DOI: 10.1002/ejoc.200900137

Molecular Tailored Histidine-Based Complexing Surfactants: From Micelles to Hydrogels

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Keywords: Peptides / Surfactants / Self-assembly / Gels / Chelates / Amphiphiles

Novel histidine-based complexing surfactants, designed as AA-His-EO_m-C_n, containing trifunctional moduli (peptidic/hydrophilic/hydrophobic) were synthesized by a modular step-by-step procedure, which allowed easy structural changes, and consequently correlations between their molecular structures and their self-assembling properties could be established. Thus, micelles or hydrogels could be obtained by simply modifying the hydrophobic tail lengths or the junction between the different moduli of the designed compounds. At low pH values, all compounds were surface active in aqueous solutions. At higher pH values, in the range 8–10, micellization took place for decyl compounds (n = 10), whereas hydrogelation occurred for longer chain lengths (n = 12, 14), and this, at very-low concentrations of surfactant (<0.3 wt.-%), could thus act as low molecular weight gelator (LMWG). The driving forces for gel formation were noncovalent intermolecular interactions such as π-stacking and hydrophobic and hydrogen-bonding interactions.

Introduction

Inspired by biologically active carnosine or carcinine (Figure 1), respectively, histidine-peptide and histidine-peptidoamine, which are known for their intrinsic antioxidant properties and their potential therapeutic effects in many diseases, for example, Wilson, Parkinson, Alzheimer, or inflammatory diseases,[1] histidine (His) di- or tripeptides appear as promising building blocks for new therapeutics and might constitute a straightforward approach for the design of bioactive formulations with direct applications in the medical field, and more particularly for oxidation stress problems.

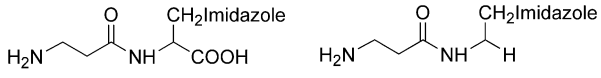

Figure 1. Structure of carnosine and carcinine.

Their antioxidant properties are directly related to the presence of the imidazole moiety and might be enhanced in the corresponding metallocomplexes.[2–4] Indeed, His-oligopeptides and peptidoamines exhibit a large variety of coordination modes toward transition-metal ions such as Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+},[5–10] and some of them, such as Cu^{2+}or Ni^{2+} complexes, are of significant biological importance. Some glycine (Gly)-peptides show similar properties.[11]

On the other side, new formulations such as soft materials known as hydrogels have gathered much attention recently, owing to their unique properties and versatile applications in many areas, from applied chemistry to biomedicine.[12,13] In this respect, a major challenge is the development of biocompatible and/or biodegradable low molecular weight (LMW) hydrogelators with well-defined chemical structures and predictable properties with respect to their polymeric counterparts. To this end, amphiphilic molecules, namely surfactants, appear as suitable candidates, as they can form well-characterized supramolecular structures. When appropriately grafted with amino acid[14,15] moieties they may conduct to stimuli-sensitive hydrogels through weak noncovalent interactions between gelator molecules and entrapped solvent molecules.

On the basis of anterior results on peptidoamines and surfactants with complexing properties,[16–18] this study concerns the synthesis and the physicochemical characterization of trimodulus compounds, designed as AA-EO_m-Alk_n, bearing (i) a hydrophobic alkyl group, (ii) a polar peptide group with complexing properties, and (iii) a variable hydrophilic junction modulus, allowing HLB (Hydrophilic Lipophilic Balance) control. Depending on the junction type between these moduli, ester or amide, three types of

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900137.

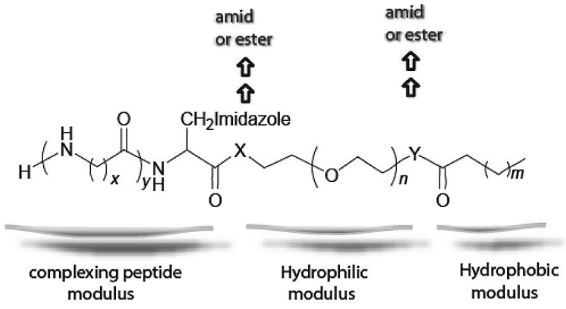
compounds were synthesized: amide–ester AA-His-a-EO_m-e-C_n, ester–amide AA-His-e-EO_m-a-C_n, and amide–amide AA-His-a-EO_m-a-C_n. Thus, by small structural modulations, intermolecular interactions might be easily tailored.

In this work our interests focus on the synthesis of new histidine-based surfactants and their self-assembling properties for the rational design of hydrogels. Moreover, as a result of their complexing properties, such systems, micelles or hydrogels, might be used for selective extraction of metallic cations, for example, Ni^{2+} or Cu^{2+}. In order to easily tailor the molecular structure, the strategy of the synthesis was based on a step-by-step coupling, mimicking peptide synthesis.

Results and Discussions

Molecular Design and Synthesis

The general structure of the target compounds is presented in Scheme 1.



Scheme 1. General structure of the three moduli target compounds.

All compounds were prepared by a modular strategy of synthesis, as described below. The first stage of synthesis corresponds to the grafting of a fatty acid onto an ethoxylated amino alcohol or a diamine by an amide or ester link. The second step consists of the grafting of the histidine carboxylic acid function onto the free moiety of the bimoduli compound, and the third step corresponds to the coupling with other amino acids.

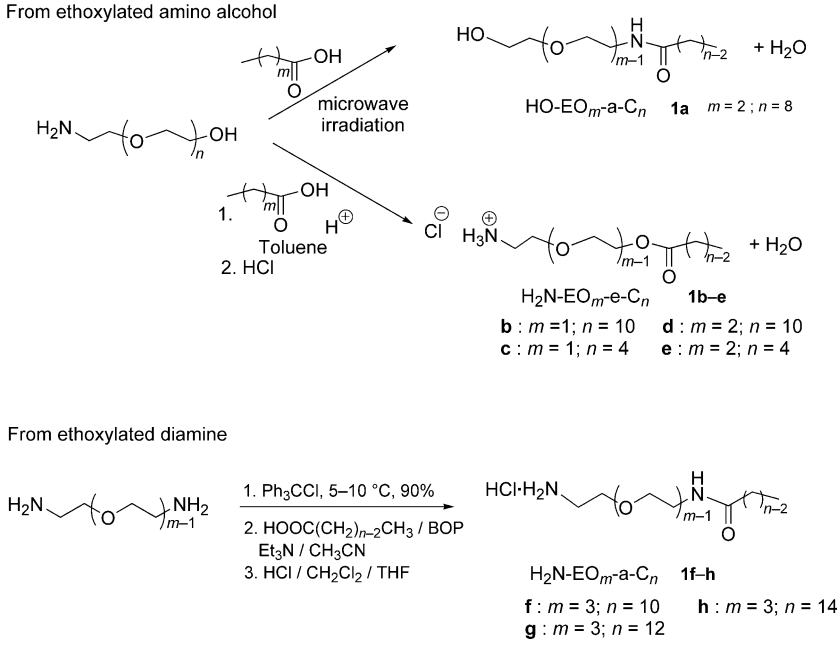
Synthesis of Bimoduli Compounds

The first step involved association of a hydrophilic part (ethoxylated amino alcohol or ethoxylated diamine) with the hydrophobic part. Three types of compounds were synthesized by variation of the links between the two parts. H_2N-EO_m-e-C_n esters and HO-EO_m-a-C_n amides were prepared from the corresponding ethoxylated amino alcohols, whereas H_2N-EO_m-a-C_n amides were prepared from ethoxylated diamines.

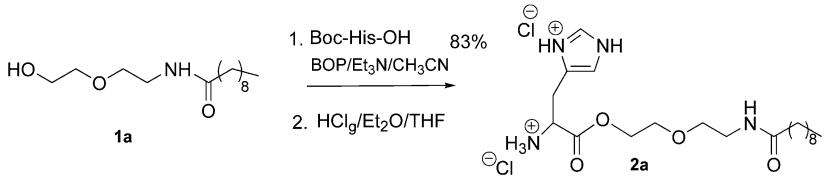
A mixture of the fatty acid and an excess amount of the ethoxylated amino alcohol, under acidic and azeotropic conditions, led mainly to esterification, whereas by microwave activation, the regioselectivity was reversed and the amidation products were predominant. Preventive protection of the ethoxylated diamine by trityl groups was necessary to obtain monoacylation (Scheme 2). Deprotection with the use of HCl was quantitative.

