Supporting Information

New Small-Molecule Alcohol Synthesis by Breaking the Space Limitation of "Aromatic Cage" in *Pseudomonas* sp. AK1 BBOX

Zhiqin Xu[a], Yaling Mo[a], Zhengwen Li[a], Shurong Ban[a*], Heng song[b,c*].

[a]School of Pharmacy, Shanxi Medical University, Taiyuan, Shanxi Province 030001, China

[b]College of Chemistry & Molecular Science, Wuhan University, Wuhan, Hubei Province 430072, China

[c]Wuhan University Shenzhen Research Institute, Shenzhen, Guangdong Province 518000, China

*Email: hengsong@whu.edu.cn; shurongban@sxmu.edu.cn

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SDS-PAGE analysis of WT-psBBOX

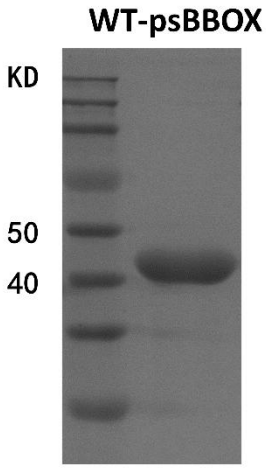


Figure S1 SDS-PAGE analysis of the WT-psBBOX (45.3 KD).

Sequence alignment[1]

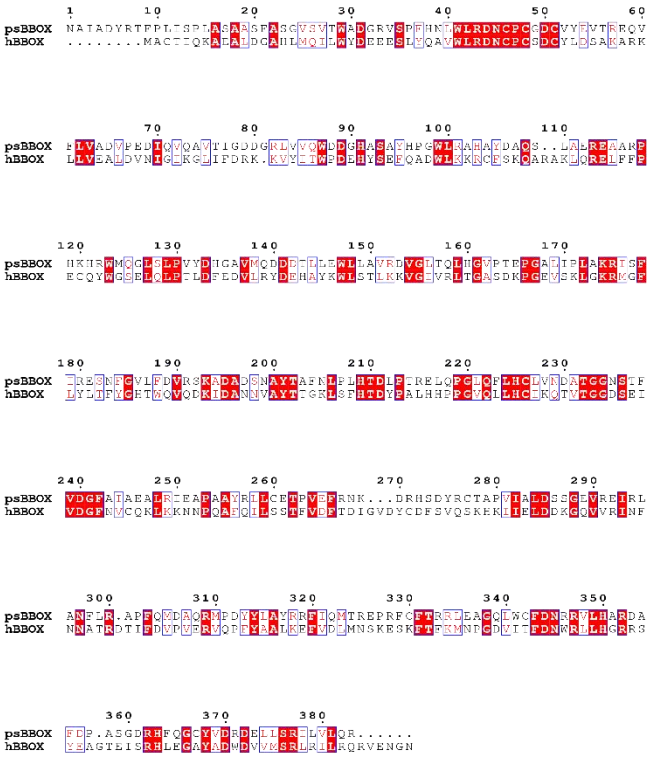


Figure S2 Sequence alignment of *Pseudomonas* sp. AK1 BBOX (psBBOX gi|231642) and human BBOX (hBBOX gi|158261239).

Homology modelling

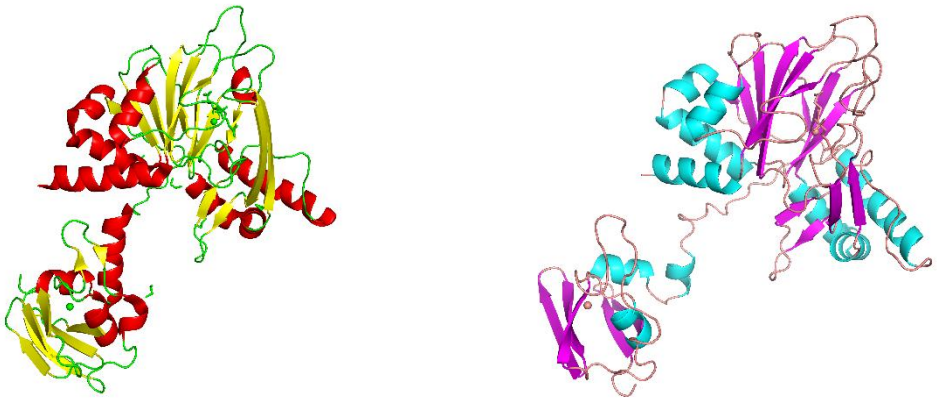


Figure S3 hBBOX template (PDB: 3O2G) (left) and psBBOX model (right).

Molecular docking

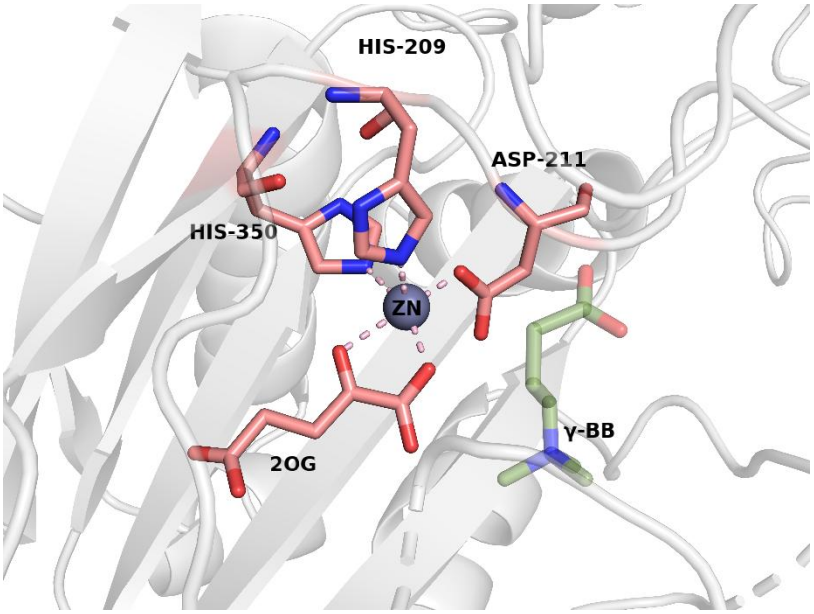


Figure S4 Catalytic center of psBBOX is composed of His-350, His-209, Asp211 and Metal ion.

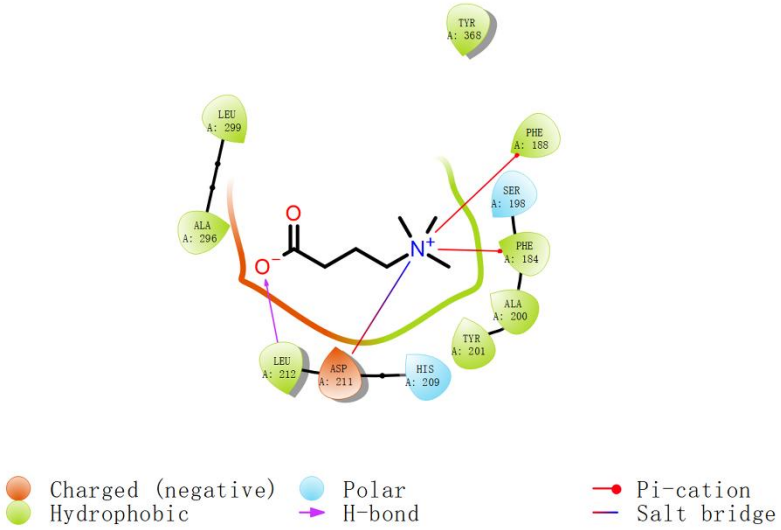


Figure S5 2D graphic of interaction between γ-BB and amino acid residues.

Construction of psBBOX mutants

Table S1 Primers used in this study

Primers	Sequence
184A-F	5'-CGAAAGCAACGCAGGCGTGCTGTTTGATGTGCG-3'
184A-R	5'-CAGCACGCCTGCGTTGCTTTCGCGAATAAAGC-3'
188A-F	5'-GGCGTGCTGGCAGATGTGCGCAGCAAAGCGG-3'
188A-R	5'-GCGCACATCTGCCAGCACGCCAAAGTTGCTTTCG-3'
201A-F	5'-GATAGCAACGCGGCAACCGCGTTTAACCTGCCGCTG-3'
201A-R	5'-GTTAAACGCGGTTGCCGCGTTGCTATCCGCATCC-3'
184Y-F	5'-GCAACTATGGCGTGCTGTTTGATGTGCG-3'
184Y-R	5'-GCACGCCATAGTTGCTTTCGCGAATAAAGC-3'
188Y-F	5'-CGTGCTGTATGATGTGCGCAGCAAAGCGGATG-3'
188Y-R	5'-GCACATCATACAGCACGCCAAAGTTGCTTTCGC-3'
201F-F	5'-CAACGCGTTTACCGCGTTTAACCTGCCGC-3'
201F-R	5'-GCGGTAAACGCGTTGCTATCCGCATCCG-3'
368A-F	5'-GCTGCGCAGTGGATCGCGATGAACTGC-3'
368A-R	5'-CGATCCACTGCGCAGCCTTGAAAATGGC-3'
184W-F	5'-GCAACTGGGGCGTGCTGTTTGATG-3'
184W-R	5'-CGCCCCAGTTGCTTTCGCGAATAAAG-3'
188W-F	5'-GCTGTGGGATGTGCGCAGCAAAGC-3'
188W-R	5'-CATCCCACAGCACGCCAAAGTTGC-3'
188G-F	5'-GCTGGGTGATGTGCGCAGCAAAG-3'
188G-R	5'-CATCACCCAGCACGCCAAAGTTGC-3'
188V-F	5'-GCTGGTTGATGTGCGCAGCAAAG-3'
188V-R	5'-CATCAACCAGCACGCCAAAGTTGC-3'

Table S2 Construction of mutants based on PCR methods

For 184A, 188A, 201A:

Reaction mixtures (20 μL)		PCR conditions	
0.6 μL	template DNA	95°C and 3 min,	
1μL	forward primer	95°C and 30 s	×30 cycles
1 μL	reverse primer	55°C and 30 s	
7.4 μL	H_2O	72°C and 4 min 30 s	
10 μL	Gloria Nova HS 2×	72°C and 5 min.	

For the other mutants:

Reaction mixtures (20 μL)		PCR conditions	
0.6 μL	template DNA	95°C and 3 min,	
1 μL	forward primer	95°C and 30 s	×30 cycles
1 μL	reverse primer	52°C and 30 s	
7.4 μL	H_2O	72°C and 4 min	
10 μL	Gloria Nova HS 2×	72°C and 5 min.	

