Supporting Information

Synthesis of Orotidine by Intramolecular Nucleosidation

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General Experimental

Orotic acid was purchased from *Alfa Aesar*. 1,1′-Carbonyldiimidazole (CDI) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) were purchased from *Oakwood Chemicals*. Pyridinium chloride and bis(trimethylsilyl)acetamide (BSA) were purchased from *Acros Organics*. Si-Amine was purchased from *SiliCycle*. Anhydrous solvents were purchased from *EMD Chemicals*. All experiments were performed under a nitrogen atmosphere. Thin layer chromatography (TLC) was performed on silica gel 60 Å F_{254} from *Angela Technologies*, and it was visualized by UV lamp and/or a stain solution of phosphomolybdic acid (PMA) in ethanol. Flash column chromatography was performed on silica gel 60 Å with particle size 35-70 μm purchased from *Acros Organics*. NMR spectra were recorded on Bruker DRX-600 or AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C). Mass spectra were measured with an Agilent ESI-TOF or ThermoElectron Finnigan LTQ ion trap mass spectrometer.

1′,2′,3′-Tri-*O*-benzoyl-5′-*O*-orotyl-D-ribofuranoside (3)

The mixture of 1,2,3-tri-*O*-benzoyl-α,β-D-ribofuranose **2**[1] (0.50 g, 1.08 mmol), anhydrous orotic acid (0.17 g, 1.08 mmol), EEDQ (0.27 g, 1.08 mmol) and *N*-methylmorpholine (0.12 mL, 1.08 mmol) in anhydrous DMF (5 mL) was stirred at 50 °C overnight. After DMF was evaporated, the residue was absorbed onto silica and purified by column chromatography (CH_2Cl_2:Acetone = 10:1 → 7:1) to give compound **3** as white solid (0.24 g, 63% based on the recovery of 0.28 g unreacted starting material). HRMS (ESI-TOF) calculated for $C_{31}H_{24}N_2NaO_{11}$ $[MNa]^+$ 623.1272, found 623.1274. TLC (CH_2Cl_2:Acetone = 10:1) $R_f = 0.26$; ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 8.02-7.97 (m, 4H, Bz), 7.88-7.87 (m, 2H, Bz), 7.70-7.63 (m, 3H, Bz), 7.53-7.49 (m, 4H, Bz), 7.46-7.44 (m, 2H, Bz), 6.54 (s, 1H, H-1′), 6.06 (dd, *J* = 6.7, 5.1 Hz, 1H, H-3′), 6.04 (s, 1H, H-5), 5.94 (d, *J* = 5.1 Hz, 1H, H-2′), 4.91-4.88 (m, 1H, H-4′), 4.86 (dd, *J* = 12.2, 3.2 Hz, 1H, H-5′), 4.45 (dd, *J* = 12.2, 4.1 Hz, 1H, H-5′); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 164.8 (Bz; C=O), 164.5 (Bz; C=O), 164.0 (Bz; C=O), 163.4, 159.8, 150.6, 140.8 (orot.), 134.0, 133.9, 129.4, 129.3, 128.9, 128.7, 128.6, 128.5 (arom.), 104.0 (orot.; C-5), 98.8 (C-1′), 79.8 (C-4′), 75.0 (C-2′), 71.0 (C-3′), 64.6 (C-5′).

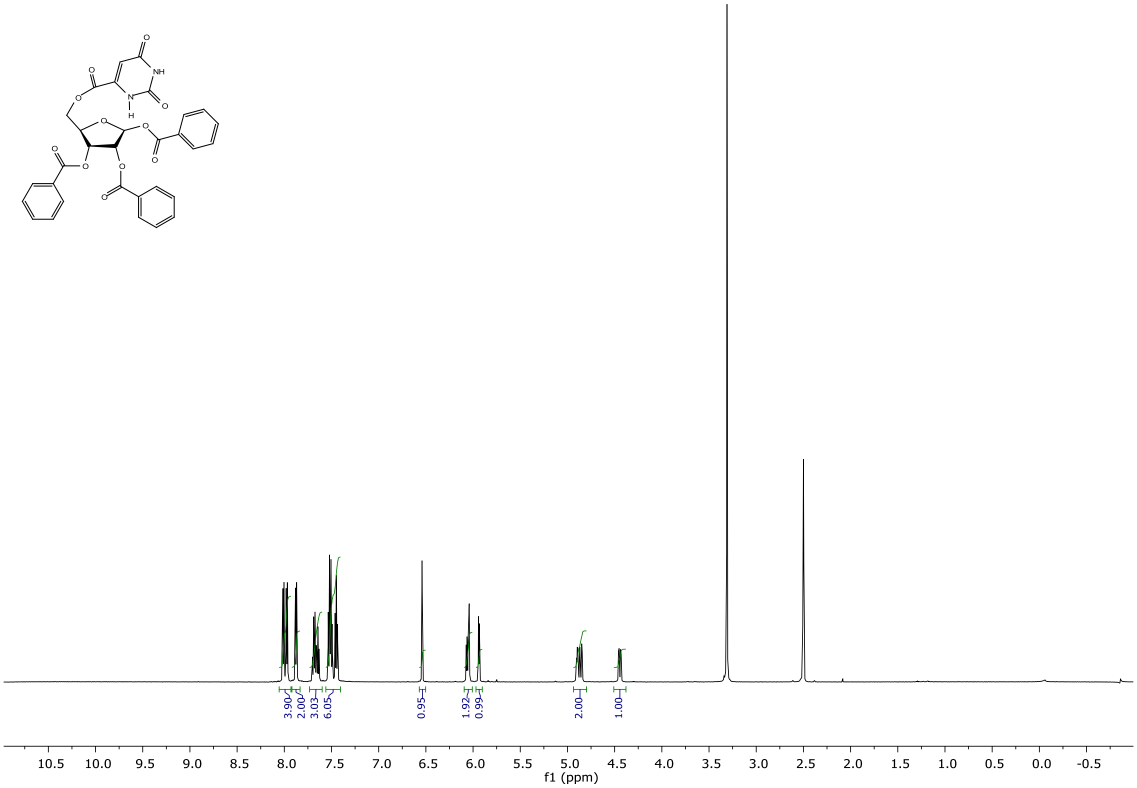

Figure S1 ^{1}H NMR of 1′,2′,3′-tri-*O*-benzoyl-5′-*O*-orotyl-D-ribofuranoside (**3**) in DMSO-d_6.

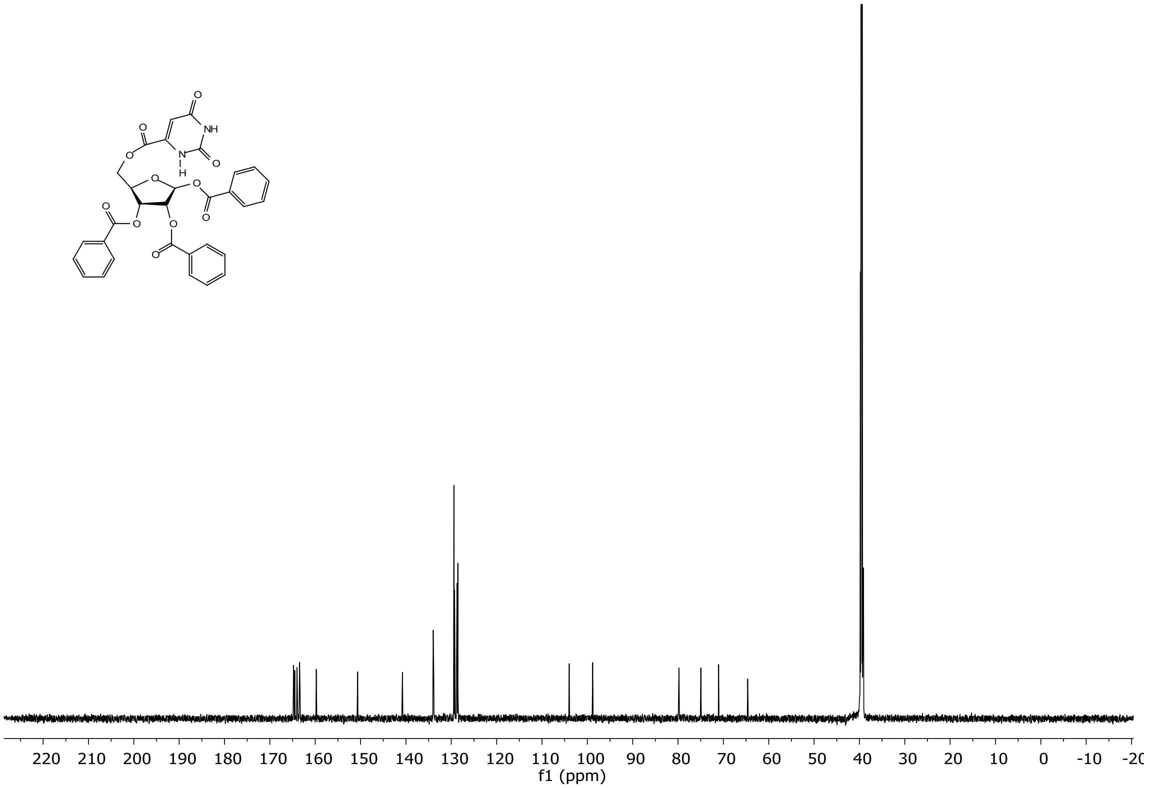

Figure S2 ^{13}C NMR of 1′,2′,3′-tri-*O*-benzoyl-5′-*O*-orotyl-D-ribofuranoside (**3**) in DMSO-d_6.

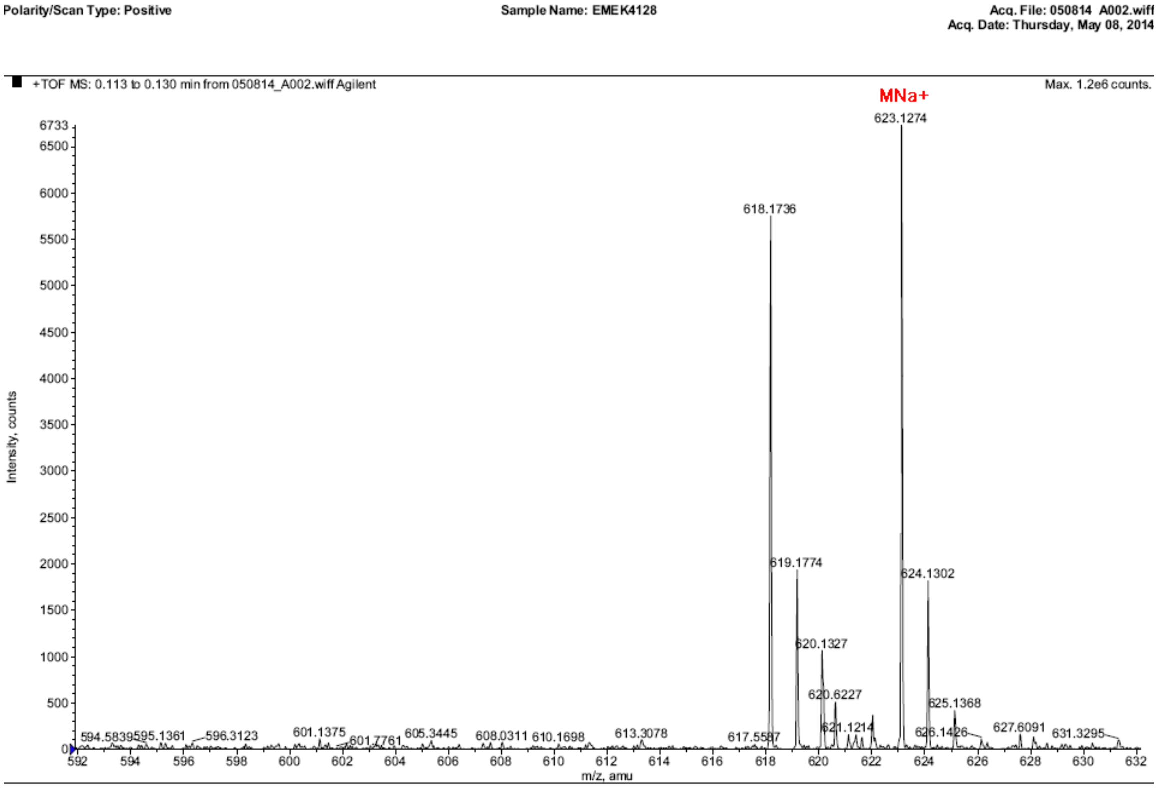

Figure S3 HRMS (ESI-TOF) of 1′,2′,3′-tri-*O*-benzoyl-5′-*O*-orotyl-D-ribofuranoside (**3**).

1-*O*-Pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (5a) was prepared according to the reported procedure.[2,3] The anomeric mixture (β:α = 2:5) could be separated. **5a**β; TLC (Hexanes:EtOAc = 2:1) $R_f = 0.43$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.24 (s, 1H, H-1), 4.76 (d, *J* = 6.0 Hz, 1H, H-2), 4.68 (d, *J* = 6.0 Hz, 1H, H-3), 4.42 (t, *J* = 5.3 Hz, 1H, H-4), 3.70 (dd, *J* = 11.9, 5.3 Hz, 1H, H-5), 3.62 (dd, *J* = 11.9, 5.3 Hz, 1H, H-5), 1.51 (s, 3H, Me), 1.33 (s, 3H, Me), 1.20 (s, 9H, *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.7 (C=O), 113.1 (quaternary C), 102.9 (C-1), 88.9 (C-4), 85.8 (C-3), 81.4 (C-2), 63.7 (C-5), 38.8 (*t*Bu; quaternary C), 27.0 (*t*Bu), 26.6 (Me), 25.0 (Me). **5a**α; TLC (Hexanes:EtOAc = 2:1) R_f = 0.26; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 6.16 (d, *J* = 4.6 Hz, 1H, H-1), 4.82 (dd, *J* = 7.3, 4.6 Hz, 1H, H-2), 4.76 (dd, *J* = 7.3, 3.6 Hz, 1H, H-3), 4.28-4.26 (m, 1H, H-4), 3.85 (dd, *J* = 12.1, 3.1 Hz, 1H, H-5), 3.71 (dd, *J* = 12.1, 3.5 Hz, 1H, H-5), 1.57 (s, 3H, Me), 1.36 (s, 3H, Me), 1.25 (s, 9H, *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.6 (C=O), 115.9 (quaternary C), 96.5 (C-1), 83.6 (C-4), 80.6 (C-2), 80.2 (C-3), 62.7 (C-5), 39.1 (*t*Bu; quaternary C), 27.2 (*t*Bu), 26.4 (Me), 25.3 (Me).

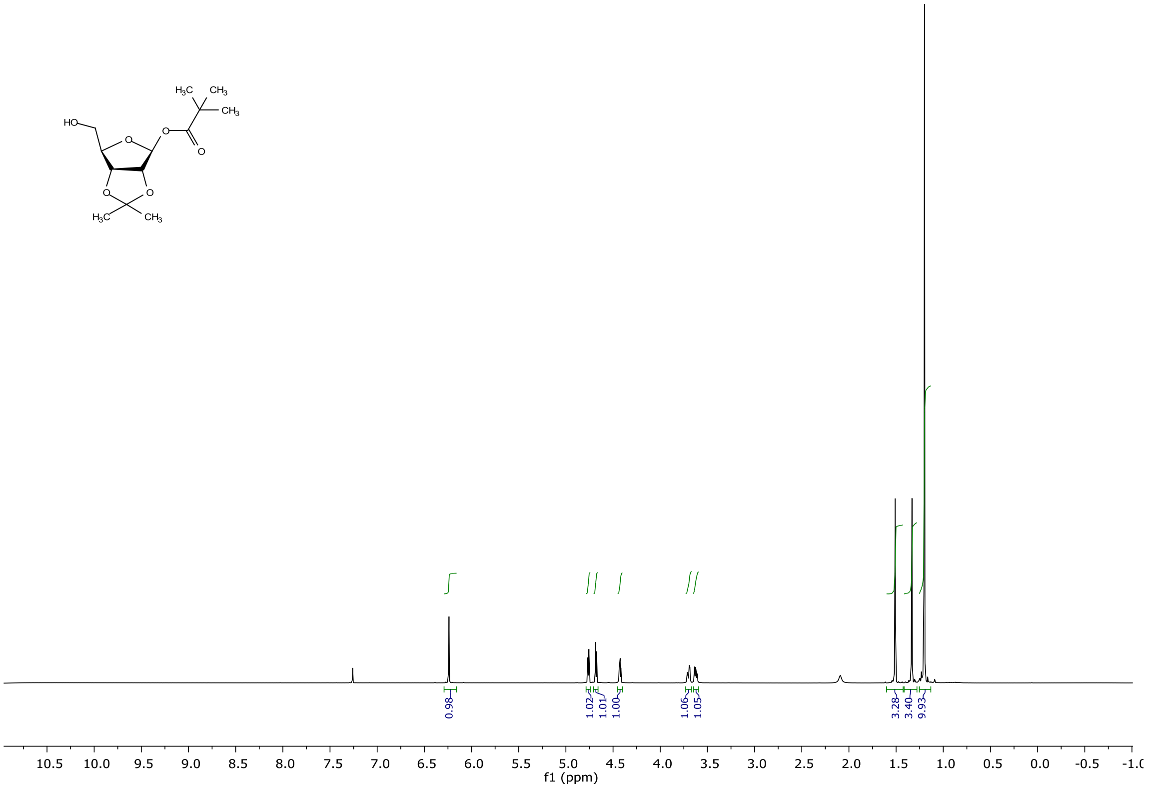

Figure S4 ^{1}H NMR of 1-*O*-pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5a**β) in CDCl$_3$.

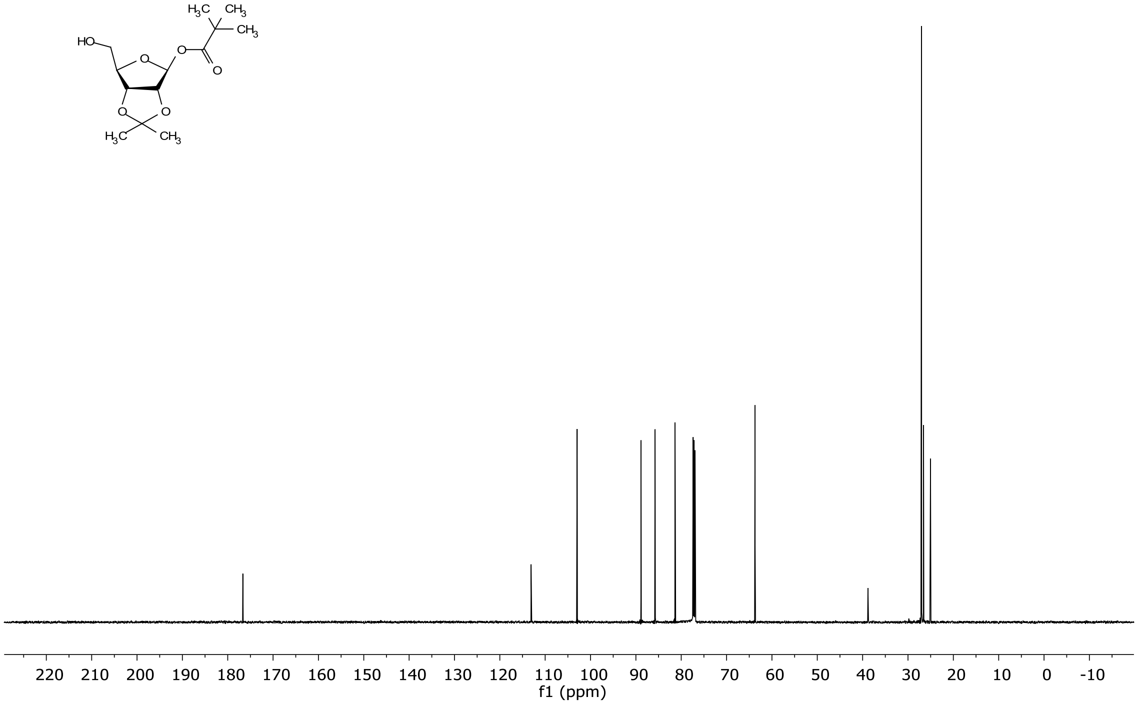

Figure S5 ^{13}C NMR of 1-*O*-pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5a**β) in CDCl$_3$.

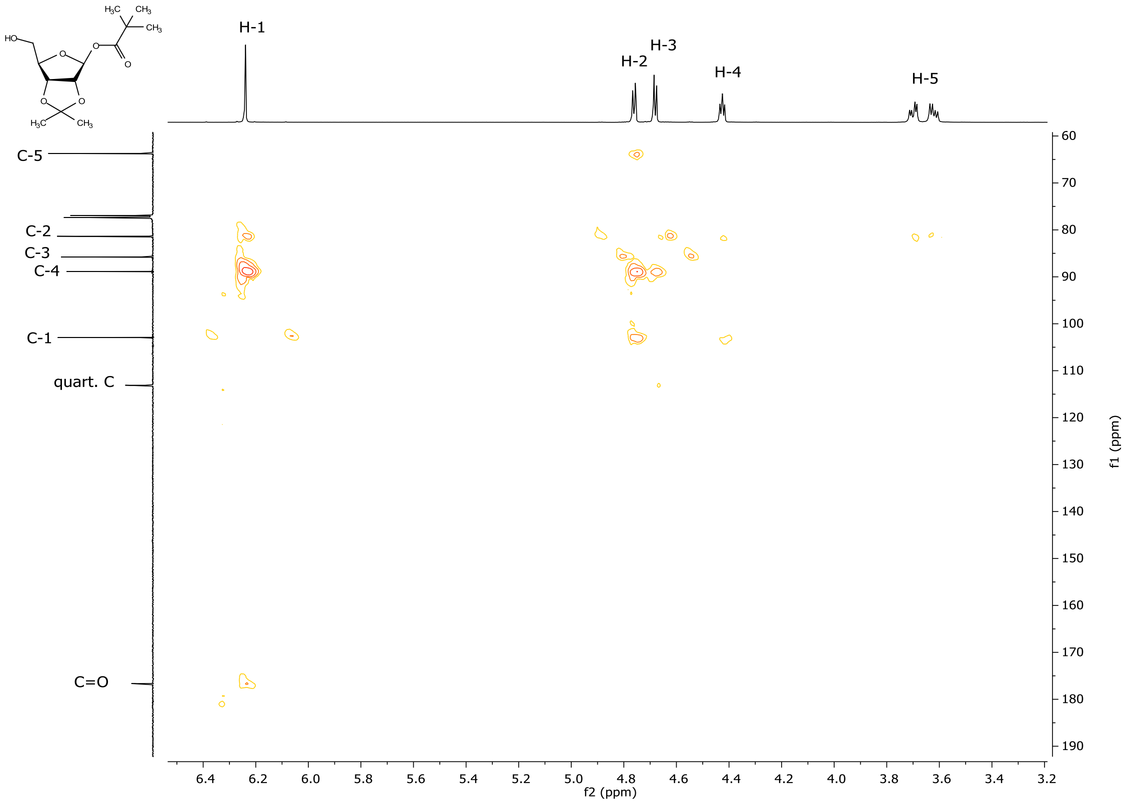

Figure S6 HMBC NMR of 1-*O*-pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5a**β) in CDCl$_3$.

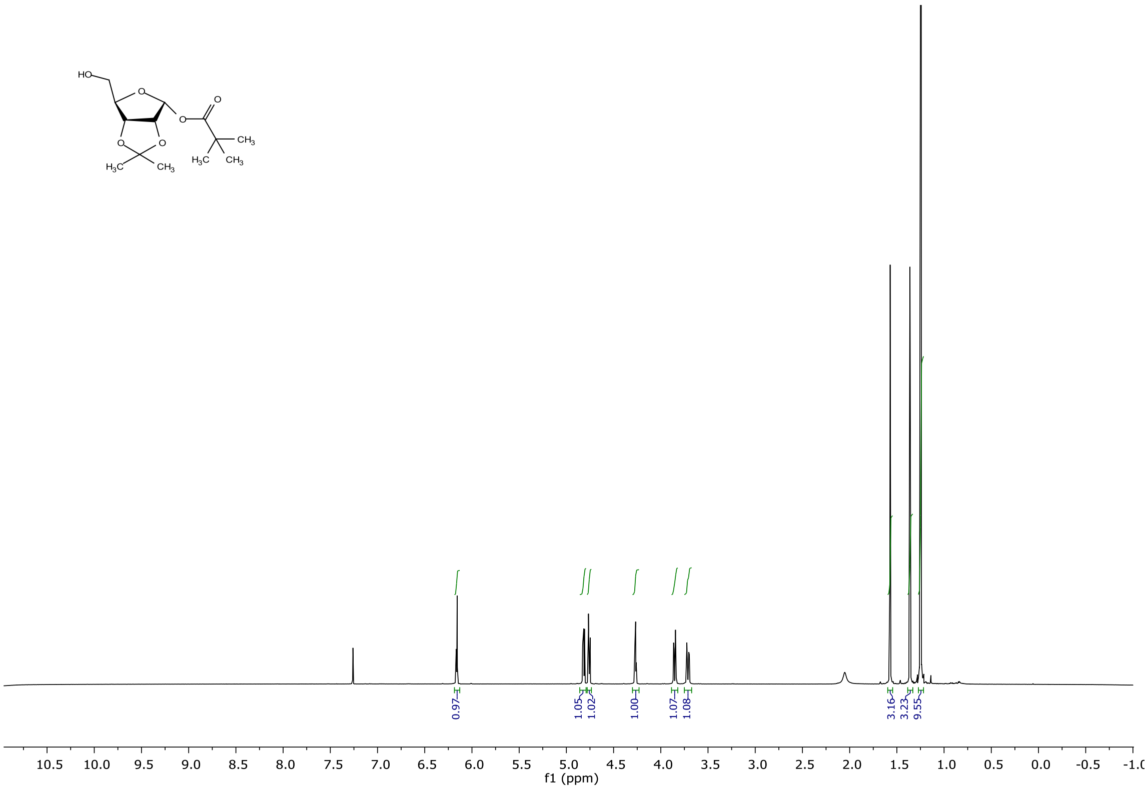

Figure S7 ^{1}H NMR of 1-*O*-pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5a**α) in CDCl$_3$.

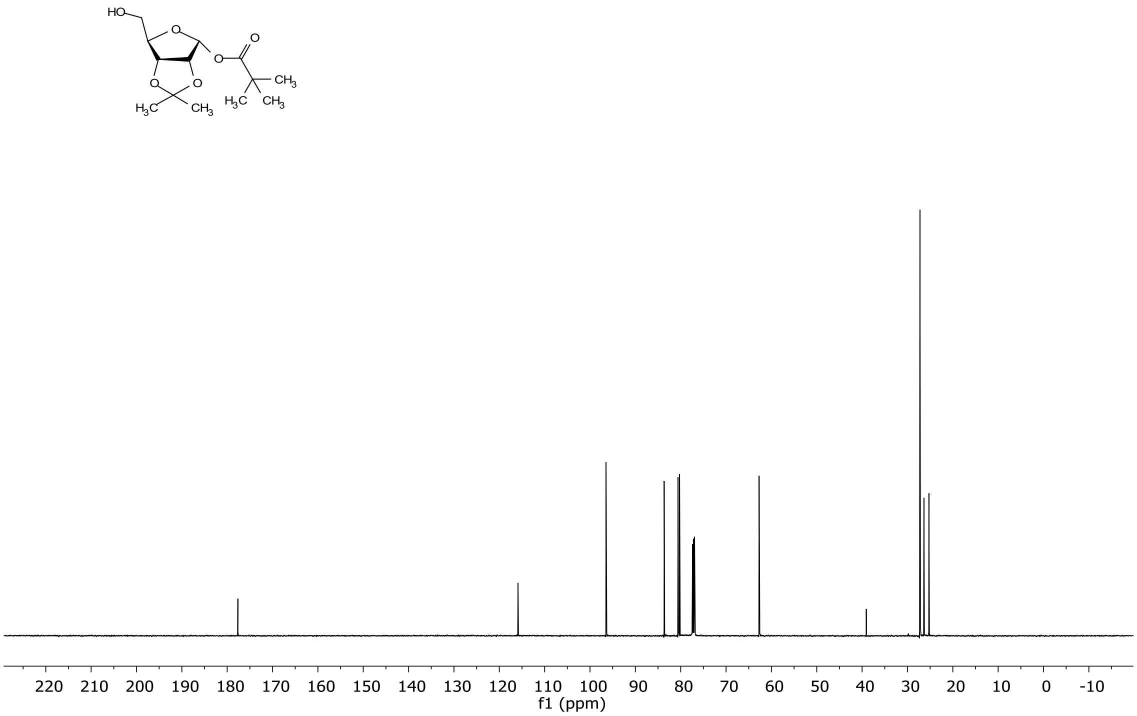

Figure S8 ^{13}C NMR of 1-*O*-pivaloyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5a**α) in CDCl$_3$.

