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Synthesis and transdermal permeation-enhancing activity of carbonate and carbamate analogs of Transkarbam 12

Tomáš Holas, Kateřina Vávrová,* Martin Šíma, Jana Klimentová and Alexandr Hrabálek

Centre for New Antivirals and Antineoplastics, Faculty of Pharmacy, Charles University, Heyrovského 1203, 50005 Hradec Králové, Czech Republic

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Abstract—Transkarbam 12 (5-(dodecyloxycarbonyl)pentylammonium-5-(dodecyloxycarbonyl)pentylcarbamate, T12) is a highly effective skin permeation enhancer. In this study, ester groups in the molecule of T12 were replaced by carbonate and carbamate ones, respectively. The in vitro permeation-enhancing activities were evaluated using porcine skin and compared with those of T12 and previously prepared series of amide, ketone, and alkyl analogs. According to the activities and behavior of the compounds in donor samples, ester group is essential for the activity of T12; its replacement not only decreases the enhancing potency, but is likely to change the mechanism of action.

1. Introduction

Transdermal permeation enhancers are compounds, which interact with skin constituents to promote drug flux. To date, a vast array of chemicals has been evaluated as enhancers, yet their inclusion into topical or transdermal formulation is limited since the underlying mechanisms of action of these agents are seldom clearly defined. For the most recent reviews on enhancers, see for example, Williams and Barry,[1] Purdon et al.,[2] and Vávrová et al.[3]

5-(Dodecyloxycarbonyl)pentylammonium-5-(dodecyloxycarbonyl)pentylcarbamate (Transkarbam 12, T12, Fig. 1) is a novel biodegradable permeation enhancer with high activity toward a variety of drugs with a wide spectrum of physicochemical properties,[4,5] low toxicity, and no dermal irritability.[4,6] The carbamate polar head of T12 is essential for the permeation-enhancing activity of the compound since the parent amino ester has been completely inactive.[4] In our effort to explain the mechanism of action of T12 and/or to prepare more active analogs, we focused on the role of ester linking group in T12 molecule. Replacement of ester with methylene and ketone groups, respectively, resulted in a loss of enhancing activity. Activities of the amide analogs were comparable to that of T12 in a lipophilic vehicle; however, in a hydrophilic one, where T12 reached its highest enhancement ratio, the amides were much less active.[7]

In this study, we aimed at synthesizing two novel series of T12 analogs, esters of carbonic or carbamic acid with an additional heteroatom introduced into the linking group (Fig. 1). The skin permeation-enhancing activity of the analogs was evaluated under the same conditions as described in our previous study[7] to allow for comparison of the whole series. Furthermore, solubility of the enhancers and their effect on solubility of the model drug, theophylline, in the donor samples were determined to gain more insights into the mode of action of this structurally novel group of enhancers.

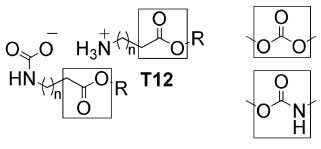

Figure 1. Transdermal permeation enhancer T12 ($n = 4$; R = $C_{12}H_{25}$) and its carbamate ($n = 4$–6; R = C_8H_{17}–$C_{12}H_{25}$), and carbonate analogs ($n = 5$, 6; R = C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$).

Keywords: Transdermal drug delivery; Cutaneous absorption; Penetration enhancer; Ammonium carbamate; Biodegradable.
* Corresponding author. Tel.: +420 495 067497; fax: +420 495 514330; e-mail: katerina.vavrova@faf.cuni.cz

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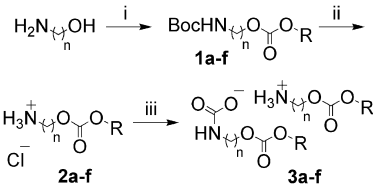
2. Results

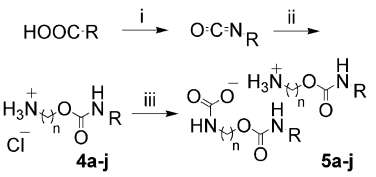
The carbonate analogs were prepared by reaction of *N*-Boc protected amino alcohol with an appropriate alkylchloroformate (Scheme 1). The protective group was removed by acid hydrolysis and the amino carbonates were subjected to a reaction with CO_2 to yield the target ammonium carbamate derivatives with carbonate groups within the chains. In case of 4-aminobutanol derivatives, that is, compounds having only 4-carbon linking chain between nitrogen and the carbonate group, no carbamate salts were obtained. Although precipitation of some material was observed at −20 °C, and we were able to isolate it at low temperature, it readily decomposed at ambient temperature and the spectra showed no presence of carbamic acid salt. Therefore, these derivatives were not evaluated for their enhancing activity.

For the preparation of the second series, the carbamate analogs, isocyanates were prepared by the Curtius rearrangement. These intermediates reacted with hydrochlorides of amino alcohols and the final products were prepared by reaction with CO_2 (Scheme 2).

Due to the low solubility and instability of the final lipophilic salts in acidic environment, characterization by NMR spectra was problematic. The spectra were recorded in $CDCl_3$ saturated with triethylamine or pyridine; however, only two members of the carbonate series, **3c** and **3f**, gave satisfactory results. The other spectra obtained in suspension were poor, showing only a weak signal of $NHCOO^-$ carbon at 163.1 ppm (d1 = 8s, ct = 9200), and could not be used for routine characterization of the products. Nevertheless, when dissolved in chloroform or similar acidic solvents, the products, that is, ammonium carbamate derivatives, decompose into carbon dioxide and the parent amino compounds, the NMR spectra of which fully confirm the structure and purity of such compounds. The presence of the ammonium carbamate was then sufficiently proved by specific N–H vibration in IR spectroscopy. For carbamic acid salts, moderately intensive band of the NH stretching vibration between 3200 and 3400 cm^{-1} was characteristic. Weak, broad band around 2150 cm^{-1} indicated presence of ammonium ions (combination band of RNH_3^+ torsion and antisymmetrical RNH_3^+ deformation). The medium-intensity band around 1650 cm^{-1} was assigned to amide I band of the carbamic acid salt.[8] Moreover, the existence of these substances in the form of an ammonium–carbamate salt was confirmed by TGA analysis, which showed that with increasing temperature, the substance releases exactly one equivalent of carbon dioxide (Fig. 2a and b). Finally, CHN analysis unambiguously confirmed the elemental composition corresponding to the proposed structures.



Scheme 1. Synthesis of the carbonate analogs. (**a**) $n = 5$, R = C_8H_{17}; (**b**) $n = 5$, R = $C_{10}H_{21}$; (**c**) $n = 5$, R = $C_{12}H_{25}$; (d) $n = 6$, R = C_8H_{17}; (**e**) $n = 6$, R = $C_{10}H_{21}$; (**f**) $n = 6$, R = $C_{12}H_{25}$. Reagents and conditions: (i) 1—Boc anhydride, NaOH, THF, 2—alkylchloroformate, pyridine, N_2 atmosphere, 0 °C; (ii) $HCl/CHCl_3$ or $CF_3COOH/CHCl_3$, 0 °C; (iii) TEA/CO_2.



Scheme 2. Synthesis of the carbamate analogs. (**a**) $n = 4$, R = C_8H_{17}; (**b**) $n = 4$, R = C_9H_{19}; (**c**) $n = 4$, R = $C_{10}H_{21}$; (**d**) $n = 4$, R = $C_{11}H_{23}$; (**e**) $n = 5$, R = $C_{10}H_{21}$; (**f**) $n = 5$, R = $C_{11}H_{23}$; (**g**) $n = 5$, R = $C_{12}H_{25}$; (**h**) $n = 6$, R = $C_{10}H_{21}$; (**i**) $n = 6$, R = $C_{11}H_{23}$; (**j**) $n = 6$, R = $C_{12}H_{25}$. Reagents and conditions: (i) 1—$SOCl_2$, 2—NaN_3, 3—60 °C; (ii) *n*-hydroxyalkylammonium chloride; (iii) TEA/CO_2.