Grafting of Histidine

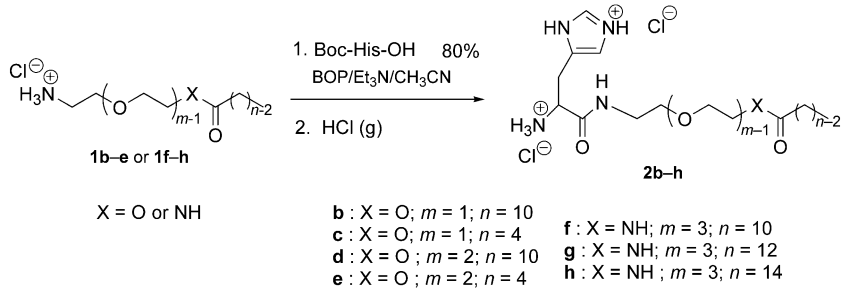
The activation of carboxylic acid by peptide coupling agents like BOP led rapidly and efficiently to the desired products; the removal of the *tert*-butyloxycarbonyl moiety was realized with gaseous HCl. The two parts were grafted by either of two methods: (i) by an ester link between histi-



Scheme 2. Synthesis of bimoduli amphiphilic compounds.



Scheme 3. Histidine grafting by an ester link.



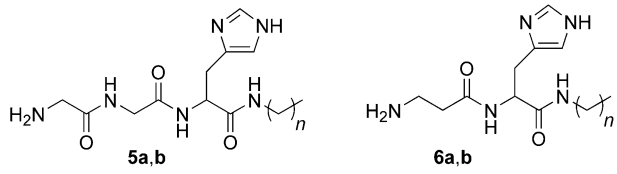
Scheme 4. Histidine grafting by an amide link.

dine and the ethoxylated amino alcohol (Scheme 3), or (ii) by an amide link between histidine and the ethoxylated amino alcohol or ethoxylated diamine (Scheme 4).

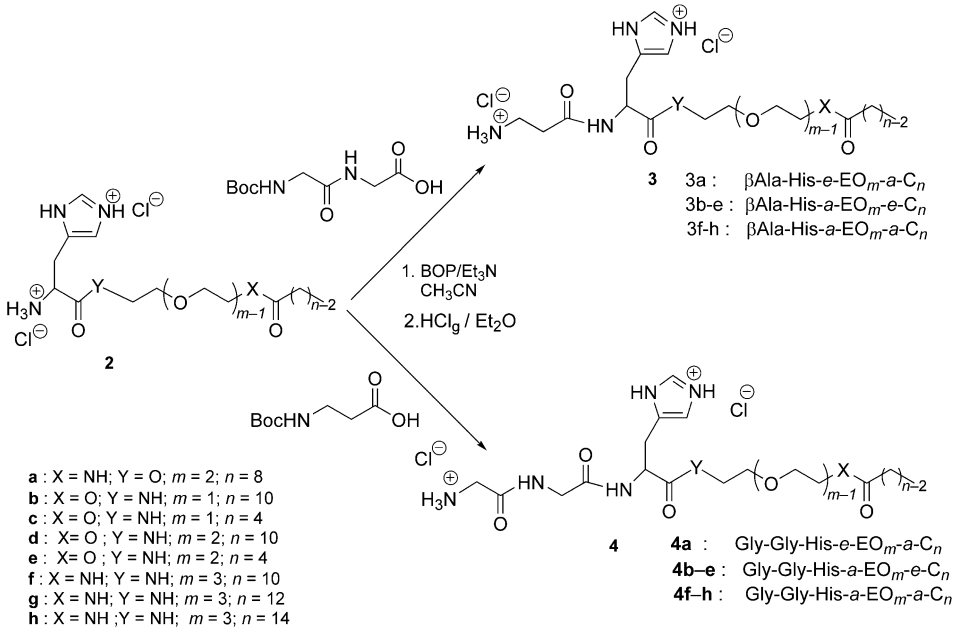
Grafting of the Peptidic Moiety

Grafting by various coupling and protection–deprotection steps led to "ester–amide" compounds designed as AA-His-e-EO$_m$-a-C$_n$ (**3a** and **4a**) or "amide–esters" designed as AA-His-a-EO$_m$-e-C$_n$ (**3b** and **4b**) or "amide–amide" compounds designed as AA-His-a-EO$_m$-a-C$_n$ (**3c** and **4c**) (Scheme 5). All compounds were obtained with good overall yields; Boc deprotection reactions were quantitative.

Similar compounds exempt of diethylene oxide moieties were investigated in this work; their synthesis will be reported elsewhere (Scheme 6).



Scheme 6. Structure of amphiphilic bimodular pseudopeptides **5** and **6** (n = 9, 11).



Scheme 5. Grafting of complexing peptide modulus/preparation of pseudopeptides AA-His-EO$_m$-C$_n$.

Complexing Properties

To prove that the presence of two moduli, hydrophilic ethylene oxide and hydrophobic alkyl chains, do not alter the complexing properties of the His-peptide moiety, protonation and metal coordination studies (with Cu^{2+} and Ni^{2+}) were performed.

Protonation

The deprotonation constants of some amphiphilic compounds were determined through titration curves of the ligands generated by pH-metry. The macroscopic protonation constants K_1 and K_2 of the free ligands are listed in Table 1. They can be approximately assigned to the protonation of the amino group and imidazole ring, respectively. The pK_a values obtained for the terminal amino group were similar to those of similar peptides (Gly-Gly-His, βAla-His) or pseudopeptides (Gly-Gly-Ha, βAla-Ha). In contrast, the pK_a of the imidazole ring of the amphiphilic peptides were lower than those of corresponding non-ethoxylated peptides or pseudopeptides. The decrease in the pK_a values could be related to the inductive attractor effect of the oxyethylene moiety, which thus alters the acidity constant of imidazole. In the case of the β-Ala derivative, deprotonation is easier than for Gly-Gly derivatives, because the electron-withdrawing effect of the carbonyl group in the β-ala residue is weaker.

Table 1. pK_a values of "amide–ester" trimoduli amphiphiles and peptidoamine analogs (T = 25 °C, I = 0.1 M).

Products	pK_1 (amino)	pK_2 (imidazole)
Gly-Gly-His-aEO$_2$e-C10 (**4d**)	7.79	6.14
Gly-Gly-His[19]	7.94	6.82
Gly-Gly-Ha[20]	7.85	6.81
β-Ala-His-aEO$_2$e-C10 (**3d**)	9.13	5.95
β-Ala-His-aEOe-C10 (**3b**)	9.24	6.20
β-Ala-His[22]	9.35	6.74
β-Ala-Ha[21]	9.21	6.84

