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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 2896–2903

Synthesis and transdermal permeation-enhancing activity of ketone, amide, and alkane analogs of Transkarbam 12

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Received 17 October 2005; revised 1 December 2005; accepted 2 December 2005
Available online 11 January 2006

Abstract—Transkarbam 12 (5-(dodecyloxycarbonyl)pentylammonium-5-(dodecyloxycarbonyl)pentylcarbamate, **T12**) is a highly active transdermal permeation enhancer. In this study, ketone, amide, and alkane analogs of **T12** have been synthesized and evaluated for their permeation-enhancing activity using porcine skin and theophylline as a model drug. Replacement of ester by methylene and ketone, respectively, led to a significant decrease of activity. Amide analogs displayed lower activity in 60% propylene glycol and were comparable to **T12** in isopropyl myristate. An intramolecular H-bond between ester and ammonium-carbamate group was suggested to be important for the permeation-enhancing activity of **T12**.

1. Introduction

Transdermal drug delivery offers numerous advantages over conventional routes of administration; however, poor permeation of most drugs across the skin barrier constitutes a serious limitation of this methodology. One of the approaches used to enlarge the number of transdermally applicable drugs uses permeation enhancers. These compounds promote drug permeation through the skin by a reversible decrease of the barrier resistance. The enhancers act by one or more ways of these possibilities: interaction with the stratum corneum lipids, interaction with protein structures and partitioning modification.[1]

We have previously reported the permeation-enhancing activity of 5-(dodecyloxycarbonyl)pentylammonium-5-(dodecyloxycarbonyl)pentylcarbamate (Transkarbam 12, **T12**, Fig. 1). The substance enhanced skin transport of a variety of drugs with a wide spectrum of physicochemical properties,[2,3] displaying low toxicity and no dermal irritability.[4] Moreover, the susceptibility of the compound to metabolic deactivation into nontoxic substances was shown in vitro using porcine esterase.

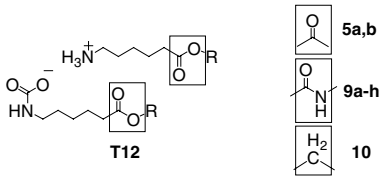

Figure 1. Transdermal permeation enhancer **T12** (R = $C_{12}H_{23}$) and its ketone (R = $C_{11}H_{21}$–$C_{12}H_{23}$), amide (R = C_6H_{13}–$C_{18}H_{37}$), and alkane analogs (R = $C_{12}H_{23}$).

Diffusion experiments have demonstrated that the ammonium-carbamate structure was responsible for the enhancing activity; the parent amino ester was inactive.[2]

To gain more information about the structure–activity relationships and, consequently, the mode of action of this promising enhancer, we aimed at investigating the role of the ester bond in the **T12** molecule. In this preliminary study, ketone, amide, and alkane analogs of **T12** were prepared and their permeation-enhancing activities evaluated.

2. Results

In this study, analogs of **T12** with the ester group replaced by amide, ketone, and methylene groups, respectively, were synthesized. The procedure for the preparation of the ketone analogs **5a**,**b** is outlined in

Keywords: Transdermal drug delivery; Percutaneous absorption; Permeation enhancer; Carbamate; Hydrogen bonding; Structure–activity relationships.
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doi:10.1016/j.bmc.2005.12.006

Scheme 1. 6-Bromohexanoyl chloride was prepared by the opening of the caprolactone ring,[5] substitution of hydroxyl with HBr, and the reaction of the carboxyl group with $SOCl_2$. The ketone was formed by reaction of the acyltributylphosphonium ion with the corresponding Grignard reagent.[6] The amino ketones were prepared by Gabriel synthesis, and the phthalimide hydrolyzed under acid catalysis.[7] The resulting ammonium chloride was alkalized, and the amine converted to the pertinent carbamate by reaction with CO_2.

The amide analogs were prepared via the mixed anhydride method[8] from 6-Boc-aminohexanoic acid. The protective group was removed by HCl, and the resulting amino amides reacted with CO_2 to form the target ammonium-carbamate salts **9a–h** (Scheme 2).

The alkane analog **10** was prepared by introducing CO_2 into the $CHCl_3$ solution of octadecylamine.

The carbamate salts were not sufficiently soluble in any solvent for recording the NMR spectra, except for $CDCl_3$ at elevated temperature, which, however, caused decomposition of the carbamate. Even the method employed previously for similar ammonium-carbamate derivatives[2,9,10] was not successful for detection of this type of carbamate. The spectra recorded in $CDCl_3$, however, confirmed the purity of the substances (as amino compounds), and presence of the carbamate structure has been confirmed by elemental analysis and FTIR spectroscopy.

In the IR spectra of the prepared compounds, the band of the NH stretching vibration of medium intensity between 3300 and 3400 cm^{-1}, characteristic of the carbamic acid salts, was observed. Weak, broad band at around 2150 cm^{-1} indicated the presence of ammonium ions (combination band of RNH_3^+ torsion and antisymmetrical RNH_3^+ deformation). The medium-intensity band at around 1650 cm^{-1} was assigned to the carbonyl stretching vibration of the carbamate group.[10]

The presence of the carbamate structure was further confirmed by thermogravimetric analysis (TGA). An example is given in Figure 2, showing a 6.9% decrease of weight of the compound **9g** at 135 °C, corresponding to an equimolar release of CO_2 from the carbamate molecule.[10]

The enhancement ratio (ER) values for the permeation of theophylline are summarized in Table 1. Theophylline has been selected as a model drug of medium polarity for comparison of the enhancing activities of the prepared compounds. This model drug has been widely studied previously using various enhancers,[11–13] skin species,[14,15] used as a tool to study skin barrier properties,[16,17] in mathematical models to predict skin permeability,[18] and for drug monitoring in premature neonates.[19] Typical permeation profiles for both tested vehicles and enhancers with the same chain length are shown in Figure 3. Propylene glycol/water 6:4 (Pg/W, Fig. 3a) has been selected to represent a hydrophilic vehicle and isopropyl myristate (IPM, Fig. 3b) a lipophilic one.

