Chapter-2

Materials and Methods
CONTENTS

2.1 General Procedures

2.2 Synthesis of Galactofuranose containing Glycan Core of *Leishmania donovani*

2.2.1 Synthesis of Manα(1,3)Man intermediate

2.2.2 Synthesis of the Galactofuranose intermediate

2.2.3 Synthesis of the Galβ(1,3)Manα(1,3)Man intermediate

2.2.4 Synthesis of Galα(1,6)Gal intermediate

2.2.5 Synthesis of Galα(1,6)Galα(1,3)Gal/intermediate

2.3 Synthesis of the headgroups of the major antigenic GIPLs (iM2, iM3 and iM4) of *Leishmania donovani*

2.3.1 Synthesis of iM2 headgroup

2.3.2 Synthesis of iM3 headgroup

2.3.3 Synthesis of iM4 headgroup

2.4 Synthesis of exogenous substrates and characterization of enzymes involved in the galactofuranose assembly in LPG biosynthesis.

2.4.1 Synthesis of exogenous substrates

2.4.1.1 Synthesis of UDP-α-Galactofuranose

2.4.1.2 Synthesis of UDP-β-Galactofuranose

2.4.2 Synthesis of exogenous acceptor

2.4.3 Characterization of enzymes involved in the galactofuranose assembly in LPG biosynthesis.

2.4.3.1 Culture of *L. donovani* promastigotes

2.4.3.2 Preparation of cell-free system of *L. donovani*

2.4.3.3 Estimation of protein

2.4.3.4 UDP-galactopyranose mutase assay

2.4.3.5 Galactofuranosyl transferase assay
2.1 General Procedures

All the reagents used in chemical syntheses and biochemical analyses were of highest purity grade available. The sources and grades of chemicals/biochemicals have been given with them at appropriate places. All anhydrous solvents were prepared in laboratory using standard procedures. Anhydrous acetonitrile, dichloromethane (CH$_2$Cl$_2$), benzene, diethyl ether, pyridine were obtained by distilling the respective solvents from calcium hydride. Toluene and methanol (MeOH) were dried over sodium metal, tetrahydrofuran was distilled from sodium/benzophenone. Anhydrous dimethylformamide (DMF) was obtained from Spectrochem (HPLC grade). Milli Q UF (Reverse Osmosed, ion exchanged and Ultra-filtered; Millipore Corporation, USA) grade water was used.

For refluxing, an oil-bath (high boiling silicone oil) was used and temperature was controlled by a variostat and a heater. Stirring of the contents of the oil-bath was done using appropriate sized magnetic bars and magnetic stirrer. For maintaining 0 to -10 °C during reactions, ice-salt mixtures in Dewar flasks (Aldrich) was used. For maintaining even lower temperatures of up to -70 °C, mixtures of ethanol-liquid N$_2$ was used. In all these cases and for room temperature reactions, stirring of the reaction mixture was done using appropriate sized magnetic bars and magnetic stirrer (Thermolyne).

For filtration of materials, Celite (Fluka) and Silica Gel (60-120 mesh, SRL) was used. For silica column chromatography, silica gel (60-120 mesh, SRL) was normally used. For flash column chromatography, silica gel (230-400 mesh, SRL) was used. The packing and eluants have been mentioned in appropriate places.

Büchi rotavapor R-114 was used for removing and concentrating solvents, which was connected to either a vacuum aspirator (Brinkmann, B-160) or to high vacuum pump (Vacuubrand, RZ2), and to a water-chiller circulator (LKB Bromma 2219 Multitemp II).

Monitoring of the progress of reactions, analysis of column fractions, identification of reaction intermediates were done by thin layer chromatography on aluminium precoated TLC plates. Glass-backed and aluminium TLC plates (Kieselgel 60 F$_{254}$) were procured from Merck. Various TLC detection systems were used.
1. Iodine vapors: Sublimed iodine crystals (Ranbaxy) were mixed with silica gel in an airtight chamber. When the chamber was full of iodine vapors, the plates were exposed to this.

2. Ultra-violet light: UV absorbing compounds were visualized by a hand-held UV lamp (Spectroline Model ENF-260C/F) employing both long and short wavelength UV.

3. Ammonium molybdate-ceric sulfate reagent: This reagent was prepared by dissolving ammonium molybdate (2.5 g) and ceric sulfate (1 g) in water (90 ml) to which conc. sulfuric acid (10 ml) was added. The plates were immersed into this solution and immediately heated with a heat-gun (Aldrich). Blue or bluish-green spots were observed against nearly white background.

The NMR spectra of compounds were obtained on a 300 MHz (for $^1$H) NMR spectrometer (Avance-DRX 300; Bruker), equipped with a 5 mm quadri-nuclear probe (QNP) and an inverse probe, using XWIN NMR software. The deuterated solvents (CDCl$_3$, CD$_3$OD, D$_2$O etc.; Merck) were used to dissolve the samples and for locking the instrument. Tetramethyl silane (TMS) was used as an internal standard for $^1$H NMR and 80% ortho-phosphoric acid as external reference for $^{31}$P-NMR. The chemical shifts have been expressed in terms of parts per million (ppm, $\delta$) relative to TMS and coupling constants ($J$-values) have been expressed in Hertz (Hz).

Molecular masses of compounds were determined by mass spectrometry. The mass spectra were obtained on a quadrupole mass spectrometer (VG Platform II; VG BioTech, Fisons Instruments, UK) using MassLynx Software. High resolution mass spectra were obtained from Penn State Intercollegiate Mass Spectrometry Center, USA.

Radioactivity operations were carried out in a fume-hood devoted to radiochemical work. Disposable items were discarded at a defined and instructed place as per the instructions for radioactivity disposal. All other necessary precautions for radioactivity handling were taken. Liquid scintillation counting of labeled materials was done using scintillation counter using preset program for individual $\beta$-emitters.
A suitable aliquot in triplicate was mixed with 5 mL scintillation fluid (Cocktail W, SRL) in scintillation vials.

**Reagents generated for different experiments**

1. Benzyl alcohol saturated with hydrogen chloride
   Ammonium chloride (50 g) in 100 mL conc. HCl was stirred in a two necked round bottom flask. Conc. sulphuric acid was added dropwise using a pressure equalizing dropping funnel. The evolved gas (hydrogen chloride gas) was first bubbled through conc. sulphuric acid and then bubbled into benzyl alcohol (kept in an ice bath). This procedure was continued for 30 min. The freshly prepared solution was stored in -20 °C and was found to be stable at this temperature for 5-6 months.

2. Acetonitrile saturated with dimethylamine gas
   Potassium hydroxide was taken in a two-necked round bottom flask. 40% aqueous dimethylamine solution was added dropwise using a pressure equalizing dropping funnel. The evolved dimethylamine gas was bubbled through anhydrous acetonitrile (kept in a -20 °C bath). This procedure was continued for 30 min. The freshly prepared solution was stored in -20 °C and was found to be stable at this temperature for 10-12 months.

3. Phosphate buffer saline (PBS)
   Sodium chloride (8 g, 0.137 mol), potassium chloride (0.2 g, 2.7 mmol), disodium hydrogen phosphate (1.13 g, 8 mmol) and potassium dihydrogen phosphate (0.19 g, 1.4 mmol) were dissolved in water (800 mL). pH was adjusted to 7.2 and volume made upto 1 L.
2.2 Synthesis of Galactofuranose containing Glycan Core of the *Leishmania donovani*

2.2.1 Synthesis of Manα(1,3)Man intermediate

(Scheme-1 & 2 of Results and Discussion)

**Benzyl-\(\alpha\)-D-mannopyranoside (1)**

A suspension of mannose (10 g, 0.055 mol) in benzyl alcohol (140 mL) was heated at 95 °C for 30 min. To this, 10 mL of benzyl alcohol saturated with hydrogen chloride (preparation described in General Procedures) was added. The reaction mixture was stirred for 6 h at 95 °C, during which the suspension had cleared up. The reaction mixture was cooled and added to diethyl ether (800 mL). There was evident turbidity but no precipitation. The solution was kept at -20 °C overnight to obtain a thick precipitate. The solution was filtered and the residue obtained was purified using silica column chromatography (20% MeOH-CH\(_2\)Cl\(_2\)) to provide compound 1 (14 g, 93%); R\(_f\) 0.4 (20% MeOH in CH\(_2\)Cl\(_2\)); \(^1^H\) NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) 7.35-7.30 (m, 5H), 4.69 (s, 1H), 4.49-4.41 (m, 2H), 3.71-3.65 (m, 2H), 3.50-3.41 (m, 4H); \(^1^C\) NMR (DMSO-d\(_6\), 75 MHz) \(\delta\) 138.26, 128.64-126.78, 99.35, 74.61, 71.43, 70.68, 67.86, 67.44, 61.86; ESMS m/z 292.88 (M+Na)

**Benzyl 4,6-\(\alpha\)-benzylidene-\(\alpha\)-D-mannopyranoside (2)**

To a solution of benzyl-\(\alpha\)-D-mannopyranoside (1, 10 g, 0.037 mol) in anhydrous DMF (50 mL) was added pTSA (25 mg) and benzaldehyde dimethyl acetal (6 mL, 0.059 mol). The reaction mixture was stirred at 60 °C for 24 h. The solution was cooled to rt. A solution of sodium bicarbonate (1g in 50 mL) was added. The reaction mixture was then heated at 100 °C till precipitation occurred. The precipitate was filtered. Addition of 50 mL of sodium bicarbonate solution to the filtrate afforded more precipitate. The precipitate was washed well with water and dried under vacuum over anhydrous P\(_2\)O\(_5\) to give compound 2 (10.5 g, 77%), R\(_f\) 0.2 (50% ethyl acetate in hexane); \(^1^H\) NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.50-7.35 (m, 10H), 5.56 (s, 1H), 4.95 (s, 1H), 4.74 (d, \(J = 12\) Hz, 1H), 4.53 (d, \(J = 12\) Hz, 1H), 4.30-4.23 (m, 1H), 4.15-4.05 (m, 2H), 3.95-3.77 (m, 3H); \(^1^C\) NMR (CDCl\(_3\), 75 MHz) \(\delta\)
Mannose pentaacetate (10 g, 0.026 mol) was dissolved in anhydrous acetonitrile saturated with dimethylamine (100 mL) at -20 °C and stirred for 2 h after which TLC confirmed disappearance of the starting material. Excess of dimethylamine was removed under reduced pressure below 30 °C and the reaction mixture concentrated to give the colorless syrupy product 3 (8.5 g, 95.5%). Rf 0.4 (70% ethyl acetate in hexane). 1H NMR (CDCl3, 300 MHz) δ 5.46-5.39 (dd, J = 2.7 Hz, 6.9 Hz, 1H), 5.35-5.23 (m, 3H), 4.30-4.22 (m, 2H), 4.16-4.09 (m, 1H), 2.15 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). ESMS m/z (M+Na)+ 371.34. The data was identical to that reported by Upreti et. al.89

