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Towards novel efficient monomeric surfactants based on serine, tyrosine and 4-hydroxyproline: synthesis and micellization properties

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ABSTRACT

The synthesis of some novel monomeric serine- and tyrosine-based cationic and 4-hydroxyproline-based anionic surfactants, having a long lipophilic alkyl chain directly attached to the nitrogen atom of the amino acid, is described. The most efficient synthetic methodologies were established: reductive amination of the corresponding 'fatty' aldehydes, followed by methylation and deprotection (serine and tyrosine) to obtain the cationic surfactants; or reductive amination followed by saponification (4-hydroxyproline) to obtain the anionic ones. All the compounds were obtained in good to excellent yields. An assessment of their micellization properties and surface activity by tensiometry showed fairly good performance levels.

1. Introduction

The interfacial and self-assembly properties of surfactants in aqueous environments have been investigated for many decades owing to their basic interest in physical chemistry, biophysics and material sciences, and enormous practical relevance.[1,2]Surfactants find widespread use in inter alia household products, food and pharmaceutical formulations, oil recovery products, paints and paper. In view of this importance, the synthesis of a great variety of these compounds, including alkyl phosphates and sulfates, alkylbenzene sulfonates, alkyl glycosides and quaternary ammonium salts, is already well established. However, the ever growing demand for environmentally friendly speciality surfactants requires the design of molecules not only with efficient interfacial properties but also with high levels of biocompatibility and biodegradability. This is particularly important for surfactants used for cosmetic or biomedical purposes (e.g., drug and gene delivery). Natural surfactants would fulfil these requirements, but the high cost of work-up is an insuperable disadvantage: the natural products are usually present in small quantities and the separation process tends to be time-consuming and inefficient.[3] Thus, surfactants containing natural structural motifs—like amino acids, sugars and fatty acids—made from renewable raw materials have emerged as alternatives to reduce the impact on the environment and to save fossil resources. The low toxicity, good biocompatibility and fast degradation of many of these surfactants are the main reasons for the increasing industrial interest in these compounds.[4–10]

With regard to amino acids, their potential as raw materials for the preparation of surfactants has been highlighted in the literature. Initially used as preservatives for medical and cosmetic applications, they have been found to possess interesting biological activities (anti-viral, anti-neoplastic, gene delivery/therapy) and excellent emulsifying properties.[5,11–15] Such monomeric surfactants can be defined as amphiphilic molecules that contain one amino acid residue as the hydrophilic part and a long chain as the lipophilic moiety. The long chain can be introduced into the amino acid structure through the α-amino, the α-COOH or the side chain groups, yielding different types of surfactants. As can be seen in Scheme 1, reaction of the amino acid with fatty acids gives the corresponding *N*-acyl derivatives **1** (fatty acid amides of amino acids), while the reaction with alkyl halides (S_N2) or aldehydes/ketones (reductive amination) gives the *N*-alkyl derivates **3**. Furthermore, the reaction of amines or alcohols on the carboxylic group of the amino acid yields the corresponding *N*-alkyl amides or esters (**2** or **4**), respectively.[5] The cationic surfactants can then be obtained by methylation of the amine functionality with an alkylating agent (**2**, **3** and **4**) while the anionic ones can be accessed by saponification (**1** and **3**). In addition, compounds of type **3** offer the possibility of preparing zwitterionic surfactants. The large variety of amino acid as well as fatty acid structures allows for a diversity of tailored molecules and, consequently, of physicochemical properties.

Most studies on the synthesis and biological evaluation of amino acid-based surfactants address arginine and lysine derivatives. Arginine-based cationic surfactants are found to be less toxic than conventional quaternary ammonium-based surfactants and to be readily biodegradable; besides, they show excellent emulsifying properties and strong antimicrobial activity.[16,17] Lysine-based anionic/non-ionic surfactants are interesting for their haemolytic

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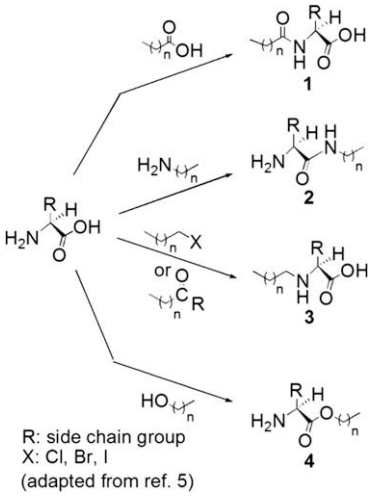


Scheme 1. Methodologies of synthesis of monomeric surfactants based on amino acids.

activities and have shown to be environmentally friendly.[18,19] In what concerns the synthesis of these amino acid-based surfactants, among the different types of linkages between the long aliphatic chain and the amino acid, the methods described refer essentially to compounds of type **1**, *N*-alkanoyl derivatives, of type **2**, *N*-alkyl amides, and of type **4**, *O*-alkyl esters.[5]

To our knowledge, besides the case of *N*-dodecyl-*N*,*N*-dimethylglycine,[20] there is only one report on the synthesis of surfactants of type **3** derived from the amino acid cysteine, which has been obtained by nucleophilic substitution with the corresponding alkyl halide.[21]

In the current work, our main aim has been to develop an efficient methodology for the synthesis of novel monomeric surfactants containing serine, tyrosine (cationic) and 4-hydroxyproline (anionic) as polar head groups, linked to a long lipophilic chain through an *N*-alkyl linkage. This work is carried out in the broader context of the search for new bio-friendly surfactants with potentially interesting physicochemical properties.[22,23] Therefore, in order to make an initial assessment of their interfacial performance, in particular with respect to some common homologous surfactants of commercial origin and widespread use, we also present here their basic physicochemical properties in aqueous solution, e.g., the Krafft temperature and critical micelle concentration.

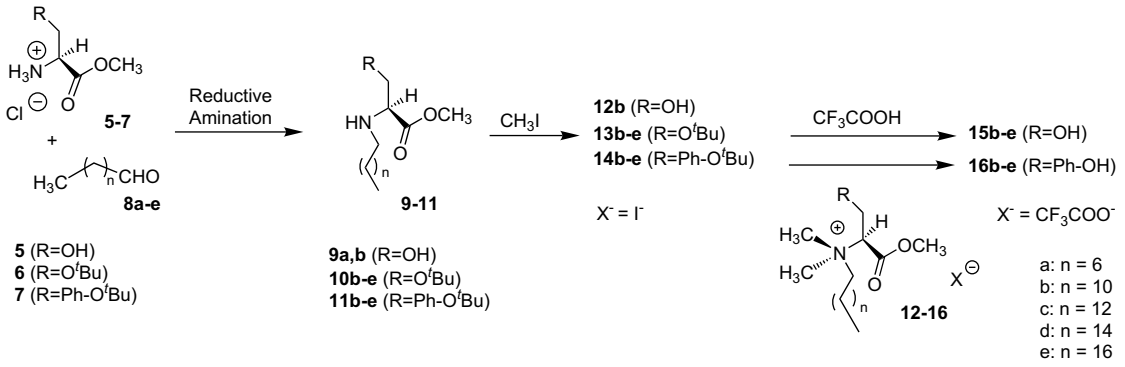
While these surfactants should be clearly interesting per se, the presence of the hydroxyl group in the side chain of these amino acids is intended to allow for the subsequent introduction of a spacer at the oxygen atoms in order to obtain dimeric structures.[24] In this context, we have performed initial studies on lysine, envisaging the preparation of the corresponding *N*,*N*′-dialkanoyl and *N*,*N*′-dialkyl derivatives (type 1 and type 3 surfactants, respectively, see Scheme 1),[25] both structures representing pseudo-gemini (monomeric double-chained) surfactants. However, with the novel monomeric surfactants herein described, new possibilities have been opened and are being explored.

2. Results and discussion

The first step in the synthesis of the proposed type 3 surfactants consists in the introduction of the alkyl chain into the amino acid. Initially, and according to a previously reported method,[26] we performed a direct alkylation using alkyl halides, but observed the formation of dialkylated products, the target compounds being isolated in very poor yields. Attempts to improve the yield modifying the reaction conditions (e.g., order of addition of reagents, use of different bases, use of various solvents and different reaction times) did not lead to satisfactory results. Consequently, the introduction of the alkyl chain into the amino acid by reductive amination of 'fatty' aldehydes was attempted (Scheme 2).[27,28] The aldehydes used were octanal (**8a**), dodecanal (**8b**), tetradecanal (**8c**), hexadecanal (**8d**) and octadecanal (**8e**), which, with exception of octanal and dodecanal (commercially available), were synthesized by oxidation of the corresponding alcohols using TPAP/NMO (yields 48–63%) according to a method described in the literature.[29] The initial studies were performed with serine methyl ester hydrochloride (**5**) and dodecanal (**8b**).

