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Investigating the Role of Specific Tear Film Lipids Connected to Dry Eye Syndrome: A Study on O-Acyl-ω-hydroxy Fatty Acids and Diesters

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ABSTRACT: Dry eye syndrome (DES) is a prevalent disease in which the tear film homeostasis is compromised. One of the main causes of DES is thought to be an alteration in the composition of the outermost layer of the tear film, the tear film lipid layer (TFLL), resulting in an increased evaporation of water from the tear film and subsequent drying of the ocular surface. Recent studies have suggested that the specific TFLL lipids, namely, O-acyl-ω-hydroxy fatty acids (OAHFAs) and diesters (DiEs), may play a role in the development of DES. However, their specific connection to DES has remained largely unknown until now because of the lack of information on their biophysical properties and their role in the TFLL. Herein, we have addressed this issue by studying the biophysical properties and evaporation resistance of a library containing 10 synthetic analogues of TFLL OAHFAs and DiEs. Our results show how the variations of chain length and polar groups affect the phase behavior of these lipids at the tear film surface. In addition, the results revealed that the OAHFAs exhibiting a liquid-expanded to solid phase transition formed films with high evaporation resistance, whereas the DiEs were found to have no evaporation resistance. Altogether, our results shed new light on the role of the OAHFAs and DiEs in the TFLL and their connection to DES, suggesting that OAHFAs are likely a key lipid class in maintaining the TFLL evaporation resistance.

1. INTRODUCTION

Dry eye syndrome (DES) is a disease of the ocular surface characterized by tear film instability, hyperosmolality, and ocular surface damage. DES has a prevalence of 10−30% in the adult population and constitutes a heavy economic burden with the estimated annual direct and indirect costs of $55 billion in the United States alone. Current topical treatments available for DES include artificial tears, lipid-based eye drops, and anti-inflammatory agents such as corticosteroids. These treatments aim to alleviate the symptoms of DES but have not been found to cure the disease. For example, lipids used within lipid-based formulations (triglycerides, phospholipids, and mineral oil) have been shown to be ineffective in forming evaporation-resistant films at the air–water interface, and corticosteroids are associated with several negative side effects in long-term use, such as ocular hypertension, cataracts, and opportunistic infections. There is a need for improved treatments which would restore the tear film to its normal state; however, the development of such treatments requires insights into the function, properties, and role of specific tear film components.

In healthy eyes, a functional tear film covers the ocular surface and protects the eye by acting as a barrier between the eye and the environment (see Figure 1). The tear film is approximately 3 μm thick and comprises two distinct layers: a mucoaqueous layer and a lipid layer. The tear film lipid layer (TFLL) is ~40 nm thin and stabilizes the entire tear film by reducing its surface tension and retarding the evaporation of water from the mucoaqueous layer. Therefore, a dysfunctional TFLL is thought to be a major underlying reason for the development of DES. This hypothesis is supported by a recent animal study in which transgenic mice with altered TFLL composition have been shown to develop DES. In humans, reduced concentrations of specific lipids such as type I and type II diesters (DiEs) as well as O-acyl-ω-hydroxy fatty acids (OAHFAs) have been associated with DES (see Figure 1). Therefore, these specific TFLL lipids may play a key role in healthy eyes and offer protection against the development of DES.

Surprisingly, there is almost no information reported on the properties of individual lipids belonging to the OAHFA and DiE categories in the scientific literature. To investigate the role of OAHFAs and DiEs in the TFLL, we set out to study the biophysical properties of model OAHFAs and type II DiEs.
2. RESULTS AND DISCUSSION

2.1. Synthesis of Model Lipids. To understand the biophysical properties of OAHFAs and DiEs, a synthetic library of molecules was needed. As shortening the chain lengths of tear film wax esters, cholesterol esters, and OAHFAs resulted in the development of DES in mice,9 molecules with varying chain lengths were selected as model compounds. In addition to varying the chain lengths of the model lipids, we chose to focus on oleic acid-derived molecules, as oleic acid is the most abundant acyl chain in OAHFAs and DiEs in the TFLL. In this study, we therefore opted to synthesize a library of structural analogues of naturally occurring OAHFAs and DiEs which would allow mapping of their biophysical properties and structure–activity relationships on an unprecedented level. The biophysical properties of the synthesized lipids were evaluated using a Langmuir trough system. In terms of understanding the role of lipids in the TFLL and their connection to DES, the spreading properties and evaporation resistance were considered to be key factors. We therefore measured surface pressure and surface potential isotherms accompanied by imaging with Brewster angle microscopy (BAM) for each synthesized lipid. Furthermore, the evaporation resistance of films formed by each lipid was determined. In addition to the effects of different structural features on the properties of the lipids, our study shows that OAHFAs have antievaporative features and may offer protection against the development of DES, whereas DiEs are able to spread and form monolayers on the aqueous surface but lack antievaporative properties.

