Nucleoterpenes of Thymidine and 2′-Deoxyinosine: Synthons for a Biomimetic Lipophilization of Oligonucleotides

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In memoriam Prof. Dr. *Friedrich Cramer*, Göttingen

2′-Deoxyinosine (**1**) and thymidine (**7**) were *N*-alkylated with geranyl and farnesyl moieties. These hydrophobic derivatives, **3a** and **3b**, and **9a** and **9b**, respectively, represent the first synthetic biomimetic nucleoterpenes and were subsequently 5′-protected and converted into the corresponding 3′-*O*-phosphoramidites, **5a** and **5b** and **11a** and **11b**, respectively. The latter were used to prepare a series of lipophilized oligonucleotide dodecamers, a part of which were additionally labelled with indocarbocyanine fluorescent dyes (Cy3 or Cy5), **18**–**23**. The insertion of the lipooligonucleotides into, as well as duplex formation at artificial lipid bilayers was studied by single-molecule fluorescence spectroscopy and fluorescence microscopy.

1. Introduction. – With the discovery of gene silencing – also of human genes – by short nucleic acids (*si*RNA) [1][2], a new therapeutic principle has evolved. This culminated in the synthesis of the so-called antagomires, short modified oligomers which are built-up from 2′-*O*-methyl-*β*-D-ribonucleosides, and which carry at both termini several phosphorothioate internucleotide linkages as well as a cholesterol tag at the 5′-end. Particularly, the latter modification renders the oligomer permeable for the cell membrane [3]. However, it has been reported that already one cholesterol moiety loads the oligomer synthesis, and its purification and handling with difficulties [4].

For these reasons, we have been searching for alternative methods for a less strong and stepped lipophilization of oligonucleotides [5]. For this purpose, a series of base-alkylated 2′-deoxyinosine- as well as 2′-deoxythymidine 2-cyanoethyl phosphoramidites, **5a** and **5b**, and **11a** and **11b**, respectively, were prepared and used for the preparation of 5′-lipophilized oligonucleotides. Principally, these phosphoramidites can be incorporated at each position of a growing nucleic acid chain by a conventional solid-phase synthesis, so that the oligonucleotide can be hydrophobized at each predetermined locus (*Fig. 1*).

The positioning of the lipophilic side chain was performed at a nucleobase atom which is involved in a *Watson–Crick* base pairing so that the resulting DNA building blocks are pure hydrophobization tools with a basic nucleoside structure. As side chains, acyclic mono- and sesquiterpenes, namely geranyl and farnesyl residues, were chosen because such residues are used in post-translational prenylation of various proteins in order to embed them within biological membranes [6].

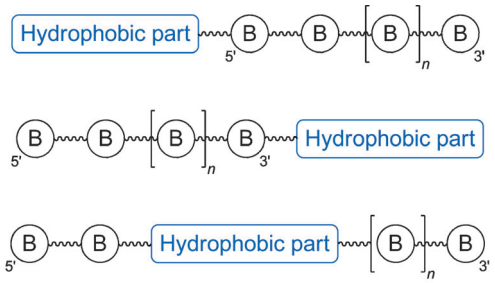
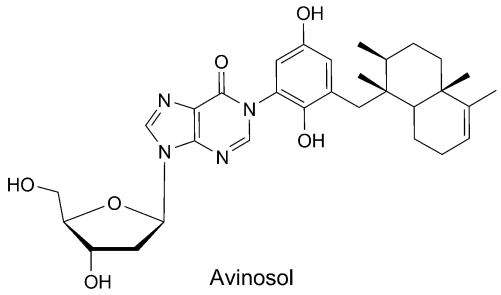


Fig. 1. *Different positionings of nucleolipids within a nucleic acid*

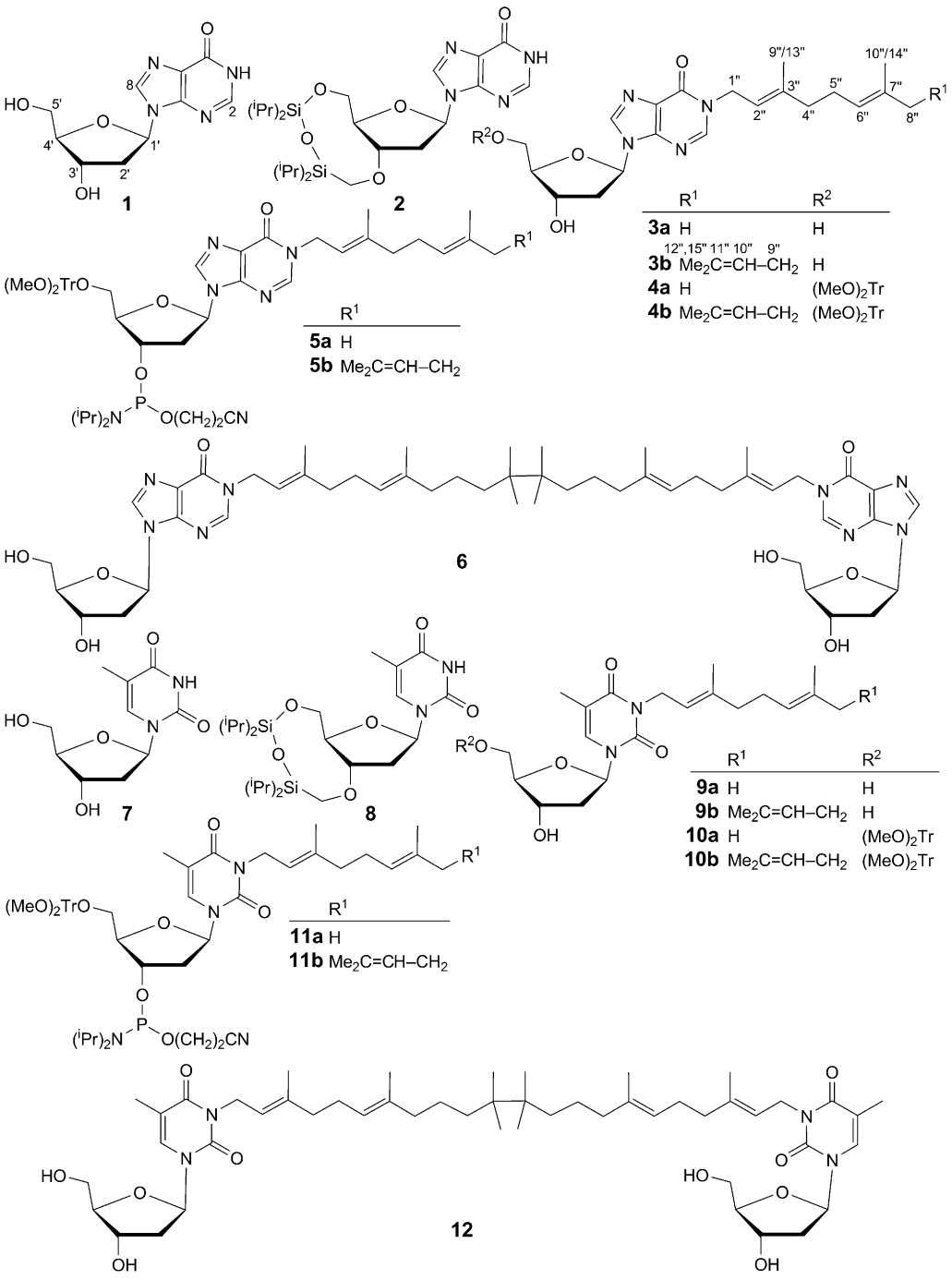
The only naturally-occurring meronucleoterpene known so far is avinosol and has been isolated from the sponge *Dysidea* sp., near Papua New Guinea in 2006. This *N(1)*-alkylated 2′-deoxyinosine derivative, carrying a benzene-1,4-diole and a sesquiterpene residue, has been shown to possess promising anti-angiogenic and antimetastatic properties [7].



Avinosol

2. Results and Discussion. – 2.1. *Synthesis.* For the preparation of *N*-geranylated and *N*-farnesylated nucleoterpenes of 2′-deoxyinosine (**1**) and thymidine (**7**), respectively, base-catalyzed alkylation in DMF with the corresponding terpenyl bromides was chosen [8]. As it has been reported that, under such conditions, also *O*-alkylation of the sugar OH groups as well as of the base occur as side reactions [9], we prepared first the 1,1,3,3-tetraisopropyldisiloxane-sugar derivatives of both 2′-deoxynucleosides, **2** and **8**, respectively. This so-called *Markiewicz* silyl clamp [10] can be easily introduced and cleaved off with Bu_4NF under mild conditions. Subsequent deprotonation of compounds **2** and **8** was performed under various reaction conditions with respect to solvent (DMF, MeCN) and base (NaH, K_2CO_3). In all cases, however, several reaction products were formed upon alkylation. In the case of 2′-deoxyinosine, a solid–liquid phase-transfer alkylation in the presence tris[(2-methoxyethoxy)ethyl]amine (MeCN, K_2CO_3) was conducted but proved to be unsuccessful. The reason for the unsteady alkylations of the silyl-protected educts might be either a shift of the silyl clamp [11] or a silyl bridging of two nucleoside molecules.

As a consequence, both unprotected 2′-deoxynucleosides, **1** and **7**, were alkylated with geranyl bromide and farnesyl bromide (DMF, K_2CO_3). In the case of 2′-deoxyinosine (**1**), a reaction time of 24 h at room temperature was sufficient for a


1
2
3a H H
3b Me2C=CH–CH2 H
4a H (MeO)2Tr
4b Me2C=CH–CH2 (MeO)2Tr
5a H
5b Me2C=CH–CH2
6
7
8
9a H H
9b Me2C=CH–CH2 H
10a H (MeO)2Tr
10b Me2C=CH–CH2 (MeO)2Tr
11a H
11b Me2C=CH–CH2
12

moderate yield (50–75%) of the products **3a** and **3b**, while for thymidine (**7**) 48 h and 40° were necessary to obtain the corresponding products **9a** and **9b** in comparable yields. In *Table 1*, the lipophilicities of the thymidine derivatives in form of their calculated log *P* values and their retention times in *RP-18* HPLC are compiled.

Table 1. *Calculated log* P *Values and Retention Times of Thymidine Derivatives in* RP-18 *HPLC* (for details, see *Exper. Part*)

Compound	log *P* Value	t_R [min]
7	-1.11 ± 0.49	1.89
9a	4.57 ± 0.61	3.34
9b	6.60 ± 0.64	7.85

Compounds **3a** and **3b**, and **9a** and **9b** represent, to our knowledge, the first examples of synthetic nucleoterpenes. All of these compounds were characterized by ^{1}H- and ^{13}C-NMR, and UV spectroscopy as well as by elemental analyses. Moreover, ESI mass spectra were recorded in the presence of 2% aqueous HCOOH. In all cases, signals corresponding to the appropriate dimeric molecules, *e.g.*, **6** and **12**, were detected. The tendency of dimerization is most pronounced in the case of the 2′-deoxyinosine nucleoterpenes.

Of the thymidine nucleoterpenes, **9a** and **9b**, the farnesylated compound **9b** exhibited a stronger dimerization tendency than the geranylated derivative **9a** (for an example, see *Fig. 2*).

Scheme 1 displays a plausible mechanism for a dimerization of a farnesylated nucleoterpene, showing only the formation of one conceivable product, namely an end-to-end dimerization. It should be kept in mind, however, that also the formations of other products, *i.e.*, either an end-to-end reaction with an intermediary hydrid rearrangement or non-end-to-end dimerization reaction, are possible.

To rule out that the synthesized nucleoterpenes, particularly the 2′-deoxyinosine derivatives **3a** and **3b**, for which predominantly dimers were detected in their ESI mass spectra, are principally dimeric molecules, as an example, a gel-permeation chromatography (GPC) was performed (THF) with compound **3b** in order to determine the molecular weight of the product. *Fig. 3* displays the elution profile of a typical chromatographic run using a light-scattering detection.

This figure clearly shows that two peaks are detectable, one of which (484 g/mol) corresponds to the calculated molar mass of compound **3b** (456.6 g/mol). However, the profile also exhibits the presence of high-molecular-weight aggregates with a mean molecular weight of 1,571,000 g/mol, corresponding to the 3,440-fold of the molar mass of the monomer. This result points to the occurrence of probably inverse micelles or liposomes with the nucleoside residues forming an inner core, and the farnesyl residues protruding into the solvent lattice.