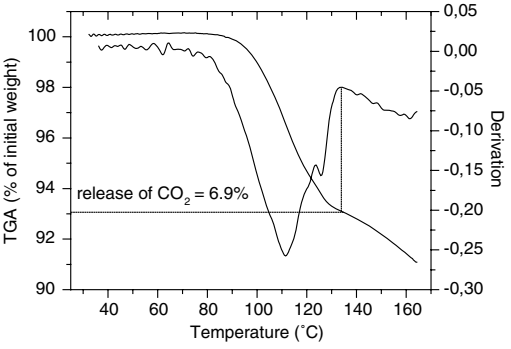
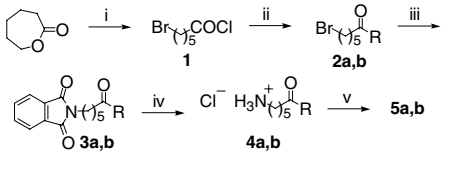


Figure 2. The TGA curve of **9g** representing an equimolar release of CO_2 from the ammonium-carbamate molecule.

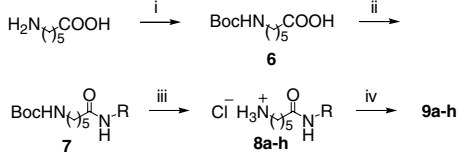
Table 1. Enhancement ratios of the **T12** analogs
R–X–$(CH_2)_5$NHCOO$^-$$H_3N^+$$(CH_2)_5$–X–R

Compound	X	R	ER ± SD	
			Pg/W	IPM
5a	–CO–	$C_{11}H_{23}$	$3.8 \pm 1.2^{*,**}$	$2.6 \pm 0.2^{*,**}$
5b		$C_{12}H_{25}$	$3.4 \pm 0.7^{*,**}$	$1.1 \pm 0.1^{**}$
9a	–CONH–	C_6H_{13}	$3.8 \pm 0.5^{*,**}$	$4.9 \pm 0.8^{*}$
9b		C_7H_{15}	$3.8 \pm 0.7^{*,**}$	$6.8 \pm 0.4^{*}$
9c		Octan-2-yl	$2.2 \pm 1.4^{**}$	$5.2 \pm 0.7^{*}$
9d		C_8H_{17}	$5.0 \pm 1.2^{*,**}$	$7.0 \pm 0.4^{*}$
9e		C_9H_{19}	$8.6 \pm 2.8^{*,**}$	$8.6 \pm 1.3^{*}$
9f		$C_{10}H_{21}$	$6.0 \pm 1.4^{*,**}$	$6.7 \pm 1.5^{*}$
9g		$C_{12}H_{25}$	$2.2 \pm 0.5^{*,**}$	$5.1 \pm 0.7^{*}$
9h		$C_{18}H_{37}$	$0.9 \pm 0.4^{**}$	$1.3 \pm 0.7^{**}$
10	$–CH_2–$	$C_{12}H_{25}$	$1.8 \pm 0.6^{**}$	$1.2 \pm 0.4^{**}$
T12	–COO–	$C_{12}H_{25}$	$22.8 \pm 1.1^{*}$	$6.6 \pm 0.5^{*}$

n = 4–6 (skin fragments from at least two animals for one compound).
* Significantly different from control (theophylline suspension in the given vehicle without an enhancer; $p < 0.05$).
** Significantly different from **T12** ($p < 0.05$).



Scheme 1. Synthesis of the ketone analogs. Reagents and conditions: a: R = $C_{11}H_{23}$; b: R = $C_{12}H_{25}$; (i) 1. HBr/H_2SO_4, 2. $SOCl_2$; (ii) Bu_3P, RMgBr; (iii) potassium phthalimide; (iv) 36% HCl, 175 °C, 10 bar; (v) TEA/CO_2.



Scheme 2. Synthesis of the amide analogs. Reagents and conditions: a: R = C_6H_{13}; b: R = C_7H_{15}; c: R = C_8H_{17}; d: R = C_8H_{17}; e: R = C_9H_{19}; f: R = $C_{10}H_{21}$; g: R = $C_{12}H_{25}$; h: R = $C_{18}H_{37}$; (i) 1. Boc_2, NaOH, 2. $KHSO_4$; (ii) $ClCOOC_2H_5$, RNH_2, TEA; (iii) $HCl_{(gas)}$; (iv) TEA/CO_2.

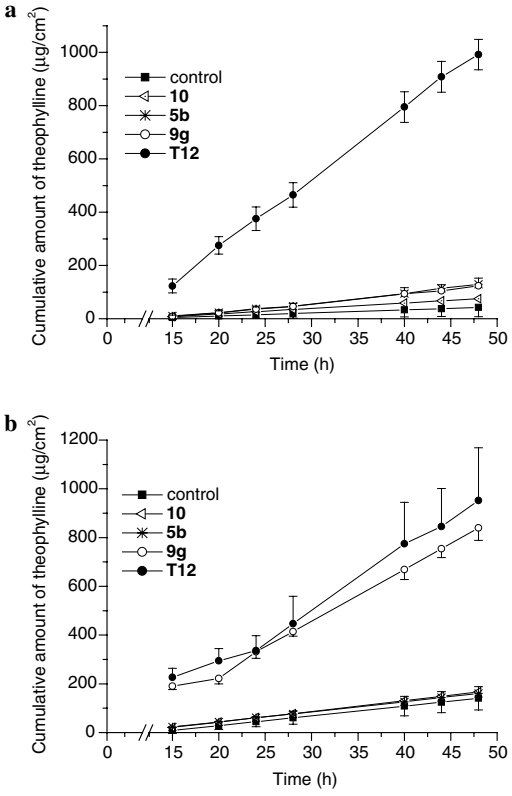


Figure 3. The permeation profiles of theophylline through the porcine skin using: (a) Pg/W and (b) IPM as the donor media and **T12**, and its analogs with the same chain length as enhancers. Control samples contained theophylline only in the pertinent vehicle.

Using Pg/W as the donor vehicle, the activity of the compounds with the same hydrocarbon chain length decreased as follows: **T12** (ester, ER = 22.8 ± 1.1) > **5b** (ketone, ER = 3.4 ± 0.7) > **9g** (amide, ER = 2.2 ± 0.5) > **10** (methylene, ER = 1.8 ± 0.6). When applied in a hydrophobic IPM suspension, the activities of amides were comparable with that of **T12** (ERs 5.1 ± 0.7 and 6.6 ± 0.5 for **9g** and **T12**, respectively), while the alkane and ketones were almost inactive.

In amide analogs, the effect of the chain length was further investigated. A parabolic relationship was found with maximum at nonyl derivatives in both vehicles. Branching of the hydrocarbon chain decreased the activity markedly; the activity of the octyl derivative **9d** and its isomer octan-2-yl **9c** applied in Pg/W was 5.0 ± 1.2 and 2.2 ± 1.4, respectively. Similar difference was observed in IPM as a vehicle (Table 1).

