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Synthesis of a new alkynylated deoxyadenosine phosphoramidite for the click reaction-mediated DNA labeling

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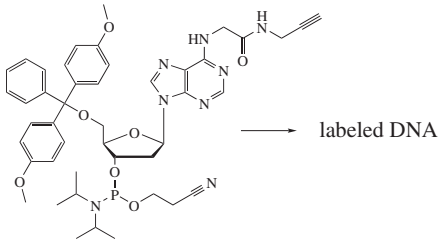
A new 5′-dimethoxytrityl N^6-alkynylated 2′-deoxyadenosine phosphoramidite has been designed and synthesized from 2′-deoxyadenosine. The applicability of this building block for the production of alkyne-modified oligodeoxynucleotide has been demonstrated. This allows one to introduce various labels into DNA using the click reaction.



Bioorthogonal molecular labeling is an important area in chemical biology and biomedical engineering, since it uses chemical reactions that do not interfere with a biological system.[1] In the past decades, numerous bioorthogonal reactions have been described,[2] an example is the azide–alkyne Huisgen cycloaddition,[3,4] which was brought back into focus after development of the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC or click reaction).[5–7] Due to many advantages of this reaction, namely harmless byproducts, high efficiency, simple operation and mild reaction conditions, it resulted in a breakthrough in the nucleic acids labeling, allowing one to introduce easily into DNA such moieties as fluorescent dyes, sugars, peptides and other reporter groups, as well as to cyclize DNA, synthesize DNA catenanes and produce nucleic acid analogues with modified nucleobases and backbones.[8,9] So far, various types of alkyne-containing DNA have been synthesised for the click reaction-mediated DNA labeling,[10–13] mainly by solid-phase chemistry, using alkynylated nucleoside phosphoramidite monomers as building blocks. Considering the nucleic acid structure, the alkynyl group can in principle be introduced into the ribose ring hydroxyl,[14] into the phosphodiester backbone[15] and into the nucleobase,[10,13] the last approach being widely used, for example in fluorescent labeling and single nuleotide polymorphism (SNP) detection.[16–18] Although several nucleobase-alkynylated DNAs have already been reported for the following use in the click reaction-mediated labeling,[10,13] the typical starting materials for the synthesis of the corresponding nucleobase-alkynylated phosphoramidites are mainly synthetic nucleosides, such as 5-iodo-2′-deoxyuridine, 7-deaza-2′-deoxy-7-iodoadenosine and 7-deaza-2′-deoxy-7-iodoguanosine.[13] With these non-natural nucleosides, their preliminary multistep synthesis is required, resulting in an increased total production cost and a greater workload.[19] In this work, we have designed and synthesized a new alternative building block based on N^6-alkynylated 2′-deoxyadenosine, using simple and commercially available 2′-deoxyadenosine as a starting material, as well as demonstrated its potential for DNA functionalization.

