Synthesis and Hybridization Properties of Oligodeoxynucleotides with Long-Chain Linkers

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New oligonucleotides with a long-chain linker (6,9-dioxa-3,12-diazatetradecane-1,14-diyl) in their backbone were synthesized, and their hybridization properties were studied by measurement of their T_m curves and fluorescence spectra. The T_m analyses revealed that these oligonucleotides could bind to their complementary strands despite the presence of the long-chain linker. We also demonstrated interesting fluorescence properties of oligodeoxynucleotides with an anthracen-9-ylmethyl group on one of the two N-atoms in the long-chain linker. The fluorescence intensity of these oligonucleotides increased upon their hybridization to the complementary strands and was sensitive to the presence of the mismatch base pairs at a specific position.

1. Introduction. – Various modifications of phosphodiester backbones have been reported in which substituents without P-atoms have been used to develop functional oligodeoxynucleotides (ODNs). These modifications included amides [1], hydroxylamines [2][3], formacetals [4–8], carbonates [9][10], ureas and carbamates [11], squaramides [12][13], and guanidines [14]. In most of these examples, the phosphate surrogates were designed to be isosteric to the phosphodiester structure so that they could connect the up and downstream nucleoside residues without distortion of the original structure. On the other hand, there are some studies of replacement of the phosphodiester backbone with long-chain linkers such as polyethylene glycols in both DNA and RNAs [15–30]. Oligonucleotides with such long-chain linkers have been developed for structural studies of hairpin loops [15–18], triplexes [17][19–21], ribozymes [22][23], gap-DNAs [24], CpG motifs [25], circular DNAs [26][27], and structured RNAs [28][29]. The duplex formation of oligonucleotides with long-chain linkers has also been described [30].

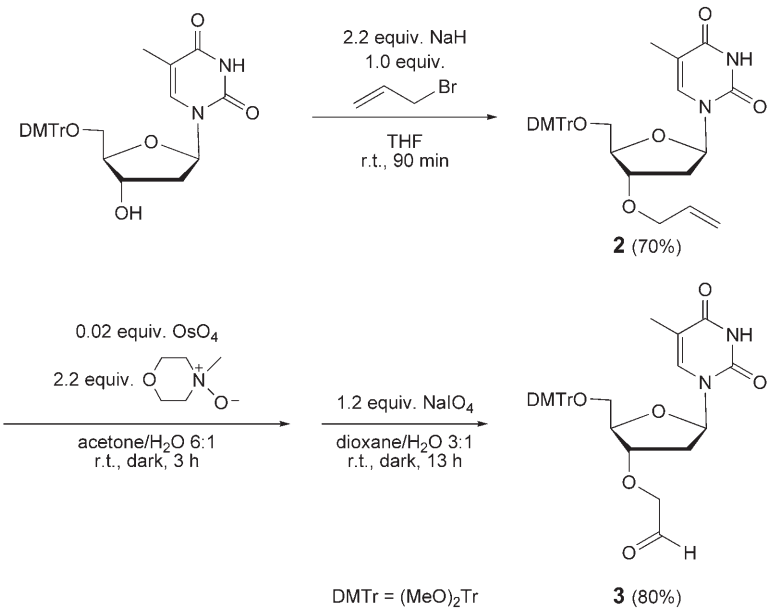
In this paper, we report the synthesis of new modified 15mer ODNs – ODN**1**, ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b** – that have a 6,9-dioxa-3,12-diazatetradecane-1,14-diyl group as a long-chain linker in their backbone (see below, *Fig. 1*), and a study of their hybridization affinity for natural-type 15mer targets ODN**4**–**9** (see below, *Fig. 2*). Because both the modified and natural-type target ODNs have the same number of nucleotide residues, the entire structure of the long-chain linker can be expected to drastically change its conformation upon binding to the targets. Such

conformation change synchronizing with the hybridization might confer interesting properties on the modified ODNs. Keeping such possibilities in mind, we synthesized and studied the properties of the modified ODNs. The T_m analyses revealed the significant affinity of ODN**1** for its complementary strand despite the presence of the long-chain linker.

We also found interesting fluorescence properties of the ODNs with an anthracen-9-ylmethyl group on their backbone (ODN**2a**,**b**, and ODN**3a**,**b**) that might be useful for the detection of single-base mismatches by fluorescence spectroscopy. To substitute the long-chain linker part with a fluorescent anthracene moiety, we designed a linker with secondary amino functions as shown below in *Fig. 1*. The introduction of the fluorescent group made it possible to monitor the hybridization of the modified ODNs by fluorescence spectroscopy.

2. Results and Discussion. – 2.1. *Synthesis of Dimer* **1**. The thymidine dimer **1** with the long-chain linker (see below, *Fig. 1*) was synthesized by the coupling of the 5′ half-unit **3** (*Scheme 1*) and the 3′ half-unit **8** (see *Schemes 2* and *3*) by reductive amination. The 5′ half-unit **3** was synthesized from 5′-*O*-(4,4′-dimethoxytrityl)thymidine by treatment with 2.2 equiv. of NaH and prop-2-en-1-yl bromide to give the 3′-*O*-propenyl derivative **2** in 70% yield (*Scheme 1*). Compound **2** was then oxidized with a catalytic amount of OsO_4 in combination with 1.2 equiv. of $NaIO_4$, giving the desired 3′-*O*-(formylmethyl) derivative **3** in 80% yield.

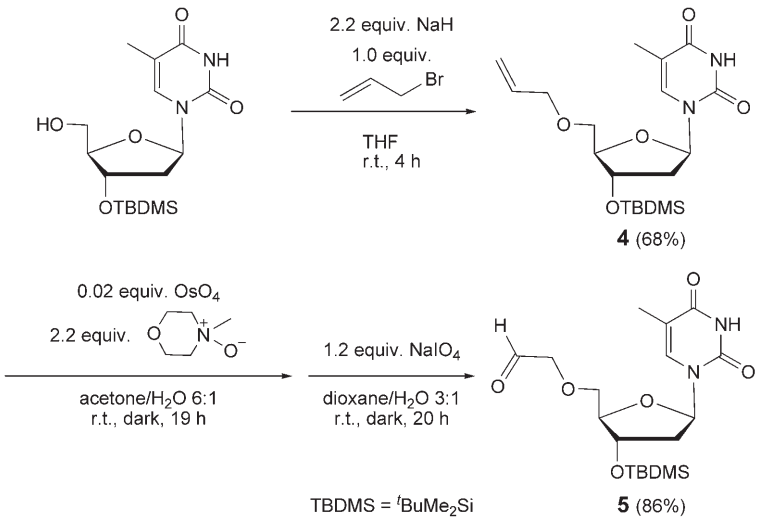
Scheme 1. *Synthesis of the 5′ Half-unit* **3**



The 3′ half-unit **8** was synthesized as shown in *Schemes 2* and *3*. We first synthesized the 5′-*O*-(formylmethyl) derivative **5** *via* **4** according to a procedure similar to that

shown in *Scheme 1* by the use of 3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine in place of 5′-*O*-(4,4′-dimethoxytrityl)thymidine (*Scheme 2*). Next, we examined the reductive amination of **5** with amine **6** [31], which proceeded most successfully when $NaBH(OAc)_3$ was used as a reducing agent in $CHCl_3$ (*Scheme 3*). In this case, compound **7** was obtained in 84% yield. The reducing agents $BH_3 \cdot$pyridine complex and $NaBH_4$ were not effective. Among several solvents tested, $CHCl_3$ gave the best results. Interestingly, the use of CH_2Cl_2 in place of $CHCl_3$ resulted in a worse result, giving the desired compound **7** in only 40% yield. Compound **7** thus obtained was treated with 3% trichloroacetic acid to give the 3′ half-unit **8** in 88% yield. Finally, compound **8** was coupled with the 5′ half-unit **3** under conditions similar to those of the above-mentioned reductive amination to furnish dimer **9** in 54% yield, which was deprotected by acid treatment to give dimer **1** in 88% yield.

Scheme 2. *Synthesis of the 5′-*O*-(Formylmethyl) Derivative* **5**

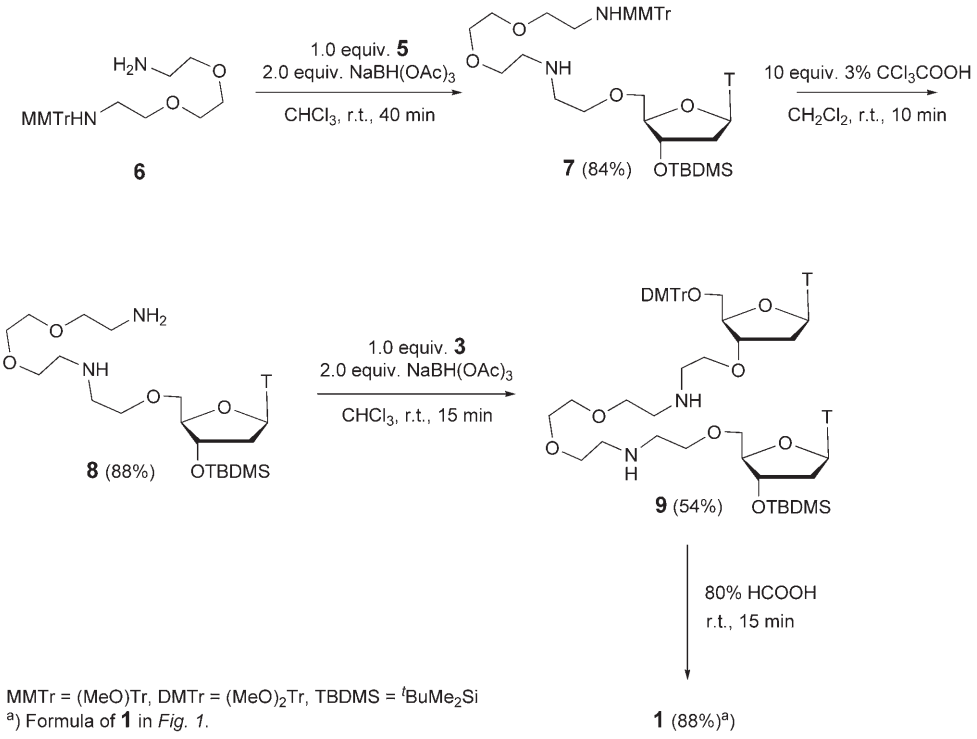


2.2. *Synthesis of Phosphoramidites.* Next, we tried to synthesize the phosphoramidite derivatives **12**, **18a**, and **18b** from the synthetic intermediates described above. Phosphoramidites **18a** and **18b** were designed to monitor the hybridization of oligodeoxynucleotides incorporating the long-chain linker by the use of the fluorescent signal of anthracene.

The phosphoramidite **12** was synthesized starting from the protected dimer **9** (*Scheme 4*). The ^{t}BuMe$_2$Si group of **9** was removed by treatment with Bu_4NF to give **10** in 91% yield. The two secondary-amine moieties were both protected with a trifluoroacetyl group by treatment with trifluoroacetic anhydride to give **11** in 83% yield, and the remaining OH group of **11** was phosphitylated by the phosphorodiamidite reagent to give **12** in 76% yield.

