# Oligoribonucleotides containing an aminoalkyl group at the N(4) atom of cytosine as precursors of new reagents for site-specific modifications of biopolymers

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A solid-phase H-phosphonate procedure was developed for the synthesis of oligoribonucleotides containing aliphatic amino linkers with different lengths at the N(4) atom of cytosine. The hybridization properties of modified oligoribonucleotides were studied. Thermal stability of the RNA—RNA* and DNA—RNA* duplexes depends on the position of modified cytosine in the chain and the type of the duplex.

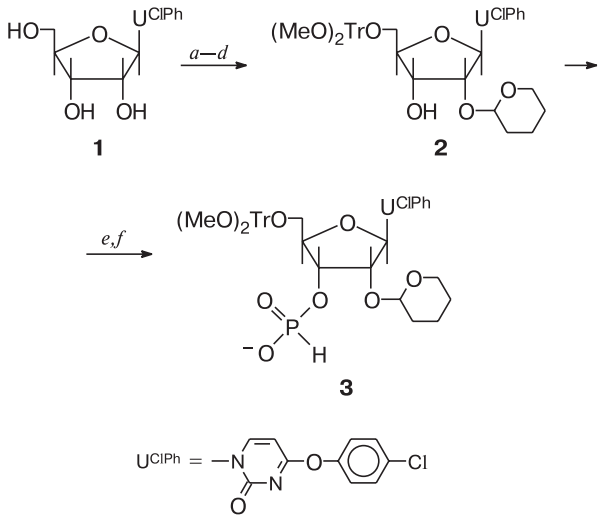
**Key words:** oligoribonucleotides, H-phosphonate synthesis, modification, cytosine, aliphatic amino linkers, duplex stability.

Oligoribonucleotides containing modified nucleotides at particular sites of the chain are used as precision tools in studies of biological processes based on interactions of RNA with nucleic acids and proteins. A convenient procedure for the modification of oligonucleotides involves the modification of heterocyclic bases.[1] Previously,[2,3] we have proposed an approach to the introduction of an aliphatic amino linker at the C(8) position of adenine or at the C(5) position of uracil. In the present study, we developed a solid-phase H-phosphonate procedure for the synthesis of oligoribonucleotides containing an aliphatic amino group at the N(4) position of cytosine and examined their properties.

## Results and Discussion

The approach under consideration is based on the insertion of a modified nucleoside, which contains the chlorophenyl group in the heterocyclic base, into an oligomer. The chlorophenyl substituent is stable under the synthesis conditions and is a good leaving group in the reactions with aliphatic diamines.[4] Oligoribonucleotides containing an aminoalkyl group at the N(4) atom of cytosine were synthesized with the use of the solid-phase H-phosphonate procedure developed by us previously.[5] The required monomeric synthon, *viz.*, 4-*O*-(4-chlorophenyl)-5´-*O*-dimethoxytrityl-2´-*O*-tetrahydropyranyluridine 3´-H-phosphonate (**3**), was prepared from 4-*O*-(4-chlorophenyl)uridine (**1**) (Scheme 1) and used in the corresponding step of the solid-phase H-phosphonate synthesis. The synthesis was carried out as described previously.[3] After the addition of the required number of nucleotide units, detritylation, and oxidation, the 4-*O*-(4-chlorophenyl)uridine-containing *N*-acyl-2´-*O*-tetrahydropyranylated oligomer (**4**), which was linked to a polymeric carrier, was treated with a 2 *M* solution of aliphatic diamine in MeOH at 42 °C for 18 h (Scheme 2). Previously, it has been demonstrated

**Scheme 1**




**Reagents:** *a*. $Pr^i_2(Cl)Si—O—Si(Cl)Pr^i_2$; *b*. DHP; *c*. $Et_4NBr/KF$; *d*. dimethoxytrityl chloride; *e*. $PCl_3/ImH/Et_3N$; *f*. $H_2O$.

