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Synthesis and Characterization of Nonconventional Surfactants of Aromatic Amino Acid–Glycerol Ethers: Effect of the Amino Acid Moiety on the Orientation and Surface Properties of These Soap-Type Amphiphiles

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Abstract: The synthesis, characterization, and surface properties of soap-type amphiphiles comprising alkyl chains of 10–16 carbon atoms linked through an ether group to a glycerol–amino acid hydrophilic head group is described. The surface properties of members of this series derived from histidine and tyrosine were compared with those of phenylalanine and tryptophan derivatives described previously and with those of conventional soaps. In all cases, the amino acid derivatives showed superior surface properties, and an interesting differentiation was discovered regarding the orientation of tryptophan derivatives.

Keywords: amphiphiles · aromatic amino acids · surface chemistry · surfactants · synthesis

Introduction

Recently, the nonconventional, ecofriendly surfactants have become of great importance in applications, such as detergency, emulsification, and wetting, as well as in other personal care formulations.[1,2] The classic soap is still considered absolutely ecological as it possesses better biodegradability and is milder to the skin than the commercial surfactants derived from petrochemicals.[2b–f] However, the fatty acid salts, namely soaps, have drawbacks, such as poor surface-active properties, low solubility in neutral cold water, and formation of insoluble calcium salts in hard water.[3] Nevertheless, the above surfactants are very suitable for the study of the orientation of amphiphiles at interfaces as well as for the aggregation and micellar formation in solutions of different pH ranges.[4–7] Moreover, soap-type surfactants including a moiety, such as a peptide linkage[8] or a 1,3-dioxolane ring[3], in addition to the hydrophilic carboxylic group, possess enhanced surface properties and solubility and have, therefore, been proposed as alternatives to the classic soaps.

As such alternatives, the two nonconventional, homologous series of carboxylic-acid salts from the aromatic amino acid tryptophan and phenylalanine conjugated to glycerol through an amide bond were recently synthesized, characterized, and studied.[9,10a] In these compounds the hydrophobic alkyl chain is conjugated to glycerol through an ether bond, giving an advantage over the hydrolysable ester bond of glycerol esters.[11,12] Although these surfactants are soap-type sodium salts, due to the carboxylic group of the amino acid, they possess very low critical micelle concentrations (CMC) and low corresponding surface tensions (γ_{cmc}), compared to conventional soaps.[3,9a] They also gave very promising results upon application as wetting agents in the mercerization of cotton, as cleansing agents, and as emulsifiers in oil/water emulsions. This encouraged us to continue the synthesis of analogous surfactants with the remaining two aromatic amino acids, that is, histidine and tyrosine, thus, completing the whole family. Moreover, the amino acids are generally key intermediates in enzymic redox reactions.[13] The tryptophan itself plays an important role in the study of structural changes of proteins and as a sensor for Ca^{2+} ions.[13] Thus, we expect that the aromatic amino acids as moieties of amphipilic molecules could facilitate these studies in aqueous solutions.

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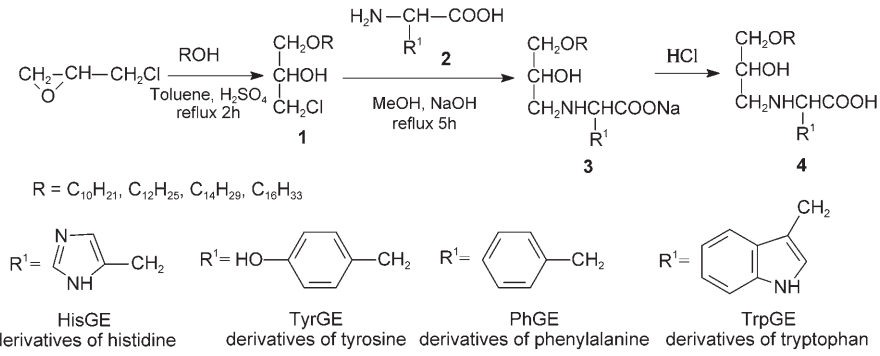
Results and Discussion

Synthesis and characterization: The new homologous series, derivatives of histidine and tyrosine with 10, 12, 14, or 16 carbon atoms in the hydrophobic alkyl chain, namely HisGE and TyrGE, were synthesized in good yields (63–72%) according to Scheme 1.

