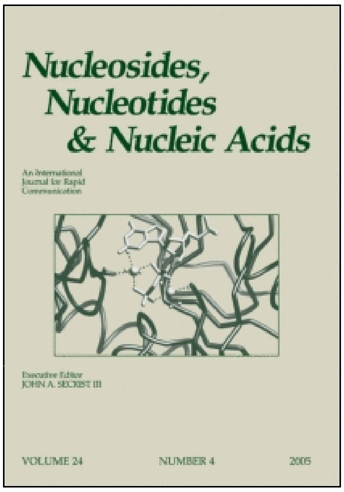

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Achiral Nucleotide Analogs: 5,5-Disubstituted Pyrimidines Related to Barbituric Acid

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ACHIRAL NUCLEOTIDE ANALOGS: 5,5-DISUBSTITUTED PYRIMIDINES RELATED TO BARBITURIC ACID

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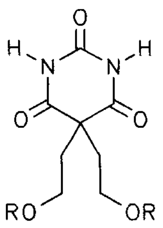
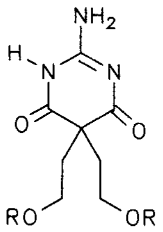
Abstract. Achiral nucleotide analogs are of potential interest in origin of life studies. Four new 5,5-diphosphoethylpyrimidine analogs were prepared by condensation of urea or guanidine with α,α-di(2-[ethoxy-1-ethoxy]ethyl)malononitrile or α,α-di(2-[ethoxy-1-ethoxy]ethyl)ethyl cyanoacetate respectively, followed by removal of the protecting groups and bisphosphorylation. Oligomers of two potentially complementary analogs have been prepared. Interactions between these oligomers appear to be weak in aqueous solution.

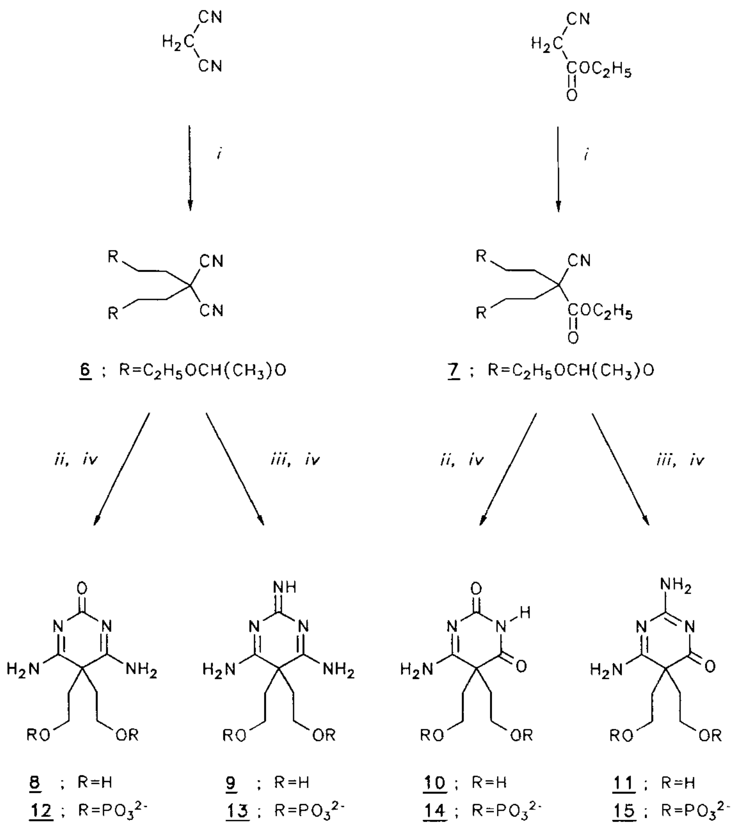
Molecular recognition via the formation of hydrogen-bonded complexes plays a central role in the design of self-replicating systems[1-3]. Barbituric acid derivatives, which can form six hydrogen bonds with two complementary 2,4,6-triaminopyrimidine analogs, have also been utilized as the basis for a supramolecular system[4]. We have shown recently that 5,5-di(2-phosphoethyl)barbituric acid **3** can be polymerized with high efficiency in aqueous solution[5].