The isolation of pure substrates from the tear film is a tedious task because of the variation of lipid compositions between patients, the challenges of collecting sufficient quantities of meibum from patients, and the large number of lipid species found in the TFLL. In this study, we therefore opted to synthesize a library of structural analogues of naturally occurring OAHFAs and DiEs which would allow mapping of their biophysical properties and structure–activity relationships on an unprecedented level. The biophysical properties of the synthesized lipids were evaluated using a Langmuir trough system. In terms of understanding the role of lipids in the TFLL and their connection to DES, the spreading properties and evaporation resistance were considered to be key factors. We therefore measured surface pressure and surface potential isotherms accompanied by imaging with Brewster angle microscopy (BAM) for each synthesized lipid. Furthermore, the evaporation resistance of films formed by each lipid was determined. In addition to the effects of different structural features on the properties of the lipids, our study shows that OAHFAs have antievaporative features and may offer protection against the development of DES, whereas DiEs are able to spread and form monolayers on the aqueous surface but lack antievaporative properties.

The isolated yields varied between 57 and 82%. The yields were slightly lower than those previously obtained by Raghunanan et al. in their synthesis of shorter DiEs;14 however, our reactions were performed under near conditions which can be considered advantageous. Although the double bond in oleic acid was reported to undergo isomerization when sulfuric acid was used as the catalyst,14 isomerization did not take place with sodium bisulfate as confirmed by the detailed nuclear magnetic resonance (NMR) spectroscopic characterization of the products. In this study, we did not seek to optimize the yields of the DiE synthesis, but we did note that increasing the amount of oleic acid had a substantial impact on the yield, although the reaction time had little influence.

With the synthesis of the DiEs completed, we turned our attention to the construction of model OAHFAs. A number of papers have recently appeared covering the synthesis of OAHFAs.15–19 We chose to use an approach similar to the one described by Balas et al.18 In their approach, the synthesis of a monoacylated diol (OAHFA analogue) was conducted directly in one step without the use of exhaustive protection—deprotection reactions as previously reported by others.18 Although the yields were excellent in the previously reported direct monoacylation reaction, we decided to investigate if this

![Scheme 1](https://example.com/scheme1.png)

**Scheme 1**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid (2.4–3 equiv), diol (n = 8, 12, 15 or 20), NaHSO₄·H₂O (3.5 mol %), 100 °C, ~0.3 mbar, 2.5 h.</td>
<td>8-DiE, n = 8; 12-DiE, n = 12; 15-DiE, n = 15; 20-DiE, n = 20</td>
<td>82%–85%</td>
</tr>
<tr>
<td>Oleic acid (1.2 equiv), diol (n = 8, 12, 15 or 20), NaHSO₄·H₂O (3.5 mol %), 100 °C, 0.3 mbar, 2.5 h.</td>
<td>12-OAHFAL, n = 11; 15-OAHFAL, n = 14; 20-OAHFAL, n = 19</td>
<td>63%–67%</td>
</tr>
<tr>
<td>Jones reagent (2.2 equiv), acetone, 0 °C, 0.5 h.</td>
<td>12-OAHFA, n = 11; 15-OAHFA, n = 14; 20-OAHFA, n = 19</td>
<td>57%–85%</td>
</tr>
</tbody>
</table>

