

DNA Containing Side Chains with Terminal Triple Bonds: Base-Pair Stability and Functionalization of Alkynylated Pyrimidines and 7-Deazapurines

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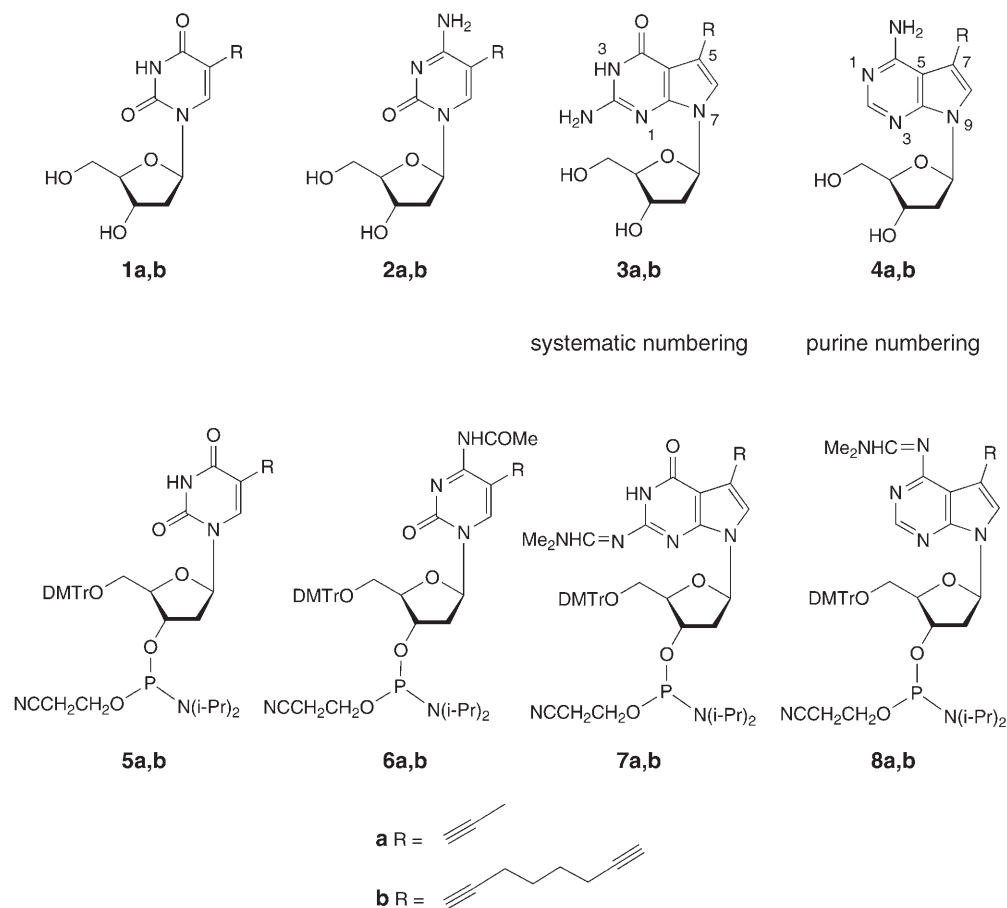
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The synthesis of a series of oligonucleotides containing 5-substituted pyrimidines as well as 7-substituted 7-deazapurines bearing diyne groups with terminal triple bonds is reported. The modified nucleosides were prepared from the corresponding iodo nucleosides and diynes by the *Sonogashira* cross-coupling reaction. They were converted into phosphoramidites and employed in solid-phase synthesis of oligonucleotides. The effect of the diyne modifications on the duplex stability was investigated. The modified nucleosides were used for further functionalization using the protocol of *Huisgen–Sharpless* [2+3] cycloaddition ('click chemistry').

Introduction. – Modified oligodeoxynucleotides (ODNs) are widely used in genomic studies as primers and probes for DNA detection and sequencing. This led to the advances in synthetic nucleic acid chemistry with modifications of the nucleobase, the sugar moiety, or the phosphodiester backbone to improve the hybridization strength, stability, and cellular uptake. It has been shown that the 5-position of pyrimidine [1][2a] and the 7-position of 7-deazapurine nucleosides [3][4] (purine numbering is used in the discussion) are the ideal positions to introduce functionalities, as these sites lie in the major groove of the DNA providing steric freedom. Among the various groups introduced in these positions, the alkynyl groups are of particular importance as they stabilize the DNA duplex structure [2a]. Aminoalkynyl groups are used to incorporate reporter groups into the DNA molecule [2b]. Oligonucleotides containing the nucleoside residues **1a–4a** carrying propynyl groups have been already synthesized using the phosphoramidites **5a–8a** as educts [5–8]. As the chain length increased from propynyl to hexynyl groups, there is an unfavorable effect on the duplex stability, which is most likely due to the increasing hydrophobic character of the alkynyl chain [9]. As the hydrophobic character is decreased by the incorporation of additional triple bonds into the side chain, this principle was now used for the modification of oligonucleotides with long linker arms. Furthermore, side chains carrying terminal C≡C bonds have the potential for further functionalization by the *Huisgen–Sharpless* [2+3] cycloaddition ('click chemistry')