3. Discussion

The novel group of skin permeation enhancers termed transkarbams was originally prepared as 6-aminohexanoic acid esters,[4] that is, open Azone[20] analogs. The compounds, however, were found to trap air carbon dioxide to form two-chain ammonium-carbamate derivatives. The direct comparison of the enhancing effect of the carbamate **T12** and the corresponding amino ester tested under argon showed that the ammonium-carbamate structure is responsible for the enhancing effect and the amino ester is completely inactive. The pH dependence of its effect further supported this finding.[2]

It was therefore hypothesized that it is only the ammonium-carbamate polar head that bears the exceptional enhancing properties of **T12** and the rest of the molecule (e.g., the chain length) only slightly modulates its activity. Further support was brought in by a series of compounds with 'reversed' ester group, that is, ω-aminoalkan-1-ol derivatives, displaying equal permeation-enhancing activity.[21]

At first, we aimed to confirm this hypothesis by evaluating a simple alkylammonium-alkylcarbamate **10**. Surprisingly, the compound was not active. Therefore, both carbamate salt and ester group in **T12** participate in its activity. The FTIR spectra of **T12** showed a doublet of the ester carbonyl vibration,[22] suggesting different hydrogen bonding of the two ester groups in the molecule. Thus, one ester group might be forming a hydrogen bond with the ammonium-carbamate group, which is somehow important for the activity of **T12**.

We have further replaced the ester group in **T12** by a ketone, which is a stronger hydrogen bond acceptor. This, however, resulted in activities an order of magnitude lower. Monosubstituted amides are both hydrogen bond donors and acceptors, and their activity was significantly decreased in Pg/W and comparable to **T12** in IPM. None of these compounds displayed a doublet of carbonyl vibrations in FTIR spectra as **T12**. The carbonyl groups of these compounds therefore do not form the same hydrogen bonding as in **T12**, which may be the reason for their decreased potency. The importance of the hydrogen-bonding ability in skin permeation enhancers has already been reported.[23,24] Interestingly, only **T12** displayed markedly different activities in Pg/W and IPM vehicles. This might be connected also with different hydrogen bonding of **T12** in these two vehicles or a synergic action of **T12** and Pg in the stratum corneum.

The parabolic relationship between the chain length of the amide analogs **9a**–**h** and their enhancement activity is in broad agreement with that found in other groups of enhancers,[21,25] suggesting a similar mode of action. Also the negative effect of chain branching confirms our previous findings.[26]

In conclusion, this preliminary study showed that not only the carbamate polar head and a suitable chain length were essential for the permeation-enhancing activity of transkarbams, but also the character of the linking group was of great importance. The reason for the difference in activities of the present compounds could be different steric ordering of the enhancer, hypothetically via an intramolecular hydrogen bond, which could bring different solubility in the donor medium, and/or a specific action within the stratum corneum. From the current comparison, ester bond seems to be the best choice for its biodegradability and high activity;

however, this unusual behavior warrants further investigation.

4. Experimental

4.1. Chemicals and instrumentation

All chemicals were purchased from Sigma–Aldrich (Schnelldorf, Germany). Silica gel 60 (230–400 mesh) for column chromatography and TLC plates (silica gel 60 F_{254}, aluminum back) were obtained from Merck (Darmstadt, Germany). IR spectra were recorded on a Nicolet Impact 400 apparatus equipped with a DTGS detector with a resolution of 4 cm^{-1}. ^1H and ^{13}C NMR spectra were measured on a Varian Mercury-Vx BB 300 instrument, operating at 300 MHz for ^1H, 75 MHz for ^{13}C. Elemental analysis (C, H, and N) was performed on a Fisons EA 1110 CHNS-O elemental analyzer. The melting point was measured with a Kofler apparatus and is uncorrected. TGA was recorded using a Stanton Redcroft TG 750 instrument.

4.2. Chemistry

The target compounds were synthesized according to Scheme 1 (ketone analogs) and Scheme 2 (amide analogs). The alkane analog was prepared by reaction of octadecylamine with CO_2.

4.2.1. 6-Bromohexanoyl chloride (1). A mixture of caprolactone (236 g; 2.07 mol), 48% HBr (520 mL; 4.6 mol), and 96% H_2SO_4 (235 mL) was heated at reflux for 3 h. Five liters of water was added, the organic layer separated, and the aqueous layer extracted several times with diethyl ether. The combined organic extracts were distilled at 150–155 °C/15 torr[5] to yield a colorless liquid 6-bromohexanoic acid (305 g, 75%). To the resulting acid (97 g, 0.5 mol) an excess of thionyl chloride (71 mL; 1 mol) was added and stirred at 50 °C for 1 h. The mixture was distilled at 113 °C/11 mbar to yield a colorless liquid **1** (98 g, 93%).

4.2.2. General procedure for the preparation of 1-bromoalkan-6-ones (2a,b)[6]. 6-Bromohexanoyl chloride **1** was dissolved in anhydrous THF, cooled to −30 °C, and Bu_3P (1.1 equiv) added under N_2 atmosphere. The resulting suspension was stirred for 30 min. An ethereal solution of the pertinent Grignard reagent (1 equiv) was added slowly to the well-stirred mixture. After stirring for 1 h at the same temperature, the reaction was quenched by addition of 100 mL of 1 M HCl. The organic layer was separated and the aqueous mixture was extracted three times with diethyl ether. The combined organic layers were washed with 1% $NaHCO_3$ and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was subjected to column chromatography (petroleum ether/ethyl acetate 3:1) and subsequent crystallization afforded the product.

4.2.2.1. 1-Bromoheptadecan-6-one (2a). White crystals; mp = 29–32 °C, yield 45%; IR (KBr): ν_{max} 2918s, 2849s, 1701s ($\nu_{(CO)}$), 1471s, 1419m ($\delta_{sci(COCH_2)}$), 1384w, 1247m, 717w cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.40 (t; 2H; J = 6.9 Hz; $BrCH_2$), 2.41 (t; 2H; J = 7.4 Hz; $COCH_2$), 2.38 (t; 2H; J = 7.5 Hz; CH_2CO), 1.87 (p; 2H; CH_2CH_2Br), 1.65–1.50 (m; 4H; CH_2CH_2-$COCH_2CH_2$), 1.49–1.36 (m; 2H; $CH_2CH_2CH_2Br$), 1.35–1.14 (m; 16H; CH_2), 0.88 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.2; 42.9; 42.4; 33.6; 32.5; 31.9; 29.6; 29.6; 29.5; 29.4; 29.3; 29.3; 27.7; 23.9; 22.8; 22.7; 14.1 ppm.