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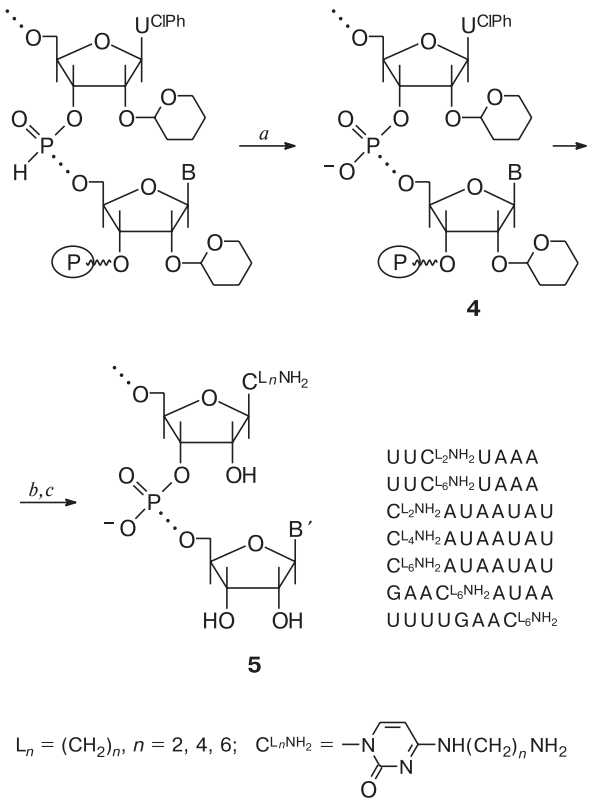
**Scheme 2**




**Reagents:** *a*. $I_2$/Py—$H_2O$; *b*. $NH_2(CH_2)_nNH_2$, $n$ = 2, 4, 6; *c*. 0.01 *M* HCl.

that under these conditions the chlorophenyl group in 4-*O*-(4-chlorophenyl)uridine (**1**) was completely replaced by the residue of aliphatic diamine. In addition, model experiments (the results are not presented) showed that the replacement of the chlorophenyl group was accompanied by deblocking of exocyclic amino groups of heterocyclic bases and removal of the oligomer from the polymeric carrier. The protective 2′-*O*-tetrahydropyranyl group was removed under standard conditions[3] and the deprotected amino-containing oligoribonucleotides were isolated by ion-exchange and reversed phase (RP) HPLC. It should be noted that the use of an acid-labile protective group (in this study, the 2′-*O*-tetrahydropyranyl group) enables one to prevent the undesirable cleavage of oligoribonucleotides under the action of amines.

If an oligomer contains several cytosine residues, the risk of the transamination of the latter under the action of aliphatic amines can be minimized using labile *N*-protective groups (for example, the phenoxyacetyl group[4]).

The proposed procedure for the synthesis of amino-containing derivatives of oligoribonucleotides allows one to prepare a family of amino-containing oligoribonucleotides, which differ only by the structure of the amino linker, starting from the same modified oligoribonucleotide precursor.

This approach was used for the synthesis of a series of modified oligoribonucleotides. Their yields and characteristics are given in Table 1. The nucleoside compositions of the resulting oligomers were confirmed by enzymatic hydrolysis followed by quantitative analysis of hydrolyzates by RP HPLC with the use of the molar absorption coefficients of specially synthesized nucleoside markers (see Table 1).

**Table 1.** Yields and characteristics of the modified oligoribonucleotides

| Oligoribonucleotide[a] | Yield[b] (%) | RP HPLC[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | $\tau$[d] /min | Spectral ratios ($A^{\lambda_1}/A^{\lambda_2}$) 250/260 | 270/260 | 280/260 | 290/260 | Nucleoside composition[e] |
| UUC$^{L_2NH_2}$UAAA | 5 (61) | 8.7 | 0.84 | 0.80 | 0.43 | 0.13 | U : C$^{L_2NH_2}$ : A = 3.1 : 1.2 : 3.0 |
| UUC$^{L_6NH_2}$UAAA | 15 (73) | 10.9 | 0.82 | 0.81 | 0.41 | 0.12 | U : C$^{L_6NH_2}$ : A = 3.2 : 0.8 : 3.0 |
| C$^{L_2NH_2}$AUAAUAU | 5 (65) | 10.6 | 0.87 | 0.87 | 0.46 | 0.11 | U : C$^{L_2NH_2}$ : A = 3.2 : 0.7 : 4.2 |
| C$^{L_4NH_2}$AUAAUAU | 6 (67) | 10.7 | 0.81 | 0.80 | 0.38 | 0.09 | U : C$^{L_4NH_2}$ : A = 3.0 : 0.8 : 4.2 |
| C$^{L_6NH_2}$AUAAUAU | 8 (70) | 11.4 | 0.80 | 0.80 | 0.38 | 0.09 | U : C$^{L_6NH_2}$ : A = 3.1 : 0.8 : 4.2 |
| GAAC$^{L_6NH_2}$AUAA | 12 (74) | 12.7 | 0.85 | 0.78 | 0.40 | 0.13 | U : C$^{L_6NH_2}$ : G : A = 1.1 : 1.0 : 1.3 : 4.7 |
| UUUUGAAC$^{L_6NH_2}$ | 12 (74) | 11.4 | 0.84 | 0.82 | 0.46 | 0.16 | U : C$^{L_6NH_2}$ : G : A = 4.0 : 1.0 : 1.1 : 1.8 |

