Oligonucleotides Containing 7-Deaza-2′-deoxyxanthosine: Synthesis, Base Protection, and Base-Pair Stability

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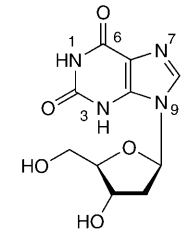
Oligonucleotides incorporating 7-deaza-2′-deoxyxanthosine (**3**) and 2′-deoxyxanthosine (**1**) were prepared by solid-phase synthesis using the phosphoramidites **6**–**9** and **16** which were protected with allyl, diphenylcarbamoyl, or 2-(4-nitrophenyl)ethyl groups. Among the various groups, only the 2-(4-nitrophenyl)ethyl group was applicable to 7-deazaxanthine protection being removed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) by β-elimination, while the deprotection of the allyl residue with Pd^0 catalyst or the diphenylcarbamoyl group with ammonia failed. Contrarily, the allyl group was found to be an excellent protecting group for 2′-deoxyxanthosine (**1**). The base pairing of nucleoside **3** with the four canonical DNA constituents as well as with 3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**4**) within the 12-mer duplexes was studied, showing that 7-deaza-2′-deoxyxanthosine (**3**) has the same universal base-pairing properties as 2′-deoxyxanthosine (**1**). Contrary to the latter, it is extremely stable at the *N*-glycosylic bond, while compound **1** is easily hydrolyzed under slightly acidic conditions. Due to the pK_a values 5.7 (**1**) and 6.7 (**3**), both compounds form monoanions under neutral conditions (95% for **1**; 65% for **3**). Although both compounds form monoanions at pH 7.0, pH-dependent T_m measurements showed that the base-pair stability of 7-deaza-2′-deoxyxanthosine (**3**) with dT is pH-independent. This indicates that the 2-oxo group is not involved in base-pair formation.

Introduction. – The 2′-deoxyxanthosine (**1**) and 2′-deoxyoxanosine (**2**) are the major products of the spontaneous nitrosative deamination of 2′-deoxyguanosine [1] (purine numbering is used throughout the discussion section). Both nucleosides are susceptible to *N*-glycosylic-bond hydrolysis, thereby forming abasic sites in the DNA helix. The 2′-deoxyxanthosine (**1**) is sensitive to acidic conditions [2] but is relatively stable at physiological pH [3][4]. Compound **1** has been prepared by enzymatic transglycosylation [2][5] or nitrosative deamination of dG under alkaline conditions [6]. An enzymatic synthesis of **1** from 2′-deoxyisoinosine has been reported by our laboratory [7]. Contrary to the labile xanthine nucleoside **1**, the glycosylic bond of 7-deaza-2′-deoxyxanthosine (**3**), which was first synthesized in our laboratory, is resistant to 'depurination' even under strong acidic conditions [8]. Both, 2′-deoxyxanthosine (**1**) and 7-deaza-2′-deoxyxanthosine (**3**), form oligonucleotide duplexes and triplexes [9–12]. Also, 2′-deoxyxanthosine (**1**) forms four-stranded (quartets) and/or six-stranded structures in aqueous solution [13]. As the recognition sites of the nucleosides **1** and **3** show the same donor–acceptor pattern as thymidine (dT), base pairs are formed with comple-



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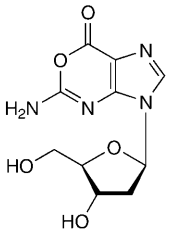
purine numbering



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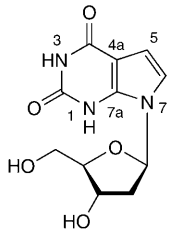

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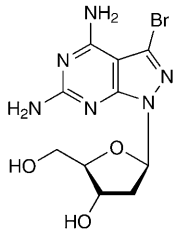
systematic numbering



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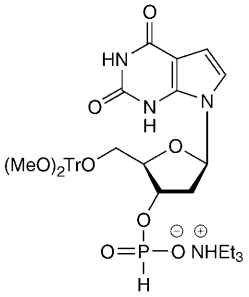

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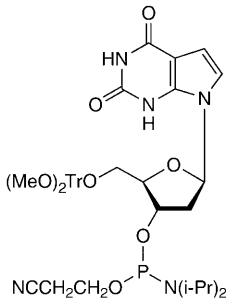

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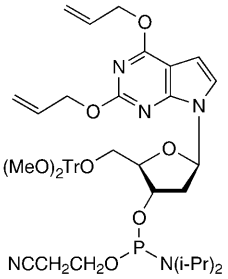

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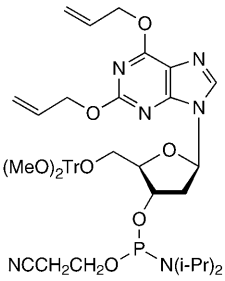

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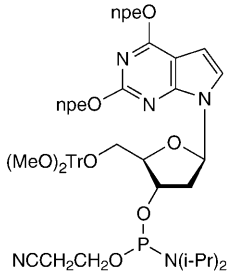


9 npe: 2-(4-nitrophenyl)ethyl

mentary nucleosides which normally pair with dU or dT. However, pairing with dA or related purine-2,6-diamine nucleosides generates purine–purine pairs which can distort the DNA-helix structure but can contribute better stacking interactions induced by an increased surface area.

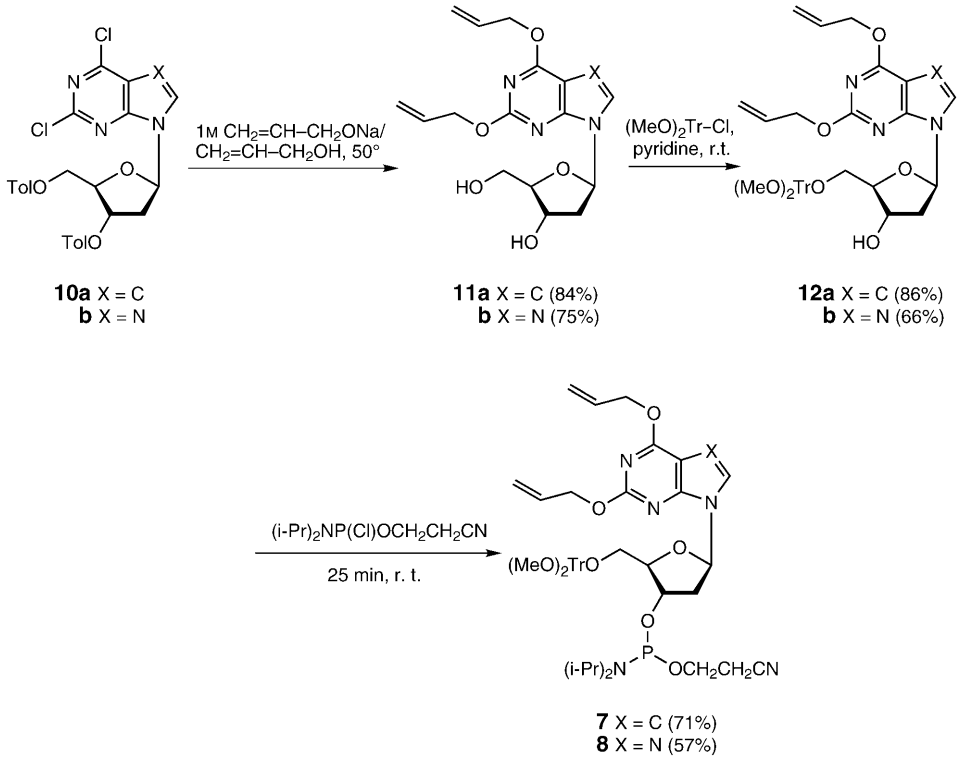
It has been reported that 2′-deoxyxanthosine (**1**) might expand the genetic code when it pairs with a pyrimidine-2,4-diamine *C*-nucleoside [14]. However, experimental data show that the enzymatic incorporation of a pyrimidine-2,4-diamine *C*-nucleoside triphosphate on a DNA template containing compounds **1** or **3** is slow compared to the canonical nucleotides [14]. Therefore, the expansion of the genetic code system by a new base pair containing compound **1** or **3** is limited due to polymerases acceptance [14]. Nevertheless, compound **1** is valuable in the structural investigation of DNA as

it is the major deamination product formed from 2′-deoxyguanosine, and compound **3** is useful for DNA-triplex and -quartet formation. Also, compounds **1** and **3** can be applied to oligonucleotide diagnostics and therapeutics as well as for primer probing. Having this in mind, efforts were made to prepare oligonucleotides containing 7-deaza-2′-deoxyxanthosine (**3**) or 2′-deoxyxanthosine (**1**). Solid-phase oligonucleotide synthesis has already been performed with **3** by using the protocol of phosphonate chemistry. In this case, the base of the 7-deaza-2′-deoxyxanthosine phosphonate **5** stayed unprotected [12][14]. As oligonucleotide synthesis by using phosphonate chemistry is now rarely undertaken, phosphoramidite building blocks which can be employed in solid-phase synthesis are needed.

For the first time, we now report on a base-protected 7-deaza-2′-deoxyxanthosine phosphoramidite (see **9**) which allowed multiple incorporations into an oligonucleotide chain with coupling yields identical to those of the canonical nucleosides. The corresponding unprotected phosphoramidite **6** does not fulfill these necessities as coupling yields were low, by-products were formed, and multiple incorporations were almost impossible [15]. The applicability of allyl, diphenylcarbamoyl, and 2-(4-nitrophenyl)-ethyl protecting groups to block the 2,6-dioxo functions of compound **3** were compared. Allyl protection was also applied to 2′-deoxyxanthosine (**1**) which has been protected earlier with one or two 2-(4-nitrophenyl)ethyl residues as protecting groups [4][9][10]. The hybridization properties of oligonucleotide duplexes containing **1** and **3** with the canonical DNA bases as well as with the 3-bromo-1-[2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**4**) were also studied.

Results and Discussion. – 1. *Nucleobase Protection and Phosphoramidite Synthesis.* Different from the large number of amino-protecting groups used in oligonucleotide synthesis, the number of oxo-protecting groups used for the nucleobase protection is limited. This results from the observation that in some of the cases, *e.g.*, for 2′-deoxyguanosine, oxo-group protection is not required when phosphoramidite chemistry is employed. However, already in the case of 2′-deoxyisoguanosine, it was necessary to protect the 2-oxo-function, otherwise the preparation of a phosphoramidite was difficult, and multiple incorporations of the modified nucleoside were impossible [16]. In the first set of experiments, the allyl group was selected as a protecting group for 7-deaza-2′-deoxyxanthosine (**3**). The *O*-allyl protection of nucleosides has already been reported by *Hayakawa, Noyori*, and co-worker [17]. The *O*-allyl protecting group is stable under standard conditions applied to oligonucleotide synthesis and deprotection. As it is sensitive to the palladium(0) complexes in the presence of nucleophiles but stable in ammonia, it represents an orthogonal protecting group among the common protecting groups used in oligonucleotide chemistry. Phosphoramidites with allyl protection were already prepared for 2′-deoxyguanosine (dG) and 2′-deoxythymidine (dT) and were used successfully in coupling reactions performed in solution or on solid-phase [17][18]. *Eschenmoser* and co-workers used the 2,6-di-*O*-allyl-protected phosphoramidite of hexopyranosylxanthine nucleoside in oligonucleotide solid-phase synthesis [19]. The same protecting group is now employed for the protection of the 2,6-dioxo functions of nucleoside **3**. For that, 2,4-dichloro-7-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]pyrrolo[2,3-*d*]pyrimidine (**10a**) was prepared [20]. It was treated with 1M $NaOCH_2CH{=}CH_2$ in allyl alcohol at 50° furnishing 2,6-bis(allyl-

Scheme 1



10a X = C
b X = N

11a X = C (84%)
b X = N (75%)

12a X = C (86%)
b X = N (66%)

7 X = C (71%)
8 X = N (57%)

oxy) compound **11a** in 84% yield. Then the 4,4′-dimethoxytrityl ($(MeO)_2Tr$) residue was introduced with 4,4′-dimethoxytrityl chloride in pyridine yielding derivative **12a**. Subsequent treatment of **12a** with 2-cyanoethyl diisopropylphosphoramidochloridite afforded phosphoramidite **7** (*Scheme 1*).

