Supporting Information

Enzymatic Synthesis of 2-Hydroxy-4*H*-Quinolizin-4-one Scaffolds by Integrating Coenzyme A Ligases and a Type III PKS from *Huperzia serrata*

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Supplementary Tables

Table S1. Data of compounds P1 and P2

No.	P1[a]		P2[b]	
	δ_H	δ_C	δ_H	δ_C
1	6.39 (1H, s)	96.5	6.37 (1H, s)	92.8
2		168.9		162.6
3	6.01 (1H, s)	95.2		101.2
4		161.8		158.9
5	8.81 (1H, br.d, J = 7.0 Hz)	127.4	8.73 (1H, br.d, J = 7.2 Hz)	126.4
6	7.34 (1H, br.t, J = 7.5 Hz)	131.2	7.26 (1H, br.t, J = 7.5 Hz)	129.1
7	6.92 (1H, br.t, J = 7.5 Hz)	114.6	6.86 (1H, br.t, J = 7.5 Hz)	113.2
8	7.43 (1H, br.d, J = 8.5 Hz)	125.6	7.45 (1H, br.d, J = 9.0 Hz)	124.6
9		144.8		140.3
3-CH_3			2.01 (3H, s)	10.1

[a] Measured in CD_3OD (500 MHz for ^1H, and 125 MHz for ^{13}C), [b] Measured in DMSO-d_6 (600 MHz for ^1H, and 150 MHz for ^{13}C).

Table S2 kinetic parameters of PcPCL

Substrate	K_M (mM)	k_{cat} (s^{-1})
phenylacetic acid *	6.1 ± 0.3	1.4 ± 0.3
p-coumaric acid*	0.47 ± 0.08	3.4 ± 0.5
p-coumaric acid	0.36 ± 0.05	2.6 ± 0.4
2-(pyridine-2-yl)acetic acid	0.26 ± 0.02	3.8 ± 0.3
2-(5-F-pyridine-2yl) acetic acid	0.56 ± 0.06	6.7 ± 0.6
2-(6-F-pyridine-2yl) acetic acid	0.92 ± 0.06	8.1 ± 0.2

* Kinetic parameters reported in literature.[1]

Supplementary Figures

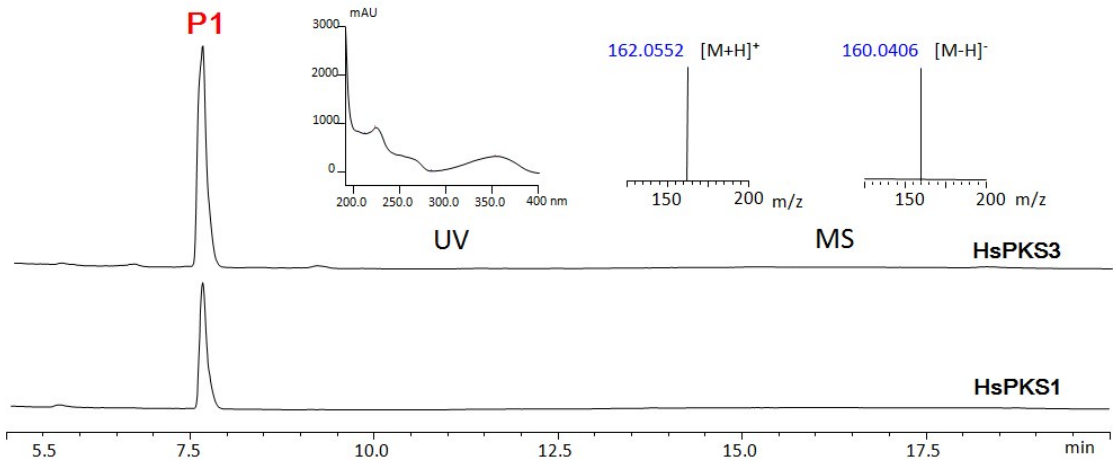

Figure S1. LC-MS charts of the enzymatic products produced by HsPKS3 and HsPKS1 from the condensation of 2-pyridylacetyl-CoA and malonyl-CoA. Data for the other eight PKSs, including HsPKS2, ErQNS1, ErQNS2, AsCHS, AsPKS1, AsPKS2, ClDCS, and ZoCURS were not included here due to the absent generation of enzymatic products. The HPLC chromatograms were measured at 230 nm, and the crude yield of the enzymatic product produced by HsPKS3 is about 2.5 times higher (calcuted by the area of the peaks presented in the HPLC charts) than that produced by HsPKS1.

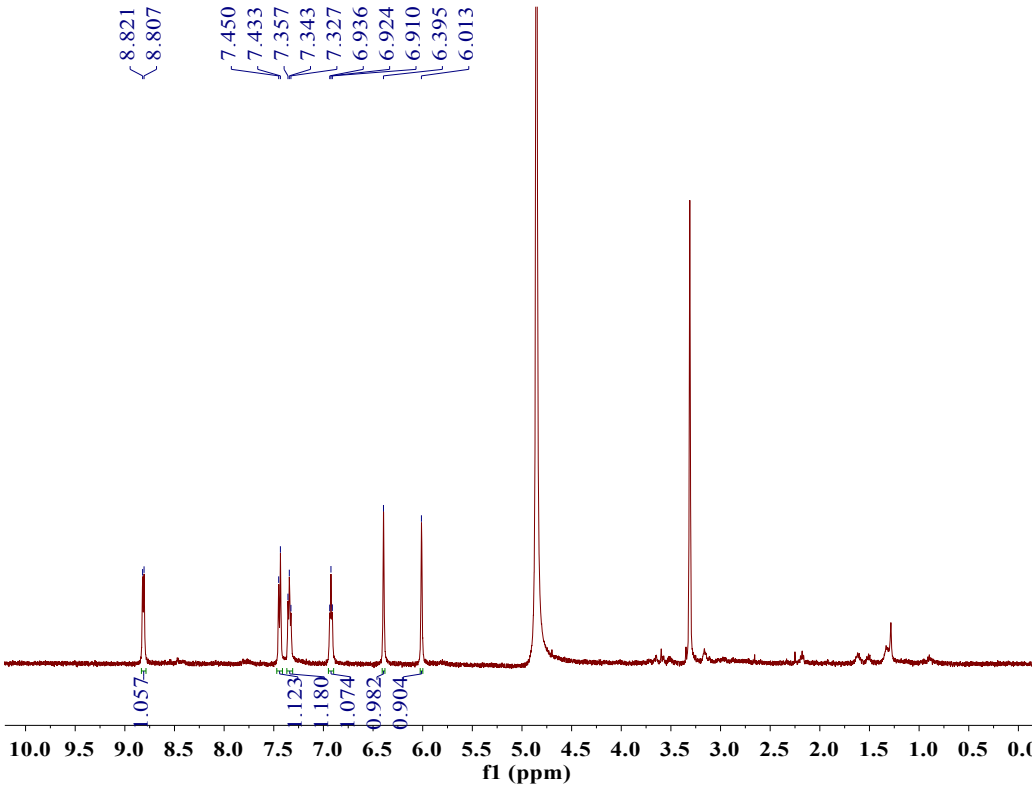

Figure S2. ^{1}H NMR spectrum of compound **P1** (in CD_3OD, 500 MHz)

