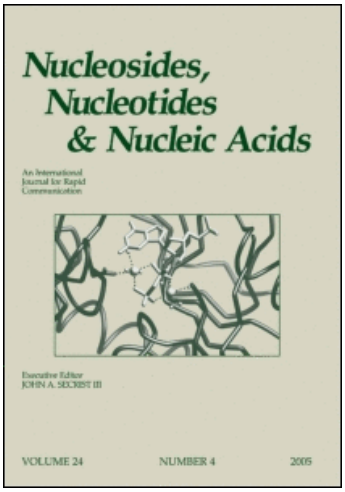

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Stereoelectronic Effects of Modified Purines on the Sugar Conformation of Nucleosides and Fluorescence Properties

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STEREOELECTRONIC EFFECTS OF MODIFIED PURINES ON THE SUGAR CONFORMATION OF NUCLEOSIDES AND FLUORESCENCE PROPERTIES

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ABSTRACT.- Conformational analyses of the sugar moieties of a series base-modified purine-2'-deoxynucleosides on the basis of vicinal [^{1}H,^{1}H] coupling constants is presented (*PSEUROT 6.2)* Fluorescence data of several 7-deaza- and 8-azapurine 2'-deoxynucleosides are given.

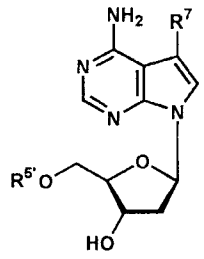
An antisense oligo(2'-deoxyribonucleotide) has to adopt A-conformation if it is bound to the RNA target. One of the characteristics of an A-DNA is the $C_{3'}$-endo pucker (N-sugar); the B-DNA exhibits a $C_{2'}$-endo pucker (S-sugar). Therefore, the synthesis of 2'-deoxyribonucleosides which show the maximal N-conformer populations of the sugar is of interest.

In 7-deazapurine 2'-deoxyribonucleosides it is found that the substituents of the pyrrole ring can influence the sugar puckering. Such conformational changes on a series of 7-(8)-substituted 7-deazapurine-2'-deoxynucleosides (formula schemes)[1-4] have been studied on the basis of vicinal [^{1}H,^{1}H] coupling constants using the *PSEUROT 6.2* program[5,6].

Calculations were performed using pseudorotational starting parameters recommended in the user's manual of the program [Φ_{max} = 36° (both N and S); P_N = 19°; P_S = 156°]. The input contained the following coupling constants: J(H1',H2'), J(H1',H2"), J(H2',H3'), J(H2",H3'), and J(H3',H4'). During the iterations either the puckering parameters (P, Φ_{max}) of the minor conformer (N) or the puckering amplitudes of both conformers were constrained. In all cases the RMS values were ≤ 0.4 Hz and the $|\Delta J_{max}| \leq 0.5$ Hz.

From the data given in the table some general trends can be deduced: Enhancement of the bulkyness of a substituent in position 8 drives the N ⇔ S equilibrium of a 7-deaza-2'-deoxyguanosine (**14**, **17**, **22**) towards S-type sugar puckering. This conformation generally correlates with the syn-conformation at the N-glycosylic bond. The nature of this effect seems to be mainly steric as a linear correlation exists between the S-type conformer population and the van der Waals radii of the 8-substituents (*Figure 1*).

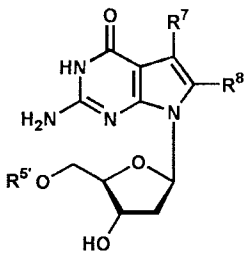
Only the sterically demanding 7-deaza-8-methyl-2'-deoxyguanosine (**22**) exhibits a noticable temperature dependence between 296 and 343 K. From a van't Hoff plot the



	1	2	3	4	5
R^7	H	H	Cl	Br	Br
$R^{5'}$	H	TP	H	H	TP

	6	7	8	9	10
R^7	I	I	CN	NO_2	Me
$R^{5'}$	H	TP	H	H	H

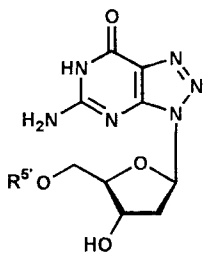
	11	12	13
R^7	C≡CH	Hexin-(1)-yl	Hexin-(1)-yl
$R^{5'}$	H	H	TP



