

Supporting Information

Nanocapsule Engineering Endows Aldolase with Robust Organic-Solvent Tolerance and Enhanced Catalytic Efficiency

Can Cui^{‡1, 2}, Xuerui Jin^{‡1, 2}, Yunzi Luo^{*1, 2, 3}

¹ Frontiers Science Center for Synthetic Biology (Ministry of Education), School of Synthetic Biology and Biomanufacturing, Tianjin University, Tianjin 300072, China

² State Key Laboratory of Synthetic Biology, Tianjin University, Tianjin, 300072, China

³ Haihe Laboratory of Sustainable Chemical Transformations, Tianjin 300192, China

* E-mail: yunzi.luo@tju.edu.cn

‡ These authors contributed equally.

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Number of figures: 17

Number of tables: 2

Contents of Supporting Information

I. Supplementary Tables

Table S1 The information of the aldolases used in this study.	S1
Table S2 The lysine information of the aldolases used in this study.	S2

II. Supplementary Figures

Figure S1 The SDS-PAGE result of the purified and modified aldolase GarL (27.4 kDa).	S3
Figure S2 Size exclusion chromatography (SEC) and SDS-PAGE of n-GarL modified with different ratios of NAS.	S4
Figure S3 Enzymatic activity of n-GarL modified with different ratios of NAS.	S5
Figure S4 Effect of external ethanol on the enzymatic reaction activity of n-GarL with varied monomer ratios.	S6
Figure S5 Validation of n-GarL by Ion Exchange Chromatography (IEC) and SDS-PAGE.	S7
Figure S6 Validation of n-7080 by Ion Exchange Chromatography (IEC) and SDS-PAGE.	S8
Figure S7 Transmission electron microscopy results of aldolase 7080 and GarL before and after modification.	S9
Figure S8 Tolerance of Aldolase Nanocapsule to high temperature.	S10
Figure S9 The relative enzyme activity improvement of n-7080 in the reaction of 1b detected by HPLC and the MS detection of the product 4b.	S11
Figure S10 The relative enzyme activity improvement of n-7080 in the reaction of 1c detected by HPLC and the MS detection of the product 4c.	S12
Figure S11 The relative enzyme activity improvement of n-7080 in the reaction of 1d detected by HPLC and the MS detection of the product 4d.	S13
Figure S12 The relative enzyme activity improvement of n-7080 in the reaction of 1e detected by HPLC and the MS detection of the product 4e.	S14
Figure S13 The relative enzyme activity improvement of n-7080 in the reaction of 1f detected by HPLC and the MS detection of the product 4f.	S15
Figure S14 The relative enzyme activity improvement of n-7080 in the reaction of 1g detected by HPLC and the MS detection of the product 4g.	S16
Figure S15 The relative enzyme activity improvement of n-7080 in the reaction of 1h detected by HPLC and the MS detection of the product 4h.	S17
Figure S16 The relative enzyme activity improvement of n-7080 in the reaction of 1i detected by HPLC and the MS detection of the product 4i.	S18
Figure S17 The relative enzyme activity improvement of n-7080 in the reaction of 1j detected by HPLC and the MS detection of the product 4j.	S19

I. Supplementary Tables

Table S1 The information of the aldolases used in this study.

Protein	Origin	Accession number	Class	Assembly	Amino acid sequences
7080	<i>Streptomyces rapamycinicus</i>	AAA57929.1	II	hexamer	MNTSPADFAARLRARRRIIGYWVVL DNPVGTERLAGLGYDYICVDAQHGL LDYKGTLGAMTAIDARGTAAGMVR VPGNDGFWIGQALDAGARGVIVPLV NTAE EAAAAVS YCRY PPLGVRSYGP MRSGLRVGPAPAESNRQVACVVMIE TAQGLANAAEICATDGLDGVYIGPSD LTIALGGRTSTDTSVAEEFEALAKV AEEARTAGIAAGIHCPDGATAAGRLA QGFTFATVSSDLAHLAQAAAHLRD ANG*
GarL	<i>Escherichia coli</i> DH10B	none	II	hexamer	MNTSPADFAARLRARRRIIGYWVVL DNPVGTERLAGLGYDYICVDAQHGL LDYKGTLGAMTAIDARGTAAGMVR VPGNDGFWIGQALDAGARGVIVPLV NTAE EAAAAVS YCRY PPLGVRSYGP MRSGLRVGPAPAESNRQVACVVMIE TAQGLANAAEICATDGLDGVYIGPSD LTIALGGRTSTDTSVAEEFEALAKV AEEARTAGIAAGIHCPDGATAAGRLA QGFTFATVSSDLAHLAQAAAHLRD ANG*

Table S2 The lysine information of the aldolases used in this study.

Protein	Lysine location	Lysine distribution
7080	Lys54	Interior
	Lys200	Surface
GarL	Lys9	Surface
	Lys11	Surface
	Lys17	Surface
	Lys66	Interior
	Lys85	Interior
	Lys103	Surface
	Lys144	Surface
	Lys197	Surface
	Lys211	Surface
	Lys249	Surface
Lys255	Surface	
Lys256	Surface	

II. Supplementary Figures

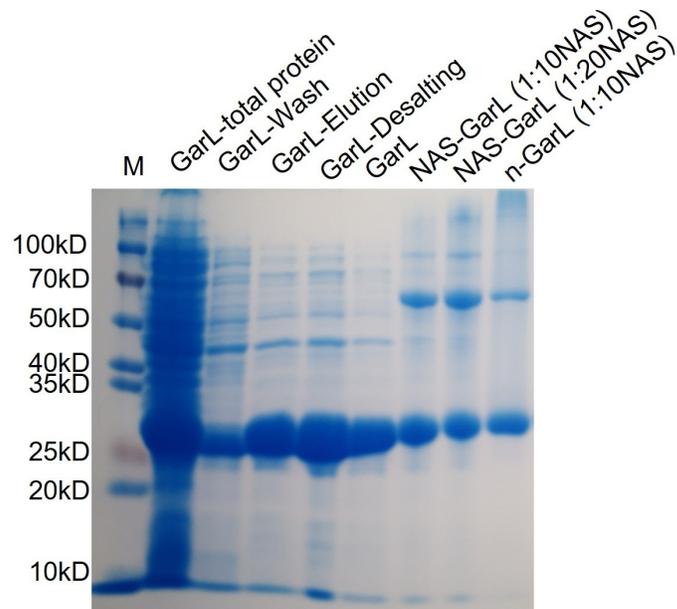


Figure S1 The SDS-PAGE result of the purified and modified aldolase GarL (27.4 kDa).

