Mitsunobu Reactions of 5-Fluorouridine with the Terpenols *Phytol* and *Nerol*: DNA Building Blocks for a Biomimetic Lipophilization of Nucleic Acids

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Dedicated to the memory of the late Prof. Dr. Dr. *Eckard Schlimme*

The cancerostatic 5-fluorouridine (5-FUrd; **1**) was sequentially sugar-protected by introduction of a 2′,3′-*O*-heptylidene ketal group (→**2**), followed by 5′-*O*-monomethoxytritylation (→**3**). This fully protected derivative was submitted to *Mitsunobu* reactions with either phytol ((*Z* and *E*)-isomer) or nerol ((*Z*)-isomer) to yield the nucleoterpenes **4a** and **4b**. Both were 5′-*O*-deprotected with 2% $Cl_2CHCOOH$ in CH_2Cl_2 to yield compounds **5a** and **5b**, respectively. These were converted to the 5′-*O*-cyanoethyl phosphoramidites **6a** and **6b**, respectively. Moreover, the 2′,3′-*O*-(1-nonyldecylidene) derivative, **7a**, of 5-fluorouridine was resynthesized and labelled at C(5′) with an *Eterneon-480* fluorophor® (→**7b**). The resulting nucleolipid was studied with respect to its incorporation in an artificial bilayer, as well as to its aggregate formation. Additionally, two oligonucleotides carrying terminal phytol-alkylated 5-fluorouridine tags were prepared, one of which was studied concerning its incorporation in an artificial lipid bilayer.

1. Introduction. – Post-biosynthetic lipophilization reactions of biomolecules such as proteins and carbohydrates are inevitable in the chemistry of life [1]. Of particular relevance in this concern is the recent discovery and biological characterization of geranylated tRNA in bacteria [2], reflecting the chemodiversity of RNA constituents. Also in biomedical chemistry, the lipophilization of compounds is of great importance. One of the major drawbacks of many chemotherapeutics is their insufficient penetration through cell membranes, as well as the crossing of the blood–brain barrier due to their high hydrophilicity. This is particularly valid for antisense and antigene oligonucleotides [3]. In the case of low-molecular-weight drugs, this kind of chemical modification is heading for the fulfilment of '*Lipinski's Rule of Five*' [4]. The rule describes molecular properties relevant for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion, and it is of high importance for drug development where a pharmacologically active lead structure is optimized stepwise for increased activity and selectivity. One part of the rule concerns the drug's partition coefficient (log *P* between *n*-octan-1-ol and H_2O) within the range of -0.4 to $+5.5$.

Meanwhile, synthesis and theranostic applications of nucleolipids and lipo-oligonucleotides are established [5]. Herein, we describe the synthesis of lipophilic derivatives of the cancerostatic nucleoside 5-fluorouridine (5-FUrd; nucleolipid), their

conversion in 5′-*O*-(2-cyanoethyl)phosphoramidites, the appending of the nucleolipids to oligonucleotides, and their incorporation in artificial lipid bilayers. For the biomimetic lipophilization of 5-FUrd, we used phytol as well as nerol which were linked to N(3) of the nucleoside applying *Mitsunobu* reaction conditions [6–15]. Nerol is a *monoterpene* found in many *essential oils* such as *lemongrass* and *hops*. Phytol is – *inter alia* – a constituent of chlorophyll with which the latter is embedded in the thylakoid membranes of chloroplasts.

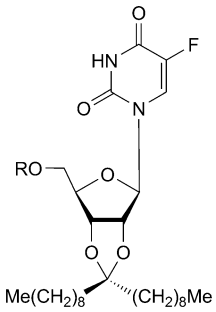
2. Results and Discussion. – 2.1. *Synthesis of the Monomers.* It has been found recently that *Mitsunobu* reactions between an alcohol and a nucleoside leads only then to a predominantly base-alkylated product, if the nucleoside is fully protected at the glyconic moiety [6].

Moreover, such a reaction requires a pK_{BH^+} value of the nucleosidic educt between 0 and 11 [16]. In principle, 5-fluorouracil (5-FU; $pK_{BH^+} = 8.0 \pm 0.1$) as well as uracil ($pK_{BH^+} = 9.4 \pm 0.1$) are, therefore, suitable for *Mitsunobu* alkylations. For these reasons, we protected compound **1** first at the 2′,3′-OH groups by reaction with heptan-4-one in the presence of $(EtO)_3CH$ and 4M HCl in 1,4-dioxane and obtained compound **2** (*Scheme*) [17]. This had been prepared before and possesses a suitably high acidic stability at the ketal group ($\tau = 130$ min in 1N aq. HCl/MeCN 1:1 (*v*/*v*)). The latter was then protected at the 5′-OH group with a (4-methoxyphenyl)diphenylmethyl (MMTs) residue ($\rightarrow$ **3**). This intermediate was next submitted to *Mitsunobu* alkylations with either phytol or nerol (PPh_3, diethyl azodicarboxylate (DEAD), THF, 0°) to furnish compounds **4a** and **4b**. Both were subsequently detritylated in 4% $Cl_2CHCOOH$ in CH_2Cl_2 at room temperature for 10 min to afford compounds **5a** and **5b** which possess significantly enhanced log *P* values compared to 5-FUrd (**1**: $\log P = -1.34 \pm 0.46$; **5a**: $\log P = +12.5 \pm 0.63$; **5b**: $\log P = +7.65 \pm 0.65$). The latter were then phosphitylated to give the phosphoramidites **6a** and **6b**, respectively.

Because it has been reported that *Mitsunobu* alkylations of nucleobases may occur at the N- and the O-atoms of the heterocycle, the ^{13}C-NMR spectra of the corresponding *O(2)*-, the *O(4)*-, and of the *N(3)*-alkylated compounds were simulated and compared with the recorded spectrum of the isolated main product (*Table*). As can be seen from the *Table*, the selected ^{13}C-NMR resonances of the recorded signals coincide mostly with those of the *N(3)*-alkylated compounds **8** and **9**.

