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Cross-Linked DNA: Propargylated Ribonucleosides as "Click" Ligation Sites for Bifunctional Azides

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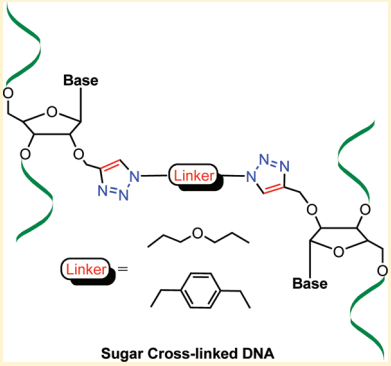
Supporting Information

ABSTRACT: 2′-*O* or 3′-*O*-propargylated adenosines and ribothymidines were used as click targets for cross-linking of oligonucleotides with aliphatic and aromatic azides. The cross-link generates a sugar modification at the 2′-*O*-ligation site. Inexpensive ribonucleosides were used as starting materials. Cross-linking of oligonucleotides was performed at internal or terminal positions. Hybridization of homodimers with two complementary single strands resulted in stable ligated DNA duplexes.



Formation of cross-links in DNA can arrest cell replication, thereby causing the death of the cell, a property that is extensively exploited in cancer chemotherapy and genetic engineering.[1] For that purpose, a wide spectrum of cross-linking agents has been reported in literature, among them are cis-platin, nitrogen mustards, and natural compounds such as psoralenes, nitrous acid, etc.[1,2] Out of various techniques for cross-linking,[3] the Cu(I)-catalyzed azide−alkyne cycloaddition "click chemistry" (CuAAC) can offer exciting prospects because of its simplicity and bio-orthogonal nature.[4]

To widen the scope of this application to chemistry, chemical biology, and nanotechnology, we recently reported on the "bis-click" technology to cross-link DNA, which is site- and template-independent.[5,6] In all of these cases base modifications were employed. Modification of the sugar moiety in nucleic acids appears to be a highly enterprise work since the hydroxyl groups of the sugar can be modified seemingly to any kind of functionalities. We envisioned the ploy to modify the sugar as a substrate unit to "bis-click" chemistry. Nucleobases,[5−7] sugar moieties,[8] and phosphate backbones[9] have been modified to introduce cross-links by various methods but to the best of our knowledge not by "click" cross-linking with bifunctional azides on the sugar moiety. This manuscript reports on the viability of the "bis-click" chemistry on the sugar moiety (Figure 1) and the effect of cross-linking on duplex stability. For that, 2′/3′-*O*-propargylated nucleosides **1**−**4** were prepared, employed in solid-phase oligonucleotide synthesis, and used for cross-linking. DNA ligation was performed using the bis-azides **5** and **6** (Figure 2).

The 2′- or 3′-*O*-propargylated adenine and ribothymidine nucleosides were synthesized.[10] The bis-azides **5** and **6**, one being aliphatic and the other being aromatic, were prepared as cross-linkers.[11] As model compounds for the "bis-click" reaction at the sugar moiety, "simple" nucleosides were used. Consequently, the reaction was performed with the 2′-*O*-propargylated derivatives, namely, nucleoside **1** was reacted with bis-azide **6** and nucleoside **3** with bis-azide **5** (Scheme 1). The obtained cross-linked nucleosides **7** (48%) and **8** (45%) were characterized. For ^{1}H NMR, ^{13}C NMR, DEPT-135, and ^{1}H−^{13}C gated-decoupled NMR spectra, as well as mass spectra, see Experimental Section and Supporting Information.

The bis-headed nucleosides **7** and **8** show relationship to other bis-headed nucleosides synthesized in our laboratory.[5,6,8f] They are good models for the study of intramolecular base stacking, which comes by virtue of their flexibility. Moreover, as these molecules contain two nucleoside functionalities, they can act as bis-substrates or bis-inhibitors of nucleoside converting enzymes, which find applications in drug discovery and medicinal chemistry.[12] To convert the bis-headed adenosine derivative **8** into inosine derivative **9**, adenosine deaminase (ADA) was used (Scheme 1).[13] The reaction was followed UV spectroscopically (Figure S1, Supporting Information). The inosine derivative **9** accessible in preparative scale was obtained in 75% yield. We anticipate that related bis-headed adenosines can be converted to the corresponding inosine derivatives as well.