[a] $L_2$ = $(CH_2)_2$, $L_4$ = $(CH_2)_4$, $L_6$ = $(CH_2)_6$.
[b] The total yield (the yield per step is given in parentheses) after two chromatographies with respect to the first nucleoside linked to the polymer.
[c] Nucleosil C-18 (5 μm, Macherey-Nagel, Germany), MeCN concentration gradient (0—20%) in 0.05 *M* $LiClO_4$.
[d] $\tau$ is the retention time.
[e] Nuclease $P_1$ in 30 *mM* NaOAc (pH 5.2), 1 *mM* $ZnSO_4$ (4 h, 37 °C) + *E.coli* alkaline phosphatase + 1—2 μL of 1% NaOH to pH 8.5 (4 h, 37 °C).

**Table 2.** Melting points (°C) of the duplexes of the modified octaribonucleotides with the complementary oligonucleotides of the ribo and deoxyribo series

| Oligoribonucleotide (5′-3′) | I | II |
|---|---|---|
| UUUUGAAC | 23 | 12 |
| UUUUGAAC$^{L_6NH_2}$ | 20 | 13 |
| GAACAUAA | 36 | 26 |
| GAAC$^{L_6NH_2}$AUAA | 35 | 24 |
| CAUAAUAU | 20 | 15 |
| C$^{L_6NH_2}$AUAAUAU | 16 | 13 |

*Note*. I, 3′-AAAACUUGUAUUAUA; II, 3′-d(AAAACTTGTATTATA); $L_6 = (CH_2)_6$. The conditions are given in the Experimental section.

With the aim of performing comparative studies of the hybridization properties of the modified oligomers, we constructed a model system consisting of the ribopentadecamer, its deoxyribo analog, and complementary oligoribonucleotides, which contain modified cytosine at the 3′- or 5′-terminus or within the chain (Table 2). In all cases, the RNA—RNA* duplexes were characterized by higher thermal stability and, simultaneously, by higher sensitivity with respect to the position of modified cytosine in the oligomer chain. Probably, this is associated with the fact that the RNA—RNA* duplex is structurally more rigid than the hybrid DNA—RNA* duplex. The replacement of one hydrogen atom in the 4-$NH_2$ group of cytosine, which is not involved in Watson—Crick interactions between the bases and is directed toward the major groove of the duplex, is unlikely to exert a steric effect on these interactions and, consequently, cannot essentially change the thermal stability of the duplex. Apparently, destabilization of the duplexes, which was observed in the case under consideration, results from a combination of the electronic effects of the protonated amino and hexamethylene fragments of the substituent.[1]

The results of the present study provide evidence that the hybridization ability of oligoribonucleotides containing an aminoalkyl group at the N(4) atom of cytosine depends on the position of the modified base in the chain and the type of the duplex.

The possibility of the insertion of reactive groups into amino-containing oligomers was exemplified by the synthesis of perfluoroarylazide derivatives of the oligoribonucleotides UUC$^{L_2NHR}$UAAA and C$^{L_6NHR}$AUAAUAU (R = —C(O)$C_6F_4N_3$) under the conditions described by us previously.[3] Oligoribonucleotide conjugates of this type can be used as new photoactivated reagents for site-directed modifications of biopolymers. In the future, we plan to study the hybridization and modifying properties of these conjugates.

To summarize, we developed a convenient procedure for the synthesis of oligoribonucleotides modified at the N(4) atom of cytosine. These compounds are precursors of new reagents for site-specific modifications of biopolymers.