1 ; R=H
3 ; R=PO_3^{2-}



2 ; R=H
4 ; R=PO_3^{2-}



Scheme 1. *i* NaH in DMF, **5**, *ii* urea/$NaOC_2H_5$ in ethanol, *iii* guanidine/$NaOC_2H_5$ in ethanol, *iv* H^+.

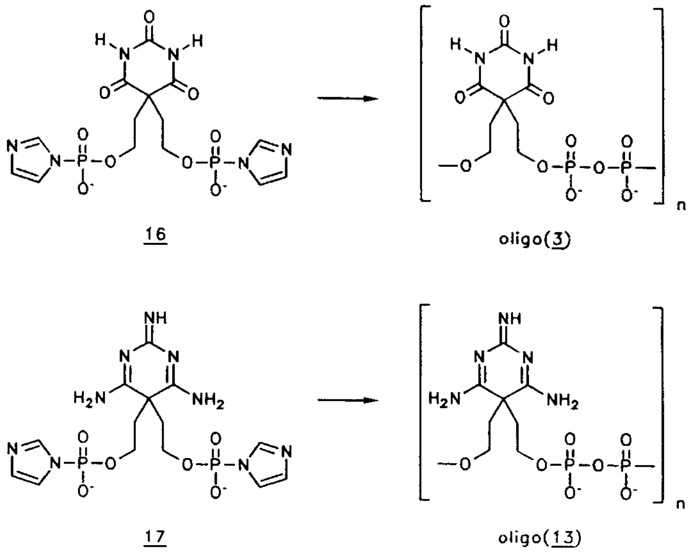
This ease of polymerization, combined with the potential for molecular recognition between barbituric acid derivatives and aminopyrimidines which are themselves derivatives of barbituric acid, suggested the possibility of studying a new model of template-directed replication based on achiral nucleotide analogs[6]. The goal of this model is to test the hypothesis that nonchiral nucleotide and oligonucleotide analogs may have been precursors of the first RNA molecules[7]. The parent compound of **3**, 5,5-di(2-hydroxyethyl)barbituric acid **1**, as well as the 2-amino analog **2**, have been synthesized previously from malonic ester[8]. In order to study all possible complementary pairs involving these analogs, we have now synthesized compounds **8** to **11** as well as the phosphorylated species **12** to **15**. (Scheme 1).

Results and Discussion

The four 5,5-disubstituted analogs **8** , **9** , **10** and **11** were prepared by condensation of urea or guanidine with dialkylated malononitrile **6** or dialkylated ethylcyanoacetate **7**. The latter compounds were obtained using a one pot synthesis in which first the disodium salt was prepared followed by dialkylation of malononitrile or ethylcyanoacetate. The disodium salt of malononitrile was prepared[9] using NaH in dimethylformamide (DMF). DMF was preferred above dimethyl sulfoxide (DMSO) because reactions in DMSO were accompanied by major side products. The two hydroxyethyl groups, acetal protected, were introduced at the α position of malononitrile using 1-bromo-2-(ethoxy-1-ethoxy)ethane **5** as alkylating agent[10], yielding **6** in 71%. The same procedure was used for dialkylation of ethylcyanoacetate to give **7** in 77%. The compounds **6** and **7** were condensed with urea or guanidine in refluxing ethanol in the presence of sodium ethanolate, followed by the removal of the protecting groups in 1 M HCl. The overall yield of the synthesis ranged from 20% for compound **8** to 53% for compound **10**. Finally the hydroxyl groups were bisphosphorylated with phosphorus oxychloride in trimethylphosphate[11] to give **12** to **15** in yields ranging from 60% to 87%. The imidazolides of **3** and **13** (**16** and **17**, respectively) were prepared by treatment with imidazole, triphenylphosphine and 2,2'-dithiodipyridine, in yields of 89 and 78%.

