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Stereospecific synthesis of sugar-1-phosphates and their conversion to sugar nucleotides

Shannon C. Timmons[a] and David L. Jakeman[a,b,*]

[a]*Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3*
[b]*College of Pharmacy, Dalhousie University, 5968 College Street, Halifax, Nova Scotia, Canada B3H 3J5*

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Abstract—As Leloir glycosyltransferases are increasingly being used to prepare oligosaccharides, glycoconjugates, and glycosylated natural products, efficient access to stereopure sugar nucleotide donor substrates is required. Herein, the rapid synthesis and purification of eight sugar nucleotides is described by a facile 30 min activation of nucleoside 5′-monophosphates bearing purine and pyrimidine bases with trifluoroacetic anhydride and *N*-methylimidazole, followed by a 2 h coupling with stereospecifically prepared sugar-1-phosphates. Tributylammonium bicarbonate and tributylammonium acetate were the ion-pair reagents of choice for the C18 reversed-phase purification of 6-deoxysugar nucleotides, and hexose or pentose-derived sugar nucleotides, respectively.

Keywords: Sugar nucleotides; Glycosyltransferases; Sugar-1-phosphates

1. Introduction

The glycosylation of an array of structurally diverse biomolecules including proteins, lipids, and many classes of natural products mediates a wide variety of important biological processes.[1–3] In contrast to protein and nucleic acid research, the development of tools to study glycosylation has been limited by the structural and functional diversity of carbohydrates, resulting in a chasm of understanding between these groups of biomolecules.[4] Recent advances in chemical glycobiology[5] have begun to bridge this gap in knowledge and innovative strategies for engineering glycosylation have been described.[6–8]

The ubiquitous enzymes responsible for the transfer of carbohydrates from sugar nucleoside diphosphate donors to various acceptors are Leloir glycosyltransferases.[9] Until recently, the membrane-associated nature of these anabolic enzymes largely limited their access for use in glycosylation studies.[10] Advances in molecular biology have facilitated the overexpression, purification, and crystallization of an increasing number of glycosyltransferases, revealing a higher degree of structural diversity than originally anticipated.[11] A second major obstacle to investigating the functional role of glycosylation in numerous biological systems using glycosyltransferases is a lack of efficient access to stereopure sugar nucleotides.[12] The recent observation that the glycosyltransferase VinC is capable of processing both α- and β-anomer of a sugar nucleotide to give glycosylated products with corresponding inversion of stereochemistry at the anomeric linkage[13] underlies the importance of stereospecific synthetic methods for the preparation of these substrates. Although the enzymatic preparation of these compounds has emerged,[14–18] the versatility available via chemical synthesis is unparalleled.

One synthetic strategy used to prepare sugar nucleotides involves the direct coupling of electrophilic sugar donors with nucleoside 5′-diphosphates.[19–21] In addition to low yields, these routes typically suffer from low anomeric diastereoselectivites, although an improvement involving neighboring group participation has recently been described.[22] A second synthetic approach

* Corresponding author. Tel.: +1 902 494 7159; fax: +1 902 494 1396; e-mail: david.jakeman@dal.ca

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used to prepare sugar nucleotides, initially developed by Moffatt and Khorana,[23] involves the coupling of sugar-1-phosphates with electrophilic nucleoside 5′-monophosphates, frequently activated as morpholidate or imidazolide esters.[24] Although improved yields have been reported using 1*H*-tetrazole as a catalyst[25] or through extensive co-evaporations with pyridine,[26] many coupling reactions of this type take several days to reach completion and result in only low to moderate yields (<50%) following purification.[27–31]

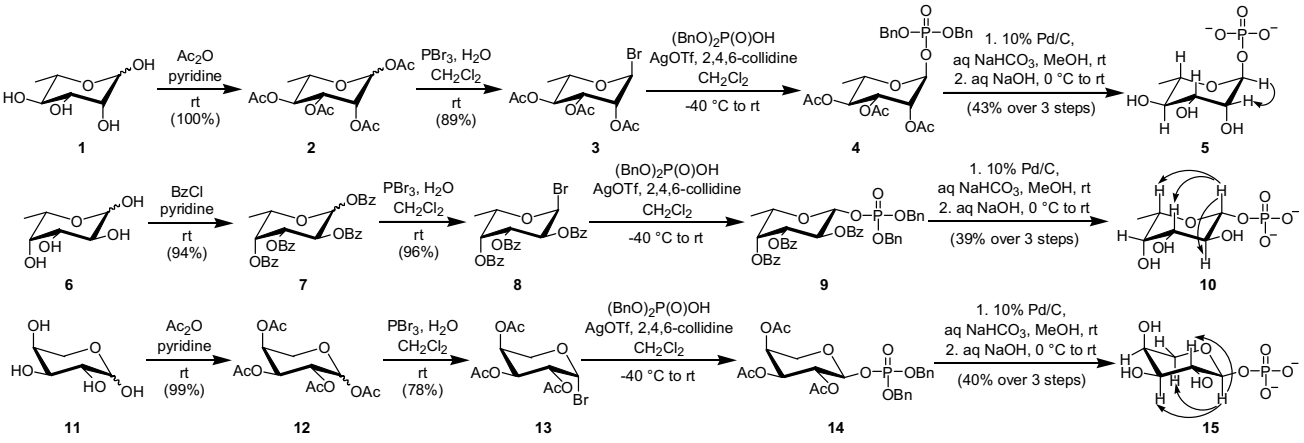
Herein, we report the synthesis and purification of eight sugar nucleotides via coupling of sugar-1-phosphates with nucleoside 5′-monophosphates activated using trifluoroacetic anhydride and *N*-methylimidazole. This activation method was first reported by Bogachev for use in the synthesis of nucleoside 5′-triphosphates,[32] and has since been used in the synthesis of UDP-α-D-galactofuranose by Marlow and Kiessling,[33] for the preparation of GDP-hexanolamine by Vincent and Gastinel[34] and in the synthesis of electron-deficient nucleoside 5′-β,γ-methylenetriphosphate analogues by Mohamady and Jakeman.[35] We also report efficient chromatographic conditions to separate the desired sugar nucleotide products from undesirable dinucleoside diphosphate byproducts that are frequently associated with these types of phosphate-coupling reactions.

