2-Aza-2′-deoxyadenosine: Synthesis, Base-Pairing Selectivity, and Stacking Properties of Oligonucleotides

Tamizi Sugiyama, Enno Schweinberger, Zygmunt Kazimierczuk, Natalya Ramzaeva, Helmut Rosemeyer, and Frank Seela*[a]

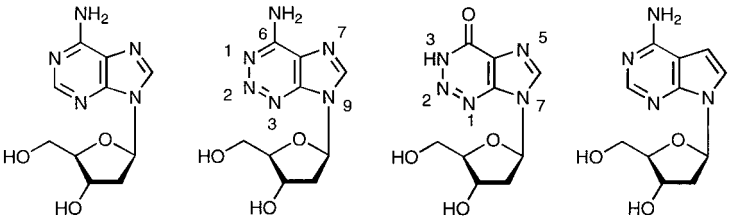
Dedicated to Prof. Dr. Wolfram Saenger on the occasion of his 60th birthday.

Abstract: 2-Aza-2′-deoxyadenosine (**2**, z^2A_d) is synthesized via its 1,N^6-etheno derivative **7** and enzymatically deaminated to 2-aza-2′-deoxyinosine (**3**). Compound **2** is converted into the phosphoramidite building block **10b**. This is employed in solid-phase oligonucleotide synthesis. The 2-azapurine base forms a strong base pair with guanine, but a much weaker one with adenine, thymine, and cytosine. Oligonucleotide duplexes with dangling nucleotide residues, such as 2-aza-2′-deoxyadenosine and 7-deaza-2′-deoxyadenosine (**4**, c^7A_d), either on one or both termini, are synthesized, and the thermal stability of the duplexes is correlated with the hydrophobic properties of the dangling nucleotide residues.

Keywords: 2-aza-2′-deoxyadenosine · DNA structures · duplex stability · nucleosides · oligonucleotides

Introduction

2-Azapurines, such as 2-azaadenine and 2-azahypoxanthine, have long been known to inhibit the growth of both microbial and mammalian cells.[1–6] However, as a consequence of the paucity of nucleoside starting materials, little is known about 2-azapurine-containing oligonucleotides. One reason for this might be the photochemical lability of such triazine compounds, which decompose upon UV irradiation to give 4,5-disubstituted imidazole nucleosides.[7] On the other hand, such compounds have been used as precursors for the preparation of 2-azapurine nucleosides. 2-Azapurine nucleosides are isosteric, but not isoelectronic, to regular purine nucleosides and possess a different dipole moment.[8] Such minor structural modifications of a nucleobase, especially when they are effected at strategic positions of the heterocyclic ring, may alter the base-pairing properties because of differences in hydrogen bonding, base pairing, and electronic properties between the analogues and the parent nucleosides. In this respect, aza[9a, 9b, 10] and deaza[11] analogues of purine nucleosides, such as compounds **2**, **3**, or **4**, are of particular value for probing nucleic acid function, structure, and stability. In this paper, we report a new and efficient synthesis of 2-aza-2′-deoxyadenosine (**2**) and its incorporation into oligonucleotides. The base-pairing properties of this minor-groove-modifying base with regular bases, as well as its effect as a dangling nucleotide on the duplex stability of oligodeoxynucleotides, are investigated.



1 **2** **3** **4**

purine numbering systematic numbering

Results and Discussion

Monomers

Synthesis: The synthesis of 2-azapurine ribonucleosides, 2′-deoxyribonucleosides, and arabino nucleosides, as well as of α-D or acyclic analogues,[12–21] is based mainly on ring-annulation reactions of imidazole nucleoside precursors (purine numbering is used throughout except for the Experimental Section). Most successful was the preparation of

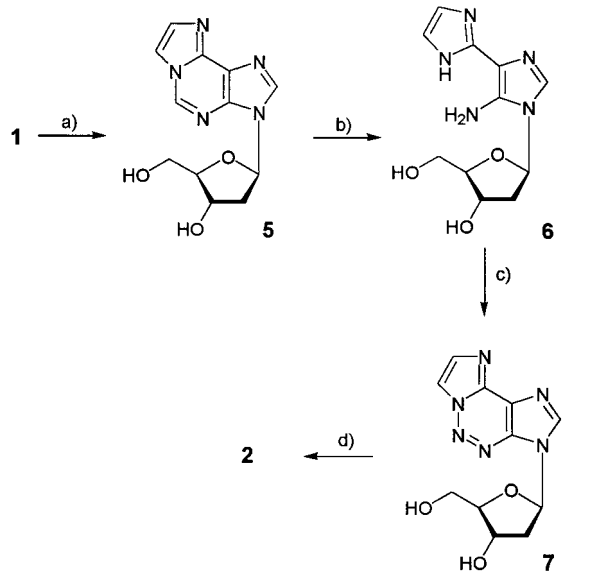
[a] Prof. Dr. Frank Seela, Prof. Dr. T. Sugiyama,[+]
Dipl.-Chem. E. Schweinberger, Prof. Dr. Z. Kazimierczuk,
Dr. N. Ramzaeva, Dr. H. Rosemeyer
Laboratorium für Organische und Bioorganische Chemie
Institut für Chemie, Fachbereich Biologie/Chemie
Universität Osnabrück, Barbarastr. 7
D-49069 Osnabrück (Germany)
Fax: (+49) 541-9692370
E-mail: fraseela@rz.uni-osnabrueck.de

[+] T. Sugiyama is on leave from the Meiji University, Kawasaki (Japan)

  0947-6539/00/0602-0369 $ 17.50+.50/0

2-azainosine by ring closure of 5-amino-1-β-D-ribofuranosylimidazol-4-carboxamide (AICA-riboside) in the presence of nitrous acid and 6 N hydrochloric acid at low temperature.[14] The glycosylation of a 2-azapurine derivative with a sugar halide has been described in only two cases.[20a, b, 21] Most versatile is the nucleobase anion glycosylation of 6-methylthio-2-azapurine under phase-transfer conditions.[21] However, this method is fraught with difficulties because different regioisomeric nucleosides are formed upon glycosylation.

We report the synthesis of 2-aza-2′-deoxyadenosine (**2**) via its 1,N^6-etheno derivative starting from 2′-deoxyadenosine (**1**) (Scheme 1). This route has been elaborated by Yamaji and



Scheme 1. Synthesis of **2**. a) $ClCH_2CHO$, 1M aq NAOC buffer, pH 4–5, 70%; b) 1N NaOH, 73%; c) $NaNO_2$, 80% aq HOAc, 53%; d) NBS, 1M aq NaOAc buffer, pH 4–5, 38%.

Kato[22] for the synthesis of alkyl derivatives and for β-D-ribofuranosyl 3′,5′-cyclic phosphates. Reaction of 2′-deoxyadenosine (**1**) with chloroacetaldehyde gave 1,N^6-etheno-2′-deoxyadenosine (εA_d, **5**),[23, 24] which was treated with aqueous sodium hydroxide to form the bis-imidazole 2′-deoxynucleoside **6**. This was treated with sodium nitrite in 80% aqueous acetic acid to give 1,N^6-etheno-2-aza-2′-deoxyadenosine (**7**). Treatment of compound **7** with *N*-bromosuccinimide in acetate buffer (pH 4.0–4.5) resulted in formation of 2-aza-2′-deoxyadenosine (**2**). The overall yield starting from 2′-deoxyadenosine was 13% over four steps; this is superior to other synthetic routes.[20a,b;21] The purity of all compounds was ascertained by ^1H and ^{13}C NMR spectra and by elemental analyses (see Table 7 in the Experimental Section). The ^{13}C NMR resonances were assigned from gated-decoupled (Table 8 in the Experimental Section) and heteronuclear-correlation spectra. The stability of 2-aza-2′-deoxyadenosine (**2**) at its *N*-glycosylic bond in aqueous hydrochloric acid (0.1M, RT), measured by UV spectrophotometry at 290 nm, is similar to that of dA (z^2A_d: $\tau = 63$ min; dA: $\tau \approx 45$ min).[25]

It had been reported[21] that **2** is a substrate for adenosine deaminase, though it possesses a lower v_{max} than adenosine. This property was used for the enzyme-catalyzed synthesis of 2-aza-2′-deoxyinosine (**3**) on a preparative scale. Compound **3** was characterized by means of UV, ^1H, and ^{13}C NMR spectra (Table 7 in the Experimental Section).

Fluorescence and conformational properties of monomers: Strong fluorescence of 1,N^6-ethenoadenine derivatives, including compound **5**, has been widely recognized and investigated.[23] Moreover, it has been reported that the electron-donating or electron-withdrawing properties of 2-substituents of 1,N^6-ethenoadenosine exert a strong influence on its fluorescence.[26] It was therefore of interest to compare the fluorescence of compound **5** with **7**. The additional nitrogen atom in the 2-position of **7** reduces the fluorescence of 1,N^6-ethenoadenosine (**5**) to about 7% (data not shown). The residual fluorescence emission spectrum of 1,N^6-etheno-2-aza-2′-deoxy-adenosine (**7**; $\lambda_{EX} = 240$ nm, H_2O) exhibits a bathochromic shift of about 80 nm relative to compound **5**; 2-aza-2′-deoxyadenosine (**2**) shows no fluorescence.

