

Supporting information

2'-Substituted 2-Amino-3-Methylpyridine Ribonucleosides in Triplex-Forming Oligonucleotides: Triplex Stability is Determined by Chemical Environment

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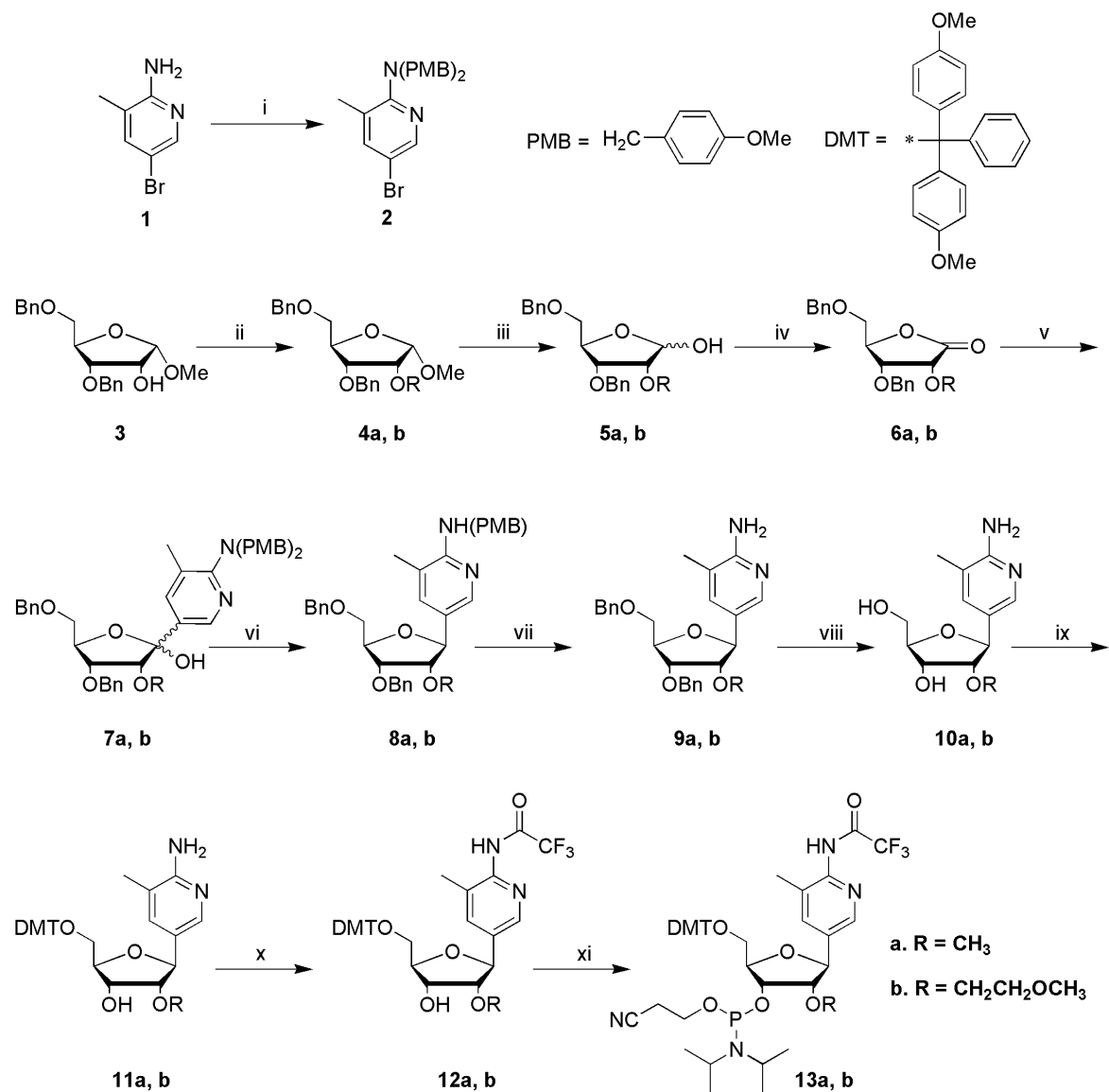
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S1. General Experimental

All reagents were purchased from Aldrich, Fluka or Lancaster and used without further purification. DNA phosphoramidite monomers, solid supports and additional reagents were purchased from Link Technologies Ltd, Glen Research or Applied Biosystems Ltd. Dichloromethane (DCM), acetonitrile (CH₃CN), *N,N*-diisopropylethylamine (DIPEA), triethylamine (Et₃N) and pyridine were distilled over calcium hydride. All reactions were carried out under an argon atmosphere using glassware that had been dried at 120°C overnight. Column chromatography was carried out under pressure using Fisher scientific DAVSIL 60A 30-70 micron silica. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F₂₅₄ (0.22mm thickness, aluminium backed). Compounds were visualized at 254 nm or stained with 10% sulfuric acid in EtOH or 2.5% *p*-anisaldehyde in EtOH solution (EtOH:H₂O = 95:5, containing 3.4% conc. sulfuric acid and 1% acetic acid). ¹H-NMR spectra were measured at 300 MHz on a Bruker AV 300 spectrometer or 400 MHz on a Bruker DPX 400 spectrometer. ¹³C-NMR spectra were measured at 75 MHz or 100 MHz on the same spectrometers. Chemical shifts are given in ppm and *J* values are given in Hz. All assignments for ¹H-NMR and ¹³C-NMR have been confirmed by H-H COSY, HMQC and HMBC. ³¹P NMR spectra were recorded on a Bruker AV 300 spectrometer at 121 MHz. MeOD-*d*⁴, MeCN-*d*³, CDCl₃ and DMSO-*d*⁶ were used as solvents. The IR spectra were obtained on ThermoNicolet 380 FT-IR spectrometer with Smart Orbit Goldengate attachment. Low resolution mass spectra were recorded in acetonitrile or methanol using the electrospray technique on a Fisons VG platform instrument. High resolution mass spectra were recorded in acetonitrile or methanol using the electrospray technique on a Bruker APEX III FT-ICR mass spectrometer. HPLC grade of MeOH or CH₃CN was used as solvents.

S2. Synthesis of dMe-MAP and dMOE-MAP phosphoramidite monomers:



Scheme S1. Reagents and conditions: (i) 4-methoxybenzyl chloride, NaH, DMF, rt, 8 h, 61%; (ii) CH_3I or $\text{BrCH}_2\text{CH}_2\text{OCH}_3$, NaH, DMF, 0 °C, 8 h, **4a** 73%, **4b** 94%; (iii) 80% AcOH, 1% conc. H_2SO_4 , 80 °C, 1 h, **5a** 77%, **5b** 94%; (iv) 4-methylmorpholine-*N*-oxide, tetrapropylammonium perruthenate, DCM, rt, 8 h, **6a** 87%, **6b** 87%; (v) **2**, *n*-BuLi, THF, -78 °C, 4 h, **7a** 69%, **7b** 65%; (vi) triethylsilane, boron trifluoride diethyletherate, DCM, -78 °C, 4 h, rt, 16 h, **8a** 91%, **8b** n.a.; (vii) CF_3COOH , rt, 5 h, **9a** 89%, **9b** 98% for two steps; (viii) **10a** $\text{Pd}(\text{OH})_2$ (20% on carbon), H_2 , EtOH, 65 °C, 12 h, 75%; **10b** $\text{Pd}(\text{OH})_2$ (20% on carbon), HCOOH/MeOH (1:1), 50 °C, overnight, 56%; (ix) DMTCl, pyridine, rt, 3 h, **11a** 98%, **11b** 60%; (x) $(\text{CF}_3\text{CO})_2\text{O}$, DIPEA, DCM, 0 °C, 2 h (or overnight), **12a** 90%, **12b** 74%; (xi) 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite, DIPEA, DCM, rt, 3 h, **13a** 70%, **13b** 92%

[Bis-*N,N*-(4-Methoxybenzyl)amino]-3-methyl-5-bromopyridine (2)

2-Amino-3-methyl-5-bromopyridine (50.5 g, 270 mmol) was dissolved in anhydrous DMF (500 ml) to which 4-methoxybenzyl chloride (100 mL, 737 mmol) was added. The solution was cooled to 0 °C before adding NaH in portions (60% dispersed in mineral oil, 29.5 g, 737 mmol). The reaction mixture was stirred at rt for 8 h after which methanol (20 mL) was added and the resulting solution was partitioned between DCM (400 mL) and water (200 mL). The organic layer was separated and the aqueous layer further extracted with DCM (200 mL × 2). The organic layers were combined and washed by water (200 mL × 3), saturated sodium bicarbonate (200 mL × 1), water (200 mL × 1) and brine (200 mL × 1). After being dried over anhydrous sodium sulfate, the solution was evaporated *in vacuo* to give an orange coloured oil. After recrystallisation (95% ethanol), a white solid **2** was obtained (70.0 g, 61%). R_f 0.85 (ethyl acetate/hexane, 2:3). $^1\text{H NMR}$: (400 MHz, CDCl_3) δ ppm 8.08 (d, $J = 2.34$ Hz, 1H, CH^6), 7.43 (d, $J = 2.4$ Hz, 1H, CH^4), 7.06 (d, $J = 8.6$ Hz, 4H, CH-Ar), 6.73 (d, $J = 8.7$ Hz, 4H, CH-Ar), 4.13 (s, 4H, CH_2), 3.70 (s, 6H, OCH_3), 2.27 (s, 3H, CH_3). $^{13}\text{C NMR}$: (100 MHz, CDCl_3) δ ppm 160.6 (C-Ar), 159.0 (C-Ar), 146.3 (CH^6), 142.0 (CH^4), 131.0 (C-Ar), 129.9 (CH-Ar), 128.1 (C-Ar), 114.1 (CH-Ar), 113.6 (C-Ar), 55.6 (OCH_3), 54.1 (CH_2), 19.1 (CH_3). IR (neat): 3000, 2954, 2929, 2834, 1572, 1509, 1448, 1393, 1364, 1300, 1243, 1167, 1033, 915, 804 cm^{-1} m/z (%) LRMS [ES^+ , MeCN]: 427.2 ($[\text{M}+\text{H}]^+$, 41%). HRMS [ES^+]: $\text{C}_{22}\text{H}_{24}\text{BrN}_2\text{O}_2$ requires 427.1013 found 427.1013

1, 2-Di-*O*-Methyl-3, 5-di-*O*-benzyl- α -D-ribofuranoside (4a)

A solution of **3**¹ (5.00 g, 14.5 mmol) in anhydrous DMF (60 ml) was cooled to 0 °C and NaH was added (60% dispersion in mineral oil, 0.87 g, 21.7 mmol). The mixture was stirred at 0 °C for 1 h before iodomethane (1.81 mL, 29.1 mmol) was added dropwise. The suspension was stirred at rt for 8 h. Upon completion, water (5 mL) was added. After the solvents were removed *in vacuo*, the residue was redissolved in DCM (100 mL) and washed with water (50 mL × 3), dilute hydrochloric acid (0.5%, 50 mL × 1), saturated sodium bicarbonate (50 mL × 1), water (50 mL × 1) and brine (50 mL × 1). After being dried over anhydrous sodium sulfate, the solution was evaporated *in vacuo* to remove the solvent and the residue was

purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to give the product as pale yellow oil (3.80 g, 73%). R_f 0.45 (ethyl acetate/hexane, 1:1). ^1H NMR: (400 MHz, CDCl_3) δ ppm 7.30-7.15 (m, 10H, CH-Ar), 4.93 (d, $J = 4.4$ Hz, 1H, CH^1), 4.61 (d, $J = 12.7$ Hz, 1H, CHH), 4.52 (d, $J = 12.7$ Hz, 1H; CHH), 4.45 (d, $J = 12.1$ Hz, 1H, CHH), 4.39 (d, $J = 12.1$ Hz, 1H, CHH), 4.18 (dd, $J = 7.3, 4.0$ Hz, 1H, CH^4), 3.81 (dd, $J = 6.7, 3.0$ Hz, 1H, CH^3), 3.59 (dd, $J = 6.7, 4.4$ Hz, 1H, CH^2), 3.41 (s, 3H, CH_3), 3.39-3.34 (m, 1H, CHH^5), 3.30 (dd, $J = 10.4, 4.2$ Hz, 1H; CHH^5), 3.36 (s, 3H, CH_3). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 138.6 (C-Ar), 138.4 (C-Ar), 128.8 (CH-Ar), 128.7 (CH-Ar), 128.1 (CH-Ar), 102.7 (CH^1), 82.6 (CH^4), 81.2 (CH^2), 75.3 (CH^3), 73.9 (CH_2), 72.8 (CH_2), 70.7 (CH_2^5), 59.0 (CH_3), 55.9 (CH_3). IR (neat): 3062, 3029, 2909, 2832, 1605, 1496, 1453, 1103, 1026, 856, 735, 697 cm^{-1} . m/z (%) LRMS [ES^+ , MeCN]: 381.3 ($[\text{M}+\text{Na}]^+$, 100%), 739.6 ($[\text{2M}+\text{Na}]^+$, 60%). HRMS [ES^+]: $\text{C}_{21}\text{H}_{26}\text{O}_5\text{Na}$ requires 381.1678 found 381.1676.

1-O-Methyl-2-O-(2-methoxyethyl)-3, 5-di-O-benzyl- α -D-ribofuranoside (4b)

A solution of **3** (6.11 g, 17.7 mmol) in anhydrous DMF (60 mL) was cooled to 0 °C and NaH was added (60% dispersion in mineral oil, 2.40 g, 60.0 mmol). The mixture was stirred at 0 °C for 1 h before 2-bromoethyl methyl ether was added dropwise (10.4 mL, 110 mmol). The suspension was stirred at rt for 8 h after which time water (5 mL) was added. After the solvents were removed *in vacuo*, the residue was redissolved in DCM (120 mL) and washed with water (60 mL \times 3), diluted hydrochloric acid (0.5%, 60 mL \times 1), saturated sodium bicarbonate (60 mL \times 1), water (60 mL \times 1) and brine (60 mL \times 1). After being dried over anhydrous sodium sulfate, the solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography (55% ethyl acetate in petroleum ether) to give the product as pale yellow oil (6.74 g, 94%). R_f 0.22 (ethyl acetate/hexane, 1:1). ^1H NMR: (300 MHz, CDCl_3) δ ppm 7.39-7.11 (m, 10H, CH-Ar), 4.91 (d, $J = 3.7$ Hz, 1H, CH^1), 4.63 (d, $J = 12.7$ Hz, 1H, CHH), 4.51 (d, $J = 12.7$ Hz, 1H, CHH), 4.45 (d, $J = 12.1$ Hz, 1H, CHH), 4.38 (d, $J = 12.1$ Hz, 1H, CHH), 4.16 (dd, $J = 6.8, 4.1$ Hz, 1H, CH^4), 3.82-3.74 (m, 2H, CH^3 , CH^2), 3.66-3.59 (m, 2H, CH_2), 3.56-3.50 (m, 2H, CH_2), 3.39 (s, 3H, CH_3), 3.41-3.27 (m, 2H, CH_2^5), 3.30 (s, 3H, CH_3). ^{13}C NMR: (75 MHz, CDCl_3) δ ppm 138.4 (C-Ar), 138.0 (C-Ar), 128.4

(CH-Ar), 128.2 (CH-Ar), 127.7 (CH-Ar), 127.6 (CH-Ar), 102.4 (CH¹), 82.0 (CH⁴), 79.7 (CH), 75.0 (CH), 73.5 (CH₂), 72.3 (CH₂), 72.1 (CH₂), 70.2 (CH₂⁵), 70.1 (CH₂), 59.1 (CH₃), 55.4 (CH₃). IR (neat): 3029, 2911, 1604, 1496, 1453, 1243, 1106, 1025, 852, 736, 697 cm⁻¹. *m/z* (%) LRMS [ES⁺, MeCN]: 425.3 ([M+Na]⁺, 100%), 827.6 ([2M+Na]⁺, 45%). HRMS [ES⁺]: C₂₃H₃₀O₆Na requires 425.1935 found 425.1942.

2-O-Methyl-3, 5-di-O-benzyl-D-ribose (5a)

A solution of **4a** (3.23 g, 9.02 mmol) in 80% acetic acid (30.0 mL) with conc. sulfuric acid (0.3 mL) was heated at 80 °C for 1 h. After addition of water (150 mL) the aqueous layer was extracted with DCM (60 mL × 3). The organic layer was combined and washed with water (60 mL × 3), saturated sodium bicarbonate (60 mL × 2), water (60 mL × 1) and brine (60 mL × 1). After being dried over anhydrous sodium sulfate, the solution was evaporated *in vacuo* to remove the solvent. The residue was purified by silica gel column chromatography (30% ethyl acetate in hexane) to afford the product as pale yellow oil (2.39 g, 77%). R_f 0.62 (acetone/hexane, 2:1, run for two times) IR (neat): 3416, 3030, 2927, 1496, 1454, 1207, 1087, 1026, 851, 735, 697 cm⁻¹. *m/z* (%) LRMS [ES⁺, MeCN]: 367.2 ([M+Na]⁺, 100%), 711.5 ([2M+Na]⁺, 30%). HRMS [ES⁺]: C₂₀H₂₄O₅Na requires 367.1521 found 367.1520.

2-O-(2-Methoxyethyl)-3, 5-di-O-benzyl-D-ribose (5b)

A solution of **4b** (6.50 g, 16.2 mmol) in 80% acetic acid (100 mL) with conc. sulfuric acid (3.5 mL) was heated at 80 °C for 1 h. Water (300 mL) was added and the aqueous layer was extracted by DCM (150 mL × 3). The organic layer was combined and washed with water (150 mL × 3), saturated sodium bicarbonate (150 mL × 2), water (150 mL × 1) and brine (150 mL × 1). After being dried over anhydrous sodium sulfate, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (50% ethyl acetate in hexane) to afford the product as pale yellow oil (5.90 g, 94%). R_f 0.67 (MeOH/ethyl acetate, 1:99, run for two times). IR (neat): 3396, 3030, 2873, 1602, 1496, 1453, 1201, 1088, 1026, 849, 737, 697 cm⁻¹. *m/z* (%) LRMS [ES⁺, CH₃CN]: 411.2 ([M+Na]⁺, 100%), 799.5 ([2M+Na]⁺, 15%). HRMS [ES⁺]: C₂₂H₂₈O₆Na requires 411.1778 found 411.1774.

