Synthesis of the carbocyclic analog of uridine 5′-(α-D-galactopyranosyl diphosphate) (UDP-Gal) as an inhibitor of β(1 → 4)-galactosyltransferase

Hideya Yuasa, Monica M. Palcic, and Ole Hindsgaul

Abstract: Uridine 5′-(5a-carba-α-D-galactopyranosyl diphosphate), the carbocyclic analog of UDP-galactose where the ring oxygen of the galactose residue is replaced by a methylene group, was chemically synthesized as a potential inhibitor of galactosyltransferases. It was found to be a competitive inhibitor of UDP-galactose:*N*-acetylglucosamine β(1 → 4)-galactosyltransferase from bovine milk (EC 2.4.1.22) with a K_i value of 58 μM, similar to the K_m value for UDP-galactose (25 μM).

Key words: carbocycles, UDP-5a-carbagalactose, galactosyltransferase, glycosyltransferase inhibitor.

Résumé : La 5′-(5a-carba-α-D-galactopyranosyl diphosphate) uridine, l'analogue carbocyclique de la UDP-galactose dans laquelle l'atome d'oxygène du résidu galactose a été remplacé par un groupe méthylène, a été synthétisée chimiquement comme un inhibiteur potentiel des galactosyltransférases. On a trouvé qu'elle est un inhibiteur compétitif de la UDP-galactose : *N*-acétylglucosamine β(1 → 4)-galactosyltransférase du lait bovin (EC 2.4.1.22) avec une valeur K_i de 58 μM, semblable à la valeur K_m de la UDP-galactose (25 μM).

Mots clés : carboxycles, UDP-5a-carbagalactose, galactosyltransférase, inhibiteur de glycosyltransférase.

[Traduit par la rédaction]

Introduction

Galactosyltransferases (Gal-T) catalyze the transfer of galactose from uridine 5′-diphosphate galactose (UDP-Gal, **1**) to growing oligosaccharide chains. Recent studies have revealed that a number of diseases are accompanied by structural changes of cell surface glycoconjugates; some of these structural changes are due to a change in Gal-T activity (1). For example, the down-regulation of Gal-T has been observed in multiple myeloma (2) and rheumatoid arthritis (3), while the up-regulation has been suggested for some liver diseases (4) and for Burkitt's lymphoma (5). Inhibitors of Gal-T could be important research tools since they might be able to regulate Gal-T activity, thereby clarifying the role of this enzyme in aberrant glycosylation. In the long term, small-molecule glycosyltransferase inhibitors may serve as drugs because they may be able to alter cell-surface glycosylation by decreasing galactosylation.

The unreactive sugar nucleotide analogs **2** (6) and **3** (7), which differ from UDP-Gal (**1**) in the diphosphate portion, have been synthesized previously and their K_i values were estimated to be 97 and 165 μM, respectively. The K_i (32 μM) of UDP is reported to be considerably lower (8), suggesting that an intact diphosphate grouping is preferred for binding. We therefore decided to synthesize the carbocyclic analog **4** of UDP-Gal, an analog expected to be unreactive while retaining an unmodified diphosphate linkage. The analog **4** might also be an inhibitor of UDP-Gal binding proteins such as the UDP-Gal transporter protein. A mutant of a cancer cell line lacking UDP-Gal transport protein does not metastasize (9).

During the course of this study, Toyokuni's group reported the synthesis of the carbocylic analog of guanosine 5′-(β-L-fucopyranosyl diphosphate) (GDP-Fuc) as an inhibitor of fucosyltransferases (FucT) and showed that the inhibitor binds to FucT comparably to GDP-Fuc (10). Here we report the synthesis of **4** and its inhibitory effect toward β(1 → 4)-Gal-T.

Results and discussion

Although two groups have reported the syntheses of 5a-carba-α-D-galactopyranose (11), the procedures used appeared cumbersome in view of the selective protection of hydroxyls required for UDP incorporation at OH-1. We therefore decided to employ the Ferrier reaction in the key step as has been frequently used in the synthesis of carbocycles (for example, see ref. 12). Initial attempts to use the Ferrier reaction for the synthesis of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-*arabino*-hex-5-enopyranoside resulted in decomposition, so we elected to use benzyl groups for hydroxyl protection. Methyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside **5** (13), obtainable in three steps from methyl β-D-galactopyranoside, was the starting material (Scheme 1).

