# Stereospecific Synthesis of 2-Deoxy-2,2-difluororibonolactone and Its Use in the Preparation of 2'-Deoxy-2',2'-difluoro-β-D-ribofuranosyl Pyrimidine Nucleosides: The Key Role of Selective Crystallization

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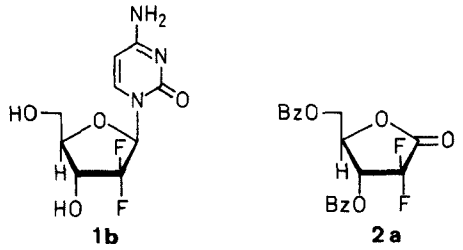
A stereospecific synthesis of 2'-deoxy-2',2'-difluorocytidine (gemcitabine), a potential anticancer agent, is described. The stereoselectivity was accomplished via two diastereoselective crystallizations, i.e. the crystallization of the key intermediate, difluororibonolactone **2a**, and the crystallization of the hydrochloride salt of gemcitabine **16b** from the anomeric mixture. Because of the availability of **2a** in large quantities, other 2'-deoxy-2',2'-difluoropyrimidine nucleosides such as 2'-deoxy-2',2'-difluorouridine **(19)** were synthesized for structure–activity relationship studies.

The synthesis of 2'-deoxy-2',2'-difluorocytidine **(1b)**,[1] a potential anticancer agent, was originally accomplished by Hertel and co-workers.[2] However, this synthesis is not suitable for kilogram scale production. We utilized the same synthetic scheme but selected benzoyl over *tert*-butyldimethylsilyl as the protecting group for hydroxyl groups. With this modification, a crucial selective crystallization is now possible, i.e., crystallization of the desired ribonolactone **2a** from a diastereomeric mixture consisting of **2a** and **2b**. Also the crystallization of nucleoside **1b** from a 1:1 anomeric mixture was accomplished. This 1:1 anomeric mixture is also a vast improvement over the **1a**:**1b** = 4:1 mixture when *tert*-butyldimethylsilyl was used as the protecting group. With these successes, a production process was found which addressed the isomeric separation difficulties.

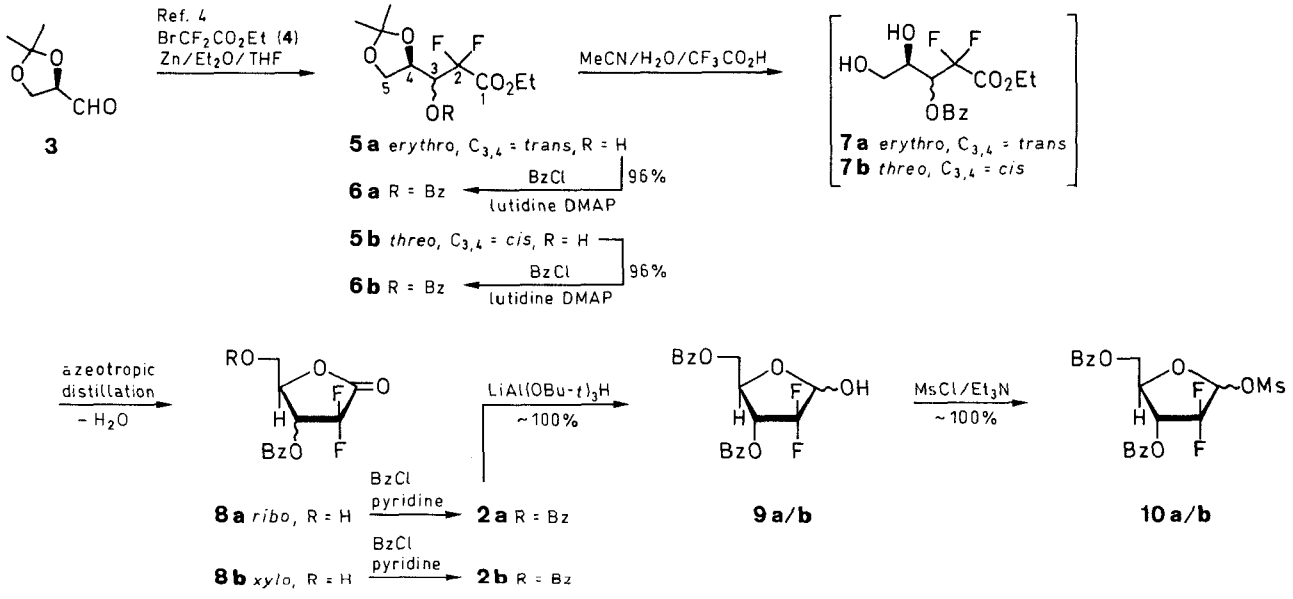


**1b**



**2a**

Treatment of (*R*)-2,3-*O*-isopropylideneglyceraldehyde **(3)**[3] with ethyl bromodifluoroacetate **(4)** produced diastereomeric isomers **5a/b**,[4] which although were deactivated by the *gem*-difluoro moiety, could be benzoylated to give **6a/b** in high yield (Scheme). The benzoyl group was introduced at this stage because it provided a UV chromophore for easy monitoring by HPLC in subsequent reactions. Hydrolysis of **6a/b** with a catalytic amount of trifluoroacetic acid in wet acetonitrile removed the isopropylidene group. The product, **7a/b** could be cyclized to lactones **8a/b** by azeotropic distillation because **7a/b** was found to be thermally stable in solution at temperatures below 100 °C. In theory, cyclization of **7a/b** could lead to either a furanosyl lactone or a pyranosyl lactone, but only the diastereomeric furanosyl lactones were detected.[5] Unlike the 2-deoxyribose which consists of an equilibrium mixture of pyranose and furanose structures, 2-deoxyribonic acid and 2-deoxy-2,2-difluororibonic acid apparently prefer γ-lactone over the δ-lactone form.[6] Benzoylation of the primary hydroxy group in **8a/b** was uneventful but selective crystallization of the desired **2a** from **2a/b** required great care because the carbonyl group in **2a** was easily solvolyzed.[7] For the same reason, washing the benzoylated mixture was prohibitive; therefore, a solvent system capable of crystallizing **2a** directly from the crude reaction mixture was needed. A




