SUPPORTING INFORMATION

Highly Stereoselective Synthesis of Lamivudine (3TC) and Emtricitabine (FTC) by a Novel N-Glycosidation Procedure

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EXPERIMENTAL PROCEDURES

General methods and materials. All chemicals and solvents were purchased with the highest degree of purity (Sigma-Aldrich, Alfa Aesar, VWR) and used without further purification. All moisture-sensitive reactions were performed under nitrogen atmosphere using oven-dried glassware. Reactions were monitored by TLC (precoated silica gel plate F254, Merck) and the products were detected by exposure to ultraviolet radiation, iodine vapor and chromic mixture. Column chromatography: Merck Kieselgel 60 (70-230 mesh); flash chromatography: Merck Kieselgel 60 (230-400 mesh). Monodimensional (1H, 13C, 19F) and bidimensional (NOESY) NMR spectra were recorded on NMR spectrometers operating at 400 MHz (Bruker DRX, Bruker AVANCE), 500 MHz (Varian Inova equipped with a VnmrJ 4.0 software) and 600 MHz (Bruker DRX equipped with a CryoProbe), using CDCl₃ solutions unless otherwise specified. The determination of β/α ratios in the N-glycosidation reactions was performed by integration of 1H NMR anomeric signals (H-5) of each pair of diastereomers at the given spectrometer (detection limit: down to 10⁻⁶ g). Combustion analyses were performed using a CHNS analyzer. Melting points are uncorrected and were determined with a capillary apparatus. Optical rotations were measured at 25 ± 2 °C in the stated solvent.

(1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5R)-5-(methylcarbonyloxy)-1,3-oxathiolane-2-carboxylate [(2R,5R)-5b]. L-Menthol glyoxylate monohydrate (10) (2.5 g, 10.86 mmol), toluene (12.5 mL) and acetic acid (0.25 mL) were mixed under stirring and the resulting mixture was heated to 120 °C, removing water azeotropically with a Dean-Stark trap. The resulting solution was concentrated under reduced pressure, to collect 5 mL of distillate, and cooled to rt; then 1,4-dithiane-2,5-diol (826 mg, 5.43 mmol) was added. The reaction mixture was refluxed for 4 h; then it was cooled to 80 °C and clarified. The filtrate was cooled to 0 °C, and a solution of triethylamine (150 μL) in n-hexane (15 mL) was added dropwise. The mixture was stirred at 0 °C for 16 h, observing the formation of a precipitate. The isolated solid was filtered, washed with a mixture of toluene and n-hexane (1:3 v/v) and dried to give the hemiacetal 5a (1.86 g, 60% o.y.) as a mixture of two stereoisomers. Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl 5-hydroxy-1,3-
oxathiolane-2-carboxylate (5a). $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 0.69 (d, $J = 6.9, 1.5$H), 0.70 (d, $J = 6.9, 1.5$H), 0.76-0.90 (m, 7H), 0.90-1.09 (m, 2H), 1.31-1.52 (m, 2H), 1.57-1.65 (m, 2H), 1.77-1.94 (m, 2H), 2.85 (bd, $J = 10.6, 1$H), 3.11 (dd, $J = 4.5, 10.0, 0.5$H), 3.12 (dd, $J = 4.7, 10.0, 0.5$H), 4.59 (dt, $J = 4.2, 11.1, 0.5$H), 4.62 (dt, $J = 4.2, 11.1, 0.5$H), 5.54 (s, 0.5H), 5.55 (s, 0.5H), 5.82-5.87 (m, 1H), 7.02 (d, $J = 8.4, 0.5$H), 7.03 (d, $J = 8.4, 0.5$H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): ppm 16.5, 16.6, 20.8, 20.9, 22.2, 23.2, 23.3, 26.0, 26.1, 31.1, 31.2, 33.4, 38.0, 38.1, 40.6, 46.6, 46.7, 74.9, 75.0, 76.4, 76.6, 101.2, 101.5, 169.4, 169.5. A solution of 5a (1.86 g, 6.52 mmol), acetic anhydride (3.75 mL, 39.75 mmol) and dichloromethane (10 mL) were mixed under nitrogen atmosphere. The solution was cooled to 0 °C and pyridine (0.95 mL, 18.20 mmol) was added dropwise under stirring. The reaction mixture was warmed to room temperature and stirred for 4 h; after completion (TLC) it was quenched by addition of water at 0 °C. The organic layer was washed with diluted HCl, dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/ethyl acetate 95:5) gave 5b (2.34 g, 91% yield) as a mixture of four diastereoisomers. A sample of 1.2 g was dissolved in 40 mL of hexane with 200 µL of TEA, and after 72 h at -20 °C the only desired trans-(2R,5R) stereoisomer was precipitated (0.50 g, 42% of the acetylated product). Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5R)-5-(methylcarbonyloxy)-1,3-oxathiolane-2-carboxylate [(2R,5R)-5b]. White crystals, m.p. 99-103 °C (hexane). [α]$_D^{25}$ –56.0 (c 3.1, CHCl$_3$). $^1$H NMR (400 MHz): $\delta$ 0.74 (d, $J = 7.0, 3$H), 0.81-0.92 (m, 7H), 0.92-1.10 (m, 2H), 1.39 (bt, $J = 11.3, 1$H), 1.44-1.55 (m, 1H), 1.67 (bd, $J = 11.4, 2$H), 1.87-1.95 (m, 1H), 1.99 (bd, $J = 11.7, 1$H), 2.09 (s, 3H), 3.15 (d, $J = 11.7, 1$H), 3.43 (dd, $J = 4.2, 11.7, 1$H), 4.71 (td, $J = 4.4, 10.9, 1$H), 5.60 (s, 1H), 6.77 (d, $J = 4.2$ Hz, 1H). $^{13}$C NMR (100 MHz): $\delta$ 16.0, 20.6, 21.0, 21.9, 23.2, 26.0, 31.3, 34.0, 37.1, 40.5, 47.0, 76.0, 79.8, 99.7, 168.5, 169.6. Anal. calcd for C$_{16}$H$_{26}$O$_5$S: C 58.16, H 7.93, S, 9.70. Found: C 58.01, H 7.96, S, 9.74.

