44. Synthesis of 4-Substituted 1*H*-Benzimidazole 2′-Deoxyribonucleosides and Utility of the 4-Nitro Compound as Universal Base

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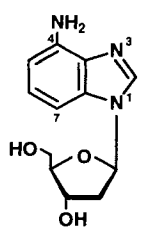
The stereoselective synthesis of 4-substituted 1*H*-benzimidazole 2′-deoxyribonucleosides is described. Regioisomeric (N^1 and N^3) β-D-deoxyribonucleosides **2a–c** and **3a–c** were formed. ^{13}C-NMR Chemical shifts of the 1*H*-benzimidazole 2′-deoxy-β-D-ribofuranosides were correlated with point charges of C-atoms as well as with *Hammett* constants of the exocyclic substituents. Phosphonate and phosphoramidite building blocks of 4-nitro-1*H*-benzimidazole 2′-deoxyribofuranoside (**2a**) were prepared (see **4a**, **b**). Oligonucleotides of the d(A_{20}) type were synthesized in which the two central dA bases were replaced by 4-nitro-1*H*-benzimidazole residues. They were hybridized with oligomeric dT and related oligomers having the other conventional bases opposite to the 4-nitro-1*H*-benzimidazole moieties. Within these duplexes (**12·13**, **12·14**, **12·15**, and **12·16**), the destabilization was almost independent of the mismatch which is required for a universal base. The thermodynamic data indicate that the 4-nitro-1*H*-benzimidazole residues do not form H-bonds with opposite bases but are stabilizing the duplex by stacking interactions and favorable entropic changes.

Introduction. – Universal bases which pair almost equally well with all four natural DNA constituents are currently under intensive investigation. Such bases are useful in primers or probes for DNA technology [1] [2]. The 2′-deoxyinosine (dI) is the classical nucleoside used for such purposes. However, it was observed that the base-pair stability of dI decreases in the order d(I·C) > d(I·A) > d(I·G) and d(I·T) with a preferential binding to dC [3] [4]. Therefore, dI cannot be considered as a universal base. The discovery that several nitro-substituted nucleosides such as 3-nitropyrrole [1], 4-, 5-, and 6-nitroindole [2], or acyclic 5-nitroindazole nucleosides [5] can act as ambiguous or 'lure' bases initiated a search for other compounds which form reasonably stable duplexes but show no base specificity. Very recently, hydrophobic fluoro- and methyl-substituted



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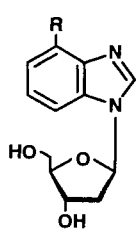
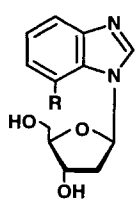
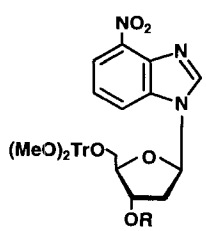
Systematic numbering



2a R = NO_2
b R = F
c R = CH_3
d R = H



3a R = NO_2
b R = F
c R = CH_3



4a R = PH(O)O$^-$ Et_3NH^+
b R = P[(i-Pr)$_2$N](OCH$_2$CH$_2$CN)

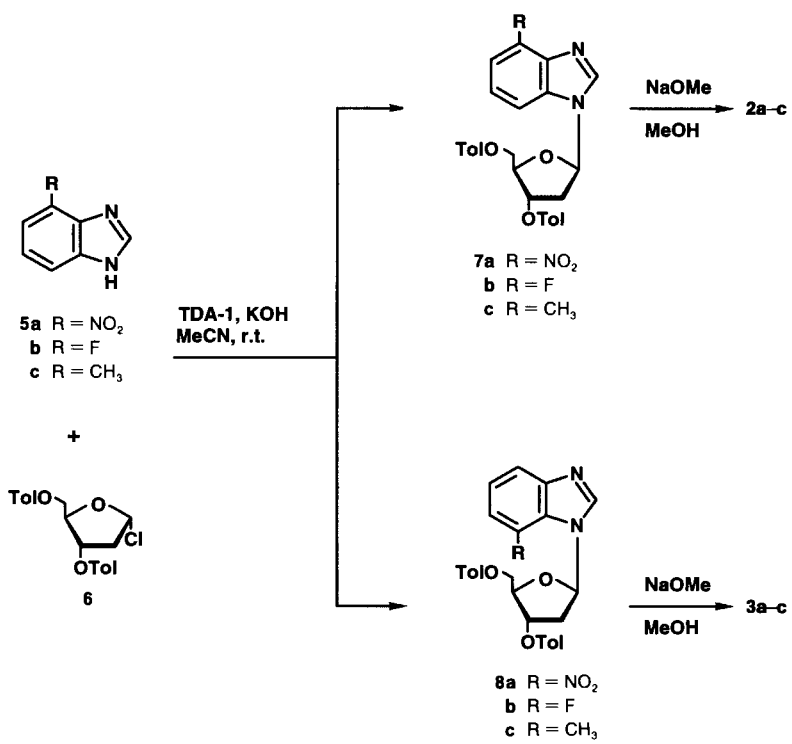
9 R = H

benzene and indole nucleosides were designed and incorporated into oligodeoxyribonucleotides [6]. All these nonpolar isosteres of natural nucleosides exhibited similar pairing properties with the four natural DNA constituents but unfortunately with decreased duplex stability.

Here, we report on the synthesis of the 4-fluoro-, 4-methyl- as well as 4-nitro-1*H*-benzimidazole 2′-deoxyribonucleosides **2a–c**. Compound **2a** was chosen as candidate for an ambiguous nucleoside and was incorporated into duplex structures. For this purpose, the DNA building blocks **4a** and **4b** were prepared and used in solid-phase oligonucleotide synthesis. The behavior of 4-nitro-1*H*-benzimidazole 2′-deoxyribonucleoside (**2a**) opposite to dT, dC, dA, and dG was studied in a series of double-stranded 20-mers.