Additionally, compound **7a** was resynthesized, for which details of preparation will be published in due course. This compound was fluorophore-labelled at 5′-OH with *Eterneon-480*® *via* its *N*-hydroxysuccinimide ester ($\rightarrow$ **7b**). The chemical structure of the fluorescence dye has not been disclosed so far. The dye-labelled cancerostatic nucleolipid was studied with respect to its incorporation in an artificial lipid bilayer composed of 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphoethanolamine (POPE)/1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) 8:2 (*w*/*w*) in decane (10 mg/ml) in a set-up shown in *Fig. 1* (see *Exper. Part* and [18][19] for details and for construction plans of the device).

From *Fig. 2*, it can be seen that, upon injection of a dilute MeCN solution of compound **7b** into the *cis* compartment of the bilayer slide, the bilayer is torn at once. As a result, compound **7b** forms high molecular-weight aggregates (micelles) which slowly mass at the *Teflon*-coated aperture annulus (*Fig. 2*, *b*–*d*).



7a R = H
7b R = *Eterneon-480®*

Scheme

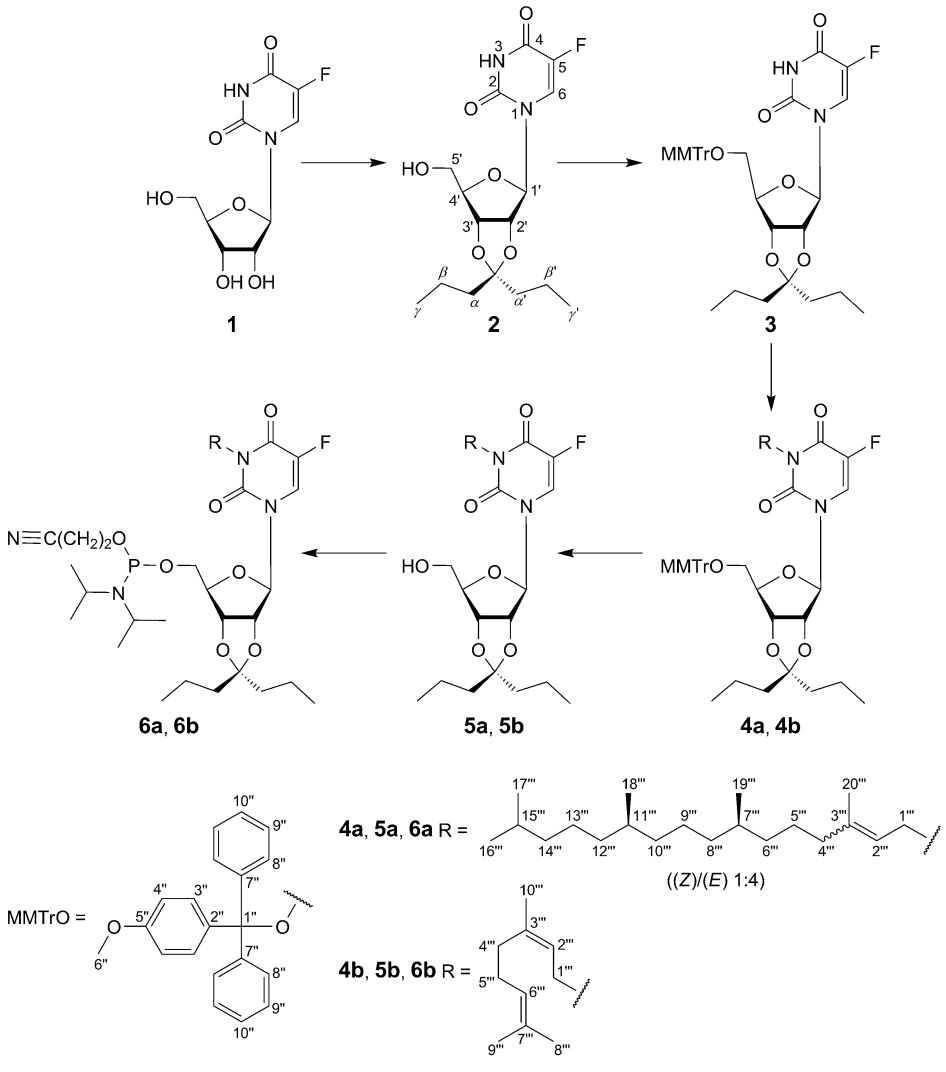


Table 1. *^{13}C-NMR Chemical Shifts of Simulated as Well as of Recorded Spectra of Various Alkylated 5-Fluorouridines*

C-Atom	*Data from Simulated Spectra*[a])			
	5-FUrd	*O(2)*-Alkylated product	*O(4)*-Alkylated product	*N(3)*-Alkylated product
C(4)	158.2	168.8	160.4	162.9
C(5)	140.2	140.0	121.0	140.2
C(6)	128.4	124.5	102.2	128.4
C(2)	150.8	155.5	155.1	151.4
C(1″)	–	55.9	57.8	38.8
C-Atom	*Data from Recorded Spectra*			
	5-FUrd	Phytol-alkylated product **5a**		Nerol-alkylated product **5b**
C(4)	157.07/156.86	156.20/155.99		156.20/156.00
C(5)	140.81/138.98	140.24/138.43		140.27/138.45
C(6)	124.95/124.67	124.64/124.37		124.63/124.35
C(2)	149.22	148.73		148.73
C(1″)	–	38.93		38.85

[a]) Data from nerol- and phytol-alkylated compounds are identical.

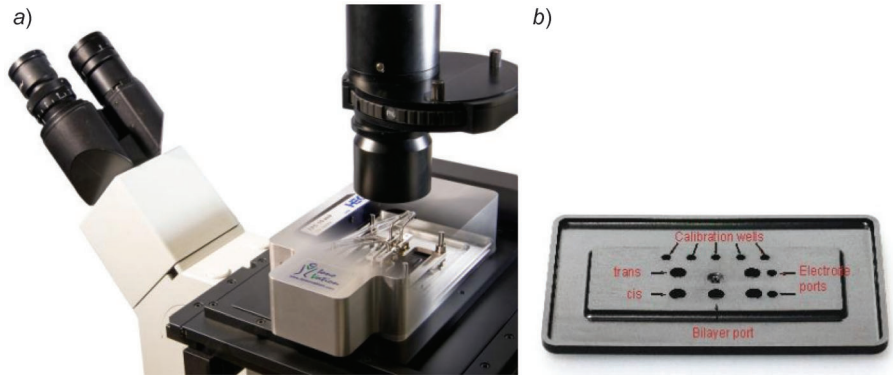


Fig. 1. a) *Stage unit of the 'Ionovation Explorer' mounted on a standard inverted fluorescence microscope.* The computer controlled perfusion unit is a side board and is not shown. b) *'Ionovation Bilayer Slide', a disposable, optical transparent microfluidic sample carrier with perfusion capabilities.* 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. Calibration wells allow optical control experiments when needed.