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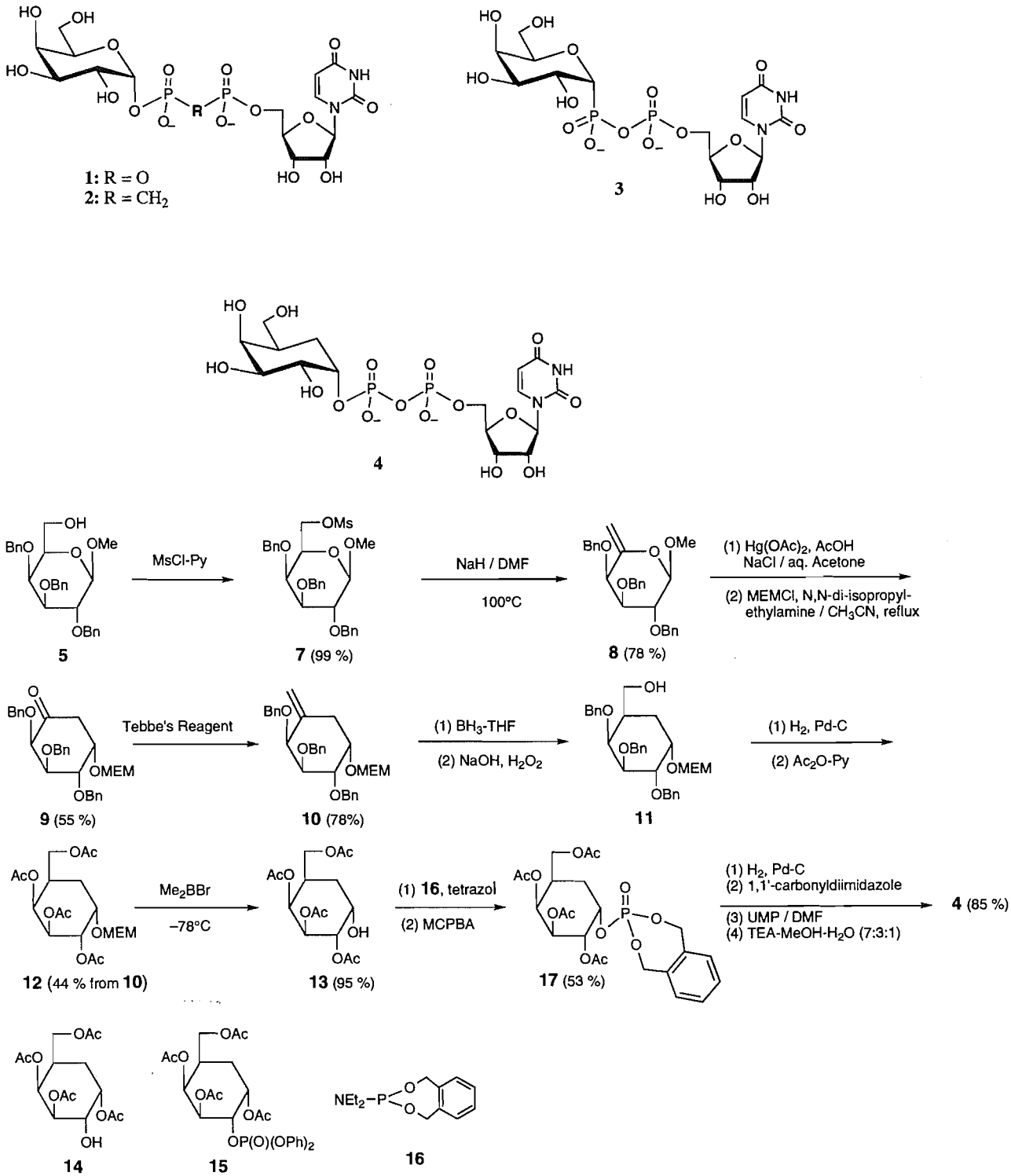
Scheme 1.

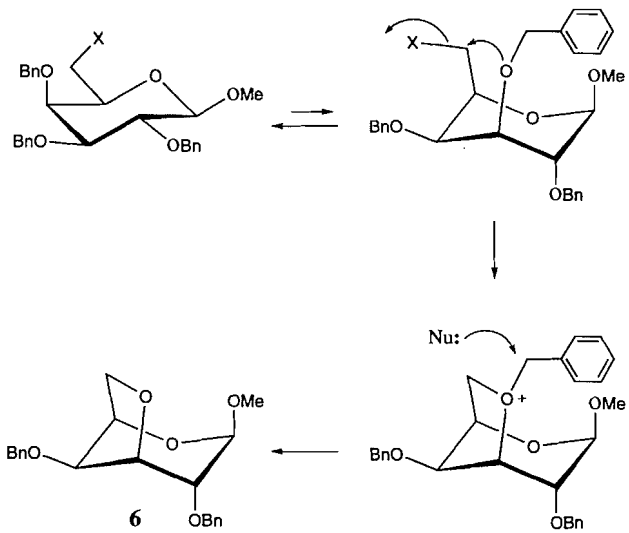


Attempts to introduce reactive leaving groups such as iodide and trifluoromethanesulfonate, for the purpose of producing alkene **8**, resulted instead in cyclization between O-3 and C-6 to give the 3,6-anhydro-sugar **6**, presumably through the mechanism shown in Scheme 2. The mesylate **7**, however, was readily prepared and reacted smoothly with sodium hydride to give **8**.

