Full Paper

Difluorotetrahydropyridothiazinone: A Selective β-Galactosidase Inhibitor

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Selective difluorination, introducing a lactame moiety (instead of an amine) and a double bond in a trihydroxy-2-thiaquinolizidine derivative reverses the selectivity of the glycosidase inhibitor – a selective inhibitor for an α-glucosidase is altered into an excellent, competitive inhibitor for a β-galactosidase.

Keywords: Galactosidase / Galactosidase inhibitor / Glycosidase inhibitor

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Introduction

Hyperglycemia and hyperlipidemia are recognized and severe health-care problems. Obesity is a major risk to develop [1, 2] coronary artery diseases and hypertension, dyslipidemia and hyperglycemia as well as cancer. Today, more than 120 million people are considered obese [3] and the market expenditures for drugs are expected [4] to increase to over US$ 120 billion by 2010. The use of iminosugars has a long tradition in the therapy of diabetes [5] and metabolic disorders [6]; miglitol (**I**, Fig. 1) is a blockbuster in this field.

Several years ago, trihydroxy-2-thiaquinolizidines were introduced [7] as a new class of bicyclic iminohexitol glycosidase inhibitors and one of these derivatives (**II**) was shown to be active only against α-glucosidases. The activity of these compounds was low as compared, for example to miglitol. Recently, we were able to show [8] that mono- or difluorination of iminosugars leads to a dramatic change of activity and selectivity.

Results and discussion

Recently, introducing a geminal difluoromethylene group at position-3 in a piperidine ring attracted notable interest. Compounds owning this feature have been shown to display pronounced activity in anti-inflammatory macrophage colony-stimulating factor-1 receptor inhibition [9] as well as novel histamine-3 receptor inverse agonists [10, 11].

Since rigidity seems to be essential for selectivity in the inhibition of glycosidases [7], we set out for the synthesis of a target compound derived from lead structure **II** but possessing a lactame moiety as well as a geminal difluoromethylene group in β-position to the ring nitrogen. An extra double bond in the ring should further reduce the flexibility of the molecule.

Well-known compound **1** [12] (Scheme 1) was deprotected [13] to afford 82% of **2**, whose hydrogenolysis [14–16] gave the 2,2-difluoro-β-D-*arabino*-hexopyranoside **3** in 94% yield. A slightly higher yield of **3** was obtained by the hydrogenolysis of **1** in the presence of Pd/C. Compound **3** is characterized by its ^{19}F-NMR spectrum by the presence of two signals at $\delta = -124.21$ and -143.19 ppm showing a large $^{2}J_{F,F} = 245.7$ Hz. In the ^{13}C-NMR spectrum, geminal difluorinated C(2) was found at $\delta = 117.0$ ppm showing $^{1}J_{C,F} = 253.6$ Hz.

Tritylation [17–19] of **3** gave the 6-*O*-trityl derivative **4** whose pivaloylation [20–22] gave 74% of fully protected **5**. Detritylation [23, 24] of **5** was performed using trimethylsilyl iodide in dry dichloromethane and **6** was obtained in almost quantitative yield. Bromination of **6** proceeded smoothly using triphenylphosphane/tetrabromomethane [25] and the corresponding 6-bromo-2,2-difluoro-hexopyranoside **7** was obtained in 98% yield (isolated).

Oxidative ring opening was performed following the original procedure of Angyal [26, 27]. Thus, treating **7** with CrO_3 (Scheme 2) in a mixture of acetic acid and acetic anhydride provided 68% of acyclic methyl hex-5-ulosonate **8**. In its ^{19}F-NMR, compound **8** shows two signals at $\delta = -111.16$ and

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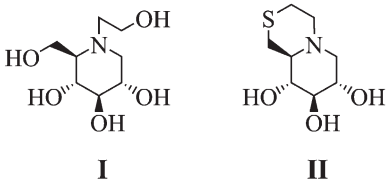
Abbreviation: dialkylaminopyridine (DMAP)



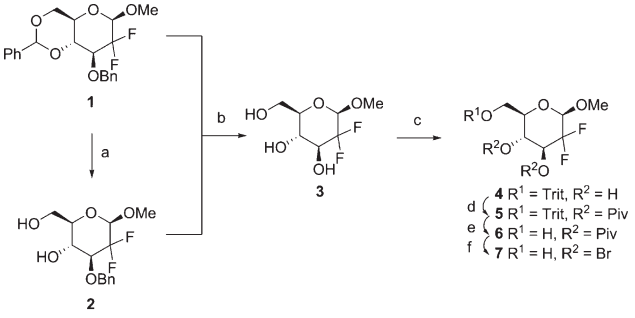
I **II**

Figure 1. Structure of α-glucosidase inhibitors miglitol **I** and a 2-thiaquinolizidine (**II**, [7]).

−118.04 ppm possessing a $^{2}J_{F,F} = 270.4$ Hz. The ^{13}C-NMR spectrum shows C(5) at $\delta = 194.7$ ppm and the ester carbonyl at $\delta = 162.2$ ppm (this signal displays two $^{2}J_{C,F}$ of 30.2 and 32.7 Hz, respectively).

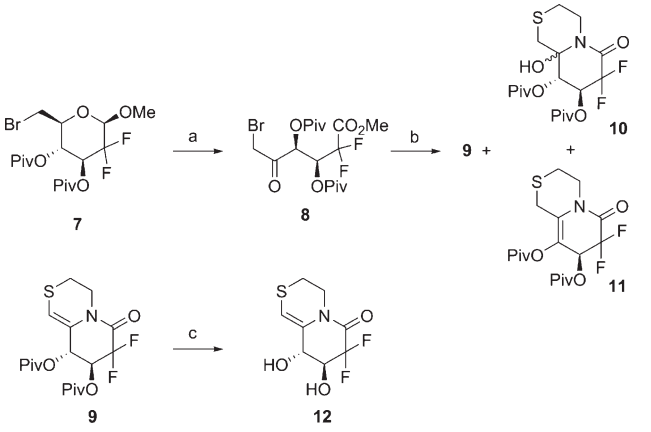
The reaction of **8** with cysteamine [7] furnished **9** as the main product. As by-products an inseparable mixture of **10** was obtained in 24% yield as well 18% of **11** resulting from an elimination reaction. The structures of **10** and **11** can be assigned unambiguously from the ^{13}C-NMR spectra. For C(5), compound **9** shows a chemical shift at $\delta = 124.9$ ppm whereas for C(5) in **11** $\delta = 149.3$ ppm is detected; C(4) is found at $\delta = 100.9$ ppm. Finally, compound **9** was deprotected using sodium methoxide in methanol [28] and **12** was obtained in 94% yield.

