Nucleic Acids Research

Oligodeoxyribonucleotides containing 1,3-propanediol as nucleoside substitute

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ABSTRACT

1,3-Propanediol was protected with one dimethoxytrityl resi-
due and converted into the methoxy- and cyanoethoxyphosphor-
amidites 2a and 2b, respectively. Solid-phase oligonucleotide
synthesis, employing the phosphoramidite 2a resulted in the
dodecamers d(CGCGAATTCGCG) (6-9), in which dA or dT residues were
replaced by 1,3-propanediol. These oligomers showed a high
tendency to form hairpins. Their phosphodiester bonds between the
3'-position of a nucleoside and the propanediol moiety was not
cleaved by snake venom phosphodiesterase.

INTRODUCTION

The excision of DNA-nucleobases, in particular purines by
proton-catalyzed hydrolysis of the N-glycosylic bond is a
wellknown phenomenon. This process can also be catalyzed by
enzymes, such as uracil or 3-methyladenine glycosylase. These
enzymes are able to repair modified DNA's by excision of a
modified nucleotide to form an apurinic or apyrimidinic site in a
DNA molecule as an intermediate [1,2].

Recently Igolen [3,4] has demonstrated that incorporation of
1-cyano-2-deoxy-ß-D-erythro-pentofuranose, as a model for a
"non-base residue" into a tridecanucleotide gives the chain enough
flexibility for a B/Z transition. So a right-handed and left-
handed conformation coexist within the same DNA-molecule, induced
by a single apurinic residue by releasing the torsional stress
within a constrained duplex after removing one base-pair.

1,3-propanediol is the acyclic analogue of the 2'-deoxy-
ribofuranose moiety mimicking the C-3/C-5 carbon chain but lacking
all other sugar carbons as well as the nucleobase. This unit
represents a fragment of the DNA-backbone which is highly flexible
but can adopt the conformations of the ß-D-2'-deoxyribofuranosyl

residue. On the other hand it cannot take part in base-pairing and base-stacking, two processes which stabilize the DNA-duplex.

In the following we report on the synthesis of appropriately protected phosphoramidites of 1,3-propanediol and their use in the synthesis of oligonucleotides containing 2a as nucleoside substitute [5].

RESULTS AND DISCUSSION

Synthesis of the Phosphoramidites 2a and 2b.

In order to employ 1,3-propanediol in solid-phase synthesis of oligonucleotides the selective protection of one of its two hydroxyl-groups had to be achieved. As protecting group the 4,4'-dimethoxytrityl residue was chosen which is compatible to the chemistry employed on commercially available automated oligonucleotide synthesizers. Employing an equimolar amount of 4,4'-dimethoxytrityl chloride and 1,3-propanediol in pyridine in the presence of a twofold molar excess of Hünig's base two tritylation products were formed at room temperature as detected by TLC. The slower migrating zone contained compound 1a, the faster zone the ditritylated compound 1b. The molar ratio of the reaction products was determined after a reaction time of 2 h with a TLC-scanner and was found to be 1 : 1. This shows that the reactivity of the hydroxyl groups of propanediol is different from that of the 3', 5'-hydroxyl functions of 2'-deoxyribo-furanosides. In that case an almost regioselective monotritylation occurred at the 5'-position due to the higher reactivity of the primary 5'-hydroxyl group. Experiments, carried out under iden-tical conditions but with a 2 : 1 excess of DMT-Cl over 1,3-propanediol gave 98% 1b and only 2% 1a. However, if a five-fold excess of 1,3-propanediol over 4,4'-dimethoxytrityl chloride was used, 80% of the monotritylated 1a and only 20% of the ditri-tylated product 1b were formed. These conditions were chosen for the preparative scale experiments and compound 1a was isolated after purification and crystallization in 68% yield. TLC and elemental analysis confirmed the purity. The structure was in agreement with ^{1}H and ^{13}C NMR data.

Two different phosphoramidites were prepared from the DMT-derivative 1a. Phosphitylation with chlorodiisopropylaminomethoxy-

phosphane in dichloromethane in the presence of N-ethyldiisopro-
pylamine yielded the phosphoramidite 2a. The procedure followed a
protocol which was originally developed by Caruthers [6]. Purifi-
cation was achieved by short column flash chromatography yielding
a colorless solid material which was characterized by ^{1}H and
^{13}C NMR spectra. The purity with respect to other phosphorous
containing impurities was assayed by ^{31}P NMR spectroscopy.
Compound 2a exhibited one ^{31}P signal at 146.2 ppm in $CDCl_3$
solution. The phosphoramidites of protected 2'-deoxyribo-
furanosides exhibit two signals at similar chemical shift, due to
the fact that these are mixtures of diastereoisomers whereas
compound 2a is a racemic mixture. The phosphoramidite 2b was
prepared in a similar fashion but using 2-cyanoethoxy-bis-
N,N-diisopropylaminophosphane [7,8,9]. Triethylamine, which was
present during the chromatographical purification of 2a was absent
during flash chromatography of 2b. The colorless amorphous residue
showed only one signal in the ^{31}P NMR spectrum.