2,3,4,6-tetra-O-acetyl mannopyranosyl trichloroacetimidate (4)
A solution of compound 2,3,4,6-tetra-O-acetyl mannopyranose (3, 8 g, 0.023 mol) in anhydrous CH2Cl2 (70 mL) was stirred at 0 °C. To this solution, trichloroacetonitrile (30 mL, 0.22 mol) was added, followed by dropwise addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2 mL, 0.013 mol). The reaction mixture was stirred at 0 °C for 4 h. The solvent was evaporated under reduced pressure and residue passed through a silica column (20-25% ethyl acetate in hexane) to give pure compound 4 (11 g, 97.3%). Rf 0.4 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 8.78 (s, 1H), 6.28 (d, 1.8 Hz, 1H), 5.47-5.44 (m, 1H), 5.42-5.38 (m, 2H), 4.33-4.09 (m, 3H), 2.19 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H) 13C NMR (CDCl3, 75 MHz) δ 169.45, 168.64, 168.55, 168.35, 159.23, 94.67, 71.56, 68.67, 67.98, 67.67, 65.26, 61.36, 21.23. ESMS m/z (M+Na)+ 515.25. The data was in accordance with that reported by Upreti et. al.89
Benzyl 3-O-(α-D-mannopyranosyl)-4,6-O-benzylidene mannopyranoside (5)

A solution of benzyl 4,6-O-benzylidene-α-D-mannopyranoside (2, 2 g, 5.6 mmol) and donor trichloroacetimidate (4, 3.3 g, 6.7 mmol) in anhydrous diethyl ether (20 mL) and anhydrous CH₂Cl₂ (4 mL) was stirred with freshly activated 4 Å molecular sieves (3 g, Fluka) under Ar atmosphere, at -40 °C for 15 min. Lewis acid catalyst BF₃.Et₂O (2 mL, 0.75 mmol) was slowly added to the cooled solution. The reaction mixture was brought to room temperature and stirring continued for 40 min. The solution was cooled to -5 °C and solid NaHCO₃ added to quench the reaction. Reaction mixture was filtered through celite. The filtrate was concentrated and residue obtained was dissolved in methanol (75 mL). To this solution, sodium methoxide (100 µL, saturated solution in methanol) was added and reaction mixture stirred at room temperature for 2 h. Amberlite 1R-120 (50 mg) was added to neutralize the reaction mixture. The solution was filtered and the filtrate concentrated to give a residue which was purified using silica column chromatography (5% MeOH in ethyl acetate) to yield compound 5 (1.25 g, 43% overall yield). R₇ 0.1 (100% ethyl acetate); ¹H NMR (CD₃OD, 300 MHz) δ 7.37-7.31 (m, 10H), 5.59 (s, 1H), 5.08 (d, J = 1.5 Hz, 1H), 4.72 (d, J = 12 Hz, 1H), 4.57 (s, 1H), 4.56 (d, J = 12 Hz, 1H), 4.17-4.05 (m, 3H), 3.93-3.89 (m, 1H), 3.88-3.62 (m, 8H); ¹³C NMR (CD₃OD, 75 MHz) δ 137.70, 137.21, 129.62-126.01, 102.11, 100.23, 99.23, 78.85, 77.85, 74.99, 72.69, 71.66, 70.99, 68.89, 68.84, 64.99, 63.24; ESMS m/z 543.46 (M+Na)⁺; HRMS (ESMS): calcd for (M+NH₄)⁺ C₂₆H₃₆N₀₁₁ 538.2288; found 538.2284.

Benzyl 3-O-(3-O-p-methoxybenzyl-α-D-mannopyranosyl)-4,6-O-benzylidene-α-D-mannopyranoside (6)

To a solution of benzyl 3-O-(α-D-mannopyranosyl)-4,6-O-benzylidene mannopyranoside (5, 1.5 g, 2.9 mmol) in anhydrous methanol (60 mL) was added dibutyltin oxide (1 g, 4.0 mmol). The reaction mixture was refluxed at 80 °C for 4 h. The solvent was removed under high vacuum and the residue was dried azeotropically with anhydrous toluene. The residue was dissolved in anhydrous THF (35 mL) and anhydrous DMF (3.5 mL). To this solution, p-methoxybenzyl chloride (468 µl, 3.45
mmol), cesium fluoride (0.615 g, 4.0 mmol), potassium iodide (0.675 g, 4.1 mmol), catalytic amounts of tetrabutyl ammonium iodide (TBAI) and tetrabutyl ammonium bromide (TBAB) were added. The reaction mixture was stirred under Ar atmosphere at rt for 36 h. The solution was concentrated and residue purified using silica column chromatography (100% ethyl acetate) to give compound 6 (1.3 g, 75%) Rf 0.25 (100% ethyl acetate); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.47-7.28 (m, 10H), 7.21 (d, J = 8.1 Hz, 2H), 6.80 (d, J = 8.1 Hz, 2H), 5.54 (s, 1H), 5.52 (s, 1H), 4.89 (s, 1H), 4.68 (d, J = 12 Hz, 1H), 4.521-4.44 (m, 3H), 4.21 (d, J = 6 Hz, 1H), 4.15-4.08 (m, 3H), 3.99 (t, J = 1.8 Hz, 1H), 3.89-3.71 (m, 7H), 3.70 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 159.47, 137.71, 137.31, 129.65-129.95, 113.89, 101.57, 100.63, 99.67, 78.80, 77.96, 75.04, 72.69, 71.47, 70.58, 69.34, 68.72, 67.64, 66.09, 63.76, 61.78; ESMS m/z 663.60 ($M+Na$)$^+$; HRMS (ESMS): calcd for ($M+NH_4$)$^+$ C$_{34}$H$_{44}$N$_6$O$_{12}$ 658.2864; found 658.2848.

**Benzyl 2,4,6-tri-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-3-O- p-methoxybenzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (7)**

Benzyl 3-O-(3-O-p-methoxybenzyl-α-D-mannopyranosyl)-4,6-O-benzylidene-α-D-mannopyranoside (6, 450 mg, 0.70 mmol) was dissolved in 1M HCl: acetone (1:9, 10 mL) and the reaction was stirred at 35 °C for 0.5 h. TLC showed complete disappearance of compound 6 to give the debenzylidenedated derivative. The solvent was evaporated using high vacuum. The reaction was made basic by adding a few drops of triethylamine (Et$_3$N). The solvent was concentrated and residue purified by filtration through silica column (30% MeOH in ethyl acetate). The fractions containing the purified debenzylidenedated derivative was dried thoroughly with anhydrous toluene and then with anhydrous pyridine. The dried residue was dissolved in anhydrous Py:anhydrous CH$_2$Cl$_2$ (1:1, 50 mL). The solution was cooled to 0 °C and benzoyl chloride (9 mL) was added dropwise. Catalytic amount of dimethyl aminopyridine (DMAP) was also added. The reaction mixture was allowed to stir at room temperature under Ar atmosphere overnight. The reaction mixture was diluted with CH$_2$Cl$_2$ (150 mL) and extracted sequentially with 0.05 N HCl (100 mL), water (100 mL), saturated NaHCO$_3$ (100 mL) and water (100 mL).
The organic layer was dried with anhydrous Na₂SO₄ and concentrated. The residue was purified using silica column chromatography (20% ethyl acetate in hexane), to afford pure compound 7 (603 mg, 76% overall yield) R_f 0.3 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.15-8.04 (m, 9H), 7.85-7.81 (m, 2H), 7.71-7.66 (m, 2H), 7.59-7.28 (m, 24H), 6.78 (d, J = 8.7 Hz, 2H), 6.43 (d, J = 8.7 Hz, 2H), 5.97 (t, J = 9.9 Hz, 1H), 5.72 (t, J = 8.7 Hz, 1H), 5.66-5.63 (m, 2H), 5.26-5.19 (m, 2H), 5.15 (d, J = 1.2 Hz, 1H), 4.76 (d, J = 12 Hz, 1H), 4.65-4.82 (m, 5H), 4.32-4.07 (m, 5H), 3.98 (d, J = 12 Hz, 1H), 3.88 (dd, J = 6.9 Hz, 1H), 3.65 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 166.06, 166.04, 165.60, 165.57, 164.90, 164.86, 158.83, 136.19, 133.51, 132.83, 129.80, 128.03, 113.41, 99.81, 96.68, 77.10, 76.34, 73.39, 71.82, 70.49, 69.91, 69.68, 68.94, 68.50, 68.29, 67.34, 62.93, 62.57, 54.94; ESMS m/z 1199.79 (M+Na⁺); HRMS (ESMS): calcd for (M+NH₄)⁺ C₆₉H₆₄NO₁₈ 1194.4123; found 1194.4126.

**Benzyl 2,4,6-tri-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (8)**

Benzyl 2,4,6-tri-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-3-O-p-methoxybenzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (7, 585 mg, 0.50 mmol) was dissolved in a cooled solution of 10% TFA in CH₂Cl₂ (30 mL), at 0 °C. The reaction was brought to room temperature and stirring continued for 20 min, after which TLC showed complete disappearance of compound 7. The reaction was quenched by dropwise addition of saturated NaHCO₃ followed by extraction of the organic layer with water. The organic layers were dried with anhydrous Na₂SO₄ and concentrated. The residue was purified using silica column chromatography (30% ethyl acetate in hexane), to afford pure compound 8 (440 mg, 83%) R_f 0.2 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.21-8.02 (m, 10H), 7.89-7.81 (m, 3H), 7.59-7.24 (m, 23H), 6.01 (t, J = 9.3 Hz, 1H), 5.73-5.69 (m, 1H), 5.63 (s, 1H), 5.17 (s, 1H), 5.09-5.05 (m, 1H), 4.78 (d, J = 12.3 Hz, 1H), 4.68-4.57 (m, 4H), 4.46 (dd, J = 5.4 Hz, 7.5 Hz, 1H), 4.40-4.25 (m, 3H), 4.20 (dd, J = 3.3 Hz, 6.6 Hz, 1H); ¹³C NMR (CDCl₃, 75 MHz) δ 166.32, 166.08, 165.97, 165.63, 165.59, 165.07, 136.19, 133.54, 133.37, 133.33, 133.18, 132.94, 132.81, 129.87-128.06, 99.44, 96.71, 76.32, 62.93, 62.57, 54.94; ESMS m/z 1199.79 (M+Na⁺); HRMS (ESMS): calcd for (M+NH₄)⁺ C₆₉H₆₄NO₁₈ 1194.4123; found 1194.4126.
2.2.2 Synthesis of the galactofuranose intermediate
(Scheme-3 of Results and Discussion)