**Scheme**

useful solvent system consisting of dichloromethane and heptane (3:2) was found to selectively crystallize **2a** even in 2000 gallon scale.[8]

The best reducing agents for the carbonyl group in **2a** were metal hydride complexes. Using lithium tri-*tert*-butoxyaluminum hydride, **9a/b** were routinely obtained in 93–95% yield. Diisobutylaluminum hydride could also be used, although a substantial amount of unreacted starting material and over-reduction were observed. In all cases, both anomers **9a/b** were produced. The preparation of crystalline mesylates **10a/b** from **9a/b** was straightforward, and the combined yield from **2a** to **10a/b** was in the range of 91–92%. The mesylates could be separated by selective crystallization to provide either anomer in high purity and, in contrast to the mesylates of common 2-deoxyribosyl sugars, they are stable in inert solvents even at temperatures as high as 125 °C.

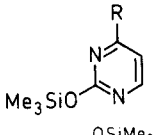
$^{13}C$ NMR spectroscopy was used to study the composition and anomeric configuration of the 2-deoxy-2,2-difluororibose derivatives. After examination of a variety of derivatives, both protected and unprotected at the C-3 or C-5 hydroxyl,[9] and in a variety of solvents, the chemical shift of the C-4 carbon was found to reliably provide information about anomeric configuration. In a pair of anomers, C-4 carbon resonance of the α-anomer was shifted about 2–3 ppm downfield relative to the β-anomer. Examples of this correlation are shown in the following Table.

**Table.** Variation of Carbon Chemical Shifts for C-4 with Anomeric Substitution

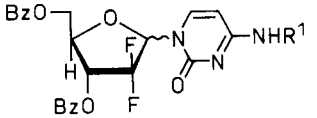
| Compound | Solvent | $^{13}C$ (C-4), α | $^{13}C$ (C-4), β | $\Delta = (\alpha-\beta)$ |
|---|---|---|---|---|
| **1a/b** | $D_2O$ | 84.36 | 81.54[a] | 2.82 |
| **10a/b** | $CDCl_3$ | 82.75 | 79.78 | 2.97 |
| **14a/b** | DMSO-$d_6$ | 79.44 | 75.71 | 4.72 |
| **16a/b** | $D_2O$ | 84.91 | 81.89 | 3.02 |

[a] Structure determined by X-ray crystallography.[2]

Pyrimidine nucleosides were made by reacting **10a/b** with silylated pyrimidine derivatives **11** or **12** under the Vorbrueggen protocol[10] to give the corresponding nucleosides **13a/b** or **14a/b**[11] in moderate yields.

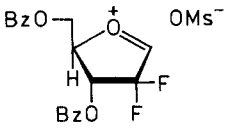


**11** R = N(OSiMe$_3$)Me
**12** R = NHSiMe$_3$
**17** R = OSiMe$_3$



**13a/b** R$^1$ = Ac
**14a/b** R$^1$ = H

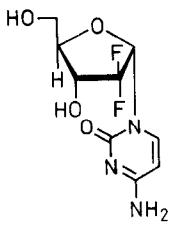
Significantly, a 1:1 mixture of the two anomeric nucleosides was obtained in both cases, regardless of the anomeric composition of the mesylates. Non-polar solvents and high temperature (80–130 °C) were important factors for the success of the glycosylation reaction because only a trace amount of the nucleosides were detected, even at pressures up to 13 kilobar,[12] when the reaction was carried out in dichloroethane at room temperature for 4 days. We believe that the reaction proceeds via an $S_N1$ pathway involving an oxonium ion intermediate **15** giving rise to a 1:1 mixture of nucleoside anomers.



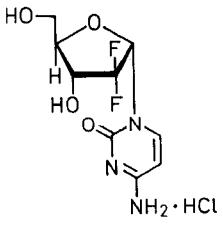
**15**

Support for this mechanism was obtained from the following studies. Except for the formation of a small amount of lactol **9a/b**, no anomeric product was found when **10b** was refluxed either in 1,2-dichloroethane alone or when one equivalent of trimethylsilyl triflate was added to the reaction mixture. Likewise, when the nucleoside **14a** (α-anomer) was resubjected to the Vorbrueggen glycosylation conditions, no anomeric product **14b** (β-anomer) was detected. Furthermore, no anomerization occurred when nucleosides **1a** and **1b** were converted to the triacetates,[13] and subjected to an anomerization procedure developed by Saneyoshi and Yamaguchi for 2′-deoxycytidine and thymidine.[14] Therefore, we concluded that the observed 1:1 β/α ratio of nucleosides was not the result of epimerization of either the mesylate starting material or the nucleoside product, but rather a consequence of the predominant reaction mechanism.

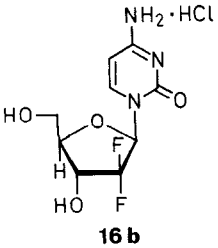
Treating **13a/b** or **14a/b** with either ammonia or sodium methoxide in methanol effectively removed the benzoyl groups. Debenzoylation in ammonia could be observed in a stepwise manner with the benzoyl group at C-3′ being removed first.[15] The completely deprotected nucleosides **1a/b** could be crystallized as hydrochloride salts by adding concentrated hydrochloric acid to the nucleoside solution in isopropyl alcohol.[16] This salt was also a 1:1 mixture of anomers **16a/b**; but **16b** could be selectively crystallized from the mixture by dissolving it in water and then adding excess acetone as counter-solvent. The selectivity of this crystallization was very high and consistent, and greater than 99% pure **16b** could be obtained after only one crystallization. Alternatively, the β-nucleoside **1b** could be selectively crystallized from the 1:1 mixture of **1a/b** or **16a/b** as the free base. The diastereomeric selectivity of this process was also very high (> 99%). Nucleoside **1b** could be reconverted to **16b** by pH control using concentrated hydrochloric acid.



