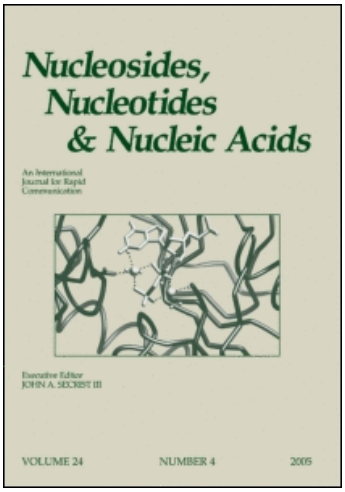

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Assignment of Anomeric Configuration of D-Ribo-, Arabino-, 2'-Deoxyribo-, and 2',3'-Dideoxyribonucleosides by Noe Difference Spectroscopy

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ASSIGNMENT OF ANOMERIC CONFIGURATION OF D-RIBO-, ARABINO-, 2′-DEOXYRIBO-, AND 2′,3′-DIDEOXYRIBONUCLEOSIDES BY NOE DIFFERENCE SPECTROSCOPY

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ABSTRACT. The anomeric configuration of D-ribo-, D-arabino, D-2′-deoxyribo-, and D-2′,3′-dideoxyribonucleosides was assigned unambiguously applying n.O.e. difference spectroscopy. For this purpose 1′-H signals were saturated and the n.O.e. factors of 4′-H, 3′-H, and 2′-H were measured.

INTRODUCTION

The assignment of anomeric configuration of rare or structurally modified nucleosides is a repeating task resulting either from the unknown stereochemistry of the natural product or from the strategy of convergent synthesis. Chemical [1,2] and biochemical methods [3], as well as CD-[4], ^{1}H- and ^{13}C-NMR measurements [5-10] are utilised to approach this problem.

Comparative analysis of ^{1}H-and ^{13}C-NMR spectra of anomeric nucleoside pairs led to empirical rules relying on different chemical shifts and/or coupling constants of the anomers. However, anisotropy- and electronic effects of furanose substituents can influence these parameters. As

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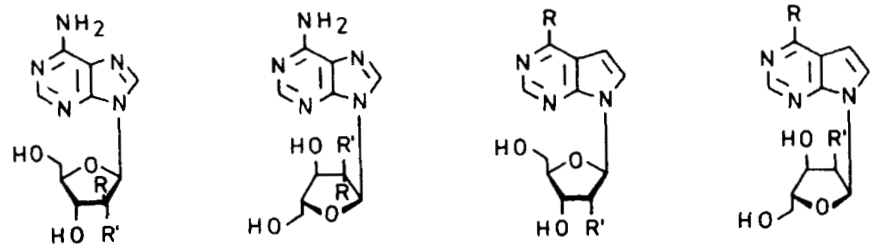
substituents may also alter the conformational equilibria (pseudorotation, syn-anti modes), the location of a signal or its coupling constants are difficult to predict. As a consequence, empirical rules derived from chemical shifts or coupling constants of anomers cannot be generally applicated for configurational assignment at C-1′. Knutsen et al. have reported that proton-proton nuclear Overhauser effects (n.O.e.) of particular sugar signals are different in case of anomeric C-nucleosides [11]. We have now measured one-dimensional n.O.e. difference spectra of a series of regular, base- and/or sugar-modified nucleosides in order to develop a general rule allowing the unambiguous assignment of the anomeric configuration.

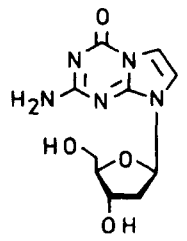
RESULTS AND DISCUSSION

The next formula scheme shows the nucleosides on which we tested scope and limitations of anomer assignment based on n.O.e. difference spectroscopy. Compounds 5-9, 11-14, and 16 were synthesized in our laboratory [3, 12-16]. Compounds 1-3, 18,19, and 22 are commercially available. Nucleosides 4 ,15, 17, and 21 were provided by other laboratories (see Exp. Section).