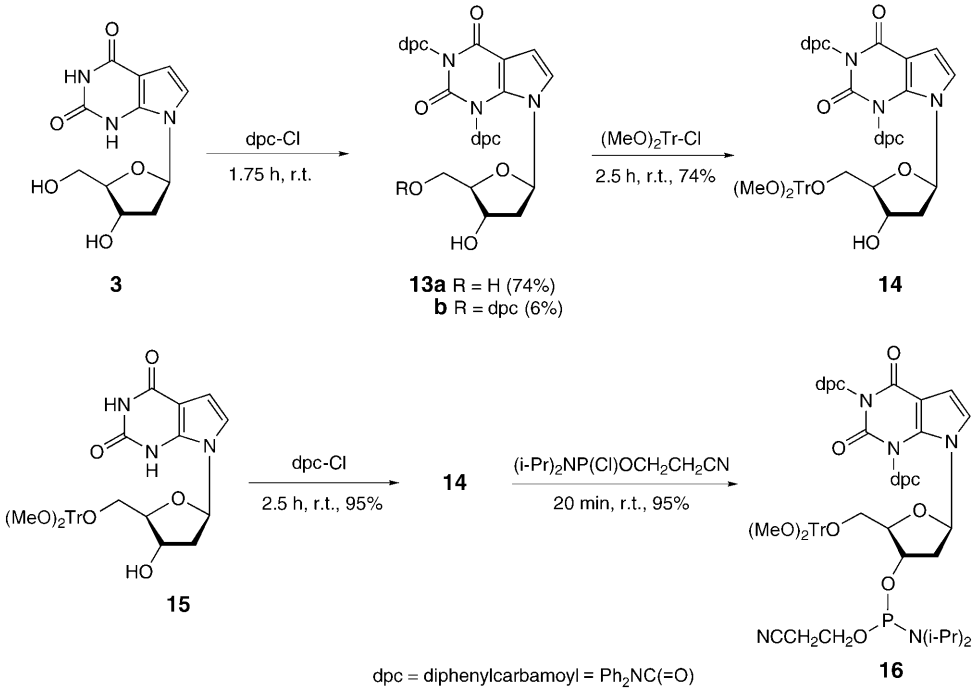
Phosphoramidite **7** was successfully employed in solid-phase synthesis resulting in coupling yields being compatible with normal phosphoramidites (see *Sect. 2*). However, the deblocking of the allyl group from the otherwise deprotected oligonucleotide failed. As it was not clear whether this was due to the 7-deazapurine system, the allyl protecting group was also used for the 2′-deoxyxanthosine (**1**) protection. The same protocol as performed with **3** was also applied to compound **1**. In this case, 2,6-dichloro-9-[2-deoxy-3,5-di-*O*-(*p*-toluoyl)-*β*-D-*erythro*-pentofuranosyl]purine (**10b**) [20] was used as precursor. Allylation of **10b** was performed in a similar way as described for **11a** affording **11b**. Then, compound **11b** was treated with 4,4′-dimethoxytrityl chloride/pyridine furnishing the 4,4′-dimethoxytrityl derivative **12b**. Subsequent treatment with 2-cyanoethyl diisopropylphosphoramidochloridite afforded phosphoramidite **8**. When this building block was used in solid-phase oligonucleotide synthesis in the same way as described for phosphoramidite **7**, the same high coupling yields were realized with **8** as observed with **7**. However, contrary to the 7-deazapurine containing oligonucleotides, the deprotection of the allyl residues from the xanthine

oligomers progressed smoothly furnishing the deprotected oligonucleotides (more information is given in *Sect. 2*). Therefore, the diphenylcarbamoyl (dpc) group was chosen next for the protection of compound **3**.

The dpc group was used earlier as oxo-protecting group for 7-deaza-2′-deoxyguanosine and 7-deaza-2′-deoxyisoguanosine [21][22]. It can be removed under standard conditions (25% aqueous NH_3 solution, 60°). Treatment of **3** with diphenylcarbamic chloride in pyridine afforded compound **13a** (74%) together with tris(diphenycarbamoyl) derivative **13b** (6%) (*Scheme 2*). Different from 7-deaza-2′-deoxyguanosine or 7-deaza-2′-deoxyisoguanosine where the reaction with diphenylcarbamic chloride occurred at the oxo group, the dpc residues of **13a** became attached to the ring N-atoms. This was confirmed by ^{13}C-NMR spectroscopy (*Table 1*). The chemical shifts of C(2) and C(6) of compound **13a** (purine numbering) appeared at similar positions as those of the nucleoside **3** or the N(1), N(3)-methylated derivative **13c** (=7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,7-dihydro-1,3-dimethyl-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione) [23] (**13a**: (δ151.1(C(2)), δ157.6(C(6)); **3**: δ150.4(C(2)), δ159.5(C(6)); **13c**: δ151.5(C(2)), δ158.1(C(6)), while for the *O*-allyl-protected compound **11a** the C(2) and C(6) signals are shifted downfield (δ160.1(C(2)) 162.7(C(6))) (see *Table 1*). Hence, the dpc residues of compound **13a** are connected to the ring N-atoms. As in related nucleosides such as 2′-deoxyuridine or 6-aza-2′-deoxyuridine the protection of the lactam N-atoms by acyl protecting groups was successfully employed in oligonucleotide synthesis [24], the synthetic route with compound **13a** was continued. The latter was treated with $(MeO)_2Tr-Cl$ in pyridine affording the 4,4′-dimethoxytrityl derivative **14** in 74% yield. To circumvent the side reaction of **3** with dpc–Cl on OH groups, the 5′-hydroxy group of **3** was protected first with the $(MeO)_2Tr$ residue resulting in the $(MeO)_2Tr$ derivative **15**. Then compound **15** was treated with dpc–Cl affording compound **14** (95% yield). Subsequently, the phosphoramidite **16** was prepared by using **14** and 2-cyanoethyl diisopropylphosphoramidochloridite (*Scheme 2*). Finally, the dpc-protected phosphoramidite **16** was employed in solid-phase oligonucleotide synthesis which resulted in coupling yields similar to those of the allyl-protected phosphoramidite **7** (see *Sect. 2*). However, also in this case, we were not able to obtain a clean deprotection product from the oligonucleotide (25% aqueous NH_3 solution, 60°).

Due to the difficulties encountered during the removal of the allyl and dpc groups from 7-deazaxanthine oligonucleotides, the 2-(4-nitrophenyl)ethyl (npe) group was selected for the protection of the oxo functionalities of **3**. It is a β-eliminating group requiring a strong base (DBU=1,8-diazabicyclo[5.4.0]undec-7-ene) to achieve deprotection. Earlier, this group was used by *Pfleiderer* and co-workers for the protection of 2′-deoxyguanosine [25]. The first solid-phase oligonucleotide synthesis with 2′-deoxyxanthosine (**1**) which was performed by *Eritja* and co-workers used mono npe-protected, *i.e.*, 6-*O*-[2-(4-nitrophenyl)ethyl]-protected phosphoramidite [9]. As this resulted in low coupling yields and by-products, *Herdewijn* and co-workers prepared the 2,6-bis-*O*-npe-protected phosphotriester which was applied to oligonucleotide solution synthesis [26]. Later, *Battersby* and co-workers reported the synthesis of the 2,6-bis-*O*-npe-protected phosphoramidite of **1** in which the 2-oxo group was protected with the npe residue by using npe–I and Ag_2CO_3 (*Königs–Knorr* conditions) [10]. Also, the 2,6-bis-*O*-npe-protected phosphoramidite of **1** was prepared by the *Mitsunobu* reaction [4]. The *Mitsunobu* conditions were now used to synthesize the bis-*O*-npe-protected

Scheme 2



phosphoramidite **9** (*Scheme 3*). For this, 7-deaza-2′-deoxyguanosine (c^7G_d) [27] was chosen as a starting material. In the initial step, the 3′,5′-hydroxy groups of c^7G_d were blocked by acetylation with Ac_2O in pyridine affording compound **17** (88% yield). Next, the 2-amino group of **17** was protected with the 4,4′-dimethoxytrityl group to give **18**. When the *Mitsunobu* reaction was performed with **18** and 2-(4-nitrophenyl)ethanol in the presence of DEAD/PPh_3 in dioxane, the $(MeO)_2Tr$ residue was cleaved from the 2-amino function, and compound **19** was isolated in only 23% yield. Therefore, the *Mitsunobu* reaction was directly applied to the 3′,5′-di-*O*-acetyl-protected compound **17** in THF yielding the 6-*O*-protected nucleoside **19** (55%). The amino group of compound **19** was transformed to an OH group with $NaNO_2$/AcOH in H_2O/acetone furnishing compound **20**. Next, a second *Mitsunobu* reaction with **20** gave the fully protected nucleoside **21**. Treatment of **21** with methanolic NH_3 yielded the 2,6-bis-*O*-[2-(4-nitrophenyl)ethyl]-protected 7-deaza-2′-deoxyxanthosine **22**. Then, compound **22** was protected with the $(MeO)_2Tr$ residue to give the derivative **23**. Subsequent treatment of **23** with 2-cyanoethyl diisopropylphosphoramidochloridite afforded the phosphoramidite **9**. The position of the npe residue of compound **22** was established by ^{13}C-NMR spectroscopy. According to *Table 1*, the chemical-shifts of C(2) and C(6) of the npe-protected compound **22** appear at almost the same δ as observed for the di-*O*-allyl nucleoside **11a** (**22**: δ160.2(C(2)), δ162.8(C(6)); **11a**: δ160.1(C(2)), 162.7(C(6))). Hence the npe residues of **22** protect the oxo functions.

To compare the applicability of the npe-protected phosphoramidite **9** in the oligonucleotide synthesis, we prepared the 2,6-*O*-unprotected phosphoramidite **6** as well.