Results and Discussion. – *4-Substituted 1*H*-Benzimidazole 2′-Deoxyribofuranosides.* The 1*H*-benzimidazole 2′-deoxyribofuranosides can be synthesized in a non-stereoselective manner [7] as well as stereospecifically [8]. Nevertheless, their convergent synthesis starting from an unsymmetrically substituted benzimidazole derivative and a suitably protected halosugar is fraught with difficulties due to the formation of N^1- and N^3-regioisomers. Recently, the stereoselective synthesis of 1,3-dideaza-2′-deoxyadenosine (**1**) and related 2′-deoxyribofuranosides was performed in our laboratory using the nucleobase-anion glycosylation [9]. In the following, the synthesis of the 4-substituted 1*H*-benzimidazole nucleosides **2b,c** and **3b,c** will be described (the systematic numbering is used

Scheme



throughout this communication). For the synthesis of the 4-fluoro- and 4-methyl compounds **2b** and **2c**, the 1*H*-benzimidazoles **5b** and **5c**, respectively, were prepared, **5b** from 2-fluoroacetanilide [10] and **5c** by condensation of 3-methyl-1,2-phenylenediamine with dichloromethyl methyl ether [11] [12].

Glycosylation of the anions of **5a–c** with the halogenose **6** was carried out in MeCN with powdered KOH in the presence of TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine; see *Scheme*). As found for **5a** [9], two regioisomers were also formed in the case of **5b** and **5c**. These were later identified as the N^1- and N^3-isomers. Compounds **7b** and **8b** were separated by flash chromatography, deprotected with NaOMe [13], and the resulting 1*H*-benzimidazole 2′-deoxyribofuranosides **2b** and **3b** were crystallized. The separation of **7c**/**8c** was not successful. Their ratio was estimated from the integrals of the H–C(2) NMR signals (**7c**, 68%; **8c**, 15%). Then, the mixture **7c**/**8c** was deprotected (NaOMe/MeOH) to give **2c**/**3c**. Also at this stage, chromatographic separation proved to be impossible. Nevertheless, the N^1-regioisomer **2c** could be crystallized from the solution; a second portion remained in the mother liquor together with the N^3-regioisomer **3c**. The anomeric configuration and the glycosylation positions of the various regioisomers were unequivocally assigned by ^{1}H-NOE difference spectroscopy (*Table 1*) [14].

Table 1. *NOE Data* [%] *of* 1H-*Benzimidazole 2′-Deoxyribofuranosides*[a])[b])

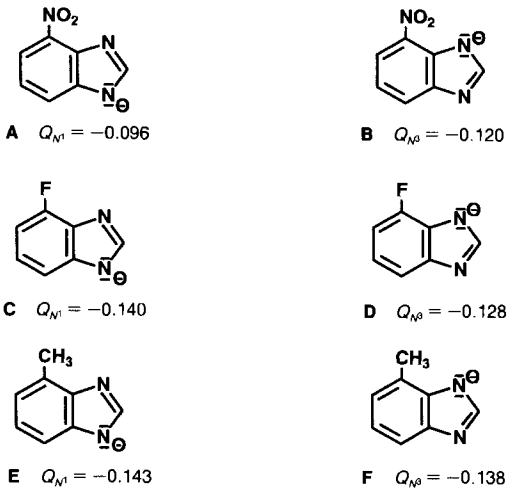
	Irrad.	NOE [%]
2b	H–C(1′)	H–C(2) (5.4); H–C(7) (6.3); H–C(4′) (2.3); H_α–C(2′) (6.0)
	H–C(2)	H–C(1′) (4.4); H–C(3′) (0.7); H_β–C(2′) (3.0)
2c	H–C(1′)	H–C(2) (5.5); H–C(7) (5.8); H–C(4′) (2.3); H_α–C(2′) (6.0)
3b	H–C(1′)	H–C(2) (2.6); H–C(4′) (2.3); H_α–C(2′) (6.3)
	H–C(2)	H–C(1′) (2.6); H–C(3′) (0.9); H_β–C(2′) (3.5)

[a]) In (D_6)DMSO at 23°. [b]) Systematic numbering.

Table 2. *Glycosylation Yields of 4-Substituted* 1H-*Benzimidazoles Under Phase-Transfer Reaction Conditions* (KOH, TDA-1, MeCN): *Influence of the 4-Substituent R on the Orientation of* N-*Substitution*

1*H*-Benzimidazole	R	N^1-Isomer [%]	N^3-Isomer [%]
5a	NO_2	**7a** (45)	**8a** (30)
5b	F	**7b** (47)	**8b** (36)
5c	Me	**7c** (68)	**8c** (15)

The ratio of regioisomers formed during the nucleobase-anion glycosylation, which proceeded under kinetic control, was influenced by the 4-substituent. According to *Table 2*, an electron-withdrawing substituent in position 4 increased the amount of the N^3-isomers, whereas the electron-donating Me group led mainly to the expected N^1-compound. Computation of the point charges (Alchemy III, *Tripos Inc.*) of the anions of **5a–c** showed that, in the cases of **5b** and **5c**, N(1) carries more negative charge than N(3) (**C** and **E** *vs.* **D** and **F**). This behavior is opposite to **5a** (see **A** *vs.* **B**). However, as these charge differences are small, steric as well as field effects of the exocyclic substituents have to be taken into account.



Although the assignment of ^{13}C-NMR data of benzimidazoles are reported [15–18], the complete assignment of ^{13}C chemical shifts of benzimidazole nucleosides is difficult, even using gated-decoupled or ^{1}H,^{13}C-correlation spectra. However, in the case of the compounds **2b** and **3b**, the ^{13}C,^{19}F couplings could be used efficiently (*Table 3*). The ^{13}C-NMR spectra of these compounds show a splitting pattern for nearly all C-atoms of the nucleobase which allows an unequivocal assignment (*Table 4*). Also the ^{19}F-NMR spectra of the deprotected compounds **2b** and **3b** as well as of the protected precursors **7b** and **8b** were measured.