We also synthesized phosphoramidite **18a** according to the procedure shown in *Scheme 5*. The alkylation of compound **7** with 9-(chloromethyl)anthracene in DMF gave **13** in 64% yield. The (MeO)Tr group of **13** was removed by treatment with 3%

Scheme 3. *Synthesis of the 3′ Half-unit* **8** *and Dimer* **9**

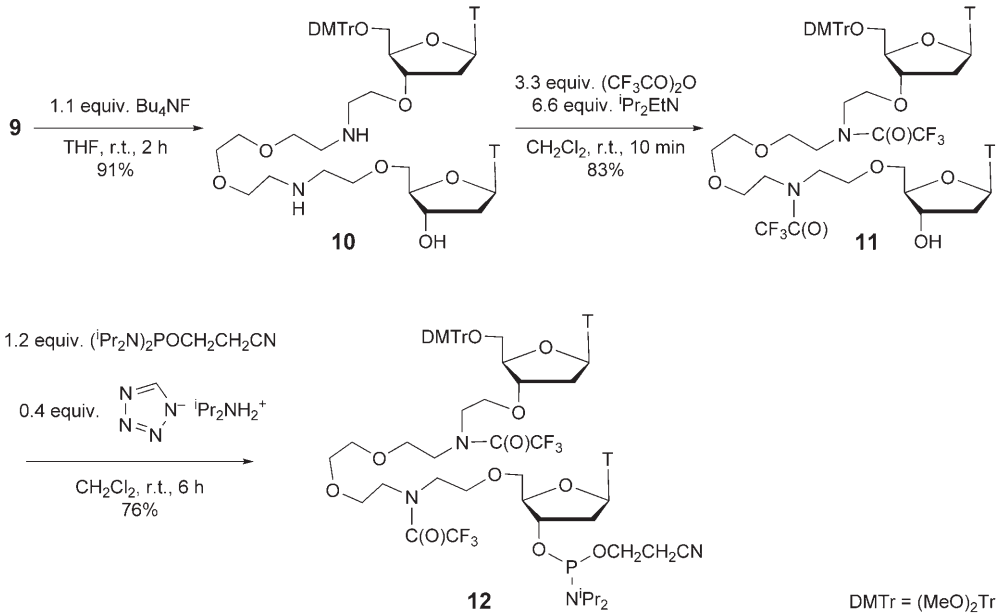


MMTr = (MeO)Tr, DMTr = $(MeO)_2$Tr, TBDMS = ^{t}BuMe_2Si
a) Formula of **1** in *Fig. 1*.

CCl_3COOH to give **14** in 93% yield. Subsequently, the primary-amine function of **14** was condensed with compound **3** by applying conditions similar to those used for the above-mentioned reductive amination, to give dimer **15** in 55% yield. Subsequently, the ^{t}BuMe_2Si group of **15** was removed by treatment with Bu_4NF (→**16**), and the secondary-amino group was protected by a CF_3CO group (→**17**). Finally, the 3′-OH group was phosphitylated to give the desired phosphoramidite **18a**. Compound **18a** was unstable under purification procedures such as gel-filtration column chromatography, probably because of the presence of the basic amine part. This compound, however, could be used successfully in the ensuing oligonucleotide synthesis without further purification.

Next, we synthesized the phosphoramidite **18b** (*Scheme 6*). In the synthesis of **18b**, we used the new protected amine **19** in place of amine **6** because the tritylthio (TrS) group [32–37] of **19** could be selectively removed by a radical reduction [34] without damaging the $(MeO)_2$TrO group at the 5′-position of the nucleoside, as described below. One of the amino groups of 2,2′-[ethane-1,2-diylbis(oxy)]bis[ethanamine] was protected by the TrS group according to the procedure reported previously [32][34]. The protected amine **19** was obtained in 55% yield and coupled with the 3′-*O*-(formylmethyl) derivative **3** by reductive amination. Alkylation of the secondary-amine function of **20** with 9-(chloromethyl)anthracene gave **21** in 77% yield.

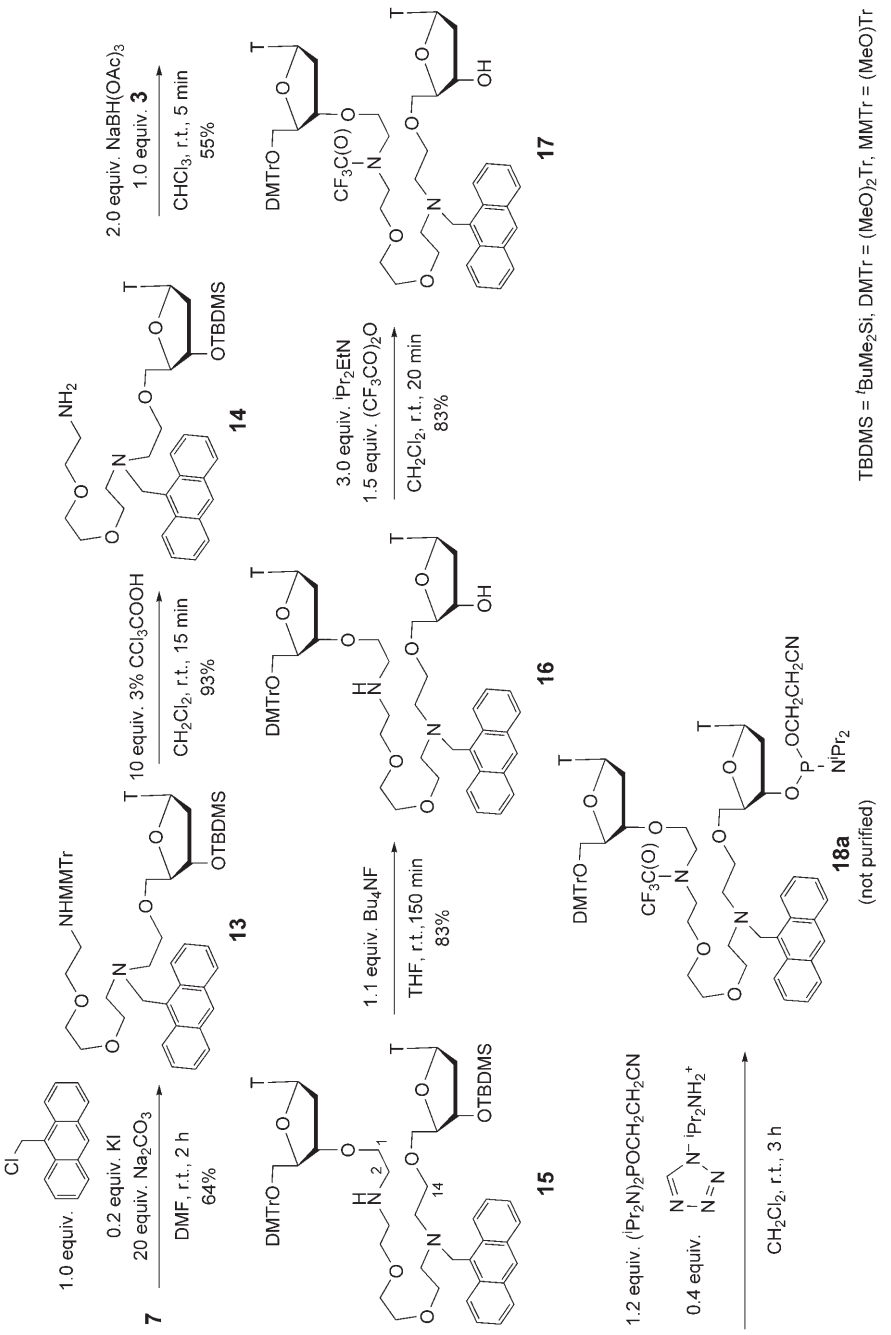
Scheme 4. *Synthesis of Phosphoramidite* **12**



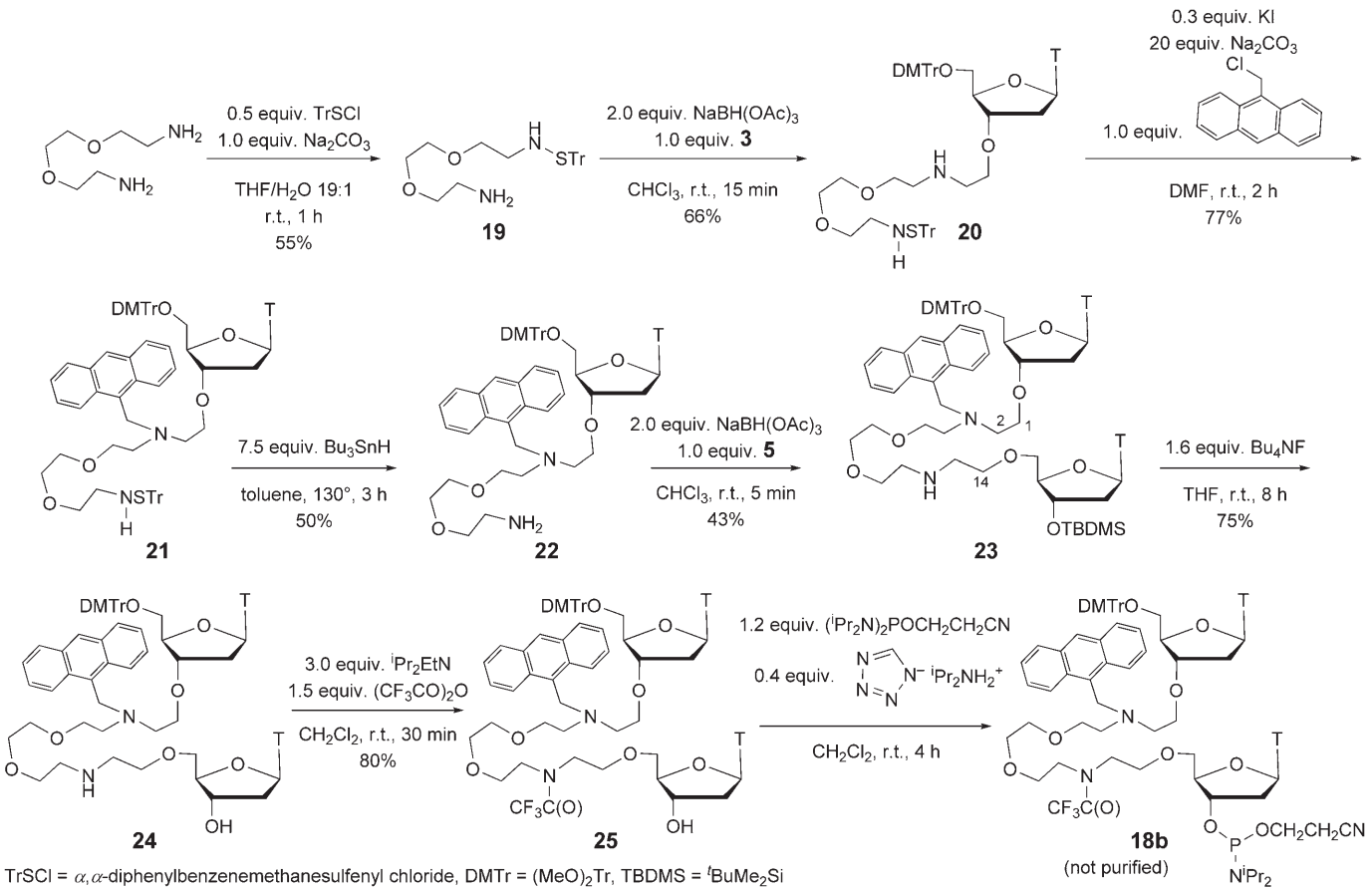
Subsequently, the TrS group was removed by reduction with tributylstannane to give **22** in 50% yield without damaging the $(MeO)_2Tr$ group [34]. The amine **22** was condensed with the 5′-*O*-(formylmethyl) derivative **5** by reductive amination to give **23** in 43% yield. The *t*BuMe$_2$Si group of **23** was removed by treatment with Bu_4NF, and the amino group was protected by a CF_3CO group. Finally, the 3′-OH group was phosphitylated to give the desired phosphoramidite **18b**. Like phosphoramidite **18a**, this compound was unstable during the purification procedure and had to be used without further purification for oligonucleotide synthesis.

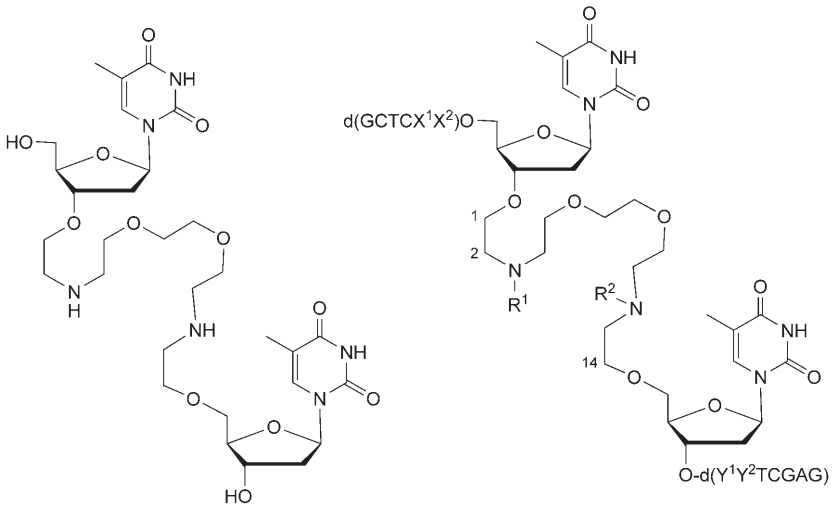
2.3. *Oligonucleotide Synthesis.* By the use of the phosphoramidites **12**, **18a**, and **18b**, the oligonucleotides ODN**1** (*Fig. 1*), ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b** (*Figs. 1* and *2*) were synthesized. The 3′ half of each DNA, 5′-d(Y^{1}Y^2 TCGAG)-3′, was synthesized on CPG (controlled-pore glass) supports with a commercially available DNA synthesizer. After the $(MeO)_2Tr$ group at the 5′-terminal nucleotide (Y^1) was removed, the CPG resin was transferred to a glass syringe. The dimer phosphoramidites with the long-chain linker (**12**, **18a**, and **18b**) were then used for further chain elongation by a manual coupling procedure. Subsequently, the CPG supports were again transferred to the reaction vessel of the DNA synthesizer, and the 5′-half part of each DNA, 5′-d(GCTCX^{1}X^2)-3′, was elongated in the synthesizer. After cleavage of the oligonucleotide from the solid support, all protecting groups at the nucleobases and phosphate groups and at the amino groups were removed by treatment with aqueous ammonia. The structures of the oligonucleotides thus obtained were confirmed by MALDI-TOF-MS analyses.