Oligomerization of compound **16** was performed in pyridine and yielded pyrophosphate-linked oligomers of **3** with chainlengths of 10 and longer (48%, Scheme 2). Compound **17** was oligomerized (yielding oligo(**13**)) in a mixture of pyridine and imidazole buffer (pH 6.5) containing $MgCl_2$ as catalyst. Oligomers with chainlengths of 10 and longer were formed in 63% yield. After treatment with alkaline phosphatase to remove terminal phosphate groups, the oligomers were fractionated on a Q-Sepharose column. The ^{31}P-NMR spectra of the oligomers showed only one signal at -11.4 (**3**) and -11.7 (**13**) ppm, respectively, from which it is concluded that only pyrophosphate bonds were formed. The ^{1}H-NMR spectra of the alkaline phosphatase-treated fractions showed the expected upfield shifts due to dephosphorylation of the terminal groups.

Oligomers (length 16-18) of **3** and **13**, which are capable of forming complementary pairs,[6] were studied by UV spectroscopy. Measurements of mixtures showed no detectable hypochromicity (Fig. 1). Because hypochromicity can largely be ascribed to stacking interactions, intramolecular as well as intermolecular,[12] we also checked for hyperchromicity as a result of the hydrolysis of oligo(**13**) with Zr(IV) and found no such effect.[13] We



Scheme 2

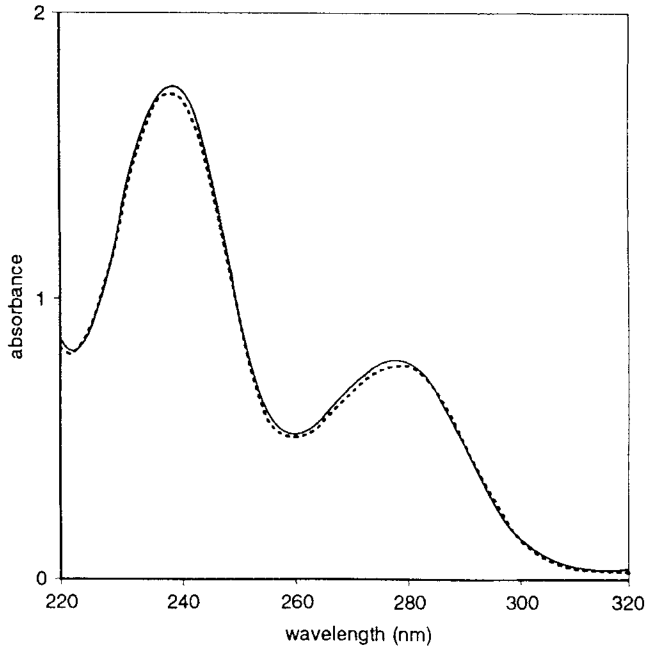


Figure 1. UV spectra at 4°C. Shown is the sum (dashed curve) of the two individual spectra of oligo(**2**) and oligo(**13**), and the spectrum of an equilmolar mixture of both oligomers (solid curve). Conditions: 0.67 mM of each oligomer (monomer equivalent), 0.1 M $MgCl_2$, 1.0 M NaCl and 0.2 M Tris HCl (pH 7.0).

therefore predict that complementary interactions between these analogs will only be observable in organic solvents, where hydrogen-bonding plays a more important role than stacking forces.

Experimental

Proton nuclear magnetic resonance spectra were recorded with a Varian EM 390 or Bruker 400 Mhz spectrometer, ^{31}P-NMR spectra (proton decoupled) with a Bruker 400 Mhz spectrometer. Infrared spectra were recorded with a Perkin-Elmer 457 spectrophotometer, ultraviolet spectra with a Beckman DU-40 spectrophotometer using cells of 1.0 or 10mm pathlength. Mass spectra were recorded on a 7070.E VG Analytical mass spectrometer using methane as chemical ionization gas at 70 ev. HPLC analyses were performed on a LiChrospher RP-18 (5 μm) column (Merck) in 0.02 M KH_2PO_4 with a linear gradient of methanol (0 - 6% over 12 minutes) at a flow rate of 1 ml/min. Peak detection was by absorbance monitoring at 254 nm. Q-Sepharose (Fast Flow), DEAE-Sephadex (A25) and alkaline phosphatase (type III from *Escherichia coli*) were purchased from Pharmacia; Dowex AG1 - X2 (Cl^-) and Dowex A6 50W-X8(H^+) from Bio-Rad Laboratories. HPLC analysis of oligomers was carried out on an RPC-5 column in 0.02 M NaOH with a linear gradient of $NaClO_4$ (0.02 M over 30 min) at 1.0 ml/min. The eluent was monitored at 240 or 280 nm.

