Lipase and metal chloride hydrate-natural deep eutectic solvents synergistically catalyze amidation reaction via multiple non-covalent bond interactions

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Number of pages: #18

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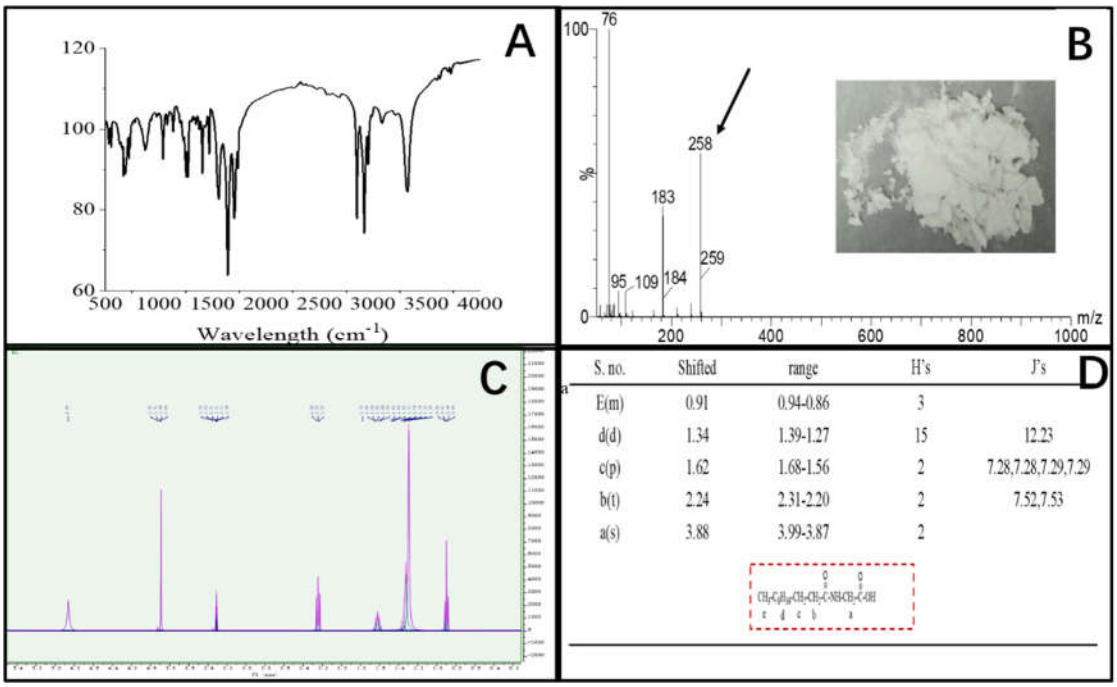

Figure S1. Characterization of Lauroyl glycine (LG)

* The peaks of 3321.92cm^{-1}, 1704.31cm^{-1}, 1643.87cm^{-1} and 1558.69cm^{-1} in Figure S1a (FT-IR) are attributed to the -NH, -C=O of -COOH; -C=O of -CONH and -C-N- respectively; Figure S1b (MS) suggested that the product showed a characteristic peak with m/z 258.1, which was consistent with (LG+H^{+}). The product was also analyzed by ^{1}HNMR, with tetramethyl silane (TMS) as the internal standard and CD_3OD as the solvent. The chemical shift values of the peaks and their assignments were shown in Figure S1 (c, d).