4.2.2.2. 1-Bromooctadecan-6-one (2b). White crystals; mp = 28–33 °C, yield 43%; IR ($CHCl_3$): ν_{max} 2927s, 2855s, 1708s ($\nu_{(CO)}$), 1464m, 1408w ($\delta_{sci(COCH_2)}$), 1375w cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.40 (t; 2H; J = 6.9 Hz; $BrCH_2$), 2.41 (t; 2H; J = 7.0 Hz; $COCH_2$), 2.38 (t; 2H; J = 7.3 Hz; CH_2CO), 1.86 (p; 2H; J = 7.2 Hz; CH_2CH_2Br), 1.66–1.49 (m; 4H; CH_2CH_2-$COCH_2CH_2$), 1.48–1.36 (m; 2H; $CH_2CH_2CH_2Br$), 1.35–1.16 (m; 18H; CH_2), 0.87 (t; 3H; J = 6.7 Hz; CH_3); ^{13}C NMR (75 MHz, CDCl3): δ 211.2; 42.9; 42.4; 33.6; 32.5; 31.9; 29.6; 29.6; 29.6; 29.5; 29.4; 29.3; 29.2; 27.7; 23.9; 22.8; 22.7; 14.1 ppm.

4.2.3. General procedure for the preparation of *N*-(6-oxoalkyl)phthalimides (3a,b)[7]. Solution of bromo ketone **2** in toluene was added to an anhydrous toluene suspension of potassium phthalimide (1.1 equiv) dropwise and heated under reflux for 3 h. The reaction mixture was kept at 4 °C overnight and precipitated, unreacted potassium phthalimide filtered off. The mixture was evaporated, dissolved in diethyl ether and extracted by 1 M NaOH solution and brine, and dried over Na_2SO_4. The resulting orange oil was crystallized from methanol to yield white crystals of **3a**,**b**.

4.2.3.1. *N*-(6-Oxoheptadecyl)phthalimide (3a). White crystals; mp = 66–69 °C, yield 95%; IR (KBr): ν_{max} 2918s, 2849s, 1772m ($\nu_{as(NC=O)}$), 1698s ($\nu_{(C=O)}$, $\nu_{s(NC=O)}$), 1614w, 1465m, 1435w, 1406m, 1374m, 1335w cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.86–7.79 (m; 2H; CH), 7.73–7.66 (m; 2H; CH), 3.66 (t; 2H; J = 7.1 Hz; NCH_2), 2.43–2.31 (m; 4H; CH_2COCH_2), 1.74–1.46 (m; 6H; CH_2), 1.40–1.15 (m; 18H; CH_2), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.2; 168.4; 133.9; 132.1; 123.1; 42.8; 42.5; 37.8; 31.9; 29.7; 29.6; 29.6; 29.4; 29.3; 29.2; 28.3; 26.4; 23.9; 23.3; 22.6; 14.1 ppm.

4.2.3.2. *N*-(6-Oxooctadecyl)phthalimide (3b). White crystals; mp = 70–72 °C, yield 91%; IR (KBr): ν_{max} 2917s, 2849s, 1773m ($\nu_{as(NC=O)}$), 1704s ($\nu_{(C=O)}$), 1694s ($\nu_{s(NC=O)}$), 1615m, 1464m, 1435w, 1404m, 1375m, 1337w cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.86–7.78 (m; 2H; CH), 7.73–7.66 (m; 2H; CH), 3.66 (t; 2H; J = 7.1 Hz; NCH_2), 2.42–2.31 (m; 4H; CH_2COCH_2), 1.74–1.46 (m; 6H; CH_2), 1.40–1.14 (m; 20H; CH_2), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.3; 168.4; 133.9; 132.1; 123.2; 42.9; 42.5; 37.8; 31.9; 29.6; 29.6; 29.6; 29.5; 29.4; 29.3; 29.2; 28.4; 26.5; 23.9; 23.3; 22.7; 14.1 ppm.

4.2.4. General procedure for the preparation of 6-oxoalkylammonium-chlorides (4a,b)[7]. *N*-(6-Oxoalkyl)phthalimide **3** was hydrolyzed by 36% HCl in an autoclave (Miniclave, Büchi AG Uster/Switzerland) at 175 °C/10 bar for 15 h. The product was mixed with toluene and evaporated to remove the residual HCl. The resulting mixture was dissolved in $CHCl_3$, filtered, and evaporated. Crystallization from ethanol/acetone yielded a white crystalline product.

4.2.4.1. 6-Oxoheptadecylammonium-chloride (4a). White crystals; mp = 124–130 °C, yield 86%; IR ($CHCl_3$): ν_{max} 2927s, 2856s, 1708s ($\nu_{(C=O)}$), 1615m ($\delta_{as(NH_3^+)}$), 1521m ($\delta_{s(NH_3^+)}$), 1466m, 1408w ($\delta_{sci(COCH_2)}$), 1377m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.24 (s; 3H; NH_3^+), 3.15–2.87 (m; 2H; $CH_2NH_3^+$), 2.47–2.32 (m; 4H; CH_2COCH_2), 1.87–1.71 (m; 2H; CH_2), 1.67–1.17 (m; 22H; CH_2), 0.87 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.3; 42.9; 42.4; 39.8; 31.9; 29.6; 29.5; 29.4; 29.3; 29.3; 27.4; 26.0; 23.8; 22.9; 22.7; 14.1 ppm.

4.2.4.2. 6-Oxooctadecylammonium-chloride (4b). White crystals; mp = 120–130 °C, yield 78%; IR ($CHCl_3$): ν_{max} 2927s, 2856s, 1708s ($\nu_{(C=O)}$), 1615m ($\delta_{as(NH_3^+)}$), 1522m ($\delta_{s(NH_3^+)}$), 1466m, 1408w ($\delta_{sci(COCH_2)}$), 1377m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.07 (s; 3H; NH_3^+), 3.13–2.97 (m; 2H; $CH_2NH_3^+$), 2.48–2.33 (m; 4H; CH_2COCH_2), 1.90–1.75 (m; 4H; CH_2), 1.67–1.46 (m; 4H; CH_2), 1.46–1.33 (m; 2H; CH_2), 1.32–1.14 (m; 16H; CH_2), 0.87 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.6; 43.0; 42.2; 39.9; 31.9; 29.7; 29.6; 29.5; 29.4; 29.3; 29.3; 27.2; 26.0; 23.9; 22.9; 22.7; 14.1 ppm.