Application of the Ferrier reaction to **8** was successful and protection of the resultant hydroxyl group as the methoxyethoxymethyl (MEM) ether afforded the cyclohexanone **9**. Conversion of the carbonyl group of **9** into the required exocyclic methylene group was achieved with Tebbe's reagent (14) to give the methylenecyclohexane **10**. Attempted Wittig reaction of **9** with methylenetriphenylphosphorane gave only decomposition products.

The hydroboration of **10** gave a complex mixture contain-

Scheme 2.



ing **11**, which was converted to **12** by hydrogenation (H_2, Pd/C) and acetylation. The coupling constants of the ring protons of **12** ($J_{1,2}$ 3.0, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0, $J_{4,5}$ ~0 Hz) are consistent with the $^4C_1$ conformation. The C-5 epimer would not be expected to adopt this $^4C_1$ conformation. Common reagents used for deprotection of the MEM group in **12**, e.g., $ZnBr_2$ and $TiCl_4$, were unsuccessful, but dimethylboron bromide, developed by Guindon et al. (15), gave a high yield of the deprotected alcohol **13**.

Phosphorylation of the hydroxyl group in **13** was first attempted using diphenylphosphorochloridate and *n*-butyllithium. However, migration of the acetyl group from O-2 to O-1 was the major reaction yielding **14** (12%) and the 2-O-phosphorylated compound **15** (51%). Even pyridine as a solvent was sufficient to cause this migration so a milder method of phosphorylation was required. We therefore employed phosphitylation (16) with 2-dimethylamino-5,6-benzo-1,3-dioxa-2-phosphepane **16** followed by oxidation with *m*-chloroperoxybenzoic acid (MCPBA) to give the desired phosphate **17**. Removal of the benzyl esters of the phosphate by hydrogenation, activation with 1,1′-carbonyldiimidazole, coupling with uridine monophosphate (UMP), and de-O-acetylation then yielded the title compound **4**, which was purified by ion-exchange chromatography.

The K_i value of **4** toward UDP-galactose:N-acetylglucosamine β(1 → 4)-galactosyltransferase from bovine milk (EC 2.4.1.22) was determined to be 58 μM using a previously reported radiochemical "Sep-Pak" assay (17) with 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside as the acceptor. This value is essentially the same as the K_m value determined for UDP-Gal (25 μM) under the same experimental conditions, and the same as the K_i value for UDP-5′-thiogalactose (ref. 18, and unpublished data). To confirm that transfer of "carba-Gal" did not occur, a preparative synthesis was attempted using 20 units of Gal-T, 4.4 mg of compound **4**, and 1.6 mg of acceptor for 5 days at room temperature. No product formation was detected by ^1H NMR spectroscopy. Compound **4** was assumed to be stable under these conditions since UDP-Gal has been successfully used in such long incubations, but this issue was not specifically addressed in this work. Certainly, it is stable in the shorter incubations or unusual kinetic behaviour would have been observed. Under these conditions, less than 1 min would be required for complete galactosylation using the natural donor, UDP-Gal. From these results, the conclusions are that the ring oxygen of galactose is not essential for recognition of UDP-Gal by the enzyme but is essential for transfer to occur. This is consistent with the generally accepted mechanism for glycosyl transfer that involves an oxocarbonium ion-like transition state (19).

Experimental

All solvents and reagents used were reagent grade and, in cases where further purification was required, standard procedures (20) were followed. All solid reactants for moisture-sensitive reactions were dried overnight over phosphorus pentoxide under high vacuum prior to use. Solution transfers where anhydrous conditions were required were done under dry nitrogen using syringes. Molecular sieves were purchased from BDH chemicals. Thin-layer chromatograms (TLC) were performed on precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and visualized by quenching of fluorescence and (or) by charring after spraying with 5% sulfuric acid in ethanol. For silica gel chromatography 40–63 μm (400–230 mesh) silica gel 60 (E. Merck No. 9385) and distilled solvents were used, and the ratio of silica gel to compound was in the range 50:1–100:1. Water was evaporated under the vacuum of an oil pump using a rotary evaporator equipped with a condenser containing acetone and Dry Ice. Organic solvents were removed on a rotary evaporator under the vacuum of a water aspirator with bath temperatures of 40°C or lower. Elemental analyses were performed by the Analytical Service Laboratory of the Chemistry Department.