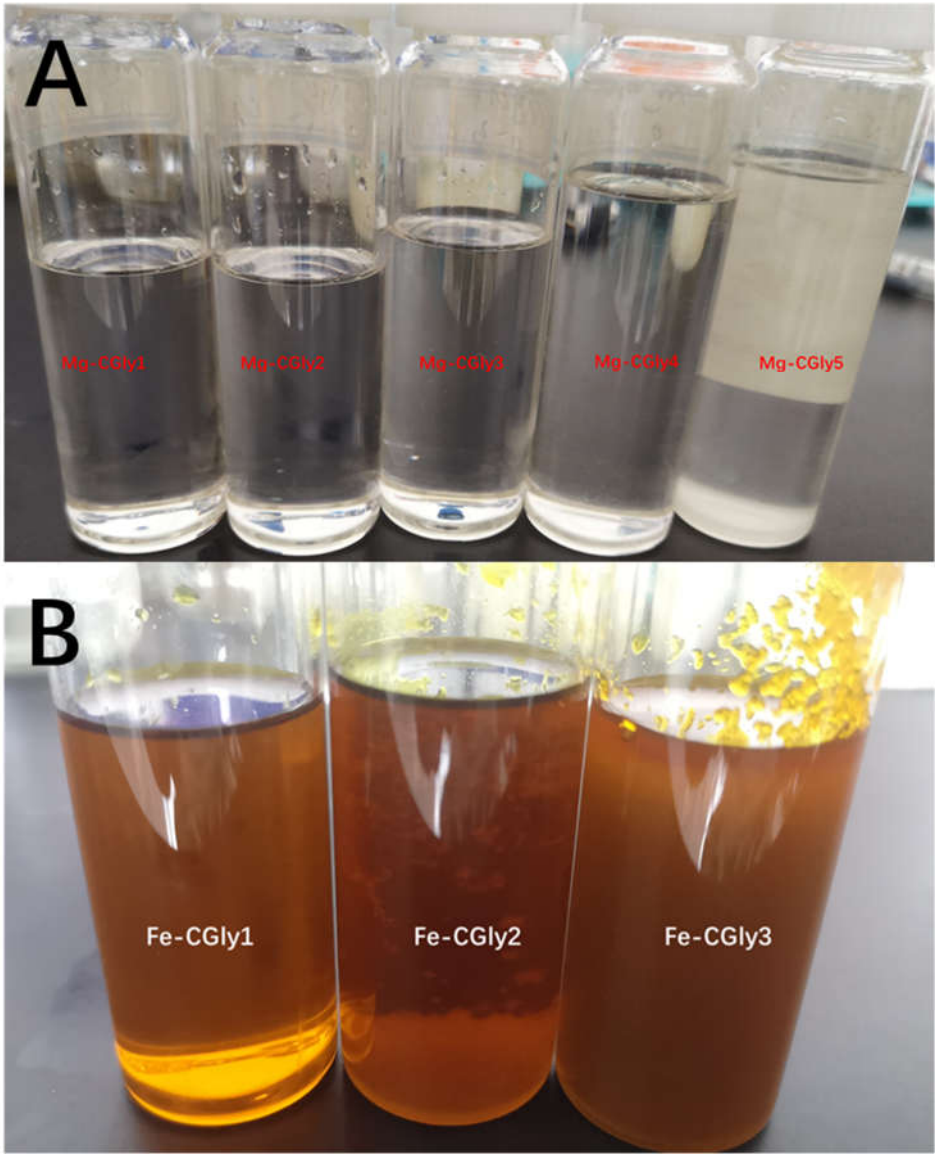

Figure S2. Construction of 3c-NADESs

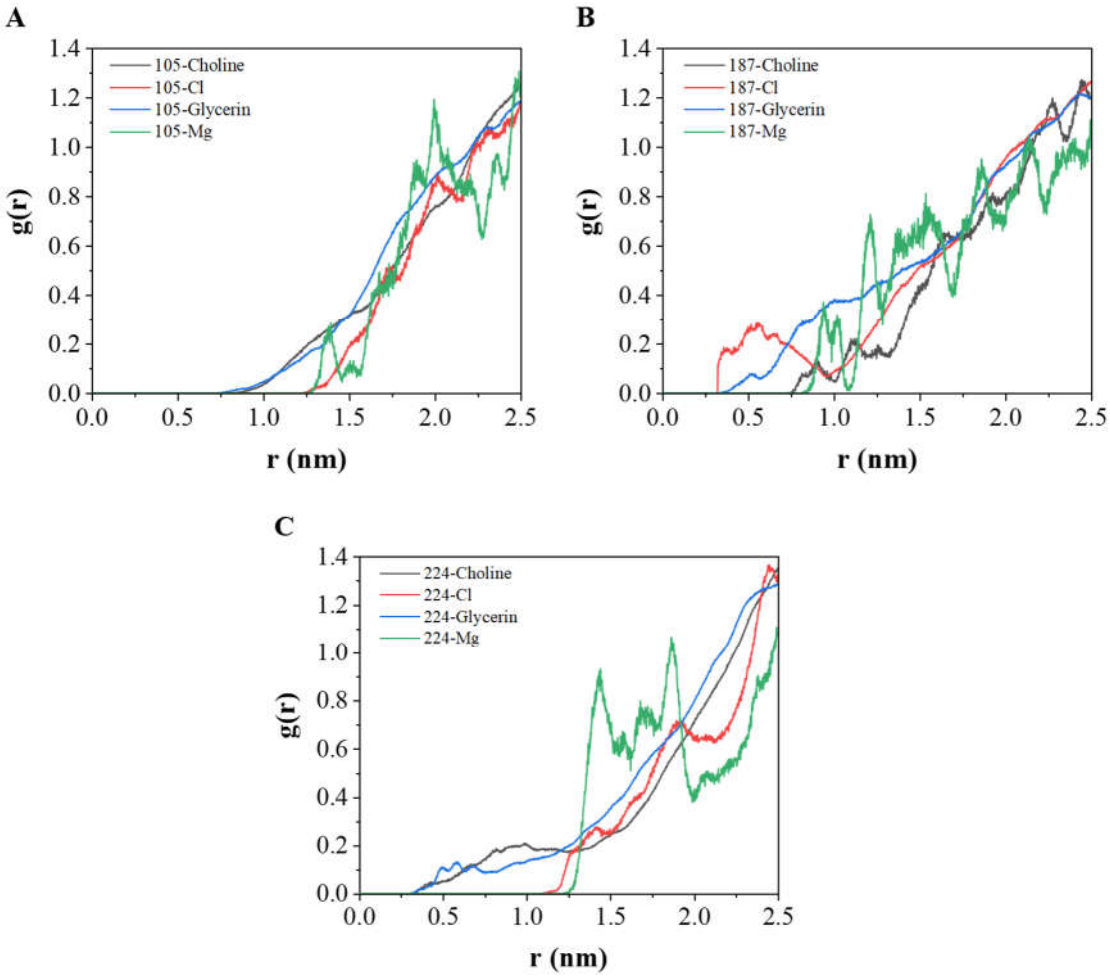

Figure S3. COM RDFs for Mg-CGly3 components around CALB catalytic triad (Ser-His-Asp)