Compound **12** was tested against several glycosidases [29] using the *p*-nitrophenolate assay. Thus, **12** showed no inhibition at all for the α-glucosidase from *Bacillus stearothermophilus*, the β-glucosidase from almonds, and the α-galactosidase from green coffee beans, but is a competitive inhibitor for a β-galactosidase from *E. coli* showing an IC_{50} of 2.01 mM. Compound **12** owns a rigid bicyclic structure and seems to be locked in its conformation (as indicated by semi-empirical PM3-calculations using the CAChe 4.0 software from Oxford Molecular) – this results in inactivity against β-glycosidases. Usually, compounds derived from 1-deoxynojirimycin, castanospermine, or swainsonine with nitrogen instead of the ring oxygen are selective inhibitors [30] for α-glucosidases. The presence of a lactame instead of an amine, however, makes **12** less attractive for α-glucosidases. The specificity of **12** is a starting point to develop even stronger but still selective inhibitors for β-galactosidases. This might be of some interest in the therapy of lysosomal storage disorders.



Reagents and conditions: a) TFA/H_2O, DCM, 25°C, 5 h, 82 %; b) Pd/C (10%), H_2 (5 at), MeOH, 50°C, 48 h, 94–97%; c) TritCl, DMAP, DCM, 40°C, 4 d, quant.; d) PivCl, pyridine, 25 → 50°C, 48 h, 74%; e) TMSI, DCM, 25°C, 12 h, 99%; f) CBr_4, PPh_3, 65°C, 4 h, 98%.

Scheme 1. Synthesis of compounds **2–7**.



Reagents and conditions: a) AcOH/Ac_2O, CrO_3, 25°C, 2 h, 68%; b) HS-$(CH_2)_2$-NH_2, 25°C: **9** (41%), **10** (24%), **11** (18%); c) NaOMe/MeOH, 25°C, 4 h, 94%.

Scheme 2. Synthesis of compounds **8–12**.

Experimental

General

Melting points are uncorrected (Leica hot stage microscope; Leica, Wetzlar, Germany), NMR spectra were recorded using the Varian spectrometers (Varian, USA) Gemini 200, Gemini 2000, or Unity 500 (δ given in ppm, J in Hz, internal Me_4Si or internal CCl_3F), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell, 20°C; Perkin-Elmer, USA), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV; Intectra GmbH, Harpstedt, Germany) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen; Thermo Scientific, Germany) instrument. TLC was performed on silica gel (Merck 5554, detection by UV absorption; Merck, Germany). The solvents were dried according to usual procedures.

Biological evaluation

The *p*-nitrophenolate assay was performed as a microtiter plate assay using a Tecan instrument. The α-glucosidase (from *Bacillus stearothermophilus*) was obtained from Sigma (96 U/mg; Sigma-Aldrich, Steinheim, Germany), β-glucosidase (almonds) was obtained from Fluka (8.92 U/mg; Sigma-Aldrich), α-galactosidase (green coffee beans) was from Sigma (45.6 U/mL) and the β-

galactosidase (165 U/mg) from Fluka. For all experiments, 0.06 M phosphate buffer (pH = 6.0) was used except for the β-glucosidase where an acetate buffer (0.05 M, pH = 5.0) was applied. Dilution factors of 6.0, 4.0, 3.0, 2.0, 1.0, 0.03, and 0.01 mg/mL were applied for the inhibitor and 1.0, 0.5, 0.1, 0.05, and 0.025 mM for the 4-nitrophenol. UV-measurement was performed at $\lambda = 415$ nm; experiments were performed at least in triplicate, resulting in a SD of 0.0187 and $R^2 = 0.99989$.

Synthesis

*Methyl-3-O-benzyl-2-deoxy-2,2-difluoro-β-D-arabino-hexopyranoside **2***

To a solution of **1** (1.05 g, 2.68 mmol) in dichloromethane (19.6 mL) and water (0.4 mL), trifluoroacetic acid in dichloromethane (30%; 12.0 mL) was added dropwise and the solution was stirred at 25°C for 5 h. After the solution was diluted with dichloromethane (70 mL) and washed with water (50 mL), the organic and aqueous phases were separated and the aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined organic phases were dried (Na_2SO_4), the solvent was removed under diminished pressure and the residue subjected to chromatography (silica gel, hexane/ethyl acetate, 5:50) to afford **2** (2.20 mmol, 82.3%) as a white solid; m. p.: 100–101°C; $[\alpha]_D = -77.60^\circ$ ($c = 0.44$, $CHCl_3$); R_f (hexane/ethyl acetate, 5:3): 0.12; IR (KBr) ν: 3569*s*, 3489*s*, 3035*m*, 2942*s*, 2875*m*, 1959*m*, 1739*m*, 1661*m*, 1572*m*, 1499*m*, 1454*s*, 1404*m*, 1368*m*, 1347*m*, 1318*m*, 1249*s*, 1228*s*, 1091*s*, 1064*s*, 1046*s*, 1002*s*, 916*m*, 864*s*, 782*s*, 751*m*, 698*s*, 648*m*, 630*m*, 544*m*, 483*m*, 424*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.37–7.30 (m, 5H, Ph), 5.00 (d, 1H, $^2J_{H'',H'} = 11.5$ Hz, C*H*''$_2$, OBn), 4.66 (d, 1H, $^2J_{H',H''} = 11.5$ Hz, C*H*′$_2$, OBn), 4.44 (d, 1H, $^3J_{1,F'} = 14.6$ Hz, H-1), 3.91 (dd, 1H, $^3J_{6B,5} = 3.4$, $^2J_{6B,6A} = 12.0$ Hz, H-6$_B$), 3.80 (dd, 1H, $^3J_{6A,5} = 4.7$, $^2J_{6A,6B} = 12.0$ Hz, H-6$_A$), 3.76 (ddd, 1H, $^3J_{4,F'} = 2.1$, $^3J_{4,5} = 9.5$, $^3J_{4,3} = 9.3$ Hz, H-4), 3.62 (s, 3H, OC*H*$_3$), 3.55 (ddd, 1H, $^3J_{3,F} = 5.1$, $^3J_{3,4} = 9.3$, $^3J_{3,F'} = 19.5$ Hz, H-3), 3.40 (ddd, 1H, $^3J_{5,6B} = 3.4$, $^3J_{5,4} = 9.5$, $^3J_{5,6A} = 4.7$ Hz, H-5) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 137.1 (C_{ar}), 128.8 (C_{ar}), 128.7 (C_{ar}), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.3 (C_{ar}), 116.1 (dd, $^1J_{2,F} = 253.9$, $^1J_{2,F} = 258.2$ Hz, C2), 99.4 (dd, $^2J_{1,F} = 19.2$, $^2J_{1,F} = 8.7$ Hz, C1), 80.6 (dd, $^2J_{3,F} = 18.2$, $^2J_{3,F} = 18.2$ Hz, C3), 75.2 (C5), 75.0 (d, $^4J_{CH2(OBn),F} = 3.4$ Hz, *C*H$_2$, OBn), 69.0 (d, $^3J_{4,F} = 8.2$ Hz, C4), 62.1 (C6), 58.1 (O*C*H$_3$) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −118.82 (dd, 1F, $^3J_{F'',3} = 5.1$, $^2J_{F'',F'} = 249.0$ Hz, F''), -138.37 (dddd, 1F, $^4J_{F',4} = 2.1$, $^3J_{F',1} = 14.6$, $^3J_{F',3} = 19.5$, $^2J_{F',F''} = 249.0$ Hz, F′) ppm; MS (ESI − MeOH) *m*/*z* (%): 305.2 $[M + H]^+$ (15), 322.2 $[M + NH_4]^+$ (75), 327.3 $[M + Na]^+$ (100), 630.8 $[M_2 + Na]^+$ (95); MS (ESI − MeOH + $LiClO_4$) *m*/*z* (%): 331.3 $[M + Li]^+$ (100), 342.7 $[M + Li, MeOH]^+$ (15), 614.9 $[M_2 + Li]^+$ (5). Anal. calcd. for $C_{14}H_{18}F_2O_5$ (304.29): C, 55.26; H, 5.96. Found: C, 55.14; H, 6.17.

