Sunfish Amphiphiles: Conceptually New Carriers for DNA Delivery

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A conceptually new class of cationic amphiphiles, Sunfish amphiphiles, designed for the delivery of genes into cells is introduced. Sunfish amphiphiles have two hydrophobic tails, connected at the 4- and the *N*-position to the cationic pyridinium headgroup. Two extreme morphologies visualised by backfolding and combining of both tails at one site (matching situation) or unfolding of the tails at distinct interaction sites at biological membranes will lead to considerable differences in morphological behaviour. The underlying rationale allows controlled release by using this morphological alteration of the Sunfish/helper-lipid/DNA complex (lipoplex). The often-excellent transfection efficiencies are probably related to these morphological changes. In addition, the Sunfish amphiphiles possess low toxicities, resulting in high cell survival after internalisation. The underlying rationale, design, synthesis and in vitro transfection potential are discussed in detail. Moreover, some physico-chemical characteristics of the Sunfish amphiphiles have been studied.

Introduction

The recent conclusion of the human genome-sequencing project offers unprecedented knowledge with regard to the genetic bias of human life. In addition, the genetic codes of a vast group of microorganisms have been determined or will be elucidated shortly. This "genetic abundance" offers a clear-cut opportunity to study, identify and understand the genetic basis of several classes of disease. Moreover, realisation of gene therapy as an integral part of disease curing strategies in the (near) future appears a realistic target, although it is still far from a general clinical reality at this moment.[1] Despite the large hurdles en route, gene therapy has established itself in a promising position as a principal method in the treatment of disorders with a genetic bias (e.g. cystic fibrosis) or in the development of therapeutic strategies for a wide range of diseases such as cancer, infectious (e.g. AIDS, hepatitis) and acquired diseases like Parkinson's disease, rheumatic arthritis and others.[2,3]

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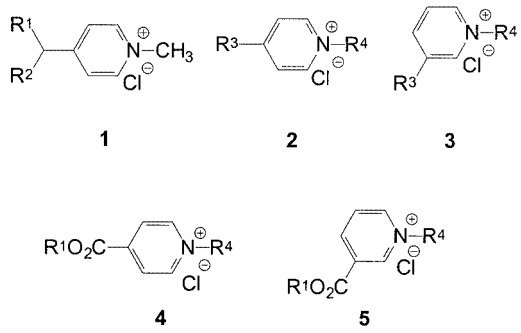
The concept of gene therapy is pleasantly straightforward and simple, and involves the introduction of engineered or foreign genetic material into target cells or organs in order to induce protein expression. Various introduction/delivery methods have been developed, mostly based upon either viral or non-viral carrier systems (vectors). At present, the most efficient methods for the transfer of genetic material (transfection) involve the use of viral vectors[4,5] (e.g. retrovirus, adenovirus), although there are strong arguments concerning the risks with regard to immunogenicity and propagation.[6,7] Synthetic cationic amphiphiles (cytofectins), mostly combined with neutral helper lipids such as dioleoylphosphatidylethanolamine (DOPE), partially circumvent these problems and have proved to be efficient non-viral carrier systems for both in vitro and in vivo delivery of DNA,[8−12] although significant improvements in transfection efficiency and toxicity are necessary.

Previously we reported on both the synthesis and the transfection potential of a class of cationic pyridinium-based amphiphiles, *S*ynthetic *A*mphiphiles *Int*erdisciplinary, SAINTs, showing good transfection ability as well as a low overall toxicity towards several eukaryotic cell types[13−15] (Scheme 1).

The synthetic protocol allows the easy introduction of structural modifications of the "basic" SAINT skeleton **1**, yielding a large variety of novel amphiphiles with altered characteristics in terms of structure-induced morphological behaviour as well as overall transfection potential and toxicity.



$R^1/R^2/R^4$: $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$
R^3: $C_{5:0}$, $C_{7:0}$, $C_{9:0}$, $C_{11:0}$, $C_{13:0}$, $C_{15:0}$, $C_{15:1}$, $C_{17:0}$, $C_{17:1}$, $C_{19:0}$, $C_{19:1}$

Scheme 1. SAINTs **1** and several Sunfish amphiphiles **2**–**5**; all present generalized structures and possible R modifications

In order to meet the criterion for efficient delivery capability, the cationic amphiphile system condenses the DNA by displacement of the positively charged counterions, provided that the proportions of each of these compounds is optimised. Besides binding to, and subsequent condensing of, the DNA, the cationic lipid is also facilitating the interaction of the complex with the negatively charged plasma membrane of the cell.[16] The neutral co-lipid is thought to act as a membrane-destabilising agent, facilitating the penetration of the DNA into the cytoplasm.[17] Crucial steps in the trafficking pathway towards the interior of the cell or nucleus include the crossing of the cell membrane by endocytosis and subsequent endosomal escape of the DNA material into the cytoplasm. Influencing the ability to interact with the cell membrane accompanied by increased ability for passage either by means of pore formation or endocytosis would positively influence this part of the sequence, whereas release from the endosomal compartment might also be facilitated by interaction with, and disruption of, the endosomal membrane.

A method of improving the DNA-delivery capabilities of synthetic, bilayer-forming amphiphiles is to induce a morphological change in the lipid/DNA complex (lipoplex) from a relatively stable bilayer vesicular phase (lamellar) into a more aggressive inverted hexagonal micellar phase (H_{II}) that is believed to facilitate the interaction with the cell membrane and/or enable escape from the endosome. The morphological changes are preferably induced only in the proximity of, or after contact with, the cell membrane, or at the endosomal level. Kinnunen and co-workers have outlined the concept in detail as a key step in membrane fusion and hemi-fusion processes, although not in a transfection context.[18,19]

This rationale can also be applied to the design of cytofectins, the so-called Sunfish amphiphiles (Scheme 1, **2**–**5**). The Sunfish concept exploits this by attaching a flexible (and functionalised) antenna to the cationic headgroup of the amphiphilic carrier rather than a methyl moiety as in the SAINT materials (Scheme 1). Such antennae are expected to participate in molecular recognition processes at the cell surface and may facilitate the transport of DNA through the cell membrane by (inducing) processes like endocytosis or poration. Moreover, if the two different tails combine at the same interface (**A**, Figure 1), bilayer vesicles (lamellar) are formed (DNA-condensing and -transporting morphology), whereas un-matching (or, more tentatively, unfolding) leads to the formation of more aggressive hexagonal micellar (H_{II}) structures (DNA-expelling morphology, **B**, Figure 1). We envisage this concept as "backfolding-induced vesicle formation" and "unfolding-induced micelle formation", provided that the two alkyl chains are both of an appropriate length (vide infra), the match-mismatch rational.

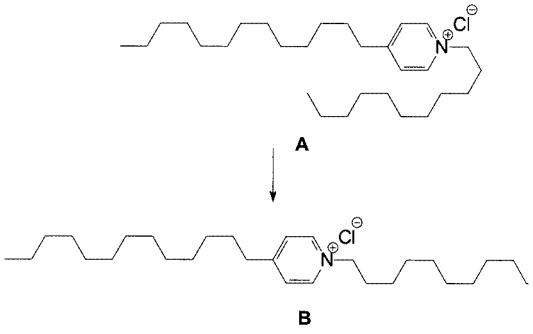


Figure 1. The Sunfish concept; backfolding of the *N*-alkyl chain leads to a lamellar (vesicular) morphology (**A**) whereas unfolding leads to an inverted hexagonal (H_{II}) phase as outlined in **B** (see text for explanation)

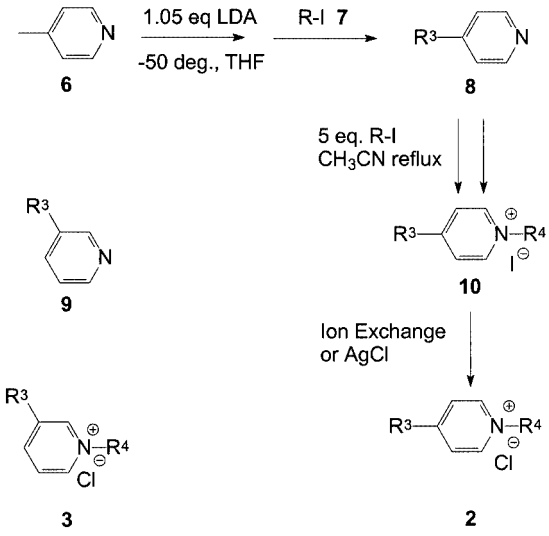
In this paper, the synthesis, characterisation and some of the physiochemical properties relevant for influencing the DNA-delivering efficiency are reported.

Results and Discussion

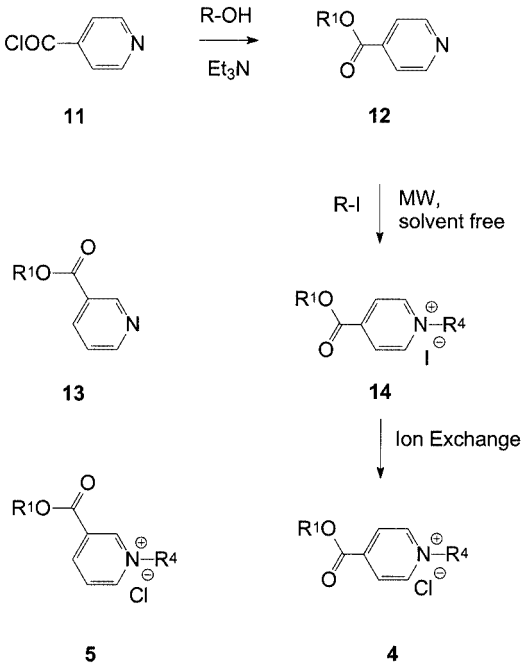
Synthesis

In analogy with the synthetic protocol used for the preparation of SAINT materials,[15,20] 4-picoline **6** was treated with one equivalent of LDA in THF at −40 °C, followed by quenching with the appropriate alkyl iodides **7** affording the mono-tailed pyridine lipids **8** in excellent yields (Scheme 2).[21] The very unpleasantly smelling products **8** were obtained analytically pure as viscous oils or low melting solids after purification by means of column chromatography (Al_2O_3, hexane/diethyl ether/chloroform gradients). This procedure prevents the formation of bis-alkylated materials, leading to separation and purification problems. Subsequent alkylation at the ring nitrogen using a large excess of alkyl iodide in polar solvents such as acetonitrile or ethanol, in analogy to the procedure used for SAINT materials, afforded the desired *N*-alkylated materials **10**, although only in low yields. Reaction time extension (up to three weeks) increased the yields to approximately 40–50 %, although the prolonged reaction periods at elev-

ated temperatures also led to significant side-product formation and degradation of the products (vide infra).



Scheme 2. Synthesis of Sunfish amphiphiles **2** and **3**; *N*-alkylation with R-I is performed using two different routes as outlined (see text for explanation)



Scheme 3. Synthesis of Sunfish ester amphiphiles **4** and **5**; *N*-alkylation with R-I is performed using microwave radiation

The rate of alkylation at the ring nitrogen can be significantly enhanced by using microwave radiation. Alkylation reactions carried out under solvent-free conditions could be taken to 90 % conversion in approximately 45 minutes, although power-supply conditions as well as the (pulsed) delivery intervals had to be chosen with care as side and decomposition reactions are also accelerated.[22]

Purification of **10** (chromatography over Al_2O_3 hexane/chloroform/methanol gradients) yielded analytically pure materials that were only moderately stable, as iodide tends to attack the pyridine ring at a significant rate. Therefore, ion exchange from iodide **10** to chloride **2** was performed using Dowex-50 or Sephadex ion-exchange columns and methanol as eluent. Replacement of the iodide anion was also possible by treatment with AgCl in refluxing acetone, although traces of silver proved difficult to remove, probably due to complex formation with the double bonds. The *meta*-substituted amphiphiles **3** were prepared following the same synthetic protocols starting from 3-picoline (not shown) via the 3-alkyl-pyridines **9**.[23]

Several (bio)-degradable amphiphiles were also prepared, based upon the hydrolytically sensitive ester analogues.[24] Esterification of isonicotionyl chloride hydrochloride **11** with long-chained alcohols afforded the corresponding pyridine esters **12**, which were purified by column chromatography (Al_2O_3, gradients of hexane/chloroform and traces of methanol, Scheme 3).[25,26]

As expected, introduction of the *N*-terminated lipid tail leading to pyridinium salts **14** (iodides) proved impossible by means of traditional synthetic methods due to the strongly decreased nucleophilicity of the ring nitrogen (electron-withdrawing inductive effect of the ester functionality) compared to the non-ester amphiphiles. Alkylation using microwave radiation, however, proceeded smoothly provided that solvent-free conditions were applied. Monitoring the progress by means of ^{1}H NMR spectroscopy on small aliquots allowed continuation until exact completion of the alkylation reaction.

Subsequent ion exchange of the iodide for chloride afforded the desired products **4** in high overall yields. The *meta*-substituted counterparts **5** were obtained starting from nicotinoyl chloride hydrochloride (not shown) via the *meta*-substituted esters **13** following the same procedure,[27] although purification appeared less easy to perform and sometimes led to significant decomposition.

In order to gain insights into the underlying concept of backfolding-induced morphological changes and to study these influences on the transfection activity, the *para*-substituted Sunfish array has been set-up with two sub-series A and B. In series A, the R^3 tail is kept at constant length ($C_{13:0}$) whereas in series B R^4 is kept constant at $C_{18:1}$ (Table 1).

A much smaller array of *meta*-substituted amphiphiles **3** was prepared (Table 2), whereas the ester-functionalised amphiphiles **4** and **5** are summarised in Table 3 and Table 4, respectively.