Enhancement activity of the prepared compounds was evaluated using theophylline as a model drug and three vehicles of different polarity, water (W), propylene glycol/water 6:4 v/v (Pg/W), and isopropyl myristate (IPM). For the enhancement ratios (ERs), see Table 1.

When applied in aqueous suspensions, the carbonates of the same chain length as T12 were approximately equally active with ERs of 1.9 ± 0.3, 1.6 ± 0.3, and 2.5 ± 0.9 for T12, **3f**, and **3c**, respectively. Shorter-chain carbonates **3a**, **b**, **d**, and **e** displayed significantly higher activity than T12 with ERs up to 5.1. Neither of the carbamates showed significant enhancement of theophylline permeation when applied in aqueous donor vehicle.

The highest ER values were obtained with Pg/W as a donor vehicle. In this vehicle, none of the novel enhancers reached the activity of T12 (22.8 ± 1.1). Incorporation of additional oxygen decreased the activity of the carbonates approximately twice (ERs from 7.1 to 12.4). The carbamates showed activities even lower (ERs from 1.7 to 6.3).

With lipophilic donor vehicle, IPM, T12 enhanced permeation of theophylline 6.6 times. Only compound **3e** showed higher ER (9.7 ± 1.4) than T12; the other carbonates were less active (ERs from 2.2 to 4.0), as well as the carbamates (ERs from 0.8 to 5.6).

The solubility of the model drug, theophylline, in the Pg/W donor sample was 35 mg/mL. Addition of the enhancers increased this value significantly, however, no difference was observed between the individual compounds (Table 2).

The solubility of T12 and its analogs with the same chain length including previously prepared ketone, amide, and alkyl analogs in the Pg/W donor samples

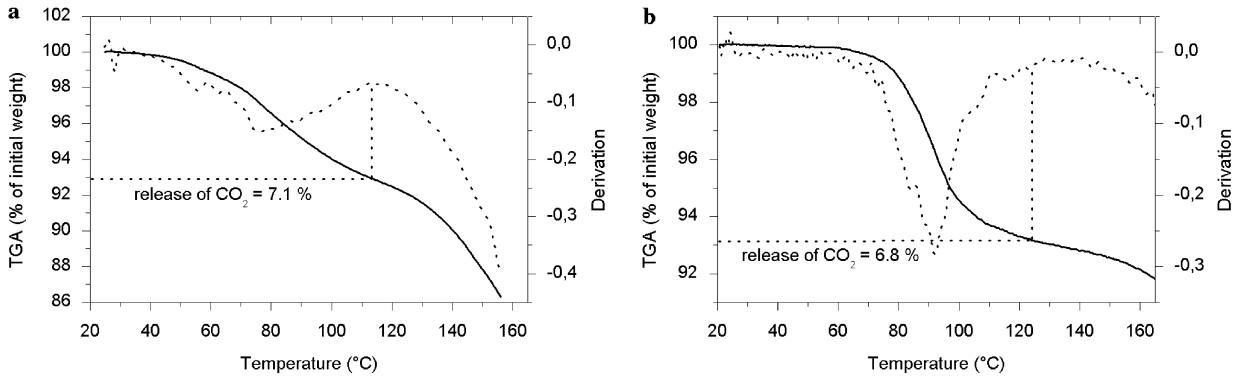


Figure 2. TGA analysis and derivation curves of compounds (a) **3b** and (b) **5f**, demonstrating an equimolar release of CO_2 from the ammonium–carbamate structure.

Table 1. ER values of the prepared carbonates **3a–f** and carbamates **5a–j** in three vehicles of different polarity (W, Pg/W, and IPM, respectively)

Compound	ER ± SD		
	W	Pg/W	IPM
3a	4.1 ± 1.0*,**	12.4 ± 2.6*	2.2 ± 0.1*
3b	5.1 ± 1.1*,**	11.8 ± 0.7*	5.5 ± 1.6*
3c	2.5 ± 0.9*	7.4 ± 0.2*	2.9 ± 0.5*
3d	4.6 ± 0.5*,**	7.1 ± 0.4*	3.4 ± 0.6*
3e	4.7 ± 0.5*,**	7.9 ± 1.6*	9.7 ± 1.4*,**
3f	1.6 ± 0.3*	8.4 ± 3.3*	4.0 ± 1.4*
5a	1.6 ± 0.5	3.2 ± 0.8*	4.8 ± 0.5*
5b	1.9 ± 0.6	6.3 ± 1.8	5.6 ± 0.3*
5c	2.1 ± 1.1	5.6 ± 0.5*	3.6 ± 0.2*
5d	1.5 ± 0.2	2.9 ± 0.4*	2.5 ± 0.2*
5e	1.5 ± 0.5	5.3 ± 0.9*	2.6 ± 0.4*
5f	1.0 ± 0.2	2.3 ± 0.5*	2.2 ± 0.2*
5g	1.2 ± 0.3	1.7 ± 0.5	1.7 ± 0.3*
5h	1.2 ± 0.2	4.3 ± 0.6*	3.1 ± 0.3*
5i	1.5 ± 0.6	3.7 ± 0.3*	1.7 ± 0.3*
5j	1.3 ± 0.3	1.9 ± 0.3	0.8 ± 0.1
T12	1.9 ± 0.3*	22.8 ± 1.1*	6.6 ± 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 ($p < 0.05$).

Table 2. Solubility of T12 and its analogs with the same chain length and their effect on the drug solubility in Pg/W

Enhancer	Linking group	ER	C_e (mg/mL)	C_0 (mg/mL)
No enhancer	—	1.0	—	35.0 ± 0.0
T12	–COO–	22.8 ± 1.1	3.5 ± 0.1	42.8 ± 1.5*
3f	–OCOO–	8.4 ± 3.3	6.4 ± 0.3	39.6 ± 0.9*
5j	–OCONH–	1.9 ± 0.3	1.6 ± 0.0	37.8 ± 0.6*
Ketone analog	–CO–	3.4 ± 0.7[a]	1.0 ± 0.1	42.7 ± 3.9*
Amide analog	–CONH–	2.2 ± 0.5[a]	1.8 ± 0.0	40.6 ± 1.5*
Alkyl analog	–CH2–	1.8 ± 0.6[a]	0.5 ± 0.1	39.6 ± 1.6*

C_e, solubility of the enhancer; C_0, solubility of theophylline.
[a] Ketone, amide, and alkyl analogs were studied in our previous work[7].
* Significantly different from Control (without an enhancer; $p < 0.05$).

was determined by potentiometric titration and is shown in Table 2. Linear correlation ($p < 0.01$; $R^2 = 0.96$) was found between the ER values, that is, the activity of the compounds, and their solubility in the donor sample, with T12 excluded from the dataset.

3. Discussion

T12 was originally prepared as an open Azone analog, dodecyl 6-aminohexanoate (DDEAC).[6] Recently, we have found that DDEAC traps carbon dioxide to form two-chain ammonium–carbamate salt, T12. Direct comparison of these compounds revealed that only T12 is responsible for the enhancing properties and DDEAC is inactive.[4] The labile ammonium–carbamate salt within the polar head was found to be essential and suggested to relate to the mode of action of T12.[9] Thus, we assumed that mainly the carbamate salt bears the enhancing activity and the ester group only modulates its solubility and/or partitioning into the stratum corneum. However, replacement of ester group by amide, ketone, and methylene, respectively, resulted in compounds with markedly decreased activity.[7]

In this study, a series of carbonate and carbamate analogs of T12 was evaluated. Both these groups have been widely used as pH-sensitive biodegradable linkers for example, in surfactants[10] or carrier lipids for gene delivery.[11,12] Although the carbonates displayed significant permeation-enhancing activities, none of the compounds reached the potency of T12 in Pg/W donor vehicle. Replacement of oxygen with nitrogen further decreased the activity, similar to amide analogs. Generally, the 10-carbon-chain compounds were the most active enhancers, which is in accordance with previous studies.[3,13] The length of the linking chain between the ammonium–carbamate group and oxygen in carbonate and carbamate group, respectively, had only a little influence. However, certain length is probably required as the carbonates with 4-carbon linking chain did not form stabile ammonium–carbamates.

