Supporting Information

Linear triglycerol-based fluorosurfactants show high potential for droplet-microfluidics-based biochemical assays

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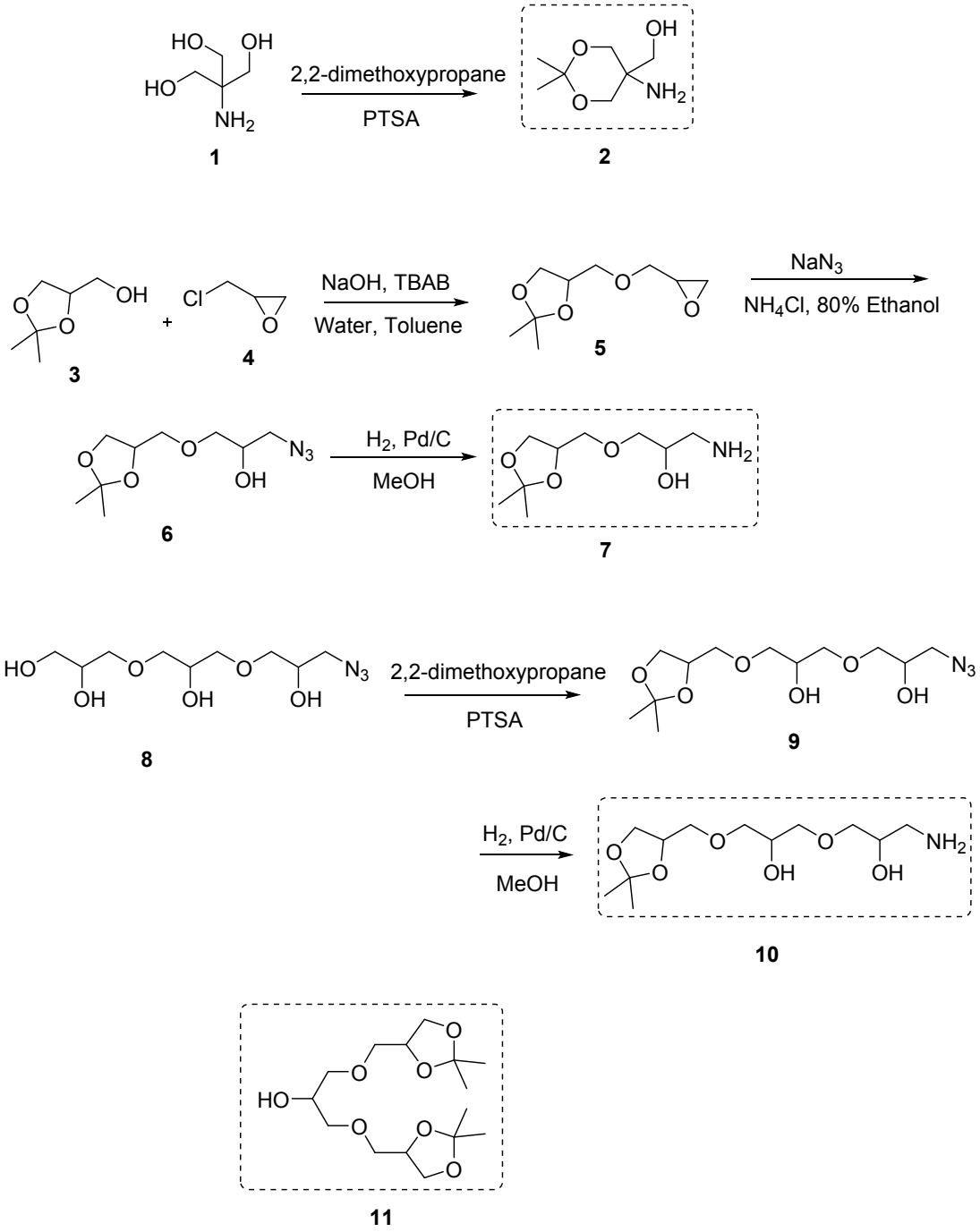
Materials. Krytox 157-FSL (MW = ~2200 g mol^{-1}) with monocarboxylic acid at the terminus was purchased from LUB SERVICE GmbH (Germany). Both HFE 7100- and HFE 7500-fluorinated oils were obtained from 3M. Dibenzylcyclooctyne (DBCO)-Sulfo-Cy3 and dibenzylcyclooctyne (DBCO)-PEG4-Biotin were bought from Jena Bioscience (Germany). All other chemicals we purchased were reagent grade. These chemicals are from Acros Organics (Belgium) and/or from Merck (Germany) unless otherwise stated. They were used as received with no further purification. Prior to conducting moisture sensitive reactions, the glassware was flame-dried, and reactions were performed under inert conditions. The fluorosurfactant solution was made at 2% by weight in the bioinert HFE-7500 oil.

Instrumentation. An ECX400 spectrometer (Jeol Ltd., Japan), or an AMX 500 spectrometer (Bruker, Switzerland) was used to record the NMR spectra. δ values in ppm were used to present the proton NMR chemical shift. To calibrate the recorded peak, the deuterated solvent peak was used. To record IR spectra, Nicolet AVATAR 320 FT-IR 5 SXC (Thermo Fisher Scientific, USA) with a DTGS detector from 4000 to 650 cm^{-1} wavenumbers was employed. Fluorescence images were taken using Leica confocal microscope (TCS SP8, Germany). During water-in-oil emulsion droplet generation by microfluidic flow focusing, a high-speed Phantom MIRO ex2 camera (Vision Research, USA) was used for brightfield imaging. OriginPro 2019b (academic version) was used to generate Boxplots and bar charts. The fluorescence intensity of dye-encapsulated and dye-free droplets was analyzed by Image J.

Synthesis of four acetal-protected head groups



Compound 2

Commercially available tris was converted to the corresponding acetonide-protected tris amine (**2**) following the protocol by Öberg et al.[1]

Compound 5

Isopropylidenglycerol-glycidylether (**5**) was synthesized using solketal (10g, 1 eq.) and epichlorohydrin (20.90 g, 3 eq.) in presence of NaOH (4.54 g, 1.5 eq.) and tetra-n-butylammonium bromide (TBAB) (10 wt% of starting material, 3.08 g) in water/toluene (1:1) solvent mixture at room temperature. Progress of the reaction was monitored by TLC using hexane/ethyl acetate (1:1) with Rf of 0.3. After completion of the reaction, the mixture was suspended in water and ethyl acetate (3x30 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was evaporated to yield the crude product, which was purified through column chromatography to give compound **5** as a white viscous liquid in 60% isolated yield (~8.5 g).

