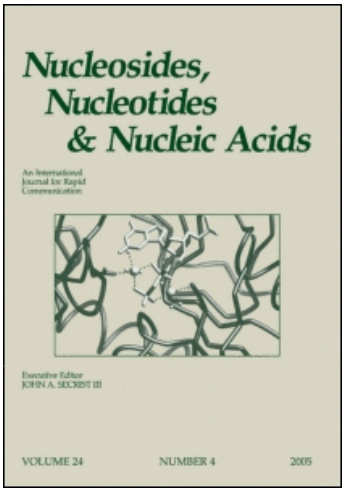

Nucleosides, Nucleotides and Nucleic Acids

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SYNTHESIS OF MODIFIED NUCLEOTIDE BUILDING BLOCKS CONTAINING ELECTROPHILIC GROUPS IN THE 2'-POSITION

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ABSTRACT: Chemical syntheses of 2'-*O*-(allyloxycarbonyl)methyladenosine, 2'-*O*-(methoxycarbonyl)methyladenosine and 2'-*O*-(2,3-dibenzoyloxy)propyluridine 3'-2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite building blocks are described. These monomers were used successfully to incorporate carboxylic acid, 1,2-diol and aldehyde functionalities into synthetic oligonucleotides.

INTRODUCTION

Chemically modified oligodeoxyribonucleotides have gained considerable importance mainly due to their potential application as modulators of gene expression, for example by an antisense mechanism.[1,2] Modifications may be introduced at base, phosphate or sugar moieties of the oligomers, primarily to alter chemical characteristics and to increase stability against degradation by nucleases. Derivatization of oligonucleotides at the nucleobases may cause interference with base-pairing and base-stacking interactions.

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† This publication is dedicated to Professor Alexander Krayevsky, an outstanding Russian scientist in the areas of bioorganic chemistry and molecular biology, who made a valuable contribution to the chemistry of nucleosides and nucleotides.

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Derivatization at the phosphodiester linkages may present solubility and chirality problems. Therefore, the functionalization of oligonucleotides at the carbohydrate moiety may provide a more suitable way for derivatization with certain reactive groups.

A growing interest in sugar functionalization of oligodeoxyribonucleotides has encouraged the development of nucleoside phosphoramidite derivatives suitable for such incorporation. Oligomers containing electrophilic groups such as carboxyl and carbonyl types can be used for covalent attachment to other nucleic acids fragments, peptides, reporter groups or for construction of other types of conjugates.[1,2] Modification of oligomers at the 2'-position allows one to introduce the modified phosphoramidite into any preselected site of the oligonucleotide chain. For this reason, we would like to report the synthesis of suitably protected 2'-*O*-(allyloxycarbonyl)methyl-5'-*O*-(4,4'-dimethoxytrityl)-N^6-benzoyladenosine, 2'-*O*-(methoxycarbonyl)methyl-5'-*O*-(4,4'-dimethoxytrityl)-N^6-benzoyladenosine and 2'-*O*-(2,3-dibenzoyloxy)propyl-5'-*O*-(4,4'-dimethoxytrityl)uridine 3'-2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite building blocks as potential sources of carboxylic acid, 1,2-diol and aldehyde functions in an oligonucleotide chain.

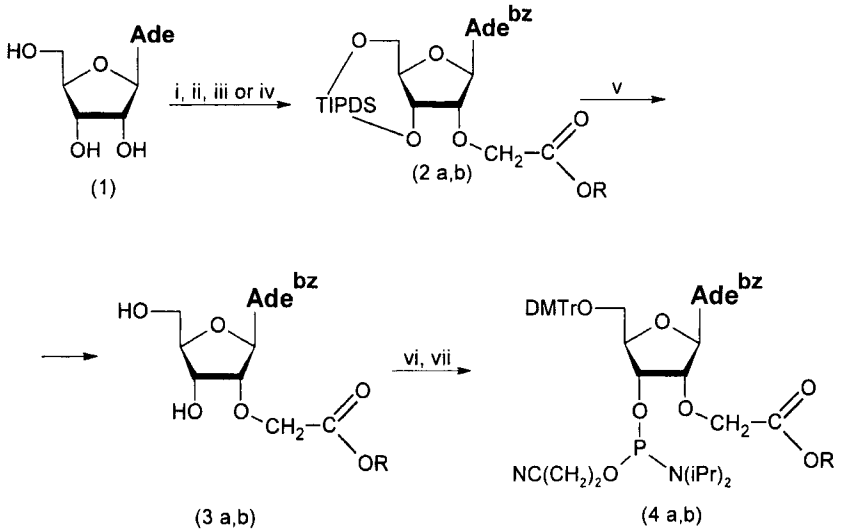
RESULTS AND DISCUSSION

The modified phosphoramidites **4a,b** and **11** (see **SCHEMES 1** and **2**) were synthesized for their incorporation into oligonucleotide chains. The 2'-carboxylic acid group needs to be protected and the protecting group must be easily introduced, stable throughout the process of automated oligonucleotide synthesis, and selectively removed in the end of assembly. Furthermore, free carboxyl groups may present problems with solubility in organic phase during the synthesis of the modified nucleoside derivatives. For these objectives, we selected methyl and allyl esters to block the carboxyl groups of the modified phosphoramidites. Each of these protecting groups can be easily removed after oligonucleotide synthesis. Moreover, selective deprotection of allyl esters using *tetrakis*(triphenylphosphine)palladium(0)[3] allows the modified, fully protected oligomer to remain support-bound and hence for subsequent reactions to be carried out on solid phase.

