Synthesis of 2′,3′-Dideoxy-2′-difluoromethyl Azanucleosides

Xiao-Long Qiu,[a] Feng-Ling Qing*[a,b]

[a] Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, P. R. China

[b] College of Chemistry and Chemical Engineering, Donghua University, 1882 West Yanan Lu, Shanghai 200051, P. R. China
Fax +86(21)64166128; E-mail: flq@mail.sioc.ac.cn

Received 30 October 2003; revised 4 December 2003

Abstract: Methyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-difluoromethylpyroglutamate (**9a**) was synthesized from *trans*-4-hydroxy-L-proline (**5**). Compound **9a** was converted to (5*S*,3*S*)-*N*-benzyloxycarbonyl-5-(*tert*-butyldimethylsilyloxymethyl-3-difluoromethyl-2-pyrrolidone (**15**) over 4 steps in 66% yield, which was used as a key intermediate for the synthesis of 2′,3′-dideoxy-2′-difluoromethyl azanucleosides

Key words: fluorine-containing nucleoside, azanucleoside, pyroglutamate, difluoromethylenation, glycosylation reaction

The small and highly electronegative fluorine atom has found many applications in medicinal and physical organic chemistry.[1] The strong carbon–fluorine bond in fluorine-containing molecules is particularly resistant to metabolic transformation,[2] which plays a great role in medical designation. Thus introduction of fluorinated groups into potential bioactive molecules such as amino acids, peptides and nucleosides attracts the attention of many chemists.

In recent years, nucleosides and nucleotides, known to be DNA and RNA subunits, have become an interestingly important subject of research in the field of pharmaceutical science,[3] and many studies have dealt with the synthesis and biological activity of novel types of nucleosides and nucleotides including fluorine-containing nucleosides. Fluorinated nucleosides are the attractive nucleoside analogues because of their antiviral and anticancer activities. Up to date, many fluorinated nucleoside analogues have been synthesized and characterized. Some highly bioactive fluorinated nucleosides have been prepared including FMAU,[4] FLT,[5] FIAC[4b] and Gemcitabine[6] (Figure 1).

Azanucleosides, in which the oxygen atom of pyranose or furanose ring is replaced by nitrogen atom, are an important class of modified nucleosides. The synthesis and biological activities of azanucleosides were summarized by Yokoyama et al in 1999.[3] However, among the many azanucleosides reported to date, only a few examples of fluorinated azanucleosides have been synthesized and investigated.[7] In connection with our studies on fluorinated amino acids, fluorinated peptides and fluorinated nucleosides (investigation on the effects of fluorinated groups on the biological activities of azanucleosides), we embarked on the synthesis of 2′,3′-dideoxy-2′-difluoromethyl azanucleosides.

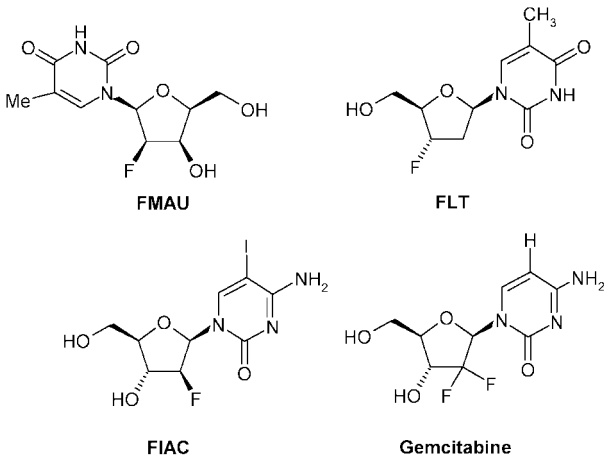
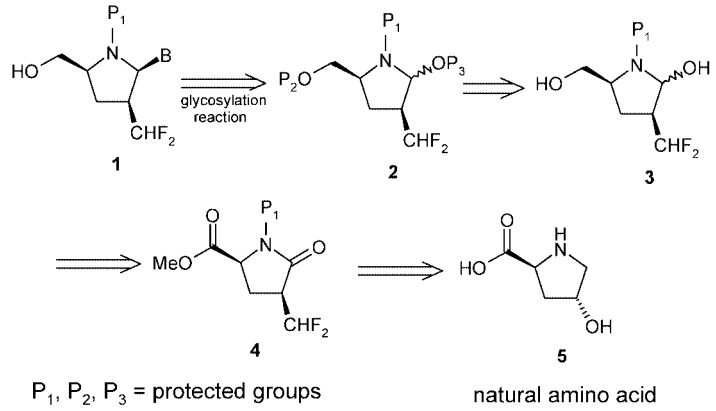


Figure 1 Several highly bioactive fluorinated nucleosides

Our retrosynthetic analysis (Scheme 1) was based on the thought that the target molecules 2′,3′-dideoxy-2′-difluoromethyl azanucleosides **1** could be prepared from protected pyrrolidine **2** by the glycosylation reactions, which could be reached from **3** by protection of two hydroxy groups of **3** step by step. Compound **3** could be prepared from the corresponding fluorinated pyroglutamate **4** by reduction of the ester and lactam with DIBAL-H in one step according to the reported methods.[8] Compound **4** could be obtained from *trans*-4-hydroxy-L-proline (**5**) using the similar methods reported recently by us.[9]

According to our retrosynthetic analysis, the methyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-difluoromethylpyroglutamate (**9a**) was synthesized first from the *trans*-4-hydroxy-L-proline (**5)** under the similar synthetic route (Scheme 2).[9] Thus the commercially available amino acid **5** was converted to the protected 4-oxo-L-proline **6** in three steps.[10] Difluoromethylenation[11] of **6** under CF_2Br_2/HMPT/Zn condition afforded the unstable compound **7** in 50% yield. However, hydrogenation of **7** with Pd/C in EtOH gave the compound **8** in 94% yield with moderate diastereoselectivity (*cis*/*trans* = 7:1). When the ester methyl group in **7** was replaced by a benzyl group, the hydrogenation proceeded with high diastereoselectivity.[12] In our opinion, this was caused by the different block effect of two groups. Finally, oxidation of the fluorinated proline

SYNTHESIS 2004, No. 3, pp 0334–0340
Advanced online publication: 26.01.2004
DOI: 10.1055/s-2004-815932; Art ID: F10303SS.pdf

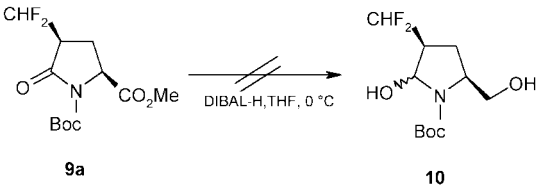


Scheme 1 Retrosynthetic analysis of 2′, 3′-dideoxy-2′-difluoromethyl-*N*-azanucleosides.