When sodium triacetoxyborohydride was used as reducing agent in the presence of NEt_3, low yields of **9** were obtained (28%, Table 1). With sodium borohydride in MeOH (entry 2) only a vestigial amount of the desired product formed (2%), but in the presence of NEt_3 the yield increased significantly (entry 3, 32%). Since the amino acid ester hydrochloride was being used, the NEt_3 was necessary to liberate the free amine from the salt, thus increasing the nucleophilicity of the nitrogen atom. In any case the yields were satisfactory and we attempted the synthesis of the target compounds using the reductive amination under solvent-free conditions, as described by Cho and Kang.[30] In this case, the aldehyde used was octanal (**8a**) whose choice was due to the fact that it is liquid at rt and allows for a better mixture of the reagents. Once again the yield was very low (13%), the work-up of the resulting mixture being the major problem. In fact, irrespective of the employed methodology, the compounds obtained in this first step were very difficult to isolate from the reaction mixture, mainly due to their strong amphiphilic character.

While performing the subsequent reaction (methylation) of the serine derivative **9b**, it was verified that the presence of the hydroxyl group in the amino acid side chain was disadvantageous,



Scheme 2. Synthesis of cationic monomeric surfactants.

Table 1
Reductive amination of aldehydes with serine, tyrosine and 4-hydroxyproline derivatives

Entry	Method[a]	Amino acid	Product	Yield/%
1	I	HSerOMe	**9b**	28
2	II_a	HSerOMe	**9b**	2
3	II_b	HSerOMe	**9b**	32
4	III	HSerOMe	**9a**	13
5	II_b	HSer(tBu)OMe	**10b**	10
6	I	HSer(tBu)OMe	**10b**	79
7		HSer(tBu)OMe	**10c**	62
8		HSer(tBu)OMe	**10d**	71
9		HSer(tBu)OMe	**10e**	84
10	I	HTyr(tBu)OMe	**11b**	81
11		HTyr(tBu)OMe	**11c**	74
12		HTyr(tBu)OMe	**11d**	61
13		HTyr(tBu)OMe	**11e**	39
14	I	HHypOMe	**18b**	85
15		HHypOMe	**18c**	54
16		HHypOMe	**18d**	76

[a] I: DCE, NEt_3 (1.5 equiv), $NaBH(OAc)_3$ (1.4–1.5 equiv); IIa: MeOH, $NaBH_4$ (1.7 equiv); IIb: MeOH, NEt_3 (1.5 equiv), $NaBH_4$ (1.7 equiv); III: NEt_3 (1 equiv), TsOH (1 equiv), $NaBH_4$ (1 equiv).

leading to a complex mixture of products, which could not be properly separated. Methylation seemed to occur not only at the nitrogen atom but also at the oxygen of the OH group. Indeed, the fractions isolated by column chromatography were not homogenous by TLC, and the NMR analyses revealed the presence of various methyl groups, which could be attributed to multiple alkylations as well as to a mixture of different alkylated products. Hence, in order to circumvent this problem, the commercially available *O*-*tert*-butyl protected serine/tyrosine methyl ester hydrochlorides, **6** and **7**, respectively, were used for the further reductive aminations.

In the case of 4-hydroxyproline, as the desired *O*-protected derivative was not commercially available, the reductive aminations were performed using **17** and the OH group of the resulting *N*-alkylated derivatives (**18b**–**d**) was protected, prior to methylation, using diphenyl-*tert*-butylchlorosilane.[31]

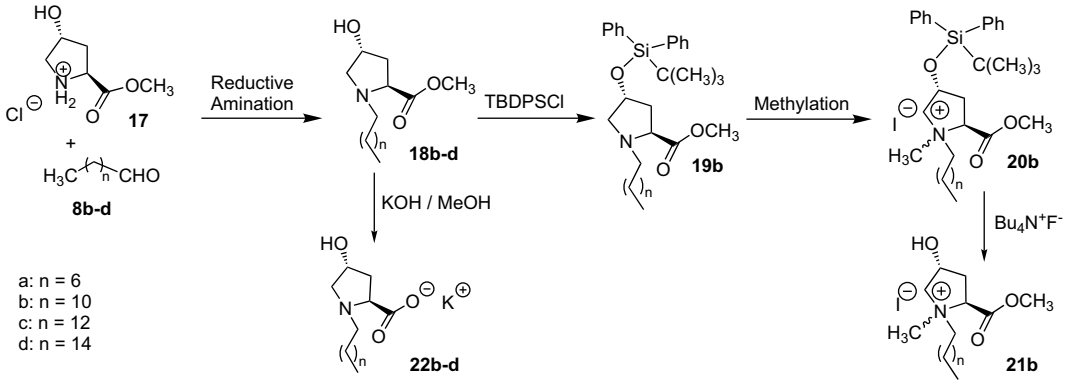
For the reaction of the *O*-protected serine derivative **6** with dodecanal (**8b**), the two best yielding methods, I and IIb, were tested. While in the case of IIb only 10% of the desired product (**10b**) formed, high yields were obtained using method I (entries 5 and 6, respectively). The reaction proceeded rapidly (6 h) and although it was not complete as confirmed by TLC, increasing the reaction time did not increase the yield. The work-up (extraction) of the reaction mixture was more efficient than when using the unprotected analogue, due to the lower hydrophilic character of the product imparted by the presence of the *tert*-butyl group, therefore the higher yields obtained in comparison to compound **9b** (entry 1). After optimization of the reaction conditions of method I all further reductive aminations were thus performed. In all cases good to excellent yields were obtained (Table 1).

The next step in the synthesis of the serine- and tyrosine-based cationic surfactants consisted in the methylation of the previously synthesized compounds **9**–**11**.[32] As already mentioned, with the serine *N*-dodecyl methyl ester (**9b**) difficulties were encountered since the excess of methyl iodide/bromide led to alkylation not only of the amine but also of the hydroxyl group. In order to avoid this competitive alkylations, only 2 mol equiv of CH_3I was used, but under these conditions the main product obtained was the mono methylated derivative, while the desired quaternary ammonium salt (**12b**) formed in only 16% yield (Table 2, entry 1). Using the *O*-*tert*-butyl protected amino acid derivatives, a large excess of the alkylating agent could be employed without concerning about competitive alkylation of the side chain OH group. After the establishment of the best reaction conditions, the yields were almost quantitative. Although an additional step, the removal of the protecting group, is necessary for the obtention of the target cationic surfactants, now as the corresponding trifluoroacetates, this methodology is by far advantageous, as the deprotection is carried out with yields in the order of 60–90% (Table 2).

In what concerns the 4-hydroxyproline derivatives, the synthesis of the corresponding cationic and anionic surfactants was aimed at. For the obtention of the cationic surfactants, we tried to establish the reaction sequence using the *N*-alkylated derivative **18b**. After protection of the OH group using *tert*-butyldiphenylchlorosilane, methylation of the resulting product (**19b**, 81%) using the previously established reaction conditions afforded the *O*-protected derivative (**20b**) in good yield (79%) (see Scheme 3). However, when deprotection was attempted using tetrabutylammonium fluoride, a complex mixture, from which no major product could be isolated, was obtained. The three different fractions isolated by column chromatography turned out to be tetrabutylammonium hydroxide, *tert*-butyldiphenylfluorosilane and a non-identifiable mixture (NMR, some peaks of the expected compound were missing). To overcome the difficulties arising from this protection/deprotection sequence the next synthetic approach was solid phase organic synthesis (SPOS). We used a 2-chlorotritylchloride resin to anchor the amino acid derivative **18b** through its OH group, so there would be no further need for protection/deprotection of this functionality. Methylation of the resin bound compound should yield the corresponding quaternary ammonium (proline) derivative, which upon cleavage with CF_3COOH should render the desired cationic surfactant (Scheme 4).[33] After work-up of the final reaction mixture we verified that the reaction occurred to a very low extent, yielding only a vestigial quantity of the target cationic surfactant. Trials to optimize the reaction conditions were not successful, in part due to the difficulty to account for the extension of each of the reaction steps. Although there are several colorimetric tests for the evaluation of the presence of functional groups on the resin-supported molecules, thus allowing for a quick and reliable monitoring of most reactions, they were not suited for the type of compounds used.[34] Chromatographic techniques, on the other hand, require the cleavage of the compound from the solid support. Furthermore, the need for a large excess of reagents inherent to this methodology constitutes a disadvantage, because of the high cost of the starting material. Despite the fact that the compounds obtained with this approach showed MS spectra compatible with the expected structures, the small quantities of product obtained (1–2 mg) were not sufficient for an NMR analysis

Table 2
Methylation and removal of the protective group (serine and tyrosine derivatives)

Reaction type	Entry	Reaction conditions	Product	Yield/%
Methylation	1	Isopropanol, CH_3I (2 mol equiv), 50–60 °C	**12b**	16
	2	DMF, K_2CO_3 (2 mol equiv),	**13b**	90
	3	18-crown-6 (cat); CH_3I	**13c**	70
	4	(4 mol equiv)	**13d**	96
	5		**13e**	88
	6		**14b**	95
	7		**14c**	52
	8		**14d**	93
	9		**14e**	66
Removal of protective group	10	CF_3COOH	**15b**	73
	11		**15c**	71
	12		**15d**	62
	13		**15e**	89
	14		**16b**	75
	15		**16c**	37
	16		**16d**	77
	17		**16e**	35



Scheme 3. Synthesis of proline-based monomeric surfactants.

to confirm the results. The SPOS methodology proved not to be suitable for this type of syntheses and was therefore abandoned.