Subsequently, the nucleoterpene **3b** was labelled with two different dyes: *i*) with a fluorenyl moiety (Fmoc) *via* a glycine spacer and *ii*) with *Texas Red*. Both reactions were performed on a small scale. First, compound **3b** was reacted at its 5′-OH group with *N*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}glycine (**13**) by using a *Steglich* esterification (DCC, DMAP) [12] (*Scheme 2*). TLC Analysis indicated the formation of three

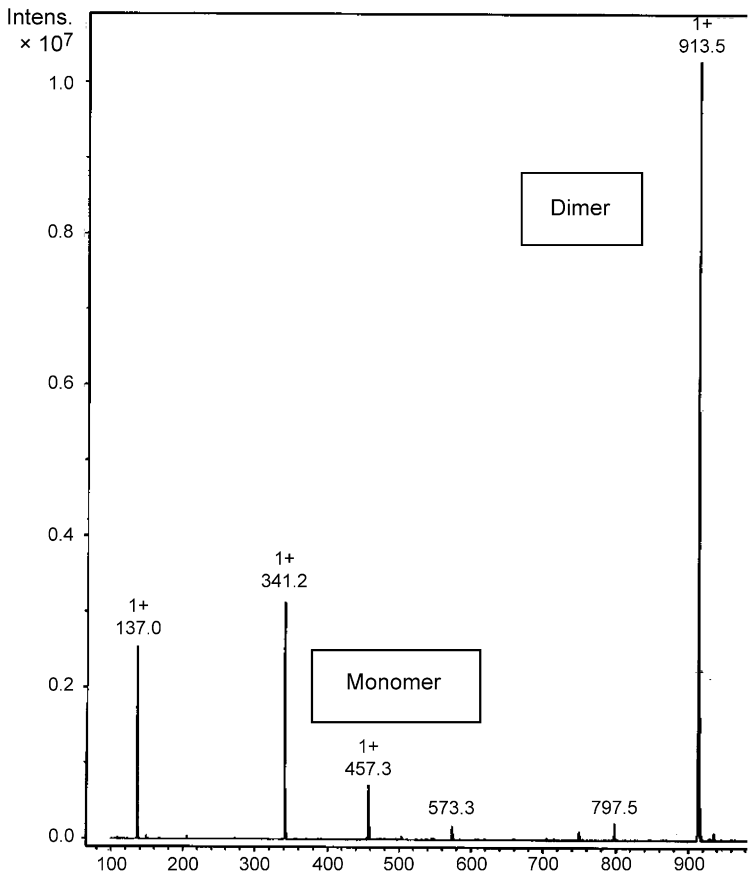


Fig. 2. *ESI-MS of compound* **3b** (ionization with 2% aq. HCOOH)

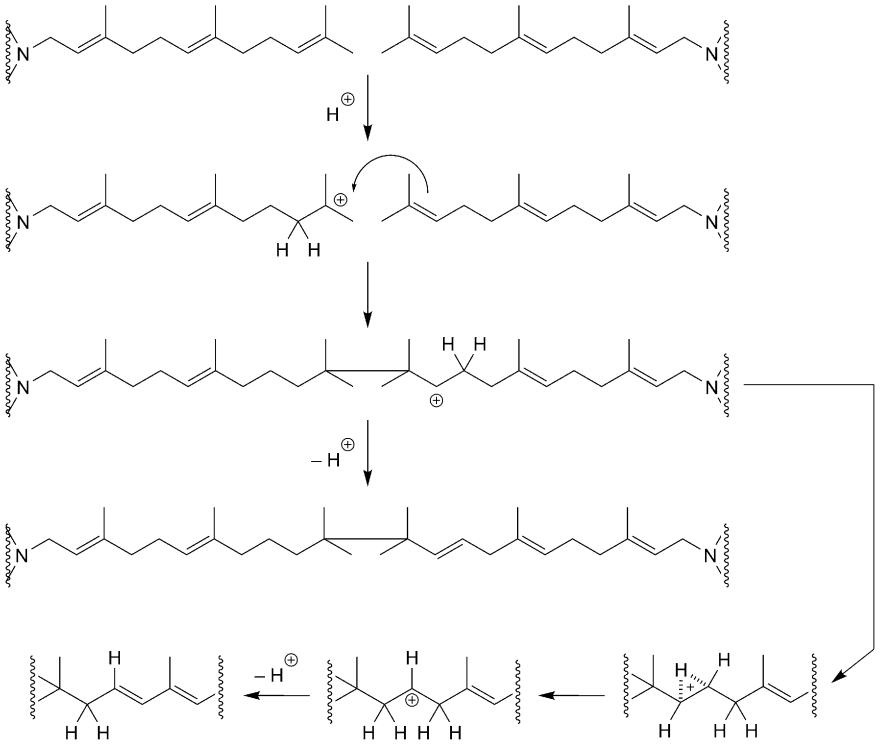
products which could be separated by chromatography. All compounds were characterized by ^{1}H- and ^{13}C-NMR as well as by UV/VIS spectroscopy. The fastest-migrating compound was identified as the 3′,5′-difluorenylated derivative **14**, and the others were the 5′- and the 3′- labelled compounds, **15** and **16**, respectively (*Scheme 2*). *Fig. 4* displays the ^{1}H-NMR low-field regions of the three derivatives **14**–**16**.

In the ^{1}H-NMR regions depicted in *Fig. 4*, the Fmoc resonances of **14** exhibit double the integral values as the signals of H–C(2) and H–C(8), establishing the double labelling.

Next, compound **3b** was coupled with sulforhodamin-101-sulfonyl chloride (*Texas Red*). After extraction and silica gel chromatography, the product **17** was obtained as a deep black, amorphous material. *Fig. 5* displays the UV/VIS spectra of educts as well as of the product.

The UV/VIS spectrum (*Fig. 5*) of the *Texas Red*-labelled derivative resembles clearly the spectrum of the dye.

Scheme 1



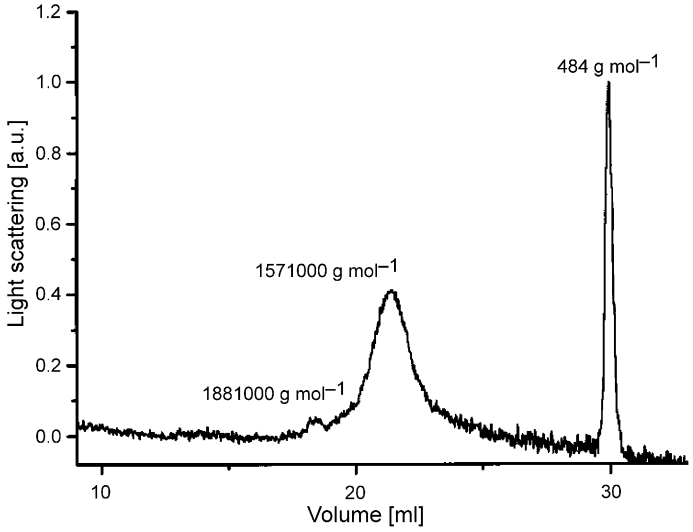
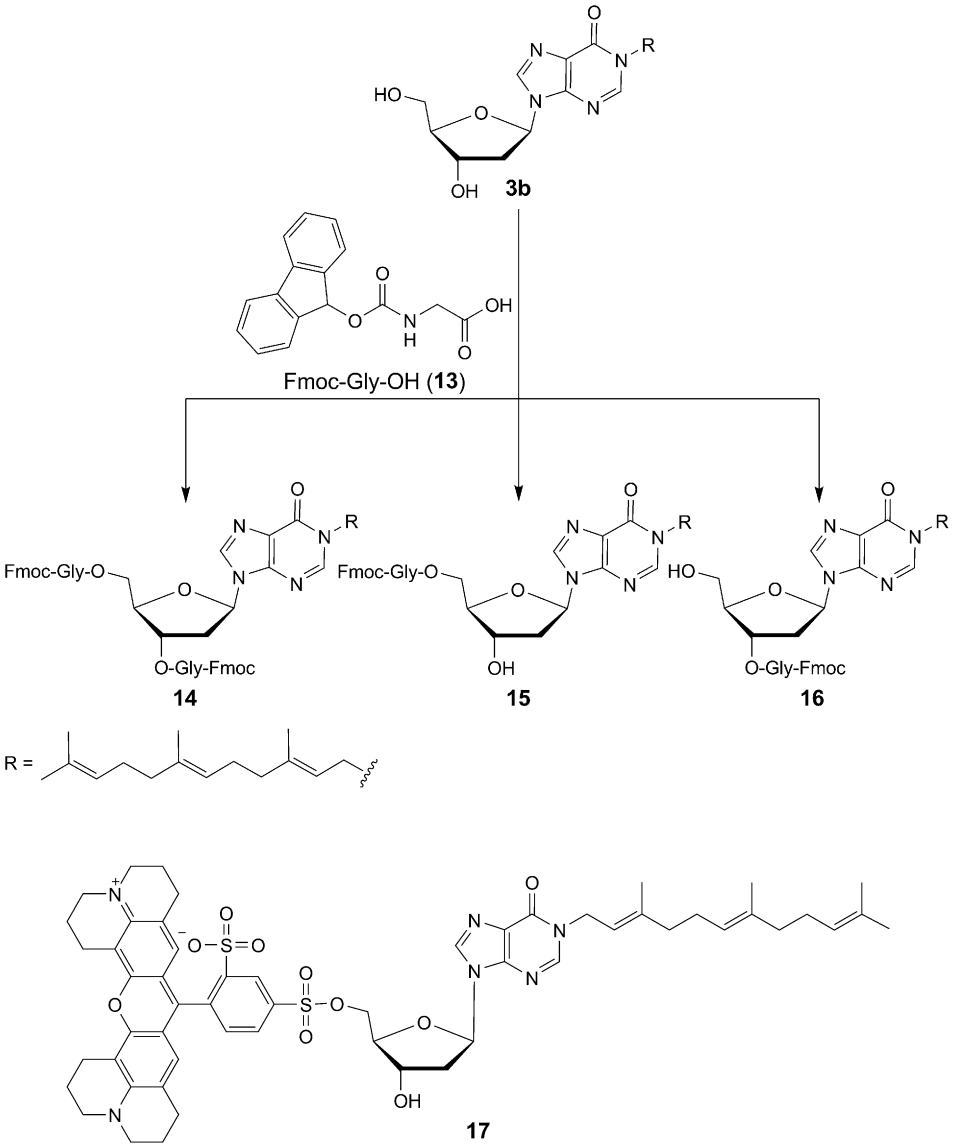


Fig. 3. *Elution profile of a gel-permeation chromatography of compound* **3b** *in THF*

Scheme 2



2.2. *Oligonucleotides.* The phosphoramidites **5b** and **11a**, and **11b** were used to prepare a series of lipophilized oligonucleotides, and their insertion into artificial lipid bilayers was studied [13–19]. The oligonucleotides synthesized and characterized by MALDI-TOF-MS are compiled in *Table 2*.

The oligonucleotides **18**–**20** contain – besides a nucleoterpene (*i.e.*, **3b** or **9a**, and **9b**) – an indocarbocyanine dye in the 5′-(n–1) position which was introduced *via* its

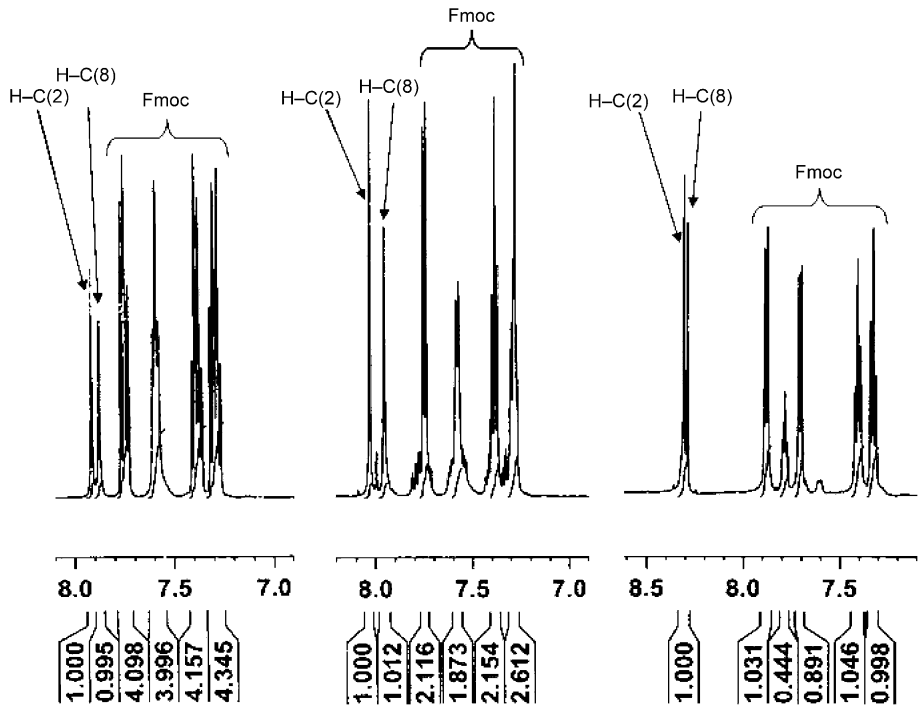


Fig. 4. *^{1}H-NMR Low-field region of compounds* **14**–**16** (from left to right)

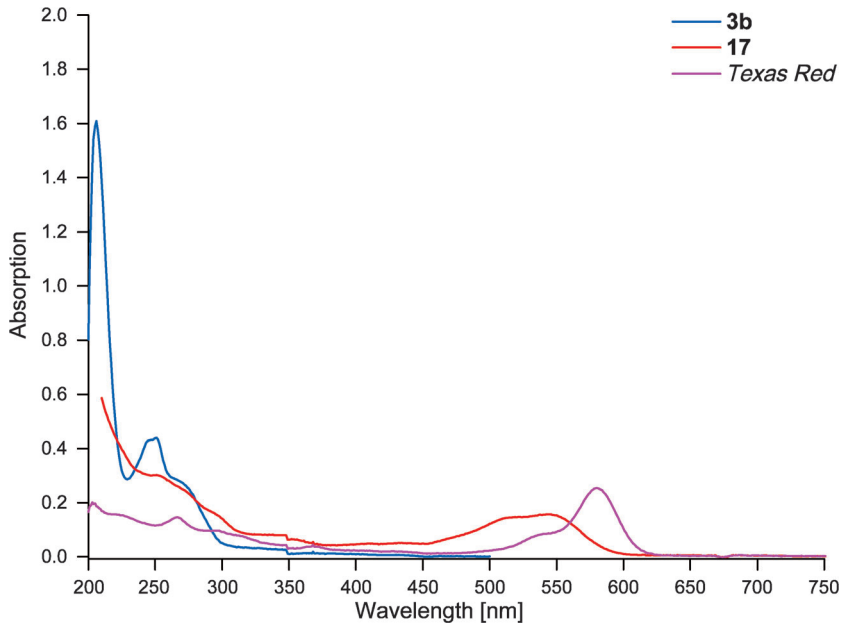


Fig. 5. *UV/VIS Spectrum of compound* **17** *as well as of the starting materials,* Texas Red, *and* **3b**

Table 2. *Sequences and MALDI-TOF Data of Oligonucleotides*

Oligonucleotide (sequence, formula No, abbreviation)			$[M+H]^+$ (calc.)	$[M+H]^+$ (found)
5′-d(**3b**-Cy3-TAG GTC AAT ACT)-3′	**18**	**KK1**	4671.6	4671.1
5′-d(**9b**-Cy3-TAG GTC AAT, ACT)-3′	**19**	**EW1**	4660.6	4659.5
5′-d(**9a**-Cy3-TAG GTC AAT, ACT)-3′	**20**	**EW2**	4592.5	4590.5
5′-d(**9b**-TAG GTC AAT, ACT)-3′	**21**	**EW3**	4153.0	4152.3
5′-d(**9b**-ATC CAG TTA TGA)-3′	**22**	**EW4**	4153.0	4152.0
5′-d(Cy5-AGT ATT GAC CTA)-3′	**23**	**EW5**	4178.1	4178.4

phosphoramidite. The oligomers **21** and **22** carry the thymidine terpene **9b** at the 5′-end, while the oligomer **23** carries a Cy5 fluorophore label and is complementary to the oligonucleotide **21** in an antiparallel strand orientation, but not to **22**.