The nucleosides **2** and **7** were analyzed by ^1H NOE difference spectroscopy to elucidate their base conformation around the *N*-glycosylic bond (Table 1). Irradiation of H-C(8) of nucleosides **2** and **7** revealed NOEs of 5.8% and 5.9% at H-C(1′). This, the NOE data on H_β-C(2′) and H-C(3′), and a graphical method[27] for calculating the *syn* and *anti* conformer populations allowed us estimate that the rotamer populations for both compounds are equal (50% *syn*, 50% *anti*). This result is identical to that obtained for 2′-deoxyadenosine, implying that the additional nitrogen in the 2-position of compounds **2** and **7** has no influence on the conformation at the *N*-glycosylic bond. There are differences, however, with respect to sugar puckering. Aided by vicinal ^3J(H,H) coupling constants of the sugar protons, the conformations of the glyconic moiety of compounds **2** and **3** were determined (Table 1). Information on the preferred sugar puckering [$^3T_{2'}(N) \Leftrightarrow {}_3T^{2'}(S)$] and the rotation about the C(4′)–C(5′) bond ($\gamma^{g+} \Leftrightarrow \gamma^{t} \Leftrightarrow \gamma^{g-}$) was obtained with the program PSEUROT 6.2[28a,b] and the method of Westhof et al.[29] For compounds **2** and **3**, *N*-conformer populations of 36% were calculated, which is 8% higher than that of 2′-deoxyadenosine.[30] Surprisingly, the distribution of rotamers about the C(4′)–C(5′) bond is strongly affected by the additional nitrogen atom(Table 1) in the case of **3**, but only slightly for

Abstract in German: *2-Aza-2′-desoxyadenosine (**2**, z^2A_d) wird über sein 1,N^6-Etheno-Derivat **7** dargestellt und enzymatisch zum 2-Aza-2′-desoxyinosin (**3**) desaminiert. Verbindung **2** wird in das Phosphoramidit **10a** für die Oligonucleotid Festphasensynthese umgewandelt. Dieses wird für die Synthese einer Reihe modifizierter Oligomere verwendet. Die 2-Azapurin Base zeigt eine starke Basenpaarung mit Guanin, eine viel schwächere jedoch mit Adenin, Thymin und Cytosin. Eine Serie von Oligonucleotiden mit jeweils einem überhängenden Nucleotid an entweder einem oder an beiden Termini—darunter 2-Aza-2′-desoxyadenosin (**2**) oder 7-Desaza-2′-desoxyadenosin (**4**)—wird synthetisiert und ihr stabilisierender Effekt auf die thermische Stabilität der Duplexe wird mit der Hydrophobie der überhängenden Nucleotide korreliert.*

0947-6539/00/0602-0370 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 2

Table 1a. ^{1}H NOE data[a] and conformer populations of compounds **2** and **7**.[b]

	Proton irradiated	Observed NOE [%]	Conformer population	
			anti [%]	*syn* [%]
7	H-C(8)	H-C(1′) [5.8]; H_β-C(2′) [2.8]; H-C(3′) [1.6]; 5′-OH [1.2]	50	50
2	H-C(8)	H-C(1′) [5.9]; H_β-C(2′) [3.4]; H-C(3′) [1.8]; 5′-OH [1.2]	50	50

[a] Measured in [D_6]DMSO. [b] Measured at 303 °C.

Table 1b. ^3J(H,H) coupling constants[a] of compounds **1**–**3**.

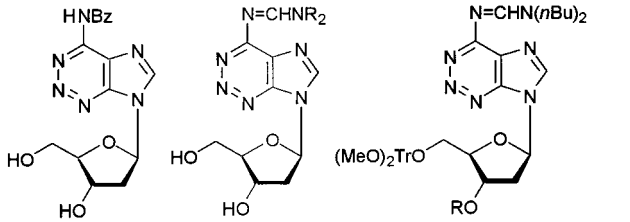
	^3J(H,H) [Hz][b]							Sugar conformation					Pseudorotational parameters			
	1′,2′	1′,2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,5″	% *N*	% *S*	% γ^{g+}	% γ^{t}	% γ^{g-}	P_N	P_S	$\Psi_{m(N)}$	$\Psi_{m(S)}$
2	6.65	6.65	6.50	4.00	3.65	3.35	4.75	36	64	55	30	15	19	160.5	36	30.2
3	6.70	6.35	6.15	3.80	3.55	4.20	6.50	36	64	26	50	24	19	162.8	36	32.7
1	7.20	6.50	6.50	3.30	3.20	3.45	4.30	28	72	59	25	16	19	160.7	36	30.3

[a] Measured in D_2O. [b] Primed and double primed locants are used to distinguish between the protons at C(2′) or C(5′); root mean square ≤ 0.4 Hz; $|\Delta J_{max}| \leq 0.4$ Hz.

2. In contrast to **2**, compound **3** exhibits an extraordinary high $\gamma^{(t)}$ population (= − sc), in which the 5′-OH group is no longer located above the ribose ring (+ g), but is twisted away from it. As this is in contrast to the results found for 2′-deoxyinosine and 7-deaza-2′-deoxyinosine,[31] it is postulated that compound **3** exists as a H–N(3) tautomer in water, but not as a H–N(1) tautomer as found for 2′-deoxyinosine.

Synthesis of a 2-aza-2′-deoxyadenosine phosphoramidite: For the preparation of oligodeoxynucleotide building blocks several amino protecting groups for nucleoside **2** were tested. Benzoylation of **2** under transient silylation[32] conditions gave compound **8**. The half-life of its deprotection reaction in concentrated aqueous ammonia at 40 °C (monitiored by UV-spectrophotometry at 275 nm) is 480 min, which is not appropriate for other nucleoside building blocks intended for solid-phase DNA synthesis.

Next, a dimethylaminomethylidene residue was introduced into **2**(→**9a**) by reaction with dimethylformamide dimethylacetal.[33,34] This protection group proved to be very labile: at room temperature it exhibits a half-life (25 % aq. NH_3, 320 nm) of only three minutes and was, therefore, abandoned. The application of higher dialkylformamide dimethylacetals solved the problem. Reaction of *N*,*N*-dimethylformamidine dimethylacetal with either diisobutylamine or di-*n*-butylamine (100 °C, 3 d) and fractionated distillation under reduced pressure according to reference [35] afforded the corresponding higher dialkylformamidine dimethyl acetals, which were treated with nucleoside **2** to give compounds **9b** and **9c**, respectively.



8 **9a**: R = CH_3 **9b**: R = *i*Bu **9c**: R = *n*Bu **10a**: R = H **10b**: R = -P[(*i*Pr)$_2$N][O(CH$_2$)$_2$CN]

Determination of their τ values in concentrated aqueous ammonia (40 °C, 325 nm) (Table 2) revealed that the di-*n*-butylaminomethylidene derivative **9c** possesses an appropriate half-life (τ=35 min), while other protecting groups proved either too stable or too labile. For these reasons compound **9c** was used for further 5′-O-4,4′-dimethoxytritylation[36] under standard reaction conditions (**10a**). Subsequent phosphitylation of compound **10a** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine[37] gave the phosphoramidite **10b**. All new compounds were characterized by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy as well as by elemental analyses (see Table 7 in the Experimental Section).

Table 2. Half-life values (τ) of protected 2′-deoxy-2-azaadenosine derivatives in aqueous NH_3 (25 %) at 40 °C.

	Half-life τ [min]
8	480
9a	3[a]
9b	100
9c	35

[a] Measured at 20 °C.

Oligonucleotides

Synthesis: Oligonucleotides were synthesized on solid phase by means of an automated synthesizer with the phosphoramidite **10b** and the phosphoramidites of regular DNA constituents. The oligonucleotides were purified by either reversed-phase or anion-exchange HPLC. Their nucleoside composition was determined by hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase[10] and partially by MALDI-TOF spectrometry (see Experimental Section and Table 6 later).

