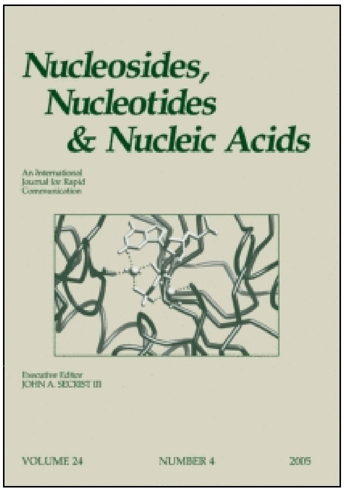

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Eugeny M. Zubin[a]; Dmitry A. Stetsenko[bc]; Tatiana S. Oretskaya[a]; Michael J. Gait[b]
[a] Chemistry Department and A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow, Russia [b] Medical Research Council Laboratory of Molecular Biology, Cambridge, UK [c] Laboratory of Molecular Biology, Medical Research Council, Cambridge, UK

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1,2-Diol and Hydrazide Phosphoramidites for Solid-Phase Synthesis and Chemoselective Ligation of 2′-Modified Oligonucleotides

Eugeny M. Zubin,[1] Dmitry A. Stetsenko,[2,*] Tatiana S. Oretskaya,[1] and Michael J. Gait[2]

[1]Chemistry Department and A.N. Belozersky Institute of Physico-Chemical Biology, M.V. Lomonosov Moscow State University, Moscow, Russia
[2]Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

ABSTRACT

The preparation of two novel 2′-*O*-alkyl phosphoramidites bearing 1,2-diol and hydrazide functions for a chemoselective ligation is described. The former amidite was used to obtain 2′-modified oligodeoxyribonucleotides, which can be later oxidised by $NaIO_4$ to generate 2′-aldehyde oligonucleotides. These were successfully conjugated to acceptor molecules. The latter amidite also showed good coupling yields, but the hydrazide function was demonstrated to be labile under basic deprotection conditions.

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

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*Correspondence: Dmitry A. Stetsenko, Laboratory of Molecular Biology, Medical Research Council, Hills Road, CB2 2QH Cambridge, UK; Fax: +44 1223 412178; E-mail: ds@mrc-lmb.cam.ac.uk.

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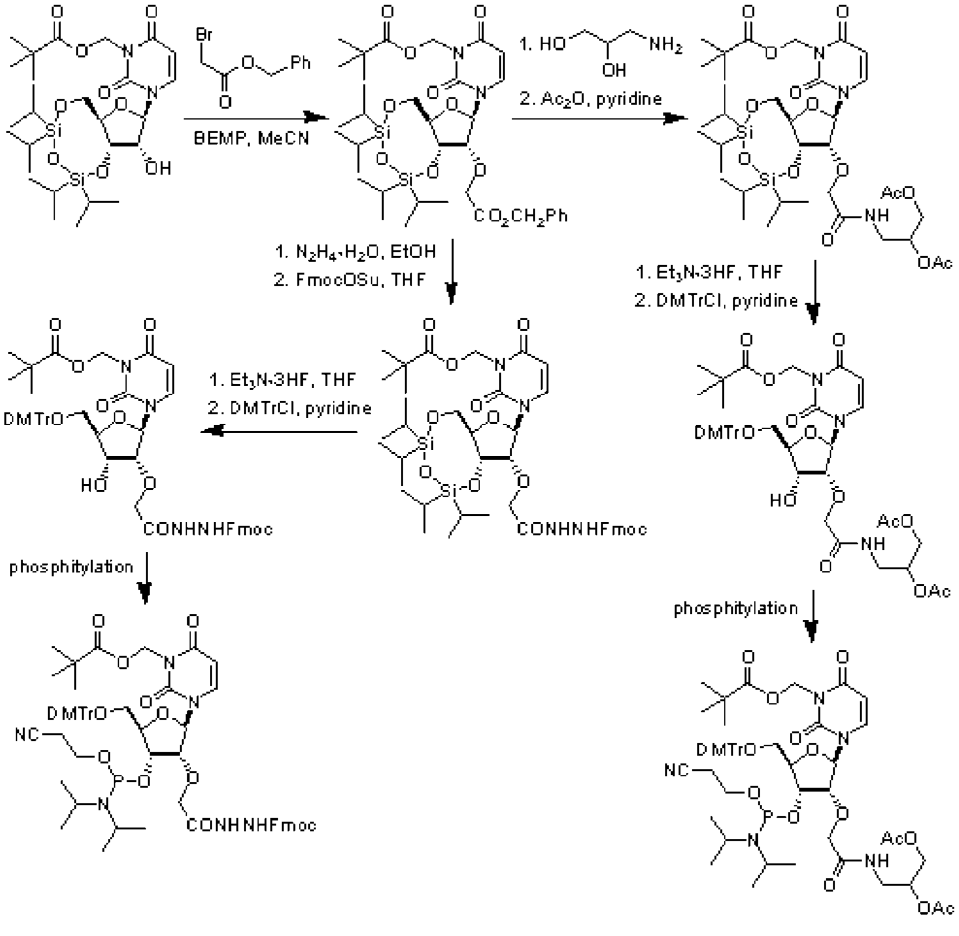
2′-*O*-Alkyloligoribonucleotides have attracted particular attention as steric block inhibitors of gene expression due to their high duplex stability with RNA and improved nuclease resistance. Conjugation of an oligonucleotide analogue to certain peptide sequences may facilitate its cellular uptake and/or affect intracellular localisation.[1] To link multiple peptides covalently to a single oligonucleotide sequence, the 2′-sugar position may offer less disturbance of duplex stability. One general method of preparation of peptide-oligonucleotide conjugates is total stepwise solid-phase synthesis.[2] An alternative way is a separate solid-phase assembly of oligonucleotide and peptide fragments, deprotection and purification followed by a chemoselective ligation mediated by mutually reactive groups introduced into each component during solid-phase assembly or post-synthetically.[2]

