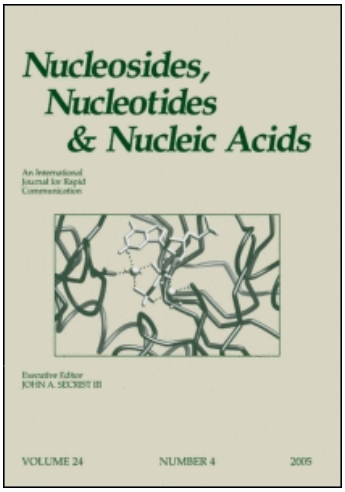

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Solid-Phase Synthesis of Oligodeoxyribonucleotide Analogues Containing 5, 6-Dihydroimidazo [1, 2-c] Pyrimidin-5-One as a Base Moiety

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SOLID-PHASE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDE ANALOGUES CONTAINING 5,6-DIHYDROIMIDAZO[1,2-c]PYRIMIDIN-5-ONE AS A BASE MOIETY

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ABSTRACT

The synthesis on a polyacrylamide resin of oligonucleotides containing 5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (X) as a base residue is described. Preliminary results on enzymatic hydrolysis of the modified octanucleotide d(TXAATTCA) (21), containing the recognition sequence for the endonuclease ECO RI where dG is replaced by dX (1), are reported.

Synthetic oligonucleotides containing specific and various modifications on the phosphate-sugar backbone or on the bases are of interest for several studies.

Phosphorothioate analogues of oligodeoxyribonucleotides have proven to be powerful tools for studies of the mechanism of action of phosphorolytic enzymes[1]. The introduction of this modification on the phosphate can also render the polymers more resistant to nucleases than the normal ones and this can offer advantages when a long-lived polymer is required[2].

Oligonucleotides containing unnatural bases can be successfully used for elucidating the mechanism of gene expression[3]

and for studies of nucleic acid-protein interactions[4]. Restriction endonucleases have been used as model due to their high specificity for DNA sequences and the simplicity of the DNA site recognized.One of the most studied systems in this field is the endodeoxyribonuclease ECO RI and its interaction with modified oligodeoxyribonucleotides[5]. We wish to report here the solid-phase synthesis of the modified octanucleotide d(TXAATTCA) (21) containing the recognition sequence for the restriction endodeoxyribonuclease ECO RI, where 2'-deoxyguanosine residue at the enzymatic cleavage site is replaced by 6-(2'-deoxy-ß-D-ribofuranosyl)-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (dX, 1).

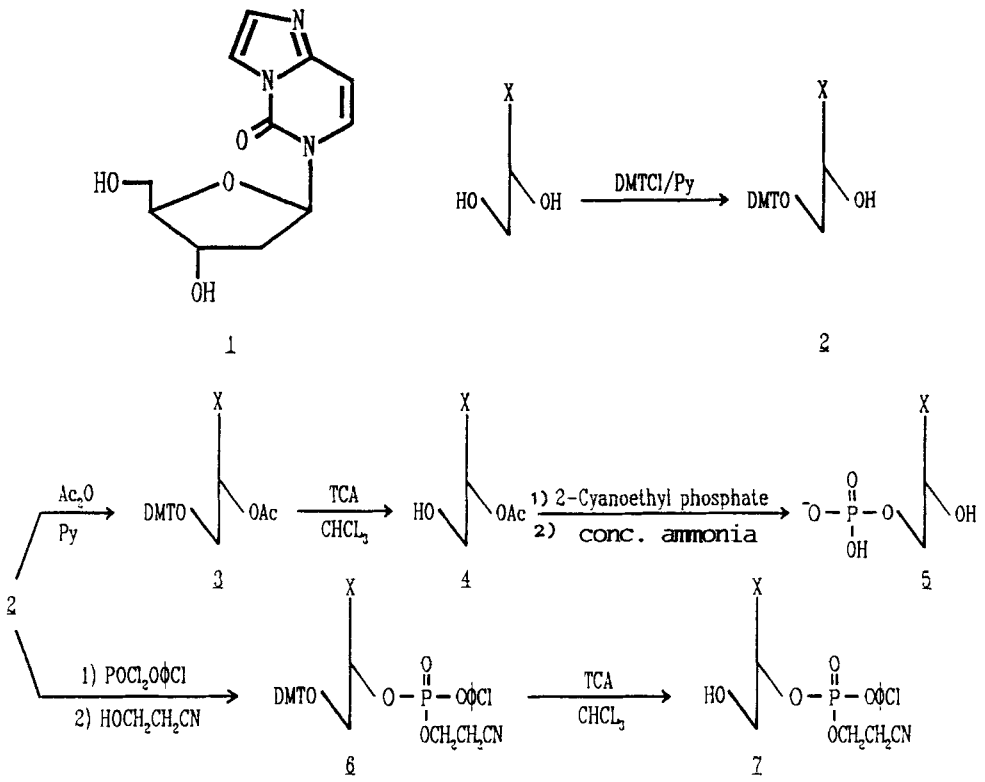
We also performed some enzymatic tests on 21 in comparison with the self complementary octamer 20 containing the recognition sequence of ECO RI.

