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One-Step Synthesis of Sugar Nucleotides

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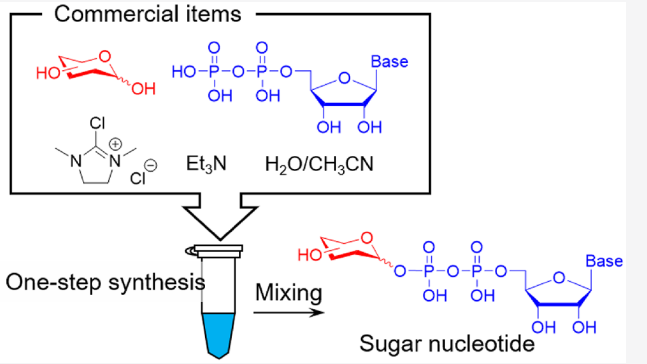
ABSTRACT: The chemical synthesis of sugar nucleotides requires a multistep procedure to ensure a selective reaction. Herein, sugar nucleotides were synthesized in one step using 2-chloro-1,3-dimethylimidazolinium chloride as the condensation reagent. The products were obtained in yields of 12−30%, and the yields were increased to 35−47% by the addition of a tuning reagent. NMR identification of the sugar nucleotides showed that mainly 1,2-*trans*-glycosides were present. The reported method represents a one-step route to sugar nucleotides from commercially available materials.



Oligosaccharides, polysaccharides, and glycoconjugates have attracted the attention of biologists due to their broad structural diversities and their roles in the modulation of a variety of biological processes.[1−3] The biosyntheses of these compounds are catalyzed by glycosyltransferases, which transfer monosaccharide residues in a step-by-step manner, both regio- and stereoselectively.[4,5] Such enzymatic reactions are extremely useful in the syntheses of oligosaccharides because they do not require multistep reactions based on chemical protection, deprotection, and selective glycosylation. To date, over 100 families of glycosyltransferases have been identified and classified based on the sequence similarities of their amino acids. In terms of the glycosyl donor substrates for glycosyltransferases, sugar nucleotides (for example, uridine 5′-diphosphate(UDP)-glucose and UDP-galactose) are consumed as the primary building blocks to build complex oligosaccharides on glycoconjugates. Therefore, sugar nucleotides are essential in the enzymatic glycosylation process catalyzed by glycosyltransferase.[6−8]

To date, the preparation of sugar nucleotides has been conducted via both chemical and enzymatic routes. More specifically, the chemical methods can be divided into two types, viz. pyrophosphate formation by the condensation of a sugar-1-phosphate with an activated nucleoside monophosphate (phosphormorpholidate or diisopropylphosphoramidite)[9−16] and the direct coupling of an activated sugar (mainly a bromide) with a nucleoside diphosphate.[17−20] Although the former reaction is particularly slow and gives poor product yields, these obstacles have been overcome by the addition of 1*H*-tetrazole[10] and metals.[21] However, the nucleoside monophosphate dimer formed as a byproduct in the condensation stage causes issues during purification by silica gel chromatography because the dimer exhibits a polarity similar to that of the desired sugar nucleotide. Moreover, the latter technique requires the use of an unstable glycosyl halide and strict anhydrous conditions, and it leads to an anomeric mixture of sugar nucleotides. However, the resulting products can be easily separated.

In contrast, enzymatic methods can yield the desired sugar nucleotides in a one-pot reaction using multiple enzymes.[22−26] First, a glycokinase catalyzes the formation of a sugar-1-phosphate from the corresponding sugar and a nucleoside triphosphate. Subsequently, a sugar nucleotide pyrophosphorylase constructs the desired sugar nucleoside diphosphate using the sugar-1-phosphate and a nucleoside triphosphate. An inorganic pyrophosphatase, which degrades the pyrophosphate formed in these reactions, is then employed to drive the overall equilibrium of the multienzymatic reaction toward the formation of the desired sugar nucleotide. This system exclusively gives the product exhibiting only the natural-type anomer configuration. However, issues regarding obtaining suitable enzymes, optimization of the enzymatic reactions, and isolation/purification of the products from the reaction mixture remain.

To address these issues related to tedious synthetic methods, the direct reaction of a monosaccharide with a nucleotide using

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Scheme 1. Synthesis of UDP-Glucose 1 via a One-Step Reaction

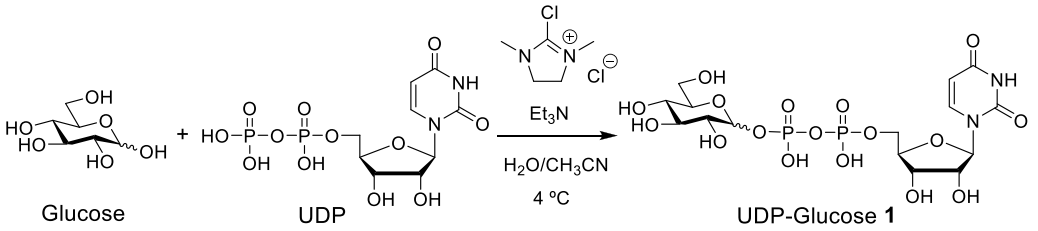
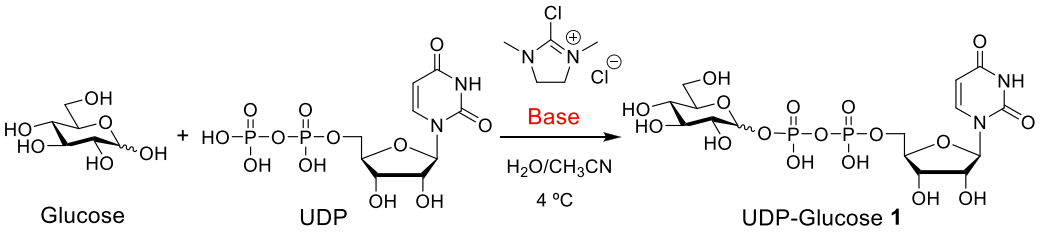