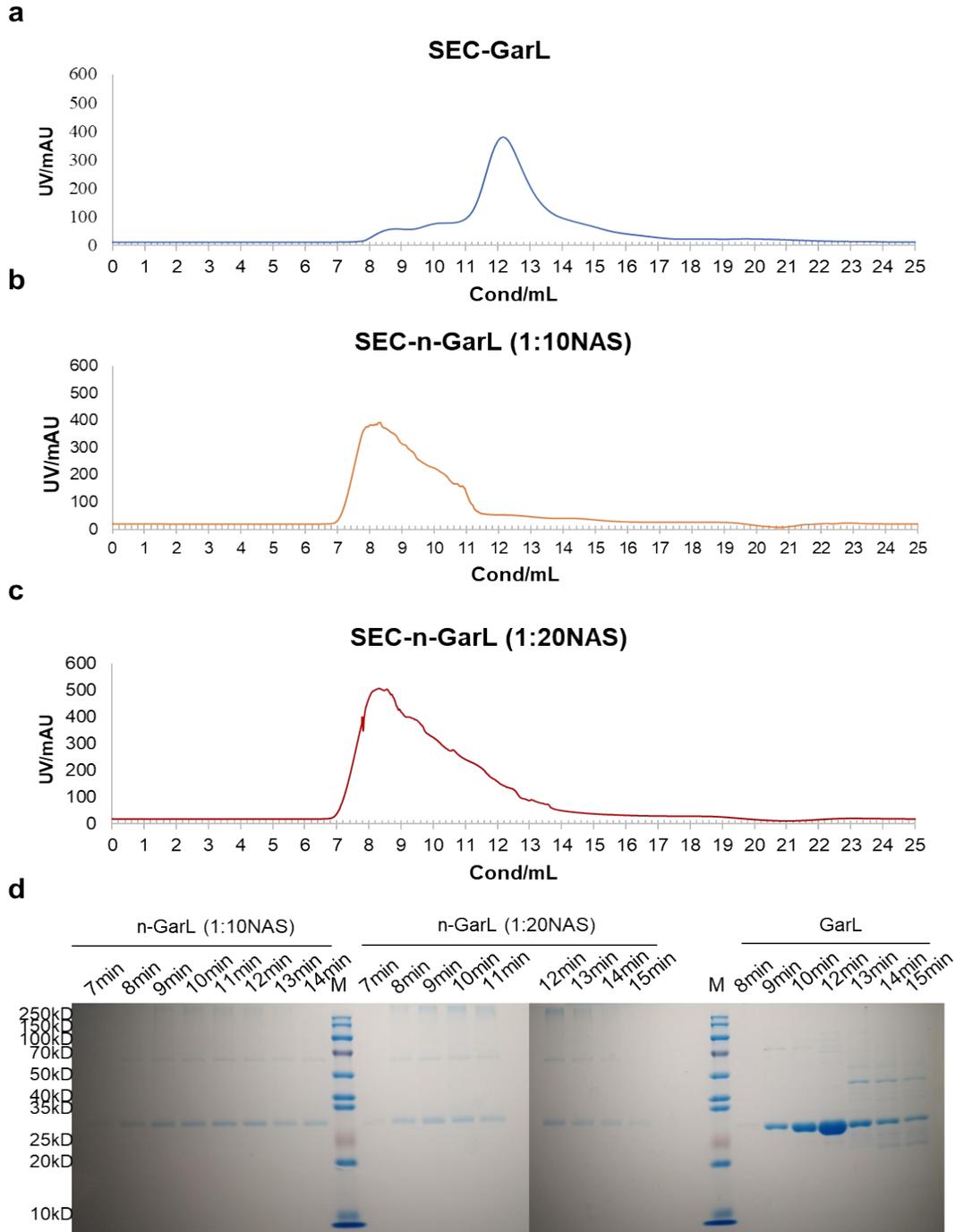
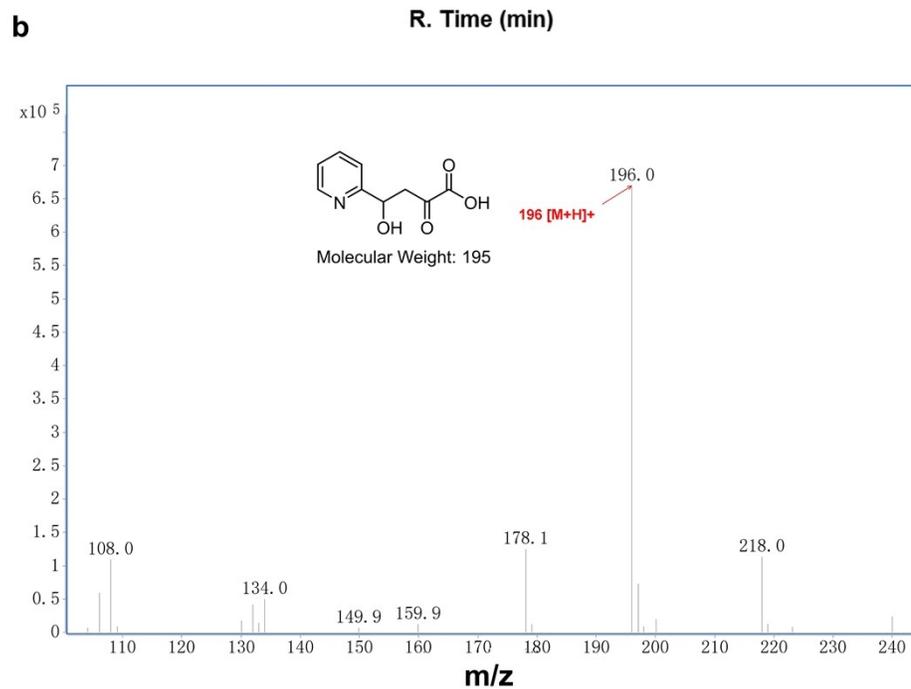
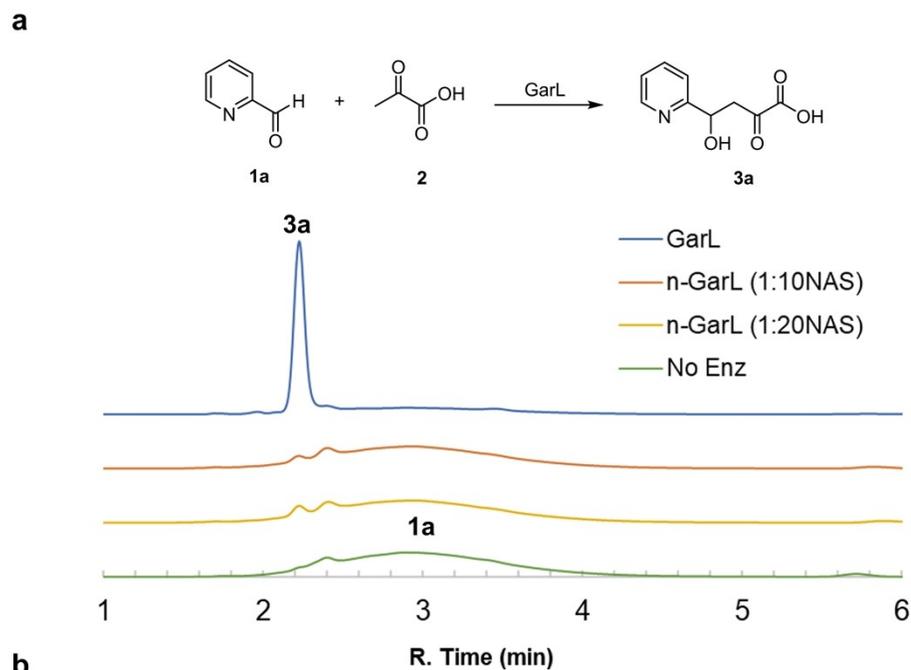
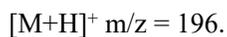


Figure S2 Size exclusion chromatography (SEC) and SDS-PAGE of n-GarL modified with different ratios of NAS.