The starting compounds, chlorides **1**, synthesized by reaction of epichlorohydrin with the corresponding fatty alcohols, reacted as alkylating agents with the amino acids **2** to give, after acidification of the initially obtained sodium salts **3**, the target amphiphiles **4** in satisfactory yields. Direct selective monoalkylation of amino acids and their esters is not generally applicable and most alkylation methods referred to in the literature deal mainly with N-methylation reactions. For alkylations other than methylation, procedures via intermediate-activated nitrophenylsulfonyl derivatives are usually employed.[14] The relative reactivity of **1** towards amino acids may be ascribed partially to the neighboring-group assistance of the hydroxyl group. Participation of the hydroxyl group has been invoked for the ease of quartenization of secondary amines with epichlorohydrin.[15,16] However, attempted alkylation reactions of **1** with a series of amino acids missing an aromatic moiety, such as alanine, glutamine, isoleucine, arginine, proline, and valine, failed to give any product, indicating that structural features of the amino acid also affect the course of the reaction.



Scheme 1. Synthesis of the aromatic amino acid–glycerol ether surfactants.

The observed differences in reactivities between aromatic and nonaromatic amino acids can be explained by examining the conformations around the bond connecting the chiral carbon and the methylene carbon. As depicted in Figure 1, on the basis of stereochemical reasons only, the less-hindered conformations II and III are expected to be the most stable. In the case of aromatic amino acids, the presence of sodium cations in the reaction medium is expected to affect further the stability of the conformers, because of the well-documented interaction of aromatic side chains of amino acids as neutral donor groups for alkali metal cations.[17–21] In conformation III, cooperation of the aryl group with the dominant carboxylate-anion donor to sodium binding is expected to cause a further stabilization. By combining steric factors and binding-metal interactions, conformer III is expected to be the most favorable. In this conformation, the amino group is less hindered and is more reactive towards a primary halide, such as **1**, in an S_N2-type substitution-reaction process.

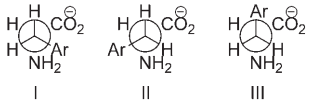


Figure 1. Conformations of aromatic amino acids around the C_a–C_b bond.

The IR spectra for all compounds **4**-HisGE and **4**-TyrGE show absorptions in the region 3400–3244 cm^{-1} due to OH and NH stretching vibration, as in the series of TrpGE and PhGE derivatives.[9,10] Furthermore, absorption peaks at 1580 and 1610 cm^{-1} suggest the existence of carboxylate anion (COO^-) and alkylamine ($^+NH_2R$) as a zwitterion.

The ^1H NMR spectra are in accordance with the proposed structures and contain the expected chemical shifts for both the alkyloxypropanol and amino acid moieties. Due to the restricted solubility of the compounds, the spectra were recorded in alkaline methanol, thus, the labile protons (OH, NH, COOH) do not appear in the spectrum. The histidine derivatives (HisGE) exhibit two singlets at $\delta = 6.83$ and 7.51–7.53 ppm for the imidazole protons, whereas the tyrosine derivatives (TyrGE) exhibit two doublets at $\delta = 6.54$–6.62 and 6.92–7.01 ppm for the aromatic-ring protons. The aliphatic-chain protons give the expected peaks in the region 0.89–1.55 ppm. The protons attached to the heteroatoms and the methylene protons next to the aromatic ring give a series of complex and broad multiplets in the region 2.45–3.85 ppm. Due to the complexity and the broadening of the multiplets, a complete assignment of these protons was not possible in most cases. Indicatively for the TyrGE-10 derivative, a more detailed assignment based on decoupling experiments carried out on a sample taken in D_2O/KOH solution was possible. Thus, the methylene protons next to the nitrogen give a multiplet at $\delta = 2.45$–2.70 ppm, the methylene protons next to the aromatic ring give a multiplet at $\delta = 2.75$–2.85 ppm, the methine proton next to the carboxyl gives an almost-triplet at $\delta = 3.26$ ppm, the methylene protons next to oxygen give a multiplet at $\delta = 3.35$–3.36 ppm, and the methine proton next to oxygen is the most deshielded and gives a symmetric multiplet centered at $\delta = 3.88$ ppm. The ^{13}C NMR spectra are clearer. All the carbons give characteristic chemical shifts close to the expected values, as assigned in the experimental part. Notably, some of the carbons give two peaks (values separated by slashes

in the experimental part) corresponding to the two possible diastereoisomeric structures. Because compounds **3** were obtained from the reaction of a racemic chloride with the L-amino acid, they must be mixtures of two diastereoisomers (DL and LL).

Surface-active properties of surfactants 4: All compounds **4** are insoluble in aqueous solution between pH 4–10. Consequently, the surface-active study of these compounds was undertaken in alkaline solution at pH 12 and it is assumed that at this pH surfactants **4** behave as carboxylates **3**, that is, as anionic surfactants.