**1a**



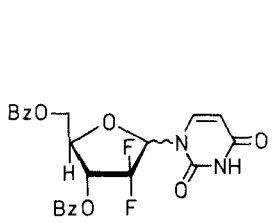
**16a**



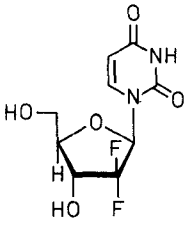
**16b**

The hydrochloride salt is the preferred final product dosage form for gemcitabine, the generic name for 2′-deoxy-2′,2′-difluorocytidine.

The glycosylation protocol could be extended to uracil as well. Thus, reacting bis trimethylsilyluracil **17** with **10a/b** produced the uridine nucleosides **18a/b** also in an approximately 1:1 ratio of anomers. The dibenzoyl protected uridine derivatives were crystalline compounds, and the desired $\beta$ anomer could also be isolated by selective crystallization from the diastereomeric mixture. Similarly, deprotection of the $\beta$-anomer **18b** with ammonia in methanol gave 2′-deoxy-2′,2′-difluorouridine **(19)**[17] in good yield.



**18a/b**



**19**

Unless otherwise noted, all chemicals were reagent grade materials from commercial suppliers and were used without further purification. All reactions were conducted under $N_2$ atmosphere except those involving water. Melting points are uncorrected. $^1H$ and $^{13}C$ NMR spectra were obtained at 250, 270, 300 or 500 MHz; assignments were derived from COSY and H/C correlation spectra and from consideration of F/C coupling patterns observed in the carbon spectra. Peaks assigned to benzoyl (Bz) protecting groups are omitted from most listings. Overlapping peaks are designated by ov. HPLC systems used in the experiments are as follows: System A: column, Zorbax CN 4.6 × 25 cm; eluent, hexane/*i*-PrOH (96:4); flowrate, 2 mL/min; detector wavelength $\lambda = 230$ nm. System B: column, Zorbax CN 4.6 × 25 cm; eluent, hexane/*i*-PrOH (92:8); flowrate, 5 mL/min; detector wavelength $\lambda = 254$ nm. System C: column, Zorbax RX 4.6 × 25 cm; eluent, gradient with MeCN and 0.1 M $NaH_2PO_4$ buffer at pH 3.0; flowrate, 1.5 mL/min; detector wavelength $\lambda = 275$ nm. System D: column, Zorbax RX 4.6 × 25 cm; eluent, 100% 0.1 M $NaH_2PO_4$ buffer at pH 3.0; flowrate, 1.0 mL/min; detector wavelength $\lambda = 275$ nm. TLC was performed on silica gel plates from EM Science; the solvent systems were: A, Hexane/EtOAc (3:1); B, $CH_2Cl_2$/MeOH (9:1).

**Ethyl (3*RS*)-3-(Benzoyloxy)-2,2-difluoro-3-(2,2-dimethyldioxolan-4-yl)propionate (6a/b):**

A mixture of ethyl (3*RS*)-2,2-difluoro-3-(2,2-dimethyldioxolan-4-yl)propionate (**5a/b**; 100.0 g, 0.39 mol), 2,6-lutidine (90.7 mL, 0.79 mol) and catalytic 4-dimethylaminopyridine (DMAP) (24.4 g, 0.20 mol) in $CH_2Cl_2$ (500 mL) was warmed to 35 °C. To this mixture was added dropwise a solution of benzoyl chloride (54.5 mL, 0.47 mol) in $CH_2Cl_2$ (500 mL) over 3 h at 33–36 °C. The mixture was cooled to r.t., and the organic phase washed successively with water, 1 N HCl, 5% $NaHCO_3$ and water (500 mL each) and then concentrated in vacuo to give **6a/b** as an oil; yield: 134.6 g (96%).

This oil was used in the following hydrolysis-cyclization reaction without further purification. For identification purposes, a sample of **5a/b** was chromatographed on a silica gel Prep-500 column with 0.5% MeOH in $CH_2Cl_2$ as eluent. A purified sample of **5a** was obtained and subjected to the same benzoylation procedure as shown above to give **6a**, also as an oil.

| | | | | |
|---|---|---|---|---|
| $C_{17}H_{20}F_2O_6$ | calc. | C 56.98 | H 5.63 | F 10.60 |
| (358.3) | found | 56.96 | 5.88 | 10.30 |

$^1H$ NMR ($CDCl_3$) for **6a**: $\delta$ = 1.29 (t, 3 H, $CH_2CH_3$), 1.31, 1.34 [2 s, 6 H, $C(CH_3)_2$], 4.06, 4.14 (2 t, 2 H, $CH_2$), 4.29 (q, 2 H, $CH_2CH_3$), 4.52 (q, 1 H, H-4), 5.87 (t, 1 H, H-3), 7.48, 7.62, 8.08 (m, 5 $H_{arom}$).

$^{13}C$ NMR ($CDCl_3$) for **6a**: $\delta$ = 13.81 ($CH_2CH_3$), 25.18, 26.12 [$C(CH_3)_2$], 63.39 ($CH_2CH_3$), 65.68 (C-5), 71.04 (C-3, $J_{C-F}$ = 22.7, 25.6 Hz), 72.63 (C-4), 110.12 [$C(CH_3)_2$], 112.88 (C-2, $J_{C-F}$ = 256, 256 Hz), 128.67, 128.76, 130.09, 133.84 ($C_{arom}$), 162.16 (C-1, $J_{C-F}$ = 31, 31 Hz), 164.69 (OCOPh).

**(D-*erythro*,D-*threo*)-2-Deoxy-2,2-difluoropentofuranos-1-ulose-3-benzoate (8a/b):**

A mixture of **6a/b** (90.2 g, 0.25 mol), MeCN (500 mL), water (25 mL, 1.39 mol) and $CF_3CO_2H$ (4.3 mL, 0.06 mol) was refluxed at 78 °C for 3 h. The hydrolyzed product **7a/b** was not isolated, but converted to **8a/b** by azeotropic distillation using a Dean–Stark water separator. The displaced MeCN was replaced with dry toluene, and the azeotropic distillation continued until the pot temperature reached 95–100 °C. The mixture was cooled to 40–45 °C and the solvent evaporated in vacuo to give **8a/b** as an oil (84 g). This oil was used for conversion to **2a/b** without further purification. Similarly, purified samples of **7a** and **8a** were obtained from **6a** by the same method.