Photon correlation spectroscopy (PCS) recordings with a light-scattering detection at an angle of 90° (*Fig. 3*) give a *Poisson*-type size distribution of the resulting particles with mean values between *ca.* 600 and 1200 nm. One exception (*rept. 4*) is probably due to a dust particle. In a second experiment, a more concentrated solution of **7b** in MeCN was injected into the *cis* compartment of the bilayer slide. In this case, it was observed that, during an incubation time of 5 min, the dye-labelled nucleolipid is immobilized within the bilayer (*Fig. 2*, *e* and *f*). *Fig. 4* shows the relative brightness intensities of the bilayer before and after addition of compound **7b**.

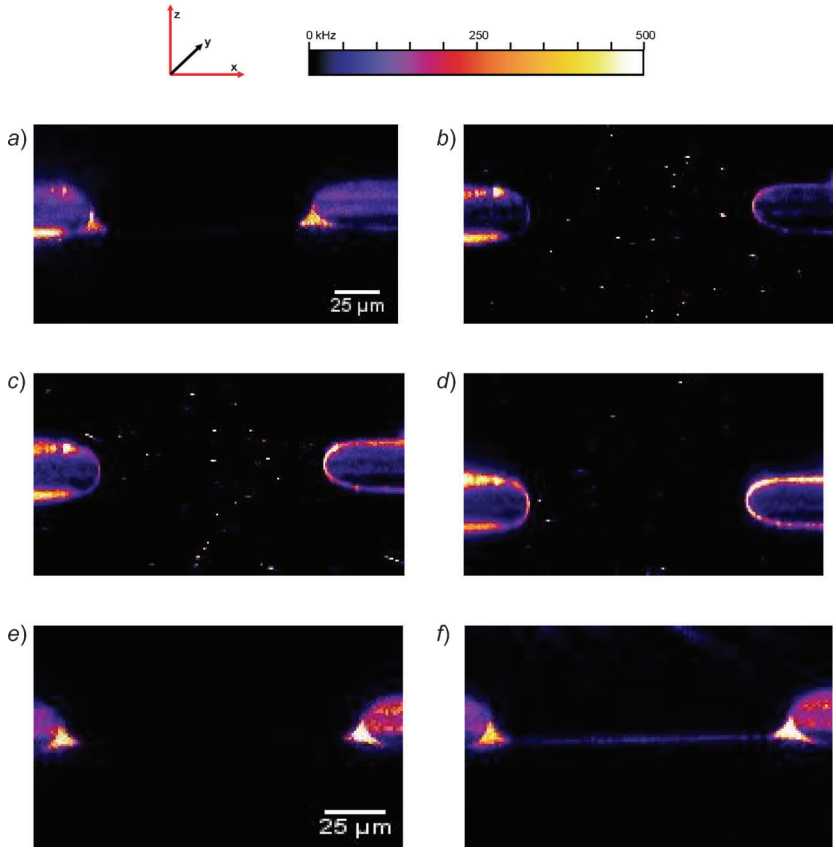


Fig. 2. *Insertion of compound* **7b** *into an artificial bilayer*. *a*) *Z*-Scan of an empty bilayer. *b*) *Z*-Scan after injection of a dilute soln. of **7b** in MeCN (1 µl) into the *cis* compartment of the slide and torn of the bilayer. *c*) *Z*-Scan after 5 min of incubation. Slowly massing of aggregates at the *Teflon* annulus. *d*) *Z*-Scan after further 5 min of incubation. Most of the aggregates are covering the *Teflon* annulus. *e*) Repeat of the experiment as in *a*. *Z*-Scan of the empty bilayer. *f*) *Z*-Scan after injection of a MeCN soln. of **7b** (1 µl) to the *cis* compartment of the slide and 5 min of incubation.

2.2. *Lipo-oligonucleotides: Synthesis and Bilayer Incorporation.* The phosphoramidite **6a** was used to prepare the following oligonucleotides with an appending nucleolipid **5a**:

5′-d(**5a**-Cy5-TAG GTC AAT ACT)-3′ (**8**)

5′-d(**5a**-TAG GTC AAT ACT)-3′ (**9**)

3′-d(ATC CAG TTA TGA)-5′ (**10**)

The cyanine-5-labelled oligomer **8** was used to study the efficiency of lipid bilayer incorporation with respect to its stability (*Fig. 5*).

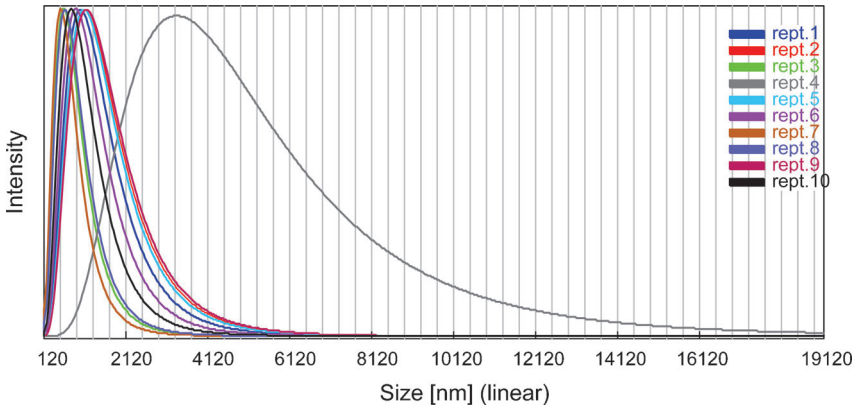


Fig. 3. *Graphical presentation of PCS measurements* (intensity *vs.* particle size) *of* **7b**-*particles* (see: *Exper. Part*). The curve of *rept. 4* is probably due to a dust particle.

