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Supplementary Materials

A novel NADP(H)-dependent 7alpha-HSDH: discovery and construction of substrate selectivity mutant by C-terminal truncation

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Abstract: 7α-Hydroxysteroid dehydrogenase (7α-HSDH) plays an important role in the biosynthesis of tauroursodeoxycholic acid (TUDCA) using complex substrate chicken bile powder as raw material. However, chicken bile powder contains 4.74% taurocholic acid (TCA), and a new by-product tauroursocholic acid (TUCA) will be produced, having the risk of causing colorectal cancer. Here, we obtained a novel NADP(H)-dependent 7α-HSDH with good thermostability from *Ursus thibetanus* gut microbiota (named St-2-2). St-2-2 could catalyze taurochenodeoxycholic acid (TCDCA) and TCA with the catalytic activity of 128.13 and 269.39 U/mg, respectively. Interestingly, by a structure-based C-terminal truncation strategy, St-2-2△C10 only remained catalytic activity on TCDCA (14.19 U/mg) and had no activity on TCA. As a result, it can selectively catalyze TCDCA in waste chicken bile powder. MD simulation and structural analysis indicated that enhanced surface hydrophilicity and improved C-terminal rigidity affected the entry and exit of substrates. Hydrogen bond interactions between different subunits and interaction changes in Phe249 of the C-terminal loop inverted the substrate catalytic activity. This is the first report on substrate selectivity of 7α-HSDH by C-terminal truncation strategy and it can be extended to other 7α-HSDHs (J-1-1, S1-a-1).

Keywords: 7α-hydroxysteroid dehydrogenase (7α-HSDH; St-2-2); St-2-2△C10; C-terminal truncation; substrate selectivity; tauroursodeoxycholic acid

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Table S1. Primers used for mutagenesis.

Primer	Sequence (5'-3')
St-2-2△C2-RP	CGCTCGAGTTAAACAGCATCCCCATAAATAGGT
St-2-2△C4-RP	CGCTCGAGTTAATCCCCATAAATAGGTGA
St-2-2△C6-RP	CGCTCGAGTTAATAAATAGGTGATGGCATACCA
St-2-2△C8-RP	CGCTCGAGTTAAGGTGATGGCATACCAAAT
St-2-2△C10-RP	CGCTCGAGTTATGGCATACCAAATCCACCT
St-2-2△*-FP	CGCGGATCCATGAAAAGAGTAGAAAATAAAG
J-1-1△C1-RP	CGCTCGAGTTATTGAACAAGGTCTCCGTATTG
J-1-1△C4-RP	CGCTCGAGTTAGTCTCCGTATTGAGGTGTTCCA
J-1-1△C6-RP	CGCTCGAGTTAGTATTGAGGTGTTCCAAGGGCA
J-1-1△C8-RP	CGCTCGAGTTAAGGTGTTCCAAGGGCATATCCA
J-1-1△**-FP	CGCGGATCCATGAGAGTAAAAGATAAAATAGCTT
S1-a-1△C2-RP	CGCTCGAGCTATCTCTCCATCATTGCAGAAAAC
S1-a-1△C4-RP	CGCTCGAGCTACATCATTGCAGAAAACTCA
S1-a-1△C6-RP	CGCTCGAGCTATGCAGAAAACTCAGCATAT
S1-a-1△C8-RP	CGCTCGAGCTAAAACTCAGCATATTGTGGA
S1-a-1△***-FP	CGCGGATCCATGAAAAAGTTAGAAGATAAAGT

* The FP of all St-2-2 mutants are the same. **The FP of all J-1-1 mutants are the same. ***The FP of all S1-a-1 mutants are the same.