5-Fluoro-2-oxo-4-(phenylcarboxamido)-1,2-dihydropyrimidine (12b). 5-Fluorocytosine (12a, 0.2 g, 1.5 mmol) was suspended in pyridine (10 mL) and the resulting mixture was cooled to 0 °C. Benzoyl chloride (1.04 mL, 9 mmol) was added dropwise and the suspension was stirred at rt for 16 h. The reaction mixture was cooled in an ice bath, treated with NH$_4$OH (1 mL) for 90 min and concentrated under reduced pressure. Chromatography of the crude residue over silica gel (dichloromethane:MeOH 85:15) provided the pure nucleobase 12b (0.36 g, 99%). $^1$H NMR
(DMSO-$d_6$, 400 MHz): $\delta$ 7.49 (t, $J = 7.7$, 2H), 7.60 (t, $J = 7.4$, 1H), 8.01 (bd, $J = 7.4$, 3H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 128.9, 129.4, 133.2, 135.0, 139.5, (d, $J = 225.9$), 152.3 (d, $J = 21.2$), 162.0, 164.9. Anal. Calcd for C$_{11}$H$_8$FN$_3$O$_2$: C 56.65, H 3.46, N 18.02. Found: C 56.52, H 3.45, N 18.08.

**N-glycosylation reaction: general procedure.** (a) Iodine (1.2 eq) was suspended in anhydrous dichloromethane (7.50 mL) under nitrogen atmosphere and the silane (1.2 eq) was added. After 15 min, the resulting mixture was cooled to 0 °C and cannulated dropwise into a solution of oxathiolane 5b (1 mmol) in anhydrous dichloromethane (0.75 mL), using additional dichloromethane (7.50 mL) for rinsing. The reaction was then stirred at the same temperature for 1 h. (b) BSA (3.25 eq when reacting with 11a or 12a; 2 eq when reacting with 11b, 11c or 12b) was added to a suspension of the nucleobase (1.3 eq) in anhydrous dichloromethane (7.50 mL) under nitrogen atmosphere. The resulting mixture was warmed to 40 °C until a clear solution was observed. The mixture (a) was cannulated dropwise into solution (b) under stirring at 0 °C, using additional dichloromethane (7.50 mL) for rinsing. The reaction mixture was then warmed to rt and stirred for 1 h. Afterwards, the reaction was quenched with few drops of saturated NaHCO$_3$ solution. The emulsion was washed with a 1 N Na$_2$S$_2$O$_3$ solution and brine and extracted with dichloromethane. The organic layer was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. Chromatography of the crude residue over silica gel (8a, 9a: dichloromethane:MeOH 95:5; 8b-c, 9b: hexane:ethyl acetate 1:1) provided the pure nucleoside (8a: 86-98%; 8b: 87-94%; 8c: 80-95%; 9a: 84-91%; 9b: 85-91%). Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-amino-2-oxo-1,2-dihydro-1-pyrimidinyl)-1,3-oxathiolane-2-carboxylate (8a). $^1$H NMR (400 MHz): $\delta$ 0.77 (d, $J = 7.0$, 3H), 0.84-0.96 (m, 7H), 0.98-1.11 (m, 2H), 1.43 (bt, $J = 12.0$, 1H), 1.48-1.57 (m, 1H), 1.71 (bd, $J = 11.3$, 2H), 1.88-1.99 (m, 1H), 2.01-2.09 (m, 1H), 3.13 (dd, $J = 6.6$, 12.1, 1H), 3.56 (dd, $J = 4.7$, 12.1, 1H), 4.76 (td, $J = 4.4$, 11.1, 1H), 5.46 (s, 1H), 5.73 (d, $J = 7.5$, 1H), 6.47 (dd, $J = 4.7$, 6.5, 1H), 8.40 (d, $J = 7.5$, 1H). $^{13}$C NMR (100 MHz): ppm 16.1, 20.7, 21.9, 23.2, 26.1, 31.5, 34.1, 36.6, 40.8, 47.1, 76.7, 78.6, 90.4, 93.8, 142.4, 155.5, 165.5, 169.8; Anal. Calcd for
C_{18}H_{27}N_3O_4S: C, 56.67; H, 7.13; N, 11.01; S, 8.41. Found: C, 56.56; H, 7.16; N, 11.05; S, 8.44. Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-[2-oxo-4-(phenylcarboxamido)-1,2-dihydro-1-pyrimidinyl]-1,3-oxathiolane-2-carboxylate (8b). \(^1\)H NMR (600 MHz): \(\delta\) 0.78 (d, \(J = 6.9, 3H\)), 0.82-0.98 (m, 7H), 0.99-1.15 (m, 2H), 1.45 (bt, \(J = 11.5, 1H\)), 1.49-1.59 (m, 1H), 1.62-1.76 (m, 2H), 1.87-1.99 (m, 1H), 2.02-2.12 (m, 1H), 3.24 (dd, \(J = 5.9, 12.3, 1H\)), 3.69 (dd, \(J = 4.7, 12.3, 1H\)), 4.80 (td, \(J = 4.2, 10.9, 1H\)), 5.54 (s, 1H), 6.42 (t, \(J = 5.3, 1H\)), 7.52 (t, \(J = 7.8, 2H\)), 7.62 (t, \(J = 7.4, 1H\)), 7.89 (d, \(J = 7.5, 2H\)), 8.69 (bs, 1H), 8.79 (d, \(J = 7.5, 1H\)). \(^{13}\)C NMR (100 MHz): ppm 16.1, 20.7, 21.9, 23.2, 26.2, 31.5, 34.0, 37.1, 40.8, 47.1, 77.0, 79.7, 90.7, 96.6, 127.5, 129.1, 133.0, 133.2, 143.6, 154.6, 154.9, 162.6, 166.3, 169.3. Anal. Calcd for C_{25}H_{31}N_2O_5S: C, 61.83; H, 6.43; N, 8.65; S, 6.60. Found: C, 61.97; H, 6.41; N, 8.62; S, 6.57. Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-[4-(methylcarboxamido)-2-oxo-1,2-dihydro-1-pyrimidinyl]-1,3-oxathiolane-2-carboxylate (8c). \(^1\)H NMR (500 MHz): \(\delta\) 0.74 (d, \(J = 6.9\) Hz, 3H), 0.80-0.95 (m, 7H), 0.96-1.11 (m, 2H), 1.41 (bt, \(J = 11.7, 1H\)), 1.46-1.56 (m, 1H), 1.68 (bd, \(J = 11.4\) Hz, 2H), 1.83-1.94 (m, 1H), 2.03 (bd, \(J = 11.6, 1H\)), 2.19 (s, 3H), 3.18 (dd, \(J = 5.8, 12.3, 1H\)), 3.65 (dd, \(J = 4.6, 12.3, 1H\)), 4.75 (td, \(J = 4.4, 11.1\) Hz, 1H), 5.55 (s, 1H), 6.40 (bt, \(J = 5.4, 1H\)), 7.41 (d, \(J = 7.3, 1H\)), 8.13 (bs, 1H), 8.71 (d, \(J = 7.3\) Hz, 1H). \(^{13}\)C NMR (100 MHz): ppm 16.0, 20.6, 21.8, 23.1, 24.9, 26.0, 31.3, 33.9, 37.0, 40.6, 46.9, 76.8, 79.6, 90.6, 96.6, 145.4, 154.9, 162.9, 169.2, 169.9. Anal. Calcd for C_{20}H_{25}N_3O_5S: C, 56.72; H, 6.90; N, 9.92; S, 7.57. Found: C, 56.88; H, 6.87; N, 9.88; S, 7.54. Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-[4-amino-5-fluoro-2-oxo-1,2-dihydro-1-pyrimidinyl]-1,3-oxathiolane-2-carboxylate (9a). \(^1\)H NMR (400 MHz): \(\delta\) 0.76 (d, \(J = 6.9, 3H\)), 0.80-0.95 (m, 7H), 0.96-1.12 (m, 2H), 1.43 (bt, \(J = 11.4, 1H\)), 1.47-1.58 (m, 1H), 1.69 (bd, \(J = 11.4, 2H\)), 1.87-1.98 (m, 1H), 2.04-2.08 (m, 1H), 3.11 (dd, \(J = 6.6, 12.1, 1H\)), 3.51 (dd, \(J = 4.7, 12.1, 1H\)), 4.78 (td, \(J = 4.4, 11.0, 1H\)), 5.44 (s, 1H), 5.82 (bs, 1H), 6.40 (dd, \(J = 1.6, 4.8, 6.4, 1H\)), 8.36 (bs, 1H), 8.46 (d, \(J = 6.6, 1H\)). \(^{13}\)C NMR (100 MHz): ppm 16.0, 20.6, 21.8, 23.1, 26.0, 31.4, 34.0, 36.2, 40.6, 47.0, 76.7, 78.6, 90.3, 125.7 (d, \(J = 33.2\)), 136.5 (d, \(J = 242.0\)), 153.8, 158.3 (d, \(J = 14.2\)), 169.7. Anal. Calcd for C_{18}H_{26}FN_3O_5S: C, 54.12; H, 6.56; N, 10.52; S, 8.03. Found: C 53.97, H 6.58, N, 10.55; S, 8.06. Data for (1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-[5-fluoro-2-oxo-4-(phenylcarboxamido)-1,2-dihydro-1-pyrimidinyl]-1,3-oxathiolane-2-carboxylate (9b). \(^1\)H NMR (600 MHz): \(\delta\) 0.78 (d, \(J = 7.0, 3H\)), 0.83-0.97 (m, 7H), 0.99-1.15 (m, 2H), 1.46 (bt, \(J = 12.2, 1H\)), 1.48-1.60 (m, 1H), 1.72 (bd, \(J = 11.3, 2H\)), 1.87-1.98 (m, 1H), 2.02-2.11 (m, 1H), 3.20 (dd, \(J = 6.9, 12.2, 1H\)), 3.50 (dd, \(J = 4.7, 12.2, 1H\)), 4.82 (dt, \(J = 4.3, 11.0, 1H\)), 5.48 (s, 1H), 6.43 (bt, \(J = 5.6, 1H\)), 7.46 (t, \(J = 7.6, 2H\)), 7.56 (t, \(J = 7.6, 1H\)), 8.27 (d, \(J = 7.6, 2H\)), 8.70 (d, \(J = 6.3, 1H\)). \(^{13}\)C NMR (100 MHz): ppm 16.0, 20.6, 21.8, 23.1, 26.0, 31.4, 33.9, 35.6, 40.6, 47.0, 77.0, 78.6, 89.7, 126.2 (d, \(J = 41.3\)), 128.3, 129.9, 133.0, 135.6, 139.7 (d, \(J = 241.7\)), 147.1,
