Oligodeoxyribonucleotides containing 1,3-propanediol as nucleoside substitute

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ABSTRACT

1,3-Propanediol was protected with one dimethoxytrityl residue and converted into the methoxy- and cyanoethoxyphosphoramidites 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-β-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 β-D-2’-deoxyribofuranosyl
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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 Hüning's base two tritylation products were formed at room temperature as detected by TLC. The slower migrating zone contained compound la, the faster zone the ditritylated compound lb. 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'-deoxyribofuranosides. 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 identical conditions but with a 2 : 1 excess of DMT-Cl over 1,3-propanediol gave 98% lb and only 2% la. However, if a five-fold excess of 1,3-propanediol over 4,4'-dimethoxytrityl chloride was used, 80% of the monotritylated la and only 20% of the ditritylated product lb were formed. These conditions were chosen for the preparative scale experiments and compound la was isolated after purification and crystallization in 68% yield. TLC and elemental analysis confirmed the purity. The structure was in agreement with 1H and 13C NMR data.

Two different phosphoramidites were prepared from the DMT-derivative la. Phosphitylation with chlorodiisopropylaminomethoxy-
phosphane in dichloromethane in the presence of N-ethylidiisopropylamine yielded the phosphoramidite 2a. The procedure followed a protocol which was originally developed by Caruthers [6]. Purification 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'-deoxyribofuranosides 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-complementary "Dickerson" dodecamer d(CGCGAATTCGCG) where the dA or dT
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 occurred upon treatment with acetic acid for 15 min and the
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 structure. 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

![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).](image-url)
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 particular 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-nitrophenol in the presence of dicyclohexylcarbodiimide to yield the p-nitrophenylester 3b. Condensation of 3b with aminopropyl-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-hydroxypropyl 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 subsequent treatment with alkaline phosphatase.
Figure 2. Normalized melting profile of d(CGCGPATTCGCG) in 1 mM 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\textsubscript{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 \mu M.

| compd.                  | T\textsubscript{m} [°C] |
|-------------------------|-------------------------|
| d(CGCGAATTCGCG)         | 65                      |
| d(CGCPATTCGCG) (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\textsubscript{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\textsubscript{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\textsubscript{m} values were expected, if melting represents strand separation. As one can see from the table, higher T\textsubscript{m} values were observed for all oligomers containing 1,3-propanediol. The strongest increase of T\textsubscript{m} values was found for the oligomers 6 and 7 in which a purine nucleoside was replaced.

Next, the T\textsubscript{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\textsubscript{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$_2$O containing 100 mM NaCl$^+$, 1 mM EDTA and 1 mM phosphate, pH 7.0.

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 $\Delta T_m$ values for of the melting curves at 260 and 280 nm in figure 5 should reflect the $\Delta T_m$ values for d(GC) melting at these two wavelengths. The about eightfold higher value of $\Delta T_m$ at 280 nm of compound 6 relative to the $\Delta T_m$ value measured at 260 nm is very similar to that of d(CG)$_3$ which exhibited a tenfold $\Delta T_m$ 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 divided 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.

nucleation 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 replacement 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'-deoxynucleosides by 1,3-propanediol within the loop region of self-complementary oligonucleotides can enhance hairpin formation. Further 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 (1H and 13C) or to external 85% phosphoric acid (31P). 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 UV254 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 (N2). Solvent systems for TLC: (A) CH2Cl2, (B) CH2Cl2-ethyl acetate-triethylamine (75:25:5), (C) CH2Cl2-CH3OH-triethylamine (95:5:5), (D) CH2Cl2-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 phosphodiesterase (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/-NH2, 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 linearly 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 LiChrosorb 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 triethylammonium 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 occurred with MeOH/water (3:2). The flow rate was 1 mL min⁻¹.

O-(4,4'-Dimethoxytrityl)-1,3-propanediol (la). 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 NaHCO₃ (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 chromatography. Isolation of the material of the main zone yielded a yellow oil of la (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): Rf 0.23, ¹H-NMR (CDCl₃) 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 OCH₃), 6.70-7.50 (m, arom. H). ¹³C-NMR (CDCl₃) δ 32.54 (C-2), 55.04 (OCH₃), 61.50 (C-3), 62.00 (C-1). Anal. Calcd. for C₂₄H₂₆O₄ (378.5): C, 76.16, H, 6.92. Found: C, 76.14, H, 6.76.

Bis-O-(4,4'-dimethoxytrityl)-1,3-propanediol (lb). 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 NaHCO₃ (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 1b (450 mg, 44%). TLC (silica gel, solvent A): Rf 0.72, 1H-NMR (CDCl3) δ 61.88 (2H, quint, H-2), 3.22 (4H, t, H-1 and H-3, J = 6.2 Hz), 3.77 (12 H, s, OCH3), 6.75-7.40 (m, arom. H). 13C NMR (CDCl3) δ 30.58 (C-2), 55.18 (OCH3), 60.18 (C-1, C-3). Anal. Calcd. for C45H44O6 (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 1a (1.51 g, 3.99 mmol) dissolved in anhydrous dichloromethane (10 mL) was preflushed with argon in a round bottom flask. Chlorodiisopropylaminomethoxyphosphate (0.99 g, 5.0 mmol) and diisopropylethylamine (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 NaHCO3 (50 mL) and extracted three times with dichloromethane. 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 2a as colorless oil (1.64 g, 76%). TLC (silica gel, solvent B), Rf 0.95, 1H NMR (CDCl3) δ 1.15 (6H, m, CH3-diisopropyl), 1.91 (2H, quint, H-2), 3.15 (2H, m, H-1), 3.30 (3H, d, POCH3, J = 13.1 Hz), 3.76 (6H, s, 2 OCH3), 3.40-4.00 (4H, m, N-CH and H-3). 13C NMR (CDCl3) δ 24.51 (CH-CH3), 32.03 (C-2, d, 3JPC-2 = 6.8 Hz), 42.73 (N-CH, d, 2JPC = 12.6 Hz), 50.19 (POCH3, d, 2JPC = 17.3 Hz), 55.00 (OCH3), 60.37 (C-1), 60.88 (C-3, d, 2JPC = 18.2 Hz) 31P NMR (CDCl3) δ 146.20. Anal. Calcd. for C31H42NO5P (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)-8-cyanoethoxy-phosphino]-1,3-propanediol (2b): Compound 1a (300 mg, 0.8 mmol) was dried by coevaporation with dry aceto-
nitrile (5 mL) and dissolved under nitrogen in dry acetonitrile (5 mL). Tetrazol (56 mg, 0.8 mmol) was added with stirring at 20°C, followed by 2-cyanoethoxy-bis-N,N-diisopropylaminophosphane (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 2b as colorless solid (290 mg, 63%).

