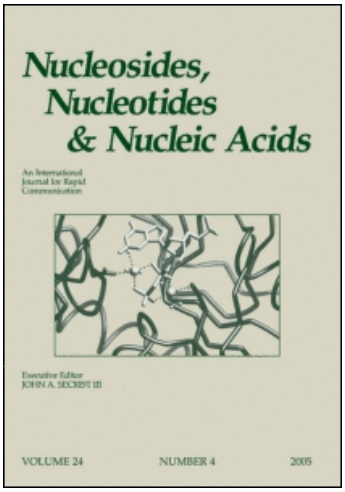

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SYNTHESIS OF NOVEL DNA ANALOGUES CONTAINING AMINOMETHYLPHOSPHONATE INTERNUCLEOSIDE LINKAGES

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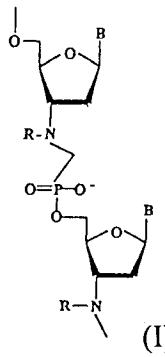
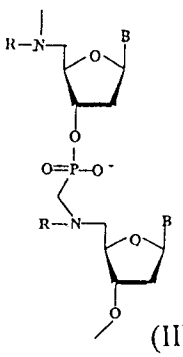
ABSTRACT: Modified oligodeoxyribonucleotides with aminomethylphosphonate bonds between nucleoside residues were prepared and investigated for their hybridisation properties toward DNA and RNA .

Last years an impressive number of oligonucleotide analogues has been developed as potential therapeutics for the antisense and antigene approach[1]. Recently, we reported the preparation of a DNA analogue, in which the natural phosphodiester bonds have been replaced by the carboxamidophosphate linkages[2] and a novel phosphonate PNA analogues containing N-2-(hydroxyethyl)aminomethyl[3], or N-2-(aminoethyl)aminomethyl phosphonate[4] backbone. We now report the preparation of a set of new aminomethyl-phosphonate DNA analogues (I-III) containing -N(R)-CH_2-$P(O_2)^-$-O- internucleoside linkages.



(I)



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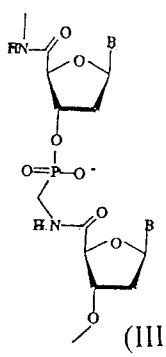

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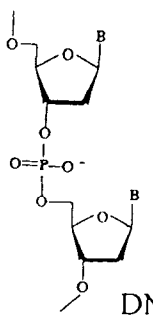
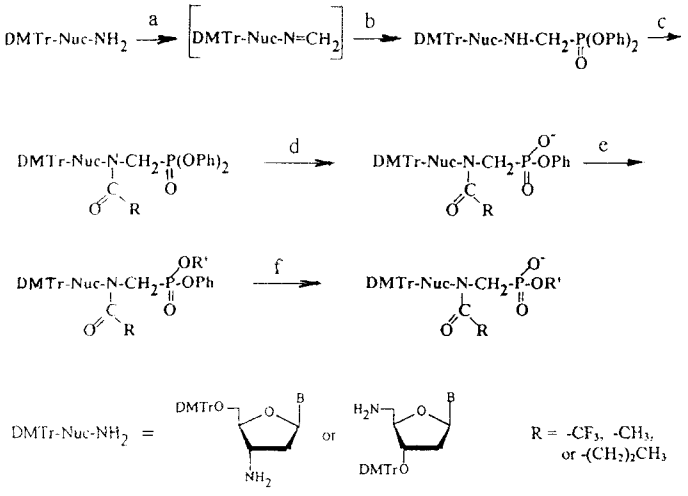


DNA

(a) R = H ; (b) R = Acyl

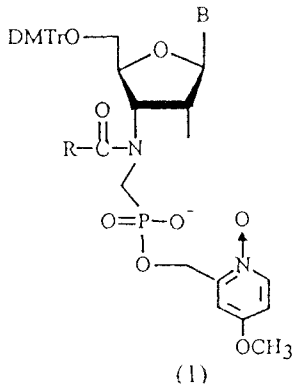


Reagents and conditions:

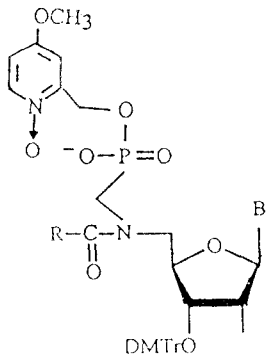
(a) CH_2O (1.2eq.) in CH_3CN, 15 min;
(b) diphenyl phosphite (1 eq.) in toluene, 75°C, 1-2 h;
(c) $(Acyl)_2O$ (3 eq.) / MeIm (3 eq.) in toluene, 1-2 h;
(d) DBU (0.5 M) in CH_3CN - H_2O (9.5 : 0.5, v/v), 15-30 min;
(e) R'-OH (1.25 eq.), TPSNT (1.5 eq.) in acetonitrile - pyridine (4:1, v/v), 10 min;
(f) DBU (0.5 M) in CH_3CN - H_2O (9.5 : 0.5, v/v), 15-30 min.