Scheme 5. *Synthesis of Phosphoramidite* **18a**



Scheme 6. *Synthesis of Phosphoramidite* **18b**





1

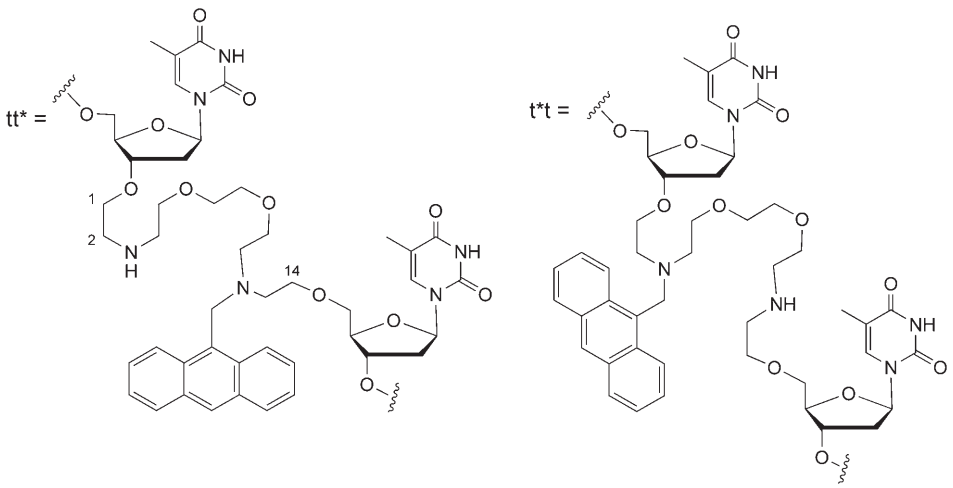
ODN**1**	X^1 = A, X^2 = G, Y^1 = G, Y^2 = A $R^1 = R^2$ = H
ODN**2a**	X^1 = A, X^2 = G, Y^1 = G, Y^2 = A R^1 = H, R^2 = anthracen-9-ylmethyl
ODN**2b**	X^1 = A, X^2 = G, Y^1 = G, Y^2 = A R^1 = anthracen-9-ylmethyl, R^2 = H
ODN**3a**	X^1 = C, X^2 = T, Y^1 = A, Y^2 = G R^1 = H, R^2 = anthracen-9-ylmethyl
ODN**3b**	X^1 = C, X^2 = T, Y^1 = A, Y^2 = G R^1 = anthracen-9-ylmethyl, R^2 = H

Fig. 1. *Structure of the dimer unit* **1** *and the oligodeoxynucleotides with long-chain linkers*

2.4. *Hybridization Properties of Oligodeoxynucleotides with Long-Chain Linkers.* We first measured the UV melting curve of the duplex of ODN**1**, *i.e.*, 5′-d(GCTCAG[tt]GATCGAG)-3′, where [tt] represents the dithymidine unit derived from **1** and introduced by means of **12**, with the complementary strand ODN**4**, *i.e.*, 5′-d(CTCGATCAACTGAGC)-3′ (*Fig. 2*). We also measured the T_m value of the duplex of the unmodified DNA strand ODN**5′**, *i.e.*, 5′-d(GCTCAGTTGATCGAG)-3′, with ODN**4** to clarify the effect of the modification on the melting temperature. Although the T_m value of the ODN**1** · ODN**4** duplex was lower by 9° than that of the natural-type duplex, as shown in the *Table*, it was clearly revealed that ODN**1** could hybridize with the target sequence despite the presence of the long-chain linker in its backbone.

Table. T_m *Values of Modified* (ODN**1**) *and Natural-Type* (ODN**5′**) *Duplexes*

	ODN**1** · ODN**4**	ODN**5′** · ODN**4**
T_m [°]	48	57



Modified DNA (5' to 3')

ODN**2a**	d(GCTCAG[tt*]GATCGAG)
ODN**2b**	d(GCTCAG[t*t]GATCGAG)
ODN**3a**	d(GCTCCT[tt*]AGTCGAG)
ODN**3b**	d(GCTCCT[t*t]AGTCGAG)

Complementary targets (3' to 5')

ODN**4**	d(CGAGTCAACTAGCTC)
ODN**5**	d(CGAGGAAATCAGCTC)
ODN**5'**	d(GAGCTAGTTGACTCG)

Mismatch targets of ODN**3a** (3' to 5')

ODN**6**	d(CGAGGAAATCCGCTC)
ODN**7**	d(CGAGGAAATTAGCTC)
ODN**8**	d(CGAGGAAACCAGCTC)
ODN**9**	d(CGCGGAAATCAGCTC)

Fig. 2. *Sequences of the oligonucleotides used in this study.* The abbreviations tt* and t*t refer to the modified thymidine dimers. ODN**4** is complementary to ODN**2a** and ODN**2b**. ODN**5** is complementary to ODN**3a** and ODN**3b**. ODN**6**–**9** are mismatched targets for ODN**3a**. The mismatch sites are indicated by underlines.

Next, we studied the hybridization properties of oligodeoxynucleotides ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b** (*Figs. 1* and *2*) in more detail by measurement of the fluorescence of the anthracene moiety of these oligonucleotides. ODN**2a** and ODN**2b** have almost identical structures, except that ODN**2a** has an anthracene-9-ylmethyl group at the N-atom of the long-chain linker closer to the 3′ terminus, as represented by [tt*] (*Fig. 2*), and ODN**2b** has the same fluorescent aromatic group at the N-atom nearer to the 5′ terminus, as represented by [t*t]. We also synthesized the oligonucleotides ODN**3a** and ODN**3b** with –CT[tt*]AG– and –CT[t*t]AG– sequences in place of the –AG[tt*]GA– and –AG[t*t]GA– sequences of ODN**2a** and ODN**2b**, respectively, to explore the effect of the nearby guanine residue on the fluorescence intensity of the anthracene moiety. ODN**4** is the complementary strand of ODN**2a** and ODN**2b**, and ODN**5** is that of ODN**3a** and ODN**3b**.

First we measured the fluorescence intensities of ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b** in their single-strand states in various pH regions. The results are shown on the left-hand side of *Fig. 3*. As the pH value of the solution increased, the fluorescence intensity decreased in all cases. These observations agree with the fact that the fluorescence of the anthracene moiety is quenched by the nearby lone pair on the N-atom, and the quenching is suppressed by protonation of the N-atom [38–43]. Moreover, comparison between ODN**2a** and ODN**2b** revealed that ODN**2a** with the anthracene moiety at the N-atom closer to the 3′ terminus showed approximately twice as much fluorescence in all pH regions. This trend was also seen for the fluorescence intensities of ODN**3a** and ODN**3b**.

As expected, ODN**2a** and ODN**2b**, having guanine residues at the nearest-neighbor positions of the tt* and t*t dimer units, showed lower fluorescence intensity in comparison with ODN**3a** and ODN**3b**, respectively. These results suggest that the fluorescence of the anthracene moiety was quenched by the guanine residue.

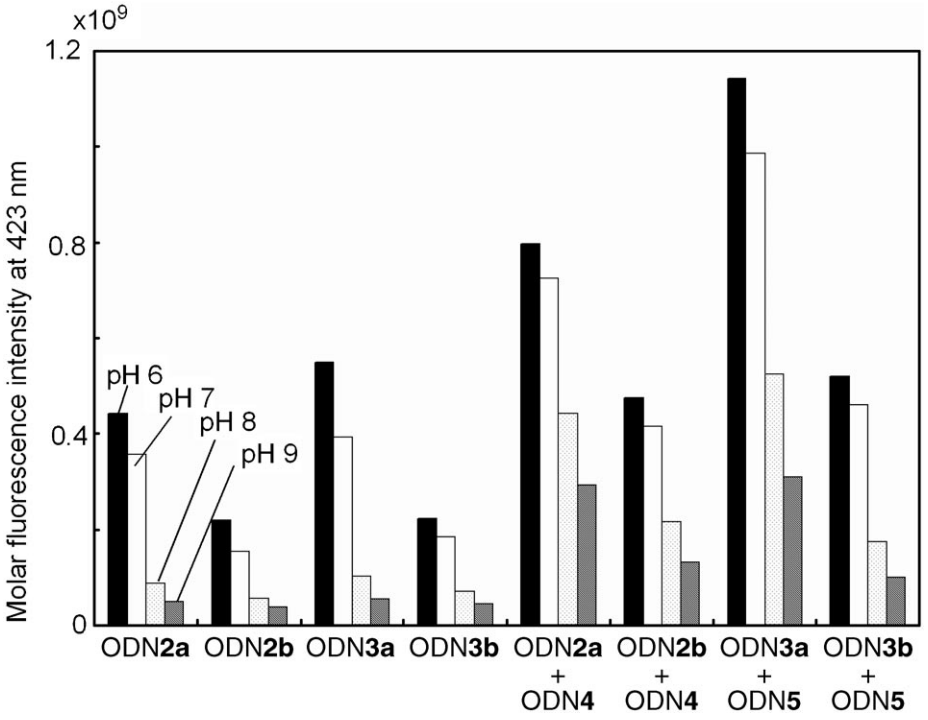


Fig. 3. *Fluorescence intensities of ODN***2a**, *ODN***2b**, *ODN***3a**, *and ODN***3b** *in the presence and absence of their complementary targets*

Next, we measured the fluorescence spectra of ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b** in the presence of their complementary strands ODN**4** and ODN**5**. Interestingly, upon addition of these complementary strands, the fluorescence intensities increased about 2-fold in all pH regions. It is well known that the fluorescence of the anthracene moiety is quenched by interaction with DNA strands

[44–46]. Therefore, the observed intensity increases suggested that the anthracene moiety on the long-chain linker was more accessible to the nucleobases in the single-strand state than in the double-strand state. *Yamana* and co-workers also reported that the fluorescence of the anthracene moiety introduced to the 2′ position of RNA derivatives increased similarly upon hybridization to the DNA and RNA targets [46]. The observed increases in fluorescence intensities suggested to use the anthracene-modified oligonucleotides reported in this study as oligonucleotide probes for hybridization sensing.

Therefore, we examined the fluorescence properties of the most strongly fluorescent ODN**3a** upon the addition of the target oligonucleotides with a mismatched base (ODN**6**–**9**), and compared the fluorescence intensity with that of the fully matched ODN**3a**·ODN**5** duplex (*Fig. 4*). Here, ODN**3a**·ODN**6** has a T·C mismatch at the third position from the tt* unit, ODN**3a**·ODN**7** has a G·T mismatch at the second position, ODN**3a**·ODN**9** has a T·C mismatch at the fourth position, and ODN**3a**·ODN**8** has an A·C mismatch at the position next to the tt* unit. Interestingly, the mismatch-containing duplexes ODN**3a**·ODN**9**, ODN**3a**·ODN**7**, and ODN**3a**·ODN**6** showed fluorescence spectra almost identical to that of the fully matched duplex ODN**3a**·ODN**5**. In contrast, the duplex ODN**3a**·ODN**8**, containing an A·C mismatch near tt* had significantly lower fluorescence intensity than the others. This observation indicates that the fluorescence intensity of the anthracene moiety in ODN**3a** is very sensitive to the loss of the *Watson–Crick* base pair only at the neighboring position. Although the detailed mechanism of this fluorescence change was not clear, the interesting properties of ODNs containing tt* might enable them to be used as modified oligonucleotide probes for mismatch detection.

