Supporting Information

Microflow-based dynamic combinatorial chemistry: A microscale synthesis and screening platform for rapid and accurate identification of bioactive molecules

Chuanhong Qiu, [a†] Zheng Fang, [b†] Lihuan Zhao, [b] Wei He, [b] Zhao Yang, [c] Chengkou Liu [b] and Kai Guo*[b, d]

[a]*School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing 211816, PR China.*

[b]*College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211816, PR China.*

[c]*College of Engineering, China Pharmaceutical University, Nanjing 210009, PR China.*

[d]*State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, Nanjing 210009, PR China*

† *These two authors contributed equally to this work.*

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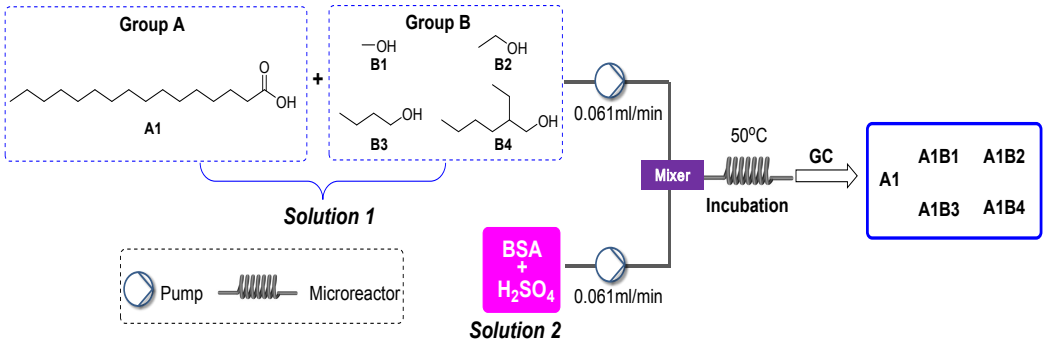
General Information

All the reagents used in this study were obtained from commercial supplier and used without further purification unless otherwise noted. The quantitative analysis was performed on a gas chromatograph system (Agilent 7890A). High resolution mass spectra (HRMS) were performed on Agilent Acrrurate-Mass Q-ToF LC/MS 6520 mass spectrometer with electron spray ionization (ESI) mode. The microflow system was supplied by Ehrfeld Mikrotechnik BTS and made up mainly of microreactors, LH25 slit-plate type mixers and plunger pumps.

Experimental procedure

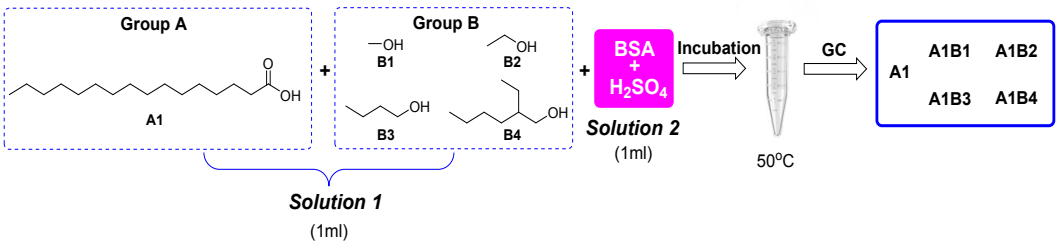
Preparation of DCL 1



Reaction conditions: solution 1: 50 mM of palmitic acid and 10 mM of alcohols in hexane, flow rate 0.061 ml/min; solution 2: 0.2 mM of sulfuric acid and 10 mM of BSA in distilled water,flow rate 0.061 ml/min. Microreactors: T= 50°C, volume = 3.66 ml, 7.32 ml, 10.98 ml, 14.64 ml

Stock mixed solution of palmitic acid (50 mM) and alcohols (10 mM), namely solution 1, was freshly prepared in hexane. Stock mixed solution of sulfuric acid (0.2 mM) and BSA (10 mM), namely solution 2, was freshly prepared in distilled water. The flow velocity of solution 1 and solution 2 was respectively 0.061mL/min. The two flows were pumped into a LH25 slit-plate type mixer, followed by entering into the microreactors with different volumes (volume = 3.66 ml, 7.32 ml, 10.98 ml, 14.64 ml) at 50 °C. After different residence time (0.5h, 1h, 1.5h, 2h), the reaction mixture was then detected by GC.