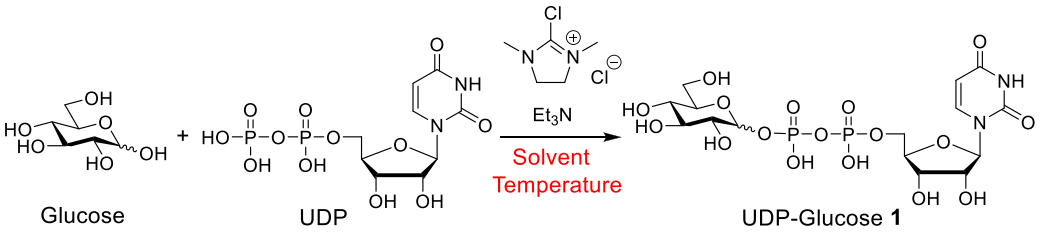
Table 1. Effect of the Base on the Condensation Reaction[a]



entry	base	solvent	yield (%)[b]
1	TEA	H_2O/CH_3CN (1:1)	28
2	DIPEA		17
3	TMA		12
4	imidazole		4.5
5	proton sponge		4.2
6	DMAP		1.3
7	DBU		nd
8	piperidine		nd
9	2,6-lutidine		nd
10	$NaHCO_3$	H_2O/CH_3CN (2:1)	nd
11	$CsCO_3$		nd
12	K_2SO_4		nd
13	$CaCl_2$		nd
14	CH_3COONa		nd

[a]Reaction conditions: glucose (11 μmol), UDP (55 μmol), DMC (36 μmol), and base (220 μmol) in H_2O/CH_3CN (66 μL) were allowed to react 4 °C for 1 h. [b]Determined by HPLC analysis; nd = not detected.

Table 2. Effects of the Reaction Solvent and Temperature on the Condensation Reaction[a]



entry	solvent	temperature (°C)	yield (%)[b]
1	H_2O	4	23
2	H_2O/CH_3CN (6:1)	4	24
3	H_2O/CH_3CN (2:1)	4	29
4	H_2O/CH_3CN (3:2)	4	27
5	H_2O/CH_3CN (2:1)	25	21
6	H_2O/THF (1:1)	4	24
7	H_2O/acetone (1:1)	4	31
8	H_2O/2-propanol (1:1)	4	6.8
9	DMF	4	0.4
10	DMSO	25	nd

[a]Reaction conditions: glucose (11 μmol), UDP (55 μmol), DMC (36 μmol), and TEA (220 μmol) in solvent (66 μL) were allowed to react for 1 h. [b]Determined by HPLC analysis; nd = not detected.

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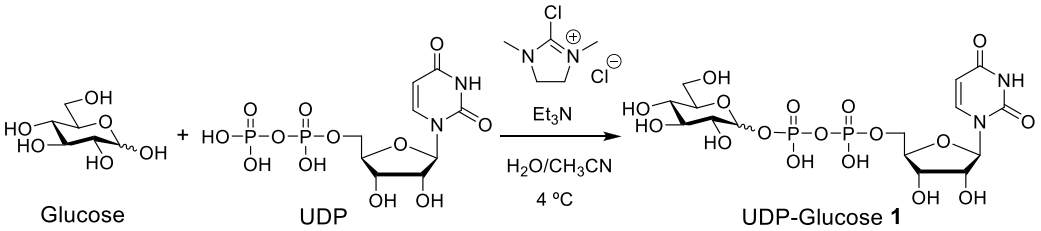
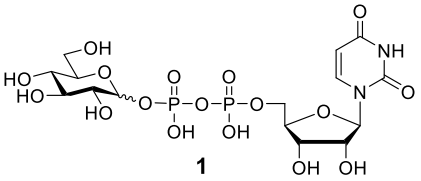
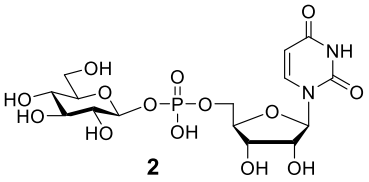
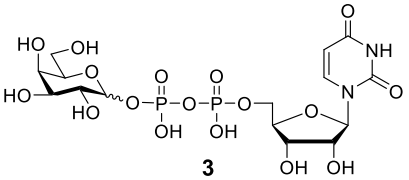
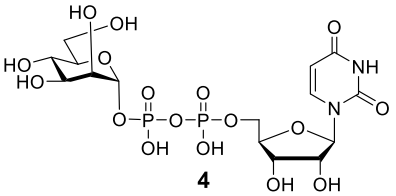
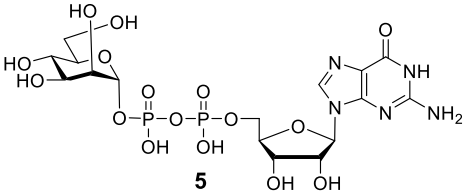
Table 3. Effect of the Number of UDP Equivalents on the Condensation Reaction[a]



entry	UDP (equiv)	TEA (equiv)	yield (%)[b]
1	5	20	29
2	3	15	25
3	1	12	28
4	0.2	10	18

[a]Reaction conditions: glucose (11 μmol), DMC (36 μmol), UDP, and TEA in H_2O/CH_3CN (2:1, 66 μL) were allowed to react at 4 °C for 1 h. [b]Determined by HPLC analysis.