Selective alkylation of nucleosides at the sugar moiety usually involves a series of protection and deprotection steps, since unwanted alkylation at the heterocyclic residue

may otherwise occur. It should be noted that selective reaction at the 2'-hydroxyl group of the ribose moiety is difficult and efficient alkylation can only be achieved by use of very reactive electrophiles. General methods of selective alkylation have been reported in a number of publications.[4-6] Some authors have suggested the use of sodium or potassium hydroxide as a base. This approach has some disadvantages, notably low yields (<18%) as well as limitations to introduction of certain types of alkyl groups. Use of a metal hydroxide as a base can cause rearrangement reactions and the formation of a number of by-products. Thus, we adopted a convenient procedure for alkylation that resulted in a reasonable yield (35%) by use of a strong organic base 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorin (BDDDP), first described by Schwesinger.[7] Our route to the 2'-O-(allyloxycarbonyl)methyl-5'-*O*-(4,4'-dimethoxytrityl)-N^6-benzoyladenosine and 2'-O-(methoxycarbonyl)methyl-5'-*O*-(4,4'-dimethoxytrityl)-N^6-benzoyladenosine 3'-phosphoramidite building blocks **4a,b** is illustrated in **SCHEME 1**, starting with adenosine **1**. N^6-Benzoyladenosine was synthesized using a standard benzoylation procedure[8] and then 3',5'-protected using the well known Markiewicz reagent.[9] The product was then converted into the corresponding 2'-*O*-(allyloxycarbonyl)methyl-N^6-benzoyladenosine **2a** and 2'-*O*-(methoxycarbonyl)methyl-N^6-benzoyladenosine **2b** derivatives using chloroacetic acid allyl ester and bromoacetic acid methyl ester, respectively. We found that alkylation could be achieved using two equivalents of both BDDDP and the corresponding alkylating agent, and the reactions proceeded in reasonable yield with reaction times of 3-5 hours. Some N^1-alkylation was also observed as a side reaction (15% yield). Alkylation involving use of a strong inorganic base resulted in hydrolysis in the case of bromoacetic acid methyl ester and to cause rearrangements in the case of chloroacetic acid allyl ester. Compounds **2a,b** were then desilylated with TBAF[10] giving the compounds **3a,b**. Each modified nucleoside was treated with 4,4'-dimethoxytrityl chloride followed by 2-cyanoethoxy-*N,N*-diisopropylaminochlorophosphine to form the desired compounds **4a,b** in excellent yield.

It is well known that an aldehyde group is not sufficiently stable under standard conditions of oligonucleotide synthesis using phosphoramidite chemistry, and therefore it must be protected. One approach to is to protect the carbonyl moiety of the aldehyde.

