Supplementary Information

Simple synthetic route to an enzyme-inspired transesterification catalyst

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Contents

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Experimental

All commercially obtained solvents and reagents were used without further purification. L-serine(99%), sodium hydroxide(97%), anhydrous methanol(99.8%), 1H-imidazole-2-carbaldehyde(97%), Sodium borohydride(99%), glacial acetic acid(99%), n-octan-1-ol(99%), dichloromethane(99.8%), pyridinium dichromate(98%), diethyl ether(99%), hexane, ethyl acetate(99.5%), Sodium triacetoxyborohydride(97%), acetonitrile(99.9%), L-histidine(99%), hydrochloric acid 37% and 1-Octanal(99%) were all purchased from the Sigma Aldrich. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F254 glass plates and flash chromatography (FC) was performed on Merck silica gel 60 (70 - 230 mesh). Visualization was achieved using short-wave UV light and/or $KMnO_4$ staining solution, followed by mild heating. Surfactants were purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC) on a Biotage SP-1 HPFC Flash Purification System using a reverse-phase Biotage SNAP Cartridge (KP-C18-HS, 60 g).

^1H, ^{13}C and ^{19}F solution-state NMR were recorded on a Varian Unity Inova 500 (500 MHz for ^1H and 125 MHz for ^{13}C) or a Varian Unity Inova AS600 (600 MHz for ^1H and 150 MHz for ^{13}C) or a Varian INOVA 400 (400 MHz for ^1H and 376 MHz for ^{19}F) spectrometers. Chemical shifts δ are reported relative to the resonance signal of ^1H or ^{13}C cores of tetramethylsilane and in ppm. The ^1H spectra were calibrated by setting the solvent peaks, caused by remaining traces of protons, to values known from the literature (δ $CHCl_3$ = 7.26 ppm, δCD_3OH = 4.87 ppm, δD_2O = 4.79 ppm). The ^{13}C spectra were calibrated by setting the Deuterium coupled solvent signal to values known from the literature (δ$CDCl_3$ = 77.16 ppm, δCD_3OD = 49.00 ppm). The coupling constants J are reported in Hz.

Single Crystal X-ray Diffraction of the ACT surfactant see *Nothling et al.*[1]

Modular synthesis
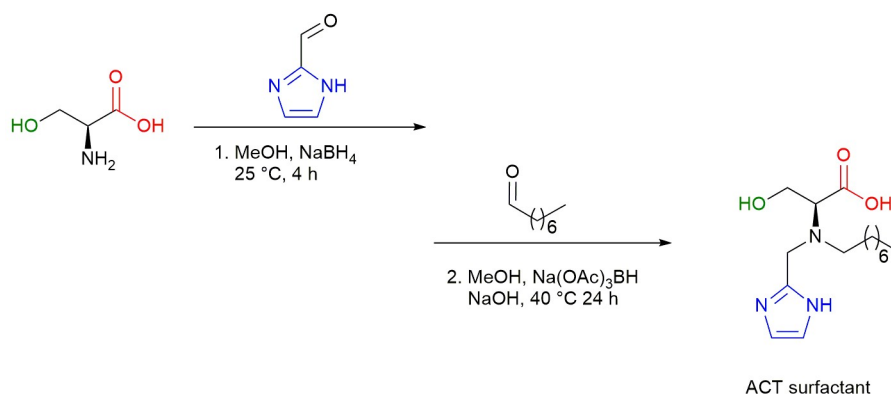
Scheme S1. Modular synthesis of ACT surfactant

Reductive amination of L-serine with 1H-imidazole-2-carbaldehyde (Preparation of ACT molecule)[1]: To a solution of L-serine (1.05 g, 10 mmol, 1.00 eq) and sodium hydroxide (420 mg, 10.5 mmol, 1.05 eq) in methanol ($c_{L\text{-serine}}$ ≈ 85 mM) was added 1H-imidazole-2-carbaldehyde (1.06 g, 11 mmol, 1.10 eq) while stirring. The resulting mixture was allowed to stir at

40°C for 30 minutes and thereafter cooled down to room temperature over 4 hours while stirring. Sodium borohydride (605.3 mg, 16 mmol, 1.60 eq) was gradually added and the reaction mixture was stirred at room temperature for 30 min. Glacial acetic acid was added dropwise until the mixture achieved approximately a pH of 4 – 5 and the resultant suspension stirred at room temperature for a another 10 minutes followed by filtration and washing with methanol yielded product as a precipitate (*L*-serine derivative were obtained as fine, white solid, yield >99%). ^{1}H-NMR (600 MHz, D_2O, 298 K): δ = 7.28 (*s*, 2H, aromatic); 4.35 (*d*, ^2J = 15.1 Hz, 1H, CHHN); 4.28 (*d*, ^2J = 15.1 Hz, 1H, CHHN); 3.96 – 3.84 (*m*, 2H, CH$_2$OH); 3.56 (*dd*, ^3J = 5.3 Hz, ^3J = 4.1 Hz, 1H, CHCO$_2^-$). MS (ESI) calculated for $C_7H_{11}N_3O_3H^+$ ([M+H]$^+$): 186.09. Found: 186.09.

Preparation of *n*-octan-1-al from *n*-octan-1-ol[1]: To a solution of *n*-octan-1-ol[50] (1.3 g, 10 mmol, 1.0 eq.) in dichloromethane ($c_{alcohol} \approx 65$ mM) was added pyridinium dichromate (4.5 g, 12 mmol, 1.2 eq.) portionwise and the mixture was stirred at room temperature for 8 hours. The obtained suspension was filtered using filter paper and a short silica pad followed by multiple washing with diethyl ether. The solvents were removed under reduced pressure and the crude product was purified by flash chromatography (hexanes/ethyl acetate = 19:1) to yield a waxy colorless solid (819 mg, 64%). ^{1}H NMR (400 MHz, CDCl$_3$, 298 K): δ = 9.74 (t, *J* = 1.9 Hz, 1H, CHO), 2.40 (td, *J* = 7.4, 1.9 Hz, 2H, CH$_2$CHO), 1.60 (m, CH$_2$, 2H), 1.35 to 1.19 (m, CH$_2$, 8H), 0.86 (t, *J* = 6.8 Hz, 3H).

Reductive amination of ACT molecule with n-Octan-1-al (Preparation of ACT surfactant): The synthesis of ACT surfactant has been published.[1] To a solution of ACT molecule (1.00 eq) and sodium hydroxide (1.05 eq.) in methanol ($c_{precursor} \approx 100$ mM) was added *n*-Octan-1-al (1.20 eq.) and sodium triacetoxyborohydride (1.40 eq.) and the resulting mixture was left for stirring at 40 °C for 2 hours. Every 2 hours, another two batch of *n*-Octan-1-al (2 × 1.20 eq.) and Sodium triacetoxyborohydride (2 × 1.40 eq.) were added to the solution, and after 4 hours, the mixture was allowed to stir at 40 °C for another 20 hours. The reaction was quenched with glacial acetic acid and the solvents were removed under reduced pressure followed by washing with the ethyl acetate and the resulting crude product was purified by RP-HPLC with an H$_2$O/acetonitrile gradient (19:1 → 1:19) to yield a pale-yellow solid (60%). ^{1}H-NMR (800 MHz, D_2O, 298 K): δ 7.22 (*s*, 2H, aromatic); 4.17 – 4.02 (*m*, 2H, ImCH$_2$N), 3.98 – 3.88 (*m*, 3H, CH$_2$OH), 3.56 (*dd*, ^3J = 5.9, ^3J = 2.0 Hz, 1H, CHCO$_2$H), 2.78 – 2.72 (*m*, 2H, NCH$_2$CH$_2$), 1.50 – 1.27 (*m*, 12H, CH$_2$), 0.98 (*t*, ^3J = 7.2 Hz, 3H, CH$_3$), (**Figure S1**). MS (ESI) calculated for $C_{15}H_{27}N_3O_3H^+$ ([M+H]$^+$): 298.21. Found: 298.24.