1 : R = H; R′ = OH
2 : R = OH; R′ = H
3 : R = H; R′ = OH
4 : R = OH; R′ = H
5 : R = Cl; R′ = OH
6 : R = NH_2; R′ = OH
7 : R = NH_2; R′ = H
8 : R = Cl; R′ = OH
9 : R = NH_2; R′ = OH
10 : R = NH_2; R′ = H

The α-anomer of 2′-deoxytubercidin (10) was prepared by solid-liquid phase-transfer glycosylation of 25 [17] with the halogenose 26 [18], pre-equilibrated in DMF. This



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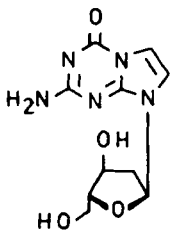

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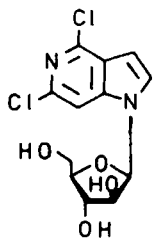

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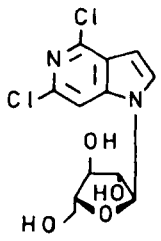

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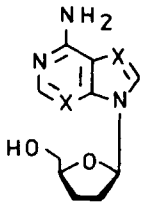
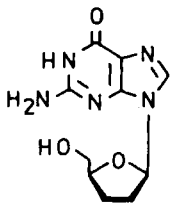


15 : X = N
16 : X = CH



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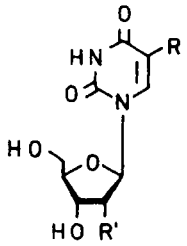
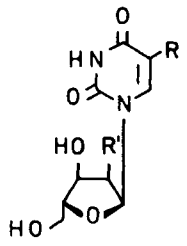


18: R = H; R' = OH
19: R = CH_3; R' = H
20: R = CH=CHBr; R' = H

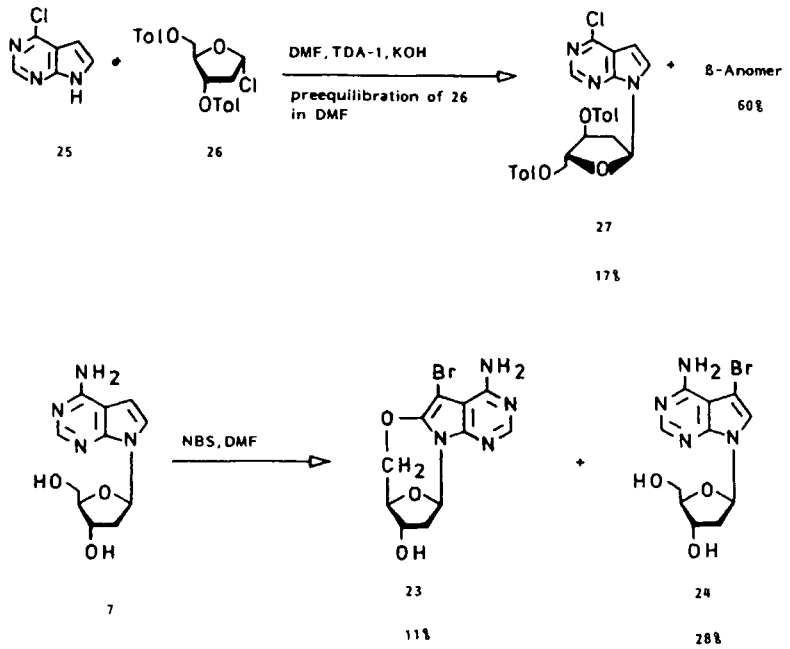


21: R = H; R' = OH
22: R = CH_3; R' = H

afforded the α-anomer 27 in 17 % yield besides 60% of its β-anomer (reaction scheme). Compound 27 was deprotected with methanolic ammonia; the chloro substituent was displaced by conc. aq. ammonia (autoclave) to give α-2′-deoxytubercidin (10) in 47% yield.