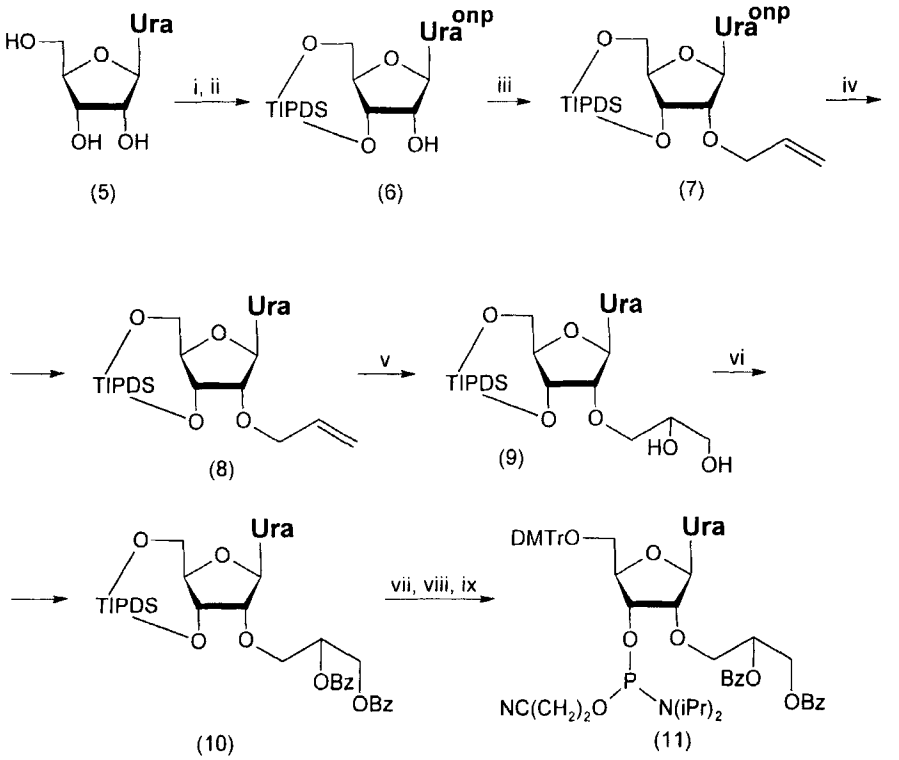


R = Allyl (a serie),
R = Me (b serie)

(i) TMSCl, BzCl, H_2O; (ii) TIPDS-Cl_2 ; (iii) chloroacetic acid allyl ester, BDDDP; (iv) bromoacetic acid methyl ester, BDDDP;(v) TBAF; (vi) DMTrCl; (vii) 2-cyanoethoxy-N,N-diisopropylamino-chlorophosphine

SCHEME 1

Such a protecting group must be stable under acidic and basic conditions and must be easily removed under very mild conditions. Therefore only a few of the common carbonyl protecting groups can be used. The most recently described protecting group: 1,3-di(3-chlorophenyl)imidazolidine which has been suggested by Matsuda *et al.*[11] is sterically hindered and is inappropriate for our application. An alternative approach relies on the synthesis of a "convertible" aldehyde precursor which can be easily released by a chemo-selective route. 1,2-Diols, which can be obtained from OsO_4-catalysed hydroxylation of alkenes, are obvious precursors that can be subsequently oxidized by treatment with periodate to give the corresponding aldehydes. Incorporation into oligonucleotides of a number of different modified nucleosides containing a 1,2-diol group has been discussed by several authors.[12-14] We carried out the synthesis of 2'-*O*-



(i) TIPDS-Cl_2; (ii) 1)TMSCl, 2) MesCl, DMAP, 3) 2-nitrophenol, DABCO, 4)TosOH; (iii) Pd^0, allylmethylcarbonate; (iv) 2-nitrobenzaldoxime, 1,1,3,3-tetramethylguanidine, (v) OsO_4, N-methylmorpholine N-oxide; (vi) BzCN; (vii) TBAF; (viii) DMTrCl; (ix) 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine

DABCO = 1,4-diazobicyclo[2.2.2]octane

onp = o-nitrophenyl

SCHEME 2

(2,3-dibenzoyloxy)propyl-5'-*O*-(4,4'-dimethoxytrityl)uridine 3'-2-cyanoethyl-*N*,*N*-diisopropyl phosphoramidite **11** using the route presented in **SCHEME 2**, starting from uridine **5**. Compound **6** was synthesized using the Markiewicz reagent followed by 2-nitrophenol.[10] Further selective and effective allylation of 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-O^4-2-nitrophenyluridine **6** with allylmethylcarbonate

and *tris*(dibenzylideneacetone) dipalladium(0) as suggested by Sproat *et al.*[15] gave the derivative **7** in a total yield of 60%. 3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-allyl-O^4-2-nitrophenyluridine **7** was then deprotected using 2-nitrobenzaldoxime and 1,1,3,3-tetramethylguanidine[10] to give compound **8**. The 1,2-diol **9** was accessible through an OsO_4-catalyzed dihydroxylation of the protected 2'-*O*-allylnucleoside **8**. To make this process more selective, we modified the method suggested by Lawrence *et al.*[16] We found that an effective oxidation of the allylic double bond by a catalytic amount of osmium tetroxide in the presence of *N*-methylmorpholine *N*-oxide proceeded in two days in very good yield (90%), with practically no oxidation of the uracil 5-6 double bond. The modified nucleoside containing a 1,2-diol at the 2'-position of the sugar moiety was protected using phenylglyoxylonitrile[17] resulting in formation of compound **10**. Standard desilylation[10] gave the 2'-*O*-(2,3-dibenzoyloxy)propyluridine, which was then reacted with 4,4'-dimethoxytrityl chloride followed by 2-cyanoethoxy-*N*,*N*-diisopropylamino chlorophosphine to give the desired compound **11** in nearly quantitative yield.

The phosphoramidites obtained were utilized in synthesis of a number of modified oligonucleotides (see **FIGURE 1**). Oligomers **I**, **III** and **IV** were synthesized as models in order to select and optimize the specific conditions of deblocking, purification and further oxidation in case of **IV**. Deblocking of modified oligonucleotides **I**, **III** and **IV** proceeded in the standard manner.[10] The oligomers obtained were then analysed and purified by reversed phase HPLC.

