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Phosphoramidite Reagents and Solid-Phase Supports Based on Hydroxyprolinol for the Synthesis of Modified Oligonucleotides

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Abstract—The synthesis of phosphoramidite reagents and solid-phase supports based on hydroxyprolinol for the introduction of the residues of biotin, lipoic acid, amino groups, and terminal acetylene groups at different positions of the oligonucleotide chain has been described. The efficiency of the reagents and supports has been confirmed by the synthesis of the corresponding modified oligonucleotides.

Keywords: modified oligonucleotides, hydroxyprolinol, phosphoramidite reagents, oligonucleotide synthesis
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INTRODUCTION

Modified oligonucleotides containing various reporter groups have found wide application in molecular biology as a convenient tool for the specific detection of sequences, in DNA sequencing, and in other modern molecular biology methods [1—3]. Biotin [4], sulfur-containing (thiol and disulfide) functional groups possessing the affinity for the surface of noble metals and fluorescent nanocrystals (quantum dots) [5], terminal alkyne for the bioconjugation through the [3 + 2] cycloaddition of azides and alkynes [6], as well as the amino group for the conjugation through carbodiimide condensation or the condensation with the use of activated esters [7], are often applied as modifications.

The introduction of a modification into the oligonucleotide chain immediately during the automated solid-phase oligonucleotide synthesis makes it possible to avoid additional condensation stages, attain regioselectivity, and prevent side reactions. Here, we describe the synthesis of new phosphoramidite reagents and the preparation of the corresponding solid-phase supports that enable one to perform this functionalization in the automated regime. The application of these reagents does not require changes in the standard protocols of synthesis, as well as deblocking, subsequent treatment, and purification of oligonucleotides.

RESULTS AND DISCUSSION

We chose (2*S*,4*R*)-4-hydroxyprolinol, a derivative of the natural amino acid (2*S*,4*R*)-4-hydroxyproline, as a structural basis for the preparation of modifying reagents and supports (Fig. 1). Hydroxyprolinol has a structural and spatial similarity to the deoxyribose residue, which is a key structural component of DNA chains. It contains a secondary amino group convenient for the attachment of a target modification, as well as the primary and secondary hydroxyl groups, which can be functionalized similarly to the hydroxyl groups of natural nucleosides with the conversion to solid-phase supports and phosphoramidite reagents for the single and multiple introduction of modifications at the 3'- and 5'-terminal and internucleotide positions of an oligonucleotide during the automated solid-phase synthesis. Hydroxyprolinol has been used earlier in the synthesis of modifying reagents for the introduction of fluorescent labels and functional groups into oligonucleotides [8—11]. In the present work, based on hydroxyprolinol, we improved the method for obtaining a universal precursor reagent and extended the range of functional groups introduced with the use of this compound.

(2*S*,4*R*)-4-Hydroxyprolinol (**II**) was obtained as a hydrochloride from the readily available (2*S*,4*R*)-4-hydroxyproline methyl ester (**I**) (Scheme 1). It is known that esters are capable of being reduced with

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Abbreviations: CPG, controlled pore glass; DIPC, diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMTr, dimethoxytrityl; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; minR, minor rotamer; majR, major rotamer; dst, diastereomer.

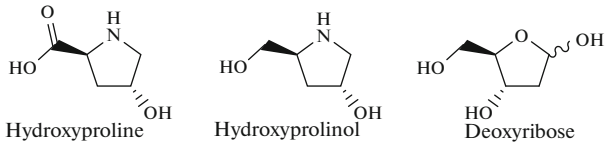
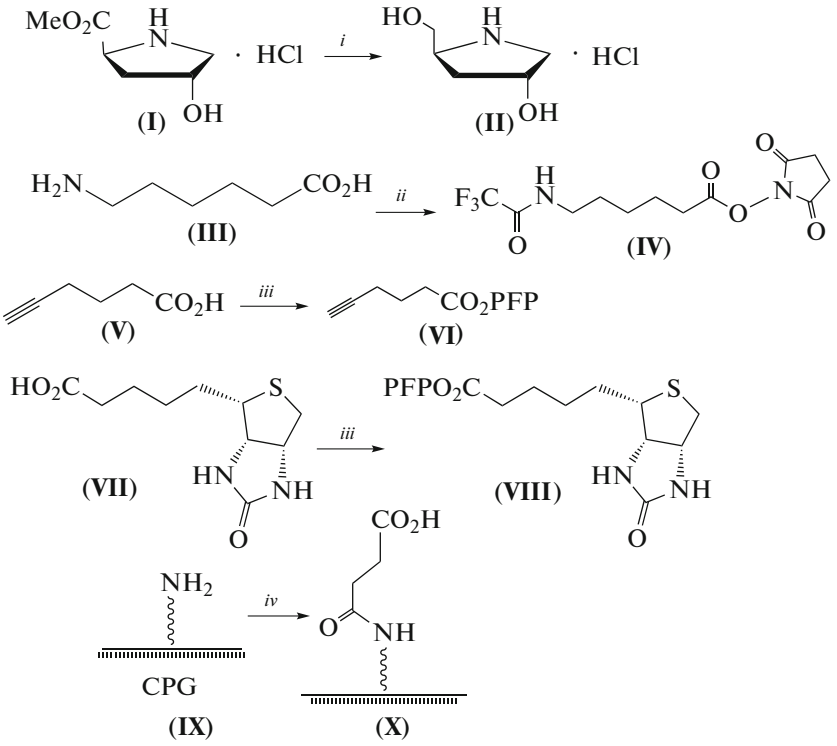


Fig. 1. Hydroxyprolinol, a structural basis of modifying reagents, and its similarity to deoxyribose.

sodium borohydride in alcoholic medium, first to aldehyde, which is quickly converted under reaction conditions to alcohol [12]. Based on this method, we successfully performed the reduction of methyl ester hydrochloride (**I**) with sodium borohydride using dioxane as the main solvent followed by the addition of methanol, which has not been described earlier. This made it possible to carry out the treatment of the reaction medium and isolation of the product without using ion-exchange resins.



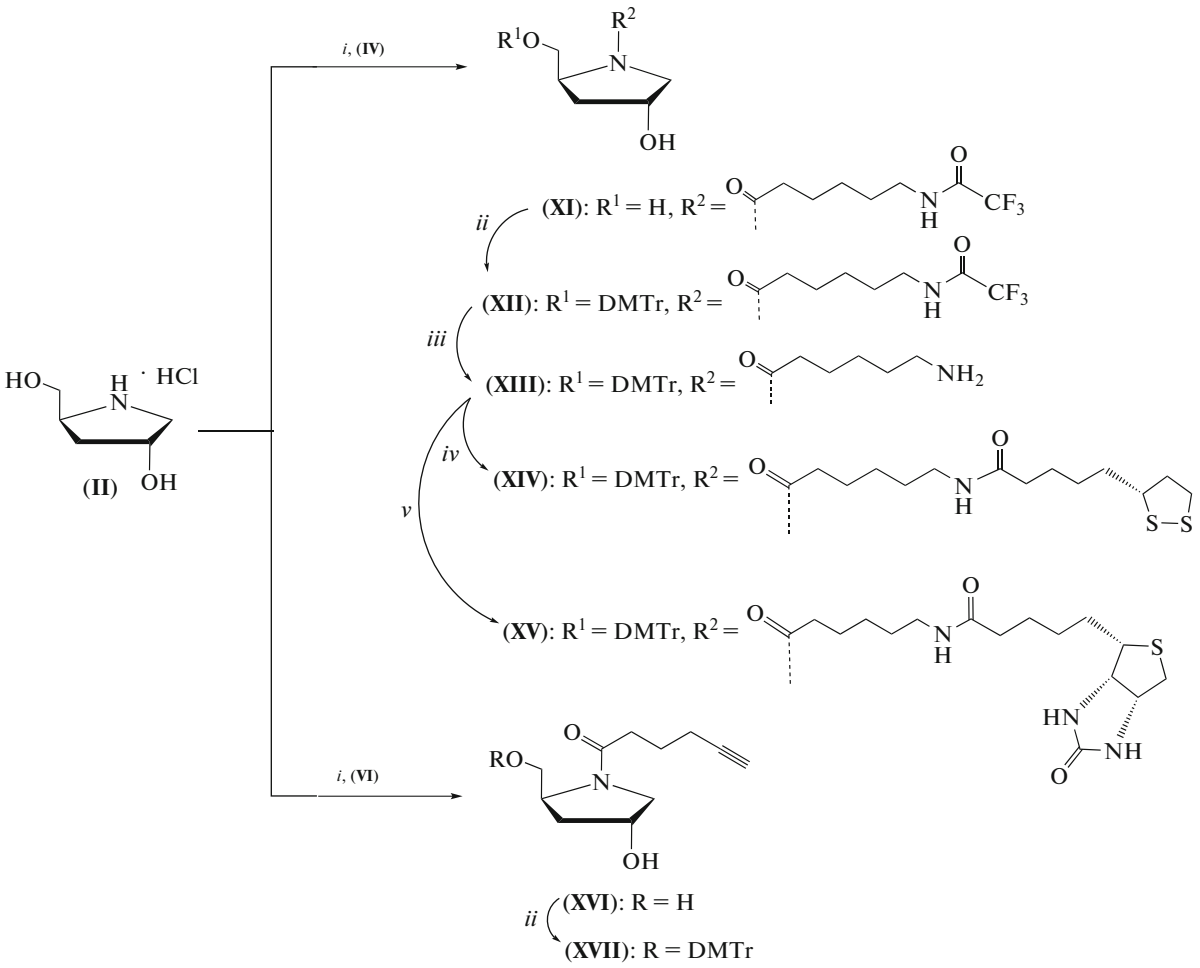
Scheme 1. Preparation of basic synthetic blocks for the assembly of target reagents. *i*: $NaBH_4$, MeOH, dioxane, 70°C; *ii*: trifluoroacetic anhydride, *N*-hydroxysuccinimide, CH_2Cl_2, 25°C; *iii*: pentafluorophenol, dicyclohexylcarbodiimide, CH_2Cl_2, 0°C; *iv*: succinic anhydride, DMAP, pyridine, 25°C.