On the other hand, 2′-deoxytubercidin (7) was brominated with N-bromosuccinimide [19] in DMF yielding 5-bromo-2′-deoxytubercidin (24) as main product. As a minor component the 5′,6-cyclo-6-oxo nucleoside 23 was isolated. Assignment of its ^{13}C-NMR shifts was made on the basis of the gated-decoupled ^{13}C-NMR spectrum. The "unusual" O-4′-exo conformation was deduced from analysis of its ^{1}H-NMR spectrum (J(1′-H,2′-H_α) = 5.0 Hz; J(1′-H,2′-H_β) = 0 Hz, Exp. Section).

Inspection of Dreiding models shows that in β-anomers with N- or S-type conformation [20] 1′-H and 4′-H are



25 26 27 17%

7 23 11% 24 28%

located in almost the same spatial proximity on the same side (α) of the sugar moiety. The 3′-H_β and 2′-H_β are positioned on the opposite face. An exeption are β-anomers with O-4′-exo conformation occuring in cyclonucleosides [21], like 23. Here, the distance between 1′-H and 4′-H is significantly larger than in the cases described above. Contrary to β-nucleosides, α-nucleosides with S-type-puckered sugar - bearing 1′-H and 3′-H on the β-side of the furanose ring - exhibit a large 1′-H-3′-H distance. A shorter one is observed for N-type-puckered sugar moieties.

Saturation of 1′-H of the β-anomers 1, 2, 5-7, 11, 13, 15-20 results in characteristic n.O.e.s (1.5 - 2.6%) of the 4′-H signal, while there is none at the 3′-H signal (Table). Irradiation of 1′-H of the cyclonucleoside 23, however, does not lead to an n.O.e. at 4′-H demonstrating the limitation of the method to molecules with a short H-1′-H-4′ distance.

TABLE: NOE-Data (%) of Compounds 1-23 upon Irradiation of 1′-H (DMSO-d_6, 23°C, 250 MHz).

	D-ribofuranosides							D-arabinofuranosides				
	1β	3α	6β	9α	5β	8α	18β	21α	2β	4α	13β	14α
4′-H	2.1	a	2.4	a	2.1	a	1.7	a	2.0	a	2.5	a
3′-H	a	1.5	a	3.5	a	1.5	a	3.7	a	3.0	a	3.5
2′-H_β	1.9	11	2.1	11	2.1	10	2.2	10	-	-	-	-
2′-H_α	-	-	-	-	-	-	-	-	10	4.1[b]	12	2.3

	D-2′-deoxyribofuranosides							D-2′,3′-dideoxy-ribofuranosides			
	7β	10α	11β	12α	19β	22α	20β	23β	15β	16β	17β[c]
4′-H	2.0	a	1.5	a	2.3	a	2.3	a	1.8	2.0	2.6
3′-H	a	1	a	a	a	1	a	a	a	a	a
2′-H_β	a	5.6	a	5.0	a	6.3	a	-	a	a	a
2′-H_α	5.6	a	5.1	a	6.3	a	5.9	5.4	6.6	7.8	6.4

a: no detectable intensity enhancement (<0.5%)
b: additional partial saturation of 2′-OH
c: measured at 50°C to avoid overlapping of 1′-H and NH_2