Solid-Phase Synthesis of the Dodecamers 6-9 containing 1,3-
Propanediol as Nucleoside Substitute.

Recently it has been reported that the "Dickerson" dodecamer
d(CGCGAATTCGCG) [10,11] shows a strong tendency to form hairpins
in dilute aqueous solution at low salt concentration [12]. These
hairpins melt at higher temperature as the corresponding duplexes
[13,14]. The equilibrium between hairpins and duplexes depends on
the oligomer concentration. High oligomer concentration favours
duplex structures, whereas low oligomer concentration gives rise
to hairpin formation. Comparison of hairpin with duplex structure
immediately shows that depending on the loop size less bases are
base-paired in the hairpin than in a duplex. Also base-stacking
may be less efficient in the hairpin loop. As a consequence
nucleosides substitutes which are located in the loop region and
do not contain a nucleobase should enhance hairpin formation, due
to their inability to participate in Watson-Crick base pairing. To
test this we have synthesized a set of oligomers derived from the
sequence d(CGCGAATTCGCG) and have compared thermodynamic data of
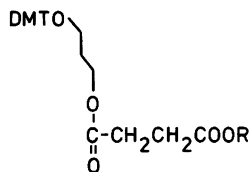these compounds with the parent "Dickerson" oligomer.

The incorporation of 1,3-propanediol into the self-complemen-
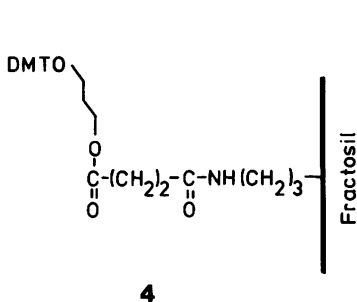tary "Dickerson" dodecamer d(CGCGAATTCGCG) where the dA or dT
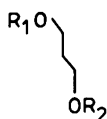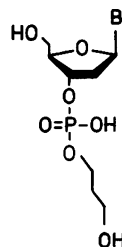
1   a: R_1 = DMT, R_2 = H
    b: R_1 = R_2 = DMT
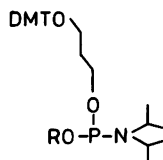
2   a: R = CH_3
    b: R = C_2H_4CN
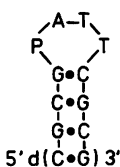
3   a: R = H
    b: R = Np

4

5   a: B = gua ^{ibu}
    b: B = ade ^{bz}
    c: B = guanine
    d: B = adenine
    e: B = thymine

6   7   8   9

5'd(CGCGPATTCGCG) 3'   d(CGCGAAPTCGCG)
3'd(GCGCTTAPGCGC) 5'   d(GCGCTPAAGCGC)

VI   VIII
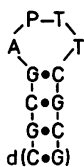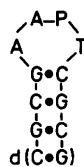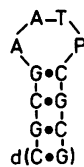
P = 1,3-propanediol residue

residues are replaced by the 1,3-propanediol was carried out on an Applied Biosystem 380 B DNA-synthesizer employing the phosphoramidite **2a**. The dodecamers **6-9** were recovered from the column as the 5'-dimethoxytritylated derivatives. After base deprotection with ammonia purification of the DMT-protected oligomers was accomplished by reverse-phase HPLC. Removal of the DMT-residues occured upon treatment with acetic acid for 15 min and the