2. Results and discussion

To facilitate the synthesis of sugar nucleotides via the *N*-methylimidazole activation route, sugar-1-phosphates derived from L-rhamnose (**1**), L-fucose (**6**), and L-arabinose (**11**) were first prepared using a similar procedure to that of Wong and co-workers[36] (Scheme 1) for the preparation of **10**. This synthetic route involved the initial preparation of acylated glycosyl bromides, which were coupled with dibenzyl phosphate in the presence of silver triflate and 2,4,6-collidine in the key phosphorylation step. Following deprotection via hydrogenolysis and treatment with aq NaOH, exclusively one sugar-1-phosphate anomer was obtained for each sugar (Table 1). The stereospecific outcome of the phosphorylation step is well known for L-fucose derivatives[36] where neighboring group participation controls the attack of the phosphate at the anomeric center, as only the bottom face of the compound would be open to attack in this circumstance, resulting in 1,2-trans stereochemistry.[22] Consistent with this mechanistic outcome, we present the first stereospecific preparation of α-L-arabinose-1-phosphate (**15**), whereupon phosphorylation of **13** only the α anomer was prepared with an observed $^{3}J_{\text{H-1,H-2}}$ value of 7.5 Hz, suggesting a $^{4}C_{1}$ chair conformation. The outcome of this phosphorylation method with acylated α-L-rhamnosyl bromide (**3**) has previously been reported incorrectly by Zhao and Thorson,[37] where the isolation of only the β diastereomer was reported. In our hands, the analysis of 1D NOE and $^{1}J_{\text{C-1,H-1}}$ data[38] for the phosphorylation reaction product indicated that exclusively the α diastereomer (**5**) was prepared. Further indirect evidence to support this assignment is the addition of nucleoside 5′-diphosphates to acylated α-D-mannosyl bromide (a similarly 1,2-trans-configured monosaccharide) resulting in the stereospecific formation of an α-configured sugar nucleotide.[22] The difficulties associated with the formation of 1,2-cis-linked glycosides from L-rhamnose[39] and D-mannose[40] also provide indirect evidence for the configuration of **5**. Thus, the phosphorylation of acylated glycosyl bromides provides an efficient stereospecific method to access 1,2-trans-linked sugar-1-phosphates.

Table 1. Characterization of sugar-1-phosphates

Product	δ H1 (ppm)	$^{3}J_{\text{H1–H2}}$ (Hz)	$^{1}J_{\text{C1–H1}}$ (Hz)
α-L-Rhamnose-1-phosphate (**5**)	5.31	1.5	170
β-L-Fucose-1-phosphate (**10**)	4.86	8.0	162
α-L-Arabinose-1-phosphate (**15**)	4.82	7.5	163

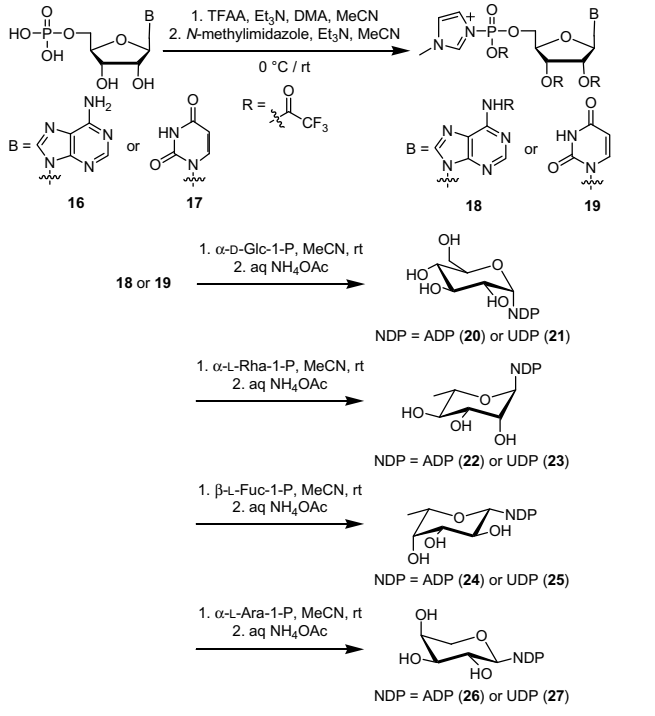


Scheme 1. Synthesis of sugar-1-phosphates (arrows indicate strong 1D NOE interactions).

The coupling of commercially available α-D-glucose-1-phosphate as well as the three synthetically prepared sugar-1-phosphates described above (**5**, **10**, and **15**) with activated nucleoside 5′-monophosphates (**18** or **19**) was accomplished via four rapid, straightforward steps (Scheme 2). Nucleoside 5′-monophosphates (AMP or UMP) in free acid form were first trifluoroacetylated using trifluoroacetic anhydride in the presence of triethylamine and *N*,*N*-dimethylaniline. After 15 min at rt, homogeneous pale yellow solutions were obtained. An advantage of using excess trifluoroacetic anhydride in this step is that it eliminates the necessity for preliminary drying of hygroscopic nucleoside 5′-monophosphates via co-evaporation with pyridine. In addition, the trifluoroacetylation conditions were sufficiently vigorous to allow the use of nucleoside 5′-monophosphates in free acid form, eliminating the requirement for preliminary conversion to an organic-soluble alkyl salt form. Following the removal of volatile reaction components, trifluoroacetylated intermediates were treated with *N*-methylimidazole to generate the desired electrophilic nucleoside 5′-monophosphates **18** and **19**. Reactions were complete after 15 min at 0–5 °C, after which time bright yellow solutions were obtained. The essentially quantitative formation of *N*-methylimidazole-activated nucleoside 5′-monophosphates was confirmed by $^{31}P\{^{1}H\}$ NMR (δ −11.0 ppm for AMP-*N*-methylimidazolide (**18**), δ −10.8 ppm for UMP-*N*-methylimidazolide (**19**)). Activated nucleoside 5′-monophosphates and sugar-1-phosphates were immediately combined and coupling reactions were monitored by analyzing $^{31}P\{^{1}H\}$ NMR spectra of reaction aliquots. A 1:2 ratio of nucleoside 5′-monophosphate:sugar-1-phosphate was used in an attempt to reduce byproducts resulting from the 'self-condensation' of nucleoside 5′-monophosphates, which results in dinucleoside diphosphate (NppN) byproducts that are sometimes difficult to chromatographically separate from sugar nucleotide products. Unreacted sugar-1-phosphates were quantitatively recovered during the purification process and were used again in future reactions following cation exchange. After 2 h at rt, NMP-*N*-methylimidazolides had been completely consumed, as observed by $^{31}P\{^{1}H\}$ NMR, and reactions were quenched with aq ammonium acetate, which facilitated the removal of remaining trifluoroacetyl protecting groups. Sugar nucleotide products were purified using automated C18 reversed-phase ion-pair chromatography.

A summary of results obtained using the aforementioned coupling procedure to prepare eight sugar nucleotides is shown in Table 2. Commercially available α-D-glucose-1-phosphate was initially employed to test the efficacy of this coupling procedure with both purine- and pyrimidine-containing nucleoside 5′-monophosphates (AMP and UMP, respectively). Following coupling and purification, ADP-α-D-glucose (**20**) and UDP-α-D-glucose (**21**) were isolated in 48% and 35% yields, respectively, over four steps, demonstrating the versatility of this method with different nucleoside bases. On extension of this coupling procedure to synthetically prepared sugar-1-phosphates α-L-rhamnose-1-phosphate (**5**), β-L-fucose-1-phosphate (**10**), and α-L-arabinose-1-phosphate (**15**), reproducible conversions to product of approximately 40–50% by $^{31}P\{^{1}H\}$ NMR were obtained in all cases.

Purification of this series of sugar nucleotides required the removal of dinucleoside diphosphates, which were the main byproducts (<50% by $^{31}P\{^{1}H\}$ NMR) in all reaction mixtures. The addition of molecular sieves in



Scheme 2. Synthesis of sugar nucleotides.

