Replacement of Canonical DNA Nucleobases by Benzotriazole and 1,2,3-Triazolo[4,5-*d*]pyrimidine: Synthesis, Fluorescence, and Ambiguous Base Pairing

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The syntheses and the fluorescence properties of 7*H*-3,6-dihydro-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2′-deoxy-*β*-D-ribonucleosides (=2′-deoxy-8-azainosine) **3** (N^3), **15** (N^2), and **16** (N^1) as well as of 1,2,3-benzotriazole 2′-*O*-methyl-*β*- or -*α*-D-ribofuranosides **6** (N^1) and **24** (N^1) are described. Also the fluorescence properties of 1,2,3-benzotriazole 2′-deoxy-*β*-D-ribofuranosides **4** (N^1) and **5** (N^2) are evaluated. From the nucleosides **3**–**6**, the phosphoramidites **19**, **26a, 26b**, and **28** are prepared and employed in solid-phase oligonucleotide synthesis. In 12-mer DNA duplexes, compound **3** shows similar ambiguous base-pairing properties as 2′-deoxyinosine (**1**), while the nucleosides **4**–**6** show strong pairing with each other and discriminate very little the four canonical DNA constituents.

Introduction. – DNA-Duplex stability does not only depend on the horizontal interactions of the bases (H-bonding), but also vertical interactions (base stacking) are of utmost importance [1–6]. These interactions are controlled by the properties of the nucleobases (regular or modified ones) but also by the structure of the sugar–phosphate backbone (*N* or *S* sugar conformation, PNA, LNA) and the environmental conditions such as counter ions and H_2O molecules. Within the series of base-modified oligonucleotides, a number of heterocyclic systems (hydrophilic or hydrophobic) were synthesized and incorporated in DNA or RNA for various purposes [6–13]. This led to the development of the so-called universal or ambiguous nucleosides which are categorized into two types: *i*) those forming base pairs by H-bonding and *ii*) those stabilizing duplexes by stacking.

The 2′-deoxyinosine (**1**) is one of the most widely used universal nucleosides for primers and probes in DNA technology. It forms H-bonds with all four natural constituents of DNA; however, a wide range of T_m values is noticed [9][10]. Earlier, we have studied the ambiguous base-pairing properties of the related 2′-deoxy-7-deazainosine (**2**) in DNA duplexes [11]. The oligonucleotides incorporating **2** show similar base-pairing properties to those of 2′-deoxyinosine (**1**), and **2** emerges also as a universal nucleoside. As almost all of the universal nucleosides decrease the T_m values of the oligonucleotide duplexes, we were looking for analogous compounds not showing such drawbacks. Recently, it was reported that an additional N-atom in the 8-position of purines leads to duplex stabilization compared to the parent purines [12–15]. This prompted us to synthesize 2′-deoxy-8-azainosine (=7*H*-3,6-dihydro-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2′-deoxy-*β*-D-ribonucleoside; **3**; systematic numbering

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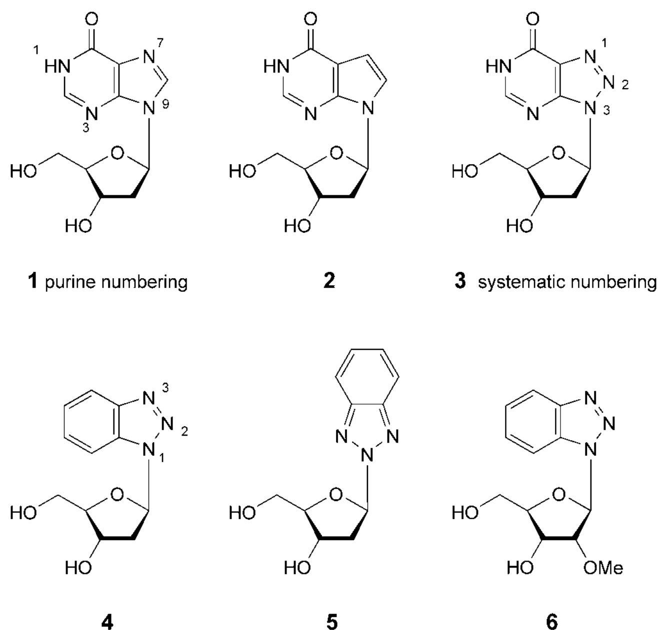
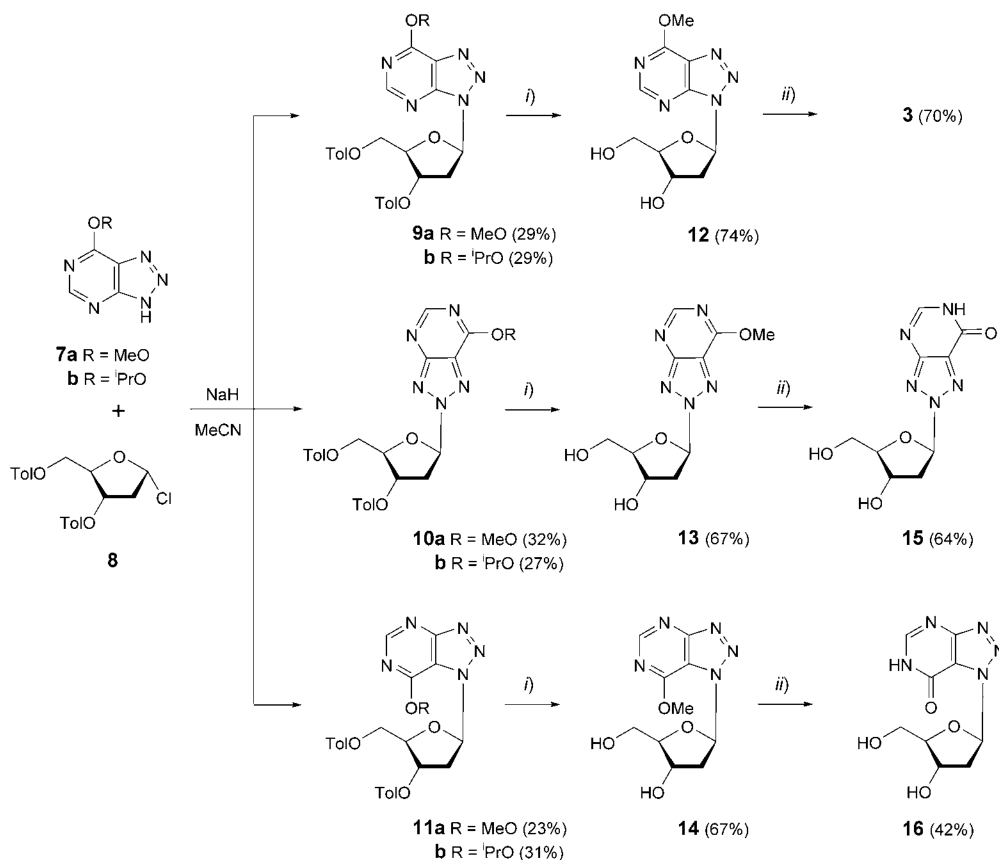
is used throughout the *General Part*; purine numbering is used exceptionally only when data are compared with those of purine analogues), and to study its potential as universal nucleoside. Likewise, related 1,2,3-benzotriazole nucleosides such as **4** and **5** as well as the 2′-methoxy derivative **6** were prepared. Compounds **4**–**6** are not able to form H-bonds but can develop stacking interactions through the benzene rings which are replacing the pyrimidinone moieties of compound **3**. Moreover, 1,2,3-benzotriazole nucleosides (=8-azapurine nucleosides) are known to be fluorescent; therefore, their fluorescence properties are of particular interest for studying the behavior in various biological systems. This communication reports on the synthesis and the fluorescence properties of the 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (=8-azapurine) and benzotriazole nucleosides **3**–**6**. Corresponding phosphoramidites were synthesized, and the base-pairing properties of oligonucleotides containing these modified nucleosides were investigated.



Results and Discussion. – 1. *Monomers.* The synthesis of 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (8-azapurine) nucleosides encounters difficulties for the reason that regioisomers as well as anomers can be formed upon glycosylation [16]. The synthesis of our target nucleoside **3** was first described by *Montgomery* [16b]: The reaction of *N*-(3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl)nonanamide with 3,5-bis-*O*-(4-chlorobenzoyl)-2-deoxy-D-ribofuranosyl chloride in the presence of Et_3N in refluxing benzene yielded an anomeric mixture, which was separated and deacylated; compound **3** was then obtained by deamination of the 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine 3-(2′-deoxy-β-D-ribofuranoside) (=2′-deoxy-8-azaadenosine) [16]. However, this route resulted in a low overall yield of **3**. Our laboratory has reported the synthesis of 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-amine 3-(2′-deoxy-β-D-ribofuranoside) *via* the amination

Scheme 1



i) 1M MeONa/MeOH (30 ml), r.t., 30 min. *ii*) 1M NaOH (30 ml), 50°, 3 h.

