A Fast Synthetic Route to GDP-Sugars Modified at the Nucleobase

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(i) General. All chemicals were obtained commercially and used as received unless stated otherwise. Chelex 100 resin was purchased from Sigma (www.sigmaaldrich.com) and used in the sodium form as purchased. TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck). Compounds were visualized by exposure to UV light. NMR spectra were recorded at 298 K on a Varian VXR 400 S spectrometer at 400 MHz (^{1}H), or on a Bruker Avance DPX-300 spectrometer at 300 MHz (^{1}H), or on a Varian Inova spectrometer at 500 MHz (^{1}H). Chemical shifts (δ) are reported in ppm and referenced to acetone (^{1}H δ 2.05, ^{13}C δ 30.83 for solutions in D_2O). Coupling constants (*J*) are reported in Hz. Resonance allocations were made with the aid of COSY experiments. Accurate electrospray ionisation mass spectra (HR ESI-MS) were obtained on a Finnigan MAT 900 XLT mass spectrometer at the EPSRC National Mass Spectrometry Service Centre, Swansea. Analytical chromatograhy (HPLC) was carried out on an Agilent 1200 machine equipped with a Supelcosil LC-18T column (25cm ×4.6mm, particle size 5μm); gradient: MeOH against 0.1M KH_2PO_4; detection (diode array detector): 254 / 280 nm. Preparative chromatography was performed on a Biologic LP chromatography system equipped with a peristaltic pump and a 254nm UV Optics Module under the following conditions:

Purification method 1 – Ion-pair chromatography was performed using Lichroprep RP-18 resin, gradient 0 – 15 % MeCN against 0.05 M TEAB over 400 mL, flow rate 3 mL/min. Product containing fractions were combined and reduced to dryness. The residue was co-evaporated repeatedly with methanol to remove residual TEAB (triethylammonium bicarbonate).

Purification method 2 – Anion exchange chromatography was performed using a Macro prep 25Q 5 mL cartridge, gradient 0 – 100 % 1M TEAB (pH 7.3) against H_2O over 20 column volumes. Product containing fractions were combined and reduced to dryness. The residue was co-evaporated repeatedly with methanol to remove residual TEAB (triethylammonium bicarbonate).

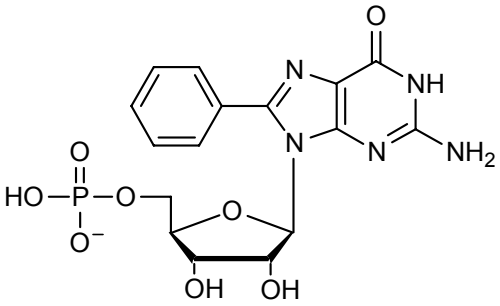
8-Bromoguanosine (**1**) and 8-bromo GMP (**2**) were prepared as previously reported.[1] For the cross-coupling of nucleotides and sugar-nucleotides, the following general procedures were applied:

General procedure A – Suzuki-Miyaura coupling of sugar-nucleotides. Na_2Cl_4Pd (2.5 mol %), TPPTS (2.5 eq. to Pd), K_2CO_3 (1.5 eq.) and arylboronic acid (1.2 eq.) were placed in a flask and purged with N_2. A solution of 8-bromo GDP-mannose **5** (1 eq.) in degassed water (3 mL) was added via a syringe. The pale yellow solution was stirred at 80°C under N_2 for 1h. Upon completion of the reaction, the solution turned dark red/brown. The reaction was cooled to room temperature and the pH adjusted to 7 with 1 % HCl solution. All solvents were removed *in vacuo* and the crude material was purified by chromatography.