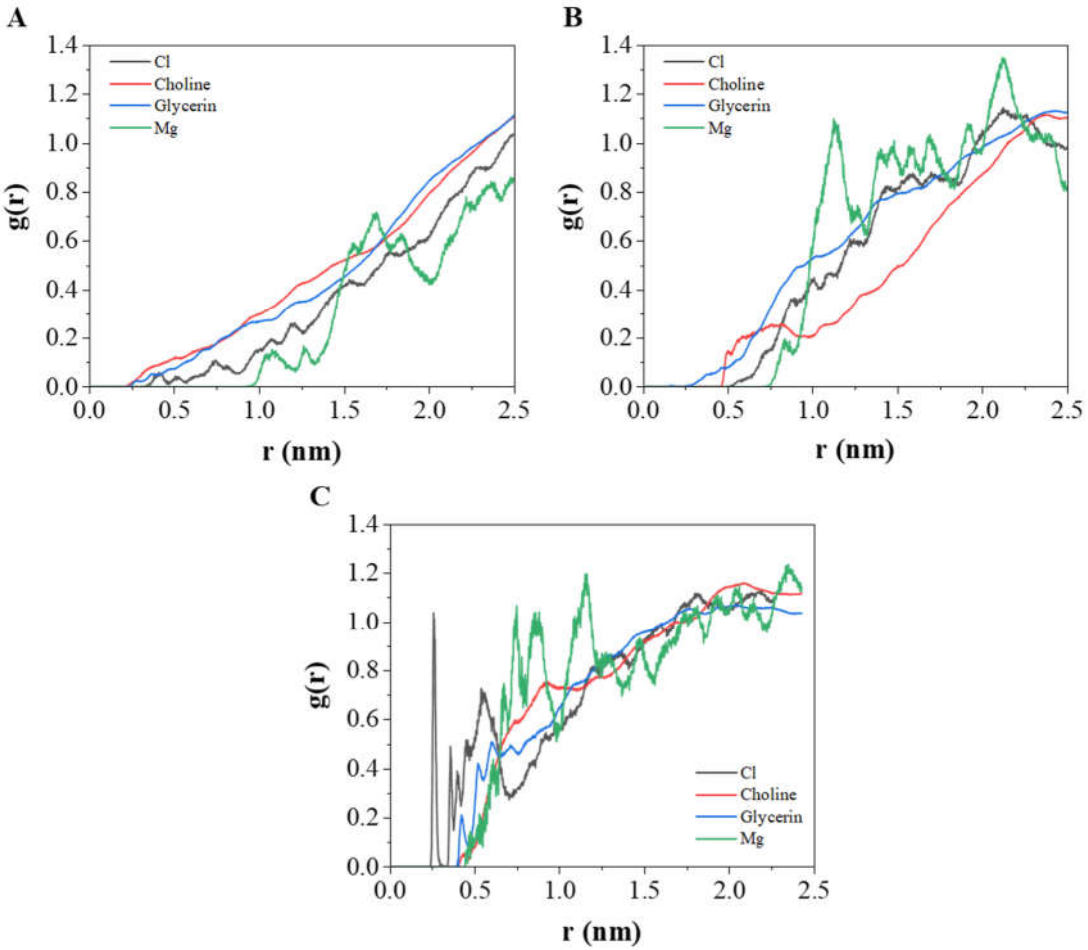

Figure S4. COM RDFs for Mg-CGly3 components around CALB large (A), medium (B) and acyl binding pocket (C)

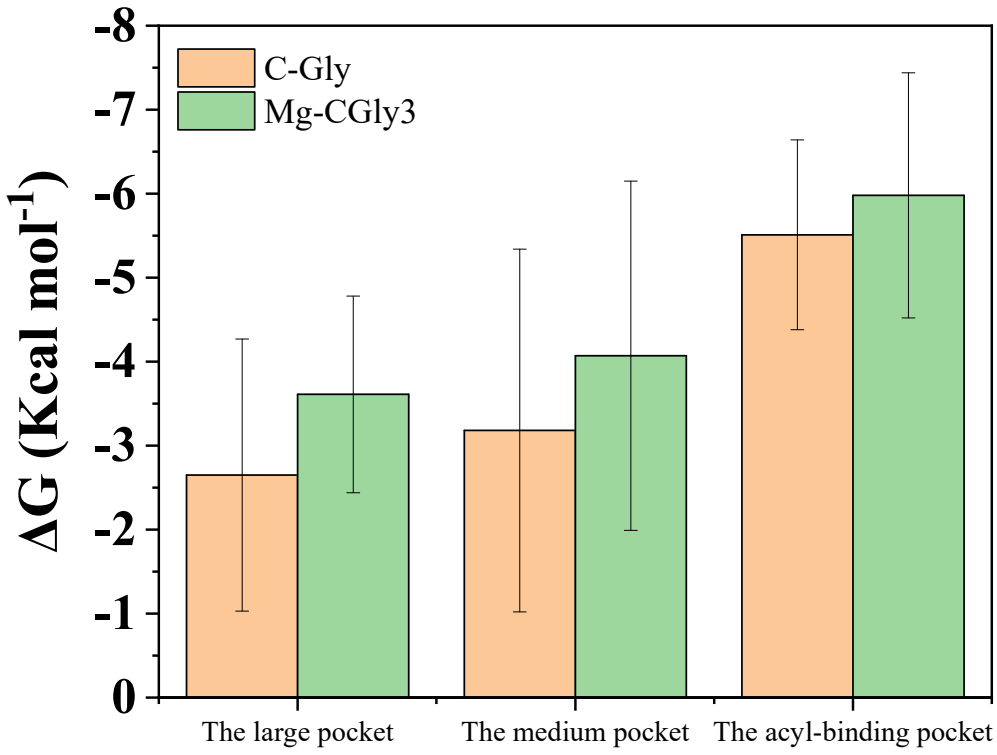

Figure S5. Combined free energy of CALB substrate-binding pocket and lauric acid in NADESs and 3c-NADESs calculated by MM-PBSA