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded at 300 MHz (Bruker AM-300) with internal tetramethylsilane (δ 0 in $CDCl_3$) or external acetone (δ 2.225 in D_2O) at ambient temperature. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75 MHz (Bruker AM-300) with either internal tetramethylsilane (δ 0 in $CDCl_3$) or external 1,4-dioxane (δ 67.4 in D_2O) as the reference standard. Phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectra were recorded at 108 MHz (Bruker AM-200) with external H_3PO_4 (85%) (δ 0 in D_2O) as the reference standard. The chemical shifts at lower field, relative to external H_3PO_4, are expressed as positive numbers while those at higher field are expressed as negative numbers. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in a 1 dm cell at ambient temperature (23 ± 1°C).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methanesulfonyl-β-D-galactopyranoside (7)

A solution of methyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**5**, 0.488 g, 1.05 mmol) and methanesulfonyl chloride (0.12 mL, 1.55 mmol) in pyridine (10 mL) was kept at room temperature for 2.5 h. Water was added to the reaction mixture, which was then extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$ and concentrated. The residue was chromatographed on silica gel (hexane – ethyl acetate 3:1) to give **7** (0.563 g, 99%) as a white solid: $[\alpha]_D$ −14.2 (*c* 1.5, $CHCl_3$); R_f 0.38 (2:1 hexane – ethyl acetate). ^1H NMR ($CDCl_3$) δ: 7.4–7.25 (m, 15H, Ar), 5.00, 4.62 (each d, 2H, J_{gem} 11.5 Hz, $PhCH_2$), 4.90, 4.75 (each d, 2H, J_{gem} 11.0 Hz,

$PhCH_2$), 4.86, 4.74 (each d, 2H, J_{gem} 12.0 Hz, $PhCH_2$), 4.33 (dd, 1H, $J_{5,6a}$ 7.0, $J_{6a,6b}$ 10.8 Hz, H-6a), 4.29 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.06 (dd, 1H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.82 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), (m, 1H, H-4), 3.65 (ddd, H-1, $J_{4,5}$ 1.0 Hz, H-5), 3.55 (d, 1H, $J_{3,4}$ 3.0 Hz, H-3), 3.55 (s, 3H, OCH_3), 2.93 (s, 3H, SO_2CH_3). Anal. calcd. for $C_{29}H_{34}O_8S$: C 64.19, H 6.32, S 5.91; found: C 63.97, H 6.30, S 6.27.

Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-L-*arabino*-hex-5-enopyranoside (8)

A mixture of **7** (0.362 g, 0.667 mmol) and 50% NaH (75 mg, 1.6 mmol, from which the oil was removed by washing with hexane prior to use) in DMF (8 mL) was stirred for 2 h at 100°C. After cooling, ice-water was added and the mixture was extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (hexane – ethyl acetate 13:1) to give **8** (0.232 g, 78%) as a syrup: $[\alpha]_D$ −9.0 (*c* 2.0, $CHCl_3$); R_f 0.33 (10:1 hexane – ethyl acetate). ^1H NMR ($CDCl_3$) δ: 7.45–7.25 (m, 15H, Ar), 4.85–4.75 (m, 4H, 2 × $PhCH_2$), 4.62 (s, 2H, $PhCH_2$), 4.46, 4.44 (each d, 2H, J_{gem} 6.0 Hz, C=CH_2), 4.43 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1), 4.02 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4), 3.99 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.62 (s, 3H, OCH_3), 3.56 (dd, 1H, H-3). Anal. calcd. for $C_{28}H_{30}$plain$6O_5$: C 75.31, H 6.77; found: C 75.12, H 6.82.

(2*R*)-2,3/4,5-2,3,4-Tribenzyloxy-5-methoxyethoxymethoxycyclohexanone (9)

Enolate **8** (0.591 g, 1.32 mmol) and mercury(II) acetate (0.758 g, 2.38 mmol) were heated to reflux in 40% aqueous acetone (28 mL) containing acetic acid (1%) for 1 h. NaCl (0.758 g, 13 mmol) was added, and the mixture was heated for a further 20 min. After cooling, the mixture was concentrated, and the residue was partitioned between CH_2Cl_2 and water. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (hexane – acetyl acetate 5:2) to give a mixture of (2*R*)-2,3/4,5-2,3,4-tribenzyloxy-5-hydroxycyclohexanone and unknown compounds as a syrup (0.615 g). A mixture of this syrup, 2-methoxyethoxymethyl chloride (0.78 mL, 6.83 mmol), and *N*,*N*-diisopropylethylamine (1.10 mL, 7.85 mmol) in CH_3CN (12 mL) was refluxed for 2 h. The mixture was concentrated and chromatographed on silica gel (hexane – ethyl acetate 3:1–5:2) to give **9** (0.376 g, 55%; R_f 0.39, 2:1 *n*-hexane – ethyl acetate) as a syrup and a mixture of unknown compounds (87 mg; R_f 0.29, the same solvent as above).