1-*O*-Benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (5b)

To a solution of **4b**[4] (3.18 g, 7.78 mmol) in dry THF (40 mL) was added triethylamine trihydrofluoride (1.52 mL, 9.34 mmol) at 0 °C and the mixture was stirred at room temperature overnight. The mixture was then diluted with EtOAc (100 mL) and washed with saturated aqueous NaHCO$_3$ (100 mL). The aqueous layer was extracted with EtOAc (70 mL). The combined organic extract was dried over Na$_2$SO$_4$ and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 3:1) to give compound **5b** (1.67 g, 73%). The anomeric mixture (β:α = 6:1) could be separated under these conditions as white solid for **5b**β (1.43g, 63%) and colorless oil for **5b**α (0.24g, 10%). **5b**β; TLC (Hexanes:EtOAc = 2:1) R$_f$ = 0.31; ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.98-7.96 (m, 2H, Bz), 7.59-7.56 (m, 1H, Bz), 7.45-7.43 (m, 2H, Bz), 6.48 (s, 1H, H-1), 4.87 (d, *J* = 6.0 Hz, 1H, H-2), 4.83 (d, *J* = 6.0 Hz, 1H, H-3), 4.47 (t, *J* = 5.5 Hz, 1H, H-4), 3.75-3.68 (m, 2H, H-5), 1.53 (s, 3H, Me), 1.35 (s, 3H, Me); ^{13}C NMR (150 MHz, CDCl$_3$) δ 165.0 (C=O), 133.8, 129.7, 129.3, 128.7 (arom.), 113.2 (quaternary C), 103.4 (C-1), 89.0 (C-4), 85.7 (C-2), 81.3 (C-3), 63.6 (C-5), 26.5 (Me), 25.0 (Me). **5b**α; TLC (Hexanes:EtOAc = 2:1) R$_f$ = 0.14; ^{1}H-NMR (600 MHz, CDCl$_3$) δ 8.10-8.09 (m, 2H, Bz), 7.57-7.56 (m, 1H, Bz), 7.46-7.43 (m, 2H, Bz), 6.47 (d, *J* = 4.5 Hz, 1H, H-1), 4.91 (dd, *J* = 7.1, 4.5 Hz, 1H, H-2), 4.82 (dd, *J* = 7.1, 3.1 Hz, 1H, H-3), 4.49-4.47 (m, 1H, H-4), 3.91 (dd, *J* = 12.1, 2.9 Hz, 1H, H-5), 3.77 (dd, *J* = 12.1, 3.1 Hz, 1H, H-5), 1.41 (s, 3H, Me), 1.35 (s, 3H, Me); ^{13}C NMR (150 MHz, CDCl$_3$) δ 165.4 (C=O), 133.4, 130.0, 129.9, 128.5 (arom.), 115.9 (quaternary C), 97.5 (C-1), 84.2 (C-4), 80.9 (C-2), 80.1 (C-3), 62.7 (C-5), 26.1 (Me), 25.3 (Me).

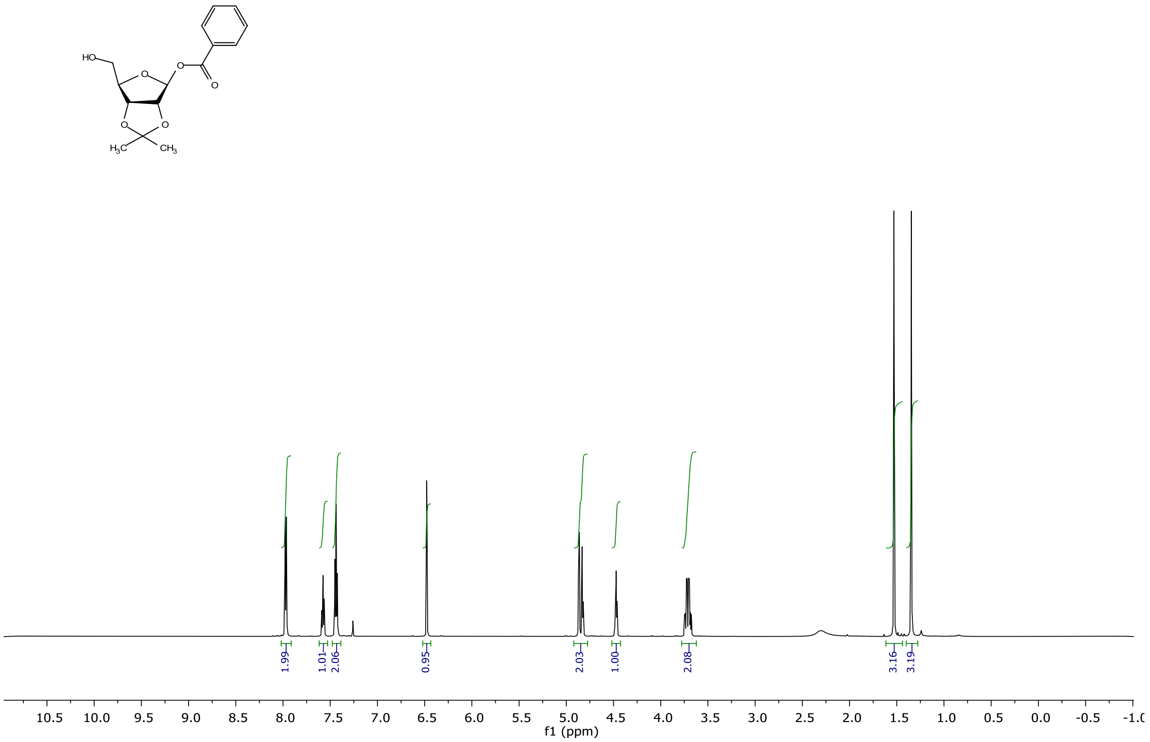

Figure S9 ^{1}H NMR of 1-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5b**β) in CDCl$_3$.

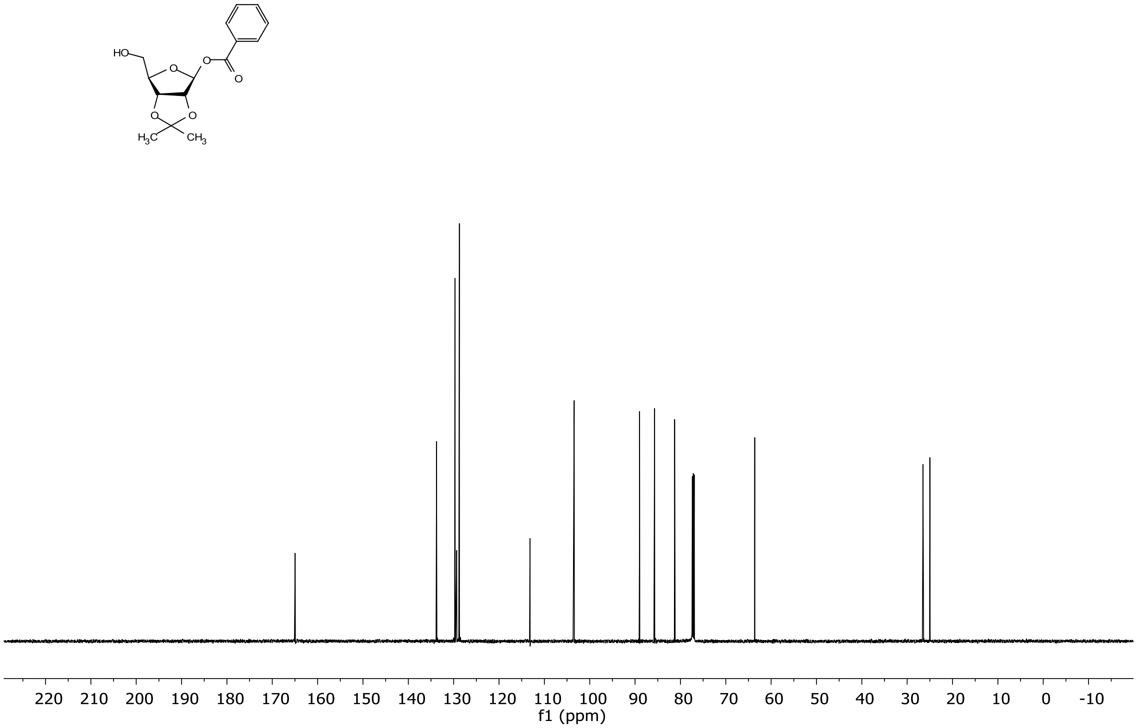

Figure S10 ^{13}C NMR of 1-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5b**β) in CDCl$_3$.

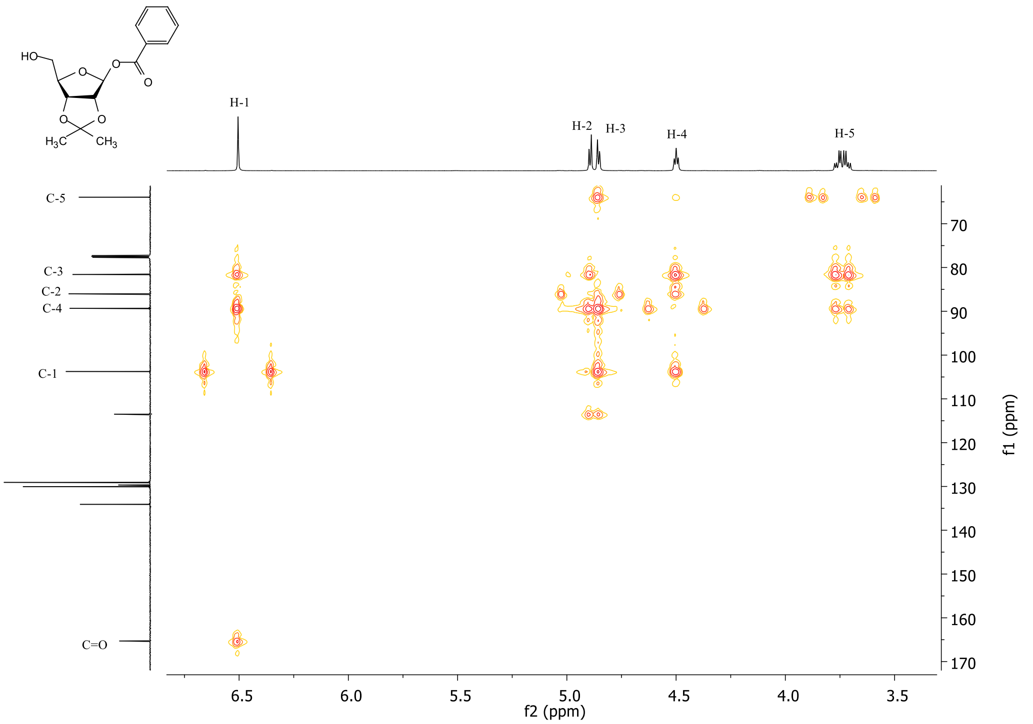

Figure S11 HMBC NMR of 1-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5b**β) in CDCl$_3$.

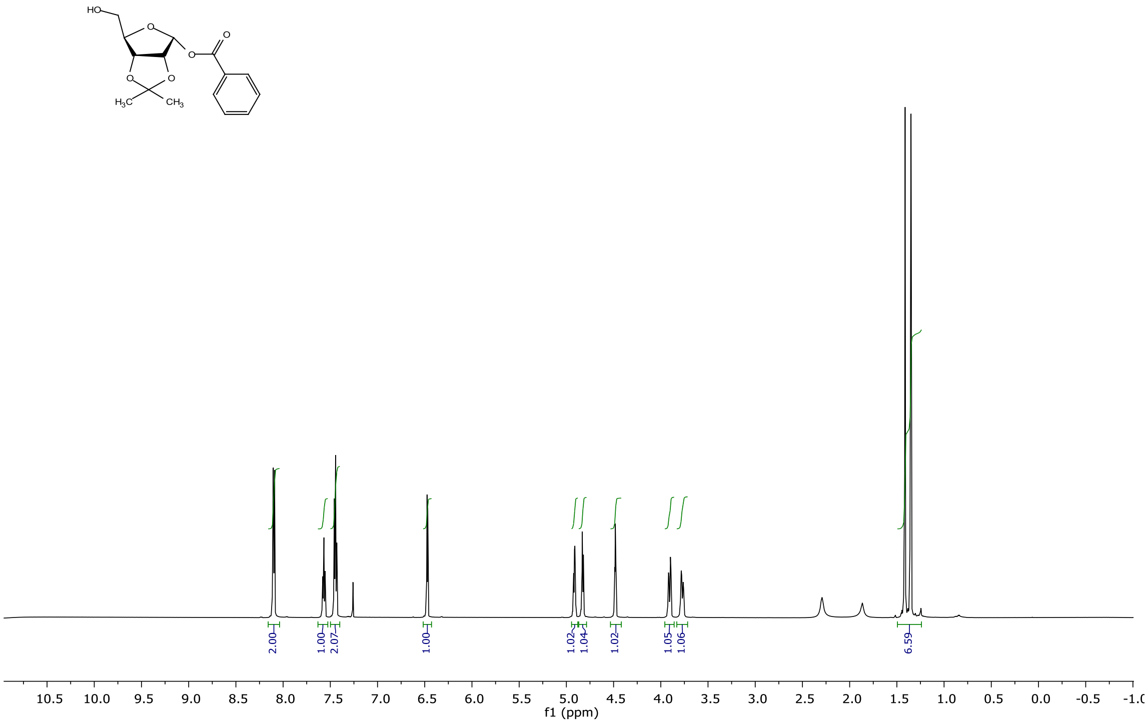

Figure S12 ^{1}H NMR of 1-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5b**α) in CDCl$_3$.

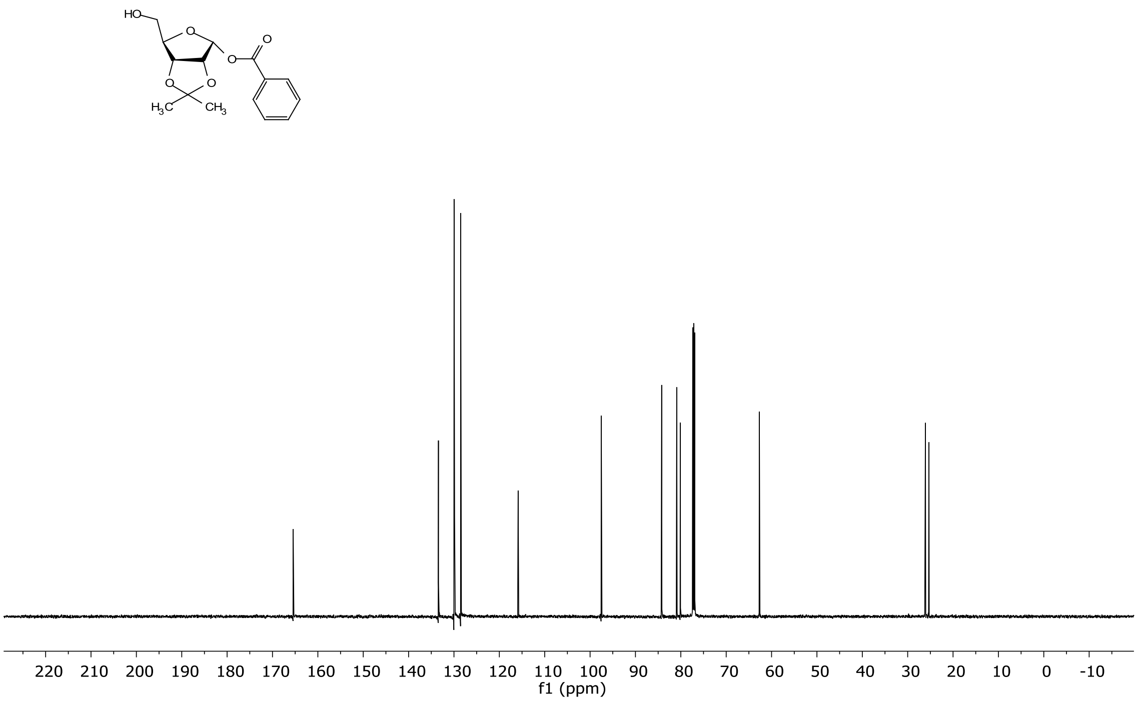

Figure S13 ^{13}C NMR of 1-*O*-benzoyl-2,3-*O*-isopropylidene-D-ribofuranoside (**5b**α) in $CDCl_3$.

1-*O*-Acetyl-2,3-*O*-isopropylidene-D-ribofuranoside (5c)

To a solution of **4c**β[5] (5.27 g, 15.2 mmol) in dry THF (80 mL) was added triethylamine trihydrofluoride (3.0 mL, 18.2 mmol) at 0 °C and the mixture was stirred at room temperature overnight. The mixture was then diluted with EtOAc (150 mL) and washed with saturated aqueous $NaHCO_3$ (150 mL). The aqueous layer was extracted with EtOAc (100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 2:1) to give compound **5c**β as syrup (3.42 g, 97%). **5c**β; TLC (Hexanes:EtOAc = 1:1) $R_f = 0.39$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.25 (s, 1H, H-1), 4.78 (d, *J* = 6.0 Hz, 1H, H-3), 4.70 (d, *J* = 6.0 Hz, 1H, H-2), 4.43 (t, *J* = 4.8 Hz, 1H, H-4), 3.72 (dd, *J* = 12.1, 4.8 Hz, 1H, H-5), 3.64 (dd, *J* = 12.1, 4.8 Hz, 1H, H-5), 2.09 (s, 3H, Ac), 1.51 (s, 3H, Me), 1.33 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.2 (C=O), 113.1 (quaternary C), 103.0 (C-1), 89.1 (C-4), 85.7 (C-3), 81.2 (C-2), 63.7 (C-5), 26.6 (Me), 25.1 (Me), 21.4 (Ac).

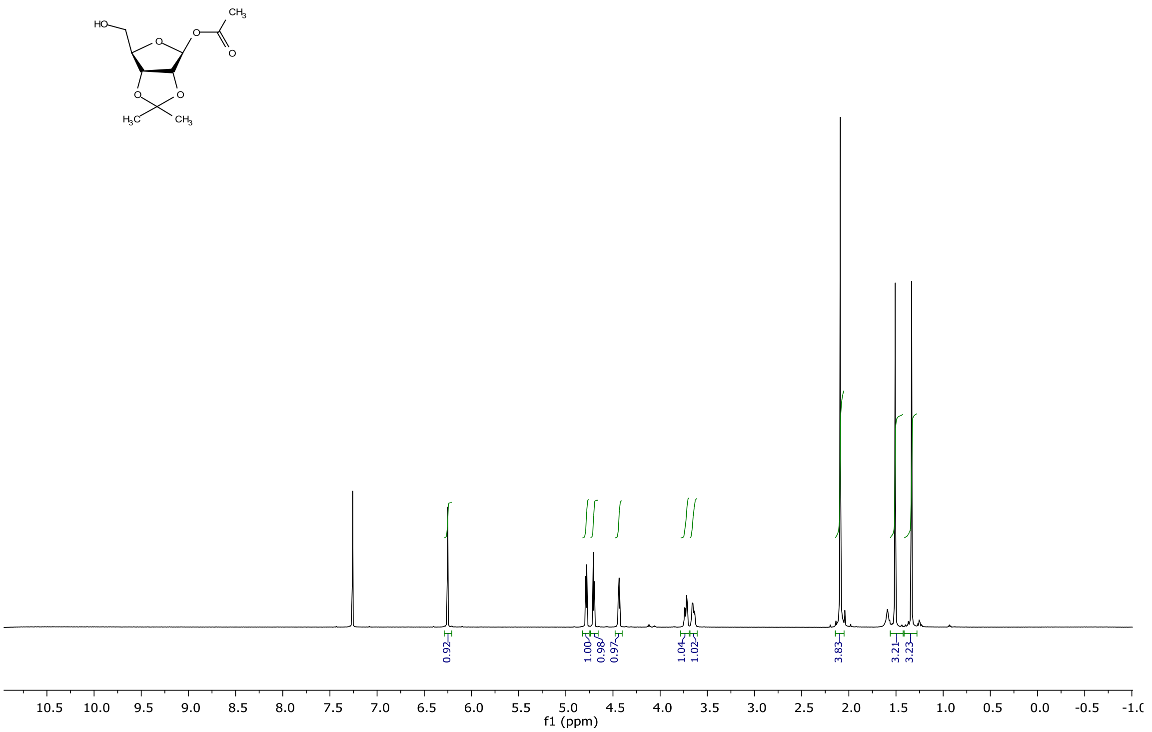

Figure S14 ^{1}H NMR of 1-*O*-acetyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**5c**β) in CDCl$_3$.

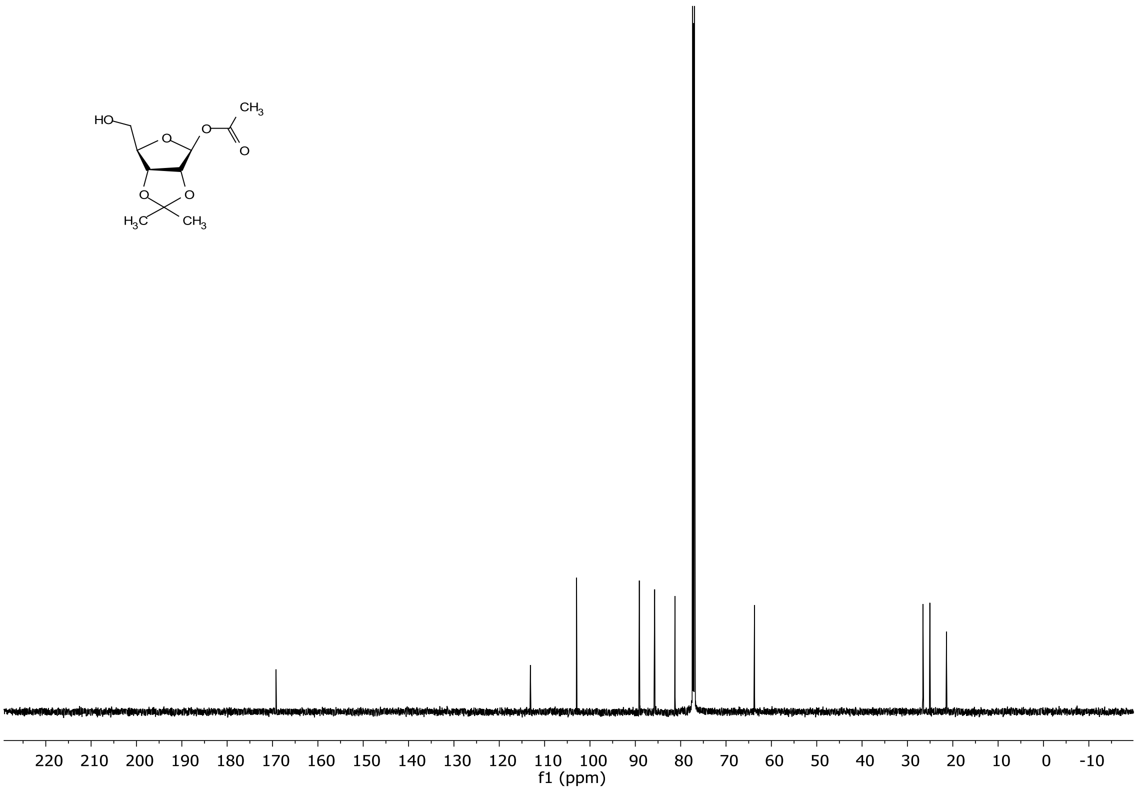

Figure S15 ^{13}C NMR of 1-*O*-acetyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**5c**β) in CDCl$_3$.

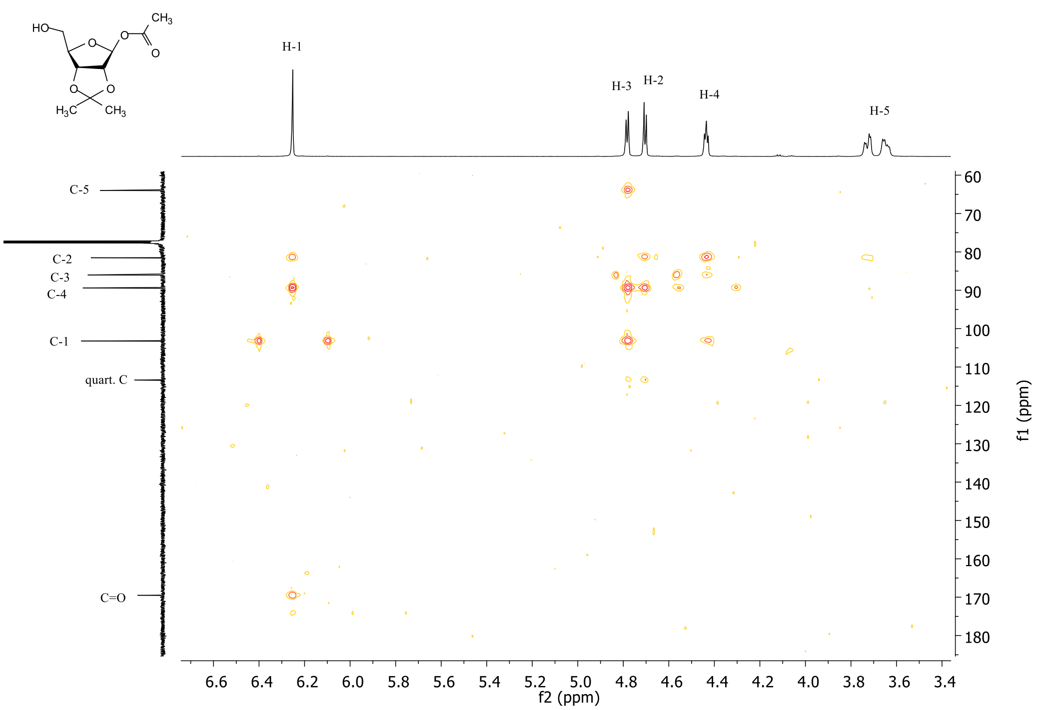

Figure S16 HMBC NMR of 1-*O*-acetyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**5cβ**) in $CDCl_3$.

1′-*O*-Pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (6a)

A slurry of orotic acid (86 mg, 0.55 mmol) and 1,1′-carbonyldiimidazole (107 mg, 0.66 mmol) in dry DMF (10 mL) was stirred at room temperature. As the reaction proceeded, slow CO_2 release was observed. After 16 h, the crude orotylimidazole was filtered, washed with acetone (10 mL), and dried under high vacuum. The crude orotylimidazole (225 mg), pre-dried pyridinium chloride[6] (83 mg, 0.72 mmol) and the compound **5a** (100 mg, 0.36 mmol) were then placed in dry acetonitrile (7 mL) and the reaction mixture was stirred overnight at room temperature. After filtration through Celite, the filtrate was concentrated and the residue was partitioned between H_2O (80 mL) and chloroform (80 mL). The aqueous layer was extracted with chloroform (2×50 mL) and combined organic extract was dried over Na_2SO_4. The crude product was absorbed onto silica while chloroform was evaporated, and purified by column chromatography (Hexanes:EtOAc = 1:2) to give compound **6a** as white solid (134 mg, 90%). The anomeric mixture (β:α = 2:5) could be separated under these conditions. HRMS (ESI-TOF) calculated for $C_{18}H_{25}N_2O_9$ $[MH]^+$ 413.1554, found 413.1550. **6a**β; TLC (Hexanes:EtOAc = 1:2) $R_f = 0.36$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.76 (bs, 1H, NH), 9.50 (bs, 1H, NH), 6.47 (s, 1H, H-5), 6.26 (s, 1H, H-1′), 4.87 (d, *J* = 5.9 Hz, 1H, H-2′), 4.77 (d, *J* = 5.9 Hz, 1H, H-3′), 4.70 (dt, *J* = 14.3, 7.1 Hz, 1H, H-4′), 4.34-4.27 (m, 2H, H-5′), 1.60 (s, 3H, Me), 1.40 (s, 3H, Me), 1.21 (s, 9H, *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.0 (Piv; C=O), 162.3 (orot.), 158.9 (orot.; 6-C=O), 151.7 (orot.), 139.7 (orot.), 113.2 (quaternary C), 105.8 (C-5), 101.6 (C-1′), 84.6 (C-4′), 83.4 (C-2′), 81.6 (C-3′), 66.1 (C-5′), 38.2 (quaternary C of *t*Bu), 26.4 (*t*Bu), 25.8 (Me), 24.5 (Me). **6a**α; TLC (Hexanes:EtOAc = 1:2) $R_f = 0.25$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.90 (bs, 1H, NH), 9.80 (bs, 1H, NH), 6.44 (s, 1H, H-5), 6.17 (d, *J* = 4.4 Hz, 1H, H-1′), 4.92 (dd, J = 6.8, 4.4 Hz, 1H, H-2′), 4.73 (dd, J = 6.8, 3.4 Hz, 1H, H-3′), 4.55 (dd, J = 13.1, 5.3 Hz, 1H, H-5′), 4.47-4.46 (m, 2H, H-4′,5′), 1.59 (s, 3H, Me), 1.38 (s, 3H, Me), 1.25 (s, 9H, *t*Bu); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.4 (Piv; C=O), 163.8 (orot.), 160.1 (orot.; 6-C=O), 151.2 (orot.), 140.0 (orot.), 116.8 (quaternary C), 105.8 (C-5), 96.3 (C-1′), 80.3 (C-4′), 80.2 (C-2′, C-3′), 66.1 (C-5′), 39.1 (quaternary C of *t*Bu), 27.2 (*t*Bu), 26.4 (Me), 25.4 (Me).

