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Efficient conjugation and preferential DNA binding of oligonucleotides containing 2′-*O*-(2-oxoethyl)arabinouridine

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Abstract—Oligodeoxyribonucleotides were synthesized that contain a novel nucleoside, 2′-*O*-(2-oxoethyl)arabinouridine. Whereas such oligonucleotides showed only a slight reduction in the T_M values of their complexes with complementary DNA, a significant destabilization was observed in the case of duplexes formed with RNA. This may be explained by the $C_{2'}$-*endo* conformation of 2′-*O*-(2,3-dihydroxypropyl)arabinouridine as demonstrated by NMR experiments in D_2O. The modified oligonucleotides were used to synthesize a number of conjugates with dyes, biotin and a *N*-modified laminin peptide, by hydrazone and oxime formation. We suggest that the 2′-arabinoaldehyde-containing DNA duplexes may be valuable tools for affinity modification of DNA-binding proteins.

Recent advances in the chemical synthesis of modified oligonucleotides have led to their wide application as diagnostic agents, and as potential therapeutics (antisense, antigene, aptamer or RNAi strategies).[1,2] Today, various oligonucleotide-based conjugates with specific properties are under development. Ligands attached to oligonucleotides are used as reporter groups (fluorescent, electrochemical or spin labels), for stabilization of double or triple helical structures (intercalators and minor groove binders) and for increasing cellular uptake (peptides, hydrophobic molecules and carbohydrates). Aldehyde–nucleophile coupling is used in peptide chemistry,[3] glycochemistry[4] and nucleic acid chemistry,[5,6] and seems to be a good alternative to traditional amide, disulfide or thioether conjugation, especially in the case of complex biomolecules. Oligonucleotides with reactive groups are effective tools for affinity modification of DNA-binding proteins.[7] Such reactive groups can be incorporated into heterocyclic base, phosphate or sugar moieties. The use of nucleosides with modified sugar residues is particularly general. The *C*2′-modification is especially appropriate since this leads to minimal distortions in oligonucleotide structure in general and is relatively straightforward to achieve chemically. It also provides an opportunity to adjust the sugar conformation towards either $C_{2'}$-*exo* or $C_{2'}$-*endo* depending on the type and configuration of the substituent, and to 'direct' a 2′-functionality to the major or minor groove.[8] The presence of a 2′-*O*-alkyl group causes local changes from the B-DNA to the A-DNA-type conformation and the substituent is placed in the minor groove.[9,10] On the other hand, it was shown that a 2′-*O*-methyl *arabino* substituent is directed into the major groove and does not significantly influence the B-like helix structure of the duplex.[11–13] This feature is particularly important in our research, since we have demonstrated that oligonucleotides containing 2′-*O*-(2-oxoethyl)uridine can be used for affinity modification of DNA-binding proteins.[14,15]

Here we report the preparation of 2′-*O*-(2,3-dihydroxypropyl)arabinouridine phosphoramidite and its incorporation into oligonucleotides. 2D COSY and NOESY NMR experiments show that the desired nucleoside adopts the $C_{2'}$-*endo* conformation in D_2O. We also describe an efficient conjugation of the 2′-arabinoaldehyde oligonucleotides with a number of fluorescent markers and a *N*-modified laminin peptide.

Keywords: Modified oligonucleotides; Conjugation; Aldehyde; Oxime; Hydrazone.
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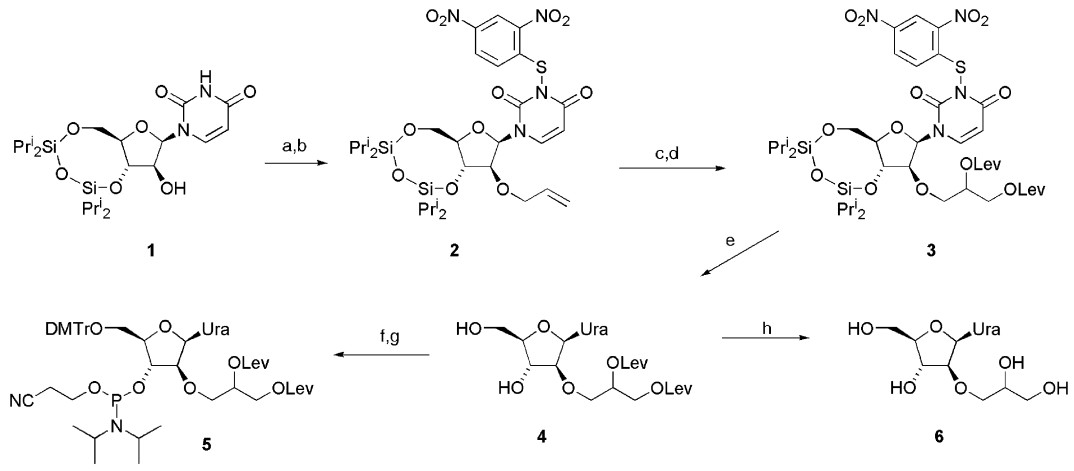