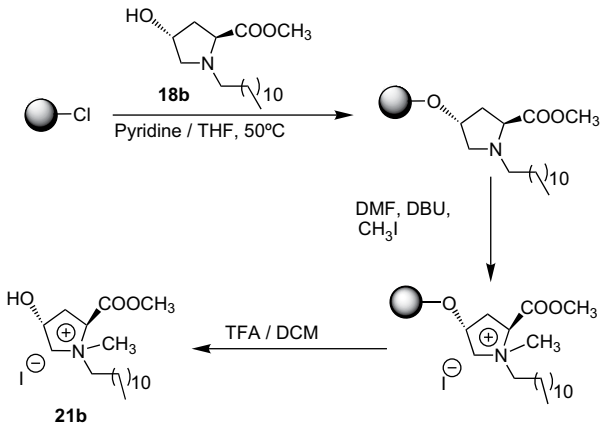
The anionic hydroxyproline-based surfactants were readily accessed by saponification of the corresponding *N*-alkyl-4-hydroxyproline methyl esters (**18**) with KOH/MeOH at rt, as described by Bodanszky and Bodanszky.[35] Although the separation process was difficult, partly due to the emulsifying properties of the resulting compounds, they could be isolated in good to excellent yields (Table 3, Scheme 3).

Concerning the stereochemistry of the compounds obtained, and taking into account the reaction conditions throughout the whole syntheses, epimerization of the chiral carbon atom cannot be excluded. However, the ^{1}H NMR spectra of one of the compounds in the presence of varying concentrations of Eu(hfc)$_3$ did not show separation of any of the peaks. Furthermore, when independent batches of the same compound were used in physicochemical measurements, no meaningful variation of the determined properties—namely the critical micelle concentration—was observed (data below). Thus, even if there is any epimerization, surface tension data strongly suggest that the composition of the final product is always the same.

The evaluation of the physicochemical properties of the synthesized surfactants has been carried out, in order to probe for their efficiency and potential as surface-active agents in action. First, it should be recalled that commercially available cationic surfactants, such as hexadecyltrimethylammonium bromide (HTAB), are known to be fairly toxic, with respect to both aquatic and cellular environments.[4,36] Anionic surfactants, such as sodium dodecyl sulfate, also suffer from similar shortcomings, albeit in a smaller magnitude than cationics.[19] Efforts to improve the toxicological properties of functional surfactants using 'natural' starting materials, while keeping a desirable interfacial performance, are therefore a relevant and challenging task, especially when it comes to direct applications.

The assessment of the basic micellization and surface action properties for selected synthesized surfactants was carried out by means of tensiometry. The C16 derivatives were chosen—C16Hyp (**22d**), C16Tyr (**16d**) and C16Ser (**15d**)—as well as the complete series of the Ser-based surfactants (C12 to C18). Figure 1 shows the surface tension versus log(concentration) curves obtained for the C16 derivatives and the commercial homologue, HTAB. Similar curves were obtained for all the other compounds. The critical micelle concentration (cmc) is the inflection point in the curve, as graphically illustrated in Figure 1 for C16Hyp. Because no minimum (or well) in the vicinity of the cmc is seen, a common fingerprint of surface-active impurities or surfactant mixtures,[37] these curves provide further evidence for the good purity of the surfactants.

In Table 4, the cmc values and other parameters of interest are listed for the selected surfactants. Values for reference anionic (SDS and SHS) and cationic (DTAB and HTAB) commercial homologues are also listed for comparison. Several relevant conclusions can be withdrawn. (i) The Krafft temperature—temperature at which the surfactant solubilizes to yield micelles—of the new surfactants is within expected values for the respective alkyl chain length. (ii) Their cmc values are without exception lower than those of the commercial homologues. For the cationic amphiphiles, the values are between two and seven times lower, whereas for the anionic compounds, 16Hyp and SHS, a 48-fold difference is observed, a remarkable value, even accounting for the different temperature and pH of measurement. (iii) The surface tension values at the cmc plateau of the new surfactants, another measure of interfacial effectiveness, are comparable to their commercial homologues, and



Scheme 4. SPOS approach to 4-hydroxyproline-based cationic surfactants.

Table 3
Saponification of *N*-alkyl-4-hydroxyproline methyl esters (**18**)

Reaction conditions	Product	Yield/%
KOH, methanol	**22b**	72
	22c	53
	22d	85

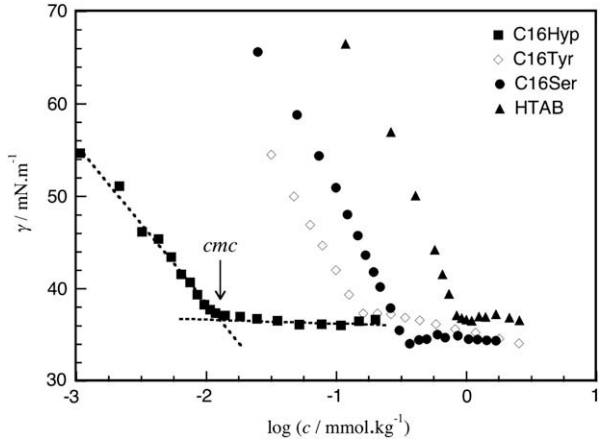


Figure 1. Surface tension versus log(concentration) curves for the hexadecyl amino acid derivatives, used for the cmc determination, as illustrated graphically for C16Hyp. The temperature is 40.0 °C, except for C16Hyp, 25.0 °C. For comparison, the curve for the commercial cationic surfactant HTAB is also shown.

in one case, C12Ser (**15b**), notably lower (10 mN m^{-1} difference with respect to DTAB). Overall, these results indicate good surface performance levels for the new compounds, and in some parameters even enhanced performance with respect to reference homologues.

It should be pointed out that in a recent report we have shown that both C12Ser and C16Ser, in combination with anionic lysine-based amphiphiles, are able to spontaneously form vesicles of long-term stability.[38] This type of vesicles, based on cationic/anionic surfactant mixtures, possess enhanced chemical and colloidal stability, and good encapsulation properties for drugs, increasingly arising as a viable alternative to classical lipid-based liposomes for drug delivery.[22,39] It is also known that cationic surfactants per se can be used for compaction and decompaction of DNA, aiming at non-viral gene delivery to cells.[14] In this context, cationic molecules based on natural sources and thus of lower toxicity should be greatly advantageous. Preliminary toxicity tests (haemolytic activity and potential ocular irritation) performed for C12Ser and C12Tyr have labelled these molecules as only slightly or moderately irritant, respectively, showing better results than, for instance, HTAB.[40]

All these observations highlight a good potential for application-related uses of the new molecules. Further studies of their self-assembly properties in aqueous solution are currently being undertaken.

Table 4
Micellization properties of amino acid-based surfactants and some reference commercial ionic homologues[a]

Surfactant	T_{Kr}/°C	T_{meas}/°C	cmc/mmol kg^{-1}	γ_{cmc}/mN m^{-1}
C16Hyp (**22d**)	<25	25.0	0.012[b]	36.5
C16Tyr (**16d**)	28.7	40.0	0.14	38.2
C12Ser (**15b**)	<25	25.0	1.87	29.2
C14Ser (**15c**)	25.7	35.0	1.16	33.0
C16Ser (**15d**)	31.2	40.0	0.34	34.7
C18Ser (**15e**)	46.7	48.0	0.049	34.3
SDS	<25	25.0	8.3[c]	40.0
SHS	n.a.	40.0	0.58[d]	n.a.
DTAB	<25	25.0	13.6	39.5
HTAB	26.0	40.0	0.84	36.5

[a] T_{Kr}, surfactant Krafft temperature; T_{meas}, temperature of cmc measurement; cmc, critical micelle concentration; γ_{cmc}, surface tension value at the cmc. Surfactant acronyms: SDS, sodium dodecyl sulfate, SHS, sodium hexadecylsulfate; DTAB, dodecyltrimethylammonium bromide; HTAB, hexadecyltrimethylammonium bromide.
[b] Measured at pH=12.0, for solubility reasons.
[c] From Ref. 2.
[d] From Ref. 37.

3. Conclusions

In this work, a straightforward and high yielding methodology for the synthesis of novel serine- and tyrosine-based cationic monomeric surfactants and 4-hydroxyproline-based anionic monomeric surfactants has been developed and optimized. Three surfactant series with even chain lengths between C12 and C18 have been synthesized. These surfactants differ from most of the reported amino acid-based ones in that they are *N*-alkyl derivatives of amino acids. Because they are prepared from natural sources, they are expected to be less toxic than conventional ones and thus of great interest for application purposes. While they structurally 'mimic' conventional amphiphiles, surface tension studies demonstrate that they possess enhanced interfacial performance, as judged from their lower cmc values in comparison to corresponding homologues and, in some cases, decreased surface tension values at the cmc.

4. Experimental

4.1. General

Amino acids were purchased from NovaBiochem. Solvents (p.a. quality) were from Merck. Both, thin layer chromatography (TLC) aluminium foil plates covered with silica 60 F_{254} (0.25 mm) and silica gel 60 (70–230 mesh ASTM) for preparative column chromatography were from Merck. TPAP was purchased from TCI, other chemicals were obtained from Sigma–Aldrich.