4.2.5. General procedure for the preparation of 6-Boc-aminohexanoic acid alkyl amides (7)[8]. 6-Boc-aminohexanoic acid (1.15 g; 5 mmol, prepared as described previously[27,28]) in 20 mL of dry 1,2-dimethoxyethane was mixed with dry TEA (5 mmol) at −15 °C. After 15 min, ethylchloroformate (5 mmol), and, after additional 10 min, 20% solution of the corresponding amine (5 mmol) in dry 1,2-dimethoxyethane were slowly added. The mixture was stirred for 2 h at laboratory temperature and then triethylammonium-chloride was filtered off. The solution was evaporated, dissolved in ethyl acetate, and washed with 10% HCl, 5% $NaHCO_3$ and twice with water. The organic layer was evaporated and the product crystallized from a mixture of diethyl ether/petroleum ether.

4.2.5.1. 6-Boc-aminohexanoic acid hexyl amide (7a). White crystals; mp = 43–50 °C, yield 83%; IR ($CHCl_3$): ν_{max} 3452m ($\nu_{(NHBoc)}$) and 3350w ($\nu_{(N-H)}$), 1708s ($\nu_{(NHC=OO)}$), 1661s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1456m, 1393m ($\delta_{(t\text{-butyl})}$), 1367s ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.74 (s; 1H; NHamide), 4.62 (s; 1H; NH), 3.22 (q; 2H; J = 6.4 Hz; CH_2Namide), 3.14–3.02 (m; 2H; CH_2NHBoc), 2.16 (t; 2H; J = 7.8 Hz; CH_2CONH), 1.63 (p; 2H; J = 7.6 Hz; CH_2), 1.53–1.14 (m; 21H; CH_2), 0.86 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0; 156.0; 79.0; 40.3; 39.5; 36.5; 31.4; 29.7; 29.5; 28.4; 26.5; 26.3; 25.3; 22.5; 14.0 ppm.

4.2.5.2. 6-Boc-aminohexanoic acid heptyl amide (7b). White crystals; mp = 65–68 °C, yield 74%; IR ($CHCl_3$): ν_{max} 3452m ($\nu_{(NHBoc)}$), 1709s ($\nu_{(NHC=OO)}$), 1661s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1457w, 1393w ($\delta_{(t\text{-butyl})}$), 1367m ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.76 (s; 1H; NHamide), 4.62 (s; 1H; NH), 3.20 (q; 2H; J = 6.5 Hz; CH_2Namide), 3.13–3.00 (m; 2H; CH_2NHBoc), 2.14 (t; 2H; J = 7.5 Hz; CH_2CONH), 1.69–1.55 (m; 2H; CH_2), 1.53–1.11 (m; 23H; CH_2), 0.84 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9; 156.0; 79.0; 40.3; 39.5; 36.6; 31.7; 29.7; 29.6; 28.9; 28.4; 26.8; 26.3; 25.3; 22.5; 14.0 ppm.

4.2.5.3. 6-Boc-aminohexanoic acid octan-2-yl amide (7c). White crystals; mp = 59–70 °C, yield 75%; IR ($CHCl_3$): ν_{max} 3452m ($\nu_{(NHBoc)}$) and 3438m and 3342w ($\nu_{(NHamide)}$), 1706s ($\nu_{(NHC=OO)}$), 1655s ($\nu_{(NHC=O)}$), 1510s ($\delta_{(NH)}$), 1456m, 1393m ($\delta_{(t\text{-butyl})}$), 1367s ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.46 (s; 1H; NHamide), 4.58 (s; 1H; NH), 3.95 (p; 1H; J = 7.0 Hz; CH), 3.16–2.99 (m; 2H; CH_2NBoc), 2.15 (t; 2H; J = 7.7 Hz; CH_2CONH), 1.63 (p; 2H; J = 7.5 Hz; CH_2), 1.54–1.14 (m; 23H; CH_2), 1.10 (d; 3H; J = 6.6 Hz; CH_3), 0.86 (t; 3H; J = 6.3 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2; 156.0; 79.1; 45.2; 40.3; 36.9; 36.6; 31.7; 29.7; 29.1; 28.4; 28.3; 26.0; 25.4; 22.5; 21.0; 14.0 ppm.

4.2.5.4. 6-Boc-aminohexanoic acid octyl amide (7d). White crystals; mp = 74–75 °C, yield 88%; IR ($CHCl_3$): ν_{max} 3452s ($\nu_{(NHBoc)}$) and 3351w ($\nu_{(N-H)}$), 1706s ($\nu_{(NHC=OO)}$), 1661s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1457w, 1393m ($\delta_{(t\text{-butyl})}$), 1367s ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.68 (s; 1H; NHamide), 4.58 (s; 1H; NH), 3.210 (q; 2H; J = 6.5 Hz; CH_2Namide), 3.13–3.01 (m; 2H; CH_2NHBoc), 2.15 (t; 2H; J = 7.6 Hz; CH_2CONH), 1.69–1.56 (m; 2H; CH_2), 1.53–1.13 (m; 25H; CH_2), 0.85 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9; 156.0; 79.0; 40.3; 39.5; 36.6; 31.7; 29.7; 29.6; 29.3; 28.9; 28.4; 26.8; 26.3; 25.3; 22.5; 14.0 ppm.

4.2.5.5. 6-Boc-aminohexanoic acid pentyl amide (7e). White crystals; mp = 73–76 °C, yield 74%; IR ($CHCl_3$): ν_{max} 3452m ($\nu_{(NHBoc)}$), 3351w ($\nu_{(NHamide)}$), 1708s ($\nu_{(NHC=OO)}$), 1660s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1457m, 1393m ($\delta_{(t\text{-butyl})}$), 1367s ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.63 (s; 1H; NHamide), 4.55 (s; 1H; NH), 3.27–3.18 (m; 2H; CH_2Namide), 3.09 (t; 2H; J = 6.0 Hz; CH_2NH), 2.17 (t; 2H; J = 7.5 Hz; CH_2CONH), 1.70–1.58 (m; 2H; CH_2), 1.54–1.17 (m; 27H; CH_2), 0.87 (t; 3H; J = 6.8 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9; 156.0; 79.1; 40.3; 39.6; 36.5; 31.8; 29.8; 29.6; 29.5; 29.3; 29.2; 28.4; 26.9; 26.3; 25.3; 22.6; 14.1 ppm.

