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# $^1$H NMR Conformational Study of Antiherpetic C5-Substituted 2′-Deoxyuridines: Insight into the Nature of Structure–Activity Relationships

Jarosław Poznański,* Krzysztof Felczak,* Tadeusz Kulikowski,* and Mieczysław Remin†,[1]
†*Division of Biophysics, Institute of Experimental Physics, Department of Physics, University of Warsaw, al. Żwirki i Wigury 93, 02-089 Warsaw, Poland; and* **Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Pawinskiego 5a, 02-106 Warsaw, Poland*

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**$^1$H NMR study and conformational analysis of a broad series of biologically important C5-substituted 2′-deoxyuridines, including alkyl, halogen, vinyl, hydroxymethyl, and hydroxy derivatives as well as nitro, formyl, trifluoromethyl, and dimethylamino substituents, is presented. A thorough analysis of chemical shifts in correlation with C5-substituent electronegativity as well as calculations by SCF semi-empirical method of the formal charge localized on C6 carbon is discussed in terms of charge distribution for electron attracting and electron donating groups. Conformation of the sugar ring is determined from proton–proton coupling constants and described in terms of pseudorotation between two main puckering domains C2′*endo (S)* and C3′*endo (N)*. Generally, electron donating groups destabilise the *N* conformation, simultaneously decreasing the mean pseudorotation amplitude. Absolute assignments of the H5′ and H5″ methylene protons in $^1$H NMR spectra permitted the unequivocal determination of molar fractions of the three classical exocyclic C4′–C5′ rotamers *gauche*$^+$, *trans*, and *gauche*$^-$, and correlation of them with the sugar ring puckering domains. Conformation about the glycosidic bond is described in terms of equilibrium between two conformational regions, *anti* and *syn*. Finally, the role of the C5-substituent in the creation of cytotoxic activity is considered on the basis of a simplified model assuming that compound activity is a function of substituent polar surface, its molecular volume, and its molecule polarity defined at the relative partition of the polar atoms.** 

The modified C(5) 2′-deoxyuridines have been investigated extensively for experimental treatment of neoplastic (1) and viral diseases (2). While only a few of the 5-substituted 2′-deoxyuridines exert effective *in vivo* antitumor properties (1), a broad range of them show potent and selective *in vitro* and/or *in vivo* antiherpetic activity (2). Among them (*E*)-5-(3,3,3-trifluoro-1-propenyl) and the (*E*)-5-(2-bromovinyl)- and (*E*)-5-(2-iodovinyl)-analogues are the most potent and selective antiherpetic compounds (3). 5-Trifluoromethyl-, 5-iodo-, and 5-ethyl analogues were used in treatment of herpetic infections in humans (4).

To elucidate the role of the C(5)-substituent in determining the structural and biological properties of 2′-deoxyuridines, we performed $^1$H NMR study and conformational analysis of a series of C(5)-substituted 2′-deoxyuridines, including alkyl, halogen, vinyl, hydroxymethyl, and hydroxy derivatives as well as nitro, formyl, trifluoromethyl, and dimethylamino substituents. This broad series provides different classes of model compounds, which differ in terms of electronic properties of the substituent. Highly resolved $^1$H NMR spectra and improved simulation methods, with an error of line positions of 0.03 Hz, made possible determination of chemical shifts and proton–proton coupling constants, with the highest possible accuracy.

A high-resolution $^1$H NMR study and detailed conformational analysis may provide insight into the question of how the nature of the C5-substituent influences the conformational properties of the base and the sugar moiety in correlation with electronic properties. An original analysis of maps of pseudorotation amplitudes of the sugar ring shows that the nature of the C5-substituent is a significant factor in the conformational characteristics. Absolute assignments of the H5′ and H5″ methylene protons in $^1$H NMR spectra permitted unequivocal determination of molar fractions of the three classical exocyclic C4′–C5′ rotamers, *gauche*$^+$, *trans,* and *gauche*$^-$, and correlation of them with the sugar ring puckering domains.

[1] To whom correspondence should be addressed. Fax: 48 (22) 8220248. E-mail: remin@asp.biogeo.uw.edu.pl.

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To gain insight into the nature of structure–activity relationships, the role of the C5-substituent in the creation of cytotoxic activity is considered on the basis of a simplified prediction model. It is assumed that compound cytotoxicity is a function of substituent polar surface, its molecular volume, and its molecule polarity defined at the relative partition of the polar atoms.

## MATERIALS AND METHODS

*Chemistry.* 5-Chloro, 5-bromo, 5-iodo 2′-deoxyuridines were prepared by a cerium(IV)-mediated halogenation procedure (5). 3′,5′-Di-*O*-acetyl-2′-deoxyuridine with appropriate lithium halide in the presence of ammonium hexanitratocerate(IV) in acetonitrile provided acetylated 5-halo-2′-deoxyuridines, which were then subjected to deacetylation catalysed by sodium methoxide in methanol. 5-Alkyl-2′-deoxyuridines, 5-ethyl (6), 5-propyl (7), 5-isopropyl (8), 5-benzyloxymethyl (9), and 5-isopropoxymethyl (10), were prepared by condensation of the appropriate persililated 5-alkyluracil with acylated 1-*O*-methyl-2-deoxyribose (6). The condensation was accomplished using trimethylsilyl triflate (TMSOTf) as the Lewis acid in acetonitrile. 5-Nitro-2′-deoxyuridine (11) was prepared by a procedure (12) involving condensation of bis-trimethylsiloxy-5-nitrouracil in the presence of copper(I) iodide in chloroform. This coupling reaction yielded predominantly $\beta$ anomer of 3′,5′-di-*O*-*p*-toluyl-5-nitrodeoxyuridine, which after deprotection gave 5-nitro-2′-deoxyuridine. 5-Hydroxymethyl-2′-deoxyuridine was prepared according to the methodology of Kahilainen *et al.* 1985 (13). 5-Hydroxy-2′-deoxyuridine (14) was prepared from 2′-deoxyuridine by one-pot bromination with elemental bromine and controlled hydrolysis under slightly alkaline conditions. 5-Hydroxy-2′-deoxyuridine was alkylated in the presence of base with allyl bromide to give 5-allyloxy-2′-deoxyuridine (15). Palladium-mediated method (16) was used for synthesis of 5-vinyl (17), 5-allyl (18), (*E*)-5-(2-cyanovinyl) (18), (*E*)-5-(1-propenyl) (18), (Z)-5-(1-propenyl) (18), (*E*)-5-(2-bromovinyl) (19), (*E*)-5-(2-carboethoxyvinyl) (20), and (*E*)-5-(2-carboxyvinyl) (19) -2′-deoxyuridines. 5-Dimethylamino-2′-deoxyuridine (21) was prepared by reaction of 5-bromo-2′-deoxyuridine with dimethylamine by reported procedure.

*Melting points* (uncorrected) were measured on a Boetius microscopic hot stage. $^1$H NMR spectra for identification procedure were recorded on a Varian 500-MHz spectrometer in $D_2O$, with DSS as internal standard or in $CDCl_3$, with tetramethylsilane as internal standard. Column chromatography was performed on Merck silica gel 60 (0.04–0.063 mm). Thin-layer chromatography (TLC) was run on Merck silica gel $F_{254}$ glass plates (DC, 20 × 20 cm, 0.25 mm, No 1.05715). 2′-Deoxyuridine, thymidine, 5-trifluoromethyl-2′-deoxyuridine, and 5-fluoro-2′-deoxyuridine were obtained from Sigma (St. Louis, MO).