SDS-PAGE analysis of mutants

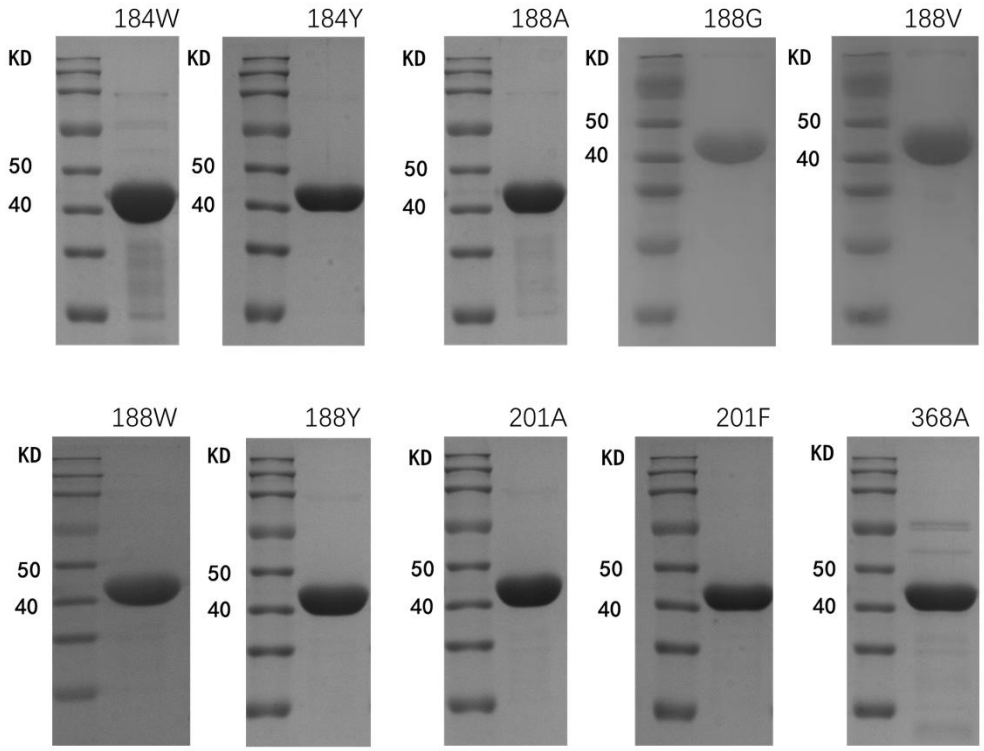


Figure S6 SDS-PAGE analysis of psBBOX mutants.

Analysis of psBBOX-188A activity

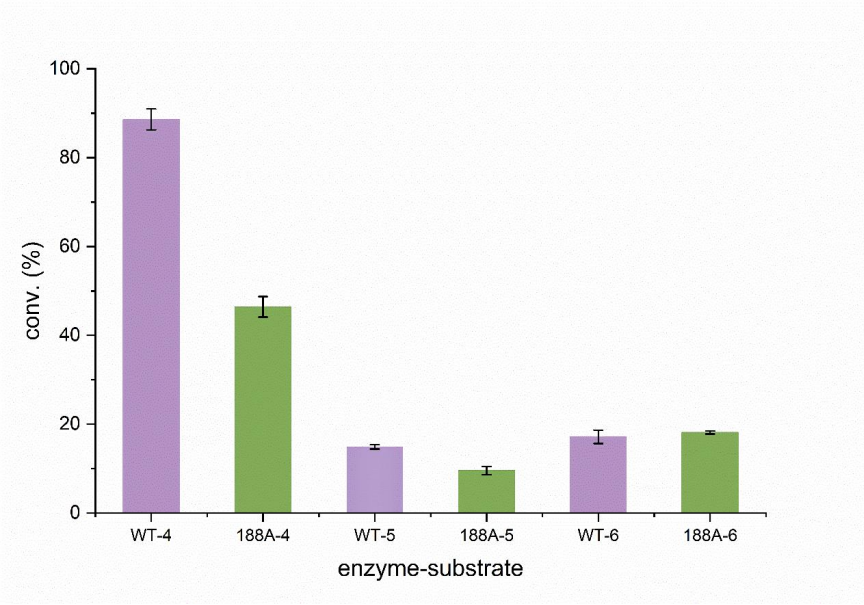


Figure S7 Comparison of conversion of quaternary ammonium analogs 4-6 catalyzed by WT and 188A.

kinetic analysis

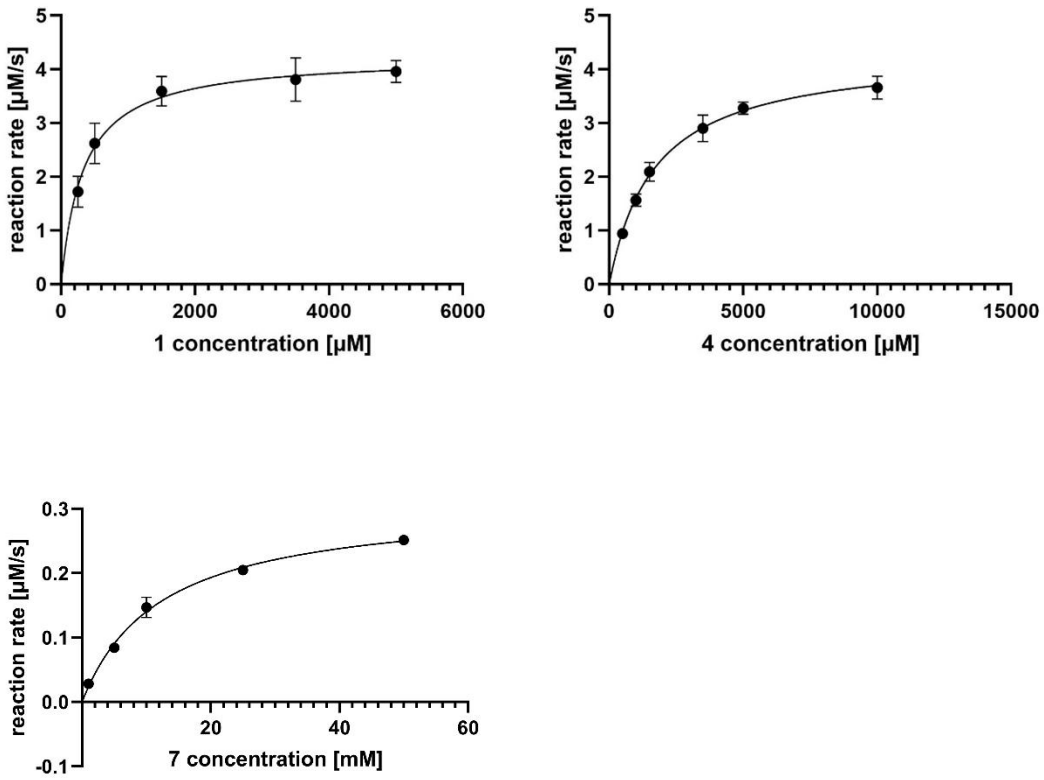


Figure S8 Dependence of reaction rates on γ-BB, 4 and 7 concentration for wt-psBBOX.

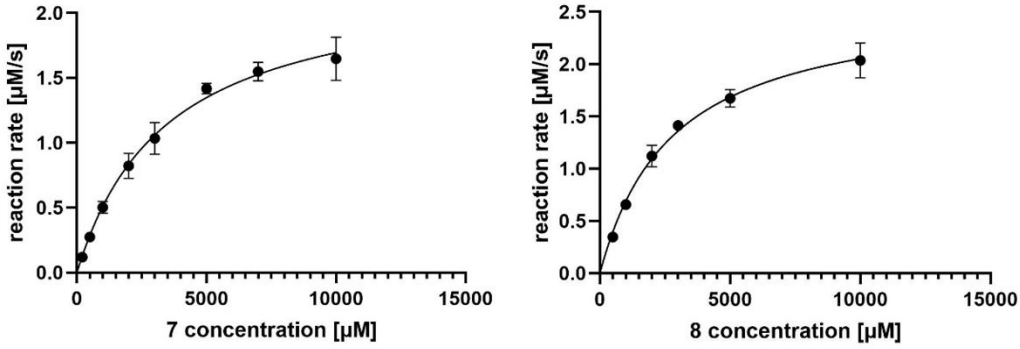


Figure S9 Dependence of reaction rates on 7 and 8 concentration for 188A mutant.

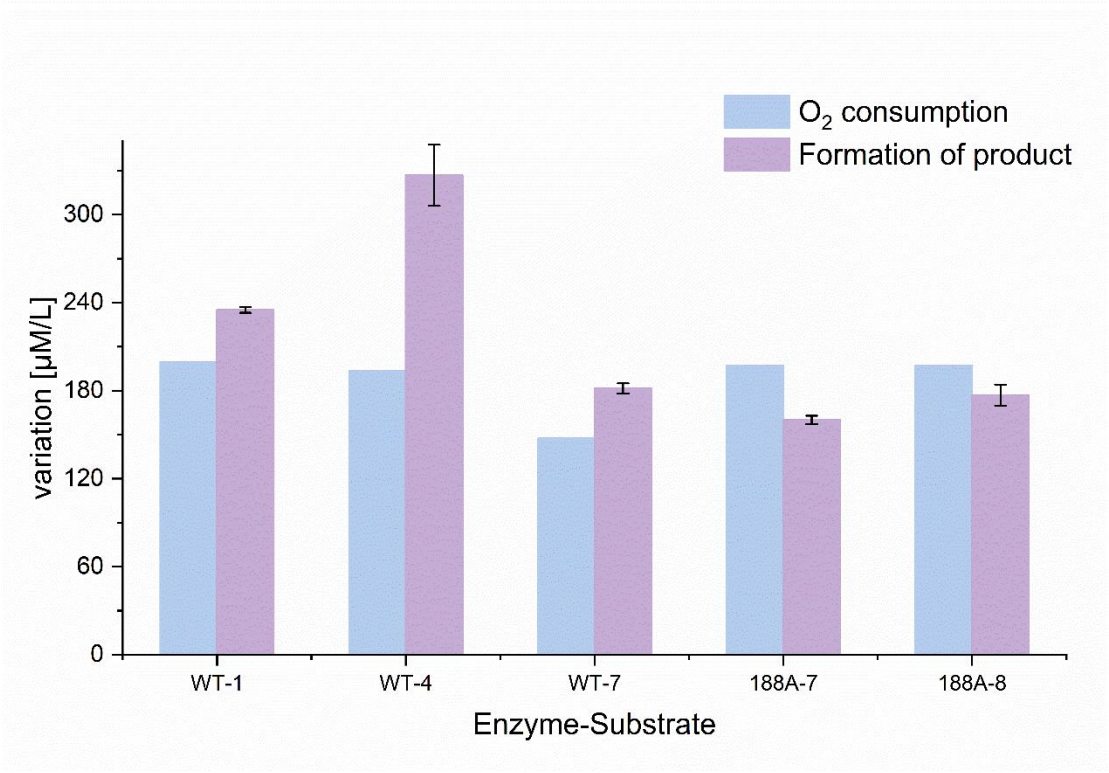
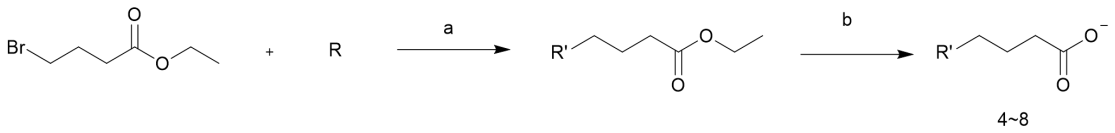


Figure S10 Ratio of oxygen consumption to product formation.

