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An efficient reagent for 5′-azido oligonucleotide synthesis

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Abstract—A new bromohexyl phosphoramidite was synthesized and used for the introduction of an azide function at the 5′-end of oligonucleotides after a treatment on solid support with sodium azide and sodium iodide. The corresponding 5′-azido oligonucleotide could be further used for 'Click' conjugation with alkyne derivatives or by Staudinger ligation.

Oligonucleotide (ODN) conjugates are widely used for various applications in biology, biotechnology, and medicine. Many methods of conjugation are reported in the literature.[1–3] An alternative approach is the use of the azide/alkyne system for conjugation. Both functions react together through a Huisgen 1,3-dipolar cycloaddition called 'click chemistry'.[4] This reaction was found to be catalyzed by Cu(I) ion,[5,6] therefore dramatically decreasing the reaction times while the interest for 'click chemistry' strongly increased and a wide range of applications for bioconjugation have been reported (for recent reviews see[7–10]). The alkyne and azide functions are mainly orthogonal with other functionalities so that the click reaction is chemoselective and can be performed in water and organic solvents. Furthermore, azido-oligonucleotides were also reported for conjugation via a Staudinger ligation.[11]

For that purpose, it is useful to develop a rapid and easy way to introduce an azido function on an oligonucleotide. A direct introduction of azide by means of a phosphoramidite derivative is impossible due to its reactivity with phosphines known as the Staudinger reaction.[12] Hence, the azide function must be introduced after oligonucleotide elongation. Two main procedures are reported in the literature. The first one uses an azidosuccinimidyl ester derivative reacting in solution with an aminoalkyl linker attached to the oligonucleotide.[13,14] The second procedure, carried out on solid support, is a 5′-iodination of the oligonucleotide with Moffatt's reagent[15] followed by iodide displacement using sodium azide.[16] The first protocol requires the introduction of an amino function and then, after deprotection, a long coupling time (4–12 h) to create an amide linkage between the ODN and the azide function. Since the coupling step is carried out in solution, this method requires long workup procedures and is time consuming. The second procedure is more convenient because it is performed on solid support. However, the method is limited due to a side reaction occurring during the formation of the 5′-iodo intermediate leading to moderate yields when the 5′-end of the ODN is a deoxyadenosine (56%).

To overcome these limitations, we present herein the use of 6-bromohexyl 2-cyanoethyl diisopropylphosphoramidite **1** as a powerful reagent for 5′-azido oligonucleotide preparation. After its coupling using a standard phosphoramidite elongation cycle, the bromine atom was displaced by sodium azide to yield quantitatively the 5′-azido hexyl oligonucleotide on solid support. Introduction of **1** was rapid and not dependent on the nature of the 5′-terminal nucleoside. Preparation of 5′-azido oligonucleotides was performed within 1 h 15 min with all reactions being carried out on solid support.

Reagent **1** was easily and rapidly synthesized from commercially available reagents in one step from 6-bromo-1-hexanol and cyanoethyl tetraisopropylphosphorodiamidite activated with diisopropylammonium