When required, solvents were dried and distilled prior to use. DCM was distilled from P_4O_{10} and Et_3N from KOH.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX250, AMX300 or AMX500 spectrometer. Chemical shifts, δ, are presented in parts per million from tetramethylsilane (TMS, 0 ppm) as internal standard for $CDCl_3$ and residual solvent peaks for other deuterated solvents. Coupling constants, *J*, are in hertz, Hz. Infrared spectra (IR) were recorded on a Bruker Tensor 27 spectrometer. ESI mass spectra were recorded on a Finnigan Surveyor instrument, equipped with mass detector Finnigan LCQ DECA XP MX (Finnigan Corp.San José, CA, USA) and API (Atmospheric Pressure Ionization) using an ESI interface (Electrospray Ionization). ESI-TOF HRMS spectra were obtained on a Microtof apparatus. The samples have been analyzed by direct injection and the spectra were obtained in positive ESI mode (*m*/*z* 50–1000). For the surface tension measurements, a DCAT11 tensiometer from Dataphysics Instruments GmbH was used (Wilhelmy plate method). Temperature was kept constant at the desired value (±0.1 °C) using a thermostated Julabo water bath. Melting points (MP)/clearing points (CP) of cationic serine and anionic proline derivatives were determined by combined DSC and polarizing light microscopy using a Setaram DSC141 calorimeter and an Olympus BX51 microscope equipped with a Linkam TMHS 600 hot-stage, respectively. The temperature values were extracted from the thermogram using the software provided by the manufacturer.

4.2. Representative procedure for reductive amination

*4.2.1. N-Dodecyl-O-tert-butyl serine methyl ester (**10b**)*

H-Ser-(^{t}Bu)-OMe]·HCl (**6**) (9.0 mmol, 1.9 g) and triethylamine (14 mmol, 2.0 mL) were dissolved in DCE (10 mL) and stirred for 15 min to liberate the amine from the salt. Then a solution of dodecanal (**8a**) (9.2 mmol, 1.7 mmol) in 20 mL of DCE was added, followed by sodium triacetoxyborohydride (14 mmol, 3.0 g). The mixture was stirred at rt under an argon atmosphere for 12 h, quenched with $NaHCO_3$ (saturated solution) and the product was extracted with ethyl acetate (3×20 mL). The ethyl acetate extracts were washed with brine and dried (Na_2SO_4). The solvent was evaporated and the crude product was subjected to column chromatography on silica gel using hexane/ethyl acetate (5:1) and DCM/MeOH (10:1) mixtures as eluents to yield the product as an uncoloured oil (yield 79%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.71 (s, 3H), 3.62–3.48 (m, 2H), 3.39 (t, 1H, J=5.4 Hz), 2.62 (dt, 1H, J=11.0, 7.2 Hz), 2.47 (dt, 1H, J=11.2, 7.2 Hz), 1.76 (s, 1H), 1.55–1.40 (m, 2H), 1.35–1.18 (br s, 18H), 1.13 (s, 9H), 0.86 (t, 3H, J=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.03 (CO), 73.12 (C), 63.01 (CH_2), 62.10 (CH_3), 48.29 (CH_2), 31.88 (CH_2), 30.12 (CH_2) [29.63, 29.60, 29.57, 29.57, 29.55, 29.47, 29.31 ($7CH_2$)], 27.32 (CH_3), 27.20 (CH_2), 22.65 (CH_2), 14.07 (CH_3). FTIR (cm^{-1}, liquid film): 3319 (s), 2955 (s), 2854 (s), 1750 (s), 1467 (s), 1364 (m), 1197 (m), 737 (m). MS (ESI, MeOH) m/z calcd 344.32 $[M+H]^+$; found 344.37.

*4.2.2. N-Tetradecyl-O-tert-butyl serine methyl ester (**10c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.75 (br s, 1H), 3.72 (s, 3H), 3.63–3.51 (m, 2H), 3.44 (t, 1H, J=5.0 Hz), 2.64 (dt, 1H, J=11.1, 7.4 Hz), 2.51 (dt, 1H, J=11.1, 7.1 Hz), 1.54–1.41 (m, 2H), 1.24 (br s, 22H), 1.13 (s, 9H), 0.86 (t, 3H, J=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.57 (CO), 73.18 (C), 62.60 (CH_2), 61.69 (CH), 51.70 (CH_3), 47.96 (CH_2), 31.89 (CH_2), 29.75 (CH_2) [29.64, 29.58, 29.54, 29.44, 29.33, ($8CH_2$)], 27.30 (CH_3), 27.17 (CH_2), 22.66 (CH_2), 14.08 (CH_3). FTIR (cm^{-1}, liquid film): 3317 (w), 2924 (s), 2853 (s), 1741 (m), 1467 (m), 1364 (m), 1197 (m), 721 (m). MS (ESI, MeOH) m/z calcd 372.35 $[M+H]^+$; found 372.87.

*4.2.3. N-Hexadecyl-O-tert-butyl serine methyl ester (**10d**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.69 (s, 3H), 3.60–3.45 (m, 2H), 3.37 (t, 1H, J=5.4 Hz), 2.61 (dt, 1H, J=11.1, 7.4 Hz), 2.46 (dt, 1H, J=11.1, 7.2 Hz), 1.77 (br s, 1H), 1.54–1.35 (m, 2H), 1.22 (br s, 26H), 1.11 (s, 9H), 0.85 (t, 3H, J=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.98 (CO), 73.07 (C), 62.97 (CH_2), 62.06 (CH), 51.55 (CH_3), 48.25 (CH_2), 31.86 (CH_2), 30.09 (CH_2) [29.63, 29.55, 29.52, 29.45, 29.30, 27.29 ($10CH_2$)], 27.17 (CH_3), 22.62 (CH_2), 14.04 (CH_3). FTIR (cm^{-1}, Nujol) 3352 (w), 2924 (s), 2853 (s), 1744 (m), 1464 (m), 1377 (m), 1232 (m), 720 (w). MS (ESI, MeOH) m/z calcd 400.38 $[M+H]^+$; found 400.47.

*4.2.4. N-Octadecyl-O-tert-butyl serine methyl ester (**10e**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.72 (s, 3H), 3.65–3.49 (m, 2H), 3.40 (t, 1H, J=5.3 Hz), 2.63 (dt, 1H, J=11.1, 7.4 Hz), 2.49 (dt, 1H, J=11.0, 7.2 Hz), 2.14 (br s, 1H), 1.55–1.41 (m, 2H), 1.24 (br s, 30H), 1.13 (s, 9H), 0.87 (t, 3H, J=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.98 (CO), 73.14 (C), 62.95 (CH_2), 62.05 (CH), 51.63 (CH_3), 48.25 (CH_2), 31.90 (CH_2), 30.08 (CH_2) [29.67, 29.59, 29.56, 29.48, 29.34 ($12CH_2$)], 27.33 (CH_3), 27.21 (CH_2), 22.66 (CH_2), 14.09 (CH_3). FTIR (cm^{-1}, Nujol) 3352 (m), 2917 (s), 2849 (s), 1745 (s), 1463 (s), 1364 (m), 1158 (s), 729 (m). MS (ESI, MeOH) m/z calcd 428.41 $[M+H]^+$; found 428.49.

*4.2.5. N-Dodecyl-O-tert-butyl tyrosine methyl ester (**11b**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.06 (d, 2H, J=8.7 Hz), 6.90 (d, 2H, J=8.4 Hz), 3.59 (s, 3H), 3.48 (t, 1H, J=7.1 Hz), 2.95 (dd, 1H, J=13.5, 6.9 Hz), 2.87 (dd, 1H, J=13.5, 7.2 Hz), 2.56 (dt, 1H, J=11.2, 7.2 Hz), 2.44 (dt, 1H, J=11.2, 7.1 Hz), 1.81–1.69 (m, 1H), 1.53–1.31 (m, 2H), 1.32 (s, 9H), 1.28–1.20 (m, 18H), 0.88 (t, 3H, J=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.24 (CO), 154.02 (C), 132.12 (C), 129.48 (CH), 124.12 (CH), 78.27 (C), 63.27 (CH), 51.45 (CH_3), 48.20 (CH_2), 39.12 (CH_2), 31.89 (CH_2), 29.98 (CH_2) [29.63, 29.61, 29.58, 29.53, 29.44, 29.32 ($6CH_2$)], 28.81 (CH_3), 27.12 (CH_2), 22.66 (CH_2), 14.09 (CH_3). FTIR (cm^{-1}, liquid film) 3328 (w), 3028 (s), 2926 (s), 2854 (s), 1739 (s), 1609 (m), 1467 (s), 1389 (s), 1164 (s), 899 (s), 723 (m). MS (ESI, MeOH) m/z calcd 420.35 $[M+H]^+$; found 420.54.

