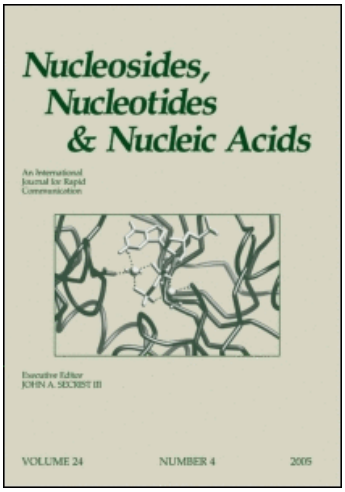


## Nucleosides, Nucleotides and Nucleic Acids

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# OLIGONUCLEOTIDES CONTAINING PYRAZOLO[3,4-d]PYRIMIDINES: 8-Aza-7-deazaadenines With Bulky Substituents in the 2- or 7-Position

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□ *The synthesis of the 2′-deoxyadenosine analogues* **1b**, **2b**, *and* **3c** *modified at the 7- and/or 2-position is described. The effect of 7-chloro and 2-methylthio groups on the duplex stability is evaluated. For that, the nucleosides* **1b**, **2b**, *and* **3c** *were converted to the corresponding phosphoramidites* **15**, **19**, *and* **22**, *which were employed in the solid-phase oligonucleotide synthesis. In oligonucleotide duplexes, compound* **1b** *forms stable base pairs with dT, of which the separated* **1b**-**dT** *base pairs contribute stronger than that of the consecutive base pairs. Compound* **2b** *shows universal base pairing properties while its N8 isomer* **3c** *forms duplexes with lower stability.*

**Keywords** Nucleosides; Oligonucleotides; Pyrazolo[3,4-d]pyrimidines; Bulky susbtituents; Base pairing; Duplex stability



## INTRODUCTION

8-Aza-7-deaza-2′-deoxyadenosine (**1a**)[1] (purine numbering is used throughout the discussion section, Figure 1) is an ideal substitute of 2′-deoxyadenosine within duplex DNA. The multiple incorporations of **1a**-dT in place of dA-dT do not cause a significant change of the $T_m$ values.[2,3] Extensive studies have been performed modifying the 8-aza-7-deaza-2′-deoxyadenosine (**1a**) at the 7-position with bulky substituents. It was shown that the 7-substituents of moderate size stabilize the duplex DNA significantly.[3–6]

In memory to my good friend the late Professor Dr. John A. Montgomery.

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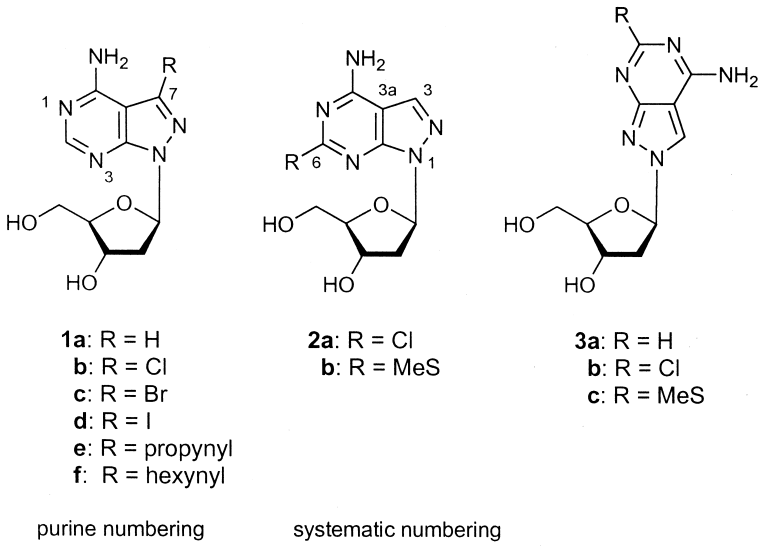



**FIGURE 1** 8-Aza-7-deazapurine-2′-deoxyribonucleosides.



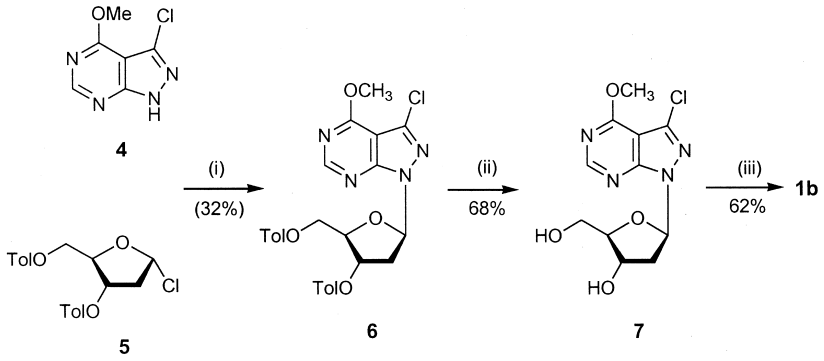
This has been demonstrated on halogenated and alkynylated nucleosides. Up to now the bromo, iodo, propynyl, and hexynyl compounds **1c-1f** have been studied and their bulky side chains were found to be accommodated well in the major groove of B-DNA.[3–6] We are further extending the series of 7-substituted compounds with the 7-chloro nucleoside **1b**. Different from the 7-substituted nucleosides, 2-substituted purine or purine like nucleobases do not show such favorable properties. 2-Substituted purine nucleosides with halogeno substituents have already been prepared by Montgomery and by others.[7–12] Unlike these nucleosides only a few pyrazolo pyrimidine nucleosides containing halogens in the 2-position have been synthesized.[13,14] As 2-substituents are located in the minor groove of B-DNA, their dimension is limited by the narrow size of this groove. Recently, the effect of 2-chloro substituent was studied by incorporating the 2-chloro-8-aza-7-deazaadenine nucleoside **2a** in duplex DNA.[14] The 2-chloro group causes a steric clash with the 2-oxo group of dT resulting in duplex destabilization. Nature uses even bulkier 2-methylthio groups to modify adenine residues in RNA[15–17]—a phenomenon which has recently been studied on the oligonucleotide level.[15] We became interested in such a modification on 8-aza-7-deazapurine nucleosides (**2b** and **3c**). Contrary to purines, an 8-aza-7-deazapurine cannot form Hoogsteen base pairs and will be devoid base pairing within duplex DNA but will allow base stacking. This manuscript reports on the incorporation and the base pairing of the 8-aza-7-deazaadenine nucleosides **1b**, **2b**, and **3c** containing oligonucleotide duplexes. For that the phosphoramidites **15**, **19**, and **22** are prepared which are employed in solid-phase synthesis.

## RESULTS AND DISCUSSION

### Synthesis and Properties of Monomers

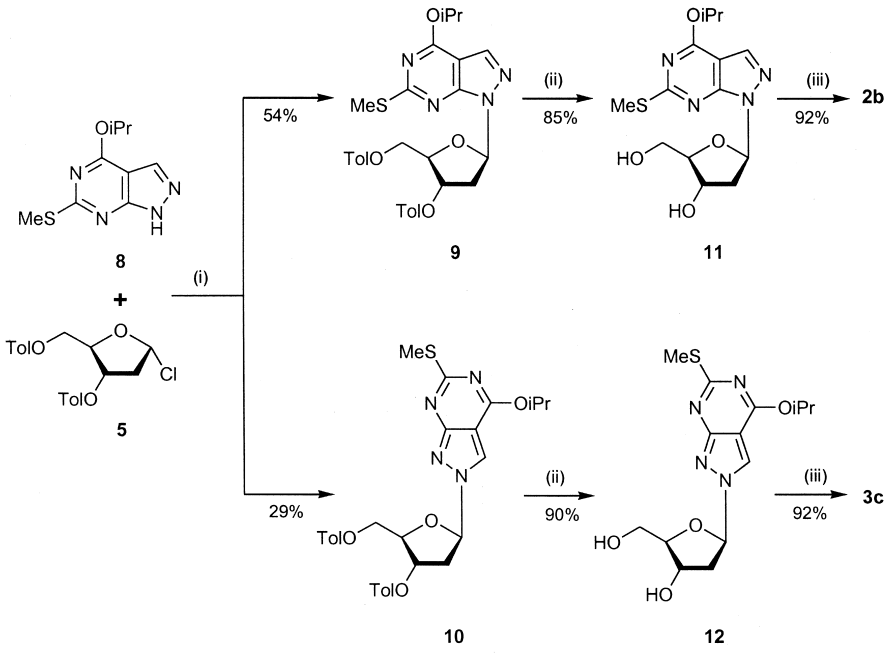
For the synthesis of the target nucleosides **1b**, **2b**, and **3c**, 8-aza-7-chloro-7-deaza-6-methoxypurine (**4**) and 8-aza-2-methylthio-7-deaza-6-isopropoxypurine (**8**) served as precursors. Treatment of 8-aza-7-deaza-6-methoxypurine[1,18] with N-chlorosuccinimide (NCS) in DMF gave **4**, while the reaction of 8-aza-2-methylthio-7-deaza-6-chloropurine[19] with 1M NaOiPr yielded **8**. Glycosylation of compound **4** under nucleobase anion conditions (KOH/TDA-1) with 2-deoxy-3,5-di-O-(p-toluoyl)-$\beta$-D-*erythro*-pentofuranosyl chloride (**5**)[20,21] afforded compound **6** as major product. Likewise, the glycosylation of **8** with **5** gave the regioisomeric $N^9$- and $N^8$-glycosylated compounds **9** and **10** in nearly 2:1 proportion.[14] The latter were deprotected with 0.1 or 0.4 M Na in corresponding alcohols to yield the nucleosides **7**, **11**, and **12** as colorless compounds.[4] Subsequently, the 6-alkoxy groups were displaced by an amino group in $NH_3$/MeOH in a steel bomb (90°C) furnishing the nucleosides **1b**, **2b**, and **3c** (Schemes 1 and 2). As 2′-deoxyribonucleosides are sensitive to acidic depurination, the stability of the *N*-glycosylic bonds of **1b**, **2b**, and **3c** were evaluated UV-spectrophotometrically in 2 N HCl at 40°C. Compound **2b** ($t_{1/2}$ 5.8 h) was much more stable on its *N*-glycosylic bond than its regioisomer **3c** ($t_{1/2}$ 3.8 h) and 8-aza-7-chloro-7-deaza-2′-deoxyadenosine **1b** ($t_{1/2}$ 2.3 h).