Scheme 1. Preparation of the phosphoramidite **5**. Reagents and conditions: (a) (1) TMSCl, Py, 10 min; (2) 2,4-dinitrophenylsulfenylchloride, Py, 3 h; (3) NH_3 aq, Py, 30 min, 75%; (b) AllBr, BDDDP, MeCN, 3 h, 79%; (c) OsO_4 (0.05 equiv), *N*-methylmorpholine *N*-oxide, THF–H_2O (2:1, v/v), overnight, 95%; (d) Lev_2O, Py, DMAP, 1 h, 88%; (e) $Et_3N(HF)_3$, THF, 1 h, 86%; (f) DMTrCl, Py, 3 h, 92%; (g) $(Pr^i_2N)_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, CH_2Cl_2, 1 h, 75%; (h) K_2CO_3, MeOH, 3 h, 90%.[16]

The target phosphoramidite **5** was synthesized by a route similar to that we published previously for the *ribo* epimer[17] (Scheme 1). However, we describe here the application of protecting groups different from those used previously, which lead to a significant increase in the overall yield. The N^3-position of 3′,5′-*O*-TIPDS-protected arabinouridine **1**[18] was blocked by a 2,4-dinitrophenylsulfenyl protecting group, cf. Alvarez et al.[19] to prevent side alkylation during the next step. 2′-*O*-Allyl ether **2** was synthesized as described earlier by Grøtli et al.,[20] then the 2′-allyloxy group was converted into the blocked *cis*-diol group by osmium tetroxide/*N*-methylmorpholine *N*-oxide oxidation followed by *O*-levulination. The formation of a diastereoisomeric mixture (65:35) was observed. The levulinyl protecting group is well-known in oligonucleotide synthesis, since it can be selectively removed by hydrazinium acetate treatment on a solid support. This method can also be applied to the solid phase synthesis of oligonucleotide conjugates.[21] The compound **3** was treated with triethylamine trihydrofluoride in THF to afford **4**. *O*-Deprotection gave us the nucleoside **6** to carry out ^{2}D NMR experiments (COSY, NOESY) on the conformation of the desired nucleoside in D_2O. A strong interaction was observed between H-1′ (δ = 6.15 ppm) and H-2′ (δ = 4.33 ppm). Furthermore, an additional cross-peak between *H*-2′ and H-4′ (δ = 3.85 ppm) confirmed the arabino configuration of nucleoside **6**. A predominant South ($C_{2'}$-*endo*) conformation was supported by the ^{1}H, ^{1}H coupling constants (i.e., $J_{1',2'}$ = 5.1 Hz, $J_{3',4'}$ = 0 Hz). Further transformations of **4** were carried out to provide the building block **5**[22] suitable for oligonucleotide synthesis. The modified oligonucleotides were prepared by the phosphoramidite approach. The sequences synthesized are shown in Table 1.

Generation of the 2′-aldehyde group was carried out by use of sodium periodate as described earlier (Scheme 2).[10] However the reaction time was prolonged up to 1.5 h due to steric hindrance. Conjugation of the 2′-arabinoaldehyde oligonucleotides with a *N*-aminooxy-modified peptide, an aromatic hydrazine and two hydrazides was achieved under previously reported conditions[10] in good yields (Table 2). The hydrazone formed from 9-hydrazinoacridine was stable under the analysis conditions but in the case of hydrazides, cya-

Table 1. MALDI-TOF MS analysis of the 2′-modified oligonucleotides synthesized and thermal stability of the duplexes formed by the 2′-modified oligonucleotides with complementary DNA and RNA targets