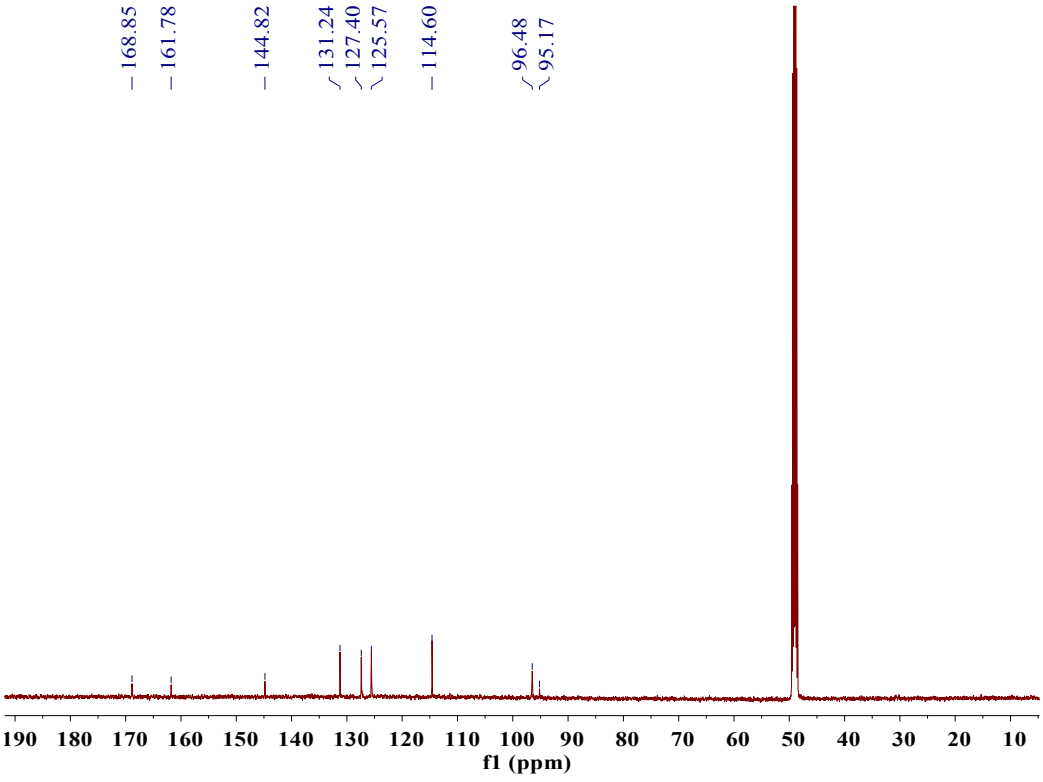

Figure S3. ^{13}C NMR spectrum of compound **P1** (in CD_3OD, 125 MHz)

