Synthesis and Duplex Stabilization of Oligonucleotides Consisting of Isonucleosides

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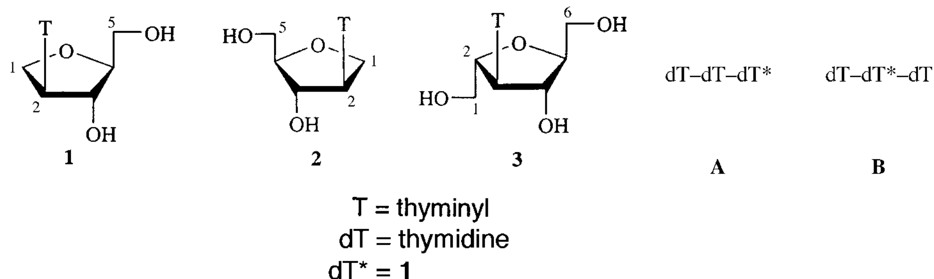
Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

Novel oligonucleotide analogues built from isonucleosides were synthesized by the phosphoramidite approach on an automated DNA synthesizer. The phosphoramidite building blocks were synthesized by phosphitylation of the corresponding protected isonucleosides. The oligonucleotide analogues **C**–**G** containing the isonucleoside **1**–**3** were studied with respect to their hybridization properties and enzymatic stability. The oligomers bearing an isonucleoside at the end of the strands all proved stable towards snake-venom phosphodiesterase, but only the oligomers **D**–**G** exhibit acceptable duplex stability when hybridized with complementary $d(A_{14})$.

Introduction. – One of the efforts to enhance the biological activity of oligonucleotides as inhibitors of gene expression has been made by improvement of their stability to nuclease digestion. Antisense oligonucleotides with phosphorothioate backbones exhibit several advantages over other forms, including relatively high nuclease resistance as well as the ability to induce the degradation of the target sequence by RNase H [1] [2]. However, phosphorothioate oligonucleotides are possibly hydrolyzed, primarily from the 3′-end and have also been shown to block the proliferation of HIV-1 in acutely infected cells in a non-sequence-specific manner [3]. Another problem in the use of such modified antisense oligonucleotides is their inefficient cellular uptake. Several techniques for the stabilization of the phosphodiester bonds of the oligonucleotides have been proposed, such as the incorporation of various chemical substituents at the 3′- and 5′-OH groups. Another approach to solve these problems has been the development of altered sugar moieties along the oligonucleotide chain [4–6]. Most of these modifications contain a five-membered sugar ring closely resembling the natural 2-deoxyribose [7–10]. Oligonucleotides consisting of hexose nucleoside analogues were reported to possess significantly increased stability towards phosphodiesterases whereby the hybridization properties are retained [11]. Recently, an 'inverse oligonucleotide' was introduced, where the backbone of the oligonucleotide consists of a phosphorylated cyclopentanediol moiety, and the heterocyclic base is bound *via* a flexible ethylene linkage [12].

Isonucleosides represent a new class of nucleoside analogues in which the nucleobase is linked to various positions of ribose other than C(1′). The torsion angles in the sugar-phosphate backbones of such oligonucleotides exhibit profound changes compared to regular oligonucleotides. These alternations in torsion angles might affect the recognition of such oligomers by nucleases. It could also be anticipated that the bases in the modified oligonucleotide retain their hybridization properties with

complementary sequences. This prompted us to study the hybridization properties and enzymatic stability of oligonucleotides bearing such isonucleosides. In this paper, we report on the synthesis of oligonucleotides consisting of 1,4-anhydro-2-deoxy-2-(thymin-1-yl)-L-arabinitol (**1**), 1,4-anhydro-2-deoxy-2-(thymin-1-yl)-D-arabinitol (**2**) and 2,5-anhydro-3-deoxy-3-(thymin-1-yl)-L-mannitol (**3**). Oligonucleotides bearing the isonucleosides **1**, **2**, or **3** show increased stability towards snake-venom phosphodiesterase (SVPDE), but only oligonucleotides built up from the isonucleoside **2** and **3** retained their hybridization properties.



