(1990/1991) provided by Harbor Branch Oceanographic Institution to N.K.G. is acknowledged. We thank Drs. Eberhard Essich and Peter McCarthy for antitumor and antimicrobial assays, respectively. We are also greateful to Mr. Dan Pentek, Yale University Instrument Center, New Haven, CT, for mass spectral data. This is Harbor Branch Oceanographic Institution Contribution No 880.

Supplementary Material Available: ^1H, ^1H–^1H COSY, COSYRCT, ^{13}C, DEPT, HETCOR, HMBC, NOESY, and 1D NOE NMR charts for 1, ^1H and ^{13}C NMR spectra for 2, and ^1H NMR spectra for 3–6 (46 pages). Ordering information is given in any current masthead page.

(^{13}C)-Substituted Erythronucleosides: Synthesis and Conformational Analysis by ^1H and ^{13}C NMR Spectroscopy

Paul C. Kline and Anthony S. Serianni*

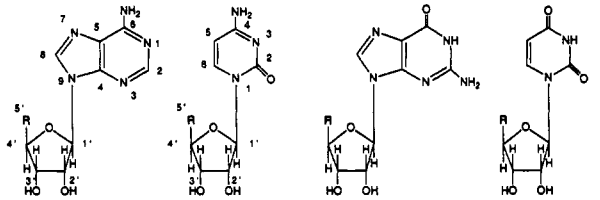
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Received August 1, 1991 (Revised Manuscript Received November 25, 1991)

The erythronucleosides, 9-β-D-erythrofuranosyladenine (**1b**), 1-β-D-erythrofuranosylcytosine (**2b**), 9-β-D-erythrofuranosylguanine (**3b**), and 1-β-D-erythrofuranosyluracil (**4b**), were synthesized with and without ^{13}C-substitution at C1′ of the furanose ring. 75-MHz ^{13}C and 620-MHz ^1H NMR spectra of **1–4b** were interpreted, in the latter case with the assistance of spectral simulation, and ^1H–^1H, ^{13}C–^1H, and ^{13}C–^{13}C spin couplings were used to assess furanose conformation. $^3J_{HH}$ data in ^2H_2O were treated by computer to determine the preferred north and south conformers, their puckering amplitudes, and their mole fractions in solution, and J_{CH} data were used to complement this analysis. A similar treatment of spin coupling data for the corresponding ribonucleosides **1–4a** was also conducted to permit a comparison of furanose conformations in both series of compounds. Results show that the removal of the exocyclic hydroxymethyl group from **1–4a**, giving **1–4b**, significantly enhances the proportion of south conformers in aqueous (^2H_2O) solution.

Introduction

The ribonucleosides adenosine (**1a**), cytidine (**2a**), guanosine (**3a**), and uridine (**4a**) are the major constituent monomers of ribonucleic acid (RNA). Three interdependent[1] conformational domains exist in **1–4a** and have been



studied by a variety of experimental techniques.[2,3] These domains include the furanose ring, the exocyclic C5′ hydroxymethyl (CH_2OH) group, and the *N*-glycoside linkage. The conformational interdependence of these domains is illustrated, for example, by the coexistence of the *gg* hydroxymethyl conformation and anti *N*-glycoside bond conformation in certain ribonucleosides which apparently results from intramolecular hydrogen bonding. Thus, while the C5′ exocyclic hydroxymethyl group is a key determinant of furanose ring conformation in nucleosides, its presence is believed to have a global effect on the conformational behavior of these molecules in solution.

The erythronucleosides (9-β-D-erythrofuranosyladenine (**1b**), 1-β-D-erythrofuranosylcytosine (**2b**), 9-β-D-erythrofuranosylguanine (**3b**), and 1-β-D-erythrofuranosyluracil (**4b**)) are structural analogues of **1–4a**. Compounds **1–4b** contain the β-D-erythrofuranosyl ring in which the C5′ exocyclic CH_2OH group found in the β-D-ribofuranosyl ring of **1–4a** is replaced by a proton (H4′*R*). Hence, a comparison of the conformational properties of **1–4b** to those of **1–4a** may assist in evaluating the general effect of furanose ring substitution at C4′ on the solution conformations of nucleosides. Substitution effects can be important in determining the chemical and biological reactivities of nucleosides. For example, Van Roey et al.[4] have proposed recently that the anti-HIV activity of 3′-azido-2′,3′-dideoxythymidine (AZT) is related to the preferred south conformation of the furanose ring in this substituted nucleoside. This observation serves to emphasize the importance of understanding the structural factors that influence furanose ring conformation in biologically important molecules.

Four vicinal ^1H–^1H spin-coupling constants ($^3J_{HH}$) are available to assess the conformation of the β-D-erythrofuranosyl ring in **1–4b**: $^3J_{H1',H2'}$, $^3J_{H2',H3'}$, $^3J_{H3',H4'S}$, and $^3J_{H3',H4'R}$. Complementary structural information is supplied by ^{13}C–^1H and ^{13}C–^{13}C spin couplings, as described previously by Cyr and Perlin[5] and Serianni and Barker,[6] and these couplings are most easily and accurately measured in (^{13}C)-substituted compounds.[7] This report describes the chemical synthesis of natural and (1′-^{13}C)-substituted erythronucleosides **1–4b**, the interpretation of their ^1H and ^{13}C NMR spectra, and a conformational analysis of the furanose rings in **1–4b** based on ^1H–^1H, ^{13}C–^1H, and ^{13}C–^{13}C spin coupling constants. The preferred erythrofuranosyl ring conformations in **1–4b** are

* Author to whom correspondence should be addressed.