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Left: schematic view of the eye, highlighting the tear film. Right: special lipid classes found within the TFLL.
The alternative approach, and the one we chose, is to study the lipids at the air–water interface resembling the aqueous layer of the tear film, and their connection to DES. Neuronally providing information on their role in the TFLL and biophysical properties on a molecular level, thus simultaneously studying the spreading behavior of each synthesized lipid at the air–water interface was studied using a Langmuir trough. Langmuir monolayers were measured at 35 °C, and the polar ester groups were expected to be oriented toward the subphase, whereas the hydrocarbon chains would orient toward the air. 8-DiE, 12-DiE, and 15-DiE readily formed a monolayer on the PBS subphase at 35 °C. In contrast, 20-DiE did not show any surface activity at 35 °C, and therefore the surface pressure and potential isotherms were recorded above its melting point (41 °C) at 35 °C. At large mean molecular areas, the DiEs (Figure 2) showed no increase in surface pressure, and fluctuations in surface potential between 0 and 300 mV were observed. This indicated the coexistence of the gas and liquid monolayer phases, which was confirmed by the corresponding BAM images, showing the fluid light and dark regions, corresponding to the liquid and gas monolayer phases, respectively (Figure 2(i)). As the film was compressed, a transition to the liquid phase occurred, accompanied by an increasing surface pressure and homogeneous film appearance in the BAM images (Figure 2(ii)). A surface pressure lift-off occurred at similar areas for 8-DiE, 12-DiE, and 15-DiE (113–125 Å²/molecule). As the lift-off area was close to that of 1,2-dioleoyl-sn-glycero-3-phosphocholine and depended weakly on the length of the diol linker, it is likely that the linker adopts a coiled conformation rather than lying flat on the water surface. Using the surface potential at lift-off, molecular dipole moments of 910, 60, 900, and 870 ± 80 mD can be calculated for 8-DiE, 12-DiE, and 15-DiE, respectively. As the measured dipole moment is approximately 2 times larger compared to the expanded films of wax esters (see the Supporting Information) and fatty acid ethyl and butyl esters, both the ester groups appear to be oriented toward the aqueous subphase at lift-off in all of the DiEs. An increase of 15–30 mV was observed in surface potential during the compression of these DiEs, indicating that no major rearrangement of the ester groups occurred before collapse. The films of

![Figure 2. Surface pressure and surface potential (mean ± standard deviation (SD)) isotherms of synthesized DiEs. Representative BAM images from 8-DiE (i–iii) are shown with a scheme describing the molecular arrangement in each case. Blue circles represent ester groups and lines represent hydrocarbon chains. 8-DiE, 12-DiE, and 15-DiE were measured at 35 °C. 20-DiE was measured at 43 °C.](https://doi.org/10.1021/acs.langmuir.8b04182)
8-DiE, 12-DiE, and 15-DiE collapsed at relatively low surface pressures (2.5–8 mN/m), shown by a surface pressure and potential plateau, as well as the appearance of bright aggregates in the BAM images (Figure 2(iii)).

In contrast to the shorter DiEs, 20-DiE showed a small increase in surface pressure (~1 mN/m) at 100 Å²/molecule. However, based on the BAM images, the 20-DiE film was partially collapsed directly after spreading (not shown). This is in line with the trend relating a decreasing collapse pressure to the increasing chain length as observed for shorter DiEs, which reflects the increased intermolecular van der Waals forces between DiEs compared to the interactions between the ester groups and water. Therefore, DiEs with 56 carbons or more are unlikely to be surface-active enough to form stable monolayers.

This is a highly relevant information considering the TFLL, as the TFLL DiEs contain 62–72 carbons. Although a majority of TFLL DiEs contain an additional double bond in the linker compared to the DiEs studied here, this is unlikely to have a strong effect on the overall van der Waals forces between the DiEs in a disordered state. Therefore, DiEs are likely to form a part of the nonpolar layer of the TFLL and require the presence of more polar lipids to spread on the aqueous tear film surface.

2.2.2. OAHFAs. OAHFAs were found to spread readily on the PBS subphase and displayed a considerably higher surface activity than DiEs. Surface pressure started increasing at ~120 Å²/molecule along with an increase in surface potential for all the studied OAHFAs. The large lift-off area (close to the lift-off area of DiEs with a corresponding linker) suggests that in the expanded phase, OAHFAs were lying flat on the subphase surface with both the ester group and the carboxylic acid group interacting with the aqueous phase (see Figure 3 scheme i). In the case of 12-OAHFA, no phase transitions were observed during compression, and a homogeneous film with increasing intensity was observed in the BAM images (Figure 3(i–iii)). This indicates that the thickness of the film increased as the molecules were packed closer together, but the film remained in a disordered liquid expanded-like phase. In contrast, 15-OAHFA and 20-OAHFA displayed a liquid–solid phase transition demonstrated by a plateau in the surface pressure starting at 70 and 100 Å²/molecule, respectively. During the plateau, formation of crystalline, high-intensity domains were observed in the BAM images (Figure 3(v)). At a limiting mean molecular area of approximately 20 Å²/molecule, corresponding to the cross-sectional area of an acyl chain, the entire film appeared to be solid (Figure 3(vi)). This phase transition was accompanied by a marked decrease in surface potential, indicating a reorientation of the electric dipoles in the molecules.

