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Synthesis of Nucleosides with 2′-Fixed Lipid Anchors and Their Behavior in Phospholipid Membranes

Oliver Kaczmarek,[a] Nicolai Brodersen,[a] Andreas Bunge,[b,c] Ludwig Löser,[b,c] Daniel Huster,[b,c] Andreas Herrmann,[d] Anna Arbuzova,[d] and Jürgen Liebscher*[a]

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Various new nucleosides bearing one or two lipophilic groups at the 2′-position have been synthesized. The lipophilic substituents were attached to a 2′-hydroxy, 2′-amino, or 2′-thio function. These lipophilic nucleosides anchor in large unilamellar POPC vesicles serving as phospholipid membrane models. The insertion of these molecules into the membranes was investigated by NMR techniques. For comparison, nucleosides with two or three lipophilic groups at the 2′-, 3′-, or 5′-positions have also been studied.

Introduction

Nucleic acids equipped with lipophilic groups (long-chain alkyl, fatty acyl, fatty alkyloxy, steroidyl, terpenyl) have gained interest because of their ability to anchor in lipid membranes and to form double strands with complementary single-stranded nucleotides. Further, the lipophilic group may enable nucleic acids to traffic through cell membranes. The former property is useful in the field of chip technology and diagnostics.[1] Membrane passage of lipophilic nucleic acids was recently applied in siRNA-mediated in vivo gene therapy.[2,3] The ability of oligonucleotides to permeate cell membranes by the introduction of lipidic structures into the oligonucleotides has been used in the development of antiviral compounds.[4–6] In all these cases the lipophilic moiety was attached either to the 5′- or 3′-OH group of the oligonucleotide by using the corresponding 3′- or 5′-lipidated nucleosides, respectively, as building blocks in the phosphoramidite method. In order to study the effect of the position of the lipidated nucleotide in an oligonucleotide strand and also the position within the lipophilic nucleotide itself (positional screening) it is necessary to develop nucleosides in which both the 3′- and 5′-positions are free. Such nucleosides can then be transformed into the corresponding 5′-DMT-protected 3′-phosphoramidites and used in automated synthesis to be introduced at any given position within the growing oligonucleotide chain. So far, only a very few examples of such compounds with free 3′- and 5′-positions have been reported.[7–10] As an alternative, nucleosides in which the lipophilic group is attached to the nucleobase could also be incorporated into any position of an oligonucleotide.[11]

Recently we showed that nucleosides with lipid moieties connected either to the nucleobase[12] or to the 5′-position[13] anchor in the phospholipid double layers of giant and large unilamellar vesicles (GUV and LUV). The corresponding oligonucleotides with two lipophilic nucleotides along the chain could be anchored in such liposomes and were shown to form double-strand DNA with complementary oligonucleotides in solution.[11,14] Membrane incorporation could be achieved in a lipid-domain specific manner being enriched in liquid-disordered domains but not in liquid-ordered domains.

As a continuation of our efforts to find suitable lipophilic nucleosides that can be introduced into any position of an oligonucleotide strand, we aimed to introduce the lipid scaffold into the 2′-position of uridine to obtain products of the general structure **1** in which X represents O, N, or S. Herein, we report the synthesis and membrane incorporation of lipophilic uridines of the type **1**. As it has previously been reported that nucleobases have a tendency to be located at the lipid/water interface of the membrane,[12,15] which renders the recognition of these membrane-associated molecules by single-stranded DNA difficult, we also studied the influence of the number of acyl chain anchors on the average localization of the nucleobases. Based on

[a] Institute of Chemistry, Humboldt University Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany
E-mail: Liebscher@chemie.hu-berlin.de
[b] Junior Research Group "Structural Biology of Membrane Proteins", Institute of Biochemistry/Biotechnology, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany
[c] Institute of Medical Physics and Biophysics, University Leipzig Härtelstr. 16–18, 04107 Leipzig, Germany
[d] Institute of Biology, Molecular Biophysics, Humboldt University Berlin, Invalidenstr. 42, 10115 Berlin, Germany
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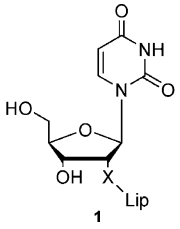
these results we conclude that the most optimal structure for nanotechnological applications is a lipophilic nucleoside with two acyl chains and a spacer between the ribose and the nucleobase moieties.



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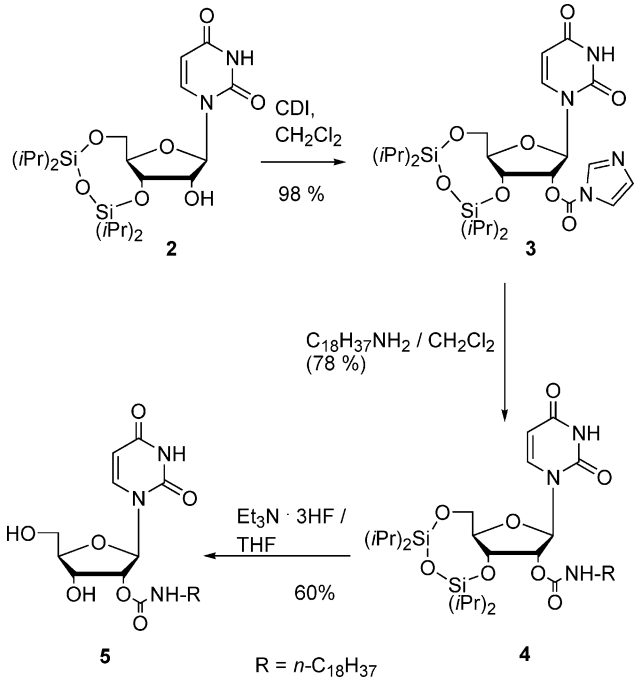
Results and Discussion

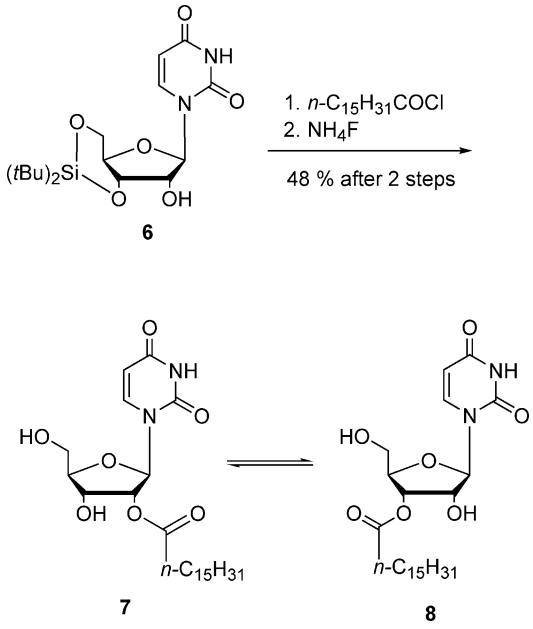
Synthesis of Lipophilic Uridines

In order to attach the lipophilic moiety to the 2′-hydroxy group of uridine two strategies were applied. First, the 3′,5′-disilylated uridine **2**[16] was used as the starting material and transformed into the imidazole-1-carboxylate **3** with carbonyldiimidazole (Scheme 1).[17] Reaction with octadecylamine and final deprotection of the resulting **4** afforded the *N*-octadecylcarbamate **5**. Use of $Et_3N{\cdot}3HF$ instead of TBAF is recommended in order to prevent migration of the carbamate group from the 2′- to the 3′-position.[17]



Scheme 1.

However, when fatty acyl groups are attached to the 2′-position of 3′,5′-diprotected uridines[18] the 2′-acyl group migrates after deprotection.[19–21] In this way, a mixture of the 2′- and 3′-palmitoyluridines **7** and **8** was obtained in a 4:9 ratio starting from **6** (Scheme 2).



Scheme 2.

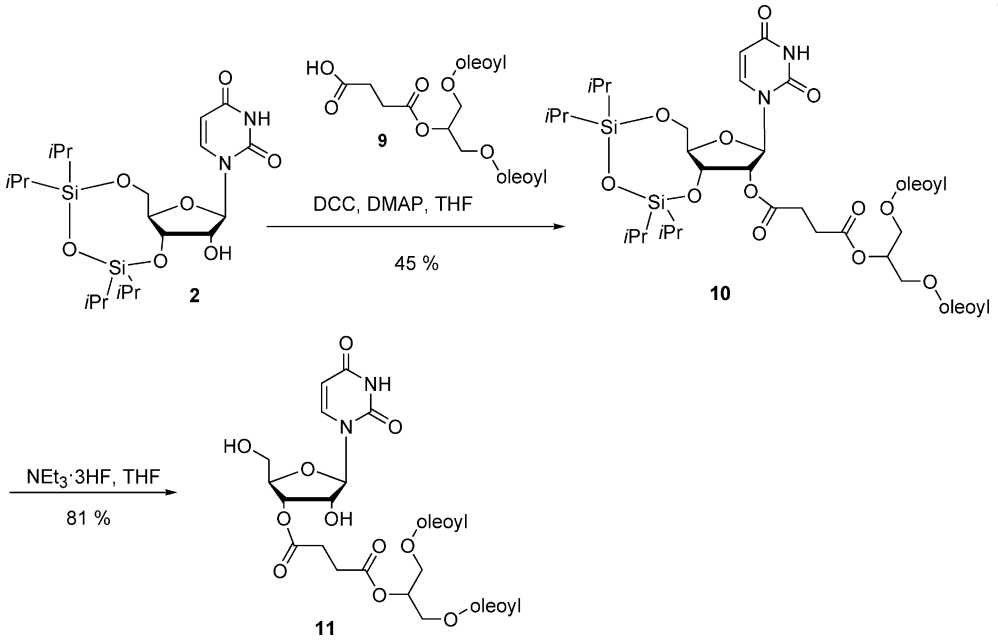
In an extreme case the 2′-[(1,3-di-*O*-oleoylglycer-2-*O*-yl)-succinyl] substituent of the acylation product **10**, obtained from the 3′,5′-protected uridine **2**, migrated completely to the 3′-position when the protective groups were removed by fluoride. Thus, product **11** was obtained as the sole product with two lipophilic groups attached to the 3′-position rather than the 2′-position (Scheme 3).

This migration is prevented when acyl groups are attached to the 2′-position of the corresponding arabinose nucleoside. Thus, Mitsunobu reaction of the 3′,5′-protected uridine **6** with succinoyl dioleoyl glycerol **9**[22] yielded the stable 2′-acyl arabinose derivative **13** in high yield after deprotection of the resulting acylation product **12** (Scheme 4). In this product two lipid anchors are tethered to the 2′-position.

