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Synthesis and Hybridization Properties of an Acyclic Achiral Phosphonate DNA Analogue

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Abstract—Protected *N*-(2-hydroxyethyl)-*N*-(nucleobase-acetyl)aminomethanephosphonic acids (**6a–d**) of all four DNA nucleobases have been prepared and oligomerized by solid-phase synthesis. Four DNA decamers containing 1–10 of these 'PPNA' monomers were prepared and evaluated by T_m measurements (medium salt) for binding to their DNA and RNA complements. One central modification reduced the binding strongly ($\Delta T_m = -10\,^{\circ}C$), but contiguous PPNA monomers gave smaller effects, and the all-PPNA decamer bound to RNA with a ΔT_m of $-1.2\,^{\circ}C$ per modification. Thus PPNA oligomers are inferior DNA and RNA binders compared to the closely related and strongly binding PNA oligomers.

Introduction

The synthesis and characterization of new oligonucleotide analogues has for several years been an area of active research due to the potential of such compounds to exert therapeutic effects via antisense or antigene mechanisms.[1,2] Modification of the oligonucleotide is necessary in order to confer resistance in vivo to nucleases which cleave natural DNA outside the nucleus.[2] Compounds with modified phosphate groups (e.g. phosphorothioates, methylphosphonates, or phosphoramidates) have been studied extensively, and more recently a number of 'dephospho' analogues, where the phosphate linkage has been substituted with, for example, amide, hydroxylamine, and formacetal groups, have been evaluated.[3] Radically changed analogues, where the whole sugar phosphate backbone is substituted, have shown poor binding to DNA or RNA, with two exceptions: 'Morpholino-DNA'[4] and 'PNA'[5] (Figure 1). Both analogues bind more strongly than unmodified DNA to complementary DNA and RNA, and PNA has been shown to be highly discriminative towards mismatches, and to form very stable PNA:DNA:PNA triplexes. Here we describe the preparation and properties of a new acyclic and achiral phosphonate nucleotide analogue, 'PPNA' (Fig. 1), which has a structure similar to PNA, but has a phosphonate linkage between the units instead of the carboxamide linkage in PNA. Compared to PNA, the analogue PPNA has a more flexible backbone and is negatively charged. This would be expected to change such properties as binding to biomolecules and solubility in water which are relevant to the use of PPNAs as therapeutic agents. Recently and independent of us, van der Laan et al.[6(a)] and Peyman et al.[6(b)] have published on the preparation of the same oligomers. However, their monomers are different from ours, and they prepared oligomers derived from pyrimidines only, by solid-phase[6(a)] or solution[6(b)] chemistries.

Results and Discussion

The monomers used to obtain PPNA oligomers were prepared as shown in Scheme 1. The pixyl protecting group was chosen to give crystalline intermediates, and the methyl protecting group on the phosphonate group was chosen to allow easy removal with thiolate reagents. The common backbone structure (**3**) was obtained by

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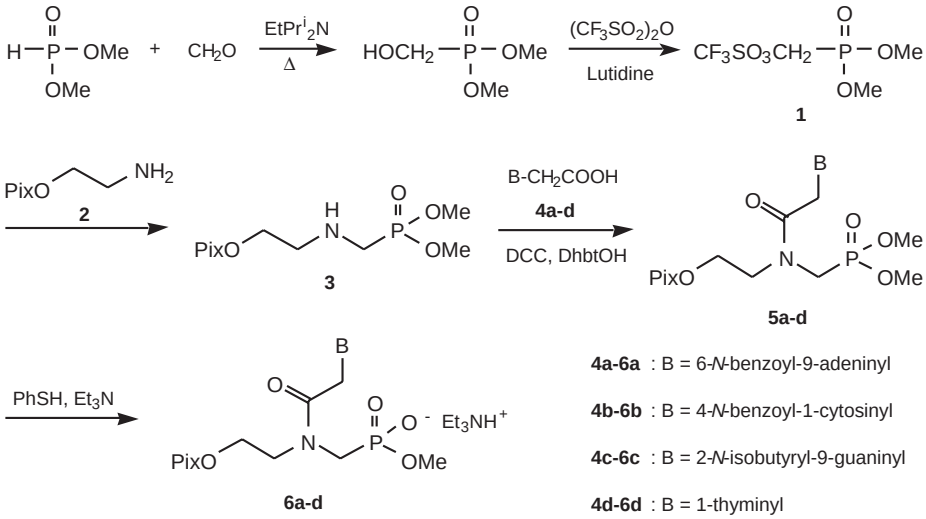


Scheme 1. Preparation of the PPNA monomers **6a**–**d**.

alkylation of 2-(pixyloxy)ethylamine (**2**) with dimethyl trifluoromethylsulfonylmethylphosphonate (**1**). Less reactive leaving groups than triflate (e.g. *p*-toluenesulfonate or oxytributylphosphonium) gave unacceptable amounts of demethylation of the phosphonate ester. Other methods to prepare **3** were also investigated (e.g. reductive alkylation of dimethyl aminomethylphosphonate with pixyloxyacetaldehyde, and Arbuzov reactions of protected *N*-chloromethylethanolamines with trimethyl phosphite), but the method given here was the easiest and gave a better yield. The backbone (**3**) was acylated with the four properly protected nucleobase-acetic acids (**4a**–**d**)[7]* to give the protected monomers (**5a**–**d**), and one of the methyl groups was finally removed with thiophenolate to give the monomers (**6a**–**d**) in good yields.

