Synthesis of oligo-2′-*O*-methylribonucleotides containing α-amino acid residues in 2′-position*

E. V. Kazanova,[a] E. M. Zubin,[a] A. V. Kachalova,[a] D. A. Stetsenko,[b] M. J. Gait,[c] and T. S. Oretskaya[a]*

[a]*Department of Chemistry , M. V. Lomonosov Moscow State University, 1 Leninskie Gory, 119992 Moscow, Russian Federation. Fax: +7 (495) 939 3181. E-mail: evkazanova@mail.ru, emzubin@mail.ru*
[b]*Supramolecular and Biomolecular Chemistry Division, School of Biomedical and Molecular Sciences, University of Surrey,Guildford, Surrey GU2 7XH, UK*
[c]*Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK*

Oligo-2′-*O*-methylribonucleotides containing residues of phenylalanine, histidine, and lysine amides were synthesized with the use of new phosphoramidites of 2′-aminoacid derivatives of uridine.

Key words: nucleosides, phosphoramidites, oligonucleotides, amino acids.

Derivatives of oligonucleotides are widely used in molecular biology and medicine due to their unique ability to bind to complementary sequences of DNA and RNA, which can result in a selective suppression of expression of certain genes. At present, an intensive research work is being carried out aimed at the design of effective therapeutic preparations based on nucleic acids.[2,3]

Elaboration of methods for the synthesis of artificial ribonucleases is one of trends in chemistry of nucleic acids.[4,5] A cell or viral RNA may serve as the potential targets, which determines the topicality of this task in the first place. Very often, in the design of artificial ribonucleases it is preferable to combine, in one molecule, an oligonucleotide complementary to the given region of RNA and a substance that catalyzes hydrolysis of certain phosphodiester bonds of the ribonucleic acid after formation of a two-stranded complex (duplex). For example, attempts were undertaken at constructing artificial ribonucleases based on oligonucleotides containing imidazole and primary aliphatic amine residues.[5,6]

It was shown earlier that in the presence of basic amino acids and short peptides containing polyamine residues a remarkable increase in the rate of cleavage of the RNA target takes place upon its treatment with ribozymes under deficiency in magnesium ions.[7] In this connection, it can be supposed that oligonucleotides bearing basic amino acid residues can also possess ribonuclease activity. Thus, the development of efficient methods for the synthesis of amino acid derivatives of oligonucleotides is quite justified. The heterocyclic bases in the catalytic sites of ribozymes are often involved into various non-covalent interactions essential for maintaining their structure and catalytic activity; the ribose residue, in particular its 2′-position, seems to be the most preferable for the modification. In the present work, a promising method of 2′-functionalization was used, which is based on the formation of a carbamate bond between primary or secondary alcohol and primary aliphatic amine upon treatment with *N*,*N*′-carbonyldiimidazole[8] or *N*,*N*′-disuccinimidyl carbonate (DSC).[9] It was noted[8,9] that oligonucleotide derivatives containing *N*-substituted carbamate bonds with involvment of the 2′-hydroxy group of uridine form duplexes with complementary DNA or RNA; these duplexes have low thermal stability. At the same time, it is known that many 2′-*O*-alkyl derivatives of oligoribonucleotides form particularly stable duplexes with complementary RNAs.[10] For example, it was shown[11] that analogous 2′-derivatives do not decrease stability of the complex of modified oligonucleotide with complementary RNA. In order to avoid the negative effect of the modification on the hybridization properties of oligonucleotides, we have developed an approach allowing separation of the 2′-*O*-position of nucleoside from the carbamate bond by an alkoxy group.

Thus, in the present work a general method was proposed for the synthesis of 2′-amino acid derivatives of oligo-2′-*O*-methylribonucleotides containing residues of phenylalanine, histidine, and lysine amides.

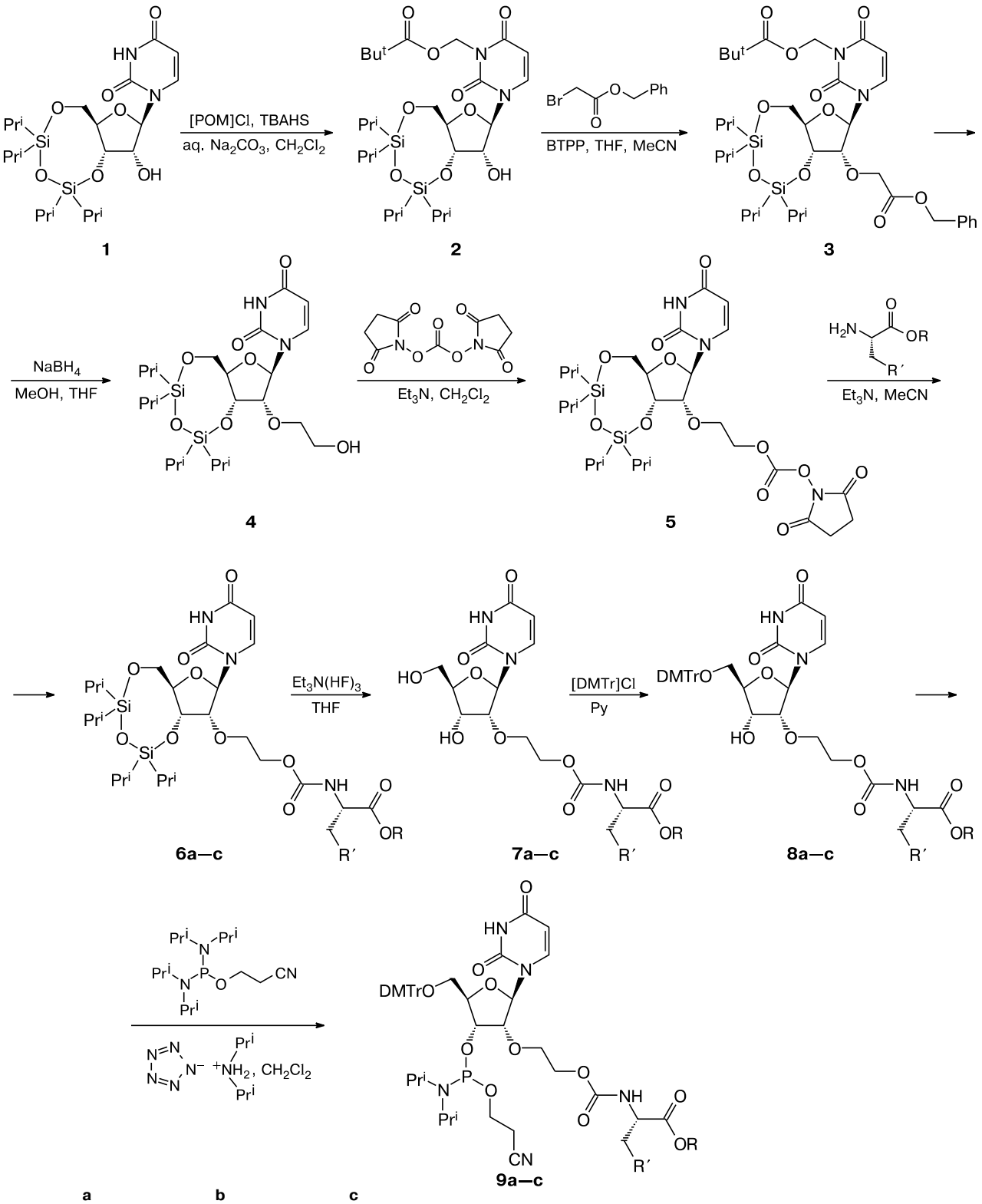
Results and Discussion

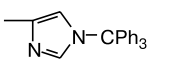
The synthesis of 3′-phosphoramidites of 2′-amino acid derivatives of uridine is illustrated in Scheme 1.

* For communication see Ref. 1.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 775—783, April, 2007.

Scheme 1



	a	b	c
R	$CH_2CH{=}CH_2$	Me	Me
R′	Ph		$(CH_2)_3NHfmoc$

Note. TBAHS is tetrabutylammonium hydrogen sulfate.

3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridine **1** was obtained from uridine by treatment with the Markevich reagent, *i.e.*, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1,3-Cl_2-TIPDS).[12] Protection of position 3 in uridine with pivaloyloxymethyl group (POM) makes it possible to selectively alkylate 2′-OH group of the ribose residue in the 3′,5′-protected nucleoside. The procedure of introduction of the pivaloyloxymethyl group[13,14] includes the intermediate protection of the 2′-hydroxy group with the trimethylsilyl protecting group, which can further be removed by mild hydrolysis under the action of TsOH. We employed phase-transfer catalysis to obtain 3-pivaloyloxymethyl derivative. In this case, no intermediate protection of the 2′-hydroxy group is required and 3-pivaloyloxymethyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)uridine **2** was obtained in 70% yield (see Ref. 15).

The next stage included formation of 3,3′,5′-protected 2′-*O*-benzyloxycarbonylmethyluridine **3**. Methods for the synthesis of 2′-*O*-alkylnucleosides are summarized in a review.[16] Earlier,[17,18] we have successfully used a procedure for 2′-*O*-alkylation in the presence of a strong organic base, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP). In the present work, we used *tert*-butylimino-tris(pyrrolidino)phosphorane (BTPP) with similar properties instead of expensive BEMP. Alkylation of compound **2** with benzyl bromoacetate in the presence of BTPP occurred selectively at the 2′-hydroxy group to yield 91% of **3**.