*5-Hydroxymethyl-2′-deoxyuridine.* To the pressure tube containing 25 ml of pyridine was added 1 g (4.38 mmol) of 2′-deoxyuridine, 3.5 g (117.7 mmol) of paraformaldehyde, and 1 g (3.75 mmol) of tetrabutylammonium fluoride (TBAF). The mixture was vigorously stirred and heated at 70°C until a complete transformation to product had occurred. The progress of the reaction was monitored by TLC on silica gel with the use of $CHCl_3$:MeOH, 8:2 (v/v), solvent system. After cooling to room temperature, the mixture was filtered through a 10-mm pad of Hyflo Super Cell, washed with ethanol, and concentrated to the oily residue. This was chromatographed on silica gel 60 using a gradient of $CHCl_3$/MeOH (100:0–70:30) as eluent, giving after recrystallization from ethanol the title compound 0.75 g (66%) mp 174–177°C (lit. (22) 176–179°C).

*Condensation procedure: 5-Isopropoxymethyl-2′-deoxyuridine.* To the stirred under argon suspension of 5-isopropoxymethyluracil (184 mg; 1 mmol) in 5 ml of dry MeCN, 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluylribofuranose (384 mg; 1 mmol) and BSTFA (0.5 ml; 1.88 mmol) were added. The mixture was stirred 30 min at 60°C. After cooling to 0°C to the clear solution TMSOTf (220:1, 1.2 mmol) was added dropwise. The reaction mixture was stirred at room temperature. Progress of the reaction was monitored by TLC using toluene/ethyl acetate (10:3, v/v) as solvent system. After standard workup, the crude product was crystallyzed from methanol. Crystalline product (250 mg) containing traces of $\alpha$-anomer afforded pure 1-(3′,5′-di-*O*-*p*-toluyl-2′-deoxy-$\beta$-D-ribofuranosyl)-5-isopropoxymethyluracil on recrystallization from methanol yields 150 mg (28%), mp 160–162°C; NMR ($CDCl_3$), $\delta$(ppm), 1.60 (6H, s, ar$CH_3$), 2.33–2.39 (1H, m, H2′), 2.42 (6H, d, ($CH_3$)$_2$ $J$ = 9.74 Hz), 2.70–2.74 (1H, m, C2″), 4.04 (2H, s, 5-$CH_2$-O), 4.56–4.58 (1H, m, H3′), 4.65–4.68 (1H, add, H5′), 4.68–4.74 (1H, add, H5″) 5.60–5.68 (1H, m, H4′), 6.42–6.45 (1H, dd, H1′) 7.62 (1H, s, H6), 7.90–7.98 (4H, m, ar), 8.1 (1H, s, N1). 100 mg of the above mentioned compound was deprotected with 5 ml of methanol saturated with ammonia at 0°C, giving after evaporation and crystallization the title compound in the yield of 52 mg (96%), mp 96–98°C. This compound was reported previously (10) but no mp was given.

*NMR experiments and data analysis.* All compounds were analysed by NMR $^1$H spectroscopy in $D_2O$ solution at *ca.* 4 mM concentration. The spectra were measured alternatively on a 500-MHz Varian UX-NMR or Bruker Aspect 2000 spectrometer with presaturation of the resting water resonance. The spectra were accumulated at 298 K with 6-kHz band width and 32K digital resolution and then processed with the help of MastRe-C program (23) using 64K zero filling and lorenzian filter resulting in 0.2-Hz line broadening. When required, precise estimation of small coupling constants (e.g. H1′-5F in 5F-dU) was carried out with a pure sine-bell filter instead of lorenzian. The line positions were determined by parabolic interpolation with an error of 0.03 Hz. Chemical shifts and coupling constants (see Table 1) were determined by the locally made program ISSSS (24) based on the LAOCOON II (25) strategy using estimated positions of the individual resonance lines. An example of the simulation is presented in Fig. 1. In the case of extremely strongly coupled systems the version of the program based on the iterative fitting to the parts of experimental spectrum was used. The furanose ring puckering analysis was done with the aid of the program based on the Altona *et al.* algorithm and parametrization (26), analogously as PSEUROT (27) does. Electron density calculations were done for the 5-substituted 1-methyl-uracil analogues using MOPAC-6.0 (28) program in PM3 parametrization (29). Structural calculations and molecular graphics were done with the help of Sybyl program using tripos forcefield (30).

## RESULTS AND DISCUSSION

### *C5-Substituent Influences the Charge Distribution*

Chemical shift of the H6 proton varies significantly upon substitution on the C5 carbon. Electron attracting groups (nitro, trifluoromethyl, formyl) cause a downfield shift of H6 resonance whereas the electron donating groups (amino, methyl, ethyl) effect in the opposite direction. As is presented in Fig. 2a, the chemical shift of H6 is a good measure of the both C5-substituent electronegativity and, as calculated by SCF semi-empirical method, the formal charge localized on C6 carbon, Q(C6). The best correlation was obtained for substituent parametrization by Hammet $\sigma_p$ constant (31). Finally it could be concluded that chemical shift of the H6 proton is a precise measure of the electron density distribution changes upon substitution on C5 position and so can be used as a value

**TABLE 1**

$^1$H Chemical Shifts and Proton–Proton Coupling Constants for a Series of 5-Substituted 2′-Deoxyuridines in $D_2O$