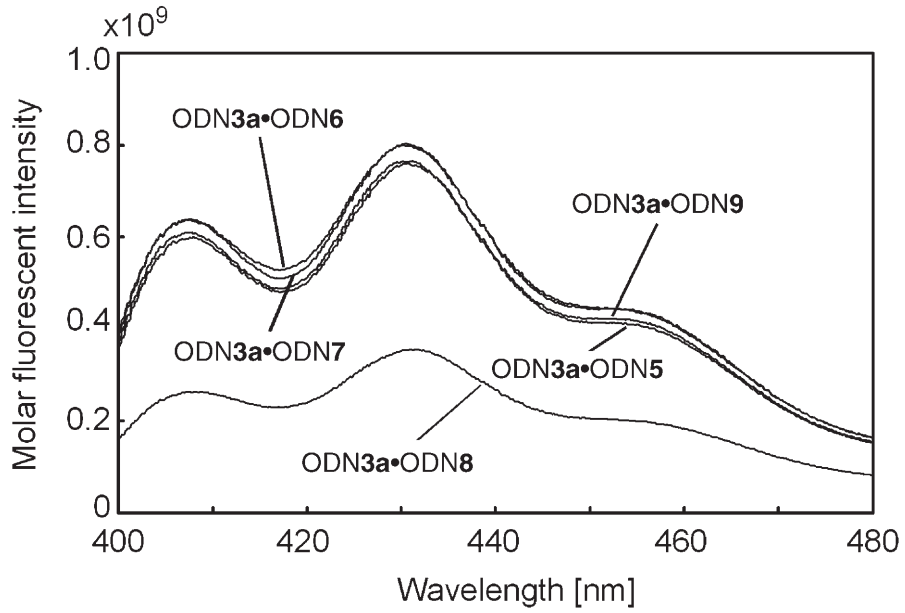


Fig. 4. *Fluorescence intensities of duplexes of* ODN**3a** *with the target strands* ODN**6**–**9**

3. Conclusions. – In this work, we synthesized new oligonucleotides with a 6,9-dioxa-3,12-diazatetradecane-1,14-diyl long-chain linker in their backbone and studied their hybridization properties by measurement of their T_m curves and fluorescence spectra. It should be noted that oligonucleotides with such long linkers could hybridize with their target strands. The T_m analysis by use of ODN**1** revealed the ability of this oligonucleotide to bind its complementary strand despite the presence of the long-chain backbone. In addition, we demonstrated the interesting fluorescence properties of oligodeoxynucleotides with an anthracen-9-ylmethyl group at one of the two N-atoms in the long-chain linker. The fluorescence intensity of the oligonucleotides ODN**2a**, ODN**2b**, ODN**3a**, and ODN**3b**, increased upon their hybridization with their complementary strands. These results indicate that the dynamic conformation change of the long-chain linker upon hybridization changes the environment around the anthracene residue so that their fluorescence increases.

We also examined the effect of the presence of mismatch base pairs on the fluorescence of ODN**3a**. Interestingly, when ODN**3a** was hybridized to the target strand ODN**8** with an A · C mismatch at the nearby site of the long-chain-linked units, the fluorescence decreased significantly. This observation suggests that the fluorescence of the anthracene moiety of ODN**3a** is very sensitive to the loss of base pairs at a specific site.

Experimental Part

1. *General*. TLC: *Merck* silica gel *60* (F_{254}) plates. Column chromatography (CC): silica gel *C-200* (*Wako Co. Ltd.*); for rapid separations a minipump for a goldfish bowl was used to attain sufficient pressure. UV Spectra: *Biospec-mini* spectrophotometer. Circular dichroism (CD) spectra: *J-725* spectrometer, 0.5 cm cell. ^{1}H-, ^{13}C-, and ^{31}P-NMR Spectra: at 500, 126, and 202 MHz, resp.; chemical shifts δ in ppm rel. to $SiMe_4$ (= 0 ppm) or (D_6)DMSO (= 2.49 ppm) for ^{1}H, and rel. to $CDCl_3$ (= 77.0 ppm) or (D_6)DMSO (= 39.7 ppm) for ^{13}C. ESI-MS: *Mariner*™; in *m*/*z*. Cyclic voltammetry: *ALS* electrochemical analyzer, model *600*A. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

*5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-3′-*O*-(prop-2-en-1-yl)thymidine* (**2**). To a soln. of 5′-*O*-bis[(4-methoxyphenyl)phenylmethyl]thymidine (22 g, 41 mmol) in THF (200 ml), NaH (3.3 g, 89 mmol) and 3-bromoprop-1-ene (3.5 ml, 41 mmol) were added. The soln. was stirred at r.t. for 90 min, then the reaction was quenched by addition of MeOH (10 ml). The soln. was diluted with AcOEt (200 ml), washed twice with brine (100 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (silica gel, hexane/$CHCl_3$ 1:1): **2** (17 g, 70%). ^{1}H-NMR (500 MHz, $CDCl_5$): 1.46 (*s*, Me–C(5)); 2.17–2.51 (*m*, 2 H–C(2′)); 3.30–3.51 (*m*, 2 H–C(5′)); 3.80 (*s*, 2 MeO); 3.90–4.02 (*m*, CH_2=CHC*H*$_2$O); 4.13–4.15 (*m*, H–C(4′)); 4.25–4.27 (*m*, H–C(3′)); 5.18–5.27 (*m*, C*H*$_2$=CHCH$_2$O); 5.82–5.90 (*m*, CH_2=C*H*CH$_2$O); 6.34–6.37 (*t*, *J* = 2.2, 1 H–C(1′)); 6.82–7.41 (*m*, 13 H of $(MeO)_2$*Tr*); 7.62 (*s*, H–C(6)); 8.23 (*s*, NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.97; 38.17; 55.41; 63.77; 70.41; 79.03; 84.31; 84.99; 87.05; 111.27; 113.41; 113.42; 117.67; 127.31; 128.15; 128.26; 130.22; 134.20; 135.50; 135.55; 135.77; 144.47; 150.24; 158.88; 163.62. ESI-MS: 604.2575 ($C_{34}H_{37}N_2NaO_7^+$; calc. 607.2420).

*5′-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-3′-(2-oxoethyl)thymidine* (**3**). To a soln. of **2** (17 g, 28 mmol) in acetone/H_2O 6:1 (230 ml), OsO_4 (7.1 ml, 570 μmol) and 4-methylmorpholine 4-oxide (13 ml; 50% in H_2O, 63 mmol) were added. The soln. was stirred in the dark at r.t. for 3 h. The solid was removed by filtration, and the filtrate was diluted with AcOEt (250 ml). The org. layer was washed once with sat. aq. $NaHCO_3$ soln. (100 ml), dried ($MgSO_4$), and concentrated. The residue was dissolved in 1,4-dioxane/H_2O 3:1 (280 ml), and to this soln. was added $NaIO_4$ (7.2 g, 34 mmol). The soln. was stirred in the dark at r.t. for 13 h. The soln. was diluted with AcOEt (300 ml), washed once with sat. aq. $NaHCO_3$

soln., dried ($MgSO_4$), and concentrated and the residue subjected to CC (silica gel, $CHCl_3$): **3** (13 g, 80%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.55 (*s*, Me–C(5)); 2.11–2.16 (*m*, 1 H–C(2′)); 2.40–2.49 (*m*, 1 H–C(2′)); 3.31–3.56 (*m*, 2 H–C(5′)); 3.80 (*s*, 2 MeO); 4.01–4.11 (*m*, 1 H, CH_2CH=O); 4.16–4.23 (*m*, H–C(3′), H–C(4′)); 6.35–6.37 (*m*, H–C(1′)); 6.83–7.40 (*m*, 13 H of (MeO)$_2$*Tr*); 7.58 (*m*, H–C(6)); 10.01–10.10 (*m*, CH$_2$C*H*=O). ^{13}C-NMR (126 MHz, $CDCl_3$): 12.01; 37.78; 55.40; 63.69; 74.67; 81.01; 84.05; 84.79; 87.18; 111.50; 113.43; 113.44; 127.32; 127.37; 128.15; 128.17; 128.20; 128.24; 130.17; 130.21; 135.37; 135.43; 135.54; 144.34; 150.38; 158.86; 158.91; 163.76; 199.45. ESI-MS: 609.2295 ($C_{33}H_{34}N_2NaO_8^+$; calc. 609.2213).

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(prop-2-en-1-yl)thymidine* (**4**). As described for **2**, with 3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine (9.8 g, 27 mmol), THF (140 ml), NaH (1.4 g, 60 mmol), and 3-bromoprop-1-ene (2.3 ml, 27 mmol) for 4 h. Workup with MeOH (10 ml), AcOEt (150 ml), and brine (100 ml) and CC (silica gel, hexane/$CHCl_3$ 3:2), gave **4** (7.3 g, 68%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.08 (*s*, Me_2Si); 0.89 (*s*, ^{t}BuSi); 1.90 (*s*, Me–C(5)); 2.04–2.08 (*m*, 1 H–C(2′)); 2.16–2.20 (*m*, 1 H–C(2′)); 3.51 (*dd*, *J* = 2.2, 10.7, 1 H–C(5′)), 3.66 (*dd*, *J* = 2.2, 10.7, 1 H–C(5′)); 3.98–4.00 (*m*, H–C(4′)); 4.05–4.07 (*m*, CH$_2$=CHCH_2O); 4.44–4.47 (*m*, H–C(3′)); 5.14–5.24 (*m*, CH_2=CHCH$_2$O); 5.89–5.96 (*m*, CH$_2$=C*H*CH$_2$O); 6.26 (*t*, *J* = 6.3, H–C(1′)); 7.66 (*s*, H–C(6)); 8.07 (*s*, NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.84; −4.65; 12.60; 18.00; 25.77; 41.43; 69.49; 71.97; 72.40; 85.06; 86.45; 110.75; 117.54; 134.07; 136.05; 150.66; 164.34. ESI-MS: 397.2357 ($C_{19}H_{33}N_2O_{16}Si^+$; calc. 397.2159).

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-(2-oxoethyl)thymidine* (**5**). As described for **3**, with **4** (7.3 g, 19 mmol), acetone/H_2O 6:1 (190 ml), OsO_4 (3.8 g, 370 μmol), and 4-methylmorpholine-4-oxide (8.5 ml; 50% in H_2O, 41 mmol) for 19 h. Then workup with AcOEt (200 ml) and sat. aq. $NaHCO_3$ soln. (150 ml), followed by treatment of the residue in 1,4-dioxane/H_2O 3:1 (190 ml), with $NaIO_4$ (4.7 g, 22 mmol) for 20 h. Workup with AcOEt (200 ml) and sat. aq. $NaHCO_3$ soln. and CC (silica gel, hexane/$CHCl_3$ 2:3 gave **5** (6.4 g, 86%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.01 (*s*, Me_2Si); 0.80–0.81 (*m*, ^{t}BuSi); 1.81 (*d*, *J* = 0.7, Me–C(5)); 2.12–2.27 (*m*, 2 H–C(2′)); 3.45–3.89 (*m*, 2 H–C(5′), CH_2CH=O); 3.97–4.02 (*m*, H–C(4′)); 4.42–4.53 (*m*, H–C(3′)); 6.29–6.37 (*m*, H–C(1′)); 7.50–7.58 (*m*, H–C(6)); 8.12 (*s*, NH(3)); 9.72 (*s*, CH$_2$C*H*=O). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.78; −4.58; 12.62; 18.05; 25.81; 41.08; 71.15; 72.23; 85.20; 86.12; 111.12; 136.08; 150.57; 164.08; 198.02. ESI-MS: 399.1714 ($C_{18}H_{31}N_2O_6Si^+$; calc. 399.1951).