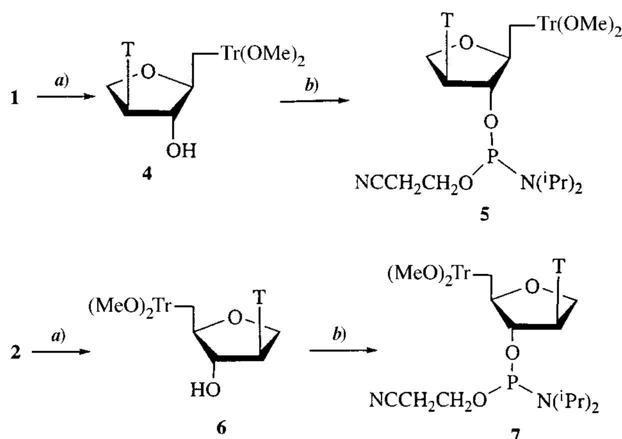
T = thyminyl
dT = thymidine
dT* = **1**

Results and Discussion. – *Monomers.* The anhydro-L-arabinitol derivative **1** ($[\alpha]_D^{20} = +17.2$ ($c = 0.702$, MeOH)) was synthesized from D-xylose [13]. We also reported on the synthesis of two trinucleotides **A** and **B** carrying compound **1** at the 3′-terminus or in the center, respectively [14][15]. It was found that **A** and **B** are both stable towards nuclease S1 in contrast to the unmodified trinucleotide. To shed some more light on the hybridization properties and the conformational requirement for duplex formation, oligonucleotides built up from structurally different isonucleosides were investigated. For this purpose, the enantiomeric anhydro-D-arabinitol **2** ($[\alpha]_D^{20} = -16.7$ ($c = 0.647$, MeOH) was synthesized from L-xylose on an analogous synthetic route [16][17]. For the synthesis of oligonucleotides consisting of the isonucleosides **1** and **2**, the latter were transformed into the building blocks **5** and **7** by dimethoxyltritylation ($\rightarrow$ **4** and **6**, respectively) and subsequent phosphitylation with 2-cyanoethyl diisopropylphosphoramidochloridite by the standard protocol (*Scheme 1*). Compounds **6** and **7** have been synthesized before [19][1]).

To understand the relationship between the structure and hybridization properties of an oligonucleotide prepared from the different isonucleosides, we synthesized 2,5-anhydro-3-deoxy-3-(thymin-1-yl)-L-mannitol (**3**) [18] which has an additional OH group at the sugar ring as compared to the isonucleoside **1**. This additional OH group may exert some influence on the hybridization properties of corresponding oligonucleotides. For the synthesis of the corresponding building block **12**, 2,5-anhydro-4-deoxy-4-(thymin-1-yl)-L-mannofuranose dimethyl acetal (**8**) [18] was used as starting material (*Scheme 2*). Compound **8** was protected selectively by a benzoyl group at OH–C(6) to give compound **9**. The latter was then converted to **10** by treatment with

[1]) In previous communications, compounds **1** and **2** were named 3-(*R*)-(thymin-1-yl)-4-(*S*)-hydroxy-5-(*R*)-hydroxymethylene-tetrahydrofuran (**1**) and 3-(*S*)-(thymin-1-yl)-4-(*R*)-hydroxy-5-(*S*)-hydroxymethylene-tetrahydrofuran (**2**).

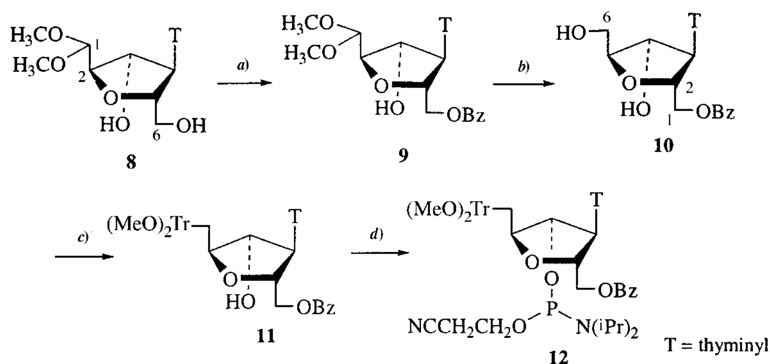
Scheme 1



T = thyminyl

a) $(MeO)_2TrCl$/pyridine ($(MeO)_2Tr$ = 4,4′-dimethoxytrityl). *b*) Cl–P(OCH_2CH_2CN)[N($^iPr)_2$]/($^iPr)_2$EtN.