General procedure B – One-pot, two-step bromination / cross-coupling of nucleotides and sugar-nucleotides. To a solution of the nucleotide or sugar-nucleotide (1 eq.) in 0.5 M NaOAc buffer (0.5 mL, pH 4.0)[2] was added saturated bromine water dropwise until the yellow colour persisted. After stirring at rt for 20 min the solution was extracted with $CHCl_3$ (3 x 5 mL) and the aqueous layer was dried *in vacuo*. A solution of Na_2Cl_4Pd (2.5 mol %), TPPTS (2.5 eq. to Pd), $PhB(OH)_2$ (1.2 eq.) and K_2CO_3 (3 eq.) in degassed H_2O (3 mL) was added, and the yellow solution was stirred under N_2 at 80°C for 1h. Upon completion of the reaction, the pH was adjusted to 7 with 1 % HCl, all solvents were removed *in vacuo*, and the crude material was purified by chromatography.

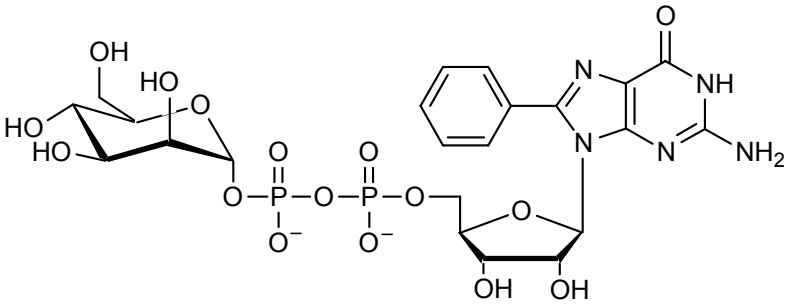
In our experience, purification of the cross-coupling products by chromatography (cf. purification methods 1 and 2), followed by treatment with Chelex 100 resin, was sufficient to remove any residual Pd**.**

(ii) 8-Phenylguanosine monophosphate (3).



3 was prepared either from 8-bromo GMP (**2**) as previously reported,[1] or from GMP (50.4 mg, 0.124 mmol) and phenylboronic acid according to general procedure B. From general procedure B, and after purification by ion pair chromatography (method 1), **3** was obtained as the triethylammonium salt in 75% yield (51.9 mg, 1.2 eq. of TEA as shown by NMR). δ_H (400 MHz, D_2O) 7.38 – 7.29 (5H, m, 5Ph), 5.56 (1H, d, J = 6.0, H-1′), 5.02 (1H, t, J = 5.9, H-2′), 4.28 (1H, dd, J = 3.6, 5.7, H-3′), 4.02 – 3.90 (3H, m, H-4′ + H_2-5′), 2.97 (7H, q, J = 7.3, CH_2), 1.05 (10H, t, J = 7.3, CH_3). δ_C (100 MHz, D_2O) 159.2, 153.8, 153.3, 150.9, 131.1, 129.8, 129.5, 128.3, 116.6, 89.6, 84.1 (d, $J_{C,P}$ = 8.4), 71.0, 70.7, 65.2 (d, $J_{C,P}$ = 4.6), 47.2, 8.8. δ_P (162 MHz, D_2O) 7.43; *m/z* (ESI) 438.0820 (monoanion, $C_{16}H_{17}O_8N_5P$ requires 438.0813).

(iii) 8-Phenylguanosine diphosphate mannose (4a).



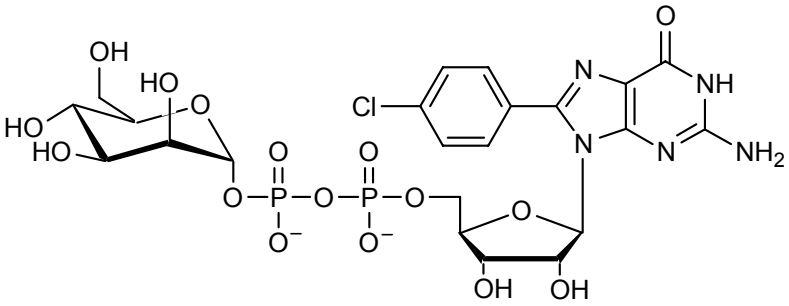
From 8-phenylguanosine monophosphate **3** – **4a** was prepared from **3**[1] (50.0 mg, 0.09 mmol) and mannose-1-phosphate[3] according to the method used for the synthesis of **5**. After purification by anion exchange chromatography (method 2) and treatment with Chelex 100 resin (Na^+ form), **4a** was obtained as a glassy solid in 40% yield (26.4 mg).

