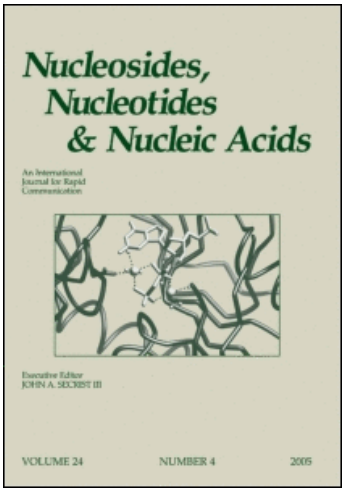

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Synthesis of 5'-O-Dimeth0Xytrityl-4-N-/6-Trifluoroacetamidohexyl/-2'-Deoxycytidine and its Application in the Synthesis of Biotin-Labeled Oligonucleotides

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SYNTHESIS OF 5'-O-DIMETHOXYTRITYL-4-N-/6-TRIFLUOROACETAMIDOHEXYL/-2'-DEOXYCYTIDINE AND ITS APPLICATION IN THE SYNTHESIS OF BIOTIN-LABELED OLIGONUCLEOTIDES.

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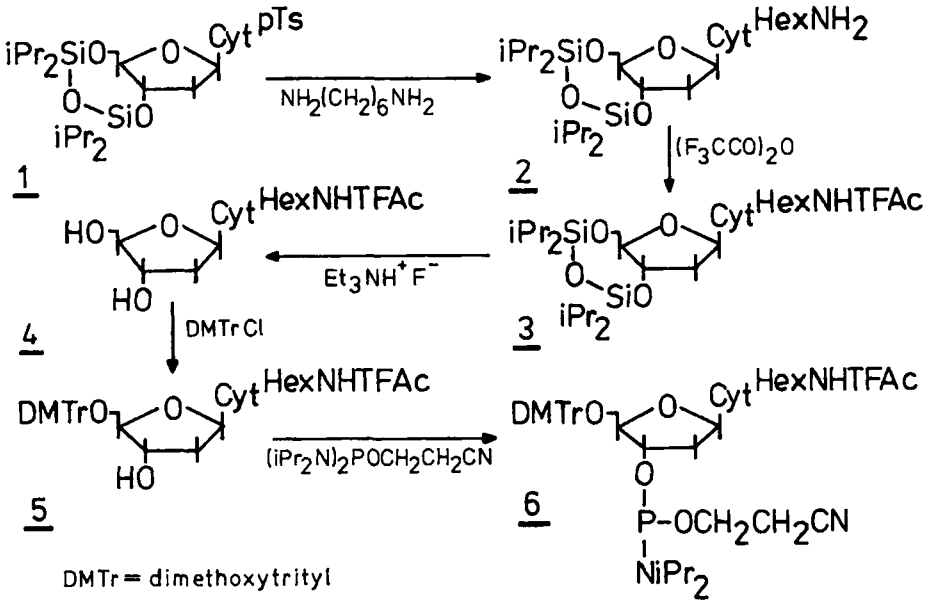
ABSTRACT: 5'3'-O-protected 4-N-tosyl-2'-deoxycytidine was converted with 1,6-diaminohexane to 4-N-/6-aminohexyl/-2'-deoxycytidine and then into 5'-O-dimethoxytrityl-4-N-/6-trifluoroacetamidohexyl/-2'-deoxycytidine. The latter was used to prepare oligonucleotides by the phosphoramidite approach.Deprotected oligomers were labeled with biotin.

Hybridization probes are useful in analysis of human, animal and plant DNA or RNA /ref. 1/. Recently, synthetic biotin-labeled oligonucleotides, as non-radioactive material, are becoming promising tools in diagnosis and research areas. The strong and specific interection of biotin and avidin or streptavidin on the one hand and easy possibility of detection formed complex by commercially available enzymatic systems on the other make this an approach of the future /refs. 2,3/.

A few different methods of incorporation of biotin into oligonucleotide chains have been described /refs. 4-7/.

This communication reports synthesis of oligonucleotides where biotin is linked by a n-hexyl chain to C-4 carbon of 5' terminal cytidine. We have found that 4-N-arylsulfonyl-2'-deoxycytidine can be converted in high yield into 4-N-aminoalkyl derivative by treatment with appropriate 1^{o} diamine under moderate conditions /ref. 8/. This made us propose a synthesis of cytidine derivative with 6-aminohexyl or other aminialkyl chains as useful linkers for biotin or other markers in oligonucleotides.

For this purpose, 5'3'-O-/tetraisopropyldisiloxane-1,3-diyl/-4-N-/p-toluenesulfonyl/-2'-deoxycytidine /1/ /ref. 8/ was converted with 1,6-diaminohexane into derivative 4-N-/6-aminohexyl/-2'-deoxycytidine /2/ followed by corresponding trifluoroacetamide /3/. The compound /3/ was desylilated by triethylammonium fluoride/TEAHF/ /ref. 9/ followed by dimethoxytritylation. The derivative /5/ was synthtized in 46% yield /calculated on 1/. The structure was confirmed by ^{1}H NMR, ^{13}C NMR and UV spectra. Next,



5'-O-dimethoxytrityl-4-N-/6-trifluoroacetamidohexyl/-2'-deoxycytidine was converted into corresponding nucleoside-3'-/diisopropylamino, 2-cyanoethoxy/ phosphine /6/ and used "in situ" in manual synthesis /refs, 10,11/ of following sequences: C^{+}GGGCGCGAGGAAGG/ 15-mer/, C^{+}GGGCGCGAGGAAGGACACCC /21-mer/, C^{+}GGCCGCTGGGCACTCCCCACCGTCCT /27-mer/ /C^{+}, 4-N-/6-aminohexyl/-2'-deoxycytidine residue/.

After complete deprotection the crude reaction mixture was treated with biotin-N-hydroxysuccinimidyl ester /ref. 6/ and the longest oligomer was converted into a biotin-labeled derivative in 71% yield. The reaction mixture was purified by 20% polyacrylamide gel electrophoresis. The purity of biotin-labeled oligonucleotides was confirmed by phosphorylation with /γ-^{32}P/ ATP and T4-kinase.

The synthetized biotin-labeled oligonucleotides are already tested as hybridization probes for potato spindle tuber viroid /PSTV/.

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