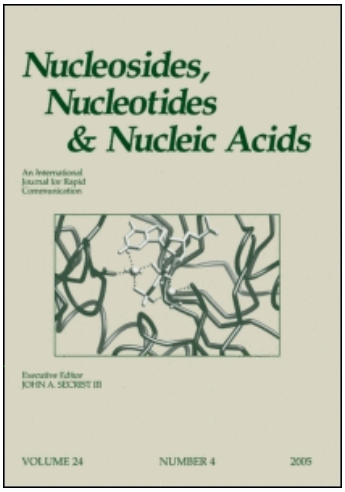

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NUCLEOSIDES AND OLIGONUCLEOTIDES WITH DIYNYL SIDE CHAINS: THE HUISGEN-SHARPLESS CYCLOADDITION "CLICK REACTION" PERFORMED ON DNA AND THEIR CONSTITUENTS

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□ *Phosphoramidite building blocks related to the four canonical DNA constituents and oligonucleotides with octadiynyl side chains (**5b–8b**) or dipropargyl ether residues (**5c**) were synthesized. Nucleosides and oligonucleotides were functionalized at the terminal triple bonds employing the Huisgen-Sharpless cycloaddition "click reaction."*

Keywords Nucleosides; phosphoramidites; octadiynyl; dipropargyl ether; Huisgen-Sharpless cycloaddition

INTRODUCTION

Among the recent advances in "click chemistry," the Cu(I)-catalyzed Huisgen [2 + 3] dipolar cycloaddition between an organic azide and an alkyne has attracted a great deal of attention. Azides and alkynes are highly energetic functional groups with a particularly narrow distribution of reactivity. The irreversible Huisgen-Sharpless cycloaddition of azides and alkynes is thermodynamically favorable by approximately 30–35 kcal/mol. The reaction is characterized by mild and simple reaction conditions and oxygen and water tolerance. It is chemoselective affording only the desired 1,2,3-triazole even in the presence of a large variety of other functional groups.

The "click" functionalization of oligonucleotides (ODNs) can be used to introduce almost any reporter group in nucleic acids. So, they can be employed as primers and probes for DNA detection, hybridization, sequencing, and nanotechnology applications. The most suitable positions to introduce

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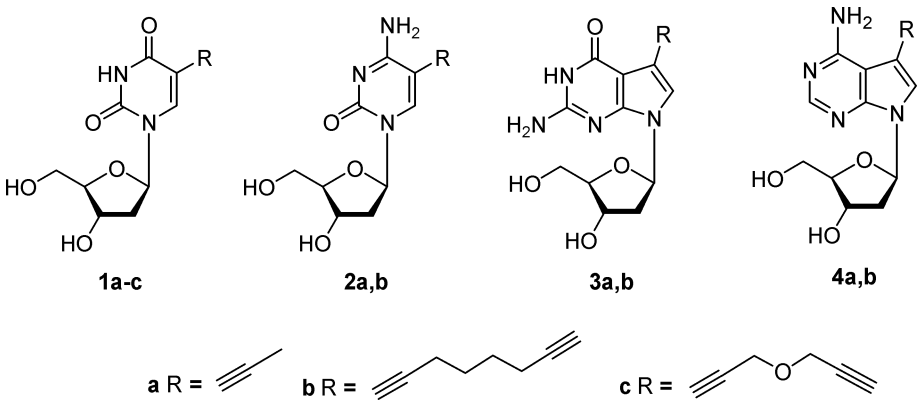


FIGURE 1

such functionalities are the 5-position of pyrimidines[1] and the 7-position of 7-deazapurines[2] (purine numbering is used). Among the various groups introduced in these positions, the alkadiynyl groups are of particular importance. They can be linked to the iodinated nucleobase by the Sonogashira cross-coupling and can be successively functionalized using the Huisgen-Sharpless "click chemistry." In our laboratory we introduced a large variety of alkynyl residues into pyrimidine and 7-deazapurine nucleosides and recently reported also octadiynyl derivatives (Figure 1).[3]

RESULTS AND DISCUSSION

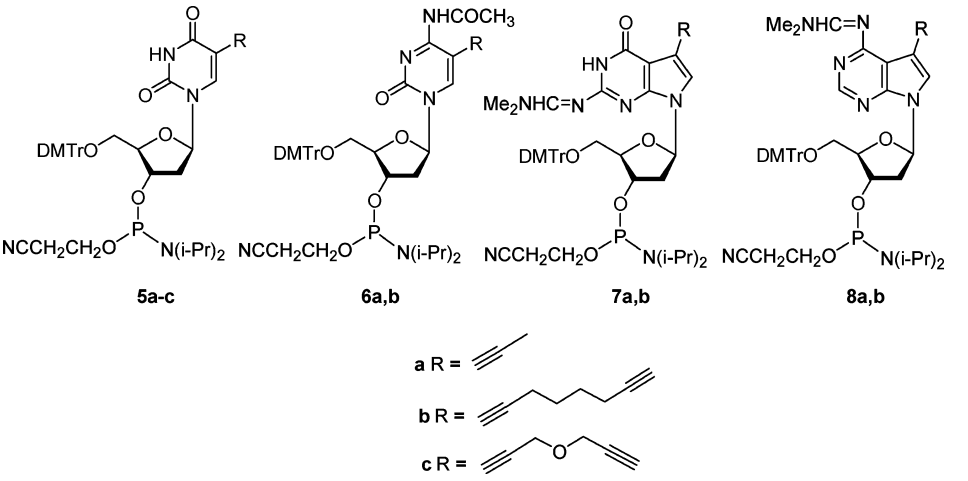
Synthesis and Properties of Monomers and ODNs