Next, the protection of the 6-amino group of the nucleosides **1b**, **2b**, and **3c** was performed. For that, the nucleosides **1b** and **2b** were treated with dimethylformamide dimethylacetal at r.t. or 50°C yielding the (dimethylamino)methylidene compounds **13** and **16** (Schemes 3 and 4).[4] The nucleoside **3c** was treated with isobutyryl chloride affording the protected compound **20**.[22] The stability of the protecting groups was determined by




(i) KOH, TDA-1, MeCN, r.t., (ii) 0.4 M NaOMe in MeOH and (iii) $NH_3$/MeOH

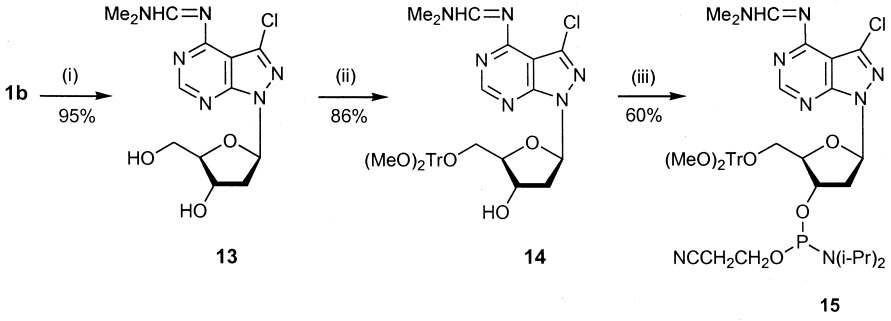
**SCHEME 1**




(i) KOH, TDA-1, MeCN, r.t., (ii) 0.1M NaOiPr in iPrOH and (iii) $NH_3$/MeOH

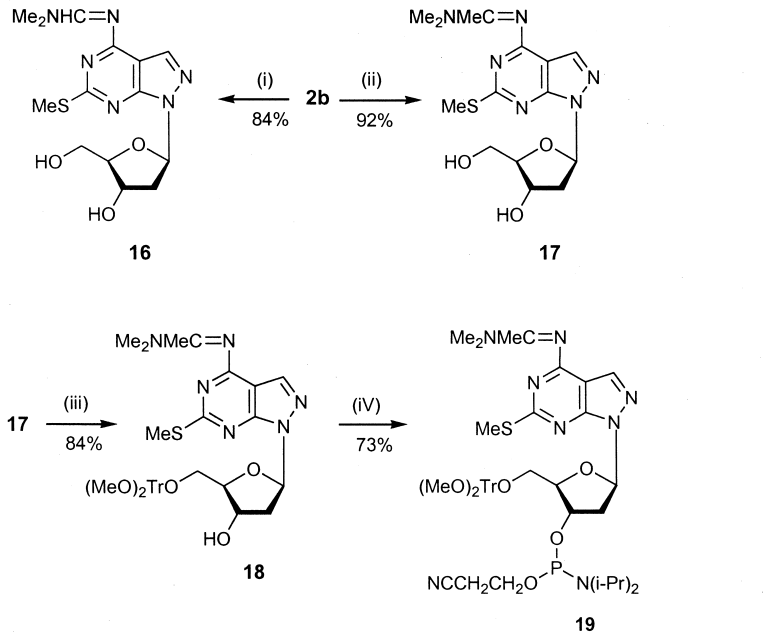
**SCHEME 2**

UV spectrophotometrically in 25% ammonia at r.t. While the formamidine derivative **13** showed a half life $\tau = 1.6$ min, at 230 nm, the compound **16** was rather labile to ammonia treatment. So acetamidine protection was employed for the amino function of **2b** which was therefore treated with dimethylacetamide dimethyl acetal in methanol at 50°C to yield compound




(i) $(Me)_2NHC(OMe)_2$, MeOH, (ii) $(MeO)_2TrCl$, pyridine and
(iii) 2-cyanoethyldiisopropylphosphoramido chloridite, $CH_2Cl_2$

**SCHEME 3**

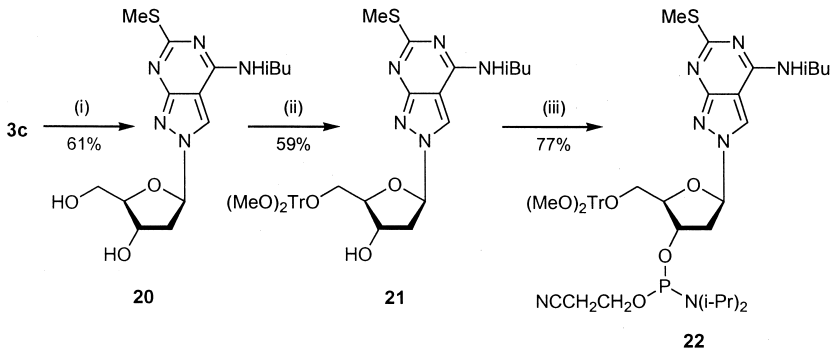



(i) $(Me)_2NHC(OMe)_2$, MeOH, (ii) $(Me)_2NMeC(OMe)_2$, MeOH, (iii) $(MeO)_2TrCl$, pyridine and (iv) 2-cyanoethyldiisopropylphosphoramido chloridite, $CH_2Cl_2$

**SCHEME 4**

**17** ($\tau$ = 21.5 min, at 320, in 25% ammonia).[23] For compound **20**, the isobutyryl group was found to be the most suitable protecting group ($\tau$ = 128 min, at 330 nm). The 5′-OH groups of the nucleosides **13**, **17**, and **20** were protected with the 4,4′-dimethoxytrityl residue → **14**, **18**, and **21** under standard conditions and the 3′-OH groups were phosphitylated to give the phosporamidites **15**, **19**, and **22.**[14] (Schemes 3–5).

All compounds were characterized by $^1$H-, $^{13}$C-, or $^{31}$P-NMR spectra (Table 1 and Experimental), as well as by elemental analysis or mass spectra. The $^{13}$C-NMR signals were assigned by gated-decoupled $^{13}$C-NMR or heteronuclear [$^1$H, $^{13}$C]-NMR correlation spectra. Nucleoside **1b** was assigned as N9 isomer according to the published literature.[4] The assignment of the glycosylation position of **2b** and **3c**, was performed on the basis of chemical shift analyses. In $^{13}$C-NMR, the carbon signal next to the glycosylation site is shifted by 8 ppm upfield when the glycosylation position changes from N9 to N8 (Table 1). This is also the case for the corresponding derivatives **9**-**12**. The introduction of the acetamidine protecting group (**17**) has a strong influence on the $^{13}$C-NMR chemical shifts of the heterocyclic system. NOE data were used to confirm the anomeric configuration as well as the glycosylation position of the nucleoside **3c**. Irradiation of **3c** on H-(7) gave NOEs




(i) i-BuCl, pyridine, (ii) $(MeO)_2TrCl$, pyridine and
(iii) 2-cyanoethyldiisopropylphosphoramido chloridite, $CH_2Cl_2$

**SCHEME 5**

at H-(1′) (8.8%), H-(2′) (1.5%), and H-(3′) (1.8%). Due to the spatial relationships of the H-atoms, the glycosylic bond of compound **3c** is $\beta$-D, and the glycosylation site is N8.

## Oligonucleotides

**Synthesis.** Oligonucleotide synthesis was performed applying the phosphoramidites **15**, **19** and **22** in solid-phase oligonucleotide chemistry in an automatized DNA synthesizer. The coupling yields were always higher than 95%. The oligonucleotides were deprotected in 25% $NH_3$ solution and purified by RP-18 HPLC. The homogeneity of the oligonucleotides was established by reversed-phase HPLC. The modified oligonucleotides were characterized by MALDI-TOF mass spectroscopy, and the detected masses were found in good agreement with the calculated values (Table 2).