*3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-*O*-[13-(4-methoxyphenyl)-13,13-diphenyl-6,9-dioxa-3,12-diazatridec-1-yl]thymidine* (**7**). To the soln. of **6** (200 mg, 502 μmol) in $CHCl_3$ (5 ml) was added **5** (211 mg, 502 μmol). To this soln. was added $NaBH(OAc)_3$ (213 mg, 1.0 mmol), and the resulting mixture was stirred at r.t. for 40 min. The soln. was diluted with AcOEt (10 ml) and washed once with sat. aq. $NaHCO_3$ soln. (10 ml). The org. layer was dried ($MgSO_4$) and concentrated and the residue subjected to CC (NH-silica gel, hexane/$CHCl_3$ 2:3): **7** (338 mg, 84%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.10 (*s*, Me_2Si); 0.91 (*s*, ^{t}BuSi); 1.92 (*s*, Me–C(5)); 2.07–2.11 (*m*, 1 H–C(2′)); 2.23–2.27 (*m*, 1 H–C(2′)); 2.36 (*t*, *J* = 5.6, 2 H, CH_2N); 2.79 (*t*, *J* = 5.3, 2 H, CH_2N); 2.84 (*t*, *J* = 6.1, 2 H, CH_2N); 3.50–3.65 (*m*, 1 H–C(5′), OCH_2CH$_2$NHCH$_2$CH_2OCH_2CH_2OCH_2CH$_2$NH); 3.72 (*dd*, *J* = 2.9, 10.8, 1 H–C(5′)); 3.79 (*s*, MeO), 3.98–4.00 (*m*, H–C(4′)); 4.42–4.44 (*m*, H–C(3′)); 6.34 (*t*, *J* = 2.8, H–C(1′)); 6.80–6.83 (*m*, 2 arom. H); 7.15–7.52 (*m*, 12 H of (MeO)*Tr*); 7.53 (*s*, H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.93; −4.76; 12.61; 17.84; 25.65; 41.06; 42.95; 48.84; 49.10; 55.01; 69.94; 70.02; 70.17; 70.31; 70.40; 71.06; 71.16; 72.09; 84.84; 86.18; 110.62; 112.97; 126.03; 127.64; 128.48; 129.72; 135.56; 138.15; 146.28; 150.61; 157.71; 164.23. ESI-MS: 803.2904 ($C_{44}H_{63}N_4O_8Si^+$; calc. 803.4415).

*5′-*O*-[2-{[2-[2-(2-Aminoethoxy)ethoxy]ethyl]amino}ethyl]-3′-*O*-[(*tert*-butyl)dimethylsilyl]thymidine* (**8**). A soln. of **7** (590 mg, 735 μmol) in 3% CCl_3COOH/CH_2Cl_2 (25 ml) was stirred at r.t. for 10 min. The soln. was diluted with $CHCl_3$ (10 ml), washed with sat. aq. $NaHCO_3$ soln. (4 × 10 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (NH-silica gel, $CHCl_3$/MeOH/Et_3N 97:3:1): **8** (350 mg, 88%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.08 (*s*, Me_2Si); 0.89 (*s*, ^{t}BuSi), 1.93 (*s*, Me–C(5)), 2.07–2.18 (*m*, 1 H–C(2′)); 2.21–2.29 (*m*, 1 H–C(2′)); 2.80–2.88 (*m*, 6 H, CH_2N); 3.38 (br., NH_2); 3.49–3.74 (*m*, 12 H, 2 H–C(5′), CH_2O); 3.98–4.00 (*m*, H–C(4′)); 4.39–4.42 (*m*, H–C(3′)); 6.27–6.30 (*t*, *J* = 6.7, H–C(1′)); 7.52 (*s*, H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.74; −4.56; 12.86; 18.06; 25.83; 41.36; 41.73; 49.25; 49.48; 70.27; 70.43; 70.60; 70.65; 71.39; 72.31; 73.34; 85.25; 86.45; 110.78; 135.85; 150.56; 160.11. ESI-MS: 531.1849 ($C_{24}H_{47}N_4O_7Si^+$; calc. 531.3214).

*5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl(6,9-dioxa-3,12-diazatetradecane-1,14-diyl)-(3′ → 5′)-3′-O-[(*tert*-butyl)dimethylsilyl]thymidine* **9**. As described for **7**, with **8** (350 mg, 677 μmol) in $CHCl_3$ (17 ml), **3** (397 mg, 677 μmol) in $CHCl_3$ (17 ml), and $NaBH(OAc)_3$ (387 mg, 1.4 mmol) for 15 min. Workup with AcOEt (50 ml) and sat. aq. $NaHCO_3$ soln. (2 × 30 ml) and CC (silica gel, $CHCl_3$/MeOH (96 : 4) gave **9** (403 mg, 54%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.08 (*s*, Me_2Si); 0.89 (*s*, ^{t}BuSi); 1.45 (*s*, 1 Me–C(5)); 1.95 (*s*, 1 Me–C(5)); 2.07–2.49 (*m*, 4 H, H–C(2′)); 2.81–2.85 (br., 4 CH_2N); 3.32 (*dd*, *J* = 3.5, 10.5, 1 H–C(5′)); 3.51 (*dd*, *J* = 2.7, 10.5, 1 H–C(5″)); 3.54–3.68 (*m*, 2 H–C(5′)), 6 CH_2O); 3.79 (*s*, 2 MeO); 3.98–4.40 (*m*, 2 H–C(3′), 2 H–C(4′)); 6.28 (*t*, *J* = 4.5, 1 H–C(1′)); 6.37 (*t*, *J* = 2.9, 1 H–C(1′)); 6.83 (*m*, 2 arom. H); 7.18–7.40 (*m*, 11 arom. H); 7.50 (*s*, 1 H–C(6)); 7.59 (*s*, 1 H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.74; −4.56; 11.91; 12.85; 18.05; 25.83; 37.94; 41.35; 49.12; 49.17; 49.21; 49.35; 55.36; 64.03; 68.85; 70.30; 70.36; 70.40; 70.53; 70.64; 71.21; 72.33; 80.44; 84.14; 84.85; 85.42; 86.48; 87.02; 110.71; 111.30; 113.38; 127.26; 128.10; 128.24; 130.18; 135.46; 135.50; 135.69; 135.93; 144.43; 150.56; 158.83; 164.01; 164.14. ESI-MS: 1101.6314 ($[M+H]^+$, $C_{57}H_{81}N_6O_{14}Si^+$; calc. 1101.5580).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl(6,9-dioxa-3,12-diazatetradecane-1,14-diyl)-(3′ → 5′)-thymidine (**10**). To a soln. of **9** (837 mg, 760 μmol) in THF (7.6 ml) was added 1M Bu_4NF in THF (836 μl), and the soln. was stirred at r.t. for 2 h. The soln. was diluted with AcOEt (10 ml), washed once with brine (10 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (NH-silica gel, $CHCl_3$/MeOH 99 : 1): **10** (680 mg, 91%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.44 (*s*, 1 Me–C(5); 1.90 (*s*, 1 Me–C(5); 2.15–2.51 (*m*, 4 H, H–C(2′)); 2.79–2.85 (*m*, 8 H, CH_2N); 3.29–3.75 (*m*, 16 H, H–C(5′), CH_2O); 3.78 (*s*, 2 MeO); 4.01 (br., 1 H–C(4′)); 4.15 (br., 1 H–C(4′)); 4.18 (br., 1 H–C(3′)); 4.50 (br., 1 H–C(3′)); 6.32–6.37 (*m*, 2 H–C(1′)); 6.79–6.83 (*m*, 4 arom. H); 7.18–7.41 (*m*, 9 arom. H); 7.57, 7.60 (2*s*, 2 H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.81; 12.72; 37.77; 40.80; 48.58; 48.70; 48.88; 55.23; 63.93; 68.53; 70.01; 70.18; 70.30; 70.36; 70.66; 80.31; 83.94; 84.64; 84.92; 85.80; 86.87; 110.56; 111.22; 113.25; 127.11; 127.97; 128.10; 130.06; 135.33; 135.37; 135.56; 135.99; 144.30; 150.76; 150.80; 158.66; 164.44; 164.49. ESI-MS: 987.5160 ($[M+H]^+$, $C_{51}H_{67}N_6O_{14}^+$; calc. 987.4715).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3,12-bis(2,2,2-trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine (**11**). To a soln. of **10** (510 mg, 507 μmol) in CH_2Cl_2 (5 ml) were added ^{i}Pr$_2$EtN (580 μl, 3.4 mmol) and $(CF_3CO)_2O$ (233 μl, 1.7 mmol), and the soln. was stirred at r.t. for 10 min. The soln. was diluted with AcOEt (10 ml), washed once with brine (10 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (silica gel, $CHCl_3$/MeOH 98 : 2): **11** (507 mg, 83%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.41–1.48 (*m*, Me–C(5)); 1.90–1.92 (*m*, Me–C(5)); 2.12–2.47 (*m*, 4 H–C(2′)); 3.29–3.76 (*m*, 4 H–C(5′), 10 CH_2 of 3′ → 5′ linker); 3.79 (*s*, 2 MeO); 4.06–4.45 (*m*, 2 H–C(3′), 2 H–C(4′)); 6.29–6.36 (*m*, 2 H–C(1′)); 6.84 (*m*, 2 arom. H); 7.21–7.40 (*m*, 11 arom. H); 7.46 (*s*, 1 H–C(6)); 7.59 (*s*, 1 H–C(6)); 9.99 (br., 2 NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 0.13; 11.89; 12.97; 12.76; 37.89; 37.86; 40.20; 40.25; 47.56; 47.92; 48.25; 48.41; 48.70; 48.80; 55.41; 63.96; 64.20; 66.56; 68.27; 68.52; 68.46; 70.02; 70.14; 70.31; 70.52; 70.70; 70.74; 70.82; 70.88; 71.26; 71.62; 81.05; 84.01; 84.13; 84.76; 85.34; 85.36; 85.41; 85.48; 85.74; 87.24; 87.22; 111.02; 111.10; 111.75; 111.79; 113.45; 115.40; 117.69; 127.39; 128.18; 128.24; 130.20; 135.34; 135.49; 135.58; 135.99; 136.11; 144.33; 150.57; 150.79; 158.93; 163.85; 163.90; 163.94. ESI-MS: 1201.4032 ($[M+Na]^+$, $C_{55}H_{65}F_6N_6NaO_{16}^+$; calc. 1201.4180).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3,12-bis(2,2,2-trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine 3′-(2-Cyanoethyl N,N-*Diisopropylphosphoramidite)* (**12**). To a soln. of **11** (193 mg, 164 μmol) in CH_2Cl_2 (660 μl) were added 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (62 μl, 196 μmol) and diisopropylammonium 1*H*-tetrazolide (11 mg, 66 μmol). The mixture was stirred at r.t. for 6 h. The soln. was diluted with AcOEt (10 ml), and washed once with sat. aq. $NaHCO_3$ soln. (10 ml), dried ($MgSO_4$), and concentrated and the residue purified by gel-filtration HPLC (AcOEt): **12** (171 mg, 76%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.18–1.20 (*m*, 2 *Me*$_2$CH); 1.44, 1.48 (2*s*, 1 Me–C(5)); 1.91, 1.94 (2*s*, 1 Me–C(5)); 2.14–2.45 (*m*, 4 H–C(2′)); 2.64 (*t*, *J* = 6.1, $POCH_2CH_2CN$); 3.27–3.78 (*m*, 4 H–C(5′), 2 Me_2CH, $POCH_2CH_2CN$, 10 CH_2 of 3′ → 5′ linker); 3.80 (*s*, 2 MeO); 4.09–4.56 (*m*, 2 H–C(3′), 2 H–C(4′)); 6.24–6.36 (*m*, 2 H–C(1′)); 6.83–6.85 (*m*, 4 arom. H); 7.18–7.37 (*m*, 9 H of $(MeO)_2Tr$); 7.38–7.47 (*m*, 2 H–C(6)); 7.58 (br., 2 NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.89; 11.95; 12.66; 19.34; 20.48; 20.52; 21.57; 24.50; 24.55; 24.59; 24.66; 24.69; 24.71;

24.74; 37.70; 37.85; 39.42; 43.34; 43.44; 46.76; 47.44; 47.67; 47.95; 48.05; 48.18; 48.23; 48.50; 48.70; 55.36; 57.92; 58.02; 58.17; 63.84; 64.10; 66.52; 68.22; 68.30; 68.41; 69.88; 69.85; 70.17; 70.44; 70.59; 70.69; 70.78; 71.14; 71.17; 80.87; 83.94; 84.06; 84.57; 84.72; 84.76; 84.82; 87.14; 111.05; 111.12; 111.50; 111.54; 113.40; 115.38; 117.67; 117.82; 117.89; 127.32; 128.12; 128.21; 130.16; 135.35; 135.48; 135.54; 136.11; 144.32; 150.51; 150.55; 150.58; 150.61; 158.87; 163.93; 164.01. ^{31}P-NMR ($CDCl_3$, 203 MHz): 149.33; 149.37. ESI-MS: 1401.5037 ($[M+H]^+$, $C_{61}H_{78}F_6N_7NaOP_{16}^+$; calc. 1401.5439).