Reduction of nucleoside precursors 8-9. A suspension of nucleoside 8b or 9b (1 mmol) in anhydrous methanol (20 mL) was heated to 40 °C and left under stirring at the same temperature for 48-72 h. The reaction mixture (containing nucleosides 8a and 9a as the main products) was then cooled to room temperature and a solution of K₂HPO₄ (3 eq) in H₂O (2 mL) was added. Hence a solution of NaBH₄ (2 eq) in H₂O (2 mL) containing 25% w/w NaOH was added to the reaction mixture. After 1 h, the reaction was quenched with dilute HCl, adjusting the pH to 4-4.5 and then to pH 7 using a saturated solution of NaHCO₃. The mixture was filtered through a Celite pad and concentrated under reduced pressure. Chromatography of the crude residue over silica gel gave the pure nucleoside (1: 95% o.y.; 2: 75% o.y.). Data for 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydro-2-pyrimidinone (3TC, 1). ¹H NMR (CD₃OD, 500 MHz): δ 3.12 (dd, J = 4.1, 12.0, 1H), 3.50 (dd, J = 5.4, 12.0, 1H), 3.86 (dd, J = 4.0, 12.5, 1H), 3.94 (dd, J = 2.8, 12.5, 1H), 5.27 (bs, 1H), 5.88 (d, J = 7.5, 1H), 6.28 (bt, J = 4.6, 1H), 8.05 (d, J = 7.5, 1H). ¹³C NMR (CD₃OD, 100 MHz): ppm 37.2, 62.5, 86.3, 86.6, 94.2, 141.6, 156.6, 166.3. Anal. Calcd for C₁₈H₁₁N₃O₃S: C, 41.91; H, 4.84; N, 18.33; S, 13.99. Found: C, 42.03; H, 4.82; N, 18.27; S, 14.02. Data for 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydro-2-pyrimidinone (FTC, 2). ¹H NMR (DMSO-d₆, 400 MHz): δ 3.10 (dd, J = 4.4, 11.9, 1H), 3.40 (dd, J = 5.6, 11.9, 1H), 3.72 (dd, J = 4.1, 12.3, 1H), 3.77 (dd, J = 3.8, 12.3, 1H), 5.17 (t, J = 3.9, 1H), 6.12 (ddd, J = 1.8, 4.8, 5.6, 1H), 7.54 (bs, 1H), 7.78 (bs, 1H), 8.17 (d, J = 7.2, 1H). ¹³C NMR (DMSO-d₆, 100 MHz): ppm 36.6, 62.2, 86.4, 86.5, 125.6, (d, J = 32.6), 136.2 (d, J = 240.6), 152.9, 157.5 (d, J = 13.4). Anal. Calcd for C₁₈H₁₁FN₃O₃S: C, 38.86; H, 4.08; N, 17.00; S, 12.97. Found: C, 38.74; H, 4.10; N, 17.05; S, 13.01.
500 MHz, DMSO-$d_6$

(mixture of two stereoisomers)
100 MHz, DMSO-$d_6$

(mixture of two stereoisomers)
400 MHz, CDCl₃

(mixture of four stereoisomers)
100 MHz, CDCl₃

(mixture of four stereoisomers)
400 MHz, CDCl₃

(2R,5R)-5b
100 MHz, CDCl₃

(2R,5R)-5b
400 MHz, CDCl₃

(2S,5S)-5b
100 MHz, CDCl₃

(2S,5S)-5b
500 MHz, CDCl₃

(2R,5R)-5b
400 MHz, CDCl₃
400 MHz, CDCl₃
400 MHz, CDCl₃
100 MHz, CDCl₃
$600 \text{ MHz, CDCl}_3$
100 MHz, CDCl₃
100 MHz, DMSO-$d_6$
376 MHz, DMSO-$d_6$
100 MHz, CDCl₃
100 MHz, CDCl₃
500 MHz, CD$_2$OD

(-)-(2S,5R)-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-cytosine (3TC)
100 MHz, CD$_3$OD
400 MHz, DMSO-$d_6$

(-)-5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-cytosine (FTC)