1-Bromo-2-(ethoxy-1-ethoxy)ethane **5**.

Ethyl vinyl ether (85 ml, 0.89 mol) was added to a stirred solution of 2-bromoethanol (84.6 g, 0.68 mol) and pyridinium p-toluenesulphonate (1.2 g) in 200 ml CH_2Cl_2 at 0°C. After 1 h the solution was washed with water, dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil was purified by distillation under reduced pressure (bp 70-73°C at 20 mm Hg) to give product **5** in a yield of 86% (115 g, 0.58 mol).
IR (neat): 2880, 2960, 1100, 1375. ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 1.1 - 1.4 (t + d, 6H, 2 × CH_3, $J_t = 7.2$ Hz, $J_d = 5.3$ Hz), 3.3 - 4.0 (m, 6H, 3 × CH_2), 4.7 - 4.8 (q, 1H, CH, J = 5.4 Hz).

α,α-Di(2-[ethoxy-1-ethoxy]ethyl)malononitrile **6**.

To a stirred suspension of NaH (1.42 g, 59.2 mmol) in 20 ml anhydrous dimethylformamide (DMF) a solution of malononitrile (1.98 g, 30 mmol) in 15 ml DMF was added dropwise at 0°C. After 15 minutes 1-bromo-2-(ethoxy-1-ethoxy)ethane (15 g, 76 mmol) was added dropwise at 0°C, and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated *in vacuo* then the residue was dissolved in dichloromethane (200 ml) and washed twice with saturated sodium bicarbonate (75 ml). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The oil was purified by short path distillation *in vacuo* (bp 94-100°C at 0.4 mm Hg) to give 6.4 g (21.4 mmol, yield 71%) of **6**.

IR (neat): 2900, 2270, 1400, 1100, 950. ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 1.2 - 1.4 (m, 12H, 4 × CH_3), 2.2 - 2.4 (t, 4H, 2 × CH_2, J = 6.0 Hz), 3.3 - 4.1 (m, 8H, 4 × CH_2O), 4.6 - 4.8 (q, 2H, 2 × CH, J = 5.3 Hz).

α,α-Di(2-[ethoxy-1-ethoxy]ethyl)ethylcyanoacetate **7**.

A mixture of ethylcyanoacetate (3.16 g, 28 mmol) in 10 ml anhydrous DMF was added dropwise to a stirred suspension of NaH (1.49 g, 62 mmol) in 20 ml anhydrous DMF at 0°C. After 30 minutes, during which time the NaH dissolves, 1-bromo-2-(ethoxy-1-ethoxy)ethane (15 g, 76 mmol) was added dropwise at 0°C. The suspension produced was stirred for 3 hours at room temperature. The solvent was evaporated under reduced pressure and the precipitate was partitioned between CH_2Cl_2 and water. The organic layer was washed with saturated sodium bicarbonate, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification was by short path distillation *in vacuo* (bp 110-116°C at 0.4 mm Hg) to give **7** as a colorless oil in 77% yield (7.45 g, 21.6 mmol).

IR (neat): 2900, 2250, 1740, 1380, 1240, 1100, 940. ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 1.2 - 1.5 (m, 15H, 5 × CH_3), 1.8 - 2.6 (m, 4H, 2 × CH_2), 3.3 - 4.0 (m, 8H, 4 × OCH_2), 4.1 - 4.4 (q, 2H, CO_2CH_2, J = 7.4 Hz), 4.6 - 4.8 (q, 2H, 2 × CH, J = 5.3 Hz).

General synthesis of compounds **8**, **9**, **10**, *and* **11**.