Table 4. Preparation of Sugar Nucleotides Using the Optimized Reaction Conditions

Sugar	Nucleotide	Sugar-nucleotide	Yield (%)	H-1 (ppm)	$J_{1,2}$ (Hz)
Glucose	UDP	 **1**	30 (α : β = 1 : 17)	α 5.62 β 5.03	α 3.6 β 7.9
Glucose	UMP	 **2**	18 (β only)	β 4.93	β 7.6
Galactose	UDP	 **3**	21 (α : β = 1 : 1.9)	α 5.66 β 4.97	α 3.6 β 7.8
Mannose	UDP	 **4**	20 (α only)	α 5.53	α 1.6
Mannose	GDP	 **5**	12 (α only)	5.48	—[a]

[a]The $J_{1,2}$ value of the broadened peak could not be determined.

a condensing agent was investigated and optimized. Herein, we report a simple and novel method for the synthesis of sugar nucleotides in a one-step reaction using commercially available reagents in H_2O/CH_3CN at 4 °C, followed by subsequent silica gel chromatographic separation of the desired products (Scheme 1).

Initially, glucose and UDP were treated with two different condensation reagents, viz. 4-(4,6-dimethoxy-1,3,5-triazin-2-

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yl)-4-methylmorpholinium chloride (DMT-MM)[27,28] and 2-chloro-1,3-dimethylimidazolinium chloride (DMC),[28−31] to promote the nonprotecting glycosidation reaction. These condensation reagents have previously been used for the synthesis of glycosides using unprotected sugars in aqueous solution; thus, they were selected for this condensation reaction, which was also carried out in an aqueous solution to ensure the dissolution of UDP. As indicated by HPLC analysis, the coupling reaction employing DMC proceeded to afford a condensation product, whereas the reaction carried out in the presence of DMT-MM appeared unsuccessful, with no product peak being observed. DMC was therefore selected for use in further reactions.

Subsequently, the effect of the base (20 equiv) was examined, whereby the condensation reaction was conducted using 3 equiv of DMC in H_2O/CH_3CN at 4 °C (Table 1). Due to a constant yield being obtained beyond a reaction time of 1 h, a comparison of the reaction yields was carried out after 1 h. More specially, the reaction using triethylamine (TEA) proceeded most efficiently to give UDP-glucose **1** in 28% yield (Table 1, entry 1). Diisopropyl ethylamine (DIPEA) and trimethylamine (TMA) gave the desired product in moderate yields (Table 1, entries 2 and 3), while the coupling reactions carried out using heterocyclic amines gave low yields (Table 1, entries 4−6), and inorganic bases produced no trace of the product (Table 1, entries 10−14). As a result, TEA was considered the preferred base for this condensation reaction.

The effect of the reaction solvent was then examined, as summarized in Table 2. Due to the presence of highly polar phosphate groups in the structure of UDP, it was poorly soluble in organic solvents, and so the use of DMF, DMSO, and H_2O mixed with various organic solvents was examined. Based on the obtained product yields, a mixture of H_2O/CH_3CN was found to be superior, with a ratio of 2:1 being optimal. In terms of the reaction temperature, the reaction carried out at 4 °C gave improved yields compared to those obtained at 25 °C.

Subsequently, the ratio of glucose to UDP was varied (Table 3), whereby the number of equivalents of UDP to glucose ranged from 5 to 0.2. Using 5, 3, or 1 equiv of UDP, similar product yields were obtained (25−29%), while 0.2 equiv of UDP gave a yield of only 18%. However, HPLC analysis indicated the presence of various byproducts, derived from UDP, which causes issues during purification. Based on the obtained results, the use of 1 equiv of UDP per glucose molecule was considered optimal.

Purification of the reaction mixture was then attempted using silica gel column chromatography as per a previous report.[16] Thus, ethyl acetate/methanol/H_2O was employed as the solvent system, and the fractions were collected. All fractions exhibiting UV absorbance originating from the uracil moiety were analyzed by HPLC, and the fractions containing only the desired product were evaporated and freeze-dried to afford a white powder. Analysis of this powder by NMR spectroscopy indicated that the anomeric configuration of the obtained UDP-glucose **1** was that of the unnatural β-configuration (J = 7.8 Hz). Since the α-isomer is commercially available, it was possible to compare its spectral properties (i.e., ^1H, ^{13}C, and ^{31}P NMR spectra) with those of the obtained product.

The optimized procedure was then applied to a range of substrates to determine the substrate scope of the reaction (Table 4). For example, uridine 5′-monophosphate (UMP) was condensed with glucose to afford UMP-glucose **2** with the β-configuration in 18% yield (Table 4, entry 2). This result indicated that monophosphates can also be coupled easily with sugars using our procedure. Subsequently, galactose was condensed with UDP to give an α- and β-mixture of UDP-galactose **3** in 21% yield, which was a higher α ratio than that obtained for UDP-glucose **1** (Table 4, entry 3). In contrast, UDP-mannose **4**, bearing the same α-linkage as the natural product,[32] was synthesized through the formation of a 1,2-*trans*-glycosidic bond, and guanosine 5′-diphosphate (GDP) was successfully coupled with mannose to afford the natural conformation of GDP-mannose **5**,[33] thereby indicating that GDP was also suitable for application in the condensation reaction.

Finally, to improve the yield of this reaction, DMC was added to the reaction mixture at hourly intervals. The resulting yields of UDP-glucose and GDP-mannose gradually increased from 17 and 10%, respectively, at 30 min, to 47 and 35%, respectively, after 4 h, as shown in Figure 1. The obtained results indicate that the nucleotides are not consumed by side reactions but rather that DMC or activated sugars are hydrolyzed in the aqueous reaction solvent prior to reacting with the nucleotides.

