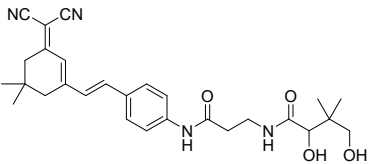
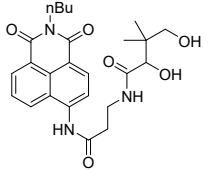
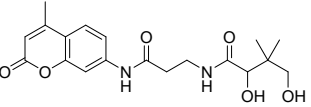
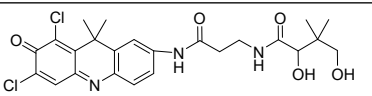
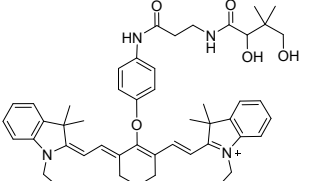


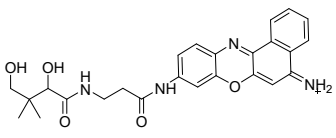
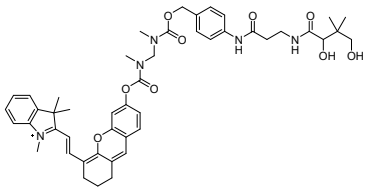
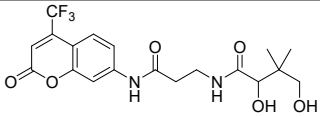
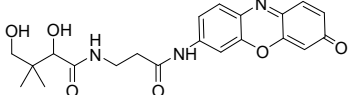
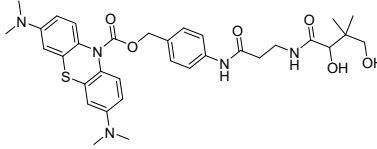
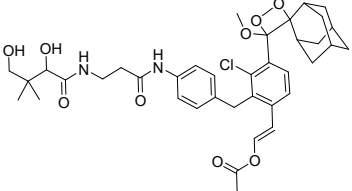
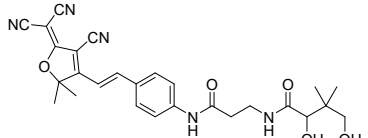
Supplementary material

Design and synthesis of fluorescent probes specific for Pantetheinase and their application in bioimaging

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Table S1 Current fluorescent probes for Pantetheinase

Probe structure	$\lambda_{\text{abs}}(\text{nm})$	$\lambda_{\text{em}}(\text{nm})$	Stokes shift	Km	Limit of detection	references
	568	645	77	0.94 ng/mL	0.37 ng/mL	32
	430	545	115	0.18 ng/mL	0.18 ng/mL	33
	340	460	120	$28 \pm 1.8 \mu\text{M}$	NO	34
	480	670	190	$4.667 \mu\text{M}$	NO	35
	730	780	50	NO	0.85 ng/mL	36

	582	628	46	78 μM	4.7 ng/mL	37
	680	710	30	84 μM	0.02 ng/mL	38
	420	510	90	NO	1 ng/mL	39
	530	590	60	16.06 μM	0.054 ng/mL	40
	665	710	45	6.855 μM	0.031 ng/mL	41
	NO	540	NO	NO	0.089 ng/mL	42
	510	608	98	15.9 μM	13.8 ng/mL	This work

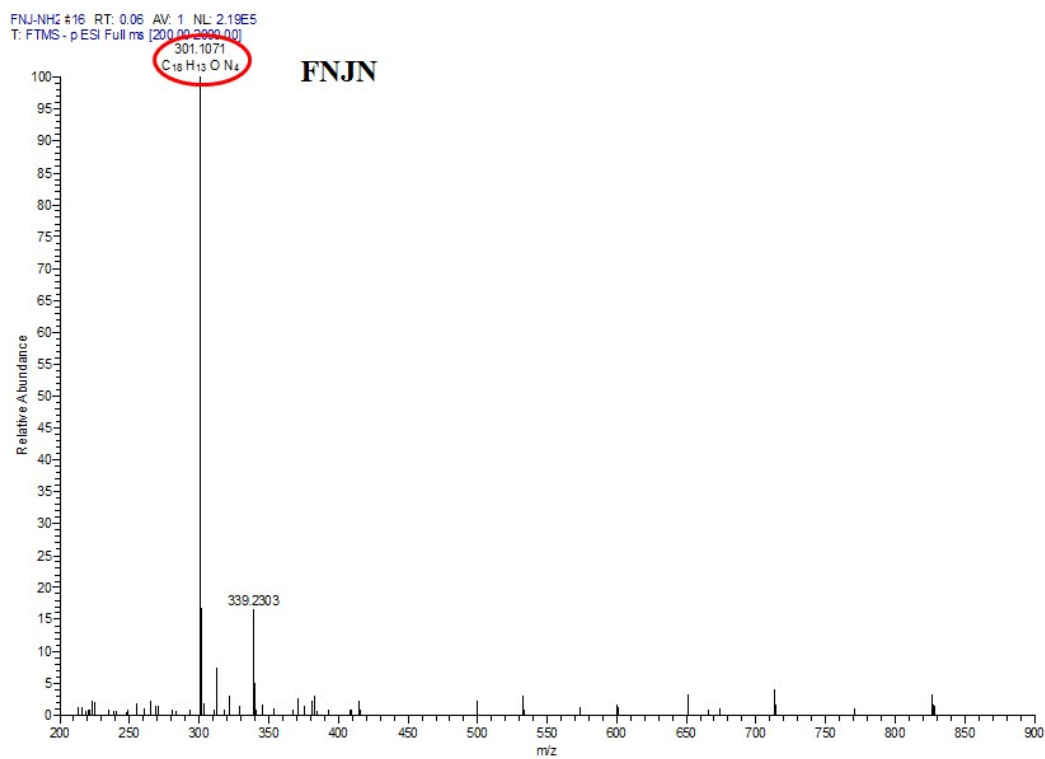


Fig. S1. HRMS after 70 min incubation with probes FNJ-PA (10 μ M) and Vanin-1 (400 ng/mL).

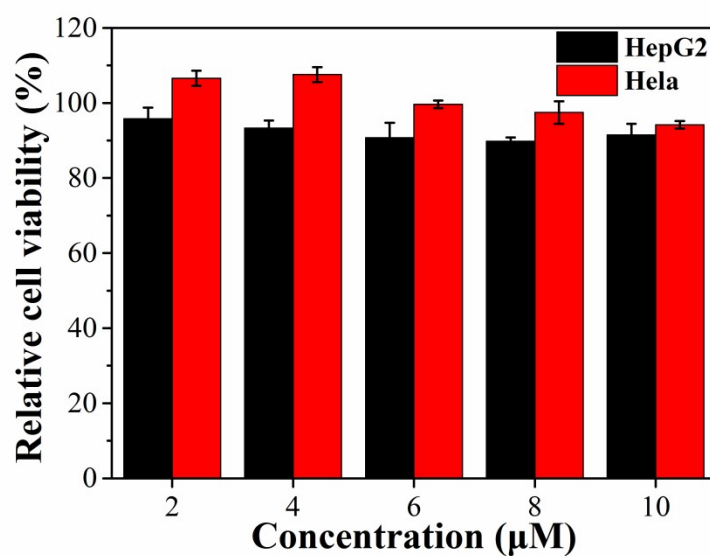


Fig. S2. Survival of HepG2, HeLa cells incubated for 24 h with different concentrations of probe FNJ-PA.