Scheme 1

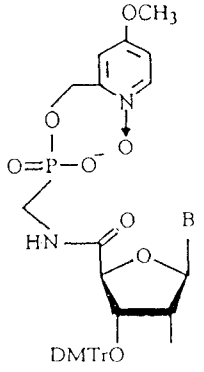
Procedures to obtain corresponding thymine and cytosine monomeric units having the combination of blocking groups compatible with the DNA phosphotriester synthesis has been developed. As a starting compounds for the synthesis of monomers (1) and (2), 5'- or 3'-aminonucleosides with the dimethoxytrityl protective group on a OH-function were



(1)

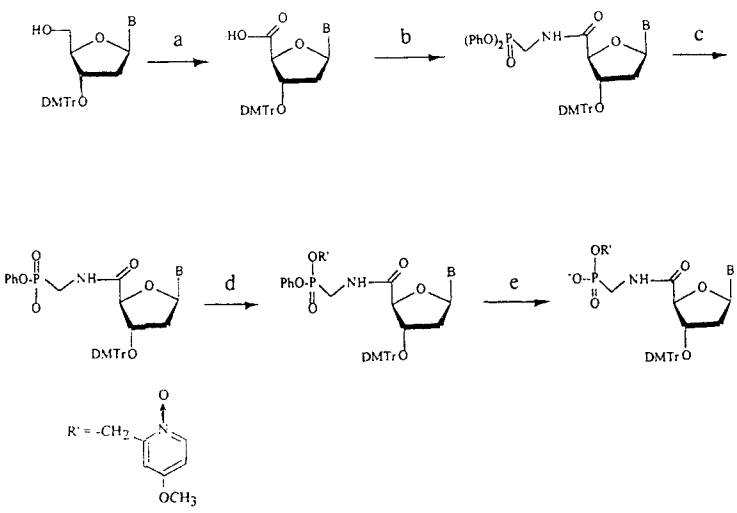


(2)



(3)

R = $-CF_3$, $-CH_3$, or $-CH_2CH_2CH_3$



Reagents and conditions:

(a) $RuCl_3.H_2O$ (cat), $K_2S_2O_8$ (4.5 eq.), 1 M KOH, 6 h[7];
(b) $(PhO)_2PO\text{-}CH_2\text{-}NH_2$ (1.1 eq.), MeIm (1.5 eq.), BOP (1.1 eq.), in CH_2Cl_2, 10 min;
(c) DBU (0.5 M), in CH_3CN - H_2O (9.5 : 0.5, v/v), 15 min;
(d) R'OH (1.25 eq.), TPSNT (1.5 eq.) in CH_3CN - pyridine (4:1, v/v), 5 min;
(e) DBU (0.5 M) in CH_3CN - H_2O (9.5 : 0.5, v/v), 30 min.

Scheme 2

chosen. Using the same chemistry as for the synthesis of phosphono-PNA analogues[3,4], we prepared several variants of the nucleoside aminomethylphosphonates (1,2) having different N-acyl blocking groups for the secondary amino function (SCHEME 1). Easy removable trifluoroacetyl blocking group was used to obtain the zwitterionic DNA analogues (Ia) and (IIa), whereas acetyl and butyryl N-blocking groups were used to prepare corresponding anionic N-acylaminomethylphosphonate analogues (Ib, IIb), in which N-acyl group is external to the internucleoside linkage. For comparison with analogues (Ib, and IIb), the preparation of the deoxyribonucleoside derivative of the type (3) for the synthesis of oligomers (III) with carboxamidomethyl phosphonate linkers, in which amide bond is a part of the internucleoside linkage, was realised by the chain of reactions shown in SCHEME 2. All monomers bear 1-oxido-4-methoxy-2-picolyl phosphonate protecting group, which could enhance the rate of the phosphonate function esterification as an effective O-nucleophilic catalyst[5,6].