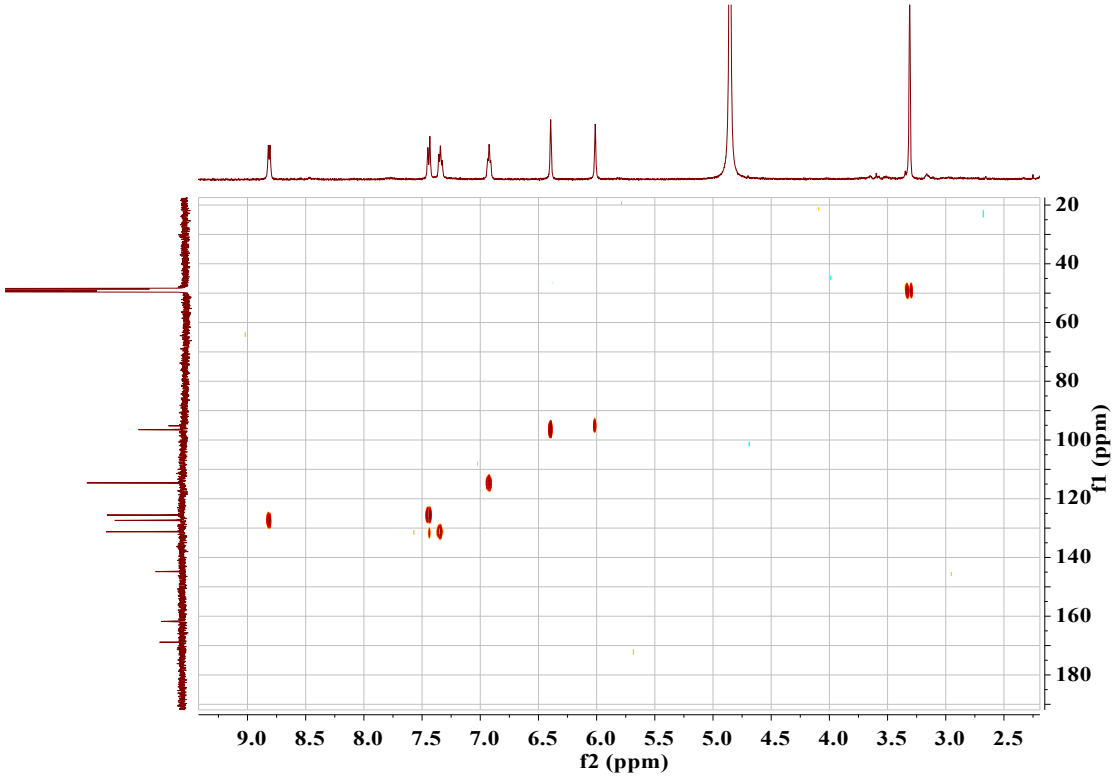

Figure S4. gHSQC spectrum of compound **P1**

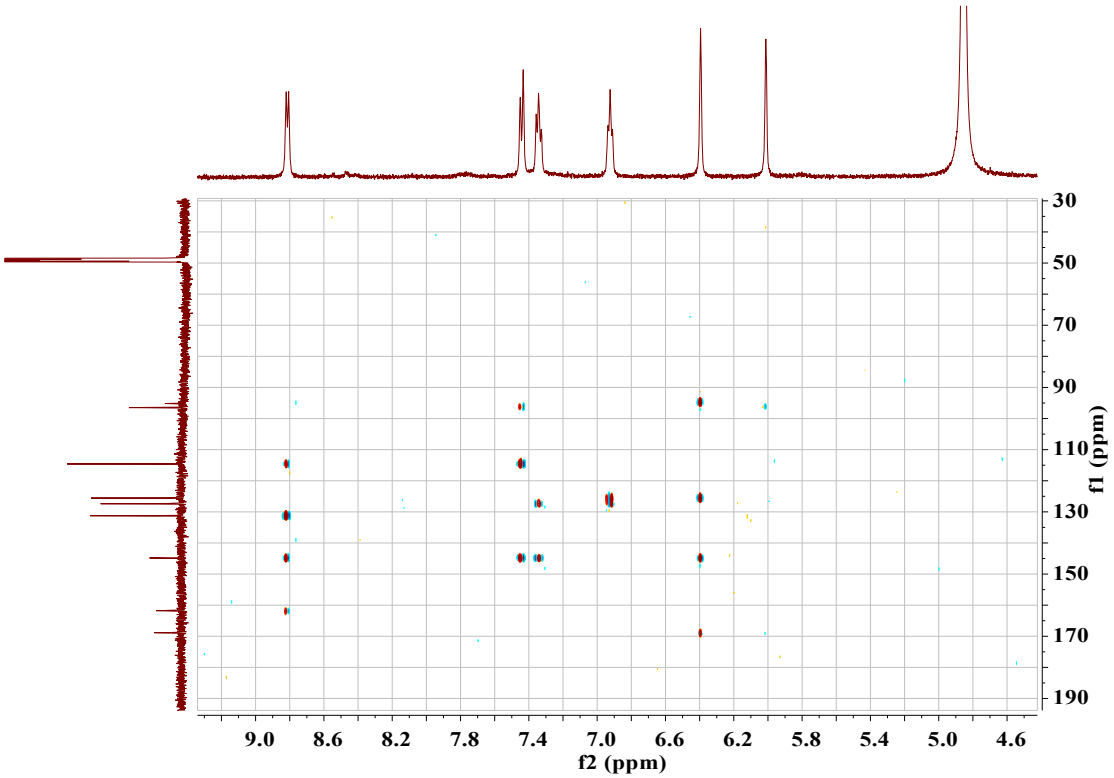

Figure S5. gHMBC spectrum of compound **P1**

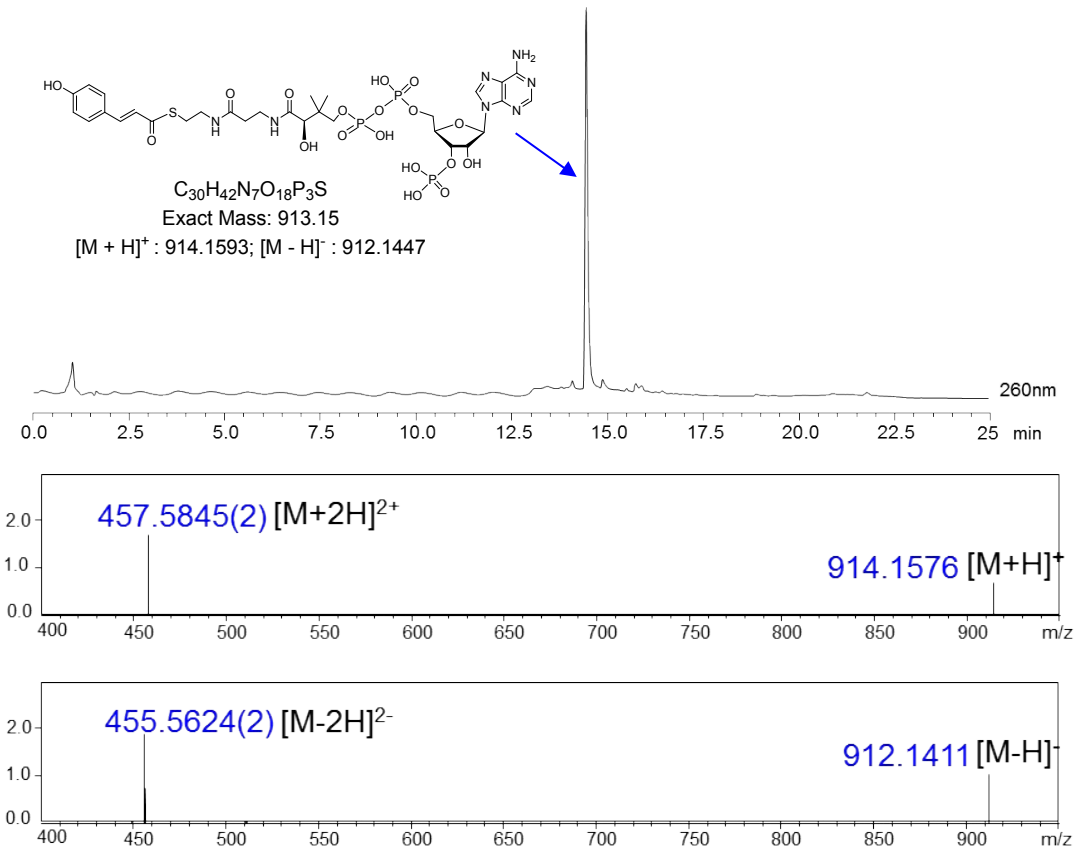