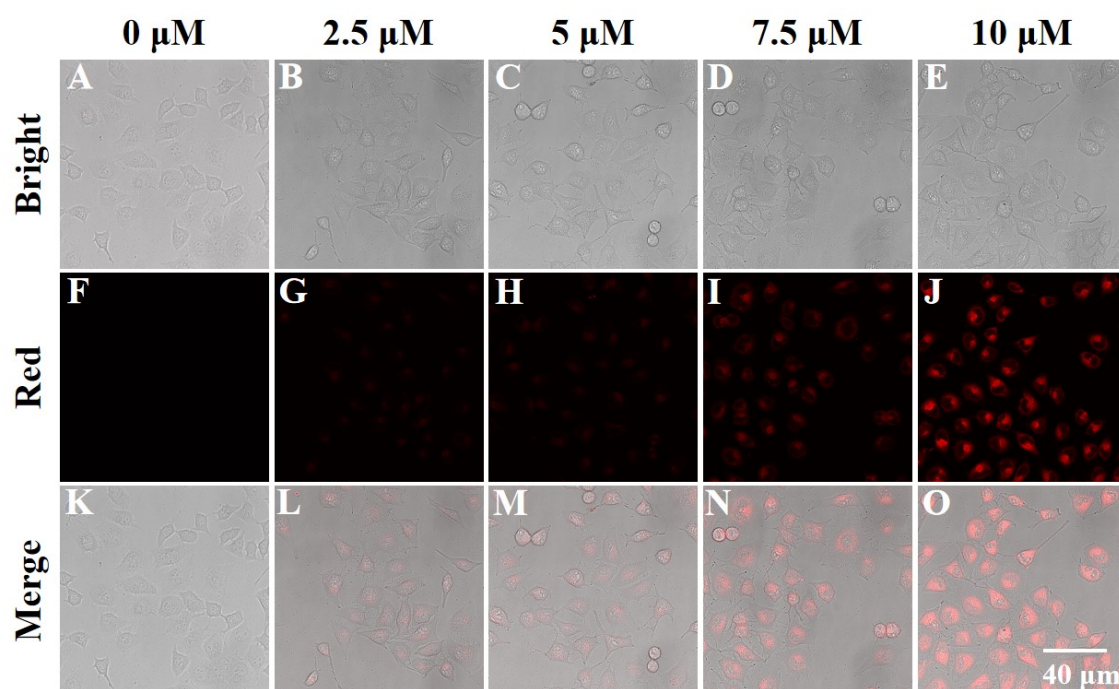


Fig. S3. (A-O) Fluorescence imaging of HepG2 cells incubated with different concentrations of probe FNJ-PA (0, 2.5, 5, 7.5, 10 μM) for 1.5 h. $\lambda_{\text{ex}} = 552 \text{ nm}$, $\lambda_{\text{em}} = 575\text{-}675 \text{ nm}$, scale bar = 40 μm.

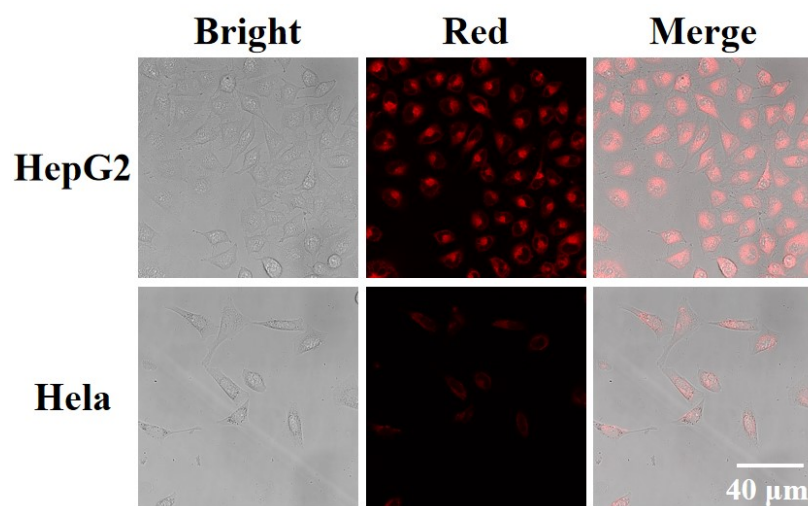


Fig. S4. Fluorescence imaging of probe FNJ-PA (10 μ M) after incubation with HepG2 and HeLa cells for 1.5 h, respectively.

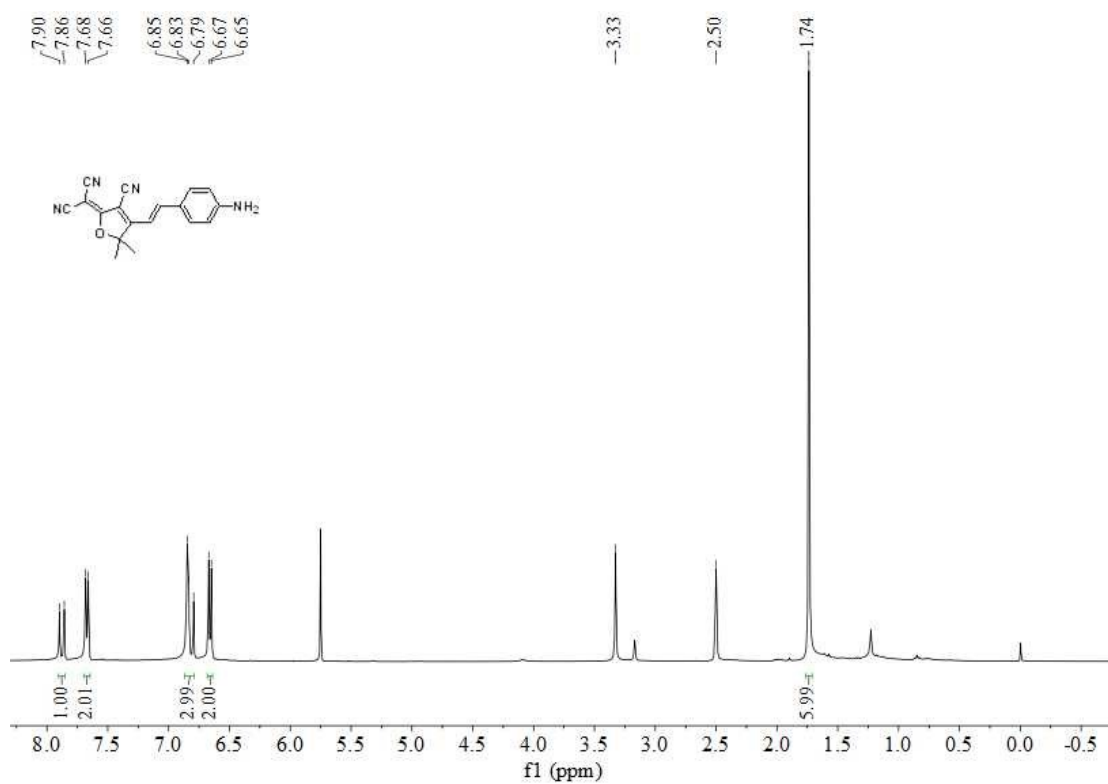


Fig. S5. ^1H NMR spectrum (DMSO- d_6) for FNJN.

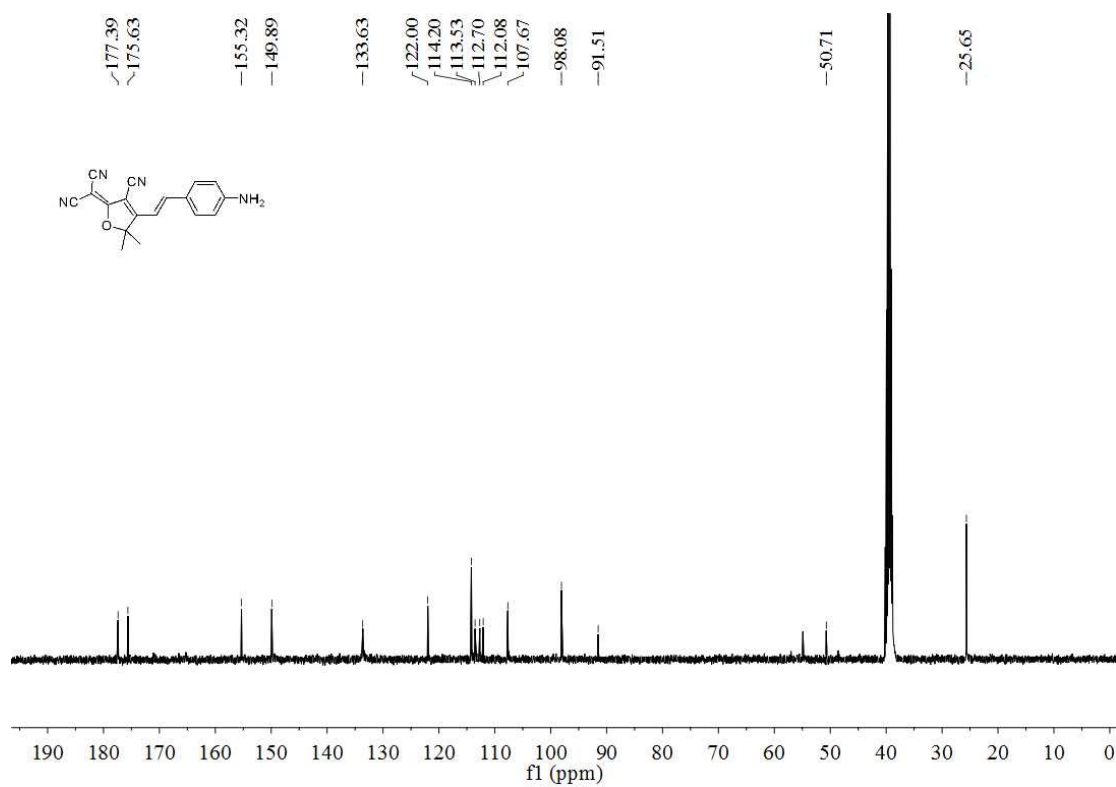


Fig. S6. ¹³C NMR spectrum (DMSO-*d*₆) for **FNJN**.