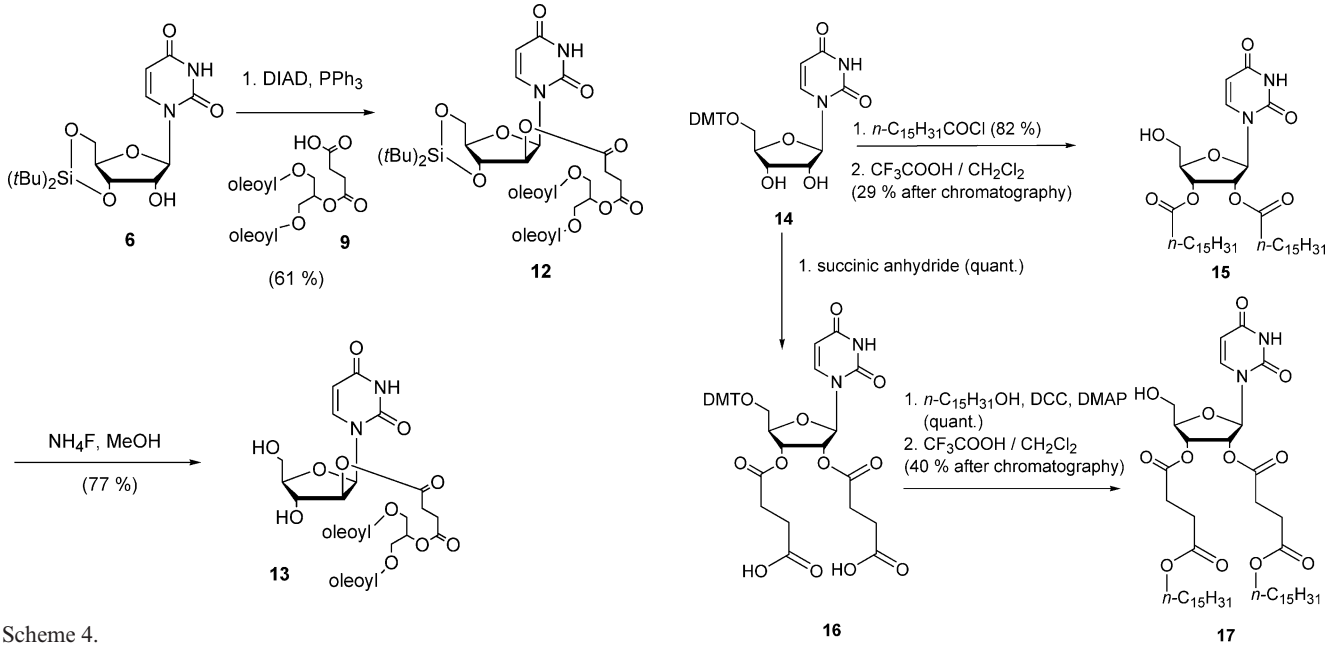
In order to investigate the effect of the number and position of lipid anchors in uridines on the membrane-anchoring behavior we also synthesized the diesters **15** and **17** by two-fold acylation of 5′-dimethoxytrityl-protected uridine **14**[23] (Scheme 5). In the latter case the succinate linkers were first introduced to yield **16**, which was then esterified with pentadecanol[24] to afford **17**.

With the same motivation the trispalmityl ester **19** was synthesized by three-fold acylation of uridine **18** with palmitoyl chloride following a literature method.[25]

Nucleosides in which lipophilic groups are attached to a 2′-amino or 2′-thio group were synthesized starting from 5′-DMT-uridine.[23] *N*- or *S*-unsubstituted 5′-DMT-protected 2′-amino-2′-deoxyuridine **21** and 2′-thio-2′-deoxyuridine **22** were obtained following a known route via the so-called 2′,2-*O*-anhydrouridine **20** which was treated with trichloroacetonitrile and subsequently with NaOH solution[8] or with (4-methoxyphenyl)methanethiol and then with TFA,[26] respectively (Scheme 6).



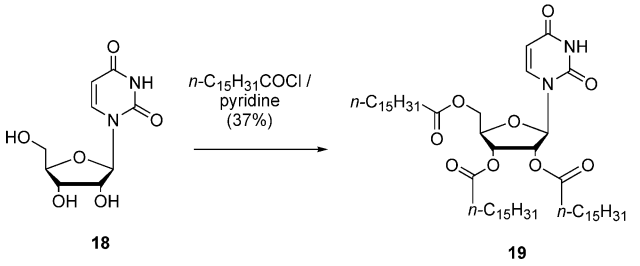
Scheme 3.



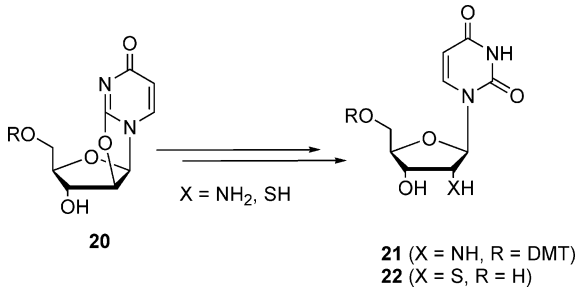
Scheme 4.

Lipidation of the 2-amino group of **21** was achieved in three different ways: *N*-Acylation, *N*-alkylation, and reductive amination (Scheme 7).[27]

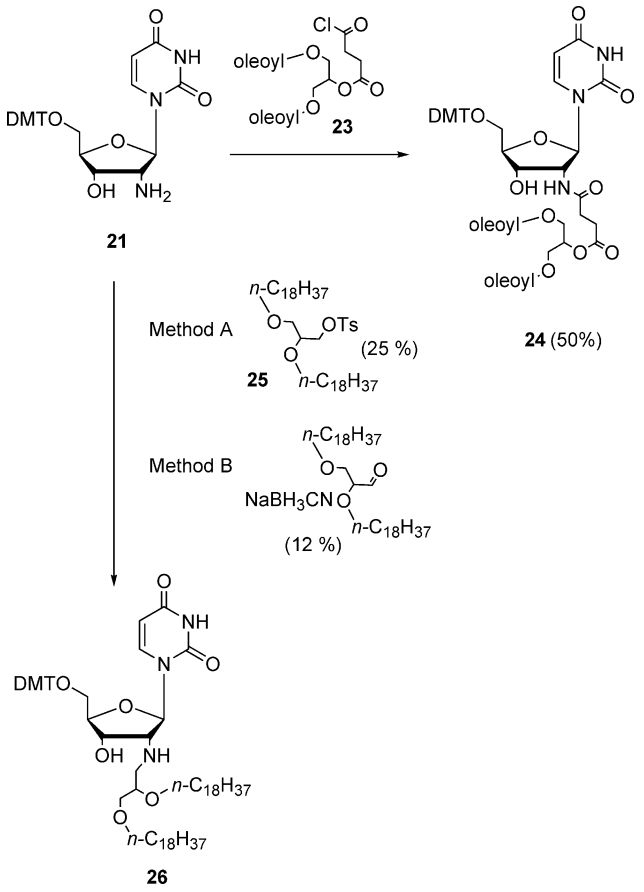
Acylation of the 2′-aminouridine **21** with the known glycerol triester **23**[28–30] afforded 50% of the expected 2′-acylaminouridine **24** in which two lipophilic oleoyl groups are tethered to the amino group. Alternatively, two lipophilic groups could be introduced into **21** (formation of **26**) by alkylation with glycerol ether tosylate **25**[31,32] obtained from the known 1,3-dioctadecylglycerol. Reductive amination of 2′,3′-dioctadecylglyceraldehyde using **21** in the



Scheme 5.



Scheme 6.



Scheme 7.

presence of sodium cyanoborohydride gave **26**.[27] The low yield of the dioctadecyloxyethyl product **26** can be attributed to problems in its isolation and purification.

Tethering lipophilic substituents at the sulfur atom of 2′-thio-2′-deoxyuridine **22** was possible without protection of the hydroxy groups. It is known from the literature that *S*-alkyl groups can be introduced into the 2′-position of **22** by *S*-alkylation with the corresponding alkyl bromides or iodides.[33,34] Here, the lipophilic thioethers **27**–**30** could be obtained in the former way. The structure of the *S*-hexadecyl thioether **27** was confirmed by X-ray crystal analysis (Figure 1). Interestingly, lipophilic and polar layers are formed in the crystal by hydrophobic interaction of the lipid chains and hydrogen bonding of the uracil moieties, respectively. The lipid layers are twisted with respect to each other by about 120°. Products **29** and **30** contain pyrene and dansyl, respectively, as the fluorescence labels. In the case of **31** two lipid groups were introduced by alkylation. Diisopropyl azodicarboxylate-mediated formation of unsymmetrical disulfides from thiols is an established way to link ligands to biological SH-containing molecules.[35] This methodology was used here (Method A) to introduce the lipophilic octa-

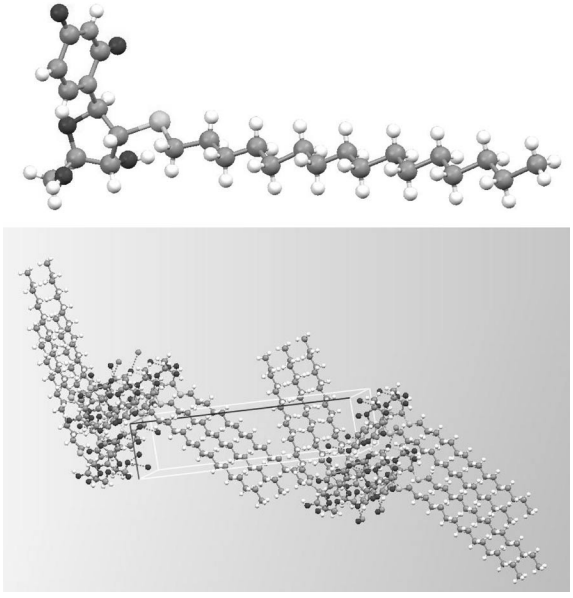
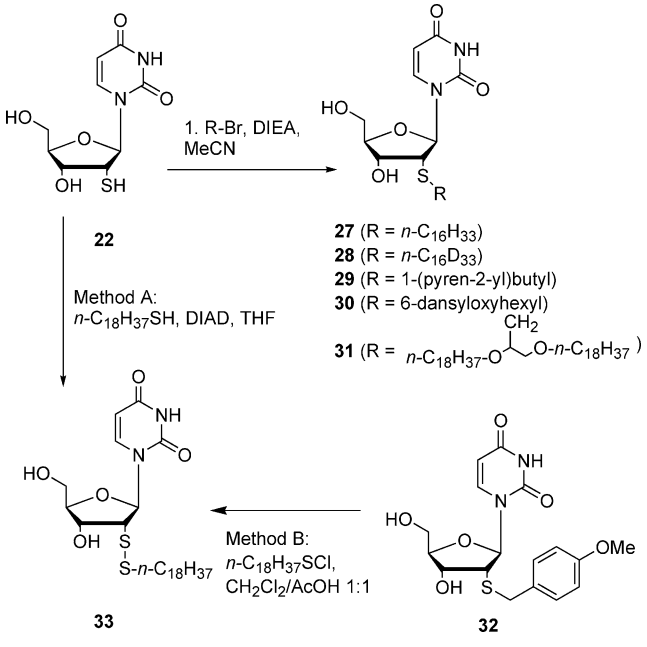


Figure 1. X-ray crystal structure of 2′-hexadecylthio-2′-deoxyuridine (**27**). The upper structure shows the single molecule and the lower panel depicts the molecular assembly in the crystal.



Scheme 8.

decylthio group to the 2′-thio-2′-deoxyuridine **22** to afford the disulfide **33**, albeit in low yield (Scheme 8). An alternative access to **33** (Method B) was achieved by treatment of the uridine 4-methoxybenyl thioether **32** with octadecylsulfenyl chloride following a literature procedure.[36]

Anchoring in Lipid Membranes

To characterize membrane insertion of selected lipophilic nucleosides we used solid-state NMR spectroscopy.

Molecules **7/8**, **15**, and **19** feature a uridine moiety in the "head group". The membrane affinity of these nucleosides is provided by one, two, or three lipophilic groups connected to the 2′-, 3′-, or 5′-positions of ribose. These structures are ideal targets to investigate the effect of the number and position of the lipophilic anchors on the membrane-binding properties of these molecules. In addition, we studied the membrane anchorage of thionucleoside **27** and the corresponding chain-deuteriated molecule **28** with one lipophilic anchor at the 2′-position as well as the membrane anchorage of **11** carrying two unsaturated anchors connected to the 3′-position via a polar spacer.

Alterations in the structural and dynamic characteristics of the phospholipid membranes after incorporation of the lipophilic nucleosides were investigated by ^{31}P and ^{2}H NMR spectroscopy.[37,38] The ^{31}P NMR spectra of POPC membranes in the presence of 20 mol-% of the five selected compounds are presented in Figure 2.