| | | Proton chemical shift (ppm) | | | | | | | | Proton–proton coupling constant (Hz) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5-Substituent | 1′ | 2′ | 2″ | 3′ | 4′ | 5′ | 5″ | 6 | 1′2′ | 1′2″ | 2′2″ | 2′3′ | 2″3′ | 3′4′ | 4′5′ | 4′5″ | 5′5″ |
| 1 | Hydrogen | 6,27 | 2,36 | 2,40 | 4,45 | 4,03 | 3,82 | 3,74 | 7,83 | 6,65 | 6,62 | −14,25 | 6,80 | 4,05 | 3,73 | 3,49 | 5,14 | −12,48 |
| 2 | Methyl | 6,29 | 2,37 | 2,37 | 4,46 | 4,01 | 3,83 | 3,76 | 7,64 | 6,76 | 6,76 | | 5,45 | 5,45 | 3,86 | 3,66 | 4,95 | −12,46 |
| 3 | Ethyl | 6,30 | 2,38 | 2,38 | 4,47 | 4,02 | 3,84 | 3,77 | 7,63 | 6,83 | 6,83 | | 5,35 | 5,35 | 3,98 | 3,40 | 4,56 | −12,53 |
| 4 | Propyl | 6,29 | 2,37 | 2,38 | 4,47 | 4,02 | 3,83 | 3,76 | 7,65 | 6,53 | 6,74 | −14,15 | 7,40 | 3,42 | 3,99 | 3,48 | 4,57 | −12,47 |
| 5 | Isopropyl | 6,32 | 2,40 | 2,40 | 4,50 | 4,05 | 3,86 | 3,79 | 7,64 | 6,61 | 6,61 | | 5,48 | 5,48 | 3,94 | 3,29 | 4,35 | −12,46 |
| 6 | Allyl | 6,29 | 2,37 | 2,38 | 4,46 | 4,02 | 3,82 | 3,75 | 7,66 | 6,70 | 6,62 | −14,22 | 7,52 | 3,49 | 4,15 | 3,46 | 4,74 | −12,50 |
| 7 | Fluor | 6,27 | 2,33 | 2,41 | 4,45 | 4,04 | 3,84 | 3,76 | 8,03 | 6,61 | 6,20 | −14,27 | 6,71 | 3,89 | 3,81 | 3,49 | 4,91 | −12,52 |
| 8 | Chlor | 6,25 | 2,36 | 2,43 | 4,46 | 4,04 | 3,85 | 3,77 | 8,16 | 6,62 | 6,48 | −14,24 | 6,68 | 4,22 | 4,22 | 3,45 | 4,69 | −12,55 |
| 9 | Brom | 6,25 | 2,37 | 2,43 | 4,46 | 4,04 | 3,85 | 3,77 | 8,23 | 6,57 | 6,57 | −14,17 | 6,68 | 4,42 | 4,30 | 3,45 | 4,68 | −12,55 |
| 10 | Iod | 6,23 | 2,37 | 2,42 | 4,46 | 4,04 | 3,86 | 3,77 | 8,38 | 6,40 | 6,45 | −14,44 | 6,79 | 4,34 | 4,22 | 3,54 | 4,48 | −12,60 |
| 11 | Trifluoromethyl | 6,24 | 2,41 | 2,48 | 4,46 | 4,07 | 3,88 | 3,78 | 8,60 | 5,77 | 6,44 | −14,19 | 6,37 | 5,25 | 4,61 | 3,06 | 4,14 | −12,69 |
| 12 | Vinyl | 6,28 | 2,40 | 2,42 | 4,48 | 4,04 | 3,86 | 3,78 | 7,99 | 6,16 | 6,75 | −14,11 | 6,43 | 4,47 | 4,37 | 3,31 | 4,37 | −12,56 |
| 13 | (*E*)-2-Bromovinyl | 6,26 | 2,38 | 2,42 | 4,46 | 4,04 | 3,85 | 3,77 | 7,90 | 6,50 | 6,58 | −12,26 | 6,70 | 4,18 | 4,08 | 3,54 | 4,69 | −12,52 |
| 14 | (*E*)-2-Cyanovinyl | 6,27 | 2,41 | 2,48 | 4,48 | 4,09 | 3,89 | 3,79 | 8,22 | 6,43 | 6,64 | −14,13 | 6,61 | 4,13 | 4,26 | 3,33 | 4,74 | −12,59 |
| 15 | (*E*)-2-Carboxyvinyl | 6,28 | 2,42 | 2,46 | 4,48 | 4,08 | 3,88 | 3,79 | 8,27 | 6,45 | 6,72 | −14,35 | 6,51 | 4,69 | 4,25 | 3,37 | 4,64 | −12,56 |
| 16 | (*E*)-2-Carboetoxyvinyl | 6,28 | 2,42 | 2,48 | 4,30 | 4,09 | 3,90 | 3,81 | 8,27 | 6,46 | 6,51 | −14,20 | 6,58 | 4,51 | 4,13 | 3,30 | 4,73 | −12,56 |
| 17 | (*E*)-1-Propenyl | 6,30 | 2,41 | 2,42 | 4,50 | 4,05 | 3,87 | 3,79 | 7,87 | 6,56 | 6,58 | −14,19 | 6,93 | 4,58 | 3,85 | 3,42 | 5,19 | −12,87 |
| 18 | (*Z*)-1-Propenyl | 6,36 | | | 4,49 | 4,07 | 3,84 | 3,77 | 7,82 | | | | | | | 3,37 | 4,53 | −12,55 |
| 19 | Hydroxymethyl | 6,29 | 2,38 | 2,41 | 4,47 | 4,04 | 3,85 | 3,77 | 7,89 | 6,71 | 6,68 | −14,23 | 6,51 | 4,17 | 4,00 | 3,40 | 4,83 | −12,54 |
| 20 | Isopropoxymethyl | 6,32 | 2,40 | 2,40 | 4,50 | 4,06 | 3,87 | 3,79 | 7,65 | 6,69 | 6,58 | −14,21 | 6,88 | 4,32 | 3,96 | 3,41 | 4,79 | −12,55 |
| 21 | Benzyloxymethyl | 6,24 | 2,32 | 2,41 | 4,45 | 4,04 | 3,83 | 3,75 | 7,87 | 6,59 | 6,51 | −14,26 | 6,61 | 4,18 | 4,12 | 3,33 | 4,78 | −12,46 |
| 22 | Metoxymethyl | 6,27 | 2,37 | 2,42 | 4,47 | 4,03 | 3,84 | 3,76 | 7,98 | 6,20 | 6,50 | | 6,10 | 4,40 | 4,00 | 3,10 | 4,60 | |
| 23 | Hydroxy | 6,31 | 2,35 | 2,37 | 4,47 | 4,03 | 3,84 | 3,77 | 7,45 | 7,02 | 6,65 | −14,25 | 6,49 | 4,10 | 3,76 | 3,52 | 5,05 | −12,47 |
| 24 | Allyloxy | 6,32 | 2,35 | 2,41 | 4,49 | 4,05 | 3,87 | 3,80 | 7,57 | 6,73 | 6,53 | −14,25 | 6,87 | 4,16 | 3,93 | 3,17 | 4,19 | −12,48 |
| 25 | Dimethylamino | 6,34 | 2,40 | 2,41 | 4,51 | 4,06 | 3,88 | 3,81 | 7,47 | 6,28 | 6,78 | −14,18 | 6,34 | 4,73 | 3,95 | 3,23 | 4,06 | −12,51 |
| 26 | Formyl | 6,25 | 2,46 | 2,56 | 4,49 | 4,14 | 3,92 | 3,82 | 8,77 | 5,60 | 6,50 | | 6,70 | 5,00 | 3,90 | 3,20 | 4,70 | |
| 27 | Nitro | 6,32 | 2,40 | 2,40 | 4,50 | 4,06 | 3,87 | 3,79 | 9,60 | 4,97 | 6,55 | −14,20 | 6,38 | 5,62 | 4,87 | 3,05 | 4,08 | −12,73 |
| 28 | Cyanurique 2′dUrd | | | | | | | | | 6,00 | 8,20 | | 7,70 | 5,50 | 5,10 | 3,50 | 6,40 | |

characterizing electronic properties of the substituted base moiety.

*Conformation about Glycosidic Bonds*

The solution conformation of nucleosides about glycosidic bond is generally described in terms of equilibrium between two conformations, *anti* and *syn* (32–34). Such a description is justified for pyrimidine nucleosides where there are two significant barriers for the rotation about glycosidic bond (35). A thorough analysis of chemical shifts of H2′ and H2″ in $\alpha$- and $\beta$-pyrimidine 2′-deoxyribonucleosides in the *anti* and *syn* conformation has been presented (33). Using thymidine as an *anti* model and 6-methyluridine as a *syn* model, it was shown that the conformational change from *anti* to *syn* leads to a large downfield (0.6 ppm) displacement of the *cis* H2′ proton and a much smaller upfield (0.1 ppm) shift of the *trans* H2″ proton. The two protons, H2′ and H2″, show similar chemical shifts (2.37 ppm) in thymidine. In a series of 5-substituted 2′-deoxyribonucleosides, H2′ and H2″ lie usually in relatively narrow ranges, 2.32–2.42 and 2.37–2.48 ppm, respectively, but in 5-formyl-2′deoxyuridine, they are slightly greater, 2.46 and 2.56 ppm, respectively. The chemical shifts of H2′ and H2″ protons thus indicate that generally these compounds exist predominately in the *anti* conformation. This interpretation is consistent with the conformation of the 5′-exocyclic group about the C4′–C5′ bond (see section "Exocyclic C4′–C5′ Rotamers").