	Oligonucleotide	MALDI-TOF MS calcd/found[a] $[M+H]^+$	T_M values of duplexes formed by 2′-modified oligonucleotides with complementary DNA or RNA[b], °C			
			d(TGAGCCTGGAG)		r(UGAGCCUGGAG)	
			$T_M \pm 0.5$, °C	ΔT_M/mod[c], °C	$T_M \pm 0.5$, °C	ΔT_M/mod, °C
I	CTCCCAGGCTCA	—	56.0	—	58.6	—
II	CTCCCAGGC***U***CA	3643.4/3645.1	52.9[a] (53.1)[d]		49.5[a] (48.7)[d]	
III	C***U***CCCAGGCTCA	3643.4/3641.3	54.3[a] (55.2)[d]	−3.9[a] (−3.2)[d]	50.3[a] (49.5)[d]	−11.0[a] (−9.1)[d]
IV	C***U***CCCAGGC***U***CA	3719.4/3718.3	49.1[a] (47.0)[d]		42.9[a] (41.0)[d]	

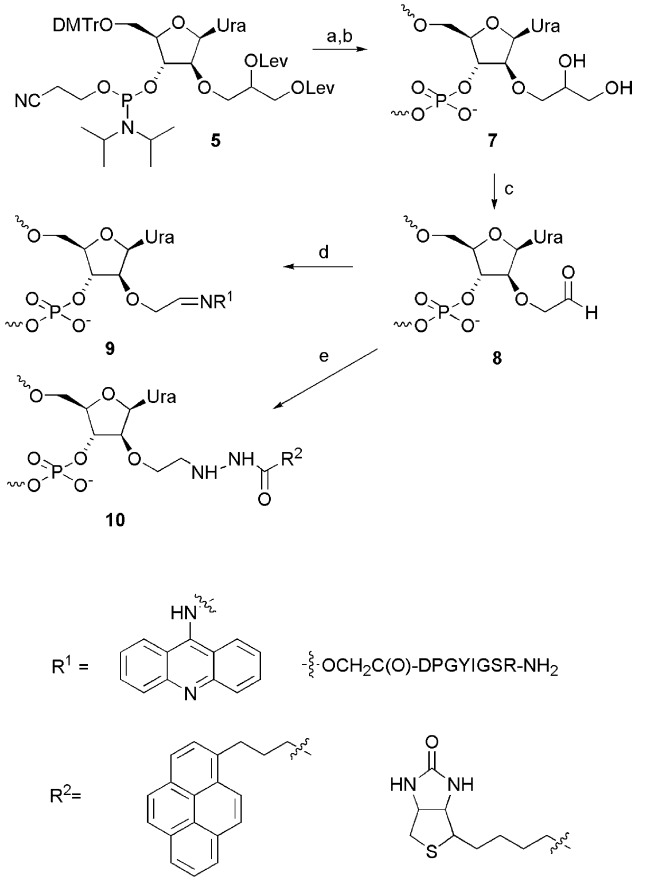
U—2′-*O*-(2,3-Dihydroxypropyl)arabinouridine or 2′-*O*-(2-oxoethyl)arabinouridine.

[a] For the 2′-diol modification.

[b] Buffer: 100 mM NaCl, 10 mM $NaHPO_4$, 1 mM EDTA, pH 7.0.

[c] $\Delta T_M/\text{mod} = \sum\{[T_M(n) - T_M(I)]/M\}/k$, where M is the number of modified nucleotides in a duplex, k is the number of the studied duplexes.

[d] For the 2′-aldehyde modification.



Scheme 2. Preparation of the 2′-aldehyde-containing oligonucleotides and their conjugates. Reagents and conditions: (a) automated oligonucleotide synthesis followed by aq NH_3, 55 °C, overnight; (b) 80% AcOH, 30 min; (c) $NaIO_4$, 0.2 M AcONa, pH 5.0, 15 min; (d) R^1NH_2 (1.5–2 equiv), 0.4 M AcONa, pH 4.5, DMSO (50% v/v), 3 h; (e) $R^2CONHNH_2$ (1.5–2 equiv), 0.4 M AcONa, pH 4.5, DMSO (50% v/v), 3 h; then $NaBH_3CN$, 30 min.

noborohydride reduction was needed. These results are in accordance with published data for hydrazone stability.[23] The conjugates were characterized by MALDI TOF MS. In all cases, the experimentally determined molecular masses were in good agreement with the calculated values (Table 2).