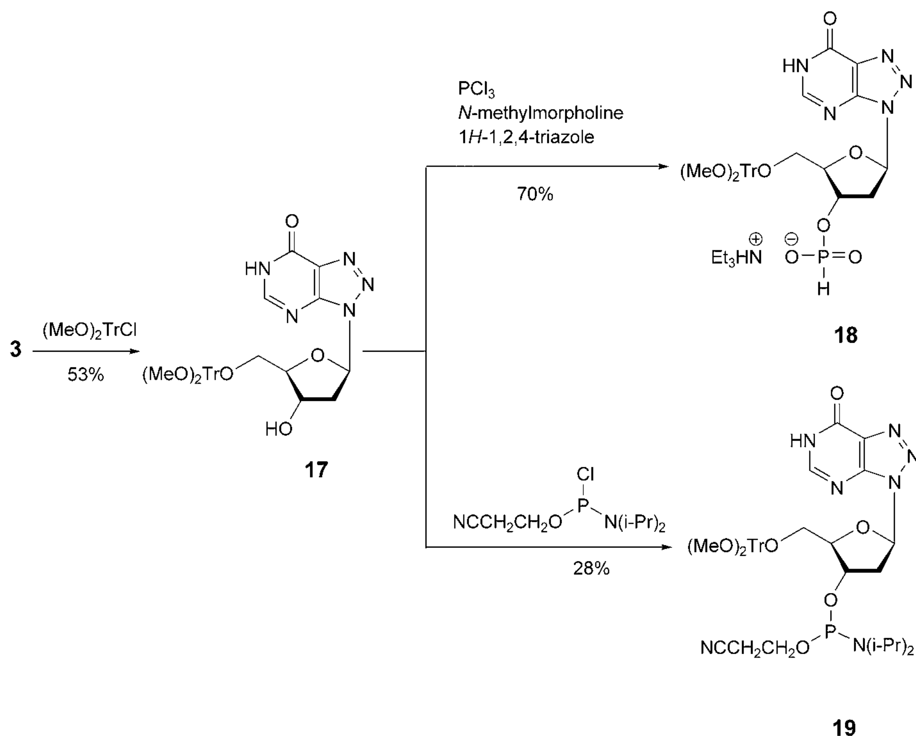
of the corresponding methoxy nucleoside [17]. The glycosylation of 7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**7a**) with 2′-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl chloride (**8**) was undertaken affording a mixture of the regioisomeric glycosylation products **9a** (N^3), **10a** (N^2), and **11a** (N^1) in a ratio of nearly 3:4:3 [17] (*Scheme 1*). As the methoxy nucleosides are susceptible for the nucleophilic displacement by OH^- ions, they are target molecules for the preparation of 7*H*-3,6-dihydro-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (=2′-deoxy-8-azainosine) nucleosides **3**, **15**, and **16**. We also tried to alter the ratio of regioisomers and opted for the glycosylation of 7-isopropoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**7b**) carrying a bulky residue in the 7-position. For that, 7-isopropoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (**7b**) was synthesized and glycosylated with pentofuranosyl chloride **8** to give a mixture of the glycosylation products regioisomeric at N(3) (**9b**, 29%), N(2) (**10b**, 27%), and

N(1) (**11b**, 31%). As the proportion was not significantly changed in favor of the N^3 isomer **9b**, the MeO derivatives were chosen for further manipulation [17].

Deprotection of compounds **9a**, **10a**, and **11a** in 1M NaOMe/MeOH afforded 7-methoxy nucleosides **12**–**14**, which, on further treatment with 1M NaOH, led to 1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2′-deoxyribonucleosides (=2′-deoxy-8-azainosine derivatives) **3**, **15**, and **16**, respectively (*Scheme 1*). The site of glycosylation was assigned by ^{13}C-NMR spectra. As the precursor methoxy compounds **9a**–**11a** used in the experiment were already assigned earlier [17], we followed the same assignment for **3**, **15**, and **16**. The ^{13}C-NMR spectra of the regioisomers **3**, **15**, and **16** show changes in the chemical shifts of the bridgehead C-atoms C(3a) and C(7a). As compared to **3** (N^3 isomer), a downfield shift of C(3a) (8 ppm) of **15** (N^2) and a strong upfield shift of C(7a) (11 ppm) for compound **16** (N^1) confirms the site of glycosylation as N(3), N(2), and N(1) for **3**, **15** and **16**, respectively. Compound **3** was also prepared enzymatically from **9a** with adenosine deaminase from calf intestinal mucosa.

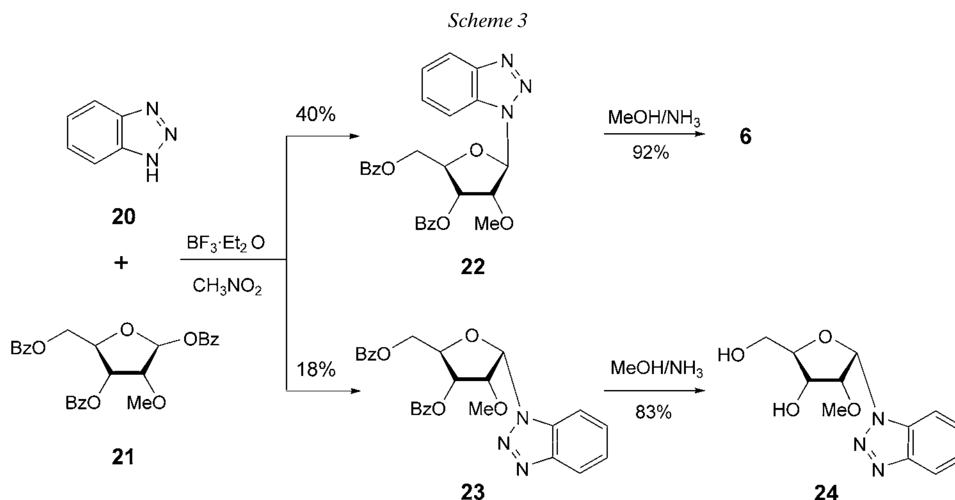
Next, nucleoside **3** was converted into the 4,4′-dimethoxytrityl derivative **17** which on further reaction with PCl_3/*N*-methylmorpholine/1*H*-1,2,4-triazole afforded the 3′-phosphonate **18**. The phosphoramidite **19** was prepared from **17** with 2-cyanoethyl diisopropylphosphoramidochloridite [13] (*Scheme* 2).

Scheme 2



Likewise, the benzotriazole nucleosides were prepared. We have already reported the synthesis of the benzotriazole 2′-deoxyribonucleosides **4** and **5** by nucleobase-anion glycosylation [18]. The glycosylation of 1*H*-1,2,3-benzotriazole (**20**) with 1,3,5-tri-*O*-benzoyl-2-*O*-methyl-D-ribofuranose (**21**) was carried out in nitromethane with $BF_3 \cdot Et_2O$ as catalyst (*Scheme 3*). In this case, the *N*[1]-glycosylation product was the only regioisomer formed, which is the result of the thermodynamic control of the reaction (high temperature); unfortunately, an anomeric mixture of the *N*[1]-glycosylated compounds **22** and **23** was obtained [19]. The *O*-benzoyl groups were removed with methanolic ammonia to give the 1-(2-*O*-methyl-β/α-D-ribofuranosyl)-1*H*,2,3-benzotriazoles **6** and **24** in a 2:1 ratio (*Scheme 3*). The site of glycosylation and the anomeric configuration of **6** and **24** were assigned on the basis of both ^{1}H-NMR and UV spectra.

Scheme 3



The UV spectra of **6** and **24** are strikingly similar. The ^{1}H-NMR spectral comparison of **6** and **24** revealed that H−C(1′) and H−C(4′) of the α-D-anomer **24** always resonate downfield from that of the β-D-anomer **6** [20] [21]. The correct identity of the anomers was assigned by ^{1}H-NMR NOE difference spectroscopy. Irradiation of H−C(1′) of **6** resulted in an NOE at H−C(4′) of 1.8%, while that of **24** gave an NOE at H−C(2′) of 7.5% and at H−C(3′) of 0.8%. Due to the similar environment of H−C(1′) and H−C(4′) in **6** and that of H−C(1′), H−C(2′), and H−C(3′) in **24**, they were assigned to be the β-D- and α-D-anomers, respectively.

Treatment of the nucleosides **4**, **5**, or **6** with 4,4′-dimethoxytrityl chloride ($(MeO)_2TrCl$) yielded the $(MeO)_2Tr$ derivatives **25a**, **25b**, and **27** (*Scheme 4*), from which the phosphoramidites **26a**, **26b**, and **28** were prepared with 2-cyanoethyl diisopropylphosphoramidochloridite (*Scheme 4*).

All monomeric compounds were characterized by elemental analysis as well as by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra (*see Exper. Part* and *Tables 1* and *2*). The ^{13}C-NMR signals were identified by gated-decoupled ^{13}C-NMR spectra or heteronuclear ^{1}H,^{13}C-NMR correlation spectra.