To provide the steric availability of functional groups being introduced during the conjugation with biomolecules and inorganic materials, it is necessary to insert a spacer between the hydroxyprolinol skeleton and the functional group. For this purpose, we chose the readily available 6-aminohexanoic acid (**III**), which was converted to activated ester (**IV**) with the simultaneous protection of the amino group by the treatment with trifluoroacetic anhydride in the presence of *N*-hydroxysuccinimide. Performing the reaction in methylene chloride substantially facilitated the treatment of the reaction mixture and the isolation of the product in comparison with the previously described protocol [13] according to which the reaction was carried out in DMF.

For the introduction of functional modifications, pentafluorophenyl esters of 5-hexynoic acid (**VI**) and

biotin (**VIII**) were synthesized. CPG porous glass covered with amino groups (**IX**) was treated with succinic anhydride to obtain the carboxylated support (**X**) (Scheme 1).

The reaction of hydroxyprolinol (**II**) with activated esters (**IV**) and (**VI**) led to amides (**XI**) and (**XVI**) from which a series of precursors of modifying reagents were further synthesized (Scheme 2).

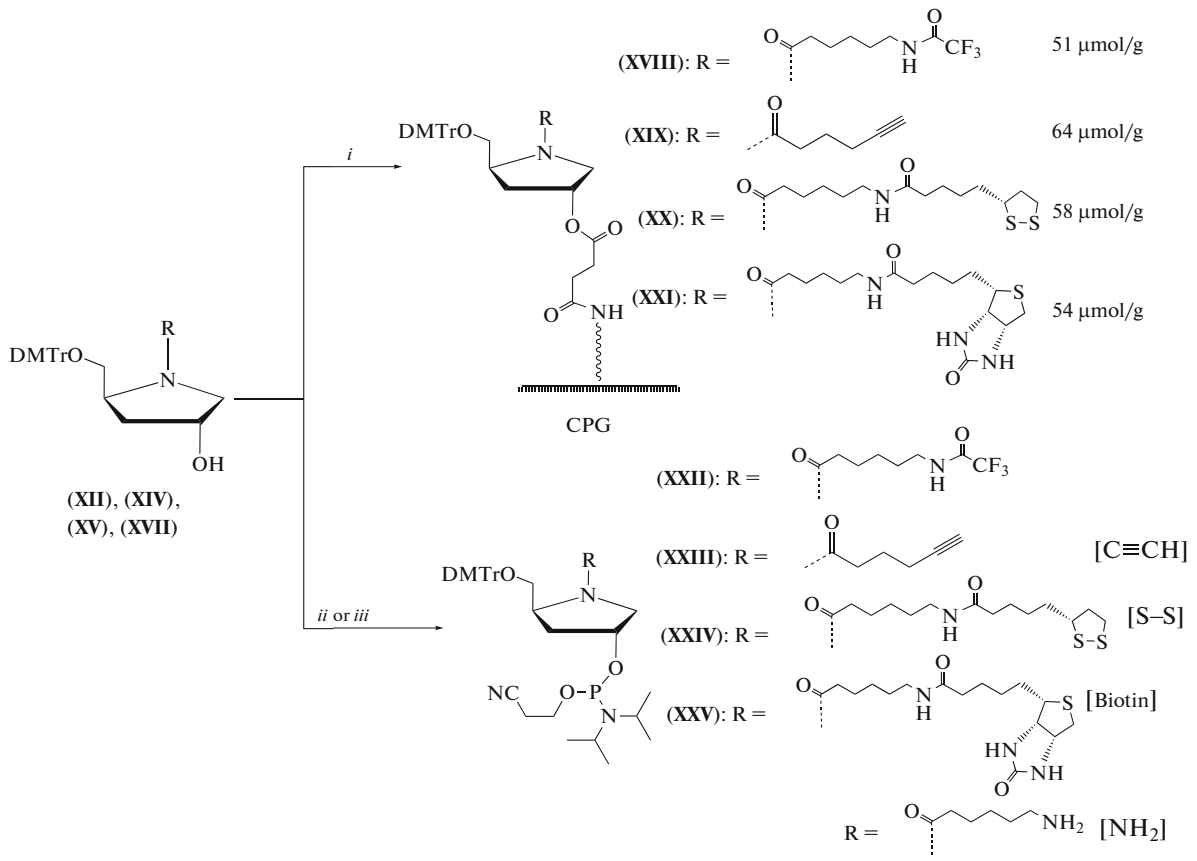


Scheme 2. Synthesis of precursor reagents based on hydroxyprolinol. *i*: Et_3N/pyridine, 25°C; *ii*: DMTr-Cl/pyridine, 25°C; *iii*: K_2CO_3, MeOH–H_2O (4 : 1), 25°C; *iv*: lipoic acid, PyBOP, CH_2Cl_2, 25°C; *v*: (**VIII**), pyridine, 25°C.

Owing to a high selectivity of 4,4'-dimethoxytrityl-chloride (DMTr-Cl) in pyridine towards primary alcohols, it is the primary hydroxyl that is predominantly tritylated in the presence of the equimolar amount of the reagent in diols (**XI**) and (**XVI**), resulting in the formation of products (**XII**) and (**XVII**).

After the protection of the primary hydroxyl, the trifluoroacetyl *N*-protecting group was removed in an alkaline medium. The resulting amine (**XIII**) was acylated with lipoic acid or the activated ester of biotin to form amides (**XIV**) and (**XV**). The lipoic acid residue was introduced by direct condensation with the amino group of compound (**XIII**), since its derivatives tend to polymerize on drying [14]. As a condensing agent, we used PyBOP.

From compounds (**XII**), (**XIV**), (**XV**), and (**XVII**), solid-phase supports and phosphoramidite reagents (**XVIII**)–(**XXV**) (Scheme 3) were prepared, which were then used in the automated DNA synthesis to obtain 5'- and 3'-modified oligodeoxyribonucleotides and sequences carrying an internal single and double modifications. The time of the condensation of modifying reagents was increased from 20 s to 5 min. Typical HPLC profiles of 5'-modified oligonucleotides carrying one modification are shown in Fig. 2.



Scheme 3. Synthesis of target phosphoramidite reagents and supports. *i*: (**X**), DIPC, DMAP, pyridine–DMF, 25°C; *ii*: *N,N*-diisopropylamino-2-cyanoethoxychlorophosphine, acetonitrile or CH_2Cl_2, 0°C; *iii*: bis(*N,N*-diisopropylamino-2-cyanoethoxy)phosphine, CH_2Cl_2, 25°C. For solid-phase supports, the capacity is given to the right; for reagents, a conditional designation of modifications is given, which is used in the table to indicate modifications being introduced into oligonucleotides.

The efficiency of condensation of the phosphoramidite reagents, calculated from the ratio of the area of the peak of a modified reagent to the area of the peak of an unmodified precursor, was 97% for the modifying phosphoramidite containing the amino group (**XXII**) (amine) and more than 99% for reagents (**XXIII**)–(**XXV**). However, due to the formation of side products during the oligonucleotide synthesis, the content of target oligonucleotides after the deblocking decreased to 82% upon modification by lipoic acid; to 99% upon modification by biotin; to 90% by modification with the aliphatic amino group; and to 96% upon the modification with a terminal acetylene group. The conjugates synthesized were purified by HPLC and characterized by MALDI-TOF mass spectrometry (Table 1).

Thus, we synthesized phosphoramidite reagents and solid-phase supports that make it possible to introduce single and multiple modifications at the 5'- and 3'-terminal positions and inside the oligonucleotide chain.

EXPERIMENTAL

The following reagents were used: PyBOP, succinic anhydride, (2*S*,4*R*)-4-hydroxyproline (Aldrich); 6-aminohexanoic acid, *N*-hydroxysuccinimide (Fluka); lipoic acid (reagent purity grade), 4,4'-dimethoxytritylchloride (BASF), (*N,N*-diisopropylamino)-(2-cyanoethoxy)chlorophosphine, bis(*N,N*-diisopropylamino)-(2-cyanoethoxy)phosphine (ALC Primetech, Belarus); aminopropyl-CPG (mean pore size 500 Å, content of amino groups 78 μmol/g; Amersham). Prior to experiments, solvents were purified by distillation and, if necessary, absolutized by standard methods.