Table 2. Yields of sugar nucleotides

Product	Coupling rxn time (h)	Yield[a,b] (%)
ADP-α-D-glucose (**20**)	2	48
UDP-α-D-glucose (**21**)	2	35
ADP-α-L-rhamnose (**22**)	2	25
UDP-α-L-rhamnose (**23**)	2	30
ADP-β-L-fucose (**24**)	2	28
UDP-β-L-fucose (**25**)	2	26
ADP-α-L-arabinose (**26**)	2	35
UDP-α-L-arabinose (**27**)	2	32

[a] Yields are reported for purified products over 4 steps from nucleoside 5′-monophosphates.

[b] Yields were determined by UV using λ_{max} 260 nm ($\varepsilon = 1.51 \times 10^4\ M^{-1}\ cm^{-1}$) for products containing adenosine and λ_{max} 261 nm ($\varepsilon = 1.01 \times 10^4\ M^{-1}\ cm^{-1}$) for products containing uridine.

the coupling step helped to minimize these byproducts, but could not completely suppress their formation. In the case of the D-glucose (**20**, **21**) and L-arabinose-containing (**26**, **27**) sugar nucleotides, tributylammonium acetate was used as an ion-pair reagent (10 mM aqueous buffer at pH 4) in C18 reversed-phase ion-pair chromatography as previously described for the purification of nucleoside 5′-β, γ-methylenetriphosphate analogues.[35] Interestingly, this ion-pair reagent did not permit the separation of L-rhamnose (**22**, **23**) and L-fucose-containing (**24**, **25**) sugar nucleotides from dinucleoside diphosphate byproducts. The purification of these four sugar nucleotides was instead achieved using tributylammonium bicarbonate as an ion-pair reagent (10 mM aqueous buffer at pH 7),[22] which enabled the separation of the desired sugar nucleotide products from NppNs. Although these two ion-pair reagents have the same cationic counterion, the difference in buffer pH could affect the interactions of reaction products with the C18 stationary phase.

3. Conclusions

The phosphorylation of acylated 1,2-*trans* glycosyl bromides offers the opportunity to prepare stereopure sugar-1-phosphates that when coupled with *N*-methylimidazole-activated nucleoside 5′-monophosphates represents a rapid and reproducible method to prepare sugar nucleotides. The moderate yields obtained using this synthetic strategy are generally comparable to yields reported using the more widely used morpholidate and imidazolide methods of nucleoside 5′-monophosphate activation and are offset by the directness of this route. The increased electrophilicity of *N*-methylimidazole-activated nucleoside 5′-monophosphates over those activated as morpholidates or imidazolides facilitates significantly shorter reaction times that may have significant utility in reactions where less reactive nucleophiles are employed. Thus, when the desired sugar-1-phosphates are readily available via commercial sources or chemical synthesis, the aforementioned coupling and purification protocol is a rapid method for the preparation of sugar nucleotides for glycosyltransferase studies.

4. Experimental

4.1. General methods

With the exclusion of certain solvents, chemicals were purchased commercially and used without further purification unless otherwise specified. Anhyd CH_2Cl_2 was purified via filtration through alumina using an Innovative Technology solvent purification system. All other solvents were of reagent grade unless otherwise noted. Analytical thin-layer chromatography was performed on glass-backed TLC plates pre-coated with silica gel (SiliCycle™, 250 μm) and compounds were detected by UV absorbance (254 nm) and/or by spraying with a $KMnO_4$ visualization soln (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5% w/v aq NaOH, 300 mL water). Analytical HPLC of purified sugar nucleotides was performed using a Hewlett Packard Series 1050 instrument equipped with an Agilent Zorbax 5 μm Rx-C18 column (150 cm × 4.6 mm). Compounds containing nucleoside base chromophores were monitored at an absorbance of 254 nm over a linear gradient from 90:10 A:B to 40:60 A:B over 8.0 min followed by a plateau at 40:60 A:B from 8.0 to 10.0 min at 1.0 mL/min, where A is an aq buffer containing 12 mM Bu_4NBr, 10 mM KH_2PO_4, and 5% v/v HPLC grade MeCN (pH 4) and B is HPLC grade MeCN (hereafter denoted as Method A). Automated C18 reversed-phase chromatography was performed using a Biotage SP1™ flash chromatography system. Hydrogenolysis was performed using a Parr Model 3911 pressure reaction apparatus. All NMR data were collected using a Bruker AVANCE 500 MHz spectrometer and 1D NOE NMR experiments were performed using a 750 ms mixing time. Chemical shifts are reported in parts per million (ppm) relative to a tetramethylsilane internal standard at 0.00 ppm for samples dissolved in $CDCl_3$, while spectra recorded in D_2O were referenced to the solvent peak at 4.79 ppm. $^{31}P\{^1H\}$ spectra were referenced relative to an external 85% aq H_3PO_4 standard at 0.00 ppm, while $^{19}F\{^1H\}$ spectra were referenced relative to an external 0.5% v/v $CF_3C_6H_5$ standard in $CDCl_3$ at −63.72 ppm. All ^1H and $^{13}C\{^1H\}$ NMR assignments were confirmed using COSY, HSQC, and/or HMBC 2D NMR experiments. Low-resolution ESIMS measurements were obtained using a ThermoFinnigan LCQ Duo ion trap instrument operating in negative ion mode. High-resolution ESIMS measurements were obtained using a Bruker Daltonics micrOTOF instrument operating in negative ion mode.

4.2. Synthetic procedures and spectroscopic data

4.2.1. 1,2,3,4-Tetra-*O*-acetyl-α/β-L-rhamnopyranose (2). L-Rhamnose monohydrate (5.00 g, 27.4 mmol) and pyridine (18 mL) were combined in a round-bottomed flask. Ac_2O (21 mL, 220 mmol) was added and the reaction mixture was stirred for 1 h at rt, after which time the reaction was deemed complete by TLC (1:1 EtOAc–hexanes, R_f product **2** = 0.60). The reaction mixture was diluted with an ice-water soln (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with 1 M aq HCl (3 × 50 mL), water (2 × 50 mL), satd aq $NaHCO_3$ (50 mL), and again with water (50 mL). After drying over Na_2SO_4 and concentration under reduced pressure, acetylated

product **2** was obtained as a pale yellow syrup in an α:β ratio of 3:1 (9.11 g, 27.4 mmol, 100% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ α *diastereomer*: 6.02 (d, $J_{1,2}$ 2.0 Hz, 1H, H-1), 5.31 (dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 10.5 Hz, 1H, H-3), 5.25 (dd, 1H, H-2), 5.12 (dd, $J_{4,5}$ 10.0 Hz, 1H, H-4), 3.94 (dq, $J_{5,6}$ 6.5 Hz, 1H, H-5), 2.17 (s, 3H, C(O)CH_3), 2.16 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.23 (d, 3H, H_3-6); β *diastereomer*: 5.84 (d, $J_{1,2}$ 1.5 Hz, 1H, H-1), 5.48 (dd, $J_{2,3}$ 3.0 Hz, 1H, H-2), 5.08 (m, 2H, H-3, H-4), 3.67 (dq, $J_{5,6}$ 6.0 Hz, 1H, H-5), 2.21 (s, 3H, C(O)CH_3), 2.10 (s, 3H, C(O)CH_3), 2.06 (s, 3H, C(O)CH_3), 2.00 (s, 3H, C(O)CH_3), 1.29 (d, 3H, H_3-6).

