Nucleolipids of the Cancerostatic 5-Fluorouridine: Synthesis, Adherence to Oligonucleotides, and Incorporation in Artificial Lipid Bilayers

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Dedicated to Prof. Dr. Dr. *h.c. Wolfgang Pfleiderer*, Konstanz, in admiration of his outstanding contributions to Organic Chemistry

5-Fluorouridine (**1a**) was converted to its *N(3)*-farnesylated nucleoterpene derivative **8** by direct alkylation with farnesyl bromide (**4**). Reaction of the cancerostatic **1a** with either acetone, heptan-4-one, nonadecan-10-one, or hentriacontan-16-one afforded the 2′,3′-*O*-ketals **2a**–**2d**. Compound **2b** was then first farnesylated (→ **5**) and subsequently phosphitylated to give the phosphoramidite **6**. The ketal **2c** was directly 5′-phosphitylated without farnesylation of the base to give the phosphoramidite **7**. Moreover, the recently prepared cyclic 2′,3′-*O*-ketal **11** was 5′-phosphitylated to yield the phosphoramidite **12**. The 2′,3′-*O*-isopropylidene derivative **2a** proved to be too labile to be converted to a phosphoramidite. All novel derivatives of **1a** were unequivocally characterized by NMR and UV spectroscopy and ESI mass spectrometry, as well as by elemental analyses. The lipophilicity of the phosphoramidite precursors were characterized by both their retention times in *RP-18* HPLC and by calculated log *P* values. The phosphoramidites **6**, **7**, and **12** were exemplarily used for the preparation of four terminally lipophilized oligodeoxynucleotides carrying a cyanine-3 or a cyanine-5 residue at the 5′-(n–1) position (*i.e.*, **14**–**17**). Their incorporation in an artificial lipid bilayer was studied by single-molecule fluorescence spectroscopy and fluorescence microscopy.

1. Introduction. – 5-Fluorouridine (**1a**) as well as its 2′-deoxy **1b** possess antitumor activity against various types of carcinomas, particularly of the breast and the gastrointestinal tract. Furthermore, promising results have been obtained in the topical treatment of premalignant keratosis of the skin and basal cell carcinomas [1][2]. The intrathecal use of 5-fluoro-2′-deoxyuridine (**1b**) has been studied for meningeal dissemination of malignant brain tumors, and it has been found that this nucleoside has an excellent antitumor activity and minimal neurotoxicity [3].

A large number of lipophilic prodrugs of 5-fluorouracil and its derivatives have been prepared and found to possess useful antitumor properties. Besides *Ftorafur* and its derivatives [4–10], recently 5-fluoro-5′-uridylic acid, its mono[2-(decyloxy)-3-(dodecylsulfanyl)propyl]ester, and its salts (*Fosfluridine*, *Fozivudintidoxil*) have been used for the treatment of intraepithelial proliferative diseases [11].

We now report the synthesis of novel lipophilic 2′,3′-*O*-ketal as well as of *N(1)*-farnesyl (=(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) derivatives (nucleolipids

and nucleoterpens) of compound **1a**, and discuss their lipophilicity as well as their stability towards acid. Moreover, 5′-*O*-(2-cyanoethylphosphoramidites) of the nucleolipids were prepared and appended to oligo(2′-deoxyribonucleotides) [12][13]. Such nucleic acid building blocks represent useful chain terminators which *i*) protect a nucleic acid from 5′-exonucleolytic degradation and *ii*) which lend the nucleic acid a strong lipophilicity, thereby enhancing its lipid membrane incorporation. For these reasons, such lipophilized oligonucleotides may be useful for gene silencing. The insertion of three model lipo-oligonucleotides, which contain an additional cyanine-3 or -5 residue at the 5′-($n-1$) position into lipid bilayer membranes, is studied by confocal fluorescence microscopy.

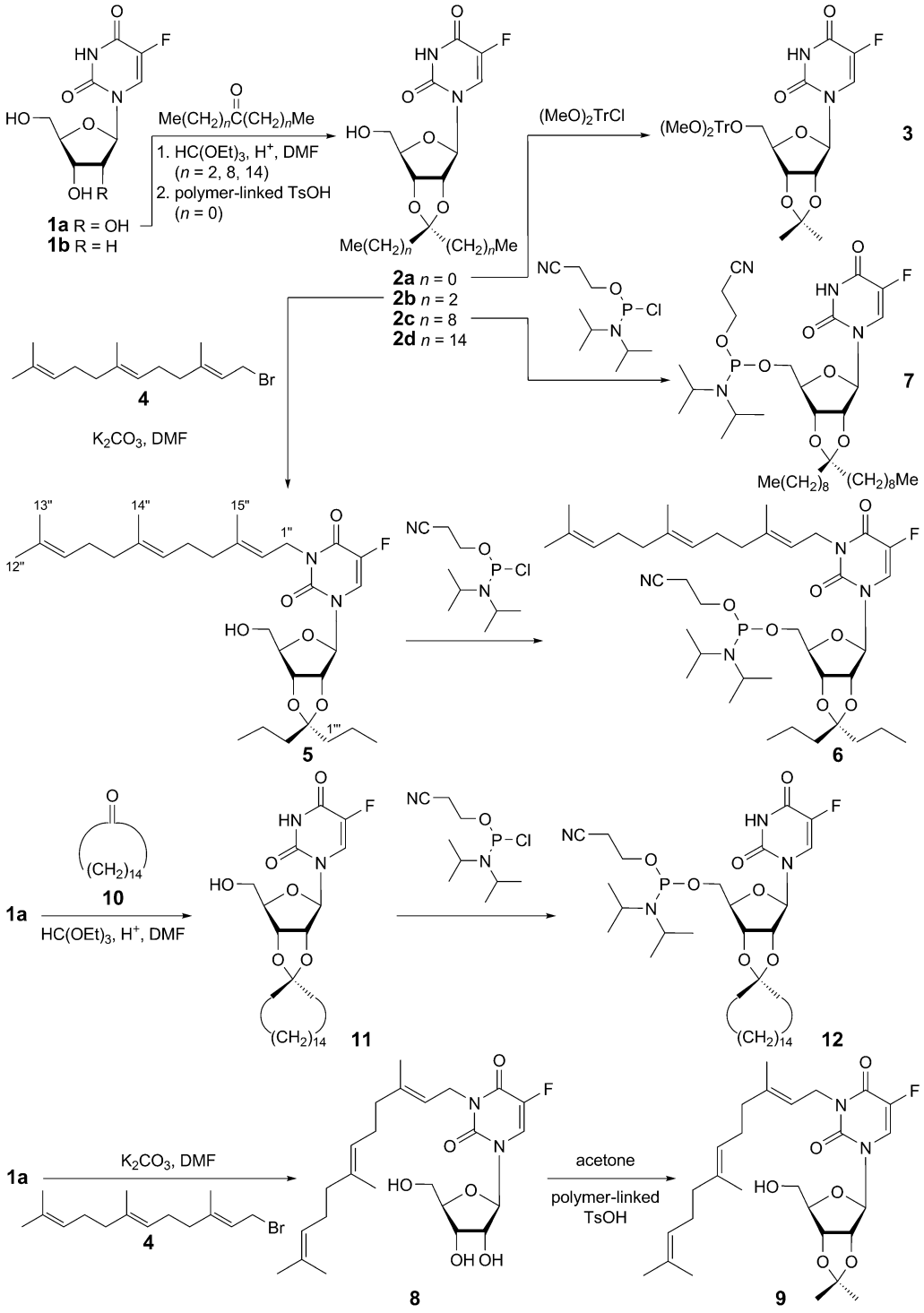
2. Results and Discussion. – 2.1. *Synthesis.* For the preparation of *N(3)*-farnesyl-5-fluorouridine (**8**), we anticipated that the protection of the glyconic OH groups would be advantageous. Therefore, first 2′,3′-*O*-isopropylidene-5-fluorouridine **2a** was prepared. For this purpose, 5-fluorouridine (**1a**) was reacted with acetone and polymer-bound TsOH as catalyst to afford the desired compound in almost quantitative yield. Subsequent reaction of **2a** with 4,4′-dimethoxytriphenylmethyl ($(MeO)_2TrCl$) chloride gave the fully sugar-protected derivative **3** (*Scheme*). However, the isopropylidene protecting group turned out to be very labile. Incubation of **2a** in 1N aqueous HCl/MeCN 1:1 (*v*/*v*), neutralization with Et_3N, and subsequent *RP-18* HPLC analysis (see *Exper. Part*) gave a half-life value, τ, of less than 1 min. Attempts to alkylate the 2′,3′-*O*-isopropylidene-protected derivative with farnesyl bromide (K_2CO_3, DMF, room temperature) were unsuccessful and led to a cleavage of the isopropylidene group. Therefore, we alkylated **1a** directly with farnesyl bromide in the presence of K_2CO_3 in DMF [12] and accepted an only moderate yield of compound **8** (43%). Subsequent introduction of an isopropylidene group gave the desired derivative **9**, also in 43% yield. Further phosphitylation of **9** at the 5′-OH group with (chloro)(2-cyanoethoxy)-(diisopropylamino)phosphine, however, led again to a cleavage of the protecting group. Therefore, for further reactions, 5-fluoro-*O*-2′,3′-(1-propylbutylidene)uridine (**2b**), described in [13], was used. In this derivative, the sugar protecting group is sufficiently stable and shows a τ value (1N aqueous HCl/MeCN 1:1 (*v*/*v*)) of 130 min. Alkylation of **2b** with farnesyl bromide (K_2CO_3, DMF, room temperature) gave, after chromatographic workup, the sugar-protected nucleoterpene **5** in 51% yield. The latter was then 5′-*O*-phosphitylated with (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine to furnish the 5′-*O*-(2-cyanoethyl phosphoramidite) **6** in 60% yield (*Scheme*).

