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An Approach Towards the Synthesis of Oligomers Containing a *N*-2-Hydroxyethyl-aminomethylphosphonate Backbone: A Novel PNA Analogue

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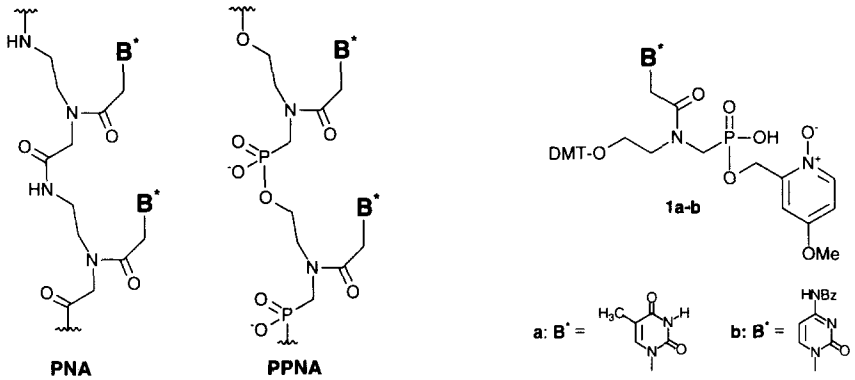
Abstract: A convenient route to the preparation of 4-methoxy-1-oxido-pyridine-2-methyl *N*-2-(4,4'-dimethoxytrityloxy)ethyl-*N*-thymin-1-yl-aminomethylphosphonate (**1a**, T*) and the corresponding N^4-benzoylcytosin-1-yl derivative **1b** (C*) is reported. These PPNA monomers proved to be suitable building blocks in a solid-support synthesis of the tetradecameric fragment (C*T*T*T*C*T*T*T*T*C*T*C*T*)dT. Copyright © 1996 Elsevier Science Ltd

In the last decade much effort has been directed towards the design and synthesis of natural and modified nucleic acids[1] that bind specifically to genes at the mRNA (antisense) or double stranded DNA (antigene) level. Recently it was revealed[2] that stacking interactions and Watson-Crick base pairing, as in B-DNA, could be effectively mimicked by replacing the deoxyribose phosphate backbone in DNA by an achiral polyamide backbone comprising *N*-(2-aminoethyl)glycine repeating units. This so-called PNA forms

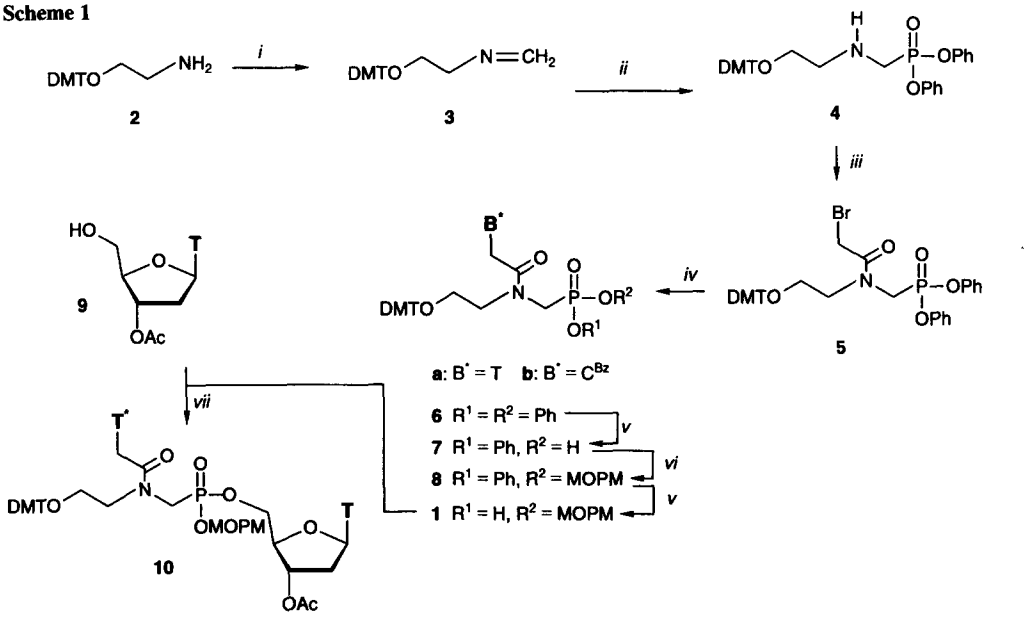


highly stable PNA-DNA(RNA) complexes in a sequence specific manner and may therefore present a promising lead to therapeutics targeting at specific genes. However, the lack of negative charge in PNA, which is one of the factors responsible for the stability of PNA-DNA(RNA) duplexes, leads to poor solubility in a physiological environment[3]. It occurred to us that replacement of the amido group in PNA by a charged phosphonate linkage would give a water soluble PNA analogue (*i.e.* PPNA).