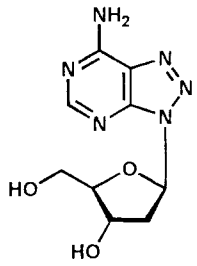
	14	15	16	17	18
R^7	H	H	Cl	H	Br
R^8	H	H	H	Cl	H
$R^{5'}$	H	TP	H	H	H

	19	20	21	22	23
R^7	I	I	Me	H	Cl
R^8	H	H	H	Me	Cl
$R^{5'}$	H	TP	H	H	H

	24	25	26	27
R^7	Hexin-(1)-yl	Hexin-(1)-yl	5-Amino-pentin-(1)-yl	5-Amino-pentin-(1)-yl
R^8	H	H	H	H
$R^{5'}$	H	TP	H	TP



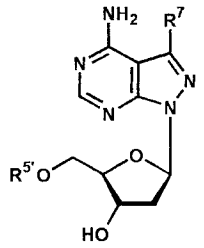
	28	29
$R^{5'}$	H	TP



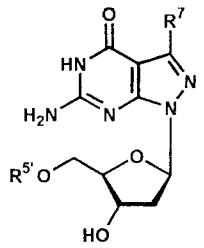
30

TP: 5'-Triphosphate

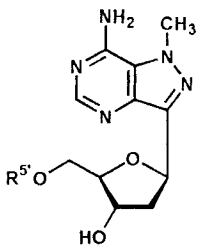
Formula Scheme 1



	31	32	33	34
R^7	H	H	I	Hexin-(1)-yl
$R^{5'}$	H	TP	H	H



	35	36	37	38
R^7	H	H	I	Hexin-(1)-yl
$R^{5'}$	H	TP	H	H



	39	40
$R^{5'}$	H	TP

Formula Scheme 2

TABLE. ^{3}J(H,H) Coupling constants of the sugar moieties and N/S-conformer populations of 2'-deoxynucleosides at 303K.

Compound	^{3}J(H,H) [Hz] 1',2'	1',2''	2',3'	2'',3'	3',4'	Conformation % N	%S
dA	7.20	6.50	6.50	3.30	3.20	28	72
1	6.60	7.60	7.00	3.00	3.00	24	76
3	7.50	6.35	6.25	3.25	3.50	30	70
4	7.05	6.55	6.60	3.15	3.40	29	71
6	6.90	6.50	6.50	3.10	3.30	29	71
8	6.80	6.55	6.25	3.65	3.45	34	66
9	6.80	6.75	6.00	4.15	3.85	38	62
11	7.50	6.40	6.20	3.20	3.40	29	71
10	8.00	6.25	6.15	3.05	3.00	26	74
12	6.70	6.45	5.90	2.95	3.45	31	69
dG[7]	7.30	6.50	6.30	3.60	3.20,	29	71
14	7.25	6.50	6.25	3.00	3.35	28	72
16	6.95	6.60	6.65	3.70	3.80	34	66
17	7.45	7.20	6.80	2.85	3.30	22	78
18	6.50	6.90	6.40	3.00	3.00	28	72
19	6.60	7.00	6.40	3.20	3.60	31	69
21	7.20	6.45	6.20	3.05	3.25	28	72
22	8.00	6.70	6.75	2.25	3.20	18	82
23	7.30	7.30	7.10	3.50	3.65	27	73
24	7.55	6.45	6.10	3.20	3.10	28	72
28	6.20	6.45	5.90	5.30	5.10	51	49
30	6.45	6.60	5.65	5.15	5.20	50	50
31	6.55	6.70	6.45	4.00	3.70	37	63
33	6.30	6.45	6.60	4.10	3.40	37	63
34	6.25	6.45	6.00	4.15	4.10	41	59
35	6.65	6.65	6.25	3.65	3.90	36	64
37	6.55	6.65	6.50	4.10	3.70	37	63
38	6.40	6.65	6.30	3.85	4.20	38	62
39	10.70	5.80	5.90	0.90	3.85	5	95

Solvent, D_2O; RMS, ≤ 0.4 Hz; $|\Delta J_{max}|$ ≤ 0.5 Hz

thermodynamics of the N ⇔ S interconversion could be estimated ($Me^8c^7G_d$: ΔH = -0.8 kcal/mol, ΔS = 0.4 cal/K mol; $Me^7c^7G_d$: ΔH = 0.2 kcal/mol, ΔS = -2.5 cal/K mol).