Synthesis of γ-BB analogues



No.	R	R'
4		
5		
6		
7		
8		

Figure S11 Synthetic routes of quaternary ammonium analogues 4~8.

Procedure a[2]

Ethyl 4-bromobutyrate (1.2equiv.); tertiary amine (1.0equiv. For (4), N,N-Dimethylethylamine; for(5), N,N-Dimethylisopropylamine; for (6), N,N-Diethylmethylamine; for (7), 1-Methylpyrrolidine; for (8), N-Methylpiperidine.) and acetone (10ml) was added to round-bottom flask, room temperature stirred for 12h. Solvents were evaporated in vacuo. Without purification, it can be directly used in the next reaction.

Procedure b[2]

3M hydrochloric acid was added until pH1, overnight at room temperature, the product was purified by cationic exchange resin.

Stereoselective synthesis of two configurations of 4a, 7a, 8a.

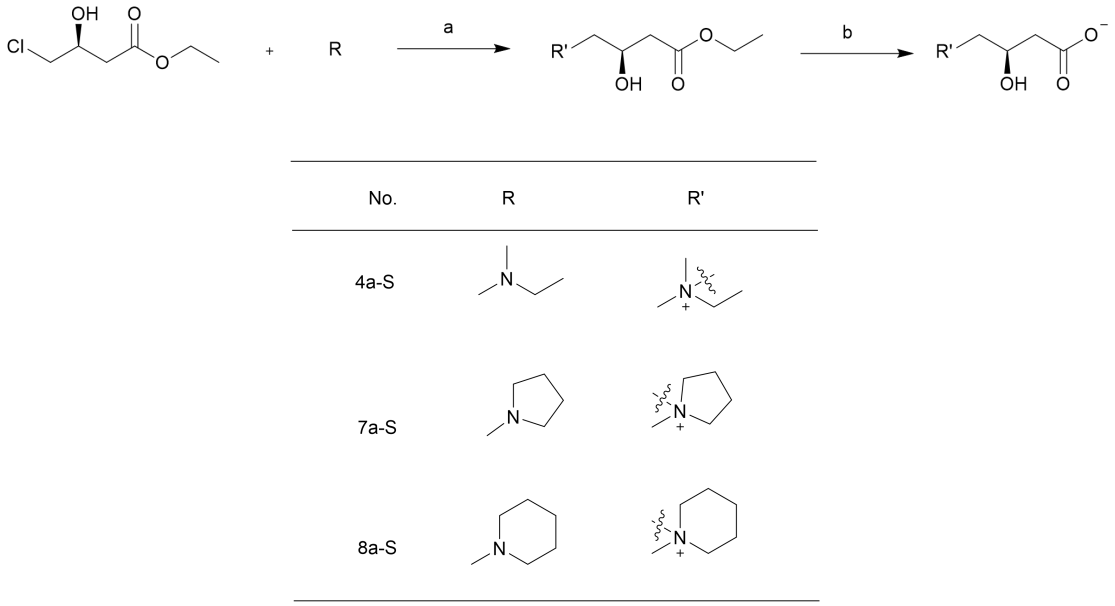


Figure S12 Synthetic routes of 4a-S, 7a-S, 8a-S.

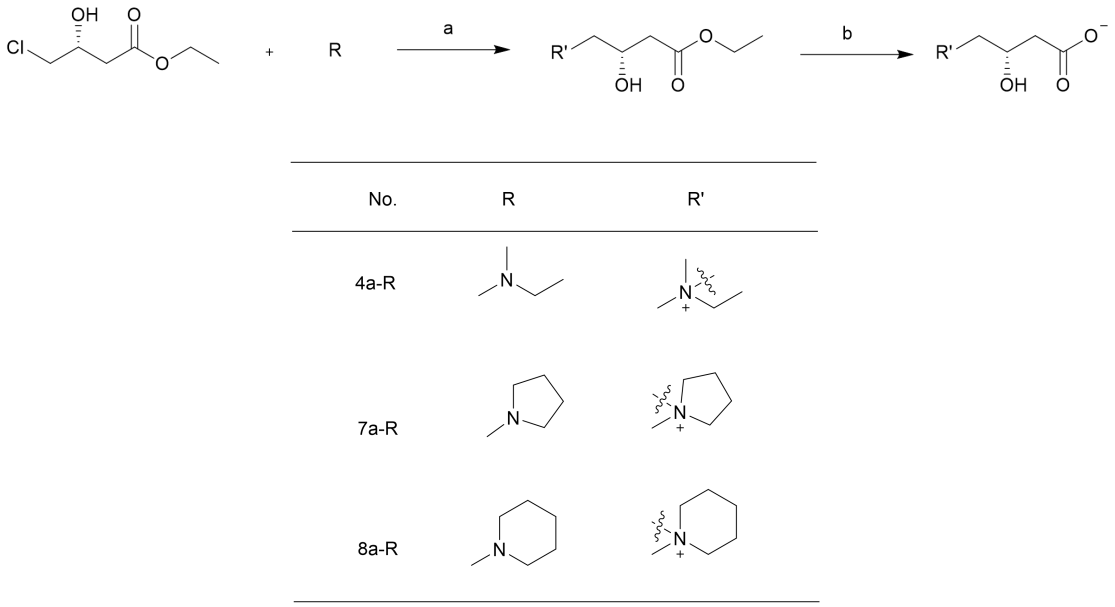


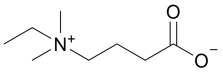
Figure S13 Synthetic routes of 4a-R, 7a-R, 8a-R.

Procedure a[3]: Adding 193mg sodium hydroxide into 3.5ml water, stir to dissolve it, cool down in the ice bath, add 2.5mmol corresponding tertiary amine and 3mmol Ethyl (R)-(+)-4-chloro-3-hydroxybutyrate or Ethyl (S)-4-chloro-3-hydroxybutyrate successively, react in the ice bath for 1h, and temperature rise to room temperature for 12h.

Procedure b[3]: Adding 3M hydrochloric acid to pH 6, purification using cationic resin.

NMR spectra

4-[ethyl(dimethyl)ammonio]butanoate；4



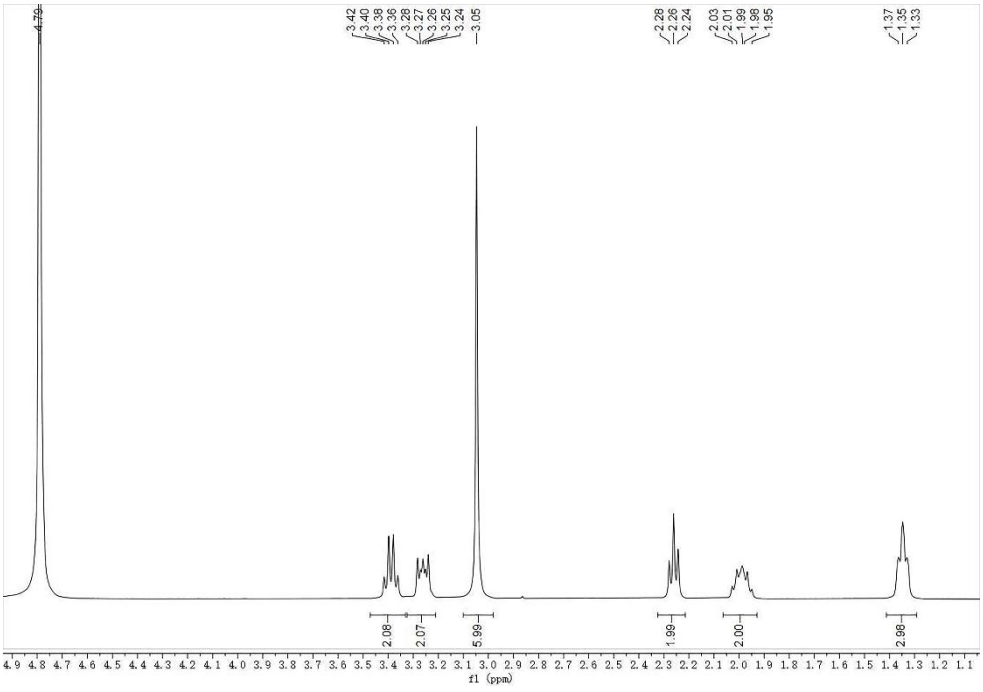


Figure S14 [1]HNMR spectra of 4.

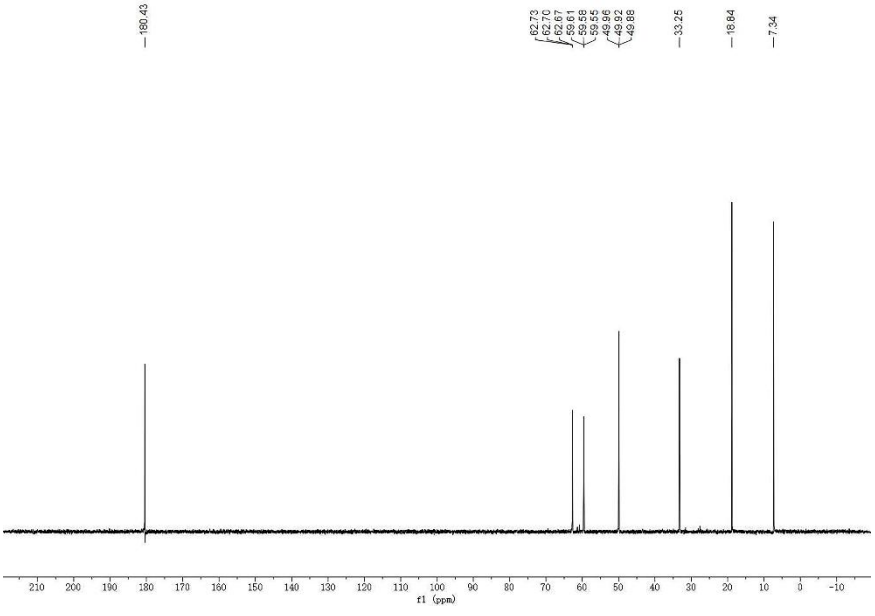
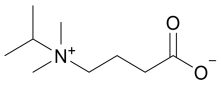


Figure S15 [13]CNMR spectra of 4.