In addition, ketalization of 5-fluorouridine (**1a**) with nonadecan-10-one [13] in the presence of $HC(OEt)_3$ and 4M HCl in 1,4-dioxane (DMF, 48 h, r.t.) gave, after workup, 5-fluoro-2′,3′-*O*-(1-nonyldecylidene)uridine (**2c**) in 68% yield, which was also 5′-*O*-phosphitylated and afforded the lipophilized 5′-*O*-(2-cyanoethyl phosphoramidite) **7** (74%). Compound **1a** was further ketalized [14] with hentriacontan-16-one, the main plant wax from expanding leek leaves [15], to yield the nucleolipid **2d**.

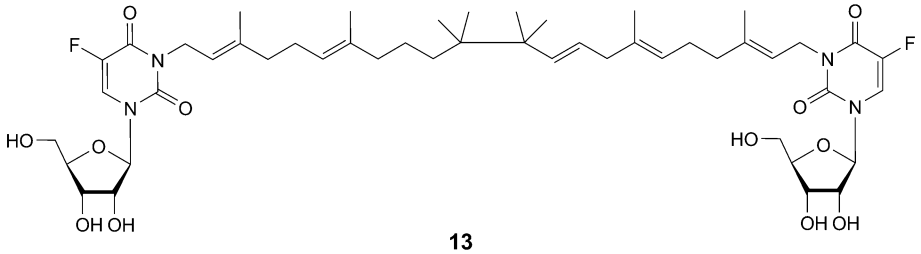
Moreover, the previously described 2′,3′-*O*-(cyclopentadecane-1,1-diyl)-5-fluorouridine (**11**) [13] was converted into its 5′-*O*-(2-cyanoethyl phosphoramidite) **12** as described above.

All novel monomeric compounds were characterized by ^1H-, ^{13}C-, and ^{31}P-NMR, and UV spectroscopy, as well as by elemental analysis or HR-ESI mass spectrometry.

Scheme


1a R = OH
1b R = H
Me(CH2)nC(CH2)nMe
1. HC(OEt)3, H+, DMF
(n = 2, 8, 14)
2. polymer-linked TsOH
(n = 0)
2a n = 0
2b n = 2
2c n = 8
2d n = 14
(MeO)2TrCl
3
4
K2CO3, DMF
5
6
7
10
HC(OEt)3, H+, DMF
11
12
K2CO3, DMF
8
acetone
polymer-linked
TsOH
9

^{1}H- and ^{13}C-NMR resonances were unequivocally assigned by gradient-selected homo- and heteronuclear correlation spectroscopy (*Bruker* pulse programs, ^{1}H,^{13}C: HSQCETGP; ^{1}H,^{1}H: COSYGPSW) as well as by DEPT-135 spectra. Since, in case of *N*-farnesylated 2′-deoxyinosine and -thymidine [12], we had detected a tail-tail dimerization of the products in the ESI-MS spectra upon ionization with 2% aqueous HCOOH, we have also expected such a reaction with compound **8** to give **13**. We were, however, unable to detect the formation of compound **13** or other dimerization products under various ESI-MS recording conditions. Moreover, the formation of stable high-molecular-weight aggregates of compound **8**, as in the case of N(1)-farnesylated 2′-deoxyinosine reported in [12], could be excluded by gel permeation chromatography (GPC).



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2.2. *Lipophilicity of the 5-Fluorouridine* (**1a**) *Derivatives.* Since the primary aim of our work is the synthesis of novel nucleolipids of 5-fluorouridine, their conversion to 5′-*O*-(2-cyanoethyl phosphoramidites), and incorporation in oligo(2′-deoxynucleotides), we studied next the lipophilicity of the novel hydrophobic nucleoside derivatives. This was accomplished in two ways: *i*) log *P* values of the compounds were calculated (*Table 1*; see *Exper. Part*) and compared with those of the unmodified nucleoside **1a**, *ii*) the chromatographic mobilities of the compounds were determined in terms of retention times (t_R [min]) by *RP-18* HPLC.

Table 1. *Calculated log* P *and RP-18 HPLC* t_R *Values of Hydrophobic 5-Fluorouridine Derivatives*

Compound	Calc. log *P*	*RP-18* HPLC t_R [min]
1a, 5-fluorouridine	-1.34 ± 0.46	1
2a	$+0.50 \pm 0.56$	unstable
8	$+6.26 \pm 0.62$	27
9	$+7.56 \pm 0.67$	24
2c	$+9.00 \pm 0.56$	>600
5	$+9.68 \pm 0.67$	87

In *Table 1*, the calculated log *P* as well as the corresponding t_R values of the various compounds are compiled. It can be seen that the calculated log *P* values vary by more than ten orders of magnitude.

2.3. *Goniometric Measurements of the Nucleolipids* **2c** *and* **11**. For both nucleolipids, goniometric measurements were performed in DMSO solution both on pendant (*Fig. 1*) and sessile drops (*Fig. 2*).

Nucleolipid concentrations varied between 0.01 and 10 mg/ml. From the measurements on pendant drops, the corresponding interfacial tension values (mN/m) were calculated by drop-shape analysis (*Fig. 1*). Measurements on sessile drops (*Fig. 2*) allow the assessment of the wetting behavior of the nucleolipid solutions by determination of the contact angle on glass plates. Inspection of *Figs. 1* and *2* reveals

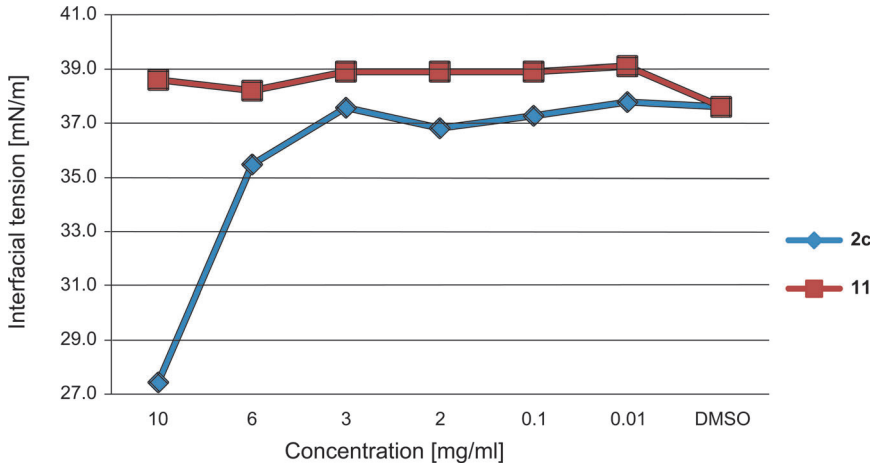

Fig. 1. *Goniometric measurements of* **2c** *and* **11** *in DMSO solutions on pendant drops*

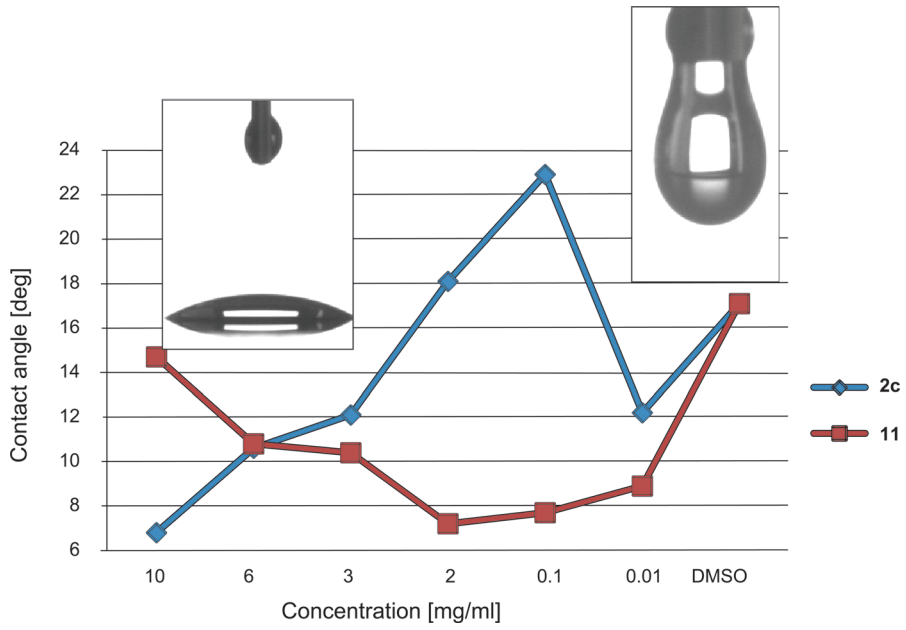

Fig. 2. *Goniometric measurements of* **2c** *and* **11** *in DMSO solutions on sessile drops*

a significantly different behavior of both nucleolipids. The reason for this remains yet unclear, but it is probably due to the structurally different ketal moieties (**2c**: acylic; **11**: cyclic).