For the oligomer synthesis we used a phosphotriester solid phase method, based on the chain elongation in the 5'- to 3'-end direction[6], on a polyacrylamide resin functionalized as described below.

The carboxyl group generated on the Sheppard's polymer (Fluka) by alkaline hydrolysis,was turned into the corresponding acyl chloride (scheme 2) by treatment with $SOCl_2$ in $CHCl_3$/pyridine[7]. The resulting resin (10), after removal of the excess reagent and solvents in vacuo, was allowed to react with a solution of the pertinent N-protected 2'-deoxynucleoside-3'-O-fully protected phosphate (7, 11, 12) in dry pyridine. Capping of the possible unreacted functional groups was performed by treatment with anhydrous ethanol in the presence of N,N'-dicyclohexylcarbodiimide (DCCI).

The loading of the resins 13-15 was estimated by spectro-

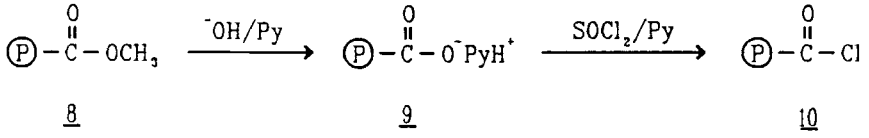
SCHEME 1



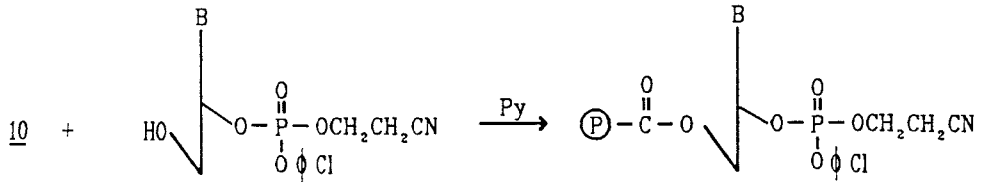
DMTCl = 4,4'-Dimethoxytriphenylmethychloride
OΦCl = 2-Chlorophenyl
TCA = Trichloroacetic acid
Py = Pyridine

scopic measurement of the nucleotidic material liberated from a weighted sample of the resin by treatment with concentrated ammonia, and resulted to be constantly in the range 0.20--0.25 meq/g.

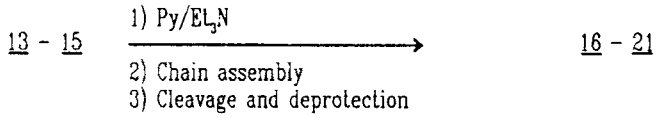
SCHEME 2



8 9 10



7, 11, 12 13 - 15



Ⓟ = Polyacrylamide resin

11 = 2'-Deoxythymidin-3-O-(2-chlorophenyl-2-cyanoethyl)phosphate
12 = N^4-Benzoyl-2'-deoxycytidin-3'-O-(2-chlorophenyl-2-cyanoethyl) phosphate
13 : B = X
14 : B = T
15 : B = C^{bz} (bz = benzoyl)

The above functionalization procedure does not require the preliminary synthesis of tne succinylnucleoside component[6,8].

To obtain the modified octamer 21 it was necessary to prepare the appropriately protected nucleotide 7 (scheme 1) starting from 6-(2'-deoxy-β-D-ribofuranosyl)-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (1) synthesized by reaction of chloroacetaldehyde with 2'-deoxycytidine according to the previously described procedure[9].