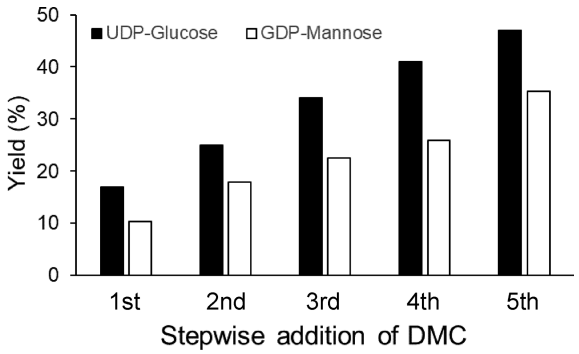

Figure 1. Synthesis of UDP-glucose and GDP-mannose with the stepwise addition of DMC. For UDP-glucose: glucose (12 μmol), UDP (4.6 equiv), DMC (3.0 equiv), and TEA (18.5 equiv) in H_2O/CH_3CN (2:1) were allowed to react at 4 °C. To the resulting solution were added DMC (3.0 equiv) and TEA (5.0 equiv) in H_2O/CH_3CN (2:1) at each step. For GDP-mannose: mannose (12 μmol), GDP (4.1 equiv), DMC (3.2 equiv), and TEA (18.5 equiv) in H_2O/CH_3CN (2:1) were allowed to react at 4 °C. To the solution were added DMC (3.0 equiv) and TEA (5.0 equiv) in H_2O/CH_3CN (2:1) at each step.

In conclusion, a facile synthetic route to sugar nucleotides was developed based on a simple condensation of sugars with nucleotides using DMC as the condensing reagent. The synthesized sugar nucleotides mainly exhibited 1,2-*trans*-glycosidic bonds, which differed from the configuration of natural sugar nucleotides. Although the reaction initially gave a low yield, this was improved by the stepwise addition of DMC to afford UDP-glucose **1** and GDP-mannose **5** in 47 and 35% yields, respectively. Further studies will focus on the application of our developed method for the formation of the 1,2-*cis* bond, which remains an ongoing challenge for carbohydrate chemists.

EXPERIMENTAL SECTION

General. ^1H NMR spectra were recorded in D_2O at 400 MHz using a Bruker AVANCE 400 Plus Nanobay instrument. ^{13}C and ^{31}P

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NMR spectra were recorded in D_2O at 101 and 162 MHz using a Bruker AVANCE 400 Plus Nanobay instrument and a Bruker AVANCE 500 instrument fitted with a cryoprobe, respectively. Chemical shifts (δ) are given in ppm and referenced to the internal solvent signal used as an internal standard. Assignments in the NMR spectra were made by first-order analysis of the spectra and were supported by $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC correlation results. High-resolution mass spectrometry was performed on a Waters Synapt G2-S HDMS spectrometer. Optical rotation measurements were carried out on a Horiba SEPA-300 instrument. Unless otherwise stated, all of the commercially available solvents and reagents were purchased from FUJIFILM Wako Pure Chemical Corporation and Merck KGaA without further purification. During purification by silica gel column chromatography, the absorbances of all fractions were measured at 262 nm using a Shimadzu UV-1280 detector. HPLC analysis was performed on a Shimadzu HPLC system equipped with a TSKgel Amide-80 column (Tosoh Bioscience, 4.6 mm × 250 mm, 5 μm). The HPLC system consisted of a system controller (CBM-20A), a diode array detector (SPD-M20A), a pump (LC-20AD), an autosampler (SIL-20AC), a column oven (CTO-20AC), and a degasser (DGU-20As).

General Procedure for Investigating the Effect of Different Bases (Table 1). The sugar (11 μmol) was dissolved in H_2O/CH_3CN (2:1, 44 μL), and 3.3 equiv of DMC (36 μmol) dissolved in H_2O/CH_3CN (2:1, 22 μL) was added. Subsequently, 10 equiv of the base (220 μmol) and 5.0 equiv of UDP (55 μmol) were added to the solution, and the mixture was stirred until all components had completely dissolved. After stirring at 4 °C for 1 h, the reaction mixture was collected and diluted 100 times with water. The diluted solution was analyzed using HPLC to calculate the product yields.

General Procedure for Investigating the Effect of Different Reaction Solvents (Table 2). The sugar (11 μmol) was dissolved in the desired solvent (2:1, 44 μL), and 3.3 equiv of DMC (36 μmol) dissolved in the desired solvent (2:1, 22 μL) was added. Subsequently, 10 equiv of Et_3N (220 μmol) and 5.0 equiv of UDP (55 μmol) were added to the solution, and the mixture was stirred until all of the components had completely dissolved. After stirring at 4 or 25 °C for 1 h, the reaction mixture was collected and diluted 100 times with water. The diluted solution was analyzed using HPLC to calculate the product yields.

General Procedure for Investigating of the Effect of UDP Equivalents (Table 3). The sugar (11 μmol) was dissolved in H_2O/CH_3CN (2:1, 44 μL), and 3.3 equiv of DMC (36 μmol) dissolved in H_2O/CH_3CN (2:1, 22 μL) was added. Subsequently, Et_3N (220, 165, or 136 μmol) and UDP (55, 34, or 11 μmol) were added to the solution, and the mixture was stirred until all of the components had completely dissolved. After stirring at 4 °C for 1 h, the reaction mixture was collected and diluted 100 times with water. The diluted solution was analyzed using HPLC to calculate the product yields.