All lipophilic nucleoside/lipid dispersions exhibit a ^{31}P axially symmetric powder pattern with an intense high-field peak and a shallow low-field shoulder typical of liquid crystalline bilayers. There are no indications of nonlamellar or isotropic phases indicating that even high concentrations of lipophilic nucleosides do not alter the structure and dynamics of the membrane head group. The width of the ^{31}P spectra is called the chemical shift anisotropy (CSA, $\Delta\sigma$). Alterations in the CSA can be attributed to changes in the dynamics or orientation of the lipid head group. Quantitative values for $\Delta\sigma$ obtained from best-fit simulations are listed in Table 1. All lipophilic nucleosides except for **15** slightly reduce the CSA compared with pure POPC membranes. The largest but still fairly modest alterations can be observed for **7/8**, **19**, and **11**.

To investigate the effect of the incorporation of lipophilic nucleosides on the packing properties in the membrane acyl chain region, ^{2}H NMR spectra of chain-perdeuteriated [D_{31}]POPC were acquired. From these spectra, smoothed order parameter profiles were extracted (Figure 3).[39] The carbon atoms are numbered starting from the carbonyl group of the perdeuteriated palmitoyl chain of [D_{31}]POPC.

All the lipophilic nucleosides increase the chain order of pure [D_{31}]POPC membranes. The lipophilic nucleoside **19** drastically increases the order of the lipids within the membrane. This can be explained by the cone-shaped molecule with a relative small head group size relative to the large acyl chain area of the three hydrocarbon chains. Moderate order parameter changes are observed for [D_{31}]POPC membranes in the presence of **7/8**, **11**, and **15**, whereas **27** has a negligible effect on the phospholipid chain order in the membrane. This can also be seen from the average order parameters reported in Table 1. As order parameters are directly related to the average length of the lipid chains, these effects can also be seen from the average order parameter and the hydrocarbon chain length reported in Table 1.

To provide information about lipid-chain dynamics, ^{2}H NMR spin-lattice relaxation measurements were conducted. For each peak doublet in the ^{2}H NMR spectra the Zeeman order longitudinal relaxation time (T_{1Z}) was determined in an inversion recovery experiment. By correlating the longitudinal relaxation rate R_{1Z} (inverse relaxation time) with the squared order parameters for the individual

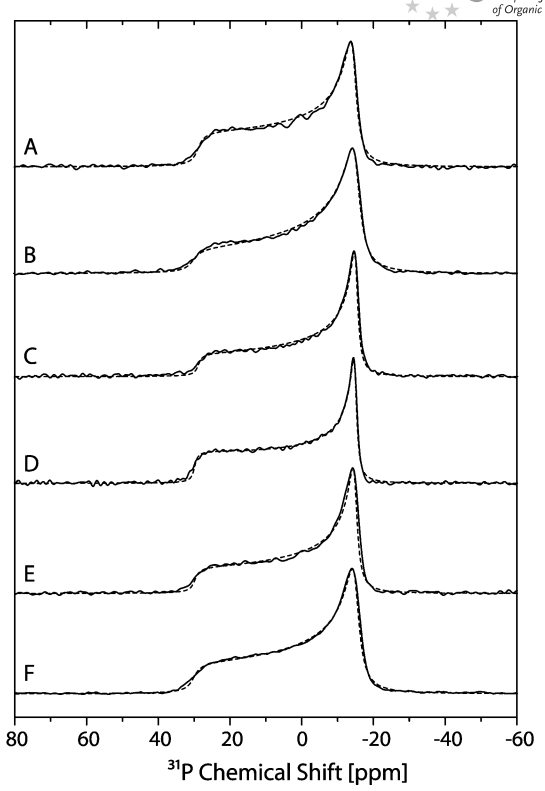


Figure 2. ^{31}P NMR spectra of POPC membranes in the presence of 20 mol-% **7/8** (A), **15** (B), **19** (C), **11** (D), **27** (E), and of pure POPC membranes as the reference (F). The measurements were conducted at a water content of 40 wt.-% and a temperature of 30 °C. The dashed lines represent best-fit simulations of the ^{31}P NMR spectra.

Table 1. Chemical shift anisotropies, average order parameters, and average chain lengths of POPC in membranes containing 20 mol-% of lipophilic nucleosides, as determined from ^{31}P and ^{2}H NMR spectra.

Sample	$\Delta\sigma$ [ppm]	S_{av}	$<L_C^*>$ [Å]
POPC	45.2	0.153	11.46
POPC/**7/8**	43.4	0.162	11.74
POPC/**11**	44.1	0.167	11.88
POPC/**15**	45.5	0.165	11.84
POPC/**19**	44.0	0.183	12.35
POPC/**27**	44.7	0.154	11.51
POPC/**28**	–	0.152	11.36

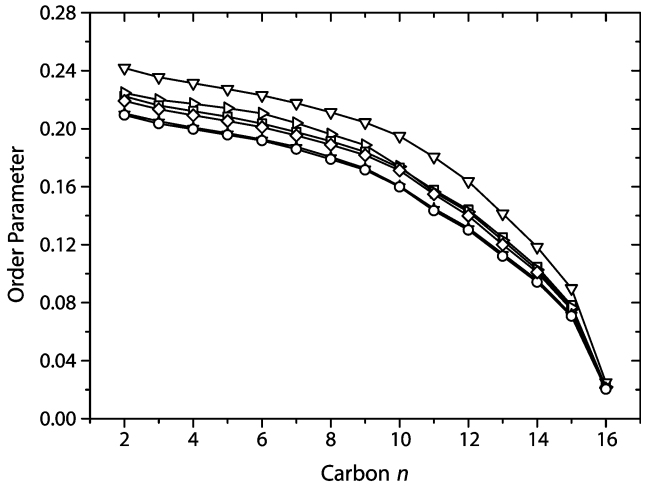


Figure 3. Smoothed order parameter profiles obtained from ^{2}H NMR spectra of [D_{31}]POPC of the samples [D_{31}]POPC (○), (**7/8**)/[D_{31}]POPC (✧), **11**/[D_{31}]POPC (), **15**/[D_{31}]POPC (□), **19**/[D_{31}]-POPC (∇), and **27**/[D_{31}]POPC (*) all at a molar ratio of 1:4 and a temperature of 30 °C.

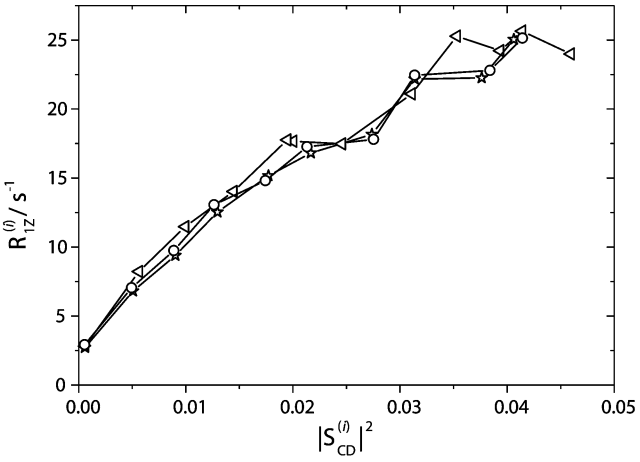


Figure 4. Dependence of the longitudinal relaxation rate $R_{1Z}^{(i)}$ on the corresponding order parameter squared for **27**/[D_{31}]POPC (*) and **28**/POPC () at a molar ratio of 1:4, and for pure [D_{31}]POPC (○) at 40 wt.-% H_2O and a temperature of 30 °C. The elastic properties of the [D_{31}]POPC membranes are unchanged in the presence of the lipophilic nucleoside **27** indicating that this molecule is well incorporated into the bilayer without interfering with the elastic properties of the membrane. This is also seen from the square-law plot of deuteriated **28** which is essentially identical to POPC in the presence and in the absence of **27**.

methylene and methyl groups, a linear dependence for saturated acyl chains is typically found for phospholipid membranes.[40,41] The slope of such a square-law plot directly relates to the elastic properties of the membrane and is inversely related to the softness of the membrane.[42] For this more time-consuming measurement, we chose the lipophilic nucleoside that showed the least membrane perturbation (**27**). To obtain information about the molecular dynamics of the host membrane in the presence of the lipophilic nucleoside as well as for the lipophilic nucleoside itself, we also studied the deuteriated analogue (**28**) of molecule **27**. The square-law plots for the samples **27**/[D_{31}]POPC, **28**/POPC, and pure [D_{31}]POPC membranes at 30 °C are shown in Figure 4.

The square-law plots of the [D_{31}]POPC membranes are identical in the presence and in the absence of **28** indicating unchanged elastic properties of the lipid matrix despite incorporation of the lipophilic nucleoside into the membrane. The chain-deuteriated **27**, that is, **28**, shows identical behavior suggesting that it is homogeneously distributed and well incorporated into the membrane.

The preferential localization of the lipophilic nucleosides within the lipid matrix was determined by ^{1}H NOESY NMR spectroscopy under magic-angle spinning conditions.[43,44] The intermolecular NOESY cross-peaks between the nucleobase/ribose moiety of the lipophilic nucleosides and the segments of the lipid molecules were integrated and cross-relaxation rates σ_{ij} were determined by using the spin-pair model. The cross-relaxation rates reflect the probability of the contacts of protons and can be used to characterize the preferential localization of the lipophilic nucleosides within the membrane.[43,44] By plotting the values of σ_{ij} between hydrogen atoms of the nucleobase/ribose and the lipid segments as a function of the membrane coordinates of such segments information about the distribution of the nucleosides parallel to the membrane normal can be collected. The lipid coordinates were obtained from a molecular dynamics simulation of POPC.[45] Because the absolute values of the cross-relaxation rates belonging to **7/8**, **11**, **15**, and **19** vary as a result of small correlation time differences normalization is needed. The nucleoside moieties of the molecules are broadly distributed along the bilayer normal illustrated in Figure 5.

The maximum of the distribution of the nucleobase/sugar moiety, that is, the highest probability of molecular contacts, is found in the lipid/water interface of the membrane for all lipophilic nucleosides. Significantly lower probabilities of contacts are found in the fatty acid chain region. The cross-relaxation rates in the lipid head group are significantly increased for **15** compared with **19**. Yet a higher probability of contacts in this region can be observed for **7/8**. The highest relative cross-relaxation rates in the lipid head group are measured for **11**. The shift of the probability distribution to the head-group region can be explained by the different number of lipophilic anchors in the three nucleosides. Nucleoside **19** carries three lipophilic groups and exhibits the highest hydrophobic energy with the most stable incorporation into the membrane and localization of the nucleobase in the upper-chain region. The nucleosides **7/8** contain one lipophilic anchor. The probability distribution of the uracil of these molecules is significantly shifted to the head-group region. The lipophilic nucleoside **15** shows a distribution with respect to the membrane that is intermediate between **7/8** and **19**. In contrast, the most probable localization of the nucleobase close to the head group is found for substance **11**. This is achieved as a result of the polar spacer between the ribose and the lipophilic anchors pushing the uracil moiety outwards.