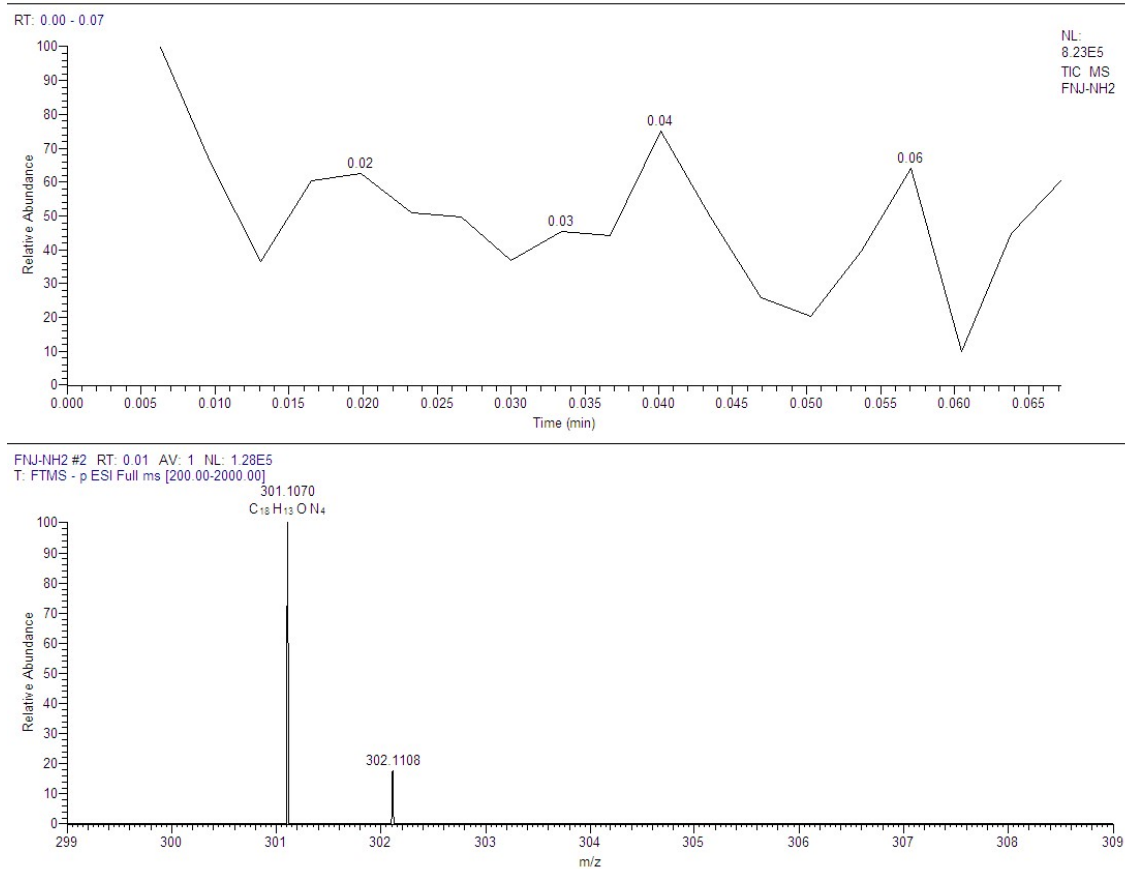


Fig. S7. HRMS spectrum of **FNJN**.

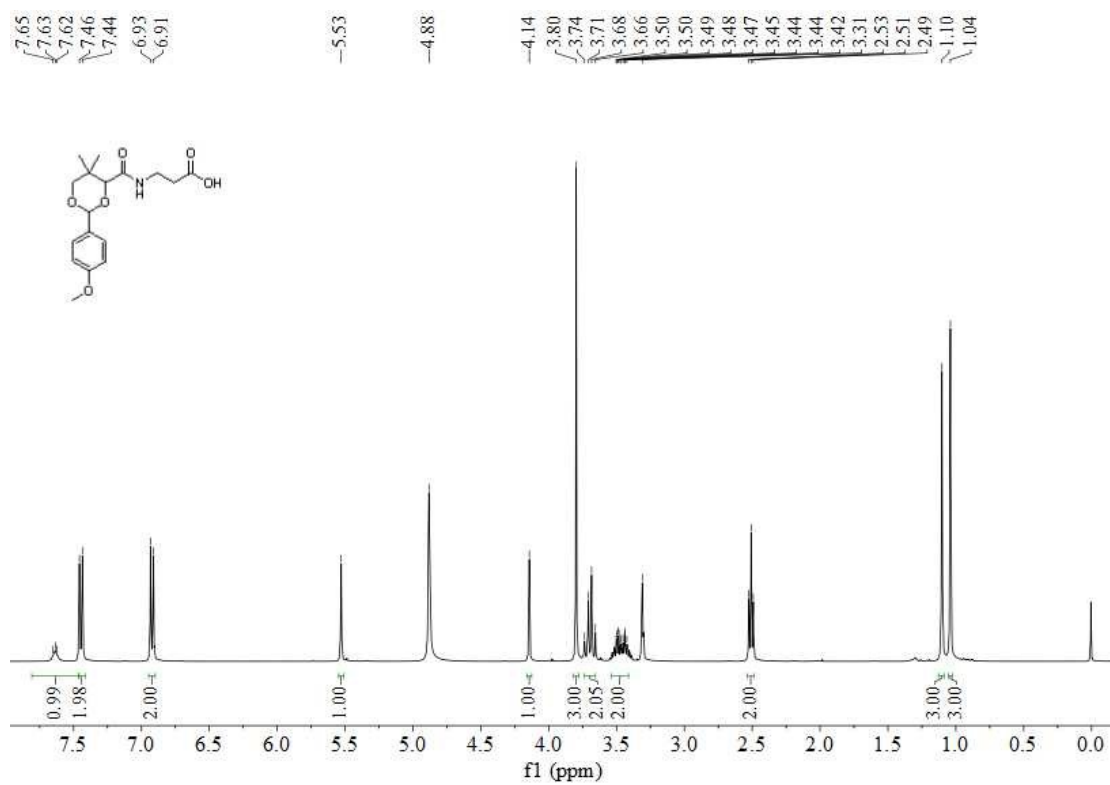


Fig. S8. ¹H NMR spectrum (CD₃OD) for **a**.