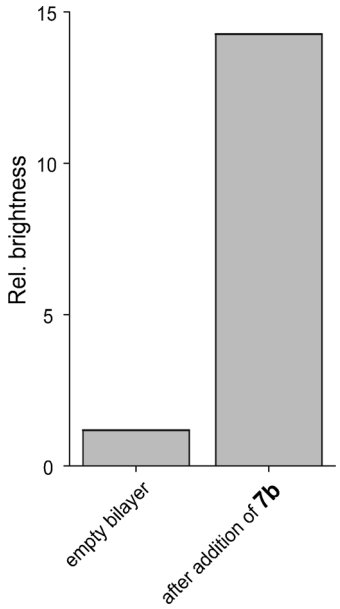


Fig. 4. *Relative bilayer brightness intensity before and after addition of compound* **7b**

As can be seen from *Fig. 5*, the oligomer **8** carrying a 5′-phytol residue is highly resistant against perfusion of the *cis* compartment of the bilayer slide; after five perfusions (30 s each, with 1 ml/min of 250 nM KCl, 10 mM MOPS/*Tris*, pH 7.0; incubation time between two consecutive perfusions, 5 min), only *ca.* 20% of **8** had been washed out.

The oligomer **9** was prepared in advance in order to study the duplex formation between this lipo-oligonucleotide and its complementary strand **10** at the lipid bilayer–water phase boundary layer using *SYBR® Green* as intercalating fluorescent

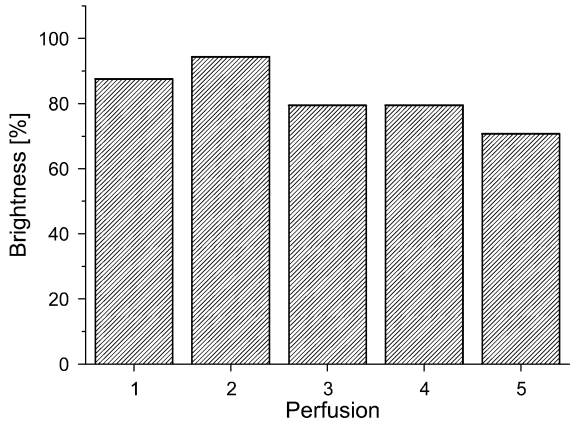


Fig. 5. *Bilayer brightness* [%] *after incorporation of the oligomer* **8** *in the lipid bilayer as a function of the perfusion number* (for details, see *Exper. Part*)

dye. The results of these experiments will be compared with analogous ones on oligonucleotides carrying other types of lipids at their 5′-end and will be published later. Such studies will be of importance for the optimization of our novel nucleic-acid biosensor technology [18]. Moreover, the properties of hybrid molecules as those presented here might be of interest regarding recent reports on similar nucleoterpenes found in nature [20][21].

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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: aluminum sheets, silica gel *60* F_{254}, 0.2-mm layer (*Merck*, Germany). M.p. *Büchi* SMP-20; uncorrected. UV Spectra: *Cary 1E* spectrophotometer (*Varian*, D-Darmstadt). NMR Spectra: *AMX-500* spectrometer (*Bruker*, D-Rheinstetten); ^{1}H: 500.14, ^{13}C: 125.76, and ^{31}P: 101.3 MHz, chemical shifts, δ in ppm rel. to TMS as internal standard for ^{1}H and ^{13}C, and external 85% H_3PO_4; *J* values in Hz. Elemental analyses (C, H, N): *VarioMICRO* instrument (*Fa. Elementar*, D-Hanau). Particle size distribution of **7b** particles were determined by photon correlation spectroscopy (PCS) with an *N5 Submicro Particle Size Analyzer* (*Beckman Coulter*, D-Krefeld). Light-scattering detection was performed at 90 deg. log *P* Values were calculated using 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).

Incorporation of Compound **7b** *in an Artificial Bilayer.* The incorporation of **7b** in an artificial bilayer was performed at a lipid mixture 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphoethanolamine (POPE)/1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC) 8:2 (*w*/*w*; 10 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 consists of PTFE (*Teflon*) 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/*Tris*; pH 7), the *cis* and *trans* compartments were linked with Ag/AgCl electrodes embedded in agarose/3M KCl. Then, a soln. of POPC/POPE (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 made-up automatically using a perfusion system (*Bilayer Explorer V01*, *Ionovation GmbH*, D-Osnabrück). The formation of a stable bilayer was monitored both 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 soln. 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), and with an emitting laser diode (635 nm), a 40× H_2O-immersion objective (*UApo 340*, 40×; NA, 1.15, *Olympus*, Tokyo), and an *Avalanche* photodiode detector (*SPCM-AQR-13-FC*, *Perkin-Elmer 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. 3* and *5*) the confocal plane was moved in 100-nm steps. Diffusion coefficients were determined by photon correlation spectroscopy (PCS) and are compiled in the *Table*.

Incorporation of the Oligonucleotide **8** *in an Artificial Bilayer.* The incorporation of the oligonucleotide **8** (concentration, 50 nM) in an artificial bilayer was principally performed as described above for compound **7b**. The laser irradiation of the cyanine-5-labelled oligomer was performed at 635 nm with an irradiation power of 200 μW. Perfusions were conducted for 30 s each, with 250 nM KCl, 10 mM MOPS/*Tris:* pH 7.0 buffer. Consecutive perfusions of the *cis* compartment of the bilayer slide were interrupted by incubation periods of 5 min.