Table 3. *^{13}C,^{19}F-Coupling Constants* [Hz] *of the 1*H*-Benzimidazole 2′-Deoxy-β-*D*-ribofuranosides* **2b** *and* **3b** *in (D$_6$)DMSO at 23°* [a])

2b		**3b**		**3b**	
^{1}J(C(4),F)	250.2	^{1}J(C(7),F)	245.7	^{3}J(C(3a),F)	4.1
^{2}J(C(5),F)	17.2	^{2}J(C(6),F)	17.9	^{4}J(C(4),F)	3.8
^{2}J(C(3a),F)	16.8	^{2}J(C(7a),F)	9.8	^{4}J(C(1′),F)	4.8
^{3}J(C(6),F)	7.3	^{3}J(C(5),F)	6.9		
^{3}J(C(7a),F)	8.8				
^{4}J(C(7),F)	3.9				

[a]) Systematic numbering.

Table 4. *^{13}C-NMR Chemical Shifts of 1*H*-Benzimidazole 2′-Deoxyribofuranosides in (D$_6$)DMSO at 23°* [a])

	C(2)	C(3a)	C(4)	C(5)	C(6)	C(7)	C(7a)
2b	142.6	132.0	153.2	107.4	123.3	107.9	135.9
2c	141.2	143.0	128.9	122.4	122.1	108.7	134.5
2d	142.2	143.8	119.6	122.7	122.1	111.4	133.0
3b	142.5	147.0	115.9	122.3	108.9	148.6	120.9
4a	145.3	137.1	135.8	118.9	122.3	118.8	138.8
7b	142.6	132.1	152.8	107.7	123.4	108.0	135.7
8b	142.9	147.3	116.3	122.8	109.4	148.6	120.8
9	145.1	137.0	135.8	118.8	122.2	118.8	138.7

Table 4 (cont.)

	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O	Me	MeO
2b	84.7	[b])	70.4	87.6	61.4	–	–	–
2c	84.4	[b])	70.5	87.4	61.5	–	16.3	–
2d	84.5	[b])	70.6	87.6	61.6	–	–	–
3b	85.7	[b])	70.3	87.7	61.4	–	–	–
4a	85.3	[b])	72.3	85.1	63.2	–	16.3	55.0
7b	85.0	36.2	74.6	81.5	64.0	165.3	21.2	–
8b	85.7	37.2	74.5	81.5	64.1	165.4	21.3	–
9	85.4	[b])	69.9	84.7	63.4	–	16.3	54.9

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

The assignment of ^{13}C-NMR data is problematic if reporter atoms such as an F-atom are absent. To approach this problem in a more general way, the correlation of ^{13}C-NMR chemical shifts with point charges and *Hammett* constants [19–21] was made. A linear dependence of the chemical shifts of C(7) with the σ_p constants of the 4-substituents was observed with a regression coefficient of $|r^2| = 0.92$. A significantly better linear correlation ($|r^2| = 0.99$) was obtained for a graph of δ(C(7)) *vs.* σ_{p+} values (*Fig. 1*). This is due to an inductive barrier at C(4). The influence of the exocyclic substituents at the benzene moiety of **1** and **2a–d** on the charge density of the imidazole ring is clearly documented by the correlation of the *Hammett* constants with the corresponding C(2) chemical shifts (*Fig. 2*). Here, the best results for **1** and **2a–d** were obtained using the σ_p values (*Fig. 2*, $|r^2| = 0.99$) indicating no inductive barrier.

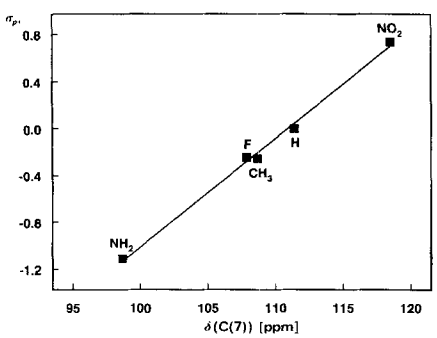


Fig. 1. Hammett *constants* (σ_{p+}) *as a function of the* ^{13}*C-NMR chemical shift of C(7) of compounds* **1** *and* **2a–d**

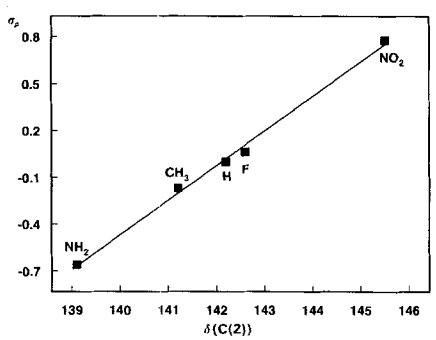


Fig. 2. Hammett *constants* (σ_p) *as a function of the* ^{13}*C-NMR chemical shift of C(2) of compounds* **1** *and* **2a–d**

Next, the point charges (Q_C) of different C-atoms (C(2), C(4), C(7a)) of benzimidazole nucleosides were correlated with the corresponding ^{13}C-NMR chemical shifts [22]. The point charges were calculated according to a method of *Gasteiger* and *Marsili* [23] [24] which is implemented into the Alchemy III molecular-modeling program (Alchemy III, release 1992, *Tripos Co.*). *Figs. 3–5* display the linear correlations of best fit for C(2), C(4), and C(7a). The graphs presented follow approximately the empirical *Spiesecke-Schneider*

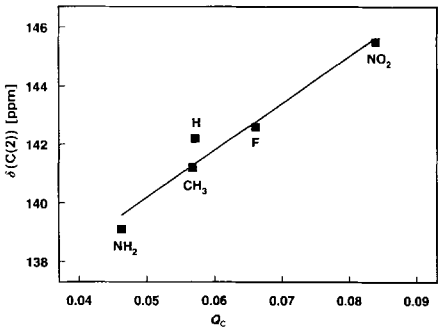


Fig. 3. *^{13}C-NMR Chemical shift of C(2) as a function of point charges* Q_C *of compounds* **1** *and* **2a–d**