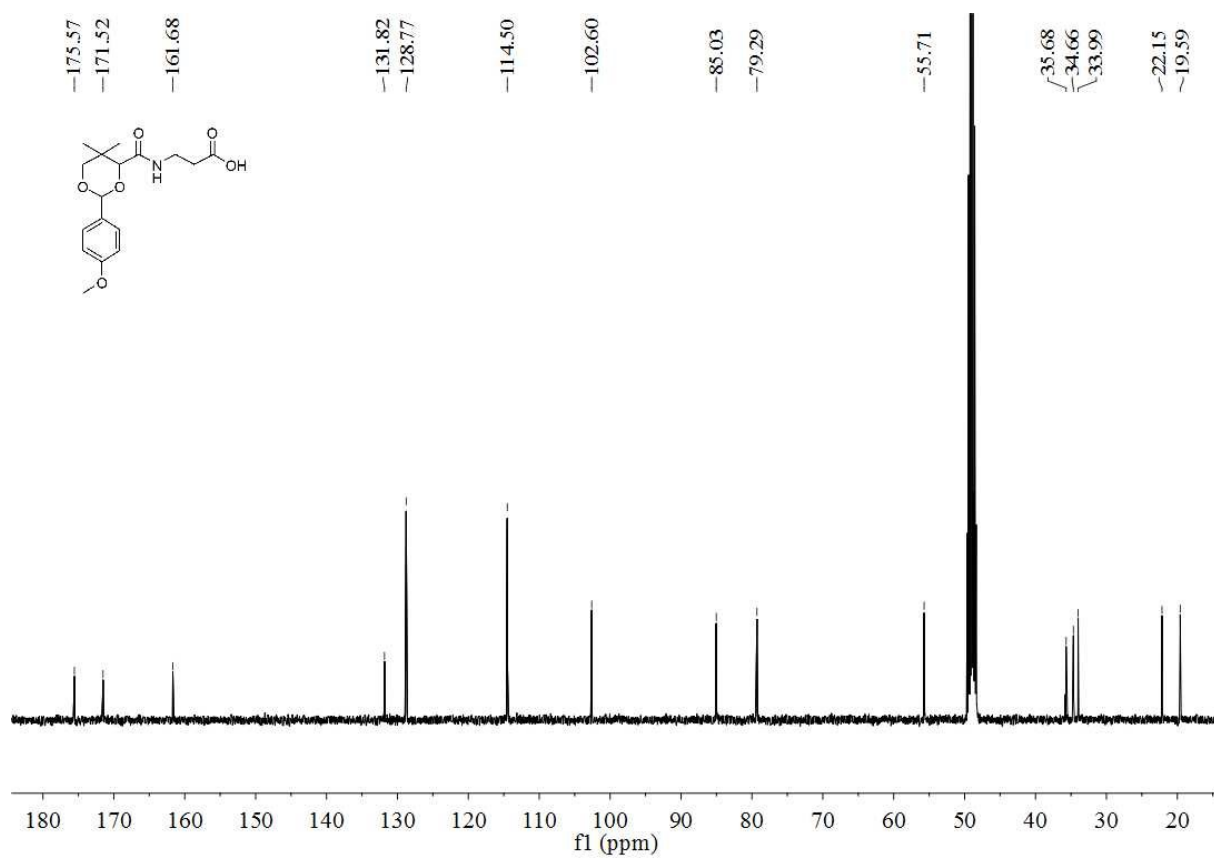
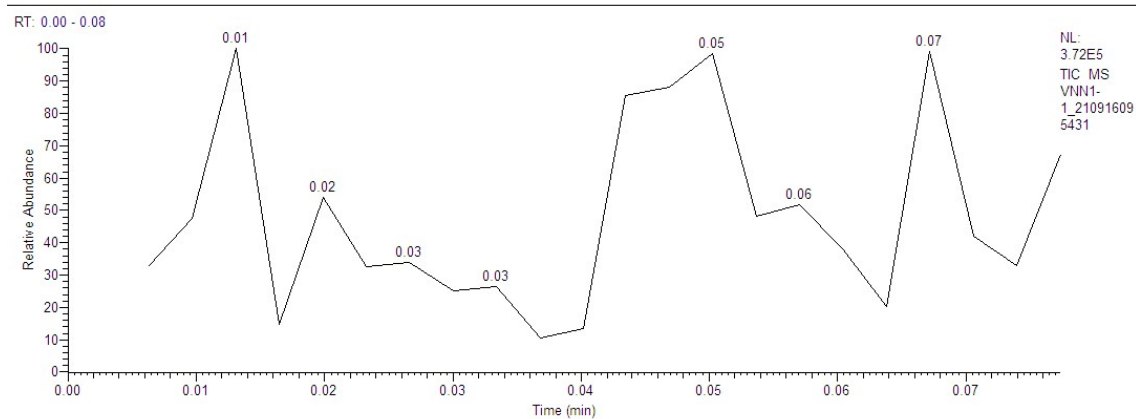


Fig. S9. ^{13}C NMR spectrum (CD₃OD) for **a**.



VNN1-1_210916095431 #2 RT: 0.01 AV: 1 NL: 2.32E4
T: FTMS + p ESI sid=12.50 Full ms [120.00-800.00]

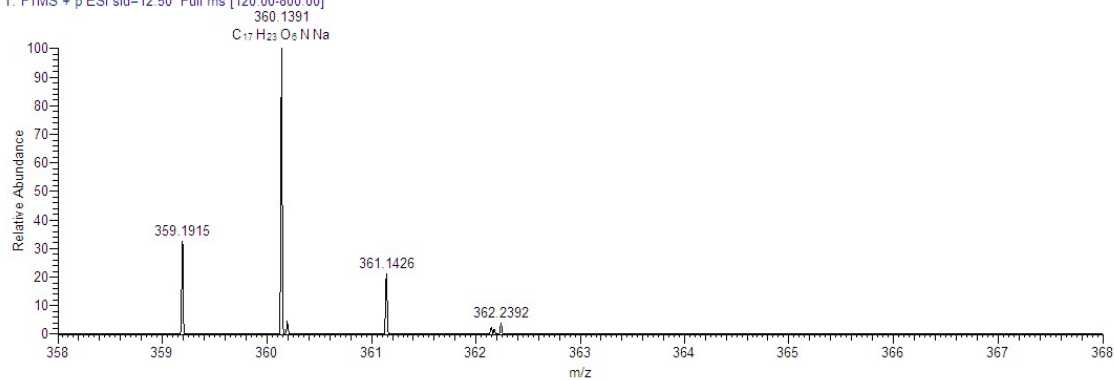


Fig. S10. HRMS spectrum of **a**.

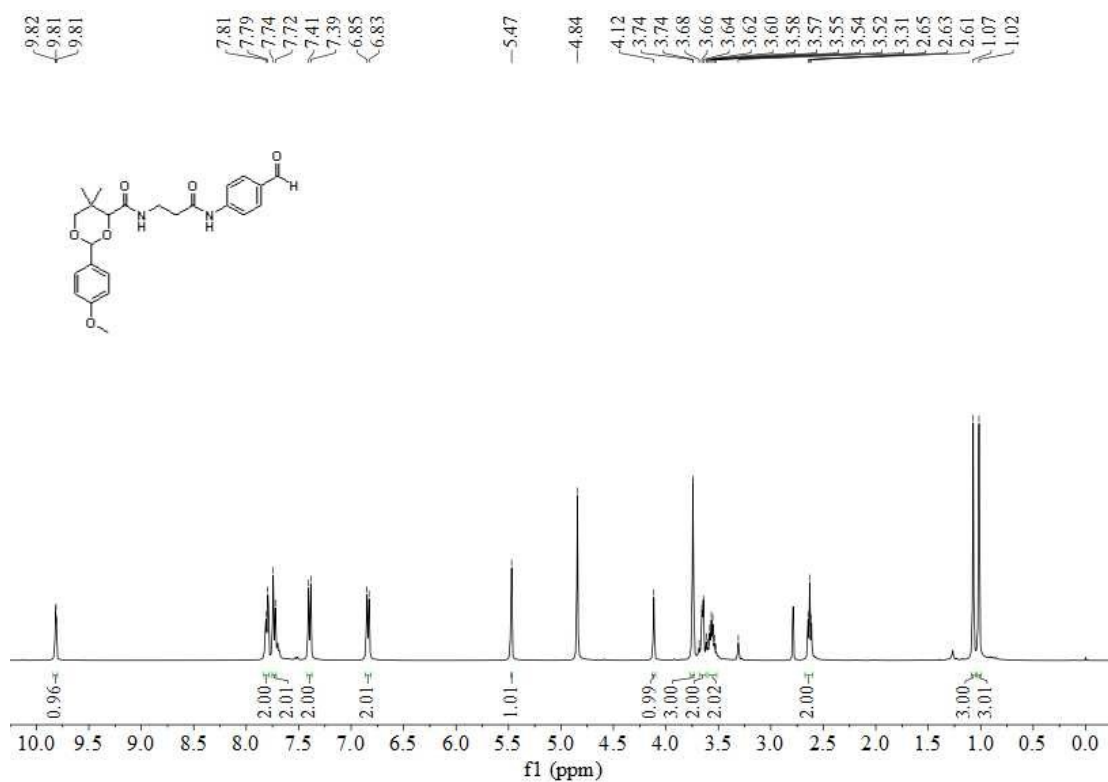


Fig. S11. ¹H NMR spectrum (CDCl₃) for **b**.