We described recently an efficient method for introduction of electrophilic groups into the 2′-position of an oligonucleotide.[3] Furthermore, we demonstrated



Scheme 1.

the possibility of a multiple peptide 2′-conjugation via oxime/thiazolidine/hydrazine chemistry.[4] Now we would like to report on the synthesis of two new 2′-*O*-alkyl phosphoramidite reagents for introduction of reactive groups for chemoselective ligation.

Initially, we used 2′-*O*-(2,3-dihydroxypropyl)uridine to introduce 2′-aldehyde function into oligonucleotides.[3] However, to maintain satisfactory duplex stability and to be able to modulate linker length, another approach to 2′-electrophilic nucleosides proved to be successful. Thus N^3-pivaloyloxymethyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[*N*-(2,3-diacetoxypropyl)aminocarbonyl]methyluridine 3′-phosphoramidite was obtained from N^3,3′,5′-*O*-protected uridine in six steps (see Sch. 1). This synthetic route was shorter and more straightforward and high-yielding than that described in.[3] The coupling efficiency of the monomer in a DNA synthesis was $>97\%$. Singly- and doubly-modified 2′-deoxyoligonucleotides were isolated, their integrity and purity assessed by HPLC and MALDI-TOF analysis. These oligonucleotides were then treated with $NaIO_4$ to generate 2′-aldehyde function as described in.[4] New 2′-aldehyde oligonucleotides were successfully conjugated to 9-hydrazinoacridine and H-Cys-Gly-OH dipeptide via hydrazone and thiazolidine linkages, respectively.

We also attempted to prepare modified oligonucleotides containing a 2′-hydrazide group which can react with aldehydes e.g. peptide *C*- or *N*-terminal aldehydes. In the main step, N^3,3′,5′-*O*-protected 2′-*O*-(benzyloxycarbonyl)methyluridine was treated with N_2H_4/EtOH to give the corresponding hydrazide derivative, which was further *N*-protected by a Fmoc group (Sch. 1). 2′-Hydrazide phosphoramidite showed good reactivity in solid-phase coupling ($>96\%$ stepwise yield). However, deprotection revealed an unexpected lability of the hydrazide function. Cleavage and deprotection carried out with conc. aq. NH_3 at 55°C overnight or at room temperature for 8–10 h and 0.4 M NaOH/MeOH did not lead to a product with the correct molecular mass.

In conclusion, we described the preparation of two novel 2′-*O*-alkyl phosphoramidites bearing 1,2-diol and hydrazide groups for a chemoselective ligation. The former phosphoramidite was used to prepare 2′-modified oligodeoxyribonucleotides, which can be later oxidised by $NaIO_4$ to generate a 2′-aldehyde group. These 2′-aldehyde oligonucleotides can be conjugated successfully to acceptor molecules. The latter phosphoramidite showed good coupling yields, but the hydrazide function was found to be labile under basic deprotection conditions.

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