2. *Fluorescence Properties.* The fluorescence of 5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one ribonucleoside (8-azaguanosine) and its 2′-deoxy de-

Scheme 4

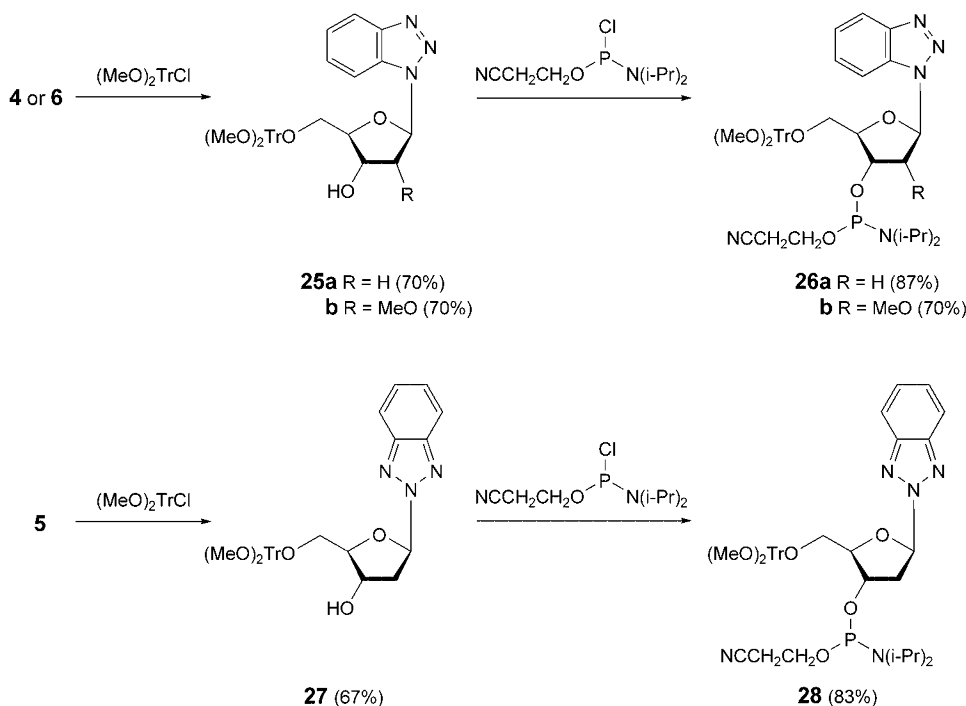


Table 1. *^{13}C-NMR Chemical Shifts of 1,2,3-Triazolo*[*4,5*-d]*pyrimidines*[a])[b])

	C(3a)	C(5)	C(7)	C(7a)	OCH	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
7b	153.4	155.5	160.1	123.8	71.1					
9b	153.9	156.5	161.9	114.8	72.1	88.2	36.3	74.0	82.2	63.5
10b	159.2	156.1	161.5	126.3	71.5	94.1	36.8	73.9	82.9	63.4
11b	150.5	156.5	160.7	125.7	63.5	82.2	35.4	74.3	85.6	71.7
3	148.6	155.3	150.1	130.0		85.2	38.2	70.6	88.5	61.9
15	156.3	148.8	157.2	131.5		93.6	[c])	70.5	88.8	62.0
16	153.2	147.0	158.3	119.0		87.7	[c])	70.6	88.7	61.8

[a]) Measured in (D_6)DMSO. [b]) Systematic numbering. [c]) Superimposed by DMSO.

rivative at neutral pH increases by several orders when the pH of the solution is raised to 11.0 [22] [23]. It was, therefore, of interest to investigate the fluorescence properties of compounds **3**, **15**, **16**, and **4**–**6** in neutral and alkaline medium (see the *Fig.* and *Table 3*): all three 3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2′-deoxyribonucleosides **3**, **15**, and **16** are fluorescent. The fluorescence of analogue **15** (N^2) at pH 7.0 is enhanced by a factor of 10 when the pH of the solution is raised to 11.0. Also, the weak fluorescence of compounds **3** and **16** at neutral pH is increased by a factor of 5–10 at pH 11.0 (see the *Fig.*). According to this, the significant fluorescence at pH 11.0 must be due to the anionic species, which was also supported by p*K* values. The

Table 2. *^{13}C-NMR Chemical Shifts of Benzotriazole Nucleosides*[a])[b])

	C(3a)	C(4)	C(5)	C(6)	C(7)	C(7a)	
4	145.5	119.2	124.3	127.6	111.1	132.4	
5	143.6	118.2	127.0	127.0	118.2	143.6	
25a	145.5	119.2	124.3	127.5	111.0	132.6	
27	144.8	118.3	127.0	127.5	112.9	143.6	
22	145.3	119.5	124.7	128.2	110.8	132.8	
23	145.1	119.0	124.0	127.4	111.8	132.9	
6	145.5	119.3	124.5	127.9	111.2	132.4	
24	145.4	118.9	124.1	127.3	112.9	132.7	
25b	145.4	119.3	124.5	127.9	113.0	132.8	
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	MeO	C=O
4	86.2	[c])	70.6	88.1	61.8		
5	93.1	[c])	70.7	88.7	62.1		
25a	85.1	[c])	70.2	85.9	63.9	54.9 (5′)	
27	92.8	[c])	70.2	85.1	62.1	54.9 (5′)	
22	87.6	80.0	71.9	81.1	63.6	58.6 (2′)	165.1, 165.4
23	87.6	80.2	71.4	81.5	64.2	59.2 (2′)	165.1, 165.5
6	88.4	86.4	69.2	82.1	61.5	57.6 (2′)	
24	88.8	85.6	69.9	81.7	61.1	58.6 (2′)	
25b	87.6	85.2	69.8	82.6	63.4	58.0 (2′), 55.0 (5′)	

[a]) Measured in (D_6) DMSO. [b]) Systematic numbering. [c]) Superimposed by DMSO.

pK values of protonation and deprotonation were determined UV-spectrophotometrically in 0.1M sodium phosphate buffer (pH 7.0). Only one pK_a was found for **3** (7.9) and **15** (8.5). Comparison of the pK_a value of **3** with that of its counterparts **1** (8.9) and **2** (9.9) establishes that the extra N-atom (N(2)) in the 1,2,3-triazolo[4,5-*d*]pyrimidine nucleosides increases their acidity. This indicates that the enhancement of fluorescence at pH 11.0 is induced by the formation of anionic species. The 1*H*-1,2,3-benzotriazole 2-(2′-deoxy-D-ribofuranoside) (**5**) exhibits a significant fluorescence (H_2O, 10^{-5} M) with an emission maximum at 368 nm (excitation at 281 nm). A quantum yield (Φ_f) of 0.1 was obtained for nucleoside **5**, which is remarkably high compared to canonical nucleosides. As expected, the fluorescence of **5** is pH-independent in the range of 7.0 to 11.0, so it is the neutral species which emits the light. The nucleosides **4** and **6** are nonfluorescent.

From the above observations, it can be noted that the N^2-glycosylated triazole nucleosides are always fluorescent while the regioisomeric glycosylation products show almost no fluorescence. In the case of the 3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one analogues, a large *Stokes* shift of *ca.* 80 nm is observed at pH 7.0 which even increases at pH 11.0. In particular, compound **15** shows a maximal shift of 121 nm at pH 11.0. A significant *Stokes* shift is also observed in case of the benzotriazole 2-(2′-deoxy-D-ribofuranoside) **5** (94 nm) which is pH-independent. Thus, nucleosides with N(2) as glycosylation site exhibit the strongest fluorescence which becomes even stronger in alkaline medium.

3. *Synthesis and Base-Pairing Properties of Oligonucleotides.* The oligonucleotide synthesis was performed on the solid phase employing the phosphoramidites **19**, **26a**, **26b**, and **28** as well as the phosphonate **18** (*Tables 4* and *5*). The coupling yield was

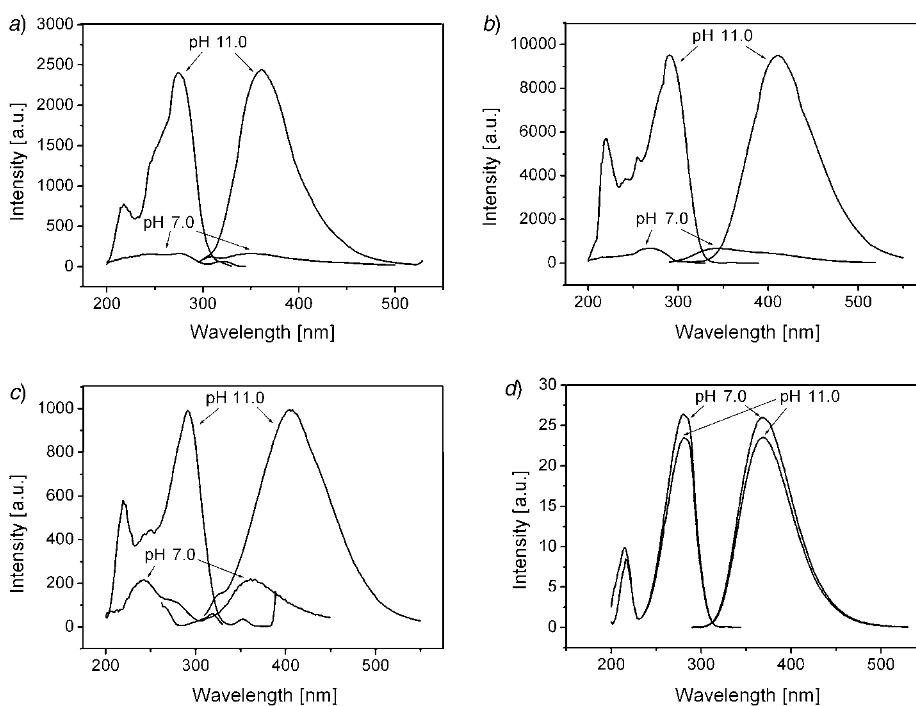


Figure. *Fluorescence spectra:* a) **3** *at pH 7.0 and 11.0* (excitation 274 nm and emission 362 nm), b) **15** *at pH 7.0* (excitation 272 nm and emission 340 nm) *and pH 11.0* (excitation 290 nm and emission 411 nm), c) **16** *at pH 7.0* (excitation 278 nm and emission 360 nm) *and pH 11.0* (excitation 291 nm and emission 404 nm), *and* d) **5** *at pH 7.0 and 11.0* (excitation 281 nm and emission 368 nm).