Benzyl ester **3** was further reduced into the known[19] alcohol **4**. The reaction was carried out with sodium borohydride in a THF—methanol mixture (5 : 1, v/v) under reflux for 3 h according to the described procedure.[20] The yield of compound **4** was 70%. It should be noted that during the reduction the removal of the pivaloyloxymethyl protecting group also takes place.

Reaction of alcohol **4** with *N*,*N*′-disuccinimidyl carbonate in the presence of Et_3N in CH_2Cl_2 gave the activated carbonate **5** in virtually quantitative yield. This compound decomposed during attempted column chromatography on silica gel, that is why it was used in the reaction with the corresponding amino acid esters (L-phenylalanine allyl ester and N^{Im}-trityl-L-histidine and methyl esters of N^{ε}-fmoc-L-lysine; fmoc stands for fluoren-9-ylmethoxycarbonyl) without chromatographic isolation. The phenylalanine derivative was chosen as a model compound for optimization of the reaction conditions. The yields of compounds **6a—c** were 89, 81, and 90%, respectively. Amino acid derivatives of uridine **6a—c** were further treated with triethylamine trihydrofluoride in THF to remove the silyl protecting group[8] resulting in compounds **7a—c** in high yields.

Protection of the 5′-hydroxy group of the nucleoside was carried out with dimethoxytrityl protecting group (DMTr) according to the known procedure.[21] Amino acid derivatives of 5′-*O*-protected nucleosides **8a—c** were obtained in 85—90% yields after chromatographic isolation. Compounds **8a—c** were further phosphitylated with bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine and diisopropylammonium tetrazolide according to the described procedure.[22] 3′-Phosphoramidites of uridine **9a—c** with phenylalanine, histidine, and lysine residues were thus obtained. The structures of all the compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ^{31}P NMR (for

Table 1. Properties of modified oligo-2′-*O*-methylribonucleotides[a]

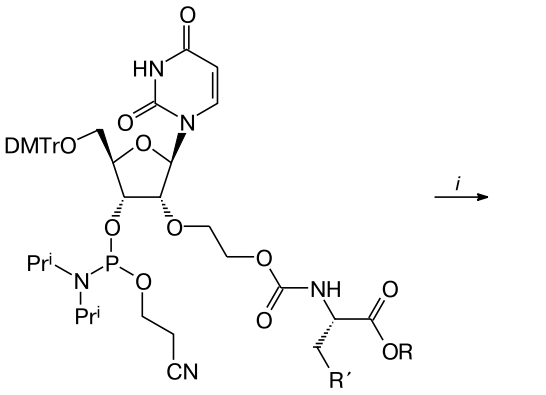
Oligonucleotide	Oligonucleotide sequence (5′→3′)	MALDI-TOF MS, Found/calculated	τ/min[b]
I	CUCCCAGGC**U**$^{\mathbf{Phe}}$CA	4117.85/4117.82	17.3
II	GUCCUU$^{\mathbf{Phe}}$ACUCC	3735.13/3736.72	15.9
III	CUCCCAGGC**U**$^{\mathbf{His}}$CA	4109.32/4109.79	16.6
IV	GUCCUU$^{\mathbf{His}}$ACUCC	3726.12/3726.71	15.8
V	CUCCCAGGC**U**$^{\mathbf{Lys}}$CAAAU	5104.02/5105.03	14.4
VI	GUCCUU$^{\mathbf{Lys}}$ACUCC	3718.97/3719.54	14.0
VII	**U**$^{\mathbf{His}}$**UU**$^{\mathbf{Lys}}$**UU**$^{\mathbf{His}}$ACUCCGUCCG	5456.99/5457.08	16.1
VIII	**U**$^{\mathbf{Lys}}$**UU**$^{\mathbf{His}}$**UU**$^{\mathbf{His}}$ACUCCGUCCG	5457.48/5457.08	16.0
IX	**U**$^{\mathbf{His}}$**UU**$^{\mathbf{Lys}}$**UU**$^{\mathbf{Lys}}$ACUCCGUCCG	5448.49/5448.12	14.6
X	**U**$^{\mathbf{His}}$**UU**$^{\mathbf{His}}$**UU**$^{\mathbf{Lys}}$ACUCCGUCC	5098.03/5098.02	15.6
XI	**U**$^{\mathbf{His}}$**UU**$^{\mathbf{His}}$**UU**$^{\mathbf{His}}$ACUCCGUCCG	5466.09/5466.04	17.7
XII	**U**$^{\mathbf{Lys}}$**UU**$^{\mathbf{His}}$**UU**$^{\mathbf{Lys}}$ACUCCGUCCG	5448.00/5448.12	14.6

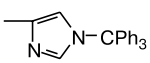
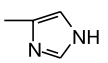
[a] **U**$^{\mathbf{Phe}}$: amide of N^{α}-{2-[uridin-2′-yl]oxyethoxy}carbonyl-L-phenylalanine, **U**$^{\mathbf{His}}$: amide of N^{α}-{2-[uridin-2′-yl]oxyethoxy}carbonyl-N^{Im}-trityl-L-histidine, **U**$^{\mathbf{Lys}}$: amide of N^{α}-{2-[uridin-2′-yl]oxyethoxy}carbonyl-N^{ε}-(9-fluorenylmethoxycarbonyl)-L-lysine; A, C, U, G are 2′-Me-derivatives of the corresponding nucleotides.

[b] Reversed-phase HPLC in an ion-pair version (conditions are given in the Experimental); τ, retention time.

phosphoramidites) spectroscopic data, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), and electrospray ionisation mass spectrometry (ESI) (see Experimental).

Scheme 2



	R	R′	R″
a	$CH_2CH{=}CH_2$	Ph	Ph
b	Me		
c	Me	$(CH_2)_3NHfmoc$	$(CH_2)_3NH_2$

i. Oligonucleotide synthesis. *ii*. 1) Conc. aq. NH_3, 55 °C; 2) 80% aq. AcOH.

Phosphoramidites of 2′-modified nucleosides were used in automated solid-phase synthesis (Scheme 2) after thorough drying *in vacuo* and without chromatographic purification. To obtain oligonucleotides, 0.2—0.5 *M* solutions of the modified phosphoramidites in anhydrous acetonitrile were used. The time of the attachment of the modified unit to a growing chain was increased up to 30—60 min. Under these conditions, the degree of conversion at the condensation stage calculated from optical absorption of the dimethoxytrityl cation was no less than 95—97%. We synthesized a series of oligo-2′-*O*-methylribonucleotides containing 2′-amino acid residues, the sequences and properties of which are summarized in Table 1.

Oligonucleotides **VII—XII** are fragments of the catalytic site of ribozyme of the hammerhead type. After the solid-phase synthesis was over, the oligonucleotides were treated with concentrated aq. ammonia at 55 °C for 12—18 h (see Scheme 2) to cleave them from the polymeric support and to remove the protecting groups. As a result, amino acid esters were converted into amides. Analysis and isolation of target products were performed by the reversed-phase HPLC owing to hydrophobic properties of the 5′-terminal dimethoxytrityl group, after that the oligonucleotides were treated with 80% aq. AcOH and analyzed by ion-pair reversed-phase HPLC (Fig. 1). The structures of all the oligonucleotides obtained were confirmed by MALDI-TOF mass spectrometry (see Table 1).

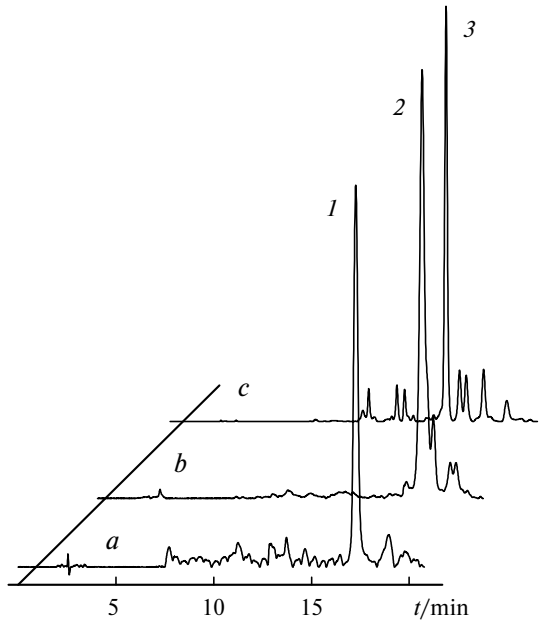


Fig. 1. Reversed-phase HPLC in ion-pair version of the reaction mixtures from automated synthesis of modified oligo-2′-*O*-methylribonucleotides: **I** (*a*, peak *1*), **III** (*b*, peak *2*), and **V** (*c*, peak *3*) (conditions are given in the Experimental).

In conclusion, we synthesized novel 2′-amino acid derivatives of uridine and the corresponding 3′-phosphoramidites. Oligo-2′-*O*-methylribonucleotides with residues of phenylalanine, histidine, and lysine amides were obtained with the use of these derivatives. The possibility of the multiple introduction of the modified units into an oligonucleotide chain was demonstrated.