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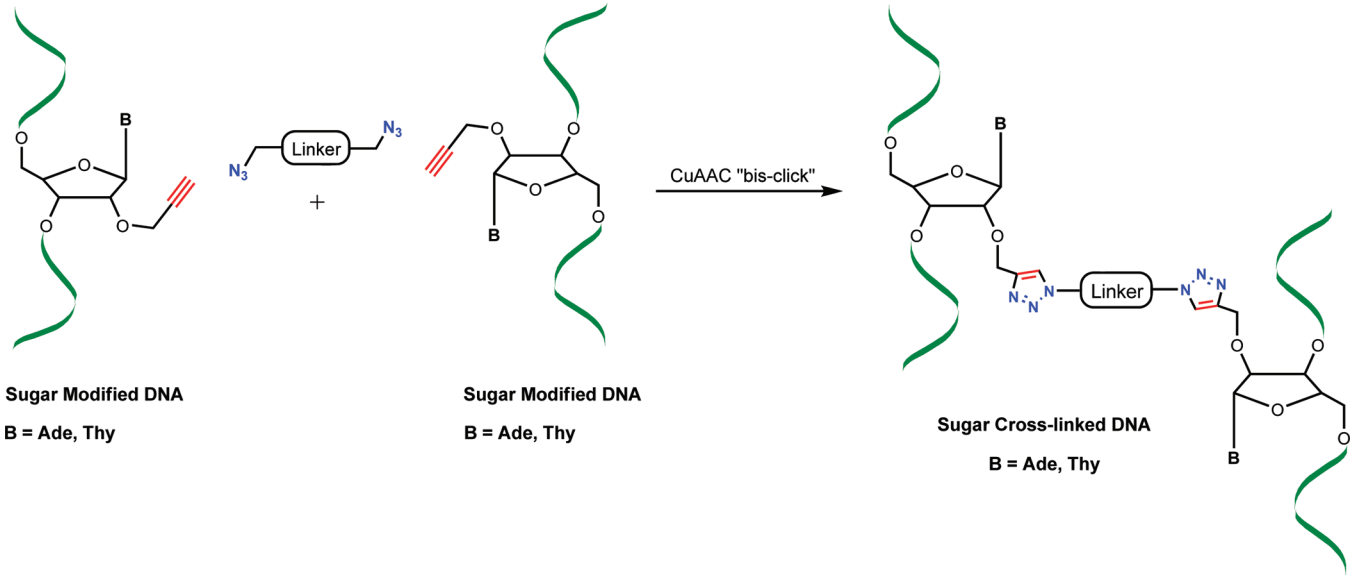

Figure 1. Schematic presentation of "bis-click" reaction on sugar modified DNA.

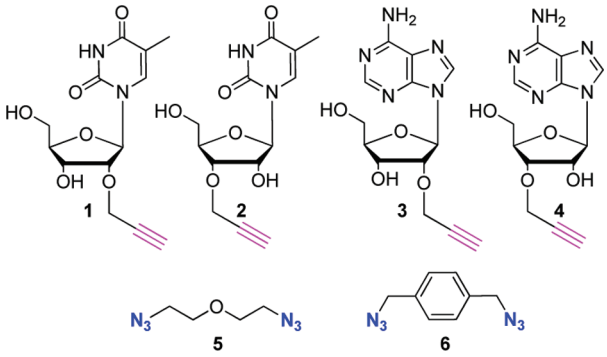

Figure 2. Alkynylated nucleosides and bis-azides used for "bis-click" cross-linking.

To investigate the performance of the reaction on oligonucleotides, the "bis-click" reaction was performed on single-stranded oligonucleotides (ODNs). For this, the phosphoramidites of nucleosides **1**–**3** were synthesized.[10] Then, a series of oligonucleotides containing the propargylated nucleoside residues **1**, **2**, and **3** at various positions were prepared on solid phase using the standard protocol of phosphoramidite chemistry. The synthesized oligonucleotides were characterized by MALDI-TOF mass spectra (experimental part) and HPLC (Supporting Information).

With 2′- and 3′-*O*-propargylated oligonucleotides in hand, the "bis-click" reaction was performed. For this, 5.0 A_{260} units of ODN **10** dissolved in water was treated with Cu-TBTA, TCEP, and $NaHCO_3$. After that the oligonucleotides were clicked together by adding the corresponding bis-azide **5** or **6**. Accordingly, a series of interstrand cross-linked oligonucleotides (ICLs) were prepared (Table 1 and Supporting Information, Figure S2). The "bis-click" reaction on ODNs is outlined in Schemes 2 and 3. The composition of cross-linked oligonucleotides was proven by MALDI-TOF spectra (Table 1) and the purity by denaturing gel electrophoresis (PAGE) (for details see Experimental Section).