Preparation of *Imz-COOH* [(R)-3-(1H-imidazol-5-yl)-2-(octylamino)propanoic acid] control structure: *L*-histidine (620.8 mg, 4.0 mmol, 1.0 eq.) was dissolved in 20 ml of methanol at 50°C followed by addition of sodium hydroxide (168 mg, 4.2 mmol, 1.1 eq.) with stirring. 1-Octanal (620 mg, 4.8 mmol, 1.2 eq.) was then added, and the reaction was stirred overnight at room temperature. NaBH$_4$ (300 mg, 8.0 mmol, 2.0 eq.) was then added, and the reaction mixture was stirred for a further 1 hour at room temperature. Concentrated hydrochloric acid (32%, ~1.0 ml) was added to quench the reaction mixture, the suspension was filtered, and the solvent was removed in vacuo. The crude product was washed with ethyl acetate and a

small amount of water and then purified via reverse-phase column chromatography (MeOH/H_2O 19:1→0:1) to yield target structure (639 mg, 2.4 mmol, 60%). ^{1}H NMR (400 MHz, MeOD): δ 8.69 (s, 1H, Im), 7.39 (s, 1H, Im), 3.89 (s, 1H, CHCOOH), 3.09 - 3.02 (m, 2H, CH_2NH), 1.73 – 1.68 (m, 2H, CH_2CH_2NH), 1.41 – 1.29 (m, 12H, CH_2), 0.92 – 0.89 (m, 3H, CH_3), (**Figure S2**); ^{13}C NMR (400 MHz, Methanol-d_4): δ= 168.9, 134.1, 127.6, 117.9, 53.4, 31.4, 28.7, 26.1, 25.8, 24.4, 22.2, 13.0, (**Figure S3**). MS (ESI) was calculated for $C_{14}H_{25}N_3O_2H^+$ ([M + H]$^+$): 268.20. Found: 268.21.

Preparation of *OH-COOH* [(S)-3-hydroxy-2-(octylamino)propanoic acid] control structure: *L*-serine (210 mg, 2.0 mmol, 1.0 eq.) was dissolved in 15 ml of methanol at 50°C followed by addition of sodium hydroxide (88 mg, 1.4 mmol, 1.1 eq.) while stirring. 1-Octanal (310 mg, 2.4 mmol, 1.2 eq.) was then added, and the reaction was stirred overnight at room temperature. $NaBH_4$ (150 mg, 4.0 mmol, 2.0 eq.) was then added, and the reaction mixture was stirred for a further 1 hour at room temperature. Concentrated hydrochloric acid (32%, ~0.7 ml) was added to quench the reaction mixture, the suspension was filtered, and solvent was removed in vacuo. The crude product was washed with ethyl acetate and then purified via reverse-phase column chromatography (MeOH/H_2O 19:1→0:1) to yield target structure (239 mg, 1.1 mmol, 54%). ^{1}H NMR (400 MHz; MeOD, 298 K): δ = 4.03 (dd, ^2J = 12.4, ^3J = 3.6 Hz, 2H, CH_2OH), 3.98 (dd, ^2J = 12.1, ^3J = 4.8 Hz, 2H, CH_2OH), 3.84 (dd, ^3J = 3 Hz, 1H, $CHCH_2OH$), 3.08 – 3.04 (m, 2H, CH_2NH), 1.73 (p, ^3J = 7.6 Hz, 2H, $CH_2CH_2CH_2$), 1.41 - 1.30 (m, 10H, CH_2), 0.92 - 0.88 (m, 3H, CH_3), (**Figure S4**); ^{13}C NMR (400 MHz, Methanol-d_4): δ= 170.4, 63.6, 60.1, 47.7, 32.9, 30.2, 27.6, 27.1, 23.7, 14.4, (**Figure S5**). MS (ESI) was calculated for $C_{11}H_{23}NO_3H^+$ ([M + H]$^+$): 218.17. Found: 218.19.

Transesterification of VTFA and methanol in the presence of ACT surfactant catalyst: Pre-dried methanol and *d*-chloroform from Sigma were again dried by passing through a column of activated basic alumina, prior to addition of reactants. In 5 mL of dry methanol, ACT surfactant (9.2 mg) was dissolved to yield a catalyst solution (6.18 x 10^{-3} M). VTFA (Initial VTFA conc. = 0.025, 0.05, 0.125, 0.25, 0.5 M) was dissolved in dry *d*-chloroform (688.7 μL) and 64.7 μL of catalyst stock solution (catalysed runs) or dry methanol (blank runs) was added. The obtained mixture was immediately observed via 400 MHz ^{1}H NMR on a Varian INOVA spectrometer. The first spectrum was obtained at ~ 3 minutes, and subsequent spectra were taken every 4 minutes for up to 12 hours. Reaction progress (% conversion) was computed by comparing reduction of the vinyl proton peaks (δ = 7.19, 5.16, 4.87 ppm) with the appearance of a methyl proton peak associated with methyl trifluoroacetate (δ = 3.91 ppm) and aldehyde proton peak associated with acetaldehyde (δ = 9.71 ppm). Parallel reaction mixtures were subjected to 400 MHz ^{19}F NMR confirming the transesterification product methyl trifluoroacetate as major product for time periods up to 1.5 hours after mixing. The concentration of hydrolysis product trifluoroacetic acid was found to be rising over a longer length of time, possibly due to hydrolysis of the transesterification product (up to 10% mol after 12 hours).

Determination of Kinetic Parameters: Michaelis-Menten equation was used to measure k_{cat} and K_m for the catalytic activity of the catalysts. Dry d-chloroform and dry methanol were used in each kinetic experiment. A dried NMR tube was added with different initial VTFA conc. = 0.025, 0.05, 0.125, 0.25, 0.5 M and 64.7 μL of catalyst stock solution (0.5 mM catalyst loading) for catalysed runs or dry methanol for the background reaction in dry CDCl$_3$ at 23 °C. The progress of the reaction was monitored by ^{1}H NMR. Integration of the starting material and product signals was used to calculate the concentration of products. After 3 minutes of mixing time, the reaction velocity was measured as the tangent to the product concentration vs. time curve. All curve and Michaelis-Menten equation were fitted using the GraphPad prism software.

Preparation of ACT surfactant solutions for DLS/^{1}H NMR: Stock solutions of ACT surfactants/methanol were prepared by mass and volumetric dilution. Individual solutions were prepared from the stock solutions (80.9 μL), incorporating methanol (0, 20, 40, 60, 80, 100, 120, 140, 160 μL) and CHCl$_3$ (or CDCl$_3$ for ^{1}H NMR) with calibrated microsyringes. Therefore, each solution contains a different amount of methanol. The solutions were agitated in sonication bath to obtain clear transparent solutions with a single phase.

Dynamic Light Scattering Measurements: Particle sizing was measured by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments) at 25 °C, using a forward scatter angle of 13° and Non-Invasive Back Scatter (NIBS) angle of 173°. The quartz cuvettes was cleaned with ethanol, then with doubly distilled water and dried with acetone; this step was critical for producing consistent and reproducible data.[2] The cuvettes was then washed twice with pure chloroform and then with the solution to be analysed before each sample was inserted into the cuvette. Before data acquisition, samples were equilibrated in the DLS instrument for 10 min at 25 °C, and where each replicate was measured 10 times to determine the average apparent diameter (d_{app}). DLS experiments were carried out at [ACT surfactant] = 0.5 mM. For each experiment, 1ml cuvettes were filled with increasing volume of methanol = 0, 20 and 60 μL, catalyst stock volume of 80.9 μL and adding sufficient chloroform to make up the final volume. The DLS instrument was not able to measure at 40 μL and higher volumes of methanol (> 60 μL). In all DLS spectra, peak greater than 1 μm corresponds to dust while the peak around 100 nm is due to unknown contamination.