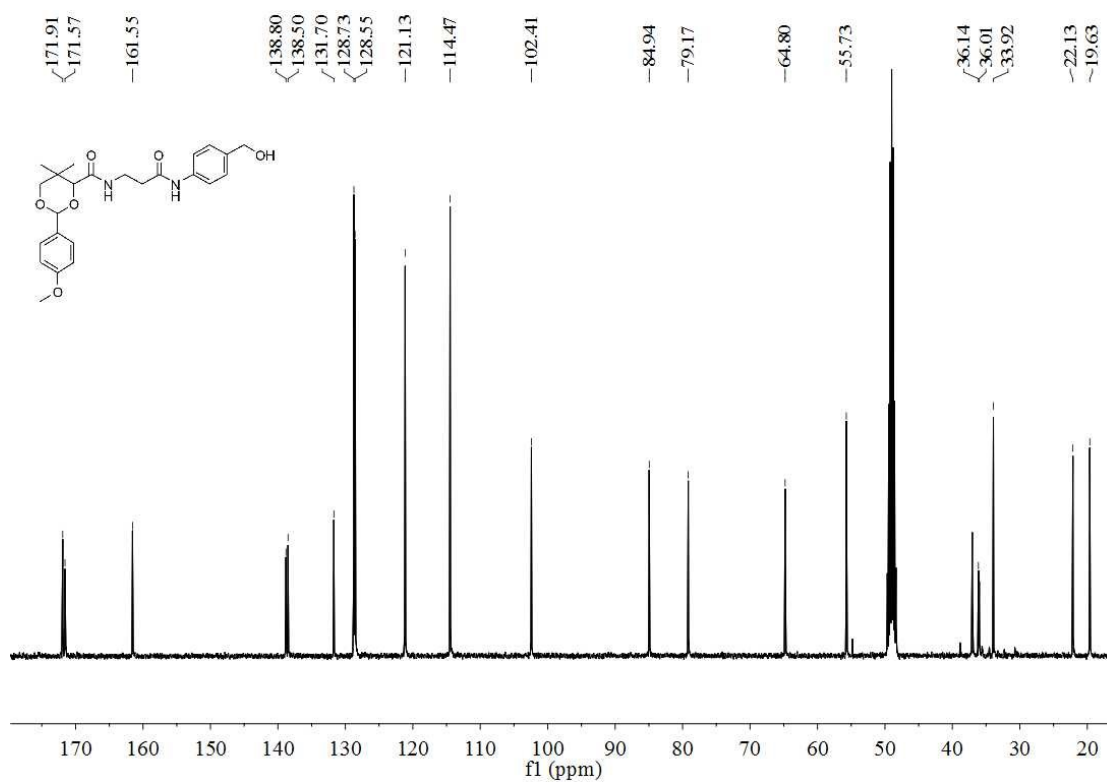
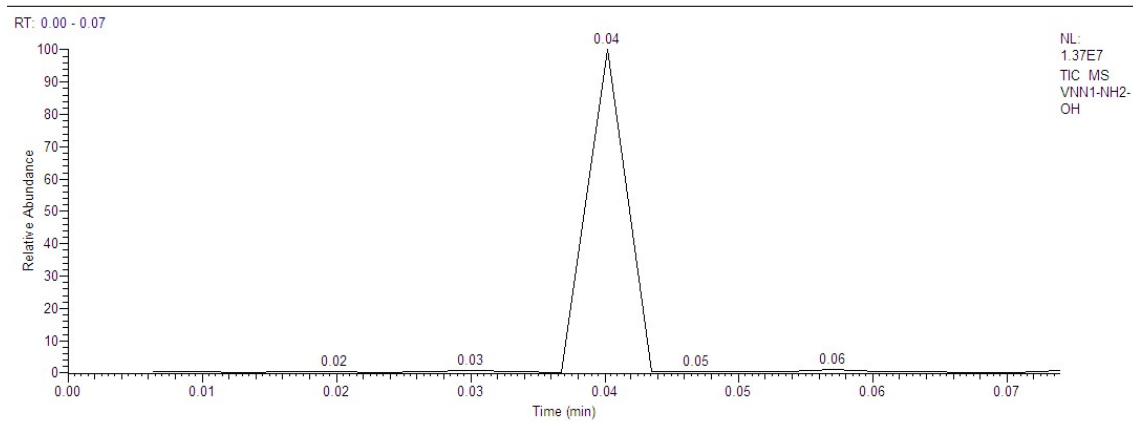


Fig. S12. ¹³C NMR spectrum (CDCl₃) for **b**.



VNN1-NH2-OH #2 RT: 0.01 AV: 1 NL: 1.04E4
T: FTMS + p ESI sid=12.50 Full ms [120.00-800.00]

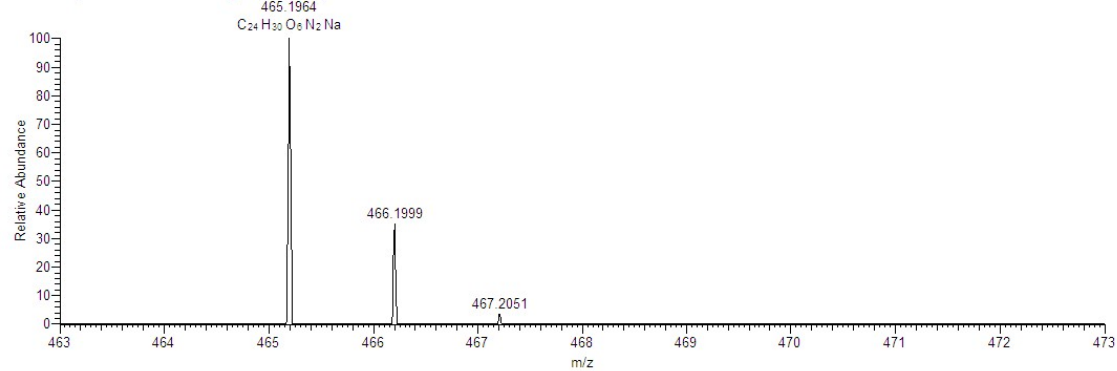


Fig. S13. HRMS spectrum of **b**.

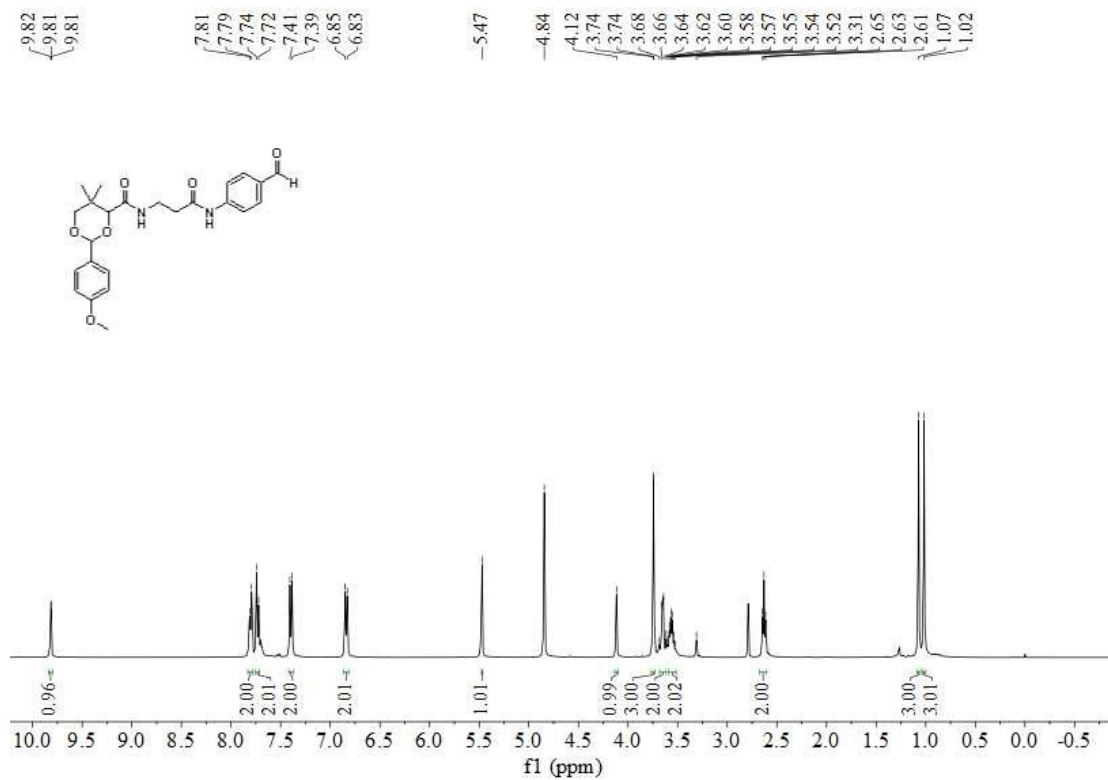


Fig. S14. ^1H NMR spectrum (CD₃OD) for **c**.