Figure 1. HPLC elution profiles of the purified oligomers 6-9
(figure a-d, gradient system II) and the enzymatically hydrolysed
oligomers 6-9 (figure e-h, solvent system I). Digestion was
performed with snake venom phosphodiesterase followed by alkaline
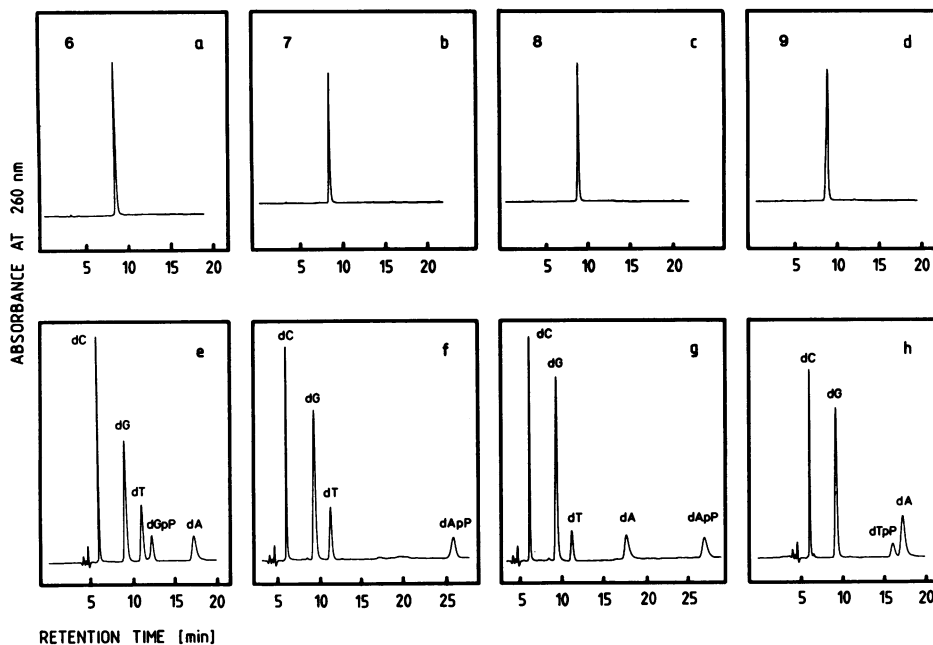phosphatase (see Experimental Section).

resulting oligomers 6-9 were purified by reverse-phase HPLC.
Samples of the main zone were lyophilized, desalted and stored
frozen at -20 °C.

Enzymatic Hydrolysis of the Oligomers 6-9.

     To confirm the structure of the dodecamers 6-9 enzymatic
hydrolysis with snake venom phosphodiesterase followed by alkaline
phosphatase was performed. Unexpected results were obtained from
the cleavage pattern of the oligonucleotides by HPLC analysis. As
can be seen from figure 1e the oligomer 6 shows a cleavage pattern
with five peaks instead of four which was expected from the struc-
ture. The fifth unknown peak has the same UV-spectrum as dG, but
shows a completely different retention time. Similar results were
obtained from the enzymatic cleavage of the dodecamer 8 (figure
1g). Also in that case an additional fifth peak was detected which
exhibited the UV-spectrum of dA but again was not the nucleoside.
In contrast to the HPLC-pattern containing five peaks only four

peaks were detected from the enzymatic cleavage products of the
oligomers 7 and 9 (figure 1f and 1h). In the latter case only
three deoxynucleosides can be detected, the missing fourth
nucleoside was derivatized which was deduced from its non-identity
to dA or dT, respectively. From the results discussed above it was
assumed that the unknown peaks formed after enzymatic hydrolysis
contain substances, in which 1,3-propanediol residues are linked
via a phosphate moiety to the 3'-hydroxyl group of the parti-
cular nucleoside.

In order to assign the structures of these hydrolysis
products we have synthesized the 3-hydroxypropyl nucleoside-
3'-phosphodiesters 5c-e. The synthesis was carried out in a
Beckman DNA synthesizer. In order to obtain the particular
hydrolysis products silica-gel bound 1,3-propanediol 4 was
prepared. For this purpose compound 1a was succinylated with
succinic anhydride to yield the monoester 3a. This was activated
with p-nitrophenole in the presence of dicyclohexylcarbodiimide to
yield the p-nitrophenylester 3b. Condensation of 3b with amino-
propyl-functionalized silica gave the modified matrix 4 which was
then employed in oligonucleotide synthesis [15]. Two protected
products (5a and 5b) were recovered after detritylation and
converted into 5c and 5d by the action of ammonia. The third
compound (5e) was obtained after detritylation. The 3-hydroxy-
propyl nucleoside-3'-phosphodiesters 5c-e were then employed
separately in HPLC analysis and compared with the corresponding
compounds obtained from enzymatic digestion. HPLC analysis showed
that the peak contents obtained from enzymatic hydrolysis were
identical to those obtained from the chemical synthesis. This
confirmed that snake venom phosphodiesterase was not able to
cleave the phosphodiester moiety between the 1,3-propanediol
phosphate and the 3'-hydroxyl-group of the particular
nucleoside. Although this enzyme is a 3'-5' exonuclease, all
other nucleotides were released from the oligomer. This shows that
the enzyme, which starts from the 3'-terminal OH-group of an
oligonucleotide [16] cannot recognize the phosphodiester bond
between the 3'-position of a nucleoside and the 1,3-propanediol
unit. As a result compounds such as 5c-e were formed after sub-
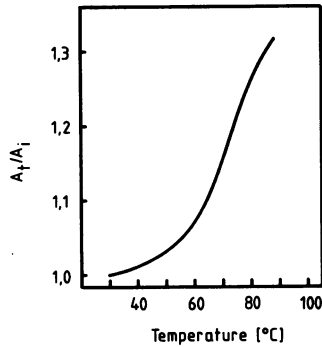sequent treatment with alkaline phosphatase.