1,2:5,6-di-0-isopropylidene galactofuranose (9)
D-Galactose (15 g, 0.083 mol) was dissolved in hot anhydrous DMF (150 mL). Anhydrous CuSO₄ (75 g, Merck) and acetone (450 mL) were added to this solution. The reaction mixture was refluxed for 24 h, after which another 25 g of anhydrous CuSO₄ and 500 mL acetone was added. Refluxing was continued for another 24 h. The reaction mixture was cooled and filtered through sintered funnel (G1 pore size) to remove copper sulphate. The filtrate was concentrated under high vacuum. Water (250 mL) was added to the residue and extracted with chloroform (5 x 50 mL). The chloroform layers were dried (anhydrous Na₂SO₄). The residue, a mixture of both galactofuranosyl and galactopyranosyl diisopropylidene derivatives (indistinguishable on TLC), was extracted with light petroleum ether (3 x 150 mL) at 35 °C to remove unwanted galactopyranosyl diisopropylidene derivative. The residue was crystallized from diethyl ether-petroleum ether to afford compound 9 (4 g, 18%) Rf 0.25 (40% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 5.86 (d, J = 4.2 Hz, 1H), 4.53 (dd, J = 1 Hz, 3 Hz, 1H), 4.12 (t, J = 4.5 Hz, 1H), 4.09-4.03 (m, 1H), 3.88-3.81 (m, 3H), 1.54 (s, 3H), 1.44 (s, 3H), 1.37 (s, 3H), 1.34 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 104.83, 87.53, 85.81, 76.08, 75.25, 65.57, 27.32, 26.63, 26.44, 25.15; ESMS m/z 282.88 (M+Na)⁺. The data was identical to that reported by Morgenlie.¹⁰⁴

1,2:5,6-di-O-isopropylidene-3-O-benzyl galactofuranose (10)
1,2:5,6-di-O-isopropylidene galactofuranose (9, 3 g, 0.011 mol) was dissolved in anhydrous THF (5 mL) and added dropwise to a stirred solution containing NaH (0.65 g, 0.0165 mmol) in anhydrous THF (2 mL). After the addition of the last alcohol, the solution was refluxed at 70 °C for 2 h. The solution was allowed to cool
and benzyl chloride (1.4 mL, 1.1eq) in anhydrous THF (2.5 mL) was added and solution refluxed overnight. THF was removed, the residue was dissolved in petroleum ether and extracted with water. The ether layers were pooled, dried over anhydrous Na₂SO₄ and concentrated. The residue was purified using silica gel chromatography (10-15% ethyl acetate in hexane) to give pure compound 10 (2.1 g, 52.5%) Rf 0.3 (40% ethyl acetate in hexane) ¹H NMR (CDCl₃, 300 MHz) δ 7.34-7.24 (m, 5H), 5.85 (d, J = 4.0 Hz, 1H), 4.65-4.63 (m, 1H), 4.59 (d, J = 12 Hz, 1H), 4.29-4.14 (m, 3H), 3.99-3.96 (m, 1H), 3.83 (dd, J = 1.2 Hz, 1H), 3.72-3.70 (m, 1H), 1.54 (s, 3H), 1.48 (s, 3H), 1.36 (s, 3H), 1.33 (s, 3H); ESMS m/z (M+Na)⁺ 372.97. The data was in accordance with that reported by Morris et. al.¹⁰⁵

1,2-O-isopropylidene-3-O-benzyl galactofuranose (11)
A solution of 1,2:5,6-di-O-isopropylidene-3-O-benzyl galactofuranose (10, 4 g, 0.011 mol) in 40% acetic acid (10 mL) was allowed to stir at room temperature for 18 h, after which TLC showed disappearance of starting material. The solution was made basic by addition of K₂CO₃. The reaction mixture was extracted with CH₂Cl₂. The organic layers were dried (anhydrous Na₂SO₄) and concentrated and purified using silica column chromatography (20% ethyl acetate in hexane) to give compound 11 (2.71g, 77%) Rf 0.1 (40% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.38-7.28 (m, 5H), 5.91 (d, J = 3.9 Hz, 1H), 4.69-4.65 (m, 1H), 4.62 (s, 1H), 4.55 (d, J = 12 Hz, 1H), 4.15-4.10 (m, 1H), 3.99 (d, J = 1 Hz, 1H), 3.83-3.51 (m, 4H), 2.81 (bs, 1H), 2.12 (bs, 1H), 1.52 (s, 3H), 1.34 (s, 3H); ESMS m/z 332.51 (M+Na)⁺. The data was in accordance with that reported by Morris et. al.¹⁰⁵

1,2-O-isopropylidene-3-O-benzyl -5,6-di-O-acetyl galactofuranose (12)
To a solution of 1,2-O-isopropylidene-3-O-benzyl galactofuranose (11, 350 mg, 1.1 mmol) in pyridine:acetic anhydride (2:1, 13 mL), DMAP (12.9 mg) was added. The reaction mixture was stirred at room temperature for 1 h, after which TLC showed disappearance of the starting material. The solvent was removed under high vacuum and the residue was filtered through a silica column and product eluted with 50% ethyl acetate in hexane to afford pure compound 12 (417 mg, 92%) Rf 0.26 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.36-7.29 (m, 5H), 5.82 (d, J
= 3.9 Hz, 1H), 4.68-4.62 (m, 2H), 4.52 (d, \( J = 12 \) Hz, 1H), 4.31 (dd, \( J = 4.2 \) Hz, 8.1 Hz, 1H), 4.16-4.04 (m, 3H), 3.88 (dd, \( J = 1 \) Hz, 4.5 Hz, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.55 (s, 3H), 1.55 (s, 3H); ESMS \( m/z \) (M+Na+H)+ 418.25. The data was in accordance with that reported by Ruda et. al.\(^{101}\)

1,2,5,6-tetra-O-acetyl-3-O-benzyl galactofuranose (13)

1,2-O-isopropylidene-3-O-benzyl-5,6-di-O-acetyl galactofuranose (12, 400 mg, 1.01 mmol) was dissolved in a trifluoroacetic acid (TFA): chloroform solution (1:10, 16.5 mL) at 0 °C. The reaction mixture was brought to room temperature and stirred for 8 h. Saturated NaHCO\(_3\) solution was added slowly to quench the reaction, followed by a chloroform-water extraction. The chloroform layers were dried (anhydrous Na\(_2\)SO\(_4\)) and concentrated. The residue was dissolved in pyridine: acetic anhydride solution (1:1, 20 mL) and stirred at room temperature for 20 min. The solvent was then removed using high vacuum and residue purified using silica column chromatography (25% ethyl acetate in hexane) to give pure compound 13 (350 mg, 78%) \( R_f 0.3 \) (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta \) 7.37-7.34 (m, 10H), 6.34 (d, \( J = 4.5 \) Hz, 1H), 6.18 (s, 1H), 5.31-5.20 (m, 4H), 4.76-4.54 (m, 5H), 4.35-3.89 (m, 9 H), 2.08-1.99 (m, 30 H); ESMS \( m/z \) 461.23 (M+Na)+. The data was in accordance with that reported by Ruda et. al.\(^{101}\)

Pentenyl 2,5,6-tri-O-acetyl-3-O-benzyl-galactofuranoside (14)

(Scheme-5 of Results and Discussion)

A solution of 1,2,5,6-tetra-O-acetyl-3-O-benzyl galactofuranose (13, 200 mg, 0.46 mmol) and 4-penten-1-ol (182 \( \mu l \), 1.79 mmol, Fluka) in 3.6 mL CH\(_2\)Cl\(_2\) was stirred with freshly activated 4 Å molecular sieves (500 mg, Fluka) under Ar atmosphere, at room temperature for 30 min. Lewis acid catalyst SnCl\(_4\) (72.7 \( \mu l \)) was slowly added to the stirred solution. Stirring was continued for 4 h, after which TLC showed only 50% consumption of starting material 13 and a new spot corresponding to a compound with less polarity. 40 \( \mu l \) of SnCl\(_4\) was added and stirring continued for 2 h, with TLC now showing 90% reaction completion. The reaction was quenched by the addition of solid NaHCO\(_3\) and Na\(_2\)SO\(_4\). The reaction mixture was filtered through celite and the filtrate concentrated. The residue was purified using silica
column chromatography (20% ethyl acetate in hexane) to afford pure compound 14 (162 mg, 77%) $R_f$ 0.5 (50% ethyl acetate in hexane); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 7.34-7.25 (m, 5H), 5.89-5.71 (m, 1H), 5.34-5.25 (m, 1H), 5.08 (s, 1H), 5.05-4.91 (m, 3H), 5.05-4.91 (m, 3H), 4.69 (d, $J = 12.3$ Hz, 1H), 4.49 (d, $J = 12.3$ Hz, 1H), 4.28 (dd, $J = 4.5$ Hz, 8.1 Hz, 1H), 4.20 (q, $J = 4.5$ Hz, 1.8Hz, 1H), 4.16-4.07 (m, 1H), 3.74 (d, $J = 6.6$ Hz, 1H), 3.70-3.61 (m, 1H), 3.47-3.38 (m, 1H), 2.16-2.08 (m, 2H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.73-1.61 (m, 2H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 170.39, 170.01, 169.61, 137.93, 137.11, 128.32, 127.93, 127.85, 114.81, 105.83, 82.94, 81.19, 80.30, 82.94, 81.19, 80.30, 72.33, 69.70, 66.79, 62.68, 60.04, 28.47, 20.77, 20.66, 20.56; ESMS $m/z$ 487.39 (M+Na$^+$); HRMS (ESMS): calcd for (M+NH$_4^+$) $^+$$C_{24}H_{36}NO_9$ 482.2390; found 482.2392.

2.2.3 Synthesis of Galβ(1,3)Manα(1,3)Man intermediate  
(Scheme-4, 5, 6 and 7 of Results and Discussion)

**Benzyl 2,4,6-tri-O-benzoyl-3-O-[2,4,6-tri-O-benzoyl-3-O-(2,5,6-tri-O-acetyl-3-O-benzyl-β-D-galactofuranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (15)**  
A solution of pentenyl 2,5,6-tri-O-acetyl-3-O-benzyl-galactofuranoside (14, 220 mg, 0.47 mmol), benzyl 2,4,6-tri-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-α-D-mannopyranoside (8, 315 mg, 0.64mmol) and N-iodosuccinimide (NIS, 138 mg, 0.613 mmol, Fluka) in anhydrous CH$_2$Cl$_2$ (7 mL) was stirred with freshly activated 4 Å molecular sieves (500 mg, Fluka) under Ar atmosphere, at room temperature and in the dark for 20 min. Lewis acid catalyst triethyl silyl trifluoromethanesulphonate (TESOTf, 50 μl, 0.22 mmol) in 1 mL anhydrous CH$_2$Cl$_2$ was slowly added to the stirred solution and stirring was continued for 1 h. The reaction was quenched with saturated NaHCO$_3$ solution and extracted with CH$_2$Cl$_2$. The CH$_2$Cl$_2$ layers were pooled, dried (anhydrous Na$_2$SO$_4$) and concentrated. The residue was purified using silica column chromatography (30% ethyl acetate in hexane) to afford pure compound 15 (272 mg, 64%). $R_f$ 0.55 (50% ethyl acetate in hexane); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.23-8.01 (m, 9H), 7.88-7.79 (m, 4H), 7.58-7.29 (m, 25H), 7.19-7.13 (m, 3H), 6.94-6.87 (m, 2H), 5.99 (t, $J = 9.6$ Hz, 1H), 5.82-
5.68 (m, 2H), 5.30-5.13 (m, 3H), 4.98 (t, J = 5.4 Hz, 1H), 4.87 (s, 1H), 4.80-4.73 (m, 2H), 4.67-4.57 (m, 4H), 4.51-4.06 (m, 10H), 3.97-3.89 (m, 1H), 3.69 (t, J = 6 Hz, 1H), 3.58 (s, 1H), 3.44 (d, J = 3.2 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H); $^1$H NMR (CDCl$_3$, 75 MHz) $\delta$ 170.19, 169.73, 168.93, 166.08, 165.59, 165.33, 164.84, 164.49, 137.25, 136.20, 133.51, 133.27, 132.98-132.72, 129.98-127.28, 102.30, 99.69, 96.82, 81.96, 81.46, 79.88, 75.85, 71.68, 71.11, 70.21, 69.87, 69.68, 69.55, 69.02, 68.61, 67.66, 66.48, 62.86, 62.61, 62.47, 20.66, 20.54, 20.47.; ESMS $m/z$ 1458.33 (M+Na$^+$); HRMS (ESMS): calcd for (M+NH$_4$)$^+$ C$_{80}$H$_{78}$N$_2$O$_{25}$ 1452.4863; found 1452.4885.