Figures 2 and 3 show plots of the surface tension (γ) versus log of surfactant concentration for all surfactant ho-

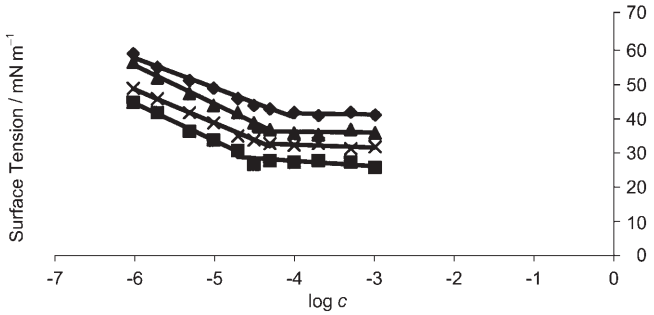


Figure 2. Surface tension versus log$c$ of compounds **4**-HisGEs in aqueous solutions at pH 12. ◆: R = $C_{10}H_{21}$, ▲: R = $C_{12}H_{25}$, ×: R = $C_{14}H_{29}$, ■: R = $C_{16}H_{33}$.

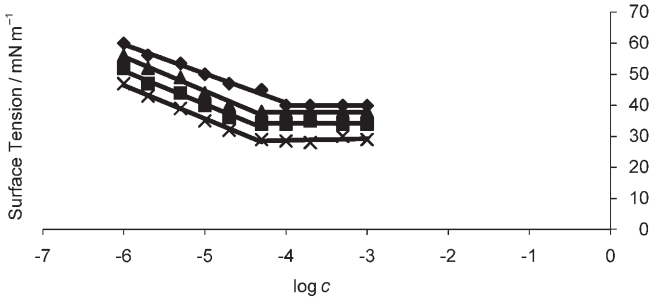


Figure 3. Surface tension versus log$c$ of compounds **4**-TyrGEs in aqueous solutions at pH 12. ◆: R = $C_{10}H_{21}$, ▲: R = $C_{12}H_{25}$, ■: R = $C_{14}H_{29}$, ×: R = $C_{16}H_{33}$.

mologues **4**-HisGE and **4**-TyrGE, derivatives of histidine and tyrosine, respectively. The absence of a minimum in the curves reflects the high purity of the new compounds. The surface-active parameters, such as critical micelle concentration (CMC), surface tension corresponding to this concentration (γ_{CMC}), and efficiency of absorption (pc_{20}) were determined from the curves of Figures 2 and 3. The values of surface excess (Γ_{max}) and area per molecule (A_{min}) were calculated by using the Gibbs adsorption Equations (1) and (2):[9a,10a,b]

$$\Gamma_{max} = -\frac{1}{2.303nRT}\left(\frac{\partial\gamma}{\partial \log c}\right) \quad (1)$$

$$A_{min} = \frac{10^{23}}{N\Gamma_{max}} \quad (2)$$

in which ($\partial\gamma/\partial\log c$) is the maximum slope in each case; T= absolute temperature; $n=1$ (in a 1:1 ionic surfactant in the presence of a 1:1 electrolyte[10b]), $R=8.31\ J\,mol^{-1}\,K^{-1}$, and N is Avogadro's number.

The CMC values of compounds **4**-HisGE and **4**-TyrGE show a fairly linear decrease as the number of methylene groups in the alkyl chain is reduced. This is expected from the increase in hydrophobicity, evident in Figure 4, that is

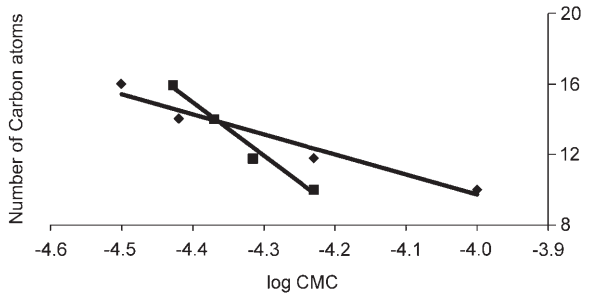


Figure 4. Relationship between log CMC (critical micelle concentration) and the alkyl-chain length of the **4**-HisGEs (■) and **4**-TyrGEs (◆).

common to analogous compounds[9a,10a] and that is observed usually in homologous series of conventional[10d] as well as nonconventional surfactants.[10a,d]

As in a conventional series of homologues, the efficiency of absorption, pc_{20}, increases as the number of carbon atoms increases. The larger the pc_{20} value, the more efficiently the surfactant is absorbed at the interface and the more efficiently it reduces surface tension. This increase is fairly linear, as seen in Figure 5 for surfactants **4**-HisGEs and **4**-TyrGEs, as it occurs in other carboxyl systems.[9a,10a]

