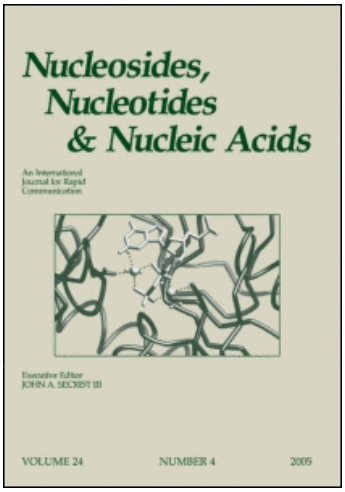

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7-DEAZA-2′-DEOXYXANTHOSINE: NUCLEOBASE PROTECTION AND BASE PAIRING OF OLIGONUCLEOTIDES

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□ *Oligonucleotides containing 7-deaza-2′-deoxyxanthosine (**1**) and 2′-deoxyxanthosine (**2**) were prepared. The 2-(4-nitrophenyl)ethyl group is applicable for 7-deazaxanthine protection that is removed with DBU by β-elimination, while the deprotection of the allyl residue with Pd (0) catalyst failed. Contrarily, the allyl group was found to be an excellent protecting group for 2′-deoxyxanthosine (**2**). The base pairing of nucleosides **1** and **2** with the four canonical DNA constituents as well as with **3** within the 12-mer duplexes is studied.*

Keywords 7-Deaza-2′-deoxyxanthosine; 2′-Deoxyxanthosine; protecting groups; phosphoramidites; oligonucleotides; duplexes; base pairing

INTRODUCTION

2′-deoxyxanthosine (**2**) is very sensitive to acidic conditions[1] while 7-deaza-2′-deoxyxanthosine (**1**), which was first synthesized in our laboratory, is resistant to "depurination" even in acidic conditions.[2] Both nucleosides **1** and **2** form DNA duplexes and triplexes and hence they can be useful for oligonucleotide diagnostics and therapeutics as well as for primer probe applications. Therefore, phosphoramidite building blocks for **1** which can be employed in solid-phase synthesis are needed. Here, we report for the first time on a base protected 7-deaza-2′-deoxyxanthosine phosphoramidite (**6**) which allows multiple incorporations with coupling yields identical to those of the canonical nucleosides. Allyl-protected phosphoramidites **4** and **5** of **1** and **2** respectively were also prepared and used in solid-phase synthesis. The hybridization properties of oligonucleotide duplexes containing **1**

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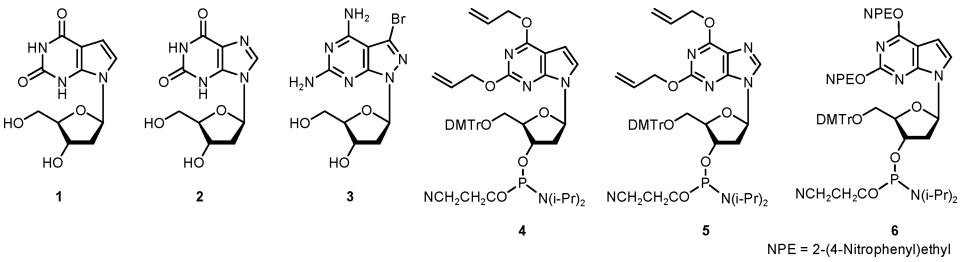
and **2** with the canonical DNA bases as well as with nucleoside (**3**) will be reported (Scheme 1).