Non-selfcomplementary oligonucleotides with random base composition: The two oligonucleotides 5′-d(TAGGTCAATACT) (**11**) and 5′-d(AGTATTGACCTA) (**12**) were constructed to form a stable hybrid with a T_m value of 47 °C. This duplex is used in our laboratory as a standard to study the influence of modified bases on duplex structure and stability.[10] As can be seen in Table 3, replacement of one central

Table 3. T_m values and thermodynamic data of oligonucleotide duplexes.[a,b]

	T_m [°C]	ΔH^o [kcal mol^{-1}][d]	ΔS^o [cal K^{-1} mol^{-1}][d]	ΔG^o_{298} [kcal mol^{-1}][d]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	47	− 82	− 230	− 10.4
5′-d(T-A-G-G-T-C-A*-A-T-A-C-T) (**13**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	42	− 85	− 245	− 9.2
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A*-G-T-T-A*-T-G-A) (**14**)	37	− 81	− 237	− 7.6
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	37	− 76	− 219	− 7.7
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A*-G-T-T-A*-T-G-A) (**14**)	28	− 72	− 214	− 5.6
5′-d(T-A-G-G-T-C-zA-zA-T-A-C-T) (**16**) 3′-d(A-T-C-C-zA-G-T-T-zA-T-G-A) (**17**)	45	− 73	− 203	− 9.6
5′-d(T-iC-A-T-A-A-iC-T-iG-iG-A-T) (**18**) 5′-d(A-G-T-A*-T-T-G-A*-C-C-T-A) (**14**)	35[c]	− 81	− 238	− 7.4
5′-d(T-iC-A-T-A-A-iC-T-iG-iG-A-T) (**18**) 5′-d(A-G-T-A-T-T-G-A-C-C-T-A) (**12**)	45[c]	− 85	− 242	− 10.3

[a] A*$_d$ = z^{2}A$_d$ = 2-aza-2′-deoxyadenosine (**2**); zA$_d$ = 8-aza-2′-deoxyadenosine; single strand concentration, 5 μM. [b] 10 mM Na-cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.0. [c] 60 mM Na-cacodylate, 100 mM $MgCl_2$, 1 M NaCl, pH 7.0. [d] 1 cal = 4.184 J.

urally occurring DNA is antiparallel. The orientation can be switched to parallel when the duplex contains isoG$_d$ – dC and or isoMe^{5}C$_d$ – dG base pairs.[38] As the pairing of dA with dT is ambiguous, any natural DNA can be hybridized in the parallel mode when the second strand contains the bases isoguanine, isocytosine, adenine, and thymine. An example, namely the duplex **18·12**, is given in Table 3. When two dA – dT base pairs are replaced by z^{2}A$_d$ – dT in this duplex (**18·14**), a reduction of the T_m value by 10 °C is observed; this is identical with the results for the corresponding antiparallel oligonucleotide duplexes.

dA – dT by a z^{2}A$_d$ – dT base pair reduces the T_m of the duplex (**13·12**) by 5 °C; exchange of two such base pairs (**11·14**) reveals a decrease in T_m of 10 °C. In this case the reduction of duplex stability evidently does not depend on the position of base-pair replacement: the duplex **15·12**, which contains two consecutive z^{2}A$_d$ – dT pairs, has the same T_m as **11·14** in which the modified base pairs are separated by three regular ones. Duplex stability is linearly decreased further when the number of z^{2}A$_d$ – dT base pairs is increased; the oligonucleotide **14·15**, which contains four modified pairs, exhibits a T_m of only 28 °C. This result is in striking contrast to findings on oligonucleotides in which dA residues are replaced by 8-aza-2′-deoxyadenosine. Replacement of up to four dA by z^{8}A$_d$ residues has no effect on duplex stability (**16·17**, Table 3).[10] This is in line with findings by us and by others that modifications within the minor groove of B-DNA, in contrast to major groove modifications, have an enormous influence on duplex structure and stability, possibly by interruption of a spine of water molecules located along the minor groove. An alternative explanation for this finding would be an electrostatic repulsion between N(2) of z^{2}A$_d$ and the 2-oxo group of dT.

The results described above stem from duplexes with antiparallel backbone orientation. However, the same result was found for oligonucleotide duplexes with a parallel strand polarity. The backbone orientation of nat-

The T_m data listed in Table 4 display another interesting feature of the base-pairing properties of z^{2}A$_d$ (**2**). Stimulated by the finding that replacement of a destabilizing central z^{2}A$_d$ – dT base pair (**13·12**) by z^{2}A$_d$ – dG (**13·19**) increases the T_m value of the oligomer back to the value of the unmodified duplex **11·12** (T_m 46 °C, Table 4), we investigated the duplex stabilities of oligomers that contain two consecutive mismatches.

For this purpose the oligodeoxynucleotides **15·12**, **15·20**, **15·21**, and **15·22** were synthesized in which two central z^{2}A$_d$ residues are placed opposite two dT, dA, dC, or dG residues. As can be seen in Table 4, in all cases, except for z^{2}A$_d$ – dG-containing duplexes, the T_m value decreased significantly, and the effect was most pronounced for the oligomer with two z^{2}A$_d$ – dC pairs (Figure 1). The oligonucleotide **15·22**, how-

Table 4. T_m values and thermodynamic data of oligonucleotide duplexes.[a,b]

	T_m [°C]	ΔH^o [kcal mol^{-1}][c]	ΔS^o [cal K^{-1} mol^{-1}][c]	ΔG^o_{298} [kcal mol^{-1}][c]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	47	− 82	− 230	− 10.4
5′-d(T-A-G-G-T-C-A*-A-T-A-C-T) (**13**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	42	− 85	− 245	− 9.2
5′-d(T-A-G-G-T-C-A*-A-T-A-C-T) (**13**) 3′-d(A-T-C-C-A-G-G-T-A-T-G-A) (**19**)	46	− 83	− 236	− 9.9
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	37	− 76	− 219	− 7.7
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A-G-A-A-A-T-G-A) (**20**)	25	− 49	− 141	− 5.7
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A-G-C-C-A-T-G-A) (**21**)	20	− 41	− 113	− 5.5
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**) 3′-d(A-T-C-C-A-G-G-G-A-T-G-A) (**22**)	46	− 74	− 206	− 10.1
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A-G-G-G-A-T-G-A) (**22**)	36	− 47	− 127	− 7.4

[a] A*$_d$ = z^{2}A$_d$ = 2-aza-2′-deoxyadenosine (**2**); single strand concentration, 5 μM. [b] 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.0. [c] 1 cal = 4.184 J.

0947-6539/00/0602-0372 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 2

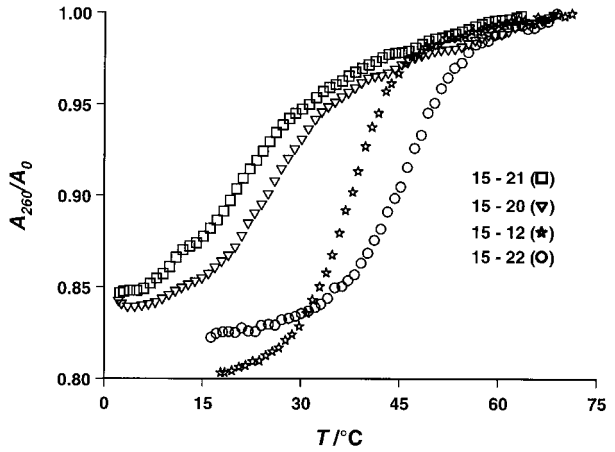


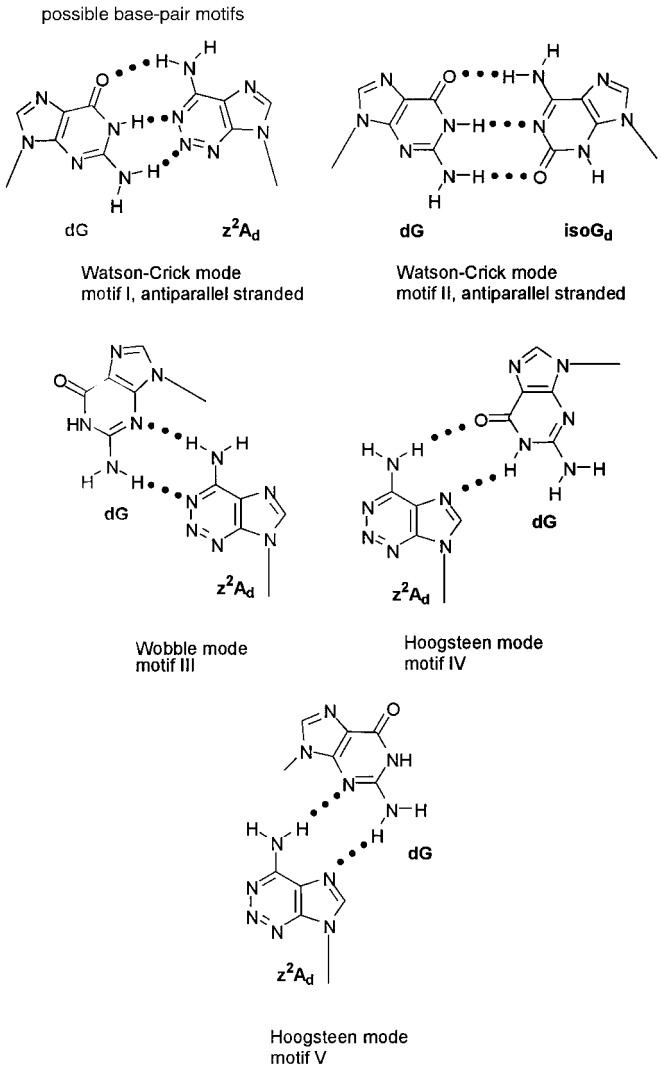
Figure 1. Melting profiles of the oligonucleotide duplexes **15·21**, **15·20**, **15·22**, and **15·12** recorded at 260 nm in 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl (pH 7.0) at an oligomer concentration of 5 μM.