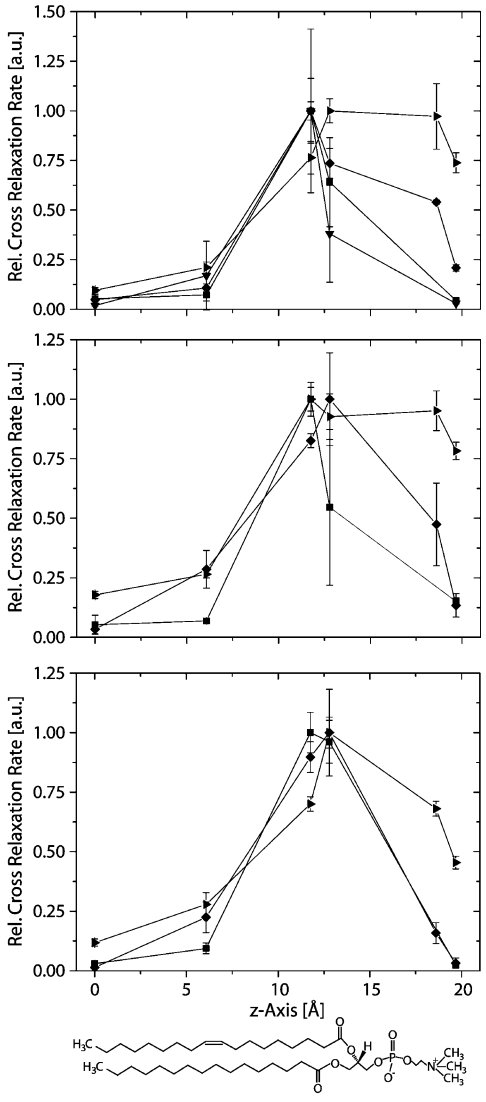


Figure 5. Relative ^{1}H NOESY cross-relaxation rates between the nucleoside moiety of (**7**/**8**) (♦), **11** (►), **15** (■), and **19** (▼) and segments of the POPC molecules within the membrane as a function of the coordinates of such lipid segments. Localization of 6-H (top) and 5-H (middle) of the uracil nucleobase and of 1′-H of the ribose with respect to the membrane normal is shown. The approximate localization of these hydrogen atoms with respect to various segments of the lipid molecules in the host membrane can be estimated from the phospholipid molecule drawn below. The measurements were carried out at a 1:4 molar ratio of lipophilic nucleoside/POPC and at a temperature of 30 °C.

Conclusions

A series of new uridines have been synthesized bearing one or two lipophilic groups in the 2′-position. The lipophilic moieties can be fixed with or without spacers at O, N, or S atoms at the 2′-position. The syntheses are straight forward and provide flexibility as far as the lipophilic groups are concerned. The lipophilic uridine products have the potential to be incorporated into any position of the oligonucleotide chain. All the lipophilic nucleosides can be incorporated into lipid membranes without inducing nonlamellar or isotropic phases. Hence, the incorporation of such nucleosides should not interfere with the integrity of the membranes causing enhanced permeability or even lysis. However, the influence of individual molecules on lipid order varies. The best-suited lipophilic nucleoside is **27** which does not seem to alter membrane structure or dynamics. The sugar/nucleobase moiety of the all molecules is located in the lipid/water interface of the membrane. An increased number of hydrocarbon chains attached to the lipophilic nucleoside drag the nucleobase deeper into the membrane. Therefore, to expose it to the aqueous environment for possible base-pairing, a molecular spacer may provide the desired property.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively, with a Bruker AC 300 in $CDCl_3$ with TMS as the internal standard. Silica gel (0.04–0.063 mm, Merck) was used for preparative column chromatography. Starting materials **3**,[17] **20**,[8] **21**,[8] and **22**[26,34] were synthesized according to literature procedures. All other materials were purchased from commercial suppliers.

Membrane Incorporation of Lipophilic Nucleosides: POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and [D_{31}]POPC (1-[D_{31}]palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. For ^{2}H, ^{31}P, and ^{1}H NOESY NMR measurements, mixtures of phospholipids and lipophilic nucleosides were prepared in a chloroform/methanol mixture (1:1 v/v). The solvent was removed by rotary evaporation and the resulting lipid film was redissolved in cyclohexane and lyophilized overnight to obtain a fluffy powder. Samples were hydrated with 40 wt.-% water and equilibrated by 10 freeze–thaw cycles and gentle centrifugation. The liposome dispersions were transferred into 4 mm high-resolution MAS rotors fitted with spherical Kel-F inserts for liquid samples or into 5 mm glass vials for static ^{2}H NMR experiments. ^{31}P NMR spectra were accumulated with a Bruker DRX 600 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) at a resonance frequency of 242.8 MHz for ^{31}P NMR by using a Hahn echo pulse sequence with a 90° pulse length of 7 μs, a Hahn echo delay of 50 μs, a spectral width of 100 kHz, and a recycle delay of 4 s. Continuous-wave proton-decoupling was applied during signal acquisition. Spectral simulations of the ^{31}P NMR line-shape were carried out to obtain the chemical shift anisotropy ($\Delta\sigma$) using a program written in Mathcad 2001.[46] ^{2}H NMR spectra were recorded with a Bruker Avance 400 NMR spectrometer at a resonance frequency of 61.5 MHz for ^{2}H by using a solid probe with a 5 mm solenoid coil. The ^{2}H NMR spectra were accumulated by using the quadrupolar echo sequence and a relaxation delay of 1 s. The two 3.1 μs $\pi/2$ pulses were separated by a 60 μs delay. ^{2}H NMR spectra were depeaked and order parameters for each methylene group in the chain were determined as described previously.[39]

For the ^{2}H NMR relaxation studies a phase-sensitive inversion recovery quadrupolar echo pulse sequence was used to obtain the spin-lattice relaxation time T_{1Z}. Spectral simulations of the ^{2}H NMR spectra were carried out to obtain the cross-relaxation rates by using a program written in Mathcad 2001.

^{1}H MAS NMR spectra were acquired at a spinning frequency of 6009 Hz with a Bruker DRX 600 NMR spectrometer by using a 4 mm HR-MAS probe. Typical $\pi/2$ pulse lengths were 9 μs. A ^{2}H

lock was used for field stability. Two-dimensional ^{1}H MAS NOESY spectra were acquired at various mixing times (between 1 and 600 ms). The dwell time of the indirect dimension was set equal to one rotor period to avoid folding of spinning sidebands into the center band region of the 2D NOESY spectra. Typically, between 400 and 500 data points were acquired in the indirect dimension with 32 scans per increment at a relaxation delay of 3.5 s. The volumes of the diagonal and the cross-peaks were integrated by using the Bruker TopSpin software package.[47] NOE build-up curves were fitted to the spin-pair model to obtain cross-relaxation rates. All spectra were acquired at a temperature of 30 °C.

CCDC-679143 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

3′,5′-*O*-[Oxybis(diisopropylsilyl)]-2′-*O*-(stearoylaminocarbonyl)uridine (4): *n*-Octadecylamine (550 mg, 2.2 mmol) was added to a solution of **3** (1.16 g, 4.0 mmol) in CH_2Cl_2 (30 mL) and the completion of reaction (from 1 h to several days) was monitored by TLC (EtOAc). After 48 h the reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with water (10 mL), 5% citric acid (10 mL), and water (10 mL), then dried ($MgSO_4$), evaporated, and the residue purified by chromatography (EtOAc) to give **4** (1.17 g, 1.6 mmol, 78%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.60 (s, 1 H, NH), 7.64 (d, J = 8.1 Hz, 1 H, 6-H), 5.87 (s, 1 H, 1′-H), 5.21 (d, J = 8.1 Hz, 1 H, 5-H), 5.26 (d, J = 5.1 Hz, 1 H, CH), 4.82 (d, J = 6.1 Hz, 1 H, CH), 4.41 (d, J = 5.1 Hz, 1 H, CH), 4.19 (m, 1 H, CH), 4.00 (m, 2 H, CH_2), 3.21 (m, 2 H, 5′-H), 1.50 (s, 2 H, CH_2), 1.26 (s, 26 H, CH_2), 1.01 (s, 24 H, –CH–CH_3), 0.89 (t, J = 6.6 Hz, 3 H, –CH_2–CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.5 (C-4), 154.6 (C=O), 149.5 (C-6), 139.6 (C-2), 102.2 (C-5), 88.8 (CH), 82.2 (CH), 75.6 (CH), 68.1 (CH), 59.8 (CH_2), 41.2 (CH_2), 31.9 (CH_2), 29.8–29.3 (CH_2), 26.7 (CH_2), 22.7 (CH_2), 17.5 (–*C*H–CH_3), 16.9 (–*C*H–CH_3), 14.1 (–CH_2–*C*H_3), 13.4 (–CH–*C*H_3), 12.9 (–CH–*C*H_3) ppm.

2′-*O*-(Stearoylaminocarbonyl)uridine (5): Triethylamine trihydrofluoride (10 mL, 2.00 mmol) was added to a solution of **4** (1.1 g, 1.5 mmol) in THF (5 mL) in a screw-top Teflon can (Nalgene) and the mixture was left for 6 h at room temperature [the completion of deprotection was checked by TLC (15% MeOH in CH_2Cl_2, v/v)] and then diluted with hexane (5 mL). The upper layer was discarded, the residue was washed with a 1:1 (v/v) toluene/hexane mixture (3 × 5 mL) by decantation and trituration in absolute EtOH (1 mL) gave the crystalline product **5** (0.48 g, 0.9 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.66 (d, J = 8.1 Hz, 1 H, 6-H), 5.91 (s, 1 H, 1′-H), 5.77 (d, J = 8.1 Hz, 1 H, 5-H), 5.26 (t, J = 5.3 Hz, 1 H, CH), 4.58 (t, J = 5.0 Hz, 1 H, CH), 4.22 (s, 1 H, CH), 3.90 (m, 2 H, CH_2), 3.17 (m, 2 H, 5′-H), 1.64 (s, 2 H, CH_2), 1.26 (s, 26 H, CH_2), 0.89 (t, J = 6.6 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.5 (C-4), 150.5 (C-6), 141.2 (C-2), 103.6 (C-5), 91.2 (CH), 84.4 (CH), 80.2 (CH), 63.8 (CH), 60.2 (CH_2), 44.2 (CH_2), 33.7 (CH_2), 30.9–29.7 (CH_2), 19.9 (CH_2), 14.1 (CH_3) ppm.

2′-*O*-(Palmitoyl)uridine (7) and 3′-*O*-(Palmitoyl)uridine (8): Uridine **6** (500 mg, 1.30 mmol), palmitoyl chloride (540 mg, 1.95 mmol), and DMAP (40 mg, 0.40 mmol) were dissolved in anhydrous pyridine (10 mL). The reaction was stirred overnight at ambient temperature. TLC analysis (CH_2Cl_2/MeOH, 20:1) showed the complete disappearance of the starting material. The pyridine was removed in vacuo and the residue was then coevaporated with toluene (3 × 50 mL). The dark-brown residue was taken up in CH_2CI_2 (100 mL) and washed with saturated brine. After drying the organic phase with $MgSO_4$ the solvent was removed in vacuo. The crude 3′,5′-diprotected product was obtained as a light-brown foam (1.06 g, ca. 1.30 mmol) and was deprotected without any further purification. It was dissolved in MeOH (20 mL) and NH_4F (1.00 g, 27 mmol) was added. The mixture was stirred at ambient temperature overnight. The volatiles were evaporated and partitioned (CH_2Cl_2/brine). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (cyclohexane/EtOAc, 2:1) to give the crystalline product (300 mg, 0.64 mmol, 48% over two steps) as a mixture of **7/8** in a 4:9 ratio.

7: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.7 (d, J = 6.0 Hz, 1 H, 6-H), 5.88 (d, J = 2.3 Hz, 1 H, 1′-H), 5.62 (d, J = 6.0 Hz, 1 H, 6-H), 5.08 (d, J = 2.1 Hz, 1 H, CH), 4.32 (m, 1 H, CH), 3.98 (m, 1 H, CH), 3.66 (m, 2 H, 5′-H), 2.32 (m, 2 H, CH_2), 1.52 (m, 2 H, CH_2), 1.2 (m, 26 H, CH_2), 0.78 (t, J = 6.8 Hz, 3 H, CH_3) ppm.