Thus, ester group, which was originally selected for its biodegradability, is essential for the activity of T12 as well as the ammonium–carbamate polar head. Third requirement is flexibility of the linking chain between these functional groups. This is documented by decreased activity of tranexamic acid derivatives, which differ from T12 only by presence of a cyclohexane ring instead of the alkyl chain.[14]

Drug permeation across the stratum corneum obeys Fick's first law ($J = D \times C_0 \times P/h$) where steady-state flux (J) is related to the diffusion coefficient (D), the drug concentration in the vehicle (C_0), the partition coefficient between the vehicle and the stratum corneum (P), and the stratum corneum thickness (h). Permeation enhancement may be achieved by increasing D (enhancer permeates into the stratum corneum and disorders the barrier structures, for example, the intercellular lipid matrix), C_0 (enhancer increases solubility of a poorly soluble drug in the vehicle), P (enhancer alters the solvent nature of the skin barrier and thus increases partitioning of a drug into the stratum corneum), or, less likely, by decreasing h (providing a more permeable shortcut).

Increasing C_0, that is, maximizing the availability of drug for absorption, is a compromise between obtaining a sufficient amount of poorly soluble drug in solution by solubilization, and maintaining or inducing a high level of thermodynamic activity to support the absorption process. In case of T12 and its analogs, the donor samples were suspensions, thus the thermodynamic activity of theophylline was at its maximum value in all samples. Addition of the evaluated enhancers increased C_0, however, this increase was too small (approximately 20%) to be responsible for the ERs observed. Moreover, there was no difference between T12 and its analogs.

Partitioning of a drug into the stratum corneum is usually increased by polar solvents like ethanol and Pg. In case of amphiphilic enhancers that enter the stratum corneum in low concentrations, this mechanism is negligible.[15]

Therefore, we may assume that the main mode of action of T12 and its analogs is increasing D, that is, a specific action within the skin barrier. Since Pg is known to increase P by changing the solvent properties of the stratum corneum, the effects of T12 and Pg would be synergic. This is consistent with the observed behavior; the highest enhancement activity of T12 has been found when applied in Pg/W donor vehicle.

Disordering of the barrier structures requires certain concentration of an enhancer in the site of action. For the carbonate, carbamate, amide, ketone, and alkyl analogs, a linear correlation was found between their solubility in the donor sample and their activity, suggesting an approximately equal extent of barrier-disordering ability. This could explain relatively high activity of the carbonate analogs compared to the other derivatives. On the other hand, T12 is poorly soluble in the vehicle; thus we hypothesize that ester group in T12 is associated with a different mechanism of action.

Previously, a doublet of ester carbonyl stretching has been observed in FTIR spectra of T12,[16] which could be explained by different hydrogen bonding of the two ester groups. No such splitting of carbonyl stretching has been observed in the analogs. It has been suggested that one ester group might be forming an intramolecular hydrogen bond between ester and carbamate group of T12.[7] Such hydrogen bond would bring different steric ordering of the molecule, which could result in different partitioning of T12 into the stratum corneum or, more probably, a specific action within the barrier compared to the analogs. The importance of hydrogen bonding for the action of the skin permeation enhancers has already been documented.[17,18]

Another possibility is that the hydrogen bond has an influence on the stability of the ammonium–carbamate polar head. Carbamic acid salts are, generally, unstable in a mildly acidic environment. It has been hypothesized that in the stratum corneum, which is acidic in nature, T12 would be decomposed releasing carbon dioxide and two molecules of DDEAC or, more precisely, its ammonium salt.[9] As DDEAC is inactive, the mode of action could be related to the released carbon dioxide or a conformational change connected to the decomposition. Carbon dioxide present in the stratum corneum could disrupt the lipid lamellae by changing the hydrogen bonding between their polar heads or create permeable pores. Studies aiming at investigating this hypothesis are in progress.

In conclusion, this work described a novel series of T12 analogs. Although none of them reached the activity of the parent compound, the results narrowed the number of the possible mechanisms of action of this highly active, nontoxic, nonirritant, and biodegradable skin permeation enhancer.

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, N) was performed on a Fisons EA 1110 CHNS-O elemental analyzer. The melting point was measured on 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 (carbonate analogs) and Scheme 2 (carbamate analogs).

4.2.1. General procedure for the preparation of carbonic acid esters (1a–f). One hundred milliliters of Boc_2O (0.24 mol) solution in THF was slowly dropped to an aqueous solution (150 mL) of an aminoalcohol (0.2 mol) and NaOH (0.2 mol) at 0 °C. The mixture was stirred overnight at room temperature. The reaction

mixture was evaporated in vacuum and extracted several times with diethyl ether. The combined organic layers were washed with diluted HCl and brine and dried over Na_2SO_4. After removal of the solvent, the purity of product was checked by TLC. The yield of Boc protected aminoalcohol was quantitative.[19]

An alkylchloroformate (4.4 mmol) was slowly added to Boc-aminoalcohol (4 mmol) solution in freshly distilled dry pyridine (8 mL) at 0 °C under N_2 atmosphere. The mixture was stirred overnight and then acidified by 10% HCl and extracted three times with diethyl ether. The combined organic layers were washed with brine and the residue was subjected to column chromatography (petroleum ether/ethyl acetate 8:2) to yield carbonic acid ester **1a**–**f**.[20]

4.2.1.1. 5-*tert*-Butoxycarbonylaminopentyl(octyl)carbonate (1a). Colorless liquid, yield 78%; IR ($CHCl_3$): υ_{max} 3455m ($\upsilon_{(N-H)}$), 1739s ($\upsilon_{(OC=OO)}$), 1709s ($\upsilon as_{(HNC=OO)}$), 1508s ($\delta_{(NH)}$), 1468m, 1457m, 1404m ($\upsilon as_{(O-C(O)-O)}$), 1393m ($\delta_{(tert\text{-}butyl)}$), 1367s ($\upsilon_{(C-O)}$), 1265sbr ($\upsilon_{(C-N,C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (s; 1H; NH), 4.10 (t; 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 3.10 (t; 2H; J = 6.9 Hz; CH_2NH), 1.59–1.73 (m; 4H; CH_2), 1.17–1.55 (m; 23H; CH_2; CH_3), 0.86 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.2, 79.1, 68.1, 67.6, 40.4, 31.7, 29.7, 29.1, 28.6, 28.3, 28.3, 25.6, 23.0, 22.6, 14.1 ppm.

4.2.1.2. 5-*tert*-Butoxycarbonylaminopentyl(decyl)carbonate (1b). Colorless liquid, yield 72%; IR ($CHCl_3$): υ_{max} 3455m ($\upsilon_{(N-H)}$), 1740s ($\upsilon_{(OC=OO)}$), 1710s ($\upsilon as_{(HNC=OO)}$), 1508s ($\delta_{(NH)}$), 1468m, 1457m, 1404m ($\upsilon as_{(O-C(O)-O)}$), 1393m ($\delta_{(tert\text{-}butyl)}$), 1367s ($\upsilon_{(C-O)}$), 1266sbr ($\upsilon_{(C-N,C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.55 (s; 1H; NH), 4.09 (t; 4H; J = 6.7 Hz; $CH_2OCOOCH_2$), 3.10 (t; 2H; J = 6.9 Hz; CH_2NH), 1.59–1.72 (m; 4H; CH_2), 1.15–1.53 (m; 27H; CH_2; CH_3), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.2, 79.1, 68.1, 67.6, 40.4, 31.8, 29.7, 29.5, 29.4, 29.2, 29.1, 28.6, 28.3, 28.3, 25.6, 23.0, 22.6, 14.0 ppm.