Saturation of 1′-H of the α-nucleosides 3, 4, 8, 9, 14, and 21 yields n.O.e.s of both, 3′-H (1.5 - 3.7%) and 2′-H_β (10 - 11%) while that of 4′-H is zero. As the table shows, the α-D-2′-deoxyribofuranosides 10, 12, and 22 exhibit only weak enhancements at 3′-H upon irradiating 1′-H (0.5%). This points to a high population of the C-2′-endo/C-3′-exo conformer (S-type) with relatively large 1′-H-3′-H distance. These findings are in agreement with the coupling constants ($J(1'\text{-H},2'\text{-H}_\alpha) \cong J(2'\text{-H}_\alpha,3'\text{-H}) \cong 3$ Hz and $J(1'\text{-H},2'\text{-H}_\beta) \cong 8$ Hz). Nevertheless, the assignment of α-configuration of 10, 12, and 22 follows from the typical n.O.e. values (5.0 - 6.3%) measured at the 2′-H_β proton upon irradiating 1′-H. According to expectations based on **the conformation and the three-spin effects, the 2′-H_β** n.O.e. values of the α-D-ribofuranosides 3, 8, 9, and 21 are significantly higher (10 - 11%). In contrast to

this, β-anomers (1, 5-7, 11, 15-20) show only weak 2′-H_β n.O.e. values (0 - 2.3%).

In conclusion, the anomeric configuration of nucleosides can unambiguously be assigned by saturating 1′-H and measurement of the n.O.e. factors at 4′-H and 3′-H. Nuclear Overhauser enhancement of the 4′-H signal proves β-configuration while an n.O.e. at 3′-H confirms α-configuration.

EXPERIMENTAL SECTION

Materials and methods see [22]. Flash chromatography was performed on silica gel 60H (Merck, FRG) at 0.5 bar. Solvent systems: (A) $CHCl_3$-MeOH, 99:1; (B) $CHCl_3$-MeOH, 9:1; (C) $CHCl_3$-MeOH, 95:5. DMF was dried by distillation with benzene and water. UV spectra were recorded on a UV 3200 spectrophotometer (Hitachi, Japan). Compounds 1-3, 18, 19, and 22 were purchased from either Pharma Waldhof (Darmstadt, West Germany) or Sigma Chemicals Co. (St. Louis, USA). Compounds 4 and 21 were generous gifts of Dr. F. Hansske (Boehringer Mannheim, West Germany), compound 20 was a gift of Prof. Dr. E. de Clercq (University of Leuven, Belgium), and compounds 15 and 17 were provided by Boehringer Mannheim (West Germany).

NMR Spectroscopy: AC-250 spectrometer with Aspect-3000 computer and array processor (Bruker, FRG). Operational frequencies: ^1H: 250.133 MHz (digital resolution: 0.275 Hz/pt), ^{13}C: 62.898 MHz (digital resolution: 0.526 Hz/pt). δ Values are in ppm relative to tetramethylsilane as internal standard. For the n.O.e. measurements the DMSO-d_6 solutions (ca. 0.1 M) were degased. Typical spectral conditions were as follows: number of data points 32 K; pre-irradiation delay: 1.6 sec, relaxation delay 4.5 sec. Compounds 3-6, 11-13, 15, and 16 were measured applying the NOEDIFF mode of the Bruker software package, all others by the NOEMULT mode (release 1987) where the lines of the multiplets were

irradiated separately and several times during the pre-irradiation to achieve a higher selectivity [23].

4-Chloro-7-(2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (27).