Gel electrophoresis demonstrated that the cross-linked oligonucleotides as a result of their increased number of negative charges migrate significantly slower than the starting materials (24-mer cross-linked oligonucleotides **21**, **23** and **26**, **27** compared to the 12-mer propargylated oligonucleotides **29** and **32**). Figure 3 shows the PAGE results of alkynylated and cross-linked oligonucleotides.

Cross-linked oligonucleotides find applications in the construction of nanostructures by which DNA machines such as DNA tweezers, walker, and stepper are formed.[14] Earlier work from Endo et al. demonstrated the potential of sugar-phosphate backbone cross-linked DNA.[15] In their studies, the cross-link uses the phosphodiester moiety as target site that was converted in a phosphotriester by the cross-linking reagent. However, phosphotriester cross-links make the backbone of DNA labile against hydrolysis, remove negative charges, and create chirality. Our cross-linked DNA is at least as stable as canonical DNA, and introduction of the cross-link at the 2′-*O*-position of the sugar moiety usually increases resistance of the molecule against hydrolysis by nucleases.

Scheme 1. Cu(I)-Catalyzed "Bis-Click" Reaction to Cross-Link 1 and 3 with Bis-azide 5 and 6 and Deamination of 8

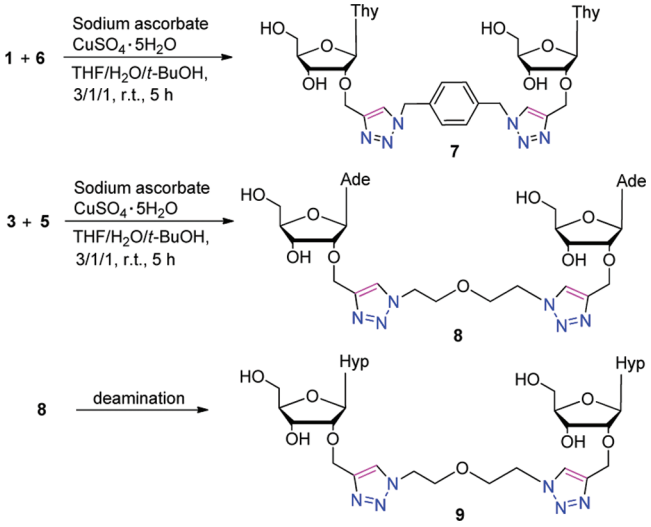

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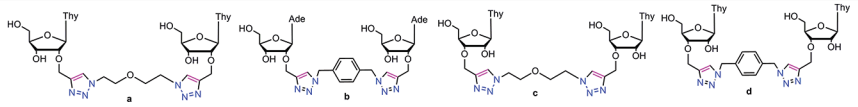
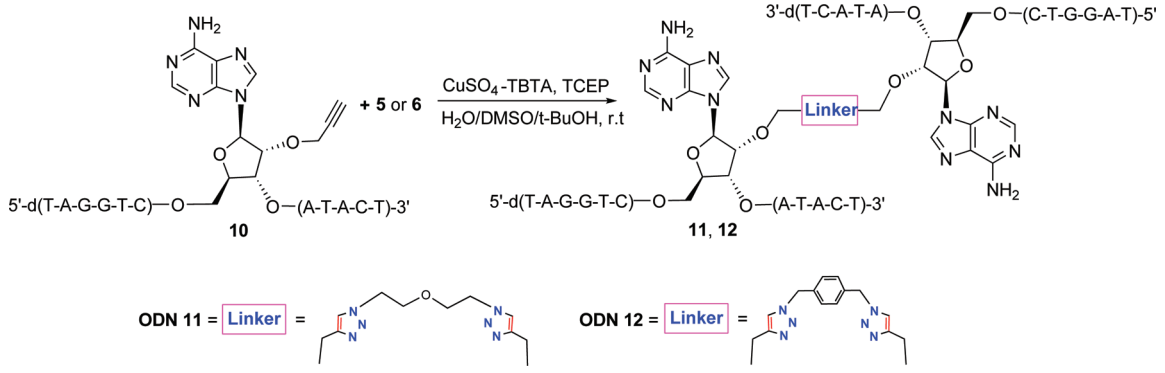
Table 1. T_m Values and Mass Spectrometric Data of Cross-Linked Oligonucleotides[a,b]