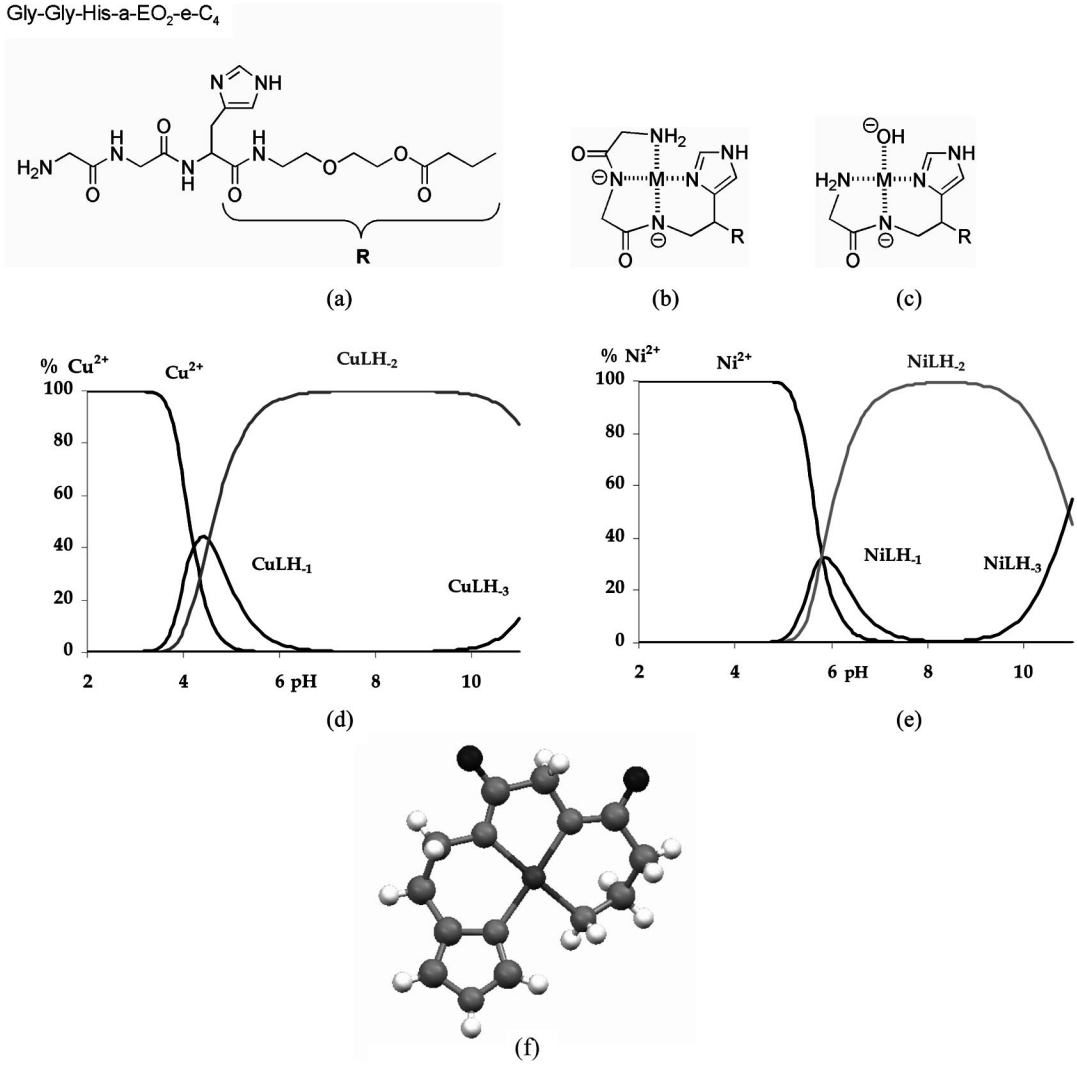


Figure 2. Chemical structures of an amphiphilic trimoduli compound (a), predominant complexes MLH$_{-2}$ of the Gly-Gly-His derivative (b) and the β-Ala-His derivative (c); species repartition diagram as a function of pH for Cu^{2+} (d), Ni^{2+} (e); and X-ray structure Cu^{2+} Gly-Gly-Ha[20] (f).

Table 2. $Cu^{2+}$and Ni^{2+} formation constants of complexes of hydrophilic Gly-Gly-His-aEO$_2$e-C4 compared to Ala-Gly-Ha and Gly-Gly-Ha (T = 25 °C, I = 0.1 M).

	Gly-Gly-His-aEO$_2$e-C4		Ala-Gly-Ha[10]		Gly-Gly-Ha[22]	
	Cu^{2+}	Ni^{2+}	Cu^{2+}	Ni^{2+}	Cu^{2+}	Ni^{2+}
MLH	–	–	11.59 (06)	10.21 (08)	12.02 (03)	10.48 (02)
ML	–	–	–	–	6.61 (04)	4.15 (04)
MLH_{-1}	3.68 (03)	–0.88 (03)	2.36 (02)	–1.68 (02)	1.65 (09)	–2.87 (12)
MLH_{-2}	–0.85 (02)	–6.67 (01)	–2.86 (01)	–7.89 (01)	–2.48 (04)	–7.99 (01)
MLH_{-3}	–12.68 (04)	–17.59 (03)	–15.12 (02)	–19.87 (02)	–14.34 (02)	–19.56 (02)
$pK^{MLH}_{MLH_{-1}}$	4.53	5.79	5.22	6.21	4.13	5.12
$pK^{MLH_{-1}}_{MLH_{-2}}$	11.83	10.92	12.26	11.98	11.86	11.57

Metal(II) Coordination

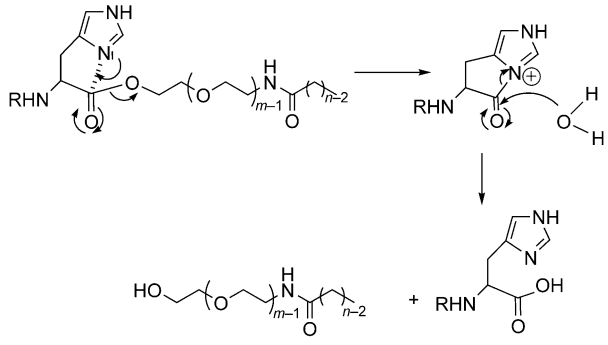
Gly-Gly-His-aEO$_2$e-C4 was used as a complexing model, as the resulting complexes are easier to assign with respect to their βAla-His counterparts. In fact, in the presence of Cu or Ni, the repartition diagram of the species shows only one major complex, namely, MLH_{-2}, existing over a large range of pH values. This is probably related to the cooperative effect of deprotonation, which provides four donor atoms that are able to fully occupy the coordination sphere of the metallic center, whereas carnosine derivatives exhibit only three donor centers and, therefore, several complexes might be formed (Figure 2).

The pH titration curve of an equimolar solution of Cu^{2+} and Gly-Gly-His-aEO$_2$e-C4 shows a sharp break (pH 5.5 to 10) after the consumption of four equivalents of base per metal ion, suggesting the deprotonation and coordination of the two nitrogen atoms of the peptidic bond besides the terminal amino and imidazole groups (in the MLH_{-2} species), analogous to the Cu^{2+}-Gly-Gly-Ha system (Table 2). The titration curve shows that only 1:1 complexes are formed for any metal-to-ligand ratio. Consequently, the formation of bis complexes can be neglected. The visible absorption spectra taken at variable pH values show the gradual formation of a dominant species at pH 5–10, with λ^{d-d}_{max} = 530 nm. At this pH, $CuLH_{-2}$ is the largely predominant species and the metal is coordinated by four nitrogen donor atoms: one amino nitrogen atom, two nitrogen atoms of the peptidic bond, and the N3 atom of the imidazole ring. In the case of Ni^{2+}, complex formation starts at higher pH values than for Cu^{2+}, but the titration curves are identical above pH 7, suggesting the formation of the same complexes in both systems. The color of Ni^{2+}-containing solutions changes from light green to yellow between pH 6 and 7, indicating the formation of diamagnetic, square-planar complexes (λ^{d-d}_{max} = 425 nm). For both systems, further deprotonation occurs above pH 10, where the MLH_{-3} complex appears, as shown by the changes in the visible spectra, indicating the deprotonation of the N1 nitrogen atom of the imidazole ring.

The Cu^{2+} complexes are more stable than Ni^{2+} complexes, which is in agreement with the Irving–Williams series. The complexes CuLH and CuL are not detected, probably as a result of the strong acidity of imidazole, but the stability of the $CuLH_{-1}$ species is higher than with Gly-Gly-Ha or Ala-Gly-Ha. These differences may be due to a phenomenon of solvation because the complex is more hydrophobic than Gly-Gly-Ha or Ala-Gly-Ha. The presence of the hydrophobic chain seems to have only a slight influence on the stability of the complexes; however, the capacity of complexation of the amphiphile (ligand) is conserved.

Amphiphilic Properties

First of all, the stability of the synthesized compounds has been assessed. The " amide–ester" compounds designed as AA-His-a-EO$_m$-e-C$_n$ (**3b-h** and **4b**–**h**) appear to be stable under acidic conditions for at least several days, whereas hydrolysis occurs under basic conditions in less than 1 h, as estimated by ^{1}H NMR spectroscopy by following the disappearance of the CH_2 signal in the position α to the ester bond (see Scheme 7 and Supporting Information). Interestingly, the position of the ester bond influences dramatically the stability of the amphiphile in water. Thus, "ester–amide" compounds, designed as AA-His-e-EO$_m$-a-C$_n$ (**3a** and **4a**) are rapidly hydrolyzed under both acidic and basic conditions. The cleavage between the ethoxylated moiety and the His residue might be related to the catalytic effect of the imidazole ring, which is in proximity to the ester link. Consequently, as a result of this low stability of the "ester–amide" compounds, their amphiphilic properties could not be determined.



Scheme 7. Hypothetical mechanism of the self-catalyzed hydrolysis of AA-His-e-EO$_m$-a-C$_n$ (**3a** and **4a**).

To increase the stability of the surfactants under both acidic and basic conditions "amide–amide" compounds were synthesized. Moreover, this provided increased hydrophilicity and additional hydrogen-bonding centers (Figure 3).