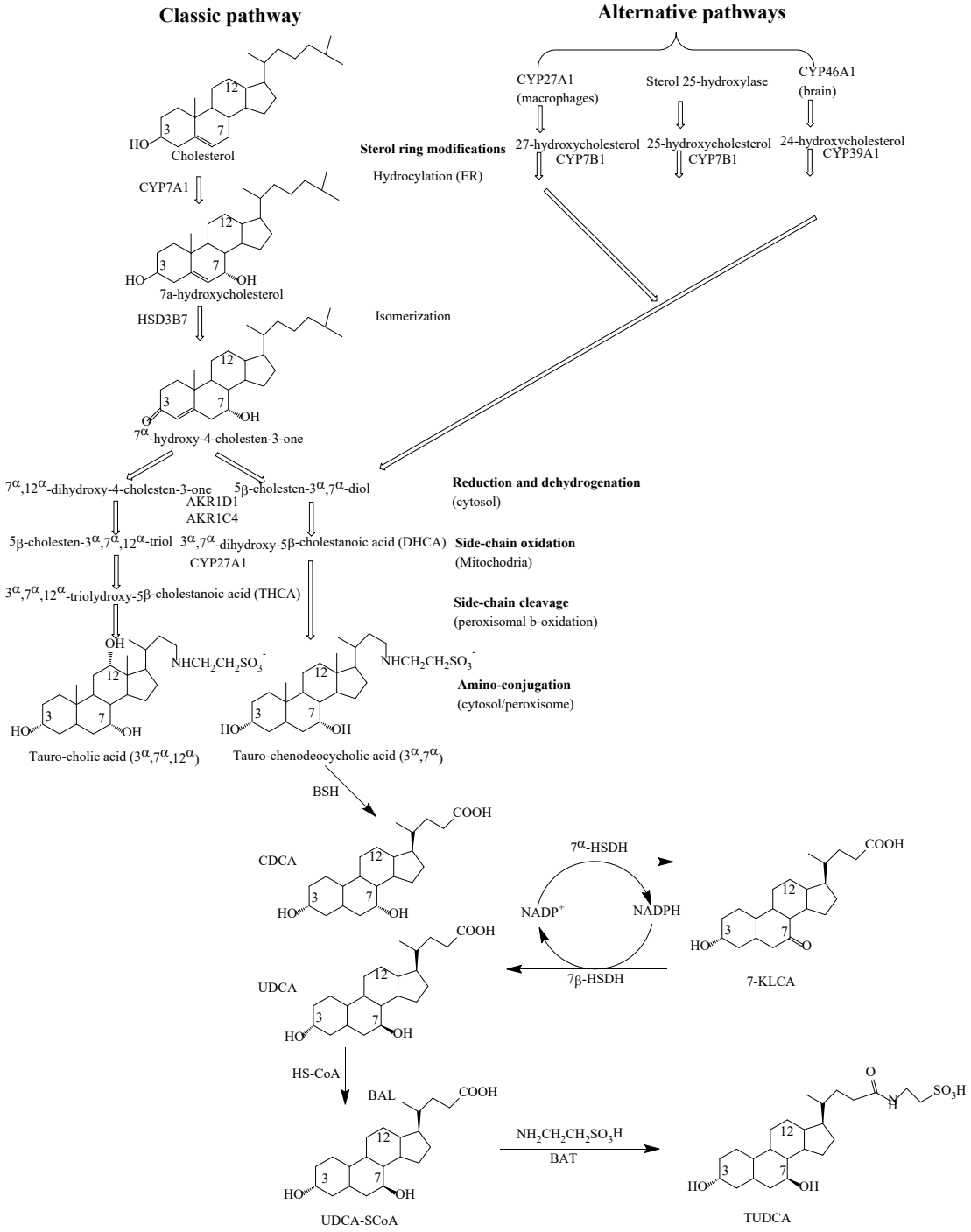

Figure S1. The metabolic pathway of TUDCA i*n vivo*[1].