8 with $RuO_2 \cdot xH_2O/NaIO_4$/EtOAc system yielded the corresponding pyroglutamate **9**. Slightly surprisingly, the two diastereoisomers of **9** could be separated by flash chromatography. *Cis*-**9a** and *trans*-**9b** were obtained in 68 and 11% yields, respectively.

With **9a** in hand, the sequential reduction of the ester and lactam in one step with DIBAL-H was carried out according to the reported methods.[8] However, when compound **9a** was treated with DIBAL-H in THF at 0 °C (Scheme 3), the reaction was complicated and the desired compound **10** was not observed. In our opinion, the existence of the difluoromethyl group may be responsible for the failure of the reaction.

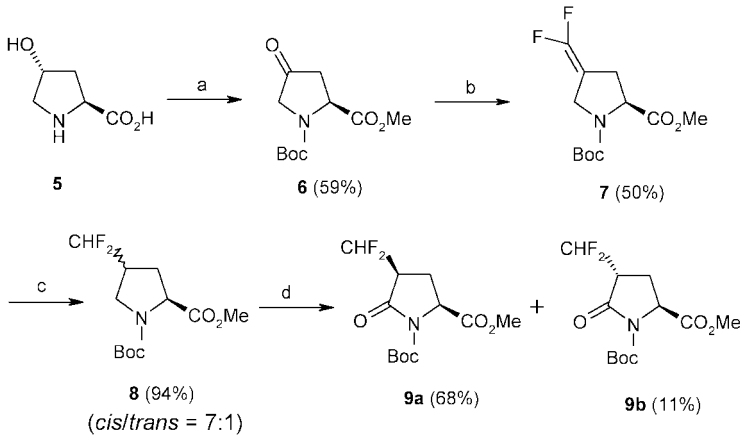
In view of above failure, we changed our strategies, that is, step by step reduction of the ester and the lactam. Thus, deprotection of *N*-Boc group of **9a** with trifluoroacetic acid in CH_2Cl_2, followed by reduction of the ester with $NaBH_4$, and further protection of the resulting hydroxyl group with TBDMSCl afforded compound **11** in 80% yield over three steps (Scheme 4). However, protection of the amino group of **11** with *tert*-butoxycarbonyl group under usual conditions only afforded the desired compound **12** in 9% yield, and the diastereoisomeric compound **13** (9%) and defluorinated compound **14** (25%) were also isolated. Fortunately, when the amino group of **11** was



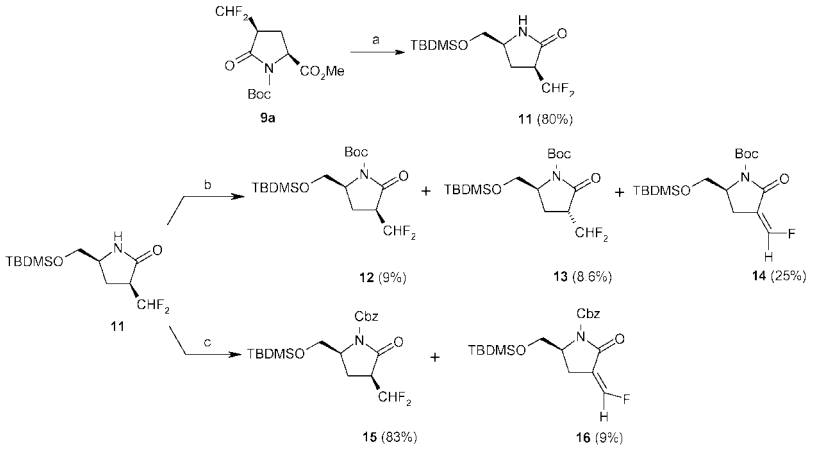
Scheme 3

protected with a benzyloxycarbonyl (Cbz) group, by Kikugawa's method,[13] the reaction proceeded smoothly. The desired compound **15** was obtained in 83% yield along with the defluorinated compound **16** in 9% yield.

Thus, reduction of compound **15** with $LiBEt_3H$ in THF at –78 °C yielded two diastereoisomers **17a** and **17b** (**17a**/**17b** = 9.2:1) in 88% yield. The two isomers could be separated on silica gel chromatography (Scheme 5). The acetylation of **17a** with acetic anhydride in CH_2Cl_2 afforded the compound **18** in 92% yield. Coupling of **18** with silylated uracil and thymine under Vorbrüggen's conditions[14] (glycosylation reaction) gave the desired silyl-protected azanucleosides. Following deprotection of the silyl protection groups with TBAF in THF afforded our target molecules **19a**, **19b**, **20a** and **20b**. The ratio of **19a**, **19b** and that of **20a, 20b** were different and opposite.



Scheme 2 Reagents and conditions: (a) i. $SOCl_2$, MeOH; ii. Boc_2O, Et_3N, DMAP, CH_2Cl_2; iii. Swern oxidation; (b) CF_2Br_2/HMPT/Zn; (c) Pd/C, H_2, EtOH, r.t., 1 atm.; (d) $RuO_2 \cdot xH_2O$, $NaIO_4$, EtOAc, H_2O.



Scheme 4 Reagents and conditions: (a) i. TFA, CH_2Cl_2; ii. $NaBH_4$, MeOH, –78 → 0 °C; iii. TBDMSCl, imidazole, DMAP, CH_2Cl_2; (b) Boc_2O, Et_3N, CH_2Cl_2, DMAP, r.t.; (c) LHMDS, CbzCl, THF, –78 °C, 10 min.

This was probably due to the different block effects of between methyl group and benzyloxycarbonyl group and between hydrogen and benzyloxycarbonyl group. The absolute configuration of compound **20b** was confirmed by X-ray (Figure 2).

In summary, we have synthesized the novel fluorinated azanucleosides: 2′,3′-dideoxy-2′-difluoromethyl azanucleosides **19a**, **19b**, **20a**, **20b** from *trans*- 4-hydroxy-L–proline (**5**). The investigations of their biological activities are in progress.