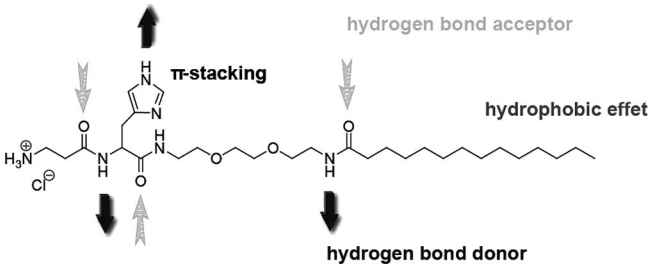


Figure 3. Possible sites of interaction between amphiphiles.

The amphiphilic properties of the synthesized compounds were evaluated by two techniques, generally used for this purpose, namely, surface tension measurements and spectrofluorimetry with the use of pyrene as a probe. For solubility reasons, the measurements were made at pH 2.

As indicated in Table 3, all compounds have critical micellar concentration (CMC) values in the same order of magnitude varying from 10^{-3} to 10^{-2} M; the small variations observed are consistent with the thermodynamic theory of self-assembling behavior: (i) When the number of ethylene oxide moieties increase, the hydrophilicity increases and consequently, the CMC value too. (ii) When the hydrophobic alkyl chain length increases, the CMC values decrease. Moreover, decyl pseudopeptides, with or without an EO spacer, decrease the surface tension to 35 mN m^{-1}. Surface tension measurements allowed us also to estimate the main area per molecule A [Equation (1)] of the adsorbed cationic surfactant, from the inverse of Γ calculated according to the Gibbs equation.

$$A = \frac{n \cdot 2.303 \cdot kT}{-\frac{d\gamma}{d\log C}} \quad \text{where } n = 1 \text{ (excess electrolyte solution)} \tag{1}$$

where Γ is the surface excess concentration (mol m^{-2}), k the Boltzmann constant, T the absolute temperature, γ the surface tension, and C the surfactant concentration. For all investigated compounds, the mean area per molecule A in the monolayer was estimated to be in the range 73–83 Å^2. These close values of area per molecule of surfactant, with variations within the limits of the experimental error, are relatively high, more than four times the minimum area per molecule for a hydrocarbon chain (18 Å^2). Therefore, it seems that the major contribution in the formation of the film is related to the peptidic moiety rather than to the flexible EO spacer or the hydrophobic tail. This can be due to interactions with the aqueous subphase and to the formation of intermolecular hydrogen bonds between the peptidic polar heads, stabilizing the Gibbs film (Figure 4).

At pH 8, surfactants behave differently, depending on the EO spacer and the alkyl chain length. Non-ethoxylated compounds precipitate independently from the alkyl chain length (C_n), whereas ethoxylated products form either micellar solutions, when n = 10, or hydrogels, when n = 12 or 14.

To evaluate their gelator properties, gel-to-solution transition temperatures were estimated by using the inversion method (Table 4). Compounds **3g**,**h** or **4g**,**h** were thus placed in small vials, and an aqueous solution of appropriate pH was added. Mixtures (6% w/v) were heated to 60 °C for 10 min and then allowed to stand for 30 min (unless

Table 3. CMC values of the various trimodulus amphiphiles in aqueous solutions.

Compound	CMC [mM]	Compound	CMC [mM]
β-Ala-His-C10 (**6a**)	2.4[a]	β-Ala-His-aEOe-C10 (**3b**)	3.3[a]
β-Ala-His-aEO$_2$e-C10 (**3d**)	5.7[a]	β-Ala-His-aEO$_3$a-C10 (**3f**)	9.8[b]
β-Ala-His-C12 (**6b**)	1.1[b]	β-Ala-His-aEO$_3$a-C12 (**3g**)	2.8[b]
Gly-Gly-His-C10 (**5a**)	5.7[a]	Gly-Gly-His-C12 (**5b**)	3.0[b]
Gly-Gly-His-aEO$_2$e-C10 (**4d**)	8.9[a]	Gly-Gly-His-aEO$_3$a-C12 (**4g**)	4.6[b]

[a] CMC values were determined by spectrofluorimetry at 25 °C, pH 2. [b] CMC values were determined by surface tension measurements at 25 °C, pH 2.

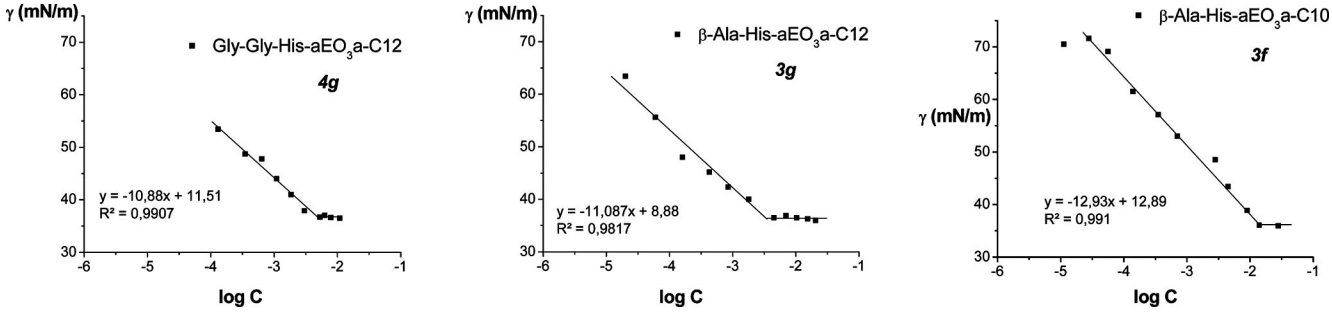


Figure 4. Surface tension curves $\gamma = f(\log C)$ at pH 2.

otherwise specified) at 20 °C. The vials were then turned upside down, and samples were considered successfully gelled if the solvent was completely immobilized. Indeed, as reported in the literature,[22] when the hot solution is cooled, the molecules start to condense and three situations are possible: (i) a highly ordered aggregation giving rise to crystals, (ii) a random aggregation resulting in an amorphous precipitate, or (iii) an aggregation process intermediate between these two, yielding a gel. In our case, the appropriate balance between hydrophilic and hydrophobic modulus, as well as the appropriate number of H-donor and H-acceptor centers seems to be responsible for the gelation phenomenon.

Table 4. Gel-to-solution transition temperatures of the trimoduli "amide–amide" amphiphiles in aqueous solutions (pH 8).

Compound	Gly-Gly-His-aEO$_3$a-C$_n$		β-Ala-His-aEO$_3$a-C$_n$	
n	12 (**4g**)	14(**4h**)	12(**3g**)	14(**3h**)
T_{gel} [°C]	26	35	31	42

Polarized optical microscopy (POM) of the hydrogel revealed the presence of entangled fibers (Figure 5). Deeper insight into the morphology of these fibers was obtained from scanning electron microscopy (SEM; Figure 6). The micrographs show bundles containing thin (less than 100 nm) aligned fibers that form the gel. The fibers are hundreds of micrometers long but less than 1 μm wide, and water molecules are trapped in the interstitial spaces. Upon gelation, it seems that 1D fiber-like self-assembly of these low-molecular weight molecules results in the formation of highly anisotropic fibrous networks. Deeper insight into the characteristics of these gels will be reported elsewhere and their use as "soft space organizers" and aligner molecules as well as the formation of supramolecular architectures through molecularly controlled self-assembling is currently under investigation.

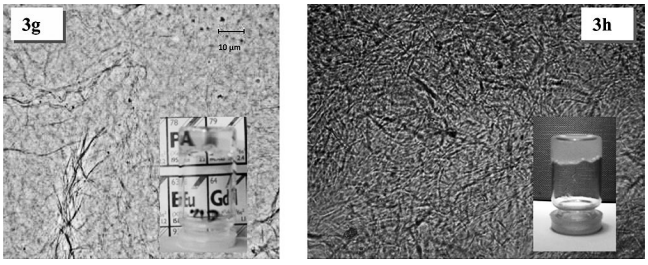


Figure 5. POM of 1% (w/v) supramolecular hydrogel of **3g** (left) and **3h** (right) indicating fiber bundles.

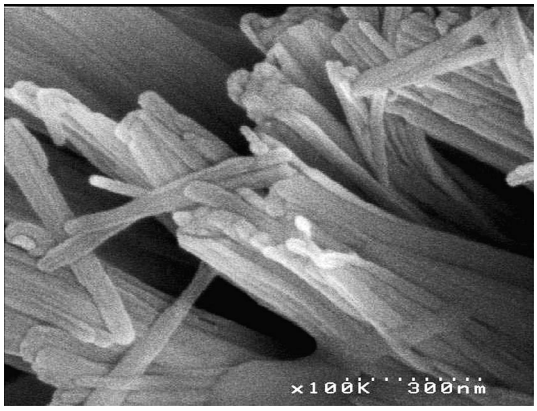


Figure 6. SEM micrographs of **3h** hydrogels after drying.