Compound 6-7

For the synthesis of mono-azido diglycerol derivative, an isopropylidenglycerol-glycidylether (5) (5 g, 1 eq) was refluxed overnight with NaN_3 (5.18 g, 3 eq) and NH_4Cl (1.43 g, 1 eq) in 80% ethanol. Progress of the reaction was monitored by TLC using methanol/dichloromethane (1:19) with Rf of 0.45. After completion of the reaction, the mixture was suspended in water and dichloromethane (3x30 mL). The combined organic layers are dried over anhydrous sodium sulfate and the solvent was evaporated to yield the crude product, which was purified through column chromatography using DCM and methanol to give compound **6** as a white viscous liquid in 85% isolated yield (5.22 g). Finally, to convert azide to amine functionalities, 10% (w/w) a palladium catalyst (10% Pd on charcoal) was used with respect to the weight of acetal-protected lDG-N_3 (6) for hydrogenation. The substrate was dissolved in dry methanol in a hydrogen reactor and pressurized to ~5 bar hydrogen atmosphere at RT for 3 days under vigorous stirring. Then the Pd/charcoal was filtered using a double-layer cellulose filter paper. This hydrogenation step was quantitative, which was monitored by TLC using methanol/dichloromethane (1:9) with Rf of 0.15. Therefore, after concentrating the solution, the acetal-protected lDG-NH_2 (**7**) was used for surfactant synthesis without further purifications.

^{1}H NMR (500 MHz, MeOD) for compound 6: δ 4.34-4.29 (p, 1H) 4.12- 4.09 (dd, 1H), 3.95-3.91 (p, 1H), 3.79-3.76 (dd, 1H), 3.58-3.56 (m, 4H), 3.43-3.34 (m, 2H), 1.44 (s, 3H), 1.38 (s, 3H); ^{13}C

NMR (126 MHz, METHANOL-D3) δ 109.28, 74.90, 72.75, 72.09, 69.43, 66.20, 53.51, 25.72, 24.32. ESI-MS m/z 231.2520 (calcd. $C_9H_{17}NaN_3O_4$ 254.2560).

^{1}H NMR (500 MHz, MeOD) for compound 7: δ 4.29-4.25 (p, 1H), 4.06-4.03 (dd, 1H), 3.74-3.69 (m, 2H), 3.53-3.47 (m, 4H), 2.76-2.60 (m, 2H), 1.38 (s, 3H), 1.33 (s, 3H). ESI-MS m/z 205.1314 (calcd. $C_9H_{19}NaNO_4$ 228.1360).

Compound 8-10

Mono-azide linear triglycerol (**8**) was synthesized following the reported method.[2] The acetonide-protected mono-azide linear triglycerol (**9**) was synthesized using compound 8 (3 g, 1 eq.) and 2,2 dimethoxypropane (5.88 g, 5 eq.) under solvent-free conditions at 60 ^{o}C for 15 h. Progress of the reaction was monitored by TLC using methanol/dichloromethane (1:9) with Rf of 0.25. After completion of the reaction, the mixture was suspended in water and dichloromethane (3x30 mL). The combined organic layers were dried over anhydrous sodium sulphate and the solvent was evaporated to yield the crude product, which had been purified through column chromatography using DCM and methanol to give compound **9** as a white viscous liquid in 75% isolated yield (2.6 g). And compound **10** was prepared according to the method mentioned above (see *compound 6-7* section for hydrogenation and work up conditions).

^{1}H NMR (500 MHz, MeOD) for compound 9: δ 4.31-4.27 (p, 1H), 4.09-4.06 (dd, 1H), 3.92-3.88 (p, 2H), 3.76-3.73 (dd, 1H), 3.59-3.49 (m, 8H), 3.40-3.31 (m, 2H), 1.41 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, METHANOL-D3) δ 109.27, 74.88, 72.64, 72.09, 69.54, 69.30, 66.21, 53.52, 25.79, 24.34. ESI-MS m/z 305.3310 (calcd. $C_{12}H_{23}NaN_3O_6$ 328.3510).

^{1}H NMR (400 MHz, MeOD) for compound 10: δ 4.28-4.23 (p, 1H), 4.06-4.02 (m, 1H), 3.89-3.84 (m, 2H), 3.73-3.69 (m, 2H), 3.56-3.45 (m, 7H), 2.92-2.59 (m, 2H), 1.37 (s, 3H), 1.32 (s, 3H). ESI-MS m/z 279.1682 (calcd. $C_{12}H_{25}NaNO_6$ 302.1660).

Compound 11

Acetal-protected tri-glycerol dendron-amine (dTG-NH_2) (**11**) was prepared following our previously described synthetic routes.[3]

Synthesis of di-block fluorosurfactants. Synthesis of all four fluorosurfactants, which consisted of a PFPE polymer chain of low molecular weight (Krytox 157-FSL) and compounds 2, 7, 10, and 11, was conducted following our earlier reported procedures.[3]

Post-modification of L-lTG fluorosurfactant. L-lTG-N_3 was prepared in two steps. In the first step, 1 equivalent of 2% L-lTG (300 mg) (w/w) solution in HFE7500 oil was stirred vigorously with an equal part of dimethylformamide (DMF). Then 1 equivalent of bromoacetic acid (19 mg) is added to it, followed by addition of 1.5 equivalent of EDC.HCl (39 mg) and 1.1 equivalent of DMAP (19 mg). The mixture was then stirred overnight at 45 ^oC. In the second step, the DMF solvent phase was removed from the reaction mixture as it phase-separates without stirring. Then DMF, an equal amount to the HFE7500, was used for washing (1X), removing the reactants and the by-products soluble in DMF. Then 10 equivalent of sodium azide (NaN_3) (90 mg) were added with respect to the obtained crude product. In this step, DMF was used roughly 2X the volume of HFE7500. The reaction was then heated at 75 ^oC for 2d. The clear organic phase was removed. Finally, repeated washing with DMF (4X) removed the salt along with the by-product, and a pure fluorosurfactant was obtained in ~91% isolated yield (~300 mg) after rotary drying under reduced pressure.

