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Comparative ^{1}H NMR chemical shift study on the stacking interaction of pyrimidine with purine and 6-methylpurine

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Abstract

The pyrimidine–purine and pyrimidine–6-methylpurine cross-interactions were compared by measuring the mutually induced concentration-dependent changes in proton chemical shifts in deuterium oxide at 35°C. The chemical shift vs. concentration profiles were analysed using a competitive dimer model. The equilibrium constants (0.41 ± 0.06 and 0.74 ± 0.05 l mol^{-1} for the pyrimidine–purine and pyrimidine–6-methylpurine hetero-associations, respectively) and the dimer shifts imply that the methylation of purine significantly influences the stacking interaction between the parent molecules of nucleic acid bases and thus their plane-to-plane association structure.

Keywords: ^{1}H NMR chemical shift; Stacking interaction; Hetero-association; Pyrimidine–purine cross-interaction; 6-Methylpurine

1. Introduction

The vertical base–base stacking interaction exhibited by polynucleotides is one of the major driving forces in the formation of stable three-dimensional nucleic acid structures in aqueous solutions [1]. The 'character' of this interaction is known to depend, among others, on the base sequence, more precisely, on the type of the interacting bases, pyrimidine or purine, and on the base modification, e.g. on the base methylation [2,3]. NMR experiments on relatively short oligonucleotide fragments in aqueous solutions provide insight into the nature of the stacking interaction because, among the solvents tested, water has the greatest propensity to restrict the base pairing via hydrogen bonding and to promote base stacking [1]. The data on dinucleoside monophosphates give information about the stacked-unstacked conformational equilibrium: the intramolecular stacking interaction is stronger in purine–purine dinucleoside monophosphates than in purine–pyrimidine (or pyrimidine–purine) dinucleoside monophosphates [4] and the methylation of the purine base(s) brings about an increase in the population of the stacked conformers [5,6]. The results of the studies on the conformation of purine–pyrimidine–purine trinucleoside diphosphates [7,8] and purine–pyrimidine–purine sequences in tetra- and pentanucleotides [9] indicate the so-called 'bulged-out' structure: the central pyrimidine residue bulges out, whereas the purine residues stack upon each other and this purine–purine 'next-nearest-neighbour stacking interaction' can be made more

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pronounced by using methylated purine base(s). The chain elongation restricts the 'next-nearest-neighbour stacking interaction' and the 'nearest-neighbour stacking interaction' is preferred in accordance with the conformational transmission along the chain. However, the methylation of an interior purine base in hexa-, octa- and decanucleotides induces local conformational and dynamic changes: the methyl group provokes hairpin formation [10], reduces the melting point of the helices [11–13] and results in a slowing down of the opening rate for the methylated purine–pyrimidine base pair and surprisingly has an even greater effect upon the life time of the adjacent unmethylated purine–pyrimidine base pair [13,14]. Each of these phenomena suggests that the purine bases and their methylated forms play a dominant role in the stacking interaction between nucleic acid bases. The question has been raised whether this dominance can be attributed to the parent molecule of the purine bases as its inherent property. A number of studies, based on the same origin of both the intramolecular stacking interaction resulting in stacked conformers and the intermolecular stacking interaction resulting in stacking-like associations, have demonstrated the strong self-association proclivity of purine, in comparison with pyrimidine, and the enhancement of this ability by its methylation [15–28]. However, there are but few quantitative results on the pyrimidine–purine hetero-association [29,30] and, to the author's knowledge, data on the hetero-association between pyrimidine and methylated purine have not yet been published in the open literature.

In order to quantify the effect of the purine methylation on the prominent pyrimidine–purine hetero-association, in this work, the pyrimidine–6-methylpurine interaction was investigated and the pyrimidine–purine interaction was reinvestigated. The proton NMR chemical shift vs. concentration profiles observed in pyrimidine–purine and pyrimidine–6-methylpurine mixed aqueous solutions were analysed using a competitive dimer model which takes into account the self-association of the counterparts. The question to be answered was whether the hetero-association parameters imply the effect of purine methylation.

2. Experimental

Several sets of deuterium oxide solutions with one and two solutes were prepared in order to yield chemical shift vs. concentration profiles. Within a set of solutions the molar concentration of one solute (monitor) was kept constant at 0.1, 0.3 and 0.5 *M* while the other (inductor) was varied from 0.0 to 0.5 *M*. Pyrimidine and purine from Sigma and 6-methylpurine from Aldrich were used without further purification.