Conclusions

In summary, we reported herein the modular synthesis of new His-based surfactants containing trifunctional building blocks. Slight modifications of each part as well as the nature of the spacer in between (ester or amide) induced considerable changes on their self-assembling properties, the results reaching from micelles to hydrogels. Moreover, we assessed the gelation properties of new LMWGs based on His-containing peptides and could show that they are due to weak noncovalent interactions, which thereby create highly anisotropic supramolecular hydrogels. As a result of their complexing properties, these compounds are suitable candidates for selective binding of biologically relevant cations such as Cu^{2+} or Ni^{2+}, and therefore, they can be used in a straightforward approach for the design of bioactive formulations and, more particularly, for oxidation stress problems.

Experimental Section

Materials: All solvents were reagent grade and used without further purification. All reagents were obtained from Aldrich and used without further purification. BOP [benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate] was supplied from Neosystem. Stock solutions of metal perchlorates (Alpha Ventron products) were standardized by complexometry.

Methods: The purity of products was checked by NMR spectroscopic measurements, elemental analysis, and acid–base titrations. ^{1}H and ^{13}C NMR spectroscopic data were recorded with a Bruker AM-400 spectrometer. SEM micrographs of dried samples were registered with a Hitachi FEG S4800 microscope.

Fluorescence emission spectra were recorded by employing an excitation wavelength of 334 nm by using pyrene as a probe, and the intensities $I1$ and $I3$ were measured at wavelengths corresponding to the first and third vibronic bands located at ca. 373 and 384 nm. The ratios $I1/I3$ were plotted as a function of the total surfactant concentration. The CMC was taken from the best fit of the partition between the micelle and water, corresponding approximately to the abrupt decrease scale $I1/I3$ ratio as represented in Figure 1 (see Supporting Information). The fluorescence measurements were recorded with a Fluorolog-3 Horiba-Jobin Yvon spectrofluorimeter.

The coordination equilibria were investigated by potentiometric titrations under an argon atmosphere. For the equilibrium measurements, a PC-controlled full automatic titration set was used, including a potentiometric titrator Metrohm (721Net titrino) and an Orion 710 pH meter (precision 0.1 mV). The ionic strength was adjusted to 0.1 mol dm^{-3} with $NaClO_4$ and the cell was thermostatted to 298.0 ± 0.1 K. Changes in pH were followed by using a Thermo (ref Orion 9103SC) combined glass electrode. For the quantitative evaluation of the data, a correlation [Equation (2)] was

used between the experimental electromotive force values (E) and the equilibrium hydrogen ion concentrations [H^+], where j_H and j_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction potential and to the possible alkaline and acidic errors of the glass electrode, and K_w is the autoprotolysis constant of water[23] ($10^{-13.75}$).

$$E = E_0 + \frac{RT}{F}\ln[H^+] + j_H[H^+] + j_{OH}[H^+]^{-1}K_W \quad (2)$$

The formation constants (β_{pqr}) for the generalized reaction given in Equation (3) were evaluated from the pH-metric titration data with the PSEQUAD computer program.[24] In this notation, L is the neutral ligand, consequently the fully protonated ligands are referred to as LH_2. The metal-promoted deprotonation of the neutral ligand or, equivalently, the formation of mixed hydroxido complexes are denoted as MLH_{-1} and MLH_{-2}. The constants were calculated from an average of six independent titrations. The metal-to-ligand ratio was varied from 1:1 to 1:4 with metal-ion concentrations between 5×10^{-3} and 2×10^{-3} mol dm^{-3}.

$$pM + qL + rH = M_pL_qH_r \quad (3)$$

***N*-[2-(2-hydroxyethoxy)ethyl]decanamide (1a):** A mixture of decanoic acid (10 mmol) and 2-(2-aminoethoxy)ethanol (1 equiv.) was homogenized by heating and then exposed to microwave irradiation for 3 min. After cooling to room temperature, the syrup was dispersed in ethyl acetate (50 mL) and successively washed with a saturated aqueous solution of $NaHCO_3$ (30 mL) and a saturated solution of NaCl (2×30 mL). The organic phase was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The white powder was recrystallized from Et_2O. Yield: 2.35 g, 90%. M.p. 70 °C. R_f (EtOH/AcOEt, 1:5) = 0.65. IR (KBr): $\tilde{\nu}$ = 2910, 1633, 1560, 1130 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.94 (t, ^3J = 6.5 Hz, 3 H, CH_3) 1.2–1.4 (m, 12 H, CH_2 fatty chain) 1.64 (m, 2 H, CH_2 in β of CO) 2.29 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 3.40 (t, ^3J = 5 Hz, 2 H, CH_2NH) 3.57 (m, 4 H,CH_2O) 3.71 (t, ^3J = 5 Hz, 2 H, CH_2OH) ppm. ^{13}C NMR (100.58 MHz, CD_3OD): δ = 15.2 (CH_3) 24–37.5 (CH_2 fatty chain) 37.9 (CH_2 in α of CO) 41.1 (CH_2NH) 63 (CH_2OH) 71.4 and 74.2 (CH_2O) 177.2 (C=O) ppm. $C_{14}H_{29}NO_3$ (261.41): calcd. C 64.32, H 11.18; found C 64.10, H 11.50.

Salts 1b–e: To a 250-mL flask equipped with a Dean Stark and containing a solution of alcanoic acid (10 mmol) in toluene was added 2-(2-aminoethoxy)ethanol (10 mmol) and *para*-toluenesulfonic acid (11 mmol). The mixture was stirred and with azeotropic reflux for 18 h. Then, room temperature was reached, and the solution was concentrated to 50% of its volume. Compounds **1b**–**e** precipitated as white powders. The precipitate was separated by filtration, washed by ethyl acetate and recrystallized from ethyl acetate. The powder was dispersed in anhydrous Et_2O (100 mL) and then treated with anhydrous HCl (g) obtained from the reaction of NaCl (40 g) with a mixture of concentrated H_2SO_4 (36 mL) and H_2O (16 mL). After removal of the solvent, a white powder was obtained.

1b: Yield: 2.12 g, 81%. M.p. 80 °C. IR (KBr): $\tilde{\nu}$ = 3080, 2921, 1739 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3) 1.2–1.4 (m, 12 H, CH_2 fatty chain) 1.6 (m, 2 H, CH_2 in β of CO) 2.28 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 3.01 (t, ^3J = 5 Hz, 2 H, $CH_2NH_3^+$) 4.32 (t, ^3J = 5 Hz, 2 H, CH_2O) ppm. ^{13}C NMR (100.58 MHz, CD_3OD): δ = 15.3 (CH_3) 24–35 (CH_2 fatty chain) 35.2 (CH_2CO) 40.2 (CH_2 NH_3^+) 67.1 (CH_2O) 176.4 (C = 0) ppm. $C_{12}H_{26}ClNO_3$ (267.78): calcd. C 53.82, H 9.78; found C 54.01, H 10.20.

1c: Yield: 1.34 g, 80%. M.p. 90 °C. IR, ^{1}H NMR, and ^{13}C NMR spectral data are identical to those of **1b**. $C_6H_{14}ClNO_2$ (168.58): calcd. C 42.99, H 8.37; found C 43.01, H 8.50.

1d: Yield: 2.21 g, 75%. IR (KBr): Data are identical to those of **1b**. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3) 1.2–1.4 (m, 12 H, CH_2 fatty chain) 1.62 (m, 2 H, CH_2 in β of CO) 2.34 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 3.12 (t, ^3J = 5 Hz, 2 H, $CH_2NH_3^+$), 3.72 (m, 4 H, CH_2O) 4.26 (t,^3J = 5 Hz, 2 H, CH_2OCO) ppm. ^{13}C NMR (100.58 Hz, CD_3OD): δ = 15.3 (CH_3) 24–35 (CH_2 fatty chain) 35.7 (CH_2 in α of CO) 41.4 ($CH_2NH_3^+$) 65.1 (CH_2OCO) 68.6 and 71.1 (CH_2O) 176.2 (C = 0) ppm. $C_{14}H_{30}ClNO_3$ (295.83): calcd. C 56.84, H 10.22; found C 57.02, H 9.99.

1e: Yield: 1.49 g, 70%. IR, ^{1}H NMR, and ^{13}C NMR spectral data are identical to those of **1d**. $C_8H_{18}ClNO_3$ (211.69): calcd. C 45.39, H 8.57; found C 45.10, H 8.35.