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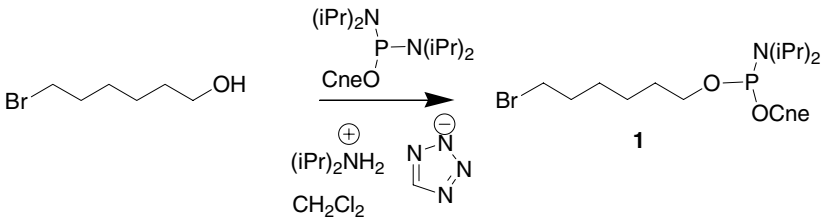
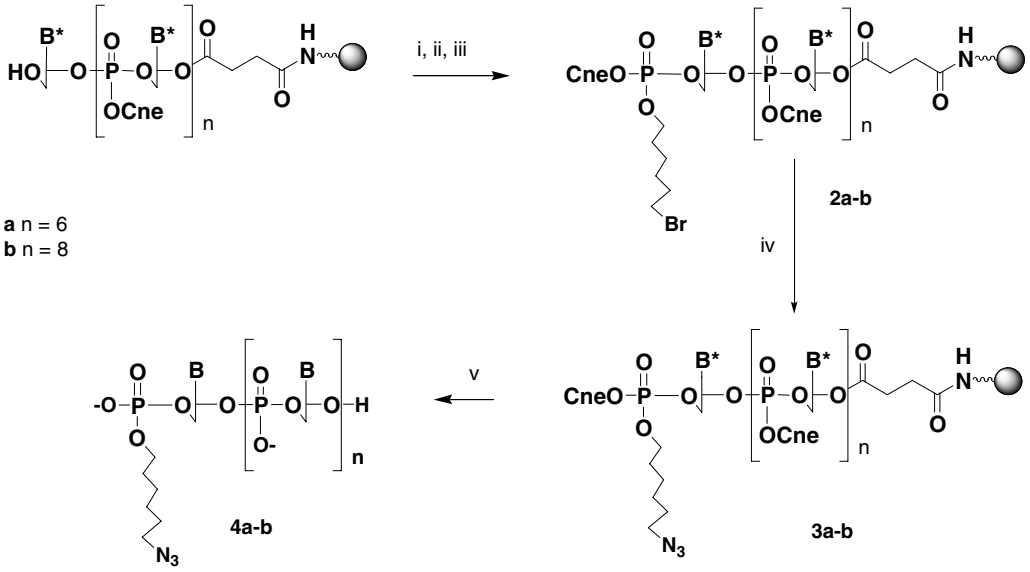


Scheme 1. Synthesis of 6-bromohexyl 2-cyanoethyl diisopropylphosphoramidite **1**, Cne = 2-cyanoethyl.

tetrazolide in dichloromethane (Scheme 1). Phosphoramidite **1** was obtained as colorless oil in 60% yield after workup and flash chromatography on silica gel.[17]

Two 5′-bromohexyloligonucleotides were synthesized (T_7 and AGACACGAT) on a DNA synthesizer using a standard phosphoramidite elongation cycle for coupling of commercial nucleoside phosphoramidites and our phosphoramidite **1** (Scheme 2).

Coupling efficiency is usually monitored by treatment of a small portion of CPG-oligonucleotide beads by ammonia affording the deprotected oligonucleotides in solution, which is analyzed by MALDI-TOF MS. However, treatment of **2a**–**b** with ammonia would substitute bromine atom to give a 5′-aminohexyl ODN. To overcome this limitation, we monitored the coupling efficiency of **1** by a direct analysis of **2a** by MALDI-TOF mass spectrometry as previously reported by us.[18,19]



Scheme 2. Synthesis of 5′-azido-oligonucleotides (**a**) T_7 and (**b**) AGACACGAT, for **2** and **3** $B^* = A^{Bz}$, C^{Bz} G^{ibu} or T for **4** B = A, C, G or T. Reagents and Conditions: (i) **1** + benzylthiotetrazole, CH_3CN; (ii) Ac_2O N–Me imidazole, THF pyridine; (iii) I_2/H_2O THF pyridine; (iv) NaN_3, NaI, DMF 1 h 65 °C; (v) NH_4OH.