NMR
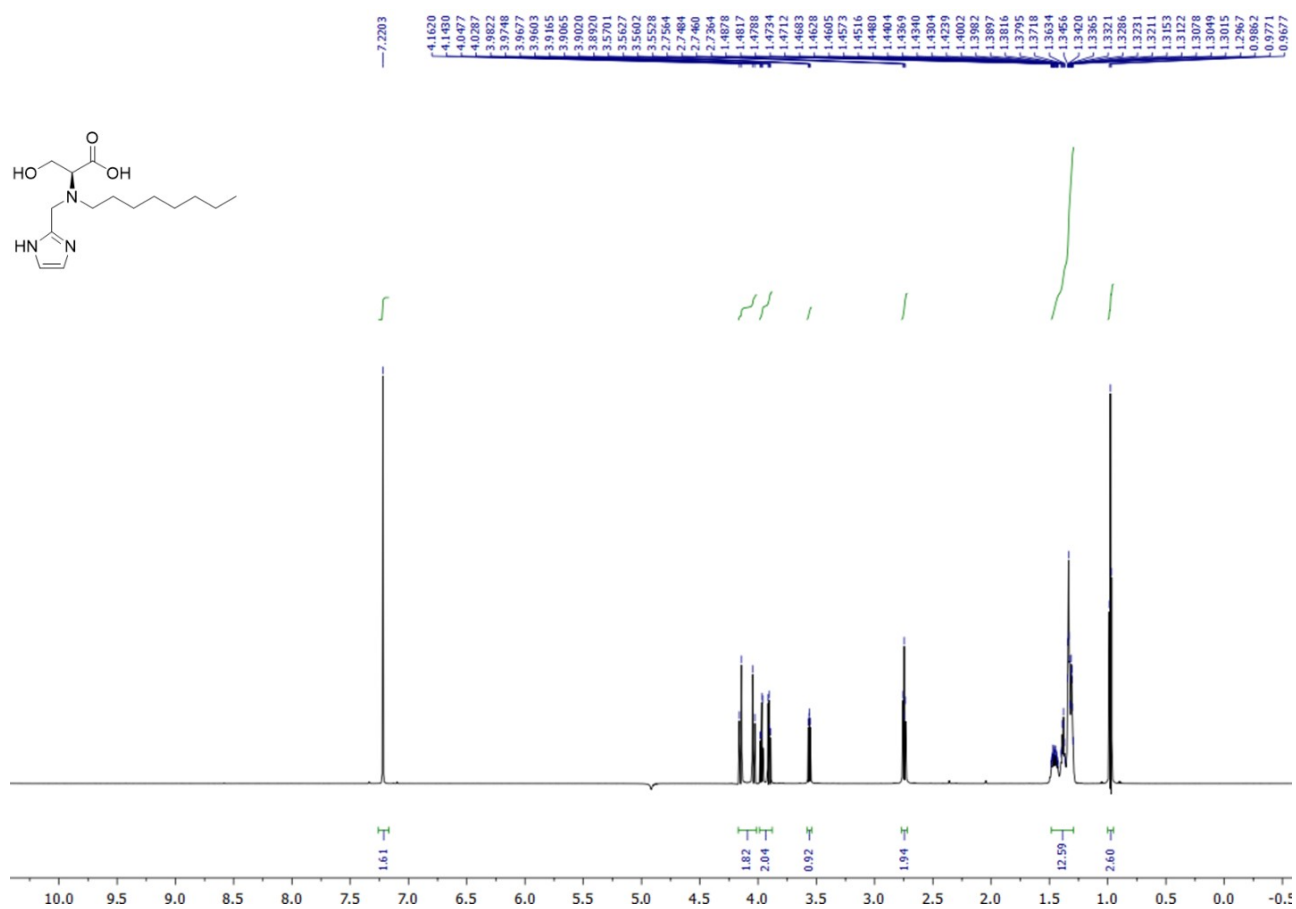
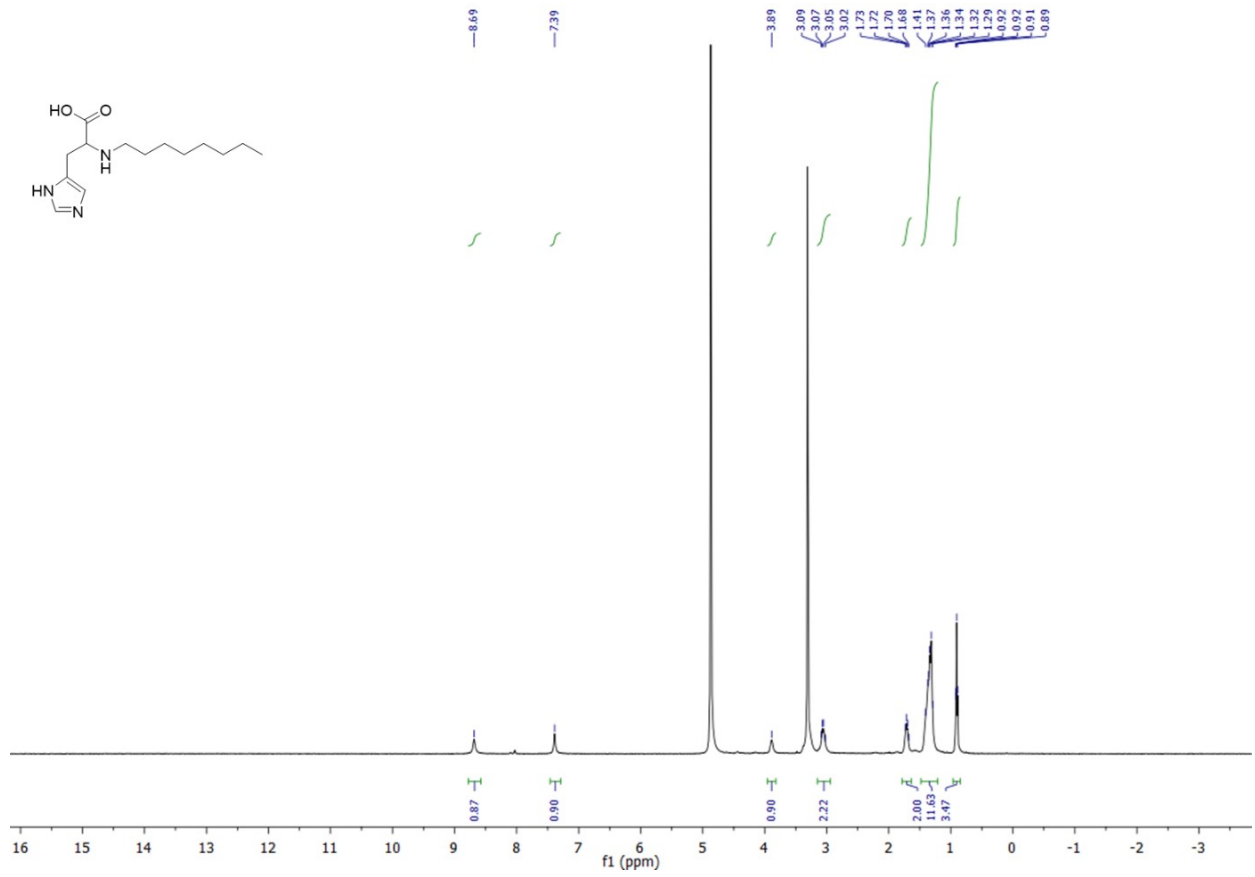**Figure S1.** ^{1}H NMR of ACT surfactant using D_2O suppression.
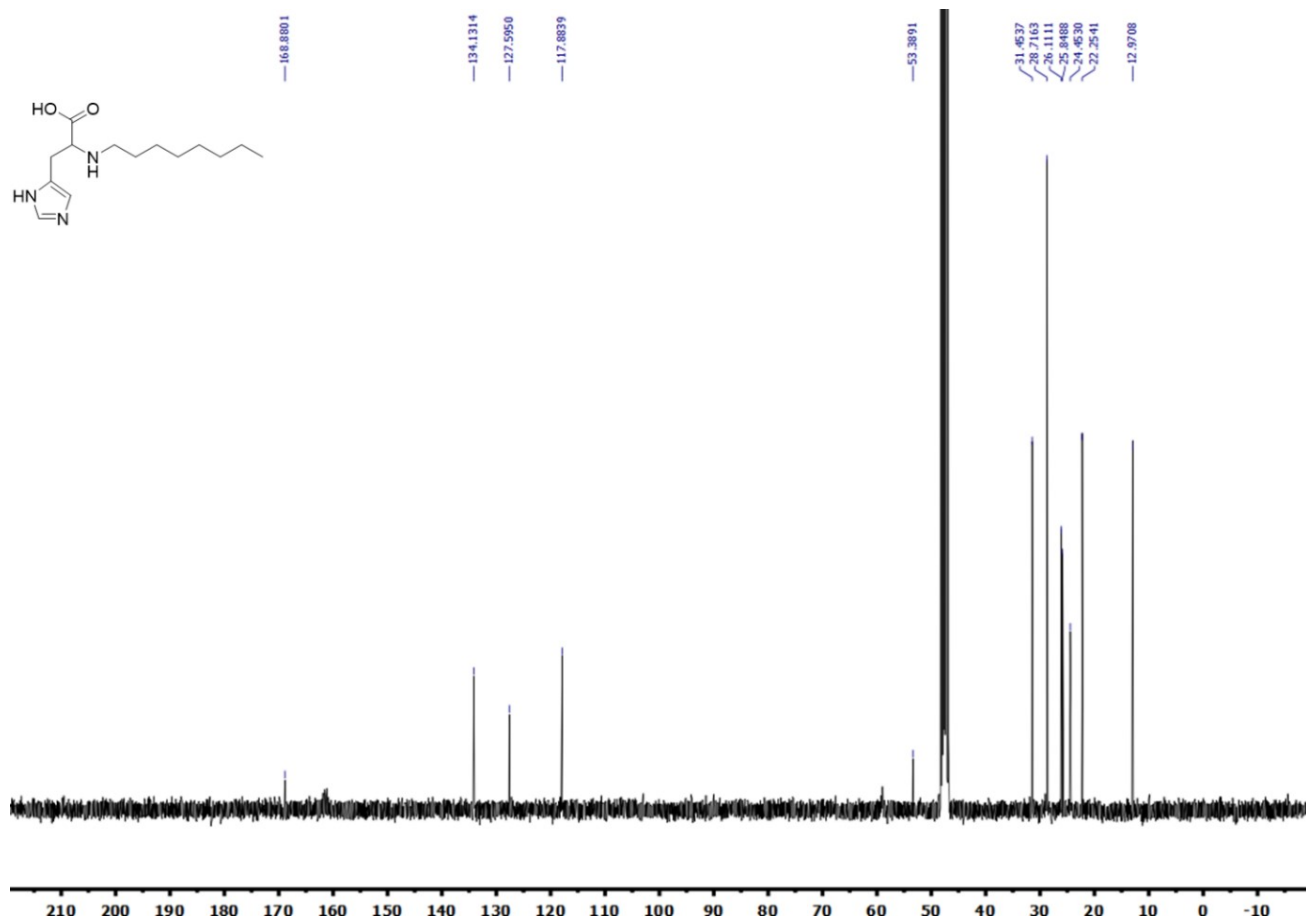
Figure S2. ^{1}H NMR of *Imz-COOH* in methanol-d_4.