Solid-phase syntheses of PPNA oligomers (Scheme 2) were performed on **7**, a normal T polystyrene support (7 μmol/g, ABI, or 22 μmol/g, Pharmacia) pretreated with a reagent (5′ Phosphate-ON, Cruachem), which enabled cleavage of 3′-unmodified oligomers from the support with aq ammonia. The strongly activating reagent, PyFNOP,[9] and NMI were used to enable coupling of the relatively unreactive phosphonate monomers (**6a**–**d**). Coupling efficiencies, estimated from the absorbance of the released Pix^+ at 375 nm, were dependent on the support type and the dryness of **6a**–**d**. For freshly dried monomers the first 3 to 4 couplings gave an efficiency of ca. 95%,† but the coupling efficiency decreased for subsequent couplings to reach ca. 65% for base 9. This was not improved by double couplings. The reason for the decrease in coupling efficiencies after a few couplings is unknown, but could be caused by aggregation of the growing oligomers. Accordingly the low-loaded ABI polystyrene support gave somewhat better coupling efficiencies after base 4. Following synthesis, the phosphonate methyl groups were removed with $\mathbf{RS}^-$, disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate,[10] in methanol-acetic acid at rt for 2 h. It was crucial to buffer the $\mathbf{RS}^-$ reagent with acetic acid to pH 5 to 7 in order to avoid excessive cleavage of the base-labile linker to the support at this step. The PPNA oligomers were then cleaved from the support and the base protecting groups removed with conc. aq ammonia at 55 °C. Four decamers, containing 1 to 10 PPNA units, were prepared and characterized (Table 1). The PPNA DNA chimera **B** and **C** were purified by ethanol precipitation and gave yields (30–40%) in reasonable accordance with the pixyl and DMT efficiencies. The 5′-pixyl PPNA oligomers **D** and **E** were purified and the

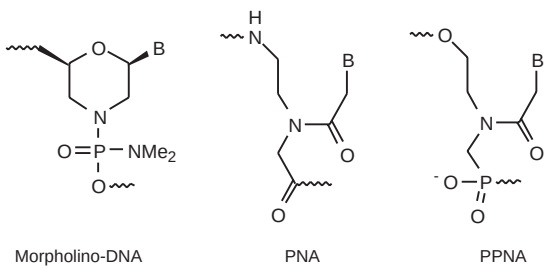


Figure 1. Structures of morpholino-DNA, PNA, and PPNA.

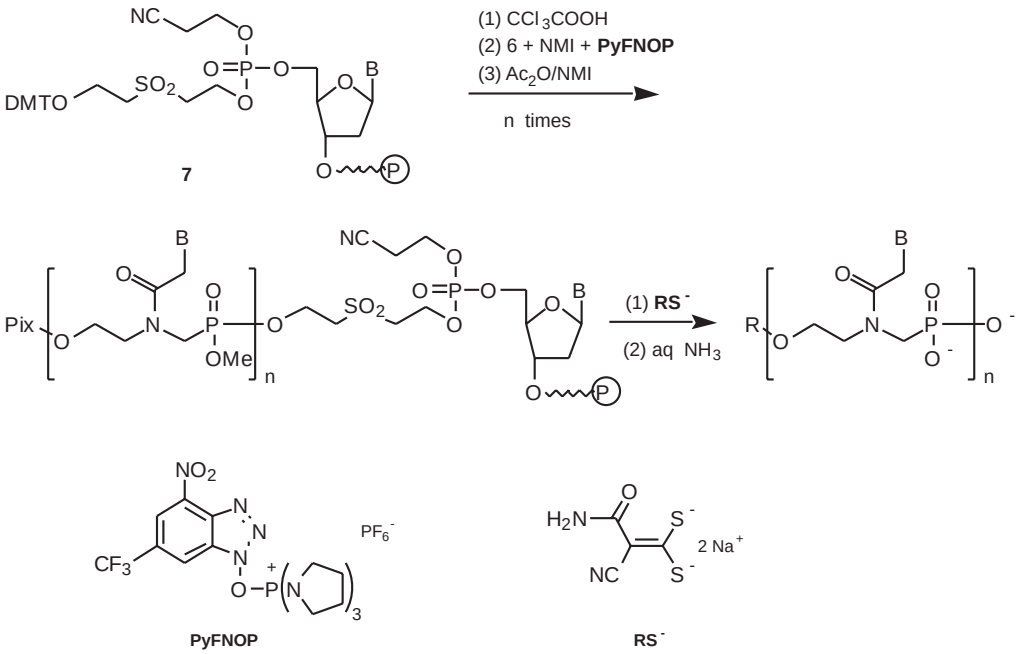
*The guanine derivative **4c** was prepared by a modified procedure of Will et al.[8]