2.4. *Synthesis of Lipo-Oligonucleotides and Their Bilayer Insertion*. First, the phosphoramidites **6** and **7**, together with a cyanine 3(Cy3)phosphoramidite, were used to prepare two oligonucleotides, **14** and **15**:

5′-d[(**5**)-(Cy3)-TAG GTC AAT ACT] (**14**) (= VO2)

5′-d[(**2c**)-(Cy3)-TAG GTC AAT ACT] (**15**) (= VO1)

The oligomers were characterized by MALDI-TOF mass spectrometry. The MS analysis revealed that, during recording the spectrum, the oligomer **14**, with a pending *N(3)*-farnesylated 5-fluorouridine residue (**5**), underwent an acid-induced deprenylation of the sesquiterpene side chain by one isoprene moiety, yielding an oligomer which carries a terminal *N(3)*-geranylated 5-fluorouridine nucleotide derivative. This was surprising, as we have successfully synthesized simultaneously corresponding oligomers which carry nucleotide residues with either pending *N(3)*-farnesylated thymidine or *N(1)*-farnesylated inosine; in those cases, no cleavage of the sesquiterpene moiety was observed during MALDI TOF analysis, which points to an electronic long-range influence of the F substituent on the farnesyl side chain.

2.4. *Bilayer Insertion of Oligonucleotides.* In the first orienting experiments, the insertion of the oligonucleotides **14** and **15** was tested at artificial bilayer membranes composed of POPE/POPC[1]) 8 : 2 (*w*/*w*) in decane (10 mg/ml) in a setup shown in *Fig. 3* (see *Exper. Part* and [16] for the detailed construction).

From *Figs. 4* and *5*, it can be seen that both lipophilized oligonucleotides are successfully incorporated in the artifical bilayer. Comparison of the brightness of the layers, however, clearly show that the oligomer **15**, carrying a double-tailed nucleolipid moiety, is inserted more efficiently. In this case, even several perfusions of 60 s each, do not lead to a significant removal of the conjugate from the bilayer, while, in the case of the oligomer **14**, the brightness of the layer decreases after two perfusions.

Moreover, the diffusion times [μs] of **14** and **15** were determined, both without and in the presence of an artificial bilayer. For the determination of the free diffusion times, the corresponding oligomer solution (50 nM) was diluted so that in the confocal measuring volume (*ca.* 10^{-15} l) only one fluorescent molecule was present. Each measurement was performed tenfold for 30 s, each. To determine the diffusion times of the lipophilized oligonucleotides **14** and **15** in the presence of a bilayer, five measuring positions above, beneath, and in the bilayer were chosen. This was necessary, because the bilayer is floating within certain limits, which makes it difficult to target it most exactly (*Fig. 3*,*a*, measuring positions 1–5). Each measurement was performed by *i*) recording reference data of a stable, blank bilayer, *ii*) addition of the oligonucleotide sample and a subsequent 25-min incubation, followed by recording the data, *iii*)

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[1]) POPE, 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phoshocholine.

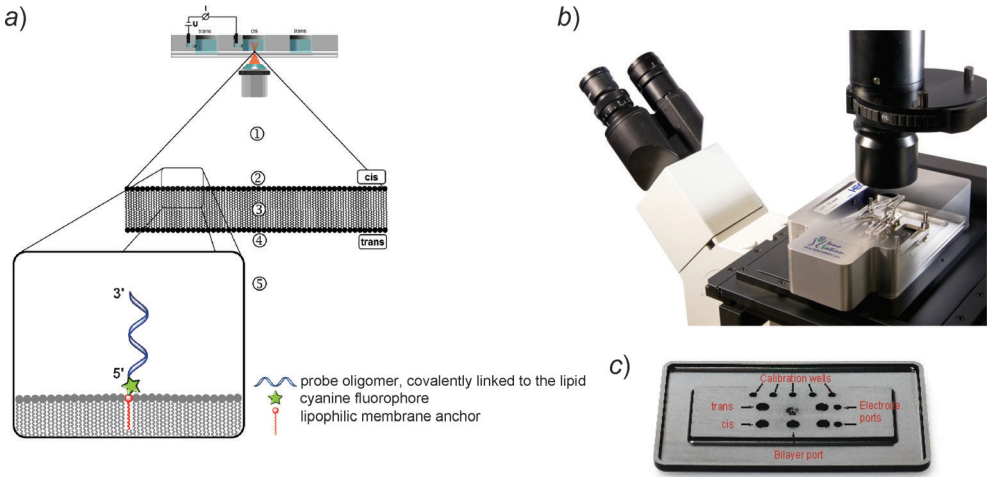


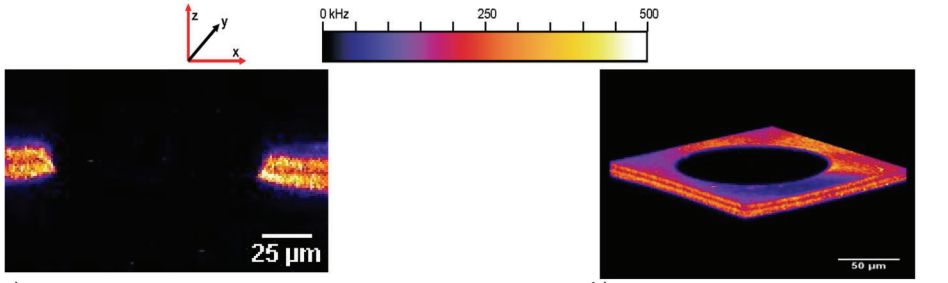
Fig. 3. *Experimental Setup*. *a*) Schematic drawing of the laser scanning microscope, the optical transparent microfluidic bilayer slide, and the lipid bilayer with incorporated double-tailed nucleolipids. The bilayer slide encloses two microfluidic channels (*cis* and *trans*), which are separated by a thin medical-grade PTFE (poly(tetrafluoroethylene)) foil. This foil hosts a central 100-μm aperture, which is located 120 μm above the coverslip and thus within the working distance of high NA (numerical aperture) objectives. It is the only connection between the *trans*- and *cis*-channel. When a lipid solution is painted across the aperture a bilayer is formed spontaneously. Electrodes in the *cis*- and *trans*-channels allow an online monitoring of the bilayer integrity, as well as electrophysiological recordings. *b*) Stage unit of the '*Ionovation Explorer*' mounted on a standard inverted fluorescence microscope. The computer-controlled perfusion unit is a sideboard and is not shown. *c*) '*Ionovation Bilayer Slide*', a disposable, optically transparent microfluidic sample carrier with perfusion capabilities. The 'Bilayer Port' gives direct access to the lipid bilayer, while both sides of the bilayer can be perfused *via* the *cis*- and *trans*-channel. Calibrations wells allow optical control experiments when needed.

recording of further data series after perfusion of the chambers. In *Table 2*, the results are collected.

It can be seen that the free diffusion time of the oligomer with a double-chained 5-fluorouridine derivative **15** is significantly longer than that of the oligomer **14**, indicating formation of a high-molecular-weight aggregate of the nucleolipid. Also near a lipid bilayer, the oligonucleotide with a **2c** residue at the 5′-terminus is incorporated

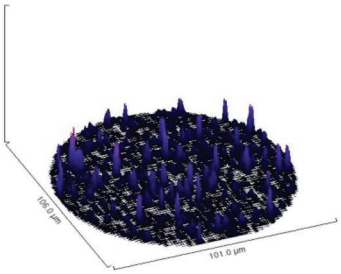
Table 2. *Diffusion Times* [μs] *of* **14** *and* **15** *without and in the Presence of a Lipid Bilayer*

Sample	Free diffusion time [μs]
15	279.67 ± 141.08
14	390.39 ± 249.05
	Diffusion times in the presence of a bilayer [μs] at measuring point 3 (*Fig. 3*,*a*)
15	33547 ± 16751, after 1. perfusion
15	12866 ± 1364, after 2. perfusion
14	75952 ± 8201, after 1 perfusion
14	53868 ± 11623, after 2. perfusion
14	19891 ± 3266, after 3. perfusion

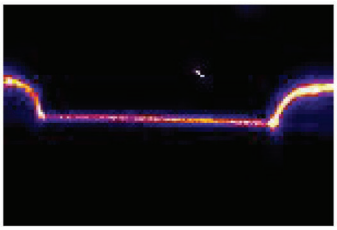


a) Z-Scan of the empty bilayer; after the bilayer has been formed, both compartments (*cis* and *trans*) were perfused (30 s, 1.1 ml/min, each).