*Methyl-2-deoxy-2,2-difluoro-β-D-arabino-hexopyranoside **3***

From **1**: A solution of **1** (550 mg, 1.40 mmol) in dry methanol (30 mL) containing palladium on charcoal (10%; 600 mg) was hydrogenated (50°C, 5.14 atm) for 24 h; additional palladium on charcoal (10%; 500 mg) was added and hydrogenation (50°C, 5.14 atm) continued for another 24 h. The solution was filtered through a pad of Celite. The filtrate was dried ($MgSO_4$), the solvent removed, and the residue was subjected to chromatography (silica gel, methanol/ethyl acetate, 10:90) to afford **3** (290 mg, 96.5%) as a white solid.

From **2**: A solution of **2** (670 mg, 2.20 mmol) in dry methanol (40 mL) containing palladium on charcoal (10%; 700 mg) was hydrogenated (50°C, 5.14 atm, 48 h). The solution was filtered through a pad of Celite. The filtrate was dried ($MgSO_4$), the solvent removed, and the residue was subjected to chromatography (silica gel, methanol/ethyl acetate, 10:90) to yield **3** (443 mg, 94.0%) as a white solid; m. p.: 190–191°C; $[\alpha]_D = -33.20^\circ$ ($c = 1.0$, MeOH); R_f (methanol/ethyl acetate, 10:90): 0.54; IR (KBr) ν: 3472*s*, 2949*s*, 1636*m*, 1455*m*, 1410*m*, 1386*m*, 1353*m*, 1327*m*, 1255*s*, 1225*s*, 1121*s*, 1072*s*, 1029*s*, 982*s*, 898*m*, 860*s*, 785*m*, 706*m*, 628*m*, 530*m*, 503*m*, 453*w* cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): $\delta = 4.54$ (*d*, 1 H, $^3J_{1,F'} = 15.2$ Hz, H-1), 3.89 (dd, 1H, $^3J_{6B,5} = 2.3$, $^2J_{6B,6A} = 12.1$ Hz, H-6$_B$), 3.68 (dd, 1H, $^3J_{6A,5} = 5.8$, $^2J_{6A,6B} = 12.1$ Hz, H-6$_A$), 3.65 (ddd, 1H, $^3J_{3,F''} = 3.8$, $^3J_{3,4} = 9.3$, $^3J_{3,F''} = 19.5$ Hz, H-3), 3.57 (s, 3H, O*CH*$_3$), 3.45 (ddd, 1H, $^3J_{4,F''} = 1.9$, $^3J_{4,5} = 9.5$, $^3J_{4,3} = 9.3$ Hz, H-4), 3.36 (ddd, 1H, $^3J_{5,6B} = 2.3$, $^3J_{5,4} = 9.5$, $^3J_{5,6A} = 5.8$ Hz, H-5) ppm; ^{13}C-NMR (125 MHz, CD_3OD) δ: 117.0 (dd, $^1J_{2,F} = 253.6$, $^1J_{2,F} = 253.6$ Hz, C2), 100.5 (dd, $^2J_{1,F} = 19.2$, $^2J_{1,F} = 27.1$ Hz, C1), 78.1 (C5), 75.3 (dd, $^2J_{3,F} = 18.9$, $^2J_{3,F} = 18.9$ Hz, C3), 70.4 (d, $^3J_{4,F} = 7.3$ Hz, C4), 62.4 (C6), 57.9 (O*C*H$_3$) ppm; ^{19}F-NMR (188 MHz, CD_3OD) δ: −124.21 (ddd, 1F, $^3J_{4,F''} = 1.9$, $^3J_{F'',3} = 3.8$, $^2J_{F'',F'} = 245.7$ Hz, F''), -143.19 (ddd, 1F, $^3J_{F',1} = 15.2$, $^3J_{F',3} = 19.5$, $^2J_{F'',F'} = 245.7$ Hz, F′) ppm; MS (ESI − MeOH) *m*/*z* (%): 237.3 $[M + Na]^+$ (100), 341.1 $[M_3 + K, H]^{2+}$ (82), 447.9 $[M_4 + K, H]^{2+}$ (28), 450.9 $[M_2 + Na]^+$ (52). Anal. calcd. for $C_7H_{12}F_2O_5$ (214.17): C, 39.26; H, 5.56. Found: C, 39.14; H, 5.79.

*Methyl-2-deoxy-2,2-difluoro-6-O-trityl-β-D-arabino-hexopyranoside **4***

To a solution of **3** (3.10 g, 14.48 mmol) and DMAP (1.00 g, 8.19 mmol) in dry pyridine (70 mL), trityl chloride (8.07 g, 28.95 mmol) was added in several portions and stirring was continued at 40°C for four days. The solvents were removed under reduced pressure and the residue was dissolved in dichloromethane (200 mL). The organic phase was washed with water (150 mL) and the aqueous phase was extracted with dichloromethane (3 × 100 mL). The combined organic phases were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product **4** was directly used in the next step; R_f (hexane/ethyl acetate, 50:50): 0.48.

