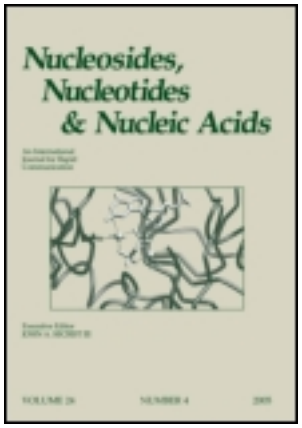


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis of Biotin-Containing Phosphoramidite Linker with Polyether Spacer Arm

Dr Alexey Kayushin [a] , Dr Alexandra Demekhina [a] , Dr Maria Korosteleva [a] , Professor Anatoly Miroshnikov [a] & Professor Alex Azhayev [b]

[a] Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

[b] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopo, Finland

Version of record first published: 02 Sep 2011.

To cite this article: Dr Alexey Kayushin, Dr Alexandra Demekhina, Dr Maria Korosteleva, Professor Anatoly Miroshnikov & Professor Alex Azhayev (2011): Synthesis of Biotin-Containing Phosphoramidite Linker with Polyether Spacer Arm, Nucleosides, Nucleotides and Nucleic Acids, 30:7-8, 490-502

To link to this article: http://dx.doi.org/10.1080/15257770.2011.587702

Nucleosides, Nucleotides and Nucleic Acids, 30:490–502, 2011

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2011.587702

SYNTHESIS OF BIOTIN-CONTAINING PHOSPHORAMIDITE LINKER WITH POLYETHER SPACER ARM

Alexey Kayushin,[1] Alexandra Demekhina,[1] Maria Korosteleva,[1] Anatoly Miroshnikov,[1] and Alex Azhayev[2]

[1]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

[2]*School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopo, Finland*

□ *A phosphoramidite linker unit, based on glycerol backbone and containing a biotin residue attached through a tetraethylene glycol spacer arm, was synthesized. DMTr-Glycidol and tetraethylene glycol were used as starting materials. After conversion of one of hydroxy groups in tetraethylene glycol into an amino group, the epoxy cycle in DMTr-glycidol was opened by this amino alcohol, resulting in the corresponding ether and some quantity of secondary amine. After attaching of biotin residue to the ether followed by phosphitylation, the desirable linker was obtained. The structure of the linker was confirmed by ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{15}N HSQC, and ^{1}H-^{15}N HMBC spectra. The resulted phosphoramidite linker unit is suitable for use in common DNA synthesizers. This approach can be used for preparation of various modifiers containing reporter groups attached to the primary amino function using conventional procedures.*

Keywords Biotin; phosphoramidite; linker; polyether spacer arm

INTRODUCTION

Oligonucleotide and polynucleotide probes containing biotin residues are widely used in diagnostics as well as in scientific research. Presently a number of various approaches for the introduction of biotin residue into oligonucleotides were reported. Biotin may be linked to the heterocyclic base of a nucleoside using a diethyleneglycol-based spacer arm.[1] But the modification of the base can change the hybridization properties of a probe. Moreover, in this construction, the biotin residue is located in the inner

Received 9 March 2011; accepted 9 May 2011.

The authors would like to thank Professor Jouko Vepsäläinen (University of Eastern Finland) and Dr. T. Balashova (Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences) for help with NMR spectra.

Address correspondence to Alexey Kayushin, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Mikluho-Maklaya st, 16/10, Moscow 117997, Russia. E-mail: kaushin@mail.ibch.ru

part of a double helix; this can prevent the formation of biotin–avidin or biotin–streptavidin complex.

The 5′-terminus of oligonucleotide can be modified with primary aliphatic amine followed by attaching biotin residue.[2] Using this method, only 5′-labeled oligonucleotides can be obtained and the additional postsynthesis step of biotinylation of an oligonucleotide is required.

An alternative approach to the synthesis of biotinylated oligonucleotides is incorporation of modifiers directly into the oligonucleotide chain. Currently, the modifiers based on 1,2-diol[3] and 1,3-diol[4] structures are most popular. Those modifiers allow inclusion of several biotin residues into any location of an oligonucleotide chain, increasing the sensitivity of hybridization analysis, and providing a possibility of 3′-terminal labeling. It should be noted that incorporation of 1,2-diol residues into an oligonucleotide chain destabilizes a duplex;[5] thus, from a practical point of view the quantity of biotin residues that can be incorporated using this approach is restricted.

Glen Research Corporation offers a product named BiotinTEG Phosphoramidite (Cat. no. 10-1955-10-1955-xx). This modifier is based on the 1,2-diol structure and was successfully used for various purposes (see, for instance, Ebhardt & Unrau; Bai et al.; Gronewold et al.[6–8]). Herewith, we describe some features of synthesis of BiotinTEG Phosphoramidite (**8**) and suggest a simple, inexpensive, and convenient approach to prepare this compound. It should be noted that this approach can be used for synthesis of modifiers containing any reporter groups (for instance, fluorescent and spin labeled), which can be attached to the primary amino group.