2-*O*-Methyl-3,5-di-*O*-benzyl-D-ribo lactone (6a)

A mixture of **5a** (2.18 g, 6.33 mmol), 4-methylmorpholine-*N*-oxide (0.850 g, 7.25 mmol), and molecular sieves (0.850 g) in anhydrous DCM (25 mL) was cooled to 0 °C before adding tetrapropylammonium perruthenate (0.0850 g, 0.240 mmol, suspended in 1 mL DCM) dropwise. The reaction mixture was stirred at rt for 8 h. After removing the solid residue by filtration, the solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (20% ethyl acetate in petroleum ether) to yield the product as pale yellow oil (1.89 g, 87%). R_f 0.67 (ethyl acetate /hexane, 1:1). ^1H NMR: (400 MHz, CDCl_3) δ ppm 7.30-7.15 (m, 10H, CH-Ar), 4.65 (d, $J = 11.9$ Hz, 1H, CHH), 4.54 (d, $J = 11.9$ Hz, 1H, CHH), 4.49-4.43 (m, 2H, CHH , CH^4), 4.40 (d, $J = 12.0$ Hz, 1H, CHH), 4.16-4.12 (m, 2H, CH^3 , CH^2), 3.61 (dd, $J = 11.0, 2.9$ Hz, 1H, CHH^5), 3.54-3.49 (m, 1H, CHH^5), 3.52 (s, 3H, CH_3). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 173.7 (C=O), 137.6 (C-Ar), 129.0 (CH-Ar), 128.5 (CH-Ar), 128.4 (CH-Ar), 128.1 (CH-Ar), 82.0 (CH^4), 77.0 (CH), 75.0 (CH), 74.2 (CH_2), 72.9 (CH_2), 69.1 (CH_2^5), 59.5 (CH_3). IR (neat): 3030, 2867, 1781, 1496, 1454, 1210, 1095, 1026, 735, 697 cm^{-1} . m/z (%) LRMS [ES^+ , MeCN]: 365.2 ($[\text{M}+\text{Na}]^+$, 100%), 707.5 ($[\text{2M}+\text{Na}]^+$, 10%). HRMS [ES^+]: $\text{C}_{20}\text{H}_{22}\text{O}_5\text{Na}$ requires 365.1365 found 365.1354.

2-*O*-(2-Methoxyethyl)-3, 5-di-*O*-benzyl-D-ribo lactone (6b)

A mixture of **5b** (3.68 g, 9.48 mmol), 4-methylmorpholine-*N*-oxide (1.70 g, 14.5 mmol), and molecular sieves (1.70 g) in anhydrous DCM (40 mL) was cooled to 0 °C before adding tetrapropylammonium perruthenate (0.368 g, 1.05 mmol, suspended in 2 mL DCM) dropwise. The reaction mixture was stirred at rt for 8 h. After removing the solid residue by filtration, the solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (25% ethyl acetate in hexane) to yield the product as pale yellow oil (3.19 g, 87%). R_f 0.52 (ethyl acetate/hexane, 1:1). ^1H NMR: (400 MHz, CDCl_3) δ ppm 7.30-7.14 (m, 10H, CH-Ar), 4.71 (d, $J = 11.8$ Hz, 1H, CHH), 4.54 (d, $J = 11.8$ Hz, 1H, CHH), 4.48-4.36 (m, 4H, CH^4 , CH^2 , CH_2), 4.26 (dd, $J = 5.6, 1.7$ Hz, 1H, CH^3), 4.01-3.95 (m, 1H, CHH), 3.76-3.70 (m, 1H, CHH), 3.62-3.48 (m, 4H, CH_2 , CH_2^5), 3.28 (s, 3H, CH_3). ^{13}C NMR: (100 MHz,

CDCl₃) δ ppm 173.8 (C=O), 137.4 (C-Ar), 137.2 (C-Ar), 128.6 (CH-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 127.6 (CH-Ar), 81.9 (s, CH⁴), 75.9 (CH²), 75.4 (CH³), 73.7 (CH₂), 72.5 (CH₂), 72.1 (CH₂), 70.1 (CH₂), 68.9 (CH₂⁵), 59.0 (CH₃). IR (neat): 3030, 2872, 1782, 1496, 1454, 1156, 1095, 1026, 735, 697 cm⁻¹. *m/z* (%) LRMS [ES⁺, MeCN]: 409.2 ([M+Na]⁺, 100%), 795.5 ([2M+Na]⁺, 50%). HRMS [ES⁺]: C₂₂H₂₆O₆Na requires 409.1622 found 409.1612.

2-[Bis-*N,N*-(4-Methoxybenzyl)amino]-3-methyl-5-(1'-hydroxyl-2'-*O*-methyl-3',5'-di-*O*-benzyl-D-ribofuranosyl)-pyridine α - and β -anomers (7a)

Compound **2** (5.83 g, 13.6 mmol) was dissolved in anhydrous THF (50 mL) and cooled to -78 °C under an argon atmosphere before adding *n*-BuLi (1.6 M solution in hexane, 8.52 mL, 13.6 mmol). The mixture was stirred at -78 °C for 2 h before adding compound **6a** dropwise (4.0 g, 11.7 mmol, dissolved in 10 mL THF). The reaction mixture was further stirred at -78 °C for 2 h before warming to rt. Saturated sodium bicarbonate (140 mL) was added to quench the reaction and the mixture was extracted by DCM (70 mL \times 3). The organic layer was combined and washed by water (70 mL \times 3), saturated sodium bicarbonate (70 mL \times 1), water (70 mL \times 1) and brine (70 mL \times 1). After being dried over anhydrous sodium sulfate, solvent was removed *in vacuo* to give amber oil, which was purified by silica gel column chromatography (32% ethyl acetate in petroleum ether) to afford the product as amber gum (5.54 g, 69%). *R_f* 0.35 (ethyl acetate/hexane, 2:3). *m/z* (%) LRMS [ES⁺, MeCN]: 691.4 ([M+H]⁺, 100%). HRMS [ES⁺]: C₄₂H₄₇O₇N₂ requires 691.3378 found 691.3367.

2-[Bis-*N,N*-(4-Methoxybenzyl)amino]-3-methyl-5-[1'-hydroxyl-2'-*O*-(2-methoxyethyl)-3',5'-di-*O*-benzyl-D-ribofuranosyl]-pyridine α - and β -anomers (7b)

Compound **2** (8.12 g, 19.0 mmol) was dissolved in anhydrous THF (40 mL) and cooled to -78 °C under an argon atmosphere before adding *n*-BuLi (1.6 M solution in hexane, 11.9 mL, 19.0 mmol) dropwise. The mixture was stirred at -78 °C for 2 h before adding **6b** (4.90 g, 12.7 mmol, dissolved in 10 mL THF) dropwise. The reaction mixture was further stirred at -78 °C for 2 h before warming to rt. Saturated sodium bicarbonate (150 mL) was added to

quench the reaction and the mixture was extracted by DCM (80 mL \times 3). The organic layers were combined and washed by water (80 mL \times 3), saturated sodium bicarbonate (80 mL \times 1), water (80 mL \times 1) and brine (80 mL \times 1). After being dried over anhydrous sodium sulfate, the solvent was removed *in vacuo* to give amber oil, which was purified by silica gel column chromatography (50% ethyl acetate in hexane) to afford the product as amber oil (6.06 g, 65%). R_f 0.25 (ethyl acetate/hexane, 2:3). m/z (%) LRMS [ES⁺, MeCN]: 735.5 ([M+H]⁺, 100%). HRMS [ES⁺]: C₄₄H₅₁N₂O₈ requires 735.3640 found 735.3656.

2-[N-(4-Methoxybenzyl)amino]-3-methyl-5-(2'-O-methyl-3',5'-di-O-benzyl- β -D-ribofuranosyl)-pyridine (8a)

Compound **7a** (1.96 g, 2.84 mmol) was dissolved in anhydrous DCM (20 mL) and cooled to -78 °C under an argon atmosphere before adding triethylsilane (1.40 mL, 8.76 mmol) and boron trifluoride diethyl etherate (1.08 mL, 8.75 mmol). The mixture was left to stir at -78 °C for 4 h then left at rt for 16 h. Saturated sodium bicarbonate (120 mL) was added to quench the reaction and the mixture was further stirred for 1 h. The mixture was then extracted by DCM (60 mL \times 3) and the organic layers were combined and washed by water (60 mL \times 3), saturated sodium bicarbonate (60 mL \times 1), water (60 mL \times 1) and brine (60 mL \times 1). After being dried over anhydrous sodium sulfate, the solution was evaporated *in vacuo* to remove solvent and the residue was purified by silica gel column chromatography (40% ethyl acetate in petroleum ether) to give the product as amber gum (1.43 g, 91%). R_f 0.29 (ethyl acetate/hexane, 2:3). m/z (%) LRMS [ES⁺, MeCN]: 555.3 ([M+H]⁺, 100%). HRMS [ES⁺]: C₃₄H₃₉O₅N₂ requires 555.2853 found 555.2848.

2-[N-(4-Methoxybenzyl)amino]-3-methyl-5-[2'-O-(2-methoxyethyl)-3',5'-di-O-benzyl- β -D-ribofuranosyl]-pyridine (8b)

Compound **7b** (6.06 g, 8.25 mmol) was dissolved in anhydrous DCM (100 mL) and cooled to -78 °C under an argon atmosphere before adding triethylsilane (7.90 mL, 49.4 mmol) and boron trifluoride diethyletherate (6.10 mL, 49.4 mmol). The mixture was left to stir at -78 °C for 4h then left at rt for 16 h. Saturated sodium bicarbonate (150 mL) was added to quench

the reaction and the mixture was further stirred for 1 h then extracted with DCM (100 mL × 3). The organic layers were combined and washed by water (100 mL × 3), saturated sodium bicarbonate (100 mL × 1), water (100 mL × 1) and brine (100 mL × 1). After being dried over anhydrous sodium sulfate, the solution was evaporated *in vacuo* to give the crude product as amber oil (10.0 g), which was used in the next reaction without further purification. R_f 0.44 (ethyl acetate/hexane, 2:3). m/z (%) LRMS [ES⁺, MeCN]: 599.5 ([M+H]⁺, 100%)

2-Amino-3-methyl-5-(2'-O-methyl-3',5'-di-O-benzyl-β-D-ribofuranosyl)- pyridine (9a)

Compound **8a** (3.33 g, 6.0 mmol) was dissolved in trifluoroacetic acid (10.0 mL) and stirred at rt for 5 h. The trifluoroacetic acid was evaporated *in vacuo* and water (100 mL) was added followed by sodium carbonate (solid) to adjust the pH to 9.5. The aqueous layer was then extracted with DCM (60 mL × 3). After drying the combined DCM extracts over anhydrous sodium sulfate, the solvent was removed and the residue was purified by silica gel column chromatography (ethyl acetate with 1% Et₃N) to give the product as amber foam (2.33 g, 89%). R_f 0.54 (MeOH/ethyl acetate, 1:15). ¹H NMR: (400 MHz, DMSO-*d*⁶) δ ppm 7.86 (s, 1H, CH⁶), 7.55-7.38 (m, 10H, CH-Ar), 7.33 (s, 1H, CH⁴), 5.79 (s, 2H, NH₂), 4.75-4.61 (m, 5H, CH¹, PhCH₂, PhCH₂), 4.26 (q, J = 3.8, 3.7, 3.7 Hz, 1H, CH^{4'}), 4.19-4.15 (m, 1H, CH^{3'}), 3.83-3.79 (m, 1H, CH^{2'}), 3.75 (dd, J = 10.6, 4.1 Hz, 1H, CHH^{5'}), 3.70 (dd, J = 10.4, 4.3 Hz, 1H; CHH^{5'}) 3.35 (s, 3H, OCH₃), 2.07 (s, 3H, CH₃). ¹³C NMR: (100 MHz, DMSO-*d*⁶) δ ppm 158.7 (C²), 144.5 (CH⁶), 138.8 (C-Ar), 138.7 (C-Ar), 135.7 (CH⁴), 128.7 (CH-Ar), 128.3 (CH-Ar), 128.0 (CH-Ar), 127.9 (CH-Ar), 127.8 (CH-Ar), 124.3, 116.0 (C^{3,5}), 85.3 (CH^{2'}), 81.3 (CH^{4'}), 80.4 (CH^{1'}), 77.9 (CH^{3'}), 72.9 (PhCH₂), 71.4 (CH₂), 71.0 (CH₂), 57.8 (OCH₃), 17.5 (CH₃). m/z (%) LRMS [ES⁺, MeCN]: 435.4 ([M+H]⁺, 100%). HRMS [ES⁺]: C₂₆H₃₁N₂O₄ requires 435.2278 found 435.2267.

2-Amino-3-methyl-5-[2'-O-(2-methoxyethyl)-3',5'-di-O-benzyl-β-D-ribofuranosyl]- pyridine (9b)

Compound **8b** (crude product, 10.0 g) was dissolved in trifluoroacetic acid (25.0 mL) and stirred at rt for 5 h. The trifluoroacetic acid was evaporated *in vacuo* and water (100 mL) was added followed by sodium carbonate (solid) to adjust to pH 9.5. The aqueous layer was

extracted with DCM (50 mL × 3). After drying the combined DCM extracts over anhydrous sodium sulfate, the solvent was removed and the residue was purified by silica gel column chromatography (2% MeOH in ethyl acetate with 0.5% Et₃N) to give the product as amber oil (3.86 g, 98% for two steps). R_f 0.34 (MeOH/ethyl acetate, 1:15, 5% Et₃N). ¹H NMR: (400 MHz, CDCl₃) δ ppm 7.90 (d, *J* = 2.1 Hz, 1H, CH⁶), 7.31-7.17 (m, 11H, CH⁴, CH-Ar), 4.76 (d, *J* = 7.1 Hz, 1H, CH¹), 4.63 (d, *J* = 12.0 Hz, 1H, PhCHH), 4.55 (d, *J* = 12.0 Hz, 1H; PhCHH), 4.53 (d, *J* = 12.0 Hz, 1H, PhCHH), 4.48 (d, *J* = 12.0 Hz, 1H; PhCHH), 4.31 (s, 2H, NH₂), 4.19 (q, *J* = 3.9, 3.9, 3.9 Hz, 1H, CH⁴), 3.98 (dd, *J* = 5.2, 3.8 Hz, 1H, CH³), 3.72 (dd, *J* = 7.1, 5.3 Hz, 1H, CH²), 3.59 (dd, *J* = 10.4, 4.0 Hz, 1H, CHH⁵), 3.56-3.34 (m, 5H, CHH⁵, CH₂CH₂), 3.31 (s, 3H, OCH₃), 2.05 (s, 3H, CH₃). ¹³C NMR: (100 MHz, CDCl₃) δ ppm 157.3 (C²), 144.6 (CH⁶), 138.6 (C-Ar), 136.5 (CH⁴), 128.8 (CH-Ar), 128.4 (CH-Ar), 128.1 (CH-Ar), 128.0 (CH-Ar), 126.7, 116.9 (C^{3,5}), 85.4 (CH²), 82.2 (CH⁴), 80.9 (CH¹), 78.1 (CH³), 73.9 (CH₂), 72.5 (CH₂), 72.4 (CH₂), 71.0 (CH₂), 70.5 (CH₂), 59.0 (OCH₃), 17.1 (CH₃). *m/z* (%) LRMS [ES⁺, MeCN]: 479.4 ([M+H]⁺, 100%). HRMS [ES⁺]: C₂₈H₃₅N₂O₅ requires 479.2540 found 479.2533.

2-Amino-3-methyl-5-(2'-*O*-methyl-β-D-ribofuranosyl)-pyridine (10a)

Compound **9a** (2.33 g, 5.36 mmol) was dissolved in ethanol (25 mL) before adding palladium hydroxide (20% on carbon, 0.700 g, 0.3 eq). The reaction mixture was stirred under a hydrogen atmosphere at 65 °C for 12 h. After filtering the catalysts the solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (8% MeOH in ethyl acetate with 1% Et₃N) to give the product as white solid (1.02 g, 75%). R_f 0.24 (MeOH/ethyl acetate, 1:5). ¹H NMR: (400 MHz, DMSO-*d*⁶) δ ppm 7.86 (s, 1H, CH⁶), 7.37 (s, 1H, CH⁴), 5.76 (s, 2H, NH₂), 4.94 (s, 1H, 3'-OH), 4.91 (s, 1H, 5'-OH) 4.61 (d, *J* = 7.4 Hz, 1H, CH¹), 4.22-4.16 (m, 1H, CH³) 3.90-3.84 (m, 1H, CH⁴), 3.68-3.55 (m, 3H, CH², CH₂⁵), 3.36 (s, 3H, OCH₃), 2.15 (s, 3H, CH₃). ¹³C NMR: (100 MHz, DMSO-*d*⁶) δ ppm 158.5 (C²), 144.4 (CH⁶), 136.0 (CH⁴), 124.7, 115.9 (C^{3,5}), 86.4 (CH²), 85.9 (CH⁴), 79.8 (CH¹), 70.1 (CH³), 62.5 (CH₂⁵), 57.7 (OCH₃), 17.6 (CH₃). *m/z* (%) LRMS [ES⁺, MeCN]: 255.2 ([M+H]⁺, 100%). HRMS [ES⁺]: C₁₂H₁₉N₂O₄ requires 255.1339 found 255.1342.

2-Amino-3-methyl-5-[2'-O-(2-methoxyethyl)- β -D-ribofuranosyl]-pyridine (10b)

Compound **9b** (1.00 g, 2.08 mmol) was dissolved in formic acid/anhydrous MeOH (1:1, 50.0 mL) before adding palladium hydroxide (20% on carbon, 0.200 g, 0.2 eq). The reaction mixture was heated at 50 °C overnight. After filtering the catalyst, the solvent was removed *in vacuo*. Ammonia solution (35%, 30.0 mL) was added and the reaction mixture was stirred for 3 h. The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (10% MeOH in ethyl acetate with 5% Et₃N) to give the product as amber oil (0.350 g, 56%). R_f 0.36 (MeOH/ethyl acetate, 1:3). ¹H NMR: (400 MHz, DMSO-*d*⁶) δ ppm 7.86 (d, *J* = 2.0 Hz, 1H, CH⁶), 7.37 (d, *J* = 1.2 Hz, 1H, CH⁴), 5.75 (s, 2H, NH₂), 4.60 (d, *J* = 7.5 Hz, 1H, CH^{1'}), 4.19-4.11 (m, 1H, CH^{3'}), 3.88 (dd, *J* = 7.9, 4.4 Hz, 1H, CH^{4'}), 3.77-3.70 (m, 2H, CHH, CH^{2'}), 3.66-3.55 (m, 3H, CH₂^{5'}, CHH); 3.53-3.48 (2H, CH₂), 3.32 (s, 3H, CH₃), 2.15 (s, 3H, CH₃). ¹³C NMR: (100 MHz, DMSO-*d*⁶) δ ppm 158.5(C²), 144.4 (CH⁶), 136.0 (CH⁴), 124.6, 115.9 (C^{3,5}), 85.8 (CH^{4'}), 85.0 (CH^{2'}), 79.8 (CH^{1'}), 71.7 (CH₂), 70.4 (CH^{3'}), 69.2 (CH₂), 62.5 (CH₂^{5'}), 58.5 (CH₃), 17.5 (CH₃). *m/z* (%) LRMS [ES⁺, MeCN]: 299.2 ([M+H]⁺, 100%). HRMS [ES⁺]: C₁₄H₂₃N₂O₅ requires 299.1601 found 299.1600.