In order not to disturb the state of the solutions (with an internal reference) and the homogeneity of the NMR magnetic field (with a capillary reference), a special procedure was adopted for the measurement of the changes in chemical shifts. In an experiment on a set of solutions (of constant monitor concentration), the sample with one solute was chosen as the reference; each of the tubes containing the mixed solutions to be measured was changed several times with the NMR reference tube and the average displacements in Hz of the appropriate signals produced the experimental data.

The ^{1}H NMR spectra were recorded at 60 MHz using a Perkin-Elmer R12A spectrometer at a probe temperature of 35°C. By following the above-described experimental procedure, the accuracy of the observed chemical shift changes proved to be about 0.1 Hz.

3. Data analysis

Experimental data on molecular hetero-association or complexation, obtained by different experimental techniques, can be analysed by a variety of mathematical models having assumptions for the number of equilibrium sites and for the stoichiometry of associations [31–37].

In the present work, the extraction of the association parameters from the concentration-dependent chemical shift changes occurring on diffusion-controlled weak molecular interactions (self- and hetero-associations) was carried out by means of dynamic NMR line-shape analysis [38,39] in the fast exchange limit at equilibrium [40,41]. Assuming simultaneous, competitive

dimer equilibria, the 'interaction network' for the hetero-association between two self-associating compounds can be represented by

$$A + A \rightleftharpoons D1\ (=A_2) \quad K_{d1} = [D1]/[A]^2$$
$$B + B \rightleftharpoons D2\ (=B_2) \quad K_{d2} = [D2]/[B]^2$$
$$A + B \rightleftharpoons C\ (=AB) \quad K_c = [C]/[A][B]$$

where K_{d1}, K_{d2} and K_c are the appropriate equilibrium constants. Under conditions of fast exchange on the NMR time scale, the observed chemical shift is the average of the chemical shifts of a proton of the monitor molecule in monomer, self-associated and hetero-associated states (δ_m, δ_d and δ_c, respectively), weighted by the fractional occupancy of these states:

$$\delta_{obs} = \frac{[A]}{[A]_0}\delta_m + 2\frac{[D1]}{[A]_0}\delta_d + \frac{[C]}{[A]_0}\delta_c \qquad (1)$$

If $[A]_0 = [A] + 2[D1] + [C]$ and $[B]_0 = [B] + 2[D2] + [C]$ correspond to the total concentrations of the monitor and inductor molecules, the concentrations of the self-associated and hetero-associated species can be determined by iteration from the following equations:

$$[D1]^2 - \left([A]_0 - [C] + \frac{1}{4K_{d1}}\right)[D1] + \tfrac{1}{4}([A]_0 - [C])^2 = 0$$

$$[D2]^2 - \left([B]_0 - [C] + \frac{1}{4K_{d2}}\right)[D2] + \tfrac{1}{4}([B]_0 - [C])^2 = 0$$

$$[D1][D2] - \frac{K_{d1}K_{d2}}{K_c^2}[C]^2 = 0$$

Furthermore, if the monomer shift is used as reference, i.e. the experimental data are corrected for the chemical shift change occurring on self-association in the reference solution (containing only the monitor molecules with the same total concentration of $[A]_0 = [A]' + 2[D1]'$ as the appropriate mixed solution), eq. (1) can be simplified and the chemical shift vs. concentration profiles can be described by the equation:

$$\Delta_{corr} = 2\frac{[D1]}{[A]_0}\Delta_d + \frac{[C]}{[A]_0}\Delta_c \qquad (2)$$

where $\Delta_{corr} = \delta_m - \delta_{obs}$, $\Delta_d = \delta_m - \delta_d$ and $\Delta_c = \delta_m - \delta_c$; Δ_{corr} is the corrected upfield shift, Δ_d and Δ_c denote the self- and hetero-association dimer shifts, respectively (a negative sign corresponds to a downfield shift).

The equilibrium constants and the dimer shifts for the hetero-associations were derived from eq. (2) by the process of non-linear least-squares fitting [42] performed on an IBM compatible microcomputer system.