First, the insertion of the oligonucleotides **18**–**20** (*i.e.*, **KK1**, **EW1**, and **EW2**, resp.) was tested at artificial bilayer membranes composed of 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 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. 6* (see *Exper. Part* and [20][21] for the detailed construction). From *Figs. 7* and *8*, it can be clearly seen that all *Cy3*-labelled lipo-oligonucleotides are inserted into the lipid bilayer, but to a different extent and with different stabilities towards perfusion. It is obvious that the oligomer carrying the *N(3)*-geranyl-thymidine nucleoterpene was inserted to the highest extent, but is washed out by one perfusion already to *ca.* 50%. The oligomers carrying farnesylated nucleosides at their 5′-end are significantly more stable towards perfusion.

Next, we studied the duplex formation between bilayer-immobilized lipo-oligonucleotides **21** and **22** (*i.e.*, **EW3** and **EW4**, resp.) with a Cy5-labelled oligomer **23** (**EW5**) which is complementary only to **21** (**EW3**) but not to **22** (**EW4**). Fluorescence microscopy (*cf. Figs. 9* and *10* and *Fig. 11*) clearly evidence duplex formation as expected for **21**·**23** but not for **22**·**23** [20].

Furthermore, the diffusion times (τ_D [ms]) of the duplex **21**·**23** were measured, both without and in the presence of an artificial bilayer (*Table 3*). For the determination of the free diffusion times, the corresponding oligomer duplex solution (50 nM) was diluted so that there was only a single fluorescent molecule in the confocal measuring volume (*ca.* 1 fl). Each measurement was repeated ten times for 30 s. To determine the diffusion times of the lipophilized oligonucleotide duplex **21**·**23** in the presence of a bilayer, two measuring positions, one above (in solution but in close proximity to the bilayer) and one within the bilayer were chosen. Each measurement was performed *i*) by recording reference data of a stable, blank bilayer, *ii*) after formation of the oligonucleotide duplex and a subsequent 30-min incubation, followed by recording of the data, *iii*) recording further data after perfusion of the *Bilayer Slide*. The results compiled in *Table 3* indicated that the diffusion of oligomer **23** as well as of the duplex **21**·**23** was fast. However, the broad diffusion-time distribution of the duplex **21**·**23** indicated aggregate formation of heterogeneous size. In the close proximity of a stable bilayer, the diffusion time increased approximately by a factor of 10. Probably, the molecules aggregate, and the aggregates interact partly with the bilayer. The

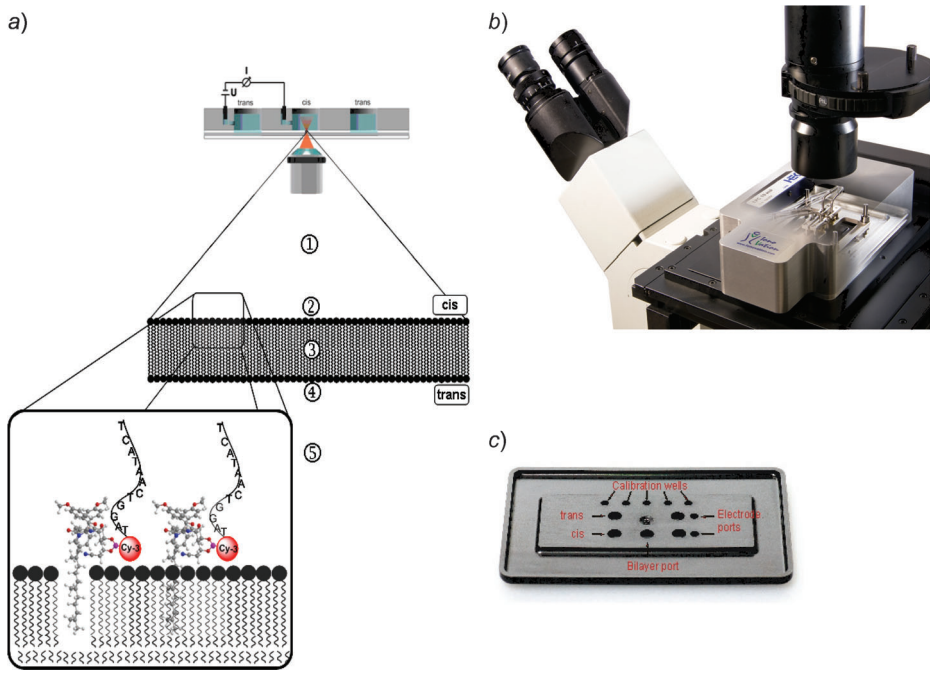


Fig. 6. *Experimental setup:* a) *schematic drawing of the laser scanning microscope, the optical transparent microfluidic bilayer slide, and the lipid bilayer with incorporated double-tailed nucleolipids.* The bilayer slide encloses two microfluidic channels (*cis* and *trans*) which are separated by a thin medical-grade polytetrafluoroethylene (PTFE) 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 (numeric apeture) objectives. It is the only connection between the *trans* and *cis* channel. When a lipid soln. 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 side board and is not shown. c) '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.

diffusion time of the bilayer-immobilized DNA duplex increased further by a factor of 10.

Further studies with other types of lipophilic nucleoside phosphoramidites, their incorporation into oligonucleotides, and their insertion into and transfer through artificial bilayer membranes are in progress.

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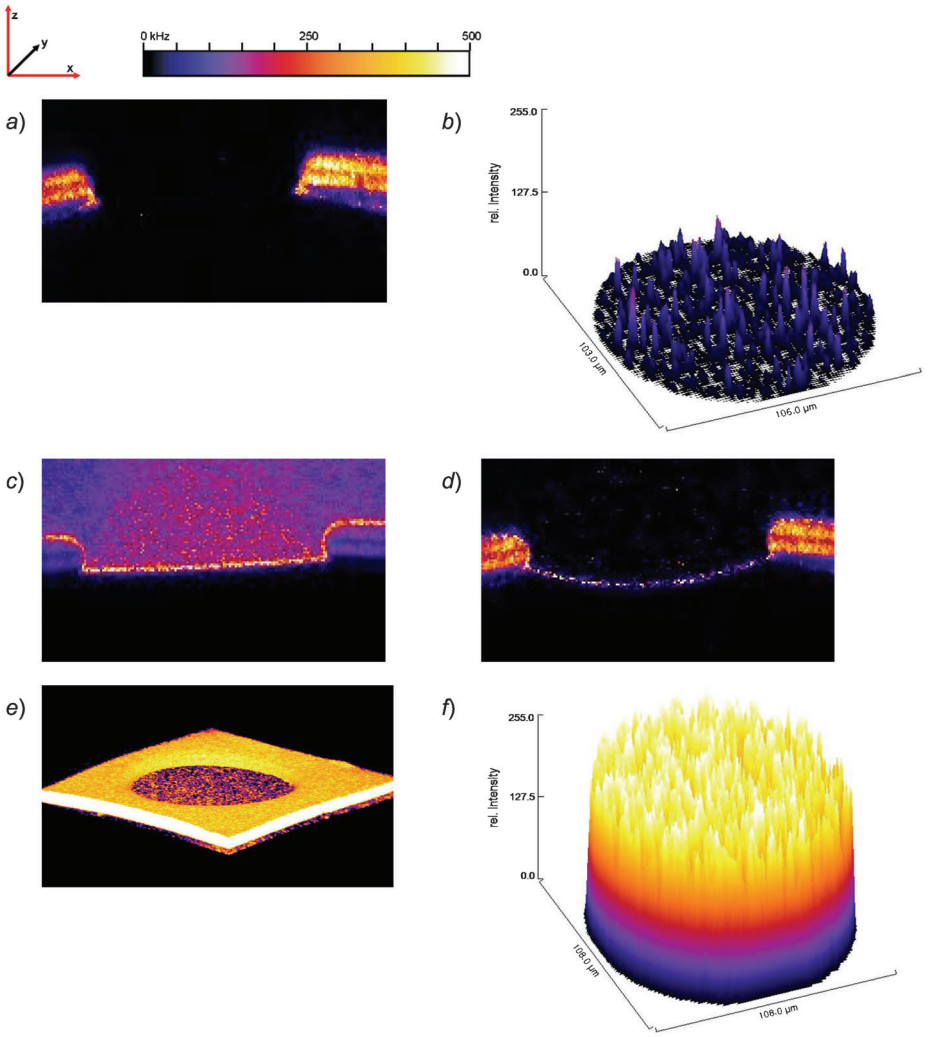


Fig. 7. *Protocol of the insertion of the oligonucleotides* **18**–**20** *into an artificial bilayer*. *a*) *z*-Scan of an empty bilayer; both channels (*cis* and *trans*) were perfused (30 s, 1.1 ml/min, each). *b*) Pixel-resolved 3D scan of an empty bilayer. *c*) *z*-Scan after addition of **18** (4 µl, 50 nM) to the *cis* channel and 25 min of incubation. *d*) *z*-Scan after first perfusion of the *cis* compartment (60 s, 1.1 ml/min). *e*) Tilted 3D view onto the bilayer, filled with **18**, after first perfusion. *f*) Pixel-resolved 3D scan of the bilayer, filled with **18**, after first perfusion. *g*) *z*-Scan after second perfusion of the *cis* compartment (60 s, 1.1 min/ml). *h*) Pixel-resolved 3D scan of the bilayer, filled with **18**, after second perfusion. *i*) *z*-Scan of the bilayer after addition of **19** (4 µl, 50 nM) to the *cis* channel and 25 min of incubation. *j*) *z*-Scan of the bilayer after first perfusion of the *cis* compartment (60 s, 1.1 ml/min). *k*) *z*-Scan of the bilayer after second perfusion of the *cis* compartment (60 s, 1.1 min/ml). *l*) *z*-Scan of the bilayer after third perfusion of the *cis* compartment (60 s, 1.1 min/ml). *m*) Tilted 3D view onto the empty bilayer. *n*) *z*-Scan of the bilayer after addition of **20** (4 µl, 50 nM) to the *cis* channel and 25 min of incubation. *o*) *z*-Scan of the bilayer, filled with **20**, after first perfusion of the *cis* compartment (60 s, 1.1 ml/min). *p*) *z*-Scan of the bilayer after second perfusion of the *cis* compartment (60 s, 1.1 min/ml).

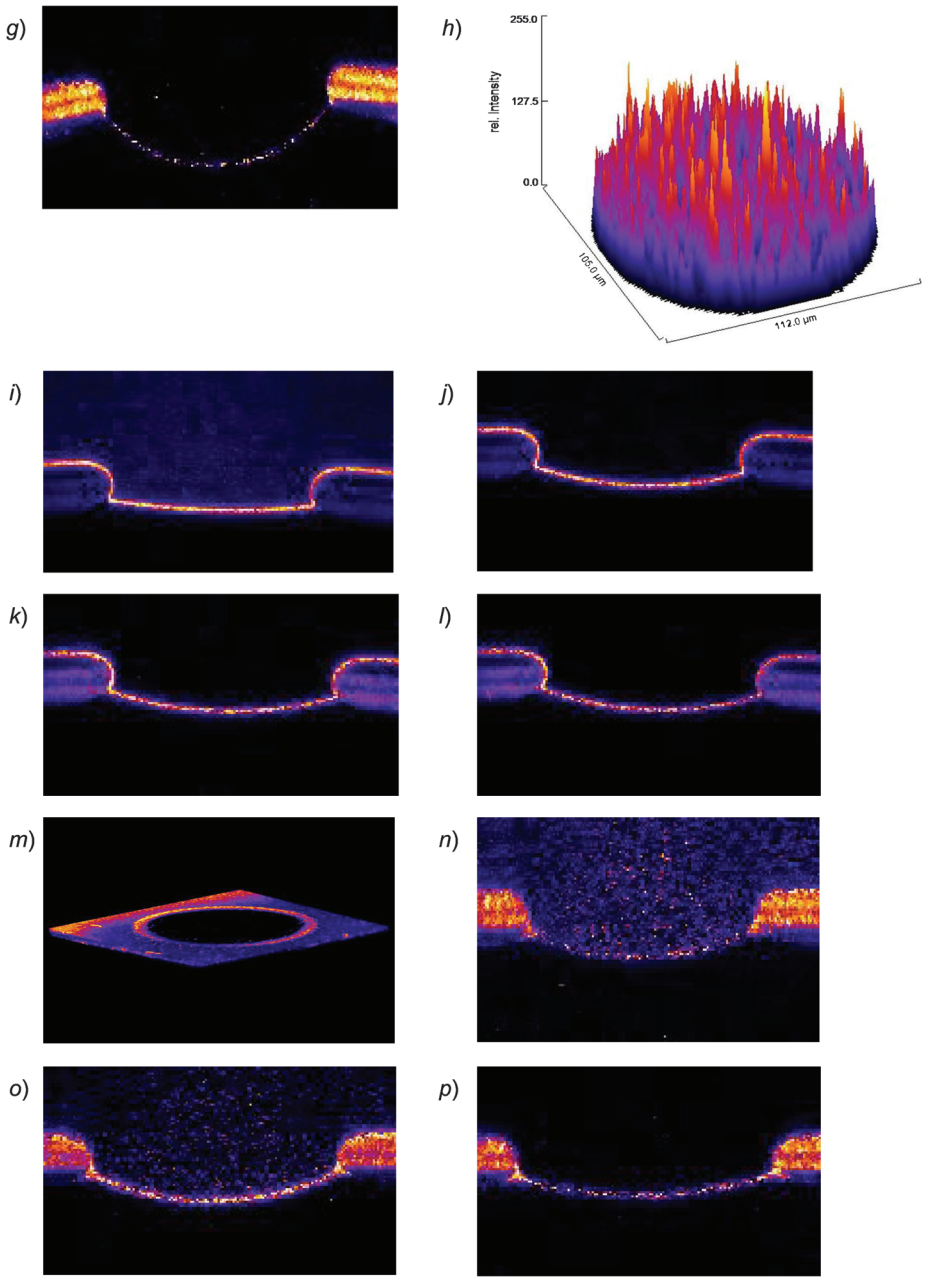


Fig. 7 (*cont.*)

Schuster for elemental analyses, Mrs. *Sandra Schwidtke* for gel-permeation chromatography, and Mrs. *Petra Bösel* for the formula drawings.