Experimental

The following reagents were used: chloromethyl pivalate ([POM]Cl), tetrabutylammonium hydrogen sulfate (TBAHS), benzyl bromoacetate, triethylamine trihydrofluoride, sodium borohydride (Aldrich); 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1,3-Cl_2-TIPDS), *tert*-butylimino-tris(pyrrolidino)phosphorane (BTPP), *N*,*N*′-disuccinimidyl carbonate (DSC), triethylamine, bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (Fluka); 4,4′-dimethoxytrityl chloride ([DMTr]Cl) (Avocado); L-phenylalanine allyl ester toluene-*p*-sulfonate (Novabiochem); N^{Im}-trityl-L-histidine methyl ester dihydrochloride (Senn Chemicals); N^{ε}-fmoc-L-lysine methyl ester hydrochloride (Bachem), 3′-phosphoramidites of 2′-*O*-methylribonucleotides (Proligo, Germany). Diisopropylammonium tetrazolide was synthesized from 1*H*-tetrazole and diisopropylamine.[20] The following anhydrous solvents were employed: dichloromethane, pyridine, acetonitrile, THF, methanol, dioxane, and hexane. Other solvents (chloroform, benzene, ethanol, and ethyl acetate) were used without additional purification.

^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Bruker DRX-500 spectrometer (500.13 MHz for ^{1}H, 75.47 MHz for ^{13}C, and 121.50 MHz for ^{31}P) in $CDCl_3$ with admixture of $CHCl_3$ serving as the internal standard. For assignment of 2D-spectra, COSY- and HSQC-procedures adapted for this spectrometer were used. Chemical shifts (δ, accuracy to within 0.01 ppm) are given relative to Me_4Si (^{1}H, ^{13}C) and 85% aq. H_3PO_4 (^{31}P). Spin-spin coupling constants (*J*, Hz) are reported with accuracy to within 0.5 Hz. MALDI-TOF mass spectra were registered on Voyager DE Biospectrometry Workstation (PerSeptive Biosystems) and Bruker Reflex IV (Bruker Daltonics) instruments. A mixture (1 : 1, v/v) of solutions of 2,6-dihydroxyacetophenone (2,6-DHAP) (40 mg mL^{-1} in methanol) and diammonium hydrogen citrate (HCD) (80 mg mL^{-1} in water) was used as a matrix for the oligonucleotides, this was prepared immediately before each measurement. A matrix based on 3-hydroxypicolinic acid was used for measurements on a Bruker Reflex IV instrument. Solutions of 2,5-dihydroxybenzoic acid (2,5-DHBA) (10 mg mL^{-1} in methanol) or 2,4,6-trihydroxyacetophenone (2,4,6-THAP) (10 mg mL^{-1} in 50% aq. acetinitrile) were used as matrixes for low-molecular-weight compounds. High-resolution ESI mass spectra were registered on a Bruker Bio-Apex II FT-ICR instrument (Bruker Daltonics). Thin-layer chromatography was performed on aluminum plates Kieselgel 60 F_{254} (Merck). Compounds absorbing UV light were visualized as dark spots against the fluorescenting background when exposed to UV light with the wavelength of 254 nm. Compounds with dimethoxytrityl group were visualized as bright yellow spots by exposing the chromatographic plates to trifluoroacetic acid vapor. Column chromatography was performed on Silica gel 60 (BDH).

3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridine (1) was obtained from uridine by a known procedure.[11]

3-Pivaloyloxymethyl-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (2) was obtained by known procedure.[15] The yield of compound **2** as a white foam was 70%, R_f 0.34 ($CHCl_3$—EtOH, 96 : 4 v/v). ^{1}H NMR ($CDCl_3$), δ: 7.82 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 5.95 (s, 2 H, $C\underline{H}_2OCOBu^t$); 5.82 (d, 1 H, H(5)); 5.75 (br.s, 1 H, H(1′)); 4.33 (br.s, 2 H, H(2′), H(3′)); 4.15 (m, 1 H, H(4′)); 3.98 (d, 1 H, H_a(5′), $J_{5'a,5'b}$ = 12.0 Hz); 3.82 (d, 1 H, H_b(5′)); 1.20 (s, 9 H, Bu^t); 1.10—0.80 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 178.00 ($CH_2O\underline{C}OBu^t$); 161.75 (C(4)); 151.50 (C(2)); 140.02 (C(6)); 101.85 (C(5)); 92.58 (C(1′)); 85.33 (C(4′)); 74.97 (C(2′)); 70.20 (C(3′)); 64.58 ($\underline{C}H_2OCOBu^t$); 61.65 (C(5′)); 38.92 ($\underline{C}(CH_3)_3$); 27.01 ($C(\underline{C}H_3)_3$); 17.09 ($CH(\underline{C}H_3)_2$); 13.39—12.85 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,5-DHBA), *m/z*: found 624.05, 641.77; calculated for $[M + Na]^+$ 623.84, for $[M + K]^+$ 639.95.

2′-*O*-Benzyloxycarbonylmethyl-3-pivaloyloxymethyl-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (3). Compound **2** (8.41 g, 14 mmol) was dried by dissolution in, and distillation off, anhydrous acetonitrile (3×50 mL) and dissolved in an anhydrous THF—MeCN mixture (300 mL, 1 : 1 v/v). Benzyl bromoacetate (5.54 mL, 35 mmol) and BTPP (11.98 mL, 39.20 mmol) were added to the resulting mixture. Completeness of the reaction was monitored by TLC ($CHCl_3$—EtOH, 97 : 3 v/v). The reaction mixture was kept for ~16 h and concentrated *in vacuo* and the residue (oil) was dried by dissolution in, and distillation off, benzene (3×50 mL). The target product was isolated by column chromatography (gradient of EtOAc in benzene, 0→2→4→6%). The yield of compound **3** as a colorless foam was 9.54 g (91%), R_f 0.31 ($CHCl_3$—EtOH, 97 : 3 v/v). ^{1}H NMR ($CDCl_3$), δ: 7.90 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.40—7.30 (m, 5 H, Ph); 5.97 (d, 1 H, $C\underline{H}_aH_bOCOBu^t$, $J_{Ha,Hb}$ = 9.5 Hz); 5.91 (d, 1 H, $CH_a\underline{H}_bOCOBu^t$); 5.81 (br.s, 1 H, H(1′)); 5.71 (d, 1 H, H(5)); 5.21 (d, 1 H, $OC\underline{H}_aH_bPh$, $J_{Ha,Hb}$ = 12.5 Hz); 5.18 (d, 1 H, $OCH_a\underline{H}_bPh$); 4.61 (d, 1 H, $OC\underline{H}_aH_bCO$, $J_{Ha,Hb}$ = 16.5 Hz); 4.48 (d, 1 H, $OCH_a\underline{H}_bCO$); 4.25 (d, 1 H, H_a(5′), $J_{5'a,5'b}$ = 13.3 Hz); 4.22 (br.s, 2 H, H(3′), H(4′)); 4.05 (s, 1 H, H(2′)); 3.98 (d, 1 H, H_b(5′)); 1.20 (s, 9 H, Bu^t); 1.10—0.80 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 177.36 ($CH_2O\underline{C}OBu^t$); 169.66 ($OCH_2\underline{C}O$); 161.61 (C(4)); 149.97 (C(2)); 138.36 (C(6)); 135.42 (*ipso*-Ph); 128.53, 128.35 (Ph); 101.10 (C(5)); 89.13 (C(1′)); 82.46 (C(2′)); 81.60 (C(4′)); 68.42 (C(3′)); 67.53 ($O\underline{C}H_2CO$); 66.56 ($O\underline{C}H_2Ph$); 64.48 ($\underline{C}H_2OCOBu^t$); 59.36 (C(5′)); 38.82 ($\underline{C}(CH_3)_3$); 27.01 ($C(\underline{C}H_3)_3$); 17.42—16.76 ($CH(\underline{C}H_3)_2$); 13.46—12.37 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,4,6-THAP), *m/z*: found 750.17, 788.63; calculated for $[M + H]^+$ 750.02, found 772.50, calculated for $[M + Na]^+$ 772.00; for $[M + K]^+$ 788.11.

2′-*O*-(2-Hydroxyethyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (4). Compound **3** (9.54 g, 12.74 mmol) was dissolved in an anhydrous THF—MeOH mixture (66 mL, 5 : 1), $NaBH_4$ (1.45 g, 38.22 mmol) was added to the solution and the reaction mixture was stirred for 3 h under reflux. Completeness of the reaction was monitored by TLC ($CHCl_3$—EtOH, 96 : 4). Then the reaction mixture was concentrated *in vacuo*, the residue was dissolved in EtOAc (100 mL) and washed with water (2×100 mL), 5% aq. $NaHCO_3$ (100 mL), and 20% aq. NaCl (100 mL) and the target product was isolated by chromatography (gradient of EtOH in $CHCl_3$, 0→1→2%); the yield of compound **4** as a white foam was 4.73 g (70%), R_f 0.27

($CHCl_3$—EtOH, 96 : 4). ^{1}H NMR ($CDCl_3$), δ: 9.75 (br.s, 1 H, H(3)); 7.76 (d, 1 H, H(6), $J_{5,6}$ = 8.1 Hz); 5.71 (s, 1 H, H(1′)); 5.68 (d, 1 H, H(5)); 4.22 (d, 1 H, H_a(5′), $J_{5'a,5'b}$ = 13.5 Hz); 4.18 (dd, 1 H, H(3′), $J_{2'3'}$ = 3.9 Hz, $J_{3'4'}$ = 9.6 Hz); 4.12 (br.d, 1 H, H(2′)); 3.95 (m, 1 H, $OC\underline{H}_2CH_2OH$); 3.92 (d, 1 H, H_b(5′)); 3.90 (m, 1 H, $OC\underline{H}_2CH_2OH$); 3.88 (d, 1 H, H(4′), $J_{4',5'}$ = 4.0 Hz); 3.71 (m, 2 H, $OCH_2C\underline{H}_2OH$); 1.10—0.90 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 163.69 (C(4)); 150.42 (C(2)); 139.22 (C(6)); 101.83 (C(5)); 89.47 (C(1′)); 82.91 (C(4′)); 81.85 (C(2′)); 73.08 ($O\underline{C}H_2CH_2OH$); 68.24 (C(3′)); 61.65 ($OCH_2\underline{C}H_2OH$); 59.31 (C(5′)); 17.50—16.82 ($CH(\underline{C}H_3)_2$); 13.48—12.59 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,4,6-THAP), *m/z*: found 531.10, 552.96, 568.91; calculated for $[M + H]^+$ 531.26, for $[M + Na]^+$ 553.24, for $[M + K]^+$ 569.21.