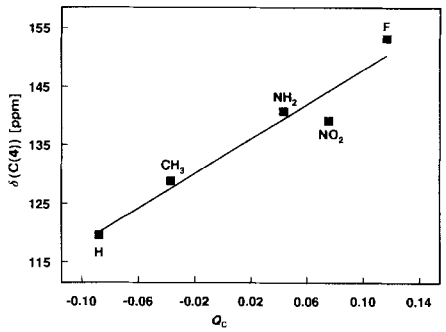


Fig. 4. *^{13}C-NMR Chemical shift of C(4) as a function of point charges* Q_C *of compounds* **1** *and* **2a–d**

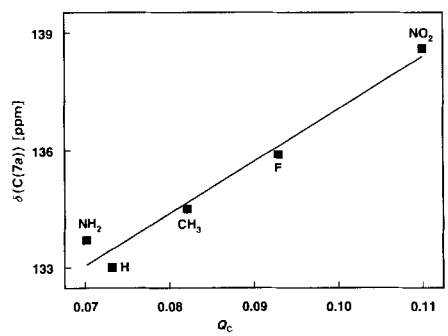


Fig. 5. *^{13}C-NMR Chemical shift of C(7a) as a function of point charges* Q_C *of compounds* **1** *and* **2a–d**

[25] relation $\Delta\delta = -160 \cdot \Delta Q_C$ which has been evaluated for aromatic compounds. For the atoms C(2), C(4), and C(7a), values between −152 and −168 ppm/e$^-$ were calculated. The data clearly demonstrate that the aromatic character of the heterocycle is significantly influenced by the exocyclic substituents.

*4-Nitro-1*H*-benzimidazole Oligodeoxyribonucleotides.* Next, the oligonucleotide building blocks **4a** and **4b** were synthesized. For this purpose, compound **2a** was converted into the 4,4′-dimethoxytrityl derivative **9** using standard protocols of preparation [26]. The reaction of **9** with tris(1*H*-1,2,4-triazol-1-yl)phosphane [26] furnished the triethylammonium phosphonate **4a** (72%). The phosphoramidite **4b** (52%; diastereoisomeric

$d(A_{20})$	$d[A_9\text{-}(c^1c^3A)_2\text{-}A_9]$	$d\{A_9\text{-}[(NO_2)^6c^1c^3A]_2\text{-}A_9\}$
10	**11**	**12**
$d(T_{20})$	$d(T_9\text{-}G_2\text{-}T_9)$	$d(T_9\text{-}A_2\text{-}T_9)$
13	**14**	**15**
$d(T_9\text{-}C_2\text{-}T_9)$	$d[G\text{-}T\text{-}A\text{-}G\text{-}(NO_2)^6c^1c^3A\text{-}(NO_2)^6c^1c^3A\text{-}T\text{-}T\text{-}C\text{-}T\text{-}A\text{-}C]$	
16	**17**	

mixture) was also prepared [27]. Both building blocks were used in automated solid-phase synthesis. After treatment with aqueous NH_3 solution, the 5′-protected oligomers were purified, detritylated, and desalted on oligonucleotide-purification cartridges (*Applied Biosystems*) to yield the oligomers **10**–**17**. The composition of **10**–**17** was determined by hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase and *RP-18* HPLC.

*Duplex Stability of Oligonucleotides with 4-Nitro-1*H*-benzimidazole Opposite to the Four Nucleobases.* To study the stability of duplexes containing 4-nitro-1*H*-benzimidazole opposite to the four naturally occurring nucleobases, the T_m values of the hybrids of **12**·**13**, **12**·**14**, **12**·**15**, and **12**·**16** were measured. In all cases, cooperative monophasic melting profiles were observed (1M NaCl, 0.1M $MgCl_2$, 60 mM Na-cacodylate, pH 7.0). *Fig. 6, a* shows a typical melting curve obtained for the duplex **12**·**13** (T_m values *Table 5*). Duplex formation was confirmed by the concentration dependence of the T_m values within a range of 10 to 1.5 μM (see *Fig. 6, b*). Hairpin formation was favoured in the case of the self-complementary oligonucleotide **17** where only the central dA residues are replaced by **2a**. This is demonstrated in *Fig. 7, b* showing the concentration-independent UV-melting profiles (2–20 μM, *Fig. 7, b*).

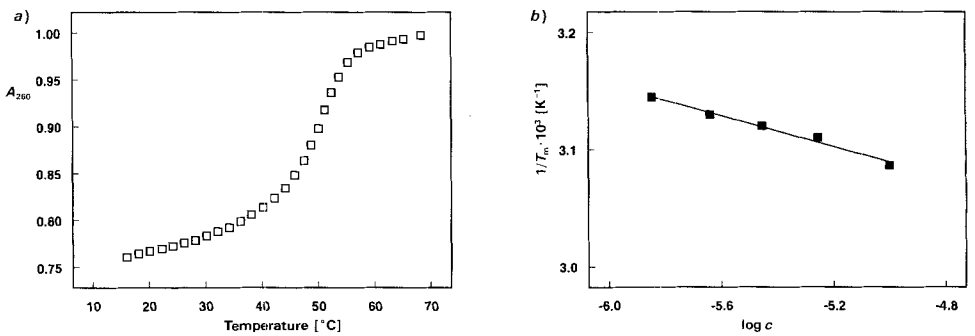


Fig. 6. a) *Normalized melting profile of* $d\{A_9\text{-}[(NO_2)^6c^1c^3A]_2\text{-}A_9\}\cdot d(T_{20})$ (**12**·**13**) *and* b) $1/T_m$ vs. *log* c *of* **12**·**13**. Measured at 260 nm in 60 mM Na-cacodylate, 1M NaCl, and 100 mM $MgCl_2$ at pH 7.0; oligomer concentration in *Fig. 6, a*, 5.0 μM of single strands.