*Methyl-2-deoxy-2,2-difluoro-3,4-di-O-pivaloyl-6-O-trityl-β-D-arabino-hexopyranoside **5***

To an ice-cold solution of **4** (4.77 g, 10.45 mmol) in pyridine (50 mL), pivaloyl chloride (3.21 mL, 26.13 mmol) was added dropwise, the solution was allowed to warm to 25°C and was stirred at this temperature for 24 h, followed by stirring at 50°C for another 24 h. The solvent was removed under reduced pressure and the remaining residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 85:15) to afford **5** (4.84 g, 74.1%) as a white solid; m. p.: 53–54°C; $[\alpha]_D = -1.53^\circ$ ($c = 0.46$, $CHCl_3$); R_f (hexane/ethyl acetate, 85:15): 0.47; IR (KBr) ν: 3455*m*, 3061*s*, 3021*s*, 2981*s*, 2943*s*, 2916*s*, 2873*s*, 1966*m*, 1753*s*, 1736*s*, 1596*m*, 1481*s*, 1449*s*, 1399*s*, 1369*s*, 1322*s*, 1281*s*, 1214*s*, 1144*s*, 1064*s*, 1035*s*, 1011*s*, 987*s*, 938*m*, 899*m*, 889*m*, 859*s*, 807*m*, 778*s*, 764*s*, 710*s*, 699*s*, 671*m*, 645*m*, 634*s*, 592*w*, 568*m*, 556*m*, 544*m*, 531*m*, 514*m*, 482*w*, 459*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.46–7.44 (m, 5H, Ph), 7.29–7.20 (m, 10 H, Ph), 5.29 (ddd, 1H, $^3J_{3,F''} = 5.1$, $^3J_{3,4} = 9.9$, $^3J_{3,F'} = 19.$ z, H-3), 5.17 (ddd, 1H, $^3J_{4,F'} = 1.0$, $^3J_{4,3} = 9.9$, $^3J_{4,5} = 10.0$ Hz, H-4), 4.60 (d, 1H, $^3J_{1,F} = 14.1$ Hz, H-1), 3.75 (ddd, 1H, $^3J_{5,6B} = 6.5$, $^3J_{5,4} = 10.0$,

$^3J_{5,6A}$ = 1.8 Hz, H-5), 3.74 (s, 3H, OCH_3), 3.24 (dd, 1H, $^3J_{6B,5}$ = 6.5, $^2J_{6B,6A}$ = 10.4 Hz, H-6_B), 3.10 (dd, 1H, $^3J_{6A,5}$ = 1.8, $^2J_{6A,6B}$ = 10.4 Hz, H-6_A), 1.18 (s, 9H, CH_3, OPiv), 0.86 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 176.9 (*C*=O, OPiv), 176.7 (*C*=O, OPiv), 143.4 ($C_{i\text{-}ar}$, trityl), 128.6 (C_{ar}), 127.8 (C_{ar}), 127.0 (C_{ar}), 114.3 (dd, $^1J_{2,F}$ = 257.1, $^1J_{2,F}$ = 257.1 Hz, C2), 99.2 (dd, $^2J_{1,F}$ = 18.4, $^2J_{1,F}$ = 26.5 Hz, C1), 86.7 (CPh_3 (trityl)), 74.2 (C5), 70.9 (dd, $^2J_{3,F}$ = 21.5, $^2J_{3,F}$ = 18.0 Hz, C3), 67.3 (d, $^4J_{4,F}$ = 6.5 Hz, C4), 62.2 (C6), 57.5 (OCH_3), 39.1 ($C(CH_3)_3$, OPiv), 38.5 ($C(CH_3)_3$, OPiv), 27.1 (CH_3, OPiv), 26.9 (CH_3, OPiv) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −122.29 (dd, 1F, $^3J_{F'',3}$ = 5.1, $^2J_{F'',F'}$ = 247.4 Hz, F''), −137.75 (dddd, 1F, $^4J_{F',4}$ = 1.0, $^3J_{F',1}$ = 14.1, $^3J_{F',3}$ = 19.7, $^2J_{F'',F'}$ = 247.4 Hz, F') ppm; MS (ESI − MeOH) *m/z* (%): 243.3 (trityl, 30), 642.0 $[M + NH_4]^+$ (23), 647.3 $[M + Na]^+$ (100), 1270.5 $[M_2 + Na]^+$ (16). Anal. calcd. for $C_{36}H_{42}F_2O_7$ (624.73): C, 69.21; H, 6.78. Found: C, 68.98; H, 6.91.

Methyl-2-deoxy-2,2-difluoro-3,4-di-O-pivaloyl-β-D-arabino-hexopyranoside ***6***

To an ice-cold solution of **5** (1.00 g, 1.60 mmol) in anhydrous dichloromethane (20 mL), trimethylsilyl iodide (858 μL, 6.00 mmol) was added dropwise and stirring was continued at this temperature for 30 min followed by stirring at 25°C for 12 h. Water (10 mL) was added and the solution was stirred at 25°C for 10 min. Dichloromethane (80 mL) was added and the solution was washed with aqueous sodium thiosulfate (10%, 50 mL). The aq. phase was extracted with dichloromethane (3 × 100 mL) and the combined organic layers were dried ($MgSO_4$). The solvent was removed under diminished pressure and the remaining residue subjected to chromatography (silica gel, hexane/ethyl acetate, 50:50) to afford **6** (610 mg, 99.7%) as a white solid; m. p.: 160–162°C; $[\alpha]_D$ = −5.54° (*c* = 0.46, $CHCl_3$); R_f (hexane/ethyl acetate, 50:50): 0.65; IR (KBr) ν: 3386*s*, 2978*s*, 2876*m*, 1747*s*, 1725*s*, 1482*m*, 1463*m*, 1399*m*, 1372*m*, 1279*m*, 1228*m*, 1147*s*, 1103*s*, 1087*s*, 1045*s*, 1001*m*, 941*w*, 911*m*, 894*w*, 860*m*, 810*w*, 766*w*, 750*w*, 663*w*, 588*w*, 542*m*, 450*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.40 (ddd, 1H, $^3J_{3,F''}$ = 5.0, $^3J_{3,4}$ = 10.0, $^3J_{3,F'}$ = 19.6 Hz, H-3), 5.17 (ddd, 1H, $^3J_{4,F'}$ = 1.1, $^3J_{4,3}$ = 10.0, $^3J_{4,5}$ = 9.8 Hz, H-4), 4.57 (d, 1H, $^3J_{1,F'}$ = 14.1 Hz, H-1), 3.64 (s, 3H, OCH_3), 3.75 (m, 1H, H-6_B), 3.62-3.56 (m, 2H, H-5, H-6_A), 1.21 (s, 9H, CH_3, OPiv), 1.16 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 177.3 (*C*=O, OPiv), 177.1 (*C*=O, OPiv), 114.1 (dd, $^1J_{2,F}$ = 257.6, $^1J_{2,F}$ = 254.7 Hz, C2), 99.2 (dd, $^2J_{1,F}$ = 19.2, $^2J_{1,F}$ = 26.9 Hz, C1), 74.4 (C5), 70.4 (dd, $^2J_{3,F}$ = 18.2, $^2J_{3,F}$ = 21.5 Hz, C3), 67.1 (C4), 61.1 (C6), 58.1 (OCH_3), 39.0 ($C(CH_3)_3$, OPiv), 38.8 ($C(CH_3)_3$, OPiv), 27.0 (CH_3, OPiv), 26.9 (CH_3, OPiv) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −122.28 (dd, 1F, $^3J_{F'',3}$ = 5.0, $^2J_{F'',F'}$ = 248.5 Hz, F''), -137.81 (dddd, 1F, $^4J_{F',4}$ = 1.1, $^3J_{F',1}$ = 14.1, $^3J_{F',3}$ = 19.6, $^2J_{F'',F'}$ = 248.5 Hz, F') ppm; MS (ESI − MeOH) *m/z* (%): 383.3 $[M + H]^+$ (9), 400.3 $[M + NH_4]^+$ (52), 405.3 $[M + Na]^+$ (27), 593.0 $[M_3 + K, H]^{2+}$ (8), 786.9 $[M_2 + Na]^+$ (100). Anal. calcd. for $C_{17}H_{28}F_2O_7$ (382.41): C, 53.40; H, 7.38. Found: C, 53.29; H, 7.52.