[10][11]. Here, we report on the synthesis of the phosphoramidite building blocks **5b–8b** related to those of the four canonical constituents of DNA. The duplex stability of the oligonucleotides carrying propynyl or octadiynyl side chains will be compared, and the application of the *Huisgen–Sharpless* ‘click chemistry’ will be demonstrated on compound **1b** derivatized with the antivirally active nucleoside AZT.



Results and Discussion. – Recently, our laboratory has reported on the construction of polymeric networks by the functionalization of the 2'-deoxyuridine side chain on a terminal alkyne [12]. For this purpose, the 5-(octa-1,7-diynyl) nucleoside **1b** containing a terminal C≡C bond was prepared from the iodo nucleoside by the *Sonogashira* cross-coupling reaction and used in the formation of cross-linked 5,5-bis-nucleosides. During this work, it became apparent that the *Sonogashira* cross-coupling performed on 2'-deoxy-5-iodouridine, 2'-deoxy-5-iodocytidine, 7-deaza-2'-deoxy-7-iodoadenosine, or 7-deaza-2'-deoxy-7-iodoguanosine forms only the mono-functionalized octadiynyl nucleosides **1b–4b**, when an excess amount of diyne is employed [13]. Reactions on both

$C\equiv C$ bonds occur, when compounds **1b–4b** are employed as intermediates for a second cross-coupling reaction. The nucleosides **1b–4b** were converted to the phosphoramidite building blocks **5b–8b**, respectively, in a similar way as reported for the propynyl compounds **5a–8a** [5–8]. All the monomers were characterized by 1H - and ^{13}C -NMR spectra as well as by elemental analyses.

A series of oligonucleotides were prepared by solid-phase synthesis employing the phosphoramidites **5b–8b** (Table). The coupling efficiency was always higher than 95%. The nucleoside composition of the oligomers was determined by the enzymatic digestion of the oligonucleotides with snake-venom phosphodiesterase and alkaline phosphatase, followed by HPLC (RP-18 column; Figure, a). MALDI-TOF Mass spectra of the oligonucleotides were recorded establishing the correct molecular weights. Next, the role of the oct-1,7-diynyl group in the duplexes **11·12** was studied by incorporating the base modified nucleosides **1b–4b** replacing dT, dC, dG, or dA. The 5-(octa-1,7-diynyl) nucleoside **1b** enhances the stability of 'dA-dT' base pair resulting in a T_m increase of $1-2^\circ$, while a stronger stabilization was observed for **2b** and **3b** ($2-3^\circ$ per modification, Table).

From the Table, it is apparent that the oct-1,7-diynyl side chain has a significant positive influence on the duplex stability when it is situated in the 5-position of 2'-deoxycytidine or in the 7-position of 7-deaza-2'-deoxyguanosine, while its effect is