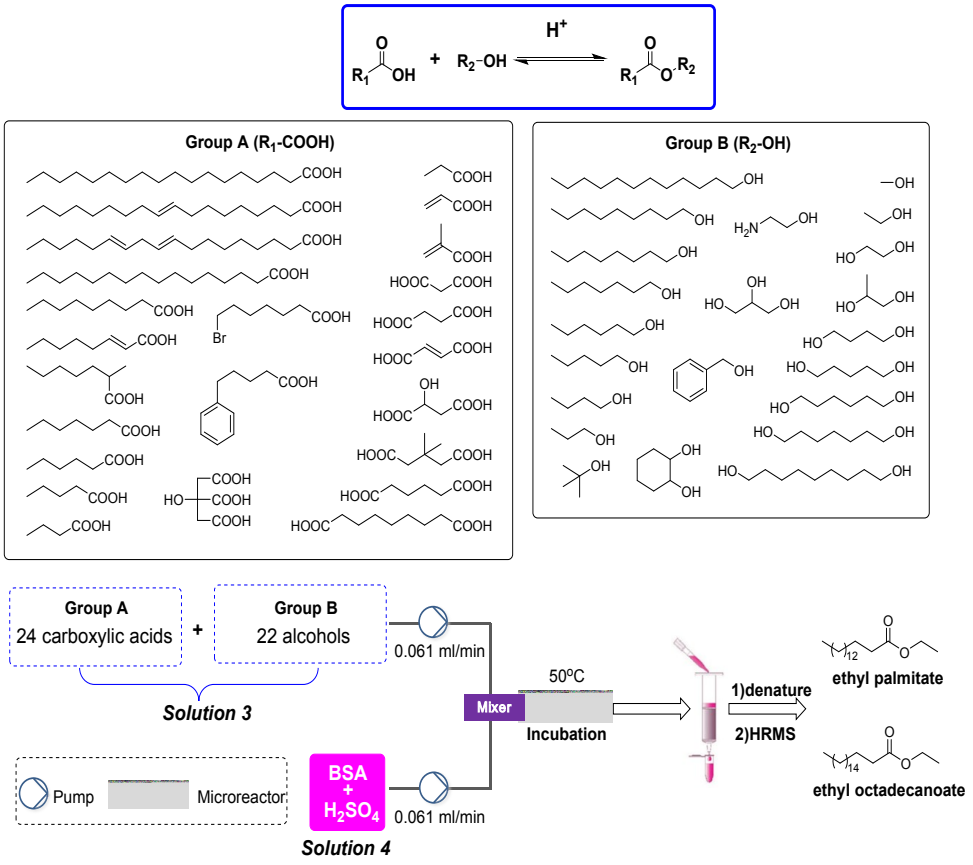
Preparation of DCL 2



Reaction conditions: solution 1: 50 mM of palmitic acid and 10 mM of alcohols in hexane, 1ml ; solution 2: 0.2 mM of sulfuric acid and 10 mM of BSA in distilled water, 1 ml; T = 50°C.

Stock mixed solution of palmitic acid (50 mM) and alcohols (10 mM), namely solution 1, was freshly prepared in hexane. Stock mixed solution of sulfuric acid (0.2 mM) and BSA (10 mM), namely solution 2, was freshly prepared in distilled water. Solution 1 (1 mL) and Solution 2 (1 mL) were combined in an Eppendorf tube, followed by stirring for different time at 50 °C. The reaction mixture was then detected by GC.

Preparation of DCL 3

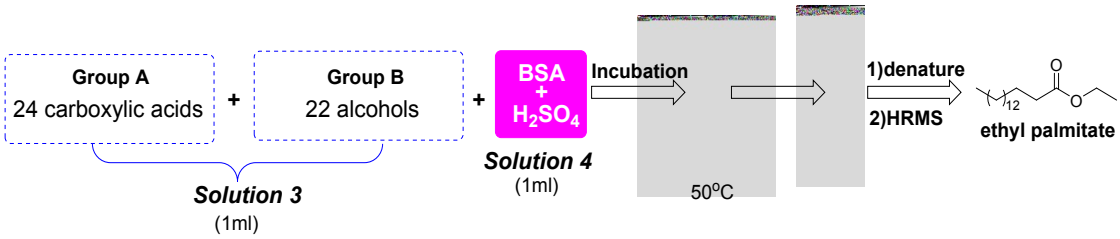


Reaction conditions: solution 3: 10 mM of carboxylic acids and 15 mM of alcohols in hexane, flow rate 0.061 ml/min; solution 4: 1.5 mM of sulfuric acid and 10 mM of BSA in distilled water,flow rate 0.061 ml/min. Microreactor:T = 50°C, volume = 7.32 ml.