*5′-O-[3-(Anthracen-9-ylmethyl)-13-(4-methoxyphenyl)-13,13-diphenyl-6,9-dioxa-3,12-diazatridec-1-yl]-3′-O-[(*tert*-butyl)dimethylsilyl]thymidine* (**13**). To a soln. of **7** (4.1 g, 5.0 mmol) in *N*,*N*-dimethylformamide (50 ml) were added 9-(chloromethyl)anthracene (1.1 g, 5.0 mmol), Na_2CO_3 (11 g, 101 mmol), and KI (167 mg, 1.0 mmol). The mixture was stirred at r.t. for 2 h. The soln. was diluted with AcOEt (200 ml), washed with brine (3 × 200 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (NH-silica gel, hexane/$CHCl_3$ 1:1): 13 (3.2 g, 64%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.04, 0.07 (2*s*, Me_2Si); 0.87 (*s*, ^{t}BuSi); 1.53 (*s*, Me–C(5)); 1.67–1.80 (*m*, 1 H–C(2′)); 2.02–2.08 (*m*, 1 H–C(2′)); 2.38–2.40 (*m*, 2 H, CH_2N); 2.81–2.95 (*m*, 4 H, CH_2N); 3.35–3.67 (*m*, 15 H, 2 H–C(5′), 5 CH_2O, MeO); 3.84 (*s*, H–C(4′)); 4.20 (*s*, H–C(3′)); 4.63 (*s*, NCH_2-anth); 6.19 (*t*, *J* = 6.3, H–C(1′)); 6.76–6.80 (*m*, 2 arom. H); 7.18–7.57 (*m*, 17 H, H–C(6), arom. H); 8.01 (*d*, *J* = 6.1, 2 arom. H); 8.40 (*s*, 1 arom. H); 8.53 (*d*, *J* = 8.8, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.75; −4.59; 12.49; 18.05; 25.83; 41.11; 43.16; 52.18; 53.54; 54.28; 55.26; 70.24; 70.30; 70.47; 70.57; 71.50; 72.63; 85.17; 86.54; 110.49; 113.17; 125.00; 125.10; 125.79; 126.26; 127.75; 127.86; 128.71; 129.18; 129.95; 130.11; 131.46; 131.54; 135.77; 138.37; 146.49; 157.93. ESI-MS: 993.3506 ($[M+H]^+$, $C_{59}H_{73}N_4O_8Si^+$; calc. 993.5198).

*5′-O-{2-{[2-[2-(2-Aminoethoxy)ethoxy]ethyl](anthracen-9-ylmethyl)amino}ethyl]-3′-O-[(*tert*-butyl)dimethylsilyl]thymidine* (**14**). As described for **8**, with **13** (1.0 g, 1.0 mmol) and 3% CCl_3COOH/CH_2Cl_2 (34 ml) for 15 min. Workup with $CHCl_3$ (30 ml) and sat. aq. $NaHCO_3$ soln. (4 × 50 ml) and CC (NH-silica gel, $CHCl_3$) gave **14** (678 mg, 93%). ^{1}H-NMR (500 MHz, $CDCl_3$): −0.01 to 0.01 (2*s*, Me_2Si); 0.83 (*s*, ^{t}BuSi); 1.53 (*s*, Me–C(5)); 1.68–1.79 (*m*, 1 H–C(2′)); 1.98–2.02 (*m*, 1 H–C(2′)); 2.78–2.90 (*m*, 6 H, CH_2N); 3.34–3.63 (*m*, 12 H, 2 H–C(5′), CH_2O); 3.81–3.82 (*m*, H–C(4′), *J* = 2.7 Hz); 4.08 (br., NH_2); 4.19–4.21 (*m*, H–C(3′)); 4.61 (*s*, NCH_2-anth); 6.18 (*t*, *J* = 6.3, H–C(1′)); 7.21 (*s*, H–C(6)); 7.40–7.53 (*m*, 4 arom. H); 7.98 (*m*, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.41–8.49 (*m*, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.76; −4.59; 12.53; 18.05; 25.82; 41.12; 41.79; 52.20; 53.67; 54.25; 70.21; 70.36; 70.41; 70.46; 70.60; 72.59; 73.51; 85.19; 86.52; 110.50; 124.99; 125.08; 125.79; 127.77; 129.19; 130.07; 131.45; 131.53; 135.76; 150.33; 163.81. ESI-MS: 721.2548 ($[M+H]^+$, $C_{39}H_{57}N_4O_7Si^+$; calc. 721.3997).

*5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[12-(anthracen-9-ylmethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′→5′)-3′-O-[(*tert*)-butyl)dimethylsilyl]thymidine* (**15**). As described for **7**, with **14** (579 mg, 804 μmol) and **3** (397 mg, 677 μmol) in $CHCl_3$ (8 ml) and $NaBH(OAc)_3$ (431 mg, 1.6 mmol) for 5 min. Workup with AcOEt (20 ml) and sat. aq. $NaHCO_3$ soln. (2 × 20 ml) and CC (silica gel, $CHCl_3$/MeOH 98:2) gave **15** (572 mg, 55%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.001, 0.025 (2*s*, Me_2Si); 0.86 (*s*, ^{t}BuSi); 1.46 (*s*, 1 Me–C(5)); 1.56 (*s*, 1 Me–C(5)); 1.71–1.78 (*m*, 1 H–C(2′)); 2.01–2.06 (*m*, 1 H–C(2′)); 2.17–2.19 (*m*, 1 H–C(2′)); 2.42–2.47 (*m*, 1 H–C(2′)); 2.72–2.94 (*m*, 8 H, CH_2N); 3.27–3.68 (*m*, 16 H, 4 H–C(5), CH_2O); 3.80 (*s*, 2 MeO); 3.84–3.85 (*m*, 1 H–C(4′)); 4.12–4.15 (*m*, 1 H–C(3′), 1 H–C(4′)); 4.20–4.23 (*m*, 1 H–C(3′)); 4.66 (*s*, NCH_2-anth); 6.18 (*t*, *J* = 7.5, 1 H–C(1′)); 6.36 (*dd*, *J* = 8.8, 5.0, 1 H–C(1′)); 6.82–6.84 (*m*, 4 arom. H); 7.21–7.36 (*m*, 9 arom. H); 7.41–7.50 (*m*, 1 H–C(6), 4 arom. H); 7.60 (*s*, 1 H–C(6)); 7.98 (*d*, *J* = 7.8, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.51 (*d*, *J* = 8.8, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.81; −4.64; 11.86; 12.48; 17.99; 25.77; 37.85; 41.04; 49.05; 40.15; 52.10; 53.51; 54.22; 55.32; 63.95; 68.73; 70.11; 70.27; 70.36; 70.39; 70.45; 70.50; 72.50; 80.34; 84.09; 84.83; 85.20; 86.45; 86.97; 110.43; 111.28; 113.35; 124.95; 125.06; 125.74; 127.22; 127.68; 128.06; 128.20; 129.11; 130.07; 130.14; 131.38; 135.42; 135.46; 135.68; 135.79; 144.39; 150.44; 150.57; 158.79; 164.07; 164.10. ESI-MS: 1291.6973 ($[M+H]^+$, $C_{72}H_{91}N_6O_{14}Si^+$; calc. 1291.6362).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[12-(anthracen-9-ylmethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′→5′)-thymidine (**16**). As described for **10**, with **15** (492 mg, 389 μmol), THF (4 ml), and Bu_4NF (112 mg, 428 μmol) for 2.5 h. Workup and CC as described gave **16** (371 mg, 83%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.44 (*s*, 1 Me–C(5)); 1.50 (*s*, 1 Me–C(5)); 1.82–2.46 (*m*, 4 H–C(2′)); 2.71–2.97 (*m*, 8 H, CH_2N); 3.25–3.30 (*m*, 1 H–C(5′)); 3.42–3.74 (*m*, 15 H, H–C(5′), CH_2O); 3.76 (*s*, 2 MeO); 3.78–3.81 (*m*, 1 H–C(3′)); 4.11–4.12 (*m*, 1 H–C(3′)); 4.13–4.28

(*m*, 2 H–C(4′)); 4.52 (*d*, *J* = 13, 1 H, CH_2N-anth); 4.62 (*d*, *J* = 13, 1 H, CH_2N-anth); 6.25 (*t*, *J* = 7.8, 1 H–C(1′)); 6.37 (*dd*, *J* = 5.9, 8.3, 1 H–C(1′)); 6.81–6.85 (*m*, 4 arom. H); 7.23–7.50 (*m*, 14 arom. H); 7.61 (*s*, 1 arom. H); 7.97 (*d*, *J* = 8.1, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.51 (*d*, *J* = 8.8, 2 H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.89; 12.45; 37.86; 40.95; 49.01; 49.07; 51.98; 53.66; 54.06; 55.31; 63.99; 68.45; 69.75; 69.89; 70.32; 70.36; 70.56; 70.74; 71.45; 80.41; 84.07; 84.80; 85.21; 86.16; 86.96; 110.30; 111.32; 113.34; 124.93; 125.07; 127.22; 127.75; 128.06; 128.20; 129.13; 129.95; 130.14; 131.37; 131.42; 135.41; 135.45; 135.68; 135.90; 144.39; 150.56; 150.69; 158.77; 164.20; 164.23. ESI-MS: 1177.6360 ($[M+H]^+$, $C_{66}H_{77}N_6O_{14}^+$; calc. 1177.5497).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[12-(anthracen-9-ylmethyl)-3-(2,2,2-trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine (**17**). As described for **11**, with **16** (365 mg, 310 μmol), CH_2Cl_2 (3 ml), $^iPr_2$EtN (160 μl, 930 μmol), and $(CF_3CO)_2O$ (65 μl, 470 μmol) for 20 min. Workup AcOEt (15 ml) and brine (10 ml) and CC (silica gel, $CHCl_3$/MeOH 99 : 1) gave **17** (326 mg, 83%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.41–1.59 (*m*, 6 H, 2 Me–C(5)); 1.79–1.83 (*m*, 1 H–C(2′)); 2.12–2.17 (*m*, 2 H–C(2′)); 2.32–2.34 (*m*, 1 H–C(2′)); 2.77–3.73 (*m*, 24 H, CH_2N, CH_2O, H–C(5′)); 3.76 (*s*, 2 MeO); 3.91–4.02 (*m*, 2 H–C(3′)); 4.03–4.21 (*m*, 2 H–C(4′)); 4.56 (*d*, *J* = 12.9, 1 H, CH_2N-anth); 4.64 (*d*, *J* = 13.4, 1 H, CH_2N-anth); 6.24–6.34 (*m*, 2 H–C(1′)); 6.83 (*m*, 4 arom. H); 7.21–7.48 (*m*, 13 H, H–C(6′), arom. H); 7.59 (*s*, 1 H–C(6′)); 7.96 (*d*, *J* = 8.3, 2 arom. H); 8.36 (*s*, 2 arom. H); 8.57 (*d*, *J* = 9.0, 2 arom. H); 9.94 (br., 2 NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.76; 11.82; 12.34; 37.43; 37.62; 40.44; 47.92; 48.08; 48.29; 48.42; 51.81; 53.50; 53.77; 55.17; 63.59; 63.85; 66.16; 67.91; 68.14; 69.63; 69.90; 70.02; 70.30; 70.56; 70.67; 71.85; 80.43; 80.48; 83.70; 83.83; 84.51; 84.99; 85.90; 86.87; 86.89; 110.37; 111.34; 111.37; 113.21; 124.84; 124. 92; 125.65; 127.13; 127.58; 127.96; 128.02; 128.97; 129.89; 129.99; 131.20; 135.15; 135.47; 135.76; 144.18; 150.59; 150.68; 150.71; 158.63; 164.17; 164.23. ESI-MS: 1273.5637 ($[M+H]^+$; calc. 1273.5320).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[12-(anthracen-9-ylmethyl)-3-(2,2,2-trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine 3′-(2-Cyanoethyl N,N-*Diisopropylphosophoramidite)* (**18a**). As described for **12**, with **17** (150 mg, 118 μmol), CH_2Cl_2 (470 μl), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (107 mg, 354 μmol), and diisopropylammonium 1*H*-tetrazolide (3.3 mg, 47 μmol) for 3 h. Workup with AcOEt (10 ml) and sat. aq. $NaHCO_3$ soln. (10 ml) yielded crude **18a**, which was used without further purification for the next oligonucleotide synthesis because of the instability on CC purification. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.14–1.31 (*m*, 12 H, Me_2CH); 1.45–1.53 (*m*, 2 Me–C(5)); 1.73–2.30 (*m*, 4 H–C(2′); 2.52–2.58 (*m*, 2 H, CH_2N); 2.73–2.90 (*m*, 4 H–C(5′)); 3.19–3.75 (*m*, 22 H, CH_2N, CH_2O); 3.77 (*s*, 2 MeO); 3.95–4.37 (*m*, 4 H, H–C(3′), H–C(4′)); 4.64–4.65 (*m*, CH_2N-anth); 6.20–6.31 (*m*, 2 H–C(1′)); 6.83 (*m*, 4 arom. H); 7.22–7.58 (*m*, 13 arom. H); 7.98 (*m*, 2 arom. H); 8.19 (*s*, 1 arom. H); 8.50–8.53 (*m*, 2 arom. H); 9.41 (*s*, 1 H, NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.61; 11.80; 11.86; 12.31; 12.37; 14.18; 15.26; 19.83; 19.89; 20.29; 20.35; 21.02; 22.21; 22.83; 23.12; 23.46; 24.37; 24.43; 24.49; 24.51; 24.55; 24.61; 37.52; 37.71; 39.63; 39.66; 39.70; 39.74; 43.15; 43.18; 43.25; 43.28; 45.53; 45.57; 45.61; 45.65; 47.98; 48.14; 48.31; 48.51; 52.02; 53.47; 53.83; 53.90; 55.22; 57.90; 58.05; 58.09; 58.24; 58.60; 58.62; 60.37; 63.65; 63.91; 65.82; 66.26; 67.97; 68.29; 69.98; 70.02; 70.17; 70.40; 70.66; 70.71; 70.80; 74.17; 74.30; 74.43; 74.56; 80.45; 80.50; 83.74; 83.90; 84.58; 84.96; 85.03; 85.13; 85.41; 85.44; 86.95; 86.97; 110.47; 110.59; 111.29; 111.31; 113.28; 117.54; 117.69; 124.90; 125.00; 125.71; 127.18; 127.20; 127.60; 127.62; 128.00; 128.11; 129.04; 130.06; 131.31; 131.38; 135.27; 135.37; 135.43; 135.58; 144.28; 150.38; 150.45; 150.51; 150.53; 158.75; 163.91; 163.94; 163.96; 171.13. ^{31}P-NMR ($CDCl_3$, 203 MHz): 149.24; 149.57. ESI-MS: 1473.5786 ($[M+H]^+$, $C_{77}H_{93}F_3N_8O_{16}P^+$; calc. 1473.6106).

