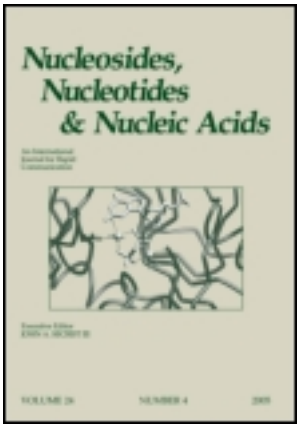

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Oligonucleotide Functionalization by a Novel Alkyne-Modified Nonnucleosidic Reagent Obtained by Versatile Building Block Chemistry

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OLIGONUCLEOTIDE FUNCTIONALIZATION BY A NOVEL ALKYNE-MODIFIED NONNUCLEOSIDIC REAGENT OBTAINED BY VERSATILE BUILDING BLOCK CHEMISTRY

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□ *A convenient synthetic strategy has been designed to prepare an alkyne-modified synthon for automated DNA synthesis. It is based on the key* O*-DMTr-protected 4-(2-hydroxyethyl)morpholin-2,3-dione and building blocks obtained by its functionalization by various aliphatic amines. A respective nonnucleosidic phosphoramidite monomer containing a terminal alkyne in the side-chain was synthesized, and corresponding oligothymidylates incorporating the modification in various positions were prepared. The presence of the alkyne group was confirmed by Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) between the functionalized oligonucleotide and an azide derivative of 7-nitro-2,1,3-benzoxadiazole.*

Keywords Nonnucleosidic phosphoramidite; fluorescent oligonucleotide probe; alkyne-azide click chemistry

INTRODUCTION

Modified oligonucleotides are widely used as molecular tools for cutting-edge research in life sciences, in particular, in molecular diagnostics. Automated solid-phase phosphoramidite DNA synthesis is an established method for oligonucleotide preparation. A majority of chemical modifications can be routinely introduced into DNA sequences during the synthesis by using nonnucleoside phosphoramidite monomers bearing the required functional groups.[1–3] It should be noted that the preparation of those monomers may be labor-consuming in some cases. Thus, the development of versatile and

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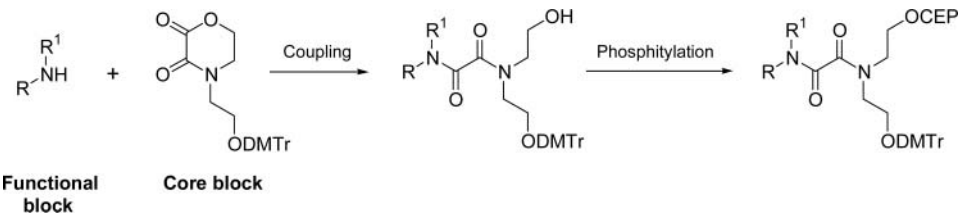
convenient methods for the preparation of nonnucleoside phosphoramidite synthons equipped with desired functionalities is an important task.

We describe herein a novel approach to the design of nonnucleosidic DNA functionalization reagents based on a merger of two methodologies: one utilizing the ring-opening of lactones by aliphatic amines [4] and another that uses activated oxalate esters[5] for oligonucleotide synthesis and derivatization. It also takes into account our previous experience in the synthesis and application of oligonucleotides with nonnucleotidic inserts.[6]

We have applied our new building block strategy for the preparation of achiral nonnucleoside phosphoramidite synthons bearing various functional groups.[7,8] In accordance with the proposed approach, two separately functionalized building blocks have been synthesized: a core block bearing functional groups for incorporation into DNA chain and a functional block bearing functional groups required to obtain oligonucleotide derivatives with specific properties. The core block can be used as a common reagent for the preparation of a family of monomers with varying functionalities. In each case, DNA modification would be accomplished by using an appropriately functionalized synthon. The structure of a functional block would be defined by the desired oligonucleotide modification. An aliphatic amine bearing the required functionality would be a suitable candidate for a functional block. Coupling of the two building blocks is performed immediately before the final phosphitylation step that yields a nonnucleosidic phosphoramidite monomer (Scheme 1).



SCHEME 1 A general scheme of building block strategy for the preparation of nonnucleoside phosphoramidite monomers. R, R^1—a desired functional group or H, CEP–2-cyanoethoxy-*N*,*N*-diisopropylaminophosphinyl.

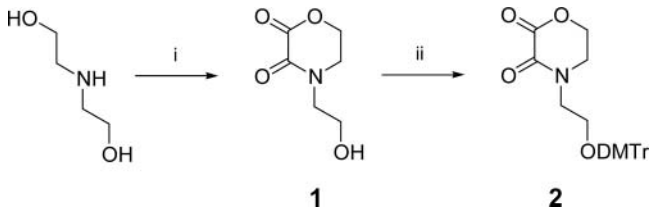
In this work, we demonstrate the applicability of our approach to the preparation of a phosphoramidite synthon suitable for the introduction of a terminal alkyne group into either terminal or internal positions within an oligonucleotide chain. The alkyne moiety is widely used in click chemistry[9] allowing for mild postsynthetic conjugation with functionalized azides via copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).

