fluoro(2,2,5,5-tetramethyltetrahydrofuran) (Figure 5) as is the case with bis(perfluoroneopentyl) ether[3,16] and other perfluorinated compounds.[26] Despite the different "through-bond" distances between trifluoromethyl and two difluoromethylene groups, it appeared that CF_3 groups were "equally" split by the four secondary fluorine atoms. On the other hand, the very complicated F–F coupling of bis(perfluoroisopropyl) ether (Figure 6) may have been due to repulsion of the intramolecular tertiary fluorine atoms; this highly branched perfluorinated ether may have a "staggered asymmetrical" structure, and the decrease in the melting point[27] could be related to this unusual structure.

Like perfluorinated ethers, perfluorinated tertiary amines are currently important materials in many electronic and medical applications.[28] Because amines dissolve well and give fair stability in anhydrous hydrogen fluoride, different classes of perfluorinated amines can be prepared from their hydrocarbon analogues by the Simons' electrochemical fluorination process.[28] In fact, this electrolytic technique is the only significant synthetic route to perfluorinated tertiary amines. However, rearrangements and degradations limit its capability to prepare highly branched perfluorinated amines. Our successful synthesis of perfluoro(1,2,2,6,6-pentamethylpiperidine) provides an improved route to perfluorinated amines. As compared with the melting point decreases observed for our novel perfluorinated fluids,[12,27] this new perfluorinated tertiary amine should be classified as an extremely structurally crowded molecule. Due to this steric advantage and the qualified physical properties, we surmise that we have obtained a promising PFSC-based "artificial blood" or "additive". Preliminary animal tests show promising results, and further studies are in process.[24] Despite the over-long retention in mammalian bodies, perfluoro(tri-*n*-propylamine) has been used as an "additive" to improve the stabilities of perfluorodecalin-based emulsions, and these "*multi*-PFSC-base" emulsions have been tested on healthy humans.[2]

Acknowledgment. We are grateful for support of this work by the Air Force Office of Scientific Research (AFOSR-82-0197). The helpful discussions with Dr. B. A. Shoulders and Mr. S. Sorey concerning the NMR interpretations and the permission to use the special $^{13}C\{^{19}F\}$ NMR probe built by Mr. O. Williams are appreciated.

Registry No. $CF_3C(CF_3)_2COCF_3$, 88995-83-9; $CF_3CF(CF_3)CF_2OCF_3$, 110719-85-2; $CF_3C(CF_3)_2COCF_2CF(CF_3)$, 88995-86-2; $[(CF_3)_2CFCF_2]_2O$, 97187-06-9; $(CF_3)_2CFOCF_2CF_3$, 56102-25-1; $[(CF_3)_2CF]_2O$, 83935-39-1; $(CF_3)_2CFOCF_2CF_2CF_3$, 83935-38-0; $[(CF_3)_3OCF_2]_2$, 110719-92-1; $(CF_3)_3CF$, 354-92-7; $(CF_3)_2CFCHF_2$, 59571-40-3; *tert*-butyl isobutyl ether, 33021-02-2; 1,2-di-*tert*-butoxyethane, 26547-47-7; perfluoro(2,2,5,5-tetramethyltetrahydrofuran), 110719-86-3; perfluoro(2,2,5-trimethyltetrahydropyran), 110719-87-4; perfluoro(2,2,5-trimethyltetrahydrofuran), 110719-88-5; 3-hydropentadecafluoro-2,2,5,5-tetramethyltetrahydrofuran, 110743-66-3; *cis*-perfluoro(2,5-dimethyltetrahydrofuran), 110719-89-6; *trans*-perfluoro(2,5-dimethyltetrahydrofuran), 110719-90-9; perfluoro(2-ethyltetrahydrofuran), 356-48-9; perfluoro(1,2,2,6-pentamethylpiperidine), 110719-91-0; perfluoro(cyclohexyl neopentyl ether), 110719-93-2; fluorine, 7782-41-4; 2,2,5,5-tetramethyltetrahydrofuran, 15045-43-9; *tert*-butyl methyl ether, 1634-04-4; 1,2,2,6,6-pentamethylpiperidine, 79-55-0; *cis*-2,5-dimethyltetrahydrofuran, 2144-41-4; 7-oxabicyclo[2.2.1]heptane, 279-49-2; isopropyl ether, 108-20-3; neopentyl phenyl ether, 2189-88-0; perfluoropentane, 678-26-2; perfluorohexanoyl fluoride, 355-38-4; *trans*-2,5-dimethyltetrahydrofuran, 2390-94-5.