(1) Davies, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 135.
(2) For a comprehensive review, see: Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(3) Van De Ven, F. J. M.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *178*, 1–38.
(4) Van Roey, P.; Salerno, J. M.; Chu, C. K.; Schinazi, R. F. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3929.
(5) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504.
(6) Serianni, A. S.; Barker, R. *J. Org. Chem.* **1984**, *49*, 3292.
(7) King-Morris, M. J.; Serianni, A. S. *J. Am. Chem. Soc.* **1987**, *109*, 3501.
(8) Kline, P. C.; Serianni, A. S. *J. Am. Chem. Soc.* **1990**, *112*, 7373.

0022-3263/92/1957-1772$03.00/0

compared to the preferred ribofuranosyl ring conformations in **1**–**4a**.

Experimental Section

Materials. Uracil, N^2-acetylguanine, cytosine, and N^6-benzoyladenine were purchased from Sigma Chemical Co., and trimethylsilyl trifluoromethanesulfonate was purchased from Aldrich Chemical Co. Hexamethyldisilazane (HMDS) was obtained from Pierce Chemical Co. Acetonitrile was refluxed for 2 h over CaH_2 and distilled immediately prior to use. Pyridine was stored over KOH for 1 week, distilled at atmospheric pressure, and stored over 4-Å molecular sieves (Linde). Potassium (^{13}C)-cyanide ($K^{13}CN$, 99 atom % ^{13}C), deuterium oxide (^2H_2O, 98 atom % ^{2}H), and DMSO-d_6 (98 atom % ^{2}H) were purchased from Cambridge Isotope Laboratories.

Instrumentation. ^{1}H-Decoupled ^{13}C NMR spectra were obtained in DMSO-d_6 at ~25 °C. High-resolution 620-MHz ^{1}H FT-NMR spectra were obtained in ^2H_2O at ~25 °C at the NMR Facility for Biomedical Studies in the Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, which is partly supported by NIH grant P41RR00292. ^{1}H spectra were resolution enhanced to improve the detection of small couplings and/or the separation of overlapping signals.

Two-dimensional ^{13}C–^{1}H chemical shift correlation spectra[9] (HETCOR), ^{1}H–^{1}H COSY spectra (absolute value),[10] and DEPT spectra[11] were obtained at ~25 °C.

Computer simulation of 620-MHz ^{1}H NMR spectra was performed using the LAOCN5 program as implemented in the FTNMR program (Vax version) available from Hare Research, Inc. of Woodinville, WA.[12]

General Preparations. N^4-Acetylcytosine was prepared as described by Codington and Fox.[13] D-Erythrose,[14] D-glyceraldehyde,[15] and D-(1-^{13}C)erythrose[16] were prepared as described previously.

1,2,3-Tri-*O*-acetyl-D-erythrofuranose (5). D-Erythrose was acetylated according to the procedure of Murray and Prokop.[17] D-Erythrose (12 g, 100 mmol) was exchanged three times with anhydrous pyridine (50 mL) by evaporation at 30 °C in vacuo. The dried syrup was dissolved in anhydrous pyridine (100 mL), and the solution was cooled to 15 °C in an ice bath. Acetic anhydride (47 mL, 0.5 mol) was added slowly to the reaction mixture, which was stored overnight at 4 °C. Ice–water (370 mL) was added to the reaction mixture, and the solution was stirred for 30 min. The mixture was extracted with $CHCl_3$ (3 × 200 mL), and the combined organic extracts were washed with ice-cold saturated aqueous $NaHCO_3$ (3 × 125 mL). The organic phase was dried over anhydrous Na_2SO_4 overnight, the suspension was filtered with suction, and the filtrate was concentrated to a syrup at 30 °C in vacuo. The syrup was distilled under reduced pressure (120–122 °C (0.25 mmHg)) in a Kugelrohr apparatus to give a colorless syrup of **5** containing both α- and β-anomers. Yield: 15.2 g, 61.8 mmol, 62% (12% α, 88% β by NMR integration). ^{13}C NMR (ring carbons) in C^2HCl_3: α-anomer, 93.0 (C1), 71.3, 70.0, 68.1; β-anomer, 98.5 (C1), 74.5, 70.3, 70.2.

β-D-Erythrofuranosylnucleosides 1–4b. The appropriate base (N^6-benzoyladenine, N^4-acetylcytosine, N^2-acetylguanine, uracil) (5.5 mmol) was suspended in HMDS (20 mL), and the suspension was refluxed under a dry N_2 atmosphere until the base dissolved (~8 h). The excess HMDS was removed by distillation at atmospheric pressure, taking precautions to exclude moisture from the reaction vessel. To ensure the removal of residual HMDS, the syrup was heated at 50 °C under vacuum (2 mmHg) for 30 min. The vacuum was released by the addition of dry N_2, and 50 mL of a 0.1 M solution of **5** in anhydrous CH_3CN was added from a glass syringe, followed by trimethylsilyl trifluoromethanesulfonate (1.45 mL, 8.0 mmol). The resulting solution was gently refluxed (**1b**, 5 h; **2b** and **4b**, 2 h; **3b**, 3 h).

After refluxing for the appropriate time, the reaction mixture was cooled to room temperature and diluted with CH_2Cl_2 (100 mL). The mixture was extracted with ice-cold saturated aqueous $NaHCO_3$ (75 mL) and dried overnight. The suspension was filtered with suction and the filtrate was concentrated at 30 °C in vacuo, giving a syrup containing the crude protected erythronucleoside.

Crude acylated erythroguanosine **3b** was further purified by flash chromatography on a column (1.5 × 27 cm) of silica gel.[18] The column was eluted (5 mL/min) with 200 mL of EtOAc followed by 1000 mL of 97:3 EtOAc/MeOH. Fractions (20 mL) were collected and assayed by thin-layer chromatography on silica gel (solvent: 9:1 CH_3CN/1,4-dioxane); spots were visualized with the use of an ultraviolet lamp operating at 254 nm. Two UV-absorbing compounds eluted in fractions 20–27 and 35–60. and these fractions were pooled and the solvent evaporated at 30 °C in vacuo, giving syrups. Fractions 35–60 contained the desired protected **3b** (see Results and Discussion).