## Experimental

The reagents and sorbents were purchased from Sigma (USA), Fluka (Switzerland), and Merck (Germany).

**4-*N*-(2-Aminoethyl)cytidine** was prepared according to a procedure described previously[6] in 33% yield. The homogeneity was 98% (RP HPLC). The spectral ratios ($\lambda_1/\lambda_2$, $A^{\lambda_1}/A^{\lambda_2}$): 250/260 0.88; 270/260 1.16; 280/260 0.96; 290/260 0.40. UV ($H_2O$), λ/nm (ε/mol$^{-1}$ L cm$^{-1}$): $\lambda^1_{max}$ 239 (5500); $\lambda^2_{max}$ 271 (7020); λ 260 (5900). $^1$H NMR ($D_2O$), δ: 7.86 (d, 1 H, H(6), $J$ = 2 Hz); 6.15 (d, 1 H, H(5), $J$ = 2 Hz); 6.00 (d, 1 H, H(1′), $J$ = 1 Hz); 4.39 (t, 1 H, H(4′), $J$ = 1 Hz); 4.31 (t, 1 H, H(3′), $J$ = 1 Hz); 4.21 (m, 1 H, H(2′)); 3.90 (m, 2 H, H(5′)); 3.79 and 3.33 (both m, 4 H, —NH(C$\underline{H}_2$)$_2$NH$_2$).

**4-*N*-(4-Aminobutyl)cytidine** was prepared according to a procedure described previously[4] in 91% yield. The homogeneity was 98% (RP HPLC). The spectral ratios ($\lambda_1/\lambda_2$, $A^{\lambda_1}/A^{\lambda_2}$): 250/260 0.86; 270/260 1.16; 280/260 0.97; 290/260 0.44. UV (EtOH), λ/nm (ε/mol$^{-1}$ L cm$^{-1}$): $\lambda_{max}$ 282 (8000); λ 260 (5800). $^1$H NMR ($CD_3OD$), δ: 7.90 (d, 1 H, H(6), $J$ = 2 Hz); 5.88—5.85 (m, 2 H, H(5), H(1′)); 4.20—3.70 (m, 5 H, H(2′), H(3′), H(4′), 2 H(5′)); 2.76 and 1.62 (both m, 8 H, —NH(C$\underline{H}_2$)$_4$NH$_2$).

**4-*N*-(6-Aminohexyl)cytidine** was prepared according to a procedure described previously[4] in 91% yield. The homogeneity was 94% (RP HPLC). The spectral ratios ($\lambda_1/\lambda_2$, $A^{\lambda_1}/A^{\lambda_2}$): 250/260 0.85; 270/260 1.18; 280/260 1.04; 290/260 0.55. UV (EtOH), λ/nm (ε/mol$^{-1}$ L cm$^{-1}$): $\lambda_{max}$ 273 (10300); λ 260 (8700). $^1$H NMR ($CD_3OD$), δ: 7.80 (d, 1 H, H(6), $J$ = 2 Hz); 5.97—5.89 (m, 2 H, H(5), H(1′)); 4.23—3.70 (m, 5 H, H(2′), H(3′), H(4′), 2 H(5′)); 2.92—2.76 and 1.70—1.32 (both m, 12 H, —NH(C$\underline{H}_2$)$_6$NH$_2$).

**4-*O*-(4-Chlorophenyl)uridine (1)** was prepared according to a procedure described previously[4] in 60% yield.

**4-*O*-(4-Chlorophenyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine** was prepared according to a procedure described previously[7] in 92% yield. $R_f$ 0.60, TLC, $CHCl_3$—MeOH (9 : 1).

**4-*O*-(4-Chlorophenyl)-2′-*O*-(tetrahydropyran-2-yl)uridine** (a mixture of diastereomers) was prepared according to a procedure described previously[7] in 41% yield. $R_f$ 0.23 and 0.34, TLC, $CHCl_3$—MeOH (9 : 1).