(27) "Melting point drop" behavior is defined as the melting point of the perfluorinated compound lower than its hydrocarbon analogue.
(28) Danielson, R. D. *Encycl. Chem. Technol.* **1979**, *10*, 874.

Antiviral Nucleosides. A Stereospecific, Total Synthesis of 2′-Fluoro-2′-deoxy-β-D-arabinofuranosyl Nucleosides

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A general, total synthesis of (2′-fluoro-2′-deoxy-β-D-arabinofuranosyl)uracils **1a–d** is described. A study of the coupling reaction between 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**7**) and silylated pyrimidines **11a–d** has resulted in a high overall yield for the five-step stereospecific synthesis of β-nucleosides.

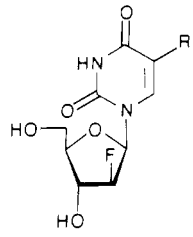
The severity of diseases like AIDS has intensified the interest in a variety of synthetic agents[1] that are effective against viral disease. A class of agents found to be active against DNA viruses was discovered in the late 1970s by Watanabe and Fox.[2] These compounds are (2′-fluoro-2′-deoxy-β-D-arabinofuranosyl)pyrimidines substituted in the 5 position, **1a–d** (Scheme I). As our interest in this class of biologically potent compounds developed, we began a search for efficient synthetic approaches.

Previous communications from this laboratory have described a simple preparation of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (**4**),[3] and its conversion to 2-fluoro-2-deoxy-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (**6**).[4] This

(1) (a) Robins, R. K. *Chem. Eng. News* January 27, **1986**, 28–40. (b) Dagooni, R. *Chem. Eng. News* December 8, **1986**, 7–14. (c) DeClerq, E. *J. Med. Chem.* **1986**, *29*, 1561.
(2) Su, T.-L.; Watanabe, K. A.; Schinazi, R. F.; Fox, J. J. *J. Med. Chem.* **1986**, *29*, 151.
(3) Howell, H. G.; Brodfuehrer, P. R.; Sapino, C. *J. Org. Chem.* **1985**, *50*, 2597.
(4) Howell, H. G.; Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, C. *J. Org. Chem.* **1985**, *50*, 3644.

