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Thiosugar nucleotide analogues: synthesis of uridine 5′-(2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranosyl diphosphate)

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Abstract

The synthesis of a novel uridine diphosphate galactose (UDP-Gal) analog, (UDP-2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranose) (**10**) is described. Compound **10** contains a sulfur in the place of oxygen at the 4-position of the galactose moiety. Compound **10** represents a protected form of a novel sugar nucleotide analog that can potentially be used during chemoenzymatic synthesis to modify complex oligosaccharides. © 2001 Published by Elsevier Science Ltd.

Keywords: Sugar nucleotides; β-(1→4)-Galactosyltransferase; Thio sugars

1. Introduction

Modified sugar nucleotide analogs are very useful tools in the regio- and stereoselective preparation of unnatural complex oligosaccharides. They are also useful in probing the active site of glycosyltransferases. Numerous glycosyltransferases that utilize sugar nucleotide donors have been cloned,[1] and very recently some of their crystal structures have been elucidated.[2–4] Recently, Breton et al.[5] analyzed the genetic sequence of several glycosyltransferases and classified them into five families. The specificity of many of these enzymes with regard to their sugar nucleotide donor and sugar acceptor substrates was found to be sufficiently flexible to allow the synthesis of unnatural or 'modified' oligosaccharides.[6] This circumvents the need for the complicated regio- and stereoselective synthesis of modified complex oligosaccharides.

As part of our ongoing effort to prepare modified sugar nucleotides as potential substrates for glycosyltransferases, we report now the synthesis of UDP-2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thiogalactose. The enzyme β-(1→4)-galactosyltransferase (GalT, EC 2.4.1.90) transfers a galactose moiety from the sugar nucleotide donor, uridine diphosphate galactose (UDP-Gal), to 2-acetamido-2-deoxy-D-glucose terminating glycoconjugates to create a new β-(1→4)-glycosidic linkage (Fig. 1). The donor substrate specificity of β-(1→4)-galactosyltransferase has been studied extensively. Several analogs have been prepared by different groups, and many of them were found to be either substrates or inhibitors of β-(1→4)-GalT. The 2-, 3-, 4-, and 6-deoxy

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Gal analogs[7–11] have been shown to serve as substrates for the enzyme, the 2-deoxy[7] being the best analog in this series, whereas the 2-deoxy-2-fluoro[12] was found to be a competitive inhibitor. Likewise, UDP-arabinose (UDP-Ara) which lacks the 6-CH_2OH arm[11] as well as the 6-fluorogalactose derivative[13] are used by the enzyme. Even some O-methylated galactose derivatives of UDP-Gal[14] were used as substrates. Several UDP-Gal analogs, where the ring oxygen was replaced by either sulfur or carbon, have been prepared by different groups. Yuasa et al.[15] reported the synthesis of UDP-5*S*-Gal, which was found to be transferred to a GlcNAc acceptor at a 5% rate compared to UDP-Gal. UDP-5*S*-GalNAc was also prepared and transferred at a 0.23% rate.[16] However, the carbocyclic analog[17] of UDP-Gal was an inhibitor of β-(1→4)-GalT, consistent with the proposed mechanism of this enzyme, which proceeds through an oxocarbonium ion-like transition state.[18]