*5-Fluoro-1-[(3a*R,*4*R,*6*R,*6a*R*)-tetrahydro-6-(hydroxymethyl)-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H,*3*H*)-dione* (**2**) [17]. Anh. *5-fluorouridine* (5-FUrd; **1**; 2 g, 7.64 mmol) was dissolved in dry DMF (30 ml). After addition of heptan-4-one (2.2 ml; 15.28 mmol), $(EtO)_3CH$ (2 ml; 11.46 mmol), and 4M HCl in 1,4-dioxane (6.8 ml), the mixture was stirred for 22 h at r.t. The raw product was partitioned between 350 ml of CH_2Cl_2 and 350 ml of conc. aq. $NaHCO_3$, and extracted. The org. layer was washed with H_2O (350 ml), dried (Na_2SO_4), filtered, and evaporated to dryness. Residual DMF was removed at 40° in high vacuum. The resulting yellowish oil was crystallized from CH_2Cl_2 at 4° overnight, and the crystals were again dried at 40° in high vacuum. Yield: 1.88 g, (69%). Colorless needles. M.p. 155°. R_f ($CHCl_3$/MeOH 9:1) 0.45. ^{1}H-NMR ((D_6)DMSO): 11.83 (*s*, H–N(3)); 8.16 (*d*, ^3J(6,F) = 5.0, H–C(6)); 5.84 (*s*, H–C(1′)); 5.14 (*t*, ^3J = 7.5, HO–C(5′)); 4.90–4.89 (*m*, H–C(2′)); 4.77–4.75 (*m*, H–C(3′)); 4.11–4.09 (*m*, H–C(4′)); 3.63–3.55 (*m*, ^2J = −10.0, CH_2(5′)); 1.68–1.64 (*m*, $CH_2(\alpha')$); 1.53–1.50 (*m*, $CH_2(\alpha)$); 1.45–1.37 (*m*, $CH_2(\beta')$); 1.33–1.24 (*m*, $CH_2(\beta)$); 0.91 (*t*, ^3J = 5.0, Me(γ')); 0.87 (*t*, ^3J = 5.0, Me(γ)). ^{13}C-NMR ((D_6)DMSO): 157.0 (*d*, ^2J(4,F) = −26.28, C(4)); 149.0 (C(2)); 139.8 (*d*, ^1J(5,F) = 230.49, C(5)); 125.9 (*d*, ^2J(6,F) = −34.70, C(6)); 116.4 (O–C–O); 91.1 (C(4′)); 86.68 (C(1′)); 83.9 (C(2′)); 80.5 (C(3′)); 61.2 (C(5′)); 38.7 (C(α'), C(α)); 16.9 (C(β')); 16.3 (C(β)); 14.1 (C(γ')); 14.0 (C(γ)).

*5-Fluoro-1-[(3a*R,*4*R,*6*R,*6a*R*)-tetrahydro-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H,*3*H*)-dione* (**3**). Compound **2** (0.5 g, 1.4 mmol) was evaporated trice from anh. pyridine and then dissolved in anh. pyridine (4 ml). Then, (4-

methoxyphenyl)(diphenyl)methyl chloride (MMTrCl; 0.53 g, 1.67 mmol) was added under N_2. The mixture was stirred for 18 h at r.t., and the reaction was then quenched by addition of MeOH (3.5 ml). After 10 min, an ice-cold aq. 5% $NaHCO_3$ soln. was added, and the mixture was extracted three times with CH_2Cl_2 (80 ml each). The combined org. layers were dried for 30 min (Na_2SO_4), filtered, evaporated, and dried in high vacuum to yield a colorless foam. Column chromatography (CC) of the residue (SiO_2 *60 H*, column: 10 × 14 cm; CH_2Cl_2/MeOH 97:3) afforded one main zone from which compound **3** (0.8 g, 91%) was isolated. Colorless foam. R_f (CH_2Cl_2/MeOH 97:3) 0.60. UV (MeOH): 270 (9.000). ^{1}H-NMR ((D$_6$)DMSO): 11.86 (*s*, H–N(3)); 8.05 (*d*, ^3J(6,F) = 4.0, H–C(6)); 7.39–7.19 (*m*, 2 H–C(3″), 4 H–C(8″), 4 H–C(9″), 2 H–C(10″)); 6.87 (*d*, ^3J = 9.0, 2 H–C(4″)); 5.80 (*s*, H–C(1′)); 4.99–4.97 (*m*, H–C(2′)); 4.66–4.64 (*m*, H–C(3′)); 4.17–4.14 (*m*, H–C(4′)); 3.74 (*s*, MeO); 3.34–3.31 (*m*, ^2J = −10.25, 1 H, CH_2(5′)); 3.14–3.12 (*m*, ^2J = −5.25, 1 H, CH_2(5′)); 1.66–1.63 (*m*, CH_2(α')); 1.50–1.47 (*m*, CH_2(α)); 1.43–1.36 (*m*, CH_2(β')); 1.28–1.21 (*m*, CH_2(β)); 0.91 (*t*, ^3J = 7.5, Me(γ')); 0.85 (*t*, ^3J = 7.5, Me(γ)). ^{13}C-NMR ((D$_6$)DMSO): 158.2 (C(5″)); 157.0 (*d*, ^2J(4,F) = −26.28, C(4)); 148.8 (C(2)); 144.1 (C(7″)); 139.9 (*d*, ^1J(5,F) = 231.5, C(5)); 134.7 (C(2″)); 129.9–127.4 (*m*, C(3″), C(8″), C(9″), C(10″)); 126.8 (*d*, ^2J(6,F) = −5.40, C(6)); 116.8 (O–C–O); 113.1 (C(4″)); 91.9 (C(1″)); 85.9 (C(4′)); 85.6 (C(1′)); 83.6 (C(2′)); 80.7 (C(3′)); 64.1 (C(5′)); 54.9 (C(6″)); 38.7 (C(α')); 38.6 (C(α)); 16.9 (C(β')); 16.3 (C(β)); 14.1 (C(γ')); 14.0 (C(γ)). Anal. calc. for $C_{36}H_{29}FN_2O_7$ (630.70): C 68.56, H 6.23, N 4.44; found: C 68.85, H 6.03, N 4.23.