ever, exhibits nearly the same T_m value as the unmodified duplex (**11·12**). This prompted us to propose a tridentate z^2A_d–dG base pair, as shown in Scheme 2 (motif I), although the alternative, bidentate pairing modes (motifs III–V Scheme 2) cannot be ruled out.[9b] The finding that the oligonucleotide duplex **15·22**, which contains two z^2A_d–dG base pairs, is as stable as the unmodified duplex (**11·12**) with two dA–dT pairs, points to a lower hydrogen bond strength between N(2) and/or the NH_2 group of z^2A_d and dG. As a control, Table 4 lists the T_m value of an oligonucleotide duplex that contains two dA–dG mismatches (**11·22**: $T_m = 36\,^\circ C$). Comparison of models of base-pair motif I (purine–purine, Scheme 2) and of dA–dT (purine–pyrimidine) reveals that, due to a short hydrogen bond between N(2) of z^2A_d and the amino group of dG, the distances between the corresponding C(1′) atoms are similar (≈11 Å).[39] The distance between the two C(1′) atoms of a dA–dG (purine–purine) mismatch (Watson–Crick pairing mode[40]) is higher (≈13 Å). As a consequence, the minor groove around a z^2A_d–dG base pair might be more shallow than that of a regular B-DNA secondary structure.

The tridentate base pair of 2-aza-2′-deoxyadenosine implies that the pairing properties of this nucleoside might be similar to those of 2′-deoxyisoguanosine ($isoG_d$), the more so as both show a similar hydrogen-bonding donor–acceptor pattern, provided one assumes a keto/H–N(3) tautomeric form of 2′-deoxyisoguanosine (Scheme 2, motif II).[41] Indeed, the latter forms a purine–purine base pair with 2′-deoxyguanosine in oligodeoxynucleotides with antiparallel strand polarity, but significantly weaker base pairs with dC, dT, and, in particular, dA.[42]

The results described above raise the possibility of lowering the high T_m value of oligonucleotides rich in dG–dC through specific replacement of dG–dC by dG–z^2A_d, which exhibits the base-pairing energy of a dA–dT base pair.

Oligonucleotides with dangling nucleotide residues: It has been reported recently that one or more dangling nucleotide units can lead to significant duplex stabilization. Moreover, hydrophobic non-nucleoside overhangs, such as naphtalene, phenanthrene, or pyrene residues, raise the T_m value of an



Scheme 2. Possible motifs for the dG-z^2A_d base pair.

oligonucleotide. It has been postulated that the surface area of overlap between the dangling aromatic ring and the terminal base pair is an important factor in duplex stabilization by π–π stacking.[43, 44]

Table 5 presents T_m data of a series of self-complementary as well as nonself-complementary oligonucleotides with dangling 2-aza- or 7-deaza-2′-deoxyadenosine residues. Data for an alternating dC–dG duplex [5′-d(C-G)$_3$, **24·24**] with two dangling dA and dT residues are given for comparison. As can be seen, two dangling dA (**1**) nucleotides enhance the duplex stability of 5′-d(C-G)$_3$ by 9 °C. The stabilizing effect of 2-aza-2′-deoxyadenosine (**2**) is identical to that of dT and hence ΔT_m is slightly lower (+7 °C) than that of **1**. The more hydrophobic 7-deaza-2′-deoxy-adenosine (c^7A_d, **4**) raises the T_m value of **24·24** slightly more than dA does: two dangling c^7A_d nucleotides on both 5′-termini give a melting temperature of 57 °C ($\Delta T_m = +11\,^\circ C$). This stabilization of 5′-d(C–G)$_3$ by dangling c^7A_d prompted us to measure its effect on the nonself-complementary duplex **11·12**, with the result that also in this case two dangling 7-deaza-2′-deoxyadenosines raise the T_m value by 6 °C.

Table 5. T_m values of oligodeoxynucleotides with different dangling nucleotides.[a,b]

	T_m [°C]	ΔH^o [kcal mol^{-1}][c]	ΔS^o [cal K^{-1} mol^{-1}][c]	ΔG^o_{298} [kcal mol^{-1}][c]
5′-d(C-G-C-G-C-G) (**24**) 3′-d(G-C-G-C-G-C) (**24**)	46	– 53	– 143	– 8.2
5′-d(**A***-C-G-C-G-C-G) (**23**) 3′-d(G-C-G-C-G-C-**A***) (**23**)	53	– 59	– 158	– 9.7
5′-d(**A**-C-G-C-G-C-G) (**25**) 3′-d(G-C-G-C-G-C-**A**) (**25**)	55	– 57	– 151	– 10.1
5′-d(**T**-C-G-C-G-C-G) (**26**) 3′-d(G-C-G-C-G-C-**T**) (**26**)	53	– 57	– 152	– 9.6
5′-d(**c^{7}A**-C-G-C-G-C-G) (**27**) 3′-d(G-C-G-C-G-C-**c^{7}A**) (**27**)	57	– 58	– 154	– 10.0
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	50	– 75	– 205	– 11.1
5′-d(T-A-G-G-T-C-A-A-T-A-C-T) (**11**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A-**c^{7}A**) (**28**)	53	– 111	– 314	– 13.4
5′-d(**c^{7}A**-T-A-G-G-T-C-A-A-T-A-C-T) (**29**) 3′-d(A-T-C-C-A-G-T-T-A-T-G-A) (**12**)	54	– 97	– 272	– 13.0
5′-d(**c^{7}A**-A-G-T-A-T-T-G-A-C-C-T-A) (**28**) 3′-d(T-C-A-T-A-A-C-T-G-G-A-T-**c^{7}A**) (**29**)	56	– 90	– 249	– 12.6

[a] $A^*_d = z^2A_d$ = 2-aza-2′-deoxyadenosine (**2**); c^7A_d: 7-deaza-2′-deoxyadenosine (**4**); single strand concentration, 5 μM. [b] 0.01 M Na_2HPO_4, 1 M NaCl, pH 7.0. [c] 1 cal = 4.184 J.

Figure 2 is a plot of $T\Delta S^o$ against ΔH^o of duplex formation for all oligodeoxyribonucleotides listed in Tables 3–5. The linear correlation indicates almost perfect enthalpy–entropy compensation.[45, 46] This means that it takes more energy to melt the highly stable, rigidly constrained base pairs than it does to melt less stable, weakly constrained base pairs. Rigid base pairs that have fewer degrees of freedom in the double helix will gain more degrees of freedom upon melting, whereas the opposite is true for less stable base pairs. For this reason a low enthalpy of duplex formation is usually accompanied by a proportionally favorable entropy effect.

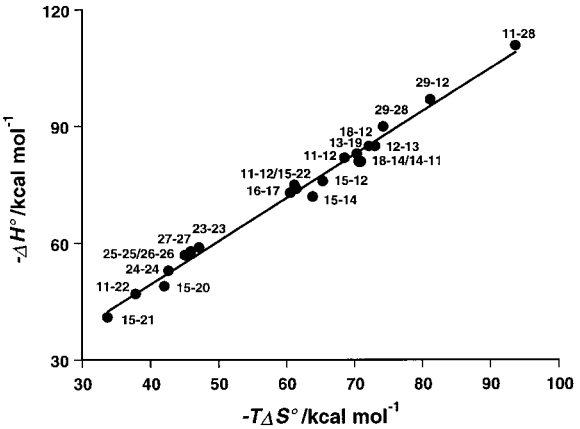


Figure 2. Plot of $-\Delta H^o$ against $-T\Delta S^o$ for the oligonucleotide duplexes listed in Tables 5, 7, and 8 (enthalpy–entropy compensation).

Our results confirm that the dangling nucleotides agglutinate the duplex by additional stacking interactions with the terminal base pairs. This occurs with bases in the same strand and, because of helical twist, with terminal bases in the opposite strand. The overhanging nucleotides do not raise the T_m value of the dodecamer **11·12** as much as that of the hexamer **24·24** because of the small contribution of additional stacking energy in **11·12** relative to the whole strand interaction energy. The model of a "drop of hydrophobic stacking glue"[43] is corroborated by the finding that only one overhanging nucleotide on either the 5′- or the 3′-terminus enhances the duplex stability only half as much as two dangling units on both sides.