The acylated erythronucleosides were dissolved in methanolic ammonia (20 mL) freshly prepared by bubbling anhydrous NH_3 through 50 mL of anhydrous MeOH at 0 °C for 10 min. The reaction mixture was kept at 25 °C for 24 h (48 h for acylated **3b**). Methanolic ammonia was removed by evaporation at 30 °C in vacuo, the residue was dissolved in distilled H_2O (100 mL), and the solution was extracted with $CHCl_3$ (3 × 50 mL) and Et_2O (3 × 50 mL).

The crude erythronucleosides were purified by chromatography on a column (1.0 cm × 10 cm) of Dowex 1 × 2 (200–400 mesh) resin in the hydroxide form.[19] The erythronucleosides **1b** and **2b** were eluted with 30% MeOH/70% CO_2-free water (v/v). For **3b** and **4b**, the column was washed with 500 mL of 30% MeOH/70% CO_2-free water (v/v) and eluted with 250 mM triethylammonium bicarbonate (TEAB), pH 9.0. Column effluents were monitored at 254 nm using an ISCO UA-5 UV detector with a Type 6 optical unit. The main UV absorbing peaks were pooled, and solvent was removed at 30 °C in vacuo. The TEAB salt was removed by evaporation from distilled water several times at 30 °C in vacuo. Compounds **1b** and **3b** were crystallized from hot distilled H_2O, and **2b** and **4b** were crystallized from hot EtOH. Yields based on **5** were as follows: **1b**, 63%; **2b**, 55%; **3b**, 25%; **4b**, 43%. Melting points (uncorr): **1b**, 230–232 °C dec; **2b**, 224–226 °C dec; **3b**, >325 °C dec; **4b**, 186–188 °C.

(1′-^{13}C)-Substituted **1–4b** were prepared by substituting D-(1-^{13}C)erythrose for D-erythrose in the above protocol. The purity of **1–4b** was assessed at >98% by ^{1}H and ^{13}C NMR spectroscopy (see supplementary material). High-resolution mass spectrometry gave the following results: **1b**, $C_9H_{11}N_5O_3$, m/e 237.0862 calcd, 237.0867 found; (1′-^{13}C)**2b**, $^{12}C_7{}^{13}CH_{11}N_3O_4$, m/e 214.0783 calcd, 214.0791 found; (1′-^{13}C)**3b**, $^{12}C_8{}^{13}CH_{11}N_5O_4$, m/e 254.0845 calcd, 254.0845 found; **4b**, $C_8H_{10}N_2O_5$, m/e 214.0590 calcd, 214.0589 found.

Results and Discussion

Synthesis of β-D-Erythrofuranosylnucleosides 1–4b. The erythronucleosides **1b**, **2b**, and **4b** have been prepared previously,[17,20,21] but the reported procedures gave low yields and multiple products, making the methods unattractive for the preparation of labeled compounds. The trimethylsilyl method developed by Vorbrüggen and coworkers[22] was used in this study to prepare **1–4b** more rapidly and with improved yields. The use of trimethylsilyl trifluoromethanesulfonate as the Friedel–Crafts catalyst,

(9) Bodenhausen, G.; Freeman, R. *J. Magn. Reson.* **1977**, *28*, 471.
(10) (a) Jeener, J. Ampere International Summer School II, Basko Polje, Yugoslavia, 1971. (b) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *42*, 164.
(11) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(12) A copy of this program may be purchased from Hare Research, Inc., 14810 216th Avenue NE, Woodinville, WA 98072.
(13) Codington, J. F.; Fox, J. J. *Methods Carbohydr. Chem.* **1963**, *2*, 114.
(14) Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 64.
(15) Perlin, A. S. *Methods Carbohydr. Chem.* **1962**, *1*, 61.
(16) Serianni, A. S.; Clark, E. L.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 79.
(17) Murray, D. H.; Prokop, J. *J. Pharm. Sci.* **1967**, *56*, 867.
(18) Still, W. C. *J. Org. Chem.* **1978**, *43*, 2923.
(19) Dekker, C. A. *J. Am. Chem. Soc.* **1965**, *87*, 4028.
(20) Coombes, R. F., Ph.D. Thesis, University of Houston, May 1972.
(21) Szekeres, G. L.; Witkowski, J. T.; Robins, R. K. *J. Carbohydrates, Nucleosides, Nucleotides* **1977**, *4*, 147.
(22) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.

Table I. ^{13}C Chemical Shifts[a] of Erythronucleosides 1–4b in DMSO-d_6

nucleus	erythro-adenosine 1b	erythro-cytidine 2b	erythro-guanosine 3b	erythro-uridine 4b
C2	152.5	155.4	153.5	150.7
C4	149.4	165.5	151.4	162.9
C5	119.3	94.0	116.3	101.8
C6	155.9	142.0	156.7	141.5
C8	140.1		136.0	
C1′	87.6	90.3	86.6	88.6
C2′	74.1	74.1	74.2	73.6
C3′	70.2	69.8	70.1	69.7
C4′	73.5	72.8	73.2	73.2

[a] Chemical shifts are reported in ppm relative to the DMSO-d_6 solvent signal (39.5 ppm) and are accurate to ±0.1 ppm.

and acetonitrile as the solvent, generally gave cleaner reaction mixtures than those obtained by previous methods.[17,20,21] Hence, this method was used to prepare 1–4b containing ^{13}C-substitution at C1′. (^{13}C)-Substituted ribonucleosides were prepared recently by a similar approach.[8]

1-*O*-Acetyl-2,3-di-*O*-benzoyl-β-D-erythrofuranose **6** was chosen initially as the protected sugar donor in the Friedel–Crafts reaction, by analogy to the method used to prepare labeled ribonucleosides,[8] but attempts to synthesize **6** failed. ^{13}C NMR analysis showed that O-benzoylation of methyl β-D-erythrofuranoside proceeded smoothly and in good yield. However, 1-O-acetylation of the di-*O*-benzoyl β-furanoside gave a complex mixture from which the desired product could not be isolated. In contrast, an anomeric mixture of **5** was easily prepared and purified as described[17] and served as an acceptable furanose donor. Although ^{13}C NMR analysis showed that preparations of **5** contained ~12% α-anomer and ~88% β-anomer, the Friedel–Crafts condensation gave erythronucleosides having the β-configuration exclusively.