*5-Fluoro-1-[(3a*R*,4*R*,6*R*,6a*R*)-tetrahydro-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]-3-[(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione* (**4a**, (*E*) + (*Z*)). Compound **3** (1 g, 1.59 mmol) was dissolved in anh. THF (10.6 ml). After addition of phytol (0.61 ml, 1.59 mmol) and Ph_3P (0.62 g, 2.38 mmol), the mixture was stirred for 5 min at r.t. under N_2 and with exclusion of light. Then, the mixture was cooled to 0°, and a 40% soln. of diethyl azodicarboxylate (DEAD) in toluene (0.69 ml; 2.38 mmol) was added dropwise within 1 min. After further 5 min of stirring at 0°, the mixture was allowed to warm to r.t., and stirring was continued for 2 h. After evaporation of the solvent in high vacuum (45°), the residue was purified by repeated CC (SiO_2, 1st column: 2 × 25 cm; AcOEt/petroleum ether (PE) 1:13; 2nd column: 2 × 18 cm; AcOEt/PE, 15:75; each solvent with 1% of Et_3N). Yield 1.1 g (76%). Colorless foam. R_f (AcOEt/PE 1:7) 0.55 and 0.60 (*E*)- and (*Z*)-isomers, resp.). UV (MeOH): 270 (9,000). ^{1}H-NMR ((D$_6$)DMSO): 8.16 (*d*, ^3J(6$_{cis}$,F) = 6.5, H$_{cis}$–C(6)); 8.25 (*d*, ^3J(6,F) = 6.5, H–C(6)(*E*)); 7.38–7.21 (*m*, 2 H–C(3″), 4 H–C(8″), 4 H–C(9″), 2 H–C(10″)); 6.84 (*d*, ^3J = 9.0, 2 H–C(4″)); 5.84 (*s*, H–C(1′)); 4.99–4.97 (*m*, H–C(2′), H–C(2‴)); 4.67–4.63 (*m*, H–C(3′)); 4.36 (*d*, ^3J = 5.0, H–C(1‴)(*Z*)); 4.33 (*d*, ^3J = 5.0, H–C(1‴)(*E*)); 4.21–4.18 (*m*, H–C(4′)); 3.72 (*s*, Me(6″)); 3.35–3.32 (*m*, H$_\alpha$–C(5′)); 3.15–3.09 (*m*, H$_\beta$–C(5′)); 2.07 (*t*, ^3J = 7.5, H–C(4‴)(*Z*)); 1.87 (*t*, ^3J = 7.5, H–C(4‴)(*E*)); 1.67 (*s*, Me(20″)); 1.66–1.63 (*m*, CH_2(α')); 1.51–1.43 (*m*, CH_2(α), H–C(15‴)); 1.42–1.30 (*m*, CH_2(β'), CH_2(5‴), H–C(7‴), H–C(11‴)); 1.28–0.98 (*m*, CH_2(β), CH_2(6‴), CH_2(8‴), CH_2(9‴), CH_2(10‴), CH_2(12‴), CH_2(13‴), CH_2(14‴)); 0.91 (*t*, ^3J = 7.0, Me(γ')); 0.84 (*t*, ^3J = 7.0, Me(γ)); 0.83–0.78 (*m*, Me(16‴), Me(17‴), Me(18‴), Me(19‴)). ^{13}C-NMR ((D$_6$)DMSO): 158.2 (C(5″)); 156.1 (*d*, ^2J(4,F) = −25.78, C(4)); 148.6 (*d*, ^4J(2,F) = 6.28, C(2)); 144.0 (C(7″)); 139.4 (*d*, ^1J(5,F) = 229.8, C(5)); 139.8 (*s*, C(3‴)(*Z*)); 139.6 (*s*, C(3‴)(*E*)); 134.7 (C(2″)); 129.9–126.7 (*m*, C(3″), C(8″), C(9″), C(10″)); 125.6 (*d*, ^2J(6,F) = −32.82, C(6)); 117.8 (C(2‴)); 116.7 (O–C–O); 113.1 (C(4″)); 93.2 (C(4′)); 86.3 (C(1″)); 86.0 (C(1′)); 83.7 (C(2′)); 80.8 (C(3′)); 64.1 (C(5′)); 54.9 (C(6″)); 39.0 (C(1‴)); 38.7 (C(α')); 38.5 (C(α)); 36.7–36.5 (*m*, C(6‴), C(8‴), C(10‴), C(12‴)); 35.8, 35.7 (2*s*, C(7‴), C(11‴)); 27.3 (C(15‴)); 24.3 (C(5‴)); 24.0 (C(9‴)); 23.6 (C(13‴)); 22.4, 22.3 (2*s*, C(16‴), C(17‴)); 19.5, 19.4 (2*s*, C(18‴), C(19‴)); 16.9(C(β')); 16.3 (C(β)); 15.9 (C(20‴)); 14.1 (C(γ')); 14.0 (C(γ)). Anal. calc for $C_{56}H_{77}FN_2O_7$ (909.22): C 73.98, H 8.54, N 3.08; found: C 73.75, H 8.57, N 2.73.

*3-[(2*Z*)-3,7-Dimethylocta-2,6-dien-1-yl]-5-fluorotetrahydro-1-[(3a*R*,4*R*,6*R*,6a*R*)-6-{[(4-methoxyphenyl)(diphenyl)methoxy]methyl}-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H*,3*H*)-dione* (**4b**). Compound **3** (1 g, 1.59 mmol) was dissolved in anh. THF (11 ml) and reacted with nerol (0.28 ml, 1.59 mmol), Ph_3P (0.62 g, 2.38 mmol), and DEAD (40% in toluene, 0.69 ml, 2.38 mmol) as described for **4a**. The raw product was purified by CC (SiO_2 *60*, 2 × 30 cm; AcOEt/PE 1:13, containing 1% of Et_3N). From the main zone, compound **4b** (0.98 g, 79%) was isolated. Colorless oil. R_f (AcOEt/PE 1:7) 0.42. UV (MeOH): 270 (11,500). ^{1}H-NMR ((D$_6$)DMSO): 8.15 (*d*, ^3J(6,F) = 6.0, H–C(6)); 7.38–7.20 (*m*, 2 H–C(3″), 4 H–C(8″), 4 H–C(9″), 2 H–C(10″)); 6.85 (*d*, ^3J = 9.0, 2 H–C(4″)); 5.84 (*s*, H–C(1′));