Figure S6. The LC-MS charts of *p*-coumaroyl-CoA produced by PcPCL

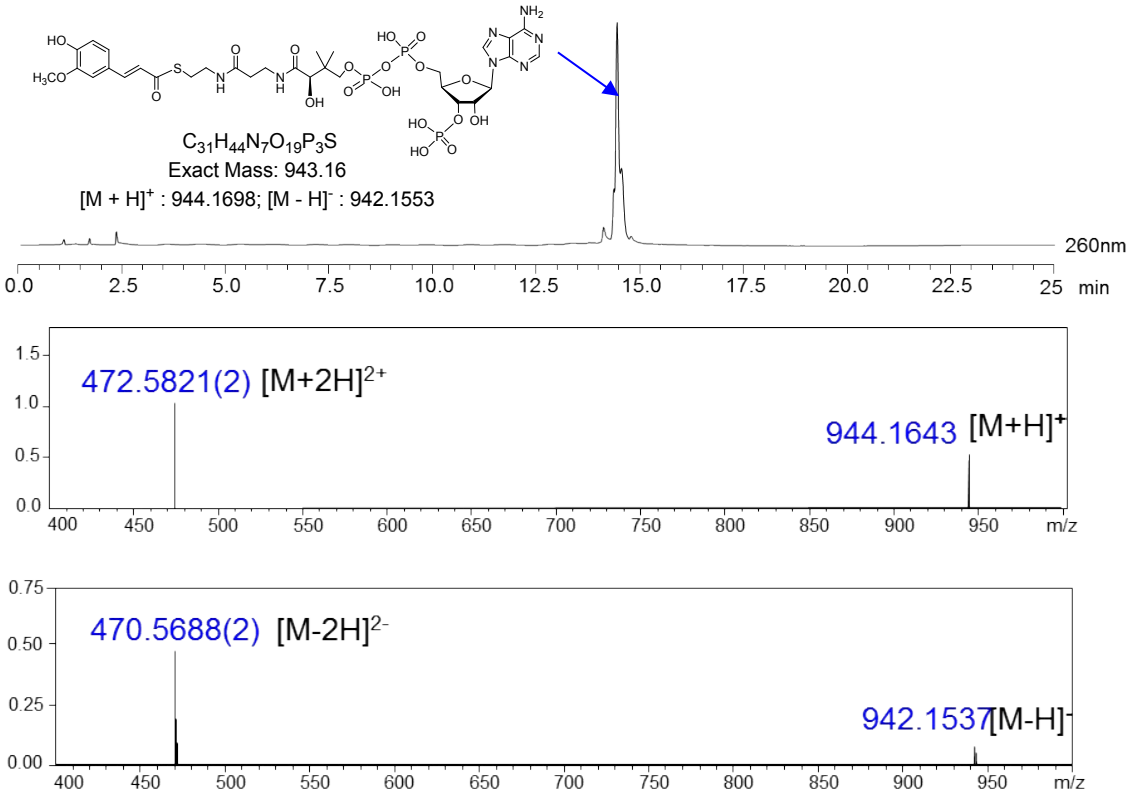

Figure S7. The LC-MS charts of feruloyl-CoA produced by PcPCL

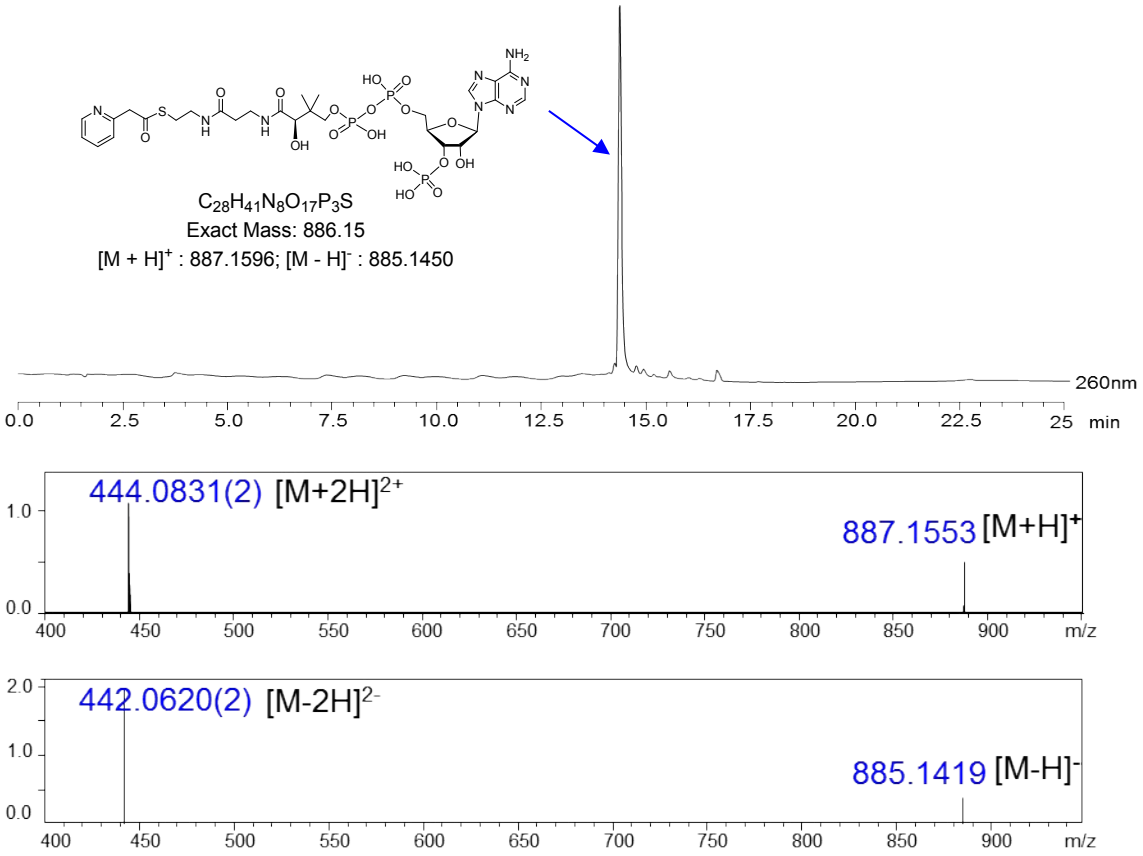

Figure S8. The LC-MS charts of 2-pyridylacetyl-CoA produced by PcPCL