Figure 2 demonstrates the stereoelectronic influence of 7-substituents on the N ⇔ S equilibrium of a series of 7-deaza-2'-deoxyadenosines (**1**, **3**, **4**, **6**, **8-11**). The figure clearly demonstrates that the higher the electron-withdrawing effect of the 7-substituent is, the more the N ⇔ S equilibrium of the sugar moieties is biased towards N-conformation[8,9]. In case of the 7,8-dichloro-substituted 7-deaza-2'-deoxyguanosine (**23**) the steric effect of the 8- and the electronic

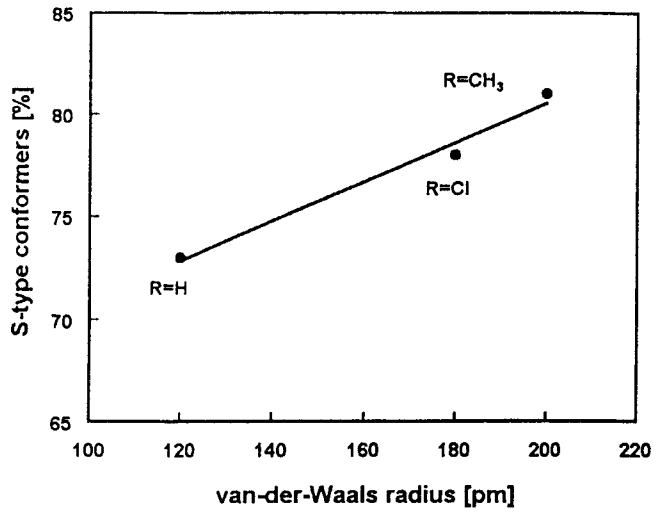


FIGURE 1. S-type conformer population of 8-substituted 7-deaza-2'-deoxyguanosines as function of the van der Waals radii of substituents.

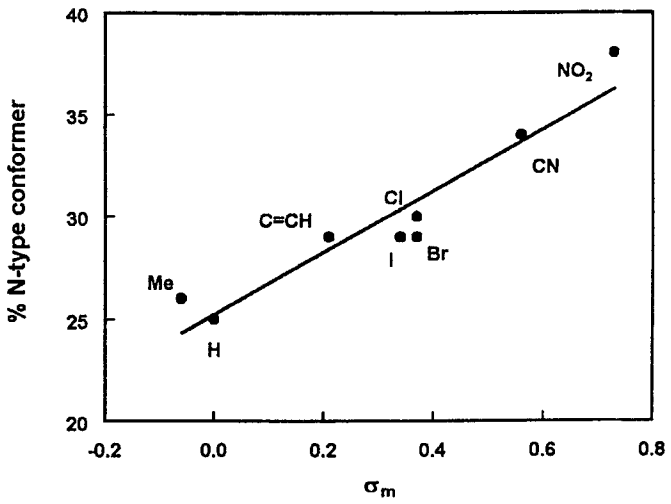


FIGURE 2. N-type conformer population of 7-substituted 7-deaza-2'-deoxyadenosines vs. the σ_m values of substituents.