Table 1. *^{13}C-NMR Chemical Shifts of 2′-Deoxyxanthosine and 7-Deaza-2′-deoxyxanthosine Derivatives*[a])

Compound[b])[c])	C(2)[d]) C(2)[d])	C(4)[d]) C(6)[d])	C(4a) C(5)	C(6) C(8)	C(7a) C(4)	C(5) C(7)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
11b[e])	159.9	160.4	116.8	140.9	153.0	–	83.3	[f])	70.6	87.7	61.6
12b[e])	159.8	160.5	117.2	[g])	152.8	–	83.7	[f])	70.7	86.0	64.3
11a	160.1	162.7	100.3	122.6	153.0	99.0	82.8	[f])	71.3	87.2	61.9
12a	160.0	162.6	100.4	[g])	152.9	98.9	83.0	[f])	70.8	85.3	64.3
13a	151.1	157.6	107.3	[g])	141.5	99.7	83.1	[f])	70.8	87.5	61.7
13b	151.0	157.7	107.2	[g])	141.4	99.9	82.9	[f])	70.7	84.2	65.8
3[h])	150.4	159.5	99.4	117.6	138.0	103.1	85.5	[f])	70.8	87.3	61.4
13c[i])	151.5	158.1	101.0	117.7	138.0	103.5	84.7	[f])	70.2	87.4	61.4
14	151.1	158.0	107.3	[g])	141.5	99.9	82.7	[f])	70.6	85.5	64.0
15	151.1	159.6	99.0	115.9	138.9	103.7	83.1	[f])	70.0	85.2	63.6
17	152.8	158.6	100.1	116.3	150.9	102.9	80.8	35.7	74.6	82.3	63.8
18	157.6	158.3	100.5	116.8	149.8	102.6	80.7	36.4	74.5	83.0	63.6
19	159.6	162.3	97.1	119.0	154.7	99.7	80.7	35.5	74.6	82.2	63.8
20	160.3	163.3	98.7	120.5	[j])	99.9	80.9	35.6	74.5	82.7	63.7
21	160.3	162.9	99.5	122.6	153.2	100.5	80.9	35.5	74.3	83.1	63.7
22	160.2	162.8	99.0	122.5	153.0	100.2	82.7	[f])	71.0	87.2	61.9
23	160.2	162.8	99.0	122.6	152.9	100.4	82.9	[f])	70.7	85.3	64.2

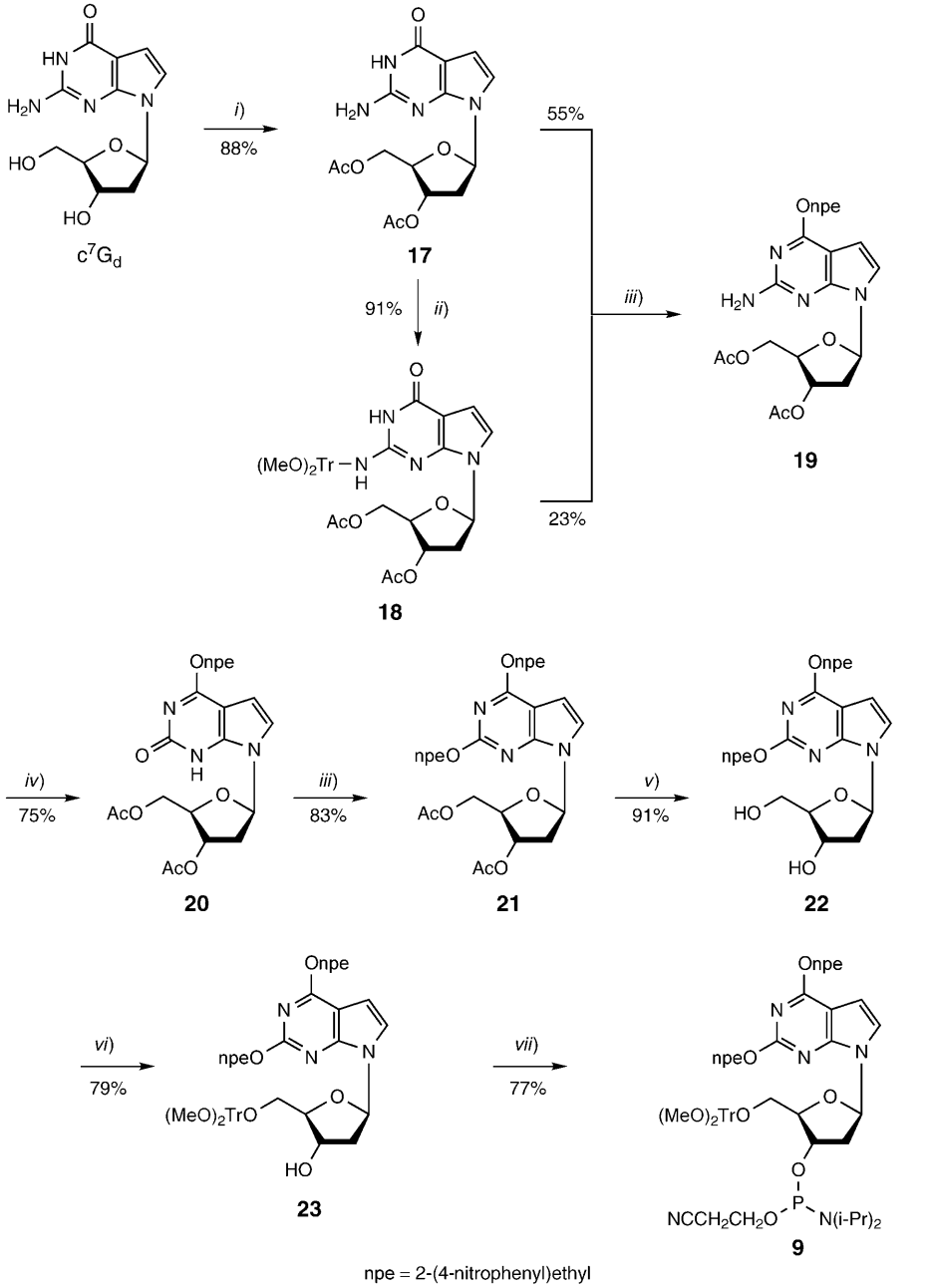
[a]) Measured in (D_6)DMSO at 25°. [b]) First heading row = systematic numbering. [c]) Second heading row = purine numbering. [d]) Tentative. [e]) Only purine numbering is used. [f]) Superimposed by (D_6)DMSO. [g]) Overlapped with aromatic signals. [h]) [8] i) [23] [j]) Not detected.

For this compound **15** was treated with 2-cyanoethyl diisopropylphosphoramidochloridite in the presence of diisopropyl(ethyl)amine which afforded the phosphoramidite **6** (*Scheme 4*).

The structures of all new compounds were confirmed by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy as well as by elemental analyses (see *Table 1* and *Exper. Part*). The ^{13}C-NMR chemical-shift assignment was made by means of gated-decoupled ^{13}C-NMR spectra as well as by comparison with the other related 7-deazapurine nucleosides [8][23]. The assignments for C(2) and C(6) are tentative. The assignment of C(1′) and C(4′) are based on the difference of the ^1J(C,H) coupling constants, which are larger for C(1′) than for C(4′) [28].

2. *Synthesis and Characterization of Oligonucleotides* **26**–**32**. For the 12-mer-duplex studies based on the reference duplex **24**·**25** (see *Sect. 3*), the oligonucleotides **26**–**40** were needed. Oligonucleotide syntheses were carried out at a 1-μmol scale following the synthesis protocol for phosphoramidite chemistry. The phosphoramidites **6**–**9** and **16** were used together with those of the phosphoramidites of canonical DNA constituents as well as that of the pyrazolo[3,4-*d*]pyrimidine nucleoside **4** [29]. The coupling yields of the oligonucleotide samples **26**–**32** are displayed in *Table 2*. From this it is apparent that the coupling steps of the modified bases proceed with a similar efficiency as those of the canonical phosphoramidites. The syntheses were performed with the $(MeO)_2$Tr-on mode. The following protocols were employed: *i*) allyl protection, *ii*) npe-protection, and *iii*) diphenylcarbamoyl protection. The deallylation was performed on the solid-support-bound oligonucleotide with [$Pd^0(PPh_3)_4$] in the presence of PPh_3,

Scheme 3



i) Ac_2O, pyridine, overnight, r.t. *ii*) $(MeO)_2Tr-Cl$, pyridine, overnight, r.t. *iii*) PPh_3, DEAD (diethyl diazenedicarboxylate), 2-(4-nitrophenyl)ethanol, THF or dioxane, 1 h, r.t. *iv*) 30% aq. AcOH/acetone 2 : 1, $NaNO_2$, 2 h, r.t. *v*) NH_3/MeOH, 2 h, r.t. *vi*) $(MeO)_2Tr-Cl$, pyridine, 1 h, r.t. *vii*) 2-Cyanoethyl diisopropylphosphoramidochloridite, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 30 min, r.t.

Scheme 4

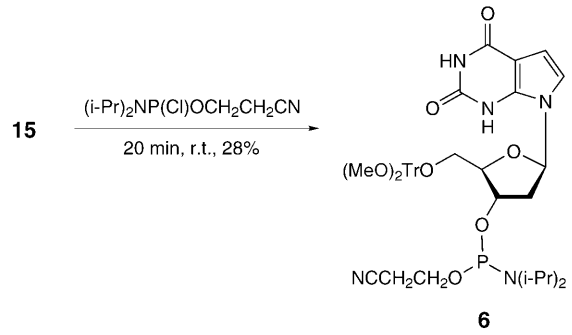


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Table 2. *Coupling Yields, Deprotection Conditions, and Molecular Masses of Oligonucleotides Obtained with Various Phosphoramidites*

Oligonucleotide	Phosphoramidite	Coupling yield [%]	Deprotection protocol	$[M^+H^+]$	
				calc.	found
[5′-d(TAG G**3**C AA**3** ACT)] (**26**=**30**) in mixture	**7** [a])	97, 98	Pd^0, r.t.	3695	[e])
[5′-d(TAG G**1**C AA**1** ACT)] (**27**)	**8** [a])	95, 94	Pd^0, r.t.	3696	3696
[5′-d(TAG GTC A**1**T ACT)] (**28**)	**8** [a])	95	Pd^0, r.t.	3662	3661
[5′-d(TAG G**3**C AA**3** ACT)] (**29**=**30**) in mixture	**16** [b])	98, 85	NH_3, 60°	3695	[e])
[5′-d(TAG G**3**C AA**3** ACT)] (**30**)	**9** [c])	100, 96	DBU in py	3695	3695
[5′-d(TAG GTC A**3**T ACT)] (**31**)	**9** [c])	96	DBU in py	3660	3660
[5′-d(TAG G**3**C AAT ACT)] (**32**)	**6** [d])	60	NH_3, 60°	3669	3670

[a]) Allyl-protected [b]) dpc-protected. [c]) npe-protected. [d]) Unprotected. [e]) See text.

and HCOOH/butylamine 1:1 as scavenger in anhydrous THF [17][18] (for details, see *Exper. Part*). The excess of the reagent was removed with 5M aq. *N*,*N*-diethyldithiocarbamate (=diethylcarbamodithioate=DDTC). For the npe-group removal, the solid-supported oligonucleotides were treated with 0.5M DBU in pyridine. The solid-support-bound oligonucleotides obtained by the allyl or the npe deprotection were then deprotected in 25% aqueous NH_3 solution at 60°. This condition was also directly applied to the dpc-protected oligonucleotides. The $(MeO)_2$Tr-protected oligomers were purified by reversed-phase HPLC. The $(MeO)_2$Tr-residues were removed with 2.5% dichloroacetic acid in CH_2Cl_2 followed by reversed-phase HPLC (*RP-18*) purification.