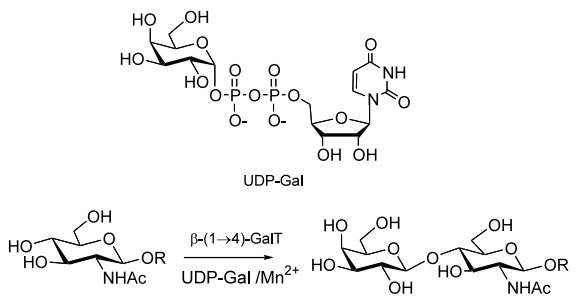
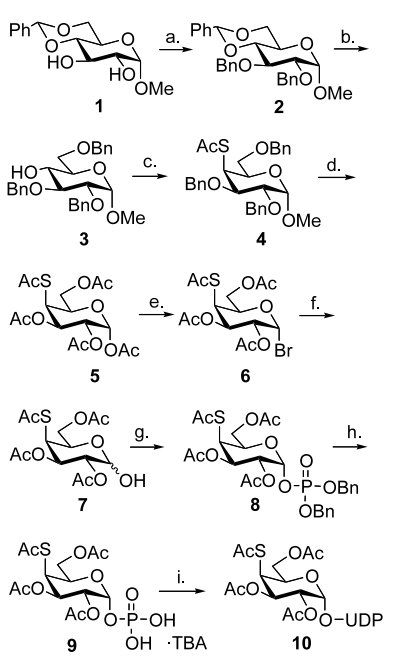


Fig. 1. β-(1→4)-Galactosyltransferase reaction.



Scheme 1. Total synthesis of title compound **10**. Reagents and conditions: (a) BnCl–NaH/DMF. (b) $NaCNBH_3$–HCl/THF. (c) Tfl_2O/Py; KSac/Et(C=O)Me. (d) $FeCl_3$–Ac_2O. (e) $TiBr_4$–CH_2Cl_2. (f) NaOAc–Ag_2CO_3. (g) BuLi/THF, TBPP. (h) Pd–C, Bu_3N, (1→4)-cyclohexadiene/EtOH. (i) UMP-morpholidate (4-morpholidine-*N*,*N*′-dicyclohexylcarbodiimide salt)/DMF.

Several of these analogs have been used to prepare unnatural oligosaccharides. Kajihara et al.[13] transferred UDP-6-deoxy-Gal to asialo agalacto α_1-acid glycoprotein, thus showing the potential application of unnatural sugar nucleotides to remodel the *N*-glycans on a glycoprotein.

Replacing a sugar hydroxyl group with a different atom has been studied, and its effect on some biological properties has been analyzed.[19,20] Sulfur-containing carbohydrates have been either discovered or prepared, and their biological properties are documented.[21] Among the naturally occuring thiosugars are 5-thio-D-mannose,[22] thiolactomycin,[23] salacinol,[24] the glucosinolates[25] such as Sinigrin and Goitrin, and the sulfoquinovoses[26,27] (6-sulfo-α-glucopyranosyl derivatives involved in the biosynthesis of chloroplasts). Our interest in thiol-containing sugar nucleotide analogs arises from the fact that, should the enzyme accept the novel sugar nucleotide analog, then we would be able to utilize the newly introduced thiol moiety to selectively modify the novel complex thiol-containing oligosaccharides. The synthetic approach is described below.

2. Results and discussion

The synthesis of the final product **10** (Scheme 1) started from the commercially available methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**). Benzylation of the 2- and 3-positions gave methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2**), which was followed immediately by a regioselective opening of the benzylidene ring using $NaCNBH_3$–ethereal HCl to give methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**3**). Benzylation at the 2- and 3-positions is required for the subsequent reduction step since a bulky

group at the 3-position is required for the regioselectivity of the ring opening.[28] The ring-opening reaction of the di-O-acetylated derivative of **1** led to several products probably due to acetyl migration. Triflation followed by thioacetylation inverted the configuration at C-4 to give methyl 4-*S*-acetyl-2,3,6-tri-*O*-benzyl-4-thio-α-D-galactopyranoside (**4**). The galacto configuration was confirmed by the splitting pattern of H-5 and H-3, since the H-4 signal was eclipsed in the benzylic proton region. In addition, the ^{13}C NMR spectrum showed the upfield shift of the C-4 signal at 46.64 ppm.[29,30]