Deprotection of the carboxylic acid function of oligonucleotides **I** and **II** containing a 2'-*O*-(methyloxycarbonyl)methyladenosine residue was carried out with 2M NaOH solution. The presence of a 2'-carboxylic acid was confirmed by reaction in aqueous solution of oligodeoxyribonucleotide **II** with ethylenediamine dihydrochloride through activation of the 2'-carboxylic acid moiety with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The reaction mixture was analysed by reversed phase HPLC (ion pair mode, see **FIGURE 2**).

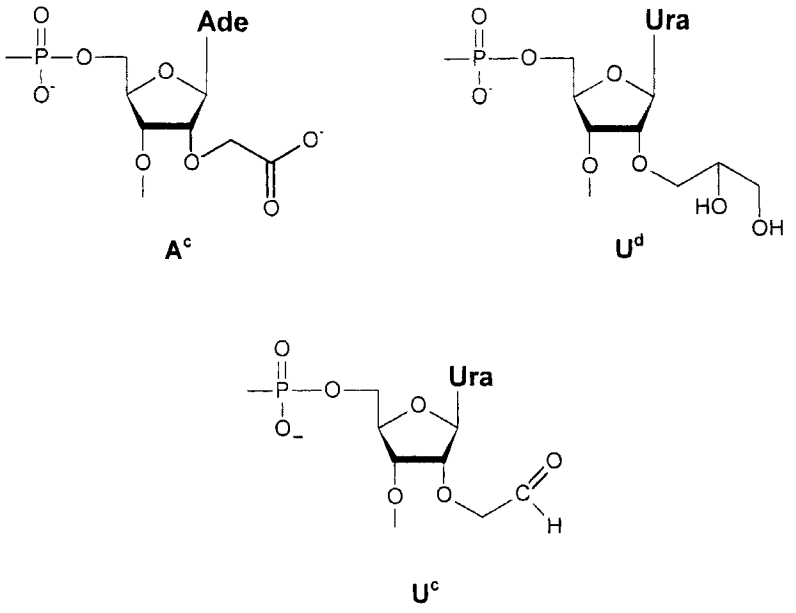
In the case of modified oligomer **III**, removal of the allyl protecting group was achieved using a solution of *tetrakis*(triphenylphosphine)palladium(0) and morpholine in dry dichloromethane. During this procedure, other protecting groups remained intact and the oligonucleotide was still linked to the polymer support. Then oligonucleotide **III** was

5'-TTTT**A**^{c}TTT-3' (I)

5'-GCTCCC**A**^{c}GGCTCAAA (II)

5'-TTTTTT**A**^{c}TTT-3' (III)

5'-TTTTTT**U**^{d}TTT-3' (IV)

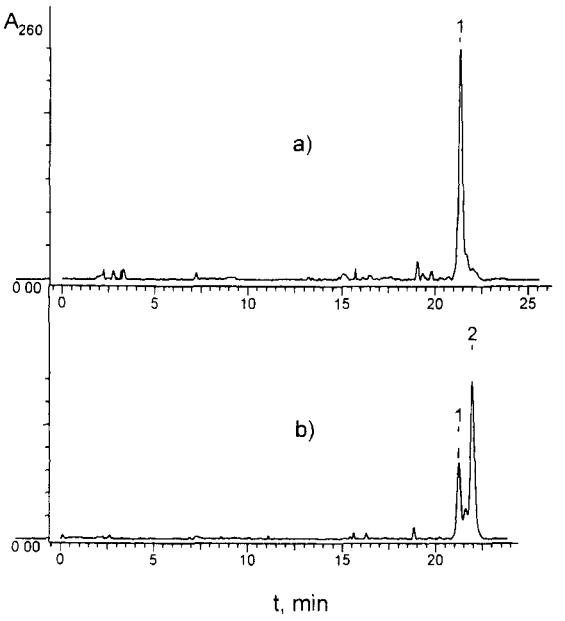


A^c U^d U^c

FIG. 1

cleaved from the support and deprotected using the standard procedure.[10] The presence of a carboxylic acid group was confirmed as described above.

The oxidation of oligonucleotide **IV** containing a deprotected and purified 1,2-diol was carried out using 5 mM $NaIO_4$ solution. Then reaction with 1M 4-nitrophenylhydrazine in 50% aqueous DMF was carried out to verify the presence of an aldehyde group. The reaction mixture was analysed by reversed phase HPLC (ion pair mode, see **FIGURE 3**).



