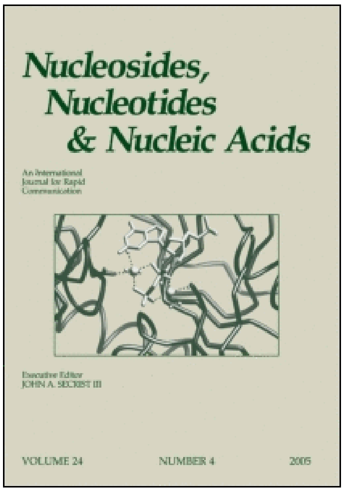

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SYNTHESIS AND CHARACTERIZATION OF PHOSPHATE-MODIFIED DNA-DIMERS WITH FLUORINE AS AN UNCHARGED LIGAND

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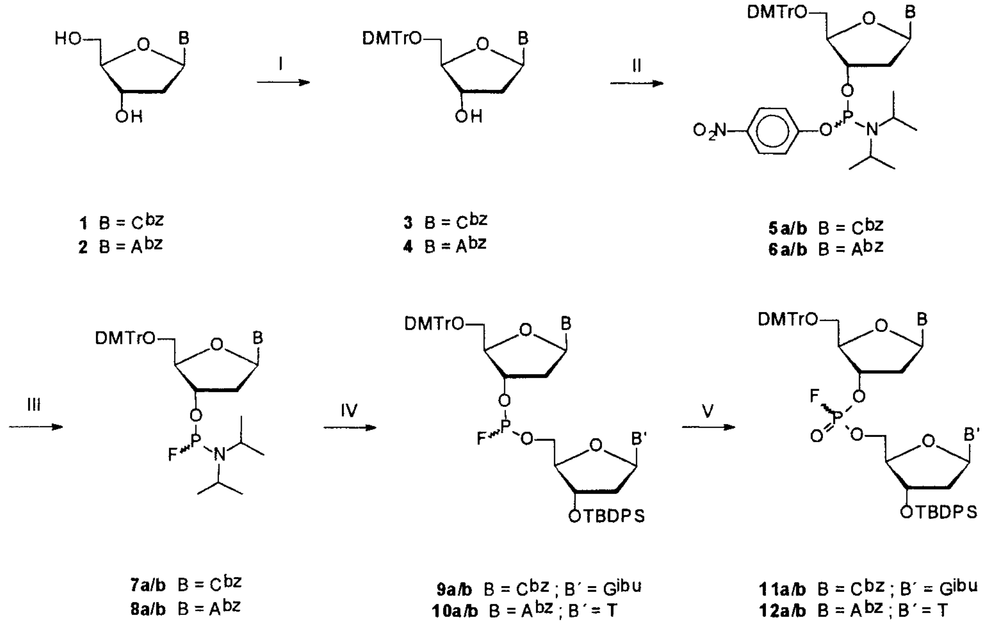
ABSTRACT: The potential of the dinucleosidylphosphorofluoridates dCp_FdG and dAp_FdT is described. Side products of the phosphoramidite coupling reaction are synthesized and characterized by ^{31}P-NMR-spectroscopy.

Oligonucleotides are the basis for an important new approach to the design of therapeutic agents, where the biological target is RNA or DNA rather than conventional *in vivo* targets such as enzymes, proteins or receptors[1]. Natural oligonucleotides suffer from a number of disadvantages such as their relatively poor uptake by cells and rapid degradation by cellular enzymes. These disadvantages have stimulated efforts to prepare backbone modified derivatives which are not hampered by these limitations. Therefore a large number of chemically modified oligonucleotides has been synthesized. Methyl-, phenyl- and benzylphosphonates[2] as well as phosphotriesters belong to the class of uncharged backbone modifications. Their increased lipophilicity facilitates the ability to penetrate easier through cell membranes. Phosphorothioates and -dithioates[3] as anionic and phosphoramidates as cationic charged modifications are although studied[4]. Several modification are used for improving cellular uptake and for increasing the stability of antisense-oligonucleotides towards extra- and intracellular enzymes.

A new class of uncharged backbone modification is represented by phosphorofluoridates. The exchange of oxygen by an electronegative fluorine ligand leads to uncharged dinucleosidylphosphorofluoridates. It is well known that a P-F single bond has a molar bond energie of 496 kJ/mol. For that it is one of the strongest bond energies at all and we expect therefore an acceptable stability for this new kind of modified