Table 3. *Spectral Properties of 1,2,3-Triazolo[4,5-*d*]pyrimidin-7-one Derivatives* **3**, **15**, *and* **16** *and of Benzotriazole Nucleoside* **5**[a])

	pH	UV absorption λ_{max} [nm]	Fluorescence λ_{max} [nm]	Quantum yield Φ_f
3	7.0	258	360	< 0.001
	11.0	270	404	0.006
15	7.0	271	340	0.003
	11.0	291	411	0.04
16	7.0	257	362	< 0.001
	11.0	274	362	0.004
5	7.0	274	368	0.1
	11.0	274	368	0.1

[a]) UV Absorption and fluorescence spectra were measured in aqueous medium. The fluorescence quantum yield (Φ_f) was determined by using quinine sulfate dihydrate in 0.1M $HClO_4$ as standard ($\Phi_f = 0.59$).

always higher than 95%. Deprotection was performed with 25% aqueous NH_3 solution (60°, 16 h), and the oligonucleotides were purified by reversed-phase HPLC. The oligonucleotides **29**–**46** were characterized by MALDI-TOF-MS, and the detected masses were in good agreement with the calculated values (*Table 6*). The duplexes 5′-

Table 4. *Comparisons of* T_m *Values and Thermodynamic Data of Oligonucleotides Containing Compounds* **1**, **2**, *or* **3** *Opposite to the Canonical Nucleosides*[a])

Duplex	T_m [°]	$\Delta G°_{310}$ [kcal/mol]	Duplex	T_m [°]	$\Delta G°_{310}$ [kcal/mol]
5′-d(TA**CC**TCAATACT)-3′ (**29**) 3′-d(AT**GG**AGTTATGA)-5′ (**30**)	46	−10.4			
5′-d(TA**CC**TCAATACT)-3′ (**29**) 3′-d(AT**11**AGTTATGA)-5′ (**31**)	36	−7.6	5′-d(TA**GG**TCAATACT)-3′ (**35**) 3′-d(AT**11**AGTTATGA)-5′ (**31**)	22	−4.9
5′-d(TA**CC**TCAATACT)-3′ (**29**) 3′-d(AT**22**AGTTATGA)-5′ (**32**)	35	−7.3	5′-d(TA**GG**TCAATACT)-3′ (**35**) 3′-d(AT**22**AGTTATGA)-5′ (**32**)	20	−4.1
5′-d(TA**CC**TCAATACT)-3′ (**29**) 3′-d(AT**33**AGTTATGA)-5′ (**33**)	36	−7.8	5′-d(TA**GG**TCAATACT)-3′ (**35**) 3′-d(AT**22**AGTTATGA)-5′ (**33**)	24	−5.8
5′-d(TA**AA**TCAATACT)-3′ (**34**) 3′-d(AT**11**AGTTATGA)-5′ (**31**)	31	−6.1	5′-d(TA**TT**TCAATACT)-3′ (**36**) 3′-d(AT**11**AGTTATGA)-5′	21	−5.2
5′-d(TA**AA**TCAATACT)-3′ (**34**) 3′-d(AT**22**AGTTATGA)-5′ (**32**)	33	−6.9	5′-d(TA**TT**TCAATACT)-3′ (**36**) 3′-d(AT**22**AGTTATGA)-5′ (**32**)	24	−5.3
5′-d(TA**AA**TCAATACT)-3′ (**34**) 3′-d(AT**33**AGTTATGA)-5′ (**33**)	25	−5.6	5′-d(TA**TT**TCAATACT)-3′ (**36**) 3′-d(AT**33**AGTTATGA)-5′ (**33**)	21	−5.7

[a]) Measured at 260 nm in 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with 5 µM single-strand concentration.

Table 5. T_m *Values and Thermodynamic Data of Oligonucleotides Containing* **4**, **5** *or* **6** *Opposite to Regular Nucleosides*[a])

Duplex	T_m [°]	$\Delta G°_{310}$ [kcal/mol]	Duplex	T_m [°]	$\Delta G°_{310}$ [kcal/mol]
5′-d(TAGG**T**CAATACT)3′ (**35**) 3′-d(ATCC**A**GTTATGA)-5′ (**37**)	50	−12.0			
5′-d(TAGG**T**CAATACT)-3′ (**35**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	36	−7.4	5′-d(TAGG**A**CAATACT)-3′ (**40**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	37	−7.4
5′-d(TAGG**C**CAATACT)-3′ (**39**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	35	−7.2	5′-d(TAGG**G**CAATACT)-3′ (**41**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	38	−7.6
5′-d(TAGG**A**CAATACT)-3′ (**40**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	37	−7.7	5′-d(TAGG**T**CAATACT)-3′ (**35**) 3′-d(ATCC**6**GTTATGA)-5′ (**43**)	36	−7.3
5′-d(TAGG**G**CAATACT)-3′ (**41**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	41	−8.4	5′-d(TAGG**C**CAATACT)-3′ (**39**) 3′-d(ATCC**6**GTTATGA)-5′ (**43**)	35	−7.0
5′-d(TAGG**T**CAATACT)-3′ (**35**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	35	−7.0	5′-d(TAGG**A**CAATACT)-3′ (**40**) 3′-d(ATCC**6**GTTATGA)-5′ (**43**)	36	−7.3
5′-d(TAGG**C**CAATACT)-3′ (**39**) 3′-d(ATCC**5**GTTATGA)5-′ (**42**)	33	−6.8	5′-d(TAGG**G**CAATACT)-3′ (**41**) 3′-d(ATCC**6**GTTATGA)-5′ (**43**)	39	−8.0
5′-d(TAGG**4**CAATACT)-3′ (**44**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	40	−8.4	5′-d(TAGG**5**CAATACT)-3′ (**45**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	40	−8.4
5′-d(TAGG**5**CAATACT)-3′ (**45**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	39	−8.0	5′-d(TAGG**6**CAATACT)-3′ (**46**) 3′-d(ATCC**4**GTTATGA)-5′ (**38**)	39	−8.1
5′-d(TAGG**6**CAATACT)-3′ (**46**) 3′-d(ATCC**6**GTTATGA)-5′ (**43**)	39	−7.8	5′-d(TAGG**6**CAATACT)-3′ (**46**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	39	−8.0
5′-d(TAGG**4**CAATACT)-3′ (**44**) 3′-d(ATCC**5**GTTATGA)-5′ (**42**)	39	−8.2			

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

Table 6. *Molecular Masses* ($[M+H]^+$) *of Oligonucleotides Measured by MALDI-TOF-MS*

Oligonucleotide	$[M+H]^+$ (calc.)	$[M+H]^+$ (found)
5′-d(A-G-T-A-T-T-G-A-**3**-**3**-T-A)-3′ (**33**)	3694.50	3693.10
5′-d(A-G-T-A-T-T-G-**4**-C-C-T-A)-3′ (**38**)	3628.41	3628.46
5′-d(A-G-T-A-T-T-G-**5**-C-C-T-A)-3′ (**42**)	3628.41	3629.58
5′-d(A-G-T-A-T-T-G-**6**-C-C-T-A)-3′ (**43**)	3658.41	3659.60
5′-d(T-A-G-G-**4**-C-A-A-T-A-C-T)-3′ (**44**)	3638.41	3639.52
5′-d(T-A-G-G-**5**-C-A-A-T-A-C-T)-3′ (**45**)	3638.41	3638.59
5′-d(T-A-G-G-**6**-C-A-A-T-A-C-T)-3′ (**46**)	3668.41	3667.85

d(TACCTCAATACT)-3′ · 3′-d(ATGGAGTTATGA)-5′ (**29** · **30**) and 5′-d(TAGGTCAATACT)-3′ · 3′-d(ATCCAGTTATGA)-5′ (**35** · **37**) were used as a standard for the evaluation of the strength of the base pairing (*Tables 4* and *5*).