**Acetyl 2,4,6-tri-O-benzoyl-3-O-[2,4,6-tri-O-benzoyl-3-O-(2,5,6-tri-O-acetyl-\(\beta\)-D-galactofuranosyl)-\(\alpha\)-D-mannopyranosyl]-\(\alpha\)-D-mannopyranoside (16)**

To a solution of coupled trisaccharide (15, 150 mg, 0.11mmol) in MeOH (5 mL), catalyst 10% Pd/C catalyst (100 mg) and ammonium formate (75 mg) was added. The reaction mixture was refluxed for 1 h, after which TLC showed the formation of a compound that corresponded to the removal of a single benzyl from the anomeric position. The catalyst was removed through celite filtration. The filtrate was concentrated and the residue was purified using silica column chromatography (45% ethyl acetate in hexane). $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 8.18-8.00 (m, 8H), 7.90-7.77 (m, 4H), 7.61-7.26 (m, 20H), 7.17-7.13 (m, 3H), 6.92-6.87 (m, 2H), 6.00 (t, J = 3.9 Hz, 1H), 5.73-5.65 (m, 2H), 5.44 (s, 1H), 5.30-5.17 (m, 3H), 4.99-4.93 (m, 1H), 4.90 (s, 1H), 4.78 (s, 1H), 4.74-4.65 (m, 1H), 4.45-4.37 (m, 2H), 4.34-4.25 (m, 4H), 4.20-4.11 (m, 1H), 3.91 (dd, J = 3.3 Hz, 8.7 Hz, 1H), 3.72-3.61 (m, 2H), 3.42 (d, J = 4.8 Hz, 1H), 1.96 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75 MHz) $\delta$ 170.22, 169.71, 168.94, 166.20, 165.65, 165.27, 164.81, 164.58, 137.25, 133.43-132.76, 129.82-127.28, 102.33, 99.56, 92.27, 81.97, 81.46, 79.84, 74.69, 71.96, 71.09, 70.19, 69.68, 69.55, 68.83, 68.68, 67.64, 66.62, 62.86, 62.76, 62.45, 20.66, 20.53, 20.48; ESMS $m/z$ 1390.78 (M+2Na$^+$); HRMS (ESMS): calcd for (M+NH$_4$)$^+$ C$_{73}$H$_{72}$N$_2$O$_{25}$ 1362.4393; found 1362.4357. The residue was dissolved in anhydrous pyridine (750 µl). The solution was cooled to 0 °C and acetic anhydride (750 µl) was added dropwise. The reaction mixture was brought to room temperature and stirred
overnight. Pyridine was removed using a high vacuum pump and the syrupy residue filtered through silica column where the major product eluted out at 30% ethyl acetate in hexane. The fractions were concentrated and analyzed using ESMS which showed a m/z of 1409 (M+Na)\(^+\) corresponding to the mass of the anomeric acetate derivative. The residue was redissolved in MeOH (5 mL). 10% Pd/C catalyst (75 mg) and ammonium formate (75 mg) was added and reaction refluxed overnight. The catalyst was removed through celite filtration. The filtrate was concentrated and residue purified using silica column chromatography (35% ethyl acetate in hexane) to give pure compound 16 (80 mg, 60% overall yield). R\(_f\) 0.45 (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 8.20 (d, \(J = 7.8\) Hz, 2H), 8.12-7.99 (m, 7H), 7.87-7.77 (m, 3H), 7.58-7.27 (m, 18H), 7.17-7.12 (m, 3H), 6.92-6.86 (m, 2H), 6.03 (t, \(J = 10.5\) Hz, 1H), 5.71-5.65 (m, 2H), 5.44 (s, 1H), 5.27-5.22 (m, 2H), 4.98-4.92 (m, 1H), 4.90 (s, 1H), 4.78 (s, 1H), 4.70-4.23 (m, 12H), 3.90 (dd, \(J = 2.4\) Hz, 9.6 Hz, 1H), 3.71-3.60 (m, 2H), 3.42 (d, \(J = 5.4\) Hz, 1H), 2.04 (s, 3H), 1.96 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H); ESMS m/z 1365.33 (M+2xNa)\(^+\)

2.2.4 Synthesis of Galα(1,6)Gal intermediate

(Scheme-8 and 9 of Results and Discussion)

**Allyl-\(\alpha\)-d-galactopyranoside (17)**

D-Galactose (10 g, 0.055 mol) was suspended in allyl alcohol (50 mL). CSA (140 mg) was added as the catalyst. The reaction mixture was heated at 90-95 °C overnight, during which the solution cleared up. The solvent was removed under high vacuum. A few drops of Et\(_3\)N were added to initially neutralize and then basify the reaction mixture. Water (300 mL) was added and then extracted with CH\(_2\)Cl\(_2\). The aqueous layer was collected and concentrated using good quality high vacuum pump. The residue was dissolved in methanol and purified using silica column chromatography (10% MeOH in CH\(_2\)Cl\(_2\)) to afford compound 17 (8.5 g, 70%) as a white fluffy powder. R\(_f\) 0.3 (15% MeOH in CH\(_2\)Cl\(_2\)); \(^1\)H NMR (D\(_2\)O, 300 MHz) \(\delta\) 5.89-5.73 (m, 1H), 5.27-5.06 (m, 2H), 4.81 (s, 3H), 4.06 (dd, \(J = 4.8\) Hz, 7.8 Hz, 1H), 3.89 (dd, \(J = 6\) Hz, 6.3 Hz, 1H), 3.82-3.73 (m, 2H), 3.68-3.64 (m, 2H), 3.59-3.53 (m, 2H); \(^1^3\)C NMR (D\(_2\)O, 75 MHz) \(\delta\) 136.01, 116.90, 99.78, 72.70, 71.67,
71.36, 70.6, 69.70, 63.22; ESMS m/z (M+Na)^+ 243.10. The data was in accordance with that reported by Casali et. al.\textsuperscript{106}

**Allyl 2,3,4-tri-O-benzyl-α-D-galactopyranoside (19)**

A solution of allyl-α-D-galactopyranoside (17, 8 g, 0.036 mol), trityl chloride (15 g, 0.053 mol, Fluka) and DMAP (34 mg) was made in anhydrous pyridine (73 mL). The solution was stirred at 80 °C for 16 h, after which TLC showed 85% disappearance of starting material and an appearance of a comparatively less polar product. The solvent was concentrated using high vacuum and residue filtered through silica in a sintered funnel and compound eluted with 70% ethyl acetate in hexane to give allyl 6-O-trityl-α-D-galactopyranoside (18) (10g, 89%) R\textsubscript{f} 0.3 (70% ethyl acetate in hexane); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ 7.43-7.21 (m, 15H), 5.91-5.73 (m, 1H), 5.25-5.07 (m, 2H), 4.81 (s, 3H), 4.10-3.85 (m, 2H), 3.82-3.59 (m, 5H), 3.45-3.39 (m,2H). To a solution of compound allyl 6-O-trityl-α-D-galactopyranoside (18, 2 g, 4.3 mmol) in anhydrous DMF (20 mL), cooled to 0 °C, NaH (60% suspension in oil, 0.41 g, 17.3 mmol, Merck) was added in small lots. Reaction mixture was stirred for 15 min and benzyl bromide (2.06 mL, 17.3 mmol, Fluka) was added dropwise to this reaction mixture. Catalytic amount of TBAI was also added. The reaction was brought to room temperature and stirring continued for 5 h. The reaction was again cooled to 0 °C and cold MeOH (1 mL) was added in order to destroy excess NaH. The reaction mixture was stirred for 30 min after which CH\textsubscript{2}Cl\textsubscript{2} (200 mL) was added. The solution was extracted with water (3 x 75 mL). The organic layers were pooled, dried (anhydrous Na\textsubscript{2}SO\textsubscript{4}) and concentrated. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2}: MeOH (1:2, 43 mL). pTSA (43 mg) was added to this solution to get pH 2 of the reaction. The reaction mixture was stirred at room temperature overnight. Et\textsubscript{3}N was added dropwise to neutralize the reaction. The reaction mixture was concentrated and residue obtained was purified using silica column chromatography (25% ethyl acetate in hexane) to give pure compound 19 (2.6 g, 82%) R\textsubscript{f} 0.2 (40% ethyl acetate in hexane); \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz) δ 7.43-7.26 (m, 15H), 6.01-5.84 (m, 1H), 5.35-5.16 (m, 3H), 5.01-4.62 (m, 7H), 4.19-3.65 (m, 6H), 3.56-3.43 (m, 1H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 75 MHz) δ 138.15, 138.13,
138.10, 133.86, 128.46-127.46, 117.90, 96.37, 79.04, 76.46, 75.15, 74.39, 73.53, 73.30, 70.39, 68.32, 62.34; ESMS m/z 513.32 (M+Na)+

**Allyl 2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranoside (20)**

To a solution of allyl 2,3,4-tri-O-benzyl-α-D-galactopyranoside (19, 2 g, 4.0 mmol) in anhydrous DMF (20 mL), cooled to 0 °C, NaH (60% suspension in oil, 0.14 g, 6.0 mmol, Merck) was added. Reaction mixture was stirred for 30 min and benzyl bromide (0.9 mL, 6 mmol, Fluka) was added dropwise to this reaction mixture. Catalytic amount of TBAI was also added. The reaction was brought to room temperature and stirring continued for 16 h. The reaction was again cooled to 0 °C and cold MeOH (0.5 mL) was added in order to destroy excess NaH. The product was isolated by a CH$_2$Cl$_2$-water extraction, where the product was obtained from the organic layer. Crude product was purified using silica column chromatography (15% ethyl acetate in hexane) affording pure compound 20 (1.6 g, 70%) R$_f$ 0.3 (20% ethyl acetate in hexane); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.45-7.31 (m, 15H), 7.25 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 6.07-5.90 (m, 1H), 5.41-5.21 (m, 2H), 5.04-4.68 (m, 7H), 4.61 (d, J = 11.4 Hz, 1H), 4.22 (dd, J = 5.4 Hz, 7.8 Hz, 1H), 4.13-3.99 (m, 5H), 3.86 (s, 3H); $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 138.14, 138.11, 138.08, 133.91, 128.39-127.30, 117.96, 113.67, 96.29, 78.97, 76.46, 75.29, 74.44, 73.38, 73.29, 70.39, 68.32, 62.56, 55.23. ESMS m/z 772.79 (M+2Na)+