The decrease in potential is likely caused by the reorientation of the less polar ester groups away from the water surface, whereas the acyl chains assume an upright orientation perpendicular to the surface in a tightly packed ordered array during the phase transition (see Figure 3 schemes v,vi). The reorientation of the carboxylic acid group is unlikely to have a large contribution to the surface potential shift, as the dipole moment of the fatty acid monolayers has been observed to be almost constant in the expanded and condensed phases. Interestingly, a similar decrease in surface potential was observed in 12-OAHFA, although no phase transition was apparent. This indicates that the shift in the surface potential was not largely affected by the crystalline ordering of the OAHFAs but represented the general orientation of ester groups away from the water surface (see Figure 3, scheme iii). As the surface potential decreases to approximately zero in all the OAHFAs, the ester group appears to have a negative contribution to the surface potential in the compressed state, indicating that the carbonyl oxygen is directed slightly toward the air upon compression.

The lower phase transition surface pressure of 20-OAHFA (1.5 mN/m) compared to 15-OAHFA (15 mN/m) can be explained by the stronger intermolecular van der Waals interactions caused by the increased chain length. By extrapolating the data presented here, we can assume that oleate-based OAHFAs with a saturated linker chain longer than 20 carbons are likely to form only solid monolayers under physiological temperatures, whereas OAHFAs with 12 or less carbons in the linker chain are likely to form only liquid phases.

Our findings are in accordance with previous studies on OAHFAs but provide several additional details and clarifications. The preliminary molecular modeling by Butovich proposed that both carboxylic acid and ester groups would interact with water. An experimental study by Schuett and...
Millar\textsuperscript{19} on the surface properties of O-oleyl-\(\omega\)-hydroxy palmitic acid suggested that the ester groups would orient away from the aqueous surface upon compression. The evidence at the time was questionable because the conclusions were drawn based on the surface pressure measurements of a single OAHFA. Nevertheless, it was interpreted that the surface pressure plateau, like that observed in 15-OAHFA (see Figure 3), is caused by the loss of ester groups from the aqueous surface. The results presented here, including the surface potential isotherms and BAM imaging, show that this interpretation is not entirely accurate. Although the surface potential measurements confirm that ester group reorientation does occur, the surface pressure plateau is caused by a liquid–solid phase transition, and reorientation of the ester groups can occur also without such a transition, like in the case of 12-OAHFA.

These results also provide insight into the role of OAHFAs in the TFLL. OAHFAs were shown to readily spread and form monolayers that are stable upon compression to high surface pressures (>30 mN/m) in physiological conditions. This indicates that OAHFAs mainly reside in the polar sublayer between the mucoaqueous layer and the nonpolar layer formed by nonpolar tear film lipids. In addition, our results show that OAHFAs can form a solid monolayer with crystalline structure. A crystalline order has also been detected by using X-ray diffraction to study meibum, the waxy secretion that forms the TFLL, on the water surface,\textsuperscript{1,32} but the lipids forming the monolayers that are stable upon compression to high surface pressures (>30 mN/m) in physiological conditions. This solid phase transition, and reorientation of the ester groups can form an ordered phase on the water surface.\textsuperscript{24} OAHFAs can form a solid monolayer with crystalline structure. A crystalline order has also been detected by using X-ray diffraction to study meibum, the waxy secretion that forms the TFLL, on the water surface,\textsuperscript{1,32} but the lipids forming the monolayers that are stable upon compression to high surface pressures (>30 mN/m) in physiological conditions. This solid phase transition, and reorientation of the ester groups can form an ordered phase on the water surface.\textsuperscript{24}

2.2.3. OAHFALS. OAHFALS showed similar surface pressure and potential isotherms as OAHFAs, and their organization at the aqueous surface is likely to be similar to that presented in the Figure 3 schemes.

In the case of 12-OAHFAL and 15-OAHFAL, initial increases in surface pressure and surface potential were observed along with a homogeneous film appearance in BAM images (Figure 4(i,iv)), indicating a liquid monolayer phase. Phase transition plateaus were observed in the surface pressure isotherms at 22 and 5 mN/m for 12-OAHFAL and 15-OAHFAL, respectively. The phase transition of 12-OAHFAL involved only a small change in mean molecular area, from 34 to 25 Å\(^2\)/molecule, and the phase boundary appeared rounded in the BAM images (Figure 4(ii,iii)), suggesting that this was likely a liquid-expanded–liquid-condensed-like phase transition. In contrast, the 15-OAHFAL phase transition started at 80 Å\(^2\)/molecule, and crystalline domains with dendritic “snowflake” patterns were observed in the BAM images during the phase transition (Figure 4(v,vi)). This is an indication of a clear liquid–solid monolayer phase transition. Similar to OAHFAs, a decrease in surface potential was observed. This is likely because of the similar reorientation of the ester groups as discussed for the OAHFAs above.