**7a:**

IR ($CHCl_3$): $\nu$ = 1775, 1733 $cm^{-1}$.

$^1H$ NMR ($CDCl_3$): $\delta$ = 1.24 (t, 3 H, $CH_3$), 3.62, 3.72 (m, 2 H, H-5), 4.12 (m, 1 H, H-4), 4.26 (m, 2 H, $CH_2CH_3$), 5.78 (m, 1 H, H-3).

$^{13}C$ NMR ($CDCl_3$): $\delta$ = 13.66 ($CH_3$), 62.38 (C-5), 63.63 ($CH_2CH_3$), 69.11 (C-4), 70.85 (C-3), 113.24 (C-2), 162.99 (C-1).

FD/MS: $m/z$ = 319 $(M + H)^+$.

**8a:**

IR ($CHCl_3$): $\nu$ = 1830, 1733 $cm^{-1}$.

$^1H$ NMR ($CDCl_3$): $\delta$ = 3.92, 4.06 (dd, 2 H, H-5), 4.71 (bs, 1 H, H-4), 5.80 (m, 1 H, H-3).

$^{13}C$ NMR: $\delta$ = 60.14 (C-5), 68.70 (C-3), 80.85 (C-4), 111.91 (C-2), 163.38 (C-1).

FAB/MS: $m/z$ = found: $[M + H]^+$ = 273.0566; calc. for $[C_{12}H_{11}O_5F_2]^+$ = 273.0575.

**(D-*erythro*,D-*threo*)-2-Deoxy-2,2-difluoropentofuranos-1-ulose-3,5-dibenzoate (2a/b):**

To **8a/b** (76.2 g, 0.28 mol) in EtOAc (500 mL) were added pyridine (44.1 g, 0.56 mol) and 4-dimethylaminopyridine (3.4 g, 28 mmol). The mixture was warmed to 65 °C, and benzoyl chloride (46.9 g, 0.33 mol) in EtOAc (500 mL) was added dropwise. After stirring for 2 h, the mixture was cooled to 5 °C and pyridine HCl was filtered off. The filtrate was treated with Darco G-60 (10 g), filtered (Hyflo Supercel), and concentrated to provide **2a/b** as an oil; 110 g (~100%). This oil was used for the crystallization study shown in the following experiment.

**Crystallization of 2-Deoxy-2,2-difluoro-D-*erythro*-pentofuranos-1-ulose-3,5-dibenzoate (2a):**

Compound **2a/b** was dissolved in $CH_2Cl_2$ (73 mL), and the solution warmed to 35 °C. To this was added heptane (110 mL) at 33–36 °C. The solution was seeded with **2a** and cooled to 5 °C. The crystals were filtered, rinsed with 50 mL of cold heptane/$CH_2Cl_2$ (3:2, 50 mL), dried at 50 °C in vacuo to provide **2a**; yield: 28.6 g (26%); mp 119–120 °C; $[\alpha]_D$ ($c$ = 1.0, $CHCl_3$) + 47.35°, $[\alpha]_{365}$ + 187.01°. HPLC assay indicated a purity of 99.6% with no contamination of the xylo isomer.

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{14}F_2O_6$ | calc. | C 60.64 | H 3.75 | F 10.10 |
| (376.3) | found | 60.42 | 3.66 | 9.98 |

UV $\lambda$ = 268 ($\varepsilon$ = 1970), 230 nm ($\varepsilon$ = 25 500).

IR ($CHCl_3$): $\nu$ = 1827.2, 1732.0, 1244.8, 1206.9 $cm^{-1}$.

$^1H$ NMR($CDCl_3$): $\delta$ = 4.69, 4.76 (m, 2 H, H-5), 4.99 (q, 1 H, H-4), 5.76 (p, 1 H, H-3).

$^{13}C$ NMR ($CDCl_3$): $\delta$ = 62.29 (C-5), 69.50 (C-3, $J_{F-F}$ = 15.7, 30.7 Hz), 78.50 (C-4, $J_{C-F}$ = 5.6 Hz), 111.57 (C-2, $J_{C-F}$ = 258, 263 Hz), 162.48 (C-1, $J$ = 32.1, 33.6 Hz).

**2-Deoxy-2,2-difluoro-D-ribofuranose-3,5-dibenzoates (9a/b):**
A solution of **2a** (1000 g, ~90% pure, 2.4–2.6 mol) in dry $Et_2O$ (8 L) and dry THF (2 L) was cooled under $N_2$ to ca. 10°C. $LiAl(OBu\text{-}t)_3H$ (782 g, 3.07 mol) was added portionwise (40 min) at < 30°C. After stirring for 1 h, MeOH (200 mL) was added slowly in 30 min followed by 1 N HCl (830 mL). The organic layer was separated, washed with 5% aq. $NaHCO_3$ and water (100 mL each), dried ($MgSO_4$) and filtered. The filtrate was concentrated to provide **9a/b** as an oily residue; yield: 930 mg (~100%). This residue was used directly in the subsequent mesylation reaction. A small sample of the residue solidified upon standing and, after trituation with MeOH, **9a/b** crystallized, mp 68–70°C; HPLC (system B) showed two close peaks at 4.20 and 4.40 min; TLC (hexane/EtOAc, 3:1), $R_f$ 0.38.

| | | | | | |
|---|---|---|---|---|---|
| $C_{19}H_{16}F_2O_6$ | calc. | C 60.32 | H 4.26 | F 10.04 | |
| (378.3) | found | 60.48 | 4.42 | 9.88 | |

*NMR of major (ca. 55%) anomer:*

$^1H$ ($CDCl_3$): $\delta$ = 3.55 (s, 1 H, OH), 4.58, 4.69 (ov, 2 H, H-5), 4.76 (m, 1 H, H-4), 5.49 (ov, 1 H, H-3), 5.47 (m, 1 H, H-1).