The course of the reaction was monitored by TLC on Kieselgel 60 F_{254} plates (Merck); for column chromatography, silica gel Kieselgel 60 (Merck) with a particle size of 40—63 μm was used. Solutions were evaporated on a rotary evaporator in the vacuum of a water-jet pump at a bath temperature of 30—50°C. IR spectra (ν, cm^{-1}, KBr tablets) were recorded on a Protégé 460 spectrometer (Nicolet). NMR spectra (δ, ppm; *J*, Hz) were recorded at 500 MHz (^1H), 125.7 MHz (^{13}C), and 202.4 MHz (^{31}P) on an AVANCE-500 spec-

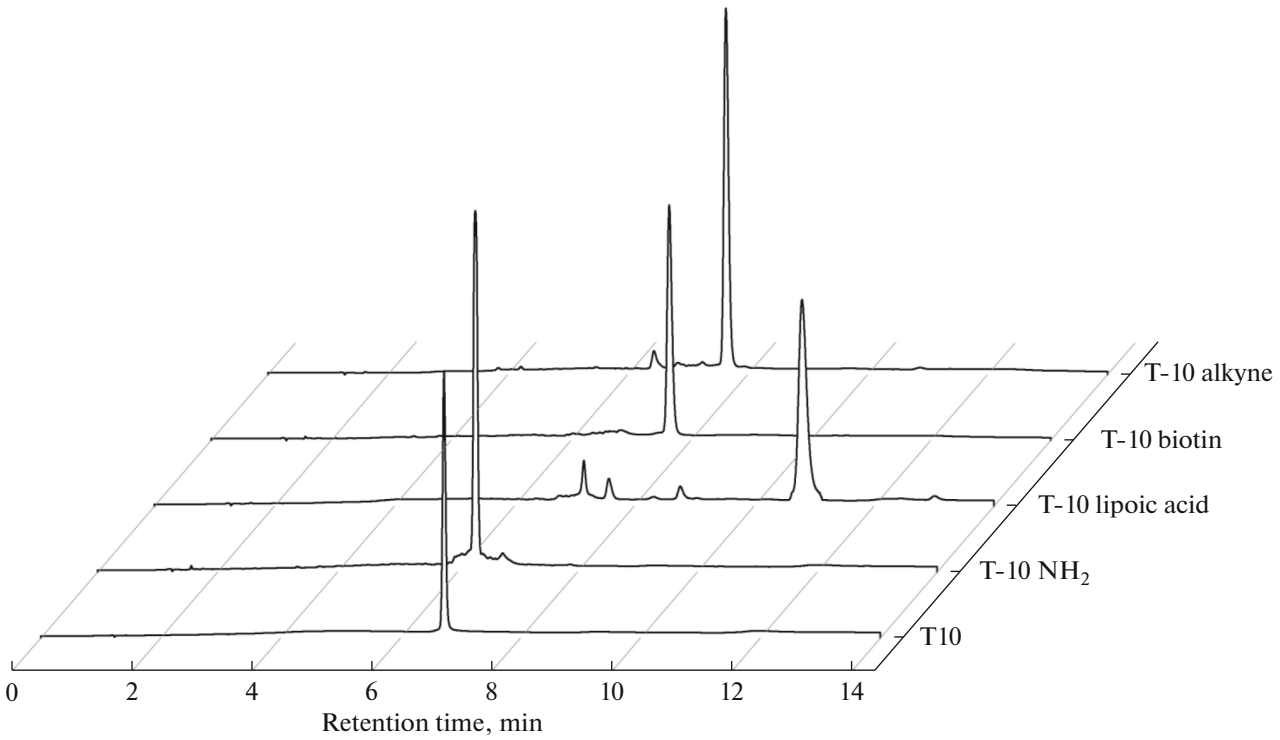


Fig. 2. Typical HPLC profiles of unpurified 5'-modified oligonucleotides.

trometer (Bruker-Biospin). NMR spectra were recorded in $CDCl_3$, unless otherwise indicated. Spectra were calibrated against residual signals of the protons of the solvent; chemical shifts are given relative to $SiMe_4$ (^1H and ^{13}C) and 85% aqueous H_3PO_4 (^{31}P). Melting temperatures were determined on a Boetius heating table (not corrected). Oligonucleotides were synthesized on a BiosSet ASM-800 device. MALDI-TOF mass spectra were recorded on a Bruker Ultraflex spectrometer.

(2*S*,4*R*)-4-Hydroxyprolinol hydrochloride (II). Sodium borohydride (57 g, 1.50 mol) was added to a suspension of hydrochloride of (2*S*,4*R*)-4-hydroxyproline methyl ester (**I**) (55 g, 0.30 mol) in dioxane (600 mL), the mixture was heated to boiling, and methanol (200 mL) was added dropwise. The reaction mixture was allowed to stand overnight at room temperature under stirring. Then, concentrated HCl (200 mL) was added, and the mixture was filtered. The filtrate was evaporated on a rotary evaporator after which 100 mL of isopropanol was added. The precipitate insoluble in alcohol was filtered, and methanol (150 mL) and concentrated HCl (20 mL) were added to the filtrate. The mixture was refluxed for 30 min and evaporated on a rotary evaporator. The residue was recrystallized from isopropanol to give 44.8 g (96%) of the product as a white powder; mp 122°C (*i*-PrOH); ^1H NMR (D_2O): 4.51–4.47 (m, 1H, C*H*OH), 3.89–3.82 (m, 1H, $HOCH_aH_b$), 3.76 (dd, 1H, J_1 12.5, J_2 3.6, $HOCH_aH_b$), 3.55 (dd, 1H, J_1 12.4, J_2 7.0, NHC*H*), 3.28 (dd, 1H, J_1 12.7, J_2 3.4, $NHCH_aH_b$), 3.16 (d, 1H, J 12.6, $NHCH_aH_b$), 1.97 (dd, 1H, J_1 14.1, J_2 6.6, $CHCH_aH_bCH$), 1.82–1.74 (m, 1H, $CHCH_aH_bCH$); ^{13}C NMR (D_2O): 70.07 (*C*HOH), 60.44 (CH_2OH), 60.29 (NH*C*H), 53.19 ($NHCH_2$), 35.21 ($CHCH_2CH$); IR: 3407.0, 3332.4, 3092.1, 2958.4, 1392.8, 1315.2, 1050.1 , 976.8.

***N*-Oxysuccinimide ester of 6-*N*-trifluoroacetylaminohexanoic acid (IV).** *N*-hydroxysuccinimide (52.7 g, 0.46 mol) was added to a suspension of 6-aminohexanoic acid (50.0 g, 0.38 mol) in methylene chloride (800 mL), and the mixture was cooled to 0°C. Pyridine (70 mL) was added under stirring, and trifluoroacetic anhydride (132.6 mL, 0.70 mol) was added dropwise for 2 h. The reaction mixture was heated to room temperature and stirred for 30 min after which it was washed with water (4 × 400 mL), a saturated $NaHCO_3$ solution (400 mL), and a saturated NaCl solution (400 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated on a rotary evaporator to dryness to yield 95.0 g (77%) of a white crystalline substance; R_f 0.43 (acetone–CH_2Cl_2, 1 : 9); mp 85°C (CH_2Cl_2); ^1H NMR: 6.68 (br s, 1H, N*H*), 3.38 (q, 2H, J 6.4, CH_2NH), 2.84 (s, 4H, $COCH_2CH_2CO$), 2.62 (t, 2H, J 7.0, $COCH_2$), 1.80 (quin, 2H, J 7.5, CH_2CH_2NH), 1.63 (quin, 2H, J 7.3, $COCH_2CH_2$), 1.49 (quin, 2H, J 7.5, $COCH_2CH_2CH_2$); ^{13}C NMR: 169.34 (2C,

Table 1. Properties of modified oligonucleotides synthesized using reagents (**XVIII**)–(**XXV**)*

Sequence (5'→3')	MALDI-TOF mass spectra, mass found/calculated
[Biotin]-TTTTTTTTTT	3496.8/3496.7
[HC≡C]-TTTTTTTTTT	3251.7/3251.6
[S-S]-TTTTTTTTTT	3458.7/3458.7
[NH_2]-TTTTTTTTTT	3269.7/3270.6
TTTTTTTTTT-[Biotin]	3496.8/3496.7
TTTTTTTTTT-[C≡CH]	3251.7/3251.6
TTTTTTTTTT-[S-S]	3458.7/3458.7
TTTTTTTTTT-[NH_2]	3270.7/3270.6
TTTTT-[Biotin]-TTTTT	3496.8/3496.7
TTTTT-[C≡CH]-TTTTT	3251.7/3251.6
TTTTT-[S-S]-TTTTT	3458.7/3458.7
TTTTT-[NH_2]-TTTTT	3270.7/3270.6
TTTTT-[Biotin][Biotin]-TTTTT	4015.0/4014.9
TTTTT-[C≡CH][C≡CH]-TTTTT	3524.8/3524.7
TTTTT-[S-S][S-S]-TTTTT	3938.9/3938.8
TTTTT-[NH_2][NH_2]-TTTTT	3561.8/3562.7

* In brackets, the type of a modification is indicated; in Scheme 3 it is shown near the formulas of the corresponding phosphoramidite reagents that make it possible to introduce these modifications during the oligonucleotide synthesis.