The hybridization properties of oligonucleotides containing 7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2’-deoxyribonucleoside (2′-deoxy-8-azainosine) **3** were investigated first and were compared to the corresponding oligomers containing either 2′-deoxyinosine (**1**) and its 7-deazapurine analogue **2**. For that, two consecutive incorporations of **3** were done replacing the dG residue of the sequence 5′-d(AGTATTGA**GG**TA) (**30**). The resulting oligomer **33** was hybridized with the complementary strands containing four canonical bases opposite to **3** (*Table 4*). From *Table 4*, it is apparent that the 3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 2′-deoxyribonucleoside **3** located opposite to dC, dG, dA, and dT behaved similar to that of 2′-deoxyinosine (**1**) and 2′-deoxy-7-deazainosine (**2**). Surprisingly, we observed a rather low stability of duplexes when compound **3** was located opposite to dA (**34** · **33**). The above observations indicate that the base-pairing modes for compound **3** against dC, dG, dA, and dT were almost the same as those of compounds **1** and **2**, and support the various base-pair motifs suggested earlier [11]. Although compound **3** did not increase duplex stability as compared to compounds **1** and **2**, its fluorescence properties, in particular the strong change going from the neutral to the alkaline medium, is of interest.

Next, the hybridization properties of the benzotriazole nucleosides **4**–**6** were investigated. For that, they were incorporated in oligonucleotides replacing the dA residue of 5′-d(AGTATTG**A**CCTA)-3′ (**37**), and for the self-pairing, the dT moiety of 5′-d(TAGG**T**CAATACT)-3′ (**35**). The pairing properties of **4**–**6** opposite the canonical nucleosides and opposite of each other were investigated (*Table 5*). For duplexes containing **4**, **5**, or **6** opposite to canonical nucleosides, a maximum stabilization of 2–5° was observed opposite to dA or dG as compared to that opposite to dC or dT. The present results indicate the preference of hydrophobic bases for the purines over the pyrimidine bases at stacking, which were also observed for other compounds by *Leonard et al.* [24]. The duplexes containing the nucleosides **4**, **5**, or **6** preferred to pair with themselves than with the natural nucleosides.

Despite of the different glycosylation sites, the nucleosides **4** and **5** paired among each other and as well as with **6**, without any discrimination. Even though, the presence of a 2′-*O*-methyl group in **6** did not destabilize the duplexes. As there is no possibility of H-bonding, the interstrand interaction in which the benzotriazole rings stack upon each other is the main driving force for the observed stability [25][26]. Interstrand stacking

can be controlled by the surface area of the nucleobases, the hydrophobicity of the residues, and their molecular polarizability. Thus, the higher molecular polarizability of guanine and adenine (14.06 and $13.72 \pm 0.5\ 10^{-24}$, resp.) over thymine and cytosine (11.77 and $10.82 \pm 0.5\ 10^{-24}$, resp.) contributes favorably to their enhanced interactions [27][28]. Likewise, an increased hydrophobicity and surface area of self-paired benzotriazole nucleobase (molecular polarizability, $13.76 \pm 0.5\ 10^{-24}$) give a *ca.* 2° stabilization over dG · dA. This implies that the natural DNA bases are not particularly effective for an interstrand stacking. Nevertheless, it is surprising that the regioisomeric benzotriazole nucleosides **4** and **5**, when paired with each other, resulted in the same degree of duplex stabilization. It seems that the DNA backbone is flexible enough to accommodate these changes.

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Experimental Part

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 $60F_{254}$, 0.2-mm layer (*VWR*, Germany). Column flash chromatography (FC): silica gel *60* (*Merck*, Germany) at 0.4 bar; sample collection with an *UltroRac-II* fraction collector (*LKB Instruments*, Sweden). Solvent systems for TLC and FC: CH_2Cl_2/MeOH 98:2 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 9:1 (*C*), CH_2Cl_2/MeOH 8:2 (*D*), petroleum ether/acetone 8:2 (*E*), petroleum ether/AcOEt 7:3 (*F*), petroleum ether/AcOEt 8:2 (*G*), petroleum ether/AcOEt 5:1 (*H*), CH_2Cl_2/acetone 98:2 (*I*), CH_2Cl_2/acetone 95:5 (*J*). 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 ^1H and ^{13}C; δ in ppm rel. to Me_4Si as internal standard, *J* values in Hz. Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller* (Göttingen, Germany).

Synthesis, Purification, and Characterization of the Oligonucleotides **29**–**46**. The oligonucleotide synthesis was performed on a DNA synthesizer, model *392* (*Applied Biosystems*, Weiterstadt, Germany). Melting curves were measured with a *Cary 1/3* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a *Cary* thermoelectric controller. The temp. was measured continuously in the reference cell with a *Pt-100* resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program. Oligonucleotide analysis was carried out by reversed-phase HPLC (*Merck-Hitachi* HPLC; 250 × 4 mm *RP-18* column; gradients of 0.1M (Et_3NH)OAc (pH 7.0)/MeCN 95:5 (*K*) and MeCN (*L*); gradient *a:* 15 min 0–50% *L* in *K*, flow rate 1 ml/min; gradient *b:* 25 min 0–20% *L* in *K*, 25–35 min 20–20% *L* in *K*, flow rate 1 ml/min. MALDI-TOF-MS: *Biflex-III* spectrometer (*Bruker Saxonia*, Leipzig, Germany).

*7-Isopropoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**7b**). A suspension of 6-chloropyrimidine-4,5-diamine (1.45 g, 10 mmol) in 1,4-dioxane (75 ml) was treated with pentyl nitrite (2.1 ml, 16 mmol) at 80–90° for 30 min. The mixture was cooled in an ice bath and concentrated to 1/3 of its volume. Then 1M Na in ^{i}PrOH (10 ml) was added, and stirring was continued at 80° for 30 min. The mixture was evaporated, the residue dissolved in H_2O (20 ml), and the soln. treated with charcoal. After filtration, the aq. soln. was acidified to pH 5 with AcOH, and the precipitating crude product was removed by filtration. The solid was then dissolved in MeOH, adsorbed on silica gel, and subjected to FC (column 20 × 5 cm, *C*): **7b** (985 mg, 55%). Colorless solid. TLC (*C*): R_f 0.33. UV (MeOH): 258 (8712). ^1H-NMR ((D_6)DMSO): 1.45 (*d*, *J* = 6.1, *Me*$_2$CHO); 5.66 (*q*, *J* = 6.1, Me$_2$*CH*O); 8.68 (*s*, H–C(5)). Anal. calc. for $C_7H_9N_5O$ (179.18): C 46.92, H 5.06, N 39.09; found: C 47.14, H 5.11, N 38.83.

Glycosylation of **7b** *with 2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-α-D-*erythro*-pentofuranosyl Chloride* (**8**). The suspension of **7b** (1.98 g, 11 mmol) in MeCN (70 ml) was treated with NaH (97%; 280 mg, 11.3 mmol), and the mixture was stirred for 15 min at r.t. Then **8** (4.27 g, 10.98 mmol) was added portionwise within 30 min, and stirring was continued for 30 min. The mixture was filtered through *Celite* and the solvent evaporated. The

resulting oil was subjected to FC (column 20 × 5 cm, *D*) and separated in three main zones. The faster migrating zone was always the N^3 isomer, zone II contained the N^2 isomer, and the slower migrating zone was the N^1 isomer.

*3-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-*D-erythro*-pentofuranosyl]-7-isopropoxy-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**9b**): Colorless foam (1.69 g, 29%). TLC (*F*): R_f 0.25. UV (MeOH): 242 (31869). ¹H-NMR ((D$_6$)DMSO): 1.45, 1.50 (2*d*, *J* = 6.0, *Me*$_2$CHO); 2.44, 2.39 (2*s*, 2 Me); 3.03 (*m*, H$_β$–C(2')); 3.57 (*m*, H$_α$–C(2')); 4.38, 4.52 (2*m*, 2 H–C(5')); 4.75 (*m*, H–C(4')); 5.64 (*m*, Me$_2$C*H*O); 5.95 (*m*, H–C(3')); 6.99 (*t*, *J* = 5.0, H–C(1')); 7.34, 7.86 (2*m*, arom. H); 8.77 (*s*, H–C(5)). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.56): C 63.27, H 5.50; N 13.18; found: C 63.49, H 5.51, N 13.08.

*2-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-*D-erythro*-pentofuranosyl]-7-isopropoxy-2*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**10b**): Colorless foam (1.58 g, 27%). TLC (*F*): R_f 0.18. UV (MeOH): 241 (34878), 270 (11958). ¹H-NMR ((D$_6$)DMSO): 1.45, 1.46 (2*d*, *J* = 6.0, *Me*$_2$CHO); 2.43, 2.37 (2*s*, 2 Me); 3.03 (*m*, H$_β$–C(2')); 3.47 (*m*, H$_α$–C(2')); 4.49, 4.61 (2*m*, 2 H–C(5')); 4.77 (*m*, H–C(4')); 5.65 (*m*, Me$_2$C*H*O); 5.95 (*m*, H–C(3')); 6.90 (*dd*, *J* = 3.8, 6.8, H–C(1')); 7.34, 7.86 (2*m*, arom. H); 8.77 (*s*, H–C(5)). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.56): C 63.27, H 5.50; N 13.18; found: C 63.20, H 5.70, N 13.05.