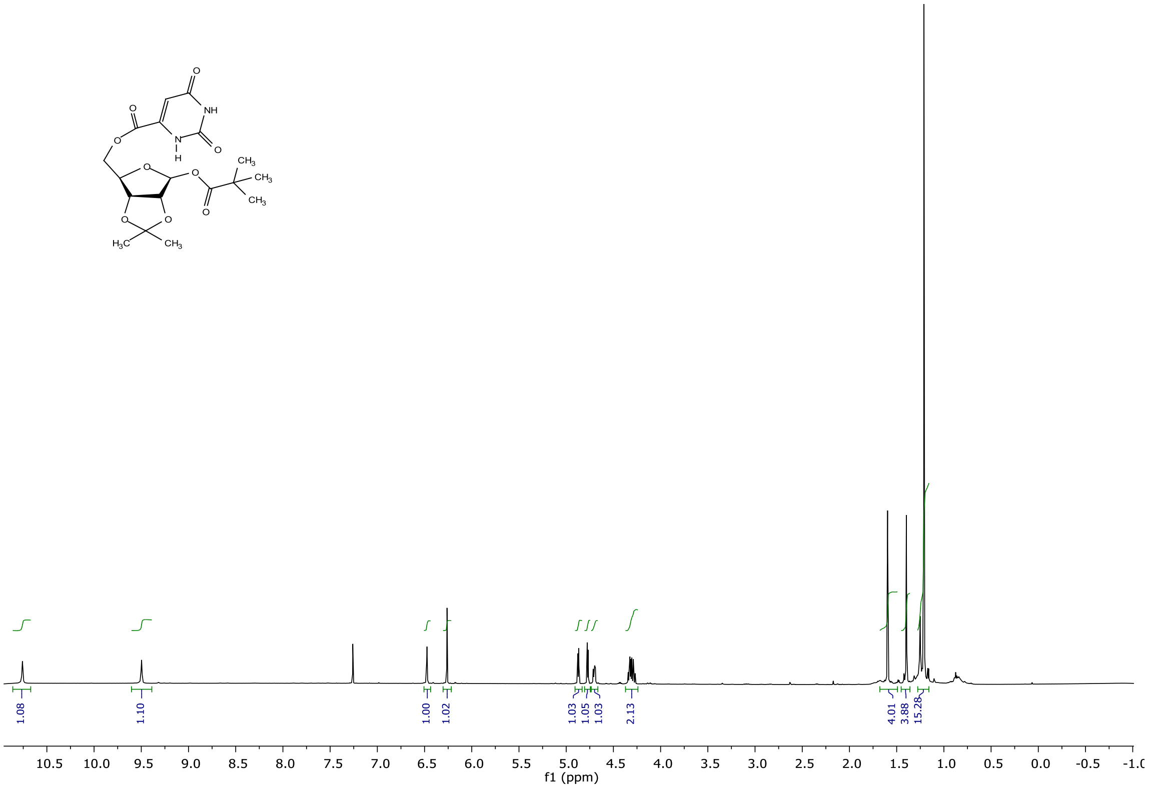

Figure S17 ^{1}H NMR of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**β) in $CDCl_3$.

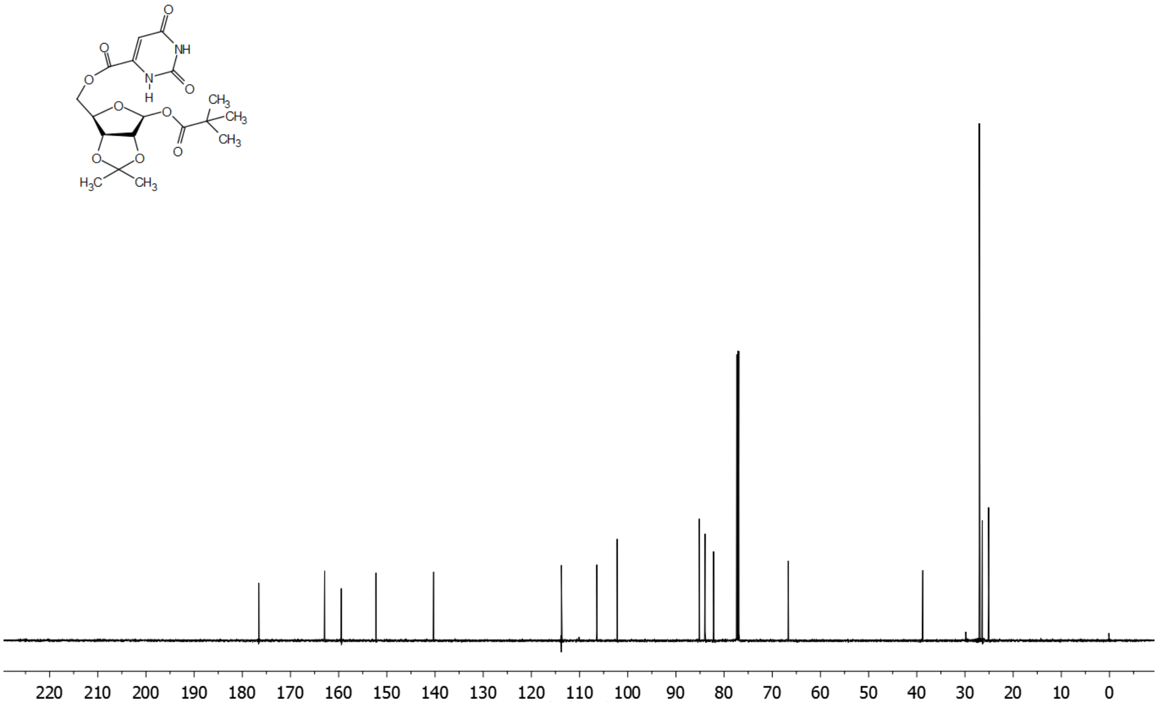

Figure S18 ^{13}C NMR of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**β) in CDCl$_3$.

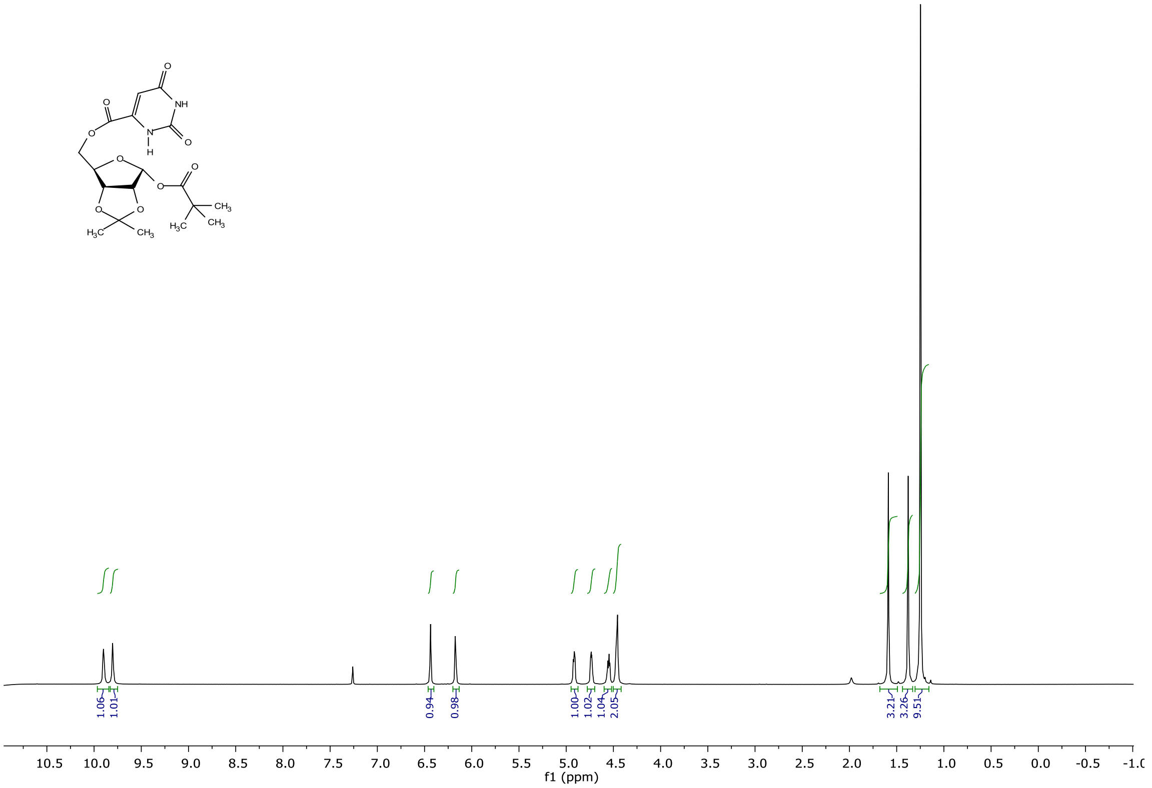

Figure S19 ^{1}H NMR of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**α) in CDCl$_3$.

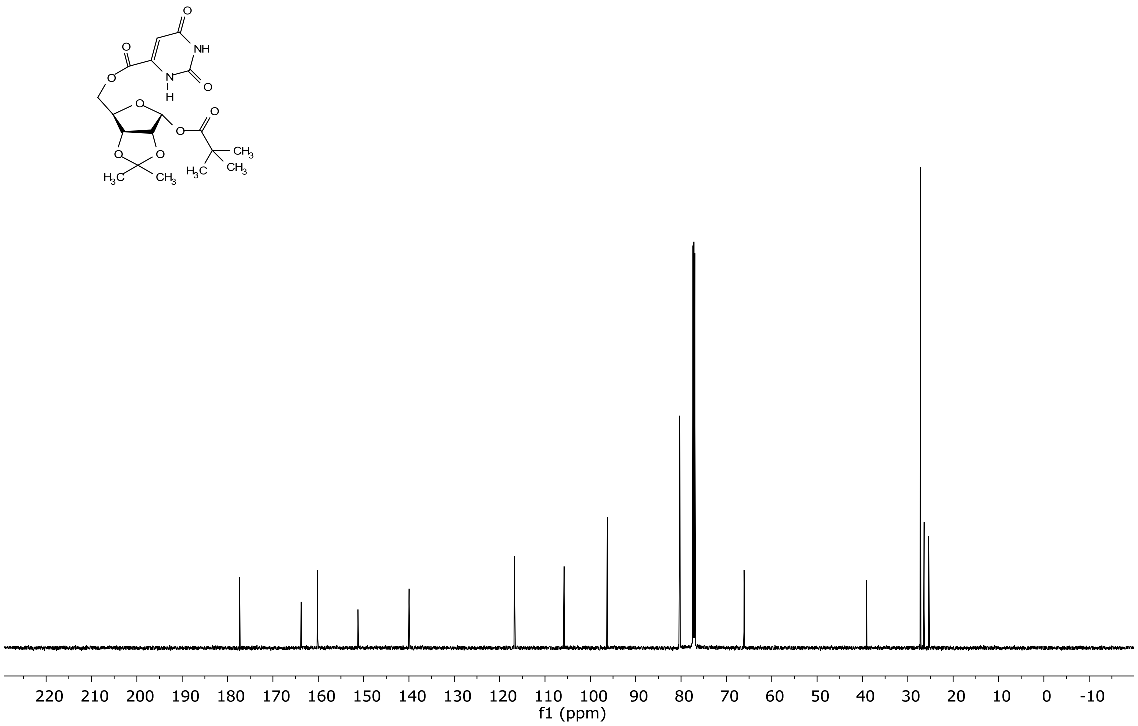

Figure S20 ^{13}C NMR of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**α) in $CDCl_3$.

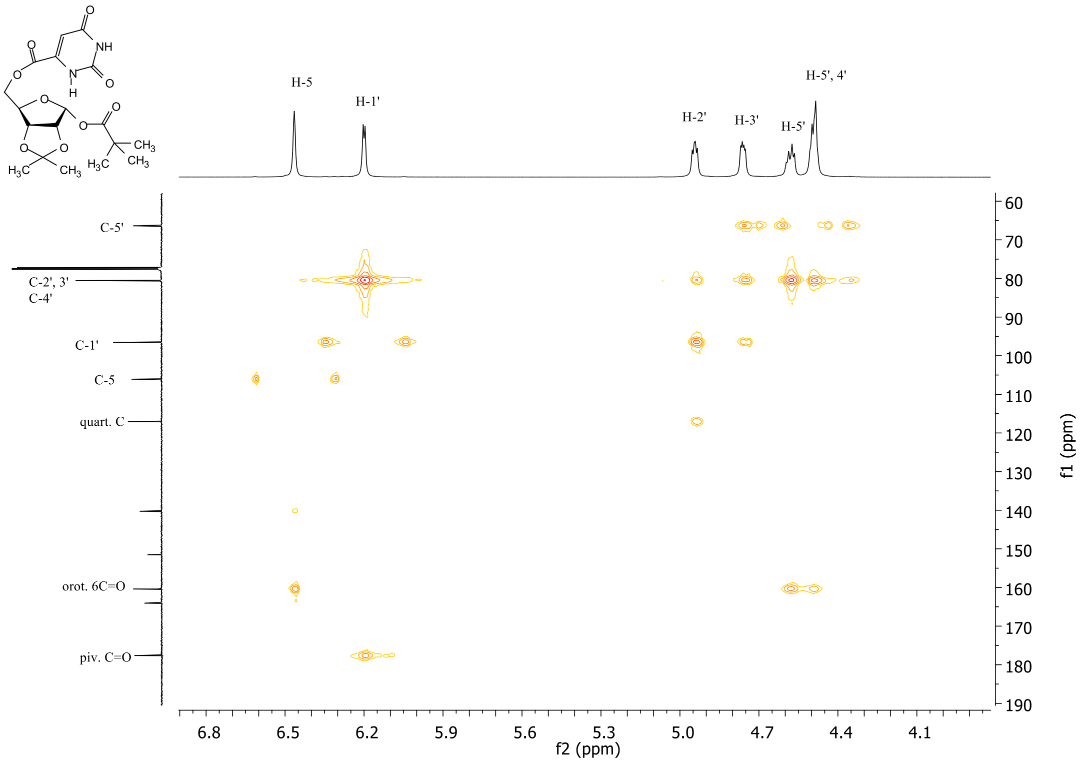

Figure S21 HMBC NMR of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**α) in $CDCl_3$.

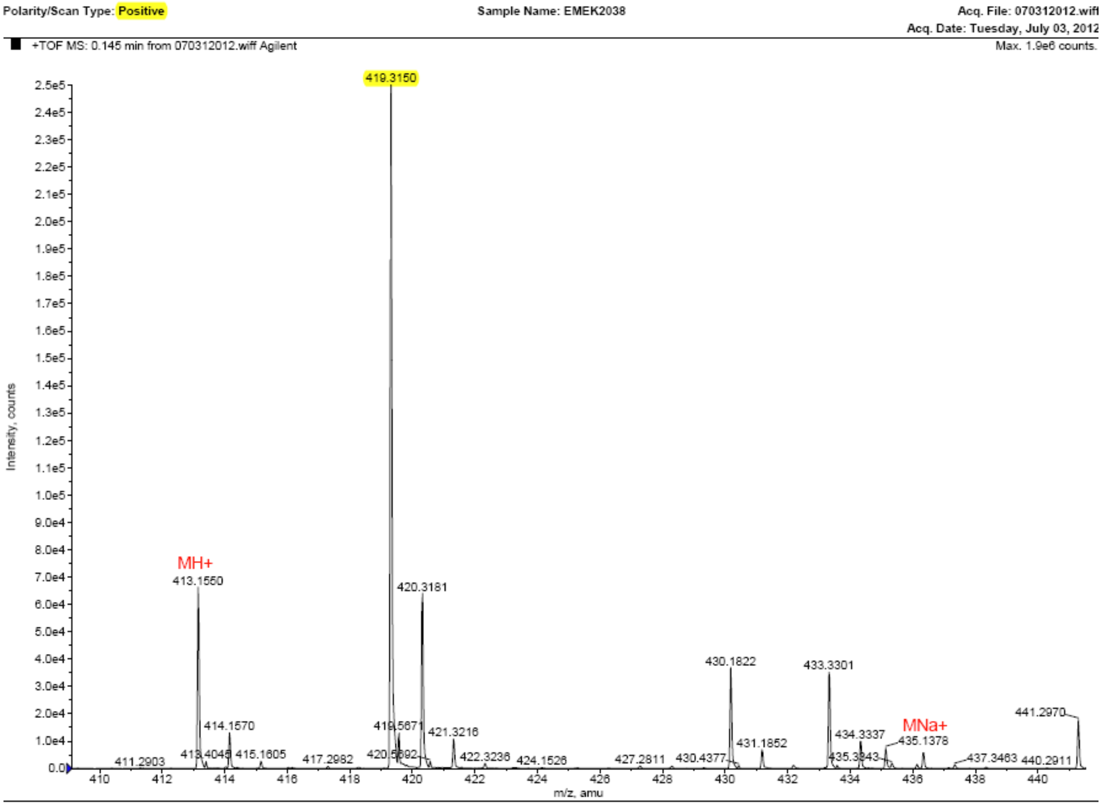

Figure S22 HRMS (ESI-TOF) of 1′-*O*-pivaloyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6a**).

1′-*O*-Benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (6b)

A slurry of orotic acid (0.58 g, 3.74 mmol) and 1,1′-carbonyldiimidazole (0.72 g, 4.42 mmol) in dry DMF (30 mL) was stirred at room temperature. As the reaction proceeded, slow CO_2 release was observed. After 16 h, pre-dried pyridinium chloride[6] (0.78 g, 6.80 mmol) and a solution of compound **5b** (1.0 g, 3.40 mmol) in DMF (10 mL) were added to the crude mixture of orotylimidazole. After being stirred for 4h at room temperature, the mixture was partitioned between H_2O (100 mL) and chloroform (100 mL). The aqueous layer was extracted with chloroform (2×70 mL) and the combined organic extract was dried over Na_2SO_4. The crude product was absorbed onto silica while chloroform was evaporated and purified by column chromatography (Hexanes:EtOAc = 1:2) to give compound **6b** as white solid (1.39 g, 94%). The anomeric mixture (β:α = 6:1) could be separated under these conditions. HRMS (ESI-TOF) calculated for $C_{20}H_{21}N_2O_9$ $[MH]^+$ 433.1242, found 433.1253. **6b**β; TLC (Hexanes:EtOAc = 1:2) $R_f = 0.34$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.36 (bs, 1H, NH), 9.37 (bs, 1H, NH), 7.99-7.97 (m, 2H, Bz), 7.61-7.59 (m, 1H, Bz), 7.48-7.45 (m, 2H, Bz), 6.49 (s, 1H, H-1′), 6.43 (s, 1H, H-5), 4.97-4.93 (m, 2H, H-2′,3′), 4.76-4.74 (m, 1H, H-4′), 4.47-4.42 (m, 2H, H-5′), 1.61 (s, 3H, Me), 1.42 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.9 (Bz; C=O), 162.9 (orot.), 159.6 (orot.; 6-C=O), 151.7 (orot.), 140.1 (orot.), 133.9, 129.8, 129.3, 128.8 (arom.), 113.9 (quaternary C), 106.2 (orot. C-5), 103.2 (C-1′), 85.4 (C-2′), 84.5 (C-4′), 81.9 (C-3′), 66.6 (C-5′), 26.5 (Me), 25.2 (Me). **6b**α; TLC (Hexanes:EtOAc = 1:2) $R_f = 0.28$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.97 (bs, 1H, NH), 9.85 (bs, 1H, NH), 8.09-8.08 (m, 2H, Bz), 7.58-7.55 (m, 1H, Bz), 7.45-7.43 (m, 2H, Bz), 6.49 (d, *J* = 4.4 Hz, 1H, H-1′), 6.47 (s, 1H, H-5), 5.01 (dd, *J* = 7.2, 4.4 Hz, 1H, H-2′), 4.79 (dd, *J* = 7.2, 3.5 Hz, 1H, H-3′), 4.68-4.66 (m, 1H, H-4′), 4.60 (dd, *J* = 11.9, 3.9 Hz, 1H, H-5′), 4.51 (dd, *J* = 11.9, 4.8 Hz, 1H, H-5′), 1.43 (s, 3H, Me), 1.36 (s, 3H, Me); ^{13}C NMR (150 MHz, CDCl$_3$) δ 165.2 (Bz; C=O), 163.9 (orot.), 160.2 (orot.; 6-C=O), 151.3 (orot.), 140.0 (orot.), 133.5, 130.0, 129.7, 128.6 (arom.), 116.8 (quaternary C), 105.8 (orot.; C-5), 97.3 (C-1′), 80.8 (C-4′), 80.7 (C-2′), 80.2 (C-3′), 66.3 (C-5′), 26.2 (Me), 25.5 (Me).

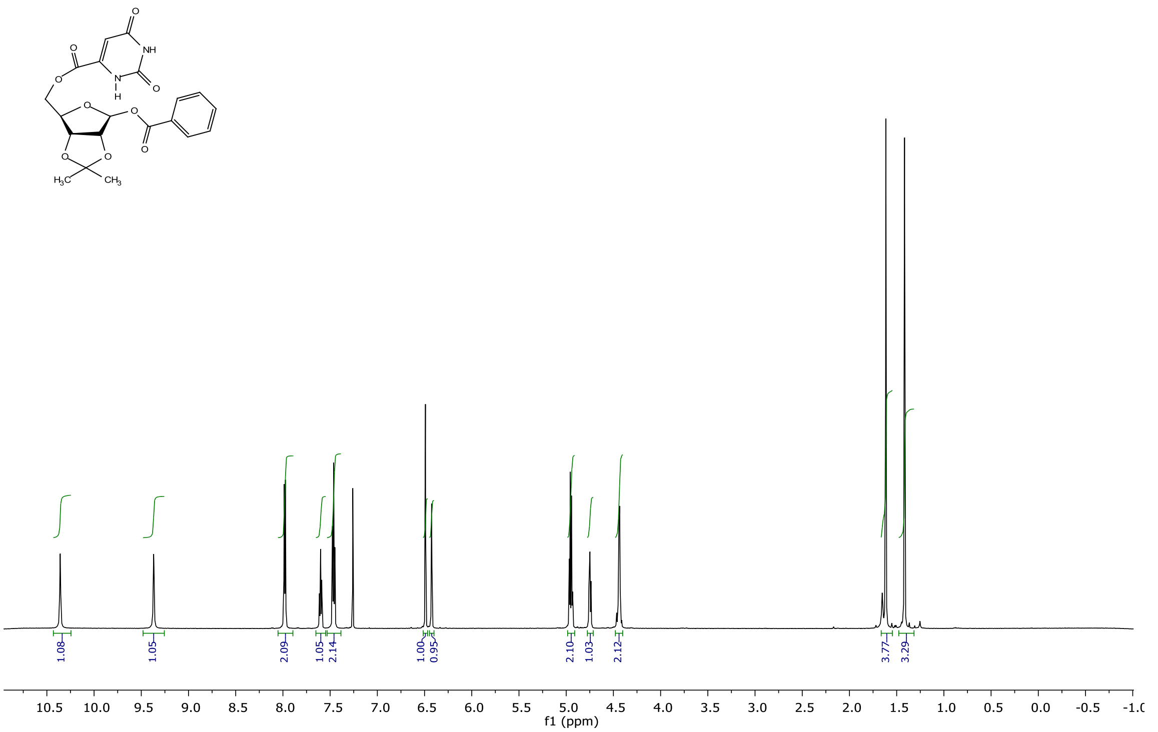

Figure S23 ^{1}H NMR of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**β) in CDCl$_3$.

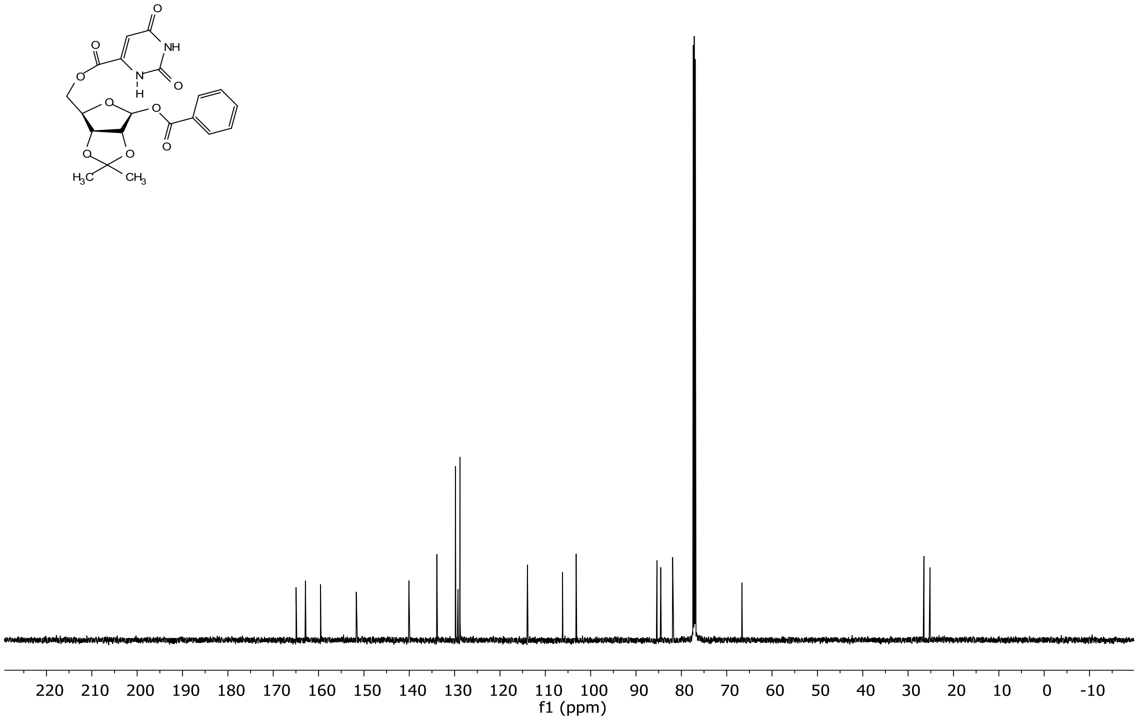

Figure S24 ^{13}C NMR of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**β) in CDCl$_3$.

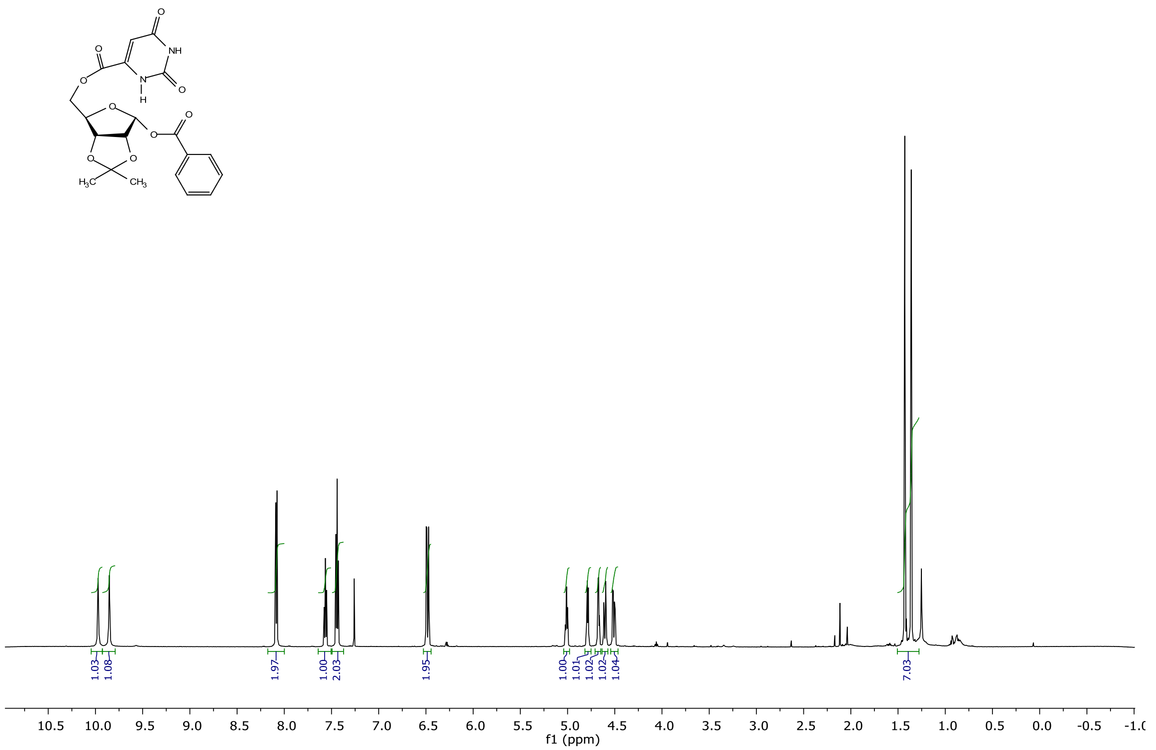

Figure S25 ^{1}H NMR of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**α) in CDCl$_3$.