*C*OCH$_2$CH$_2$*C*O), 168.38 (1C, *C*O$_2$), 157.30 (q, *J* 36.7, *C*OCF$_3$), 115.82 (q, *J* 287.6, *C*F$_3$), 39.45 (*C*H$_2$NH), 30.77 (CO*C*H$_2$), 28.08 (*C*H$_2$CH$_2$NH), 25.56 (2C, CO*C*H$_2$*C*H$_2$CO), 25.41 (COCH$_2$*C*H$_2$), 24.02 (COCH$_2$CH$_2$*C*H$_2$); IR: 3330.6, 1815.5, 1792.2, 1747.7, 1704.7, 1563.2, 1205.4, 1177.1, 1152.17, 1065.4, 1045.4, 873.9, 727.3, 655.0.

Pentafluorophenyl ester of hex-5-ynoic acid (VI) was synthesized by the method described earlier [15]. The product was obtained as a mobile yellow liquid. Yield: 5.44 g (98%); R_f 0.74 (toluene); ^{1}H NMR: 2.84 (t, *J* 7.4, 2H, OCC*H*$_2$), 2.36 (dt, 2H, J_1 6.9, J_2 2.6, C*H*$_2$C≡CH), 2.03 (t, 1H, *J* 2.6, C≡C*H*), 1.99 (quin, 2H, *J* 7.2, CH$_2$C*H*$_2$CH$_2$); ^{13}C NMR: 169.01 (*C*O), 82.43 (*C*≡CH), 69.76 (C≡*C*H), 31.8 (CO*C*H$_2$), 23.31 (*C*H$_2$C≡CH), 17.61 (CH$_2$*C*H$_2$CH$_2$); IR: 1788.3, 1520.9, 1144.7, 1104.4, 1069.9, 1003.9, 996.7, 645.4.

Pentafluorophenyl ester of biotin (VIII) was synthesized by the method described earlier [16]. The product was obtained as a white powder-like substance. Yield: 9.96 g (73%); R_f 0.45 (MeOH–CH_2Cl_2, 1 : 5); mp 187°C (petroleum ether–CH_2Cl_2, 1 : 1); ^{1}H NMR (acetone-d_6): 5.91 (s, 1H, N*H*), 5.75 (s, 1H, N*H*), 4.53–4.40 (m, 1H, C*H*NH), 4.38–4.33 (m, 1H, C*H*NH), 3.28–3.23 (m, 1H, SC*H*), 2.95 (dd, 1H, J_1 12.5, J_2 5.0, SC*H*$_a$H$_b$), 2.82 (t, 2H, *J* 7.5, COC*H*$_2$), 2.71 (d, 1H, *J* 12.5, SCH$_a$*H*$_b$), 1.87–1.79 (m, 2H, COCH$_2$C*H*$_2$), 1.73–1.63 (m, 2H, C*H*$_2$CHS), 1.62–1.52 (m, 2H, COCH$_2$CH$_2$C*H*$_2$); ^{13}C NMR (acetone-d_6): 62.41 (*C*HNH), 60.76 (*C*HNH), 56.37 (S*C*H), 41.08 (S*C*H$_2$), 33.38 (*C*H$_2$CO), 29.16 (COCH$_2$*C*H$_2$), 28.98 (*C*H$_2$CHS), 25.47 (COCH$_2$CH$_2$*C*H$_2$); IR: 3254.0, 2940.2, 2872.2, 2850.2, 1794.9, 1708.4, 1520.5, 1469.5, 1112.6, 1094.6, 1078.4, 1021.5, 1005.9, 987.9.

Preparation of a support containing carboxyl groups (X) based on CPG. DMAP (1.08 g, 8.8 mmol) and succinic anhydride (3.0 g, 30.0 mmol) were added to a suspension of the amino-containing support (2.5 g) (aminopropyl-CPG, 500Å, the content of amino groups 78 μmol/g) in pyridine (67 mL). The mixture was allowed to stand at room temperature for 48 h, and the support was filtered, washed successively with pyridine, acetonitrile, methylene chloride, and ether (50 mL each), and dried in vacuo.

(2*S*,4*R*)-4-Hydroxy-2-hydroxymethyl-1-(6-*N*-trifluoroacetylaminohexanoyl) pyrrolidine (XI). Triethylamine (55 mL) was added dropwise under stirring to a suspension of hydrochloride (**II**) (30.7 g, 0.20 mol) in pyridine (600 mL). Then, the *N*-oxysuccinimide ester of 6-(*N*-(trifluoroacetyl)amino)hexanoic acid (**IV**) (71.3 g, 0.22 mol) was added, and the mixture was left to stand overnight under stirring. Pyridine was evaporated on a rotary evaporator, and the residue was dissolved in ethyl acetate (900 mL), washed with an equal volume of a saturated NaCl solution, and dried over anhydrous sodium sulfate. Ethyl acetate was distilled away. The product was purified by column chromatography on silica gel using 5% ethanol in CH_2Cl_2 as an eluent. Compound (**XI**) was obtained as a colorless

oil. Yield: 46.6 g (72%); R_f 0.55 (EtOH–CH_2Cl_2, 1 : 4); ^{1}H NMR (CD_3CN): 7.47 (br s, 1H, *H*OCH$_2$), 5.34 (1H, d, *J* 8.1, *H*OCH), 4.28 (1H, br s, N*H*), 4.20–4.13 1H, (m, C*H*OH), 3.57–3.49 (m, 2H, HOCH_2), 3.45–3.42 (m, 1H, NC*H*), 3.39 (t, *J* 11.4, 2H, CH_2NH), 3.22–3.17 (m, 2H, NCH_2), 2.26–2.11 (m, 3H, COCH_2, CHCH_aH$_b$CH), 1.61–1.50 (m, 3H, CHCH$_a$$H_bCH, CH_2CH_2$NH), 1.47 (quin, 2H, *J* 7.2, COCH$_2$CH_2), 1.26 (quin, 2H, *J* 7.7, COCH$_2$CH$_2$CH_2); ^{13}C NMR (CD_3CN): 173.98 (*C*O), 68.44 (*C*HOH), 66.07 (*C*H$_2$OH), 59.43 (N*C*H), 55.76 (N*C*H$_2$), 38.92 (*C*H$_2$NH), 36.32 (CO*C*H$_2$), 34.35 (CH*C*H$_2$CH), 27.83 (*C*H$_2$CH$_2$NH), 25.62 (COCH$_2$*C*H$_2$), 23.52 (COCH$_2$CH$_2$*C*H$_2$); IR: 3301.6, 2941.6, 1711.5, 1618.0, 1566.0, 1445.4, 1210.7, 1188.6, 1156.7.

(2*S*,4*R*)-4-Hydroxy-2-(4,4'-dimethoxytrityloxymethyl)-1-(6-*N*-trifluoroacetylaminohexanoyl)pyrrolidine (XII). 4,4'-Dimethoxytritylchloride (40.6 g, 0.12 mol) was added to a solution of compound (**XI**) (32.6 g, 0.10 mol) in pyridine (300 mL) under stirring, and the mixture was allowed to stand at room temperature overnight. The solvent was evaporated in a rotary evaporator after which the residue was dissolved in ethyl acetate (300 mL) and washed with an equal volume of NaCl. The organic phase was separated and dried over anhydrous sodium sulfate. Ethyl acetate was distilled away. After the purification of the product by column chromatography on silica gel using 1% triethylamine in CH_2Cl_2 as an eluent, compound (**XII**) was obtained as a white amorphous powder. Yield: 39.1 g (62%); R_f 0.28 (Et_3N–EtOH–CH_2Cl_2, 1 : 3 : 96); ^{1}H NMR: 7.37–7.33 (m, 2H, Ar*H*) 7.28–7.22 (m, 6H, Ar*H*), 7.21–7.16 (m, 1H, Ar*H*), 6.83–6.77 (m, 4H, Ar*H*), 4.64–4.58 (br s, 1H, N*H*), 4.42–4.34 (br s, 1H, *H*O), 4.14–4.08 (m, 1H, C*H*OH), 3.76 (s, 6H, OCH_3), 3.67 (dd, 1H, J_1 10.9, J_2 4.9, DMTrOCH_aH$_b$), 3.48 (dd, 1H, J_1 10.7, J_2 2.3, DMTr-OCH$_a$$H_b$), 3,41 (dd, 1H, J_1 9.3, J_2 4.5, CH_aH$_b$NH), 3.38–3.33 (m, 1H, NC*H*), 3,31 (q, 1H, *J* 6.4, CH$_a$$H_b$NH), 3.15–3.11 (m, 1H, NCH_aH$_b$), 3.08 (q, 1H, *J* 7.3, NCH$_a$$H_b$), 2.36–2.29 (m, 1H, COC$H_aH_b$), 2.27–2.16 (m, 2H, COCH$_a$$H_b$, CHC$H_aH_b$CH), 1.67–1.61 (m, 1H, CHCH$_a$$H_b$CH), 1.59 (quin, 2H, *J* 7.2, CH_2CH$_2$NH), 1.50 (quin, 1H, *J* 7.2, COCH$_2$CH_aH$_b$), 1.42–1.33 (m, 3H, COCH$_2$CH$_a$$H_b$, COCH$_2CH_2CH_2$); ^{13}C NMR: 172.49 (0.4C, *C*O, minR), 171.66 (0.6C, *C*O, majR), 158.33 (2C, OCH$_3$), 144.99, 136.15, 135.63, 129.93 (4C), 127.98 (2C), 127.70 (2C), 126.69, 113.15 (Ar$_3$*C*O), 112.98 (4C), 85.82 (*C*H$_2$ODMTr), 70.36 (*C*HOH), 63.37 (N*C*H$_2$), 55.76 (O*C*H$_3$), 55.22 (O*C*H$_3$), 55.15 (N*C*H), 39.15 (*C*H$_2$NH), 36.60 (CO*C*H$_2$), 34.42 (CH*C*H$_2$CH), 28.09 (*C*H$_2$CH$_2$NH), 25.98 (COCH$_2$*C*H$_2$), 23.35 (COCH$_2$CH$_2$*C*H$_2$); IR: 3415.1, 2928.8, 1718.4, 1625.1, 1610.7, 1509.4, 1445.4, 1251.5, 1176.5, 1067.2, 1032.5.