Attempted acetolysis of **4** using sulfuric acid–acetic anhydride–acetic acid was shown by previous groups[29,30] to give the 4-thiofuranose derivatives. In our case, the reaction gave a mixture of pyranose and furanose products that could not be chromatographically resolved. Instead, it was found that the cleavage of *O*-benzyl groups using $FeCl_3$[31–33] also effected a concomitant cleavage of the *O*-methyl ether.[34] Treatment of **4** with 8 equivalents of $FeCl_3$ in acetic anhydride at 80 °C for two days gave exclusively the α-anomer of the pentaacetate derivative, **5**. The reaction time proved to be critical (two days instead of one day) for the removal of all benzyl groups (as monitored on TLC). The 3-*O*-benzyl group was found to be the most resistant. Bromination of **5** using $TiBr_4$[35] gave 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranosyl bromide (**6**), which was then hydrolyzed to give an α,β mixture of 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α,β-D-galactopyranose (**7**) in a 2:1 ratio.

Phosphorylation of **7** was achieved using BuLi and tetrabenzylpyrophosphate (TBPP) at low temperature to provide 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranosyl dibenzyl phosphate (**8**) as previously shown by other groups.[7,13,14] Removal of the benzyl groups of **8** was accomplished by transfer hydrogenolysis to give 2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranosyl phosphate mono-tributylammonium salt (**9**). Compound **9** was then coupled to UMP-morpholidate to give uridine 5′-(2,3,6-tri-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranosyl diphosphate) (**10**). Purification of **10** was accomplished using preparative reversed phase (RP) HPLC.

Compound **10** represents the protected form of the sugar nucleotide donor analog UDP-4S-Gal. Removal of the acetyl groups of **10** under basic conditions led to decomposition due to hydrolysis of the phosphodiester linkage. Alternatively, the thiol group could be trapped during the O,S-deacetylation using 2,2′-dithioldipyridine, resulting in a stable, reducible intermediate that is readily deprotected by reduction prior to enzyme transfer. The full details of this procedure will be reported separately along with the results of chemoenzymatic synthesis using this and related analogs.

3. Experimental

General Methods.—^1H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker NMR (AVANCE DRX500 or AVANCE DPX300). Samples in $CDCl_3$ or acetone-d_6 used 1% Me_4Si as an internal standard, whereas samples prepared in CD_3OD, acetone-d_6 or D_2O used acetone as an internal standard. ^{31}P NMR spectra were recorded using neat H_3PO_4 as an external standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. High-resolution mass spectra (HRMS) were recorded using a magnetic sector (Micromass, model 70-250S) by chemical ionization with NH_3. All reactions were monitored by TLC on aluminum sheets precoated with Silica Gel 60 F_{254} (Alltech) (0.2 mm thickness) visualized by UV light or by charring with 10% H_2SO_4 in MeOH. Flash column chromatography was carried out using silica gel (40 μm) (Scientific Adsorbents). All reaction solvents were dried prior to use according to standard procedures. Organic solvents were evaporated on a rotary evaporator under water aspirator vacuum with bath temperatures of 35–40 °C. Whenever anhydrous conditions were required, the reactions were conducted under dry nitrogen, and the reagent transfer was performed using hypodermic syringes. Silica gel chromatography solvents were of HPLC grade. All reagents were purchased from Aldrich. Uridine 5′-monophosphomorpholidate was purchased from Sigma Chemical Co.

Analytical RP-HPLC experiments were carried out using a C_{18} column (Microsorb-MW, 25 cm × 4.6 mm) on an Isco model 2350 pump equipped with a 2360 gradient programmer, while preparative RP-HPLC purification steps were carried out using a Prosphere C_{18} column (Alltech, 25 cm × 22 mm).