RESULTS AND DISCUSSION

Preparation of Click Chemistry Components

Synthesis of the Core Block

Synthesis of the core block **2** was carried out in two high-yielding steps (Scheme 2). A reaction between diethanolamine and diethyl oxalate results in a key lactone 4-(2-hydroxyethyl)morpholin-2,3-dione (**1**).

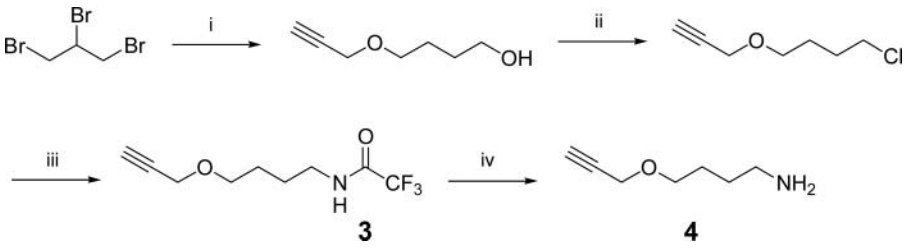


SCHEME 2 Synthesis of the core block. (i) diethyl oxalate, Pr^{i}OH; (ii) DMTrCl,pyridine.

Very small excess of 4,4′-dimethoxytrityl chloride (DMTrCl) (1.05 equiv.) was enough to quantitatively convert **1** into a monodimethoxytritylated core block (**2**). A convenient extraction without column chromatography was sufficient. Trace amount of DMTrOH in **2** does not interfere with further reactions (data not shown). It should be noted that extraction with aq $NaHCO_3$ results in quantitative hydrolysis of the lactone **2**. To avoid this, phosphate buffer (0.3 M KH_2PO_4, pH 6.5) was used as an aqueous phase.

Synthesis of an Alkyne Functional Block

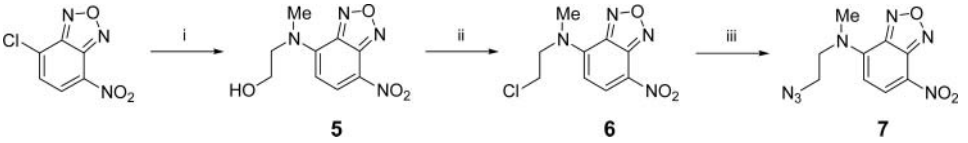
Synthesis of a functional block 4-(2-propynyloxy)butylamine bearing the terminal alkyne group was carried out in accordance with a previously published protocol[10] starting from 1,4-butanediol and 1,2,3-tribromopropane (Scheme 3).



SCHEME 3 Synthesis of the alkyne-containing functional block 4-(2-propynyloxy)butylamine: (i) 1,4-butanediol, KOH, Bu^{t}OH; (ii) $SOCl_2$; (iii) CF_3CONH_2, Cs_2CO_3, DMF; (iv) aq NaOH.

Synthesis of an Azide-Functionalized Fluorophore

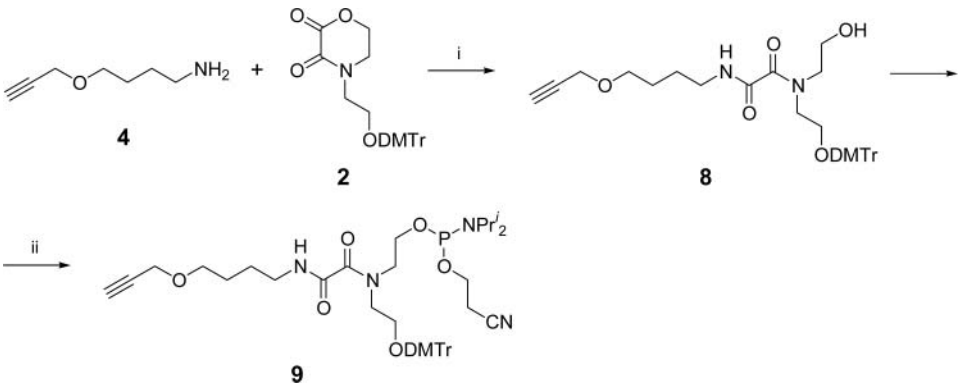
As a fluorophore for the postsynthetic labeling of oligonucleotides via the CuAAC reaction, we have used an azido derivative of 7-nitro-2,1,3-benzoxadiazole.[11] The compound was prepared by a series of transformations from commercially available 4-chloro-7-nitro-2,1,3-benzoxadiazole (Scheme 4). The overall yield of the reaction was acceptable due to optimization of procedures.



SCHEME 4 Synthesis of *N*-(2-azidoethyl)-*N*-methyl-7-nitro-2,1,3-benzoxadiazol-4-ylamine. (i) *N*-methylaminoethanol, EtOH, 20°C, 40 minutes; (ii) $PhSO_2Cl$, pyridine, 90°C, 5 hours; (iii) NaN_3, DMSO, 65°C, 8 hours.

Preparation of an Alkyne-Functionalized Phosphoramidite Synthon

The reaction between the core block (**2**) and functional block (**4**) has produced an alkyne-functionalized alcohol **8**. Noteworthy, such a conversion does not require any condensing agents. After the phosphitylation of the alcohol (**8**), we have obtained the required phosphoramidite monomer **9** for the incorporation of a terminal alkyne into oligonucleotides (Scheme 5).