(2*S*,4*R*)-1-(6-Aminohexanoyl)-4-hydroxy-2-(4,4′-dimethoxytrityloxymethyl)pyrrolidine (XIII). Water (50 mL) and K_2CO_3 (4.75 g, 34.42 mmol) were added to a solution of compound (**XII**) (4.16 g, 6.62 mmol) in methanol (200 mL) under stirring. The mixture was allowed to stand overnight at room temperature after which methanol was evaporated in a rotary evaporator, and the residue was dissolved in water (50 mL) and extracted with methylene chloride (4 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated under low pressure. The residue was chromatographed on silica gel in a 1% Et_3N/CH_2Cl_2 system using a gradient of ethanol (the content of ethanol was increased by 1% after each 100 mL of the eluent). The product was obtained as a pale yellow substance in the form of amorphous powder. Yield: 2.89 g (82%); R_f 0.10 (Et_3N–EtOH–CH_2Cl_2, 1 : 20 : 79); ^{1}H NMR (DMSO-d_6): 7.30–7.22 (m, 4H, Ar*H*), 7.19–7.12 (m, 5H, Ar*H*), 6.88–6.80 (m, 4H, Ar*H*), 4.38–4.32 (m, 1H, C*H*OH), 4.14–4.04 (m, 1H, DMTr-OCH_aH$_b$), 3.69 (s, 6H, O*C*H$_3$), 3.56–3.52 (m, 1H, DMTr-OCH$_a$$H_b$), 3.24–3.19 (m, 1H, NC*H*), 3.16–3.08 (m, 1H, NCH_aH$_b$), 2.98–2.90 (m, 1H, NCH$_a$$H_b$), 2.51 (t, 1H, *J* 7.15, COCH_aH$_b$), 2.17 (t, 1H, *J* 7.2, COCH$_a$$H_b$), 2.04–1.94 (m, 1H, C$H_aH_bNH_2$), 1.94–1.84 (m, 1H, CH$_a$$H_bNH_2$), 1.83–1.76 (m, 1H, CHC$H_aH_b$CH), 1.48–1.38 (m, 1H, CHCH$_a$$H_b$CH), 1.38–1.30 (m, 2H, C$H_2CH_2NH_2$), 1.30–1.20 (m, 2H, COCH$_2$CH_2), 1.13–1.05 (m, 1H, COCH$_2$CH$_2$CH_aH$_b$), 1.02 (t, 1H, *J* 7.0, COCH$_2$CH$_2$CH$_a$$H_b$); ^{13}C NMR (DMSO-$d_6$): 171.47 (*C*O), 158.55 (2C), 145.67, 136.44, 136.32, 130.17 (4C), 128.36 (2C), 128.15 (2C), 127.17 (1C), 113.69 (5C), 85.69 (*C*H$_2$ODMTr), 69.15 (*C*HOH), 63.92 (N*C*H$_2$), 55.59 (3C, O*C*H$_3$, N*C*H), 41.45 (*C*H$_2$NH$_2$), 36.87 (CO*C*H$_2$), 34.73 (CH*C*H$_2$CH), 32.34 (*C*H$_2$CH$_2$NH), 26.64 (COCH$_2$*C*H$_2$), 24.86 (COCH$_2$CH$_2$*C*H$_2$); IR: 2926.6, 2856.4, 1608.1, 1508.8, 1444.4, 1301.2, 1249.6, 1174.8, 1071.3, 1031.7, 827.4.

(2*S*,4*R*)-4-Hydroxy-2-(4,4'-dimethoxytrityloxymethyl)-1-(6-(*N*-(5-((*R*)-1,2-dithiolan-3-yl)pentanoylamino)hexanoyl)pyrrolidine (XIV). DIPEA (1.80 mL) was added to a solution of lipoic acid (1.08 g, 5.26 mmol) and PyBOP (2.60 g, 5.00 mmol) in DMF (50 mL) under stirring. After 10 min, a solution of amine (**XIII**) (2.80 g, 5.26 mmol) in DMF (25 mL) was added, and the mixture was left for 1 h at room temperature. The reaction mixture was diluted with water (300 mL) and extracted with an equal volume of chloroform. The organic phase was washed with water (2 × 300 mL), a saturated $NaHCO_3$ solution (300 mL), and a saturated NaCl solution (300 mL). The mixture was dried over sodium sulfate and chromatographed on silica gel using acetonitrile as an eluent. The product was obtained as a yellow oily liquid, which is polymerized on long-term storage in a dry

state. Yield: 3.6 g (95%); R_f 0.50 (Et_3N–EtOH–CH_2Cl_2, 1 : 10 : 89); ^{1}H NMR (DMSO-d_6): 7.12–7.04 (m, 4H, Ar*H*), 7.00–6.94 (m, 5H, Ar*H*), 6.67–6.62 (m, 4H, Ar*H*), 4.19–4.13 (m, 1H, C*H*OH), 3.95–3.86 (m, 1H, SC*H*), 3.50 (s, 6H, OCH_3), 3.40–3.32 (m, 2H, DMTrOCH_2), 3.06–3.00 (m, 1H, NC*H*), 2.96–2.90 (m, 2H, NCH_2), 2.90–2.82 (m, 1H, SCH_aH$_b$), 2.81–2.72 (m, 1H, SCH$_a$$H_b$), 2.20–2.12 (m, 1H, SCH$_2CH_aH_b$), 1.98 (t, 1H, *J* 7.30, CH_aH$_b$NHCO), 1.85–1.75 (m, 3H, CH$_a$$H_b$NHCO, NCOC$H_2$), 1.64–1.56 (m, 2H, HNCOCH_2), 1.52–1.48 (m, 2H, SCH$_2$CH$_a$$H_b$, CHC$H_aH_b$CH), 1.46–1.48 (m, 1H, CHCH$_a$$H_b$CH), 1.34–1.20 (m, 4H, SCHCH_2CH$_2$CH_2), 1.20–1.12 (m, 2H, CH_2CH$_2$NH), 1.12–0.86 (m, 6H, SCHCH$_2$CH_2CH$_2$, NCOCH$_2$CH_2, NCOCH$_2$CH$_2$CH_2); ^{13}C NMR (DMSO-d_6): 171.66 (N*C*O), 170.82 (HN*C*O), 162.26 (2C), 145.03, 135.83, 135.67, 129.56 (4C), 127.74 (2C), 127.53 (2C), 126.54, 113.06 (5C), 85.06 (DMTr-O*C*H$_2$), 68.54 (*C*HOH), 63.28 (N*C*H$_2$), 56.11 (S*C*H), 54.94 (4C(O*C*H$_3$)$_2$, N*C*H, S*C*H$_2$), 45.80 (SCH$_2$*C*H$_2$), 38.21 (*C*H$_2$NH), 36.25 (NCO*C*H$_2$), 35.20 (CH*C*H$_2$CH), 30.71 (HNCO*C*H$_2$), 29.06 (SCH*C*H$_2$CH$_2$CH$_2$), 28.30 (*C*H$_2$CH$_2$NH), 26.17 (HNCOCH$_2$*C*H$_2$), 25.91 (NCOCH$_2$*C*H$_2$), 25.05 (HNCOCH$_2$CH$_2$*C*H$_2$), 24.14 (NCOCH$_2$CH$_2$*C*H$_2$); IR: 2931.4, 2863.0, 1643.5, 1621.4, 1609.2, 1508.6, 1444.0, 1249.9, 1176.4, 1072.9, 1032.5, 828.6.