Preparation of methyl 2,3,6-tri-O-*benzyl*-α-D-*glucopyranoside (**3**).*—Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (Aldrich) (**1**) (4.0 g, 14.3 mol) was dissolved in 150 mL of dry DMF. NaH (3.0 g, 60% oil suspension) was added to the reaction, and the mixture was kept at 0 °C for 30 min with continuous stirring, then brought to rt, at which time benzyl bromide (3.5 mL) was added dropwise. The reaction was monitored by TLC using 2:1 (v/v) hexane–EtOAc as the mobile phase. The reaction was allowed to proceed for 10 h, and then it was quenched by the addition of MeOH (10 mL). The mixture was washed with hexane (2 × 50 mL), diluted with 400 mL of CH_2Cl_2, washed with 1 N $NaHCO_3$ (2 × 100 mL) and water (2 × 100 mL), concentrated under vacuum pump, and dried for 8 h at 35 °C. The product solidified upon cooling to rt to give 6.2 g (13.6 mmol, 94%) of **2**. ^{1}H NMR ($CDCl_3$, ext. Me_4Si, 300 MHz): δ 7.32–7.48 (m, 15 H, aromatic), 5.58 (s, 1 H, Ph–C*H*–), 4.60–4.95 (m, 5 H, H-1, 4 benzylic), 4.30 (dd, 1 H, H-4, $J_{3,4}$ 9.6, $J_{4,5}$ 10.3), 4.18 (pseudo t, 1 H, H-3, J 9.6), 3.86 (ddd, 1 H, H-5, $J_{4,5}$ 10.3, $J_{5,6}$ 4.1), 3.72 (pseudo t, 1 H, H-6), 3.61 (pseudo t, 1 H, H-6′) 3.58 (dd, 1 H, H-2). 3.40 (s, 3 H, OMe)

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**2**) (4.5 g, 9.7 mmol) was dissolved in 100 mL of dry THF, followed by the addition of sodium cyanoborohydride (8.0 g, 0.13 mol). The reaction was stirred at rt until the sodium cyanoborohydride was completely dissolved. HCl (1 M in Et_2O) was added dropwise to the reaction at rt until the evolution of gas stopped. (Caution! HCN–extreme poison). The reaction was monitored by TLC using 3:1 (v/v) hexane–EtOAc. After completion, the mixture was diluted with CH_2Cl_2 (400 mL), washed with 1.0 N $NaHCO_3$ (100 mL), water (100 mL) and concentrated. The desired product was obtained using flash chromatography on silica gel (3:1 hexane–EtOAc). Fractions corresponding to the product (detected by charring and UV) were collected and concentrated at 35 °C to give 4.3 g (9.3 mmol, 95%) of **3**. ^{1}H NMR ($CDCl_3$, ext. Me_4Si, 300 MHz): δ 7.28–7.40 (m, 15 H, aromatic), 4.51–5.07 (m, 8 H, H-1, 6 benzylic, 4-OH, $J_{1,2}$ 3.6), 3.88 (pseudo t, 1 H, H-3, J 10.3), 3.58–3.75 (m, 4 H, H-4, H-5, H-6, H-6′), 3.54 (dd, 1 H, H-2, $J_{1,2}$ 3.6, $J_{2,3}$ 10.3), 3.40 (s, 3 H, OMe). ^{13}C NMR (125 MHz): δ 138.73, 137.93, 137.92, 128.60, 128.46, 128.35, 128.12, 128.00, 127.85, 127.62, 98.15 (C-1), 81.38, 79.51, 75.42, 73.55, 73.15, 70.55, 69.77, 69.41, 55.23. HRMS: Calcd, 464.2199; found, 464.2185.

Methyl 2,3,6-tri-O-*benzyl-4*-S-*acetyl-4-thio*-α-D-*galactopyranoside (**4**).*—Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**3**) (3.5 g, 7.6 mmol) was dissolved in 50 mL of dry CH_2Cl_2 along with 4 mL of dry Py. Trifluoromethanesulfonic anhydride (5 mL in 10 mL of dry CH_2Cl_2) was added dropwise while stirring and cooling to −10 °C. After 1 h the reaction was diluted with CH_2Cl_2 (100 mL), washed with 2 M HCl (50 mL), satd $NaHCO_3$ (50 mL), and water (50 mL). The organic extract was then concentrated to give a crude mixture that was used for the next step without further purification.