Cross-linked ODNs	M.W. calcd. M.W. found	T_m[c] [°C]	Cross-linked ODNs	M.W. calcd. M.W. found	T_m[c] [°C]
5'-d(TAG GTC AA**T** ACT) **18** 5'-d(TAG GTC AA**T** ACT)	7553 7552	(**17•18•17**) 42.0	5'-d(TAG GTC AA**T*** ACT) **19** 5'-d(TAG GTC AA**T*** ACT)	7585 7584	(**17•19•17**) 41.5
5'-d(**T**AG GTC AAT ACT) **20** 5'-d(**T**AG GTC AAT ACT)	7553 7554	(**17•20•17**) 50.0	5'-d(**T***AG GTC AAT ACT) **21** 5'-d(**T***AG GTC AAT ACT)	7585 7585	(**17•21•17**) 50.0
3'-d(AT**C** CAG TTA TGA) **22** 3'-d(AT**C** CAG TTA TGA)	7553 7552	(**16•22•16**) 51.0	3'-d(AT***C** CAG TTA TGA) **23** 3'-d(AT***C** CAG TTA TGA)	7585 7584	(**16•23•16**) 51.0
5'-d(TAG GTC **A**AT ACT) **11** 5'-d(TAG GTC **A**AT ACT)	7553 7557	(**17•11•17**) 45.0	5'-d(TAG GTC **A***AT ACT) **12** 5'-d(TAG GTC **A***AT ACT)	7585 7588	(**17•12•17**) 42.5
5'-d(T**A**G GTC AAT ACT) **24** 5'-d(T**A**G GTC AAT ACT)	7553 7545	(**17•24•17**) 47.0	5'-d(T**A***G GTC AAT ACT) **25** 5'-d(T**A***G GTC AAT ACT)	7585 7570	(**17•25•17**) 47.0
3'-d(ATC C**A**G TTA TGA) **26** 3'-d(ATC C**A**G TTA TGA)	7553 7555	(**16•26•16**) 38.5	3'-d(ATC C**A***G TTA TGA) **27** 3'-d(ATC C**A***G TTA TGA)	7585 7585	(**16•27•16**) 38.5
5'-d(TAG GTC AA**T**# ACT) **14** 5'-d(TAG GTC AA**T**# ACT)	7553 7559	(**17•14•17**) 43.5	5'-d(TAG GTC AA**T**§ ACT) **15** 5'-d(TAG GTC AA**T**§ ACT)	7585 7591	(**17•15•17**) 42.0



[a] **T-T** corresponds to **a**, **A-A** corresponds to **8**, **T*-T*** corresponds to **7**, **A*-A*** corresponds to **b**, **T#-T#** corresponds to **c**, and **T§-T§** corresponds to **d**. **16** corresponds to 5′-d(TAGGTCAATACT), and **17** corresponds to 3′-d(ATCCAGTTATGA). [b] The structures of all cross-linked oligonucleotides are shown in the Supporting Information, Figure S2. [c] Measured at 260 nm in a 1 M NaCl solution containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 2.5 μM single-strand concentration.

Scheme 2. CuAAC "Bis-Click" Cross-Linking Reaction of 2′-*O*-Propargylated ODN 10 with Bis-azides 5 and 6



Scheme 3. CuAAC "Bis-Click" Cross-Linking Reaction of 3′-*O*-Propargylated ODN 13 with Bis-azides 5 and 6

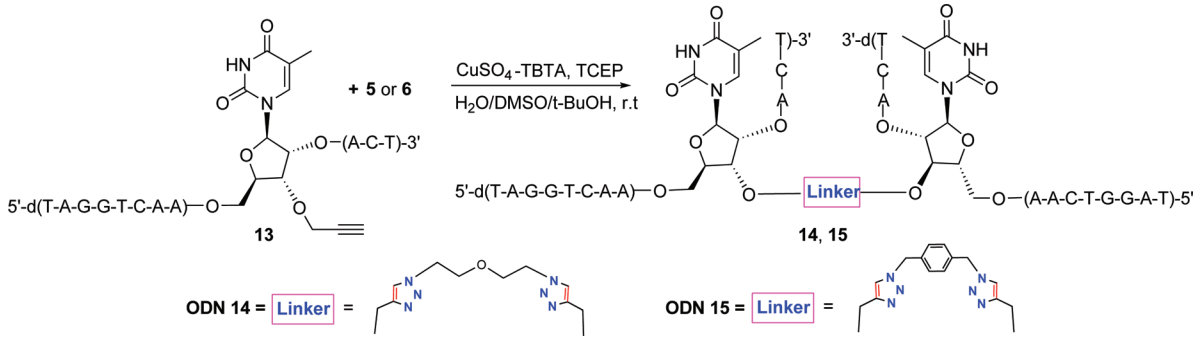


Table 1 shows that the cross-linked homodimers hybridize well with the complementary strands 5′-d(TAG GTC AAT ACT) (ODN **16**) and 3′-d(ATC CAG TTA TGA) (ODN **17**) to form double helices cross-linked by sugar linkages. The introduction at the 2′-*O*-position keeps the duplex DNA intact and has only a minor effect on duplex stability. This