***N*$^{\alpha}$-{2-[3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters 6a—c (general procedure).** Compound **4** (0.53 g, 1 mmol) was dried by dissolution in, and distillation off, anhydrous CH_2Cl_2 (3×25 mL) and dissolved in anhydrous CH_2Cl_2 (20 mL), *N*,*N*′-disuccinimidyl carbonate (0.64 g, 2.5 mmol) and triethylamine (0.35 mL, 2.5 mmol) were added to the solution, which was stirred for 24 h at ~20 °C. Completeness of the reaction was monitored by TLC in EtOAc (R_f 0.71). The reaction mixture was concentrated *in vacuo*, the residue (oil) was dissolved in EtOAc (50 mL) and washed with 2% aq. $NaHCO_3$ (5×50 mL), water (3×50 mL), and 20% aq. NaCl (3×50 mL). The organic phase was dried with anhydrous Na_2SO_4, the desiccant was filtered off, and the solvent was evaporated. The residue was dried by dissolution in, and distillation off, anhydrous CH_2Cl_2 (3×25 mL) and thoroughly dried *in vacuo*. Then compound **5** obtained as a white foam was dissolved in anhydrous MeCN (20 mL) and the corresponding amino acid ester (1.15 mmol) and triethylamine (0.16 mL, 1.15 mmol) were added with stirring. The reaction mixture was stirred for 5 h, diluted with EtOAc (50 mL) and washed with water (50 mL), 5% aq. citric acid (2×50 mL), water (50 mL), 5% aq. $NaHCO_3$ (2×50 mL), and 20% aq. NaCl (50 mL). The organic phase was dried with anhydrous Na_2SO_4, the desiccant was filtered off, the solvent was evaporated, the residue (oil) was dried by dissolution in, and distillation off, $CHCl_3$ (3×25 mL) and the target product was isolated by column chromatography.

***N*$^{\alpha}$-{2-[3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridin-2′-yl]oxyethoxy}carbonyl-L-phenylalanine allyl ester (6a).** After chromatography ($CHCl_3$), compound **6a** (1.72 g, 89%) was obtained as a white foam, R_f 0.52 ($CHCl_3$—EtOAc, 2 : 1). ^{1}H NMR ($CDCl_3$), δ: 8.50 (br.s, 1 H, N$\underline{H}$CH); 7.86 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.5 (m, 3 H, *m*,*p*-Ph); 7.15 (d, 2 H, *o*-Ph, *J* = 7.3 Hz); 5.85 (m, 1 H, $CH_2C\underline{H}{=}CH_2$); 5.62 (d, 1 H, H(5)); 5.30 (d, 1 H, CH=C$\underline{H}_a$H$_b$, J_{CH,CH_a} = 17.0 Hz); 5.25 (s, 1 H, H(1′)); 5.23 (d, 1 H, CH=CH$_a\underline{H}_b$, J_{CH,CH_b} = 9.5 Hz); 4.68 (dt, 1 H, NHC$\underline{H}$CH$_2$, $J_{NH,CH}$ = 7.0 Hz, J_{CH,CH_2} = 5.5 Hz); 4.55 (d, 1 H, H(2′), $J_{2',3'}$ = 5.0 Hz); 4.30 (m, 2 H, H(4′), H_a(5′)); 4.12 (m, 2 H, $OCH_2C\underline{H}_2OCO$); 4.00 (m, 2 H, H(3′), H_b(5′)); 3.90 (m, 2 H, $OC\underline{H}_2CH_2OCO$); 3.11 (dd, 1 H, CHC$\underline{H}_a$H$_b$Ph, $J_{Ha,Hb}$ = 13.0 Hz); 3.08 (dd, 1 H, CHCH$_a\underline{H}_b$Ph); 1.20—0.90 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 171.42 ($\underline{C}$OOAllyl); 163.24 (C(4)); 156.00 (O$\underline{C}$ONH); 149.88 (C(2)); 139.32 (C(6)); 135.96 (*ipso*-Ph); 131.43 ($\underline{C}H{=}CH_2$); 129.56, 128.69, 127.05 (Ph); 119.04 (CH=$\underline{C}H_2$); 101.48 (C(5)); 89.26 (C(1′)); 83.29 (C(2′)); 81.60 (C(4′)); 69.89 ($O\underline{C}H_2CH_2OCO$); 68.58 (C(3′)); 65.96 ($OCH_2\underline{C}H_2OCO$); 64.74 ($\underline{C}H_2CH{=}CH_2$); 59.38 (C(5′)); 54.74 (NH$\underline{C}$H); 38.21 (CH$\underline{C}H_2$Ph); 17.51—16.87 ($CH(\underline{C}H_3)_2$); 13.40—12.57 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,4,6-THAP), *m/z*: found 762.08, calculated for $[M + H]^+$ 762.34.

***N*$^{\alpha}$-{2-[3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridin-2′-yl]oxyethoxy}carbonyl-*N*^{Im}-trityl-L-histidine methyl ester (6b).** After chromatography ($CHCl_3$ + 1% of pyridine), compound **6b** (2.87 g, 81%) was obtained as a white foam, R_f 0.41 (EtOAc). ^{1}H NMR ($CDCl_3$), δ: 11.40 (br.s, 1 H, H(3)); 7.80 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.64 (s, 1 H, H^{Im}(2)); 7.32 (m, 9 H, Ph); 7.10 (m, 6 H, Ph); 6.80 (br.d, 1 H, N$\underline{H}$CH, $J_{NH,CH}$ = 6.5 Hz); 6.52 (s, 1 H, H^{Im}(5)); 5.85 (s, 1 H, H(1′)); 5.62 (d, 1 H, H(5)); 4.55 (ddd, 1 H, NHC$\underline{H}CH_2$, J_{CH,CH_2} = 4.0 Hz, 6.0 Hz); 4.25 (m, 1 H, H_a(5′)); 4.22 (m, 1 H, $OCH_2C\underline{H}_2OCO$); 4.20, 4.00 (both m, 1 H each, H(2′), H(4′)); 4.18 (m, 1 H, H(3′)); 4.10 (m, 2 H, $OC\underline{H}_2C\underline{H}_2OCO$); 4.00 (m, 1 H, H_b(5′)); 3.90 (m, 1 H, $OC\underline{H}_2CH_2OCO$); 3.50 (s, 3 H, $OC\underline{H}_3$); 3.18 (dd, 1 H, CHC$\underline{H}_a$H$_b$Im, $J_{Ha,Hb}$ = 14.0 Hz); 3.09 (dd, 1 H, CHCH$_a\underline{H}_b$Im); 1.20—0.90 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 172.11 ($\underline{C}OOCH_3$); 163.71 (C(4)); 156.77 (O$\underline{C}$ONH); 150.72 (C(2)); 142.13 (*ipso*-Ph); 138.88 (C^{Im}(2)); 138.73 (C(6)); 135.79 (C^{Im}(4)); 129.86, 128.17 (Ph); 120.18 (C^{Im}(5)); 101.95 (C(5)); 89.30 (C(1′)); 84.25 (C(2′)); 81.77 (C(4′)); 76.57 ($\underline{C}(Ph)_3$); 70.12 ($O\underline{C}H_2CH_2OCO$); 68.64 (C(3′)); 65.50 ($OCH_2\underline{C}H_2OCO$); 59.45 (C(5′)); 54.57 (NH$\underline{C}$H); 52.03 ($O\underline{C}H_3$); 29.80 (CH$\underline{C}H_2$Im); 17.55—16.95 ($CH(\underline{C}H_3)_2$); 13.50—12.64 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,4,6-THAP), *m/z*: found 968.56, calculated for $[M + H]^+$ 968.43, 990.65, 1007.01; for $[M + Na]^+$ 990.41, for $[M + K]^+$ 1007.35.