**Oligonucleotide Duplex Stability.** Among the 8-aza-7-deaza-2′-deoxyadenosines, the 7-substituted Br, I, propynyl and hexynyl nucleosides (**1c-1f**) have shown a positive effect on the duplex stability.[3–6] In this regard, the contribution of a 7-chloro substituent (**1b**) is unknown. For that purpose, a series of oligonucleotides were synthesized replacing dA at the various positions by **1b** in the duplex 5′-d(TAGGTCAATACT)•3′-d(ATCCAGTTATGA) (**23•24**) (Table 3).

A systematic evaluation of the duplexes with an increasing number of **1b** reveals the importance of different positions of incorporation to the thermal stability (Table 3). Thus, a single incorporation of **1b** at the central position ($\rightarrow$ **25**) stabilizes the dA-dT base pair by 2°C, while a consecutive incorporation ($\rightarrow$ **26**) gives a stabilization of 1.5°C per modification. In another sequence ($\rightarrow$ **27**), two incorporations of **1b** at different positions led

**TABLE 1** $^{13}$C-NMR Chemical Shifts of Pyrazolo[3,4-d]pyrimidine Nucleosides[a]

| Compound[b] | C(3) | C(3a) | C(4) | C(6) | C(7a) | MeS | $OCH_3/2CH_3$ |
|---|---|---|---|---|---|---|---|
| **4** | 130.9 | 98.9 | 162.9 | 156.1 | 156.3 | | |
| **6** | 132.3 | 100.3 | 163.1 | 156.8 | 155.5 | | 54.7 |
| **7** | 131.6 | 100.1 | 163.0 | 156.6 | 155.4 | | 54.6 |
| **1b** | 131.7 | 97.4 | 157.2 | 157.7 | 154.5 | | |
| **8** | 131.9 | 99.0 | 168.3 | 161.5 | 156.8 | 13.7 | 21.6 |
| **9** | 132.9 | 100.2 | 169.5 | 161.5 | 155.7 | 13.7 | 21.5 |
| **10** | 125.5 | 100.5 | 168.5 | 163.0 | 161.5 | 13.7 | 21.5 |
| **11** | 133.5 | 100.1 | 169.1 | 161.5 | 155.6 | 13.7 | 21.5 |
| **2b** | 133.3 | 98.2 | 168.9 | 157.0 | 154.3 | 13.3 | |
| **12** | 124.4 | 100.2 | 168.1 | 163.0 | 161.3 | 13.6 | 21.5 |
| **3c** | 125.7 | 100.4 | 169.6 | 161.1 | 159.2 | 14.0 | |
| **13** | 133.4 | 104.0 | 161.9 | 156.4 | 155.1 | | |
| **14** | 133.4 | 104.0 | 161.9 | 156.4 | 155.1 | | 54.8 |
| **16** | 133.9 | 105.4 | 168.5 | 161.3 | 155.2 | 13.7 | |
| **17** | 133.8 | 105.7 | 168.5 | 160.8 | 154.9 | 13.6 | |
| **18** | 133.7 | 105.7 | 168.5 | 160.8 | 154.8 | 13.6 | 54.9 |
| **20** | 129.8 | 100.6 | 167.7 | 161.0 | 153.7 | 13.3 | 19.1 |
| **21** | 129.7 | 100.9 | 167.5 | 161.1 | 153.7 | 13.4 | 19.1 |
| | C(1′) | C(2′) | C(3′) | C(4) | C(5) | | |
| **6** | 84.3 | 35.2 | 78.4 | 87.6 | 63.6 | | |
| **7** | 84.2 | 37.8 | 70.6 | 87.7 | 62.0 | | |
| **1b** | 83.7 | 37.7 | 70.7 | 87.6 | 62.2 | | |
| **9** | 81.3 | 35.2 | 74.6 | 84.2 | 63.9 | | |
| **10** | 90.3 | 36.7 | 74.4 | 82.2 | 63.8 | | |
| **11** | 83.9 | 37.7 | 70.9 | 87.6 | 62.3 | | |
| **2b** | 83.5 | 37.7 | 71.1 | 87.4 | 62.4 | | |
| **12** | 90.6 | [c] | 70.3 | 88.3 | 61.6 | | |
| **3c** | 91.2 | [c] | 71.7 | 89.2 | 63.1 | | |
| **13** | 83.7 | 37.7 | 70.7 | 87.6 | 62.2 | | |
| **14** | 83.6 | 38.0 | 70.4 | 85.1 | 64.0 | | |
| **16** | 83.7 | 37.7 | 71.1 | 87.5 | 62.5 | | |
| **17** | 83.7 | 37.7 | 71.1 | 87.5 | 62.5 | | |
| **18** | 83.6 | 38.0 | 70.8 | 85.2 | 64.4 | | |
| **20** | 90.6 | [c] | 70.7 | 88.5 | 62.1 | | |
| **21** | 90.1 | [c] | 70.2 | 86.1 | 63.8 | | |

[a]Measured in DMSO-$d_6$.
[b]Systematic numbering.
[c]Superimposed by DMSO-$d_6$.

to a stronger stabilization (3°C per modification). A combination of consecutive and two separated incorporations in a same sequence (→ **28**) reflect to the stability and contribute 1.75° per modification. From the above observation, it can be concluded that the incorporation of **1b** stabilizes the duplexes significantly and the separated base pairs contribute stronger than that of the consecutive ones. The increase in the duplex stability can be attributed to the stacking of the nearest neighbors and the flexibility obtained to the 7-Cl substituent so as to accommodate well in the major groove.

**TABLE 2** Molecular Masses Determined by MALDI-TOF Mass Spectroscopy

| Oligonucleotides | [$M$ H$^+$] (calc.) | [$M$ H$^+$] (found) |
|---|---|---|
| 5′-d(TAGGTC**1b**ATACT) (**25**) | 3680 | 3679 |
| 5′-d(TAGGTC**1b1b**TACT) (**26**) | 3714 | 3714 |
| 3′-d(ATCC**1b**GTT**1b**TGA) (**27**) | 3714 | 3713 |
| 5′-d(T**1b**GGTC**1b1b**T**1b**CT) (**28**) | 3783 | 3783 |
| 5′-d(TAGGTCA**1a**TACT) (**36**) | 3645 | 3645 |
| 5′-d(TAGGTCA**2b**TACT) (**41**) | 3691 | 3691 |
| 5′-d(TAGGTCA**3c**TACT) (**45**) | 3691 | 3692 |

Next, the self-complementary oligonucleotides containing alternating bases or base tracts were studied. Earlier investigations on oligonucleotides with modified bases showed that self-complementary duplexes formed by alternating 5′-d(A-T)$_n$ are extraordinarily sensitive to base modifications. It results from the particular structure of these oligomers.[24] For that reason, the self-complementary oligomer 5′-d(**1b**-dT)$_6$ was evaluated. When compared to 5′-d(**1c**-dT)$_6$, 5′-d(**1d**-dT)$_6$, 5′-d(**1e**-dT)$_6$, and 5′-d(**1f**-dT)$_6$ duplexes[4–6] (Table 4), the $T_m$ values of the alternating duplexes differ significantly from each other. From Table 4, it is apparent that the oligonucleotides with alternating 7-substituted **1b-1f** show a stabilization of 15–30° over unsubstituted **1a**. Thermal stability increases from **1a-1e** with the exception of **1f**. We attribute the increase in $T_m$ values from **1a-1e** to the bulkiness of the 7-substituted groups (Table 5).[25] Being most bulky, the 7-hexynyl substituted compound **1f** (Table 5) does not lead to a duplex stabilization compared to

**TABLE 3** $T_m$ Values and Thermodynamic Data of Oligonucleotides Containing Compd. **1b**[a]

| Duplexes | | $T_m$ (°C) | $\Delta T_m$ (°) per modification | $\Delta H°$ (kcal/mol) | $\Delta S°$ (cal/mol K) | $\Delta G°_{310}$ (kcal/mol) |
|---|---|---|---|---|---|---|
| 5′-d(TAGGTCAATACT) | **23** | 47 | | −84 | −236 | −10.6 |
| 3′-d(ATCCAGTTATGA) | **24** | | | | | |
| 5′-d(TAGGTC**1b**ATACT) | **25** | 49 | +2.0 | −93 | −264 | −11.2 |
| 3′-d(ATCCAGTTATGA) | **24** | | | | | |
| 5′-d(TAGGTC**1b1b**TACT) | **26** | 50 | +1.5 | −89 | −250 | −11.5 |
| 3′-d(ATCCAGTTATGA) | **24** | | | | | |
| 5′-d(TAGGTCAATACT) | **23** | 53 | +3.0 | −90 | −252 | −12.1 |
| 3′-d(ATCC**1b**GTT**1b**TGA) | **27** | | | | | |
| 5′-d(TAG GTC**1b**ATACT) | **25** | 54 | +2.3 | −95 | −265 | −12.7 |
| 3′-d(ATCC**1b**GTT**1b**TGA) | **27** | | | | | |
| 5′-d(TAGGTC**1b1b**TACT) | **26** | 56 | +2.25 | −92 | −253 | −12.9 |
| 3′-d(ATCC**1b**GTT**1b**TGA) | **27** | | | | | |
| 5′-d(T**1b**GGTC**1b1b**T**1b**CT) | **28** | 54 | +1.75 | −92 | −256 | −12.4 |
| 3′-d(ATCCAGTTATGA) | **24** | | | | | |
| 5′-d(T**1b**GGTC**1b1b**T**1b**CT) | **28** | 58 | +1.8 | −95 | −261 | −13.9 |
| 3′-d(ATCC**1b**GTT**1b**TGA) | **27** | | | | | |

[a]Measured in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 $\mu$M single-strand concentration.