The allyl protocol *i*) was successfully employed for the oligonucleotides **27** and **28** containing 2′-deoxyxanthosine (**1**) while the deprotection of the *O*-allyl-protected 7-deaza-2′-deoxyxanthosine residues failed (→ **26** in mixture). As the 7-deazapurine nucleobases have a significantly lower redox potential than the corresponding purine compounds, destructive oxidation by a Pd^{II} procedure during the deallylation is the most likely process causing this failure. The npe deprotection protocol *ii*) was successfully applied for the oligonucleotides **30** and **31** containing 7-deaza-2′-deoxyxanthosine

(**3**) as it was reported for 2′-deoxyxanthosine [4][10]. The dpc deprotection protocol *iii*) which was applied only to oligonucleotides containing 7-deaza-2′-deoxyxanthosine (**3**) failed as the protecting groups could not be removed from the protected precursor of oligomer **29**.

Next the oligonucleotides were characterized by MALDI-TOF mass spectrometry (*Table 2*). Correct masses (reflector mode) were found for the oligonucleotides **27** and **28** containing 2′-deoxyxanthosine (**1**) prepared *via* allyl protection (*Table 2*). In both oligonucleotides, apart from the main peaks, mass peaks with low intensity were observed which corresponds to the release of one or two xanthine bases and the addition of one or two Na^+ ions. The depurination of the xanthine moiety is expected as 2′-deoxyxanthosine is susceptible to acidic conditions (matrix used for MALDI-TOF: 3-hydroxypicolinic acid).

The mixture obtained from the allyl removal expected to give oligonucleotide **26** containing compound **3** showed masses of 3694, 3713, and 3731 Da. While the low mass corresponds to the deprotected molecule **26**, the higher masses represent molecules which were oxidized (addition of 19 and 37 Da with respect to the deprotected **26**). The complex reaction mixtures obtained by the deprotection of the dpc groups expected to give oligomer **29** containing compound **3** gave MALDI-TOF spectra with masses of the deprotected oligomer **29** as well as of partially deprotected compounds. Correct masses were obtained for oligonucleotides **30** and **31** containing compound **3** prepared by the npe protection. No additional peaks caused by 'depurination' or oxidation were detected. This results from the high glycosylic-bond stability of compound **3** under acidic conditions. Similar observations have been already reported for oligonucleotides incorporating 7-deaza-2′-deoxyadenosine or 7-deaza-2′-deoxyguanosine residues which are also not susceptible to 'depurination' [30]. As these nucleosides could be used as surrogates for the canonical purine compounds in enzyme catalyzed DNA incorporation, the sensitivity of MALDI-TOF spectroscopy was significantly increased by using these modified analogs [30]. Thus, the 2-(4-nitrophenyl)ethyl (npe) group proves to be an ideal protecting group for the protection of 2,6-dioxo functions of 7-deaza-2′-deoxyxanthosine (**3**), while the allyl group represents an orthogonal protecting group for 2′-deoxyxanthosine (**1**) and not for 7-deaza-2′-deoxyxanthosine (**3**).

Also, the composition of the oligonucleotides containing **1** and **3** was determined by reversed-phase HPLC (*RP-18*) after tandem hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase in 0.1M *Tris*·HCl buffer (pH 8.3) (*Fig. 1, Exper. Part*). According to *Fig. 1* the canonical as well as the modified residues were detected. From the peak area, the nucleoside ratio was determined which was in agreement with the composition of the oligonucleotides. On the lipophilic reversed-phase column, 7-deaza-2′-deoxyxanthosine (**3**) shows a lower mobility than that of 2′-deoxyxanthosine (**1**), which corresponds to its increased hydrophobic character.

3. *Base Pairing of Oligonucleotides.* Next, the duplex stability of the oligonucleotides was investigated. Oligonucleotides containing 2′-deoxyxanthosine (**1**) have been reported, and their base pairing was discussed [9][10]. As nothing is known about the duplex stability of oligonucleotides containing 7-deaza-2′-deoxyxanthosine (**3**), we studied this behavior with respect to the 12-mer duplex 5′-d(TAG GTC AAT ACT) (**24**)·3′-d(ATC CAG TTA TGA) (**25**) which is used as reference in most of

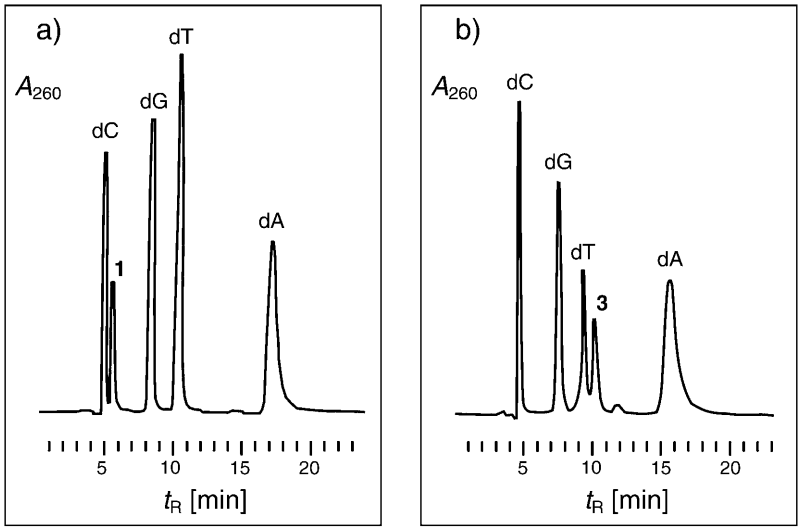

Fig. 1. *Reversed-phase HPLC profiles after the enzymatic hydrolysis of* a) *oligonucleotide* **28** *containing* **1** *and* b) *oligonucleotide* **30** *containing* **3** *with snake-venom phosphodiesterase and alkaline phosphatase in 0.1*M Tris·*HCl buffer* (pH 8.3). Eluent: *A*, flow rate 0.7 ml/min (*A* = 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95 : 5).

our studies [29]. In the first strand, **24**, one dA residue located near the centre of the molecule was replaced by compounds **3** or by **1** for comparison. The second strand, **25**, was modified in such a way that the four canonical bases were located opposite to compound **1** or **3**. The T_m values are given in *Table 3*.

According to *Table 3*, the incorporation of the nucleosides **1** or **3** opposite to the four canonical nucleosides resulted in a destabilization of the duplex structure compared to unmodified duplex **24**·**25**. All T_m values are now in a similar range. Nevertheless, the melting temperatures are higher when 7-deaza-2′-deoxyxanthosine (**3**) is located opposite to dT or dG or dA than to dC. A similar behavior was observed for oligonucleotide duplexes containing 2′-deoxyxanthosine (**1**), except that **1** shows a lower T_m opposite to dA (*Table 3*). This melting behavior fulfils the requirements of universal nucleosides. The T_m values of duplexes containing a classical universal nucleoside, namely 5-nitro-1*H*-indole 2′-deoxy-β-D-ribofuranoside (**N**) which solely contributes base stacking to duplex stability are shown for comparison [31][32]. Other universal nucleosides, namely 2′-deoxyinosine as well as 7-deaza-2′-deoxyinosine [33] which are related to compounds **1** and **3** show a similar behavior. However, the base pairs of the 2′-deoxyinosines with dC are always stronger than those with the other canonical residues [33]. The T_m decrease of duplexes within universal base pairs formed by 2′-deoxyxanthosine (**1**) and 7-deaza-2′-deoxyxanthosine (**3**) compared to 2′-deoxyinosine [33] can be explained by the spatial demand of the 2-oxo group. Related observation were already made on dA·dT base pairs substituted with halogen atoms at the 2-position.

Possible base pairs of 7-deaza-2′-deoxyxanthosine with dG, dA, and dC (motif **I**, **II,** and **III** resp.) are depicted in *Fig. 2*. Pairing motifs for 2′-deoxyxanthosine (**1**) have

Table 3. T_m *Values and Thermodynamic Data of Oligonucleotide Duplexes Containing 2′-Deoxyxanthosine* (**1**) *and 7-Deaza-2′-deoxyxanthosine* (**3**)[a])

Duplexes	T_m [°]	ΔT_m [°]	$\Delta G°_{310}$ [kcal/mol]	$\Delta S°$ [cal/K mol]	$\Delta H°$ [kcal/mol]
5′-d(TAG GTC AAT ACT)-3′ (**24**) 3′-d(ATC CAG TTA TGA)-5′ (**25**)	47	0	−10.6	−253	−89
5′-d(TAG GTC A**1**T ACT)-3′ (**28**) 3′-d(ATC CAG T**T**A TGA)-5′ (**33**)	42	−5	−9.1	−232	−81
5′-d(TAG GTC A**1**T ACT)-3′ (**28**) 3′-d(ATC CAG T**G**A TGA)-5′ (**34**)	40	−7	−8.6	−209	−73
5′-d(TAG GTC A**1**T ACT)-3′ (**28**) 3′-d(ATC CAG T**A**A TGA)-5′ (**35**)	39	−8	−8.2	−226	−78
5′-d(TAG GTC A**1**T ACT)-3′ (**28**) 3′-d(ATC CAG T**C**A TGA)-5′ (**36**)	36	−11	−7.2	−196	−68
5′-d(TAG GTC A**3**T ACT)-3′ (**31**) 3′-d(ATC CAG T**T**A TGA)-5′ (**33**)	42	-5	−10.3	−208	−75
5′-d(TAG GTC A**3**T ACT)-3′ (**31**) 3′-d(ATC CAG T**G**A TGA)-5′ (**34**)	40	−7	−10.0	−348	−118
5′-d(TAG GTC A**3**T ACT)-3′ (**31**) 3′-d(ATC CAG T**A**A TGA)-5′ (**35**)	43	−4	−10.7	−206	−75
5′-d(TAG GTC A**3**T ACT)-3′ (**31**) 3′-d(ATC CAG T**C**A TGA)-5′ (**36**)	38	−9	−9.3	−226	−80
5′-d(TAG GTC A**N**T ACT)-3′ (**37**) 3′-d(ATC CAG T**T**A TGA)-5′ (**33**)	42[b])	−5	−8.7	−173	−63
5′-d(TAG GTC A**N**T ACT)-3′ (**37**) 3′-d(ATC CAG T**G**A TGA)-5′ (**34**)	41[b])	−6	−8.2	−163	−59
5′-d(TAG GTC A**N**T ACT)-3′ (**37**) 3′-d(ATC CAG T**A**A TGA)-5′ (**35**)	44[b])	−3	−8.9	−181	−65
5′-d(TAG GTC A**N**T ACT)-3′ (**37**) 3′-d(ATC CAG T**C**A TGA)-5′ (**36**)	45[b])	−2	−9.6	−210	−75
5′-d(TAG GTC A**1**T ACT)-3′ (**28**) 3′-d(ATC CAG T**4**A TGA)-5′ (**38**)	47	0	−11.6	−237	−85
5′-d(TAG GTC A**3**T ACT)-3′ (**31**) 3′-d(ATC CAG T**4**A TGA)-5′ (**38**)	46	−1	−10.9	−192	−71
5′-d(TAG G**1**C AA**1** ACT)-3′ (**27**) 3′-d(ATC C**4**G TT**4** TGA)-5′ (**39**)	49	+2	−11.9	−211	−77
5′-d(TAG G**3**C AA**3** ACT)-3′ (**30**) 3′-d(ATC C**4**G TT**4** TGA)-5′ (**39**)	49	+2	−12.1	−199	−74
5′-d(TAG GT**C** AA**T** ACT)-3′ (**40**) 3′-d(ATC C**4**G TT**4** TGA)-5′ (**39**)	59	+12	−13.9	[c])	[c])

[a]) Measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration. [b]) Measured in 1M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM single-strand concentration, **N** = 5-nitro-1 *H*-indole 2′-deoxy-β-D-ribofuranoside [32]. [c]) Not reported [29].

already been reported [9][34]. Similar modes are expected for 7-deaza-2′-deoxyxanthosine (**3**). However, as we observed a significant T_m increase when compound **3** pairs with dA (*Table 3*), a change from the *Watson–Crick* to the *Hoogsteen* mode might be the reason for this behavior (*Fig. 2*, motif **IV**).