Preparation of the Sugar Nucleotides. *Uridine 5′-Diphospho-αβ-D-glucopyranose, 1.* D-Glucose (27 mg, 150 μmol) was dissolved in D_2O/CH_3CN (2:1, 400 μL), and DMC (76 mg, 450 μmol) dissolved in D_2O/CH_3CN (2:1, 200 μL) was added to the resulting solution. Subsequently, Et_3N (251 μL, 1.80 mmol) and UDP (68 mg, 152 μmol) were added to the solution, and the mixture was stirred at 4 °C for 3 days. The reaction mixture was then divided into 351 μL portions for analysis and 500 μL for separation. The 500 μL aliquot was purified by silica gel chromatography using a solvent composition and gradient of 9:2:1 → 8:2:1 → 6:2:1 (v/v/v) ethyl acetate/methanol/H_2O. The absorbance of each fraction was measured at 262 nm, and the combined fractions were evaporated to give UDP-glucose **1** (15 mg, 30%, α/β = 1:17): ^1H NMR (400 MHz, D_2O) δ 7.95 (d, 1.0H, $J_{5'',6''}$ = 8.0 Hz, H-6″), 6.00−5.98 (m, 2.1H, H-1′, H-5′′), 5.62 (dd, 0.1H, $J_{1\alpha,P}$ = 7.2 Hz, $J_{1\alpha,2}$ = 3.6 Hz, H-1α), 5.03 (dd, 1.0H, $J_{1\beta,P}$ = 7.9 Hz, $J_{1\beta,2}$ = 7.9 Hz, H-1β), 4.40−4.36 (m, 2.1H, H-2′, H-3′), 4.29−4.18 (m, 3.2H, H-4′, H-5a′, H-5b′), 3.92 (dd, 1.0H, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a), 3.72 (dd, 1.0H, $J_{5,6b}$ = 6.4 Hz, H-6b), 3.57−3.50 (m, 2.1H, H-3, H-5), 3.42−3.37 (m, 2.1H, H-2, H-4); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 166.2 (C-4″), 151.8 (C-2′′), 141.6 (C-6′′), 102.7 (C-5′′), 97.9 (C-1), 88.4 (C-1′), 83.2 (C-4′), 76.5, 75.2 (C-3, C-5), 73.7 (C-2′), 73.6 (C-2), 69.7 (C-3′), 69.4 (C-4), 65.0 (C-5′), 60.8 (C-6); ^{31}P NMR (162 MHz, D_2O) δ −11.37 (d, $J_{P,P}$ = 16.2 Hz), −13.11 (d); HRMS (ESI/Q-TOF) m/z $[M - H]^-$ calcd for $C_{15}H_{23}N_2O_{17}P_2^-$ 565.0477, found 565.0475; $[\alpha]_D^{26.4}$ −11.7 (10^{-1} deg cm^2 g^{-1}) (c 3.216 × 10^{-3}, H_2O).

Uridine 5′-Monophospho-β-D-glucopyranose, 2. D-Glucose (16 mg, 87 μmol) was dissolved in H_2O/CH_3CN (2:1, 200 μL), and DMC (46 mg, 269 μmol) dissolved in H_2O/CH_3CN (2:1, 150 μL) was added to the resulting solution. Subsequently, Et_3N (148 μL, 1.06 mmol) and UMP (32 mg, 87 μmol) were added to the solution, and the mixture was stirred at 4 °C for 1 day. The reaction mixture was then purified by silica gel chromatography using a solvent composition and gradient of 9:2:1 → 8:2:1 → 6:2:1 → 5:2:1 → 4:2:1 → 3:2:1 (v/v/v) ethyl acetate/methanol/H_2O. The absorbance of each fraction was measured at 262 nm, and the combined fractions were evaporated to give UMP-glucose **2** (9 mg, 18%): ^1H NMR (400 MHz, D_2O) δ 7.95 (d, 1.0H, $J_{5'',6''}$ = 8.1 Hz, H-6″), 6.00−5.95 (m, 2.0H, H-1′, H-5′′), 4.93 (dd, 1.0H, $J_{1,P}$ = 7.6 Hz, $J_{1,2}$ = 7.6 Hz, H-1), 4.39−4.35 (m, 2.0H, H-2′, H-3′), 4.29−4.14 (m, 3.0H, H-4′, H-5a′, H-5b′), 3.92 (dd, 1.0H, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a), 3.74 (dd, 1.0H, $J_{5,6b}$ = 5.6 Hz, H-6b), 3.56−3.49 (m, 2.0H, H-3, H-5), 3.44−3.34 (m, 2.0H, H-2, H-4); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 166.2 (C-4″), 151.8 (C-2′′), 141.6 (C-6′′), 102.5 (C-5′′), 97.8 (C-1), 88.5 (C-1′), 83.2 (C-4′), 76.4, 75.3 (C-3, C-5), 73.7 (C-2′), 73.5 (C-2), 69.7 (C-3′), 69.3 (C-4), 64.8 (C-5′), 60.6 (C-6); ^{31}P NMR (162 MHz, D_2O) δ −1.81 (d, $J_{P,P}$ = 4.86 Hz); HRMS (ESI/Q-TOF) m/z $[M - H]^-$ calcd for $C_{15}H_{22}N_2O_{14}P_1^-$ 485.0814, found 485.0802.

Uridine 5′-Diphospho-αβ-D-galactose, 3. D-Galactose (25 mg, 139 μmol) was dissolved in H_2O/CH_3CN (2:1, 600 μL), and DMC (70 mg, 416 μmol) dissolved in H_2O/CH_3CN (2:1, 50 μL) was added to the resulting solution. Subsequently, Et_3N (232 μL, 1.80 mmol) and UDP (62 mg, 139 μmol) were added to the solution, and the mixture was stirred at 4 °C for 1 d. The reaction mixture was purified by silica gel chromatography using a solvent composition and gradient of 9:2:1 → 8:2:1 → 7:2:1 → 6:2:1 (v/v/v) ethyl acetate/methanol/H_2O. The absorbance of each fraction was measured at 262 nm, and the combined fractions were evaporated. The obtained powders were identified and then collected to afford UDP-galactose **3** (16 mg, 21%, α/β = 1:1.9). UDP-galactose **3** (α:β = 1:13): ^1H NMR (400 MHz, D_2O) δ 7.96 (d, 1.1H, $J_{5'',6''}$ = 8.1 Hz, H-6″), 6.00−5.98 (m, 2.2H, H-1′, H-5′′), 5.66 (dd, 0.1H, $J_{1,P}$ = 7.2 Hz, $J_{1,2}$ = 3.6 Hz, H-1α), 4.97 (br d, 1.0H, $J_{1,P}$ = 7.8 Hz, $J_{1,2}$ = 7.8 Hz, H-1β), 4.41−4.38 (m, 2.2H, H-2′, H-3′), 4.30−4.20 (m, 3.3H, H-4′, H-5a′, H-5b′), 4.04−4.03 (br d, 0.1H, H-4α), 3.94−3.93 (br d, 1.0H, H-4β), 3.85−3.69 (m, 4.2H, H-3β, H-5β, H-6aβ, H-6bβ, H-2α, H-3α), 3.63 (dd, 1.2H, $J_{2,3}$ = 10.0 Hz, H-2β, H-6aα, H-6bα); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 166.3 (C-4″), 151.9 (C-2′′), 141.6 (C-6′′), 102.7 (C-5′′), 98.5 (C-1β), 88.3 (C-1′), 83.3, 83.2 (C-4′), 75.9 (C-5), 73.7 (C-2′), 72.3 (C-3), 71.3, 71.2 (C-2), 69.7 (C-3′), 68.6 (C-4), 65.1, 65.0 (C-5′), 61.2 (C-6), 46.7 (NCH_2), 8.2 (CH_3); ^{31}P NMR (162 MHz, D_2O) δ −11.51 (d, $J_{P,P}$ = 14.6 Hz), −13.82 (d); HRMS (ESI/Q-TOF) m/z $[M - H]^-$ calcd for $C_{15}H_{23}N_2O_{17}P_2^-$ 565.0477, found 565.0478.