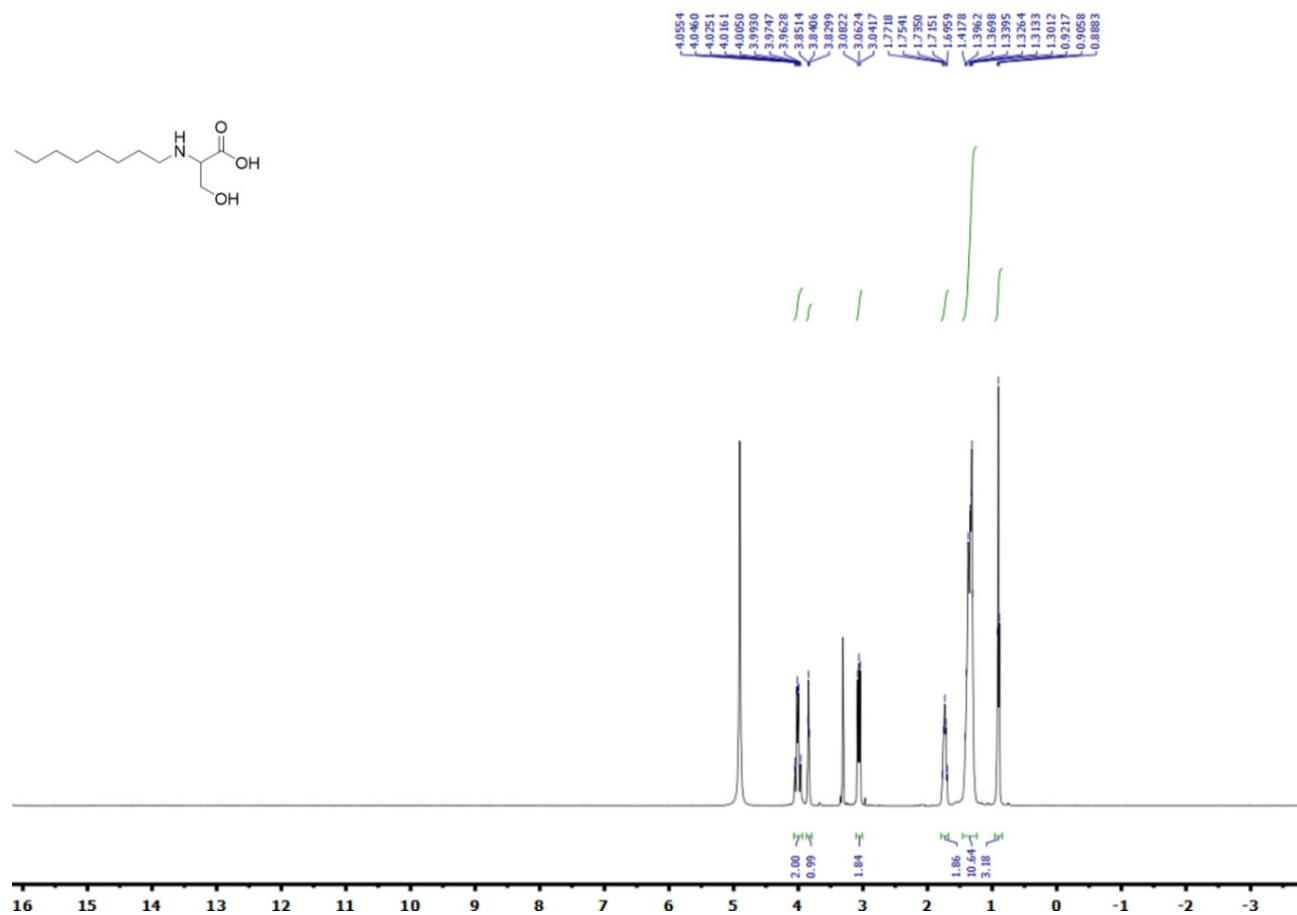**Figure S3.** ^{13}C NMR of *Imz-COOH* in methanol-d_4.
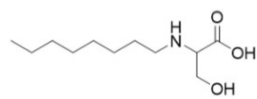
Figure S4. ^{1}H NMR of *OH-COOH* in methanol-d_4.
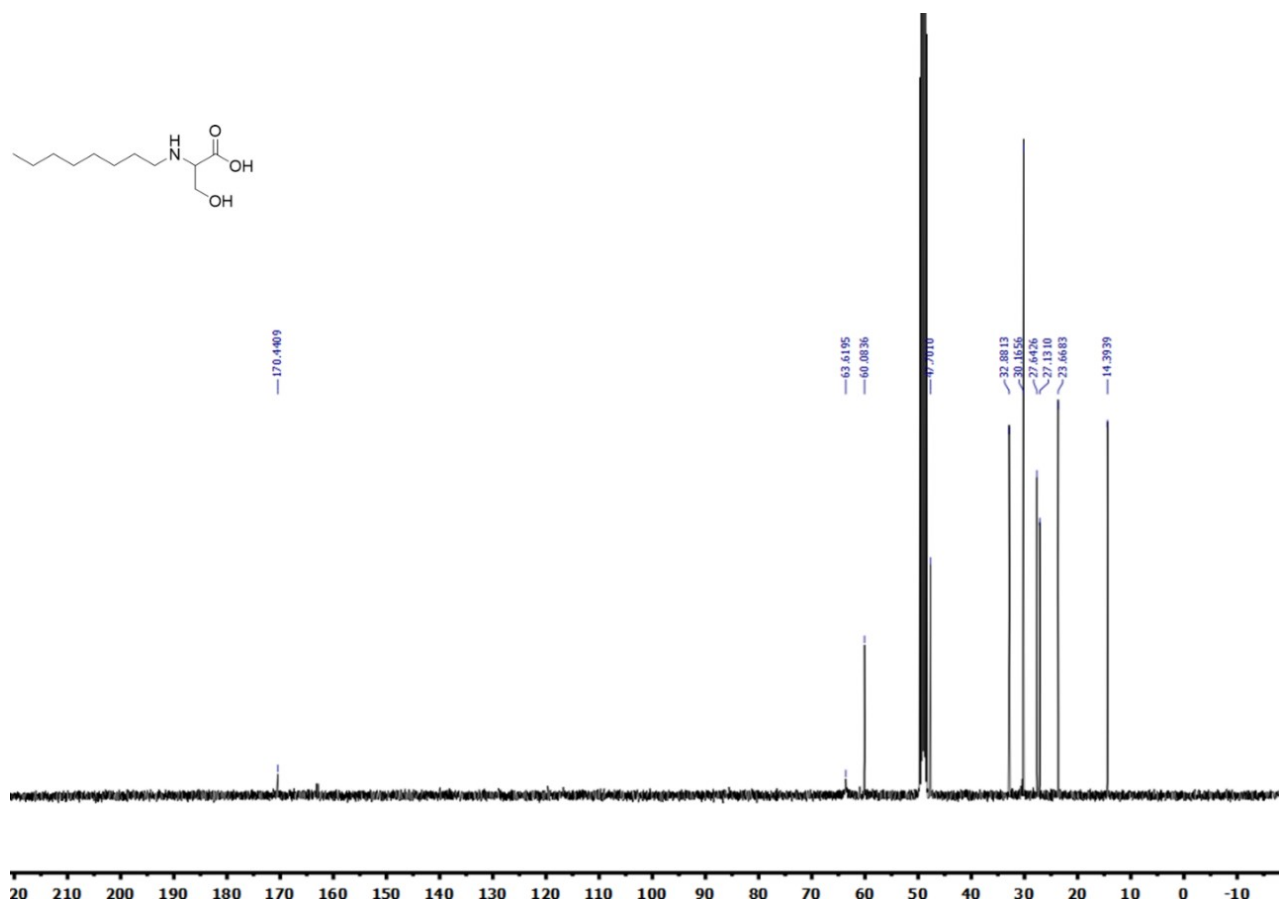
Figure S5: ^{13}C NMR of *OH-COOH* in methanol-d_4.