Some Characteristics of the Sunfish Amphiphilic Aggregates

Backfolding of the R^3 or R^4 Tails

In order to establish a working hypothesis for the appropriate (minimal) lengths of the R^3 and R^4 tails to reach a backfolded or ultimately a matched situation of the two tails, NMR studies were performed using three model compounds **15**–**17** (Scheme 4).[28]

The use of model compounds appeared necessary, as "normal" Sunfish amphiphiles of either series did not allow

Table 1. Match and mismatch *para*-substituted Sunfish **2** series A and B; in series A the R^3 tail is kept constant ($C_{13:0}$), whereas in series B R^4 is kept at a constant length ($C_{18:1}$)

M&M *para* series A			M&M *para* series B		
R^3	$C_{13:0}$		R^3	CH_3	**2i**
R^4	$C_{6:0}$	**2a**		$C_{3:0}$	**2j**
	$C_{8:0}$	**2b**		$C_{5:0}$	**2k**
	$C_{10:0}$	**2c**		$C_{7:0}$	**2l**
	$C_{12:0}$	**2d**		$C_{9:0}$	**2m**
	$C_{14:0}$	**2e**		$C_{11:0}$	**2n**
	$C_{16:0}$	**2f**		$C_{15:0}$	**2o**
	$C_{18:0}$	**2g**		$C_{17:0}$	**2p**
	$C_{18:1}$	**2h**		$C_{19:0}$	**2q**
				$C_{19:1}$	**2r**
			R^4	$C_{18:1}$	

Table 2. Match and mismatch *meta*-substituted Sunfish **3**

Sunfish	R^3	R^4
3a	$C_{13:0}$	$C_{18:1}$
3b	$C_{15:0}$	$C_{18:1}$
3c	$C_{17:0}$	$C_{18:1}$
3d	$C_{19:0}$	$C_{18:1}$
3e	$C_{19:1}$	$C_{18:1}$
3f	$C_{13:0}$	$C_{16:0}$
3g	$C_{13:0}$	$C_{16:1}$

Table 3. Match and mismatch *para*-substituted ester Sunfish **4**

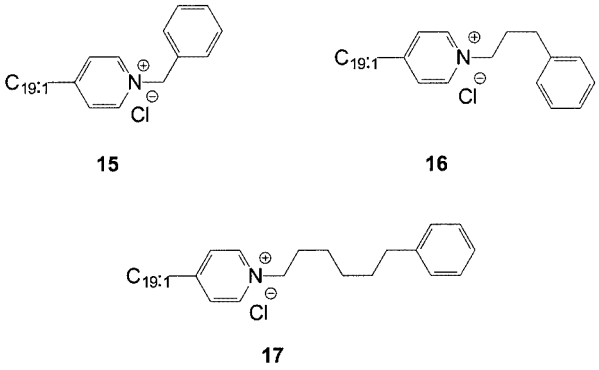
Sunfish	R^1	R^4
4a	$C_{12:0}$	$C_{12:0}$
4b	$C_{12:0}$	$C_{18:0}$
4c	$C_{12:0}$	$C_{18:1}$
4d	$C_{14:0}$	$C_{12:0}$
4e	$C_{14:0}$	$C_{18:0}$
4f	$C_{14:0}$	$C_{18:1}$
4g	$C_{16:0}$	$C_{12:0}$
4h	$C_{16:0}$	$C_{18:0}$
4i	$C_{16:0}$	$C_{18:1}$
4j	$C_{18:1}$	$C_{18:1}$

Table 4. Match and mismatch *meta*-substituted ester Sunfish **5**

Sunfish	R^1	R^4
5a	$C_{12:0}$	$C_{18:0}$
5b	$C_{12:0}$	$C_{18:1}$
5c	$C_{14:0}$	$C_{18:0}$
5d	$C_{14:0}$	$C_{18:1}$
5e	$C_{16:0}$	$C_{18:0}$
5f	$C_{16:0}$	$C_{18:1}$

unambiguous assignment of the resonances to either of the chains R^3 or R^4 and, hence, no statements were justified with regard to the backfolding processes monitored.

Backfolding of the *N*-terminated alkyl tails and subsequent π-stacking of the phenyl and pyridine moieties occurred for chains containing six carbons (**17**) or more in the linker unit, as established by means of chemical shift anisotropy studies (shielding effects; see Figure 2). Also NOE-difference and ROESY NMR spectroscopic techniques clearly indicated dipolar contacts between the aromatic phenyl protons and the pyridine moiety solely for C_6 or longer linker entities. Moreover, the experiments also revealed the highly dynamic character of the backfolding process, as suggested by significant NOE-exchange (EXSY, not shown). This dynamic behaviour suggests that the backfolding process can likely be influenced by small effects, such as counterions, temperature, solvents or combinations thereof, as well as by the presence of co-lipids.[29]



Scheme 4. Model compounds **15**−**17** used for preliminary study of backfolding ability as a function of chain length

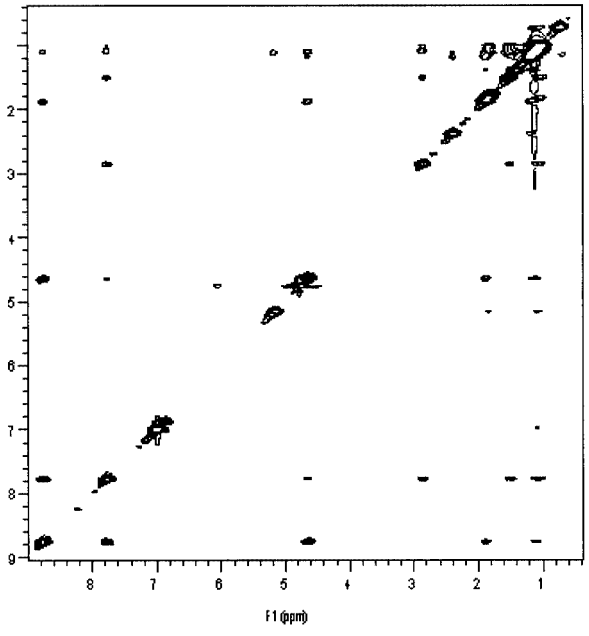


Figure 2. 2D NOE spectrum of **17** in D_2O; clearly visible dipolar contacts between the pyridine protons and the linker unit suggests significant backfolding of the C_6-phenyl moiety

Comprehensive NMR studies are currently being performed with the aim to gain a detailed insight into the mor-

phology and dynamics of these types of amphiphiles and lipoplexes thereof.[30]

Sunfish/DNA Binding

Cationic lipids are designed to bind, condense, protect and deliver DNA into the cell's interior. In order to establish the ability of Sunfish amphiphiles to bind DNA plasmid material, the retardation on 1 % agarose gel after complexation with Sunfish amphiphiles alone or in equimolar mixtures with DOPE has been determined. In this assay, free or partially bound DNA migrates into the gel and is visible as a fluorescent-labelled band. No band is observed for the completely bound DNA since the DNA polymer is not accessible for ethidium bromide. On the basis of the DNA binding results, Sunfish amphiphiles could be divided into three groups: a) Sunfish amphiphiles which bind DNA efficiently without DOPE (e.g. **2h**, **2k**, **2r**), b) Sunfish amphiphiles which need a helper lipid such as DOPE to bind DNA completely (e.g. **2d**, **2f**, **2q**), and c) amphiphiles like **2g**, which are not able to bind DNA effectively even in the presence of DOPE. These differences between the classes of Sunfish amphiphiles can be explained in terms of the fluidity of the bilayer formed upon vesicle formation. Cationic lipids that are in a fluidic state during the electrostatic lipid-DNA interaction on one hand and lipids that form more stiff bilayers on the other are the two extreme situations, with all kinds of intermediate morphologies in between. The presence of an unsaturated bond in the alkyl chains causes extensive disorder, and the more fluidic behaviour observed for these amphiphiles could explain the ability of these Sunfish amphiphiles to bind DNA even without DOPE as a helper lipid (**2h**, **2k** and **2r**). The second group of Sunfish amphiphiles (**2d**, **2f**, **2q**) does not have this ability because their long and/or saturated alkyl chains tend to form a more rigid bilayer morphology. These Sunfish amphiphiles need the assistance of DOPE, which functions more or less as a fluidiser. The extreme case, **2g**, which bears the longest alkyl chain ($C_{18:0}$) and clearly leads to the formation of the most rigid morphological entity, needs both the helper lipid DOPE and higher temperatures (about 60 °C, data not shown) to obtain complete DNA binding. This improvement of DNA binding by the application of both conditions was also reflected in higher transfection efficiencies (vide infra).

Sunfish/DNA Aggregation Properties

For adequate DNA binding, Sunfish amphiphiles capable of forming vesicles are preferable.[31] Therefore, the aggregation properties of Sunfish amphiphiles in equimolar mixtures with DOPE (in vitro transfection conditions) were studied using particle sizing in order to determine the sizes of the aggregates. The results for a selected group of Sunfish amphiphiles from series A and B are summarised in Table 5.

Table 5. Aggregation properties of Sunfish amphiphiles from series A and B mixed with DOPE (1:1) at room temperature (see text for discussion)

Sunfish	Size of the aggregates in nm	
	First population	*Second population*
Series A		
2a	100 ± 2	–
2d	130 ± 4	1900 ± 150
2f	210 ± 30	3000 ± 565
2g	170 ± 13	1400 ± 220
2g (60 °C)	80–190 ± 42	–
2h	140 ± 4	–
Series B		
2k	120–240 ± 72	–
2n	220–320 ± 53	–
2p	330 ± 40	–
2q	460 ± 25	4000 ± 660
2r	160 ± 5	–

For Sunfish amphiphiles of series **A** with only saturated alkyl chains, hydration and sonication of the Sunfish/DOPE (1:1) film for **2a**, with the shortest R^4 alkyl chain C_6, resulted in the formation of a homogeneous, single population of small particles (likely vesicles) with a size of about 100 nm. Elongation of R^4 to C_{12}, C_{16} or C_{18} resulted in incomplete vesicle formation, characterised by the presence of larger aggregates in the micrometer range besides a population of vesicles with sizes ranging from 130 nm to 210 nm. These findings can be rationalised by the lower mobility of the longer R^4 alkyl chains, resulting in a lower ability of the R^4 chains to fold back and form vesicles. Warming up of **2g**, for example, resulted in the formation of a single population of small vesicles (80–190 nm). The introduction of an unsaturated bond into the R^3 alkyl chain or both alkyl chains (series B) apparently ensured the fluidity as well as enhanced mobility of the R^4 alkyl chain, since all the Sunfish amphiphiles tested so far formed single populations of vesicles with sizes ranging from 120 to 330 nm. The only exception was **2q**, with the longest saturated R^3 alkyl chain, which contained a small population of aggregates in the micrometer range after sonication.

On the basis of these results it is concluded that complete vesicle formation was only ensured when the Sunfish amphiphiles contain short R^3 and R^4 alkyl chains (like e.g. **2a**) or at least one double bond in the R^4 alkyl chain combined with R^3 saturated alkyl chains shorter than C_{19}.

Aggregation Properties of Sunfish/DOPE/DNA Complexes

The electrostatic and possibly hydrophobic interactions between the cationic lipid and DNA are accompanied by the disintegration of vesicular structures on the one hand and the condensation of DNA on the other. As a consequence, larger lipoplexes are formed, suggesting the aggregation and possibly fusion of lipid membranes in the presence of DNA. Aggregation of Sunfish/DOPE/DNA aggregates, resulting in increased average particle sizes, can readily be monitored by particle-sizing techniques. The data for

selected Sunfish amphiphiles as obtained 10 min and 4 hours (duration of in vitro transfection) after the complexation with DNA are summarised in Table 6.

Table 6. Size of the Sunfish/DOPE/DNA complexes prepared in HBS buffer and diluted in culture medium at room temperature.

Sunfish	Size of aggregates [nm] with the highest frequency of appearance	
	10 minutes	*4 hours*
Series A		
2a	450 ± 75	1340 ± 200
2d	1200 ± 94	–
2f	950 ± 135	1700 ± 53
2g	1400 ± 150	1700 ± 62
2g (60 °C)	400 ± 80	1000 ± 100
2h	1250 ± 40	–
Series B		
2k	400 ± 80	950 ± 80
2n	600 ± 55	1250 ± 180
2q	1250 ± 35	1500 ± 220
2r	600 ± 85	1100 ± 25

Addition of plasmid DNA to a Sunfish/DOPE (1:1) mixture under the conditions used for the in vitro transfection studies generated large aggregates which grew further and reached sizes of above one micrometer within 10 minutes. Generally, if the starting lipid generated single populations of relatively small vesicles (e.g. **2k**, **2g** at 60 °C, **2a**), the aggregates formed after 10 min were smaller than those for Sunfish amphiphiles, which leads to incomplete vesicle formation (**2d**, **2f**, **2q**). However, these lipoplexes grew to particles of micrometer size after 4 h, stressing the dynamic character of the lipoplex formation.

Although for in vitro as well as ex vivo applications aggregation often correlates with transfection activity, the presence of aggregates is not compatible with the in vivo applications and is, moreover, only one of the factors determining the transfection. For optimised results, further optimisation of the lipid formulation is therefore desired.

Manipulation of the Morphology by Physical Methods

As mentioned above, temperature is an important parameter in the manipulation of Sunfish amphiphile morphology. As an example of this effect, **2g** can be mentioned, for which sonication at 60 °C resulted in an improvement of the vesicle formation and, consequently, also DNA binding (vide supra). Both improvements are also reflected in an improvement of the transfection efficiency (100 % increase for COS-7 and 35 % for HepG2).