Figure 2. Normalized melting profile of d(CGCGPATTCGCG) in 1mM phosphate buffer, pH 7.0 containing 100 mM NaCl and 1 mM EDTA at an oligomer concentration of 4 µM. A_t/A_i is the ratio of absorbance at 280 nm at a given temperature (t) to the initial temperature (i).

Melting Profiles and Structural Properties of the Oligomers 6-9.

To study the influence of the 1,3-propanediol moiety on the secondary structure of the DNA-fragments, the melting behaviour of the dodecamer d(CGCGAATTCGCG) and of the oligomers 6-9 was compared. The experiments were carried out at an oligomer concentration of 4 µM in 1 mM phosphate buffer, pH 7.0 containing 1 mM EDTA and 100 mM NaCl. Figure 2 shows a typical example of the
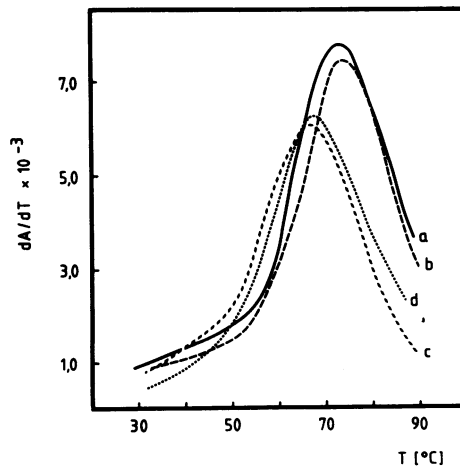

Figure 3. Differential melting curves of the oligomers 6-9 (a-d) in 1 mM phosphate buffer, pH 7.0 containing 100 mM NaCl and 1 mM EDTA at an oligomer concentration of 4 µM.

Table: T_m-values at 280 nm of the dodecamers 6-9 in 1 mM phosphate buffer, pH 7.0 containing 1 mM EDTA and 100 mM NaCl at an oligomer concentration of 4 μM.

compd.		T_m [°C]
d(CGCGAATTCGCG)		65
d(CGCGPATTCGCG)	(6)	73
d(CGCGAPTTCGCG)	(7)	74
d(CGCGAAPTCGCG)	(8)	67
d(CGCGAATPCGCG)	(9)	68

cooperative melting of the oligomer 6 at 280 nm. The T_m values at 280 nm of the oligomers 6-9 were obtained from the differential melting profiles which are depicted in figure 3. Data including the T_m of d(CGCGAATTCGCG) [14] are shown in the table. As the oligomers 6-9 missed either one adenine or thymidine base of the "Dickerson" dodecamer, decreased T_m values were expected, if melting represents strand separation. As one can see from the table, higher T_m values were observed for all oligomers containing 1,3-propanediol. The strongest increase of T_m values was found for the oligomers 6 and 7 in which a purine nucleoside was replaced.

Next, the T_m values (280 nm) of compound 6 were determined at different oligomer concentrations. From the graph of figure 4 it can be seen that melting was independent of the strand concentration. This led to the conclusion that this transition results from the melting of hairpins into single strands [12,14].

It has been reported earlier that a biphasic melting of the "Dickerson" dodecamer can be observed at 260 nm at low salt concentration [12,14]. Therefore, in a further experiment the melting curves of the oligomer 6 and the "Dickerson" dodecamer were compared at this wavelength (melting curves a and b of figure 5). In contrast to the "Dickerson" dodecamer, the oligomer 6 exhibited only monophasic transitions both at 260 and 280 nm (c). Moreover, there was no difference between the T_m values at these different wavelength, which was in contrast to the "Dickerson"
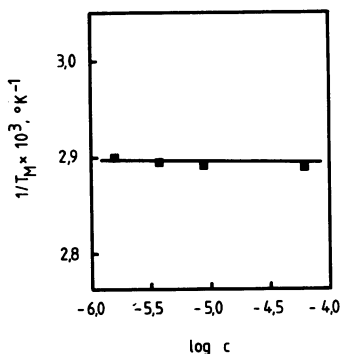
Figure 4. Plot of $1/T_m$ vs. log c for the oligomer 6 in H_2O containing 100 mM NaCl, 1 mM EDTA and 1 mM phosphate, pH 7.0.

oligomer [14]. From this difference it was concluded that hairpins as well as duplexes are present in phosphate buffer pH 7.0 within a range of 1-100 mM NaCl concentration. As compound 6 did show the same melting temperature at 260 nm (67 °C) and 280 nm (68 °C) which were independent of the strand concentration, it was clear that only one species, namely hairpins, were present in solution.