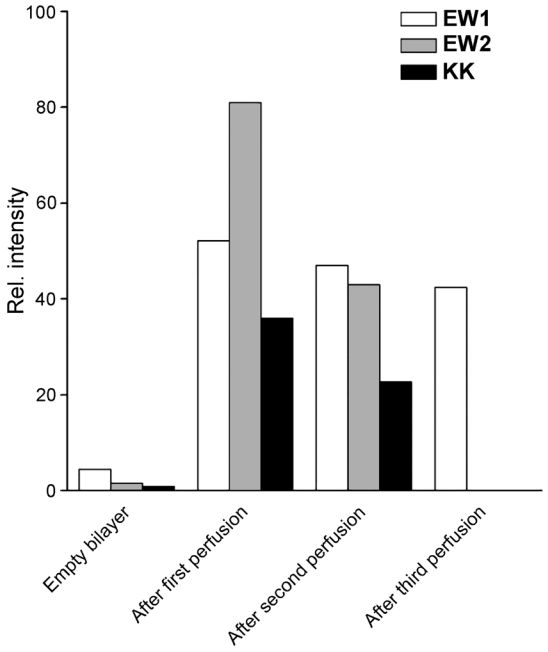


Fig. 8. *Relative bilayer brightness as a function of the perfusion number*

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. Column chromatography (CC) and flash chromatography (FC, 0.5 bar) were performed on silica gel *60*. 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 (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; *J* values in Hz. ESI-MS: *Bruker Daltronics Esquire HCT* instrument (*Bruker Daltronics*, D-Leipzig); ionization performed with a 2% aq. HCOOH soln. Elemental analyses (C, H, N) of crystallized compounds: *VarioMICRO* instrument (Fa. *Elementar*, D-Hanau). Gel-permeation chromatography (GPC): three columns with a light-scattering detector (*Dawn Helios*) and an RI detector (*Optilab rEX*, Wyatt), the results were evaluated and displayed with the program ASTRA 5.3.4, version 14. 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).

Oligonucleotide Incorporation in Artificial Bilayers [20]. The incorporation of the oligonucleotides **18**–**22** in artificial lipid bilayers was performed using a lipid mixture of 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (POPC; 8:2 (*w*/*w*); 10 mg/ml of decane). The horizontal bilayers were produced automatically within the '*Bilayer Slides*' using an '*Ionovation Explorer*' (*Ionovation GmbH*, D-Osnabrück). After pre-filling with buffer (250 mM KCl, 10 mM MOPS/*Tris*, pH 7), the '*Bilayer Slide*' was inserted into the stage unit of the '*Ionovation Explorer*'. The Ag/AgCl electrodes were mounted, and, after addition of 0.2 µl of POPE / POPC to the *cis* compartment, the automated bilayer production was started. The '*Ionovation Explorer*' uses a modified painting technique, where the air–water interface paints the lipid across the aperture.

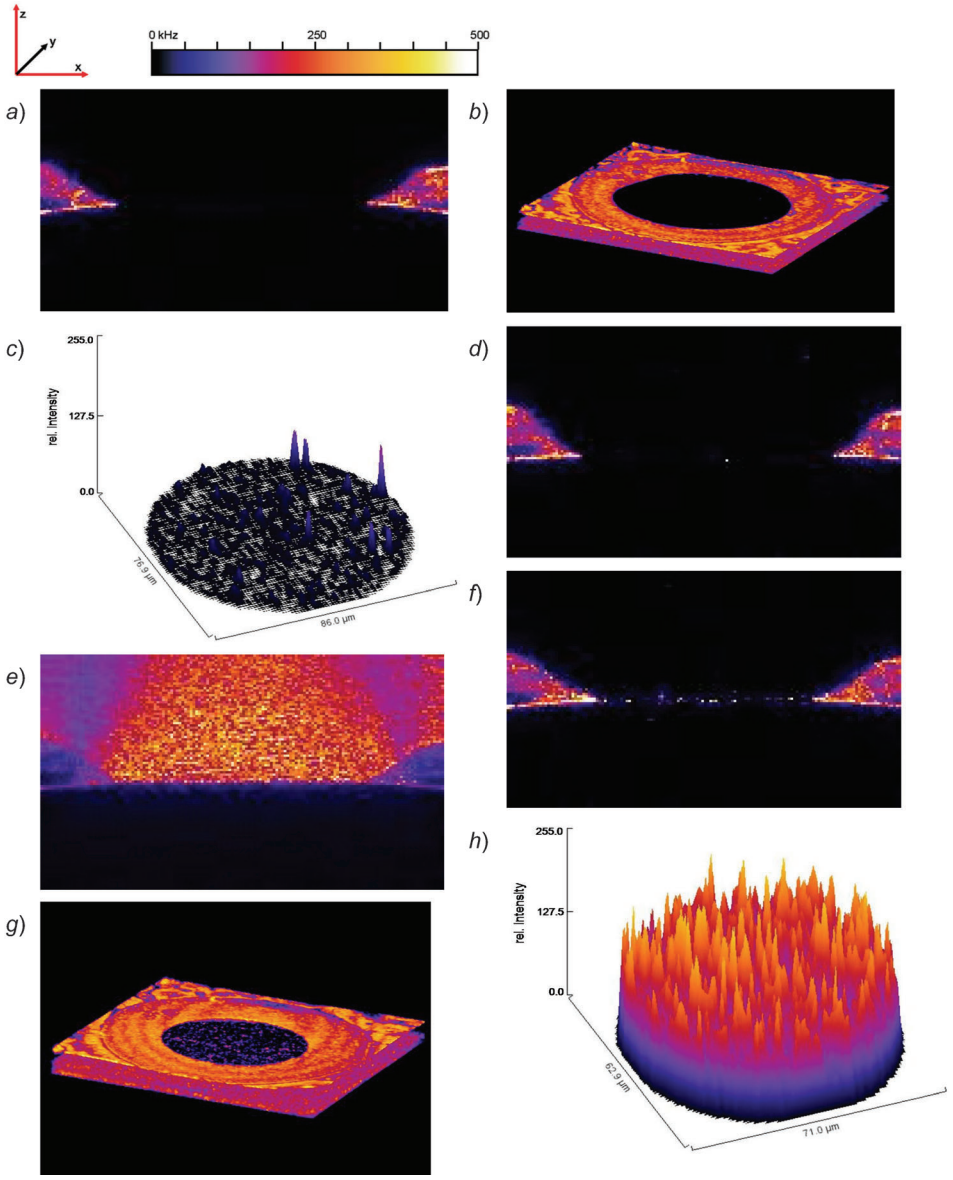


Fig. 9. *Chronological protocol of duplex formation of the oligonucleotide EW3 with the complementary, Cy3-labelled oligomer EW5 at an artificial lipid bilayer–aq. buffer boundary, followed by a perfusion. a*) *z*-Scan of an empty bilayer. *b*) Tilted 3D view on an empty bilayer. *c*) Pixel-resolved 3D scan on an empty bilayer. *d*) *z*-Scan after addition of the oligomer **EW3** (6 µl, 500 nM) to the *cis* compartment and 60 min of incubation. *e*) *z*-Scan after addition of the Cy5-labelled oligomer (6 µl, 50 nM) to the *cis* compartment and 60 min of incubation. *f*) *z*-Scan after perfusion of the *cis* compartment (30 s, 1.1 ml/min). *g*) Tilted 3D view on the bilayer as in (*f*). *h*) Pixel-resolved 3D scan of the bilayer as in (*f*).

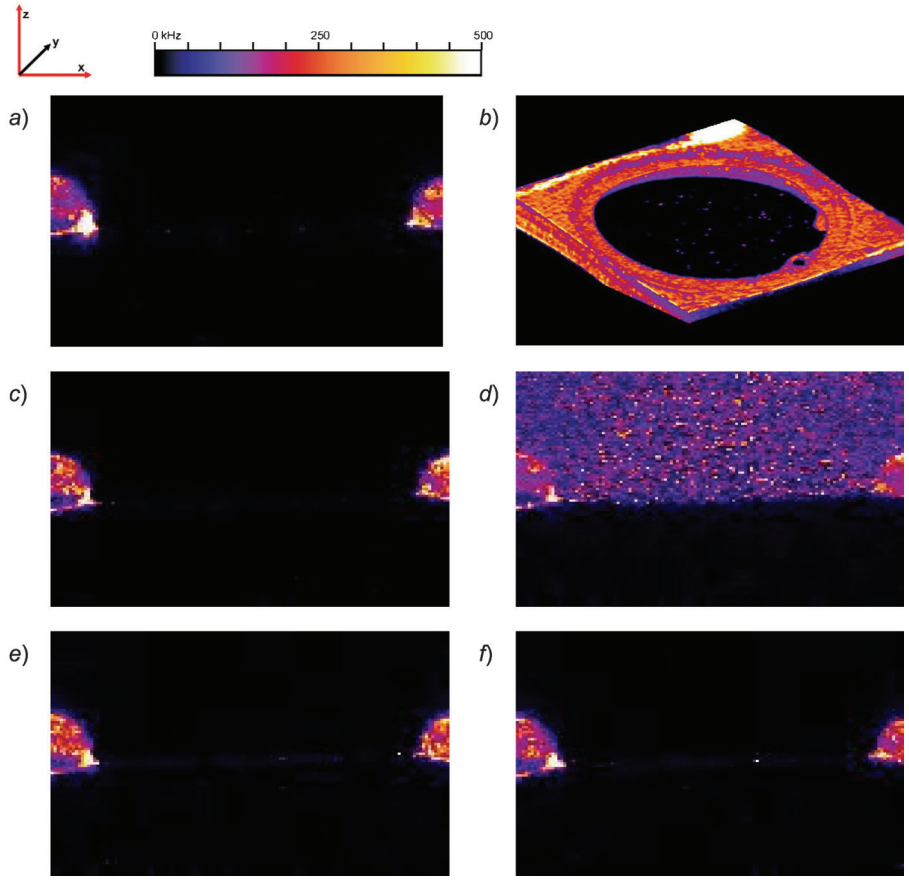


Fig. 10. *Chronological control experiment with the non-complementary oligonucleotides* **EW4** *and* **EW5**. *a*) *z*-Scan of an empty bilayer. *b*) Tilted 3D view on an empty bilayer. *c*) *z*-Scan after addition of the oligomer **EW4** (6 µl, 500 nM) to the *cis* compartment, and 50 min of incubation and perfusion (30 s, 1.1 ml/min). *d*) *z*-Scan after addition of the Cy5-labelled oligonucleotide (6 µl, 50 nM) to the *cis* compartment and 40 min of incubation. *e*) *z*-Scan after perfusion of the *cis* compartment (30 s, 1.1 ml/min). *f*) *z*-Scan after further incubation for 20 min and two perfusions of the *cis* compartment (60 s, 1.1 ml/min, each).

The bilayer formation was monitored *via* capacitance measurements. When a stable bilayer was established (< 50 pF), the corresponding oligonucleotide soln. was injected into the *cis* compartment of the '*Bilayer Slide*'. During the incubation time of 25 min, the bilayer integrity was monitored by the '*Ionovation Explorer*' through continuous capacitance measurements.

A confocal laser scanning microscope (*Insight Cell 3D*, *Evotec Technologies GmbH*, D-Hamburg), equipped with a 635-nm emitting laser diode (*LDH-P-635*, *PicoQuant GmbH*, D-Berlin), a $40\times H_2O$-immersion objective (*UApo 340*, $40\times$; NA (numeric aperture) 1.15, *Olympus*, Tokyo, Japan), and an *Avalanche* photodiode detector (*SPCM-AQR-13-FC*, *Perkin-Elmer Optoelectronics*, Fremont, CA, USA), was used for the optical measurements. Fluorescence irradiation was obtained with an excitation laser power of 200 ± 5 µW. 2D- and 3D scans were performed by scanning the confocal laser spot in *x,y* direction with a rotating beam scanner and movement of the objective in *Z* direction. The movement in

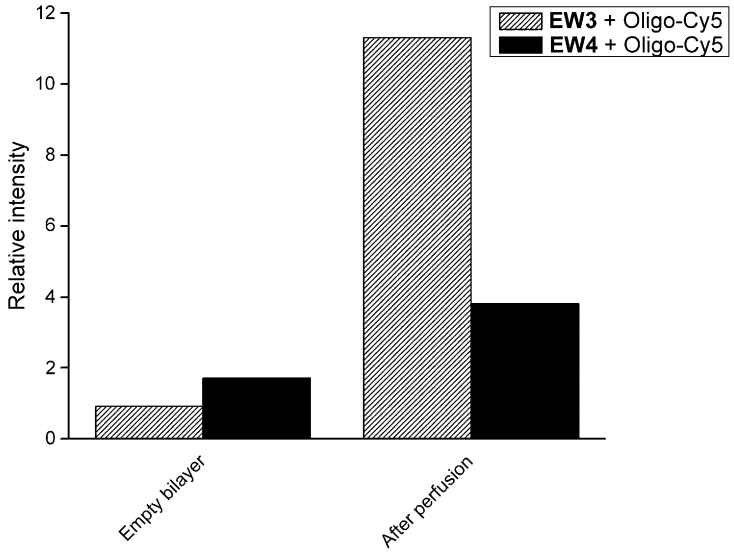
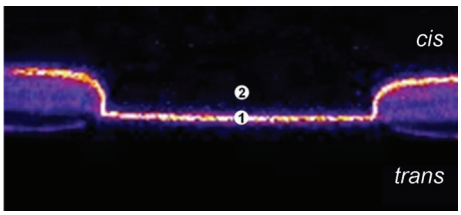


Fig. 11. *Relative bilayer brightness*

Table 3. *Diffusion times* (τ_D [ms]) *of* **21**·**23** *without and in the Presence of a Lipid Bilayer*. Location: 1, bilayer; 2, solution in close proximity to the bilayer.