A solution of compound 25 [17] (500 mg, 3.26 mmol) in anhydrous DMF (30 mL) containing powdered KOH (400 mg, 7.07 mmol) and tris-(2-(2-methoxyethoxy)ethyl)amine (TDA-1, 50 µL, 0.16 mmol) was stirred at room temperature under nitrogen for 15 min. 2-Deoxy-3,5-di-O-p-toluoyl-α-D-erythro-pentofuranosyl chloride [18] (1.4 g, 3.61 mmol) which had previously been aged by stirring in anhydrous DMF for 30 min was added while stirring was continued for another 30 min. Insoluble material was removed by filtration and the filtrate evaporated to give an oil which was purified by flash-chromatography on silica gel 60H (column: 40 x 6 cm, solvent A). From the first zone the β-anomer (990 mg, 60%) was obtained as colorless needles from EtOH; m.p. 118°C; [15]: m.p. 118°C. From the second zone compound 27 (285 mg, 17%) was isolated as colorless foam upon evaporation. TLC (solvent A) R_f 0.5; UV (MeOH) λ_{max} 225, 240 nm (34.800, 30.000). ^{1}H-NMR (DMSO-d_6) δ 2.85 (m, 2′-H_b), 3.12 (m, 2′-H_a), 4.51 (m, 5′-H), 4.94 (m, 4′-H), 5.64 (m, 3′-H), 6.74 (d, J = 3.8 Hz, 5-H), 6.81 (dd, J = 4.5, 7.2 Hz, 1′-H), 7.30 (d, J = 8 Hz, tol-H), 7.33 (d, J = 8 Hz, tol-H), 7.71 (d, J = 8 Hz, tol-H), 7.91 (d, J = 8 Hz, tol-H), 8.00 (d, J = 3.8 Hz, 6-H), 8.64 (s, 2-H). ^{13}C-NMR (DMSO-d_6) δ 150.8 (C-2), 150.5 (C-4), 117.5 (C-4a), 99.4 (C-5), 128.4 (C-6), 150.3 (C-7a), 85.1 (C-1′), 37.5 (C-2′), 74.9 (C-3′), 83.0 (C-4′), 64.3 (C-5′), 21.3 (tol-CH_3). Anal. calcd. for $C_{27}H_{24}N_3O_5Cl$ (506.0): C 64.09, H 4.78, N 8.31. Found: C 64.09, H 4.81, N 8.30.

4-Amino-7-(2-deoxy-α-D-erythro-pentofuranosyl)-7H-pyrrolo-[2,3-d]pyrimidine (10).

Compound 27 (250 mg, 0.49 mmol) was dissolved in methanolic ammonia (10 mL, saturated at 0°C) and stirred at

room temperature for 24 h. After evaporation of the solvent the residue was dissolved in 25% aqueous ammonia and stirred at 100°C for 12 in an autoclave. After evaporation of the solvent the residue was purified by flash-chromatography on silica gel 60H (column: 10 x 6 cm, solvent B) to yield compound 10 (58 mg, 47%) as colorless foam upon evaporation. TLC (solvent B): R_f 0.2. UV (MeOH) λ_{max} 270 nm (10.100). ^{1}H-NMR (DMSO-d_6) δ 2.15 (m, 2′-H_b), 2.74 (m, 2′-H_a), 3.44 (m, 5′-H), 4.00 (m, 4′-H), 4.26 (m, 3′-H), 4.80 (t, J = 5.7 Hz, 5′-OH), 5.70, J = 4.6 Hz, 3′-OH), 6.48 (dd, J = 3.6, 7.0 Hz, 1′-H, 6.58 (d, J = 3.5 Hz, 5-H), 7.03 (s, NH_2), 7.52 (d, J = 3.5 Hz, 6-H, 8.06 (s, 2-H). ^{13}C-NMR (DMSO-d_6) δ 151.6 (C-2), 157.6 (C-4), 102.7 (C-4a), 99.5 (C-5), 122.4 (C-6), 149.7 (C-7a), 82.9 (C-1′), 40.2 (C-2′), 70.9 (C-3′), 79.3 (C-4′), 61.9 (C-5′). Anal. calcd. for $C_{11}H_{14}N_4O_3$ (250.3): C 52.79, H 5.64, N 22.39. Found: C 52.89, H 5.79, N 22.23.

4-Amino-5-bromo-6,5′-cyclo-7-(2-deoxy-β-D-erythropentofuranosyl)-6-oxo-7H-pyrrolo[2,3-d]pyrimidine (24) and 4-Amino-5-bromo-7-(2-deoxy-β-D-erythro-pentofuranosyl)-7H-pyrrolo[2,3-d]pyrimidine (23).