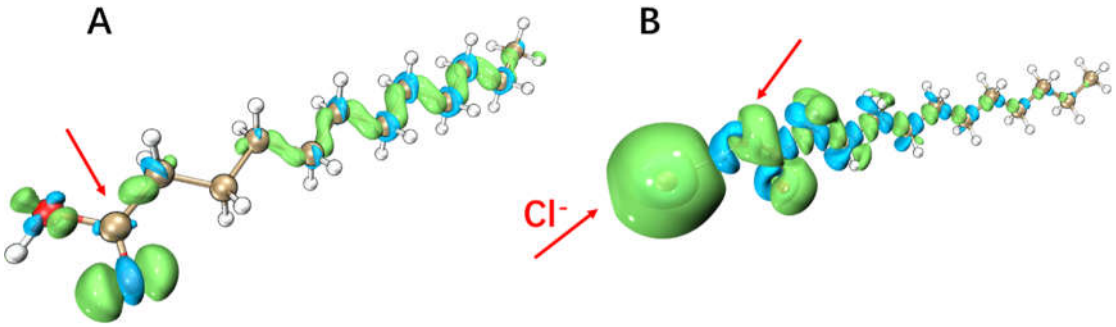

Figure S6. The effect of chloride ions on the Fukui function of lauric acid

*The Fukui function of lauric acid was calculated via Multiwfn software, and the isosurface value is 0.007.

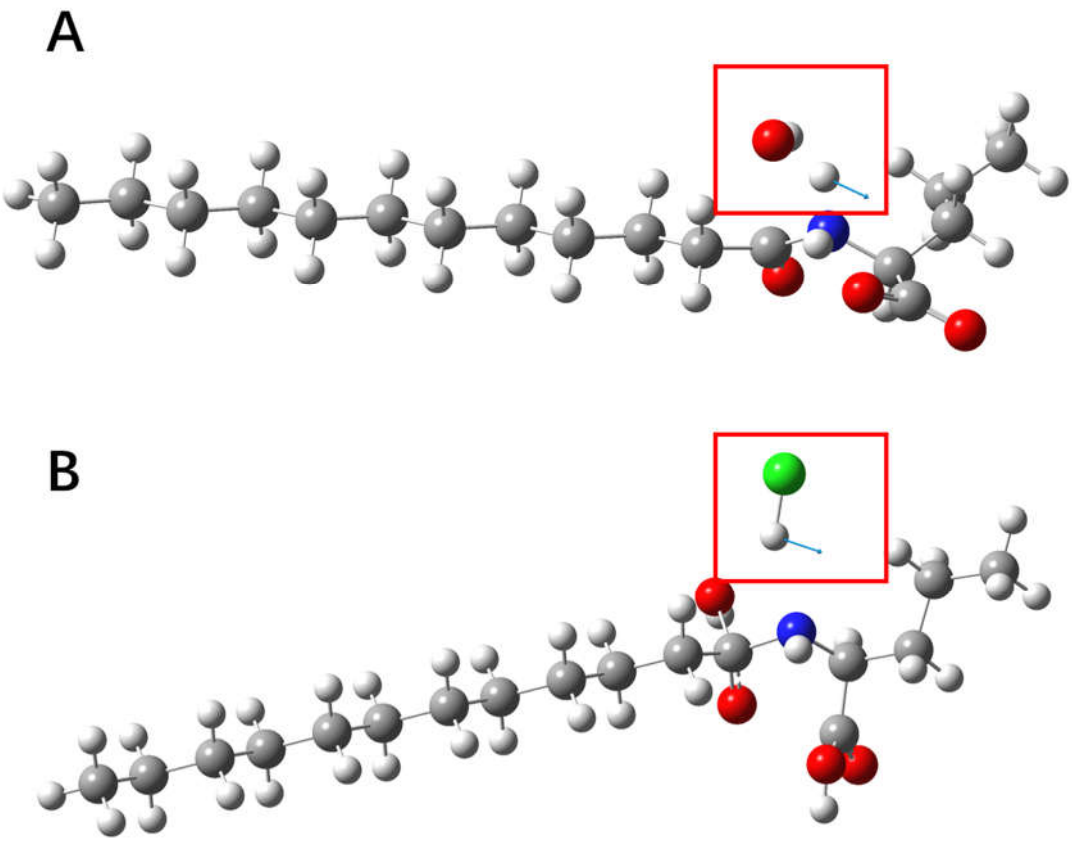

Figure S7. The harmonic vibrational frequency of the amidation reaction of lauric acid and glycine in C-Gly (A) and Mg-CGly3 (B)