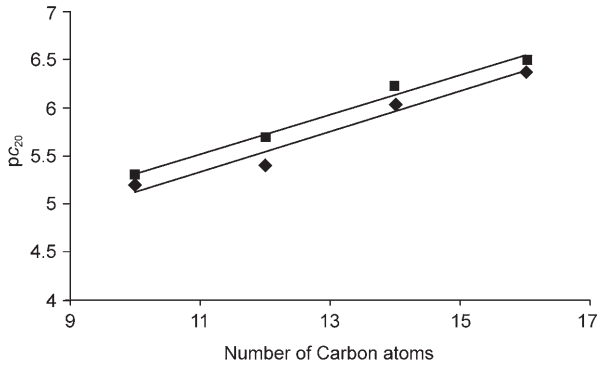


Figure 5. Plots of pc_{20} versus the number of carbon atoms in surfactants **4**-HisGEs (■) and **4**-TyrGEs (◆) (pc_{20}=log of surfactant concentration required to reduce the surface tension of the solvent by 20 mN m^{-1}).

All the physicochemical parameters of the new surfactants HisGE and TyrGE, and for comparison, those of TrpGE and PhGE synthesized previously, are listed in Table 1.

Table 1. Surface-active parameters for surfactants **4** of all four homologous series in aqueous solution at pH 12 and $T=25\,°C$.

Compound	CMC [$\times 10^5$ mol dm^{-3}]	γ_{CMC} [mN m^{-1}]	pc_{20}	A_{min} [Å^2]	T_K [°C]
R = $C_{10}H_{21}$					
TrpGE[a]	36.00	33.00	4.70	67.00	50
TyrGE	10.00	40.00	5.30	91.90	52
PhGE[b]	4.80	32.30	6.15	97.00	50
HisGE	5.80	42.20	5.20	100.00	54
R = $C_{12}H_{25}$					
TrpGE[a]	3.80	32.00	5.50	51.00	56
TyrGE	5.80	38.00	5.70	80.00	52
PhGE[b]	3.50	30.00	6.39	103.00	51
HisGE	4.80	36.90	5.40	100.00	54
R = $C_{14}H_{29}$					
TrpGE[a]	2.00	30.50	6.10	64.00	52
TyrGE	3.80	34.00	6.20	106.00	53
PhGE[b]	1.00	28.75	7.00	103.00	52
HisGE	4.31	32.50	6.00	104.50	55.5
R = $C_{16}H_{33}$					
TrpGE[a]	1.70	30.00	6.30	66.00	53
TyrGE	3.10	28.00	6.50	114.00	54.5
PhGE[c]	0.91	28.40	7.05	118.00	53
HisGE	3.70	28.10	6.4	106.00	56

[a] Values from ref. [9a]. [b] Values from ref. [10a]. [c] Value from ref. [10a], for the member with R = $C_{15}H_{31}$.

According to the molecular structure, the most hydrophobic surfactants were expected to be those of the PhGE series. This is evident from the data in Table 1, which shows that for the same alkyl chain, phenylalanine derivatives have the lowest CMC and γ_{cmc}, and the highest pc_{20} values.

The new surfactants have much lower CMC values than the typical soaps. Thus, in alkaline solution, the CMC of the sodium laurate is approximately 2×10^{-2} M,[3,9a] whereas the CMCs of all the compounds studied are of the order 10^{-5} M. These values are also much lower than sodium salts of *N*-acylamino acids.[8a] This behavior is probably due to the presence of the aromatic moiety of the amino acids, which increases significantly the hydrophobicity of the new surfactants and their ability to create micelles in the bulk solution at very low concentrations. This is very effective in applications of detergency and wetting.[3] The γ_{cmc} of the members with 12 carbon atoms in the hydrophobic alkyl chain ranges between 30 and 38 mN m^{-1}; the lowest value (30 mN m^{-1}) is that of the most hydrophobic PhGE derivative, and the value of the sodium laurate is 37.5 mN m^{-1}.[3] Similarly, the Krafft point, known as the temperature at which the solubility is equal to the CMC and can be regarded as the temperature at which micelles become soluble,[3,10b] ranges between 50 and 56 °C for all members of the four homologous series. These values are higher than the value 19 °C for the abovementioned soap under the same conditions,[3] in accordance with the higher hydrophobicity and consequently lower solubility of the studied compounds.

Area per molecule (A_{min}) and orientation of surfactants at the air/water surface: Surprisingly, as evident from Table 1, TrpGE derivatives have the lowest A_{min} values, in a range 51–67 Å^2 close to that of sodium laurate (69 Å^2[3]), whereas all the derivatives of the three other amino acids have higher A_{min} values in a range 80–118 Å^2. However, according to the hydrophobicity of the new surfactants, it could be expected that the PhGEs should posses the lowest A_{min} values, as they are more hydrophobic.

