A fast synthetic route to GDP-sugars modified at the nucleobase†

Alice Collier and Gerd K. Wagner*

Received (in Cambridge, UK) 18th September 2007, Accepted 10th October 2007
First published as an Advance Article on the web 17th October 2007
DOI: 10.1039/b714379f

The direct structural modification of GDP-mannose *via* the bromination and Suzuki–Miyaura cross-coupling of the unprotected sugar–nucleotide, to produce 8-substituted fluorescent analogues of GDP-mannose.

GDP (guanosine diphosphate)-mannose (Fig. 1) is a natural substrate for many biologically important and therapeutically relevant glycoprocessing enzymes. GDP-mannose-dependent mannosyltransferases, for example, are essential for the viability and virulence of many human pathogens, including *Mycobacterium tuberculosis*, *Candida albicans* and *Trypanosoma brucei*. Important examples include the mycobacterial mannosyltransferases PimA[1] and PimB,[2] the fungal α-1,2-mannosyltransferases Mnt1p and Mnt2p,[3] and dolicholphosphate mannose synthase (DPMS), a mannosyltransferase required for the biosynthesis of glycosylphosphatidyl inositol (GPI) anchors in the protozoon *Trypanosoma brucei*.[4] Mannosyltransferases therefore represent promising new targets for anti-infective therapy, and structural analogues of their natural donor substrate, GDP-mannose, are of great interest as inhibitor candidates and biochemical tools for the investigation of this enzyme class.

With the view to developing a general mannosyltransferase assay, we recently required fluorescent analogues of GDP-mannose. The α-1,2-mannosyltransferase Kre2p from yeast, a homologue of the *Candida* Mnt1p enzyme, is one of only two mannosyltransferases whose structure has been solved to date.[5] An analysis of the Kre2p structure in complex with GDP suggested that the nucleotide binding domain may be able to accommodate GDP-mannose derivatives with additional substituents at the nucleobase. This observation inspired the design of a series of novel GDP-mannose analogues bearing a fluorescent marker at position 8 (Fig. 1).

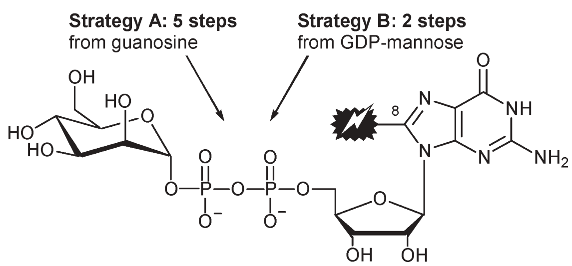


Fig. 1 Synthetic strategies towards GDP-mannose analogues with a fluorescent label in position 8.

Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, NR4 7TJ, UK. E-mail: g.wagner@uea.ac.uk; Fax: +44 (0)1603 592003; Tel: +44 (0)1603 593889

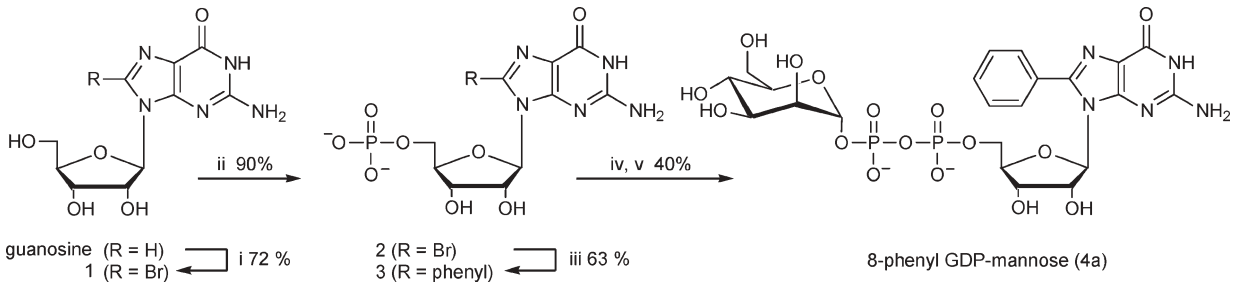
† Electronic supplementary information (ESI) available: Experimental procedures. See DOI: 10.1039/b714379f