	Position	τ_D [ms]
23	Diffusion (in solution without bilayer)	0.24±0.1
21·**23**		0.12±0.1
21·**23**	1	26.6±2.0
21·**23**	2	2.39±0.3

all directions was piezo-controlled which allows a nm-precise positioning. For the 2D pictures (*z*-scans; *Figs. 7*, *9*, and *10*, resp.), the confocal plane was moved in 100-nm steps.

From the fluorescence signals of single molecules which pass the laser spot, the diffusion constants can be calculated by means of fluorescence correlation analysis. The diffusion times of the fluorescent oligonucleotides within and in the proximity of the bilayer were measured at overall five different positions above, below, and within the layer (*Fig. 6,a*). At each point five 30-s measurements were conducted. In summary, each measurement protocol consisted of: *i*) a reference scan of the stable (empty) bilayer; *ii*) addition of the sample with 30-min of incubation, followed by a scan series; *iii*) additional scan series, each after a 1st and 2nd perfusion (60 s each).

Subsequently, the cyanine-5-labelled oligonucleotide **23** (50 nM, 6 µl) was injected into the *cis* compartment of the '*Bilayer Slides*' containing either membrane-bound **21** or membrane-bound **22**. After

an equilibration time of 60 min, the *cis* channel was perfused repeatedly for 30 s (1.1 ml/min), and the bilayers were inspected by confocal fluorescence microscopy.

RP-18 HPLC. RP-18 HPLC was carried out with 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 655 A*), a controller (*Model L-5000*), and an integrator (*Model D-2000*). Solvent: MeCN/0.1M $Et_3NH^+Ac^-O$ (35 : 65 (*v*/*v*), pH 7.0).

*1,9-Dihydro-9-[(6a*R*,8*R*,9a*S*)-tetrahydro-2,2,4,4-tetra(propan-2-yl)-6*H*-furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]-6*H*-purin-6-one* (**2**). Anh. *2′-deoxyinosine* (**1**; 1.01 g, 4 mmol) was suspended in dry pyridine (40 ml), and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.39 g; 4.4 mmol) was added under moisture exclusion. After stirring for 24 h at r.t., the solvent was evaporated, and the residue was partitioned between AcOEt and H_2O (80 ml, 1 : 1 (*v*/*v*)). The org. layer was washed twice with 1M HCl (80 ml), followed by H_2O, conc. aq. $HaHCO_3$, and brine (80 ml, each). After drying (Na_2SO_4, 1 h) the solvent was evaporated, and the residue was chromatographed (column: 6 × 10 cm, $CHCl_3$/MeOH 9 : 1 (*v*/*v*)). From the main zone, **2** (1.96 g, 99%) was isolated. Colorless amorphous material. M.p. 210°. R_f ($CHCl_3$/MeOH 9 : 1 (*v*/*v*)) 0.56. UV (MeOH): 244 (12,250); ε_{260}, 7,500. ^{1}H-NMR ((D$_6$)DMSO): 12.33 (*s*, NH); 8.18 (*s*, H–C(2)); 7.96 (*s*, H–C(8)); 6.27 (*dd*, $J(1',2'_\alpha)$ = 8.0, $J(1',2'_\beta)$ = 3.0, H–C(1′)); 4.94 (*ddd*, $J(3',2'_\alpha) = J(3',2'_\alpha) = J(3',4')$ = 7.5, H–C(3′)); 3.92–3.91 (*m*, CH_2(5′)); 3.93–3.80 (*m*, H–C(4′)); 2.80 (*ABdd*, J_{AB} = – 13.0, $J(2'_\beta,1')$ = 7.5, $J(2'_\beta,3')$ = 3.0, H_β–C(2′)); 2.60–2.53 (*m*, H_α–C(2′)); 1.08–1.01 (*m*, 4 *Me*$_2$*CH*). ^{13}C-NMR ((D$_6$)DMSO): 156.5 (C(6)); 147.4 (C(4)); 145.5 (C(2)); 138.7 (C(8)); 124.7 (C(5)); 84.5 (C(1′)); 82.1 (C(4′)); 71.2 (C(3′)); 62.4 (C(5′)); 38.7 (C(2′)); 17.1 (8 Me); 12.33 (4 CH). Anal. calc. for $C_{22}H_{38}N_4O_5Si_2$ (494.732): C 53.41, H 7.74, N 11.32; found: C 53.21, H 7.78, N 11.23.

*2′-Deoxy-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**3b**). Anh. **1** (1.01 g, 4 mmol) was suspended in anh., amine-free DMF and heated on a H_2O bath (55°). Then, anh. K_2CO_3 (1.44 g, 10.4 mmol) was added, and the mixture was stirred for 10 min. After cooling to r.t., farnesyl bromide (1.32 g, 4.4 mmol) was added dropwise under N_2. After stirring for 24 h at r.t., the solvent was evaporated, and the residue was dried in high vacuum. Chromatography (column: 6 × 14 cm; $CHCl_3$/MeOH 9 : 1 (*v*/*v*)) gave one main zone from which after evaporation of the solvent **3b** (1.28 g, 74%) was isolated. Amorphous solid. R_f ($CHCl_3$/MeOH 95 : 5, (*v*/*v*)) 0.42. log *P:* 3.40 ± 0.94. UV (MeOH): 250 (10,150); ε_{260} 7,000. ^{1}H-NMR ((D$_6$)DMSO): 8.34 (*s*, H–C(2)); 8.30 (*s*, H–C(8)); 6.28 (*dd*, $J(1',2'_\alpha) = J(1',2'_\beta)$ = 7.0, H–C(1′)); 5.28 (*t*, $J(2'',1'')$ = 7.5, H–C(2″)); 5.35 (*d*, $J(OH_{3'},3')$ = 7.5, HO–C(3′)); 5.03–4.98 (*m*, H–C(6″), H–C(10″)); 4.91 (*t*, $J(OH_{5'},5')$ = 6.5, HO–C(5′)); 4.60 (*d*, $J(1'',2'')$ = 7.5, CH_2(1″)); 4.38 (*ddd*, $J(3',2_\beta)$ = 9.0, $J(3',2_\alpha) = J(3',4')$ = 3.5, H–C(3′)); 3.87–3.85 (*m*, H–C(4′)); 3.60–3.49 (*m*, H–C(5′)); 2.63–2.58 (*m*, H_β–C(2′)); 2.28 (*ABdd*, J_{AB} = – 13.5, $J(2'_\alpha,1')$ = 6.5, $J(2'_\alpha,3')$ = 3.5, H_α–C(2′)); 2.05–2.02 (*m*, CH_2(8″)); 2.00–1.97 (*m*, CH_2(9″)); 1.94–1.92 (*m*, CH_2(5″)); 1.87–1.84 (*m*, CH_2(4″)); 1.77 (*s*, Me(13″)); 1.60 (*s*, Me(12″)); 1.512, 1.507 (2*s*, Me(14″), Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 155.7 (C(6)); 148.0 (C(4)); 147.0 (C(2)); 140.1 (C(3″)); 139.0 (C(8)); 134.7 (C(7″)); 130.6 (C(11″)); 124.0 (C(6″)); 123.8 (C(5)); 123.4 (C(10″)); 119.4 (C2″)); 88.0 (C(1′)); 83.6 (C(4′)); 70.7 (C(3′)); 61.6 (C(5′)); 43.2 (C(1″)); 39.5 (C(2′)); 39.1 (C(8″)); 38.8 (C(4″); 26.1 (C(5″)); 25.6 (C(12″)); 25.4 (C(9″)); 17.5 (C(15″)); 16.2 (C(14″)); 15.8 (C(13″)). Anal. calc. for $C_{25}H_{36}N_4O_4$ (456.58): C 65.76, H 7.95, N 1227; found: C 65.42, H 8.06, N 12.04.