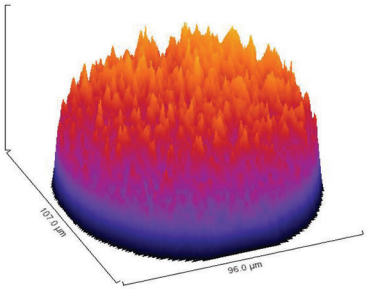
b) Tilted 3D view on an empty bilayer.



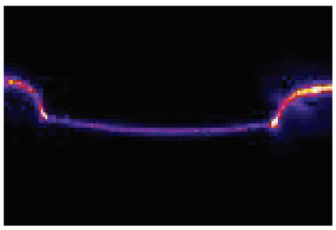
c) Pixel-resolved 3D scan of an empty bilayer.



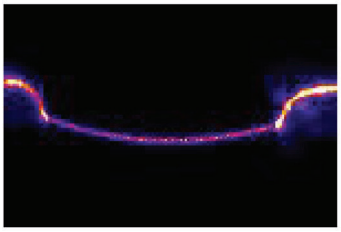
d) Z-Scan after addition of the oligomer **14** (6 µl, 50 nM) to the *cis* compartment, followed by 25 min of incubation.



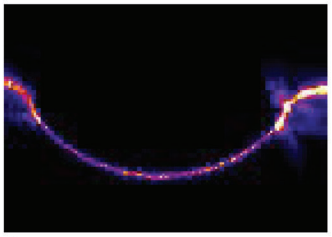
e) Pixel-resolved 3D scan of the oligomer-filled bilayer.



f) Z-Scan after 1. perfusion of the *cis* compartment (60 s, 1.1 ml/min)

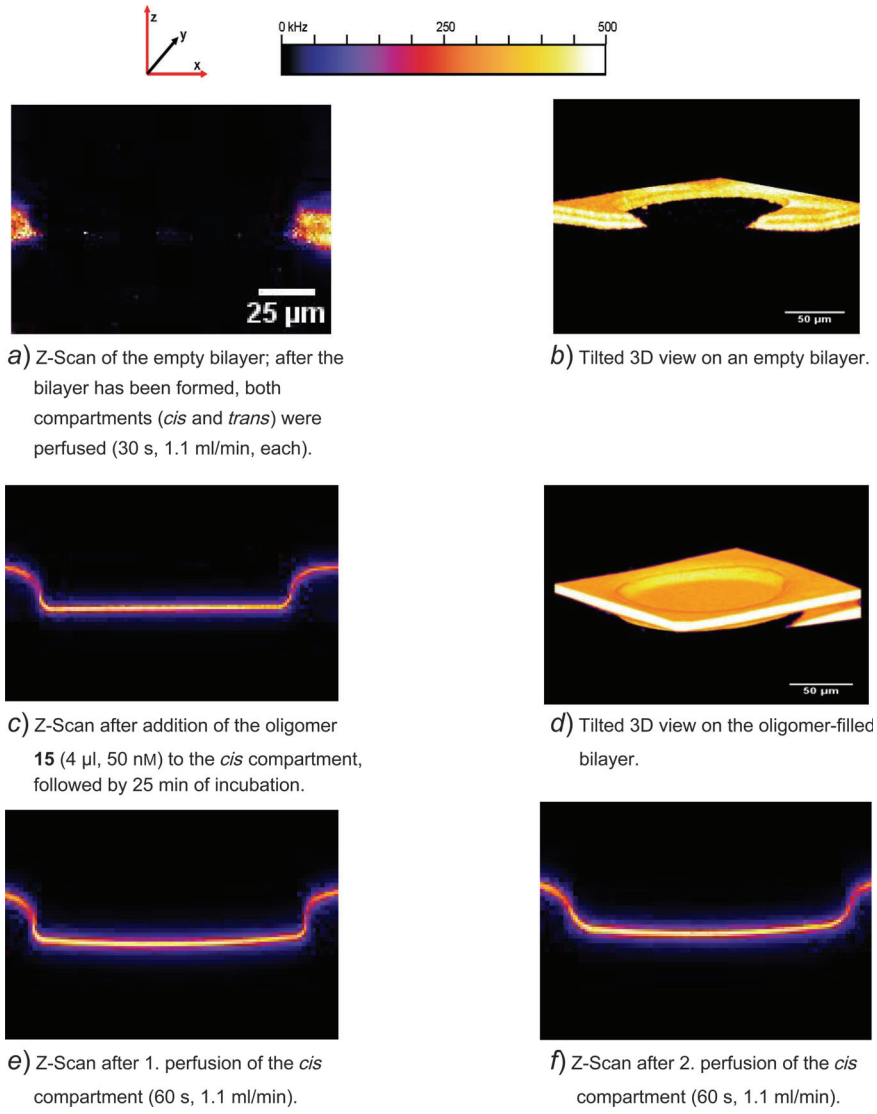


g) Z-Scan after 2. perfusion of the *cis* compartment (60 s, 1.1 ml/min).



h) Z-Scan after 3. perfusion of the *cis* compartment (60 s, 1.1 ml/min).

Fig. 4. *Chronological protocol of the bilayer insertion of the oligomer* **14**, *followed by three perfusions of the* cis-*compartment of the bilayer slide* (irradiation wavelength, 635 nm)



a) Z-Scan of the empty bilayer; after the bilayer has been formed, both compartments (*cis* and *trans*) were perfused (30 s, 1.1 ml/min, each).

b) Tilted 3D view on an empty bilayer.

c) Z-Scan after addition of the oligomer **15** (4 µl, 50 nM) to the *cis* compartment, followed by 25 min of incubation.

d) Tilted 3D view on the oligomer-filled bilayer.

e) Z-Scan after 1. perfusion of the *cis* compartment (60 s, 1.1 ml/min).

f) Z-Scan after 2. perfusion of the *cis* compartment (60 s, 1.1 ml/min).

Fig. 5. *Chronological protocol of the bilayer insertion of the oligomer* **15**, *followed by two perfusion of the* cis-*compartment of the bilayer slide* (irradiation wavelength, 635 nm)

more strongly in the bilayer and exhibits, therefore, a significantly higher diffusion time compared to that of **14**.

The above described results prompted us to repeat the experiments with the corresponding lipo-oligonucleotides **16** and **17** by using cyanine 5 as fluorophore at the $(n-1)$ position.

5′-d[(**2c**)-(Cy5)-TAG GTC AAT ACT] (**16**) (= VO3)

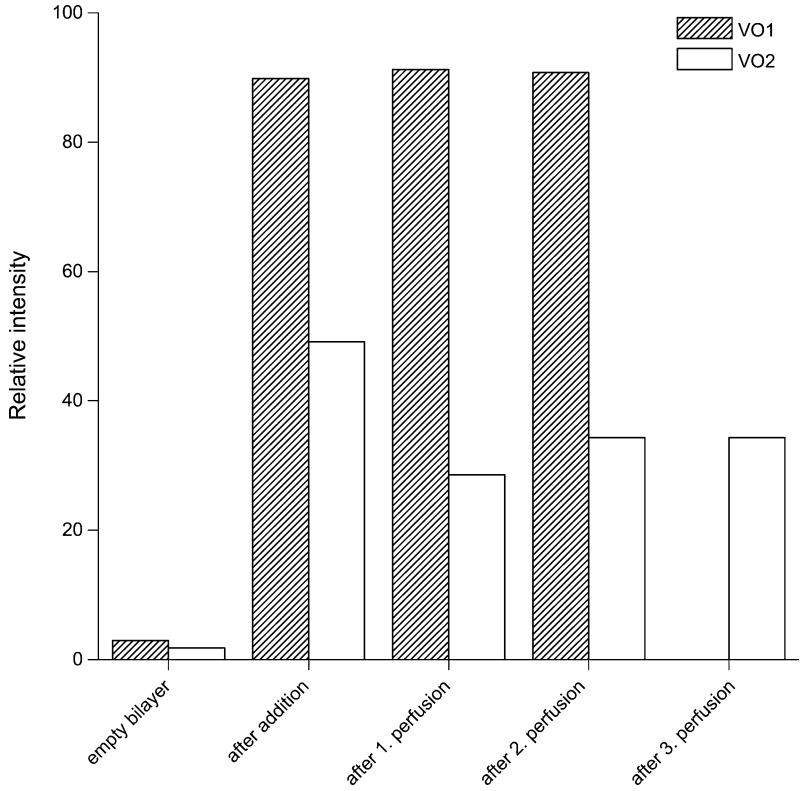


Fig. 6. *Relative bilayer brightness as a function of the perfusion number after insertion of the oligonucleotides* **14** *and* **15**

5′-d[(**11**)-(Cy5)-TAG GTC AAT ACT] (**17**) (= EM1)

The lipo-oligonucleotide **16** also carries the nucleolipid **2c**; the oligomer **17** bears the nucleolipid **11** with a cyclic ketal moiety, which has been reported in [13]. A detailed comparison of the bilayer incorporation and stability of a series of lipo-oligonucleotides carrying the above described as well as other nucleolipids will be published soon. Moreover, the cytostatic/cytotoxic activity of 5-fluorouridine nucleolipids, described herein, against human HT-29 colon carcinoma cells was meanwhile reported [17].