4-[isopropyl(dimethyl)ammonio]butanoate；5



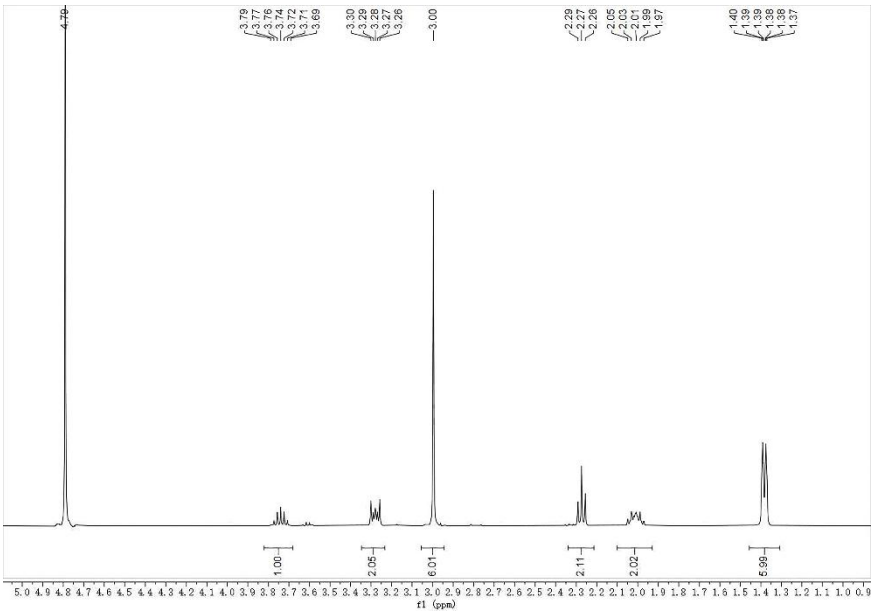


Figure S16 [1]HNMR spectra of 5.

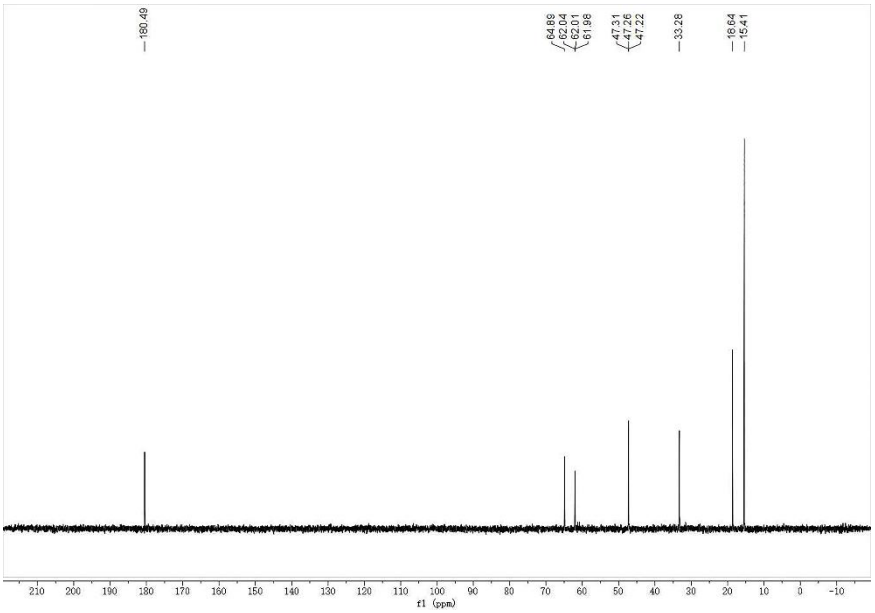
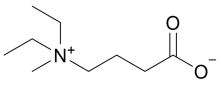


Figure S17 [13]CNMR spectra of 5.

4-[diethyl(methyl)ammonio]butanoate；6



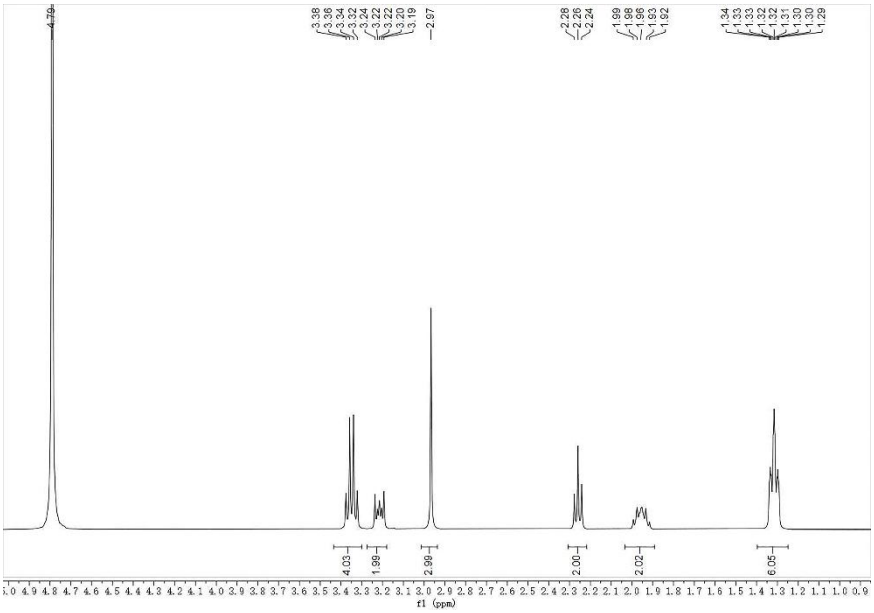


Figure S18 [1]HNMR spectra of 6.

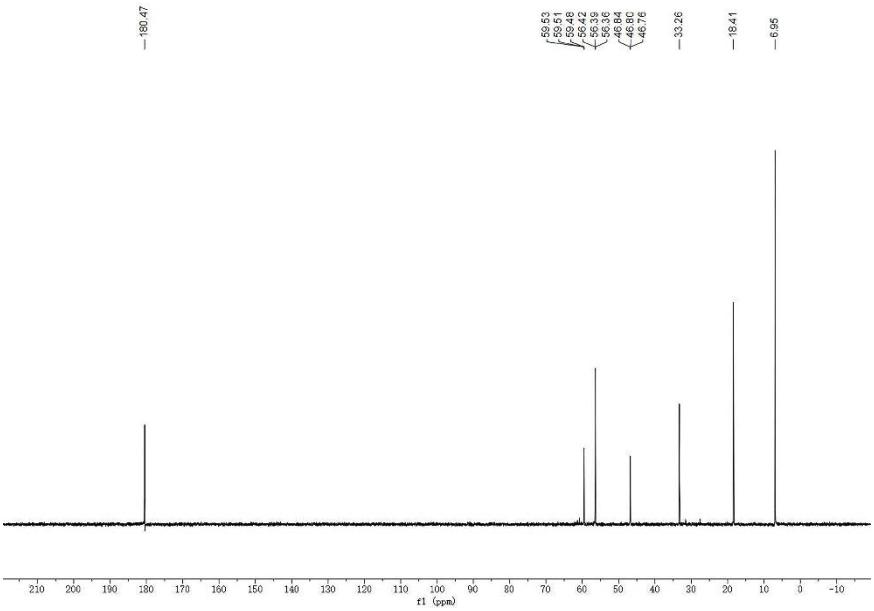


Figure S19 [13]CNMR spectra of 6.

4-(1-methylpyrrolidin-1-ium-1-yl)butanoate; 7

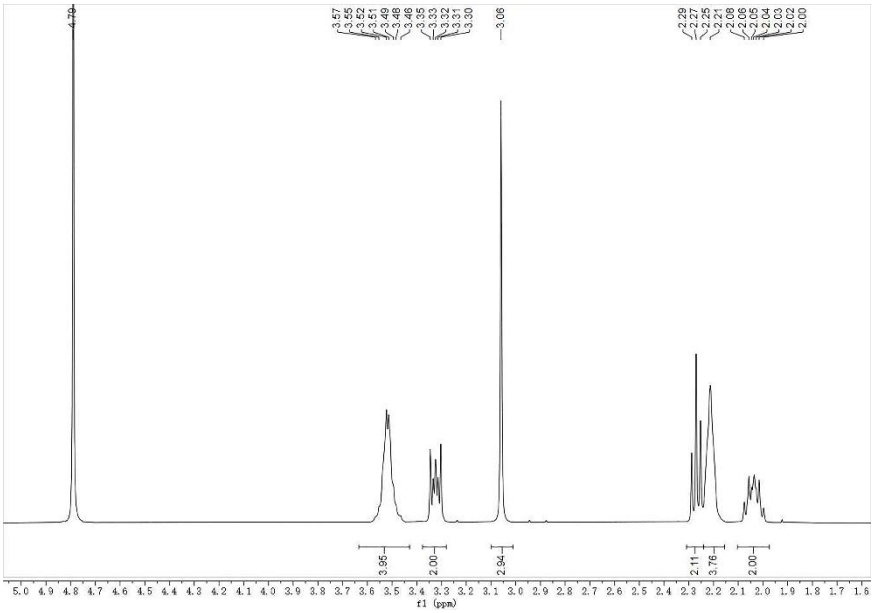


Figure S20 [1]HNMR spectra of 7.

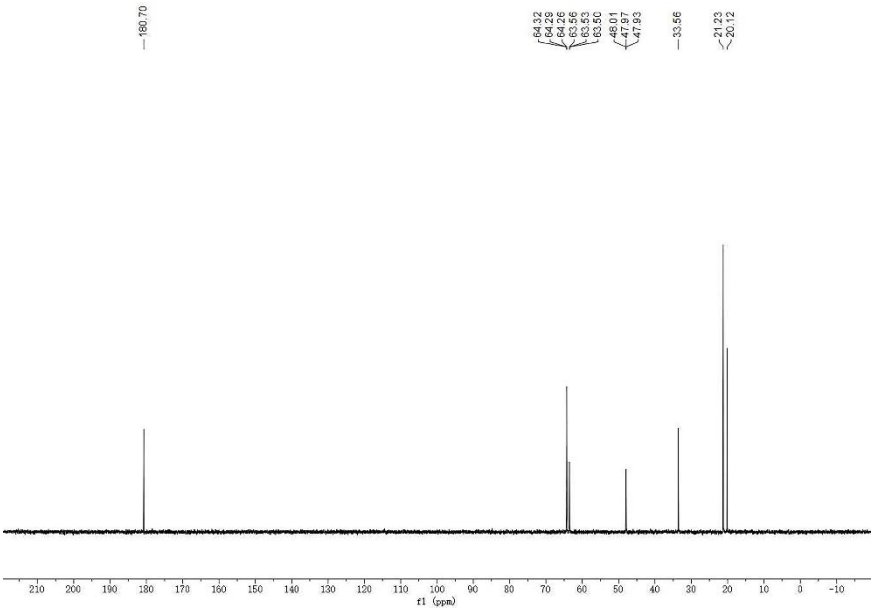


Figure S21 [13]CNMR spectra of 7.