$^{13}C$ ($CDCl_3$): $\delta$ = 63.31 (C-5), 72.11 (C-3, $J_{C-F}$ = 17.9, 35.6 Hz), 79.65 (C-4), 96.24 (C-1, $J_{C-F}$ = 23.4, 42.0 Hz), 121.58 (C-2, $J_{C-F}$ = 249, 272 Hz).

NMR of minor (ca. 45%) anomer:

$^1H$ ($CDCl_3$): $\delta$ = 3.82 (s, 1 H, OH), 4.45 (ov, 1 H, H-4), 4.62 (ov, 2 H, H-5), 5.35 (dd, 1 H, H-3), 5.73 (ov, 1 H, H-1).

$^{13}C$ ($CDCl_3$): $\delta$ = 64.33 (C-5), 71.52 (C-3, $J_{C-F}$ = 16.6, 28.8 Hz), 77.40 (C-4), 96.00 (C-1, $J_{C-F}$ = 24.4, 36.7 Hz), 121.09 (C-2, $J_{C-F}$ = 257, 280 Hz).

**2-Deoxy-2,2-difluoro-D-ribofuranose-3,5-dibenzoate-1-methanesulfonate (10a/b):**
To a solution of **9a/b** (1679 g, 4.44 mol) in dry $CH_2Cl_2$ (17 L) at 0°C, was added $Et_3N$ (875 mL) followed by $MeSO_2Cl$ (421 mL, 1.2 eq) at < 28°C. The mixture was stirred at 23°C for 2 h and then washed with 12 L each of 1 N HCl, 5% $NaHCO_3$ and water, dried ($MgSO_4$) and filtered. After stripping off most of the solvent, **10a/b** (4022 g) was obtained as a reddish-brown oil. HPLC (system B) showed two peaks at 8.48 and 10.40 min; TLC (system A), $R_f$ 0.76, 0.48. The oil was used for the subsequent glycosylation without further purification.

Analytically pure samples of **10a** (α-anomer) and **10b** (β-anomer) were obtained from **10a/b** by selective crystallization from $CH_2Cl_2$–$Et_2O$.

**10a:** mp 88–89°C; $[\alpha]_D$ (*c* = 1.01, $CHCl_3$) + 84.2°; $[\alpha]_{365}$ + 302.0°.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_{20}H_{18}O_8SF_2$ | calc. | C 52.63 | H 3.98 | F 8.33 | S 7.02 | |
| (456.4) | found | 52.92 | 3.82 | 8.33 | 7.30 | |

$^1H$ NMR ($CDCl_3$): $\delta$ = 3.17 (s, 3 H, $CH_3$), 4.66, 4.76 (m, 2 H, 5 H), 4.84 (q, 1 H, H-4), 5.57 (dd, 1 H, H-3), 6.13 (d, 1 H, H-1).

$^{13}C$ NMR ($CDCl_3$): $\delta$ = 40.22 ($CH_3$), 62.51 (C-5), 71.03 (C-3), $J_{C,F}$ = 18.3, 38.5 Hz), 82.75 (C-4), 99.59 (C-1, $J_{C,F}$ = 25.5, 48.3 Hz), 122.24 (C-2, $J_{C,F}$ = 259, 286 Hz).

**10b:** mp 114–115°C; $[\alpha]_D$ (*c* = 1.01, $CHCl_3$) – 40.7°, $[\alpha]_{365}$ – 90.8°.

| | | | | |
|---|---|---|---|---|
| $C_{20}H_{18}O_8SF_2$ | calc. | C 52.63 | H 3.98 | F 8.33 |
| (456.4) | found | 52.38 | 3.94 | 8.51 |

$^1H$ NMR ($CDCl_3$): $\delta$ = 3.02 (s, 3 H, $CH_3$), 4.61, 4.74 (m, 2 H, H-5), 4.65 (ov, 1 H, H-4), 5.93 (dt, 1 H, H-3), 6.04 (d, 1 H, H-1).

$^{13}C$ NMR ($CDCl_3$): $\delta$ = 40.35 ($CH_3$), 63.03 (C-5), 69.66 (C-3), 79.78 (C-4), 98.84 (C-1), 120.60 (C-2).

**2′-Deoxy-2′,2′-difluorocytidines (16 a/b) from 10a/b and 11:**
A slurry of bis(trimethylsilyl)-*N*-acetylcytosine[18] (**11**; 2.92 kg, 13 mol) and $Me_3SiOTf$ (2.9 kg, 13 mol) in 1,2-dichloroethane (30 L) was stirred at 23°C. After 1 h at 30°C, **10a/b** (3.94 kg, 8.6 mol) in 1,2-dichloroethane (10 L) was added, and the mixture refluxed (83°C) overnight. When no **10a/b** could be detected by HPLC (system C, retention time for: **10a**, 24.8 min; **10b**, 25.6 min; **13a**, 15.6 min; for **13b**, 16.8 min) or TLC (system B), the reflux was stopped. The mixture was cooled to 40°C, washed with water (2 × 24 L), 5% $NaHCO_3$ (1 × 24 L), brine (1 × 24 L), dried ($MgSO_4$), and then filtered. The filtrate was concentrated to provide a foam containing the desired **13a/b**.

Compound **13a/b** was deprotected as follows. MeOH (38 L) was added to dissolve the foam. The solution was cooled to 12°C and 1 kg (7.1 molar equiv) of $NH_3$ gas was bubbled into the solution in ~2 h. The mixture was stirred overnight at r.t. Solvent and volatile constituents were stripped off to produce a gum weighing 4.16 kg. This gum was dissolved in water (8 L), and extracted with EtOAc (8 L). The organic layer was separated, washed with water (8 L). The combined aqueous layers were treated with Actibon C (790 g) and filtered through a pad of Hyflo. The water was stripped off to give a yellow colored gum weighing 1.73 kg. The HPLC (system D: **1a**, 7.4 min; **1b**, 11.9 min) assay disclosed that it contained **1a/b** in approximately equal amounts. This gum was slurried in *i*-PrOH (8 L) and warmed to 70°C, and conc. HCl (2 L) added in one portion. Immediately all solids dissolved, and the solution began to crystallize. The crystals were filtered, rinsed with an ice-cold solution of *i*-PrOH (3 L) and heptane (2 L), and then vacuum dried; yield: 1.27 kg (49.2%). HPLC assay showed that it contained 47.3% of **16b** and 52.7% **16a**.