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Experimental Part

General. All chemicals were purchased from *Sigma–Aldrich* (D-Deisenhofen) or from *TCI* – Europe (B-Zwijndrecht). Solvents were of laboratory grade and were distilled before use. TLC: Aluminium sheets, silica gel (SiO_2) *60* F_{254}, 0.2-mm layer (*Merck*, Germany). M.p. *Büchi SMP-20*; uncorrected. UV Spectra: *Cary 1E* spectrophotometer (*Varian*, D-Darmstadt); λ_{max} (ε) in nm ([M^{-1} cm^{-1}]). NMR Spectra (incl. ^{1}H-DOSY spectra): *AMX-500* spectrometer (*Bruker*, D-Rheinstetten); ^{1}H: 500.14, ^{13}C: 125.76, and ^{31}P: 101.3 MHz; chemical shifts in ppm rel. to TMS as internal standard for ^{1}H and ^{13}C nuclei, and external 85% H_3PO_4 for ^{31}p; *J* values in Hz. ESI-MS: *Bruker Daltronics Esquire HCT* instrument (*Bruker Daltronics*, D-Leipzig); ionization with a 2% aq. HCOOH soln. Elemental analyses (C, H, N) of crystallized compounds: *VarioMICRO* instrument (Fa. *Elementar*, D-Hanau). The log *P* values were calculated with the program suite ChemSketch (version 12.0, provided by *Advanced Chemistry Developments Inc.*; Toronto, Canada; http://www.acdlabs.com. Oligonucleotides were synthesized, purified, and characterized (MALDI-TOF MS) by *Eurogentec* (Eurogentec S. A., Liege Science Park, B-Seraing).

RP-18 *HPLC. RP-18* HPLC Analysis was carried out on a 250 × 4 mm *RP-18* column (*Merck*, Germany) on a *Merck-Hitachi* HPLC apparatus with one pump (*Model 655A-12*) connected with a proportioning valve, a variable-wavelength monitor (*Model 655A*), a controller (*Model L-5000*), and an integrator (*Model D-2000*); solvent: MeCN/0.1M $Et_3NH^+AcO^-$ 35 : 65 (*v*/*v*; pH 7.0).

Oligonucleotide Incorporation in Artificial Bilayers. The incorporation of the oligonucleotides **14** and **15** in artificial bilayers was performed with a lipid mixture POPE/POPC[1]) (8 : 2 (*w*/*w*); 100 mg/ml of decane). For the preparation of the horizontal bilayers, planar slides (*Ionovation GmbH*, D-Osnabrück) were used. These slides contain chambers for *cis*- and *trans*-compartments as well as electrode access (see *Fig. 1*). The main body of the slides contains PTFE (= poly(tetrafluoroethylene) foil (thickness, 25 μm) with an aperture of *ca.* 100 μm diameter. This foil separates the chamber into the *cis*- and *trans*-compartments which are only connected by the aperture. After filling of the chamber with buffer (250 mM KCl, 10 mM MOPS (= 3-morpholinopropanesulfonic acid)/*Tris*, pH 7), the *cis*- and *trans*-compartments were linked with Ag/AgCl electrodes – embedded in agarose/3M KCl). Then, a soln. of the POPC/POPE[1]) lipid mixture (0.2 μl) was applied onto the aperture of the PTFE foil using a *Hamilton* syringe (*Hamilton*, CH-Bonaduz). A small *Faraday* cage shielded the bilayer and the electrodes from HF-electrical noise. Next, a bilayer was formed automatically using a perfusion system (*Bilayer Explorer V01*, *Ionovation GmbH*, D-Osnabrück). The formation of a stable bilayer was monitored optically using a laser scanning microscope (*Insight Cell 3D*, *Evotec Technologies GmbH*, D-Hamburg) as well as electrically by capacity measurements. When a stable bilayer had been obtained (capacity, 50–75 pF), the corresponding oligonucleotide soln. (50 nM, 4 μl) was injected into the *cis*-compartment of the chip. During an incubation time of 25 min, the intactness of the bilayer was electro-physiologically controlled using a headstage *EPC 10 USB* with a patch clamp amplifier (software: Patchmaster, *HEKA Elektronik Dr. Schulze GmbH*, D-Lambrecht). The following optical pictures of fluorescence fluctuations were obtained with a confocal laser scanning microscope (*Insight Cell 3D*, *Evotec Technologies GmbH*, D-Hamburg), equipped with a He-Ne laser (543 nm), a 40 × water-immersion objective (*UApo 340*, 40 ×; NA, 1.15, *Olympus*, Tokyo, Japan), and an *Avalanche* photodiode detector (*SPCM-AQR-13-FC*, *PerkinElmer Optoelectronics*, Fremont, USA). Fluorescence irradiation was obtained with a laser power of 200 ± 5 μW. 2D and 3D scans were performed by scanning the confocal spot in *XY* direction with a rotating beam scanner and movement of the objective in *Z* direction. The movement in both directions was piezo-controlled which allows a nm-precise positioning. For the 2D pictures (*Z*-scans, *Figs. 4* and *5*), the confocal plane was moved in 100 nm steps.

From the fluorescence signals of single molecules which pass the excitation volume, the diffusion constants can be calculated. The diffusion times of the fluorescent oligonucleotides within and near the bilayer, were measured at five different positions, above, below and within the layer. At each point, five measurements, for 30 s each, were taken. In summary, each measuring protocol was as follows: *i*) a reference scan of the stable (empty) bilayer; *ii*) addition of the sample with 25 min of incubation, followed by a scan series; *iii*) additional scan series, each after 1st, 2nd, and 3rd perfusion (60 s each).

Goniometric Measurements of Nucleolipids. Goniometric measurements with the nucleolipids **2c** and **11** were performed with a drop-shape analyzer (*DSA 100*, *KRÜSS GmbH*, D-Hamburg) in a closed chamber. Concentrations of the nucleolipid solns. were 0.01, 0.1, 1, 3, 5, and 10 mg/ml of DMSO. From a drug-shape analysis of pendant drops, the interfacial tension values (mN/m) were automatically calculated and plotted *vs.* the concentration. Contact angle measurements on sessile drops were conducted on glass-purified plates in the same concentration range.

5-Fluoro-2′,3′-O-*(1-methylethylidene)uridine* (=*5-Fluoro-1-[(3a*R,*4*R,*6*R,*6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4*-d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H,*3*H*)-dione*; **2a**). Anh. *5-fluorouridine* (**1a**; 1.0 g, 3.82 mmol; dried for 48 h at 75° over $CaCl_2$ under high vacuum) was suspended in dry acetone (200 ml). To this suspension, polymer-linked TsOH (15.24 g, 38.1 mmol) was added, and the mixture was stirred at r.t. for 1 h. Subsequently, the polymer-bound acid was filtered off, and the filtrate was evaporated to a small volume, whereupon the crude product crystallized. This was filtered and recrystallized from $CHCl_3$/MeOH 97:3 to give 1.12 g (97%) of pure **2a**. Colorless needles. TLC (SiO_2, $CHCl_3$): R_f 0.77. M.p. 196–197° ([18], 197–199°). ^{1}H-NMR ((D$_6$)DMSO): 11.866 (*d*, ^4J(NH,F) = 5.0, NH); 8.182 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 5.840 (*d*, ^3J(H–C(1′),H–C(2′)) = 1.5, H–C(1′)); 4.887 (*dd*, ^3J(H–C(2′),H–C(1′)) = 2.5, ^3J(H–C(2′),H–C(3′)) = 6.5, H–C(2′)); 4.767 (*dd*, ^3J(H–C(3′),H–C(4′)) = 3.5, ^3J(H–C(3′),H–C(2′)) = 6.5, H–C(3′)); 4.114 (*Ψq*, ^3J(H–C(4′),H–C(3′)) = 3.5, ^3J(H–C(4′),CH_2(5′)) = 4.0, H–C(4′)); 3.642 (*dd*, ^3J(H_a–C(5′),H–C(4′)) = 4.0, ^2J(H_a–C(5′),H_b–C(5′)) = −12, H_a–C(5′)); 3.588 (*dd*, ^2J(H_b–C(5′),H–C(4′)) = 4.5, ^3J(H_b–C(5′),H_a–C(5′) = −12, H_b–C(5′)), 1.493 (*s*, 3 H_{endo}–C(α')), 1.296 (*s*, 3 H_{exo}–C(α)). ^{13}C-NMR ((D$_6$)DMSO): 157.02 (*d*, ^2J(C(4),F) = 26.2, C(4)); 148.947 (C(2)); 139.898 (*d*, ^1J(C(5),F) = 230.14, C(5)); 125.782 (*d*, ^2J(C(6),F) = 34.6, C(6)); 112.91 (C(acetal)); 90.948 (C(1′)); 86.502 (C(4′)); 83.740 (C(2′)); 80.215 (C(3′)); 61.127 (C(5′)); 26.982 (C_{endo}(α')); 25.147 (C_{exo}(α)). HR-ESI-MS: 305.10 ($[M+H]^+$, $C_{12}H_{16}FN_2O_6^+$; calc. 303.914).