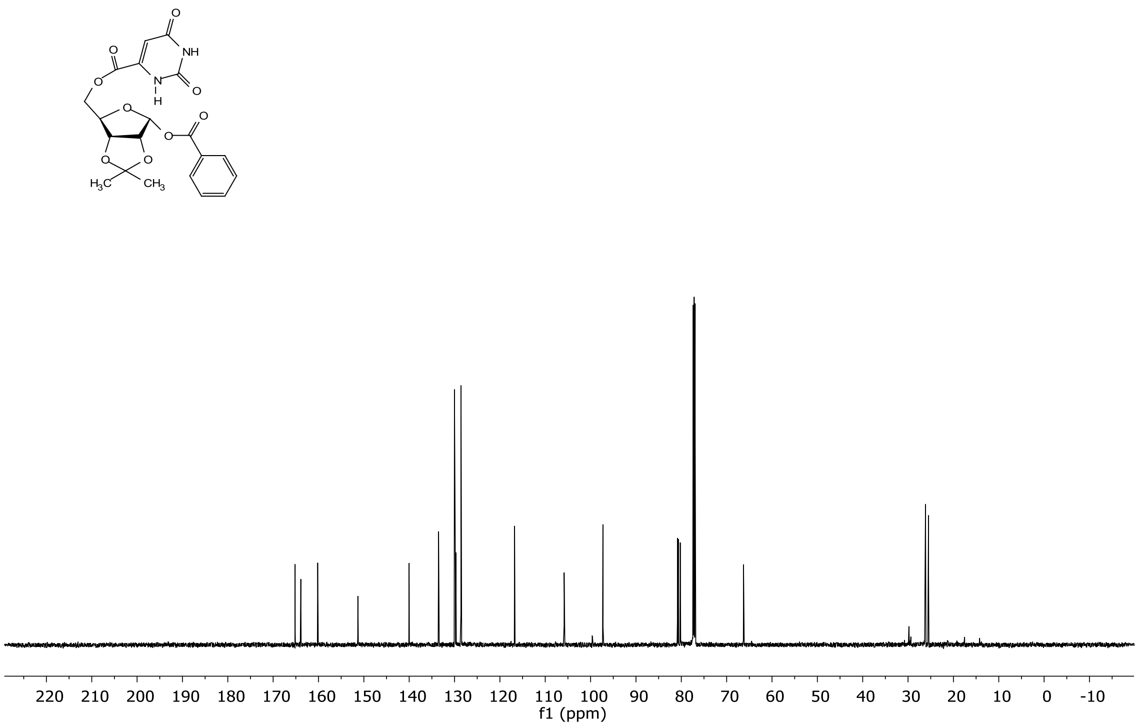

Figure S26 ^{13}C NMR of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**α) in $CDCl_3$.

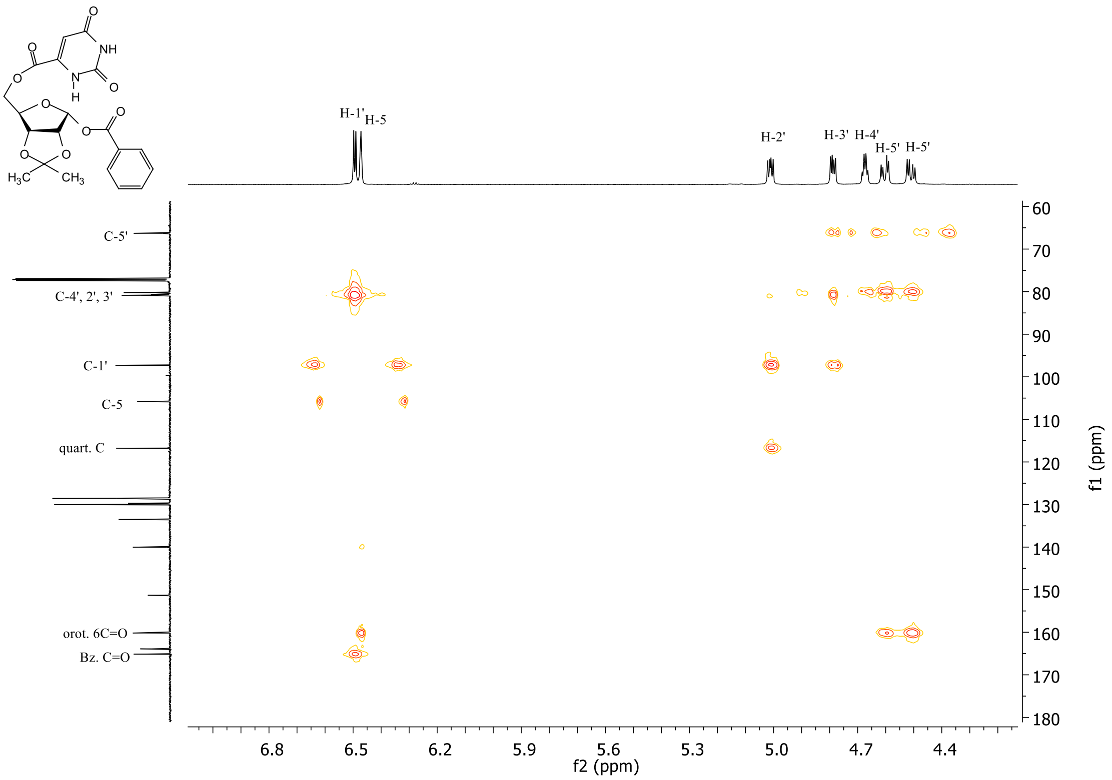

Figure S27 HMBC NMR of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**α) in $CDCl_3$.

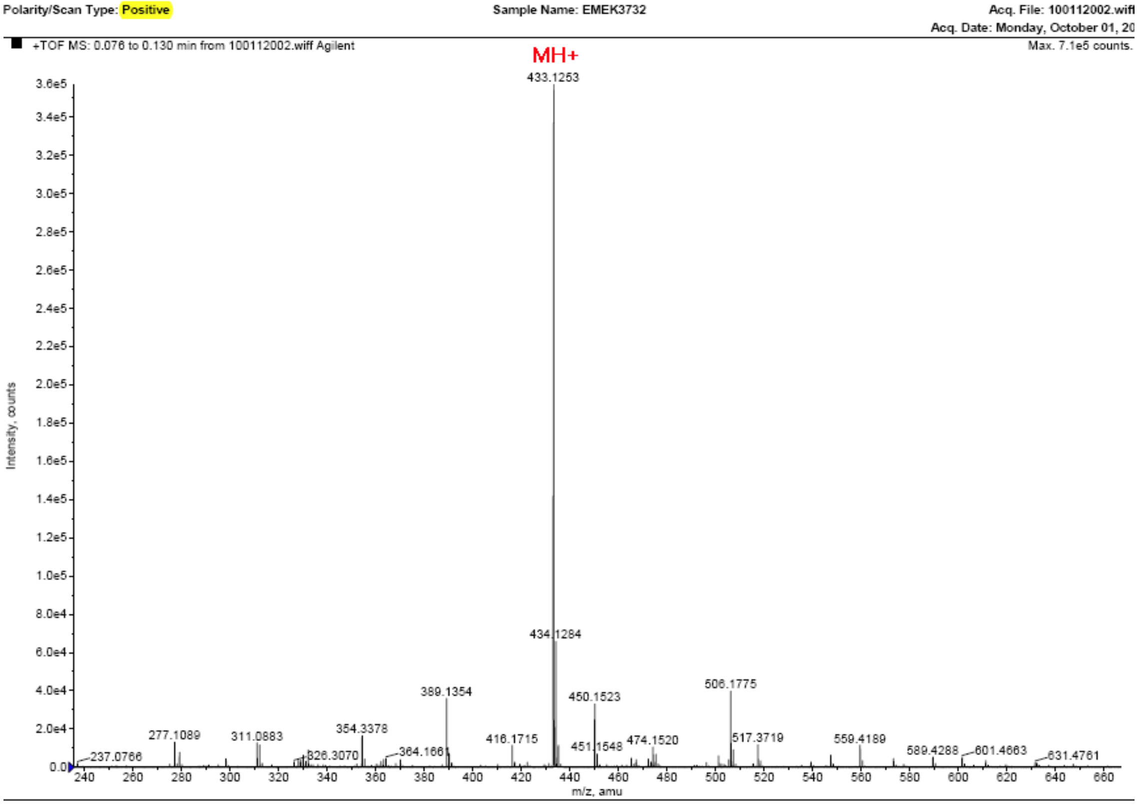

Figure S28 HRMS (ESI-TOF) of 1′-*O*-benzoyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**6b**).

1′-*O*-Acetyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (6c)

From 5c; A slurry of orotic acid (2.37 g, 15.16 mmol) and 1,1′-carbonyldiimidazole (2.90 g, 17.91 mmol) in dry DMF (180 mL) was stirred at room temperature. As the reaction proceeded, slow CO_2 release was observed. After 16 h, pre-dried pyridinium chloride[6] (3.18 g, 27.56 mmol) was added to the crude mixture of orotylimidazole and the resulting mixture was stirred for 10 min. A solution of compound **5c** (3.20 g, 13.78 mmol) in DMF (20 mL) was then added. After being stirred overnight at room temperature, the mixture was partitioned between H_2O (200 mL) and chloroform (200 mL). The aqueous layer was extracted with chloroform (3×150 mL) and the combined organic extract was dried over Na_2SO_4. The crude product was absorbed onto silica while chloroform was evaporated and purified by column chromatography (Hexanes:EtOAc = 1:3) to give compound **6c** as white solid (5.03g, 98%).

From 9; Compound **9** (107 mg, 0.33 mmol) and DMAP (8 mg, 0.06 mmol) were placed in dry pyridine (3.3 mL). To this, acetic anhydride (62 µL, 0.65 mmol) was added at room temperature. After being stirred overnight at room temperature, the mixture was partitioned between chloroform (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The aqueous layer was extracted with chloroform (2×10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 1:3) to give compound **6c** (77 mg, 64%). HRMS (ESI-TOF) calculated for $C_{15}H_{19}N_2O_9$ $[MH]^+$ 371.1085, found 371.1083; TLC (Hexanes:EtOAc =

1:3) R_f = 0.24; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 10.57 (bs, 1H, NH), 9.72 (bs, 1H, NH), 6.48 (t, *J* = 1.7 Hz, 1H, H-5), 6.25 (s, 1H, H-1′), 4.85 (d, *J* = 5.9 Hz, 1H, H-3′), 4.78 (d, *J* = 5.9 Hz, 1H, H-2′), 4.67 (dd, *J* = 7.4, 5.9 Hz, 1H, H-4′), 4.44 (dd, *J* = 11.5, 5.9 Hz, 1H, H-5′), 4.30 (dd, *J* = 11.5, 7.4 Hz, 1H, H-5′), 2.08 (s, 3H, Ac), 1.56 (s, 3H, Me), 1.38 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.3 (Ac; C=O), 163.2 (orot.), 159.6 (orot.; 6-C=O), 151.9 (orot.), 140.3 (orot.), 113.8 (quarternary C), 106.2 (C-5), 102.2 (C-1′), 85.2 (C-2′), 84.5 (C-4′), 81.8 (C-3′), 66.6 (C-5′), 26.5 (Me), 25.1 (Me), 21.3 (Ac).

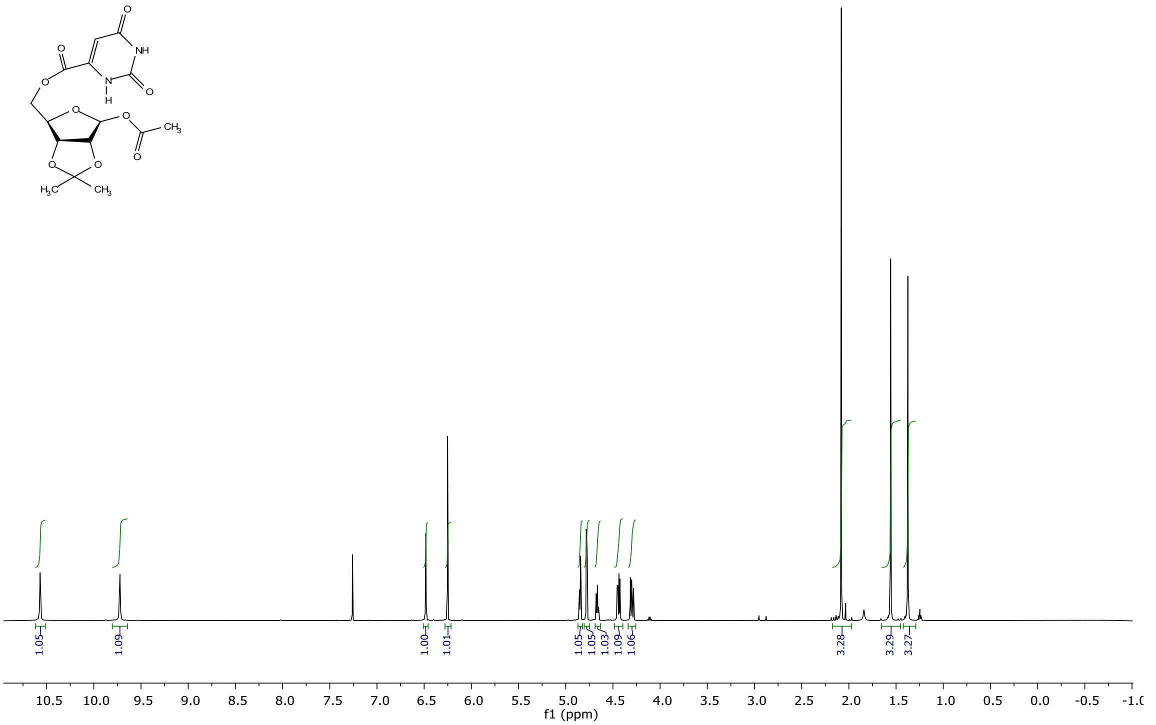

Figure S29 ^{1}H NMR of 1′-*O*-acetyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-β-D-ribofuranoside (**6c**β) in $CDCl_3$.

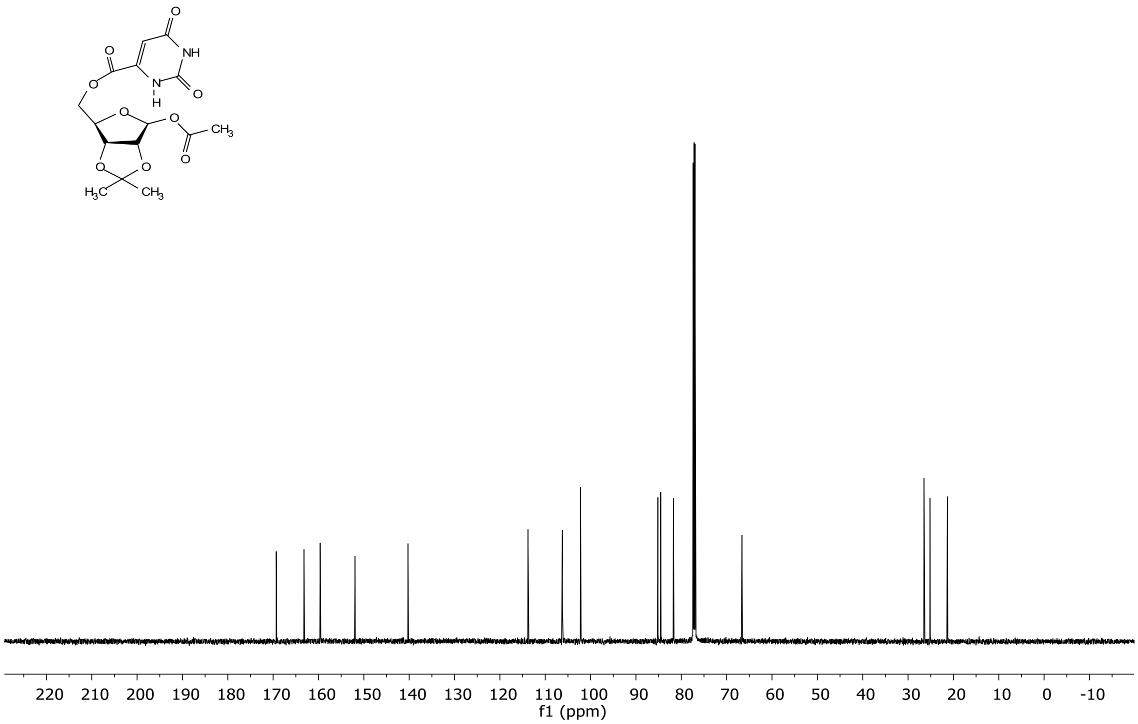

Figure S30 ^{13}C NMR of 1′-*O*-acetyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-β-D-ribofuranoside (**6c**β) in CDCl$_3$.

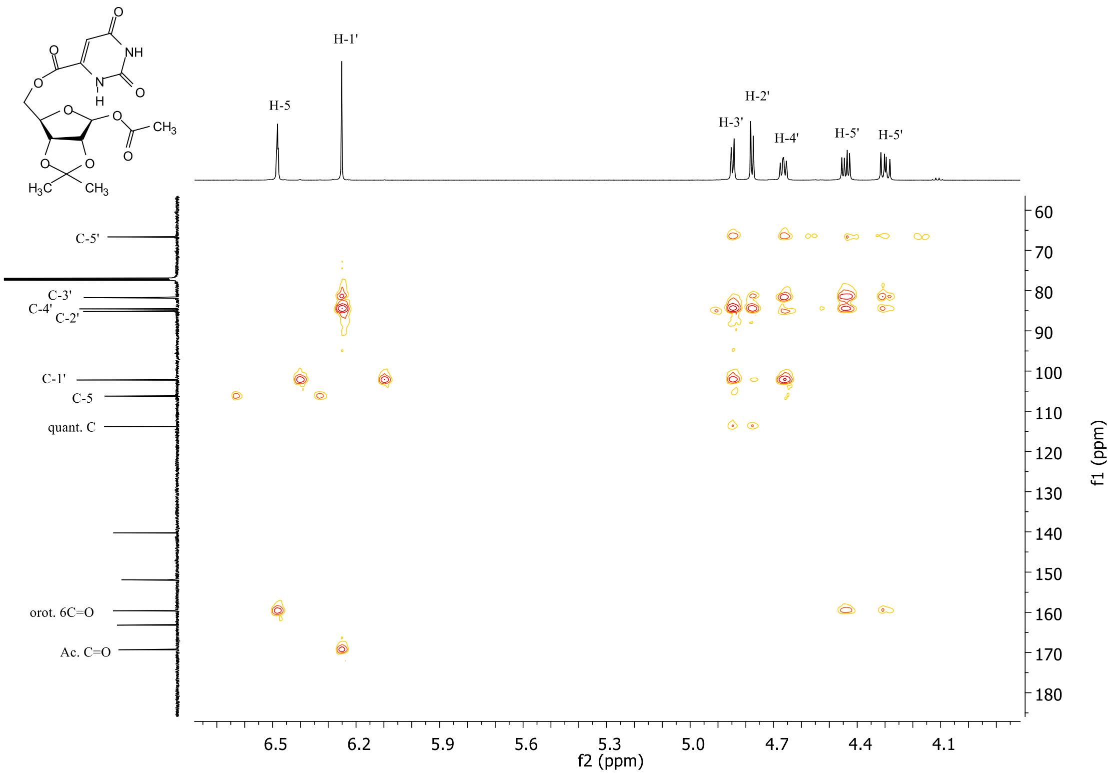

Figure S31 HMBC NMR of 1′-*O*-acetyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-β-D-ribofuranoside (**6c**β) in CDCl$_3$.

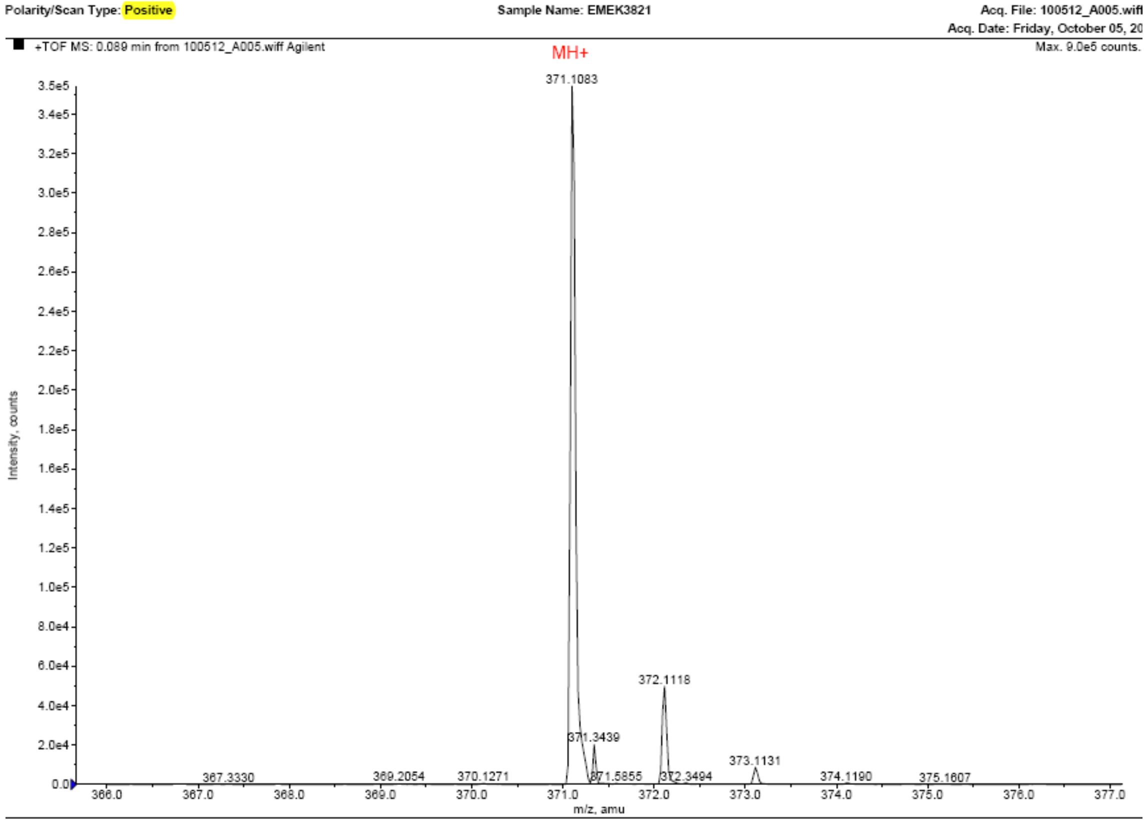

Figure S32 HRMS (ESI-TOF) of 1′-*O*-acetyl-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-β-D-ribofuranoside.

2′,3′-*O*-Isopropylideneorotidine 5′-lactone (7)

From 6c; To a solution of compound **6c** (2.26 g, 6.10 mmol) in dry acetonitrile (610 mL) was added bis(trimethylsilyl)acetamide (3.3 mL, 13.42 mmol) and the mixture was stirred at room temperature for 1h. After the addition of trimethylsilyl trifluoromethanesulfonate (0.55 mL, 3.05 mmol), the reaction mixture was stirred at 50 °C for 2 days. The reaction was quenched by the addition of $NaHCO_3$ (0.4 g). After being stirred for 30 min, the mixture was filtered and concentrated. The crude was purified by column chromatography (CH_2Cl_2:Acetone = 4:1) to give compound **7** as white solid (1.43 g, 76%).

From 15; To a solution of compound **15** (193 mg, 0.40 mmol) in dry acetonitrile (40 mL) was added bis(trimethylsilyl)acetamide (0.44 mL, 1.76 mmol) and the mixture was stirred at room temperature for 1h. After the addition of trimethylsilyl trifluoromethanesulfonate (72 μL, 0.40 mmol), the reaction mixture was stirred at 50 °C for 20 h. The reaction was quenched by the addition of $NaHCO_3$ (0.1 g). After being stirred for 30 min, the mixture was filtered and concentrated. The crude was purified by column chromatography (CH_2Cl_2:Acetone = 4:1) to give compound **7** as white solid (66 mg, 53%); HRMS (ESI-TOF) calculated for $C_{13}H_{15}N_2O_7$ $[MH]^+$ 311.0874, found 311.0864. TLC (CH_2Cl_2:Acetone = 4:1) $R_f = 0.53$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 5.75 (s, 1H, H-5), 5.73 (s, 1H, H-1′), 5.11 (d, J = 5.9 Hz, 1H, H-2′), 4.67 (dd, J = 10.4, 5.2 Hz, 1H, H-4′), 4.62 (d, J = 5.2 Hz, 1H, H-3′), 4.56 (dd, J = 10.4, 5.9 Hz, 1H, H-5′), 4.19-4.16 (m, 1H, H-5′), 1.43 (s, 3H, Me), 1.28 (s, 3H, Me); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.2 (orot.; 6-

C=O), 162.5 (orot.), 149.6 (orot.), 142.9 (orot.), 111.7 (quaternary C), 102.8 (C-5), 94.8 (C-1′), 86.2 (C-2′), 85.2 (C-4′), 79.6 (C-3′), 66.0 (C-5′), 26.1 (Me), 24.6 (Me).

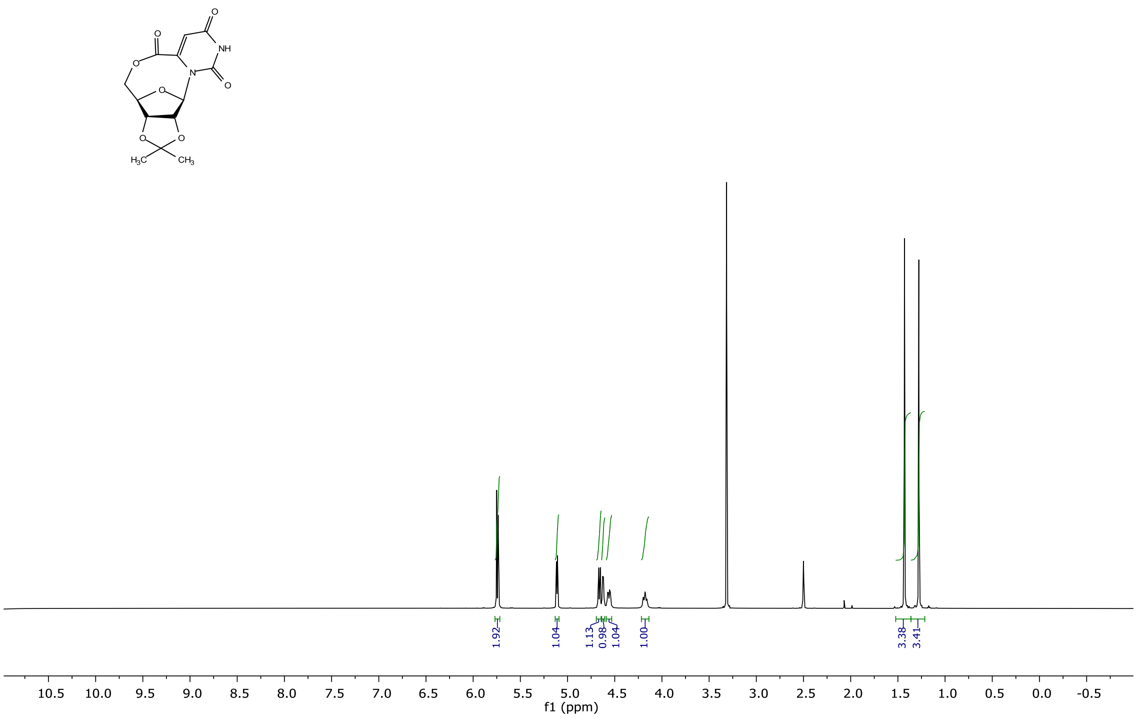

Figure S33 ^{1}H NMR of 2′,3′-*O*-isopropylideneorotidine 5′-lactone (**7**) in DMSO-d_6.