†The ABI support gave the best results; CPG-based supports gave unsatisfactory coupling efficiencies (about 70%).

pixyl group removed on an Oligo–Pak column (Millipore), and the products isolated by lyophilization. However, the isolated yields were only 18% for **D** and 2% for **E** for unknown reasons. The purities were estimated by capillary gel electrophoresis to be ca. 90% for **B**–**D**, and ca. 55% for the fully modified **E** (Fig. 2).

Thermal melting temperature measurements (Table 1) against the complementary DNA or RNA decamer at medium salt concentrations showed that the oligonucleotides modified with PPNA hybridized poorly to both DNA and RNA. One PPNA unit in the middle of the DNA decamer **B** gave a T_m depression of ca. 10 °C, whereas the depression was smaller for PPNA units placed at the ends of the decamers **C** and **D** (ΔT_m −2 to −5 °C per modification). The fully modified decamer **E** did not give a deflection on the melting curve against DNA (T_m less than 10 °C), but bound to the RNA complement with a T_m of 26 °C (ΔT_m −1.2 °C per modification).

These results show that although one PPNA unit in a DNA strand disturbs the helix structure severely, contiguous PPNA units influence the duplex stability to a lesser degree, and a fully modified PPNA strand is able to bind to RNA with only a moderate T_m depression per modification. The PNA analogue of **E** (H-GTA GAT CAC T-NH_2) binds to its DNA complement with a T_m of 49 °C and to its RNA complement with a T_m of 54 °C



Scheme 2. Solid-phase synthesis of PPNA oligomers.

Table 1. PPNA oligomers prepared and their characterization by MALDI TOF MS and hybridization data

	Oligomer[a]	MALDI TOF MS[b]	T_m[c]	T_m[d]
A	5′-GTA GAT CAC T-3′	—	39.5	37.5
B	5′-GTA GAT CAC T-3′	3022.7 (3023.5)	30.0	27.0
C	5′-GTA GAT CAC T-3′	3104.9 (3101.5)	24.0	27.0
D	5′-GTA GAT CAC T-3′	3020.4 (3021.5)	32.0	—
E	5′-GTA GAT CAC T-3′	3095.0 (3094.6)	< 10	26.0

[a]Deoxyribonucleotides modified with PPNA units at the underlined poisition.
[b]Found (calcd for monoanions with H^+ as the counterions).
[c]Melting temperatures (°C) against the DNA complement, 5′ = AGT GAT CTA C-3′, ca. 5 μM in each strand, buffer 140 mM MaCl, 10 mM Na_2HPO_4, 1 mM EDTA, pH 7.2. The values were obtained by increasing the temperature from 5 to 70 °C and were reproducible within 1 °C.
[d]Melting temperature against the RNA complement, 5′-AGU GAU CUA C-3′, conditions as above.