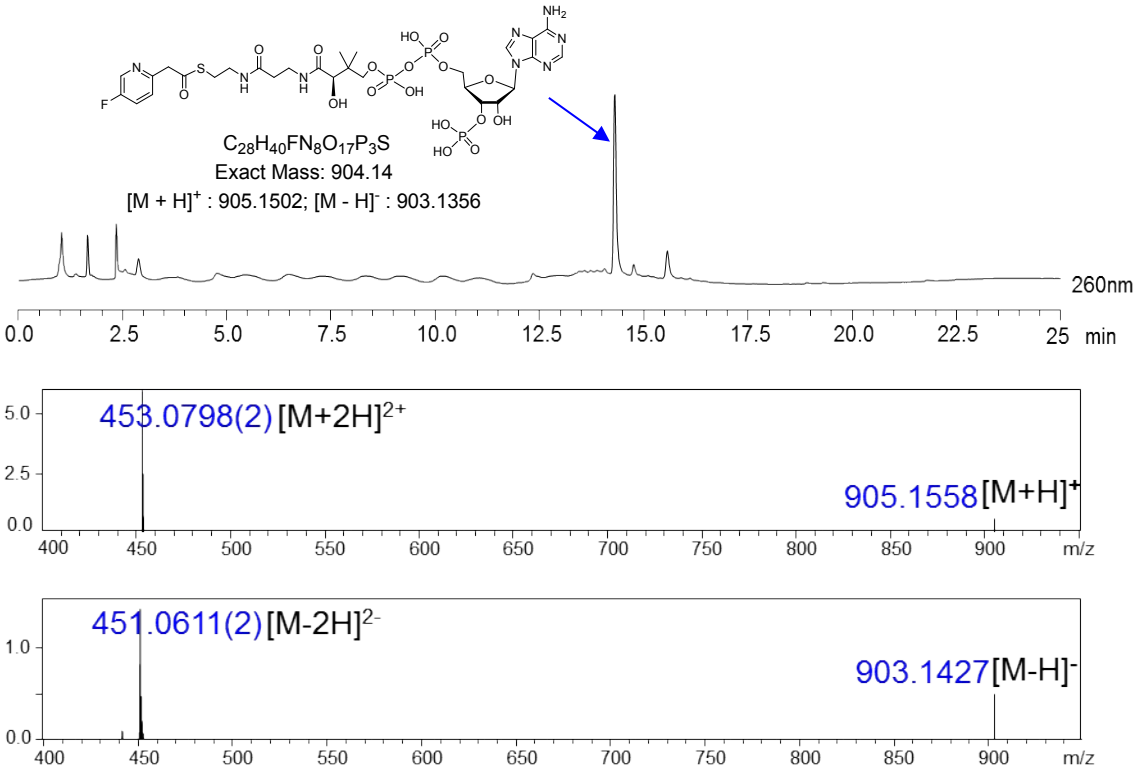

Figure S9. The LC-MS charts of 2-(5-F-pyridine-2yl) acetyl-CoA produced by PcPCL

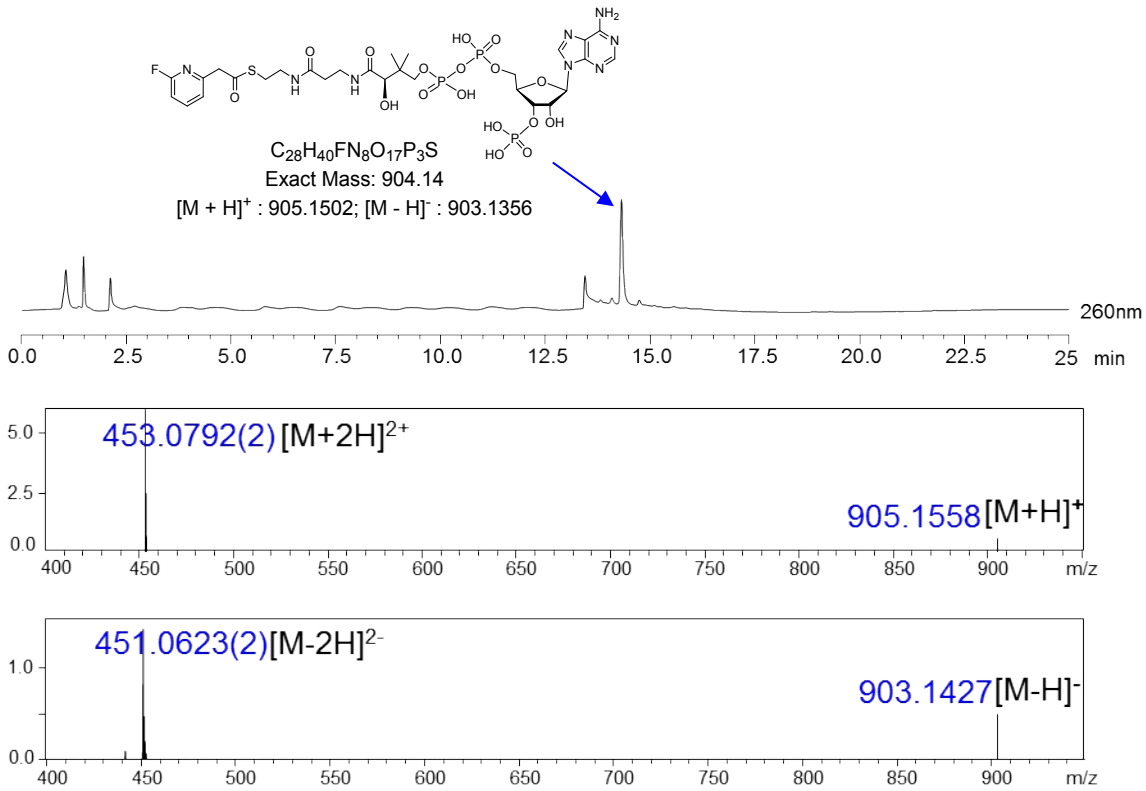

Figure S10. The LC-MS charts of 2-(6-F-pyridine-2yl) acetyl-CoA produced by PcPCL