5′-O-*[Bis(4-methoxyphenyl)(phenyl)methyl]-5-fluoro-2′,3′*-O-*(1-methylethylidene)uridine* (=*1-[(3a*R,*4*R,*6*R,*6a*R*)-6-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-2,2-dimethyltetrahydrofuro[3,4*-d*][1,3]dioxol-4-yl]-5-fluoropyrimidine-2,4(1*H,*3*H*)-dione*; **3**). Compound **2a** (317.3 mg, 1.05 mmol) was dried by repeated evaporation with anh. pyridine and then dissolved in dry pyridine (6 ml). Then, (4,4′-dimethoxytriphenyl)methyl chloride (397.5 mg, 1.15 mmol) was added, and the mixture was stirred for 3 h under N_2 at r.t. Subsequently, the reaction was quenched by addition of 30 ml of a 5% aq. $NaHCO_3$ soln. The mixture was washed three times with $CHCl_3$ (50 ml each), and the combined org. layers were dried (Na_2SO_4), filtered, and evaporated to dryness. The oily residue was chromatographed (SiO_2 (6.5 × 13 cm); $CHCl_3$/MeOH 98:2) to give 207 mg (32%) of colorless **3**. TLC (SiO_2, $CHCl_3$/MeOH 98:2): R_f 0.37. ^{1}H-NMR[2]) ((D$_6$)DMSO): 11.891 (*d*, ^4J(NH,F) = 4.0, NH); 8.02 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 7.394–7.367, 7.305–7.205, 6.883–6.854 (3*m*, 13 H of DMTr); 5.840 (*d*, ^3J(H–C(1′),H–C(2′)) = 1.5, H–C(1′)); 4.969 (*dd*, ^3J(H–C(2′),H–C(1′)) = 2.0, ^3J(H–C(2′),H–C(3′)) = 6.5, H–C(2′)); 4.685 (*dd*, ^3J(H–C(3′),H–C(4′)) = 4.5, ^3J(H–C(3′),H–C(2′)) = 6.0, H–C(3′)); 4.143 (*Ψq*, ^3J(H–C(4′),H–C(3′)) = 3.0, ^3J(H–C(4′),CH_2(5′)) = 4.0, H–C(4′)); 3.736, 3.732 (2*s*, 2 MeO); 3.310 (*dd*, ^3J(H_a–C(5′),H–C(4′)) = 6.9, ^2J(H_a–C(5′),H_b–C(5′)) = −10.5, H_a–C(5′)); 3.120 (*dd*, ^2J(H_b–C(5′), H–C(4′)) = 3.3, ^3J(H_b–C(5′),H_a–C(5′) = −10.5, H_b–C(5′)); 1.470 (*s*, 3 '*endo*'-Me), 1.268 (*s*, 3 '*exo*'-Me). ^{13}C-NMR[2]) ((D$_6$)DMSO): 158.054, 158.026 (2 C(5″)); 156.90 (*d*, ^2J(C(4),F) = 26.2, C(4)); 148.775 (C(2)); 144.607 (C(7″)); 139.849 (*d*, ^1J(C(5),F) = 231.4, C(5)); 135.99 (C(2″)); 135.284, 135.225 (2 C(9″)); 129.591, 129.533 (2 C(8″)); 127.704, 127.540 (2 C(3″)); 126.616 C(10″)); 126.368 (*d*, ^2J(C(6),F) = 34.5, C(6)); 113.218 (C(ketal)); 113.102 (C(4″)); 91.511 (C(1′)); 85.690 (C(4′)); 85.396 (C(1″)); 83.483 (C(2′)); 80.223 (C(3′)); 63.737 (C(5′)); 54.926, 54.905 (2 MeO); 26.898 ('*endo*'-Me); 25.152 ('*exo*'-Me). HR-ESI-MS: 604.481 ($[M+H]^+$, $C_{33}H_{33}FN_2O_8^+$; calc. 604.622).

*5-Fluoro-3-[(2*E,*6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]uridine* (=*1-[(2*R,*3*R,*4*S,*5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-fluoro-3-[(2*E,*6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]pyrimidine-2,4(1*H,*3*H*)-dione*; **8**). Anh. **1a** (1.048 g, 4 mmol) was dissolved in anh. DMF (24 ml), and dry K_2CO_3 (1.44 g, 10.64 mmol) was added. After stirring for 10 min at r.t., farnesyl bromide (1.4 ml, 4.4 mmol) was added dropwise under N_2 . The mixture was stirred for further 24 h at r.t. Then, the K_2CO_3

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[2]) Double-primed numbers refer to the C-atoms of the bis(4-methoxyphenyl)(phenyl)methyl (DMTr) groups.

was filtered off and washed with CH_2Cl_2. The filtrate was evaporated and dried overnight in high vacuum. The residue was chromatographed (SiO_2 (6.5 × 15 cm); CH_2Cl_2/MeOH 9:1) to give **8** (0.78 g, 43%). Colorless oil. TLC (SiO_2 (6.5 × 15 cm); CH_2Cl_2/MeOH 9:1): R_f 0.57. UV (MeOH): 267 (8.800). ^{1}H-NMR[3]) ((D$_6$)DMSO): 8.381 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 5.787 (*dd*, ^3J(H–C(1′),H–C(2′)) = 1.5, ^5J(H–C(1′),F) = 4.5, H–C(1′)); 5.388 (*d*, ^3J(HO–C(2′),H–C(2′)) = 5.0, HO–C(2′)); 5.249 (*t*, ^3J(HO–C(5′),CH$_2$(5′)) = 4.5, HO–C(5′)); 5.130 (*t*, ^3J(H–C(2″),H–C(1″) = 6.5, H–C(2″)); 5.082 (*m*, ^3J(HO–C(3′),H–C(3′) = 5.5, HO–C(3′), CH$_2$(1″)); 4.407 (*m*, ^3J = 5.5, H–C(6″), 2 H–C(10″)); 4.043–3.977 (*m*, H–C(2′,3′)); 3.871 (*Ψquint.*, ^3J(H–C(4′),H–C(3′) = 5.0, ^3J(H–C(4′),CH$_2$(5′) = 2.5, H–C(4′)); 3.704 (*ddd*, ^3J(H$_a$–C(5′),H–C(4′)) = 4.5, ^2J(H$_a$–C(5′),H$_b$–C(5′)) = −12.0, ^3J (H$_a$–C(5′),HO–C(5′)) = 2.5, H$_a$–C(5′)); 3.592 (*ddd*, ^3J(H$_b$–C(5′),H–C(4′)) = 5.0, ^2J(H$_b$–C(5′),H$_a$–C(5′)) = −12.0, ^3J (H$_b$–C(5′),HO–C(5′)) = 3.0, H$_b$–C(5′)); 2.057–1.888 (*m*, CH$_2$(8″,9″,5″,4″)); 1.741 (*s*, Me(13″)); 1.629 (*s*, Me(14″)); 1.548 (*s*, Me(15″)); 1.535 (*s*, Me(12″)). ^{13}C-NMR[3]) ((D$_6$)DMSO): 156.073 (*d*, ^2J(C(4),F) = 26.2, C(4)); 148.931 (C(2)); 139.364 (*d*, ^1J(C(5),F) = 231.4, C(5)); 139.344 (C(3″)); 134.534 (C(7″)); 130.537 (C(11″)); 124.004 (C(6″)); 123.432 (*d*, ^2J(C(6),F) = 35.0, C(6)); 118.170 (C(2″)); 89.250 (C(4′)); 84.550 (C(1′)); 73.845 (C(3′)); 69.028 (C(2′)); 60.046 (C(5′)); 39.108, 38.999, 38.838 (C(1″,4″,8″)); 26.099 (C(5″)), 25.645 (C(9″)); 25.369 (C(12″)); 17.426 (C(15″)); 16.076 (C(13″)); 15.706 (C(14″)). HR-ESI-MS: 467.10 ([*M*+H]$^+$, $C_{24}H_{36}FN_2O_6^+$; calc. 466.543); 335.2 (*N(3)*-farnesyl-5-fluorouracil). Anal calc. for $C_{24}H_{35}FN_2O_6$ (466.543): C 61.79, H 7.56, N 6.00; found: C 61.53, H 7.38, N 5.86.