The mixture was dissolved in 60 mL of 2-butanone containing 2.0 g of potassium thioacetate and then refluxed at 70 °C for 1.5 h. After cooling, the insolubles were removed by filtration, and the filter paper was washed with 2-butanone (30 mL). The combined filtrate and washings were concentrated using a water aspirator at 35 °C to a dry, dark solid which was then purified by flash chromatography using 3:1 (v/v) hexane–EtOAc as the mobile phase to give (2.7 g, 4.9 mmol) of **4** (68%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.27–7.40 (m, 15 H, aromatic), 4.45–4.90 (m, 8 H, 6 benzylic, H-1, H-4), 4.31 (pseudo t, 1 H, H-5, $J_{4,5}$ 1.9, $J_{5,6}$ 6.3, $J_{5,6'}$ 6.8), 4.19 (dd, 1 H, H-3, $J_{3,4}$ 4.3, $J_{2,3}$ 10.3), 3.61 (dd, 1 H, H-6), 3.53 (dd, 1 H, H-6′), 3.47 (dd, 1 H, H-2, $J_{2,3}$ 10.0, $J_{1,2}$ 3.7), 3.43 (s, 3 H, OMe), 2.39 (s, 3 H, SAc). ^{13}C NMR (acetone-d_6, 125 MHz): δ 194.42, 138.43, 138.08, 137.97, 128.37, 128.34, 128.32, 127.95, 127.88, 127.71, 127.65, 127.61, 127.60, 98.54 (C-1), 77.93, 76.14, 72.81, 72.45,

71.21, 70.49, 67.92, 54.43, 47.31, 29.94. HRMS: Calcd, 540.2076; found, 540.2081.

1,2,3,6-Tetra-O-*acetyl-4*-S-*acetyl-4-thio-α-D-galactopyranoside* (**5**).—Methyl 4-*S*-acetyl-2,3,6-tri-*O*-benzyl-4-thio-α-D-galactopyranoside (**4**) (2.1 g, 4.0 mmol) was dissolved in 20 mL of Ac_2O, along with 5.12 g of anhyd $FeCl_3$. The reaction was stirred at 80 °C for 2 days. After cooling, the reaction was quenched with water (5 mL) and evaporated to dryness using a vacuum pump at 35 °C. The residue was redissolved in CH_2Cl_2, and TLC analysis (4:1 v/v toluene–EtOAc) revealed a single major product with R_f 0.45. The organic phase was washed with water, concentrated to dryness using a water aspirator, and then purified using flash chromatography on silica gel, eluting with 4:1 (v/v) toluene–EtOAc to yield 1.1 g of **5** (2.7 mmol, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.31 (d, 1 H, H-1, $J_{1,2}$ 3.7), 5.51 (dd, 1 H, H-3, $J_{3,4}$ 4.3, $J_{2,3}$ 10.8), 5.17 (dd, 1 H, H-2, $J_{1,2}$ 3.7, $J_{2,3}$ 10.8), 4.58 (pseudo t, 1 H, H-5, $J_{4,5}$ 1.8, $J_{5,6}$ 6.3, $J_{5,6'}$ 6.8), 4.40 (dd, 1 H, H-4, $J_{3,4}$ 4.3, $J_{4,5}$ 1.7), 4.08–4.20 (m, 2 H, H-6 and H-6′), 2.41 (s, 3 H, SAc), 2.17, 2.05, 2.02, 1.98 (each s, each 3 H, each OAc). ^{13}C NMR (125 MHz): δ 193.81 (SAc), 170.88, 170.41, 170.17, 169.28 (each OAc), 90.08 (C-1), 69.78 (C-5), 68.15 (C-3), 67.96 (C-2), 63.69 (C-6), 46.64 (C-4), 31.21 (SAc), 21.29, 21.11, 20.91 (3OAc). HRMS: Calcd, 406.0934; found, 406.0913.