Stock mixed solution of carboxylic acids (10 mM) and alcohols (15 mM), namely solution 3, was freshly prepared in hexane prior to use. Stock mixed solution of sulfuric acid (1.5 mM) and BSA (10 mM), namely solution 4, was freshly prepared in distilled

water prior to use. The flow velocity of solution 3 and solution 4 was respectively 0.061mL/min. The two flows were pumped into a LH25 slit-plate type mixer, followed by entering into a 7.32 ml microreactor at 50 °C. After a residence time of 1 h, about 25 μL of the reaction mixture was collected and purified by a suitable size-exclusion column. MeOH (75 μL) was added into the eluent and the mixture was centrifuged at 1000 g for 4 min, and then the supernatant was detected by HRMS.

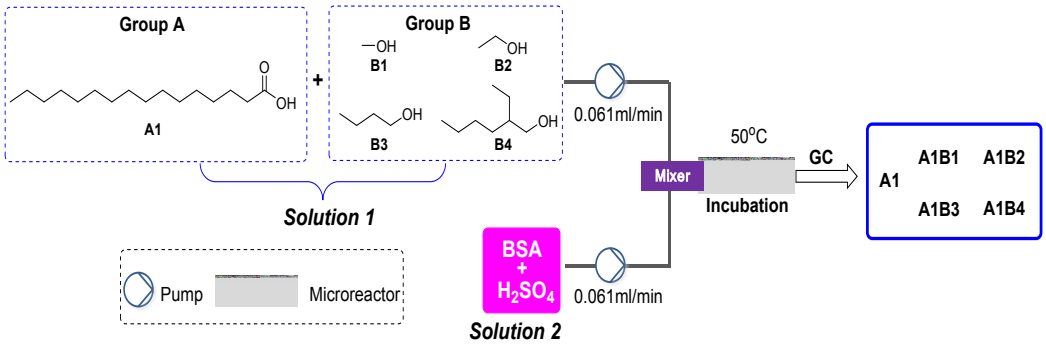
Preparation of DCL 4



Reaction conditions: solution 3: 10 mM of carboxylic acids and 15 mM of alcohols in hexane, 1 ml; solution 4: 1.5 mM of sulfuric acid and 10 mM of BSA in distilled water, 1 ml. T = 50°C, 12h.

Stock mixed solution of carboxylic acid (50 mM) and alcohols (60 mM), namely solution 3, was freshly prepared in hexane prior to use. Stock mixed solution of sulfuric acid (1.5 mM) and BSA (10 mM), namely solution 4, was freshly prepared in distilled water prior to use. Solution 3 (1 mL) and Solution 4 (1 mL) were combined in an Eppendorf tube, followed by stirring for 12h at 50 °C. About 25 μL of the reaction mixture was taken out and purified by a suitable size-exclusion column. MeOH (75 μL) was added into the eluent and the mixture was centrifuged at 1000 g for 4 min, and then the supernatant was detected by HRMS.

GC chromatograms of dynamic combinatorial library (DCL 1)



Reaction conditions: solution 1: 50 mM of palmitic acid and 10 mM of alcohols in hexane, flow rate 0.061 ml/min; solution 2: 0.2 mM of sulfuric acid and 10 mM of BSA in distilled water,flow rate 0.061 ml/min. Microreactors: T= 50°C, volume = 3.66 ml, 7.32 ml, 10.98 ml, 14.64 ml

Table S1: GC peak area (A%) of each compound at different residence time in microflow mode

In microflow mode				
Entry	0.5h	1h	1.5h	2h
A1	8.85%	9.73%	8.05%	7.92%
A1B1	18.77%	9.67%	9.56%	9.43%
A1B2	38.96%	58.74%	60.30%	60.18%
A1B3	17.88%	10.88%	10.85%	10.99%
A1B4	15.53%	10.97%	11.24%	11.47%