**Isolation of 16a and 16b from a 16a/b Mixture:**
The crude HCl salt **16a/b** (1260 g) from above was dissolved in water (6 L) at 50°C. The solution was cooled to r.t. and acetone (72 L) was added. After stirring for 2 h, the crystals were filtered, rinsed with acetone and dried at 40°C to give **16b**; yield: 463 g; mp 287–292°C (dec.). HPLC (system D) analysis showed that it contained 98% of **16b** and a trace amount of **16a**.

**16b**: $[\alpha]_D$ (*c* = 1.0, $D_2O$) + 48.0°; $[\alpha]_{365}$ + 257.9°.

| | | | | | | |
|---|---|---|---|---|---|---|
| $C_9H_{12}ClF_2N_3O_4$ | calc. | C 36.07 | H 4.04 | Cl 11.83 | F 12.68 | N 14.02 |
| (299.7) | found | 36.20 | 4.00 | 11.87 | 12.60 | 13.86 |

$^1H$ NMR ($D_2O$): $\delta$ = 3.87, 4.02 (dd, dd, 2 H, H-5′), 4.12 (m, 1 H, H-4′), 4.39 (m, 1 H, H-3′), 6.24 (t, 1 H, H-1′), 6.29 (d, 1 H, H-5), 8.02 (d, 1 H, H-6).

$^{13}C$ NMR ($D_2O$): $\delta$ = 60.12 (C-5′), 69.95 (C-3′), 81.89 (C-4′), 85.38 (C-1′), 96.38 (C-5), 122.89 (C-2′), 144.75 (C-6), 149.12 (C-2), 160.23 (C-4).

UV (water, neutral): $\lambda$ = 268 ($\varepsilon$ = 9360), 232 nm ($\varepsilon$ = 7960); UV (water, acidic): $\lambda$ = 275 nm ($\varepsilon$ = 13500), 210 nm ($\varepsilon$ = 1020); UV (water, basic): $\lambda$ = 269 ($\varepsilon$ = 9300), 230 nm ($\varepsilon$ = 8100 sh).

The mother liquor usually contained 18% of **16b** and 82% **16a**. Using HP-20 column with water/MeOH as eluents, the content of **16b** in certain fractions could be increased to > 50%, and more **16b** could be recovered using the same crystallization procedure. From those fractions containing more α-anomer, crystalline **16a** was obtained; $[\alpha]_D$ (*c* = 1.02, MeOH) + 11.96°; $[\alpha]_{365}$ – 104.71°.

UV (water, neutral): $\lambda$ = 269 nm ($\varepsilon$ = 9170).

$^1H$ NMR ($D_2O$): $\delta$ = 3.79 and 3.94 (dd, dd, 2 H, H-5′), 4.42 (m, 1 H, H-4′), 4.56 (m, 1 H, H-3′), 6.28 (d, 1 H, H-5), 6.35 (m, 1 H, H-1′), 7.94 (d, 1 H, H-6).

$^{13}C$ NMR ($D_2O$): $\delta$ = 60.70 (C-5′), 70.58 (C-3′), 84.91 (C-4′), 86.02 (C-1′), 96.06 (C-5), 123.06 (C-2′), 144.62 (C-6), 149.35 (C-2), 160.33 (C-4).

**Conversion of 16a/b to 16b via 1b:**
A sample of **16a/b** (7.5 g, 25 mmol, containing 47.8% β-anomer and 51.0% α-anomer) was dissolved in water (pH 4, 15 mL) at 55°C. After dissolution of the solids, more aq. 2 N NaOH solution was added to bring the pH to 8.5. The mixture was then cooled to r.t. and seeded with **1b**. After stirring for 1 h at 0°C, the crystalline solids were filtered, rinsed twice with ice-cold water, and then vacuum dried at 45°C to give **1b**; yield 2.74 g (78.2%). HPLC indicated that it contained 100% the desired β-anomer; $[\alpha]_D$ (*c* = 0.96, MeOH) + 71.51°; $[\alpha]_{365}$ + 425.36°.

| | | | | | |
|---|---|---|---|---|---|
| $C_9H_{11}F_2N_3O_4$ | calc. | C 41.07 | H 4.21 | F 14.44 | N 15.27 |
| (299.7) | found | 41.04 | 4.27 | 14.61 | 16.21 |

IR (KBr): $\nu$ = 3485, 3338, 1651, 1620, 1033 $cm^{-1}$.

UV (EtOH, neutral): $\lambda = 268$ ($\varepsilon = 8560$), 234 nm ($\varepsilon = 7810$); UV (EtOH, acidic): $\lambda = 275$ ($\varepsilon = 12200$), 210 nm ($\varepsilon = 9530$); UV (EtOH, basic): $\lambda$ 269 ($\varepsilon = 9300$), 206 nm ($\varepsilon = 55000$).

$^{13}$C NMR ($D_2O$): $\delta$ = 58.95 (C-5'), 68.43 (C-3'), 80.41 (C-4'), 83.49 (C-1'), 94.53 (C-5), 123.02 (C-2'), 140.76 (C-6), 154.65 (C-2), 165.61 (C-4).

Compound **1b** was converted to its HCl salt **16b** as follows: A sample of **1b** (2.0 g) was added to water (20 mL) at 55 °C at pH 3.5 to facilitate the solubilization, and then lowered to 0.5 by adding conc. HCl. As the solution was cooled to 40 °C, crystallization occured. After stirring at 0 °C for 1 h, the crystals were filtered, and vacuum dried (50 °C) to give **16b** weighing 1.95 g. HPLC analysis indicated a purity of 99.9 %.

