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Bis- and Tris-Alkyne Phosphoramidites for Multiple 5′-Labeling of Oligonucleotides by Click Chemistry

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Three new phosphoramidites exhibiting two or three alkyne functions were prepared and introduced at the 5′ end of oligonucleotides. The resulting bis- and tris-alkyne oligonucleotides were conjugated with acetylthiohexyl, ferrocenecarboamide hexyl, or carbohydrate-propyl azides by using click chemistry (CuAAC) to afford polyconjugated oligonucleotides. Conjugations were performed either in solution or on solid support with high efficiency.

Introduction

Oligonucleotide conjugates are widely used for various applications in biology, biotechnology, and medicine. Several applications require multiple labeling of oligonucleotides. This is the case of multiple redox tags for enhanced signals by electrochemistry,[1–3] of the introduction of several carbohydrates for cluster effects,[4–7] or several sulfur atoms for immobilization on a gold surface.[8,9] To address this point, the use of a "trebler" phosphoramidite for the introduction of three new alcohol functions for subsequent couplings has been reported, and this method allows oligonucleotide dendrimers,[10] glycoconjugates,[11] or multiple thiol anchor conjugates[8] to be synthesized. As an alternative, we present herein the synthesis of three new phosphoramidites containing two or three alkyne functions (Figure 1). Indeed, the azide alkyne 1,3-dipolar cycloaddition catalyzed by copper(I) (CuAAC)[12,13] is a very efficient and popular method used to label biomolecules, especially oligonucleotides.[14] Although many kinds of alkyne phosphoramidites have been reported in the literature, only a few of them contain multiple alkyne functions.[15–19] In contrast, numerous alkyne phosphoramidites can be incorporated several times, but in these cases, the labels could not be close to each other.[14,20] Finally, our strategy allows the multiple 5′-end labeling either on solid support or in solution; hence, it is possible to introduce base-sensitive molecules.

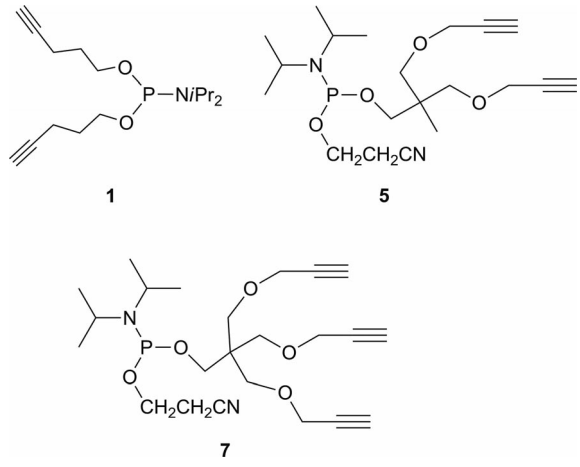
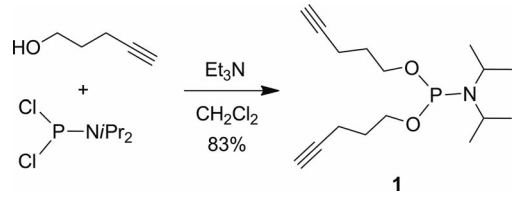


Figure 1. Structure of bis-alkyne phosphoramidites **1** and **5** and tris-alkyne phosphoramidite **7**.

Results and Discussion

Phosphoramidites **1** and **5** both contain two alkyne functions and phosphoramidite **7** contains three alkyne functions (Figure 1). Compound **1** was prepared in one step by reaction of diisopropylphosphoramidous dichloride with pent-4-yn-1-ol (2 equiv.) in the presence of triethylamine. It was isolated in 83% yield after purification by flash chromatography (Scheme 1).



Scheme 1. Synthesis of bis-pent-4-ynyl phosphoramidite **1**.

Bis-alkyne derivative **5** was synthesized by bis-alkylation of 1-(4,4′-dimethoxytrityloxy)-2,2-bis(hydroxymethyl)propane (**2**)[21] with propargyl bromide in the presence of so-

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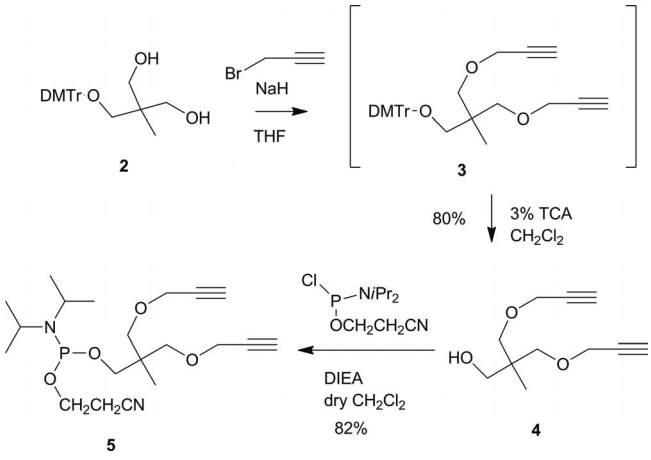
dium hydride in THF to afford **3**, which was directly engaged in the next step (Scheme 2). The DMTr group was removed by treatment with a solution of 3% trichloroacetic acid in dichloromethane to give **4**, which was purified by silica gel flash chromatography (80%, two steps). Finally, pure compound **4** was phosphitylated by using diisopropyl cyanoethyl chlorophosphoramidite in the presence of diisopropylethylamine in dry dichloromethane to afford expected phosphoramidite **5** bearing two alkyne functions in 82% yield (66% overall yield).