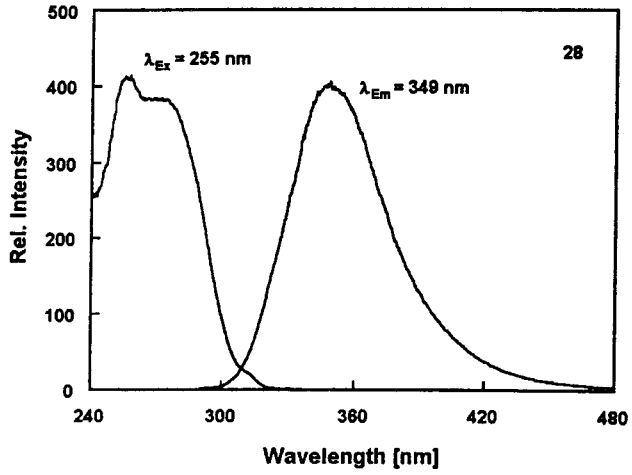


FIGURE 3. Fluorescence spectrum (excitation and emission) of 8-aza-2'-deoxyguanosine (**28**) in H_2O (c = 10^{-5} M).

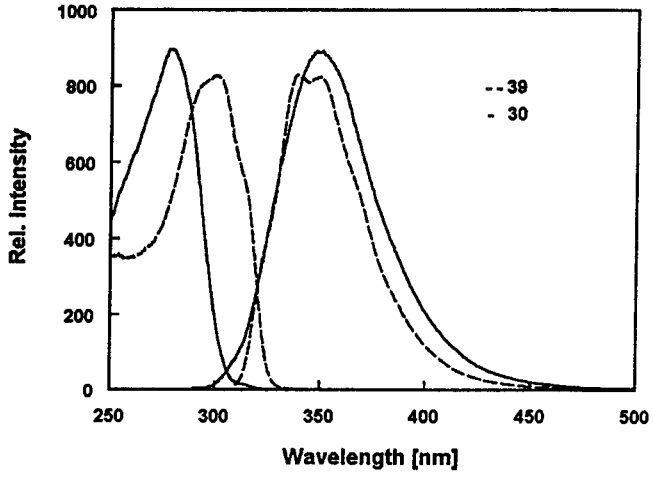


FIGURE 4. Fluorescence spectra of N(1)-methyl-2'-deoxyformycin A (**39**) and 8-aza-2'-deoxyadenosine in H_2O (**30**)(c = 10^{-5} M, each).

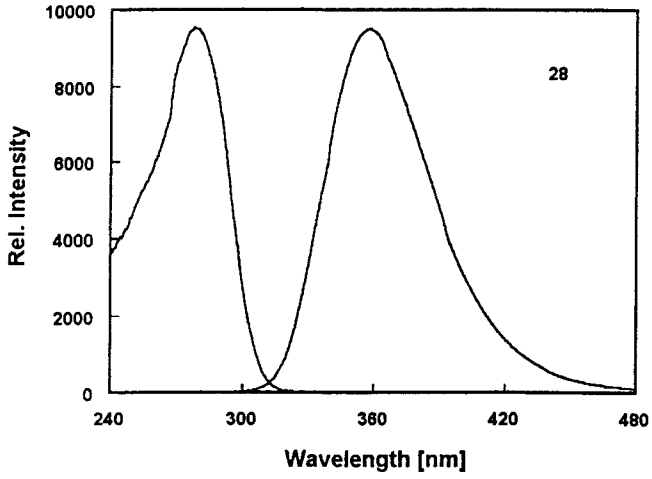


FIGURE 5. Fluorescence spectrum (exication and emission) of 8-aza-2'-deoxyguanosine (**28**) at pH 11 ($c = 10^{-5}$ M).

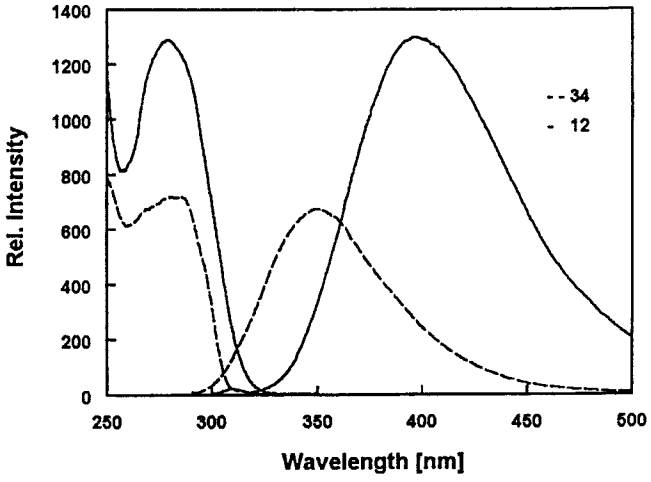


FIGURE 6. Fluorescence spectra of the 7-hexin(1)-yl compounds **12** and **34** in H_2O ($c = 10^{-5}$ M, each).