*5-Fluoro-2′,3′-*O*-(1-methylethylidene)-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]uridine* (= *5-Fluoro-1-[(3a*R*,4*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione*; **9**). Compound **8** (0.25 g, 0.5 mmol) was suspended in anh. acetone, and polymer-linked TsOH (1.0 g, 2.5 mmol) was added. The mixture was stirred for 1 h at r.t. The polymer-bound acid was filtered off, and the filtrate was evaporated and dried overnight in high vacuum. Chromatography (SiO_2 (6.5 × 15 cm); CH_2Cl_2/MeOH 95:5) gave, after evaporation of the main zone, **9** (780 mg, 43%). Colorless oil. TLC (SiO_2, CH_2Cl_2/MeOH 95:5): R_f 0.57. UV (MeOH): 267 (8.900). ^{1}H-NMR[3]) ((D$_6$)DMSO): 8.237 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 5.89 (*d*, ^3J(H–C(1′),H–C(2′) = 1.0, H–C(1′)); 5.210 (*t*, ^3J(H–C(2″),CH$_2$(1″) = 5.0, H–C(2″)); 5.127 (*t*, ^3J(HO–C(5′),CH$_2$(5′)) = 6.5, HO–C(5′)); 5.062–5.027 (*m*, CH$_2$(1″)); 4.875–4.857 (*m*, H–C(6″)); 4.779–4.760 (*m*, H–C(10″)); 4.405–4.393 (*m*, H–C(2′,3′)); 4.148 (*Ψq*, ^3J(H–C(4′), CH$_2$(5′)) = 3.5; ^3J(H–C(4′),H–C(3′)) = 3.5, H–C(4′)); 3.666–3.562 (*m*, ^2J(H$_a$–C(5′),H$_b$–C(5′)) = 15.0, CH$_2$(5′)); 2.059–1.889 (*m*, CH$_2$(8″,9″,5″,4″)); 1.739 (*s*, Me(13″)); 1.630 (*s*, Me(14″)); 1.550 (*s*, Me(15″)); 1.535 (*s*, Me(12″)); 1.491 (*s*, 3 '*endo*'-Me), 1.289 (*s*, 3 '*exo*'-Me). ^{13}C-NMR[3]) ((D$_6$)DMSO): 156.021 (*d*, ^2J(C(4),F) = 29.3, C(4)); 148.631 (C(2)); 139.271 (C(3″)); 139.273 (*d*, ^1J(C(5),F) = 230.1, C(5)); 134.439 (C(7″)); 130.447 (C(11″)); 124.298 (*d*, ^2J(C(6),F) = 37.2, C(6)); 123.914 (C(6″)); 123.387 (C(10″)); 118.031 (C(2″)); 112.728 (C(acetal)); 91.726 (C(1′)); 86.547 (C(4′)); 83.803 (C(2′)); 80.100 (C(2′)); 60.942 (C(5′)); 39.082 (C(1″)); 38.915 (C(4″)); 38.710 (C(8″)); 26.834 (C(5″)); 26.010 (C(9″)); 25.520 ('*endo*'-Me); 25.275 ('*exo*'-Me); 25.003 (C(12″)); 17.330 (C(15″)); 15.993 (C(14″)); 15.610 (C(13″)). HR-ESI-MS: 507.60 ([*M*+H]$^+$, $C_{27}H_{40}FN_2O_6^+$; calc. 507.61); 335.2 (*N(1)*-farnesyl-5-fluorouracil).

*5-Fluoro-2′,3′-*O*-(1-propylbutylidene)-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]uridine* (= *5-Fluoro-1-[(3a*R*,4*R*,6a*R*)-6-(hydroxymethyl)-2,2-dipropyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione*; **5**). *5-Fluoro-2′,3′-*O*-(1-propylbutylidene)uridine* (**2b**; 358.3 mg, 1 mmol) [13] was dissolved in anh. DMF (6 ml), and dry K_2CO_3 (360 mg, 2.6 mmol) was added. After stirring for 10 min, farnesyl bromide (0.35 mmol, 1.1 mmol) was added dropwise under N_2. Stirring at r.t. was continued for further 24 h. Subsequently, the K_2CO_3 was filtered off and washed with CH_2Cl_2. The filtrate was evaporated, and the residue was dried overnight in high vacuum. Chromatography (SiO_2 (6.5 × 15 cm); CH_2Cl_2, 95:5) gave **5** (285 mg, 51%). Colorless oil. TLC (SiO_2; CH_2Cl_2/MeOH 95:5): R_f 0.71. UV (MeOH): 267 (11.340). ^{1}H-NMR[3]) ((D$_6$)DMSO): 8.222 (*d*, ^3J(H–C(6),F) = 6.5, H–C(6)); 5.890 (*d*, ^3J(H–C(1′),H–C(2′)) = 1.0, H–C(1′)); 5.169 (*t*, ^3J(H–C(2″),H–C(1″)) = 5.0, H–C(2″)); 5.121 (*t*, ^3J(HO–C(5′),CH$_2$(5′) = 6.5, HO–C(5′)); 5.040–5.032 (*m*, CH$_2$(1″)); 4.878 (*dd*, ^3J(H–C(2′),H–C(1′) = 6.5, ^3J(H–C(2′),H–C(3′) = 3.0), H–C(2′)); 4.765 (*dd*, ^3J(H–C(3′),H–C(2′) = 3.0, ^3J(H–C(3′),H–C(4′) = 6.5, H–C(3′)); 4.144–4.130 (*m*, ^3J(H–C(4′),

[3]) Double-primed numbers refer to the C-atoms of the farnesyl side chain.

H–C(3′) = 7.0, H–C(4′)); 3.644–3.547 (*m*, ^2J(H_a–C(5′),H_b–C(5′) = −14.0, CH_2(5′)); 2.039–1.859 (*m*, CH_2(8″,9″,5″,4″)); 1.733 (*s*, Me(13″)); 1.679–1.647 (‘*endo*’-CH_2(1‴); 1.624 (*s*, Me(12″)); 1.543 (*s*, Me(14″)); 1.527 (*s*, Me(15″)); 1.510–1.493 (‘*exo*’-CH_2(1‴)); 1.434–1.387 (‘*endo*’-CH_2(2‴)); 1.295–1.248 (‘*exo*’-CH_2(2‴)); 0.911 (*t*, ^3J(‘*endo*’-Me(3‴), ‘*endo*’-CH_2(2‴) = 7.5, ‘*endo*’-Me(3‴)); 0.862 (*t*, ^3J(‘*exo*’-Me(3‴), ‘*exo*’-CH_2(2‴) = 7.5, ‘*exo*’-Me(3‴)). ^{13}C-NMR[3]) ((D_6)DMSO): 156.135 (*d*, ^2J(C(4),F) = 25.9, C(4)); 148.735 (C(2)); 139.348 (*d*, ^1J(C(5),F) = 228.5, C(5)); 139.318 (C(3″)); 134.504 (C(7″)); 130.517 (C(11″)); 124.482 (*d*, ^2J(C(6),F) = 34.8, C(6)); 123.989 (C(6″)); 123.470 (C(10″)); 118.137 (C(2″)); 116.394 (C(ketal)); 92.069 (C(4′)); 86.882 (C(1′)); 84.092 (C(3′)); 80.549 (C(2′)); 61.185 (C(5′)); 39.104, 39.074 (2 C(1‴); 38.775, 38.716, 38.592 (C(1″,4″,8″)); 26.090 (C(5″); 25.584 (C(9″)); 25.349 (C(12″)); 17.403 (C(13″)); 16.892 (C(2‴)); 16.222 (C(2‴)); 16.063 (C(14″)); 16.063 (C(15″)); 14.041, 14.006 (C(*γ*′), C(*γ*)). Anal. calc. for $C_{31}H_{47}FN_2O_6$ (562.713): C 66.17, H 8.82, N, 4.98; found: C 66.12, H 8.39, N 4.82.

5-Fluoro-2′,3′-O-*(1-nonyldecylidene)uridine* (= *5-Fluoro-1-[(3a*R,*4*R,*6*R,*6a*R*)-6-(hydroxymethyl)-2,2-dinonyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H,*3*H*)-dione*; **2c**). Anh. **1a** (1.0 g, 3.82 mmol) was dissolved in anh. DMF (15 ml), and nonadecan-10-one (2.16 g, 7.64 mmol) was added. After addition of $HC(OEt)_3$ (1.0 g, 5.73 mmol) and 4M HCl in 1,4-dioxane (3.4 ml), the mixture was stirred for 48 h at r.t. Then, the mixture was partitioned between $CHCl_3$ (350 ml) and a sat. aq. $NaHCO_3$ soln. (50 ml). The org. layer was washed three times with H_2O (100 ml each), and the aq. layers were re-extracted with CH_2Cl_2 (25 ml). The combined org. phases were dried (Na_2SO_4), filtered, and evaporated. The residue was dried overnight in high vacuum and then chromatographed (SiO_2 (6 × 12 cm); CH_2Cl_2/MeOH 95:5) to give **2c** (1.38 g, 68%). Colorless oil. TLC (SiO_2, CH_2Cl_2/MeOH 95:5): R_f 0.56. UV (MeOH): 265 (9.860). ^{1}H-NMR[4]) ((D_6)DMSO): 11.843 (*d*, ^4J(NH,F) = 5.0, NH); 8.156 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 5.832 (*d*, ^3J(H–C(1′),H–C(2′)) = 1.5, H–C(1′)); 5.141 (*t*, ^3J(HO–C(5′), CH_2(5′)) = 5.0, HO–C(5′)); 4.883 (*dd*, ^3J(H–C(2′),H–C(1′)) = 2.0, ^3J(H–C(2′),H–C(3′)) = 7.0, H–C(2′)); 4.742 (*dd*, ^3J(H–C(3′),H–C(2′)) = 7.0, ^3J(H–C(3′),H–C(4′)) = 4.0, H–C(3′)); 4.101–4.081 (*m*, H–C(4′)); 3.631–3.544 (*m*, 2 H–C(5′)) = −13.5, CH_2(5′)); 1.677–1.646 (*m*, ‘*endo*’-CH_2(1″)); 1.525–1.502 (*m*, ‘*exo*’-CH_2(1″)); 1.386–1.359 (*m*, ‘*endo*’-CH_2(2″)); 1.251–1.238 (*m*, 13 CH_2(ketal)); 0.865–0.842 (*m*, 2 Me(9″)). ^{13}C-NMR[4]) ((D_6)DMSO): 156.970 (*d*, ^2J(C(4),F) = 23.4, C(4)); 148.893 (C(2)); 139.008 (*d*, ^1J(C(5),F) = 230.2, C(5)); 125.894 (*d*, ^2J(C(6),F) = 36.2, C(6)); 116.569 (C(ketal)); 91.050 (C(1′)); 86.657 (C(4′)); 83.858 (C(3′)); 80.449 (C(2′)); 61.205 (C(5′)); 41.685 (C_{endo}(1″)); 36.330 (C_{exo}(1″)); 36.271, 31.150, 29.052, 28.982, 28.764, 28.708, 28.541, 28.465, 23.467, 23.150, 22.832, 21.956 (12 CH_2); 13.451 (2 C(8″)). Anal. calc. for $C_{28}H_{47}FN_2O_6$ (526.681): C 63.85, H 8.99, N 5.32; found: C 63.78, H 8.80, N 5.15.