As observed previously in the synthesis of ribonucleosides,[8] the guanine-containing nucleoside **3b** proved to be the most difficult erythronucleoside to synthesize. The condensation reaction yielded two major products as determined by ^{13}C NMR analysis of the crude acylated product. These compounds were separated by flash chromatography on silica gel, deblocked with methanolic ammonia, and analyzed by ^1H and ^{13}C NMR spectroscopy. Using the criteria of Garner and Ramakanth,[23] the compound migrating more slowly on silica gel was identified as the desired N^9-linked isomer. The H8, C1′, C4, and C8 signals of this isomer resonated upfield, and the C5 and the N6 amino proton signals resonated downfield, of the corresponding signals of the faster-moving compound, the latter being assigned to the N^7-linked isomer. Regardless of the reaction time, the ratio of N^7- to N^9-linked products remained constant at ~1:1.3.

^{13}C NMR Signal Assignments. The ^{13}C signal assignments for 1–4b were made in DMSO-d_6 (Table I) due to their limited solubility in ^2H_2O. The assignments of the anomeric C1′ signals (~88 ppm) were made by analogy to related assignments reported previously in ribonucleosides and confirmed in ^{13}C NMR spectra of the (1′-^{13}C)-substituted compounds. The C2′ signals were assigned by observing the large splittings of these signals in ^{13}C NMR spectra of the (1′-^{13}C)-substituted compounds due to the presence of $^1J_{C1',C2'}$ (~44 Hz). The remaining C3′ and C4′ signals were distinguished with the use of DEPT spectra.[11] For 1–4b in DMSO-d_6, $\delta_{C1'} > \delta_{C2'} > \delta_{C4'} > \delta_{C3'}$ (Table I).

(23) Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294.

Table II. ^1H Chemical Shifts[a] of Erythronucleosides 1–4b and Methyl β-D-Erythrofuranoside 7 in ^2H_2O

nucleus	erythro-adenosine 1b	erythro-cytidine 2b	erythro-guanosine 3b	erythro-uridine 4b	O-glycoside[b] 7
H2	8.22				
H5		6.05		5.95	
H6		7.65		7.69	
H8	8.31		8.00		
H1′	6.02	5.81	5.87	5.84	4.90
H2′	4.94	4.50	4.88	4.52	4.04
H3′	4.54	4.41	4.51	4.41	4.35
H4′*R*	4.56	4.42	4.53	4.43	4.11
H4′*S*	4.09	4.00	4.06	4.02	3.81

[a] Chemical shifts are reported in ppm relative to the internal HO^{2}H signal (4.80 ppm) and are accurate to ±0.01 ppm. Values for 1b, 2b, and 4b were verified by computer simulation. [b] Data taken from ref 6.

Table III. ^1H–^1H, ^{13}C–^1H, and ^{13}C–^{13}C Spin-Coupling Constants[a] in Erythronucleosides 1b, 2b, and 4b and Methyl β-D-Erythrofuranoside 7

coupled nuclei	compd 1b	2b	4b	7[b]
H1′, H2′	6.7 (6.2)[c]	5.5 (4.0)	5.9 (4.5)	2.9
H2′, H3′	4.6 (5.3)	4.7 (5.2)	4.7 (5.3)	4.8
H3′, H4′*R*	3.8	4.3	4.1	5.0
H3′, H4′*S*	1.7 (3.3)	3.2 (6.0)	2.3 (5.5)	3.5
H4′*R*, H4′*S*	−10.4	−10.1	−10.1	−9.8
H5, H6		7.5 (7.6)	8.0 (8.1)	
C1′, H1′	166.0 (165.6)	169.2 (170.3)	169.5 (170.1)	
C1′, H2′	4.1 (3.2)			2.4
C1′, H3′	5.2 (~5.1)			3.9
C1′, H4′*R*	1.7			0.6
C1′, H4′*S*	3.7 (1.3)			4.8
C1′, H6		3.0 (2.8)	3.4 (2.7)	
C1′, C2′	43.4 (42.5)	43.9 (42.9)	43.9 (43.0)	47.6
C1′, C3′	3.3 (3.3)	3.2 (3.8)	3.4 (3.8)	2.1
C1′, C4′	0 (0.8)	0 (0.8)	0 (0.9)	0
C1′, C8	2.3 (1.8)			

[a] Coupling constants are reported in Hz and are accurate to ±0.1 Hz. ^1H–^1H couplings in 1b, 2b, and 4b were determined by computer simulation of 620-MHz ^1H NMR spectra. In 1b, 2b, and 4b, ^1H–^1H and ^{13}C–^1H couplings were obtained in ^2H_2O, and ^{13}C–^{13}C couplings were obtained in DMSO-d_6. [b] Data (in ^2H_2O) taken from ref 6. [c] Values in parentheses are corresponding couplings in the ribonucleosides (in ^2H_2O) reported in ref 8.

The assignments of the base carbon signals of 1–4b were made by analogy to those made in 1–4a. Protonated base carbon assignments were further supported by HETCOR spectra[9] using the base proton assignments discussed below.