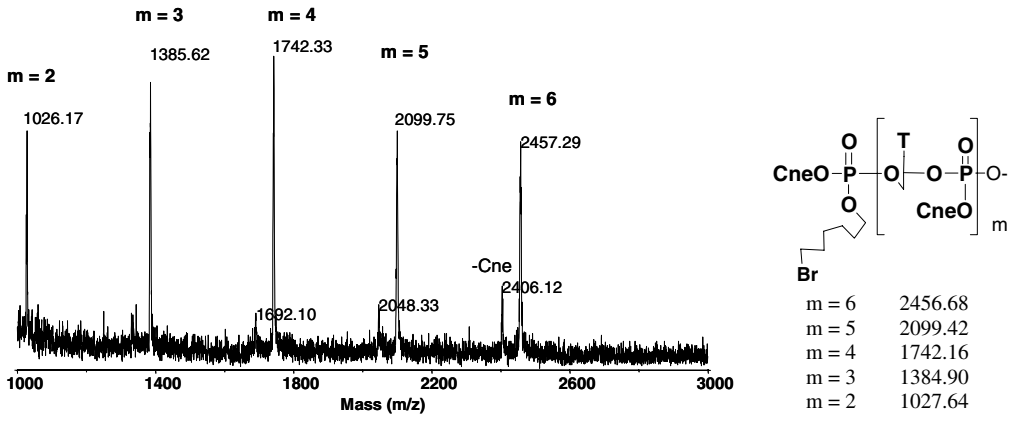


Figure 1. MALDI-TOF MS spectra of **2a** and schematic representation of formed ions and their calculated mass.

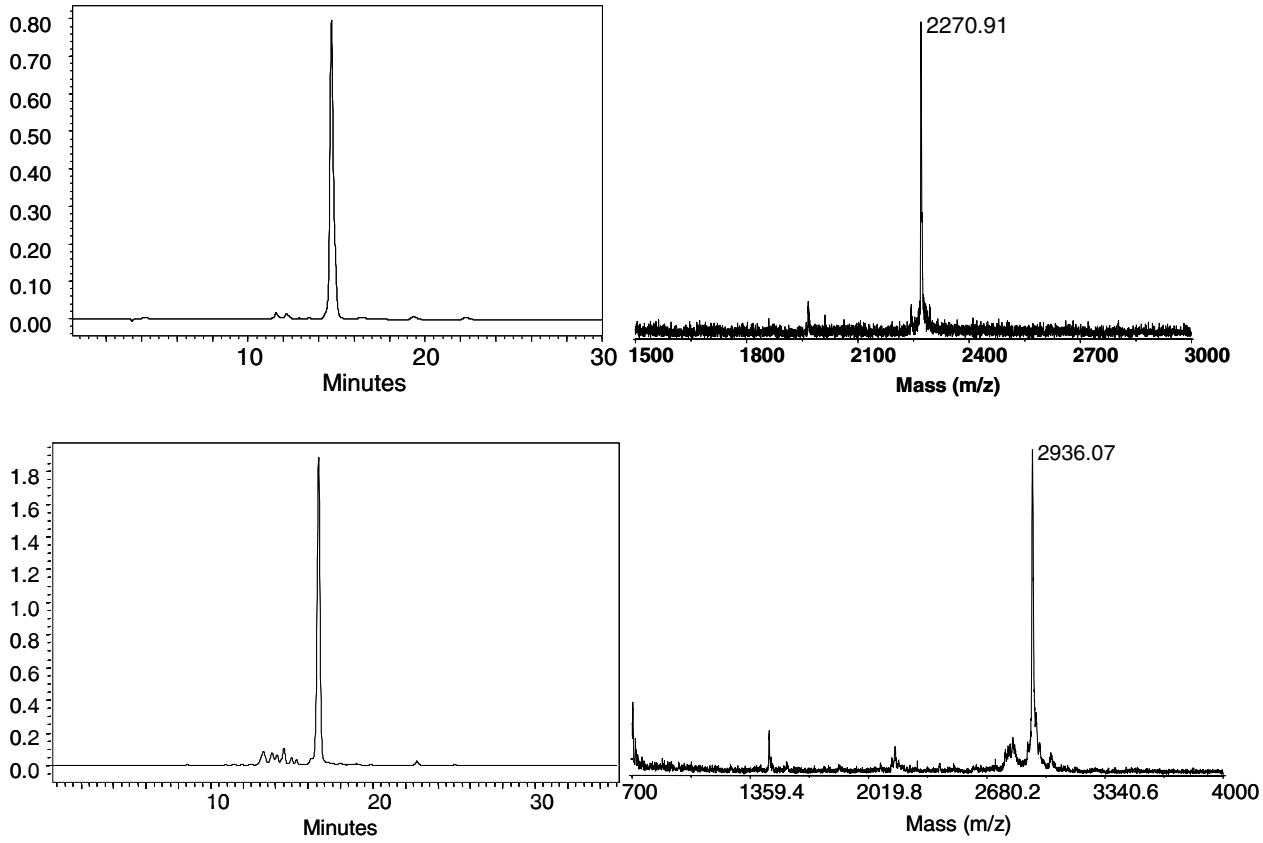


Figure 2. HPLC profiles and MALDI-TOF MS spectra of crude **4a** (top) and crude **4b** (bottom). MS negative mode for **4a**: $C_{76}H_{103}N_{17}O_{50}P_7$ calc. 2271.56, found 2270.91; **4b**: $C_{94}H_{121}N_{41}O_{52}P_9$ calc. 2936.01, found 2936.07.