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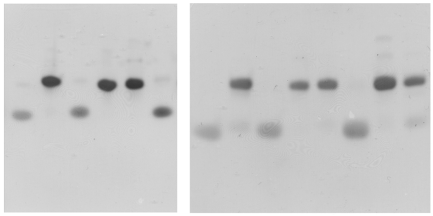

Figure 3. Denaturing polyacrylamide gel electrophoresis of alkynylated and cross-linked oligonucleotides.

phenomenon is similar to helices ligated by their side chain at the nucleobases.[16] At the center of the duplex, cross-linking is slightly destabilizing, while for cross-linking at the termini of the helices stabilization was observed. So, cross-links introduced into the sugar moiety do not perturb the helix structure. When the sequential propargylation and cross-linking was performed at the 3′-*O*-position, the resulting 2′-5′-oligonucleotides (**17·14·17**, **17·15·17**) are much more destabilized compared to corresponding 3′-5′ connected counterparts (Table 1), which results from the unusual phosphodiester linkage.

In conclusion, our "bis-click" protocol is efficient and proceeds almost quantitatively with no side product formation when identical oligonucleotide strands are used. In analogy to nucleobase cross-linking,[5,6] the method has the potential to ligate nonidentical strands in a stepwise way. Our methodology is applicable to nucleosides and oligonucleotides bearing propargyl residues at the sugar moiety. The cross-link can be introduced at any position of an oligonucleotide chain. A diversity of bis-azides can be applied to cross-link oligonucleotides. This protocol has the potential to construct oligonucleotide−peptide, −carbohydrate, or −lipid conjugates by a stepwise procedure. The method is applicable for the construction of larger assemblies, which are accessible by a combination of covalent cross-linking and noncovalent assembly by hydrogen bonding.

EXPERIMENTAL SECTION

General Methods and Materials. All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40−60 μM) at 0.4 bar. Adenosine deaminase (EC 3.5.4.4), Type V from bovine spleen. Solution in 50% glycerol, 50 mM potassium phosphate, pH 6.0, 160 units per mL. The adenosine deaminase was diluted with 0.06 M Sørensen buffer (pH 7.0). A 40-fold diluted solution (0.004 units per μL) was used. The substrate was dissolved in the same buffer after the deaminase (1 μL, 0.004 units) was added. The UV absorbance was detected and recorded at certain wavelengths where the UV spectra have the maximal changes. UV spectra: λ_{max} in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. NMR spectra: measured at 300.15 MHz for ^1H, 75.48 MHz for ^{13}C, and 121.52 MHz for ^{31}P. The *J* values are given in Hz; δ values in ppm relative to Me_4Si as internal standard. For NMR spectra recorded in DMSO, the chemical shift of the solvent peak was set to 2.50 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR.

The syntheses of oligonucleotides were performed on a DNA synthesizer at a 1 μmol scale (trityl-on mode) using the phosphoramidites **S4**−**S6** and the standard phosphoramidite building blocks following the synthesis protocol for 3′-*O*-(2-cyanoethyl)-phosphoramidites. After cleavage from the solid support, the oligonucleotides were deprotected in 25% aqueous ammonia solution for 12−16 h at 60 °C. The purification of the "trityl-on" oligonucleotides was carried out on reversed-phase HPLC (RP-18 column; gradient system I). The purified "trityl-on" oligonucleotides were treated with 2.5% $Cl_2CHCOOH/CH_2Cl_2$ for 5 min at 0 °C to remove the dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC (gradient II). The oligomers were desalted on a short column using distilled water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). Then, the solvent was evaporated using a SpeedVac evaporator to yield colorless solids that were frozen at −24 °C. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear negative mode (experimental section).

Reversed-phase HPLC was carried out on a 250 mm × 4 mm RP-18 LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller, and an integrator. Gradients used for HPLC chromatography: A = MeCN; B = 0.1 M $(Et_3NH)OAc$ (pH 7.0)/ MeCN, 95:5. Conditions: (I) 3 min 15% A in B, 12 min 15−50% A in B, and 5 min 50−10% A in B, flow rate 0.8 mL min^{-1}; (II) 0−25 min 0−20% A in B, flow rate 0.8 mL min^{-1}; (III) 0−15 min 0−20% A in B, 15−18 min 20−40% A in B, flow rate 0.8 mL min^{-1}.