Scheme 2



a) BzCl/pyridine. *b*) 1. 1% HCl in THF/H_2O 1:1; 2. $NaBH_4$. *c*) $(MeO)_2TrCl$/pyridine ($(MeO)_2Tr$ = 4,4′-dimethoxytrityl). *d*) Cl–P(OCH_2CH_2CN)[N($^iPr)_2$]/($^iPr)_2$EtN.

1% HCl in THF/H_2O 1:1 and $NaBH_4$ (yield 97%). The phosphoramidite **12** was obtained by the same procedure as described for **5** and **7** (see above).

Oligonucleotides. The solid-phase synthesis of oligonucleotides was carried out on an automated DNA synthesizer (*Perkin Elmer*, model *381 A*) using a standard *Perkin Elmer* cycle (1-μmol scale). The tetradecamers **C**–**E** were synthesized with an average coupling yield of 95% for building block **5**, 90% for building block **7**, and 82% for building block **12** (*Table 1*). Oligonucleotides **F** and **G** carrying the isonucleosides **1** and **3** at a different position were also synthesized. The oligomers were purified by HPLC (*Zorbax*® ion-exchange column) and desalted (*Sephadex-G-15* column). The compo-

Table 1. *Yields of Novel Oligonucleotides* **C**–**G**, *Determined by Dimethoxytrityl Cation Photometry*

	Oligomer	Total yield [%]	Yield of isonucleoside incorporation [%]
C	5′-d[(**1**)$_{13}$-T]-3′	69.4	95.2
D	5′-d[(**2**)$_{13}$-T]-3′	54.6	90.2
E	5′-d[(**3**)$_{13}$-T]-3′	6.8	82.5
F	5′-d[**1**-(T)$_{13}$]-3′	54.7	90.2
G	5′-d[**3**-(T)$_{13}$]-3′	44.3	50.5

Table 2. *HPLC*[a]) *and MALDI-TOF-MS Data of Oligonucleotides* **C**–**G** (see *Table 1*)

		C	**D**	**E**	**F**	**G**
HPLC:	retention time [min]	58.6	58.4	51.5	62.6	59.7
	peak area [%]	72.5	72.0	8.5	62.9	43.3
MALDI-TOF-MS:	*m/z* ion	4215.5 $[M+Na-H]^+$	4194.4 M^+	4584.5 M^+	4193.5 $[M-H]^+$	4246.4 $[M+Na-H]^+$

[a]) Eluting conditions of HPLC: buffer *A*, 0.02M NaH_2PO_4 + 20% MeCN (*v*/*v*); buffer *B*, 0.66M NaCl in buffer *A*; gradient elution from 35% *B* to 90% *B*, flow rate 1.0 ml/min.

sition of the oligomers was verified by matrix-assisted laser-desorption mass spectrometry (MALDI-TOF-MS) (*Table 2*).

The hybridization of the novel oligonucleotide analogues **C**–**G** towards their complementary strand d(A_{14}) was determined by measuring the UV melting profiles from which the corresponding T_m values were calculated by means of the SWIFT T_m software. The results are shown in the *Figure* as well as in *Table 3*.

Table 3. *Melting Temperatures* T_m *of Duplexes*[a])

	T_m [°]	ΔT_m [°][b])
d(A_{14})·d(T_{14})	38.2	–
d(A_{14})·5′-d[(**1**)$_{13}$-T]-3′ (=d(A_{14})·**C**)	< 20	–
d(A_{14})·5′-d[(**2**)$_{13}$-T]-3′ (=d(A_{14})·**D**)	32.3	– 5.9
d(A_{14})·5′-d[(**3**)$_{13}$-T]-3′ (=d(A_{14})·**E**)	33.5	– 4.7
d(A_{14})·5′-d[**1**-(T)$_{13}$]-3′ (=d(A_{14})·**F**)	36.3	– 1.9
d(A_{14})·5′-d[**3**-(T)$_{13}$]-3′ (=d(A_{14})·**G**)	36.9	– 1.3

[a]) 0.14M NaCl, 0.01M Na_2HPO_4, pH 7.2, 1.0 mM EDTA. Oligonucleotide single-strand conc., 2 μM). [b]) ΔT_m relative to d(A_{14})·d(T_{14}).