**TABLE 4** $T_m$ Values and Thermodynamic Data of Self-complimentary Oligonucleotides Containing the Nucleosides **1a-f**[a]

| Duplexes | | $T_m$ (°C) | $\Delta T_m$ (°) per modification | $\Delta H°$ (kcal/mol) | $\Delta S°$ (cal/mol K) | $\Delta G°_{310}$ (kcal/mol) |
|---|---|---|---|---|---|---|
| 5′-d[(A-T)$_6$]$_2$-3′ | **29•29** | 33 | | −45 | −125 | −6.3 |
| 5′-d[(**1a**-T)$_6$]$_2$-3′ | **30•30** | 36 | 0.25 | — | — | — |
| 5′-d[(**1b**-T)$_6$]$_2$-3′ | **31•31** | 51 | 1.5 | −56 | −152 | −9.3 |
| 5′-d[(**1c**-T)$_6$]$_2$-3′ | **32•32** | 52 | 1.6 | −59 | −157 | −9.7 |
| 5′-d[(**1d**-T)$_6$]$_2$-3′ | **33•33** | 56 | 1.9 | −61 | −163 | −10.5 |
| 5′-d[(**1e**-T)$_6$]$_2$-3′ | **34•34** | 66 | 2.75 | −91 | −246 | −14.5 |
| 5′-d[(**1f**-T)$_6$]$_2$-3′ | **35•35** | 56 | 1.9 | −61 | −163 | −10.6 |

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

the 7-propynyl residue **1e**. This suggests that the major groove can accommodate the bulky 7-substituents up to a certain size, thereafter it does reflect to the stability. Thus, the size of the 7-substituted groups plays an important role to the stabilization of the duplexes. Moreover, all the halogenated compounds increase the duplex stability compared to **1a**.

In contrary to the 7-substituted compounds **1a-1f**, 2-substituted compounds influence the base pairing property as they are located in the core of the helix. Earlier, the contribution of the 2-chloro group of 2-chloro-8-aza-7-deaza-2′-deoxyadenosine (**2a**) has been evaluated incorporating it in a duplex **23•24**.[14] The 2-chloro substituent causes steric clash with the 2-oxo group of dT as well as with the 2-amino group of dG and results to the destabilization. Besides that, it shows the stacking effect opposite to dC and dA. Recent studies on the RNA duplexes containing 2-methylthio adenosine and its derivatives have shown that 2-methylthio groups enhance the stacking interactions with adjacent base pairs.[15] Therefore, it was anticipated that the base pairing strength opposite to dC and dA can be maximized with the large size 2-MeS group (Table 5), which could lead to universal base pairing properties. For that, a single incorporation of 2-methylthio-8-

**TABLE 5** The Molar Volume (Size) of Nucleobases of **1a-f** and **2a**, **2b**, or **3c**

| Substituent | Molar volume ($cm^3$) | Van der Waals radii (Å) |
|---|---|---|
| H (**1a**) | 83.8 ± 3.0 | 1.20 (H) |
| 7-Cl (**1b**) or 2-Cl (**2a**) | 95.7 ± 3.0 | 1.80 (Cl) |
| 7-Br (**1c**) | 100.0 ± 3.0 | 1.95 (Br) |
| 7-I (**1d**) | 105.8 ± 3.0 | 2.15 (I) |
| 7-Propynyl (**1e**) | 118.3 ± 5.0 | — |
| 7-Hexynyl (**1f**) | 167.4 ± 5.0 | — |
| 2-MeS (**2b** or **3c**) | 116.2 ± 5.0 | — |

**TABLE 6** $T_m$ Values and Thermodynamic Data of Oligonucleotides Incorporating the Nucleosides **1a**, **2a**, and **2b** Opposite to the Canonical Nucleosides

| Duplexes | | $T_m$ (°C)[a] | $\Delta T_m$ (°C)[a] | $\Delta H°$ (kcal/mol) | $\Delta S°$ (cal/mol·K) | $\Delta G°_{310}$ (kcal/mol) |
|---|---|---|---|---|---|---|
| 5′-d(TAGGTCA**A**TACT) | **23** | 50 | | −90 | −252 | −12.0 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**1a**TACT) | **36** | 51 | +1 | −73 | −204 | −10.2 |
| 3′-d(ATCCAGT**T**ATGA) | **25** | | | | | |
| 5′-d(TAGGTCA**1a**TACT) | **36** | 36 | −15 | −64 | −181 | −7.5 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**1a**TACT) | **36** | 38 | −13 | −64 | −181 | −8.0 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**1a**TACT) | **36** | 44 | −6 | −70 | −196 | −9.0 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |
| 5′-d(TAGGTCA**2a**TACT) | **40** | 44 | −6 | −76 | −213 | −9.5 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**2a**TACT) | **40** | 31 | −19 | −58 | −166 | −6.7 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**2a**TACT) | **40** | 38 | −12 | −71 | −203 | −8.1 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**2a**TACT) | **40** | 38 | −12 | −60 | −167 | −7.9 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |
| 5′-d(TAGGTCA**2b**TACT) | **41** | 41 | −9 | −73 | −206 | −8.7 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**2b**TACT) | **41** | 37 | −13 | −67 | −192 | −7.6 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**2b**TACT) | **41** | 40 | −10 | −69 | −196 | −8.2 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**2b**TACT) | **41** | 39 | −11 | −73 | −209 | −8.0 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |
| 5′-d(TAGGTCA**S**TACT) | **42** | 33 | −17 | −47 | −129 | −7.0 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**S**TACT) | **42** | 34 | −16 | −52 | −146 | −7.1 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**S**TACT) | **42** | 34 | −16 | −51 | −141 | −7.2 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**S**TACT) | **42** | 33 | −17 | −48 | −131 | −7.0 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |

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

aza-7-deaza-2′-deoxyadenosine **2b** or **3c** was made replacing dA residue in the sequence **23**, leading to oligonucleotides **41** and **45** (Tables 6 and 7). The data for the 2-chloro compound **2a** used for the comparison is taken from the previously published article.[14]

According to the $T_m$ values shown in Table 6, the 2-methylthio nucleoside **2b** behaves almost identically towards all the DNA constituents (dA, dG, dT, and dC) and shows universal base pairing. $T_m$ values are generally 10–12°C lower than that of parent duplex **23•24**. As compared to the 2-chloro nucleoside **2a**, a degree of stabilization of **2b** against dC is 6°C higher and

**TABLE 7** $T_m$ Values and Thermodynamic Data of Oligonucleotides Incorporating the Nucleosides **3a**, **3b**, and **3c** Opposite to the Canonical Nucleosides

| Duplexes | | $T_m$ (°C)[a] | $\Delta T_m$ (°C)[a] | $\Delta H°$ (kcal/mol) | $\Delta S°$ (cal/mol·K) | $\Delta G°_{310}$ (kcal/mol) |
|---|---|---|---|---|---|---|
| 5′-d(TAGGTCAA**T**ACT) | **23** | 50 | | −90 | −252 | −12.0 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**3a**TACT) | **43** | 43 | −7 | −74 | −210 | −9.1 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**3a**TACT) | **43** | 44 | −6 | −79 | −226 | −9.3 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**3a**TACT) | **43** | 44 | −6 | −72 | −202 | −9.4 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**3a**TACT) | **43** | 46 | −4 | −75 | −209 | −9.8 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |
| 5′-d(TAGGTCA**3b**TACT) | **44** | 40 | −10 | −76 | −216 | −8.4 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**3b**TACT) | **44** | 46 | −4 | −83 | −233 | −10.3 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**3b**TACT) | **44** | 46 | −4 | −81 | −227 | −10.1 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**3b**TACT) | **44** | 41 | −9 | −77 | −221 | −8.8 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |
| 5′-d(TAGGTCA**3c**TACT) | **45** | 39 | −11 | −53 | −147 | −8.0 |
| 3′-d(ATCCAGT**T**ATGA) | **24** | | | | | |
| 5′-d(TAGGTCA**3c**TACT) | **45** | 47 | −3 | −77 | −218 | −9.7 |
| 3′-d(ATCCAGT**C**ATGA) | **37** | | | | | |
| 5′-d(TAGGTCA**3c**TACT) | **45** | 46 | −4 | −77 | −219 | −9.4 |
| 3′-d(ATCCAGT**A**ATGA) | **38** | | | | | |
| 5′-d(TAGGTCA**3c**TACT) | **45** | 39 | −11 | −50 | −134 | −8.0 |
| 3′-d(ATCCAGT**G**ATGA) | **39** | | | | | |

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



that against dA is 2°C higher. When **2b** is incorporated against dC, it behaves like **1a**, and against dA it gives a 2°C stabilization over that of **1a**. This is a direct result of a contribution of the 2-methylthio group to the stability by enhanced stacking interactions. When there is face-face base pairing, the 2-MeS group should accommodate in the minor grove and could result in labile duplexes. Based on the earlier reports of the purine polynucleotides: the 2-methylthio group of purine polynucleotides alters the Watson-Crick base pairs due to the steric repulsion with the 2-oxo group of heterocyclic rings and stabilizes the duplex by Hoogsteen base pairing. But this is not the case in **2b** as it cannot form the Hoogsteen base pairs.[17] Therefore, we believe that when compound **2b** pairs opposite to canonical bases, the base moiety might turn around protruding the 2-MeS group into the major groove which results in a stabilization by simple stacking interaction. This is also supported by the abasic residue **S** showing $T_m$ values of ~33°C against the canonicals. Thus, the presence of a 2-methylthio group of **2b** harmonizes

the base pairing and stabilizes the DNA duplexes by stacking interactions resulting in universal base pairing properties.