2-Amino-3-methyl-5-[2'-O-methyl-5'-O-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (11a)

Compound **10a** (0.103 g, 0.405 mmol) was dissolved in anhydrous pyridine (5 mL) and DMTrCl (0.164 g, 0.48 mmol, dissolved in 2 mL pyridine) was added dropwise. The reaction mixture was stirred at rt for 3 h, after which time MeOH (2 mL) was added to quench the reaction. After the solvent was removed *in vacuo*, the residue was purified by silica gel column chromatography (5% MeOH in ethyl acetate with 1% pyridine) to give the product as yellow gum (0.222 g, 98%). R_f 0.88 (MeOH/ethyl acetate, 1:6). ¹H NMR: (400 MHz, DMSO-*d*⁶) δ ppm 7.89 (s, 1H, CH⁶), 7.57-7.31 (m, 10H, CH-Ar, CH⁴), 7.00 (d, *J* = 8.8 Hz, 4H, CH-Ar), 5.81 (s, 2H, NH₂), 5.03 (d, *J* = 5.8 Hz, 1H, 3'-OH), 4.71 (d, *J* = 6.8 Hz, 1H, CH^{1'}), 4.20-4.14 (m, 1H, CH^{3'}) 4.06-4.01 (m, 1H, CH^{4'}), 3.85 (s, 6H, CH₃), 3.67 (dd, *J* = 6.6, 5.4 Hz, 1H, CH^{2'}), 3.40 (s, 3H, CH₃), 3.28 (dd, *J* = 10.1, 3.0 Hz, 1H, CHH^{5'}), 3.23 (dd, *J* =

10.1, 5.0 Hz, 1H; $\text{CHH}^{\text{5'}}$), 2.07 (s, 3H, CH_3). ^{13}C NMR: (100 MHz, $\text{DMSO-}d^6$) δ ppm 156.1 (C^2), 144.2 (CH^6), 136.6 (CH^4), 135.7 (CH-Ar), 130.2 (CH-Ar), 128.3 (CH-Ar), 128.2 (CH-Ar), 127.1 (CH-Ar), 124.4 (CH-Ar), 113.6 (CH-Ar), 86.5 ($\text{CH}^{2'}$), 83.8 ($\text{CH}^{4'}$), 80.1 ($\text{CH}^{1'}$), 70.3 ($\text{CH}^{3'}$), 64.4 ($\text{CH}_2^{\text{5'}}$), 57.9 (CH_3), 55.5 (CH_3), 17.3 (CH_3). m/z (%) LRMS[ES^+ , MeCN]: 557.3 ($[\text{M}+\text{H}]^+$, 100%). HRMS [ES^+]: $\text{C}_{33}\text{H}_{37}\text{N}_2\text{O}_6$ requires 557.2646 found 557.2644

2-Amino-3-methyl-5-[2'-O-(2-methoxyethyl)-5'-O-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (11b)

Compound **10b** (0.546 g, 1.83 mmol) was dissolved in anhydrous pyridine (20 mL) and DMTrCl (0.930 g, 2.74 mmol, dissolved in 3 mL pyridine) was added dropwise. The reaction mixture was stirred at rt for 3 h after which time MeOH (5 mL) was added to quench the reaction. After removing the solvent in *vacuo*, the residue was purified by silica gel column chromatography (5% MeOH in ethyl acetate with 2% pyridine) to give the product as pale yellow solid (0.660 g, 60%). R_f 0.68 (MeOH/ethyl acetate, 1:3). ^1H NMR: (400 MHz, $\text{MeOD-}d^4$) δ ppm 7.45 (s, 1H, CH^6), 7.38-6.96 (m, 10H, CH^4 , CH-Ar), 6.74 (d, $J = 8.8$ Hz, 4H, CH-Ar), 4.62 (d, $J = 7.4$ Hz, 1H, $\text{CH}^{1'}$), 4.12 (dd, $J = 4.8, 3.1$ Hz, 1H, $\text{CH}^{3'}$) 3.96 (dd, $J = 6.3, 3.1$ Hz, 1H, $\text{CH}^{4'}$), 3.84 (dd, $J = 7.3, 5.1$ Hz, 1H, $\text{CH}^{2'}$), 3.66 (s, 6H, CH_3), 3.63-3.57 (m, 1H, CHH), 3.55-3.49 (m, 1H, CHH), 3.45-3.35 (m, 2H, CH_2), 3.21 (s, 3H, CH_3), 3.28 (dd, $J = 10.3, 3.0$ Hz, 1H, $\text{CHH}^{\text{5'}}$), 3.15 (dd, $J = 10.2, 3.7$ Hz, 1H; $\text{CHH}^{\text{5'}}$), 2.21 (s, 3H, CH_3). ^{13}C NMR: (100 MHz, $\text{MeOD-}d^4$) δ ppm 138.6 (CH^6), 131.8 (CH^4), 130.3 (CH-Ar), 129.8 (CH-Ar), 129.6 (CH-Ar), 129.2 (CH-Ar), 128.3 (CH-Ar), 126.7 (CH-Ar), 114.5 (CH-Ar), 87.2 ($\text{CH}^{2'}$), 86.2 ($\text{CH}^{4'}$), 81.6 ($\text{CH}^{1'}$), 73.5 (CH_2), 72.8 ($\text{CH}^{3'}$), 71.3 (CH_2), 65.8 ($\text{CH}_2^{\text{5'}}$), 59.6 (CH_3), 56.1 (CH_3), 17.1 (CH_3). m/z (%) LRMS [ES^+ , MeCN]: 601.4 ($[\text{M}+\text{H}]^+$, 100%). HRMS [ES^+]: $\text{C}_{35}\text{H}_{41}\text{N}_2\text{O}_7$ requires 601.2908 found 601.2897.

2-(N-Trifluoroacetamido)-3-methyl-5-[2'-O-methyl-5'-O-4,4-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (12a)

Compound **11a** (0.060 g, 0.108 mmol) was dissolved in anhydrous DCM (5 mL) and DIPEA

(0.300 mL, 1.72 mmol) was added. The reaction was cooled to 0 °C before trifluoroacetic anhydride (0.100 mL, 0.720 mmol) was added dropwise to the reaction mixture which was stirred at 0 °C for 2 h. Water (5 mL) was added to quench the reaction. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (70% ethyl acetate in petroleum ether with 1% pyridine) to give the product as pale yellow gum (0.063 g, 90%). R_f 0.61 (ethyl acetate/hexane, 10:1). $^1\text{H NMR}$: (400 MHz, $\text{DMSO-}d^6$) δ ppm 8.47 (s, 1H, NH), 7.95-7.87 (m, 1H, CH^6), 7.56-7.32 (m, 10H, CH^4 , CH-Ar), 7.00 (d, $J = 8.8$ Hz, 4H, CH-Ar), 5.19 (d, $J = 5.8$ Hz, 1H, 3'-OH), 4.98 (d, $J = 6.4$ Hz, 1H, CH^1), 4.22 (dd, $J = 10.0, 5.0$ Hz, 1H, CH^3), 4.18-4.11 (m, 1H, CH^4), 3.85 (s, 6H, CH_3), 3.79-3.74 (m, 1H, CH^2), 3.47 (s, 3H, CH_3), 3.35 (dd, $J = 10.3, 2.8$ Hz, 1H, CHH^5), 3.30 (dd, $J = 10.3, 4.7$ Hz, 1H; CHH^5), 2.22 (s, 3H, CH_3). $^{13}\text{C NMR}$: (100 MHz, $\text{DMSO-}d^6$) δ ppm 159.3 (C^2), 146.1 (C=O), 138.6 (CH^6), 130.2 (CH^4), 128.3 (CH-Ar), 128.2 (CH-Ar), 127.2 (CH-Ar), 124.4 (CH-Ar), 113.7 (CH-Ar), 86.8 (CH^2), 84.3 (CH^4), 79.2 (CH^1), 70.4 (CH^3), 64.4 (CH_2^5), 57.8 (CH_3), 55.5 (CH_3), 17.3 (CH_3). m/z (%) LRMS [ES^+ , MeCN]: 675.3 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+]: $\text{C}_{35}\text{H}_{36}\text{F}_3\text{N}_2\text{O}_7$ requires 653.2469 found 653.2470.

2-(*N*-Trifluoroacetamido)-3-methyl-5-[2'-*O*-(2-methoxyethyl)-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (12b)

Compound **11b** (0.350 g, 0.580 mmol) was dissolved in anhydrous DCM (20 mL) and DIPEA (1.00 mL, 5.70 mmol) was added. The reaction solution was cooled 0 °C before trifluoroacetic anhydride (0.350 mL, 2.50 mmol) was added to the reaction dropwise. The mixture was stirred at 0 °C overnight then water (3 mL) was added to quench the reaction. After the solvent was removed *in vacuo*, the residue was purified by silica gel column chromatography (70% ethyl acetate in hexane with 2.5% pyridine) to give pale yellow foam (0.300 g, 74%). R_f 0.54 (ethyl acetate/hexane, 10:1). $^1\text{H NMR}$: (400 MHz, CDCl_3) δ ppm 8.05 (s, 1H, NH), 7.82 (s, 1H, CH^6), 7.37-7.08 (m, 10H, CH^4 , CH-Ar), 6.74 (d, $J = 8.8$ Hz, 4H, CH-Ar), 4.79 (d, $J = 7.9$ Hz, 1H, CH^1), 4.21-4.14 (m, 2H, CH^3 , CH^4), 3.81-3.72 (m, 2H, CH^2 , CHH), 3.72 (s, 6H, CH_3), 3.61 (s, 1H, 3'-OH), 3.58-3.49 (m, 2H, CHH , CHH), 3.44-3.34 (m, 2H, CHH , CHH^5), 3.33 (s, 3H, CH_3), 3.23 (dd, $J = 10.3, 3.5$ Hz, 1H, CHH^5),

2.17 (s, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 159.0 ($\underline{\text{C}}^2$), 145.1 ($\underline{\text{C}}=\text{O}$), 139.5 ($\underline{\text{CH}}^6$), 130.5 ($\underline{\text{CH}}^4$), 128.6 ($\underline{\text{CH}}\text{-Ar}$), 128.3 ($\underline{\text{CH}}\text{-Ar}$), 127.4 ($\underline{\text{CH}}\text{-Ar}$), 113.6 ($\underline{\text{CH}}\text{-Ar}$), 86.6 ($\underline{\text{CH}}^2$), 85.3 ($\underline{\text{CH}}^4$), 78.7 ($\underline{\text{CH}}^1$), 72.2 ($\underline{\text{CH}}_2$), 71.4 ($\underline{\text{CH}}^3$), 70.4 ($\underline{\text{CH}}_2$), 64.5 ($\underline{\text{CH}}_2^5$), 59.4 ($\underline{\text{CH}}_3$), 55.6 ($\underline{\text{CH}}_3$), 18.0 ($\underline{\text{CH}}_3$). m/z (%) LRMS [ES^+ , MeCN]: 719.3 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+]: $\text{C}_{37}\text{H}_{39}\text{F}_3\text{N}_2\text{O}_8\text{Na}$ requires 719.2551 found 719.2553.

2-(*N*-Trifluoroacetamido)-3-methyl-5-[(2'-*O*-methyl-3'-*O*-(2-cyanoethyl-*N,N*-diisopropyl-amino)phosphanyl)-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl)]-pyridine (13a)

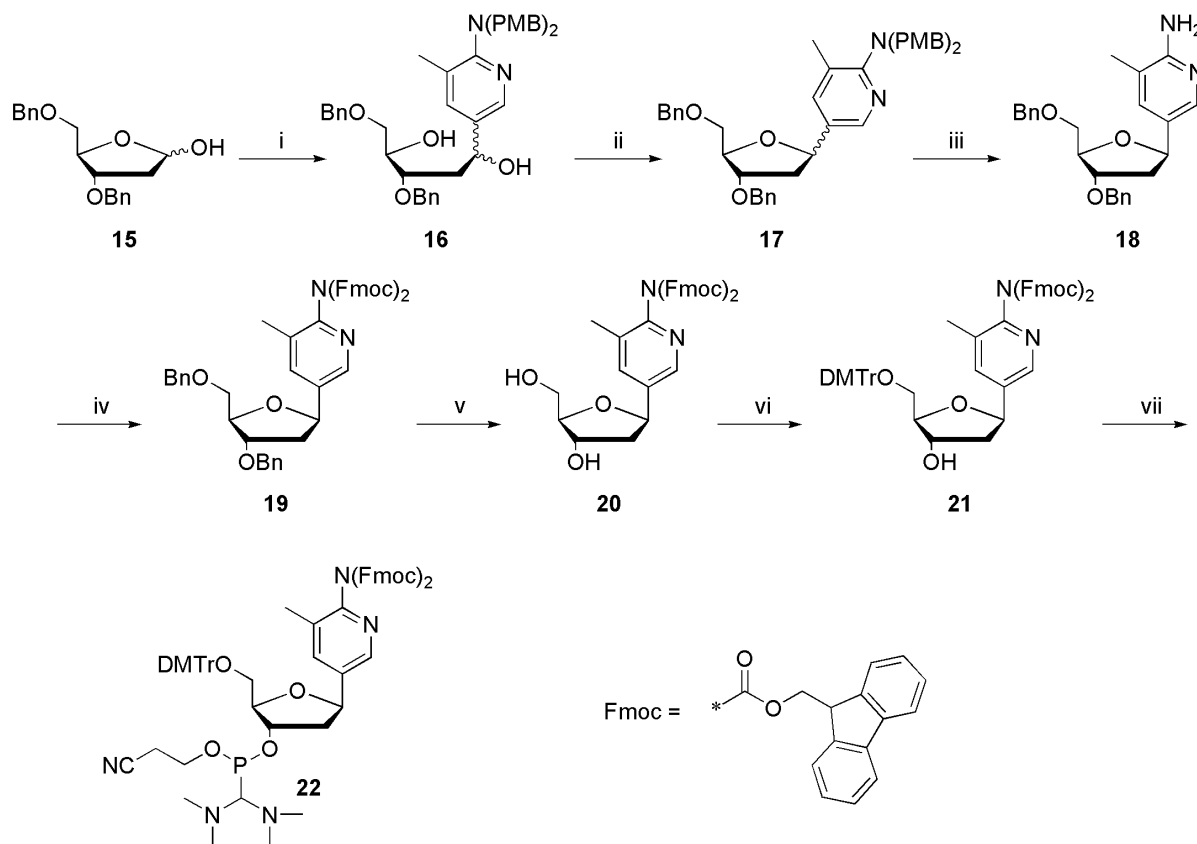
Compound **12a** (0.048 g, 0.073 mmol) was dissolved in anhydrous DCM (5 mL). DIPEA (0.30 mL, 1.72 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphine (0.050 mL, 0.224 mmol) were added and the mixture was stirred under an argon atmosphere for 3 h. The reaction mixture was quenched by saturated potassium chloride (5 mL) before anhydrous DCM (20 mL) was added. The organic layer was separated under an argon atmosphere and then the solvent evaporated *in vacuo*. The residue was purified under an argon atmosphere by silica gel column chromatography (50% ethyl acetate in hexane with 1% pyridine) to yield the product as pale yellow gum (0.0440 g, 70%). R_f 0.88 (ethyl acetate/hexane, 1:3). ^1H NMR: (400 MHz, $\text{DMSO}-d^6$) δ ppm 8.49 (s, 1H, $\underline{\text{NH}}$), 7.97*, 7.95* (d, 1H, $\underline{\text{CH}}^6$), 7.57-7.31 (m, 10H, $\underline{\text{CH}}^4$, $\underline{\text{CH}}\text{-Ar}$), 7.03-6.97 (m, 4H, $\underline{\text{CH}}\text{-Ar}$), 5.02-4.95 (m, 1H, $\underline{\text{CH}}^1$), 4.46-4.38 (m, 1H, $\underline{\text{CH}}^3$), 4.37-4.32*, 4.30-4.26* (m, 1H, $\underline{\text{CH}}^4$), 3.96-3.88 (m, 1H, $\underline{\text{CH}}^2$), 3.96-3.88*, 3.76-3.59* (m, 2H, $\text{NCC}\underline{\text{H}}_2$), 3.85 (s, 6H, $\underline{\text{CH}}_3$), 3.76-3.59 (m, 2H, NCH), 3.47*, 3.43* (s, 3H, $\underline{\text{CH}}_3$), 3.45-3.27 (m, 2H, $\underline{\text{CH}}_2^5$), 2.93-2.87*, 2.70-2.65* (m, 2H, POCH_2), 2.23*, 2.22* (s, 3H, $\underline{\text{CH}}_3$), 1.27-1.18*, 1.12-1.06* (m, 12H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, $\text{DMSO}-d^6$) δ ppm 159.9 ($\underline{\text{C}}^2$), 146.4 ($\underline{\text{C}}=\text{O}$), 138.3 ($\underline{\text{CH}}^6$), 130.2 ($\underline{\text{CH}}^4$), 130.1 ($\underline{\text{CH}}\text{-Ar}$), 128.3 ($\underline{\text{CH}}\text{-Ar}$), 128.2 ($\underline{\text{CH}}\text{-Ar}$), 128.1 ($\underline{\text{CH}}\text{-Ar}$), 127.2 ($\underline{\text{CH}}\text{-Ar}$), 124.3 ($\underline{\text{CH}}\text{-Ar}$), 113.7 ($\underline{\text{CH}}\text{-Ar}$), 86.3* 85.8* ($\underline{\text{CH}}^2$), 83.8 ($\underline{\text{CH}}^4$), 79.2*, 79.1* ($\underline{\text{CH}}^1$), 72.5*, 72.4* ($\underline{\text{CH}}^3$), 64.1*, 63.7* ($\underline{\text{CH}}_2^5$), 59.3*, 59.2*, ($\underline{\text{CH}}_2$), 58.5*, 58.4* ($\underline{\text{CH}}_3$), 55.5 ($\underline{\text{CH}}_3$), 43.2-43.1*, 43.0-42.9* ($\underline{\text{CH}}$), 24.8*, 24.6* ($\underline{\text{CH}}_3$), 20.3*, 20.2* ($\underline{\text{CH}}_2$), 17.3 ($\underline{\text{CH}}_3$). ^{31}P NMR (121 MHz, $\text{DMSO}-d^6$): δ ppm 150.45, 150.33. m/z (%) LRMS [ES^+ , MeCN]: m/z 875.4 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+]: $\text{C}_{44}\text{H}_{52}\text{F}_3\text{N}_4\text{O}_8\text{PNa}$ requires

875.3367 found 875.3354.