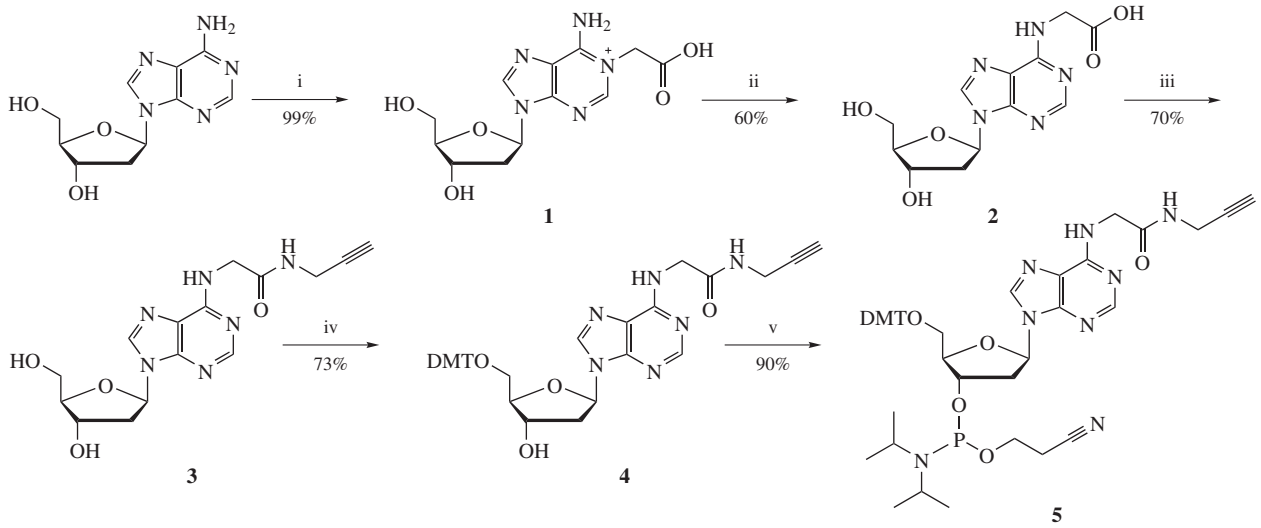
The synthetic route is shown in Scheme 1. 2′-Deoxyadenosine reacted with iodoacetic acid to give *N*^{1}-carboxymethyl-2′-deoxyadenosine **1**,† and the following Dimroth rearrangement afforded *N*^{6}-carboxymethyl-2′-deoxyadenosine **2**.‡ Subsequently, N^6-alkynylated 2′-deoxyadenosine **3**§ was obtained by amidation of compound **2** by propargylamine. The 5′-hydroxyl group of compound **3** was protected by 4,4′-dimethoxytritylchloride (DMT–Cl), and finally the protected nucleoside was converted into N^6-alkynylated deoxyadenosine cyanoethyl phosphoramidite **5**¶ by phosphitylation.

† N^1-*carboxymethyl-2′-deoxyadenosine* **1**. Yield 99%. ^{1}H NMR (600 MHz, D_2O) δ: 2.54–2.58 (m, 1H), 2.81 (ddd, 1H, *J* 13.12 Hz), 3.73 (d, 2H, CH_2, *J* 25.31 Hz), 4.07–4.10 (m, 1H), 4.59 (dd, 1H, *J* 6.16 Hz), 4.85 (s, 2H, CH_2COOH), 4.96 (s, 1H, OH), 5.03 (s, 1H, OH), 6.47 (t, 1H, *J* 4.76 Hz), 8.38 (s, 1H, HetAr–H), 8.43 (s, 1H, HetAr–H). ^{13}C NMR (100 MHz, D_2O) δ: 38.96, 53.02, 61.20, 70.68, 84.76, 87.39, 119.31, 143.13, 146.52, 147.67, 148.19, 170.50. MS (EI, ion trap), *m/z*: 310.2 $[M]^+$ (calc. for $C_{12}H_{16}N_5O_5$, *m/z*: 310.1).

‡ N^6-*carboxymethyl-2′-deoxyadenosine* **2**. Yield 60%. ^{1}H NMR (600 MHz, D_2O) δ: 2.55–2.59 (m, 1H), 2.81 (ddd, 1H), 3.80 (d, 1H, *J* 4.32 Hz), 3.85 (d, 1H, *J* 3.13 Hz), 3.97 (s, 2H, CH_2COOH), 4.09 (d, 2H, CH_2, *J* 8.47 Hz), 4.19 (q, 1H, *J* 3.34 Hz), 4.65 (m, 1H), 4.91 (d, 1H, OH, *J* 9.50 Hz), 5.06 (d, 1H, OH, *J* 24.60 Hz), 6.42 (t, 1H, *J* 6.87 Hz), 7.92 (s, 1H, HetAr–NH), 8.15 (s, 1H, HetAr–H), 8.24 (s, 1H, HetAr–H). ^{13}C NMR (100 MHz, D_2O) δ: 39.00, 61.12, 61.61, 71.18, 84.50, 87.31, 139.60, 144.25, 149.27, 152.22, 170.97, 179.79. MS (EI, ion trap), *m/z*: 310.2 $[M+H]^+$, 307.9 $[M–H]^–$ (calc. for $C_{12}H_{15}N_5O_5$, *m/z*: 309.2).