^1H NMR Signal Assignments. The ^1H signal assignments for 1–4b in ^2H_2O are given in Table II. The H1′ signals were distinguished by their characteristic chemical shift (5.89 ± 0.09 ppm) and by the observation of the large $^1J_{C1',H1'}$ (168.2 ± 1.9 Hz) (Table III) in ^1H NMR spectra of the (1′-^{13}C)-substituted compounds. The H4′*R* and H4′*S* signals were assigned by noting the presence in each multiplet of a ~10 Hz ^1H–^1H spin-coupling constant characteristic of $^2J_{HH}$ for the endocyclic methylene protons of erythrofuranosyl rings.[6] The stereochemical assignments of H4′*R* (H4′ trans to O3′) and H4′*S* (H4′ cis to O3′) are based on the syn-upfield rule[24a] that predicts H4′*S* to be

(24) (a) Anteunis, M.; Danneels, D. *Org. Magn. Reson.* **1975**, *7*, 345. (b) In addition to splittings from $^2J_{HH}$ and $^3J_{HH}$, additional small splittings in the signals of the furanosyl ring protons of 1–4b were observed and attributed to the presence of long-range coupling ($^4J_{HH}$), but the specific couplings were not identified. (c) A computer analysis of the 620-MHz ^1H NMR spectrum of 3b in ^2H_2O was not performed, and thus furanose conformation in 3b is based solely on the magnitude of $^3J_{H1',H2'}$.

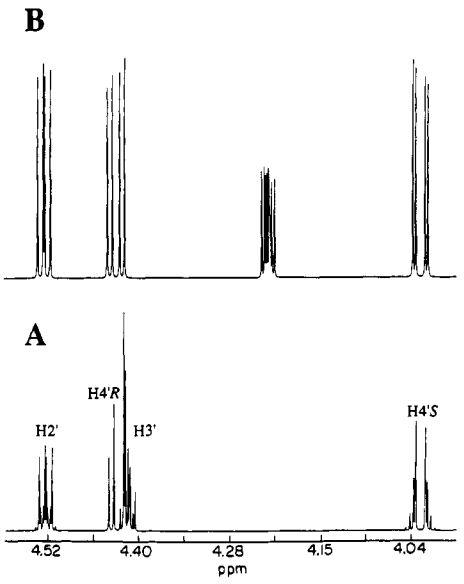


Figure 1. (A) Computer-simulated ^{1}H NMR spectrum (620 MHz) of **1b** using chemical shift and ^{1}H–^{1}H coupling data in Tables II and III. This spectrum is virtually identical to the experimental spectrum with the exception of small splittings due to $^4J_{HH}$[24b] and is non-first-order due to the similar chemical shifts of the mutually coupled H3′ and H4′*R*. (B) By moving the H3′ signal between the H4′*R* and H4′*S* signals, the simulated spectrum becomes essentially first-order.

more shielded than H4′*R*.[6] The remaining H2′ and H3′ signals were assigned via ^{1}H–^{1}H COSY spectra.[10] For **1–4b** in ^2H_2O, $\delta_{H1'} > \delta_{H2'} > \delta_{H4'R} \approx \delta_{H3'} > \delta_{H4'S}$ (Table II).

In **2b** and **4b**, the H5 and H6 signals were distinguished since H6 is commonly coupled to C1′ (~3.2 Hz) in (1′-^{13}C)pyrimidine nucleosides, whereas H5 is not.[8] These assignments were consistent with ^{13}C–^{1}H chemical shift and ^{1}H–^{1}H coupling correlations derived from HETCOR and COSY spectra, respectively.

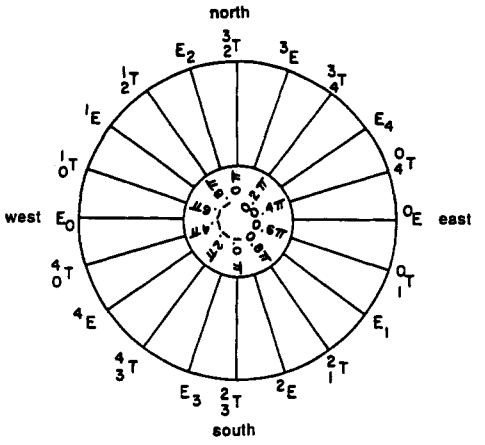
In **1b**, the H8 signal was distinguished from the H2 signal by incubating a sample of **1b** in ^2H_2O for 8 h at 70 °C and noting which signal was reduced in intensity (H8) due to ^{1}H–^{2}H exchange. The H8 signal of **3b** was assigned by analogy to the related assignment in **3a**.

Spin-Coupling Constants. The 620-MHz ^{1}H NMR spectra of **1–4b** in ^2H_2O are complex, and only that of **1b** approaches first order. This non-first-order behavior is caused by the similar chemical shifts of H3′ and H4′*R*, which are coupled to one another (Figure 1). However, spectral simulation allowed the measurement of all available ^{1}H–^{1}H spin couplings,[24b] and several ^{13}C–^{1}H spin couplings, in **1b**, **2b**, and **4b** (Table III).[24c] In addition, several ^{13}C–^{13}C spin couplings were measured from ^{13}C NMR spectra (Table III).

As observed previously in ribonucleosides,[8] $^1J_{C1',H1'}$ appears to depend on base structure, with couplings ~2 Hz smaller in **1b** than in **2b** and **4b** (Table III). In **2b** and **4b**, C1′ is coupled to H6, whereas in **1b**, no coupling is observed between C1′ and H8, despite the fact that these coupling pathways are structurally related. $^1J_{C1',C2'}$ is similar for all three erythronucleosides (43.7 ± 0.3 Hz) and appears somewhat larger in erythronucleosides than in ribonucleosides (42.8 ± 0.3 Hz), but solvent (DMSO-d_6 vs ^2H_2O) may account for this difference. $J_{C1',C3'}$ and $J_{C1',C4'}$ are comparable in magnitude in **1–4a** and **1–4b** despite differences in solvent, but an interpretation of these values is complicated by dual pathway contributions (see below).