4.2.5.6. 6-Boc-aminohexanoic acid decyl amide (7f). White crystals; mp = 59–61 °C, yield 47%; IR ($CHCl_3$): ν_{max} 3452m ($\nu_{(NHBoc)}$), 3350w ($\nu_{(NHamide)}$), 1708s ($\nu_{(NHC=OO)}$), 1661s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1457m, 1393m ($\delta_{(t\text{-butyl})}$), 1367s ($\nu_{(C-O)}$) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.63 (s; 1H; NHamide), 4.58 (s; 1H; NH), 3.26–3.17 (m; 2H; CH_2Namide), 3.14–3.03

(m; 2H; CH_2NH), 2.16 (t; 2H; J = 7.6 Hz; CH_2CONH), 1.70–1.57 (m; 2H; CH_2), 1.54–1.19 (m; 29H; CH_2), 0.86 (t; 3H; J = 7.0 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9; 156.0; 79.1; 40.3; 39.6; 36.5; 31.9; 29.7; 29.6; 29.5; 29.3; 28.4; 26.9; 26.3; 25.3; 22.6; 14.1 ppm.

4.2.5.7. 6-Boc-aminohexanoic acid dodecyl amide (7g). White crystals; mp = 68–70 °C, yield 48%; IR ($CHCl_3$): ν_{max} 3451m ($\nu_{(NHBoc)}$), 3350w ($\nu_{(NHamide)}$), 1708s ($\nu_{(NHC=OO)}$), 1661s ($\nu_{(NHC=O)}$), 1511s ($\delta_{(NH)}$), 1457m, 1393m ($\delta_{(t\text{-butyl})}$), 1368s ($\nu_{(C-O)}$) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.64 (s; 1H; NHamide), 4.56 (s; 1H; NH), 3.26–3.17 (m; 2H; CH_2Namide), 3.14–3.03 (m; 2H; CH_2NH), 2.16 (t; 2H; J = 7.5 Hz; CH_2CONH), 1.70–1.58 (m; 2H; CH_2), 1.54–1.17 (m; 33H; CH_2), 0.87 (t; 3H; J = 6.8 Hz; CH_3); ^{13}CNMR (75 MHz, $CDCl_3$): δ 172.9; 156.0; 79.1; 40.3; 39.6; 36.5; 31.9; 29.8; 29.6; 29.6; 29.5; 29.3; 29.3; 28.4; 26.9; 26.3; 25.3; 22.7; 14.1 ppm.

4.2.5.8. 6-Boc-aminohexanoic acid octadecyl amide (7h). White crystals; mp = 69–74 °C, yield 37%; IR (KBr): ν_{max} 3359br m, 3323br m, 1687s ($\nu_{(NHC=OO)}$), 1637s ($\nu_{(NHC=O)}$), 1538s ($\delta_{(NH)}$), 1469m, 1390m ($\delta_{(t\text{-butyl})}$), 1366m ($\nu_{(C-O)}$) cm^{-1}.

4.2.6. General procedure for the preparation of 5-alkylcarbamoylpentylammonium-chlorides (8). The Boc-derivatives **7a**–**h** were dissolved in dry $CHCl_3$ and deprotected by introducing dry HCl into the mixture for 1 h. The dissolved HCl was removed by flushing the mixture with dry N_2. Crystallization from ethanol/diethyl ether gave white crystalline products. The yields were quantitative.

4.2.6.1. 5-Hexylcarbamoylpentylammonium-chloride (8a). White crystals; mp = 157–159 °C; IR (Nujol): ν_{max} 3276s ($\nu_{(N-H)}$), 1640s ($\nu_{(NHC=O)}$), 1560m ($\delta_{(NH)}$), 1523m ($\delta_{(NH_3^+)}$), 1146m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s; 3H; NH_3^+), 7.84 (t; 1H; J = 4.9 Hz; NH), 2.98 (q; 2H; J = 5.9 Hz; CH_2NH), 2.70 (t; 2H; J = 7.2 Hz; $CH_2NH_3^+$), 2.03 (t; 2H; J = 6.8 Hz; CH_2), 1.61–1.13 (m; 14H; CH_2), 0.84 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.8; 38.6; 35.3; 31.2; 29.3; 27.0; 26.3; 25.8; 25.0; 22.3; 14.2 ppm.

4.2.6.2. 5-Heptylcarbamoylpentylammonium-chloride (8b). White crystals; mp = 159–164 °C; IR (Nujol): ν_{max} 3296s ($\nu_{(N-H)}$), 1633s ($\nu_{(NHC=O)}$), 1562m ($\delta_{(NH)}$), 1524w ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s; 3H; NH_3^+), 7.85 (t; 1H; J = 5.0 Hz; NH), 2.98 (q; 2H; J = 6.4 Hz; CH_2NH), 2.70 (t; 2H; J = 7.8 Hz; $CH_2NH_3^+$), 2.03 (t; 2H; J = 7.1 Hz; CH_2), 1.62–1.11 (m; 16H; CH_2), 0.84 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.8; 38.6; 35.3; 31.5; 29.4; 28.6; 27.0; 26.6; 25.8; 25.0; 22.3; 14.2 ppm.

4.2.6.3. 5-(Octan-2-ylcarbamoyl)pentylammonium-chloride (8c). White crystals; mp = 129–131 °C; IR (Nujol): ν_{max} 3270m ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1548m ($\delta_{(NH)}$), 1523m ($\delta_{(NH_3^+)}$), 1153w cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (s; 3H; NH_3^+), 7.63 (d; 1H; J = 8.4 Hz; NH), 3.69 (h; 1H; J = 6.6 Hz; CH), 2.70 (t; 2H; J = 7.1 Hz; $CH_2NH_3^+$), 2.01 (t; 2H; J = 7.2 Hz; CH_2), 1.60–1.39 (m; 4H; CH_2), 1.38–1.09 (m; 12H; CH_2), 0.84 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.2; 44.1; 38.8; 36.3; 35.4; 31.5; 28.8; 26.9; 25.9; 25.7; 25.1; 22.3; 21.1; 14.2 ppm.

4.2.6.4. 5-Octylcarbamoylpentylammonium-chloride (8d). White crystals; mp = 161–165 °C; IR (Nujol): ν_{max} 3280m ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1547m ($\delta_{(NH)}$), 1524m ($\delta_{(NH_3^+)}$), 1152w cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s; 3H; NH_3^+), 7.84 (t; 1H; J = 5.2 Hz; NH), 2.98 (q; 2H; J = 6.5 Hz; CH_2NH), 2.71 (t; 2H; J = 7.5 Hz; $CH_2NH_3^+$), 2.03 (t; 2H; J = 7.2 Hz; CH_2), 1.62–1.12 (m; 18H; CH_2), 0.85 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.8; 38.6; 35.3; 31.5; 29.4; 29.2; 28.6; 27.0; 26.6; 25.8; 25.0; 22.3; 14.2 ppm.