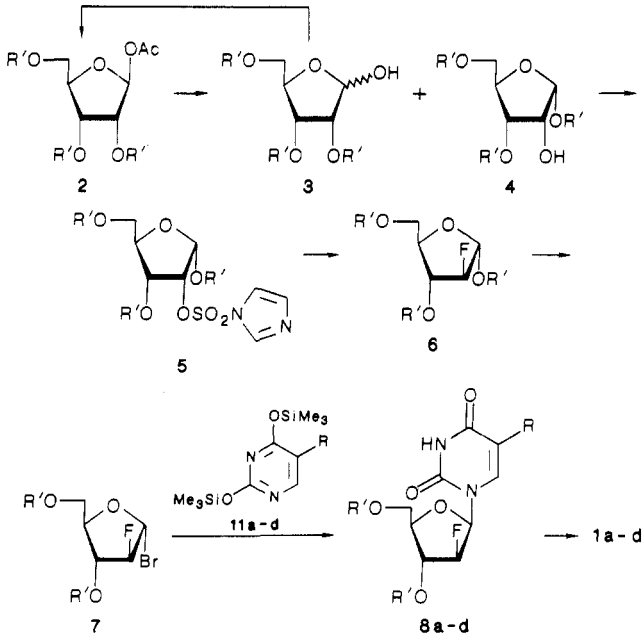
0022-3263/88/1953-0085$01.50/0

Scheme I



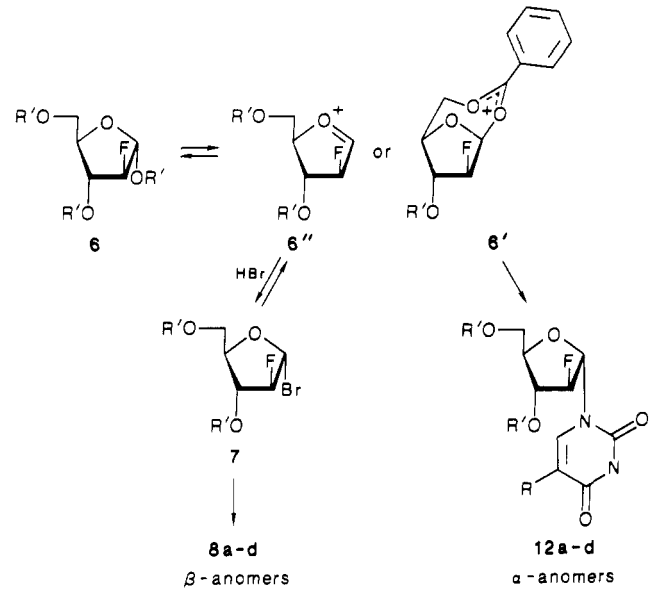
1a, R = H
b, R = CH_3
c, R = CH_2CH_3
d, R = I

Scheme II[a]

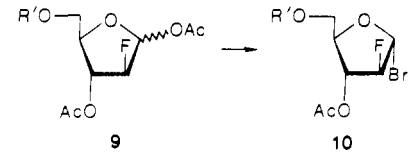


[a] R′ = benzoyl.

Scheme III



Scheme IV



formation of the nucleoside bond.

In accordance with the literature procedure,[5] a solution of the silylated pyrimidine **11a–d** in CH_3CN was reacted with a solution of bromo sugar **7** in CH_2Cl_2 for 1 week at room temperature. The β/α ratio of the product varied between 4/1 and 9/1 from run to run. When this reaction was performed at reflux in acetonitrile alone, the β/α ratio was 3/1 and in refluxing dichloromethane alone, the anomer ratio was 9/1. Reactions done at 20 °C and at elevated temperature in the same solvent have indicated that the rate of the reaction is temperature-dependent. The coupling reaction proceeds much faster at elevated temperatures; however, the anomer ratio appears unaffected (at 20–80 °C).

It had been suggested by Fox,[7a] based on work reported by Prystas,[7b] that proper control of the reaction conditions should allow one to effect stereocontrol of nucleoside formation. In fact, Fox and Ritzmann[8] found that the β/α ratio varied with the solvent: 1/1 in acetonitrile, 3/1 in dichloromethane, and 6/1 dichloroethane. Recent reports[9] have stated that coupling reactions in chloroform show a preference for β-nucleosides (β/α = 3/1). Walker and co-workers[10] further investigated the factors controlling the anomer ratio by studying the condensation of 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosyl chloride. These authors concluded that α-halo sugars exclusively form β-nucleosides via an S_N2 mechanism and α-nucleosides form as a result of anomerization to β-halo sugar followed

report now describes the coupling of **6** (via the 1-α-bromo **7**) with silylated pyrimidines which completed a stereospecific, high-yield route to the known[5] blocked, β-nucleosides **8a–d** (Scheme II). When the bromination method reported by Fox[5] was employed with **6**, a single bromo anomer was obtained in quantitative yield. The anomeric proton of **7** was assigned to a doublet at 6.7 ppm (J = 11.9 Hz) in the NMR spectrum; however, no coupling was observed between $H_{1'}$ and $H_{2'}$. This material, assigned the α-configuration, exhibited couplings typical of an anomeric proton vicinal-cis to fluorine (9–12 Hz) and vicinal-trans to another proton (~0 Hz). 1,3,5-Tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-ribofuranose (**6**), similarly, showed a doublet at 6.7 ppm (J = 9.5 Hz) for its anomeric proton.[3,6]

It could be assumed that the α-benzoyl compound **6** could produce **7** via 1,5-benzoxonium ion **6′** or oxygen-C_1-onium ion **6″** (Scheme III). Both of these would allow the exclusive formation of an α-bromo anomer. When the anomeric mixture **9** was brominated, only the α-anomer **10** was observed to form (Scheme IV). A plausible rationale to explain these results is formation of the oxygen-C_1-onium ion and preferential attack of bromide ion from the α-face. Similar mechanistic discussions have appeared in the literature previously[7] concerning the

(5) (a) Reichman, U.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1975**, *42*, 233. (b) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.