A correlation of chemical shifts of the H2′ and H2″ protons (Fig. 2b) proves the existence of two separate correlation patterns distinguishing the electron donating substituents (methyl, ethyl, propyl, isopropyl, vinyl, allyl, propenyl, hydroxymethyl, isopropoxymethyl, and amino; lower correlation pattern) from the others (upper correlation line). This clearly indicates an electron-density-driven change of the glycoside bond angle equilibrium, proving different mechanisms of charge distribution for electron attracting and electron donating groups (36).

*Conformation of the Sugar Moiety*

This is usually described in terms of pseudorotation between two main puckering domains, C2′*endo* (S) and C3′*endo* (N) (37–42). The relative populations of the two are derived from the proton–proton vicinal coupling constants (40), based on Altona's parameterization (39) of the Karplus relations, assuming average regions of particular conformations in the solid state (43). In particular the populations S and N are estimated from the equation $S = (J_N - J_{exp})/(J_N - J_S)$,

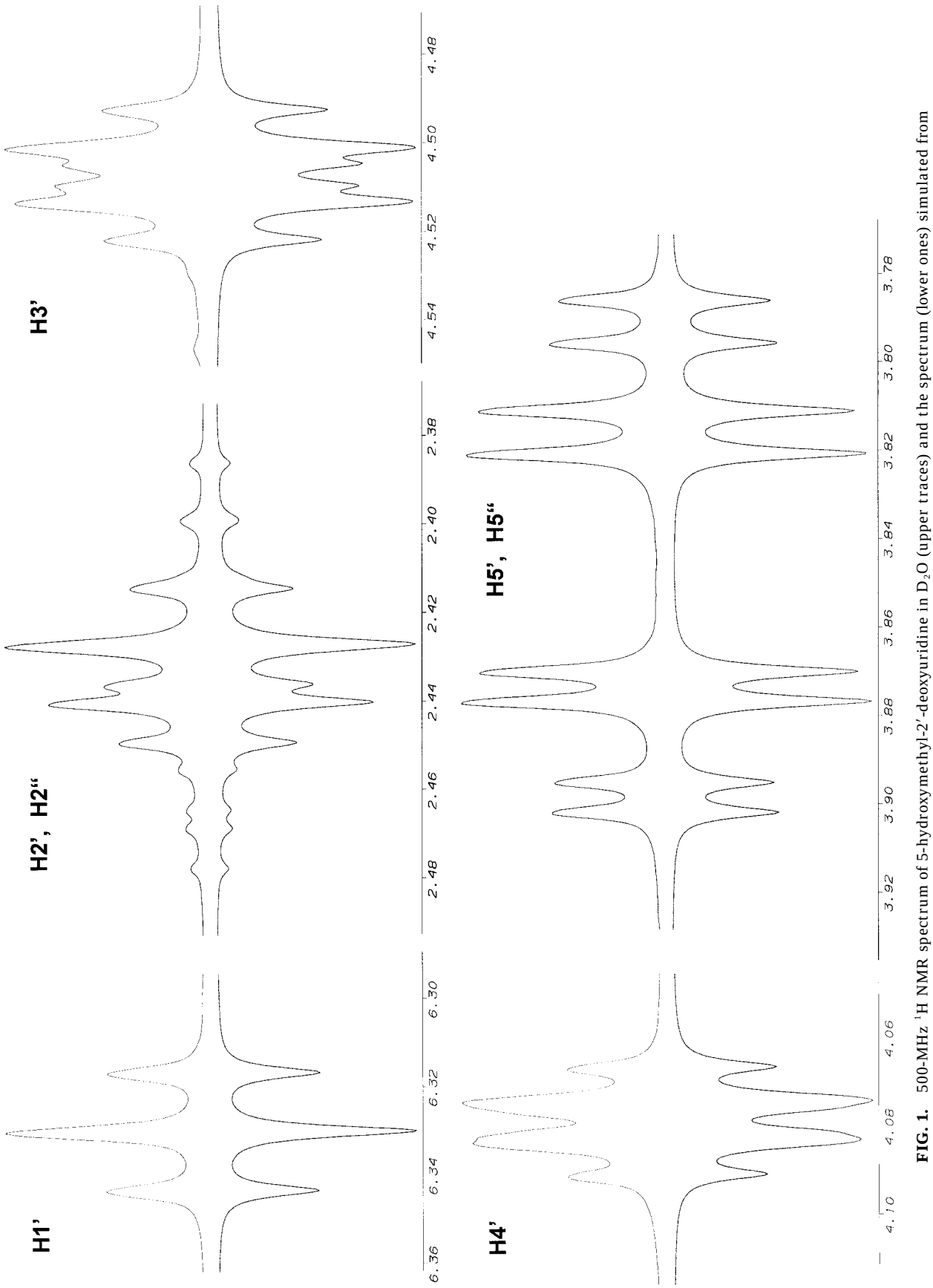


**FIG. 1.** 500-MHz $^1$H NMR spectrum of 5-hydroxymethyl-2′-deoxyuridine in $D_2O$ (upper traces) and the spectrum (lower ones) simulated from the data in Table 1.