THF was distilled from sodium metal. CH_2Cl_2 and pyridine were distilled from CaH_2. All the melting points and optical rotations are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 300 spectrometer. ^{19}F NMR spectra were recorded on a Bruker AM 300 spectrometer ($CFCl_3$ as external standard and low field is positive). Chemical shifts (δ) are reported in ppm, and coupling constants (*J*) are in Hz.

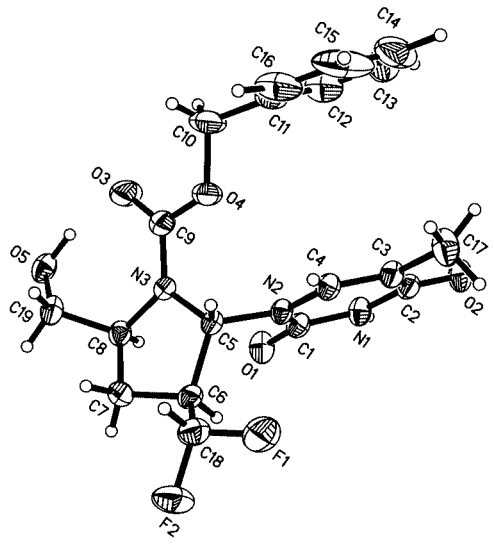
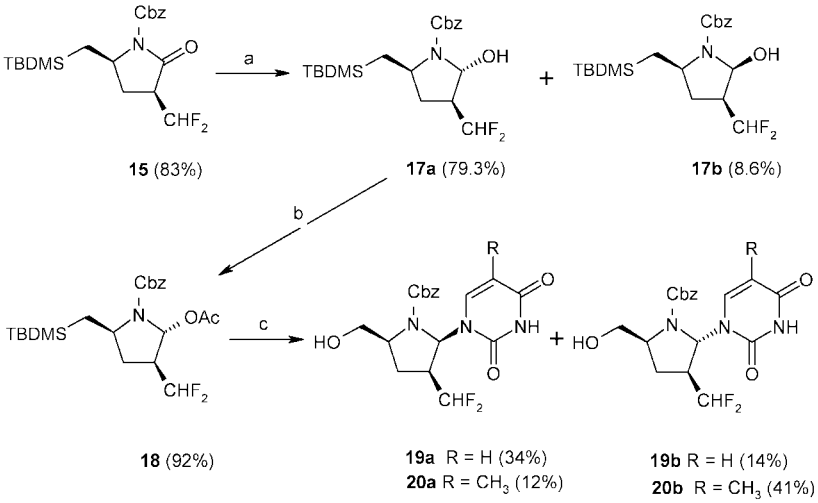


Figure 2 X-ray crystal structure of **20b**.



Scheme 5 Reagents and conditions: (a) $LiBEt_3H$, THF, –78 °C; (b) Ac_2O, DMAP, pyridine; (c) i. silylated uracil, or silylated thymine, TMSOTf, MeCN; ii. TBAF, THF.

Methyl (2*S*)-*N*- *tert*-Butoxycarbonyl-4-oxoprolinate (6)

$SOCl_2$ (6.5 mL, 89.58 mmol) was added dropwise to an ice-cold solution of *trans*-4-hydroxy-L-proline (**5**; 10.00 g, 76.26 mmol) in MeOH (100 mL) under anhydrous conditions. After the addition, the mixture was heated to reflux for 2 h. Then the reaction mixture was stirred overnight at r.t. The mixture was then concentrated in vacuo to afford a crude white solid (13.78 g), which was used without further purification. To a mixture of the white solid and DMAP (2.0 g, 16.39 mmol) was added CH_2Cl_2 (110 mL) followed by Et_3N (25 mL). The mixture was cooled in an ice-bath and a solution of Boc_2O (20.0 g, 91.6 mmol) in CH_2Cl_2 (50 mL) was added dropwise and it was stirred overnight at r.t. The reaction was then quenched with H_2O (100 mL) and the pH of the mixture was adjusted to 2–3 by the addition of dil. HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). Concentration and purification by column chromatography (hexane–EtOAc, 5:1, 2:1) afforded a light yellow oil (15.715 g). Oxalyl chloride (9.0 mL, 102.7 mmol) was added to a mixture of anhyd CH_2Cl_2 (100 mL) and DMSO (9.5 mL, 133.97 mmol) at –78 °C. After 10 min, a solution of above yellowish oil (15.715 g) in CH_2Cl_2 (120 mL) was added dropwise at a rate by keeping the reaction temperature below –60 °C. After the addition, the mixture was stirred at –78 °C for 2 h, then Et_3N (28 mL) was added dropwise. After the addition, the mixture was gradually warmed to r.t. and quenched with H_2O (60 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). Concentration and purification by silica gel chromatography (hexane–EtOAc, 2:1) gave **6** as a yellowish oil; yield: 11 g (59% from **5**).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.83–4.70 (1 H, dd, *J* = 9.9, 9.9 Hz), 3.91–3.88 (2 H, d, *J* = 7.8 Hz), 3.76 (3 H, s), 2.98–2.88 (1 H, m), 2.62–2.55 (1 H, m), 1.48, 1.46 (9 H, 2s).

Methyl (2*S*)-*N*-*tert*-Butoxycarbonyl-4-difluoromethyleneprolinate (7)

CF_2Br_2 (3.9 mL, 42.69 mmol) and HMPT (8.0 mL, 42.32 mmol) were added at 0 °C to a solution of **6** (4.85 g, 19.46 mmol) in THF (100 mL). The mixture was warmed to r.t. and stirred for 30 min. Zinc dust (2.85 g, 43.85 mmol) and HMPT (100 μL) were added. The mixture was heated to reflux for 20 min, then H_2O and Et_2O were added. The mixture was separated and the aqueous layer was extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with sat. aq $CuSO_4$ solution, H_2O and brine, and dried (Na_2SO_4). Concentration and purification on silica gel chromatography (hexane–EtOAc, 15:1) gave **7**; oil; yield: 2.777 g (50%); $[a]_D^{20}$ –27.2 (*c* = 1.45, $CHCl_3$).

IR (film): 1788, 1753, 1708, 1480, 1396, 1170 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.57–4.43 (1 H, dd, *J* = 9.6, 9.3 Hz), 4.18–4.04 (2 H, m), 3.75 (3 H, s), 3.00–2.87 (1 H, m), 2.70–2.64 (1 H, m), 1.48, 1.43 (9 H, 2s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –88.01 to –88.44 (1 F, m), –90.97 to –91.23 (1 F, m).

