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RE: **Synthesis of a Novel Hemifluorinated Glucose Based Surfactant**
By Dalton Smedley and Matt Vollmer

Dear Dr. Glaser,

Thank you very much for your response to our paper *Synthesis of a Novel Hemifluorinated Glucose Based Surfactant.* In response to the reviewer's comments and suggestions, we have made a number of changes that, overall improve the quality of the paper greatly.

Major Changes

[M.1] A section of previously not available information was added to the supporting information regarding the spectral characterization of the fluorinated analog we synthesized in the process of this report. Additionally the raw surface tension data was also provided in the supporting information as well. As requested in **[2.3]**

[M.2] As reviewer 2 had suggested the spectral data was initially quite difficult to read. A strong effort was made to clarify all of the data so that it is legible and neatly organized with the supporting information.

[M.3] Both reviewer 9 and 4 were concerned with the lack of material citing the "denaturing propensity," of the surfactants references 10 and 11 were added to substantiate that claim. See **[1.11]** and **[2.4].**

[M.4] A lot of ambiguity was left in the schemes of this paper. Scheme 1 was redone to establish the differences between the 3 compared surfactants. This was additionally explained in the scheme as well **[2.1]**. Also a lot of concern was raised with the scheme concerning the synthesis as well and further gone to explain it below the scheme as well. **[2.2]**

[M.5] The concept on the use of THAM as a polar head group was also discussed it was further clarified that THAM is not a glucose derivative, but essentially a framework for altering the polarity of the head group.

Response to Reviewer 9

[1.1] Reviewer 1 requested that sentence 2 be reworded so that the flow of the sentence was adjusted to better clarify the point. We completely agree that this sentence did not correctly clarify the purpose of the ethyl tail. This was changes to, "As opposed to the solely fluorinated analog, H2F6 was synthesized with an ethyl "tail," to improve the interaction between the surfactant and membrane proteins."

[1.2] Reviewer requested the abstract final sentence were adjusted to clarify the contents of the paper, as the wording of the sentence was very ambiguous. Changes to the end of the abstract were provided to promote better fluency and flow.

[1.3] Reviewer 1 requested that the sentence the importance of pharmaceuticals were changed. The part of the sentence regarding pharmaceuticals was removed to remove the ambiguity.

[1.4] Reviewer suggested that spacing after all paragraphs was changed to 0 pt. this was done.

[1.5] As suggested, H2F6-Diglu was added to the first sentence of the third paragraph of the introduction to provide clarity.

[1.6] trishydroxymehtyl acrylamidomethane was put into parentheses to provided sentence clarity, as suggested

[1.7] The reviewer suggested that we reword the second "paragraph" of the sentence, this was simply combined with the synthesis of the hydrophobic tail, as it was originally meant to be.

[1.8] In results and discussion "need" was changed to "needed"

[1.9] As suggested the third sentence of the results and discussion was reworded to clarify point.

[1.10] References were adjusted so that the volume was italicized

[1.11] Bibliography was altered so that the references followed the correct format. Additionally the second and third references in the bibliography were moved to the reference section, as they were references not the work that this paper was originally published on.

Response to Reviewer 4

[2.1] As pointed out be the review the first scheme not only is not explained but is rather ambiguous. A new scheme was added and explained.

[2.2] Additionally the retrosynthetic route provided in the synthesis section was misleading to this reviewer. We agree that it is open to interpretation, thus we have redone the scheme to apply directly to the synthesis of H2F6- Diglu as well as information for F6-Diglu specifically. Additionally this scheme was rewrote as well.

[2.3] Reviewer 3 would like to have seen a plot of surface tension vs. concentration of the surfactant this was added to the supporting information as the results are the only thing that have any relevance to the discussion of the surfactant effectiveness.

[2.4] As previously requested (and changed) the propensity to denature proteins was cited in the results and discussion see **[1.11]**

[2.5] The reviewer argues that, "No methods are really of calculating Gibb's free energy," The ambiguity of this statement lead us to believe that we should expand on the meaning of the Gibbs energy a sentence was added after the equation in results and discussion.

[2.6] Reviewer also had concerned about pharmaceuticals and genome percentages this was previously changes see **[1.3]**

[2.7] Reviewer notices that in the abstract H2FG-diglu was shown as H_2F_6 this was adjusted to be uniform with the rest of the paper.

[2.8] As prompted by the reviewer a major change was added, the choice as THAM as the polar head group was discussed. This is further express in major changes.

[2.9] As suggested by reviewer 1, the first sentence of the conclusions as well as the second sentence of the results and discussion, were altered **[1.9]** and **[1.8]**

Response to Reviewer 2

[3.1] The reviewer asked that the spectra were altered so that they were more clear, a real attempt to do so was attempted, however with the fact that this is a new novel surfactant, and that we do not have access to any other spectra, they were made as clear as possible. Also address in major revisions.

[3.2] Reviewer mentions a run on sentence in the abstract. We are assuming that they wanted to address the last sentence of the abstract, this was previously addressed in [**1.2]**

[3.3] The review recommend that Scheme 3 title head be put above the scheme, this was done as well.

[3.4] The reviewer asked that information about the Wilhemy plate method was discussed more, reference 7 (D. Teeters; M. A. Andersen, D. C. Thomas. Formation Wettability Studies that Incorporate the Dynamic Wilhelmy Plate Technique. *Oil-Field Chemistry* **1989**, 560-576), discusses this explicitly. Additionally there is not place for the physical methods to be incorporated into this paper, as it is not a physical chemistry paper, or a methods paper for that matter.

[3.5] The reviewer mentions that synthesis should not be its own title head, we agree and have changed that.

Again, thank you greatly for your consideration on our paper. We would also like to thank the Peer reviewers for their attentiveness and attention to detail while reviewing our manuscript, as they have helped to greatly improve the strength of the paper.

Sincerely,

Smedley and Vollmer

Synthesis of a Novel Hemifluorinated Glucose Based Surfactant

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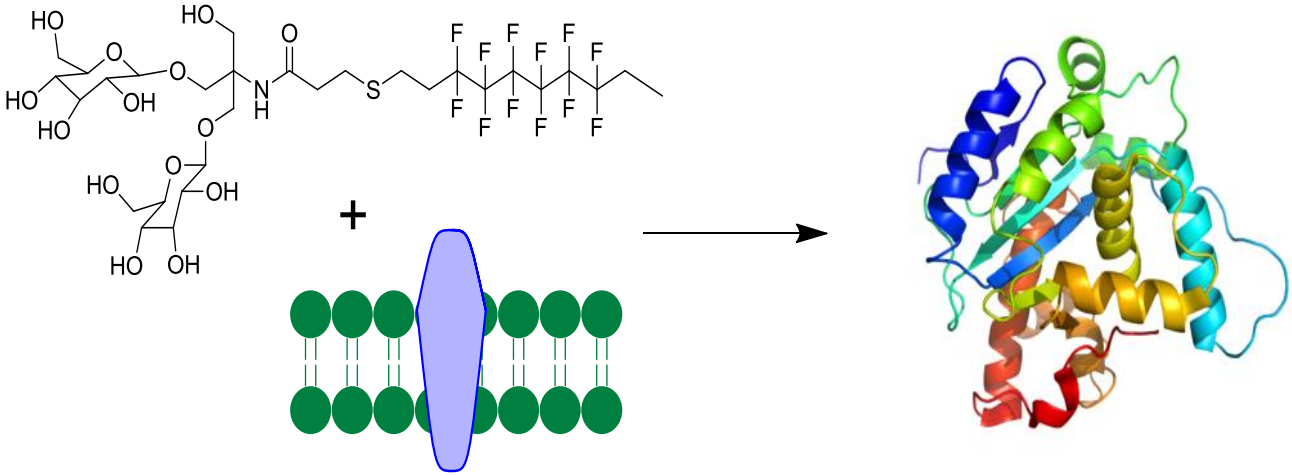
Abstract