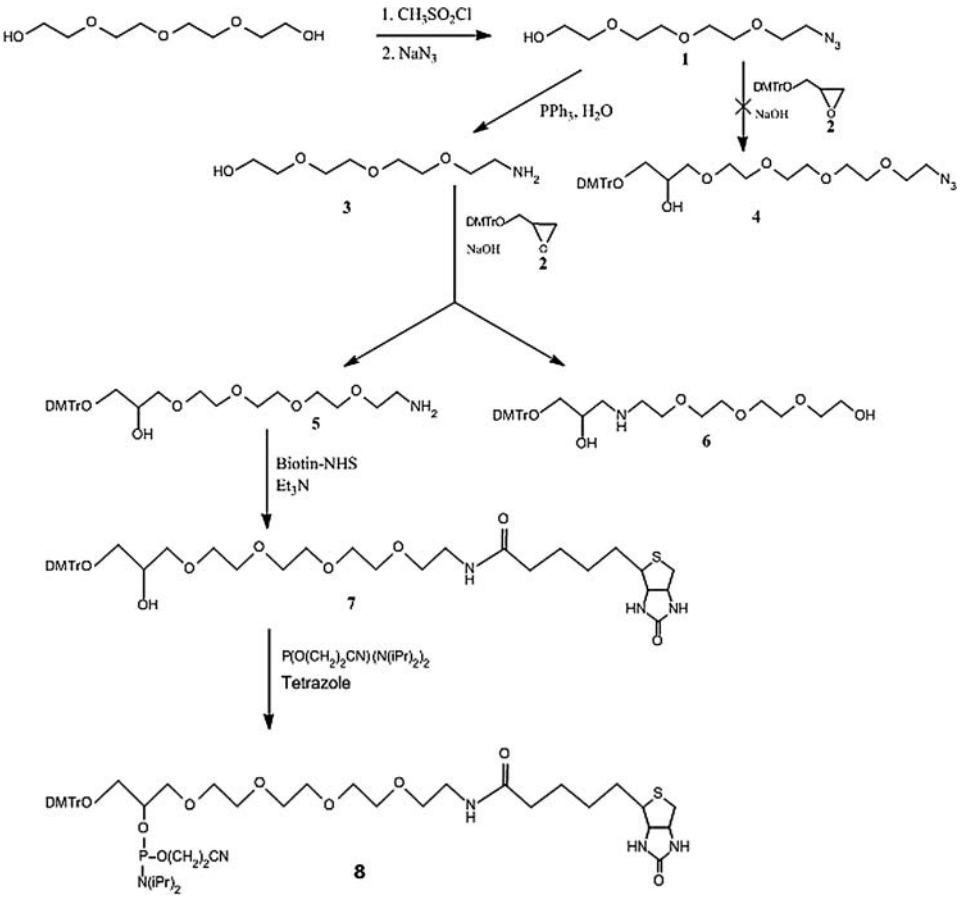
RESULTS AND DISCUSSION

The scheme for preparing **8** is shown in Scheme 1.

It is known that the epoxy cycle can be opened by primary alcohols in the presence of bases yielding ethers of primary alcohols (see, for instance, Chitwood & Freure[9]). So we have used 1-dimethoxytrityl-2,3-epoxypropanol (DMTr-glycidol) (**2**) and tetraethylene glycol as starting materials. DMTr-Glycidol has been synthesized in accordance with Zhang et al.[10] with some modifications, allowing an increase in the total yield of the product.

Using a monomethyl ether of ethylene glycol as a model, we have found that the epoxy cycle is not opened in the presence of K_2CO_3, sodium *tert*-butylate, or sodium. But in the presence of NaOH and under heating at 80°C in dioxane the reaction proceeds well. We have used these conditions in the further synthesis.

Azido alcohol **1** has been synthesized by reaction of tetraethylene glycol with methanesulfonylchloride followed by treatment with sodium azide. In general, we used conditions described in Bertozzi and Bednarski,[11] with some enhancements, which allow a decrease in the quantity of a side



SCHEME 1 The synthesis of biotin-containing phosphoramidite linker with a polyether spacer arm.

product—diazido derivative. After purification by chromatography on silica gel, **1** was obtained with a 72% yield.

Our next plan was to prepare compound **4** from **1** and **2**. After standard reduction of azido function to amino function, we expected to obtain a synthon suitable for the attachment of biotin, resulting in compound **7**. However, we have found that azide **1** is decomposed upon heating with NaOH in dioxane, leading to some unknown substances. Therefore, azide **1** has been converted into amine **3** using PPh_3 in aqueous dioxane (yield 95%).

Any attempts to protect the amino group in **3** before reaction with **2** were unsuccessful. We could not use protective groups [such as *tert*-butyloxycarbonyl (BOC)] that require acidic conditions for removal due to the presence of an acid labile protective group in **5**. We have tested a number of acyl protective groups (residues of acetic, monochloroacetic, dichloroacetic, 2,4-dichlorophenoxyacetic, benzoic, and 4-chlorobenzoic

acids), and all of them proved to be unstable under heating with NaOH in dioxane. So we decided to perform the reaction of 2 and **3** without protection of an amino group.

The reaction of **2** and **3** under the conditions mentioned above (NaOH, dioxane, 80°C, 10 hours) resulted in a mixture of two compounds that can be separated by chromatography on silica gel. Both compounds had molecular masses expected for **5** and very similar ^{1}H NMR spectra. It appeared obvious that both products were resulted from two competing reactions of epoxide **2** with the hydroxy group or amino group in **3**, resulting in **5** or **6**, respectively. In order to unambiguously assign structures to isomeric **5** and **6**, some chemical methods have been used.

First, both compounds were exhaustively acetylated. A mass spectrum of an acetyl derivative of **5** contained signals with m/z 676.37 and 692.33, corresponding to the $[M + Na]^+$ and $[M + K]^+$ of diacetyl derivative of **5** (676.31 and 692.28). A mass spectrum of acetyl derivative of **6** contained signals with m/z 718.41 and 734.39, corresponding to $[M + Na]^+$ and $[M + K]^+$ of triacetyl derivative of **6** (718.32 and 734.29). This confirms the expected structure of **5** and **6** (primary amine and primary alcohol, respectively). Second, **5** (in contrast to **6**) reacts with salicylic aldehyde under conditions described in Khomutov et al.[12] The latter reaction is specific for primary amino groups and additionally confirms the structure of **5**. The ratio of **5** to **6** was nearly 6:1.

Compound **7** was synthesized by reaction of **5** and N-oxysuccinimide ester of biotin (Biotin-NHS) in the presence of Et_3N in dimethylformamide (DMF) at room temperature. We have modified the traditional approach to the synthesis of Biotin-NHS[13] using conditions of another reaction and another method of recrystallization. As a result, we obtained a purer substance (m.p. 206°C–207°C in contrast with 196°C–200°C in Becker et al.[13]).