For the installation of the fluorophore, we wanted to use a flexible synthetic strategy that allowed the rapid generation of a family of GDP-mannose analogues, and the assessment of their fluorescent and biological properties. While the target GDP-sugars can, in principle, be synthesised from the corresponding guanosine building blocks (Fig. 1, Strategy A), the direct structural modification of the parent sugar-nucleotide represents a more efficient and elegant strategy (Fig. 1, Strategy B). This approach is non-trivial due to the limited stability of sugar-nucleotides, their poor solubility in organic solvents and the presence of several unprotected functional groups. Previously reported examples for the modification of unprotected sugar-nucleotides, either at the sugar[6] or the nucleobase,[7] are limited to the generation or introduction of a single, specific functionality (*e.g.* a carboxylic acid or azide group), and lack the flexibility required for the efficient creation of structural diversity.

Recently, we and others have developed suitable Suzuki–Miyaura reaction[8] conditions for the direct modification of adenine and guanine nucleosides[9] and nucleotides[10,11] in aqueous media. Herein, we describe the synthesis of GDP-mannose analogues with aryl and heteroaryl substituents at position 8 of the nucleobase *via* the direct cross-coupling of an unprotected sugar-nucleotide precursor. Our synthesis is short, robust and versatile, and to the best of our knowledge represents the first example of the Pd-catalysed cross-coupling of a sugar-nucleotide. We also report the fluorescent properties of these novel GDP-mannose analogues.

In our initial synthesis of the prototypical 8-substituted GDP-mannose derivative **4a** from guanosine, 8-phenyl GMP (**3**)[11] was employed as the central building block (Scheme 1). For the formation of the pyrophosphate bond, **3** was activated as the phosphoromorpholidate and reacted with mannose-1-phosphate in the presence of $MnCl_2$ using an adaptation of Lee *et al.*'s method.[12] Although this approach gave us access to one of our target compounds, it involved several lengthy purification steps, and the overall yield of **4a** was low (16% from guanosine). This prompted us to explore an alternative synthetic route centred around the cross-coupling of 8-bromo GDP mannose in the final step (Scheme 2).

Conversion of 8-bromo GMP (**2**) into the morpholidate and reaction with mannose-1-phosphate provided 8-bromo-GDP-mannose (**5**), the precursor for the cross-coupling reaction, in good yield. To our delight, **5** then underwent reaction with phenylboronic acid under our optimised Suzuki conditions[11] to give 8-phenyl GDP-mannose (**4a**) within 1 h. The reaction was conveniently monitored by RP-HPLC, and no significant decomposition was observed. As expected, the presence of the phenyl



Scheme 1 *Reagents and conditions:* (i) Br_2/H_2O, rt, 20 min; (ii) $POCl_3$, proton sponge, MeCN, 2–4 °C, 2 h; (iii) Na_2Cl_4Pd (2.5 mol%), TPPTS, $PhB(OH)_2$, K_2CO_3, H_2O, 80 °C, 1 h; (iv) morpholine, dipyridyl disulfide, PPh_3, DMSO, rt, 1 h; (v) mannose-1-phosphate, $MnCl_2$, $MgSO_4$, dry formamide, rt, 6 d.

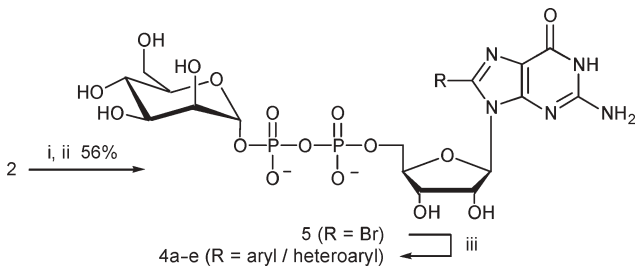
substituent in **4a** resulted in a prolonged RP-HPLC retention time and a red-shift of the UV absorbance maximum due to the extended conjugated system (λ_{max} **5**: 262 nm; **4a**: 278 nm). The cross-coupling product, **4a**, was obtained in 48% yield after purification by ion pair chromatography, and fully characterised by ^1H, ^{13}C and ^{31}P NMR, and HRMS.†

To test the scope of our conditions, and to gain access to a small library of fluorescent GDP-mannose analogues, we next reacted **5** with various fluorogenic aryl and heteroarylboronic acids (Scheme 2 and Table 1). The reactions with simple substituted phenylboronic acids (**4b** and **4c**) were complete within 1 h. Although the reactions with the furanyl and pyrenyl boronic acids (**4d** and **4e**) did not go to completion within that time, the cross-coupling products **4d** and **4e** were easily separated from the residual starting material by ion pair chromatography and obtained in moderate to good yield.

