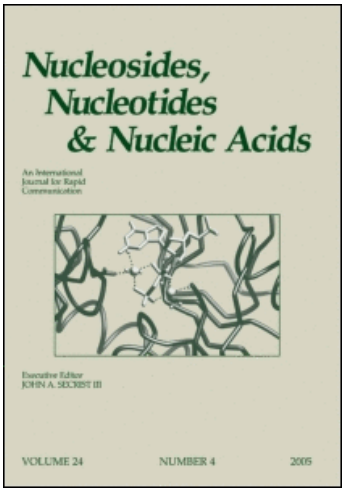

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PREPARATION OF 2′-HYDRAZINO OLIGONUCLEOTIDES AND THEIR REACTION WITH ALDEHYDES AND 1,3-DIKETONES

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□ *Oligodeoxyribonucleotides that contain a hydrazino nucleoside, 2′-O-(2-hydrazinoethyl)uridine were prepared and shown to react with aldehydes or 1,3-diketones with the formation of hydrazones or pyrazoles, respectively. The method may be applicable for the preparation of oligonucleotide-peptide conjugates.*

Keywords Oligonucleotide; conjugation; aldehyde; 1,3-diketone; hydrazone; pyrazole

INTRODUCTION

Addition-elimination reactions of the carbonyl group can be a useful way to oligonucleotide conjugates that contain a range of pendant groups.[1] Conjugation via the 2′-OH of ribose offers further advantages.[2] Recently, we have developed an efficient and specific method for the 2′-conjugation that involves a hydrazino group linked to the 2′-position of uridine.[3]

RESULTS AND DISCUSSION

Previously, we have shown that oligonucleotides containing 2′-*O*-(2-oxoethyl)uridine[4] are useful reagents for conjugation with hydrazines, hydrazides, alkoxyamines, and β-aminothiols.[5] Here we describe the

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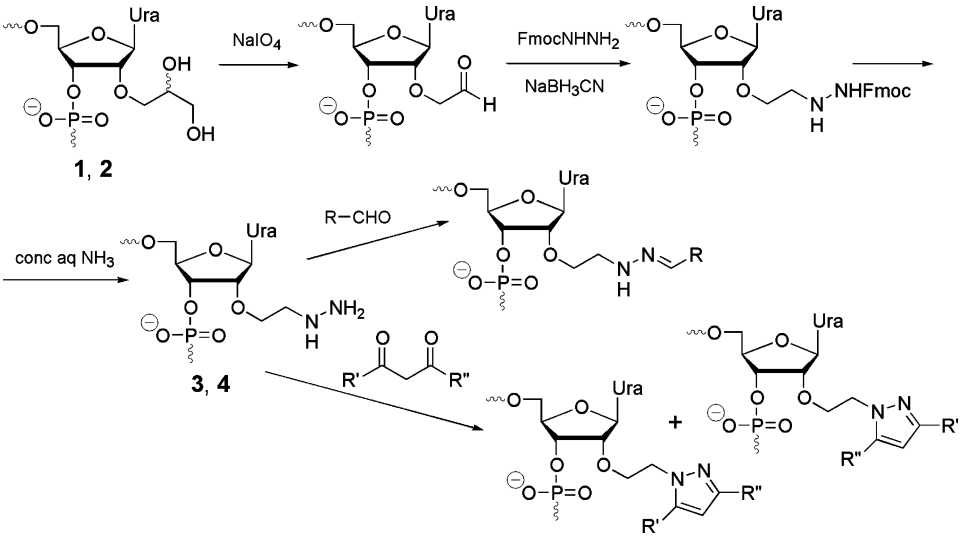
TABLE 1 Sequences and MALDI-TOF MS analysis of the 2′-modified oligonucleotides

	Oligonucleotide sequence, 5′-to-3′[a]	MALDI-TOF MS, $[M+H]^+$ calc./ found
1	CU^dCCCAGGCTCA	3658.4/ 3660.1
2	CU^dCCCAGGCU^dCA	3734.5/ 3735.9
3	CU^hCCCAGGCTCA	3642.4/ 3645.2
4	CU^hCCCAGGCU^hCA	3701.5/ 3702.1

[a] U^d – 2′-*O*-(2,3-dihydroxypropyl)uridine, U^h – 2′-*O*-(2-hydrazinoethyl)uridine

conversion of the 2′-aldehyde group into a 2′-hydrazine and an efficient reaction of the 2′-hydrazino oligonucleotides with aldehydes or 1,3-diketones.[3]