Binding of the modified oligonucleotides containing 2′-*O*-(2,3-dihydroxypropyl)arabinouridine to the complementary DNA and RNA sequences was investigated by their UV absorbance melting behaviour. Incorporation of 2′-*O*-(2-oxoethyl)arabinouridine into an oligodeoxyribonucleotide had only a slightly destabilizing effect on the T_M values of the complexes with DNA (Table 1). It should be noted that here we have used the correspondingly modified uridine derivative and that replacement of thymidine by 2′-deoxyuridine itself would be expected to decrease the T_M by ca. 0.5 °C.[24] Nonetheless, the destabilization was significantly stronger in the case of a complementary RNA (Table 1). Selective stabilization of the duplexes with a DNA target as compared to an RNA target may be explained by the *S*-conformation of the nucleoside and thus preferential B-type helix formation by the modified oligonucleotide.

Since incorporation of the modified nucleoside into oligodeoxyribonucleotides has only a marginal effect on the T_M values of their complexes with DNA targets, we believe these reactive oligomers may be useful for affinity modification of DNA-binding proteins. The investigation of cross-linking of the 2′-arabinoaldehyde oligonucleotides to transcription factor NF-kB is in progress and will be reported in due course.

Acknowledgements

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Table 2. Isolated yields and MALDI-TOF MS analysis of the conjugates of the 2′-modified oligonucleotides

Conjugated molecule	Oligonucleotide		
	II	**III**	**IV**
Biotin hydrazide	74[a] (3853.7/3853.9)[b]	79[a] (3853.7/3855.1)[b]	69[a] (4140.0/4142.1)[b]
Pyrenebutyric hydrazide	82[a] (3897.3/3893.7)[b]	—	74[a] (4228.1/4223.5)[b]
9-Hydrazinoacridine	84[a] (3802.6/3800.9)[b]	85[a] (3802.6/3803.0)[b]	62[a] (4036.8/4038.1)[b]
H_2NOCH_2CO-DPGYIGSR-NH_2	65[a] (4528.3/4531.0)[b]	—	50[a] (5490.3/5489.2)[b]

[a] Isolated yield of the conjugate.
[b] MALDI-TOF MS: calcd/found $[M+H]^+$.

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16. TMS—trimethylsilyl, Py—pyridine, All—allyl, BDDDP—2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin, Lev—levulinyl, DMAP—4-dimethylaminopyridine, DMTr—4,4′-dimethoxytrityl.
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22. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.68 (d, 1H, H-5, $J_{5,6}$ = 7.2 Hz), 7.45–7.20, 6.85–6.73 (m, 13H, DMTr), 6.23 (t, 1H, H-1′, $J_{1',2'}$ = 3.0 Hz), 5.58 (d, 1H, H-6), 5.32 (m, 1H, CH_2CH), 4.45–4.28 (m, 2H, H-2′, $CHCH_2$a), 4.17–4.12 (m, 2H, H-4′, $CHCH_2$b) 3.89–3.79 (m, 3H, H-3′, CH_2CH), 3.73 (s, 6H, DMTr), 3.52 (d, 1H, H-5′a, $J_{5'a,5'b}$ = 12.1 Hz), 3.47 (d, 1H, H-5′b), 2.78 (m, 2H, CO_2CH_2), 2.66 (m, 2H, CH_2CO), 2.54 (t, 2H, CH_2CN), 2.15 (s, 6H, $COCH_3$) 1.1 (s, 12H, CH_3CH); ^{13}C NMR ($CDCl_3$, δ, ppm): 206.63 (C═O, Lev), 172.41 (OC═O, Lev), 162.59 (C-4), 158.67 (DMTr), 150.54 (C-2), 144.43 (DMTr), 140.45 (C-6), 132.28, 130.19, 128.29, 127.96, 127.12, 117.74 (DMTr), 117.62 (CN), 113.22, 112.11 (DMTr), 103.05 (C-5), 100.04 (C-1′), 87.16 (C-4′), 86.47 (C-2′), 84.64 (C-3′), 83.04 (DMTr), 68.27 (C-2″), 65.49 (C-1″), 62.27 (C-5′), 62.04 (C-3″), 59.92 ($POCH_2$), 55.32 (DMTr), 42.79 (PNCH), 37.51 (CH_2COCH_3), 29.92 (CO_2CH_2), 29.76 ($COCH_3$), 26.11 (CH_3, Pr^i), 20.26 (CH_2CN); ^{31}P NMR (ppm): 150.40, 151.73; MALDI-MS: calcd $[M+H]^+$ = 1016.1. Found 1016.1.
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