Denaturing polyacrylamide gel electrophoresis (PAGE) was performed on oligonucleotides. PAGE analysis was carried out on a 20% polyacrylamide gel (acrylamide/bisacrylamide 19:1 with 7 M urea; 10 × 10 cm in 0.1 M tris-borate-EDTA (TBE) buffer containing 20 mM $MgCl_2$, pH 8.4). A 30 min prerun was performed in TBE buffer at rt. From a stock solution of 40 A_{260} units in 200 μL, a portion of 2 μL was added to 5 μL of distilled water. Then, 10 μL of gel loading buffer (TBE) containing formamide (1:1) was added, and the oligonucleotide solution was loaded onto the gel. Electrophoresis was performed at rt for 3.5 h at a constant field strength of 12 V/cm. The gel was stained with 0.02% methylene blue for 20 min and was then incubated in water for 1 h to remove excess dye; the electropherograms were then scanned.

Melting curves were measured with a UV−vis spectrophotometer equipped with a thermoelectrical controller with a heating rate of 1 °C/min. ESI-TOF mass spectra of the nucleosides were measured with a Micro-TOF spectrometer. Molecular masses of oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear negative mode with 3-hydroxypicolinic acid (3-HPA) as a matrix.

The formed duplexes with their respective T_m values and the MALDI-TOF analysis of the corresponding modified single strands are as follows: 5′-d(TAG GTC AA**1** ACT) (**28**), MW calcd 3696, MW found 3697, 47.5 °C for duplex **28·17**; 5′-d(**1**AG GTC AAT ACT) (**29**), MW calcd 3696, MW found 3699, 50.0 °C for duplex **29·17**; 3′-d(A**1**C CAG TTA TGA) (**30**), MW calcd 3696, MW found 3697, 50.0 °C for duplex **16·30**; 5′-d(T**3**G GTC AAT ACT) (**31**), MW calcd 3696, MW found 3697, 48.5 °C for duplex **17·31**; 5′-d(TAG GTC **3**AT ACT) (**10**), MW calcd 3696, MW found 3697, 48.0 °C for duplex **10·17**; 3′-d(ATC C**3**G TTA TGA) (**32**), MW calcd 3696, MW found 3700, 46.5 °C for duplex **16·32**; 5′-d(TAG GTC AA**2** ACT) (**13**), MW calcd 3696, MW found 3697, 45.0 °C for duplex **13·17**. The T_m values were measured at 260 nm in a 1 M NaCl solution containing 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0) with 2.5 μM single-strand concentration.

Bis-ribothymidine Click Adduct 7. To a solution of compound **1** (70 mg, 0.23 mmol) and **6** (22 mg, 0.12 mmol) in THF/H_2O/*t*-BuOH (3:1:1, 3 mL) was added a freshly prepared 1 M solution of sodium ascorbate (232 μL, 0.23 mmol) in water, followed by the addition of copper(II) sulfate pentahydrate 7.5% in water (201 μL, 0.06 mmol), and the reaction mixture was stirred at room temperature for 5 h. After completion of the reaction (monitored by TLC), the reaction mixture was evaporated, and the residue was applied to flash chromatography (FC) (silica gel, CH_2Cl_2/MeOH, 85:15). From the main zone compound **7** (45 mg, 48%) was isolated as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 80:20): R_f 0.3. λ_{max} (MeOH)/nm 260 (ε/$dm^3\ mol^{-1}\ cm^{-1}$ 19 000), 266 (20 200). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.73 (s, 6H, 2 × CH_3), 3.43−3.66 (m, 4H, 2 × C5′-H), 3.84 (d, *J* = 3.6 Hz, 2H, 2 × C4′-H), 4.04 (t, *J* = 5.1 Hz, 2H, 2 × C3′-H), 4.14−4.15 (m, 2H, 2 × C2′-H), 4.58−4.69 (m, 4H, 2 × CH_2), 5.18 (br s, 4H, 2 × C3′-OH, 2 × C5′-OH), 5.54 (s, 4H, 2 × CH_2), 5.86 (d, *J* = 5.1 Hz, 2H, 2 × C1′-H), 7.28 (s, 4H, phenyl-H), 7.72 (s, 2H, 2 × triazole-H), 8.10 (s, 2H, 2 × C6-H), 11.32 (s, 2H, 2 × NH). ^{13}C