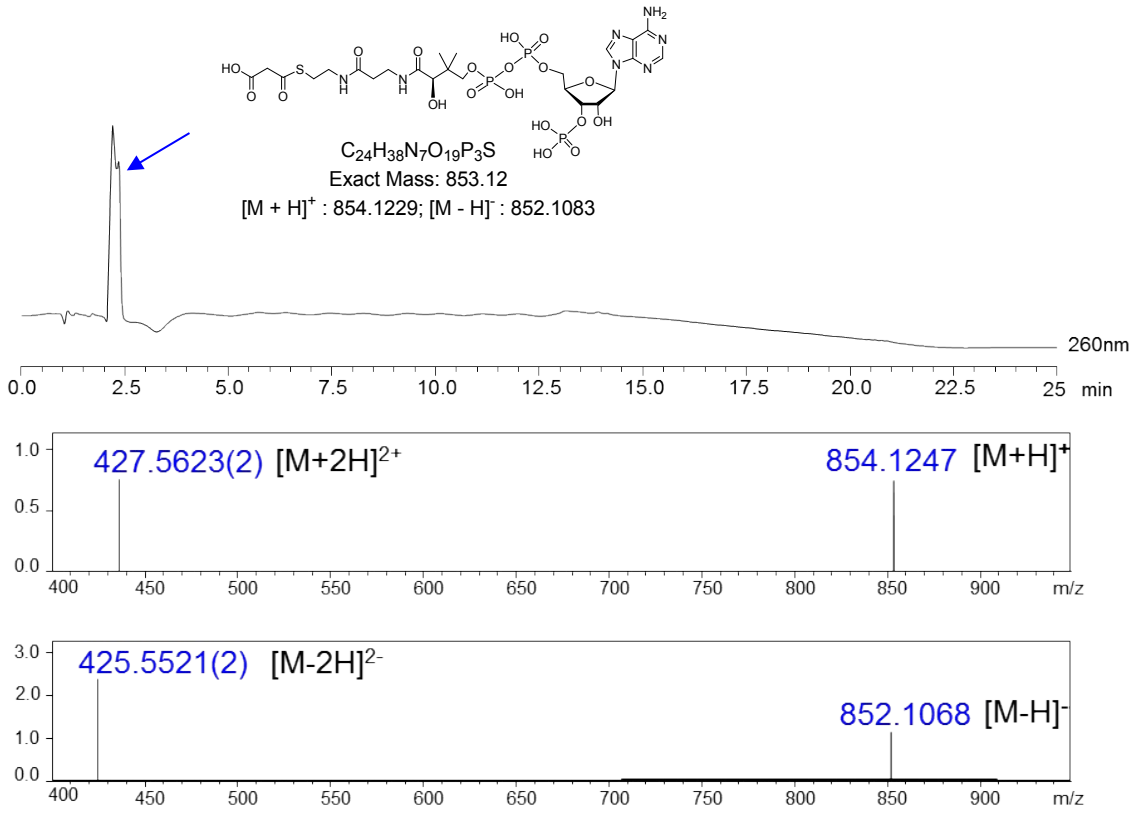

Figure S11. The LC-MS charts of the malonyl-CoA produced by AtMatB

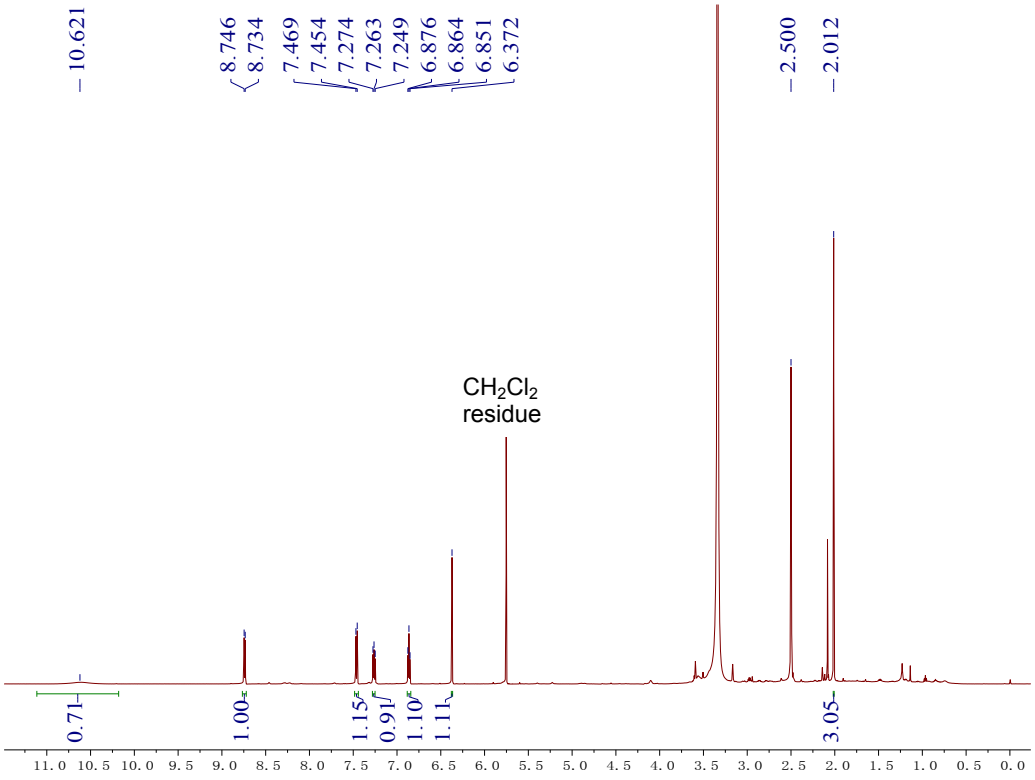

Figure S12. ^{1}H NMR spectrum of compound **P2** (in DMSO-d_6, 600 MHz)