Another factor that affects the aggregation properties and transfection efficiency of cationic lipids is serum. For in vivo applications, the effect of serum on the transfection efficiency must be either low or positive. From this point of view, the important observation has been made that most of the well-performing Sunfish amphiphiles are serum resistant, even in the presence of high serum concentrations of up to 50 %. Still more importantly, the interesting observation was made that the Sunfish amphiphiles with a lower transfection activity become more active in the presence of serum. For example, **2g** reached a threefold higher activity in the gene delivery into HepG2 cells in the presence of 50 % serum (up to levels several times that of Lipofectamine). A fundamental study has been set up in order to identify the rational behind this effect. Measurements are currently being performed in order to establish which of the characteristics of Sunfish/DNA complexes (the lipoplex structure, zeta potential or the size of lipoplex particles) correlates best with its activity in the presence of serum.

Transfection Experiments

In order to assess the ability of the prepared amphiphiles to deliver plasmids, transfection studies were undertaken on two eukaryotic cell-lines with strongly different transfection sensitivity and using three types of helper lipids.

Generally, the nature of the helper lipid determined the efficiency of Sunfish-mediated delivery of Green Fluorescent Plasmid (GFP) into COS-7 and HepG2 cells to a significant extent. Three different helper-lipids were applied: DOPE, DOPC and cholesterol. As anticipated, the results obtained using the COS-7 cell line showed a markedly different trend than the results obtained on HepG2 cells.

Using the COS-7 cell line, results of the series A- and B-mediated transfection can be generalized as follows:

The use of DOPE or cholesterol as helper lipid resulted in generally somewhat lower transfection efficiencies than DOPC-mediated transfection.

The presence of an unsaturated moiety in the alkyl chain as well as an increased length of the alkyl chain had a favourable effect on the transfection activity of the Sunfish amphiphiles. The most efficient were those Sunfish amphiphiles with both alkyl chains unsaturated, **2r** being the most efficient.

Amphiphiles containing one longer unsaturated alkyl chain in combination with saturated, shorter alkyl chains like **2f**, **2m** and **2n** were, though less effective than **2r**, the most effective in transfection. The efficiency, however, turned out to be cell-line- as well as helper-lipid-dependent.

Using the HepG2 cell line, the transfection results reflected:

Cholesterol as helper lipid resulted in the best transfection results. Sunfish amphiphiles with one or two unsaturated alkyl tail(s) gave higher transfection efficiencies.

Series A and B Amphiphiles 2 on COS-7

Elongation of the R^4 tail lengths in series A from C_6 to C_{16} (all saturated, R^3 tail is kept constant as $C_{13:0}$) leads to an increase in the transfection efficiency on the COS-7 cell line provided that DOPE or cholesterol is used as the helper lipid. Surprisingly, the maximum in the DOPE series is reached with **2f** (C_{16}), whereas upon application of cholesterol as helper lipid the maximal transfection efficiency is obtained with a somewhat shorter R^4 tail length (C_{14}; **2e**). Application of DOPE for **2g** (C_{18}) and **2h** ($C_{18:1}$) shows a backfall in transfection and an increase upon the introduc-

tion of an unsaturated bond in **2h**. The use of DOPC leads to a more balanced and generally a more or less tail-length-independent transfection ability. The generally observed increase in transfection efficiency with increasing R^4 tail length clearly fits into the rational of matching and mismatching: the longer the R^4 tail the more matching is obtained at a constant R^3 length (vide supra). Using cholesterol (series A), generally low transfection efficiencies were found, although **2r** ($C_{19:1}-C_{18:1}$) stands out, with a transfection level nearly reaching that of **2e**. Surprisingly, the application of cholesterol leads to a sharp initial increase in transfection up to $C_{14:0}$ (**2e**), followed by a drop in efficiency thereafter.

For series B (**2i**−**2r**), R^4 is $C_{18:1}$ and R^3 variable, the results were found to be similar to the results obtained on series A. If DOPE is used as helper lipid, the only exception is **2i**, which contains a methyl "tail" ($C_{1:0}$), yielding unexpectedly high levels of efficiency (ca. 17 %). The series ranging from **2j** to **2n** shows a gradual increase of transfection efficiency as a function of the increase of the R^3 tail length from $C_{3:0}$ to $C_{11:0}$, followed by a sharp decrease for **2o**, **2p** and **2q**. A large improvement was observed by the introduction of an unsaturation in the tail (**2q** to **2r**, $C_{19:0}$ to $C_{19:1}$), reaching a more than double efficiency compared to the rest of the series and nearly four times the level of Lipofectamine.[31] Application of DOPC as a helper lipid does not show an impressive improvement in transfection efficiency and a more or less tail-length-independent behaviour is observed, with an overall average transfection efficiency, except for **2r**, which shows an almost fivefold higher transfection efficiency than Lipofectamine but at the cost of a significant toxicity increase. The results are summarised in Table 7.

Table 7. Series A- and B-mediated transfection of COS-7 cells expressed as percentage GFP positive cells and cell survival (%); ratio SF to lipid 1:1 and SF to DNA 2.5:1; transfection values compared to Lipofectamine (LA)

Amphiphile	DOPE	DOPC	Cholesterol
LA	11 (16)	–	–
2a	1 (82)	19 (75)	3 (76)
2b	2 (82)	22 (72)	2 (71)
2c	1 (88)	19 (75)	6 (78)
2d	4 (84)	24 (50)	26 (57)
2e	6 (83)	16 (72)	36 (65)
2f	25 (85)	22 (65)	3 (67)
2g	6 (80)	25 (71)	8 (70)
2h	9 (84)	15 (75)	18 (78)
2i	17 (90)	24 (76)	1 (94)
2j	9 (85)	19 (77)	1 (92)
2k	12 (74)	22 (79)	1 (90)
2l	10 (80)	14 (75)	3 (89)
2m	16 (83)	4 (75)	5 (93)
2n	31 (86)	3 (85)	10 (87)
2o	11 (86)	8 (92)	9 (94)
2p	7 (85)	17 (93)	5 (93)
2q	8 (87)	14 (88)	2 (93)
2r	33 (79)	50 (57)	30 (91)

Series A and B Amphiphiles 2 on HepG2

The elongation of the R^4 tail length from C_6 to C_{16} or C_{18} (all saturated, R^3 tail kept constant at $C_{13:0}$, series A) using the HepG2 cell line gives rise to a more diffuse behaviour in terms of transfection efficiency. If DOPE is used as helper lipid, a balanced efficiency is obtained regardless of the R^4 tail length with two exceptions: **2a** (C_6) and **2h** ($C_{18:1}$). The latter reaches almost eight times the efficiency of Lipofectamine. If cholesterol is applied as helper lipid, a slight increase is found upon increasing the R^4 tail length, reaching its maximum value for **2g** (C_{18}) and **2h** ($C_{18:1}$). The cholesterol-mediated transfection efficiency reaches levels of approximately eight times the transfection activity of Lipofectamine. This is the highest efficiency in an absolute sense within the helper lipid series. The most interesting results were obtained when DOPC was applied as the helper lipid, although the outcome is complex and therefore difficult to interpret (only series A and **2r** were measured). Generally, higher levels of efficiency, up to five times that of Lipofectamine, are reached using **2r** (series B, $C_{19:1}-C_{18:1}$), **2a** (C_6), **2c** (C_{10}), **2d** (C_{12}) and **2h** ($C_{18:1}$).

For the DOPC and cholesterol-supported series B, elongation of the R^3 tail length from C_1 to C_{19} while R^4 is kept constant at $C_{18:1}$, leads to a highly complex behaviour, both in terms of transfection efficiency and survival of the cells. If DOPE is applied as helper lipid, low efficiencies are observed for **2i**−**2m** (C_1-C_9), with a sudden increase from **2m/n** to **2o** ($C_{11}-C_{15}$) followed by a decrease upon further elongation of the R^3 tails. For this series, survival of the cells is relatively low, although it improves slightly with an increase of the R^3 tail length. The cholesterol series B showed the expected increase of transfection efficiency upon increase of the R^3 tail length, similar to the behaviour of series A. Surprisingly, series B showed the highest cell survival for the cholesterol series, whereas series A showed the highest cell survival for the DOPE series. These results are summarised in Table 8.

Meta and Ester Amphiphiles 3, 4 and 5 on COS-7

Compared to the *para* series A and B, the *meta*-substituted amphiphiles **3** showed a complex behaviour in terms of transfection ability. For amphiphiles **3c** and **3e** rather high activities were found, whereas **3d** strangely falls far back and appears not to fit into the series. For the *para*-esters **4**, the highest transfection efficiencies were found for **4b**, **4e** and **4h**, all containing saturated C_{18} tails. These findings are in agreement with earlier findings on SAINT esters, which also showed the highest efficiencies upon application of saturated tails, in contrast to all of the non-ester amphiphiles (either SAINT or Sunfish).[24]

In contrast to the *meta*-substituted amphiphiles **3**, the *meta*-substituted esters **5** showed an expected gradual increase of transfection efficiency upon elongation of the R^1 tail and also showed a preference for unsaturated tails (compared to amphiphiles **4**). Apparently, *meta*-substitution eliminates the "need" for saturation in transfection terms as found for other pyridinium esters (vide supra). For

Table 8. Series A- and B-mediated transfection of HepG2 cells expressed as percentage GFP positive cells and cell survival (%); ratio SF to lipid 1:1 and SF to DNA 2.5:1; transfection values compared to Lipofectamine (LA)

Amphiphile	DOPE	DOPC	Cholesterol
LA	13 (13)	–	–
2a	52 (54)	12 (21)	50 (46)
2b	26 (56)	26 (70)	35 (44)
2c	19 (69)	29 (69)	49 (40)
2d	21 (68)	31 (68)	36 (41)
2e	17 (69)	16 (70)	52 (42)
2f	14 (63)	9 (38)	75 (37)
2g	15 (65)	14 (64)	92 (42)
2h	68 (84)	27 (60)	83 (46)
2i	4 (24)	–	27 (63)
2j	1 (25)	–	22 (66)
2k	1 (31)	–	26 (70)
2l	1 (29)	–	37 (60)
2m	6 (40)	–	25 (70)
2n	29 (52)	–	46 (68)
2o	63 (45)	–	51 (66)
2p	45 (40)	–	49 (67)
2q	–	–	39 (79)
2r	20 (70)	46 (62)	57 (47)

the amphiphiles **3**, **4** and **5** very high cell survival is observed, with outstanding cell survival results for the *meta*-substituted esters **5**. The results are summarised in Table 9.

Table 9. Meta and ester series-mediated transfection of COS-7 cells expressed as percentage GFP positive cells and cell survival (%); ratio SF to DOPE 1:1 and SF to DNA 2.5:1; transfection values compared to Lipofectamine (LA)

Amphiphile	*trans.* (cell surv.)	Amphiphile	*trans.* (cell surv.)
LA	11 (16)	**4e**	27 (63)
3a	17 (75)	**4f**	20 (79)
3b	27 (86)	**4g**	16 (80)
3c	31 (85)	**4h**	29 (51)
3d	12 (86)	**4i**	19 (63)
3e	31 (85)	**5a**	13 (77)
3f	12 (86)	**55**	14 (88)
4a	14 (83)	**5c**	17 (87)
4b	41 (77)	**5d**	22 (89)
4c	24 (86)	**5e**	17 (89)
4d	24 (70)	**5f**	24 (87)

Conclusions

A conceptually new class of cationic amphiphiles is presented that is capable of altering the morphology of the lipoplex as a function of the "status" along the transfection pathway. A protective lamellar morphology is present during the condensation and transport of the DNA, whereas a more aggressive hexagonal morphology is formed upon contact with the cell membrane. Moreover, this morphological alteration facilitates both the release as well as expulsion of the DNA payload into the cell's interior.

The synthetic protocol allows relatively easy introduction of substituent tails and functionalities, such as esters or *N*-PEG-functionalized amphiphiles. This ease envisages a "tailor-made production" of amphiphiles for a diverse range of applications, such as different cell lines (in vitro) or, ultimately, diseases (in vivo). A large array of amphiphiles was synthesised in order to be able to study the underlying concept of backfolding and unfolding. Although most amphiphiles were designed for these purposes in the first place, excellent transfection activities for two cell lines (COS-7 and HepG2) were observed for several candidates, mostly combined with very low toxicity. Toxicity often accompanies the use of cationic lipids to such an extent that their application appears pointless.

Experimental Section

General: All reactions were carried out using dried solvents under a nitrogen atmosphere in oven-dried glassware. Microwave-accelerated experiments were carried out in a CEM Discovery synthesis-dedicated microwave oven with power and pulse-interval settings as indicated below. For column chromatography, Al_2O_3 (activity II-III), prepared by adding the indicated amount of water to Merck aluminium oxide 90 active neutral (activity I) was used with the indicated eluent system(s). Melting points (uncorrected) were determined using a Stuart Scientific SMP1 melting point apparatus. Several compounds displayed liquid-crystalline behaviour, in these cases no melting points are reported. NMR spectra were recorded on Varian Gemini 200 and Varian VXR 300 spectrometers operating at 200 and 300 MHz for the proton channels, respectively. NMR experiments monitoring the degradation pathways were performed on a Varian Unity-Plus 500 operating at 500 MHz for the proton channel. Standard Varian (pulse) programs were used for the recording and the development/analysis of the multidimensional spectra. Mass spectra were recorded using a Nermag R-3010 triple quadrupole mass spectrometer equipped with an in-house built atmospheric-pressure ionisation source and ion-spray interface. Elemental analyses were carried out in the Analytical Department of the University of Groningen. Accurate elemental analyses were sometimes difficult to obtain due to the presence of longer and shorter carbon chain homologues in the alkyl-chain starting amphiphiles (technical grade), as evidenced by electron-spray mass spectroscopy.