**2',2-Difluoro-2'-deoxycytidine-3',5'-dibenzoate (14a/b) from 10a/b and 12:**

To freshly prepared bis(trimethylsilyl)cytosine (**12**; 90 mmol, prepared from 10 g of cytosine) in 1,1,2-trichloroethane[19] (200 mL) was added $Me_3SiOTf$ (19.5 g, 87.7 mmol). The mixture was stirred at r.t. for 30 min. Mesylates **10a/b** (25 g, 54.8 mmol) were then added, and the mixture was refluxed at 113 °C for 18 h. The reaction was followed by TLC (system B) or HPLC (system C), which indicated a mixture containing $\alpha/\beta$ nucleoside (1.4 : 1.0). The mixture was cooled to r.t. and concentrated in vacuo to give a residue. The residue was dissolved in EtOAc (300 mL) and washed 3 times with water and once with 5 % $NaHCO_3$ solution. The layers were separated and the organic slurry was concentrated to half volume, filtered, and vacuum dried to give a white solid; yield 22.49 (86.9 %) HPLC (system C: **14a**, 11.1 min; **14b**, 12.4 min) showed that the mixture contained 43.8 % **14b** and 56.2 % **14a**.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $C_{23}H_{19}F_2N_3O_6$ (471.4) | calc. | C 58.60 | H 4.06 | F 8.06 | H 8.91 |
| | found | 58.45 | 4.25 | 8.18 | 8.79 |

This mixture was used directly for the conversion to its HCl salts **(16a/b)** as shown in the previous two experiments.

Since **14b** was much less soluble in MeOH than **14a**, it was possible to obtain pure **14b** by slurrying **14a/b** in warm (30–35 °C) MeOH and collecting the insoluble **14b** (HPLC assay 98 %). A sample of **16a** was recovered from the filtrate (purity 97 %).

**14a:**

$^1$H NMR (DMSO-$d_6$): $\delta$ = 4.75 (m, 2 H, H-5'), 5.26 (q, 1 H, H-4'), 5.96 (d, 1 H, H-5), 6.05 (p, 1 H, H-3'), 6.70 (t, 1 H, H-1'), 7.60 (ov, 2 H, $NH_2$), 7.86 (ov, 1 H, H-6).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ = 63.74 (C-5'), 72.31 (C-3'), 79.44 (C-4'), 84.75 (C-1'), 94.88 (C-5), 122.49 (C-2'), 141.04 (C-6), 154.88 (C-2), 165.95 (C-4).

**14b:**

$^1$H NMR (DMSO-$d_6$): $\delta$ = 4.74 (m, 1 H, H-4'), 4.79 (m, 2 H, H-5'), 5.84 (d, 1 H, H-5), 5.88 (m, 1 H, H-3'), 6.44 (t, 1 H, H-1'), 7.56 (m, 2 H, $NH_2$), 7.68 (ov, 1 H, H-6).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ = 63.46 (C-5'), 71.80 (C-3'), 75.71 (C-4'), 84.64 (C-1'), 95.12 (C-5), 121.86 (C-2'), 141.93 (C-6), 154.48 (C-2), 165.87 (C-4).

Deprotection of **14a/b** to **1a/b** could be effected by $NH_3$ in $CH_3OH$, and the conversion of **1a/b** to **16b** by conc. HCl and *i*-PrOH similar to the procedures described in the procedures above.

**Preparation of 2'-Deoxy-2',2'-difluorouridine-3',5'-dibenzoate (18a/b):**

A solution of bis(trimethylsilyl)uracil (**17**; 2.29 g, 8.9 mmol) in 1,2-dichloroethane was treated with $Me_3SiOTf$ (1.72 mL, 8.9 mmol) at 30 °C for 10 min. $\alpha$-Mesylate **10a** (2.71, 5.95 mmol) was added, and the mixture refluxed at 83 °C for 5 h. The solvent was stripped off, and the resulting gum was dissolved in EtOAc (100 mL). This solution was washed with water (2 × 100 mL), 5 % $NaHCO_3$ and brine (100 mL each), dried ($Na_2CO_3$), filtered, and evaporated to a tan-yellow foam (3.11 g). HPLC (system D) showed that the foam contained **18a/b** ($\alpha$ : $\beta$ = 1.43 : 1; retention time: $\alpha$, 11.3 min; $\beta$, 12.19 min). This foam was crystallized once from $CH_2Cl_2$ to give a mixture enhanced in $\beta$-anomer (3 : 1). Second crystallization from the same solvent, raised the $\beta$ to $\alpha$ ratio to ca. 9 to 1. This material was used for the deprotection study shown below; mp 193–195 °C.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $C_{23}H_{18}F_2N_2O_7$ (472.4) | calc. | C 58.48 | H 3.84 | F 8.04 | N 5.93 |
| | found | 58.21 | 3.86 | 8.25 | 5.80 |

$^1$H NMR ($CDCl_3$) for the major component: $\delta$ = 4.59 (q, 1 H, H-4'), 4.69, 4.83 (dd, dd, 2 H, H-5'), 5.67 (dd, 1 H, H-3'), 6.39 (q, 1 H, H-1'), 7.49 (d, 1 H, H-5), 8.06 (d, 1 H, H-6), 8.17 (s, 1 H, NH).

$^{13}$C NMR ($D_2O$): $\delta$ = 58.95 (C-5'), 68.43 (C-3'), 80.41 (C-4'), 83.49 (C-1'), 94.53 (C-5), 123.02 (C-2'), 140.76 (C-6), 154.65 (C-2), 165.61 (C-4).

FDMS: $m/z = 473$ $(M + H)^+$.

**2'-Deoxy-2',2'-difluorouridine (19):**

Gaseous $NH_3$ (ca 1 g) was introduced over a 2 min period into a solution of **18a/b** ($\beta/\alpha$ = 9 : 1, 200 mg, 0.42 mmol) in MeOH (50 mL) at 5 °C. After stirring the mixture at 23 °C for 23 h, TLC (system B) showed the absence of starting material, and only trace $\alpha$-anomer was detected. The solvent and volatile constituents were removed, and the resulting oil was partitioned between EtOAc and water (50 mL each). The EtOAc layer was rinsed with water (40 mL) and the water layers were combined and evaporated to dryness to afford **19** as a clear oil; 100 mg (90 %).