(6) Ness, R. K.; Fletcher, H. G. *J. Am. Chem. Soc.* **1956**, *78*, 4710.

(7) (a) Watanabe, K. A.; Hollenberg, D. H.; Fox, J. J. *J. Carbohydr. Nucleosides Nucleotides* **1974**, *1*, 1. (b) Prystas, M.; Farkos, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1965**, *30*, 3213.

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(9) (a) Grienyl, H.; Bodenteich, M.; Hayden, W.; Wanek, E.; Streicher, W.; Statz, P.; Bachmayer, H.; Ghazzouli, I.; Rosenwirth, B. *J. Med. Chem.* **1985**, *28*, 1679. (b) Grienyl, H.; Hayden, W.; Schwartz, W.; Bachmayer, H.; Rosenwirth, B. *Eur. J. Med. Chem. Chim. Ther.* **1985**, *20*, 105.

(10) Hubbard, A. J.; Jones, A. S.; Walker, R. T. *Nucleic Acids Res.* **1984**, *12*, 6827.

Table I. Anomer Ratios of Nucleosides Prepared in Solvents of Different Polarity

solvent	reactn temp, °C	dielectric[a] constant	reactn time, h	β/α[b] 10a	10b	10c	10d
CH_3CN	82	36–38	5	4	3	2	2
CH_2Cl_2	40	8–9	44	8.5	8.5	8.5	14
$CHCl_3$	62	4–5	36	20	19	16	29
CCl_4	76	2–3	60	39	36	41	34

[a] Gordon, A. V.; Ford, R. A. *A Handbook of Practical Data, Techniques and References*; John Wiley and Sons: New York. [b] β/α ratio as determined by HPLC analysis.[12a]

by S_N2 displacement. Walker found that a solution of the α-chloro sugar in dichloromethane produced "significant quantities" of β-chloro sugar in 3 h. When bromo sugar **7** was refluxed (18 h) in dry acetonitrile, its NMR spectrum remained unchanged, and a sample in dichloromethane was likewise unchanged after 30 days. We therefore concluded that in our coupling of sugar **7** anomerization was an unlikely factor. The formation of α-nucleosides without anomerization of the 1-halo compound can be explained by an ionic pathway.[11] If this pathway (Scheme III) is correct, solvents of lower dielectric constants would favor the S_N2 products whereas higher dielectric solvents would favor products formed from a 1,5-benzoxonium ion or an oxygen-C_1-onium ion.

The results for the condensation of **7** with pyrimidines in solvents of different polarity indicate that the highest β/α anomer ratios were obtained with solvents of lower dielectric constant (Table I). The intervention of an ionic species to produce α-nucleosides is consistent with these results and those reported by Prystas.[7b] The β/α ratio from a coupling reaction in CCl_4 (reflux, 6 days) was a remarkable 60/1 (98% β), furnishing a recrystallized yield of pure β-anomer of 73%. The corresponding chloroform coupling (complete after 18 h at reflux) gave crude product with β/α ratio of 20/1 (95% β) and a 76% isolated yield of β-anomer.

Removal of the blocking groups was accomplished with excess ammonia in methanol; however, when the hydrolysis of **8a–d** was monitored by HPLC[12b] an intermediate was observed. The intermediate was identified by NMR as 1-(5′-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil and the reaction was continued until the hydrolysis was complete. This result suggests that careful control of the reaction conditions might allow selective hydrolysis of the 3′-benzoyl ester.

In summary, commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) was converted to 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose (**4**). In two more steps **4** was converted to **6**[4] and then taken to the bromo compound **7**. The coupling of **7** with various silylated pyrimidines, **11a–d**, under the conditions described gave high yields of the desired β-nucleosides. Simple removal of the blocking groups gave the nucleoside analogues **1a–d**. In the specific case of FEAU, **1c**, the overall yield was 32% (for the five-step process). A key step in the synthesis is the stereospecific formation of 3,5-di-*O*-benzoyl-2-fluoro-2-deoxy-α-D-arabinofuranosyl bromide (**7**) which allows the subsequent reaction to be performed in a stereospecific manner.