4.2.2. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (3). 1,2,3,4-Tetra-*O*-acetyl-α/β-L-rhamnopyranose (**2**) (3.11 g, 9.36 mmol) was dissolved in CH_2Cl_2 (30 mL) in a round-bottomed flask that was stoppered with a septum and cooled to 0–5 °C in an ice-water bath. Phosphorus tribromide (1.50 mL, 15.9 mmol) and water (1.01 mL, 56.2 mmol) were added dropwise to this soln. After 10 min, the reaction mixture was warmed to rt and stirred for 2 h, after which time the reaction was deemed complete by TLC (7:13 EtOAc–hexanes, R_f product **3** = 0.72).[†] The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water (2 × 80 mL), satd aq $NaHCO_3$ (80 mL), and saturated aq NaCl (2 × 80 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford an orange syrup (2.95 g, 8.35 mmol, 89% yield). ^{1}H and ^{13}C{^{1}H} NMR data were consistent with that of Thorson and co-workers.[41]

4.2.3. α-L-Rhamnose-1-phosphate (5). A mixture of dibenzyl phosphate (2.22 g, 7.98 mmol), silver triflate (1.86 g, 7.25 mmol), and 2,4,6-collidine (1.25 mL, 9.43 mmol) was dissolved in anhyd CH_2Cl_2 (8.5 mL) in a round-bottomed flask. This soln was stirred under a nitrogen atmosphere in the absence of light for 1 h. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**3**) (2.56 g, 7.25 mmol) was dissolved in anhyd CH_2Cl_2 (7.0 mL) in a second round-bottomed flask under a nitrogen atmosphere and both flasks were cooled to −40 °C in a dry ice-acetone bath. The contents of the second round-bottomed flask were then added dropwise via cannula to the first flask and the resulting reaction mixture was stirred for 4 h at −40 °C under a nitrogen atmosphere in the absence of light. After 4 h, no more dry ice was added and the reaction mixture was allowed to slowly warm to rt while stirring overnight. After 12 h at rt, TLC analysis (7:13 EtOAc–hexanes, R_f dibenzylphosphorylated product **4** = 0.27) revealed that the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and the organic layer was washed with water (25 mL), saturated aq K_2CO_3 (2 × 25 mL), and again with water (2 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. It was found that exclusively one diastereomer of dibenzylphosphorylated product **4** had been prepared and the only byproducts visible in the ^{1}H NMR spectrum of the crude product were 2,4,6-collidine and a small amount of hydrolyzed glycosyl bromide. To streamline the purification procedure, the crude dibenzylphosphorylated product was used directly in the next deprotection step where 2,4,6-collidine and the small amount of hydrolyzed glycosyl bromide were easily extracted into the organic layer after hydrogenolysis of the benzyl groups protecting the phosphate. In preparation for hydrogenolysis, 10% Pd/C (700 mg) and aq $NaHCO_3$ (12 mL, 1 M) were first added to the hydrogenolysis jar. The crude protected sugar-1-phosphate was subsequently dissolved in HPLC grade MeOH (30 mL), added to the jar, and subjected to hydrogenolysis under a 50 psi hydrogen atmosphere for 1 h, after which time no starting material was visible by TLC. The reaction mixture was filtered through a short plug of Celite® in a scintillated funnel (rinsing with HPLC grade MeOH) to remove the Pd/C catalyst. The filtrate was concentrated under reduced pressure and partitioned between CH_2Cl_2 (30 mL) and water (30 mL). To remove the acetyl groups protecting the monosaccharide, the aqueous layer was diluted with water (total vol 20 mL) and cooled to 0–5 °C in an ice-water bath; 1 M aq NaOH (17 mL) was added dropwise and the reaction mixture was allowed to warm slowly to rt over 3 h. The pH was then carefully adjusted to 7.0–7.5 with cold 1 M aq acetic acid and the reaction mixture was concentrated to ~5 mL in volume and applied to an Amberlite® IR-120 (H^+) cation exchange column (free acid form). The sugar-1-phosphate was eluted with H_2O and fractions containing the desired product were pooled. The pH of these fractions was immediately adjusted to 7.0–7.5 with 1 M aq NH_4OH and the sugar-1-phosphate was concentrated under reduced pressure and lyophilized. Trituration with ethanol followed by vortexing and centrifugation at 4 °C facilitated the successful removal of the remaining NH_4OAc. After concentration under reduced pressure, the sugar-1-phosphate was re-dissolved in water (~2 mL) and lyophilized a second time, affording α-L-rhamnose-1-phosphate (**5**, diammonium salt) as a white solid (868 mg, 3.12 mmol, 43% yield over 3 steps). ^{1}H and ^{13}C{^{1}H} NMR data were consistent with that of Zhao and Thorson, which was reported referring to the product as the β diastereomer.[37] However, coupled ^{13}C–^{1}H HSQC: $^1J_{C\text{-}1,H\text{-}1}$ 170 Hz confirms an α-linkage; ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 6): δ 1.34 (s); LRMS (ESI$^-$) for $C_6H_{13}O_8P$ (free acid, 244.1) = *m*/*z* 243.0 [M−H]$^-$.

[†] Glycosyl bromide **3** degrades on silica gel TLC plates, presumably to the corresponding hemiacetals, which had an R_f of 0.41 in 7:13 EtOAc–hexanes.

4.2.4. 1,2,3,4-Tetra-*O*-benzoyl-α/β-L-fucopyranose (7). L-Fucose (2.00 g, 12.2 mmol) and pyridine (40 mL) were combined in a round-bottomed flask and the resulting mixture was cooled to 0–5 °C in an ice-water bath. Benzoyl chloride (7.20 mL, 62.1 mmol) was added dropwise and the ice-water bath was removed. The reaction mixture was stirred for 3 h at rt, after which time the reaction was deemed complete by TLC (7:13 EtOAc–hexanes, R_f product **7** = 0.64). The reaction mixture was poured into an ice-water mixture (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with cold aq HCl (3 × 200 mL, 1 M), water (200 mL), satd aq $NaHCO_3$ (200 mL), and saturated aq NaCl (200 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford a light brown solid in an α:β ratio of 15:1 (6.68 g, 11.5 mmol, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ α *diastereomer*: 8.17–7.23 (m, 20H, Ph), 6.87 (d, $J_{1,2}$ 3.5 Hz, 1H, H-1), 6.08 (dd, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.3 Hz, 1H, H-3), 5.99 (dd, 1H, H-2), 5.90 (dd, $J_{4,5}$ 1.0 Hz, 1H, H-4), 4.64 (br q, $J_{5,6}$ 6.5 Hz, 1H, H-5), 1.32 (d, 3H, H_3-6).