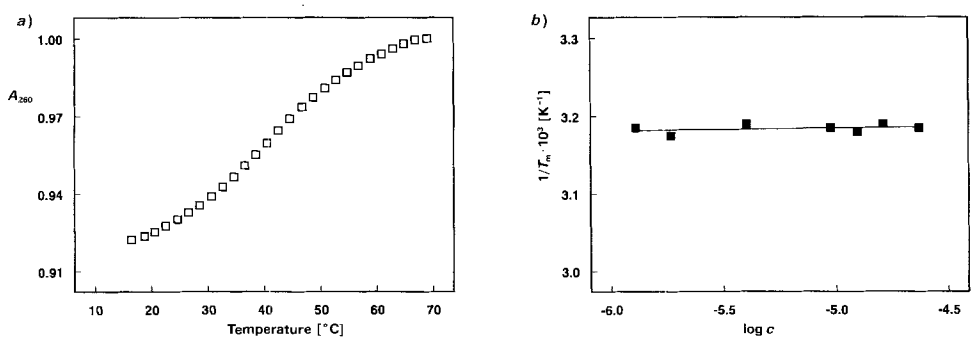


Fig. 7. a) *Normalized melting profile of* $d[G\text{-}T\text{-}A\text{-}G\text{-}(NO_2)^6c^1c^3A\text{-}(NO_2)^6c^1c^3A\text{-}T\text{-}T\text{-}C\text{-}T\text{-}A\text{-}C]$ (**17**; same buffer as in *Fig. 6*; oligomer concentration, 5.0M) *and* b) $1/T_m$ vs. *log* c *of* **17** (conditions, see *Fig. 6*)

Table 5. T_m *Values and Thermodynamic Data of the Oligomers* **10**–**20**

	T_m [°C]	h_{mel} [%]	ΔH [kcal/mol]	ΔS [cal/K · mol]
10·13 [34]	60.0	40	−190.1	−571
11·13 [29]	54.0	22	−179.2	−549
12·13	51.0	24	−160.3	−495
12·14	46.5	28	−140.6	−440
12·15	45.5	26	−128.9	−405
12·16	44.0	26	−122.8	−388
17	41.0	8	−24.1	−77

[a]) Determined at 298 K.

From *Table 5* it can be seen that the incorporation of two 4-nitro-1*H*-benzimidazole residues in the centre of the duplex **12·13** reduced the T_m value by 9° compared to the parent oligomer **10·13** (T_m 60°). Furthermore, the incorporation of **2a** opposite to dT, dG, dA, or dC (**12·13**, **12·14**, **12·15**, or **12·16**) shows no significant differences in the T_m values (*Table 5*). This behavior would satisfy the requirements of an ambiguous base. As it was of interest whether the relatively weak destabilization is the result of enthalpic or entropic changes, the thermodynamic parameters (ΔH, ΔS) of the duplexes **12·13**, **12·14**, **12·15**, and **12·16** were calculated. A two-state model for helix-coil transition was used [28] (*Table 5*).

According to *Table 5*, the ΔH values decrease in all cases, whereas a favorable term of entropy compensates this destabilization partly. The behavior of base-modified nucleosides not having the ability to form a *Watson-Crick*- or *Hoogsteen*-type base pair is still unclear. It is very likely that a nitropyrrole, nitroindole, or a nitrobenzimidazole base is stacked within an oligonucleotide duplex, and that bound H_2O is liberated during duplex formation. Contrary to regular nucleic-acid constituents, the enthalpy necessary for the removal of the H_2O from the bases is not gained upon duplex formation. According to *Table 5*, a loss of enthalpy is observed in the cases of the base-modified compounds which reflects that base pairing between the universal and the normal base does not exist. Opposite to these findings, the loss of enthalpy is only moderate if 4-amino-1*H*-benzimidazole residues (**1**) are incorporated in oligodeoxyribonucleotides (**11·13**) [29] (see *Table 5*). Apparently, 4-amino-1*H*-benzimidazole can undergo base pairing under formation of one H-bond (amino group of 1,3-dideazaadenine and carbonyl group of dT). The gain of entropy upon duplex formation is typical for all regular oligonucleotides. The more favorable term in case of the universal bases may result from their hydrophobic character. The nitro-1*H*-benzimidazole residues of the single-stranded oligonucleotide can bind H_2O at the imidazole N-atom as well as at the NO_2 groups. Apparently, the amount of H_2O expelled during duplex formation is larger than in the case of a regular base pair.

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Experimental Part

General. See [30]. The phosphonates were purchased from *Sigma*, St. Louis, and CPG (30–50 μmol of immobilized protected 2′-deoxyribonucleoside/g of solid support) from *Milligene*, Eschborn, Germany. Oligonucleotide synthesis was carried out on a DNA synthesizer, model *380 B*, *Applied Biosystems*, Weiterstadt, Germany. Melting curves were measured with a *Cary 1E* UV/VIS spectrophotometer (*Varian*, Australia) equipped with a thermoelectrical controller. The actual temp. was measured in the reference cell with a Pt-100 resistor (linear temp. increase from 15 to 70° with 0.5°/min). The enzymatic hydrolysis of the oligomers was carried out as described [30]. The mixture was analysed on reversed-phase HPLC (*RP-18*, solvent system II; see below). Quantification of the material was made on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituents (λ_{260}: dA 15400, dC 7300, dG 11700, dT 8800, $(NO_2)^6c^1c^3A_d$ 1500). HPLC: a gradient consisting of 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5 (*A*) and MeCN (*B*) was used; gradient, 20 min 0–20% *B* in *A*, flow rate 1.0 ml/min. Solvent systems for flash chromatography (FC) and TLC: CH_2Cl_2/AcOEt 8:2 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 9:1 (*C*), CH_2Cl_2/MeOH 5:1 (*D*), CH_2Cl_2/Et_3N 98:2 (*E*), CH_2Cl_2/MeOH/Et_3N 95:3:2 (*F*), CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5 (*G*), CH_2Cl_2/MeOH/Et_3N 90:8:2 (*H*), CH_2Cl_2/AcOEt/Et_3N 80:18:2 (*I*). NMR Spectra: *Bruker-AC-250* and *-AMX-500* spectrometers, δ in ppm rel. to Me_4Si (^1H, ^{13}C) and CF_3Cl (^{19}F) as internal standards, *J* in Hz. Point charges were calculated using the Alchemy III program (release version 1992; *Tripos Co.*, St. Louis).