(2*S*,4*R*)-4-Hydroxy-2-(4,4'-dimethoxytrityloxymethyl)-1-(6-(*N*-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-ylpentanoylamino)-hexanoyl)-pyrrolidine (XV). Triethylamine (1.7 g, 16.8 mmol) was added dropwise under stirring to a solution of amine (**XIII**) (8.51 g, 16 mmol) in DMF (62 mL). Then, a solution of pentafluorophenyl ester of biotin (6.9 g, 16.8 mmol) in DMF (50 mL) was added dropwise for 40 min. The reaction mixture was left to stand overnight under stirring after which it was diluted with methylene chloride (300 mL) and washed with water (6 × 500 mL), a saturated $NaHCO_3$ solution (500 mL), and a saturated NaCl solution (500 mL). The mixture was dried over anhydrous sodium sulfate, evaporated to dryness, and chromatographed in a system of 1% triethylamine in CH_2Cl_2 using a gradient of ethanol. The product was obtained as pale yellow foam. Yield: 11.84 g (97%); R_f 0.53 (Et_3N–EtOH–CH_2Cl_2, 1 : 20 : 79); ^{1}H NMR: 7.38–7.30 (m, 2H, Ar*H*), 7.26–7.12 (m, 7H, Ar*H*), 6.86–6.71 (m, 4H, Ar*H*), 6.63–6.54 (br s, 1H, N*H*CONH), 5.94–5.84 (br s, 1H, NHCON*H*), 4.54–4.47 (m, 1H, C*H*NH), 4.47–4.38 (br s, 1H, O*H*), 4.38–4.31 (m, 1H, C*H*NH), 4.27–4.12 (m, 1H, C*H*OH), 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.63–3.58 (br s, 1H, N*H*CH$_2$), 3.54–3.46 (m, 2H, DMTr-OCH_2), 3.43–3.37 (m, 1H, NC*H*), 3.25–3.12 (m, 2H, CH_2NH), 3.12–3.04 (m, 3H, SC*H*, NCH_2), 2.85–2.77 (m, 1H, SCH_aH$_b$), 2.74–2.64 (m, 1H, SCH$_a$$H_b$), 2.40–2.31 (m, 1H, CHC$H_aH_b$CH), 2.25–2.10 (m, 4H, NCOCH_2, HNCOCH_2), 2.10–1.94 (m, 1H, CHCH$_a$$H_b$CH), 1.77–1.53 (m, 6H, SCHCH_2CH$_2$CH_2, CH_2CH$_2$NH), 1.53–1.47 (m, 2H, NCOCH$_2$CH_2), 1.47–1.31 (m, 4H, NCOCH$_2$CH$_2$CH_2, SCHCH$_2$CH_2); ^{13}C NMR: 173.59 (NH*C*OCH$_2$), 172.94 (0.3C, N*C*OCH$_2$, minR), 172.36 (0.7C, N*C*OCH$_2$, majR), 163.95 (NH*C*ONH), 158.30 (2C), 145.06, 136.27, 136.13, 129.94 (4C), 128.03 (2C), 127.70 (2C), 126.66 (C), 113.12 (Ar$_3$*C*O), 112.88 (4C), 85.64 (*C*H$_2$O-DMTr), 69.98 (*C*HOH), 63.35 (N*C*H$_2$), 63.03 (*C*HNH), 61.64 (*C*HNH), 60.07 (S*C*H), 55.22 (N*C*H), 55.17 (2C, OCH_3), 45.79 (S*C*H$_2$), 38.65 (*C*H$_2$NH), 35.74 (NCO*C*H$_2$), 34.72 (CH*C*H$_2$CH), 33.05 (HNCO*C*H$_2$), 28.88 (HNCOCH$_2$*C*H$_2$), 28.72 (*C*H$_2$CHS), 27.96 (*C*H$_2$CH$_2$NH), 26.28 (NCOCH$_2$*C*H$_2$), 25.68 (HNCOCH$_2$CH$_2$*C*H$_2$), 24.00 (NCOCH$_2$CH$_2$*C*H$_2$); IR: 3385.4, 3284.9, 2924.6, 2854.7, 1702.7, 1630.9, 1608.6 , 1508.7, 1444.5, 1301.4, 1248.9, 1174.6, 1155.8, 1068.8, 1029.4, 583.1.

(2*S*,4*R*)-4-Hydroxy-2-hydroxymethyl-1-(hex-5-inoyl)pyrrolidine (XVI). Triethylamine (3.57 g, 35.3 mmol) was added dropwise under stirring to a suspension of (2*S*,4*R*)-4-hydroxyprolinol hydrochloride (**II**) (2.7 g, 17.7 mmol) in pyridine (60 mL). Then, the pentafluorophenyl ester (**VI**) (5.4 g, 19.4 mmol) was added, and the mixture was left overnight at room temperature under stirring. Pyridine was evaporated on a rotary evaporator, and the residue was dissolved in ethyl acetate (60 mL) and washed with an equal volume of a saturated NaCl solution. The mixture was evaporated to dryness, and the residue was chromatographed on silica gel in a system of 5% ethanol in CH_2Cl_2 to give 2.39 g (64%) of colorless crystals; R_f 0.61 (EtOH–CH_2Cl_2, 1 : 4); mp 79°C (CH_2Cl_2); ^{1}H NMR: 5.45 (dd, 1H, J_1 8.7, J_2 2.0, *H*OCH$_2$), 4.46–4.41 (br s, 1H, *H*OCH), 4.36–4.29 (m, 1H, HOC*H*), 3.67 (ddd, 1H, J_1 11.7, J_2 8.6, J_3 2.2, HOCH_aH$_b$), 3.59–3.57 (m, 2H, NC*H,* NCH_aH$_b$), 3.69 (ddd, 1H, J_1 11.7, J_2 7.6, J_3 1.8, HOCH$_a$$H_b$), 2.87 (d, 1H, *J* 3.6, NCH$_a$$H_b$), 2.48 (dt, 1H, J_1 16.0, J_2 7.3, COCH_aH$_b$), 2.41 (dt, 1H, J_1 16.0, J_2 7.4, COCH$_a$$H_b$), 2.29 (td, 2H, J_1 6.8, J_2 2.7, CH_2C≡CH), 2.09 (dddd, 1H, J_1 13.6, J_2 7.4, J_3 2.4 , J_4 1.4, CHCH_aH$_b$CH), 1.98 (t, 1H, *J* 2.7, C≡C*H),* 1.86 (quin, 2H, *J* 7.1, CH_2CH$_2$C≡CH), 1.71 (ddd, 1H, J_1 13.7, J_2 9.2, J_3 4.6, CHCH$_a$$H_b$CH); ^{13}C NMR: 173.98 (*C*O), 83.64 (*C*≡CH), 69.24 (C≡*C*H), 69.15 (*C*HOH), 66.75 (*C*H$_2$OH), 59.98 (N*C*H), 56.19 (N*C*H$_2$), 37.15 (CH*C*H$_2$CH), 33.57 (CO*C*H$_2$), 23.41 (*C*H$_2$C≡CH), 17.80 (CH$_2$*C*H$_2$CH$_2$); IR: 3406.4, 3229.3, 2943.5, 2932.8, 2900.9, 1618.5, 1450.3, 1424.4, 1405.7, 1384.4, 1334.0, 1323.4, 1250.8 , 1214.3, 1195.6, 1080.8, 1061.0, 978.9, 859.0, 850.6, 711.9, 660.8, 538.8.

(2*S*,4*R*)-4-Hydroxy-2-(4,4'-dimethoxytrityloxymethyl)-1-hex-5-ynoylpyrrolidine (XVII). Dimethoxytritylchloride (4.06 g, 12 mmol) was added to a solu-