General Procedure for the Preparation of 8 and 9: A solution of diisopropylamine (5.05 g, 49.9 mmol) in 300 mL of dry THF was cooled to 0 °C under a nitrogen atmosphere. Then, 31.3 mL of an *n*-butyllithium/hexane solution (1.6 M in hexane, 50.0 mmol) was added dropwise and the resulting reaction mixture was stirred for an additional 30 min at 0 °C. Subsequently, 4-picoline (**6**; 4.50 g, 48.5 mmol) was added dropwise at −50 °C under careful temperature control. After completion, the orange-yellow solution was stirred for another 30 min at −50 °C. Thereafter, 1-iodoalkane/alkene (**7**; 48.5 mmol) in 25 mL of dry THF was added dropwise at −50 °C. The bright red solution was stirred for an additional 60 min at −50 °C and at room temperature overnight. The reaction was quenched by adding 300 mL of a saturated NH_4Cl solution, the organic layer was separated and the aqueous layer extracted with three portions of diethyl ether (50 mL). The combined organic

fractions were washed twice with H_2O (100 mL) and brine (200 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to yield viscous brown oils or crystalline amphiphiles. The products were purified by means of column chromatography over 300 g of neutral Al_2O_3 (act. III, freshly prepared) using *n*-hexane/diethyl ether as gradient eluent mixture (100:0 > 80:20) yielding the 4-alkylpyridines **8** as colourless viscous oils or crystalline materials. The crystalline materials were crystallised from acetonitrile or acetone. The *meta*-substituted amphiphiles **9** were prepared from 3-picoline (not shown).

4-Propylpyridine (8a):[33,34] ^{1}H NMR ($CDCl_3$): δ = 0.94 (t, ^3J = 7.2 Hz, 3 H), 1.68 (br. m, 2 H), 2.58 (t, ^3J = 7.4 Hz, 2 H), 7.10 (d, $^3J_{AB}$ = 6.4 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR: δ = 12.1, 21.9, 35.7, 122.4, 148.0, 149.9 ppm. Mass calcd. for $C_8H_{11}N$ 121.18; found 121 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-Pentylpyridine (8b):[33,34] ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, ^3J = 6.6 Hz, 3 H), 1.35 (br. m, 4 H), 1.61 (br. m, 2 H), 2.59 (t, ^3J = 7.6 Hz, 2 H), 7.10 (d, $^3J_{AB}$ = 4.4 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 4.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.3, 20.8, 28.3, 29.7, 33.5, 122.2, 147.9, 150.0 ppm. Mass calcd. for $C_{10}H_{15}N$ 149.27; found 149 [M^+].

4-Heptylpyridine (8c):[34] ^{1}H NMR ($CDCl_3$): δ = 0.82 (t, ^3J = 4.4 Hz, 3 H), 1.24 (br. m, 8 H), 1.55 (br. m, 2 H), 2.54 (t, ^3J = 5.2 Hz, 2 H), 7.04 (d, $^3J_{AB}$ = 4.0 Hz, 2 H), 8.42 (d, $^3J_{AB}$ = 4.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 21.1, 27.5, 27.6, 28.8, 30.2, 33.7, 122.3, 148.1, 150.2 ppm. Mass calcd. for $C_{12}H_{19}N$ 177.29; found 177 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-Nonylpyridine (8d):[34,35] ^{1}H NMR ($CDCl_3$): δ = 0.82 (t, ^3J = 4.4 Hz, 3 H), 1.25 (br. m, 12 H), 1.57 (br. m, 2 H), 2.54 (t, ^3J = 5.2 Hz, 2 H), 7.04 (d, $^3J_{AB}$ = 3.6 Hz, 2 H), 8.42 (d, $^3J_{AB}$ = 3.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 21.1, 27.6, 27.7, 27.8, 27.9, 28.7, 30.3, 33.6, 122.2, 148.0, 150.0 ppm. Mass calcd. for $C_{14}H_{23}N$ 205.34; found 205 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-Undecylpyridine (8e):[36] ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 6.3 Hz, 3 H), 1.27 (br. m, 16 H), 1.61 (br. m, 2 H), 2.59 (t, ^3J = 3.8 Hz, 2 H), 7.09 (d, $^3J_{AB}$ = 5.6 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 5.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.8, 27.9, 28.0, 28.1, 28.8, 30.4, 33.7, 122.3, 128.1, 150.2 ppm. Mass calcd. for $C_{16}H_{27}N$ 233.40; found 233 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-Tridecylpyridine (8f):[37] ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 4.4 Hz, 3 H), 1.25 (br. m, 20 H), 1.61 (br. m, 2 H), 2.59 (t, ^3J = 7.8 Hz, 2 H), 7.09 (d, $^3J_{AB}$ = 4.8 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 4.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.1, 27.6, 27.8, 27.9, 28.0, 28.1, 28.8, 30.4, 33.7, 33.8, 122.3, 148.0, 150.1 ppm. Mass calcd. for $C_{18}H_{31}N$ 261.45; found 261 [M^+], 106 [McLafferty rearrangement].

4-Pentadecylpyridine (8g):[38] ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 6.4 Hz, 3 H), 1.23 (br. m, 24 H), 1.61 (br. m, 2 H), 2.56 (t, ^3J = 7.7 Hz, 2 H), 7.09 (d, $^3J_{AB}$ = 4.4 Hz, 2 H), 8.46 (d, $^3J_{AB}$ = 4.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 28.0, 28.2, 28.8, 30.4, 33.7, 122.4, 148.0, 150.3 ppm. Mass calcd. for $C_{20}H_{35}N$ 289.50; found 289 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-Heptadecylpyridine (8h):[35] ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H), 1.26 (br. m, 28 H), 1.62 (br. m, 2 H), 2.60 (t, ^3J = 7.7 Hz, 2 H), 7.10 (d, $^3J_{AB}$ = 4.4 Hz, 2 H), 8.48 (d, $^3J_{AB}$ = 4.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 28.0, 28.2, 28.8, 30.4, 33.7, 122.4, 148.1 ppm. Mass calcd. for $C_{22}H_{39}N$ 317.56; found 317 [M^+], 106 [McLafferty rearrangement].

4-Nonadecylpyridine (8i):[39] ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, ^3J = 6.4 Hz, 3 H), 1.25 (br. m, 32 H), 1.61 (br. m, 2 H), 2.60 (t, ^3J = 7.6 Hz, 2 H), 7.10 (d, $^3J_{AB}$ = 4.4 Hz, 2 H), 8.48 (d, $^3J_{AB}$ = 4.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 28.0, 28.2, 28.8, 30.4, 33.7, 122.4, 148.1 ppm. Mass calcd. for $C_{24}H_{43}N$ 345.61; found 345 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

4-[(*Z*)-10-Nonadecenyl]pyridine (8j): ^{1}H NMR ($CDCl_3$): δ = 0.81 (t, ^3J = 6.9 Hz, 3 H), 1.15−1.35 (br. m, 26 H), 1.52−1.58 (br. m, 2 H), 1.82−1.98 (br. m, 4 H), 2.52 (t, ^3J = 7.7 Hz, 2 H), 5.21−5.38 (m, 2 H), 7.02 (d, $^3J_{AB}$ = 5.5 Hz, 2 H), 8.41 (d, $^3J_{AB}$ = 5.5 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 21.8, 24.1, 25.7, 27.7, 27.7, 27.8, 28.0, 28.2, 28.8, 30.4, 31.1, 66.4, 122.4, 123.1, 128.3, 128.4, 128.8, 148.1 ppm. $C_{24}H_{41}N$: calcd. C 83.90, H 12.03, N 4.08; found C 83.87, H 12.07, N 4.04. Mass calcd. for $C_{24}H_{41}N$ 343.59; found 343 [M^+], 106 [McLafferty rearrangement]. The *cis*/*trans* ratio at the double bond was unchanged (85:15) based upon ^{1}H NMR integration of the protons at δ = 5.25 and 5.33 ppm, which are broad singlets when selectively homonuclear decoupled at about δ = 1.90 ppm.

3-Tridecylpyridine (9a):[40] ^{1}H NMR ($CDCl_3$): δ = 0.87 (br. t, 3 H), 1.25 (br. m, 20 H), 1.61 (br. m, 2 H), 2.59 (t, 2 H), 7.20 (dd, ^3J = 7.8 Hz, ^3J = 4.49 Hz, 1 H), 7.45 (d, ^3J = 7.8 Hz, 1 H), 8.42 (m's, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.6, 27.8, 27.9, 28.0, 28.1, 29.6, 30.4, 31.5, 121.7, 134.2, 145.7, 148.5 ppm. EI-MS (electrospray) calcd. for $C_{18}H_{31}N$ 261.45; found 261 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

3-Pentadecylpyridine (9b)[23]**:** ^{1}H NMR ($CDCl_3$): δ = 0.88 (br. t, 3 H), 1.27 (br. m, 24 H), 1.62 (br. m, 2 H), 2.61 (t, 2 H), 7.23 (dd, ^3J = 7.6 Hz, ^3J = 4.98 Hz, 1 H), 7.51 (d, ^3J = 7.6 Hz, 1 H), 8.45 (m's, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 27.9, 28.1, 28.2, 29.7, 30.4, 31.5, 121.7, 134.2, 145.7, 148.5 ppm. EI-MS (electrospray) calcd. for $C_{20}H_{35}N$ 289.50; found 289 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

3-Heptadecylpyridine (9c): ^{1}H NMR ($CDCl_3$): δ = 0.89 (br. t, 3 H), 1.26 (br. m, 28 H), 1.65 (br. m, 2 H), 2.60 (t, 2 H), 7.26 (dd, 1 H), 7.49 (dd, ^3J = 7.3 Hz, ^3J = 4.76 Hz, 1 H), 8.45 (m's, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 28.0, 28.1, 28.2, 29.7, 30.4, 31.5, 121.7, 134.3, 145.7, 148.5 ppm. $C_{22}H_{39}N$: calcd. C 83.21, H 12.38, N 4.41; found C 83.12, H 12.34, N 4.43. EI-MS (electrospray) calcd. for $C_{22}H_{39}N$ 317.56; found 317 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

3-Nonadecylpyridine (9d): ^{1}H NMR ($CDCl_3$): δ = 0.89 (br. t, 3 H), 1.26 (br. m, 32 H), 1.60 (br. m, 2 H), 2.61 (t, 2 H), 7.21 (dd, ^3J = 7.8 Hz, ^3J = 4.90 Hz, 1 H), 7.49 (d, ^3J = 7.8 Hz, 1 H), 8.44 (m's, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.7, 27.9, 28.0, 28.2, 29.7, 30.4, 31.5, 121.7, 134.3, 145.6, 148.5 ppm. $C_{24}H_{43}N$: calcd. C 83.41, H 12.54, N 4.05; found C 83.32, H 12.45, N 4.16. EI-MS (electrospray) calcd. for $C_{24}H_{43}N$ 345.61; found 345 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

3-[(*Z*)-10-Nonadecenyl]pyridine (9e): ^{1}H NMR ($CDCl_3$): δ = 0.88 (br. t, 3 H), 1.28 (br. m, 22 H), 1.62 (br. m, 2 H), 2.02 (m, 4 H),

2.61 (t, 2 H), 5.35 (m, 2 H), 7.20 (dd, 1 H), 7.49 (dd, ^3J = 7.8 Hz, ^3J = 4.64 Hz, 1 H), 8.44 (m's, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 25.7, 27.7, 27.8, 27.9, 28.0, 28.1, 28.3, 29.7, 30.4, 31.1, 31.5, 121.7, 128.3, 128.4, 128.9, 134.3, 145.6, 148.4 ppm. $C_{24}H_{41}N$: calcd. C 83.90, H 12.03, N 4.08; found C 83.72, H 11.81, N 4.12. EI-MS (electrospray) calcd. for $C_{24}H_{41}N$ 343.60; found 343 [M^+], 106 [stepwise McLafferty rearrangement], 93 [concerted McLafferty rearrangement].

General Procedure for the Synthesis of 2 and 3. Procedure 1:[15] A solution of 4-alkylpyridine **8** (1.0 equivalent) and 1.05 equivalents of 1-iodoalkane/alkene **7** in acetonitrile (10 mL/0.5 g 4-alkylpyridine) was heated to 90 °C for at least 24 hours. The colour of the solution changed during the night from colourless to red. After the reaction had progressed to approximately 75 % conversion, the mixture was concentrated and the crude iodide salt **10** purified using column chromatography (Al_2O_3 activity III, eluent hexane > CH_2Cl_2 > CH_2Cl_2/MeOH, 95:5). Directly thereafter, the counterion was exchanged on a Sephadex DEAE ion-exchange column with methanol as eluent. Finally, the crude chloride **2** was purified by means of column chromatography (Al_2O_3 activity III column, eluent hexane > CH_2Cl_2 > CH_2Cl_2/MeOH, 95:5) yielding **2** as highly viscous oils or waxy solid materials.

Procedure 2: A mixture of 4-alkylpyridine **8** (1.0 equivalent) and 1.05 equivalents of 1-iodoalkane/alkene **7** was placed in a microwave oven and carefully deoxygenated. The mixture was reacted using pulsed intervals (2 × 10 min of 40 Watt, 10 min hold time and 2 × 10 min of 25 Watt and 10 min hold time, sequence repeated until completion based on ^{1}H NMR spectrum of the crude mixture), resulting in a very viscous dark-red oil. The material was purified as described under procedure 1. The corresponding *meta*-substituted amphiphiles **3** were prepared from the alkylpyridines **9**.

