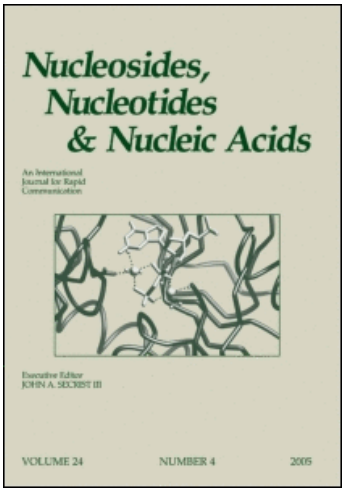

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A GENERAL APPROACH FOR THE HYDROXY GROUP FUNCTIONALIZATION OF SYNTHETIC OLIGONUCLEOTIDES

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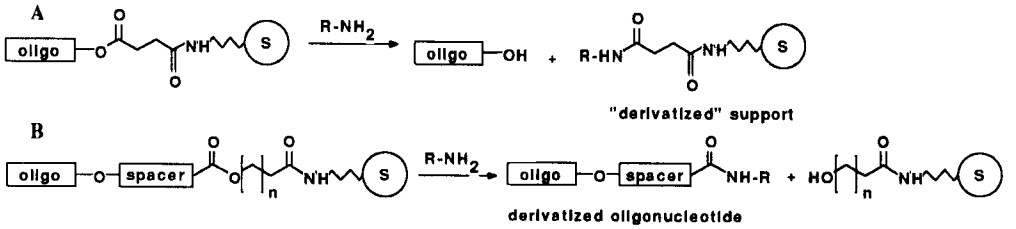
Abstract: Generally applicable non-nucleosidic solid supports and phosphoramidite building blocks that enable attachment of various tethers to the hydroxy groups of oligonucleotides were prepared. The key feature of the structure of these reagents is an ester bond of moderate reactivity which allows postsynthetic introduction of tethers by reaction with substituted alkylamines.

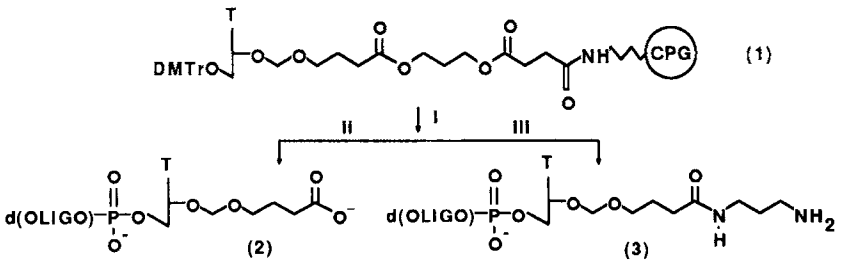
Oligonucleotides bearing either a reporter group or a chemical cross-linker at their terminus are widely used in molecular biology as diagnostic probes and regulators of gene expression. Moreover, 3'-modified oligonucleotides are rather resistant towards the action of nucleases, and hence they have found usage as antisense inhibitors. Usually non-nucleosidic building blocks or solid supports, both containing a spacer arm with a protected functional group serve as tools to introduce the desired functionality at the appropriate terminus of the oligonucleotide.[1] A more general approach enables the introduction of various functional groups by postsynthetic derivatization of oligonucleotide 3'- or 5'-phosphates[2] or thiophosphates.[2,3]

Building blocks containing a readily displaceable group offer a most versatile approach to the preparation of oligonucleotide conjugates, since a single moiety may be exploited to introduce a variety of tether groups. The applicability of this methodology has been demonstrated with base-modified nucleotidic building blocks.[4] Pyrimidinone 2'-deoxynucleoside residues bearing a leaving group at the C4 position allow the introduction of various nucleophiles into the oligonucleotide. The same approach has also been exploited for 5'-labeling by using 4,4'-dimethoxy-4"-(succinimidooxycarbonyl)trityl group instead of the conventional 4,4'-dimethoxytrityl group.[5]

A number of methods have been reported that allow a release of natural and modified oligonucleotides anchored to a solid support by 3'-*O*-succinyl linkage. Aqueous alkali, ammonia, methylamine,[6] ethanolamine[7] and ethane-1,2-diamine[8] cleave out the 3'-deprotected oligonucleotide from the solid support, which simultaneously gets derivatized



Scheme 1.



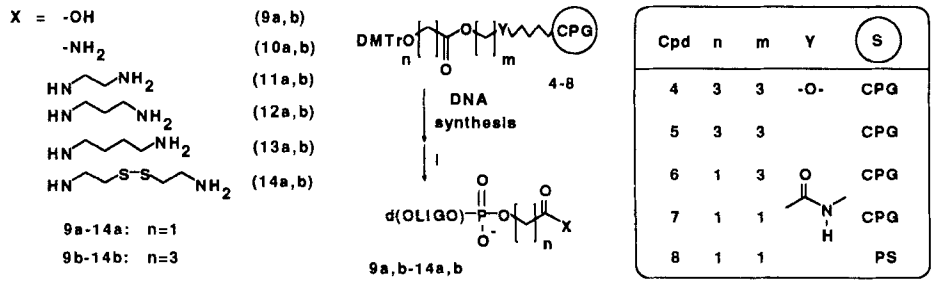
Scheme 2. i: DNA synthesis; **ii:** aq. NaOH, then $NH_3 \cdot H_2O$; **iii: 1.** $N_2H_4 \cdot H_2O$/AcOH/Py; **2.** propane-1,3-diamine/EtOH.

(Scheme 1, **A**). Thus, an alkoxycarbonyl group attached by an appropriate spacer to some hydroxy function of the oligonucleotide seemed to be an attractive alternative for terminal functionalization with nucleophilic reagents (Scheme 1, **B**).