Considering these results it seems likely that not only the overlap integral between the overhanging aromatic system and that of the terminal base pair affects the stabilization, but also the hydrophobicity of the dangling nucleobase. A comparison of the retention times of nucleosides **1**–**3** and dT on an RP-18 HPLC column (Figure 3) shows that the hydrophobicities of these compounds are in the following order: $dT \approx z^2A_d$ (**2**) $< dA < c^7A_d$ (Table 6). Duplex stabilization by the dangling nucleotides increases in the same order(Table 5).

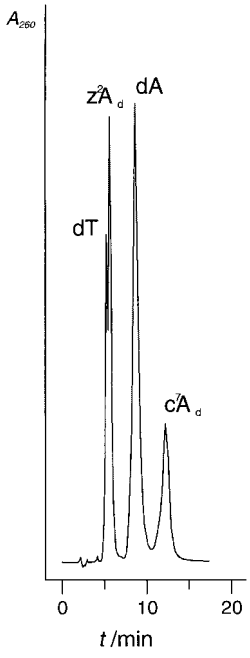


Figure 3. RP-18 HPLC profile of dT, dA, z^2A_d (**2**) and c^7A_d (**4**); for details see Experimental Section.

Experimental Section

Monomers: Flash chromatography (FC): at 0.5 bar with silica gel 60 (Merck, Darmstadt, Germany). Solvent systems for FC and TLC: CH_2Cl_2/MeOH 85:15 (A), EtOAc/MeOH 3:1 (B), CH_2Cl_2/MeOH 80:20 (C), CH_2Cl_2/HOAc/MeOH 17:1:3 (D), CH_2Cl_2/MeOH 9:1 (E), CH_2Cl_2/acetone 85:15 (F). Samples were collected with an UltroRac II fractions collector (LKB Instruments, Sweden). Melting points: Büchi SMP-20 apparatus (Büchi, Switzerland). UV spectra: U 3200 spectrophotometer (Hitachi, Japan). NMR spectra:

0947-6539/00/0602-0374 $ 17.50+.50/0 Chem. Eur. J. 2000, 6, No. 2

Table 6. Retention times,[a] yields, and relative molecular masses determined by MALDI-TOF mass spectra of some oligonucleotides.

	Retention time [min]	Yield [A_{260} units]	M^+ (calcd) [Da]	M^+ (found) [Da]
5′-d(T-A-G-G-T-C-A*-A*-T-A-C-T) (**15**)	12.5	27	3646	3647
5′-d(A-G-T-A*-T-T-G-A*-C-C-T-A) (**14**)	12.8	31	3646	3644
5′-d(T-A-G-G-T-C-A*-A-T-A-C-T) (**13**)	12.5	35	3645	3643
5′-d(A*-C-G-C-G-C-G) (**23**)	13.2	8	2106	2109
5′-d(c^7A-C-G-C-G-C-G) (**27**)	13.4	3	2106	2107
5′-d(A-C-G-C-G-C-G) (**25**)	13.5	3	2107	2109
5′-d(T-C-G-C-G-C-G) (**26**)	13.1	5	2097	2095
5′-d(A-G-T-A-A-A-G-A-C-C-T-A) (**20**)	13.2	25	3663	3663
5′-d(A-G-T-A-C-C-G-A-C-C-T-A) (**21**)	13.7	27	3614	3614
5′-d(A-G-T-A-G-G-G-A-C-C-T-A) (**22**)	13.8	21	3695	3698
5′-d(c^7A-T-A-G-G-T-C-A-A-T-A-C-T) (**29**)	14.1	27	3957	3955
5′-d(c^7A-A-G-T-A-T-T-G-A-C-C-T-A) (**28**)	14.3	15	3957	3956

[a] Determined on a NucleoPac ion-exchange HPLC column as described above.

AC250 and AMX500 spectrometers (Bruker, Germany); δ values are relative to internal Me_4Si or external H_3PO_4 (Tables 7 and 8). Fluorescence spectra were recorded in H_2O on a F-4500 fluorescence spectrophotometer (Hitachi, Japan). Microanalyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany).

Oligonucleotides: Oligonucleotides were synthesized with a ABI 392 DNA synthesizer (Applied Biosystems, Germany) according to standard protocol in "trityl-off" mode, except for unmodified oligodeoxynucleotides which were synthesized in "trityl-on" mode. The coupling yields of modified phosphoramidites were on average 95% (trityl conductivity monitoring). The detritylated-modified oligomers were purified by ion-exchange chromatography on a Dionex Nucleopac PA-100 HPLC column (4 × 250 mm, P/N 043010, Dionex, Idstein, Germany) by means of the following gradient: 5 min 5% 0.01M NaOH/1.5M aqueous LiCl (X) in 0.01M NaOH (Y); 25 min 5–30% Y in X; 10 min 30–5% Y in X; 5 min 5% Y in X. Ion-exchange HPLC apparatus: L-4250 UV/VIS detector, L-6250 intelligent pump, and D-2500 integrator (Merck–Hitachi, Germany). The tritylated unmodified oligonucleotides were purified by RP-18 HPLC with the following apparatus and procedure: 250 × 4 mm RP-18 column (Merck, Germany); Merck–Hitachi HPLC apparatus consisting of a 655 A-12 liquid chromatograph with a 655A variable-wavelength UV monitor and a D-2000 Chromato-Integrator (Merck–Hitachi, Darmstadt, Germany); gradients of 0.1M $(Et_3NH)OAc$ (pH 7.0)/MeCN 95:5 (U) and MeCN (V); gradient I: 0–50 min 0–50% V in U, flow rate 1 mL min^{-1}; gradient II: 0–20 min 0–20% V in U; 20–40 min 20–40% V in U, flow rate 1 mL min^{-1}. Detritylation was performed by treating the purified oligomers with a 2.5% dichloroacetic acid solution in CH_2Cl_2 (1 mL) for 5 min. After neutralization with Et_3N, evaporation to dryness, followed by coevaporation with MeOH, oligomers were again purified by RP-18 HPLC on the above-mentioned device. Gradient: 0–30 min 0–20% V in U, 30–35 min 20% V in U, 35–40 min 20–0% V in U, 40–45 min 0% V in U. Subsequent desalting for all oligonucleotides was performed on an RP-18 HPLC column (4 × 100 mm) and the apparatus as described above. Solvent for adsorption: H_2O, solvent for desorption: MeOH/H_2O 3:2. General flow rate: 1 mL min^{-1}. MALDI-TOF Mass spectra of the oligonucleotides were recorded by Mrs. Julia Gross (Institute of Medical Physics and Biophysics, Westfälische Wilhelms-Universität, Münster) on a home-built apparatus with UV laser irradiation at 337 nm for 3 ns.

Enzymatic hydrolysis of the oligomers was performed as in reference [10], but with a flow rate of 0.6 mL min^{-1}. Quantification of the constituents was based on peak areas, which were divided by the extinction coefficients of the nucleoside (ε_{260} values: dA 15400, dC 7300, dG 11400, dT 8800, z^2A_d 8200). The snake venom phosphodiesterase (EC 3.1.15.1, Crotallus durissus) and alkaline phosphatase (EC 3.1.3.1, E. coli) used for the enzymatic hydrolysis of oligonucleotides were generous gifts of the Roche Diagnostics.

Determination of melting curves and thermodynamics: Absorbance as a function of temperature profiles were measured on Cary 1 or 1E spectrophotometers (Varian, Australia) with a Cary thermoelectrical controller. The T_m values were measured in the reference cell with a Pt-100 resistor. Thermodynamic data (ΔH^o, ΔS^o, ΔG^o_{298}) were calculated with the program MeltWin 3.0.[47]

3-(2-Deoxy-β-D-erythropentofuranosyl)-3*H*-imidazo[2,1-i]purine (1,N^6-etheno-2′-deoxyadenosine, 5): 2′-Deoxyadenosine monohydrate (**1**; 5.0 g, 20 mmol) was dissolved in aqueous sodium acetate buffer (1M, pH 4.5–5.0, 110 mL) by warming to 40–50 °C. Chloroacetaldehyde (50% aqueous solution, 7.7 mol L^{-1}; 25 mL) was added to the solution, and the reaction mixture was stirred for 70 h at room temperature. The yellow solution was evaporated to dryness, and the residue was dissolved in MeOH and filtered to remove inorganic salt. After washing with MeOH, the combined filtrate and washings were concentrated in vacuo at 40–50 °C. The residue was purified by FC (silica gel 60H, column: 20 × 6 cm). Elution with CH_2Cl_2/MeOH (85:15) gave a main fraction from which, after evaporation of the solvent and subsequent crystallization from MeOH/EtOAc, compound **5** (3.86 g, 70%) was isolated in the form of colorless crystals. M.p. 138–141 °C; TLC (silica gel, EtOAc/MeOH, 3:1): $R_f = 0.4$; UV (MeOH): λ_{max} (ε) = 275 (7300), 265 (7600), 258 (6600), 229 nm (35700); ^{1}H NMR ([D$_6$]DMSO) $\delta = 2.39$ (m, 1H, H_α-C(2′)), 2.70 (m, 1H, H_β-C(2′)), 3.57 (m, 1H, H_a-C(5′)), 3.67 (m, 1H, H_b-C(5′)), 3.88 (m, 1H, H-C(4′), 4.43 (m, 1H, H-C(3′)), 4.99 (t, ^3J(H,H) = 5.2 Hz, 1H, 5′-OH), 5.38 (d, ^3J(H,H) = 3.8 Hz, 1H, 3′-OH), 6.47 (pseudo t, ^3J(H,H) = 6.2 Hz, 1H, H-C(1′)), 7.55 (s, 1H, H-C(11)), 8.07 (s, 1H, H-C(10), 8.53 (s, 1H, H-C(2)), 9.29 (s, 1H, H-C(8)). For further analytical data see ref. [24].