2-(*N*-Trifluoroacetamido)-3-methyl-5-[2'-*O*-(2-methoxyethyl)-3'-*O*-(2-cyanoethyl-*N,N*-diisopropyl-amino)phosphanyl)-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (13b)

Compound **12b** (0.410 g, 0.589 mmol) was dissolved in anhydrous DCM (10 mL). To this was added DIPEA (0.225 mL, 1.29 mmol) and 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite (0.150 mL, 0.670 mmol) and the mixture was stirred under an argon atmosphere for 3 h. To the reaction was added saturated potassium chloride solution (10 mL) then anhydrous DCM (30 mL). After extraction under an argon atmosphere the organic layer was separated and the solvent evaporated *in vacuo*. The residue was purified under an argon atmosphere by silica gel column chromatography (50% ethyl acetate in hexane with 2.5% triethylamine) to yield the product as pale yellow gum (0.487 g, 92%). R_f 0.78 (ethyl acetate/hexane, 1:3). ^1H NMR: (400 MHz, CDCl_3) δ ppm 8.05*, 8.04* (d, 1H, NH), 7.90*, 7.88* (d, 1H, CH^6), 7.41-7.10 (m, 10H, CH^4 , CH-Ar), 6.81-6.68 (m, 4H, CH-Ar), 4.84-4.79 (m, 1H, $\text{CH}^{1'}$), 4.38-4.17 (m, 2H, $\text{CH}^{3'}$, $\text{CH}^{4'}$), 3.93-3.79 (m, 1H, $\text{CH}^{2'}$), 3.93-3.79*, 3.64-3.39* (m, 2H, NCCH_2), 3.72*, 3.71* (d, 6H, CH_3), 3.64-3.39 (m, 6H, CH , CH , CH_2CH_2), 3.21(s, 3H, CH_3), 3.39-3.13 (m, 2H, $\text{CH}_2^{5'}$), 2.62-2.56*, 2.28-2.22* (m, 2H, POCH_2), 2.19*, 2.18* (d, 3H, CH_3), 1.16-1.07*, 0.99-0.92* (m, 12H, CH_3). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 158.6 (C^2), 139.1 (CH^6), 130.1, 130.0, 128.6, 128.3, 127.9, 127.0, 113.2 (CH^4 , CH-Ar), 86.2 ($\text{CH}^{2'}$), 85.3 ($\text{CH}^{4'}$), 78.4 ($\text{CH}^{1'}$), 71.8 (CH_2), 71.0 ($\text{CH}^{3'}$), 70.0 (CH_2), 64.1 ($\text{CH}_2^{5'}$), 59.0 (CH_3), 55.2 (CH_3), 24.6 (CH_3). ^{31}P NMR (121 MHz, CDCl_3): δ ppm 150.06, 150.01. m/z (%) LRMS [ES^+ , MeCN]: 919.7 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+]: $\text{C}_{46}\text{H}_{56}\text{F}_3\text{N}_4\text{O}_9\text{PNa}$ requires 919.2629 found 919.3631.

S3. Synthesis of dMAP phosphoramidite monomer^{2,3}



Scheme S2. Reagents and conditions: (i) 2-[*N,N*-bis(4-methoxybenzyl)]amino-3-methyl-5-bromopyridine (**2**), *n*-BuLi, THF, -35 °C, 0.5h; -30 °C, 1h; rt, 4.5h, 92%; (ii) Bu₃P, TMAD, THF, rt, 6h, 88%; (iii) CF₃COOH/DCM, rt, 7h, 99%; (iv) Fmoc-Cl (in CH₃CN), pyridine, 0 °C, rt, 2h, 96% recovering; (v) BCl₃, DCM, -78 °C, 6h; methanol, -75 °C, 0.5h; 4 °C, 17.5h, 79%; (vi) DMTrCl, pyridine, rt, 3.5h, 87% recovering; (vii) 2-cyanoethyl-*N,N*-diisopropyl chlorophosphoramidite, DIPEA, DCM, rt, 2h, 50%.

2-[*N,N*-bis(4-methoxybenzyl)]-amino-3-methyl-5-(1'-hydroxyl-2'-deoxy-3',5'-di-*O*-benzyl-1-*D*-ribofuranosyl)-pyridine *R*- and *S*- anomers (**16**)

A solution of 2-[*N,N*-bis(4-methoxybenzyl)]amino-3-methyl-5-bromopyridine (**2**, 53.8 g, 126 mmol) in anhydrous THF (180 mL) was cooled to -35 °C before dropwise addition of *n*-butyllithium (1.6 M solution in hexanes, 79.0 mL, 126 mmol). The reaction mixture was left to stir at -35 °C for 0.5 h, before dropwise addition of a solution of

2'-deoxy-3',5'-di-O-benzyl-D-ribo-1,4-lactol⁴ (**15**, 15.8 g, 50.4 mmol) in anhydrous THF (50 mL). The reaction mixture was then left to stir at -30 °C for 1 h, after which it was warmed to rt for 4.5 h. After solvent was removed *in vacuo*, the brown-orange residue was quenched with water and extracted with ethyl acetate. The organic phases were combined and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (50% ethyl acetate in DCM) to afford the product (epimeric mixture, **R** and **S**) as a brown foam (30.7 g, 92%). **R**-anomer: 0.35; **S**-anomer: 0.25 (ethyl acetate/hexane, 1:1). **R**-anomer: ¹H NMR: (400 MHz, CDCl₃) δ ppm 8.11 (d, *J* = 2.0 Hz, 1H, CH⁶), 7.43 (d, *J* = 2.0 Hz, 1H, CH⁴), 7.37-7.29 (m, 10H, CH-Ar), 7.17 (d, *J* = 8.5 Hz, 4H, CH-Ar), 6.80 (d, *J* = 8.5 Hz, 4H, CH-Ar), 4.88 (br t, *J* = 6.5 Hz, 1H, CH¹), 4.63-4.59 (m, 2H, CH₂), 4.56 (s, 2H, CH₂), 4.23 (s, 4H, CH₂), 4.03-3.98 (m, 1H, CH⁴), 3.81 (dd, *J* = 11.0, 5.5 Hz, 1H, CH³), 3.76 (s, 6H, CH₃), 3.65 (dd, *J* = 9.5, 3.5 Hz, 1H, CHH⁵), 3.60 (dd, *J* = 9.5, 6.5 Hz, 1H, CHH⁵), 3.17 (bs, 1H, 1'-OH), 2.86 (bs, 1H, 4'-OH), 2.37 (s, 3H, CH₃), 2.00 (br t, *J* = 6.0 Hz, 2H, CH₂²). ¹³C NMR: (100 MHz, CDCl₃) δ ppm 160.9 (C²), 158.5 (C-Ar), 142.9 (CH⁶) 138.0 (C-Ar), 137.9 (C-Ar), 137.3 (CH⁴), 134.2 (C-Ar), 131.2 (C-Ar), 130.9 (C-Ar), 129.6 (CH-Ar), 128.6 (CH-Ar), 128.6 (CH-Ar), 128.4 (CH-Ar), 128.2 (CH-Ar), 128.1 (CH-Ar), 128.0 (CH-Ar), 125.8 (C-Ar), 113.7 (CH-Ar), 77.4 (CH³), 73.6 (CH₂), 72.6 (CH₂), 71.8 (CH⁴), 71.1 (CH₂⁵), 68.9 (CH¹), 55.2 (OCH₃), 53.8 (CH₂), 39.5 (CH₂²), 18.8 (CH₃). *m/z* (%) LRMS [ES⁺, MeOH]: 663 ([M+H]⁺, 100%). **S**-anomer: ¹H NMR: (400 MHz, CDCl₃) δ ppm 8.12 (d, *J* = 2.5 Hz, 1H, CH⁶), 7.43 (d, *J* = 2.0 Hz, 1H, CH⁴), 7.36-7.28 (m, 10H, CH-Ar), 7.17 (d, *J* = 8.5 Hz, 4H, CH-Ar), 6.81 (d, *J* = 8.6 Hz, 4H, CH-Ar), 4.89 (dd, *J* = 8.5, 3.0 Hz, 1H, CH¹), 4.61-4.51 (m, 4H, CH₂), 4.23 (s, 4H, CH₂), 4.05 (dd, *J* = 10.6, 5.5 Hz, 1H, CH⁴), 3.76 (br s, 7H, OCH₃, OCH₃, CH³), 3.61-3.58 (m, 2H, CH₂⁵), 2.38 (s, 3H, CH₃), 2.13 (br dt, *J* = 15.1, 8.5 Hz, 1H, CHH²), 1.92 (dt, *J* = 15.0, 3.5 Hz, 1H, CHH²). ¹³C NMR: (100 MHz, CDCl₃) δ ppm 161.0 (C²), 158.5 (C-Ar), 143.1 (CH⁶), 137.8 (C-Ar), 137.8 (C-Ar), 137.8 (CH⁴), 134.2 (C-Ar), 134.0 (C-Ar), 131.1 (C-Ar), 130.9 (C-Ar), 129.6 (CH-Ar), 128.6 (CH-Ar), 128.5 (CH-Ar), 128.2 (CH-Ar), 128.1 (CH-Ar), 128.0 (CH-Ar), 127.9 (CH-Ar), 125.8 (C-Ar), 113.6 (CH-Ar), 78.7 (CH³), 73.5 (CH₂), 72.1 (CH₂), 71.6 (CH⁴), 70.9 (CH₂⁵), 69.5 (CH¹), 55.2 (OCH₃), 53.8 (CH₂), 39.0 (CH₂²), 18.8

($\underline{\text{C}}\text{H}_3$). m/z (%) LRMS [ES^+ , MeOH]: 663 ($(\text{M}+\text{H})^+$, 100%). HRMS [ES^+]: $\text{C}_{41}\text{H}_{47}\text{N}_2\text{O}_6$ requires 663.3429 found 663.3421.

2-[*N,N*-Bis-(4-Methoxybenzyl)]amino-3-methyl-5-(2'-deoxy-3',5'-di-*O*-benzyl-D-ribofuranosyl)-pyridine α - and β - anomers (17)

A solution of **16** (12.5 g, 18.8 mmol) in anhydrous THF (86.0 mL) was cooled to 0 °C, before addition of Bu_3P (7.44 mL, 30.2 mmol) followed by TMAD (5.19 g, 30.2 mmol). The reaction mixture was left to stir at rt for 6 h, after which the reaction mixture was filtered and the filtrate concentrated *in vacuo*. The residue was quenched with water and extracted with ethyl acetate. The organic phases were combined and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (25% ethyl acetate in petroleum ether) to afford the product (10.8 g, 88%, $\alpha/\beta=2:3$) as yellow oil. R_f α -anomer: 0.58; β -anomer: 0.61 (ethyl acetate/hexane, 1:1). α -anomer: ^1H NMR: (400 MHz, CDCl_3) δ ppm 8.09 (d, $J = 2.0$ Hz, 1H, $\underline{\text{C}}\text{H}^6$), 7.59 (s, 1H, $\underline{\text{C}}\text{H}^4$), 7.36-7.28 (m, 10H, $\underline{\text{C}}\text{H}$ -Ar), 7.15 (d, $J = 8.5$ Hz, 4H, $\underline{\text{C}}\text{H}$ -Ar), 6.80 (d, $J = 8.5$ Hz, 4H, $\underline{\text{C}}\text{H}$ -Ar), 5.02 (t, $J = 8.0$ Hz, 1H, $\underline{\text{C}}\text{H}^{1'}$), 4.63-4.52 (m, 4H, $\underline{\text{C}}\text{H}_2$), 4.36 (dd, $J = 9.0, 4.5$ Hz, 1H, $\underline{\text{C}}\text{H}^4$), 4.26 (dd, $J = 10.6, 6.5$ Hz, 1H, $\underline{\text{C}}\text{H}^{3'}$), 4.22 (s, 4H, $\underline{\text{C}}\text{H}_2$), 3.78 (s, 6H, OCH_3), 3.61 (m, 2H, $\underline{\text{C}}\text{H}_2^{5'}$), 2.61 (dt, $J = 13.0, 6.5$ Hz, 1H, $\underline{\text{C}}\text{H}\text{H}^{2' \alpha}$), 2.36 (s, 3H, $\underline{\text{C}}\text{H}_3$), 2.05 (ddd, $J = 13.6, 8.0, 6.0$ Hz, 1H, $\underline{\text{C}}\text{H}\text{H}^{2' \beta}$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 161.2 ($\underline{\text{C}}^2$), 158.6 ($\underline{\text{C}}$ -Ar), 143.5 ($\underline{\text{C}}\text{H}^6$), 138.3 ($\underline{\text{C}}\text{H}^4$), 138.1 ($\underline{\text{C}}$ -Ar), 137.9 ($\underline{\text{C}}$ -Ar), 131.8 ($\underline{\text{C}}$ -Ar), 131.1 ($\underline{\text{C}}$ -Ar), 129.6 ($\underline{\text{C}}\text{H}$ -Ar), 128.5 ($\underline{\text{C}}\text{H}$ -Ar), 127.8 ($\underline{\text{C}}\text{H}$ -Ar), 125.9 ($\underline{\text{C}}$ -Ar), 113.7 ($\underline{\text{C}}\text{H}$ -Ar), 83.0 ($\underline{\text{C}}\text{H}^4$), 80.9 ($\underline{\text{C}}\text{H}^{3'}$), 78.2 ($\underline{\text{C}}\text{H}^{1'}$), 73.6 ($\underline{\text{C}}\text{H}_2$), 71.9 ($\underline{\text{C}}\text{H}_2$), 71.0 ($\underline{\text{C}}\text{H}_2^{5'}$), 55.3 (OCH_3), 53.7 ($\underline{\text{C}}\text{H}_2$), 40.7 ($\underline{\text{C}}\text{H}_2^{2'}$), 18.9 ($\underline{\text{C}}\text{H}_3$). NOE (400 MHz, CDCl_3): irradiation at δ 8.09 (H-C(6)) produced NOE enhancement at δ 5.02 (H-C(1')); irradiation at δ 7.59 (H-C(4)) produced NOE enhancements at δ 5.02 (H-C(1')), 2.36 (H-C(CH_3)), 2.05 (H-C($2' \alpha$)), the cross-peak between the H^4 and the higher field $\text{H}^{2' \alpha}$ resonance signals is stronger than that between H^4 and $\text{H}^{1'}$; irradiation at δ 5.02 (H-C(1')) produced NOE enhancements at δ 8.09 (H-C(6)), 7.59 (H-C(4)), 3.61 (H-C(5')), 2.61 (H-C($2' \beta$)), the cross-peak between the $\text{H}^{1'}$ and lower field $\text{H}^{2' \beta}$ resonance signals is very strong, and the cross-peak between $\text{H}^{1'}$ and H^6 resonance signals is stronger

than that between $H^{1'}$ and H^4 ; irradiation at δ 3.61 (H-C(5')) produced NOE enhancements at δ 5.02 (H-C(1')), 4.60 (H-C(Bn 5')), 4.36 (H-C(4')). HRMS [ES⁺]: C₄₁H₄₅N₂O₅ requires 645.3323 found 645.3324. β -anomer: ¹H NMR: (400 MHz, CDCl₃) δ ppm 8.06 (d, J = 2.0 Hz, 1H, CH⁶), 7.40 (s, 1H, CH⁴), 7.29-7.21 (m, 10H, CH-Ar), 7.10 (d, J = 8.5 Hz, 4H, CH-Ar), 6.74 (d, J = 9.0 Hz, 4H, CH-Ar), 5.02 (dd, J = 10.5, 4.5 Hz, 1H, CH^{1'}), 4.53 (s, 2H, CH₂), 4.50 (s, 2H, CH₂), 4.23 (m, 1H, CH^{4'}), 4.16-4.12 (m, 5H, CH^{3'}, CH₂, CH₂), 3.68 (s, 6H, OCH₃), 3.60 (dd, J = 10.0, 4.0 Hz, 1H, CHH^{5'}), 3.53 (dd, J = 10.0, 5.0 Hz, 1H, CHH^{5'}), 2.27 (m, 4H, CHH^{2'}_a, CH₃), 1.89 (ddd, J = 13.0, 11.0, 6.0 Hz, 1H, CHH^{2'} _{β}). ¹³C NMR (100 MHz, CDCl₃): δ 161.3 (C²), 158.5 (C-Ar), 143.6 (CH⁶) 138.2 (C-Ar), 138.1 (C-Ar), 137.7 (CH⁴), 131.1 (C-Ar), 130.6 (C-Ar), 129.5 (C-Ar), 128.5 (CH-Ar), 128.4 (CH-Ar), 127.7 (CH-Ar), 127.7 (CH-Ar), 127.6 (C-Ar), 125.7 (C-Ar), 113.6 (CH-Ar), 83.9 (CH^{4'}), 81.7 (CH^{3'}), 78.3 (CH^{1'}), 73.5 (CH₂), 71.2 (CH₂), 71.1 (CH₂^{5'}), 55.2 (OCH₃), 53.7 (CH₂), 40.8 (CH₂^{2'}), 18.8 (CH₃). NOE (400 MHz, CDCl₃): irradiation at δ 8.06 (H-C(6)) produced NOE enhancement at δ 5.02 (H-C(1')); irradiation at δ 7.40 (H-C(4)) produced NOE enhancements at δ 5.02 (H-C(1')), 2.27 (H-C(CH₃)), 1.89 (H-C(2' _{β})), the cross-peak between H^4 and higher field $H^{2'_{\beta}}$ resonance signals is stronger than that between H^4 and $H^{1'}$; irradiation at δ 5.02 (H-C(1')) produced NOE enhancements at δ 8.06 (H-C(6)), 7.40 (H-C(4)), 2.27 (H-C(2'_a)), 1.89 (H-C(2' _{β})), the cross peak between $H^{1'}$ and H^6 resonance signals is stronger than that between $H^{1'}$ and H^4 , and the cross peak between $H^{1'}$ and $H^{2'_a}$ resonance signals is stronger than that between $H^{1'}$ and $H^{2'_{\beta}}$; irradiation at δ 3.60 (H-C(5')) produced NOE enhancements at δ 4.53 (H-C(Bn 5')), 3.53 (H-C(5')), the cross-peak between $H^{5'}$ and $H^{5'}$ resonance signals is stronger than that between $H^{5'}$ and Bn-CH₂; irradiation at δ 3.53 (H-C(5')) produced NOE enhancements at δ 3.60 (H-C(5')), 1.89 (H-C(2' _{β})), the cross-peak between $H^{5'}$ and $H^{5'}$ resonance signals is stronger than that between $H^{5'}$ and higher field $H^{2'_{\beta}}$ m/z (%) LRMS [ES⁺, MeOH]: 645 ([M+H]⁺, 100%).