- a. SEC result of GarL (Loading sample: 5 mg); b. SEC result of n-GarL (1:10NAS) (Loading sample: 5 mg); c. SEC result of n-GarL (1:20NAS) (Loading sample: 5 mg); d. SDS-PAGE result of the corresponding fraction collections.



ed by GarL and n-GarL; **b**. The electrospray ionization mass spectrometry (MS) detection of **3a**:



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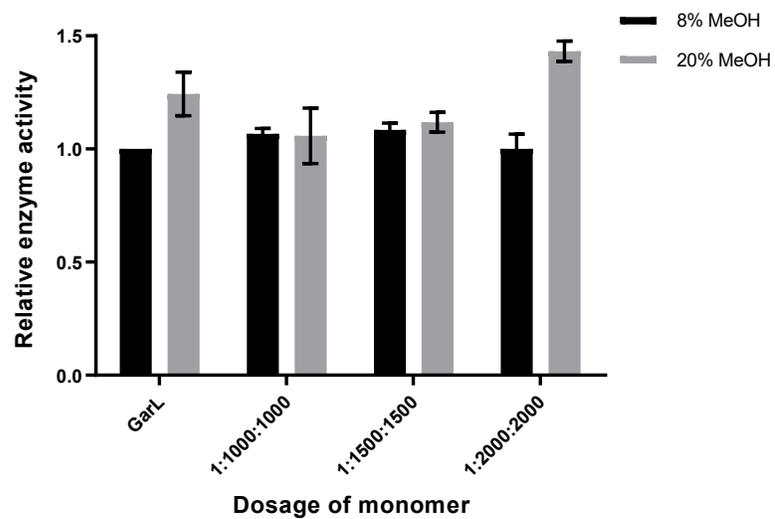


Figure S4 Effect of external ethanol on the enzymatic reaction activity of n-GarL with varied monomer ratios.

GarL-WT was used as the control, and the reaction system contained 8% methanol as the substrate solvent.

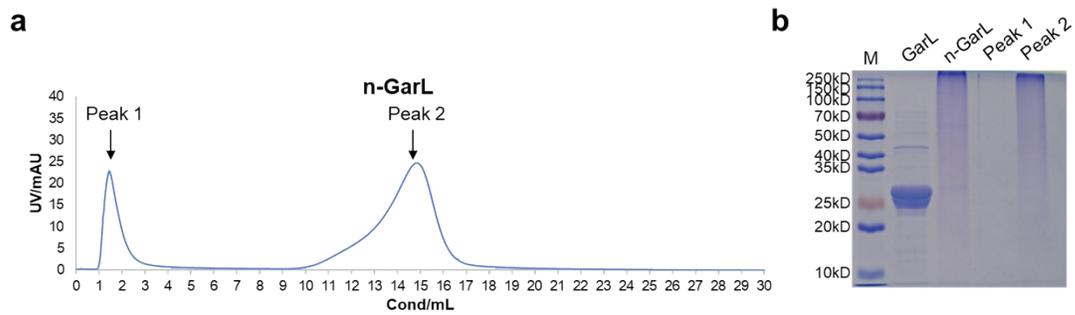


Figure S5 Validation of n-GarL by Ion Exchange Chromatography (IEC) and SDS-PAGE.
a. IEC result of n-GarL (Loading sample: 500 μ g); **b.** SDS-PAGE result of the corresponding fraction collections.

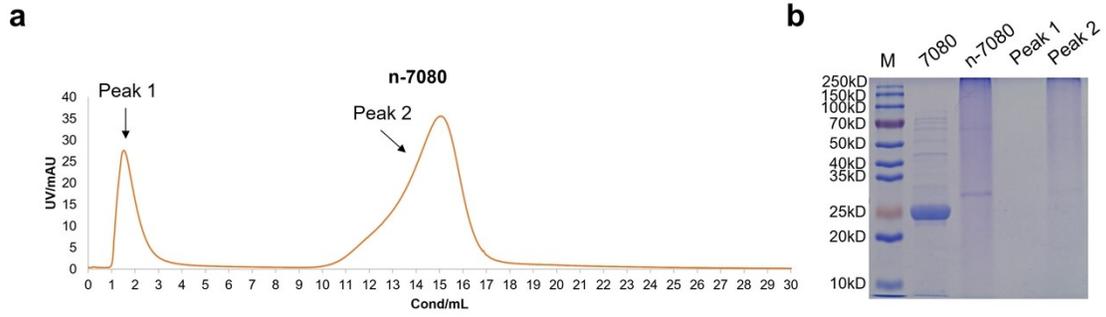


Figure S6 Validation of n-7080 by Ion Exchange Chromatography (IEC) and SDS-PAGE.

a. IEC result of n-7080 (Loading sample: 500 μ g); **b.** SDS-PAGE result of the corresponding fraction collections.