*1-[2-Deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-*D-erythro*-pentofuranosyl]-7-isopropoxy-1*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine* (**11b**): Colorless foam (1.81 g, 31%). TLC (*F*): R_f 0.09, UV (MeOH): 240 (34383), 271 (8383). ¹H-NMR ((D$_6$)DMSO): 1.45, 1.50 (2*d*, *J* = 6.0, *Me*$_2$CHO); 2.44, 2.39 (2*s*, 2 Me); 3.03 (*m*, H$_β$–C(2')); 3.57 (*m*, H$_α$–C(2')); 4.38, 4.52 (2*m*, 2 H–C(5')); 4.76 (*m*, H–C(4')); 5.64 (*m*, Me$_2$C*H*O); 5.95 (*m*, H–C(3')); 7.00 (*t*, *J* = 5.0, H–C(1')); 7.36, 7.86 (2*m*, arom. H); 8.77 (*s*, H–C(5)). Anal. calc. for $C_{28}H_{29}N_5O_6$ (531.56): C 63.27, H 5.50; N 13.18; found: C 63.35, H 5.61, N 13.15.

Deprotection of Compounds **9a**–**11a**. A soln. of **9a**, **10a**, or **11a** (1.26 g, 2.5 mmol) in 1M NaOMe/MeOH (30 ml) was stirred at r.t. for 30 min. The mixture was adsorbed on silica gel and subjected to FC (column 35 × 5 cm, *B*).

*3-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-7-methoxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**12**): Colorless solid (494 mg, 74%). TLC (*B*): R_f 0.10. ¹H-NMR ((D$_6$)DMSO): 2.51 (*m*, H$_β$–C(2')); 3.09 (*m*, H$_α$–C(2')); 3.41, 3.57 (2*m*, 2 H–C(5')); 3.92 (*m*, H–C(4')); 4.23 (*s*, MeO); 4.59 (*m*, H–C(3')); 4.72 (*t*, *J* = 5.7, OH–C(5')); 5.41 (*d*, *J* = 4.6, OH–C(3')); 6.73 (*t*, *J* = 6.2, H–C(1')); 8.80 (*s*, H–C(5)). Anal. calc. for $C_{10}H_{13}N_5O_4$ (267.24): C 44.94, H 4.90, N 26.21; found: C 44.82, H 4.92, N 26.04.

*2-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-7-methoxy-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**13**): Colorless solid (450 mg, 67%). TLC (*B*): R_f 0.05. ¹H-NMR ((D$_6$)DMSO): 2.55 (*m*, H$_β$–C(2')); 2.91 (*m*, H$_α$–C(2')); 3.45, 3.58 (2*m*, 2 H–C(5')); 3.97 (*m*, H–C(4')); 4.20 (*s*, MeO); 4.56 (*m*, H–C(3')); 4.71 (*t*, *J* = 5.4, OH–C(5')); 5.41 (*d*, *J* = 4.8, OH–C(3')); 6.64 (*dd*, *J* = 4.0, 6.8, H–C(1')); 8.77 (*s*, H–C(5)). Anal. calc. for $C_{10}H_{13}N_5O_4$ (267.24): C 44.94, H 4.90, N 26.21; found: C 45.05, H 4.82, N 26.09.

*1-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-7-methoxy-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidine* (**14**): Colorless solid (449 mg, 67%). TLC (*C*): R_f 0.22. ¹H-NMR ((D$_6$)DMSO): 2.49 (*m*, H$_β$–C(2')); 3.11 (*m*, H$_α$–C(2')); 3.33, 3.49 (2*m*, 2 H–C(5')); 3.92 (*m*, H–C(4')); 4.22 (*s*, MeO); 4.56 (*m*, H–C(3')); 4.65 (*t*, *J* = 5.6, OH–C(5')); 5.42 (*d*, *J* = 4.4, OH–C(3')); 6.64 (*t*, *J* = 5.8, H–C(1')); 8.76 (*s*, H–C(5)). Anal. calc. for $C_{10}H_{13}N_5O_4$ (267.24): C 44.94, H 4.90, N 26.21; found: C 45.07, H 4.98, N 26.10.

8-Aza-2'-deoxyinosines **3**, **15**, *and* **16**. A soln. of compound **12**, **13**, or **14** (668 mg, 2.5 mmol) in 1N NaOH (30 ml) was stirred for 3 h at 50°. The mixture was cooled to r.t. and neutralized with AcOH. The soln. was diluted with H_2O (250 ml) and subjected to *Amberlit XAD-2* (20–50 mesh) (column 5 × 35 cm). The column was washed with H_2O (300 ml) and eluted with H_2O/^{i}PrOH 9 : 1. Evaporation and repetitive co-evaporation with acetone afforded the isomeric 8-aza-2'-deoxyinosines **3**, **15**, and **16**.

*3-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**3**): Colorless solid (443 mg, 70%). TLC (D): R_f 0.33. UV (MeOH): 256 (8200). ¹H-NMR ((D$_6$)DMSO): 2.44 (*m*, H$_β$–C(2')); 2.98 (*m*, H$_α$–C(2')); 3.38, 3.55 (2*m*, 2 H–C(5')); 3.90 (*m*, H–C(4')); 4.54 (*m*, H–C(3')); 4.78 (br. *s*, OH–C(5')); 5.43 (br. *s*, OH–C(3')); 6.56 (*dd*, *J* = 6.1, 6.0, H–C(1')); 8.30 (*s*, H–C(5)); 12.80 (br. *s*, NH). Anal. calc. for $C_9H_{11}N_5O_4$ (253.21): C 42.69, H 4.38, N 27.66; found: C 42.82, H 4.56, N 27.39.

*2-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-2,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**15**): Colorless solid (405 mg, 64%). TLC (*D*): R_f 0.33. UV (MeOH): 271 (9400). ¹H-NMR ((D$_6$)DMSO): 2.46 (*m*, H$_β$–C(2')); 2.83 (*m*, H$_α$–C(2')); 3.39, 3.55 (2*m*, 2 H–C(5')); 3.91 (*m*, H–C(4')); 4.52 (*m*, H–C(3')); 4.80 (br. *s*, OH–C(5')); 5.45 (br. *s*, OH–C(3')); 6.49 (*dd*, *J* = 4.2, 6.7, H–C(1')); 8.16 (*s*, H–C(5)); 12.40 (br. *s*, NH). Anal. calc. for $C_9H_{11}N_5O_4$ (253.21): C 42.69, H 4.38, N 27.66; found: C 42.82, H 4.56, N 27.39.

*1-(2-Deoxy-β-*D-erythro*-pentofuranosyl)-1,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**16**): Colorless solid (267 mg, 42%). TLC (*D*): R_f 0.33. UV (MeOH): 255 (7000). ¹H-NMR ((D$_6$)DMSO): 2.45

(*m*, $H_β$–C(2')); 2.99 (*m*, $H_α$–C(2')); 3.38, 3.52 (2*m*, 2 H–C(5')); 3.90 (*m*, H–C(4')); 4.51 (*m*, H–C(3')); 4.75 (*s*, OH–C(5')); 5.43 (*s*, OH–C(3')); 6.46 (*dd*, *J* = 5.8, 6.0, H–C(1')); 8.13 (*s*, H–C(5)). Anal. calc. for $C_9H_{11}N_5O_4$ (253.21): C 42.69, H 4.38, N 27.66; found: C 42.82, H 4.56, N 27.39.

Enzymatic Synthesis of **3** from **9a**. The 7-methoxy compound **9a** (1.34 g, 2.66 mmol) was disolved in H_2O (500 ml) and treated with adenosine-deaminase ADA (from calf intestine; soln. in glycerol, 50 μl). After stirring for 16 h at 30°, the soln. was evaporated followed by co-evaporation with acetone. The crude product was purified by FC (silica gel, column 3 × 10 cm, *C*): **3** (529 mg, 79%). Colorless solid. Data: identical with that described above.

*3-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one* (**17**). A suspension of **3** (771 mg, 3.05 mmol) in anh. pyridine (3 ml) was stirred with $(MeO)_2TrCl$ (1.23 g, 3.63 mmol) for 1 h. The reaction was quenched by the addition of AcOEt (20 ml), followed by brine (10 ml). The mixture was extracted twice with AcOEt (20 ml, each), the combined org. layer washed with H_2O (10 ml), dried ($MgSO_4$), and evaporated and the residue purified by FC (column 6 × 20 cm, gradient *A* → *B*): **17** (901 mg, 53%). Colorless foam. TLC (*E*): R_f 0.17. ^{1}H-NMR ((D_6)DMSO): 2.50 (*m*, $H_β$–C(2')); 2.98–3.32 (*m*, $H_α$–C(2'), 2 H–C(5')); 3.72, 3.73 (2*s*, 2 MeO); 4.04 (*m*, H–C(4')); 4.62 (*m*, H–C(3')); 5.44 (*s*, OH–C(3')); 6.63 (*dd*, *J* = 3.6, 6.9, H–C(1')); 8.28 (*s*, H–C(5)); 6.73–7.28 (*m*, 13 arom. H); 12.79 (br. *s*, NH). Anal. calc. for $C_{30}H_{29}N_5O_6$ (555.58): C 64.85, H 5.26, N 12.61; found: C 64.76, H 5.25, N 12.51.