2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranose (21)

Allyl 2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranoside (20, 1.5 g, 2.5 mmol) was dissolved in DMSO (50 mL). Potassium tert-butoxide (3.3 g) was added to this solution. The reaction mixture was stirred at 60 °C for 1.5 h. The reaction was quenched with ice, followed by extraction with ethyl acetate and water. The organic layers were pooled, dried (anhydrous Na$_2$SO$_4$) and concentrated. The residue was resuspended in 1M HCl:acetone (1:9, 100 mL) and reaction mixture kept at 40 °C for 1.5 h, after which TLC showed completion of reaction. The solution was cooled in ice and Et$_3$N was added to neutralize the reaction. The solvent was evaporated and residue purified using silica column chromatography.
(20% ethyl acetate in hexane) to give pure compound 21 (1 g, 70%) Rf 0.37 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 7.37-7.27 (m, 15H), 7.19 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 5.27 (d, J = 3.6 Hz, 1H), 4.96-4.88 (m, 2H), 4.86-4.70 (m, 5H), 4.60-4.54 (m, 2H), 4.46-4.38 (m, 2H), 4.18-4.08 (m, 2H), 4.02 (dd, J = 3.3 Hz, 6 Hz, 1H), 3.96-3.86 (m, 2H), 3.79 (s, 3H); 13C NMR (CDCl3, 75 MHz) δ 138.91-138.80, 129.49-127.44, 113.63, 91.73, 78.51, 74.76, 74.59, 73.32, 72.94, 72.65, 69.41, 68.53, 55.14.; ESMS m/z 593.27 (M+Na)+

Allyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (22)

To a solution of 2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranose (21, 1g, 2mmol) in anhydrous CH2Cl2 (10 mL), freshly activated and finely powdered potassium carbonate (1g) and trichloroacetonitrile (0.9 mL) were added. The reaction was vigorously stirred for 6h at room temperature. TLC showed 60% reaction completion to the β-trichloroacetimidate derivative of compound 21. The reaction mixture was diluted with CH2Cl2 and filtered through celite and the filtrate concentrated. The β-configuration of the trichloroacetimidate derivative was authenticated by 1H NMR (CDCl3, 300 MHz) δ 8.64 (s, 1H), 7.35-7.26 (m, 15H), 7.19 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.73 (d, J = 7.8 Hz, 1H), 4.97-4.87 (m, 2H), 4.81 (d, J = 12 Hz, 1H), 4.38 (d, J = 12 Hz, 1H), 3.94 (d, J = 2.4 Hz, 1H), 3.79 (s, 3H), 3.73 (t, J = 6 Hz, 1H), 3.67-3.66 (m, 2H). The trichloroacetimidate donor thus obtained was then mixed with compound 19 (500 mg, 1.02 mmol). The trichloroacetimidate donor and the acceptor 19 were dried thoroughly with anhydrous toluene, and finally dissolved in anhydrous diethyl ether (15 mL). The solution was stirred with freshly activated powdered 4 Å molecular sieves (500 mg, Fluka) under Ar atmosphere at −20 °C for 30 min. Lewis acid catalyst TMSOTf (1.5 mL, 0.033 M solution in diethyl ether) was slowly added to the stirred solution and stirring was continued for 1 h at −20 °C. The reaction was quenched using solid NaHCO3 and the solution filtered through celite. The filtrate was concentrated and the residue was purified using silica column chromatography (12.5% ethyl acetate in hexane) to give pure coupled product 22 (497 mg, 47%) Rf
0.49 (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.41-7.14 (m, 33H), 6.82 (d, \(J = 8.4\) Hz, 2H), 5.98-5.80 (m, 1H), 5.29-5.10 (m, 2H), 4.95-4.49 (m, 13H), 4.14-3.87 (m, 9H), 3.76 (s, 3H), 3.72-3.65 (m, 1H), 3.58-3.39 (m, 3H); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz) \(\delta\) 139.83-138.52, 133.91, 129.39-127.31, 117.96, 113.71, 98.15, 96.10, 78.97, 78.93, 77.10, 76.22, 75.31, 74.83, 74.69, 74.54, 73.38, 73.21, 73.14, 72.98, 72.64, 69.24, 68.16, 68.10, 67.15, 55.13; ESMS \(m/z\) 1043.65 (M+H)

\(\text{2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-\(\alpha\)-D-galactopyranosyl)-\(\alpha\)-D-galactopyranose (23)}\)

Allyl \(2,3,4\)-tri-\(O\)-benzyl-6-\(O\)-(2,3,4-tri-\(O\)-benzyl-6-\(O\)-p-methoxybenzyl-\(\alpha\)-D-galactopyranosyl)-\(\alpha\)-D-galactopyranoside (22, 300 mg, 0.287 mmol) was dissolved in DMSO (30 mL) and potassium tert-butoxide (500 mg) was added to this solution. The reaction mixture was stirred at 60 °C for 1.5 h. The reaction was quenched with ice, followed by extraction with ethyl acetate and water. The organic layers were pooled, dried (anhydrous Na\(_2\)SO\(_4\)) and concentrated. The residue was dissolved in acetone:water (3:1, 16 mL). To this solution, HgCl\(_2\) (80 mg) and HgO (80 mg) were added. The reaction was stirred at room temperature for 10 min. TLC showed completion of reaction. The reaction mixture was immediately filtered through silica column to get pure compound 23 (245 mg, 85%) \(R_f\) 0.4 (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.32-7.16 (m, 32H), 7.13 (d, \(J = 8.4\) Hz, 2H), 6.77 (d, \(J = 8.4\) Hz, 2H), 5.14 (d, \(J = 3.3\) Hz, 1H), 4.89-4.42 (m, 12H), 4.34-4.19 (m, 3H), 4.07 (t, \(J = 5.1\) Hz, 1H), 3.97-3.81 (m, 9H), 3.70 (s, 3H), 3.69-3.68 (m, 3H), 3.49-3.39 (m, 3H); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz) \(\delta\) 138.73-138.32, 129.82-127.25, 113.79, 98.43, 91.59, 78.67, 78.63, 75.28, 75.01, 74.63, 74.58, 74.01, 73.45, 73.14, 72.89, 71.98, 70.11, 68.76, 68.15, 67.15, 55.39; ESMS \(m/z\) 1026.25 (M+Na)

\(\text{2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-\(\alpha\)-D-galactopyranosyl)-\(\beta\)-D-galactopyranosyl trichloroacetimidate (24)}\)

To a solution of 2,3,4-tri-\(O\)-benzyl-6-\(O\)-(2,3,4-tri-\(O\)-benzyl-6-\(O\)-p-methoxybenzyl-\(\alpha\)-D-galactopyranosyl)-\(\alpha\)-D-galactopyranose (23, 200 mg, 0.2 mmol) in anhydrous CH\(_2\)Cl\(_2\) (2 mL), freshly activated and finely powdered potassium carbonate (0.2 g)
and trichloroacetonitrile (200 µL) were added. The reaction was vigorously stirred for 6 h at room temperature. TLC showed 50% reaction completion to the β-trichloroacetimidate derivative 24. The reaction mixture was filtered through celite to remove potassium carbonate. The filtrate was concentrated and thoroughly dried. The residue would be coupled directly to the acceptors as we could see considerable degradation of the trichloroacetimidate during silica column purification. Rf 0.55 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) showed that 55% of the product was in the β configuration. δ at 8.49 (s, 1H) and 5.73 (d, J = 7.8 Hz, 1H) were the characteristic peaks.

**Thiophenyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-acetyl-α-D-galactopyranosyl)-α-D-galactopyranoside (26)**

(Scheme-12 of Results and Discussion)

2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranose (23, 10 mg, 0.01 mmol) was dissolved in anhydrous pyridine (250 µL) and the solution was cooled to 0 °C. To this acetic anhydride (250 µL) was added and the reaction mixture was allowed to stir at room temperature overnight. The solvent was removed using high vacuum and column filtered to give the anomeric acetate derivative acetyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranoside 25 (9 mg, 0.008 mmol). Compound 25 was dissolved in anhydrous CH2Cl2 (50 µL) and 10 µL thiophenol was added. The solution was cooled to 0 °C. BF3·Et2O (10 µL) was added to the reaction mixture and stirred for 45 min. The reaction was quenched with solid NaHCO3. ESMS analysis [ESMS m/z 997.63 (M+Na)+] at this stage showed that the anomeric acetate group had been exchanged with thiophenol group, but the PMB group at the 6-OH position had also been cleaved off. It was then decided to protect the position using an acetate group. The reaction mixture was filtered and the filtrate concentrated. The residue was redissolved in anhydrous pyridine (250 µL) and acetic anhydride (250 µL) at 0 °C and the reaction was allowed to stir overnight at room temperature. The solvent was evaporated and the residue purified using silica column chromatography (25% ethyl acetate in hexane)
to give pure compound 26 (8.3 mg, 82%) \( R_f 0.2 \) (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta 7.39-7.27 \) (m, 33H), \( 5.71 \) (d, \( J = 4.5 \) Hz, 1H), 5.02-4.89 (m, 6H), 4.85-4.59 (m, 15H), 4.39-4.25 (m, 2H), 4.16-4.04 (m, 2H), 3.98-3.73 (m, 4H), 3.62-3.42 (m, 5H), 1.92 (s, 3H); ESMS \( m/z \) 1039.69 (M+Na)

\textbf{2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-acetyl-\( \alpha \)-D-galactopyranosyl)-\( \alpha \)-D-galactopyranosyl bromide (27)}

(Scheme-13 of Results and Discussion)

Acetyl 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-\( \alpha \)-D-galactopyranosyl)-\( \alpha \)-D-galactopyranoside (25, 5mg, 0.005 mmol) was dissolved in anhydrous CH\(_2\)Cl\(_2\) (100 \( \mu \)L) at 0 °C. To this solution, 33% HBr-AcOH (20 \( \mu \)L) was added. It was kept at 4 °C overnight, after which TLC showed disappearance of starting material. The solution was extracted with saturated NaHCO\(_3\) solution and the CH\(_2\)Cl\(_2\) layers were combined, dried and evaporated to give the corresponding bromide 27. This was directly used in the next step.