MS (ESI) : *m*/*z* = 300.2 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{12}H_{17}F_2NNaO_4$: 300.1018; found: 300.1009.

Anal. Calcd for $C_{12}H_{17}F_2NO_4$: C, 51.99; H, 6.14; N, 5.05. Found: C, 51.58; H, 6.28; N, 5.51.

Methyl (2*S*)-*N*-*tert*-Butoxycarbonyl-4-difluoromethylprolinate (8)

5% Pd/C (1.00 g) was added to a solution of **7** (2.777 g, 10.03 mmol) in EtOH (100 mL). Then the mixture was hydrogenated overnight at r.t. and 1 atm. After filtration and removal of the EtOH in vacuo, the residue was purified by flash chromatography (hexane–EtOAc, 10:1) to afford **8** as a colorless oil; yield: 2.626 g (94%).

IR (film): 1751, 1706, 1480, 1396, 1165 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.02–6.21 (1 H, m), 4.38–4.26 (1 H, m), 3.77–3.66 (4 H, m), 3.52–3.44 (1 H, m), 2.73–2.67 (1 H, m), 2.53–2.43 (1 H, m), 2.07–1.97 (1 H, m), 1.47, 1.41 (9 H, 2 s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –120 (m).

MS (EI): *m*/*z* = 280 (M^+ + 1), 178 (M^+ – Boc, 27), 57 (100).

Anal. Calcd for $C_{12}H_{19}F_2NO_4$: C, 51.62; H, 6.81; N, 5.02. Found: C, 51.76; H, 7.03; N, 4.82.

Methyl (2*S*,4*S*)-*N*-*tert*-Butoxycarbonyl-4-difluoromethylpyroglutamate (9a) and Methyl (2*S*,4*R*)-*N*-*tert*-Butoxycarbonyl-4-difluoromethylpyroglutamate (9b)

To a solution of $NaIO_4$ (4.780 g, 22.34 mmol) in H_2O (50 mL) was added $RuO_2 \cdot xH_2O$ (240 mg, 1.73 mmol) under N_2. The resulting greenish-yellow solution was stirred for 5 min, followed by addition of **8** (2.435 g, 8.727 mmol) in EtOAc (35 mL) in one portion. The mixture was stirred vigorously at r.t. Additional aliquots of 10% aq $NaIO_4$ were added to maintain a yellow-colored solution during the reaction. After 30 h, the resulting mixture was then diluted with EtOAc and filtered. The filtrate was washed with sat. aq $NaHSO_3$ solution. The organic layer was washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo, the resulting residue was purified by silica gel chromatography (hexane–EtOAc, 10:1, then 7:1) to give **9b** (less polar) and **9a** (more polar).

9b

White solid; yield: 280 mg (11%); mp 102–103 °C; $[\alpha]_D^{20}$ –17.5 (*c* = 0.78, $CHCl_3$).

IR (film): 1781, 1745, 1706, 1687, 1324 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.41–6.04 (1 H, td, *J* = 54.6, 2.1 Hz), 4.68–4.64 (1 H, dd, *J* = 2.4, 2.4 Hz), 3.80 (3 H, s), 3.27–3.19 (1 H, m), 2.62–2.50 (1 H, m), 2.21–2.14 (1 H, m), 1.50 (9 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –123.97 to –125.20 (1 F, ddd, *J* = 287.0, 55.0, 6.0 Hz), –127.19 to –128.49 (1 F, ddd, *J* = 286.0, 55.0, 26.0 Hz).

MS (ESI): *m*/*z* = 316 (M^+ + Na).

Anal. Calcd for $C_{12}H_{17}F_2NO_5$: C, 49.15; H, 5.80; N, 4.78. Found: C, 49.12; H, 5.57; N, 4.52.

9a

White solid; yield: 1.745 g (68%); mp 112–114 °C; $[\alpha]_D^{20}$ –30.7 (*c* = 0.46, $CHCl_3$).

IR (film): 1776, 1750, 1699, 1478 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.37–5.99 (1 H, td, *J* = 55.0, 2.4 Hz), 4.65–4.60 (1 H, dd, *J* = 6.6, 6.0 Hz), 3.80 (3 H, s), 3.19–3.09 (1 H, m), 2.61–2.49 (1 H, m), 2.32–2.22 (1 H, m), 1.51 (9 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –121.70 to –122.0 (1 F, ddd, *J* = 287.0, 55.0, 7.6 Hz), –125.20 to –126.53 (1 F, ddd, *J* = 285.0, 55.0, 25.0 Hz).

MS (EI): *m*/*z* 278 (M^+ – 15, <1), 192 (M^+ – Boc, 24), 57 (100).

Anal. Calcd for $C_{12}H_{17}F_2NO_5$: C, 49.15; H, 5.80; N, 4.78. Found: C, 49.14; H, 5.91; N, 4.65.

(5*S*,3*S*)-5-*tert*-Butyldimethylsiloxymethyl-3-difluoromethylpyrrolidin-2-one (11)

TFA (0.88 mL) was added dropwise to a solution of **9a** (771 mg, 2.63 mmol) in CH_2Cl_2 (30 mL) at –78 °C. The mixture was then warmed to r.t. and stirred overnight. The reaction mixture was cooled to 0 °C and quenched with sat. aq $NaHCO_3$ (40 mL). The organic phase was separated and the aqueous phase was extracted

with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo, the residue was dissolved in MeOH (20 mL) and the solution was cooled to –78 °C and $NaBH_4$ (120 mg, 3.16 mmol) was added. The mixture was then warmed to 0 °C and stirred for 2 h. The reaction was quenched with conc. HCl and the mixture was then filtered and concentrated to give a yellowish solid. To a cooled solution of DMAP (30 mg, 0.25 mmol) and imidazole (718 mg, 10.56 mmol) in CH_2Cl_2 (10 mL) was added a solution of the above yellowish solid in DMF (3 mL). Then a solution of TBDMSCl (1.230 g, 8.16 mmol) in CH_2Cl_2 (10 mL) was added dropwise. After the mixture was stirred overnight at r.t., it was quenched with sat. aq NH_4Cl in an ice-bath. The organic phase was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent in vacuo, the resulting residue was purified by flash chromatography (hexane–EtOAc, 5:1, then 1:1) to give **11**; colorless oil; yield: 587 mg (80%); $[\alpha]_D^{20}$ +60.9 (*c* = 0.89, $CHCl_3$).