Conformational Analysis. Pseudorotation[25] describes

Chart I



the conformational dynamics of furanose rings in which 20 idealized nonplanar conformers (10 envelope (E), 10 twist (T)) (Chart I) interconvert via a cyclic pathway (the pseudorotational itinerary[25]). Conformer interconversion occurs via small, systematic endocyclic bond rotations, and thus the planar form is not required as an intermediate in the process. The structure of each conformer can be described by two parameters, phase angle (*P*) and puckering amplitude (τ_m).[25] Furanose conformational dynamics of nucleosides in solution is commonly described by a two-state model in which a limited number of conformers in the north (N) and south (S) domains of the pseudorotational itinerary (Chart I) are preferred, and interconversion between these domains is rapid. The presence of this conformational averaging can, therefore, complicate the analysis of NMR parameters such as chemical shifts and coupling constants,[6,26a] but several approaches have been developed to assess furanose ring conformation based on these parameters.

Inspection of the data in Table III reveals that $^3J_{H1',H2'}$ is larger in **1–4b** than in corresponding **1–4a**, indicating a change in the relative populations of N and S conformers. deLeeuw and Altona have reported a graphical method relating this coupling in **1–4a** to the percent S conformer in solution.[26b] Since the β-D-erythrofuranosyl ring is structurally related to the β-D-ribofuranosyl ring, this method was applied to **1–4b** using $^3J_{H1',H2'}$ values of 6.7 (**1b**), 5.5 (**2b**), 6.5 (**3b**), and 5.9 Hz (**4b**) to give the following percentages of S conformers: **1b**, 91%; **2b**, 70%; **3b**, 84%; **4b**, 76%. These results indicate that the furanose rings in **1–4b** highly prefer a south conformation (Chart I) and that this preference is more pronounced when the base is a purine.

These conclusions were tested more rigorously using a computer program,[27] PSEUROT, that calculates the phase angles for the preferred north (*P*(N)) and south (*P*(S)) populations, their puckering amplitudes (τ_m(N) and (τ_m-(S)), and their mole fractions (X_N and X_S) from furanose $^3J_{HH}$ values (Table IV). Calculations performed on **1b**, **2b**, and **4b** gave X_S values of 0.95, 0.79, and 0.86, respectively (Table IV), which are somewhat larger than those derived from the singular analysis of $^3J_{H1',H2'}$ described above.

While $^3J_{HH}$ have been used extensively in conformational analyses of furanose rings, J_{CH} and J_{CC} can provide valuable complementary information in these studies.[6,7,28]

(25) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.

(26) (a) Jardetzky, O. *Biochem. Biophys. Acta* **1980**, *621*, 227. (b) deLeeuw, F. A. A. M.; Altona, C. *J. Chem. Soc., Perkin Trans. 2* **1982**, 375.

(27) de Leeuw, F. A. A. M.; Altona, C. *QCPE* **1983**, *8*, 463.

Table IV. Computer Analysis[a] of Vicinal ^1H–^1H Spin-Coupling Constants in Erythronucleosides 1b, 2b, and 4b and Methyl β-D-Erythrofuranoside 7

coupled protons	1b	2b	4b	7[c]
1′, 2′	6.7 (6.7)	5.6 (5.5)	5.9 (5.9)	3.2 (2.9)
2′, 3′	4.8 (4.6)	4.6 (4.7)	4.6 (4.7)	5.4 (4.8)
3′, 4′*R*	3.8 (3.8)	4.3 (4.3)	4.1 (4.1)	5.2 (5.0)
3′, 4′*S*	1.7 (1.7)	3.3 (3.2)	2.3 (2.3)	3.6 (3.5)
P(N)	−14.9	24.1	−24.9	−3.2
τ_m(N)	40°	40°	40°	36°
P(S)	180.1	186.4	187.9	202.2
τ_m(S)	40°	40°	40°	36°
X_S	0.95	0.79	0.86	0.65
rms error	0.079	0.069	0.063	0.357

[a] Analysis was performed using the PSEUROT program described in ref 27. Values in parentheses are the experimentally observed $^3J_{HH}$ values (Table III). [b] The parameters *P*(N), τ_m(N), *P*(S), τ_m(S), and X_S are defined in the text. [c] See ref 36.

Although there are no $^3J_{C,C}$ values within the erythrofuranosyl ring that are easily interpreted due to the dual pathway problem,[29] three useful J_{CH} are available from (1′-^{13}C)-substituted 1–4b: $^3J_{C1',H3'}$, $^3J_{C1',H4'R}$, and $^3J_{C1',H4'S}$. Although the dependence of $^3J_{CH}$ on molecular dihedral angle has not been defined as well as that for $^3J_{HH}$, these couplings may be examined qualitatively for their consistency with conformational conclusions based on $^3J_{HH}$. In the highly preferred $^2T_3$ conformation (*P*(S) = 180.1, Table IV) of the erythrofuranosyl ring of **1b** deduced from $^3J_{HH}$ (see above), the dihedral angle between C1′ and H3′ is large (~160 °C), and thus $^3J_{C1',H3'}$ should be approaching its maximal value. The observed coupling of 5.2 Hz (Table III) is consistent with this expectation. This dihedral angle is smaller in N conformers and thus if N conformers were present in solution in significant proportions, a smaller value of $^3J_{C1',H3'}$ would be expected. Furthermore, in the $^2T_3$ conformation, the dihedral angle between C1′ and H4′*R* is ~100°, whereas that between C1′ and H4′S is ~140°. Thus, $^3J_{C1',H4'R}$ should be smaller than $^3J_{C1',H4'S}$. In **1b**, $^3J_{C1',H4'S}$ and $^3J_{C1',H4'R}$ are 3.7 and 1.7 Hz, respectively (Table III).