**4-*O*-(4-Chlorophenyl)-5′-*O*-dimethoxytrityl-2′-*O*-(tetrahydropyran-2-yl)uridine (2)** (a mixture of diastereomers) was prepared according to a procedure described previously[7] in 93% yield. $R_f$ 0.60 and 0.70, TLC, hexane—$CHCl_3$—MeOH (4.5 : 4.5 : 1.0). $^1$H NMR ($CDCl_3$), δ: 1.50—1.86 (m, 6 H, (C$\underline{H}_2$)$_3$CH$_2$OCH in THP); 3.55 (m, 3 H, (CH$_2$)$_3$C$\underline{H}_2$OC$\underline{H}$ in THP); 3.77 (s, 6 H, OMe); 3.92 (m, 2 H, H(5′)); 4.10, 4.30—4.40, and 4.86 (all m, 1 H each, H(2′), H(3′), H(4′)); 5.68 (d, 1 H, H(5), $J$ = 8 Hz); 5.88 and 6.25 (both d, 1 H, H(1′), $J$ = 2 Hz, $J$ = 5 Hz); 6.80—7.50 (m, 17 H, C$_6\underline{H}_4$Cl, C$_6\underline{H}_4$OMe, C$_6\underline{H}_5$); 8.40 and 8.50 (both d, 1 H, H(6), $J$ = 8 Hz, $J$ = 8 Hz). The assignment of the signals in the spectra was made as described previously.[3,4]

**4-*O*-(4-Chlorophenyl)-5′-*O*-dimethoxytrityl-2′-*O*-(tetrahydropyran-2-yl)uridine 3′-H-phosphonate (3)** (a mixture of diastereomers) was prepared according to a procedure described previously[8] in 73% yield. $R_f$ 0.25, TLC, MeCN—$H_2O$ (6 : 4). $^{31}P$ NMR (Py—MeCN), δ: 1.5, 1.7, $J_{P,H}$ = 610 Hz.

**Reactions of 4-*O*-(4-chlorophenyl)uridine (1) with aliphatic diamines** were carried out by keeping compound **1** in a 2 *M* solution of diamine in methanol at 42 °C for 18 h.[4] Then water was added, the reaction mixture was neutralized with AcOH, and the completeness of the transformation of compound **1** into the corresponding 4-*N*-alkylaminocytidines was analyzed by RP HPLC.

**H-Phosphonate synthesis of modified oligoribonucleotides** was carried out with the use of modified synthon **3** analogously to a procedure reported previously.[3] *N*,2′-*O*-Protected chlorophenyluridine-containing oligoribonucleotide **4** linked to a polymeric carrier was treated with diamine under the conditions described above. The modified oligoribonucleotides were salted out and isolated as described previously.[3] The homogeneity of the oligomers was 92—99% (RP HPLC).

**Thermal denaturation of oligonucleotide duplexes** was studied in a buffer solution containing 100 *mM* NaCl, 10 *mM* sodium cacodylate Na, and 1 *mM* $Na_2H_2$edta (pH 7.4, the concentration of each oligonucleotide component was $1.3 \cdot 10^{-5}$ *M*) on an instrument equipped with a temperature-controlled optical cell based on a UV detector of a Milikhrom liquid chromatograph (Nauchpribor, Russia) at the wavelength of 270 nm. The rate of heating was 0.7—1.0 deg $min^{-1}$.

**4-Azidotetrafluorobenzamide derivatives of oligoribonucleotides** were synthesized by the reactions of amino-containing oligomers with *N*-hydroxysuccinimide ester of 4-azidotetrafluorobenzoic acid as described previously.[3] The oligoribonucleotide $UUC^{L_2NHC(O)C_6F_4N_3}UAAA$: the yield was 82%; according to the data from RP HPLC, the homogeneity was 92%, the retention time was 13.4 min (the retention time of $UUC^{L_2NH_2}UAAA$ was 8.7 min); the spectral ratios ($\lambda_1/\lambda_2$, $A^{\lambda_1}/A^{\lambda_2}$): 250/260 0.82; 270/260 0.79; 280/260 0.41; 290/260 0.13. The oligoribonucleotide $C^{L_6NHC(O)C_6F_4N_3}AUAAUAU$: the yield was 85%; according to the data from RP HPLC, the homogeneity was 95%; the spectral ratios ($\lambda_1/\lambda_2$, $A^{\lambda_1}/A^{\lambda_2}$): 250/260 0.80; 270/260 0.81; 280/260 0.38; 290/260 0.11.

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