Owing to the sequence of the dodecamers 6-9 the melting of the hairpin structures results exclusively in an opening of d(GC) base pairs. The transition from a possible duplex (VI, VII, etc.) into a hairpin should show an exclusive opening of d(AT) base pairs while d(GC) base pairs are only reorganized, intermolecular hydrogen bonds are replaced by intramolecular ones. The d(GC) melting can be observed almost exclusively around 280 nm whereas the d(AT) melting curve shows an isosbestic point at this wavelength [17,18]. In contrast, d(GC) and d(AT) melting is observed at 260 nm. As the hairpin 6 does not contain d(AT) base pairs the $\Delta\varepsilon$ values for of the melting curves at 260 and 280 nm in figure 5 should reflect the $\Delta\varepsilon$ values for d(GC) melting at these two wavelengths. The about eightfold higher value of $\Delta\varepsilon$ at 280 nm of compound 6 relative to the $\Delta\varepsilon$ value measured at 260 nm is very similar to that of $d(CG)_3$ which exhibited a tenfold $\Delta\varepsilon$ at 280 nm compared to that at 260 nm [19].

As reported earlier [14], the melting curve of the "Dickerson" dodecamer was not reversible at 260 nm at low salt concentration. This hysteresis was explained with difficulties arising from
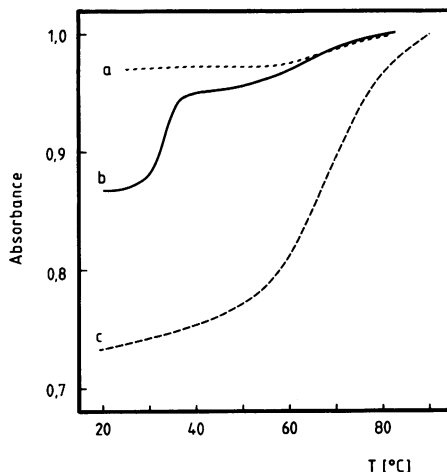
Figure 5. Normalized melting profiles (absorbance at the actual temperature devided by the absorbance at 90 °C) of d(CGCGAATTCGCG) (b), d(CGCGPATTCGCG) (6) (a) at 260 nm and 6 (c) at 280 nm measured in 1 mM phosphate buffer, pH 7.0 containing 1 mM NaCl and 1 mM EDTA at an oligomer concentration of 4 µM.

nucleoation of d(GC) base pairs within the hairpin. As compound 6 did not show this behaviour a more flexible structure of the hairpin 6 compared to the "Dickerson" dodecamer is the direct consequence. The absence of four hydrogen bonds within possible duplex structures such as VI etc., which results from the replace- ment of dA or dT residues by 1,3-propanediol and the reduction of torsional stress within the loop region may account for preferred hairpin formation.

In conclusion, it is shown that the replacement of 2'-deoxy- nucleosides by 1,3-propanediol within the loop region of self- complementary oligonucleotides can enhance hairpin formation. Fur- ther experiments employing "artificial" apurinic or apyrimidinic sites in DNA are in progress.

EXPERIMENTAL SECTION

Elemental analysis were performed by Microanalytisches Labor Beller (Göttingen, FRG). NMR spectra were recorded on a Bruker WM 250 spectrometer, values are in ppm relative to tetramethylsilane as internal standard (^{1}H and ^{13}C) or to external 85% phosphoric acid (^{31}P). Chemical shifts are

positive when downfield from the appropriate standard. UV
spectra were recorded on a Uvicon 810 spectrophotometer
(Kontron, Switzerland). Thin-layer chromatography (TLC) was
performed on silica gel SIL G-25 UV_{254} plates (Macherey-
Nagel, FRG). Quantitative TLC was carried out on a Shimadzu
CS-930 TLC scanner, connected with a Shimadzu DR-2 data
recorder. Flash chromatography was performed with silica gel
60 H (Merck, FRG) at 0.9 bar (N_2). Solvent systems for TLC:
(A) CH_2Cl_2, (B) CH_2Cl_2-ethyl acetat-triethylamine
(75:25:5), (C) CH_2Cl_2-CH_3OH-triethylamine (95:5:5), (D)
CH_2Cl_2-ethyl acetate (95:5). Pyridine and N-diisopropyl-
ethylamine were distilled from KOH and stored over 4 A
molecular sieves. Dioxane and DMF were filtered through a bed
of aluminium oxide (Woelm basic, grade I).
Tetrazole, 4-dimethylaminopyridine and trichloroacetic acid
were sublimated under reduced pressure. Snake venom phospho-
diesterase (EC 3.1.4.1., Crotallus durissus) and alkaline
phosphatase (EC 3.1.3.1., E.coli) were products of Boehringer
Mannheim (FRG). Fractosil (70 µmol of immobilized 2'-deoxy-
nucleoside/g of solid support) and macroporous silica
(Fractosil 500/-NH_2, 150 µmol/g) were purchased from
Biosyntech (FRG).