The synthesis of oligonucleotides containing 2′-*O*-(2,3-dihydroxypropyl)uridine was performed as described.[4,5] The dihydroxypropyl group was then oxidized to a 2′-aldehyde group by $NaIO_4$ followed by the addition of 9-fluorenylmethyl carbazate and $NaBH_3CN$ (Scheme 1). The Fmoc-protected oligonucleotides were purified by reverse-phase HPLC. Deprotection by conc aq NH_3 (20°C, 1 h) led to the 2′-hydrazino oligonucleotides (Table 1), which were then treated with aliphatic or aromatic aldehydes or 1,3-diketones to give oligonucleotide conjugates (Table 2).



SCHEME 1 Synthesis of 2′-hydrazino oligonucleotides and their conjugates with aldehydes and 1,3-diketones.

In the case of some of the 1,3-diketones, two products were formed (Table 2). MALDI-TOF MS recorded the same molecular mass for both products. The difference in retention time may be a result of the incomplete conversion of the intermediate mono-hydrazone (faster-eluting peak) into the pyrazole (slower-eluting peak). The water molecule could have been eliminated during MALDI-TOF analysis. Such an explanation is supported

TABLE 2 Yields and MALDI-TOF MS of the conjugates of the 2′-hydrazino oligonucleotides

Aldehyde or 1,3-diketone	Oligonucleotide (Table 1)	
	3	**4**
Pentanal	82[a] (3717.5/ 3717.2)[b]	73[a] (3822.6/ 3826.2)[b]
4-Methoxybenzaldehyde	93[a] (3767.5/ 3769.1)[b]	78[a] (3922.7/ 3924.0)[b]
1-Pyrenecarboxaldehyde	89[a] (3842.7/ 3845.2)[b]	81[a] (4117.0/ 4117.1)[b]
OCHCO-LLK amide	85[a] (4073.9/ 4075.2)[b]	69[a] (4625.5/ 4626.3)[b]
OCHCO-LLGKV amide	82[a] (4244.1/ 4247.0)[b]	65[a] (4883.9/ 4887.3)[b]
$(MeCO)_2CH_2$	70[a] (3690.5/ 3693.5)[b]	60[a] (3852.6/ 3856.1)[b]
$MeCOCH_2COCF_3$	19+57[c] (3744.5/ 3740.4)[b]	67[d] (3960.6/ 3960.1)[b]
$(CF_3CO)_2CH_2$	67+5[c] (3798.4/ 3800.6)[b]	78[d] (4030.5/ 4033.1)[b]
$MeCOCH_2CO(CH_2)_2CO_2H$	69[d] (3779.5/ 3782.1)[b]	54[d] (3930.7/ 3932.4)[b]

[a]Yields are based on the RP-HPLC signal areas; [b]MALDI-TOF MS, $[M+H]^+$, $[M+Na]^+$ or $[M+K]^+$, calc./ found; [c]Yields of faster- and slower-moving conjugates are given; [d]After heating at 55°C overnight.

by the ratio of the HPLC signal areas and the gradual disappearance of the faster-eluting peak. In the case of 2,4-pentanedione, only a single peak of the pyrazole conjugate was observed (Table 2). Further heating of the isolated faster-moving products in aqueous solution (55°C overnight) led to their complete conversion into the pyrazole conjugate. Reaction with 4,6-dioxoheptanoic acid (Table 2) produced a broadened peak, which could be explained by the formation of two regioisomeric 3,5-disubstituted pyrazoles (Scheme 1).

All the conjugates were sufficiently stable at slightly acidic (pH 3–4) and neutral pH (<10% hydrolysis after 24 h at pH 3.0), but partial hydrolysis of hydrazones under basic conditions (pH 9–10) was observed. The pyrazole conjugates were unchanged after 24 h over the pH range 3–10.

CONCLUSIONS

We have developed an efficient method for the synthesis of 2′-hydrazino oligonucleotides from the corresponding 2′-aldehydo derivatives. The resulting compounds were shown to react with various aldehydes or 1,3-diketones. We have demonstrated the stability of the hydrazone conjugates at neutral and mildly acidic pH whilst the pyrazole conjugates are stable over the pH range studied. The method could be applied for the preparation of oligonucleotide-peptide conjugates that are useful compounds for cell delivery studies.

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