Experimental Section

NMR spectra were recorded on a Bruker AM 360 spectrometer at a proton frequency of 360 MHz and chemical shifts are expressed in parts per million. HPLC analysis were performed on a Hewlett-Packard 1084B instrument using a reverse-phase C-18 column. Elemental analysis were performed by the Analytical Research Department of Bristol-Myers Pharmaceutical Research and Development, Syracuse, NY.

1,3,5-Tri-*O*-benzoyl-α-D-ribofuranose (4). A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) (400 g, 0.793 mol) and 2 L of CH_2Cl_2 was stirred at ca. 5 °C, and 70.6 g (0.873 mol) of hydrogen bromide gas added slowly from a cylinder.[13] The reaction was judged to be complete when the NMR signal for the CH_3 of the starting material integrated to less than 2 mol %. At this point (less than 2 h), 800 mL of H_2O was added and the mixture was stirred vigorously for 1 h. The organic layer was separated, dried ($MgSO_4$), concentrated (to ca. 1.5 L), and cooled to ca. 5 °C. Heptane (2.5 L) was added slowly with stirring. The resulting slurry was stirred for 2 h and the crystals were collected. The product was washed on the filter (3 × 200 mL, 2/1, heptane/CH_2Cl_2), giving 215 g of **4** (59%): mp 140–141 °C; NMR (CD_2Cl_2) 7.55–8.25 (m, 15 H, Ar H), 6.2 (d, 1 H, J = 4 Hz, C_1), 5.45–5.65 (m, 1 H, C_3), 4.85–4.50 (m, 4 H, $C_{2,4,5}$), 2.81 (d, 1 H, OH, J = 9 Hz). Anal. ($C_{26}H_{22}O_8$) C, H.

The filtrate was vacuum concentrated, giving the anomeric mixture 2,3,5-tri-*O*-benzoyl-D-ribofuranose (**3**) as a syrup which was used to prepare **2**.

1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2). The syrupy 2,3,5-tri-*O*-benzoyl-D-ribofuranose (300 g, 0.649 mol) was dissolved in 370 mL of acetic anhydride and 160 mL acetic acid and then cooled to 0–5 °C. Sulfuric acid (53 mL, 1.0 mol) was added dropwise over 0.5 h. After an additional 0.5 h the ice bath was removed and the reaction continued at 20 °C for 1 h. The mixture was added to 4.8 L of cold water and stirred for 0.5 h, and the product was collected. The crude material was recrystallized from 2.4 L of hot isopropyl alcohol, giving 241 g of **2** (74%). Spectral data were identical with the commercially obtained material.[14]

2,4-Bis-*O*-(trimethylsilyl)-5-ethyluracil (11c). A suspension of 5-ethyluracil[15] (57.2 g, 0.408 mol), hexamethyldisilazane (96.5 mL, 0.457 mol), ammonium sulfate (4.83 g, 0.037 mol), and 1.45 L of CH_3CN was stirred at reflux 3 h. The solution was evaporated under reduced pressure to an oil which was used without further treatment.

3,5-Di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl Bromide (7). A solution of 159.3 g (0.343 mol) of 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (**6**), 725 mL of CH_2Cl_2, and 200 mL (0.74 mol) of 30% HBr in acetic acid was stirred at 20 °C for 20 h. The reaction mixture was washed (H_2O, 2 × 1.2 L; saturated $NaHCO_3$ solution, 2 × 1.2 L), dried (Na_2SO_4),

(11) After our manuscript was prepared, a report appeared in the literature which proposed a similar explanation for the formation of α-glycosides from α-pyranosyl bromides. Fei, C. P.; Chan, T. H. *Tetrahedron Lett.* **1987**, *28*, 849.

(12) (a) HPLC on reverse phase (C-18 IBM column) at 50% CH_3CN, H_2O and 2 mL/min at 254-nm UV detection was used to monitor the coupling reactions. Under these conditions the retention times were 0.95 min for 5-ethyluracil, 4.9 min for **12c**, 5.7 min for **8c**, and 10.1 min for **7**. (b) The hydrolysis reactions were generally worked up after 65 h when the HPLC analysis indicated less than 2 area percent of the incomplete hydrolysis product remained. HPLC performed as above gave retention times of 5.7 min for **8c**, 1.45 min for 5-benzoyl, and 0.9 min for **1c**.