Alkylamido[2-Aminoethoxyethoxy]ethyl Hydrochlorides (1f–h)

Step 1: Amino-ethoxy-ethoxy-ethyl-triphenyl-methylamine: In a 500-mL flask placed in an ice bath, chlorotriphenylmethane (7.5 g, 27 mmol, 1 equiv.) was added in small portions, over 1 h, to a solution of (ethylendioxy)bisethylamine (47.4 g, 320 mmol, 12 equiv.) in chloroform (200 mL). During addition, the reaction mixture was stirred at 5–10 °C. Then, room temperature was reached, and the solution was washed with a saturated solution of NaCl (2×100 mL) and dried with $MgSO_4$. The solvent was then removed under vacuum, and the crude product was purified by silica gel chromatography (AcOEt/MeOH) to give a colorless solid (9.4 g, 24 mmol, 90%). IR (KBr): $\tilde{\nu}$ = 3382, 1120, 1094, 743, 703 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 1.35 (br. s, 3 H, NH and NH_2), 2.02 (t, ^3J = 5.2 Hz, 2 H, CH_2 in α of NH_2), 2.53 (t, ^3J = 5.2 Hz, 2 H, CH_2 in α of NH), 3.20–3.50 (m, 8 H, CH_2), 7.00–7.50 (m, 15 H, Ar-H) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 42.0 (CH_2 in α of NH_2), 43.3 (CH_2 in α of NH), 70.4, 70.6, 71.5, 73.6 (CH_2), 126.5, 128.1, 129.0 (Ar), 146.4 (C_q) ppm. $C_{25}H_{30}N_2O_2$ (392.61): calcd. C 76.48, H 7.70; found C 76.95, H 7.50.

Step 2: Condensation of Fatty Acid: To a 250-mL flask was added a solution of the fatty acid (10 mmol, 1 equiv.) in CH_3CN (50 mL), BOP (4.42 g, 10 mmol, 1 equiv.), triethylamine (2.02 g, 20 mmol, 2 equiv.), and amino-ethoxy-ethoxy-ethyl-triphenyl-methylamine (3.9 g, 10 mmol, 1 equiv.). The pH of the solution was brought to ca. 8 by the addition of triethylamine, if necessary. The reaction mixture was stirred at room temperature for 15 h. Evaporation of the solvent under reduced pressure afforded an orange viscous liquid, which was solubilized in ethyl acetate (30 mL) and washed successively with HCl (0.1 N, 20 mL), saturated $NaHCO_3$ (20 mL), and saturated NaCl (2×20 mL). Then, the organic phase was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The triphenylmethyl moiety was removed after treatment with a mixture of CH_2Cl_2 (15 mL), THF (40 mL), and HCl (37%, 40 mL). The solvent mixture was then removed, and the crude material was purified by recrystallization from CH_3CN. The white powder was dried under vacuum to give **1f**–**h**.

1f: Yield: 2.35 g, 69%. IR (KBr): $\tilde{\nu}$ = 3256, 2921, 1633 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD): δ = 0.94 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.20–1.40 (m, CH_2 fatty chain), 1.64 (m, 2 H, CH_2 in β of CO), 2.23 (t, ^3J = 6.5 Hz, 2 H, CH_2 in α of CO), 2.96 (t, ^3J = 5 Hz, 2 H, CH_2 in α of NH_3^+), 3.4 (t, ^3J = 5 Hz, 2 H, CH_2 in α of NH), 3.58 (t, ^3J = 5 Hz, 2 H, CH_2 in β of NH_3^+), 3.63 (t, ^3J = 5 Hz, 2 H, CH_2 in β of NH), 3.68 (s, 4 H, CH_2-O) ppm. ^{13}C NMR

(100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 22–38 (8 CH_2), 40.9 and 41.5 (CH_2 in α of NH_3^+ and CH_2 in α of NH), 68–72 (4 CH_2-O), 177.2 (C=O) ppm. $C_{16}H_{35}ClN_2O_3$ (338.9): calcd. C 56.70, H 10.41; found C 56.23, H 9.95. M.p. 156 °C.

1g: Yield: 2.80 g, 76%. M.p. 120 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **1f**. $C_{18}H_{39}ClN_2O_3$ (366.97): calcd. C 58.91, H 10.71; found C 59.23, H 10.84.

1h: Yield: 3.38 g, 85%. M.p. 80 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **1f**. $C_{20}H_{43}ClN_2O_3$ (398.06).

Decylamido-ethoxy-ethyl Histidinoate Hydrochloride (2a): To a 250-mL flask was added a solution of Boc-His (2 g, 7.8 mmol, 1 equiv.) in CH_3CN (50 mL), BOP (3.45 g, 7.8 mmol, 1 equiv.), triethylamine (2.36 g, 23.4 mmol, 3 equiv.), and **1a** (7.8 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 15 h. Evaporation of the solvent under reduced pressure afforded a syrupy liquid that was solubilized in ethyl acetate (30 mL) and washed successively with HCl (0.1 N, 20 mL), saturated solution of NaCl (20 mL), saturated solution of $NaHCO_3$ (20 mL), and saturated solution of NaCl (2 × 20 mL). Then, the organic phase was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The white powder was washed with diethyl ether. Powder was dispersed in anhydrous ether and then treated with anhydrous HCl (g) obtained from the reaction of concentrate H_2SO_4 (30 mL) and NaCl (40 g) in water (10 mL). The final product was a hygroscopic powder. Yield: 3.03 g, 83%. $C_{20}H_{38}Cl_2N_4O_4$ (469.37): calcd. C 51.17, H 8.15; found C 50.67, H 7.95. M.p. 130 °C. IR (KBr): $\tilde{\nu}$ = 3310, 2921, 1739, 1642, 1121 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.94 (t, ^3J = 6.5 Hz, CH_3) 1.2–1.4 (m, 12 H, CH_2 fatty chain) 1.63 (m, 2 H, CH_2 in β of CO) 2.24 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 3.40 (m, 2 H, CH_2 Im) 3.47 (t, ^3J = 5 Hz, CH_2NH) 3.73 (t, ^3J = 5 Hz, CH_2O) 4.3–4.6 (m, 3 H, CH_2OCO, CH His) 7.59 (s, 1 H, CH Im) 7.74 (s, 1 H, CH Im) ppm.

Alkyl-amido-ethoxy-ethoxy-ethylamidohistidine Dihydrochloride (2b–h): To a 250-mL flask was added a solution of Boc-His (2 g, 7.8 mmol, 1 equiv.) in CH_3CN (50 mL), BOP (3.45 g, 7.8 mmol, 1 equiv.), triethylamine (2.36 g, 23.4 mmol, 3 equiv.), and **2b–h** (7.8 mmol, 1 equiv.). The reaction mixture was stirred at room temperature for 15 h. Compounds **2g–h** precipitated as white powders. The precipitate was separated by filtration, washed with CH_3CN (50 mL) and Et_2O (50 mL) and then recrystallized from acetonitrile. Compounds **2b–f** did not precipitate. Evaporation of the solvent under reduced pressure afforded a syrupy liquid that was solubilized in ethyl acetate (30 mL) and washed successively with HCl (0.1 N, 20 mL), saturated solution of NaCl (20 mL), saturated solution of $NaHCO_3$ (20 mL), and saturated solution of NaCl (2 × 20 mL). Then, the organic phase was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The mixture was dried with $MgSO_4$, the solvent was removed under reduced pressure, and the white powder was washed with Et_2O and filtered. For all compounds, the powders were dispersed in anhydrous ether and then treated with anhydrous HCl (g) obtained from the reaction of concentrated H_2SO_4 (30 mL) and NaCl (40 g) in water (10 mL). The final products were hygroscopic white solids.

2b: Yield: 2.73 g, 82%. M.p. 120 °C. IR (KBr): $\tilde{\nu}$ = 3304, 2921, 1739, 1648 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.34 (t, ^3J = 7 Hz, 2 H, CH_2 in α of CO), 3.01 (t, ^3J = 5 Hz, 2 H, CH_2NH), 3.37 (m, 2 H, CH_2 Im), 4.24 (t, ^3J = 7 Hz, 1 H, CH-NH_3^+), 4.32 (t, ^3J = 5 Hz, 2 H, CH_2OCO) 7.02 (s, 1 H, CH=C), 7.45 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH3), 23–36 (CH_2 fatty chain), 35.2 (CH_2CO), 54.3 ($CHNH_3^+$), 55.2 (CH_2NHCO), 63.8 (CH_2OCO), 118.82 (CH=C), 134 (C=NH), 137.18 (C=CH) 175 and 176 (C=O) ppm. $C_{18}H_{34}Cl_2N_4O_3$ (425.40): calcd. C 50.82, H 8.06; found C 49.98, H 7.62.