SCHEME 5 A scheme for the preparation of an alkyne-functionalized phosphoramidite. (i) DMAP, pyridine; (ii) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite, 1*H*-tetrazole, *N*,*N*-diisopropylethylamine, MeCN.

The obtained monomer **9** is derived from diethanolamine, which is a convenient scaffold for construction of nonnucleosidic inserts for oligonucleotide functionalization. There are two published approaches to prepare diethanolamine-based nonnucleosidic synthons. One requires

prior functionalization of the amino group of diethanolamine by a desired substituent followed by dimethoxytritylation and phosphitylation of the obtained symmetrical diol.[12–14] Another approach utilizes initial *O*-monodimethoxytritylation of diethanolamine followed by *N*-function alization and phosphitylation.[15] In some cases, additional temporary protection for another hydroxyl group of diethanolamine is required to increase the yield of the desired synthon. Approaches of both type require the preparation of intermediate mono-*O*-protected diol precursors that are accompanied by inevitable formation of substantial amount of di-*O*-substituted byproduct.

Our scheme of conversion of diethanolamine into compound **1** solves this problem by simultaneous *N*-functionalization and *O*-activation. The core precursor **2** already has a temporary ester protecting group for one of the two hydroxyl group of diethanolamine, which is stable under neutral conditions and yet able to react with primary and secondary aliphatic amines without any additional activation.[7] Highly specific acylation of an appropriately functionalized aliphatic amine with concomitant deprotection of the hydroxyl group followed by final phosphitylation step furnishes the desired phosphoramidite synthon. The results obtained allow us to conclude that the functionalized synthon obtained by our approach could be inserted into any position of an oligonucleotide chain by standard automated synthesis protocol.

Synthesis of Alkyne-Tethered Oligonucleotides and Their Fluorescent Labeling via Click Chemistry

By the use of monomer **9** (Scheme 5), we have been able to obtain model oligonucleotides functionalized with alkyne-containing nonnucleotidic inserts at either internal or terminal position and an oligonucleotide post-synthetically labeled with an azide-tethered fluorophore.

It was shown that compound **9** effectively reacts with the growing DNA chain under standard coupling conditions in a DNA synthesizer. A series of oligothymidylates bearing the alkyne group at either terminal or internal position was obtained in good yields including an example of three consecutive incorporations of the modification. All the oligonucleotides after deprotection followed by RP-HPLC purification were analyzed for homogeneity by denaturing gel electrophoresis visualized by staining with Stains-All®. MALDI-TOF mass-spectrometry was applied to analyze the molecular mass of the products obtained (Table 1).

Various conditions for cleaving the oligomers from solid support and deprotecting them were utilized. There was no degradation of the alkyne-containing oligothymidylates when either mild (*tert*-butylamine in aq methanol) or standard (conc aq ammonia) protocols were applied. The only exception was the case of the 5′-modified oligonucleotide which was freed from the 5′-terminal DMTr group before alkaline treatment. A product with

TABLE 1 MALDI TOF analysis of the oligonucleotides prepared

		Results of MALDI-mass analysis (found values), *m/z*			
Sequence	Exact mass[4]	Positive mode	Ion form	Negative mode	Ion form
XT_6[1]	2412	2149.1	$[M - DMTr + K]^+$	2411.0	$[M - H]^-$
XT_6[2]	2110	2149.0	$[M + K]^+$	2109.3	$[M - H]^-$
XT_6[3]	2110	1930.5	$[M + H]^+$	1928.0	$[M - H]^-$
X_3T_6[1]	3108	3111.9	$[M + H]^+$	3107.1	$[M - H]^-$
T_3XT_6	3022	3024.6	$[M + H]^+$	3024.4	$[M - H]^-$
T_3XT_3	2110	2111.0	$[M + H]^+$	2108.7	$[M - H]^-$
T_3YT_3	2373	2374.6	$[M + H]^+$	2373.8	$[M - H]^-$

[1] Synthesized in the DMTr-on mode and analyzed by MALDI-TOF after purification without manual detritylation.

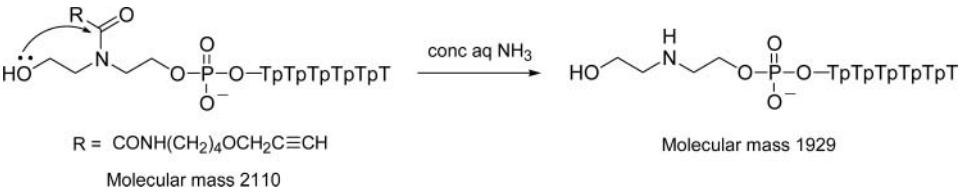
[2] Synthesized in the DMTr-on mode and analyzed by MALDI-TOF after purification and manual detritylation.

[3] Synthesized in the DMTr-off mode and analyzed by MALDI-TOF after purification.