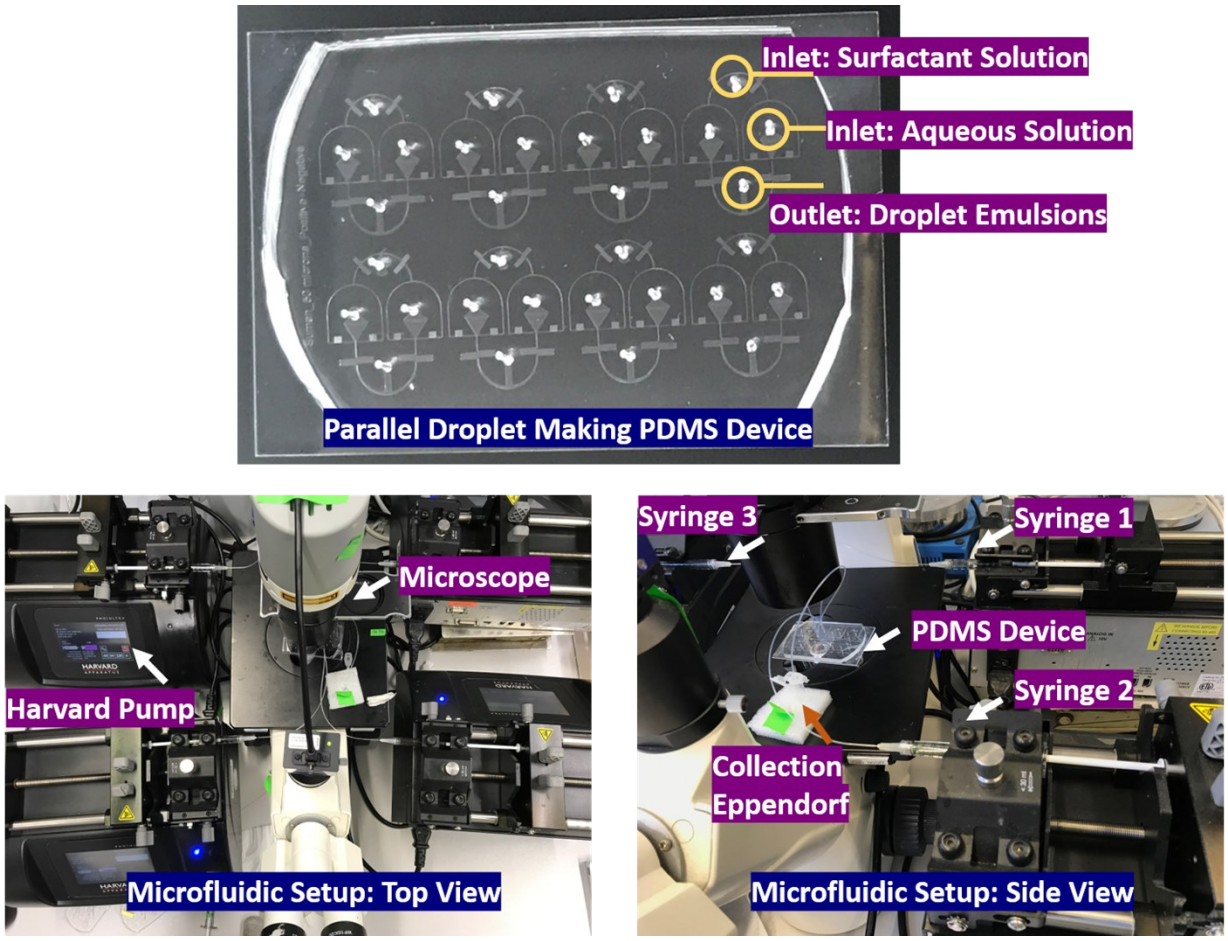


Figure S1. Images showing a parallel droplet making PDMS device (image at the top panel) and a microfluidics setup (images at the bottom panel). The PDMS device has eight parallel droplet making channels each with two inlets for aqueous streams, one inlet for surfactant solution, and one outlet for droplet collections. The top view of the microfluidics setup shows four Harvard pumps and an inverted microscope mounted with a fast camera. The side view shows three syringes that were filled with reagents for droplet generation. Two syringes contained aqueous reagents and one syringe contained surfactant solution. A 0.5 mL Eppendorf tube was used to collect the emulsion droplets. The PDMS device was placed on the microscope stage for monitoring droplet generation via the fast camera which was synchronized with the computer software that captured live video. Inlet and outlet of the channel were connected with the syringe needles via PE20 tubing (dimension: I.D. 0.38 mm, O.D. 1.09 mm; order no. 427406; BECTON DICKINSON).

Figure S2. TLC analysis of PFPE-COOH, PFPE-dTG, PFPE-lTG, PFPE-lDG, and PFPE-Tris from left to right, respectively. A mixture of 20% methanol (v/v) and HFE-7100 was used as a mobile phase to run the TLC.

Interfacial tension measurement. The interfacial tension of the fluorosurfactants was measured following a previously reported protocol.[4] Briefly, we measured the interfacial tensions using a drop shape analyzer (Krüss, DSA30). We introduced an inner phase consisting of a surfactant-loaded HFE 7500 solution into a continuous phase of deionized water (18.2 M "Ohms" cm, Direct Q Merck Millipore system) via a syringe needle, and at 1 s intervals we monitored the interfacial tension between the oil drop and the water phase. For each measurement, we introduced a suitable volume of the oil phase (typically around 5 - 10 microliters) so that the oil drop broke off from the needle after 25 s or more due to gravity, which confirmed that the plateau of the interfacial tension value had been reached before the drop pinched off. We report the interfacial tension value right before the break-off of the drop. We performed five measurements using 1% (w/w) surfactant in HFE7500 to obtain the average interfacial tension value.

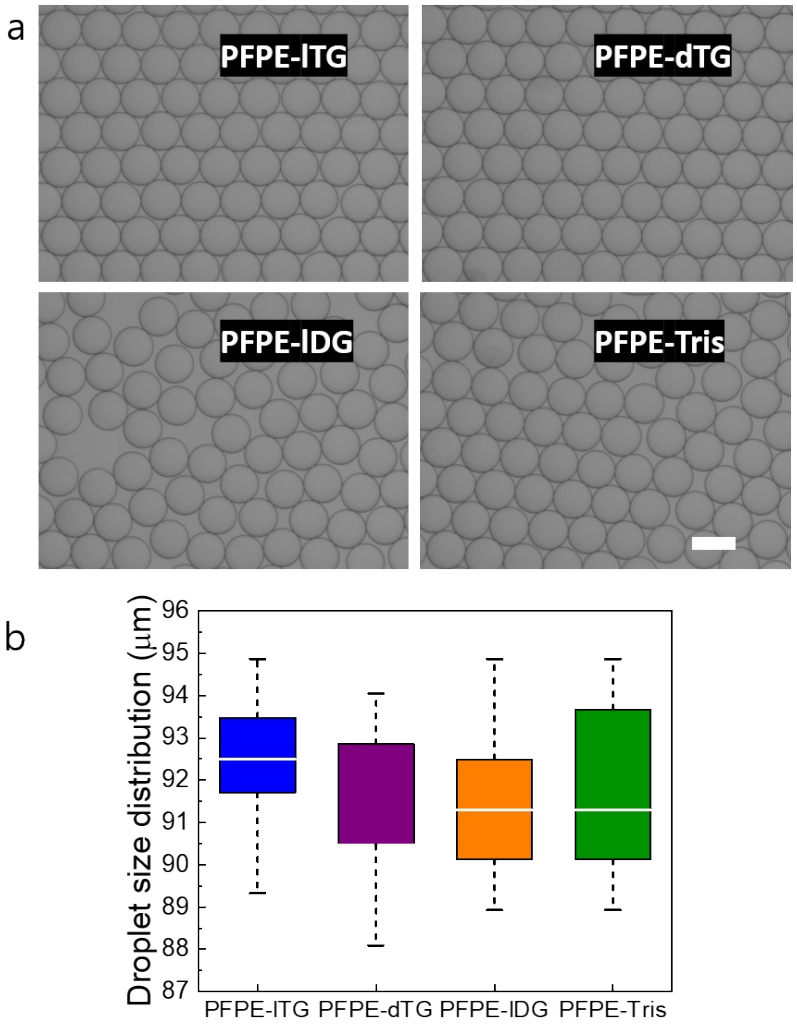


Figure S3. (a) Micrographs showing size distribution of the droplets stabilized with PFPE-lTG, PFPE-dTG, PFPE-lDG, and PFPE-Tris, respectively. Droplets were generated using the parallel droplet making device. (b) Boxplot of droplet size distribution after 24 h incubation of the droplets at room temperature. To obtain the mean average droplet diameter value, 25 droplets were measured by Image J line profiling tool. Scale bar, 100 µm.