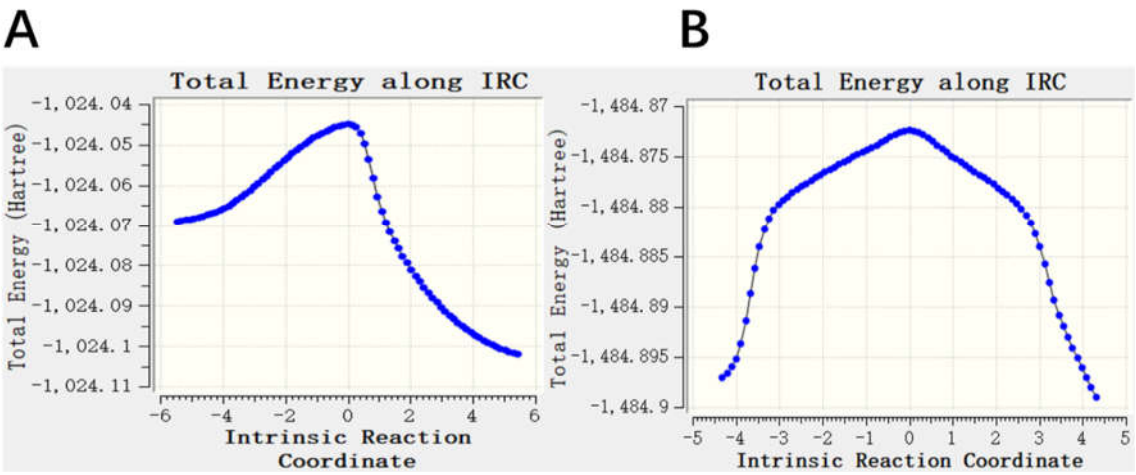

Figure S8. The intrinsic reaction coordinates (IRC) of the amidation reaction of lauric acid and glycine in C-Gly (A) and Mg-CGly3 (B)

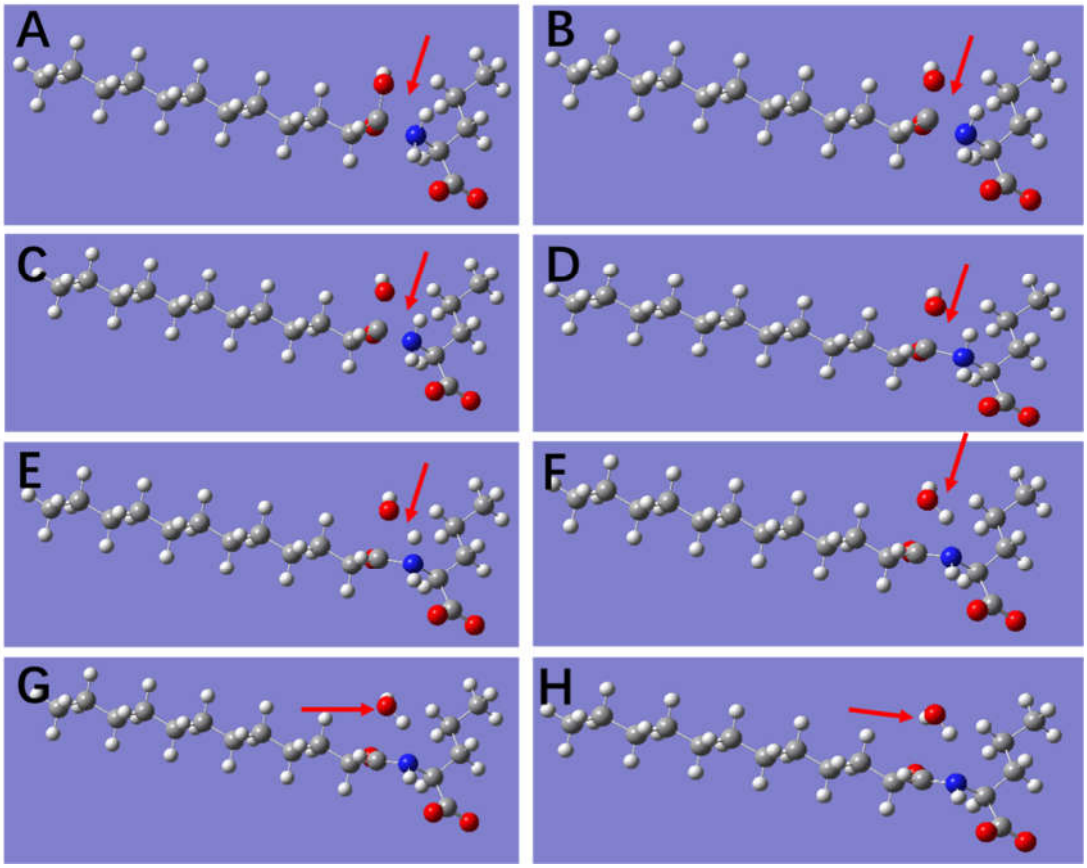

Figure S9. The reaction path of the amidation reaction of lauric acid and glycine in C-Gly calculated via IRC method.