8: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.8 (d, J = 6.0 Hz, 1 H, 6-H), 5.78 (d, J = 2.3 Hz, 1 H, 1′-H), 5.64 (d, J = 6.0 Hz, 1 H, 6-H), 5.06 (d, J = 2.1 Hz, 1 H, CH), 4.35 (m, 1 H, CH), 3.98 (m, 1 H, CH), 3.66 (m, 2 H, 5′-H), 2.32 (m, 2 H, CH_2), 1.52 (m, 2 H, CH_2), 1.2 (m, 26 H, CH_2), 0.78 (t, J = 6.8 Hz, 3 H, CH_3) ppm. **7/8:** ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.7 (C=O), 176.4 (C=O), 173.8 (C=O), 171.7 (C=O), 164.1 (C-4), 151.1 (C-6), 147.0 (C-6), 141.1 (C-2), 139.1 (C-2), 113.5 (C-5), 102.5 (C-5), 89.8 (CH), 88.1 (CH), 84.9 (CH), 83.4 (CH), 72.9 (CH), 72.6 (CH), 68.5 (CH), 61.3 (CH_2), 60.5 (CH_2), 53.9 (CH_2), 37.5 (CH_2), 35.7 (CH_2), 34.0 (CH_2), 33.9 (CH_2), 33.7 (CH_2), 31.8 (CH_2), 29.6–28.9 (CH_2), 27.2 (CH_2), 25.5 (CH_2), 24.8 (CH_2), 22.5 (CH_2), 20.9 (CH_2), 13.9 (CH_3) ppm.

2′-*O*-[1,3-Bis(oleoyloxy)isopropyloxysuccinyl]-3′,5′-*O*-[oxybis(diisopropylsilyl)]uridine (10): A solution of **2** (0.75 g, 1.5 mmol) in dry THF (20 mL), 1,3-bis(oleyloxy)isopropyl succinate (1.25 g, 1.7 mmol), DCC (0.35 g, 1.7 mmol), and DMAP (0.5 g, 0.40 mmol) were stirred under argon at ambient temperature for 3 days. The volatiles were evaporated and the residue was purified by column chromatography (cyclohexane/EtOAc, 3:1) to give the product **10** as colorless oil (0.76 g, 0.68 mmol, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.2 (s, 1 H, NH), 7.7 (d, J = 7.9 Hz, 1 H, 6-H), 5.79 (s, 1 H, 1′-H), 5.39 (m, 1 H, 5-H), 5.33 (m, 4 H, –C*H*=C*H*–), 5.24 (m, 1 H, CH), 4.26 (s, 1 H, CH), 4.20 (m, 4 H, CH_2), 3.98 (m, 2 H, CH_2), 3.65 (m, 2 H, CH_2), 2.71 (m, 4 H, CH_2), 2.66 (m, 2 H, CH_2), 2.29 (t, J = 11.9 Hz, 8 H, CH_2), 1.97 (d, J = 8.9 Hz, 2 H, CH_2), 1.58 (m, 34 H, CH_2), 1.04 (m, 24 H, –CH–CH_3), 0.85 (t, J = 6.4 Hz, 6 H, –CH_2–CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.23 (C=O), 171.07 (C=O), 170.07 (C=O), 163.26 (C-4), 149.68 (C-6), 139.31 (C-2), 129.97 (–*C*H=CH–), 129.69 (–*C*H=CH–), 102.15 (C-5), 88.53 (CH), 82.11 (CH), 77.49 (CH), 77.07 (CH), 76.64 (CH), 75.63 (CH), 69.55 (CH), 67.63 (CH), 61.85 (CH_2), 59.50 (CH_2), 33.96 (CH_2), 31.89 (CH_2), 29.75 (CH_2), 29.69 (CH_2), 29.51 (CH_2), 29.30 (CH_2), 29.16 (CH_2), 29.10 (CH_2), 27.20 (CH_2), 27.15 (CH_2), 24.80 (CH_2), 22.67 (CH_2), 17.41 (–*C*H–CH_3), 17.32 (–*C*H–CH_3), 17.25 (–*C*H–CH_3), 17.19 (–*C*H–CH_3), 16.91 (–*C*H–CH_3), 16.80 (–*C*H–CH_3), 16.75 (–*C*H–CH_3), 16.70 (–*C*H–CH_3), 14.10 (–CH_2–CH_3), 13.38 (–CH–CH_3), 12.87 (–CH–CH_3) ppm.

3′-*O*-[1,3-Bis(oleoyloxy)isopropyloxysuccinyl]uridine (11): Triethylamine trihydrofluoride (0.2 mL, 1.22 mmol) was added to a solution of **10** (0.68 g, 0.61 mmol) in THF (5 mL) in a screw-top Teflon can (Nalgene) and the mixture was left at room temperature for 2 h [completion of deprotection was checked by TLC (cyclohexane/EtOAc, 1:1, v/v)] and then diluted with water (5 mL). The organic layer was washed with water (3 × 2 mL), dried with Na_2SO_4, and evaporated to give the crystalline product **11** (0.47 g, 0.49 mmol, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (d, J = 8.1 Hz, 1 H, 6-H), 5.83 (d, J = 8.1 Hz, 1H. 5-H), 5.73 (s, 1 H, 1′-H), 5.36 (m, 1 H, CH), 5.33 (m, 4 H, –C*H*=C*H*–), 5.24 (m, 1 H, CH), 4.64 (s, 1

H, CH), 4.28 (s, 1 H, CH), 4.20 (m, 4 H, CH_2), 3.91 (m, 2 H, CH_2), 2.73 (m, 4 H, CH_2), 2.69 (m, 2 H, CH_2), 2.32 (t, J = 11.9 Hz, 8 H, CH_2), 2.00 (d, J = 8.9 Hz, 4 H, CH_2), 1.58 (m, 2 H, CH_2), 1.27 (m, 32H, –CH_2–), 0.88 (t, J = 6.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.37 (C=O), 171.98 (C=O), 171.70 (C=O), 171.57 (C=O), 163.57 (C-4), 150.92 (C-6), 141.61 (C-2), 129.95 (–*C*H=CH–), 129.65 –*C*H=CH–), 102.59 (C-5), 90.61 (CH), 83.47 (CH), 73.54 (CH), 72.90 (CH), 69.68 (CH), 61.72 (CH_2), 33.94 (CH_2), 31.84 (CH_2), 29.70 (CH_2), 29.65 (CH_2), 29.46 (CH_2), 29.26 (CH_2), 29.13 (CH_2), 29.07 (CH_2), 29.03 (CH_2), 27.16 (CH_2), 27.12 (CH_2), 24.76 (CH_2), 22.62 (CH_2), 14.07 (CH_3) ppm.

1-{2′-*O*-[1,3-Bis(oleoyloxy)isopropylsuccinyl]-3′,5′-*O*-(di-*tert*-butylsilanediyl)-(β-D-arabinofuranos-1-yl)}uracil (12): DIAD (0.14 mL) was added slowly to a solution of **6** (290 mg, 0.75 mmol), **9** (650 mg, 0.90 mmol), and PPh_3 (230 mg, 0.90 mmol) in dry THF (1.5 mL) until the red color had vanished. After 16 h at ambient temperature the volatiles were evaporated and the residue was purified by column chromatography (cyclohexane/EtOAc, 1:1) to give **12** (510 mg, 0.46 mmol, 61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.21 (d, J = 7.9 Hz, 1 H, 6-H), 6.45 (s, 1 H, 1′-H), 5.65 (d, J = 8.0 Hz, 1 H, 5-H), 5.60 (m. 1 H, CH), 5.27 (m, 4 H, –C*H*=C*H*–), 4.70 (m, 1 H, CH), 4.59 (m, 1 H, CH), 4.41 (m, 1 H, CH), 4.21 (m, 2 H, CH_2), 4.05 (m, 4 H, CH_2), 2.59 (s, 2 H, CH_2), 2.24 (t, J = 6 Hz, 4 H, CH_2), 1.95 (m, 8 H, CH_2), 1.52 (m, 4 H, CH_2), 1.26 (s, 40 H, CH_2), 1.00 (s, 18 H, C–CH_3), 0.80 (t, J = 6.8 Hz, 3 H, CH_2–*C*H_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.3 (C=O), 171.7 (C=O), 171.4 (C=O), 163.0 (C-4), 156.4 (C-6), 149.8 (C-2), 130.0 (–*C*H=CH–), 129.7 (–*C*H=CH–), 102.8 (C-5), 84.7 (CH), 81.6 (CH), 70.0 (CH), 69.6 (CH), 67.2 (CH_2), 65.8 (CH_2), 64.2 (CH_2), 61.8 (CH_2), 60.4 (CH_2), 51.8 (CH_2), 33.9 (CH_2), 31.8 (CH_2), 29.7–26.6 (CH_2), 25.8 (CH_2), 22.7 (CH_2), 21.9 (CH_2), 21.8 (CH_2), 21.0 (CH_2), 20.1 (CH_2), 19.7 (*C*–CH_3), 15.2 (C–*C*H_3) 14.1 (CH_2–*C*H_3) ppm.

1-{2′-*O*-[1,3-Bis(oleoyloxy)isopropyloxysuccinyl]-(β-D-arabinofuranos-1-yl)}uracil (13): NH_4F (1.00 g, 27 mmol) was added to a solution of **12** (510 mg, 0.46 mmol) in MeOH (20 mL). The reaction mixture was stirred overnight at ambient temperature, the volatiles were evaporated, and the remainder was partitioned (CH_2Cl_2/brine). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (cyclohexane/EtOAc, 1:1) to give **13** (330 mg, 0.35 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.0 (s, 1 H, NH), 7.54 (d, J = 7.5 Hz, 1 H, 6-H), 6.53 (s, 1 H, 1′-H), 5.76 (d, J = 3.0 Hz, 1 H, 5-H), 5.71 (d, J = 7.9 Hz, 1 H, CH), 5.27 (m, 4 H, –C*H*=C*H*–), 4.70 (m, 1 H, CH), 4.59 (m, 1 H, CH), 4.32 (m, 1 H, CH), 4.12 (m, 2 H, CH_2), 4.10 (m, 4 H, CH_2), 2.25 (t, J = 7.2 Hz, 2 H, CH_2), 1.92 (m, 8 H, CH_2), 1.54 (m, 4 H, CH_2), 1.19 (s, 40 H, CH_2), 0.81 (t, J = 6.8 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.4 (C=O), 171.8 (C=O), 171.6 (C=O), 163.7 (C-4), 151.0 (C-6), 140.1 (C-2), 130.0 (–*C*H=CH–), 129.7 (–*C*H=CH–), 102.6 (C-5), 90.7 (CH), 81.9 (CH), 74.7 (CH), 70.1 (CH), 69.9 (CH), 68.1 (CH_2), 63.7 (CH_2), 61.8 (CH_2), 33.9 (CH_2), 31.8 (CH_2), 29.7–29.1 (CH_2), 27.2 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 21.9 (CH_2), 14.1 (CH_3) ppm.