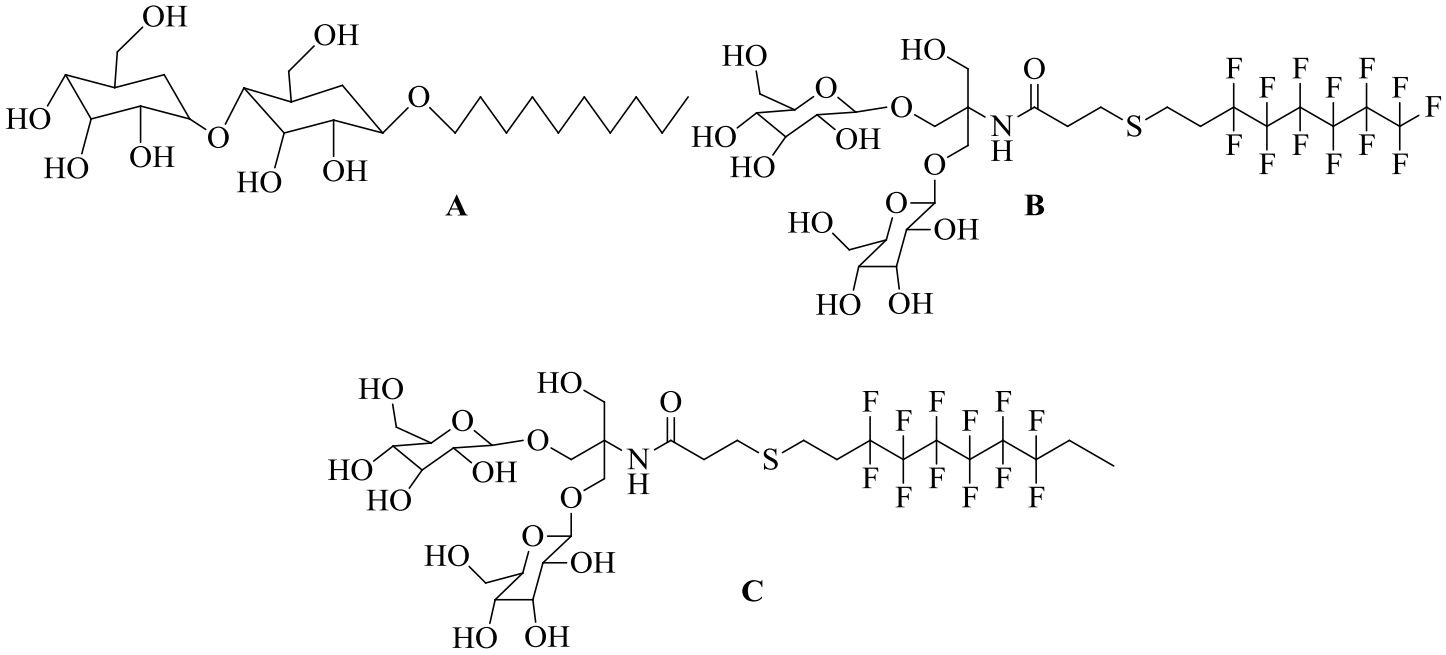
We herein report the synthesis and characterization of a novel hemifluorinated surfactant *N-1,1-Di[(2',3',4',6'-tetra-O-acetyl-ß-D-glucopyranosyl)oxymeth-* yl]acetoxyethyl-4-thia-7,7,8,8,9,9,10,10,11,11,12,12-dodecafluoro- tetradecanamide, named with the shorthand H2F6-Diglu. As opposed to the solely fluorinated analog, H2F6 was synthesized with an ethyl "tail," to improve the interaction between the surfactant and membrane proteins. These new novel surfactant will then alternatively be compared to current fluorinated surfactants (also synthesized in this paper, F6-Diglu) as well as the non-fluorinated alkyl chain analog, dodecyl maltoside (DDM). The methodology of dynamic light scattering (DLS) was then applied to gather the information available to form this report. Specifically; Critical Micelle Concentration (CMC), surface tensions at the CMC (γCMC), surface excess concentration at CMC (Γ max), Gibbs free energy of micelle formation ($\Delta G_m°$), and finally the average aggregate size formed. These values will then be combined in an effort to explain the qualities of H2F6-Diglu and F6-Diglu that make them good candidates for usage as detergents in membrane isolation and crystallography. Additionally a new synthesis for these novel surfactants is provided as well.



Introduction

The study of membrane proteins is of the upmost importance to the fields of biology, chemistry, medicine, as well as biochemistry. Given that 20-40% of encoded genomes are composed of membrane proteins, obtaining proteins that are soluble, active, and stable for study is of unparalleled urgency and importance.[1] This isolation is commonly done using a variety of detergents which bind where lipids would typically bind, in order to isolate them.[2,3,4] This is done through the dissociating effects of detergents; however, this is very difficult to control. Often, the proteins are rendered unusable due to denaturing by the detergent.

This flaw of already produced detergents was initially intended to be solved with the fluorination of an alkyl chain named fluorinated surfactants (FS).[5,6] However, these fluorinated chains were far too lipophilic, and thus to improve their affinity for the hydrophobic trans-membrane surface of membrane proteins, an ethyl "tail" was added to the already synthesized