Wenzel and *Nair* [19] reported the synthesis of isonucleoside **2** and found that the oligodeoxynucleotides carrying this compound exhibit high resistance towards exonucleases. They used the isonucleoside **2** (isodT) and its analogue isodA to construct a self-complementary oligomer, on which it was shown that even replacement of the innermost regular nucleosides by both isodT and isodA did not impair a regular base pairing [19]. We synthesized oligomer **D** build up completely from the isonucleoside **2** (except the dT residue at the 3′-end) and investigated its duplex formation with d(A_{14}). It was found that oligomer **D** could form a duplex with the complementary d(A_{14}); the T_m value showed a slight decrease compared with that of the parent duplex d(A_{14})·d(T_{14}).

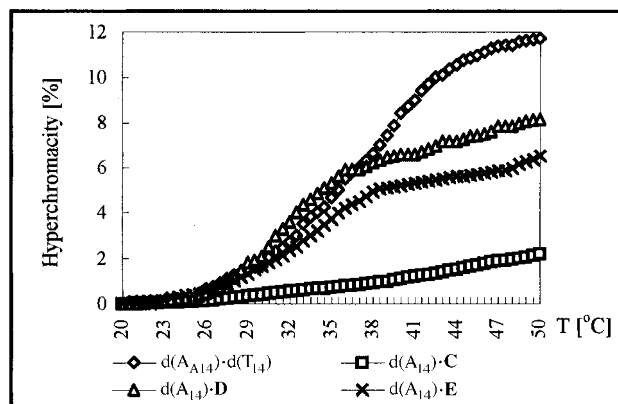


Figure. *Melting curves of the oligonucleotides* $d(T_{14})$, **C**, **D**, *and* **E**, *hybridized with* $d(A_{14})$

We have recently reported the profound changes of torsion angles in the sugar-phosphate backbones of the trinucleotides **A** and **B** [14] [15]. In the case of oligomer **D**, the same torsion angles in each nucleotide unit make the bases to array in one direction, and therefore, it is possible to form much stronger *Watson-Crick* base pairs. This conclusion is supported by the finding that the torsion angles in each nucleotide within the oligomer **C** are opposite to those in the oligomer **D**. Therefore, it is not surprising that no cooperative melting was observed for a mixture of the oligomer **C** with $d(A_{14})$. Interestingly, oligomer **E** could form a stable duplex with $d(A_{14})$, the T_m value being even higher than that of the duplex $d(A_{14}) \cdot$ **D**.

Recently, *Beier et al.* reported on the synthesis of all four members of the family of pentopyranosyl-(2′-4′) oligonucleotides and found generally stronger *Watson-Crick* base pairing [20]. They reported that the extraordinary pairing strength must be a consequence of the extensive steric constraint exerted on the phosphodiester group when flanked at the 2′-junction not only by the nucleobase, but also by the 3′-OH group in an equatorial position. This constraint may preorganize the single strands towards an optimal conformation for a pairing with the opposite strand [20]. In this context, it is interesting to see that the additional hydroxymethyl group in the building blocks **3** from which the oligomer **E** is formed leads obviously to a common type of duplex structure. Comparing the T_m values of duplexes $d(A_{14}) \cdot$ **F** and $d(A_{14}) \cdot$ **G** shows clearly that the higher stability of the latter is due to the additional hydroxymethyl group in the building block **3**.

Finally, the enzymatic hydrolysis of the oligomers **C** – **G** and $d(T_{14})$ was studied. The results show a significant resistance of the phosphodiester bonds of **C** – **G** towards snake-venom phosphodiesterase while $d(T_{14})$ was completely hydrolyzed under the same conditions (see *Exper. Part*).

We thank Prof. *J. R. Peng* for the MALDI-TOF mass spectra. Financial support by the *National Natural Science Foundation of China* is gratefully acknowledged.