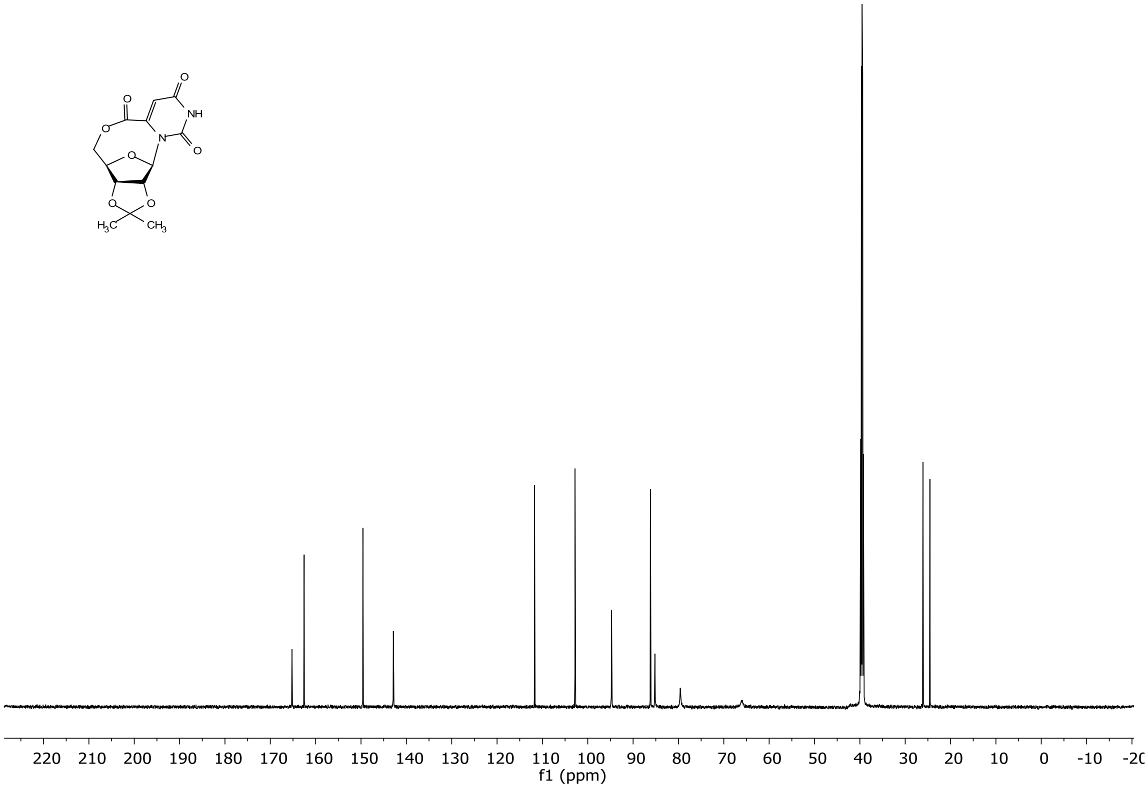

Figure S34 ^{13}C NMR of 2′,3′-*O*-isopropylideneorotidine 5′-lactone (**7**) in DMSO-d_6.

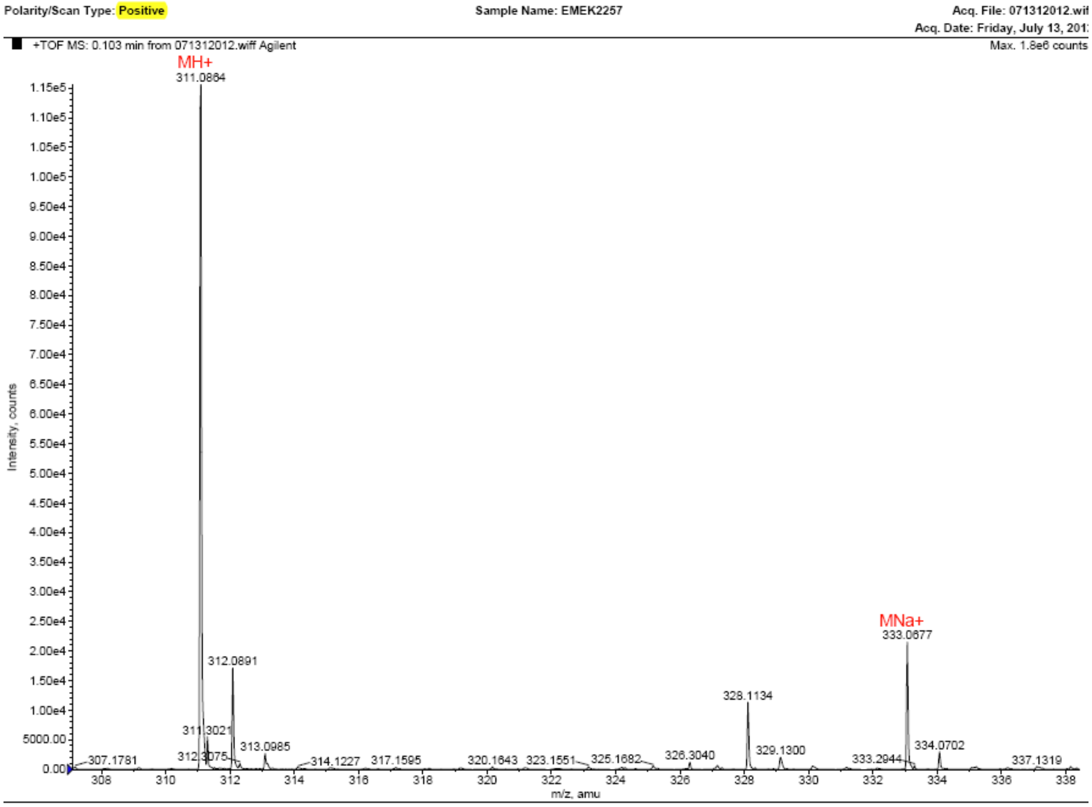

Figure S35 HRMS (ESI-TOF) of 2′,3′-*O*-isopropylideneorotidine 5′-lactone (**7**).

1′-Hydroxy-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (9)

A slurry of orotic acid (0.28 g, 1.8 mmol) and 1,1′-carbonyldiimidazole (0.34 g, 2.1 mmol) in dry DMF (10 mL) was stirred at room temperature. As the reaction proceeded, slow CO_2 release was observed. After 16 h, the crude orotylimidazole was filtered, washed with acetone (10 mL), and dried under high vacuum. The crude orotylimidazole (0.53g), pre-dried pyridinium chloride[6] (0.28 g, 2.4 mmol) and 2,3-*O*-isopropylidene-D-ribofuranoside[2] (0.23 g, 1.2 mmol) were then placed in dry acetonitrile (20 mL) and the reaction mixture was stirred overnight at room temperature. After filtration through Celite, the filtrate was concentrated and the residue was partitioned between H_2O (80 mL) and chloroform (80 mL). The aqueous layer was extracted with chloroform (2×50 mL) and combined organic extract was dried over Na_2SO_4. The crude product was absorbed onto silica while chloroform was evaporated, and purified by column chromatography (Hexanes:EtOAc = 1:1) to give compound **9** as white solid (0.27g, 68%). HRMS (ESI-TOF) calculated for $C_{13}H_{17}N_2O_8$ $[MH]^+$ 329.0979, found 329.0976. TLC (Hexanes:EtOAc = 1:3) $R_f = 0.27$; ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.77 (bs, 1H, OH), 6.12 (d, J = 1.9 Hz, 1H, H-5), 5.26 (d, J = 3.3 Hz, 1H, H-1′), 4.87-4.83 (m, 1H, H-3′), 4.59-4.49 (m, 1H, H-2′), 4.35-4.26 (m, 3H, H-4′,5′,5″), 1.39 (s, 3H, Me), 1.26 (s, 3H, Me); ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.8 (orot.), 159.9 (orot.; 6-C=O), 150.8 (orot.), 141.3 (orot.), 111.4 (quaternary C), 103.9 (C-5), 102.3 (C-1′), 85.7 (C-2′), 82.5 (C-3′), 81.6 (C-4′), 67.0 (C-5′), 26.4 (Me), 24.8 (Me).

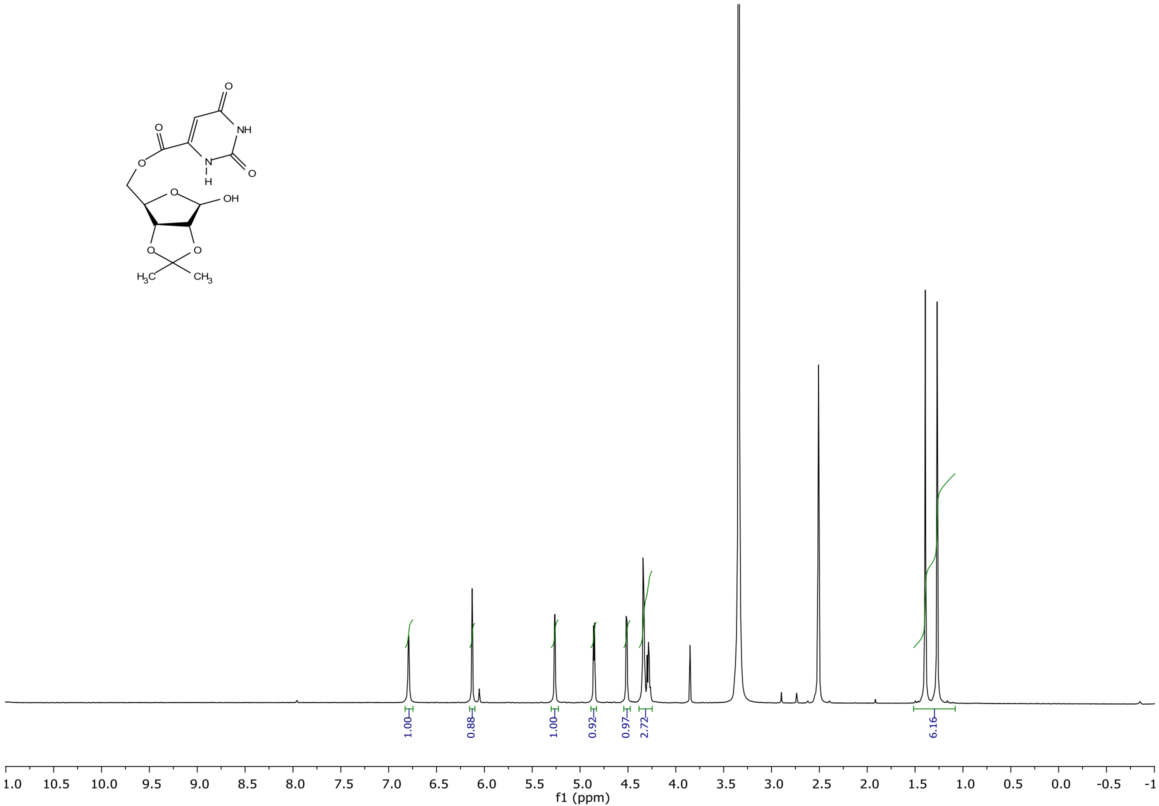

Figure S36 ^{1}H NMR of 1′-hydroxy-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**9**) in DMSO-d_6.

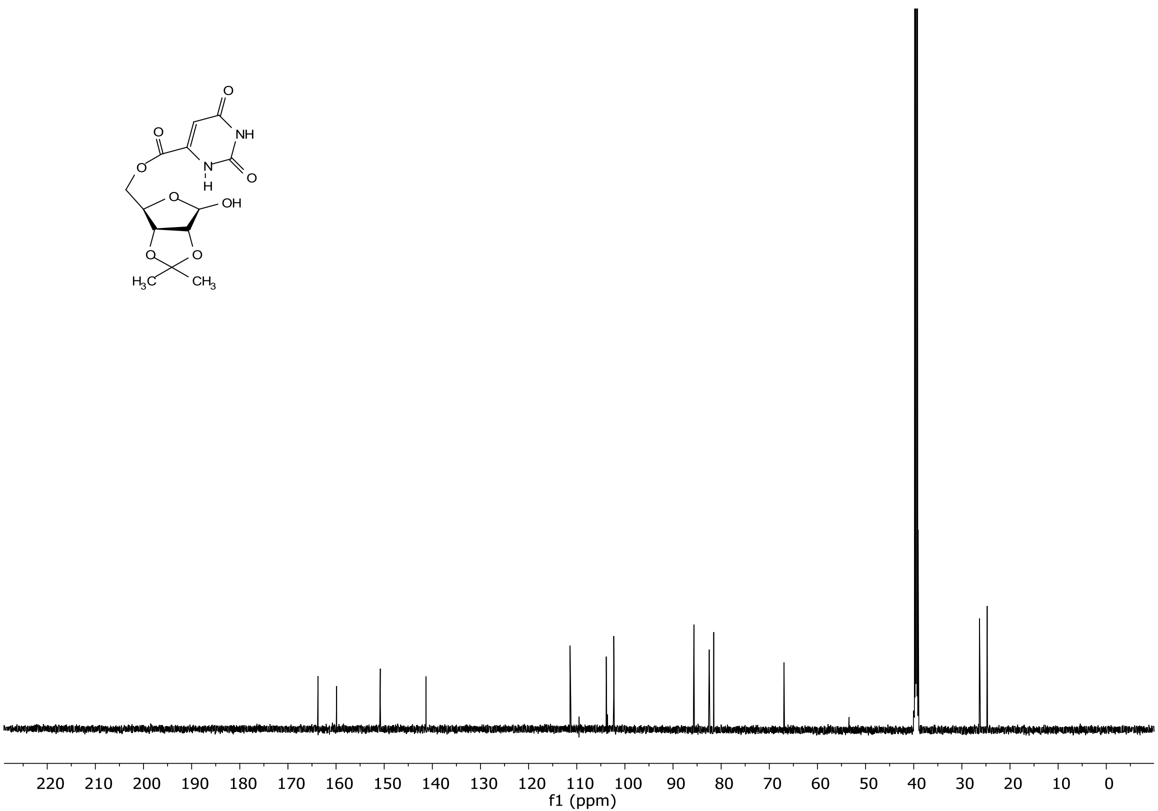

Figure S37 ^{13}C NMR of 1′-hydroxy-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**9**) in DMSO-d_6.

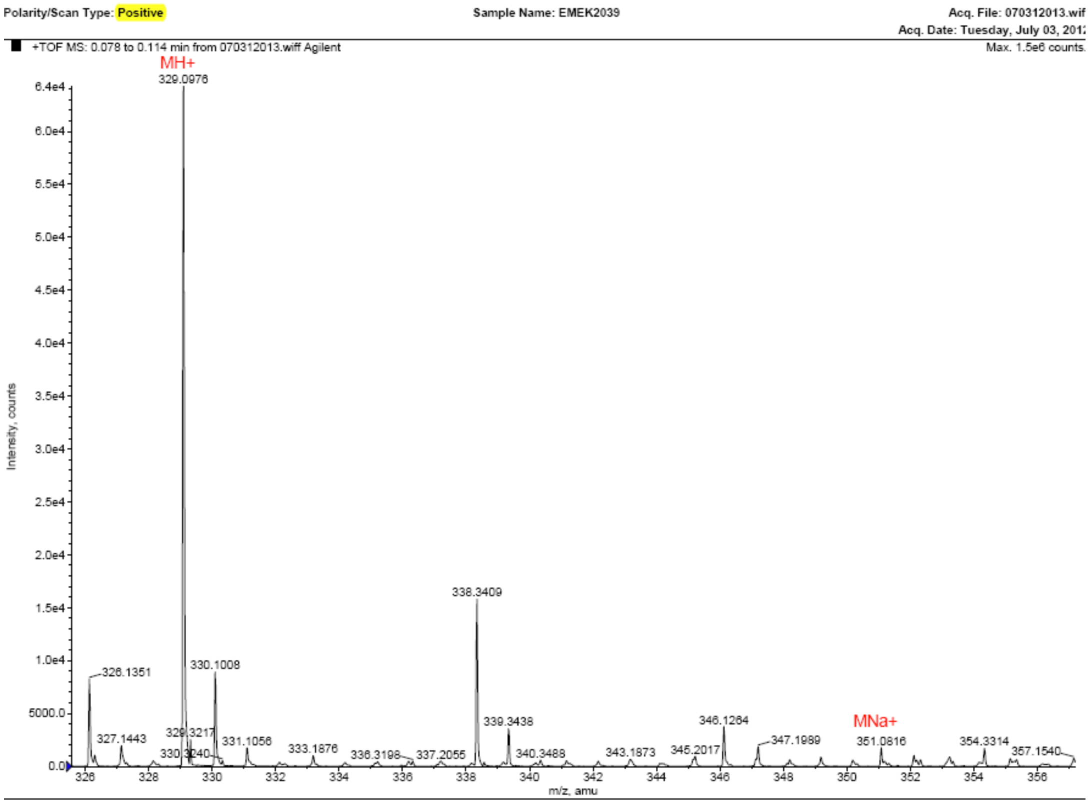

Figure S38 HRMS (ESI-TOF) of 1′-hydroxy-2′,3′-*O*-isopropylidene-5′-*O*-orotyl-D-ribofuranoside (**9**).

Orotidine methyl ester (10)

Method A To a solution of compound **7** (1.42 g, 4.57 mmol) in anhydrous MeOH (45 mL) was added 0.2 eq of 1 M NaOMe in MeOH (0.92 mL, 0.92 mmol) and the mixture was stirred at room temperature overnight. While the mixture was concentrated, the residue was absorbed onto silica and purified by column chromatography (CH_2Cl_2:Acetone = 3:1→1:1) to give 2′,3′-*O*-isopropylideneorotidine methyl ester as white foam (1.42 g, 91%). HRMS (ESI-TOF) calculated for 2′,3′-*O*-isopropylideneorotidine methyl ester, $C_{14}H_{19}N_2O_8$ $[MH]^+$ 343.1136, found 343.1147. TLC (CH_2Cl_2:Acetone = 4:1) $R_f = 0.29$; -^{1}H-NMR (600 MHz, $CDCl_3$) δ 9.91 (bs, 1H, NH), 6.12 (s, 1H, H-5), 5.82 (d, *J* = 2.4 Hz, 1H, H-1′), 5.22 (dd, *J* = 6.6, 2.4 Hz, 1H, H-2′), 4.96 (dd, *J* = 6.6, 4.7 Hz, 1H, H-3′), 4.18-4.16 (m, 1H, H-4′), 3.95 (s, 3H, OMe), 3.88 (dd, *J* = 12.1, 3.2 Hz, 1H, H-5′), 3.78 (dd, *J* = 12.1, 4.7 Hz, 1H, H-5′), 1.54 (s, 3H, Me), 1.35 (s, 3H, Me); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.1 (orot.), 161.8 (orot.; 6-C=O), 150.8 (orot.), 145.1 (orot.), 114.7 (quaternary C), 106.7 (orot.; C-5), 93.8 (C-1′), 87.8 (C-4′), 84.2 (C-2′), 80.2 (C-3′), 62.6 (C-5′), 54.2 (OMe), 27.3 (Me), 25.4 (Me).

2′,3′-*O*-isopropylideneorotidine methyl ester (1.34 g, 3.91 mmol) was then treated with aqueous 60% trifluoroacetic acid (v/v, 15mL) at 0 °C and the mixture was stirred at 4 °C overnight. The mixture was further stirred at room temperature for 5h. The volatiles were then co-evaporated with EtOH (3×20 mL) and the residue was absorbed onto silica and purified by column chromatography (CH_2Cl_2:MeOH = 20:1→10:1) to give compound **10** as white foam (0.94 g, 80%).

Method B The compound **7** (173 mg, 0.56 mmol) and $In(OTf)_3$ (3 mg, 1 mol%) were placed in a Biotage microwave process tube with a stir-bar, and 3 mL of CH_3CN/H_2O (9:1, v/v) was added. The resulting mixture was heated under microwave conditions at 120 °C for 30 min. After being concentrated, the residue was taken up into CH_2Cl_2 (5 mL) and MeOH (2 mL). Then, Si-Amine (20 mg) was added to remove metal residues. The mixture was stirred for 10 min, filtered and the filtrate was concentrated to dryness. The residue was dissolved in anhydrous MeOH (20 mL), and 1M NaOMe (0.11 mL) was added. The mixture was stirred at room temperature overnight. While the mixture was concentrated, the residue was absorbed onto silica and purified by column chromatography (CH_2Cl_2:MeOH = 20:1) to give compound **10** as white foam (112 mg, 67% over two steps). HRMS (ESI-TOF) calculated for **10**, $C_{11}H_{15}N_2O_8$ $[MH]^+$ 303.0823, found 303.0822. TLC (CH_2Cl_2:MeOH = 20:1) $R_f = 0.20$; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 5.96 (d, *J* = 2.2 Hz, 1H, H-5), 5.38 (d, *J* = 4.3 Hz, 1H, H-1′), 5.17 (br s, 1H, OH), 4.93 (br s, 1H, OH), 4.69 (br s, 1H, OH), 4.45 (dd, *J* = 6.2, 4.3 Hz, 1H, H-2′), 3.93 (t, *J* = 6.2 Hz, 1H, H-3′), 3.85 (s, 3H, OMe), 3.68-3.65 (m, 1H, H-4′), 3.57 (dd, *J* = 11.7, 3.9 Hz, 1H, H-5′), 3.41 (dd, *J* = 11.7, 6.5 Hz, 1H, H-5′); ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.0 (orot.; 6-C=O), 162.8 (orot.), 150.5 (orot.), 146.4 (orot.), 104.7 (C-5), 93.9 (C-1′), 85.4 (C-4′), 72.0 (C-2′), 69.8 (C-3′), 62.1 (C-5′), 54.4 (OMe).

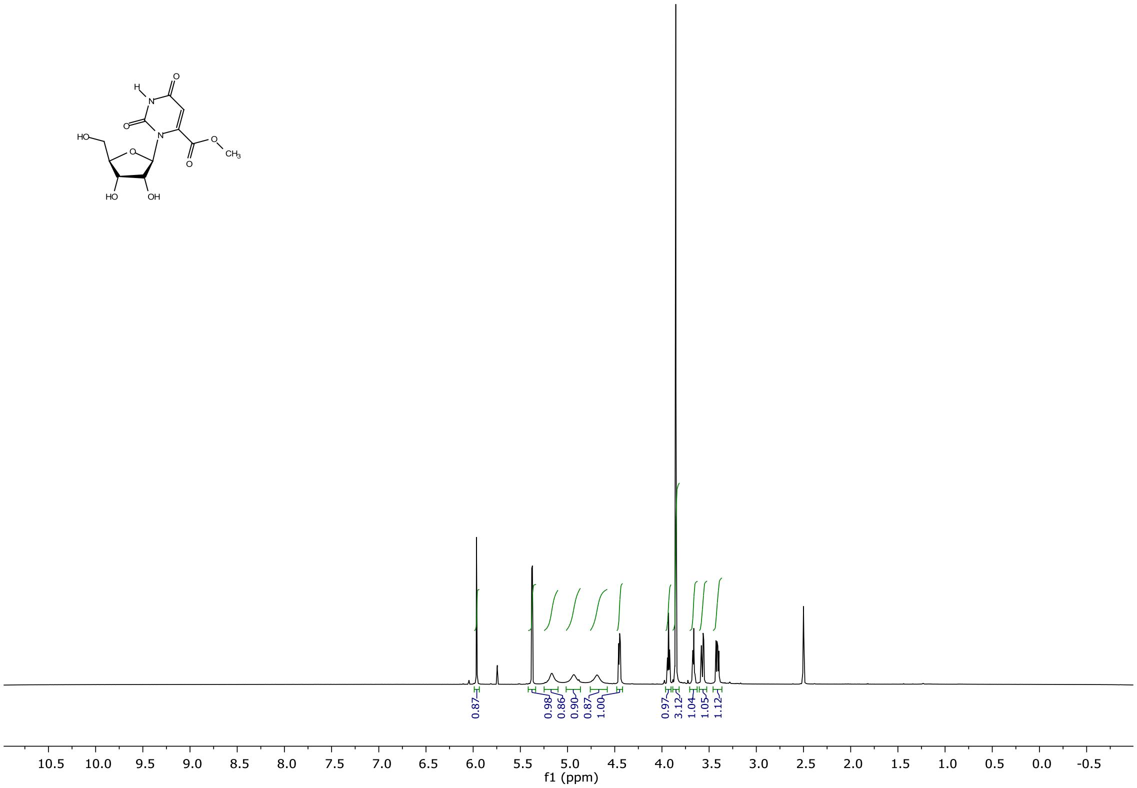

Figure S39 ^{1}H NMR of orotidine methyl ester (**10**) in DMSO-d_6.

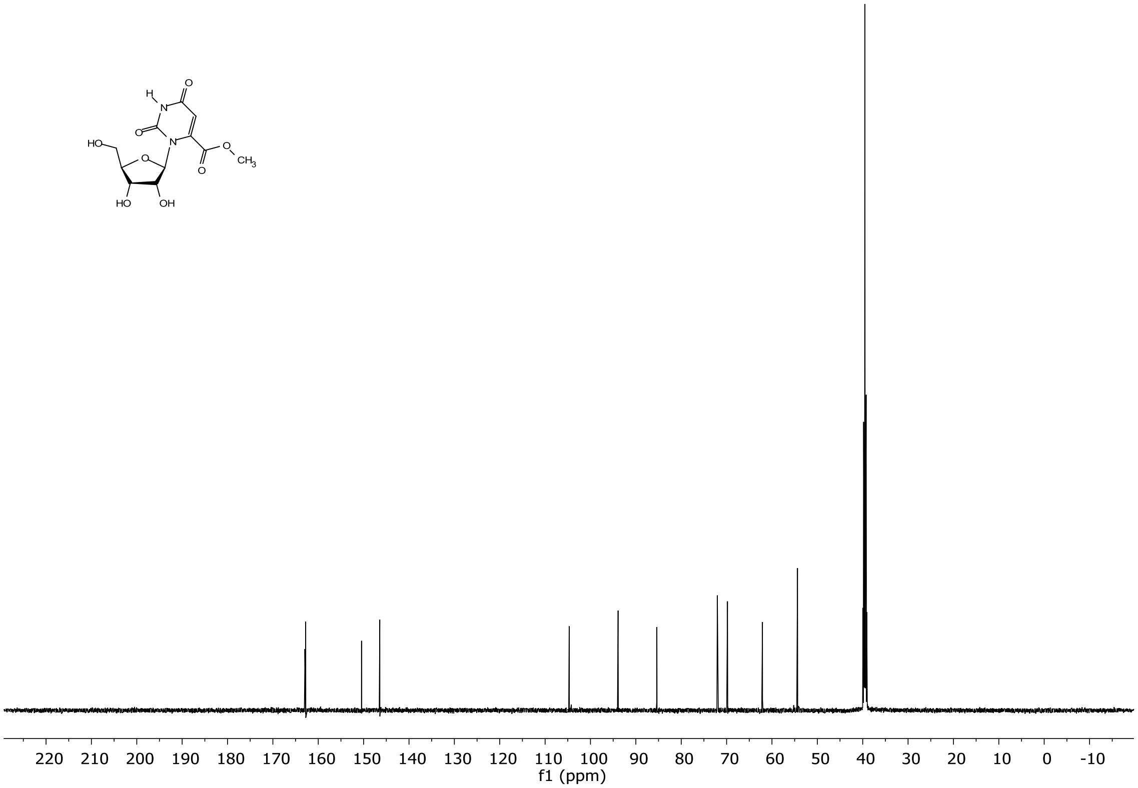

Figure S40 ^{13}C NMR of orotidine methyl ester (**10**) in DMSO-d_6.

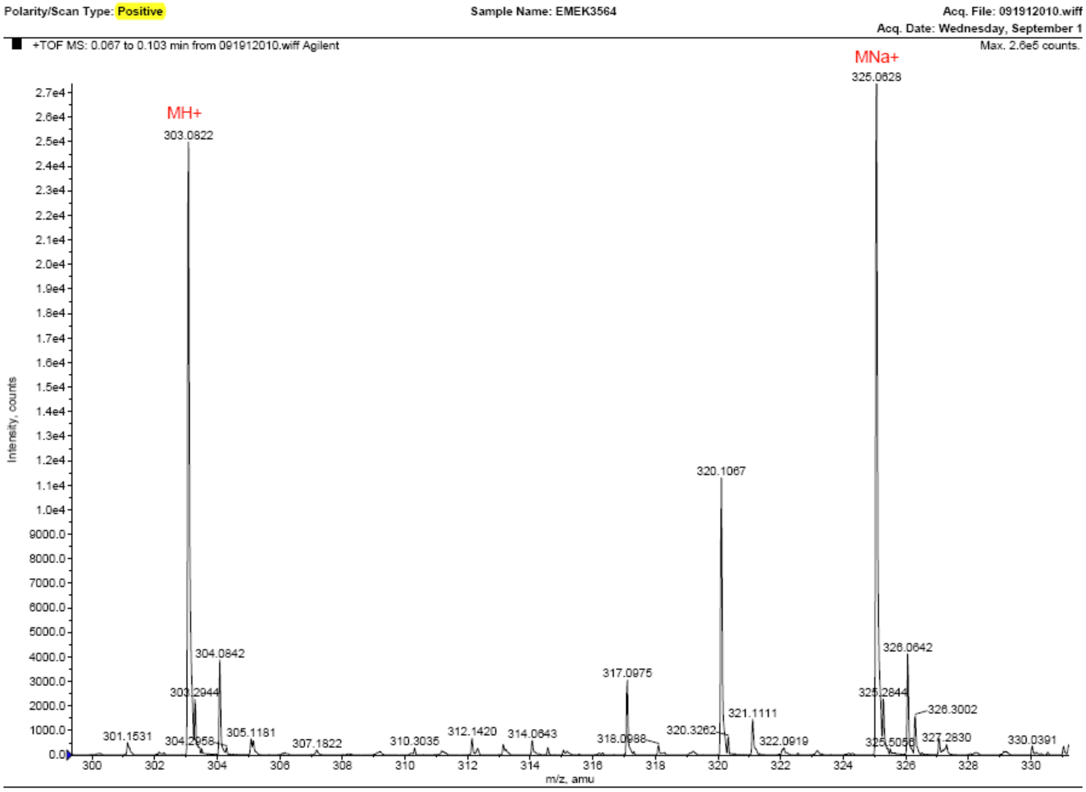

Figure S41 HRMS (ESI-TOF) of orotidine methyl ester (**10**).