$^1$H NMR ($D_2O$): $\delta$ = 3.83, 3.98 (dd, d, 2 H, H-5'), 4.04 (m, 1 H, H-4'), 4.36 (dt, 1 H, H-3'), 5.87 (d, 1 H, H-5), 6.17 (t, 1 H, H-1'), 7.74 (d, 1 H, H-6).

$^{13}$C NMR ($D_2O$) $\delta$ = 62.19 (C-5'), 72.08 (C-3', $J_{C,F}$ = 19.3, 27.2 Hz), 83.35 (C-4', $J_{C,F}$ = 7.1 Hz), 86.82 (C-1'), $J_{C,F}$ = 24.4, 40.0 Hz), 105.17 (C-5), 124.85 (C-2', $J_{C,F}$ = 257. 261 Hz), 144.02 (C-6), 153.88 (C-2), 168.30 (C-4).

FAB/FD: $m/z$ = found: $[M + H]^+ = 265.0630$; calc. for $[C_9H_{11}N_2O_5F_2]^+ = 265.0636$.

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(4) We thank Dr. L. W. Hertel and Mr. S. J. Kroin of Lilly Research Laboratories for providing us with an authentic sample of **5a/b**.

(5) The carbonyl stretching frequency of pyranosyl lactone would be expected at $\nu$ = 1770–1790 $cm^{-1}$ instead of 1828 $cm^{-1}$ for **2a**.

(6) Ferrier, P.; Collins, P. *Monosaccharide Chemistry*; Penguin: Harmondsworth, Middlesex, England; 1972, p. 84.

(7) Several monosubstituted benzoyl compounds have been used as the hydroxyl protecting group, but none of the corresponding lactones are crystalline compounds. The difluorolactones cannot be purified by column chromatography because the lactone ring is easily opened; thus the benzoyl protecting group indeed occupies an unique place in this synthesis.

(8) A second solvent system, composed of $CH_2Cl_2$/*i*-PrOH (10:1) could also be used. However, since this system contained alcohol, it was not reliable in production scales.

(9) The C-3, 4 and 5 carbons of sugar **10a/b** correspond to the C-3′, 4′ and 5′ carbons of nucleosides **1a/b**, **14a/b** and **16a/b**.

(10) Vorbrueggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234.

(11) Compound **14a/b** was first prepared as its isotopomer by Dr. W.J. Wheeler of Lilly Research Laboratories. He found that the compounds, in contrast to **13a/b**, were poorly soluble in organic solvents and could be isolated by filtration.

(12) We thank Dr. J.S. Ward of Lilly Research Laboratories in helping us carry out the high-pressure experiment.

(13) For triacetate of **1a**: mp 199–199.5 °C; for triacetate of **1b**: mp 120–122 °C.

(14) Saneyoshi, M.; Yamaguchi, T. *Chem. Pharm. Bull.* **1984**, *32*, 1441.

(15) Deprotection of **14b** with $NH_3$/MeOH initially leads to the 5′-monobenzoyl β-nucleoside, as indicated by FAB/MS [$(M + H)^+$, $m/z = 368$, $(C_{16}H_{15}F_2N_3O_5 + H)^+$] and by $^1H$ and $^{13}C$ NMR data. The 3′-hydroxyl resonance (6.49 ppm in DMSO-$d_6$) disappeared on addition of $D_2O$ to the solution.

$^1H$ NMR (DMSO-$d_6$): $\delta$ = 4.18 (m,1 H, H-4′), 4.35 (m, 1 H, H-3′), 4.54, 4.68 (dd, d, 2 H, H-5′), 5.72 (d, 1 H, H-5), 6.21 (t, 1 H, H-1′),6.49 (d, 1 H, 3′-OH), 7.43, 7.54 (2s, 2 H, $NH_2$), 7.69 (t, 1 H, H-6).

$^{13}C$ NMR (DMSO-$d_6$): $\delta$ = 63.25 (C-5′), 70.25 (C-3′), 77.52 (C-4′), 84.08 (C-1′), 94.75 (C-5), 122.65 (C-2′), 141.06 (C-6), 154.38 (C-4), 165.43 (C-2).

(16) Replacing conc. HCl with 48% aq HBr solution converts the nucleoside to a 1:1 mixture of the two anomeric nucleosides as the HBr salt. Recrystallization of the mixture with the same water-acetone system also produces the β-anomer in pure form.

(17) Hertel et al[2] also prepared the uridine nucleosides using the *tert*-butyldimethylsilyl protected difluorodeoxyribonyl mesylate. Again, the ratio was ca. 4 to 1 against the β nucleoside.

(18) Bistrimethylsilyl-*N*-acetylcytosine was prepared by heating *N*-acetylcytosine in hexamethyldisilazane at ca. 125 °C in the presence of a catalytic amount of $(NH_4)_2SO_4$. The ratio of bis to mono silylated species was assayed by GC/MS: Varian 3400; DB-1 column 30 m × 0.25 μm film thickness; helium flow rate, 2 mL/m at r.t.; split ratio, 25:1; injector temperature, 250 °C; initial temperature, 90 °C; ramp rate, 10 deg/m; final temperature, 280 °C; scan rate, 1 scan/s; ion source temperature, 170 °C; chemical ionization reagent gas $NH_3$; $5 \times 10^{-2}$ Torr; Nermag R3010 triple quadrupole. The authors thank Dr. A.J. Breau of Lilly Research Laboratories for developing the assay and providing the results.

(19) Other solvents, such as 1,2-dichloroethane and xylenes, can be used to replace trichloroethane. If dichloroethane is used, the reflux temperature is 83 °C, and it takes ca. 48 h for the glycosylation to complete. If xylenes are used, the reaction temperature is controlled at 125 °C, and the reaction time is ca. 3 h. The reaction time in trichloroethane is ca. 5 h. The overnight reflux quoted in this experiment was done for the purpose of monitoring and evaluating the course of the reaction.