From 8-bromoguanosine diphosphate mannose **5** – **4a** was prepared from **5** (16.9 mg, 0.025 mmol) and phenylboronic acid according to general procedure A. After purification by ion pair chromatography (method 1, fractions 28-29) and treatment with Chelex 100 resin (Na^+ form), **4a** was obtained as a glassy solid in 48% yield (11.0 mg).

From guanosine diphosphate mannose – **4a** was prepared from guanosine diphosphate mannose (10.0 mg, 0.016 mmol) and phenylboronic acid according to general procedure B. The crude

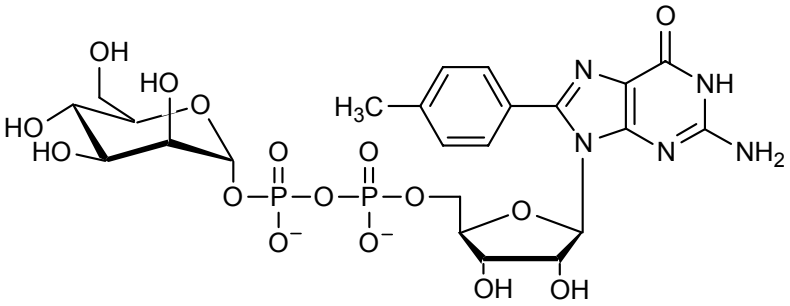
residue was purified first by ion pair chromatography (method 1) and then on an anion exchange cartridge (method 2). The glassy residue obtained from repeated evaporations with MeOH was dissolved in H_2O and treated with Chelex 100 resin (Na^+ form) to give **4a** as the sodium salt in 44% yield (6.2 mg). δ_H (400 MHz; D_2O) 7.38 (5H, m, phenyl), 5.61 (1H, d, J = 6.1, H-1′), 5.35 (1H, d, J = 7.8, H-1″), 5.12 (1H, t, J = 5.9, H-2′), 4.36 (1H, dd, J = 3.3, 5.6, H-3′), 4.15 (1H, m, H-5′a), 4.06 (2H, m, H-4′, H-5′b), 3.89 (1H, m, H-2″), 3.76 (1H, dd, J = 3.4, 9.7, H-3″), 3.70 (2H, m, H-5″, H-6″a), 3.54 (2H, m, H-6″b, H-4″); δ_C (100 MHz; D_2O) 160.7, 159.4, 153.7, 152.8, 150.1, 130.4, 129.2, 128.8, 127.8, 116.0, 96.2 (d, $J_{c,p}$ = 7.9), 88.7, 83.0 (d, $J_{c,p}$ = 11.5), 73.4, 70.0 (d, $J_{c,p}$ = 11.9), 69.6, 66.2, 65.0 (d, $J_{c,p}$ = 7.1), 60.5, 58.7; δ_P (121 MHz; D_2O) -7.75 (d, J = 20.4 Hz), -10.18 (d, J = 21.4); *m/z* (ESI) 680.1012 (monoanion, $C_{22}H_{28}N_5O_{16}P_2$ requires 680.1012).

(iv) 8-(4-Chlorophenyl)guanosine diphosphate mannose (4b).