4. Results and discussion

The mutually induced proton chemical shift changes observed in pyrimidine–purine and pyrimidine–6-methylpurine aqueous systems were standardized by means of a correction procedure: the concentration-dependent changes were related to the extrapolated monomer shifts of the appropriate component. The standardized or corrected experimental data are presented in Figs. 1–3; the data sets are translated along the ordinate in such a way that the intercepts correspond to the self-association upfield shifts of the references. Otherwise, the monomer shifts calculated from the self-association parameters [43,44] were found to be 548.1, 528.6 and 455.8 Hz for the pyrimidine protons (H-2, H-4,6 and H-5, respectively), 537.8, 548.6 and 515.8 Hz for the purine protons (H-2, H-6 and H-8, respectively) and 527.1, 168.9 and 510.4 Hz for the 6-methylpurine protons (H-2, 6-Me and H-8, respectively) from an external 0.05 *M* deuterium oxide solution of DSS (at 60 MHz).

The chemical shift (of the monitor molecule) vs. concentration (of the inductor molecule) profiles in Fig. 1 show that, at 0.1 *M* monitor concentration, the counterparts induce different upfield shifts for the different protons of the same molecule in the pyrimidine–purine and pyrimidine–6-methylpurine mixed solutions and these

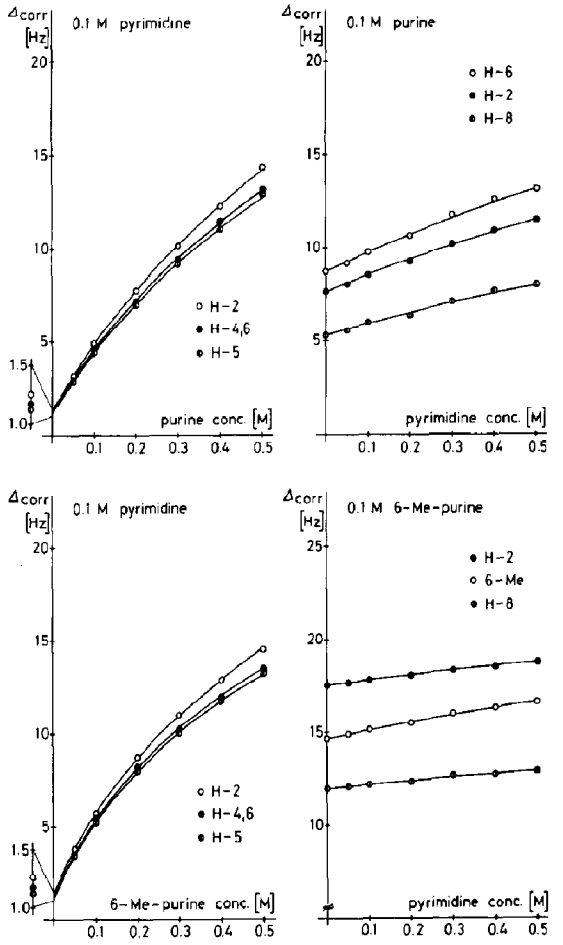

Fig. 1. Chemical shift vs. concentration profiles for the non-exchangable protons in pyrimidine–purine and pyrimidine–6-methylpurine mixed deuterium oxide solutions at a fixed concentration of the 'monitor' molecule. Corrected experimental (symbols) and fitted (solid lines) upfield shifts relative to the extrapolated monomer shifts in pure solutions; temperature, 35°C.

selective chemical shift changes reflect a saturation tendency. The saturation character of the profiles is confirmed by the convergence of the data sets obtained at different monitor concentrations as demonstrated for the H-2 protons of the three molecules in Fig. 2. It is remarkable, that, in the pyrimidine–6-methylpurine mixed solutions, the sign of the chemical shift changes induced by pyrimidine on the H-2 proton of 6-methylpurine alters at a monitor concentration between 0.1 and 0.3 *M*: on addition of the inductor component, the monitor signal moves upfield at lower concentrations and downfield at higher concentrations. The exact value of this 'sign-reversing' monitor concentration depends also on the inductor concentration. The same effect induced by pyrimidine can be seen for the 6-Me and H-8 protons of 6-methylpurine in Fig. 3, in comparison with the profiles measured in pyrimidine–purine mixed solutions for the H-6 and H-8 protons of purine. Similar findings have been interpreted as the breakdown of the self-association structure [45]. Indeed, assuming a competition between the self-association and the hetero-