Methyl-6-bromo-2,6-dideoxy-2,2-difluoro-3,4-di-O-pivaloyl-β-D-arabino-hexopyranoside ***7***

To an ice-cold solution of **6** (610 mg, 1.60 mmol) in pyridine (10 mL), triphenylphosphane (837 mg, 3.19 mmol) and CBr_4 (794 mg, 2.39 mmol) were slowly added and the solution was stirred at 0°C for 10 min followed by stirring at 65°C for another 4 h. The solution was cooled to 25°C, methanol (10 mL) was added and the solvents were removed under reduced pressure. The remaining residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 85:15) to afford **7** (698 mg, 98.2%) as a white solid; m. p.: 160–163°C; $[\alpha]_D$ = −1.89° (*c* = 0.32, $CHCl_3$); R_f (hexane/ethyl acetate, 85:15): 0.39; IR (KBr) ν: 3449*m*, 2972*m*, 2946*m*, 1746*s*, 1733*s*, 1630*w*, 1482*m*, 1461*m*, 1400*m*, 1372*m*, 1281*m*, 1232*m*, 1148*s*, 1068*s*, 1033*m*, 1007*m*, 981*w*, 962*w*, 901*w*, 860*m*, 798*w*, 770*w*, 686*w*, 652*w*, 562*w*, 536*w*, 502*w*, 473*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.34 (ddd, 1H, $^3J_{3,F''}$ = 5.1, $^3J_{3,4}$ = 9.6, $^3J_{3,F'}$ = 19.1 Hz, H-3), 5.13 (ddd, 1H, $^3J_{4,F'}$ = 1.1, $^3J_{4,3}$ = 9.6, $^3J_{4,5}$ = 9.6 Hz, H-4), 4.57 (d, 1H, $^3J_{1,F}$ = 13.7 Hz, H-1), 3.81 (dt, 1H, $^3J_{5,(6B,6A)}$ = 5.2, $^3J_{5,4}$ = 9.6 Hz, H-5), 3.67 (s, 3H, OCH_3), 3.40 (d, 2H, $^3J_{(6B,6A),5}$ = 5.2 Hz, H-6_B, H-6_A), 1.20 (s, 9H, CH_3, OPiv), 1.17 (*s*, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 176.9 (*C*=O, OPiv), 176.4 (*C*=O, OPiv), 113.9 (dd, $^1J_{2,F}$ = 255.0, $^1J_{2,F}$ = 257.7 Hz, C2), 99.0 (dd, $^2J_{1,F}$ = 19.4, $^2J_{1,F}$ = 27.4 Hz, C1), 74.0 (C5), 70.2 (dd, $^2J_{3,F}$ = 18.5, $^2J_{3,F}$ = 22.1 Hz, C3), 69.4 (d, $^3J_{4,F}$ = 6.8 Hz, C4), 57.96 (OCH_3), 39.0 ($C(CH_3)_3$, OPiv), 38.8 ($C(CH_3)_3$, OPiv), 29.9 (C6), 27.0 (CH_3, OPiv), 26.9 (CH_3, OPiv) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −122.80 (dd, 1F, $^3J_{F'',3}$ = 5.1, $^2J_{F'',F'}$ = 249.0 Hz, F''), −137.3 (dddd, 1F, $^4J_{F',4}$ = 1.1, $^3J_{F',1}$ = 13.7, $^3J_{F',3}$ = 19.1, $^2J_{F'',F'}$ = 249.0 Hz, F') ppm; MS (ESI − MeOH) *m/z* (%): 445.1 and 447.1 $[M + H]^+$ (10 each), 462.2 and 464.2 $[M + NH_4]^+$ (50 each), 467.2 and 469.1 $[M + Na]^+$ (60 each), 685.9 $[M_{A3} + K, H]^{2+}$ (6), 910.6 $[M_2 + Na]^+$ (58), 912.5 $[M_A + M_B + Na]^+$ (100), 914.5 $[M_2 + Na]^+$ (54). Anal. calcd. for $C_{17}H_{27}BrF_2O_6$ (445.30): C, 45.85; H, 6.11. Found: C, 45.68; H, 6.17.

Methyl-6-bromo-2,6-dideoxy-2,2-difluoro-3,4-di-O-pivaloyl-D-arabino-hex-5-ulosonate ***8***