*4.2.6. N-Tetradecyl-O-tert-butyl tyrosine methyl ester (**11c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 2H, J=8.4 Hz), 6.83 (d, 2H, J=8.4 Hz), 3.52 (s, 3H), 3.46 (t, 1H, J=7.1 Hz), 2.92 (dd, 1H, J=13.5, 6.5 Hz), 2.81 (dd, 1H, J=13.5, 7.5 Hz), 2.55–2.34 (m, 2H), 1.43–1.30 (m, 2H), 1.25 (s, 9H), 1.22–1.14 (m, 22H), 0.87 (t, 3H, J=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.83 (CO), 154.09 (C), 131.83 (C), 129.52 (CH), 124.16 (CH), 78.31 (C), 62.94 (CH), 51.53 (CH_3), 47.95 (CH_2), 38.78 (CH_2), 34.19 (CH_2), 31.91 (CH_2) [29.64, 29.59, 29.53, 29.46, 29.41, 29.34, 29.27, 29.13 ($8CH_2$)], 28.81 (CH_3), 27.09 (CH_2), 22.67 (CH_2), 14.09 (CH_3). FTIR (cm^{-1}, liquid film) 3166 (w), 2954 (s), 2919 (s), 2850 (s), 1742 (s), 1508 (m), 1465 (m), 1365 (m), 1165 (m), 1093 (m), 900 (s). MS (ESI, MeOH) m/z calcd 448.38 $[M+H]^+$; found 448.58.

*4.2.7. N-Hexadecyl-O-tert-butyl tyrosine methyl ester (**11d**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05 (d, 2H, J=8.4 Hz), 6.89 (d, 2H, J=8.4 Hz), 3.58 (s, 3H), 3.47 (t, 1H, J=7.1 Hz), 2.94 (dd, 1H, J=13.4, 6.8 Hz), 2.86 (dd, 1H, J=13.4, 7.4 Hz), 2.55 (dt, 1H, J=11.1, 7.3 Hz), 2.43 (dt, 1H, J=11.1, 7.1 Hz), 1.65 (br s, 1H), 1.49–1.36 (m, 2H), 1.31 (s, 9H), 1.29–1.20 (m, 26H), 0.87 (t, 3H, J=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.25 (CO), 154.02 (C), 132.13 (C), 129.48 (CH), 124.12 (CH); 78.26 (C); 63.28 (CH); 51.45 (CH_3); 48.21 (CH_2); 39.13 (CH_2); 31.90 (CH_2); 30.00 (CH_2); [29.67, 29.64, 29.59; 29.45; 29.34 (10 CH_2)]; 28.81 (CH_3); 27.13 (CH_2); 22.67 (CH_2); 14.09 (CH_3). FTIR (cm^{-1}, liquid film) 3329 (w); 3028 (s); 2925 (s); 2854 (s); 1739 (s); 1507 (s); 1467 (s); 1369 (s); 1236 (s); 1164 (s); 1018 (m); 899 (s). MS (ESI, MeOH) m/z calcd 476.41 $[M+H]^+$; found 476.62.

*4.2.8. N-Octadecyl-O-tert-butyl tyrosine methyl ester (**11e**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 2H, J=8.4 Hz), 6.83 (d, 2H, J=8.7 Hz), 3.52 (s, 3H), 3.41 (t, 1H, J=7.1 Hz), 2.88 (dd, 1H, J=13.5, 6.6 Hz), 2.79 (dd, 1H, J=13.5, 7.2 Hz), 2.48 (dt, 1H, J=13.5, 7.2 Hz), 1.57 (br s, 1H), 1.41–1.30 (m, 2H), 1.25 (s, 9H), 1.23–1.15 (m, 30H), 0.87 (t, 3H, J=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.24 (CO), 154.01 (C), 132.12 (C), 129.47 (CH), 124.12 (CH), 78.27 (C), 63.27 (CH), 51.45 (CH_3), 48.20 (CH_2), 39.12 (CH_2), 31.90 (CH_2), 29.98 (CH_2) [29.67, 29.64, 29.59, 29.54, 29.44, 29.33 ($12CH_2$)], 28.80 (CH_3), 27.13 (CH_2), 22.66 (CH_2), 14.08 (CH_3). FTIR (cm^{-1}, liquid film) 3329 (w), 2924 (s), 2853 (s), 1739 (s), 1507 (s), 1467 (s), 1365 (s), 1234 (s), 1164 (s), 1018 (m), 899 (s). MS (ESI, MeOH) m/z calcd 504.44 $[M+H]^+$; found 504.70.

*4.2.9. N-Dodecyl proline methyl ester (**18b**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.50–4.40 (m, 1H), 3.69 (s, 3H), 3.49 (t, 1H, J=7.8 Hz), 3.40 (dd, 1H, J=9.9, 5.7 Hz), 2.69–2.58 (m, 1H) 2.50 (br s, 1H), 2.47–2.38 (m, 2H), 2.25–2.15 (m, 1H), 2.07–1.99 (m, 1H), 1.36–1.52 (m, 2H), 1.22 (br s, 18H), 0.85 (t, 3H, J=6.9 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.00 (CO), 70.42 (CH), 64.29 (CH), 61.31 (CH_2), 54.39 (CH_2), 51.80 (CH_3), 39.55 (CH_2), 31.91 (CH_2) [29.61, 29.47, 29.31, 28.30 (7 CH_2)], 27.42 (CH_2), 22.68 (CH_2), 14.11 (CH_3). FTIR (cm^{-1}, liquid film) 3522 (sharp), 3341 (br), 2853 (s), 1733 (s), 1472 (s), 1377 (m), 1221 (s). MS (ESI, MeOH) m/z calcd 314.27 $[M+H]^+$; found 314.44.

*4.2.10. N-Tetradecyl proline methyl ester (**18c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.46–4.36 (m, 1H), 3.65 (s, 3H), 3.46 (t, 1H, J=7.7 Hz), 3.37 (dd, 1H, J=10.2, 5.7 Hz, 1H), 2.79 (br s, 1H), 2.66–2.49 (m, 1H), 2.46–2.38 (m, 2H), 2.22–2.10 (m, 1H), 2.08–1.94 (m, 1H), 1.47–1.34 (m, 2H), 1.18 (br s, 22H), 0.87–0.77 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.05 (CO), 70.25 (CH), 64.34 (CH), 61.32

(CH_2), 54.48 (CH_2), 51.77 (CH_3), 39.48 (CH_2), 31.88 (CH_2) [29.63, 29.54, 29.45, 29.32, 28.29 ($9CH_2$)], 27.40 (CH_2), 22.65 (CH_2), 14.07 (CH_3). FTIR (cm^{-1}, liquid film) 3341 (br), 2918 (s), 2850 (s), 1736 (s), 1469 (s), 1193 (s), 721 (m). MS (ESI, MeOH) *m*/*z* calcd 342.30 $[M+H]^+$; found 342.50.

*4.2.11. N-Hexadecyl proline methyl ester (**18d**)*

^1H NMR ($CDCl_3$, 300 MHz) δ 4.54–4.42 (m, 1H), 3.71 (s, 3H), 3.51 (t, 3H, *J*=7.7 Hz), 3.43 (dd, 1H, *J*=10.1, 5.6 Hz), 2.66 (dt, 1H, *J*=12.3, 8.0 Hz), 2.52–2.38 (m, 2H), 2.29–2.14 (m, 1H), 2.12–2.00 (m, 2H), 1.54–1.40 (m), 1.25 (br s, 26H), 0.88 (t, 3H, *J*=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.19 (CO), 70.36 (CH), 64.36 (CH), 61.39 (CH_2), 54.54 (CH_2), 51.77 (CH_3), 39.55 (CH_2), 31.90 (CH_2) [29.67, 29.60, 29.56, 29.48, 29.33, 28.40 ($11CH_2$)], 27.41 (CH_2), 22.66 (CH_2), 14.09 (CH_3). FTIR (cm^{-1}, liquid film) 3521 (sharp), 2914 (s), 2850 (s), 1730 (s), 1470 (s), 1376 (m), 1220 (s), 718 (m). MS (ESI, MeOH): *m*/*z* calcd 370.33 $[M+H]^+$; found 370.55.

4.3. Representative procedure for methylation

*4.3.1. N-(2-tert-Butyloxy-1-methyloxycarbonyl)ethyl-N-dodecyl-N,N-dimethylammonium iodide (**13b**)*

To a solution of *N*-dodecyl-*O*-*tert*-butyl serine methyl ester **(10b)** (3.0 mmol, 1.0 g) in DMF or dry acetone (5 mL) were added potassium carbonate (6.0 mmol, 0.80 g), 18-crown-6 (cat) and iodomethane (12.0 mmol, 0.74 mL). The mixture was stirred at rt for 6–12 h until the reactants were consumed (TLC). The solvent was evaporated to give the crude ammonium salt, which was taken up in ethyl acetate (3×20 mL) and washed with sodium thiosulfate (10%) (3×20 mL). The ethyl acetate layer was dried (Na_2SO_4) and after removal of the solvent product **13b** was obtained as a white powder (yield 90%): ^1H NMR ($CDCl_3$, 300 MHz) δ 5.32 (m, 1H), 4.11 (s, 2H), 3.92–3.69 (m, 2H), 3.80 (s, 3H), 3.51 (s, 3H), 3.48 (s, 3H), 1.87–1.68 (m, 2H), 1.32–1.21 (m, 18H), 1.15 (s, 9H), 0.84 (t, 3H, *J*=6.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.88 (CO), 75.12 (C), 71.91 (CH), 65.52 (CH_2), 58.99 (CH_2), 53.20 (CH_3), 51.08 (CH_3), 50.84 (CH_3), 31.77 (CH_2) [29.46, 29.32, 29.27, 29.19, 28.95 ($6CH_2$)], 27.04 (CH_3), 26.13 (CH_2), 22.86 (CH_2), 22.55 (CH_2), 13.99 (CH_3). FTIR (cm^{-1}, liquid film) 2923 (s), 2853 (s), 1759 (w), 1463 (s), 1377 (s). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 372.35; found 372.57.