The 5'-hydroxy group of 1 was protected by treatment with 4,4'-dimethoxytriphenylmethyl chloride (DMTCl) in pyridine and the resulting DMT-derivative (2) was phosphorylated with

2-chlorophenylphosphorodichloridate in pyridine and successively treated with excess of 3-hydroxypropionitrile in order to obtain the fully protected intermediate 6, which was detritylated by treatment with trichloroacetic acid (10% in $CHCl_3$) to obtain the 5'-hydroxy derivative 7. Alternatively compound 2 was acetylated and successively detritylated to afford compound 4.

To establish the suitability of the building blocks containing the modified base (4, 7, 13) in the phosphotriester synthesis, we first prepared the modified trinucleotides d(TXC) (17), d(XTC) (18) and d(CTX) (19) using the functionalized supports 14, 13 and 15, respectively. We did not observe any significative difference in the coupling yields, when 4, 7 and 13 were used instead of the corresponding reagents containing the natural bases. Moreover trinucleotides resulted to be stable during the final treatment with concentrated ammonia.

For the synthesis of the two octamers we used 50 mg of the functionalized support 14 and performed 6 cycles using the appropriate N-protected 5'-hydroxy-2'-deoxynucleoside-3'-O--fully protected phosphate. In the last cycle N-benzoyl-3'-O--benzoyl-2'-deoxyadenosine was added in order to obtain a 3'--hydroxy oligomer.

After complete deprotection and release from the resin by reaction with concentrated ammonia, the recovered oligomers were analyzed and purified by HPLC using an anion exchange (Partisil 10 SAX, Whatman) and a reverse-phase (Lichrosorb RP-18, Merck) column, successively.

The size and the composition of the octamers were ensured by analysis of the ^{1}HNMR spectra and by checking the chain elongation step by step.

The oligomers 20 and 21 were further characterized by nuclease S1 digestion followed by analysis of the products by

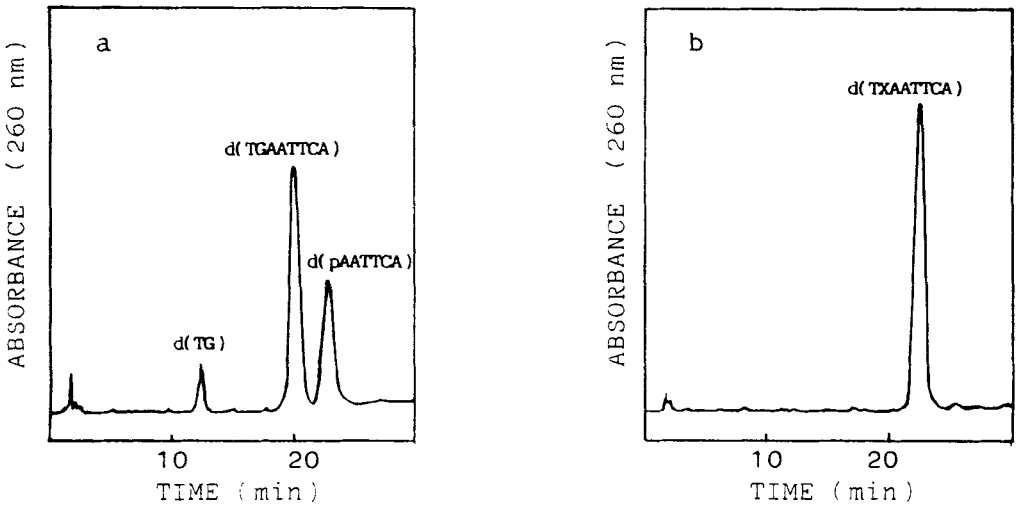


Fig. 1. HPLC analyses on a Partisil 10 SAX column of d(TGAATTCA) (a) and d(TXAATTCA) (b) after digestion with endodeoxyribonuclease ECO RI.