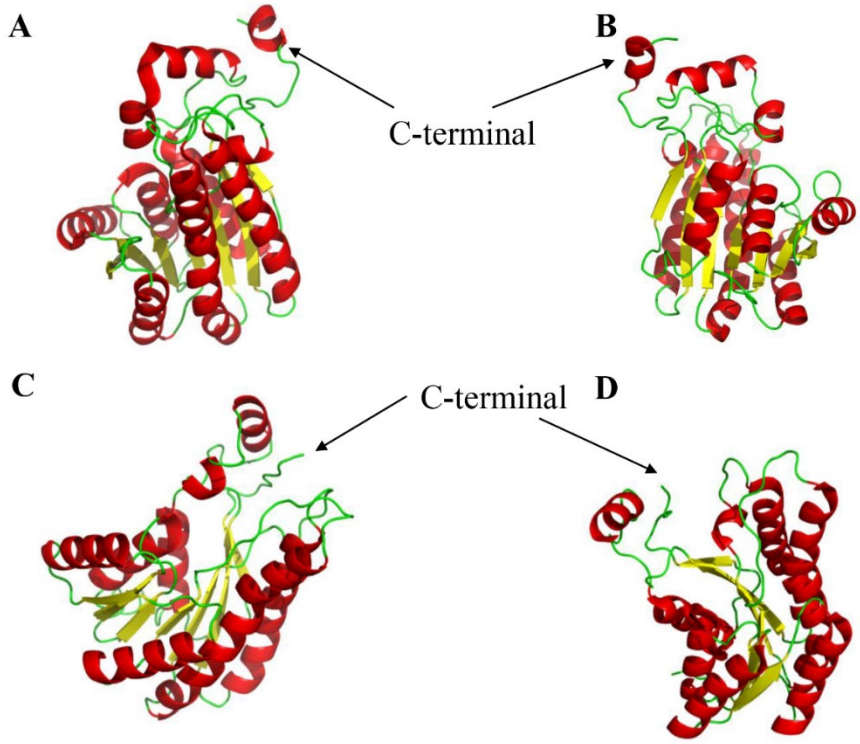

Figure S2. Structural comparison of 7α-HSDHs. The structure of 7α-HSDHs from *Clostridium sardiniense* (PDB code: 5EPO)、St-2-2、*Escherichia coli* (PDB code: 1FMC) and *Brucella Melitensis* (PDB code: 3GAF).

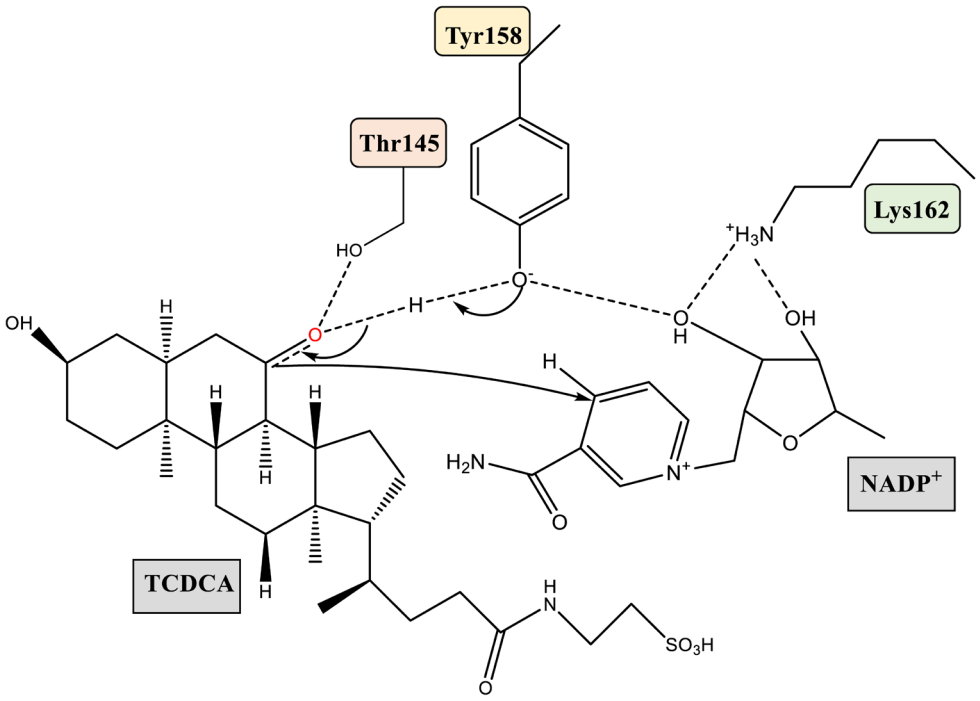

Figure S3. Reaction mechanism for the reduction of TCDCA to T-7-KLCA by 7α-HSDH and the role of T-Y-K residues at the catalytic center. Black arrows show hypothesized proton transfer during the reaction. Picture modified on the base of a scheme at Tanaka et al.[2].