Experimental Part

General. All solvents were dried and distilled prior to use. Evaporations were carried out under reduced pressure with a bath temp. 45°. Solid-phase synthesis of oligonucleotides was carried out on a DNA synthesizer (*Perkin-Elmer 381 A*) using phosphoramidite chemistry. TLC: silica gel *GF-254* (*Qing-Dao Chemical Company*, China); detection by UV. Column chromatography (CC): silica gel (200–300 mesh; *Qing-Dao Chemical Company*, China). HPLC: *Waters Delta Prep 3000*, *Zorbax*® ion-exchange oligo column, semi-prep. 9.4 × 250 mm. Optical rotations: Perkin-Elmer-243 B polarimeter. UV Spectra: *Pharmacia-LKB-Biochrom-4060* spectrophotometer. NMR Spectra: *Varian-VXR-300* or *Bruker-DPX-400* instruments with $SiMe_4$ as internal standard: δ in ppm, *J* in Hz. MS: *ZAB-HS*, *KYKY-ZHP-5*, and *LDI1700* instruments (*Linear Scientific Inc.*), MALDI-TOF with the latter; *m/z* (% rel. int.).

*1,4-Anhydro-2-deoxy-5-*O*-(dimethoxytrityl)-2-(thymin-1-yl)-*L*-arabinitol* (**4**). To a soln. of **1** (0.30 g, 1.18 mmol) in pyridine (35 ml) at 0°, dimethoxytrityl chloride (0.45 g) was added. The soln. was stirred for 26 h at r.t. After evaporation, the mixture was purified by CC (silica gel, petroleum ether/$CHCl_3$/AcOEt 8:1:1 → 0:1:1), with 0.5% Et_3N added): 61% of **4**. Colorless foam. ^{1}H-NMR ((D_6)DMSO): 1.62 (*s*, Me–C(5′)); 2.43 (*dd*, *J* = 5.6, 11.2, 1 H); 3.14 (*dd*, *J* = 4.8, 8.4, 1 H); 3.18 (*dd*, *J* = 2.4, 8.4); 3.74 (*s*, (*Me*O)$_2$Tr); 3.78 (*ddd*, *J* = 2.0, 4.8, 7.2, 1 H); 3.96 (*dd*, *J* = 3.6, 7.8, 1 H); 4.15 (*t*, *J* = 4.4, 1 H); 4.80 (*dt*, *J* = 3.6, 10.4, 1 H); 5.63 (*s*, OH); 6.89 (*m*, 4 H); 7.21–7.45 (*m*, 9 H); 7.23 (*s*, H–C(6′)); 11.22 (*s*, H–N(3′)). FAB-MS: 545 ([*M* + H]$^+$). HR-MS: 544.2209 ($C_{31}H_{32}N_2O_7^+$; calc. 544.2210).

*1,4-Anhydro-2-deoxy-5-*O*-(dimethoxytrityl)-2-(thymin-1-yl)-*L*-arabinitol 3-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5**). Compound **4** (0.20 g, 0.37 mmol) was dried by repeated co-evaporation with MeCN (3 × 2 ml) and dissolved in anh. THF (2.5 ml) under Ar. To this soln. (^{i}Pr)$_2$EtN (0.66 ml) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.14 ml) were added, and the mixture was stirred at 0° for 10 min. Then, stirring was continued for 30 min at r.t. The mixture was cooled to 0° and quenched by dropwise addition of MeOH (0.96 ml). After stirring for 10 min, O_2-free AcOEt (18 ml) was added and the org. soln. washed with 5% aq. $NaHCO_3$ soln. (2 × 5.0 ml) and H_2O (5.0 ml), dried (Na_2SO_4), and evaporated to an oily residue. Purification was carried out by CC (silica gel, cyclohexane/$CHCl_3$/AcOEt 5:1:1 → 1:1:1, with 0.3% Et_3N) added: 79% of **5**. Colorless foam. ^{1}H-NMR ((D_6)DMSO): 0.81 (*d*, *J* = 8.8, 3 H); 0.96 (*d*, *J* = 8.8, 3 H); 1.01 (*d*, *J* = 9.6, 3 H); 1.06 (*d*, *J* = 9.6, 3 H); 1.56 (*s*, Me–C(5′)); 2.40 (*m*, 3 H); 2.60 (*m*, 3 H); 3.65 (*m*, 2 H); 3.72 (*s*, 6 H); 3.90 (*m*, 1 H); 4.06 (*m*, 2 H); 4.41 (*m*, 1 H); 4.93 (*m*, 1 H); 6.87 (*d*, 4 H); 7.28 (*m*, 6 H); 7.23 (*s*, H–C(6′)); 7.37 (*t*, 2 H); 7.49 (*d*, 1 H); 11.32 (*s*, H–N(3′)). ^{31}P-NMR ((D_6)DMSO): 149.9, 150.1. FAB-MS: 745 ([*M* + H]$^+$). HR-MS: 744.3289 ($C_{40}H_{49}N_4O_8P^+$; calc. 744.3288).