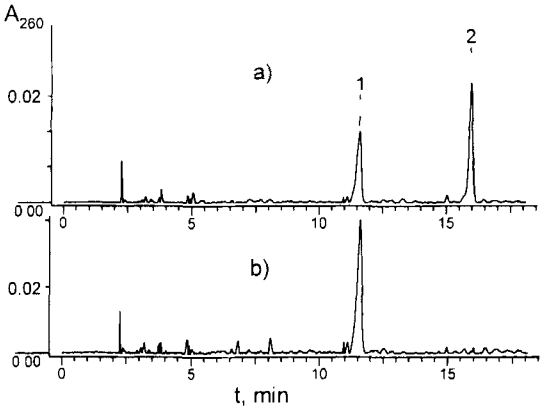
Reversed phase HPLC analysis (ion pair mode) of EDC-induced transformation of carboxylic acid function containing oligonucleotide (II) with ethylenediamine dihydrochloride in water solution (see **Experimental Section** for details): a) reaction mixture; b) reaction mixture co-injected with purified oligonucleotide (II), peak 1 - reaction product, peak 2 - purified oligonucleotide (II)

FIG. 2

In conclusion, we have described a new approach to the synthesis of modified oligodeoxyribonucleotides containing either carboxylic acid, 1,2-diol or aldehyde functions at the 2'-position of a sugar moiety. The presence of the functional groups was confirmed by chemical transformations. Further investigations into the chemical and physical chemical properties of such modified oligomers are in progress.

EXPERIMENTAL SECTION

Materials and general procedures: 2'-Deoxyribonucleoside 5'-*O*-(4,4'-dimethoxytrityl) 3'-2-cyanoethyl-*N*,*N*-diisopropylamino phosphoramidites for



Reversed phase HPLC analysis (ion pair mode) of reaction mixture resulting from transformation of oligonucleotide (IV) with 4-nitrophenylhydrazine followed by $NaIO_4$ oxidation (see **Experimental Section** for details): a) reaction mixture, peak 1 - oligomer (IV) after $NaIO_4$ oxidation, peak 2 – reaction product; b) purified oligomer (IV)

FIG. 3

oligonucleotide synthesis were purchased from Glen Research. All reagents for nucleoside chemistry were purchased from Fluka. TLC was carried out on silica gel TLC plates "Kieselgel 60 F_{254}" (Merck). Column chromatography was carried out on silica gel "ICN Silica 32-63, 60A" (ICN). ^1H NMR spectra were measured at 30^0C using a Bruker DRX-500 spectrometer (500.13 MHz for ^1H and 125.76 MHz for ^{13}C) and 2-5 mM solutions. Tetramethylsilane was used as the external standard. 2D spectra involved use of adapted COSY and HMQC techniques. ^{13}C spectra were measured using broad band proton decoupling. Chemical shifts are accurate to within 0.01 ppm for ^1H and ^{13}C. CSSI are accurate to within 0.25 Hz. Mass spectra (MS) were recorded on a Finnigan MAT LCQ mass spectrometer. Reversed phase HPLC analysis and purification of 5'-*O*-(4,4'-dimethoxytrityl)-protected oligonucleotides was carried out on a Tracor instrument using 4x250 mm column of DIAKS-130-CETYL (6 mm); buffer A: 0.1 M ammonium acetate (pH 7); buffer B: 0.1 M ammonium acetate, 40% MeCN, (pH 7); gradient from 0 to

100% of buffer B in 60 min; flow rate 1 ml/min; temperature 45 °C. Oligomers were analysed by reversed phase HPLC (ion pair mode) (Waters) on a DIAKS-130-CETYL column (6 mm, 4x250 mm) with a logarithmic gradient [0 - 45.9% B (1 min); 45.9 - 49.2% B (1 min); 49.2 - 53.6% B (3 min); 53.6 - 56.9% B (5 min); 56.9 - 60.2 B (10 min); 60.2 - 62.1% B (10 min); 62.1 - 63.5% B (10 min)] whereby a separation of oligomers with a retention time step of 1 min/unit is carried out; mobile phase A: water/MeCN (95:5 v/v), 2 mM tetrabutylammonium dihydrogen phosphate, 48 mM KH_2PO_4, pH 7; mobile phase B: water/MeCN (60:40 v/v), 2 mM tetrabutylammonium dihydrogen phosphate, 48 mM KH_2PO_4, pH 7; flow rate 1 ml/min, and temperature 45°C.

N^6-Benzoyladenosine was synthesized as described.[8] N^6-Benzoyladenosine and uridine were protected with the Markiewicz reagent as described[10]. 3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-O^4-2-nitrophenyluridine **6** was obtained using the method suggested by Sproat *et al.*[10]

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-(allyloxycarbonyl)methyl-N^6-benzoyladenosine ***2a***. 3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyl adenosine (2.45 g, 4 mmol) was dissolved in dry acetonitrile (8 ml) under an argon atmosphere. To this solution BDDDP (2 ml, 8 mmol) and chloroacetic acid allyl ester (8 mmol) were added with stirring and exclusion of moisture. Silica gel TLC in ethanol/chloroform (1:19 v/v) showed complete reaction after 5 hours. The reaction mixture was evaporated to dryness *in vacuo*. The dark red oil was dissolved in $CHCl_3$ (30 ml) and washed with water (30 ml). The aqueous phase was then washed with $CHCl_3$ (2x20 ml). The combined organic layers were dried (Na_2SO_4), filtered and solvent was removed *in vacuo*. The red oil was purified by column chromatography on silica gel, eluting with $CHCl_3$. The title compound was obtained as a white solid (1.0 g, 35 %). R_f 0.45 (ethanol/chloroform 3:97 v/v).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-(methoxycarbonyl)methyl-N^6-benzoyladenosine ***2b*** was synthesized as described above for **2a** using bromoacetic acid methyl ester (reaction time 3 h). The desired compound was obtained as a white solid (0.98 g, 35 %). R_f 0.43 (ethanol/chloroform 3:97 v/v).