***N*$^{\alpha}$-{2-[3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridin-2′-yl]oxyethoxy}carbonyl-*N*$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)-L-lysine methyl ester (6c).** After chromatography in a gradient of EtOH in $CHCl_3$ (0→10%), compound **6c** (3.09 g, 90%) was obtained as a white foam, R_f 0.42 ($CHCl_3$—EtOAc, 1 : 1). ^{1}H NMR ($CDCl_3$), δ: 9.35 (br.s, 1 H, H(3)); 7.90 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.77 (d, 2 H, H_{fmoc}(4), H_{fmoc}(5), $J_{3,4} = J_{5,6}$ = 7.8 Hz); 7.60 (d, 2 H, H_{fmoc}(1), H_{fmoc}(8), $J_{1,2} = J_{7,8}$ = 7.5 Hz); 7.48, 7.38 (both t, 2 H each, H_{fmoc}(2), H_{fmoc}(3), H_{fmoc}(6), H_{fmoc}(7)); 5.85 (br.d, 1 H, N$\underline{H}$CH, $J_{NH,CH}$ = 7.0 Hz); 5.80 (s, 1 H, H(1′)); 5.68 (d, 1 H, H(5)); 5.00 (br.t, 1 H, $CH_2N\underline{H}$); 4.45 (d, 2 H, $COOC\underline{H}_2CH$, J_{CH,CH_2} = 7.5 Hz); 4.35 (m, 1 H, NHC$\underline{H}$); 4.32 (m, 1 H, $OCH_2C\underline{H}_2OCO$); 4.25 (m, 4 H, H(3′), H_a(5′), $OCH_2C\underline{H}_2OCO$, H_{fmoc}(9)); 4.15 (d, 1 H, H(4′), $J_{4',5'}$ = 5.0 Hz); 4.10 (m, 1 H, $OC\underline{H}_2CH_2OCO$); 3.95 (m, 1 H, H_b(5′)); 3.90 (m, 2 H, H(2′), $OC\underline{H}_2CH_2OCO$); 3.70 (s, 3 H, $OC\underline{H}_3$); 3.20 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2NH$); 1.80 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2NH$); 1.60—1.50, 1.50—1.30 (both m, 2 H each, $CH_2C\underline{H}_2C\underline{H}_2CH_2NH$); 1.20—0.90 (m, 28 H, Pr^i). ^{13}C NMR ($CDCl_3$), δ: 173.15 ($\underline{C}OOCH_3$); 163.51 (C(4)); 156.68, 156.31 ($\underline{C}$(O)N)); 150.23 (C(2)); 144.04, 141.36 (C_{fmoc}(4a), C_{fmoc}(9a)); 139.42 (C(6)); 127.69, 127.06 (C_{fmoc}(2), C_{fmoc}(3)); 125.09 (C_{fmoc}(4)); 119.98 (C_{fmoc}(1)); 101.72 (C(5)); 89.27 (C(1′)); 83.48 (C(2′)); 81.62 (C(4′)); 70.07 ($OCH_2\underline{C}H_2OCO$); 68.75 (C(3′)); 66.64 ($COO\underline{C}H_2CH$); 64.76 ($O\underline{C}H_2CH_2OCO$); 59.42 (C(5′)); 53.71 (NH$\underline{C}$H); 52.37 ($O\underline{C}H_3$); 47.32 (C_{fmoc}(9)); 40.53 ($CH_2CH_2CH_2\underline{C}H_2NH$); 32.02, 29.54 ($\underline{C}H_2CH_2\underline{C}H_2CH_2NH$); 22.38 ($CH_2\underline{C}H_2CH_2CH_2NH$); 17.55—16.93 ($CH(\underline{C}H_3)_2$); 13.44—12.62 ($\underline{C}H(CH_3)_2$). MS (MALDI-TOF, 2,4,6-THAP), *m/z*: found 939.71, 961.38, 977.86; calculated for $[M + H]^+$ 939.42, for $[M + Na]^+$ 961.41, for $[M + K]^+$ 977.38.

***N*$^{\alpha}$-{2-[Uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters 7a—c (general procedure).** *N*$^{\alpha}$-{2-[3′,5′-*O*-(Tetraisopropyldisiloxan-1,3-diyl)uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters **6a—c** (1 mmol) were dissolved in anhydrous THF (5 mL) and triethylamine trihydrofluoride (0.326 mL, 2 mmol) was added with stirring. After 2—3 h, the mixture was diluted with EtOAc (50 mL) and washed sequentially with 2% aq. $NaHCO_3$ (2×50 mL), water (50 mL), 5% aq. citric acid (2×50 mL), 20% aq. NaCl (50 mL) and the target product was isolated.

***N*$^{\alpha}$-{2-[Uridin-2′-yl]oxyethoxy}carbonyl-L-phenylalanine allyl ester (7a).** After chromatography in a gradient of EtOH in $CHCl_3$ (0→2→5%), compound **7a** (0.45 g, 91%) was obtained as a white foam, R_f 0.15 (EtOAc). ^{1}H NMR ($CDCl_3$), δ: 9.55 (br.s, 1 H, H(3)); 7.82 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.32 (m, 3 H, *m*,*p*-Ph); 7.15 (d, 2 H, *o*-Ph, *J* = 7.3 Hz); 5.80 (ddd, 1 H, $CH_2C\underline{H}{=}CH_aH_b$, J_{CH,CH_a} = 16.5 Hz, J_{CH,CH_b} = 10.0 Hz, J_{CH,CH_2} = 6.5 Hz); 5.79 (s, 1 H, H(1′)); 5.75 (br.s, 1 H, N<u>H</u>CH); 5.70 (d, 1 H, H(5)); 5.30 (d, 1 H, $CH{=}C\underline{H}_aH_b$); 5.25 (d, 1 H, $CH{=}CH_a\underline{H}_b$); 4.60 (q, 1 H, NHC<u>H</u>CH_2, $J_{NH,CH}$ = J_{CH,CH_2} = 7.5 Hz); 4.57 (d, 2 H, $C\underline{H}_2CH{=}CH_2$); 4.22 (m, 2 H, $OCH_2C\underline{H}_2OCO$); 4.20 (m, 1 H, H(4′)); 4.10 (d, 1 H, H(2′), $J_{2',3'}$ = 5.5 Hz); 4.05 (m, 3 H, H(3′), $OC\underline{H}_2CH_2OCO$); 3.90 (m, 1 H, H_a(5′)); 3.75 (m, 1 H, H_b(5′)); 3.09 (d, 1 H, $CHC\underline{H}_aH_bPh$, J_{H_a,H_b} = 13.5 Hz); 3.05 (d, 1 H, $CHCH_a\underline{H}_bPh$). ^{13}C NMR ($CDCl_3$), δ: 171.75 (<u>C</u>OOAllyl); 163.70 (C(4)); 155.91 (O<u>C</u>ONH); 150.49 (C(2)); 141.34 (C(6)); 135.80 (*ipso*-Ph); 131.39 (<u>C</u>H=CH_2); 129.34, 128.68, 127.21 (Ph); 119.20 (CH=<u>C</u>H_2); 102.33 (C(5)); 89.43 (C(1′)); 84.71 (C(2′)); 82.12 (C(4′)); 69.63 (O<u>C</u>H_2CH_2OCO); 68.58 (C(3′)); 66.27 ($OCH_2\underline{C}H_2OCO$); 64.11 (<u>C</u>$H_2CH{=}CH_2$); 60.95 (C(5′)); 55.04 (NH<u>C</u>H); 38.11 ($CH\underline{C}H_2Ph$). MS (MALDI-TOF, 2,5-DHBA), *m*/*z*: found 542.58, 558.59; calculated for $[M + Na]^+$ 542.49, for $[M + K]^+$ 558.60.

***N*$^{\alpha}$-{2-[Uridin-2′-yl]oxyethoxy}carbonyl-*N*^{Im}-trityl-L-histidine methyl ester (7b).** After chromatography in a gradient of EtOH in $CHCl_3$ (0→1→2→5→7→10%) + 1% of pyridine, compound **7b** (2.05 g, 95%) was obtained as a white foam, R_f 0.21 ($CHCl_3$—EtOH, 9 : 1). ^{1}H NMR ($CDCl_3$), δ: 11.45 (br.s, 1 H, H(3)); 7.70 (m, 1 H, H(6)); 7.50 (s, 1 H, H^{Im}(2)); 7.32 (m, 9 H, Ph); 7.10 (m, 6 H, Ph); 6.51 (s, 1 H, H^{Im}(5)); 6.40 (d, 1 H, N<u>H</u>CH, $J_{NH,CH}$ = 7.5 Hz); 5.70 (d, 1 H, H(5), $J_{5,6}$ = 8.0 Hz); 5.56 (d, 1 H, H(1′), $J_{1',2'}$ = 3.0 Hz); 4.78 (q, 1 H, NHC<u>H</u>); 4.60 (m, 1 H, $OCH_2C\underline{H}_2OCO$); 4.41—4.06 (m, 4 H, H(2′), H(3′), H(4′), $OCH_2C\underline{H}_2OCO$); 3.90 (d, 1 H, H_a(5′), $J_{5'a,5'b}$ = 11.0 Hz); 3.85 (m, 2 H, $OC\underline{H}_2CH_2OCO$); 3.70 (d, 1 H, H_b(5′)); 3.50 (s, 3 H, $OC\underline{H}_3$); 3.09 (m, 2 H, $CHC\underline{H}_2Im$). ^{13}C NMR ($CDCl_3$), δ: 171.73 (<u>C</u>$OOCH_3$); 163.48 (C(4)); 155.78 (O<u>C</u>ONH); 151.18 (C(2)); 142.06 (*ipso*-Ph); 138.74 (C(6)); 138.74 (C^{Im}(2)); 135.67 (C^{Im}(4)); 129.70, 128.17 (Ph); 119.91 (C^{Im}(5)); 102.87 (C(5)); 91.02 (C(1′)); 85.95 (C(2′)); 81.07 (C(4′)); 75.63 (<u>C</u>$(Ph)_3$); 69.91, 69.52 (C(3′), O<u>C</u>H_2CH_2OCO); 65.39 ($OCH_2\underline{C}H_2OCO$); 62.02 (C(5′)); 54.60 (NH<u>C</u>H); 52.15 (O<u>C</u>H_3); 30.03 ($CH\underline{C}H_2Im$). MS (MALDI-TOF, 2,5-DHBA), *m*/*z*: found 726.58, 748.68, 764.49; calculated for $[M + H]^+$ 726.28, for $[M + Na]^+$ 748.73, for $[M + K]^+$ 764.23.