Uridine 5′-Diphospho-α-D-mannopyranose, 4. D-Mannose (27 mg, 149 μmol) was dissolved in H_2O/CH_3CN (2:1, 400 μL), and DMC (77 mg, 454 μmol) dissolved in H_2O/CH_3CN (2:1, 200 μL) was added to the resulting solution. Subsequently, Et_3N (251 μL, 1.80 mmol) and UDP (67 mg, 150 μmol) were added to the solution, and the mixture was stirred at 4 °C for 1 day. The reaction mixture was then divided into 351 μL and 500 μL portions for analysis and separation, respectively. The 500 μL portion was purified by silica gel chromatography using a solvent composition and gradient of 9:2:1 → 8:2:1 → 6:2:1 → 5:2:1 → 4:2:1 → 3:2:1 → 2:2:1 (v/v/v) ethyl acetate/methanol/H_2O. The absorbance of each fraction was measured at 262 nm, and the combined fractions were evaporated to give UDP-mannose **4** (10 mg, 20%): ^1H NMR (400 MHz, D_2O) δ 7.96 (d, 1.0H, $J_{5'',6''}$ = 8.0 Hz, H-6″), 6.00−5.97 (m, 2.0H, H-1′, H-5′′), 5.53 (dd, 1.0H, $J_{1,2}$ = 1.6 Hz, $J_{1,P}$ = 7.6 Hz, H-1), 4.39−4.35 (m, 2.0H, H-2′, H-3′), 4.30−4.17 (m, 3.0H, H-4′, H-5a′, H-5b′), 4.06−4.04 (m, 1.0H, H-2), 3.94−3.84 (m, 3.0H, H-3, H-5, H-6a), 3.77 (dd,

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1.0H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6b), 3.69 (dd, 1.0H, $J_{5,6b}$ = 9.6 Hz, H-4); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 166.3 (C-4″), 151.9 (C-2″), 141.6 (C-6″), 102.6 (C-5″), 96.5 (C-1), 88.5 (C-1′), 83.2, 83.1 (C-4′), 73.7 (C-2′, C-5), 70.2 (C-2), 69.8 (C-3), 69.6 (C-3′), 66.4 (C-4), 64.9 (C-5′), 60.8 (C-6); ^{31}P NMR (162 MHz, D_2O) δ −11.56 (d, $J_{P,P}$ = 13.0 Hz), −13.91 (d); HRMS (ESI/Q-TOF) m/z $[M - H]^-$ calcd for $C_{15}H_{23}N_2O_{17}P_2^-$ 565.0477, found 565.0466.

Guanosine 5′-Diphospho-α-D-mannopyranose, 5. D-Mannose (13 mg, 72 μmol) was dissolved in H_2O/CH_3CN (2:1, 400 μL), and DMC (35 mg, 207 μmol) dissolved in H_2O/CH_3CN (2:1, 60 μL) was added to the resulting solution. Subsequently, Et_3N (110 μL, 789 μmol) and GDP (33 mg, 67 μmol) were added to the solution, and the mixture was stirred at 4 °C for 1 h. The reaction mixture was then purified by silica gel chromatography using a solvent composition and gradient of 9:2:1 → 8:2:1 → 7:2:1 → 6:2:1 → 5:2:1 → 4:2:1 → 3:2:1 (v/v/v) ethyl acetate/methanol/H_2O. The absorbance of each fraction was measured at 262 nm, and the combined fractions were evaporated to give GDP-mannose **5** (5 mg, 12%): ^1H NMR (400 MHz, D_2O) δ 8.13 (s, 1.0H, H-8″), 5.92 (d, 1.0H, $J_{1',2'}$ = 5.9 Hz, H-1′), 5.48 (br s, 1.0H, H-1), 4.48 (br t, 1.0H, $J_{3',4'}$ = 3.8 Hz, H-3′), 4.33 (br s, 1.0H, H-4′), 4.18 (br s, 2.0H, H-5a′, H-5b′), 4.01 (br s, 1.0H, H-2), 3.90−3.82 (m, 3.0H, H-3, H-5, H-6a), 3.74−3.62 (m, 2.0H, H-4, H-6b); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 154.0 (C-2″), 96.5 (C-1), 86.9 (C-1′), 83.8 (C-4′), 73.7 (C-2′), 73.6 (C-5), 70.4 (C-3′), 70.2 (C-2), 69.8 (C-4), 66.4 (C-3), 65.3 (C-5′), 60.8 (C-6); ^{31}P NMR (162 MHz, D_2O) δ −11.57 (d, $J_{P,P}$ = 13.0 Hz), −13.92 (dd); HRMS (ESI/Q-TOF) m/z $[M - H]^-$ calcd for $C_{16}H_{24}N_5O_{16}P_2^-$ 604.0699, found 604.0684.