(13) In smaller reactions, the amount of HBr gas added directly into the solution was difficult to measure. To avoid excess, a saturated HBr–CH_2Cl_2 solution was formed at 0–5 °C and titrated, it was usually found to be 0.4 M. The volume necessary to provide 1 to 1.5 equiv of HBr was then used in the reaction.

(14) Pouchert, C. J.; Campbell, J. R. *The Aldrich Library of NMR Spectra, VII*, 38C. The NMR and IR spectra of purchased material were directly compared to synthetic material and found to be identical.

(15) The 5-ethyluracil was prepared by Ms. S. I. Hauck and Mr. K. M. Shih according to Ravinder, K.; Gebhard, K.; Siegfried, E.; Bernd, H. *J. Pharm. Sci.* **1980**, *69*, 531.

and concentrated to give 143 g (98%) of oil which was used without further purification: NMR ($CDCl_3$) 8.2–7.4 (m, 10 H, Ar H), 6.7 (d, 1 H, $J_{H1,F}$ = 11.9 Hz, C_1), 5.6 (d, 1 H, $J_{H2,F}$ = 50 Hz, C_2), 5.5 (dd, 1 H, J = 22 Hz, 3 Hz, C_3), 4.75 (m, 3 H, C_4, C_5).

1-(3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (8c). A solution of 2,4-bis-*O*-(trimethylsilyl)-5-ethyluracil (**11c**) (0.408 mol), 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**7**) (0.343 mol), and 1.7 L of $CHCl_3$ (alcohol free) was stirred at reflux for 20 h.[12a] The cooled reaction mixture was washed (H_2O, 2 × 2 L) and dried (Na_2SO_4), and the solvent was removed at reduced pressure. The solid product was recrystallized from 1.5 L of hot, absolute ethyl alcohol to give 126 g of **8c** (76.2%): mp 155–157 °C; NMR ($CDCl_3$) 8.7 (s, 1 H, C=CH), 8.2–7.2 (m, 10 H, Ar H), 6.34 (dd, 1 H, J = 3 Hz, $J_{H1',F}$ = 22 Hz, $C_{1'}$), 5.63 (dd, 1 H, J = 3 Hz, $J_{H3',F}$ = 17.7 Hz, $C_{3'}$), 5.31 (dd, 1 H, J = 3 Hz, $J_{H2',F}$ = 50 Hz, $C_{2'}$), 4.8 (m, 2 H, $C_{5'}$), 4.7 (dd, 1 H, $C_{4'}$), 2.19 (q, 2 H, J = 7 Hz, CH_2), 0.95 (t, 3 H, J = 7 Hz, CH_3). Anal. ($C_{25}H_{23}N_2O_7F$) C, H, N.

1-(2′-Deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil, FEAU (1c). A solution of 275 mL (12.75 mol) of liquified ammonia and 1.72 L of methyl alcohol was stirred at ca. 0 °C. The solid 1-(3′,5′-di-*O*-benzoyl-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (**8c**), 172 g (0.356 mol), was added portionwise over 5 to 10 min. The ice bath was removed and the slurry stirred for 65 h (HPLC monitoring indicated only a trace of the incomplete hydrolysis product, 5-benzoyl[12b] remained). The solvent was removed and the crude product recrystallized from 700 mL of boiling CH_3CN. After crystallization at 0 °C, 4 h, the product was removed by filtration, washed with cold CH_3CN (2 × 100 mL), and dried to give **1c**, 90.8 g (93%): mp 171–173 °C; NMR ($NaOD/D_2O$) 7.5 (s, 1 H, C=CH), 6.3 (dd, 1 H, $J_{H,F}$ = 16 Hz, $C_{1'}$), 5.16 (m, 1 H, $J_{H2',F}$ = 52 Hz, $C_{2'}$), 4.4 (m, 1 H, $J_{H3',F}$ = 19.9 Hz, $C_{3'}$), 4.0–3.77 (m, 3 H, $C_{4'}$, $C_{5'}$), 2.27 (q, 2 H, J = 7.5 Hz, CH_2), 1.05 (t, 3 H, J = 7.5 Hz, CH_3). Anal. ($C_{11}H_{15}N_2O_5F$) C, H, N.