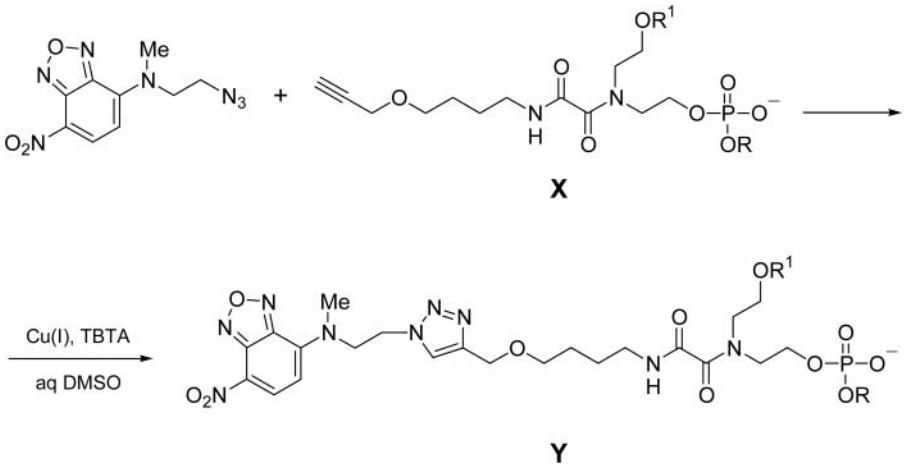
[4] The sum of atomic masses of the most common isotope of each element in the compound calculated using ISISDraw® software.

X, Y—nonnucleosidic insert introduced into the oligonucleotide using phosphoramidite **9** before (X) and after (Y) click reaction. For structures see Scheme 7.

the expected mass was not detected during RP-HPLC purification. A similar result has been observed earlier with oligonucleotides modified at the 5′-end by a substituted aminoethanol residue.[14] We ascribe the observed degradation of the oxalamide proximal to *O*-unprotected ethanolamine during alkaline deprotection step to anchimeric effect (Scheme 6). In contrast with previous results,[14] we have been able to identify the product of such a base-catalyzed degradation. It is the oligonucleotide derivative bearing the 5′-terminal *N*-deacylated diethanolamine monophosphate, i.e. the product of oxalamide bond cleavage assisted by the neighboring free hydroxyl group. In contrast, di-*O*-phosphorylated *N*-oxalyl diethanolamine in the middle of an oligonucleotide is not sensitive to alkaline treatment. MALDI-TOF data provide the evidence of the reaction (see Table 1 and footnotes therein). However, intact 5′-alkyne-tethered oligonucleotides can be obtained by retaining the 5′-terminal DMTr group during ammonia deprotection step and removing it afterwards by aqueous acetic acid treatment (80% AcOH, 10 minutes, ambient temperature) (Table 1). The product is stable under neutral condition and can be easily purified by RP-HPLC.



SCHEME 6 Degradation of the oxalyl diethanolamine unit at the 5′-end during ammonia deprotection.



SCHEME 7 Scheme of click chemistry conjugation. R—oligonucleotide, R^1 —oligonucleotide or hydrogen.

Conjugation of Alkyne-Functionalized Oligonucleotide with Azide-Tethered Fluorophore

An alkyne-tethered oligonucleotide containing the nonnucleotidic insert in the middle of the sequence T_3XT_3 was chosen for the postsynthetic conjugation with a model azide by click chemistry. A deprotected and desalted

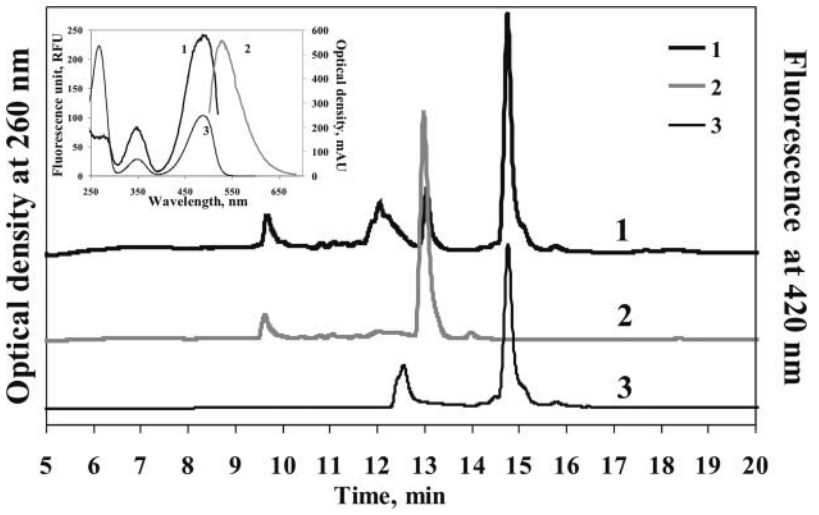


FIGURE 1 HPLC profiles of oligonucleotide derivatives: lines 1 and 3— labeled oligonucleotide T_3YT_3; line 2—alkyne-modified precursor oligonucleotide T_3XT_3. Lines 1 and 2 recorded at 260 nm. Line 3 recorded by fluorescence detector λ_{em} 550 nm, λ_{ex} 450 nm. **Insert:** Spectral analysis of the oligonucleotide T_3YT_3. Fluorescence spectra (left axis), excitation (λ_{em} 540 nm, line 1), and emission (λ_{ex} 480 nm, line 2). Line 3: UV-Vis spectrum of the product obtained by click reaction (right axis).

sample of alkyne-modified oligomer reacts very efficiently with azidofluorophore (**7**). The reaction was carried out in the presence of TBTA (*tris*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine) ligand (for full protocol see Experimental section). According to gel electrophoresis (data not shown) and HPLC, the yield of click conjugation was ~90%. MALDI-TOF, UV/VIS and fluorescence spectra allow us to conclude that the fluorophore was successfully incorporated into the model oligonucleotide (Scheme 7, Figure 1, Table 1).