¹⁹F NMR

(a)

(b)

-75.00 -75.05 -75.10 -75.15 -75.20 -75.25 -75.30 -75.35 -75.40 -75.45 -75.50 -75.55 -75.60 -75.65 -75.70 -75.75 -75.80 -75.85 -75.90 -75.95 -76.00 -76.05 -76.1(
(ppm)
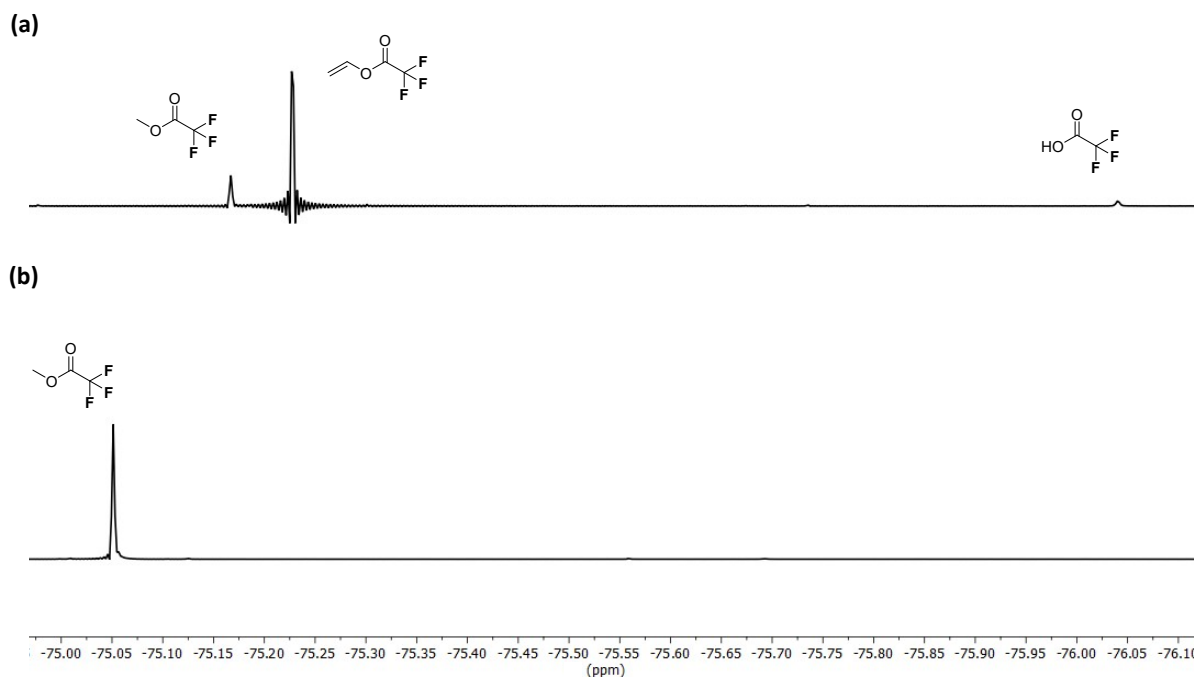
Figure S6: ¹⁹F NMR of reaction mixture (1 mol% catalyst, 0.05 M VTFA, 2M MeOH, *d*-CDCl₃), (a) after 10 minutes of mixing, (b) after 1h minutes of mixing. The product peak shifted over a one-hour period. The appearance of a small peak due to the side hydrolysis reaction was observed (trifluoroacetic acid near -76.05 ppm).

Michaelis-Menten kinetic parameters

Catalyst	k_{cat} and k_{unc} (s^{-1})	K_M (M)	k_{cat}/k_{unc}	k_{cat}/K_M (s^{-1}M^{-1})
ACT surfactant	3.302	1.061	4.81 x 10^4	3.11
Imz-COOH	0.549	0.802	8.00 x 10^3	6.84 x 10^{-1}
OH-COOH	0.312	0.429	4.54 x 10^3	7.27 x 10^{-1}
Background	0.0000686			

Table S1. Michaelis-Menten kinetic parameters of the ACT catalysts and the first-order rate constant for background transesterification.