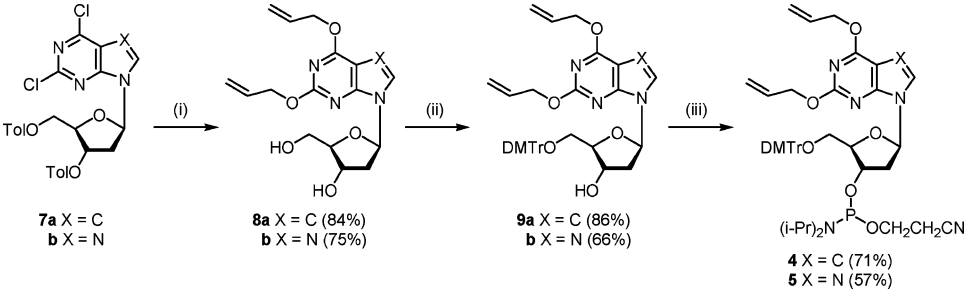


SCHEME 1

RESULTS AND DISCUSSION

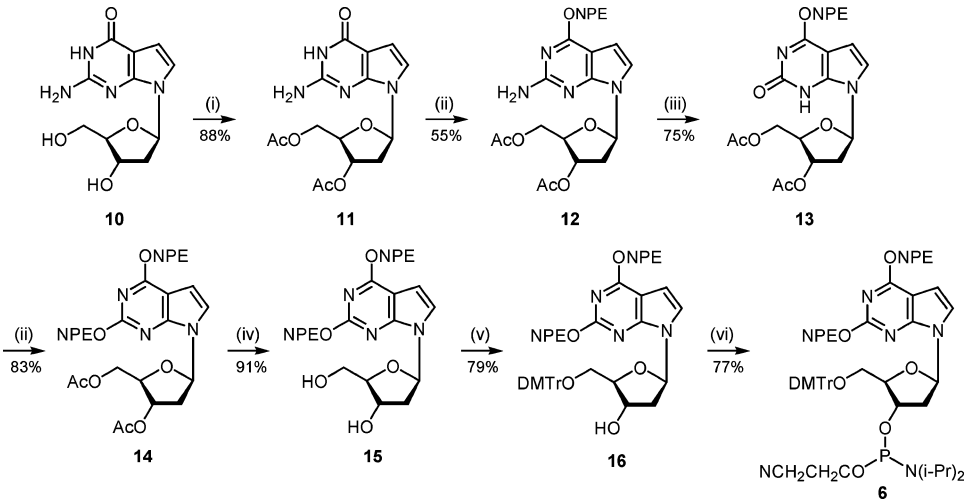
Synthesis of the Phosphoramidites

As an allyl group is sensitive to palladium(0) complexes in the presence of nucleophiles[3] and stable in ammonia, it represents an orthogonal protecting group among the common protecting groups used for oxo-protection. Therefore, this group was selected for the protection of oxo-groups **1** and **2**. For that purpose, compounds **7a**,**b** were treated with 1 M $NaOCH_2CH{=}CH_2$ in allyl alcohol at 50°C furnishing 2,6-bis(allyloxy) compounds **8a**,**b** and the DMT protecting group was introduced with 4,4′-dimethoxytrityl chloride in pyridine yielding the derivatives **9a**,**b** respectively. Subsequent treatment of **9a**,**b** with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite afforded phosphoramidites **4** and **5** of **1** and **2** respectively (Scheme 2). These phosphoramidites gave >95% coupling yields during solid-phase synthesis. The allyl residues from the oligonucleotides containing **2** were deprotected successfully by Pd(0) complex while the oligonucleotides containing **1** undergo oxidation by Pd(0) catalyst.



SCHEME 2 ***Reagents and conditions***: (i) 1 M $CH_2{=}CH\text{-}CH_2ONa/CH_2{=}CH\text{-}CH_2OH$, 50°C; (ii) DMTr-Cl, pyridine, r.t.; (iii) $(i\text{-}Pr)_2NP(Cl)OCH_2CH_2CN$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2.

Due to the difficulties in the deprotection of allyl groups, the 2-(4-nitrophenyl)ethyl (NPE) group was used for the 2,6-dioxo-protection of **1** which is the β-eliminating group that requires a strong base (DBU) for deprotection.[4] For that purpose, the 3′,5′-hydroxyl groups of **10** were blocked by acetylation affording compound **11**. The *Mitsunobu* reaction was performed with **11** and 2-(4-nitrophenyl)ethanol in the presence of DEAD/PPh_3 in THF yielding the 6-oxo-protected compound **12**. The amino group of **12** was transformed into a hydroxyl group with $NaNO_2$/CH_3COOH in a water/acetone mixture furnishing compound **13**. Next, a second *Mitsunobu* reaction was performed on **13** furnishing the fully protected nucleoside **14**. Treatment of **14** with methanolic NH_3 yielded the 2,6-bis(2-(4-nitrophenyl)ethyl)-protected 7-deaza-2′-deoxyxanthosine **15**. Then, compound **15** was protected with the DMT residue to give the derivative **16**. Subsequent treatment of **16** with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite afforded the phosphoramidite **6** (Scheme 3). The phosphoramidite **6** gave >95% coupling yield during solid-phase synthesis and the NPE residues from the oligonucleotides containing **1** were deprotected successfully using 0.5 M DBU in pyridine.