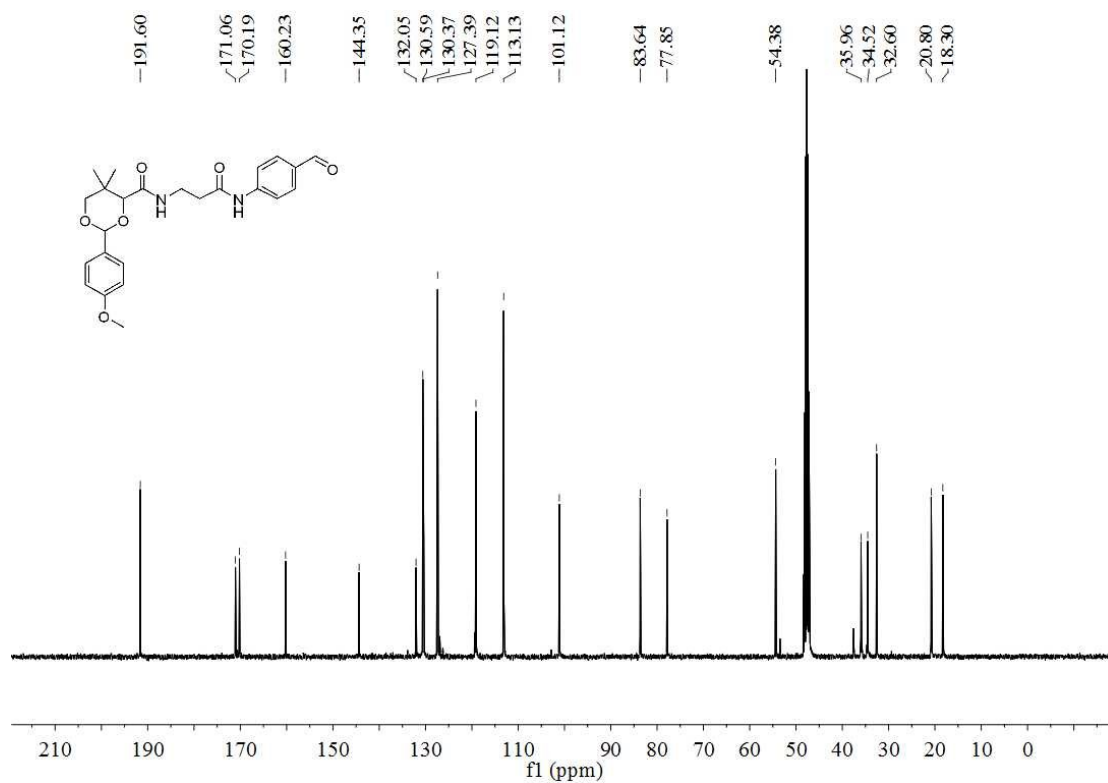
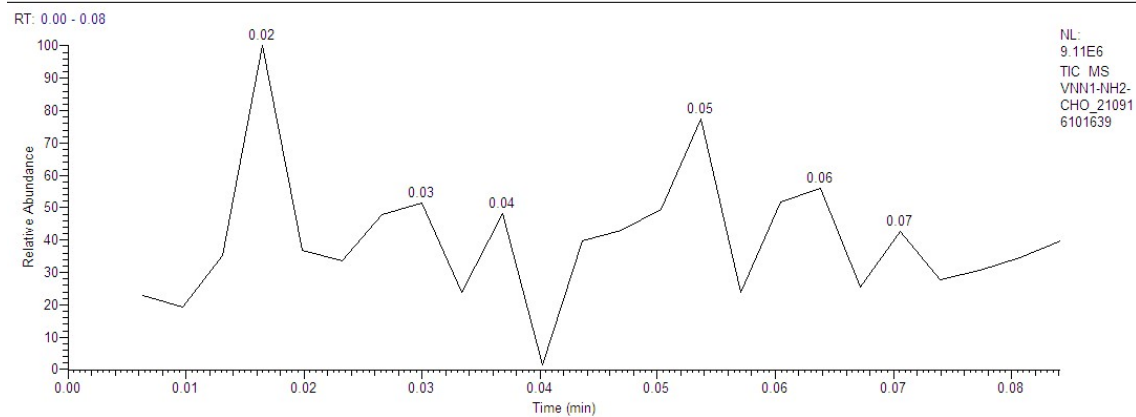


Fig. S15. ¹³C NMR spectrum (CD₃OD) for **c**.



VNN1-NH2-CHO_210916101639 #2 RT: 0.01 AV: 1 NL: 1.73E5
T: FTMS - p ESI Full ms [200.00-800.00]

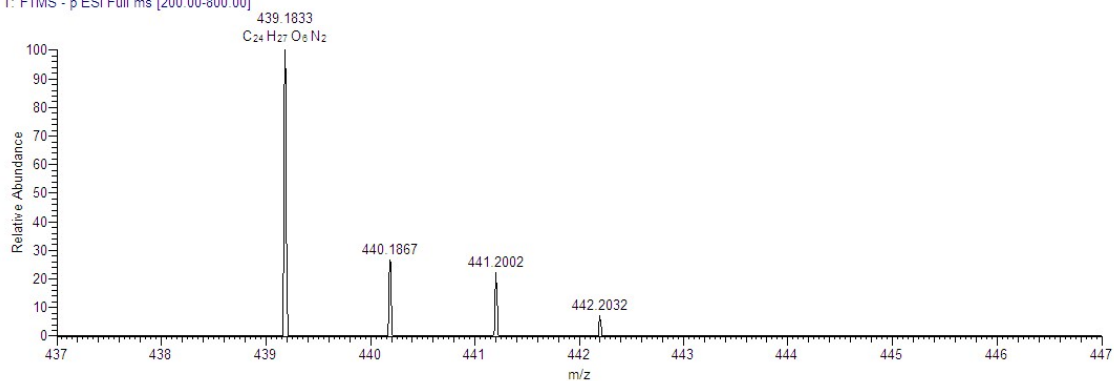


Fig. S16. HRMS spectrum of **c**.

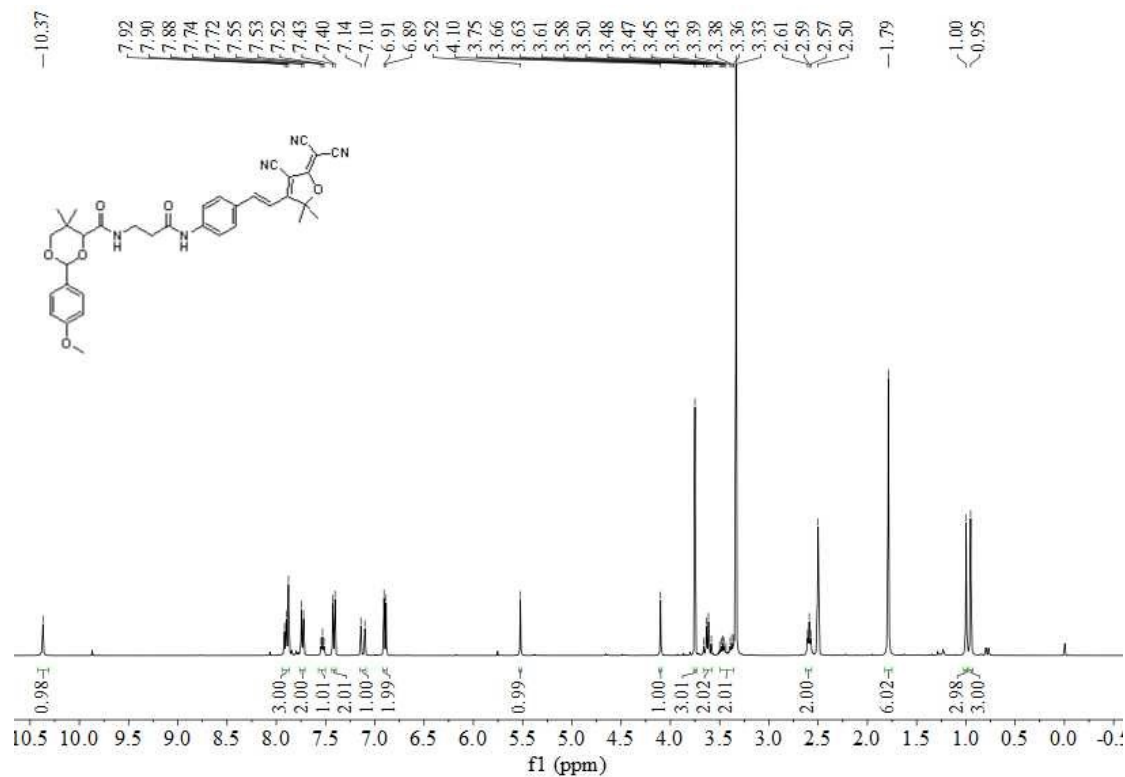


Fig. S17. ^1H NMR spectrum (DMSO- d_6) for **d**.