Effect of the Interval Addition of DMC. *Uridine 5′-Diphospho-αβ-D-glucopyranose.* D-Glucose (2.2 mg, 12 μmol) was dissolved in H_2O/CH_3CN (2:1, 44 μL), and DMC (6.0 mg, 36 μmol) dissolved in H_2O/CH_3CN (2:1, 22 μL) was added to the resulting solution. Subsequently, Et_3N (31 μL, 222 μmol) and UDP (25 mg, 56 μmol) were added to the solution, and the mixture was stirred at 4 °C for 30 min. After this time, an aliquot (5.0 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (5.4 mg, 32 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (5.0 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (6.1 mg, 36 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (5.0 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (5.6 mg, 33 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (5 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (5.2 mg, 31 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (5 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. More specifically, the sample aliquots for HPLC analysis were diluted with H_2O/CH_3CN (2:1, 245 μL) prior to injection. The analytical results gave the following yields 30 min = 17%; 90 min = 25%; 150 min = 34%; 210 min = 41%; 270 min = 47%.

Guanosine 5′-Diphospho-α-D-mannopyranose. D-Mannose (2.2 mg, 12 μmol) was dissolved in H_2O/CH_3CN (2:1, 132 μL), and DMC (6.4 mg, 38 μmol) dissolved in H_2O/CH_3CN (2:1, 66 μL) was added to the resulting solution. Subsequently, Et_3N (31 μL, 222 μmol) and GDP (24 mg, 49 μmol) were added to the solution, and the mixture was stirred at 4 °C for 30 min. After this time, an aliquot (10 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (6.6 mg, 39 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (10 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (6.7 mg, 40 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (10 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (6.4 mg, 38 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (10 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. To the remaining reaction mixture was added DMC (6.7 mg, 40 μmol) dissolved in H_2O/CH_3CN (2:1, 10 μL) and Et_3N (9.3 μL, 67 μmol), and the mixture was allowed to stir at 4 °C for a further 60 min. After this time, an aliquot (10 μL) of the reaction mixture was collected for analysis by HPLC and yield determination. More specifically, the sample aliquots for HPLC analysis were diluted with H_2O/CH_3CN (2:1, 240 μL) prior to injection. The analytical results gave the following yields 30 min = 10%; 90 min = 18%; 150 min = 22%; 210 min = 26%; 270 min = 35%.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01943.

^1H, ^{13}C, and ^{31}P NMR, $^1H-^1H$ COSY, $^1H-^{13}C$ HMQC, and ESI-MS spectra for all products (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DMC, 2-chloro-1,3-dimethylimidazolinium chloride; UDP, uridine 5′-diphosphate; UMP, uridine 5′-monophosphate; GDP, guanosine 5′-diphosphate