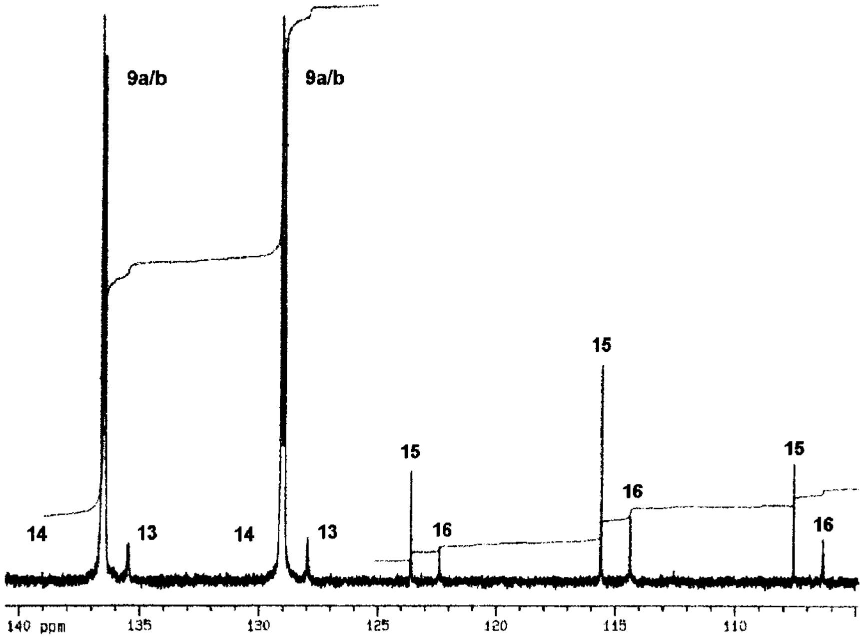


I) 4,4′-dimethoxytritylchloride, dimethylaminopyridine, NEt_3, pyridine, CH_3OH, rt, 2 h; II) *N*,*N*-di*iso*propyl-di-*O*-(p-nitrophenoxy)-phosphane, 1,8-diazabicyclo-[5.4.0]-undec-7-ene, dry tetrahydrofurane, rt, 2 h; III) tetra-*n*-butylammonium fluoride, dry tetrahydrofurane, rt, 2 h; IV) 3′-*O*-TBDPS protected nucleosides, 1H-tetrazole (6 eq.), dry CH_3CN, rt, 10 min.; V) *tert*-butylhydroperoxide, dry CH_3CN, rt, 15 min.

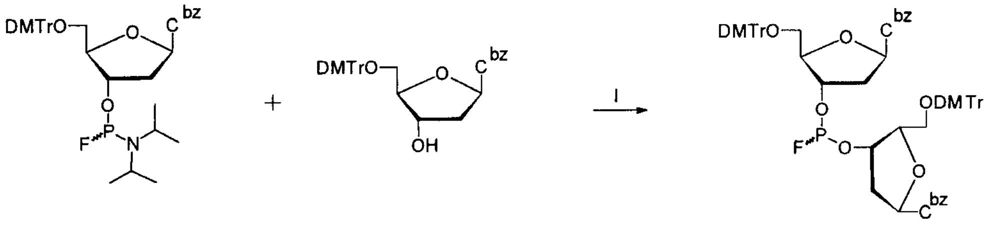
FIGURE 1: Synthesis of phosphorofluoridates dCp_FdG **11a/b** and dAp_FdT **12a/b**

deoxynucleotides. Dabkowski et al. have worked out an efficient synthesis for these new analogues of nucleotides[7]. Here we present the synthesis of two novel dinucleosidyl-phosphorofluoridates: a dCp_FdG and dAp_FdT dimer are synthesized and characterized by ^{31}P-NMR spectroscopy.

The synthetic pathway leading to the dinucleosidylphosphorofluoridates **11a/b** and **12a/b** is shown in figure 1. After protection of the 2′-deoxynucleosides at the 5′-position and at the nucleobases[5] the unprotected 3′-hydroxyl group was phosphitylated with *N*,*N*-di*iso*propylamino-di-*O*-(p-nitrophenoxy)-phosphane. The phosphitylating reagent was obtained in two steps starting by the conversion of phosphorus trichloride with two equivalents of *N*,*N*-di*iso*propylamine. Treatment with two equivalents of p-nitrophenol in the presence of triethylamine leaded to *N*,*N*-di*iso*propylamino-di-*O*-(p-nitrophenoxy)-phosphane[6].