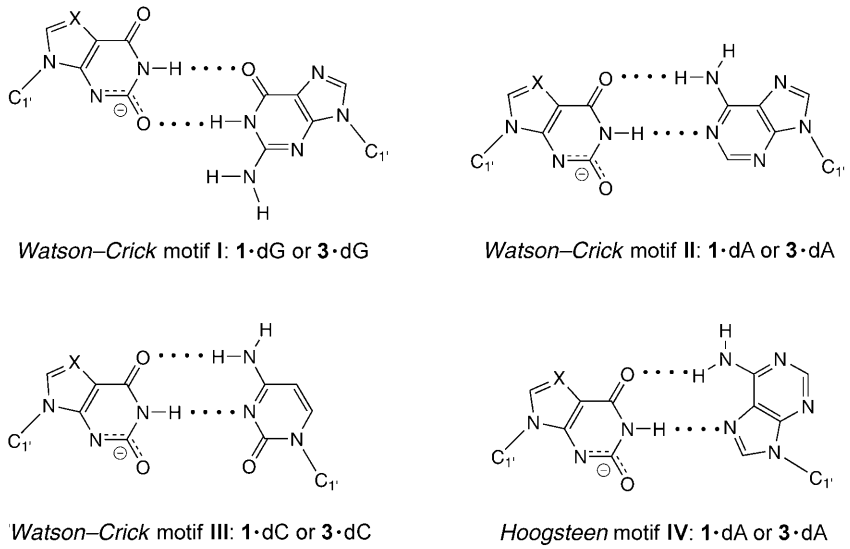

Fig. 2. *Base-pair motifs for compounds* **3** (X = CH) *or* **1** (X = N) *with dG, dC, and dA*

The oxo groups play an important role in the properties and functions of 2′-deoxyxanthosine (**1**) or its 7-deaza analogue **3**. The excellent review of *Shugar* and co-workers has already discussed many aspects of the special properties of the xanthine and the xanthosine molecule [34]. While all canonical nucleosides are neutral under physiological conditions, the xanthosine exists predominantly as monoanion. The pK_a value refers to the deprotonation of H–N(3) leading to a mesomeric stabilized 2-oxo anion of compound **1**. According to its pK_a of 5.7, *ca.* 95% of the 2′-deoxyxanthosine molecules are monoanions at pH 7.0. Thus it was suggested that not the neutral molecule but the monoanion contributes to base pairing. The situation becomes even more complicated as two different motifs can be formed due to the donor and acceptor pattern of the molecules involved in base pairing. *Fig. 3* shows possible base-pair motifs for 2′-deoxyxanthosine (**1**) as well as for 7-deaza-2′-deoxyxanthosine (**3**) with dT within duplex DNA.

Earlier, we determined a pK_a value of 7.2 for 7-deaza-2′-deoxyxanthosine (**3**). A recent experiment changed this to 6.7. This value is 1 pK_a unit higher than that of **1** (pK_a 5.7), with the consequence that significantly less molecules are monoanions at pH 7.0 in the case of **3**. The single-crystal X-ray structure of the neutral 7-deaza-2′-deoxyxanthosine (**3**) has been reported, which was crystallized under slightly acidic conditions [35]. Consequently, we measured the T_m values of the duplex **31**·**33** containing one **3**·dT base pair at three different pH values (pH 5.5, 7.0, and 8.0) under the conditions described in *Table 3*. The T_m value of **31**·**33** (42°) at pH 7.0 was not changed either under alkaline or acidic conditions. This is in contrast to the results observed for a related molecule with a similar pK_a value (6-aza-2′-deoxyuridine, pK_a 6.8). A significant increase of the T_m value was observed for oligonucleotide-duplex formation when changing from neutral to acidic conditions [24]. However, the 2-oxo group

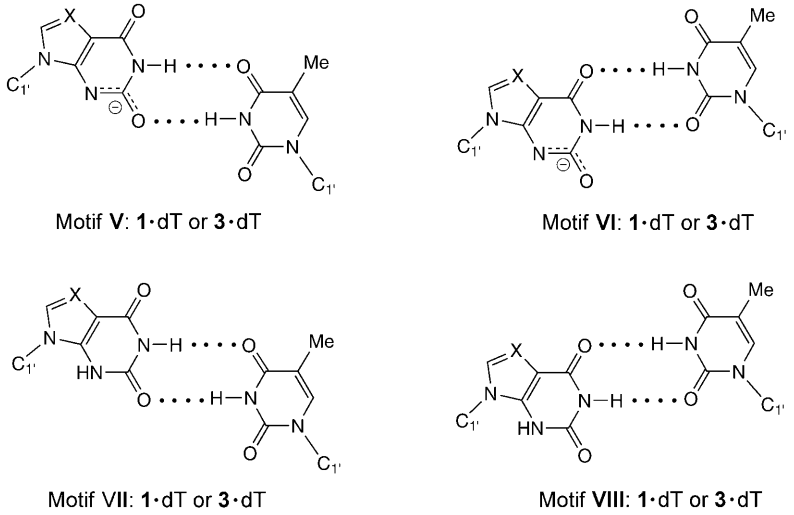

Fig. 3. *Base-pair motifs for compounds* **3** (X = CH) *or* **1** (X = N) *with dT*

might not be directly involved in H-bonding as shown in the motifs **VI** and **VIII** (*Fig. 3*); thus, this behavior becomes understandable.

Oligonucleotides containing the nucleosides **1** and **3** were also hybridized with those incorporating the diamine nucleoside **4** (3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine) [29]. A single incorporation of **1** against **4** in the place of the dA·dT pair stabilized the duplex **28**·**38** to the level of the unmodified duplex **24**·**25** (T_m 47°) and hence, base pair **1**·**4** is as stable as the dA·dT base pair. Two modifications enhanced the stability to 49° (duplex **27**·**39**) showing the nearest neighbor-influence on the base-pair stability. Also, a similar experiment was carried out with 7-deaza-2′-deoxyxanthosine (**3**). The corresponding duplex **31**·**38** showed a similar T_m (46°). Also two incorporations of **3** in duplex **30**·**39** resulted in the same T_m increase (49°) as that observed for **1**. This T_m increase results mainly from the Br-substitution and not from the presence of the additional 2-amino function. This was already verified earlier in related duplex structures [29]. The higher stability of the dA·dT base pair including that of a 'pyrazolo-dA' with dT than that of dA with compound **1** or **3** might result from a distortion of the helix axis by the purine·purine pair. On the other hand, the larger surface area of 'purine' residues can cause better stacking. It stays to proof if duplexes containing the base pair motif **IX** (*Fig. 4*) exclusively are more stable than those formed by dA·dT pairs.

Conclusions. – Among various groups (allyl, diphenylcarbamoyl, 2-(4-nitrophenyl)ethyl) studied for the oxo-group protection of the phosphoramidite of 7-deaza-2′-deoxyxanthosine (**3**) only the 2-(4-nitrophenyl)ethyl (npe) group was found to be effective. It was removed from CPG-bound oligonucleotide by DBU in pyridine. The allyl group which works efficiently for the protection of 2′-deoxyxanthosine (**1**) caused difficulties in the case of 7-deaza-2′-deoxyxanthosine (**3**) due to the oxidation of the 7-deazapurine base in the presence of Pd-catalyst. Although it was possible to incorporate

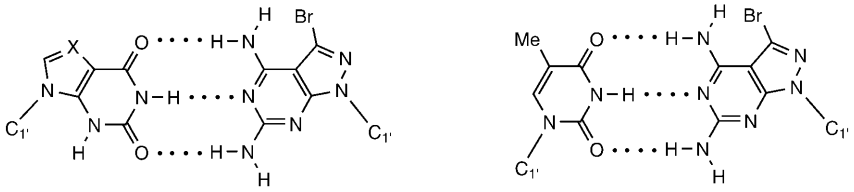

Fig. 4. *Base-pair motifs suggested for compounds* **3** (X=CH) *or* **1** (X=N) *with* **4** *and dT with* **4**

one 7-deaza-2′-deoxyxanthosine residue without base protection in a moderate yield (see **32** in *Table 2*), multiple incorporations failed. Oligonucleotides containing several 7-deaza-2′-deoxyxanthosine (**3**) residues required oxo-group protecting, preferentially by the npe residue. Compound **3** can act as universal nucleoside as it forms almost equally stable base pairs with the canonical DNA constituents as reported for 2′-deoxyxanthosine (**1**) or the corresponding 2′-deoxyinosine derivatives. According to its pK_a value (6.7), a significant amount of **3** forms a monoanion at pH 7.0 in the monomeric state as well as when it is part of the oligonucleotide chain. Surprisingly, no change of the duplex stability was observed when the pH value was changed from 5.5 to 8.0. As the related 6-aza-2′-deoxyuridine (pK_a=6.8) shows a significant T_m change, it is concluded that the 2-oxo group of nucleoside **3** does not participate in the base pairing. The nucleosides **1** and **3** pair with 3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*- pyrazolo[3,4-*d*]pyrimidine-4,6-diamine (**4**) resulting in duplexes which are as stable as those containing dA·dT pairs. This results from the influence of the Br-substituents and not from the additional amino group as it was already demonstrated with compounds incorporating base pairs of **4** with dT [29]. It is expected that duplexes are further stabilized when the nucleoside **3** bears 7-halogen or 7-alkynyl substituents [36].

Experimental Part

1. *General*. All chemicals were purchased from *Aldrich, Sigma*, or *Fluka* (*Sigma-Aldrich Chemie GmbH,* Deisenhofen, Germany). Solvents were of laboratory grade. TLC: aluminium sheets, silica gel *60* F_{254}, 0.2-mm layer (*VWR*, Germany). Column flash chromatography (FC): silica gel *60* (*VWR*, Germany) at 0.4 bar; sample collection with an *UltroRac-II* fractions collector (*LKB Instruments*, Sweden). Melting temp.: *Cary-1/3*-UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectrical controller; continuous temp. measurement in the reference cell with a *Pt-100* resistor. UV Spectra: *U-3200* spectrometer (*Hitachi*, Tokyo, Japan); λ_{max} (ε) in nm. NMR Spectra: *Avance-250* or *AMX-500* spectrometers (*Bruker*, Karlsruhe, Germany), at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm relative to Me_4Si as internal standard or external 85% H_3PO_4 for ^{31}P, *J* values in Hz. Elemental analyses were performed by the *Mikroanalytisches Laboratorium Beller* (Göttingen, Germany).