*2′-Deoxy-1-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]inosine* (**3a**). Compound **3a** was prepared and worked up from **1** (1.01 g, 4 mmol) and geranyl bromide (0.96 g, 4.4 mmol) as described for **3b**. Yield: 0.80 g (51%). R_f ($CHCl_3$/MeOH 95 : 5 (*v*/*v*)) 0.41. UV (MeOH): 250 (10,450); ε_{260} 7,800. log *P:* 1.37 ± 0.93. ^{1}H-NMR ($CDCl_3$): 8.15 (*s*, H–C(2)); 8.01 (*s*, H–C(8)); 6.39 (*dd*, $J(1',2'_\beta)$ = 7.5, $J(1',2'_\alpha)$ = 6.5, H–C(1′)); 5.37–5.32 (*m*, H–C(2″)); 5.08 (*t*, $J(OH_{3'},3')$, HO–C(3′)); 4.80 (*t*, $J(6'',5'')$ = 6.5, H–C(6″)); 4.70 (*d*, $J(1'',2'')$ = 6.5, CH_2(1″)); 4.20–4.19 (*m*, H–C(3′)); 4.01–3.89 (*m*, H–C(4′), CH_2(5′)); 2.86–2.80 (*m*, H_β–C(2′)); 2.46 (*ABdd*, J_{AB} = – 13.5, $J(2'_\alpha,1')$ = 5.5, $J(2'_\alpha,3')$ = 3.0, H_α–C(2′)); 2.14–2.08 (*m*, CH_2(5″), CH_2(4″)); 1.83 (*s*, Me(9″)); 1.70 (*s*, Me(8″)); 1.62 (Me(10″)). ^{13}C-NMR ($CDCl_3$): 156.2 (C(6)); 148.5 (C(4)); 147.5 (C(2)); 140.6 (C(3″)); 139.5 (C(8)); 131.5 (C(7″)); 124.3 (C(6″)); 124.2 (C(5)); 119.9 (C2″)); 88.4 (C(1′)); 84.1 (C(4′)); 71.1 (C(3′)); 62.1 (C(5′)); 43.7 (C(1″)); 39.7 (C(2′)); 39.3 (C(8″)); 39.0 (C(4″); 26.2 (C(5″)); 25.8 (C(8″)); 18.0 (C(14″)); 16.7 (C(13″)). Anal. calc. for $C_{20}H_{28}N_4O_4$ (388.46): C 61.84, H 7.27, N 14.42; found: C 61.72, H 7.31, N 14.31.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-2′-deoxy-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**4b**). Compound **3b** (0.46 g, 1.0 mmol) was co-evaporated twice from dry pyridine (1 ml, each) and then dissolved in anh. pyridine (5 ml). After addition of 1,1′-[chloro(phenyl)methanediyl]-bis(4-methoxybenzene) (0.40 g, 1.15 mmol), the mixture was stirred for 24 h at r.t. under N_2. Then, the reaction was quenched by addition of MeOH (3 ml). After addition of aq. 5% $NaHCO_3$ (30 ml), the aq. phase was extracted three times with CH_2Cl_2 (30 ml each), and the combined org. layers were dried (Na_2SO_4, 1 h) and filtered. Chromatography (column: 6 × 10 cm; $CHCl_3$/MeOH 96 : 4 (*v*/*v*)) gave one main zone from which compound **4b** (0.46 g, 61%) was isolated. Slightly yellowish glass. M.p. 68°. R_f ($CHCl_3$/MeOH 96 : 4 (*v*/*v*)) 0.13. UV (MeOH): 235 (38,200); ε_{260} 14,150. log *P:* 9.81 ± 0.96. ^{1}H-NMR ((D$_6$)DMSO): 8.23 (*s*, H–C(2)); 8.16 (*s*, H–C(8)); 7.32–7.31 (*m*, H–C(4) and H–C(2,6) of Ph); 7.23–7.17 (*m*, 2 H–C(2,6) of 4-MeO–C$_6$H$_4$, H–C(3,5) of Ph); 6.82–6.78 (*m*, 2 H–C(3,5) of 4-MeO–C$_6$H$_4$); 6.31 (*dd*, *J*(1′,2′$_\alpha$) = *J*(1′,2′$_\beta$) = 6.5, H–C(1′)); 5.33 (br. *s*, HO–C(3′)); 5.24 (*t*, *J*(2″,1″) = 7.0, H–C(2″)); 5.02 (*t*, *J*(10″,9″) = 6.0, H–C(10″)); 4.98 (*t*, *J*(6″,5″) = 6.5, H–C(6″)); 4.63–4.55 (*m*, H–C(1″)); 4.40 (br. *s*, H–C(3′)); 4.00–3.97 (*m*, H–C(4′)); 3.71 (*s*, 2 MeO); 3.20–3.12 (*m*, CH_2(5′)); 2.74 (*ABdd*, J_{AB} = – 13.5, *J*(2′$_\beta$,1′) = *J*(2′$_\beta$,3′) = 6.0, H$_\beta$–C(2′)); 2.32 (*ABdd*, J_{AB} = – 13.5, *J*(2′$_\alpha$,1′) = 5.5, *J*(2′$_\alpha$,3′) = 6.5, H$_\alpha$–C(2′)); 2.05 (*t*, *J*(8″,9″) = 6.5, CH_2(8″)); 2.00 (*t*, *J*(9″,8″) = 6.5, CH_2(9″)); 1.93 (*t*, *J*(5″,4″) = 7.5, CH_2(5″)); 1.85 (*t*, *J*(4″,5″) = 7.5, CH_2(4″)); 1.77 (*s*, Me(13″)); 1.59 (*s*, Me(12″)); 1.50 (*s*, Me(14″), Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 157.9 (2 MeO–C); 155.7 (C(6)); 147.7 (C(2)); 146.9 (C(4)); 144.7 (C(1) of Ph); 140.0 (C(3″)); 139.1 (C(8)); 135.5 (2 C(1) of 4-MeO–C$_6$H$_4$); 134.6 (C(7″)); 130.5 (C(11″)); 129.5 (2 C(2,6) of 4-MeO–C$_6$H$_4$); 129.5 (C(3,5) of Ph); 127.6 (C(2,6) of Ph); 126.5 (C(4) of Ph); 123.9 (C(6″)); 123.9 (C(10″)); 123.4 (C(5)); 119.3 (C(2″)); 113.0 (2 C(3,5) of 4-MeO–C$_6$H$_4$); 85.9 (*C*Ar$_3$); 85.4 (C(1′)); 83.4 (C(4′)); 70.5 (C(3′)); 64.0 (C(5′)); 54.9 (2 MeO); 43.1 (C(2′)); 39.0 (C(8″)); 38.9 (C(4″)); 38.8 (C(1″)); 26.0 (C(9″)); 25.6 (C(5″)); 25.3 (C(12″)); 17.4 (C(15″)); 16.1 (C(14″)); 15.7 (C(13″)). Anal. calc. for $C_{46}H_{54}N_4O_6$ (758.94): C 72.80, H 7.17, N 7.38; found: C 72.53, H 7.14, N 7.27.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-2′-deoxy-1-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]inosine* (**4a**). Compound **4a** was prepared from **3a** (0.39 g, 1.0 mmol) and worked up as described for **4b**. Yield: 0.48 g (69%). Colorless glass. M.p.: 74°. R_f ($CHCl_3$/MeOH 96 : 4, *v*/*v*) 0.19. UV (MeOH): 235 (32,820); ε_{260} 12,791. log *P:* 7.77 ± 0.94. ^{1}H-NMR ((D$_6$)DMSO): 8.25 (*s*, H–C(2)); 8.18 (*s*, H–C(8)); 7.28–2.22 (*m*, H–C(4) and H–C(2,6) of Ph); 2.28–7.20 (*m*, 2 H–C(2,6) of 4-MeO–C$_6$H$_4$, H–C(3,5) of Ph); 6.82–6.79 (2 H–C(3,5) of 4-MeO–C$_6$H$_4$); 6.32 (*dd*, *J*(1′,2′$_\alpha$) = *J*(1′,2′$_\beta$) = 6.5, H–C(1′)); 5.46–5.34 (*m*, HO–C(3′)); 5.24 (*t*, *J*(2″,1″) = 6.5, H–C(2″)); 5.01 (*t*, *J*(6″,5″) = 6.0, H–C(6″)); 4.61–4.59 (*m*, H–C(1″)); 4.41–4.40 (*m*, H–C(3′)); 3.99–3.98 (*m*, H–C(4′)); 3.72–3.71 (*m*, 2 MeO); 3.21–3.12 (*m*, H–C(5′)); 2.75 (*ABdd*, J_{AB} = – 13.0, *J*(2′$_\beta$,1′) = *J*(2′$_\beta$,3′) = 6.5, H$_\beta$–C(2′)); 2.34 (*ABdd*, J_{AB} = – 13.0, *J*(2′$_\alpha$,1′) = 6.0, *J*(2′$_\alpha$,3′) = 6.5, H$_\alpha$–C(2′)); 2.04–1.99 (*m*, CH_2(5″), CH_2(4″)); 1.77 (*s*, Me(9″)); 1.57 (*s*, Me(8″)); 1.51 (*s*, Me(10″)). ^{13}C-NMR ((D$_6$)DMSO): 158.0 (2 MeO–C); 155.7 (C(6)); 147.7 (C(2)); 147.0 (C(4)); 144.7 (C(1) of Ph); 140.1 (C(3″)); 139.1 (C(8)); 135.5 (2 C(1) of 4-MeO–C$_6$H$_4$); 130.9 (C(11″)); 129.56 (2 C(2,6) of 4-MeO–C$_6$H$_4$); 129.55 (C(3,5) of Ph); 127.7 (C(2,6) of Ph); 126.5 (C(4) of Ph); 124.0 (C(6″)); 123.6 (C(5)); 119.3 (C(2″)); 113.0 (2 C(3,5) of 4-MeO–C$_6$H$_4$); 85.9 (*C*Ar$_3$); 85.4 (C(1′)); 83.4 (C(4′)); 70.5 (C(3′)); 64.0 (C(5′)); 54.9 (2 MeO); 43.1 (C(2′)); 38.7 (C(4″)); 38.7 (C(1″)); 25.7 (C(5″)); 25.3 (C(8″)); 17.4 (C(14″)); 16.1 (C(13″)). Anal. calc. for $C_{41}H_{46}N_4O_6$ (690.827): C 71.28, H 6.71, N 8.11; found: C 70.94, H 6.67, N 7.93.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-3′-O-{(2-cyanoethoxy)[di(propan-2-yl)amino]phosphanyl}-2′-deoxy-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**5b**). Compound **4b** (100 mg, 0.13 mmol) was co-evaporated twice with CH_2Cl_2 and then dissolved in CH_2Cl_2 (5 ml). After addition of EtN^iPr_2 (42 µl, 0.24 mmol) and (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine (= 2-cyanoethyl dipropan-2-ylphosphoramidochloridoite; 52 µl, 0.24 mmol), the mixture was stirred for 15 min (!) at r.t. under N_2. Then, ice-cold 5% aq. $NaHCO_3$ was added (4 ml), and the mixture was extracted three times with CH_2Cl_2 (8 ml, each). The combined org. layers were dried (Na_2SO_4, 10 min), filtered, and the solvent was evaporated (< 25°). FC (column: 2 × 8 cm; CH_2Cl_2/acetone 8 : 2 (*v*/*v*)) gave **5b** (98 mg, 78%). Amorphous material. R_f (diastereoisomers; CH_2Cl_2/acetone 8 : 2 (*v*/*v*)) 0.64, 0.76. log *P:* 12.86 ± 1.11. ^{31}P-NMR ($CDCl_3$): 148.9; 149.0.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-3′-O-{(2-cyanoethoxy)[di(propan-2-yl)amino]phosphanyl}-2′-deoxy-1-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]inosine* (**5a**). Compound **5a** was prepared and worked up from **4a** (100 mg, 0.14 mmol) as described for **5b**. Yield: 79 mg (61%). Amorphous material

(diastereoisomers; CH_2Cl_2/acetone 8:2 (*v*/*v*)) R_f 0.64, 0.76. log *P:* 10.82 ± 1.10. ^{31}P-NMR ($CDCl_3$):148.9; 149.0.

*5-Methyl-1-[(6a*R,*8*R,*9a*S*)-tetrahydro-2,2,4,4-tetra(propan-2-yl)-6*H-*furo[3,2-*f*][1,3,5,2,4]trioxadisilocin-8-yl]pyrimidine-2,4(1*H,*3*H*)-dione* (**8**). Anh. *thymidine* (**7**; 0.242 g, 1 mmol) was dissolved in dry pyridine (10 ml), and 1,3-dichloro-1,1,3,3-tetra(propan-2-yl)disiloxane (0.347 g, 1.1 mmol) was added. The mixture was stirred for 24 h at r.t. After evaporation of the solvent, the residue was partitioned between AcOEt and H_2O (80 ml; 1:1 (*v*/*v*)). The org. layer was washed twice with cold 1M aq. HCl and H_2O (20 ml, each), followed by sat. aq. $NaHCO_3$ and brine. After drying (anh. Na_2SO_4) and filtration, the soln. was evaporated to dryness. Chromatography (column: 2 × 10 cm, $CHCl_3$/MeOH 9:1 (*v*/*v*)) gave, after evaporation of the main zone, **8** (0.476 g, 98%). Colorless solid. M.p. 174°. R_f (Si_2O; $CHCl_3$/MeOH 9:1 (*v*/*v*)) 0.9. UV(MeOH): 265 (12.040). ^{1}H-NMR ((D_6)DMSO): 11.33 (*s*, NH); 7.40 (*d*, *J*(Me,6) = 1.0, H–C(6)); 6.00 (*dd*, *J*(1′,2′$_\alpha$) = *J*(1′,2′$_\beta$) = 5.0, H–C(1′)); 4.56 (*ddd*, *J*(3′,2′$_\alpha$) = *J*(3′,2′$_\beta$) = *J*(3′,4′) = 7.5, H–C(3′)); 3.99 (*ABd*, J_{AB} = − 12.2, *J*(5′$_b$,4′) = 5.5, H_b–C(5′)); 3.93 (*ABd*, J_{AB} = − 12.0, *J*(5′$_a$,4′) = 3.25, H_a–C(5′)); 3.70 (*ddd*, *J*(4′,3′) = 7.5, *J*(4′,5′$_b$) = 5.5, *J*(4′,5′$_a$) = 3.25, H–C(4′)); 2.44–2.39 (*m*, H_β–C(2′)); 2.33–2.27 (*m*, H_α–C(2′)); 1.76 (*d*, *J*(Me,6) = 0.5, Me); 1.09–0.99 (*m*, 4 *Me*$_2$C*H*). ^{13}C-NMR: ((D_6)DMSO): 163.7 (C(4)); 150.1 (C(2)); 136.2 (C(6)); 109.3 (C(5)); 84.2 (C(1′)); 83.2 (C(4′)); 70.3 (C(3′)); 61.7 (C(5′)); 38.5 (C(2′)); 17.3, 17.2, 17.2, 17.1, 17.0, 16.9, 16.8, 17.3–16.8 (4 *Me*$_2$CH); 12.7–12.0 (4 Me$_2$C*H*); 12.1 (Me). Anal. calc. for $C_{22}H_{40}N_2O_6Si_2$ (484.73): C 54.51, H 8.32, N 5.78; found: C 54.54, H 8.21, N 5.68.

*3,4-Dihydro-2′-deoxy-3-[(2*E,*6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]thymidine* (**9b**). Anh. **7** (0.97 g, 4 mmol) was dissolved in amine-free, anh. DMF (20 ml), and dry K_2CO_3 (1.2 g, 10.4 mmol) was added. Then, (*E*,*E*)-farnesyl bromide (0.87 ml, 4.4 mmol) was added dropwise within 10 min under N_2, and the mixture was stirred for 48 h at 40°. After filtration, the mixture was then partitioned between CH_2Cl_2 and H_2O (100 ml; 1:1 (*v*/*v*)), the org. layer was separated and dried (anh. Na_2SO_4). After filtration and evaporation of the solvent, the residue was dried in high vacuum. Subsequent gradient chromatography (column: 6 × 10 cm; 1. CH_2Cl_2/MeOH 95:5 (*v*/*v*); 2. CH_2Cl_2/MeOH 9:1 (*v*/*v*)) gave, after evaporation of the main zone, **9b** (0.76 g, 44%). R_f (CH_2Cl_2/MeOH 9:1 (*v*/*v*)) 0.5; R_f (Si_2O; CH_2Cl_2/MeOH 95:5 (*v*/*v*)) 0.3. UV (MeOH): 266 (9,660). log *P* = 6.60 ± 0.64. ^{1}H-NMR ((D_6)DMSO): 7.75 (*d*, *J*(Me,6) = 1.26, H–C(6)); 6.20 (*dd*, *J*(1′,2′$_\alpha$) = *J*(1′,2′$_\beta$) = 7.0, H–C(1′)); 5.20 (*d*, *J*(OH$_{3'}$,3′) = 4.5, HO–C(3′)); 5.10 (*t*, *J*(2″,1″) = 6.5, H–C(2″)); 5.05–5.01 (*m*, (H–C(6″), H–C(10″)); 5.00 (*dd*, *J*(OH$_{5'}$,5′$_b$) = *J*(OH$_{5'}$,5′$_a$) = 5.2, HO–C(5′)); 4.39 (*d*, *J*(1″,2″) = 7.0, CH_2(1″)); 4.24 (*ddd*, *J*(4′,3′) = 3.8, *J*(4′,5′$_b$) = 4.0, *J*(4′,5′$_a$) = 4.0, H–C(4′)); 3.78 (*ddd*, *J*(3′,2′$_\alpha$) = *J*(3′,2′$_\beta$) = *J*(3′,4′) = 3.8, H–C(3′)); 3.60 (*ABdd*, J_{AB} = − 12.0, *J*(5′$_b$,4′) = 4.0, *J*(5′$_b$,OH$_{5'}$) = 5.2, H_b–C(5′)); 3.57–3.53 (*m*, H_a–C(5′)); 2.04–1.86 (*m*, (CH_2(4″), CH_2(5″), CH_2(8″), CH_2(9″)); 1.81 (*d*, *J*(Me,6 = 1.0, CH_3)); 1.74 (*s*, Me(13″)); 1.63 (*s*, Me(12″)); 1.55 (*s*, Me(14″)); 1.52 (*s*, Me(15″)). ^{13}C-NMR ((D_6) DMSO): 162.4 (C(4)); 150.2 (C(2)); 138.7 (C(3″); 134.7 (C(6)); 134.5 (C(7″)); 130.6 (C(11″)); 124.1 (C(6″)); 123.6 (C(10″)); 119.0 (C(2″)); 108.5 (C(5)); 87.4 (C(1′)); 84.8 (C(4′)); 70.3 (C(3′)); 61.2 (C(5′)); 40.1 (C(2′)); 39.2 (C(1″)); 38.9 (C(8″)); 38.6 (C(4″)); 26.2 (C(5″)); 25.7 (C(9″)); 25.4 (C(12″)); 17.5 (C(15″)); 16.1 (C(14″)); 15.8 (C(13″)); 12.8 (Me). Anal. calc. for $C_{25}H_{38}N_2O_5$ (446.58): C 67.24, H 8.58, N 6.27; found: C 67.39, H 8.56, N 5.90.