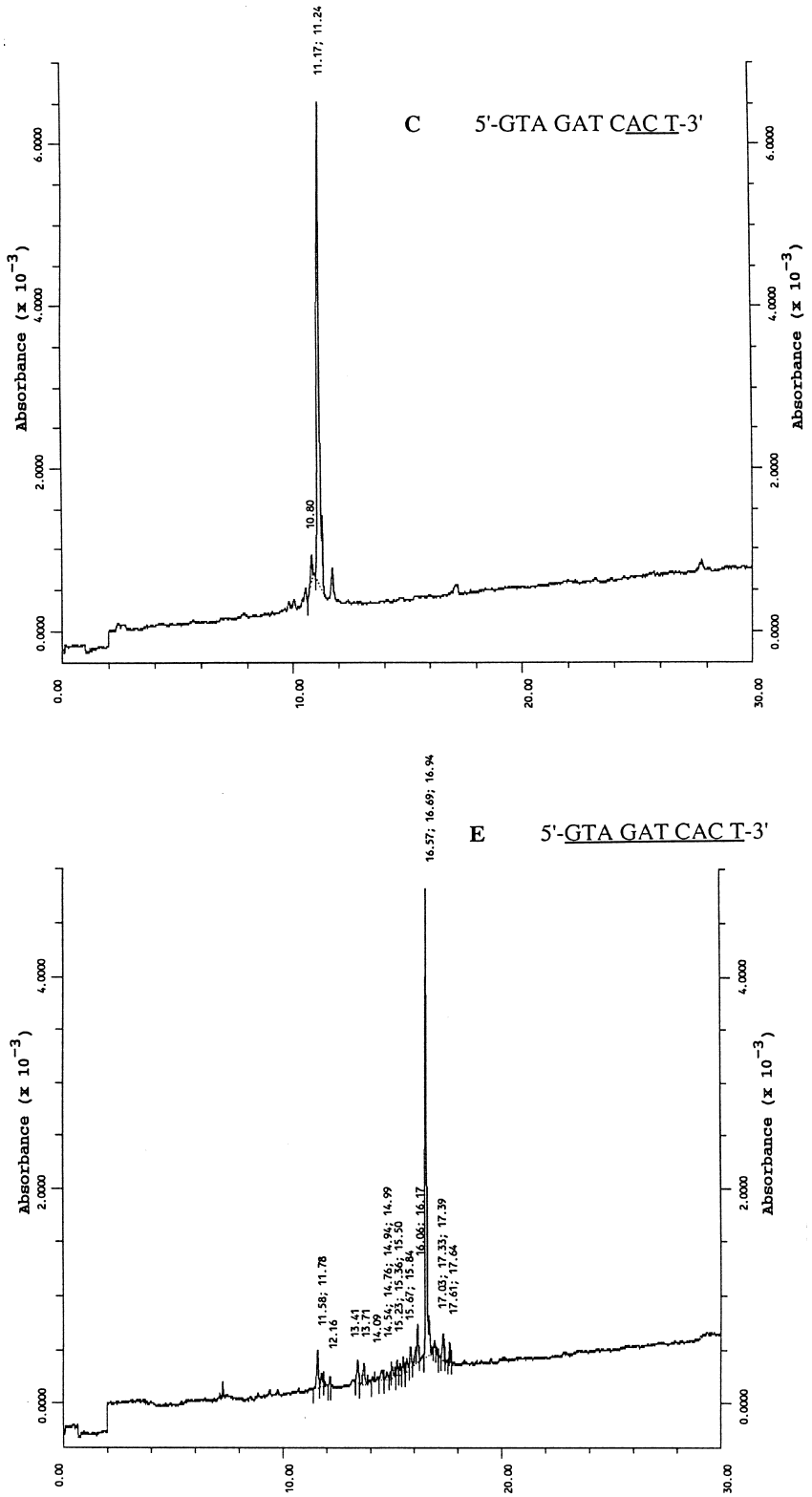


Figure 2. Capillary gel electrophoresis electropherograms of purified **C** and **E**.

(at 100 mM NaCl),[11] which show that substitution of the carboxamide groups of the PNA backbone with phosphonate groups severely compromise the hybridization to DNA and RNA. Whether the reason for this poor binding of PPNA oligomers, compared to PNA, is charge repulsion, a more flexible backbone (increased entropy loss), or a combination of these effects, are not known from the limited data of this work. The only other data on the binding properties of PPNA oligomers is T_m measurements of a T_9-mer against $d(A)_9$ at high salt conditions (1 M NaCl, 20 mM $MgCl_2$), where a T_m of 21 °C was found.[6(b)] This is close to the T_m of 22 °C measured for the corresponding DNA–DNA hybrid and indicate that charge repulsions between PPNA oligomers and DNA, which is of lesser importance at such high salt conditions, is a major determinant of the poor hybridization.

Conclusion

An efficient synthetic route to the PPNA monomers (**6a–d**) derived from the four DNA nucleobases has been developed. Oligomerization by solid phase synthesis was possible using the strong activator PyFNOP + NMI, although the coupling yields were low after the first four to five couplings. Decamers containing 1 to 10 PPNA monomers were prepared and shown by T_m measurements (medium salt) to bind less strongly than unmodified DNA to their DNA and RNA complements. Compared to the structurally related PNA oligomers, the negative charge and/or the higher flexibility of the PPNA oligomers thus makes them much poorer DNA and RNA binders.

Experimental

Dicyclohexylcarbodiimide (DCC), dimethyl phosphite, ethyldiisopropylamine, 3-hydroxy-1,2,3-benzotriazin-4-one (DhbtOH), *N*-(2-hydroxyethyl)phthalimide, and trifluoromethanesulfonic anhydride were from Aldrich, 9-chloro-9-phenylxanthene (pixyl chloride) and hydrazine monohydrate from Fluka, and paraformaldehyde from Merck. Solvents were HPLC grade from LAB-SCAN and were dried over molecular sieves (Grace 4 Å). 6-*N*-benzoyl-9-adeninylacetic acid.[7] 4-*N*-benzoyl-1-cytosinylacetic acid,[7] 2-*N*-isobutyryl-9-guaninylacetic acid,[8] and 1-thyminylacetic acid[7] were prepared according to literature methods. TLC was run on Merck 5554 silica 60 aluminium sheets, column chromatography on Merck 9385 silica 60 (0.040–0.063 mm). ^{31}P NMR spectra were run on a JEOL FX 90 Q spectrometer, ^1H NMR spectra on a Bruker 250 MHz or a Varian Unity 400 MHz spectrometer, FAB MS data were obtained on a JEOL HX 110/110 Mass Spectrometer, and MALDI TOF MS data on a HP G2025A LD-TOF spectrometer. Melting temperature measurements were performed on a Gilford Response II spectrometer or a Perkin–Elmer Lambda 20 spectrometer with a PTP 6 controller and UV TempLab software.