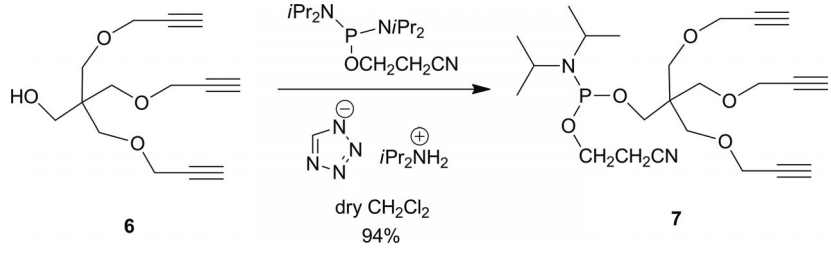
Scheme 2. Synthesis of bis-alkyne phosphoramidite **5**.

The preparation of tris-alkyne phosphoramidite **7**[22] proceeded similarly with the use of tris-propargyl pentaerythritol derivative **6** prepared from pentaerythritol according to the literature[23] and 2-cyanoethyl tetraisopropylphosphorodiamidite activated with diisopropylammonium tetrazolide in dry dichloromethane (94%, Scheme 3).

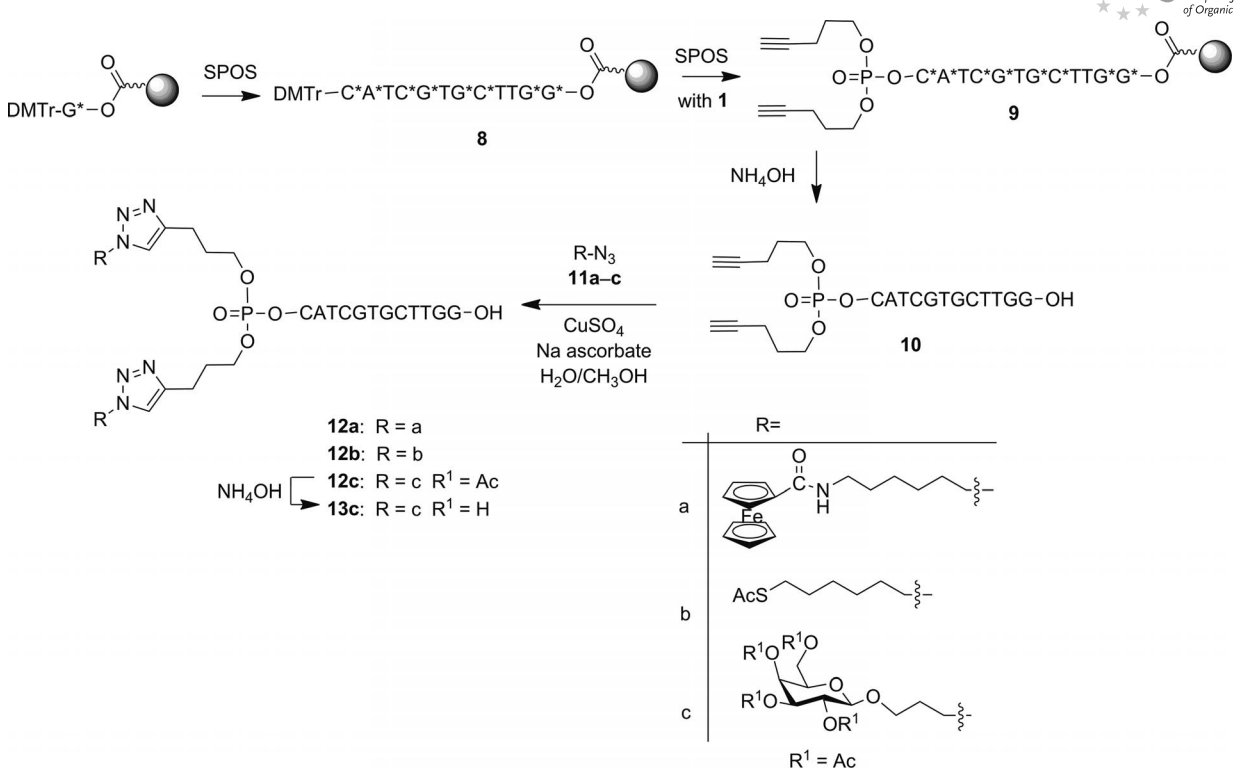
Oligonucleotide (12-mer) **8** was synthesized by a standard phosphoramidite protocol on a DNA synthesizer by using a commercially available solid support and nucleoside phosphoramidites. Then, bis-pent-4-ynyl phosphoramidite **1** was coupled at the 5′-end according to the same elongation cycle, except a coupling time of 60 s was used to ensure good coupling. The expected solid-supported 5′-bis-pent-4-ynyl 12-mer **9** was deprotected and released from the solid support by treatment with ammonia (55 °C overnight) to afford **10** (Scheme 4). The CuAAC reaction was carried out on part of crude bis-alkyne oligonucleotide **10** with azide derivatives **11a** and **11b** (11 equiv.) exhibiting a ferrocenamide hexyl and an *S*-acetylthiohexyl group, respectively, whereas the CuAAC reaction with tetraacetyl-β-D-galactopyranoside propyl azide **11c**[24] was performed with purified **10**. Copper(I) was generated in situ by using $CuSO_4$ (5 equiv.) reduced by sodium ascorbate (25 equiv.) in a mixture of water/methanol (1:1). The reactions proceeded at room temperature and were finished within 2.5 h. We observed that 5 equiv. of copper was required for a rapid CuAAC reaction and avoided degradation of the the oligonucleotide without the use of a Cu^I chelator like tris-(benzyltriazolylmethyl)amine (TBTA).[25] Furthermore, we worked with a degassed aqueous solution and avoided the presence of oxygen that would lead to degradation of the oligonucleotide. The mixture was desalted by size-exclusion chromatography (SEC), which also removed the excess amount of the azide derivatives. The HPLC profiles of the crude showed very clean reactions with the formation of expected bis-conjugated oligonucleotides **12a**–**c** with a high purity (Figure 2). Bis-conjugates **12a** and **12b** were obtained from crude **10** and purified by C_{18} HPLC. The yields were between 80 and 87%, respectively. For the synthesis of **13c**, we started with purified **10**, which was conjugated with **11c** leading to pure conjugate **12c** after SEC. Finally, the acetyl groups on the galactose moieties of **12c** were removed by ammonia treatment (1 h) affording pure bis-galactosyloligonucleotide **13c** (93%) without further purification (Figure 2). Each conjugate was characterized by MALDI-TOF mass spectrometry (see the Supporting Information).