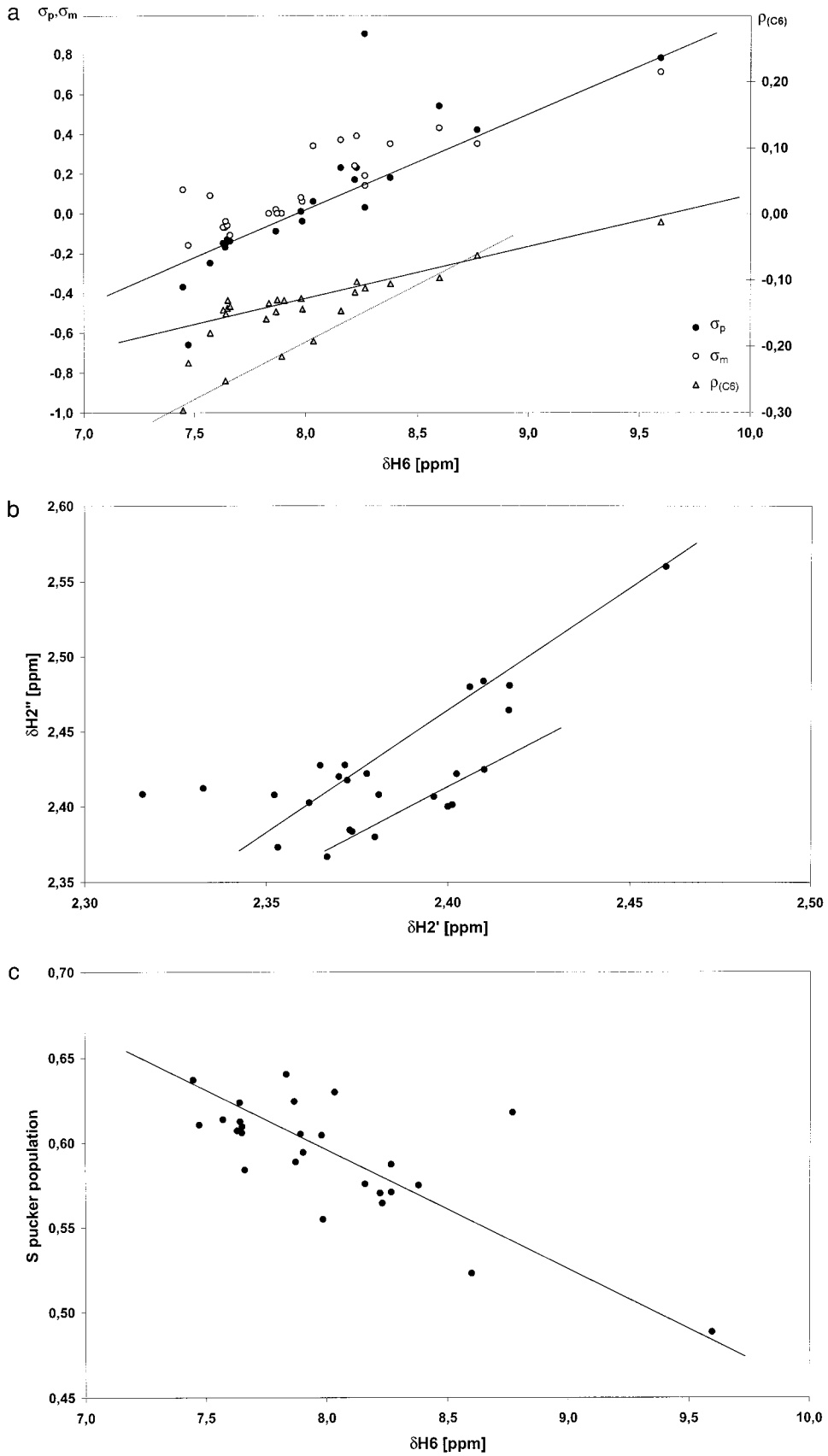


**FIG. 2.** (a) Correlation of Hammett constants, meta and para, and the SCF semi-empirical charge localized on C6 carbon, with chemical shift of the H6 proton. (b) Correlations of chemical shifts of the H2′ and H2″ protons. (c) Correlation of the molar fraction of S (C2′*endo*) pucker with the chemical shift of H6. (d) Correlation of the pseudorotation puckering amplitude with the chemical shift of H6. (e) Correlation of molar fractions of the C4′–C5′ rotamers, with the chemical shift of H6. (f) Correlation of the proportions of combinations of sugar ring puckers and C4′–C5′ rotamers, $F_{12} = Ng^{+}/Nt$ vs $F_{34} = Sg^{+}/St$.

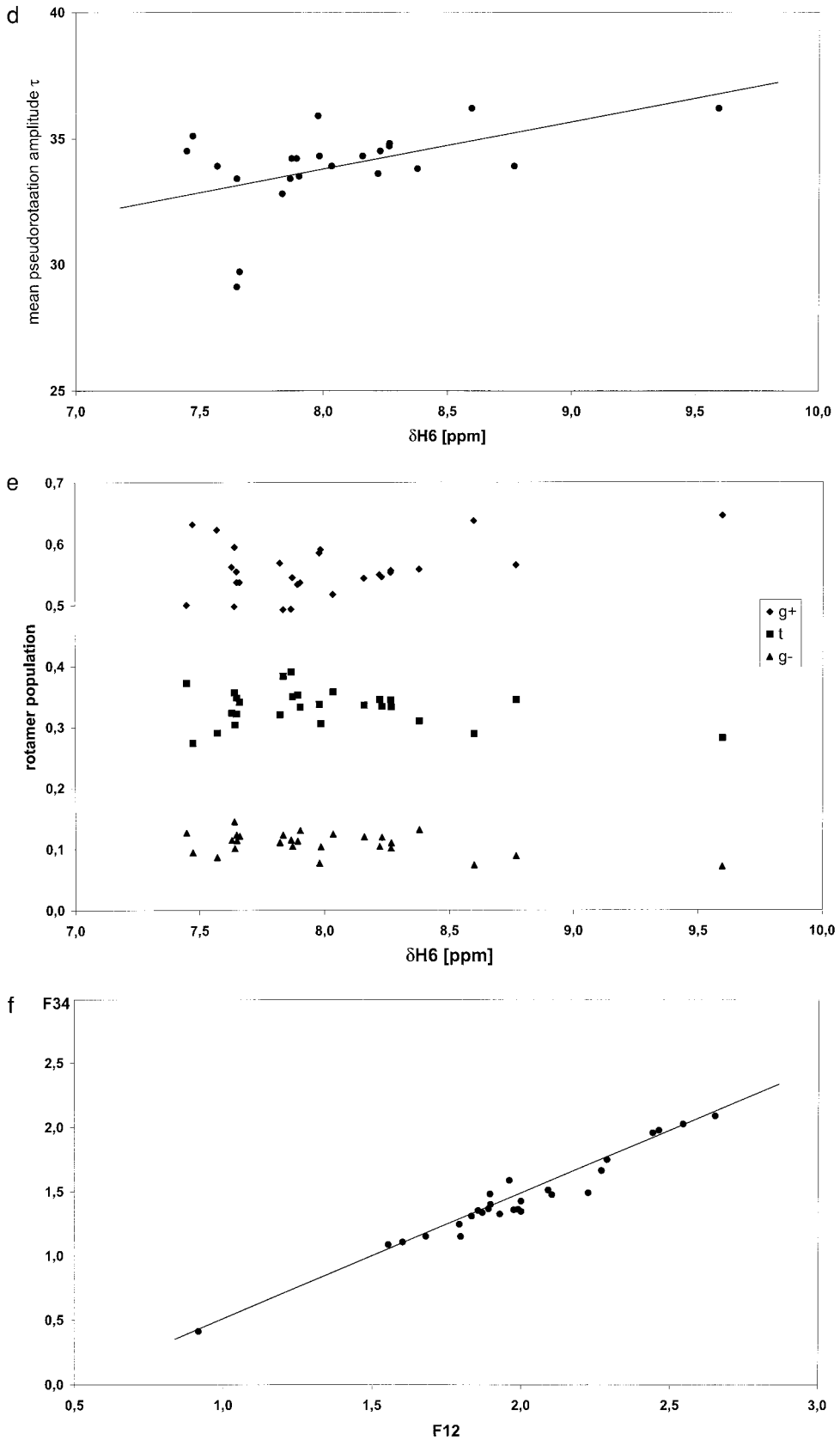


**FIG. 2**—*Continued*

**TABLE 2**

Conformational Parameters, Molar Fractions of Sugar Ring Puckers, S and N, and C4′–C5′ Rotamers, $g^+$, t, and $g^-$, and Their Combinations, $Ng^+$, Nt, $Sg^+$, St, and $Sg^-$, and Proportions, $F_{12} = Ng^+/Nt$ and $F_{34} = Sg^+/St$, as Well as Pseudorotation Amplitude and Molar Fraction of the N Pucker, N(5), as Derived from Full Pseudorotation Analysis, and Also Electronic Parameters, Hammett Constants of C(5)-Substituents and Electron Density Localized at C6 Carbon, Q(C6)