2′,3′-*O*,*O*-Dipalmitoyl-5′-*O*-(4,4′-dimethoxytrityl)uridine and 2′,3′-*O*,*O*-Dipalmitoyluridine (15): Palmitoyl chloride (1.65 g, 6.00 mmol) and DMAP (30 mg, 0.25 mmol) were added in one batch to a solution of 5′-*O*-(4,4′-dimethoxytrityl)uridine (**14**) (1.10 g, 2.00 mmol) in dry pyridine (25 mL) at 0 °C. The reaction mixture was warmed to room temp. and the completion of the reaction was monitored by TLC (cyclohexane/EtOAc, 1:3). After 2 h the reaction mixture was concentrated in vacuo and partitioned between diethyl ether (200 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil. Purification by column chromatography (SiO_2) eluting with cyclohexane/EtOAc (1:3 with 1% Et_3N) gave 2′,3′-*O*-dipalmitoyl-5′-*O*-(4,4′-dimethoxytrityl)uridine (1.66 g, 1.66 mmol, 82%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.02 (s, 1 H, NH), 7.76 (d, J = 6 Hz, 1 H, 6-H), 7.16–7.25 (m, 9 H, CH_{ar}), 6.82–6.88 (m, 4 H, CH_{ar}), 6.25 (d, J = 6 Hz, 1 H, 1′-H), 5.61 (d, J = 3.9 Hz, 1 H, 5-H), 5.41 (m, 2 H, CH),4.22 (s, 1 H, CH), 3.80 (s, 6 H, OCH_3), 3.50 (s, 2 H, CH_2), 2.36 (m, 4 H, CH_2), 2.04 (m, 8 H, CH_2), 1.63 (t, J = 6.99 Hz, 4 H, CH_2), 1.13 (s, 40 H, CH_2), 0.90 (t, J = 6.42 Hz, 6 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.9 (C=O), 164.2 (C-4), 159.5 (C-6), 150.4 (C-2), 143.8 (C_q), 139.9 (C_q), 134.9 (C_q), 130.2–127.0 (CH_{ar}), 125.3 (CH_{ar}), 113.7 (CH_{ar}), 113.1 (CH_{ar}), 102.9 (C-5), 87.6 (CH), 85.6 (CH), 82.2 (CH), 72.8 (CH), 71.0 (CH), 60.4 (CH_2), 55.2 (OCH_3), 33.9 (CH_2), 31.9 (CH_2), 29.7–27.1 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 14.2 (–*C*H_2–*C*H_3) ppm.

A solution of 5′-*O*-(4,4′-dimethoxytrityl)-protected **15** (1.66 g, 1.66 mmol) in 80% CF_3COOH (15 mL) was stirred at ambient temperature for 15 h. The reaction was diluted with CH_2Cl_2 (20 mL), carefully washed with saturated aq. $NaHCO_3$, and dried with $MgSO_4$. Silica gel flash chromatography with a short column using cyclohexane/EtOAc (2:1) gave the product **15** as a white solid (310 mg, 0.43 mmol, 29%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.11 (s, 1 H, NH), 7.68 (d, J = 8 Hz, 1 H, 6-H), 5.99 (d, J = 5.7 Hz, 1 H, 1′-H), 5.79 (d, J = 7.82 Hz, 1 H, 5-H), 5.41 (m, 2 H, CH), 4.12 (s, 1 H, CH), 3.85 (q, J = 1.9, 11.5 Hz, 2 H, CH_2), 2.32 (m, 4 H, CH_2), 1.54 (t, J = 6.99 Hz, 4 H, CH_2), 1.18 (s, 40 H, CH_2), 0.80 (t, J = 6.42 Hz, 6 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.9 (C=O), 172.5 (C=O), 163.2 (C-4), 150.4 (C-2), 103.2 (C-5), 87.7 (CH), 83.6 (CH), 72.9 (CH), 70.9 (CH), 61.8 (CH_2), 34.0 (CH_2), 33.8 (CH_2), 31.9 (CH_2), 29.7–29.1 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 14.2 (CH_3) ppm.

5′-*O*-(4,4′-Dimethoxytrityl)-2′,3′-*O*,*O*-disuccinyluridine (16): 5′-*O*-(4,4′-Dimethoxytrityl)uridine (1.10 g, 2.00 mmol), succinic anhydride (0.60 g, 6.00 mmol), and DMAP (120 mg, 1.00 mmol) were dissolved in anhydrous pyridine (10 mL). The reaction was stirred for 3 days at ambient temperature. TLC analysis (EtOAc/MeOH, 9:1) showed the complete disappearance of the starting material. Pyridine was removed in vacuo and the residue was then co-evaporated with toluene (3 × 50 mL). The dark-brown residue was taken up in CH_2CI_2 (100 mL) and washed with saturated brine. The organic phase was dried with $MgSO_4$ and concentrated in vacuo to provide the crude product **14** as light-brown foam (1.56 g, 2.00 mmol, quant. yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.0 (s, 1 H, COOH), 8.6 (s, 1 H, NH), 7.6 (d, J = 8.1 Hz, 1 H, 6-H), 7.29–7.08 (m, 9 H, CH_{ar}), 6.76 (m, 4 H, CH_{ar}), 6.15 (d, J = 6.8 Hz, 1 H, 1′-H), 5.56 (m, 2 H, 5-H, CH), 5.41 (m, 1 H, CH), 4.18 (s, 1 H, CH), 3.72 (s, 6 H, O*C*H_3), 3.40 (m, 2 H, CH_2), 2.58 (s, 8 H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4 (C=O), 171.3 (C=O), 163.7 (C-4), 158.8 (C-2), 143.9 (C_q), 140.0 (C-6), 139.7 (C_q), 139.4 (CH_{ar}), 134.9 (CH_{ar}), 134.7 (CH_{ar}), 130.2 (CH_{ar}), 130.0 (CH_{ar}), 129.1–127.1 (CH_{ar}), 125.3 (CH_{ar}), 113.4 (CH_{ar}), 113.2 (CH_{ar}), 103.1 (C-5), 87.6 (CH), 85.7 (CH), 82.1 (CH), 72.9 (CH), 71.7 (CH), 62.8 (CH_2), 55.3 (OCH_3), 29.1–28.3 (CH_2) ppm.

5′-*O*-(4,4′-Dimethoxytrityl)-2′,3′-*O*,*O*-bis(pentadecyloxysuccinyl)-uridine and 2′,3′-*O*,*O*–Bis(pentadecyloxysuccinyl)uridine (17): The disuccinate **16** (1.20 g, 1.60 mmol), pentadecanol (0.23 g, 0.80 mmol), DCC (0.19 g, 0.90 mmol), and DMAP (0.02 g, 0.20 mmol) were dissolved in anhydrous toluene (10 mL). The reaction was stirred for 3 days under argon at ambient temperature. The volatiles were evaporated and the residue was purified by column chromatography ($CHCl_3$/MeOH, 9:1, with 1% Et_3N) to give

the 5′-DMT-protected **17** (0.44 g, 0.38 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.9 (s, 1 H, NH), 7.7 (d, J = 8.1 Hz, 1 H, 6-H), 7.31–7.18 (m, 9 H, CH_{ar}), 6.85 (m, 4 H, CH_{ar}), 6.3 (d, J = 5.7 Hz, 1 H, 1′-H), 5.62 (m, 2 H, 5-H, CH), 5.31 (m, 1 H, CH), 4.26 (s, 1 H, CH), 4.09 (m, 4 H, CH_2), 3.81 (s, 6 H, OCH_3), 3.65 (m, 2 H, CH_2), 2.71 (s, 8 H, CH_2), 1.62 (m, 4 H, CH_2), 1.28 (s, 48 H, CH_2), 0.90 (t, J = 6.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1 (C=O), 171.5 (C=O), 171.3 (C=O), 162.9 (C-4), 158.8 (C-2), 143.9 (C_q), 140.7 (C-6), 139.9 (C_q), 139.5 (CH_{ar}), 137.9 (CH_{ar}), 134.9 (CH_{ar}), 134.7 (CH_{ar}), 130.2 (CH_{ar}), 130.0 (CH_{ar}), 129.5 (CH_{ar}), 129.0 (CH_{ar}), 128.2–127.0 (CH_{ar}), 125.3 (CH_{ar}), 113.4 (CH_{ar}), 113.2 (CH_{ar}), 113.0 (CH_{ar}), 102.9 (C-5), 87.6 (CH), 85.7 (CH), 82.1 (CH), 81.4 (CH), 73.1 (CH), 71.3 (CH), 65.9 (CH_2), 65.1 (CH_2), 63.1 (CH_2), 62.7 (CH_2), 55.2 (OCH_3), 33.8 (CH_2), 32.8 (CH_2), 32.0 (CH_2), 29.7–28.6 (CH_2), 25.9 (CH_2), 25.8 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 21.5 (CH_2) 14.1 (–CH_2–CH_3) ppm.

A solution of 5′-DMT-protected **17** (0.44 g, 0.38 mmol) in dry CH_2Cl_2 (80 mL) was stirred for 5 min. After adding trifluoroacetic acid (2.4 mL) the reaction mixture was stirred for 30 min and quenched with MeOH (3.0 mL). The reaction was diluted with CH_2Cl_2 (20 mL). The solution was washed carefully with saturated $NaHCO_3$ and dried with $MgSO_4$. Flash chromatography with a short column using $CHCl_3$/MeOH (9:1) with 1% Et_3N gave the product **17** as a white solid (150 mg, 0.17 mmol, 40%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.1 (s, 1 H, NH), 7.7 (d, J = 8.1 Hz, 1 H, 6-H), 6.00 (d, J = 5.3 Hz, 1 H, 1′-H), 5.78 (d, J = 8.1 Hz, 1 H, 5-H), 5.52 (m, 1 H, CH), 4.23 (s, 1 H, CH), 4.09 (m, 4 H, CH_2), 3.92 (m, 2 H, CH_2), 2.71 (s, 8 H, CH_2), 1.62 (m, 4 H, CH_2), 1.26 (s, 48 H, CH_2), 0.88 (t, J = 6.4 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2 (C=O), 171.6 (C=O), 171.4 (C=O), 163.1 (C-4), 150.4 (C-2), 141.1 (C-6), 103.1 (C-5), 88.5 (CH), 83.5 (CH), 73.1 (CH), 71.2 (CH), 65.1 (CH_2), 61.7 (CH_2), 31.9 (CH_2), 29.7–28.6 (CH_2), 25.9 (CH_2), 22.7 (CH_2), 14.1 (–CH_2–CH_3) ppm.

2′,3′,5′-*O*,*O*,*O*-Tripalmitoyluridine (19): Palmitoyl chloride (10.00 mL, 45.00 mmol) was added dropwise to a solution of uridine (2.44 g, 10.00 mmol) in dry pyridine (130 mL). After 4 h pyridine was removed in vacuo and the residue was crystallized (EtOH) to give **19** (3.55 g, 3.70 mmol, 37%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.93 (s, 1 H, NH), 7.82 (d, J = 8 Hz, 1 H, 6-H), 6.05 (d, J = 5.7 Hz, 1 H, 1′-H), 5.77 (m, 3 H, 5-H, CH), 4.59 (s, 1 H, CH), 4.40 (m, 2 H, CH_2), 4.19 (s, 1 H, CH), 2.32 (m, 6 H, CH_2), 1.61 (t, J = 6.99 Hz, 6 H, CH_2), 1.24 (s, 60 H, CH_2), 0.86 (t, J = 6.3 Hz, 9 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.8 (C=O), 163.7 (C-4), 151.1 (C-2), 141.6 (C-6), 102.8 (C-5), 90.7 (CH) 83.5 (CH), 80.2 (CH), 73.2 (CH), 61.8 (CH_2), 34.0 (CH_2), 31.9 (CH_2), 29.7–29.1 (CH_2), 24.7 (CH_2), 22.7 (CH_2), 14.1 (CH_3) ppm.