*3-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 3'-(2-Cyanoethyl diisopropylphosphoramidite)* (**19**). To a soln. of **17** (580 mg, 1.04 mmol) and $^iPr_2EtN$ (0.27 ml, 1.57 mmol) in anh. THF (15 ml), 2-cyanoethyl diisopropylphosphoramidochloridite (0.35 ml, 1.55 mmol) was added dropwise. After stirring at r.t. for 70 min, the reaction was quenched by the addition of a 5% aq. $NaHCO_3$ soln. (5 ml). The aq. layer was extracted with CH_2Cl_2 (2 × 10 ml), the combined org. layer washed with brine (10 ml), dried ($MgSO_4$), and evaporated, and the resulting oil subjected to FC (column 9 × 3 cm, *C*): **19** (224 mg, 28%). Colorless foam. TLC (*E*): R_f 0.50. ^{31}P-NMR ($CDCl_3$): 150.1, 150.2.

*3-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one 3'-(Triethylammonium Phosphonate)* (**18**). To a soln. of PCl_3 (0.22 ml, 2.52 mmol) and 4-methylmorpholine (2.75 ml, 25.0 mmol) in CH_2Cl_2 (10 ml), 1*H*-1,2,4-triazole (553 mg, 8.0 mmol) was added. The soln. was cooled to 0°, then **17** (278 mg, 0.50 mmol) in CH_2Cl_2 (15 ml) was added dropwise at r.t., and stirring was continued for 30 min. The mixture was poured into 1M $(Et_3NH)HCO_3$ (= TBK; pH 8.0, 25 ml), the aq. layer extracted with CH_2Cl_2 (3 × 20 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue subjected to FC (column 15 × 3 cm, *D*). Evaporation of the main zone afforded a colorless oil, which was dissolved in CH_2Cl_2. The soln. was extracted with aq. 0.1M TBK (10 × 20 ml), dried, and evaporated: **18** (250 mg, 70%). Colorless foam. TLC (*D*): R_f 0.15. ^{31}P-NMR ((D_6)DMSO): 1.12 (*dd*, *J* = 5.87, 9.1).

*Glycosylation of 1*H*-1,2,3-Benzotriazole* (**20**) *with 1,3,5-tri-*O*-Benzoyl-2-*O*-methyl-α-*D*-ribofuranose* (**21**). A soln. of **21** (2.74 g, 5.75 mmol) and 1*H*-1,2,3-benzotriazole (**20**; 850 mg, 7.14 mmol) in dry nitromethane (160 ml) was brought to reflux for 15 min. Then $BF_3 \cdot Et_2O$ (1.32 ml, 10.7 mmol) was added, and the mixture was refluxed for further 15 min, cooled, and evaporated. The resulting oil was dissolved in CH_2Cl_2 (50 ml), the soln. washed with 5% $NaHCO_3$ soln. (20 ml), dried (Na_2SO_4), and evaporated, and the residue purified by FC (column 20 × 3 cm, *I*): **22** (1.1 g, 40%; less polar) and **23** (500 mg, 18%; more polar).

*1-(3,5-Di-*O*-benzoyl-2-*O*-methyl-β-*D*-ribofuranosyl)-1*H*-1,2,3-benzotriazole* (**22**): Colorless foam. TLC (*I*): R_f 0.5. UV (MeOH): 231 (25500), 275 (5200), 282 (5100). ^{1}H-NMR ((D_6)DMSO): 3.39 (*s*, MeO–C(2')); 4.51 (*m*, 2 H–C(5')); 4.82 (*m*, H–C(4')); 5.17 (*m*, H–C(2')); 6.0 (*m*, H–C(3')); 6.82 (*d*, *J* = 4.11, H–C(1')); 7.43–8.13 (*m*, 14 arom. H). Anal. calc. for $C_{26}H_{23}N_3O_6$ (473.48): C 65.95, H 4.90, N 8.87; found: C 65.99, H 4.96, N 8.78.

*1-(3,5-Di-*O*-benzoyl-2-*O*-methyl-α-*D*-ribofuranosyl)-1*H*-1,2,3-benzotriazole* (**23**): Colorless foam. TLC (*I*): R_f 0.42. UV (MeOH): 232 (26300), 275 (5500), 282 (5400). ^{1}H-NMR ((D_6)DMSO): 3.07 (*s*, MeO–C(2')); 4.67–4.75 (*m*, H–C(4'), 2 H–C(5')); 5.25 (*m*, H–C(2')); 5.84 (*m*, H–C(3')); 7.19 (*d*, *J* = 5.41, H–C(1')); 7.46–8.10 (*m*, 14 arom. H). Anal. calc. for $C_{26}H_{23}N_3O_6$ (473.48): C 65.95, H 4.90, N 8.87; found: C 66.05, H 5.01, N 8.82.

*1-(2-*O*-Methyl-β-*D*-ribofuranosyl)-1*H*-1,2,3-benzotriazole* (**6**). Compound **22** (600 mg, 1.27 mmol) in methanolic ammonia (60 ml) was stirred overnight in a sealed bottle. The mixture was evaporated and the residue subjected to FC (column 12 × 3 cm, *C*): **6** (310 mg, 92%). Colorless oil. TLC (*C*): R_f 0.5. UV (MeOH): 255 (6200), 282 (3500). ^{1}H-NMR ((D_6)DMSO): 3.33 (*s*, MeO–C(2')); 3.59 (*m*, 2 H–C(5')); 4.05 (*m*, H–C(4')); 4.46 (*m*, H–C(2')); 4.56 (*m*, H–C(3')); 4.97 (*t*, *J* = 4.8, OH–C(5')); 5.36 (*d*, *J* = 4.4, OH–C(3')); 6.46 (*d*, *J* =

5.1, H−C(1′)); 7.42–7.56 (2′*t*', H−C(5), H−C(6)); 8.07, 8.10 (*d*, H−C(4), H−C(7)). Anal. calc. for $C_{12}H_{15}N_3O_4$ (265.27): C 54.33, H 5.70, N 15.84; found: C 54.38, H 5.75, N 15.73.

*1-(2-*O*-Methyl-α-*D*-ribofuranosyl)-1*H*-1,2,3-benzotriazole* (**24**). As described for **6**, **23** (300 mg, 0.63 mmol) in methanolic ammonia (30 ml) gave **24** (139 mg, 83%). Colorless oil. TLC (*C*): R_f 0.5. UV (MeOH): 256 (6200), 282 (3800). ^{1}H-NMR ((D$_6$)DMSO): 2.99 (*s*, MeO−C(2′)); 3.50, 3.65 (2*m*, 2 H−C(5′)); 4.22 (*m*, H−C(4′)); 4.31–4.37 (*m*, H−C(2′), H−C(3′)); 4.97 (*t*, *J* = 5.6, OH−C(5′)); 5.36 (*d*, *J* = 6.6, OH−C(3′)); 6.87 (*d*, *J* = 4.7, H−C(1′)); 7.37–7.56 (2′*t*', H−C(5), H−C(6)); 7.94, 8.8.06 (2*d*, H−C(4), H−C(7)). Anal. calc. for $C_{12}H_{15}N_3O_4$ (265.27): C 54.33, H 5.70, N 15.84; found: C 54.56, H 5.91, N 15.92.

*1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-*O*-methyl-β-*D*-ribofuranosyl}-1*H*-1,2,3-benzotriazole* (**25b**). Compound **6** (200 mg, 0.75 mmol) was dried by repeated co-evaporation with anh. pyridine and suspended in dry pyridine (3 ml), to which (MeO)$_2$TrCl (320 mg, 0.94 mmol) was added. The mixture was stirred for 24 h, quenched by the addition of 5% aq. $NaHCO_3$ soln. (10 ml), and extracted with CH_2Cl_2 (3 × 10 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue subjected to FC (column 12 × 3 cm, *J*): **25b** (300 mg, 70%). Colorless foam. TLC (*J*): R_f 0.45. UV (MeOH): 235 (23700), 276 (6600). ^{1}H-NMR ((D$_6$)DMSO): 2.94, 3.15 (2*m*, 2 H−C(5′)) 3.47 (*s*, MeO−(2′)); 4.16 (*m*, H−C(4′)); 4.62–4.66 (*m*, H−C(2′), H−C(3′)); 5.36 (*d*, *J* = 4.4, OH−C(3′)); 6.58 (*d*, H−C(1′)); 6.67–7.20 (*m*, 13 arom. H); 7.42, 7.55 (2′*t*′, H−C(5), H−C(6)); 8.00, 8.11 (*d*, H−C(4), H−C(7)). Anal. calc. for $C_{33}H_{33}N_3O_6$ (567.63): C 69.83, H 5.86, N 7.40; found: C 69.94, H 5.97, N 7.39.