| | Conformar population | | | | | | | | | | | Hammett constant | | Sugar ring analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5-Substituent | S | g+ | t | g− | Ng+ | Nt | Sg+ | St | Sg− | F12 | F34 | *Meta* | *Para* | $\tau$ | N(5) | N(1) | R.M.S. | Q(C6) |
| Hydrogen | 0,64 | 0,49 | 0,38 | 0,12 | 0,22 | 0,14 | 0,27 | 0,25 | 0,12 | 1,60 | 1,10 | 0,00 | 0,00 | 32,8 | 0,36 | 0,36 | 0,25 | −0,14 |
| Methyl | 0,62 | 0,50 | 0,36 | 0,14 | 0,24 | 0,13 | 0,26 | 0,22 | 0,14 | 1,80 | 1,15 | −0,07 | −0,17 | | | 0,38 | | −0,25 |
| Ethyl | 0,61 | 0,56 | 0,32 | 0,11 | 0,27 | 0,13 | 0,30 | 0,20 | 0,11 | 2,09 | 1,51 | −0,07 | −0,15 | | | 0,39 | | −0,15 |
| Propyl | 0,61 | 0,55 | 0,32 | 0,12 | 0,27 | 0,13 | 0,29 | 0,19 | 0,12 | 2,11 | 1,48 | −0,06 | −0,13 | 29,1 | 0,36 | 0,39 | 0,39 | −0,14 |
| Isopropyl | 0,61 | 0,59 | 0,30 | 0,10 | 0,27 | 0,12 | 0,33 | 0,19 | 0,10 | 2,29 | 1,75 | −0,04 | −0,15 | | | 0,39 | | −0,15 |
| Allyl | 0,58 | 0,54 | 0,34 | 0,12 | 0,27 | 0,14 | 0,26 | 0,20 | 0,12 | 1,93 | 1,32 | −0,11 | −0,14 | 29,7 | 0,36 | 0,42 | 0,40 | −0,14 |
| Fluor | 0,63 | 0,52 | 0,36 | 0,12 | 0,24 | 0,13 | 0,28 | 0,23 | 0,12 | 1,79 | 1,24 | 0,34 | 0,06 | 33,9 | 0,36 | 0,37 | 0,43 | −0,19 |
| Chlor | 0,58 | 0,54 | 0,34 | 0,12 | 0,28 | 0,14 | 0,26 | 0,19 | 0,12 | 1,98 | 1,36 | 0,37 | 0,23 | 34,3 | 0,39 | 0,42 | | −0,15 |
| Brom | 0,56 | 0,55 | 0,33 | 0,12 | 0,29 | 0,15 | 0,26 | 0,19 | 0,12 | 1,99 | 1,36 | 0,39 | 0,23 | 34,5 | 0,41 | 0,44 | 0,22 | −0,10 |
| Iod | 0,57 | 0,56 | 0,31 | 0,13 | 0,29 | 0,13 | 0,27 | 0,18 | 0,13 | 2,23 | 1,49 | 0,35 | 0,18 | 33,8 | 0,41 | 0,43 | 0,33 | −0,11 |
| Trifluoromethyl | 0,52 | 0,64 | 0,29 | 0,07 | 0,34 | 0,14 | 0,30 | 0,15 | 0,07 | 2,46 | 1,98 | 0,43 | 0,54 | 36,2 | 0,48 | 0,48 | 0,28 | −0,10 |
| Vinyl | 0,55 | 0,59 | 0,31 | 0,10 | 0,31 | 0,14 | 0,28 | 0,17 | 0,10 | 2,27 | 1,66 | 0,06 | −0,04 | 34,3 | 0,43 | 0,45 | 0,23 | −0,14 |
| (*E*)-2-Bromovinyl | 0,59 | 0,54 | 0,33 | 0,13 | 0,27 | 0,14 | 0,27 | 0,20 | 0,13 | 2,00 | 1,35 | | | 33,5 | 0,39 | 0,41 | 0,26 | −0,13 |
| (*E*)-2-Cyanovinyl | 0,57 | 0,55 | 0,35 | 0,10 | 0,28 | 0,15 | 0,27 | 0,20 | 0,10 | 1,89 | 1,37 | 0,24 | 0,17 | 33,6 | 0,40 | 0,43 | 0,28 | −0,12 |
| (*E*)-2-Carboxyvinyl | 0,57 | 0,56 | 0,33 | 0,11 | 0,29 | 0,14 | 0,27 | 0,19 | 0,11 | 2,00 | 1,42 | 0,14 | 0,90 | 34,8 | 0,42 | 0,43 | 0,13 | −0,11 |
| (*E*)-2-Carboetoxyvinyl | 0,59 | 0,55 | 0,34 | 0,10 | 0,27 | 0,14 | 0,28 | 0,20 | 0,10 | 1,90 | 1,40 | 0,19 | 0,03 | 34,7 | 0,41 | 0,41 | 0,24 | −0,11 |
| (*E*)-1-Propenyl | 0,62 | 0,49 | 0,39 | 0,11 | 0,23 | 0,15 | 0,26 | 0,24 | 0,11 | 1,56 | 1,09 | 0,02 | −0,09 | 33,4 | 0,40 | 0,38 | 0,32 | −0,15 |
| (*Z*)-1-Propenyl | | 0,57 | 0,32 | 0,11 | | | | | | | | | | | | | | −0,16 |
| Hydroxymethyl | 0,61 | 0,53 | 0,35 | 0,11 | 0,26 | 0,14 | 0,28 | 0,21 | 0,11 | 1,84 | 1,31 | 0,00 | 0,00 | 34,2 | 0,38 | 0,39 | 0,16 | −0,22 |
| Isopropoxymethyl | 0,61 | 0,54 | 0,35 | 0,11 | 0,25 | 0,14 | 0,28 | 0,21 | 0,11 | 1,87 | 1,33 | | | 33,4 | 0,39 | 0,39 | 0,26 | −0,13 |
| Benzyloxymethyl | 0,59 | 0,54 | 0,35 | 0,10 | 0,27 | 0,14 | 0,28 | 0,21 | 0,10 | 1,86 | 1,35 | | | 34,2 | 0,39 | 0,41 | 0,26 | −0,13 |
| Metoxymethyl | 0,60 | 0,58 | 0,34 | 0,08 | 0,26 | 0,13 | 0,32 | 0,20 | 0,08 | 1,96 | 1,59 | 0,08 | 0,01 | 35,9 | 0,40 | 0,40 | 0,27 | −0,13 |
| Hydroxy | 0,64 | 0,50 | 0,37 | 0,13 | 0,23 | 0,14 | 0,27 | 0,24 | 0,13 | 1,68 | 1,15 | 0,12 | −0,37 | 34,5 | 0,35 | 0,36 | 0,11 | −0,30 |
| Allyloxy | 0,61 | 0,62 | 0,29 | 0,09 | 0,27 | 0,11 | 0,35 | 0,18 | 0,09 | 2,44 | 1,96 | 0,09 | −0,25 | 33,9 | 0,38 | 0,39 | 0,27 | −0,18 |
| Dimethylamino | 0,61 | 0,63 | 0,27 | 0,09 | 0,28 | 0,11 | 0,35 | 0,17 | 0,09 | 2,65 | 2,09 | −0,16 | −0,66 | 35,1 | 0,41 | 0,39 | 0,10 | −0,23 |
| Formyl | 0,62 | 0,57 | 0,34 | 0,09 | 0,25 | 0,13 | 0,32 | 0,21 | 0,09 | 1,90 | 1,48 | 0,35 | 0,42 | 33,9 | 0,36 | 0,38 | 0,43 | −0,06 |
| Nitro | 0,49 | 0,65 | 0,28 | 0,07 | 0,37 | 0,14 | 0,28 | 0,14 | 0,07 | 2,54 | 2,03 | 0,71 | 0,78 | 36,2 | 0,54 | 0,51 | 0,30 | −0,01 |
| Cyanurique 2′dUrd | 0,46 | 0,36 | 0,52 | 0,12 | 0,26 | 0,28 | 0,10 | 0,24 | 0,12 | 0,92 | 0,41 | | | 28,7 | 0,56 | 0,54 | 0,32 | |

where $J_{exp}$ is the experimentally determined coupling constant $J_{3',4'}$, and $J_N = 8.4$ Hz and $J_S = 1.1$ Hz are the limiting values of $J_{3',4'}$ for deoxyribosides, calculated from corresponding average phase angles, $P_N$, 14° and $P_S$, 169°, and puckering amplitudes, $\tau_N$, 37° and $\tau_S$, 38°, in the solid state (43). The results for the S and N molar fractions are presented in Table 2. C2′*endo* (S) is generally preferred (50–65%) in a whole series.