■ REFERENCES

(1) Bertozzi, C. R.; Kiessling, L. L. Chemical Glycobiology. *Science* **2001**, *291*, 2357−2364.
(2) Gabius, H.-J. *The Sugar Code: Fundamentals of Glycosciences*; Wiley-VCH: Weinheim, Germany, 2011.
(3) Varki, A.; Cummings, R. D.; Esko, J. D.; Stanley, P.; Hart, G. W.; Aebi, M.; Darvill, A. G.; Kinoshita, T.; Packer, N. H.; Prestegard, J. H.; Schnaar, R. L.; Seeberger, P. H. *Essentials of Glycobiology*, 3rd ed.; Cold Spring Harbor Laboratory Press: New York, 2017.
(4) Breton, C.; Šnajdrová, L.; Jeanneau, C.; Koča, J.; Imberty, A. Structures and mechanisms of glycosyltransferases. *Glycobiology* **2006**, *16*, 29R−37R.
(5) Lairson, L. L.; Henrissat, B.; Davies, G. J.; Withers, S. G. Glycosyltransferases: structures, functions, and mechanisms. *Annu. Rev. Biochem.* **2008**, *77*, 521−555.
(6) Sears, P.; Wong, C. H. Toward automated synthesis of oligosaccharides and glycoproteins. *Science* **2001**, *291*, 2344−2350.
(7) Murata, T.; Usui, T. Enzymatic synthesis of oligosaccharides and neoglycoconjugates. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 1049−1059.
(8) Villadsen, K.; Martos-Maldonado, M. C.; Jensen, K. J.; Thygesen, M. B. Chemoselective reactions for the synthesis of glycoconjugates from unprotected carbohydrates. *ChemBioChem* **2017**, *18*, 574−612.
(9) Roseman, S.; Distler, J. J.; Moffatt, J. G.; Khorana, H. G. Nucleoside Polyphosphates. XI. An Improved General Method for the Synthesis of Nucleotide Coenzymes. Syntheses of Uridine-5′, Cytidine-5′ and Guanosine-5′, Diphosphate Derivatives. *J. Am. Chem. Soc.* **1961**, *83*, 659−663.
(10) Wittmann, V.; Wong, C.-H. 1*H*-Tetrazole as Catalyst in Phosphomorpholidate Coupling Reactions: Efficient Synthesis of GDP-Fucose, GDP-Mannose, and UDP-Galactose. *J. Org. Chem.* **1997**, *62*, 2144−2147.
(11) Marlow, A. L.; Kiessling, L. L. Improved Chemical Synthesis of UDP-Galactofuranose. *Org. Lett.* **2001**, *3*, 2517−2519.
(12) Dinev, Z.; Wardak, A. Z.; Brownlee, R. T.; Williams, S. J. A convenient gram-scale synthesis of uridine diphospho ($^{13}C_6$) glucose. *Carbohydr. Res.* **2006**, *341*, 1743−1747.
(13) Gold, H.; van Delft, P.; Meeuwenoord, N.; Codee, J. D. C.; Filippov, D. V.; Eggink, G.; Overkleeft, H. S.; van der Marel, G. A. Synthesis of Sugar Nucleotides by Application of Phosphoramidites. *J. Org. Chem.* **2008**, *73*, 9458−9460.
(14) Wagner, G. K.; Pesnot, T.; Field, R. A. A survey of chemical methods for sugar-nucleotide synthesis. *Nat. Prod. Rep.* **2009**, *26*, 1172−1194.
(15) Tanaka, H.; Yoshimura; Jørgensen, M. R.; Cuesta-Seijo, J. A.; Hindsgaul, O. A Simple Synthesis of Sugar Nucleoside Diphosphates by Chemical Coupling in Water. *Angew. Chem., Int. Ed.* **2012**, *51*, 11531−11534.
(16) Miyagawa, A.; Takeuchi, S.; Itoda, S.; Toyama, S.; Kurimoto, K.; Yamamura, H.; Ito, Y. Chemical synthesis and isolation of UDP-2-deoxy glucose and galactose. *Synth. Commun.* **2016**, *46*, 1790−1795.
(17) Ernst, C.; Klaffke, W. Chemical Synthesis of Uridine Diphospho-D-xylose and UDP-L-arabinose. *J. Org. Chem.* **2003**, *68*, 5780−5783.
(18) Arlt, M.; Hindsgaul, O. Rapid chemical synthesis of sugar nucleotides in a form suitable for enzymic oligosaccharide synthesis. *J. Org. Chem.* **1995**, *60*, 14−15.
(19) Timmons, S. C.; Jakeman, D. L. Stereoselective chemical synthesis of sugar nucleotides via direct displacement of acylated glycosyl bromides. *Org. Lett.* **2007**, *9*, 1227−1230.
(20) Peltier, P.; Daniellou, R.; Nugier-Chauvin, C.; Ferrières, V. Versatile synthesis of rare nucleotide furanoses. *Org. Lett.* **2007**, *9*, 5227−5230.
(21) Dabrowski-Tumanski, P.; Kowalska, J.; Jemielity, J. Efficient and Rapid Synthesis of Nucleoside Diphosphate Sugars from Nucleoside Phosphorimidazolides. *Eur. J. Org. Chem.* **2013**, *11*, 2147−2154.
(22) Roseman, S. Enzymatic synthesis of cytidine 5′-monophospho-sialic acids. *Proc. Natl. Acad. Sci. U. S. A.* **1962**, *48*, 437−441.
(23) Jiang, J.; Biggins, J. B.; Thorson, J. S. A general enzymatic method for the synthesis of natural and "unnatural" UDP-and TDP-nucleotide sugars. *J. Am. Chem. Soc.* **2000**, *122*, 6803−6804.
(24) Oka, T.; Jigami, Y. Reconstruction of de novo pathway for synthesis of UDP-glucuronic acid and UDP-xylose from intrinsic UDP-glucose in *Saccharomyces cerevisiae*. *FEBS J.* **2006**, *273*, 2645−2657.
(25) Chen, Y.; Thon, V.; Li, Y.; Yu, H.; Ding, L.; Lau, K.; Qu, J.; Hie, L.; Chen, X. One-pot three-enzyme synthesis of UDP-GlcNAc derivatives. *Chem. Commun.* **2011**, *47*, 10815−10817.
(26) Muthana, M. M.; Qu, J.; Li, Y.; Zhang, L.; Yu, H.; Ding, L.; Malekan, H.; Chen, X. Efficient one-pot multienzyme synthesis of UDP-sugars using a promiscuous UDP-sugar pyrophosphorylase from *Bifidobacterium longum* (BLUSP). *Chem. Commun.* **2012**, *48*, 2728−2730.
(27) Kunishima, M.; Kawachi, C.; Monta, J.; Terao, K.; Iwasaki, F.; Tani, S. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride: an efficient condensing agent leading to the formation of amides and esters. *Tetrahedron* **1999**, *55*, 13159−13170.
(28) Noguchi, M.; Tanaka, T.; Gyakushi, H.; Kobayashi, A.; Shoda, S. I. Efficient synthesis of sugar oxazolines from unprotected *N*-acetyl-2-amino sugars by using chloroformamidinium reagent in water. *J. Org. Chem.* **2009**, *74*, 2210−2212.
(29) Tanaka, T.; Kikuta, N.; Kimura, Y.; Shoda, S. I. Metal-catalyzed stereoselective and protecting-group-free synthesis of 1,2-*cis*-glycosides using 4,6-dimethoxy-1,3,5-triazin-2-yl glycosides as glycosyl donors. *Chem. Lett.* **2015**, *44*, 846−848.
(30) Tanaka, T.; Matsumoto, T.; Noguchi, M.; Kobayashi, A.; Shoda, S. I. Direct transformation of unprotected sugars to aryl 1-thio-*β*-glycosides in aqueous media using 2-chloro-1,3-dimethylimidazolinium chloride. *Chem. Lett.* **2009**, *38*, 458−459.
(31) Novoa, A.; Barluenga, S.; Serba, C.; Winssinger, N. Solid phase synthesis of glycopeptides using Shoda's activation of unprotected carbohydrates. *Chem. Commun.* **2013**, *49*, 7608−7610.
(32) Nakajima, K.; Kizuka, Y.; Yamaguchi, Y.; Hirabayashi, Y.; Takahashi, K.; Yuzawa, Y.; Taniguchi, N. Identification and characterization of UDP-mannose in human cell lines and mouse organs: Differential distribution across brain regions and organs. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 401−407.
(33) Jensen, J. W.; Schutzbach, J. S. The biosynthesis of oligosaccharide-lipids. Partial purification and characterization of mannosyltransferase II. *J. Biol. Chem.* **1981**, *256*, 12899−12904.

https://dx.doi.org/10.1021/acs.joc.0c01943
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