Bis-alkyne phosphoramidite **5** and tri-alkyne phosphoramidite **7** were introduced at the 5′ end of oligonucleotide **14** under conditions similar to those used for **1**, leading to bis- and tris-alkynyl solid-supported oligonucleotides **15** and **16**, respectively (Scheme 5). Half of the amount of each was treated with concentrated ammonia (55 °C, overnight) for deprotection and release, affording, in solution, 5′-bis- and 5′-tris-propargyl oligonucleotides **17** and **18**, respectively. Each alkynylated oligonucleotide (100 nmol) was conjugated with *S*-acetylthiohexyl azide **11b** (5.5 equiv./alkyne) by CuAAC with the use of $CuSO_4$ (5 equiv.) and sodium ascorbate (25 equiv.) in methanol/water. The reactions were complete in 1 and 2 h leading to bis- and tris-*S*-acetylthiohexyl conjugates **19b** and **20b**, respectively. The mixtures were desalted by SEC and purification was performed by HPLC (73 and 78%). The conjugates were characterized by MALDI-TOF MS.

Similarly, solid-supported bis-alkynyl oligonucleotide **15** was conjugated with ferrocenyl azide derivative **11a**. However, we observed a strong degradation of the bis-ferrocenyl oligonucleotide due to the ammonia treatment likely



Scheme 3. Synthesis of tris-alkyne phosphoramidite **7**.



Scheme 4. Synthesis of oligonucleotides bis-conjugated with ferrocenecarboamide hexyl, *S*-acetylthiohexyl, or galactose propyl motifs by using bis-pent-4-ynyl phosphoramidite **1**. SPOS: solid-phase oligonucleotide synthesis. Conditions: (1) 3% trichloroacetic acid (TCA) in CH_2Cl_2; (2) phosphoramidite derivative + benzylthiotetrazole (BTT); (3) Ac_2O, NMe-imidazole, 2,6-lutidine; (4) 0.1 M I_2 THF/H_2O/pyridine. Asterisk (*) represents protecting group on nucleobase (benzoyl for A and C or isobutyryl for G).