*A-H indicates: 0, 10, 20, 30, 40, 50, 60 and 80 steps of IRC path

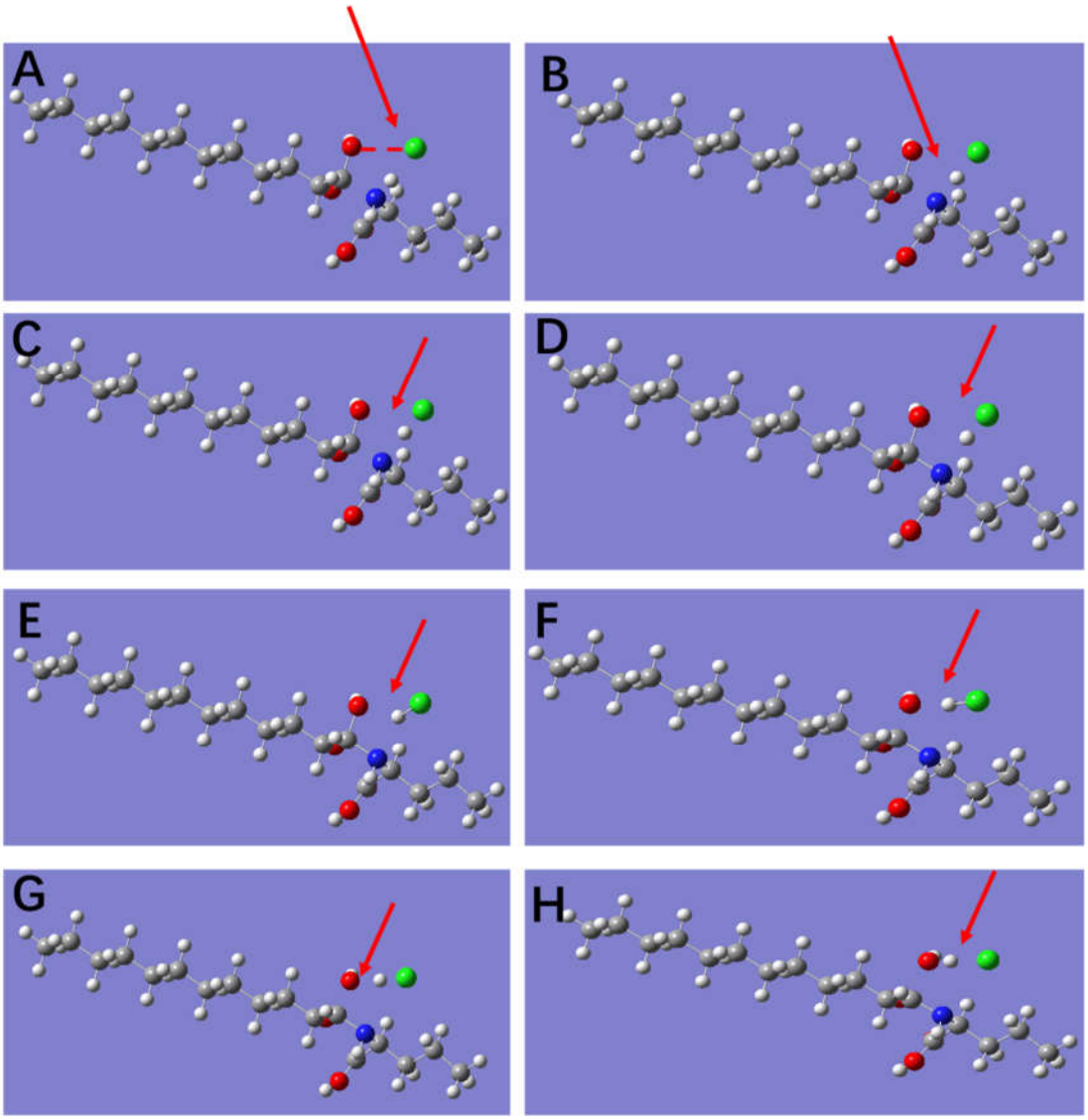

Figure S10. The reaction path of the amidation reaction of lauric acid and glycine in Mg-CGly3 calculated via IRC method.