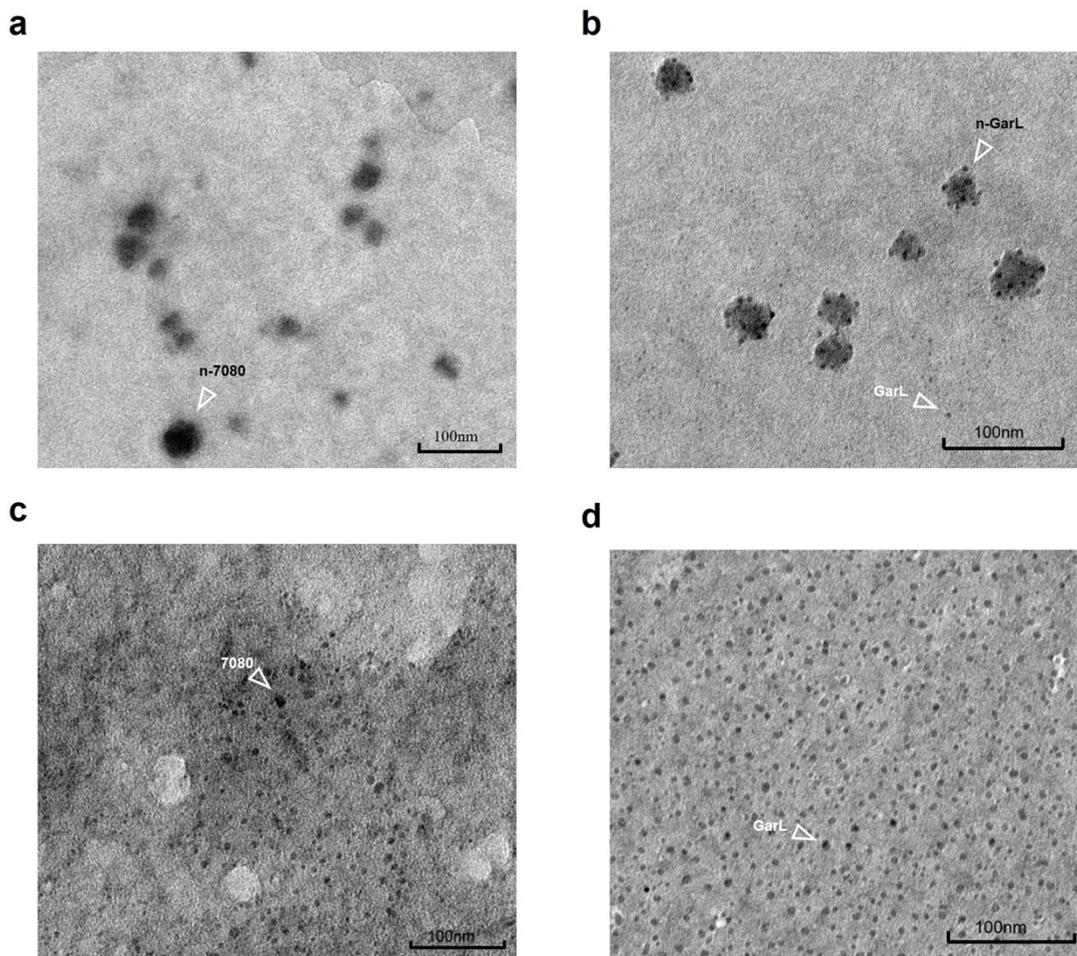


Figure S7 Transmission electron microscopy results of aldolase 7080 and GarL before and after modification.

- a.** TEM image of n-7080 (the scale bar is 100 nm); **b.** TEM image of n-GarL (the scale bar is 100 nm);
c. TEM image of 7080 (the scale bar is 100 nm); **d.** TEM image of GarL (the scale bar is 100 nm).

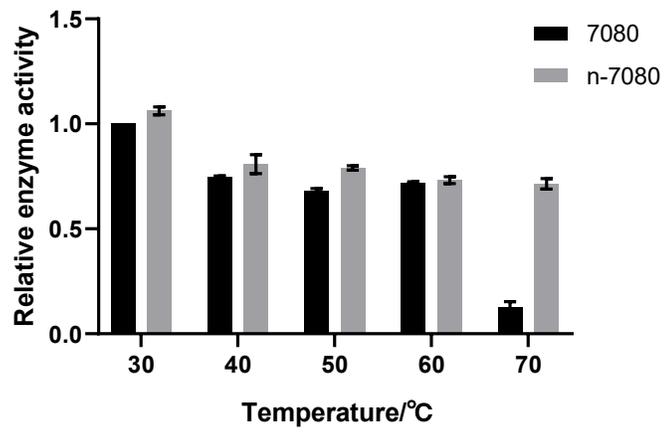


Figure S8 Tolerance of Aldolase Nanocapsule to high temperature.

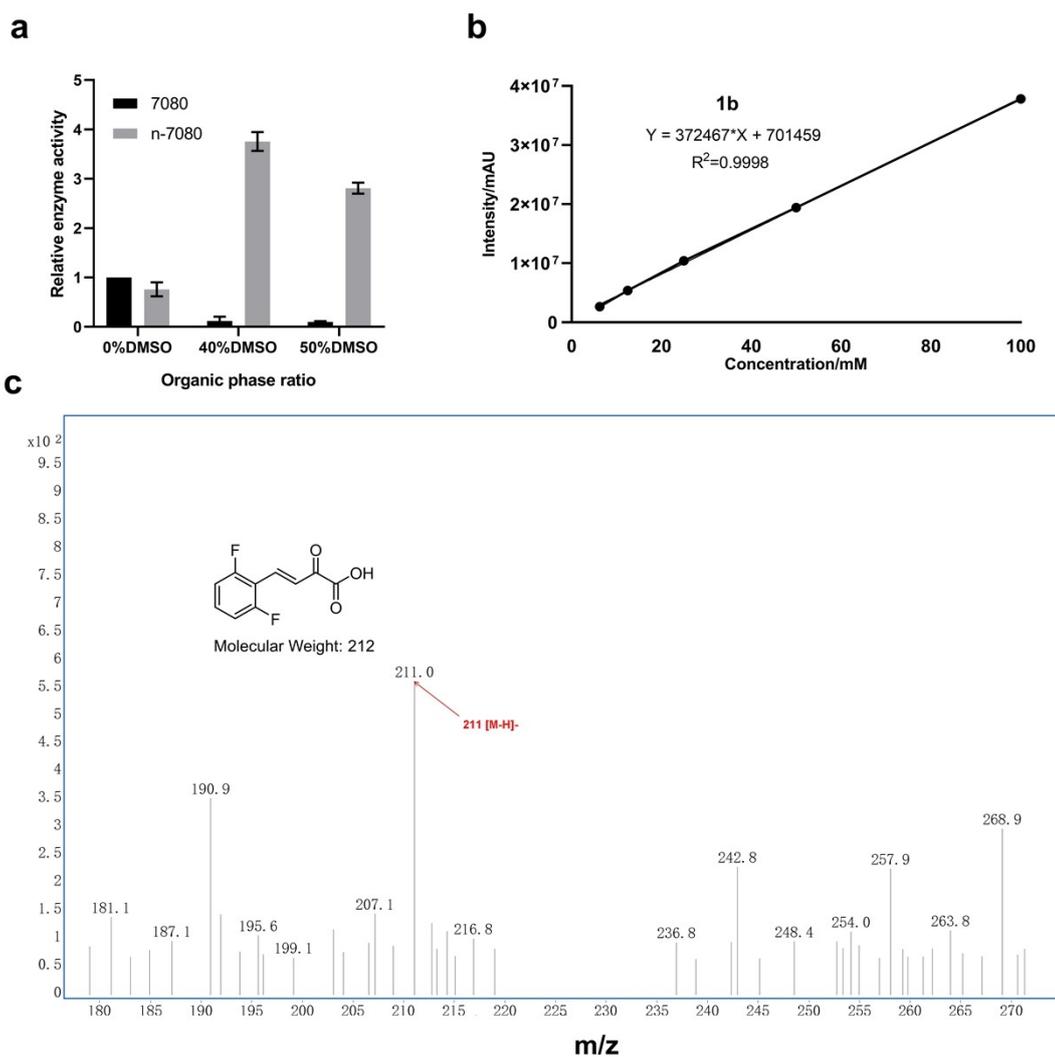


Figure S9 The relative enzyme activity improvement of n-7080 in the reaction of **1b** detected by HPLC and the MS detection of the product **4b**.
a. The relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **b.** HPLC standard curve of **1b**; **c.** MS results of **4b**: $[M-H]^-$ $m/z = 211$.