2-Amino-3-methyl-5-(2'-deoxy-3',5'-di-*O*-benzyl- β -D-ribofuranosyl)-pyridine (18)

To a solution of **17** (4.71 g, 7.30 mmol, β -anomer) in anhydrous DCM (17 mL) was added trifluoroacetic acid (17.0 mL) and the resulting solution was left to stir at rt for 5.5 h, more

trifluoroacetic acid (8.0 mL) was added and the reaction mixture left to stir for a further 1.5 h after which solvent was removed *in vacuo*. The residue was redissolved in water and sodium carbonate (solid) was added until > pH 8.0, extraction with DCM followed. The organic layers were combined and dried over anhydrous sodium sulfate. After filtration, the solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (70% ethyl acetate in hexane) to afford the product as mustard yellow oil (2.92 g, 99%). R_f 0.43 (MeOH/ethyl acetate, 1:19). $^1\text{H NMR}$: (400 MHz, CDCl_3) δ ppm 7.81 (s, 1H, CH^6), 7.41 (s, 1H, CH^4), 7.35-7.29 (m, 10H, CH-Ar), 5.50 (br s, 2H, NH_2), 5.00 (dd, $J = 10.6, 5.0$ Hz, 1H, CH^1), 4.58 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.28-4.25 (m, 1H, CH^4), 4.18 (d, $J = 6.0$ Hz, 1H, CH^3), 3.64 (dd, $J = 10.0, 4.0$ Hz, 1H, CHH^5), 3.58 (dd, $J = 10.0, 5.0$ Hz, 1H, CHH^5), 2.29 (dd, $J = 13.0, 5.0$ Hz, 1H, CHH^2), 2.08 (s, 3H, CH_3), 1.91 (ddd, $J = 13.0, 10.6, 5.5$ Hz, 1H, CHH^2). $^{13}\text{C NMR}$: (100 MHz, CDCl_3) δ ppm 156.2 (C^2), 139.8 (CH^6) 138.2 (C-Ar), 138.1 (C-Ar), 137.8 (CH^4), 128.6 (CH-Ar), 128.5 (CH-Ar), 127.9 (CH-Ar), 127.8 (CH-Ar), 127.8 (CH-Ar), 127.7 (CH-Ar), 127.1 (C-Ar), 118.4 (C-Ar), 84.0 (CH^4), 81.7 (CH^3), 78.0 (CH^1), 73.6 (CH_2), 71.2 (CH_2), 71.2 (CH_2^5), 40.7 (CH_2^2), 16.9 (CH_3). m/z (%) LRMS [ES^+ , MeOH]: 405 ($[\text{M}+\text{H}]^+$, 100%). HRMS [ES^+]: $\text{C}_{25}\text{H}_{29}\text{N}_2\text{O}_3$ requires 405.2173 found 405.2175.

2-[*N,N*-Bis-(Fmoc)]amino-3-methyl-5-(2'-deoxy-3',5'-di-*O*-benzyl- β -D-ribofuranosyl)-pyridine (19)

A solution of **18** (1.08 g, 2.66 mmol) in anhydrous pyridine (7.0 mL) was cooled to 0 °C before careful addition of Fmoc-Cl (5.51 g, 21.3 mmol) in anhydrous acetonitrile (5.0 mL). The reaction mixture was left to stir at 0 °C for 2 h after which solvent was reduced *in vacuo*. The residue was then partitioned between water and DCM. The organic phases were combined and dried over anhydrous sodium sulfate. After filtration, the solution was evaporated *in vacuo* and the residue was purified by silica gel column chromatography (40% ethyl acetate in hexane) to give the product as white foam (0.612 g, 96% recovering). R_f 0.39 (ethyl acetate/hexane, 1:1). $^1\text{H NMR}$: (400 MHz, CDCl_3) δ ppm 8.21 (d, $J = 2.0$ Hz, 1H, CH^6), 7.66 (d, $J = 7.6$ Hz, 4H, CH-Ar), 7.56 (d, $J = 2.0$ Hz, 1H, CH^4), 7.43-7.28 (m, 17H, CH-Ar), 7.20 (m, 5H, CH-Ar), 5.23 (dd, $J = 10.5, 5.0$ Hz, 1H, CH^1), 4.64 (s, 2H, CH_2), 4.63

(s, 2H, $\underline{\text{CH}}_2$), 4.49-4.40 (m, 5H, $\underline{\text{CH}}_2$, $\underline{\text{CH}}_2$, $\underline{\text{CH}}^4$), 4.25 (d, $J = 5.5$ Hz, 1H, $\underline{\text{CH}}^3$), 4.09-4.06 (m, 2H, $\underline{\text{CH}}$, $\underline{\text{CH}}$), 3.73 (dd, $J = 10.0, 4.0$ Hz, 1H, $\underline{\text{CHH}}^5$), 3.67 (dd, $J = 10.0, 5.0$ Hz, 1H, $\underline{\text{CHH}}^5$), 2.45 (dd, $J = 12.0, 5.5$ Hz, 1H, $\underline{\text{CHH}}^2$ α), 1.96 (ddd, $J = 13.5, 11.0, 6.0$ Hz, 1H, $\underline{\text{CHH}}^2$ β), 1.83 (s, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 151.6 ($\underline{\text{C}}\text{-Ar}$), 148.9 ($\underline{\text{C}}^2$), 144.9 ($\underline{\text{CH}}^6$), 144.5 ($\underline{\text{C}}\text{-Ar}$), 143.4 ($\underline{\text{C}}\text{-Ar}$), 141.7 ($\underline{\text{C}}\text{-Ar}$), 141.3 ($\underline{\text{C}}\text{-Ar}$), 138.4 ($\underline{\text{C}}\text{-Ar}$), 138.2 ($\underline{\text{C}}\text{-Ar}$), 138.1 ($\underline{\text{C}}\text{-Ar}$), 137.6 ($\underline{\text{CH}}^4$), 131.3 ($\underline{\text{C}}\text{-Ar}$), 128.7 ($\underline{\text{CH}}\text{-Ar}$), 128.6 ($\underline{\text{CH}}\text{-Ar}$), 128.0 ($\underline{\text{CH}}\text{-Ar}$), 127.9 ($\underline{\text{CH}}\text{-Ar}$), 127.8 ($\underline{\text{CH}}\text{-Ar}$), 127.8 ($\underline{\text{CH}}\text{-Ar}$), 127.2 ($\underline{\text{CH}}\text{-Ar}$), 127.2 ($\underline{\text{CH}}\text{-Ar}$), 120.2 ($\underline{\text{CH}}\text{-Ar}$), 120.1 ($\underline{\text{CH}}\text{-Ar}$), 84.3 ($\underline{\text{CH}}^4$), 81.7 ($\underline{\text{CH}}^3$), 77.9 ($\underline{\text{CH}}^1$), 73.8 ($\underline{\text{CH}}_2$), 71.4 ($\underline{\text{CH}}_2$), 71.3 ($\underline{\text{CH}}_2^5$), 68.8 ($\underline{\text{CH}}_2$), 46.7 ($\underline{\text{CH}}$), 41.5 ($\underline{\text{CH}}_2^2$), 16.8 ($\underline{\text{CH}}_3$). m/z (%) LRMS [ES^+ , MeOH]: 849 ($[\text{M}+\text{H}]^+$, 100%). HRMS [ES^+]: $\text{C}_{55}\text{H}_{48}\text{N}_2\text{O}_7\text{Na}$ requires 871.3354 found 871.3367.

2-[*N,N*-Bis-(Fmoc)]amino-3-methyl-5-(2'-deoxy- β -D-ribofuranosyl)-pyridine (20)

A solution of **19** (1.04 g, 1.22 mmol) in anhydrous DCM (3.7 mL) was cooled to -78 °C before careful dropwise addition of boron trichloride (1.0 M in DCM, 3.70 mL, 3.70 mmol). The reaction mixture was left to stir at -78 °C for 6 h after which methanol (100 mL) was added. The resulting solution was left to stir at -75 °C for 0.5 h after which it was left to stand at 4 °C for 17.5 h. Saturated sodium bicarbonate solution was added until pH 7.0 obtained. Solvent was removed *in vacuo* and the residue partitioned between DCM and water. The organic phases were combined and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (8% methanol in DCM) to give the product as cream foam (0.542 g, 79% recovering). R_f 0.43 (MeOH/DCM, 1:9). ^1H NMR: (400 MHz, $\text{DMSO}-d^6$) δ ppm 8.17 (s, 1H, $\underline{\text{CH}}^6$), 7.77 (d, $J = 7.0$ Hz, 4H, $\underline{\text{CH}}\text{-Ar}$), 7.62 (s, 1H, $\underline{\text{CH}}^4$), 7.35 (m, 4H, $\underline{\text{CH}}\text{-Ar}$), 7.28-7.17 (m, 8H, $\underline{\text{CH}}\text{-Ar}$), 5.18-5.13 (m, 2H, 3'-OH, $\underline{\text{CH}}^1$), 4.85 (t, $J = 5.5$ Hz, 1H, 5'-OH), 4.44 (m, 4H, $\underline{\text{CH}}_2$), 4.31 (m, 1H, $\underline{\text{CH}}^3$), 4.10-4.07 (m, 2H, $\underline{\text{CH}}$, $\underline{\text{CH}}$), 3.92-3.91 (m, 1H, $\underline{\text{CH}}^4$), 3.61-3.51 (m, 2H, $\underline{\text{CH}}_2^5$), 2.23 (dd, $J = 11.5, 5.0$ Hz, 1H, $\underline{\text{CHH}}^2$ α), 1.89 (td, $J = 12.6, 5.5$ Hz, 1H, $\underline{\text{CHH}}^2$ β), 1.73 (s, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, $\text{DMSO}-d^6$) δ ppm 150.7 ($\underline{\text{C}}\text{-Ar}$), 148.0 ($\underline{\text{C}}^2$), 144.3 ($\underline{\text{CH}}^6$), 143.7 ($\underline{\text{C}}\text{-Ar}$), 143.1 ($\underline{\text{C}}\text{-Ar}$), 140.6 ($\underline{\text{C}}\text{-Ar}$), 138.8 ($\underline{\text{C}}\text{-Ar}$), 137.4 ($\underline{\text{CH}}^4$), 130.4 ($\underline{\text{C}}\text{-Ar}$), 128.9 ($\underline{\text{CH}}\text{-Ar}$), 128.6 ($\underline{\text{CH}}\text{-Ar}$), 128.4 ($\underline{\text{CH}}\text{-Ar}$), 128.2 ($\underline{\text{CH}}\text{-Ar}$), 127.6 ($\underline{\text{CH}}\text{-Ar}$), 127.3

(CH-Ar), 126.9 (C-Ar), 125.9(CH-Ar), 125.2 (CH-Ar), 124.7 (C-Ar), 121.4 (CH-Ar), 120.0 (CH-Ar), 88.1 (CH^{4'}), 76.8 (CH^{1'}), 72.5 (CH^{3'}), 68.0 (CH₂), 62.4 (CH₂^{5'}), 45.9 (CH), 43.4 (CH₂^{2'}), 16.0 (CH₃). *m/z* (%) LRMS [ES⁺, MeOH]: 691 ([M+Na]⁺, 100%). HRMS [ES⁺]: C₄₁H₃₆N₂O₇Na requires 691.2415 found 691.2407.

2-[*N,N*-Bis-(Fmoc)]amino-3-methyl-5-[2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-pyridine (21)

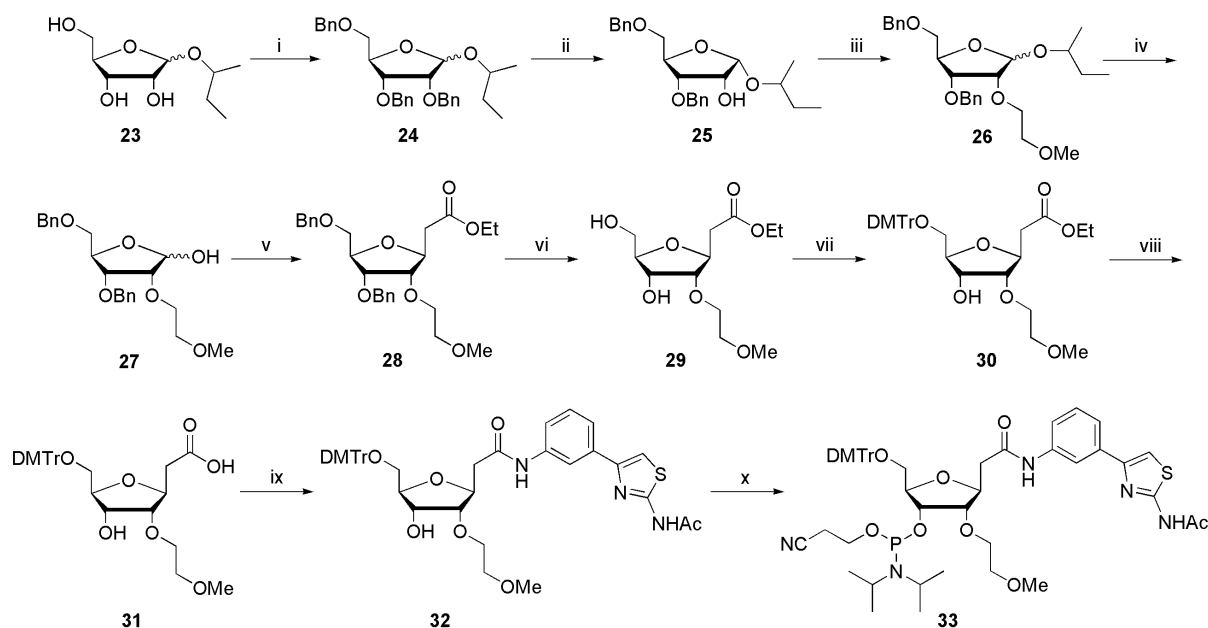
Co-evaporation of **20** (0.700 g, 1.05 mmol) with anhydrous pyridine was carried out under high vacuum and left to dry overnight under high vacuum. To a solution of **20** (0.700 g, 1.05 mmol) in anhydrous pyridine (2.5 mL) was added a solution of DMTrCl (0.488 g, 1.44 mmol, dissolved in 3.0 mL pyridine) dropwise and the reaction mixture was left to stir at rt for 3.5 h, after which solvent was reduced to half in *vacuo*. Methanol (2 mL) and triethylamine (1 mL) were added and the reaction mixture partitioned between DCM and saturated sodium bicarbonate solution. The organic phases were combined and dried over anhydrous sodium sulfate. After filtration, solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography (60% ethyl acetate in hexane with 0.5% pyridine) to afford the product as white foam (0.221 g, 87% recovering). *R_f* 0.15 (ethyl acetate/hexane, 1:1, with 0.5 % pyridine). ¹H NMR: (400 MHz, DMSO-*d*⁶) δ ppm 8.15 (d, *J* = 2.0 Hz, 1H, CH⁶), 7.74 (d, *J* = 7.5 Hz, 4H, CH-Ar), 7.57 (d, *J* = 1.5 Hz, 1H, CH⁴), 7.45 (d, *J* = 7.0 Hz, 4H, CH-Ar), 7.32-7.19 (m, 17H, CH-Ar), 6.83 (d, *J* = 8.5 Hz, 4H, CH-Ar), 5.26 (d, *J* = 4.5 Hz, 1H, 3'-OH), 5.20 (dd, *J* = 10.0, 5.5 Hz, 1H, CH^{1'}), 4.44 (m, 4H, CH₂), 4.26 (m, 1H, CH^{3'}), 4.08-4.05 (m, 3H, CH, CH, CH^{4'}), 3.67 (s, 6H, OCH₃), 3.20 (d, *J* = 4.5 Hz, 2H, CH₂^{5'}), 2.31-2.27 (m, 1H, CHH^{2'_w}), 2.00-1.90 (m, 1H, CHH^{2'_p}), 1.66 (s, 3H, CH₃). ¹³C NMR: (100 MHz, DMSO-*d*⁶) δ ppm 158.0 (C-Ar), 150.6 (C-Ar), 148.1 (C²), 144.9 (C-Ar), 144.2 (CH⁶), 143.1 (C-Ar), 140.6 (C-Ar), 138.5 (C-Ar), 137.2 (CH⁴), 135.7 (C-Ar), 135.6 (C-Ar), 130.4 (C-Ar), 129.7 (CH-Ar), 127.8 (CH-Ar), 127.7 (CH-Ar), 127.6 (CH-Ar), 126.9 (CH-Ar), 126.7 (CH-Ar), 124.7 (CH-Ar), 120.0 (CH-Ar), 113.2 (CH-Ar), 86.3 (CH^{4'}), 85.5 (Ar₃C), 76.9 (CH^{1'}), 72.5 (CH^{3'}), 67.9 (CH₂), 64.6 (CH₂^{5'}), 54.9 (OCH₃), 45.9 (CH), 43.6 (CH₂^{2'}), 16.0 (CH₃). *m/z* (%) LRMS [ES⁺, MeOH]: 993 ([M+Na]⁺, 100%). HRMS [ES⁺]: C₆₂H₅₅N₂O₉ requires 971.3902 found

971.3898.

2-[*N,N*-Bis-(Fmoc)]amino-3-methyl-5-(2'-deoxy-3'-*O*-(2-cyanoethyl-*N,N*-diisopropyl-amino)phosphanyl)-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl)-pyridine (22)

To a solution of **21** (0.387 g, 0.398 mmol) in anhydrous DCM (2.3 mL) was added anhydrous DIPEA (0.140 mL, 0.797 mmol) followed by dropwise addition of 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.110 mL, 0.483 mmol) under an argon atmosphere and the resultant solution left to stir at rt for 2 h. The reaction mixture was transferred via needle and syringe to a separating funnel containing anhydrous DCM. The reaction mixture was washed with saturated potassium chloride solution (10 mL) and the organic phase was dried over anhydrous sodium sulfate. After filtration, the filtrate was evaporated *in vacuo* under argon to give following purification by silica gel column chromatography (50% ethyl acetate in hexane with 0.5% pyridine) under argon pressure, the product as white foam (0.231 g, 50 %). R_f 0.27 (ethyl acetate/hexane, 1:1, with 0.5 % pyridine). $^1\text{H NMR}$: (400 MHz, MeCN- d^3) δ ppm 8.04 (s, 1H, CH⁶), 7.66 (d, $J = 7.5$ Hz, 4H, CH-Ar), 7.51-7.45 (m, 3H, CH-Ar, CH⁴), 7.38-7.19 (m, 19H, CH-Ar), 6.83-6.80 (m, 4H, CH-Ar), 5.20 (dd, $J = 9.0, 4.0$ Hz, 1H, CH¹), 4.59-4.56 (m, 1H, CH³), 4.47 (m, 4H, CH₂, CH₂), 4.29-4.26 (m, 1H, CH⁴), 4.05-4.02 (m, 2H, CH, CH), 3.87-3.84 (m, 1H, OCHH), 3.75-3.74 (m, 1H, OCHH), 3.69 (m, 7H, OCH₃, OCH₃, CH), 3.33 (t, $J = 4.5$ Hz, 1H, CHH⁵), 3.30 (d, $J = 4.5$ Hz, 1H, CHH⁵), 2.68 (t, $J = 6.0$ Hz, 1H, CHHCN), 2.58 (t, $J = 6.0$ Hz, 1H, CHHCN), 2.55-2.43 (m, 1H, CHH^{2'}_a), 2.10-2.04 (m, 1H, CHH^{2'}_b), 1.58 (s, 3H, CH₃), 1.23-1.13 (m, 12H, CH₃). $^{13}\text{C NMR}$: (100 MHz, MeCN- d^3) δ ppm 159.7 (C-Ar), 151.9 (C²), 149.6 (C-Ar), 146.2 (C-Ar), 145.3 (CH⁶), 144.4 (C-Ar), 142.1 (C-Ar), 139.0 (C-Ar), 139.0 (C-Ar), 138.3 (CH⁴), 131.9 (C-Ar), 131.1 (CH-Ar), 129.1 (CH-Ar), 129.0 (CH-Ar), 128.9 (CH-Ar), 128.6 (CH-Ar), 128.1 (CH-Ar), 128.0 (CH-Ar), 126.9 (CH-Ar), 125.6 (CH-Ar), 124.8 (CH-Ar), 121.0 (CH-Ar), 114.0 (CH-Ar), 87.1^{*}, 86.7^{*} (CH⁴), 78.6^{*}, 78.5^{*} (CH¹), 77.0^{*}, 76.4^{*} (CH³), 68.8 (CH₂), 65.4^{*}, 65.3^{*} (CH₂⁵), 59.5 (OCH₂), 55.9 (OCH₃), 47.3 (CH), 44.1 (CH), 44.0^{*}, 43.7^{*} (CH₂^{2'}), 24.9 (CH₃), 21.1 (CH₂CN), 16.7 (CH₃). $^{31}\text{P NMR}$ (121 MHz, MeCN- d^3): δ ppm 148.90, 148.75. m/z (%) LRMS [ES⁺, MeOH]: 1171 ([M + H]⁺, 100%).