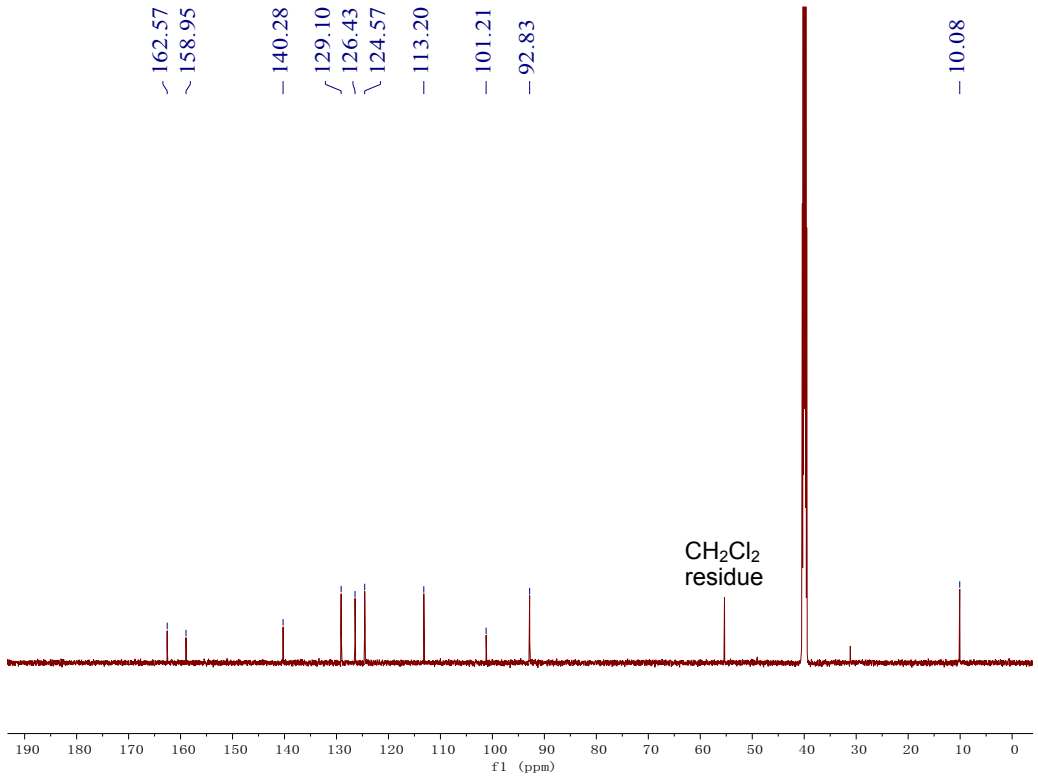

Figure S13. ^{13}C NMR spectrum of compound **P2** (in DMSO-d_6, 150 MHz)

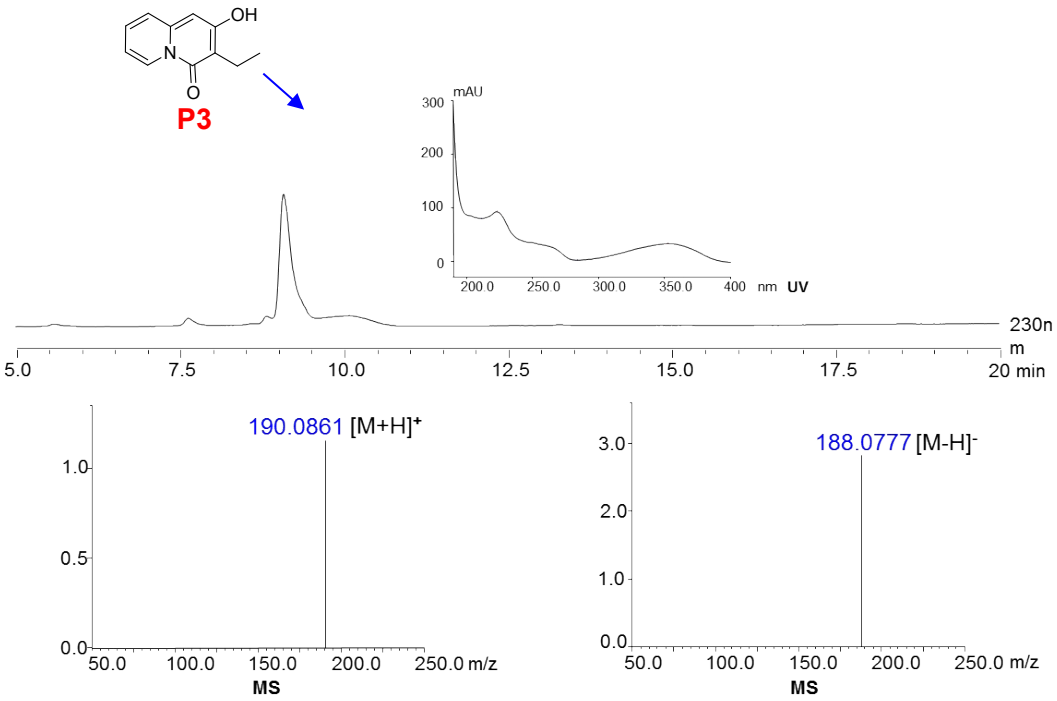

Figure S14. LC-MS charts of the enzymatic product **P3** produced by one-pot reaction

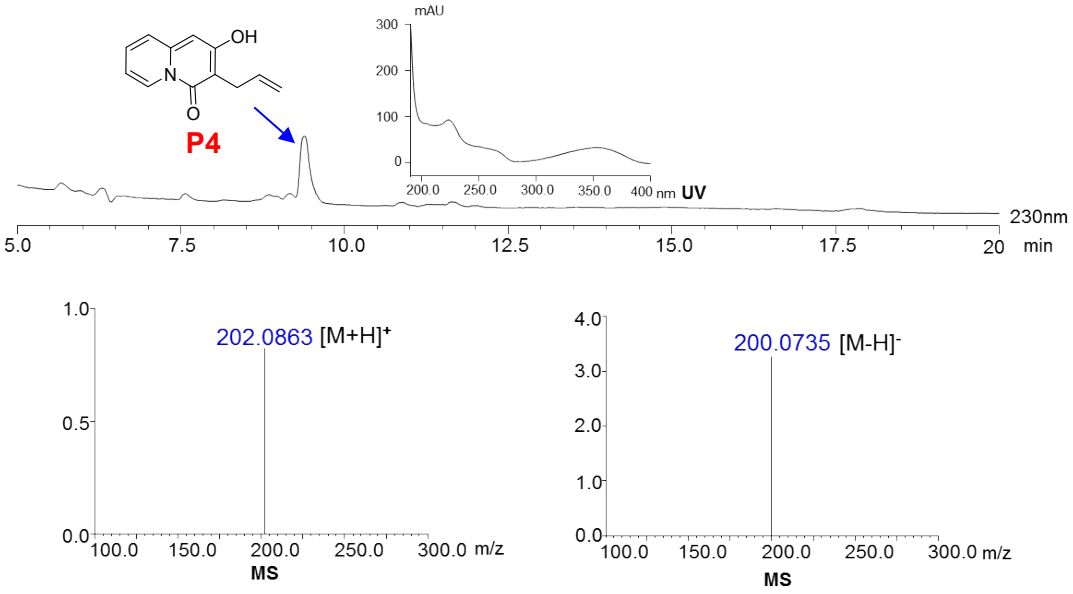

Figure S15. LC-MS charts of the enzymatic product **P4** produced by one-pot reaction