To a refluxing solution of sodium ethanolate (10.8 mmol), prepared from 0.25 g sodium in 40 ml anhydrous ethanol, was added α,α-di(2-[ethoxy-1-ethoxy]ethyl)malononitrile (2.0 g, 6.7 mmol), or α,α-di(2-[ethoxy-1-ethoxy]ethyl)ethylcyanoacetate (2.0 g, 5.8 mmol) followed by urea (0.44 g, 7.3 mmol) or guanidine.HCl (0.69 g, 7.3 mmol) After 4 hours the mixture was neutralized with 6 M HCl and partitioned between CH_2Cl_2 and water. The organic layer was washed with water, dried ($MgSO_4$) and evaporated to dryness. The hydroxyl protecting groups were hydrolyzed by treating the residue with 1 M HCl (20 ml) for 5 minutes. HCl was removed by evaporation *in vacuo* and treating with Dowex OH$^-$. Purification was by recrystallization in ethanol.

4,6-Diamino-5,5-di(2-hydroxyethyl)-2-oxopyrimidine **8**.

Yield; 410 mg (1.9 mmol, 28%), ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.1 - 2.2 (t, 4H, 2 × CH_2, J = 6.6 Hz), 3.3 - 3.4 (t, 4H, 2 × OCH_2, J = 6.6 Hz). UV (H_2O) λ (nm) (ϵ): λ_{max} = 227.5 (18260), 265 (6560) in 0.1 M NaOH, λ_{max} = 254 (8550) in 0.1 M HCl. MS, m/z (rel. int.): 198 (100), M^+ - NH_2; 167 (27.2), M^+ - NH_2 - CH_2OH; 155 (53.5), M^+ - NH_2 - NCOH. Anal. Calcd. for $C_8H_{14}N_4O_3$. 0.69 HCl: C, 40.18; H, 6.19; N, 23.42. Found: C, 40.79; H, 6.19; N, 23.08.

2,4,6-Triamino-5,5-di(2-hydroxyethyl)pyrimidine **9**.

Yield: 850 mg (4.0 mmol, 60%), ^{1}H-NMR (D_2O + NaOD, external TMS) δ ppm: 2.1 - 2.2 (t, 4H, 2 × CH_2, J = 7.5 Hz), 3.2 - 3.5 (t, 4H, 2 × OCH_2, J = 7.5 Hz). UV (H_2O) λ (nm) (ϵ): λ_{max} = 231.5 (15500), 281 (6310) in 0.1 M NaOH, λ_{max} = 239.5 (18200), 278.5 (7790) in 0.1 M HCl. MS, m/z (rel. int.): 197 (97.3), M^+ - NH_2; 166 (18.3), M^+ - NH_2 - CH_2OH; 155 (28.8), M^+ - NH_2 - $NCNH_2$. Anal. Calcd. for $C_8H_{14}N_5O_2$. 1.45 HCl: C, 36.34; H, 5.89; N, 26.48. Found: C, 37.07; H, 6.24; N, 25.78.

6-Amino-5,5-di(2-hydroxyethyl)-2,4-dioxopyrimidine **10**.

Yield: 0.860 g (4.0 mmol, 69%), ^{1}H-NMR (D_2O , external TMS) δ ppm: 2.2 - 2.7 (m, 4H, 2 × CH_2), 3.6 - 3.7 (t, 4H, 2 × OCH_2, J = 6.0 Hz). UV (H_2O) λ (nm) (ϵ): λ_{max} = 253.0 (6270) in 0.1 M NaOH. MS, m/z (rel. int.): 199 (100), M^+ - NH_2; 168 (27.1) M^+ - NH_2 - CH_2OH; 156 (64.9) M^+ - NH_2 - CONH. Anal. Calcd. for $C_8H_{13}N_3O_4$. 0.35 HCl: C, 42.15; H, 5.90; N, 18.43. Found: C, 42.74; H, 6.03; N, 18.15.

2,6-Diamino-5,5-di(2-hydroxyethyl)-4-oxopyrimidine **11**.

Yield: 0.490 g (2.3 mmol, 40%), ^{1}H-NMR (D_2O + NaOD, external TMS) δ ppm: 2.0 - 2.7 (m, 4H, 2 × CH_2), 3.5 - 3.6 (t, 4H, 2 × OCH_2, J = 5.3 Hz). UV (H_2O) λ (nm) (ϵ): λ_{max} = 230 (17000), 268.5 (7460) in 0.1 M NaOH, λ_{max} = 257.5 (12500) in 0.1 M HCl. MS. m/z (rel. int.): 198 (100), M^+ - NH_2; 167 (18.6), M^+ - NH_2 - CH_2OH; 156 (63.6), M^+ - NH_2 - $NCNH_2$. Anal. Calcd. for $C_8H_{14}N_4O_3$. 1.57 HCl: C, 35.45; H, 5.80; N, 20.67. Found: C, 35.31; H, 6.18; N, 20.75.