9: $[\alpha]_D$ +59.8 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 7.4–7.15 (m, 15H, Ar), 4.83, 4.42 (each d, 2H, J_{gem} 12.5 Hz, $PhCH_2$), 4.75, 4.55 (each d, 2H, J_{gem} 12.5 Hz, $PhCH_2$), 4.72, 4.54 (each d, 2H, J_{gem} 12.0 Hz, $PhCH_2$), 4.71 (s, 2H, OCH_2O), 4.37 (dd, 1H, $J_{2,3}$ 3.0, $J_{2.6a}$ 0.5 Hz, H-2), 4.27 (ddd, 1H, $J_{4,5}$ 3.0, $J_{5,6a}$ 10.0, $J_{5.6b}$ 6.0 Hz, H-5), 3.99 (dd, 1H, $J_{3,4}$ 5.0 Hz, H-3), 3.95 (ddd, 1H, $J_{4,6b}$ 1.0 Hz, H-4), 3.69–3.64, 3.54–3.49 (each m, 4H, OCH_2CH_2O), 3.37 (s, 3H, OCH_3), 2.80 (ddd, 1H, $J_{6a,6b}$ 13.2 Hz, H-6a), 2.73 (ddd, 1H, H-6b); ^{13}C NMR δ: 204.6 (C-1), 138.1, 137.9, 128.4, 128.3, 127.9, 127.8, 127.7 (Ar), 94.6 (OCH_2O), 81.5, 77.9, 76.9, 73.8 (C-2, C-3, C-4, C-5), 73.5, 72.6, 71.7 ($PhCH_2$), 67.2 (OCH_2), 59.1 (OCH_3), 42.6 (C-6). Anal. calcd. for $C_{31}H_{36}O_7$: C 71.52, H 6.97; found: C 71.52, H 6.94.

(1*S*)-1,2/3,4-2,3,4-Tribenzyloxy-1-methoxyethoxymethoxy-5-methylenecyclohexane (10)

To a stirred solution of **9** (0.749 g, 1.44 mmol) in toluene–THF (9/3 mL/mL) containing pyridine (7 μL) was slowly added a 2 M solution of Tebbe's reagent ($Cp_2TiCH_2AlClMe_2$) in toluene (3.08 mL, 6.16 mmol) at −40°C. After stirring for 0.5 h at −40°C, the temperature was slowly increased to room temperature over 1.5 h. After addition of 0.3 M NaOH (0.1 mL) (gas was evolved), Et_2O and $MgSO_4$ were added to the reaction mixture. Insoluble material was removed by filtration with Celite and the filtrate was concentrated. The residue was chromatographed on silica gel (hexane – ethyl acetate 5:2) to give **10** (0.585 g, 78%) as a syrup: $[\alpha]_D$ +5.5 (*c* 1.5, $CHCl_3$); R_f 0.53 (2:1 *n*-hexane – ethyl acetate). ^1H NMR ($CDCl_3$) δ: 7.4–7.25 (m, 15H, Ar), 5.03 (d, 2H, C=CH_2), 4.81–4.34 (m, 8H, 3 × $PhCH_2$ and OCH_2O), 4.12 (m, 2H, H-1 and H-4), 3.98 (dd, 1H, $J_{1,2}$ 3.0, $J_{2,3}$ 9.0 Hz, H-2), 3.83 (dd, 1H, $J_{3,4}$ 3.5 Hz, H-3), 3.70, 3.50 (each m, 4H, OCH_2CH_2O), 3.38 (s, 3H, OCH_3), 2.5–2.35 (m, 2H, H-6a and H-6b), ^{13}C NMR δ: 141.2 (C-5), 139.0, 138.5 (C=CH_2 and Ar), 128.3, 128.2, 127.8, 127.7, 127.4, 127.3 (Ar), 94.8 (OCH_2O), 78.9, 78.7, 73.6 (C-1, C-2, C-3, C-4), 73.3, 72.6, 71.7 ($PhCH_2$), 69.7, 66.9 (OCH_2CH_2O), 59.0 (OCH_3), 33.9 (C-6). Anal. calcd. for $C_{32}H_{38}O_6$: C 74.11, H 7.38; found: C 73.72, H 7.49.