*4-Fluoro-1*H*-benzimidazole* (**5b**) [10]. ^1H-NMR ((D_6)DMSO): 7.01 (*dd*, *J* = 8.0, 11.1, H–C(5)); 7.20 (*d't'*, *J* = 5.0, 8.0, H–C(6)); 7.42 (*d*, *J* = 8.0, H–C(7)); 8.30 (*s*, H–C(2)); 12.84 (*s*, NH–C(4)). ^{19}F-NMR ((D_6)DMSO): −132.0.

*1-[2-Deoxy-3,5-bis-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-4-fluoro-1*H*-benzimidazole* (**7b**) *and 1-[2-Deoxy-3,5-bis-*O*-(4-toluoyl)-β-*D*-*erythro*-pentofuranosyl]-7-fluoro-1*H*-benzimidazole* (**8b**). A suspension of KOH (380 mg, 6.8 mmol), TDA-1 (90 mg, 0.3 mmol), and **5a** (310 mg, 2.3 mmol) in dry MeCN (50 ml) was stirred at r.t. for 10 min. After addition of 2-deoxy-3,5-bis-*O*-(4-toluoyl)-α-D-erythro-pentofuranosyl chloride [31] (**6**; 0.93 g, 2.4 mmol), stirring was continued for 15 min. After filtration and evaporation, the residue was applied to FC (silica gel, column 25 × 4.5 cm, *A*).

From the faster-migrating zone, **7b** was isolated as colorless foam (470 mg, 42%). Crystallization from Et_2O afforded colorless needles. M.p. 121–122°. TLC (*A*): R_f 0.6. ^1H-NMR ((D_6)DMSO): 2.40 (*s*, Me); 2.43 (*s*, Me); 2.81 (*m*, H_α–C(2′)); 3.09 (*m*, H_β–C(2′)); 4.61 (*m*, H–C(4′), H–C(5′)); 5.76 (*d*, *J* = 6.1, H–C(3′)); 6.62 (*dd*, *J* = 5.8, 8.3, H–C(1′)); 7.03–7.18 (*m*, H–C(5), H–C(6)); 7.32–7.42 (2*d*, *J* = 8.1, arom. H); 7.60 (*d*, *J* = 7.8, H–C(7)); 7.85–8.02 (2*d*, *J* = 8.1, arom. H); 8.55 (*s*, H–C(2)). ^{19}F-NMR ((D_6)DMSO): −131.3. Anal. calc. for $C_{28}H_{25}FN_2O_5$ (488.52): C 68.84, H 5.16, N 5.73; found: C 68.91, H 5.18, N 5.65.

From the slower-migrating zone, a colorless foam (350 mg, 31%) of **8b** was isolated. TLC (*A*): R_f 0.5. ^1H-NMR ((D_6)DMSO): 2.36 (*s*, Me); 2.40 (*s*, Me); 2.83 (*m*, H_α–C(2′)); 3.08 (*m*, H_β–C(2′)); 4.49–4.58 (*m*, H–C(4′), H–C(5′)); 5.71 (*m*, H–C(3′)); 6.65 (*t'*, *J* = 6.9, H–C(1′)); 7.11–7.23 (*m*, H–C(5), H–C(6)); 7.27–7.38 (*d*, *J* = 8.1, arom. H); 7.55 (*d*, *J* = 7.7, H–C(7)); 7.81–7.95 (2*d*, *J* = 7.7, arom. H); 8.60 (*s*, H–C(2)). ^{19}F-NMR ((D_6)DMSO): −132.2. Anal. calc. for $C_{28}H_{25}FN_2O_5$ (488.52): C 68.84, H 5.16, N 5.73; found: C 68.95, H 5.16, N 5.73.

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-4-fluoro-1*H*-benzimidazole* (**2b**). Compound **7b** (490 mg, 1.0 mmol) was dissolved in MeOH (100 ml)/1M NaOMe/MeOH (2 ml) and stirred for 48 h at r.t. After evaporation, the residue was chromatographed (silica gel, column 20 × 4 cm, *D*) yielding a colorless oil. Crystallization from MeOH/H_2O afforded **2b** (200 mg, 81%). Colorless crystals. M.p. 134–135°. TLC (*D*): R_f 0.55. UV (MeOH): 245 (6200). ^1H-NMR ((D_6)DMSO): 2.34 (*ddd*, *J* = 3.4, 6.0, 13.2, H_α–C(2′)); 2.61 (*'q'*, *J* = 6.6, H_β–C(2′)); 3.58 (*m*, H–C(5′)); 3.90 (*dd*, *J* = 4.1, 7.5, H–C(4′)); 4.42 (*m*, H–C(3′)); 5.01 (*m*, OH–C(5′)); 5.39 (*m*, OH–C(3′)); 6.39 (*t'*, *J* = 6.5, H–C(1′)); 7.05 (*dd*, *J* = 8.0, 11.1, H–C(5)); 7.24 (*d't'*, *J* = 4.9, 8.0, H–C(6)); 7.57 (*d*, *J* = 8.0, H–C(7)); 8.53 (*s*, H–C(2)). ^{19}F-NMR ((D_6)DMSO): −131.5.