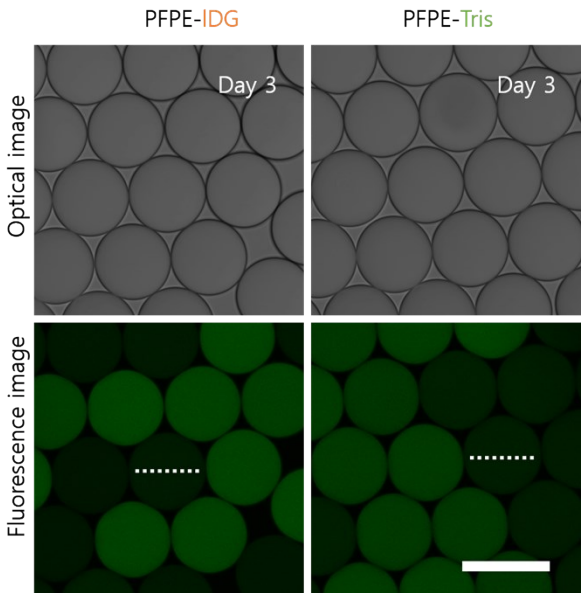


Figure S4. Micrographs showing the inter-droplet diffusion of a fluorescence dye sodium fluorescein salt after 72 h where two populations of empty and dye-containing droplets were stabilized with PFPE-lDG or PFPE-Tris. Two representative initially empty droplets are shown by the dotted lines characteristic for intensity measurements by using Image J plot profiling tool. Scale bar, 100 µm.

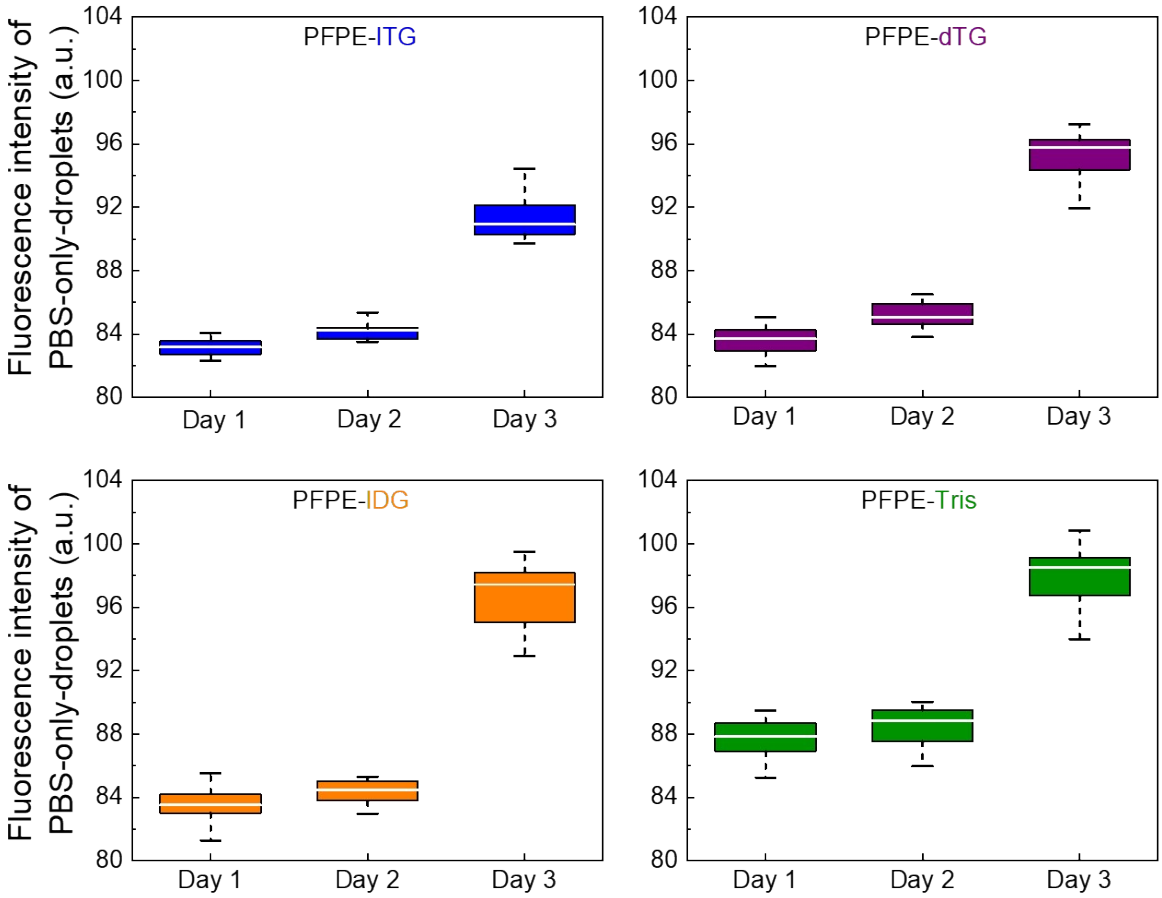


Figure S5. Boxplots showing inter-droplet diffusion kinetics for each fluorosurfactant over a period of three days. A parallel droplet making device was used to generate a mixture consisting of equal amounts of PBS-only-droplets and 3 μM sodium fluorescein dye-containing droplets. The PBS-only-droplets were imaged at day 1, day 2, and day 3. ~30 randomly selected PBS-only-droplets were analyzed to perform quantitative analysis of the fluorescence intensity at the indicated time points. The median lines in the boxplots are represented by the white center line, the blue boxes represent the interquartile range, and the whiskers represent the non-outliers.