2. *Synthesis, Deprotection, and Purification of the Oligonucleotides.* The oligonucleotide syntheses were carried out in an *ABI-392-08*-DNA synthesizer (*Applied Biosystems*, Weiterstadt, Germany) on a 1-μmol scale following the synthesis protocol for 3′-cyanoethyl phosphoramidites (user manual for the *392-DNA* synthesizer, *Applied Biosystems*, Weiterstadt, Germany). The coupling efficiency was always higher than 95%. The following deprotection protocols were used for the deprotection of the oligonucleotides.

i) *Deprotection of* O*-Allyl-Protected Oligonucleotides with a* Pd^0 *Complex Followed by Treatment with 25% Aqueous* NH_3 *Solution.* After the completion of the oligonucleotide synthesis, the columns were washed with Ar-flushed THF and disassembled. The supported oligonucleotides were treated at r.t. with a THF soln. of $[Pd^0(PPh_3)_4]$ (*ca.* 3 equiv./allylic group), PPh_3 (*ca.* 25 equiv./allylic group), and excess of allyl scavenger HCOOH/butylamine 1:1 (1.5 ml) for 30 min at r.t. [17][18][37]. Then the mixture was centrifuged, and the soln. was discarded. The supports were washed with THF, acetone, 5M aq. DDTC, and finally with distilled H_2O (2× each). The deprotection of other base-protecting groups was achieved by 25% NH_3 soln. as described below.

ii) Deprotection of O*-npe-Protected Oligonucleotides with DBU in Pyridine Followed by Treatment with 25% Aqueous* NH_3 *Solution.* Solid-supported oligonucleotides were treated with 0.5M DBU in pyridine at r.t. for 24 h [9][25]. The solvent was evaporated in a *Speed-Vac* evaporator, and the residue was treated with 25% aq. NH_3 soln. under standard conditions.

The oligonucleotides obtained by the treatment with Pd^0 or DBU were cleaved from the solid support and treated with 25% aq. NH_3 soln. at 60° for 14–16 h. The oligonucleotides without 2′-deoxyxanthosine (**1**) and 7-deaza-2′-deoxyxanthosine (**3**) residues were deprotected only in 25% aq. NH_3 soln. at 60° for 14–16 h. The completely deprotected oligomers were purified by HPLC. Purification of 5′-dimethoxytrityl oligomers was performed by reversed-phase HPLC (*RP-18*) with the following solvent gradient system: *A* = 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5; *B* = MeCN; 3 min 20% *B* in *A*, 15 min 50% *B* in *A*, and 25 min 20% *B* in *A*, with a flow rate of 0.8 ml/min. The soln. was dried and treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 5 min at 0° to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC with the gradient: within 20 min 0 → 20% *B* in *A*, with a flow rate of 0.8 ml/min. The oligomers were desalted (*RP-18*, silica gel) and lyophilized on a *Speed-Vac* evaporator to yield colorless solids which were frozen at −24°.

The enzymatic hydrolysis of the oligonucleotides was performed as described by *Seela* and *Becher* [29] with snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli* from *Roche Diagnostics GmbH*, Germany) in 0.1M *Tris*·HCl buffer (pH 8.3), which was carried out on reversed-phase HPLC (0–20 min *A*, *A* = 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5). Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleosides ((ε_{260}): dT 8800, dC 7300, dA 15400, dG 11700, **1** 8400, **3** 4800). The molecular masses of the oligonucleotides were determined by MALDI-TOF *Biflex-III* mass spectrometer (*Bruker-Saxonia*, Leipzig, Germany) with 3-hydroxypicolinic acid (3-HPA) as a matrix.

*7-[2-Deoxy-β-*D-erythro*-pentofuranosyl]-2,4-bis(prop-2-enyloxy)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**11a**). A suspension of **10a** [20] (500 mg, 0.92 mmol) in 1M sodium prop-2-enolate (50 ml) was stirred at 50° for 3 h. The soln. was cooled to r.t. and applied on the top of the column (silica gel, 2.5×15 cm, CH_2Cl_2 (→ prop-2-en-1-ol), then CH_2Cl_2/Me_2CO 8:2): **11a** (240 mg, 84%) Colorless solid. TLC (silica gel, CH_2Cl_2/Me_2CO 8:2): R_f 0.28. UV (MeOH): 259 (5000), 271 (5000). ^{1}H-NMR ((D_6)DMSO): 2.22 (*m*, H_α–(2′)); 2.49 (*m*, H_β–(2′)); 3.51 (*m*, CH_2(5′)); 3.81 (*m*, H–C(4′)); 4.35 (*m*, H–C(3′)); 4.83–4.99 (*m*, 2 CH_2, OH–C(5′)); 5.22–5.44 (*m*, 2 = CH_2, OH–C(3′)); 6.07 (*m*, 2 = CH); 6.47 (*m*, H–C(1′), H–C(5)); 7.40 (*d*, *J* = 3.71, H–C(6)). Anal. calc. for $C_{17}H_{21}N_3O_5$ (347.37): C 58.78, H 6.09, N 12.10; found: C 58.92, H 6.04, N 12.02.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D-erythro*-pentofuranosyl]-2,4-bis(prop-2-enyloxy)-7*H*-pyrrolo[2,3-*d*]pyrimidine* (**12a**). Compound **11a** (790 mg, 2.27 mmol) was dried by co-evaporation with anh. pyridine (2×10 ml) and dissolved in anh. pyridine (30 ml). To this soln. was added 4,4′-dimethoxytrityl chloride (1.149 mg, 3.405 mmol) while stirring, and stirring was continued for 3 h. The mixture was then partitioned between H_2O and CH_2Cl_2, the org. layer washed with H_2O and brine (each 2×50 ml), the solvent evaporated and the residue subjected to FC (silica gel, 3×12 cm, CH_2Cl_2/Me_2CO 95:5): **12a** (880 mg, 59%). Colorless solid. TLC (silica gel, CH_2Cl_2/Me_2CO 97.5:2.5): R_f 0.41. UV (MeOH): 273 (11500). ^{1}H-NMR ((D_6)DMSO): 2.23 (*m*, H_α–C(2′)); 2.59 (*m*, H_β–C(2′)); 3.14 (*m*, CH_2(5′)); 3.71 (*2s*, 2 MeO); 3.94 (*m*, H–C(4′)); 4.38 (*m*, H–C(3′)); 4.80 (*m*, CH_2); 4.97 (*d*, *J* = 5.46, CH_2); 5.25–5.45 (*m*, 2 = CH_2, OH–C(3′)); 6.08 (*m*, 2 = CH); 6.46 (*m*, H–C(1′), H–C(5)); 6.78–6.84 (*m*, arom. H); 7.21–7.25 (*m*, arom. H); 7.34–7.37 (*m*, H–C(6), arom. H). Anal. calc. for $C_{38}H_{39}N_3O_7$ (649.73): C 70.25, H 6.05, N 6.47; found: C 70.18, H 5.94, N 6.39.

7-[2-Deoxy-5-O-*(4,4′-dimethoxytrityl)-β*-D-erythro-*pentofuranosyl]-2,4-bis(prop-2-enyloxy)-7*H-*pyrrolo[2,3*-d*]pyrimidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**7**). To a soln. of **12a** (150 mg, 0.23 mmol) in anh. CH_2Cl_2 (5 ml), diisopropylethylamine ((i-Pr)$_2$EtN; 67 µl, 0.39 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (55.5 µl, 0.25 mmol) were added under Ar. After stirring for 30 min, 5% aq. $NaHCO_3$ soln. (25 ml) was added, and the mixture was extracted with CH_2Cl_2 (2×25 ml). The org. layer was dried (Na_2SO_4), the solvent evaporated, and the residue subjected to FC (silica gel, 1.5×7 cm, CH_2Cl_2/Me_2CO 95:5): **7** (140 mg, 71%). Colorless foam. TLC (silica gel, CH_2Cl_2/Me_2CO 97.5:2.5): R_f 0.71. ^{31}P-NMR ($CDCl_3$): 149.63; 149.96.

2′-Deoxy-2,6-bis-O-*(prop-2-enyloxy)xanthosine* (**11b**). As described for **11a**, with **10b** [20] (500 mg, 0.92 mmol) and 1M sodium prop-2-enolate (50 ml): **11b** (210 mg, 75%). Colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.47. UV (MeOH): 244 (8100), 265 (12200). ^{1}H-NMR ((D$_6$)DMSO): 2.25 (*m*, H$_\alpha$–C(2′)); 2.73 (*m*, H$_\beta$–C(2′)); 3.53 (*m*, CH_2(5′)); 3.85 (*m*, H–C(4′)); 4.41 (*m*, H–C(3′)); 4.85–5.02 (*m*, 2 CH_2, OH–C(5′)); 5.25–5.44 (*m*, 2=CH_2, OH–C(3′)); 6.09 (*m*, 2=CH); 6.31 (*m*, H–C(1′)); 8.37 (*s*, H–C(8)). Anal. calc. for $C_{16}H_{20}N_4O_5$ (348.35): C 55.17, H 5.79, N 16.08; found: C 55.20, H 5.90, N 16.02.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)-2,6-bis*-O-*(prop-2-enyloxy)xanthosine* (**12b**). As described for **12a**, with **11b** (686 mg, 1.96 mmol) and 4,4′-dimethoxytrityl chloride (800 mg, 2.36 mmol): **12b** (850 mg, 66%). Colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.27. UV (MeOH): 265 (19700). ^{1}H-NMR ((D$_6$)DMSO): 2.34 (*m*, H$_\alpha$–(2′)); 2.88 (*m*, H$_\beta$–(2′)); 2.90–3.27 (*m*, CH_2(5′)); 3.70, 3.72 (*2s*, 2 MeO); 4.00 (*m*, H–C(4′)); 4.48 (*m*, H–C(3′)); 4.67 (*m*, CH_2); 5.02 (*d*, *J*=2.61, CH_2); 5.23–5.47 (*m*, 2=CH, OH–C(3′)); 6.01, 6.13 (*m*, 2=CH); 6.36 (*t*, *J*=6.09, 6.34, H–C(1′)); 6.71–6.79 (*m*, arom. H); 7.17–7.31 (*m*, arom. H); 8.28 (*s*, H–C(8)). Anal. calc. for $C_{37}H_{38}N_4O_7$ (650.72): C 68.29, H 5.89, N 8.61; found: C 68.38, H 6.00, N 8.76.

2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)-2,6-bis*-O-*(prop-2-enyloxy)xanthosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**8**). As described for **7**, with **12b** (300 mg, 0.46 mmol), anh. CH_2Cl_2 (5 ml), (i-Pr)$_2$EtN (134 µl, 0.78 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (111 µl, 0.50 mmol). Workup with 5% aq. $NaHCO_3$ soln. (30 ml) and CH_2Cl_2 (2×30 ml), followed by FC, as described for **7**:**8** (339 mg, 86%). Colorless foam. TLC (silica gel, CH_2Cl_2/Me_2CO 95:5): R_f 0.60. ^{31}P-NMR ($CDCl_3$): 150.02; 150.19.

*7-(2-Deoxy-β-*D-erythro-*pentofuranosyl)-1,3-bis(diphenylcarbamoyl)-1,7-dihydro-2*H-*pyrrolo[2,3*-d*]pyrimidine-2,4(3*H*)-dione* (**13a**) *and 7-[2-Deoxy-5*-O-*(diphenylcarbamoyl)-β*-D-erythro-*pentofuranosyl]-1,3-bis(diphenylcarbamoyl)-1,7-dihydro-2*H-*pyrrolo[2,3*-d*]pyrimidine-2,4(3*H*)-dione* (**13b**).