In contrast to the shorter OAHFALS, 20-OAHFAL formed a solid monolayer phase directly at 35 °C, as can be seen from the sharp increase in surface pressure close to 20 Å\(^2\)/molecule and the solid crystalline domains observed in the BAM images (Figure 4(vii, viii)). Interestingly, the surface potential of 20-OAHFAL also increased with compression and reached a markedly higher value (~260 mV) compared to the shorter OAHFALS (100–120 mV). This indicates that the molecular conformation in the solid phase of 20-OAHFAL, formed directly by spreading from the solvent, differs from the solid phases formed by compression, possibly because of a different tilt angle or conformation of the ester groups.

The polar head group effects can be evaluated by comparing OAHFALS with the equivalent OAHFAs. The results show that the phase transitions occur at lower surface pressure and higher area in OAHFALS. This is likely because of the lower acidity of the hydroxyl group compared to the carboxylic acid group, which is partially charged under the conditions employed. The negative charges on the carboxyl groups cause repulsion between neighboring molecules, lowering the tendency of OAHFAs to pack tightly together compared to OAHFALS.

2.3. Evaporation Resistance. For the TFLL to effectively prevent the excess evaporation of water leading to drying of the ocular surface, two conditions should be fulfilled. First, the lipids need to be able to spread effectively to cover the mucoaqueous tear film, once the eyelids are opened after a blink. Second, the lipid film must effectively retard the passage of water molecules through the film. As all the synthesized lipids apart from 20-DiE formed stable monolayers, we then measured the evaporation resistance of each lipid at several different mean molecular areas in the 12–30 Å\(^2\)/molecule range using a modified version of the method proposed by Langmuir and Schaefer.\textsuperscript{53} A very low evaporation resistance (<0.2 s/cm) was observed for all DiEs, 12-OAHFA, and 12-OAHFAL (Figure 5). This
was expected for the DiEs because 8-DiE, 12-DiE, and 15-DiE collapsed at low surface pressures and did not form condensed films, whereas 20-DiE did not spread as a monolayer at 35 °C. 12-OAHFA did not form a condensed phase, which likely explains the lack of evaporation resistance. Interestingly, 12-OAHFAL also showed no evaporation resistance, although a transition to a condensed phase was observed for this lipid. It is likely that the condensed phase of 12-OAHFAL is more disordered compared to the longer OAHFAs and OAHFALs, as suggested by its larger area of collapse (26 Å²/molecule) and the accompanying BAM images (Figure 4(ii,iii)).

A clear evaporation resistance, however, was observed for the longer OAHFAs and OAHFALs. The evaporation resistance increased sharply and leveled off at a mean molecular area of approximately 18 Å²/molecule for all these lipids, corresponding to the solid phase of each lipid. Formation of a tightly packed hydrocarbon layer is necessary to significantly slow the permeation of water through lipid monolayers. A higher evaporation resistance (5 s/cm) was observed for 20-OAHFA than 15-OAHFA (2 s/cm). This is in line with the previous observations for a wide array of evaporation-resistant lipids, which show that an increasing chain length is associated with a higher evaporation resistance. In contrast, a lower evaporation resistance was observed for 20-OAHFAL than 15-OAHFAL. This is likely because of the absence of an expanded phase in 20-OAHFAL, resulting in a poor spreading and an incomplete coverage of the aqueous subphase surface.

Taken together, these results show that OAHFAs and OAHFALs form evaporation-resistant monolayers, and evaporation resistance appears to be maximized by increasing the chain length of the molecules but only up to the point that an expanded monolayer phase still exists. To consider the potential clinical relevance of the measured evaporation resistances, we can calculate the reduction in evaporation in typical conditions by using the model by Cerretani et al. While standing still or walking, an evaporation resistance of 2 s/cm would slow down the evaporation from the ocular surface by 33–50%, whereas that of 5 s/cm would result in a 60–80% lower evaporation rate. These kinds of reductions are clearly clinically relevant, suggesting that OAHFAs may have a central role in maintaining the evaporation resistance of the TFLL. This also underlies using TFLL thickness measurements as a diagnosis method for DES, as most of the TFLL thickness is made up by nonpolar lipids. However, a monolayer of OAHFAs is enough to form an effective evaporation-resistant layer, but the evaporation resistance is lost completely if the surface concentration of OAHFAs decreases below a critical limit (see Figure 5). This would imply that the quantity and quality of OAHFAs and other lipids participating in the formation of the polar lipid sublayer are more important than the TFLL thickness. The evaporation resistance originating from the polar lipid sublayer would also explain why the TFLL thickness only affects the water evaporation rate from the ocular surface in cases where the TFLL is almost completely absent, as well as the clinical reports conflicting the idea of reduced evaporation through thick lipid layer regions.