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-amino-4-(imidazol-2″-yl)imidazole (6): Compound **5** (3.85 g, 14 mmol) was treated with aqueous NaOH (1N, 60 mL) at room temperature overnight. The reaction mixture was adjusted to pH 7 by addition of aqueous HCl (2N) and concentrated to a syrup. This was dissolved in absolute MeOH, and the precipitated NaCl was filtered of and washed with MeOH. Filtrate and washings were combined and evaporated. The residue was purified by FC (silica gel 60H, column: 20 × 6 cm). Elution with CH_2Cl_2/MeOH (C) afforded a main zone from which compound **6** (2.70 g, 73%) was obtained as a colorless foam, which was used for subsequent reactions without further purification. An analytical sample was crystallized from MeOH/EtOAc to give colorless spherical crystals. M.p. 91–93 °C (decomp); TLC (silica gel, CH_2Cl_2/HOAc/MeOH, 17:1:3): $R_f = 0.22$; UV (MeOH): λ_{max} (ε) = 271 nm (12800); ^{1}H NMR ([D$_6$]DMSO) $\delta = 2.21$ (m, 1H, H_α-C(2′)), 2.47 (m, 1H, H_β-C(2′)), 3.57 (m, 2H, H_2-C(5′)), 3.84 (m, 1H, H-C(4′)), 4.36 (m, 1H, H-C(3′)), 6.00 (pseudo t, ^3J(H,H) = 6.5 Hz, 1H, H-C(1′)), 6.60 (br s, NH_2), 7.13 (s, 2H, H-C(4) + H-C(5)), 7.55 (s, 1H, H-C(2)), 8.16 (s, NH).

3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-diimidazo[1,2-c:4′,5′-e][1,2,3]-triazine (1,N^6-etheno-2-aza-2′-deoxyadenosine, 7): A solution of compound **6** (4.50 g, 17 mmol) in 80% aqueous HOAc was treated with sodium nitrite (1.17 g, 17 mmol) in an ice-water bath for 1 h. The reaction mixture was evaporated to a syrup. This was dissolved in H_2O and evaporated repeatedly to remove HOAc. The residue was purified by FC (silica gel 60H, column, 20 × 6 cm). Elution with CH_2Cl_2/MeOH (85:15) afforded compound **7** (2.50 g, 53%) upon evaporation. M.p. 151–152 °C (decomp); TLC (silica gel, CH_2Cl_2–MeOH, 4:1): $R_f = 0.5$; UV (MeOH): λ_{max} (ε) = 282 (3100), 268 (3200), 238 nm (37900); ^{1}H NMR ([D$_6$]DMSO) $\delta = 2.54$ (m, 1H, H_α-C(2′)), 2.85 (m, 1H, H_β-C(2′)), 3.97 (m, 2H, H_2-C(5′)), 4.00 (m, 1H,

Table 7. ^{13}C NMR data of nucleosides.[a]

	C(2)[b]	C(4)[b]	C(5)[b]	C(6)[b]	C(8)[b]	C(10)[b]	C(11)[b]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O/=CH	CH_3	OCH_3	CH_2/CH
dA	152.9	149.4	120.0	156.7	140.3	–	–	83.8	39.6	70.9	87.8	61.7	–	–	–	–
5	139.7	140.6	123.1	138.1	137.1	132.8	112.2	83.9	[c]	70.7	88.0	61.7	–	–	–	–
6	–	142.8	109.3	137.7	163.0	137.1	119.1	83.7	[c]	70.5	87.4	61.3	–	–	–	–
7	–	137.7	126.4	133.1	145.0	133.5	115.3	85.0	[c]	70.2	88.3	61.2	–	–	–	–
2	–	146.3	116.6	152.5	143.0	–	–	84.5	[c]	70.6	88.3	61.5	–	–	–	–
3[d]	–	146.4	124.8	160.6	142.9	–	–	85.8	39.6	71.2	87.9	61.6	–	–	–	–
8	–	149.5	123.7	147.3	146.7	–	–	84.8	39.6	70.3	88.3	61.3	165.8	–	–	–
9a	–	148.3	123.0	155.0	144.7	–	–	84.6	39.6	70.5	88.1	61.5	158.4	34.6	–	–
9b	–	148.3	123.0	155.1	144.7	–	–	84.6	39.6	70.5	88.1	61.5	158.4	13.6	–	19.6, 19.1
														13.5		30.3, 28.6
																51.1, 44.6
9c	–	148.3	123.0	155.2	144.8	–	–	84.6	39.6	70.5	88.1	61.4	159.3	19.9	–	26.6, 25.7
														19.4		59.0, 52.1
10a	–	148.3	123.1	155.1	144.7	–	–	84.3	[c]	70.2	86.0	63.8	158.2	13.6	54.9	19.6, 19.1
														13.5		30.3, 28.6
																51.1, 44.6

[a] Measured in [D_6]DMSO at 303 K. [b] Purine numbering. [c] Superimposed by the signals of [D_6]DMSO. [d] D_2O.

Table 8. *J*(H,C) coupling constants [Hz] of nucleosides.[a]

	2	**6**	**7**
J(C(2),H-C(2))[b]	213.9		
J(C(2),H-C(10))[b]	3.9		
J(C(4),H-C(8))[b]	5.4	7.5	5.8
J(C(4),H-C(1′))[b]			1.9
J(C(5),H-C(8))[b]	11.6		11.9
J(C(6),H-C(11))[b]	13.0		11.3
J(C(6),H-C(10))[b]	5.4		5.9
J(C(6),H-C(2))[b]	2.3		
J(C(8),H-C(8))[b]	215.4	213.9	216.1
J(C(8),H-C(1′))[b]			3.7
J(C(10),H-C(10))[b]	189.5	192.5	192.2
J(C(10),H-C(11))[b]	10.3		9.8
J(C(11),H-C(11))[b]	196.9	213.2	200.5
J(C(11),H-C(10))[b]	16.6	10.4	16.4
J(C(1′),H-C(1′))	166.7	164.4	167.7
J(C(1′),H-C(3′))	5.3	4.8	4.6
J(C(1′),H-C(8))[b]	2.6		3.4
J(C(1′),H-C(4′))	2.0		3.3
J(C(3′),H-C(3′))	149.0	149.2	149.4
J(C(4′),H-C(4′))	148.4	147.4	147.9
J(C(5′),H-C(5′))	141.4	140.3	140.3

[a] Measured in [D_6]DMSO at 303 K. [b] Purine numbering.

H-C(4′)), 4.50 (m, 1H, H-C(3′)), 4.96 (t, ^3J(H,H) = 5.4 Hz, 1H, 5′-OH), 5.41 (d, ^3J(H,H) = 4.3 Hz, 1H, 3′-OH), 6.69 (pseudo t, ^3J(H,H) = 6.3 Hz, 1H, H-C(1′)), 7.85 (d, ^3J(H,H) = 1.1 Hz, 1H, H-C(11)), 8.75 (d, ^3J(H,H) = 1.1 Hz, 1H, H-C(10)), 8.95 (s, 1H, H-C(8)); $C_{11}H_{12}N_6O_3$ (276.25): calcd C 47.83, H 4.38, N 30.42; found C 47.71, H 4.32, N 30.32.

4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-imidazo[4,5-d]-[1,2,3]triazine (2-aza-2′-deoxyadenosine, 2): Compound **7** (0.56 g, 2 mmol) was dissolved in aqueous sodium acetate buffer (1M, pH 4.0–4.5, 120 mL) by warming to 40–50 °C. To this solution *N*-bromosuccinimide (2.8 g, 16 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was evaporated and purified on a Dowex 1 × 8 ion exchange column (3 × 12 cm, OH^- form). Elution with H_2O (250 mL) gave compound **2** (0.19 g, 38%) as colorless needles that decomposed above 185 °C. The reaction product was identical with an authentic sample in all respects.[21]

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-imidazo[4,5-d][1,2,3]triazin-4-one (2-aza-2′-deoxyinosine, 3): Compound **2** (19 mg, 0.076 mmol) was dissolved in H_2O and adenosine deaminase (2 μg, from calf intestine, dissolved in glycerole) was added. The reaction mixture was stirred for 18 h at room temperature until **2** had completely disappeared (UV monitoring) and was then evaporated to dryness in a SpeedVac concentrator. UV (H_2O): λ_{max} (ε) = 247 (5500), 290 nm (6200); ^{1}H NMR (D_2O): δ = 2.49 (m, 1H, H_α-C(2′)), 2.75 (m, 1H, H_β-C(2′)), 3.41, 3.50 (2m, 2H, H_2-C(5′)), 4.03 (m, 1H, H-C(4′)), 4.51 (m, 1H, H-C(3′)), 6.43 (pseudo t, ^3J(H,H) = 3.2 Hz, 1H, H-C(1′)), 8.31 (s, 1H, H-C(8)).