1-Hexyl-4-tridecylpyridinium Chloride (2a):[37] ^{1}H NMR ($CDCl_3$): δ = 0.91 (br. m, 6 H), 1.33 (br. m, 26 H), 1.73 (br. m, 2 H) 2.03 (br. m, 2 H), 2.86 (t, ^3J = 7.6 Hz, 2 H), 4.93 (t, ^3J = 7.6 Hz, 2 H), 7.83 (d, $^3J_{AB}$ = 6.7 Hz, 2 H), 9.39 (d, $^3J_{AB}$ = 6.7 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.4, 12.6, 20.8, 21.2, 24.2, 27.6, 27.7, 27.8, 27.9, 28.0, 28.1, 29.6, 30.4, 34.4, 59.7, 126.4, 143.0, 161.5 ppm. $C_{24}H_{44}ClN$: calcd C 75.53, H 11.63, N 3.67; found C 75.54, H 11.83, N 3.74. EI-MS (electrospray) calcd. for $C_{24}H_{44}ClN$ 382.07; found 346.5 [M − Cl].

1-Octyl-4-tridecylpyridinium Chloride (2b): ^{1}H NMR ($CDCl_3$): δ = 0.85 (br. m, 6 H), 1.27 (br. m, 30 H), 1.69 (br. m, 2 H), 2.02 (br. m, 2 H), 2.85 (t, ^3J = 7.6 Hz, 2 H), 4.94 (t, ^3J = 7.6 Hz, 2 H), 7.82 (d, $^3J_{AB}$ = 6.4 Hz, 2 H), 9.42 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 12.6, 21.0, 21.2, 24.6, 27.5, 27.5, 27.6, 27.7, 27.8, 27.9, 28.1, 28.1, 30.1, 30.4, 30.4, 34.4, 59.7, 126.4, 143.0, 161.4 ppm. $C_{26}H_{48}ClN$: calcd. C 76.22, H 11.82, N 3.42; found C 76.16, H 12.05, N 3.46. EI-MS (electrospray) calcd. for $C_{26}H_{48}ClN$ 410.13; found 374.6 [M − Cl].

1-Decyl-4-tridecylpyridinium Chloride (2c): ^{1}H NMR ($CDCl_3$): δ = 0.86 (br. m, 6 H), 1.24 (br. m, 34 H), 1.68 (br. m, 2 H), 2.05 (br. m, 2 H), 2.87 (t, ^3J = 7.3 Hz, 2 H), 4.94 (t, ^3J = 7.5 Hz, 2 H), 7.82 (d, $^3J_{AB}$ = 6.5 Hz, 2 H), 9.37 (d, $^3J_{AB}$ = 6.5 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.6, 27.6, 27.7, 27.8, 27.9, 28.1, 30.3, 30.4, 34.4, 59.8, 126.4, 142.9, 161.5 ppm. $C_{28}H_{52}ClN{\cdot}H_2O$: calcd. C 73.72, H 11.93, N 3.07; found C 73.75, H 11.86, N 3.18. EI-MS (electrospray) calcd. for $C_{28}H_{52}ClN$ 438.18; found 402.4 [M − Cl].

1-Dodecyl-4-tridecylpyridinium Chloride (2d): ^{1}H NMR ($CDCl_3$): δ = 0.81 (t, ^3J = 4.4 Hz, 2 H), 1.31 (br. m, 38 H), 1.63 (br. m, 2 H), 1.93 (br. m, 2 H), 2.80 (t, ^3J = 5.0 Hz, 2 H), 4.86 (t, ^3J = 5.0 Hz, 2 H), 7.75 (d, $^3J_{AB}$ = 4.4 Hz, 2 H), 9.27 (d, $^3J_{AB}$ = 4.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.6, 27.7, 27.8, 28.0, 28.1, 28.1, 30.4, 34.4, 59.9, 126.4, 143.8, 161.6 ppm. $C_{30}H_{56}ClN{\cdot}1^1/_2H_2O$: calcd. C 73.05, H 12.06, N 2.84; found C 73.47, H 12.27, N 2.96. EI-MS (electrospray) calcd. for $C_{30}H_{56}ClN$ 466.23; found 430.2 [M − Cl].

1-Tetradecyl-4-tridecylpyridinium Chloride (2e): ^{1}H NMR ($CDCl_3$): δ = 0.86 (br. m, 6 H), 1.27 (br. m, 42 H), 1.67 (br. m, 2 H), 1.98 (br. m, 2 H), 2.85 (t, ^3J = 7.7 Hz, 2 H), 4.91 (t, ^3J = 7.5 Hz, 2 H), 7.83 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.38 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.6, 27.7, 27.7, 27.8, 28.1, 30.4, 34.4, 59.7, 126.4, 143.0, 161.4 ppm. $C_{32}H_{60}ClN{\cdot}2H_2O$: calcd. C 72.48, H 12.16, N 2.64; found C 72.51, H 12.22, N 2.73. EI-MS (electrospray) calcd. for $C_{32}H_{60}ClN$ 494.29; found 458.2 [M − Cl].

1-Hexadecyl-4-tridecylpyridinium Chloride (2f): ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, ^3J = 6.3 Hz, 6 H), 1.26 (br. m, 46 H), 1.69 (br. m, 2 H), 2.00 (br. m, 2 H), 2.85 (t, ^3J = 7.7 Hz, 2 H), 4.90 (t, ^3J = 7.3 Hz, 2 H), 7.83 (d, $^3J_{AB}$ = 6.8 Hz, 2 H), 9.39 (d, $^3J_{AB}$ = 6.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.6, 27.7, 27.7, 27.8, 28.1, 30.4, 34.4, 59.7, 126.4, 143.0, 161.4 ppm. $C_{34}H_{64}ClN{\cdot}{}^1/_2H_2O$: calcd. C 76.86, H 12.33, N 2.64; found C 76.84, H 12.57, N 2.72. EI-MS (electrospray) calcd. for $C_{34}H_{64}ClN$ 522.34; found 486.5 [M − Cl].

1-Octadecyl-4-tridecylpyridinium Chloride (2g): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 4.4 Hz, 6 H), 1.27 (br. m, 50 H), 1.65 (br. m, 2 H), 1.95 (br. m, 2 H), 2.83 (t, ^3J = 5.2 Hz, 2 H), 4.94 (t, ^3J = 4.8 Hz, 2 H), 7.82 (d, $^3J_{AB}$ = 4.0 Hz, 2 H) 9.37 (d, $^3J_{AB}$ = 4.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.6, 27.7, 27.8, 27.9, 28.1, 28.2, 30.4, 34.4, 59.8, 126.4, 142.9, 161.5 ppm. $C_{36}H_{68}ClN$: calcd. C 76.07, H 12.41, N 2.46; found C 76.35, H 12.74, N 2.58. EI-MS (electrospray) calcd. for $C_{36}H_{68}ClN$ 550.40; found 514.8 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-tridecylpyridinium Chloride (2h): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 6 H), 1.28 (br. m, 44 H), 1.67 (br. m, 2 H), 2.09 (br. m, 6 H), 2.87 (t, ^3J = 7.8 Hz, 2 H), 4.96 (t, ^3J = 7.2 Hz, 2 H), 5.35 (br. m, 2 H), 7.81 (d, $^3J_{AB}$ = 6.0 Hz, 2 H), 9.38 (d, $^3J_{AB}$ = 6.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.6, 27.7, 27.8, 28.1, 30.4, 34.4, 59.8, 126.3, 128.1, 128.5, 142.9 ppm. $C_{36}H_{66}ClN{\cdot}2H_2O$: calcd. C 73.99, H 12.07, N 2.40; found C 73.98, H 12.03, N 2.47. EI-MS (electrospray) calcd. for $C_{36}H_{66}ClN$ 548.38; found 512.3 [M − Cl].

4-Methyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2i): ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, ^3J = 6.4 Hz, 3 H), 1.24 (br. m, 22 H), 1.97 (br. m, 6 H), 2.64 (s, 3 H), 4.90 (t, ^3J = 7.2 Hz, 2 H), 5.30 (br. m, 2 H), 7.60 (d, $^3J_{AB}$ = 6.4 Hz, 2 H), 9.39 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 20.7, 21.1, 24.6, 25.6, 25.7, 27.6, 27.7, 27.8, 28.0, 28.2, 28.3, 30.4, 30.4, 31.1, 59.7, 127.3, 128.1, 128.5, 142.9, 159.1 ppm. $C_{24}H_{42}ClN{\cdot}{}^1/_2H_2O$: calcd. C 74.09, H 11.14, N 3.60; found C 74.46, H 11.24, N 3.69. EI-MS (electrospray) calcd. for $C_{24}H_{42}ClN$ 380.06; found 344.2 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-propylpyridinium Chloride (2j): ^{1}H NMR ($CDCl_3$): δ = 0.83 (t, ^3J = 6.4 Hz, 3 H), 0.96 (t, ^3J = 7.2 Hz, 3 H), 1.22 (br. m, 24 H), 1.71 (br. m, 2 H), 1.96 (br. m, 6 H), 2.82 (t, ^3J = 7.6 Hz, 2 H), 4.89 (t, ^3J = 7.2 Hz, 2 H), 5.29 (br. m, 2 H), 7.86 (d, $^3J_{AB}$ = 6.4 Hz, 2 H), 9.52 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.1, 12.6, 21.1, 21.4, 24.6, 25.6, 27.6, 27.7, 27.8, 28.0, 28.2, 28.2, 30.4, 30.4, 31.1, 36.2, 59.6, 126.5, 128.1, 128.5, 128.9, 143.1, 161.0 ppm. $C_{26}H_{46}ClN$: calcd. C 76.52, H

11.36, N 3.43; found C 76.32, H 11.48, N 3.52. EI-MS (electrospray) calcd. for $C_{26}H_{46}ClN$ 408.11; found 372.2 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-pentylpyridinium Chloride (2k): ^{1}H NMR ($CDCl_3$): δ = 0.75 (br. m, 6 H), 1.20 (br. m, 26 H), 1.59 (br. m, 2 H), 1.89 (br. m, 6 H), 2.74 (t, ^3J = 7.4 Hz, 2 H), 4.78 (br. m, 2 H), 5.21 (br. m, 2 H), 7.79 (d, $^3J_{AB}$ = 6.4 Hz, 2 H), 9.49 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.2, 12.5, 20.7, 21.0, 24.5, 25.6, 27.5, 27.6, 27.7, 28.1, 28.9, 29.6, 30.3, 30.3, 31.0, 34.2, 59.4, 75.6, 126.5, 128.0, 128.4, 143.1, 161.2 ppm. $C_{28}H_{50}ClN{\cdot}2H_2O$: calcd. C 71.22, H 11.53, N 2.97; found C 70.66, H 11.06, N 3.06. EI-MS (electrospray) calcd. for $C_{28}H_{50}ClN$ 436.16; found 400.6 [M − Cl].

4-Heptyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2l): ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 6.4 Hz, 6 H), 1.27 (br. m, 30 H), 1.68 (br. m, 2 H), 1.99 (br. m, 6 H), 2.86 (t, ^3J = 7.6 Hz, 2 H), 4.94 (t, ^3J = 7.2 Hz, 2 H), 5.32 (br. m, 2 H), 7.83 (d, $^3J_{AB}$ = 6.8 Hz, 2 H), 9.45 (d, $^3J_{AB}$ = 6.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 12.6, 21.0, 21.2, 24.6, 25.6, 25.7, 27.4, 27.6, 27.7, 27.8, 28.0, 28.1, 28.2, 28.3, 30.0, 30.4, 30.5, 31.1, 34.4, 59.7, 126.4, 128.1, 128.5, 143.0, 161.4 ppm. $C_{30}H_{54}ClN{\cdot}^1/_2H_2O$: calcd. C 76.14, H 11.71, N 2.96; found C 76.36, H 11.73, N 3.04. EI-MS (electrospray) calcd. for $C_{30}H_{54}ClN$ 464.22; found 428.2 [M − Cl].

4-Nonyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2m): ^{1}H NMR ($CDCl_3$): δ = 0.80 (br. m, 6 H), 1.19 (br. m, 38 H), 1.61 (br. m, 2 H), 1.92 (br. m, 6 H), 2.80 (t, ^3J = 7.8 Hz, 2 H), 4.85 (t, ^3J = 7.2 Hz, 2 H), 5.26 (br. m, 2 H), 7.83 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.53 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 21.2, 21.1, 24.6, 25.7, 27.6, 27.6, 27.7, 28.0, 28.1, 28.2, 28.3, 30.2, 30.3, 30.4, 31.0, 34.3, 59.5, 75.7, 126.5, 128.1, 128.4, 143.2, 161.2 ppm. $C_{32}H_{58}ClN{\cdot}2H_2O$: calcd. C 72.75, H 11.83, N 2.65; found C 72.72, H 12.01, N 2.68. EI-MS (electrospray) calcd. for $C_{32}H_{58}ClN$ 492.27; found 456.6 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-undecylpyridinium Chloride (2n): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 6 H), 1.27 (br. m, 38 H), 1.71 (br. m, 2 H), 1.99 (br. m, 6 H), 2.88 (t, ^3J = 7.8 Hz, 2 H), 5.34 (br. m, 2 H), 7.82 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.39 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.6, 27.6, 27.7, 27.8, 28.1, 30.4, 34.4, 59.8, 126.3, 128.1, 128.5, 142.9, 161.5 ppm. $C_{34}H_{62}ClN{\cdot}^1/_2H_2O$: calcd. C 77.15, H 12.00, N 2.65; found C 76.93, H 11.98, N 2.65. EI-MS (electrospray) calcd. for $C_{34}H_{62}ClN$ 520.33; found 484.5 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-pentadecylpyridinium Chloride (2o): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 6 H), 1.26 (br. m, 46 H), 1.71 (br. m, 2 H), 2.00 (br. m, 6 H), 2.87 (t, ^3J = 7.6 Hz, 2 H), 4.96 (t, ^3J = 7.2 Hz, 2 H), 5.33 (br. m, 2 H), 7.82 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.39 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.7, 27.8, 28.2, 30.4, 34.4, 59.8, 126.3, 128.1, 128.5, 142.9, 161.5 ppm. $C_{38}H_{70}ClN{\cdot}11/2H_2O$: calcd. C 76.01, H 11.75, N 2.33; found C 76.05, H 11.96, N 2.31. EI-MS (electrospray) calcd. for $C_{38}H_{70}ClN$ 576.43; found 540.3 [M − Cl].

