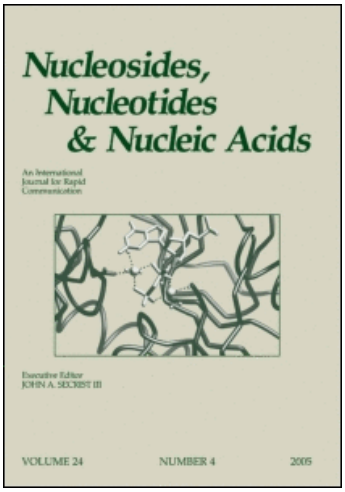

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SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES CONTAINING AN ALIPHATIC AMINO LINKER ARM AT SELECTED ADENINE BASES AND DERIVATIZATION WITH BIOTIN

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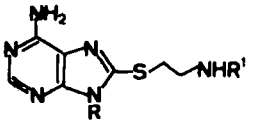
Abstract. The chemical synthesis and characterization of oligodeoxyribonucleotides with a "linker arm" attached to the 8-position of some adenine bases are presented. Derivatization with biotin is described and evaluation of these modified DNA fragments as non-radioactive hybridization probes is briefly discussed.

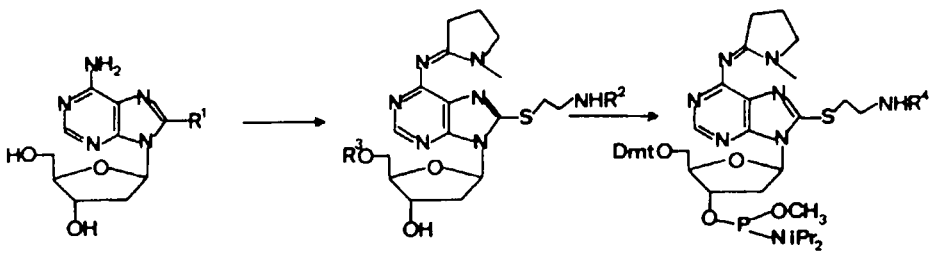
Introduction. Nucleic acid probe hybridization is a powerful tool for the detection, identification and potentially the isolation of specific nucleotide sequences. Recently, a great deal of effort has been devoted to develop methods for the replacement of radioactive labels on DNA probes with stable and safe non-radioactive reporter groups such as biotin or fluorescent groups. Various chemical methods have been described for the attachement of non radioactive tags to oligonucleotides, either at the 5' end[1,2,3] or at selected modified bases[4] or at random sites[5]. Enzymatic incorporation of biotin into DNA by nick-translation using a biotinylated dUTP derivative[6] or by other procedures[7,8] is well documented.

We wish to report a method for the preparation of oligonucleotide probes derivatized with a primary amino alkyl side arm attached to the 8-position of selected adenine bases as in 1a. We describe the synthesis of the fully protected nucleoside phosphoramidite 2 and its incorporation into DNA fragments using standard oligodeoxyribonucleotide synthesis methodology.



1a R' = H
1b R' = biotinyl
1c R' = caproylamidobiotinyl

We show that DNA fragments containing the modified base 1a can be labelled with biotin and used as hybridization probes. This approach enables to control site of attachement and number of labels. Other potential applications are the preparation of enzyme-DNA conjugates[9] and the immobilization of DNA onto solid matrices.



3 R^1 = H
4 R^1 = Br
5a R^1 = $S(CH_2)_2NHCOCH_3$
5b R^1 = $S(CH_2)_2NHCOCF_3$

	R^2	R^3=H	R^3=Dmt
	$COCH_3$	6a	7a
	$COCF_3$	6b	7b
	Fmoc	6c	7c

2a R^4 = $COCH_3$
2b R^4 = $COCF_3$
2c R^4 = Fmoc

Results. The nucleoside phosphoramidites 2a[10] and 2b were prepared in five steps from 2'-deoxyadenosine(3). The synthesis of 2c required seven steps. Bromination of 3 afforded 4 (80%) which was quantitatively transformed in 5a or 5b by nucleophilic substitution of the 8-bromo substituent[11] using the preformed thiolate salt (NaH in DMF) of N-acetylcysteamine or N-trifluoroacetylcysteamine respectively. The 6-amino group was then protected as an amidine-type derivative[12] 6 a,b in 95% yield. A different route was followed for 6c because of the instability of the Fmoc group under amidine synthesis conditions : the trifluoroacetamide group in 6b was hydrolyzed (NH_3 in water-THF) and replaced by Fmoc (Fmoc chloride, pyridine) to give 6c in 35% yield. Selective 5'-O protection of 6a-c with 4,4'-dimethoxytrityl chloride in pyridine[13] afforded the nucleosides 7a-c (90-95%). The phosphoramidites 2a-c were prepared from 7a-c using a published procedure[14] in 76-92% yield[15]. All phosphoramidites 2a-c were used to construct the oligonucleotides mentioned in the Table by automated published methodology[16]. The thiophenol deprotection step was omitted and capping reactions (Ac_2O, DMAP, lutidine) were not performed when 2c was used. The yields for the coupling cycles were in the range 96-99% for 2a and 2c, 80-90% for 2b. The isolation and purification of oligonucleotides

TABLE : Selected examples of oligodeoxyribonucleotides containing 8-(aminoethyl)thioadenine base (Z = 1a)

5'	CTGZAACCGGCGAAG
5'	CTGZZACCGGCGAAG
5'	CTGZAACCGGCGZAG
5'	CTGZZZCCGGCGAAG
5'	CTGZZZCCGGCGZZG

was as reported earlier[17]. The trifluoroacetyl and Fmoc protective groups are cleaved off during the standard ammonia deprotection whereas the acetyl group is stable. Further attempts to hydrolyse the N-acetyl group by sodium hydroxide failed. As a result, 2c is the building block of choice for the synthesis of oligonucleotides containing 1a.

The melting curves of DNA duplexes containing 1a are conventional. Each base 1a induces a slight destabilization of 1°C on the Tm. The selectivity of hybridization is not changed.

Attachment of biotin to 1a was done by reaction of the oligonucleotide with the N-hydroxysuccinimide ester of either biotin or caproylamidobiotin, overnight in neutral aqueous DMF buffer, to give 1b or 1c respectively in nearly quantitative yield. Biotinylated oligonucleotides are purified from polyacrylamide gel electrophoresis or affinity chromatography on avidin agarose[1]. Biotin derivatization slows the migration rate on polyacrylamide electrophoresis : the differences observed for the oligonucleotides of the Table suggest that one biotin residue is attached to each of the bases 1a.

Hybridization of biotinylated oligonucleotides to dot blots of target plasmid DNA immobilized on nylon membranes is under study. For type 1b oligonuleotides, the detection limit is of 10 fmol of DNA using the polyalkaline phosphatase-base colorimetric assay[18]. This sensitivity is 5 times lower than that reported for 5'-biotinylated oligonucleotides[1]. We are currently assessing the importance of the length of the linker arm by studying type 1c oligonucleotides. We are also investigating the influence of the number of biotin labels.

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