5.12 (*t*, ^3J = 6.0, H–C(2‴)); 5.01, 5.00 (2*s*, H–C(2′), H–C(6‴)); 4.66 (*t*, ^3J = 4.5, H–C(3′)); 4.30 (*pseudo-quint.*, ^3J = 7.5, CH_2(1‴)); 4.22–4.20 (*m*, H–C(4′)); 3.73 (*s*, MeO); 3.35–3.31 (*m*, H_α–C(5′)); 3.13–3.11 (*m*, H_β–C(5′)); 2.14–2.05 (*m*, CH_2(4‴), CH_2(5‴)); 1.67–1.59 (*m*, CH_2(α'), Me(8‴), Me(9‴), Me(10‴)); 1.50–1.46 (*m*, CH_2(α)); 1.43–1.41 (*m*, CH_2(β')); 1.27–1.22 (*m*, CH_2(β)); 0.92 (*t*, ^3J = 7.0, Me(γ')); 0.87 (*t*, ^3J = 7.0, Me(γ)). ^{13}C-NMR ((D$_6$)DMSO): 158.2 (C(3″)); 156.1 (*d*, ^2J (4,F) = −25.09, C(4)); 148.6 (C(7″)); 144.1 (*d*, ^4J(2,F) = 25.09, (C(2)); 148.6 (C(7″)); 144.1 (*d*, ^1J(5,F) = 229.6, C(5)); 139.4 (C(3‴)); 134.7 (C(2″)); 131.1 (C(7‴)); 129.9–126.8 (C(3″), C(8″), C(9″), C(10″)); 125.7 (*d*, ^2J(6,F) = 32.57, C(6)); 123.8 (C(2‴)); 118.8 (C(6‴)); 116.7 (O–C–O); 113.1 (C(4″)); 93.4 (C(4′)); 86.2 (C(1″)); 86.0 (C(1′)); 83.8 (C(2′)); 80.9 (C(3′)); 64.2 (C(5′)); 54.9 (C(6″)); 38.9 (C(1‴)); 38.7 (C(α')); 38.5 (C(α)); 31.6 (C(4‴)); 26.3 (C(5‴)); 25.4 (C(9‴)); 22.9 (C(10‴)); 17.4 (C(8‴)); 16.9 (C(β')); 16.2 (C(β)); 14.0 (C(γ'), C(γ)). Anal. calc. for $C_{46}H_{55}FN_2O_7 \cdot 0.5\ C_6H_{12}$ (809.02): C 72.75, H 7.60, N 3.46; found: C 72.48, H 7.43, N 3.28.

*5-Fluoro-1-[(3a*R*,4*R*,6*R*,6a*R*)-tetrahydro-6-(hydroxymethyl)-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]-3-[(7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione* (**5a**, (*E*)+(*Z*)). Compound **4a** ((*E*)+(*Z*); 200 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (4.5 ml). Then, 4.5 ml of a 4% soln. of $Cl_2CHCOOH$ in CH_2Cl_2 was added dropwise. The mixture was stirred for 10 min at r.t. and then washed with H_2O, until the aq. phase became neutral. The layers were separated by centrifugation, and the org. phase was evaporated to dryness. The residue was dissolved in a small volume of AcOEt, adsorbed to a small amount of SiO_2 subjected to CC (SiO_2, 2 × 15 cm; AcOEt/PE, 1:4) to yield, from the main zone, **5a** (87 mg, 62%). R_f (AcOEt/PE 1:4) 0.41. UV (MeOH): 270 (10,600). ^{1}H-NMR ((D$_6$)DMSO): 8.23 (*d*, ^3J(6,F) = 6.5, H–C(6)); 5.89 (*s*, H–C(1′)); 5.18–5.11 (*m*, H–C(5′), H–C(2″)); 4.90–4.88 (*m*, H–C(2′), H–C(2″)); 4.78–4.76 (*m*, H–C(3′)); 4.40 (*d*, ^3J = 5.0, H–C(1″)(*Z*)); 4.39 (*d*, ^3J = 5.0, H–C(1″)(*E*)); 4.15–4.14 (*m*, H–C(4′)); 3.62–3.60 (*m*, H_α–C(5′)); 3.59–3.57 (*m*, H_β–C(5′)); 2.12 (*t*, ^3J = 7.5, H–C(4″)(*Z*)); 1.92 (*t*, ^3J = 7.5, H–C(4″)(*E*)); 1.71 (*s*, Me(20″)); 1.68–1.65 (*m*, CH_2(α')); 1.53–1.47 (*m*, CH_2(α), H–C(15″)); 1.46–1.31 (*m*, CH_2(β'), CH_2(5″), H–C(7″), H–C(11″)); 1.29–1.04 (*m*, CH_2(β), CH_2(6″), CH_2(8″), CH_2(9″), CH_2(10″), CH_2(12″), CH_2(13″), CH_2(14″)); 0.91 (*t*, ^3J = 7.5, Me(γ')); 0.86 (*t*, ^3J = 7.5, Me(γ)); 0.85–0.80 (*m*, Me(16″), Me(17″), Me(18″), Me(19″)). ^{13}C-NMR ((D$_6$)DMSO): 157.9 (*d*, ^2J(4,F) = −46.38, C(4)); 148.7 (*d*, ^4J(2,F) = 3.77, C(2)); 139.3 (*d*, ^1J(5,F) = 228.75, C(5)); 140.0 (*s*, C(3″)(*Z*)); 139.6 (*s*, C(3″)(*E*)); 124.5 (*d*, ^2J(6,F) = −34.58, C(6)); 118.7 (*s*, C(2″)(*Z*)); 118.0 (*s*, C(2″)(*E*)); 116.4 (O–C–O); 92.1 (C(4′)); 86.9 (C(1′)); 84.1 (C(2′)); 80.5 (C(3′)); 61.2 (C(5′)); 38.7 (C(1″)); 38.6 (C(α')); 38.5 (C(α)); 36.6–36.5 (*m*, C(6″), C(8″), C(10″), C(12″)); 35.8, 35.7 (2*s*, C(7″), C(11″)); 27.3 (C(15″)); 24.2 (C(5″)); 24.2 (C(9″)); 24.0 (C(13″)); 22.4, 22.3 (2*s*, C(16″), C(17″)); 19.50, 19.45 (2*s*, C(18″), C(19″)); 16.9 (C(β')); 16.2 (C(β)); 15.9 (C(20″)); 14.1 (C(γ')); 14.0 (C(γ)). Anal. calc. for $C_{36}H_{61}FN_2O_6$ (636.88): C 67.89, H 9.65, N 4.40; found: C 67.61, H 9.79, N 4.29. log *P* = +12.5 ± 0.63.