  Melting experiments. The melting experiments were carried
out in a thermostatically controlled cell holder with a
Shimadzu 210-A UV-spectrophotometer connected with a Kipp and
Zonen BD 90 recorder. The increase of absorbance at the
appropriate wavelength as a function of time was recorded
while the temperature of the solution was increased linearily'
with time at a rate of 20°C/h using a Lauda PM-350 programmer
and a Lauda RCS 6 bath equipped with a R 22 unit (MGW Lauda,
FRG). The actual temperature was measured in the reference
cell with a Pt-resistor.

  HPLC separation. High performance liquid chromatography
was carried out on a 4 x 250 + 4 x 25 mm (10µm) RP-18 LiChro-
sorb column (Merck) using a Merck-Hitachi HPLC apparatus with
one pump (model 655A-12) connected with a proportioning valve,
a variable wavelength monitor (model 655A), and a controller
(model L-5000), connected with an integrator (model D-2000).

The solvent systems and gradients consisting of 0.1 M tri-
ethylammonium acetate, ph 7.0 (A) and acetonitrile (B) were
used in the following order: gradient I: 10 min (25-40% B),
gradient II: 10 min (10-20% B), flow rates of gradient I and
II: 1 mL min^{-1}. Solvent I: 6% B, flow rate, 0.6 mL min^{-1}.
For desalting of the oligomers a 25x4 mm RP-18 cartridge was
loaded with the particular oligomer,dissolved in water.
Elution (5 min) of salt was accomplished with water, elution
of the oligomer occured with MeOH/water (3:2). The flow rate
was 1 mL min^{-1}.

O-(4,4'-Dimethoxytrityl)-1,3-propanediol (1a). To a
stirred solution of 4,4'-dimethoxytrityl chloride (2.05 g,
6.05 mmol) and N-ethyldiisopropylamine (1.55 g, 12 mmol) in
anhydrous pyridine (30 mL) 1,3-propanediol (2.28 g, 30.0 mmol)
was added. Stirring was continued for 3 h under nitrogen at
room temperature and the reaction was monitored on TLC (silica
gel, solvent A). 5% aqueous NaHCO$_3$ (50 mL) was added to the
solution and the resultant was extracted with dichloromethane
(100 mL). The combined organic layers were washed with water,
dried over sodium sulfate, filtered and the solvent was
evaporated. The residue was applied to a 12 x 5 cm column
(silica gel 60 H, solvent A) and separated by flash chromato-
graphy. Isolation of the material of the main zone yielded a
yellow oil of 1a (1.56 g, 68%), which crystallized from
ether/n-hexane at -18 °C after one week. M.p.: 69-70°C. TLC
(silica gel, solvent A): R$_f$ 0.23, ^{1}H-NMR (CDCl$_3$) δ 1.84
(2H, quint, H-2), 2.26 (1H, s, OH), 3.26 (2H, t, H-1, J = 6
Hz), 3.77 (8H, m, H-3, 2 OCH$_3$), 6.70-7.50 (m, arom. H).
^{13}C-NMR (CDCl$_3$) δ 32.54 (C-2), 55.04 (OCH$_3$), 61.50
(C-3), 62.00 (C-1). Anal. Calcd. for C$_{24}$H$_{26}$O$_4$ (378.5):
C, 76.16, H, 6.92. Found: C, 76.14, H, 6.76.

Bis-O-(4,4'-dimethoxytrityl)-1,3-propanediol (1b). A
solution of 4,4'-dimethoxytrityl chloride (1,0 g, 3 mmol)
and N-ethyldiisopropylamine (387 mg, 3 mmol) in absolute
pyridine (10 mL) was stirred and 1,3-propanediol (114 mg, 1.5
mmol) was added. After 2 h the reaction mixture was poured
into 5% aqueous NaHCO$_3$ (50 mL) and the resultant was
extracted three times with dichloromethane (100 mL, each). The

combined organic layers were washed, and dried over sodium
sulfate, filtered and the solvent was evaporated. The residue
was applied to a 12 x 5 cm column (silica gel 60H, solvent A)
and separated by flash chromatography. Isolation of the
material of the main zone yielded a colorless foam of <u>1b</u> (450
mg, 44%). TLC (silica gel, solvent A) : R_f 0.72, ^{1}H-NMR
(CDCl$_3$) δ1.88 (2H, quint, H-2), 3.22 (4H, t, H-1 and H-3,
J = 6.2 Hz), 3.77 (12 H, s, OCH$_3$), 6.75-7.40 (m, arom. H).
^{13}C NMR (CDCl$_3$) δ 30.58 (C-2), 55.18 (OCH$_3$), 60.18
(C-1, C-3). Anal. Calcd. for C$_{45}$H$_{44}$O$_6$ (680.8) : C,79.39,
H, 6.51. Found : C, 79.13, H, 6.54.