4.2.6.5. 5-Nonylcarbamoylpentylammonium-chloride (8e). White crystals; mp = 165–167 °C; IR (Nujol): ν_{max} 3292s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1561m ($\delta_{(NH)}$), 1523m ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 8.05 (s; 3H; NH_3^+), 7.83 (t; 1H; J = 5.5 Hz; NH), 3.03–2.94 (m; 2H; CH_2NH), 2.72 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 2.04 (t; 2H; J = 7.4 Hz; CH_2), 1.61–1.41 (m; 4H; CH_2), 1.39–1.14 (m; 16H; CH_2), 0.85 (t; 3H; J = 6.9 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.8; 38.6; 35.3; 31.5; 29.4; 29.2; 29.0, 28.9; 26.9; 26.6; 25.7; 25.0; 22.3; 14.2 ppm.

4.2.6.6. 5-Decylcarbamoylpentylammonium-chloride (8f). White crystals; mp = 162–165 °C; IR (Nujol): ν_{max} 3297s ($\nu_{(N-H)}$), 1633s ($\nu_{(NHC=O)}$), 1560m ($\delta_{(NH)}$), 1523m ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 8.02 (s; 3H; NH_3^+), 7.83 (t; 1H; J = 5.5 Hz; NH), 2.99 (q; 2H; J = 6.5 Hz; CH_2NH), 2.72 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 2.04 (t; 2H; J = 7.4 Hz; CH_2), 1.60–1.41 (m; 4H; CH_2), 1.39–1.14 (m; 18H; CH_2), 0.85 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.5; 38.3; 35.1; 31.3; 29.1; 29.0; 28.9; 28.7; 28.7; 26.7; 26.4; 25.5; 24.8; 22.1; 13.9 ppm.

4.2.6.7. 5-Dodecylcarbamoylpentylammonium-chloride (8g). White crystals; mp = 160–164 °C; IR (Nujol): ν_{max} 3295s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1561m ($\delta_{(NH)}$), 1523m ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 8.07 (s; 3H; NH_3^+), 7.84 (t; 1H; J = 5.5 Hz; NH), 2.99 (q; 2H; J = 6.5 Hz; CH_2NH), 2.72 (t; 2H; J = 7.7 Hz; $CH_2NH_3^+$), 2.04 (t; 2H; J = 7.1 Hz; CH_2), 1.61–1.41 (m; 4H; CH_2), 1.39–1.15 (m; 22H; CH_2), 0.84 (t; 3H; J = 6.9 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 171.9; 38.8; 38.6; 35.3; 31.5; 29.4; 29.2; 29.0; 29.0; 27.0, 26.7; 25.8; 25.0; 22.3; 14.2 ppm.

4.2.6.8. 5-Octadecylcarbamoylpentylammonium-chloride (8h). White crystals; mp = 144–149 °C; IR (Nujol): ν_{max} 3294s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1559m ($\delta_{(NH)}$), 1523w ($\delta_{(NH_3^+)}$), 1152m cm^{-1}.

4.2.7. General procedure for the preparation of carbamates (5, 9, and 10). The appropriate ammonium-chlo-

ride was dissolved in water, alkalized by 1.5 equiv of TEA, and extracted three times with diethyl ether. The ethereal extracts were dried over Na_2SO_4 and then dry CO_2 was slowly bubbled through the solution for approximately 20 min. The precipitated carbamate was filtered off through dense filter paper and dried in vacuo over P_4O_{10}.

4.2.7.1. 6-Oxoheptadecylammonium-6-oxoheptadecylcarbamate (5a). White crystals; mp = 82–86 °C, yield 59%; IR (Nujol): ν_{max} 3307m ($\nu_{(N-H)}$), 2196wbr, 1705s ($\nu_{(C=O)}$), 1696w, 1640m ($\nu_{(NHC=OO^-)}$), 1610m ($\delta_{(NH_3^+)}$) cm^{-1}; CHN analysis for $C_{35}H_{70}N_2O_4$ (found/calculated): 71.68/72.11; 12.00/12.10; 4.86/4.81.

4.2.7.2. 6-Oxooctadecylammonium-6-oxooctadecylcarbamate (5b). White crystals; mp = 78–85 °C, yield 49%; IR (Nujol): ν_{max} 3305m ($\nu_{(N-H)}$), 2183wbr, 1705s ($\nu_{(C=O)}$), 1641m ($\nu_{(NHC=OO^-)}$), 1609m ($\delta_{(NH_3^+)}$) cm^{-1}; CHN analysis for $C_{37}H_{74}N_2O_4$ (found/calculated): 72.82/72.73; 12.22/12.21; 4.57/4.58.

4.2.7.3. 5-Hexylcarbamoylpentylammonium-5-hexylcarbamoylpentylcarbamate (9a). White crystals; mp = 93–97 °C, yield 70%; IR (Nujol): ν_{max} 3294s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1559m ($\delta_{(NH)}$), 1523w ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; CHN analysis for $C_{25}H_{52}N_4O_4$ (found/calculated): 63.37/63.52; 11.45/11.09; 11.89/11.85.

4.2.7.4. 5-Heptylcarbamoylpentyammonium-5-heptylcarbamoylpentylcarbamate (9b). White crystals; mp = 93–97 °C, yield 64%; IR (Nujol): ν_{max} 3294s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1559m ($\delta_{(NH)}$), 1523w ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; CHN analysis for $C_{27}H_{56}N_4O_4$ (found/calculated): 65.33/64.76; 11.63/11.27; 11.41/11.19.

4.2.7.5. 5-(Octan-2-ylcarbamoyl)pentylammonium-5-(octan-2-ylcarbamoyl)pentylcarbamate (9c). White crystals; mp = 75–80 °C, yield quantitative; IR (Nujol): ν_{max} 3294s ($\nu_{(N-H)}$), 1632s ($\nu_{(NHC=O)}$), 1559m ($\delta_{(NH)}$), 1523w ($\delta_{(NH_3^+)}$), 1152m cm^{-1}; CHN analysis for $C_{29}H_{60}N_4O_4$ (found/calculated): 65.66/65.87; 11.56/11.44; 10.24/10.59.

4.2.7.6. 5-Octylcarbamoylpentylammonium-5-octylcarbamoylpentylcarbamate (9d). White crystals; mp = 96–98 °C, yield 60%; IR (Nujol): ν_{max} 3356s($\nu_{(N-HCOO^-)}$), 3232s ($\nu_{(CON-H)}$), 2121w, 1626s ($\nu_{(NHC=O)}$), 1560s,br ($\delta_{(NH)}$) cm^{-1}; CHN analysis for $C_{29}H_{60}N_4O_4$ (found/calculated): 65.49/65.87; 11.62/11.44; 10.42/10.59.