4.2.1.3. 5-*tert*-Butoxycarbonylaminopentyl(dodecyl)carbonate (1c). Colorless liquid, yield 62%; IR ($CHCl_3$): υ_{max} 3455m ($\upsilon_{(N-H)}$), 1740s ($\upsilon_{(OC=OO)}$), 1710s ($\upsilon as_{(HNC=OO)}$), 1507s ($\delta_{(NH)}$), 1467m, 1457m, 1403m ($\upsilon as_{(O-C(O)-O)}$), 1393m ($\delta_{(tert\text{-}butyl)}$), 1367s ($\upsilon_{(C-O)}$), 1267sbr ($\upsilon_{(C-N,C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.57 (s; 1H; NH), 4.08 (t; 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 3.09 (t; 2H; J = 6.9 Hz; CH_2NH), 1.56–1.73 (m; 4H; CH_2), 1.14–1.56 (m; 31H; CH_2; CH_3), 0.86 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.3, 79.1, 68.1, 67.6, 40.4, 31.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 28.6, 28.3, 28.3, 25.6, 23.0, 22.6, 14.1 ppm.

4.2.1.4. 6-*tert*-Butoxycarbonylaminohexyl(octyl)carbonate (1d). Colorless liquid, yield 75%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (s; 1H; NH), 4.09 (t; 4H; J = 6.6 Hz; $CH_2OCOOCH_2$), 3.08 (t; 2H; J = 6.9 Hz; CH_2NH), 1.59–1.72 (m; 4H; CH_2), 1.13–1.50 (m; 25H; CH_2; CH_3), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.4, 79.1, 68.0, 67.7, 40.5, 31.8, 29.8, 29.5, 29.4, 29.2, 28.6, 28.3, 26.3, 25.6, 25.4, 22.6, 14.1 ppm.

4.2.1.5. 6-*tert*-Butoxycarbonylaminohexyl(decyl)carbonate (1e). Colorless liquid, yield 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (s; 1H; NH), 4.09 (t; 4H; J = 6.8 Hz; $CH_2OCOOCH_2$), 3.08 (t; 2H; J = 7.0 Hz; CH_2NH), 1.57–1.70 (m; 4H; CH_2), 1.17–1.52 (m; 29H; CH_2; CH_3), 0.86 (t; 3H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.4, 79.1, 68.0, 67.7, 40.5, 31.8, 29.9, 29.5, 29.4, 29.2, 29.2, 28.6, 28.5, 28.4, 26.3, 25.6, 25.4, 22.6, 14.1 ppm.

4.2.1.6. 6-*tert*-Butoxycarbonylaminohexyl(dodecyl)carbonate (1f). Colorless liquid, yield 52%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (s; 1H; NH), 4.10 (t; 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 3.08 (t; 2H; J = 7.0 Hz; CH_2NH), 1.56–1.72 (m; 4H; CH_2), 1.14–1.52 (m; 33H; CH_2; CH_3), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 155.3, 79.1, 68.0, 67.7, 40.5, 31.7, 29.9, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 28.4, 28.4, 26.3, 25.6, 25.4, 22.6, 14.0 ppm.

4.2.2. General procedure for the preparation of 5-alkoxycarbonyloxypentylammonium salts (2a–f). *Method A:*[21] Ten equivalents of CF_3COOH was added dropwise in a solution of compound **1a**–**c** in dry $CHCl_3$ at 0 °C. The reaction was monitored by TLC (petroleum ether/ethyl acetate 8:2). The reaction mixture was evaporated in vacuum and the residual CF_3COOH was removed by a stream of N_2 to yield yellowish liquid **2a**–**c** (quant.).

Method B: Dry HCl was slowly introduced into a solution of compound **1d**–**f** (4 mmol) in 10 mL of dry $CHCl_3$ at −10 °C for 30 min. The reaction was monitored by TLC (petroleum ether/ethyl acetate 8:2). The reaction mixture was evaporated in vacuum and crystallized from $CHCl_3$/diethyl ether. The yield of white crystalline compound **2d**–**f** was approximately 75%.

4.2.2.1. 5-Octyloxycarbonyloxypentylammonium-trifluoroacetate (2a). Colorless liquid, IR ($CDCl_3$): υ_{max} 1741s ($\upsilon_{(OC=OO)}$), 1676s ($\upsilon as_{(CF_3COO^-)}$), 1529m $\delta s_{NH_3^+}$, 1469m, 1459m, 1436m, 1407m ($\upsilon as_{(O-C(O)-O)}$), 1380w, 1269s ($\upsilon_{(C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ7.50 (s; 3H; NH_3^+), 4.06–4.17 (m; 4H; $CH_2OCOOCH_2$), 2.94–3.08 (m; 2H; $CH_2NH_3^+$), 1.59–1.78 (m; 6H; CH_2), 1.17–1.53 (m; 12H; CH_2), 0.87 (t; 3H; J = 6.8 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.0, 161.6, 161.1, 160.5, 155.5, 121.0, 117.3, 113.5, 109.4, 68.5, 67.3, 40.1, 31.7, 29.1, 29.1, 28.5, 27.8, 26.8, 25.6, 22.6, 22.4, 14.1 ppm.

4.2.2.2. 5-Decyloxycarbonyloxypentylammonium-trifluoroacetate (2b). Colorless liquid, ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (s; 3H;), 4.11 (t; NH_3^+ 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 2.92–3.06 (m; 2H; $CH_2NH_3^+$), 1.59–1.77 (m; 6H; CH_2), 1.18–1.51 (m; 16H; CH_2), 0.87 (t; 3H; J = 6.8 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.2, 161.7, 161.2, 160.7, 155.4,

121.3, 117.5, 113.7, 109.8, 68.5, 67.3, 40.0, 31.8, 29.5, 29.5, 29.3, 29.2, 28.6, 27.9, 26.8, 25.6, 22.6, 22.4, 14.1 ppm.

4.2.2.3. 5-Dodecycarbonyloxypentylammonium-trifluoroacetate (2c). Colorless liquid, ^{1}H NMR(300 MHz, $CDCl_3$): δ 7.36 (s; 3H; NH_3^+), 4.08–4.18 (m; 4H; $CH_2OCOOCH_2$), 2.98–3.10 (m; 2H; $CH_2NH_3^+$), 1.59–1.78 (m; 6H; CH_2), 1.17–1.51 (m; 20H; CH_2), 0.87 (t; 3H; J = 6.7 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.2, 161.7, 161.2, 160.7, 155.6, 121.3, 117.5, 113.7, 109.8, 68.6, 67.4, 40.3, 31.9, 29.6, 29.5, 29.5, 29.3, 29.2, 28.5, 27.8, 26.8, 25.6, 22.7, 22.3, 14.1 ppm.

4.2.2.4. 6-Octyloxycarbonyloxyhexylammonium-chloride (2d). Mp = 88–90 °C, IR ($CHCl_3$): υ_{max} 1739s ($\upsilon_{(OC=OO)}$), 1614m ($\delta as_{NH_3^+}$), 1522m ($\delta s_{NH_3^+}$), 1468m, 1404m ($\upsilon as_{(O-C(O)-O)}$), 1380w, 1268s ($\upsilon_{(C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ8.23 (s; 3H; NH_3^+), 4.09 (t; 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 2.90–3.06 (m; 2H; $CH_2NH_3^+$), 1.58–1.83 (m; 6H; CH_2), 1.18–1.49 (m; 14H; CH_2), 0.86 (t; 3H; J = 6.8 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.3, 68.1, 67.5, 39.8, 31.7, 29.1, 29.1, 28.6, 28.3, 27.4, 26.0, 25.6, 25.1, 22.6, 14.0 ppm.