2c: Yield: 1.70 g, 75%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **2b**. $C_{12}H_{22}Cl_2N_4O_3$ (341.24): calcd. C 42.24, H 6.50; found C 41.98, H 6.85.

2d: Yield: 2.92 g, 80%. M.p. 125 °C, ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.34 (t, ^3J = 7.5 Hz, 2 H, CH_2 in α of CO), 3.12 (t, ^3J = 5 Hz, 2 H, CH_2NH), 3.37 (m, 2 H, CH_2 Im), 4.26 (t, ^3J = 5 Hz, 2 H, CH_2OCO) 4.34 (t, ^3J = 7 Hz, 1 H, CH-NH_3^+), 6.97 (s, 1 H, CH=C), 7.51 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 23–36 (CH_2 fatty chain), 34.7 (CH_2CO), 42.7 ($CHNH_3^+$), 55.6 (CH_2NHCO), 63.8 (CH_2OCO), 70–71 (CH_2O) 118.2 (CH=C), 133.4 (C=NH), 137.2 (C=CH) 175 and 176 (C=O) ppm. $C_{20}H_{37}Cl_2N_4O_4$ (468.44): calcd. C 51.25, H 7.95; found C 50.98, H 7.62.

2e: Yield: 2.02 g, 70%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **2d**. $C_{14}H_{26}Cl_2N_4O_3$ (369.14): calcd. C 45.53, H 7.10; found C 45.35, H 7.42.

2f: Yield: 3.16 g, 81%. M.p. 170 °C. IR (KBr): $\tilde{\nu}$ = 3291, 2921, 1648, 1633 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.94 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.63 (m, 2 H, CH_2 in β of CO), 2.25 (t, ^3J = 7 Hz, 2 H, CH_2 in α of CO), 3.4–3.7 (m, 12 H, CH_2), 4.28 (t, ^3J = 6.7 Hz, 1 H, CH-NH_3^+), 7.58 (s, 1 H, CH=C), 8.97 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 22–38 (CH_2 long chain and CH_2 of His), 40.9 and 41.5 (2 CH_2 in α of NH), 55.7 (CH-NH_3^+), 68–72 (4 CH_2-O), 118.2 (CH=C), 133.4 (CH=N), 137.2 (C_q), 175–176 (2 C=O) ppm. $C_{21}H_{43}Cl_2N_5O_4$ (500.46): calcd. C 50.39, H 8.66; found C 49.98, H 8.22.

2g: Yield: 3.73 g, 88%. M.p. 134 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **2f**. $C_{24}H_{47}Cl_2N_5O_4$ (540.53): calcd. C 53.33, H 8.76; found C 53.25, H 8.45.

2h: Yield: 3.99 g, 90%. M.p. 112 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **2f**. $C_{26}H_{51}Cl_2N_5O_4$ (568.58): calcd. C 54.92, H 9.04; found C 54.55, H 8.65.

Alkyl-amido-ethoxy-ethoxy-ethylamido-β-alanyl-histidine Dihydrochloride (3a–h): The procedure was similar to that used for the preparation of **2b–f**. Boc-β-alanine was used to replace Boc-His.

3a: Yield: 3.29 g, 82%. M.p. 150 °C. IR (KBr): $\tilde{\nu}$ = 3250, 2921, 1737, 1646, 1620, 1122 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.92 (t, ^3J = 6.5 Hz, 3 H, CH_3) 1.2–1.4 (m, 12 H, $6CH_2$) 1.62 (m, 2 H, CH_2 in β of CO) 2.24 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 2.72 (t, ^3J = 7.5 Hz, 2 H, CH_2CO β-alanine) 3.15–3.30 (m, 4 H, CH_2NHCO and CH_2 Im) 3.44 and 3.57 (t, ^3J = 5 Hz, CH_2O) 3.70 (t, ^3J = 7.5 Hz, 2 H, $CH_2NH_3^+$) 4.30 (t, ^3J = 6.5 Hz, CH_2OCO) 4.76 (t, ^3J = 7.5 Hz, CH His), 7.41 and 7.88 (s, 1 H, CH Im) ppm. $C_{23}H_{43}Cl_2N_5O_5$ (543.34): calcd. C 50.82, H 7.97; found C 50.35, H 7.60.

3b: Yield: 3.11 g, 82%. M.p. 146 °C, IR (KBr): $\tilde{\nu}$ = 3240, 2926, 1739, 1648, 1621 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.48 (t, ^3J = 7.5 Hz, 2 H, CH_2 in α of CO), 2.72 (t, ^3J = 7.5 Hz, 2 H, CH_2CO β-alanine) 3.29 (m, 2 H, CH_2 Im), 3.41 (t, ^3J = 5 Hz, 2 H, CH_2NH), 3.70 (t, ^3J = 7 Hz, 1 H, CH-NH_3^+), 4.33 (m, 2 H, CH_2OCO) 4.74 (t, ^3J = 7.5 Hz, 1 H, CH His) 7.02 (s, 1 H, CH=C), 7.45 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 23–36 (CH_2 fatty chain), 37.7 ($CHNH_3^+$), 52.3 (CH His), 55.3 (CH_2NHCO), 63.6

(CH_2OCO), 120.3 (CH=C), 130.2 (C=NH), 147 (C=CH), 170 and 176 (C=O) ppm. $C_{21}H_{40}Cl_2N_5O_4$ (500.28): calcd. C 50.41, H 8.06, N 14.11; found C 50.61, H 7.87, N 13.92.

3c: Yield: 2.51 g, 78%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **3b**. $C_{15}H_{27}Cl_2N_5O_4$ (412.32): calcd. C 43.70, H 6.60; found C 44.01, H 6.45.

3d: Yield: 3.56 g, 84%. M.p. 150 °C. ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.48 (t, ^3J = 7.5 Hz, 2 H, CH_2 in α of CO), 2.72 (t, ^3J = 7.5 Hz, 2 H, CH_2NH), 3.15–3.30 (m, 4 H, CH_2 Im and CH_2NHCO), 3.42 and 3.57 (t, ^3J = 5 Hz, 2 H, CH_2O) 3.70 (t, ^3J = 7.5 Hz, 2 H, $CH_2NH_3^+$) 4.25 (t, ^3J = 6.5 Hz, 2 H, CH_2OCO) 4.75 (t, ^3J = 7 Hz, 1 H, CH-NH_3^+), 7.41 (s, 1 H, CH=C), 7.88 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 23–36 (CH_2 fatty chain), 37.7 ($CH_2NH_3^+$), 39.2 (CH_2OCO) 52.3 ($CHNH_3^+$), 63.5 (CH_2NHCO), 70 and 71 (CH_2O) 120.1 (CH=C), 130.2 (C=NH), 147.1 (C=CH) 170 and 176 (C=O) ppm. $C_{23}H_{43}Cl_2N_5O_5$ (543.54): calcd. C 50.82, H 7.97, N 12.96; found C 50.91, H 7.88, N 12.89.

3e: Yield: 2.57 g, 72%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **3d**.$C_{17}H_{31}Cl_2N_5O_5$ (456.37): calcd. C 44.74, H 6.85; found C 45.10, H 6.45.

3f: Yield: 3.98 g, 87%. M.p. 220 °C. IR (KBr): $\tilde{\nu}$ = 3245, 2916, 1681, 1643, 1623 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.94 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.33 (m, CH_2 fatty chain), 1.63 (m, 2 H, CH_2 in β of CO), 2.23 (t, ^3J = 7 Hz, 2 H, CH_2 in α of CO), 2.74 (m, 2 H, CH_2 in β of NH_3^+), 3.20–3.40 (m, 8 H, 2 CH_2NH, CH_2 Im and $CH_2NH_3^+$), 3.58 (t, ^3J = 5 Hz, 2 H, CH_2), 3.66 (s, 4 H, CH_2), 4.75 (t, ^3J = 6.6 Hz, 1 H, CH), 7.44 (s, 1 H, CH=C), 8.89 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.5 (CH_3), 24–38 (CH_2), 41.4 (CH_2-NH_3^+), 54.5 (CH), 71–72 (4 CH_2-O), 119.2 (CH=C), 131.7 (CH=N), 135.7 (C_q), 172–177 (3 C=O) ppm. $C_{25}H_{48}Cl_2N_6O_5$ (583.60): calcd. C 51.45, H 8.29, N 14.40; found C 50.97, H 8.18, N 14.12. $C_{25}H_{48}Cl_2N_5O_5$ (586.96): calcd. C 51.15, H 8.29, N 14.40; found C 50.97, H 8.18, N 14.12.