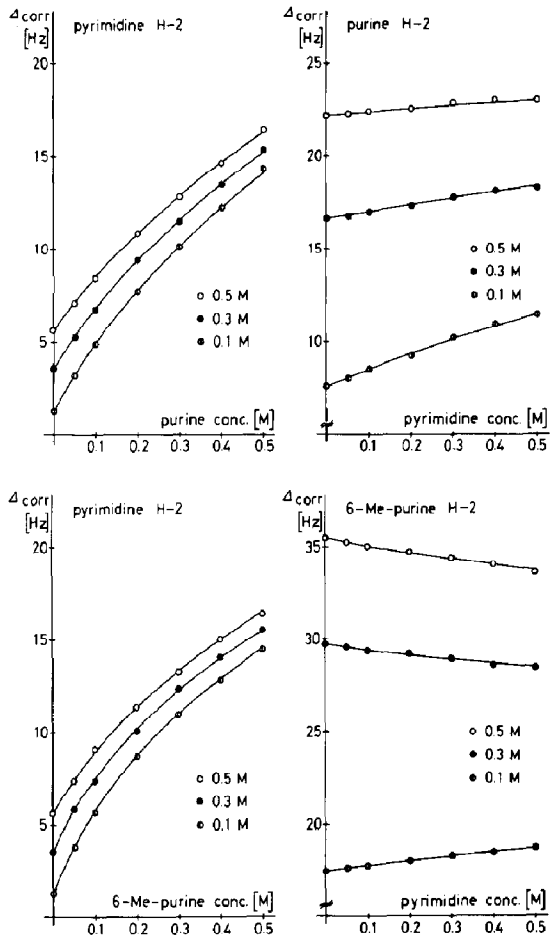

Fig. 2. Chemical shift vs. concentration profiles for the H-2 protons of pyrimidine, purine and 6-methylpurine in pyrimidine–purine and pyrimidine–6-methylpurine mixed deuterium oxide solutions at different concentrations of the 'monitor' molecule. Corrected experimental (symbols) and fitted (solid lines) upfield shifts relative to the extrapolated monomer shifts in pure solutions; temperature, 35°C.

association, pyrimidine forming hetero-association with and restricting the self-association of 6-methylpurine can result in downfield or virtual paramagnetic shifts. However, in general, the profile-shape over the concentration range investigated, monotonic increasing or monotonic decreasing or having an extreme value, is determined by the relationship of the self- and hetero-association parameters.

The former qualitative statements become more obvious if we compare the more substantial association parameters obtained by fitting eq. (2) to the experimental data sets. These parameters with their standard deviations are listed in Table 1.

The values of the individual hetero-association constants, calculated for each of the non-exchangable protons of the interacting molecules, and those of the average hetero-association constants, calculated separately for the counterparts, suggested to use for comparison the so-called overall average equilibrium constants which proved to be 0.41 ± 0.06 and 0.74 ± 0.05 l mol^{-1} for the pyrimidine–purine and pyrimidine–6-methylpurine hetero-association, respectively. These parameters refer to the fact, that the methylation of purine significantly enhances the association ability between pyrimidine and purine.

The positive values of the upfield dimer shifts, i.e. the mutual diamagnetic shielding effects, implicate plane-to-plane or stacking-like hetero-association structures [46]. The dimer shifts, particularly those for the ring protons which, corresponding to the monitor–inductor relationship, can be ordered in an increasing series of 6-methylpurine–pyrimidine < purine–pyrimidine < pyrimidine–6-methylpurine < pyrimidine–purine, reflect the more extended shielding regions of purines and the less extended shielding region of pyrimidine. (It is noteworthy, that the appropriate dimer shifts in the self-associations are smaller than those induced by purines and greater than those induced by pyrimidine in the hetero-associations.) The fact that the dimer shifts for the pyrimidine–6-methylpurine association are smaller than those for the pyrimidine–purine as-

Table 1

Equilibrium constants in l mol^{-1} and upfield dimer shifts in Hz (at 60 MHz) for the self-associations (K_d and Δ_d) of pyrimidine [43], purine [44] and 6-methylpurine [44] in pure solutions and for the hetero-associations (K_c and Δ_c) of pyrimidine with purine and 6-methylpurine in mixed solutions, calculated from the concentration-dependent self-induced and mutually induced chemical shift changes using dimer models (solvent, deuterium oxide; temperature, 35°C)