\textbf{2.2.5 Synthesis of Gal\( \alpha \)(1,6)Gal\( \alpha \)(1,3)Gal\( \beta \) intermediate}

(Scheme-14 and 15 of Results and Discussion)

\textbf{1,2:5,6-di-O-isopropylidene-3-O-trifluoromethanesulphonyl-\( \beta \)-D-galactofuranose (28)}

A solution of anhydrous CH\(_2\)Cl\(_2\) (1.6 mL) and anhydrous pyridine (33 \( \mu \)L) was cooled to \(-10 \) °C. A solution of triflic anhydride (67 \( \mu \)L, 0.4 mmol) in anhydrous CH\(_2\)Cl\(_2\) (0.8 mL) was added to this solution. A thick white precipitate was formed. The suspension was allowed to stir for 10 min. A solution of 1,2:5,6-di-O-isopropylidene galactofuranose (9, 50 mg, 0.2 mmol) in anhydrous CH\(_2\)Cl\(_2\) (0.8 mL) was added to this reagent. The reaction was allowed to stir at \(-10 \) °C for 1.5 h. TLC showed complete consumption of starting material. The solvent was evaporated and the residue purified using silica column chromatography (10% ethyl acetate in hexane) to give pure compound 28 (61 mg, 81%). \( R_f 0.6 \) (40% ethyl acetate in hexane). \(^1\)H NMR (CDCl\(_3\), 300 MHz) \( \delta 5.94 \) (d, \( J = 4.0 \) Hz, 1H), 5.45-5.34 (m, 1H),

58
4.53-4.33 (m, 1H), 4.12-4.05 (m, 2H), 3.88-3.81 (m, 2H), 1.55 (s, 3H), 1.42 (s, 3H), 1.35 (s, 3H), 1.31 (s, 3H). The ESMS data of this compound is not available due to lability of the triflate, but peak corresponding to (M-CF$_3$+Na)$^+$ 346.32 was observed sometimes. The data was identical to that reported by Binkley et. al. $^{107}$

1,2:5,6-di-O-Isopropylidene-3-O-[2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranosyl] galactofuranose (29)

2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranose (23, 10 mg, 0.01 mmol) was dissolved in a DMF:HMPA (1:2, 123 μL) and the solution is cooled to 7 °C. Sodium hydride (1 mg) was added to this solution and the reaction mixture was stirred for 10 min. The freshly prepared triflate (28, 6 mg, 0.015 mmol), dissolved in DMF:HMPA (1:2, 77 μL) was now added to this solution and the temperature of the reaction mixture was reduced to -12 °C. The reaction was allowed to stir at -12 °C for 15 h. The reaction was quenched with methanol. Ethyl acetate was added, followed by extraction with water. The organic layers were dried (anhydrous Na$_2$SO$_4$) and concentrated. The residue was purified using silica column chromatography (20% ethyl acetate in hexane) to give pure compound 29 (4 mg, 33%) $R_f$ 0.47 (40% ethyl acetate in hexane); $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.42-7.20 (m, 33H), 6.80 (d, $J = 8.4$ Hz, 2H), 5.53 (dd, $J = 6$ Hz, 9.3 Hz, 1H), 5.37-5.35 (m, 1H), 5.04-5.01 (m, 1H), 4.95-4.83 (m, 2H), 4.76-4.47 (m, 9H), 4.37-4.29 (m, 3H), 4.15-3.96 (m, 6H), 3.87 (s, 3H), 3.86-3.70 (m, 4H), 3.17-3.07 (m, 1H), 1.55 (s, 3H), 1.49 (s, 3H), 1.36 (s, 3H), 1.32 (s, 3H); ESMS m/z 1267.81 (M+Na)$^+$

2-O-Benzoyl-5,6-di-O-isopropylidene-D-galactonolactone (30)

D-galactono-1,4-lactone (1 g, 5 mmol) was dissolved in acetone: acetone dimethyl acetal (3:1, 14 mL). The reaction was cooled to 0 °C. To this solution, conc. H$_2$SO$_4$ (10 μL) was added. The reaction was stirred at 0 °C for 30 min. The pH was made neutral using liquor ammonia. The suspension was filtered and the filtrate was concentrated. The residue was dissolved in anhydrous pyridine (5 mL). The solution
was cooled to -12 °C. Benzyol chloride (0.8 mL, 5.6 mmol) was added to the solution slowly over 30 min, and stirring was continued for another 30 min. The resulting mixture was poured into ice water (100 mL) and stirred with CH₂Cl₂. The organic layer was washed sequentially with 5% HCl, water and saturated NaHCO₃. The organic layer was dried (anhydrous Na₂SO₄) and concentrated. Purification of compound using silica gel chromatography (15% ethyl acetate in hexane) afforded pure compound 30 (351 mg, 20%) Rf 0.14 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.13 (d, J = 7.5 Hz, 2H), 7.69-7.45 (m, 3H), 5.51 (d, J = 8.1 Hz, 1H), 4.63 (t, J = 7.5 Hz, 1H), 4.46-4.39 (m, 1H), 4.33 (dd, J = 3.3 Hz, 3.9 Hz, 1H), 4.21-4.14 (m, 1H), 4.11-4.04 (m, 1H), 1.47 (s, 3H), 1.40 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) 168.49, 167.12, 134.17, 130.19, 128.57, 110.31, 79.71, 77.50, 73.91, 73.45, 64.93, 25.94, 25.36; ESMS m/z 348.93 (M+Na+H⁺). The data was identical to that reported earlier.¹⁰⁸

2-0-Benzoyl-5,6-di-O-isopropylidene-3-O-[2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-α-D-galactopyranosyl] galactonolactone (31)

A solution of 2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl trichloroacetimidate (24, 16 mg, 0.014 mmol) and 2-0-benzoyl-5,6-di-O-isopropylidene-D-galactonolactone (30, 5 mg, 0.015 mmol) in anhydrous diethyl ether (0.5 mL) was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere, at 0 °C for 20 min. Lewis acid catalyst, trimethyl silyl triflouromethanesulphonate (TMSOTf, 60 μL, 0.06M in diethyl ether) was slowly added to the cooled solution. The reaction mixture was stirred for 2 h. The reaction mixture was quenched with Et₃N and filtered through celite. The filtrate was concentrated and the residue purified using silica gel chromatography (20% ethyl acetate in hexane) to afford pure compound 31 (11 mg, 55%) Rf 0.26 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.14 (d, J = 7.5 Hz, 2H), 8.00 (d, J = 7.5 Hz, 2H), 7.60-7.20 (m, 32H), 6.83 (d, J = 8.1 Hz, 2H), 5.80 (d, J = 9.0 Hz, 1H), 5.41 (d, J = 7.8 Hz, 1H), 5.23 (s, 1H), 4.87 (d, J = 11.1 Hz, 1H), 4.78-4.46 (m, 15H), 4.29-4.22 (m, 3H), 3.96-3.81 (m, 7H), 3.70 (s, 3H), 3.55-3.31
(m, 4H), 1.39 (s, 3H), 1.32 (s, 3H); ESMS m/z 1329.54 (M+Na)^+; m/z 1324.88 (M+H\textsubscript{2}O)^+

2-O-Benzoyl-5,6-di-O-isopropylidene-3-O-[2,3,4-tri-O-benzyl-6-O-(2,3,4-tri-O-benzyl-6-O-p-methoxybenzyl-\alpha-D-galactopyranosyl)-\alpha-D-galactopyranosyl]galactofuranose (32)

The trisaccharide (31, 6.5 mg, 0.005 mmol) was dissolved in methanol (650 \mu L) and cooled to 0 to -5°C. After 10 min stirring, NaBH\textsubscript{4} (6.5 mg) was added slowly to the reaction. The reaction mixture was allowed to stir at this temperature for 40 min, after which TLC confirmed absence of the compound 31 and a new more polar compound was seen. The reaction was quenched by adding 30% acetic acid in methanol. The solvent was evaporated and the residue purified through column. The product eluted out in 25% ethyl acetate in hexane; ESMS m/z 1331.79 (M+Na)^+
2.3 Synthesis of the headgroups of the major antigenic GIPLs (iM2, iM3 and iM4) of *Leishmania donovani*

2.3.1 Synthesis of iM2 headgroup

(Scheme-16 of Results and Discussion)

**Benzyl 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl) mannopyranoside (34)**

A solution of benzyl 4,6-O-benzylidene-α-D-mannopyranoside (2, 3 g, 8.4 mmol) and donor trichloroacetimidate (4, 6 g, 12.6 mmol) in anhydrous CH₂Cl₂ (25 mL) was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere at 0 °C for 20 min. TMSOTf (3 mL, 0.033 M solution in diethyl ether) was slowly added to the cooled solution. The solution was stirred at rt for 45 min, TLC after which showed consumption of the acceptor 2. Solid NaHCO₃ added to quench the reaction. Reaction mixture was filtered through celite. The filtrate was concentrated and residue obtained was analyzed to be benzyl 3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl) mannopyranoside (33, 1.5 g). The benzylidene group was found cleaved. Compound 33 was subjected to acetylation by dissolving in anhydrous pyridine:acetic anhydride (1:1, 15 mL) at 0 °C. The solution was brought to room temperature and allowed to stir overnight. The solvent was removed using high vacuum and residue purified using silica column chromatography (40% ethyl acetate in hexane) to give pure compound 34 (1.6 g, 88%) R₇ 0.4 (60% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.36-7.29 (m, 5H), 5.35-5.19 (m, 5H), 5.01-4.97 (m, 2H), 4.91 (d, J = 1.5 Hz, 1H), 4.68 (d, J = 12 Hz, 1H), 4.51 (d, J = 12 Hz, 1H), 4.28-4.17 (m, 4H), 4.11-4.00 (m, 3H), 2.19 (s, 3H), 2.12 (s, 3H), 2.11 (s, 6H), 2.04 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 170.54, 170.51, 170.27, 169.87, 169.75, 169.72, 169.43, 136.11, 128.53-128.18, 98.72, 96.70, 77.11, 74.57, 70.81, 69.92, 68.11, 67.71, 65.90, 62.47, 62.33, 60.27, 20.91, 20.78, 20.69, 20.65, 20.61, 20.50, 20.41; ESMS m/z 772.81 (M+2Na)⁺
2.3.2 Synthesis of iM3 headgroup

(Scheme-17 of Results and Discussion)

**Benzyl 3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2-O-benzyl-4,6-O-benzylidene mannopyranoside (35)**

A solution of benzyl 3-O-(α-D-mannopyranosyl)-4,6-O-benzylidene mannopyranoside (5, 0.765 g, 1.47 mmol) in anhydrous DMF (7.5 mL) was cooled to 0 °C. NaH (60% suspension in paraffin oil, 300 mg, 8.085 mmol) was added to this solution in small lots. After stirring the reaction mixture for 30 min at 0 °C, BnBr (1 mL, 8.085 mmol) was added dropwise. Catalytic amount of TBAI was also added. The reaction was brought to room temperature and stirred for 3.5 h followed by quenching of reaction with cold methanol (1 mL). The reaction mixture was diluted with CH₂Cl₂ (100 mL), followed by washing with water (3 x 20 mL). The organic layers were pooled, dried (with anhydrous Na₂SO₄) and concentrated. The residue obtained was purified using silica column chromatography (15-20% ethyl acetate in hexane) to give pure product 35 (0.9 g, 74%) Rf 0.45 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.47-7.06 (m, 32H), 5.57 (s, 1H), 5.40 (s, 1H), 4.91-4.80 (m, 2H), 4.67-4.55 (m, 10H), 4.19-3.75 (m, 5H), 3.81-3.71 (m, 4H), 3.68-3.49 (m, 3H); ESMS m/z 994.96 (M+Na)⁺; HRMS (ESMS): calcd for (M+NH₄)⁺ C₆₁H₆₅NO₁₁ 990.4792; found 990.4478

**Benzyl 3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2-O-benzyl mannopyranoside (36)**

Benzyl 3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2-O-benzyl-4,6-O-benzylidene mannopyranoside (35, 600 mg, 0.62 mmol) was dissolved in methanol (5 mL). To this solution, pTSA was added so that the pH of the solution was around 2. The reaction mixture was stirred at room temperature overnight. Methanol was removed under vacuum, and residue redissolved in CH₂Cl₂, followed by extraction with saturated NaHCO₃. The CH₂Cl₂ layers are dried (anhydrous Na₂SO₄) and concentrated to give compound 36 (360 mg, 72%) Rf 0.3 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.32-7.15 (m, 27H), 5.4 (d, J = 2.1 Hz, 1H),
4.85 (t, $J = 1.5$ Hz, 1H), 4.81 (s, 1H), 4.72-4.40 (m, 11H), 4.09-3.99 (m, 2H), 3.89-3.84 (m, 3H), 3.81-3.75 (m, 4H), 3.70 (dd, $J = 2.1$ Hz, 10.2 Hz, 1H), 3.64-3.58 (m, 2H); ESMS $m/z$ 906.74 (M+Na)$^+$; HRMS (ESMS): calcd for (M+NH$_4$)$^+$ $C_{54}H_{62}NO_{11}$ 900.4323; found 900.4303.