To a soln. (7-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione) [8] (**3**; 200 mg, 0.75 mmol) in anh. pyridine (5 ml) was (i-Pr)$_2$EtN (400 µl, 2.33 mmol) and diphenylcarbamic chloride (490 mg, 2.11 mmol) while stirring. After 100 min, 5% aq. $NaHCO_3$ soln. (60 ml) was added, and the mixture was extracted with CH_2Cl_2 (3×50 ml). The combined org. phase was dried (Na_2SO_4), the solvent evaporated, and the residue co-evaporated with toluene (3×50 ml) and subjected to FC (silica gel, 10×3 cm, CH_2Cl_2/Me_2CO 85:15 (100 ml), then CH_2Cl_2/MeOH 9:1 (400 ml)): less polar **13b** (37 mg, 6%) and more polar **13a** (364 mg, 74%). Data of **13b**: Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.4. ^{1}H-NMR ((D$_6$)DMSO): 2.12–1.97 (*m*, CH_2(2′)); 4.01 (*m*, H–C(4′)); 4.32–4.24 (*m*, H–C(3′), CH_2(5′)); 5.47 (*d*, *J*=4.2, OH–C(3′)); 6.46 (*t*, *J*=6.8, H–C(1′)); 6.70 (*d*, *J*=3.8, H–C(5)); 7.12 (*d*, *J*=3.8, H–C(6)); 7.54–7.22 (*m*, arom. H).

Data of **13a**: TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.2. UV (MeOH): 226 (48000). ^{1}H-NMR ((D$_6$)DMSO): 2.50 (br. *s*, CH_2(2′)); 3.54 (*m*, CH_2(5′)); 3.84 (br. *s*, H–C(4′)); 4.38 (br. *s*, H–C(3′)); 4.96 (*t*, *J*=5.2, OH–C(5′)); 5.35 (*d*, *J*=3.9, OH–C(3′)); 6.54 (*t*, *J*=6.7, H–C(1′)); 6.77 (*d*, *J*=3.5, H–C(5)); 7.51–7.32 (*m*, arom. H); 7.86 (*d*, *J*=3.6, H–C(6)). Anal. calc. for $C_{37}H_{31}N_5O_7$ (657.67): C 67.57, H 4.75, N 10.65; found: C 67.37, H 4.91, N 10.30.

7-[2-Deoxy-5-O-*(4,4′-dimethoxytrityl)-β*-D-erythro-*pentofuranosyl]-1,3-bis(diphenylcarbamoyl)-1,7-dihydro-2*H-*pyrrolo[2,3*-d*]pyrimidine-2,4(3*H*)-dione* (**14**). *Method A*: Compound **15** (380 mg, 0.67 mmol) was dissolved in anh. pyridine (44 ml) while stirring at r.t. To this soln., (i-Pr)$_2$EtN (325 µl, 1.87 mmol) and diphenylcarbamic chloride (432 mg, 1.86 mmol) was added. The mixture was stirred for 2.5 h at r.t. and then poured into ice-cold aq. of 5% $NaHCO_3$ soln. (80 ml). The mixture was extracted with CH_2Cl_2

(3×50 ml), the combined org. phase dried (Na_2SO_4), the solvent evaporated, and the residue co-evaporated with toluene (50 ml×3) and subjected to FC (silica gel, 10×3 cm, CH_2Cl_2/Me_2CO 98:2 (250 ml), then CH_2Cl_2/Me_2CO 95:5 (320 ml): **14** (608 mg, 95%). Colorless foam.

Method B: To a soln. of **13a** (50 mg, 0.08 mmol) in anh. pyridine (6.4 ml) was added 4,4′-dimethoxytrityl chloride (123 mg, 0.36 mmol) while stirring at. r.t. After stirring for 2.5 h, the reaction was quenched with MeOH (3 ml) and 5% aq. $NaHCO_3$ soln. (30 ml) was added. The mixture was extracted with CH_2Cl_2 (3×20 ml), the combined org. phase dried (Na_2SO_4), the solvent evaporated, and the residue adsorbed on silica gel (10 g) and subjected to FC (silica gel, 10×1.5 cm, CH_2Cl_2 (10 ml), then CH_2Cl_2/MeOH 98:2 (100 ml), and CH_2Cl_2/MeOH 95:5 (200 ml): **14** (54 mg, 74%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.7. UV (MeOH): 226 (66600). ^{1}H-NMR ((D$_6$)DMSO): 2.36–2.34 (*m*, H$_\alpha$–(2′)); 2.60–2.54 (*m*, H$_\beta$–(2′)); 3.16 (*m*, CH_2(5′)); 3.68 (*s*, 2 MeO); 3.96 (*m*, H–C(4′)); 4.39 (*m*, H–C(3′)); 5.42 (*d*, *J*=4.5, OH–C(3′)); 6.57 (*t*, *J*=6.8, H–C(1′)); 6.74 (*d*, *J*=3.7, H–C(5)); 6.84–6.79 (*m*, arom H); 7.54–7.20 (*m*, arom. H); 7.67 (*d*, *J*=3.6, H–C(6)). Anal. calc. for $C_{58}H_{49}N_5O_9$ (960.05): C 72.56, H 5.14, N 7.29; found: C 72.63, H 5.50, N 7.51.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl)-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]-pyrimidin-2,4-(3*H*)-dione* (**15**). Compound **3** [8] was dried by repeated co-evaporation of anh. pyridine and dissolved in anh. pyridine (65 ml). To this suspension, 4,4′-dimethoxytrityl chloride (1.25 g, 3.69 mmol) was added and stirred for 2.5 h at r.t. The reaction was quenched by adding MeOH (3 ml). To this soln., 5% aq. $NaHCO_3$ soln. was added (100 ml). The mixture was extracted with CH_2Cl_2 (3×50 ml), the org. phase dried (Na_2SO_4), the solvent evaporated, the residue dissolved in CH_2Cl_2, and the soln. adsorbed on silica gel (10 g) and subjected to FC (silica gel, CH_2Cl_2/MeOH 98:2 (350 ml), then CH_2Cl_2/MeOH 9:1 (150 ml)): **15** (800 mg, 75%). Colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.5. UV (MeOH): 235 (27400), 273 (9300). ^{1}H-NMR ((D$_6$)DMSO): 2.27 (*m*, H$_\alpha$–(2′)); 2.42–2.39 (*m*, H$_\beta$–(2′)); 3.09 (*m*, CH_2(5′)); 3.73 (*s*, 2 MeO); 3.87 (*m*, H–C(4′)); 4.30 (*m*, H–C(3′)); 5.33 (*d*, *J*=4.3, OH–C(3′)); 6.24 (*t*, *J*=5.9, H–C(1′)); 6.28 (*d*, *J*=3.5, H–C(5)); 6.79 (*d*, *J*=3.5, H–C(6)); 6.87–6.83 (*m*, arom. H); 7.36–7.20 (*m*, arom. H); 10.68 (*s*, NH); 11.71 (*s*, NH). Anal. calc. for $C_{32}H_{31}N_3O_7$ (569.60): C 67.48, H 5.49, N 7.38; found: C 67.29, H 5.61, N 7.32.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-1,3-bis(diphenylcarbamoyl)-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**16**). As described for **7**, with **14** (200 mg, 0.21 mmol), CH_2Cl_2 (10 ml), (i-Pr)$_2$EtN (49 µl, 0.28 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (60 µl, 0.27 mmol) for 20 min. Workup with 5% aq. $NaHCO_3$ soln. (30 ml) and CH_2Cl_2 (3×20 ml) as described for **7**. FC (silica gel, 7×1.5 cm, CH_2Cl_2/Me_2CO 98:2 (6 ml), then CH_2Cl_2/Me_2CO 95:5 (25 ml)) afforded **16** (230 mg, 95%). Colorless foam. TLC (silica gel, CH_2Cl_2/Me_2CO 95:5): R_f 0.4. ^{31}P-NMR ($CDCl_3$): 149.84; 150.01.

*7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-*D*-*erythro*-pentofuranosyl]-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]-pyrimidin-2,4(3*H*)-dione 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**6**). As described for **7**, with **15** (390 mg, 0.68 mmol), anh. THF (5 ml) instead of CH_2Cl_2, (i-Pr)$_2$EtN (357 µl, 2.05 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (200 µl, 0.90 mmol) for 20 min. After evaporation (no extraction), the residue was subjected to FC (silica gel, 10×1.5 cm, CH_2Cl_2/Me_2CO 9:1 (20 ml), then CH_2Cl_2/Me_2CO 4:1 (20 ml), and CH_2Cl_2/Me_2CO 1:1 (20 ml)). Evaporation (temp. $\leq 30°$) of the main zone afforded **6** (150 mg, 28%). Colorless foam. TLC (silica gel, CH_2Cl_2/Me_2CO 1:1): R_f 0.7. ^{31}P-NMR ($CDCl_3$): 149.57; 149.98.

*2-Amino-7-(3,5-di-*O*-acetyl-2-deoxy-β-*D*-erythro-pentofuranosyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one* (**17**). To a suspension of 7-deaza-2′-deoxyguanosine (c^7G_d) [27] (2 g, 7.51 mmol) in anh. pyridine (35 ml) was added Ac_2O (3 ml, 31.7 mmol) and the mixture was stirred overnight at r.t. The reaction was quenched by additon of MeOH (1 ml). Then the mixture was poured into 5% aq. $NaHCO_3$ soln. (100 ml) and extracted with CH_2Cl_2 (3×100 ml), the combined org. phase dried (Na_2SO_4), the solvent evaporated, and the residue subjected to FC (silica gel, 5×12 cm, CH_2Cl_2/MeOH 9:1): **17** (2.31 g, 88%). Colorless solid. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.42. UV (MeOH): 259 (14600). ^{1}H-NMR ((D$_6$)DMSO): 2.05, 2.07 (*2s*, 2 MeCO); 2.33 (*m*, H$_\alpha$–(2′)); 2.68 (*m*, H$_\beta$–(2′)); 4.18 (*m*, H–C(4′), CH_2(5′)); 5.21 (*d*, *J*=5.25, H–C(3′)); 6.03 (*m*, H–C(1′), H–C(5), NH_2); 6.90 (*d*, *J*=4.12, H–C(6)); 10.42 (*s*, NH). Anal. calc. for $C_{15}H_{18}N_4O_6$ (350.33): C 51.43, H 5.18, N 15.99; found: C 51.50, H 5.14 N 15.86.