The syntheses of GDP-mannose analogues **4a–e** from **5** are, we believe, the first examples of the successful cross-coupling of a sugar-nucleotide. With regard to analogue preparation, this novel synthetic route is considerably more flexible and efficient than our initial synthesis of **4a**. In order to access a family of 8-substituted GDP-mannose derivatives, the low yielding and time consuming pyrophosphate bond-forming step had to be carried out only once, and the target compounds **4a–e** were obtained in generally good overall yields (27–46% from **2**).

At this stage, time spent on the purification of the sugar-nucleotide intermediates and products considerably exceeded the rather short times required for their actual preparation. In order to minimize the number of lengthy purification steps in the sequence, we decided to investigate the possibility of combining the bromination and cross-coupling of GDP-mannose in a one-pot, two-step procedure. Thus, we expected to be able to produce analogues of GDP-mannose from a commercially available starting material within one day, ideally with only one purification step required.

To identify suitable conditions for the one-pot, two-step procedure (Scheme 3), we initially used GMP as a model substrate. First, we optimised conditions for the bromination reaction. While guanosine was easily brominated with saturated bromine water (Scheme 1), application of the same conditions[13] to the bromination of GMP, in our hands, led to cleavage of the glycosidic bond. Unsurprisingly, attempts to brominate GMP in a sodium carbonate buffer (pH 9), a suitable medium for the subsequent Suzuki cross-coupling step, were equally unsuccessful. Finally, GMP was successfully brominated with saturated bromine water in a sodium acetate buffer (pH 4). After extraction of excess

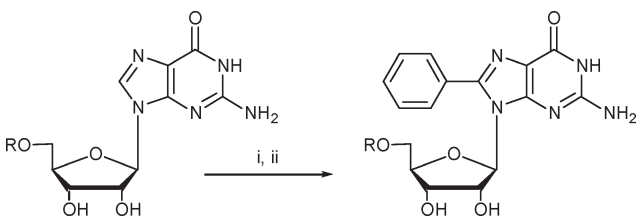


Scheme 2 *Reagents and conditions:* (i) morpholine, dipyridyl disulfide, PPh_3, DMSO, rt, 1 h; (ii) mannose-1-phosphate, $MnCl_2$, $MgSO_4$, dry formamide, rt, 6 d; (iii) Na_2Cl_4Pd (2.5 mol%), TPPTS, $R–B(OH)_2$, K_2CO_3, H_2O, 80 °C, 1 h. For substituents R and yields, see Table 1.

Table 1 Suzuki–Miyaura coupling of 8-bromo-GDP-mannose (**5**) with various aryl and heteroarylboronic acids ($R–B(OH)_2$)

Product	R	Yield[a] (%)
4a	Phenyl	48
4b	4-Chlorophenyl	82
4c	4-Methylphenyl	82
4d	Furan-2-yl	57
4e	Pyren-1-yl	73

[a] Isolated yields.



Scheme 3 *Reagents and conditions:* (i) Br_2/H_2O, NaOAc buffer (0.5 M, pH 4.0), rt, 20 min; (ii) Na_2Cl_4Pd (2.5 mol%), TPPTS, $PhB(OH)_2$, K_2CO_3, H_2O, 80 °C, 1 h. For substituents R and yields, see Table 2.

Table 2 One-pot synthesis of 8-phenyl GMP, GDP and GDP-mannose

Product	R	Yield[a] (%)
3	$(PO_3)^{2-}$	75
6	$(PO_3)_2^{3-}$	50
4a	$Man–(PO_3)_2^{2-}$	44

[a] Isolated yields.

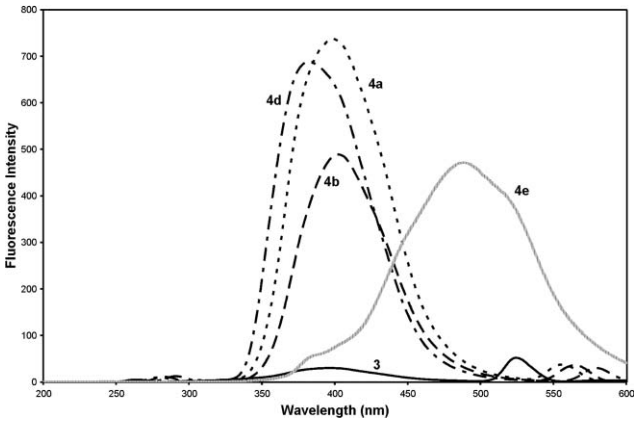


Fig. 2 Fluorescence emission spectra for GDP-mannose analogues **3**, **4a**, **4b**, **4d** and **4e** in H_2O.