N-*{2-[2-(2-Aminoethoxy)ethoxy]ethyl}-α,α-diphenylbenzenemethanesulfenamide* (**19**). To the soln. of 2,2′-[(ethane-1,2-diylbis(oxy)]bis[ethanamine] (2.0 ml, 14 mmol) in THF/H_2O 19 : 1 (270 ml) were added Na_2CO_3 (1.4 g, 14 mmol) and *α,α*-diphenylbenzenemethanesulfenyl chloride (2.1 g, 6.8 mmol). The soln. was stirred at r.t. for 1 h and then diluted with AcOEt (500 ml). The org. layer was washed once with sat. aq. $NaHCO_3$ soln. (500 ml), dried ($MgSO_4$), and concentrated and the residue subjected to CC (silica gel, $CHCl_3$/MeOH 96 : 4): **19** (1.6 g, 55%). ^{1}H-NMR (500 MHz, $CDCl_3$): 2.66–2.70 (*m*, 2 H, CH_2N); 2.81–2.85 (*m*, 2 H, CH_2N); 3.29–3.13 (*t*, *J* = 5.4, 2 H, CH_2O); 3.46–3.49 (*m*, 4 H, CH_2O); 3.53–3.55 (*m*, 2 H, CH_2O); 7.21–7.48 (*m*, 15 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 41.58; 51.93; 69.89;

69.98; 70.05; 70.36; 70.75; 73.21; 73.24; 126.05; 126.44; 127.59; 128.49; 129.74; 144.06. ESI-MS: 423.6831 ($[M+H]^+$, $C_{25}H_{31}N_2O_2S^+$; calc. 423.2106).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-(14,14,14-triphenyl-6,9-dioxa-13-thia-3,12-diazatetradec-1-yl)thymidine (**20**). As described for **7**, with **3** (1.8 g, 3.0 mmol), $CHCl_3$ (30 ml), **19** (1.3 g, 3.0 mol), and $NaBH(OAc)_3$ (1.3 mg, 6.0 mmol) for 15 min. Workup with $CHCl_3$ (20 ml) and (1 × 20 ml) and CC (NH-silica gel, hexane/$CHCl_3$ 1:4) gave **20** (2.0 g, 66%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.46 (*s*, Me–C(5)); 2.16–2.22 (*m*, 1 H–C(2′)); 2.49–2.53 (*m*, 1 H–C(2′)); 2.61–2.81 (*m*, 6 H, CH_2N); 3.07–3.61 (*m*, 12 H, 2 H–C(5′), CH_2O); 3.75 (*s*, 2 MeO); 4.12–4.13 (*m*, H–C(4′)); 4.18–4.19 (*m*, H–C(3′)); 6.35 (*dd*, *J* = 5.6, 7.8, H–C(1′)); 6.83–7.48 (*m*, 28 H of $(MeO)_2$*Tr* and TrS); 7.61 (*s*, H–C(6)). ^{13}C-NMR (126 MHz, $CDCl_3$): 12.12; 38.14; 49.36; 49.43; 52.39; 55.52; 64.17; 69.22; 70.42; 70.51; 70.79; 70.84; 71.22; 80.49; 84.30; 85.04; 87.17; 111.43; 113.56; 126.89; 127.42; 128.04; 128.27; 128.42; 128.96; 130.21; 130.37; 135.66; 135.71; 135.86; 144.53; 144.65; 150.85; 159.00; 164.42. ESI-MS: 993.2842 ($[M+H]^+$, $C_{58}H_{65}N_4O_9S^+$; calc. 993.4472).

3′-O-[3-(Anthracen-9-ylmethyl)-14,14,14-triphenyl-6,9-dioxa-13-thia-3,12-diazatetradec-1-yl]-5′-O-[bis(4-methoxyphenyl)phenylmethyl]thymidine (**21**). As described for **13**, with **20** (2.0 g, 2.0 mmol), of *N,N*-dimethylformamide (20 ml), 9-(chloromethyl)anthracene (450 g, 2.0 mmol), Na_2CO_3 (4.2 g, 40 mmol), and KI (33 mg, 594 μmol) for 2 h. Workup with AcOEt (100 ml) and brine (3 × 100 ml) and CC as described gave **21** (1.8 g, 77%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.44 (*s*, Me–C(5)); 1.95–2.03 (*m*, 1 H–C(2′)); 2.22–2.37 (*m*, 1 H–C(2′)); 2.59–2.84 (*m*, 6 H, CH_2N); 2.95–3.54 (*m*, 12 H, 2 H–C(5′), CH_2O); 3.75 (*s*, 2 MeO); 3.88–3.91 (*m*, H–C(4′), H–C(3′)); 4.62 (*s*, NCH_2-anth); 6.22–6.26 (*dd*, *J* = 5.6, 8.3, H–C(1′)); 6.77–7.46 (*m*, 32 H of $(MeO)_2$*Tr*, TrS, and anth); 7.54 (*s*, H–C(6)); 7.94 (*m*, 2 arom. H); 8.02 (br., 1 NH(3)); 8.38 (*s*, 1 arom. H); 8.57 (*m*, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.90; 11.92; 37.89; 52.10; 52.15; 53.84; 54.23; 55.27; 55.29; 63.89; 68.52; 70.11; 70.14; 70.20; 70.61; 70.98; 79.93; 84.14; 84.83; 86.92; 111.15; 113.31; 113.32; 124.94; 125.26; 125.68; 126.26; 126.66; 127.19; 127.55; 127.82; 128.04; 128.19; 128.75; 129.01; 129.99; 130.14; 130.49; 131.39; 131.46; 135.46; 144.30; 144.43; 150.41; 158.76; 163.98. ESI-MS: 1183.5185 ($[M+H]^+$, $C_{73}H_{75}N_4O_9S^+$; calc. 1183.5254).

3′-O-{2-{[2-[2-(2-Aminoethoxy)ethoxy]ethyl](anthracen-9-ylmethyl)amino}ethyl}-5′-O-[bis(4-methoxyphenyl)phenylmethyl]thymidine (**22**). To a soln. of **21** (1.7 g, 1.4 mmol) in toluene (14 ml) was added tributylstannane (2.9 ml, 11 mmol), and the mixture was stirred at 130° for 3 h. The mixture was subjected to CC (NH-silica gel, $CHCl_3$): **22** (647 mg, 50%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.49 (*s*, Me–C(5)); 1.97–2.29 (*m*, 2 H–C(2′)); 2.77–2.86 (*m*, 6 H, CH_2N); 3.10–3.54 (*m*, 12 H, 2 H–C(5′), CH_2O); 3.72 (*s*, 2 MeO); 3.91–3.93 (*m*, H–C(4′), H–C(3′)); 4.61 (*s*, CH_2N-anth); 6.29–6.31 (*t*, *J* = 6.3, H–C(1′)); 6.77–7.46 (*m*, 17 H of $(MeO)_2$*Tr* and anth); 7.51 (*s*, H–C(6)); 7.91 (*m*, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.52 (*m*, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 13.70; 16.22; 27.03; 28.20; 37.71; 41.56; 52.02; 53.76; 54.06; 55.14; 63.80; 68.35; 70.00; 70.10; 70.18; 73.25; 79.84; 83.88; 84.70; 86.74; 113.16; 113.18; 124.80; 125.13; 125.54; 127.02; 127.41; 127.90; 128.07; 128.87; 128.97; 130.01; 130.37; 131.26; 131.32; 135.39; 144.38; 158.61. ESI-MS: 909.2227 ($[M+H]^+$, $C_{54}H_{61}N_4O_9^+$; calc. 909.4439).