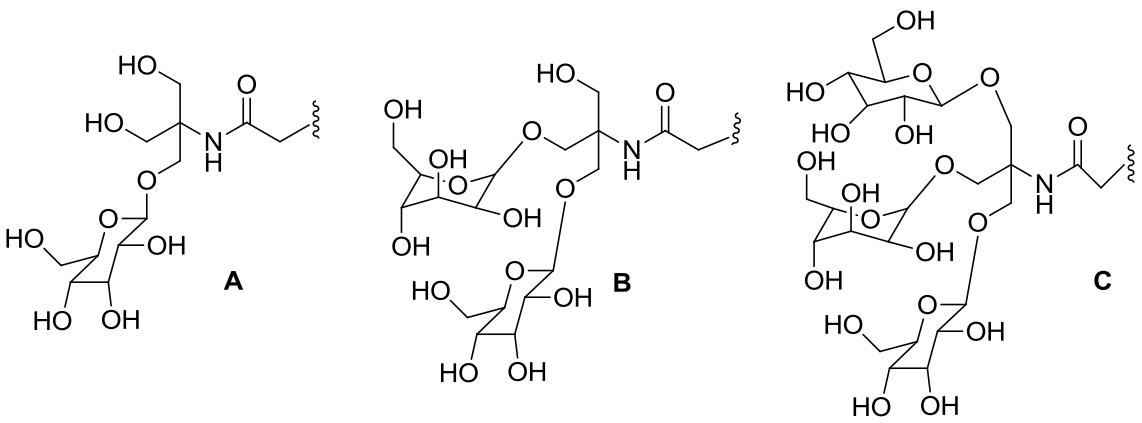
Scheme 1: Above are displayed the surfactants to be compared in this paper. From A-C, we see dodecyl maltoside (A), the fluorinated compound this research was originally based upon F6-Diglucose (B), as well as the surfactant discussed in this paper H2F6-diglucose (C), the hemiflourinated analog of B.

FS.[6] These hemifluorinated tails gave rise to the name hemifluorinated surfactants (HFs), Hemifluorinated surfactants were then combined with THAM (2-Amino-2-hydroxymethyl-propane-1,3-diol), to act as a backbone for a glucose based surfactant. The addition of the THAM thus allowed for the choice of the head group to counteract the insolubility of membrane proteins in water seen traditionally in FS. Another Advantage of the THAM head is that the degree of addition could be well controlled as well giving rise to a mono, dual, and triglucosylated head group.

We herein report the results of the new synthesis of a novel modified hemifluorinated surfactant H2F6-Diglu, shown in Scheme 1. This surfactant shall be compared between previously synthesized fully fluorinated surfactants with diglucosylated polar heads as well as commercially available dodecyl maltoside, also previewed in Scheme 1.[5,6]

Materials and Methods

Synthesis of H2F6-Diglu and F6-Diglu. The synthesis of this surfactant was achieved by the synthesis of two different moieties that are conjoined through a carbon – sulfur – carbon bond. The polar head group of the molecule was synthesized from THAM (trishydroxymethyl acrylamidomethane). This allowed for the selection of different polar ability through monoglucosylated, diglucosylated, or triglucosylated THAM derivatives referenced in Scheme 2.



Scheme 2: A representation of the selective ability of the polar head region. The polarity of the surfactant can be well controlled by the addition of 1 to 3 units of glucose. Working from left to right we see A) a monoglycosylated polar head B) a diglycosylated polar head and C) the Triglycosylated polar head.

The next step is the synthesis of the Hemi-Fluorinated hydrophobic tail. Originally the bis monoethylenation of di-iodoperfluorohexane resulted in 1,10-diiodo-3,3,4,4,5,5,6,6,7,7,8,8-dodefluorodecane. A mono-reduction of this product was achieved by combining the product with $HSi(SiMe_3)_3$ and the presence of a radical catalyst. This was then combined with potassium thio-acetate (1.5 equiv) in anhydrous DMF to form a thiol that was capable of conjoining with the polar head group. A condensation of the chosen THAM derivate combined with the hemifluorinated thiol provided satisfactory yield ranging between 50-62%. For this particular paper we will focus on the hemifluorinated diglucosylated surfactant denoted in short hand H2F6-Diglu. These choices of reagents lead to the combination of a variety of different products with different polar affinities and thus different abilities' as surfactants for the isolation of membrane proteins.

Characterization of DDM, H2F6-Diglu, and F6-diglu. The previously synthesized hemifluorinated surfactants were characterized by a few different methods. First the surface tension measurements were taken and thus provided the following: critical micelle formation concentration (CMC), surface tension at CMC (γ_{CMC}), and the surface excess concentration at

CMC (Γmax). All of this data was gathered through the plot of surface tension versus log([surfactant]), (additional information can be found in supporting information). The surface tensions were measured through the Wilhelmy plate technique.[7] Additionally from the equilibrium constant, Gibbs free energy and thus the favored ability of micelle formation was determined.[2]

The final measure of these surfactants will be their micellar free energy denoted $\Delta G_m°$ kJ/mol. The effectiveness of a fully fluorinated diglucosylated surfactant (F_6-Diglu), a hemifluorinated diglucosylated surfactant (H_2F_6-DiGlu), and a generic mass produced surfactant dodecyl maltoside (DDM) were then explored. Results for all tests run on the three surfactants are shown in Table 1.

Table 1:

Surface Active Data for F6-Diglu, H2F6-Diglu, and DDM

Surfactant	CMC(mM)	γCMC (mN/m)	Γ max 10-12 (mol/mm2)b	$\Delta G_m°$ kJ/mol
F6-Diglu	.113	28.3	1.79	-15.34
H2F6-Diglu	.347	36.0	1.5	-12.56
DDM[8]	.15	35.75	3.32	-14.64

Results and Discussion

As seen by the following equation, the micellar free energy, the spontaneity of a surfactant to form micelles, is directly related to the critical micelle concentration.[9]

$$\Delta G_m° = RTIn(CMC/55.5)$$

This indicates that the higher the concentration needed in bulk to form micelles is very important to ability of the surfactant to act as a detergent on a membrane protein. As the value $\Delta G_m°$ related a more negative energy to a higher propensity to form micelles. As shown, micelle formation for

F6-Diglucose was more favorable. This is evident by the -15.34 kJ/mol of micelle formation. This is shortly followed by dodecyl maltoside with a free energy formation of -14.64 kJ/mol and finally followed by the least favored micelle formation H2F6-Diglucose, showing a free energy of -12.56.

Although the $\Delta G_m°$ of a surfactant is important, the propensity of that surfactant to denature the protein is just as, if not more, important. It is important to note that none of the stated surfactants have been reported to denature membrane proteins completely.[10, 11]

The ability of DDM to form micelles is an advantage, but the major side effect of this is the aggregation that occurs due to the micelle formation. This over time results in the denaturing of the protein, thus a detergent that forms micelle readily without rapid aggregate is an advantageous in the solubilization and isolation of membrane proteins. The hemi-fluorinated surfactants provide this advantage. The average aggregation results in aggregates no more than 6.5nm. However, the average rate is smaller when compared to that of DDM, with a reported aggregate size of 8.2nm.[5] Yet, the other large micelle forming detergents, such as DDM, create poly disperse aggregates ranging around 25nm in diameter. Thus the fact that the hemi-fluorinated surfactant can readily form micelles as well as avoid large aggregation makes them a suitable contender for the observation and isolation of membrane proteins.

Conclusions

It has been established that the study of more efficient detergents for the isolation of membrane proteins for various fields of study is important. As shown above, there has been an advance as to better chemical detergents that can help prevent the problems that arise with the use of its predecessors such as dodecyl maltoside and fluorinated surfactants. With the addition of an ethyl tail to the fluorinated surfactant creating the new hemifluorinated surfactant, the

compound may become less favorable, in terms of free energy, but it also has a higher micelle surface tension as well as the ability to form micelles without the set back of aggregation.