Molecular dynamics simulations of ACT surfactant

ACT surfactant in 10% MeOH/Chloroform solution

Method

All simulations were performed using GROMACS 2019.3[3] with the GROMOS 54a7 force field[4]. United atom parameters and co-ordinates for the solvents (chloroform (ATB ID 1307), methanol (ATB ID 15607)), VTFA (ATB ID 479693) and ACT surfactant (without charge, ATB ID 479692) were developed using the Automated Topology Builder (ATB) and are publicly available on the ATB website.[5] All simulations were performed in a 10 nm^3 box. Here, VTFA and ACT surfactant were added to a pre-equilibrated box of chloroform containing 10% methanol. All concentrations were determined relative to experimental ratios of components at 200 times the concentration used in experimental conditions to enable sampling. This is in accord with previous investigations of the behaviour of ACT surfactant in a micellar solution.[1] Simulations were performed using an NPT ensemble with periodic boundary conditions. The temperature of all systems was set to 300 K, which was controlled using a v-rescale thermostat ($\tau P = 0.1$ ps). Isotropic pressure coupling was implemented using the Berendsen barostat ($\tau P = 0.5$ ps and isothermal compressibility = 4.5×10^{-5} bar). Long-range electrostatics were calculated using the particle-mesh Ewald method (1nm cutoff). All systems were minimized using a steepest descent for 10,000 steps and equilibrated for 2 fs to stabilise system density. The LINCS algorithm was used to constrain covalent bond lengths**.** Production simulations were run for 500 ns with a 2 fs timestep. For each system, replicate systems were prepared in triplicate, where each replicate system contained a unique random distribution of molecules to remove bias. To initiate each system, random velocities were assigned. Using frames collected every 0.1 ns, data were analysed using inbuilt GROMACS 2019.3[3] analysis tools and the python package MDTraj[6]. Images of trajectory frames were produced using VMD.[7] Coordinates of the first and last frames of each trajectory may be found on github at https://github.com/OMaraLab/ACT-Chlor-MeOH/
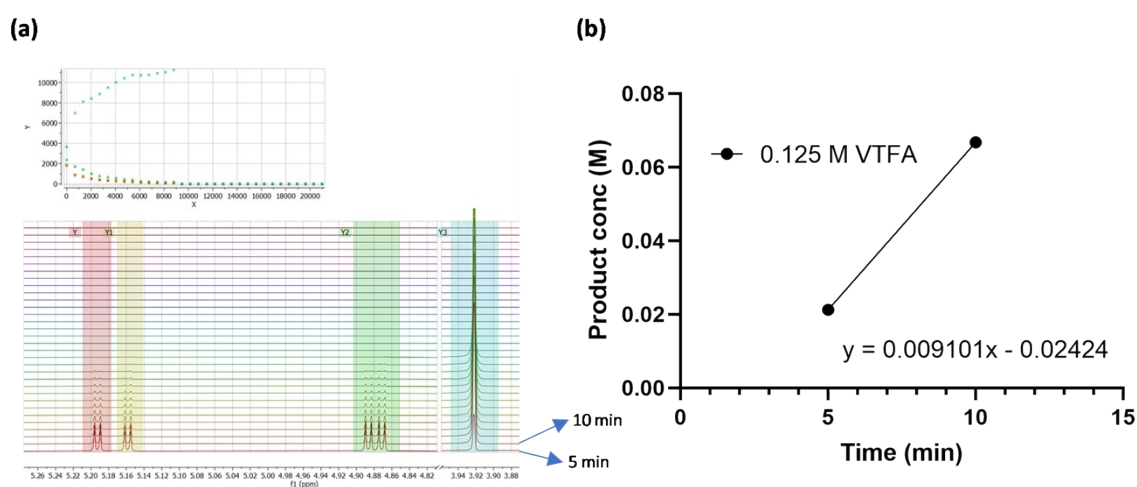
Kinetic profile of ACT surfactant
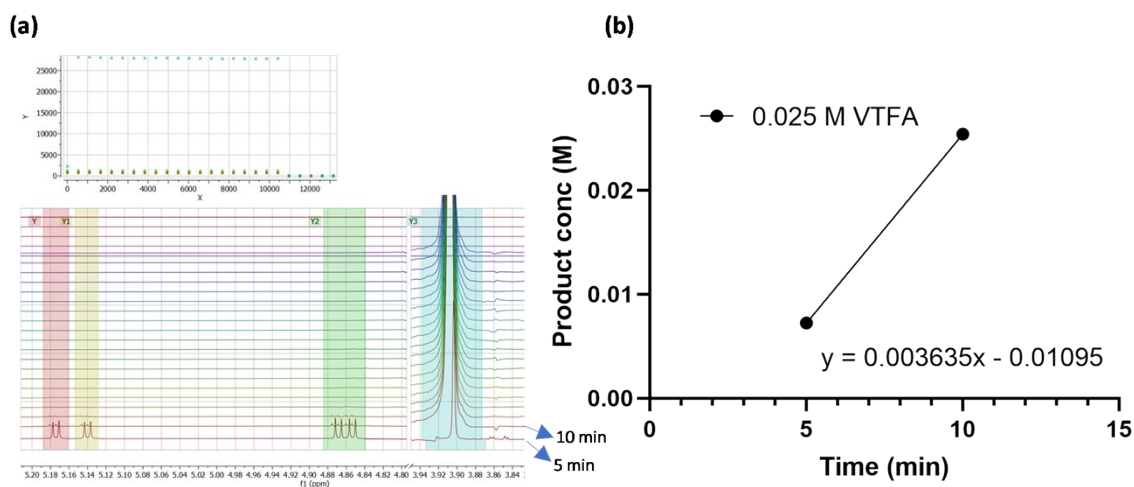
Figure S7: Kinetic profile of ACT surfactant catalysed transesterification of 0.025 M VTFA with methanol, (a) ^{1}H NMR spectra of the reaction mixture of ACT surfactant (1 mol%), VTFA (0.025 M) and methanol (2 M) in *d*-chloroform with time. The first spectrum was acquired at 5 minutes, while subsequent spectra were acquired at interval of 5 minutes. Different coloured regions show the integral area (red, yellow and green corresponds to vinyl proton peaks while blue corresponds to methyl proton peaks of methyl trifluoroacetate), (b) Linear regression for initial velocity measurements taken at 5 and 10 minutes.
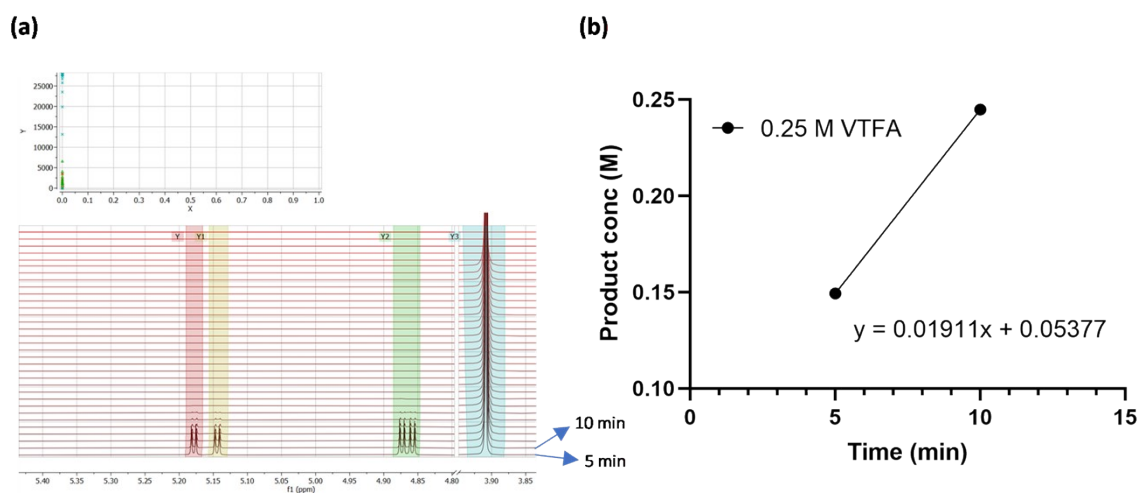
(a)

(b)
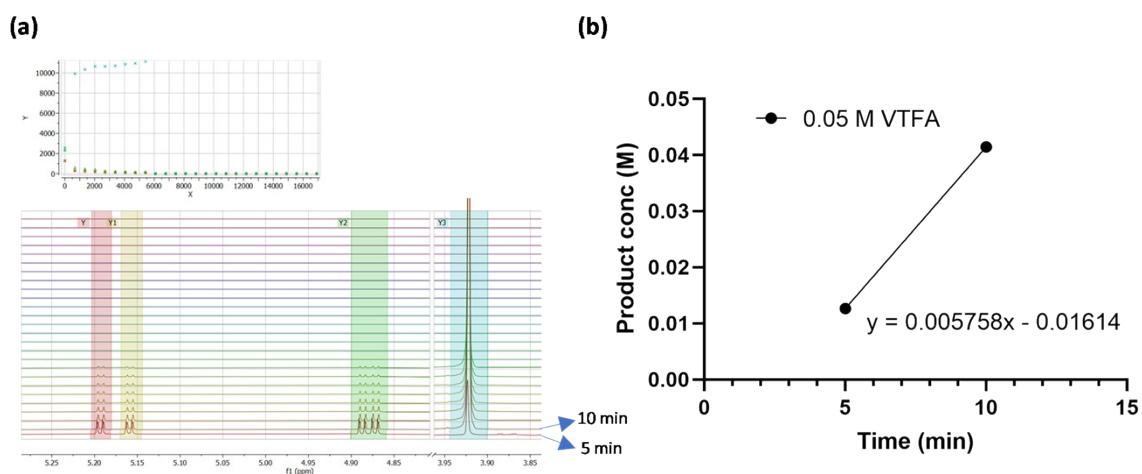
Figure S8: Kinetic profile of ACT surfactant catalysed transesterification of 0.05 M VTFA with methanol, (a) ^{1}H NMR spectra of the reaction mixture of ACT surfactant (1 mol%), VTFA (0.05 M) and methanol (2 M) in *d*-chloroform with time. The first spectrum was acquired at 5 minutes, while subsequent spectra were acquired at interval of 5 minutes. Different coloured regions show the integral area (red, yellow and green corresponds to vinyl proton peaks while blue corresponds to methyl proton peaks of methyl trifluoroacetate), (b) Linear regression for initial velocity measurements taken at 5 and 10 minutes.
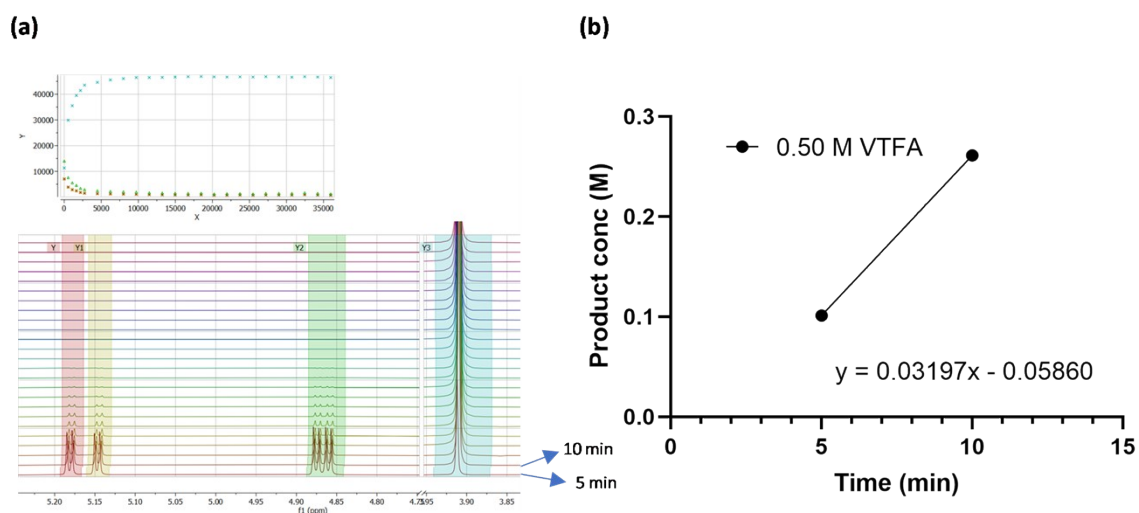
(a)

(b)

Figure S9: Kinetic profile of ACT surfactant catalysed transesterification of 0.125 M VTFA with methanol, (a) ^{1}H NMR spectra of the reaction mixture of ACT surfactant (1 mol%), VTFA (0.125 M) and methanol (2 M) in *d*-chloroform with time. The first

spectrum was acquired at 5 minutes, while subsequent spectra were acquired at interval of 5 minutes. Different coloured

regions show the integral area (red, yellow and green corresponds to vinyl proton peaks while blue corresponds to methyl

proton peaks of methyl trifluoroacetate), (b) Linear regression for initial velocity measurements taken at 5 and 10 minutes.