Table 3 Fluorescence spectroscopic properties of GDP-mannose analogues

Compound	λ_{ex}/nm	λ_{em}/nm	Stokes shift[a]/cm^{-1}
5	262	—	—
4a	278	398	10846
4b	282	402	10585
4c	280	396	10462
4d	290	382	8305
4e	240	488	21175

[a] Stokes shift = $(1/\lambda_{excitation} - 1/\lambda_{emission})$.

bromine with chloroform and removal of all solvents, the crude residue was used for the Suzuki cross-coupling. When we applied our standard conditions (1.5 equivalents of base), no reaction was initially seen. We attributed this lack of cross-coupling reactivity to the neutral pH of the aqueous solution. Indeed, upon increasing the number of equivalents of base to a total of three (pH 9), the cross-coupling reaction went to completion in 20 min. No decomposition products were observed throughout, and purification by ion pair chromatography gave **3** in 75% isolated yield. This one-pot procedure was then also applied successfully to GDP and GDP-mannose (Table 2, products **6** and **4a**).‡ The lower yields obtained for **6** and **4a** reflect the increased fragility of the GDP and GDP-mannose substrates, and these reactions needed further purification to remove minor decomposition products.

8-Alkynylated and -arylated purine nucleosides have been reported as useful fluorescent probes.[14] Similarly, our novel 8-substituted GDP-mannose analogues possess interesting fluorescent properties, which can be modulated by the nature of the substituent in position 8 (Fig. 2 and Table 3). As expected, 8-bromo GDP-mannose (**5**) is non-fluorescent. The phenyl-substituted analogues **4a–c** show only subtle differences in their fluorescence characteristics, with λ_{em} around 400 nm and similar Stokes shifts. A slight blue-shift was observed in the fluorescence emission of 8-furanyl GDP-mannose **4d**, while the attachment of the bulky, fluorescent pyrene group (**4e**) resulted in a pronounced red-shift into the visible region.

In summary, we have developed a fast and flexible synthetic route to novel 8-substituted GDP-mannose analogues with interesting fluorescent properties. From a single synthetic precursor, our protocol provides easy access to a set of structurally diverse target molecules. These sugar-nucleotides will be useful as fluorescent probes for GDP-mannose-utilising enzymes, and investigations into their biological activity are currently underway.

We thank the University of East Anglia for a studentship (to A. C.), the EPSRC for financial support (First Grant EP/D059186/1), the EPSRC National Mass Spectrometry Service Centre, Swansea, for the recording of mass spectra, and Rob Field and Mark Searcey for helpful discussions.