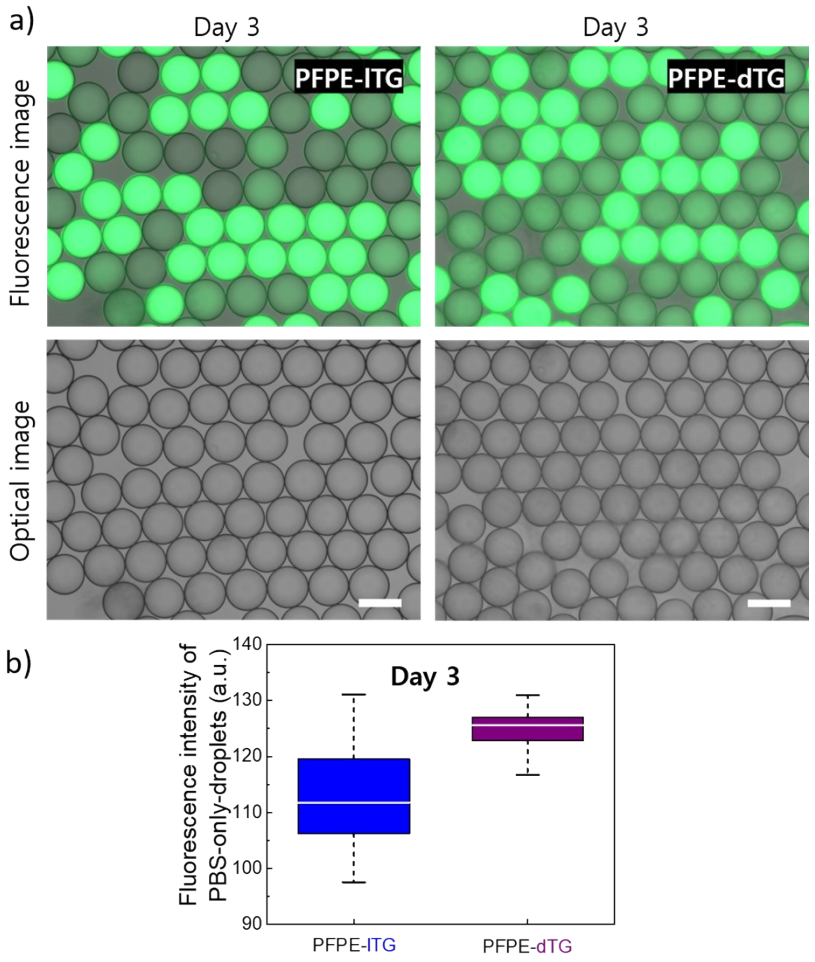


Figure S6. Micrographs showing the inter-droplet diffusion of a less-leaky fluorescence dye sodium fluorescein salt at day 3 where two populations of empty and dye-containing droplets (10 μM) were stabilized with PFPE-lTG or PFPE-dTG. ~35 randomly selected PBS-only-droplets were analyzed to perform the quantitative analysis of the fluorescence intensity at day 3. The median lines in the boxplots are represented by the white center line, the boxes represent the interquartile range, and the whiskers represent the non-outliers. Scale bar, 100 μm.

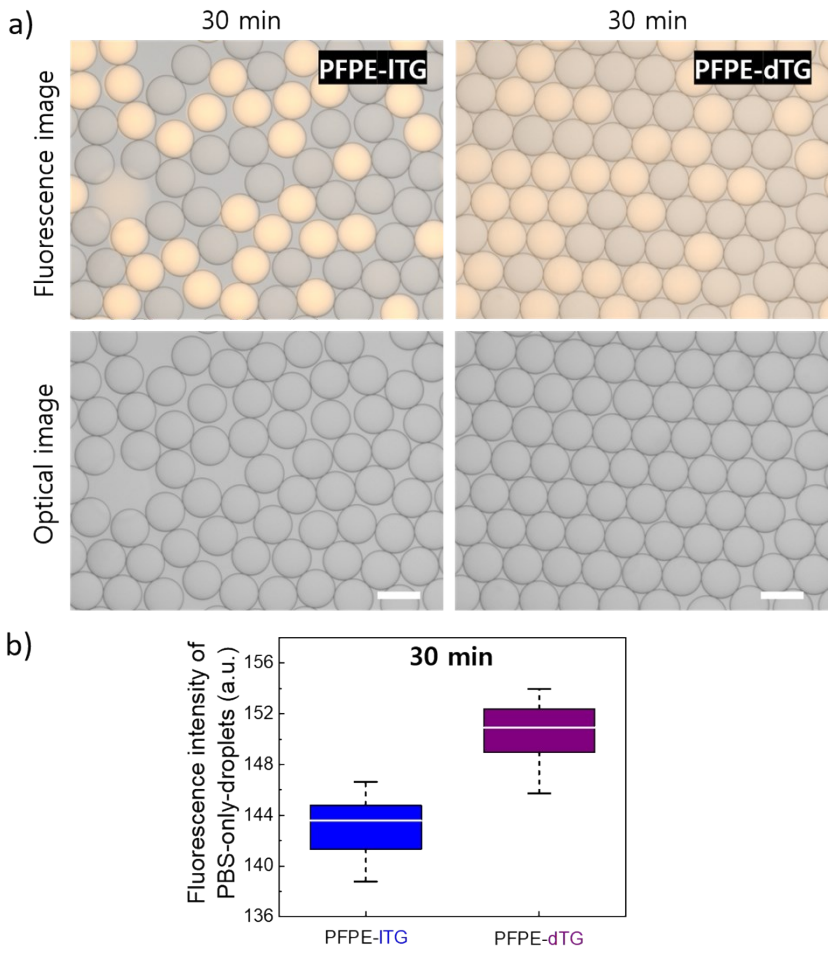


Figure S7. Micrographs showing the inter-droplet diffusion of a more-leaky fluorescence dye resorufin sodium salt after 30 min where two populations of empty and dye-containing droplets (2 μM) were stabilized with PFPE-lTG or PFPE-dTG. ~35 randomly selected PBS-only-droplets were analyzed to perform the quantitative analysis of the fluorescence intensity after 30 min. The median lines in the boxplots are represented by the white center line, the boxes represent the interquartile range, and the whiskers represent the non-outliers. Scale bar, 100 μm.

PDMS device fabrication and flow rates of aqueous and oil streams. The microfluidic PDMS devices were prepared according to our reported protocol with no further modification.[3] The flow rates of oil and aqueous streams for single droplet making device were 600 μL/h and 300 μL/h, respectively. The flow rates of oil and aqueous streams for parallel droplet making device were 1200 μL/h and 300 μL/h, respectively.

Inter-droplet molecular transport assay. For inter-droplet molecular transport experiment the parallel droplet maker, flow rates for continuous oil phase, two aqueous phases, and emulsion droplet incubation conditions were used following previously reported methods with some modifications depicted below.[3] For imaging, the emulsion droplets were drawn by capillary force into a hollow rectangular capillary tube (dimension: ID 0.1 x 2.0 x 50 mm; supplier CM Scientific (UK)) and the open ends were then sealed with grease followed by fixing on microscope cover glass (thickness: 0.13-0.16 mm). The droplets were fluorescently imaged either using Leica confocal microscope (TCS SP8, Germany) or using ZEISS fluorescence microscope at days 0, 1, 2, and 3. Imaging using Leica confocal microscope and ZEISS fluorescence microscope was conducted with the same settings for all experiments. The shutter intensity was 3.99% and the line average was set to 1 while using the confocal microscope. The exposure time for imaging with ZEISS fluorescence microscope was set to 2.5 s. The fluorescence intensity of the PBS-only-droplets was measured either by line profile tool of Leica analysis software or by Image J using the oval selection tool for mean intensity measurement.