It should be noted that mobilities of Biotin-NHS and **7** on silica gel are very similar; thus, we added a little bit of NH_4OH to the reaction mixture after completing of the reaction to destroy an excess of Biotin-NHS.

The structure of **7** was proved by ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC spectra. On the basis of these spectra, structure of the studied molecule was unambiguously proved to be **7** because in the ^{1}H spectrum there are three amide protons (–CONH–) present. As follows from the ^{1}H-^{13}C HMBC spectrum, two of these amide protons are incorporated into the biotin ring and the third one is located between the biotin side chain carbonyl moiety and the polyethylene glycol chain ($-OCH_2CH_2\underline{NH}COCH_2CH_2-$).

Finally, the target phosphoramidite **8** has been synthesized using a bis(diisopropylamino)-2-cyanoethoxyphosphine as a phosphitylating agent and tetrazole as an activator. Compound **8** was isolated by reversed-phase column chromatography as a white foam with a yield of 68%. The structure of **8** was fully characterized and confirmed by various ^{1}H-X correlated

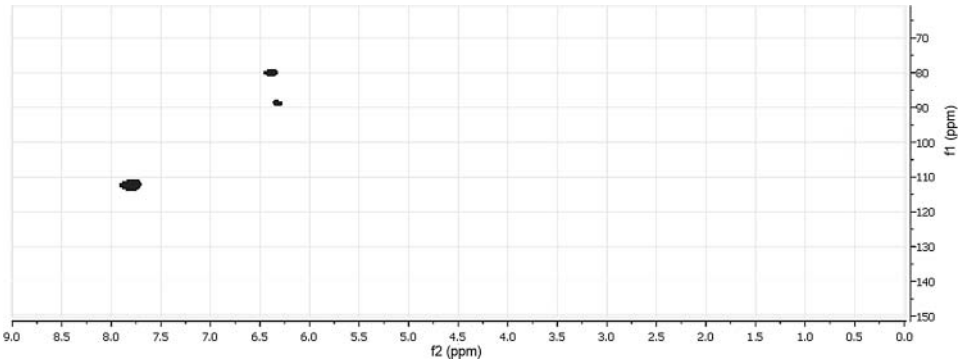


FIGURE 1 ^{1}H-^{15}N HSQC spectrum of **8** (DMSO-d_6).

NMR spectra. The most important details of those spectra are described below.

In Figure 1, the ^{1}H-^{15}N HSQC spectrum of **8** is shown. Only three crosspeaks are observed. Two of them (6.38/79.98 and 6.32/88.77) correspond to NH groups of biotin. As can be seen from Figure 2 (^{1}H-^{15}N HMBC spectrum of **8**), N3 of biotin (δ 79.98) correlates with H3 (δ 6.38) and H4 of biotin (δ 3.09), whereas N1 (δ 88.77) correlates with H1 (δ 6.32) and H6

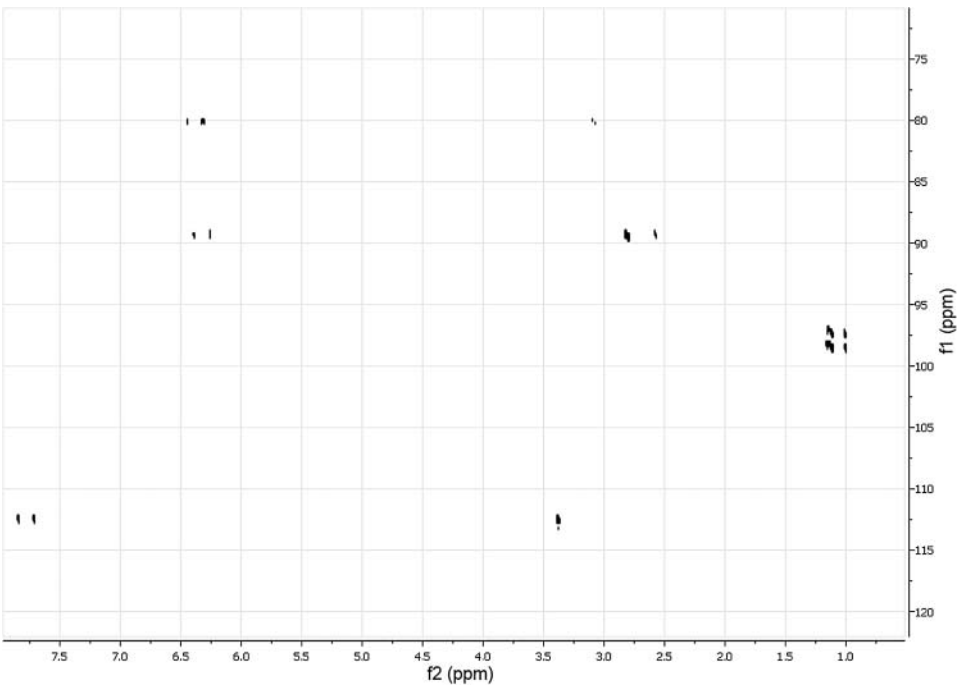


FIGURE 2 ^{1}H-^{15}N HMBC spectrum of **8** (DMSO-d_6).