General procedure for the phosphorylation of compounds **1**,**2** *and* **8**-**11**.

To a suspension of 100 mg of each of the compounds in 2 ml trimethylphosphate, 0.25 ml phosphorus oxychloride was added dropwise at 0°C. The mixture was stirred for 2 hours at 0°C, during which time a clear solution was formed. To the reacting mixture was added 30 ml of a cold solution of triethylamine bicarbonate (TEAB, 2 M, pH 8) at -20°C. The solvent was evaporated under reduced pressure. Purification was by column chromatography on DEAE-Sephadex using a TEAB gradient of 0.05 M to 0.5 M at pH 8.

5,5-Di(2-phosphoethyl)-2,4,6-trioxopyrimidine **3**.

Yield: 71%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: -0.330 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.302 - 2.330 (t, 4H, 2 × CH_2, J = 5.5 Hz), 3.775 - 3.815 (q, 4H, 2 × OCH_2, J = 5.4 Hz). UV (H_2O) λ (nm): λ_{max} = 238 in 0.1 M NaOH.

2-Amino-5,5-di(2-phosphoethyl)-4,6-dioxopyrimidine **4**.

Yield: 24%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: 0.909 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.503 - 2.540 (t, 4H, 2 × CH_2, J = 7.3 Hz) 3.742 - 3.797 (q, 4H, 2 × OCH_2, J = 7.3 Hz). UV (H_2O) λ_{max} 266.5 in 0.1 M NaOH, λ_{max} 263.0 in 0.1 M HCl.

4,6-Diamino-5,5-di(2-phosphoethyl)-2-oxopyrimidine **12**.

Yield: 60%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: -0.3387 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.552 - 2.579 (t, 4H, 2 × CH_2, J = 5.3 Hz), 3.843 - 3.883 (q, 4H, 2 × OCH_2, J = 5.4 Hz). UV (H_2O) λ (nm): λ_{max} = 226.5, 264.5 in 0.1 M NaOH, λ_{max} = 254.0 in 0.1 M HCl.

2,4,6-Triamino-5,5-di(2-phosphoethyl)pyrimidine **13** .

Yield: 81%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: 0.0282 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.441 - 2.470 (t, 4H, 2 × CH_2, J = 5.8 Hz), 3.743 - 3.786 (q, 4H, 2 × OCH_2, J = 5.8 Hz). UV (H_2O) λ (nm): λ_{max} = 238, 279 in 0.1 M NaOH, λ_{max} = 238.5, 276.5 in 0.1 M HCl.

6-Amino-5,5-di(2-phosphoethyl)-2,4-dioxopyrimidine **14** .

Yield: 87%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: -0.1677 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.209 - 2.257 (2 x t, 1.14H, 1.14 x CH', $J_{H',H''}$ = -14.4

Hz, $J_{H',OCH}$ = 4.6 Hz), 2.288 - 2.316 (t, 1.71H, 0.86 x CH' + 0.86 x CH", $J_{H,OCH}$ = 5.7 Hz), 2.454 - 2.525 (2 x dd, 1.14H, 1.14 x CH", $J_{H",H'}$ = -14.4 Hz, $J_{H",OCH}$ = 8.3 and 5.8 Hz), 3.737 - 3.814 (m, 4H, 2 x OCH_2). UV (H_2O) λ (nm): λ_{max} = 250 in 0.1 M NaOH.

2,6-Diamino-5,5-di(2-phosphoethyl)-4-oxopyrimidine **15** .

Yield: 72%. ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: -0.0160 (s). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.232 - 2.289 (2 × t, 2H, 2 × CH', $J_{H',H"}$ = - 14.8 Hz, $J_{H',OCH}$ = 4.7 Hz), 2.498 - 2.568 (2 × q, 2H, 2 × CH", $J_{H",H'}$ = - 14.8 Hz, $J_{H",OCH}$ = 8.4 and 5.0 Hz), 3.714 - 3.832 (m, 4H, 2 × OCH_2). UV (H_2O) λ (nm): λ_{max} = 231.5, 269 in 0.1 M NaOH, λ_{max} = 258.5 in 0.1 M HCl.