effect of the 7-substituent compensate each other, so that for this compound the distribution of N- and S-conformers is almost the same as for the unsubstituted 7-deaza-2'-deoxyguanosine (**14**).

Electron-withdrawing substituents in position 7 of 7-deazapurine 2'-deoxynucleosides preform the sugar moiety towards a conformation (N-type) which they also adopt in an A-DNA.

Determination of the syn- and anti conformer populations of compounds **1**, **3**, **4**, **6**, and **8-11** by 1D-NOE difference spectroscopy according to ref.[10](data not shown) shows a clear tendency: the higher the anti-conformer population - the lower the population of S-type sugar puckered molecules (**9**: 72% anti, 62 % S; **1**: 64 % anti, 76 % S) [11].

Besides the data of 7-deazapurine 2'-deoxynucleosides the table contains the vicinal [^{1}H,^{1}H] coupling constants as well as sugar puckering data of a series of 8-aza-7-deazapurine-[12-14] and 8-azapurine 2'-deoxynucleosides[15,16](formula scheme 2: **31**, **33-35**, **37**, **38**; formula scheme 1: **28**, **30**). As can be seen, the 8-aza-7-deazapurine 2'-deoxynucleosides (pyrazolo[3,4-d]pyrimidine 2'-deoxyribonucleosides) exhibit generally an 8-10% higher N-conformer population (37-41% N-type) which is due to the increased π-electron deficiency. This is in contrast to N(1)-methyl-2'-deoxyformycin A (**39**) which represents a pyrazolo[4,3-d]pyrimidine 2'-deoxyribonucleoside. This nucleoside shows a 95% S-conformer population - a result which is caused by the absence of an anomeric effect leading to a pseudoequatorial orientation of the C-nucleobase.

Going from 8-aza-7-deazapurine- to 8-azapurine 2'-deoxynucleosides[15,16] (formula scheme 1: **28**, **30**) enhances the N-type conformer population further: these compounds exhibit an almost equal distribution of N- and S-type puckered sugar moities which makes them to interesting candidates for the synthesis of A-type DNA constructs.

The figures 3-6 show the fluorescence spectra (excitation and emission) of some of the aza- and deazapurine 2'-deoxynucleosides (**12**, **28**, **30**, **34**, and **39**).

As can be seen, both 8-azapurine 2'-deoxynucleosides (**28**, **30**) exhibit significant fluorescence with emission maxima at about 350 nm. The relative intensity of 8-aza 2'-deoxyadenosine (**30**) (H_2O, c = 10 µM) is almost identical with that of N(1)-methyl 2'-deoxyformycin A (**39**) (*Figure 4*) [17] while that of 8-aza 2'-deoxyguanosine (**28**) exhibits only half of the intensity. The fluorescence of the latter, however, is increased by a factor of 20 when the pH of the solution is raised to 11.0 (*Figure 5*). *Figure 6* represents a comparison of the fluorescence spectra of the 7-hexin(1)-yl -substituted 2'-deoxyadenosine derivatives **12** and **34**. It is interesting to note that both compounds exhibit almost identical excitation spectra but completely different emission spectra: 7-[hexin(1)-yl]-7-deaza-2'-deoxyadenosine (**12**) shows an emission at 400 nm with double the intensity of that of 8-aza-7-[hexin(1)-yl]-7-deaza-2'-deoxyadenosine (**34**).

Out of each series several compounds were converted into their 5'-triphosphates (**2**, **5**, **7**, **13**, **15**, **20**, **25**, **27**, **29**[18], **32**, **36**, and **40**). All could be successfully incorporated into DNA by different matrix-dependent DNA polymerases[19].

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