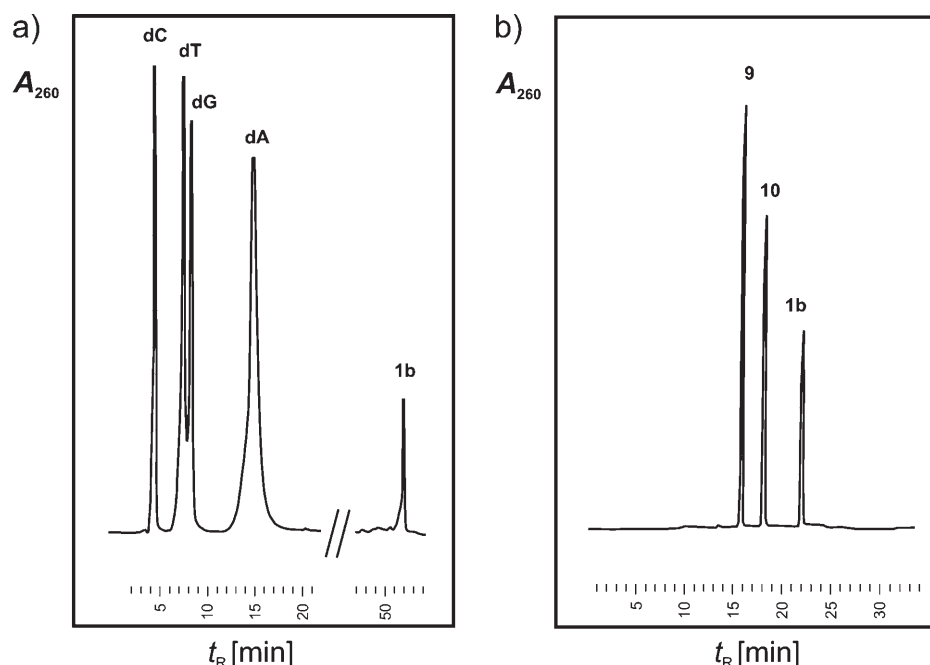


Figure. a) HPLC Profile of the reaction products obtained after enzymatic hydrolysis of the oligomer 5'-d-(TAG G1bC AA1b ACT) with snake venom phosphodiesterase and alkaline phosphatase in 1M Tris HCl buffer (pH 8.0) at 37° . Column: LiChrosphere 100 RP-18 ($5\ \mu m$). The following solvent systems were used: MeCN (A) and 0.1M AcO(Et₃NH)/MeCN 95:5 (B). Gradient: 25 min 100% B, 60 min 0–40% A in B. b) HPLC Profile of the nucleoside mixture of **1b**, **9**, and **10**. Gradient: 35 min 0–50% A in B.

Table. T_m Values of Oligonucleotide Duplexes Containing the Nucleosides **1a–3a** and **1b–3b**^{a)}

| Duplex | T_m | Duplex | T_m |
|--|-------|--|-------|
| 5'-d(TAG GTC AAT ACT) (11) | 50 | 5'-d(TAG GTC AAT ACT) (11) | 50 |
| 3'-d(ATC CAG TTA TGA) (12) | | 3'-d(ATC CAG TTA TGA) (12) | |
| 5'-d(TAG G 1b c AAT ACT) (13) | 52 | 5'-d(TAG G 1a c AAT ACT) (20) | 52 |
| 3'-d(ATC CAG TTA TGA) (12) | | 3'-d(ATC CAG TTA TGA) (12) | |
| 5'-d(TAG GTC AAT ACT) (11) | 53 | 5'-d(TAG GTC AAT ACT) (11) | 52 |
| 3'-d(ATC CAG 1b1b a TGA) (14) | | 3'-d(ATC CAG 1a1a a TGA) (21) | |
| 5'-d(TAG GT 2b AAT ACT) (15) | 53.5 | 5'-d(TAG GT 2a AAT ACT) (22) | 53 |
| 3'-d(ATC CAG TTA TGA) (12) | | 3'-d(ATC CAG TTA TGA) (12) | |
| 5'-d(TAG GT 2b AAT A 2b T) (16) | 54 | 5'-d(TAG GT 2a AAT A 2a T) (23) | 54 |
| 3'-d(ATC CAG TTA TGA) (12) | | 3'-d(ATC CAG TTA TGA) (12) | |
| 5'-d(TA 3b GTC AAT ACT) (17) | 52 | 5'-d(TA 3a GTC AAT ACT) (24) | 50 |
| 3'-d(ATC CAG TTA TGA) (12) | | 3'-d(ATC CAG TTA TGA) (12) | |
| 5'-d(TA 3b 3b TC AAT ACT) (18) | 58 | 5'-d(TA 3a 3a TC AAT ACT) (25) | 55 |
| 3'-d(ATC CA 3b TTA T 3b A) (19) | | 3'-d(ATC CA 3a TTA T 3a A) (26) | |

^{a)} Measured at 260 nm in 1M NaCl, 100 mM MgCl₂, and 60 mM Na-cacodylate (pH 7.0) with 5 μ M + 5 μ M single-strand concentration.