Desilylation of compounds **2a,b** was carried out as described.[10]

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(allyloxycarbonyl)methyl-N^6-benzoyladenosine was obtained from **3a** by standard dimethoxytritylation procedure[8] (0.87 g, 90 %). ^{1}H-NMR

($CDCl_3$, δ, ppm): 8.45 (s, 1H, H8), 8.07 (s, 1H, H2), 7.58-7.53, 7.27-7.16, 6.77-6.73, (m, 20H, 4,4'-dimethoxytrityl, 3'-OH, 6-NH, Ph), 5.90 (m, 1H, -CH=), 5.78 (d, 1H, H1', $J_{1',2'}$=7.0Hz), 5.35 (dd, 1H, =CH_2a, $J_{=CH2a,\ =CH2b}$ =1.8Hz), 5.31 (dd, 1H, =CH_2b), 5,16 (d, 2H, -CH_2COO-), 4.73 (t, 1H, H2'), 4.67 (dd, 2H, -OCH_2), 4.41-4.36 (m, 2H, H3', H4'), 3.80 (s, 6H, H-methoxy), 3.44 (dd, 1H, H-5'a, $J_{5'a,5'b}$=11.8Hz), 3.27 (dd, 1H, H-5'b).

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(methoxycarbonyl)methyl-N^6-benzoyladenosine was obtained from **3b** as described[8] (0.85 g, 90 %). ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.48 (s, 1H, H8), 8.09 (s, 1H, H2), 7.57-7.55, 7.27-7.05, 6.77-6.75 (m, 20H, 4,4'-dimethoxytrityl, 3'-OH, 6-NH, Ph), 5.95 (d, 1H, H1', $J_{1',2'}$=7.0Hz), 5,16 (d, 2H, -CH_2COO-), 4.73 (t, 1H, H2'), 4.42-4.39 (m, 2H, H3', H4'), 3.79 (s, 6H, OCH_3), 3,76 (d, 3H, -$COOCH_3$), 3.42 (dd, 1H, H-5'a, $J_{5'a,5'b}$=11.8Hz), 3.26 (dd, 1H, H-5'b).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-allyl-O^4-2-nitrophenyluridine ***7*** was obtained from **6** as described[15] (3.37 g, 90%). MALDI MS: calc. $C_{30}H_{46}N_3O_9Si_2$ 647.8, found m/z 647.2 ($M+H^+$).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-allyluridine ***8*** was obtained from **7** using 2-nitrobenzaldoxime and 1,1,3,3-tetramethylguanidine as described[10] (2.32 g, 85%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 9.35 (d_b, 1H, NH), 7.92 (d, 1H, H6, $J_{5,6}$=8.1Hz), 5.95 (m, 1H, -CH=), 5.76 (m, 1H, H1') 5.69 (m, 1H, H5), 5.40 (dd, 1H, =CH_2a, $J_{=CH2a,=CH2b}$=1.8Hz), 5.20 (dd, 1H, =CH_2b), 4.39 (m, 2H, -CH_2-), 4.27-4.00 (m, 5H, H2', H3', H4', H5'), 1.12-0.95 (m, 28H, H-iPr).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-(2,3-dihydroxypropyl)uridine ***9***. 3',5'-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-*O*-allyluridine **8** (2.1 g, 4 mmol) was dissolved in dry THF (45 ml). *N*-Methylmorpholine *N*-oxide (0.65 g, 4.8 mmol) in water (20 ml) and OsO_4 (0.04 mg, 0.16 mmol) in dry THF (5 ml) were then added. The solution was stirred for 4 h at room temperature for 2 days at 4 °C. When TLC (ethanol/chloroform 1:9 v/v) showed complete reaction, it was quenched by the addition of saturated aqueous $Na_2S_2O_3$ solution and diluted with $CHCl_3$ (20 ml). The mixture was washed with saturated $NaHCO_3$ solution (20 ml) and water (20 ml). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a gradient of ethanol in chloroform (0 to 6%). The pure product was obtained as a white solid (2.25 g, 90%), R_f 0.4

(ethanol/chloroform 1:9 v/v). ^{1}H-NMR ($CDCl_3$, δ, ppm): 9.80 (d_b, 1H, NH), 7.88 (d, 1H, H6, $J_{5,6}$=8.1Hz), 5.74 (d, 1H, H1'), 5.72 (d, 1H, H5), 4.27-3.74 (m, 11H, H2', H3', H4', H5', -CH_2- , -C<u>H</u>(OH)-, -C<u>H$_2$</u>OH, -CH(O<u>H</u>)-, -CH_2O<u>H</u>), 1.26-0.98 (m, 28H, H-iPr).