However, this is not the case with the unusually linked nucleoside **3c** (Table 7). Compound **3c** does not show universal base pairing properties of the nucleoside **3a** and forms two sets of duplexes showing similar stabilities. It gives maximum stabilization when located opposite dA and dC than those opposite dG and dT. The effect of the 2-MeS group of the $N^8$ isomer **3c** is exactly similar to that of the 2-chloro group of **3b**. The base pair motifs of **3b** are already suggested by He et al.[14] According to that, the 2-MeS group of the nucleoside **3c** must be located in the major groove of DNA helices. In case of **3c**-dC or **3c**-dA base pairs, the 2-MeS substituent is well accommodated in the major groove and has enough steric freedom during its interaction with the substituents of the cognate base. This may result to the maximum stabilization opposite to dC or dA. By contrast, when **3c** is situated opposite to dT or dG, the 2-MeS group clashes with the oxo groups of dT or dG resulting in 6–7°C lower $T_m$ values than that of **3c**-dC or **3c**-dA base pairs. Note that all the duplexes are more stable than those containing the abasic residue **S** (Table 6).



## EXPERIMENTAL

### General

All chemicals were purchased from Aldrich, Acros, Sigma, or Fluka (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade.Thin layer chromatography (TLC) silica gel 60 $F_{254}$ (0.2 mm) (VWR, Damstadt, Germany) and flash chromatography (FC) with 0.4 bar on silica gel 60 H (VWR, Darmstadt, Germany). NMR Spectra were measured on an Avance-DPX-250 spectrometer (Bruker, Germany), 250.13 MHz for $^1$H, 62.89MHz for $^{13}$C, 101.256Hz for $^{31}$P, $\delta$ values are in ppm related to internal $SiMe_4$ ($^1$H, $^{13}$C) or external $H_3PO_4$. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany).

**3-Chloro-4-methoxy-1*H*-pyrazolo[3,4-d]pyrimidine (4).** N-Chlorosuccinimide (5.0 g, 37.45 mmol) was added to a solution of 4-methoxy-1H-pyrazolo[3,4-d]pyrimidine[1,18] (2.81 g, 18.72 mmol) in anh. DMF (50 mL). After stirring for 32 h at 50°C, the solvent was removed in vacuo. The residue was applied to FC (column 50 × 3.5 cm). Elution was performed with $CH_2Cl_2$/MeOH, 98:2 (300 mL) followed by 95:5 (300 mL) affording compound **4** as a white solid (2.4 g, 69%). TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.57; UV (MeOH) $\lambda_{max}$ 246 ($\varepsilon$ 6600); $^1$H-NMR (DMSO-$d_6$) $\delta$ 4.10 (s, 3 H, $OCH_3$), 8.56 (s, 1 H, H-6), 14.20 (s, 1 H, NH); Anal. Calc. for $C_6H_5N_4OCl$ (184.58): C 39.04, H 2.73, N 30.35, Cl 19.21, found: C 39.34, H 2.86, N 30.65, Cl 19.34.

**3-Chloro-1-[2-deoxy-3,5-di-*O*-(p-toluoyl)-*β*-D-*erythro*-pentofuranosyl]-4-methoxy-1*H*-pyrazolo[3,4-d]pyrimidine (6).** To a suspension of compound **4** (370 mg, 2.01 mmol) in MeCN (50 mL), KOH (85%, 214 mg, 3.81 mmol) and TDA-1 (*tris*[2-(2-methoxyethoxy)ethyl]amine, 35 $\mu$L, 0.11 mmol) were added. After stirring at r.t. for 10 min, 2-deoxy-3, 5-di-*O*-p-toluoyl-$\alpha$-D-*erythro*-pentofuranosyl chloride (**5**)[20,21] (1.00 g, 2.57 mmol) were added, and stirring was continued for another 30 min.[4] Insoluble material was filtered off, the solvent evaporated, and the residue subjected to FC (column 40 × 2.5 cm). Elution was performed with petroleum ether/ethyl acetate, 3:1 (300 mL), 2:1 (300 mL), and 1:1 (300 mL). Compound **6** was crystallized from petroleum ether/ethyl acetate yielding colorless needles (344 mg, 32%). TLC (petroleum ether/ethyl acetate 2:1): $R_f$ 0.6; UV (MeOH) $\lambda_{max}$ 241 ($\varepsilon$ 23,500); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.37, 2.40 (2s, 6 H, 2 $CH_3$), 2.78 (m, 1 H, $H_\alpha$-2′), 3.18 (m, 1 H, $H_\beta$-2′), 4.13 (s, 3 H, $OCH_3$), 4.47 (m, 2 H, H-5′), 4.56 (m, 1 H, H-4′); 5.81 (m, 1 H, H-3′), 6.82 (t, 1 H, $J$ = 6.20, H-1′), 7.30–7.96 (m, 8 H, arom), 8.68 (s, 1 H, H-6); Anal. Calc. for $C_{27}H_{25}N_4O_6Cl$ (536.96): C 60.39, H 4.69, N 10.43; found: C 60.42, H 4.78, N 10.39.



**3-Chloro-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-d]pyrimidine (7).** Compound **6** (343.9 mg, 0.64 mmol) was stirred for 4 h in 0.4 M NaOMe/MeOH (150 mL). The solution was evaporated to dryness, and the residue was subjected to FC (column 40 × 2.5 cm, $CH_2Cl_2$/MeOH 9:1). Compound **7** (131 mg, 68%) was obtained as a white solid. TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.6; UV (MeOH) $\lambda_{max}$ 271 ($\varepsilon$ 7400), 246 ($\varepsilon$ 9100); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.31 (m, 1 H, $H_\alpha$-2′), 2.80 (m, 1 H, $H_\beta$-2′), 3.48 (m, 2 H, H-5′), 3.82 (m, 1 H, H-4′), 4.12 (s, 3 H, $OCH_3$), 4.43 (m, 1 H, H-3′), 4.71 (t, 1 H, $J$ = 5.77, OH-5′), 5.33 (d, 1 H, $J$ = 4.69, OH-3′), 6.62 (t, 1 H, $J$ = 6.67, H-1′), 8.67 (s, 1 H, H-6); Anal. Calc. for $C_{11}H_{13}N_4O_4Cl$ (300.70): C 43.94, H 4.36, N 18.63; found: C 44.04, H 4.26, N 18.56.

**4-Amino-3-chloro-1-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo [3,4-d]pyrimidine (1b).** Compound **7** (435.9 mg, 1.45 mmol) was suspended in sat. $NH_3$/MeOH (0°C) solution (200 mL) and the reaction mixture were stirred at 90°C for 4 h in a steel bomb. After cooling the solvent was removed in vacuo, and the residue was applied to FC (column 40 × 2.5 cm, $CH_2Cl_2$/MeOH 95:5). Compound **1b** (256 mg, 62%) was obtained as a white solid. TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.3; UV (MeOH) $\lambda_{max}$ 228 ($\varepsilon$ 7400), 260 ($\varepsilon$ 7500), 280 ($\varepsilon$ 10,300); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.24 (m, 1 H, $H_\alpha$-2′), 2.75 (m,1 H, $H_\beta$-2′), 3.47 (m, 2 H, H-5′), 3.78 (m, 1 H, H-4′), 4.39 (m, 1 H, H-3′), 4.76 (t, 1 H, $J$ = 5.55, OH-5′), 5.29 (d, 1 H, $J$ = 4.36, OH-3′), 6.51 (t, 1 H, $J$ = 6.30, H-1′), 7.18, 8.07 (br, 2 H, $NH_2$), 8.23 (s, 1 H, H-6); Anal. Calc. for $C_{10}H_{12}N_5O_3Cl$ (285.69): C 42.04, H 4.23, N 24.51; found: C 42.20, H 4.16, N 24.39.