5,5-Di(2-imidazolylphosphoethyl)-2,4,6-trioxopyrimidine **16**.

A solution of **3** (500 μmol) in anhydrous DMSO (2.0 ml) and triethylamine (TEA, 300 μl) was added dropwise to an anhydrous mixture of DMF (3.0 ml) and TEA (300 μl), containing imidazole (170 mg, 2.5 mmol), triphenylphosphine (673 mg, 2.5 mmol) and 2,2'-dithiodipyridine (551 mg, 2.5 mmol), which was stirred for 2 h under argon at room temperature. The product was precipitated by addition of an anhydrous mixture of acetone (50 ml), diethylether (50 ml), TEA (4 ml) and a saturated solution of $NaClO_4$ in acetone (0.5 ml). The suspension was centrifuged, the supernatant was removed and the residue was washed three times with an anhydrous mixture of acetone-diethylether (1:1) and finally twice with anhydrous diethylether. After drying under vacuum, product **16** was obtained as a white powder (211 mg, 443 μmol, 89%). ^{31}P-NMR (DMSO-d6) δ ppm: -8.314 (s). ^{1}H-NMR (d_6-DMSO, external TMS) δ ppm: 1.869 - 1.902 (t, 4H, 2xCH_2, J = 5.16 Hz), 3.368 (s (broad), 2xOCH_2), 6.803, 6.967, 7.513 (3xs, 6H, 6xCH (imidazole)). The identity of product **16** was confirmed by HPLC after hydrolysis with sodium acetate.

2,4,6-Triamino-5,5-di(2-imidazolylphosphoethyl)pyrimidine **17**.

The triethylammonium salt of compound **13** (390 μmol) was converted into a tributylammonium salt on Dowex AG50WX8 (tributylammonium form) using a mixture of water and ethanol. The eluent was evaporated to dryness, coevaporated with ethanol and the residue was dried under vacuum. The tributylammonium salt was dissolved in DMSO (2.0 ml) and added dropwise to an anhydrous solution of imidazole (2.66 g, 39 mmol), triphenylphosphine (525 mg, 1.95 mmol) and 2,2'-dithiodipyridine (430 mg, 1.95 mmol) in

DMF (4.0 ml), which was stirred for 2 h under argon at room temperature. The product was worked up as described for compound **16** and obtained as a white powder (1.45 mg, 306 μmol, 78%). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.30 - 2.41 (t, 4H, 2xCH_2, J = 5.25 Hz), 3.52 - 3.71 (q, 4H, 2xOCH_2, J = 5.25 Hz), 7.05, 7.11, 7.78 (3xs, 6H, 6xCH (imidazole). The identity of product **17** was confirmed by HPLC after hydrolysis with sodium acetate.

General treatment of compounds **3** *,* **4** *and* **12** *to* **15** *with alkaline phosphatase.*

The compounds (0.05 μmol) were treated with 0.1 unit of alkaline phosphatase in 100 μl Tris HCl (0.04 M, pH 8) containing 0.02 M $MgCl_2$ at 37°C. Samples were taken after 5, 15, 60 and 120 minutes and analyzed by HPLC on a C-18 reverse phase column. Degradation of the bisphosphates produced, sequentially, the monophosphates and the dephosphorylated compounds.

General treatment of compounds **16** *and* **17** *with sodium acetate.*

The compounds (0.05 μmol) **16** and **17** were incubated with sodium acetate (0.1 M, pH 4.0) at 50°C. Samples were taken after 5, 15 and 60 minutes and analyzed by HPLC on a C-18 reverse phase column. The imidazole groups were hydrolyzed sequentially, producing compounds with one and ultimately two free phosphate groups (**3** or **13**).