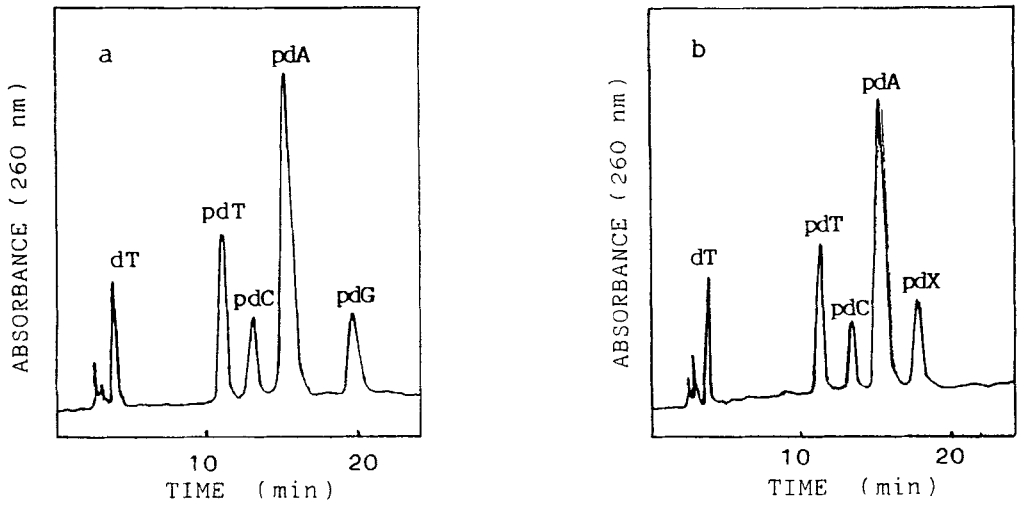


Fig. 2. HPLC analyses on a Partisil 10 SAX column of the product of enzymatic digestion of d(TGAATTCA) (a) and d(TXAATTCA) (b) with exonuclease III.

HPLC. d(TGAATTCA) (20) yielded dT, dpG, dpC, dpT and dpA in the ratio 1, 1, 1, 2 and 3 respectively whereas d(TXAATTCA) (21) furnished dT, dpX, dpC, dpT, and dpA in the ratio 1, 1, 1, 2, and 3, respectively.

To identify the reaction products of the enzymatic cleavages, we synthesized the nucleoside-5'-phosphate 5, the dinucleotide 16 and the hexamer-5'-phosphate d(pAATTCA) (22), to be used as standards in the HPLC analyses. Nucleotide 5 was prepared by reaction of 3'-O-acetylderivative 4 with 2-cyanoethylphosphate[10] in the presence of DCCI in pyridine and successive treatment with concentrated ammonia for 1.5 h at 50 °C. The hexamer 22 was prepared by a solid-phase synthesis according to the previously described procedure[11].

Prior to enzymatic tests with ECO RI and exonuclease III, which both require polydeoxyribonucleotides in duplex form, we ensured that the octamers 20 and 21 form a double strand structure under appropriate salt conditions. Cooperative melting for these oligomers was observed, thus indicating that a double strand-single strand transition takes place. The oligomer 21 exhibits a lower Tm value (18 °C) than that of 20 (22 °C).

Enzymatic digestion of 20 with ECO RI (48 h at 12 °C) induced ca. 40 % cleavage of this oligomer to give two products: d(TG) and d(pAATTCA) (22) as deduced from HPLC analysis (fig. 1-a) in comparison with authentic samples.

On the other hand the octamer 21, incubated in the same conditions as 20, was not significantly cleaved within 48 h (HPLC profile is reported in fig. 1-b).

Vice versa both octamers were digested almost at the same extent by exonuclease III (48 h, 12 °C) yielding dT and nucleoside-5'-phosphates in a ratio which corresponds well with their base compositions (fig. 2 a-b).

The above results demonstrated that the replacement of dG with dX at the recognition site of ECO RI completely inhibits or at least drastically reduces the rate of the enzymatic hydrolysis. On the contrary the digestion with S1 and exonuclease III is not affected by the presence of the unnatural base in the oligonucleotide sequence.

EXPERIMENTAL

General procedure

The ^{1}H NMR spectra were recorded with a Bruker WM 270 instrument. Chemical shifts are expressed in p.p.m. on the respect of the residue solvent signal. UV spectra were taken on a Perkin-Elmer lambda 7 spectrophotometer.