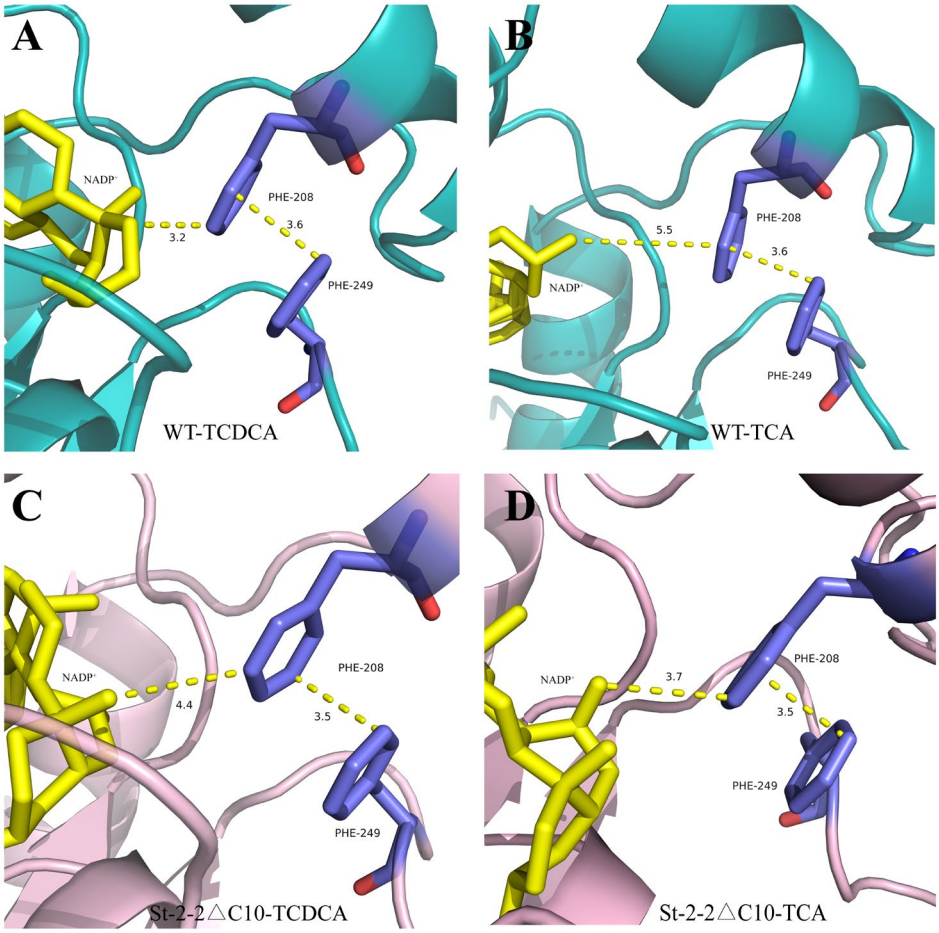

Figure S4. Interaction analysis of substrate and amino acid residues at C-terminal. (A) WT-TCDCA-NADP+, (B) WT-TCA-NADP+ (C) St-2-2△C10-TCDAC-NADP+, (D) St-2-2△C10-TCA-NADP+. NADP+ is colored in yellow stick. WT and St-2-2△C10 are colored in cyan and pink, respectively. The yellow dotted line indicates the distance between the substrate and neighbor amino acid residues. Amino acids are shown as stick models.