IR (film): 3223, 3111, 1701, 1256 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 6.51 (1 H, s), 6.35–5.97 (1 H, td, *J* = 55.0, 2.4 Hz), 3.79–3.72 (1 H, m), 3.69–3.64 (1 H, dd, *J* = 4.2, 4.2 Hz), 3.47–3.41 (1 H, dd, *J* = 5.1, 5.4 Hz), 3.06–2.93 (1 H, m), 2.31–2.20 (1 H, m), 1.95–1.85 (1 H, m), 0.88 (9 H, s), 0.05 (6 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –122.78 to –124.02 (1 F, ddd, *J* = 284.0, 55.0, 3.1 Hz), –126.26 to –127.56 (1 F, ddd, *J* = 283.0, 57.0, 27.0 Hz).

MS (ESI): *m*/*z* = 302 (M^+ + Na).

Anal. Calcd for $C_{12}H_{23}F_2NO_2Si$: C, 51.61; H, 8.24; N, 5.02. Found: C, 51.56; H, 8.14; N, 4.80.

(5*S*,3*S*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-3-difluoromethyl-2-pyrrolidone (12), (5*S*,3*R*)-*N*-*tert*-Butoxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-3-difluoromethyl-2-pyrrolidone (13), and (5*S*)-*N*-*tert*-Butoxycarbonyl)-5-*tert*-butyldimethylsilyloxymethyl-3-[(*Z*)-fluoromethylidene]-2-pyrrolidone (14)

To a cooled solution of **11** (664 mg, 2.38 mmol), DMAP (126 mg, 1.03 mmol) and Et_3N (1.5 mL) in CH_2Cl_2 (15 mL) was added Boc_2O (1.283 g, 5.87 mmol) in CH_2Cl_2 (5 mL) dropwise. Then the mixture was warmed to r.t. and stirred overnight. The reaction was quenched with sat. aq NH_4Cl and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with dil. HCl, brine and dried (Na_2SO_4). After removal of the solvent in vacuo, the residue was purified by flash chromatography (hexane–EtOAc, 20:1, then 15:1) to give **12** (most polar), **13** (less polar) and **14** (more polar).

12

White solid; yield: 82 mg (9%); mp 83–85 °C; $[\alpha]_D^{20}$ –52.3 (*c* = 0.81, $CHCl_3$).

IR (film): 1766, 1689, 1474 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 6.34–5.95 (1 H, td, *J* = 56.0, 3.0 Hz), 4.15–4.10 (1 H, m), 3.91–3.86 (1 H, dd, *J* = 5.4, 5.1 Hz), 3.76–3.72 (1 H, dd, *J* = 3.0, 2.7 Hz), 3.11–3.00 (1 H, m), 2.28–2.21 (2 H, m), 1.52 (9 H, s), 0.86 (9 H, s,), 0.05, 0.04 (6 H, 2 s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –121.18 to –122.41 (1 F, ddd, *J* = 284.1, 8.5, 9.0 Hz), –123.71 to –124.36 (1 F, ddd, *J* = 284.3, 22.9, 14.7 Hz).

MS (ESI): *m*/*z* = 402.2 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{17}H_{31}F_2NO_4NaSi$: 402.1883; found: 402.1874.

13

Colorless oil; yield: 78 mg (9%); $[\alpha]_D^{20}$ –55.0 (*c* = 0.26, $CHCl_3$).

IR (film): 1791, 1755, 1720, 1473, 1369 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 6.39–6.01 (1 H, td, *J* = 56.8, 2.1 Hz), 4.24–4.21 (1 H, d, *J* = 9.3 Hz), 4.04–3.99 (1 H, dd, *J* = 3.0, 2.4 Hz), 3.71–3.67 (1 H, dd, *J* = 2.7, 1.8 Hz), 3.40–3.31 (1 H, m), 2.43–2.32 (1 H, m), 2.14–2.07 (1 H, m), 1.52 (9 H, s), 0.88 (9 H, s), 0.05, 0.04 (6 H, 2s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –118.67 to –119.98 (1 F, ddd, *J* = 283.6, 27.6, 26.5 Hz), –112.99 to –123.22 (1 F, ddd, *J* = 283.97, 6.1, 6.3 Hz).

MS (ESI): *m*/*z* = 402.1 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{17}H_{31}F_2NO_4NaSi$: 402.1883; found: 402.1892.

Anal. Calcd for $C_{17}H_{31}F_2NO_4Si$: C, 53.83; H, 8.18; N, 3.69. Found: C, 53.51; H, 8.26; N, 3.52.

14

White solid; yield: 216 mg (25%); mp 32–34 °C; $[\alpha]_D^{20}$ –31.5 (*c* = 1.36, $CHCl_3$).

IR (film): 1718, 1687, 1473, 1366 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.57–7.29 (1 H, dt, *J* = 79.5, 2.6 Hz), 4.25–4.19 (1 H, m), 3.85–3.81 (1 H, dd, *J* = 3.9, 3.6 Hz), 3.67–3.64 (1 H, dd, *J* = 1.8, 2.1 Hz), 2.84–2.70 (2 H, m), 1.53 (9 H, s), 0.84 (9 H, s), 0.09–0.04 (6 H, m).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –123.57 to –123.86 (1 F, d, *J* = 80.5 Hz).

MS (EI): *m*/*z* = 360 (M^+ + 1, 1), 57 (100).

Anal. Calcd for $C_{17}H_{30}FNO_4Si$: C, 56.82; H, 8.36; N, 3.90. Found: C, 56.79; H, 8.23; N, 3.65.

(5*S*,3*S*)-*N*-Benzyloxycarbonyl-5-*tert*-butyldimethylsilyloxymethyl-3-difluoromethyl-2-pyrrolidone (15) and (5*S*)-*N*-Benzyloxycarbonyl-5-*tert*-butydimethylsilyloxymethyl-3-[(*Z*)-fluoromethylidene]-2-pyrrolidone (16)

To a solution of **11** (220 mg, 0.79 mmol) in THF (10 mL) at –78 °C was added LHMDS (0.79 mL, 1 M in THF, 0.79 mmol) dropwise. After the mixture was stirred for 10 min, CbzCl (0.17 mL, 1.19 mmol) was added dropwise. The mixture was stirred for further 10 min at –78 °C and quenched with sat. aq NH_4Cl (6 mL). The mixture was warmed to r.t. and extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent, the resulting residue was purified by flash chromatography (hexane–EtOAc, 10:1, then 7:1) to give **15** and **16**.