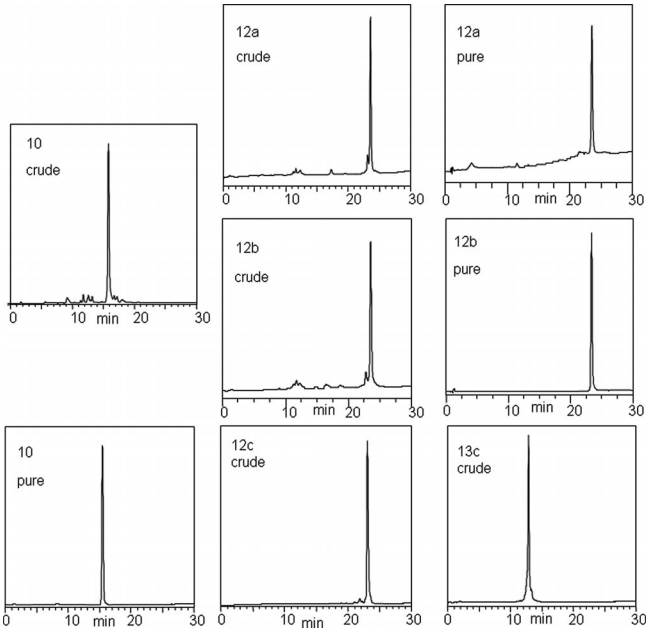
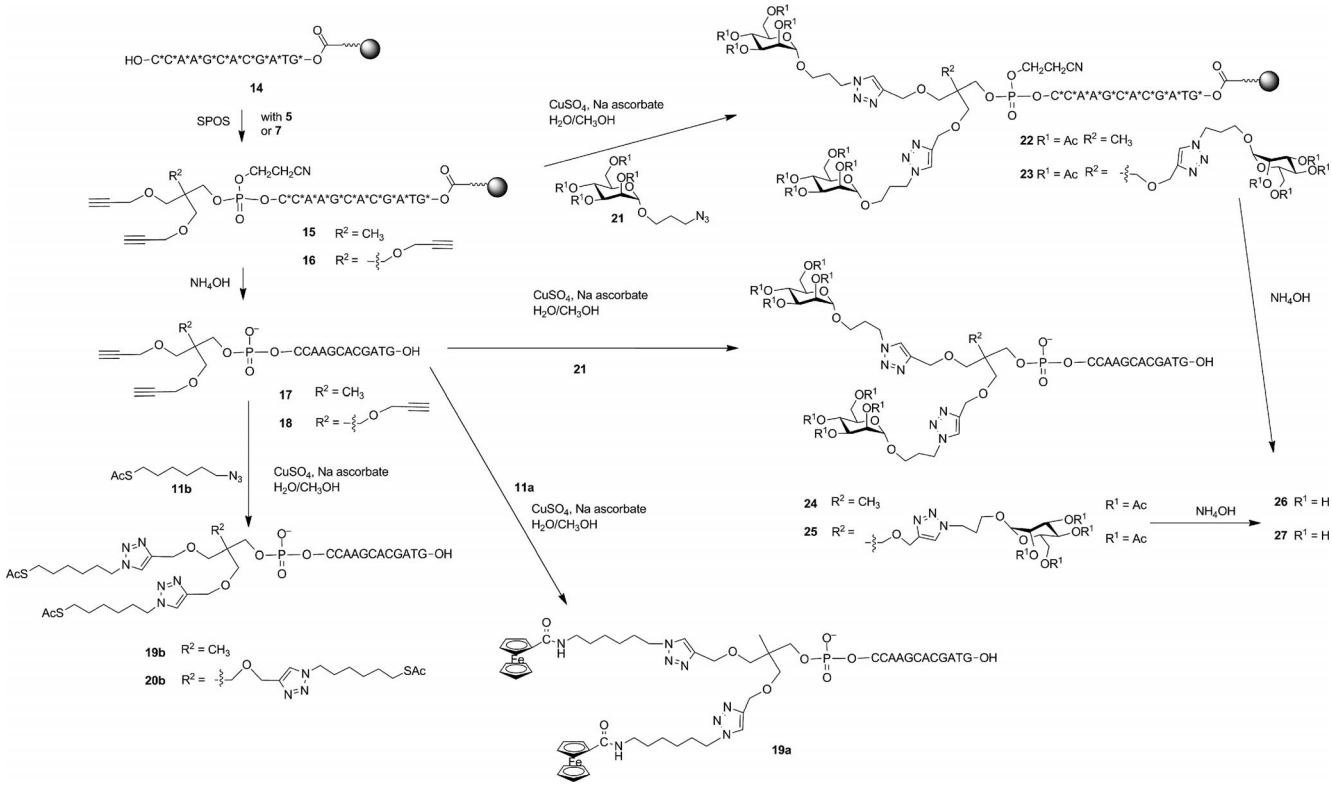


Figure 2. HPLC profiles of bis-alkyne oligonucleotide **10** and its conjugates with **11a**–**c**.