*3-[(2*Z*)-3,7-Dimethylocta-2,6-dien-1-yl]-5-fluoro-1-[(3a*R*,4*R*,6*R*,6a*R*)-tetrahydro-6-(hydroxymethyl)-2,2-dipropylfuro[3,4-*d*][1,3]dioxol-4-yl]pyrimidine-2,4(1*H*,3*H*)-dione* (**5b**). Compound **4b** (1.25 g, 1.63 mmol) was detritylated and purified as described for **5a**. CC (SiO_2, 2 × 7.5 cm, AcOEt/PE 1:4) gave one main zone from which **5b** (0.528 g, 66%) was obtained. Colourless oil. R_f (AcOEt/PE 1:4) 0.25. UV (MeOH): 270 (9900). ^{1}H-NMR ((D$_6$)DMSO): 8.23 (*d*, ^3J(6,F) = 7.0, H–C(6)); 5.90 (*s*, H–C(1′)); 5.18–5.13 (*m*, H–C(2′), H–C(2″), H–C(6″)); 4.90–4.88 (*m*, HO–C(5′)); 4.77–4.75 (*m*, H–C(3)); 4.40 (*d*, ^3J = 7.0, CH_2(1″)); 4.15 (*dt*, ^3J = 3.5, H–C(4′)); 3.62–3.57 (*m*, CH_2(5′)); 2.18–2.06 (*m*, CH_2(4″), CH_2(5″)); 1.68–1.66 (*m*, CH_2(α'), Me(9″), Me(10″)); 1.59 (*s*, Me(8″)); 1.53–1.49 (*m*, CH_2(α)); 1.44–1.39 (*m*, CH_2(β')); 1.30–1.52 (*m*, CH_2(β)); 0.93 (*t*, ^3J = 7.0, Me(γ')); 0.91 (*t*, ^3J = 7.0, Me(γ)). ^{13}C-NMR ((D$_6$)DMSO): 156.1 (*d*, ^2J(4,F) = −25.65, C(4)); 148.7 (C(2)); 139.4 (*d*, ^2J(5,F) = 228.76, C(5)); 139.5 (C(3″)); 131.1 (C(7″)); 124.5 (*d*, ^1J(6,F) = 34.8, C(6)); 123.8 (C(2″)); 118.9 (C(6″)); 116.4 (O–C–O); 92.1 (C(4′)); 86.9 (C(1′)); 84.1 (C(2′)); 80.5 (C(3′)); 61.2 (C(5′)); 38.7 (C(α')); 38.6 (C(α)); 31.6 (C(4″)); 25.9 (C(5″)); 25.4 (C(9″)); 22.9 (C(10″)); 17.4 (C(8″)); 16.9 (C(β')); 16.2 (C(β)); 14.02 (C(γ')); 13.97 (C(γ)). Anal. calc. for $C_{26}H_{39}FN_2O_6$ (494.60): C 63.14, H 7.95, N 5.66; found: C 63.08, H 8.13, N 5.69. log *P* = +7.65 ± 0.65.

*2-Cyanoethyl [(3a*R*,4*R*,6*R*,6a*R*)-6-[5-Fluoro-2,4-dioxo-3-[(7*R*,11*R*)-3,4-dihydro-3,7,11,15-tetramethylhexadec-2-en-1-yl]pyrimidin-1(2*H*)-yl]-2,2-dipropyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]methyl Di(propan-2-yl)phosphoramidite* (**6a**, (*E*)+(*Z*)). Compound **5a** (*E*)+(*Z*); (0.2 g, 0.314 mmol) was evaporated three times from anh. CH_2Cl_2 and then dissolved in anh. CH_2Cl_2 (12 ml). Thereupon, EtN^iPr_2 (*Hünig*'s base; 101.5 µl, 0.597 mmol) and chloro(2-cyanoethyl)(diisopropyl)phosphine (126 µl,

0.565 mmol) were added under N_2. The mixture was stirred at r.t. for exactly 15 min. After addition of an ice-cold 5% aq. $NaHCO_3$ soln. (10 ml), the mixture was extracted three times with CH_2Cl_2 (5 ml each). The combined org. layers were dried (Na_2SO_4) for 1 min under N_2 and with cooling. After filtration, the soln. was evaporated to dryness. The residue was subjected to flash chromatography (0.5 bar, SiO_2, 2 × 10 cm; CH_2Cl_2/acetone 85:15) within *ca.* 20 min. Evaporation of the main zone afforded **6a** (0.178 g, 68%). Colorless oil. R_f (CH_2Cl_2/acetone, 85:15): 0.96. ^{31}P-NMR ($CDCl_3$): 149.93; 149.75.

*2-Cyanoethyl [(3a*R*,4*R*,6*R*,6a*R*)-6-{3-[(2*Z*)-3,7-Dimethylocta-2,6-dien-1-yl]-5-fluoro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl}-2,2-dipropyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl]methyl Di(propan-2-yl)phosphoramidite* (**6b**). Compound **5b** (0.2 g, 0.405 mmol) was phosphitylated and purified as described for **6a** to give **6b**. Yield: 255 mg (91%). Colorless oil. R_f (CH_2Cl_2/acetone, 85:15): 0.96. ^{31}P-NMR ($CDCl_3$): 149.84; 149.66.

*5-Fluoro-1-[(3a*R*,4*R*,6a*R*)-tetrahydro-6-(hydroxymethyl)-2,2-dinonylfuro[3,4-*d*][1,3]dioxol-4-yl]-pyrimidine-2,4(1*H*,3*H*)-dione* (**7a**) [17]. Anh. **1** (1.0 g, 3.82 mmol) was dissolved in anh. DMF, and nonadecan-10-one (2.16 g, 7.64 mmol) was added. After addition of $(EtO)_3CH$ (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 CH_2Cl_2 (175 ml) and a sat. aq. $NaHCO_3$ soln. The org. layer was washed three times with H_2O (25 ml, each). Both aq. phases were back-extracted with CH_2Cl_2. All combined org. layers were evaporated to dryness and dried overnight in high vacuum. The residue was chromatographed twice (SiO_2, 6.5 × 12 cm; CH_2Cl_2/MeOH 95:5). Evaporation of the corresponding main zone afforded **7a** (1.38 g, 68%). Colorless oil. R_f (CH_2Cl_2/MeOH 95:5) 0.56. All anal. data were identical to those reported in [17].

Small-Scale Labelling of Compound **7a** *with the* N*-Hydroxysuccinimide Ester of* Eterneon 480® (→ **7b**). *Eterneon 480®* (5 mg, 0.0095 mmol) and **7a** (5.3 mg, 0.0095 mmol) were both dissolved in MeCN (1.5 ml, each). The soln. of **7a** was added dropwise to the fluorophore soln. under N_2 and under exclusion of light within 5 min. The mixture was stirred for 26 h at r.t. The product was purified by CC (SiO_2, 2 × 19 cm; CH_2Cl_2/MeOH 99:1) to afford **7b**. Deep red solid. R_f (CH_2Cl_2/MeOH 99:1) 0.5.

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