When all vicinal coupling constants were determined, a full pseudorotation analysis was performed. Mean pseudorotation amplitude was determined as follows. For the two-state model of equilibrium between the N and S puckers, five parameters, $X_N$, $\tau_N$, $\tau_S$, $\phi_N$ and $\phi_S$, are used to fit five coupling constants values $^3J_{1'2'}$ $^3J_{1'2''}$, $^3J_{2'3'}$, $^3J_{2''3'}$, $^3J_{3'4'}$, to the experimental values (Table 2). The analysis of the maps representing $\chi^2$ value (defined as the sum of squared deviation between calculated and experimental coupling constants) when $\tau_N$ and $\tau_S$ are fixed is presented in Fig. 3. It demonstrates that regions of the lowest $\chi^2$ provide a best fit and are located close to the $\tau_N + \tau_S = 2\tau$ line. Thus $\tau$ can be interpreted as the mean pseudorotation amplitude of the sugar moiety. The populations of the N and S puckering domains do not vary upon $\tau_N$, $\tau_S$ change, so they are determined from the five-parameter model.

Finally, the two parameters, S and $\tau$, provide conformational characteristics of the sugar moiety. As presented in Table 2, both parameters vary with substitution of the C5 carbon. Generally, electron donating groups stabilize the S conformation, lowering the mean pseudorotation amplitude. The latter agrees with the hypothesis (36, 44) of the role played in conformational equilibrium by unfavorable interaction of the C6 $\pi$ orbital with the nonbonded orbital of O4′ occupied by an electron pair. In this point of view, an increase of $\pi$-electron density located on C6 disables large movements of O4′, which lowers $\tau$. Analogously, electron attracting C5-substituents decrease the $\pi$-electron density on C6, enabling larger pseudorotation amplitude.

Figures 2c and 2d show correlation of the two parameters, S and $\tau$, with the chemical shift of H6, which represents a measure of both C5-substituent electronegativity and a formal charge localized on the C6 carbon, as calculated by SCF semi-empirical method. In principle, postulated mechanism of $\pi$(C6)–$n$(O4′) orbital interaction distinguish between N and S puckering forms. In the S one, O4′ oxygen is pointed toward C6, which decreases conformational flexibility (in terms of pseudorotational

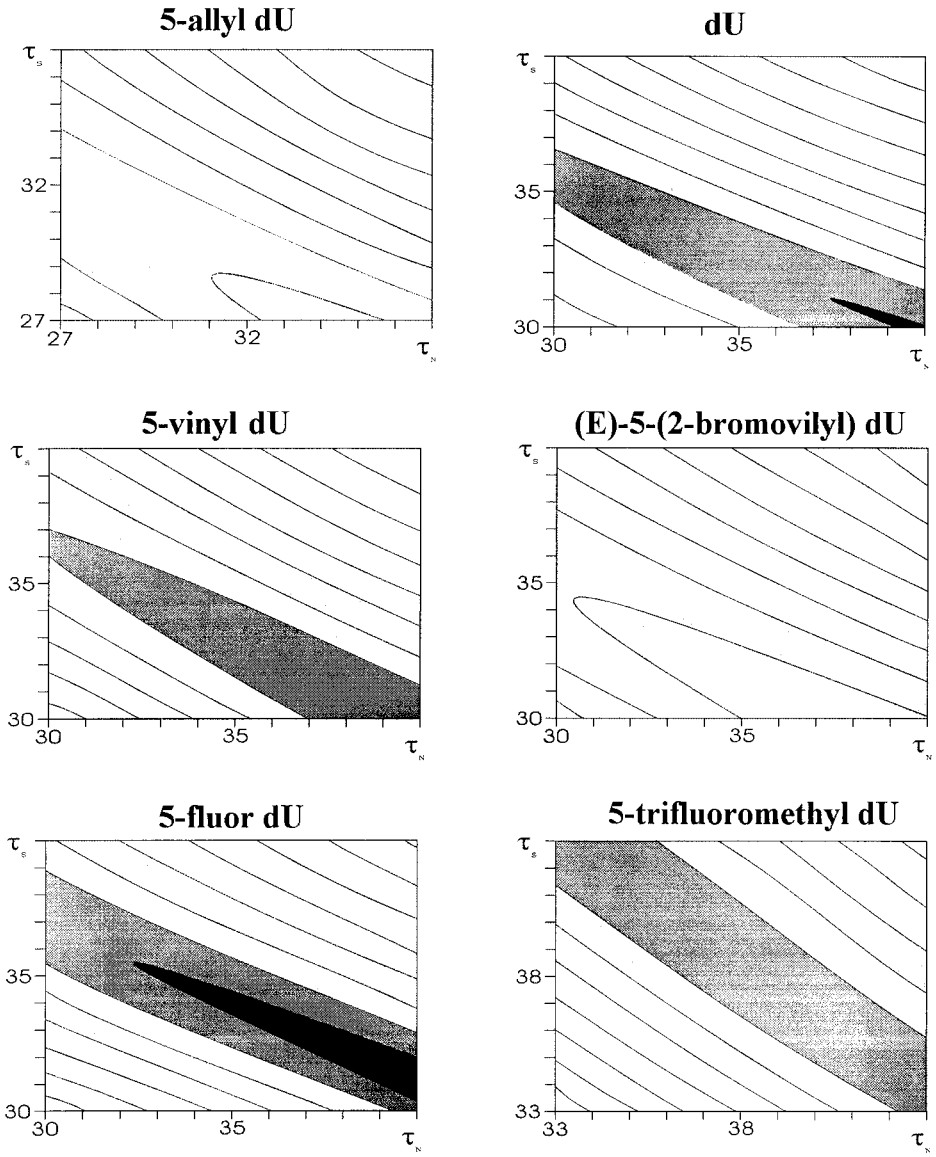


**FIG. 3.** Pseudorotation analysis of the sugar puckering based on the five experimental proton–proton coupling constants values, ${}^3J_{1'2'}$ ${}^3J_{1'2''}$, ${}^3J_{2'3'}$, ${}^3J_{2''3'}$, ${}^3J_{3'4'}$. Maps represent $\chi^2$ of the model fit as a function of a given pseudorotation amplitudes, $\tau_N$ and $\tau_S$, of the N and S sugar puckers. Estimated sugar pucker falls in the region of the lowest $\chi^2$ representing the best fit.

amplitude, $\tau$) whereas in the N one, larger $\tau$ values are possible. As a result, the positive correlation of $\tau$ and population of N pucker form is observed ($R$ = 0.5); but $R$ was increased up to 0.9 for two separate subgroups attributed mainly to more and less polar substituents (figure not presented), which agrees with earlier conformational analysis (42).

