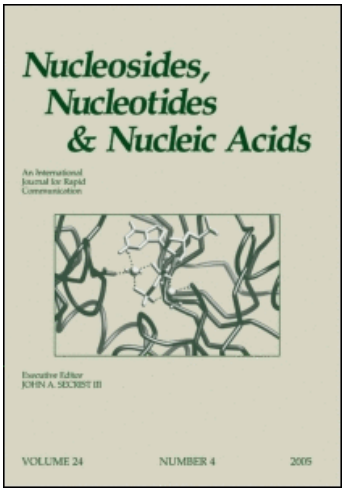

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NOVEL METHOD FOR THE SYNTHESIS OF 2′-PHOSPHORYLATED OLIGONUCLEOTIDES

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□ *We have developed a new method for the preparation of oligodeoxyribonucleotides and oligo(2′-O-methylribonucleotides) that contain a 2′-phosphorylated ribonucleoside residue, and optimized it to avoid 2′-3′-isomerization and chain cleavage. Structures of the 2′-phosphorylated oligonucleotides were confirmed by MALDI-TOF MS and enzymatic digestion, and the stability of their duplexes with DNA and RNA was investigated. 2′-Phosphorylated oligonucleotides may be useful intermediates for the introduction of various chemical groups for a wide range of applications.*

Keywords 2′-Modification; oligodeoxyribonucleotides; oligo(2′-*O*-methylribonucleotides); 2′-phosphate; solid-phase synthesis

INTRODUCTION

2′-Functionalization of oligonucleotides is a convenient approach for the design of nucleic acid conjugates.[1,2] It provides a chance for the precise positioning of a pendant group along the sequence, may cause a minimal distortion of the duplex structure, and maintains the 3′- and 5′-termini free for other chemical or enzymatic reactions. Recently, we have published a preliminary communication on the preparation of the 2′-phosphorylated oligodeoxyribonucleotides and their conjugates.[3] Here we would like to

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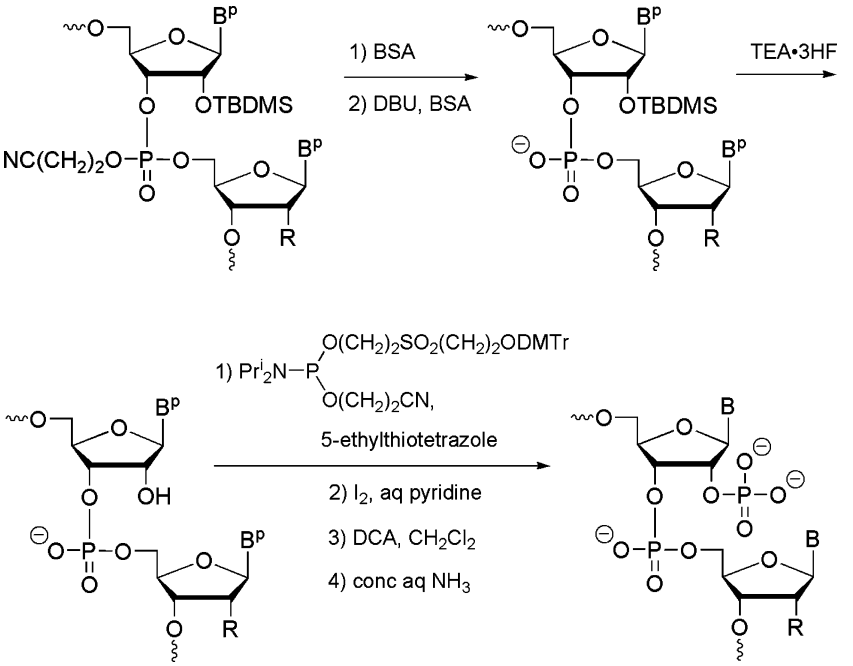
report on the further refinement of this method and its extension to oligo(2′-*O*-methylribonucleotides).

RESULTS AND DISCUSSION

2′-Phosphorylated oligonucleotides have been prepared previously via ribonucleoside 3′-phosphoramidites with the 2′-phosphotriester group.[4] The authors have had to address the 2′-3′-migration of the phosphotriester group and slow coupling of the 2′-phosphorylated 3′-phosphoramidites. Additionally, to introduce the 2′-phosphate group into any position within an oligonucleotide sequence by this method, all four of the corresponding phosphoramidites have to be prepared by a complex multi-step synthesis.

To circumvent these problems, we have adopted a solid-phase approach that uses commercially available 2′-O-TBDMS-ribonucleosides. Oligonucleotide synthesis is started on polystyrene support and a 2′-O-TBDMS-ribonucleoside is incorporated at an appropriate position (Scheme 1). At the end of the assembly, the 5′-O-DMTr group is removed and the 5′-OH is capped by acetylation. Because in the presence of the free 2′-OH the vicinal phosphotriester migrates more easily and gives more chain cleavage than the corresponding phosphodiester,[5]



SCHEME 1 Synthesis of 2′-phosphorylated oligonucleotides. B^p/B = *N*-protected/unprotected nucleobase, R = H or OMe, BSA – *N*,*O*-bis(trimethylsilyl)acetamide, DBU – 1,8-diazabicyclo[5.4.0]undec-7-ene, DCA – dichloroacetic acid, DMTr – 4,4′-dimethoxytrityl, TBDMS – *tert*-butyldimethylsilyl, TEA·3HF – triethylamine trihydrofluoride.