2,3,6-Tri-O-*acetyl-4*-S-*acetyl-4-thio-α,β-D-galactopyranose* (**7**).—1,2,3,6-Tetra-*O*-acetyl-4-*S*-acetyl-4-thio-α-D-galactopyranoside (**5**) (930 mg, 2.3 mmol) was dissolved in CH_2Cl_2 (20 mL) and EtOAc (2 mL). $TiBr_4$ (3.12 g, 8.5 mmol) was added, and the reaction mixture was stirred at rt under nitrogen. The progress of the reaction was monitored using TLC (2:1 v/v hexane–EtOAc), which demonstrated a complete conversion in 6 h of the α-acetate into the faster moving α-D-galactopyranosyl bromide (**6**). Sodium acetate (1.2 g) was added, and the reaction mixture was stirred for 15 min. The mixture was then filtered over Celite, and the Celite pad was washed with CH_2Cl_2 (20 mL). The organic phase was then washed with 100 mL of cold water and evaporated to dryness using a water aspirator. The crude product was dissolved in 30 mL of acetone containing 1 mL of water. Silver carbonate (0.64 g) was then added, and the mixture was stirred for 6 h at rt with the exclusion of light. The progress of the reaction was monitored by TLC using 2:1 (v/v) hexane–EtOAc to reveal the formation of one major product (R_f 0.33). The mixture was then filtered at rt and washed with acetone (30 mL), and the solution was evaporated to dryness. Final purification was accomplished using flash chromatography on silica gel (2:1 v/v hexane–EtOAc) to give 430 mg of **7** (1.2 mmol, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.59 (dd, 1 H, H-3α, $J_{3,4}$ 4.3, $J_{2,3}$ 10.7), 5.48 (broad d, 1 H, H-1α, $J_{1,2}$ 3.3), 5.32 (s, 1.5 H, –OH), 5.25 (dd, 0.5 H, H-3β, $J_{3,4}$ 4.3, $J_{2,3}$ 10.0), 4.99 (dd, 1 H, H-2α, $J_{2,3}$ 10.7, $J_{1,2}$ 3.7), 4.88 (dd, 0.5 H, H-2β, $J_{2,3}$ 10.3, $J_{1,2}$ 9.9), 4.66–5.75 (m, 1.5 H, H-5α, H-1β), 4.38 (dd, 1 H, H-4α, $J_{3,4}$ 4.3, $J_{4,5}$ 1.8), 4.32 (dd, 0.5 H, H-4β, $J_{3,4}$ 4.5, $J_{4,5}$ 1.5), 4.11–4.27 (m, 2.5 H, H-6,6′ (α,β), H-5β), 2.41 (s, 3 H, SAc), 2.11, 2.07, 1.99 (each s, each 3 H, each OAc). ^{13}C NMR ($CDCl_3$, 125 MHz) (only α anomer shown): δ 194.03 (SAc), 170.79, 170.64, 170.31 (OAc), 91.18, 70.01, 67.20, 67.00, 64.22, 60.83, 47.33, 31.21 (SAc), 21.49, 21.22, 21.07. HRMS: Calcd, 364.0828; found, 364.0824.

2,3,6-Tri-O-*acetyl-4*-S-*acetyl-4-thio-α-D-galactopyranosyl-1-phosphate mono-tributylammonium salt* (**9**).—Tetrabenzylpyrophosphate (TBPP) was prepared by reacting 2 equiv of dibenzyl phosphate with 1 equiv of DCC in toluene at rt with stirring for 5 h. The dicyclohexylurea was removed by gravity filtration, the filtrate was dried at rt using a vacuum pump, and the TBPP solidified upon freezing as a white powder.