Figure S1. 0.5h in DCL1

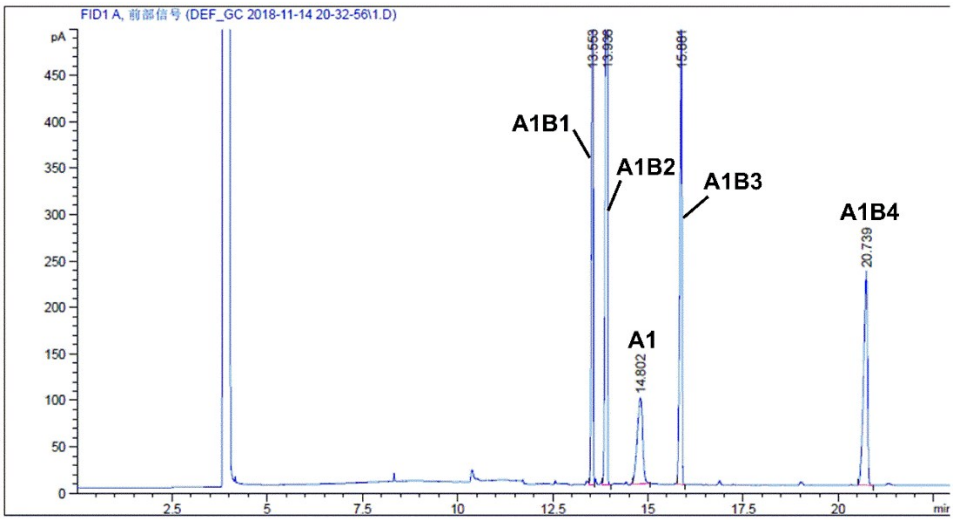


Figure S2. 1h in DCL1

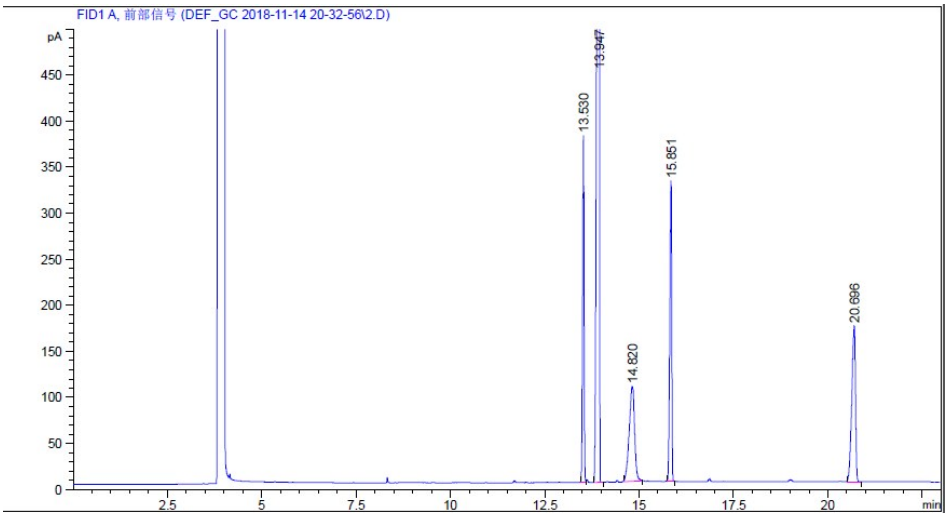


Figure S3. 1.5h in DCL1

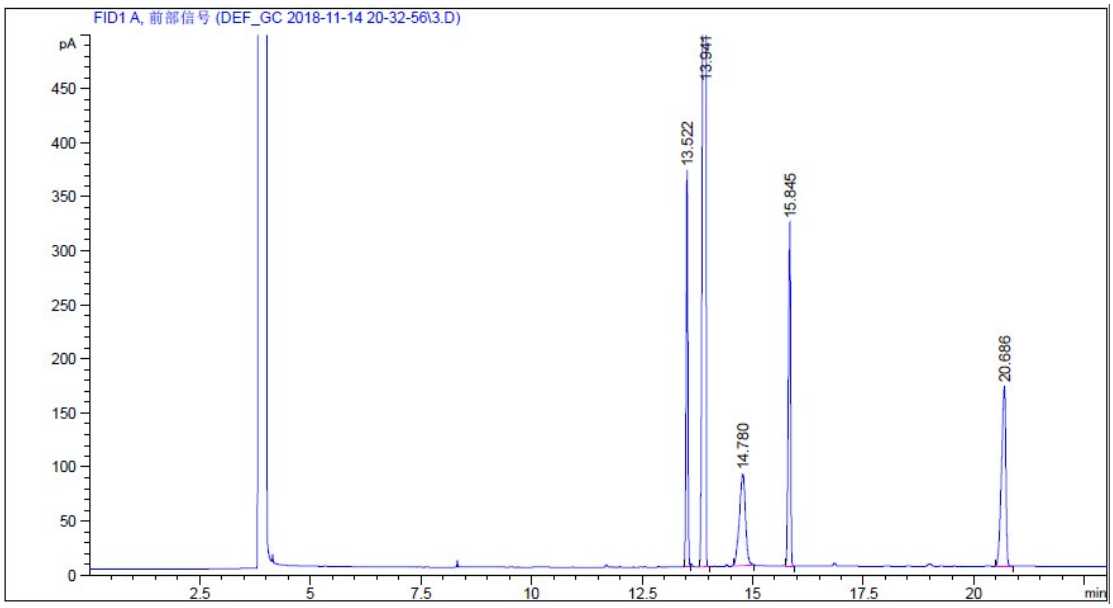
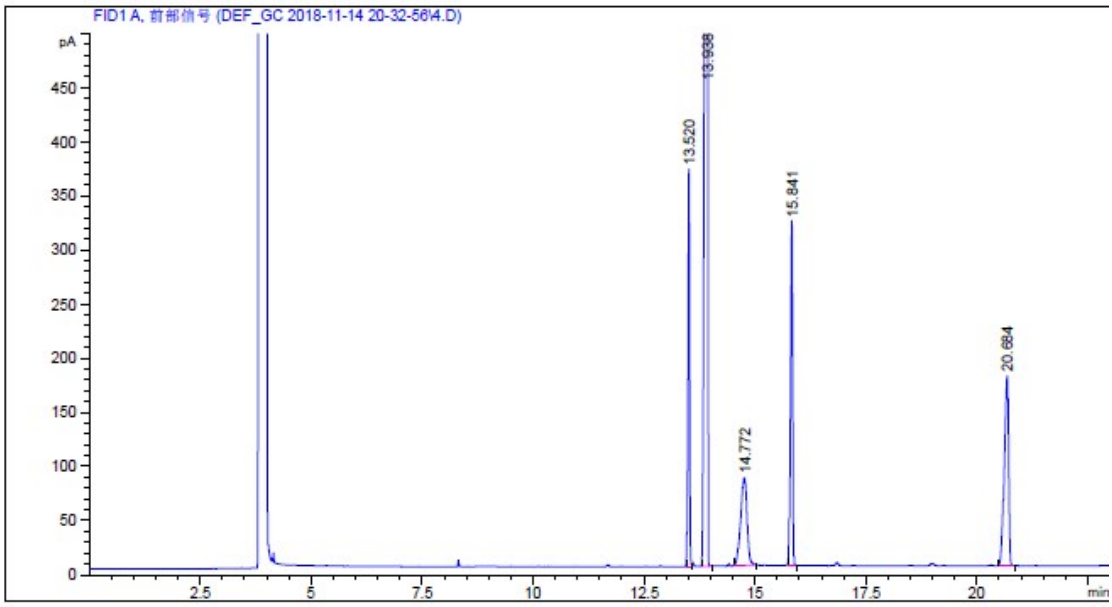
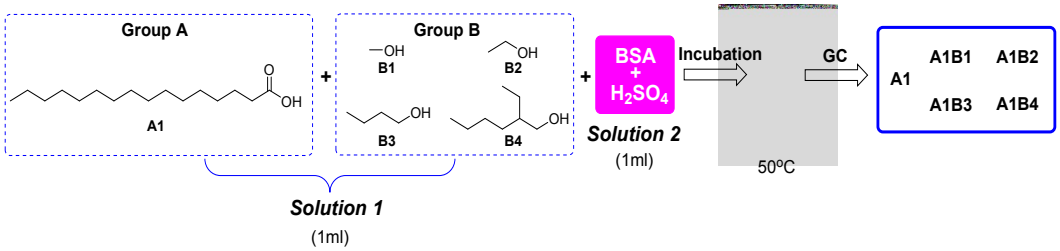


Figure S4. 2h in DCL1



GC chromatograms of dynamic combinatorial library (DCL 2)



Reaction conditions: solution 1: 50 mM of palmitic acid and 10 mM of alcohols in hexane, 1ml ; solution 2: 0.2 mM of sulfuric acid and 10 mM of BSA in distilled water, 1 ml; T = 50°C.