tion of compound (**XVI**) (2.3 g, 10.9 mmol) in pyridine (36 mL) under stirring, and the mixture was allowed to stand overnight under stirring. Pyridine was evaporated after which the residue was dissolved in ethyl acetate (60 mL) and washed with an equal volume of a saturated NaCl solution. The organic phase was evaporated to dryness, and the residue was chromatographed on silica gel in a system of 1% triethylamine in CH_2Cl_2. The product was obtained as white foam. Yield: 3.7 g (66%); R_f 0.23 (Et_3N–EtOH–CH_2Cl_2, 1 : 3 : 96); ^{1}H NMR: 7.38–7.32 (m, 2H, Ar*H*), 7.30–7.21 m, 6H, Ar*H*), 7.21–7.15 (m, 1H, Ar*H*), 6.84–6.76 (m, 2H, Ar*H*), 4.67–4.60 (br s, 0.7H, *H*OCH, majR), 4.51–4.45 (br s, 0.3H, *H*OCH, minR), 4.41–4.34 (m, 0.7H, HOC*H*, majR), 4.20–4.13 (m, 0.3H, HOC*H*, minR), 3.77 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.73 (dd, 1H, J_1 11.0, J_2 5.2, HOCH_aH$_b$), 3.49 (dd, 1H, J_1 11.0, J_2 3.4, HOCH$_a$$H_b$), 3.46–3.38 (m, 1H, NC*H*), 3.17–3.01 (m, 2H, NCH_2), 2.43 (dt, 1H, J_1 15.7, J_2 7.4, *COCH*$_a$H$_b$), 2.39 (dt, 1H, J_1 15.7, J_2 7.4, COCH$_a$$H_b$), 2.28 (td, 2H, J_1 6.7, J_2 2.5, CH_2C≡CH), 1.96 (t, *J* 2.7, 1H, C≡C*H*), 1.85 (quin, *J* 7.2, 2H, CH_2CH$_2$C≡CH), 1.83–1.69 (m, 1H, CHCH_aH$_b$CH), 1.43–1.35 (m, 1H, CHCH$_a$$H_b$CH); ^{13}C NMR: 171.86 (0.4C, *C*O, minR), 171.07 (0.6C, *C*O, majR), 158.51 (0.6C, minR), 158.34 (1.3C, majR), 145.03 (0.6C, majR), 144.55 (0.4C, minR), 136.19 (1C), 135.65 (1C), 129.94 (4C), 128.02 (1.4C, majR), 127.99 (0.6C, minR), 127.87 (0.7C, minR), 127.72 (1.3C, majR), 126.89 (0.3C, minR), 126.68 (0.7C, majR), 113.15 (Ar$_3$*C*O), 113.01 (4C), 85.82 (DMTrO*C*H$_2$), 83.90 (*C*≡CH), 70.38 (C≡*C*H), 69.05 (*C*HOH), 63.44 (N*C*H), 55.73 (N*C*H$_2$), 55.16 (2C, O*C*H$_3$), 36.66 (CH*C*H$_2$CH), 33.37 (CO*C*H$_2$), 23.48 (*C*H$_2$C≡CH), 17.93 (CH$_2$*C*H$_2$CH$_2$); IR: 3288.2, 2932.7, 1608.3, 1508.9, 1460.8, 1444.4, 1301.4, 1249.7, 1175.1, 1154.0, 1111.4, 1074.4, 1031.7, 828.3, 701.7, 582.9.

Solid-Phase Supports (XVIII)–(XXI) for the Preparation of Oligonucleotides Modified at the 3'-Position. A General Method

The support containing carboxyl groups (**X**) (0.50 g), DMAP (30 mg), and diisopropylcarbodiimide (420 μL, 2.70 mmol) were added to a solution (0.4 mmol) of one of precursor reagents (**XII**), (**XIV**), (**XV**), or (**XVII**) in 6 mL of a pyridine–DMF mixture 1 : 1. The mixture was left to stand for 72 h at room temperature under periodic stirring after which a solution of pentafluorophenol (0.15 g) in pyridine (0.5 mL) was added, and the mixture was allowed to stand overnight. The glass was filtered and suspended in a solution of piperidine in pyridine. After 5 min, the glass was filtered again, washed with acetonitrile (11 mL), and suspended in a solution containing *N*-methylimidazole (0.45 mL), acetic anhydride (0.25 mL), and 2,6-lutidine (0.25 mL) in acetonitrile (4 mL). After 2 h, the support was filtered, washed with acetonitrile (30 mL), methylene chloride (30 mL), and ether (30 mL), and dried in vacuo. From the absorbance of the dimethoxytrityl cation in a solution of trifluoroacetic acid (3%) in CH_2Cl_2, the load of solid-phase supports was determined, which was for different samples from 51 to 64 μmol/g.

(2*S*,4*R*)-4-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyloxy)-2-(4,4'-dimethoxytrityloxymethyl)-1-(6-*N*-trifluoroacetylamino)hexanoyl-pyrrolidine (XXII). Diisopropylethylamine (1.23 g, 9.6 mmol) was added to a solution of alcohol (**XII**) (5.48 g, 8.7 mmol) in anhydrous methylene chloride (16 mL) under stirring, argon was blown through the mixture, and the mixture was cooled to 0°C. *N*,*N*-Diisopropylamino-2-cyanoethoxychlorophosphine (2.27 g, 9.6 mmol) was added to the cooled solution. The mixture was stirred and heated to room temperature for 1 h. Methylene chloride (80 mL) was added to the solution, and the mixture was washed with a saturated $NaHCO_3$ solution (100 mL) and a saturated NaCl solution (100 mL). The organic phase was evaporated to dryness, and the product was purified by column chromatography in a system of 2% triethylamine in CH_2Cl_2. The product was obtained as snow-white amorphous foam. Yield: 6.5 g (90%); R_f 0.49 (Et_3N–acetone–toluene 1 : 10 : 39); ^{1}H NMR (CD_3CN): 7.67–7.58 (br s, 1H, N*H*), 7.41–7.37 (m, 2H, Ar*H*), 7.32–7.28 (m, 2H, Ar*H*), 7.28–7.24 (m, 4H, Ar*H*), 7.24–7.19 (m, 1H, Ar*H*), 6.88–6.82 (m, 4H, Ar*H*), 4.75–4.64 (m, 0.7H, C*H*OP, majR), 4.62–4.52 (m, 0.2H, C*H*OP, minR), 4.24–4.18 (m, 0.7H, NCH_aH$_b$, majR), 4.12–4.06 (m, 0.2H, NCH_aH$_b$, minR), 3.82–3.68 (m, 9H, (OCH_3)$_2$, DMTOCH_2, NCH$_a$$H_b$), 3.64–3.55 (m, 2H, OC$H_2CH_2$CN), 3.32 (ddd, 1H, J_1 17.8, J_2 9.1, J_3 4.6, NC*H*), 3.28–3.20 (m, 2H, CH_2NH), 3.04 (ddd, 1H, J_1 11.9, J_2 9.1, J_3 3.0, CHCH_aH$_b$CH), 2.65–2.59 (m, 2H, OCH$_2$CH_2CN), 2.29–2.23 (m, 1H, CHCH$_a$$H_b$CH), 2.23–2.13 (m, 4H, COCH_2, N(C*H*(CH$_3$)$_2$)$_2$), 1.60–1.42 (m, 4H, COCH$_2$CH_2, CH_2CH$_2$NH), 1.39–1.30 (m, 2H, COCH$_2$CH$_2$CH_2), 1.18 (d, 3H, *J* 1.3, NCHCH_3), 1.16 (d, 3H, *J* 1.3, NCHCH_3), 1.16 (d, 3H, *J* 2.0, NCHCH_3), 1.14 (d, 3H, *J* 2.0, NCHCH_3); ^{31}P NMR (CD_3CN): 148.14 (sext, 0.38P, *J* 8.8, dst 1/majR), 147.85 (sext, 0.25P, *J* 9.0, dst 1/minR + dst 2/minR), 147.43 (sext, 0.37P, *J* 9.2, dst 2/majR).

(2*S*,4*R*)-4-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyloxy)-2-(4,4'-dimethoxytrityloxymethyl)-1-(hex-5-ynoyl)-pyrrolidine (XXIII). Diisopropylethylamine (0.73 g, 5.7 mmol) was added under stirring to a solution of alcohol (**XVII**) (2.42 g, 4.7 mmol) in anhydrous methylene chloride (10 mL), argon was blown through the mixture, and the mixture was cooled to 0°C. *N*,*N*-Diisopropylamino-2-cyanoethoxychlorophosphine (1.34 g, 5.7 mmol) was added to the cooled solution, and the mixture was stirred and heated to room temperature for 1 h. Methylene chloride (40 mL) was added, and the solution was washed

with a saturated $NaHCO_3$ solution (50 mL) and a saturated NaCl solution (50 mL). The organic phase was evaporated to dryness, and the product was purified by column chromatography in a system of 2% triethylamine in toluene. The product was obtained as a dense oily liquid. Yield: 2.73 g (81%); R_f 0.48 (Et_3N–acetone–toluene 1 : 10 : 39); ^1H NMR (CD_3CN): 7.41–7.36 (m, 2H, Ar*H*), 7.33–7.13 (m, 7H, Ar*H*), 6.88–6.82 (m, 4H, Ar*H*), 4.76–4.65 (m, 0.7H, C*H*OP, majR), 4.62–4.52 (m, 0.2H, C*H*OP, minR), 4.24–4.18 (m, 0.7H, NCH_aH_b, majR), 4.13–4.07 (m, 0.2H, NCH_aH_b, minR), 3.82–3.66 (m, 9H, $(OCH_3)_2$, $DMTrOCH_2$, NCH_aH_b), 3.64–3.53 (m, 2H, OCH_2CH_2CN), 3.33 (ddd, 1H, J_1 17.5, J_2 9.2 Hz, J_3 4.4, NC*H*), 3.02 (ddd, 1H, J_1 12.1, J_2 9.1 Hz, J_3 3.0, $CHCH_aH_bCH$), 2.65–2.59 (m, 2H, OCH_2CH_2CN), 2.40–2.31 (m, 2H, $CHCH_aH_bCH$, $NCH(CH_3)_2$), 2.25–2.20 (m, 2H, $COCH_2$), 2.20–2.14 (m, 2H, $NCH(CH_3)_2$, C≡C*H*), 2.13–2.01 (m, 2H, $CH_2C{\equiv}CH$), 1.75 (quin, 1H, *J* 7.2, $COCH_2CH_2$, dst 1), 1.74 (quin, 1H, *J* 7.2, $COCH_2CH_2$, dst 2), 1.19–1.12 (m, 12H, $N(CH(CH_3)_2)_2$); ^{31}P NMR (CD_3CN): 147.46 (sext, 0.39P, *J* 8.9, dst 1/majR), 147.19 (sept, 0.25P, *J* 8.4, dst 1/minR + dst 2/minR), 146.88 (sext, 0.36P, *J* 9.1, dst 2/majR).