2-Fluoro-2-deoxy-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl Bromide (7) Stock Solution. A solution of 9.29 g (20.0 mmol) of **6** and 42 mL of dry CH_2Cl_2 was stirred at ca. 20 °C and 11.7 mL (43.4 mmol) of 30% hydrogen bromide in acetic acid added. The solution was stirred for 18 h in a stoppered flask, then washed (H_2O, 2 × 60 mL, and saturated $NaHCO_3$ solution, 2 × 60 mL), and dried ($MgSO_4$), and the solvent was evaporated at reduced pressure to give 8.48 g of a light yellow oil. The material was found to be pure **8a** (NMR). The oil was diluted with CH_2Cl_2 to exactly 50 mL in a volumetric flask. The concentration of **7** was calculated as 0.4 M and portions of this solution were used for the general coupling procedure as described below.

2,4-Bis-*O*-(trimethylsilyl)pyrimidine 11a–d Stock Solution. A stock solution of each of the four uracils was prepared in this way; a mixture of 75 mL of CH_3CN, 4.75 mL of hexamethyldisilazane, 0.25 g of ammonium sulfate, and 2.24 g (20 mmol) of uracil was stirred at reflux 18 h. The solution of the bis-silylated pyrimidine was calculated to be 0.252 M and used as described below.

General Procedure for the Coupling Reaction. A 4.4-mL portion of the silylated uracil solution (1.1 mmol) was evaporated to dryness and dissolved in 2 mL of the appropriate, dry, solvent (CH_3CN, CH_2Cl_2, $CHCl_3$ or CCl_4). A 2.5-mL portion of the 0.4 M solution of **7** (1.0 mmol) was evaporated to dryness and dissolved in 2 mL of the same solvent as above. The solution of **7** was then added to the solution of the silylated pyrimidine **11a–d**. The flask that had contained **7** was rinsed with 1 mL of the same solvent and added to the reaction (total volume 5 mL). The reaction was stirred at reflux and samples were taken at intervals to determine when the reaction was complete. When HPLC[12a] indicated that **7** was consumed, the HPLC area count of each isomer was used to calculate the anomer ratios listed in Table I.

Acknowledgment. We are very grateful to Professor A. I. Meyers for discussions and advice concerning this manuscript.

Registry No. **1c**, 83546-42-3; **2**, 6974-32-9; α-**3**, 79439-67-1; β-**3**, 67525-66-0; **4**, 22224-41-5; **6**, 97614-43-2; **7**, 97614-44-3; **8c**, 95740-18-4; **11c**, 31167-05-2; 5-ethyluracil, 4212-49-1.

Enzymatic α/β Inversion of C-3 Hydroxyl of Bile Acids and Study of the Effects of Organic Solvents on Reaction Rates

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Enzymatic α/β inversion of the C-3 hydroxyl of numerous bile acids containing different numbers of hydroxyl groups in the skeleton and side chains of different lengths has been carried out. Inversion was obtained in two steps through the sequential use of the commercial enzymes 3α- and 3β-hydroxysteroid dehydrogenase, employed in the free form or immobilized on Eupergit C. The transformations were practically quantitative and the products more than 98% pure. NAD was regenerated in situ with the pyruvate/lactic dehydrogenase system and NADH with the formate/formate dehydrogenase system. The effects of product inhibition on reaction rates and the favorable effects produced by low concentrations (7–10%, v/v) of ethyl acetate and ethanol were also examined.

Introduction

The NAD(P)-dependent oxidoreductases[2] have been successfully used for the regio-, stereo-, and (or) enantiospecific oxidoreduction of the hydroxyl keto groups of a variety of compounds.[3] In particular, hydroxysteroid dehydrogenases have been employed for preparative-scale transformations of bile acids and neutral steroids in aqueous and in organic media.[4] The potential use of these

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(2) Abbreviations: NAD, β-nicotinamide adenine dinucleotide; K_m, Michaelis constant; K_i, product inhibition constant; V_{max}, maximal enzymatic velocity; DTT, dithiothreitol.

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