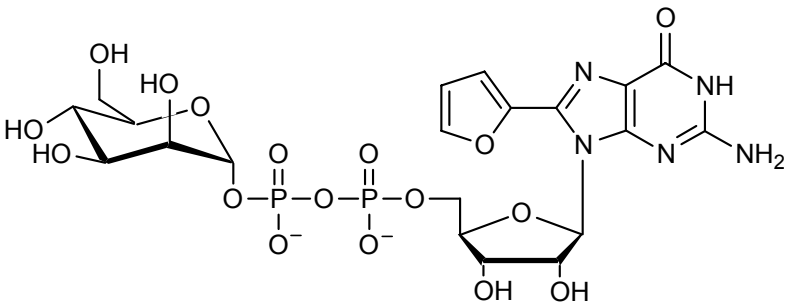
4b was prepared from **5** (15.9 mg, 0.022 mmol) and 4-chlorophenylboronic acid according to general procedure A. After purification by ion pair chromatography (method 1, fractions 41-48) and treatment with Chelex 100 resin (Na^+ form), **4b** was obtained as a glassy solid in 82% yield (13.3 mg). δ_H (400 MHz; D_2O) 7.39 (4H, s, phenyl), 5.58 (1H, d, J = 6.3, H-1′), 5.34 (1H, d, J = 7.9, H-1″), 5.18 (1H, t, J = 6.0, H-2′), 4.40 (1H, dd, J = 3.3, 5.7, H-3′), 4.16 (1H, m, H-5′a), 4.04 (2H, d, J = 7.8, H-4′, H-5′b), 3.88 (1H, m, H-2″), 3.75 (1H, dd, J = 3.4, 9.8, H-3″), 3.69 (2H, m, H-5″, H-6″a), 3.56 (1H, dd, J = 5.9, 12.8, H-6″b), 3.50 (1H, t, J = 9.8, H-4″); δ_C (125 MHz; D_2O) 130.4, 128.7, 96.0, 88.4, 82.9, 73.2, 69.8, 69.4, 66.0, 64.8, 60.4; δ_P (121 MHz; D_2O) -7.67 (d, J = 21.1 Hz), -10.09 (d, J = 20.3); *m/z* (ESI) 714.0621 (monoanion, $C_{22}H_{27}{}^{35}ClN_5O_{16}P_2$ requires 714.0622).

(v) 8-(4-Methylphenyl)guanosine diphosphate mannose (4c).



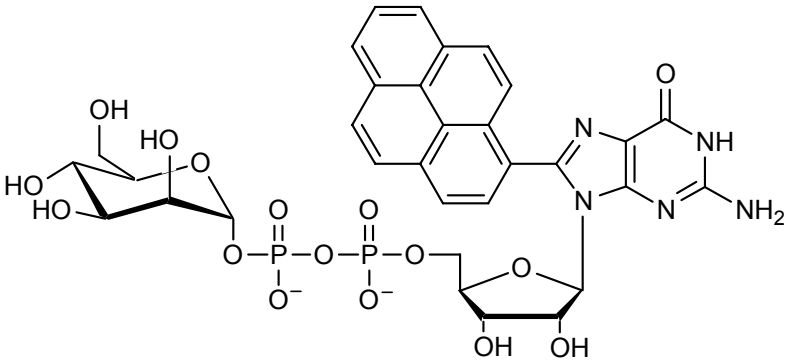
4c was prepared from **5** (15.7 mg, 0.022 mmol) and 4-methylphenylboronic acid according to general procedure A. After purification by ion pair chromatography (method 1, fractions 38-42) and treatment with Chelex 100 resin (Na^+ form), **4c** was obtained as a glassy solid in 82% yield (13.1 mg). δ_H (400 MHz; D_2O) 7.33 (2H, d, J = 8.2, phenyl), 7.22 (2H, d, J = 8.4, phenyl), 5.61 (1H, d, J = 6.3, H-1′), 5.35 (1H, d, J = 7.9, H-1″), 5.16 (1H, t, J = 6.0, H-2′), 4.39 (1H, dd, J = 3.3, 5.6, H-3′), 4.15 (1H, m, H-5′a), 4.04 (2H, m, H-4′, H-5′b), 3.88 (1H, dd, J = 2.0, 3.3, H-2″), 3.75 (1H, dd, J = 3.4, 9.8, H-3″), 3.68 (2H, m, H-5″, H-6″a), 3.54 (2H, m, H-6″b, H-4″), 2.25 (3H, s, methyl); δ_C (125 MHz; D_2O) 129.0, 128.8, 95.9, 88.4, 82.8, 73.0, 69.7, 69.4, 66.1, 64.7, 60.2, 20.0; δ_P (121 MHz; D_2O) -7.64 (d, J = 21.2 Hz), -10.09 (d, J = 20.3); *m/z* (ESI) 694.1171 (monoanion, $C_{23}H_{30}N_5O_{16}P_2$ requires 694.1168).