The synthesis of the phosphoramidite building blocks **5a–8b** carrying propynyl or octadiynyl has been recently reported for all canonical bases. As it was observed that the lipophilic character of a side chain has a favorable influence on the duplex stability, we extended the work to compounds

TABLE 1 T_m values of oligonucleotide duplexes containing the nucleoside **1a–c**, **2b**, **3b**[a]

Duplex	T_m [°C]	Duplex	T_m [°C]
5′-d(TAG GTC AAT ACT) (**17**)	50	5′-d(TAG G**1a**C AAT ACT) (**21**)	52
3′-d(ATC CAG TTA TGA) (**18**)		3′-d(ATC CAG TTA TGA) (**18**)	
5′-d(TAG G**1c**C AAT ACT) (**19**)	52	5′-d(TAG G**1b**C AAT ACT) (**22**)	52
3′-d(ATC CAG TTA TGA) (**18**)		3′-d(ATC CAG TTA TGA) (**18**)	
5′-d(TAG GTC AAT ACT) (**17**)	53	5′-d(TAG GT**2b** AAT ACT) (**23**)	54
3′-d(A**1c**C CAG TTA **1c**GA) (**20**)		3′-d(ATC CAG TTA TGA) (**18**)	
5′-d(TAG G**1c**C AAT ACT) (**19**)	53	5′-d(TA**3b3b**TCAATACT) (**24**)	55
3′-d(A**1c**C CAG TTA **1c**GA) (**20**)		3′-d(AT C CAGTTAT**3b**A) (**18**)	

[a]Measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration.