Oligomerization of compound **16** *to produce oligo(***3***).*

Compound **16** (140 μmol) was suspended in a mixture of pyridine (370 μl) and water (6 μl) and incubated for 4 days at 50°C. The reaction mixture was evaporated and coevaporated twice with water, after which the reaction was analyzed by HPLC on an RPC-5 column. The residue was treated with a solution of Tris HCl (6.0 ml, 0.04 M, pH 8.0) containing 0.02 M $MgCl_2$ and 20 units alkaline phosphatase to remove free phosphate groups. After stirring for 4 h at 37°C the reaction was quenched by addition of EDTA (240 μl, 1.0 M, pH 9.0) and the mixture was diluted with a TEAB solution (200 ml, 0.1 M, pH 8). Separation of the oligomers was over a Q-Sepharose column with a linear gradient of TEAB (0.1-2.0 M, pH 8). Yield ($n>18$) as determined by UV was 27.1 μmol (19%). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.208-2.238 (t, CH_2 (without phosphate), J = 5.9 Hz), 2.311 (s (broad), 4H, 2xCH_2), 3.516 - 3.542 (t, CH_2 (without phosphate), J = 5.9 Hz, 3.831 (s (broad), 4H, 2xOCH_2). ^{31}P-NMR (D_2O, external trimethylphosphate) δ ppm: -11.383 (s (broad)).

Oligomerization of compound 17 to produce oligo(13).

A cold and fresly prepared solution of compound 17 (175 μmol) in imidazole buffer (300 μl, 1.0 M, pH 6.5) was added to a suspension of $MgCl_2.6H_2O$ (1.22 mg, 0.6 mmol) in pyridine (1.2 ml). A clear solution was formed and the reaction mixture was incubated for 5 days at 37°C. The reaction was quenched by addition of an EDTA solution (600 μl, 1.0 M, pH 9.0), followed by evaporation of the reaction mixture and coevaporation twice with water. The reaction was analyzed by HPLC on an RPC-5 column. The residue was treated with a solution of Tris HCl (9.0 ml, 0.04 M, pH 8.0) containing 0.02 M $MgCl_2$ and 20 units alkaline phosphatase to remove free phosphate groups. After stirring for 4 h at 37°C the reaction was quenched with EDTA (360 μl, 1.0 M, pH 9.0) and the mixture was diluted with a TEAB solution (200 ml, 0.02 M, pH 8). Separation of the oligomers was over a Q-Sepharose column with a linear gradient of TEAB (0.1-1.0 M, pH 8). Yield (n>18) as determined by UV was 43 μmol (25%). ^{1}H-NMR (D_2O, external TMS) δ ppm: 2.329 - 2.363 (t, CH_2 (without phosphate), J = 5.8 Hz), 2.452 - 2.480 (t, 4H, $2xCH_2$, J = 5.6 Hz), 3.483 - 3.519 (t, OCH_2 (without phosphate), J = 5.8 Hz), 3.813 (s (broad), 4H, $2xOCH_2$). ^{31}P-NMR (D_2O, external trimethyl phosphate) δ ppm: -11.740 (s (broad)).

UV-measurements.

Absorbance spectra were measured for solutions of oligo(3), oligo(13) and of an equimolar mixture of the two oligomers. The chainlength of the oligomers was 16-18. Three series of experiments were performed in which the concentrations of the oligomers were 0.1, 0.67 and 1.0 mM (theoretical monomer equivalent). The solutions contained $MgCl_2$ (0.1 M), NaCl (1.0 M) and Tris HCl (0.2 M, pH 7.0). Every series of experiments was performed at 4, 20 and 40°C. To ensure complete complex formation the reaction mixtures were stored overnight at 4°C, followed by 10 minutes at the appropriate temperature before the absorbance spectra were recorded.

Hydrolysis of oligo(13) with $ZrCl_4$ for determination of hyperchromicity.

All reactions were performed in 2.0 ml polyethylene tubes (Eppendorf). To each tube a solution of oligo(13) (0.2 μmol, chainlength 16-18) in sodium acetate buffer (2.0 ml, 0.04 M, pH 4.5) was added, followed by $ZrCl_4$ (8 μl, 1M). The tubes were mixed and incubated at 50°C for 0, 1, 2.5 and 5 h. UV absorbance spectra were measured, after which the reactions were quenched with EDTA (1.0 M, 24 μl), neutralized with NaOH (10

M, 15 μl) and filtered. The degree of degradation was determined by HPLC on an RPC-5 column.

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