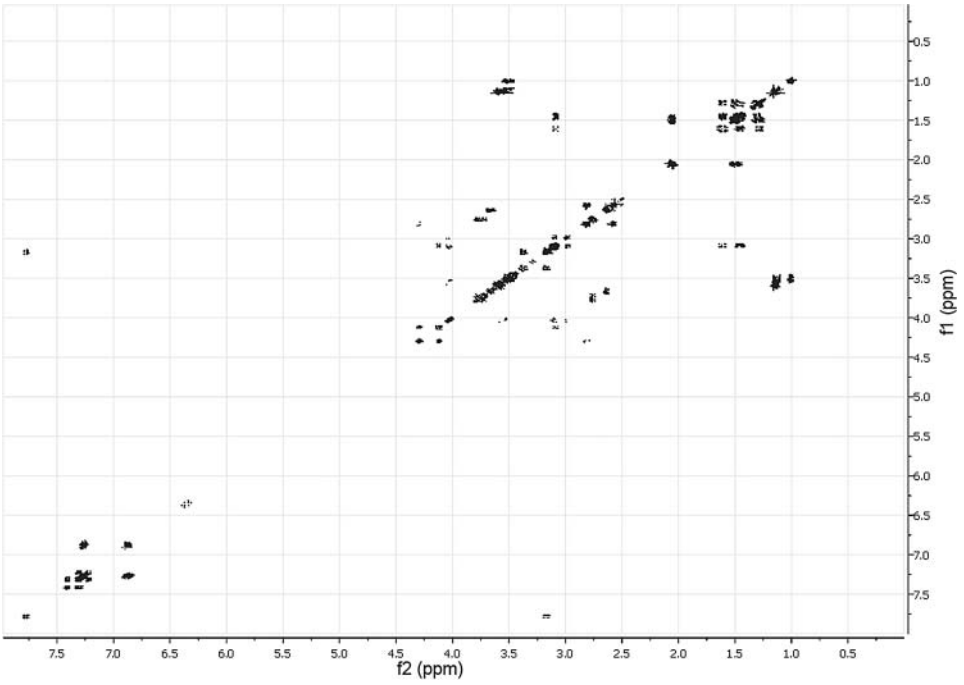


FIGURE 3 ^{1}H-^{1}H COSY spectrum of **8** (DMSO-d_6).

(δ 2.81 and 2.57). All this confirms that **8** does contain the unsubstituted biotin ring system.

The third crosspeak (7.78/112.47) corresponds to the NH-group between the carbonyl group of the biotin side chain and polyether chain ($-OCH_2CH_2\underline{NH}C(O)CH_2CH_2CH_2-$). This nitrogen correlates with protons in CH_2 group in polyether chain (δ 3.38 and 3.17). From the ^{1}H-^{1}H COSY spectrum (see Figure 3), it is clear that protons with δ 7.78, 3.17, and 3.38 form an isolated spin system. In accordance with the ^{1}H-^{13}C HMBC spectrum (see Figure 4), the carbon atom of the carbonyl group (δ 172.53) correlates with the following protons: $-OCH_2CH_2NHC(O)CH_2\underline{CH_2}CH_2-$ (δ 1.50), $-OCH_2CH_2NHC(O)\underline{CH_2}CH_2CH_2-$ (δ 2.06), $-O\underline{CH_2}CH_2\underline{NH}C(O)CH_2CH_2CH_2-$ (δ 7.78), and $-OCH_2\underline{CH_2}NHC(O)CH_2CH_2CH_2-$ (δ 3.17). All this confirms the correctness of attaching of a biotin moiety to the polyether chain.

The presence of the (diisopropyl)phosphoroamidite system in **8** is confirmed by the ^{1}H-^{15}N HMBC spectrum (see Figure 2). Crosspeaks between CH_3 protons (δ 1.15, 1.12, and 1.01) and ^{15}N (δ 98.43 and 97.45) are observed. Two signals of ^{15}N are caused by the presence of a chiral P-atom. Those results confirm the expected structure of this part of molecule.

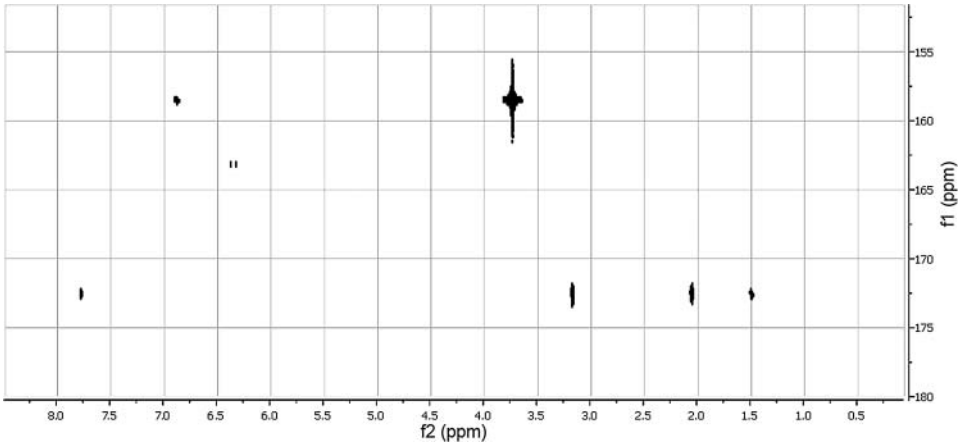


FIGURE 4 Part of ^{1}H-^{13}C HMBC spectrum of **8** (DMSO-d_6).

CONCLUSION

We developed a preparative method of synthesis of the phosphoramidite unit, which is based on glycerol backbone and contains a biotin residue attached through a tetraethylene glycol spacer arm. It was found that the epoxy cycle in DMTr-glycidol can be opened by amino alcohol, resulting in a mixture of both ether and secondary amine. Using this generic approach, various modifiers containing reporter groups, attached via primary amino function, can be prepared. Those modifiers can be used for incorporation of several reporter groups into the oligonucleotide chain without affecting the nucleic base structure.