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NMR (DMSO-d_6, 75.4 MHz): δ 163.7 (C4), 150.6 (C2), 144.1 (triazole), 128.4 (C6), 124.2 (triazole), 109.3 (C5), 85.9 (C1′), 85.0 (C4′), 80.4 (C3′), 68.3 (C2′), 62.8 (C5′), 60.6 (CH_2), 52.4 (CH_2). $^1H-^{13}C$ Coupling constants in Hz (DMSO-d_6, 75.4 MHz): 161.1 [^1J (C6, H-C6)], 8.0 [^3J (C2, H-C6)], 5.2 [^2J (C5, H-C6)], 9.6 [^3J (C4, H-C6)], 196.2 [^1J (triazole, H-C5)], 9.6 [^2J (triazole C4, H-C5)], 168.8 [^1J (C1′, H-C1′)], 143.7 [^1J (C2′, H-C2′)], 148.7 [^1J (C3′, H-C3′)], 146.6 [^1J (C4′, H-C4′)], 140.8 [^1J (C5′, H-C5′)]. ESI-TOF calcd for $C_{34}H_{40}N_{10}O_{12}Na$ (M + Na^+) 803.2725; m/z found 803.2719.

Bis-adenosine Click Adduct 8. To a solution of compound **3** (150 mg, 0.49 mmol) and **5** (38 mg, 0.24 mmol) in THF/H_2O/*t*-BuOH (3:1:1, 3 mL) was added a freshly prepared 1 M solution of sodium ascorbate (484 μL, 0.5 mmol) in water, followed by the addition of copper(II) sulfate pentahydrate 7.5% in water (419 μL, 0.12 mmol), and the reaction mixture was stirred at room temperature for 5 h. After completion of the reaction (monitored by TLC), the reaction mixture was evaporated, and the residue was applied to flash chromatography (FC) (silica gel, CH_2Cl_2/MeOH, 80:20). From the main zone compound **8** (85 mg, 45%) was isolated as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 60:40): R_f 0.3. λ_{max} (MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 19 100). ^1H NMR (DMSO-d_6, 300 MHz): δ 3.56 (br s, 2H, 2 × C5′-H), 3.66−3.71 (m, 6H, 2 × CH_2, 2 × C5′-H), 4.00−4.01 (m, 2H, 2 × C4′-H), 4.33−4.38 (m, 6H, 2 × CH_2, 2 × C3′-H), 4.50−4.54 (m, 2H, 2 × C2′-H), 4.60−4.69 (m, 4H, 2 × CH_2), 5.37 (br s, 2H, 2 × C5′-OH), 5.46 (br s, 2H, 2 × C3′-OH), 6.00 (d, J = 6.0 Hz, 2H, 2 × C1′-H), 7.34 (br s, 4H, 2 × NH_2), 7.77 (s, 2H, 2 × triazole), 8.12 (s, 2H, 2 × C8-H), 8.29 (s, 2H, 2 × C2-H). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 156.1 (C6), 152.5 (C2), 148.9 (C4), 143.4 (triazole), 139.7 (C8), 124.2 (triazole), 119.2 (C5), 86.4 (C4′), 86.0 (C1′), 80.3 (C3′), 69.0 (C2′), 68.4 (CH_2), 62.8 (C5′), 61.5 (CH_2), 49.1 (CH_2). $^1H-^{13}C$ Coupling constants in Hz (DMSO-d_6, 75.4 MHz): 11.3 [^3J (C6, H-C2)], 199.2 [^1J (C2, H-C2)], 216.5 [^1J (C8, H-C8)], 11.4 [^3J (C5, H-C8)], 11.5 [^3J (C4, H-C2)], 196.4 [^1J (triazole, H-C5)], 9.4 [^2J (triazole C4, H-C5)], 165.2 [^1J (C1′, H-C1′)], 144.3 [^1J (C2′, H-C2′)], 149.5 [^1J (C3′, H-C3′)], 148.5 [^1J (C4′, H-C4′)], 141.9 [^1J (C5′, H-C5′)]. ESI-TOF calcd for $C_{30}H_{38}N_{16}O_9Na$ (M + Na^+) 789.2906; m/z found 789.2900.