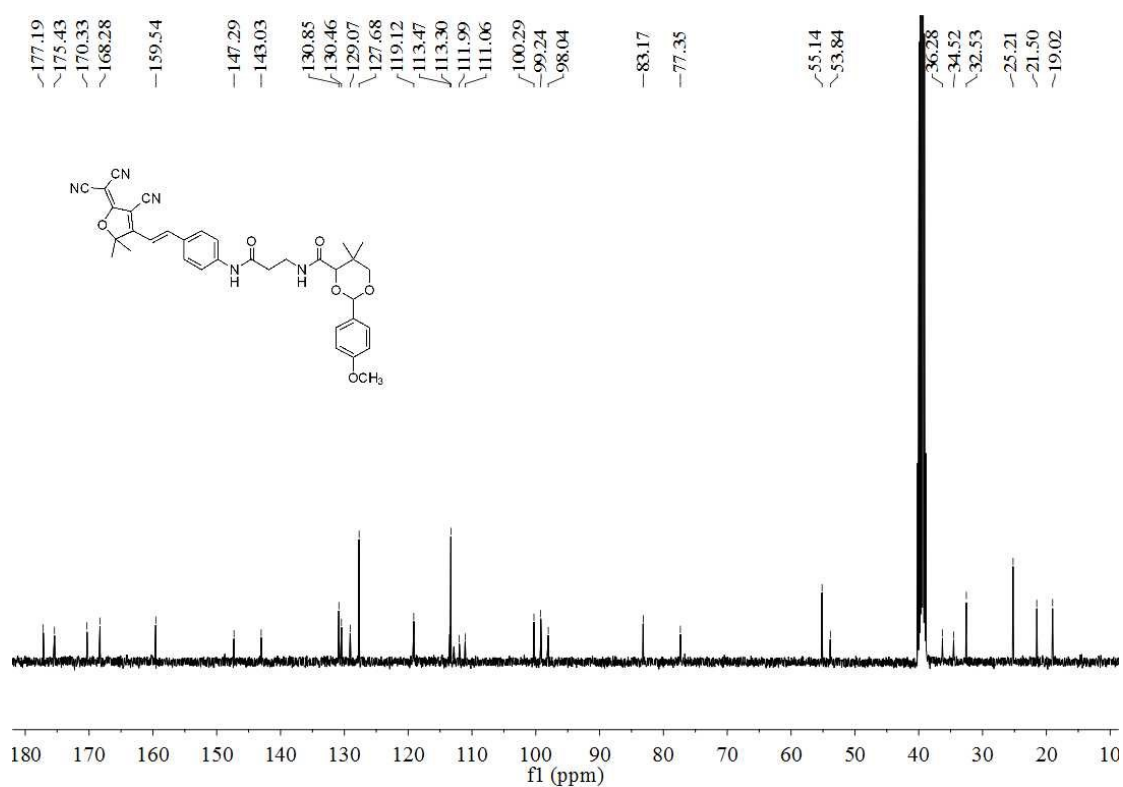
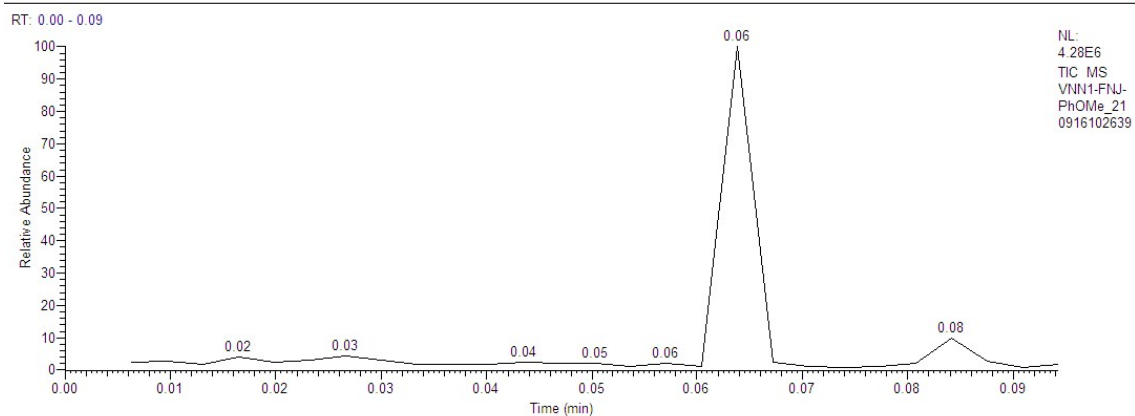


Fig. S18. ^{13}C NMR spectrum (DMSO- d_6) for **d**.



VNN1-FNJ-PhOMe_210916102639 #2 RT: 0.01 AV: 1 NL: 2.13E4
T: FTMS + p ESI sid=12.50 Full ms [500.00-1000.00]

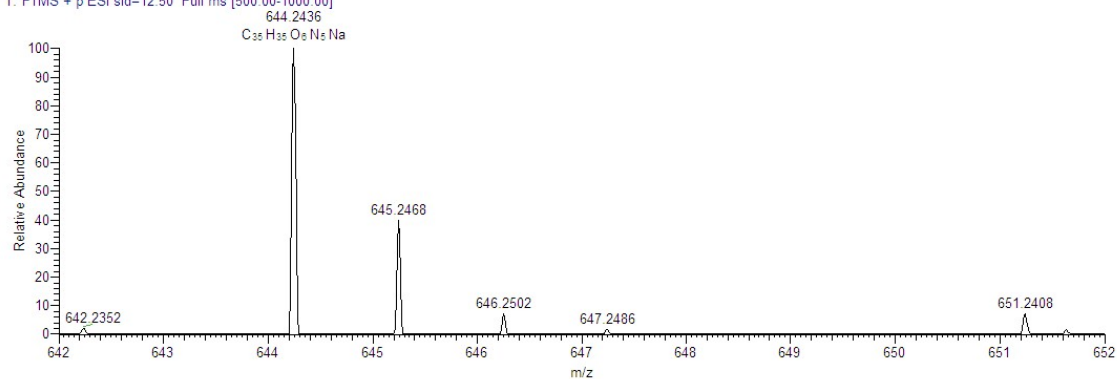


Fig. S19. HRMS spectrum of **d**.