Protons of monitor molecules	Inductor molecules					
	Pyrimidine		Purine		6-Methylpurine	
	K_d	Δ_d	K_c	Δ_c	K_c	Δ_c
Pyrimidine	0.091 ± 0.006 [a]		0.40 ± 0.02 [a]		0.73 ± 0.03 [a]	
H-2	0.096 ± 0.011	72.0 ± 2.8	0.39 ± 0.01	111.5 ± 2.6	0.73 ± 0.02	94.4 ± 1.6
H-4,6	0.085 ± 0.007	66.7 ± 2.5	0.40 ± 0.02	102.4 ± 3.4	0.74 ± 0.03	87.7 ± 2.2
H-5	0.092 ± 0.010	63.9 ± 2.2	0.40 ± 0.02	100.9 ± 3.8	0.71 ± 0.03	86.0 ± 2.2
	K_c	Δ_c	K_d	Δ_d		
Purine	0.42 ± 0.08 [a]		0.70 ± 0.02 [a]			
H-2	0.41 ± 0.07	40.6 ± 2.0	0.68 ± 0.02	68.8 ± 1.8		
H-6	0.43 ± 0.05	46.2 ± 1.6	0.72 ± 0.02	78.7 ± 1.5		
H-8	0.40 ± 0.10	28.1 ± 2.3	0.71 ± 0.02	47.8 ± 1.0		
	K_c	Δ_c			K_d	Δ_d
6-Methylpurine	0.74 ± 0.07 [a]				2.41 ± 0.08 [a]	
H-2	0.74 ± 0.07	32.6 ± 0.7			2.46 ± 0.07	66.8 ± 1.7
6-Me	0.73 ± 0.05	32.0 ± 0.4			2.34 ± 0.07	55.8 ± 1.7
H-8	0.76 ± 0.08	22.7 ± 0.6			2.42 ± 0.05	45.7 ± 0.8

[a] Average equilibrium constants.

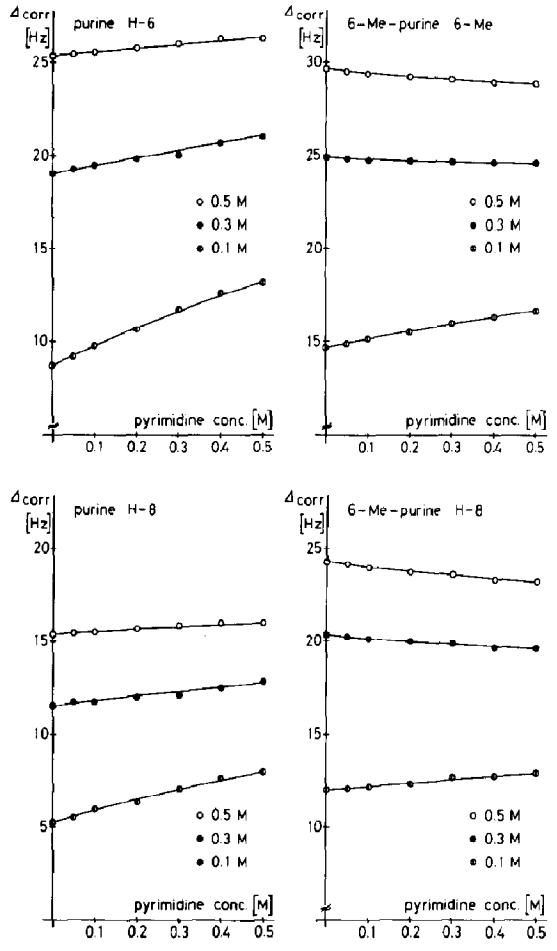

Fig. 3. Pyrimidine induced chemical shift changes for the H-6 and H-8 protons of purine and for the 6-Me and H-8 protons of 6-methylpurine in pyrimidine–purine and pyrimidine–6-methylpurine mixed deuterium oxide solutions at different concentrations of purines. Corrected experimental (symbols) and fitted (solid lines) upfield shifts relative to the extrapolated monomer shifts in pure solutions; temperature, 35°C.

sociation is a probable consequence of the steric demand of the methyl group. Relating the 6-methylpurine–pyrimidine dimer shifts to the purine–pyrimidine ones and the pyrimidine–6-methylpurine dimer shifts to the pyrimidine–purine ones (for the ring protons only), the ratios indicate that the pyrimidine induced shielding effect decreases to a higher degree (by about 20%) and the shielding effect induced by the purine counterparts decreases to a lesser degree (by about 15%). This finding can be attributed to the higher ring-current intensity of 6-methylpurine, in accordance with the conclusions drawn from the dimer shifts of the purine–6-methylpurine association [44]. However, in the case of the pyrimidine–6-methylpurine association the 'shielding-decreasing' steric effect overcomes the 'shielding-increasing' ring-current effect.