*1-{5-O-[Bis(4-methoxyphenyl)phenylmethyl]-2-*O*-methyl-β-*D*-ribofuranosyl}-1*H*-1,2,3-benzotriazole 3′-(2-Cyanoethyl diisopropylphosphoramidite)* (**26b**). To a soln. of **25b** (100 mg, 0.18 mmol) and $^iPr_2EtN$ (60 µl, 0.34 mmol) in anh. CH_2Cl_2 (2 ml), 2-cyanoethyl diisopropylphosphoramidochloridite (65 µl, 0.29 mmol) was added at r.t. After stirring for 25 min, the mixture was diluted with CH_2Cl_2 (10 ml) and quenched by adding a 5% $NaHCO_3$ soln. (20 ml). Then, the aq. layer was extracted with CH_2Cl_2 (3 × 15 ml), the combined org. layer dried (Na_2SO_4) and evaporated, and the resulting oil subjected to FC (column 10 × 3 cm, *I*): **26b** (95 mg, 70%). Colorless foam. TLC (*I*): R_f 0.5. ^{31}P-NMR ($CDCl_3$): 151.8, 152.2.

*1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl)-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-1*H*-1,2,3-benzotriazole* (**25a**). Compound **4** (250 mg, 1.06 mmol) was dried by repeated co-evaporation with anh. pyridine and suspended in dry pyridine (2 ml). The soln. was stirred in the presence of *N*,*N*-dimethylpyridin-4-amine (8 mg, 0.07 mmol) and (MeO)$_2$TrCl (398 mg, 1.18 mmol) for 5 h [13]. The mixture was diluted with 5% aq. $NaHCO_3$ soln. (10 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue subjected to FC (column 12 × 3 cm, *B*). Colorless foam (400 mg, 70%). TLC (*B*): R_f 0.44. UV (MeOH): 236 (23300), 275 (6900). ^{1}H-NMR ((D$_6$) DMSO): 2.49 (*m*, H_α−C(2′)); 3.05 (*m*, H_β−C(2′)); 3.02, 3.46 (*m*, 2 H−C(5′)); 3.67, 3.68 (2*s*, 2 MeO)); 4.05 (*m*, H−C(4′)); 4.62 (*m*, H−C(3′)); 5.49 (*d*, OH−C(3′)); 6.83 (*m*, H−C(1′)); 6.61–7.20 (*m*, arom. H); 7.48, 7.59 (2‘*t*’, H−C(5), H−C(6)); 8.01, 8.1 (2*d*, H−C(4), H−C(7)). Anal. calc. for $C_{32}H_{31}N_3O_5$ (537.61): C 71.49, H 5.81, N 7.82; found: C 71.42, H 5.80, N 7.84.

*2-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-2*H*-1,2,3-benzotriazole* (**27**). As described for **25a**, with **5** (250 mg, 1.06 mmol), *N*,*N*-dimethylpyridin-4-amine (8 mg, 0.07 mmol), (MeO)$_2$TrCl (398 mg, 1.18 mmol), and dry pyridine (2 ml) for 5 h: **27** (383 mg, 67%). Colorless foam. TLC (*B*): R_f 0.38. UV (MeOH): 234 (19600), 279 (12500). ^{1}H-NMR ((D$_6$)DMSO): 2.51 (*m*, H_α−C(2′)); 2.96 (*m*, H_β−C(2′)); 3.07, 3.46 (*m*, 2 H−C(5′)); 3.65, 3.68 (2*s*, 2 MeO); 4.06 (*m*, H−C(4′)); 4.70 (*m*, H−C(3′)); 5.48 (*d*, *J* = 5.1, OH−C(3′)); 6.75 (*m*, H−C(1′)); 6.66–7.27 (*m*, arom. H); 7.45, 7.47 (2′*t*’, H−C(5), H−C(6)); 7.91, 7.94 (2*d*, H−C(4), H−C(7)). Anal. calc. for $C_{32}H_{31}N_3O_5$ (537.61): C 71.49, H 5.81, N 7.82; found: C 71.58, H 5.75, N 7.84.

*1-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosy}-1*H*-1,2,3-benzotriazole 3′-(2-Cyanoethyl diisopropylphosphoramidite)* (**26a**). As described for **26b**, with **25a** (150 mg, 0.28 mmol), $^iPr_2EtN$ (92 µl, 0.53 mmol), 2-cyanoethyl diisopropylphosphoramidochloridite (92 µl, 0.41 mmol), and anh. CH_2Cl_2 (2 ml) at r.t. for 30 min: **26a** (180 mg, 87%). Colorless foam. TLC (*I*): R_f 0.41. ^{31}P-NMR ($CDCl_3$): 150.1, 150.4.

*2-{5-*O*-[Bis(4-methoxyphenyl)phenylmethyl]-2-deoxy-β-*D*-*erythro*-pentofuranosyl}-2*H*-1,2,3-benzotriazole 3′-(2-Cyanoethyl diisopropylphosphoramidite)* (**28**). As described for **26a**, with **27** (150 mg, 0.28 mmol), $^iPr_2EtN$ (92 µl, 0.53 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (92 µl, 0.41 mmol): **28** (170 mg, 83%). Colorless foam. TLC (*I*): R_f. 0.41. ^{31}P-NMR ($CDCl_3$): 150.0, 150.2.

REFERENCES

[1] J. D. Watson, F. H. C. Crick, *Nature (London)* **1953,** *171*, 737.
[2] K. J. Breslauer, R. Frank, H. Blöcker, L. A. Marky, *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3746.
[3] C. R. Cantor, P. R. Schimmel, in 'Biophysical Chemistry Part III: The Behaviour of Biological Macromolecules', Ed. W. H. Freeman, San Francisco, 1980, p. 1117.
[4] M.-H. Yu, J. S. Weissman, P. S. Kim, *J. Mol. Biol.* **1995**, *249*, 388.
[5] M. H. Werner, A. M. Gronenborn, G. M. Clore, *Science (Washington, D.C.)* **1996**, *271*, 778.
[6] B. A. Schweitzer, E. T. Kool, *J. Am. Chem. Soc.* **1995**, *117*, 1863; K. M. Guckian, B. A. Schweitzer, R. X.-F. Ren, C. J. Sheils, P. L. Paris, D. C. Tahmassebi, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 8182; E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem., Int. Ed.* **2000**,*39*, 990.
[7] D. Loakes, D. M. Brown, S. Linde, F. Hill, *Nucleic Acids Res.* **1995**, *23*, 2361; D. Loakes, D. M. Brown, *Nucleic Acids Res.* **1994**, *22*, 4039.
[8] J. C. Morales, E. T. Kool, *Nat. Struct. Biol.* **1998**, *5*, 950; S. Moran, R. X.-F. Ren, E. T. Kool, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10506.
[9] F. H. Martin, M. M. Castro, F. Aboul-ela, I. Tinoco Jr., *Nucleic Acids Res.* **1985**, *13*, 8927.
[10] Y. Kawase, S. Iwai, H. Inoue, K. Miura, E. Ohtsuka, *Nucleic Acids Res.* **1986**,*14*, 7727.
[11] F. Seela, C. Mittelbach, *Nucleosides Nucleotides* **1999**, *18*, 425.
[12] F. Seela, H. Debelak, *Nucleic Acids Res.* **2000**, *28*, 3224.
[13] F. Seela, A. Jawalekar, *Helv. Chim. Acta* **2002**, *85*, 1857.
[14] F. Seela, G. Becher, *Chem. Commun.* **1998**, 2017.
[15] F. Seela, G. Becher, *Helv. Chim. Acta* **1999**, *82*, 1640.
[16] a) R. D. Elliot, J. A. Montgomery, *J. Med. Chem.* **1976**, *19*, 1186; b) J. A. Montgomery, H. J. Thomas, *J. Med. Chem.* **1972**, *15*, 305.
[17] Z. Kazimierczuk, U. Binding, F. Seela, *Helv. Chim. Acta* **1989**, *72*, 1527.
[18] Z. Kazimierczuk, F. Seela, *Helv. Chim. Acta* **1990**, *73*, 316.
[19] H. B. Cottam, D. B. Wasson, H. C. Shih, A. Raychaudhuri, G. Di Pasquale, D. A. Carson, *J. Med. Chem.* **1993**, *36*, 3424.
[20] J. A. Montgomery, H. J. Thomas, *J. Org. Chem.* **1962**, *36*, 1971.
[21] F. Jeannot, G. Gosselin, C. Mathe, *Org. Biomol. Chem.* **2003**, *1*, 2096.
[22] J. Wierzchowski, B. Wielgus-Kutrowska, D. Shugar, *Biochim. Biophys. Acta* **1996**, *1290*, 9.
[23] H. Rosemeyer, M. Zulauf, N. Ramzaeva, G. Becher, E. Feiling, K. Mühlegger, I. Münster, A. Lohmann, F. Seela, *Nucleosides Nucleotides* **1997**, *16*, 821.
[24] N. J. Leonard, *Acc. Chem. Res.* **1979**, *12*, 423.
[25] J. S. Lai, E. T. Kool, *J. Am. Chem. Soc.* **2004**, *126*, 3040.
[26] S. Matsuda, A. A. Henry, P. G. Schultz, F. E. Romesberg, *J. Am. Chem. Soc.* **2003**, *125*, 6134.
[27] L. C. Sowers, B. Ramsay Shaw, W. D. Sedwick, *Biochem. Biophys. Res. Commun.* **1987**, *148*, 790.
[28] H. Rosemeyer, F. Seela, *J. Chem. Soc., Perkin Trans. 2* **2002**, 746.

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