4.2.2.5. 6-Decyloxycarbonyloxyhexylammonium-chloride (2e). Mp = 96–97 °C, IR ($CHCl_3$): υ_{max} 1740s ($\upsilon_{(OC=OO)}$), 1615m ($\delta as_{NH_3^+}$), 1522m ($\delta s_{NH_3^+}$), 1467m, 1404m ($\upsilon as_{(O-C(O)-O)}$), 1380w, 1267s ($\upsilon_{(C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (s; 3H; NH_3^+), 4.08 (t; 4H; J = 7.0 Hz; $CH_2OCOOCH_2$), 2.91–3.05 (m; 2H; $CH_2NH_3^+$), 1.57–1.84 (m; 6H; CH_2), 1.17–1.50 (m; 18H; CH_2), 0.85 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.3, 68.0, 67.4, 39.7, 31.8, 29.4, 29.4, 29.2, 29.2, 28.6, 28.3, 27.4, 26.0, 25.6, 25.0, 22.6, 14.0 ppm.

4.2.2.6. 6-Dodecylcarbonyloxyhexylammonium-chloride (2f). Mp = 100–102 °C, IR ($CHCl_3$): υ_{max} 1740s ($\upsilon_{(OC=OO)}$), 1615m ($\delta as_{NH_3^+}$), 1522m ($\delta s_{NH_3^+}$), 1467m, 1404m ($\upsilon as_{(O-C(O)-O)}$), 1380w, 1266s ($\upsilon_{(C-O)}$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (s; 3H; NH_3^+), 4.09 (t; 4H; J = 6.5 Hz; $CH_2OCOOCH_2$), 2.90–3.05 (m; 2H; $CH_2NH_3^+$), 1.57–1.83 (m; 6H; CH_2), 1.17–1.47 (m; 22H; CH_2), 0.85 (t; 3H; J = 7.0 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.3, 68.1, 67.4, 39.8, 31.8, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.6, 28.3, 27.4, 26.0, 25.6, 25.1, 22.6, 14.1 ppm.

4.2.3. General procedure for the preparation of alkyl isocyanates. An acyl chloride (prepared from the corresponding carboxylic acid by reaction with $SOCl_2$ and purified by vacuum distillation, Table 3) in acetone was added dropwise to a saturated aqueous solution of NaN_3 (1.4 equiv) at temperature not exceeding 5 °C. The organic upper phase was removed by a syringe, slowly dropped to dry toluene at 60 °C, and stirred at the same temperature for 1 h. Toluene was evaporated in vacuum and the residue was purified by distillation under reduced pressure (Table 3) yielding 65–75% of alkyl isocyanate (40% for undecylisocyanate).[22]

Table 3. Boiling points of the prepared acyl chlorides and alkyl isocyanates

R=	Bp (RCOCl)	Bp (RNCO)
Octyl	118 °C/45 mbar	114 °C/50 mbar
Nonyl	133 °C/35 mbar	128 °C/40 mbar
Decyl	145 °C/36 mbar	138 °C/35 mbar
Undecyl	163 °C/40 mbar	150 °C/36 mbar
Dodecyl	121 °C/5 mbar	114 °C/5 mbar

4.2.4. General procedure for the preparation of alkylcarbamoyloxyalkylammonium-chlorides 5a–j. *n*-Hydroxyalkylammonium chloride in acetonitrile was added to alkyl isocyanate (1.05 equiv) and heated under reflux for 12 h. Precipitated solid was filtered off and recrystallized from ethanol/acetonitrile to yield white crystalline compound **4a**–**j**.[23]

4.2.4.1. 4-Octylcarbamoyloxybutylammonium-chloride (4a). Mp = 135–138 °C, yield 90%; IR (nujol): υ_{max} 3334m ($\upsilon_{(N-H)}$), 2034, 1685s ($\upsilon_{(C=O)}$), 1611w ($\delta as_{NH_3^+}$), 1537m ($\delta_{(N-H)}$), 1275m, 1252m, 1145m; ^{1}H NMR (300 MHz, DMSO): δ 8.13 (s; 3H; NH_3^+), 7.18 (t; 1H; J = 5.2 Hz; NH), 3.97 (t; 2H; J = 6.0 Hz; CH_2O), 2.88–2.97 (m; 2H; CH_2NH), 2.79 (t; 2H; J = 6.8 Hz; $CH_2NH_3^+$), 1.84 (p; 2H; J = 6.4 Hz; CH_2), 1.09–1.43 (m; 14H; CH_2), 0.84 (t; 3H; J = 6.2 Hz; CH_3); ^{13}C NMR(75 MHz, DMSO): δ 156.3, 61.2, 40.4, 36.4, 31.5, 29.6, 28.9, 28.9, 28.7, 27.1, 26.5, 22.3, 14.2 ppm.

4.2.4.2. 4-Nonylcarbamoyloxybutylammonium-chloride (4b). Mp = 120–123 °C, yield 86%; IR (nujol): υ_{max} 3333m ($\upsilon_{(N-H)}$), 2034, 1686s ($\upsilon_{(C=O)}$), 1611w (NH_3^+), 1542m ($\delta_{(N-H)}$), 1298m, 1269m, 1246m, 1145m; ^{1}H NMR (300 MHz, DMSO): δ 8.12 (s; 3H; NH_3^+), 7.16 (t; 1H; J = 5.5 Hz; NH), 3.97 (t; 2H; J = 6.0 Hz; CH_2O), 2.92 (q; 2H; J = 6.2 Hz; CH_2NH), 2.79 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 1.84 (p; 2H; J = 6.4 Hz; CH_2), 1.05–1.43 (m; 16H; CH_2), 0.83 (t; 3H; J = 6.1 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 156.3, 61.2, 40.4, 36.4, 31.5, 29.6, 29.2, 29.1, 29.0, 28.9, 27.0, 26.5, 22.3, 14.2 ppm.

4.2.4.3. 4-Decylcarbamoyloxybutylammonium-chloride (4c). Mp = 128–131 °C, yield 78%; IR (nujol): υ_{max} 3334m ($\upsilon_{(N-H)}$), 2021, 1684s ($\upsilon_{(C=O)}$), 1598w ($\delta_{as(NH3+)}$), 1531m ($\delta_{(N-H)}$), 1286m, 1262m, 1240m, 1143m; ^{1}H NMR (300 MHz, DMSO): δ 7.81 (s; 3H; NH_3^+), 7.18 (t; 1H; J = 5.5 Hz; NH), 3.96 (t; 2H; J = 5.8 Hz; CH_2O), 2.92 (q; 2H; J = 6.2 Hz; CH_2NH), 2.79 (t; 2H; J = 7.2 Hz; $CH_2NH_3^+$), 1.84 (p; 2H; J = 6.5 Hz; CH_2), 1.11–1.41 (m; 18H; CH_2), 0.83 (t; 3H; J = 5.8 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 156.3, 61.2, 40.4, 36.4, 31.5, 29.6, 29.2, 29.2, 29.0, 28.9, 27.0, 26.5, 22.3, 14.2 ppm.