6-[5'-O-(4,4'-dimethoxytriphenylmethyl)-2'-deoxy-ß-D-ribofuranosyl]-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (2)

To a solution of 1 (1.0 mmol, 250 mg) and 4-dimethylaminopyridine (0.05 mmol, 6 mg) in anhydrous pyridine (5 ml) 4,4'-dimethoxytriphenylmethyl chloride (1.3 mmol, 440 mg) and triethylamine (1.4 mmol) were added and the resulting solution was kept for 1 h at 70 °C. The reaction was stopped by addition of water (2 ml) and the resulting solution was evaporated to dryness in vacuo. The residue, dissolved in $CHCl_3$, was purified by silica gel column chromatography using increasing amounts of MeOH in $CHCl_3$-pyridine (99.8:0.2). The fractions eluted with $CHCl_3$-pyridine/MeOH 97:3 yielded 470 mg of pure 2 (85% yield). UV (λ max in MeOH) 273 nm (ε = 13500), 241 nm (ε=14000). ^{1}H NMR (CD_3OD) : δ 7.74 (1H, d, J= 1.8 Hz, H-2), 7.62 (1H, d, J = 7.7 Hz, H-7); 7.48 - 6.80 (14 H, complex signal, aromatic protons and H-3); 6.60 (1H, dd, J= 6 and 6 Hz H-1'); 6.38 (1H, d, J = 7.7 Hz, H-8); 4.62 (1H, m, H-3'); 4.09 (1H, m, H-4'); 3.81 (6H, s, 2 OCH_3); 3.53 (2H, m, H_2-5'); 2.52 (1H, m, H_a-2'); 2.38 (1H, m, H_b-2').

6-[5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-O-acetyl-2'-deoxy--ß-D-ribofuranosyl]-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (3)

To a solution of 2 (0.5 mmol, 275 mg) in dry pyridine (5 ml) acetic anhydride (21 mmol, 2 ml) was added and the mixture kept overnight at room temperature. After addition of MeOH (2 ml) at 0°C, the solution was evaporated to dryness in vacuo and the residue was purified by silica gel column chromatography using increasing amounts of MeOH in $CHCl_3$-pyridine (99.8 : 0.2). The fractions eluted with $CHCl_3$-pyridine/MeOH 98 : 2 afforded 265 mg of pure 3 (90 % yield). UV (λ max in $CHCl_3$) 273 nm (ε = 14500), 240 nm (ε = 1500). ^{1}H NMR ($CDCl_3$): δ 7.72 (1H, d, J = 1.8 Hz, H-2); 7.60 (1H, d, J = 7.7 Hz, H-7); 7.48 - 6.80 (14H, complex signal, aromatic protons and H-3); 6.63 (1H, dd, J = 6 and 5.8 Hz, H-1'); 6.33 (1H, d, J = 7.7 Hz, H-8); 5.48 (1H, m, H-3'); 4.22 (1H, m, H-4'); 3.79 (6H, s, 2 OCH_3); 3.52 (2H, m, H_2-5'); 2.63-2.40 (2H, complex signal, H_2-2'); 2.10 (3H, s, CH_3CO).

6-(3'-O-acetyl-2'-deoxy-ß-D-ribofuranosyl)-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (4).

Compound 3 (0.5 mmol, 300 mg) dissolved in $CHCl_3$ (5 ml) was treated with 5 ml of a solution of 20 % trichloroacetic acid in $CHCl_3$ at 0°C. After 15 min the reaction was stopped by addition of pyridine (2 ml) and the mixture was washed with $NaHCO_3$ aqueous (x3) and H_2O (x3).

The organic layer was evaporated to dryness in vacuo and the residue was chromatographed on a silica gel column using increasing amounts of MeOH in $CHCl_3$. The fractions eluted with $CHCl_3$/MeOH 96:4 yielded 130 mg of pure 4 (88 % yield). UV (λ max in $CHCl_3$) 271 nm (ε = 11700). ^{1}H NMR

($CDCl_3$) : δ 7.64 (1H, d, J = 1.8 Hz, H-2); 7.64 (1H, d, J = 7.7 Hz, H-7); 7.39 (1H, d, J = 1.8 Hz, H-3); 6.66 (1H, d, J = 7.7 Hz, H-8); 6.53 (1H, dd, J = 6 and 6 Hz, H-1'); 5,42 (1H, m, H-3'); 4.19 (1H, m, H-4'); 3.97 (2H, m, H_2-5'); 2.50 (2H, m, H_2-2'); 2.14 (3H, s, CH_3CO).

6-(5'-O-phosphate-2'-deoxy-ß-D-ribofuranosyl)-5,6-dihydroimidazo-[1,2-c]pyrimidin-5-one (5).