***N*$^{\alpha}$-{2-[Uridin-2′-yl]oxyethoxy}carbonyl-*N*$^{\varepsilon}$-(9-fluorenylmethoxycarbonyl)-L-lysine methyl ester (7c).** After chromatography in a gradient of EtOH in $CHCl_3$ (0→1→2→3→4→5→6→7%), compound **7c** (1.97 g, 86%) was obtained as a white foam, R_f 0.21 ($CHCl_3$—EtOH, 9 : 1). ^{1}H NMR ($CDCl_3$), δ: 9.75 (br.s, 1 H, H(3)); 7.85 (d, 1 H, H(6), $J_{5,6}$ = 8.1 Hz); 7.71, 7.58 (both m, 2 H each, H_{fmoc}(1), H_{fmoc}(4), H_{fmoc}(5), H_{fmoc}(8)); 7.48, 7.38 (both m, 2 H each, H_{fmoc}(2), H_{fmoc}(3), H_{fmoc}(6), H_{fmoc}(7)); 5.95 (br.s, 1 H, N<u>H</u>CH); 5.79 (s, 1 H, H(1′)); 5.68 (d, 1 H, H(5)); 5.25 (br.s, 1 H, $CH_2N\underline{H}CO$); 4.50—3.70 (m, 12 H, H(2′), H(3′), H(4′), H(5′), $OCH_2C\underline{H}_2OCO$, NHC<u>H</u>, $CH_2CH_2CH_2C\underline{H}_2NH$, $COOC\underline{H}_2CH$, H_{fmoc}(9)); 3.68 (s, 3 H, $OC\underline{H}_3$); 3.10 (m, 2 H, $OC\underline{H}_2CH_2OCO$); 2.30—1.20 (m, 6 H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2NH$). ^{13}C NMR ($CDCl_3$), δ: 173.28 (<u>C</u>$OOCH_3$); 163.85 (C(4)); 156.53 (<u>C</u>(O)N); 150.61 (C(2)); 143.98, 141.33 (C_{fmoc}(1a), C_{fmoc}(4a), C_{fmoc}(5a), C_{fmoc}(9a), C(6)); 127.74, 127.09 (C_{fmoc}(2), C_{fmoc}(3), C_{fmoc}(6), C_{fmoc}(7)); 125.06 (C_{fmoc}(1), C_{fmoc}(8)); 120.03 (C_{fmoc}(4), C_{fmoc}(5)); 102.34 (C(5)), 89.15 (C(1′)); 84.81, 82.05 (C(2′), C(4′)); 69.50, 68.74, 67.16, 64.00 (O<u>C</u>$H_2\underline{C}H_2OCO$, C(3′), $COO\underline{C}H_2CH$); 61.02 (C(5′)); 53.85, 52.56 (O<u>C</u>H_3, DMTr, NH<u>C</u>H); 47.29 (C_{fmoc}(9)); 40.58 ($CH_2CH_2CH_2\underline{C}H_2NH$); 31.83, 29.36 (<u>C</u>$H_2CH_2\underline{C}H_2CH_2NH$); 22.51 ($CH_2\underline{C}H_2CH_2CH_2NH$). MS (ESI), *m*/*z*: found 697.2694, 719.2523; calculated for $[M + H]^+$ 697.2721, for $[M + Na]^+$ 719.2540.

***N*$^{\alpha}$-{2-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters 8a—c (general procedure).** *N*$^{\alpha}$-{2-[Uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters **7a—c** (1 mmol) were dried by dissolution in, and distillation off, anhydrous pyridine (3×25 mL) and dissolved in anhydrous pyridine (15 mL) and, DMTrCl (0.356 g, 1.05 mmol) was added to the solution with stirring. Completeness of the reaction was monitored by TLC ($CHCl_3$—EtOH, 95 : 5). After 24 h, the reaction mixture was concentrated *in vacuo*, the residue was dissolved in EtOAc (30 mL), washed with 2% aq. $NaHCO_3$ (2×30 mL) and 20% aq. NaCl (30 mL) and the target product was isolated.

***N*$^{\alpha}$-{2-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl]oxyethoxy}carbonyl-L-phenylalanine allyl ester (8a).** After chromatography in a gradient of $CHCl_3$ in benzene (0→30→50→70%) + 1% of pyridine, then in a gradient of EtOH in $CHCl_3$ (0→1→2→4%) + 1% of pyridine, compound **8a** (0.71 g, 85%) was obtained, R_f 0.29 ($CHCl_3$—EtOH, 95 : 5). ^{1}H NMR ($CDCl_3$), δ: 8.80 (br.s, 1 H, H(3)); 8.03 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.41—7.22 (m, 12 H, Ar); 7.12 (d, 2 H, *o*-Ph, DMTr, *J* = 8.0 Hz); 6.82 (d, 4 H, *m*-An*, *J* = 8.5 Hz); 5.92 (s, 1 H, H(1′)); 5.83 (ddd, 1 H, $CH_2C\underline{H}{=}CH_2$, J_{CH,CH_a} = 16.4 Hz, J_{CH,CH_b} = 11.0 Hz, J_{CH,CH_2} = 6.0 Hz); 5.41 (br.d, 1 H, N<u>H</u>CH, $J_{NH,CH}$ = 8.0 Hz); 5.31 (d, 1 H, H(5)); 5.27 (m, 2 H, CH=C<u>H</u>$_2$); 4.62 (q, 1 H, NHC<u>H</u>CH_2, J_{CH,CH_2} = 8.0 Hz); 4.61 (m, 2 H, $C\underline{H}_2CH{=}CH_2$); 4.44 (br.t, 1 H, H(3′), $J_{2'3'}$ = 5.0 Hz); 4.29 (m, 3 H, $OCH_2C\underline{H}_2OCO$, O<u>H</u>(3′)); 4.11 (m, 1 H, $OC\underline{H}_2CH_2OCO$); 4.08 (m, 1 H, H(4′)); 3.90 (m, 1 H, H(2′)); 3.85 (m, 1 H, $OC\underline{H}_2CH_2OCO$); 3.79 (s, 6 H, $OC\underline{H}_3$, DMTr); 3.52 (s, 2 H, H(5′)); 3.10 (d, 2 H, $CHC\underline{H}_2Ph$). ^{13}C NMR ($CDCl_3$), δ: 164.25 (<u>C</u>OOAllyl); 163.13 (C(4)); 158.72 (*p*-An); 155.88 (O<u>C</u>ONH); 150.16 (C(2)); 144.43 (*ipso*-Ph, DMTr); 139.93 (C(6)); 135.68, 135.37, 135.13 (*ipso*-Ph, *ipso*-An); 131.40—127.20 (<u>C</u>H=CH_2, arom.); 119.17 (CH=<u>C</u>H_2); 113.34 (*m*-An); 102.12 (C(5)); 87.71 (C(1′)); 87.06 (<u>C</u>$(Ar)_3$); 83.16 (C(2′), C(4′)); 69.75 (O<u>C</u>H_2CH_2OCO); 68.37 (C(3′)); 66.19 (<u>C</u>$H_2CH{=}CH_2$); 63.78 ($OCH_2\underline{C}H_2OCO$); 61.11 (C(5′)); 55.30 (O<u>C</u>H_3); 54.90 (NH<u>C</u>H); 38.19 ($CH\underline{C}H_2Ph$). MS (ESI), *m*/*z*: found 844.3054, calculated for $[M + Na]^+$ 844.3057.