*A-H indicates: 0, 10, 20, 30, 40, 50, 60 and 80 steps of IRC path

Table S1. Construction of NADESs, the HBD and HBA are given in molar ratios

S. no.	HBA	HBD	HBA：HBD	Fluidity at 363K
1	Betaine	xylitol	1:2	yes[a]
2	Betaine	Malic acid	1:2	no
3	Betaine	Citric acid	1:1	no
4	Betaine	Glycerin	1:2	yes
5	Betaine	Urea	1:2	yes
6	Choline chloride	xylitol	1:2	yes[a]
7	Choline chloride	Malic acid	1:1	yes
8	Choline chloride	glucose	3:2	no
9	Choline chloride	Trehalose	1:3	no
10	Choline chloride	Glycerin	1:2	yes
11	Choline chloride	Urea	1:1	yes[a]
12	Choline chloride	Lactic acid	1:10	yes
13	Proline	Sorbitol	1:1	no
14	Proline	Glycerin	1:2	yes
15	Proline	Urea	1:2	no
16	Lactic acid	Glycine	1:3	yes
17	Malic acid	Glucose	1:3	yes[a]
18	Malic acid	Sucrose	1:3	no
19	Malic acid	Glycerin	1:1	yes

* HBA:HBD are given in molar ratios; yes[a] was liquid, but high viscous

Table S2. Second screening round of NADES

S. no.	HBA	HBD	HBA：HBD	Fluidity at 363 K
1	Betaine	xylitol	1:1	yes[a]
2	Betaine	xylitol	2:1	NA
3	Betaine	Urea	1:1	yes
4	Betaine	Urea	2:1	no
5	Choline chloride	xylitol	1:1.5	yes
6	Choline chloride	xylitol	1:1	no
7	Choline chloride	Trehalose	3:1	yes[a]
8	Choline chloride	Trehalose	1:1	no
9	Choline chloride	Urea	1:2	yes
10	Choline chloride	Urea	1:1.5	no
11	Lactic acid	Glycine	1:1	NA
12	Lactic acid	Glycine	3:1	NA
13	Lactic acid	Glycine	6:1	NA
14	Lactic acid	Glycine	9:1	yes[a]
15	Malic acid	Glucose	1:2	NA
16	Malic acid	Glucose	1:1	yes

* HBA:HBD are given in molar ratios; yes[a] was liquid, but high viscous; NA represents that NADESs cannot be formed

Table S3. Construction of NADESs, the C-GLy and metal chloride hydrates are given in mole fractions

Mole fraction of C-Gly	Mole fraction of metal chloride hydrates			
	$MgCl_2 \cdot 6H_2O$	Abbreviation	$FeCl_3 \cdot 6H_2O$	Abbreviation
0.9	0.1	Mg-CGly1	0.1	Fe-CGly1
0.8	0.2	Mg-CGly2	0.2	Fe-CGly2
0.7	0.3	Mg-CGly3	0.3	Fe-CGly3
0.6	0.4	Mg-CGly4	0.4	Fe-CGly4
0.5	0.5	Mg-CGly5	0.5	Fe-CGly5

Table S4. The different parameters and levels used during the optimization process

Identifier	Parameter	Level 1	Level 2	Level 3
A	Time (h)	72	96	120
B	Temperature (K)	313	323	333
C	Enzyme (mg)	50	75	100
D	Fatty acids: amino acids	2:1	1.5:1	1:1

Table S5. L9 orthogonal array showing the controllable parameters for the optimization of the enzymatic synthesis of LG process in Mg-CGly3

Trail[#]	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

*For detailed description of parameters A-D and levels 1-3, please refer Table S4, four controllable were listed, to make the results easier to be analyzed, a L9 assay were adopted. A total number of 27 runs (9 trails*3repetitions) were needed to arrive at a conclusion.