*Exocyclic C4′–C5′ Rotamers*

The original absolute assignment of the H5′ and H5″ protons in $^1$H NMR spectra was based on the deshielding effect of the phosphate group on H5′ and H5″ in 3′-monophosphates of uridine and pseudouridine (45). It was showed that the H5′ and H5″ spectral region shows a similar characteristic spectral pattern: $\delta(H5') > \delta(H5'')$ and $J_{4',5'} < J_{4',5''}$ in a number of other nucleosides and nucleotides (45). It appears reasonable therefore to assume that for all these nucleosides where this pattern is observed the more shielded proton is identifiable as H5″. This absolute assignment has been extensively used and discussed (32, 39, 46). This assignment was also corroborated by Richie and Perlin by stereospecific deuteration of adenosine and its derivatives (47).

Absolute assignments of the H5′ and H5″ methylene protons in $^1$H NMR spectra and measurements of

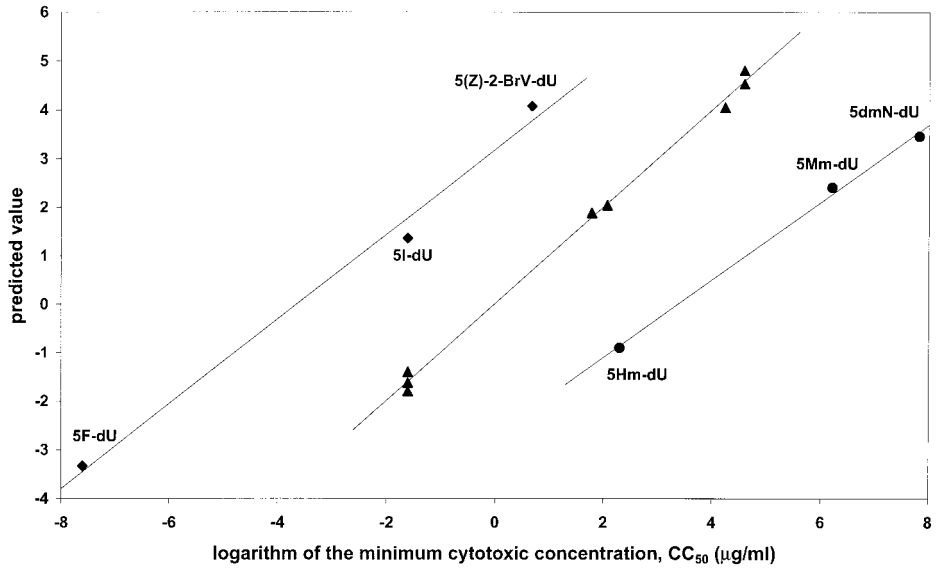


**FIG. 4.** *X* axis: Minimum cytotoxic concentration, $CC_{50}$, a concentration required to reduce viability of primary rabbit kidney cells by 50%. $CC_{50}$ values were adopted from Ref. 49. *Y* axis: A value predicted from structural parameters of C5-substituent. This is assumed that compound activity is a function of substituent polar surface, its molecular volume, and its molecule polarity defined at the relative partition of the polar atoms. Abbreviations: 5F-dU: 2′-deoxy-5-fluorouridine; 5I-dU: 2′-deoxy-5-iodouridine; 5(*Z*)-2BrV-dU: 2′-deoxy-5-bromovinyluridine; 5Hm-dU: 2′-deoxy-5-hydroxymethyluridine; 5Mm-dU: 2′-deoxy-5-methoxymethyluridine; 5dmN-dU: 2′-deoxy-5-dimethylaminouridine.

the $J_{4',5'}$ and $J_{4',5''}$ proton–proton coupling constants permitted unequivocal determination of molar fractions of the three classical exocyclic C4′–C5′ rotamers, *gauche*$^+$, *trans*, and *gauche*$^-$ ($g^+$, t and $g^-$), and their correlation with the sugar ring puckering domains, C2′*endo* (S) and C3′*endo* (N) (40, 41, 48). The molar fractions of all significant combinations of sugar puckers, N and S, and exocyclic C4′–C5′ rotamers, $g^+$, t, and $g^-$, viz., $Ng^+$, Nt, $Sg^+$, St, and $Sg^-$, are determined according to Ref. 40. $F_{12}$ and $F_{34}$ represent proportions $Ng^+$/Nt and $Sg^+$/St, respectively. The results are given in Table 2. A significant preference (50–60%) of the *gauche*$^+$ rotamer of the 5′-exocyclic group, is characteristic for the *anti* conformation (41, 48). In the *syn* conformation usually a strong destabilization of the *gauche*$^+$ rotamer is observed (41, 48).

Analysis of the conformational data, as presented in Table 2, leads to the conclusion that electron attracting groups stabilise *gauche*$^+$ rotamer and destabilize both *trans* and *gauche*$^-$ rotamers. The mechanism is based on the electrostatic interaction between O5′ and H6, stabilizing *gauche*$^+$ rotamer. Electron attracting groups lower the net charge on H6, increasing O5′–H6 interaction which stabilize *gauche*$^+$ rotamer (see Fig. 2e). Linearity of the general correlation $F_{12}$ *vs* $F_{34}$ (see Fig. 2f) proves existence of the common mechanism of 5′-$CH_2OH$ group rotamers stabilization, demonstrating domination of the *anti* conformation about the glycoside bond.

### *A Simplified Prediction Model of Compound Cytoxicity*

Assuming that compound activity is a function of substituent polar surface, $S_{pol}$, its molecular volume, $V_{mol}$, and molecule polarity, P, defined at the relative partition of the polar atoms (e.g., nitrogen, oxygen, and exchangable protons), the simplified model in form 1 was tested,

$$\ln k_I = aS_{pol} + bV_{mol} + cP + d,$$

where $k_I$ is a minimum inhibitory concentration for cell proliferation or cellular DNA synthesis (49); a, b, c, d parameters to be estimated. The results obtained for 5-hydroxy, ethyl, propyl, isopropyl, formyl, nitro, chloro, and (*E*)-2-bromovinyl derivatives are labeled by solid triangles in Fig. 4. The group of polar derivatives (hydroxymethyl, metoxymethyl, dimethylamino) is underestimated whereas activity of some halogen derivatives is overestimated.

## FINAL REMARKS

Conformational characteristics of the sugar moiety and 5′-exocyclic group are in agreement with a dominance of the *anti* conformation. C2′*endo* (S) and *gauche*$^+$ are generally preferred in the whole series of compounds. The induced changes of conformation populations of the sugar ring and 5′-exocyclic group are not very large and do not usually exceed 25%, depend-

ing on the nature of the C5-substituent. Nevertheless, the results show several interesting interrelationships between electronic and conformational properties in this series of compounds.

The original conformational analysis of maps of pseudorotation amplitudes of the sugar ring shows that the nature of the C5-substituent is a significant factor for conformational characteristics. Generally, electron donating groups destabilize the N conformation, simultaneously decreasing the mean pseudorotation amplitude. This indicates the role of unfavorable interaction of the C6 $\pi$ orbital with the nonbonded orbital of O4′, occupied by an electron pair, in conformational equilibrium of the sugar moiety. In this point of view, an increase of $\pi$-electron density located on C6 (especially in S conformation) disables large movements of O4′, resulting in lowering of $\tau$. In opposition, electron attracting C5-substituents decrease the $\pi$-electron density on C6, enabling larger pseudorotation amplitude.

To gain insight into the nature of structure–activity relationships, the role of the C5-substituent in the creation of cytotoxic activity is considered on the basis of a simplified prediction model. It is assumed that compound cytotoxicity is a function of substituent polar surface, its molecular volume, and its molecule polarity defined at the relative partition of the polar atoms. The direct interaction of C5-substituent with model primary rabbit kidney cells is probably moderated by subtle changes of electronic and conformational properties.

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