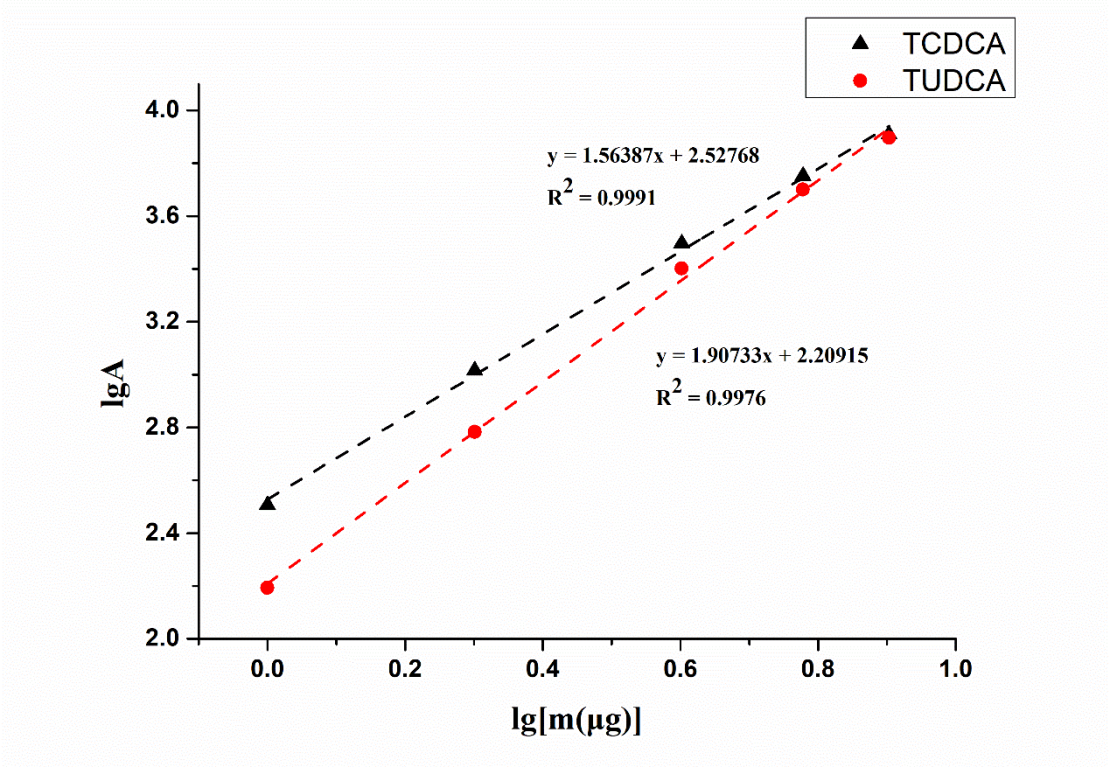

Figure S5. HPLC calibration diagram.