2,3,4-Tri-*O*-acetyl-4-*S*-acetyl-4-thio-α,β-D-galactopyranose (**7**) (210 mg, 0.58 mmol) was dissolved in 4 mL of dry THF. Butyllithium (390 μL, 0.63 mmol, from a 1.6 M solution in hexane) was added to **7** at −78 °C. The mixture was stirred for 5 min, at the end of which time, 974 mg (1.74 mmol) of TBPP (dissolved in 1 mL dry THF) was added dropwise over a period of 5 min. The reaction was then allowed to proceed at −60 °C for 30 min. After being brought to rt, the reaction mixture was diluted with Et_2O (30 mL) and washed with satd $NaHCO_3$ (2 × 10 mL) and water (2 × 10

mL). The organic phase was then dried and purified over silica gel (4 × 10 cm) using 2:1 hexane–EtOAc (v/v, containing 1% v/v Et_3N) as the mobile phase. The fractions (detected by both charring, using MeOH containing 15% H_2SO_4, and UV) corresponding to the product were collected and dried to give 90 mg of **8** (0.14 mmol, 26% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28–7.39 (m, 10 H, aromatic), 5.92 (dd, 1 H, H-1, $J_{1,2}$ 3.5, $J_{1,P}$ 7.1), 5.53 (dd, 1 H, H-3, $J_{2,3}$ 10.3, $J_{3,4}$ 4.3), 4.99–5.11 (m, 5 H, H-2, 4 benzylic), 4.60 (pseudo t, 1 H, H-5, $J_{4,5}$ 1.9, $J_{5,6}$ 6.3, $J_{5,6'}$ 6.8), 4.38 (dd, 1 H, H-4, $J_{4,5}$ 1.9, $J_{3,4}$ 4.4), 4.09 (m, 2 H, H-6, H-6′), 2.41 (s, 3 H, SAc), 1.99, 1.90, 1.89 (each s, each 3 H, 3 OAc). ^{13}C NMR (125 MHz): δ 193.60 (SAc), 170.40, 170.34, 170.14 (each OAc), 135.66, 128.36–129.15, 94.82 (C-1), 70.12, 70.02, 69.40, 68.90, 67.48, 63.72, 46.61 (C-4), 31.18, 21.08, 20.90, 20.79. ^{31}P NMR ($CDCl_3$, 120 MHz): δ −1.55. HRMS: Calcd, 624.1430; found, 624.1439.

The product (50 mg, 80 μmol) was debenzylated by transfer hydrogenolysis in EtOH (2 mL) with 50 mg of 10% Pd–C, 12 μL of Bu_3N, and 0.2 mL of 1,4-cyclohexadiene at 0 °C. The reaction was continuously monitored by TLC using 1:1 (v/v) EtOAc–hexane and 65:35:5 (v/v/v) CH_2Cl_2–MeOH–H_2O. The first benzyl group was removed more rapidly than the second benzyl group, which required the addition of more catalyst (20 mg) every 2 h to achieve complete hydrogenolysis. The mixture was filtered by gravity, the filter paper was washed with EtOH (5 mL), and the solution was dried under vacuum on a water aspirator to give 35 mg of **9** (56 μmol, 70%). ^{1}H NMR (MeOH-d_4, 1% acetone, 300 MHz): δ 5.62 (dd, 1 H, H-1, $J_{1,2}$ 3.6, $J_{1,P}$ 7.9), 5.39 (dd, 1 H, H-3, $J_{2,3}$ 10.3, $J_{3,4}$ 4.3), 4.86 (dt, 1 H, H-2, $J_{1,2}$ 3.6, $J_{2,3}$ 10.3, $J_{2,P}$ 2.2), 4.72 (pseudo t, 1 H, H-5, $J_{4,5}$ 1.2, $J_{5,6}$ 6.3, $J_{5,6'}$ 6.8), 4.39 (pseudo d, 1 H, H-4, $J_{4,5}$ 1.4, $J_{3,4}$ 4.4), 4.05 (m, 2 H, H-6, H-6′), 2.35 (SAc), 2.01, 1.96, 1.91 (3 × OAc) Bu_3N: δ 3.10 (t, 6 H, $CH_3CH_2CH_2CH_2N$), 1.65 (m, 6 H, $CH_3CH_2CH_2CH_2N$), 1.37 (m, 6 H, $CH_3CH_2CH_2CH_2N$), 0.92 (t, 9 H, $CH_3CH_2CH_2CH_2N$). ^{31}P NMR (120 MHz): δ −0.54 ppm. ESIMS Calcd, 443.0; found, 443.0.