Because the area per molecule is mainly determined from the cross-sectional area of the hydrophilic group at the interface, these findings suggest that the cross-sectional area is smaller in tryptophan derivatives. For the PhGE and TyrGE derivatives, the orientation 1 in Figure 6 is suggested, in

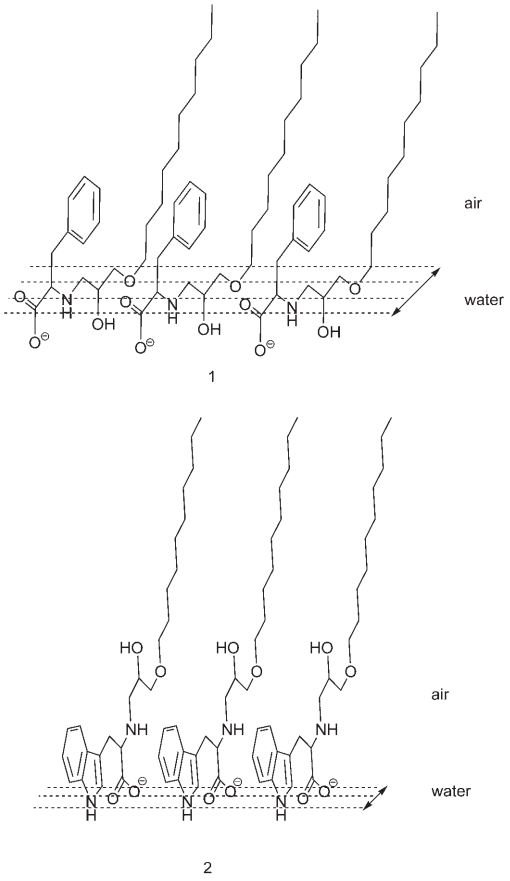


Figure 6. Orientation of surfactants PhGE (1) and TrpGE (2) at the air/water surface.

which all hydrophilic centers are in contact with the aqueous layer. In contrast, for TrpGE derivatives, the orientation 2 is the most probable, in which the indole moiety is in contact with the aqueous phase. Indole has been proved to be an effective polar head group for vesicle formation, and tryptophan residues are also known to work as membrane anchors in proteins.[22–25]

Concerning the HisGE members, orientiations like those of TrpGE members would be adopted, in which the imidazole ring, similarly to indole, is in conduct with water

through one of its nitrogen atoms (Figure 6, example 2). However, A_{min} values of HisGE are closer to those of PhGE and TyrGE, indicative of orientations similar to those of PhGE and TyrGE (Figure 6, example 1) Moreover, to the best of our knowledge, imidazole derivatives do not exhibit behavior analogous to that of indole and tryptophan at water/air interfaces.

Conclusion

The synthesis of glycerol ether–amino acid surfactants containing the aromatic amino acids tryptophan, phenylalanine, tyrosine, and histidine was completed easily in a two-step procedure with very good yields. The ability to apply this simple synthetic procedure for aromatic amino acids, in contrast to aliphatic amino acids that failed to give analogous derivatives, was attributed to the complexation of the amino acid aromatic moiety with alkali ions, which stabilized favorable conformations for substitution reactions.

The new amphiphiles could be characterized as environmentally friendly as they possess, on one hand, the glycerol skeleton and an aromatic amino acid, both existing in natural products available from renewable sources, and on the other hand, a biodegradable linear alkyl chain. Given the biocompatibility of the above surfactants, they are promising candidates for several food, cosmetic, and pharmaceutical applications, especially in conditions of extreme pH and temperature, due to their stable ether bond compared with the hydrolysable ester bond of glycerol esters. Furthermore, by incorporating the amino acids in a micellar environment they could serve as model compounds for theoretical investigations of proteins and peptides in aqueous solutions.

Initial studies of their surface behavior revealed very good surface properties. Thus, the critical micelle concentrations and surface tensions of all members of the four homologous series are significantly lower than the corresponding values of the typical soaps. These findings enhance further the expected applicabilities of these novel compounds in the above-mentioned areas. Another very interesting result was that the values of area per molecule of surfactant for derivatives of tryptophan were significantly lower that those for derivatives of the amino acids phenylalanine, tyrosine, and histidine. A suggestion was made that the more condensed packing of tryptophan derivatives at the water/air surface is due to a different orientation of these molecules, whereby the indole functions as a polar head group with the NH directed toward the water face, in agreement with the known peculiar role of tryptophan residues in stabilizing membrane proteins.

Experimental Section

Materials and methods: Pure epichlorohydrin, aliphatic alcohols with 10, 12, 14, or 16 carbon atoms, pure toluene, sulfuric acid, absolute methanol, and butanol (for analysis) were supplied by Riedel de Häen (Salze, Germany); chloroform and hexane by Merck (Darmstadt, Germany); histidine, tyrosine, glutamic acid, alanine, glutamine, isoleucine, arginine, proline, valine, and glutaminic acid by Panreac PRS (Barcelona, Spain), and silica gel plates by Riedel de Häen.