We here report the preparation of the *N*-(thymin-1-yl)- and *N*-(N^4-benzoylcytosin-1-yl)-*N*-2-hydroxyethyl-aminomethylphosphonate building units **1a-b**, the 4-methoxy-1-oxido-pyridine-2-methyl

(MOPM) group of which will facilitate the introduction of the phosphonate linkages[4,5] in PPNA. The use of the PPNA building units **1a-b** is further illustrated in a solid-support synthesis of the tetradecameric fragment (C*T*T*T*C*T*T*T*T*C*T*C*T*)dT **15**.

Scheme 1

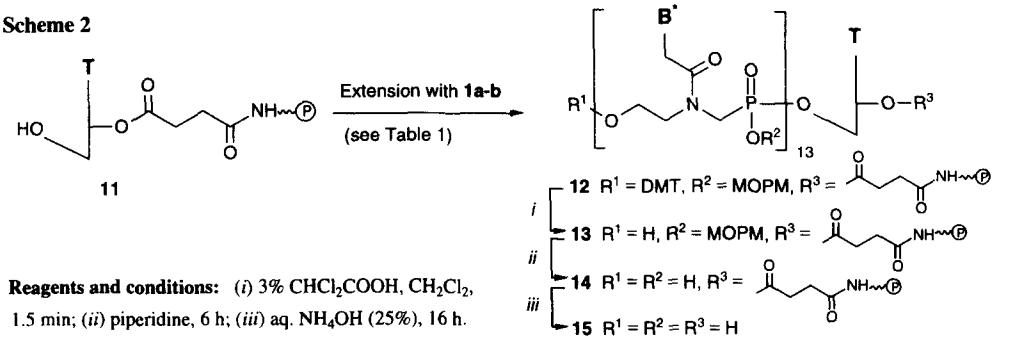


Reagents and Conditions: (*i*) CH_2O (1.2 eq) in ethyl acetate, 15 min; (*ii*) diphenyl phosphite (1.0 eq), toluene, 75°C, 2 h; (*iii*) Bromoacetic anhydride (1.0 eq), *N*-Me-morpholine (1.0 eq) in toluene (80% based on **2**); (*iv*) Thymine (1.2 eq), DBU (1.2 eq) [or N^4-benzoylcytosine (1.2 eq), NaH (1.2 eq)] in dimethylformamide, 75°C, 1 h (**6a**: 75%, **6b**: 65%); (*v*) 0.4 M DBU in CH_3CN/H_2O (95/5), 15-30 min (90-95%); (*vi*) MOPM-OH (2.0 eq), TPS-Cl (2.0 eq), 4-methoxy-1-oxido-pyridine (6.0 eq) in CH_3CN, 30 min (70-75%); (*vii*) TPS-Cl (1.5 eq) in CH_3CN/C_6H_5N (4/1, v/v), 1 min (75%).

The preparation of the required PPNA building units **1a-b** could be realized by the sequence of reactions depicted in Scheme 1. Condensation of 2-(4,4'-dimethoxytrityloxy)ethylamine (**2**)[6] in ethyl acetate with a slight excess of formaldehyde gave, after workup, the crude imino derivative **3**. Treatment of **3** at elevated temperature with an equimolar amount of diphenyl phosphite[7] led, as gauged by ^{31}P-NMR spectroscopy, to a near quantitative formation of the diphenyl phosphonate derivative **4** (δ_p = 20.2 ppm). Acylation of crude **4** with bromoacetic anhydride[8] in the presence of *N*-methyl-morpholine yielded, after purification by flash chromatography, homogeneous **5**[9]. Reaction of the intermediate bromoacetyl derivative **5** with thymine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted, after purification, in the isolation of homogeneous **6a** (B^* = T)[9] in 75% yield. Similarly, treatment of **5** with N^4-benzoylcytosine, using sodium hydride as a base, gave the corresponding cytosinyl derivative **6b** (B^* = C^{Bz})[9] in 65% yield. Transformation of **6a,b** into the PPNA building block units **1a,b**, carrying the catalytic 4-methoxy-1-oxido-pyridine-2-methyl phosphonate protecting group, entailed the following three-step procedure. Conversion[10] of the individual diphenyl phosphonates **6a,b** under the influence of DBU-H_2O led to corresponding monophenyl phosphonates **7a,b**[9]. Condensation[11] of the latter compounds with 2-hydroxymethyl-4-methoxy-1-oxido-pyridine (MOPM-OH) under the agency of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) and

4-methoxy-1-oxido-pyridine led to compounds **8a,b**[9]. Finally, removal[11] of the phenyl phosphonate protecting group from both **8a,b** with DBU-H_2O proceeded smoothly to give, after purification, **1a**[9] and **1b**[9] in an overall yield of 65% and 60%, respectively.