(1*S*)-1,2/3,5-2,3,4-Tribenzyloxy-5-hydroxymethyl-1-methoxyethoxymethoxycyclohexane (11)

To a stirred solution of **10** (1.205 g, 2.32 mmol) in THF (23 mL) was slowly added a 1 M solution of BH_3–THF complex (2.63 mL) at 0°C. The reaction mixture was allowed to warm to 20°C and stirred for 17 h. EtOH (4.8 mL) was added to the mixture at 0°C followed by 3 M NaOH (8.1 mL) and 30% H_2O_2 (4.8 mL) and the mixture was stirred for 5 h at room temperature. After addition of 1 M NaOH, the mixture was extracted three times with Et_2O. The combined organic layers were concentrated and the residue chromatographed on silica gel (hexane – ethyl acetate 1:1) to give **11**, contaminated with unknown impurities (0.940 g; R_f 0.25, 1:1 hexane – ethyl acetate), as a syrup, which was used for the next reaction without further purification.

(1*S*)-1,2/3,4,5-2,3,4-Triacetoxy-5-acetoxymethyl-1-methoxyethoxymethoxycyclohexane (12)

Syrupy **11** (0.94 g), described above, in MeOH (50 mL) was stirred under H_2 (1 atm = 101.3 kPa) in the presence of 5% Pd/C (0.882 g) for 5 h at room temperature, then filtered, and concentrated. The residue was chromatographed on silica gel (CH_2Cl_2–MeOH 8:1). The fractions containing the major product (R_f 0.22, 8:1 CH_2Cl_2–MeOH) were collected and concentrated to give a white solid (0.350 g), which was acetylated with acetic anhydride (3 mL) and pyridine (3 mL). The product obtained after evaporation and aqueous extraction was chromatographed on silica gel (hexane – ethyl acetate 1:1) to give **12** (0.444 g, 44% from **10**) as a syrup: $[\alpha]_D$ +38.9 (*c* 1.1, $CHCl_3$); R_f 0.27 (1:1 hexane – ethyl acetate). ^1H NMR ($CDCl_3$) δ: 5.54 (bs, 1H, H-4), 5.30 (dd, 1H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.0 Hz, H-3), 5.14 (dd, 1H, $J_{1,2}$ 3.0 Hz, H-2), 4.78, 4.74 (each d, 2H, J_{gem} 7.5 Hz, OCH_2O), 4.22 (m, 1H, H-1), 3.96 (dd, 1H, J_{gem} 11.0, J_{vic} 9.5 Hz, C*H*HOAc), 3.87 (dd, 1H, J_{vic} 6.0 Hz, CH*H*OAc), 3.72, 3.54 (each m, 4H, OCH_2CH_2O), 3.38 (s, 3H, OCH_3), 2.51 (m, 1H, H-5), 2.10, 2.06, 2.04, 1.98 (each s, each 3H, OAc), 1.85 (ddt, 1H, $J_{5,6e}$ 3.8, $J_{4,6e}$ 1.5, $J_{6a,6e}$ 1.40 Hz, H-6e), 1.63 (m, 1H, H-6a). Anal. calcd. for $C_{19}H_{30}O_{11}$: C 52.53, H 6.96; found: C 52.45, H 7.04.

(1*S*)-1,2/3,4,5-2,3,4-Triacetoxy-5-acetoxymethyl-1-hydroxycyclohexane (13)

To a stirred solution of **12** (0.165 g, 0.38 mmol) in CH_2Cl_2 (5 mL) was added bromo dimethylborane (0.13 mL, 1.33 mmol) at −78°C. After 1 h, the mixture was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$, and concentrated. The residue was chromatographed on silica gel (hexane – ethyl acetate 1:1) to give **13** (0.125 g, 95%) as a syrup: $[\alpha]_D$ +35.3 (*c* 0.8, $CHCl_3$); R_f 0.5 (1:2 benzene – acetate). ^1H NMR ($CDCl_3$) δ: 5.54 (bs, 1H, H-4), 5.31 (dd, 1H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.0 Hz, H-3), 5.19 (dd, 1H, $J_{1,2}$ 3.0 Hz, H-2), 4.26 (m, 1H, H-1), 3.98 (dd, 1H, J_{gem} 11.0, J_{vic} 9.0 Hz, C*H*HOAc), 3.87 (dd, 1H, J_{vic} 6.0 Hz, CH*H*OAc), 2.60 (m, 1H, H-5), 2.11 (s, 6H, 2 × Ac), 2.04, 1.99 (each s, each 3H, Ac), 1.83 (ddt, 1H, $J_{5,6e} = J_{6e,1}$ 4.0, $J_{4,6e}$ 1.5, $J_{6a,6b}$ 14.0 Hz, H-6e), 1.70 (m, 1H, H-6a). Anal. calcd. for $C_{15}H_{22}O_9$: 52.02, H 6.40; found: C 51.83, H 6.62.