*4.3.2. N-(2-tert-Butyloxy-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-tetradecylammonium iodide (**13c**)*

^1H NMR ($CDCl_3$, 300 MHz) δ 5.54 (t, 1H, *J*=3.0 Hz), 4.14–4.07 (m, 2H), 4.00–3.65 (m, 2H), 3.77 (s, 3H), 3.47 (s, 3H), 3.43 (s, 3H), 1.84–1.63 (m, 2H), 1.29–1.13 (m, 22H), 1.12 (s, 9H), 0.86–0.76 (m, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.62 (CO), 74.94 (C), 71.55 (CH), 65.49 (CH_2), 58.81 (CH_2), 53.04 (CH_3), 51.04 (CH_3), 50.84 (CH_3), 31.19 (CH_2) [29.34, 29.30, 29.16, 29.12, 29.04, 28.88, 28.77 ($8CH_2$)], 26.87 (CH_3), 25.94 (CH_2), 22.72 (CH_2), 22.37 (CH_2), 13.82 (CH_3). FTIR (cm^{-1}, liquid film) 2923 (s), 2854 (s), 1744 (w), 1463 (s), 1377 (s). MS (ESI, MeOH) positive ESI *m*/*z* calcd 400.38; found 400.61.

*4.3.3. N-(2-tert-Butyloxy-1-methyloxycarbonyl)ethyl-N-hexadecyl-N,N-dimethylammonium iodide (**13d**)*

^1H NMR ($CDCl_3$, 250 MHz) δ 5.31 (t, 1H, *J*=3.0 Hz), 4.15–4.00 (m, 2H), 3.95–3.65 (m, 2H), 3.78 (s, 3H), 3.51 (s, 3H), 3.48 (s, 3H), 1.90–1.60 (m, 2H), 1.19 (br s, 26H), 1.13 (s, 9H), 0.84 (t, 3H, *J*=6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.73 (CO), 75.05 (C), 71.70 (CH), 65.52 (CH_2), 58.95 (CH_2), 53.14 (CH_3), 51.14 (CH_3), 50.92 (CH_3), 31.72 (CH_2) [29.50, 29.46, 29.41, 29.27, 29.23, 29.16, 28.89 ($10CH_2$)], 26.98 (CH_3), 26.06 (CH_2), 22.85 (CH_2), 22.49 (CH_2), 13.93 (CH_3). FTIR (cm^{-1}, liquid film) 2924 (s), 2853 (s), 1743 (m), 1471 (m), 1367 (m), 1238 (m), 721 (w). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 428.41; found 428.66.

*4.3.4. N-(2-tert-Butyloxy-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-octadecylammonium iodide (**13e**)*

^1H NMR ($CDCl_3$, 250 MHz) δ 5.32–5.12 (m, 1H), 4.05 (sl, 2H), 3.92–3.62 (m, 2H), 3.73 (s, 3H), 3.47 (s, 3H), 3.44 (s, 3H), 1.92–1.58 (m, 2H), 1.40–1.10 (m, 30), 1.08 (m, 9H), 0.85–0.70 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.76 (CO), 71.76 (C), 70.44 (CH), 65.52 (CH_2), 58.96 (CH_2), 53.15 (CH_3), 51.15 (CH_3), 50.92 (CH_3), 31.74 (CH_2) [29.52, 29.47, 29.43, 29.29, 29.24, 29.17, 28.90 ($12CH_2$)], 26.99 (CH_3), 26.07 (CH_2), 22.86 (CH_2), 22.50 (CH_2), 13.93 (CH_3). FTIR (cm^{-1}, liquid film) 2920 (s), 2851 (s), 1741 (m), 1470 (m), 1368 (m), 1236 (m), 720 (w). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 456.44; found 456.71.

*4.3.5. N-(2-tert-Butyloxyphenyl-1-methyloxycarbonyl)ethyl-N-dodecyl-N,N-dimethylammonium iodide (**14c**)*

^{1}NMR ($CDCl_3$, 300 MHz) δ 7.21 (d, 2H, *J*=8.4 Hz), 6.94 (d, 2H, *J*=8.4 Hz), 4.54 (dd, 1H, *J*=11.7, 4.2 Hz), 3.95–3.77 (m, 1H), 3.76–3.61 (m, 2H), 3.67 (s, 3H), 3.55 (s, 3H), 3.50 (s, 3H), 3.16 (t, 1H, *J*=12.3 Hz), 1.93–1.72 (m, 2H), 1.32 (s, 9H), 1.25 (br s, 18H), 0.88 (t, 3H, *J*=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.10 (C), 155.20 (C), 130.19 (CH), 126.63 (C), 124.52 (CH), 78.72 (C), 73.63 (CH), 65.23 (CH_2), 53.04 (CH_3), 50.73 (CH_3), 49.99 (CH_3), 32.59 (CH_2), 31.80 (CH_2) [29.51, 29.38, 29.28, 29.23, 29.07 ($6\times CH_2$)], 28.73 (CH_3), 26.04 (CH_2), 22.82 (CH_2), 22.58 (CH_2), 14.03 (CH_3). FTIR (cm^{-1}, Nujol) 2923 (s), 2853 (s), 1743 (s), 1610 (m), 1508 (s), 1460 (s), 1366 (s), 1300 (m), 1239 (m), 1239 (s), 1174 (s), 897 (s), 827 (m), 723 (m). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 448.38; found 448.55.

*4.3.6. N-(2-tert-Butyloxyphenyl-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-tetradecylammonium iodide (**14c**)*

^1H NMR (DMSO-d_6, 250 MHz) δ 7.15 (d, 2H, *J*=8.5 Hz), 6.94 (d, 2H, *J*=8.5 Hz), 4.49 (dd, 1H, *J*=12.0, 3.5 Hz), 3.58–3.24 (m, 2H), 3.51 (s, 3H), 3.24 (s, 3H), 3.21 (s, 3H), 3.24–3.02 (m, 2H), 1.85–1.63 (m, 2H), 1.26 (s, 9H), 1.25 (br s, 22H), 0.90–0.80 (m, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.86 (CO), 154.39 (C), 130.03 (CH), 127.88 (C), 123.79 (CH), 77.93 (C), 73.30 (CH), 63.86 (CH_2), 52.84 (CH_3), 48.77 (CH_3), 48.68 (CH_3), 31.19 (CH_2) [28.92, 28.81, 28.61 (9 CH_2)], 28.41 (CH_3), 25.58 (CH_2), 21.99 (CH_2), 21.65 (CH_2), 13.85 (CH_3). FTIR (cm^{-1}, liquid film) 2924 (s), 2852 (s), 1744 (s), 1558 (m), 1507 (s), 1466 (s), 1366 (s), 1238 (s), 1164 (s), 898 (s). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 476.41; found 476.59.

*4.3.7. N-(2-tert-Butyloxyphenyl-1-methyloxycarbonyl)ethyl-N-hexadecyl-N,N-dimethylammonium iodide (**14d**)*

^1H NMR (DMSO-d_6, 250 MHz) δ 7.15 (d, 2H, *J*=8.3 Hz), 6.94 (d, 2H, *J*=8.5 Hz), 4.49 (dd, 1H, *J*=11.8, 3.5 Hz), 3.58–3.39 (m, 3H), 3.51 (s, 3H), 3.24 (s, 3H), 3.21 (s, 3H), 3.14–3.02 (m, 1H), 1.83–1.63 (m, 2H), 1.27 (s, 9H), 1.23 (br s, 26H), 0.90–0.78 (m, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.83 (CO), 154.37 (C), 130.02 (CH), 127.86 (C), 123.77 (CH), 77.92 (C), 73.29 (CH), 63.85 (CH_2), 52.83 (CH_3), 48.77 (CH_3), 48.69 (CH_3), 31.19 (CH_2) [28.95, 28.91, 28.81, 28.65, 28.60, 28.36 ($11CH_2$)], 28.41 (CH_3), 25.58 (CH_2), 21.99 (CH_2), 21.66 (CH_2), 13.85 (CH_3). FTIR (cm^{-1}, Nujol) 2920 (s), 2851 (s), 1754 (s), 1507 (m), 1469 (s), 1365 (m), 1269 (m), 1164 (m), 855 (s), 720 (m). MS (ESI, MeOH) positive ESI: *m*/*z* calcd 504.44; found 504.69.