15

White solid; yield: 270 mg (83%); mp 111–112 °C; $[\alpha]_D^{20}$ –53.4 (*c* = 0.41, $CHCl_3$).

IR (film): 2960, 1715, 1307, 1275 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.44–7.33 (5 H, m), 6.36–5.97 (1 H, td, *J* = 55.8, 3.0 Hz), 5.35–5.23 (2 H, m), 4.21–4.19 (1 H, m), 3.91–3.86 (1 H, dd, *J* = 5.1, 4.5 Hz), 3.75–3.70 (1 H, dd, *J* = 2.4, 2.1 Hz), 3.12 (1 H, m), 2.32–2.25 (2 H, m), 0.84 (9 H, s), 0.07 (6 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –121.53 to –122.76 (1 F, ddd, *J* = 285.8, 9.7, 7.8 Hz), –123.38 to –124.67 (1 F, ddd, *J* = 285.7, 24.8, 20.6 Hz).

MS(ESI): *m*/*z* = 436 (M^+ + Na).

Anal. Calcd for $C_{20}H_{29}F_2NO_4Si$: C, 58.11; H, 7.02; N, 3.39. Found: C, 58.12; H, 6.96; N, 3.13.

16

White solid; yield: 29 mg (9%); mp 47–48 °C; $[\alpha]_D^{20}$ –37.0 (*c* = 0.40, $CHCl_3$).

IR (film): 2957, 1738, 1712, 1685, 1294 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.61–7.44 (1 H, dt, *J* = 46.4, 1.7 Hz), 7.43–7.32 (5 H, m), 5.36–5.26 (2 H, m), 4.31–4.27 (1 H, m), 3.85–3.80 (1 H, dd, *J* = 3.9, 3.9 Hz), 3.66–3.62 (1 H, dd, *J* = 1.8, 2.1 Hz), 2.81–2.75 (2 H, m), 0.82 (9 H, s), 0.07 (6 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –122.39 to –122.69 (1 F, dt, *J* = 80.2, 3.6 Hz).

MS (ESI): *m*/*z* = 416 (M^+ + Na), 394.1 (M^+ + 1).

Anal. Calcd for $C_{20}H_{28}FNO_4Si$: C, 61.07; H, 7.12; N, 3.56. Found: C, 61.16; H, 7.35; N, 3.31.

Benzyl (2*R*,3*S*,5*S*)-5-*tert*-Butyldimethylsilyloxymethyl-3-difluoromethyl-2hydroxypyrrolidine-1-cabboxylate (17a) and Benzyl (2*S*,3*S*,5*S*) -5- *tert*-Butyldimethylsilyloxymethyl-3-difluoromethyl-2-hydroxypyrrolidine-1-carboxylate (17b)

To a solution of **15** (670 mg, 1.622 mmol) in THF (20 mL) at –78 °C was added $LiBEt_3H$ (6.4 mL, 1 M in THF, 6.4 mmol) dropwise. The mixture was stirred at –78 °C for 3.5 h. The reaction was then quenched with H_2O (5 mL) and warmed up to r.t. The mixture was extracted with CH_2Cl_2 (3 × 60 mL) and the combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent, the resulting residue was purified by flash chromatography (hexane–EtOAc, 15:1, then 7:1) to give **17a** (less polar) and **17b** (more polar).

17a

Light yellow oil; yield: 534 mg (79%); $[\alpha]_D^{20}$ –39.7 (*c* = 0.99, $CHCl_3$).

IR (film): 3422, 2957, 1709, 1406, 1097 cm^{-1}.

^1H NMR (300 MHz, acetone-d_6): δ = 7.38–7.30 (5 H, m), 6.00–5.64 (1 H, td, *J* = 56.1, 7.2 Hz), 5.34 (1 H, br), 5.24–5.08 (2 H, m), 4.21–4.08 (1 H, m), 3.79–3.74 (1 H m), 3.57–3.46 (1 H, m), 2.48–2.41 (1 H, m), 2.21–2.01 (2 H, m), 0.89, 0.86 (9 H, 2 s), 0.09, 0.07 (6 H, 2 s).

^{19}F NMR (282 MHz, acetone-d_6): δ = –118.05 to –119.41 (1 F, m), –127.23 to –128.35 (1 F, m).

MS (ESI): *m*/*z* = 438 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{20}H_{31}F_2NNaO_4Si$: 438.1883; found: 438.1899.

17b

Light yellow oil; yield: 58 mg (9%); $[\alpha]_D^{20}$ –53.3 (*c* = 0.45, $CHCl_3$).

IR (film): 3238, 1698, 1498, 1355, 1081 cm^{-1}.

^1H NMR (300 MHz, acetone-d_6): δ = 7.43–7.31 (5 H, m), 6.26–5.86 (1 H, td, *J* = 56.6, 6.3 Hz), 5.40 (1 H, br), 5.27–5.04 (3 H, m), 4.04–3.98 (1 H, m), 3.79–3.77 (1 H, br), 2.54–3.44 (2 H, m), 2.04–1.99 (1 H, m), 0.87 (9 H, s), 0.04, (6 H, s).

^{19}F NMR (282 MHz, acetone-d_6): δ = –119.89 to –121.19 (1 F, m), –123.41 to –125.20 (1 F, m).

MS (ESI): *m*/*z* = 438 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{20}H_{31}F_2NNaO_4Si$: 438.1883; found: 438.1885.

Benzyl (2*R*,3*S*,5*S*)-2-Acetyloxy-5-*tert*-butyldimethylsilyloxymethyl-3-difluoromethylpyrrolidine-1-carboxylate (18)

To a mixture of **17a** (534 mg, 1.286 mmol), DMAP (20 mg, 0.164 mmol), pyridine (1.60 mL, 19.78 mmol) in CH_2Cl_2 (20 mL) was added Ac_2O (1.21 mL, 11.95 mmol) dropwise. After the mixture was stirred overnight at r.t., the reaction was quenched with sat. aq $NaHCO_3$. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with dil. HCl, brine and dried (Na_2SO_4). After removal of the solvent, the resulting residue was purified by silica gel chromatography (hexane–EtOAc, 15:1, then 7:1) to give **18** (540 mg, 92%); white solid; yield: 540 mg (92%); mp 87–88 °C; $[\alpha]_D^{20}$ –56.4 (*c* = 0.67, $CHCl_3$).