4-Heptadecyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2p): ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 6.0 Hz, 6 H), 1.25 (br. m, 52 H), 1.71 (br. m, 2 H), 1.98 (br. m, 6 H), 2.86 (t, ^3J = 7.6 Hz, 2 H), 4.95 (t, ^3J = 7.1 Hz, 2 H), 5.33 (br. m, 2 H), 7.79 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.35 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.7, 27.8, 27.9, 28.2, 30.4, 34.4, 59.8, 126.3, 128.1, 128.5, 142.9, 161.5 ppm. $C_{40}H_{74}ClN{\cdot}H_2O$: calcd. C 77.18, H 12.31, N 2.25; found C 76.97, H 12.33, N 2.23. EI-MS (electrospray) calcd. for $C_{40}H_{74}ClN$ 604.49; found 568.3 [M − Cl].

4-Nonadecyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2q): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.6 Hz, 6 H), 1.26 (br. m, 54 H), 1.74 (br. m, 2 H), 1.99 (br. m, 6 H), 2.88 (t, ^3J = 7.6 Hz, 2 H), 4.97 (t, ^3J = 7.2 Hz, 2 H), 5.34 (br. m, 2 H), 7.81 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.36 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.6, 27.6, 27.8, 28.2, 30.4, 34.4, 59.9, 126.3, 128.1, 128.5, 142.9 ppm. $C_{42}H_{78}ClN{\cdot}^1/_2H_2O$: calcd. C 76.48, H 12.38, N 2.12; found C 76.70, H 12.23, N 2.10. EI-MS (electrospray) calcd. for $C_{42}H_{78}ClN$ 632.54; found 596.3 [M − Cl].

4-[(*Z*)-10-Nonadecenyl]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (2r): ^{1}H NMR ($CDCl_3$): δ = 0.87 (br. m, 6 H), 1.35 (br. m, 54 H), 1.68 (br. m, 2 H), 2.10 (br. m, 10 H), 2.88 (t, ^3J = 7.7 Hz, 2 H), 4.96 (t, ^3J = 7.3 Hz, 2 H), 5.34 (br. m, 4 H), 7.81 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.35 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.8, 28.0, 28.2, 30.4, 34.4, 126.3, 128.2, 128.5, 142.9 ppm. $C_{42}H_{76}ClN$: calcd. C 80.01, H 12.15, N 2.22; found C 79.58, H 12.06, N 2.25. EI-MS (electrospray) calcd. for $C_{42}H_{76}ClN$ 630.53; found 594.6 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-3-tridecylpyridinium Chloride (3a): ^{1}H NMR ($CDCl_3$): δ = 0.86 (br. t, 6 H), 1.26 (br. m, 40 H), 1.68 (br. m, 4 H), 1.97 (m, 6 H), 2.87 (t, 2 H), 5.03 (t, 2 H), 5.32 (m, 2 H), 8.02 (dd, $^3J_{AB}$ = 8.1, $^3J_{AB}$ = 5.9 Hz, 1 H), 8.20 (d, $^3J_{AB}$ = 8.1 Hz, 1 H), 9.24 (s, 1 H), 9.52 (d, $^3J_{AB}$ = 5.9 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.7, 24.6, 25.7, 27.6, 27.7, 27.0, 28.0, 28.1, 29.0, 30.4, 30.6, 31.1, 31.3, 60.4, 126.4, 128.1, 128.5, 141.6, 142.9 ppm. $C_{36}H_{66}ClN{\cdot}H_2O$: calcd. C 76.34, H 12.10, N 2.47; found C 76.09, H 12.10, N 2.42. EI-MS (electrospray) calcd. for $C_{36}H_{66}ClN$ 548.38; found 512.7 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-3-pentadecylpyridinium Chloride (3b): ^{1}H NMR ($CDCl_3$): δ = 0.86 (br. t, 6 H), 1.25 (br. m, 44 H), 1.68 (br. m, 4 H), 1.98 (d, 6 H), 2.88 (t, 2 H), 5.03 (t, 2 H), 5.32 (m, 2 H), 8.03 (dd, $^3J_{AB}$ = 8.1, $^3J_{AB}$ = 5.9 Hz, 1 H), 8.20 (d, $^3J_{AB}$ = 8.1 Hz, 1 H), 9.23 (s, 1 H), 9.52 (d, $^3J_{AB}$ = 5.9 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.6, 27.7, 27.8, 28.0, 28.2, 29.0, 30.4, 30.6, 31.3, 60.4, 126.4, 128.1, 128.5, 142.6, 142.9 ppm. $C_{38}H_{70}ClN{\cdot}H_2O$: calcd. C 76.78, H 12.21, N 2.36; found C 76.78, H 12.27, N 2.28. EI-MS (electrospray) calcd. for $C_{38}H_{70}ClN$ 576.43; found 540.2 [M − Cl].

3-Heptadecyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (3c): ^{1}H NMR ($CDCl_3$): δ = 0.88 (br. t, 6 H), 1.26 (br. m, 48 H), 1.74 (br. m, 4 H), 2.00 (d, 6 H), 2.90 (t, 2 H), 4.95 (t, 2 H), 5.34 (m, 2 H), 8.03 (dd, $^3J_{AB}$ = 8.3, $^3J_{AB}$ = 6.1 Hz, 1 H), 8.24 (d, $^3J_{AB}$ = 8.3 Hz, 1 H), 9.11 (s, 1 H), 9.22 (d, $^3J_{AB}$ = 6.1 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 25.7, 27.6, 27.8, 28.0, 28.2, 28.9, 30.4, 31.3, 60.6, 126.4, 128.1, 128.5, 142.8, 143.3 ppm. $C_{40}H_{74}ClN{\cdot}H_2O$: calcd. C 77.18, H 12.31, N 2.25; found C 77.24, H 12.46, N 2.18. EI-MS (electrospray) calcd. for $C_{40}H_{74}ClN$ 604.49; found 568.6 [M − Cl].

3-Nonadecyl-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (3d): ^{1}H NMR ($CDCl_3$): δ = 0.88 (br. t, 6 H), 1.25 (br. m, 52 H), 1.65 (br. m, 4 H), 2.00 (m, 6 H), 2.89 (t, 2 H), 5.04 (t, 2 H), 5.32 (m, 2 H), 8.00 (dd, $^3J_{AB}$ = 7.8, $^3J_{AB}$ = 6.1 Hz, 1 H), 8.19 (d, $^3J_{AB}$ = 7.8 Hz, 1 H), 9.15 (s, 1 H), 9.52 (d, $^3J_{AB}$ = 6.1 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 27.5, 27.8, 28.2, 29.0, 30.4, 30.6, 31.3, 60.5, 126.3, 142.8 ppm. $C_{42}H_{78}ClN{\cdot}H_2O$: calcd. C 77.54, H 12.31, N 2.25; found C 77.35, H 12.59, N 2.11. EI-MS (electrospray) calcd. for $C_{42}H_{78}ClN$ 632.54; found 597.1 [M − Cl].

3-[(*Z*)-10-Nonadecenyl]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (3e): ^{1}H NMR ($CDCl_3$): δ = 0.85 (br. t, 6 H), 1.25 (br. m, 44 H), 1.70 (br. m, 4 H), 2.00 (s, 10 H), 2.87 (t, 2 H), 5.03 (t, 2 H), 5.32

(m, 4 H), 8.01 (dd, $^3J_{AB}$ = 7.6, $^3J_{AB}$ = 5.1 Hz, 1 H), 8.19 (d, $^3J_{AB}$ = 7.6 Hz, 1 H), 9.18 (s, 1 H), 9.51 (d, $^3J_{AB}$ = 5.1 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 25.7, 27.6, 27.8, 28.0, 28.2, 29.0, 30.4, 30.6, 31.1, 31.3, 126.4, 128.1, 128.2, 128.4, 142.9 ppm. $C_{42}H_{76}ClN{\cdot}1^1/_4H_2O$: calcd. C 77.25, H 12.12, N 2.07; found C 77.34, H 12.15, N 2.07. EI-MS (electrospray) calcd. for $C_{42}H_{76}ClN$ 630.53; found 594.7 [M − Cl].

1-Hexadecyl-3-tridecylpyridinium Chloride (3f): ^{1}H NMR ($CDCl_3$): δ = 0.87 (br. t, 6 H), 1.27 (br. m, 46 H), 1.72 (m, 2 H), 2.03 (m, 2 H), 2.89 (t, 2 H), 5.03 (t, 2 H), 8.01 (dd, $^3J_{AB}$ = 8.1, $^3J_{AB}$ = 5.9 Hz, 1 H), 8.20 (d, $^3J_{AB}$ = 8.1 Hz, 1 H), 9.16 (s, 1 H), 9.47 (d, $^3J_{AB}$ = 5.9 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 27.5, 27.8, 28.1, 28.9, 30.4, 31.3, 126.3, 142.9 ppm. $C_{34}H_{64}ClN{\cdot}1^3/_4H_2O$: calcd. C 73.73, H 12.28, N 2.53; found C 73.81, H 11.99, N 2.46. EI-MS (electrospray) calcd. for $C_{34}H_{64}ClN$ 522.34; found 486.2 [M − Cl].

1-[(*Z*)-11-Hexadecenyl]-3-tridecylpyridinium Chloride (3g): ^{1}H NMR ($CDCl_3$): δ = 0.87 (br. m, 6 H), 1.25 (br. m, 38 H), 1.65 (br. m, 2 H), 2.02 (br. m, 6 H), 2.86 (t, ^3J = 7.8 Hz, 2 H), 4.97 (t, ^3J = 7.4 Hz, 2 H), 5.28 (br. m, 2 H), 8.02 (br. m, 1 H), 8.19 (br. m, 1 H), 9.12 (s, 1 H), 9.42 (d, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.5, 12.6, 12.9, 19.0, 20.8, 21.1, 24.6, 25.4, 25.5, 25.7, 27.5, 27.7, 27.8, 28.0, 28.1, 28.2, 28.9, 30.4, 30.5, 31.2, 60.5, 126.5, 127.7, 128.3, 130.0, 141.5, 142.3, 142.6, 142.9 ppm. $C_{34}H_{62}ClN$: calcd. C 78.48, H 12.01, N 2.69; found C 78.52, H 11.82, N 2.57. EI-MS (electrospray) calcd. for $C_{34}H_{62}ClN$ 520.33; found 484.1 [M − Cl].

General Procedure for the Synthesis of 12 and 13:[35] A solution of isonicotionyl chloride hydrochloride (**11**; 2.0 g, 11.24 mmol) and Et_3N (25 mmol) in 50 mL of CH_2Cl_2 was cooled to 0 °C. Alkyl alcohol (11.24 mmol) dissolved in 25 mL of CH_2Cl_2 was added dropwise and the reaction was allowed to react for 60 min. Subsequently, the reaction mixture was refluxed overnight. The mixture was cooled to 0 °C and filtered, the filtrate was concentrated at reduced pressure yielding yellow viscous oils. The crude product mixture was purified by column chromatography (Al_2O_3 act. III, freshly prepared, 75 g typically) using hexane as eluent to remove impurities and (excess) starting materials. The material was brought onto the column dissolved in a minimum amount of $CHCl_3$. Changing the solvent to hexane/$CHCl_3$ (gradient, 100:0 > 80:20 and a few drops of Et_3N) afforded the products **12** as colourless waxy oils that sometimes partly solidified upon standing or as white solid materials. 3-(Alkoxycarbonyl)pyridines **13** were prepared using the same protocol starting from nicotinoyl chloride hydrochloride (not show).

Dodecyl Isonicotinate (12a): ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, ^3J = 6.2 Hz, 3 H), 1.31 (m, 14 H), 1.71 (m, 2 H), 4.34 (t, ^3J = 6.6 Hz, 2 H), 7.84 (m, 2 H), 8.77(m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 27.7, 27.8, 28.0, 28.1, 28.1, 30.4, 64.5, 121.3, 136.1, 149.1, 163.7 ppm. EI-MS (electrospray) calcd. for $C_{18}H_{29}NO_2$ 291.43; found 291 [M], 124 [Pyr-CO_2-].

Tetradecyl Isonicotinate (12b): ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, ^3J = 6.2 Hz, 3 H), 1.31 (m, 22 H), 1.73 (m, 2 H), 4.34 (t, ^3J = 6.6 Hz, 2 H, 2 H), 7.84 (m, 2 H), 8.77(m, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 27.7, 27.8, 28.0, 28.1, 28.2, 30.4, 64.5, 121.3, 136.1, 149.1, 163.7 ppm. EI-MS (electrospray) calcd. for $C_{20}H_{33}NO_2$ 319.49; found 319 [M], 124 [Pyr-CO_2-].