Orotidine (1)

Compound **10** (101 mg, 0.334 mmol) was dissolved in CH_3CN/H_2O (1:1, v/v, 6 mL) and crushed NaOH (27 mg, 0.668 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature overnight. The pH was adjusted with IR-120 (H^+) to pH 3 (pH paper which was pre-wet by H_2O used). After filtration, the filtrate was concentrated to dryness without heating to give orotidine **1** (103.6 mg, quantitative yield). HRMS (ESI-TOF) in negative mode calculated for $C_{10}H_{11}N_2O_8^-$ $[M\text{-}H]^-$ 287.0521, found 287.0524. TLC (*i*PrOH:H_2O:NH_4OH = 17:2:1) $R_f = 0.42$; ^{1}H-NMR (600 MHz, D_2O) δ 5.77 (s, 1H, H-5), 5.55 (d, *J* = 3.5 Hz, 1H, H-1′), 4.74 (dd, *J* = 6.7, 3.5 Hz, 1H, H-2′), 4.35 (t, *J* = 6.7 Hz, 1H, H-3′), 3.95 (td, *J* = 6.7, 3.0 Hz, 1H, H-4′), 3.88 (dd, *J* = 12.4, 3.0 Hz, 1H, H-5′), 3.76 (dd, *J* = 12.4, 6.7 Hz, 1H, H-5′); ^{13}C NMR (151 MHz, D_2O) δ 166.8, 165.7, 154.4, 150.7, 98.1, 94.1, 83.2, 71.5, 68.6, 61.0.

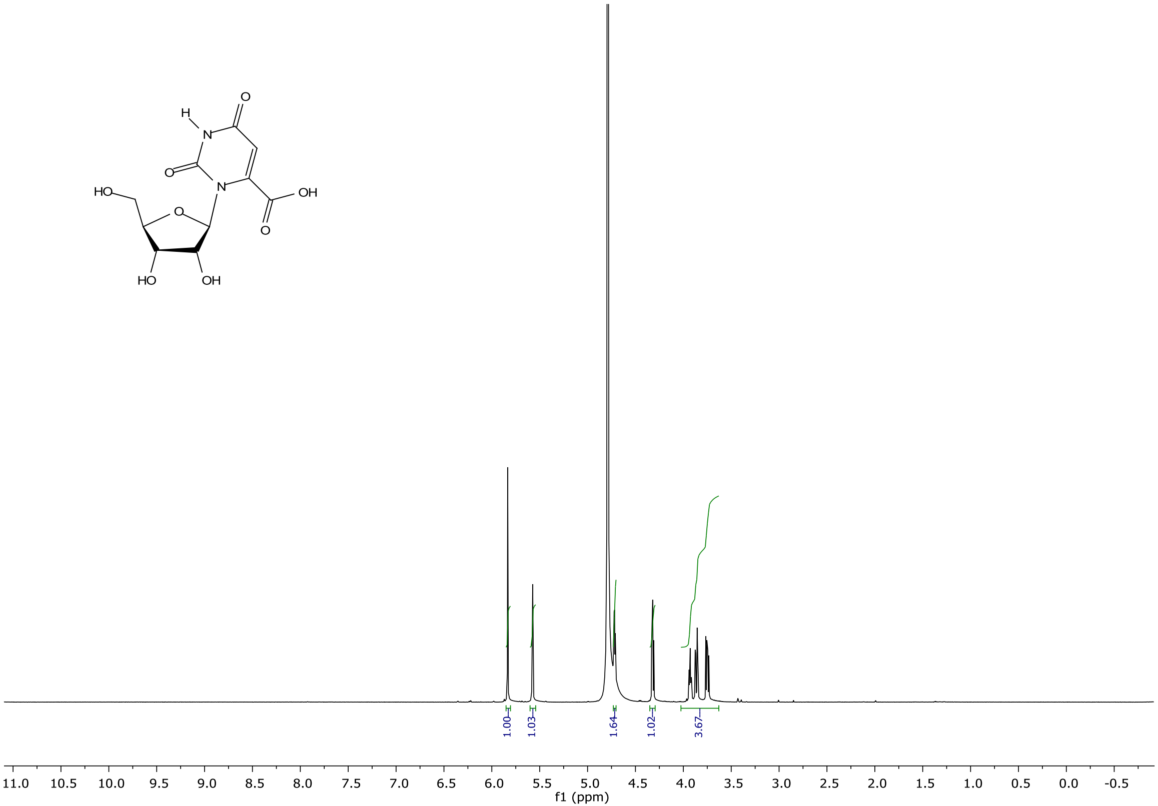

Figure S42 ^{1}H NMR of orotidine (**1**) in D_2O.

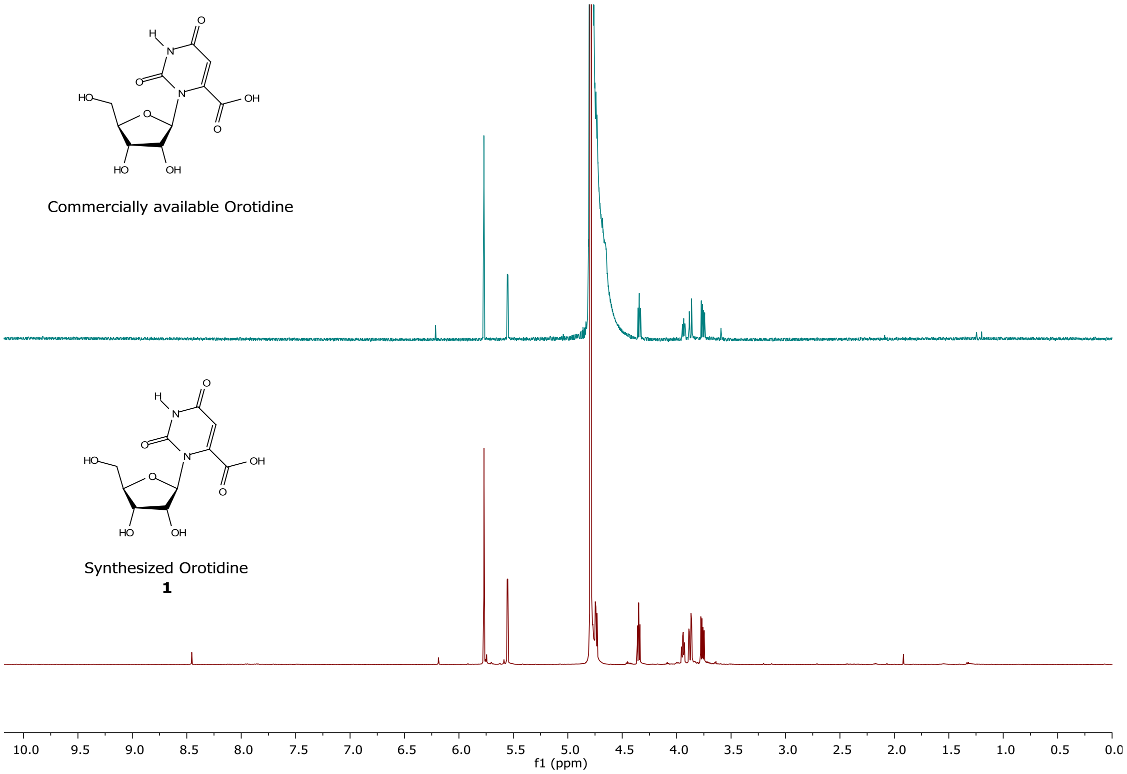

Figure S43 ^{1}H NMR comparison of synthesized orotidine (**1**) with commercially available orotidine (Sigma-Aldrich) in D_2O.

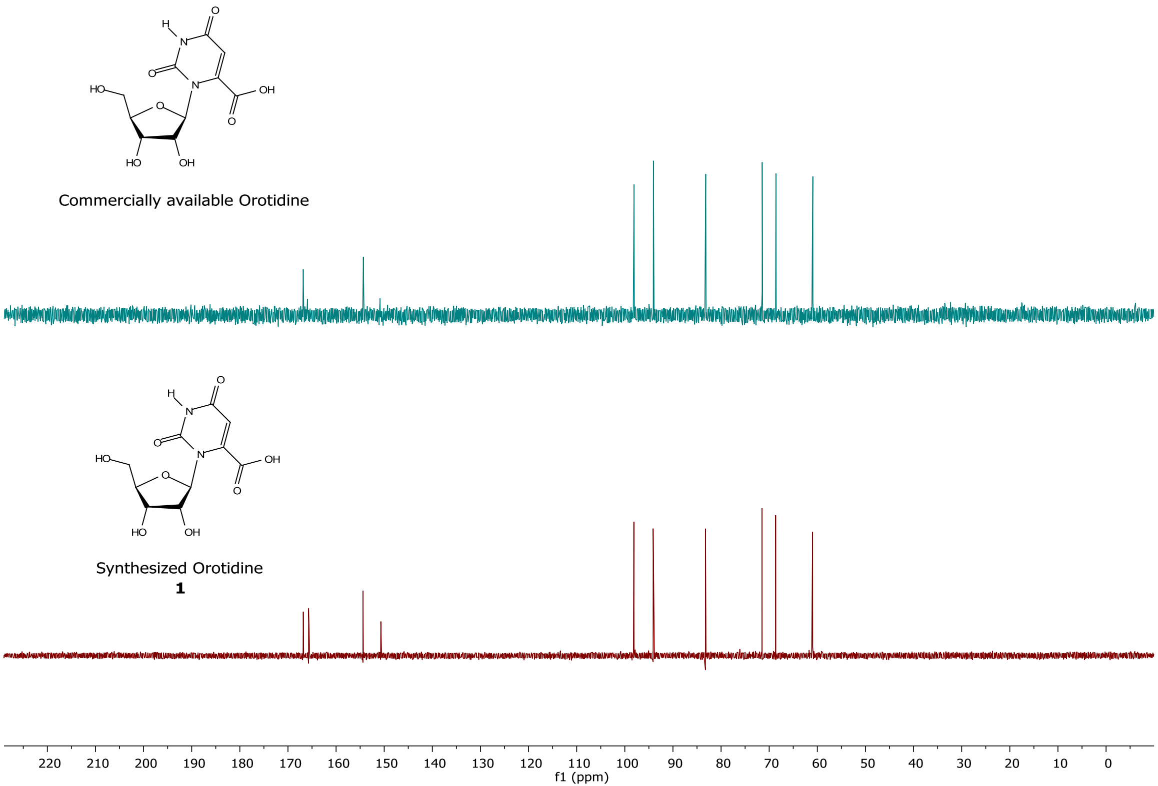

Figure S44 ^{13}C NMR comparison of synthesized orotidine (**1**) with commercially available orotidine (Sigma-Aldrich) in D_2O.

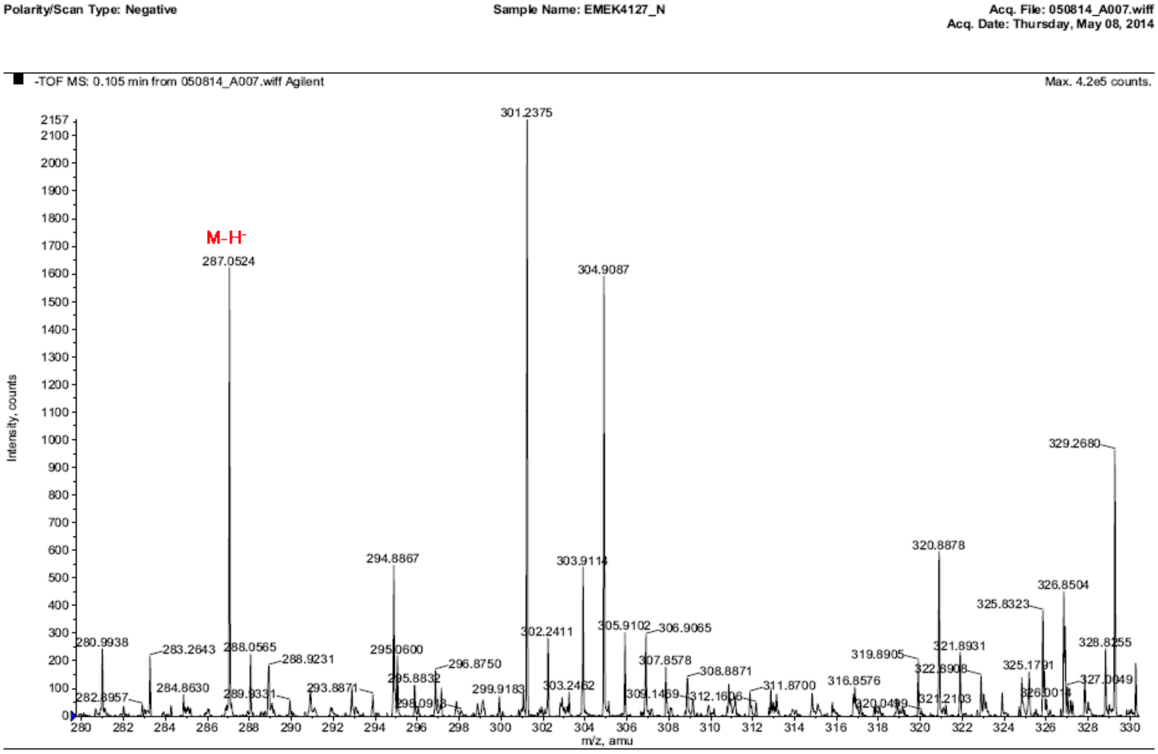

Figure S45 HRMS (ESI-TOF) of orotidine (**1**).

N(3)-(benzoyl)-methyl orotate (11)

Methyl orotate[7] (3.40g, 20.0 mmol) was placed in $CH_3CN/(i\text{Pr})_2NEt$ (200 mL, 5:2, v/v), and benzoyl chloride (5.1 mL, 44.0 mmol) was added dropwise at 0 °C. The mixture was then stirred at room temperature overnight to give N(1), N(3)-dibenzoyl derivative. After concentrated, the residue was dissolved in EtOAc (100 mL) and stirred with 0.25 M K_2CO_3 in dioxane/H_2O (100 mL, 1:1, v/v) for 5h. The mixture was extracted with EtOAc (2×150 mL), dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 5:1→1:1) to give compound **11** as light brown solid (2.80 g, 51.0%). HRMS (ESI-TOF) calculated for $C_{13}H_{11}N_2O_5$ $[MH]^+$ 275.0662, found 275.0662. TLC (Hexanes:EtOAc = 2:1) $R_f = 0.11$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.60 (bs, NH), 7.95-7.93 (m, 2H), 7.69-7.66 (m, 1H), 7.53-7.50 (m, 2H), 6.50 (s, 1H), 4.00 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.0, 162.4, 160.5, 148.8, 139.3, 135.6, 131.2, 130.6, 129.4, 105.1, 54.4.

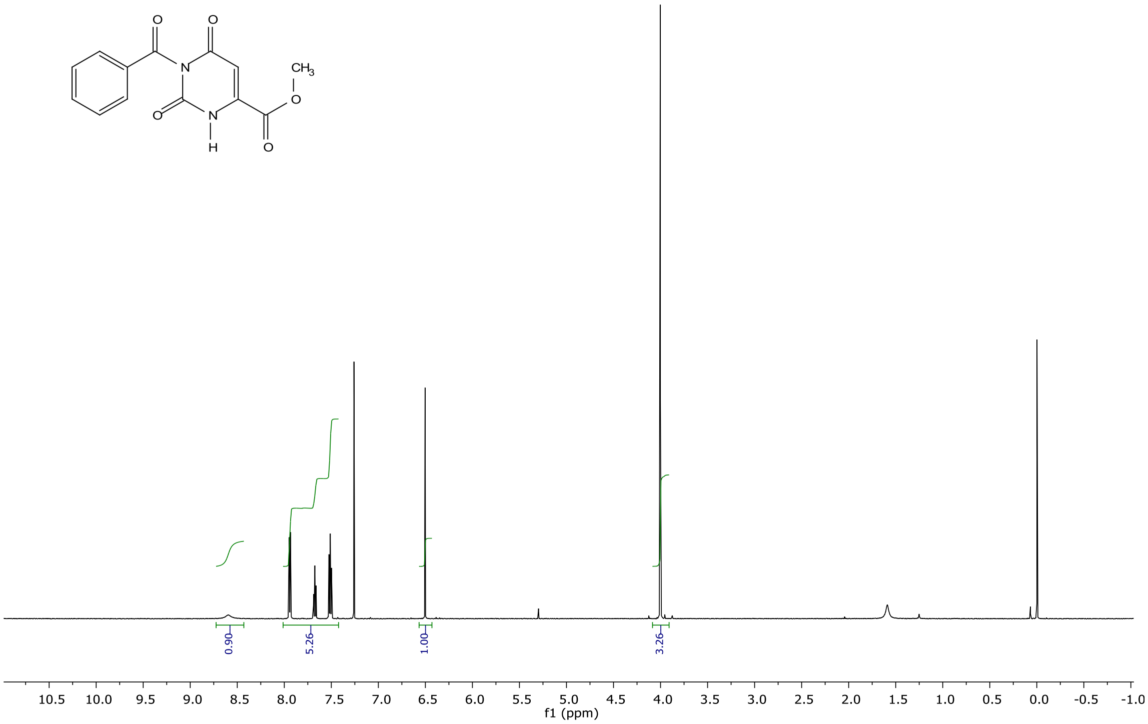

Figure S46 ^{1}H NMR of N(3)-(benzoyl)-methyl orotate (**11**) in $CDCl_3$.

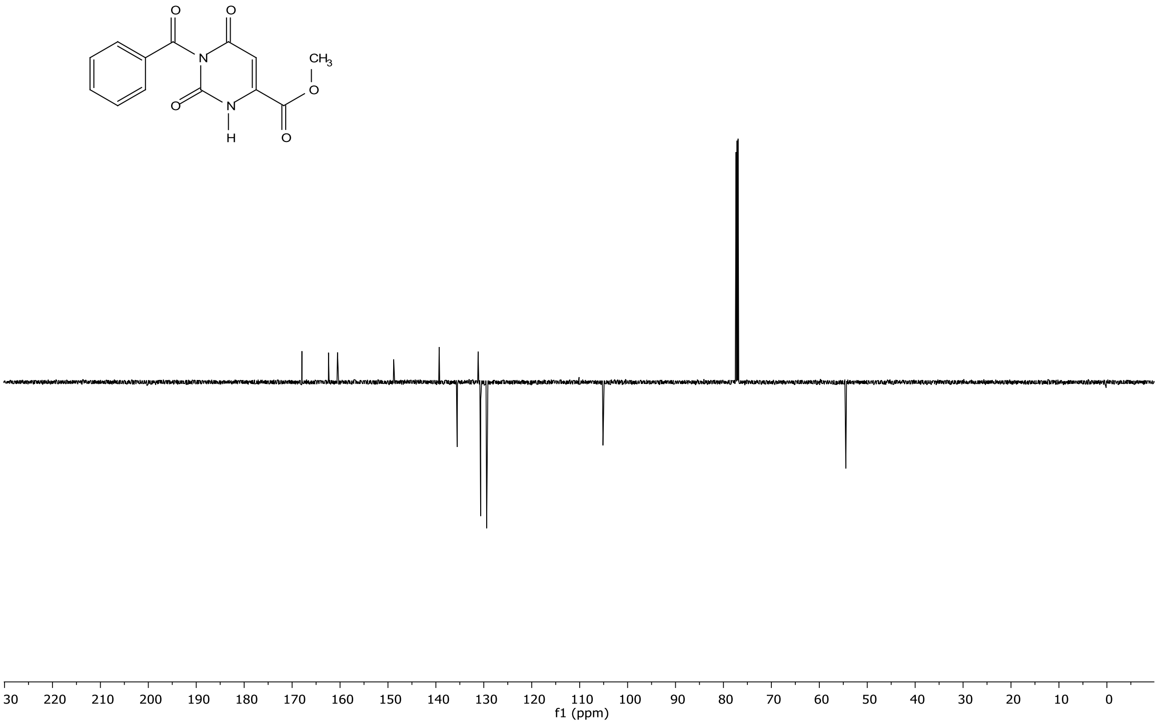

Figure S47 ^{13}C NMR of N(3)-(benzoyl)-methyl orotate (**11**) in CDCl$_3$.

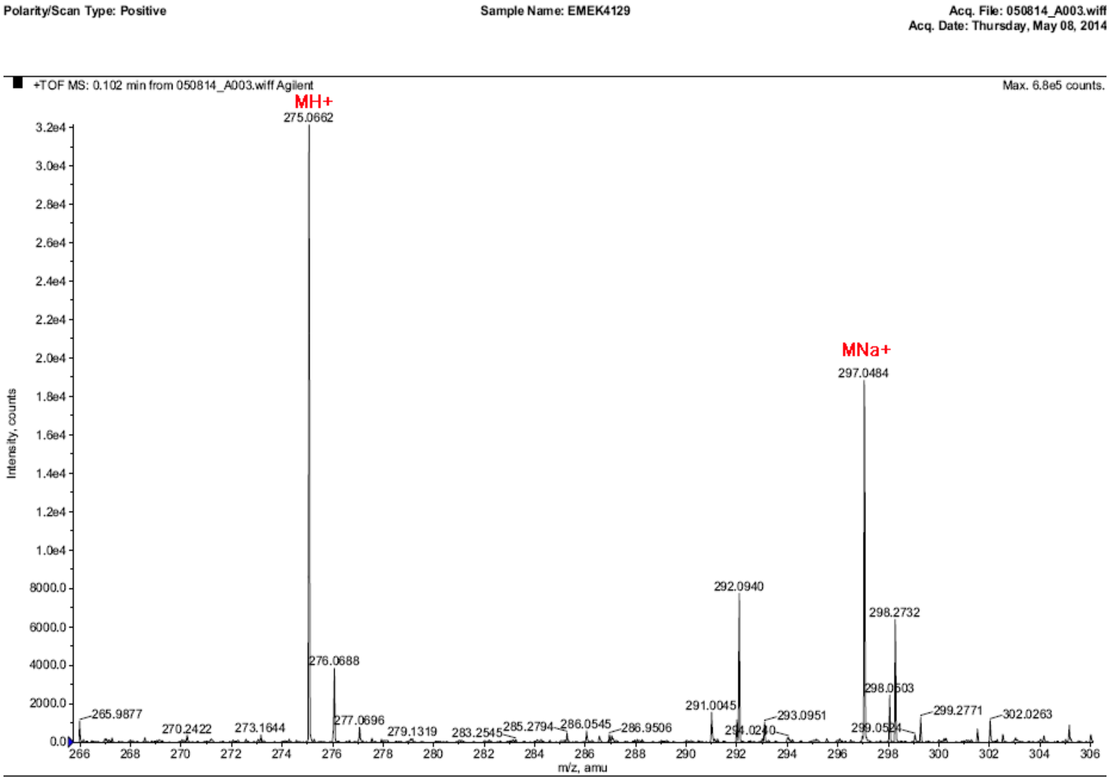

Figure S48 HRMS (ESI-TOF) of N(3)-(benzoyl)-methyl orotate (**11**) in CDCl$_3$.

N(1)-(benzoyl)-methyl orotate

For comparison purpose, N(1)-(benzoyl)-methyl orotate was synthesized according to the known procedure for the synthesis of N(1)-benzoyluracil.[8] To a suspension of methyl orotate[7] (116 mg, 0.68 mmol) in CH_3CN/pyridine (6 mL, 5:1, v/v) was added benzoyl chloride (83 µL, 0.72 mmol) dropwise at 0 °C. The mixture was then stirred at room temperature for 3h. After concentrated, the residue was partitioned between EtOAc (10 mL) and H_2O (10 mL). The aqueous layer was extracted with EtOAc (2×10 mL). The combined organic layer was dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 5:1→1:1) to give N(1)-(benzoyl)-methyl orotate as white solid (21 mg, 25% based on the recovery of 63.9 mg unreacted starting material) and compound **11** (26 mg, 31% based on the recovery of unreacted starting material). TLC for N(1)-(benzoyl)-methyl orotate (Hexanes:EtOAc = 2:1) $R_f = 0.17$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.97 (bs, NH), 7.94-7.91 (m, 2H), 7.68-7.64 (m, 1H), 7.54-7.49 (m, 2H), 6.49 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.0, 162.0, 161.0, 149.4, 143.1, 135.0, 132.5, 130.4, 129.2, 108.4, 54.2.

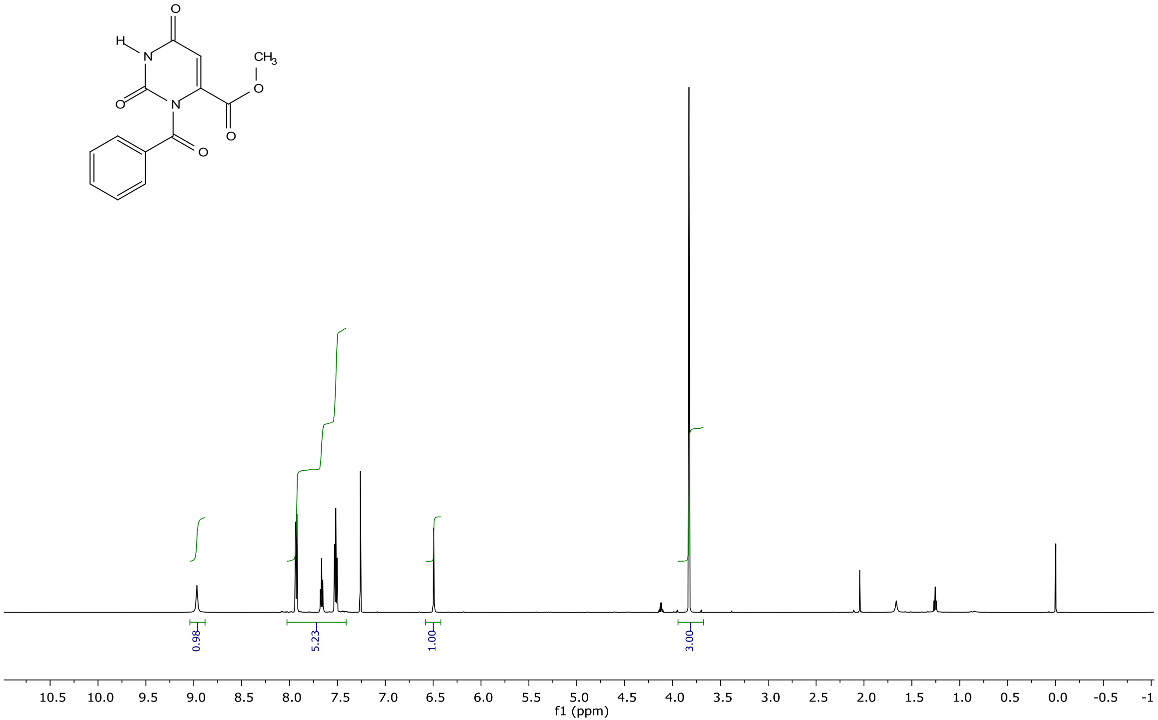

Figure S49 ^{1}H NMR of N(1)-(benzoyl)-methyl orotate in $CDCl_3$.

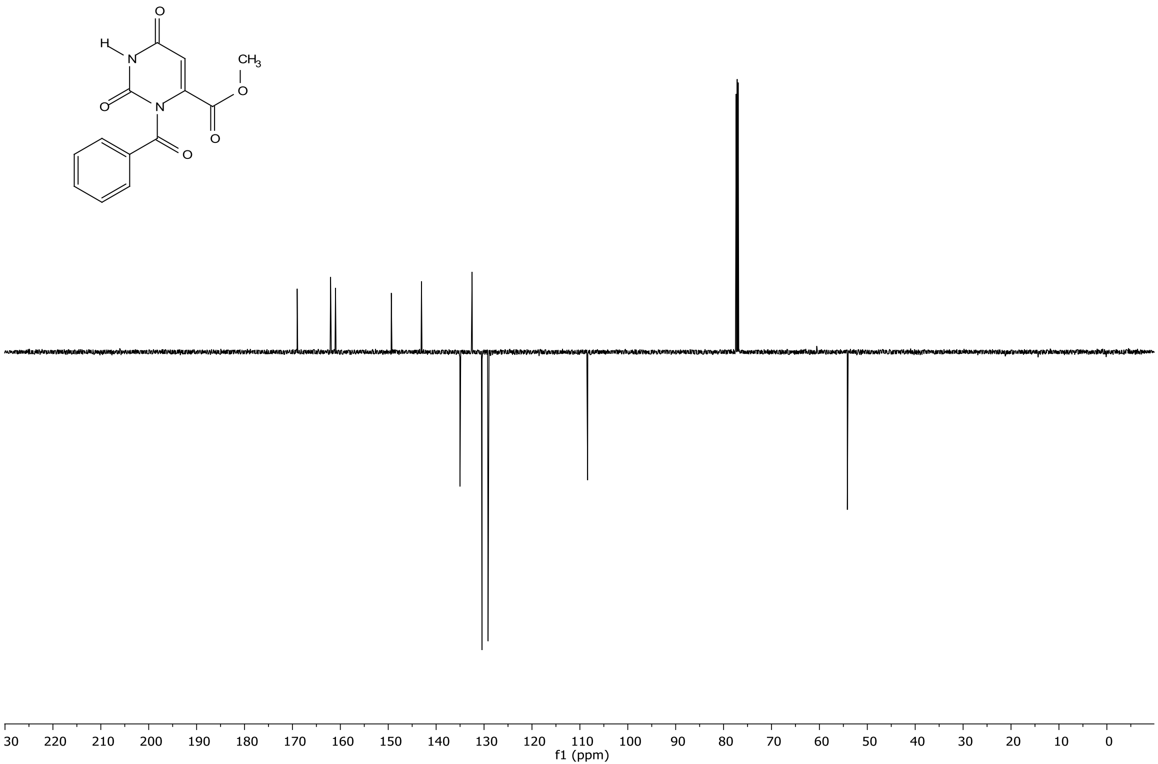

Figure S50 ^{13}C NMR of N(1)-(benzoyl)-methyl orotate in $CDCl_3$.