4-(Benzoylamino)-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-imidazo-[4,5-d][1,2,3]triazine (8): Compound **2** (125 mg, 0.5 mmol) was coevaporated twice with anhydrous pyridine. The residue was suspended in anhydrous pyridine and treated with trimethylsilyl chloride (0.5 mL, 4 mmol). After few minutes of stirring, a clear solution was formed. The reaction mixture was stirred at room temperature for 2 h. Next, benzoyl chloride (0.25 mL, 2 mmol) was added, and stirring was continued for another 2 h. The reaction mixture was cooled in an ice-water bath, and H_2O (1 mL) was added. After 10 min the reaction mixture was treated with aqueous concentrated NH_3 (0.8 mL) and left for a further 30 min. The mixture was then evaporated to dryness, treated with H_2O, and extracted with EtOAc (3 × 20 mL). The combined extracts were dried (Na_2SO_4) and purified over a silica gel column (3 × 15 cm). Elution was performed with CH_2Cl_2 (150 mL), followed by CH_2Cl_2/MeOH (9:1). The nucleoside-containing fractions were evaporated to dryness, and compound **8** was crystallized from MeOH/H_2O to yield colorless needles (135 mg, 76%). M.p. 208–210 °C; TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f = 0.31; UV (10% MeOH in water): λ_{max} (ε) = 233 (15600), 276 nm (16400); ^{1}H NMR ([D_6] DMSO): δ = 2.97 (2m, 2H, H_2-C(2′)), 3.69 (m, 2H, H_2-C(5′)), 4.00 (m, 1H, H-C(4′)), 4.57 (m, 1H, H-C(3′)), 5.07 (t, ^3J(H,H) = 4.8 Hz, 1H, 5′-OH), 5.49 (d, ^3J(H,H) = 4.0 Hz, 1H, 3′-OH), 6.72 (t, ^3J(H,H) = 6.5 Hz, 1H, H-C(1′)), 7.61–7.78 (m, 4H, aromatic-H), 8.16 (d, 2H, aromatic-H), 9.09 (s, 1H, H-C(8)), 11.84 (s, 1H, N-H); $C_{16}H_{16}N_6O_4$ (356.3): calcd C 52.93, H 4.41, N 23.38; found C 52.68, H 4.39, N 23.07.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(dimethylamino)methylidene]-amino}-7*H*-imidazo[4,5-d][1,2,3]triazine (9a): *N*,*N*-Dimethylformamide dimethylacetal (120 mg, 0.5 mmol) was added to a stirred suspension of compound **2** (63 mg, 0.25 mmol) in MeOH (5 mL). Stirring was continued for 2 h at room temperature. The reaction mixture was evaporated to dryness, and the residue was adsorbed on silica gel. Flash chromatography on a silica gel column (3 × 10 cm) with CH_2Cl_2 (100 mL) followed by CH_2Cl_2/MeOH (9:1) afforded colorless needles (MeOH/H_2O, 65 mg, 85%). M.p. 173–175 °C; TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f = 0.28; UV (10% MeOH in H_2O): λ_{max} (ε) = 234 (13250), 319 nm (29500); ^{1}H NMR ([D_6] DMSO): δ = 2.84 (2m, 2H, H-C(2′)), 3.17, 3.25 (2s, 2H, N-CH_3), 3.60 (m, 2H, H_2-C(5′)), 3.92 (m, 1H, H-C(4′)), 4.47 (m, 1H, H-C(3′)), 5.05 (t, ^3J(H,H) = 4.9 Hz, 1H, 5′-OH), 5.39 (d, ^3J(H,H) = 4.0 Hz, 1H, 3′-OH), 6.55 (t, ^3J(H,H) = 6.6 Hz, 1H, H-C(1′)), 8.79 (s, 1H, N=CH), 9.08 (s, 1H, H-C(8)); $C_{12}H_{17}N_7O_3$ (307.3): calcd C 46.90, H 5.58, N 31.90; found C 46.55, H 5.68, N 31.66.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(diisobutylamino)methylidene]-amino}-7*H*-imidazo[4,5-d][1,2,3]triazine (9b): The procedure described for **9a** was used, but with *N*,*N*-diisobutylformamide dimethylacetal. Colorless crystals (72%); m.p. 138–140 °C; TLC (silica gel, CH_2Cl_2/MeOH, 9:1):

0947-6539/00/0602-0376 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 2

$R_f = 0.40$; UV (10% MeOH in water): λ_{max} (ε) = 236 (10100), 325 nm (25850); ^{1}H NMR ([D$_6$]DMSO): $\delta = 0.88$, 0.94 (2d, 12H, CH$_3$), 1.95, 2.20 (2m, 2H, CH), 2.80 (2m, 2H, H$_2$-C(2′)), 3.30–3.74 (m, 6H, H$_2$-C(5′), 2CH$_2$), 4.00 (m, 1H, H-C(4′)), 4.45 (m, 1H, H-C(3′)), 5.03 (t, ^3J(H,H) = 4.9 Hz, 1H, 5′-OH), 5.37 (d, ^3J(H,H) = 4.0 Hz, 1H, 3′-OH), 6.54 (t, ^3J(H,H) = 6.4 Hz, 1H, H-C(1′)), 8.78 (s, 1H, N=CH), 9.10 (s, 1H, H-C(8)); $C_{18}H_{29}N_7O_3 \cdot 0.5\,H_2O$ (400.5): calcd C 53.99, H 7.55, N 24.48; found C 53.65, H 7.62, N 24.11.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-4-{[(di-*n*-butylamino)methylidene]-amino}-7*H*-imidazo[4,5-d][1,2,3]triazine (9c): The procedure desribed for **9a** was followed, but *N*,*N*-di-*n*-butylformamide dimethylacetal was used. Colorless needles (75%); m.p. 107–109° C. TLC (silica gel, CH_2Cl_2/MeOH, 9:1): $R_f = 0.42$; UV (10% MeOH in water): λ_{max} (ε) = 235 (10200), 325 nm (25700); ^{1}H NMR ([D$_6$]DMSO): $\delta = 0.93$ (t, 6H, CH$_3$), 1.33, 1.64, 3.70 (3m, 12H, $-CH_2-$), 2.45, 2.80 (2m, 2H, H$_2$-C(2′)), 3.60 (m, 2H, H$_2$-C(5′)), 3.92 (m, 1H, H-C(4′)), 4.48 (m, 1H, H C(3′)), 5.04 (t, ^3J(H,H) = 5.8 Hz, 1H, 5′-OH), 5.39 (d, ^3J(H,H) = 4.0 Hz, 1H, 3′-OH), 6.55 (pseudo t, ^3J(H,H) = 6.3 Hz, 1H, H-C(1′)), 8.78 (s, 1H, N=CH), 9.08 (s, 1H, H-C(8)); $C_{18}H_{29}N_7O_3$ (391.5): calcd C 55.23, H 7.47, N 25.05; found C 55.36, H 7.66, N 24.97.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-*n*-butylamino)methylidene]amino}-7*H*-imidazo[4,5-d][1,2,3]-triazine (10a): Compound **9c** (390 mg, 1 mmol) was coevaporated twice with pyridine. The oily residue was dissolved in anhydrous pyridine (6 mL). Next, 4,4′-dimethoxytriphenylmethyl chloride (450 mg, 1.3 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. Thereupon, MeOH (0.2 mL) was added, and stirring was continued for 15 min. The reaction mixture was poured into of an aqueous solution of $NaHCO_3$ (5%, 15 mL). This was extracted with CH_2Cl_2 (2 × 30 mL). The combined extracts were dried over Na_2SO_4 and evaporated, and the residue was adsorbed on silica gel. This was purified by chromatography over a silica gel 60H column (4 × 14 cm) with a CH_2Cl_2/acetone gradient (0 → 25% of acetone, total volume, 600 mL). The nucleoside-containing fractions were pooled and evaporated to obtain compound **10a** as solid foam (560 mg, 81%). TLC: (silica gel, CH_2Cl_2/acetone, 85:15): $R_f = 0.15$; ^{1}H NMR ([D6] DMSO): $\delta = 0.93$ (t, 6H, CH$_3$), 1.34, 1.63, 3.75 (3m, 12H, $-CH_2-$), 2.95 (2m, 2H, H-C(2′)), 3.51 (m, 2H, H$_2$-C(5′)), 3.63, 3.69 (2s, 6H, OCH$_3$), 4.01 (m, 1H, H-C(4′)), 4.59 (m, 1H, H-C(3′)), 5.45 (d, ^3J(H,H) = 4.1 Hz, 1H, 3′-OH), 6.57 (pseudo t, ^3J(H,H) = 6.2 Hz, 1H, H-C(1′)), 6.60–7.30 (m, 13H, phenyl-H), 8.71 (s, 1H, N=CH), 9.07 (s, 1H, H-C(8)); $C_{39}H_{47}N_7O_5$ (693.8): calcd C 67.51; H 6.83; N 14.13; found C 67.15, H 6.82, N 14.13.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-{[(di-*n*-butylamino)methylidene]amino}-7*H*-imidazo[4,5-d]-[1,2,3]triazine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite] (10b): *N*,*N*-Diisopropylethylamine (145 μL, 0.88 mmol) and chloro(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine (143 μL, 0.62 mmol) were added under an argon atmosphere to a solution of compound **10a** (300 mg, 0.43 mmol) in anhydrous CH_2Cl_2 (20 mL). After stirring for 20 min at room temperature, an aqueous solution of $NaHCO_3$ (5%, 15 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, column 5 × 10 cm, CH_2Cl_2/acetone, 85:15) gave a mixture of diastereoisomers of **10b** (300 mg, 78%). TLC (silica gel, CH_2Cl_2/acetone, 85:15): $R_f = 0.71$, 0.80; ^{31}P NMR ($CDCl_3$): 149.962, 150.223.