Notes and references

‡ **8-Phenyl-GDP-mannose** (**4a**). To a solution of GDP-mannose (10 mg, 0.016 mmol) in 0.5 M NaOAc buffer (0.5 mL, pH 4.0) was added, dropwise, saturated bromine water until the yellow colour persisted. After stirring at rt for 20 min, the solution was extracted with $CHCl_3$ (3 × 5 mL) and the aqueous layer dried *in vacuo*. A solution of Na_2Cl_4Pd (0.1 mg, 0.4 μmol), triphenylphosphine trisulfonate (TPPTS; 0.6 mg, 1 μmol), $PhB(OH)_2$ (2.3 mg, 0.019 mmol) and K_2CO_3 (6.6 mg, 0.048 mmol) in degassed H_2O (3 mL) was added, and the yellow solution stirred under N_2 at 80 °C for 1 h. Upon completion of the reaction, the pH was adjusted to 7 with 1% aqueous HCl and all solvents were removed *in vacuo*. The crude residue was purified, first by ion pair chromatography (0–15% MeCN against 0.05 M TEAB; triethylammonium bicarbonate), and then on an anion exchange cartridge (0–100% 1 M TEAB against H_2O). The glassy residue obtained from repeated evaporations with MeOH was dissolved in H_2O and treated with Chelex 100 resin to give **4a** as the sodium salt in 44% yield (6.2 mg). δ_H (400 MHz; D_2O, acetone): 7.38 (5H, m, phenyl), 5.61 (1H, d, J = 6.1 Hz, H-1′), 5.35 (1H, d, J = 7.8 Hz, H-1″), 5.12 (1H, t, J = 5.9 Hz, H-2′), 4.36 (1H, dd, J = 3.3 and 5.6 Hz, H-3′), 4.15 (1H, m, H-5′a), 4.06 (2H, m, H-4′ and H-5′b), 3.89 (1H, m, H-2″), 3.76 (1H, dd, J = 3.4 and 9.7 Hz, H-3″), 3.70 (2H, m, H-5″ and H-6″a) and 3.54 (2H, m, H-6″b and H-4″); δ_C (100 MHz; D_2O, acetone): 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 Hz), 88.7, 83.0 (d, $J_{c,p}$ = 11.5 Hz), 73.4, 70.0 (d, $J_{c,p}$ = 11.9 Hz), 69.6, 66.2, 65.0 (d, $J_{c,p}$ = 7.1 Hz), 60.5 and 58.7; δ_P (121 MHz; D_2O, H_3PO_4) −7.75 (d, J = 20.4 Hz), −10.18 (d, J = 21.4 Hz). *m/z* (ESI) 680.1012 $[M]^-$; $C_{22}H_{28}N_5O_{16}P_2$ requires 680.1012.

1 J. Kordulakova, M. Gilleron, K. Mikusova, G. Puzo, P. J. Brennan, B. Gicquel and M. Jackson, *J. Biol. Chem.*, 2002, **277**, 31335.
2 M. L. Schaeffer, K. H. Khoo, G. S. Besra, D. Chatterjee, P. J. Belisle and J. M. Inamine, *J. Biol. Chem.*, 1999, **274**, 31625.
3 C. A. Munro, S. Bates, E. T. Buurman, H. B. Hughes, D. M. MacCallum, G. Bertram, A. Atrih, M. A. J. Ferguson, J. M. Bain, A. Brand, S. Hamilton, C. Westwater, L. M. Thomson, A. J. P. Brown, F. C. Odds and N. A. R. Gow, *J. Biol. Chem.*, 2005, **280**, 1051.
4 T. K. Smith, A. Crossman, J. S. Brimacombe and M. A. J. Ferguson, *EMBO J.*, 2004, **23**, 4701.
5 Y. D. Lobsanov, P. A. Romero, B. Sleno, B. Yu, P. Yip, A. Herscovics and P. L. Howell, *J. Biol. Chem.*, 2004, **279**, 17921.
6 M. Rejzek, B. Mukhopadhyay, C. Q. Wenzel, J. S. Lam and R. A. Field, *Carbohydr. Res.*, 2007, **342**, 460; J. J. Li and T. D. H. Bugg, *Chem. Commun.*, 2004, 182.
7 T. Szumilo, R. R. Drake, J. L. York and A. D. Elbein, *J. Biol. Chem.*, 1993, **268**, 17943.
8 A. Suzuki, *Chem. Commun.*, 2005, 4759.
9 N. Amann and H. A. Wagenknecht, *Synlett*, 2002, 687; E. C. Western, J. R. Daft, E. M. Johnson, P. M. Gannett and K. H. Shaughnessy, *J. Org. Chem.*, 2003, **68**, 6767; N. Kohyama, T. Katashima and Y. Yamamoto, *Synthesis*, 2004, 2799; A. Collier and G. K. Wagner, *Synth. Commun.*, 2006, **36**, 3713.
10 P. Capek, R. Pohl and M. Hocek, *Org. Biomol. Chem.*, 2006, **4**, 2278.
11 A. Collier and G. K. Wagner, *Org. Biomol. Chem.*, 2006, **4**, 4526.
12 J. Lee, H. Churchil, W. B. Choi, J. E. Lynch, F. E. Roberts, R. P. Volante and P. J. Reider, *Chem. Commun.*, 1999, 729.
13 S. H. Chu, C. Y. Shiue and M. Y. Chu, *J. Med. Chem.*, 1975, **18**, 559.
14 A. G. Firth, I. J. S. Fairlamb, K. Darley and C. G. Baumann, *Tetrahedron Lett.*, 2006, **47**, 3529; N. J. Greco and Y. Tor, *Tetrahedron*, 2007, **63**, 3515.