*4.3.8. N-(2-tert-Butyloxyphenyl-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-octadecylammonium iodide (**14e**)*

^1H NMR ($CDCl_3$, 300 MHz) δ 7.18 (d, 2H, *J*=8.4 Hz), 6.91 (d, 2H, *J*=8.4 Hz), 4.52 (dd, 1H, *J*=11.7, 4.2 Hz), 3.94–3.78 (m, 1H), 3.77–3.58 (m, 2H), 3.66 (s, 3H), 3.53 (s, 3H), 3.47 (s, 3H), 3.13 (t, 1H, *J*=12.3 Hz), 1.92–1.72 (m, 2H), 1.29 (s, 9H), 1.23 (br s, 30H), 0.85 (t, 3H, *J*=6.6 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.89 (CO), 154.46 (C), 130.07 (CH), 127.91 (C), 123.84 (CH), 77.98 (C), 73.38 (CH), 63.95 (CH_2), 52.86 (CH_3), 48.74 (CH_3), 48.83 (CH_3), 31.26 (CH_2) [29.01, 28.88, 28.72, 28.66 ($13CH_2$)], 28.44 (CH_3), 25.06 (CH_2), 22.06 (CH_2), 21.74 (CH_2), 13.87 (CH_3). FTIR (cm^{-1}, Nujol) 2921 (s), 2851 (s), 1754 (s), 1507 (w),

1468 (s), 1377 (m), 1268 (m), 1164 (m), 895 (m). MS (ESI, MeOH): positive ESI: *m*/*z* calcd 532.47; found 532.70.

4.4. Representative procedure for removal of the protective group

*4.4.1. N-Dodecyl-N-(2-hydroxy-1-methyloxycarbonyl)ethyl-N,N-dimethylammonium trifluoroacetate (**15b**)*

Deprotection was accomplished by stirring *N*-dodecyl-*N*,*N*-dimethyl-*N*-(1-methyloxycarbonyl-2-*tert*-butyloxy)ethylammonium iodide (**13b**) with TFA (3–5 mL) for 36 h at rt. The mixture was treated with ethyl ether until total removal of TFA and then subjected to column chromatography on silica gel, using a DCM/methanol (5:1) mixture as eluent. The product (**15b**) was isolated as an uncoloured oil (73%), which was recrystallized from AcOEt/Hexane (1:1) to yield white crystals. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.69 (br s, 1H), 4.41–4.25 (m), 3.84 (s, 3H), 3.83–3.41 (m, 3H), 3.41 (s, 6H), 1.80–1.66 (m, 2H), 1.43–1.14 (m, 18H), 0.88 (t, 3H, *J*=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.51 (CO), 73.35 (CH), 65.84 (CH_2), 58.73 (CH_2), 53.33 (CH_3), 50.94 (CH_3), 50.04 (CH_3), 31.82 (CH_2) [29.50, 29.49, 29.33, 29.23, 28.91 ($6CH_2$)], 26.09 (CH_2), 22.60 (CH_2), 22.45 (CH_2), 14.03 (CH_3). FTIR (cm^{-1}, Nujol) 3180 (br), 2923 (s), 2853 (s), 1744 (s), 1672 (s), 1458 (s), 1377 (s), 1172 (m). HRMS (ESI-TOF) calcd for $C_{18}H_{38}NO_3$ requires 316.2846; found 316.2850. Clearing point: 91.0–91.7 °C.

*4.4.2. N-(2-Hydroxy-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-tetradecylammonium trifluoroacetate (**15c**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.78–4.68 (m, 1H), 4.38 (d, 1H, *J*=13.8 Hz), 4.28 (dd, 1H, *J*=13.8, 6.3 Hz), 3.82 (s, 3H), 3.71 (dt, 1H, *J*=12.4, 4.7 Hz), 3.55 (dt, 1H, *J*=12.5, 4.5 Hz), 3.40 (s, 6H), 1.95–1.55 (m, 2H), 1.40–1.17 (m, 22H), 0.86 (t, 3H, *J*=6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.56 (CO), 73.40 (CH), 65.88 (CH_2), 58.71 (CH_2), 53.37 (CH_3), 51.02 (CH_3), 50.07 (CH_3), 31.86 (CH_2) [29.61, 29.59, 29.52, 29.36, 29.30, 29.26, 28.94 ($8CH_2$)], 26.12 (CH_2), 22.63 (CH_2), 22.49 (CH_2), 14.06 (CH_3). FTIR (cm^{-1}, Nujol) 3401 (br), 2922 (s), 2853 (s), 1744 (s), 1672 (s), 1458 (m), 1377 (w), 1251 (s). HRMS (ESI-TOF) calcd for $C_{20}H_{42}NO_3$ requires 344.3159; found 344.3153. Clearing point: 91.0–94.2 °C.

*4.4.3. N-Hexadecyl-N-(2-hydroxyl-1-methyloxycarbonyl)ethyl-N,N-dimethylammonium trifluoroacetate (**15d**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.70 (dd, 1H, *J*=6.6, 2.7 Hz), 4.34 (dd, 1H, *J*=13.9, 2.6 Hz), 4.23 (dd, 1H, *J*=13.8, 6.6 Hz), 3.77 (s, 3H), 3.66 (dt, 1H, *J*=12.5, 4.8 Hz), 3.49 (dt, 1H, *J*=12.6, 4.8 Hz), 3.36 (s, 3H), 3.35 (s, 3H), 1.81–1.51 (m, 2H), 1.36–1.07 (m, 26H), 0.81 (t, 3H, *J*=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.61 (CO), 73.46 (CH), 65.92 (CH_2), 58.92 (CH_2), 53.39 (CH_3), 51.10 (CH_3), 50.06 (CH_3), 31.89 (CH_2) [29.65, 29.63, 29.60, 29.54, 29.38, 29.33, 29.27, 28.95 ($10CH_2$)], 26.14 (CH_2), 22.65 (CH_2), 22.50 (CH_2), 14.08 (CH_3). FTIR (cm^{-1}, Nujol) 3168 (br), 2953 (s), 2922 (s), 2852 (s), 1744 (m), 1671 (s), 1458 (s), 1377 (m), 1173 (m). HRMS (ESI-TOF) calcd for $C_{22}H_{46}NO_3$ requires 372.3472; found 372.3475. Melting point: 92.3–94.3 °C.

*4.4.4. N-(2-Hydroxy-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-octadecylammonium trifluoroacetate (**15e**)*

^{1}H NMR ($CDCl_3$, 300MHz) δ 4.74 (dd, 1H, *J*=6.6, 2.7 Hz), 4.34 (dd, 1H, *J*=13.8, 2.7 Hz), 4.23 (dd, 1H, *J*=13.9, 6.8 Hz), 3.77 (s, 3H), 3.67 (dt, 1H, *J*=12.6, 4.9 Hz), 3.48 (dt, 1H, *J*=12.6, 5.1 Hz), 3.36 (s, 6H), 1.82–1.50 (m, 2H), 1.36–1.10 (m, 30H), 0.81 (t, 3H, *J*=6.8 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.66 (CO), 73.52 (CH), 65.97 (CH_2), 59.02 (CH_2), 53.42 (CH_3), 51.19 (CH_3), 50.08 (CH_3), 31.90 (CH_2) [29.68, 29.65, 29.61, 29.55, 29.39, 29.34, 29.28, 28.96 ($12CH_2$)], 26.16 (CH_2), 22.67 (CH_2), 22.51 (CH_2), 14.09 (CH_3). FTIR (cm^{-1}, Nujol) 3174 (br), 2919 (s), 2852 (s), 1744 (s), 1671 (s), 1469 (s), 1377 (m), 1173 (s), 718 (m). HRMS (ESI-TOF) calcd for $C_{24}H_{50}NO_3$ requires 400.3785; found 400.3788. Melting point: 95.0–97.3 °C.

*4.4.5. N-Dodecyl-N-(2-hydroxyphenyl-1-methyloxy-carbonyl)-ethyl-N,N-dimethylammonium trifluoroacetate (**16b**)*

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.92–6.74 (m, 4H), 4.32–4.17 (m, 1H), 3.44 (s, 3H), 3.50–3.16 (m, 3H), 3.22 (s, 6H), 2.94 (t, 1H, *J*=11.9 Hz), 1.72 (br s, 2H), 1.25 (br s, 18H), 0.88 (t, 3H, *J*=6.6 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.31 (CO), 157.51 (C), 130.32 (CH), 122.04 (C), 116.30 (CH), 74.73 (CH), 64.89 (CH_2), 53.00 (CH_3), 49.56 (CH_3), 48.92 (CH_3), 31.99 (CH_2), 31.87 (CH_2) [29.57, 29.41, 29.30, 28.99 ($6CH_2$)], 26.09 (CH_2), 22.64 (CH_2), 22.47 (CH_2), 14.07 (CH_3). FTIR (cm^{-1}, Nujol) 3472 (br), 3043 (w), 2926 (s), 2856 (s), 1746 (s), 1683 (s), 1518 (s), 1457 (m), 1379 (m), 1242 (s), 1131 (s), 832 (s), 719 (m). HRMS (ESI-TOF) calcd for $C_{24}H_{42}NO_3$ requires 392.3159; found 392.3169.