4.2.5. 2,3,4-Tri-*O*-benzoyl-α-L-fucopyranosyl bromide (8). Following the procedure used for the preparation of glycosyl bromide **3**, 1,2,3,4-tetra-*O*-benzoyl-α/β-L-fucopyranose (**7**) (1.04 g, 1.79 mmol) was reacted with phosphorus tribromide (285 μL, 3.02 mmol) and water (190 μL, 10.5 mmol) in CH_2Cl_2 (6 mL). The aforementioned protocol afforded 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide (**8**) (7:13 EtOAc–hexanes, R_f = 0.78)[‡] as a pale orange syrup (928 mg, 1.72 mmol, 96% yield). ^{1}H and ^{13}C{^{1}H} NMR data were consistent with that of Wong and co-workers.[36]

4.2.6. β-L-Fucose-1-phosphate (10). Following the procedure used for the preparation of α-L-rhamnose-1-phosphate (**5**), 2,3,4-tri-*O*-benzoyl-α-L-fucopyranosyl bromide (**8**) (1.82 g, 5.16 mmol) was reacted with dibenzyl phosphate (1.58 g, 5.68 mmol), silver triflate (1.33 g, 5.16 mmol), and 2,4,6-collidine (890 μL, 6.71 mmol) in anhyd CH_2Cl_2 (11.0 mL). Hydrogenolysis was performed on the crude dibenzylphosphorylated product (**9**) (7:13 EtOAc–hexanes, R_f = 0.22), as previously described using 10% Pd/C (500 mg) and aq $NaHCO_3$ (8.4 mL, 1 M) in HPLC grade MeOH (20 mL). Deacetylation was accomplished by diluting the resulting sugar-1-phosphate with water (total vol 15 mL) and adding aq NaOH (12 mL, 1 M). After purification, cation exchange, and lyophilization as described for **5**, β-L-fucose-1-phosphate (**10**, diammonium salt) was obtained as a white solid (559 mg, 2.01 mmol, 39% yield over 3 steps). ^{1}H and ^{13}C{^{1}H} NMR data were consistent with that of Hindsgaul and co-workers[42] as well as Barker and co-workers.[43] Coupled ^{13}C-^{1}H HSQC: $^1J_{C\text{-}1,H\text{-}1}$ 162 Hz; ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 6): δ 1.37 (s); LRMS (ESI^-) for $C_6H_{13}O_8P$ (free acid, 244.1) = *m*/*z* 243.1 $[M-H]^-$.

4.2.7. 1,2,3,4-Tetra-*O*-acetyl-α/β-L-arabinopyranose (12). Following the procedure used for the preparation of acetylated derivative **2**, L-arabinose (3.00 g, 20.0 mmol) was reacted with Ac_2O (15.1 mL, 160 mmol) in pyridine (12.9 mL). The aforementioned protocol afforded 1,2,3,4-tetra-*O*-acetyl-α/β-L-arabinopyranose (**12**) (7:13 EtOAc–hexanes, R_f = 0.45) as a pale yellow syrup in an α:β ratio of 2:3 (6.32 g, 19.9 mmol, 99% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ α *diastereomer*: 5.67 (d, $J_{1,2}$ 7.0 Hz, 1H, H-1), 5.30 (m, 1H, H-2), 5.31 (m, 1H, H-4), 5.12 (dd, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, 1H, H-3), 3.95 (m, 2H, H_2-5), 2.14 (s, 3H, C(O)CH_3), 2.13 (s, 3H, C(O)CH_3), 2.07 (s, 3H, C(O)CH_3), 2.05 (s, 3H, C(O)CH_3); β *diastereomer*: 6.35 (d, $J_{1,2}$ 3.0 Hz, 1H, H-1), 5.34 (m, 1H, H-2), 4.39 (m, 1H, H-4), 4.24 (m, 1H, H-3), 3.95 (m, 2H, H_2-5), 2.16 (s, 3H, C(O)CH_3), 2.15 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3).

4.2.8. 2,3,4-Tri-*O*-acetyl-β-arabinopyranosyl bromide (13). Following the procedure used for the preparation of glycosyl bromide **3**, 1,2,3,4-tetra-*O*-acetyl-α/β-L-arabinopyranose (**12**) (2.50 g, 7.86 mmol) was reacted with phosphorus tribromide (1.20 mL, 12.7 mmol) and water (850 μL, 47.2 mmol) in CH_2Cl_2 (25 mL). The aforementioned protocol afforded 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**13**) (7:13 EtOAc–hexanes, R_f = 0.73)[§] as a white solid (2.09 g, 6.16 mmol, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (d, $J_{1,2}$ 4.0 Hz, 1H, H-1), 5.41 (dd, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, 1H, H-3), 5.40 (m, 1H, H-4), 5.09 (dd, 1H, H-2), 4.21 (br d, $J_{5a,5b}$ 13.5 Hz, 1H, H-5a), 3.94 (dd, 1H, H-5b), 2.16 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ 170.1 (*C*(O)CH_3), 170.0 (*C*(O)CH_3), 169.8 (*C*(O)CH_3), 89.7 (C-1), 68.0 (C-2), 67.9 (C-4), 67.6 (C-3), 64.7 (C-5), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.6 (C(O)*C*H_3).

4.2.9. α-L-Arabinose-1-phosphate (15). Following the procedure used for the preparation of β-L-fucose-1-phosphate (**5**), 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**13**) (1.75 g, 5.16 mmol) was reacted with dibenzyl phosphate (1.58 g, 5.68 mmol), silver triflate (1.33 g, 5.16 mmol), and 2,4,6-collidine (890. μL, 6.71 mmol) in anhyd CH_2Cl_2 (11.0 mL). Hydrogenolysis

[‡] Glycosyl bromide **8** degrades on silica gel TLC plates, presumably to the corresponding hemiacetals, which had an R_f of 0.41 in 7:13 EtOAc–hexanes.

[§] Glycosyl bromide **13** degrades on silica gel TLC plates, presumably to the corresponding hemiacetals, which had an R_f of 0.17 in 7:13 EtOAc–hexanes.

was performed on the crude dibenzylphosphorylated product (**14**) (7:13 EtOAc–hexanes, $R_f = 0.31$), as previously described using 10% Pd/C (500 mg) and aq $NaHCO_3$ (8.4 mL, 1 M) in HPLC grade MeOH (20 mL). Deacetylation was accomplished by diluting the resulting sugar-1-phosphate with water (total vol 15 mL) and adding 1 M NaOH (12 mL, 1 M). After purification, cation exchange, and lyophilization as described for **5**, α-L-arabinose-1-phosphate (**15**, diammonium salt) was obtained as a white solid (544 mg, 2.06 mmol, 40% yield over 3 steps). ^{1}H NMR (500 MHz, D_2O, pH 6): δ 4.82 (dd, $J_{1,P}$ 8.0 Hz, $J_{1,2}$ 7.5 Hz, 1H, H-1), 3.96 (m, 1H, H-4), 3.95 (m, 1H, H-5a), 3.73 (dd, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.0 Hz, 1H, H-3), 3.70 (br d, $J_{5a,5b}$ 12.0 Hz, 1H, H-5b), 3.61 (dd, 1H, H-2); ^{13}C{^{1}H} NMR (126 MHz, D_2O, pH 6): δ 97.8 (C-1), 72.2 (C-3), 71.9 (C-2), 68.1 (C-4), 66.1 (C-5); Coupled ^{13}C–^{1}H HSQC: $^1J_{C\text{-}1,H\text{-}1}$ 163 Hz; ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 7): δ 2.45 (s). HRESIMS$^-$ (free acid): m/z 229.0 $[M-H]^-$.