SCHEME 3 ***Reagents and conditions***: (i) $(Ac)_2O$, pyridine, overnight, r. t.; (ii) PPh_3, DEAD, 2-(4-nitrophenyl)ethanol, THF, 1 h r. t.; (iii) 30% aq. AcOH/acetone (2:1), $NaNO_2$, 2 h. r. t.; (iv) NH_3/MeOH, 2 h. r. t.; (v) DMTr-Cl, pyridine, 1 h. r. t.; (vi) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, $(i\text{-}Pr)_2NEt$, DCM, 30 min r. t.

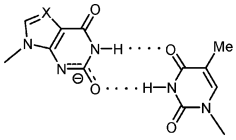
Base Pairing Properties of 7-Deaza-2′-Deoxyxanthosine (1)

According to the T_m values depicted in Table 1 it can be seen that the 7-deaza-2′-deoxyxanthosine (**1**) can act as a universal nucleoside as it forms almost equally stable base pairs with the canonical DNA constituents as

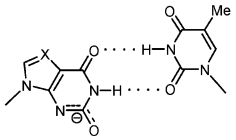
TABLE 1 T_m-Values of oligonucleotides duplexes

Duplex	T_m[°C]	Duplex	T_m[°C]
5′-d(TAG GTC A**2**T ACT) (**17**)	40	5′-d(TAG GTC A**1**T ACT) (**25**)	40
3′-d(ATC CAG T**G**A TGA) (**18**)		3′-d(ATC CAG T**G**A TGA) (**26**)	
25′-d(TAG GTC A**2**T ACT) (**17**)	39	5′-d(TAG GTC A**1**T ACT) (**25**)	43
3′-d(ATC CAG T**A**A TGA) (**19**)		3′-d(ATC CAG T**A**A TGA) (**27**)	
5′-d(TAG GTC A**2**T ACT) (**17**)	42	5′-d(TAG GTC A**1**T ACT) (**25**)	42
3′-d(ATC CAG T**T**A TGA) (**20**)		3′-d(ATC CAG T**T**A TGA) (**28**)	
5′-d(TAG GTC A**2**T ACT) (**17**)	36	5′-d(TAG GTC A**1**T ACT) (**25**)	38
3′-d(ATC CAG T**C**A TGA) (**21**)		3′-d(ATC CAG T**C**A TGA) (**29**)	
5′-d(TAG GTC A**2**T ACT) (**17**)	47	5′-d(TAG GTC A**1**T ACT) (**25**)	46
3′-d(ATC CAG T**3**A TGA) (**22**)		3′-d(ATC CAG T**3**A TGA) (**30**)	
5′-d(TAG G**2**C AA**2** ACT) (**23**)	49	5′-d(TAG G**1**C AA**1** ACT) (**31**)	49
3′-d(ATC C**3**G TT**3** TGA) (**24**)		3′-d(ATC C**3**G TT**3** TGA) (**32**)	

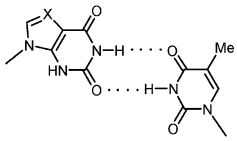
Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0)



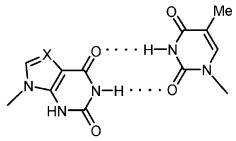
Motif I: 1/ **2** - dT



Motif II: **1/2** - dT



Motif III: **1/2** - dT



Motif IV: **1/2** - dT

reported for 2′-deoxyxanthosine (**2**). Also from its pK_a value (6.7) a significant amount of 7-deaza-2′-deoxyxanthosine forms a mono anion at pH 7.0 in the monomeric state as well as when it is a part of the oligonucleotide chain (Motif **I** - **IV**).[5] Surprisingly, no change in the duplex stability was observed when the pH value was changed from 5.5 to 8.0. It is concluded that the 2-oxo-group of nucleoside **1** does not participate in the base pairing (Motif **IV**). Nucleosides **1** and **2** pair with 3-bromo-1-[2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*- pyrazolo[3,4-*d*]pyrimidin-4,6-diamine (**3**) resulting in duplexes which are as stable as those containing dA-dT pairs. This results from the influence of the bromo substituents and not from the additional amino group as it was already demonstrated on compounds incorporating base pairs of **3** with dT.[6] It is also expected that duplexes are further stabilized when nucleoside **1** bears the 7-halogen or 7-alkynyl substituents.[7]

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