IR (film): 1718, 1399, 1086 cm^{-1}.

^1H NMR (300 MHz, acetone-d_6): δ = 7.39–7.35 (5 H, m), 6.86–5.84 (1 H, d, *J* = 5.1 Hz), 6.18–5.78 (1 H, td, *J* = 56.6, 6.0 Hz), 5.15–5.12 (2 H, br), 4.01–3.91 (3 H, m), 2.88–2.84 (1 H, m), 2.25–2.18 (2 H, m), 2.00 (3 H, s), 0.90 (9 H, s), 0.03 (6 H, s).

^{19}F NMR (282 MHz, acetone-d_6): δ = –115.47 to –116.71 (1 F, dd, *J* = 52.45, 60.9 Hz), –120.02 to –125.67 (1 F, m).

MS (ESI): *m*/*z* 480.1 (M^+ + Na).

HRMS (ESI): *m*/*z* calcd for $C_{22}H_{33}F_2NNaO_5Si$: 480.1988; found: 480.1991.

Anal. Calcd for $C_{22}H_{33}F_2NO_5Si$: C, 57.77; H, 7.22; N, 3.06. Found: C, 58.01; H, 7.30; N, 2.80.

Benzyl (2*R*,3*S*,5*S*)-5-Hydroxymethyl-2-[2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3-(difluoromethyl)pyrrolidine-1-carboxylate (19a) and Benzyl (2*S*,3*S*,5*S*)-5-Hydroxymethyl-2-[2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3-(difluoromethyl)pyrrolidine-1-carboxylate (19b); Typical Procedure

To a stirred solution of **18** (306 mg, 0.669 mmol) and uracil (220 mg, 1.96 mmol) in anhyd MeCN (30 mL) was added *N*,*O*-bis (trimethylsilyl)acetamide (1.0 mL, 3.03 mmol). The reaction mixture was stirred under reflux for 30 min. After cooling to 0 °C, TMSOTf (0.33 mL, 1.58 mmol) was added dropwise and the solution was stirred at r.t. for further 30 min. The reaction was quenched with cold sat. aq $NaHCO_3$ and the resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent, the resulting residue was purified by flash chromatography (hexane–EtOAc, 4:1, 3:1 then 2:1) to give two compounds, the less polar compound (90 mg, white foam) and the more polar compound (150 mg, white foam). A stirred solution of the above less polar compound (90 mg) in THF (10 mL) was treated with 1 M solution of TBAF (0.24 mL, 0.24 mmol) at 0 °C. After stirring at r.t. for 8.5 h, the reaction was quenched with H_2O and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). After removal of the solvent, the resulting residue was purified by flash chromatography (hexane–EtOAc, 1:1) to give **19a**. The more polar compound was also treated with TBAF under the same conditions to give **19b**.

19a

White foam; yield: 64 mg (34% from **18**); $[\alpha]_D^{20}$ +15.3 (*c* = 0.59, $CHCl_3$).

IR (film): 3444, 3203, 3061, 1687, 1465 cm^{-1}.

^1H NMR (300 MHz, methanol-d_4): δ = 8.46 (1 H, br), 7.25 (5 H, br), 6.45–6.42 (1 H, d, *J* = 7.2 Hz), 6.15–5.78 (1 H, t, *J* = 55.2 Hz), 5.56–5.53 (1 H, d, *J* = 7.8 Hz), 5.18–4.92 (2 H, m), 4.37 (1 H, br), 3.89–3.84 (1 H, t, *J* = 8.6 Hz), 3.65–3.61 (1 H, d, *J* = 12.0 Hz), 3.13–2.93 (1 H, m), 2.39–2.27 (1 H, m), 2.11–2.02 (1 H, m).

^{19}F NMR (282 MHz, methanol-d_4): δ = –117.61 to –118.84 (1 F, m), –125.93 to –127.15 (1 F, m).

^{13}C NMR (75.5 MHz, methanol-d_4): δ = 166.1, 156.2, 152.6, 143.7, 137.3, 129.6, 129.3, 128.9, 116.0 (t, *J* = 239.8 Hz), 102.2, 70.6, 68.8, 61.5, 60.8, 46.3 (t, *J* = 22.7 Hz), 24.8.

MS (EI): *m*/*z* 395 (M^+, <1), 284 (M^+ – uracil, 12), 91 (84), 43 (100).

HRMS (EI): *m*/*z* calcd for $C_{14}H_{16}F_2NO_3$ (M^+ – uracil): 284.1098; found: 284.1105.

19b
White foam; yield: 38 mg (14% from **18**); $[\alpha]_D^{20}$ –75.4 (*c* = 0.67, $CHCl_3$).

IR (film): 3424, 3201, 3063, 1690, 1463, 1409 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 9.52 (1 H, br), 7.32–7.26 (5 H, m), 6.85–6.83 (1 H, d, *J* = 6.6 Hz), 6.17–5.79 (1 H, t, *J* = 56.3 Hz), 5.65 (1 H, br), 5.38–5.37 (1 H, d, *J* = 5.1 Hz), 5.25–5.21 (1 H, d, *J* = 11.4 Hz), 4.96–4.92 (1 H, br), 4.37 (1 H, br), 3.97–3.93 (1 H, d, *J* = 12.0 Hz), 3.73–3.69 (1 H, br), 3.02 (1 H, br), 2.50 (1 H, br), 1.80 (1 H, br).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –120.69 to –121.57 (1 F, m), –123.58 to –124.95 (1 F, m).

MS (EI): *m*/*z* = 395 (M^+, <1), 364 (M^+ – CH_2OH, <1), 284 (M^+ – uracil, 24), 91 (100).

HRMS (EI): *m*/*z* calcd for $C_{14}H_{16}F_2NO_3$ (M^+ – uracil): 284.1098; found: 284.1147.