4.2.10. AMP-*N*-methylimidazolide (18). All steps were conducted under a nitrogen atmosphere. Adenosine 5′-monophosphate (free acid, 35 mg, 0.10 mmol) was stirred in anhyd MeCN (300 μL) in a round-bottomed flask. Anhyd Et_3N (140 μL, 1.01 mmol) and anhyd *N*,*N*-dimethylaniline (50 μL, 0.40 mmol) were added and the resulting mixture was cooled to 0–5 °C in an ice-water bath. Trifluoroacetic anhydride (140 μL, 0.99 mmol) and anhyd MeCN (100 μL) were combined in a second round-bottomed flask, similarly cooled to 0–5 °C, and subsequently added to the first heterogeneous mixture via cannula. The ice-water bath was removed and the reaction mixture was stirred for 15 min at rt, after which time all AMP appeared to dissolve and excess trifluoroacetic anhydride and trifluoroacetic acid were carefully removed via concentration of the reaction mixture under reduced pressure. The resulting pale yellow syrup was re-dissolved in anhyd MeCN (100 μL) and cooled to 0–5 °C in an ice-water bath. In a third round-bottomed flask, *N*-methylimidazole (24 μL, 0.30 mmol) and Et_3N (70 μL, 0.51 mmol) were dissolved in anhyd MeCN (100 μL) and cooled to 0–5 °C. The contents of this third flask were then added via cannula to the newly trifluoroacetylated AMP soln and the resulting reaction mixture was stirred at 0–5 °C for 15 min to generate the desired *N*-methylimidazole-activated AMP donor (**18**), which was bright yellow in color. The essentially quantitative formation of this species was confirmed by ^{31}P{^{1}H} NMR as **18** appeared as a singlet with a distinctive −11.0 ppm chemical shift, which was consistent with the NMR data reported by Mohamady and Jakeman.[35] This electrophile was used directly in coupling reactions with various sugar-1-phosphates as described in the preparation of **20**, **22**, **24**, and **26**.

4.2.11. UMP-*N*-methylimidazolide (19). Following the procedure used for the preparation of AMP-*N*-methylimidazolide (**18**), uridine 5′-monophosphate (free acid, 32 mg, 0.10 mmol) was treated to the identical conditions as those described for **18** to prepare UMP-*N*-methylimidazolide (**19**). The essentially quantitative formation of this species was confirmed by ^{31}P{^{1}H} NMR as the UMP-*N*-methylimidazolide donor (**19**) appeared as a singlet with a distinctive −10.8 ppm chemical shift, which was consistent with the NMR data reported by Mohamady and Jakeman[35] as well as Marlow and Kiessling.[33] This electrophile was used directly in coupling reactions with various sugar-1-phosphates as described in the preparation of **21**, **23**, **25**, and **27**.

4.2.12. ADP-α-D-glucose (20). ADP-α-D-glucose (**20**). All synthetic steps were conducted under a nitrogen atmosphere. Adenosine 5′-monophosphate (free acid, 35 mg, 0.10 mmol) was stirred in anhyd MeCN (300 μL) in a round-bottomed flask. Anhyd Et_3N (140 μL, 1.01 mmol) and anhyd *N*,*N*-dimethylaniline (50 μL, 0.40 mmol) were added and the resulting mixture was cooled to 0–5 °C in an ice-water bath. Trifluoroacetic anhydride (140 μL, 0.99 mmol) and anhyd MeCN (100 μL) were combined in a second round-bottomed flask, similarly cooled to 0–5 °C, and subsequently added to the first heterogeneous mixture via cannula. The ice-water bath was removed and the reaction mixture was stirred for 15 min at rt, after which time all AMP appeared to dissolve and excess trifluoroacetic anhydride and trifluoroacetic acid were carefully removed via concentration of the reaction mixture under reduced pressure. The resulting pale yellow syrup was re-dissolved in anhyd MeCN (100 μL) and cooled to 0–5 °C in an ice-water bath. In a third round-bottomed flask, *N*-methylimidazole (24 μL, 0.30 mmol) and Et_3N (70 μL, 0.51 mmol) were dissolved in anhyd MeCN (100 μL) and cooled to 0–5 °C. The contents of this third flask were then added via cannula to the newly trifluoroacetylated AMP soln and the resulting reaction mixture was stirred at 0–5 °C for 15 min to generate the desired *N*-methylimidazole-activated AMP donor (**18**), which was bright yellow in color. The essentially quantitative formation of this species was confirmed by ^{31}P{^{1}H} NMR as **18** appeared as a singlet with a distinctive −11.0 ppm chemical shift. α-D-Glucose-1-phosphate (tributylammonium salt (1.45 equiv), 106 mg, 0.200 mmol) was dissolved in anhyd MeCN (1.0 mL) in a separate round-bottomed flask. 3 Å Molecular sieves were added and the resulting mixture was stirred for 15 min at rt. The soln of AMP-*N*-methylimidazolide was subsequently added via cannula and reaction progress was monitored using ^{31}P{^{1}H} NMR by taking small aliquots of the reaction mixture (~50 μL) and diluting with anhyd MeCN (~500 μL). After 2 h, all

AMP-*N*-methylimidazolide (**18**) had been consumed or degraded and the reaction mixture was quenched with 250 mM aq NH_4OAc (3 mL). The resulting aqueous soln was extracted with HPLC-grade CH_2Cl_2 (3 mL) to remove organic-soluble reaction components. The product-containing aqueous layer was purified via automated C18 reversed-phase ion-pair chromatography using a Biotage® 25M (25 mm × 15 cm) column (solvent system = 100:0 A:B (2 CV), linear gradient to 7:3 A:B (5 CV), 1:1 A:B (5 CV), linear gradient to 7:3 A:B (5 CV), 1:1 A:B (8 CV), where A = an aqueous soln containing 10 mM tributylamine and 30 mM glacial acetic acid and B = HPLC grade MeOH; flow-rate = 25 mL min^{-1}; UV detection at 215 and 254 nm). Fractions containing the desired sugar nucleotide product were adjusted to pH 7 using 1 M aq NH_4OH and concentrated under reduced pressure to a volume of ~5 mL. The sugar nucleotide product was then passed through an Amberlite® IR-120 (H^+) cation exchange column (free acid form) and acidic fractions were immediately neutralized to pH 7 using 1 M aq NH_4OH. Following concentration under reduced pressure, the sugar nucleotide was re-dissolved in water (~2 mL) and lyophilized. Trituration with ethanol followed by vortexing and centrifugation at 4 °C facilitated the successful removal of remaining NH_4OAc. After concentration under reduced pressure, the sugar nucleotide product was re-dissolved in water (~2 mL) and lyophilized a second time to afford **20** as a white solid in 48% yield by UV spectroscopy (λ_{max} 260 nm, $\varepsilon = 1.51 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H and $^{13}C\{^1H\}$ NMR data were consistent with that of Lee and co-workers.[44] $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.1 (d, $J_{P,P}$ 20.8 Hz), −12.7 (d); t_R **20** = 5.58 min (Method A); HRESIMS$^-$ (*m*/*z*): calcd for $C_{16}H_{24}N_5O_{15}P_2$ 588.0750, found 588.0772.