*4.4.6. N-(2-Hydroxyphenyl-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-tetradecylammonium trifluoroacetate (**16c**)*

^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.54 (s, 1H), 7.02 (d, 2H, *J*=8.5 Hz), 6.72 (d, 2H, *J*=8.5 Hz), 4.41 (dd, 1H, *J*=11.9, 3.4 Hz), 3.55 (s, 3H), 3.65–3.31 (m, 3H masked by the peak of water from DMSO), 3.22 (s, 3H), 3.19 (s, 3H), 3.02(t, 1H, *J*=12.5 Hz), 1.73 (br s, 2H), 1.24 (br s, 22H), 0.85 (t, 3H, *J*=6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.28 (CO), 157.39 (C), 130.29 (CH), 122.08 (C), 116.30 (CH), 74.72 (CH), 64.91 (CH_2), 54.05 (CH_3), 49.67(CH_3), 48.99 (CH_3), 31.90 (CH_2), 29.67 (CH_2) [29.64, 29.60, 29.44, 29.33, 28.99 ($8CH_2$)], 26.08 (CH_2), 22.66 (CH_2), 22.44 (CH_2), 14.08 (CH_3). FTIR (cm^{-1}, Nujol) 3472 (br), 3044 (m), 2920 (s), 2854 (s), 1747 (s), 1684 (s), 1519 (s), 1467 (s), 1388 (m), 1175 (s), 830 (s). HRMS (ESI-TOF) calcd for $C_{26}H_{46}NO_3$ requires 420.3472; found 420.3487.

*4.4.7. N-(2-Hydroxyphenyl-1-methyloxycarbonyl)ethyl-N-hexadecyl-N,N-dimethylammonium trifluoroacetate (**16d**)*

^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.56 (s, 1H), 7.02 (d, 2H, *J*=8.5 Hz), 6.72 (d, 2H, *J*=8.3 Hz), 4.41 (dd, 1H, *J*=11.9, 3.4 Hz), 3.55 (s, 3H), 3.66–3.32 (m, 3H masked by the peak of water from DMSO), 3.23 (s, 3H), 3.19 (s, 3H), 3.02 (t, 1H, *J*=12.3 Hz), 1.73 (br s, 2H), 1.24 (br s, 26H), 0.85 (t, 3H, *J*=6.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.30 (CO), 157.40 (C), 130.35 (CH), 122.11 (C), 116.30 (CH), 74.75 (CH), 64.89 (CH_2), 53.04 (CH_3), 49.75 (CH_3), 49.01 (CH_3), 31.90 (CH_2), 29.69 (CH_2) [29.66, 29.64, 29.61, 29.45, 29.34, 29.01 ($10CH_2$)], 26.11(CH_2), 22.66 (CH_2), 22.49 (CH_2), 14.08 (CH_3). FTIR (cm^{-1}, Nujol) 3455 (br), 2923 (s), 2854 (s), 1740 (s), 1682 (s), 1613 (s), 1467 (s), 1388 (m), 1175 (s), 830 (s). HRMS (ESI-TOF) calcd for $C_{28}H_{50}NO_3$ requires 448.3785; found 448.3788.

*4.4.8.. N-(2-Hydroxyphenyl-1-methyloxycarbonyl)ethyl-N,N-dimethyl-N-octadecylammonium trifluoroacetate (**16e**)*

^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.52 (br s, 1H), 7.02 (d, 2H, *J*=8.3 Hz), 6.71 (d, 2H, *J*=8.5 Hz), 4.41 (dd, 1H, *J*=11.8, 3.3 Hz), 3.55 (s, 3H), 3.60–3.30 (m, 3H masked by the peak of water from DMSO), 3.22 (s, 3H), 3.19 (s, 3H), 3.02 (t, 1H, *J*=12.3 Hz), 1.73 (br s, 2H), 1.23 (br s, 30H), 0.85 (t, 3H, *J*=6.4 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.85 (CO), 156.70 (C), 130.34 (CH), 123.02 (C), 115.40 (CH), 73.60 (CH), 63.36 (CH_2), 52.91 (CH_3), 48.69 (CH_3), 48.61 (CH_3), 31.17 (CH_2), 31.00 (CH_2) [28.91, 28.79, 28.64, 28.57, 28.34 ($12CH_2$)], 25.57 (CH_2), 21.97 (CH_2), 21.64 (CH_2), 13.82 (CH_3). FTIR (cm^{-1}, liquid film) 3455 (br), 2921 (s), 2854 (s), 1740 (s), 1682 (s), 1517 (s), 1454 (s), 1379 (m), 1201 (s), 823 (s). HRMS (ESI-TOF) cal. for C30H54NO3 requires 477.4176; found 477.4194.

4.5. Representative procedure for saponification of *N*-alkyl-4-hydroxyproline methyl esters

*4.5.1. Sodium N-dodecyl-4-hydroxyprolinate (**22b**)*

Saponification was accomplished by stirring *N*-dodecyl proline methyl ester (**18b**) with NaOH/H_2O (1.5 equiv) at rt until total consumption of reagents (24 h). Upon addition of AcOEt to the resulting solution, product **22b** precipitated and was collected by

suction filtration as white crystals (yield 72%): ^{1}H NMR (CD_3OD, 300 MHz) δ 4.50–4.30 (m, 1H), 4.04 (dd, 1H, J=10.5, 7.5 Hz), 3.77 (dd, 1H, J=12.6, 4.5 Hz), 3.34–3.22 (m, 1H), 3.18–3.00 (m, 2H), 2.38 (dd, 1H, J=13.5, 7.4 Hz), 2.09 (ddd, J=13.5, 10.7, 4.5 Hz), 1.85 (s, 1H), 1.72–1.59 (m, 2H), 1.40–1.10 (m, 18H), 0.85 (t, J=6.6 Hz). ^{13}C NMR (CD3OD, 75 MHz) δ 173.40 (CO), 70.75 (CH), 70.32 (CH), 62.90 (CH_2), 59.14 (CH_2), 40.07 (CH_2), 33.11 (CH_2) [30.80, 30.71, 30.57, 30.52, 30.32, 27.62, 27.00 ($8CH_2$)], 23.78 (CH_2), 14.50 (CH_3). FTIR (cm^{-1}, Nujol) 3216 (br), 3036 (s), 2914 (s), 2835 (s), 1617 (s), 1473 (m), 1398 (s). HRMS (ESI-TOF) calcd for $C_{17}H_{34}NO_3$ requires 300.2533; found 300.2537.Clearing point: 185.0–187.3 °C.

*4.5.2. Potassium N-tetradecyl-4-hydroxyprolinate (**22c**)*

^{1}H NMR (CD_3OD, 300 MHz) δ 4.51–4.44 (m, 1H), 4.13–4.02 (m, 1H), 3.79 (dd, J=12.5, 4.4 Hz), 3.38–3.24 (m, 2H), 3.33–3.05 (m, 2H), 2.47–2.36 (m, 1H), 2.17–2.04 (m, 1H), 1.76–1.64 (m, 2H), 1.42–1.20 (m, 22H), 0.92–0.84 (m, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 173.32 (CO), 70.79 (CH), 70.34 (CH), 62.91 (CH_2), 59.22 (CH_2), 40.06 (CH_2), 33.12 (CH_2) [30.80, 30.79, 30.70, 30.52, 30.29, 30.29, 27.57, 26.96 ($10CH_2$)], 23.78 (CH_2), 14.49 (CH_3). FTIR (cm^{-1}, Nujol) 3206 (br), 2922 (s), 2852 (s), 1617 (m), 1463 (m), 721 (w). HRMS (ESI-TOF) calcd for $C_{19}H_{38}NO_3$ requires 328.2846; found 328.2857. Clearing point: 187.0–188.0 °C.

*4.5.3. Potassium N-hexadecyl-4-hydroxyprolinate (**22d**)*

^{1}H NMR (CD_3OD, 300 MHz) δ 4.52–4.40 (m), 4.07 (dd, 1H, J=10.8, 7.5 Hz), 3.77 (dd, 1H J=12.5, 4.4 Hz), 3.39–3.22 (m, 1H), 3.21–3.00 (m, 2H), 2.40 (dd, 1H, J=13.6, 7.4 Hz), 2.08 (ddd, 1H, J=13.5, 10.8, 4.5 Hz), 1.74–1.59 (m, 2H), 1.40–1.19 (m, 26H), 0.91–0.80 (m, 3H). ^{13}C NMR (CD_3OD, 75 MHz) δ 173.34 (CO), 70.78 (CH), 70.33 (CH), 62.88 (CH_2), 59.19 (CH_2), 40.08 (CH_2), 33.13 (CH_2) [30.84, 30.81, 30.72, 30.57, 30.53, 30.31, 27.60, 26.99 ($12CH_2$)], 23.78 (CH_2), 14.50 (CH_3). FTIR (cm^{-1}, Nujol) 3208 (br), 3040 (s), 2850 (s), 1618 (s), 1464 (s), 721 (m). HRMS (ESI-TOF) calcd for $C_{21}H_{42}NO_3$ requires 356.3159; found 356.3169. Clearing point: 174.6–176.5 °C.

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Supplementary data

NMR and HRMS spectra of final target compounds. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.03.043.

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