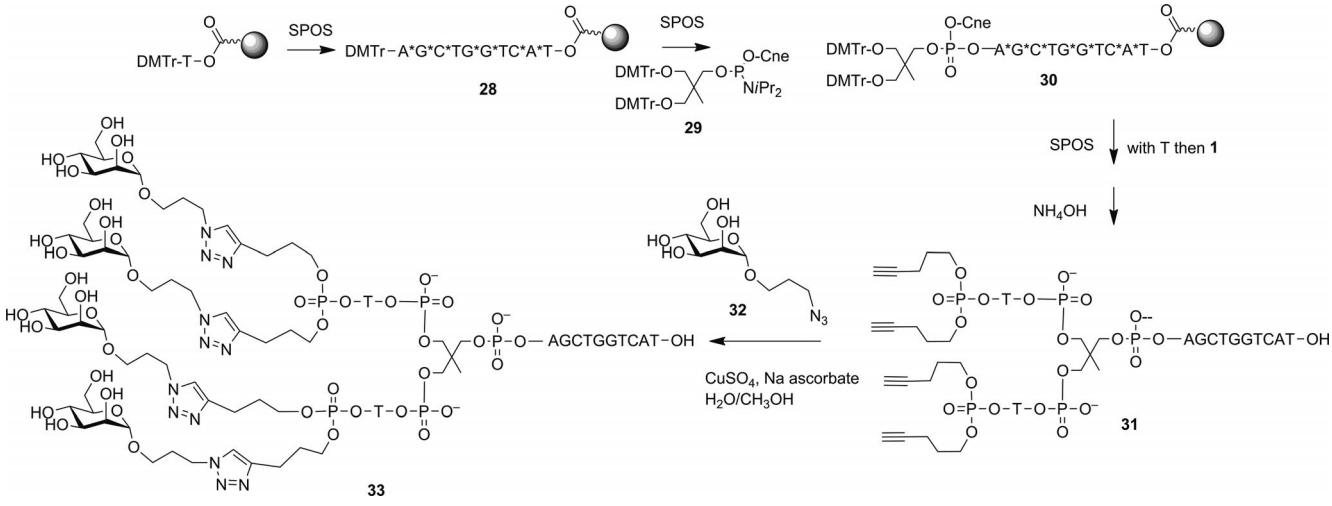
through a reaction on the ferrocenyl moiety. Thus, the CuAAC reaction was carried out in solution by using bis-alkynyl oligonucleotide **17** and ferrocenyl azide derivative **11a** affording expected bis-ferrocenyl oligonucleotide **19a** (70%). (see the Supporting Information). This result showed that this kind of ferrocenyl derivative must be conjugated in solution to avoid degradation during basic treatment.

Mannose moieties were introduced on an oligonucleotide working either on solid support or in solution (Scheme 5). To this end, solid-supported bis- and tris-alkyne oligonucleotides **15** and **16** were treated with tetraacetyl β-D-mannose propyl azide **21**[26] by using $CuSO_4$ (1 equiv.) and sodium ascorbate (5 equiv.) for 45 min at 60 °C to afford **22** and **23**, respectively, which were treated with ammonia for deprotection and release from the solid support to give bis- and tris-mannosylated oligonucleotide **26** and **27**, respectively. In parallel, a CuAAC reaction was performed in solution with bis- and tris-alkynyl oligonucleotides **17** and **18** by using azide **21** for 1 h at room temperature leading to acetylated conjugates **24** and **25**, which were desalted by SEC and treated with ammonia for 1 h to give bis- and tris-mannosylated oligonucleotide **26** and **27**, respectively. Both CuAAC reactions performed on solid support and in solution yielded similar results with an HPLC purity of the crude material between 77 and 84%. Each conjugate was characterized by MALDI-TOF mass spectrometry.

Finally, to extend the scope of multiple conjugations, a last conjugate exhibiting four alkyne functions was synthesized on a DNA synthesizer by derivatization and introduction of two molecules of bis-pentynyl phosphoramidite (**1**, Scheme 6). Thus, solid-supported oligonucleotide **28** was



Scheme 5. Synthesis of bis- and tri-alkynyl oligonucleotides by using **5** and **7** phosphoramidites, respectively, and their conjugates by CuAAC using ferrocenyl, acetylthiohexyl, or mannosyl azide derivatives.



Scheme 6. Synthesis of tetramannosylated oligonucleotide conjugate.

synthesized and special phosphoramidite **29**,[27] prepared from tris-(hydroxymethyl)ethane and bearing two hydroxy functions protected with a dimethoxytrityl group, was introduced to afford **30**. Then a thymidine phosphoramidite was introduced on each hydroxy group, as linkers, and finally two phosphoramidite moieties **1** were introduced leading to tetraalkyne oligonucleotide **31** after ammonia treatment. The CuAAC reaction was performed in solution with β-D-mannose propyl azide **32**[24] (4 equiv./alkyne) by using $CuSO_4$ (5 equiv.) and sodium ascorbate (25 equiv.) in methanol and water affording tetramannosyl oligonucleotide conjugate **33**, which was desalted by SEC and purified by HPLC.

Conclusions

Using three new phosphoramidite bearing two or three alkyne functions, we were able to synthesize several oligonucleotides conjugated with thiohexyl group, a ferrocenyl

moiety, or carbohydrate residues by using their azide derivatives. These phosphoramidites contribute to the elaboration of new building blocks for the multiple conjugation of oligonucleotides by the very efficient CuAAC reaction.