4-(N-methylpiperidinium)-butyrate; 8

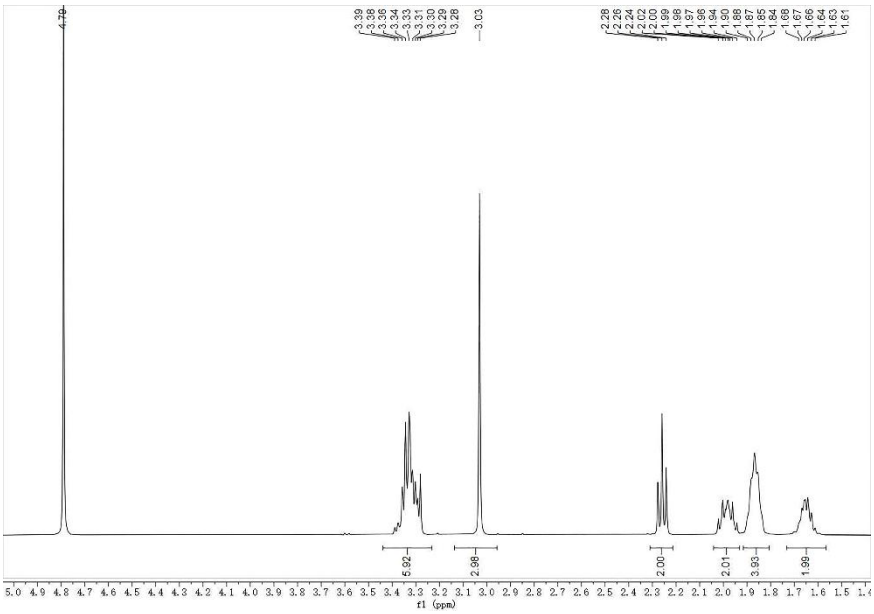


Figure S22 ^{1}HNMR spectra of 8.

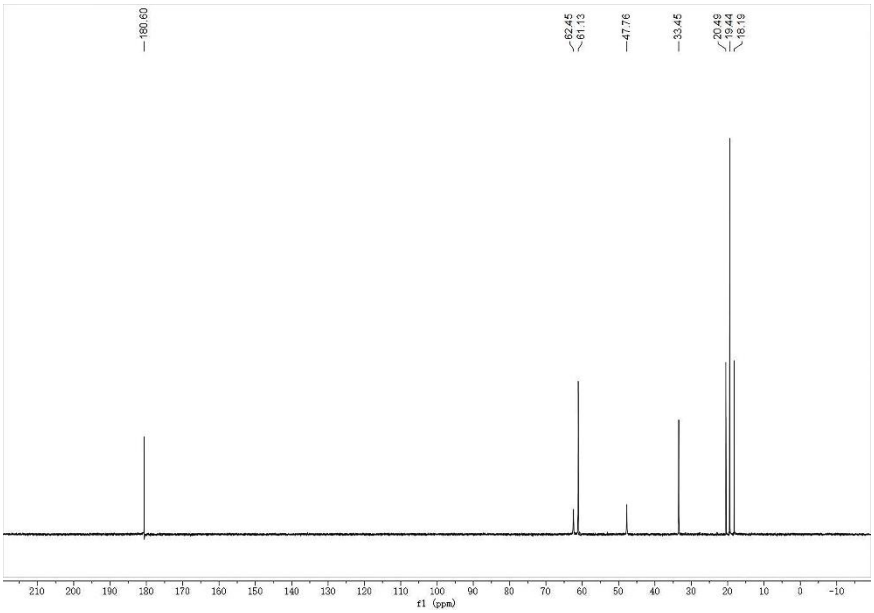
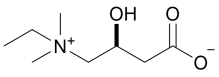


Figure S23 ^{13}CNMR spectra of 8.

4-(ethyldimethylammonio)-3-hydroxybutanoate; 4a



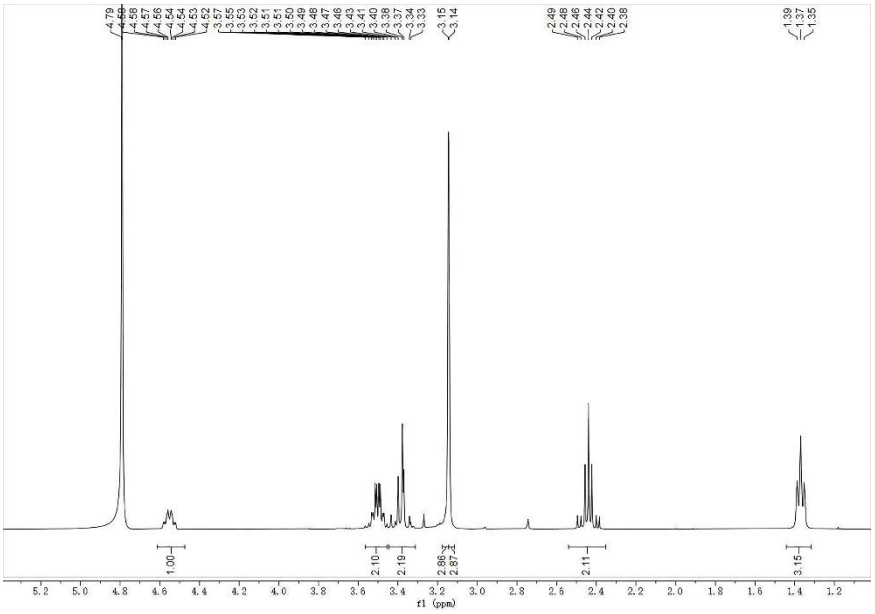


Figure S24 [1]HNMR spectra of 4a.

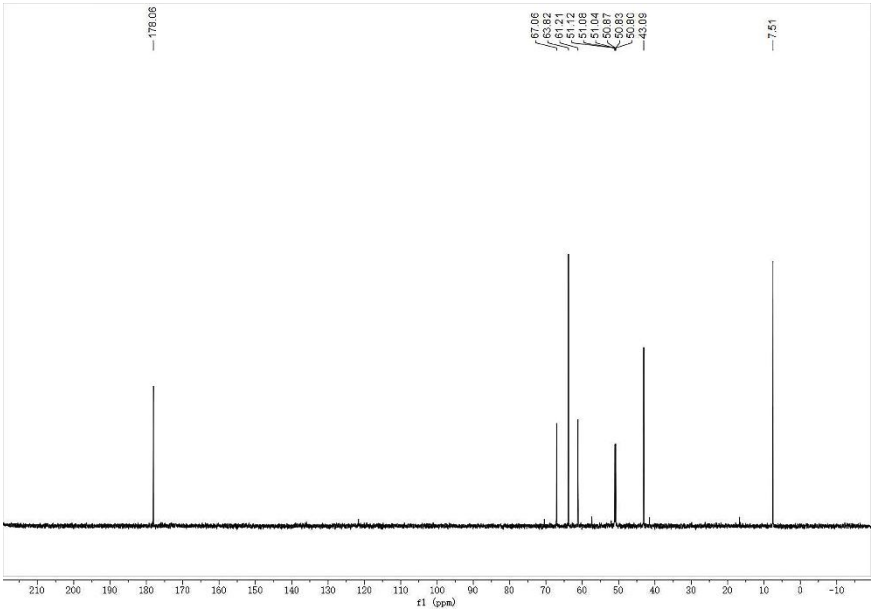
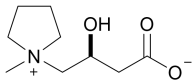


Figure S25 [13]CNMR spectra of 4a.

3-hydroxy-4-(1-methylpyrrolidin-1-ium-1-yl)butanoate; 7a



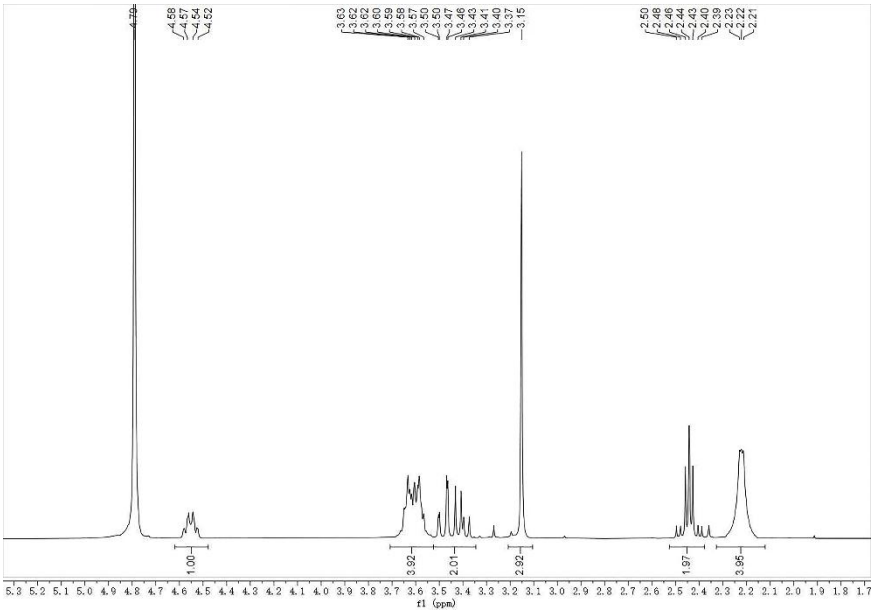


Figure S26 [1]HNMR spectra of 7a.

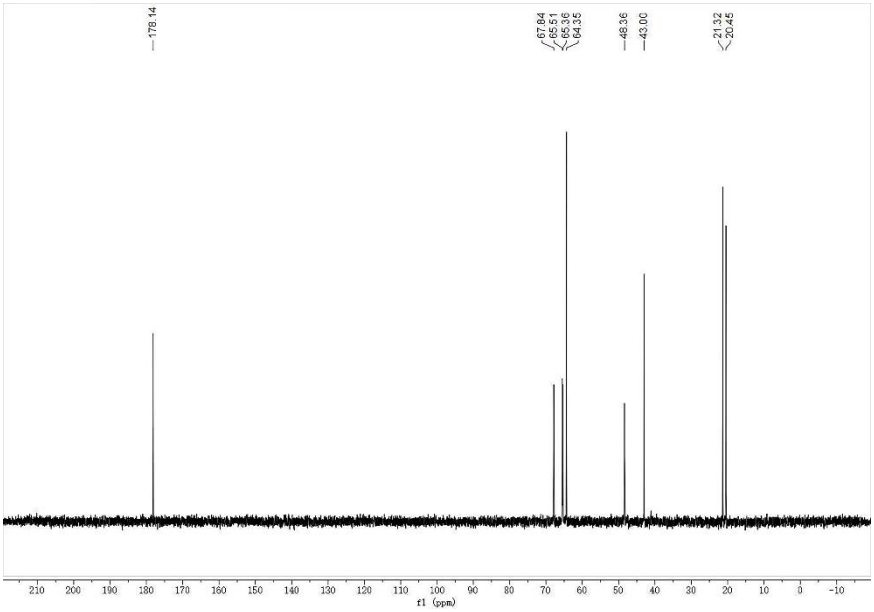
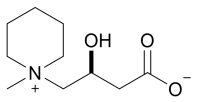


Figure S27 [13]CNMR spectra of 7a.