(2*S*,4*R*)-4-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyloxy-2-)-2-(4,4'-dimethoxytrityloxymethyl)-1-[6-*N*-5-((*R*)-1,2-dithiolan-3-ylpentanoylamino)hexanoyl]pyrrolidine (XXIV). Diisopropylethylamine (166 μL 0.97 mmol) was added to a solution of (**XIV**) (0.34 g, 0.88 mmol) in anhydrous acetonitrile (3 mL). The mixture was purged with argon, cooled to 0°C, and *N*,*N*-diisopropylamino-2-cyanoethoxy-chlorophosphine (0.23 g, 0.97 mmol) was quickly added under stirring. The solution was purged with argon once more and heated to room temperature after which it was diluted with methylene chloride (20 mL) and washed with a saturated $NaHCO_3$ solution (10 mL) and a saturated NaCl solution (2 × 10 mL). The organic phase was dried over anhydrous sodium sulfate, evaporated in a rotary evaporator, and chromatographed on silica gel using 1% triethylamine in CH_2Cl_2 as an eluent. Fractions were evaporated, and the residue was dissolved in benzene (3.5 mL) and lyophilized to give a pale-yellow oily substance. Yield: 325 mg (40%); R_f 0.80 (Et_3N–CH_2Cl_2–MeCN, 2 : 49 : 49); ^1H NMR (CD_3CN): 7.39–7.33 (m, 2H, Ar*H*), 7.30–7.16 (m, 7H, Ar*H*), 6.86–6.78 (m, 4H, Ar*H*), 6.50–6.39 (m, 1H, N*H*), 4.24–4.16 (m, 1H, C*H*OP), 3.78–3.68 (m, 8H, $(OCH_3)_2$, $DMTrOCH_2$), 3.60–3.42 (m, 4H, OCH_2CH_2CN, NC*H*, SC*H*), 3.39–3.32 (m, 1H, NCH_aH_b), 3.32–3.21 (m, 1H, NCH_aH_b), 3.19–3.02 (m, 4H, CH_2NH, SCH_2), 2.64–2.58 (m, 2H, OCH_2CH_2CN), 2.44–2.34 (m, 1H, $SCH_2CH_aH_b$), 2.27–2.12 (m, 5H, $N(CH(CH_3)_2)_2$, $NCOCH_2$, $CHCH_aH_bCH$), 2.09–2.04 (m, 2H, $NHCOCH_2$), 1.88–1.79 (m, 2H, $CHCH_aH_bCH$, $SCH_2CH_aH_b$), 1.61–1.47 (m, 6H, CH_2CH_2NH, $SCHCH_2CH_2CH_2$), 1.47–1.39 (m, 2H, $NCOCH_2CH_2CH_2$), 1.39–1.27 (m, 4H, $SCHCH_2CH_2CH_2$, $NCOCH_2CH_2CH_2$), 1.16–1.08 (m, 12H, $N(CH(CH_3)_2)_2$); ^{31}P NMR (CD_3CN): 164.96 (sext, 0.36P, *J* 9.0, dst 1/majR), 164.69 (sext, 0.26P, *J* 8.3, dst 1/minR + dst 2/minR), 164.28 (sext, 0.37P, *J* 9.2, dst 2/majR).

(2*S*,4*R*)-4-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyloxy)-2-(4,4'-dimethoxytrityloxymethyl-1-{6-*N*-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno-[3,4-d]imidazol-4-ylpentanoyl)-aminohexanoyl}pyrrolidine (XXV). Bis(*N*,*N*-diisopropylamino)-2-(cyanoethoxy)phosphine (2.36 g, 7.83 mmol) and 5-(ethylthio)-1*H*-tetrazole (68 mg, 0.522 mmol) were added to a solution of (**XV**) (3.96 g, 5.22 mmol) in methylene chloride (7 mL). The reaction mixture was left to stand overnight at room temperature after which it was diluted with methylene chloride (40 mL) and washed with equal volumes of a saturated $NaHCO_3$ and NaCl solutions. The mixture was dried over anhydrous sodium sulfate and chromatographed on silica gel in a system of 5% triethylamine in acetone. The product was obtained as colorless amorphous foam. Yield: 3.92 g (78%); R_f 0.19 (Et_3N–acetone, 1 : 19); ^1H NMR (CD_3CN): 7.41–7.36 (m, 2H, Ar*H*), 7.32–7.23 (m, 6H, Ar*H*), 7.23–7.19 (m, 1H, Ar*H*), 6.88–6.82 (m, 4H, Ar*H*), 6.53 (t, 0.7H, *J* 5.7, N*H*$COCH_2$, majR), 6.49 (t, 0.2H, *J* 6.2, N*H*$COCH_2$, minR), 5.53–5.48 (br s, 1H, NHCON*H*), 5.20–5.15 (br s, 1H, N*H*CONH), 4.74–4.64 (m, 0.7H, C*H*OP, majR), 4.62–4.51 (m, 0.2H, C*H*OP, minR), 4.39–4.35 (m, 1H, C*H*NH), 4.24–4.18 (m, 1H, C*H*NH), 3.82–3.66 (m, 9H, $(OCH_3)_2$, $DMTrOCH_2$, NC*H*), 3.66–3.53 (m, 2H, OCH_2CH_2CN), 3.32 (ddd, 1H, J_1 17.8, J_2 9.2, J_3 4.6, NCH_aH_b), 3.17–3.06 (m, 3H, CH_2NH, SC*H*), 3.03 (ddd, J_1 12.1, J_2 9.1, J_3 3.0, 1H, NCH_aH_b), 2.85 (ddd, 1H, J_1 12.7, J_2 5.0, J_3 1.1, SCH_aH_b), 2.65–2.57 (m, 3H, OCH_2CH_2CN, SCH_aH_b), 2.29–2.22 (m, 2H, $CHCH_2CH$), 2.22–2.13 (m, 2H, $N(CH(CH_3)_2)_2$), 2.13–2.03 (m, 4H, $NCOCH_2$, $NHCOCH_2$), 1.71–1.50 (m, 6H, $SCHCH_2CH_2CH_2$, CH_2CH_2NH), 1.50–1.41 (m, 2H, $NCOCH_2CH_2$), 1.41–1.29 (m, 4H, $NCOCH_2CH_2CH_2$, $SCHCH_2CH_2$), 1.18–1.13 (m, 12H, $N(CH(CH_3)_2)_2$); ^{31}P NMR (CD_3CN): 147.46 (sext, 0.36P, *J* 9.1, dst 1/majR), 147.18 (sept, 0.26P, *J* 8.4, dst 1/minR + dst 2/minR), 146.75 (sext, 0.38P, *J* 9.1, dst 2/majR).

Oligonucleotide synthesis was performed on a solid-phase support loaded with 200 nmol of an anchor group in an automatic regime using standard phosphoramidites (dA^{Bz}, dT, dC^{Bz}, dG^{Ibu}) according to the protocols of the manufacturers of the device. The condensation of terminal phosphoramidites (**XXII**)–(**XXV**) was carried out for 5 min using 0.1 M solutions of phosphoramidites in absolute acetonitrile. The

detachment of an oligonucleotide from the support and the deblocking were performed with concentrated (28%) ammonia (0.75 mL, overnight/60°C). After the deblocking, the resulting solution was evaporated, and the residue was dissolved in water (500 μL) and purified by reversed-phase HPLC. The starting eluent (solution A) was 0.1 M $AcONEt_4$ + 5% MeCN. The supplementary solution (solution B) was MeCN. Chromatography was carried out using a gradient of 2% solution B/min. Detection was with a flow-through UV detector at λ 260 nm. Collected oligonucleotide solutions were evaporated, and the residue was dissolved in water (1 mL) and desalted on NAP-10 columns (Amersham Biosciences). The concentration of an oligonucleotide was determined spectrophotometrically by measuring the absorption at 260 nm. The efficiency of the condensation of hydroxyprolinol phosphoramidite reagents was estimated from the HPLC data. During the registration of mass spectra of oligonucleotides, a mixture of solutions of 2,6-dihydroxyacetophenone (40 mg in 1 mL of methanol) and disubstituted ammonium citrate (80 mg in 1 mL of an aqueous 50% acetonitrile solution) 1 : 1 (v/v) was used as a matrix for ionization, which was prepared immediately before each measurement.

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