Benzyl (2*R*,3*S*,5*S*)-5-Hydroxymethyl-2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3-difluoromethylpyrrolidine-1-carboxylate (20a) and Benzyl (2*S*,3*S*,5*S*)-5-Hydroxymethyl-2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3-difluoromethylpyrrolidine-1-carboxylate (20b)
Compounds **20a** and **20b** were prepared as white foams from compound **18** (272 mg, 0.59 mmol) and thymine (230 mg, 1.82 mmol) using the same conditions as described for compounds **19a** and **19b**.

20a
White foam; yield: 29 mg (12%); $[\alpha]_D^{20}$ +28.0 (*c* = 0.19, $CHCl_3$).

IR (film): 3441, 3193, 3065, 1687 and 1471 cm^{-1}.

^1H NMR (300 MHz, MeOH-d_4): δ = 8.40 (1 H, br), 7.24 (5 H, br), 6.43 (1 H, br), 6.12–5.75 (1 H, t, *J* = 55.1 Hz), 5.18–4.91 (2 H, m), 4.44 (1 H, br), 3.89–3.84 (1 H, t, *J* = 8.7 Hz), 3.64–3.60 (1 H, d, *J* = 11.7 Hz), 3.11–2.91 (1 H, m), 2.42–2.30 (1 H, m), 2.10–2.01 (1 H, m), 1.74 (3 H, s).

^{19}F NMR (282 MHz, MeOH-d_4): δ = –115.58 to –116.75 (1 F, m), –125.04 to –126.17 (1 F, m).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 166.4, 156.2, 152.8, 139.5, 137.4, 129.6, 129.3, 128.9, 116.1 (t, *J* = 239.4 Hz), 111.0, 70.3, 68.7, 61.5, 60.7, 46.4 (t, *J* = 22.3 Hz), 24.8, 12.5.

MS (EI): *m*/*z* = 409 (M^+, <1), 284 (M^+ – thymine, 27), 91 (100).

MS (ESI): *m*/*z* = 410.1 (M^+ + 1).

HRMS (ESI): *m*/*z* calcd for $C_{19}H_{21}F_2N_3NaO_5$ (M^+ + Na,): 432.1341; found: 432.1360.

20b
White foam; yield: 101 mg (41%); $[\alpha]_D^{20}$ –58.8 (*c* = 0.40, $CHCl_3$).

IR (film): 3418, 3196, 1714, 1674, 1458, 1026 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 9.07 (1 H, br), 7.35–7.24 (5 H, m), 6.62 (1 H, br), 6.14–5.78 (1 H, t, *J* = 54.8 Hz), 5.64 (1 H, br), 5.27–5.23 (1 H, d, *J* = 11.4 Hz), 4.93–4.89 (1 H, d, *J* = 11.1 Hz), 4.41–4.39 (1 H, br), 3.96–3.92 (1 H, d, *J* = 12.3 Hz), 3.75–3.70 (1 H, dd, *J* = 5.1, 3.6 Hz), 3.07–2.98 (1 H, br), 2.52–2.43 (1 H, m), 1.93–1.70 (2 H, m), 1.63 (3 H, s).

^{19}F NMR (282 MHz, $CDCl_3$): δ = –120.30 to –121.67 (1 F, m), –123.49 to –125.01 (1 F, m).

MS (EI) : *m*/*z* = 409 (M^+, <1), 284 (M^+ – thymine, 18), 91 (100).

MS (ESI) : *m*/*z* = 410 (M^+ + 1), 432.2 (M^+ + Na).

HRMS (EI): *m*/*z* calcd for $C_{14}H_{16}F_2NO_3$ (M^+ – thymine,): 284.1098; found: 284.1115.

HRMS (ESI): *m*/*z* calcd for $C_{19}H_{21}F_2N_3NaO_5$ (M^+ + Na,): 432.1341; found: 432.1336.

Acknowledgment

We thank the National Natural Science Foundation of China for financial support.

Reference

(1) (a) Wilkinson, J. A. *Chem. Rev.* **1992**, *92*, 505. (b) *Organofluorine Compounds in Medicinal Chemistry and Biomedical Application*; Filler, R.; Kobayashi, Y.; Yagupolskii, L. M., Eds.; Elsevier: Amsterdam, **1993**.
(2) Harper, D. B.; O'Hagan, D. *Nat. Prod. Rep.* **1994**, *11*, 123.
(3) Yokoyama, M.; Momotake, A. *Synthesis* **1999**, 1541.
(4) (a) Chu, C. K.; Ma, T.; Shanmuganathan, K.; Wang, C.; Xiang, Y.; Pai, S. B.; Yao, G. Q.; Sommadossi, J. P.; Cheng, Y. C. *Antimicrob. Agents Chemother.* **1995**, *39*, 979. (b) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.
(5) (a) Etzold, G.; Hintsche, R.; Kowollik, G.; Langen, P. *Tetrahedron* **1971**, *27*, 2463. (b) Balzarini, J.; Baba, M.; Pauwells, R.; Hetdewijn, P.; De Clercq, E. *Biochem. Pharmacol.* **1988**, *37*, 2847.
(6) (a) Kotra, L. P.; Xiang, Y.; Newton, M. G.; Schinazi, R. F.; Cheng, Y.-C.; Chu, C. K. *J. Med. Chem.* **1997**, *40*, 3635. (b) Hertel, L. W.; Kroin, J. S.; Misner, J. W.; Tustin, J. M. *J. Org. Chem.* **1988**, *53*, 2406.
(7) Qing, F.-L.; Yu, J.; Fu, X.-K. *Collect. Czech. Chem. Commun.* **2002**, *67*, 1267.
(8) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron Lett.* **1993**, *34*, 5743.
(9) Qiu, X.-L.; Qing, F.-L. *J. Org. Chem.* **2003**, *68*, 3614.
(10) (a) Lewis, A.; Wilkie, J.; Rutherford, T. J.; Gani, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3777. (b) O'Connel, C. E.; Rowell, C. A.; Ackermann, K.; Garcia, A. M.; Lewis, M. D.; Kowalczyk, J. J. *Chem. Pharm. Bull.* **2000**, *48*, 740.
(11) Motherwell, W. B.; Tozer, M. J.; Ross, B. C. *J. Chem. Soc., Chem. Commun.* **1989**, 1437.
(12) Qiu, X.-L.; Qing, F.-L. *J. Org. Chem.* **2002**, *67*, 7162.
(13) Li, H.; Sakamoto, T.; Kato, M.; Kikugawa, Y. *Synth. Commun.* **1995**, *24*, 4045.
(14) (a) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234. (b) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256.