**3-Chloro-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(dimethylamino) methylidene]amino}-1*H*-pyrazolo[3,4-d]pyrimidine (13).** A solution of compound **1b** (200 mg, 0.7 mmol) in MeOH (20 mL) was treated with *N,N*-dimethylformamide dimethyl acetal (1.62 mL, 12.1 mmol) for 20 min at r.t. After evaporation, the residue was applied to FC (column 12 × 3.0 cm, $CH_2Cl_2$/MeOH 95:5). Compound **13** was isolated as foam (226 mg, 95%). TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.45; UV (MeOH) $\lambda_{max}$ 320 ($\varepsilon$ 32,000); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.27 (m, 1 H, $H_\alpha$-2′), 2.76 (m,1 H, $H_\beta$-2′), 3.19, 3.24 (2s, 6 H, $Me_2N$), 3.46 (m, 2 H, H-5′), 3.81 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 4.76 (t, 1 H, $J = 5.66$, OH-5′), 5.29 (d, 1 H, $J = 4.50$, OH-3′), 6.56 (t, 1 H, $J = 6.39$, H-1′), 8.45 (s, 1 H, H-6), 8.95 (s, 1 H, N=CH); Anal. Calc. for $C_{13}H_{17}N_6O_3Cl$ (340.77): C 45.82, H 5.03, N 24.66; found: C 46.13, H 5.10, N 24.12.

**3-Chloro-1-(2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-1*H*-pyrazolo [3,4-d]pyrimidine (14).** To a solution of compound **13** (180.0 mg, 0.53 mmol) in dry pyridine (1.5 mL) was added 4,4′-dimethoxytriphenylmethyl chloride (217.0 mg, 0.64 mmol). After stirring at r.t. for 2 h, the mixture was poured into ice-cold 3% aq. $NaHCO_3$ soln. (5 mL), the aq. layer was extracted with $CH_2Cl_2$ (2 × 50 mL), the combined organic layers were dried ($Na_2SO_4$), filtered and evaporated. The residue was applied to FC (column 12 × 3 cm, $CH_2Cl_2$/MeOH 95:5). Compound **14** was isolated as colorless foam (291 mg, 86%). TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.5; UV (MeOH) $\lambda_{max}$ 233 ($\varepsilon$ 28,700), 320 ($\varepsilon$ 29,500); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.31 (m, 1 H, $H_\alpha$-2′), 2.76 (m,1 H, $H_\beta$-2′), 3.18, 3.23 (2s, 6 H, $Me_2N$), 3.07 (m, 2 H, H-5′), 3.68 (2s, 6 H, 2 MeO), 3.92 (m, 1 H, H-4′), 4.53 (m, 1 H, H-3′), 5.35 (d, 1 H, $J =$ 4.85, OH-3′), 6.59 (2d, 1 H, $J = 4.13$, H-1′), 6.71–7.32 (m, 13 H, arom), 8.49 (s, 1 H, H-6), 8.95 (s, 1 H, N=CH); Anal. Calc. for $C_{34}H_{35}N_6O_5Cl$ (643.13): C 63.50, H 5.49, N 13.07; found: C 63.47, H 5.38, N 12.89.

**3-Chloro-1-(2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl)-4-{[(dimethylamino)methylidene]amino}-1*H*-pyrazolo [3,4-d]pyrimidine 3′-*O*-[(2-Cyanoethyl) *N,N*-Diisopropylphosphoramidite] (15).** To a solution of compound **14** (237.8 mg, 0.37 mmol) in dry $CH_2Cl_2$ (20 mL) anh. *N,N*-diisopropylethylamine (0.118 mL, 0.68 mmol) and chloro(2-cyanoethoxy) (*N,N*-diisopropylamino)phosphine (0.118 mL, 0.53 mmol) were added under Ar. After stirring for 30 min, the reaction mixture was diluted with $CH_2Cl_2$ (20 mL), and 5% aq. $NaHCO_3$ (10 mL) was added. The mixture was extracted with $CH_2Cl_2$ (2 × 10 mL), the organic layers were dried ($Na_2SO_4$), filtered, and evaporated. The residue was applied to FC (column 12 × 2 cm, $CH_2Cl_2$/acetone, 92:8). Compound

**15** was isolated as foam (187 mg, 60%). TLC ($CH_2Cl_2$/acetone 9:1): $R_f$ 0.6, 0.7. $^{31}$P-NMR ($CDCl_3$) $\delta$ 149.64, 149.53.

**6-Methylthio-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (8).** A solution of 6-methylthio-4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine[19] (5.0 g, 24.91 mmol) in 1 M sodium isopropoxide in isopropanol (200 mL) was refluxed for 2 h. Upon cooling NaCl was precipitated with $Et_2O$ and was filtered off. The filtrate was neutralized with acetic acid and evaporated to dryness. The residue was dissolved in 200 mL of methanol. An addition of ice-cold water precipitated out **8** as pale yellow needles (4.10 g, 73%). TLC ($CH_2Cl_2$/MeOH 95:5): $R_f$ 0.48. UV (MeOH) $\lambda_{max}$ 278 ($\varepsilon$ 12,300), 236 ($\varepsilon$ 18,600); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.36, 1.39 (2s, 6 H, 2 $CH_3$), 2.52 (s, 3 H, $CH_3S$), 5.54 (m, 1 H, CH), 8.04 (s, 1 H, H-3), 13.72 (br s, 1 H, NH); Anal. Calc. for $C_9H_{12}N_4OS$ (224.28): C 48.20, H 5.39, N 24.98; found: C 48.18, H 5.25, N 24.81.

**1-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-$\beta$-D-*erythro*-pentofuranosyl]-6-methylthio-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (9).** A solution of **8** (500 mg, 2.23 mmol) and KOH (500 mg, 8.91 mmol) was stirred in MeCN for 15 min and followed by the addition of TDA-1 (81 $\mu$L, 0.25 mmol). After 15 min 1-chloro-2-deoxy-3,5-di-*O*-toluoyl-$\alpha$-D-*erythro*-pentofuranose (**5**)[20,21] (1.04 g, 2.68 mmol) was added in portions. The reaction mixture was stirred for another 15 min and filtered. The filtrate was concentrated and applied to FC (column 15 × 3 cm, $CH_2Cl_2$/MeOH 98:2). The fast migrating zone furnished **9** as colorless foam (688 mg, 54%). TLC ($CH_2Cl_2$/MeOH 98:2): $R_f$ 0.6. UV (MeOH) $\lambda_{max}$ 279 ($\varepsilon$ 15,700), 238 ($\varepsilon$ 49,300); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.39 (br s, 6 H, 2 $CH_3$), 2.39 (br s, 6 H, 2 $CH_3O$), 2.58 (s, 3 H, $CH_3S$), 2.79 (m, 1 H, $H_\alpha$-2′), 3.37 (m, $H_\beta$-2′, $D_2O$), 4.40–4.52 (m, 3 H, H-4′, H-5′), 5.53 (m, 1 H, CH), 5.86 (m, 1 H, H-3′), 6.76 (m, 1 H, H-1′), 7.33–7.98 (m, 8 H, arom), 8.19 (s, 1 H, H-3); Anal. Calc. for $C_{30}H_{32}N_4O_6S$ (576.66): C 62.48, H 5.59, N 9.72; found: C 62.69, H 5.47, N 9.58.

**2-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-$\beta$-D-*erythro*-pentofuranosyl]-6-methylthio-4-isopropoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine (10).** The slow migrating zone afforded **10** as colorless foam (375 mg, 29%). TLC ($CH_2Cl_2$/MeOH 98:2): $R_f$ 0.5. UV (MeOH) $\lambda_{max}$ 282 ($\varepsilon$ 11,700), 240 ($\varepsilon$ 51,100): $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.34, 1.36 (2s, 6 H, $(CH_3)_2CH$), 2.35, 2.40 (2s, 6 H, 2 $CH_3O$), 2.54 (s, 3 H, $CH_3S$), 2.79 (m, 1 H, $H_\alpha$-2′), 3.18 (m, 1 H, $H_\beta$-2′), 4.43–4.64 (m, 3 H, H-4′, H-5′), 5.49 (m, 1 H, OCH), 5.87 (m, 1 H, H-3′), 6.50 (t, 1 H, $J$ = 5.43, H-1′), 7.22–7.95 (m, 8 H, arom), 8.70 (s, 1 H, H-3); Anal. Calc. for $C_{30}H_{32}N_4O_6S$ (576.66): C 62.48, H 5.59, N 9.72; found: C 62.68, H 5.56, N 9.78.

**1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-6-methylthio-4-isopropoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (11).** A suspension of **9** (3 g, 5.20 mmol) in 200 mL of 0.1 M NaOiPr was stirred for 15 min. The clear solution was evaporated and applied to FC (column 12 × 3 cm). Stepwise elution with 2% MeOH in $CH_2Cl_2$, followed by 10% MeOH in $CH_2Cl_2$, gave **11** as a colorless solid (1.5 g, 85%). TLC ($CH_2Cl_2$/MeOH 95:5): $R_f$ 0.25. UV (MeOH) $\lambda_{max}$ 279 ($\varepsilon$ 14,500), 238 ($\varepsilon$ 18,800); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.37, 1.39 (2s, 6 H, 2 $CH_3$), 2.58 (s, 3 H, $CH_3S$), 2.28 (m, 1 H, $H_\alpha$-2′), 2.82 (m, 1 H, $H_\beta$-2′), 3.49 (m, 2 H, H-5′), 3.80 (m, 1 H, H-4′), 4.45 (m, 1 H, H-3′), 4.71 (m, 1 H, OH-5′), 5.30 (m, 1 H, OH-3′), 5.56 (m, 1 H, CH), 6.61 (t, 1 H, $J$ = 6.36, H-1′), 8.15 (s, 1 H, H-3); Anal. Calc. for $C_{14}H_{20}N_4O_4S$ (340.40): C 49.40, H 5.92, N 16.46; found: C 49.68, H 5.83, N 16.29.