    1-O-(4,4'-Dimethoxytrityl)-3-O-[(N,N-diisopropylamino)
methoxyphosphino]-1,3-propanediol (2a). Compound <u>1a</u> (1.51 g,
3.99 mmol) dissolved in anhydrous dichloromethane (10mL) was
preflushed with argon in a round bottom flask. Chlorodiiso-
propylaminomethoxyphosphane (0.99 g, 5.0 mmol) and diisopro-
pylethylamine (1 mL, 5.0 mmol) were added to the stirred
solution by a syringe. Stirring was continued for 30 min at
room temperature. Then the solution was poured into 5% aqueous
NaHCO$_3$ (50 mL) and extracted three times with dichloro-
methane. The organic layer was dried over anhydrous sodium
sulfate, filtered and evaporated. The residue was purified by
flash chromatography on silica gel 60H (column 30 x 3.5 cm,
solvent B) yielding the phosphoramidite <u>2a</u> as colorless oil
(1.64 g, 76%). TLC (silica gel, solvent B), R_f 0.95, ^{1}H
NMR (CDCl$_3$) δ 1.15 (6H, m, CH$_3$-diisopropyl), 1.91 (2H,
quint, H-2), 3.15 (2H, m, H-1), 3.30 (3H, d, POCH$_3$, J = 13.1
Hz), 3.76 (6H, s, 2 OCH$_3$), 3.40-4.00 (4H, m, N-C<u>H</u> and H-3).
^{13}C NMR (CDCl$_3$) δ 24.51 (CH-C<u>H</u>$_3$), 32.03 (C-2, d,
^{3}J$_{PC-2'}$= 6.8 Hz), 42.73 (N-C<u>H</u>, d, ^{2}J$_{PCH}$= 12.6 Hz),
50.19 (POC<u>H</u>$_3$, d, ^{2}J$_{POCH_3}$= 17.3 Hz), 55.00 (OCH$_3$),
60.37 (C-1), 60.88 (C-3, d, ^{2}J$_{PC-3}$= 18.2 Hz) ^{31}P NMR
(CDCl$_3$) δ 146.20. Anal. Calcd. for C$_{31}$H$_{42}$NO$_5$P
(539.8) : C, 68.99, H, 7.84, N, 2.59. Found: C, 68.78, H,
7.81, N, 2.54.

    1-O-(4,4'-Dimethoxytrityl)-3-O-[(N,N-diisopropylamino)-
β-cyanoethoxy-phosphino]-1,3-propanediol (2b): Compound <u>1a</u>
(300 mg, 0.8 mmol) was dried by coevaporation with dry aceto-

nitrile (5 mL) and dissolved under nitrogen in dry acetoni-
trile (5 mL). Tetrazol (56 mg, 0.8 mmol) was added with
stirring at 20°C, followed by 2-cyanoethoxy-bis-N,N-diiso-
propylaminophosphane (240 mg, 0.8 mol). After 30 min a
precipitate of diisopropylammonium tetrazolide was removed by
filtration and the solution was reduced to a small volume.
Flash chromatography (solvent D, saturated with nitrogen)
yielded the phosphoramidite <u>2b</u> as colorless solid (290 mg,
63%). TLC (silica gel, solvent D) R_f = 0.90, ^{31}P-NMR
(CDCl$_3$) δ 145.8 ppm.

   <u>1-O-(4,4'-Dimethoxytrityl)-3-O-succinyl-1,3-propanediol</u>
<u>(3a)</u>. Compound <u>1a</u> (480 mg, 1.2 mmol) and dimethylamino-
pyridine (150 mg, 1.2 mmol) dissolved in pyridine were treated
with succinic anhydride (250 mg, 2.5 mmol). The mixture was
stirred for 36 h (room temperature) and the reaction was
monitored by TLC (silica gel, solvent C). Water (2 mL) was
added and the mixture was concentrated at reduced pressure.
After removal of pyridine by coevaporation with toluene, the
residue was taken up in dichloromethane and the organic layer
was washed with an aqueous solution of 10% citric acid
followed by water. The organic layer was dried over sodium
sulfate, filtered and the solvent was evaporated. The oily
residue was applied to a 10 x 5 cm column (silica gel 60H,
solvent C) and separated by flash chromatography. Compound <u>3a</u>
was isolated from the main zone as colorless solid (516 mg,
90%). TLC (silica gel, solvent C): R_f 0.55, UV (MeOH), λ max
235, 274, 280 mm. ^{1}H-NMR (CDCl$_3$) δ 1.87 (2H, quint, H-2),
2.54 (4H, m, CH$_2$), 3.12 (2H, t, H-1, J = 6 Hz), 3.75 (3H, s,
OCH$_3$), 4.21 (2H, t, H-3, J = 6.5 Hz), 6.7-7.41 (m, arom. H),
9.72 (1H, s, COOH). ^{13}C NMR (CDCl$_3$) δ 29.27-29.58 (C-2,
C-2', C-3'), 55.18 (OCH$_3$), 59.68 (C-3), 62.18 (C-1),
172.54 (COOR), 176.57 (COOH).