3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-2'-O-(2,3-dibenzoyloxy)propyl-uridine ***10*** was obtained as described[17] as a white solid (2.62 g, 85%), R_f 0.6 (ethanol/chloroform 1:9 v/v), ^{1}H-NMR ($CDCl_3$, δ, ppm): 8.78 (d_b, 1H, NH), 8.1 (m, 4H, o-Ph) 7.91 (d, 1H, H6), 7.6 (m, 2H, p-Ph), 7.45 (m, 4H, m-Ph), 5.78 (m, 1H, H1'), 5.71 (m, 2H, H5, -C<u>H</u>(OBz)-), 4.78-4.70 (m, 3H, H3', H5'), 4.30-4.17 (m, 2H, -C<u>H$_2$</u>OBz), 4.02-3.77 (m, 4H, -CH_2-, H2', H4'), 1.15-0.97 (m, 28H, H-iPr).

2'-O-(2,3-dibenzoyloxy)propyluridine was obtained by desilylation from **10** as described[10] (1.53 g, 85%), R_f 0.25 (ethanol/chloroform 1:9 v/v). ^{1}H-NMR ($(CD_3)_2CO$, δ, ppm): 9.79 (d_b, 1H, NH,), 8.10 (m, 1H, H6, $J_{5,6}$=8.1 Hz), 8.05-8.01 (m, 4H, o-Ph), 7.62-7.60 (m, 2H, p-Ph), 7.50-7.47 (m, 4H, m-Ph), 6.00 (d, 1H, H1', $J_{1',2'}$=7.00 Hz), 5.65 (m, 1H, -C<u>H</u>(OBz)-, $J_{-CH2a-,-CH(OBz)-}$=6.88 Hz), 5.54 (m, 1H, H5), 4.79 (td, 1H, -CH_2a-, $J_{-CH2a-,-CH2b-}$=11.3 Hz), 4.67 (m, 1H, -CH_2b-, $J_{-CH2b-,-CH-}$=2.94 Hz), 4.42 (k_b, 1H, H3', $J_{3',4'}$=6.50 Hz), 4.26 (k, 1H, -CH_{2a}OBz, $J_{-CH-,-CH2aOBz}$=6.88 Hz, $J_{-CH2aOBz-,-CH2bOBz}$=12.0 Hz), 4.23 (m, 1H, H2', $J_{2',3'}$=7.00 Hz), 4.20 (k, 1H, -CH_{2b}OBz, $J_{-CH-,-CH2bOBz}$=2.94 Hz), 4.02 (ddd, 1H, H4', $J_{4',5'a}$=6.04 Hz), 3.87 (t, 1H, H5'a, $J_{5'a,5'b}$=12.35 Hz), 3.81 (t, 1H, H5'b, $J_{4',5'a}$=1.01 Hz), 3.45 (m, 2H, 3'-OH, 5'-OH).

^{13}C-NMR ($(CD_3)_2CO$, δ, ppm): 166.38 {2'-O-[2,3-(O<u>Bz</u>)$_2$Pr], C=O}, 164.7 {2'-O-[2,3-(O<u>Bz</u>)$_2$Pr], C=O}, 164.0 (C-4), 151.39 (C-2), 141.20 (C-6), 134.05 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-4), 130.98 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-2, C-6), 130.85 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-2, C-6), 130.39 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-1), 130.33 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-1, 2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-3, C-5), 129.41 (2'-O-(2,3-(O<u>Bz</u>)$_2$Pr), C-3, C-5), 102.30 (C-5'), 88.55 (C-2'), 85.89 (C-1'), 85.66 (C-4'), 83.95 (C-3'), 72.00 (2'-O-(2,3-(OBz)$_2$<u>Pr</u>), C-2), 69.86 (2'-O-(2,3-(OBz)$_2$<u>Pr</u>),C-1), 64.06 (2'-O-(2,3-(OBz)$_2$<u>Pr</u>), C-3), 59.37 (C-5').

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(2,3-dibenzoyloxy)propyluridine was obtained as a white solid form using standard dimethoxytritylation procedure[8] (2.24 g, 95%), R_f 0.3 (ethanol/chloroform 1:19 v/v). ^{1}H-NMR ($CDCl_3$, δ, ppm): 9.79 (d_b, 1H, NH), 8.1-8.05, 7.65-7.25, 6.95-6.85 (m, 19H, Ph, 4,4'-dimethoxytrityl, H6), 5.92 (d, 1H, H1'), 5.75 (m, 1H, -CH(OBz)-), 5.3 (d, 1H, H5), 4.8 (dd, 1H, -CH_{2a}OBz), 4.72 (m, 1H, 3'-OH), 4.62 (m,

1H, -CH_{2b}OBz), 4.50 (m, 1H, H3'), 4.40 (m, 1H, -CH_{2a}-), 4.10-4.00 (m, 3H, -CH_{2b}-, H-2', H-4'), 3.85 (s, 6H, CH_3O), 3.62 (m, 1H, H-5'a), 3.55 (m, 1H, H-5'b).