(1*S*)-1,2/3,4,5-2,3,4-Triacetoxy-5-acetoxymethyl-1-(2-oxo-5,6-benzo-1,3,2-dioxaphosphep-2-yloxy)-cyclohexane (17)

To a solution of **13** (165 mg, 0.476 mmol) and tetrazole (107 mg, 1.53 mmol) in CH_2Cl_2 (3 mL) was added 2-dimethylamino-5,6-benzo-1,3-dioxa-2-phosphepane **15** (180 mg, 0.752 mmol) and the mixture was stirred at room temperature for 2 h. The reaction mixture was then cooled to −40°C and the solution of MCPBA (208 mg) in CH_2Cl_2 (3 mL) was added. The stirring was continued for 10 min at −40°C and for another 40 min at 0°C. The reaction mixture was diluted with CH_2Cl_2 and washed consecutively with aqueous $NaHSO_4$ and aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel (hexane – ethyl acetate 1:2) to give **17** (147 mg, 53%) as a foam: $[\alpha]_D$ +53.6 (*c* 0.8, $CHCl_3$); R_f 0.17 (1:2 hexane – ethyl acetate). ^1H NMR ($CDCl_3$) δ: 7.4–7.25 (m, 4H, Ar), 5.57 (m, 1H, H-4), 5.4–5.1 (m, 7H, H-2, H-3, 2 × $ArCH_2$), 4.00 (dd, 1H, J_{gem} 11.5, J_{vic} 9.5 Hz, C*H*HOAc), 3.88 (dd, 1H, J_{vic} 6.0 Hz, CH*H*OAc), 2.12 (s, 6H, 2 × Ac), 2.06, 2.01 (each s, each 3H, Ac), 1.81 (m, 1H, H-6a), 1.62 (m, 1H, H-6b); ^{13}C NMR δ: 170.7, 170.3, 170.2, 170.0 ($COCH_3$), 135.0, 134.9, 129.2, 128.8, 128.75 (Ar), 74.7 (d, $J_{C\text{-}1,P}$ 5.3 Hz, C-1), 69.5, (d, $J_{C\text{-}2,P}$ 3.8 Hz, C-2), 69.2, 68.1 (C-3, C-4), 68.6, 68.5 (2 × $ArCH_2$), 62.7 (CH_2OAc), 32.7 (C-5), 28.0 (C-6), 20.9, 20.7 ($COCH_3$); ^{31}P NMR δ: −0.15. Anal. calcd. for $C_{23}H_{29}O_{12}P$: C 52.28, H 5.53; found: 52.16, H 5.62.

Uridine 5′-(5a-carba-α-D-galactopyranosyl diphosphate) (4)