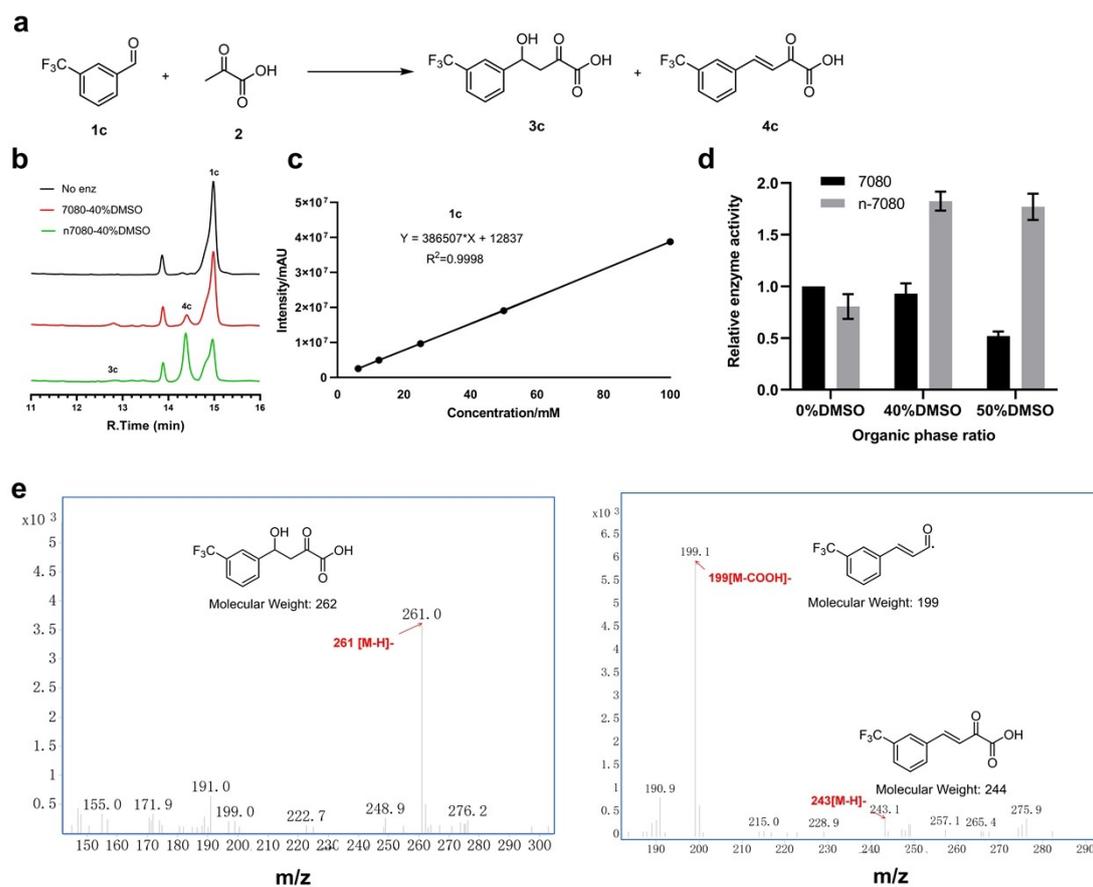


Figure S10 The relative enzyme activity improvement of n-7080 in the reaction of 1c detected by HPLC and the MS detection of the product 4c.

a. The aldol addition of 3-(trifluoromethyl) benzaldehyde (1c) and sodium pyruvate (2); **b.** HPLC detection of enzyme reactions in 40% DMSO of 1c; **c.** HPLC standard curve of 1c; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of 3c: [M-H]⁻ m/z = 261; MS results of 4c: [M-H]⁻ m/z = 243.

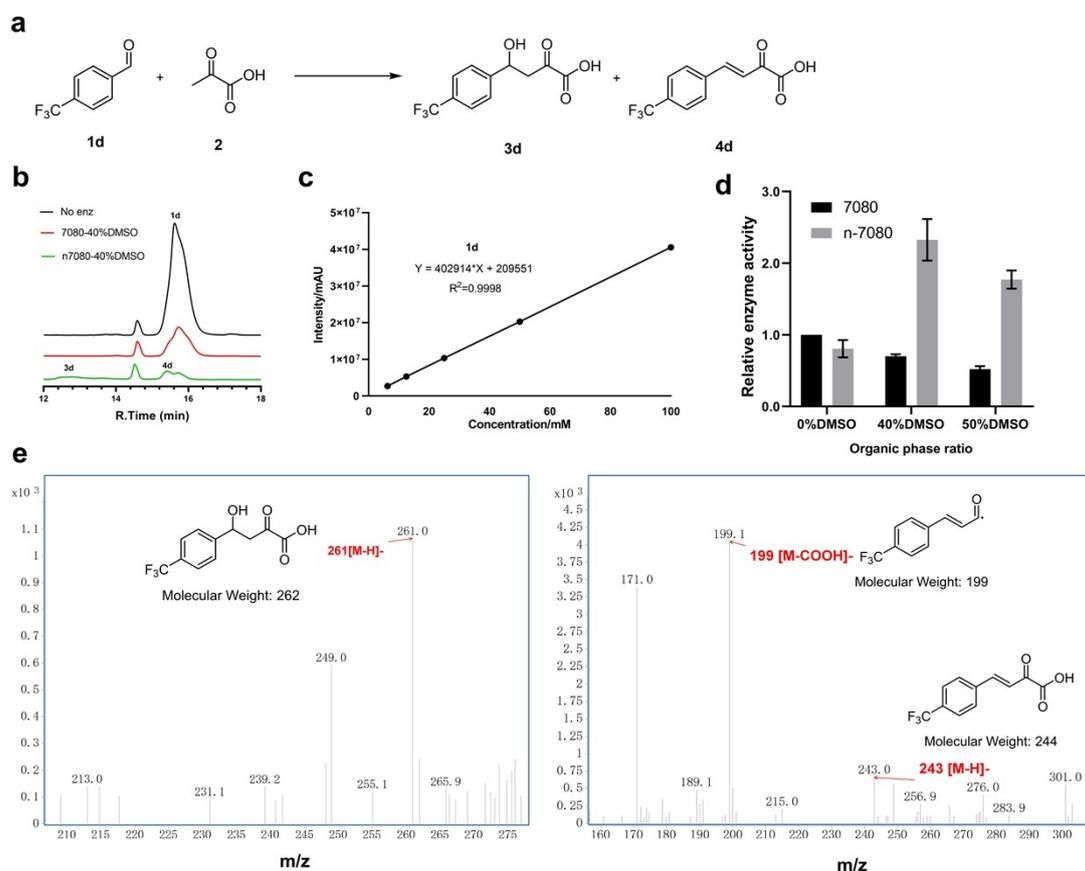


Figure S11 The relative enzyme activity improvement of n-7080 in the reaction of 1d detected by HPLC and the MS detection of the product 4d.

a. The aldol addition of 4-(trifluoromethyl) benzaldehyde (**1d**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40% DMSO of **1d**; **c.** HPLC standard curve of **1d**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3d**: [M-H]⁻ m/z = 261; MS results of **4d**: [M-H]⁻ m/z = 243.