2′-Amino-2′-*N*-[1,3-bis(oleoyloxy)isopropyloxysuccinyl]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine (24): Acyl chloride **23** was prepared from 1,3-bis(oleoyloxy)isopropyl succinate which was synthesized according to the literature[28–30] as follows. The acid (6.9 g, 9.5 mmol) was dissolved in dry DCM (10 mL) and DMF (0.1 mL) whilst stirring. The solution was cooled to 0 °C and oxalyl chloride (1 mL, 10.0 mmol) was added. The solution was stirred for 3 h and then the solvent was evaporated. The residue was dried in vacuo. NaOAc (0.64 g, 7.7 mmol) and water (3 mL) were added to a solution of **21** (0.42 g, 0.77 mmol) in THF (5 mL). Compound **23** (0.57 g, 0.77 mmol) was dissolved in dry THF (3 mL) and slowly added to the stirred emulsion of **21**. After 1 h and 30 min the reaction mixture was quenched with a saturated $NaHCO_3$ solution (15 mL). The aqueous phase was extracted with diethyl ether (30 mL) three times. The combined organic phases were concentrated in vacuo and the residue purified by column chromatography (MeOH/DCM, 1:30, 1% Et_3N, R_f = 0.5). Compound **24** (0.48 g, 0.38 mmol, 50%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65 (d, J = 8.9 Hz, 1 H, 6-H), 7.29 (m, 10 H, CH_{ar}), 6.90 (m, 4 H, CH_{ar}), 6.18 (d, J = 8.1 Hz, 1 H, 1′-H), 5.44 (d, J = 8.9 Hz, 1 H, 5-H), 5.36 (m, 4 H, –C*H*=C*H*–), 4.66 (d, J = 5.3 Hz, 1 H, CH), 4.54 (s, 1 H, CH), 4.24 (m, 5 H, CH_2, CH), 3.81 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.42 (m, 2 H, 5′-H), 2.33 (t, J = 7.6 Hz, 4 H, CH_2), 2.02 (m, J = 6.2 Hz, 8 H, CH_2), 1.61 (m, 4 H, CH_2), 1.29 (m, 40 H, CH_2), 0.90 (t, J = 6.9 Hz, 6 H, –CH_2–C*H*$_3$) ppm. ^{13}C NMR (75 MHz, CDCl3): δ = 173.6 (–O–C=O), 173.5 (–NH–C=O), 172.8 (–NH–C=O), 172.6 (C-4), 158.7 ($C_{q,ar}$), 158.6 ($C_{q,ar}$), 151.4 (C-2), 144.1 ($C_{q,ar}$), 139.5 (C-6), 135.3 ($C_{q,ar}$), 135.0 ($C_{q,ar}$), 130.2 (–*C*H=CH–), 130.1 (–*C*H=CH–), 130.0 (–CH_{ar}–), 129.7 (–*C*H=CH–), 129.2 (–*C*H=CH–), 128.2 (CH_{ar}), 128.1 (CH_{ar}), 127.9 (CH_{ar}), 127.8 (CH_{ar}), 127.1 (CH_{ar}), 127.0 (CH_{ar}), 113.4 (CH_{ar}), 113.3 (CH_{ar}), 113.1 (CH_{ar}), 103.1 (C-5), 87.1 (–*C*q–), 85.4 (CH), 81.5 (CH), 71.6 (CH), 69.9 (CH), 63.8 (CH_2), 61.6 (CH_2), 55.2 (OCH_3), 34.0 (CH_2), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 24.8 (CH_2), 22.7 (CH_2), 14.1 (–CH_2–*C*H$_3$) ppm. HRMS: calcd. for $C_{73}H_{105}{}^{39}KN_3O_{14}{}^+$ 1286.7228; found 1286.7233.

2′-*N*-[2,3-Bis(octadecyloxy)propylamino]-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine (26). Method A: The tosylate **25** was prepared according to the literature.[31,32] Nucleoside **21** (0.42 g, 0.37 mmol) was dissolved in THF (4.5 mL) and **25** (1.46 g, 1.94 mmol) was added whilst stirring. DIPEA (0.3 mL) was added to the solution and the solvent slowly evaporated at 80 °C to give a melt. The temperature was raised to 150 °C and the melt was stirred for 4 h. The mixture was cooled to room temperature and the crude material was purified by silica-gel chromatography (EtOAc/cyclohexane, 1:12 and then MeOH/$CHCl_3$, 1:30, 1% Et_3N, R_f = 0.7). Product **26** (0.091 g, 0.08 mmol, 25%) was obtained as a colorless oil which solidifies after standing in a refrigerator. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (s, 1 H, 6-H), 7.27 (m, 8 H, CH_{ar}), 6.85 (m, 5 H, CH_{ar}), 5.96 (m, 1 H, 1′-H), 5.39 (s, 1 H, 5-H), 4.29 (m, 2 H, CH), 3.81 (s, 6 H, OCH_3), 3.57 (s, 1 H, CH), 3.46 (m, 9 H, CH, CH_2), 2.84 (m, 2 H, CH_2), 1.57 (m, 4 H, CH_2), 1.27 (s, 60 H, CH_2), 0.89 (s, 6 H, –CH_2–C*H*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.7 (C-4), 158.7 ($C_{q,ar}$), 150.7 (C-2), 144.2 ($C_{q,ar}$), 144.1 ($C_{q,ar}$), 140.1 (C-6), 135.2 ($C_{q,ar}$), 135.0 ($C_{q,ar}$), 130.1 (CH_{ar}), 128.0 (CH_{ar}), 113.3 (CH_{ar}), 102.3 (C-5), 88.1 (CH), 88.0 (C-1′, CH), 87.1 (C_q), 86.1 (CH), 85.8 (CH), 71.9 (CH_2), 71.8 (CH_2), 70.9 (CH_2), 70.6 (CH_2), 70.5 (CH_2), 70.4 (CH, C-3′), 66.4 (CH, C-2′), 63.6 (CH_2), 55.2 (OCH_3), 49.4 (CH_2, C-5′), 31.9 (CH_2), 30.0 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 22.7 (CH_2), 14.1 (–CH_2–*C*H$_3$) ppm. HRMS: calcd. for $C_{69}H_{110}N_3O_9{}^+$ 1124.8242; found 1124.8235.

Method B: A solution of NaCN·BH_3 (20 mg, 0.32 mmol) in MeOH (5 mL) was added o a solution of **21** (500 mg, 0.92 mmol) and 2,3-bis(octadecyloxy)propanal (590 mg, 1.00 mmol) in a mixture of MeOH (15 mL) and cyclohexane (10 mL) at 0 °C over 30 min. The volatiles were evaporated and the residue was partitioned (Et_2O/brine). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give a yellow oil. Purification by column chromatography ($CHCl_3$/MeOH, 60:1 → 30:1) gave the product **26** (120 mg, 0.11 mmol, 12%) as a white solid (identical NMR spectra).

2′-(Hexadecylthio)-2′-deoxyuridine (27): DIEA (0.87 mL, 1.50 mmol) and hexadecyl bromide (0.45 mL, 1.5 mmol) were added to a solution of **22** (260 mg, 1.00 mmol) in MeCN (5 mL). After 16 h the reaction mixture was partitioned (CH_2Cl_2/$NaHCO_3$). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 9:1) to give **27** (300 mg, 0.62 mmol, 62%). Crystallization

(CH_2Cl_2) gave an analytically pure compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.2 (s, 1 H, NH), 7.76 (d, J = 8.0 Hz, 1 H, 6-H), 5.81 (d, J = 8.1 Hz, 1 H, 1′-H), 5.73 (d, J = 8.1 Hz, 1 H, 5-H), 4.30 (d, J = 4.2 Hz, 1 H, CH), 4.11 (s, 1 H, CH), 3.88 (q, J = 11.9, 20.1 Hz, 1 H, CH), 3.40 (m, 2 H, 5′-H), 2.50 (t, J = 7.35 Hz, 2 H, CH_2), 1.53 (t, J = 7.17 Hz, 2 H, CH_2), 1.28 (s, 26 H, CH_2), 0.84 (t, J = 6.06 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.8 (C-4), 150.8 (C-2), 141.7 (C-6), 102.8 (C-5), 90.2 (CH), 86.3(CH), 71.4 (CH), 62.2 (CH_2), 53.9 (CH), 34.1 (CH_2), 32.7 (CH_2), 32.1 (CH_2), 31.8 (CH_2), 29.9–28.1 (CH_2), 22.6 (CH_2), 14.3 (–CH_2–CH_3) ppm.

2′-([D_{33}]Hexadecylthio)-2′-deoxyuridine (28): DIEA (1.25 mL, 2.13 mmol) and [D_{33}]hexadecyl bromide (730 mg, 2.13 mmol) were added to a solution of **22** (370 mg, 1.42 mmol) in MeCN (10 mL). After 16 h the reaction mixture was partitioned (CH_2Cl_2/$NaHCO_3$). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 9:1) to give **28** (500 mg, 0.97 mmol, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.4 (s, 1 H, NH), 7.76 (d, J = 8.0 Hz, 1 H, 6-H), 5.80 (d, J = 8.1 Hz, 1 H, 1′-H), 5.68 (d, J = 8.1 Hz, 1 H, 5-H), 4.25 (d, J = 4.2 Hz, 1 H, CH), 4.20 (s, 1 H, CH), 3.82 (q, J = 11.9, 20.1 Hz, 1 H, CH), 3.30 (m, 2 H, 5′-H) ppm. ^{13}C NMR (75 MHz, CDCl3): δ = 163.8 (C-4), 150.8 (C-2), 141.7 (C-6), 102.8 (C-5), 90.2 (CH), 86.3 (CH), 71.4 (CH), 62.2 (CH_2), 53.9 (CH) ppm.

2′-[4-(Pyren-2-yl)butylthio]-2′-deoxyuridine (29): DIEA (0.87 mL, 1.5 mmol) and 2-(4-bromobutyl)pyrene (500 mg, 1.5 mmol) were added to a solution of **22** (260 mg, 1.00 mmol) in MeCN (15 mL). After 16 h the reaction mixture was partitioned (CH_2Cl_2/$NaHCO_3$). The organic layer was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (CH_2Cl_2→CH_2Cl_2/MeOH, 10:1) to give **29** (430 mg, 0.83 mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.6 (s, 1 H, NH), 8.12 (m, 8 H, CH_{ar}), 7.77 (d, J = 8.3 Hz, 1 H, 6-H), 5.88 (d, J = 7.8 Hz, 1 H, 1′-H), 5.72 (d, J = 8.1 Hz, 1 H, 5-H), 4.25 (s, 1 H, CH), 3.97 (m. 3 H, CH, 5′-H), 2.77 (m, 2 H, CH, 5′-H), 2.52 (m, 2 H, CH_2), 1.63 (m, 4 H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1 (C-4), 159.8 (C_{ar}), 150.9 (C-2), 141.8 (C-6), 136.1 (C_{ar}), 132.3–123.2 (C_{ar}), 102.8 (C-5), 90.4 (CH), 86.1 (CH), 71.2 (CH), 62.1 (CH_2), 53.9 (CH), 35.0 (CH_2), 32.1 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 29.0 (CH_2) ppm.

2′-(6-Dansyloxyhexylthio)-2′-deoxyuridine (30): DIEA (0.43 mL, 0.75 mmol) and 6-bromohexyl 5-(dimethylamino)naphthalene-1-sulfonate (420 mg, 1.0 mmol) were added to a solution of **22** (130 mg, 0.50 mmol) in MeCN (10 mL). After 16 h the reaction mixture was partitioned (CH_2Cl_2/$NaHCO_3$). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (CH_2Cl_2→CH_2Cl_2/MeOH, 20:1) to give **30** (200 mg, 0.33 mmol, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.6 (s, 1 H, NH), 7.87 (d, J = 8.3 Hz, 1 H, 6-H), 7.4 (m, 6 H, CH_{ar}), 5.88 (d, J = 7.8 Hz, 1 H, 1′-H), 5.70 (d, J = 8.1 Hz, 1 H, 5-H), 4.1 (s, 1 H, CH), 3.97 (m, 2 H, CH), 3.80 (m, 1 H, CH), 3.66 (m, 3 H, CH, 5′-H), 2.57 (m, 6 H, CH_3), 2.52 (m, 2 H, CH_2), 1.53 (m, 8 H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.3 (C-4), 150.9 (C-2), 141.6 (C-6), 132.4 (C_{ar}), 131.9 (C_{ar}), 130.7 (C_{ar}), 128.7 (C_{ar}), 102.7 (C-5), 89.6 (CH), 86.2 (CH), 72.3 (CH), 71.2 (CH), 71.0 (CH), 70.8 (CH), 62.1 (CH_2), 53.9 (CH), 45.5 (CH_3), 42.5 (CH_3), 35.2 (CH_2), 33.8 (CH_2), 32.4 (CH_2), 31.7 (CH_2), 31.6 (CH_2), 30.3 (CH_2), 29.5–27.5 (CH_2), 26.1 (CH_2) ppm.