Scheme 2



Reagents and conditions: (*i*) 3% $CHCl_2COOH$, CH_2Cl_2, 1.5 min; (*ii*) piperidine, 6 h; (*iii*) aq. NH_4OH (25%), 16 h.

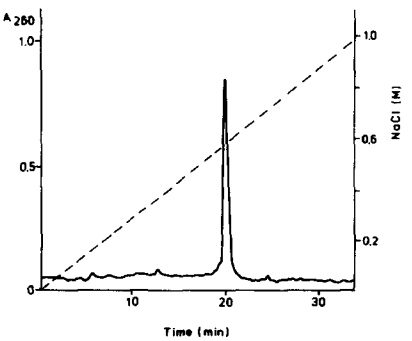
Prior to the intended solid-support synthesis of the tetradecameric fragment **15**, the rate of the phosphonylation of the thymidine derivative **9** with **1a** was monitored by ^{31}P-NMR spectroscopy. It was established that the TPS-Cl mediated condensation went to completion within 1 min. Workup and purification gave the homogeneous dimer **10** (δ_p = 22.7 and 23.1 ppm) in 75% yield. The favourable outcome of the latter condensation was a stimulus to assemble tetradecamer **15** using a fully automated DNA synthesizer. The assembly of the target oligomer **15** comprises (see Scheme 2) extension of the thymidine derivative **11**, immobilized to controlled pore glass *via* a succinyl linker, with the PPNA units **1a-b**[12]. Thus, sequential elongation of immobilized **11** with the appropriate units **1a-b** following the stepwise protocol summarized in Table 1 afforded, after thirteen elongation cycles, the fully protected and immobilized fragment **12**. The coupling efficiency of each elongation cycle was higher than 96%, as gauged spectrophotometrically by the released DMT-cation. Immobilized **12** was deblocked and released from the solid-support by the following three-step procedure. Acidolysis of the DMT group (R^1) from **12**, and subsequent removal of the MOPM group (R^2) in **13** with neat piperidine[5], led to partially protected and immobilized **14**. Finally, *N*-debenzoylation and release from the solid-support was effected by ammonolysis of **14**. Purification of the resulting crude product by ion-exchange chromatography (Q-Sepharose) and subsequent desalting (Sephadex G-25) gave tetradecameric fragment **15**, the homogeneity and identity of which was established by fast protein liquid chromatography (FPLC, Figure 1)[13] as well as mass spectro-

Table 1: Chemical steps involved in each elongation cycle of PPNA

Step	Manipulation	Solvents and reagents[a]	Time (min)
1	Detritylation	3% $CHCl_2COOH$ in CH_2Cl_2	1.5
2	Wash	CH_3CN	3.0
3	Coupling	**1a-b**[b], TPS-Cl[c] in CH_3CN/ C_6H_5N (4/1, v/v)	5.0
4	Wash	CH_3CN	30.0
5	Capping	Ac_2O/*N*-Me-imidazole/Collidine /THF, (2/3/2/32, v/v/v/v)	0.5
6	Wash	CH_3CN	2.0

[a] Reactions were performed on 28 mg (1 µmole) of resin. [b] 0.08 M **1a** (or **1b**) in CH_3CN/C_6H_5N (4/1, v/v), 5 eq. [c]0.25 M TPS-Cl in CH_3CN/C_6H_5N, (4/1, v/v), 15 eq.

Figure 1: FPLC pattern of purified **15**[13]



scopy (MALDI-TOF).

In conclusion, the successful assembly of tetradecameric fragment **15** presented in this paper may open the way for a general solid-support synthesis of homogeneous PPNA.

A full report on the solid-support synthesis and biochemical properties of homogeneous PPNA will be published in due course.

