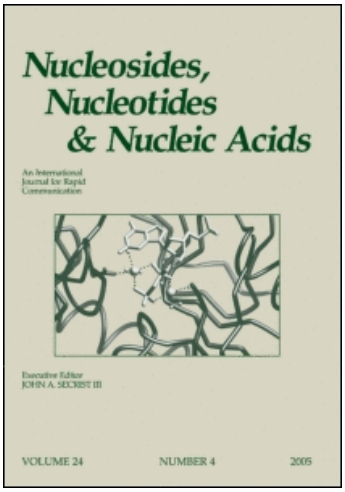

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Synthesis of 2′-Modified Oligonucleotides Containing Aldehyde or Ethylenediamine Groups

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ABSTRACT

Oligonucleotides carrying 2′-aldehyde groups were synthesized and coupled to peptides containing an *N*-terminal cysteine, aminooxy or hydrazide group to give peptide-oligonucleotide conjugates in good yield. The synthesis of a novel phosphoramidite reagent for the incorporation of 2′-*O*-(2,3-diaminopropyl)uridine into oligonucleotides was also described. Resultant 2′-diaminooligonucleotides may be useful intermediates in further peptide conjugation studies.

Key Words: Oligonucleotide; Peptide; Conjugate; Phosphoramidite; Aldehyde.

Oligonucleotides and their analogues have been studied for more than two decades as specific inhibitors of gene expression. Oligonucleotide conjugates, especially those with certain peptides, were reported to possess improved cell-specific targeting

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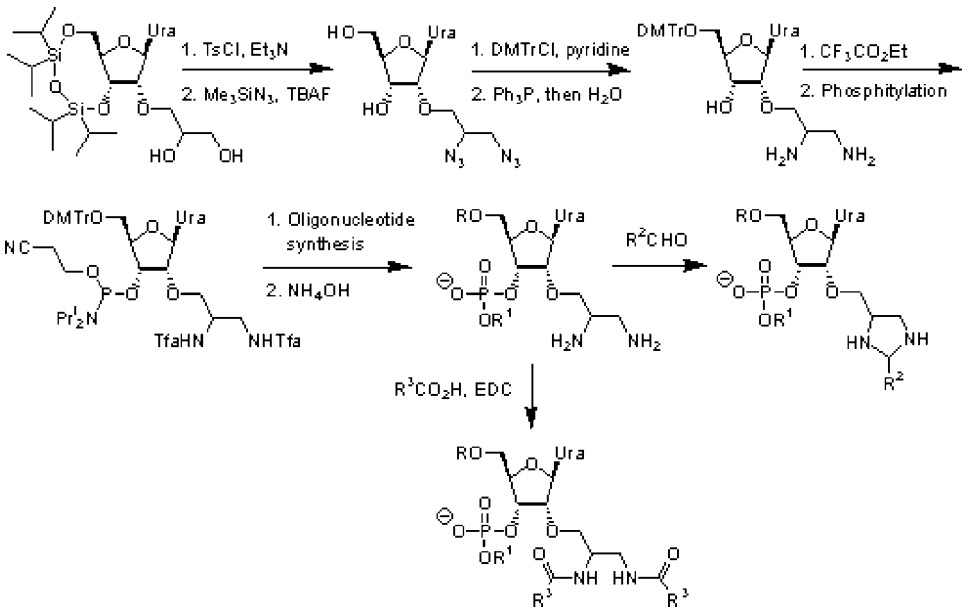
and cellular uptake efficiency.[1,2] Several methods of chemical synthesis of peptide-oligonucleotide conjugates have been developed to date.[3] The two most popular synthetic approaches are total stepwise solid phase synthesis and coupling of separately prepared peptide and oligonucleotide fragments in solution. Some recent advances in total solid phase synthesis look promising, but no routine procedure has emerged so far. A more general approach is the separate solid-phase assembly of oligonucleotide and peptide fragments, deprotection (and purification if necessary) followed by a solution phase chemoselective ligation mediated by mutually reactive groups introduced into each component during solid phase assembly or postsynthetically. Recently we described the preparation of 2′-modified oligonucleotides containing 2′-*O*-(2-oxopropyl)uridine.[4] These electrophilic oligonucleotides were successfully conjugated to various *O*-alkylhydroxylamines, hydrazines and 1,2-aminothiols including fluorescent labels (pyrene, acridine), reporter groups (biotin), and *N*-terminally modified peptides.[5] The chemistry provides the possibility of introducing multiple peptides into defined positions within an oligonucleotide sequence. Resulting linkages, oxime and thiazolidine, are sufficiently stable over the wide pH range, whilst hydrazones have to be reduced to hydrazines to add stability. It has been shown that 2′-modifications, especially peptides, do not adversely affect conjugate binding to target RNA, which is essential for antisense activity.[5]

To explore alternative conjugation chemistries, we recently obtained 2′-*O*-(2,3-diaminopropyl)uridine and incorporated it into oligonucleotides. Hydroxy groups of 3′,5′-tetraisopropyldisiloxane-1,3-diyl-2′-*O*-(2,3-dihydroxypropyl)uridine (Sch. 1) were converted into azido groups first by reaction with *p*-toluenesulfonyl chloride and tertiary amine followed by trimethylsilyl azide/tetra-*n*-butylammonium fluoride



Scheme 1. R, R^1 = unprotected oligonucleotide chain; R^2, R^3 = conjugated molecule(s). Tfa-trifluoroacetyl.

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treatment with concomitant deprotection of Markiewicz's 3′,5′-silyl group. The primary hydroxy group was 4,4′-dimethoxytritylated, and azido groups were then reduced under Staudinger reaction conditions. Resulting amino groups were protected by trifluoroacetylation, and the 3′-phosphoramidite obtained was incorporated successfully into oligodeoxyribonucleotides. These 2′-(2,3-diaminopropyl) oligonucleotides can be useful intermediates for conjugate synthesis since they can be either acylated by a carboxylic acid derivative or react with an aldehyde with a concomitant imidazolidine formation (see Sch. 1).

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