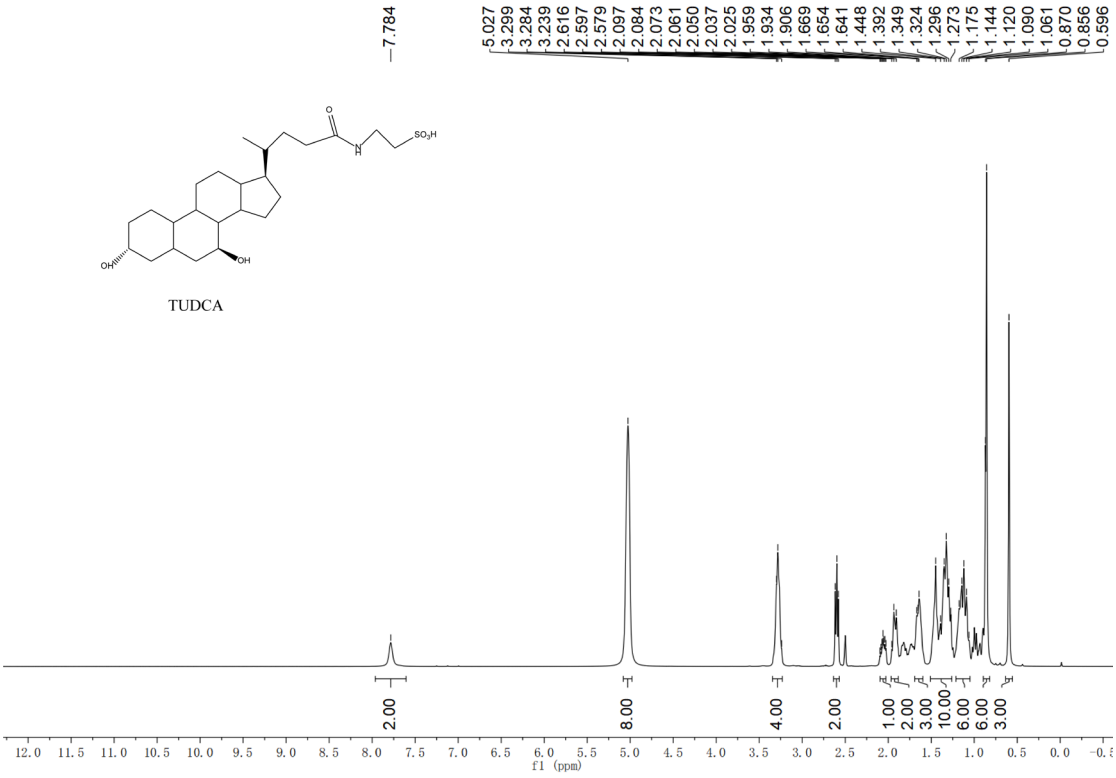

Figure S6. ^{1}H NMR of TUDCA prepared by enzymatic cascade reaction.

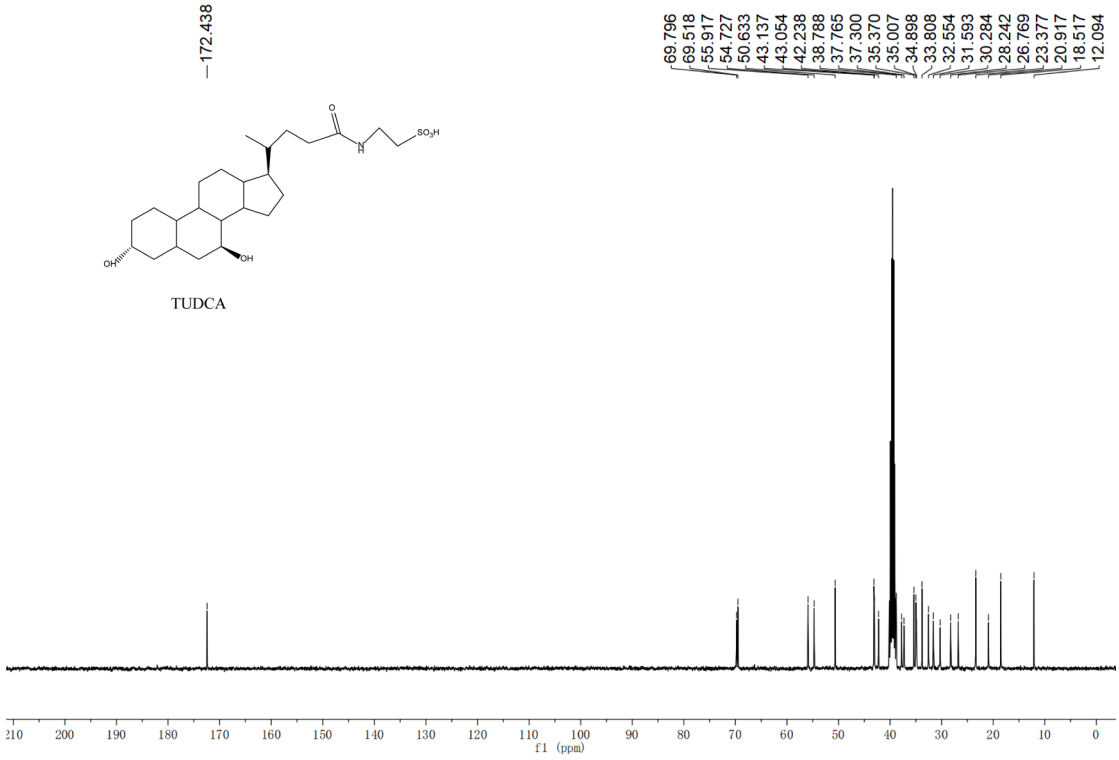

Figure S7. ^{13}C NMR of TUDCA prepared by enzymatic cascade reaction.

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