Experimental Section

Bis(pent-4-ynyl) *N*,*N*-Diisopropylphosphoramidite (1): To a solution of anhydrous pent-4-yn-1-ol (5.4 mmol, 500 μL) and anhydrous Et_3N (6 mmol, 826 μL) in dry CH_2Cl_2 (5 mL), stirred at 0 °C under an atmosphere of argon, was added a solution of diisopropylphosphorodichloridite (2 mmol, 550 mg) in dry CH_2Cl_2 (5 mL). The resulting mixture was stirred for 4 h at room temperature, diluted with CH_2Cl_2 (100 mL), filtered to remove the triethylammonium chloride salt, and washed with $NaHCO_3$ (1 × 150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (0 to 50% CH_2Cl_2 in cyclohexane containing 10% triethylamine) to afford the title compound (0.50 g, 83%) as a clear oil. R_f = 0.60 (cyclohexane/AcOEt/Et_3N, 5:4:1). ^{1}H NMR (300 MHz, CD_3CN): δ = 1.18 (d, J = 6.8 Hz, 12 H, 4 CH_3), 1.73–1.82 (m, 4 H, 2 $CH_2CH_2CH_2$), 2.16–2.19 (t, J = 2.6 Hz, 2 H, 2 CCH), 2.29 (dt, J_d = 2.6 Hz, J_t = 7.1 Hz, 4 H, 2 CH_2CH_2CCH), 3.54–3.77 (m, 6 H, 2 OCH_2, 2 $CHMe_2$) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 14.2, 23.6, 29.8, 42.3, 61.2, 68.6, 83.5 ppm. ^{31}P NMR (121 MHz, CD_3CN): δ = 146.25 ppm. HRMS (ESI+): calcd. for $C_{16}H_{29}O_2NP$ $[M + H]^+$ 298.1915; found 298.1936.

1-(4,4′-Dimethoxytrityloxymethyl)-2,2-bis(propargyloxymethyl)propane (3): To a cold solution of 1-(4,4′-dimethoxytrityloxymethyl)-2,2-bis(hydroxymethyl)propane[21] (13.25 mmol, 5.6 g) in anhydrous THF (200 mL) was added NaH (60% in oil, 200 mmol, 8.0 g), and after 10 min propargyl bromine (80% in toluene, 106 mmol, 11.8 mL) was added. The mixture was stirred at 40 °C overnight. Then, the reaction was cooled to 0 °C, quenched with H_2O (20 mL), and poured into H_2O (400 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL), and the organic layers were pooled, washed with brine, and concentrated. The crude oil was used directly for the next step or purified by silica gel column chromatography (50 to 100% CH_2Cl_2 in cyclohexane) to afford the title compound as a clear oil. R_f = 0.6 (cyclohexane/AcOEt, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (s, 3 H, CCH_3), 2.30 (t, J = 2.3 Hz, 2 H, CCH), 2.89 (s, 2 H, CCH_2ODMTr), 3.37 (d, J = 3.6 Hz, 4 H, CCH_2Opropargyl), 3.8 (s, 6 H, OCH_3), 4.0 (d, J = 2.4 Hz, 4 H, OCH_2CC), 6.72–7.36 (m, 13 H, aromatic) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.8, 40.7, 55.1, 58.6, 64.7, 72.9, 74.0, 80.2, 85.4, 112.9, 126.5, 127.6, 128.3, 130.2, 136.4, 145.4, 158.3 ppm. HRMS (ESI+): calcd. for $C_{32}H_{34}O_5Na$ $[M + H]^+$ 521.2304; found 521.2308.

2,2-Bis(propargyloxymethyl)propan-1-ol (4): To a cold solution of [1-(4,4′-dimethoxytrityloxy)-2,2-bis(propargyloxymethyl)propane (13.25 mmol, crude oil) in CH_2Cl_2 (100 mL), whilst stirring, was added a solution of 3% TCA in CH_2Cl_2 (100 mL). The red mixture was stirred for 15 min and quenched by the slow addition of an aqueous saturated solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL), and the organics layers were pooled, dried (Na_2SO_4), and concentrated. The crude oil was purified by silica gel column chromatography (0% to 5% MeOH in CH_2Cl_2) to afford **4** (2.1 g, 80%, two steps) as a brown oil. R_f = 0.25 (cyclohexane/AcOEt, 7:3). R_f = 0.5 (MeOH/CH_2Cl_2, 5:95). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (s, 3 H, CH_3), 2.37 (t, J = 2.4 Hz, 2 H, CCH), 3.43 (d, J = 1.5 Hz, 4 H, CCH_2Opropargyl), 3.5 (s, 2 H, CCH_2OH), 4.08 (dd, J = 2.4 Hz, 4 H, OCH_2CC) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.3, 40.4, 58.7, 68.3, 74, 74.4, 79.7 ppm. HRMS (ESI+): calcd. for $C_{11}H_{17}O_3$ $[M + H]^+$ 197.1175; found 197.1178.

2,2-Bis(propargyloxymethyl)propyl 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (5): To a solution of anhydrous 2,2-bis(propargyloxymethyl)propan-1-ol (**4**; 2.7 mmol, 530 mg) and diisopropylethylamine (4.05 mmol, 705 μL) in anhydrous CH_2Cl_2 (30 mL) was added 2-cyanoethyl chlorophosphoroamidite (3.25 mmol, 720 μL). The resulting mixture was stirred for 45 min at room temperature. Then, the reaction was quenched with H_2O (1 mL), diluted with CH_2Cl_2 (100 mL), and washed with $NaHCO_3$ (3 × 40 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (0 to 30% CH_2Cl_2 in cyclohexane containing 5% triethylamine) to afford **5** (0.87 g, 82%) as clear oil. R_f = 0.5 (cyclohexane/AcOEt/Et_3N, 7:2:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.02 (s, 3 H, CH_3C), 1.23 (d, J = 6.8 Hz, 12 H, CH_3 *i*pr), 2.45 (t, J = 2.4 Hz, 2 H, CCH), 2.69 (t, J = 6.4 Hz, 2 H, CH_2CN), 3.44 (s, 4 H, CCH_2Opropargyl), 3.51–3.91 [m, 6 H, CH(*i*pr), OCH_2CH_2, CCH_2OP], 4.17 (d, J = 2.4 Hz, 4 H, CH_2CCH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.3, 20.4, 24.5, 24.7, 40.8, 43.0, 43.2, 58.2, 58.4, 58.6, 66.0, 72.5, 74.1, 80.0, 117.6 ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 147.7 ppm. HRMS (ESI+): calcd. for $C_{20}H_{34}N_2O_4P$ $[M + H]^+$ 397.2256; found 397.2246