* An is anisoyl.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl]oxyethoxy}carbonyl-N^{Im}-trityl-L-histidine methyl ester (8b). After chromatography in a gradient of $CHCl_3$ in benzene (0→30→50→70%) + 1% of pyridine, then in a gradient of EtOH in $CHCl_3$ (0→1→2→3→5%) + 1% of pyridine, compound **8b** (2.75 g, 87%) was obtained, R_f 0.23 ($CHCl_3$—EtOH, 95 : 5). ^{1}H NMR ($CDCl_3$), δ: 9.90 (br.s, 1 H, H(3); 8.00 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.61 (s, 1 H, H^{Im}(2)); 7.41—7.20 (m, 22 H, Ar); 7.09 (m, 2 H, *o*-Ph); 6.85 (d, 4 H, *m*-An, J = 8.0 Hz); 6.59 (s, 1 H, H^{Im}(5)); 6.55 (d, 1 H NHCH, $J_{NH,CH}$ = 7.5 Hz); 5.90 (br.s, 1 H, H(1′)); 5.27 (d, 1 H, H(5)); 4.57 (q, 1 H, NHCH); 4.42 (t, 1 H, H(3′), $J_{2',3'} = J_{3',4'}$ = 5.0 Hz); 4.38 (m, 1 H, OCH_2CH_2OCO); 4.18 (m, 1 H, OCH_2CH_2OCO); 4.12 (m, 1 H, H(4′)); 4.05 (m, 1 H, OCH_2CH_2OCO); 3.95 (d, 1 H, H(2′)); 3.89 (m, 1 H, OCH_2CH_2OCO); 3.80 (s, 6 H, OCH_3, DMTr); 3.58 (s, 3 H, OCH_3); 3.50 (br.s, 2 H, H(5′)); 3.10 (m, 2 H, $CHCH_2Im$). ^{13}C NMR ($CDCl_3$), δ: 171.50 ($COOCH_3$); 163.50 (C(4)); 158.70 (*p*-An); 156.20 (OCONH); 150.30 (C(2)); 144.47 (*ipso*-Ph, DMTr); 141.63 (*ipso*-Ph, Tr); 139.95 (C(6)); 138.12 (C^{Im}(2)); 135.20, 135.10, 134.90 (*ipso*-An, C^{Im}(4)); 130.23, 130.17, 129.74, 128.42, 128.30, 128.21, 128.03, 127.75, 127.14 (Ar); 120.13 (C^{Im}(5)); 102.18 (C(5)); 87.71 (C(1′)); 83.19 (C(4′)); 82.94 (C(2′)); 69.58 (OCH_2CH_2OCO); 68.56 (C(3′)); 63.78 (OCH_2CH_2OCO); 61.44 (C(5′)); 55.29 (OCH_3, DMTr); 54.34 (OCH_3); 52.33 (NHCH); 29.33 ($CHCH_2Im$). MS (ESI), *m/z*: found 1028.4099, 1050.3917, 1066.3630; calculated for $[M + H]^+$ 1028.4082, for $[M + Na]^+$ 1050.3901, for $[M + K]^+$.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl]oxyethoxy}carbonyl-N^{ε}-(9-fluorenylmethoxycarbonyl)-L-lysine methyl ester (8c). After chromatography in a gradient of $CHCl_3$ in benzene (0→30→50%) + 1% of pyridine, then in a gradient of EtOH in $CHCl_3$ (0→1→3%) + 1% of pyridine, compound **8c** (2.78 g, 90%) was obtained, R_f 0.19 ($CHCl_3$—EtOH, 95 : 5). ^{1}H NMR ($CDCl_3$), δ: 9.35 (br.s, 1 H, H(3)); 8.00 (d, 1 H, H(6), $J_{5,6}$ = 8.0 Hz); 7.70 (d, 2 H, H_{fmoc}(4), H_{fmoc}(5), $J_{3,4} = J_{5,6}$ = 8.0 Hz); 7.56 (d, 2 H, H_{fmoc}(1), H_{fmoc}(8), $J_{1,2} = J_{7,8}$ = 8.0 Hz); 7.40—7.30 (m, 13 H, Ar, H_{fmoc}(2), H_{fmoc}(3), H_{fmoc}(6), H_{fmoc}(7)); 6.82 (d, 4 H, *m*-An, J = 8.5 Hz); 5.89 (s, 1 H, H(1′)); 5.70 (d, 1 H, NHCH, $J_{NH,CH}$ = 8.4 Hz); 5.30 (br.d, 1 H, H(5)); 5.15 (br.t, 1 H, CH_2NH); 4.40 (m, 2 H, $COOCH_2CH$); 4.39 (m, 1 H, H(3′)); 4.35 (m, 1 H, H_{fmoc}(9)); 4.22 (m, 2 H, OCH_2CH_2OCO); 4.20 (m, 1 H, NHCH); 4.05 (m, 1 H, H(4′)); 3.95 (m, 1 H, OCH_2CH_2OCO); 3.90 (d, 1 H, H(2′), $J_{2',3'}$ = 5.0 Hz); 3.88 (m, 1 H, OCH_2CH_2OCO); 3.75 (s, 6 H, OCH_3, DMTr); 3.70 (s, 3 H, OCH_3); 3.50 (s, 2 H, H(5′)); 3.15 (m, 2 H, $CH_2CH_2CH_2CH_2NH$); 1.40—1.20 (m, 6 H, $CH_2CH_2CH_2CH_2NH$). ^{13}C NMR ($CDCl_3$), δ: 173.06 ($COOCH_3$); 163.52 (C(4)); 158.73 (*p*-An); 156.65, 156.32 (C(O)N); 150.41 (C(2)); 144.44, 144.02 (C_{fmoc}(9a); *ipso*-Ph, DMTr); 141.33 (C_{fmoc}(4a)); 139.94 (C(6)); 135.37 (*ipso*-An); 135.14 (*ipso*-An, DMTr); 131.00, 130.23, 129.21, 128.89, 128.19, 128.04, 127.70, 127.18, 127.06 (Ar); 125.06 (C_{fmoc}(1)); 119.99 (C_{fmoc}(4)); 113.32 (*m*-An); 102.21 (C(5)); 87.68 (C(1′)); 83.14 (C(4′)); 82.89 (C(2′)); , 69.52 (OCH_2CH_2OCO); 68.46 ($COOCH_2CH$); 66.49 (C(3′)); 63.65 (OCH_2CH_2OCO); 61.23 (C(5′)); 55.29 (OCH_3, DMTr); 53.76 (NHCH); 52.52 (OCH_3); 47.32 (C_{fmoc}(9)); 40.46 ($CH_2CH_2CH_2CH_2NH$); 31.96, 29.31 ($CH_2CH_2CH_2CH_2NH$); 22.36 ($CH_2CH_2CH_2CH_2NH$). MS (ESI), *m/z*: found 1021.3822, 1037.3653; calculated for $[M + Na]^+$ 1021.3847, for $[M + K]^+$ 1037.3587.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)uridin-2′-yl]oxyethoxy}carbonyl-L-amino acid esters 9a—c (general procedure). N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl]oxyethoxy}-carbonyl-L-amino acid esters **8a—c** (1 mmol) were dried by dissolution in, and distillation off, anhydrous CH_2Cl_2 (3×20 mL) and dissolved in anhydrous CH_2Cl_2 (10 mL). Diisopropylammonium tetrazolide (0.26 g, 1.5 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.41 mL, 1.3 mmol) were added to the solution, which was stirred for ~16 h. After the reaction was over (TLC in $CHCl_3$—Et_3N, 98.5 : 1.5), the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 20% aq. NaCl (2×20 mL). The organic phase was dried with Na_2SO_4. The desiccant was filtered off, the solvent was evaporated, the residue was dried until a foam formed, hexane was added and this was kept for 18—24 h at −20 °C. The solvent was decanted and the product was dried *in vacuo*.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)uridin-2′-yl]oxyethoxy}carbonyl-L-phenylalanine allyl ester (9a). The yield of compound **9a** as a white foam was 0.84 g (96%), R_f 0.51 ($CHCl_3$—Et_3N, 98.5 : 1.5). ^{1}H NMR ($CDCl_3$), δ: 8.07, 8.01 (both d, 1 H each, H(6), $J_{5,6}$ = 8.0 Hz); 7.43—7.20 (m, 24 H, Ar); 7.13 (m, 4 H, *o*-Ph, DMTr); 6.84 (br.d, 8 H, *m*-An, J = 8.7 Hz); 5.96, 5.92 (both s, 1 H each, H(1′)); 5.85 (m, 2 H, $CH_2CH=CH_2$); 5.69, 5.56 (both br.d, 2 H each, H(5′)); 5.33 (br.s, 2 H, NHCH); 5.21 (m, 4 H, $CH=CH_2$); 4.65 (m, 2 H, $NHCHCH_2$); 4.61 (m, 2 H, $CH_2CH=CH_2$); 4.44 (m, 1 H, H(3′)); 4.29—4.11 (m, 6 H, OCH_2CH_2OCO, H(2′), H(4′)); 3.90 (br.s, 4 H, OCH_2CH_2OCO); 3.79 (s, 12 H, OCH_3, DMTr); 3.78—3.40 (s, 8 H, H(5′), OCH_2CH_2OCO, CH_2CH_2CN); 3.10 (br.d, 4 H, $CHCH_2Ph$); 2.77—2.42 (m, 8 H, CH_2CH_2CN, $CH(CH_3)_2$); 1.38—0.88 (m, 24 H, $CH(CH_3)_2$). ^{31}P ($CDCl_3$), δ: 151.495, 150.702. MS (MALDI-TOF, 2,6-DHAP—HCD), *m/z*: found 1022.93, calculated for $[M + H]^+$ 1023.09.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)uridin-2′-yl]oxyethoxy}carbonyl-N^{Im}-trityl-L-histidine methyl ester (9b). The yield of compound **9b** as a white foam was 3.17 g (97%), R_f 0.47 ($CHCl_3$—Et_3N, 98.5 : 1.5). ^{1}H NMR ($CDCl_3$), δ: 7.93, 7.85 (both d, 1 H each, H(6), $J_{5,6}$ = 8.0 Hz); 7.62, 7.58 (both d, 1 H each, H^{Im}(2)); 7.41—7.20 (m, 44 H, Ar); 7.12 (m, 4 H, *o*-Ph); 6.85 (d, 8 H, *m*-An, J = 8.0 Hz); 6.50 (s, 2 H, H^{Im}(5)); 5.70 (br.s, 1 H, H(1′)); 5.29 (d, 2 H, H(5)); 4.54 (m, 2 H, NHCH); 4.47—3.85 (m, 10 H, H(3′), OCH_2CH_2OCO, H(4′), H(2′)); 3.80 (s, 12 H, OCH_3, DMTr); 3.77—3.58 (m, 4 H, OCH_2CH_2OCO); 3.53 (s, 6 H, OCH_3); 3.49—3.39 (m, 8 H, H(5′), CH_2CH_2CN); 3.10 (m, 4 H, $CHCH_2Im$); 2.79—2.49 (m, 8 H, CH_2CH_2CN, $CH(CH_3)_2$); 1.31—1.05 (m, 24 H, $CH(CH_3)_2$). ^{31}P ($CDCl_3$), δ: 151.548, 150.596. MS (MALDI-TOF, 2,6-DHAP—HCD), *m/z*: found 1228.65, calculated for $[M + H]^+$ 1228.52.