A solution of **17** (54 mg, 0.102 mmol) in MeOH (4 mL) was stirred under H_2 (1 atm) in the presence of 5% Pd/C (24 mg) for 2 h at room temperature, then filtered and concentrated to give a syrup (R_f 0.70, 60:35:1 dichloromethane–methanol–water), which was dissolved in dry acetone (2 mL) and 1,1′-carbonyldiimidazole (50 mg, 0.246 mmol) was added. The mixture was stirred for 18 h at room temperature. When the reaction was complete, as monitored by TLC (the imidazolide has R_f 0.25, 3:1 $CHCl_3$–MeOH), MeOH (5 μL) was added and stirring continued for a further 20 min. The mixture was concentrated and dried over phosphorus pentoxide in vacuo for 6 h. Meanwhile, uridine 5′-monophosphate disodium salt (74 mg, 0.20 mmol) was passed slowly through a column of Dowex 50 × 8 (H^+) resin and the column was thoroughly washed with water. The eluate was concentrated and trioctylamine (94 μL, 2.15 mmol) was added. The mixture was coevaporated three times with DMF and a solution of the imidazolide in DMF (2.5 mL) was added. The mixture was stirred for 18 h at room temperature and monitored by TLC (the pyrophosphate coupling product has R_f 0.17, 60:40:3 CH_2Cl_2–MeOH–water). The solvent was evaporated and MeOH (3.5 mL), water (1.5 mL), and triethylamine (0.5 mL) were added. After 7 h, the reaction mixture was slowly passed through a column of Dowex 1 × 2-200 (3 × 12 cm, HCO_3^-) and the column was washed with 40 mM ammonium bicarbonate. The product was then eluted using a linear gradient from 40 to 700 mM ammonium bicarbonate (1.1 L) at 23°C. The column was at 23°C at a flow rate of 1.6 mL/min. Each fraction (12 mL) was monitored by UV absorption at 280 nm. Fractions of the product were pooled and concentrated. The residue was dissolved into water and remaining ammonium bicarbonate was removed by adding Dowex 50 × 8 (H^+) until the pH became less than 7.0 (CO_2 gas was evolved). After removing the resin by filtration, the filtrate was concentrated to 5 mL, passed through a Millex-SR 2 μM filter, and lyophilized to give **4** (49 mg, 85%) as a white solid: R_f 0.33 (7:3:1 i-propanol–water – conc. ammonium hydroxide). ^1H NMR (D_2O) δ: 7.97 (d, 1H, $J_{5,6}$ 8.3 Hz, H-6), 5.99 (d, 1H, $J_{1',2'}$ 3.0 Hz, H-1′), 5.98 (d, 1H, H-5), 4.62 (m, 1H, H-1″), 4.09 (m, 1H, H-4″), 3.78 (dd, 1H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 3.0 Hz, H-3″), 3.68 (dt, $J_{1'',2''} = J_{2'',P}$ 3.0 Hz, H-2″), 3.62 (dd, 1H, $J_{5'',6a''}$ 8.0, $J_{6a'',6b''}$ 11.5 Hz, H-6a″), 3.52 (d, 1H, $J_{5'',6b''}$ 6.5 Hz, H-6b″), 2.14 (m, 1H, H-5″), 1.94, 1.55 (each m, 2H, ring methylene); ^{13}C NMR δ: 167.1 (C-4), 152.7 (C-2), 142.5 (C-6), 103.5 (C-5), 89.3 (C-1′), 84.1 (d, $J_{C\text{-}4',P}$ 8.4 Hz, C-4′), 76.5 (d, $J_{C\text{-}1'',P}$ 5.5 Hz, C-1″), 74.6, 72.4, 70.6, 70.5 (C-2′, C-3′, C-3″, C-4″), 65.7 (d, $J_{C\text{-}5',P}$ 5.5 Hz, C-5′), 63.2 (C-6″), 37.1 (C-5″), 27.7 (ring methylene of carbagalactose); ^{31}P NMR δ: −10.8 (d, $J_{P,P}$ 20.6 Hz, P attached to uridine), −11.2 (d, P attached to carbagalactose).

Inhibitory assay

Bovine milk β(1 → 4) galactosyltransferase was isolated by chromatography on UDP-hexanolamine and α-lactalbumin Sepharose (21, 22). Kinetic studies were carried out, as previously described (17), in 0.5 mL plastic microcentrifuge tubes that contained 420 μM 8-methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranoside acceptor, 76 000 dpm of UDP-6[^{3}H] Gal, 0.01–1.1 mM UDP-Gal, 0.5 micro-units of enzyme, 250 mM NaCl, 8 mM $MnCl_2$, and 80 mM sodium cacodylate buffer, pH 7.5, in a total volume of 55 μL. After incubation for 20 min at 37°C, the samples were diluted to 500 μL with water and applied to C-18 reverse-phase cartridges. The cartridges were washed with 50–70 mL of water to removed unreacted donor and then the radiolabelled product eluted with 5 mL of methanol into 20 mL plastic scintillation vials. Ecolite (+) cocktail (10 mL) was added to the vials and the amount of product formed was quantitated by counting in a Beckman LS 1801 scintillation counter. Data were analyzed using nonlinear regression analysis of the Michaelis–Menten equation with Sigma Plot to give a K_m for the donor UDP-Gal of 25 μM under these conditions. The K_i for compound **3** was estimated by carrying out kinetic measurements with same range of donor concentrations with 100 μM of **3** added to all of the assay mixtures. Compound **3** was a competitive inhibitor of the galactosyltransferase with a K_i of 58 μM using the equa-

tion $K_m(app) = K_m(1 + [I]/[K_i]$ where $K_m = K_m$ for UDP-Gal, $K_m(app)$ is the K_m in the presence of inhibitor, and [I] is the inhibitor concentration (100 μMm).

No product was formed in incubations that contained 20 units of galactosyltransferase in 1.0 mL of 100 mM sodium cacodylate buffer, pH 7.5, 4.4 mg of compound **3**, and 1.6 mg of 8-methoxycarbonyl octyl 2-acetamido-2-deoxy-β-D-glucopyranoside acceptor after 5 days of reaction at ambient temperature. Only unreacted acceptor was re-isolated on Sep Pak cartridges as evidenced by ^{1}H NMR spectroscopy.

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