Uridine 5′-(2,3,6-tri-O-*acetyl-4*-S-*acetyl-4-thio-α-D-galactopyranosyl diphosphate)* ***(10)***. —UMP-morpholidate (4-morpholine-*N*,*N*′-dicyclohexylcarboximide salt) (100 mg, 0.14 mmol) was coevaporated with dry DMF (3 × 3 mL), and a solution in DMF (3 mL) was added to a solution of **9** (15 mg, 23.8 μmol) in dry DMF (2 mL). (Compound **9** had also been previously dried by coevaporation with dry DMF (3 × 3 mL)). The coupling was performed at rt with stirring under an atmosphere of nitrogen for 16 h. The progress of the reaction was monitored by injecting 0.1 μL of the reaction mixture onto an analytical C_{18} RP-HPLC column and eluting with a gradient of 0% to 35% MeCN in 10 mM ammonium acetate (pH 5.0) buffer over 30 min, while monitoring Abs_{262nm} to detect the product, **10**, eluting at approximately 20 min. The reaction mixture was dried under vacuum using a vacuum pump and redissolved in 3 mL distilled water. Purification was accomplished by injecting 500 μL of the aqueous mixture onto a preparative C_{18} RP-HPLC column. The column was eluted at 10 mL/min with the MeCN–ammonium acetate (pH 5.0) gradient described above as the mobile phase. Fractions corresponding to the product were pooled from multiple runs and repeatedly concentrated under vacuum using a vacuum pump while heating at 30 °C. The residual ammonium acetate was removed by repeated freeze-drying. Analytical chromatographic analysis of the purified product produced a single peak eluting at 20 min using the gradient described above. The conjugation and purification yield was calculated using an ε_{262nm} of 9000 M^{-1} cm^{-1} for uridine. This calculation gave 5.3 μmol of **10** (22%). ^{1}H NMR (D_2O, 500 MHz): δ 7.95 (d, 1 H, H-6 of uridine, $J_{5,6}$ 8.1), 5.91–5.98 (not resolved m, 2 H, H-5 of uridine, H-1 ribose, $J_{5,6}$ 8.1, $J_{1,2}$ 4.6), 5.72 (dd, 1 H, H-1 pyranose, $J_{1,2}$ 4.6, $J_{1,P}$ 7.7), 5.50 (dd, 1 H, H-3 pyranose, $J_{2,3}$ 10.7, $J_{3,4}$ 4.3), 5.01 (dt, 1 H, H-2 pyranose, $J_{2,3}$ 10.3, $J_{2,P}$ 2.2, $J_{1,2}$ 4.6), (obscured by H_2O, H-5), 4.39 (dd, 1 H, H-4 pyranose, $J_{3,4}$ 4.3, $J_{4,5}$ 1.9), 4.32 (m, 2 H, H-2, H-3 furanose), 4.24 (m, 2 H, H-5, H-5′ furanose), 4.15–4.20 (m, 3 H, H-4 furanose, H-6, H-6′ pyranose), 2.42 (s, 3 H, SAc), 2.12, 2.04, 1.97 (each s, each 3 H,

3 × OAc), morpholine signals at 3.75 and 3.42 (each t, each 8 H). ^{31}P NMR (200 MHz): δ −10.32, −12.47 (each d, J 19.6 Hz). ESIMS: Calcd, 749.0 [M − H$^+$], found, 749.0.

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