*2′-Deoxy-3-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]-3,4-dihydrothymidine* (**9a**). Anh. **7** (0.97 g, 4 mmol) was reacted and worked up with geranyl bromide (0.87 ml, 4.4 mmol) as described for **9b**. Chromatography (Si_2O, column: 6 × 10 cm; CH_2Cl_2/MeOH 9:1 (*v*/*v*)) gave, after evaporation of the main zone, **9a** (0.86 g, 2.27 mmol). Yellowish amorphous solid. R_f (CH_2Cl_2/MeOH 9:1, *v*/*v*) 0.4. UV (MeOH): 266 (7,579). log *P:* 4.57 ± 0.61. ^{1}H-NMR ((D_6)DMSO): 7.75 (*d*, *J*(Me,6) = 1.3, H–C(6)); 6.21 (*dd*, *J*(1′,2′$_\alpha$) = *J*(1′,2′$_\beta$) = 7.0, H–C(1′)); 5.20 (*d*, *J*(OH$_{3'}$,3′) = 4.5, HO–C(3′)); 5.11 (*t*, *J*(2″,1″) = 6.75, H–C(2″)); 5.04–5.01 (*m*, H–C(6″)); 4.99 (*dd*, *J*(OH$_{5'}$,5′$_b$) = *J*(OH$_{5'}$,5′$_a$) = 5.2, HO–C(5′)); 4.39 (*d*, *J*(1″,2″) = 6.5, CH_2(1″)); 4.24 (*ddd*, *J*(4′,3′) = 3.5, *J*(4′,5′$_b$) = *J*(4′,5′$_a$) = 4.0, H–C(4′)); 3.78 (*ddd*, *J*(3′,2′$_\alpha$) = *J*(3′,2′$_\beta$) = *J*(3′,4′) = 3.8, H–C(3′)); 3.61 (*ABdd*, J_{AB} = − 12.0, *J*(5′$_b$,4′)) = *J*(5′$_b$,OH$_{5'}$) = 4.0, H_b–C(5′)); 3.55 (*ABdd*, J_{AB} = − 12.0, *J*(5′$_a$,4′) = 4.0, *J*(5′$_a$,OH$_{5'}$) = 5.0, H_a–C(5′)); 2.11 (*ABdd*, J_{AB} = − 12.0, *J*(2′$_\alpha$,1′) = 7.0, *J*(2′$_\alpha$,3′) = 4.8, H_α–C(2′)); 2.09 (*ABdd*, J_{AB} = − 12.0, *J*(2′$_\beta$,1′) = 7.0, *J*(2′$_\beta$,3′) = 4.8, H_β–C(2′)); 2.02–1.99 (*m*, CH_2(4″)); 1.95–1.93 (*m*, CH_2(5″)); 1.82 (*d*, *J*(Me, 6 = 1.0, Me)); 1.74 (*s*, Me(9″)); 1.61 (*s*, Me(8″)); 1.53 (*s*, Me(10″)). ^{13}C-NMR ((D_6)DMSO): 162.3 (C(4)); 150.2 (C(2)); 138.7 (C(3″)); 134.6 (C(6)); 130.8 (C(7″)); 123.7 (C(6″)); 118.8 (C(2″)); 108.4 (C(5)); 87.3 (C(1′)); 84.7 (C(4′)); 70.2 (C(3′)); 61.2 (C(5′)); 40.1 (C(2′)); 39.4

(C(4″)); 39.1 (C(1″)); 25.8 (C(5″)); 25.3 (C(8″)); 17.4 (C(10″)); 16.1 (C(9″)); 12.8 (Me). Anal. calc. for $C_{20}H_{30}N_2O_5$ (378.46): C 63.47, H 7.99, N 7.40; found: C 63.26, H 7.98, N 7.19.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-2′-deoxy-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]-3,4-dihydrothymidine* (**10b**). Compound **9b** (0.45 g, 1 mmol) was co-evaporated twice with anh. pyridine (1 ml, each) and then dissolved in anh. pyridine (5 ml). 1,1′-[Chloro(phenyl)methanediyl]bis(4-methoxybenzene) (0.39 g, 1.15 mmol) was added under N_2, and the mixture was stirred for 24 h at r.t. Then, the reaction was quenched by addition of MeOH (3 ml). After 10 min, ice-cold 5% aq. $NaHCO_3$ was added, and the soln. was extracted with CH_2Cl_2. The org. layer was dried (Na_2SO_4), filtered, and the solvent was evaporated. The residue was dried in high vacuum until a yellowish foam formed. Chromatography (column: 6×10 cm; CH_2Cl_2/MeOH 99:1 (*v*/*v*)) gave, after evaporation of the main zone, **10b** (0. 48 g, 65%). Yellowish glass. R_f (CH_2Cl_2/MeOH 99:1 (*v*/*v*)) 0.4. UV (MeOH): 232 (26,900), 268 (13,400). log *P:* 12.17 ± 0.64. ^{1}H-NMR ((D$_6$)DMSO): 7.54 (*d*, *J*(Me,6) = 0.9, H–C(6)); 7.38 (*d*, $J(2_{Ph}/6_{Ph},3_{Ph}/5_{Ph})$ = 7.25, H–C(2,6) of Ph); 7.30 (*t*, $J(4_{Ph},3_{Ph}/5_{Ph})$ = 7.4, H–C(4) of Ph); 7.26–7.24 (*m*, H–C(2,6) of 2 4-MeO–C_6H_4, H–C(3,5) of Ph); 6.88 (*d*, $J(3_{4MeOPh}/5_{4MeOPh},2_{4MeOPh}/6_{4MeOPh})$ = 8.2, H–C(3,5) of 2 4-MeO–C_6H_4); 6.24 (*dd*, $J(1',2'_\alpha) = J(1',2'_\beta)$ = 6.62, H–C(1′)); 5.30 (*d*, $J(OH_{3'},3')$ = 4.4, HO–C(3′)); 5.12–5.10 (*m*, (H–C(2″)); 5.04–5.02 (*m*, H–C(6″), H–C(4) of Ph); 4.40 (*d*, *J*(1″,2″) = 5.0, CH_2(1″)); 4.33–4.31 (*m*, H–C(4′)); 3.92–3.88 (*m*, H–C(3′)); 3.73 (*s*, 2 MeO); 3.25–3.17 (*m*, CH_2(5′)); 2.26–2.17 (*m*, CH_2(2′)); 2.03–2.00 (*m*, CH_2(5″)); 1.99–1.93 (*m*, CH_2(8″), CH_2(9″)); 1.91–2.88 (*m*, CH_2(4″)); 1.74 (*s*, Me(5)); 1.61 (*s*, Me(13″)); 1.53 (*s*, Me(12″)); 1.52 (*s*, Me(14″)); 1.49 (*s*, Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 161.2 (C(4)); 157.1 (2 MeO–C); 149.1 (C(2)); 143.6 (C(1) of Ph); 137.7 (C(3″)); 134.4 (2 C(1) of 4-MeO–C_6H_4); 133.4 (C(6)); 129.5 (C(7″)); 128.6 (C(2,6) of 4-MeO–C_6H_4); 126.8 (C(3,5) of Ph); 126.6 (C(2,6) of Ph); 125.7 (C(4) of Ph); 123.0 (C(6″)); 122.5 (C(10″)); 117.8 (C(2″)); 112.2 (2 C(3,5) of 4-MeO–C_6H_4); 107.7 (C(5)); 84.8 (Ar_3C); 84.5 (C(1′)); 83.7 (C(4′)); 69.4 (C(3′)); 62.6 (C(5′)); 54.0 (2 MeO); 39.1 (C(2′)); 37.8 (C(4″)); 37.6 (C(1″)); 25.1 (C(5″)); 24.6 (C(9″)); 25.3 (C(8″)); 16.4 (C(15″)); 15.1 (C(14″)); 14.7 (C(13″)); 11.3 (Me). Anal. calc. for $C_{46}H_{56}N_2O_7$ (748.95): C 73.77, H 7.54, N 3.74; found: C 73.39, H 7.38, N 3.74.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-2′-deoxy-3-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]-3,4-dihydrothymidine* (**10a**). Compound **9a** (0.38 g, 1 mmol) was dried with anh. pyridine, reacted with 1,1′-[Chloro(phenyl)methanediyl]bis(4-methoxybenzene) (0.39 g, 1.15 mmol), and worked up as described for **10b**. Yield: 0.39 g (57%) of **10a**. Yellowish foam. R_f (CH_2Cl_2/MeOH 99:1 (*v*/*v*)) 0.57. UV (MeOH): 231 (24,770), 268 (12,200). log *P:* 10.14 ± 0.61. ^{1}H-NMR ((D$_6$)DMSO): 7.55 (*d*, *J*(Me,6) = 0.95, H–C(6)); 7.38 (*d*, $J(2_{Ph}/6_{Ph},3_{Ph}/5_{Ph})$ = 7.25, H–C(2,6) of Ph); 7.31 (*t*, $J(4_{Ph},3_{Ph}/5_{Ph})$ = 7.4, H–C(4) of Ph); 7.26–7.24 (*m*, H–C(2,6) of 2 4-MeO–C_6H_4, H–C(3,5) of Ph); 6.89 (*d*, $J(3_{4MeOPh}/5_{4MeOPh},2_{4MeOPh}/6_{4MeOPh})$ = 8.9, H–C(3,5) of 2 4-MeO–C_6H_4); 6.25 (*dd*, $J(1',2'_\alpha) = J(1',2'_\beta)$ = 6.8, H–C(1′)); 5.30 (*d*, $J(OH_{3'},3')$ = 4.4, HO–C(3′)); 5.13–5.10 (*m*, H–C(2″)); 5.04–5.01 (*m*, H–C(6″)); 4.40 (*d*, *J*(1″,2″) = 5.0, CH_2(1″)); 4.34–4.31 (*m*, H–C(4′)); 3.90 (*ddd*, $J(3',2'_\alpha) = J(3',2'_\beta) = J(3',4')$ = 3.9, H–C(3′)); 3.74 (*s*, 2 MeO); 3.24–3.18 (*m*, CH_2(5′)); 2.28–2.16 (*m*, CH_2(2′)); 2.03–1.99 (*m*, CH_2(5″)); 1.95–1.91 (*m*, CH_2(4″)); 1.74 (*s*, Me(5)); 1.60 (*s*, Me(9″)); 1.53 (*s*, Me(8″)); 1.50 (*s*, Me(10″)). ^{13}C-NMR (D$_6$)DMSO): 162.3 (C(4)); 158.1 (2 MeO–C); 150.1 (C(2)); 144.6 (C(1) of Ph); 144.6 (C(3″)); 135.4 (2 C(1) of 4-MeO–C_6H_4); 134.3 (C(6)); 130.8 (C(7″)); 129.7 (2 C(2,6) of 4-MeO–C_6H_4); 127.8 (C(3,5) of Ph); 127.6 (C(2,6) of Ph); 126.7 (C(4) of Ph); 123.8 (C(6″)); 118.8 (C(2″)); 113.2 (2 C(3,5) of 4-MeO–C_6H_4); 108.7 (C(5)); 85.8 (CAr_3); 85.5 (C(1′)); 84.7 (C(4′)); 70.4 (C(3′)); 63.6 (C(5′)); 55.0 (2 MeO); 40.1 (C(2′)); 30.9 (C(4″)); 38.6 (C(1″)); 25.8 (C(5″)); 25.3 (C(8″)); 17.4 (C(10″)); 16.1 (C(9″)); 12.3 (Me). Anal. calc. for $C_{41}H_{48}N_2O_7$ (680.83): C 72.33, H 7.11, N 4.11; found: C 72.16, H 7.00, N 3.84.

*5′-O-[Bis(4-methoxyphenyl)(phenyl)methyl]-3′-O-{(2-cyanoethoxy)[di(propan-2-yl)amino]phosphanyl}-2′-deoxy-3-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]-3,4-dihydrothymidine* (**11b**). Compound **10b** (0.32 g, 0.3 mmol) was co-evaporated twice from dry CH_2Cl_2 and dissolved in CH_2Cl_2 (15 ml). Then, EtN^iPr_2 (126 µl, 0.72 mmol) and (chloro)(2-cyanoethoxy)(diisopropylamino)phosphine (156 µl, 0.72 mmol) were added under N_2. The mixture was stirred for 20 min at r.t., and the reaction was then quenched by addition of ice-cold 5% aq. $NaHCO_3$ (12 ml). The raw product was extracted with CH_2Cl_2, and the soln. was dried (Na_2SO_4) for 2 min, filtered, and evaporated to dryness (bath temp.: $>25°$), followed by drying in high vacuum for 5 min. FC (column: 2×10 cm; CH_2Cl_2/acetone 8:2 (*v*/*v*), with 8 drops of Et_3N per l, total time of chromatography >15 min) afforded **11b** (0.27 g, 67%). Colorless.

Stored at −20°. R_f (diasteoisomers; CH_2Cl_2/acetone 8:2, *v*/*v*) 0.87, 0.97. ^{31}P-NMR ($CDCl_3$): 149.1 (P_R); 148.5 (P_S).

*5′-*O*-[Bis(4-methoxyphenyl)(phenyl)methyl]-3′-*O*-{(2-cyanoethoxy)[di(propan-2-yl)amino]phosphanyl}-2′-deoxy-3-[(2*E*)-3,7-dimethylocta-2,6-dien-1-yl]-3,4-dihydrothymidine* (**11a**). Compound **11a** was prepared from **10a** (0.2 g, 0.3 mmol) as described for **11b**. Yield: 0.25 g (97%). Colorless foam. R_f (diastereoisomers; CH_2Cl_2/acetone 8:2 (*v*/*v*)) 0.84, 0.94. ^{31}P-NMR ($CDCl_3$): 149.0 (P_R); 148.5 (P_S).