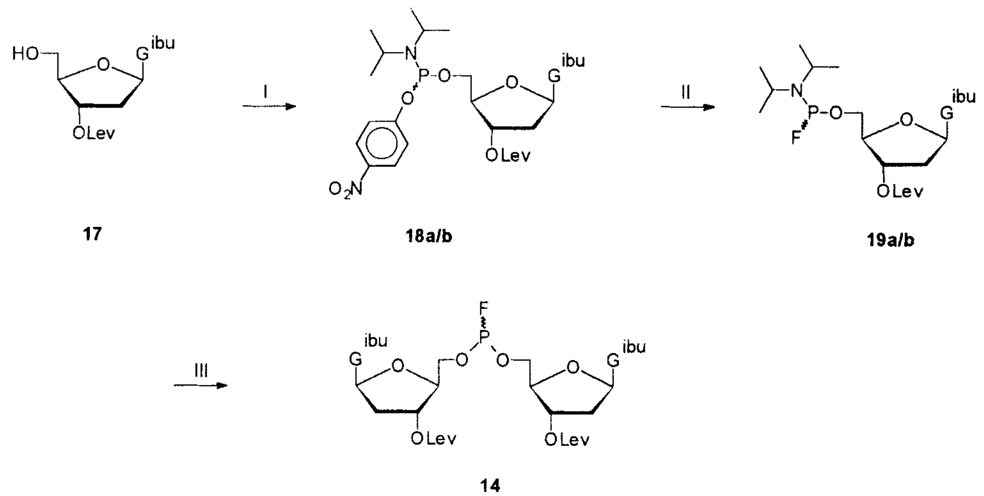
SCHEME 1: ^{31}P-NMR-spectra of the phosphoramidite coupling reaction



7 a/ b 3 13

I) 1H-tetrazole (6 eq.), dry CH_3CN, rt, 10 min.

FIGURE 2: Synthesis of symmetrical 3′-dCp$_F$dC-3′-dimer



I) *N,N*-di*iso*propyl-di-*O*-(p-nitrophenoxy)-phosphane, 1,8-diazabicyclo-[5.4.0]-undec-7-ene, dry tetrahydrofurane, rt, 2 h; II) tetra-*n*-butylammonium fluoride, dry tetrahydrofurane, rt, 2 h; III) **17**, 1H-tetrazole (6 eq.), dry CH_3CN, rt, 10 min.

FIGURE 3: Synthesis of symmetrical 5′-$\mathrm{dGp_FdG}$-5′-dimer

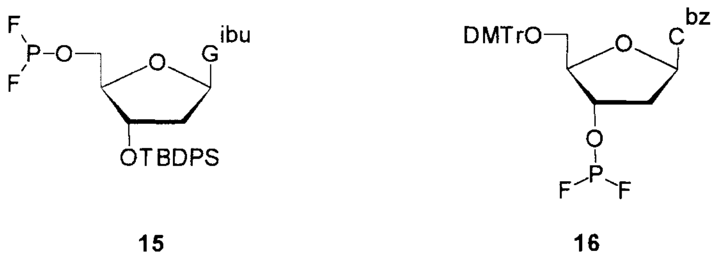


FIGURE 4: symmetrical phoshorodifluoridites

The phosphitylation step proceeded with 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) as a base with over 90 % yield. The resulting phosphoramidites **5a/b** and **6a/b** were easily converted into the phosphorofluoridites **7a/b** and **8a/b**. As fluorinating reagent served a solution of tetra-*n*-butylammonium fluoride (TBAF) in THF. The phosphorofluoridites could be characterized due to their remarkable stability. The *N,N*-diisopropylamino-phoshorofluoridites **7a/b** and **8a/b** were coupled with 3′-*O*-TBDPS protected nucleosides to yield the threevalent $\mathrm{dCp_FdG}$ **9a/b** and $\mathrm{dAp_FdT}$ **10a/b** dimers. For successful coupling 6 equivalents of 1H-tetrazole were used[7].

Oxidation under anhydrous conditions with *tert*-butylhydroperoxide (TBHP) led to the desired dinucleosidylphosphorofluoridates **11a/b** and **12a/b** which are characterized by ^{31}P-NMR-spectroscopy.

The phosphoramidite coupling reaction gave rise to side products (scheme 1). The symmetrical dinucleosidylphosphorofluoridite 3′-dCp$_F$dC-3′ **13** was synthesized analogously to the 3′-5′-dimer (figure 2).

The synthesis of 5′-dGp$_F$dG-5′-dimer **14** needed phosphitylation at the 5′-position and instead of the fluorine labile silyl group the levulinyl group to protect the 3′-hydroxyl function (figure 3). Characterization was carried out by ^{31}P-NMR-spectroscopy. Another class of potential side products are the phosphorodifluoridites (figure 4). In order to discriminate between 2′-deoxyguanosine-5′-*O*-phosphorodifluoridite **15** and 2′-deoxycytidine-3′-*O*-phosphorodifluoridite **16** a $^{3}J_{P\text{-}H}$ coupling ^{31}P-NMR experiment was carried out.

In summary, the described synthesis for the dCp$_F$dG and dAp$_F$dT dimers is quite efficient in all reaction steps. We unambiguously determined the formed side products during the phosphoramidite coupling reaction to get a better understanding of the possible reaction mechanism. The potential of fluorine modified dimers seems take less as building blocks for oligonucleotide synthesis than as potential targets to carry out substitution reactions at phosphorus[8].

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