Conjugation of azide-substituted 2,1,3-benzoxadiazole with alkyne-tethe red oligomer slightly increases the hydrophobicity of the oligonucleotide. UV-VIS spectra of the product obtained display two characteristic maxima at 350 and 490 nm, which are typical for substituted 2,1,3-benzoxadiazole. Emission and excitation spectra of the labeled conjugate show the expected fluorescent behavior of the incorporated fluorophore.[11]

CONCLUSIONS

The data obtained show that our building block approach to the preparation of nonnucleosidic reagents is both versatile and convenient. A novel alkyne-modified phosphoramidite monomer suitable for terminal as well as internal oligonucleotide modification by achiral diethanolamine units was synthesized. The proposed synthetic scheme is efficient and allows for high-yielding preparation of the target product. The synthesis of a functional block which is an alkyne-functionalized aliphatic amine would be the most time-consuming step of the synthesis. The core lactone block can be prepared in bulk quantity and stored until needed. The yields are commonly in the range of 60–90% (see Experimental section). Alkyne-functionalized oligothymidylates and a conjugate with an azide-containing fluorescent dye were obtained. These results together with our earlier reports[7,8] indicate that a promising technique for DNA modification has been designed. It could be easily applied for the synthesis of various functionalized nonnucleosidic phosphoramidites. This would be the aim of our further work, which would be published in due course.

EXPERIMENTAL

General Information

All the reagents and solvents used were of the highest available purity obtainable from their respective commercial supplier. 1,2,3-Tribromopro pane, cesium carbonate, trifluoroacetamide, thionyl chloride, 1,4-butane diol, benzenesulfonyl chloride, diethanolamine were purchased from Sigma-Aldrich (USA). 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl, 98%),

1*H*-tetrazole, *N*,*N*-diisopropylethylamine, diethyl oxalate were from Acros Organics (USA). 4-*N*,*N*-dimethylaminopyridine, triethylamine, *N*-methylimidazole were bought from Fluka (Switzerland). 4,4′-Dimethoxytrityl chloride was from ChemGenes (USA). Sodium azide was from Serva (Germany). Nucleoside 3′-β-cyanoethyl-*N*,*N*-diisopropyl phosphoramidites were obtained from Glen Research (USA). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite was a generous gift of Dr V. A. Ryabinin (LMC, ICBFM SB RAS). Solvents were dried by standard methods and stored over either 3 Å molecular sieves or calcium hydride pellets.

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Bruker AV-300, AV-400, and DRX-500 NMR spectrometers. Chemical shifts (δ) are reported in ppm relative to residual solvent signals. For ^{31}P NMR, external standard of 85% aq H_3PO_4 was used. Coupling constant (J) values are reported in Hertz (Hz), and signal multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app.t (apparent triplet), and br (broad). Matrix-assisted laser desorption ionization—time of flight (MALDI-TOF) mass spectra were run on Reflex III, Autoflex Speed (Bruker, Germany) with 3-hydroxypicolinic acid as a matrix. DFS High Resolution GC-MS and Finnigan MAT 8200 mass spectrometers were also used for molecular mass measurements. Processing of mass analysis results was performed using mMass software.[16] Elemental analyses were performed on EURO EA 3000. The presence of the azide group was confirmed by recording IR spectra on a Bruker Tensor 27 IR-spectrometer. Emission and excitation spectra were recorded on Cary Eclipse (Agilent, USA) using 10^{-6} M solution of the oligonucleotide T_3YT_3. Processing of analytical results was performed using Spin-Works software. Spectra were recorded at the Center for Collective Use of Analyses of Organic Compounds and Materials NIOC SB RAS.

Thin layer chromatography was carried out using DC-Alufolien Kieselgel 60 F_{254} TLC plates (Merck, Germany) in appropriate solvent mixtures and was visualized by UV irradiation and stained by ninhydrin (0.1% in EtOH) or 10% sulfuric acid. Column chromatography was carried out on a 100 cm^3 column using Kieselgel 60 (Merck, Germany).

Oligonucleotide synthesis. Oligonucleotides were synthesized on an automated DNA synthesizer ASM-800 (Biosset, Russia) using standard nucleoside phosphoramidites (Glen Research, USA) at 0.1 M concentration in absolute MeCN. Alkyne-modified nonnucleotidic monomer **9** was used at 0.15 M concentration in absolute MeCN. During condensation of the modified monomer, the volumes of activator (0.5 M 1*H*-tetrazole in absolute MeCN) and monomer **9** were increased from 20 to 80 μL, and condensation time was increased from 1 to 3 minutes as compared with the standard method. The removal from support and deprotection of the oligonucleotides were carried out either by a standard way (*ca.* 33% aq ammonia, 20°C, 1 hour) or with *tert*-butylamine–methanol–water (1:1:2 v/v/v) for 5 hours at 37°C.