(vi) 8-(Furan-2-yl)guanosine diphosphate mannose (4d).



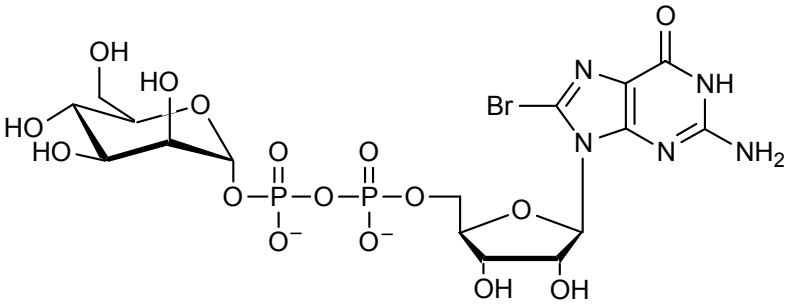
4d was prepared from **5** (16.2 mg, 0.022 mmol) and furan-2-ylboronic acid according to general procedure A. After purification by ion pair chromatography (method 1, fractions 27-31) and treatment with Chelex 100 resin (Na^+ form), **4d** was obtained as a glassy solid in 57% yield (11.5 mg). δ_H (400 MHz; D_2O) 7.58 (1H, d, J = 1.6, furanyl), 6.90 (1H, d, J = 3.5, furanyl), 6.51 (1H, dd, J = 1.8, 3.5, furanyl), 6.03 (1H, d, J = 6.1, H-1′), 5.34 (1H, d, J = 7.9, H-1″), 5.24 (1H, t, J = 5.9, H-2′), 4.47 (1H, dd, J = 3.2, 8.8, H-3′), 4.10 (3H, m, H-4′, H_2-5′), 3.88 (1H, m, H-2″), 3.75 (1H, dd, J = 3.4, 9.7, H-3″), 3.69 (2H, m, H-5″, H-6″a), 3.53 (2H, m, H-6″b, H-4″); δ_P (121 MHz; D_2O) -7.67 (d, J = 20.4 Hz), -10.10 (d, J = 21.3); *m/z* (ESI) 670.0798 (monoanion, $C_{20}H_{26}N_5O_{17}P_2$ requires 670.0804).

(vii) 8-(Pyren-1-yl)guanosine diphosphate mannose (4e).



4e was prepared from **5** (15.5 mg, 0.021 mmol) and pyrene-1-boronic acid according to general procedure A. After purification by ion pair chromatography (method 1, fractions 87-88) and treatment with Chelex 100 resin (Na^+ form), **4e** was obtained as a glassy solid in 73% yield (10.6 mg). δ_H (400 MHz; D_2O) 7.97 (9H, m), 5.32 (2H, m), 4.27 (1H, m), 4.07 (2H, m), 3.86 (3H, m), 3.75 (1H, m), 3.68 (2H, m), 3.53 (2H, m); δ_P (121 MHz; D_2O) -7.69 (d, J = 20.8 Hz), -10.11 (d, J = 20.3); *m/z* (ESI) 804.1331 (monoanion, $C_{32}H_{32}N_5O_{16}P_2$ requires 804.1325).

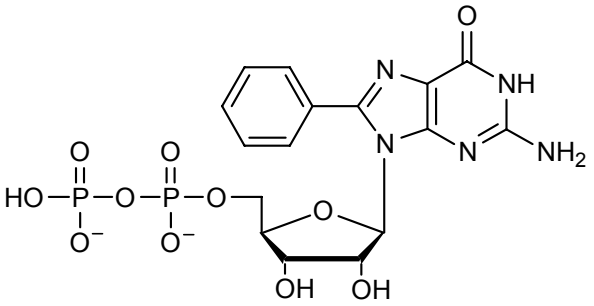
(viii) 8-Bromoguanosine Diphosphate mannose (5).