EXPERIMENTAL

General Materials and Methods

Unless otherwise noted, the materials were obtained from commercial suppliers and used without any purification. Tetraethylene glycol, dimethoxytritylchloride, methanesulfonylchloride, sodium azide, N,N′-dicyclohexylcarbodiimide, 1*H*-tetrazole, and triphenylphosphine were purchased from Fluka (Buchs, Switzerland). N-oxysuccinimide was obtained from Fluka and recrystallized from ethyl acetate. Glycidol and biotin were purchased from Aldrich (St. Louis, Mo, USA). Bis(diisopropylamino)-2-cyanoethoxyphosphine was obtained from Metkinen Chemistry (Turku, Finland). Triethylamine and acetonitrile were distilled over calcium hydride. Diethyl ether and dioxane were distilled over sodium. Thin layer chromatography (TLC) was performed on silica gel plates (TLC Silica gel 60 F_{254}, Merck, Darmstadt, Germany). Column chromatography was performed on Silica gel 60 (Fluka) or on Separon SGX C18

60 (Tessek Ltd., Prague, Czech Republic). High performance liquid chromatography (HPLC) was performed on the Waters system (Waters 1525, Waters 2489, Breeze 2) using column Nova Pack C18, 4.6 × 150 mm. NMR spectra were recorded on Bruker AMX-400 and Bruker Avance II 700 spectrometers (Bruker BioSpin, Rheinstetten, Germany) in DMSO-d_6 or $CDCl_3$ at 30°C. Chemical shifts in ppm (δ) are measured relative to the residual solvent signals as internal standards ($CDCl_3$ 7.26, DMSO-d_6 2.50). Coupling constants (J) are measured in Hz. Mass spectra MALDI-TOF were recorded on Ultraflex TOF/TOF (Bruker Daltonics, Bremen, Germany) using THAP as a matrix. Mass spectra [electrospray ionization (ESI)] were recorded on Esquire3000 Plus (Bruker Daltonics, Germany). Infrared (IR) spectra were recorded on Hitachi 270-30 Infrared Spectrophotometer (Hitachi, Tokyo, Japan).

Syntheses

1-Azido-11-Hydroxy-3,6,9-Trioxaundecane (1)

Tetraethylene glycol (16.8 mL, 103 mmol) and anhydrous triethylamine (20 mL, 252 mmol) were dissolved in 100 mL of diethyl ether and cooled in an ice bath. A solution of methanesulfonylchloride (4.0 mL, 52 mmol) in 16 mL of diethyl ether was added dropwise under cooling during 2 hours. The reaction mixture was allowed to warm up to the room temperature, the solvent was evaporated, and the residue was coevaporated with ethanol. The resulting oil was dissolved in 120 mL of ethanol, 7.210 g (111 mmol) of sodium azide was added, and the suspension was heated at reflux for 10 hours. The reaction progress was controlled by TLC in chloroform–methanol, 9:1.

The crystals were filtered off, the filtrate was concentrated, and the residue was dissolved in 30 mL of semi-saturated aqueous NaCl. The resulting solution was washed by diethyl ether, and the ether layer was washed by semi-saturated aqueous NaCl (5 × 25 mL). The aqueous layers were combined and washed by chloroform (4 × 100 mL). The organic layers were combined, dried by sodium sulfate, and concentrated to oil. The desirable product was purified by column chromatography, column 3.5 × 31 cm, elution by chloroform (300 mL) followed by 3% methanol in chloroform, flow rate 6.8 mL/minute. Yield 8.102 g (37 mmol, 72%), viscous oil. ^{1}H NMR ($CDCl_3$, 400 MHz): 3.72 (m, 2H, $-\underline{CH_2}-OH$), 3.67–3.64 (m, 10H, $-O-\underline{CH_2}-\underline{CH_2}-O-$, $-\underline{CH_2}-CH_2-N_3$), 3.60 (m, 2H, $-\underline{CH_2}-CH_2-OH$), 3.37 (t, 2H, $J = 5.3$, $-CH_2-\underline{CH_2}-N_3$), 2.47 (bs, 1H, $-OH$). MALDI (m/z): [$(M + Na)^+$] calculated for $C_8H_{17}N_3O_4Na$ 242.11, found 241.97; [$(M + K)^+$] calculated for $C_8H_{17}N_3O_4K$ 258.08, found 257.97.

DMTr-Glycidol (2)

Glycidol (2.4 mL, 36 mmol) and triethylamine (7.5 mL, 54 mmol) were dissolved in 50 mL of dioxane. Dimethoxytritylchloride (10.170 g, 30 mmol)

was dissolved in dioxane, the solution was concentrated up to the beginning of a precipitate formation, and the resulting solution was added to a solution of glycidol and triethylamine under argon and cooled in an ice bath. The reaction progress was controlled by TLC in dichloromethane. In 1 hour, 1.5 mL of methanol was added to the reaction mixture. In 1 hour, the precipitate was filtered off, the filtrate was concentrated, the residue was twice coevaporated with chloroform, and the residue was dissolved in chloroform and washed by 50 mL of 0.1 M triethylammonium bicarbonate (TEAB). The aqueous layer was washed by chloroform (3 × 50 mL), and combined organic layers were washed by 0.1 M TEAB (2 × 50 mL), dried by Na_2SO_4, and concentrated. The desired product was isolated by column chromatography (2.5 × 33 cm, elution by dichloromethane, flow rate 6.8 mL/minute). Yield 10.529 g (28 mol, 93%), viscous oil. IR (thin film): 2910, 1590, 1430, 1260, 1100, 800, 710 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): 7.38 (m, 2H, DMTr), 7.28 (m, 7H, DMTr), 6.89 (m, 4H, DMTr), 3.74 (s, 6H, −O−$\underline{CH_3}$), 3.27 (m, 2H, −$\underline{CH_2}$−O−DMTr), 3.12 (m, 1H, CH), 2.71 (dd, 1H, J = 4.3, 5.0, −O−$\underline{CH_2}$−CH−), 2.56 (dd, 1H, J = 2.7, 5.1, −O−$\underline{CH_2}$−CH−).