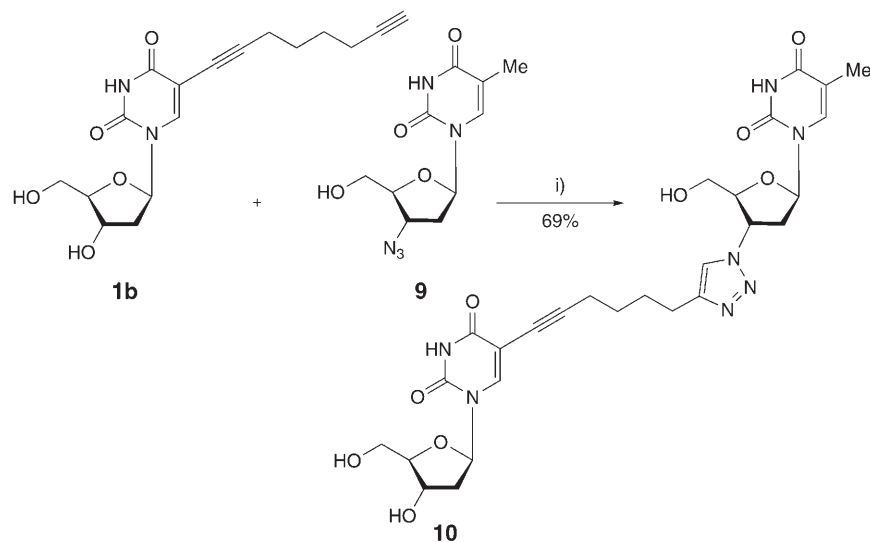
smaller in the case of 2'-deoxyuridine. The most probable reason for this is the formation of a tridentate base pair permitting only very small distortions in the major groove for the linker, whereas the bidentate AT base pair is flexible leading to some sort of freedom for the major-groove functional moieties. The oct-1,7-diynyl residue almost behaves like a propynyl residue, in few sequences it dominates the propynyl group.

The nucleosides **1b–4b** containing terminal triple bonds can be used for further functionalization employing the protocol of the *Huisgen–Sharpless* [2+3] cycloaddition ('click chemistry') [10][11]. As an example, the compound **1b** was used, which undergoes a cycloaddition reaction with AZT (**9**) to form the triazole product **10** (Figure, *b*, Scheme 1). The reaction is performed in aqueous solution at room temperature in the presence of CuSO₄ and sodium ascorbate. NMR Spectra of compound **10**¹⁾ confirmed the bis-nucleoside structure.

Conclusions and Outlook. – Octa-1,7-diynyl nucleosides such as **1b–4b** can be synthesized from the corresponding iodo compounds and octadiyne by the *Sonogashira* cross-coupling reaction as described in [13]. The conversion into the phosphoramidites **5b–8b** and their application in solid-phase oligonucleotides were accomplished according to the standard protocols of oligonucleotide chemistry. The octa-1,7-diyne side chain is stable under conditions of the oligonucleotide synthesis, which was confirmed by MALDI-TOF mass spectrometry as well as by enzymatic digestion with the formation of the monomeric nucleosides. The potential of the cycloaddition

¹⁾ Selected ¹H-NMR chemical shifts for **10** (measured in (D₆)DMSO): 2.10 (s, Me–C(5'')); 6.10 (t, H–C(1'')); 6.40 (t, H–C(1''')); 7.89 (s, 1 H, triazole); 8.06 (s, H–C(6'')); 8.13 (s, H–C(6)); 11.36 (s, HN(3'')), 11.63 (s, HN(3)) (unprimed: alkynyl base, '': sugar moiety of the alkynyl base, '': dT base, ''': sugar dT).

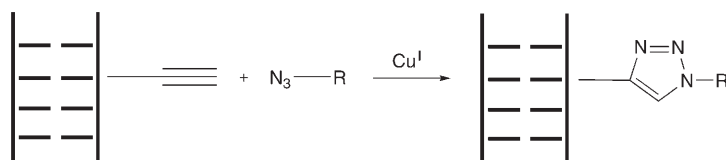
Scheme 1



i) CuSO_4 , Sodium ascorbate, THF/ H_2O , r.t., 30 h

reaction has been demonstrated on the reaction of the nucleoside **1b** with AZT. The methods described above allow the variation of the length and the structure of the side chains, and the structure of the nucleobase (\rightarrow pyrazolo[3,4-*d*]pyrimidines). The azido component is also variable regarding functionalization allowing the introduction of various reporter groups or the cross linking of nucleosides. The cycloaddition reactions demonstrated for a nucleoside are appropriate for nucleotides (mono-, di-, and triphosphates) as well as to oligonucleotides carrying the octa-1,7-diynyl side chain (Scheme 2). This principle is applicable to the solution chemistry as well as to the solid phase modification including gold surfaces or nanoparticles functionalized by α,ω -azido-sulfanylalkanes [14][15]. The method has the potential to find application in nanotechnology.

Scheme 2. Schematic View of the Cycloaddition Reaction Performed on Duplex DNA



R = Variable residues

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