With the use of this new surfactant, it is possible to isolate membrane proteins in a more stable and less denaturing micelle. Thus facilitating the study of membrane protein function, and contributing to the science of protein structure and therefore biology as a whole. The micelles formed with the use of the fluorinated surfactant are created with higher surface tension and more readily making the possibility of keeping these proteins longer without denaturing far more likely.

Supplemental Material Available: The appendix contains a table of contents listing sections with page numbers. The appendix also includes supplemental information such as a detailed description of the synthesis process along with a scheme of retrosynthetic pathways for obtaining a specific type of hemifluorinated surfactant chain. Spectroscopic characterization is included (Fluorine, Carbon, and Hydrogen).

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[10] Propyl-Ended Hemifluorinated Surfactants: Synthesis and Self-Assembling Properties. Maher Abla, Grégory Durand, and Bernard Pucci. *J. Org. Chem*. **2011**, *76 (7),* 2084-2093.

[11] Polidori, A.; Presset, M.; Lebaupain, F.; Ameduri, B.; Popot, J.-L.; Breyton, C.; Pucci, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5827–5831.

Appendix: Supporting Information

A Comparative Approach to the Effectiveness of DDM and Hemifluorinated Surfactants

Dalton Smedley, Matt Vollmer

Department of Chemistry University of Missouri

Table of Contents

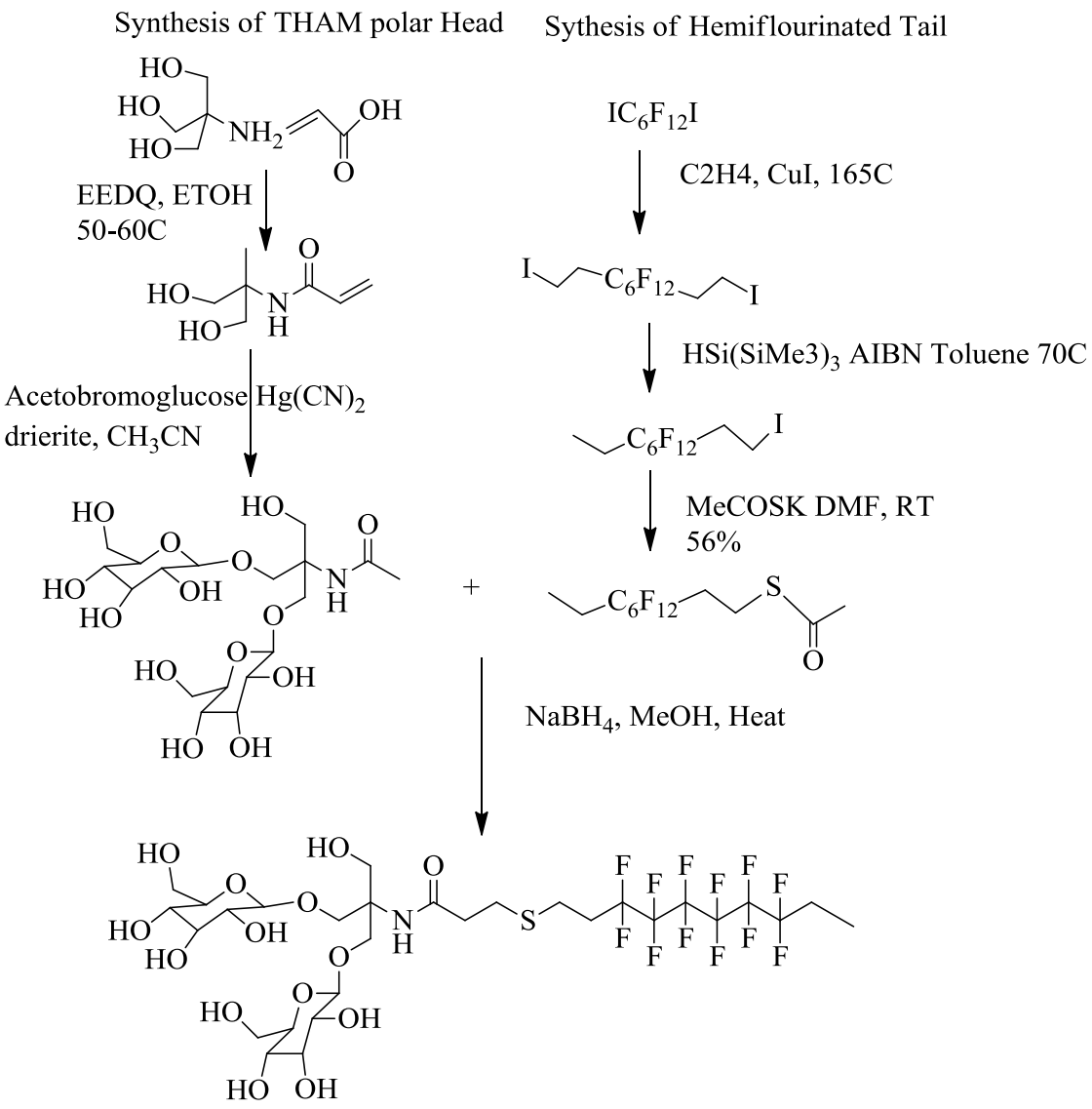
* The ^{1}H,^{13}C, ^{19}F and DEPT NMR sequences were performed at 250, 62.86 and 235 MHz for ^{1}H,^{13}C, ^{19}F experiments respectively. Chemical shifts are given in parts per million relative to the solvent residual peak of CD_3OD.

Synthesis

The first steps involved in the synthesis of a hemi fluorinated surfactant chain is the synthesis of reactants which will ultimately form our chosen surfactant. First a derivative of THAM must be formed. To begin, two of the three hydroxyl groups of the THAM were protected using 2,2-dimethoxypropane to lead after purification. Through acetylation, followed by removal of 1,3-acetonide in acidic conditions, we are given the next product. Next, this product must undergo diglucosylation in the presence of tetra-O-acetyl-D-glucopyranosyl bromide, in excess, under ultrasonic activation. This reagent is then treated with a solution of sodium methoxide and 100% methanol, leaving all hydroxyl groups replaced with beta-D-glucose or another R group.

The second and largest task is the synthesis of hemifluorinated thioacetate. It starts with $HSi(SiMe_3)_3$ used with a catalytic amount of AlBN. This allows silica gel purification of a mixture of 1-iodo 3,3,4,4,5,5,6,6,7,7,8,8 dodecafluorodecane, unreacted diiodo hemifluorinated compound, and some bis reduced compound; however, the 1-iodo the chosen product and will be used for the continuing steps. The diiodo may be recovered and used as well with use of toluene and heating of about 70^oC. Next the iodide bond must be replaced with a thiol bond. To do this the monoiodide was reacted with potassium thioacetate, with 1.5 equivalent, in anhydrous DMF giving the desired compound of the hemifluorinated chain attached to a thiol.