N^{α}-{2-[5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl)uridin-2′-yl]oxyethoxy}carbonyl-N^{ε}-(9-fluorenylmethoxycarbonyl)-L-lysine methyl ester (9c). The yield of compound **9c** was 3.16 g (95%), R_f 0.45 ($CHCl_3$—Et_3N 98.5 : 1.5). ^{1}H NMR ($CDCl_3$), δ: 8.07, 7.99 (both d, 1 H each, H(6), $J_{5,6}$ = 8.0 Hz); 7.76 (d, 4 H, H_{fmoc}(4), H_{fmoc}(5), $J_{3,4} = J_{5,6}$ = 8.0 Hz); 7.60 (d, 4 H, H_{fmoc}(1), H_{fmoc}(8), $J_{1,2} = J_{7,8}$ = 8.0 Hz); 7.43—7.30 (m, 26 H, Ar, H_{fmoc}(2), H_{fmoc}(3), H_{fmoc}(6), H_{fmoc}(7)); 6.84 (m, 8 H, *m*-An);

6.02, 5.98 (both br.s, 1 H each, NHCH); 5.67 (s, 2 H, H(1′)); 5.27 (br.d, 2 H, H(5)); 5.05 (m, 2 H, CH_2NH); 4.69—3.82 (m, 22 H, $COOCH_2CH$, H_{fmoc}(9), OCH_2CH_2OCO OCH_2CH_2OCO, NHCH, H(2′), H(3′), H(4′)); 3.78 (s, 12 H, OCH_3, DMTr); 3.70 (s, 6 H, OCH_3); 3.60—3.39 (m, 8 H, H(5′), CH_2CH_2CN); 3.19 (m, 4 H, $CH_2CH_2CH_2CH_2NH$); 2.79—2.42 (m, 8 H, CH_2CH_2CN, $CH(CH_3)_2$); 1.84—1.43 (m, 6 H, $CH_2CH_2CH_2CH_2NH$) 1.42—0.84 (m, 24 H, $CH(CH_3)_2$). ^{31}P ($CDCl_3$), δ: 151.495, 150.596. MS (MALDI-TOF, 2,6-DHAP—HCD), *m/z*: found 1199.39, calculated for $[M+H]^+$ 1199.51.

Synthesis of modified oligo-2′-*O*-methylribonucleotides was carried out on an ABI 394 automatic synthesizer according to a standard protocol with commercial reagents and solvents. Controlled-pore glass (LCAA-CPG-500) was used as a polymeric support. The specific loading of the polymer with the first nucleoside unit was 37—60 μmol g^{-1}. Solutions of 3′-phosphoramidites of 2′-*O*-methylribonucleosides (0.1 *M* in anhydrous MeCN) and solutions of 3′-phosphoramidites of modified nucleosides **9a—c** (0.2—0.5 *M*) were used. The coupling time for the modified monomers was increased to 30—60 min. The conversion at this stage was a little smaller than for 3′-phosphoramidites of 2′-*O*-methylribonucleosides, being no less than 95—97%.

After the solid-phase synthesis was over, the oligonucleotides were treated with concentrated aq. ammonia for 12—18 h at 55 °C resulting in cleavage of oligonucleotides from the solid-phase support and removal of protecting groups. Removal of the 5′-terminal dimethoxytrityl group and deprotection of the imidazole ring of hystidine was carried out by treatment with 80% aq. AcOH at ~20 °C (30 min and few days respectively).

Analysis and isolation of oligonucleotides. Analysis of the reaction mixtures obtained in the synthesis of oligonucleotides and isolation of the target products with dimethoxytrityl protecting group at 5′-end were performed by the reversed-phase HPLC on a Tracor chromatograph (Holland). A 4.6×250 mm column with Luna C-18(2) Phenomenex absorbent (with the particle size of 5 μm) was used. Conditions of separation: the column temperature 45 °C; eluents: buffer A, 0.1 *M* ammonium acetate, pH 7; buffer B, 0.02 *M* ammonium acetate in 80% aq. acetonitrile, pH 7; the linear gradient of acetonitrile 0—100% in 60 min; the elution rate, 1 mL min^{-1}. Analysis of the reaction mixtures and monitoring of the product purity after isolation was performed by the reversed-phase HPLC in an ion-pair version on a Waters chromatograph (USA) with an elution step of 0.5 or 1 nucleotide unit per minute. A 4.6×250 mm column with Luna C-18(2) Phenomenox absorbent (with the particle size of 5 μm) was used. Conditions of analytical separation: the column temperature 45 °C; eluents: buffer A, 5% aq. acetonitrile, 48 *mM* potassium-phospate buffer containing 2 mM of tetrabutylammonium dihydrogen phosphate, pH 7.0; buffer B, 40% aq. acetonitrile, 48 mM potassium-phosphate buffer containing 2 mmoles of tetrabutylammonium dihydrogen phosphate, pH 7.0; the logarithmic gradient of acetonitrile concentration: 0—43.6% B (1 min), 43.6—46.9% B (1 min), 46.9—51.2% B (3 min), 51.2—54.5% B (5 min), 54.5—57.7% B (10 min), 57.7—59.6% B (10 min), 59.6—61.0% B (10 min); the eluent consumption: 1 mL min^{-1}. The isolated oligonucleotides were desalted by ultrafiltration with the use of Microcon-3 microconcentrators (Millipore Inc.).

This work was financially supported by the Russian Federation President Council for Grants (Program for the State Support of Young Philosophy Doctors, Grant MK-2744.2005.4), Wellcome Trust Fund (Grant CRIG 069419), and Russian Foundation for Basic Research (Project No. 06-04-49196).

References

1. E. M. Zubin, D. A. Stetsenko, A. V. Kachalova, M. J. Gait, and T. S. Oretskaya, *Nucleic Acids Symp. Ser.*, 2005, **49**, 347.
2. M. Manoharan, *Antisense Nucleic Acid Drug Dev.*, 2002, **12**, 103.
3. S. T. Crooke, *Annu. Rev. Med.*, 2004. **55**, 61.
4. V. N. Sil'nikov and V. V. Vlasov, *Usp. Khim.*, 2001, **70**, 562 [*Russ. Chem. Revs*, 2001, **70** (Engl. Transl.)].
5. T. Niittymäki and H. Lönnberg, *Org. Biomol. Chem.*, 2006, **4**, 15.
6. S. K. Silverman, *Nucleic Acids Res.*, 2005, **33**, 6151.
7. K. Stolze, S. C. Holmes, D. J. Earnshow, M. Singh, D. Stetsenko, D. Williams, and M. J. Gait, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 3007.
8. V. A. Korshun, D. A. Stetsenko, and M. J. Gait, *J. Chem. Soc., Perkin Trans 1*, 2002, 1092.
9. M. Prhavc, E. A. Lesnik, V. Mohan, and M. Manoharan, *Tetrahedron Lett.*, 2001, **42**, 8777.
10. S. M. Freier and K. H. Altmann, *Nucleic Acids Res.*, 1997, **25**, 4429.
11. T. S. Zatsepin, D. A. Stetsenko, A. A. Arzumanov, E. A. Romanova, M. J. Gait, and T. S. Oretskaya, *Bioconjugate Chem.*, 2002, **13**, 822.
12. B. Beijer, M. Grøtli, M. Douglas, and B. Sproat, *Nucleosides Nucleotides*, 1994, **13**, 1905.
13. M. Sekine and T. Hata, *J. Am. Chem. Soc.*, 1986, **108**, 4581.
14. M. Grøtli, R. Eritja, and B. Sproat, *Tetrahedron*, 1997, **53**, 11317.
15. A. Kachalova, E. Zubin, D. Stetsenko, T. Oretskaya, and M. Gait, *Org. Biomol. Chem.*, 2004, **2**, 2793.
16. T. S. Zatsepin, E. A. Romanova, and T. S. Oretskaya, *Usp. Khim.*, 2002, **71**, 586 [*Russ. Chem. Revs*, 2002, **71** (Engl. Transl.)].
17. A. V. Kachalova, T. S. Zatsepin, E. A. Romanova, D. A. Stetsenko, M. J. Gait, and T. S. Oretskaya, *Nucleosides, Nucleotides & Nucleic Acids*, 2000, **19**, 1693.
18. *ChemFiles (Fluka)* 2003, **3**, http://www.sigmaaldrich.com/Drands/Fluka_Riedel_Home/Literature/ChemFiles/Vol_3_No_1.html.
19. N. Dobson, D. G. McDowell, D. J. French, L. J. Brown, J. M. Mellor, and T. Brown, *Chem. Commun.*, 2003, 1234.
20. K. Soai, H. Oyamada, M. Takase, and A. Ookawa, *Bull. Chem. Soc. Jpn*, 1963, **57**, 1948.
21. M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Am. Chem. Soc.*, 1962, **84**, 430.
22. M. H. Caruthers, A. D. Barone, S. L. Beaucage, D. R. Dodds, E. F. Fisher, L. J. McBride, M. Matteucci, Z. Stabinsky, and J.-Y. Tang, *Methods Enzymol.*, 1987, **154**, 287.

Received May 26, 2006;
in revised form December 25, 2006