*2,5-Anhydro-6-*O*-benzoyl-4-deoxy-4-(thymin-1-yl)-*L*-mannofuranose Dimethyl Acetal* (**9**). To a soln. of 2,5-anhydro-4-deoxy-4-(thymin-1-yl)-L-mannofuranose dimethyl acetal (**8**; 2.60 g, 8.23 mmol; colorless syrup) in pyridine (30 ml) at 0°, benzoyl chloride (0.94 ml, 1.14 g, 8.09 mmol) was added. The soln. was stirred at r.t. for 28 h. After evaporation, the product was isolated by CC (silica gel, petroleum ether/$CHCl_3$/AcOEt 1:1:1): 2.46 g (80%) of **9**. Colorless syrup. ^{1}H-NMR ((D_6)DMSO): 1.76 (*s*, Me–C(5)); 3.85 (*m*, 1 H); 3.96 (*m*, 1 H); 4.33 (*m*, 2 H); 4.44 (*dd*, *J* = 6.0, 12.8, H–C(2)); 4.50 (*d*, *J* = 6.0, H–C(1)); 4.79 (*m*, 1 H); 5.64 (*d*, *J* = 5.4, OH–C(3)); 7.50 (*d*, *J* = 8.0, 2 H_o (Bz)); 7.62 (*s*, H–C(6′)); 7.68 (*t*, *J* = 8.0, H_p (Bz)); 7.94 (*dd*, *J* = 8.0, 1.0, 2 H_m (Bz)); 11.33 (*s*, H–N(3′)). FAB-MS: 421 ([*M* + H]$^+$). Anal. calc. for $C_{20}H_{24}N_2O_8 \cdot$ AcOEt (420.4): C 56.69, H 6.31, N 5.51; found: C 56.84, H 6.17, N 4.95.

*2,5-Anhydro-1-*O*-benzoyl-3-deoxy-3-(thymin-1-yl)-*L*-mannitol* (**10**). To a soln. of **9** (2.5 g, 5.95 mmol) in THF (50 ml) and H_2O (50 ml), CF_3COOH (1.0 ml) was added. The soln. was refluxed for 26 h. After cooling and neutralization, $NaBH_4$ (0.50 g) was added. The mixture was stirred for 1.5 h at r.t., then cooled, and neutralized. The product was purified by CC (silica gel, $CHCl_3$/AcOEt/MeOH 10:10:0 → 10:10:1): 1.90 g (97%) of **10**. Colorless syrup. ^{1}H-NMR ((D_6)DMSO): 1.76 (*s*, Me–C(5′)); 3.53 (*dd*, *J* = 6.0, 7.5, 1 H); 3.62 (*m*, 1 H); 3.80 (*m*, 1 H); 4.28 (*m*, 2 H); 4.32 (*m*, 2 H); 4.86 (*m*, 2 H); 5.64 (*d*, *J* = 5.5, OH–C(4)); 7.51 (*d*, *J* = 8.0, 2 H_o (Bz)); 7.64 (*s*, H–C(6′)); 7.67 (*t*, *J* = 8.0, H_p (Bz)); 7.94 (*dd*, *J* = 8.0, 1.0, 2 H_m, (Bz)); 11.31 (*s*, H–N(3′)). FAB-MS: 399 ([*M* + Na]$^+$). Anal. calc. for $C_{18}H_{20}N_2O_7$ (376.4): C 57.44, H 5.36, N 7.44; found: C 57.50, H 5.48, N 7.57.