Droplet-PCR. Droplet-PCR is performed according to our previously reported protocol with no further modification.[3]

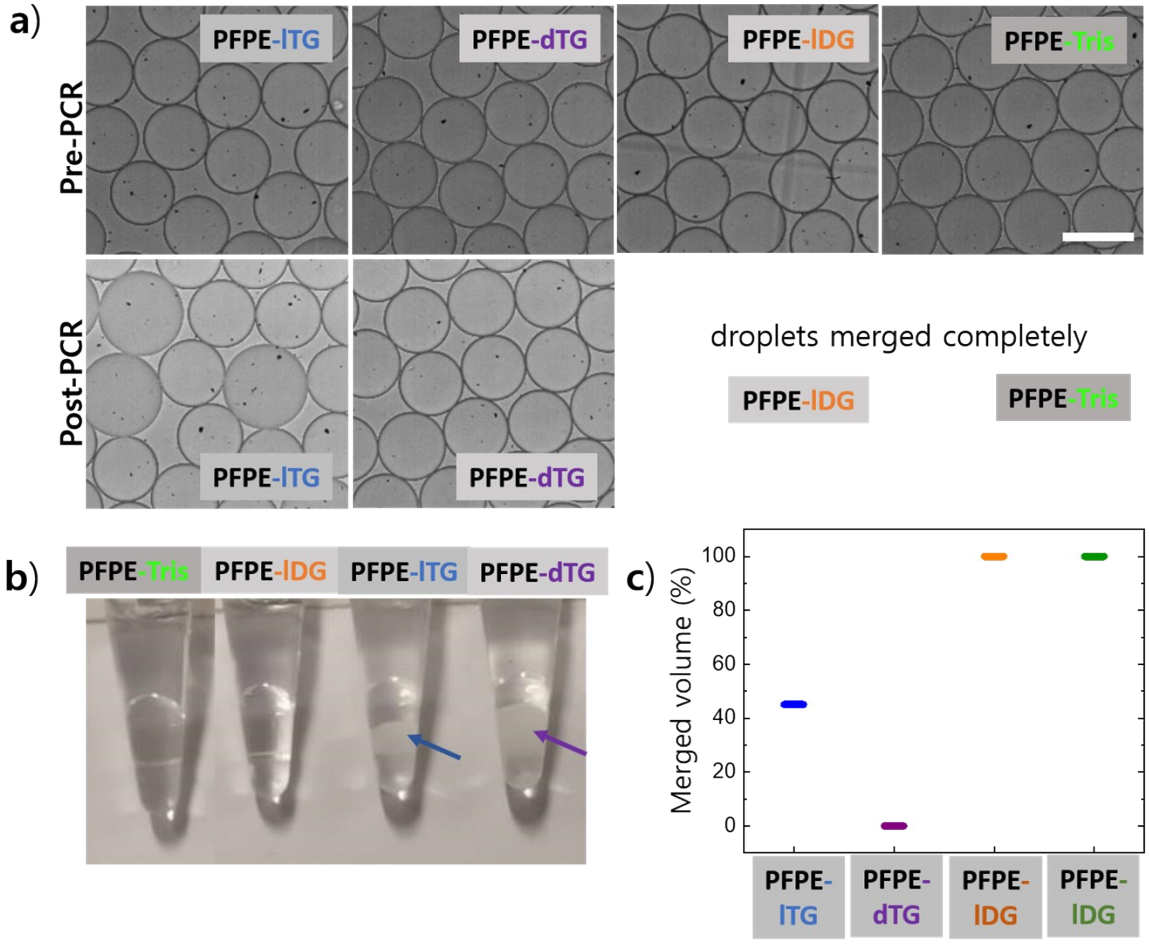


Figure S8. Micrographs (a) and (b) show the droplet size distribution before and after PCR reaction. Droplets stabilized with the linear tri-glycerol, the dendritic tri-glycerol, the di-glycerol, and the tris-based surfactants showed no merging during incubation at 4 °C (pre-PCR condition). By contrast, after 35 cycles of PCR reaction, droplets stabilized with the L-dDG or the L-dTris showed complete merging and droplets stabilized with the L-lTG showed about 50% merging with the rest being polydisperse in size. However, no merging is seen when droplets are stabilized with the L-dTG. c) A quantitative measurement of the merged-droplet volume. Note, there is no error bar as two independent measurements showed the same result. Scale bar: 100 μm.

From-Droplet fishing. For from-Droplet-fishing assay, stock solutions of DBCO-Sulfo-Cy3, DBCO-PEG4-Biotin, and Cy®5-streptavidin were prepared following the suppliers' guidelines. MQ water was used to prepare a working solution of each compound. Prior to encapsulation, a immunocomplex consisting of 5 µM DBCO-PEG4-Biotin (8 eq.) and 0.63 µM Cy®5-streptavidin (1 eq.) was prepared in 500 µL MQ water and the freshly prepared solution was stirred in a 2 mL glass vial equipped with a magnetic stirrer bead at 200 rpm for three hour at 25 °C. The flow-focusing nozzle used in the single droplet maker was 35 µm x 50 µm. Droplets were produced using the flow rates of 600 µL/h for the continuous phase (2% surfactant (w/w) solution in HFE 7500 oil) and 300 µL/h for the aqueous phase (dye or protein complex containing MQ water).