2-Cyanoethyl 2,2,2-Tris(propargyloxymethyl)ethyl *N*,*N*-Diisopropylphosphoramidite (7): To a solution of anhydrous 2,2,2-tris(propargyloxymethyl)ethan-1-ol (**6**;[23] 2.8 mmol, 700 mg) and diisopropylammonium tetrazolide (1.4 mmol, 240 mg) in anhydrous CH_2Cl_2 (10 mL) was added 2-cyanoethyl tetraisopropylphosphorodiamidite (3.4 mmol, 1.1 mL). The resulting mixture was stirred for 5 h at room temperature, diluted with CH_2Cl_2 (50 mL), and washed with brine (2 × 150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by silica gel column chromatography (15 to 50% CH_2Cl_2 in cyclohexane containing 5% triethylamine) to afford **7** (1.0 g, 94%) as clear oil. R_f = 0.45 (cyclohexane/CH_2Cl_2/Et_3N, 6:3:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.18–1.20 (dd, J = 7 Hz, 12 H, 4 CH_3), 2.41 (t, J = 2.3 Hz, 3 H, 3 CCH), 2.63–2.67 (m, 2 H, CH_2CN), 3.53 (s, 6 H, 3 CCH_2O), 3.55–3.67 (m, 4 H, $POCH_2C$, 2 $CHMe_2$), 3.81–3.89 (m, 2 H, OCH_2CH_2), 4.14 (d, J = 2.3 Hz, 6 H, 3 OCH_2CCH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 24.7, 43.2, 45.1, 58.2, 58.4, 58.7, 68.6, 74.2, 80.0, 117.73 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 147.6 ppm. HRMS (ESI+): calcd. for $C_{23}H_{36}N_2O_5P$ $[M + H]^+$ 451.2362; found 451.2373.

General Procedure for the Synthesis of Oligonucleotides: Oligonucleotides were synthesized on a 1 μmol-scale on a DNA synthesizer (ABI 394) starting from a commercially available solid support by standard phosphoramidite chemistry. For the coupling step, benzylmercaptotetrazole (BMT) was used as the activator (0.3 M in anhydrous CH_3CN), commercially available nucleoside phosphoramidites (0.075 M in anhydrous CH_3CN) was introduced with a 20 s coupling time, alkyne phosphoramidite **1** (0.2 M in anhydrous CH_3CN) was introduced with a 60 s coupling time, and alkyne phosphoramidites **5** and **7** (0.1 M in anhydrous CH_3CN) was introduced with a double coupling of 40 s each. The capping step was performed with acetic anhydride using commercial solutions (Cap A/Ac2O, pyridine, THF 10:10:80 and Cap B/10% *N*-methylimidazole in THF) for 15 s. Oxidation was performed with a commercial solution of iodide (0.05 M I_2 in THF/pyridine/water, 90:5:5) for 13 s. Detritylation was performed with 3% TCA in CH_2Cl_2 for 35 s.

General Procedure for the Deprotection of Solid-Supported Oligonucleotides: CPG beads were treated with concentrated aqueous am-

monia (1.5 mL) for 18 h at room temperature for **9** or for 5 h at 55 °C for **15** and **16**. The supernatant was withdrawn and the solvents were evaporated to dryness. The residue was dissolved in water.

General Procedure for CuAAC Reaction

In Solution: To bis- or tris-alkyne oligonucleotide (1 equiv.) in H_2O (50 μL) and azide derivative (4 equiv./alkyne in MeOH) was added a freshly prepared and degassed aqueous solution of $CuSO_4$ (5 equiv.), sodium ascorbate (25 equiv.), and water/MeOH (1:1) up to a volume of 200 μL. The tube containing the resulting preparation was sealed and magnetically stirred for 1 h at room temperature. Upon completion of the reaction, the solution was desalted on NAP10 and the solvents were evaporated.

On Solid Support: To the solid-supported bis- or tris-alkyne oligonucleotide (1 equiv.) in H_2O (50 μL) and azide derivative (4 equiv./alkyne in CH_3OH) was added a freshly prepared and degassed aqueous solution of $CuSO_4$ (1 equiv.), sodium ascorbate (5 equiv.), and water/MeOH up to 200 μL. The tube containing the resulting preparation was sealed and placed for 45 min at 60 °C in an oil bath under magnetic stirring. After the reaction, the CPG beads were filtered off, washed with H_2O (3×2 mL) and MeOH (3×2 mL), and dried.