The PSEUROT program was also used to calculate furanose conformational parameters for the ribonucleosides 1–4a using $^3J_{HH}$ data reported previously[8] (Table V). A comparison of these data with those obtained for **1b**, **2b**, and **4b** (Table IV) shows that X_S increases upon replacement of the exocyclic C5′ hydroxymethyl group in 1–4a with a proton, and this increase is observed in both purine and pyrimidine nucleosides, with approximate increases of 0.20 and 0.37, respectively. However, the *difference* in the behavior of purine and pyrimidine nucleosides with respect to the relative populations of N and S conformers is conserved in erythro- and ribonucleosides. Specifically, X_S is greater when the base is a purine in both series of compounds, suggesting that the exocyclic hydroxymethyl group in 1–4a is not a key determinant of this effect.

Values of *P*(N) and *P*(S) differ in both series of compounds. In 1–4a, *P* values are skewed toward east conformers (i.e., values lie to the right of the N/S vertical axis), whereas in erythronucleosides, *P* values are in general skewed toward west conformers (i.e., values lie to the left to the vertical N/S axis). This difference may reflect different pathways of N/S interconversion in 1–4a and 1–4b. In 1–4a, steric interactions between the C1′ and C4′ substituents destabilize N/S interconversion via west conformers, and thus N/S interconversion occurs primarily via east forms.[2,30] Since the bulky hydroxymethyl substituent is absent in 1–4b, interconversion via west conformers may become more favored.

Table V. Computer Analysis[a] of Vicinal ^1H–^1H Spin-Coupling Constants in Ribonucleosides 1–4a

coupled protons	1a	2a	3a	4a
1′, 2′	6.1 (6.2)	3.9 (4.0)	5.9 (6.0)	4.4 (4.5)
2′, 3′	5.4 (5.3)	5.3 (5.2)	5.5 (5.4)	5.4 (5.3)
3′, 4′	3.2 (3.3)	6.0 (6.0)	3.5 (3.6)	5.4 (5.5)
P(N)	19.1	21.8	20.4	22.2
τ_m(N)	38°	38°	38°	38°
P(S)	153.3	147.8	150.9	146.7
τ_m(S)	38°	38°	38°	38°
X_S	0.77	0.42	0.74	0.50
rms error	0.115	0.068	0.080	0.077

[a] Analysis was performed using the PSEUROT program described in ref 27. Values in parentheses are the experimentally observed $^3J_{HH}$ values reported in ref 8. [b] The parameters *P*(N), τ_m(N), *P*(S), τ_m(S), and X_S are defined in the text.

The relative stabilities of N and S conformers of nucleosides will be influenced by several structural factors, including the preferred quasi-equatorial orientation of the C4′–C5′ bond, the "gauche effect"[31–33] as applied to endo- and exocyclic oxygen substituents, and the orientation of the *N*-glycoside bond.[34] In N conformers of 1–4a, O3′ and O4′ are anti, O2′ and O4′ (and O2′ and O3′) are gauche, and the C4′–C5′ and C1′–N1(N9) bonds are quasi-equatorial and quasi-axial, respectively. In S conformers of 1–4a, O3′ and O4′ (and O2′ and O3′) are gauche, O2′ and O4′ are anti, and the C4′–C5′ and C1′–N1(N9) bonds are quasi-axial and quasi-equatorial, respectively. If the gauche effects involving O2′, O3′, and O4′ cancel in N/S interconversion, then only the C4′–C5′ and C1′–N1(N9) bond orientations control the proportions of N and S conformers. It is clear that the addition of a hydroxymethyl substituent at C4′ (β-D-erythrofuranosyl to β-D-ribofuranosyl) increases the proportion of N conformers in solution, since these conformers allow the preferred quasi-equatorial orientation of the C4′–C5′ bond. Interestingly, the C1–N1(N9) bond appears to prefer a quasi-equatorial orientation when the C4′–C5′ effect is removed (i.e., in 1–4b). This observation appears to contradict a recent study[34] indicating that the *N*-glycoside bond, in the absence of other effects, prefers a quasi-axial orientation.

It should also be appreciated that furanose, hydroxymethyl, and *N*-glycoside (syn, anti) conformations in nucleosides are interdependent.[2,30] In general, the N-anti and S-anti conformers are favored in pyrimidine ribo- and 2′-deoxyribonucleosides. In the corresponding purine nucleosides, however, N-anti competes with S-anti and S-syn. Since N/S distribution is altered by converting 1–4a to 1–4b, changes in the proportions of syn–anti conformers may be expected, but this feature was not examined in this study.

The conformational properties of methyl β-D-erythrofuranoside 7 appear to be notably different than those of the β-D-erythrofuranosyl ring in 1–4b, as reflected in the different spin couplings observed in these compounds

(28) Vuorinen, T.; Serianni, A. S. *Carbohydr. Res.* **1990**, *209*, 13.
(29) The magnitudes of these couplings are determined by two competing coupling pathways, thus complicating an analysis. For example, in 1–4b, $J_{C1',C3'}$ is affected by the C1′–C2′–C3′ and C1′–O4′–C4′–C3′ pathways.
(30) Pearlman, D. A.; Kim, S.-H. *J. Biomol. Struct. Dyn.* **1985**, *3*, 99.
(31) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.
(32) Zefirov, N. S.; Samoshin, V. V.; Subbotin, O. A.; Baranenkov, V. I.; Wolfe, S. *Tetrahedron* **1978**, *34*, 2953.
(33) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer: New York, 1983.
(34) Koole, L. H.; Buck, H. M.; Nyilas, A.; Chattopadhyaya, J. *Can. J. Chem.* **1987**, *65*, 2089.