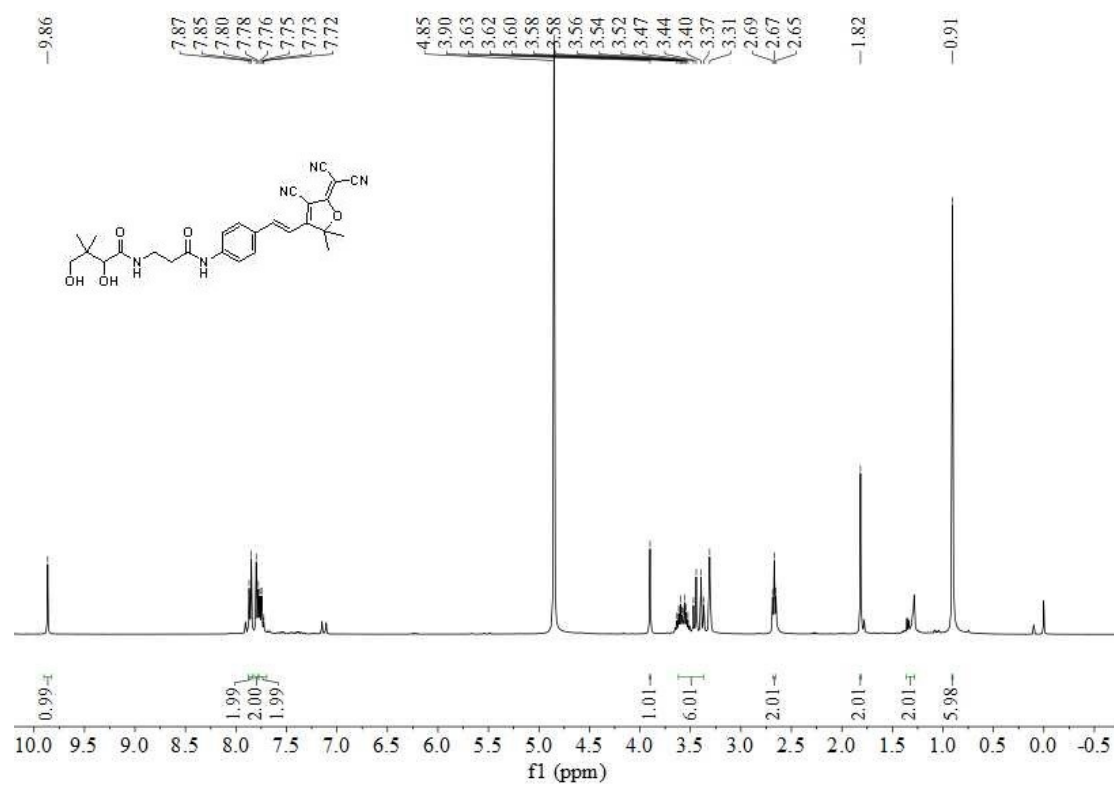


Fig. S20. ¹H NMR spectrum (CD₃OD) for **FNJ-PA**.

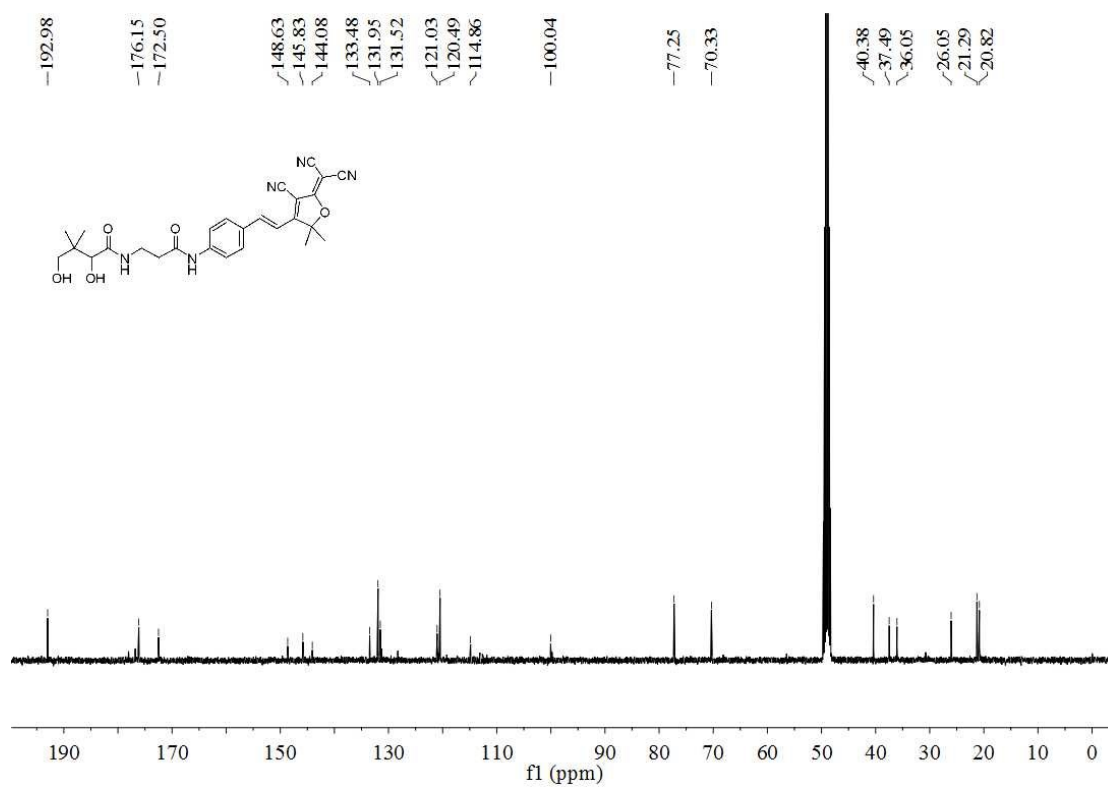


Fig. S21. ¹³C NMR spectrum (CD₃OD) for **FNJ-PA**.

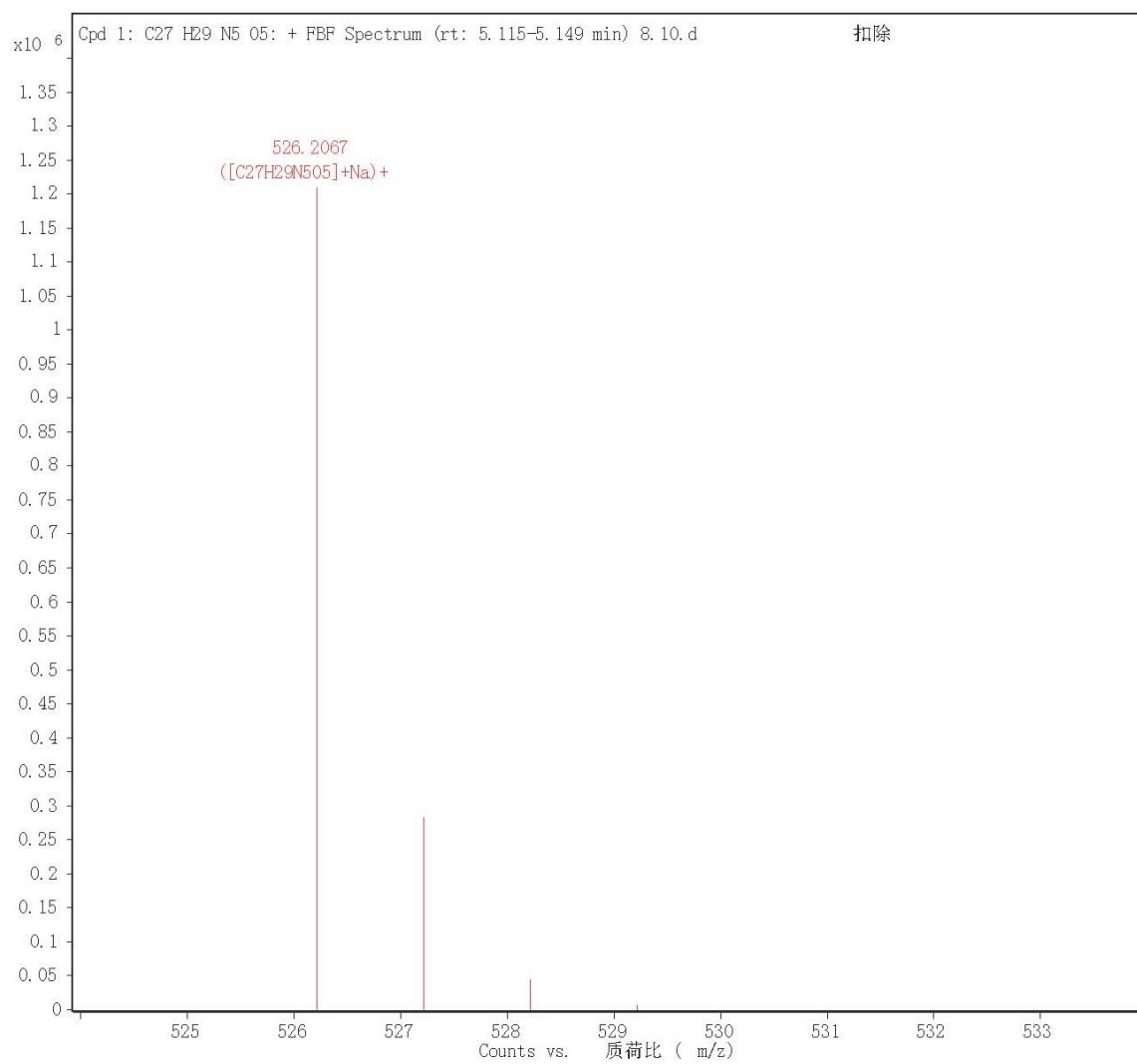


Fig. S22. HRMS spectrum of **FNJ-PA**.