Dimethyl trifluoromethylsulfonyloxymethylphosphonate (1). A mixture of dimethyl phosphite (2.75 g, 2.30 mL, 25 mmol), paraformaldehyde (0.75 g, 25 mmol CH_2O), and ethyldiisopropylamine (0.25 g, 0.34 mL, 1.9 mmol) was heated under N_2 in an oil-bath at 140 °C for ca. 2 min when an exothermic reaction occurred to give dimethyl hydroxymethylphosphonate (δ_P 26, ca. 92% pure). The oil was dissolved under N_2 in a mixture of dry dichloromethane (100 mL) and 2,6-lutidine (4.4 mL, 38 mmol), cooled in an acetone/dry-ice bath, and trifluoromethanesulfonic anhydride (7.1 g, 25 mmol) in dichloromethane (100 mL) and pre-cooled to −78 °C was added in one portion. The rection mixture was stirred for 2.5 h at −78 °C and then at rt overnight. The resulting solution of **1** (δ_P 14.2, ca. 80% pure) was used directly in the preparation of dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]aminomethylphosphonate (**3**).

2-(9-Phenyl-9-xanthenyloxy)ethylamine (2). *N*-(2-Hydroxyethyl)phthalimide (3.8 g, 20 mmol) was dried by evaporation from dry pyridine (20 mL) and redissolved in dry pyridine (20 mL) under N_2. Pixyl chloride (5.3 g, 18 mmol) was added and the reaction mixture stirred at rt for 4 h. The mixture was poured into ice water (450 mL), and the gum that precipitated was dissolved in dichloromethane (100 mL). The dichloromethane solution was washed with sat aq $NaHCO_3$ (50 mL), brine (50 mL), dried (Na_2SO_4), and the solvent removed in vacuo to give a colourless foam. The foam was dissolved in a mixture of dichloromethane (100 mL) and methanol (20 mL), hydrazine monohydrate (6 mL, 120 mmol) was added, and the reaction mixture was stirred for 16 h at rt. The phthalazine formed was removed by filtration, the solution was washed with brine (30 mL), and the solvent removed in vacuo to give a clear oil. The oil was dissolved in dry ether (50 mL), filtered, and the solvent removed in vacuo to give the product as a colourless powder (5.2 g, 95%), mp 102–104 °C, pure according to TLC and ^1H NMR. TLC $R_f = 0.15$ (CH_2Cl_2:MeOH 95:5 v/v). ^1H NMR ($CDCl_3$) δ_H 7.33–6.92 (m, 13H, pixyl), 2.90 (t, $J = 5.3$, 2H, OCH_2CH_2N), 2.70 (t, $J = 5.3$, 2H, OCH_2CH_2N), 1.2 (br. s, 2H, NH).

Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]aminomethylphosphonate (3). A solution of 2-(9-phenyl-9-xanthenyloxy)ethylamine (**2**) (6.3 g, 21 mmol) in dry dichloromethane (45 mL) and ethyldiisopropylamine (7.8 mL, 45 mmol), pre-cooled to −10 °C, was added to the above solution of dimethyl trifluoromethylsulfonyl-

oxymethylphosphonate (**1**) at −10 °C under N_2. The reaction mixture was stirred at 0 °C for 2 h, washed with sat aq $NaHCO_3$ (2×50 mL), brine (50 mL), dried (Na_2SO_4), and evaporated in vacuo to a yellow oil which was purified on a silica column (7.5×14 cm) eluted with CH_2Cl_2:EtOAc:MeOH:Et_3N 60:33.7:6:0.3 v/v/v/v. The fractions containing the product were evaporated in vacuo and the residue recrystallized from ethyl acetate: pentane 1:1 (32 mL) to give pure **3** (6.6 g, 71%), colourless crystals, mp 81–85 °C. TLC R_f=0.22 (CH_2Cl_2: MeOH, 95:5). NMR ($CDCl_3$) δ_P 28.0, δ_H 7.32–6.93 (m, 13H, pixyl), 3.71 (d, J=10.6, 6H, $POCH_3$), 2.99 (t, J=5.2, 2H, OCH_2CH_2N), 2.84 (d, J=12.6, 2H, PCH_2), 2.73 (t, J=5.2, 2H, OCH_2CH_2N). FAB$^-$ MS 424.2 (M−CH3$^+$, calcd 424.1). Anal. ($C_{24}H_{26}NO_5P$), calcd C: 65.60, H: 5.96, N: 3.19; found C: 65.36, H: 5.98, N: 3.14%.

Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(6-*N*-benzoyl-9-adeninylacetyl)aminomethylphosphonate (5a). Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]aminophosphonate (**3**) (0.44 g, 1 mmol) was dissolved in a mixture of dry DMF (6 mL) and dry dichloromethane (6 mL) and cooled to 0 °C under N_2. Dry triethylamine (0.28 mL, 2 mmol), DCC (0.29 g, 1.4 mmol), and DhbtOH (0.18 g, 1.1 mmol) were added, followed by 6-*N*-benzoyl-9-adeninylacetic acid (**4a**) (0.33 g, 1.1 mmol). The reaction mixture was stirred under N_2 for 1 h at 0 °C followed by 20 h at rt. The reaction mixture was diluted with dichloromethane (75 mL), filtered, and the filtrate washed with sat aq $NaHCO_3$ (2×25 mL), brine (25 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was purified on a silica column (4×14 cm) eluted with CH_2Cl_2:EtOAc:MeOH:Et_3N 60:33:7:0.1 v/v/v/v. The fractions containing the product were evaporated in vacuo and lyophilized from dry acetonitrile to give **5a** as a colourless powder (0.54 g, 75%). The product was pure according to TLC and NMR. TLC R_f=0.16 (EtOAc:MeOH:Et_3N 9:1:0.1 v/v/v). NMR (DMSO-d_6; this and the following compounds exist at rt as two C(O)N rotamers; chemical shifts and coupling constants for the minor rotamer are given in brackets) δ_P 23.9 (23.7), δ_H 11.15 (s, 1H, NH), 8.58 (8.67) (s, 1H, H2), 8.20 (8.23) (s, 1H, H8), 8.07 (d, J=7.1, 2H, bz), 7.67–7.54 (m, 3H, bz), 7.41–7.10 (m, 13H, pixyl), 5.45 (5.40) (s, 2H, CH_2CO), 3.66 (4.17) (d, J=11.2 (9.7), 2H, PCH_2), 3.58 (3.80) (d, J=10.8 (10.7), 8H, $POCH_3$ + OCH_2CH_2N (hidden under the $POCH_3$ doublets)), 3.29 (3.08) (t, J=4.8 (5.2), 2H, OCH_2CH_2N). FAB$^+$MS 719.1 (M + H$^+$, calcd 719.2), 741.0 (M + Na$^+$, calcd 741.2).

Methyl triethylammonium *N*-[2-(9-phenyl-9-xanthenyloxy)-ethyl]-*N*-(6-*N*-benzoyl-9-adeninylacetyl)aminomethylphosphonate (6a). Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(6-*N*-benzoyl-9-adeninylacetyl)aminomethylphosphonate (**5a**) (0.36 g, 0.5 mmol) was dissolved under N_2 in a mixture of dry pyridine (2 mL), and dry triethylamine (1.4 mL), the solution degassed in vacuo, and thiphenol (0.50 mL, 5 mmol) added. After 1 h at rt the solution was added dropwise to vigorously stirred dry ether (100 mL). The precipitate was isolated by filtration, washed several times with dry ether, and dried by lyophilization twice from dry acetonitrile to give the product (**6a**) as a colourless powder (0.35 g, 87%). NMR ($CDCl_3$) δ_P 15.1 (16.1). NMR (DMSO-d_6) δ_P 12.8 (13.3), δ_H 11.1 (s, 1H, NH), 10.7 (s, 1H, NH), 8.63 (8.57) (s, 1H, H2), 8.13 (8.16) (s, 1H, H8), 8.05 (d, J=7.3, 2H, Bz), 7.65–7.52 (m, 3H, Bz), 7.37–7.08 (m, 13H, pixyl), 5.49 (5.35) (s, 2H, CH_2CO), 3.58 (3.93) (t, J=5.2, 2H, OCH_2CH_2N), 3.48 (d, J=9.7, 2H, PCH_2), 3.44 (3.25) (d, J=10.3 (9.9), 3H, $POCH_3$), ca. 3.02 (3.21) (m, 2H, OCH_2CH_2N, partly hidden), 3.00 (q, J=7.3, 6H, Et_3NH^+), 1.15 (t, J=7.3, 9H, Et_3NH^+). FAB$^-$ MS 703.4 (M−Et_3NH^+, calcd 703.2).

Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(4-*N*-benzoyl-1-cytosinylacetyl)aminomethylphosphonate (5b). This compound was prepared in the same way as **5a** from 4-*N*-benzoyl-1-cytosinylacetic acid (**4b**) (0.30 g, 1.1 mmol), yield 0.40 g (58%), pure according to TLC and NMR. NMR ($CDCl_3$) δ_P 23.4 (23.2). NMR (DMSO-d_6) δ_H 11.19 (11.21) (s, 1H, NH), 9.02 (d, J=8.1, 2H, bz), 7.82 (7.85) (d, J=7.1, 1H, H6), 7.65–7.50 (m, 3H, Bz), 7.41–7.11 (m, 14H, pix + H5), 4.82 (4.88) (s, 2H, CH_2CO), 3.72 (4.08) (d, J=8.0 (9.8), PCH_2), 3.59 (3.74) (d, J=10.6 (10.8), 6H, $POCH_3$), 3.7 (3.6) (partly hidden, 2H, OCH_2CH_2N), 3.24 (3.09) (t, J ca. 4, OCH_2CH_2N). FAB$^+$MS 695.2 (M + H$^+$, calcd 695.7), 717.2 (M + Na$^+$, calcd 717.7).

Methyl triethylammonium *N*-[2-(9-phenyl-9-xanthenyloxy)-ethyl]-*N*-(4-*N*-benzoyl-1-cytosinylacetyl)aminomethylphosphonate (6b). Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(4-*N*-benzoyl-1-cytosinylacetyl)aminomethylphosphonate (**5b**) (0.35 g, 0.5 mmol) was demethylated as described for **6a**. Yield 0.35 g (90%). NMR (DMSO-d_6) δ_P 12.6 (13.0), δ_H 11.2, 10.8 (2 x s, br, NH), 8.02 (d, J ca. 8, 2H, bz), 7.80–7.75 (m, 1H, H6), 7.64–7.50 (m, 3H, bz), 7.40–7.10 (m, 14H, pixyl + H5), 4.99 (4.79) (s, 2H, CH_2CO), 3.84–3.17 (m, 9H, $POCH_3$ + PCH_2 + OCH_2CH_2N), 3.00 (q, J ca. 7, 6H, Et_3NH^+), 1.16 (t, J ca. 7, 9H, Et_3NH^+). FAB$^-$ MS 679.4 (M−Et_3NH^+, calcd 679.2).

Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(2-*N*-isobutyryl-9-guaninylacetyl)aminomethylphosphonate (5c). This compound was prepared in the same way as **5a** from 2-*N*-isobutyryl-9-guaninylacetic acid (**4c**) (0.31 g, 1.1 mmol), but with increased reaction time (42 h), yield 0.44 g (63%), pure according to TLC and NMR. TLC (CH_2Cl_2:EtOAc:MeOH:Et_3N, 55:30:15:0.1) R_f=0.43. NMR (DMSO-d_6) δ_P 24.0 (23.9), δ_H 12.06 (12.08) (s,

1H, NH), 11.56 (11.51) (s, 1H, NH), 7.55 (7.69) (s, 1H, H8), 7.42–7.11 (m, 13H, pixyl), 5.12 (5.15) (s, 2H, CH_2CO), 3.74 (4.12) (d, $J=11.0$ (9.9), 2H, PCH_2), 3.58 (3.75) (d, $J=10.8$ (10.8), 6H, $POCH_3$), ca. 3.75 (3.53) (t, $J=(5.6)$, 2H, OCH_2CH_2N (the signals from the major rotamer were hidden)), 3.26 (3.07) (t, $J=5.1$ (5.6), 2H, OCH_2CH_2N), 2.76 (sept, $J=6.8$, 1H, *i*-butyryl), 1.10 (t, $J=6.8$, 6H, *i*-butyryl). FAB$^+$MS 701.2 ($M+H^+$, calcd 701.2), 723.2 ($M+Na^+$, calcd 723.2).

Methyl triethylammonium *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(2-*N*-isobutyryl-9-guaninylacetyl)aminomethylphosphonate (6c). Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(2-*N*-isobutyryl-9-guaninylacetyl)-aminomethylphosphonate (**5c**) (0.35 g, 0.5 mmol) was demethylated as described for **6a**. Yield 0.37 g (93%) as a colourless powder. NMR (DMSO-d_6) δ_P 14.1 (13.1), δ_H 12.06 (12.04) (s, 1H, NH), 11.88 (11.85) (s, 1H, NH), 10.05 (br s, 1H, NH^+), 7.65 (7.52) (s, 1H, H8), 7.39–7.08 (m, 13H, pixyl), 5.23 (5.06) (s, 2H, CH_2CO), 3.9–3.0 (m, $POCH_3+PCH_2+OCH_2CH_2N+HOD$), 3.04 (q, $J=7.2$, 6H, Et_3NH^+), 2.76 (sept, $J=6.8$, 1H, *i*-butyryl), 1.17 (t, $J=7.2$, 9H, Et_3NH^+), 1.09 (t, $J=6.8$, 6H, *i*-butyryl). FAB$^-$MS 685.3 ($M-Et_3NH^+$, calcd 685.2).

Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(1-thyminylacetyl)aminomethylphosphonate (5d). This compound was prepared in the same way as **5a** from 1-thymidylacetic acid (**4d**) (0.20 g, 1.1 mmol), yield 0.42 g (70%), pure according to TLC and NMR. Recrystallization from EtOAc gave white crystals (0.33 g, 55%). TLC $R_f=0.2$ (CH_2Cl_2:MeOH 95:5 v/v). NMR ($CDCl_3$) δ_P 23.5 (22.9). NMR (DMSO-d_6) δ_H 11.29 (s, 1H, NH), 7.41–7.03 (m, 14H, pixyl+H6), 4.69 (4.67) (s, 2H, CH_2CO), 3.58 (3.72) (d, $J=10.8$, 6H, $POCH_3$), 3.65 (3.5) (partly hidden, 2H, OCH_2CH_2N), 3.63 (4.02) (d, $J=11.3$ (9.7), 2H, PCH_2), 3.19 (3.06) (t, J ca. 5, 2H, OCH_2CH_2N), 1.70 (1.76) (s, 3H, CH_3). FAB$^+$MS 606.5 ($M+H^+$, calcd 606.2), 628.5 ($M+Na^+$, calcd 628.2). Anal. ($C_{31}H_{32}N_3O_8P$), calcd C: 61.48, H: 5.33, N: 6.94; found C: 61.42, H: 5.24, N: 6.84%.

Methyl triethylammonium *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(1-thyminylacetyl)aminomethylphosphonate (6d). Dimethyl *N*-[2-(9-phenyl-9-xanthenyloxy)ethyl]-*N*-(1-thyminylacetyl)aminomethylphosphonate (**5d**) (0.30 g, 0.5 mmol) was demethylated as described for **6a**. Yield 0.28 g (80%) as a colourless powder, pure according to TLC and NMR. TLC $R_f=0.19$ (CH_2Cl_2:EtOAc:MeOH:Et_3N, 49:38:12:1, v/v/v/v). NMR (DMSO-d_6) δ_P 13.4 (14.4), δ_H 11.22 (11.26) (s, 1H, T-NH), 10.7 (s, br, 1H, NH), 7.39–7.01 (m, 14H, pixyl+H6), 4.77 (4.66) (s, 2H, CH_2CO), 3.76–3.12 (m, 9H, $OCH_2CH_2N+POCH_3+PCH_2$), 3.00 (q, J ca. 7, 6H, Et_3NH^+), 1.74 (1.69) (s, CH_3), 1.16 (t, J ca. 7, 9H, Et_3NH^+). FAB$^-$MS 590.2 ($M-Et_3NH^+$, calcd 590.2).

Preparation of oligomers. Couplings were performed in 0.6 to 0.7 μmol scale (Pharmacia Primer Support T, ca. 22 μmol/g) or 0.2 μmol scale (ABI polystyrene T support, ca. 7 μmol/g). The DNA segments of the chimera were prepared by standard phosphoramidite chemistry. For **E** and the chimera **C** the support was first modified to give **7** by one coupling with a phosphoramidite reagent (5′ Phosphate-ON, Cruachem). The couplings with **6a–d** were performed manually by injecting (pr μmol of support) a freshly made solution of 20 μmol of **6**, 120 μmol of PyFNOP, and 300 μmol of NMI in CH_3CN:CH_2Cl_2 (1:1, 100 μL). After 15 min coupling the column was drained for the coupling mixture and connected to a DNA synthesizer (ABI 380), and washed with acetonitrile (2× 0.4 mL) and methanol (3×0.4 mL), followed by capping and removal of the pixyl group by standard 1 μmol cycle procedures. Following synthesis the support in the column was dried in a stream of air, and treated in the column for 2 h at rt with a solution of disodium 2-cyanoethylene-1,1-dithiolate trihydrate (130 mg) in methanol (0.5 mL), buffered with conc. acetic acid (14 μL). After repeated washing with methanol and drying the column was opened and the support treated with conc. aq NH_3:EtOH (3:1) for 6 to 18 h at 55 °C. The supernatant, after centrifugation, was collected with a syringe and the support washed twice with H_2O:EtOH (3:1). The combined solutions were evaporated to a small volume to remove EtOH, diluted with H_2O, and the oligomer purified by standard EtOH-precipitation for **B** and **C**, and standard reverse-phase purification with removal of the Pix groups (Oligo–Pak columns, Milligen) for **D** and **E**. The yield of purified oligomer was 26 OD (40%) for **B**, 22 OD (32%) for **C**, 12 OD (18%) for **D**, and 1.5 OD (2%) for **E**.

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