§ N^6-*(propargylcarbamoylmethyl)-2′-deoxyadenosine* **3**. Yield 70%. ^{1}H NMR (600 MHz, CD_3OD) δ: 2.58 (s, 1H), 2.62 (s, 1H), 2.99–3.01 (m, 2H, CH_2), 3.05–3.08 (m, 2H, CH_2), 3.11 (d, 2H, CH_2, *J* 6.22 Hz), 3.76 (d, 1H, *J* 2.17 Hz), 3.89–3.96 (m, 1H), 6.12 (s, 1H, NH), 6.23 (t, 1H, *J* 5.37 Hz), 7.45–7.47 (m, 1H, HetAr–NH), 8.65 (s, 1H, HetAr–H). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.12, 20.98, 29.05, 29.62, 55.17, 60.32, 61.97, 63.60, 87.66, 113.08, 113.36, 127.83, 129.04, 129.87, 158.75. MS (EI, ion trap), *m/z*: 384.4 $[M+K]^+$ (calc. for $C_{15}H_{18}N_6O_4$, *m/z*: 346.3).

¶ *5′-Dimethoxytrityl-*N^6*-(propargylcarbamoylmethyl)-2′-deoxyadenosin-3′-*O*-yl 2-cyanoethyl* N,N*-diisopropylphosphoramidite* **5**. Yield 90%. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 1.20 (s, 12H, Me), 2.38 (d, 1H, *J* 6.52 Hz), 2.52–2.54 (m, 1H), 2.57–2.62 (m, 2H), 2.67 (td, 2H, CH_2, *J* 6.17 Hz), 3.43 (dq, 2H, CH_2, *J* 18.32 Hz), 3.67 (s, 2H, CH_2), 3.69 (s, 6H, OMe), 3.92–3.99



Scheme 1 *Reagents and conditions:* i, ICH_2COOH, phosphate buffer saline pH 7.0, 60 °C, 8 h; ii, 5 M aq. NaOH, H_2O, 70 °C, 2 h; iii, $NH_2CH_2C{\equiv}CH$, HATU, DIPEA, DMF, room temperature, 24 h; iv, DMT-Cl, DMAP, pyridine, CH_2Cl_2, room temperature, 24 h; v, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2, room temperature, 6 h.

In the Dimroth rearrangement leading to compound **2**, the adjustment of pH to 11–12 prior to the reaction was crucial. After the reaction completion, pH was adjusted to 3 and the precipitated solid was filtered off to give product **2**. The product was characterized at first by LC/MSD Ion Trap. In the positive ion EI mode, the molecular weight of compound **2** was determined for $[M+H]^+$ as 310.2 Da, which was consistent with the calculated value 309.2 Da. Contrary to that, for compound **1** in the positive ion mode there was almost no difference between the found (310.2 Da) and calculated (310.1 Da) values of molecular weight. This is attributed to the positive charge of compound **1**, since it is known that molecules normally carry only one positive charge in the positive ion mode.[20] Moreover, in the negative ion mode compound **2** exhibited a molecular weight of 307.9 Da for $[M–H]^+$, while compound **1** did not appear at all. These results indicated that the Dimroth rearrangement took place and the obtained product was compound **2** rather than compound **1**. Compounds **1** and **2** were further characterized by ^1H NMR spectroscopy. The chemical shift of the CH_2 moiety of the carboxymethyl group in compound **1** is 4.85, while that in compound **2** is 3.97, this is attributed to the different electron withdrawing effects of substituents in compounds **1** and **2**. Thus, compound **2** was successfully prepared *via* the S_N2 reaction and the Dimroth rearrangement in ~60% yield for the two steps, which was higher than the reported one[21] where the same compound **2** was synthesized from 6-chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine *via* S_NAr reaction with glycine ethyl ester and the subsequent ester hydrolysis.

Table 1 The influence of reagent and solvent on the yield of compound **3** (20–25 °C, 24 h).