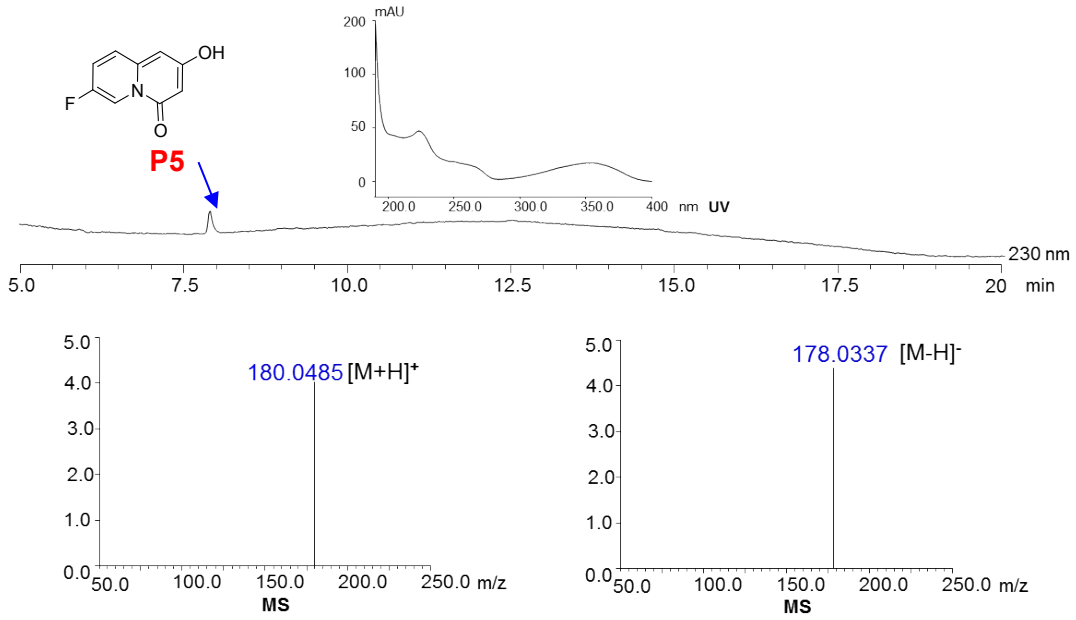

Figure S16. LC-MS charts of the enzymatic product **P5** produced by one-pot reaction

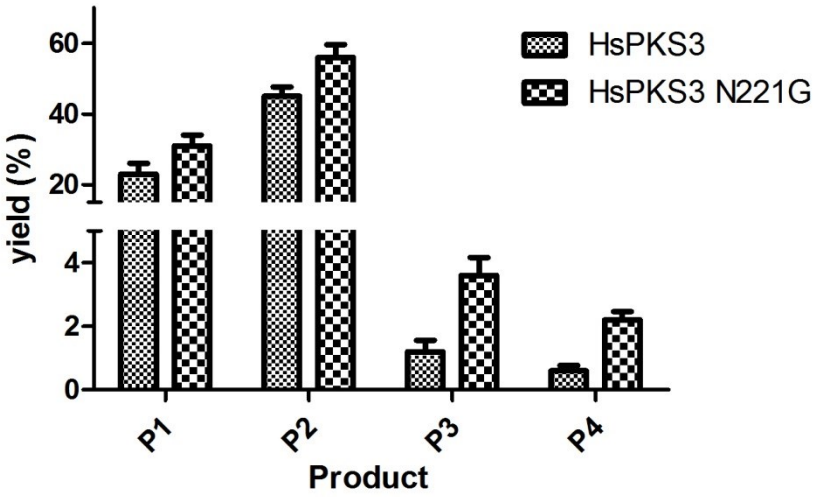

Figure S17. Comparison of **t**he yields of compounds **P1**-**P4** produced by wild HsPKS3 and HsPKS3 N221G (experiments were performed in triplicate, and the S.D. is shown).

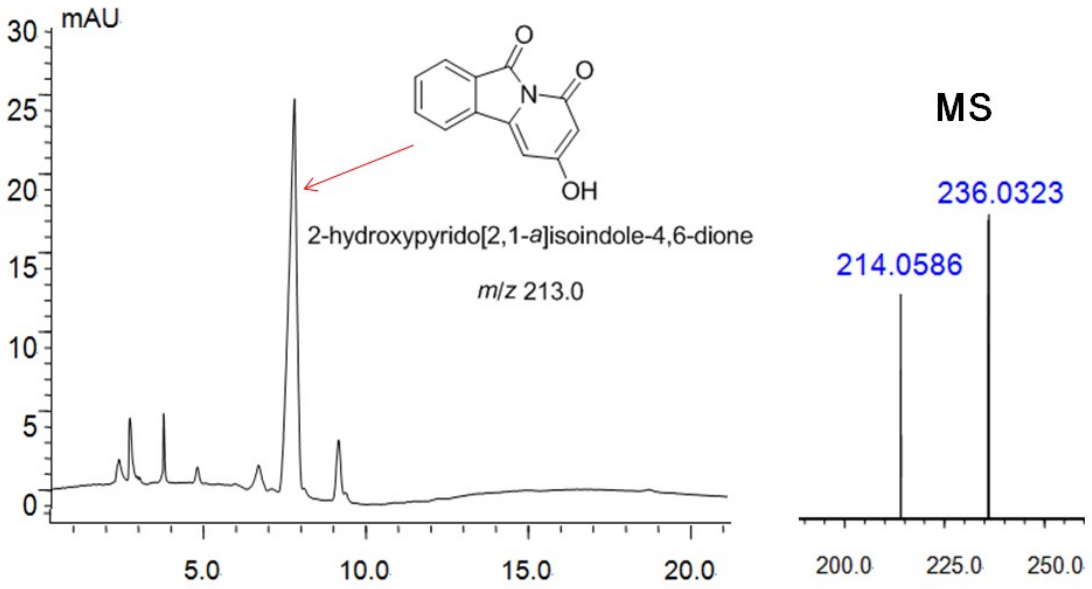

Figure S18. HsPKS3 N221G catalyzed the formation of 2-hydroxypyrido[2,1-*a*]isoindole-4,6-dione which has been reported to be synthesized by HsPKS1 from the condensation of 2-carbamoylbenzoyl-CoA and two molecules of malonyl-CoA.[2] In contrast, the wild HsPKS3 could not accept the bulky 2-carbamoylbenzoyl-CoA.[3]

References

1 M. J. Koetsier, P. A. Jekel, M. A. Van den Berg, R. A. Bovenberg, D. B. Janssen, *Biochem. J.* 2009, **417**, 467.

2 H. Morita, M. Yamashita, S. P. Shi, T. Wakimoto, S. Kondo, R. Kato, S. Sugio, T. Kohno, I. Abe, *Proc. Natl. Acad. Sci. U. S. A.* 2011, **108**, 13504.

3 J. Wang, X. H. Wang, X. Liu, J. Li, X. P. Shi, Y. L. Song, K.W. Zeng, L. Zhang, P. F. Tu, S. P. Shi, Org. Lett. 2016, 18, 3550.