Finally, the synthesis involving the connection of the thiol end of the hemifluorinated chain with alkene of the THAM derivative. With the use of $NaBH_4$ in a methanol solution with reagents, the thiolether bond was formed with the application of heat giving the desired hemifluorinated surfactant chain. A brief diagram showing retrosynthetic pathways is shown in scheme 3.



Scheme 3: mechanism of entire synthesis of H2F6-Diglu on the left the synthesis of the polar head is shown starting from THAM and ending with the diglucosylated polar network. On the right we see the synthesis of the hemiflourinted tail starting with the flurodecane and ending with a thioacetate. These were inevitably combined in NaBH4 and Methanol (while heated) to give the final product. The only difference in the synthesis of the fluorinated analog was the starting material on the hemiflourinated synthesis route.

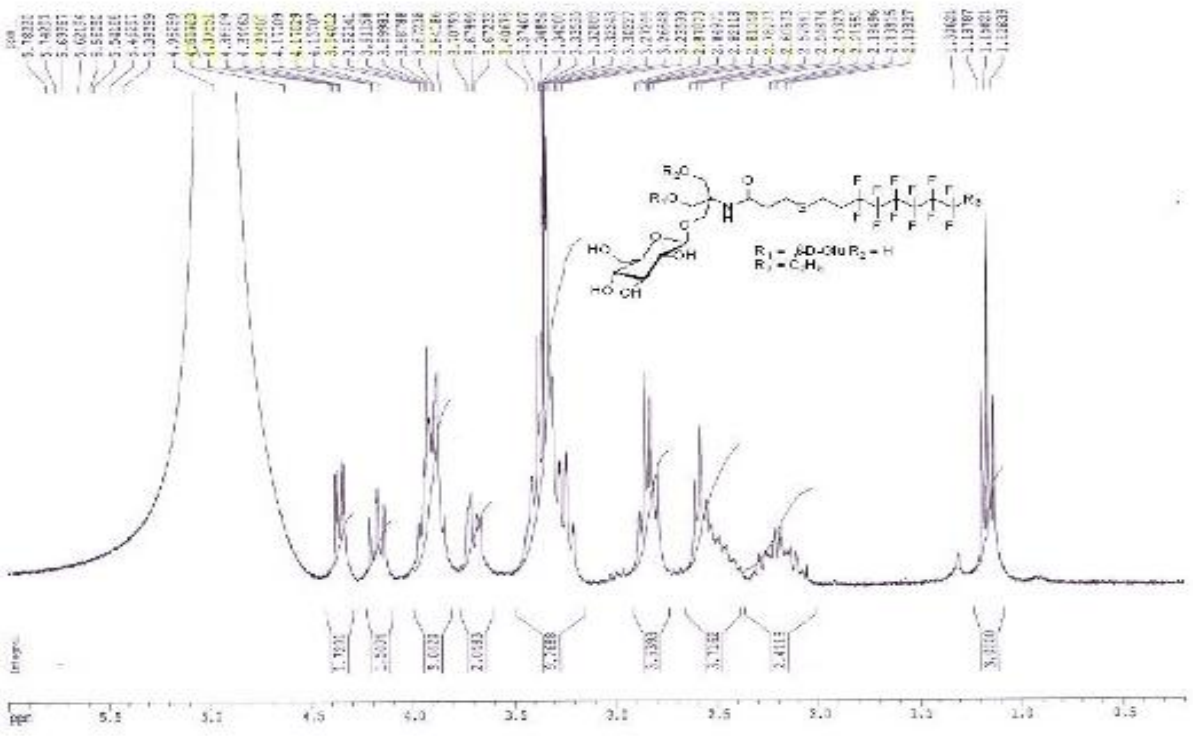


Figure S22. ^{1}H NMR spectrum of H_2F_6-Diglu **12** (CD_3OD)

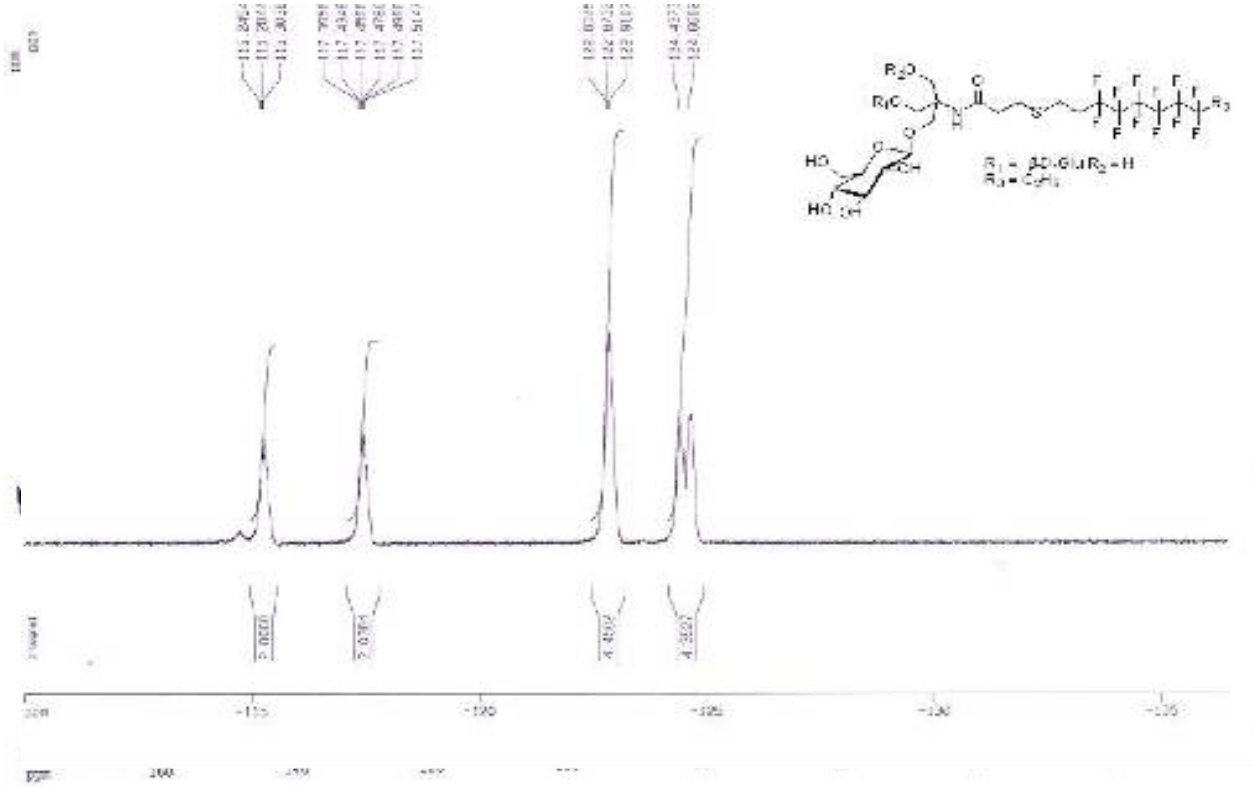


Figure S24. ^{19}F NMR spectrum of H_2F_6-Diglu **12** (CD_3OD)