Reagent	Solvent	Yield (%)	Reagent	Solvent	Yield (%)
EDC	H_2O	20	HATU/DIPEA	DMF	71
EDC/NHS	H_2O	41	DCC/DMAP	DMF	67
EDC/HOBt	DMF	48			

(m, 2H), 4.02–4.07 (m, 2H, CH_2), 4.09–4.14 (m, 2H, CH_2, *J* 9.75 Hz), 4.28–4.31 (m, 1H), 5.20 (s, 1H), 6.34 (s, 1H), 6.68–6.72 [m, 4H, (Het)Ar–H], 7.08–7.11 [m, 5H, (Het)Ar–H], 7.20–7.40 [m, 7H, (Het)Ar–H, HetAr–NH, NH], 7.87 [d, 1H, (Het)Ar–H, *J* 15.18 Hz], 8.24 [s, 1H, (Het)-Ar–H]. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.04, 20.00, 22.57, 22.91, 24.54, 28.80, 31.50, 40.42, 41.44, 45.21, 47.39, 55.16, 58.08, 86.47, 113.01, 116.81, 126.83, 127.73, 128.09, 130.00, 135.47, 142.27, 144.35, 152.12, 158.42, 161.77, 165.72, 175.82. MS (EI, ion trap), *m/z*: 849.8 $[M+H]^+$, 871.8 $[M+Na]^+$ (calc. for $C_{45}H_{53}N_8O_7P$, *m/z*: 848.8).

To increase the yield of amidation, as another key step in our synthetic route, and to simplify its post-treatment, five coupling reagents combinations and the corresponding solvents were tested (Table 1). Compound **3** was obtained in a relatively high yield using HATU/DIPEA (71%) or DCC/DMAP (67%), however, when the EDC-containing coupling reagents were used, the yield was relatively low, namely 20, 41 and 48%. In addition, when DCC was used as a coupling reagent, the resulting DCU was difficult to remove after the reaction completion. As follows from the structure of compound **3**, the purine ring generates steric hindrance for the coupling reaction between the carboxyl group and propargylamine. However, compared with EDC, HATU has been reported to be a more efficient coupling reagent, affording a higher yield for the sterically hindered examples,[22] and our experimental results are consistent with this evidence. Therefore, HATU was chosen as a reagent for the amidation step.

By dimethoxytritylation and phosphitylation, compound **3** was converted to the final product **5** in a good overall yield. Taking into account that poly(dA) oligonucleotides labeled with pyrene were used for the investigation of DNA conformation,[23,24] we chose poly(dA) as a template to clarify whether compound **5** could be incorporated into DNA. The poly(dA)-type DNA [Figure 1(*a*)] was synthesized on CPG support by phosphoramidite approach using a DNA/RNA synthesizer. After deprotection with ammonia, the crude product was purified by reversed phase HPLC and characterized by ESI MS (calculated mass 6279.2; found mass 6278.8) [Figure 1(*b*)].

In summary, a new N^6-alkynylated deoxyadenosine cyanoethyl phosphoramidite was designed and synthesized using 2′-deoxy-

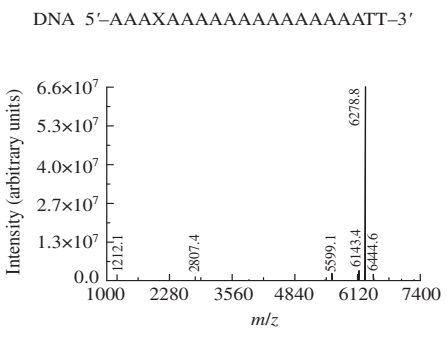


Figure 1 DNA containing alkynylated deoxyadenosine denoted as X: (*a*) sequence and (*b*) mass spectrum.

adenosine as a starting material *via* a five-step reaction. The total yield of the final product was 27%. The synthesized phosphoramidite building block was incorporated into a specific site of the oligodeoxynucleotide sequence to give alkyne-containing DNA, with a future prospect to use it in the click reaction-mediated DNA labeling and functionalization.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.09.008.

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