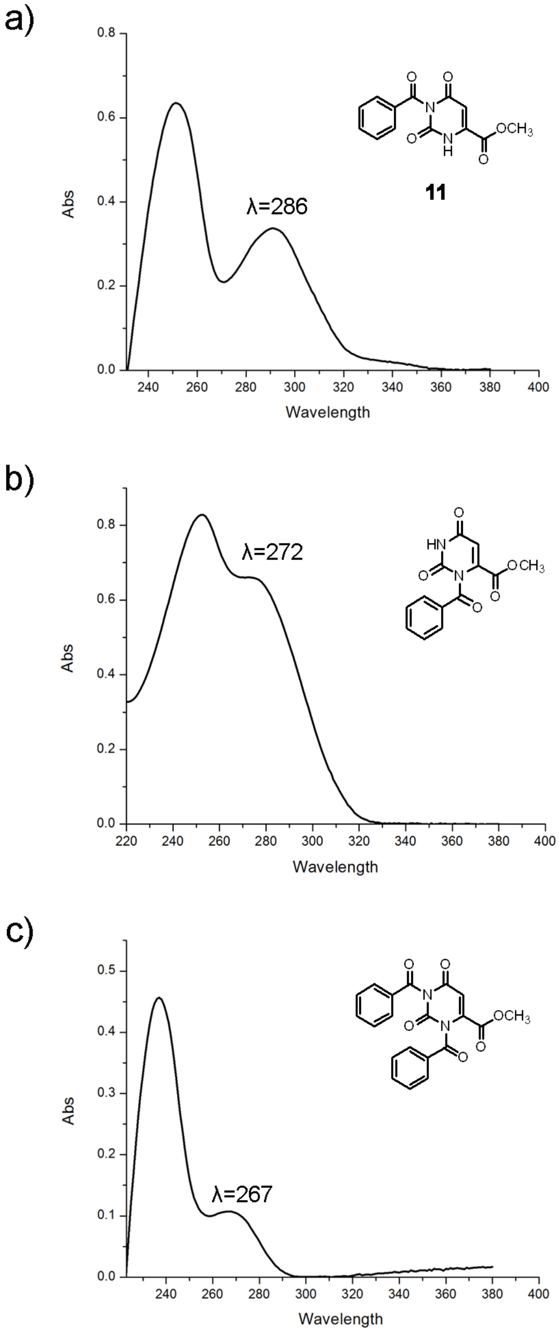

Figure S51 UV absorption spectra of **a)** N(3)-(benzoyl)-methyl orotate **11**, **b)** N(1)-(benzoyl)-methyl orotate and **c)** N(1),N(3)-(dibenzoyl)-methyl orotate in MeOH (the position of benzoyl group at the N(3) positioin in **11** was confirmed by the comparison with the wavelength of orotate moiety of N(1)-benzoylated and N(1),N(3)-dibenzoylated derivatives. The orotate moiety of **11** showed higher wavelength (λ_{max}=286) than those of N(1)-benzoylated derivative (λ_{max}=272) and N(1),N(3)-dibenzoylated derivative (λ_{max}=267) due to the electron delocalization resulting in lower transition energy. The UV λ_{max} of **11** is similar to that of N(3) substituted orotidine (isoorotidine **14**, λ_{max}=281). See page S45, figure S64).

N(3)-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-methyl orotate (12)

To a solution of 1-*O*-Ac-2,3,5-tri-*O*-Bz-β-D-ribofuranose (103 mg, 0.20 mmol) and N(3)-(benzoyl)-methyl orotate **11** (60.3 mg, 0.22 mmol) in dry CH_3CN (10 mL) was added bis(trimethylsilyl)acetamide (54 µL, 0.22 mmol) at room temperature and the mixture was stirred at room temperature for 1h. After the addition of trimethylsilyl trifluoromethanesulfonate (40 µL, 0.22 mmol), the reaction mixture was stirred at room temperature overnight. The reaction was quenched by the addition of $NaHCO_3$ (50 mg) and concentrated. The residue was partitioned between H_2O (10 mL) and EtOAc (10 mL). The aqueous layer was extracted with EtOAc (2×10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated. The crude was purified by column chromatography (Hexanes:EtOAc = 2:1→CH_2Cl_2:Acetone = 20:1) to give compound **12** as white foam (88 mg, 72%). HRMS (ESI-TOF) calculated for $C_{32}H_{27}N_2O_{11}$ $[MH]^+$ 615.1609, found 615.1609. TLC (CH_2Cl_2:Acetone = 20:1) $R_f = 0.22$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.23 (br, NH), 8.09-8.06 (m, 2H), 7.98-7.95 (m, 2H), 7.91-7.87 (m, 2H), 7.55-7.48 (m, 3H), 7.41-7.29 (m, 6H), 6.57 (d, *J* = 2.4 Hz, 1H, H-1′), 6.43 (s, 1H, H-5), 6.16 (dd, *J* = 6.7, 2.4 Hz, 1H, H-2′), 6.12 (dd, *J* = 8.1, 6.7 Hz, 1H, H-3′), 4.77 (dd, *J* = 11.8, 3.6 Hz, 1H, H-5′), 4.73-4.67 (m, 1H, H-4′), 4.77 (dd, *J* = 11.8, 5.9 Hz, 1H, H-5′), 3.99 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.4, 165.6, 165.3, 162.3, 160.6, 149.8, 138.4, 133.6, 133.5, 133.2, 130.0, 129.9, 129.9, 129.8, 129.2, 128.5, 128.5, 128.4, 104.9, 86.9, 79.4, 73.9, 71.4, 64.1, 54.2.

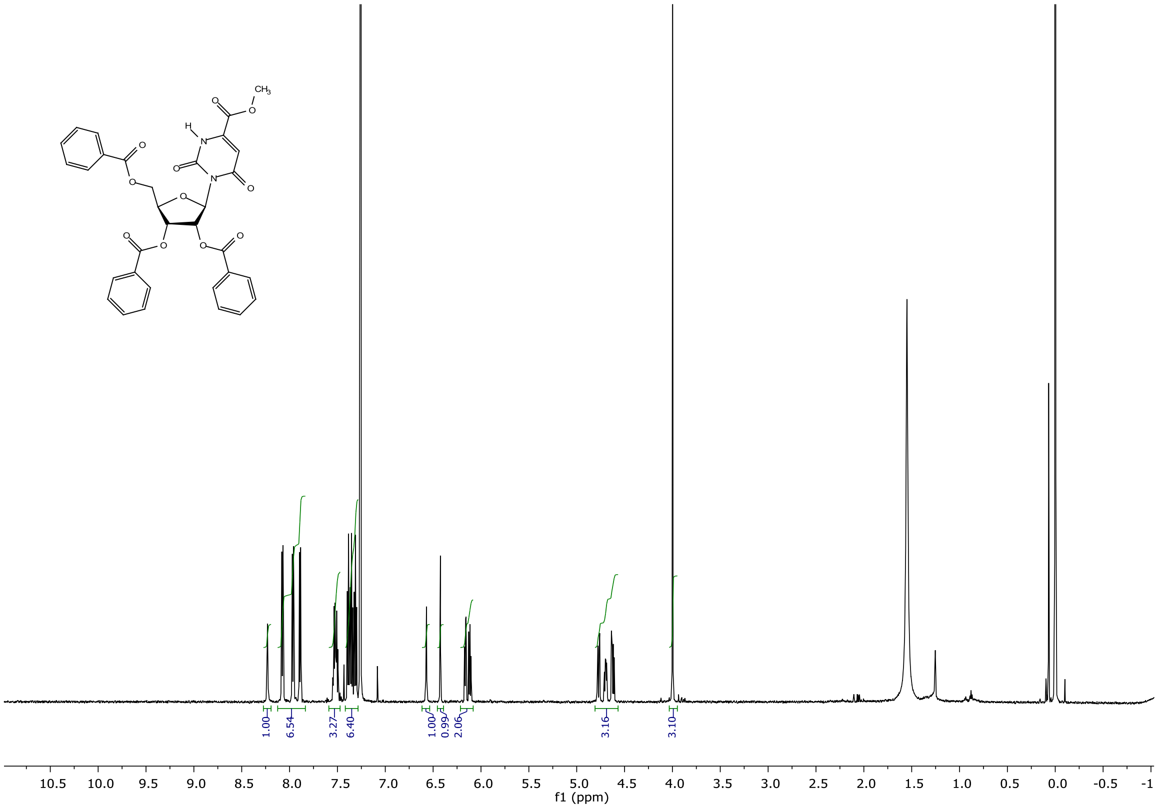

Figure S52 ^{1}H NMR of N(3)-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-methyl orotate (**12**) in $CDCl_3$.

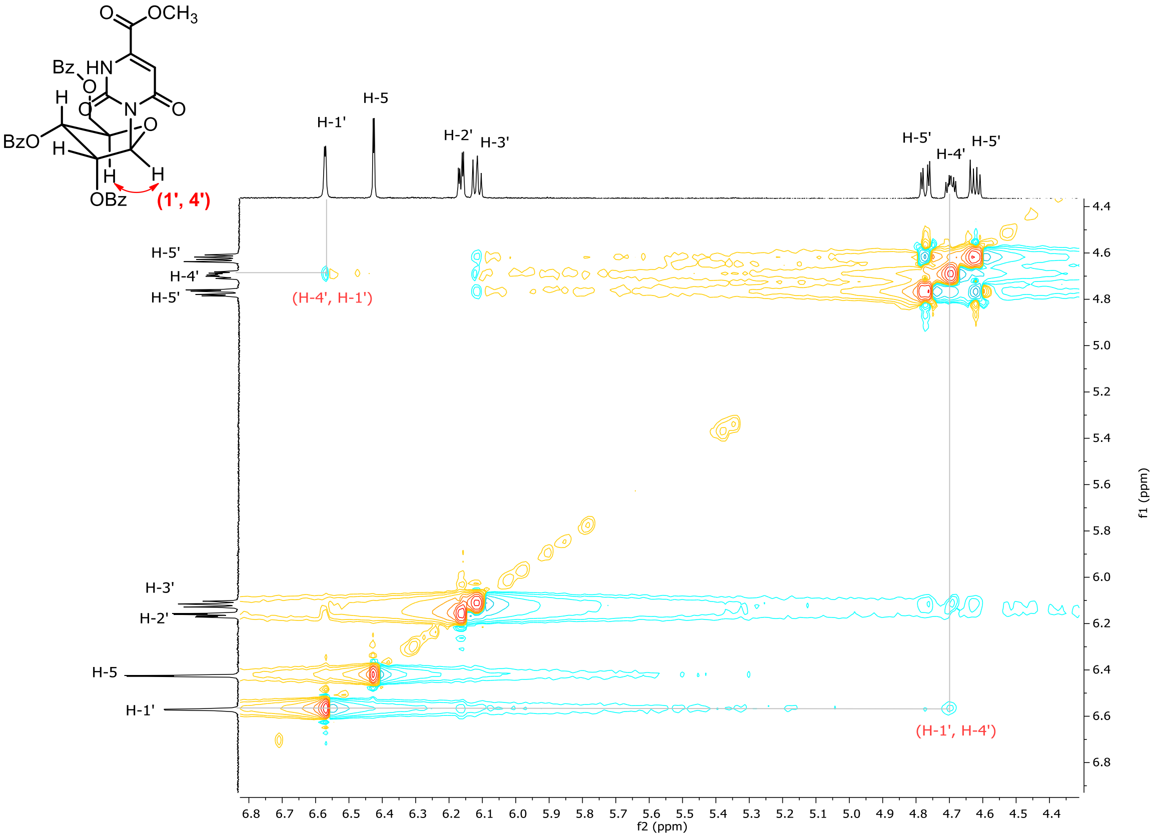

Figure S53 NOESY NMR of N(3)-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-methyl orotate (**12**) in CDCl$_3$.

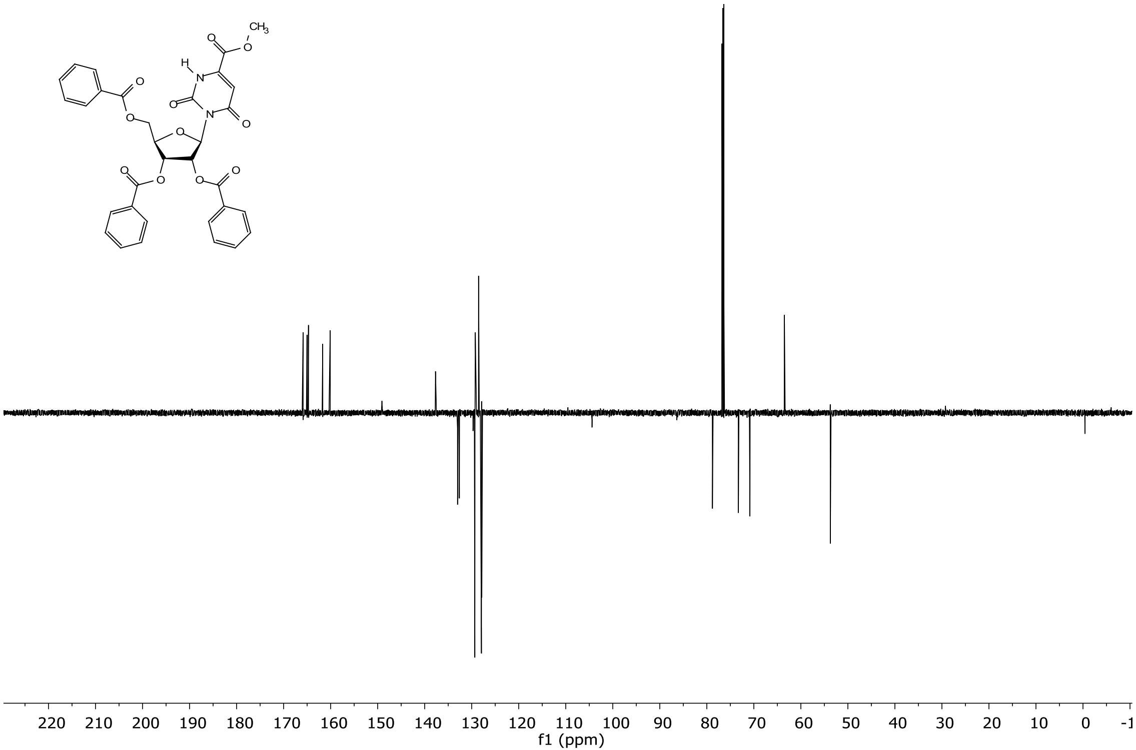

Figure S54 ^{13}C APT NMR of N(3)-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-methyl orotate (**12**) in CDCl$_3$.

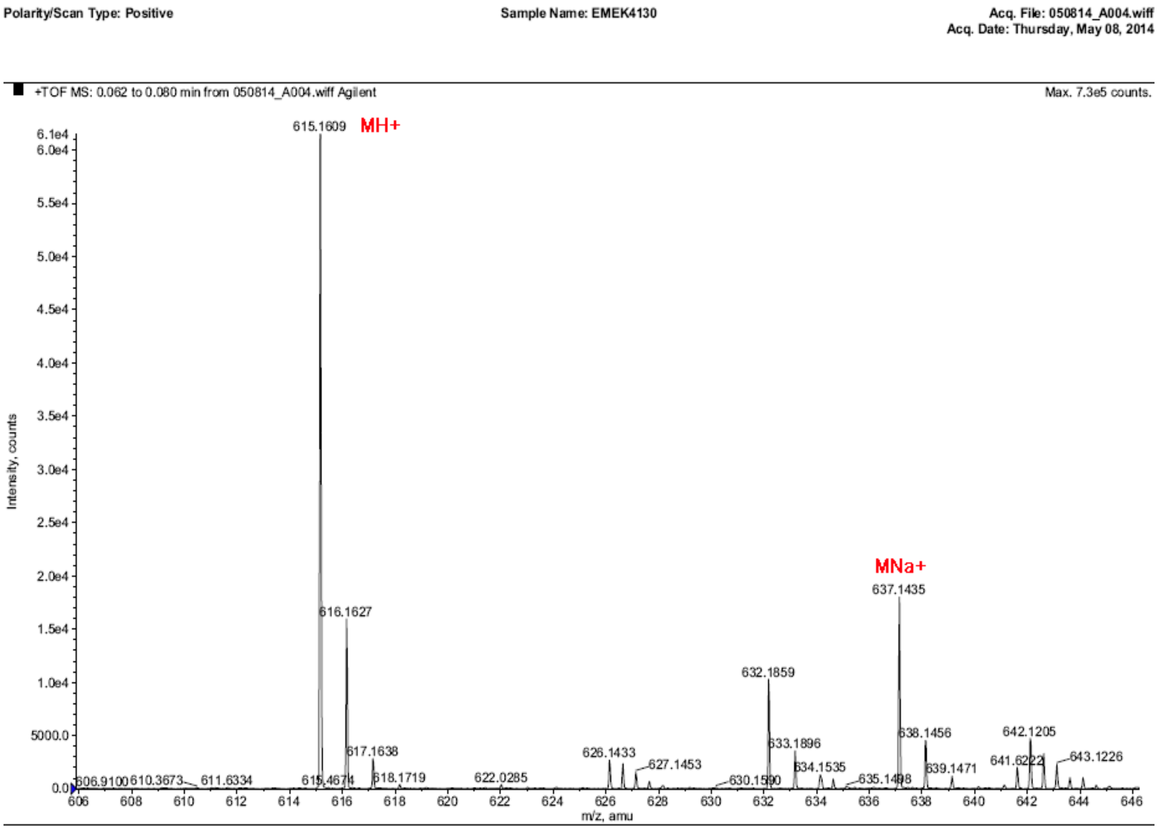

Figure S55 HRMS (ESI-TOF) of N(3)-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-methyl orotate (**12**).

Isoorotidine methyl ester (13)

To a solution of compound **12** (100 mg, 0.163 mmol) in dry MeOH (8 mL) was added 1 M NaOMe in MeOH (0.1 mL) at room temperature. After stirring overnight at room temperature, the pH of mixture was adjusted to 7 by the addition of IR-120 (H^+) (pH paper which was pre-wet by H_2O used). The mixture was filtered and the filtrate was concentrated. The residue was purified by column chromatography (CH_2Cl_2:MeOH = 20:1) to give compound **13** as white foam (45 mg, 92%). HRMS (ESI-TOF) calculated for $C_{11}H_{15}N_2O_8$ $[MH]^+$ 303.0823, found 303.0824. TLC (CH_2Cl_2:MeOH = 20:1) R_f = 0.23; -^{1}H-NMR (600 MHz, DMSO-*d*$_6$ with D_2O) δ 6.15 (s, 1H, H-5), 6.02 (d, *J* = 3.7 Hz, 1H, H-1′), 4.45 (dd, *J* = 6.3, 3.7 Hz, 1H, H-2′), 4.08 (t, *J* = 6.3 Hz, 1H, H-3′), 3.81 (s, 3H), 3.69 (td, *J* = 6.3, 3.4 Hz, 1H, H-4′), 3.59 (dd, *J* = 11.9, 3.4 Hz, 1H, H-5′), 3.41 (dd, *J* = 11.9, 6.3 Hz, 1H, H-5′); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 162.7 (orot.; 6-C=O), 160.6 (orot.), 150.3 (orot.), 140.4 (orot.), 103.1 (C-5), 87.8 (C-1′), 84.5 (C-4′), 70.9 (C-2′), 70.2 (C-3′), 62.3 (C-5′), 53.6 (OMe).

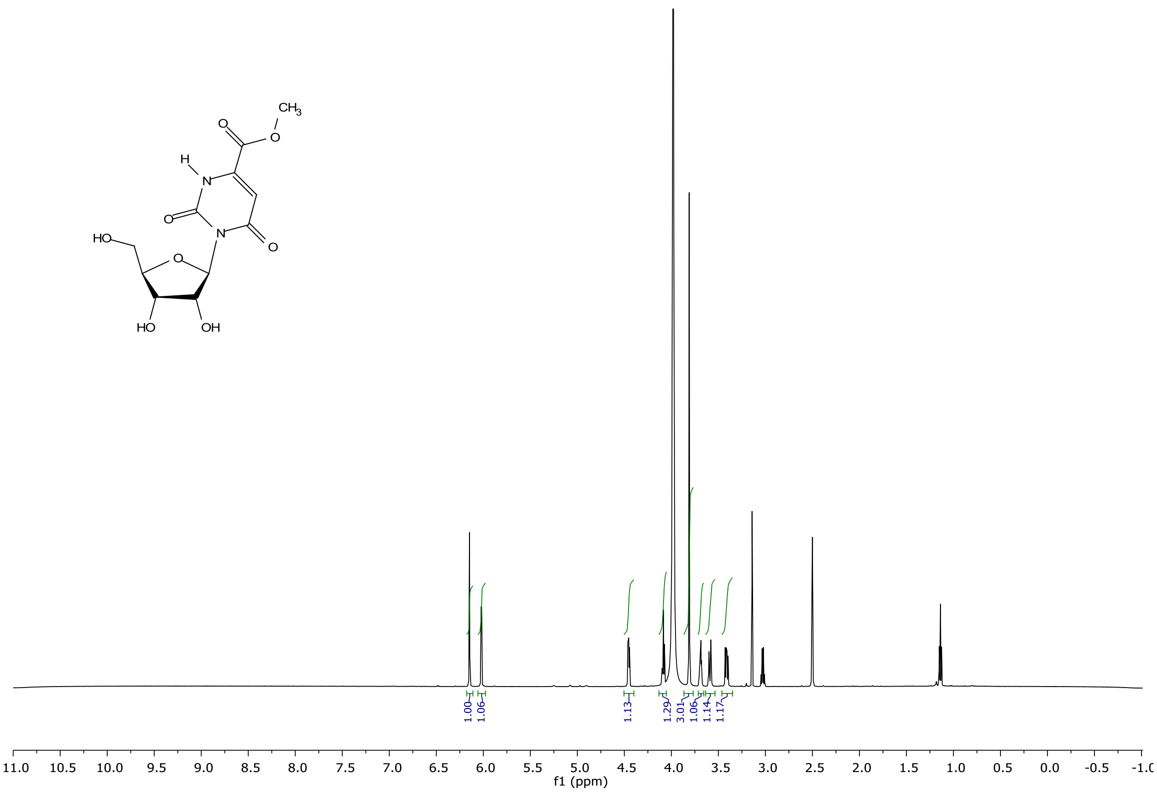

Figure S56 ^{1}H NMR of isoorotidine methyl ester (**13**) in DMSO-d_6.

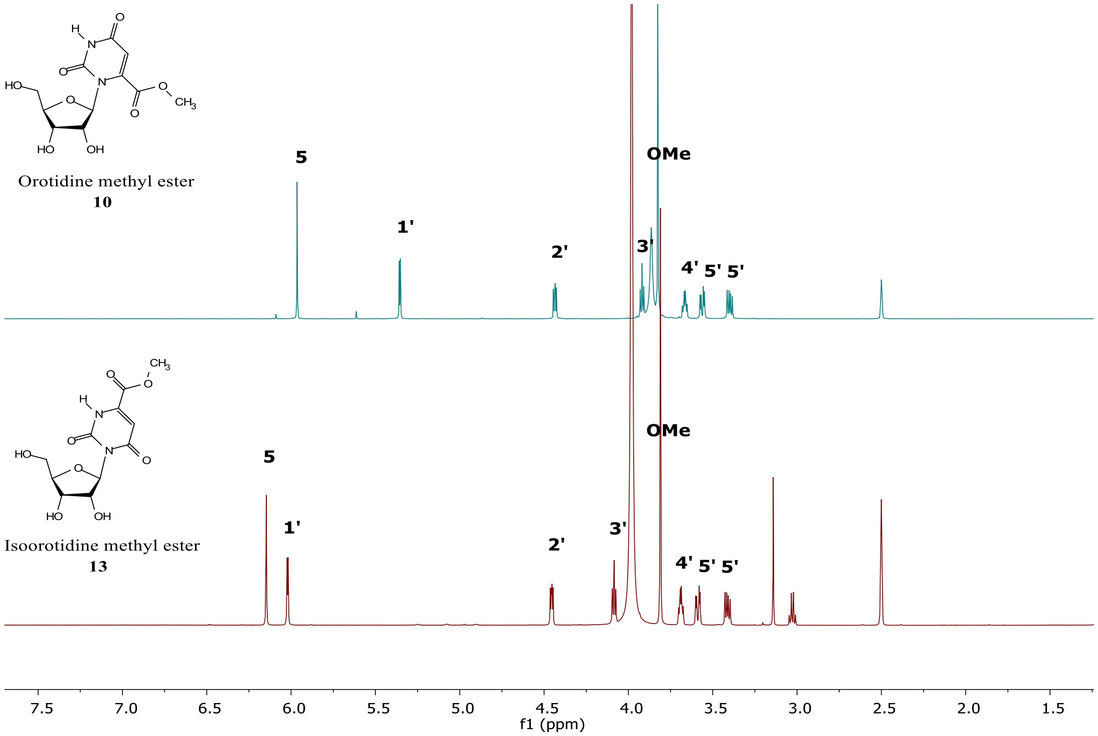

Figure S57 ^{1}H NMR comparison of orotidine methyl ester (**10**) with isoorotidine methyl ester (**13**) in DMSO-d_6.

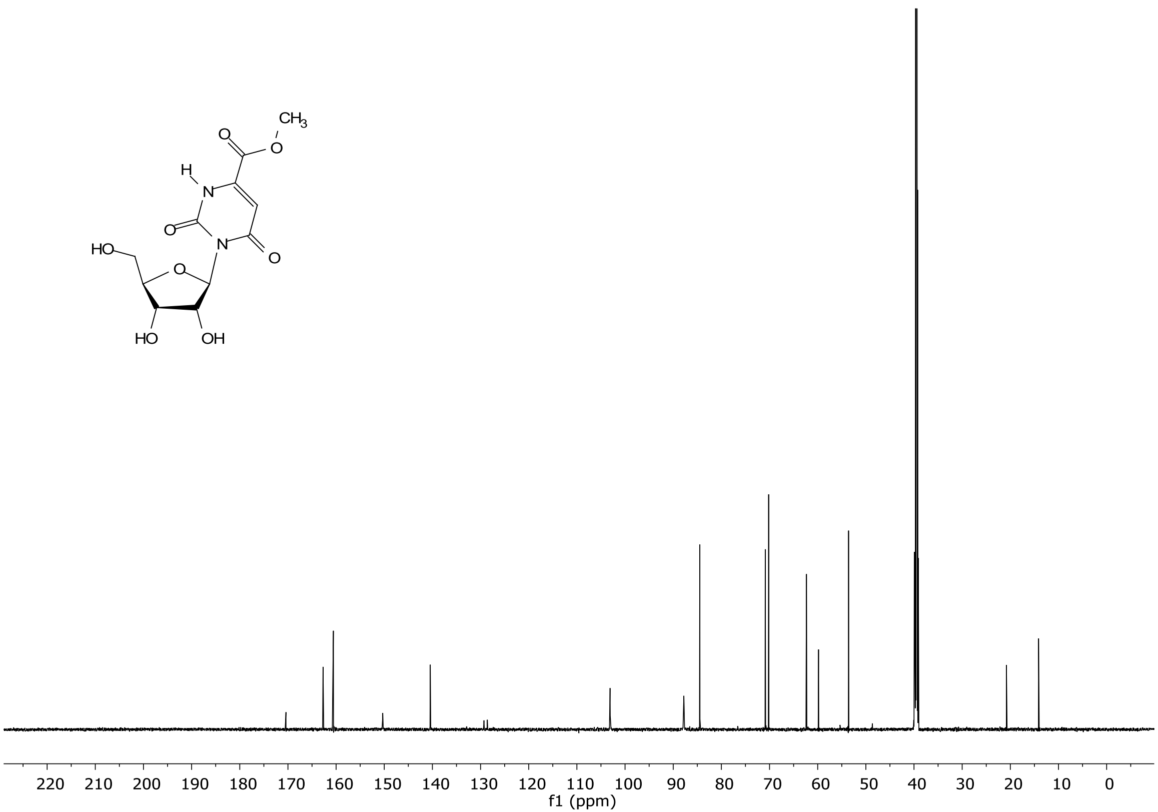

Figure S58 ^{13}C NMR of isoorotidine methyl ester (**13**) in DMSO-d_6.