Bis-inosine Click Adduct 9. Compound **8** (20 mg, 0.02 mmol) was dissolved in Sørensen buffer (pH 7.0, 2 mL). To this solution was added the enzyme adenosine deaminase (EC 3.5.4.4), type V from bovine spleen, solution in 50% glycerol, 50 mM potassium phosphate, pH 6.0, 160 units per mL, 40-fold diluted (0.004 units per μL) with the same buffer (3 μL). The reaction was monitored by UV (for time scan see Figure S1, Supporting Information). After completion the reaction mixture was evaporated to dryness, and the residue was dissolved in a mixture of MeOH and CH_2Cl_2 (v/v; 3:7). The supernatant liquid obtained after centrifuging was applied to preparative TLC on silica coated TLC plates. The solution was divided into two parts, and each part was adsorbed in two different TLC plates (20 cm × 10 cm) and run for separation. After separation on TLC, the band with the compound was scratched, and the silica was washed with a mixture of CH_2Cl_2/MeOH, (70:30) and dried under reduced pressure to obtain **9** as colorless solid (15 mg, 75%). TLC (silica gel, CH_2Cl_2/MeOH, 70:30): R_f 0.25. ^1H NMR (DMSO-d_6, 300 MHz): δ 3.48−3.57 (m, 2H, 2 × C5′-H), 3.58−3.74 (m, 6H, 2 × CH_2, 2 × C5′-H), 3.96−3.99 (m, 2H, 2 × C4′-H), 4.35 (t, J = 3.3 Hz, 2H, 2 × C3′-H), 4.39−4.42 (m, 4H, J = 4.8 Hz, 2 × CH_2), 4.50−4.57 (m, 4H, 2 × CH_2), 4.66−4.69 (m, 2H, CH_2), 5.35 (br s, 2H, 2 × C5′-OH), 5.98 (d, J = 6.0 Hz, 2H, 2 × C1′-H), 7.81 (s, 2H, 2 × triazole), 8.05 (s, 2H, 2 × C8-H), 8.26 (s, 2H, 2 × C2-H). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ 156.7 (C6), 148.0 (C4), 146.1 (C2), 143.4 (triazole), 138.5 (C8), 124.4 (C5), 124.3 (triazole), 86.2 (C4′), 85.6 (C1′), 80.8 (C3′), 68.8 (C2′), 68.4 (CH_2), 62.8 (C5′), 61.1 (CH_2), 49.0 (CH_2). $^1H-^{13}C$ Coupling constants in Hz (DMSO-d_6, 75.4 MHz): 6.9 [^3J (C6, H-C2)], 205.8 [^1J (C2, H-C2)], 215.2 [^1J (C8, H-C8)], 11.3 [^3J (C5, H-C8)], 14.4 [^3J (C4, H-C2)], 196.5 [^1J (triazole, H-C5)], 9.9 [^2J (triazole C4, H-C5)], 167.1 [^1J (C1′, H-C1′)], 147.9 [^1J (C2′, H-C2′)], 147.6 [^1J (C3′, H-C3′)], 148.4 [^1J (C4′, H-C4′)], 143.0 [^1J (C5′, H-C5′)]. ESI-TOF calcd for $C_{30}H_{38}N_{16}O_9Na$ (M + Na^+) 791.2586; m/z found 791.2580.

General Procedure for Cross-Linking Oligonucleotides. To the solution of oligonucleotide **10** (5.0 A_{260} units, 50 μmol) in 20 μL of water were added a mixture of a $CuSO_4$−TBTA ligand complex (50 μL of 20 mmol solution of $CuSO_4$ in H_2O/*t*-BuOH/DMSO (1:1:3) and 50 μL of 20 mmol solution of TBTA in *t*-BuOH/DMSO (1:3), tris(carboxyethyl)phosphine (TCEP; 50 μL of 20 mmol solution in H_2O), sodium bicarbonate ($NaHCO_3$, 50 μL of 200 mmol solution in H_2O), and the bis-azide **5** or **6** (2.5 μL of 20 mmol respective azide stock solution in THF/H_2O (1:1) for azide **5** and in THF for azide **6**) and 30 μL of DMSO, and then the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated in a Speed Vac, dissolved in 0.3 mL of bidistilled water, and centrifuged for 20 min at 14 000 rpm. The supernatant was collected and further purified by reversed-phase HPLC, using the following gradient: 0−15 min 0−20% B in A, 15−18 min 20−40% B in A, 18−25 min 40−0% B in A, flow rate 0.8 mL min^{-1}; A: 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 95:5; B: MeCN) to give the corresponding cross-linked oligonucleotide **11** or **12** (2.5 A_{260} units, 50%). The other cross-linked oligonucleotides were prepared accordingly in 50−60% yields.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of phosphoramidites, HPLC purification profiles of oligonucleotides, structures of oligonucleotides, ^1H and ^{13}C NMR, DEPT-135, and $^1H-^{13}C$ gated decoupled spectra of the nucleoside derivatives and click conjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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