Thin layer chromatography was employed to monitor the progress of all the reactions by using butanol/acetic acid/ethanol/water 4:2:3:3 as eluent. Compounds **1** were visualized by spraying the TLC plates with a solution of 20% sulfuric acid in methanol, followed by charring on a hot plate for a few minutes. For surfactants **4**, a ninhydrin solution (3 g ninhydrin dissolved in a mixture of 3 mL acetic acid and 97 mL butanol) was applied until it turned violet.

A Perkin–Elmer 2004 II analyzer was used for all elemental analyses. IR spectra were recorded by using a Perkin–Elmer FTIR (Spectrum One) spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75.5 MHz, both by using a Bruker 300 AM spectrometer in (CD_3OD/NaOH) solutions. Chemical shifts are reported in parts per million (δ, in ppm) downfield from tetramethylsilane. All melting points were determined by using a hot-stage Koffler apparatus and were uncorrected.

Synthetic procedures

*General procedure for the synthesis of 3-alkyloxy-1-chloropropan-2-ols **1***: In a round-bottomed flask, aliphatic alcohol (0.01 mmol), epichlorohydrin (0.01 mmol), and concentrated sulfuric acid (0.1 mL) were stirred under reflux in dried toluene for 2 h. The reaction was monitored by TLC. The reaction mixture was evaporated under vacuum and purified by performing silica gel column chromatography (Chromagel 60 A CC, 70–230 mesh) using an eluent of hexane/chloroform→chloroform (3:1→100% $CHCl_3$). Pure compounds **1** were obtained as oily liquids in yields of 40–45%. Their elemental analyses and ^{1}H NMR spectra are described elsewhere.[9a,10a]

General procedure for the synthesis of N-*[3-(alkyloxy)-2-hydroxypropyl] amino acids **4***: A solution of absolute methanol containing **1** and amino acid (ratio, 1:1.5 mmol) and sodium hydroxide (0.5 g, pH 12) was stirred under reflux for 5–6 h. The reaction was monitored on a TLC plate sprayed with ninhydrin solution. The solution was then adjusted with 10% HCl to pH 3 for tyrosine and pH 4–5 for histidine. These were the most appropriate conditions so that the final compounds could be precipitated as white solids without being contaminated by the unreacted amino acid. The solids were filtered, washed three to four times with distilled water, solubilized in alkaline methanol, and reprecipitated with 10% HCl at the exact pH. The final products were obtained analytically pure as white solids.

Under the same conditions, the reactions with the aliphatic amino acids alanine, glutamine, isoleucine, arginine, proline, and valine failed to give any products.

***N*-[3-(Decyloxy)-2-hydroxypropyl]tyrosine (TyrGE-10)**: M.p. 187–188 °C; yield 66%; ^{1}H NMR (CD_3OD/KOH): δ = 0.89 (t, J = 7.1 Hz, 3H; CH_3), 1.29 (m, 14H; $(CH_2)_7CH_3$), 1.52 (m, 2H; $CH_2(CH_2)_7CH_3$), 2.45–3.84 (m, 10H; CH_2OCH_2, CH_2NH, $CHOH$, NHCHCOOH, CH_2Ar), 6.62 (d, J = 7.6 Hz, 2H; Ar-H), 7.01 ppm (d, J = 7.6 Hz, 2H; Ar-H); ^{13}C NMR (CD_3OD/KOH): δ = 14.4 (CH_3), 23.7, 27.2, 30.4, 30.5, 30.6, 30.7 and 33.0 ($(CH_2)_8CH_3$), 40.3 (CH_2Ar), 52.3/52.4 (CH_2NH), 67.4/67.7, 70.4/70.7, 71.9/72.6 and 74.7/74.9 (CH_2OCH_2, CHOH, NHCHCOOH), 119.7/119.8, 124.8/124.9, 131.0/131.1 and 166.6 (C-Ar), 182.0/182.41 ppm (COOH); IR (KBr): $\tilde{\nu}_{max}$ = 3400–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{22}H_{37}NO_5 \cdot 2H_2O$: C 61.25, H 9.51, N 3.25; found: C 61.40, H 9.50, N 3.20.