7-(3,5-Di-O-*acetyl-2-deoxy-β*-D-erythro-*pentofuranosyl)2-[(4,4′-dimethoxytrityl)amino]-3,7-dihydro-4*H-*pyrrolo[2,3*-d*]pyrimidin-4-one* (**18**). To a soln. of **17** (2 g, 5.70 mmol) in anh. pyridine was added 4,4′-dimethoxytrityl chloride (2.03 g, 6.0 mmol). After stirring overnight at r.t., the mixture was poured into 5% aq. $NaHCO_3$ soln. (100 ml) and extracted with CH_2Cl_2 (3×150 ml). The combined org. phase was dried (Na_2SO_4), the solvent evaporated, and the residue subjected to FC (silica gel, 5×12 cm, CH_2Cl_2/$(CH_3)_2CO$ 98:2, then 95:5): **18** (3.4 g, 91%). Colorless foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.51. UV (MeOH): 268 (15800), 285 (14600). ^{1}H-NMR ((D$_6$)DMSO): 1.78 (*m*, H$_\alpha$–(2′)); 2.02, 2.08 (*2s*, 2 MeCO); 2.16 (*m*, H$_\beta$–(2′)); 3.71 (*s*, 2 MeO); 4.06 (*m*, H–C(4′), CH_2(5′)); 5.02 (*d*, *J*=5.35, H–C(3′)); 5.56 (*dd*, *J*=5.57, 5.42, H–C(1′)), 6.24 (*d*, *J*=3.55, H–C(5)); 6.73 (*d*, *J*=3.57, H–C(6)); 6.73–7.33 (*m*, arom. H); 7.42 (*s*, NH); 10.40 (*s*, NH). Anal. calc. for $C_{36}H_{36}N_4O_8$ (652.69): C 66.25, H 5.56, N 8.58; found: C 66.31, H 5.62, N 8.60.

2-Amino-7-(3,5-di-O-*acetyl-2-deoxy-β*-D-erythro-*pentofuranosyl)-4*-O-*[2-(4-nitrophenyl)ethyl]-7*H-*pyrrolo[2,3*-d*]pyrimidine* (**19**). *Method A*: A soln. of **18** (2 g, 3.06 mmol) in abs. dioxane (10 ml) was evaporated. The residue was dissolved in abs. dioxane (20 ml), and to this soln. were added PPh_3 (6.4 g, 24.8 mmol), 2-(4-nitrophenyl)ethanol (4.12 g , 24.8 mmol) and DEAD (4.23 g, 24.8 mmol). The mixture was stirred for 1 h at. r.t. The solvent was evaporated and the residue subjected to FC (silica gel, 5×12 cm, Et_2O). The main fraction was rechromatographed: **19** (350 mg, 23%). Yellow foam.

Method B: To a soln. of **17** (3.5 g, 10.0 mmol) in anh. THF (75 ml) were added PPh_3 (8.4 g, 33.03 mmol), DEAD (5.82 g, 33.03 mmol), and 2-(4-nitrophenyl)ethanol (5.61 g, 33.03 mmol). After stirring for 1 h at. r.t., the mixture was worked up as described for *Method A*: **19** (2.75 g, 55%). Yellow foam. TLC (Et_2O): R_f 0.33. UV (MeOH): 261 (19100), 279 (16700). ^{1}H-NMR ((D$_6$)DMSO): 2.04, 2.08 (*2s*, 2 MeCO); 2.34 (*m*, H$_\alpha$–(2′)); 2.75 (*m*, H$_\beta$–(2′)); 3.21 (*t*, *J*=6.45, 6.75, CH_2); 4.18 (*m*, H–C(4′), CH_2(5′)); 4.59 (*t*, *J*=6.40, 6.55, OCH_2); 5.23 (*d*, *J*=5.45, H–C(3′)); 6.31 (*m*, H–C(1′), H–C(5), NH_2); 7.07 (*d*, *J*=3.70, H–C(6)); 7.60 (*d*, *J*=8.50, arom. H); 8.15 (*d*, *J*=8.5, arom. H). Anal. calc. for $C_{23}H_{25}N_5O_8$ (499.47): C 55.31, H 5.05, N 14.02; found: C 55.40, H 5.00, N 13.96.

7-(3,5-Di-O-*acetyl-2-deoxy-β*-D-erythro-*pentofuranosyl)-4*-O-*[2-(4-nitrophenyl)ethyl]-1,7-dihydro-2*H-*pyrrolo[2,3*-d*]pyrimidin-2-one* (**20**). To a soln. of **19** (3 g, 6.0 mmol) in 30% aq. AcOH/Me_2CO 2:1, (375 ml) was added $NaNO_2$ (10 g, 140 mmol) in H_2O (25 ml). After stirring for 2 h at r.t., the solvent was evaporated and the residue subjected to FC (silica gel, 5×12 cm, CH_2Cl_2/MeOH 99:1, then 97:3): **20** (2.25 g, 75%). Slightly yellow foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.38. UV (MeOH): 277 (17000). ^{1}H-NMR ((D$_6$)DMSO): 2.04, 2.08 (*2s*, 2 MeCO); 2.40 (*m*, H$_\alpha$–(2′)); 2.73 (*m*, H$_\beta$–(2′)); 3.23 (*t*, *J*=6.32, 6.42, CH_2); 4.22 (*m*, H–C(4′), CH_2(5′)); 4.65 (*t*, *J*=6.28, 6.40, OCH_2); 5.24 (*d*, *J*=5.20, H–C(3′)); 6.39 (*m*, H–C(1′), H–C(5)); 7.23 (*d*, *J*=2.82, H–C(6)); 7.60 (*d*, *J*=8.62, arom. H); 8.16 (*d*, *J*=6.90, arom. H); 11.43 (*s*, NH). Anal. calc. for $C_{23}H_{24}N_4O_9$ (500.46): C 55.20, H 4.83, N 11.20; found: C 55.38, H 4.85, N 11.15.

7-(3,5-Di-O-*acetyl-2-deoxy-β*-D-erythro-*pentofuranosyl)-2,4-bis*-O-*[2-(4-nitrophenyl)ethyl]-7*H-*pyrrolo[2,3*-d*]pyrimidine* (**21**). As described for **19** (*Method B*), with **20** (1.6 g, 3.19 mmol), anh. THF (50 ml), PPh_3 (3.4 g, 13.0 mmol), DEAD (2.26 g, 13.0 mmol), and 2-(4-nitrophenyl)ethanol (2.17 g, 13.0 mmol): **21** (1.7 g, 82%). Yellow foam. TLC (Et_2O): R_f 0.5. UV (MeOH): 272 (32400). ^{1}H-NMR ((D$_6$)DMSO): 2.01, 2.08 (*2s*, 2 MeCO); 2.41 (*m*, H$_\alpha$–(2′)); 2.88 (*m*, H$_\beta$–(2′)); 3.22 (*m*, 2 CH_2); 4.20 (*m*, H–C(4′), CH_2(5′)); 4.58 (*t*, *J*=6.27, 6.25, OCH_2); 4.67 (*t*, *J*=6.14, 6.42, OCH_2); 5.31 (*d*, *J*=5.45, H–C(3′)); 6.43 (*m*, H–C(1′), H–C(5)); 7.35 (*d*, *J*=3.67, H–C(6)); 7.59 (*m*, arom. H); 8.16 (*m*, arom. H). Anal. calc. for $C_{31}H_{31}N_5O_{11}$ (649.60): C 57.32, H 4.81, N 10.78; found: C 57.40, H 4.90, N 10.66.

7-(2-Deoxy-β-D-erythro-*pentofuranosyl)-2,4-bis*-O-*[2-(4-nitrophenyl)ethyl]-7*H-*pyrrolo[2,3*-d*]pyrimidine* (**22**). Compound **21** (1.5 g, 2.31 mmol) was dissolved in sat. NH_3/MeOH and stirred for 3 h at r.t. The solvent was evaporated and the residue subjected to FC (silica gel, 5×12 cm, CH_2Cl_2/MeOH 95:5): **22** (1.19 g, 91%). Yellow foam. TLC (CH_2Cl_2/Me_2CO, 8:2): R_f 0.45. UV (MeOH): 272 (26400). ^{1}H-NMR ((D$_6$)DMSO): 2.14 (*m*, H$_\alpha$–(2′)); 2.44 (*m*, H$_\beta$–(2′)); 3.21 (*m*, 2 CH_2); 3.49 (*m*, CH_2(5′)); 3.80 (*m*, H–C(4′)); 4.33 (*m*, H–C(3′)); 4.57 (*t*, *J*=6.27, 6.18, OCH_2); 4.66 (*t*, *J*=6.11, 6.18, OCH_2); 4.92 (*t*, *J*=4.74, 5.12, OH–C(5′)); 5.29 (*d*, *J*=3.07, OH–C(3′)); 6.39 (*d*, *J*=2.66, H–C(5)); 6.46 (*t*, *J*=7.04, 6.60, H–C(1′)); 7.38 (*d*, *J*=2.90, H–C(6)); 7.60 (*m*, arom. H); 8.15 (*m*, arom. H). Anal. calc. for $C_{27}H_{27}N_5O_9$ (565.53): C 57.34, H 4.81, N 12.38; found: C 57.40, H 4.90, N 12.27.

7-[2-Deoxy-5-O-*(4,4′-dimethoxytrityl)-β*-D-erythro-*pentofuranosyl]-2,4-bis*-O-*[2-(4-nitrophenyl)-ethyl]-7*H-*pyrrolo[2,3*-d*]pyrimidine* (**23**). To a soln. of **22** (200 mg, 0.35 mmol) in anh. pyridine (3 ml) was added 4,4′-dimethoxytrityl chloride (169 mg, 0.5 mmol) while stirring at r.t. After stirring for 3 h at r.t., 5% aq. $NaHCO_3$ soln. (50 ml) was added, and the mixture was extracted with CH_2Cl_2 (3×50 ml). The org. phase was dried (Na_2SO_4), the solvent evaporated, and the residue dissolved in CH_2Cl_2, adsorbed on silica gel (10 g), and subjected to FC (silica gel, 3×12 cm, CH_2Cl_2/Me_2CO 95:5): **23** (243 mg, 79%). Slightly yellow foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.75. UV (MeOH): 272 (34100). ^{1}H-NMR ((D$_6$)DMSO): 2.26 (*m*, H$_\alpha$–(2′)); 2.56 (*m*, H$_\beta$–(2′)); 3.12–3.22 (*m*, 2 CH_2, CH_2(5′)); 3.69 (*s*, 2 MeO); 3.92 (*m*, H–C(4′)); 4.37 (*m*, H–C(3′)); 4.52 (*m*, OCH_2); 4.66 (*t*, *J*=6.16, 6.41, OCH_2); 5.36 (*d*, *J*=4.02, OH–C(3′)); 6.37 (*d*, *J*=3.61, H–C(5)); 6.46 (*t*, *J*=6.46, 6.77, H–C(1′)); 6.76–6.82 (*m*, arom. H); 7.18–7.35 (*m*, H–C(6), arom. H); 7.56 (*m*, arom. H); 8.13 (*m*, arom. H). Anal. calc. for $C_{48}H_{45}N_5O_{11}$ (867.90): C 66.43, H 5.23, N 8.07; found: C 66.52, H 5.20, N 7.95.

7-[2-Deoxy-5-O-*(4,4′-dimethoxytrityl)-β*-D-erythro-*pentofuranosyl]-2,4-bis*-O-*[2-(4-nitrophenyl)-ethyl] 7*H-*pyrrolo[2,3*-d*]pyrimidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**9**). As described for **7**, with **23** (100 mg, 0.11 mmol), anh. THF (3 ml) instead of CH_2Cl_2, (i-Pr)$_2$EtN (39 µl, 0.16 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (43 µl, 0.15 mmol) for 30 min. After evaporation (no extraction), the residue was subjected to FC (silica gel, 1.5×8 cm, CH_2Cl_2/Me_2CO 99:1): **9** (150 mg, 77%). Colorless foam. TLC (silica gel, CH_2Cl_2/Me_2CO 97.5:2.5): R_f 0.61. ^{31}P-NMR ($CDCl_3$): 149.71; 149.96.

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