**Benzyl 3-0-(2,3,4,6-tetra-O-benzyl-$\alpha$-D-mannopyranosyl)-2,4-di-O-benzyl mannopyranoside (37)**

To a solution of benzyl 3-0-(2,3,4,6-tetra-O-benzyl-$\alpha$-D-mannopyranosyl)-2-O-benzyl mannopyranoside (36, 350 mg, 0.4 mmol) in anhydrous pyridine (10 mL), trityl chloride (197 mg, 0.71 mmol) and few crystals of DMAP were added. The solution was stirred at 80 °C for 16 h, after which TLC showed disappearance of starting material and an appearance of a comparatively less polar product. The solvent was concentrated using high vacuum and residue extracted with CH$_2$Cl$_2$ and water. The CH$_2$Cl$_2$ layers were pooled, dried (anhydrous Na$_2$SO$_4$) and concentrated. The residue was dried thoroughly with toluene and redissolved in anhydrous DMF (6 mL) and the solution cooled to 0 °C. NaH (40 mg, 2 eq) was added to this solution, followed by the dropwise addition of benzyl bromide (200 µL). Catalytic amount of TBAI was also added. The reaction was stirred at room temperature for 3.5 h followed by quenching of reaction with cold methanol (0.5 mL). Excess of DMF was removed using high vacuum and the residue dissolved in CH$_2$Cl$_2$ (50 mL) was extracted with water (3 x 10 mL). The CH$_2$Cl$_2$ layers were pooled, dried (with anhydrous Na$_2$SO$_4$) and concentrated. The residue was dissolved in CH$_2$Cl$_2$: Methanol (1:2, 10 mL). pTSA was added till pH 2 was attained. The reaction mixture was stirred at room temperature overnight, neutralized with Et$_3$N to quench the reaction. The solvents were evaporated and residue purified using column chromatography (20% ethyl acetate in hexane) to give pure compound 37 (175 mg, 45% overall yield) R$_f$ 0.4 (40% ethyl acetate in hexane). $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.31-7.18 (m, 35H), 5.27 (s, 1H), 4.93-4.86 (m, 2H), 4.73-4.45 (m, 16H), 4.25 (dd, $J = 3$ Hz, 6.9 Hz, 1H), 4.73-4.45 (m, 16H), 3.97-3.91 (m, 6H), 3.78-3.67 (m, 7H). $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 138.67, 138.37, 138.25, 138.20, 138.09, 137.09, 127.09, 127.72-127.25, 128.43-128.05, 100.00, 96.96, 79.69, 78.49, 77.69,
Benzyl O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-(1,3)-O-[(2,3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1,6)]-2,4-di-O-benzyl-α-D-mannopyranoside (38) (the iM3 headgroup)

A solution of benzyl 3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2,4-di-O-benzyl mannopyranoside (37, 75 mg, 0.0772 mmol) and 2,3,4,6-tetra-O-acetyl mannopyranosyl trichloroacetimidate (4, 75.9 mg, 0.1543 mmol) in 4.2 mL anhydrous CH₂Cl₂ was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere at room temperature for 15 min. The reaction mixture was then cooled to -30 °C and TMSOTf (70 μL in 2 mL anhydrous CH₂Cl₂) was slowly added to the cooled solution. Reaction was stirred for 15 min, after which TLC showed complete disappearance of the donor. The reaction was quenched with pyridine (1 mL) and filtered through celite and the filtrate concentrated. The residue was purified using silica column chromatography (25% ethyl acetate in hexane) to give pure compound 38 (69 mg, 70%). Rₐ 0.47 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 7.32-7.20 (m, 33H), 7.19-7.18 (m, 8H), 5.40 (dd, J = 3.3 Hz, 6.9Hz, 1H), 5.33-5.29 (m, 2H), 5.27 (s, 1H), 5.23 (s, 1H), 4.88 (d, 16.8 Hz, 1H), 4.81 (s, 1H), 4.67-4.62 (m, 3H), 4.60-4.54 (m, 5H), 4.51 (t, J = 3 Hz, 1H), 4.48-4.47 (m, 2H), 4.45 (s, 2H), 4.24-4.15 (m, 3H), 4.12-4.03 (m, 3H), 3.96-3.71 (m, 11H), 3.66 (d, J = 3 Hz, 2H), 2.12 (s, 3H), 1.04 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 170.58-169.62, 138.72, 138.41, 138.36, 138.22, 138.13, 138.08, 137.15, 128.90-128.38, 100.00, 97.46, 96.32, 79.74, 78.62, 77.56, 77.13, 75.44, 75.01, 75.91, 74.65, 74.51, 73.23, 72.60, 72.28, 72.12, 71.53, 69.41, 69.01, 68.78, 68.39, 66.41, 66.11, 62.24, 29.59, 20.77-20.58.; ESMS m/z 1326.10 (M+Na+H)⁺; HRMS (ESMS): calcd for (M+NH₄)⁺ C₇₅H₈₆NO₂₀ 1320.5743; found 1320.5774.
2.3.3 Synthesis of IM4 headgroup
(Scheme-18 and 19 of Results and Discussion)

1,3,4,6-Tetra-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (39)
A solution of mannose trichloroacetimidate donor (4, 282 mg, 0.57 mmol) and commercially available 1,3,4,6-tetra-O-acetyl-β-D-mannopyranose (100 mg, 0.287 mmol) in anhydrous CH2Cl2 (5.6 mL) was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere for 30 min. The reaction mixture was cooled to -30 °C and TMSOTf (80 μL in 2.8 mL anhydrous CH2Cl2) was slowly added to the cooled solution. Reaction was stirred for 15 min, after which TLC showed complete disappearance of the donor. The reaction was quenched with pyridine (1.2 mL and filtered through celite and the filtrate concentrated. The residue was purified using silica column chromatography (50% ethyl acetate in hexane) to give pure compound 39 (328 mg, 60%). Rf 0.2 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 5.78 (d, J = 1.2 Hz), 5.48 (dd, J = 3.3 Hz, 6.9 Hz, 1H), 5.39-5.29 (m, 2H), 5.12 (dd, J = 3 Hz, 6.6 Hz, 1H), 5.00 (d, J = 1.8 Hz, 1H), 4.42-4.35 (m, 1H), 4.30-4.10 (m, 6H), 3.80-3.76 (m, 1H), 2.15-2.02 (7 x s, 21H); ESMS m/z 659.23 (M+Na)⁺. The data was identical to that reported earlier by Upreti et. al.89

3,4,6-Tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl trichloroacetimidate (40)
The mannobiose octaacetate 1,3,4,6-tetra-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranose (39, 200 mg, 0.3 mmol) was dissolved in anhydrous acetonitrile saturated with dimethylamine (15 mL) at -20 °C and stirred for 2 h after which TLC confirmed disappearance of the starting material. Excess of dimethylamine was removed under reduced pressure below 30 °C and the reaction mixture concentrated. The residue was dissolved in anhydrous CH2Cl2 (2.2 mL) at 0 °C. Trichloroacetonitrile (300 μL) and DBU (15 μL) were added successively. The reaction mixture was stirred at 0 °C for 1 h, after which TLC showed complete consumption of the starting material. The solvent was evaporated under reduced
pressure and the residue chromatographed on silica gel (40% ethyl acetate in hexane) to give disaccharide donor 40 (174.6 mg, 76%) Rf 0.27 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 8.71 (s, 1H), 6.42 (d, J = 1.86 Hz, 1H), 5.49-5.27 (m, 5H), 4.99 (s, 1H), 4.29-4.05 (m, 7H), 2.24-2.01 (7 x s, 21H). The data of this compound matched with that reported earlier by Upreti et al. 89

Benzyl O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1,2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1,6)-O-[(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-(1,3)]-2,4-di-O-benzyl-α-D-mannopyranoside (41) (the iM4 headgroup) (Scheme-19 of Results and Discussion)

A solution of benzyl 3-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2,4-di-O-benzyl mannopyranoside (37, 40 mg, 0.0411 mmol) and 3,4,6-tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl trichloroacetimide (40, 64 mg, 0.0822 mmol) in 2.2 mL anhydrous CH2Cl2 was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere, at room temperature for 30 min. The reaction mixture was cooled to -30 °C and TMSOTf (53 µl in 1 mL anhydrous CH2Cl2) was slowly added to the cooled solution. Reaction was stirred for 15 min, after which TLC showed complete disappearance of the donor. The reaction was quenched with pyridine (0.75 mL) and filtered through celite and the filtrate concentrated. The residue was purified using silica column chromatography (40% ethyl acetate in hexane) to give pure compound 41 (55 mg, 85%). Rf 0.29 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 7.18-7.32 (m, 35H), 5.08 (d, J = 1.2 Hz, 1H), 4.84 (s, 1H), 4.77 (d, J = 1.2 Hz, 1H), 4.74 (s, 1H), 4.74-4.70 (m, 1H), 4.62 (s, 2H), 4.58-4.40 (m, 11 H), 4.26-4.05 (m, 12H), 3.95-3.84 (m, 6H), 3.79-3.61 (m, 5H), 2.09 (s, 6H), 2.06 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 6H); 13C NMR (CDCl3, 75 MHz) δ 170.85, 170.38, 170.15, 169.69, 169.63, 169.35, 169.27, 138.69, 138.36, 138.33, 138.20, 138.14, 138.08, 137.12, 128.37-126.78, 99.99, 99.19, 98.46, 96.62, 79.71, 78.75, 78.48, 77.42, 75.40, 74.90, 74.87, 74.65, 74.50, 73.19, 72.63, 73.19, 72.63, 72.26, 72.23, 72.08, 71.90, 70.04, 69.71, 69.33, 68.92, 68.80, 68.46, 66.61, 66.26, 66.03, 62.24, 61.91, 20.73-20.54; ESMS m/z
1615.72 (M+Na+H)+; HRMS (ESMS): calcd for (M+NH₄)⁺ C₈₇H₁₀₂NO₂₈ 1608.6588; found 1608.6549

2.4 Synthesis of exogenous substrates and characterization of enzymes involved in the galactofuranose assembly in LPG biosynthesis.