5-Fluoro-2′,3′-O-*(1-pentadecylhexadecylidene)uridine* (= *5-Fluoro-1-[(3a*R,*4*R,*6*R,*6a*R*)-6-(hydroxymethyl)-2,2-dipentadecyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H,*3*H*)-dione*; **2d**). To anh. **1a** (1 g, 3.82 mmol) in THF (30 ml) were added TsOH (0.156 g; 0.9 mmol), hentriacontan-16-one (0.37 g, 8.22 mmol), and $HC(OEt)_3$ (0.7 ml, 4.01 mmol) in THF (*ca.* 30ml). The mixture was refluxed for 24 h. Then, the reaction was quenched by addition of Et_3N (0.22 ml, 1.59 mmol), and the mixture was poured into an ice-cold aq. 5% $NaHCO_3$ soln. (20 ml) and stirred for 15 min. Then, the aq. layer was washed with CH_2Cl_2 and the org. layer was separated, dried (Na_2SO_4), filtered, and evaporated. The residue was triturated with MeOH. The precipitate was filtered off and dried overnight in high vacuum. Yield: 0.258 g (0.4 mmol, 49%). TLC (SiO_2, CH_2Cl_2/MeOH 95:5): R_f 0.6. ^{1}H-NMR[4]) ((D_6)DMSO): 11.688 (*s*, NH); 8.107 (*d*, ^3J(F,H–C(6)) = 7.0, H–C(6)); 5.848 (*d*, ^3J(H–C(1′),H–C(2′)) = 1.3, H–C(1′)); 5.015 (*t*, ^3J(HO–C(5′),CH_2(5′)) = 5.0, HO–C(5′)); 4.881 (*dd*, ^3J(H–C(2′),H–C(1′)) = 2.5, ^3J(H–C(2′), H–C(3′)) = 6.5, H–C(2′)); 4.761 (*dd*, ^3J(H–C(3′),H–C(2′)) = 6.5, ^3J(H–C(3′),H–C(4′)) = 3.0, H–C(3′)); 4.101 (*Ψdd*, ^3J(H–C(4′),H–C(3′)) = 3.5, ^3J(H–C(4′),H–C(5′)) = 7.5, H–C(4′)); 3.612 (*m*, J_{AB} = −12.0, CH_2(5′)); 1.681 (*m*, ‘*endo*’-CH_2(1″)); 1.533 (*m*, ‘*exo*’-CH_2(1″)); 1.398 (*m*, ‘*endo*’-CH_2(2″)); 1.286 (*m*, 25 H of CH_2); 0.865 (*m*, 2 Me). ^{13}C-NMR[4]) ((D_6)DMSO): 156.722 (*d*, ^2J(F,C(4)) = 26.3, C(4)); 148.690 (C(2)); 139.662 (*d*, ^1J(F,C(5)) = 230.0, C(5)); 125.504 (*d*, ^2J(F,C(6)) = 35.8, C(6)); 116.471 (C(ketal)); 90.938 (C(1′)); 86.507 (C(4′)); 83.708 (C(3′)); 80.296 (C(2′)); 61.075 (C(5′)); 36.307 (C(1″)); 36.065 (C(1″)); 30.897, 28.767, 28.711, 28.592, 28.567, 28.469, 28.442, 28.268, 23.164, 22.616, 21.654, (CH_2); 13.451 (2 Me). HR-ESI-MS: 694.89 ($[M+H]^+$, $C_{40}H_{71}FN_2O_6^+$; calc. 695.99).

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[4]) Double-primed numbers refer to the C-atoms of the ketal moiety.

5′-O-{[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphino}-5-fluoro-2′,3′-O-(1-nonyldecylidene)uridine (*=2-Cyanoethyl [(3a*R,*4*R,*6*R,*6a*R*)-6-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,2-dinonyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]methyl Bis(1-methylethyl)phosphoramidoite*; **7**). Anh. **2c** (205.4 mg, 0.39 mmol) was dissolved in dry CH_2Cl_2 (15 ml). Then, EtN^iPr_2 (*Hünig's* base, 125 µl, 0.72 mmol) and (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine (156 µl, 0.69 mmol) were added under N_2. The mixture was stirred for 15 min at r.t., and then an ice-cold 5% aq. $NaHCO_3$ soln. (12 ml) was added. The mixture was extracted three times with cold CH_2Cl_2, the combined org. layers were dried (Na_2SO_4), filtered, and evaporated on a rotary evaporator (bath temp., 25°). Chromatography (SiO_2 (2 × 8 cm); CH_2Cl_2/acetone 8:2) gave one main zone from which **7** (210 mg, 74%) was obtained. Colorless oil. TLC (MeOH/acetone 8:2): R_f 0.96. HR-ESI-MS: 727.658 ($[M+H]^+$, $C_{37}H_{64}FN_2O_7P^+$; calc. 727.899). ^{31}P-NMR ($CDCl_3$): 150.73, 149.75.

*5′-O-{[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphino}-5-fluoro-2′,3′-O-(1-propylbutylidene)-3-[(2*E,*6*E*)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl]uridine* (*=2-Cyanoethyl [(3a*R,*4*R,*6*R,*6a*R*)-6-{5-Fluoro-2,4-dioxo-3-[(2*E,*6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]-3,4-dihydropyrimidin-1(2*H*)-yl}-2,2-dipropyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]methyl Bis(1-methylethyl)phosphoramidoite*; **6**). Anh. **5** (256 mg, 0.45 mmol) was 5′-phosphitylated with EtN^iPr_2 (*Hünig's* base, 147 µl, 0.85 mmol) and (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine (181 µl, 0.80 mmol) and worked up as described for **7**. Chromatography (SiO_2 (2 × 8 cm); CH_2Cl_2/MeOH 8:2) gave one main zone from which **6** (208 mg, 60%) was obtained. Colorless oil. TLC (CH_2Cl_2/MeOH 8:2): R_f 0.95. HR-ESI-MS: 763.65 ($[M+H]^+$, $C_{40}H_{64}FN_4O_7P^+$; calc. 763.931). ^{31}P-NMR ($CDCl_3$): 149.86, 149.71.

5′-O-{[Bis(1-methylethyl)amino](2-cyanoethoxy)phosphino}-2′,3′-O-cyclopentadecylidene-5-fluorouridine (*=2-Cyanoethyl [(3a′*R,*4′*R,*6′*R,*6a′*R*)-6′-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrospiro[cyclopentadecane-1,2′-furo[3,4-*d*][1,3]dioxol]-4′-yl]methyl Bis(1-methylethyl)phosphoramidoite*; **12**). Anh. **11** (256 mg, 0.45 mmol) [13] was 5′-phosphitylated with EtN^iPr_2 (*Hünig's* base, 147 µl, 0.85 mmol) and (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine (181 µl, 0.80 mmol) and worked up as described for **7**. Chromatography (SiO_2 (2 × 8 cm); CH_2Cl_2/MeOH 8:2) gave one main zone from which **12** (208 mg, 60%) was obtained. Colorless oil. TLC (CH_2Cl_2/MeOH 8:2): R_f 0.95. ^{31}P-NMR ($CDCl_3$): 150.11, 149.57.

Oligonucleotides. The synthesis of the oligonucleotides and their MS spectrometric analysis were performed by *Eurogentec S.A.*, Liège Science Park, Belgium. MALDI-TOF-MS: **14**: 4706.8 ($[M+H^+]$, calc. 4777.7), **15**: 4739.1 ($[M+H^+]$, calc. 4740.7), **16**: 4755.0 ($[M+H^+]$, calc. 4.757.8), **17**: 4.696.9 ($[M+H^+]$, calc. 4.700.6).

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