*1-(2-Deoxy-β-*D*-*erythro*-pentofuranosyl)-7-fluoro-1*H*-benzimidazole* (**3b**). As described for **2b**, **8b** (490 mg, 1.0 mmol) was deprotected and purified: colorless crystals (MeOH/H_2O) of **3b** (210 mg, 83%). M.p. 130–131°. UV (MeOH): 245 (6800). ^1H-NMR ((D_6)DMSO): 2.38 (*ddd*, *J* = 3.8, 6.0, 13.4 H_α–C(2′)); 2.59 (*'q'*, *J* = 6.7, H_β–C(2′)); 3.54 (*m*, H–C(5′)); 3.88 (*dd*, *J* = 4.5, 7.6, H–C(4′)); 4.36 (*m*, H–C(3′)); 4.96 (*t*, *J* = 5.3, OH–C(5′)); 5.36 (*d*, *J* = 4.2, OH–C(3′)); 6.46 (*t'*, *J* = 6.6, H–C(1′)); 7.12 (*dd*, *J* = 7.9, 11.9, H–C(6)); 7.21 (*d't'*, *J* = 5.2, 7.9, H–C(5)); 7.52 (*d*, *J* = 7.9, H–C(4)); 8.58 (*s*, H–C(2)). ^{19}F-NMR ((D_6)DMSO): −133.9. Anal. calc. for $C_{12}H_{13}FN_2O_3$ (252.25): C 57.14, H 5.19, N 11.02; found: C 57.19, H 5.16, N 11.07.

4-Methyl-1 H-*benzimidazole* (**5c**) [11] [12]. ^{1}H-NMR ($CDCl_3$): 2.63 (*s*, Me); 7.11 (*d*, *J* = 7.2, H–C(5)); 7.21 ('*t*', *J* = 7.6, H–C(6)); 7.51 (*d*, *J* = 8.0, H–C(7)); 8.17 (*s*, H–C(2)); 10.10 (*s*, NH).

*1-[2-Deoxy-3,5-bis-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-4-methyl-1* H-*benzimidazole* (**7c**) *and 1-[2-Deoxy-3,5-bis-*O*-(4-toluoyl)-β-*D-erythro-*pentofuranosyl]-7-methyl-1* H-*benzimidazole* (**8c**). To a soln. of **5c** (500 mg, 3.8 mmol) in anh. MeCN (100 ml), TDA-1 (100 mg, 0.3 mmol) and powdered KOH (530 mg, 9.5 mmol) were added. The mixture was stirred for 10 min at r.t. Then, **6** (1.54 g, 4.0 mmol) was added, and stirring was continued for 15 min. After filtration and evaporation, the residue was applied to FC (silica gel, column 25 × 4.5 cm, *B*) yielding **7c**/**8c** (1.5 g, 83%). Colorless foam. TLC (*B*): R_f 0.6. Anal. calc. for $C_{29}H_{28}N_2O_5$ (484.56): C 71.89, H 5.82, N 5.78; found: C 71.91, H 5.76, N 5.69.

*1-[2-Deoxy-β-*D-erythro-*pentofuranosyl]-4-methyl-1* H-*benzimidazole* (**2c**) *and 1-[2-Deoxy-β-*D-erythro-*pentofuranosyl]-7-methyl-1* H-*benzimidazole* (**3c**). The mixture **7c**/**8c** (1.5 g, 3.10 mmol) was treated with 1M NaOMe/MeOH. After evaporation, the residue was crystallized from H_2O: **2c**. Colorless crystals. M.p. 133–134°. TLC (*C*): R_f 0.3. UV (MeOH): 282 (2400), 272 (sh, 2900), 249 (7000). ^{1}H-NMR ((D_6)DMSO): 2.26–2.34 (*ddd*, *J* = 3.3, 6.0, 13.3, H_α–C(2')); 2.55 (*s*, Me); 2.65 (*m*, H_β–C(2')); 3.59 (*dd*, *J* = 4.7, 9.5, H–C(5')); 3.90 (*dd*, *J* = 4.3, 7.6, H–C(4')); 4.42 (*m*, H–C(3')); 5.01 (*t*, *J* = 5.3, OH–C(5')); 5.40 (*d*, *J* = 4.1, OH–C(3')); 6.35 ('*t*', *J* = 7.5, H–C(1')); 7.05 (*d*, *J* = 7.2, H–C(5)); 7.16 ('*t*', *J* = 7.7, H–C(6)); 7.51 (*d*, *J* = 8.0, H–C(7)); 8.43 (*s*, H–C(2)). Anal. calc. for $C_{13}H_{16}N_2O_3$ (248.3): C 62.89, H 6.50, N 11.28; found: C 62.86, H 6.54, N 11.07.

From the mother liquor, **2c**/**3c** was obtained.

*1-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-4-nitro-1* H-*benzimidazole* (**9**). A soln. of **2a** (315 mg, 1.1 mmol) in anh. pyridine (7 ml) was treated with 4,4'-dimethoxytriphenylmethyl chloride (535 mg, 1.6 mmol). Stirring was continued for 1 h at r.t. Then, 5% aq. $NaHCO_3$ soln. (10 ml) was added and the mixture extracted with CH_2Cl_2 (3 × 10 ml). The combined org. layers were dried (Na_2SO_4), evaporated, and co-evaporated with toluene, and the residue was applied to FC (silica gel, 25 × 3 cm, *G*). The material of the main zone was evaporated, dissolved in CH_2Cl_2 (2 ml), and precipitated from hexane/Et_2O 1:1: Colorless, amorphous powder (510 mg, 78%). TLC (*C*): R_f 0.4. ^{1}H-NMR ((D_6)DMSO): 2.48 (*m*, H_α–C(2')); 2.87 (*m*, H_β–C(2')); 3.14 (*m*, H–C(5')); 3.72 (*m*, MeO); 4.05 (*m*, H–C(4')); 4.51 (*m*, H–C(3')); 5.49 (*d*, *J* = 4.4, OH–C(3')); 6.54 ('*t*', *J* = 5.9, H–C(1')); 6.74–7.26 (*m*, arom. H); 7.41 ('*t*', *J* = 8.2, H–C(6)); 8.10 (*d*, *J* = 8.0, H–C(5)); 8.23 (*d*, *J* = 8.1, H–C(7)); 8.75 (*s*, H–C(2)). Anal. calc. for $C_{33}H_{31}N_3O_7$ (581.6): C 68.15, H 5.37, N 7.22; found: C 68.08, H 5.51, N 7.14.