S4. Synthesis of 2'-methoxyethoxy-S phosphoramidite monomer⁵⁻⁹



Scheme S3. Reagents and conditions: i) benzyl bromide, NaH, DMF, -10°C to rt, overnight, 61%; ii) SnCl₄, DCM, rt, 1h, 78%; iii) Br(CH₂)₂OMe, NaH, DMF, -10°C to rt, 2.5h, 94%; iv) CF₃CO₂H, H₂O, DCM, -10°C to rt, 4h, 90%; v) PPh₃CHCO₂Et, THF, reflux, 3h, then EtONa, EtOH, rt, 3h, 44%; vi) Pd(OH)₂ (10% on carbon), H₂, MeOH, rt, 20h, 97%; vii) DMTrCl, DMAP, Pyridine, rt, 4h, 76%; viii) NaOH (1M), THF, reflux, 4h, 76%; ix) 2-acetamido-4-(3-aminophenyl)thiazole, EDC.HCl, DMF, rt, 4h, 36%; x) 2-cyanoethyl-*N,N*-diisopropylchloro phosphoramidite, DIPEA, DCM, rt, 2h, 58%.

1-*O*-Isobutyl-2,3,5-*O*-tribenzyl-*D*-ribofuranoside α - and β - anomers (24)

A solution of 1-*O*-Isobutyl-*D*-ribose⁹ (**23**, 10.0 g, 48.5 mmol) in DMF (160 mL) was cooled down to -10 °C and stirred for 15 min before adding NaH (11.6 g, 0.291 mol) in several portions. The reaction was stirred at -10 °C for 30 min before adding benzyl bromide (34.5 mL, 291 mmol). The reaction was stirred under argon overnight at rt and quenched by adding MeOH (50 mL). The organic layer was washed with a saturated solution of sodium bicarbonate (100 mL), brine (100 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using a gradient of ethyl acetate in hexane (from 0 to 15%) to give the product as clear oil (14.2 g, 61%, mixture of α - and β - anomers). *R_f*- α -anomer: 0.66; β -anomer: 0.63 (ethyl acetate/hexane, 3:7). α -anomer: ¹H NMR: (400 MHz, CDCl₃) δ ppm 7.36-7.22 (m, 15H, CH-Ar), 5.12-5.08

(dd, $J = 12, 4$ Hz, 1H, $\underline{\text{CH}}^1$), 4.63-4.44 (m, 6H, $\underline{\text{CH}}_2$), 4.34-4.28 (m, 1H, $\underline{\text{CH}}^4$), 3.99-3.95 (m, 1H, $\underline{\text{CH}}^3$), 3.83-3.81 (dd, $J = 4.5, 1.5$ Hz, 1H, $\underline{\text{CH}}$), 3.68-3.57 (m, 2H, $\underline{\text{CH}}_2^5$), 3.54-3.48 (m, 1H, $\underline{\text{CH}}^2$), 1.49-1.33 (m, 2H, $\underline{\text{CH}}_2$), 1.09-1.03 (m, 3H, $\underline{\text{CH}}_3$), 0.84-0.77 (m, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 138.3 ($\underline{\text{C}}\text{-Ar}$), 129.0 ($\underline{\text{CH}}\text{-Ar}$), 128.2 ($\underline{\text{CH}}\text{-Ar}$), 128.0 ($\underline{\text{CH}}\text{-Ar}$), 104.9 ($\underline{\text{CH}}^1$), 80.3 ($\underline{\text{CH}}^4$), 80.1 ($\underline{\text{CH}}^3$), 78.8 ($\underline{\text{CH}}^2$), 75.6 ($\underline{\text{CH}}$), 73.6 ($\underline{\text{CH}}_2$), 71.7 ($\underline{\text{CH}}_2^5$), 29.9 ($\underline{\text{CH}}_2$), 20.85 ($\underline{\text{CH}}_3$), 9.9 ($\underline{\text{CH}}_3$). m/z (%) LRMS [ES^+ , MeOH]: 499.4 ($[\text{M}+\text{Na}]^+$, 100%), 975.9 ($[\text{2M}+\text{Na}]^+$, 22%). β -anomer: ^1H NMR: (400 MHz, CDCl_3) δ ppm 7.26-7.12 (m, 15H, $\underline{\text{CH}}\text{-Ar}$), 5.09-5.05 (dd, $J = 12, 4$ Hz, 1H, $\underline{\text{CH}}^1$), 4.43-4.34 (m, 6H, $\underline{\text{CH}}_2$), 4.24-4.18 (m, 1H, $\underline{\text{CH}}^4$), 3.89-3.85 (m, 1H, $\underline{\text{CH}}^3$), 3.73-3.71 (dd, $J = 4.5, 1.5$ Hz, 1H, $\underline{\text{CH}}$), 3.58-3.47 (m, 2H, $\underline{\text{CH}}_2^5$), 3.44-3.38 (m, 1H, $\underline{\text{CH}}^2$), 1.39-1.23 (m, 2H, $\underline{\text{CH}}_2$), 1.05-0.97 (m, 3H, $\underline{\text{CH}}_3$), 0.74-0.67 (m, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 137.9 ($\underline{\text{C}}\text{-Ar}$), 128.3 ($\underline{\text{CH}}\text{-Ar}$), 127.4 ($\underline{\text{CH}}\text{-Ar}$), 102.7 ($\underline{\text{CH}}^1$), 80.2 ($\underline{\text{CH}}^4$), 80.0 ($\underline{\text{CH}}^3$), 78.6 ($\underline{\text{CH}}^2$), 74.1 ($\underline{\text{CH}}$), 73.1 ($\underline{\text{CH}}_2$), 71.3 ($\underline{\text{CH}}_2^5$), 28.6 ($\underline{\text{CH}}_2$), 18.5 ($\underline{\text{CH}}_3$), 9.5 ($\underline{\text{CH}}_3$). m/z (%) LRMS [ES^+ , MeOH]: 499.5 ($[\text{M}+\text{Na}]^+$, 100%), 975.8 ($[\text{2M}+\text{Na}]^+$, 25%). HRMS [ES^+] $\text{C}_{30}\text{H}_{36}\text{O}_5\text{Na}$ requires 499.2460 found 499.2456.

1-*O*-Isobutyl-3,5-*O*-dibenzyl- α -D-ribofuranoside (25)

Compound **24** (2.80 g, 5.87 mmol) was dissolved in DCM (20 mL) before SnCl_4/DCM solution (1M, 5.87 mL, 5.87 mmol) was added dropwise. The reaction was stirred at rt for 1 h. Saturated sodium bicarbonate solution (50 mL) was added to quench the reaction. The organic layer was further washed with brine (50 mL), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography using a gradient of ethyl acetate in hexane (from 0 to 40%) to give the product as clear oil (1.77 g, 78%). R_f 0.54 (ethyl acetate/hexane, 3:7). ^1H NMR (400 MHz, $\text{DMSO-}d^6$) δ ppm 7.36-7.27 (m, 10H, $\underline{\text{H}}\text{-Ar}$), 5.09-4.99 (d, $J = 4$ Hz, 1H, $\underline{\text{CH}}^1$), 4.64-4.49 (m, 4H, $\underline{\text{CH}}_2$), 4.09-4.00 (m, 1H, $\underline{\text{CH}}^4$), 3.99-3.92 (m, 1H, $\underline{\text{CH}}^2$), 3.60-3.56 (m, 1H, $\underline{\text{CH}}^3$), 3.49-3.44 (m, 1H, $\underline{\text{CH}}$), 3.48-3.42 (m, 2H, $\underline{\text{CH}}_2^5$), 1.47-1.37 (m, 2H, $\underline{\text{CH}}_2$), 1.22-1.11 (d, $J = 6$ Hz, 3H, $\underline{\text{CH}}_3$), 0.92-0.86 (m, 3H, $\underline{\text{CH}}_3$). ^{13}C NMR (100 MHz, $\text{DMSO-}d^6$) δ ppm 138.6 ($\underline{\text{C}}\text{-Ar}$), 128.2 ($\underline{\text{CH}}\text{-Ar}$), 127.4 ($\underline{\text{CH}}\text{-Ar}$), 127.3 ($\underline{\text{CH}}\text{-Ar}$), 101.3 ($\underline{\text{CH}}^1$), 81.1 ($\underline{\text{CH}}^4$), 77.4 ($\underline{\text{CH}}^3$), 73.9

(CH), 72.8 (CH₂), 71.8 (CH₂), 71.4 (CH²), 70.8 (CH₂⁵), 30.1 (CH₂), 21.6 (CH₃), 10.5 (CH₃).
m/z (%) LRMS [ES⁺, MeOH]: 409.4 ([M+Na]⁺, 100%), 795.9 ([2M+Na]⁺, 39%). HRMS
[ES⁺] C₂₃H₃₀O₅Na requires 409.1991 found 409.1989.

1-*O*-Isobutyl-2-*O*-methoxyethyl-3,5-*O*-dibenzyl-*D*-ribofuranoside (26)

A solution of **25** (1.85 g, 4.81 mmol) in DMF (16 mL) was cooled down to -10 °C before sodium hydride (60% in mineral oil, 0.482 g, 12.0 mmol) was added portionwise. The reaction mixture was stirred at the same temperature for 30 min before 2-bromomethyl ethyl ether (1.13 mL, 12.0 mmol) was added. The solution was further stirred at -10 °C for 2 h before EtOH (20 mL) was added to quench the reaction. After DCM (20 mL) was added, the organic layer was separated and washed with water (50 mL), saturated sodium bicarbonate solution (50 mL), brine (50 mL) and dried over anhydrous sodium sulfate. After filtration, solvent was removed in *vacuo* and the residue was purified by silica gel column chromatography with a gradient of ethyl acetate in hexane (from 0 to 40%) to give the product (2.00 g, 94%) as clear oil and a mixture of α and β anomers. *R_f* α -anomer: 0.29; β -anomer: 0.27 (ethyl acetate/hexane, 2:8). α -anomer: ¹H NMR (400 MHz, CDCl₃) δ ppm 7.27-7.02 (m, 10H, H-Ar), 5.12-5.11 (d, *J* = 4 Hz, 1H, CH¹), 4.78-4.75 (d, *J* = 12 Hz, 1H, CH), 4.57-4.44 (m, 4H, CH₂), 4.24-4.21 (q, *J* = 4 Hz, 1H, CH⁴), 3.90-3.37 (m, 11H, CH₂⁵, CH², CH₂, CH₃, CH³, CH₂), 1.71-1.60 (m, 2H, CH₂), 1.28-1.26 (d, *J* = 6 Hz, 3H, CH₃), 0.94-0.90 (t, *J* = 8 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ ppm. 138.0 (C-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.0 (CH-Ar), 99.4 (CH¹), 81.4 (CH⁴), 79.9 (CH³), 76.1 (CH), 75.9 (CH²), 73.8 (CH₂), 72.7 (CH₂), 72.6 (CH₂), 70.5 (CH₂⁵), 59.5 (CH₃O), 29.7 (CH₂), 19.5 (CH₃), 10.6 (CH₃) *m/z* (%) LRMS [ES⁺, MeOH]: 467.3 ([M+Na]⁺, 100%). β -anomer: ¹H NMR (400 MHz, CDCl₃) δ ppm 7.24-6.99 (m, 10H, H-Ar), 5.10-5.06 (d, *J* = 4 Hz, 1H, CH¹), 4.68-4.63 (d, *J* = 12 Hz, 1H, CH), 4.41-4.35 (m, 4H, CH₂), 4.14-4.09 (q, *J* = 4 Hz, 1H, CH⁴), 3.75-3.28 (m, 11H, CH₂⁵, CH², CH₂, CH₃, CH³, CH₂), 1.53-1.33 (m, 2H, CH₂), 1.17-1.06 (d, *J* = 8 Hz, 3H, CH₃), 0.87-0.81 (t, *J* = 8 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ ppm. 139.0 (C-Ar), 128.8 (CH-Ar), 128.7 (CH-Ar), 128.1 (CH-Ar), 101.4 (CH¹), 81.7 (CH⁴), 80.0 (CH³), 76.1 (CH²), 76.8 (CH), 73.8 (CH₂), 72.2 (CH₂), 72.6 (CH₂), 70.7 (CH₂), 70.5

($\underline{\text{C}}\text{H}_2^5$), 59.5 ($\underline{\text{C}}\text{H}_3\text{O}$), 30.5 ($\underline{\text{C}}\text{H}_2$), 21.7 ($\underline{\text{C}}\text{H}_3$), 10.9 ($\underline{\text{C}}\text{H}_3$) m/z (%) LRMS [ES^+ , MeOH]: 467.4 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+] $\text{C}_{26}\text{H}_{36}\text{O}_6\text{Na}$ requires 467.2410 found 467.2398.

2-O-methoxyethyl-3,5-O-dibenzyl-D-ribose α - and β - anomers (27)

A solution of **26** (0.150 g, 0.340 mmol) in DCM (1.0 mL) was cooled down to -10°C before trifluoroacetic acid (0.262 mL) was added dropwise, followed by the addition of water (0.02 mL). The reaction mixture was stirred for 4 h before adding triethylamine (1.0 mL) and DCM (5 mL). The organic layer was washed with a saturated solution of sodium bicarbonate (5 mL), brine (5 mL) and dried over anhydrous sodium sulfate. After filtration, solvent was removed in *vacuo* and the residue was purified by silica gel column chromatography with a gradient of ethyl acetate in hexane (up to 50%) to give the product (0.119 g, 90%) as clear oil. R_f 0.43 (ethyl acetate/hexane, 6:4). ^1H NMR: (400 MHz, $\text{DMSO}-d^6$) δ ppm 7.36-7.26 (m, 10H, $\underline{\text{H}}\text{-Ar}$), 6.52-6.51 (d, $J = 4$ Hz, $\underline{\text{C}}\text{H}^{1\alpha}$, 70%), 5.64-5.62 (d, $J = 8$ Hz, $\underline{\text{C}}\text{H}^{1\beta}$, 30%), 5.23-5.21 (m, $\underline{\text{C}}\text{H}^{4\beta}$, 30%), 5.14-5.13 (m, $\underline{\text{C}}\text{H}^{4\alpha}$, 70%), 4.63-4.46 (m, 4H, $\underline{\text{C}}\text{H}_2$), 4.13- 3.98 (m, $\underline{\text{C}}\text{H}^{3\beta}$, 30%), 3.98-3.94 (m, $\underline{\text{C}}\text{H}^{3\alpha}$, 70%), 3.74-3.43 (m, 7H, $\underline{\text{C}}\text{H}_2$, $\underline{\text{C}}\text{H}^{2\alpha\beta}$, $\underline{\text{C}}\text{H}_2^{5\alpha\beta}$), 3.26 (s, 3H, $\underline{\text{C}}\text{H}_3$). ^{13}C NMR: (100 MHz, $\text{DMSO}-d^6$) δ ppm 137.8 ($\underline{\text{C}}\text{-Ar}$), 127.6 ($\underline{\text{C}}\text{H}\text{-Ar}$), 127.2 ($\underline{\text{C}}\text{H}\text{-Ar}$), 126.9 ($\underline{\text{C}}\text{H}\text{-Ar}$), 98.9 ($\underline{\text{C}}\text{H}^{1\alpha}$), 95.3 ($\underline{\text{C}}\text{H}^{1\beta}$), 81.8 ($\underline{\text{C}}\text{H}^{4\alpha}$), 79.8 ($\underline{\text{C}}\text{H}^{4\beta}$), 79.6 ($\underline{\text{C}}\text{H}^{3\alpha}$), 78.5 ($\underline{\text{C}}\text{H}^{3\beta}$), 78.4 ($\underline{\text{C}}\text{H}^{2\alpha}$), 77.3 ($\underline{\text{C}}\text{H}^{2\beta}$), 72.8 ($\underline{\text{C}}\text{H}_2$), 72.7 ($\underline{\text{C}}\text{H}_2$), 72.4 ($\underline{\text{C}}\text{H}_2$), 70.7 ($\underline{\text{C}}\text{H}^{5\alpha}$), 68.5 ($\underline{\text{C}}\text{H}_2^{5\beta}$), 58.57 ($\underline{\text{C}}\text{H}_3\text{O}$). m/z (%) LRMS [ES^+ , MeOH]: 411.3 ($[\text{M}+\text{Na}]^+$, 100%), 799.8 ($[\text{2M}+\text{Na}]^+$, 10%). HRMS [ES^+] $\text{C}_{22}\text{H}_{28}\text{O}_6\text{Na}$ requires 411.1784 found 411.1785.

Ethyl-(2-O-methoxyethyl-3,5-O-dibenzyl- β -D-ribofuranosyl)-acetate (28)

To a solution of compound **27** (4.64 g, 12.0 mmol) in THF (40 mL), was added ethyl-(triphenyl-phosphoranylidene) acetate (5.10 g, 14.4 mmol). The reaction mixture was heated to reflux for 3 h. Solvent was removed in *vacuo* and the residue was redissolved in EtOH (40 mL). Traces of sodium ethoxide were added before the mixture was stirred at rt for 3 h. The solvent was concentrated to half in *vacuo* and DCM (20mL) was added. The organic layer was separated and washed with a saturated solution of sodium bicarbonate (100 mL), brine (100 mL) and dried over anhydrous sodium sulfate. After filtration, the filtrate was

evaporated in *vacuo* and the residue was purified by silica gel column chromatography using a gradient of ethyl acetate in hexane (up to 30%) to give the product as white foam (2.38 g, 44%). R_f 0.67 (ethyl acetate/hexane, 6:4). ^1H NMR: (400 MHz, CDCl_3) δ ppm 7.32-7.23 (m, 10H, $\underline{\text{H-Ar}}$), 4.61-4.45 (m, 4H, $\underline{\text{CH}_2}$), 4.36-4.32 (m, 1H, $\underline{\text{CH}}^1$), 4.17-4.08 (m, 3H, $\underline{\text{CH}_2}$, $\underline{\text{CH}}^4$), 3.92-3.90 (t, $J = 4$ Hz, 1H, $\underline{\text{CH}}^3$), 3.70-3.57 (m, 3H, $\underline{\text{CH}_2}$, $\underline{\text{CH}}^2$), 3.52-3.50 (t, $J = 4$ Hz, 2H, $\underline{\text{CH}_2}$), 3.48- 3.47 (m, 2H, $\underline{\text{CH}_2}^5$), 3.32 (s, 3H, $\underline{\text{CH}_3}$), 2.63-2.48 (m, 2H, $\underline{\text{CH}_2}$), 1.23-1.29 (t, $J = 8$ Hz, 3H, $\underline{\text{CH}_3}$). ^{13}C NMR: (100 MHz, CDCl_3) δ ppm 171.0 ($\underline{\text{C=O}}$), 138.3 ($\underline{\text{C-Ar}}$), 138.1 ($\underline{\text{C-Ar}}$), 128.5 ($\underline{\text{CH-Ar}}$), 128.4 ($\underline{\text{CH-Ar}}$), 128.1 ($\underline{\text{CH-Ar}}$), 127.8 ($\underline{\text{CH-Ar}}$), 127.7 ($\underline{\text{CH-Ar}}$), 82.3 ($\underline{\text{CH}}^1$), 82.0 ($\underline{\text{CH}}^4$), 77.5 ($\underline{\text{CH}}^3$), 77.0 ($\underline{\text{CH}}^2$), 72.5 ($\underline{\text{CH}_2}$), 72.6 ($\underline{\text{CH}_2}$), 72.2 ($\underline{\text{CH}_2}$), 70.6 ($\underline{\text{CH}_2}$), 70.1 ($\underline{\text{CH}_2}^5$), 61.0 ($\underline{\text{CH}_2}$), 59.4 ($\underline{\text{CH}_3\text{-O}}$), 39.2 ($\underline{\text{CH}_2}$), 14.6 ($\underline{\text{CH}_3}$). m/z (%) LRMS [ES^+ , MeOH]: 481.4 ($[\text{M}+\text{Na}]^+$, 100%), 939.9 ($[\text{2M}+\text{Na}]^+$, 14%). HRMS [ES^+] $\text{C}_{26}\text{H}_{34}\text{O}_7\text{Na}$ requires 482.2202 found 481.2194.