4.2.13. UDP-α-D-glucose (21). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), α-D-glucose-1-phosphate (tributylammonium salt (1.45 equiv), 106 mg, 0.200 mmol) was coupled with UMP-*N*-methylimidazolide (**19**) (~0.10 mmol) to prepare UDP-α-D-glucose (**21**). After purification as described for **20**, UDP-α-D-glucose (**21**, diammonium salt) was obtained as a white solid in 35% yield by UV spectroscopy (λ_{max} 261 nm, $\varepsilon = 1.01 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H and $^{13}C\{^1H\}$ NMR data were consistent with that of Lee and co-workers.[44] $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.1 (d, $J_{P,P}$ 20.8 Hz), −12.8 (d); t_R **21** = 5.49 min (Method A); HRESIMS$^-$ (*m*/*z*): calcd for $C_{15}H_{23}N_2O_{17}P_2$ 565.0477, found 565.0428.

4.2.14. ADP-α-L-rhamnose (22). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), α-L-rhamnose-1-phosphate (**5**) (tributylammonium salt (1.48 equiv), 104 mg, 0.200 mmol) was coupled with AMP-*N*-methylimidazolide (**18**) (~0.10 mmol) to prepare ADP-α-L-rhamnose (**22**). Following the extraction as described for **20**, the product-containing aqueous layer was concentrated under reduced pressure and re-dissolved in aq tributylammonium bicarbonate buffer (~2 mL, 10 mM) in preparation for purification via C18 reversed-phase ion-pair chromatography. The aq tri-butylammonium bicarbonate buffer (10 mM) was prepared by adding tributylamine (Fluka, >99.5% purity, 10 mmol/L) to H_2O and bubbling CO_2 (obtained from the sublimation of dry ice) through the soln, which was cooled in an ice bath, until all tributylamine appeared to dissolve (~3 h). The resulting aq buffer was chilled in a 4 °C fridge if not used directly following its preparation. Automated C18 reversed-phase ion-pair chromatography was performed using a Biotage 25M (25 mm × 15 cm) C18 reversed-phase column (solvent system = 100:0 A:B (2 CV) followed by a linear gradient to 3:2 A:B (15 CV) and a plateau at 3:2 A:B (2 CV), where A = aq tributylammonium bicarbonate buffer (10 mM, pH 6) and B = HPLC grade MeOH; flow-rate of 25 mL/min; UV detection at 215 and 254 nm). Fractions containing the desired sugar nucleotide were combined and concentrated under reduced pressure to ~5 mL in volume. This soln was applied to a column filled with Dowex® 50W-X8 cation exchange resin (Na^+ form, 100–200 mesh) and the sugar nucleotide product was eluted with H_2O. Fractions containing the desired product were concentrated under reduced pressure and lyophilized to afford ADP-α-L-rhamnose (**22**, disodium salt) as a white solid in 25% yield by UV spectroscopy (λ_{max} 260 nm, $\varepsilon = 1.51 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H NMR (500 MHz, D_2O, pH 6): δ 8.55 (s, 1H, H-8), 8.29 (s, 1H, H-2), 6.15 (d, $J_{1',2'}$ 6.0 Hz, 1H, H-1′), 5.43 (dd, $J_{1'',P}$ 7.5 Hz, $J_{1'',2''}$ 1.5 Hz, 1H, H-1″), 4.77 (dd, $J_{2',3'}$ 4.5 Hz, 1H, H-2′), 4.53 (dd, $J_{3',4'}$ 4.0 Hz, 1H, H-3′), 4.40 (m, 1H, H-4′), 4.22 (m, 2H, H-5a′, H-5b′), 4.03 (m, 1H, H-2″), 3.90 (dq, $J_{4'',5''}$ 10.0 Hz, $J_{5'',6''}$ 6.5 Hz, 1H, H-5″), 3.87 (dd, $J_{2'',3''}$ 3.5 Hz, $J_{3'',4''}$ 9.5 Hz, 1H, H-3″), 3.41 (dd, 1H, H-4″), 1.24 (d, 3H, H_3-6″); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O, pH 6): δ 155.0 (C-6), 154.3 (C-2), 150.9 (C-4), 140.9 (C-8), 118.8 (C-5), 96.4 (C-1″), 87.1 (C-1′), 84.1 (C-4′), 74.4 (C-2′), 72.1 (C-4″), 70.5 (C-3′), 70.4 (C-2″), 69.7 (C-5″), 69.6 (C-3″), 65.2 (C-5′), 16.8 (C-6″); $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.4 (d, $J_{P,P}$ 20.2 Hz), −13.8 (d); t_R **22** = 5.67 min (Method A); HRESIMS$^-$ (*m*/*z*): calcd for $C_{16}H_{24}N_5O_{14}P_2$ 572.0800, found 572.0816.

4.2.15. UDP-α-L-rhamnose (23). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), α-L-rhamnose-1-phosphate (**5**) (tributylammonium salt (1.48 equiv), 104 mg, 0.200 mmol) was coupled with UMP-*N*-methylimidazolide (**19**) (~0.10 mmol) to prepare UDP-α-L-rhamnose (**23**). After purification as described for **22**, UDP-α-L-rhamnose (**23**, diammonium

salt) was obtained as a white solid in 30% yield by UV spectroscopy (λ_{max} 261 nm, $\varepsilon = 1.01 \times 10^4\ M^{-1}\ cm^{-1}$). The reported ^1H NMR data are consistent with the selected data reported by Barber and Behrman.[45] ^1H NMR (500 MHz, D_2O, pH 6): δ 7.99 (d, $J_{5,6}$ 8.0 Hz, 1H, H-6), 6.02 (m, 2H, H-1′, H-5), 5.47 (dd, $J_{1'',P}$ 7.5 Hz, $J_{1'',2''}$ 1.5 Hz, 1H, H-1″), 4.41 (m, 2H, H-2′, H-3′), 4.33 (m, 1H, H-4′), 4.25 (m, 2H, H-5a′, H-5b′), 4.08 (m, 1H, H-2″), 3.95 (dq, $J_{4'',5''}$ 9.5 Hz, $J_{5'',6''}$ 6.5 Hz, 1H, H-5″), 3.91 (dd, $J_{2'',3''}$ 3.5 Hz, $J_{3'',4''}$ 10.0 Hz, 1H, H-3″), 3.48 (dd, 1H, H-4″), 1.33 (d, 3H, H_3-6″); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O, pH 6): δ 167.5 (C-4), 152.9 (C-2), 141.5 (C-6), 102.8 (C-5), 96.4 (C-1″), 88.4 (C-1′), 83.2 (C-4′), 73.8 (C-2′), 72.1 (C-4″), 70.5 (C-2″), 70.4 (C-3″), 69.7 (C-5″), 69.6 (C-3′), 65.0 (C-5′), 16.8 (C-6″); $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.5 (d, $J_{P,P}$ 20.4 Hz), −13.9 (d); t_R **23** = 5.58 min (Method A); HRESIMS$^-$ (m/z): calcd for $C_{15}H_{23}N_2O_{16}P_2$ 549.0528, found 549.0485.