Hexadecyl Isonicotinate (12c): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H), 1.25 (br. m, 26 H), 1.77 (br. m, 2 H), 4.35 (t, ^3J = 6.6 Hz, 2 H), 7.84 (d, ^3J = 4.5 Hz, 2 H), 8.79 (d, ^3J = 4.5 Hz, 2 H) ppm. ^{13}C NMR($CDCl_3$): δ12.59, 21.16, 24.43, 27.04, 27.72, 27.84, 27.98, 28.04, 30.39, 64.41, 121.28, 136.08, 149.03, 163.59 ppm. Mass calcd. for $C_{22}H_{37}NO_2$ 347.54; found 348.5 [M + H], 695.7 [2M + H].

(*Z*)-9-Octadecenyl Isonicotinate (12d): ^{1}H NMR ($CDCl_3$): δ = 0.80 (t, ^3J = 5.9 Hz, 3 H), 1.15−1.42 (br. m, 22 H), 1.71 (m, 2 H), 1.87−2.01 (br. m, 4 H), 4.28 (t, ^3J = 6.7 Hz, 2 H), 5.22−5.38 (br. m, 2 H), 7.77 (d, $^3J_{AB}$ = 5.9 Hz, 2 H), 8.79 (d, $^3J_{AB}$ = 5.9 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0, 22.6, 25.8, 27.0, 27.1, 28.5, 29.1, 29.2, 29.3, 29.4, 29.6, 29.6, 31.8, 32.5, 65.8, 122.7, 129.6, 129.9, 137.5, 150.4, 165.0 ppm. Mass calcd. for $C_{24}H_{39}NO_2$ 373.58; found 374.3 [M + H], 747.6 [2M + H].

Dodecyl Nicotinate (13a):[41] Low melting white solid, M.p. 26 °C. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H), 1.38 (br. m, 18 H), 1.78 (br. m, 2 H), 4.35 (t, ^3J = 6.6 Hz, 2 H), 7.39 (br. m, 1 H), 8.29 (br. m, 1 H), 8.78 (br. m, 1 H), 9.24 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 27.1, 27.7, 27.8, 28.0, 28.1, 28.1, 30.4, 64.1, 121.7, 135.5, 149.4, 151.8 ppm. EI-MS calcd. for $C_{18}H_{29}NO_2$ 291.43; found 291 [M^+].

Tetradecyl Nicotinate (13b):[41] White solid, M.p. 35 °C. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H), 1.26 (br. m, 22 H), 1.79 (br. m, 2 H), 4.36 (t, ^3J = 6.6 Hz, 2 H), 7.38 (br. m, 1 H), 8.30 (br. m, 1 H), 8.78 (br. m, 1 H), 9.24 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 27.1, 27.7, 27.8, 28.0, 28.1, 28.1, 30.4, 64.1, 121.8, 135.6, 149.3, 151.7 ppm. EI-MS calcd. for $C_{20}H_{33}NO_2$ 319.49; found 319 [M^+].

Hexadecyl Nicotinate (13c):[42] White solid, M.p. 47 °C. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H), 1.23 (br. m, 26 H), 1.76 (br. m, 2 H), 4.36 (t, ^3J = 6.6 Hz, 2 H), 7.40 (br. m, 1 H), 8.28 (br. m, 1 H), 8.77 (br. m, 1 H), 9.24 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.5, 27.1, 27.7, 27.8, 28.0, 28.1, 28.2, 30.4, 64.1, 121.8, 135.5, 149.4, 151.8 ppm. EI-MS calcd. for $C_{22}H_{37}NO_2$ 347.54; found 347.54 [M^+].

General Procedure for the Synthesis of 4 and 5:[35] 4-(Alkoxycarbonyl)pyridine **12** (0.97 mmol) and 1-iodoalkane/alkene (3−5 mmol) were placed in a microwave oven and carefully deoxygenated. The mixture was reacted with continuous progress control (^{1}H NMR) using pulsed intervals [2 × 10 min of 45 Watt, 10 min hold time, 2 × 10 min of 25 Watt, repeated until the conversion had evolved to approx. 85 %]. This process takes about 45 minutes, resulting in a reddish very viscous material. The crude product mixture was purified by recrystallisation from acetone/acetonitrile (2:3) yielding the pure products as white solid materials. The material was used immediately for ion exchange due to the very low stability of the iodide salts applying exactly the same protocol as described for non-ester Sunfish amphiphiles **2** and **3** (vide supra). For the synthesis of 1-alkyl-3-(alkoxycarbonyl)pyridines **5**, 3-(alkoxycarbonyl)pyridines **13** where used as starting materials.

1-Dodecyl-4-[(dodecyloxy)carbonyl]pyridinium Chloride (4a): ^{1}H NMR ($CDCl_3$): δ = 0.88 (m, 6 H), 1.27 (m, 36 H), 1.80 (m, 2 H), 2.06 (m, 2 H), 4.44 (t, ^3J = 6.7 Hz, 2 H), 5.20 (t, ^3J = 7.1 Hz, 2 H), 8.48(d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.82(d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.4, 26.9, 27.8, 28.1, 30.4, 66.4, 126.0, 145.1 ppm. $C_{30}H_{54}ClNO_2{\cdot}1^3/_4H_2O$: calcd. C 69.46, H 10.98, N 2.70; found C 69.52, H 10.97, N 2.77. EI-MS (electrospray) calcd. for $C_{30}H_{54}ClNO_2$ 496.22; found 460.6 [M − Cl].

4-[(Dodecyloxy)carbonyl]-1-octadecylpyridinium Chloride (4b): ^{1}H NMR ($CDCl_3$): δ = 0.87 (m, 6 H), 1.25 (m, 48 H), 1.79 (m, 2 H), 2.04 (m, 2 H), 4.42 (t, ^3J = 6.6 Hz, 2 H), 5.23 (t, ^3J = 7.5 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.86 (d, $^3J_{AB}$ = 6.8 Hz, 2 H)

ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 26.9, 27.6, 27.7, 27.9, 28.2, 30.4, 30.8, 61.0, 66.3, 125.9, 145.2, 159.9 ppm. $C_{36}H_{66}ClNO_2 \cdot 1^3/_4H_2O$: calcd. C 71.72, H 11.45, N 2.32; found C 71.76, H 11.43, N 2.26. EI-MS (electrospray) calcd. for $C_{36}H_{66}ClNO_2$ 580.38; found 544.7 [M − Cl].

4-[(Dodecyloxy)carbonyl)]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (4c): ^1H NMR ($CDCl_3$): δ = 0.87 (m, 6 H), 1.26 (m, 48 H), 1.79 (m, 2 H), 2.00 (m, 2 H), 4.42 (t, ^3J = 6.5 Hz, 2 H), 5.20 (m, 2 H), 5.33 (m, 2 H), 8.48 (d, $^3J_{AB}$ = 6.1 Hz, 2 H), 9.91 (d, $^3J_{AB}$ = 6.1 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.7, 27.8, 28.1, 30.4, 30.7, 31.1, 61.0, 66.3, 125.9, 128.1, 128.5, 143.0, 145.3, 159.9 ppm. $C_{36}H_{64}ClNO_2 \cdot H_2O$: calcd. C 72.50, H 11.15, N 2.35; found C 72.34, H 10.98, N 2.41 %: EI-MS $C_{36}H_{64}ClNO_2$ 578.36; found 542.6 [M − Cl].

1-Dodecyl-4-[(tetradecyloxy)carbonyl]pyridinium Chloride (4d): ^1H NMR ($CDCl_3$): δ = 0.86 (m, 6 H), 1.25 (m, 40 H), 1.78 (m, 2 H), 2.03 (m, 2 H), 4.42 (t, *J* = 6.6 Hz, 2 H), 5.19 (t, *J* = 7.3 Hz, 2 H), 8.46 (d, $^3J_{AB}$ = 6.6 Hz, 2 H), 9.86 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.1, 24.3, 24.6, 27.7, 27.8, 28.2, 30.4, 30.6, 60.9, 66.1, 126.0, 142.8, 145.4, 160.0 ppm. $C_{32}H_{58}ClNO_2 \cdot 1H_2O$: calcd. C 70.88, H 11.15, N 2.58; found C 70.73, H 10.98, N 2.56. EI-MS (electrospray) calcd. for $C_{32}H_{58}ClNO_2$ 524.27; found 488.6 [M − Cl].

1-Octadecyl-4-[(tetradecyloxy)carbonyl]pyridinium Chloride (4e): ^1H NMR ($CDCl_3$): δ = 0.85 (m, 6 H), 1.24 (m, 52 H), 1.78 (m, 2 H), 2.02 (m, 2 H), 4.41 (t, ^3J = 6.6 Hz, 2 H), 5.12 (t, ^3J = 7.5 Hz, 2 H), 8.46 (d, $^3J_{AB}$ = 5.6 Hz 2 H), 9.83 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.1, 24.3, 24.6, 26.9, 27.6, 27.7, 27.8, 27.9, 28.0, 28.1, 30.4, 30.7, 61.0, 66.2, 125.9, 142.9, 145.3, 160.0 ppm. $C_{38}H_{70}ClNO_2 \cdot H_2O$: calcd. C 72.86, H 11.58, N 2.24; found C 72.58, H 11.37, N 2.18. EI-MS (electrospray) calcd. for $C_{38}H_{70}ClNO_2$ 608.43; found 572.7 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-[(tetradecyloxy)carbonyl]pyridinium Chloride (4f): ^1H NMR ($CDCl_3$): δ = 0.87 (m, 6 H), 1.26 (m, 48 H), 1.77 (m, 2 H), 2.00 (m, 2 H), 4.43 (t, ^3J = 6.6 Hz, 2 H), 5.21 (t, ^3J = 7.2 Hz, 2 H), 5.24 (m, 2 H), 8.48 (d, $^3J_{AB}$ = 6.8 Hz 2 H), 9.83 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 26.9, 27.9, 28.2, 30.4, 30.7, 66.4, 125.9, 145.1 ppm. $C_{38}H_{68}ClNO_2 \cdot 1H_2O$: calcd. C 73.09, H 11.30, N 2.24; found C 72.85, H 11.01, N 2.32. EI-MS (electrospray) calcd. for $C_{38}H_{68}ClNO_2$ 606.42; found 570.6 [M − Cl].

1-Dodecyl-4-[(hexadecyloxy)carbonyl]pyridinium Chloride (4g): ^1H NMR ($CDCl_3$): δ = 0.86 (m, 6 H), 1.25 (m, 44 H), 1.80 (m, 2 H), 2.04 (m, 2 H), 4.41 (t, ^3J = 6.6 Hz, 2 H), 5.15 (t, ^3J = 7.1 Hz, 2 H), 8.47 (d, $^3J_{AB}$ = 6.1 Hz, 2 H), 9.77 (d, $^3J_{AB}$ = 6.4 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 26.9, 27.6, 27.7, 27.9, 28.0, 28.1, 28.2, 30.4, 30.7, 61.0, 66.3, 125.9, 142.9, 145.3, 160.0 ppm. $C_{34}H_{62}ClNO_2 \cdot {}^1/_2\ H_2O$: calcd. C 72.75, H, 11.31, N 2.50; found C 72.85, H 11.33, N 2.46. EI-MS (electrospray) calcd. for $C_{34}H_{62}ClNO_2$ 552.32; found 516.6 [M − Cl].

4-[(Hexadecyloxy)carbonyl]-1-octadecylpyridinium Chloride (4h): ^1H NMR ($CDCl_3$): δ = 0.88 (m, 6 H), 1.26 (m, 56 H), 1.71 (m, 2 H), 2.06 (m, 2 H), 4.44 (t, ^3J = 6.6 Hz, 2 H), 5.20 (t, ^3J = 7.1 Hz, 2 H), 8.49 (d, $^3J_{AB}$ = 5.9 Hz, 2 H), 9.79 (d, $^3J_{AB}$ = 6.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 26.9, 27.9, 28.2, 30.4, 66.4, 126.0, 145.1 ppm. $C_{40}H_{74}ClNO_2 \cdot {}^1/_2H_2O$: calcd. C 74.43, H 11.71, N 2.17; found C 74.22, H 11.50, 2.16. EI-MS (electrospray) calcd. for $C_{40}H_{74}ClNO_2$ 636.49; found 600.7 [M − Cl].

4-[(Hexadecyloxy)carbonyl]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (4i): ^1H NMR ($CDCl_3$): δ = 0.89 (m, 6 H), 1.27 (m, 52 H), 1.81 (m, 2 H), 2.02 (m, 2 H), 4.44 (t, ^3J = 6.8 Hz, 2 H), 5.21 (t, ^3J = 7.1 Hz, 2 H), 5.35 (m, 2 H), 8.49 (d, $^3J_{AB}$ = 6.35 Hz, 2 H), 9.83 (d, $^3J_{AB}$ = 6.1 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.7, 27.8, 28.2, 30.4, 30.7, 61.1, 66.4, 126.0, 128.6, 145.1 ppm. $C_{40}H_{72}ClNO_2 \cdot 1/2H_2O$: calcd. C 74.66, H 11.43, N 2.18; found C 74.69, H 11.50, N 2.16. EI-MS (electrospray) calcd. for $C_{40}H_{72}ClNO_2$ 634.47; found 598.8 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-4-{[(*Z*)-9-octadecenyloxy]carbonyl}pyridinium Chloride (4j): ^1H NMR ($CDCl_3$): δ = 0.82 (dt, ^3J = 7.0 Hz, 6 H), 1.20−1.45 (br. m, 22 H), 1.48−1.54 (br. m, 20 H), 1.72−1.83 (m, 2 H), 1.90−2.08 (br. m, 12 H), 4.67 (t, ^3J = 6.9 Hz, 2 H), 5.09 (br. t, ^3J = 7.2 Hz, 2 H), 5.20−5.45 (m, 4 H), 8.58 (d, $^3J_{AB}$ = 5.8 Hz, 2 H), 9.61 (d, $^3J_{AB}$ = 5.8 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.7, 27.8, 28.0, 28.2, 30.4, 30.7, 31.1, 31.3, 61.5, 66.3, 125.9, 128.5, 128. 9, 143.0, 145.3, 159.9 ppm. $C_{43}H_{76}ClNO_2 \cdot 1H_2O$: calcd. C 74.58, H 11.35, N 2.02; found C 74.32, H 11.01, N 1.93. EI-MS (electrospray) calcd. for $C_{43}H_{76}ClNO_2$ 674.54; found 639.09 [M − Cl].