3-hydroxy-4-(1-methylpiperidin-1-ium-1-yl)butanoate; 8a



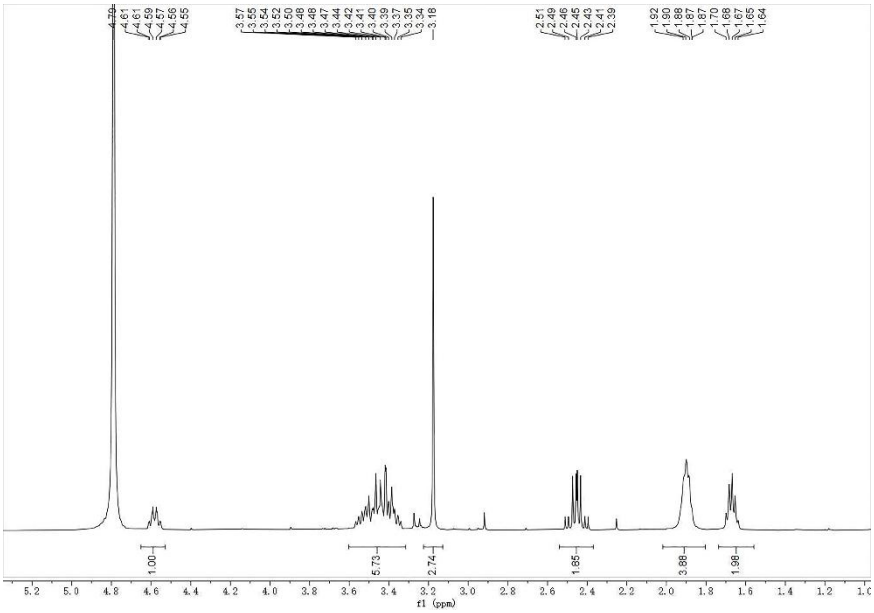


Figure S28 [1]HNMR spectra of 8a.

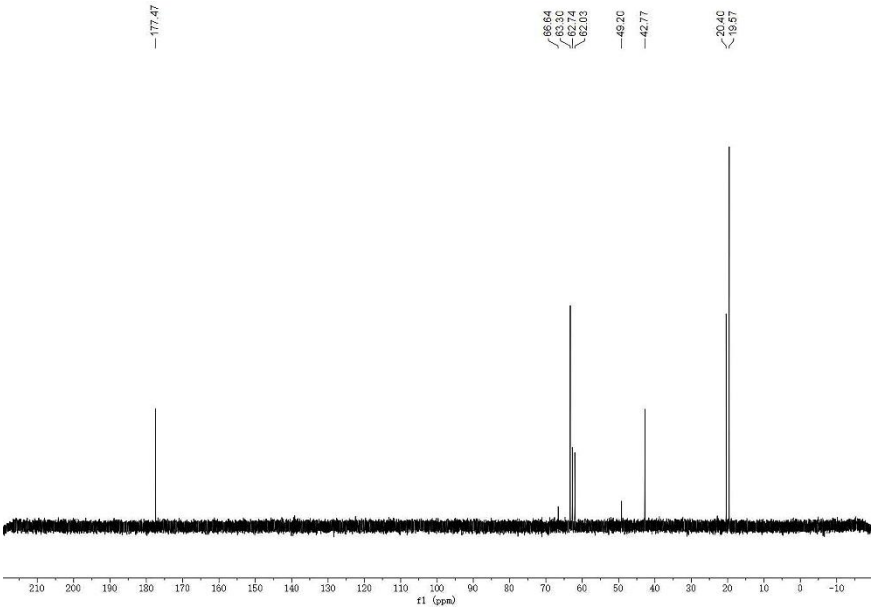
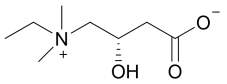


Figure S29 [13]CNMR spectra of 8a.

(R)-4-(ethyldimethylammonio)-3-hydroxybutanoate; 4a-R



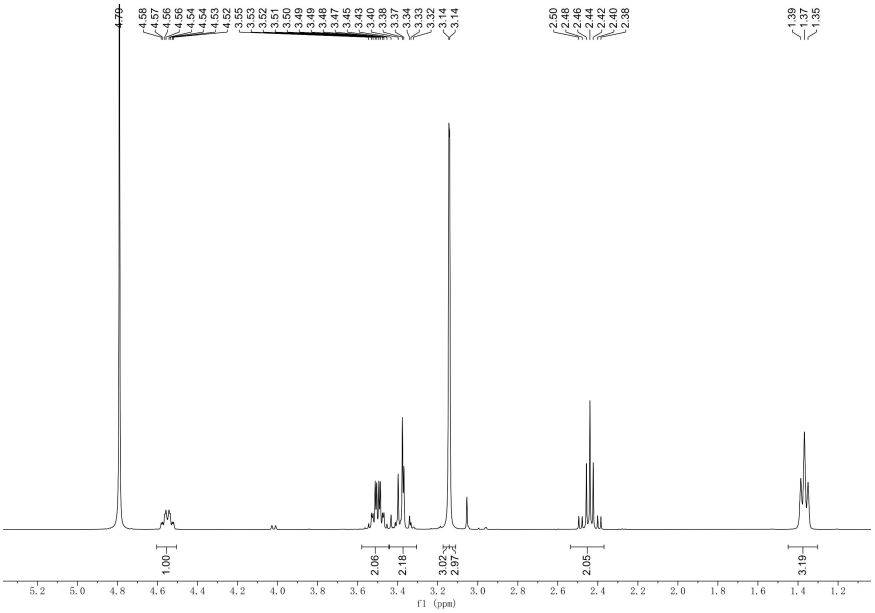


Figure S30 [1]HNMR spectra of 4a-R.

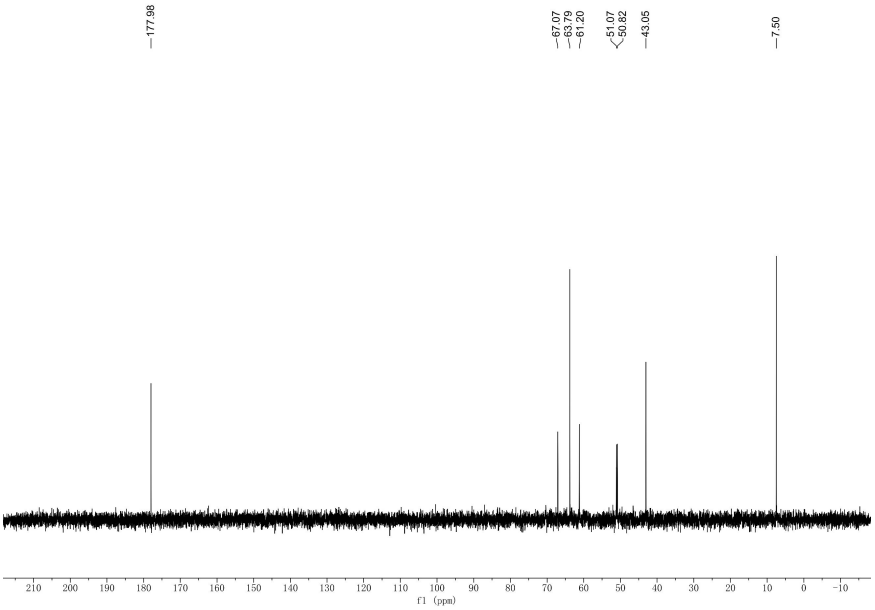


Figure S31 [13]CNMR spectra of 4a-R.

(S)-4-(ethyldimethylammonio)-3-hydroxybutanoate; 4a-S

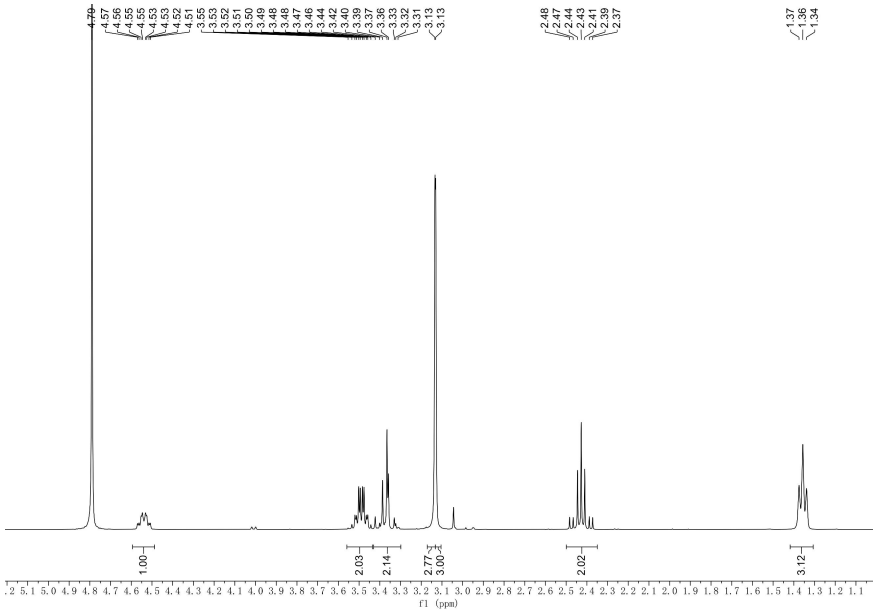


Figure S32 [1]HNMR spectra of 4a-S.

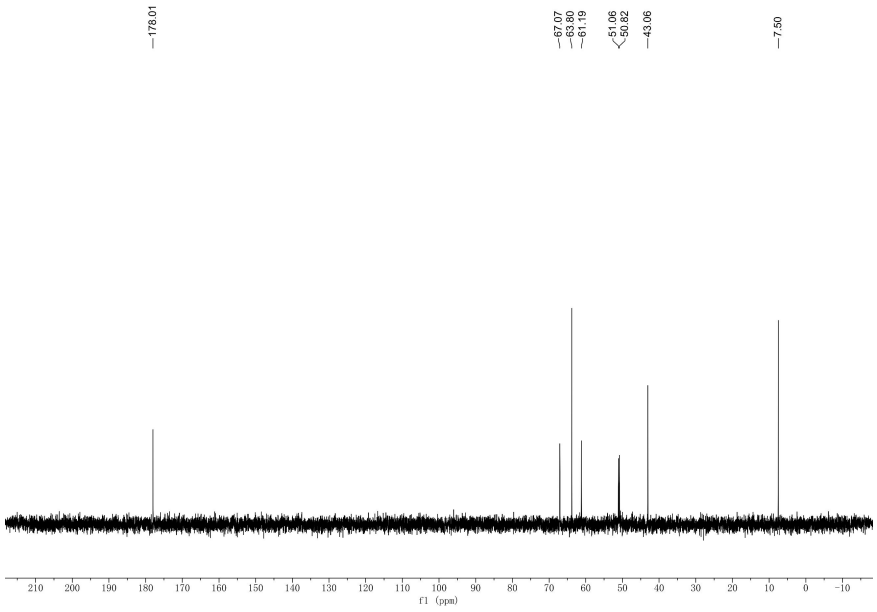
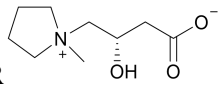


Figure S33 [13]CNMR spectra of 4a-S.