Table S2: GC peak area (A%) of each compound at different reaction time in batch mode

Entry	6h	18h	24h
A1	8.72%	15.49%	16.55%
A1B1	20.50%	7.64%	7.57%
A1B2	44.27%	61.03%	60.43%
A1B3	12.83%	8.54%	8.42%
A1B4	13.67%	7.27%	7.01%

Figure S5. 6h in DCL2

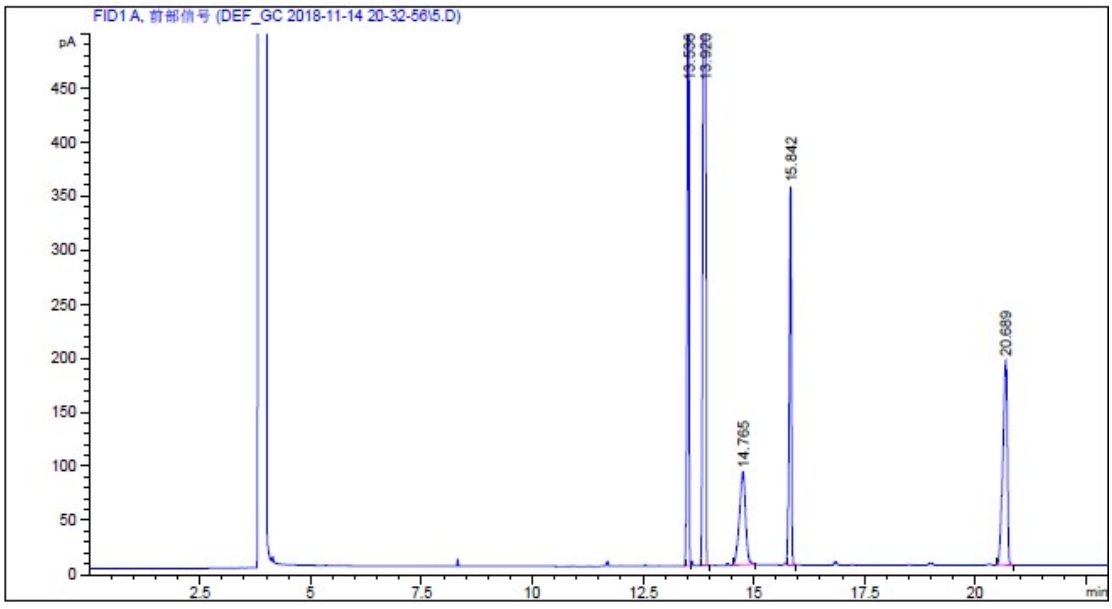


Figure S6. 12h in DCL2

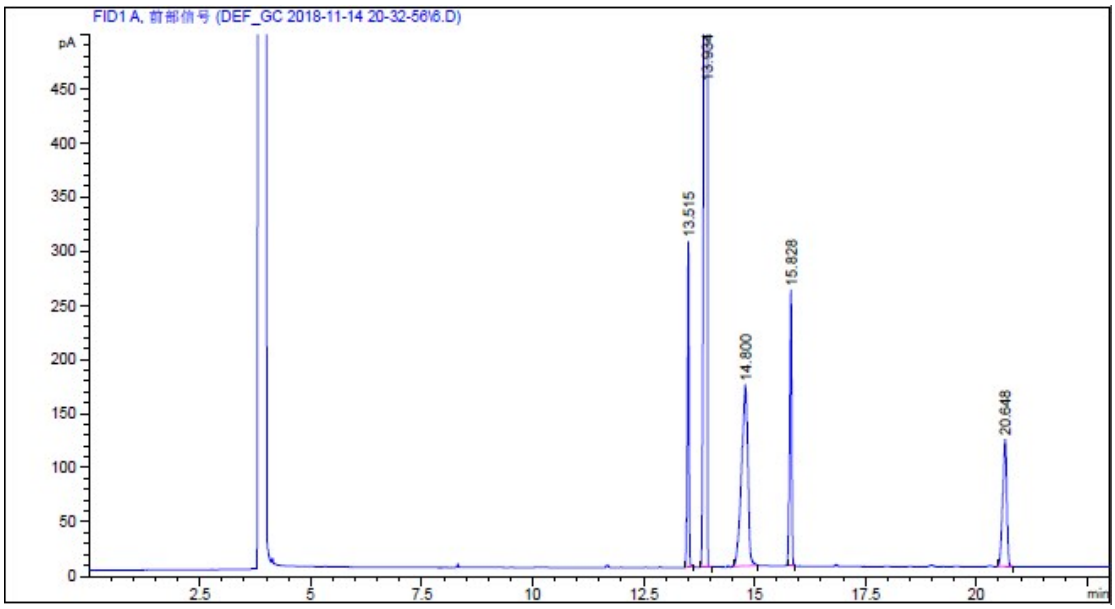


Figure S7. 18h in DCL2

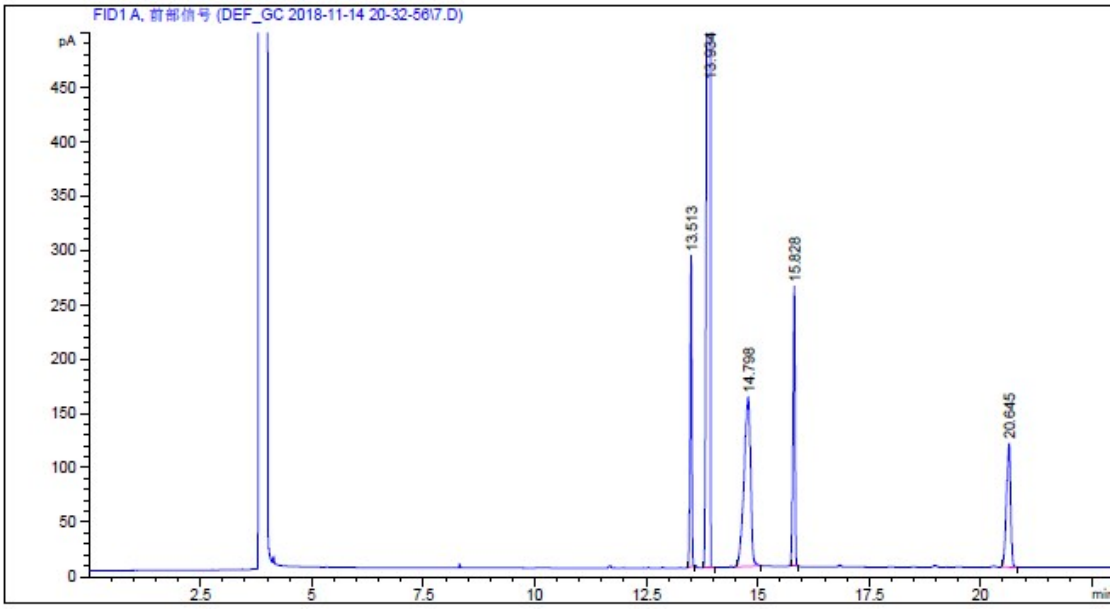
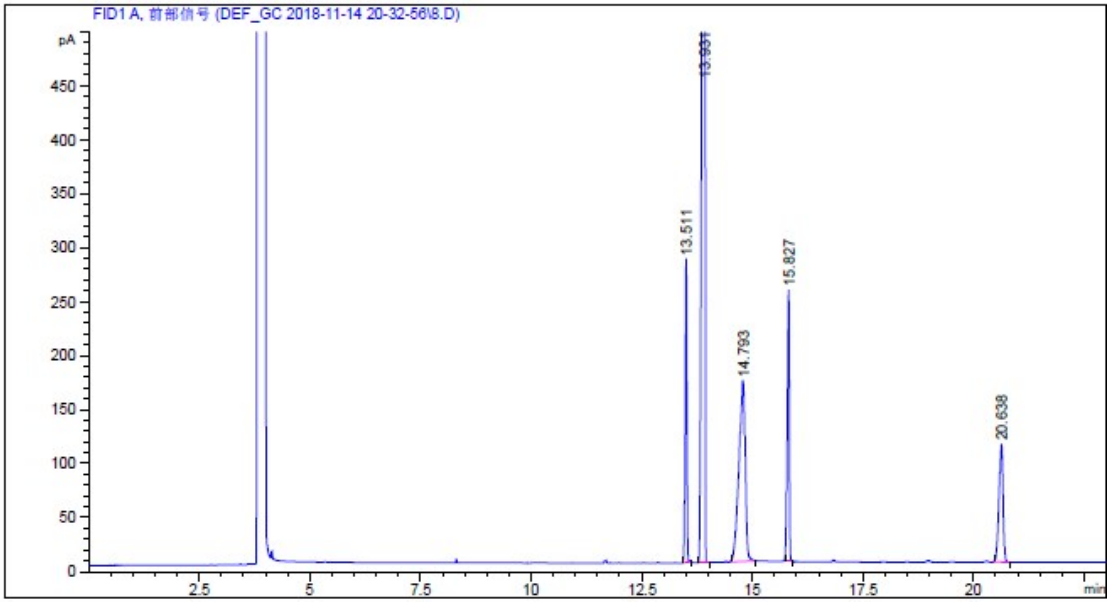
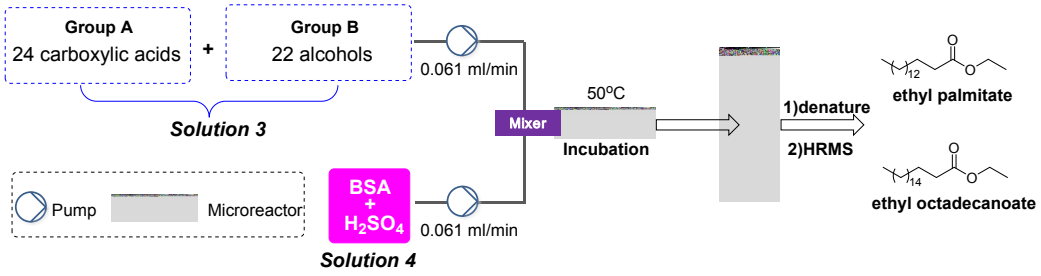