3-[(Dodecyloxy)carbonyl]-1-octadecylpyridinium Chloride (5a): White solid, M.p. 80−95 °C. ^1H NMR ($CDCl_3$): δ = 0.82 (t, ^3J = 4.4 Hz, 6 H), 1.94 (br. m, 48 H), 1.76 (br. m, 2 H), 2.01 (br. m, 2 H), 4.40 (t, ^3J = 4.6 Hz, 2 H), 5.08 (t, ^3J = 4.8 Hz, 2 H), 8.27 (br. m, 1 H), 8.85 (d, ^3J = 5.4 Hz, 1 H), 9.08 (s, 1 H), 10.50 (d, ^3J = 4.4 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ12.6, 21.2, 24.3, 24.6, 26.9, 27.5, 27.7, 27.9, 28.0, 28.2, 30.4, 30.5, 61.5, 66.3, 127.7, 129.2, 142.6, 143.2, 145.4, 148.8, 159.5 ppm. $C_{36}H_{66}ClNO_2$: calcd. C 74.50, H 11.46, N 2.41; found C 74.75, H 11.72, N 2.37. EI-MS (electrospray) calcd. for $C_{36}H_{66}ClNO_2$ 580.38; found 544.2 [M − Cl].

3-[(Dodecyloxy)carbonyl]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (5b): ^1H NMR ($CDCl_3$): δ = 0.86 (t, ^3J = 6.4 Hz, 6 H), 1.25 (br. m, 40 H), 1.81 (br. m, 2 H), 1.95 (br. m, 6 H), 4.44 (t, ^3J = 6.9 Hz, 2 H), 5.15 (t, ^3J = 7.5 Hz, 2 H), 5.32 (br. m, 2 H), 8.38 (br. m, 1 H), 8.88 (d, ^3J = 8.0 Hz, 1 H), 9.20 (s, 1 H), 10.48 (d, ^3J = 6.2 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.5, 27.7, 27.8, 28.1, 28.1, 30.4, 30.5, 61.4, 66.2, 127.7, 128.5, 129.1, 142.7, 143.2, 148.7, 159.5 ppm. $C_{36}H_{64}ClNO_2 \cdot 1H_2O$: calcd. C 72.26, H 11.45, N 2.34; found C 72.24, H 11.19, N 2.24. EI-MS (electrospray) calcd. for $C_{36}H_{64}ClNO_2$ 578.36; found 542.6 [M − Cl].

1-Octadecyl-3-[(tetradecyloxy)carbonyl]pyridinium Chloride (5c): White solid, m.p. 77−85 °C. ^1H NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.4 Hz, 6 H), 1.26(br. m, 52 H), 1.79 (br. m, 2 H), 2.09 (br. m, 2 H), 4.46 (t, ^3J = 6.8 Hz, 2 H), 5.14 (t, ^3J = 7.4 Hz, 2 H), 8.36 (m, 1 H), 8.91 (d, ^3J = 8.0 Hz, 1 H), 9.15 (s, 1 H), 10.55 (d, 6 H, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 26.9, 27.5, 27.9, 28.0, 28.2, 30.4, 61.5, 66.3, 95.5, 127.7, 142.6, 143.1, 148.8, 159.4 ppm. $C_{38}H_{70}ClNO_2 \cdot {}^1/_4H_2O$: calcd. C 74.46, H 11.59, N 2.29; found C 74.54, H 11.72, N 2.20. EI-MS (electrospray) calcd. for $C_{38}H_{70}ClNO_2$ 607.51; found 572.2 [M − Cl].

1-[(*Z*)-9-Octadecenyl]-3-[(tetradecyloxy)carbonyl]pyridinium Chloride (5d): ^1H NMR ($CDCl_3$): δ = 0.87 (t, ^3J = 6.3 Hz, 6 H), 1.25 (br. m, 44 H), 1.85 (br. m, 2 H), 2.06 (br. m, 6 H), 4.44 (t, ^3J = 6.9 Hz, 2 H), 5.16 (t, ^3J = 7.4 Hz, 2 H), 5.33 (br. m, 2 H), 8.35 (m, 1 H), 8.90 (d, ^3J = 8.0 Hz, 1 H), 9.19 (s, 1 H), 10.56 (d, ^3J = 5.2 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.5, 27.7, 27.8, 28.0, 28.1, 30.4, 30.4, 31.1, 61.4, 66.1, 127.8, 128.1, 128.5, 129.1, 142.9, 143.2, 148.5, 159.6 ppm. $C_{38}H_{68}ClNO_2 \cdot H_2O$: calcd. C 73.09, H 11.30, N 2.24; found C 72.81, H 11.45, N 2.25. EI-MS calcd. for $C_{38}H_{68}ClNO_2$ 606.42; found 570.5 [M − Cl].

3-[(Hexadecyloxy)carbonyl]-1-octadecylpyridinium Chloride (5e): White solid, m.p. 84−100 °C. ^{1}H NMR ($CDCl_3$): δ = 0.82 (t, ^3J = 6.4 Hz, 6 H), 1.20 (br. m, 56 H), 1.75 (br. m, 2 H), 2.01 (br. m, 2 H), 4.40 (t, ^3J = 6.8 Hz, 2 H), 5.08 (t, ^3J = 7.6 Hz, 2 H), 8.28 (br. m, 1 H), 8.85 (d, ^3J = 8.2 Hz, 1 H), 9.08 (s, 1 H), 10.49 (d, ^3J = 6.2 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.6, 24.6, 26.9, 27.5, 27.7, 27.9, 28.0, 28.2, 30.4, 61.5, 66.3, 127.7, 129.2, 142.6, 143.2, 148.8, 159.5 ppm. $C_{40}H_{74}ClNO_2$: calcd. C 75.48, H 11.72, N 2.20; found C 75.30, H 11.85, N 2.15. EI-MS (electrospray) calcd. for $C_{40}H_{74}ClNO_2$ 635.54; found 600.2 [M − Cl].

3-[(Hexadecyloxy)carbonyl]-1-[(*Z*)-9-octadecenyl]pyridinium Chloride (5f): White solid, m.p. 68−75 °C. ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, ^3J = 6.3 Hz, 6 H), 1.24 (br. m, 48 H), 1.84 (br. m, 2 H), 1.99 (br. m, 6 H), 4.43 (t, ^3J = 6.9 Hz, 2 H), 5.16 (t, ^3J = 7.4 Hz, 2 H), 5.32 (br. m, 2 H), 8.39 (br. m, 1 H), 8.90 (d, ^3J = 8.2 Hz, 1 H), 9.23 (s, 1 H), 10.50 (d, ^3J = 5.8 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.3, 24.6, 25.7, 26.9, 27.7, 27.8, 28.0, 28.1, 28.2, 30.4, 30.5, 31.1, 61.4, 66.2, 127.7, 128.1, 128.5, 129.1, 142.8, 143.2, 148.6, 159.5 ppm. $C_{40}H_{72}ClNO_2 \cdot {}^3/_4H_2O$: calcd. C 74.27, H 11.55, N 2.16; found C 74.27, H 11.55, N 2.16. EI-MS (electrospray) calcd. for $C_{40}H_{72}ClNO_2$ 634.47; found 598.6 [M − Cl].

1-Benzyl-4-[10-(*Z*)-nonadecenyl]pyridinium Bromide (15): ^{1}H NMR ($CDCl_3$): δ = 0.79 (t, ^3J = 7.01 Hz, 3 H), 1.09−1.36 (m, 24 H), 1.55 (q, ^3J = 7.0 Hz, 2 H), 1.84−2.05 (m, 4 H), 2.73 (t, ^3J = 6.9 Hz, 2 H), 5.23−5.36 (br. m, 2 H), 6.08 (br. s, 2 H), 7.28 (dd, $^3J_{AB}$ = 7.0, ^4J = 3.0 Hz, 3 H), 7.56 (dd, $^3J_{AB}$ = 7.0, ^4J = 3.0 Hz), 7.65 (d, $^3J_{AB}$ = 7.1 Hz, 2 H), 9.33 ($^3J_{AB}$ = 7.1 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.9, 22.4, 28.9 (s), 28.9 (s), 29.1, 29.1, 29.3, 29.4, 31.6, 31.7, 32.8, 35.6, 63.9, 128.9, 126.1, 127.6, 128.2, 128.3, 139.4, 144.0, 163.0 ppm. EI-MS (electrospray) calcd. for $C_{31}H_{46}BrN$ 512.617; found 432.7 [M − Br].

4-[10-(*Z*)-Nonadecenyl]-1-(4-phenylbutyl)pyridinium Bromide (16): ^{1}H NMR (CD_3OD): δ = 0.89 (t, ^3J = 7.0 Hz, 3 H), 1.09−1.40 (m, 26 H), 1.58−1.84 (m, 4 H), 1.89−2.13 (m, 4 H), 2.68 (t, ^3J = 6.9 Hz, 2 H), 2.97 (t, ^3J = 8.0 Hz, 2 H), 4.56 (br. m, 2 H), 5.23−5.36 (m, 2 H), 7.09−7.36 (m, 5 H), 7.92 (d, $^3J_{AB}$ = 7.1 Hz, 2 H), 8.79 (d, $^3J_{AB}$ = 7.1 Hz, 2 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 13.9, 22.1, 27.3, 28.4, 28.7, 28.9, 29.0, 30.2, 31.3, 34.4, 34.6, 59.7, 125.8, 127.6, 128.2, 129.3, 141.4, 143.9, 162.6 ppm. EI-MS (electrospray) calcd. for $C_{34}H_{52}BrN$ 554.698; found 474.8 [M − Br].

4-[10-(*Z*)-Nonadecenyl]-1-(6-phenylhexyl)pyridinium Bromide (17): ^{1}H NMR (CD_3OD): δ = 0.81 (t, ^3J = 6.0 Hz, 3 H), 1.13−1.40 (br. m, 30 H), 1.56 (t, ^3J = 8.0 Hz, 2 H), 1.64 (t, ^3J = 7.0 Hz, 2 H), 1.84−2.04 (br. m, 4 H), 2.52 (t, ^3J = 8.0 Hz, 2 H), 2.86 (t, ^3J = 7.9 Hz, 2 H), 4.46 (t, ^3J = 6.7 Hz, 2 H), 5.22−5.36 (m, 2 H), 7.01−7.18 (m, 5 H), 7.86 (d, $^3J_{AB}$ = 7.0 Hz, 2 H), 8.73 (d, $^3J_{AB}$ = 7.0 Hz) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.6, 21.2, 24.4, 25.7, 27.0, 27.7, 27.8, 28.1, 28.2, 29.5, 30.3, 31.1, 34.2, 34.4, 59.7, 124.2, 126.3, 126.8, 126.9, 128.2, 142.9, 161.5 ppm. EI-MS (electrospray) calcd. for $C_{36}H_{56}BrN$ 582.751; found 502.8 [M − Br].

Transfection Experiments

Preparation of Vesicles: A solution of amphiphile, alone or in a 1:1 molar ratio with the helper lipid in a minimum amount of CH_2Cl_2 or methanol, was concentrated under a stream of nitrogen. Residual solvent was removed under vacuum and the resulting lipid film was hydrated in water to a total lipid concentration of 1 mM, sonicated at room temperature (or higher if stated) to clarity using a Branson Sonifier Cell Disrupter B15. The solution was used immediately.

Transfections: A 4.7 kb plasmid containing the *E. coli* GFP (PEGFP-N1 Clontech, Palo Alto, CA, USA) was used as the reporter gene. DNA was isolated from *E. coli* using a Qiagen Plasmid Kit (QIAGEN® Inc., USA). The plasmid concentration was determined by measuring the absorption at λ = 260 nm using the relation 1.0 OD = 50 μg·mL^{-1}. Typically, the OD_{260}/OD_{280} ratio was 1.95. COS-7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM® Gibo®, The Netherlands) containing 7 % of fetal calf serum, 2 mM of L-glutamine, 100 units/mL of penicillin and 100 mg/mL of streptomycin at 37 °C in CO_2/air (1:19). Cells (1×10^5 cells/well) were seeded in 12-well plates and allowed to grow overnight. The complex of Sunfish/helper lipid (1:1) with PEGFP-N1 was prepared at a charge ratio 2.5:1 (15 nmol lipid and 1 μg DNA), in 100 μL of 10 mM HEPES buffer (pH 7.4, 150 mM NaCl). After 10−15 min incubation at room temperature, the lipoplex was diluted in 1 mL DMEM medium and 0.5 mL of the mixture was added to the cells and incubated for 4 h at 37 °C. The percentage of GFP-expressing cells was measured by FACS (Fluorescence Activated Cell Sorting) after 48 h.

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