Acknowledgements

We thank Mrs. E. Feiling for excellent technical assistance and Mrs. Julia Gross, University of Münster, for recording the MALDI-TOF spectra. Financial support by the Deutsche Forschungsgemeinschaft, and Roche Diagnostics GmbH is gratefully acknowledged.

[1] D. W. Wooley, E. Shaw, *J. Biol. Chem.* **1951**, *189*, 401–410.
[2] E. Shaw, D. W. Wooley, *J. Biol. Chem.* **1952**, *194*, 641–654.
[3] J. J. Biesele, *Cancer* **1952**, *5*, 787–791.
[4] A. Fjelde, *Z. Krebsforsch.* **1956**, *61*, 364–367.
[5] R. Guthrie, W. N. Lu, *Arch. Biochem. Biophys.* **1964**, *108*, 398–402.
[6] J. G. Cappuccino, M. George, P. C. Merker, G. S. Tarnowski, *Cancer Res.* **1964**, *24*, 1243–1248.
[7] Z. Kazimierczuk, F. Seela, *Liebigs Ann. Chem.* **1991**, 695–697.
[8] N. C. Sanyal, R. P. Ojha, M. Roychoudhury, *J. Theor. Biol.* **1991**, *150*, 277–286.
[9] a) J. A. Montgomery, A. G. Laseter, A. T. Shortnacy, S. J. Clayton, H. J. Thomas, *J. Med. Chem.* **1975**, *18*, 564–567; b) M. Acedo, E. DeClercq, R. Eritja, *J. Org. Chem.* **1995**, *60*, 6262–6269.
[10] F. Seela, I. Münster, U. Löchner, H. Rosemeyer, *Helv. Chim. Acta* **1998**, *81*, 1139–1155.
[11] F. Seela, A. Kehne, *Biochemistry* **1987**, *26*, 2232–2238.
[12] M. A. Stevens, H. W. Smith, G. B. Brown, *J. Am. Chem. Soc.* **1960**, *82*, 3189–3193.
[13] a) J. A. Montgomery, H. J. Thomas, *J. Chem. Soc. Chem. Commun.* **1969**, 458; b) J. A. Montgomery, H. J. Thomas, *J. Med. Chem.* **1972**, *15*, 182–187.
[14] M. Kawana, G. A. Ivanovics, R. J. Rousseau, R. K. Robins, *J. Med. Chem.* **1972**, *15*, 841–843.
[15] R. P. Panzica, L. B. Townsend, *J. Heterocycl. Chem.* **1972**, *9*, 623–628.
[16] G. A. Ivanovics, R. J. Rousseau, M. Kawana, P. C. Srivastava, R. K. Robins, *J. Org. Chem.* **1974**, *39*, 3651–3654.
[17] J. I. Andres, R. Herrenz, M. T. Garcia Lopez, *J. Heterocycl. Chem.* **1984**, *21*, 1221–1224.
[18] B. Rayner, C. Tapiero, J. L. Imbach, *J. Heterocycl. Chem.* **1982**, *19*, 593–596.
[19] A. Parkin, M. R. Harden, *J. Heterocycl. Chem.* **1982**, *19*, 33–40.
[20] a) V. I. Ofitserov, V. C. Mokrushin, I. A. Korbukh, M. N. Preobrazhenskaya, Z. V. Pushkareva, *Zh. Org. Khim.* **1975**, *11*, 909–910; b) V. A. Bakulev, V. S. Mokrushin, V. I. Ofitserov, Z. V. Pushkareva, A. N. Grishakov, *Khim. Geterotsikl. Soedin.* **1979**, *6*, 836–838.
[21] Z. Kazimierczuk, F. Seela, *Liebigs Ann. Chem.* **1990**, 647–651.
[22] N. Yamaji, M. Kato, *Chem. Lett.* **1975**, 311–314.
[23] J. A. Secrist III, J. R. Barrio, N. J. Leonard, G. Weber, *Biochemistry* **1972**, *11*, 3499–3506.
[24] S. C. Srivastava, S. K. Raza, R. Misra, *Nucleic Acids Res.* **1994**, *22*, 1296–1304.
[25] F. Seela, Y. He, C. Wei, unpublished results.
[26] K.-F. Yip, K.-C. Tsou, *J. Org. Chem.* **1975**, *40*, 1066–1070.
[27] H. Rosemeyer, G. Toth, B. Golankiewicz, Z. Kazimierczuk, W. Bourgeois, U. Kretschmer, H.-P. Muth, F. Seela, *J. Org. Chem.* **1990**, *55*, 5784–5790.
[28] a) J. van Wijk, C. Altona, *PSEUROT 6.2—A program for the conformational analysis of the five-membered rings*, University of Leiden, **1993**; b) C. A. G. Haasnoot, F. A. A. M. de Leeuw, C. Altona, *Tetrahedron* **1980**, *36*, 2783–2792.
[29] E. Westhof, O. Röder, I. Croneiss, H.-D. Lüdemann, *Z. Naturforsch. C* **1975**, *30*, 131–140.
[30] H. Rosemeyer, M. Zulauf, N. Ramzaeva, G. Becher, E. Feiling, K. Mühlegger, I. Münster, A. Lohmann, F. Seela, *Nucleosides Nucleotides* **1997**, *16*, 821–828.
[31] N. Ramzaeva, C. Mittelbach, F. Seela, *Helv. Chim. Acta* **1999**, *82*, 12–18.
[32] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.
[33] J. Zemlicka, *Collect. Czech. Chem. Commun.* **1963**, *28*, 1060–1062.
[34] L. J. McBride, R. Kierzek, S. L. Beaucage, M. H. Caruthers, *J. Am. Chem. Soc.* **1986**, *108*, 2040–2048.
[35] B. C. Froehler, M. D. Matteucci, *Nucleic Acids Res.* **1983**, *11*, 8031–8036.
[36] H. Schaller, G. Weimann, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc.* **1963**, *85*, 3821–3827.
[37] S. L. Beaucage, M. Caruthers, *Tetrahedron Lett.* **1981**, *22*, 1859–1862.
[38] F. Seela, C. Wei, *Helv. Chim. Acta* **1997**, *80*, 73–85.
[39] M. Goodman, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10493–10495.
[40] X. Zhang, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1998**, *120*, 6625–6626, and references cited therein.
[41] R. Krishnamurthy, S. Pitsch, M. Minton, C. Miculka, N. Windhab, A. Eschenmoser, *Angew. Chem.* **1996**, *108*, 1619–1623; *Angew. Chem. Int Ed. Engl.* **1996**, *35*, 1537–1541.

[42] F. Seela, C. Wei, *Helv. Chim. Acta* **1999**, *82*, 726 – 745.
[43] E. T. Kool, *Chem. Rev.* **1997**, *97*, 1473 – 1487.
[44] 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 – 8183.
[45] J. Petruska, M. F. Goodman, *J. Biol. Chem.* **1995**, *270*, 746 – 750.
[46] M. S. Searle, D. H. Williams, *Nucleic Acids Res.* **1993**, *21*, 2051 – 2056.
[47] J. A. McDowell, D. H. Turner, *Biochemistry* **1996**, *35*, 14077 – 14089.

Received: April 30, 1999 [F1758]

0947-6539/00/0602-0378 $ 17.50+.50/0