*2,5-Anhydro-1-*O*-benzoyl-3-deoxy-5-*O*-(dimethoxytrityl)-3-(thymin-1-yl)-*L*-mannitol* (**11**). As described for **4**, from **10**: 77% of **11**. Colorless foam. ^{1}H-NMR ((D_6)DMSO): 1.70 (*s*, Me–C(5′)); 3.14 (*m*, 2H); 3.70 (*s*, (*Me*O)$_2$Tr); 4.03 (*m*, 1 H); 4.27 (*dd*, *J* = 8.0, 1 H); 4.40 (*m*, 3 H); 4.82 (*m*, 1 H); 5.66 (*d*, *J* = 7.2, OH–C(4)); 6.87 (*d*, *J* = 11.6, 4 H); 7.17–7.29 (*m*, 7 H); 7.27 (*s*, H–C(6′)); 7.42 (*d*, *J* = 11.6, 2 H); 7.52 (*dd*, 2 H_m (Bz)); 7.63 (*dd*, H_p (Bz)); 7.97 (*d*, *J* = 10.4, 2 H_o (Bz)); 11.22 (*s*, H–N(3′)). FAB-MS: 679 ([*M* + H]$^+$). HR-MS: 678.2580 ($C_{39}H_{38}N_2O_9^+$; calc. 678.2577).

*2,5-Anhydro-1-*O*-benzoyl-3-deoxy-5-*O*-(dimethoxytrityl)-3-(thymin-1-yl)-*L*-mannitol 4-(2-Cyanoethyl Diisopropylphosphoramidite)* (**12**). As described for **5**, from **11**; 77% of **12**. White foam. ^{1}H-NMR ((D_6)DMSO): 0.80 (*d*, *J* = 6.6, 3 H, *Me*$_2$CH); 0.92 (*d*, *J* = 6.6, 3 H); 1.02 (*t*, *J* = 7.2, 6 H, *Me*$_2$CH); 1.19 (*m*, 2 H); 1.67 (*d*, Me–C(5′)); 2.64 (*t*, *J* = 6.0, 1 H); 3.24 (*m*, 2 H); 3.40 (*m*, 2 H); 3.61 (*q*, *J* = 6.6, 1 H); 3.72 (*s*, 6 H); 4.19 (*m*, 1 H); 4.48 (*m*, 2 H); 4.54 (*m*, 2 H); 4.97 (*m*, *J* = 8.1, 1H); 6.87 (*m*, 4 H); 7.29 (*m*, 7 H); 7.42 (*t*, 2H); 7.53 (*t*, 2 H); 7.66 (*dd*, H_p (Bz)); 7.71 (*s*, H–C(6′)); 7.98 (*dd*, 2 H); 11.35 (*s*, H–N(3′)). ^{31}P-NMR ((D_6)DMSO): 149.3, 149.5. FAB-MS: 880 ([*M* + H]$^+$). HR-MS: 878.3547 ($C_{48}H_{55}N_4O_{10}P^+$; calc. 878.3656).

Solid-Phase Synthesis of the Oligonucleotides **C**–**G**. Oligonucleotide synthesis was performed on a 1.0-μmol scale on a DNA synthesizer model **381 A** (*Perkin-Elmer*) applying regular phosphoramidite chemistry. The oligomers were purified by HPLC (*Zorbax*® ion exchanger; column 3.4 × 250 mm; elution: solvent system *A*, 0.02M NaH_2PO_4/MeCN 4 : 1; solvent system *B*, 0.66M NaCl in *A*). Desalting was performed on a *Sephadex-G-15* column. For retention times, composition, yields, and molecular masses (MALDI-TOF-MS), see *Tables 1* and *2*.

Melting Profiles of Oligonucleotides. The melting curves of the oligonucleotide duplexes were measured in 0.14M NaCl, 0.01M Na_2HPO_4, and 1 mM EDTA (pH 7.2) between 15 and 75° (temp. increase, 0.5°/min) at 260 nm; single-strand concentration, 2 μM each.

Enzymatic Oligonucleotide Hydrolysis. Oligonucleotides (*ca.* 0.4 A_{260} units; *ca.* 2 μM) in 1 ml 0.1M *Tris* · HCl (pH 8.6), 0.1M NaCl, and 14 mM $MgCl_2$ were hydrolyzed by snake-venom phosphodiesterase (0.0024 units/ml). The reaction was followed UV-spectrophotometrically at 260 nm.

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Received August 20, 1999