**2-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-6-methylthio-4-isopropoxy-2*H*-pyrazolo[3,4-*d*]pyrimidine (12).** As described for **11**, compound **10** (3 g, 5.20 mmol) gave **12** as a colorless solid (1.6 g, 90%). TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.48. UV (MeOH) $\lambda_{max}$ 282 ($\varepsilon$ 10,800), 240 ($\varepsilon$ 19,700), 227 ($\varepsilon$ 17,500); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.35, 1.38 (2s, 6 H, $(CH_3)_2CH$), 2.51 (s, 3 H, $CH_3S$), 2.34 (m, 1 H, $H_\alpha$-2′), 2.62 (m, 1 H, $H_\beta$-2′), 3.61 (m, 2 H, H-5′), 3.89 (m, 1 H, H-4′), 4.40 (m, 1 H, H-3′), 4.95 (m, 1 H, OH-5′), 5.35 (m, 1 H, OH-3′), 5.52 (m, 1 H, $(CH_3)_2CH$), 6.27 (t, 1 H, $J$ = 6.11, H-1′), 8.71 (s, 1 H, H-3); Anal. Calc. for $C_{14}H_{20}N_4O_4S$ (340.40): C 49.40, H 5.92, N 16.46; found: C 49.37, H 5.80, N 16.41.

**4-Amino-1-[2-deoxy-β-D-*erythro*-pentofuranosyl]-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidine (2b).** A suspension of **11** (200 mg, 0.59 mmol) in methanol, saturated with dry ammonia (100 mL), was stirred in a glass sealed bottle for 5 days at r.t. The clear solution was adsorbed on silica gel and applied to FC (column 15 × 3 cm, $CH_2Cl_2$/MeOH 9:1). Compound **2b** was isolated as a colorless solid (160 mg, 92%). TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.48. UV (MeOH) $\lambda_{max}$ 278 ($\varepsilon$ 14,600), 242 ($\varepsilon$ 28,800); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.48 (s, 3 H, $CH_3S$), 2.25 (m, 1 H, $H_\alpha$-2′), 2.81 (m, 1 H, $H_\beta$-2′), 3.51 (m, 2 H, H-5′), 3.79 (m, 1 H, H-4′), 4.42 (m, 1 H, H-3′), 4.73 (t, 1 H, $J$ = 5.75, OH-5′), 5.27 (d, 1 H, $J$ = 4.47, OH-3′), 6.50 (t, 1 H, $J$ = 6.5, H-1′), 7.71, 7.88 (m, 2 H, $NH_2$), 8.05 (s, 1 H, H-3); Anal. Calc. for $C_{11}H_{15}N_5O_3S$ (297.33): C 44.43, H 5.08, N 23.55; found: C 44.48, H 5.03, N 23.46.

**4-Amino-2-[2-deoxy-β-D-*erythro*-pentofuranosyl]-6-methylthio-2*H*-pyrazolo[3,4-*d*]pyrimidine (3c).** As described for **2b**, compound **3c** was obtained from **12** (200 mg, 0.59 mmol) as a colorless solid (160 mg, 92%). TLC ($CH_2Cl_2$/MeOH 95:5): $R_f$ 0.5. UV (MeOH) $\lambda_{max}$ 278 ($\varepsilon$ 11,500), 240 ($\varepsilon$ 21,500);$^1$H-NMR (DMSO-$d_6$) $\delta$ 2.51 (s, 3 H, $CH_3S$), 2.32 (m, 1 H, $H_\alpha$-2′), 2.62 (m, 1 H, $H_\beta$-2′), 3.43–3.57 (m, 2 H, H-5′), 3.89 (m, 1 H, H-4′), 4.37 (m,

1 H, H-3′), 4.87 (m, 1 H, OH-5′), 5.31 (d, 1 H, $J$ = 4.31, OH-3′), 6.26 (t, 1 H, $J$ = 6.14, H-1′), 7.69 (m, 2 H, $NH_2$), 8.42 (s, 1 H, H-3); Anal. Calc. for $C_{11}H_{15}N_5O_3S$ (297.33): C 44.43, H 5.08, N 23.55; found: C 44.55, H 5.02, N 23.46.

**1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-4{[(dimethylamino)methylidene]amino}-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidine (16).** A solution of compound **2b** (250 mg, 0.84 mmol) in MeOH was stirred with *N,N*-dimethylformamide dimethylacetal (1.4 g) at 50°C for 6 h. The solution was evaporated and applied to FC (column 20 × 3 cm, $CH_2Cl_2$/MeOH 95:5) to give compound **16** as colorless solid (250 mg, 84.4%). TLC ($CH_2Cl_2$/MeOH 95:5): $R_f$ 0.4. UV (MeOH) $\lambda_{max}$ 315 ($\varepsilon$ 17,400), 292 ($\varepsilon$ 18,300), 272 ($\varepsilon$ 16,400), 245 ($\varepsilon$ 18,700); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.55 (s, 3 H, $CH_3S$), 2.28 (m, 1 H, $H_\alpha$-2′), 2.85 (m, 1 H, $H_\beta$-2′), 3.15, 3.23 (2s, 6 H, 2 $CH_3$), 3.38–3.54 (m, 2 H, H-5′), 3.82 (m, 1 H, H-4′), 4.44 (m, 1 H, H-3′), 4.74 (t, 1 H, $J$ = 5.72, OH-5′), 5.30 (d, 1 H, $J$ = 4.44, OH-3′), 6.56 (t, 1 H, $J$ = 6.43, H-1′), 8.06 (s, 1 H, N=CH), 8.86 (s, 1 H, H-3); Anal. Calc. for $C_{14}H_{20}N_6O_3S$ (352.41): C 47.71, H 5.72, N 23.85; found: C 47.41, H 5.80, N 23.67.

**1-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-4{[(dimethylamino)ethylidene]amino}-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidine (17).** A solution of compound **2b** (150 mg, 0.50 mmol) in MeOH was stirred with *N,N*-dimethylacetamide dimethylacetal (1 mL) at 40°C for 16 h. The solution was evaporated and applied to FC (column 20 × 3 cm, $CH_2Cl_2$/acetone 8:2) to give compound **17** as colorless solid (170 mg, 92%). TLC ($CH_2Cl_2$/acetone 8:2): $R_f$ 0.38. UV (MeOH) $\lambda_{max}$ 219 ($\varepsilon$ 13,800), 269 ($\varepsilon$ 12,400), 246 ($\varepsilon$ 20,300); $^1$H-NMR (DMSO-$d_6$) $\delta$ 2.26 (s, 3 H, $CH_3$), 2.51 (s, 3 H, $CH_3S$), 2.27 (m, 1 H, $H_\alpha$-2′), 2.85 (m, 1 H, $H_\beta$-2′), 3.15, 3.23 (2s, 6 H, 2 $CH_3$), 3.38–3.54 (m, 2 H, H-5′), 3.82 (m, 1 H, H-4′), 4.44 (m, 1 H, H-3′), 4.71 (t, 1 H, $J$ = 5.70, OH-5′), 5.27 (d, 1 H, $J$ = 4.41, OH-3′), 6.56 (t, 1 H, $J$ = 6.43, H-1′), 7.96 (s, 1 H, H-3); Anal. Calc. for $C_{15}H_{22}N_6O_3S$ (366.44): C 49.17, H 6.05, N 22.93; found: C 49.26, H 6.10, N 22.78.

**1-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4{[(dimethylamino)ethylidene]amino}-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidine (18).** Compound **17** (125 mg, 0.34 mmol) in 2 mL of pyridine was stirred with $(MeO)_2$TrCl (150 mg, 0.44 mmol) at r.t. for 2.5 h. The reaction was quenched by the addition of 5% $NaHCO_3$ solution (10 mL) and extracted with dichloromethane (3 × 15 mL). The organic layer was dried over $Na_2SO_4$ and evaporated. The resulting residue was subjected to FC (column 15 × 3 cm, $CH_2Cl_2$/acetone 9:1) to give compound **18** as colorless foam (192 mg, 84%). TLC ($CH_2Cl_2$/acetone 9:1): $R_f$ 0.55. UV (MeOH) $\lambda_{max}$ 282 ($\varepsilon$ 14,300), 273 ($\varepsilon$ 14,600), 237 ($\varepsilon$ 33,600); $^1$H-NMR

(DMSO-$d_6$) $\delta$ 2.22 (s, 3 H, $CH_3$), 2.51 (s, 3 H, $CH_3S$), 2.32 (m, 1 H, $H_\alpha$-2′), 2.84 (m, 1 H, $H_\beta$-2′), 3.15, 3.23 (2s, 6 H, 2 $CH_3$), 3.02–3.15 (m, 2 H, H-5′), 3.71, 3.72 (2s, 6 H, 2 $CH_3O$), 3.93 (m, 1 H, H-4′), 4.56 (m, 1 H, H-3′), 5.31 (d, 1 H, $J = 4.83$, OH-3′), 6.58 (m, 1 H, H-1′), 7.90 (s, 1 H, H-3), 6.74–7.32 (m, 13 H, arom); Anal. Calc. for $C_{36}H_{40}N_6O_5S$ (668.81): C 64.65, H 6.03, N 12.57; found: C 65.04, H 6.04.