Modified oligonucleotides were purified by reverse-phased HPLC on an Agilent 1200 series chromatograph using a gradient of acetonitrile (0–40%) in 0.02 M triethylammonium acetate, pH 7 for 30 minutes, flow rate of 2 cm^3 min^{-1} using a Zorbax SB-C18 (5 μm) column (4.6 × 150 mm). Absorption spectra were recorded at wavelengths from 220 to 600 nm.

4-(2-Hydroxyethyl)morpholin-2,3-dione (1). Diethanolamine (24.9 g, 0.24 mol) was dried by coevaporation in isopropanol (3 × 10 cm^3), dissolved in isopropanol (20 cm^3), and added dropwise for a period of 3 hours to a well-stirred solution of diethyl oxalate (32 cm^3, 0.2 mol) in isopropanol (48 cm^3) at ambient temperature. The resulting milky solution was stirred for 12 hours. The precipitate was collected on a sintered glass filter and washed with isopropanol (2 × 10 cm^3). Final drying under reduced pressure afforded **1** (86%) as a white powder. TLC (CH_2Cl_2—EtOH 1:1 v/v): $R_f = 0.6$. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.49 (2H, t, $J = 5.1$, OCH_2CH_2N); 3.71 (2H, t, $J = 5.1$, OCH_2CH_2N); 3.53 (2H, t, $J = 5.5$, NCH_2CH_2OH); 3.43 (2H, t, $J = 5.5$, NCH_2CH_2OH). ^{13}C-NMR (300 MHz, DMSO-d_6, δ, ppm): 45.99, 49.45, 58.25, 65.84, 154.01, 157.77.

4-[2-(4,4′-Dimethoxytrityloxy)ethyl]morpholin-2,3-dione (2). Compound **1** (1.55 g, 9.85 mmol) was dried by coevaporation with absolute pyridine (3 × 10 cm^3) and dissolved in abs. pyridine (30 cm^3). 4,4′-Dimethoxytrityl chloride (3 g, 8.85 mmol) was added to the solution, and the reaction mixture was stirred at ambient temperature for 3 hours. Then the solution was evaporated to an oil, dissolved in 200 cm^3 of CH_2Cl_2, and the organic layer was washed with freshly prepared saturated $NaHCO_3$ solution (3 × 200 cm^3), dried over anhydrous Na_2SO_4, filtered, and evaporated to an oil. Compound **2** was used in the next reaction without purification. TLC (CH_2Cl_2 – EtOH 96:4 v/v): $R_f = 0.7$ (purity ~95%). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.85–8.66 (13H, m, Ar*H*); 4.49 (2H, t, $J = 4.9$, OCH_2CH_2N); 3.73 (3H, s, OMe); 3.68 (2H, t, $J = 4.9$, OCH_2CH_2N); 3.59 (2H, t, $J = 5.3$, NCH_2CH_2ODMTr); 3.16 (2H, t, $J = 5.3$, NCH_2CH_2ODMTr).

***N*-[4-(2-Propynyloxy)butyl]trifluoroacetamide (3)**. Potassium hydroxide (100 g, 1.78 mol) was dissolved in 500 cm^3 of *tert*-butanol containing 144 g (1.6 mol) of 1,4-butanediol. The mixture was stirred vigorously under reflux while 153 g (0.55 mol) of 1,2,3-tribromopropane were added dropwise. The reaction mixture was stirred at 80°C for 36 hours, then allowed to cool down to ambient temperature. Precipitated KBr was filtered off and washed twice with *tert*-butanol. The washes were added to the filtrate, evaporated under reduced pressure at 60°C, and the resulting oil was cooled and filtered. Thionyl chloride (300 cm^3) was slowly added to the crude 4-hydroxybutyl propargyl ether. The reaction mixture was stirred for 18 hours at ambient temperature then refluxed for 6 hours and filtered. The filtrate was evaporated and distilled under reduced pressure. The crude 4-chlorobutyl propargyl ether (32.3 g) was collected at 70–74°C (13 torr). Trifluoroacetamide (11.3 g,

0.1 mol), 14.66 g (0.1 mol) of crude 4-chlorobutyl propargyl ether, and 32.6 g (0.1 mol) of cesium carbonate were mixed in 100 cm^3 of DMF and stirred for 6 days at ambient temperature. The reaction mixture was cooled to −12°C, and salts were filtered off, DMF was evaporated, and the residue was distilled under reduced pressure. The fraction boiling at 45°C (0.5 torr) was collected and redistilled. The yield of **3** was 7.44 g (13.2% from 1,2,3-tribromopropane), bp 95–97°C (0.5 torr). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.24 (1H, br. t, N*H*); 4.09 (2H, d, HC≡CC*H*$_2$, $J = 2.4$); 3.53 (2H, t, OC*H*$_2$CH$_2$CH$_2$CH$_2$, $J_t = 5.7$); 3.33 (2H, dt, OCH$_2$CH$_2$CH$_2$C*H*$_2$, $J_t = 6.0$, $J_d = 6.2$); 2.40 (1H, t, *H*C≡C, $J = 2.3$); 1.67–1.61 (4H, m, OCH$_2$C*H*$_2$C*H*$_2$CH$_2$). ^{13}C NMR (100.6 MHz, $CDCl_3$, δ, ppm): 157.15; 117.2; 79.2; 77.2; 69.3; 57.9; 39.49; 26.6; 25.67. Mass spectra were recorded on a DFS High Resolution GC-MS spectrometer. Calculated for $C_9H_{12}F_3NO_2$ m/z 223.0815. Found m/z 184.0582 ($C_6H_9F_3NO_2^+$). There is a fragment with m/z 39 in the spectrum, so we have assumed that it is a breakdown product of the molecular ion.