(a)   **(b)**

Figure S10: Kinetic profile of ACT surfactant catalysed transesterification of 0.25 M VTFA with methanol, (a) ^{1}H NMR spectra

of the reaction mixture of ACT surfactant (1 mol%), VTFA (0.25 M) and methanol (2 M) in *d*-chloroform with time. The first

spectrum was acquired at 5 minutes, while subsequent spectra were acquired at interval of 5 minutes. Different coloured

regions show the integral area (red, yellow and green corresponds to vinyl proton peaks while blue corresponds to methyl

proton peaks of methyl trifluoroacetate), (b) Linear regression for initial velocity measurements taken at 5 and 10 minutes.

(a)   **(b)**

Figure S11: Kinetic profile of ACT surfactant catalysed transesterification of 0.5 M VTFA with methanol, (a) ^{1}H NMR spectra

of the reaction mixture of ACT surfactant (1 mol%), VTFA (0.5 M) and methanol (2 M) in *d*-chloroform with time. The first

spectrum was acquired at 5 minutes, while subsequent spectra were acquired at interval of 5 minutes. Different coloured

regions show the integral area (red, yellow and green corresponds to vinyl proton peaks while blue corresponds to methyl proton peaks of methyl trifluoroacetate), (b) Linear regression for initial velocity measurements taken at 5 and 10 minutes.
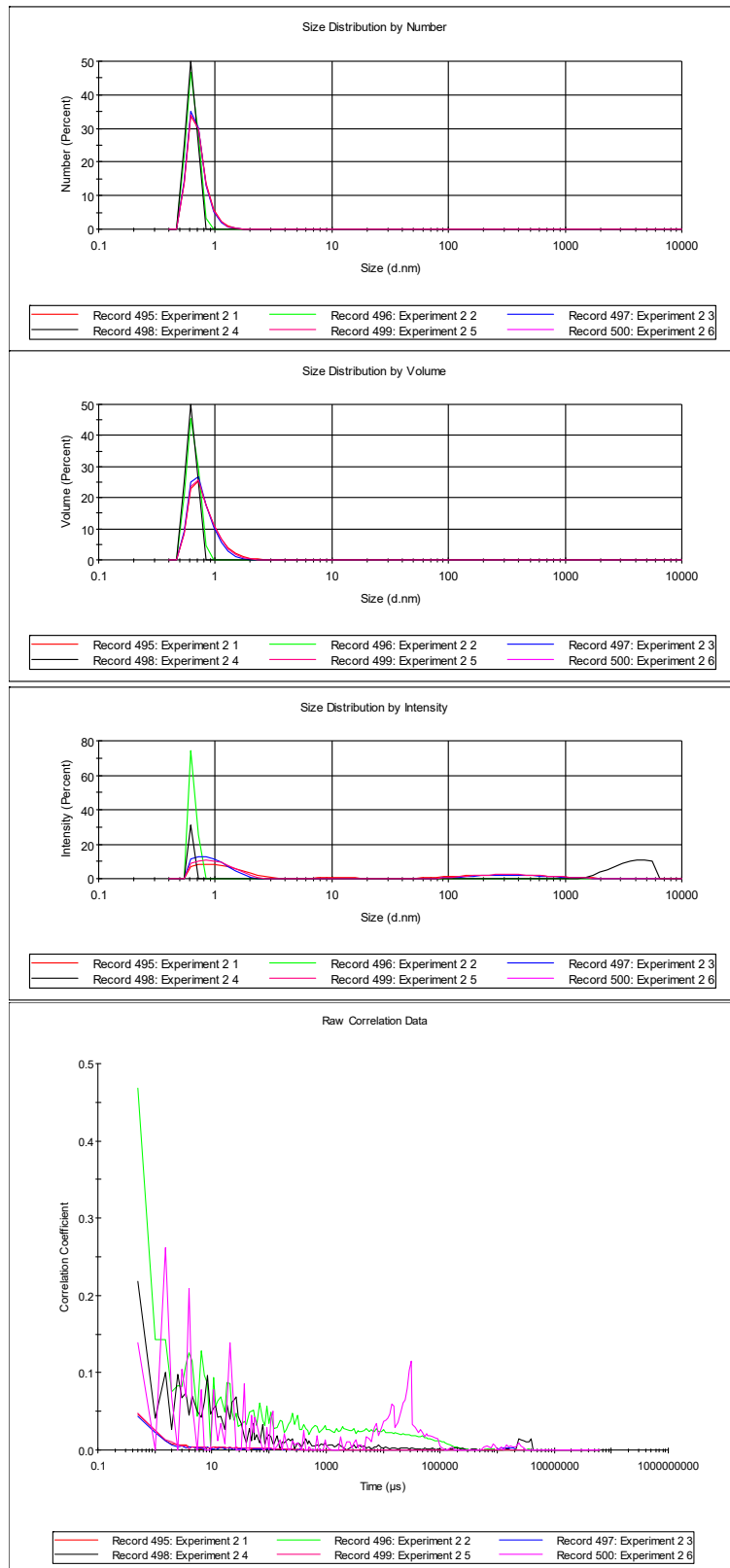
DLS and ^{1}H NMR