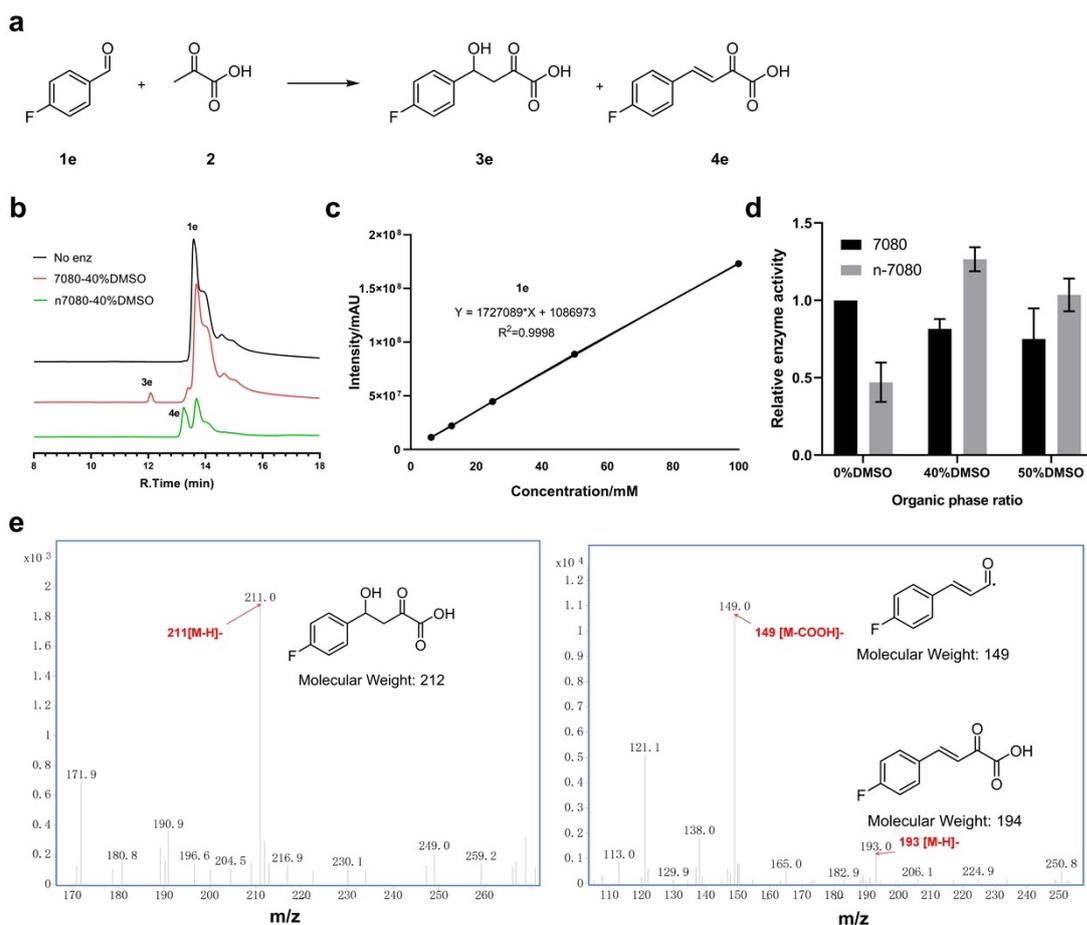


Figure S12 The relative enzyme activity improvement of n-7080 in the reaction of 1e detected by HPLC and the MS detection of the product 1e.

a. The aldol addition of 4-fluorobenzaldehyde (1e) and sodium pyruvate (2); **b.** HPLC detection of enzyme reactions in 40% DMSO of 1e; **c.** HPLC standard curve of 1e; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of 3e: [M-H]⁻ m/z = 211; MS results of 4e: [M-H]⁻ m/z = 193.

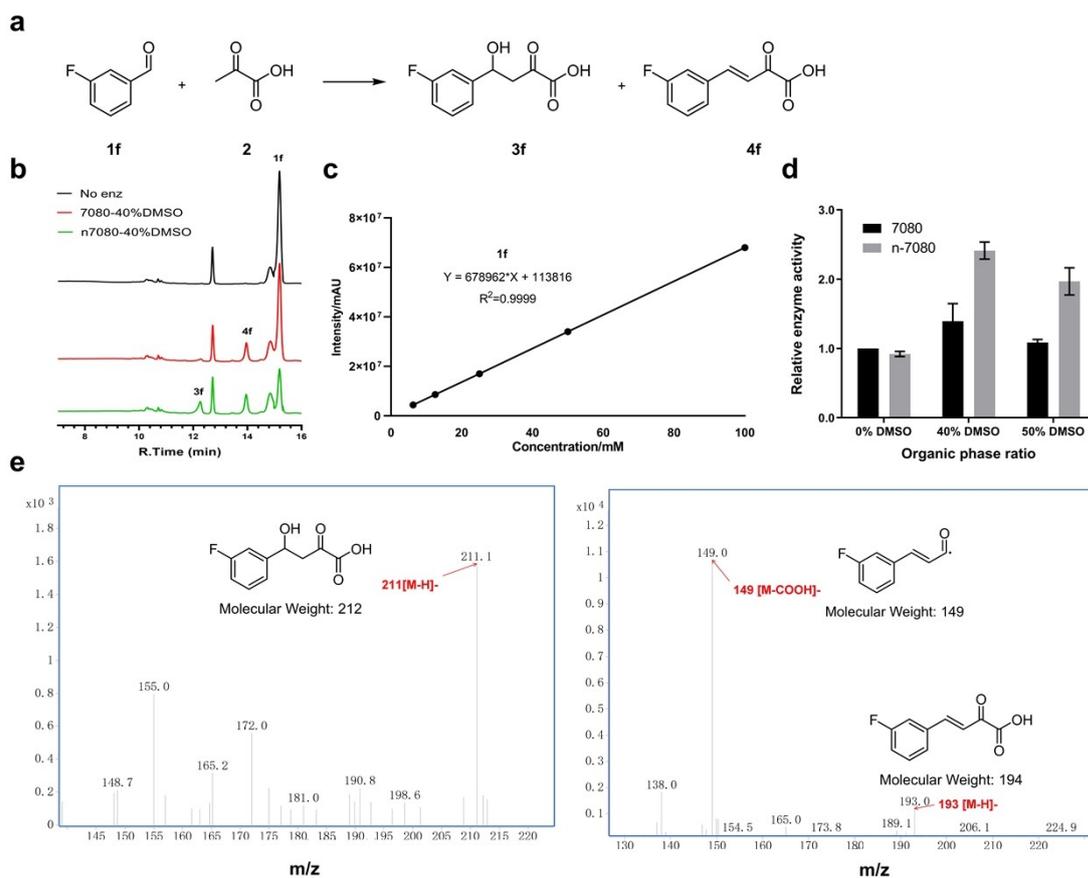


Figure S13 The relative enzyme activity improvement of n-7080 in the reaction of 1f detected by HPLC and the MS detection of the product 4f.

a. The aldol addition of 3-fluorobenzaldehyde (**1f**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40% DMSO of **1f**; **c.** HPLC standard curve of **1f**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3f**: [M-H]⁻ m/z = 211; MS results of **4f**: [M-H]⁻ m/z = 193.