(R)-3-hydroxy-4-(1-methylpyrrolidin-1-ium-1-yl)butanoate; 7a-R



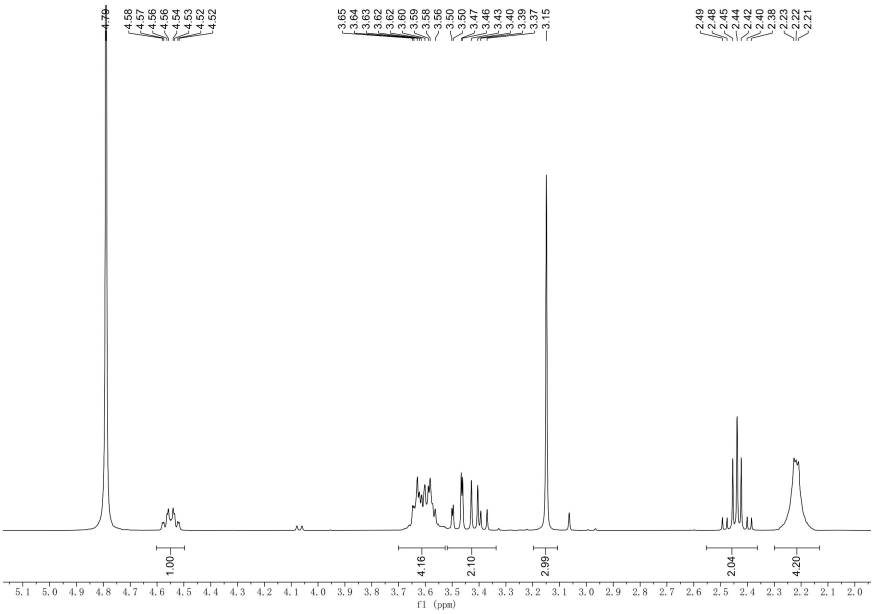


Figure S34 [1]HNMR spectra of 7a-R.

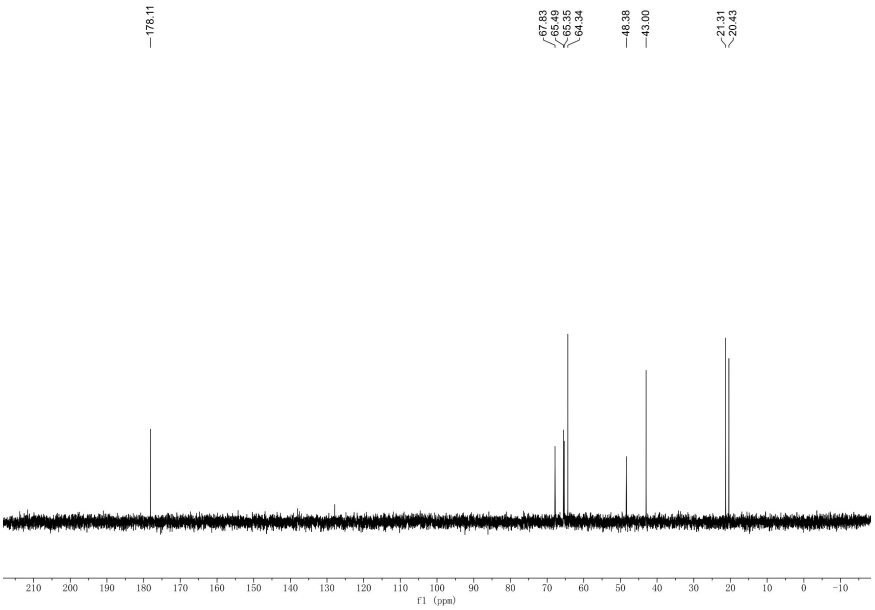
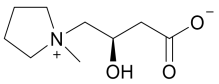


Figure S35 [13]CNMR spectra of 7a-R.

(S)-3-hydroxy-4-(1-methylpyrrolidin-1-ium-1-yl)butanoate; 7a-S



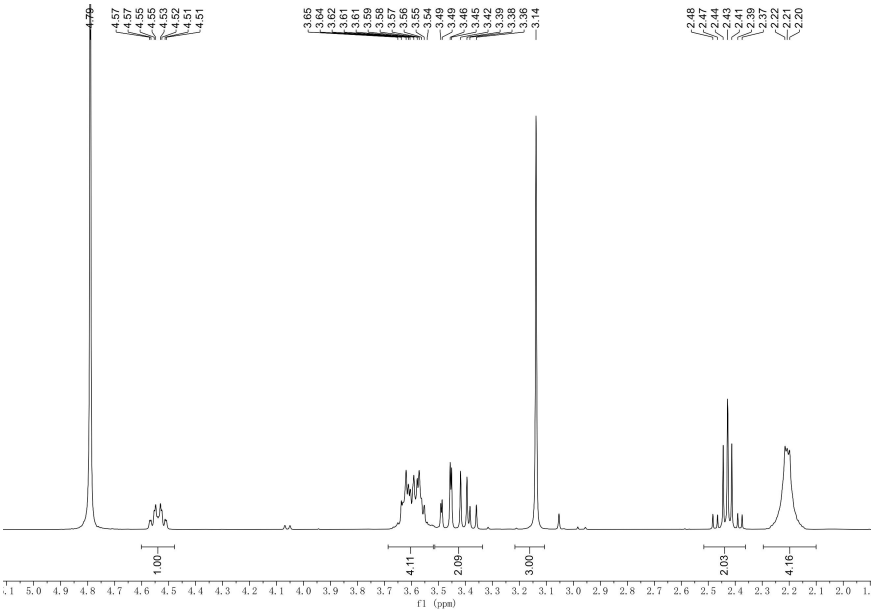


Figure S36 [1]HNMR spectra of 7a-S.

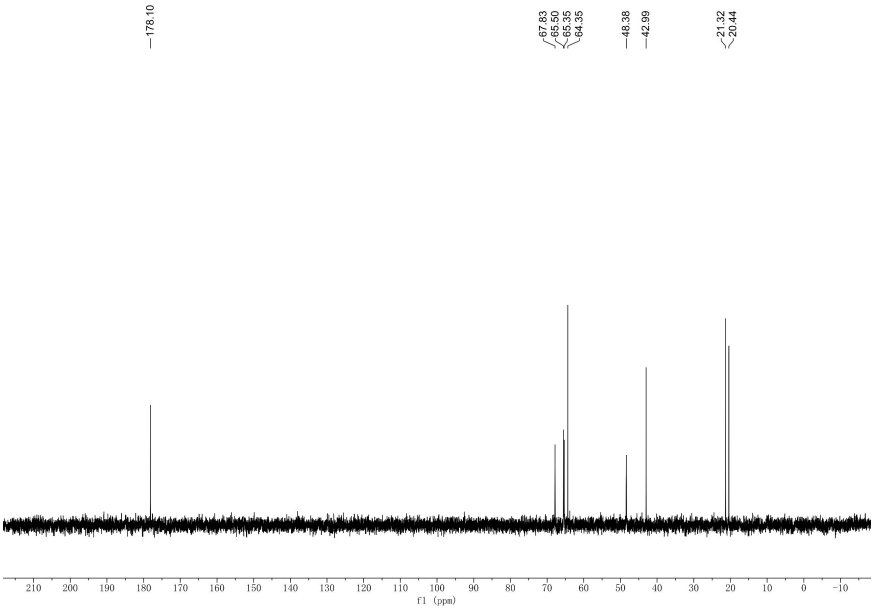
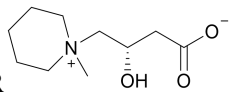


Figure S37 [13]CNMR spectra of 7a-S.

(R)-3-hydroxy-4-(1-methylpiperidin-1-ium-1-yl)butanoate; 8a-R



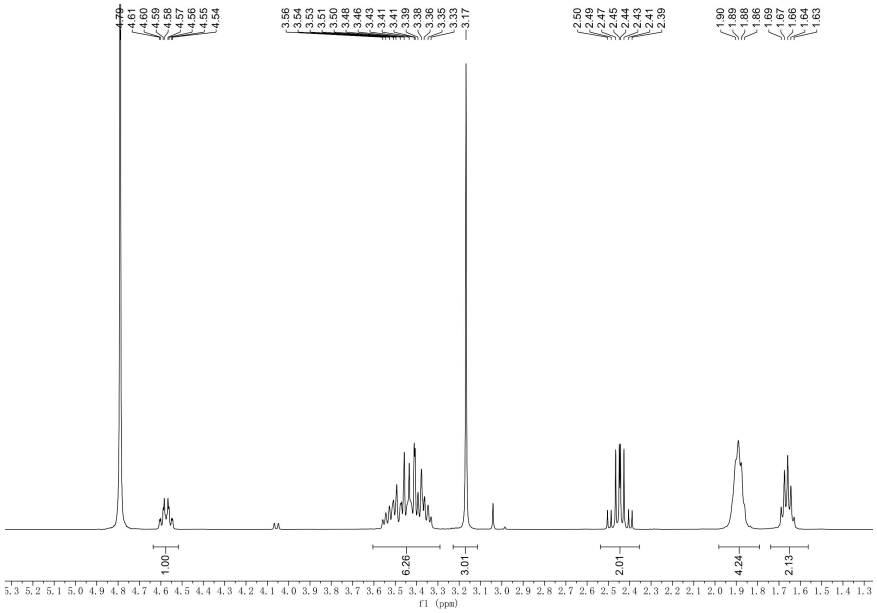


Figure S38 [1]HNMR spectra of 8a-R.

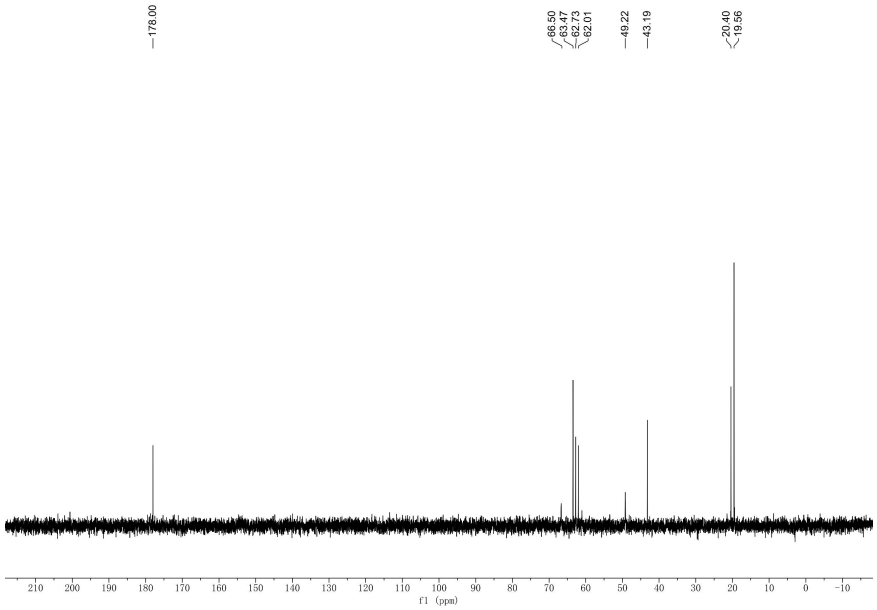
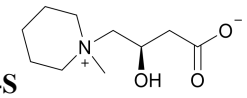


Figure S39 [13]CNMR spectra of 8a-R.