Ethyl-(2-*O*-methoxyethyl- β -D-ribofuranosyl)-acetate (29)

Compound **28** (2.38 g, 5.20 mmol) was dissolved in MeOH, $\text{Pd}(\text{OH})_2/\text{C}$ (10% on carbon, 0.365 g) was added and the reaction mixture is degassed three times (vacuum-hydrogen) before leaving the suspension stirred at rt for 20 h under hydrogen atmosphere. Upon completion the reaction mixture is filtered through celite and washed with DCM. The organic layer was washed with H_2O (100 mL), a saturated solution of sodium bicarbonate (100 mL), brine (100 mL), dried over anhydrous sodium sulfate. After filtration solvent was removed in *vacuo* and the product was used in the next step without further purification (1.40 g, 97%). R_f 0.21 (ethyl acetate/hexane, 6:4). ^1H NMR (400 MHz, $\text{MeOD-}d^4$) δ ppm 4.13-4.0 (m, 3H, $\underline{\text{CH}}^1$, $\underline{\text{CH}_2}$), 3.90-3.88 (t, $J = 4$ Hz, 1H, $\underline{\text{CH}}^4$), 3.82-3.46 (m, 7H, $2\times\underline{\text{CH}_2}$, $\underline{\text{CH}}^3$, $\underline{\text{CH}_2}^5$, $\underline{\text{CH}}^2$), 3.32 (s, 3H, $\underline{\text{CH}_3}$), 2.71-2.46 (m, 2H, $\underline{\text{CH}_2}$), 1.21-1.17 (t, $J = 8$ Hz, 3H, $\underline{\text{CH}_3}$). ^{13}C NMR (100 MHz, $\text{MeOD-}d^4$) δ ppm 173.3 ($\underline{\text{C=O}}$), 86.4 ($\underline{\text{CH}}^1$), 84.2 ($\underline{\text{CH}}^4$), 77.6 ($\underline{\text{CH}}^3$), 73.6 ($\underline{\text{CH}_2}$), 72.0 ($\underline{\text{CH}}^2$), 71.1 ($\underline{\text{CH}_2}$), 63.8 ($\underline{\text{CH}_2}$), 62.0 ($\underline{\text{CH}_2}^5$), 59.1 ($\underline{\text{CH}_3\text{O}}$), 40.0 ($\underline{\text{CH}_2}$), 14.5 ($\underline{\text{CH}_3}$). m/z (%) LRMS [ES^+ , MeOH]: 301.3 ($[\text{M}+\text{Na}]^+$, 100%), 579.5 ($[\text{2M}+\text{Na}]^+$, 30%). HRMS [ES^+] $\text{C}_{12}\text{H}_{22}\text{O}_7\text{Na}$ requires 301.1263 found 301.1258.

Ethyl-[2-O-methoxyethyl-5-O-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-acetate (30)

29 (1.45 g, 5.21 mmol) and DMAP (0.318 g, 2.60 mmol) were dissolved in pyridine (10 mL) before a solution of DMTCl (2.10 g, 6.25 mmol) in pyridine (7 mL) was added dropwise. The reaction mixture was stirred at rt for 4 h. Methanol (10 mL) and triethylamine (1 mL) were added and the solvent was removed in *vacuo*. The residue was redissolved in DCM, the organic layer was washed with a saturated solution of sodium bicarbonate (50 mL), water (50 mL), brine (50 mL), and dried over anhydrous sodium sulfate. After filtration, solvent was removed in *vacuo* and the residue was purified by silica gel column chromatography using a gradient of ethyl acetate in hexane (up to 60%) to give the product as white foam (2.29 g, 76%). R_f 0.41 (ethyl acetate/hexane, 6:4). $^1\text{H NMR}$: (400 MHz, CDCl_3) δ ppm 7.37-7.09 (m, 9H, $\underline{\text{H-Ar}}$), 6.74-6.72 (m, 4H, $\underline{\text{H-Ar}}$), 4.24-4.19 (q, $J = 8$ Hz, 1H, $\underline{\text{CH}}^1$), 4.11-4.04 (m, 3H, $\underline{\text{CH}}_2$, $\underline{\text{CH}}^4$), 3.96-3.94 (m, 1H, $\underline{\text{CH}}^3$), 3.78-3.74 (m, 1H, $\underline{\text{CH}}^2$), 3.70 (s, 6H, $\underline{\text{CH}}_3\text{-Ar}$), 3.58-3.47 (m, 2H, $\underline{\text{CH}}_2$), 3.43-3.39 (m, 2H, $\underline{\text{CH}}_2$), 3.30 (s, 3H, $\underline{\text{CH}}_3$), 3.21-3.00 (m, 2H, $\underline{\text{CH}}_2^5$), 2.59-2.57 (m, 2H, $\underline{\text{CH}}_2$), 1.18-1.15 (t, $J = 8$ Hz, 3H, $\underline{\text{CH}}_3$). $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ ppm 171.0 ($\underline{\text{C=O}}$), 158.6 ($\underline{\text{C-Ar}}$), 145.1 ($\underline{\text{C-Ar}}$), 136.3 ($\underline{\text{C-Ar}}$), 130.3 ($\underline{\text{CH-Ar}}$), 128.5 ($\underline{\text{CH-Ar}}$), 128.4 ($\underline{\text{CH-Ar}}$), 127.9 ($\underline{\text{CH-Ar}}$), 126.8 ($\underline{\text{CH-Ar}}$), 113.2 ($\underline{\text{CH-Ar}}$), 86.2 ($\underline{\text{C-Ar}}$), 84.1 ($\underline{\text{CH}}^1$), 83.5 ($\underline{\text{CH}}^4$), 77.15 ($\underline{\text{CH}}^3$), 72.2 ($\underline{\text{CH}}_2$), 71.2 ($\underline{\text{CH}}^2$), 70.22 ($\underline{\text{CH}}_2$), 64.3 ($\underline{\text{CH}}_2$), 62.0 ($\underline{\text{CH}}_2^5$), 59.1 ($\underline{\text{CH}}_3\text{O}$), 55.32 ($\underline{\text{CH}}_3\text{-Ar}$), 39.1 ($\underline{\text{CH}}_2$), 14.4 ($\underline{\text{CH}}_3$). m/z (%) LRMS [ES^+ , MeOH]: 603.6 ($[\text{M}+\text{Na}]^+$, 100%), 1184.3 ($[\text{2M}+\text{Na}]^+$, 60%). HRMS [ES^+] $\text{C}_{33}\text{H}_{40}\text{O}_9\text{Na}$ requires 603.2570 found 603.2552.

[2-O-methoxyethyl-5-O-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]-acetic acid (31)

Compound **30** (2.22 g, 3.82 mmol) was dissolved in THF before a 1M solution of sodium hydroxide (15.3 mL, 15.3 mmol) was added. The reaction mixture was heated to reflux for 4 h. pH as was adjusted to neutrality with hydrochloric acid (1M) added dropwise. The solvent was removed in *vacuo* and the residue was purified by silica gel column chromatography using a gradient of methanol in DCM (up to 30%) to give the product as white foam (1.60 g, 76%). R_f 0.28 (methanol/DCM, 1:19). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ ppm 7.41-7.10 (m, 9H, $\underline{\text{CH-Ar}}$), 6.77-6.75 (m, 4H, $\underline{\text{CH-Ar}}$), 4.28-4.23 (m, 1H, $\underline{\text{CH}}^1$), 4.03-4.01 (m, 1H, $\underline{\text{CH}}^4$),

3.97-3.95 (m, 1H, CH^3), 3.80-3.66 (m, 2H, CH_2), 3.60-3.41 (m, 2H, CH_2), 3.71 (s, 6H, CH_3 -Ar), 3.48-3.46 (m, 1H, CH^2), 3.31 (s, 3H, CH_3), 3.22-3.06 (m, 2H, CH_2^5), 2.56-2.54 (m, 2H, CH_2). ^{13}C NMR (100 MHz, CDCl_3) δ ppm 181.1 ($\text{C}=\text{O}$), 158.8 (C -Ar), 145.4 (C -Ar), 136.7 (C -Ar), 130.6 (CH -Ar), 128.7 (CH -Ar), 128.2 (CH -Ar), 127.1 (CH -Ar), 113.5 (CH -Ar), 86.4 (C -Ar), 84.0 (CH^1), 83.8 (CH^4), 77.5 (CH^3), 71.3 (CH_2), 70.1 (CH^2), 68.8 (CH_2), 64.9 (CH_2^5), 59.4 (CH_3O), 55.6 (CH_3 -Ar), 45.4 (CH_2). m/z (%) LRMS [ES^+ , MeOH]: 575.5 ($[\text{M}+\text{Na}]^+$, 100%), 1128.2 ($[\text{2M}+\text{Na}]^+$, 26%). HRMS [ES^+] $\text{C}_{31}\text{H}_{36}\text{O}_9\text{Na}$ requires 575.2257 found 575.2252.

***C*-(2'-*O*-methoxyethyl-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl-1')-*N*-[4-(2-*N*-acetyl-thiazole-4-yl)-phenyl]-acetamide (32)**

To a solution of **31** (1.25 g, 2.40 mmol) in DMF (5 mL) was added *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl, 0.920 g, 4.80 mmol). The solution was stirred at rt for 15 min before a solution of 2-acetamido-4-(3-aminophenyl)thiazole in DMF⁵ (0.448 g, 1.92 mmol, dissolved in 3 mL DMF) was added. The reaction mixture was stirred at rt for 4 h and quenched with methanol (5 mL). Diluted with DCM, the organic layer was washed with a saturated solution of sodium bicarbonate (30 mL), brine (30 mL) and dried over anhydrous sodium sulfate. After filtration, the residue was purified by silica gel column chromatography with a gradient of ethyl acetate in hexane (up to 30%) to give the product as white foam (0.665 g, 36%). R_f 0.31 (ethyl acetate/hexane, 2:8). ^1H NMR (400 MHz, CDCl_3) δ ppm 10.15 (s, 1H, NH), 8.42 (s, 1H, NH), 7.87-7.86 (d, $J = 4$ Hz, 1H, H -Ar), 7.42-7.06 (m, 12H, H -Ar), 6.88-6.87 (d, $J = 4$ Hz, 1H, H -Ar), 6.70-6.67 (m, 4H, H -Ar), 4.21-4.18 (m, 1H, CH^1), 4.06-4.02 (m, 2H, CH_2), 3.79-3.75 (m, 1H, CH^3), 3.69-3.52 (m, 3H, CH_2 , CH^4), 3.63 (s, 6H, CH_3), 3.49-3.47 (m, 2H, CH_2), 3.42-3.38 (m, 1H, CH^2), 3.29 (s, 3H, CH_3), 2.75-2.55 (m, 2H, CH_2^5), 1.95 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3) δ ppm 181.1 ($\text{C}=\text{O}$), 169.3 ($\text{C}=\text{O}$), 158.9 (C -Ar), 149.5 (C -Thiazol), 145.3 (C -Ar), 138.9 (C -Phenyl), 136.4 (C -Ar), 135.5 (C -Phenyl), 130.6 (CH -Ar), 130.4 (CH -Ar), 129.7 (CH -Ar), 128.8 (CH -Ar), 128.6 (CH -Ar), 128.3 (CH -Ar), 127.3 (CH -Phenyl), 120.0 (CH -Phenyl), 118.2 (CH -Phenyl), 113.6 (CH -Ar), 108.5 (CH -Thiazol), 86.7 (C -Ar),

85.1 ($\underline{\text{C}}\text{H}^1$), 83.9 ($\underline{\text{C}}\text{H}^4$), 77.4 ($\underline{\text{C}}\text{H}^3$), 72.2 ($\underline{\text{C}}\text{H}_2$), 71.0 ($\underline{\text{C}}\text{H}^2$), 70.5 ($\underline{\text{C}}\text{H}_2$), 64.5 ($\underline{\text{C}}\text{H}_2^5$), 59.4 ($\underline{\text{C}}\text{H}_3\text{O}$), 55.6 ($\underline{\text{C}}\text{H}_3\text{-Ar}$), 42.3 ($\underline{\text{C}}\text{H}_2$), 23.4 ($\underline{\text{C}}\text{H}_3\text{-Ac}$). m/z (%) LRMS [ES^+ , MeOH]: 790.6 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+] $\text{C}_{42}\text{H}_{45}\text{N}_3\text{O}_9\text{SNa}$ requires 790.2774 found 790.2956.

***C*-{2'-*O*-methoxyethyl-3'-*O*-[(2-cyanoethyl-*N,N*-diisopropyl-amino)-phosphanyl]-5'-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl-1')}-*N*-[4-(2-*N*-acetyl-thiazole-4-yl)-phenyl]-acetamide (33)**

Compound **32** (0.582 g, 0.760 mmol) was dissolved in anhydrous DCM (2.5 mL). DIPEA (0.530 mL, 3.00 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.254 mL, 1.14 mmol) were added and the reaction mixture was stirred under argon for 2h. After being diluted with DCM, the organic layer was washed with saturated solution of potassium chloride and dried over anhydrous sodium sulfate under argon atmosphere. After filtration, the residue was purified by silica gel column chromatography with a gradient of ethyl acetate in hexane (from 60% to 80%). A further precipitation of the product dissolved in DCM into hexane gave the product as white foam (0.573 g, 58%). R_f 0.33 (ethylacetate/hexane, 8:2). ^1H NMR (300 MHz, MeCN- d^3) δ ppm 9.93 (bs, 1H, $\underline{\text{N}}\underline{\text{H}}$), 8.48 (s, 1H, $\underline{\text{N}}\underline{\text{H}}$), 8.15 (bs, 1H, $\underline{\text{H}}\text{-Ar}$), 7.57-7.16 (m, 13H, $\underline{\text{H}}\text{-Ar}$), 6.82-6.78 (m, 4H, $\underline{\text{H}}\text{-Ar}$), 4.33-4.22 (m, 1H, $\underline{\text{C}}\underline{\text{H}}^1$), 4.09-4.03 (m, 1H, $\underline{\text{C}}\underline{\text{H}}^4$), 3.89-3.77 (m, 3H, $\underline{\text{C}}\underline{\text{H}}_2$, $\underline{\text{C}}\underline{\text{H}}^3$), 3.70 (s, 6H, $\underline{\text{C}}\underline{\text{H}}_3\text{-Ar}$), 3.66-3.44 (m, 6H, $\underline{\text{C}}\underline{\text{H}}_2$, $\underline{\text{C}}\underline{\text{H}}_2$, $\underline{\text{C}}\underline{\text{H}}$, $\underline{\text{C}}\underline{\text{H}}$), 3.30 (s, 3H, $\underline{\text{C}}\underline{\text{H}}_3$), 3.30-2.98 (m, 1H, $\underline{\text{C}}\underline{\text{H}}^2$), 2.77-2.37 (m, 6H, $\underline{\text{C}}\underline{\text{H}}_2$, $\underline{\text{C}}\underline{\text{H}}_2$, $\underline{\text{C}}\underline{\text{H}}_2^5$), 2.18 (s, 3H, $\underline{\text{C}}\underline{\text{H}}_3$), 1.14-1.12 (d, $J = 6$ Hz, 6H, $\underline{\text{C}}\underline{\text{H}}_3$), 1.00-0.97 (d, $J = 8$ Hz, 6H, $\underline{\text{C}}\underline{\text{H}}_3$). ^{31}P NMR (121 MHz, MeCN- d^3): δ ppm 149.16, 148.73. m/z (%) LRMS [ES^+ , CH_3CN]: 990.8 ($[\text{M}+\text{Na}]^+$, 100%). HRMS [ES^+] $\text{C}_{51}\text{H}_{62}\text{N}_5\text{O}_{10}\text{SPNa}$ requires 990.3853 found 990.3833.

S5. Oligonucleotide synthesis purification and analysis

Oligonucleotide synthesis was carried out on an Applied Biosystems 394 automated DNA/RNA synthesizer using a standard 0.2 μmol or 1.0 μmol phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. The capping step was omitted when *N*2-trifluoroacetyl-protected MAP phosphoramidites were incorporated. All 2-cyanoethyl phosphoramidite monomers were dissolved in anhydrous CH_3CN to a

concentration of 0.1 M immediately prior to use. The coupling time for normal A, G, C, and T monomers was 30 s and this was extended to 6 min for all modified monomers. Stepwise coupling efficiencies and overall yields were determined by automated trityl cation conductivity monitoring and in all cases were > 98.0%. The oligonucleotides attached to the synthesis columns were treated with 20% diethylamine in acetonitrile for 20 min then washed with acetonitrile (5 x 1 mL). This procedure removes cyanoethyl groups from the phosphotriesters and scavenges the resultant acrylonitrile, preventing cyanoethyl adducts being formed at the primary amines of MAP or at the amino group of 2'-aminoethoxy-5-methyl-uridine. After this, cleavage of oligonucleotides from the solid support and deprotection were achieved by exposure to concentrated aqueous ammonia for 12 h at room temperature. Purification of oligonucleotides was carried out by reversed-phase HPLC on a Gilson system using a Brownlee Aquapore column (C8, 8 mm x 250 mm, 300 Å pore) with a gradient of CH₃CN in NH₄OAc increasing from 0% to 100% buffer B over 30 min with a flow rate of 4 mL/min (buffer A: 0.1 M NH₄OAc, pH 7.0; buffer B: 0.1 M NH₄OAc with 50% CH₃CN, pH 7.0). Elution of oligonucleotides was monitored by ultraviolet absorption at 298 nm. After HPLC purification, oligonucleotides were desalted using NAP-10 Sephadex columns (GE Healthcare) according to the manufacturer's instructions.