4.2.16. ADP-β-L-fucose (24). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), β-L-fucose-1-phosphate (**10**) (tributylammonium salt (1.53 equiv), 106 mg, 0.200 mmol) was coupled with AMP-*N*-methylimidazolide (**18**) (~0.10 mmol) to prepare ADP-β-L-fucose (**24**). After purification as described for **22**, ADP-β-L-fucose (**24**, diammonium salt) was obtained as a white solid in 28% yield by UV spectroscopy (λ_{max} 260 nm, $\varepsilon = 1.51 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H NMR (500 MHz, D_2O, pH 6): δ 8.55 (s, 1H, H-8), 8.30 (s, 1H, H-2), 6.18 (d, $J_{1',2'}$ 6.0 Hz, 1H, H-1′), 4.95 (dd, $J_{1'',P}$ 8.0 Hz, $J_{1'',2''}$ 8.0 Hz, 1H, H-1″), 4.82 (dd, $J_{2',3'}$ 5.5 Hz, 1H, H-2′), 4.59 (dd, $J_{3',4'}$ 3.5 Hz, 1H, H-3′), 4.44 (m, 1H, H-4′), 4.26 (m, 2H, H-5a′, H-5b′), 3.79 (br q, $J_{5'',6''}$ 6.5 Hz, 1H, H-5″), 3.74 (br d, $J_{3'',4''}$ 3.0 Hz, 1H, H-4″), 3.68 (dd, $J_{2'',3''}$ 10.0 Hz, 1H, H-3″), 3.59 (dd, $J_{1'',2''}$ 7.5 Hz, 1H, H-2″), 1.25 (d, 3H, H_3-6″); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O, pH 6): δ 155.7 (C-6), 153.0 (C-2), 149.3 (C-4), 139.9 (C-8), 118.7 (C-5), 98.4 (C-1″), 86.8 (C-1′), 84.0 (C-4′), 74.3 (C-2′), 72.5 (C-3″), 71.5 (C-5″), 71.2 (C-4″), 71.1 (C-2″), 70.5 (C-3′), 65.3 (C-5′), 15.4 (C-6″); $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.1 (d, $J_{P,P}$ 20.8 Hz), −12.9 (d); t_R **24** = 5.84 min (Method A); HRESIMS$^-$ (m/z): calcd for $C_{16}H_{24}N_5O_{14}P_2$ 572.0800, found 572.0794.

4.2.17. UDP-β-L-fucose (25). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), β-L-fucose-1-phosphate (**10**) (tributylammonium salt (1.53 equiv), 106 mg, 0.200 mmol) was coupled with UMP-*N*-methylimidazolide (**19**) (~0.10 mmol) to prepare UDP-β-L-fucose (**25**). After purification as described for **22**, UDP-β-L-fucose (**25**, diammonium salt) was obtained as a white solid in 26% yield by UV spectroscopy (λ_{max} 261 nm, $\varepsilon = 1.01 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H and $^{13}C\{^1H\}$ NMR data were consistent with that of Augé and co-workers.[46] $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.2 (d, $J_{P,P}$ 20.2 Hz), −12.9 (d); t_R **25** = 5.60 min (Method A); HRESIMS$^-$ (m/z): calcd for $C_{15}H_{23}N_2O_{16}P_2$ 549.0528, found 549.0503.

4.2.18. ADP-α-L-arabinose (26). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), α-L-arabinose-1-phosphate (**15**) (tributylammonium salt (1.49 equiv), 102 mg, 0.200 mmol) was coupled with AMP-*N*-methylimidazolide (**18**) (~0.10 mmol) to prepare ADP-α-L-arabinose (**26**). After purification as described for **20**, ADP-α-L-arabinose (**26**, diammonium salt) was obtained as a white solid in 35% yield by UV spectroscopy (λ_{max} 260 nm, $\varepsilon = 1.51 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H NMR (500 MHz, D_2O, pH 6): δ 8.17 (s, 1H, H-8), 7.94 (s, 1H, H-2), 6.03 (d, $J_{1',2'}$ 6.0 Hz, 1H, H-1′), 4.92 (dd, $J_{1'',P}$ 7.5 Hz, $J_{1'',2''}$ 8.0 Hz, 1H, H-1″), 4.81 (dd, $J_{2',3'}$ 5.5 Hz, 1H, H-2′), 4.66 (dd, $J_{3',4'}$ 3.5 Hz, 1H, H-3′), 4.47 (m, 1H, H-4′), 4.32 (m, 2H, H-5a′, H-5b′), 4.04 (m, 1H, H-4″), 4.03 (dd, $J_{4'',5a''}$ 2.5 Hz, $J_{5a'',5b''}$ 12.5 Hz, 1H, H-5a″), 3.83 (m, 2H, H-3″, H-5b″), 3.79 (dd, $J_{2'',3''}$ 10.0 Hz, 1H, H-2″); $^{13}C\{^1H\}$ NMR (126 MHz, D_2O, pH 6): δ 155.7 (C-6), 152.9 (C-2), 149.2 (C-4), 139.9 (C-8), 118.7 (C-5), 98.7 (C-1″), 86.9 (C-1′), 84.0 (C-4′), 74.3 (C-2′), 71.9 (C-2″), 71.2 (C-3″), 71.1 (C-3′), 68.0 (C-4″), 66.5 (C-5″), 65.3 (C-5′); $^{31}P\{^1H\}$ NMR (202 MHz, D_2O, pH 6): δ −11.1 (d, $J_{P,P}$ 20.2 Hz), −13.0 (d); t_R **26** = 5.34 min (Method A); HRESIMS$^-$ (m/z): calcd for $C_{15}H_{22}N_5O_{14}P_2$ 558.0644, found 558.0613.

4.2.19. UDP-α-L-arabinose (27). Following the procedure used for the preparation of ADP-α-D-glucose (**20**), α-L-arabinose-1-phosphate (**15**) (tributylammonium salt (1.49 equiv), 102 mg, 0.200 mmol) was coupled with UMP-*N*-methylimidazolide (**19**) (~0.10 mmol) to prepare UDP-α-L-arabinose (**27**). After purification as described for **20**, UDP-α-L-arabinose (**27**, diammonium salt) was obtained as a white solid in 32% yield by UV spectroscopy (λ_{max} 261 nm, $\varepsilon = 1.01 \times 10^4\ M^{-1}\ cm^{-1}$). ^1H, $^{13}C\{^1H\}$, and $^{31}P\{^1H\}$ NMR data were consistent with that of Ernst and Klaffke.[19] t_R **27** = 5.37 min (Method A); HRESIMS$^-$ (m/z): calcd for $C_{14}H_{21}N_2O_{16}P_2$ 535.0372, found 535.0322.

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Supplementary data

^{1}H, ^{13}C, and ^{31}P spectra for sugar-1-phosphates **5**, **10**, and **15** as well as sugar nucleotides **21**–**27** (45 pages) are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.01.046.

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