Supporting Information (see footnote on the first page of this article): Preparation of **11a** and **11b** and HPLC profiles and mass spectrometry data of the oligonucleotide conjugates.

Acknowledgments

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[1] S. D. Vernon, D. H. Farkas, E. R. Unger, V. Chan, D. L. Miller, Y. P. Chen, G. F. Blackburn, W. C. Reeves, *Bmc Infect. Dis.* **2003**, 3.
[2] G. Chatelain, A. Meyer, F. Morvan, J. J. Vasseur, C. Chaix, *New J. Chem.* **2011**, *35*, 893–901.
[3] G. Chatelain, M. Ripert, C. Farre, S. Ansanay-Alex, C. Chaix, *Electrochim. Acta* **2012**, *59*, 57–63.
[4] Y. Singh, O. Renaudet, E. Defrancq, P. Dumy, *Org. Lett.* **2005**, *7*, 1359–1362.
[5] C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J. P. Cloarec, J. P. Praly, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2006**, *71*, 4700–4702.
[6] Y. Chevolot, C. Bouillon, S. Vidal, F. Morvan, A. Meyer, J. P. Cloarec, A. Jochum, J. P. Praly, J. J. Vasseur, E. Souteyrand, *Angew. Chem.* **2007**, *119*, 2450; *Angew. Chem. Int. Ed.* **2007**, *46*, 2398–2402.
[7] T. Yamada, C. G. Peng, S. Matsuda, H. Addepalli, K. N. Jayaprakash, M. R. Alam, K. Mills, M. A. Maier, K. Charisse, M. Sekine, M. Manoharan, K. G. Rajeev, *J. Org. Chem.* **2011**, *76*, 1198–1211.
[8] Z. Li, R. C. Jin, C. A. Mirkin, R. L. Letsinger, *Nucleic Acids Res.* **2002**, *30*, 1558–1562.
[9] K. W. Plaxco, N. Phares, R. J. White, *Anal. Chem.* **2009**, *81*, 1095–1100.
[10] M. S. Shchepinov, I. A. Udalova, A. J. Bridgman, E. M. Southern, *Nucleic Acids Res.* **1997**, *25*, 4447–4454.
[11] M. Dubber, J. M. J. Frechet, *Bioconjugate Chem.* **2003**, *14*, 239–246.
[12] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
[13] C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
[14] T. Carell, P. M. E. Gramlich, C. T. Wirges, A. Manetto, *Angew. Chem.* **2008**, *120*, 8478; *Angew. Chem. Int. Ed.* **2008**, *47*, 8350–8358.
[15] F. Morvan, A. Meyer, A. Jochum, C. Sabin, Y. Chevolot, A. Imberty, J. P. Praly, J. J. Vasseur, E. Souteyrand, S. Vidal, *Bioconjugate Chem.* **2007**, *18*, 1637–1643.
[16] V. R. Sirivolu, P. Chittepu, F. Seela, *ChemBioChem* **2008**, *9*, 2305–2316.
[17] L. Moni, G. Pourceau, J. Zhang, A. Meyer, S. Vidal, E. Souteyrand, A. Dondoni, F. Morvan, Y. Chevolot, J. J. Vasseur, A. Marra, *ChemBioChem* **2009**, *10*, 1369–1378.
[18] F. Seela, H. Xiong, S. Budow, *Tetrahedron* **2010**, *66*, 3930–3943.
[19] F. Seela, S. A. Ingale, *J. Org. Chem.* **2010**, *75*, 284–295.
[20] A. V. Ustinov, I. A. Stepanova, V. V. Dubnyakova, T. S. Zatsepin, E. V. Nozhevnikova, V. A. Korshun, *Russ. J. Bioorg. Chem.* **2010**, *36*, 401–445.
[21] J. Lietard, A. Meyer, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2008**, *73*, 191–200.
[22] F. Morvan, A. Meyer, G. Pourceau, S. Vidal, Y. Chevolot, E. Souteyrand, J.-J. Vasseur, *Nucleic Acids Symp. Ser.* **2008**, 47–48; F. Morvan, A. Meyer, J.-J. Vasseur, S. Vidal, J.-P. Cloarec, Y. Chevolot, E. Souteyrand, US2011245478, **2011**.
[23] A. Mollard, I. Zharov, *Inorg. Chem.* **2006**, *45*, 10172–10179.
[24] J. A. F. Joosten, V. Loimaranta, C. C. M. Appeldoorn, S. Haataja, F. A. El Maate, R. M. J. Liskamp, J. Finne, R. J. Pieters, *J. Med. Chem.* **2004**, *47*, 6499–6508.
[25] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Org. Lett.* **2004**, *6*, 2853–2855.
[26] W. Hayes, H. M. I. Osborn, S. D. Osborne, R. A. Rastall, B. Romagnoli, *Tetrahedron* **2003**, *59*, 7983–7996.
[27] E. R. Wijsman, D. Filippov, A. R. P. M. Valentijn, G. A. van der Marel, J. H. van Boom, *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 397–401.

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