1-Amino-11-Hydroxy-3,6,9-Trioxaundecane (3)

To the solution of **1** (5.900 g, 27 mmol) in 40 mL of dioxane, 4 mL of water and 7.770 g (27 mmol) of triphenylphosphine were added and the reaction mixture was incubated for 10 hours at room temperature. The reaction progress was controlled by TLC in chloroform–methanol, 7:1; the chromatograms were visualized using 0.3% solution of ninhydrin in methanol or by heating. The reaction mixture was concentrated up to minimal volume, and the residue was dissolved in 100 mL of water and left at 5°C for 10 hours. The crystals were filtered off, and the filtrate was concentrated and dried. Yield 4.943 r (26 mmol, 95%), viscous oil. ^1H NMR ($CDCl_3$, 400 MHz): 3.69 (m, 2H, −$\underline{CH_2}$−OH), 3.65–3.63 (m, 8H, −O−$\underline{CH_2}$−$\underline{CH_2}$−O), 3.58 (m, 2H, −O−$\underline{CH_2}$−CH_2−OH), 3.51 (t, 2H, J = 5.2, −$\underline{CH_2}$−CH_2−NH_2), 2.84 (t, 2H, J = 5.3, C$\underline{H_2}$−NH_2), 2.41 (bs, 3H, −OH, −NH_2). MALDI (m/z): [(M + H)$^+$] calculated for $C_8H_{20}NO_4$ 194.14, found 193.95; [(M + Na)$^+$] calculated for $C_8H_{19}NNaO_4$ 216.12, found 215.93; [(M + K)$^+$] calculated for $C_8H_{19}KNO_4$ 232.10, found 231.92.

1-Amino-14-Hydroxy-15-(O-DMTr)-3,6,9,12-Tetraoxapentadecane (5)

Compound **3** (15.456 g, 80.0 mmol) was twice coevaporated with dioxane up to a minimal volume; the reaction flask was filled with argon after each evaporation. Compound **2** (15.040 g, 40.0 mmol) was twice coevaporated with dioxane up to a minimal volume; the reaction flask was filled with argon after each evaporation. The residue was dissolved in 100 mL of dioxane, added dropwise to the solution of **3**, and 0.500 g (12.5 mmol) of powdered NaOH were added to the mixture. The reaction was

kept at 80°C for 10 hours. The reaction progress was controlled by TLC in dichloromethane and in dioxane-NH_4OH (95:5); the chromatograms were visualized using 0.3% solution of ninhydrin in methanol or by heating. The reaction mixture was concentrated; the residue was dissolved in 200 mL of dichloromethane and washed by 10% aqueous citric acid and by semi-saturated aqueous $NaHCO_3$. Organic layer was dried by Na_2SO_4 and concentrated. The desired product was isolated by column chromatography (3.5 × 36 cm, elution by gradient of methanol in chloroform, 10%–30%, 1600 mL, flow rate 6.8 mL/min.) Yield 6.056 g (10.6 mmol, 27%), viscous oil. ^{1}H NMR (DMSO-d_6, 400 MHz): 7.42 (m, 2H, DMTr), 7.32 (m, 7H, DMTr), 6.90 (m, 4H, DMTr), 4.81 (bs, 1H, CH−OH), 3.77-3.71 (m, 1H, −CH−OH, 6H, −O−CH_3), 3.50–3.45 (m, 12H, −O−CH_2−CH_2−O−), 3.42–3.38 (m, 4H, −O−CH_2−CH−OH, −O−CH_2−CH_2−NH_2), 2.95 (m, 2H, DMTr−O−CH_2−CH), 2.87 (m, 2H, −CH_2−NH_2), 1.89 (bs, 2H, −CH_2−NH_2). MALDI (m/z): [(M + H)$^+$] calculated for $C_{32}H_{44}NO_8$ 570.31, found 570.30; [(M + Na)$^+$] calculated for $C_{32}H_{43}NNaO_8$ 592.29, found 592.28; [(M + K)$^+$] calculated for $C_{32}H_{43}KNO_8$ 608.26, found 608.26.

Side product **6**: Yield 1.024 g (1.8 mmol, 2%), viscous oil. ^{1}H NMR (DMSO-d_6, 400 MHz): 7.39 (m, 2H, DMTr), 7.26 (m, 7H, DMTr), 6.88 (m, 4H, DMTr), 4.84 (br, 1H, OH), 4.51 (br, 1H, OH), 3.74 (m, 6H, −O−CH_3), 3.71 (m, 1H, −CH−OH), 3.50–3.49 (m, 12H, −O−CH_2−CH_2−O−, −CH_2−NH−, −CH_2−OH), 2.91 (m, 2H, DMTr−O−CH_2−CH−), 1.15 (m, 1H, NH). MALDI (m/z): [(M + H)$^+$] calculated for $C_{32}H_{44}NO_8$ 570.31, found 570.40; [(M + Na)$^+$] calculated for $C_{32}H_{43}NNaO_8$ 592.29, found 592.37; [(M + K)$^+$] calculated for $C_{32}H_{43}KNO_8$ 608.26, found 608.36.

N-Oxysuccinimide Ester of Biotin (Biotin-NHS)

Biotin (9.000 g, 36.9 mmol) was suspended in 300 mL of DMF. N-oxysuccinimide (4.242 g, 36.9 mmol) and N,N'-dicyclohexylcarbodiimide (7.590 g, 36.9 mmol) were added to the suspension, and the reaction mixture was kept at room temperature under stirring. In 24 hours, 1 mL of water was added, the precipitate was filtered off, the filtrate was concentrated up to the beginning of precipitate formation, and the residue was recrystallized from mixture of DMF and isopropanol (nearly 7:3) under heating up to 100°C. The crystals were washed by isopropanol and diethyl ether and dried. Yield 9.879 g (28.9 mmol, 79%). m.p. 206–207°C. ^{1}H NMR (DMSO-d_6, 400 MHz): 6.38 (s, 1H, NH of biotin), 6.33 (s, 1H, NH of biotin), 4.31 (m, 1H, H-6a of biotin), 4.15 (m, 1H, H-3a of biotin), 3.10 (m, 1H, H-4 of biotin), 2.85 (d, 1H, J = 5.1, H-6 of biotin), 2.81 (bs, 4H, CH_2 of succinimide), 2.67 (t, 2H, J = 7.4, −C(O)−CH_2−CH_2), 2.59 (d, 1H, J = 12.4, H′-6 of biotin), 1.69–1.38 (6H, −CH_2−CH_2−CH_2−). MALDI (m/z): [(M +

H)$^+$] calculated for $C_{14}H_{20}N_3O_5S$ 342.11, found 342.10; [(M + Na)$^+$] calculated for $C_{14}H_{19}NaN_3O_5S$ 364.09, found 364.10; [(M + K)$^+$] calculated for $C_{14}H_{19}KN_3O_5S$ 380.07, found 381.40.