8-bromoguanosine monophosphate[1] (155.6 mg, 0.20 mmol) was dissolved in dry DMSO (0.5 mL) and co-evaporated with dry DMF (3 x 2 mL). Morpholine was added (105 μL, 1.20 mmol) and the reaction was stirred at rt for 5 min. Dipyridyl disulfide (134.7 mg, 0.61 mmol) was added and stirring continued for 5 min. Finely ground triphenylphosphine (160.9 mg, 0.61 mmol) was added and stirring continued for 2 hr. The product phosphoromorpholidate was precipitated by dropwise addition of 0.1 M NaI solution in acetone. The white precipitate was collected by filtration and washed with acetone. The crude morpholidate was then used without further purification. The morpholidate and mannose-1-phosphate[3] (triethylammonium salt; 77 mg, 0.18 mmol) were dissolved in dry pyridine (5 mL) and evaporated to dryness several times. $MgSO_4$ (42 mg, 0.35 mmol) and dry 0.2 M $MnCl_2$ in formamide (0.4 mL) were added and the solution stirred at rt under N_2 for six days. The product sugar-nucleotide was precipitated by dropwise addition of MeCN, and the supernatant was removed. The red oily residue was purified by anion

exchange chromatography (method 2). The thin film obtained was dissolved in H_2O (5 mL) and treated with Chelex 100 resin (Na^+ form). The resin was filtered off and washed with water. The combined filtrate was reduced to dryness to yield 70.4 mg (56 %) of **5** as a glassy solid. δ_H (400 MHz; D_2O) 5.79 (1H, d, J = 6.2, H-1′), 5.34 (1H, d, J = 7.8, H-1″), 5.16 (1H, t, J = 5.9, H-2′), 4.47 (1H, dd, J = 3.3, 5.5, H-3′), 4.12 (2H, m, H-4′, H-5′a), 4.04 (1H, m, H-5′b), 3.88 (1H, dd, J = 2.0, 3.2, H-2″), 3.75 (1H, dd, J = 3.4, 9.8, H-3″), 3.69 (2H, m, H-5″, H-6″a), 3.55 (2H, m, H-6″b, H-4″); δ_C (100 MHz; D_2O) 157.5, 153.2, 152.6, 123.8, 116.5, 96.1 (d, $J_{c,p}$ = 5.7 Hz), 89.3, 83.3 (d, $J_{c,p}$ = 9.2 Hz), 73.4, 70.3, 70.0 (d, $J_{c,p}$ = 9.3 Hz), 69.8, 69.5, 66.1, 64.9 (d, $J_{c,p}$ = 5.4 Hz), 60.4; δ_P (121 MHz; D_2O) -7.92 (d, J = 20.7 Hz), -10.27 (d, J = 21.7); *m/z* (ESI) 681.9796 (monoanion, $C_{16}H_{23}{}^{79}BrN_5O_{16}P_2$ requires 681.9804).

(ix) 8-Phenylguanosine-diphosphate (6).



6 was prepared from GDP (10.0 mg, 0.020 mmol) and phenylboronic acid according to general procedure B. After purification by ion pair chromatography (method 1) and anion exchange chromatography (method 2) and treatment with Chelex 100 resin (Na^+ form), **6** was obtained as a glassy solid in 50% yield (9.0 mg). δ_H (400 MHz; D_2O) 7.47 (5H, m, phenyl), 5.60 (1H, d, J = 5.7, H-1′), 5.09 (1H, t, J = 5.8, H-2′), 4.52 (1H, m, H-3′), 4.06 (3H, m, H-4′, 2× H-5′); δ_P (121 MHz; D_2O) -2.42 (d, J = 22.0 Hz), -6.83 (d, J = 22.3). *m/z* (ESI) 518.0485 (monoanion, $C_{16}H_{18}N_5O_{11}P_2$ requires 518.0484).

References

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[2]T. S. Lin, J. C. Cheng, K. Ishiguro and A. C. Sartorelli, *J. Med. Chem.*, 1985, **28**, 1481.

[3]G. M. Watt, S. L. Flitsch, S. Fey, L. Elling and U. Kragl, *Tetrahedron Asymmetry*, 2000, **11**, 621.