4-(2-Propynyloxy)butylamine (4). *N*-[4-(2-Propynyloxy)butyl]trifluoroa cetamide **3** (160 mg, 0.72 mmol) was dissolved in 1 cm^3 of 1 M NaOH and incubated for 2 hours at ambient temperature. The reaction mixture was evaporated to 0.2 cm^3 under reduced pressure, and the amine **4** was extracted with diethyl ether. After removal of ether in vacuo, the product was obtained with 58% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 4.06 (d, 2H, HC≡CC*H*$_2$, $J = 2.34$); 3.47 (t, 2H, OC*H*$_2$CH$_2$CH$_2$CH$_2$, $J = 6.1$); 2.65 (t, 2H, OCH$_2$CH$_2$CH$_2$C*H*$_2$, $J = 7.0$); 2.36 (t, 1H, *H*C≡C, $J = 2.4$); 1.60–1.50 (m, 2H, OCH$_2$CH$_2$C*H*$_2$CH$_2$); 1.48–1.40 (m, 2H, OCH$_2$C*H*$_2$CH$_2$CH$_2$). ^{13}C NMR (75.47 MHz, $CDCl_3$, δ, ppm): 79.67; 73.96; 69.7; 57.76; 41.6; 30.05; 26.59. MALDI-TOF: calculated for $C_7H_{13}NO$ $[M+H]^+$ 127.10, observed 128.10.

2-(*N*-Methyl-*N*-7-nitro-2,1,3-benzoxadiazol-4-ylamino)ethanol (5). A mixture of 0.99 g (5.0 mmol) 4-chloro-7-nitro-2,1,3-benzoxadiazole, 0.56 cm^3 (7.0 mmol) 2-methylaminoethanol in 10 cm^3 of EtOH was stirred at 20°C for 40 minutes. The orange precipitate was collected by filtration and dried to yield 0.92 g (78%) of **5**. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 8.51 (d, 1H, H6, $J = 9.0$); 6.49 (d, 1H, H5, $J = 9.0$); 4.50 (s, 2H, C*H*$_2$OH); 3.99 (t, 2H, CH$_2$C*H*$_2$N, $J = 6.0$); 3.50 (s, 3H, NMe). MS (Finnigan MAT 8200): calculated for $C_9H_{10}N_4O_4$ m/z 238, observed m/z 238.

***N*-2-Chloroethyl-*N*-methyl-7-nitro-2,1,3-benzoxadiazol-4-ylamine (6)**. A mixture of 0.5 g (2.1 mmol) of **5**, 0.51 cm^3 (4.0 mmol) benzenesulfonyl chloride, and 10 cm^3 pyridine was stirred at 90°C for 5 hours. After cooling, the mixture was poured into crushed ice–water. The orange precipitate was collected by filtration and dried to yield 0.42 g (82%) of **6**. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 8.51 (d, 1H, H6, $J = 9.0$); 6.49 (d, 1H, H5, $J = 9.0$); 4.50 (s, 2H, C*H*$_2$Cl); 3.99 (t, 2H, CH$_2$C*H*$_2$N, $J_1 = 6.00$, $J_2 = 6.00$); 3.50 (s, 3H, Me). MS (Finnigan MAT 8200): calculated for $C_9H_9ClN_4O_3$ m/z 256, observed m/z 256.

***N*-2-Azidoethyl-*N*-methyl-7-nitro-2,1,3-benzoxadiazol-4-ylamine (7)**. A mixture of 0.3 g (1.2 mmol) *N*-(2-chloroethyl)-*N*-methyl-7-nitro-2,1,3-benzoxadiazol-4-ylamine **6**, 0.13 g (2.0 mmol) sodium azide, and 5 cm^3 DMSO was stirred at 65°C for 8 hours. After cooling, the mixture was poured into crushed ice – water. Orange precipitate was collected by filtration and dried to yield 0.22 g (74%) of **7**. IR: N_3 2100 cm^{-1}. Elemental analysis (%): calculated for $C_9H_9N_7O_3$ C, 41.06; H, 3.42; N, 37.26; found C, 40.97; H, 3.30; N, 37.73. ^{1}H NMR (500, DMSO-d_6, δ, ppm): 8.51 (d, 1H, H6, $J = 9.0$); 6.48 (d, 1H, H5, $J = 9.0$); 4.34 (br.s, 2H, CH_2N$_3$); 3.77 (t, 2H, CH_2NMe, $J_1 = 6.00$, $J_2 = 6.00$); 3.48 (s, 3H, Me). MS (DFS High Resolution GC-MS): calculated for $C_9H_{10}N_7O_3$ m/z 263, observed m/z 263.