4.2.7.7. 5-Nonylcarbamoylpentylammonium-5-nonylcarbamoylpentylcarbamate (9e). White crystals; mp = 94–100 °C, yield 63%; IR (Nujol): ν_{max} 3372m ($\nu_{(N-HCOO^-)}$), 3255m ($\nu_{(CON-H)}$), 1656m ($\nu_{(NHC=OO^-)}$), 1628s ($\nu_{(NHC=O)}$), 1556s,br ($\delta_{(NH)}$) cm^{-1}; CHN analysis for $C_{31}H_{64}N_4O_4$ (found/calculated): 67.04/66.86; 11.94/11.58; 10.17/10.06.

4.2.7.8. 5-Decylcarbamoylpentylammonium-5-decylcarbamoylpentylcarbamate (9f). White crystals; mp = 98–102 °C, yield 57%; IR (Nujol): ν_{max} 3369m ($\nu_{(N-HCOO^-)}$), 3255m ($\nu_{(CON-H)}$), 2144w, 1656w ($\nu_{(NHC=OO^-)}$), 1628s ($\nu_{(NHC=O)}$), 1556s,br ($\delta_{(NH)}$) cm^{-1}; CHN analysis for $C_{33}H_{68}N_4O_4$ (found/calculated): 67.88/67.76; 11.73/11.72; 9.67/9.58.

4.2.7.9. 5-Dodecylcarbamoylpentylammonium-5-dodecylcarbamoylpentylcarbamate (9g). White crystals; mp = 95–100 °C, yield 61%; IR (Nujol): ν_{max} 3369m ($\nu_{(N-HCOO^-)}$), 3253s ($\nu_{(CON-H)}$), 2128w, 1654m ($\nu_{(NHC=OO^-)}$), 1626s ($\nu_{(NHC=O)}$), 1553s,br ($\delta_{(NH)}$) cm^{-1}; CHN analysis for $C_{37}H_{76}N_4O_4$ (found/calculated): 69.48/69.33; 11.64/11.95; 8.93/8.74.

4.2.7.10. 5-Octadecylcarbamoylpentylammonium-5-octadecylcarbamoylpentylcarbamate (9h). The starting ammonium chloride was insoluble in water; therefore, it was dissolved in chloroform, mixed with TEA and water, and extracted with chloroform. White crystals; mp = 93–105 °C, yield 49%; IR (Nujol): ν_{max} 3369m ($\nu_{(N-HCOO^-)}$), 3253s ($\nu_{(CON-H)}$), 2128w, 1654m ($\nu_{(NHC=OO^-)}$), 1626s ($\nu_{(NHC=O)}$), 1553s,br ($\delta_{(NH)}$) cm^{-1}; CHN analysis for $C_{49}H_{100}N_4O_4$ (found/calculated): 73.18/72.72; 11.94/12.45; 6.93/6.92.

4.2.8. Octadecylammonium-octadecylcarbamate (10). Octadecylamine was dissolved in dry $CHCl_3$ and treated with CO_2 likewise. White crystals; mp = 85–88 °C, yield 71%; IR (Nujol): ν_{max} 3331s ($\nu_{(N-H)}$), 2152w, 1647m ($\nu_{(NHC=OO^-)}$), 1567s ($\delta_{(NH)}$), 1314s, 1157m, 816w cm^{-1}; CHN analysis for $C_{37}H_{78}N_2O_2$ (found/calculated): 75.91/76.22; 13.45/13.48; 4.69/4.80.

4.3. Skin preparation

Porcine ears were purchased from a local slaughterhouse. The full-thickness dorsal skin was collected and hairs were removed using a clipper. The skin was then immersed in 0.05% sodium azide solution in saline for 5 minutes for preservation. The skin fragments were stored vacuum-sealed at −18 °C for maximum of 2 months. The skin samples were thawed immediately before use.

4.4. Permeation experiments

The permeation-enhancing activities of the prepared compounds were evaluated in vitro using the Franz diffusion cells and theophylline as a model permeant. The donor samples were prepared by dispersing theophylline (5%) and the tested enhancer (1%) in Pg/W 3:2 v/v or IPM. At this concentration, both theophylline and the enhancers were suspended, with partial dissolution. The control samples were prepared likewise in the respective vehicle without addition of the enhancers. The suspensions were stirred at 50 °C for 5 min, and allowed to equilibrate at 37 °C for 24 h, and re-dispersed before application on the skin. The skin was cut into fragments and mounted into the cells dermal side down to leave a diffusion area of 1 cm^2. The acceptor compartment of the cells was filled with ca 17 mL of phosphate-buffered saline at pH 7.4 with 0.03% sodium azide as a preservative and allowed to equilibrate at 32 °C for 30 min. The donor sample of 200 μL volume was applied on the skin surface and occluded with a cover glass. The acceptor phase was kept at 32 °C with stirring

throughout the experiment. Samples of the acceptor phase of 0.6 mL volume were withdrawn at predetermined intervals over 48 h and replaced with fresh acceptor phase.

4.5. HPLC determination of theophylline

Theophylline in the acceptor phase samples was determined by HPLC using an ECOM LCP high-pressure pump, an ECOM autosampler, a Merck LiChroCART 250-4 column with LiChrospher 100, RP 18, 5 μm, a SpectraPhysics 8440 UV detector, and a CSW 1.7 integrating software. Methanol/0.1 M NaH_2PO_4 4:6 v/v at pH 5.55 was used as a mobile phase at a flow rate of 1.2 mL/min. The effluent was monitored at 272 nm. The retention time of theophylline was 3.3 ± 0.1 min.

4.6. Data treatment

The cumulative amount of theophylline having penetrated the skin, corrected for the acceptor sample replacement, was plotted against time. The steady state flux ($\mu g/cm^2/h$) was calculated from the linear region of the plot. The ER value was calculated as the ratio of the flux of theophylline with an enhancer and the flux of the permeant alone.

The data are presented as means ± SD ($n = 4$–6) obtained using the skin fragments from at least two animals. The statistical significance of the differences was analyzed using Student's *t*-test. A value of $p < 0.05$ was considered significant.

Acknowledgments

This work was supported by the Grant Agency of the Czech Republic (GACR 203/04/P042) and the Ministry of Education of the Czech Republic (MSM0021620822).

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