Figure S23. ^{13}C NMR spectrum of H_2F_6-Diglu **12** (CD_3OD)

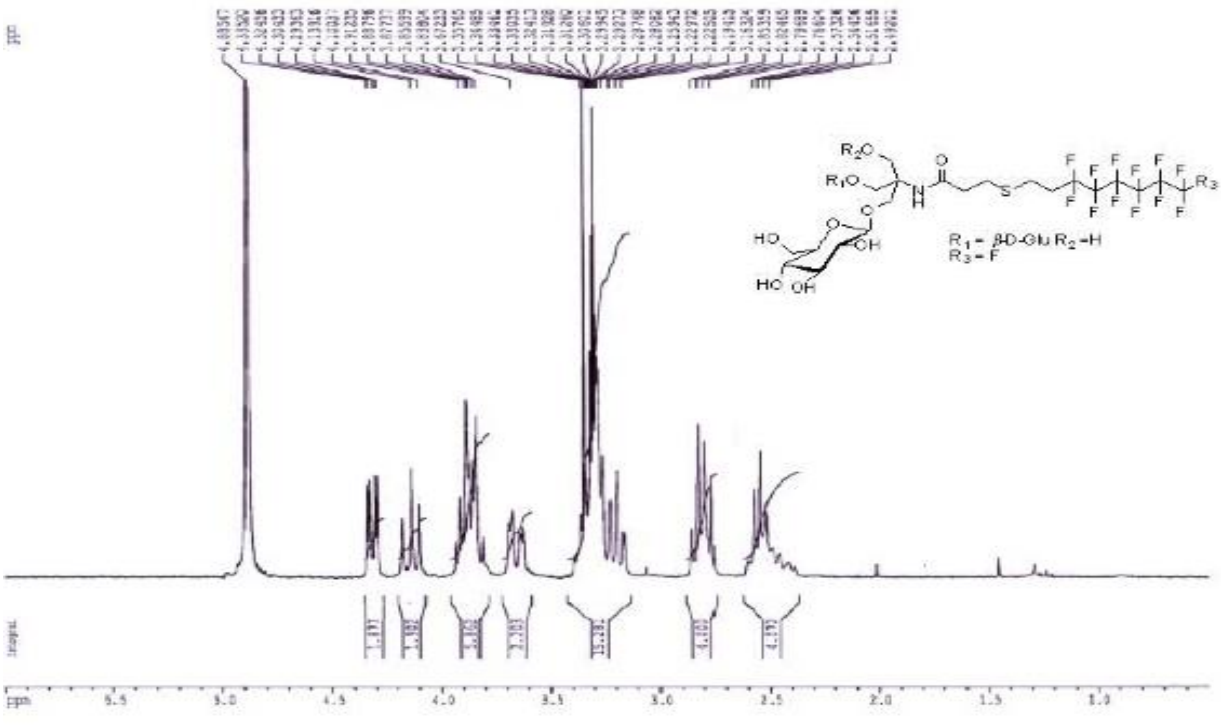


Figure S16. ^{1}H NMR spectrum of F_6-Diglu **10** (CD_3OD)

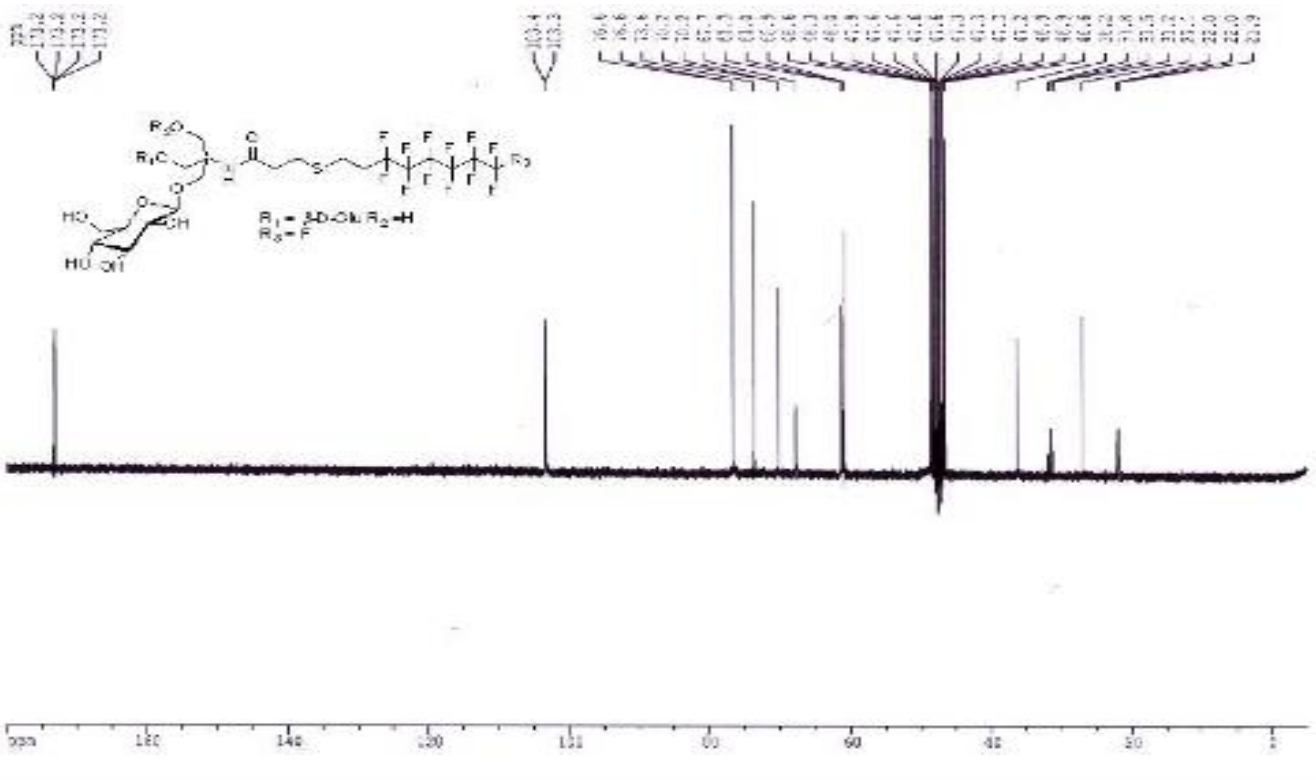


Figure S17. ^{13}C NMR spectrum of F_6-Diglu **10** (CD_3OD)