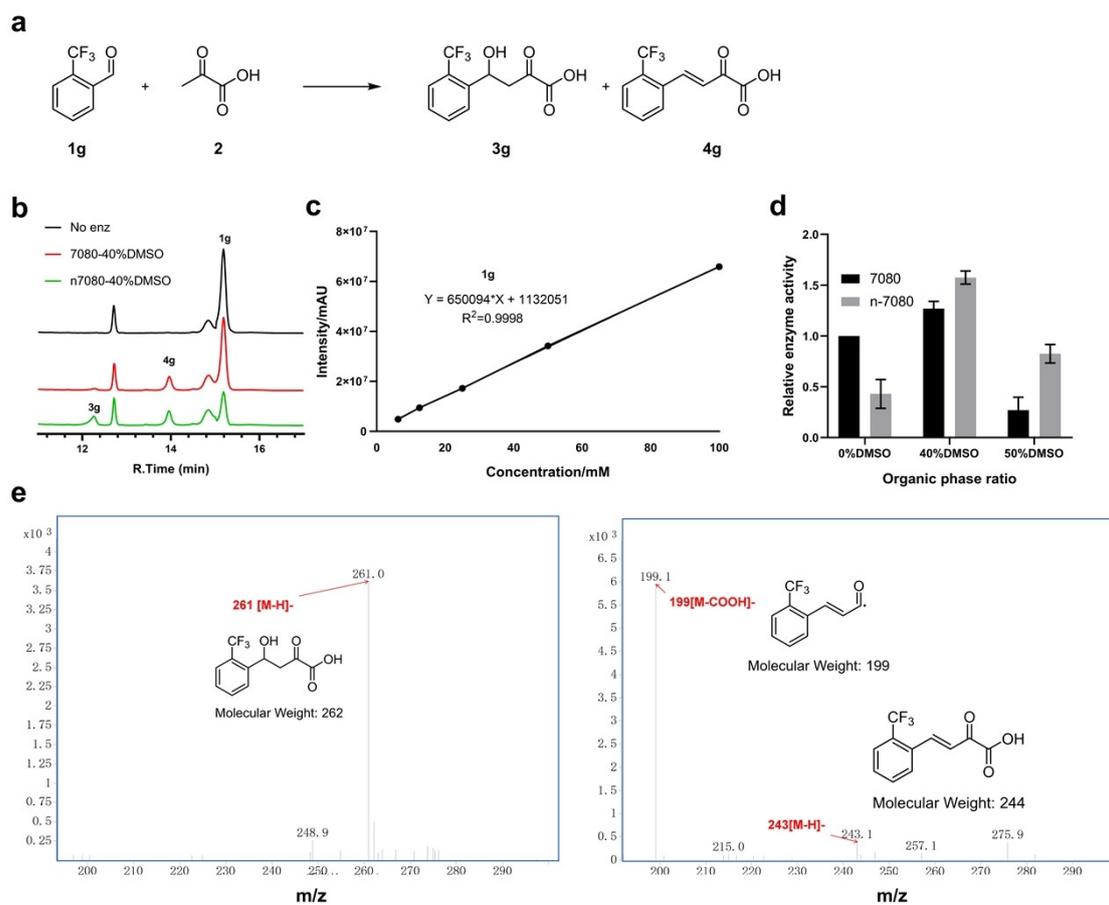


Figure S14 The relative enzyme activity improvement of n-7080 in the reaction of 1g detected by HPLC and the MS detection of the product 4g.

a. The aldol addition of 2-(trifluoromethyl)benzaldehyde (**1g**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40% DMSO of **1g**; **c.** HPLC standard curve of **1g**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3g**: [M-H]⁻ m/z = 261; MS results of **4g**: [M-H]⁻ m/z = 243.

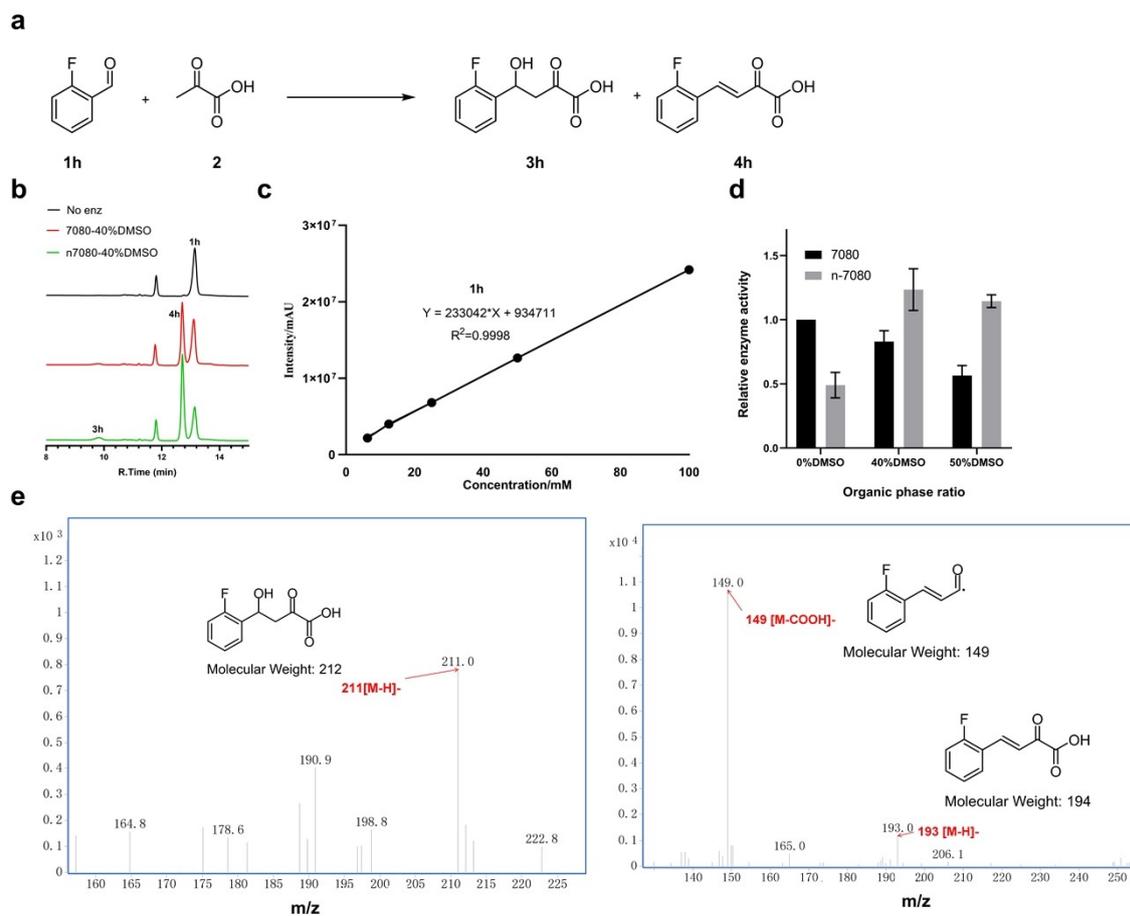


Figure S15 The relative enzyme activity improvement of n-7080 in the reaction of 1h detected by HPLC and the MS detection of the product 4h.

a. The aldol addition of 2-fluorobenzaldehyde (**1h**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40%DMSO of **1h**; **c.** HPLC standard curve of **1h**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3h**: [M-H]⁻ m/z = 211; MS results of **4h**: [M-H]⁻ m/z = 193.