A solution of acetic acid (19.0 mL) and acetic anhydride (2.0 mL) under argon was stirred at 50°C for 2 h and cooled to 25°C. To a solution of **7** (698 mg, 1.57 mmol) the above described solution was added at 25°C and the mixture was stirred at this temperature for 10 min. Then, chromium(VI) oxide (1.09 g, 10.91 mmol) was added and the suspension was stirred at 25°C for two hours. The solution was filtered through a thin layer of silica gel and diluted with ethyl acetate (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), the solvent was removed under reduced pressure and the remaining residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 85:15) to afford **8** (490 mg, 68.1%) as a colorless oil; $[\alpha]_D$ = +18.93° (*c* = 0.48, $CHCl_3$); R_f (hexane/ethyl acetate, 85:15): 0.44; IR (KBr) ν: 2977*m*, 2876*m*, 1749*s*, 1482*m*, 1462*m*, 1399*m*, 1368*m*, 1275*m*, 1230*m*, 1121*s*, 1038*m*, 941*m*, 805*m*, 765*m*, 522*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.87 (d, 1H, $^3J_{4,3}$ = 1.6 Hz, H-4), 5.75 (ddd, 1H, $^3J_{3,F''}$ = 6.8, $^3J_{3,4}$ = 1.6, $^3J_{3,F'}$ = 16.7 Hz, H-3), 4.08 (d, 1H, $^2J_{6B,6A}$ = 13.4 Hz, H-6_B), 4.02 (d, 1H, $^2J_{6A,6B}$ = 13.4 Hz, H-6_A), 3.88 (s, 3H, $COOCH_3$), 1.26 (s, 9H, CH_3, OPiv), 1.18 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 194.7 (*C*=O (C5)), 176.7 (*C*=O, OPiv), 176.5 (*C*=O, OPiv), 162.2 (dd, $^2J_{C,F}$ = 30.2, $^2J_{C,F}$ = 32.7 Hz, *C*=O ($COOCH_3$)), 112.0 (dd, $^1J_{2,F}$ = 255.3, $^1J_{2,F}$ = 262.0 Hz, C2), 71.1 (C4), 69.1 (dd, $^2J_{3,F}$ = 23.0, $^2J_{3,F}$ = 30.8 Hz, C3), 53.9 ($COOCH_3$), 38.9 ($C(CH_3)_3$, OPiv), 38.8 ($C(CH_3)_3$, OPiv), 30.8 (C6), 26.8 (CH_3, OPiv), 26.8 (CH_3, OPiv) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −111.16 (dd, 1F, $^3J_{F'',3}$ = 6.8, $^2J_{F'',F'}$ = 270.4 Hz, F''), −118.04 (dd, 1F, $^3J_{F',3}$ = 16.7, $^2J_{F'',F'}$ = 270.4 Hz, F') ppm; MS (ESI − MeOH) *m/z* (%): 459.1 and 461.1 $[M + H]^+$ (20 each), 476.2 and 478.1 $[M + NH_4]^+$ (40 each), 481.2 and 483.1 $[M + Na]^+$ (90 each), 508.0 and 510.0 $[M + NH_4, MeOH]^+$ (40 each), 513.1 and 515.1

$[M + Na, MeOH]^+$ (89 each), 708.9 $[M_{A2} + M_B + K, H]^{2+}$ (15), 938.5 $[M_{A2} + Na]^+$ (55), 940.5 $[M_A + M_B + Na]^+$ (100), 942.5 $[M_{B2} + Na]^+$ (55). Anal. calcd. for $C_{17}H_{25}BrF_2O_7$ (459.29): C, 44.46; H, 5.49. Found: C, 44.31, H, 5.64.

(8S, 9R)-7,7-Difluoro-6-oxo-3,4,6,7,8,9-hexahydropyrido[2.1-c][1,4]thiazine-8,9-diylbis(2,2-dimethylpropanoate) ***9****, (8S, 9S, 9aR,S)-7,7-difluoro-9a-hydroxy-6-oxo-octahydropyrido[2.1-c][1,4]thiazine-8,9-diyl-bis(2,2-dimethylpropanoate)* ***10****, and (8S)-7,7-difluoro-6-oxo-1,3,4,6,7,8-hexahydropyrido[2.1-c][1,4]thiazine-8,9-diylbis(2,2-dimethylpropanoate)* ***11***

To a solution of **8** (1.55 g, 3.38 mmol) in absolute methanol (60 mL), 2-aminoethanethiol (318 mg, 4.12 mmol) was added and the solution was stirred at 25°C for 24 h. The solvent was removed under reduced pressure and the remaining residue subjected to chromatography (silica gel, hexane/ethyl acetate, 5:3) to afford **9** (590 mg, 41.3%), **10** (340 mg, 23.8%), and **11** (260 mg, 18.2%).

Data for compound ***9***

White solid; m. p.: 167–168°C; $[\alpha]_D = -14.36^\circ$ ($c = 0.36$, $CHCl_3$); R_f (hexane/ethyl acetate, 5:3): 0.78; IR (KBr) ν: 3467*s*, 2973*s*, 2934*s*, 2876*s*, 1752*s*, 1706*s*, 1630*m*, 1483*s*, 1458*m*, 1408*s*, 1372*m*, 1316*m*, 1279*s*, 1225*s*, 1196*s*, 1147*s*, 1073*s*, 1040*s*, 1013*m*, 991*m*, 932*m*, 903*m*, 892*m*, 878*m*, 859*m*, 838*m*, 821*m*, 795*m*, 770*m*, 759*m*, 747*m*, 706*w*, 678*w*, 577*w*, 545*m*, 532*m*, 501*m*, 479*m*, 441*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.79 (s, 1H, H-6), 5.56 (dd, 1H, $^3J_{4,3} = 5.6$, $^3J_{4,F}$' = 5.2 Hz, H-4), 5.42 (ddd, 1H, $^3J_{3,F''} = 8.7$, $^3J_{3,4} = 5.6$, $^3J_{3,F}$' = 6.8 Hz, H-3), 4.63 (ddd, 1H, $^3J_{1'',2}$' = 3.5, $^3J_{1'',2''} = 5.0$, $^2J_{1'',1}$' = 13.5 Hz, H-1''), 3.67 (m, 1H, H-1'), 3.05–3.02 (m, 2H, H-2'', H-2'), 1.21 (s, 9H, CH_3, OPiv), 1.20 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 176.5 (*C*=O, OPiv), 175.7 (*C*=O, OPiv), 157.7 (dd, $^2J_{C,F} = 4.7$, $^2J_{C,F} = 4.3$ Hz, *C*=O (*C*ONR)), 124.9 (C5), 108.8 (C6), 108.4 (dd, $^1J_{2,F} = 249.3$, $^1J_{2,F} = 252.9$ Hz, C2), 68.1 (C4), 67.7 (dd, $^2J_{3,F} = 19.5$, $^2J_{3,F} = 29.5$ Hz, C3), 39.7 (C1'), 39.1 (*C*(CH_3)$_3$, OPiv), 39.1 (*C*(CH_3)$_3$, OPiv), 27.1 (*C*H_3, OPiv), 27.1 (*C*H_3, OPiv), 27.0 (*C*H_3, OPiv), 27.0 (*C*H_3, OPiv), 26.9 (*C*H_3, OPiv), 25.3 (C2') ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −106.94 (dd, 1F, $^3J_{F'',3} = 8.7$, $^2J_{F'',F}$' = 299.1 Hz, F''), −114.79 (ddd, 1F, $^4J_{F}$',$_4 = 5.2$, $^3J_{F}$',$_3 = 6.8$, $^2J_{F'',F}$' = 299.1 Hz, F') ppm; MS (ESI − MeOH) *m*/*z* (%): 423.1 $[M + NH_4]^+$ (8), 428.1 $[M + Na]^+$ (100), 832.8 $[M_2 + Na]^+$ (12). Anal. calcd. for $C_{18}H_{25}F_2O_5NS$ (405.46): C, 53.32; H, 6.22; N, 3.46; S, 7.91. Found: C, 53.08; H, 6.32; N, 3.39; S, 7.79.