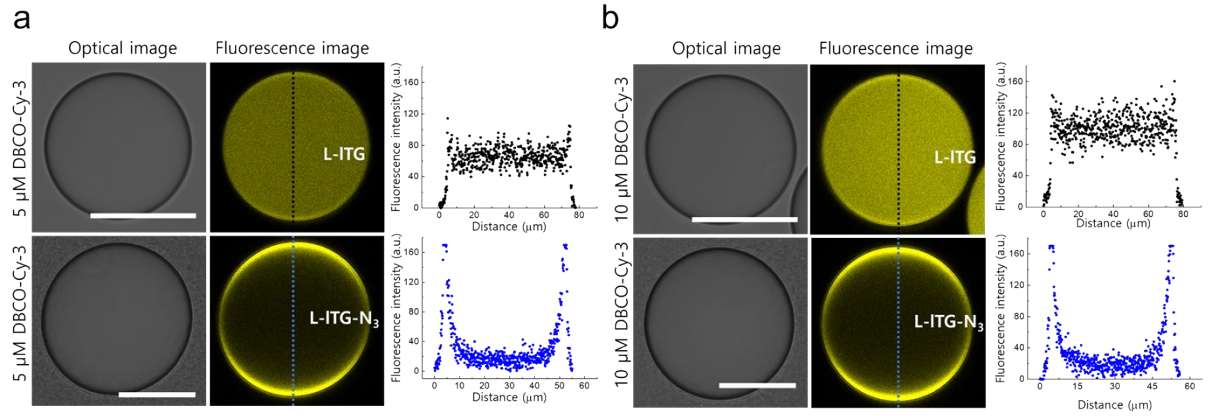


Figure S9. Confocal fluorescent images of the dye-encapsulated droplets. (a) droplets with 5 µM DBCO-Cy-3 and (b) droplets with 5 µM DBCO-Cy-3. Droplets were stabilized with either L-lTG (top panel) or L-lTG-N_3 (bottom panel) surfactant. Unlike L-lTG, L-lTG-N_3 enabled from-droplet fishing of the dye under both conditions, as indicated by the strong fluorescence intensity at the droplet interfaces. Image J plot profiling tool was used to measure the fluorescence signal across the water-in-oil droplets stabilized with either L-lTG or L-lTG-N_3 surfactant. Scale bars: 50 µm (top panel) and 25 µm (bottom panel).

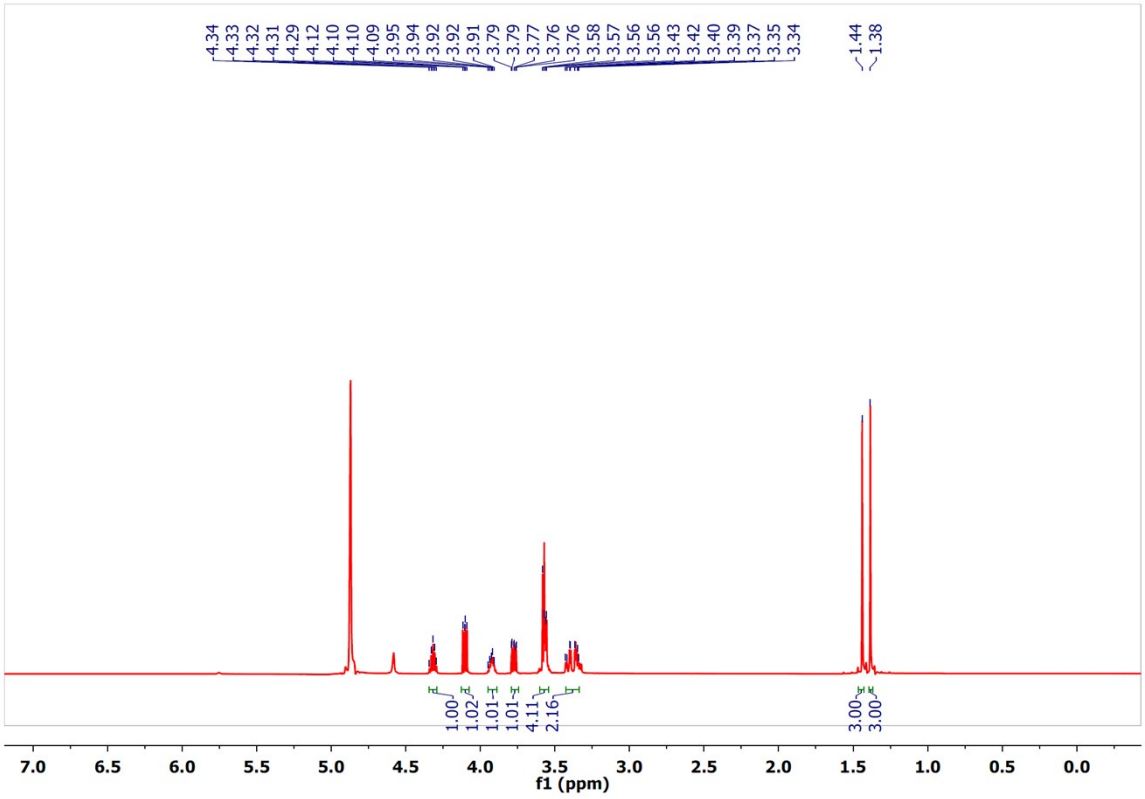


Figure S10. ^{1}H NMR (500 MHz, MeOD) of compound **6**.

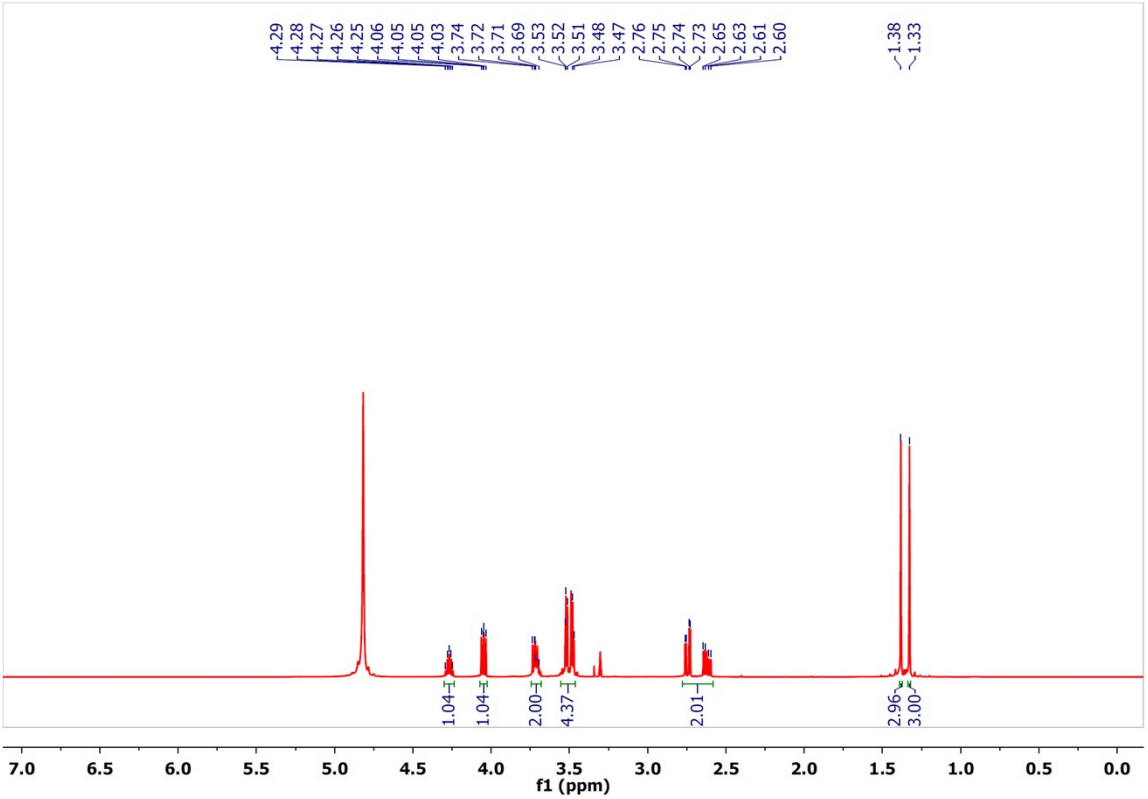


Figure S11. ^{1}H NMR (500 MHz, MeOD) of compound **7**.