4.2.4.4. 4-Undecylcarbamoyloxybutylammonium-chloride (4d). Mp = 131–134 °C, yield 80%; IR ($CHCl_3$): υ_{max} 3453w ($\upsilon_{(N-H)}$), 1712s ($\upsilon_{(C=O)}$), 1604w (NH_3^+), 1537m ($\delta_{(N-H)}$), 1417m, 1363m; ^{1}H NMR (300 MHz, DMSO): δ 8.23 (s; 3H; NH_3^+), 7.17 (t; 1H; J = 4.9 Hz; NH), 3.96 (t; 2H; J = 5.8 Hz; CH_2O), 2.91 (q; 2H; J = 5.8 Hz; CH_2NH), 1.85–2.72 (m; 2H; $CH_2NH_3^+$),

1.85 (p; 2H; J = 6.3 Hz; CH_2), 1.07–1.42 (m; 20H; CH2), 0.83 (t; 3H; J = 5.8 Hz; CH_3); ^{13}C NMR (75 MHz, DMSO): δ 156.3, 61.2, 40.4, 36.4, 31.6, 29.6, 29.3, 29.2, 29.0, 29.0, 27.0, 26.5, 22.4, 14.2 ppm.

4.2.4.5. 5-Decylcarbamoyloxypentylammonium-chloride (4e). Mp = 127–133 °C, yield 79%; IR (KBr): υ_{max} 3327m ($\upsilon_{(N–H)}$), 2957w, 2922s, 2852s, 1686s ($\upsilon_{(C=O)}$), 1623w (NH_3^+), 1541m ($\delta_{(N–H)}$), 1468w, 1284m, 1263m ($\upsilon_{(N\text{-}CO\text{-}O)}$), 1145m; ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s; 3H; NH_3^+), 5.16 (t; 1H; J = 5.5 Hz; NH), 4.04 (t; 2H; J = 6.3 Hz; CH_2O), 3.12 (q; 2H; J = 6.6 Hz; CH_2NH), 3.01 (t; 2H;J = 7.6 Hz; $CH_2NH_3^+$), 1.83 (p; 2H; J = 7.6 Hz; $CH_2NH_3^+$), 1.56–1.70 (m; 2H; CH_2), 1.47 (p; 4H; CH_2), 1.16–1.36 (m; 14H; CH_2), 0.87 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.2, 41.0, 39.8, 31.9, 30.0, 29.5, 29.3, 28.2, 27.1, 26.8, 22.9, 22.6, 14.1 ppm.

4.2.4.6. 5-Undecylcarbamoyloxypentylammonium-chloride (4f). Mp = 141–145 °C, yield 77%; IR (KBr): υ_{max} 3328m ($\upsilon_{(N–H)}$), 2957w, 2922s, 2852s, 1687s ($\upsilon_{(C=O)}$), 1623w (NH_3^+), 1541s ($\delta_{(N–H)}$), 1468m, 1276m, 1258m ($\upsilon_{(N–CO–O)}$), 1145m; ^1H NMR (300 MHz, $CDCl_3$): δ 8.20 (s; 3H; NH_3^+), 5.17 (t; 1H; J = 5.5 Hz; NH), 4.03 (t; 2H; J = 5.9 Hz; CH_2O), 3.11 (q; 2H; J = 6.6 Hz; CH_2NH), 3.01 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 1.82 (p; 2H; J = 7.7 Hz; $CH_2NH_3^+$), 1.56–1.70 (m; 2H; CH_2), 1.47 (p; 4H; CH_2), 1.20–1.33 (m; 16H; CH_2), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.2, 41.0, 39.8, 31.9, 30.0, 29.6, 29.6, 29.3, 28.2, 27.0, 26.8, 23.0, 22.6, 14.1 ppm.

4.2.4.7. 5-Dodecylcarbamoyloxypentylammonium-chloride (4g). Mp = 128–130 °C, yield 76%; IR (KBr): υ_{max} 3331m ($\upsilon_{(N–H)}$), 2957w, 2921s, 2851s, 1687s ($\upsilon_{(C=O)}$), 1621w (NH_3^+), 1540m ($\delta_{(N–H)}$), 1468m, 1378w, 1270m, 1252m ($\upsilon_{(N\text{-}CO\text{-}O)}$), 1145m; ^1H NMR (300 MHz, $CDCl_3$): δ 8.21 (s; 3H; NH_3^+), 5.16 (t; 1H; J = 5.5 Hz; NH), 4.04 (t; 2H; J = 6.2 Hz; CH_2O), 3.11 (q; 2H; J = 6.6 Hz; CH_2NH), 3.01 (t; 2H; J = 7.6 Hz; $CH_2NH_3^+$), 1.70–1.89 (p; 2H; $CH_2NH_3^+$), 1.56–1.70 (m; 2H; CH_2), 1.47 (p; 4H; CH_2), 1.18–1.35 (m; 18H; CH_2), 0.86 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.2, 41.0, 39.8, 31.9, 30.0, 29.6, 29.6, 29.5, 29.3, 28.2, 27.1, 26.8, 23.0, 22.8, 14.1 ppm.

4.2.4.8. 6-Decylcarbamoyloxyhexylammonium-chloride (4h). Mp = 135–138 °C, yield 80%; IR ($CHCl_3$): υ_{max} 3453m ($\upsilon_{(N–H)}$), 2956m, 2929s, 2857s, 1706s ($\upsilon_{(C=O)}$), 1616w (NH_3^+), 1519s ($\delta_{(N–H)}$), 1467m, 1378w; ^1H NMR (300 MHz, $CDCl_3$): δ 8.23 (s; 3H; NH_3^+), 4.96 (t; 1H; J = 5.5 Hz; NH), 4.02 (t; 2H; J = 6.3 Hz; CH_2O), 3.13 (q; 2H; J = 6.5 Hz; CH_2NH), 3.00 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 1.70–1.83 (m; 2H; $CH_2NH_3^+$), 1.55–1.67 (m; 2H; CH_2), 1.16–1.54 (m; 20H; CH_2), 0.87 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.4, 41.0, 39.8, 31.9, 30.0, 29.5, 29.3, 28.6, 27.3, 26.8, 26.0, 25.3, 22.6, 14.1 ppm.

4.2.4.9. 6-Undecylcarbamoyloxyhexylammonium-chloride (4i). Mp = 134–137 °C, yield 91%; IR ($CHCl_3$): υ_{max} 3452m ($\upsilon_{(N–H)}$), 2928s, 2856s, 1707s ($\upsilon_{(C=O)}$), 1613w (NH_3^+), 1519s ($\delta_{(N–H)}$), 1467m, 1378w; ^1H NMR (300 MHz, $CDCl_3$): δ 8.23 (s; 3H; NH_3^+), 4.96 (t; 1H; J = 5.6 Hz; NH), 4.02 (t; 2H; J = 6.3 Hz; CH_2O), 3.13 (q; 2H; J = 6.6 Hz; CH_2NH), 3.00 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 1.78 (p; 2H; J = 7.3 Hz; $CH_2NH_3^+$), 1.55–1.68 (m; 2H; CH_2), 1.16–1.54 (m; 22H; CH_2), 0.87 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.4, 41.0, 39.8, 31.9, 30.0, 29.6, 29.6, 29.3, 28.6, 27.4, 26.8, 26.0, 25.3, 22.7, 14.1 ppm.

4.2.4.10. 6-Dodecylcarbamoyloxyhexylammonium-chloride (4j). Mp = 120–123 °C, yield 80%; IR ($CHCl_3$): υ_{max} 3452m ($\upsilon_{(N–H)}$), 2928s, 2856s, 1706s ($\upsilon_{(C=O)}$), 1616w (NH_3^+), 1519s ($\delta_{(N–H)}$), 1466m, 1378w; ^1H NMR (300 MHz, $CDCl_3$): δ 8.25 (s; 3H; NH_3^+), 4.90–5.02 (m; 1H; NH), 3.97–4.13 (m; 2H; CH_2O), 3.06–3.19 (m; 2H; CH_2NH), 3.00 (t; 2H; J = 7.4 Hz; $CH_2NH_3^+$), 1.70–1.84 (m; 2H; $CH_2NH_3^+$), 1.54–1.68 (m; 2H; CH_2), 1.16–1.54 (m; 24H; CH_2), 0.87 (t; 3H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8, 64.4, 41.0, 39.8, 31.9, 30.0, 29.6, 29.6, 29.3, 28.6, 27.4, 26.8, 26.0, 25.3, 22.7, 14.1 ppm.