2′-[2,3-Bis(octadecyloxy)propylthio]-2′-deoxyuridine (31): DIEA (1.74 mL, 3.00 mmol) and 1-[3-bromo-2-(octadecyloxy)propoxy] octadecane (1.98 g, 3.00 mmol) were added to a solution of **22** (500 mg, 2.00 mmol) in MeCN (10 mL)/CH_2Cl_2 (10 mL). After 16 h the reaction mixture was partitioned (CH_2Cl_2/$NaHCO_3$). The organic layer was dried ($MgSO_4$), evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 9:1) to give **31** (220 mg, 0.28 mmol, 14%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.56 (s, 1 H, NH), 7.66 (d, J = 8.0 Hz, 1 H, 6-H), 5.76 (d, J = 8.1 Hz, 1 H, 1′-H), 5.67 (d, J = 8.1 Hz, 1 H, 5-H), 4.30 (d, J = 4.2 Hz, 1 H, CH), 4.17 (s, 1 H, CH), 3.98 (q, J = 11.9, 20.1 Hz, 1 H, CH), 3.76 (m, 7 H, CH, CH_2), 3.42 (m, 2 H, 5′-H), 2.55 (t, J = 7.35 Hz, 1 H, CH), 1.52 (m, 4 H, CH_2), 1.19 (s, 60 H, CH_2), 0.84 (t, J = 6.06 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4 (C-4), 150.6 (C-2), 142.3 (C-6), 102.8 (C-5), 91.4 (CH), 86.3 (CH), 71.4 (CH), 62.2 (CH_2), 53.9 (CH), 34.1 (CH_2), 32.4 (CH_2), 31.9 (CH_2), 31.8 (CH_2), 29.9–28.1 (CH_2), 22.7 (CH_2), 14.1 (–CH_2–CH_3) ppm.

2′-(Octadecyldisulfanyl)-2′-deoxyuridine (33). Method A: DIAD (0.40 mL, 3.00 mmol) was added to a solution of **22** (780 mg, 3.00 mmol) in dry THF (80 mL). TLC analysis ($CHCl_3$/MeOH, 9:1) showed the complete disappearance of the starting material after 16 h. Octadecylthiol (17.2 g, 60 mmol) was added and the reaction mixture was refluxed for 72 h. The volatiles were evaporated and the residue was purified by column chromatography ($CHCl_3$/EtOH, 9:1) to give **33** (0.40 g, 0.74 mmol, 25%). ^{1}H NMR (300 MHz, [D_6]DMSO/$CDCl_3$): δ = 7.70 (d, J = 8.1 Hz, 1 H, 6-H), 6.02 (d, J = 8.3 Hz, 1 H, 1′-H), 5.6 (d, J = 8.1 Hz, 1 H, 5-H), 4.34 (d, J = 2.1 Hz, 1 H, CH), 3.68 (m, 3 H, CH, 5′-H), 2.50 (m, 2 H, CH_2), 1.55 (t, J = 6.2 Hz, 2 H, CH_2), 1.12 (s, 28 H, CH_2), 0.75 (t, J = 6.2 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO/$CDCl_3$): δ = 164.1 (C-4), 150.8 (C-2), 141.6 (C-6), 102.5 (C-5), 90.1 (CH), 86.4 (CH), 72.2 (CH), 61.7 (CH_2), 58.6 (CH), 38.9 (CH_2), 31.6 (CH_2), 29.3–28.2 (CH_2), 22.4 (CH_2), 13.7. (CH_3) ppm.

Method B: A solution of 2′-(4-methoxybenzylthio)-2′-deoxyuridine (**32**) (0.57 g, 1.50 mmol) in CH_2Cl_2 (20 mL) and a solution of hexadecylsulfenyl chloride (2.41 g, 7.5 mmol) in CH_2Cl_2 (20 mL) were added carefully to a flask containing CH_2Cl_2 (65 mL) and AcOH (65 mL) at 0 °C. TLC analysis (CH_2Cl_2/MeOH, 9:1) showed the complete disappearance of the starting material after 62 h. The volatiles were evaporated and the residue was purified by column chromatography (CH_2Cl_2→CH_2Cl_2/MeOH, 9:1) to give **33** (0.30 g, 0.55 mmol, 37%).

Supporting Information (see also the footnote on the first page of this article): Synthesis of the starting materials, NMR spectra of new products.

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[1] I. Pfeiffer, F. Höök, *J. Am. Chem. Soc.* **2004**, *126*, 10224–10225.
[2] J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R. K. Pandey, T. Racie, K. G. Rajeev, I. Rohl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan, H. P. Vornlocher, *Nature* **2004**, *432*, 173–178.
[3] C. Lorenz, P. Hadwiger, M. John, H. P. Vornlocher, C. Unverzagt, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4975–4977.

[4] K. S. Ahn, W. Ou, J. Silver, *Virology* **2004**, *330*, 50–61.
[5] R. Chillemi, D. Aleo, G. Granata, S. Sciuto, *Bioconj. Chem.* **2006**, *17*, 1022–1029.
[6] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1993**, *49*, 1925–1963.
[7] S. T. Crooke, US Pat. 6094, **2000** [*Chem. Abstr.* **2000**, *133*, 173003].
[8] D. P. C. McGee, A. VaughnSettle, C. Vargeese, Y. S. Zhai, *J. Org. Chem.* **1996**, *61*, 781–785.
[9] M. Manoharan, L. A. Johnson, K. L. Tivel, R. H. Springer, P. D. Cook, *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2765–2770.
[10] M. Manoharan, K. L. Tivel, T. P. Condon, L. K. Andrade, I. BarberPeoch, G. Inamati, S. Shah, V. Mohan, M. J. Graham, C. F. Bennett, S. T. Crooke, P. D. Cook, *Nucleosides Nucleotides* **1997**, *16*, 1129–1138.
[11] A. Kurz, A. Bunge, A. K. Windeck, M. Rost, W. Flasche, A. Arbuzova, D. Strohbach, S. Mueller, J. Liebscher, D. Huster, A. Herrmann, *Angew. Chem. Int. Ed.* **2006**, *45*, 4440–4444.
[12] H. A. Scheidt, W. Flasche, C. Cismas, M. Rost, A. Herrmann, J. Liebscher, D. Huster, *J. Phys. Chem. B* **2004**, *108*, 16279–16287.
[13] N. Brodersen, J. Li, O. Kaczmarek, A. Bunge, L. Löser, D. Huster, A. Herrmann, J. Liebscher, *Eur. J. Org. Chem.* **2007**, in press.
[14] A. Bunge, A. Kurz, A. K. Windeck, T. Korte, W. Flasche, J. Liebscher, A. Herrmann, D. Huster, *Langmuir* **2007**, *23*, 4455–4464.
[15] S. Bonaccio, D. Capitani, A. L. Segre, P. Walde, P. L. Luisi, *Langmuir* **1997**, *13*, 1952–1956.
[16] K. Furusawa, K. Ueno, T. Katsura, *Chem. Lett.* **1990**, 97–100.
[17] V. A. Korshun, D. A. Stetsenko, M. J. Gait, *J. Chem. Soc. Perkin Trans. 1* **2002**, 1092–1104.
[18] K. Furusawa, K. Ueno, T. Katsura, *Chem. Lett.* **1990**, 97–100.
[19] H. Neumann, V. E. Shashoua, J. C. Sheehan, A. Rich, *Proc. Natl. Acad. Sci. U.S.A* **1968**, *61*, 1207–1214.
[20] G. A. R. Johnston, *Austr. J. Chem.* **1968**, *21*, 513–519.
[21] C. B. Reese, D. R. Trentham, *Tetrahedron Lett.* **1965**, *6*, 2467–2472.
[22] T. P. Prakash, A. M. Kawasaki, A. S. Fraser, G. Vasquez, M. Manoharan, *J. Org. Chem.* **2002**, *67*, 357–369.
[23] E. Moyroud, E. Biala, P. Strazewski, *Tetrahedron* **2000**, *56*, 1475–1484.
[24] N. Usman, K. K. Ogilvie, M. Y. Jiang, R. J. Cedergren, *J. Am. Chem. Soc.* **1987**, *109*, 7845–7854.
[25] M. A. Zinni, L. E. Iglesias, A. M. Iribarren, *Biotechnol. Lett.* **2002**, *24*, 979–983.
[26] K. J. Divakar, C. B. Reese, *J. Chem. Soc. Perkin Trans. 1* **1982**, 1625–1628.
[27] B. R. B. N. Kalra, V. S. Parmar, J. Wengel, *Org. Biomol. Chem.* **2004**, *2*, 2885–2887.
[28] R. O. Feuge, T. L. Ward, *J. Am. Oil Chem. Soc.* **1960**, *37*, 291–294.
[29] B. Gaucher, M. Rouquayrol, D. Roche, J. Greiner, A.-M. Aubertin, P. Vierling, *Org. Biomol. Chem.* **2004**, *2*, 345–357.
[30] G. K. E. Scriba, *Arch. Pharm. (Weinheim, Ger.)* **1993**, *326*, 477–481.
[31] M. Kates, N. Z. Stanacev, T. H. Chan, *Biochemistry* **1963**, *2*, 394.
[32] K. Odashima, K. Tohda, S. Yoshiyagawa, S. Yamashita, M. Kataoka, Y. Umezawa, *Heterocycles* **1998**, *47*, 847–856.
[33] S. F. Wnuk, *Tetrahedron* **1993**, *49*, 9877–9936.
[34] L. Zhu, P. S. Lukeman, J. W. Canary, N. C. Seeman, *J. Am. Chem. Soc.* **2003**, *125*, 10178–10179.
[35] S. F. Wnuk, E. Lewandowska, D. R. Companioni, P. I. Garcia Jr, J. A. Secrist III, *Org. Biomol. Chem.* **2004**, *2*, 120–126.
[36] S. Porcher, M. Meyyappan, S. Pitsch, *Helv. Chim. Acta* **2005**, *88*, 2897–2909.
[37] J. Seelig, *Biochim. Biophys. Acta* **1978**, *515*, 105.
[38] J. H. Davis, *Biochim. Biophys. Acta* **1983**, *737*, 117.
[39] D. Huster, K. Arnold, K. Gawrisch, *Biochemistry* **1998**, *37*, 17299–17308.
[40] T. P. Trouard, T. M. Alam, M. F. Brown, *J. Chem. Phys.* **1994**, *101*, 5229–5261.
[41] A. Vogel, C. P. Katzka, H. Waldmann, K. Arnold, M. F. Brown, D. Huster, *J. Am. Chem. Soc.* **2005**, *127*, 12263–12272.
[42] D. Otten, M. F. Brown, K. Beyer, *J. Phys. Chem. B* **2000**, *104*, 12119–12129.
[43] D. Huster, K. Arnold, K. Gawrisch, *J. Phys. Chem. B* **1999**, *103*, 243–251.
[44] H. A. Scheidt, D. Huster, *Acta Pharmacol. Sin.* **2008**, *29*, 35–49.
[45] S. Feller, personal communication.
[46] *Mathcad 2001*, MathSoft Engineering & Education, Inc., Cambridge, MA (USA), **2001**.
[47] *TopSpin 2.0*, Bruker Biospin, Karlsruhe, Germany.

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