TFOs were analysed by negative mode electrospray MS on a Fisons VG platform spectrometer in water with triisopropylamine (0.02%). Hairpin duplexes were analysed by MALDI-TOF using a ThermoBioAnalysis Dynamo MALDI-TOF spectrometer in positive ion mode.¹⁰ The mass data can be found in Table S1.

S6. Ultraviolet triplex melting studies

To determine triplex melting temperatures (T_m), UV melting studies were carried out on a Varian Cary 400 scan UV-visible spectrophotometer using Hellma SUPRASIL synthetic quartz 10 mm path length cuvettes, monitoring at 280 nm with a DNA duplex concentration of 1.0 μM and a volume of 1.2 mL. Samples were prepared as follows: The third strand and

the duplex were mixed in a 3:1 ratio in 2 mL Eppendorf tubes then lyophilized before suspending in 1.2 mL of the appropriate buffer solution (10 mM sodium acetate with 2mM spermine containing 200 mM NaCl, pH 5.5; 10 mM sodium acetate, pH 5.5 containing 200 mM NaCl or 10 mM sodium phosphate, pH 6.2, 6.6, 7.0, 7.5 or 8.0 containing 200 mM NaCl). The samples were then filtered into the cuvettes with Kinesis regenerated cellulose 13 mm, 0.45 μ m syringe filters. The UV melting protocol involved initial denaturation by heating to 80 °C at 10 °C /min followed by annealing by cooling to 15 °C at 0.5 °C /min, then maintaining at 15 °C for 20 min before starting the melting experiment which involved heating from 15 °C to 80 °C at 0.5 °C /min, holding at 80 °C for two min then cooling to 15 °C at 0.5 °C /min. At least two successive melting curves were measured. The T_m values were calculated using Cary Win UV thermal application software, taking an average of the melting curves.

S7. Ultraviolet duplex melting studies

To determine duplex melting temperatures UV melting studies were carried out at 260 nm with a complementary single DNA strand concentration of 1.0 μ M and a volume of 1.2 mL. Samples were prepared as follows: The TFO and the single-stranded DNA were mixed in a 1.00:1.05 ratio in 2 mL Eppendorf tubes then lyophilized before dissolving in 1.2 mL of sodium phosphate, pH 7.0 containing 200 mM NaCl. The samples were then filtered into the cuvettes with Kinesis regenerated cellulose 13 mm, 0.45 μ m syringe filters. The UV melting protocol involved initial denaturation by heating to 84 °C at 10 °C /min followed by annealing by cooling to 12 °C at 1.0 °C /min, then maintaining at 12 °C for 20 min before starting the melting experiment which involved heating from 12 °C to 84 °C at 1.0 °C /min, holding at 84 °C for two min then cooling to 12 °C at 1.0 °C /min. Three successive melting curves were measured. The T_m values were calculated using Cary Win UV thermal application software, taking an average of the three melting curves.

S8. DNase I footprinting

The target sequence for the DNase I footprinting experiments with the TFOs was prepared by

ligating the sequence

5'-GATCAAAAACAAAGCAAAAAGCGGAAAAAGCAACTGTATGC (TFO target site underlined) into the BamHI site of pUC19. The resulting clone contained a dimer of this sequence with the two copies oriented in opposite directions, enabling simultaneous visualization of the pyrimidine (lower site) and purine (upper site) strands. The radiolabelled footprinting fragment was prepared by digesting the plasmid with EcoRI and HindIII and labeling the insert at the 3'-end of the EcoRI site with α -[³²P]-dATP using reverse transcriptase. DNase I digestion was performed as previously described.¹¹ Radiolabelled DNA (1.5 μ L) was incubated overnight with 3 μ L oligonucleotide (dissolved in 50 mM sodium acetate pH 5.0 containing 2.5 mM MgCl₂). Samples were digested with DNase I (0.01 units/mL, dissolved in 20 mM NaCl, 2 mM MgCl₂, 2 mM MnCl₂) for 2 minutes before stopping the reaction by adding 4 μ L of formamide containing 10 mM EDTA. The products of the reaction were separated on 10% polyacrylamide gels containing 8M urea. The gel was fixed, dried and exposed to a phosphorimager screen overnight.

S9. Serum stability studies^{12, 13}

Oligonucleotides TFO-16, TFO-17, TFO-18 or TFO-19 (1.4 OD₂₆₀) were freeze-dried and dissolved in sodium phosphate buffer (80 μ L, 10 mM phosphate, pH 7.0, 200 mM NaCl). A 10 μ L aliquot from this solution was removed, mixed with 5 μ L of the same buffer and used as a negative control. Fetal bovine serum (10.5 μ L) and sodium phosphate buffer (24.5 μ L, 10 mM phosphate, pH 7.0, 200 mM NaCl) were added into the remaining 70 μ L of the solution, which was then vortexed and incubated at 37 °C. At each time point (1h, 3h, 6h, 12h and 24h) 15 μ L was taken and stored in a freezer at -20 °C until all the samples were collected. Formamide (15 μ L) was added to each sample and all were vortexed, heated at 80 °C for 5min and cooled in ice before loaded on a denaturing 20% polyacrylamide gel for electrophoresis. The gels were visualized in “epi short wave UV” mode by Genesnap software V7.08 on Syngene G-Box gel imager.

X-ray crystallography

X-ray Data were collected on a Bruker Nonius KappaCCD with a Mo rotating anode generator and standard data collection and processing procedures were followed. Absolute structures were not determined experimentally but inferred from the synthetic procedures.

Crystal data for 2-amino-3-methyl-5-(2'-*O*-methyl- β -D-ribofuranosyl)pyridine (10a)

$M = 254.28$, Hexagonal, $a = 25.4843(4)$, $c = 4.97850(10)$ Å, $U = 2800.11(8)$ Å³, $T = 120(2)$ K, space group $R3$, $Z = 9$, 12291 reflections measured, 1428 unique reflections ($R_{int} = 0.0461$). The final R_I values were 0.0342 ($I > 2\sigma(I)$). The final $wR(F_2)$ values were 0.0720 ($I > 2\sigma(I)$). The final R_I values were 0.0383 (all data). The final $wR(F_2)$ values were 0.0747 (all data).

Crystal data for 2-[*N*-(phenoxyacetyl)amino]-5-(2'-deoxy- β -D-ribofuranosyl)pyridine (14)

$M = 344.36$, Monoclinic, $a = 10.1283(3)$, $b = 8.5022(3)$, $c = 11.0211(4)$ Å, $\beta = 117.1590(10)^\circ$, $U = 844.42(5)$ Å³, $T = 120(2)$ K, space group $P21$, $Z = 2$, 8823 reflections measured, 1594 unique reflections ($R_{int} = 0.1598$). The final R_I values were 0.0461 ($I > 2\sigma(I)$). The final $wR(F_2)$ values were 0.1109 ($I > 2\sigma(I)$). The final R_I values were 0.0515 (all data). The final $wR(F_2)$ values were 0.1152 (all data).

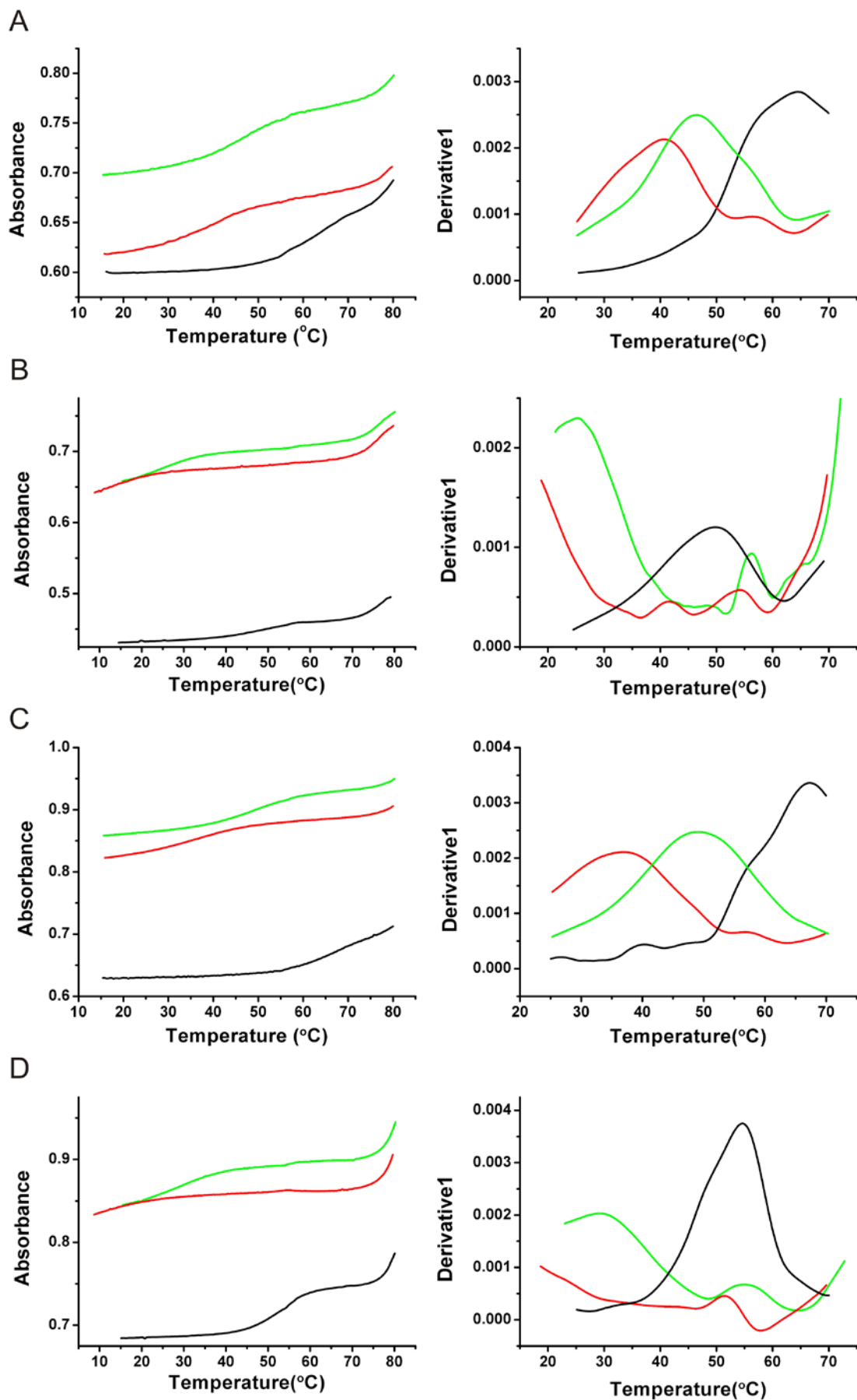


Figure S1. UV melting curves (left) and derivatives (right) of TFO-1, TFO-2, TFO-3, TFO-6, TFO-7, TFO-8 with the corresponding target hairpin duplexes. A) TFO-1 (green), TFO-2 (red) and TFO-3 (black) at pH 5.5 containing 2mM spermine; B) TFO-1 (green), TFO-2 (red) and TFO-3 (black) at pH 5.5; C) TFO-6 (green), TFO-7 (red) and TFO-8 (black) at pH 5.5 containing 2mM spermine; D) TFO-6 (green), TFO-7 (red) and TFO-8 (black) at pH 5.5. Experiments were performed in 10 mM sodium acetate pH 5.5 containing 200 mM NaCl (with or without 2 mM spermine). The concentration ratio of TFOs/target duplex was 3.0 μM :1.0 μM for UV melting.

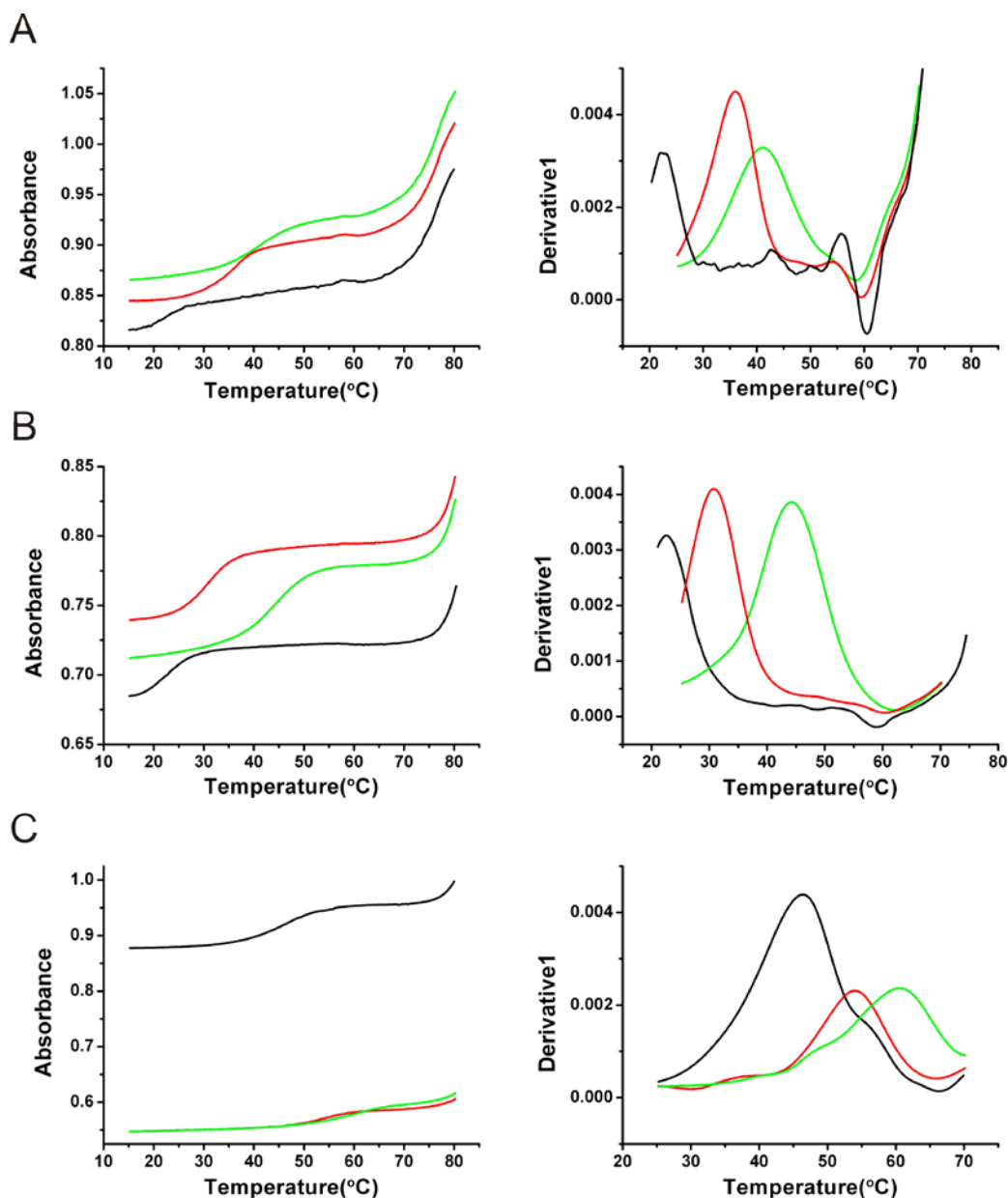


Figure S2. UV melting curves (left) and derivatives (right) of TFO-3, TFO-4, TFO-5, TFO-8, TFO-9, TFO-10, TFO-13, TFO-14 and TFO-15 with the corresponding target hairpin duplexes. A) TFO-3 (black), TFO-4 (red) and TFO-5 (green) at pH 7.0; B) TFO-8 (black), TFO-9 (red) and TFO-10 (green) at pH 7.0; C) TFO-13 (black), TFO-14 (red) and TFO-15 (green) at pH 7.0. Experiments were performed in 10 mM sodium phosphate pH 7.0 containing 200 mM NaCl. The concentration ratio of TFOs/target duplex was 3.0 μM :1.0 μM for UV melting.

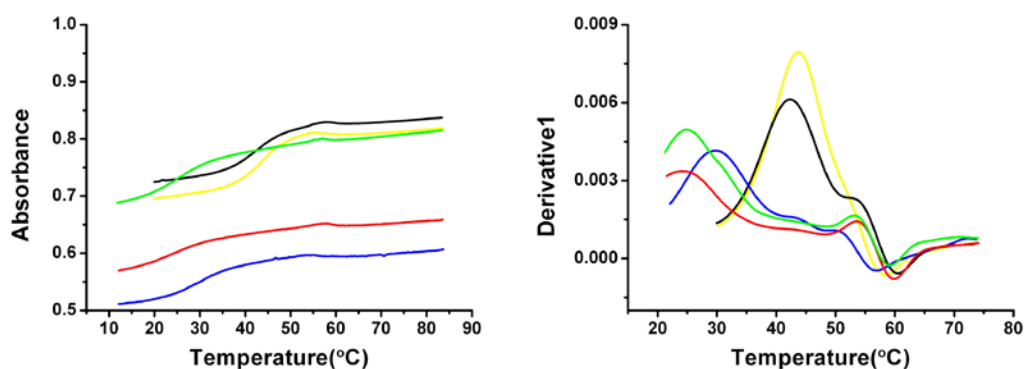


Figure S3. UV melting curves (left) and derivatives (right) of TFO-11-15 with their Watson-Crick complementary single strand DNA at pH 7.0: TFO-11 (green), TFO-12 (red), TFO-13 (black), TFO-14 (yellow), TFO-15 (blue). Experiments were performed in 10 mM sodium phosphate pH 7.0 containing 200 mM NaCl. The concentration ratio of TFOs/single strand DNA was 1.00 μM :1.05 μM for UV melting.

Table S1. MS analysis of single strand DNA, target hairpin duplexes and TFOs

Oligonucleotide	MS (Da)	
ID	Calculated	Found
Single strand DNA	6569	6568
Duplex-1	11400	11404
Duplex-2	13255	13256
TFO-1	5244	5247
TFO-2	5420	5424
TFO-3	5136	5139
TFO-4	5192	5194
TFO-5	5124	5127
TFO-6	4991	4992
TFO-7	5330	5334
TFO-8	5082	5082
TFO-9	5138	5138
TFO-10	5070	5070
TFO-11	5500	5500
TFO-12	5676	5676
TFO-13	5392	5292
TFO-14	5448	5449
TFO-15	5380	5380
TFO-16	6094	6094
TFO-17	6358	6358
TFO-18	5914	5913
TFO-19	5932	5931

Mass spectra of triplex forming oligonucleotides (TFOs) were recorded by negative mode electrospray on a Fisons VG platform spectrometer in water with triisopropylamine (2 μ L of 1% solution in MeOH). Hairpin duplexes were analysed by MALDI-TOF using a ThermoBioAnalysis Dynamo MALDI-TOF spectrometer in positive ion mode with oligo-dT as reference.

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