4.2.5. General procedure for the preparation of the carbamic acid salts 3a–f and 5a–j. Ammonium salt **2a–f** and **5a–j**, respectively, was dissolved in water, alkalized by 1.5 equiv of triethylamine, and extracted three times with diethyl ether. The ethereal extracts were dried over Na_2SO_4 and then dry CO_2 slowly bubbled through the solution for approximately 20 min. The precipitated ammonium–carbamate was filtered off through dense filter paper and dried in vacuum over P_4O_{10}.

4.2.5.1. 5-Octyloxycarbonyloxypentylammonium-5-octyloxycarbonyloxypentylcarbamate (3a). Mp = 45–47 °C, yield 50%; IR (nujol): υ_{max} 3381m ($\upsilon_{(N–H)}$), 2178wbr, 1742s ($\upsilon_{(OC=OO)}$), 1652w, 1591m, 1533m ($\delta s_{NH_3^+}$), 1271s ($\upsilon_{(C–O,C–N)}$); CHN analysis for $C_{29}H_{58}N_2O_8$ (found/calculated): 61.66/61.89, 10.62/10.39, 5.10/4.98.

4.2.5.2. 5-Decyloxycarbonyloxypentylammonium-5-decyloxycarbonyloxypentylcarbamate (3b). Mp = 48–53 °C, yield 62%; IR (nujol): υ_{max} 3381w ($\upsilon_{(N–H)}$), 2180wbr, 1742s ($\upsilon_{(OC=OO)}$), 1652w, 1591m, 1534m ($\delta s_{NH_3^+}$), 1269s ($\upsilon_{(C–O,C–N)}$), 958m; CHN analysis for $C_{33}H_{66}N_2O_8$ (found/calculated): 63.58/64.04, 10.76/10.75, 4.58/4.53.

4.2.5.3. 5-Dodecyloxycarbonyloxypentylammonium-5-dodecyloxycarbonyloxypentylcarbamate (3c). Mp = 56–59 °C, yield 43%; IR (nujol): υ_{max} 3383m ($\upsilon_{(N–H)}$), 2177wbr, 1742s ($\upsilon_{(OC=OO)}$), 1653w, 1592m, 1533m ($\delta s_{NH_3^+}$), 1272s ($\upsilon_{(C–O,\ C–N)}$), 1258w, 1168w, 953m; ^1H NMR (300 MHz, $CDCl_3$+triethylamine (TEA)): δ 10.57 (s; 3H; NH_3^+), 4.60 (s; 1H; NH), 4.04 (t; 8H; J = 6.7 Hz; CH_2COCH_2), 2.99–3.11 (m; 2H; $CH_2NH_3^+$), 2.58–2.71 (t; 2H; J = 7.0 Hz; CH_2NH), 1.10–1.67 (m; 52H; CH_2), 0.81 (t; 6H; J = 6.6 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$+TEA): δ 162.0, 155.3, 67.9, 67.7, 41.1, 31.8, 30.1, 29.5, 29.4, 29.4, 29.2, 29.1, 28.5, 28.3, 25.6, 23.0, 22.5, 14.0 ppm; CHN analysis for $C_{37}H_{74}N_2O_8$ (found/calculated): 66.24/65.84, 11.22/11.05, 4.15/4.15.

4.2.5.4. 6-Octyloxycarbonyloxyhexylammonium-6-octyloxycarbonyloxyhexylcarbamate (3d). Mp = 40–47 °C, yield 49%; IR (nujol): υ_{max} 3326m ($\upsilon_{(N-H)}$), 2106wbr, 1748s ($\upsilon_{(OC=OO)}$), 1683w, 1630w, 1556m, 1260s ($\upsilon_{(C-O,C-N)}$), 950m; CHN analysis for $C_{31}H_{62}N_2O_8$ (found/calculated): 62.65/63.02, 10.68/10.58, 4.89/4.74.

4.2.5.5. 6-Decyloxycarbonyloxyhexylammonium-6-decyloxycarbonyloxyhexylcarbamate (3e). Mp = 52–55 °C, yield 55%; IR (nujol): υ_{max} 3239m ($\upsilon_{(N-H)}$), 2192wbr, 1748s ($\upsilon_{(OC=OO)}$), 1650w, 1631w, 1579m, 1268s ($\upsilon_{(C-O,C-N)}$); CHN analysis for $C_{35}H_{70}N_2O_8$ (found/calculated): 64.42/64.98, 10.96/10.91, 4.35/4.33.

4.2.5.6. 6-Dodecyloxycarbonyloxyhexylammonium-6-dodecyloxycarbonyloxyhexylcarbamate (3f). Mp = 54–57 °C, yield 54%; IR (nujol): υ_{max} 3239m ($\upsilon_{(N-H)}$), 2192wbr, 1748s ($\upsilon_{(OC=OO)}$), 1649w, 1630w, 1577m, 1277s ($\upsilon_{(C-O,C-N)}$); ^{1}H NMR (300 MHz, $CDCl_3$+pyridine (Py)): δ6.91 (s; 3H; NH_3^+), 4.64 (s; 1H; NH), 4.05 (t; 8H; J = 6.5 Hz; CH_2COCH_2), 3.00 (t; 2H; J = 6.7 Hz; $CH_2NH_3^+$), 2.71 (t; 2H; J = 7.0 Hz; CH_2NH), 1.14–1.67 (m; 56H; CH_2), 0.82 (t; 6H; J = 6.5 Hz; CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, Py): δ 162.2, 155.2, 67.9, 67.5, 41.3, 40.0, 31.8, 30.2, 29.5, 29.5, 29.4, 29.4, 29.2, 29.1, 28.5, 28.4, 26.5, 26.2, 25.6, 25.4, 25.2, 22.5, 14.0 ppm; CHN analysis for $C_{39}H_{78}N_2O_8$ (found/calculated): 66.13/66.63, 11.48/11.18, 3.88/3.98.

4.2.5.7. 4-Octylcarbamoyloxybutylammonium-4-octylcarbamoyloxybutylcarbamate (5a). Mp = 83–86 °C, yield 72%; IR (nujol): υ_{max} 3366m ($\upsilon_{(N-H)}$), 3337s ($\upsilon_{(N-H)}$), 3272wbr, 2183wbr, 1682s ($\upsilon_{(C=O)}$), 1622w, 1584m, 1563m, 1538m, 1523m ($\delta_{(NH)}$), 1276m, 1249m, 1150m; CHN analysis for $C_{27}H_{56}N_4O_6$ (found/calculated): 60.80/60.87, 10.68/10.59, 11.01/10.52.

4.2.5.8. 4-Nonylcarbamoyloxybutylammonium-4-nonylcarbamoyloxybutylcarbamate (5b). Mp = 80–83 °C, yield 80%; IR (nujol): υ_{max} 3365m ($\upsilon_{(N-H)}$), 3336s ($\upsilon_{(N-H)}$), 3270wbr, 2180wbr, 1683s ($\upsilon_{(C=O)}$), 1657w, 1625w, 1585m, 1563m, 1539m, 1522m ($\delta_{(NH)}$),1270m, 1244m, 1149m; CHN analysis for $C_{29}H_{60}N_4O_6$ (found/calculated): 61.67/62.11, 11.09/10.78, 10.40/9.99.