Small-Scale Coupling of Compound **3b** *with* i) N-*[(9*H*-Fluoren-9-ylmethoxy)carbonyl]glycine* (**13**) *to Yield Compounds* **14**–**16** *and* ii) *Sulforhodamin 101 Sulfonyl Chloride to Yield Compound* **17**.

i) Compound **13** (65.4 mg, 0.22 mmol) was dissolved in CH_2Cl_2 (20 ml) and 4-(dimethylamino)pyridine (DMAP; 5 mg) and **3b** (100 mg, 0.22 mmol) were added. The mixture was cooled to 0°, and *N*,*N*′-dicyclohexylcarbodiimide (DCC; 45.5 mg, 0.22 mmol) in CH_2Cl_2 (2 ml) were added dropwise. After 5 min, the mixture was allowed to warm to r.t., and stirring was continued overnight. Then, further portions of **13**, DMAP, and DCC (30 mol-%, each) were added, and stirring was continued. After a total reaction time of 48 h, the suspension was filtered, and the filtrate was evaporated to dryness. Chromatography (column: 2 × 15 cm; CH_2Cl_2/acetone 6:4 (*v*/*v*)) afforded three main zones from which the following fluorene-labelled nucleolipids were obtained upon evaporation of the solvent.

*(2*R*,3*S*,5*R*)-2-{[({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}acetyl)oxy]methyl}-5-{6-oxo-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]-1,6-dihydro-9*H*-purin-9-yl}tetrahydrofuran-3-yl {[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}acetate* (**14**). R_f ($CHCl_3$/MeOH 96:4 (*v*/*v*)) 0.56. UV (MeOH): 263 (45,200); ε_{260} 44,200. log *P:* 11.67 ± 1.22. ^{1}H-NMR ($CDCl_3$): 7.92 (*s*, H–C(2)); 7.88 (*s*, H–C(8)); 7.77–7.73 (*m*, H–C(4,5) of 2 Fmoc); 7.59 (*d*, $J(1_{Fmoc}/8_{Fmoc},2_{Fmoc}/7_{Fmoc})$, H–C(1,8) of 2 Fmoc); 7.41–7.36 (*m*, H–C(3,6) of 2 Fmoc); 7.32–7.27 (*m*, H–C(2,7) of 2 Fmoc); 6.23 (*dd*, $J(1',2'_\alpha)=J(1',2'_\beta)=7.0$, H–C(1′)); 5.67 (br. *s*, NH); 5.60–5.59 (*m*, H–C(3′)); 5.52 (br. *s*, NH); 5.33–5.31 (*m*, H–C(2″)); 5.08–5.06 (*m*, H–C(6″), H–C(10″)); 4.70–4.60 (*m*, H–C(1″)); 4.44–4.40 (*m*, CH_2 of 2 Fmoc, CH_2(5′)); 4.33–4.32 (*m*, H–C(4′)); 4.26–4.20 (*m*, H–C(9) of 2 Fmoc); 4.04–4.01 (*m*, CH_2 of Gly); 3.06–3.01 (*m*, H_β–C(2′)); 2.55–2.53 (*m*, H_α–C(2′)); 2.10–2.04 (*m*, CH_2(8″), CH_2(9″), CH_2(5″)); 1.95–1.92 (*m*, CH_2(4″)); 1.80 (*s*, Me(13″)); 1.60 (*s*, Me(12″)); 1.59 (*s*, Me(14″)); 1.27 (*s*, Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 169.9 (CO of Gly); 156.5 (CO of Fmoc); 155.6 (C(6)); 148.1 (C(2)); 147.1 (C(4)); 143.7 (C(8a,9a) of Fmoc); 140.7 (C(4a,4b) of Fmoc); 140.1 (C(3″)); 139.0 (C(8)); 134.6 (C(7″)); 130.5 (C(11″)); 127.5 (C(3,6) of Fmoc); 127.0 (C(4,5) of Fmoc); 125.1 (C(2,7) of Fmoc); 124.0 (C(6″)); 123.4 (C(10″)); 120.0 (C(5)); 119.9 (C(1,8) of Fmoc); 119.3 (C(2″)); 83.3 (C(1′); 81.5 (C(4′)); 74.7 (C(3′)); 65.8 (CH_2 of Fmoc); 55.8 (C(5′)); 47.4 (C(9) of Fmoc); 46.5 (CH_2 of Gly); 43.3 (C(1″)); 39.0 (C(2′)); 38.7 (C(8″)); 35.9 (C(4″)); 26.0 (C(5″)); 25.3 (C(12″)); 24.4 (C(9″)); 17.4 (C(15″)); 16.2 (C(14″)); 15.7 (C(13″)).

*2′-Deoxy-5′-*O*-{*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]glycyl}-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**15**). R_f ($CHCl_3$/MeOH 96:4, *v*/*v*) 0.31. UV (MeOH): 263 (27,800); ε_{260} 27,100. log *P:* 7.57 ± 1.15. ^{1}H-NMR ((D$_6$)DMSO): 8.31 (*s*, H–C(2)); 8.29 (*s*, H–C(8)); 7.88 (*d*, $J(4_{Fmoc}/5_{Fmoc},3_{Fmoc}/6_{Fmoc})=7.5$, H–C(4,5) of Fmoc); 7.71 (*d*, $J(1_{Fmoc}/8_{Fmoc},2_{Fmoc}/7_{Fmoc})=7.5$, H–C(1,8) of Fmoc); 7.41 (*dd*, $J(3_{Fmoc}/6_{Fmoc},2_{Fmoc}/7_{Fmoc})=J(3_{Fmoc}/6_{Fmoc},4_{Fmoc}/5_{Fmoc})=8.0$, H–C(3,6) of Fmoc); 7.33 (*dd*, $J(2_{Fmoc}/7_{Fmoc},3_{Fmoc}/6_{Fmoc})=J(2_{Fmoc}/7_{Fmoc},1_{Fmoc}/8_{Fmoc})=8.0$, H–C(2,7) of Fmoc); 6.29 (*dd*, $J(1',2'_\beta)=8.5$, $J(1',2'_\alpha)=7.0$, H–C(1′)); 5.40 (*d*, $J(OH_3,3')=5.5$, H–C(3′)); 5.26 (*t*, $J(2'',1'')=7.0$, H–C(2″)); 5.01 (*t*, $J(6'',5'')=7.0$, H–C(6″)); 5.04–4.99 (*m*, H–C(10″)); 4.60 (*d*, $J(1'',2'')=6.5$, H–C(1″)); 4.34 (*d*, $J(CH_{2Fmoc},9_{Fmoc})=7.0$, H–$CH_2$ of Fmoc); 4.27–4.24 (*m*, H–C(4′)); 4.08–7.07 (*m*, H–C(9) of Fmoc); 3.85 (*d*, $J(CH_{2Gly},NH)=6.0$, CH_2 of Gly); 3.66–3.55 (*m*, CH_2(5′)); 2.88 (*ABdd*, $J_{AB}=13.0$, $J(2'_\beta,3')=6.0$, $J(2'_\beta,3')=8.0$, H_β–C(2′)); 2.34 (m_c (superimposed by the solvent signals, H_α–C(2′)); 2.06 (*t*, $J(8'',9'')=7.5$, CH_2(8″)); 1.99 (*t*, $J(9'',8'')=7.5$, CH_2(9″)); 1.93 (*t*, $J(5'',4'')=7.5$, CH_2(5″)); 1.86 (*t*, $J(4'',5'')=7.5$, CH_2(4″)); 1.78 (*s*, Me(13″)); 1.60 (*s*, Me(12″)); 1.52 (*s*, Me(14″)); 1.51 (*s*, Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 170.0 (CO of Gly); 156.4 (CO of Fmoc); 155.7 (C(6)); 148.0 (C(2)); 147.0 (C(4)); 143.7 (C(8a,9a) of Fmoc); 140.6 (C(4a,4b) of Fmoc); 140.0 (C(3″)); 139.0 (C(8)); 134.6 (C(7″)); 130.5 (C(11″)); 127.5 (C(3,6) of Fmoc); 127.0 (C(4,5) of Fmoc); 125.1 (C(2,7) of Fmoc); 124.0 (C(6″)); 123.8 (C(10″)); 123.4 (C(5)); 120.0 (C(1,8) of Fmoc); 119.3 (C(2″)); 84.1 (C(1′)); 83.3 (C(4′)); 70.4 (C(3′)); 65.8 (CH_2 of Fmoc); 64.0 (C(5′)); 46.5 (C(9) of Fmoc); 43.2 (CH_2 of Gly); 42.0 (C(1″)); 39.9 (C(2′)); 39.7 (C(8″)); 39.6 (C(4″)); 26.0 (C(5″)); 25.5 (C(9″)); 25.3 (C(12″)); 17.4 (C(15″)); 16.1 (C(14″)); 15.7 (C(13″)).

2′-Deoxy-3′-O-*{*N-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]glycyl}-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**16**). R_f ($CHCl_3$/MeOH 96:4, *v*/*v*) 0.20. UV (MeOH): 263 (27,730); ε_{260} 27,100. log *P:* 7.73 ± 0.98. ^{1}H-NMR ($CDCl_3$): 8.03 (*s*, H–C(2)); 7.96 (*s*, H–C(8)); 7.75 (*d*, $J(4_{Fmoc}/5_{Fmoc},3_{Fmoc}/6_{Fmoc})$ = 7.5, H–C(4,5) of Fmoc); 7.58 (*d*, $J(1_{Fmoc}/8_{Fmoc},2_{Fmoc}/7_{Fmoc})$ = 7.5, H–C(1,8) of Fmoc); 7.38 (*dd*, $J(3_{Fmoc}/6_{Fmoc},2_{Fmoc}/7_{Fmoc}) = J(3_{Fmoc}/6_{Fmoc},4_{Fmoc}/5_{Fmoc})$ = 7.5, H–C(3,6) of Fmoc); 7.30–7.27 (*m*, H–C(2,7) of Fmoc); 6.34 (*dd*, $J(1',2'_\beta) = J(1',2'_\alpha)$ = 6.5, H–C(1′)); 5.74–5.73 (*m*, HO–C(3′)); 5.32 (*t*, $J(2'',1'')$ = 6.5, H–C(2″)); 5.08–5.07 (*m*, H–C(10″)), H–C(6″)); 4.76–4.75 (*m*, H–C(3′)); 4.67–4.64 (*m*, H–C(1″)); 4.44–4.39 (*m*, CH_2 of Fmoc); 4.22–4.20 (*m*, H–C(4′), H–C(9) of Fmoc); 4.00–3.99 (*m*, CH_2 of Gly); 3.78–3.75 (*m*, CH_2(5′)); 3.60 (br. *s*, NH); 2.81–2.78 (*m*, H_β–C(2′)); 2.55–2.54 (*m*, H_α–C(2′)); 2.18–2.03 (*m*, CH_2(8″), CH_2(9″), CH_2(5″)); 1.99–1.96 (*m*, CH_2(4″)); 1.80 (*s*, Me(13″)); 1.68 (*s*, Me(12″)); 1.60 (*s*, Me(14″)); 1.59 (*s*, Me(15″)). ^{13}C-NMR ((D$_6$)DMSO): 169.7 (CO of Gly); 156.5 (CO of Fmoc); 155.6 (C(6)); 148.0 (C(2)); 147.0 (C(4)); 143.7 (C(8a,9a) of Fmoc); 140.7 (C(4a,4b) of Fmoc); 140.0 (C(3″)); 138.8 (C(8)); 134.6 (C(7″)); 130.5 (C(11″)); 127.5 (C(3,6) of Fmoc); 127.2 (C(4,5) of Fmoc); 125.1 (C(2,7) of Fmoc); 124.0 (C(6″)); 123.8 (C(10″)); 121.3 (C(5)); 120.0 (C(1,8) of Fmoc); 119.3 (C(2″)); 85.1 (C(1′); 83.5 (C(4′)); 75.5 (C(3′)); 65.8 (CH_2 of Fmoc); 61.4 (C(5′)); 46.5 (C(9) of Fmoc); 43.3 (CH_2 of Gly); 42.3 (C(1″)); 39.0 (C(2′)); 38.7 (C(8″)); 36.8 (C(4″)); 26.0 (C(5″)); 24.5 (C(9″)); 25.3 (C(12″)); 17.4 (C(15″)); 16.1 (C(14″)); 15.7 (C(13″)).

ii) *2′-Deoxy-5′*-O-*{[4-(2,3,6,7,12,13,16,17-octahydro-1*H*,5*H*,11*H*,15*H*-pyrido[3,2,1-*ij*]quinolizino[1′,9′:6,7,8]chromeno[2,3-*f*]quinolin-4-ium-9-yl)-3-sulfonatophenyl]sulfonyl}-1-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl]inosine* (**17**). Compound **3b** (2.74 mg, 6.0 μmol) was dissolved in anh. pyridine (3 ml), and sulforhodamin 101 sulfonyl chloride (2.5 mg, 4.0 μmol) was added. The mixture was stirred under N_2 for 48 h. Then, H_2O (10 ml) was added, and the mixture was extracted once with CH_2Cl_2. The aq. layer was separated and evaporated to dryness. CC (column: 2 × 10 cm; CH_2Cl_2/MeOH 1:1 (*v*/*v*)) gave, after evaporation of the solvent, **17**. Black, amorphous material. R_f ($CHCl_3$/MeOH 96:4, (*v*/*v*)) 0.56. UV (MeOH): 252 (18,200); ε_{260} 16,400.

Oligonucleotides. The synthesis of the oligonucleotides **18**–**23** and their MS analysis were performed by *Eurogentec S.A.*, Liège Science Park, Belgium.

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