A solution of 0.4 mmol (120 mg) of 4 in dry pyridine (5 ml) was treated with 2-cyanoethylphosphate pyridinium salt (1 mmol, 309 mg) in the presence of DCCI (3 mmol, 620 mg), under stirring for 15 h at room temperature. Then 2 ml of water were added and the mixture was kept at room temperature for 30 min. The mixture was evaporated to dryness in vacuo and the residue taken up in 5 ml of water to remove the dicyclohexylurea by filtration. The filtrate, evaporated to dryness in vacuo, was treated with 5 ml of concentrated ammonia for 1.5h at 50°C. The mixture was taken to dryness, dissolved in water and chromatographed by HPLC on a RP-18 column (25 cm, 10 mm i.d., flow rate 1.5 ml/min). Elution with a liner gradient of CH_3CN in triethylammonium acetate 0.1 M, pH 7, afforded 75 mg of 5 (65 % yield). UV (λ max in H_2O) 270 nm (ε = 10800). ^1H NMR (D_2O) : δ 7.74 (1H, bs, H-2); 7.72 (1H, d, J= 7.8 Hz, H-7); 7.38 (1H, bs, H-3); 6.79 (1H, d, J= 7.8 Hz, H-8); 6.55 (1H, dd, J = 6 and 6 Hz, H-1'); 4.58 (1H, m, H-3'); 4.19 (1H, m, H-4'); 4.03 (2H, m, H_2-5'); 2.42 (2H, m,H_2-2').

6-[5'-O-(4,4'-dimethoxytriphenylmethyl)-3'-O-(2-chlorophenyl-2-cyanoethyl)phosphate-2'-deoxy-ß-D-ribofuranosyl]-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (6).

To a stirred solution of 2 (0.5 mmol, 275 mg) in dry pyridine (5 ml), 4 mmol (0.7 ml) of 2-chlorophenylphosphoro-

dichloridate dissolved in 4 ml of dry pyridine were added and the mixture kept at room temperature. After 30 min TLC analysis (silica gel, eluent $CHCl_3$/MeOH 95:5) showed the complete conversion of 2 into a base-line product; then 25 mmol (1.8 ml) of 3-hydroxypropionitrile were added. After 1 h at room tempeature the mixture, concentrated to an oil under reduced pressure and dissolved in $CHCl_3$, was washed with H_2O (x3). The organic layer, evaporated to dryness in vacuo, was chromatographed on a silica gel column using increasing amounts of MeOH in $CHCl_3$-pyridine (99.8 : 0.2). The fractions eluted with $CHCl_3$-pyridine/MeOH 97:3 yielded 300 mg of pure 6 (75 % yield), as a mixture of diastereoisomers. UV (λ max in $CHCl_3$) 273 nm (ε = 15000). ^{1}H NMR ($CDCl_3$) : δ 7.73 (1H, d, J= 1.8 Hz, H-2); 7.54 (1H, d, J= 7.7 Hz, H-7); 7.48-6.79 (18H, complex signal, aromatic protons and H-3); 6.67 and 6.64 (1H, dd's, J= 6 and 6 Hz, H-1'); 6.28 (1H, d, J=7.7 Hz, H-8); 5.39 (1H, m, H-3'); 4.43-4.30 (3H, overlapped signals, OCH_2CH_2CN and H-4'); 3.80 (6H, s, 2 OCH_3); 3.53 (2H, m, H_2-5'); 3.08 - 2.42 (4H, overlapped signals, OCH_2CH_2CN and H_2-2').

6-[3'-O-(2-chlorophenyl-2-cyanoethyl)phospathe-2'-deoxy-ß-D--ribofuranosyl]-5,6-dihydroimidazo[1,2-c]pyrimidin-5-one (7).

Compound 6 (0.3 mmol, 240 mg) dissolved in $CHCl_3$ (4 ml) was treated with trichloroacetic acid under the conditions as described to obtain 4 from 3. The crude product was chromatographed on a silica gel column using increasing amounts of MeOH in $CHCl_3$. The fractions eluted with $CHCl_3$/MeOH 93:7 afforded 130 mg of 7 (89 % yield) as a mixture of diastereoisomers. UV (λmax in $CHCl_3$) 271 nm (ε = 10500); ^{1}H NMR ($CDCl_3$) : δ 7.72 (1H, d, J = 1.7 Hz, H-2); 7.56 (1H, d, J = 8.1 Hz, H-7); 7.49 - 7.13 (5H, complex signal, aromatic protons and H-3); 6.64 (1H, d, J = 8.1 Hz, H-8); 6.50 (1H, m,

H-1'); 5.38 (1H, m, H-3'); 4.52-4.26 (3H, overlapped signals, OCH_2CH_2CN and H-4'); 3.92 (2H, m, H_2-5'); 2.84 (2H, t, J= 5.8 Hz, OCH_2CH_2CN); 2.76- 2.50 (2H, complex signal, H_2-2').