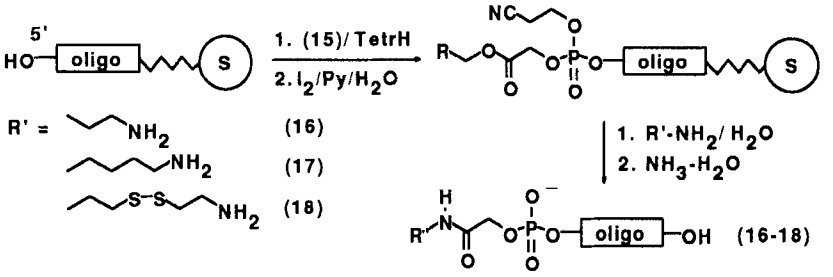
Synthesis of 3'-Modified Oligonucleotides. To elaborate an approach that enables introduction of various functional groups as a routine postsynthetic procedure, 3'-functionalization of oligonucleotides has been systematically studied. Our first attempt[9] involved preparation of a modified solid support **1**. Oligonucleotides assembled on **1** were released either by aqueous alkali or propane-1,3-diamine, giving rise to 3'-carboxyalkyl- (**2**) or 3'-aminoalkyl- (**3**) derivatized oligonucleotides (Scheme 2).

Next, several solid supports **4-8**, each containing ester bond of different reactivity, were prepared.[10,11] All of them were stable under the conditions of oligonucleotide chain assembly. Release of the immobilized oligonucleotides with either aqueous ammonia or alkali created ω-carboxamidoalkyl- and ω-carboxyalkyl groups, respectively (Scheme 3). The deprotected target oligonucleotides (**9-10a,b**) were obtained in 95-100% yield.

Introduction of amino tails to the 3'-terminus may be in principle achieved by treating the oligonucleotides attached to the solid support with α,ω-diamines. However, the solid

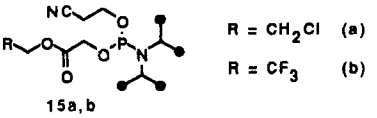


Scheme 3. i: **X** = OH: aq. NaOH, then $NH_3 \cdot H_2O$; **X** = NH_2: $NH_3 \cdot H_2O$; **X** = others, for **4,5**: **1.** $N_2H_4 \cdot H_2O$/AcOH/Py (1:4); **2.** for **11b,12b**: 50% α,ω-diamine/*i*PrOH; for **13b,14b**: 50% α,ω-diamine + 0.5 M DBU/*i*PrOH or Py; **X** = others, for **6-8**: 1.0-1.5 M aq. α,ω-diamines, then NH_3H_2O.

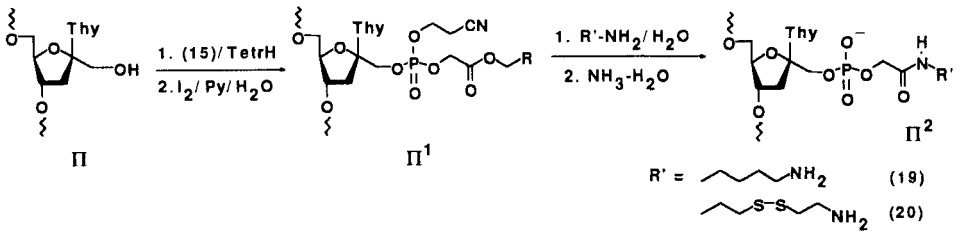


Scheme 4.

supports **4,5** were reactive only towards 50% α,ω-diamine solutions.[10,11] Accordingly, to avoid a transamination of the N^3-benzoylated cytosine residues, their debenzoylation with hydrazinium acetate had to be done as the initial deprotection step.[8] By contrast, treatment with diluted α,ω-diamines can be applied to **6-8**. Although transamination still markedly competes in organic solvents, it is suppressed by using aqueous α,ω-diamines. The only side product is the 3'-carboxyalkyl derivative **9a,b**, the amount of which, after optimization of the reaction conditions, did not exceed 4-15 %. The supports of choice, **7,8**, showed, upon treatment with 1.0-1.5 M aq. α,ω-diamines, the yields of 3'-aminoalkyl oligonucleotides **11a-14a** ranging in 88-93%.[11]



Synthesis of 5'-Modified Oligonucleotides. The next task was to apply the corresponding methodology to the 3'-terminal derivatization of the other termini of synthetic oligonucleotides. Two phosphoramidite reagents **15a,b** were prepared and tested as precursors for functional groups (Scheme 4).[12] Their reactivity towards amines was



Scheme 5.

found to be basically the same as that of **7,8**. However, lower concentration of α,ω-diamines (0.1 and 0.5 M aq. α,ω-diamines for **15a,b**, respectively) can be used for the introduction of functional groups at 5'-terminus in a high yield (90%).

Synthesis of 1'-Modified Oligonucleotides. Recently we reported on preparation of oligonucleotides containing either several aminoalkyl groups or fluorescein labels[13] by using a 3'-deoxy-β-**D**-psicothymidine[14] building block.[15] Further improvement of derivatization at 1'-hydroxy function of 3'-deoxypsicothymidine moiety Π consists of the use of phosphoramidite **15b** (Scheme 5).[12] After assembly of the oligonucleotide and a selective deprotection of 1'-hydroxy groups, coupling with phosphoramidite **15b** gave **Π¹**. Subsequent aminolysis led to oligonucleotides (**19,20**) containing fragments **Π²** in the middle of chain. No difference in the yields of 1'- and 5'-modified oligonucleotides was observed. For the attachment of two 1'-tethers, higher α,ω-diamine concentrations (0.5 M) were preferred. Under these conditions the desired functional groups were introduced in moderate yield (85%).

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