Data for compound ***10***

White solid; m. p.: 132–133°C; $[\alpha]_D = -38.49^\circ$ ($c = 0.49$, $CHCl_3$); R_f (hexane/ethyl acetate, 5:3): 0.55; IR (KBr) ν: 3424*m*, 2974*m*, 2937*m*, 2876*m*, 1751*s*, 1684*s*, 1483*m*, 1462*m*, 1433*m*, 1399*m*, 1372*m*, 1278*m*, 1233*m*, 1148*s*, 1122*s*, 1064*m*, 1043*m*, 1007*m*, 980*m*, 944*w*, 1064*m*, 1043*m*, 1007*m*, 980*m*, 944*w*, 894*m*, 879*w*, 864*w*, 836*w*, 822*w*, 796*w*, 758*w*, 540*m*, 520*w*, 464*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.86 (ddd, 1H, $^3J_{3,F''} = 17.2$, $^3J_{3,4} = 11.0$, $^3J_{3,F'} = 6.3$ Hz, H-3a), 5.71 (d, 1 H, $^3J_{4,3} = 11.3$ Hz, H-4b), 5.50 (ddd, 1H, $^3J_{3,F''} = 15.4$, $^3J_{3,4} = 11.3$, $^3J_{3,F'} = 6.2$ Hz, H-3b), 5.45 (d, 1H, $^3J_{4,3} = 11.0$ Hz, H-4a), 4.72 (ddd, 1H, $^3J_{1b'',2b'} = 2.6$, $^3J_{1b'',2b''} = 5.4$, $^2J_{1b'',1b'} = 14.4$ Hz, H-1b''), 4.58 (ddd, 1H, $^3J_{1a'',2a'} = 3.0$, $^3J_{1a'',2a''} = 3.0$, $^2J_{1a'',1a'} = 14.4$ Hz, H-1a''), 3.48 (ddd, 1H, $^3J_{1b',2b'} = 1.9$, $^3J_{1b',2b''} = 4.3$, $^2J_{1b',1b''} = 14.4$ Hz, H-1b'), 3.27 (ddd, 1H, $^3J_{1a',2a'} = 1.6$, $^3J_{1a',2a''} = 3.1$, $^2J_{1a',1a''} = 14.3$ Hz, H-1a'), 3.11 (d, 1H, $^2J_{6B,6A} = 13.7$ Hz, H-6b''), 2.92 (d, 1H, $^2J_{6B,6A} = 14.0$ Hz, H-6a''), 2.82 (ddd, 1H, $^3J_{2b'',1b'} = 4.3$, $^3J_{2b'',1b''} = 2.6$, $^2J_{2b'',2b'} = 13.7$ Hz, H-2b''), 2.78 (ddd, 1H, $^3J_{2b',1b'} = 5.4$, $^3J_{2b',1b''} = 1.9$, $^2J_{2b',2b''} = 13.7$ Hz, ethylene-H-2b'), 2.75 (ddd, 1H, $^3J_{2a'',1a'} = 3.1$, $^3J_{2a'',1a''} = 3.0$, $^2J_{2a'',2a'} = 13.8$ Hz, ethylene-H-2a''), 2.65 (ddd, 1H, $^3J_{2a',1a'} = 1.6$, $^3J_{2a',1a''} = 3.0$, $^2J_{2a',2a''} = 13.8$ Hz, H-2a'), 2.57 (dd, 1H, $^3J_{6B,H} = 2.1$, $^2J_{6B,6A} = 14.0$ Hz, H-6a''), 2.45 (dd, 1H, $^3J_{6B,H} = 1.9$, $^2J_{6B,6A} = 13.7$ Hz, H-6b''), 1.22 (s, 9H, CH_3, OPiv), 1.21 (s, 9H, CH_3, OPiv), 1.21 (s, 9H, CH_3, OPiv), 1.20 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 176.9 (*C*=O, OPiv), 176.7 (*C*=O, OPiv), 176.5 (*C*=O, OPiv), 175.9 (*C*=O, OPiv), 161.5 (dd, $^2J_{C,F} = 28.7$, $^2J_{C,F} = 26.3$ Hz, *C*=O (*C*ONR)b), 109.7 (dd, $^1J_{2,F} = 246.6$, $^1J_{2,F} = 252.3$ Hz, C2a), 109.6 (dd, $^1J_{2,F} = 246.6$, $^1J_{2,F} = 252.3$ Hz, C2b), 82.2 (C5b), 80.9 (C5a), 68.6 (d, $^2J_{4,F} = 9.6$ Hz, C4a), 68.3 (d, $^2J_{4,F} = 9.5$ Hz, C4b), 66.4 (dd, $^2J_{3,F} = 16.8$, $^2J_{3,F} = 23.5$ Hz, C3a), 66.1 (dd, $^2J_{3,F} = 16.8$, $^2J_{3,F} = 23.5$ Hz, C3b), 40.0 (C1'a), 39.5 (C1'b), 39.1 (*C*(CH_3)$_3$, OPiv), 39.1 (*C*(CH_3)$_3$, OPiv), 39.0 (*C*(CH_3)$_3$, OPiv), 39.0 (*C*(CH_3)$_3$, OPiv), 37.3 (C6a), 36.0 (C6b), 27.1 (*C*H_3, OPiv), 27.0 (*C*H_3, OPiv), 26.9 (*C*H_3, OPiv), 26.9 (*C*H_3, OPiv), 26.6 (C2'), 26.3 (C2') ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −111.54 (dd, 1F, $^3J_{F'',3} = 17.2$, $^2J_{F'',F'} = 281.8$ Hz, Fa''), −111.12 (dd, 1F, $^3J_{F'',3} = 15.4$, $^2J_{F'',F'} = 299.1$ Hz, Fb''), −114.79 (dd, 1F, $^3J_{F',3} = 6.3$, $^2J_{F'',F'} = 281.8$ Hz, Fa'), −113.99 (dd, 1F, $^3J_{F',3} = 6.2$, $^2J_{F'',F'} = 299.1$ Hz, Fb') ppm; MS (ESI − MeOH) *m*/*z* (%): 424.0 $[M + H]^+$ (10), 441.3 $[M + NH_4]^+$ (12), 446.2 $[M + Na]^+$ (36), 654.6 $[M_3 + K, H]^{2+}$ (4), 868.9 $[M_2 + Na]^+$ (100). Anal. calcd. for $C_{18}H_{27}F_2O_6NS$ (423.48): C, 51.05; H, 6.43; N, 3.31; S, 7.57. Found: C, 50.93; H, 6.52; N, 3.25; S, 7.59.