***N*-[3-(Dodecyloxy)-2-hydroxypropyl]tyrosine (TyrGE-12)**: M.p. 185–186 °C; yield 70%; ^{1}H NMR (CD_3OD/KOH): δ = 0.89 (t, J = 7.1 Hz, 3H; CH_3), 1.29 (m, 18H; $(CH_2)_9CH_3$), 1.55 (m, 2H; $CH_2(CH_2)_9CH_3$), 2.56–3.75 (m, 10H; CH_2OCH_2, CH_2NH, $CHOH$, NHCHCOOH, CH_2Ar), 6.54 (d, J = 7.3 Hz, 2H; Ar-H), 6.92 ppm (d, J = 7.3 Hz, 2H; Ar-H); ^{13}C NMR (CD_3OD/KOH): δ = 14.4 (CH_3), 23.7, 24.4, 27.0, 27.2, 30.4, 30.5, 30.6, 30.7 and 33.1 ($(CH_2)_{10}CH_3$), 40.3 (CH_2Ar), 52.3/52.4 (CH_2NH), 67.4/67.7, 70.4/70.7, 71.8/72.6 and 74.7/74.9 (CH_2OCH_2, CHOH, NHCHCOOH), 119.8/119.9, 124.8/124.9, 131.1/131.2 and 166.9 (C-Ar), 182.1/182.5 ppm (COOH); IR (KBr): $\tilde{\nu}_{max}$ = 3400–3244 (HO and NH), 1580 (COO^-),

1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{24}H_{41}NO_5$: C 68.08, H 9.69, N 3.30; found: C 68.18, H 9.74, N 3.23.

***N*-[2-Hydroxy-3-(tetradecyloxy)propyl]tyrosine (TyrGE-14)**: M.p. 180–181 °C; yield 63 %; ^{1}H NMR (CD_3OD/KOH): $\delta = 0.89$ (t, $J = 7.1$ Hz, 3H; CH_3), 1.29 (m, 22H; $(CH_2)_{11}CH_3$), 1.54 (m, 2H; $CH_2(CH_2)_{11}CH_3$), 2.52–3.85 (m, 10H; CH_2OCH_2, CH_2NH, CHOH, NHCHCOOH, CH_2Ar), 6.56 (d, $J = 7.9$ Hz, 2H; Ar-H), 6.99 ppm (d, $J = 7.9$ Hz, 2H; Ar-H); ^{13}C NMR (CD_3OD/KOH): $\delta = 14.4$ (CH_3), 23.7, 27.1, 30.4, 30.5, 30.6, 30.7, 30.8 and 33.0 ($(CH_2)_{12}CH_3$), 40.2 (CH_2Ar), 52.2/52.4 (CH_2NH), 67.3/67.7, 70.4/70.6, 72.6 and 74.6/74.8 (CH_2OCH_2, CHOH, NHCHCOOH), 119.6/119.7, 124.9/125.0, 131.0/131.1 and 166.28/166.33 (C-Ar), 182.0/182.3 ppm (COOH); IR (KBr): $\tilde{\nu}_{max} = 3400$–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{26}H_{45}NO_5 \cdot 2H_2O$: C 64.06, H 10.13, N 2.87; found: C 64.14, H 10.08, N 3.00.

***N*-[3-(Hexadecyloxy)-2-hydroxypropyl]tyrosine (TyrGE-16)**: M.p. 184–185 °C; yield 65 %; IR (KBr): $\tilde{\nu}_{max} = 3400$–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{28}H_{49}NO_5 \cdot 2H_2O$: C 65.24, H 10.29, N 2.71; found: C 65.50, H 10.31, N 2.66.

***N*-[3-(Decyloxy)-2-hydroxypropyl]histidine (HisGE-10)**: M.p. 170–171 °C; yield 70 %; ^{1}H NMR (CD_3OD/KOH): $\delta = 0.89$ (t, $J = 7.1$ Hz, 3H; CH_3), 1.29 (m, 14H; $(CH_2)_7CH_3$), 1.52 (m, 2H; $CH_2(CH_2)_7CH_3$), 2.59–3.78 (m, 10H; CH_2OCH_2, CH_2NH, CHOH, NHCHCOOH, CH_2Im), 6.83 (s, 1H; Im-H), 7.52 ppm (s, 1H; Im-H); ^{13}C NMR: $\delta = 14.5$ (CH_3), 23.7, 27.2, 30.5, 30.6, 30.8, 31.7 and 33.1 ($(CH_2)_8CH_3$, CH_2Im), 52.4 (CH_2NH), 65.6, 70.7, 72.6 and 74.9 (CH_2OCH_2, CHOH, NHCHCOOH), 121.1, 134.1 and 136.0 (C-Im), 181.5 ppm (COOH); IR (KBr): $\tilde{\nu}_{max} = 3400$–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{19}H_{35}N_3O_4$: C 61.79, H 9.49, N 11.38; found: C 61.87, H 9.55, N 11.12.