TABLE 1 2′-Phosphorylated oligonucleotides and their properties

Oligonucleotide sequence,5′ − 3′[a]	MALDI-TOF, calc./ found	Yield, %[b]	IE-HPLC retention time, min[c]		T_m, °C (ΔT_m, °C)[e]	
			2′-Phosphorylated oligonucleotide	After AP[d] treatment	DNA	RNA
TTT$\underline{U^p}$TTTTT	2757.7/ 2757.4	25	17	14	—	—
GCATCAAGC$\underline{U^p}$CCAGGC	4933.1/4933.2	18	42	30	47.0 (−4.0)	47.3 (−2.2)
GCA$\underline{U^p}$CAAGCTCCAGGC	4933.1/4933.0	18	42	30	45.9 (−5.1)	46.0 (−3.5)
GCATCAAGCAGC$\underline{U^p}$CCAGGC	5865.7/5864.2	16	—	—	68.0 (−2.0)	—
GCA$\underline{U^p}$CAAGCAGCTCCAGGC	5865.7/5863.8	16	—	—	69.0 (−1.0)	—
$G^mC^mA^mU^mC^mA^mA^mG^mC^m\underline{U^p}C^mC^mA^mG^mG^m$dC	5339.5/5338.1	24	32	27	42.3 (−6.1)	62.9 (−3.3)
$G^mC^mA^m\underline{U^p}C^mA^mA^mG^mC^mU^mC^mC^mA^mG^mG^m$dC	5339.5/5338.1	24	32	27	46.7 (−1.7)	61.1 (−5.1)
$G^mC^mA^mU^mC^mA^mA^mG^mC^mU^mC^mC^m\underline{A^p}G^mG^m$dC	5339.5/5337.2	26	—	—	—	—
$G^mC^m\underline{A^p}U^mC^mA^mA^mG^mC^mU^mC^mC^mA^mG^mG^m$dC	5339.5/5335.9	35	—	—	—	—

[a] $\underline{U^p}$ or $\underline{A^p}$ – 2′-phosphorylated ribonucleotide, N^m – 2′ − *O*-methylribonucleotide; [b] Isolated yield after purification as quantified by A_{260}; [c] Ion-exchange HPLC, 4.6 × 250 mm Polysil SA column (“Teor. Praktika”, Russia), 0–0.4M KH_2PO_4, 20% MeCN, 50 min; [d] AP – alkaline phosphatase. [e] Conditions: 0.1M NaCl, 10 mM Na-cacodylate, pH 7.4, 1 mM Na_2EDTA; [oligonucleotide] = [target] = $1.3 \cdot 10^{-5}$ M, target: r(GCCUGGAGCUUGAUGC) or d(GCCTGGAGCTTGATGC) or d(TGCCTGGAGCTGCTTGATGC); ΔT_m is the difference between the T_m for the duplexes of the unmodified and the 2′-phosphorylated oligonucleotide.

the 2-cyanoethyl groups were removed before 2′-O-TBDMS deprotection by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA).[4] The 2′-OH group was then desilylated by triethylamine trihydrofluoride treatment[6] and phosphorylated with 2-[2-(4,4′-dimethoxytrityloxy)ethylsulfonyl]ethyl-(2-cyanoethyl)-(*N*,*N*-diisopropyl)-phosphoramidite[7] and 5-ethylthiotetrazole followed by aqueous iodine oxidation. The yield of the 2′-phosphorylation can be easily monitored by the DMTr cation release.[7] The 2′-phosphorylated oligonucleotides obtained were fully deprotected and isolated by the usual means, their molecular masses established by MALDI-TOF MS (Table 1).

The presence of the 2′-phosphate group in the oligonucleotides has been confirmed by ion-exchange HPLC after alkaline phosphatase treatment (Table 1). No 2′-3′-migration products were detected by analytical HPLC after hydrolysis of a model nonanucleotide TTT$\underline{U^{p}}$TTTTT by alkaline phosphatase followed by nuclease P1 (the ratio pU:pT:T = 1:7:1).

Next, the thermal stability of the duplexes formed by the 2′-phosphorylated deoxy- and 2′-*O*-methyl oligonucleotides has been investigated (Table 1). The 2′-phosphate produced a sequence-dependent and largely detrimental effect on the stability of a duplex with either DNA or RNA, probably due to electrostatic repulsion of an extra negative charge of the group.

Finally, the stability of the 3′-5′-phosphodiester bond adjacent to the 2′-phosphate group has been demonstrated at pH 6.0–9.0. The rate of hydrolysis of the 2′-phosphorylated oligonucleotides by nuclease P1, snake venom phosphodiesterase, and in the presence of fetal calf serum was the same as for their unmodified analogs.

CONCLUSIONS

We have shown that 2′-phosphorylated oligonucleotides can be prepared efficiently by solid-phase 2′-phosphorylation of a ribonucleoside incorporated into the sequence. The derivatives described may be useful for the design of novel oligonucleotide conjugates as instruments of molecular biology, potential antisense and anti-gene reagents, and probes for sensitive detection of nucleic acids by hybridization and others.

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