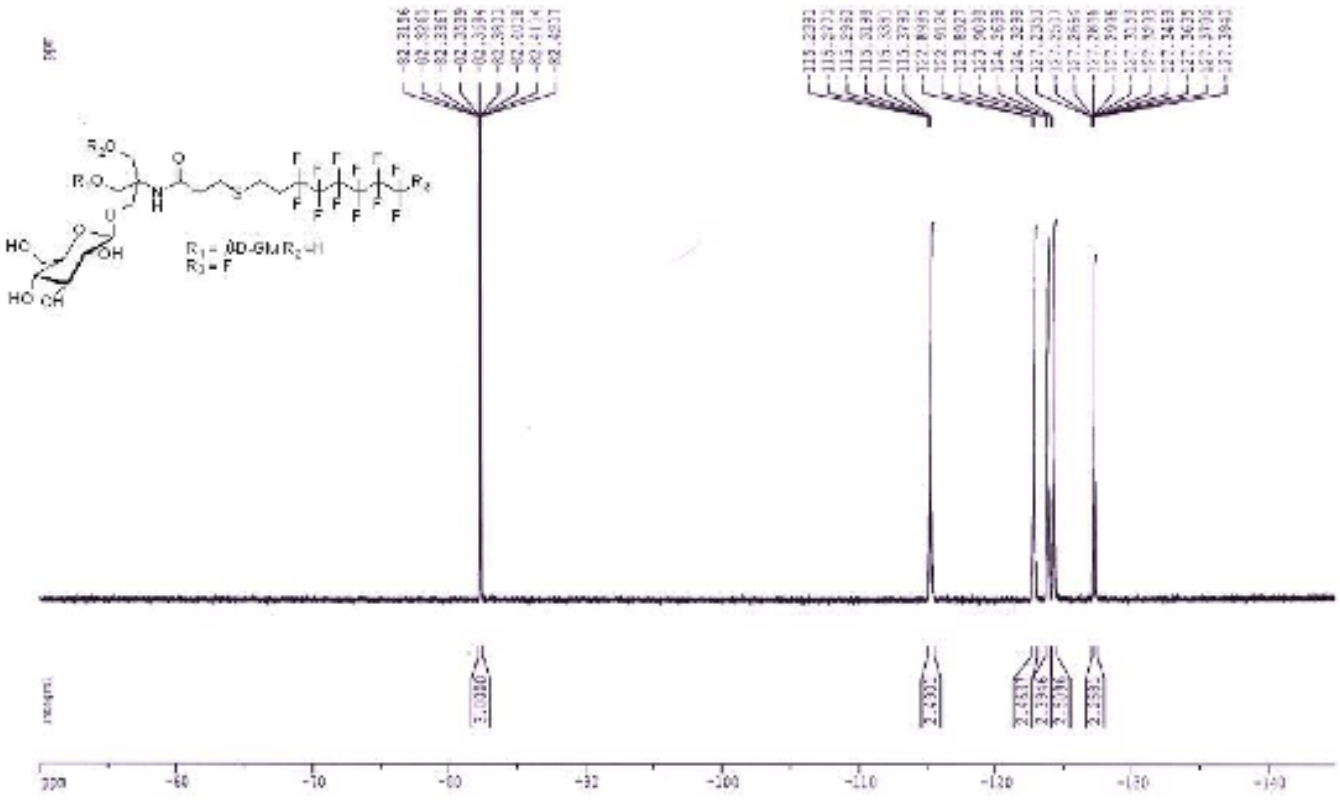


Figure S18. ^{19}F NMR spectrum of F_6-Diglu **10** (CD_3OD)

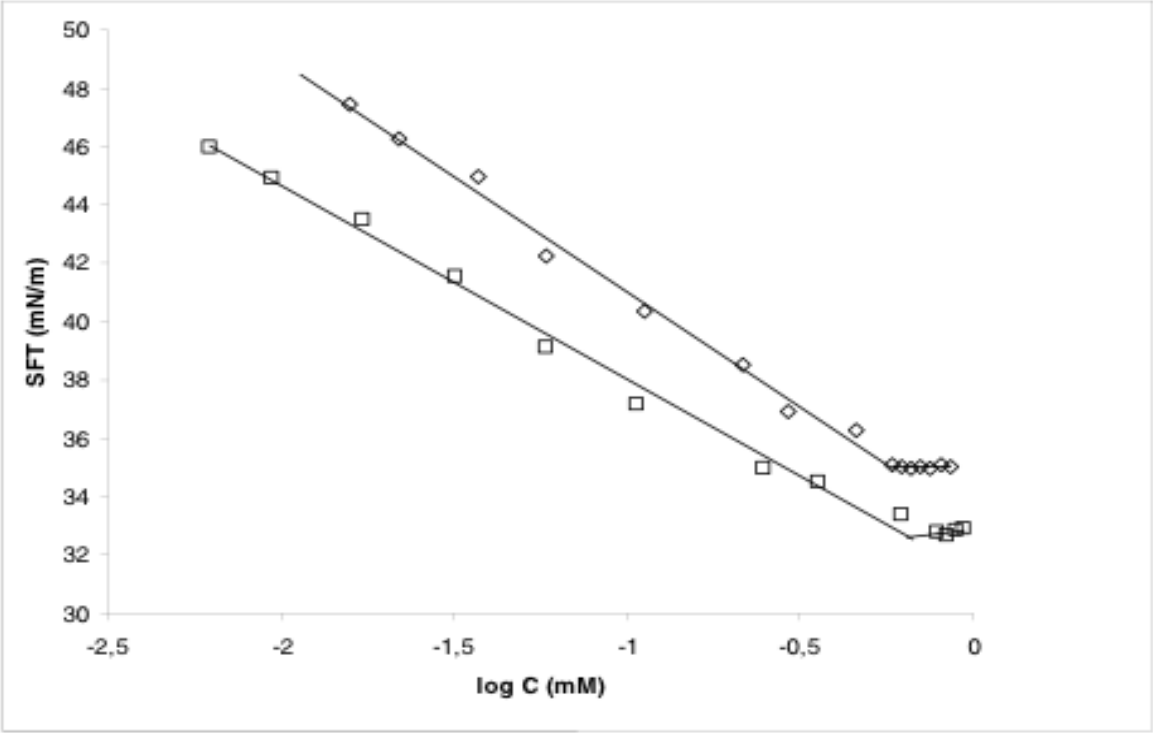


Figure S41. Surface tension vs. log C plot for H_2F_6-Diglu (◊) and H_2F_6-Triglu (□).