Compound 7 (500 mg, 2 mmol) was dissolved in anhydrous DMF (10 mL) and a solution of N-bromosuccinimide (360 mg, 2 mmol) in DMF (10 mL) was added dropwise. After stirring for 30 min at room temperature the solvent was evaporated and the residue purified by flash-chromatography on silica gel 60H (column: 25 x 6 cm, solvent C). From the faster migrating zone compound 23 (72 mg, 11%) was obtained as colorless crystals; m.p. 215 - 218 °C (MeOH). TLC (solvent B) R_f 0.55. UV (MeOH) λ_{max} 282 nm (9.300). ^{1}H-NMR (DMSO-d_6) δ 8.10 (s, 2-H), 6.72 (t, J(1′-H,2′-H_α) = 5.0 Hz, 1′-H), 6.61 (s, br., NH_2), 5.33 (d, J = 4.0 Hz, 3′-OH), 4.63 (d, J(3′-H,4′-H) = 1.4 Hz, 3′-H), 4.60 (dd, J(5′-H,4′-H) = 1.4 Hz, J(5′-H,5′′-H) = 12.5 Hz, 5′-H), 4.41 (s, br., 4′-H), 3.86 (d, J(5′′-H,5′-H) = 12.5 Hz, 5′′-H), 2.39 (t, J(2′-H_α,

1′-H) = 5.0 Hz, 2′-H_2). ^{13}C-NMR (DMSO-d_6) δ 156.2 (d, ^3J(C-4, 2-H) = 11 Hz, C-4), 151.5 (d, ^1J(C-2,2-H) = 198.7 Hz, C-2), 144.7 (t, ^3J(C-6, 5′-H_2) = 8 Hz, C-6), 143.8 (d, ^3J(C-7a,2-H) = 11 Hz, C-7a), 98.5 (s, C-4a), 87.5 (d, ^1J(C-4′,4′-H) = 153.5 Hz, C-4′), 82.1 (d, ^1J(C-1′,1′-H) = 169.5 Hz, C-1′), 76.7 (t, ^1J(C-5′5′-H_2) = 150.6 Hz, C-5′), 71.5 (d, ^1J(C-3′,3′-H) = 148.7 Hz, C-3′), 69.8 (s, C-5), 43.3 (t, ^1J(C-2′,2′-H) = 133.5 Hz, C-2′). Anal. calcd. for $C_{11}H_{11}N_4O_3Br$ (327.1): C 40.39, H 3.39, N 17.13. Found: C 40.58, H 3.54, N 17.07.

From the slower migrating zone compound 24 (180 mg, 28%) were obtained as colorless needles; m.p. 170 - 172 °C (MeOH). TLC (solvent A) R_f 0.50. UV (MeOH) λ_{max} 280 nm (9.500). ^{1}H-NMR (DMSO-d_6) δ 8.10 (s, 2-H), 7.63 (s, 6-H), 6.80 (s, br., NH_2), 6.51 (dd, J(1′-H,2′-H_α) = 7.9 Hz, J(1′-H,2′-H_β) = 1.8 Hz, 1′-H), 5.27 (d, J = 4.0 Hz, 3′-OH), 5.04 (t, J = 5.5 Hz, 5′-OH), 4.33 (m, 3′-H), 3.81 (m, 4′-H), 3.53 (m, 5′-H_2), 2.45 (m, 2′-H_β), 2.18 (m, 2′-H_α). ^{13}C-NMR (DMSO-d_6) δ 157.0 (C-4), 152.5 (C-2), 149.3 (C-7a), 121.5 (C-6), 101.0 (C-4a), 87.5 (C-4′), 86.8 (C-5), 83.0 (C-1′), 71.0 (C-3′), 61.9 (C-5′), 39.8 (C-2′). Anal. calcd. for $C_{11}H_{13}N_4O_3Br$ (329.2): C 40.14, H 4.00, N 17.02. Found: C 40.29, H 3.96, N 17.08.

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