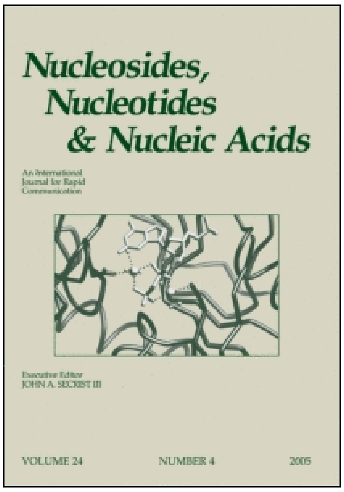

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HYBRIDIZATION PROBES WITH DEOXYINOSINE, DEOXYXANTHOSINE OR DEOXYNEBULARINE AT AMBIGUOUS CODON POSITIONS

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Abstract.

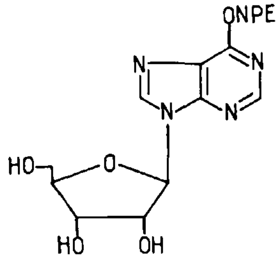
Successful incorporation of p-nitrophenylethyl protected deoxyinosine and deoxyxanthosine and of deoxynebularine into oligonucleotides following a triester strategy, is demonstrated.

Isolation of clones coding for a certain protein is often based on probing a cDNA or genomic library with oligonucleotides corresponding to a known sequence of that protein. Owing to redundancies in the genetic code, mixed-sequence oligonucleotide probes have to be used to represent all possible combinations. To avoid this problem one has started the search for an ideal base analog which would substitute for all four natural nucleosides without much destabilization.

Probes with the phenyl ring as a base substitution failed[1], but deoxyinosine has been reported to be a successful candidate[2]. We present here the synthesis of probes with deoxyinosine, deoxyxanthosine and deoxynebularine, which are used for probing for the proline rich protein (PRP) of the rat[3].

Deoxynebularine was made out of deoxyadenosine by reductive deamination[4] in 48 % overall yield. Tritylation with monomethoxytritylchloride in pyridine and phosphorylation with o-chlorophenylphosphodichloridate gave the required building block. The glycosidic bond is reasonably stable : 80 % acetic acid cleaves off 75 % of the base only after 24 h, while $ZnBr_2$ 1 M with triazole 0.02 M in CH_2Cl_2/isopropanol[5] causes depurination for 30 % after 24 h.

As deoxyinosine is claimed to give side reactions on the lactam system[6], we used the deoxyinosine monomer 4 protected on O^6 with the



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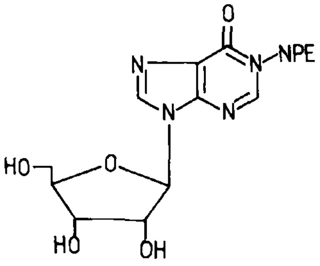

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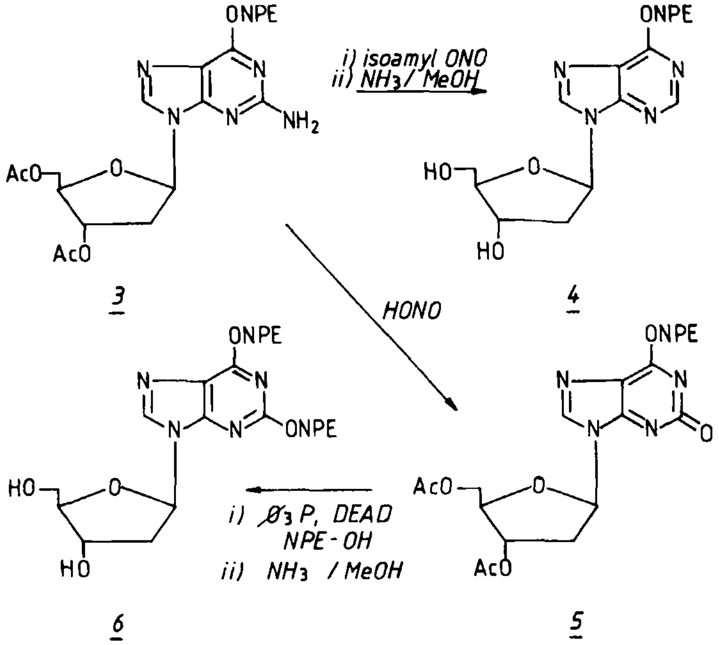

p-nitrophenylethyl group This protecting group has been introduced previously for protection of the lactam function of deoxyguanosine.[7]. Introduction of this group on acylated inosine with a Mitsunobu reaction, resulted in a 55 % yield of the desired product 1 and 24 % of its N^1 isomer 2. Structures were proven by NMR, IR and analogy with the known N^1- and O^6-methylinosine, synthesized also with a Mitsunobu reaction.

Synthesis and purification of 1 and subsequent deoxygenation is a complicated way to prepare 4. As protection of O^6 on fully acylated deoxyguanosine is straightforward[7], we decided to start synthesis from the 3',5'-di-O-acylated deoxyguanosine. Out of 3 (prepared in 64 % yield) as well the deoxyinosine 4 as the deoxyxanthosine derivative 6 could be synthesized fairly easy.

Reductive deamination of 3',5'-di-O-acetyl,O^6-p-nitrophenylethyl deoxyguanosine 3 gave 60 % of the deoxyinosine derivative according to TLC analysis of the crude mixture, and 20 % of the deoxyxanthosine derivative 5. Ammonolysis and column purification afforded 49 % (based on 3) of 4.

The O^6-protected deoxyxanthosine 5 was also formed out of 3 with nitrous acid at 5°C in aceton-water[8]. Introduction of the second NPE group went fast (10 min) and ammonolysis gave only the O^2,O^6-protected deoxyxanthosine 6. The base protecting groups of 4 and 6 can be removed either by the action of DBU in pyridine or with a 1 M solution of tetramethylguanidine-syn-2-nitrobenzaldoxime at 70°C.

Heptamers of the sequence TITIITT were made, enzymatically degraded and analysed by HPLC to confirm the incorporation of all three nucleosides without any side reactions.

Next 23 mers have been synthesized with a phosphotriester method[9] as probes for the proline rich protein of the rat. The same sequence was synthesized three times, with either deoxyinosine, deoxyxanthosine or deoxynebularine at the 6 ambiguous positions.

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