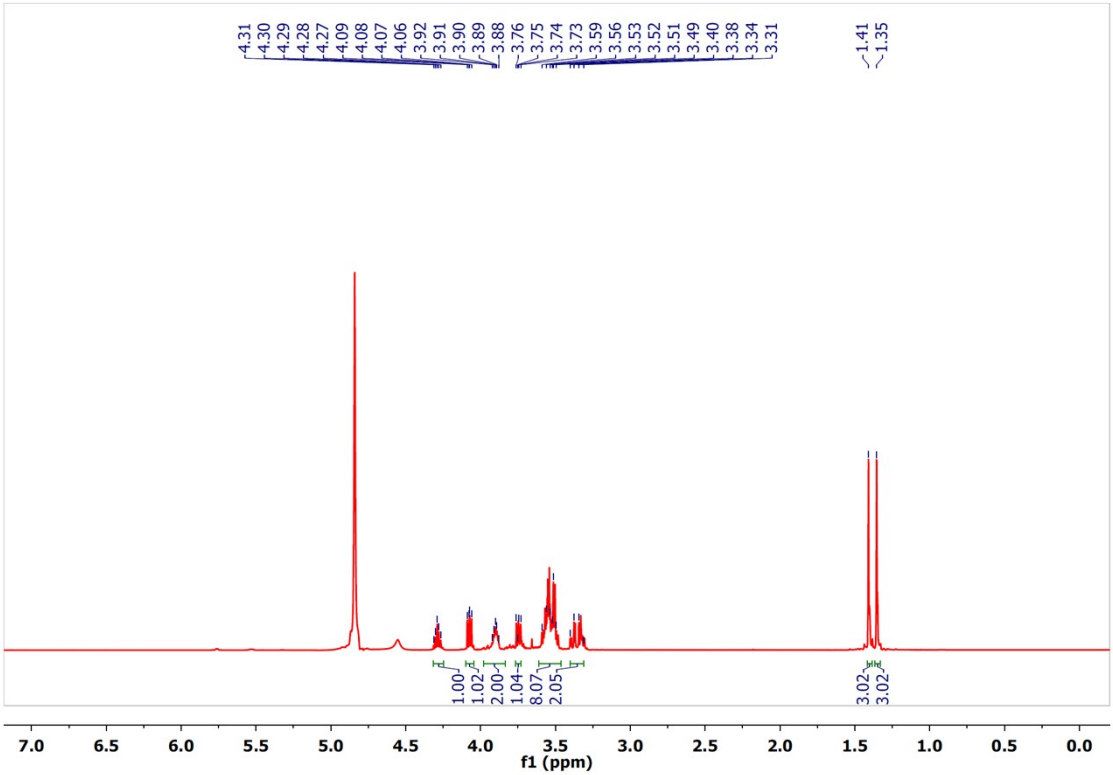


Figure S12. ^{1}H NMR (500 MHz, MeOD) of compound **9**.

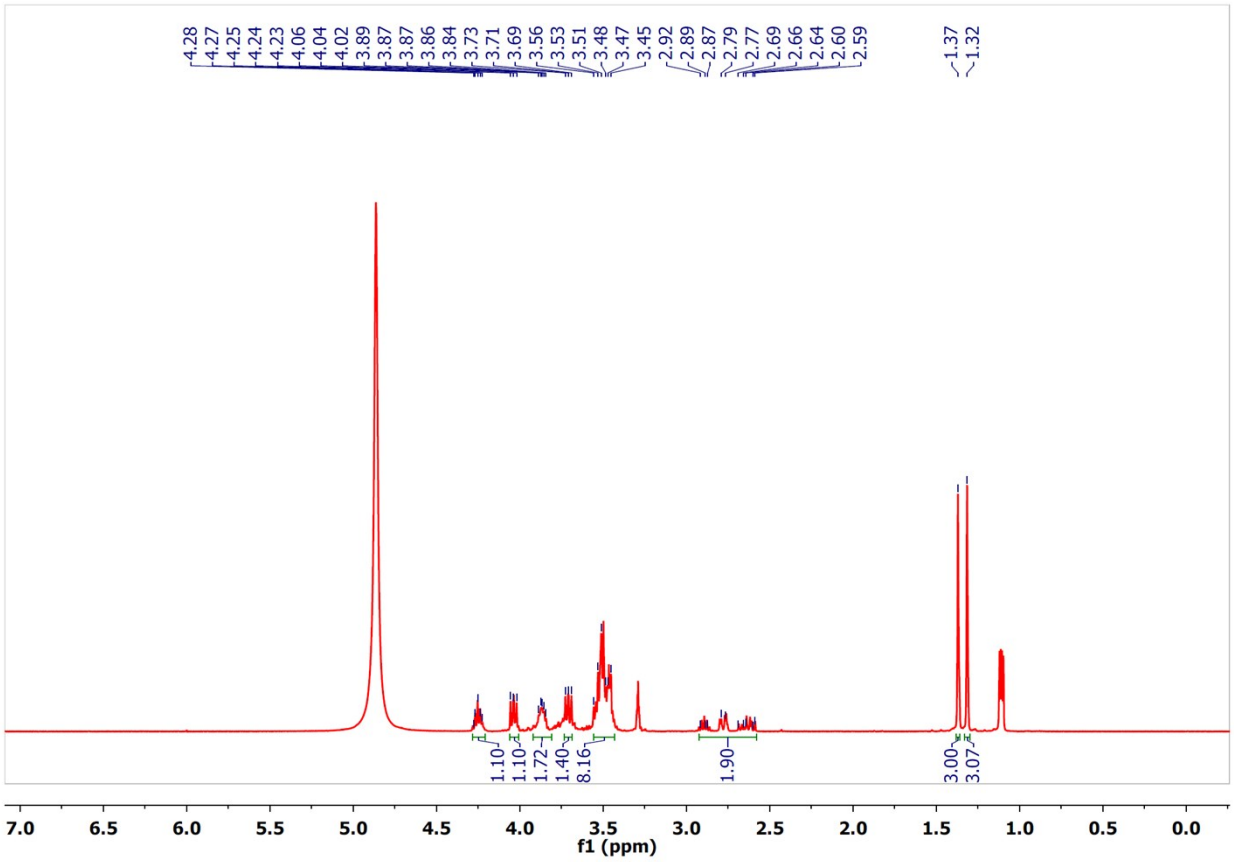


Figure S13. ^{1}H NMR (400 MHz, MeOD) of compound **10**.

References

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