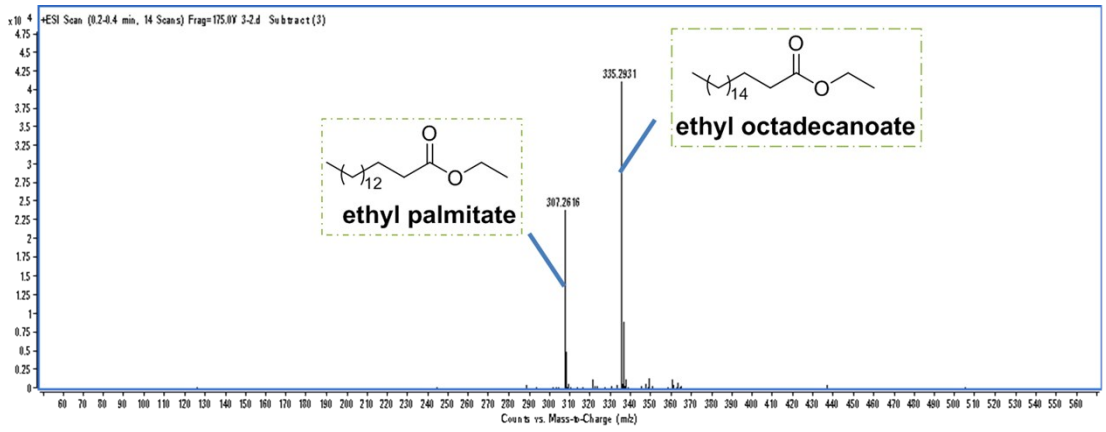
Figure S8. 24h in DCL2



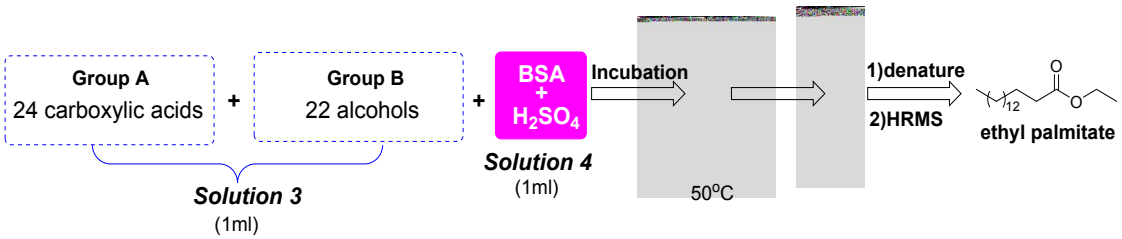
HRMS of solution from DCL 3



Reaction conditions: solution 3: 10 mM of carboxylic acids and 15 mM of alcohols in hexane, flow rate 0.061 ml/min; solution 4: 1.5 mM of sulfuric acid and 10 mM of BSA in distilled water,flow rate 0.061 ml/min. Microreactor:T = 50°C, volume = 7.32 ml.



HRMS of solution from DCL 4



Reaction conditions: solution 3: 10 mM of carboxylic acids and 15 mM of alcohols in hexane, 1 ml; solution 4: 1.5 mM of sulfuric acid and 10 mM of BSA in distilled water, 1 ml. T = 50°C, 12h.