TABLE. One elongation cycle for the solid phase phosphotriester synthesis of aminomethylphosphonate DNA analogues (I-III)

Step	Solvents and reagents*	Time(min)
1. Detritylation	3%DCA in dichloromethane	1.5
2. Wash	Acetonitrile	3.0
3. Coupling	0.08 M P-component; 0.25 M TPSNT in acetonitrile - pyridine (4:1, v/v)	5-10
4. Wash	Acetonitrile	0.5
5. Capping	Ac_2O - MeIm - collidine - THF (1 : 1.6 : 1 : 16.4, v/v/v/v)	1.0
6. Wash	Acetonitrile	2.0

*Reactions were performed using 30 mg of CPG support (1 μmol of the first nucleoside).

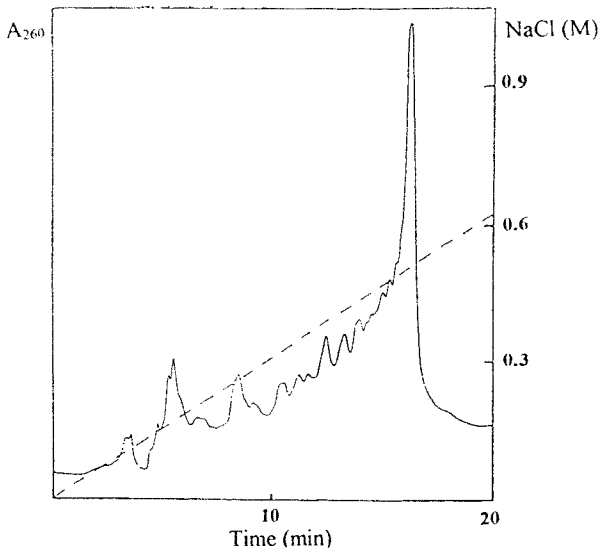


FIG 1. Analysis of the crude modified $(T^*)_{15}$ oligomer of the type (III) by anion-exchange FPLC on Pharmacia Mono-Q column with a flow rate 0.5 ml/min using a gradient of NaCl in 0.01 M NaOH (pH=12).

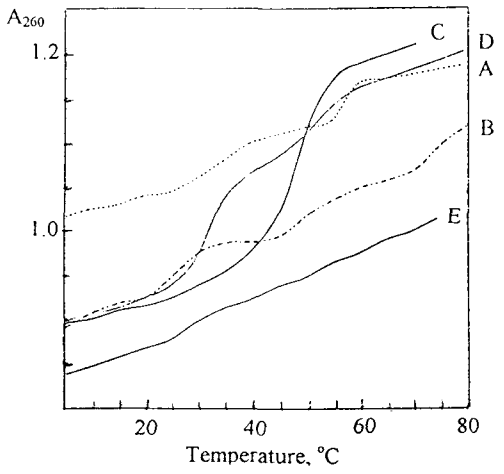


FIG.2. The melting temperature curves [in 0.02 M Tris-HCl (pH=7.5) / 0.2 M NaCl] of the hybrides consisting of an oligo-T^*_{15} analogue and its unmodified complemendary ribo-A_{15} target [A- analogue (IIa); B - analogue (IIb) and C - analogue (III)]; that of two unmodified complementary oligonucleotides (D) and single-stranded A_{15} oligomer (E).

The monomers obtained were applied for the automated solid phase synthesis of the aminomethylphosphonate linked thymidine and deoxycytidine containing oligonucleotide analogues. As a support, we used long chain alkylamine CPG functionalized with 5'-(DMTr)T-3'-O-succinate for the derivatives (1) and with 5'-O-succinyl-T(DMTr)-3'[6] for the derivatives (2) and (3). The conditions for one step of chain elongation are summarised in the Table. The coupling efficiency of each elongation cycle was 95-97% depending on the monomer used, as gauged spectrophoto-metrically by releasing dimethoxytrityl cation. After the completion of the synthesis and the removal of the terminal trityl group, the support was treated with triethylammonium thiophenolate to remove P-protecting group[5] and then with concentrated ammonia (20°C, 16 h). The deprotected oligomers were isolated by preparative denaturing PAGE, or by anion-exchange FPLC (FIG.1) and desalted.

The preliminary investigations of these modified oligomers properties revealed that they are more stable to the exonuclease degradation than oligonucleotides bearing phosphodiester bonds. The oligomers with carboxamidomethyl phosphonate ester linkages (III) hybridized to DNA and RNA similarly to unmodified oligonucleotides and significantly better than modified oligomers of types (I) and (II) (FIG.2).

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