3. CONCLUSIONS

We set out to study the biophysical properties of specific TFLL lipids (OAHFAs and DiEs) to shed light on their role in the TFLL and their connection to DES. To map the structure–activity relationship for these lipids on an unprecedented level, a library containing 10 structural analogues of naturally occurring OAHFAs and DiEs was successfully synthesized and utilized in the biophysical testing. The lipid library contained variations in chain lengths and functional groups as these had previously been indicated to be of importance. In the surface chemical testing, we focused on analyzing the spreading behavior and molecular arrangements of these lipids at the tear–air interface, as well as the evaporation resistance of the monolayers formed, as these two factors are essential to healthy eyes and are potentially compromised in patients suffering from DES.

In the case of DiEs, increasing the linker length did not alter the phase behavior, instead, it led to a decrease in surface activity, likely rendering the DiEs with more than 20 carbons in the linker chain nonsurface-active under physiological conditions. This implies that tear film DiEs are part of the nonpolar lipid pool and require polar lipids to form stable films on the water surface.

In contrast, several different surface phases were observed for the more polar OAHFAs and OAHFALs. The OAHFAs and OAHFALs with 12-carbon linkers remained in a liquid monolayer phase upon compression, although a liquid condensed phase transition was apparent in 12-OAHFAL. A liquid–solid phase transition was observed in 15-OAHFA, 20-OAHFA, and 15-OAHFAL, whereas 20-OAHFA formed a solid phase directly upon spreading. By using surface potential measurements, we were able to confirm that during the compression of the OAHFA and OAHFAL films, a conforma-
tional change occurs, where the chain orients upright, detaching the ester group from the water surface and orienting it slightly toward the air. Furthermore, we showed that OAHFAs and OAHFALs form evaporation-resistant films, with 20-OAHFA having the highest evaporation resistance (5 s/cm). This supports the hypothesis that OAHFAs are key lipids in ensuring the proper function of the TFLL. Increasing the chain length of OAHFAs was found to lead to predominantly solid films. Therefore, the double bond found in the linker chain of the ultra-long-chain OAHFAs found in the TFLL may be important in maintaining their effective spreading on the aqueous surface.

Because of their surface activity, evaporation resistance, and structural similarity to naturally occurring tear film lipids, the OAHFAs and OAHFALs described herein are potential candidates for improved lipid-based dry eye interventions aiming to restore the proper function of the TFLL. A further benefit of these model compounds is that their synthetic routes are short (one to two steps) when compared to the multistep synthetic pathways (≥10 synthetic steps) required for the synthesis of naturally occurring OAHFAs.25,26 Although this study elucidated the role of OAHFAs and DiEs in the TFLL, further studies are needed to investigate their interactions with the major tear film lipids. One of the major components, wax esters, resemble OAHFAs structurally and have also been found to form evaporation-resistant films but exhibit limited spreading properties.24,26 It is possible that OAHFAs interact with wax esters to form an evaporation-resistant layer at the interface of the mucous layer and bulk of the nonpolar TFLL.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b04182.

Experimental protocols and analytical data (PDF)

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Author Contributions

The research was planned and the manuscript written through contributions of all authors. All authors have given their approval on the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS**

BAM, Brewster angle microscope; DES, dry eye syndrome; DiE, diester; ESI, electrospray ionization; DQF-COSY, double-quantum filtered correlation spectroscopy; HRMS, high-resolution mass spectrometry; HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum coherence; MS, mass spectrometry; NMR, nuclear magnetic resonance; OAHFA, O-acyl-ω-hydroxy fatty acid; OAHFAL, O-acyl-ω-hydroxy fatty alcohol; rt, room temperature; TAG, triacylglycerides; TFLL, tear film lipid layer; TMS, tetramethylsilane; TOF, time-of-flight spectrometer; WE, wax ester

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