*1-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-4-nitro-1* H-*benzimidazole 3'-(Triethylammonium Phosphonate)* (**4a**). To a soln. of PCl_3 (190 µl, 2.2 mmol) and *N*-methylmorpholine (2.3 ml, 21.0 mmol) in dry CH_2Cl_2 (15 ml), 1,2,4-1*H*-triazole (450 mg, 6.5 mmol) was added at r.t. under stirring. After 30 min, the soln. was cooled to 0°, and **9** (250 mg, 0.4 mmol) in dry CH_2Cl_2 (7 ml) was added within 10 min. The mixture was stirred for another 40 min at r.t., then hydrolyzed with 1M aq. $(Et_3NH)HCO_3$ buffer (20 ml, pH 8.0), and extracted with CH_2Cl_2 (3 × 20 ml). The combined org. layers were dried (Na_2SO_4) and evaporated. FC (silica gel, column 15 × 4 cm, 0.5 l *E*, 0.5 l *F*, then *H*), washing with 0.1M $(Et_3NH)HCO_3$, drying (Na_2SO_4), and co-evaporation with CH_2Cl_2 yielded a colorless foam (230 mg, 72%). TLC (*C*): R_f 0.2. ^{1}H-NMR ((D_6)DMSO): 1.12 (*t*, *J* = 7.2, $(MeCH_2)_3N$); 2.63 (*m*, H_α–C(2')); 2.90 (*m*, H_β–C(2')); 2.99 (*m*, $(MeCH_2)_3N$); 3.16 (*m*, H–C(5')); 3.68 (*s*, MeO); 4.21 (*m*, H–C(4')); 4.91 (*m*, H–C(3')); 6.50 ('*t*', *J* = 6.2, H–C(1')); 6.66 (*d*, *J* = 598, PH); 6.73–7.24 (*m*, arom. H); 7.31 ('*t*', *J* = 8.0, H–C(6)); 8.06 (*d*, *J* = 8.0, H–C(5)); 8.21 (*d*, *J* = 8.0, H–C(7)); 8.68 (*s*, H–C(2)). ^{31}P-NMR ((D_6)DMSO): 1.6 (^1J(P,H) = 598; ^3J(P,H) = 8.9). Anal. calc. for $C_{39}H_{47}N_4O_9P$ (746.8): C 62.73, H 6.34, N 7.50; found: C 62.81, H 6.42, N 7.48.

*1-[2-Deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-β-*D-erythro-*pentofuranosyl]-4-nitro-1* H-*benzimidazole 3'-[(2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**4b**). To a soln. of **9** (300 mg, 0.5 mmol) and $(i\text{-}Pr)_2EtN$ (400 µl, 2.4 mmol) in anh. CH_2Cl_2 (7 ml) under Ar, chloro(2-cyanoethoxy)(diisopropylamino)phosphane (320 µl, 1.4 mmol) was added under stirring at r.t. Stirring was continued for 1 h, the mixture diluted with CH_2Cl_2 (15 ml) and quenched by addition of 5% aq. $NaHCO_3$ soln. (15 ml), the aq. layer extracted with CH_2Cl_2 (3 × 15 cm), the combined org. layers dried (Na_2SO_4) and evaporated, and the yellow oil applied to FC (silica gel, column 15 × 4 cm, *I*): pale yellow foam (210 mg, 52%). TLC (*I*): R_f 0.7, 0.8. ^{1}H-NMR ($CDCl_3$): 1.07–1.26 (*m*, Me_2CH); 2.41 (*t*, *J* = 6.3, $NCCH_2CH_2$); 2.58 (*m*, H_α–C(2')); 2.61 (*t*, *J* = 6.1, $NCCH_2CH_2$); 2.73 (*m*, H_β–C(2')); 3.58 (*m*, H–C(5')); 3.74 (*s*, MeO); 4.09 (*m*, H–C(4')); 4.75 (*m*, H–C(3')); 6.33 ('*t*', *J* = 6.4, H–C(1')); 6.76–7.23 (*m*, arom. H); 7.35 ('*t*', *J* = 7.6, H–C(6)); 7.99 (*d*, *J* = 8.1, H–C(5)); 8.10 (*d*, *J* = 8.1, H–C(7)); 8.27 (*s*, H–C(2)). ^{31}P-NMR ($CDCl_3$): 149.7.

Solid-Phase Synthesis of the Oligodeoxyribonucleotides **10**–**17**. The oligomers **10**–**17** were synthesized on a 1-µmol scale using the 3'-phosphonates of $[(MeO)_2Tr]bz^6A_d$, $[(MeO)_2Tr]ib^2G_d$, $[(MeO)_2Tr]bz^4C_d$, and $[(MeO)_2Tr]T_d$ as well as **4a**. The syntheses and deprotection of the oligonucleotides followed a slightly modified

Table 6. *Retention Times and Yields of Oligonucleotides*

	12	**14**	**15**	**16**	**17**
Retention time [min][a])	19.7	19.0	19.3	18.8	19.2
Yields [%][b])	11	18	17	20	38

[a]) The retention times refer to gradient II [34].
[b]) The yields were calculated on the basis of silica-gel-bound nucleosides.

protocol of the DNA synthesizer for 3′-phosphonates [32] [33]. Yields and retention times (gradient II) of the oligomers **12**, and **14**–**17** are shown in *Table 6*.

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