TLC (silica gel, solvent D) \( R_f = 0.90 \), \( ^{31} \text{P-NMR} \) (CDCl\(_3\)) \( \delta 145.8 \) ppm.

1-O-(4,4’-Dimethoxytrityl)-3-O-succinyl-1,3-propanediol (3a). Compound 1a (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 3a was isolated from the main zone as colorless solid (516 mg, 90%). TLC (silica gel, solvent C): \( R_f 0.55 \), UV (MeOH), \( \lambda_{max} \) 235, 274, 280 nm. \( ^1 \text{H-NMR} \) (CDCl\(_3\)) \( \delta 1.87 \) (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). \( ^{13} \text{C NMR} \) (CDCl\(_3\)) \( \delta 29.27-29.58 \) (C-2, C-2’, C-3’), 55.18 (OCH\(_3\)), 59.68 (C-3), 62.18 (C-1), 172.54 (COOR), 176.57 (COOH).

Preparation of silica-linked 1,3-propanediol (4). To a stirred solution of compound 3a (48 mg, 0.1 mmol) in anhydrous p-dioxane/pyridine (95:5, 200 \( \mu \)L) 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 \( \mu \)L) 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 NH₂-groups/g polymer support) was preswollen in DMF (1 mL) and treated with the solution of the p-nitrophosphoryl 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 dimethoxytritanol was 70 (cm²·μmol⁻¹) at 498 nm.

Solid-phase synthesis of the 3-hydroxypropyl nucleoside-3'-phosphodiesters 5c-e and the dodecamers 6-9. The oligomers 6-9 were synthesized on an Applied Biosystem Synthesizer model 380 B employing OCH₃-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 OCH₃-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 NH₄OH 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 derivatives. 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 incubation 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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REFERENCES

1. Lindahl, T. in "Methods in Enzymology", Vol.65, Grossman, L., and Moldave, K. eds., Academic Press, (1980), pp. 284-290.

2. Riazuddin, S. in "Methods in Enzymology", Vol.65, Grossman, L., and Moldave, K. eds., Academic Press, (1980), pp. 290-295

3. Pochet, S., Huynh-Dinh, T., Neumann, J.M., Tran-Dinh, S., Taboury, J., Taillandier, E., and Igolen, J. (1985) Tetrahedron Lett., 26, 2085-2088.

4. Pochet, S., Huynh-Dinh, T., Neumann, J.M., Tran-Dinh, S., Adam, S., Taboury J., Taillandier, E., and Igolen, J. (1986) Nucleic Acids Res., 14, 1107-1126.

5. Preliminary data were presented at the 7. International Round Table "Nucleosides, Nucleotides and their Biological Applications", Konstanz, and are reported by Seela, F., and Kaiser, K., (1986) Nucleosides and Nucleotides, in press.
6. McBride, L.J., and Caruthers, M.H. (1983) Tetrahedron Lett. 24, 245-248.
7. Sinha, N.D., Biernat, J., McManus, J., and Köster, H. (1984) Nucleic Acids Res. 12, 4539-4557.
8. Nielson, J., Taagaard, M., Marugg, J.E., van Boom, J.H., and Dahl, O. (1986) Nucleic Acids Res. 14, 7391-7403.
9. Nielson, J., Marugg, J.E., Taagaard, M., van Boom, J.H., and Dahl, O. (1986) Recl. Trav. Chim. Pays-Bas. 105, 33-34.
10. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R.E. (1980) Nature, 287, 755-758.
11. Dickerson, R.E., and Drew, H.R. (1981) J. Mol. Biol. 149, 761-786.
12. Marky, L.A., Blumenfield, K.S., Kozlowsky, S., and Breslauer, K.J. (1983) Biopolymers, 22, 1247-1257.
13. Xodo, L.E., Manzini, G., Quadrifoglio, F., van der Marel, G.A., and van Boom, J.H. (1986) Nucleic Acids Res. 14, 5389-5398.
14. Seela, F., and Kehne, A. (1987) Biochemistry, in press.
15. Caruthers, M.H. (1982) in Chemical and Enzymatic Synthesis of Gene Fragments, Gassen, H.G., and Lang, A., eds., pp 71-79, Verlag Chemie, Weinheim.
16. Laskowski, M. in "Methods in Enzymology", Vol.65, Grossman, L., and Moldave, K. eds., Academic Press, (1980), pp. 276-284.
17. Blake, R.D., and Lefoley, S.G. (1978) Biochim. Biophys. Acta, 518, 233-246.
18. Felsenfeld, G., and Sandeen, G. (1962) J. Mol. Biol. 5, 587-610.
19. Seela, F., and Driller, H., (unpublished data).
20. Beaucage, S.L., and Caruthers, M.H. (1981) Tetrahedron Lett. 22, 1859-1862.