*5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3-(anthracen-9-ylmethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-3′-O-[(*tert*-butyl)dimethylsilyl]thymidine* (**23**). As described for **7**, with **22** (647 mg, 713 μmol) and **5** (284 mg, 713 μmol) in $CHCl_3$ (7 ml) and $NaBH(OAc)_2$ (302 mg, 1.4 mmol) for 5 min. Workup with $CHCl_3$ (10 ml) and brine (1 × 20 ml) and CC (silica gel, $CHCl_3$/MeOH 96:4) gave **23** (393 mg, 43%). ^{1}H-NMR (500 MHz, $CDCl_3$): 0.10 (*s*, Me_2Si); 0.82 (*s*, ^{t}BuSi); 1.41 (*s*, 1 Me–C(5)); 1.84 (*s*, 1 Me–C(5)); 1.91–2.23 (*m*, 4 H–C(2′)); 2.70–2.81 (*m*, 8 H, CH_2N); 3.02–3.61 (*m*, 16 H, 4 H–C(5′), CH_2O); 3.69 (*s*, 2 MeO); 3.82–3.99 (*m*, 2 H–C(4′), 1 H–(3′)); 4.32 (*m*, 1 H–C(3′)); 4.56 (*s*, CH_2N-anth); 6.29–6.31 (*m*, 2 H–C(1′)); 6.71–7.41 (*m*, 17 H of $(MeO)_2$*Tr* and anth); 7.48 (*s*, 1 H–C(6)); 7.87 (*m*, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.47 (*m*, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): −4.83; −4.65; 11.88; 12.69; 17.94; 25.74; 37.81; 40.99; 48.65; 48.89; 52.06; 53.74; 54.22; 63.81; 68.34; 69.66; 70.01; 70.07; 70.29; 70.42; 70.55; 72.09; 79.89; 84.03; 84.70; 85.25; 86.17; 86.83; 110.76; 111.17; 113.24; 124.88; 125.18; 125.63; 127.10; 127.48; 127.97; 128.10; 128.93; 130.00; 130.06; 130.39; 131.30; 131.37; 135.38; 135.59; 135.97; 144.35; 150.55; 150.62; 158.67; 164.18. ESI-MS: 1291.6952 ($[M+H]^+$, $C_{72}H_{91}N_6O_{14}Si^+$; calc. 1291.6362).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3-(anthracen-9-ylmethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine (**24**). As described for **10**, with **23**

(609 mg, 472 μmol), THF (5 ml), and Bu_4NF (197 mg, 755 μmol) for 8 h. Workup and CC as described gave **24** (419 mg, 75%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.45 (*s*, 1 Me–C(5)); 1.88 (*s*, 1 Me–C(5)); 1.96–2.32 (*m*, 4 H–C(2′)); 2.72–2.85 (*m*, 8 H, CH_2N); 3.07–3.66 (*m*, 16 H, 4 H–C(5′), CH_2O); 3.74 (*s*, 2 MeO); 3.86–3.98 (*m*, 2 H–C(4′), 1 H–C(3′)); 4.44 (*m*, 1 H–C(3′)); 4.61 (*s*, CH_2N anth); 6.26–6.30 (*m*, 2 H–C(1′)); 6.77–7.46 (*m*, 17 H of $(MeO)_2$*Tr* and anth); 7.54 (*s*, H–C(6)); 7.92 (*d*, *J* = 8.3, 2 arom. H); 8.36 (*s*, 1 arom. H); 8.48 (*d*, *J* = 8.8, 2 arom. H). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.93; 12.77; 37.84; 40.52; 48.67; 48.88; 52.11; 53.81; 54.29; 55.29; 63.88; 68.42; 69.98; 70.08; 70.12; 70.24; 70.40; 70.53; 71.20; 79.98; 84.09; 84.76; 84.94; 85.39; 86.90; 110.78; 111.26; 113.28; 113.29; 1224.95; 125.23; 125.70; 127.16; 127.54; 128.03; 128.15; 128.98; 130.11; 130.43; 131.34; 131.42; 135.43; 135.67; 135.97; 144.40; 150.68; 150.74; 158.71; 164.28; 164.34. ESI-MS: 1177.4326 ($[M+H]^+$, $C_{66}H_{77}N_6O_{14}^+$; calc. 1177.5497).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3-(anthracen-9-ylmethyl)-12-(trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine (**25**). As described for **11**, with **24** (406 mg, 345 μmol), CH_2Cl_2 (4 ml), ^{i}Pr$_2$EtN (177 μl, 1.0 mmol), and $(CF_3CO)_2O$ (72 μl, 517 μmol) for 30 min. Workup with AcOEt (20 ml) and brine (20 ml) and CC (silica gel, $CHCl_3$/MeOH 99 : 1) gave **25** (350 mg, 80%). ^{1}H-NMR (500 MHz, $CDCl_3$): 1.46 (*s*, 1 Me–C(5)); 1.88 (*s*, 1 Me–C(5)); 2.00–2.31 (*m*, 4 H–C(2′)); 2.77–2.84 (*m*, 4 H, CH_2N); 3.08–3.72 (*m*, 20 H, CH_2N, CH_2O); 3.74 (*s*, 2 MeO); 3.77–3.98 (*m*, 2 H–C(4′), 1 H–C(3′)); 4.29–4.34 (*m*, 1 H–C(3′)); 4.61 (*s*, CH_2N-anth); 6.23–6.30 (*m*, 2 H–C(1′)); 6.77–7.47 (*m*, 17 H of $(MeO)_2$*Tr* and anth); 7.56 (*s*, H–C(6)); 7.93 (*d*, *J* = 8.8, 2 arom. H); 8.38 (*s*, 1 arom. H); 8.57 (*d*, *J* = 8.3, 2 arom. H); 9.60 (*s*, 2 NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.91; 12.69; 12.73; 37.86; 40.17; 40.33; 47.31; 47.70; 48.14; 52.06; 53.90; 54.14; 54.22; 55.30; 63.91; 67.96; 68.36; 69.83; 70.01; 70.09; 70.28; 70.49; 70.67; 70.95; 71.22; 71.42; 80.12; 84.16; 84.81; 85.00; 85.15; 85.25; 85.47; 86.95; 110.96; 111.35; 111.37; 113.30; 113.31; 125.00; 125.18; 125.37; 125.75; 127.20; 127.61; 128.05; 128.16; 128.29; 129.04; 129.10; 130.13; 130.33; 131.36; 131.43; 135.40; 135.74; 136.00; 144.38; 150.64; 150.67; 150.70; 150.72; 158.74; 164.05; 164.12; 164.17; 164.23; 164.28. ESI-MS: 1273.5462 ($[M+H]^+$, $C_{68}H_{76}F_3N_6O_{15}^+$; calc. 1273.5320).

5′-O-[Bis(4-methoxyphenyl)phenylmethyl]-3′-O-dephosphinicothymidylyl[3-(anthracen-9-ylmethyl)-12-(trifluoroacetyl)-6,9-dioxa-3,12-diazatetradecane-1,14-diyl]-(3′ → 5′)-thymidine 3′-(2-Cyanoethyl N,N-*Diisopropylphosphoramidite)* (**18b**). As decribed for **12**, with **25** (150 mg, 118 μmol), CH_2Cl_2 (470 μl), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (85 mg, 282 μmol), and diisopropylammonium 1*H*-tetrazolide (3.3 mg, 47 μmol) for 4 h. Workup with AcOEt (10 ml) and sat. aq. $NaHCO_3$ soln. (10 ml) yielded crude **18b**, which was used without further purification for the next oligonucleotide synthesis because of the instability on CC purification. ^{1}H-NMR (500 MHz, $CDCl_3$): 1.15–1.31 (*m*, 12 H, Me_2CH); 1.46–1.91 (*m*, 2 Me–C(5)); 1.97–2.42 (*m*, 4 H–C(2′)); 2.58–2.62 (*m*, 2 H, CH_2N); 2.74–2.86 (*m*, 4 H–C(5′)); 3.08–3.84 (*m*, 22 H, CH_2N, CH_2O); 3.75 (*s*, 2 MeO); 3.88–4.49 (*m*, 4 H, H–C(3′), H–C(4′)); 4.63 (*s*, CH_2N-anth); 6.23–6.33 (*m*, 2 H–C(1′)); 6.77–8.53 (*m*, 24 H, 2 H–C(6), 22 arom. H); 9.13 (*s*, NH(3)). ^{13}C-NMR (126 MHz, $CDCl_3$): 11.98; 12.73; 2048; 20.53; 21.14; 22.32; 22.93; 22.96; 23.06; 23.22; 23.56; 24.48; 24.53; 24.57; 24.64; 24.67; 24.70; 24.73; 37.86; 39.38; 43.31; 43.29; 43.39; 45.62;45.66; 47.34; 47.56; 47.80; 47.92; 47.99; 52.16; 53.91; 54.30; 55.32; 57.97; 58.03; 58.13; 58.18; 63.91; 67.81; 68.04; 68.35; 68.48; 69.41; 69.59; 70.10; 70.21; 70.26; 70.51; 70.72; 70.91; 71.04; 73.28; 73.41; 73.82; 73.98; 80.03; 80.06; 84.15; 84.81; 85.08; 85.11; 85.19; 85.24; 85.27; 86.94; 111.05; 111.13; 111.16; 111.22; 111.25; 113.31; 113.32; 116.03; 117.77; 117.83; 124.99; 125.23; 124.72; 127.20; 127.59; 128.05; 128.19; 129.04; 130.14; 130.41; 130.44; 131.38; 131.46; 135.45; 135.62; 135.70; 135.72; 135.99; 136.03; 144.42; 150.42; 150.49; 150.54; 150.57; 158.77; 163.85; 163.94; 163.96. ^{31}P-NMR ($CDCl_3$, 203 MHz): 149.60; 149.78. ESI-MS: 1473.5905 ($[M+H]^+$, $C_{77}H_{92}F_3N_8O_{16}P^+$; calc. 1473.6399).

Oligonucleotide Synthesis. Phosphoramidites **12**, **18a**, and **18b** and the commercially available deoxynucleoside phosphoroamidites were used. The 3′-half of each DNA, d(Y^1Y^2 TCGAG), was synthesized on a CPG support by use of an *ABI-392* DNA synthesizer. After the $(MeO)_2$Tr group at the 5′-terminal nucleotide (Y^1) was removed, the CPG was transferred to a glass syringe. The appropriate phosphoramidite, **12**, **18a**, or **18b**, was coupled to the free 5′-OH group according to the following manual coupling procedure: *1*) Coupling: 20 equiv. of phosphoramidite, 80 equiv. of 1*H*-tetrazole, MeCN (250 μl), 5 min. *2*) Capping: 1.1M Ac_2O, 133 mM *N,N*-dimethylpyridin-4-amine in pyridine, 2 min. *3*) Oxidation: 0.1M I_2 in pyridine/H_2O 9 : 1. Subsequently, the CPG supports were transferred to the column for the DNA synthesizer, and the 5′-half parts of each DNA, d(GCTCX^{1}X^2), were elongated on the

synthesizer. After the cleavage from the solid supports, the protecting groups at the nucleobases and phosphates and the CF_3CO groups at the amino groups were removed by treatment with aq. ammonia overnight. The desired oligonucleotides were purified by anion-exchange HPLC (*Genpak Fax*, 10 mM Na_3PO_4 applying of the gradient 1.0M NaCl → 10 mM Na_3PO_4 in 1%/min). The structures of the oligonucleotides were confirmed by the MALDI-TOF-MS.

5′-d(GCTCAG[tt]GATCGAG)-3′:(ODN**1**) yield 16%. MALDI-TOF-MS: 4750.6 (calc. 4745.0).

5′-d(GCTCAG[tt]GATCGAG)-3′*:(ODN**2a**) yield 12%. MALDI-TOF-MS: 4932.0 (calc. 4933.1).

*5′-d(GCTCAG [t*t] GATCGAG)-3′*:(ODN**2b**) yield 11%. MALDI-TOF-MS: 4931.6 (calc. 4933.1).

5′-d(GCTCCT[tt]AGTCGAG)-3′*:(ODN**3a**) yield 9%, MALDI-TOF-MS: 4882.8 (calc. 4884.1).

*5′-d(GCTCCT[t*t]AGTCGAG)-3′*:(ODN**3b**) yield 19%. MALDI-TOF-MS: 4883.0 (calc. 4884.1).

Fluorescence Spectra. For the natural-type ODNs, the ε_{260} were calculated according to [47]. The ε_{260} of an ODN incorporating a long-chain linker without the anthracene moiety was assumed to be identical to that of the natural-type ODN having the same nucleotide sequences. In the case of an ODN having an anthracene moiety, the ε_{260} was estimated as the sum of ε_{260} of the ODN and 5500. ODN**1**: 145700; ODN**2a**: 151200; ODN**2b**: 151200; ODN**3a**: 143800; ODN**3b**: 143800. The sample ODNs were dissolved in 0.1M *Tris* · HCl, 0.1M NaCl. ODN concentrations are described below. The directly observed fluorescent intensities were divided by these concentrations to give the fluorescent intensities of the hypothetical 1M solns. and used to draw *Figs. 3* and *4*.

Experiment shown in *Fig. 3:* Single strand ODN**2a**, 104 nM; ODN**2b**, 102 nM; ODN**3a**, 140 nM; ODN**3b**, 140 nM. Double strand ODN**2a** · ODN**4**, 125 nM; ODN**2b** · ODN**4**: 120 nM; ODN**3a** · ODN**5**, 121 nM; ODN**3b** · ODN**5**, 120 nM. The measurements were carried out at 10°.

Experiment shown in *Fig. 4:* ODN**3a** · ODN**5**, 120 nM; ODN**3a** · ODN**6**, 130 nM; ODN**3a** · ODN**7**, 130 nM; ODN**3a** · ODN**8**, 130 nM; ODN**3a** · ODN**9**, 80 nM. The measurements were carried out at pH 8.0.

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