4.2.5.9. 4-Decylcarbamoyloxybutylammonium-4-decylcarbamoyloxybutylcarbamate (5c). Mp = 83–86 °C, yield 78%; IR (nujol): υ_{max} 3369m ($\upsilon_{(N-H)}$), 3341s ($\upsilon_{(N-H)}$), 3274wbr, 2184wbr, 1683s ($\upsilon_{(C=O)}$), 1656w, 1625w, 1584m, 1563m, 1538m, 1522m ($\delta_{(NH)}$), 1257m, 1238m, 1149m; CHN analysis for $C_{31}H_{64}N_4O_6$ (found/calculated): 63.03/63.23, 11.28/10.95, 9.95/9.51.

4.2.5.10. 4-Undecylcarbamoyloxybutylammonium-4-undecylcarbamoyloxybutylcarbamate (5d). Mp = 73–76 °C, yield 65%; IR (nujol): υ_{max} 3367m ($\upsilon_{(N-H)}$), 3338s ($\upsilon_{(N-H)}$), 3274wbr, 2182wbr, 1684s ($\upsilon_{(C=O)}$), 1654, 1623w, 1582m, 1538m, 1521m ($\delta_{(NH)}$), 1254m, 1233w, 1148m; CHN analysis for $C_{33}H_{68}N_4O_6$ (found/calculated): 63.79/64.25, 11.22/11.11, 9.47/9.08.

4.2.5.11. 5-Decylcarbamoyloxypentylammonium-5-decylcarbamoyloxypentylcarbamate (5e). Mp = 77–79 °C, yield 89%; IR (nujol): υ_{max} 3362s ($\upsilon_{(N-H)}$), 3350s ($\upsilon_{(N-H)}$), 1687s ($\upsilon_{as(C=O)}$), 1641m ($\upsilon_{(NHC=OO^-)}$), 1574w, 1530s, 1493m, 1258s, 1144m; CHN analysis for $C_{33}H_{68}N_4O_6$ (found/calculated): 64.37/64.25, 11.35/11.11, 9.25/9.08.

4.2.5.12. 5-Undecylcarbamoyloxypentylammonium-5-undecylcarbamoyloxypentylcarbamate (5f). Mp = 77–80 °C, yield 85%; IR (nujol): υ_{max} 3355ws ($\upsilon_{(N-H)}$), 1686s ($\upsilon_{as(C=O)}$), 1650w ($\upsilon_{(NHC=OO^-)}$), 1617m, 1537s, 1496m, 1257m, 1143m; CHN analysis for $C_{35}H_{72}N_4O_6$ (found/calculated): 65.14/65.18, 11.43/11.25, 8.73/8.69.

4.2.5.13. 5-Dodecylcarbamoyloxypentylammonium-5-dodecylcarbamoyloxypentylcarbamate (5g). Mp = 76–80 °C, yield 90%; IR (nujol): υ_{max} 3364s ($\upsilon_{(N-H)}$), 3353s ($\upsilon_{(N-H)}$), 1687s ($\upsilon_{as(C=O)}$), 1652m ($\upsilon_{(NHC=OO^-)}$), 1617m, 1576w, 1532s, 1493m, 1249m, 1143m; CHN analysis for $C_{37}H_{76}N_4O_6$ (found/calculated): 66.20/66.03, 11.77/11.38, 8.50/8.32.

4.2.5.14. 6-Decylcarbamoyloxyhexylammonium-6-decylcarbamoyloxyhexylcarbamate (5h). Mp = 86–87 °C, yield 83%; IR (nujol): υ_{max} 3331s ($\upsilon_{(N-H)}$), 3246w ($\upsilon_{(N-H)}$), 2210wbr, 1684s ($\upsilon_{as(C=O)}$), 1655w ($\upsilon_{(NHC=OO^-)}$), 1629w, 1578m, 1537s, 1529s, 1149m; CHN analysis for $C_{35}H_{72}N_4O_6$ (found/calculated): 65.76/65.18, 11.32/11.25, 8.58/8.69.

4.2.5.15. 6-Undecylcarbamoyloxyhexylammonium-6-undecylcarbamoyloxyhexylcarbamate (5i). Mp = 82 °C, yield 84%; IR (nujol): υ_{max} 3329s ($\upsilon_{(N-H)}$), 3245m ($\upsilon_{(N-H)}$), 1684s ($\upsilon_{as(C=O)}$), 1655m ($\upsilon_{(NHC=OO^-)}$), 1629w, 1578m, 1538s, 1255s, 1149m; CHN analysis for $C_{37}H_{76}N_4O_6$ (found/calculated): 65.47/66.03, 11.45/11.38, 8.28/8.32.

4.2.5.16. 6-Dodecylcarbamoyloxyhexylammonium-6-dodecylcarbamoyloxyhexylcarbamate (5j). Mp = 74–76 °C, yield 87%; IR (nujol): υ_{max} 3332s ($\upsilon_{(N-H)}$), 3246s ($\upsilon_{(N-H)}$), 1684s ($\upsilon_{as(C=O)}$), 1655w ($\upsilon_{(NHC=OO^-)}$), 1615w, 1577m, 1538s, 1251s, 1149m; CHN analysis for $C_{39}H_{80}N_4O_6$ (found/calculated): 66.35/66.81; 11.59/11.50; 7.86/7.99.

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 min 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. Donor samples

Donor samples were prepared by suspending theophylline (5%) and/or the tested enhancer (1%) in water (W), propylene glycol/water 3:2 (Pg/W) or isopropyl myristate (IPM). The suspensions were stirred for

5 min at 50 °C and then were left at 37 °C for 24 h to equilibrate. Before application to the skin, the samples were carefully resuspended.

Supernatants from the Pg/W donor samples, prepared as described above, were filtered through 0.22 μm filter. The filtrate was appropriately diluted with the mobile phase and analyzed for theophylline solubility by HPLC. Each sample was analyzed four times.

Solubility of the enhancers in the donor sample was determined by a potentiometric titration. The pertinent enhancer (1%) was dispersed in Pg/W (3:2) and treated like the donor samples for the permeation experiment. After equilibration, the supernatants from the samples were filtered through 0.22 μm filter. Six microliters of each filtrate was diluted with 10 mL of water, acidified to pH 3 by 0.1 M HCl, and titrated with 0.1 M NaOH to pH 10. Enhancer concentration was calculated from the consumption of NaOH between two inflections. For each enhancer, three samples were separately prepared and analyzed.

4.5. Permeation experiments

The skin permeability was evaluated in vitro using the Franz diffusion cells and theophylline as a model permeant. Skin samples ca 2×2 cm (obtained from the same porcine ear for one experiment) were mounted into the Franz diffusion cells to leave an area of 1 cm^2. The acceptor compartments of the cells were filled with ca 17 mL of phosphate-buffered saline, pH 7.4, with 0.03% sodium azide as a preservative and allowed to equilibrate for 30 min at 32 °C. The precise volume of the acceptor phase in each cell was measured and included into calculation. Then 200 μL of a donor sample was applied and occluded with a cover glass. The acceptor phase was stirred and tempered at 32 °C throughout the experiment. Samples of the acceptor phase (0.6 mL, replaced with fresh acceptor phase) were withdrawn at seven predetermined intervals during 48 h.

4.6. HPLC determination

Theophylline in both donor and acceptor phase samples was determined by HPLC using the LCP high-pressure pump (ECOM, Prague, Czech Republic), autosampler (ECOM, Prague, Czech Republic), LiChroCART 250-4 column (LiChrospher 100, RP 18. 5 μm, Merck, Darmstadt, Germany), an SP 8440 UV detector (Spectra Physics), and CSW 1.7 integrating software, Methanol/0.1 M NaH_2PO_4 6:4 v/v was used as the 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.

4.7. Data analysis

The cumulative amount of theophylline having penetrated the skin, corrected for the acceptor sample replacement, was plotted against time. The steady-state flux (μg/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.

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