Biotin Derivative of 5 (7)

Compound **5** (10.759 g, 18.9 mmol) was dissolved in 170 mL of DMF and triethylamine (2.9 mL, 20.8 mmol) and Biotin-NHS (7.090 g, 20.8 mmol) were added to the solution. The reaction progress was controlled by TLC in chloroform–methanol, 7:3. In 1 hour, 2.5 mL of aqueous 25% NH_4OH was added to the reaction mixture; in 10 minutes, the mixture was concentrated, the residue was dissolved in chloroform, washed by water (3 × 100 mL), dried by Na_2SO_4, and concentrated. The target product was isolated by column chromatography (3.5 × 36 cm, gradient of methanol in chloroform, 0%–12%, 1100 mL, flow rate 10.2 mL/minute). Yield 10.083 g (12.6 mmol, 68%), white foam. ^{1}H NMR (DMSO-d_6, 400 MHz): 7.76 (t, 1H, J = 5.6, $-CH_2-\underline{NH}-C(O)-CH_2-$), 7.40 (m, 2H, DMTr), 7.25 (m, 7H, DMTr), 6.88 (m, 4H, DMTr), 6.37 (s, 1H, NH of biotin), 6.31 (s, 1H, NH of biotin), 4.80 (d, 1H, J = 5.4, $-CH-\underline{OH}$), 4.30 (m, 1H, H-6a of biotin), 4.12 (m, 1H, H-3a of biotin), 3.76–3.73 (m, 1H, $-\underline{CH}-OH$, 6H, $-O-\underline{CH_3}$), 3.49–3.47 (m, 12H, $-O-\underline{CH_2}-\underline{CH_2}-O-$), 3.40–3.37 (m, 4H, $-O-\underline{CH_2}-CH-OH$, $-O-\underline{CH_2}-CH_2-\underline{NH}-$), 3.18 (m, 2H, $-O-CH_2-\underline{CH_2}-\underline{NH}-$), 3.12–2.95 (m, 3H, H-4 of biotin, DMTr$-O-\underline{CH_2}-CH-$), 2.81 (dd, 1H, J = 12.4, J = 5.1, H-6 of biotin), 2.58 (d, 1H, J = 12.4, H′-6 of biotin), 2.06 (t, 2H, J = 7.4, $-C(O)-\underline{CH_2}-CH_2-$), 1.69–1.21 (m, 6H, $-CH_2-CH_2-CH_2-$). MALDI (m/z): [(M + Na)$^+$] calculated for $C_{42}H_{57}N_3NaO_{10}S$ 818.37, found 818.35; [(M + K)$^+$] calculated for $C_{42}H_{57}KN_3O_{10}S$ 834.34, found 834.32.

Phosphoramidite of 7 (8)

Compound **7** (200 mg, 0.25 mmol) was dissolved in 10 mL of acetonitrile and evaporated in vacuo up to nearly 2 mL; the reaction flask was filled with argon after evaporation. The 0.45 M solution of tetrazole (0.17 mL, 0.08 mmol) followed by a solution of bis(diisopropylamino)-2-cyanoethoxyphosphine in 0.1 mL acetonitrile (0.12 mL, 0.40 mmol) were added. The reaction progress was controlled by HPLC (gradient 75%–85% acetonitrile in 0.1 M triethylammonium acetate, pH 7.5, 15 minutes, flow rate 0.5 mL/minute, 250 nm). In 1 hour, the reaction mixture was evaporated up to dryness, the residue was dissolved in dichloromethane, and the solution was washed by semi-saturated aqueous $NaHCO_3$; the organic layer was dried by Na_2SO_4, evaporated up to dryness, and the residue was dissolved in 40% aqueous acetonitrile. The desired product was isolated by column chromatography on Separon SGX C18 [8 × 210 mm, elution by a gradient of acetonitrile in water (40%–100%) plus 0.05% Et_3N, 100 mL, flow rate 3 mL/minute]. The solution containing the target product was concentrated

