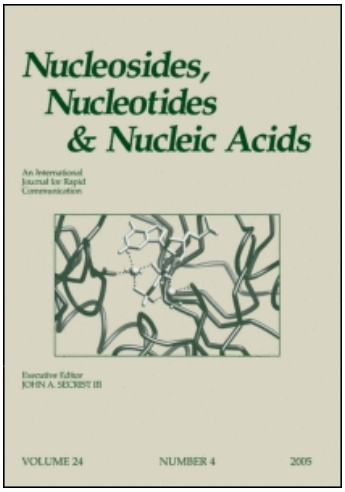

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Synthesis and Application of N(6)-Phenoxycarbonyl-deoxy-adenosine Derivatives in Oligonucleotide Probes Chemistry

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SYNTHESIS AND APPLICATION OF N(6)-PHENOXYCARBONYL-DEOXY-ADENOSINE DERIVATIVES IN OLIGONUCLEOTIDE PROBES CHEMISTRY

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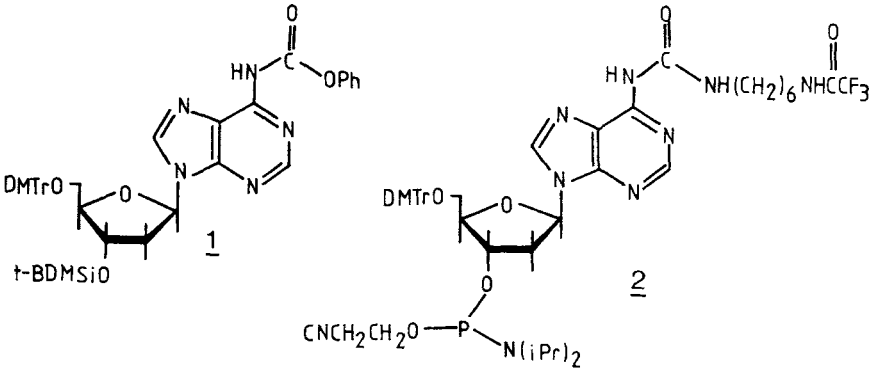
Abstract: N(6)-phenoxycarbonyl-deoxyadenosine derivatives are offered as new reactive intermediates for preparation of linker-containing oligonucleotide probes and modified LCAA-CPG supports.

During last years much effort has been undertaken in construction of oligonucleotide probes containing nucleoside units bearing reactive linkers, mainly of alkylamine type /1-3/. Here we would like to communicate on the synthesis and application of N(6)-phenoxycarbonyl-deoxyadenosine derivatives in oligonucleotide probes chemistry.

Deoxyadenosine was 5'-O-dimethoxytritylated (70% yield) with use of 6-nitroquinoline as a base /4/. Under such conditions no tritylation of exo-amine function was observed. Subsequent 3'-O-silylation with tBDMSi-chloride (dioxane-imidazole, 4 days,room temp. 95% yield) and treatment with crystalline phenoxycarbonyltetrazole /5/ led quantitatively to the 3',5'-di-O-protected-N(6)-phenoxycarbonyldeoxyadenosine 1 /6/ (85%) as a highly reactive intermediate /7,8/. Reaction of the latter with 1,6-diaminohexane gave (80%) a derivative bearing alkylamine-chain linked to nebularin-6-yl residue via, ammonia-resistant, N^1, N^2-disubstituted urea system /9/. Hexylamine protection with trifluoroacetic anhydride (70%), removal of 3'-O-TBDMSi group with $Et_3NH^+F^-$ (94%) and subsequent treatment with bis-(N,N-diiso-propylamino)(2-cyanoethoxy)phosphine gave (as lyophilisate, 95% yield) requested phosphoramidite 2 /10/.



Phosphoramidite 2 was applied for automated DNA synthesis to obtain precursor-oligonucleotides bearing free aminohexyl linker capable to react with reagents introducing reporter probes /3/. Below are examples of oligdeoxyonucleotide probes obtained and HPLC-purified:

- Fluorescein-*linker*-N(CAC CAC CAC CAC CAC) ; DNA-fingerprinting probe
- Biotin-*linker*-N(GTA AAA CGA CGG CCA GT) ; M13-sequencing primer
- thiol-*linker*-N(CCT AGT GGA GGA AAG A) ; 16s rRNA probe

N(6)-phenoxycarbonyldeoxyadenosine derivatives e.g. 1 react readily with LCAA-CPG (loading 32 μmole/g) to obtain anchored (ammonia-stable) CPG-linked oligonucleotides. When appropriate linker is to be derived from threonine, formation of detachable (ammonia labile) CPG-linked oligonucleotide is expected as in the case of hypermodified ureidonucleside t^6A /11/. Potential application of the latter approach to the solid support-aided synthesis of RNA-branch is investigated.

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6. 1 - was isolated by flash column chromatography on silica gel using methanol gradient in chloroform or used in situ after careful hydrolysis of an excess of phenoxycarbonyltetrazole as described /7/; characterized via its 3',5'-di-O-acetyl derivative, compare /5/, H NMR (90 MHz, $CDCl_3$); δ(ppm) 9.31(1H,s,N^6-H,rapid exchange in D_2O),8.90(1H,s,H-8),8.30(1H,s,H-2),7.50(5H,m,phenyl),6.40(t,1,H-1').
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9. reaction of 3'-O-acetyl analogue with conc.ammonia (50°C,48hrs) led only to removal of 3'-O-protection, showing stability of N_1,N_2-disubstituted urea system, easily detectable due to characteristic λ_{max} at 300 nm in a basic solution (pH 12.5).
10. 2 - H NMR (90 MHz, $CDCl_3$); δ(ppm) 10.30(1H,m,N-H,exchange in D_2O), 9,40(1H,s,N^6-H,rapid exchange in D_2O), 8.67(1H,s,H-8), 8.20 (1H,s,H-2), 6.37(1H,t,H-1'), 3.75(6H,s,Tr-OMe), 1.44-1.36(12H,br-m hexyl), 0.88(15,br-s,tBDMSi); P31 NMR (32.6 MHz, ext.85% H_3PO_4) δ(ppm) 148.43.
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