(S)-3-hydroxy-4-(1-methylpiperidin-1-ium-1-yl)butanoate; 8a-S



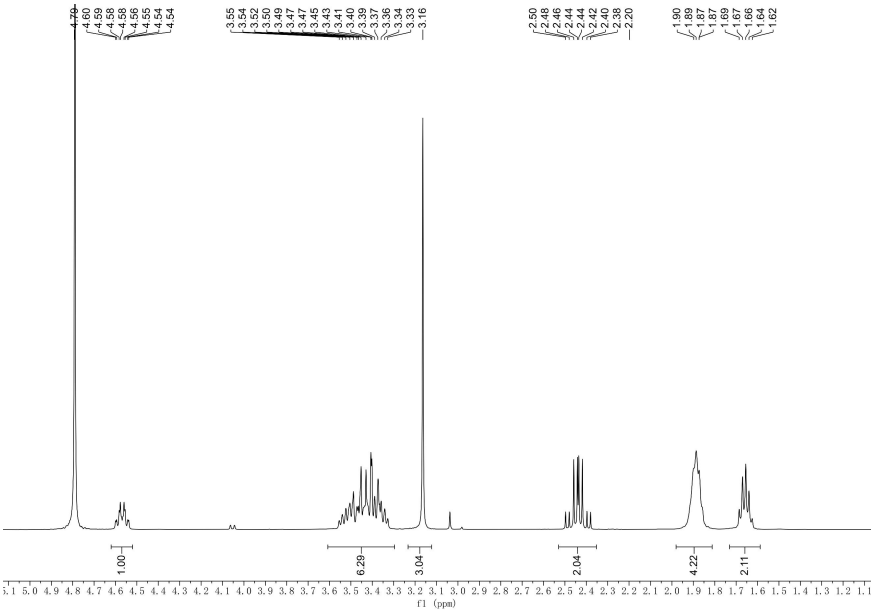


Figure S40 [1]HNMR spectra of 8a-S.

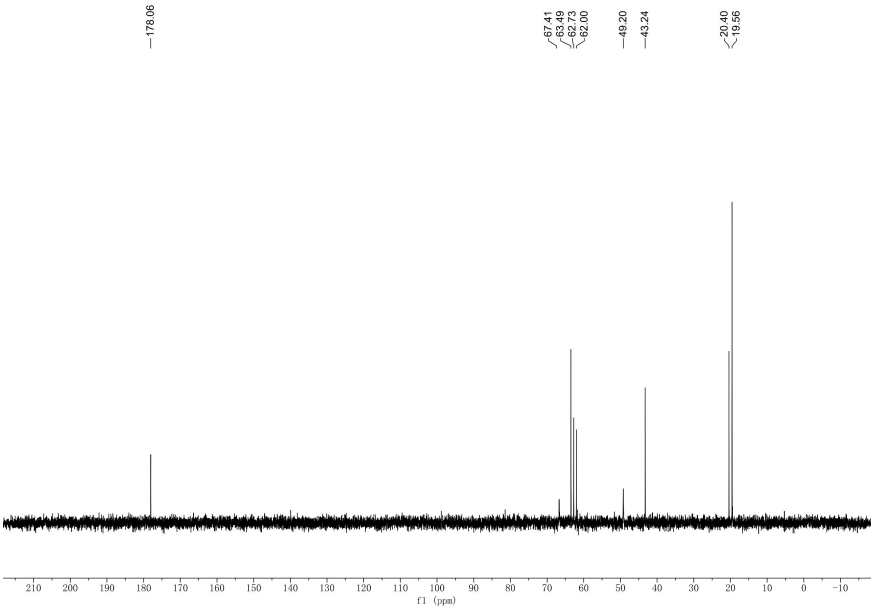


Figure S41 [13]CNMR spectra of 8a-S.

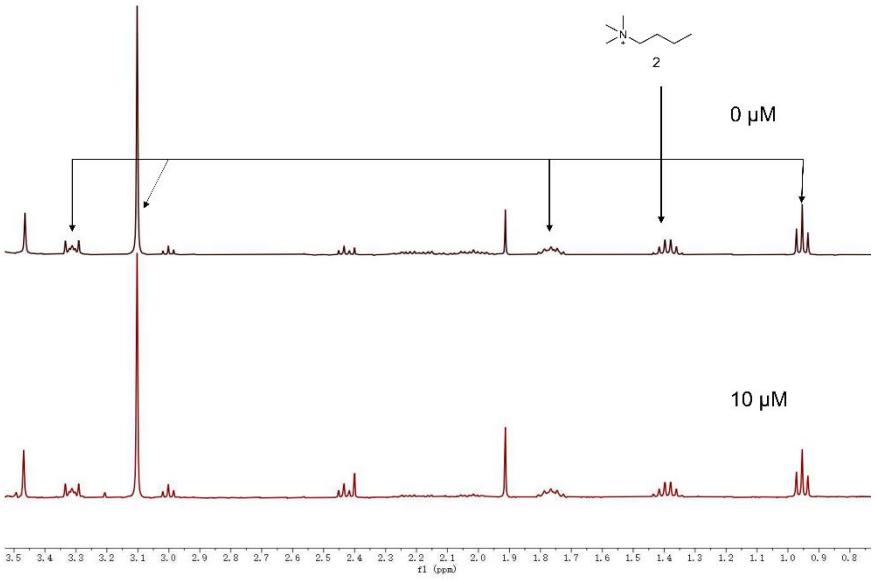


Figure S42 ^{1}H NMR monitoring of the 2 hydroxylation by psBBOX.

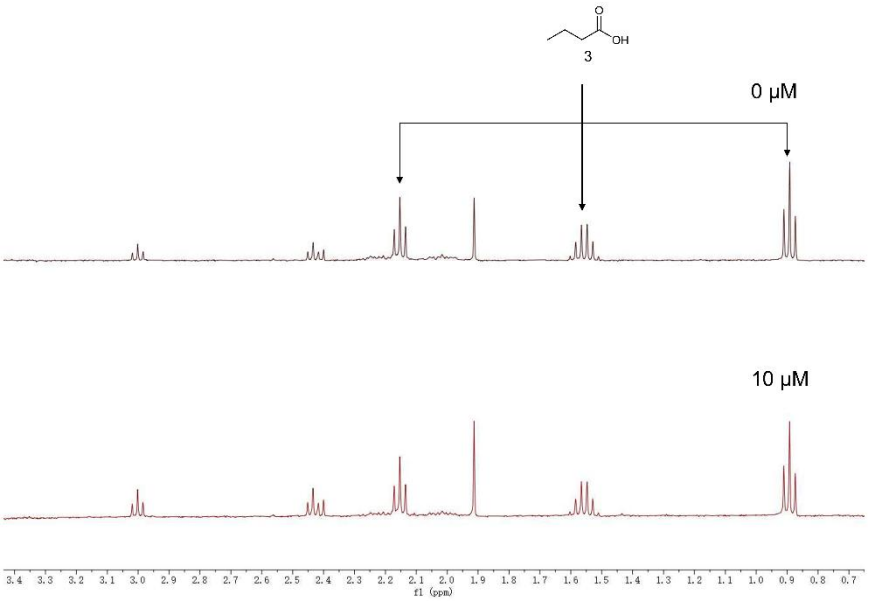


Figure S43 ^{1}H NMR monitoring of the 2 hydroxylation by psBBOX.

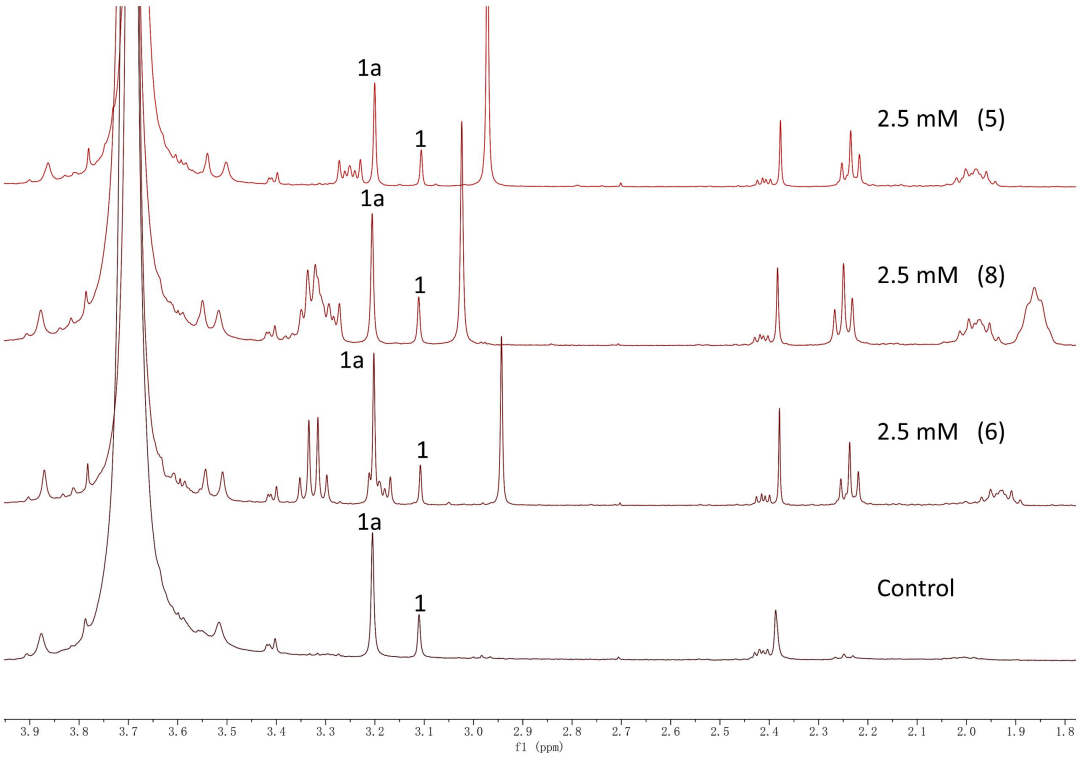


Figure S44 The inhibitory effect of (5), (6), (8) on the transformation of original substrate (1).

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