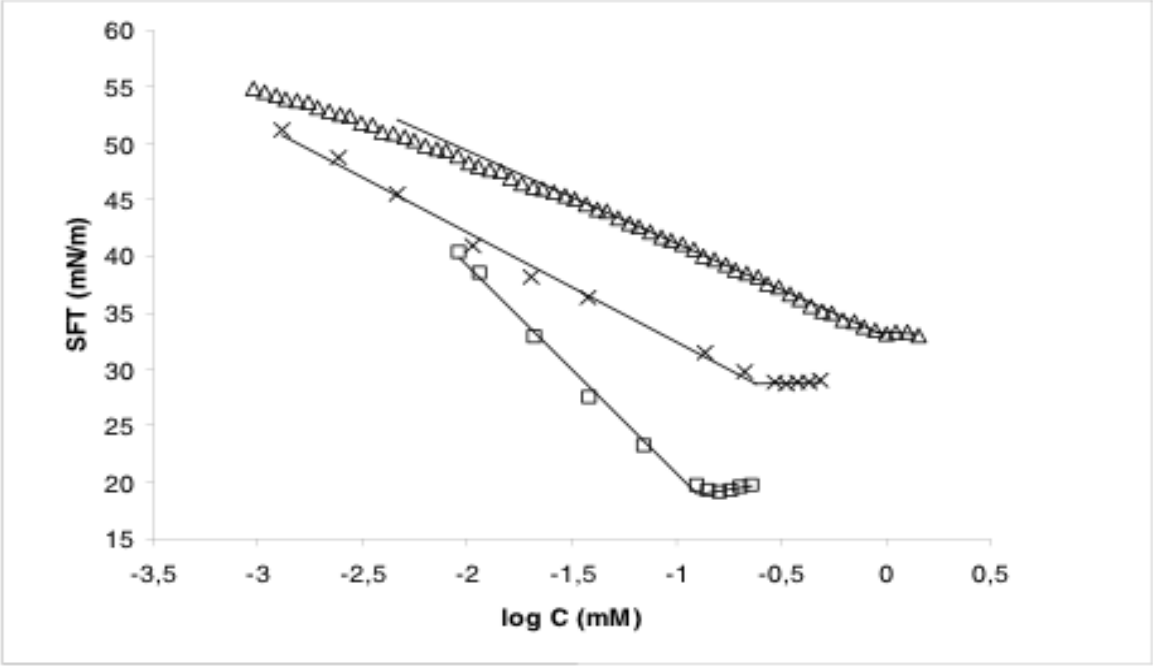


Figure S42. Surface tension vs. log C plot for F_6-Monoglu (□), F_6-Diglu (×) and F_6-Triglu (Δ).

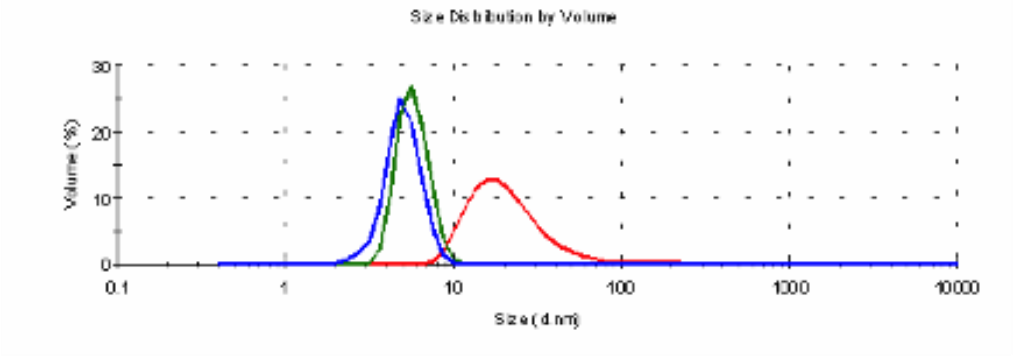


Figure S38. Hydrodynamic diameter distribution statistical plot (by volume) from DLS for: F_6-Monoglu **9** (red); F_6-Diglu **10** (green); F_6-Triglu **11** (blue); at 4mM.

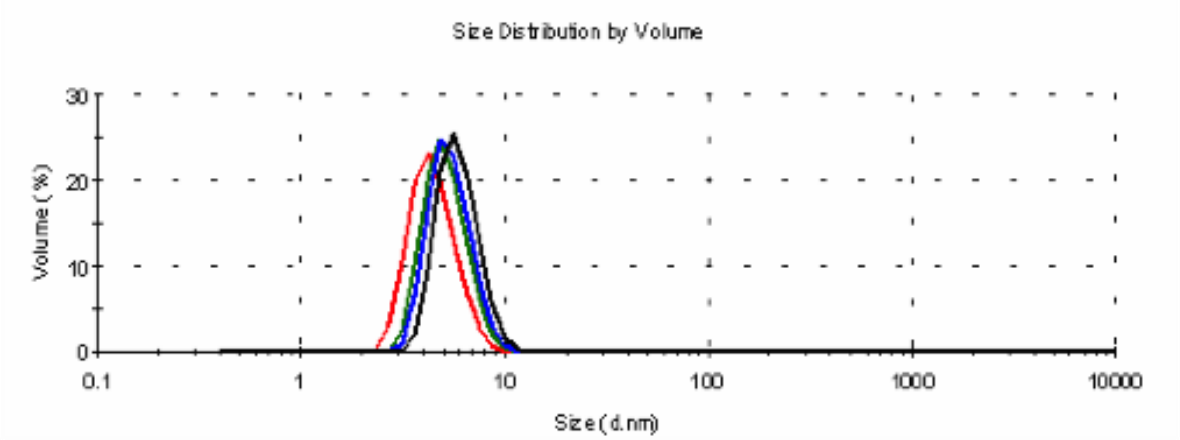


Figure S39. Hydrodynamic diameter distribution statistical plot (by volume) from DLS for: H_{10}-Diglu **14** (blue); H_{10}-Triglu **15** (red); H_{12}-Diglu **16** (black); H_{12}-Triglu **17** (green) at 5mM.

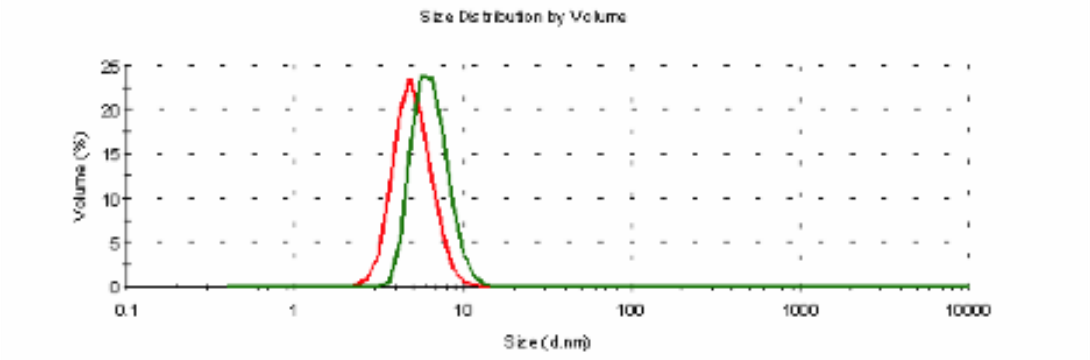


Figure S40. Hydrodynamic diameter distribution statistical plot (by volume) from DLS for: H_2F_6-Diglu **12** (green); H_2F_6-Triglu **13** (red) at 5mM.

Bibliography

The original work that this paper is based upon is cited below:

Glucose-Based Surfactants with Hydrogenated, Fluorinated, or Hemifluorinated Tails: Synthesis and Comparative Physical-Chemical Characterization. Maher Abla, Grégory Durand, and Bernard Pucci. J. Org. Chem. *2008* 73 *(21), 8142-8153*