^{13}C-NMR ($CDCl_3$, δ, ppm): 166.38 {2'-O-[2,3-(OBz)$_2$Pr], C=O}, 164.7 {2'-O-[2,3-(OBz)$_2$Pr], C=O}, 164.0 (C-4), 151.39 (C-2), 141.20 (C-6), 134.05 (2'-O-(2,3-(OBz)$_2$Pr), C-4), 130.98 (2'-O-(2,3-(OBz)$_2$Pr), C-2, C-6), 130.85 (2'-O-(2,3-(OBz)$_2$Pr), C-2, C-6), 130.39 (2'-O-(2,3-(OBz)$_2$Pr), C-1), 130.33 (2'-O-(2,3-(OBz)$_2$Pr), C-1, 2'-O-(2,3-(OBz)$_2$Pr), C-3, C-5), 129.41 (2'-O-(2,3-(OBz)$_2$Pr), C-3, C-5), 102.30 (C-5'), 88.55 (C-2'), 85.89 (C-1'), 85.66 (C-4'), 83.95 (C-3'), 72.00 (2'-O-(2,3-(OBz)$_2$Pr),C-2), 69.86 (2'-O-(2,3-(OBz)$_2$Pr),C-1), 64.06 (2'-O-(2,3-(OBz)$_2$Pr), C-3), 59.37 (C-5').

Compounds **4a,b** and **11** were obtained by the reaction of corresponding nucleoside derivatives with 2-cyanoethoxy-*N*,*N*-diisopropylaminochlorophosphine as described.[8]

Oligonucleotide Synthesis. Oligodeoxynucleotides were assembled on a ABI 380B DNA Synthesizer by the cyanoethyl phosphoramidite method following manufacturer recommendations. 0.4 μmol scale columns were used throughout. For couplings with modified phosphoramidites **4a,b** and **11**, a 0.15 M concentration in anhydrous MeCN was used and the coupling time was increased to 15 min. Trityl ON configuration was applied in each final cycle.

*Deprotection of carboxylic acid function of oligonucleotides **I**, **II***. The methyl ester was hydrolyzed by treating support-bound oligomers with 2 M NaOH for 30 min at RT and then with 2 M ammonium acetate solution for 10 min. After that oligonucleotides were cleaved from the support and deprotected using concentrated ammonia overnight at 55 °C.

*Deprotection of carboxylic acid function of oligonucleotide **III***. The allyl ester was deprotected from the support-bound oligodeoxynucleotide by treatment with a solution of morpholine (15 μl) and *tetrakis*(triphenylphosphine)palladium(0) (10 μg) in CH_2Cl_2 (20 μl) for 2 h at room temperature. The supernatant was then decanted off and the support was rinsed with CH_2Cl_2 (2x50 μl). After that the oligonucleotide was cleaved from the support and deprotected as described earlier.

The presence of a carboxylic acid group in oligonucleotides **(I), (II), (III)** was confirmed as stated below.

Reaction of modified oligodeoxyribonucleotide containing 2'-carboxylic acid group with ethylenediamine hydrochloride. The solution of oligomer **II (or III)** (0.15 OD_{260}) was evaporated to dryness *in vacuo* and redissolved in water (40 μl). Then ethylenediamine dihydrochloride (5.3 mg, 0.04 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (3 mg, 0.015 mmol) were added, and the mixture was vortexed. After six hours incubation at RT the reaction mixture was precipitated with 2M $LiClO_4$ solution (200 μl) and acetone (1000 μl) and then analysed using reversed phase HPLC (ion pair mode).

*Oxidation of 1,2-diol group of modified oligomer **IV** to aldehyde and reaction with 4-nitrophenylhydrazine.* The dried oligonucleotide (0.6 OD_{260}) was dissolved in 0.1 M acetate buffer (50 μl), pH 4.6. Then 5 mM $NaIO_4$ solution (10 μl) was added, and the reaction mixture was incubated at RT for 20 minutes. 5 mM glycerol (5 μl) was added and after 10 minutes of incubation the mixture was evaporated *in vacuo*. The residue was dissolved in 0.1M phosphate buffer (40 μl), pH 7.5 followed by addition of 0.05 M 4-nitrophenylhydrazine in DMF (40 μl). The mixture was incubated for 2 h at 55 °C. A 0.5 M solution of $NaBH_4$ (4 μl) was added four times at 30 min intervals at room temperature. After that 4 M sodium acetate buffer (20 μl), pH 4.6 was added. The mixture was diluted with water (100 μl) and organic by-products were extracted with CH_2Cl_2 (2x200 μl). Then the mixture was desalted on a NAP-5 (Pharmacia) column and analysed by reversed phase HPLC (ion pair mode).

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