***N*-[3-(Dodecyloxy)-2-hydroxypropyl]histidine (HisGE-12)**: M.p. 173–174 °C; yield 68 %; ^{1}H NMR (CD_3OD/KOH): $\delta = 0.89$ (m, 3H; CH_3), 1.28 (m, 18H; $(CH_2)_9CH_3$), 1.54 (m, 2H; $CH_2(CH_2)_9CH_3$), 2.58–3.78 (m, 10H; CH_2OCH_2, CH_2NH, CHOH, NHCHCOOH, CH_2Im), 6.83 (s, 1H; Im-H), 7.51 ppm (s, 1H; Im-H); ^{13}C NMR: $\delta = 14.5$ (CH_3), 23.7, 27.2, 30.5, 30.6, 30.8, 31.6, 31.7 and 33.1 ($(CH_2)_{10}CH_3$, CH_2Im), 52.4 (CH_2NH), 65.6/65.7, 70.5/70.7, 72.6 and 74.6/74.9 (CH_2OCH_2, CHOH, NHCHCOOH), 121.0, 134.1 and 136.0 (C-Im), 181.5 ppm (COOH); IR (KBr): $\tilde{\nu}_{max} = 3400$–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{21}H_{39}N_3O_4$: C 63.48, H 9.82, N 10.58; found: C 63.39, H 9.56, N 10.30.

***N*-[2-Hydroxyl-3-(tetradecyloxy)propyl]histidine (HisGE-14)**: M.p. 169–17 °C; yield 72 %; ^{1}H NMR (CD_3OD/KOH): $\delta = 0.89$ (m, 3H; CH_3), 1.28 (m, 22H; $(CH_2)_{11}CH_3$), 1.54 (m, 2H; $CH_2(CH_2)_{11}CH_3$), 2.50–3.79 (m, 10H; CH_2OCH_2, CH_2NH, CHOH, NHCHCOOH, CH_2Im), 6.83 (s, 1H; Im-H), 7.53 ppm (s, 1H; Im-H); ^{13}C NMR: $\delta = 14.5$ (CH_3), 23.7, 27.2, 30.4, 30.6, 30.7, 31.4, 31.5 and 33.0 ($(CH_2)_{12}CH_3$, CH_2Im), 52.3 (CH_2NH), 65.5/65.6, 70.4/70.7, 72.6 and 74.6/74.8 (CH_2OCH_2, CHOH, NHCHCOOH), 121.1, 133.8/133.9 and 135.7 (C-Im), 181.4/181.6 ppm (COOH); IR (KBr): $\tilde{\nu}_{max} = 3400$–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{23}H_{43}N_3O_4$: C 64.94, H 10.12, N 9.88; found: C 65.00, H 10.10, N 9.76.

***N*-[3-(Hexadecyloxy)-2-hydroxypropyl]histidine (HisGE-16)**: M.p. 172–173 °C; yield 70 %; ^{1}H NMR (CD_3OD/KOH): $\delta = 0.89$ (t, $J = 6.9$ Hz, 3H; CH_3), 1.27 (m, 26H; $(CH_2)_{13}CH_3$), 1.54 (m, 2H; $CH_2(CH_2)_{13}CH_3$), 2.59–3.85 (m, 10H; CH_2OCH_2, CH_2NH, CHOH, NHCHCOOH, CH_2Im), 6.83 (s, 1H; Im-H), 7.52 ppm (s, 1H; Im-H); ^{13}C NMR: $\delta = 14.5$ (CH_3), 23.7, 27.2, 30.4, 30.6, 30.8, and 33.1 ($(CH_2)_{14}CH_3$, CH_2Im), 52.4 (CH_2NH), 65.7/65.8, 70.4/70.7, 72.6 and 74.6/74.8 (CH_2OCH_2, CHOH, NHCHCOOH), 120.9, 134.2 and 136.4 (C-Im), 181.8 ppm (COOH); IR (KBr): $\tilde{\nu}_{max} =$ 3400–3244 (HO and NH), 1580 (COO^-), 1610 cm^{-1} (NH); elemental analysis calcd (%) for $C_{25}H_{47}N_3O_4$: C 66.23, H 10.38, N 9.27; found: C 66.33, H 10.53, N 9.50.

Surface tension measurements: Surface tension measurements at equilibrium (γ) were determined by using a KSV Sigma 70 (Helsinki, Finland) tensiometer fitted with a Wilhelmy plate. All the solutions containing different concentrations of compounds **4** were prepared at pH 12 by using deionized water and 1 N sodium hydroxide and were stored in closed glass bottles for 24 h before measurement. For the very dilute solutions (10^{-6} M), an aging time of 30 min was used, whereas for the most concentrated solutions (10^{-3} M), the time was 15 min. The surface tension of deionized water at pH 12 was found to be 74 mN m^{-1} at 25 °C.

Krafft point measurements: The Krafft point was measured by preparing 1 wt % of each surfactant in alkaline water (pH 12) and observing with the naked eye the temperature at which the solution became clear.[3]

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