General procedure for preparation of the solid supports 13-15.

The freshly prepared resin 10 (100 mg) was treated with a solution of 7 (11,12) (0.15 mmol) in dry pyridine (2ml) under stirring at room temperature for 12 h. The resulting polymer 13 (14,15), washed with dry pyridine, was treated with anhydrous ethanol (0.8 ml) in the presence of DCCI (250 mg) in dry pyridine (3 ml) under shaking at room temperature for 15h. It was successively washed with dry pyridine (10x2min), dry $CHCl_3$ (5x2 min) and dry Et_2O (5 x 2min), and dried in vacuo. The loading of the resin 13 (14,15) was measured by UV spectroscopy after removing the nucleotidic material of a weighted sample by treatment with concentrated ammonia at 50°C for 5h; it ranged between 0.20-0.25 meq/g.

Solid-phase synthesis and purification of the oligomers 16-21.

The functionalized support 13 (14, 15) (50 mg, 0.012 meq) was swollen by addition of dry pyridine and then treated with Et_3N/pyridine (1:1) at 50°C for 30 min. After washing with pyridine (10 x 0.5 min) the resin was left in contact with a solution of N-protected-2'-deoxynucleoside-3'-O-(2-chlorophenyl-2-cyanoethyl)phosphate (0.18 mmol) and MSNT (0.35 mmol) in dry pyridine (2.5 ml) for 20 min at 50 °C. After washing with pyridine (10 x 0.5 min) the second cycle was begun. Cycles were repeated until the required length was reached. In the last cycle the appropriate N-protected-3'-O-benzoyl-2'-deoxynucleoside (0.18 mmol) was used. The final resin was treated with 5 ml of concentrated ammonia for 3.5 h a 50 °C. The supernatant was filtered and the resin was

washed with EtOH/water (1:1, 3 x 3 ml). The combined filtrate and washings were taken to dryness, dissolved in water (2 ml) and chromatographed by HPLC on a Partisil 10 SAX column (Whatman, 25 cm, 4.6 mm i.d., 10 μm flow rate 1.5 ml/ min) eluted with a linear gradient of KH_2PO_4, KCl, 5% EtOH, pH 6.8 from 1 mM (eluent A) to 0.25 M (eluent B) at room temperature.

Oligomer		Retention time (min)	Gradient
d(TG)	16	11.0	100% H_2O→100% A in 30 min
d(TXC)	17	15.5	0 → 40% B in 30 min
d(XTC)	18	14.0	" "
d(CTX)	19	12.0	" "
d(TGAATTCA)	20	20.0	30 → 100% B in 30 min
d(TXAATTCA)	21	23.0	" "
d(pAATTCA)	22	22.5	" "

16. ^{1}H NMR (D_2O): δ 7.94 [1H, s, H-8 (G)]; 7.31 [1H, bs, H-6 (T)]; 6.12 and 5.95 (1H each, dd, 2xH-1'); 1.80 [3H, bs, CH_3-C-5 (T)].

17. ^{1}H NMR (D_2O, 400 MHz) : δ 7.68 [1H, d, J = 7.6 Hz, H-6 (C)]; 7.56 [1H, bs, H-2 (X)]; 7.37 [1H, d, J = 7.9 Hz, H-7 (X)]; 7.28 [1H, bs, H-6 (T)]; 7.18 [1H, bs, H-3 (X)]; 6.56 [1H, d, J = 7.9 Hz, H-8 (X)]; 6.32, 6.11 and 5.93 (1H each, dd, 3xH-1'); 5.86 [1H, d, J = 7.6 Hz, H-5 (C)]; 1.63 [3H, bs, CH_3-C-5 (T)].

18. ^{1}H NMR (D_2O) : δ 7.81 [1H, d, J = 7.6 Hz, H-6 (C)]; 7.68 [1H, bs, H-2 (X)]; 7.58 [1H, bs, H-6 (T)]; 7.56 [1H, d, J = 7.9 Hz, H-7 (X)]; 7.36 [1H, bs, H-3 (X)]; 6.67 [1H, d, J = 7.9 Hz, H-8 (X)]; 6.35, 6.22 and 6.14 (1H each, dd, 3xH-1'); 5.99 [1H, d, J=7.6 Hz, H-5 (C)]; 1.77 [3H, bs, CH_3-C-5 (T)].