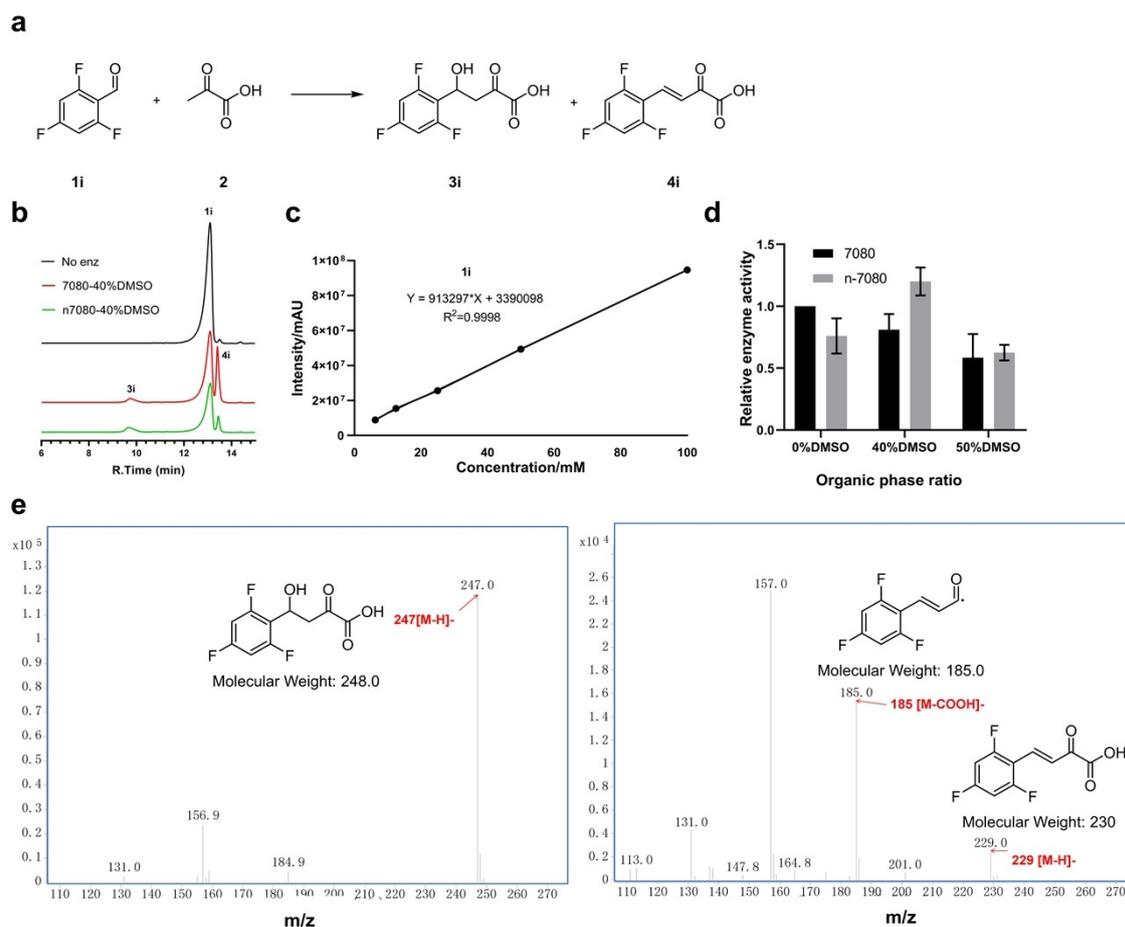


Figure S16 The relative enzyme activity improvement of n-7080 in the reaction of 1i detected by HPLC and the MS detection of the product 4i.

a. The aldol addition of 2,4,6-trifluorobenzaldehyde (**1i**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40% DMSO of **1i**; **c.** HPLC standard curve of **1i**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3i**: [M-H]⁻ m/z = 247; MS results of **4i**: [M-H]⁻ m/z = 229.

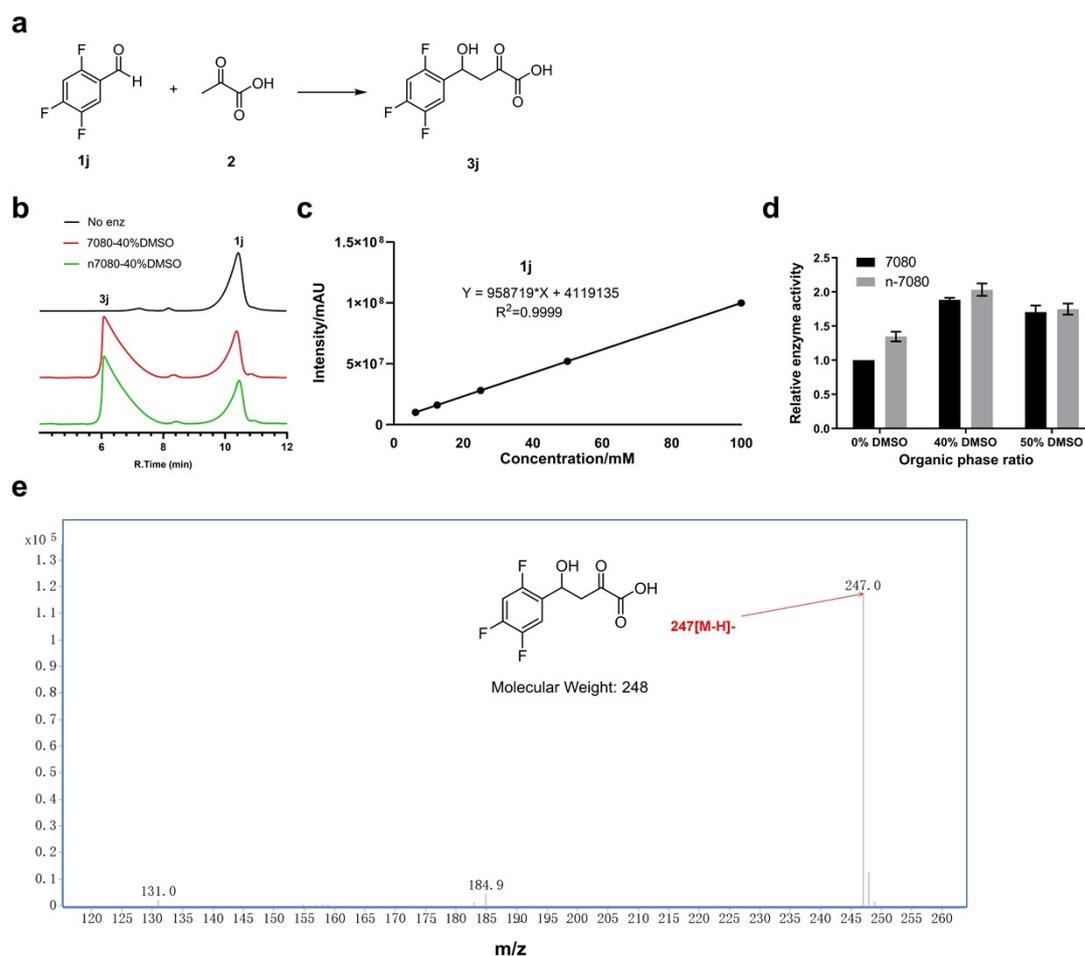


Figure S17 The relative enzyme activity improvement of n-7080 in the reaction of 1j detected by HPLC and the MS detection of the product 4j.

a. The aldol addition of 2,4,5-trifluorobenzaldehyde (**1j**) and sodium pyruvate (**2**); **b.** HPLC detection of enzyme reactions in 40% DMSO of **1j**; **c.** HPLC standard curve of **1j**; **d.** The ratio of relative enzyme activity of 7080 and n-7080 at different DMSO concentrations; **e.** MS results of **3j** [M-H]⁻ m/z = 247.