and dried in vacuo. Yield 169 mg (0.17 mmol, 68%), white foam. ^{1}H NMR (DMSO-d_6, 700 MHz): 7.78 (t, 1H, J = 5.5, $-CH_2-NH-C(O)-CH_2-$), 7.41 (m, 2H, DMTr), 7.27 (m, 7H, DMTr), 6.87 (m, 4H, DMTr), 6.38 (s, 1H, NH of biotin), 6.32 (s, 1H, NH of biotin), 4.29 (m, 1H, H-6a of biotin), 4.12 (m, 1H, H-3a of biotin), 4.02 (m, 1H, $-O-CH_2-CH-CH_2$), 3.75–3.72 (m, 7H, $-O-CH_3$, $-CH_2-CH_2-CN$), 3.67 (m, 1H, $-CH_2-CH_2-CN$), 3.62 (m, 1H, $-O-CH_2-CH-CH_2-$), 3.60–3.49 (m, 4H, $-CH-CH_2-O-$, $CH_3-CH-CH_3$, $CH_3-CH-CH_3$), 3.49–3.44 (m, 12H, $-O-CH_2-CH_2-O-$), 3.38 (m, 2H, $-O-CH_2-CH_2-NH-$), 3.17 (m, 2H, $-O-CH_2-CH_2-NH-$), 3.14–2.96 (m, 3H, DMTr$-O-CH_2-CH-$, H-4 of biotin), 2.81 (dd, 1H, J = 12.4, J = 5.1, H-6 of biotin), 2.76 (m, 1H, $-CH_2-CH_2-CN$), 2.64 (m, 1H, $-CH_2-CH_2-CN$), 2.57 (dd, 1H, J = 12.4, H'-6 of biotin), 2.06 (t, 2H, J = 7.4, $-C(O)-CH_2-CH_2-$), 1.64–1.22 (m, 6H, $-CH_2-CH_2-CH_2-$), 1.13 (m, 9H, CH_3), 1.01 (d, 3H, J = 6.8, CH_3). ^{13}C NMR (DMSO-d_6): 158.70, 145.30, 136.21, 130.08, 128.14, 127.06, 113.60, 85.63, 55.38 (DMTr); 64.14 (DMTr$-O-CH_2-$); 72.45 (DMTr$-O-CH_2-CH-$), 71.77 ($-CH_2-CH-CH_2-O-CH_2-$), 119.16, 58.58, 43.01, 24.71, 24.52, 20.13 (diisopropylamino-2-cyanoethoxyphosphoroamidite segment); 69.53–70.13 (polyether chain), 38.88 ($-O-CH_2-CH_2-NH-C(O)-CH_2-$), 172.53 ($-CH_2-CH_2-NH-C(O)-CH_2-CH_2-$), 35.43 ($-NH-C(O)-CH_2-CH_2-CH_2-CH_2-$), 25.60 ($-NH-C(O)-CH_2-CH_2-CH_2-CH_2-$), 28.46 ($-NH-C(O)-CH_2-CH_2-CH_2-CH_2-$), 25.66 ($-NH-C(O)-CH_2-CH_2-CH_2-CH_2-$), 163.11 (C-2 of biotin), 61.39 (C-3a of biotin), 55.70 (C-4 of biotin), 40.24 (C-6 of biotin), 59.67 (C-6a of biotin). ^{15}N NMR (DMSO-d_6): 112.47 ($-CH_2-CH_2-NH-C(O)-CH_2-CH_2-$), 98.43, 97.45 ($-N(i-Pr)_2$), 88.77 (N-3 of biotin), 79.98 (N-1 of biotin). ^{31}P NMR (DMSO-d_6): 151.41, 150.82 (relative to 80% H_3PO_4). ESI-MS (m/z): [$(M + Na)^+$] calculated for $C_{51}H_{74}N_5NaO_{11}PS$ 1018.5, found 1018.2, [$(M + K)^+$] calculated for $C_{51}H_{74}KN_5O_{11}PS$ 1034.5, found 1034.3.

REFERENCES

1. Pieles, U.; Sproat, B.S.; Lamm, G.M. A protected biotin containing deoxycytidine building block for solid phase synthesis of biotinyated oligonucleotides. *Nucleic Acids Res.* **1990**, 18, 4355–4360.
2. Nelson, P.S.; Sherman-Gold, R.; Leon, R. A new and versatile reagent for incorporating multiple primary aliphatic amines into synthetic oligonucleotides. *Nucleic Acids Res.* **1989**, 17, 7179–7186.
3. Misiura, K.; Durrant, I.; Evans, M.R.; Gait, M.J. Biotinyl and phosphotyrosinyl phosphoramidite derivatives useful in the incorporation of multiple reporter groups on synthetic oligonucleotides. *Nucleic Acids Res.* **1990**, 18, 4345–4354.
4. Nelson, P.S.; Kent, M.; Muthini, S. Oligonucleotide labeling methods 3. Direct labeling of oligonucleotides employing a novel, non-nucleosidic, 2-aminobutyl-1,3-propanediol backbone. *Nucleic Acids Res.* **1992**, 20, 6253–6259.

5. Nielsen, P.; Dreiøe, L.H.; Wengel, J. Synthesis and evaluation of oligodeoxynucleotides containing acyclic nucleosides: introduction of three novel analogues and a summary. *Bioorg. Med. Chem.* **1995**, 3, 19–28.
6. Ebhardt, H.A.; Unrau, P.J. Characterizing multiple exogenous and endogenous small RNA populations in parallel with subfemtomolar sensitivity using a streptavidin gel-shift assay. *RNA* **2009**, 15, 724–731.
7. Bai, X.; Li, Z.; Jockusch, S.; Turro, N.J.; Ju, J. Photocleavage of a 2-nitrobenzyl linker bridging a fluorophore to the 5′ end of DNA. *PNAS* **2003**, 100, 409–413.
8. Gronewold, T.M.; Baumgartner, A.; Quandt, E.; Famulok, M. Discrimination of single mutations in cancer-related gene fragments with a surface acoustic wave sensor. *Anal. Chem.* **2006**, 78, 4865–4871.
9. Chitwood, H.C.; Freure, B.T. The reaction of propylene oxide with alcohols. *J. Am. Chem. Soc.* **1946**, 68(4), 680–683.
10. Zhang, L.; Peritz, A.E.; Carroll, P.J.; Meggers, E. Synthesis of glycol nucleic acids. *Synthesis* **2006**, 4, 645–653.
11. Bertozzi, C.R.; Bednarski, M.D. The synthesis of heterobifunctional linkers for the conjugation of ligands to molecular probes. *J. Org. Chem.* **1991**, 56, 4326–4329.
12. Khomutov, A.R.; Shvetsov, A.S.; Vepsäläinen, J.J.; Kritzyn, A.M. Novel acid-free cleavage of *N*-(2-hydroxyarylidene) protected amines. *Tetrahedron Lett.* **2001**, 42, 2887–2889.
13. Becker, J.M.; Wilchek, M.; Katchalski, E. Irreversible inhibition of biotin transport in yeast by biotinyl-p-nitrophenyl ester. *Proc. Nat. Acad. Sci. USA.* **1971**, 68, 2604–2607.