19. ^{1}H NMR (D_2O) : δ 7.60 [1H, d, J = 7.6 Hz, H-6 (C)]; 7.53

[1H, bs, H-2 (X)]; 7.42 [1H, d, J = 7.9 Hz, H-7 (X)]; 7.35 [1H, bs, H-6 (T)]; 7.15 [1H, bs, H-3 (X)]; 6.58 [1H, d, J = 7.9 Hz, H-8 (X)]; 6.35, 5.98 and 5.93 (1H each, dd, 3xH-1'); 5.75 [1H, d, J = 7.6 Hz, H-5 (C)]; 1.59 [3H, bs, CH_3-C-5 (T)].

20. ^{1}H NMR (D_2O) : δ 8.35, 8.19. 8.12, 8.11, 8.10, 7.82 and 7.78 [1H each, s, 3xH-2 and 3 H-8 (A) and H-8 (G)]; 7.38 [1H, d, J = 7.6 Hz, H-6 (C)]; 7.26, 7.26 and 7.07 [1H each, bs, 3xH-6 (T)]; 5.85 [1H, d, J = 7.6 Hz, H-5 (C)]; 1.63, 1.60 and 1.55 [3H each, bs, 3 CH_3-C-5 (T)].

21. ^{1}H NMR (D_2O, 400 MHz) : δ 8.26, 8.16, 8.07, 8.00, 7.68 and 7.59 [1H each, s, 3 H-2 and 3 H-8 (A)]; 7.49 [1H, d, J=7.6 Hz, H-6 (C)]; 7.46 [1H, bs, H-2 (X)]; 7.45, 7.36 and 7.28 [1H each, bs, 3 H-6 (T)]; 7.25 [1H, bs, H-3 (X)]; 7.21 [1H, d, J = 7.8 Hz, H-7 (X)]; 6.53 [1H, d, J = 7.8 Hz, H-8 (X)]; 5.87 [1H, d, J = 7.6 Hz, H-5 (C)]; 1.70, 1.71 and 1.57 [3H each, bs, 3 CH_3-C-5 (T)].

Melting curves

The melting curves were measured in teflon stopped cuvettes with 1 cm light path lenght in a thermostatically controlled cell holder. The increase of absorbance at 260 nm as function of time was recorded while the temperature of the solution (100 mM tris/HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5) was increased linearly at a rate of 15 °C/h.

Enzymatic hydrolysis of the oligomer 20 , 21

Endodeoxyribonuclease ECO RI : the oligomers 20,21 (2.0 A_{260} units each), dissolved in 500 μl of 100 mM tris/HCl buffer 50 mM NaCl, 10 mM $MgCl_2$ at pH 7.5, were incubated with ECO RI (600 units) at 12 °C. Aliquots were analyzed at diffe-

rent intervals of time by HPLC using a Partisil 10 SAX column; eluent A: H_2O, eluent B: 0.25 M KH_2PO_4, KCl, 5% EtOH, pH 6.8; gradient: 0 to 6% B in 10 min, then 100% B.

Exonuclease III: the oligomers 20-21 (2.0 A_{260} units each) dissolved in 500 μl of 50 mM tris/HCl buffer, 5 mM $MgCl_2$, pH 7.5, were digested with exonuclease III (500 units) at 12°C. Aliquots were analyzed at different intervals of time by HPLC using a Partisil 10 SAX column eluted with a linear gradient from 0 to 60% B in 60 min; eluent A: 1 mM KH_2PO_4,KCl 5% EtOH pH 6.8; eluent B: 0.25 M KH_2PO_4, KCl, 5% EtOH pH 6.8.

Nuclease S1 : at the oligomers 20-21 (1.5 A_{260} units each), dissolved in 500 μl of 280 mM NaCl, 50 mM NaOAc buffer pH 4.6, 4.5 mM $ZnSO_4$ containing 20 g/ml of DNA carrier, 250 units of nuclease S1 were added and the mixture was incubated at 37°C. After 30 min the resulting mixture was analyzed by HPLC, as previously described for exonuclease digestion.

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