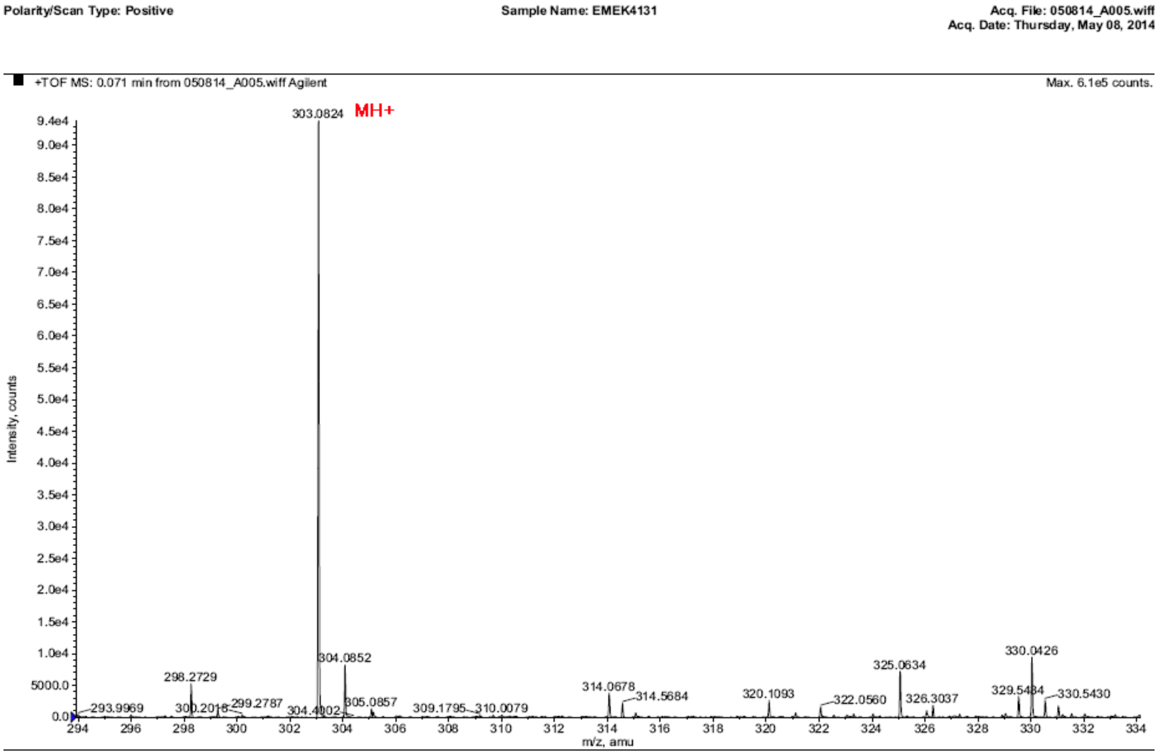

Figure S59 HRMS (ESI-TOF) of isoorotidine methyl ester (**13**).

Isoorotidine (14)

Compound **13** (59 mg, 0.195 mmol) was dissolved in CH_3CN/H_2O (1:1, v/v, 3 mL) and crushed NaOH (16 mg, 0.400 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature overnight. The pH was adjusted with IR-120 (H^+) to pH 4 (pH paper which was pre-wet by H_2O used). After filtration, the filtrate was concentrated to dryness without heating to give isoorotidine **14** (60.5 mg, quantitative yield). HRMS (ESI-TOF) in negative mode calculated for $C_{10}H_{11}N_2O_8^-$ $[M-H]^-$ 287.0521, found 287.0523. TLC (*i*PrOH:H_2O:NH_4OH = 17:2:1) $R_f = 0.30$; ^{1}H-NMR (600 MHz, D_2O) δ 6.25 (d, *J* = 3.4 Hz, 1H, H-1′), 6.23 (s, 1H, H-5), 4.75 (dd, *J* = 6.7, 3.4 Hz, 1H, H-2′), 4.44 (t, *J* = 6.7 Hz, 1H, H-3′), 3.98 (dt, *J* = 6.7, 3.0 Hz, 1H, H-4′), 3.89 (dd, *J* = 12.3, 3.0 Hz, 1H, H-5′), 3.75 (dd, *J* = 12.3, 6.7 Hz, 1H, H-5′); ^{13}C NMR (151 MHz, D_2O) δ 165.5, 164.3, 151.1, 146.1, 100.7, 87.4, 82.9, 70.9, 69.1, 61.1.

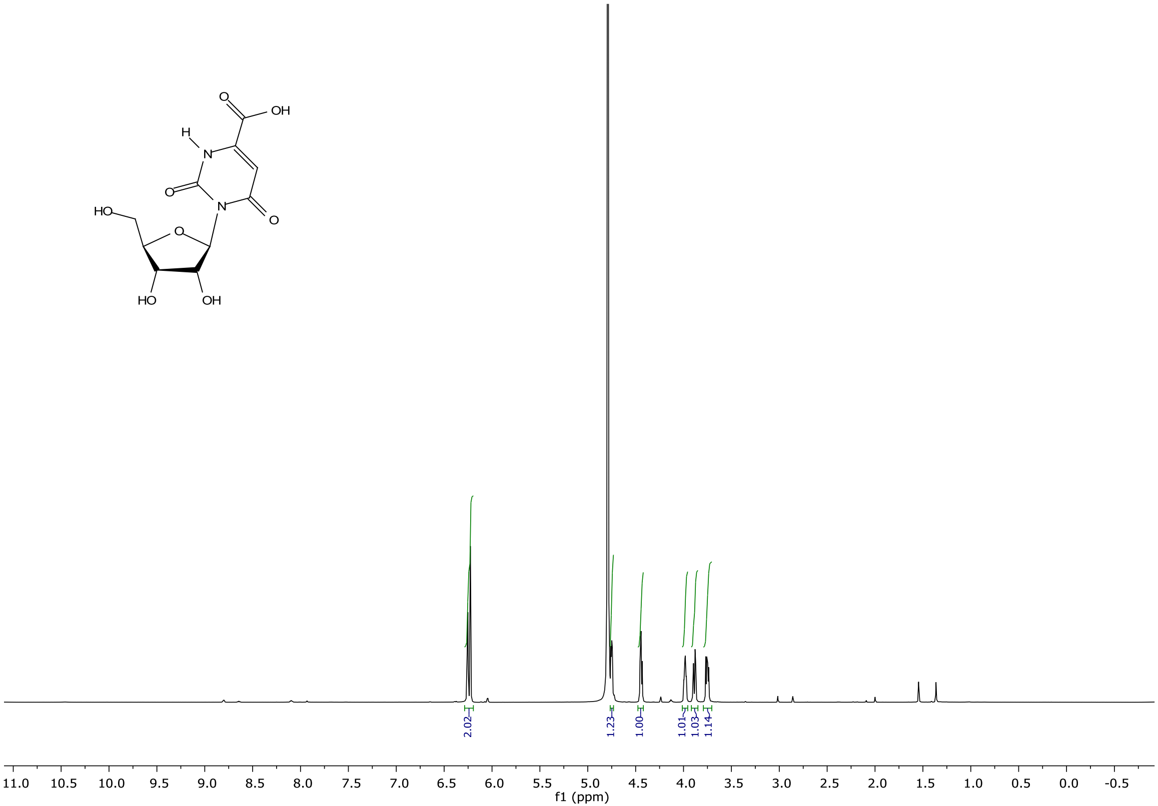

Figure S60 ^{1}H NMR of isoorotidine (**14**) in D_2O.

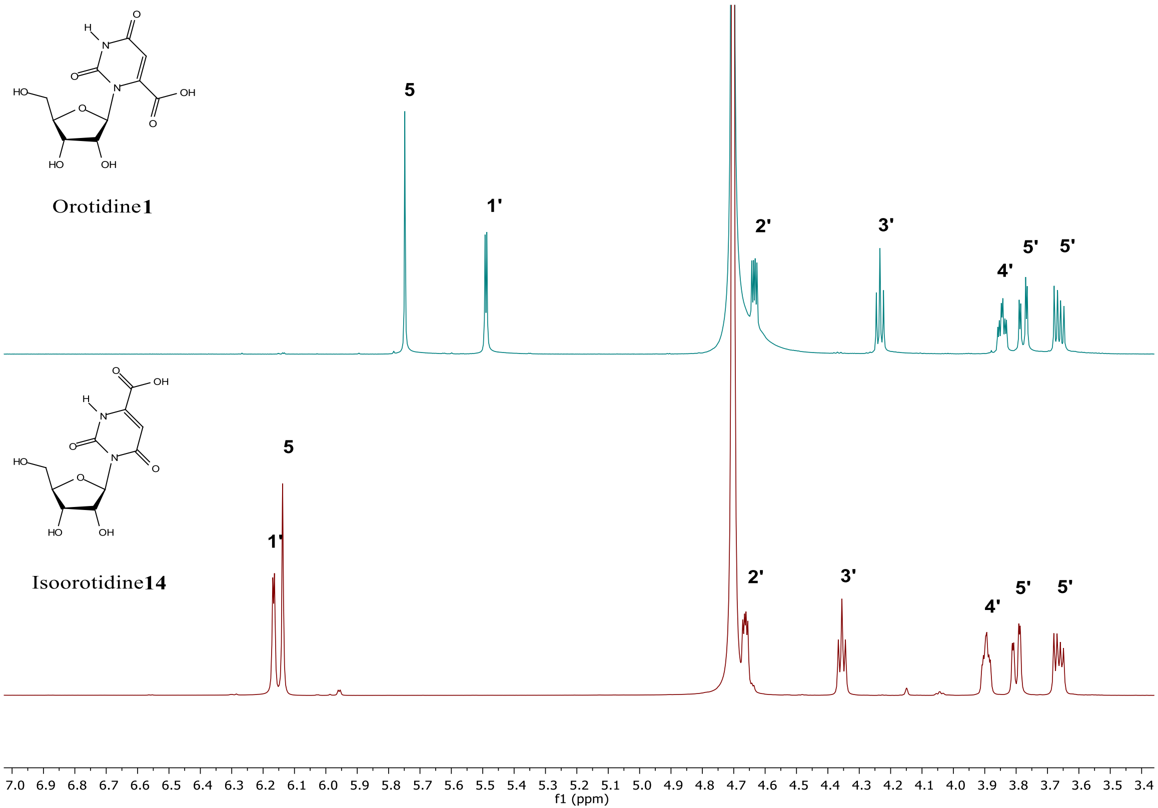

Figure S61 ^{1}H NMR comparison of orotidine (**1**) with isoorotidine (**14**) in D_2O.

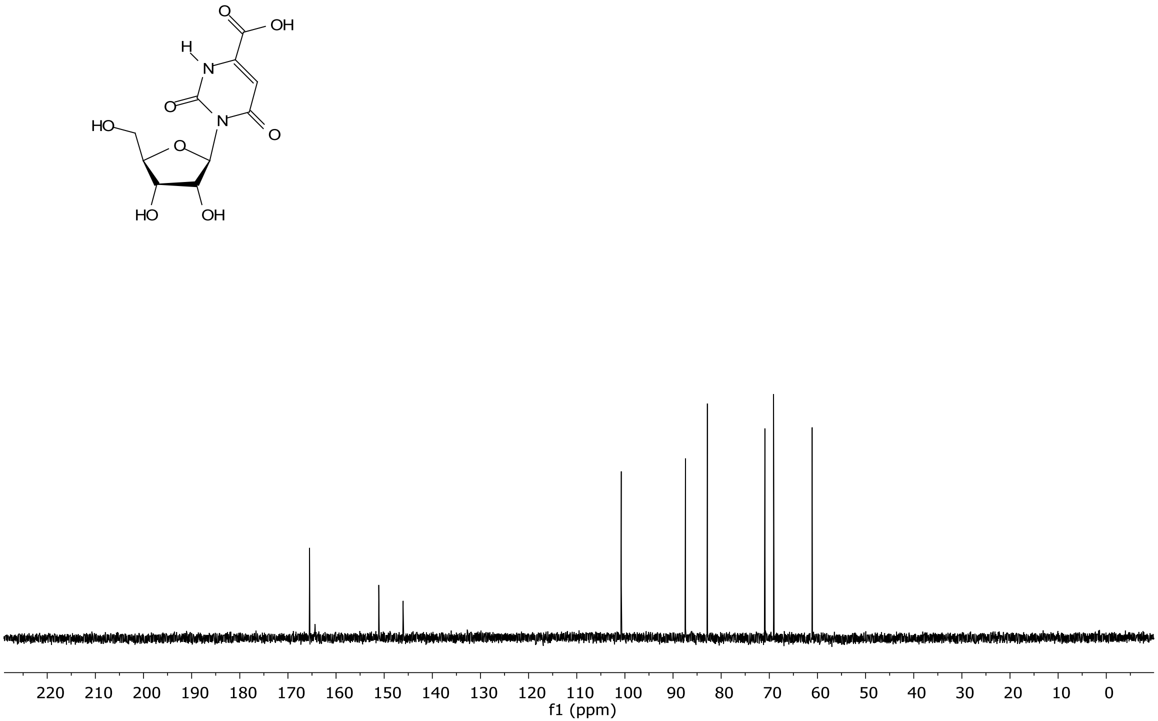

Figure S62 ^{13}C NMR of isoorotidine (**14**) in D_2O.

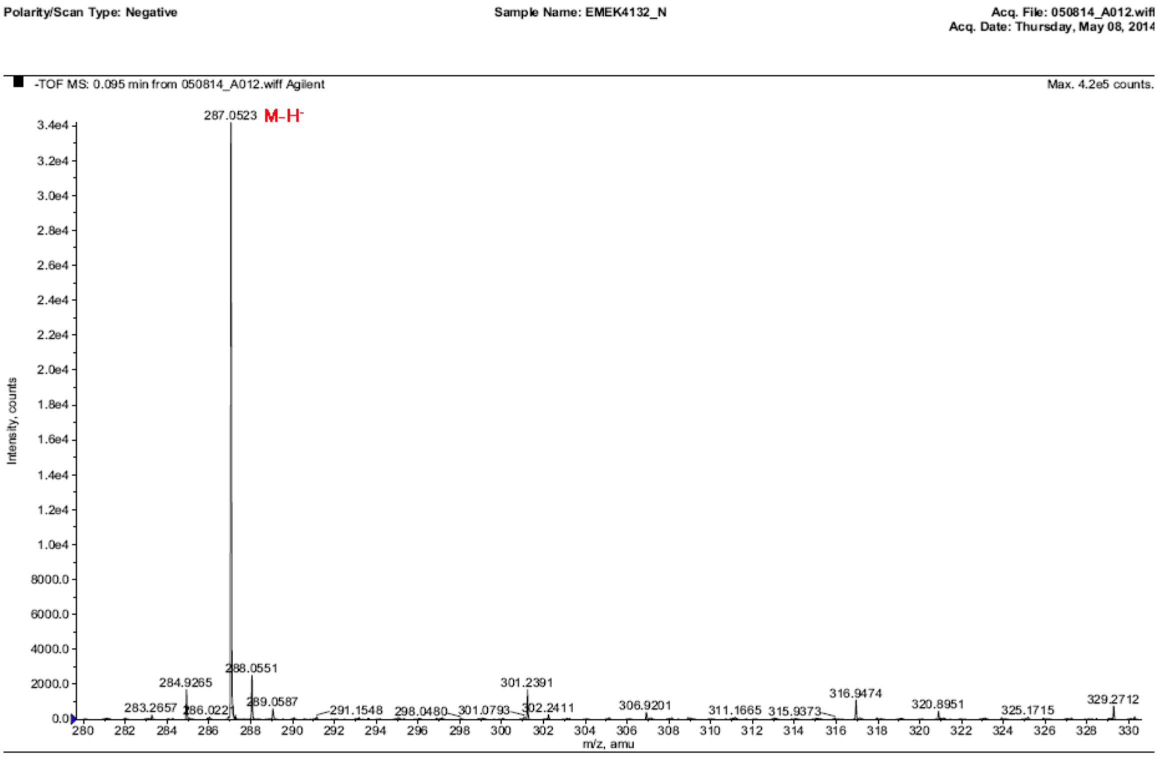

Figure S63 HRMS (ESI-TOF) of isoorotidine (**14**).

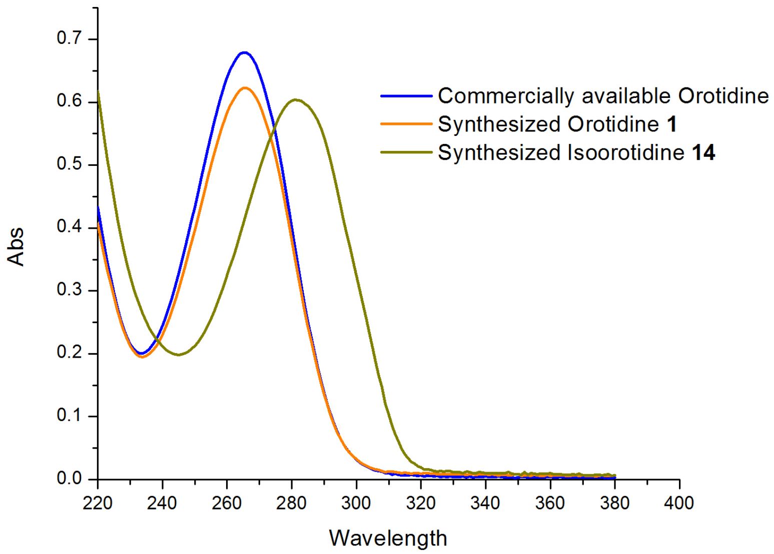

Figure S64 UV absorption spectra of commercially available orotidine (Sigma-Aldrich, Cat. No. 090505, λ_{max} = 265 nm), synthesized orotidine **1** (λ_{max} = 265 nm) and isoorotidine **14** (λ_{max} = 281 nm) taken in H_2O.

1′,5′-*O*-Diorotyl-2′,3′-*O*-isopropylidene-D-ribofuranoside (15)

A slurry of orotic acid (2.54 g, 15.77 mmol) and 1,1′-carbonyldiimidazole (2.56 g, 15.77 mmol) in dry DMF (80 mL) was stirred at room temperature. As the reaction proceeded, slow CO_2 release was observed. After 16 h, pre-dried pyridinium chloride[6] (3.04 g, 26.30 mmol) and a solution of compound **8** (1.0 g, 5.26 mmol) in DMF (20 mL) were added to the crude mixture of orotylimidazole. The mixture was stirred at room temperature overnight. After filtration through Celite, the filtrate was concentrated. The residue was absorbed onto silica and purified by column chromatography (100% EtOAc → CH_2Cl_2:MeOH = 20:1) to give compound **15** as white solid (2.13 g, 87%). HRMS (ESI-TOF) calculated for $C_{18}H_{19}N_4O_{11}$ $[MH]^+$ 467.1045, found 467.1046. TLC (CH_2Cl_2:MeOH = 20:1) $R_f = 0.19$; ^1H NMR (600 MHz, DMSO-d_6) δ 6.16 (s, 1H), 6.12 (s, 1H), 6.06 (s, 1H), 5.06-5.01 (m, 1H), 4.99-4.94 (m, 1H), 4.61 (t, *J* = 6.6 Hz, 1H), 4.44 (dd, *J* = 11.7, 7.6 Hz, 1H), 4.36 (dd, *J* = 11.8, 5.7 Hz, 1H), 1.44 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.7, 163.6, 159.8, 158.8, 150.9, 150.8, 141.0, 140.9, 112.2, 104.3, 104.0, 103.6, 85.2, 84.2, 80.4, 65.8, 26.2, 24.7.

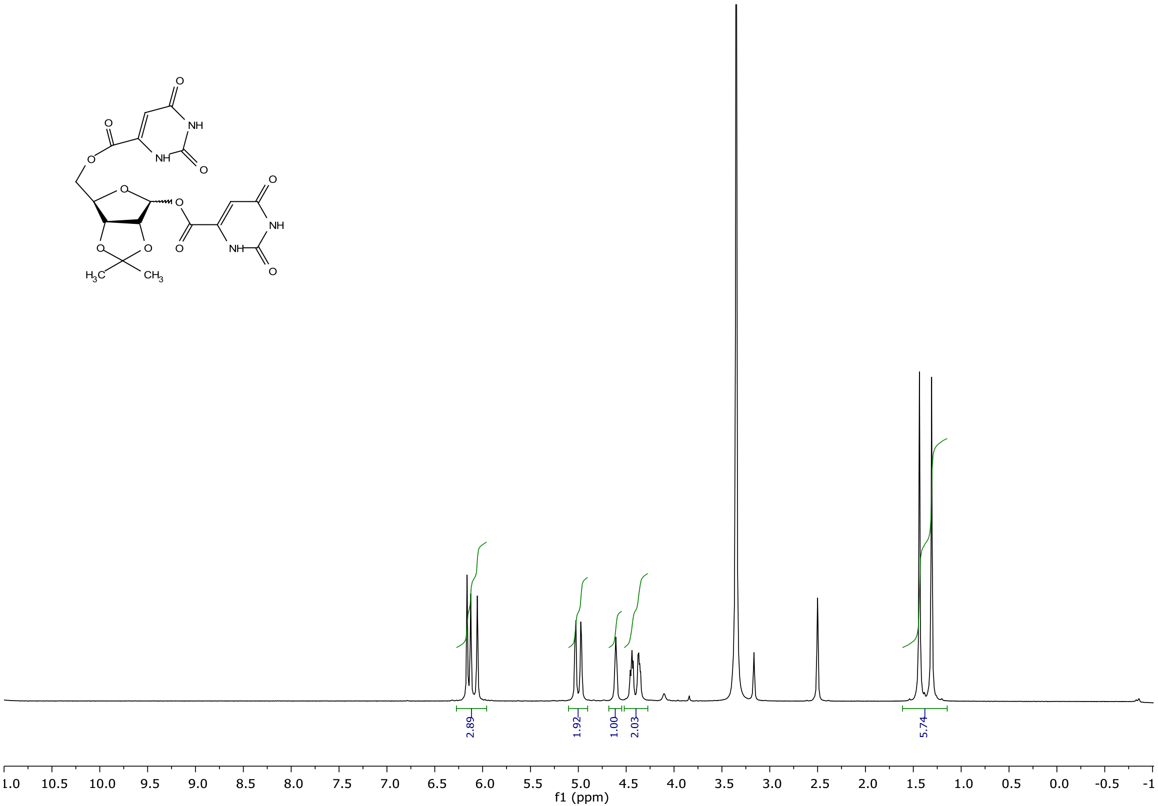

Figure S65 ^1H NMR of 1′,5′-*O*-diorotyl-2′,3′-*O*-isopropylidene-D-ribofuranoside (**15**) in DMSO-d_6.

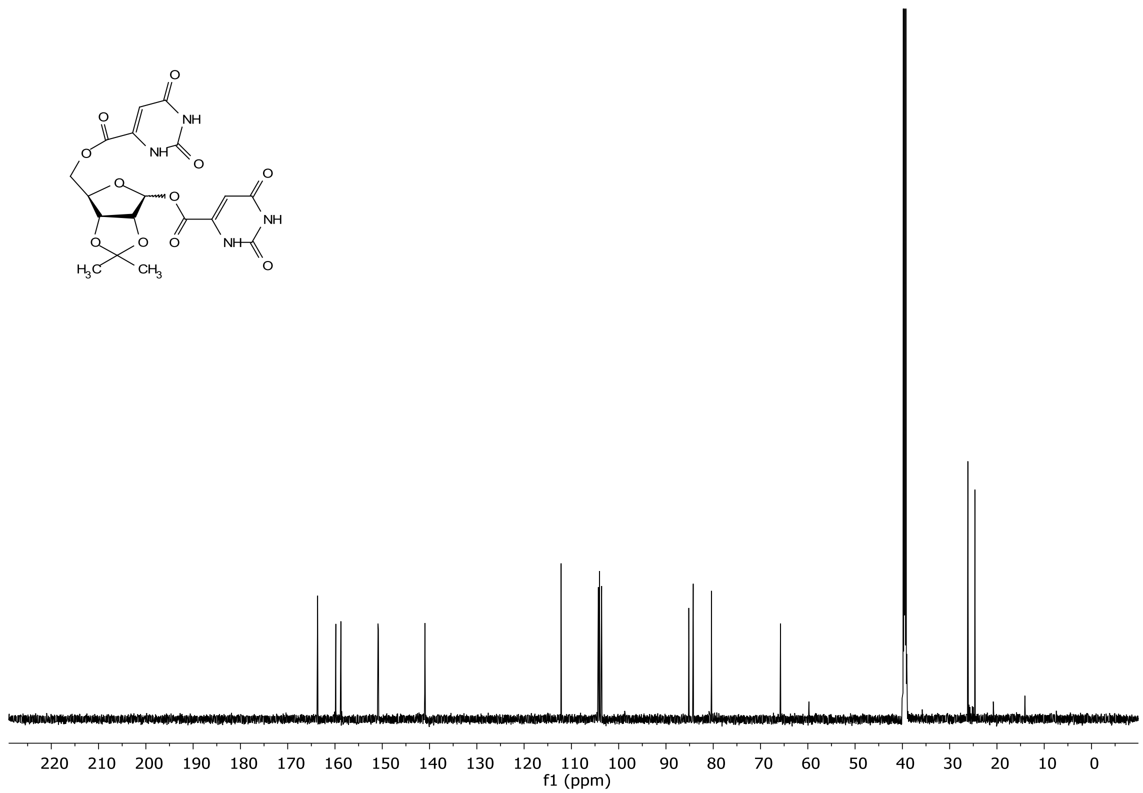

Figure S66 ^{13}C NMR of 1′,5′-*O*-diorotyl-2′,3′-*O*-isopropylidene-D-ribofuranoside (**15**) in DMSO-d_6.

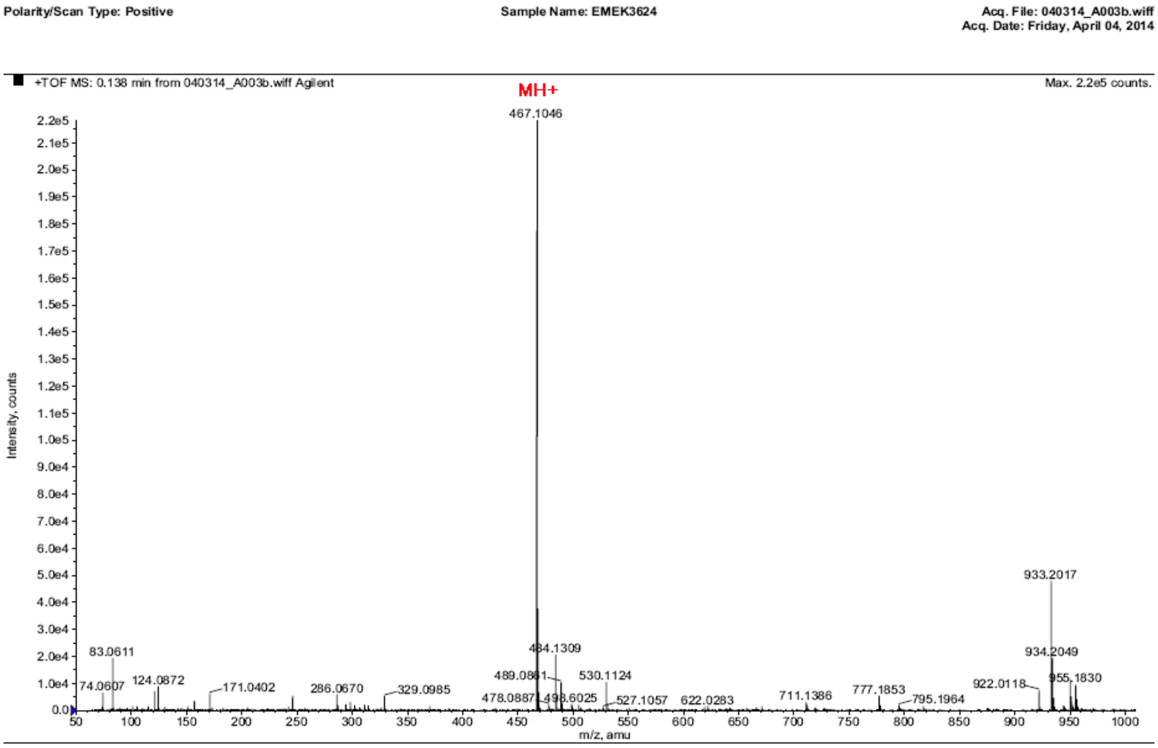

Figure S67 HRMS (ESI-TOF) of 1′,5′-*O*-diorotyl-2′,3′-*O*-isopropylidene-D-ribofuranoside (**15**).

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