Data for compound ***11***

White solid; m. p.: 257–258°C; $[\alpha]_D = -56.95^\circ$ ($c = 0.43$ g, $CHCl_3$); R_f (hexane/ethyl acetate, 5:3): 0.20; IR (KBr) ν: 3429*m*, 2978*m*, 2934*m*, 2874*m*, 1744*s*, 1652*m*, 1561*s*, 1525*m*, 1480*m*, 1448*m*, 1420*m*, 1400*m*, 1336*m*, 1303*m*, 1276*m*, 1229*m*, 1211*m*, 1151*s*, 1057*m*, 1036*m*, 971*m*, 909*m*, 852*m*, 820*w*, 754*m*, 541*m*, 449*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.72 (dd, 1H, $J_{6B,H} = 0.9$, $^2J_{6B,6A} = 6.6$ Hz, H-6$_B$), 5.60 (m, 1H, H-6$_A$), 5.55 (ddd, 1H, $^3J_{3,F''} = 5.2$, $^3J_{3,4} = 6.5$, $^3J_{3,F'} = 13.8$ Hz, H-3), 3.72–3.69 (m, 2H, H-1$_B$, H-1'), 2.90–2.88 (m, 2H, H-2'', H-2'), 1.22 (s, 9H, CH_3, OPiv), 1.19 (s, 9H, CH_3, OPiv) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 178.5 (*C*=O, OPiv), 176.3 (*C*=O, OPiv), 176.0 (dd, $^2J_{C,F} = 19.0$, $^2J_{C,F} = 19.0$ Hz, *C*ONR), 149.3 (C5), 109.6 (dd, $^1J_{2,F} = 251.4$, $^1J_{2,F} = 251.8$ Hz, C2), 100.9 (C4), 69.6 (dd, $^2J_{3,F} = 21.1$, $^2J_{3,F} = 26.9$ Hz, C3), 68.4 (C6), 43.7 (C1'), 39.3 (*C*(CH_3)$_3$, OPiv), 39.1 (*C*(CH_3)$_3$, OPiv), 27.1 (*C*H_3, OPiv), 27.0 (*C*H_3, OPiv), 23.0 (C2') ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −115.18 (dd, 1F, $^3J_{F'',3} = 5.2$, $^2J_{F'',F'} = 280.0$ Hz, F''), −117.95 (dd, 1F, $^3J_{F',3} = 13.8$, $^2J_{F'',F'} = 280.0$ Hz, F') ppm; MS (ESI − MeOH) *m*/*z* (%): 406.2 $[M + H]^+$ (35), 428.2 $[M + Na]^+$ (50), 627.6 $[M_3 + K, H]^{2+}$ (3), 832.9 $[M_2 + Na]^+$ (100); MS (ESI − MeOH) *m*/*z* (%): 404.6 $[M - H]^-$ (100). Anal. calcd. for $C_{18}H_{25}F_2O_5NS$ (405.46): C, 53.32; H, 6.22; N, 3.46; S, 7.91. Found: C, 53.21; H, 6.41; N, 3.28; S, 7.78.

(8S, 9R)-7,7-Difluoro-8,9-dihydroxy-3,4,8,9-tetrahydropyrido[2.1-c][1,4]thiazine-6(7H)-one ***12***

To a solution of **9** (200 mg, 0.49 mmol) in absolute methanol (20 mL), sodium methoxide (30 mg, 0.56 mmol) was added in small portions and the solution was stirred for 4 h at 25°C. The solvent was evaporated under reduced pressure and the remaining residue was subjected to chromatography (silica gel,

methanol/ethyl acetate, 20:80) to afford **12** (110 mg, 94.0%) as a white solid; m. p.: >250°C (decomposition); $[\alpha]_D = -75.00^\circ$ ($c = 0.18$, MeOH); R_f (hexane/ethyl acetate, 50:50): 0.33; IR (Film) ν: 3387*m*, 3314*m*, 3080*w*, 1664*s*, 1614*m*, 1425*m*, 1404*m*, 1369*m*, 1348*m*, 1277*w*, 1256*w*, 1209*w*, 1192*w*, 1157*w*, 1142*w*, 1132*w*, 1090*w*, 1065*m*, 1051*m*, 1020*m*, 999*m*, 906*w*, 879*m*, 839*m*, 825*w*, 812*m*, 793*w*, 748*w* cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ: 5.90 (s, 1H, H-6), 4.27 (ddd, 1H, $^3J_{4,F''} = 0.9$, $^3J_{4,3} = 3.8$, $^3J_{4,F'} = 8.0$ Hz, H-4), 4.22 (ddd, 1H, $^3J_{1'',2'} = 3.8$, $^3J_{1'',2''} = 5.9$, $^2J_{1'',1'} = 13.5$ Hz, H-1″), 3.94–3.88 (m, 2H, H-3, H-1′), 3.03 (m, 2H, H-2″, H-2′) ppm; ^{13}C-NMR (125 MHz, CD_3OD) δ: 159.4 (dd, $^2J_{C,F} = 29.8$, $^2J_{C,F} = 29.7$ Hz, *C*ONR), 129.3 (C5), 111.0 (dd, $^1J_{2,F} = 245.7$, $^1J_{2,F} = 249.5$ Hz, C2), 103.9 (C6), 70.9 (dd, $^2J_{4,F} = 19.7$, $^2J_{4,F} = 23.0$ Hz, C3), 69.5 (dd, $^2J_{3,F} = 2.9$, $^2J_{3,F} = 5.8$ Hz, C4), 39.7 (C1′), 24.2 (C2′) ppm; ^{19}F-NMR (188 MHz, $CDCl_3$) δ: −110.68 (ddd, 1F, $^3J_{F'',4} = 0.9$, $^3J_{F'',3} = 9.1$, $^2J_{F'',F'} = 290.6$ Hz, F″), −116.05 (ddd, 1F, $^4J_{F',4} = 8.0$, $^3J_{F',3} = 11.7$, $^2J_{F'',F'} = 290.6$ Hz, F′) ppm; MS (ESI − MeOH) *m/z* (%): 238.4 $[M + H]^+$ (8), 255.2 $[M + NH_4]^+$ (49), 260.2 $[M + Na]^+$ (92), 291.8 $[M + Na, MeOH]^+$ (100), 493.9 $[M_4 + K, H]^+$ (10), 496.8 $[M_2 + Na]^+$ (18). Anal. calcd. for $C_8H_9F_2SNO_3$ (237.22): C, 40.51; H, 3.82; N, 5.90; S, 13.52. Found: C, 40.39; H, 3.95; N, 5.85; S, 13.67.

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