Experiment 1
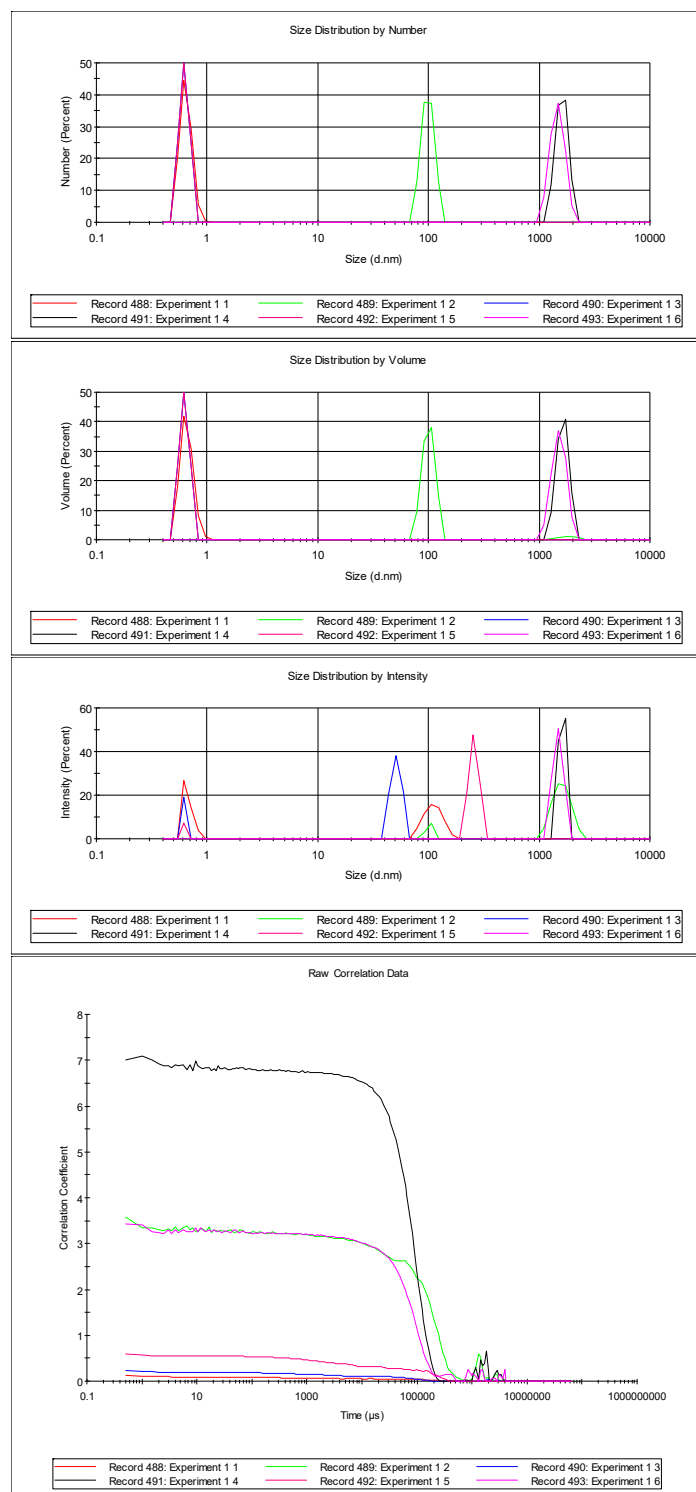
Figure S12: DLS spectra and correlation coefficient of ACT surfactants at 25 °C containing 0 μL methanol, 80.9 μL ACT surfactant stock solution and adding sufficient chloroform to make up the final volume of 1ml.
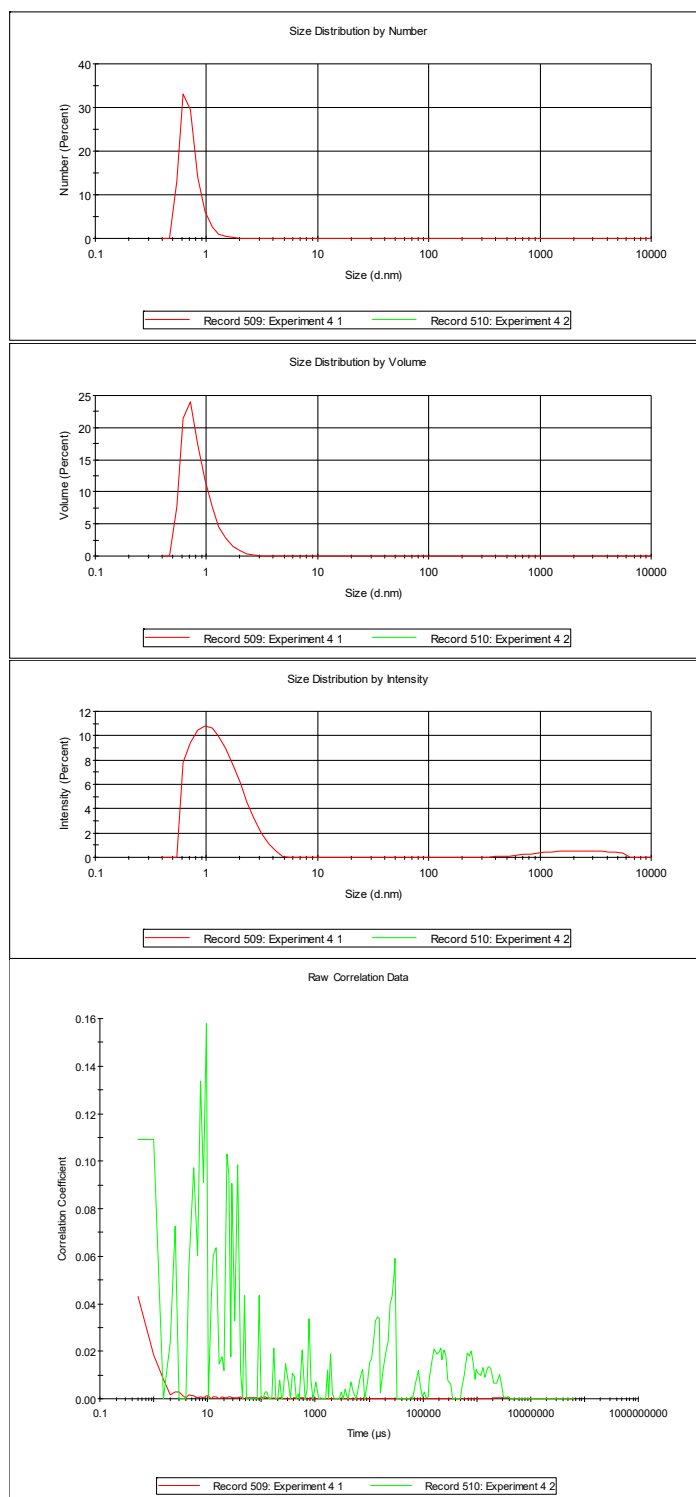
Experiment 2

Figure S13: DLS spectra and correlation coefficient of ACT surfactants at 25 °C containing 20 µL methanol, 80.9 µL ACT surfactant stock solution and adding sufficient chloroform to make up the final volume of 1ml.
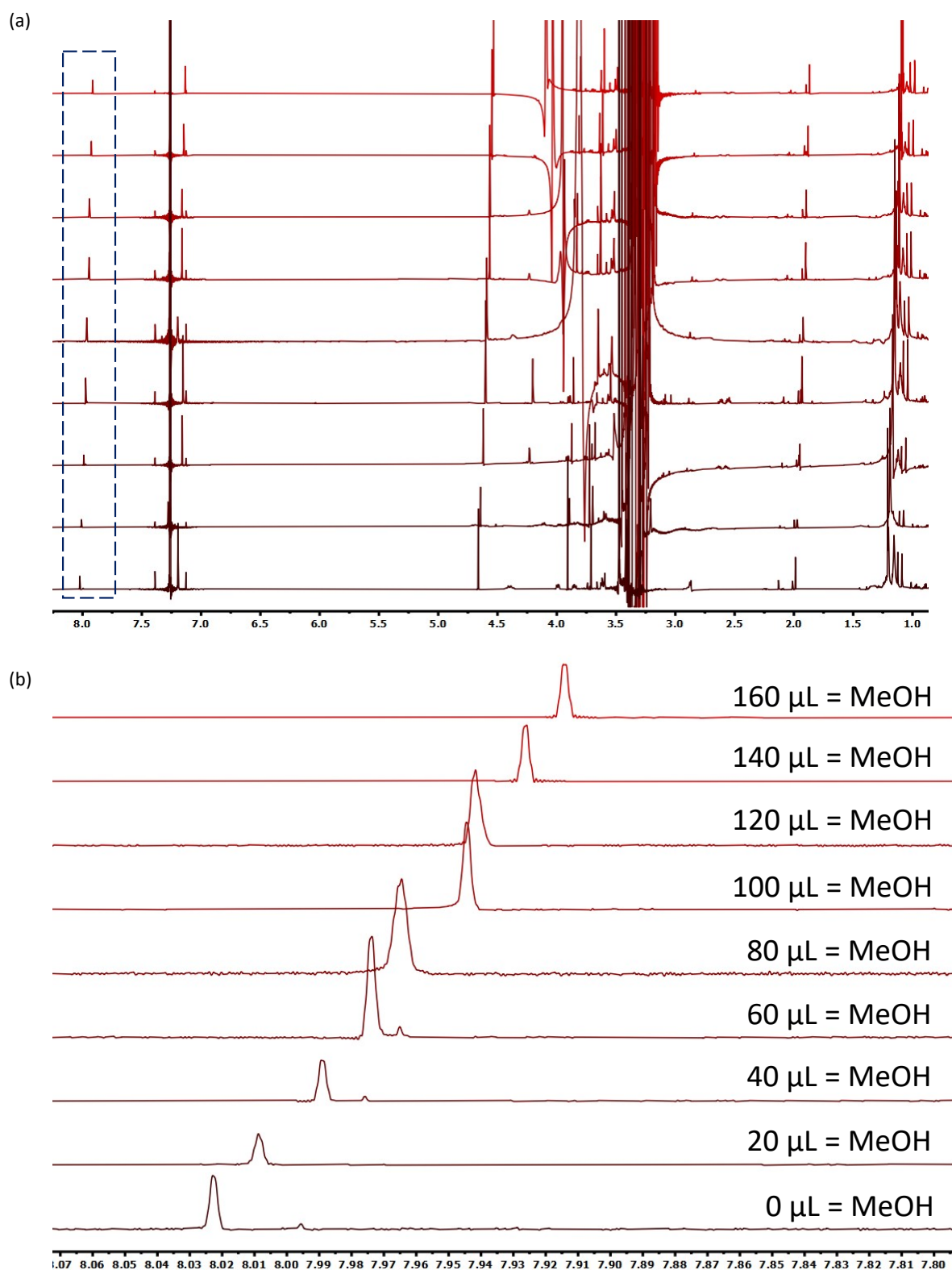
Experiment 3

Figure S14: DLS spectra and correlation coefficient of ACT surfactants at 25 °C containing 60 µL methanol, 80.9 µL ACT surfactant stock solution and adding sufficient chloroform to make up the final volume of 1ml.

Figure S15: ^{1}H NMR spectra of ACT surfactants clusters in methanol/chloroform obtained by varying the methanol content at 25 °C, [ACT surfactant] = 0.5 mM, (a) the dotted blue line highlights the ^{1}H NMR signals from the imidazole proton, (b) ^{1}H NMR spectra in the region 7.80-8.06 ppm showing shifting of H in imidazole peak with increasing methanol content.

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