3g: Yield: 4.17 g. 87%. M.p. 198 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **3f**. $C_{27}H_{52}Cl_2N_6O_5$ (611.65): calcd. C 53.02, H 8.57, N 13.74; found C 52.92, H 8.49, N 13.68.

3h: Yield: 4.61 g, 92%. M.p. 224 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **3f**. $C_{29}H_{56}Cl_2N_6O_5$ (639.70): calcd. C 54.45, H 8.82, N 13.14; found C 54.62, H 8.92, N 13.29.

Alkyl-amido-ethoxy-ethoxy-ethylamidoglycyl-glycyl-histidine Dihydrochloride (4a–h): The procedure was similar to that used for the preparation of **3**. Boc-glycyl-glycine was used to replace Boc-β-Alanine.

4a: Yield: 3.70 g, 81%. M.p. 140 °C. IR (KBr): $\tilde{\nu}$ = 3225, 2921, 1739, 1683, 1653, 1618 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.92 (t, ^3J = 6.5 Hz, 3 H, CH_3) 1.2–1.4 (m, 12 H, $6CH_2$) 1.61 (m, 2 H, CH_2 in β of CO) 2.22 (t, ^3J = 7.5 Hz, 2 H, CH_2CO) 3.35 (m, 2 H, CH_2 Im) 3.45 (t, ^3J = 5 Hz, 2 H, CH_2O) 3.58 and 3.71 (t, ^3J = 5 Hz, 4 H, CH_2O) 3.77 (s, 2 H, CH_2 Gly) 3.99 (s, 2 H, $CH_2NH_3^+$ Gly) 4.32 (m, 2 H, CH_2OCO) 4.79 (m, 1 H, CH His) 7.54 and 7.74 (s, 1 H, CH Im) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.2 (CH_3), 24–36 (CH_2 fatty chain), 42.6 (CH_2NHCO) 44.2 (CH_2-NH_3^+ Gly), 53.8 (CH His), 66.8 (CH_2OCO) 71 and 72 (CH_2-O), 119.8 (CH=C), 131.4 (CH=N), 135.9 (C_q), 169 and 172 (4 C=O) ppm. $C_{24}H_{44}Cl_2N_6O_6$ (586.62): calcd. C 49.14, H 7.56; found C 49.40, H 7.60.

4b: Yield: 3.42 g, 81%. M.p. 149 °C. IR (KBr): $\tilde{\nu}$ = 3380, 2921, 1738, 1686, 1649, 1626 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.21 (t, ^3J = 7.5 Hz, 2 H, CH_2 in α of CO), 3.29 (m, 2 H, CH_2 Im), 3.41 (t, ^3J = 5 Hz, 2 H, CH_2NH), 3.78 (s, 2 H, CH_2 Gly), 4.12 (s, 2 H, $CH_2NH_3^+$) 4.34 (m, 2 H, CH_2OCO) 4.45 (m, 1 H, CH His) 7.42 (s, 1 H, CH=C), 7.83 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 23–36 (CH_2 fatty chain and CH_2 Gly), 44.1 ($CHNH_3^+$), 52.3 (CH His), 55.3 (CH_2NHCO), 63.6 (CH_2OCO), 120.3 (CH=C), 130.2 (C=NH), 147.1 (C=CH) 170 and 176 (C=O) ppm. $C_{22}H_{40}Cl_2N_6O_5$ (542.29): calcd. C 48.72, H 7.43, N 15.58; found C 48.62, H 7.38, N 15.32.

4c: Yield: 2.85 g, 80%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **4b**.$C_{16}H_{28}Cl_2N_6O_5$ (455.34): calcd. C 42.20, H 6.20; found C 42.25, H 6.45.

4d: Yield: 3.73 g, 82%. M.p. 152 °C. ^1H NMR (400 MHz, CD_3OD): δ = 0.90 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.2–1.4 (m, CH_2 fatty chain), 1.62 (m, 2 H, CH_2 in β of CO), 2.21 (t, ^3J = 7.5 Hz, 2 H, CH_2 in α of CO), 3.30 (m, 2 H, CH_2 Im), 3.45 (t, ^3J = 5 Hz, CH_2NHCO) 3.58 and 3.71 (t, ^3J = 5 Hz, 4 H, CH_2O) 3.77 (s, 2 H, CH_2 Gly) 3.99 (s, 2 H, $CH_2NH_3^+$ Gly) 4.32 (m, 2 H, CH_2OCO) 4.82 (m, 1 H, CH His), 7.44 (s, 1 H, CH=C), 7.84 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.3 (CH_3), 23–36 (CH_2 fatty chain and CH_2 Gly) 39.2 (CH_2OCO) 43.7 ($CHNH_3^+$) 53.3 (CH His) 63.5 (CH_2NHCO) 70 and 71 (CH_2O) 120.1 (CH=C) 130.0 (C=NH), 147.0 (C=CH) 170–176 (C=O) ppm. $C_{24}H_{44}Cl_2N_6O_6$ (583.50): calcd. C 49.40, H 7.60, N 14.40; found C 48.92, H 7.48, N 14.12.

4e: Yield: 2.81 g, 72%. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **4d**. $C_{18}H_{32}Cl_2N_6O_6$ (499.39): calcd. C 43.29, H 6.46; found C 43.76, H 6.82.

4f: Yield: 4.22 g, 86%. M.p. 219 °C. IR (KBr): $\tilde{\nu}$ = 3286, 2916, 1685–1620 cm^{-1} (4 ν_{CO}). ^1H NMR (400 MHz, CD_3OD): δ = 0.89 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.20–1.40 (m, CH_2 fatty chain), 1.60 (m, 2 H, CH_2 in β of CO), 2.23 (t, ^3J = 7 Hz, 2 H, CH_2 in α of CO), 3.10–3.45 (m, 6 H, 2 CH_2NH and CH_2 Im), 3.50–3.60 (m, 4 H, CH_2 in β of NH), 3.63 (s, 4 H, CH_2O), 3.81 (s, 2 H, CH_2 Gly), 3.94 (s, 2 H, $CH_2NH_3^+$), 4.28 (t, ^3J = 6.7 Hz, 1 H, CH), 7.42 (s, 1 H, CH=C), 8.83 (s, 1 H, CH=N) ppm. ^{13}C NMR (100.58 MHz, $CDCl_3$): δ = 15.2 (CH_3), 24–38 (CH_2), 44.5 (CH_2-NH_3^+), 54.5 (CH), 71–72 (4 CH_2-O), 119.4 (CH=C), 131.8 (CH=N), 137.2 (C_q), 169–178 (4 C=O) ppm. $C_{26}H_{49}Cl_2N_7O_6$ (626.62): calcd. C 49.84, H 7.88, 15.65; found C 50.07, H 7.99, N 15.76. $C_{26}H_{49}Cl_2N_7O_6$ (630.05): calcd. C 49.56, H 7.84, N 15.65; found C 50.07, H 7.99, N 15.76.

4g: Yield: 4.36 g, 85%. M.p. 210 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **4f**. $C_{28}H_{53}Cl_2N_7O_6$ (654.68): calcd. C 51.37, H 8.16, N 14.98; found C 51.02, H 8.36, N 14.88.

4h: Yield: 4.82 g, 90%. M.p. 169 °C. IR, ^1H NMR, and ^{13}C NMR spectral data are identical to those of **4f**. $C_{30}H_{57}Cl_2N_7O_6$ (682.73): calcd. C 52.78, H 8.42, N 14.36; found C 52.67, H 8.48, N 14.26.

Supporting Information (see footnote on the first page of this article): Dependence of relative fluorescence intensity I/I_0 of pyrene on different "amide–ester" surfactant concentrations; hydrolysis of the "amide–ester" trimoduli amphiphiles as followed by ^1H NMR spectroscopy; temperature-dependent ^1H NMR spectra of **3h**.

Acknowledgments

This work is supported by the Institute Jean Barriol, France (project PRIME 2008). We would like to thank the "Ministère de l'En-

seignement supérieur et de la Recherche" for a PhD grant to P. G. and N. D., and we would also like to thank M. J. Stébé for polarized optical micrographs and useful discussions.

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Received: February 11, 2009
Published Online: July 6, 2009