   <u>Preparation of silica-linked 1,3-propanediol (4)</u>.
To a stirred solution of compound <u>3a</u> (48 mg, 0.1 mmol) in
anhydrous p-dioxane/pyridine (95:5, 200 µL) p-nitrophenol
(14 mg, 0.1 mmol) was added. The resultant was treated with
dicyclohexylcarbodiimide (20.6 mg, 0.1 mmol) in p-dioxane
(20 µL) under stirring and the reaction was monitored by TLC

(silica gel, solvent C). After a reaction time of 3 h (room temperature) dicyclohexyl urea was removed by centrifugation and the supernatant containing 3b was used for the coupling with amino-functionalized silica. Fractosil 500 (Merck, 150 µmol NH$_2$-groups/g polymer support) was preswollen in DMF (1 mL) and treated with the solution of the p-nitrophenyl ester 3b in p-dioxane. Triethylamine (200 µL) was added and the mixture was shaken for 4 h at room temperature. After addition of acetic anhydride (60 µL) the shaking was continued for another 30 min. The modified silica was filtered, washed with DMF, EtOH, and diethyl ether and dried in vacuo. The amount of immobilized ligand was determined by treatment of 4 (10 mg) with 0.1 M p-toluene sulfonic acid (1 mL) in acetonitrile. Measurement of the absorbance of the supernatant after centrifugation yielded 77 µmol/g polymer support. The extinction coefficient of an acid solution of dimethoxy-tritanol was 70 (cm$^2 \cdot$µmol^{-1}) at 498 nm.

Solid-phase synthesis of the 3-hydroxypropyl nucleoside-3'-phosphodiesters 5c-e and the dodecamers 6-9. The oligo-mers 6-9 were synthesized on an Applied Biosystem Synthesizer model 380 B employing OCH$_3$-phosphoramidite chemistry [20]. The synthesis was carried out on solid support using columns with 1 µmol immobilized 2'-deoxynucleoside on CPG. The synthesis followed a reaction cycle of detritylation,coupling, oxidation, and capping. To cleave the OCH$_3$-protecting group the thiophenol reaction was carried out on solid support. The dodecamers were recovered from the DNA synthesizer as the 5'-dimethoxytritylated derivatives. After treatment with NH$_4$OH for 16 h at 60 °C to cleave base protecting groups, the 5'-dimethoxytritylated oligomers were purified by reverse-phase HPLC (gradient system I) and detritylated by the action of 80 % acetic acid for 15 min. After removal of the acid by evaporation, the resulting oligomer was dissolved in water (5 mL) and the tritylcarbinol was extracted with diethyl ether (4 x 5 mL). The detritylated products were purified by HPLC (gradient system II). Samples of the main zone were lyophilized and desalted by reverse-phase HPLC on a 25 x 4 mm RP 18 cartridge using solvent system II. After evaporation and

lyophilization the oligomers were dissolved in 1 mL water and stored frozen at -20 °C. Yield: Between 30 and 40 A_{260}-units. The compounds 5c-e were synthesized on a Beckman DNA-Synthesizer employing OCH_3-phosphoramidite chemistry using columns loaded with 1 μmol silicalinked 1,3-propanediol (4). The 3-hydroxypropyl nucleoside-3'-phosphodiesters were recovered from the synthesizer as the detritylated deriva-tives. After demethylation with thiophenol and removal of the N-protecting groups (25 % NH_4OH, at 60 °C for 16 h) in the case of 5c and 5d purification was carried out by reverse-phase HPLC (solvent system I).

Enzymatic hydrolysis of the oligomers 6-9.The oligomer (about 0.4 A_{260} units) was dissolved in 0.1 M TRIS/HCl buffer, pH 8.5, (0.5 mL) and digested with snake venom phosphodiesterase (5 μg) for 2 h at 37 °C. Further incu-bation with alkaline phosphatase (5 μg, 37 °C, 1 h) yielded a mixture of nucleosides (figure 1e-h). After separation on HPLC (solvent system I) quantification was made at 260 nm on the basis of the peak areas and extinction coefficients of the nucleosides (ε_{260}: dG 11700, dC 7300, dA 15400, dT 8800).

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