The spectra showed a typical fragmentation pattern of the supported oligonucleotide due to the cleavage of the P–O–C-5′ bond of the phosphotriester linkages leading to the formation of ODN fragments with a 3′-cyanoethyl phosphodiester group. A pattern of mass peaks that differ from one another by a nucleotide residue was generated in agreement with the ODN sequence from the 5′- to the 3′-end except from the initial 3′-nucleoside directly linked to the solid support. Hence, the peak at $m/z = 2457.29$ corresponds to the fully protected 5′-bromohexyl T_6pOCne ion (Fig. 1). Fragment ions bearing the bromolinker were always visualized indicating that **1** was coupled with a high efficiency.

Bromohexyl ODNs were then converted into azidohexyl ODNs by treatment of **2a**–**b** with a solution of sodium azide (100 mM) and sodium iodide (100 mM) in dry DMF (2 mL), for 1 h 15 min at 65 °C.[20] Finally, **3a**–**b** were treated with ammonia affording 5′-azidohexyl-ODNs **4a**–**b** in the solution phase. HPLC profiles of the crude mixture showed a single peak corresponding to **4a**–**b** as characterized by MALDI-TOF MS (Fig. 2). A similar efficiency was observed with the heteropolymer **4b** containing a deoxyadenosine at the 5′-end.

In conclusion, we designed an easily accessible reagent to obtain efficiently and rapidly 5′-azido-oligonucleotides by coupling a bromohexyl phosphoramidite after elongation of the ODN chain followed by a treatment with sodium azide in the presence of sodium iodide. The corresponding 5′-azido oligonucleotides will be used for conjugation through Huisgen 1,3-dipolar cycloaddition[13,14] or Staudinger ligation[11] as previously reported.

References and notes

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17. *Synthesis of 6-bromohexyl 2-cyanoethyl diisopropylphosphoramidite* **1**. To a solution of 2-cyanoethyl tetraisopropylphosphorodiamidite (570 μL, 1.8 mmol) and diisopropylammonium tetrazolide (154 mg, 0.9 mmol) in anhydrous dichloromethane (5 mL) was added anhydrous 6-bromo-1-hexanol (325 mg, 1.8 mmol). The resulting mixture was stirred for 5 h at room temperature, diluted with ethyl acetate (30 mL) and washed with brine (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified by flash column chromatography (silica gel; cyclohexane with 3% Et_3N) affording phosphoramidite **1** (400 mg, 60% yield) as a colorless oil. TLC (cyclohexane/CH_2Cl_2/Et_3N; 5/4/1; v/v/v) R_f: 0.5; ^{31}P NMR (CD_3CN, 80 MHz): δ 148.5 ppm, ^1H NMR ($CDCl_3$, 200 MHz): δ 1.17–1.21 (d, 12H, CH_3), 1.44–1.88 (m, 8H, CH_2), 2.62–2.69 (m, 2H, CH_2CN), 3.39–3.87 (m, 8H, CH_2O, CH_2Br, $CHMe_2$).
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20. General protocol for azidation. Solid supported 5′-bromohexyl ODN (1 μmol) was treated with a solution of NaN_3 (13 mg, 200 μmol) and NaI (30 mg, 200 μmol) in dry DMF (2 mL). Using two syringes, this solution was pushed back and forth several times during 1 min and the column was placed at 65 °C for 1 h 15 min with regular agitation (30 s every 15 min). Column was washed with DMF, CH_2Cl_2 and flushed with nitrogen for drying.