**1-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4{[(dimethylamino)ethylidene]amino}-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidine 3′-[(2-Cyanoethyl)-N,N-diisopropyl-phosphoramidite] (19).** To a soln. of compd. **18** (90 mg, 0.14 mmol) and $(iPr)_2EtN$ (30 μL, 0.17 mmol) in anh. $CH_2Cl_2$ (2 mL), 2-cyanoethyl diisopropylphosphoramidochloridite (50 μL, 0.29 mmol) was added at r.t.. After stirring for 20 min, the mixture was diluted with $CH_2Cl_2$ (10 mL) and quenched by adding a 5% aq. $NaHCO_3$ soln. (20 mL). Then, the aq. layer was extracted with $CH_2Cl_2$ (3 × 15 mL), the combined org. layer dried over $Na_2SO_4$ and evaporated. The resulting oil was applied to FC (column 10 × 3 cm, $CH_2Cl_2$/acetone 95:5) affording colorless foam (85 mg, 73%). TLC ($CH_2Cl_2$/acetone 95:5): $R_f$ 0.52, 0.48. $^{31}$P-NMR ($CDCl_3$) $\delta$ 149.9, 149.6.

**2-[2-Deoxy-β-D-*erythro*-[pentofuranosyl]-4{[isobutyryl]amino}-6-methylthio-2*H*-pyrazolo[3,4-*d*]pyrimidine (20).** Compound **3c** (200 mg, 0.67 mmol) was repeatedly evaporated with pyridine and suspended in pyridine (2 mL). TMSCl (1.15 mL, 9.06 mmol) was added and stirred for 30 min, followed by the addition of isobutyryl chloride (0.14 mL, 1.34 mmol) and stirring was continued for 2 h at r.t. The reaction mixture was cooled in an ice-bath, 1 mL $H_2O$ was added. After 5 min, 1 mL 25% aq. $NH_3$-soln. was added, and the solution stirred for 15 min more. The solution evaporated to dryness, dissolved in 10 mL $H_2O$, and extracted with $CH_2Cl_2$ (3 × 15 mL).The organic layer was dried over $Na_2SO_4$, evaporated and applied to FC (column 15 × 3 cm, $CH_2Cl_2$/MeOH 95:5). Compound **20** was obtained as colorless foam (150 mg 60.69%). TLC ($CH_2Cl_2$/MeOH 95:5): $R_f$ 0.46. UV (MeOH) $\lambda_{max}$ 297 ($\varepsilon$ 10,800), 245 ($\varepsilon$ 16,400), 216 ($\varepsilon$ 14,000); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.13, 1.14 (2s, 6 H, 2 $CH_3$), 2.52 (s, 3 H, $CH_3S$), 2.34 (m, 1 H, $H_\alpha$-2′), 2.67 (m, 1 H, $H_\beta$-2′), 2.89 (m, 1 H, $CH(CH_3)_2$), 3.48–3.61 (m, 2 H, H-5′), 3.92 (m, 1 H, H-4′), 4.45 (m, 1 H, H-3′), 4.86 (t, 1 H, $J = 5.55$, OH-5′), 5.32 (d, 1 H, $J = 4.4$, OH-3′), 6.41 (t, 1 H, $J = 5.8$, H-1′), 8.91 (s, 1 H, H-3), 11.2 (s, 1 H, NH); Anal. Calc. for $C_{15}H_{21}N_5O_4S$ (367.42): C 49.03, H 5.76, N 19.06; found: C 48.96, H 5.65, N 18.89.

**2-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4{[isobutyryl]amino}-6-methylthio-2*H*-pyrazolo[3,4-*d*]pyrimidine (21).** To a stirred solution of **20** (100 mg, 0.27 mmol ) in anhydrous

pyridine (2 mL) was added 4-dimethylaminopyridine (25 mg, 0.20 mmol), followed by $(MeO)_2TrCl$ (125 mg, 0.37 mmol ). The reaction was worked up as described for **18**. Compound **21** was obtained as colorless foam (107 mg, 59%). TLC ($CH_2Cl_2$/acetone 9:1): $R_f$ 0.62. UV (MeOH) $\lambda_{max}$ 275 ($\varepsilon$ 12,200), 236 ($\varepsilon$ 30,300); $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.13, 1.14 (2s, 6 H, 2 $CH_3$), 2.53 (s, 3 H, $CH_3S$), 2.34 (m, 1 H, $H_\alpha$-2′), 2.83 (m, 1 H, $H_\beta$-2′), 2.86 (m, 1 H, $CH(CH_3)_2$), 3.10–3.31 (m, 2 H, H-5′), 3.67, 3.69 (2s, 6 H, 2 $CH_3O$), 3.98 (m, 1 H, H-4′), 4.50 (m, 1 H, H-3′), 5.36 (d, 1 H, $J = 5.0$, OH-3′), 6.45 (m, 1 H, H-1′), 8.94 (s, 1 H, H-3), 11.21 (s, 1 H, NH), 6.70-7.29 (m, 13 H, arom); Anal. Calc. for $C_{36}H_{39}N_5O_6S$ (669.79): C 64.56, H 5.87, N 10.46; found: C 64.60, H 5.99, N 10.40.

**2-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-*β*-D-*erythro*-pentofuranosyl]-4{[isobutyryl]amino}-6-methylthio-2*H*-pyrazolo[3,4-*d*]pyrimidine 3′-[(2-Cyanoethyl)-N,N-diisopropyl-phosphoramidite] (22).** As described for **19**, with **21** (70 mg, 0.11 mmol), $(iPr)_2EtN$ (25 $\mu$L, 0.145 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (50 $\mu$L, 0.22 mmol): **22** (70 mg, 77%). Colorless foam. TLC ($CH_2Cl_2$/acetone 95:5): $R_f$ 0.60, 0.58. $^{31}$P-NMR ($CDCl_3$) $\delta$ 150.1, 150.4.

## Oligonucleotides

The synthesis was performed with DNA solid phase synthesizer, Model 392 (Applied Biosystems, Weiterstadt, Germany) with "trityl-on" mode. The oligonucleotides were purified and desalted by HPLC with RP-18 (5 $\mu$m, 4 × 250 mm and 4 × 125 mm) (LiChrospher®, Merck, Germany), lyophilized with SpeedVac centrifuge (Savant Instruments, Farmingdale, New York). MALDI-TOF spectra were measured with Biflex III spectrometer (Bruker Saxonia, Leipzig, Germany). Melting curves were measured with a Cary 1/1E UV/VIS spectrophotometer (Varian, Australia) equipped with a thermoelectrical controller; the actual temperature was measured in the reference cell with a PT-100 resistor. UV-spectra were measured on U-3200 spectrophotometer (Hitachi, Japan). The oligonucleotides were cleaved from the solid support by 25% aq. $NH_3$-soln., and purified by HPLC with the gradient: 3 min 20% B in A, 12 min 20–40% B in A with a flow rate of 1.0 mL/min (*A*, 0.1 M ($Et_3NH$) OAc (pH 7.0)/MeCN 95:5; *B*, MeCN). They were detritylated with 2.5% dichloroacetic acid in dichloromethane, lyophilized, and coevaporated with methanol. The residue was dissolved in bidistilled water and purified by HPLC with the gradient 20 min 0–20% B in A with a flow rate of 1 mL/min. The purified oligonucleotides were desalted with water and eluted with water/methanol (3:2). The oligonucleotides were characterized by MALDI-TOF mass spectrometry (Table 2).

## CONCLUSION

The 8-aza-7-deazaadenine 2′-deoxyribonucleosides **1b** with a chloro residue at the 7-position strengthen the dA-dT base pair stability, as it was shown for related oligonucleotides containing bromine or iodine at that position. On the other hand, 2-chloro or 2-methylthio groups have an unfavorable influence on the duplex stability, leading to harmonization of base pair stability when **2b** is located opposite to dC and dA. Thus, this nucleoside can be considered as a universal nucleoside. A universal base pairing was also reported for the $N^8$-glycosylated nucleoside. However, the ambiguous base pairing of **3c** is not so striking. It forms two sets of duplex stability showing a maximum stabilization against dA or dC than that of dG or dT.

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25. Molar volume values were calculated using the program ChemSketch (version 4.55, provided by Advanced Chemistry Developments Inc., Toronto, Canada; http:/www.acdlabs.com).