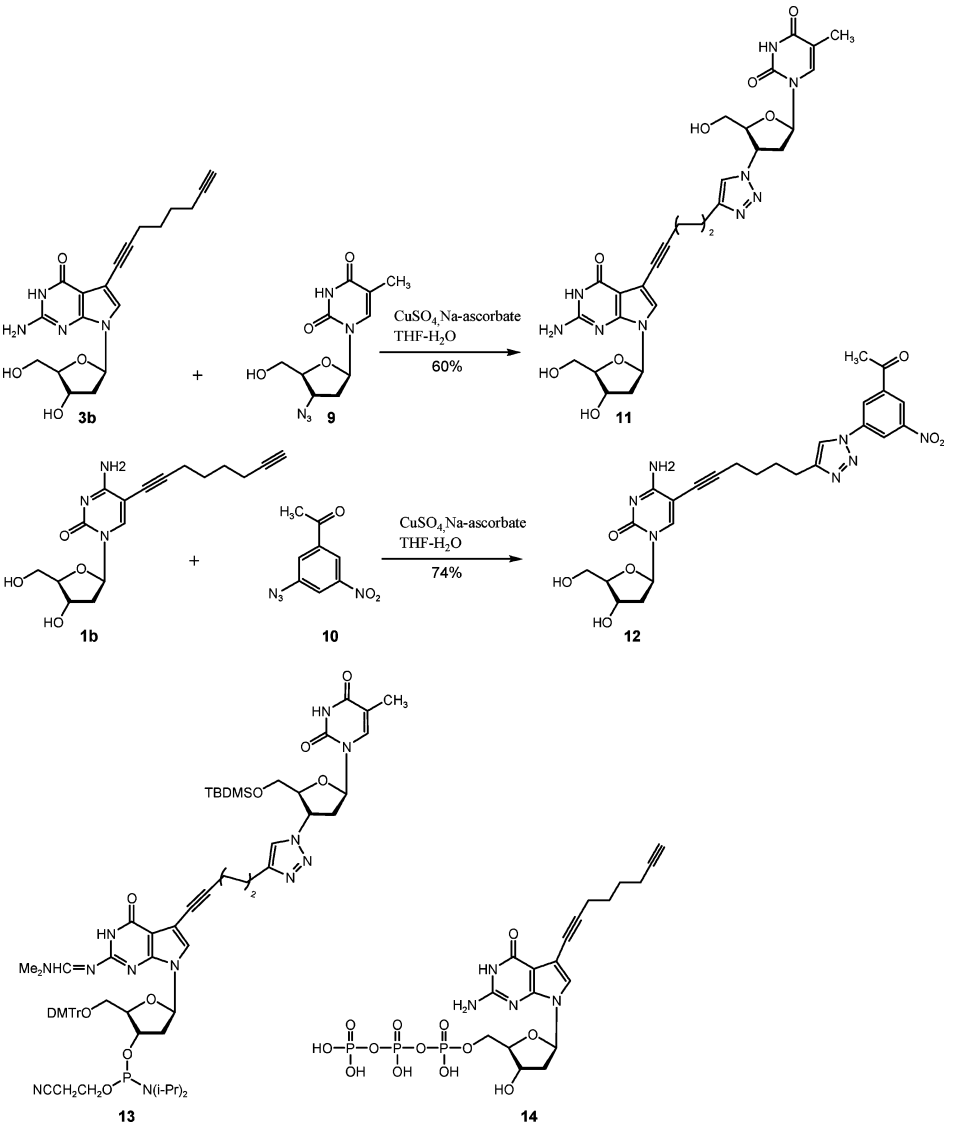
SCHEME 1

bearing the dipropargyl ether side chain **1c**, **5c**.[4,5] They were prepared by the same protocol as described for the octadiynyl residues.

Recently, we studied the influence of octadiynylated nucleosides on DNA duplex stability.[3] It is clear that the oct-1,7-diynyl side chain has a significant positive influence on the duplex stability when it is situated at the 5-position of 2′-deoxycytidine or at the 7-position of 7-deaza-2′-deoxyguanosine, while its effect is smaller in the case of 2′-deoxyuridine. To study the effect of more lipophilic dipropargyl ether moiety at the 5-position of 2′-deoxyuridine, nucleoside **1c** was studied. From Table 1 it is concluded that the dipropargyl ether group enhances the duplex stability by 1–2°C per modification and shows slightly favorable properties compared to octadiynyl side chains. A further advantage is their low lipophilicity which shows a positive affect by multiple incorporations.

Huisgen-Sharpless Functionalization

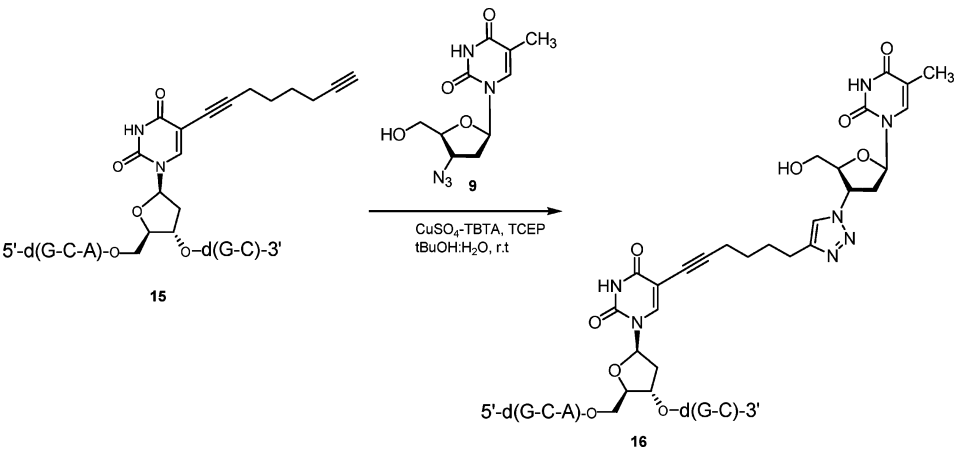
Two strategies have been applied to couple azides with the alkynylated nucleosides. One strategy consists of the pre-modification where azides were coupled to the alkynylated nucleosides. As an example, we functionalized the octadiynylated nucleosides **3b** and **1b** with the antivirally active AZT **9** or other aromatic azide **10** using the "click reaction" to form products **11** and **12**. From the intermediate **11** corresponding phosphoramidite **13** can be prepared (Scheme 2) and used later in solid phase synthesis of diynylated oligonucleotides. The second strategy was the post-modification in which AZT or other azides were coupled to the DNA containing alkynylated nucleosides. For this purpose a 6-mer oligonucleotide **15** containing one 5-octa-1,7-diynyl 2′-deoxyuridine in the place of dT was used as a starting



SCHEME 2

material for the reaction with AZT in the presence of $CuSO_4$-TBTA complex and TCEP as a reducing agent in 10% tBuOH:H_2O, to form the "click oligonucleotide" **16** (Scheme 3). The oligonucleotides were further purified by reversed-phase HPLC using the RP-18 column and were characterized by MALDI-TOF mass spectrometry as well as by the enzymatic digestion with snake-venom phosphodiesterase and alkaline phosphatase.

One more advantage of 7-substituted 7-deazapurines is the acceptance of these molecules by DNA polymerases. The triphosphate of 7-deazapurine



SCHEME 3 Schematic view of the "click reaction" performed on oligonucleotide **15.**

nucleoside **14** can be used as an efficient substitute for sequencing at the single molecule level.[6]

Outlook

The synthesis of four different canonical nucleosides and their phosphoramidite building blocks containing different terminal alkynyl side chains has been achieved and the effect of these chains on DNA duplex stability has been demonstrated. Such nucleosides and oligonucleotides are used for further functionalization using the Huisgen-Sharpless "click reaction." This method can be employed to allow the introduction of any kind of reporter groups into DNA.

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