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6. Compound **2** was readily prepared by tritylation of commercially available *N*-(hydroxyethyl)phtalimide with DMT-Cl (1.1 eq) in pyridine and subsequent treatment of the tritylated product with hydrazine (2.0 eq) in 80% overall yield.
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9. Relevant analytical data for **1a**, **1b**, **5**, **6a**, **6b**, **7a**, **7b**, **8a** and **8b**. **1a**: ^{31}P NMR data ($CDCl_3$): δ = 15.0 and 15.7 ppm; ^{13}C NMR data ($CDCl_3$): δ = 12.0 (CH_3, thymine), 46.7 (NCH_2), 48.4 (PCH_2), 55.0 (OCH_3, DMT), 56.3 (OCH_3, MOPM), 60.7 (CH_2, MOPM); EI (*m/z*): 760 $[M+H]^+$. **1b**: ^{31}P NMR data ($CDCl_3$): δ = 15.0 and 15.4 ppm; ^{13}C NMR data ($CDCl_3$): δ = 46.6 (NCH_2), 48.4 (PCH_2), 55.1 (OCH_3, DMT), 56.2 (OCH_3, MOPM), 60.9 (CH_2, MOPM), 96.6 (C-5); EI (*m/z*): 848 $[M+H]^+$. **5**: ^{31}P NMR data ($CDCl_3$): δ = 14.8 ppm. **6a**: ^{31}P NMR data ($CDCl_3$): δ = 14.6 ppm; ^{13}C NMR data ($CDCl_3$): δ = 11.5 (CH_3, thymine), 46.7 (NCH_2), 47.6 (PCH_2), 54.7 (OCH_3, DMT), 119.8 (CH, Ph). **6b**: ^{31}P NMR data ($CDCl_3$): δ = 14.6 ppm; ^{13}C NMR data ($CDCl_3$): δ = 46.6 (NCH_2), 47.4 (PCH_2), 54.5 (OCH_3, DMT), 96.6 (C-5), 120.0 (CH, Ph). **7a**: ^{31}P NMR data ($CDCl_3$): δ = 11.0 and 11.1 ppm; ^{13}C NMR data ($CDCl_3$): δ = 11.9 (CH_3, thymine), 46.5 (NCH_2), 47.7 (PCH_2), 54.5 (OCH_3, DMT), 119.6 (CH, Ph). **7b**: ^{31}P NMR data ($CDCl_3$): δ = 11.4 and 11.6 ppm; ^{13}C NMR data ($CDCl_3$): δ = 46.7 (NCH_2), 47.6 (PCH_2), 55.0 (OCH_3, DMT), 96.8 (C-5), 120.1 (CH, Ph). **8a**: ^{31}P NMR data ($CDCl_3$): δ = 19.2 ppm; ^{13}C NMR data ($CDCl_3$): δ = 12.0 (CH_3, thymine), 46.3 (NCH_2), 47.5 (PCH_2), 54.6 (OCH_3, DMT), 56.0 (OCH_3, MOPM), 119.6 (CH, Ph). **8b**: ^{31}P NMR data ($CDCl_3$): δ = 19.3 ppm; ^{13}C NMR data ($CDCl_3$): δ = 46.5 (NCH_2), 47.5 (PCH_2), 54.6 (OCH_3, DMT), 56.2 (OCH_3, MOPM), 96.4 (C-5), 119.7 (CH, Ph).
10. To a stirred solution of **6a** (or **6b**) (1.0 mmol) in a mixture of CH_3CN/H_2O (95/5, v/v, 10 mL) was added DBU (600 µL, 4.0 mmol). After 15 min at 20°C, the reaction mixture was evaporated to dryness. The residue was coevaporated with CH_3CN, redissolved in CH_2Cl_2 (15 mL) and precipitated from diethyl ether (200 mL). The precipitate was collected by centrifugation and dried *in vacuo* to give **7a** (or **7b**).
11. To a solution of **7a** (or **7b**) (1.0 mmol) and 4-methoxy-1-oxido-pyridine (0.75 g, 6.0 mmol) in dry CH_3CN (7 mL) was added TPS-Cl (0.61 g, 2.0 mmol). After stirring for 5 min, 2-hydroxymethyl-4-methoxy-1-oxido-pyridine (0.31 g, 2.0 mmol) in CH_3CN (3 mL) was added. The mixture was stirred for another 30 min, quenched with aqueous $NaHCO_3$ (M, 10 mL) and extracted with CH_2Cl_2 (2x 50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The phosphonate diester **8a** (or **8b**) was purified by silica gel colomn chromatography (eluent: CH_2Cl_2/CH_3OH, 10/0 to 9/1, v/v). Subsequently the phenyl group was selectively removed by treatment of **8a** (or **8b**) with 0.4 M DBU in CH_3CN/H_2O (95/5, v/v, 10 mL) for 30 min to give **1a** (or **1b**).
12. The solid-support synthesis was carried out on a Pharmacia Gene Assembler using preloaded 5-*O*-DMT-dT-succinyl-CPG (loading 35 µmole/g, purchased from Millipore) as support.
13. FPLC analysis was carried out on a Pharmacia mono-Q HR 5/5 column (anion exchange). Gradient elution was performed at 20°C by building up a gradient starting with buffer A (0.01 M NaOH, pH = 12) and applying buffer B (0.01 M NaOH, 1.2 M NaCl, pH = 12.0) at a flow rate of 2.0 mL/min.

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