***N*-4-(2-Propynyloxy)butyl-*N*′-2-(4,4′-dimethoxytrityloxy)ethyl-*N*′-2-hydroxyethyl oxalamide (8)**. 4-(2-Propynyloxy)butylamine **4** (250 mg, 1.96 mmol) and DMAP (465 mg, 3.9 mmol) were dried by coevaporation with absolute pyridine (3 × 5 cm^3) and dissolved in abs. pyridine (20 cm^3). Compound **2** (1530 mg, 3.3 mmol) was added to the solution, and the reaction was stirred at ambient temperature overnight. Then the solution was evaporated to an oil, dissolved in 100 cm^3 CH_2Cl_2, organic layer washed with 0.3 M KH_2PO_4, pH 7 (3 × 100 cm^3), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by flash chromatography on a silica gel column eluted with CH_2Cl_2 + 1% Et_3N yielding **8** as a yellow foam (58%). TLC (CH_2Cl_2 – EtOH 96:4 v/v): R_f = 0.4. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.84–8.65 (13H, Ar); 4.08 (2H, m, HC≡CCH_2O); 3.73 (6H, s, OMe); 3.35–3.63 (8H, m, NCH_2CH_2OH + NCH_2CH$_2$ODMTr + HC≡CCH$_2$OCH_2); 3.05–3.17 (4H, m, CH_2NH + CH_2ODMTr); 2.07 (1H, s, HC≡C); 1.4–1.55 (4H, m, CH$_2$CH_2CH_2CH$_2$). ^{13}C NMR ((DMSO-d_6, δ, ppm): 25.52; 26.33; 38.01; 45.56; 48.22; 50.67; 55.01; 57.29; 58.22; 59.59; 61.03; 62.12; 68.74; 76.90; 80.49; 85.58; 85.75; 113.22; 126.66; 127.64; 127.87; 129.62; 135.59; 144.89; 158.05; 162.93; 163.41; 164.43; 164.94.

***N*-4-(2-Propynyloxy)butyl-*N*′-2-(4,4′-dimethoxytrityloxy)ethyl-*N*′-2-(2-cyanoethoxy-*N*,*N*-diisopropylaminophosphinyloxy)ethyl]oxalamide (9).** 1*H*-Tetrazole (35 mg, 1.2 mmol) and DIPEA (0.54 cm^3, 3.1 mmol) were dissolved in anhydrous MeCN (2.5 cm^3), 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (0.44 cm^3, 1.3 mmol) was added dropwise, and the mixture was stirred for 30 minutes at ambient temperature. Compound **8** (409 mg, 0.52 mmol) was dried by coevaporation with anhydrous MeCN (3 × 5 cm^3), dissolved in anhydrous MeCN (2.5 cm^3), and added to the solution of 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite. The mixture was stirred at ambient temperature for 2 hours, then diluted with CH_2Cl_2 (100 cm^3), organic layer washed with 0.3 M KH_2PO_4, pH 7 (3 × 100 cm^3), dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. Crude product was purified by flash chromatography on silica gel column eluted with toluene + 1% Et_3N yielding **9** as a yellow foam

(59%). TLC (toluene – EtOAc 75:25 v/v): R_f = 0.8. ^{1}H NMR (300 MHz, acetone-d_6, δ, ppm): 6.85–7.91 (13H, Ar); 4.12 (2H, m, HC≡CCH_2O); 3.79 (6H, s, OMe); 3.46–3.77 (8H, m, NCH_2CH_2OP + NCH_2CH$_2$ODMTr + HC≡CCH$_2$OCH_2 + CH_2CH$_2$CN + (CH$_3$)$_2$CH); 3.23–3.35 (4H, m, CH_2NH + CH_2ODMTr); 2.71–2.93 (2H, m, CH_2CN); 2.31 (1H, s, HC≡C); 1.54–1.69 (4H, m, CH$_2$CH_2CH_2CH$_2$); 1.16–1.24 (12H, m, (CH_3)$_2$CH). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 19.37; 23.55; 23.62; 25.51; 26.27; 38.14; 42.25; 42.51; 46.93; 47.93; 54.18; 57.02; 57.87; 58.33; 60.01; 61.00; 61.85; 62.07; 62.39; 68.64; 74.24; 85.66; 112.58; 117.63; 126.17; 126.21; 127.28; 127.62; 129.51; 135.55; 144.77; 144.86; 158.23; 161.41; 161.70; 162.98; 163.15. ^{31}P NMR (300 MHz, acetone-d_6, δ, ppm): 148.67, 148.72.

Click labeling. Copper(I)-catalyzed alkyne-azide [3+2]cycloaddition was carried out in accordance with published protocol.[17] To 25 μL of 6.7 mM oligonucleotide in water, 15 μL of azide **7** (11.5 mM, 1 equiv) in DMSO was added followed by 10 μL aq $CuSO_4$ (840 mM, 50 equiv), 4.4 mg TBTA (50 equiv), 10 μL aq ascorbic acid (840 mM, 50 equiv) and 20 μL of 0.2 M triethylammonium acetate, pH 7. After incubation overnight oligonucleotide material was precipitated by 2% $LiClO_4$ in acetone, and the product was isolated by RP-HPLC (Fig. 1).

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