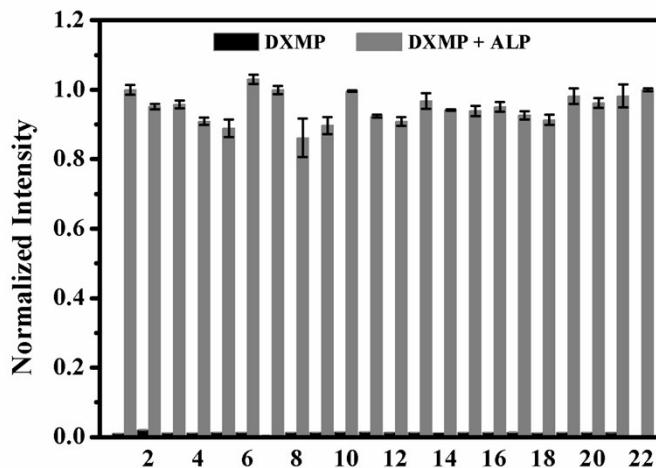


Figure S10. Fluorescence responses of DXMP (10 μ M) toward ALP in the presence of some representative analytes. Black bars represent the addition of a single analyte including: 1. none, 2. ACP, 3. PDE, 4. AchE, 5. β -gal, 6. Trypsin, 7. HRP, 8. COD, 9. LOD, 10. Mg^{2+} , 11. Zn^{2+} , 12. NO_2^- , 13. SO_4^{2-} , 14. ClO^- , 15. H_2O_2 , 16. Cys, 17. Hcy, 18. GSH, 19. β -alanine, 20. Glua, 21. Gly, 22. ALP. Gray bars represent the subsequent addition of ALP to the mixture. ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 600/640$ nm, Slits: 5 nm/5 nm)

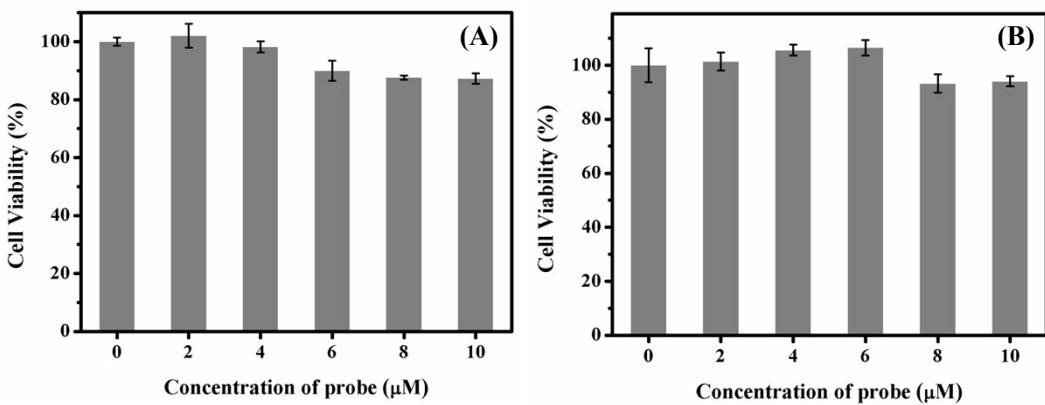


Figure S11. Cell viability of (A) HepG2 and (B) LO2 cells incubated with DXMP (0-10 μM) in culture medium (containing 1% DMSO, v/v) for 24 h at 37 °C.

Table S1. ALP detection in human serum samples by using Alkaline Phosphatase Assay Kit assay and DXMP.

Sample No.	Alkaline Phosphatase Assay Kit assay ^[a]		DXMP ^[b]	
	ALP level (U/L)	RSD (%)	ALP level (U/L)	RSD (%)
1 ^[c]	35.9 ± 2.6	4.1	39.4 ± 3.4	3.4
2 ^[c]	49.6 ± 3.1	3.6	51.5 ± 3.8	2.6
3 ^[c]	54.7 ± 5.2	2.9	57.3 ± 4.2	3.9

Note: [a] When ALP level was determined by using Alkaline Phosphatase Assay Kit, the serum samples were 50-fold diluted. [b] When ALP level was determined by using DXMP, the serum samples were 5-fold diluted. [c] Clinical samples (serum samples from healthy males with normal ALP levels, as tested by the clinical laboratory of the School Hospital of Hunan Normal University).