(Table III). Previous NMR[6] and computational[35] studies have indicated that 7 is conformationally heterogeneous, with N and S conformers having comparable stabilities. A computer analysis of the $^3J_{HH}$ values for 7 gave X_S = 0.65, which is significantly smaller than X_S determined for **1b**, **2b**, and **4b** (Table IV).[36] Thus, the conversion of the *O*-glycoside 7 to 1-**4b** significantly alters furanose ring conformation, as noted previously in the ribonucleosides.[8] The conformational heterogeneity observed in 7 is reduced in the erythronucleosides 1-**4b** by the selective stabilization of S conformers.

Acknowledgment. This work was supported by the National Institutes of Health (GM 33791), the Research Corporation (10028), and Omicron Biochemicals, Inc. The authors thank Drs. T. K. Mishra and J. Dadok of the NMR Facility For Biomedical Studies, Department of Chemistry, Carnegie Mellon University, for their assistance in obtaining the 620-MHz ^{1}H NMR spectra.

Supplementary Material Available: 300-MHz ^{1}H NMR spectra of (1'-^{13}C)**1b**, **2b**, (1'-^{13}C)**2b**, **4b**, and (1'-^{13}C)**4b** and 75-MHz ^{1}H-decoupled ^{13}C NMR spectra of **3b** and **4b** (6 pages). Ordering information is given on any current masthead page.

(35) Serianni, A. S.; Chipman, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 5297.

(36) The rms error for this calculation (Table IV) is higher than those for the nucleosides, and thus the calculated parameters should be considered less reliable.

A New Reagent for the Removal of the 4-Methoxybenzyl Ether: Application to the Synthesis of Unusual Macrocyclic and Bolaform Phosphatidylcholines

Normand Hébert, Alain Beck,† R. Bruce Lennox,* and George Just*

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Canada H3A 2K6

Received July 26, 1991

The total synthesis of two novel polymerizable phosphatidylcholines has been accomplished using 3-(4-methoxybenzyl)-*sn*-glycerol **10** as starting material. Diacylation of **10** with 13-tetradecynoic acid followed by oxidative coupling of the alkynes gives the 32-membered glycerol macrocycle **17**. Sequential acylation of **10** with palmitic acid and 15-hexadecynoic acid followed by oxidative coupling gives the bolaform **16**, tethered at the 2-position of the glycerol. A new method for the cleavage of 4-methoxybenzyl ethers using dimethylboron bromide at −78 °C in dichloromethane is described. 1,3-Diacetylenes, 1,4-dienes, and esters are stable under the experimental conditions, and the migration of acyl chains from secondary to primary positions is totally suppressed. The diacylglycerols are then efficiently converted into the corresponding phosphatidylcholines by tetrazole-catalyzed phosphitylation with 2-cyanoethyl 2-bromoethyl *N,N*-diisopropylamino phosphite, oxidation, and treatment with trimethylamine to simultaneously displace the bromide and eliminate the cyanoethyl group.

Introduction

Phospholipids constitute the main structural component of cell membranes. Due to their amphiphilic nature, they associate into aggregate structures such as vesicles, micelles, and bilayers. In biological phospholipid assemblies, the nature of the lipid components affects the function and dynamics of the membrane.[1] For example, the outer leaflet of plasma membranes is richer in phosphatidylcholines (PCs), while the inner leaflet is richer in phosphatidylethanolamines (PEs) and phosphatidylinositol (PIs).[2] In addition to differences in the polar portion, there is also a large difference in the length and degree of saturation of the lipid chains, both between monomers and between the primary and secondary positions on the glycerol backbone itself. Notably, unsaturated lipids, e.g., arachidonic acid, are almost exclusively esterified at the secondary position of the glycerol. A host of enzymes exist within the cell for the processing and turnover of membrane phospholipids.[3] Among the important members of this class is phospholipase A_2 (PLA_2), which releases arachidonate (and other fatty acids) at the 2-position of phosphatidylcholines for the production of eicosanoids, and phospholipase C, which cleaves PIs to release the important intracellular second messengers inositol triphosphate and diacylglycerol.

It is known that both the nature and the physical state of the phospholipids are important factors that determine the rate of enzymatic hydrolysis.[4] In order to gain understanding of this dependence at the molecular level with respect to the mechanism of PLA_2, we required a series of modified phospholipids with substituents on the 2-acyl chain. In a previous paper we described the synthesis of chain-substituted phosphatidylcholines by the coupling of a diacylglycerol with a 2-bromoethyl 2-cyanoethyl phosphoramidite with acid catalysis.[5] This strategy has several advantages over the standard methodology.[6] Firstly, the use of a mild acid catalyst prevents acyl migration in the diacylglycerol. Secondly, the intermediate phosphate triester is neutral and can be purified at this stage using

† Current address: Medafor: "Le Platon", Parc d'Innovation, 67400 Illkirch, France.

(1) Gennis, R. B. *Biomembranes: Molecular Structure and Function*; Springer-Verlag: New York, 1989.
(2) Zubay, G. L. *Biochemistry*; Addison-Wesley: Reading, MA, 1983.
(3) Bishop, W. R.; Bell, R. M. *Ann. Rev. Cell Biol.* **1988**, *4*, 579.
(4) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257. Achari, A.; Scott, D.; Barlow, P.; Vidal, J. C.; Otwinowski, Z.; Brunie, S.; Sigler, P. B. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 441.
(5) Hébert, N.; Just, G. *J. Chem. Soc., Chem. Commun.* **1990**, 1497.
(6) Bogomolov, O. V.; Kaplun, A. P.; Shvets, V. I. *Russ. Chem. Rev.* **1988**, *57*, 382. Stepanov, A. E.; Schvets, V. I. *Chem. Phys. Lipids* **1986**, *41*, 1. Ramirez, F.; Marecek, J. F. *Synthesis* **1985**, 449.

0022-3263/92/1957-1777$03.00/0