The individual dimer shifts give further details about the hetero-association structures. As the protons of the pyrimidine part of purines are more shielded than those of the imidazole parts, the associations can be described by an association centre over/under the six-membered rings. The most shielded ring protons of pyrimidine and purine, H-2 and H-6, respectively, and the fairly large value of the dimer shift for the methyl protons of 6-methylpurine, 'feeling' almost the same shielding effect as the H-2 proton, suggest that the C-2 carbon atom of pyrimidine and the C-6 carbon atoms of purines are nearer to (or spend more time in the neighbourhood of) the association axis perpendicular to the planes of the interacting molecules. This molecular arrangement support the dominant role of the steric demand of the methyl group in the pyrimidine–6-methylpurine association: as pyrimidine overlaps the six-membered ring of 6-methylpurine at its methylated site, the methyl group increases the distance between the counterparts to such an extent that the steric effect leads to a considerable diminution in the dimer shifts.

The enhancement of the pyrimidine–purine hetero-association constant caused by the purine methylation, which can be ascribed to the higher polarizability of 6-methylpurine [47], and the slightly oriented plane-to-plane association structures reaffirm that a part of the stacking interaction is the dipolar interaction.

5. Conclusions

Although, the dimer decomposition model, applied here to interpret the mutually induced diamagnetic and virtual paramagnetic chemical shift changes in mixed solutions, outlines an average dimer structure substituting for the probably various actual association structures, the association parameters appear to be suitable for following

some of the consequences of chemical modifications in the counterparts. Thus, on the basis of the concept that the vertical stacking interaction between nucleic acid bases can be modelled and simulated by the interaction of monomeric bases and base analogues in aqueous media [1,48–50], the changes in the pyrimidine–purine hetero-association parameters caused by the purine methylation may refer to alterations in molecular recognition through the effect of base methylation on the local conformational and dynamic properties of nucleic acids.

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References

1 C. Altona, Recl. Trav. Chim. Pays-Bas 101 (1982) 413.
2 P.O.P. Ts'o, Basic principles in nucleic acid chemistry, Vols. 1–3 (Academic Press, New York, 1974).
3 W. Saenger, Principles of nucleic acid structure (Springer, New York, 1984).
4 B.W. Bangerter and S.I. Chan, J. Am. Chem. Soc. 91 (1969) 3910.
5 A.J. Hartel, P.P. Lankhorst and C. Altona, Eur. J. Biochem. 129 (1982) 343.
6 Y.T. van den Hoogen, P.P. Lankhorst, P. Gijsman, A.J. Hartel, J.H. van Boom and C. Altona, Eur. J. Biochem. 171 (1988) 143.
7 C.-H. Lee and I. Tinoco, Jr., Biophys. Chem. 11 (1980) 283.
8 P.P. Lankhorst, C.M. Groeneveld, G. Wille, J.H. van Boom, C. Altona and C.A.G. Haasnoot, Recl. Trav. Chim. Pays-Bas 101 (1982) 253.
9 Y.T. van den Hoogen, S.J. Treurniet, H.C. P.F. Roelen, E. de Vroom, G.A. van der Marel, J.H. van Boom and C. Altona, Eur. J. Biochem. 171 (1988) 155.
10 L.J. Rinkel, G.A. van der Marel, J.H. van Boom and C. Altona, Eur. J. Biochem. 163 (1987) 287.
11 E. Quignard, G.V. Fazakerley, R. Téoule, A. Guy and W. Guschlbauer, Eur. J. Biochem. 152 (1985) 99.
12 G.V. Fazakerley, R. Téoule, A. Guy, H. Fritzsche and W. Guschlbauer, Biochemistry 24 (1985) 4540.
13 L.J. Rinkel, G.A. van der Marel, J.H. van Boom and C. Altona, Eur. J. Biochem. 163 (1987) 275.
14 E. Quignard, R. Téoule and G.V. Fazakerley, Nucleic Acids Res. 13 (1985) 7829.
15 P.O.P. Ts'o, I.S. Melvin and A.C. Olson, J. Am. Chem. Soc. 85 (1963) 1289.
16 P.O.P. Ts'o and S.I. Chan, J. Am. Chem. Soc. 86 (1964) 4176.
17 S.I. Chan, M.P. Schweizer, P.O.P. Ts'o and G.K. Helmkamp, J. Am. Chem. Soc. 86 (1964) 4182.
18 B. Pullman, P. Claverie and J. Caillet, C.R. Acad. Sci. 260 (1965) 5387.
19 S.J. Gill, M. Downing and G.F. Sheats, Biochemistry 6 (1967) 272.
20 G.K. Helmkamp and N.S. Kondo, Biochim. Biophys. Acta 157 (1968) 242.
21 P. Pörschke and F. Eggers, Eur. J. Biochem. 26 (1972) 490.
22 M.G. Marenchic and J.M. Sturtevant, J. Phys. Chem. 77 (1973) 544.
23 H. Sapper and W. Lohmann, Biophys. Struct. Mech. 4 (1978) 327.
24 D.M. Cheng, L.S. Kan, P.O.P. Ts'o, C. Giessner-Prettre and B. Pullman, J. Am. Chem. Soc. 102 (1980) 525.
25 H. Lönnberg, J. Ylikoski and A. Vesala, J. Chem. Soc., Faraday Trans. I 80 (1984) 2439.
26 H. Sterk and H. Gruber, J. Am. Chem. Soc. 106 (1984) 2239.
27 I. Stokkeland and P. Stilbs, Biophys. Chem. 22 (1985) 65.
28 P. Martel, J. Phys. Chem. 89 (1985) 230.
29 H. Lönnberg and J. Ylikoski, Acta Chem. Scand. Ser. B 39 (1985) 417.
30 F. Aradi and A. Földesi, Magn. Reson. Chem. 25 (1987) 892.
31 H.-H. Paul, H. Sapper and W. Lohmann, Z. Naturforsch., Teil C 33 (1978) 870.
32 P.R. Young and N.R. Kallenbach, J. Mol. Biol. 145 (1981) 785.
33 P.L. Laugaa, A. Delbarre and B.P. Roques, Biochimie 63 (1981) 967.
34 K. Weller, H. Schütz and I. Petri, Biophys. Chem. 19 (1984) 289.
35 R. Rymdén and P. Stilbs, Biophys. Chem. 21 (1985) 145.
36 J.A. Chudek, R. Foster and R.L. MacKay, J. Chem. Soc., Faraday Trans. I 84 (1988) 1737.
37 E.T. Adams, Jr., Biophys. Chem. 34 (1989) 143.
38 J. Sandström, Dynamic NMR spectroscopy (Academic Press, New York, 1982).
39 R.R. Ernst, G. Bodenhausen and A. Wokaun, Principles of nuclear magnetic resonance in one and two dimensions (Clarendon Press, Oxford, 1987).
40 J.-L. Dimicoli and C. Hélène, J. Am. Chem. Soc. 95 (1973) 1036.
41 K.A. Connors, Binding constants. The measurement of molecular complex stability (Wiley, New York, 1987).
42 P.R. Bevington, Data reduction and error analysis for the physical sciences (McGraw-Hill, New York, 1969).
43 F. Aradi, Magn. Reson. Chem. 28 (1990) 246.

44 F. Aradi, Magn. Reson. Chem. 28 (1990) 1040.
45 M.P. Schweizer, S.I. Chan and P.O.P. Ts'o, J. Am. Chem. Soc. 87 (1965) 5241.
46 C. Giessner-Prettre and B. Pullman, Q. Rev. Biophys. 20 (1987) 113.
47 B. Pullman, J. Chem. Phys. 43 (1965) S233.
48 P. Cieplak and P.A. Kollman, J. Am. Chem. Soc. 110 (1988) 3734.
49 N.G. Williams, L.D. Williams and B.R. Shaw, J. Am. Chem. Soc. 111 (1989) 7205.
50 L.X. Dang and P.A. Kollman, J. Am. Chem. Soc. 112 (1990) 503.