2.4.1 Synthesis of exogenous substrates

2.4.1.1 Synthesis of UDP-α-Galactofuranose

(Scheme-20 of Results and Discussion)

**Methyl-O-D-galactofuranose (42)**

To a solution of d-galactose (3.24 g, 18 mmol) in anhydrous methanol (100 mL), ferric chloride (2.91 g, 18 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, after which it was refluxed at 80 °C for 4 h. After cooling down the solution to room temperature, a little celite and saturated NaHCO₃ (10 g) were added to precipitate ferric chloride as ferric hydroxide. The reaction mixture was stirred for 1 h at room temperature. The precipitate was filtered through a celite pad and the filtrate was concentrated. TLC analysis of the residue showed that it contained a mixture of α,β-methyl galactofuranoside and α,β-methyl galactopyranoside. Careful silica column chromatography yielded β-methyl galactofuranoside (160 mg) and a mixture of α and β- methyl galactofuranoside 42 (1.3 g, 37%). Rᵣ 0.324 (α isomer) and Rᵣ 0.405 (β isomer) (isopropanol:ammonia:water 7:2:1). The data for the β isomer; ^1^H NMR (D₂O, 300 MHz) δ 4.80 (s, 1H), 4.06-3.91 (m, 2H), 3.88-3.81 (m, 1H), 3.77-3.52 (m, 4H), 3.30 (s, 3H); ^1^C NMR (D₂O, 75 MHz) δ 108.89, 82.92, 81.59, 77.26, 70.96, 62.99, 53.94. The ^1^C NMR data for the α isomer; ^1^C NMR (D₂O, 75 MHz) δ 102.33, 81.90, 76.20, 74.74, 72.89, 62.63, 54.48.

**Methyl O-2,3,5,6-tetra-O-benzoyl-D-galactofuranoside (43)**

Methyl-O-D-galactofuranose (42, 1 g, 5.2 mmol) was dissolved in anhydrous pyridine (10 mL). To this solution, benzoyl chloride (3.6 mL, 25.77 mmol) was added dropwise. Catalytic amount of DMAP was also added. The reaction was stirred overnight at room temperature. Ice was added to the reaction mixture and
extracted with CH₂Cl₂. The CH₂Cl₂ layers were dried (anhydrous Na₂SO₄) and concentrated. The residue was purified using silica column chromatography (10-15% ethyl acetate in hexane) to afford pure compound 43 (2.98 g, 95%) R₁ 0.26 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.16-7.87 (m, 8H), 7.63-7.27 (m, 13H), 6.13-6.05 (m, 1H), 5.63 (d, J = 4.8 Hz, 1H), 5.65 (s, 1H), 5.21 (s, 1H), 4.81-4.74 (m, 1H), 3.47 (s, 3H). The spectral data of this compound was found identical to that previously reported.¹¹⁰

**Acetyl O-2,3,5,6-tetra-O-benzoyl-D-galactofuranoside (44)**

To methyl O-2,3,5,6-tetra-O-benzoyl-D-galactofuranoside (43, 2.5 g, 4.1 mmol), 0.5% H₂SO₄ acetic anhydride (10 mL) was added slowly at 0 °C. The reaction mixture was allowed to stir for 1.5 h at 0 °C, after which TLC showed complete consumption of starting material 43. The reaction was quenched with saturated NaHCO₃ solution and ice cold water, followed by extraction with ethyl acetate. The ethyl acetate layers were dried (anhydrous Na₂SO₄) and concentrated. The residue was column purified (15-20% ethyl acetate in hexane) to afford pure compound 44 (2 g, 77%). R₁ 0.3 (50% ethyl acetate in hexane); ¹H NMR (CDCl₃, 300 MHz) δ 8.04-7.79 (m, 10H), 7.48-7.12 (m, 12H), 6.55 (s, 1H), 5.76 (d, J = 4.2 Hz, 1H), 5.64 (s, 1H), 4.87-4.69 (m, 4H), 2.06 (s, 3H); ¹³C NMR (CDCl₃, 75 MHz) δ 168.96, 165.88, 165.62, 165.39, 133.54, 133.40, 133.20, 132.95, 129.91-128.23, 99.38, 88.42, 83.69, 81.29, 70.34, 63.52, 20.70. The NMR and MS data of this compound was found to be similar to that reported by Marlow et. al.¹¹⁰ and Tsvetkov et. al.¹¹¹

**Dibenzyl 2,3,5,6-tetra-O-benzoyl- α-D-galactofuranosyl phosphate (45)**

To a stirred solution of acetyl O-2,3,5,6-tetra-O-benzoyl-D-galactofuranoside (44, 2 g, 3.1 mmol) in anhydrous CH₂Cl₂ (15 mL) at 0 °C, 33% HBr-AcOH (5 mL) was added dropwise. The reaction mixture was stirred for 3.5 h. TLC analysis showed the complete conversion of compound 44 to its bromide derivative. The reaction mixture was diluted with CH₂Cl₂, and extracted with ice cold water and saturated NaHCO₃. The CH₂Cl₂ layers were dried (anhydrous Na₂SO₄) and concentrated. ¹H NMR (CDCl₃, 300 MHz) δ 8.14-8.04 (m, 4H), 7.96 (d, J = 8.4 Hz, 2H), 7.83 (d, J =
8.4 Hz, 2H), 7.64-7.21 (m, 13H), 6.64 (s, 1H), 6.23-6.14 (m, 1H), 5.89 (s, 1H), 5.68 (d, J = 4.5 Hz, 1H), 4.96 (t, J = 3.9 Hz, 1H), 4.73 (d, J = 5.7 Hz, 2H); 13C NMR (CDCl3, 75 MHz) δ 165.90, 165.53, 165.49, 165.08, 133.68, 133.56, 133.30, 133.04, 129.97-129.65, 128.48-128.27, 88.28, 85.57, 84.74, 69.53, 63.27. The residue was dried thoroughly with anhydrous toluene and was resuspended in anhydrous toluene (10 mL) with freshly activated 4 Å molecular sieves. To this stirred solution, dibenzyl phosphate (1.3 g, 4.65 mmol, Fluka) and anhydrous Et3N (650 µl) were added and the reaction mixture was stirred under Ar atmosphere, at room temperature overnight. Toluene was evaporated under high vacuum and the residue purified using silica column chromatography (35% ethyl acetate in hexane) to afford pure compound 45 (1.45 g, 55%). Rf 0.36 (50% ethyl acetate in hexane); 1H NMR (CDCl3, 300 MHz) δ 8.08 (d, J = 7.8 Hz, 2H), 7.97-7.86 (m, 6H), 7.52-7.12 (m, 20H), 7.05-7.00 (m, 2H), 6.30 (t, J = 5.4 Hz, 1H), 6.13 (t, J = 7.2 Hz, 1H), 5.79 (q, J = 5.4 Hz, 5.4 Hz, 1H), 5.72-5.66 (m, 1H), 5.04-4.65 (m, 6H), 4.62-4.53 (m, 1H); 13C NMR (CDCl3, 75 MHz) δ 165.72, 165.49, 165.41, 165.29, 133.49, 133.47, 133.11, 132.95, 129.93-127.51; 31P NMR δ -2.45; ESMS m/z 879.24 (M+Na)+. The NMR and MS data of this compound was found similar to that reported by Marlow et al.110 and Tsvetkov et al.111

α-D-Galactofuranosyl bis-(triethylammonium) phosphate (46)110,111

To a solution of dibenzyl 2,3,5,6-tetra-O-benzoyl-α-D-galactofuranosyl phosphate (45, 1.45 g, 1.7 mmol) in ethyl acetate (20 mL), Et3N (1.6 mL) and hydrogenation catalyst 10% Pd/C (200 mg) were added. The reaction mixture was stirred under H2 atmosphere for 16 h, after which TLC and ESMS analysis showed that both the benzyl groups had been removed. The catalyst was removed by celite filtration and the filtrate was concentrated to give 2,3,5,6-tetra-O-benzoyl-α-D-galactofuranosyl bis-(triethylammonium) phosphate (1.1 g, 96%); 1H NMR (CDCl3, 300 MHz) δ 8.13 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 2H), 7.95-7.86 (m, 4H), 7.48-7.40 (m, 4H), 7.35-7.27 (m, 8H), 6.19 (t, J = 6.6 Hz, 1H), 5.85 (q, J = 6 Hz, 3 Hz, 1H), 5.62 (q, J = 4.5 Hz, 2.1 Hz, 1H), 4.86 (dd, J = 3.6 Hz, 8.4 Hz, 2H), 4.76-4.68 (m, 2H), 4.60 (t, J = 5.4 Hz, 1H), 3.24 (q, J = 7.5 Hz, 12H), 1.28 (t, J = 7.2 Hz, 18H); 13C NMR
(CDCl$_3$, 75 MHz) $\delta$ 165.90, 165.80, 165.68, 165.36, 133.18, 132.95, 132.80, 130.12-128.14, 95.88 (d, $J = 4.12$ Hz), 77.14, 76.64, 74.03, 71.56, 62.94, 46.56, 8.15; ESMS (negative ion) $m/z$ 675.55 (M-H$^-$). The residue was redissolved in MeOH-water-Et$_3$N (5:2:1; v/v/v; 64 mL) and the reaction was allowed to stir at room temperature for 72 h. TLC and ESMS analysis showed that all the benzoyl groups had been deprotected. The solvent was removed and the residue was purified by a quick silica column filtration (60% MeOH- CH$_2$Cl$_2$, 1% Et$_3$N) to afford pure compound 46 (367 mg, 83%). $R_f$ 0.46 (ethanol: ammonia: water, 5:3:1); $^1$H NMR (D$_2$O, 300 MHz) $\delta$ 5.43-5.31 (m, 1H), 4.06 (t, $J = 7.8$ Hz, 1H), 3.99-3.91 (m, 1H), 3.68-3.40 (m, 4H), 3.02 (q, $J = 7.5$ Hz, 12H), 1.30 (t, $J = 7.2$ Hz, 18H); $^{13}$C NMR (D$_2$O, 75 MHz) $\delta$ 96.65 (d, $J = 6.8$ Hz), 81.39, 76.48 (d, $J = 6.9$ Hz), 73.52, 71.77, 62.27, 46.60, 8.15. $^{31}$P NMR $\delta$ 2.48; ESMS (negative ion) $m/z$ 258.95 (M-H$^-$).

Uridine 5-(α-D-galactofuranosyl diphosphate) bis-(triethylammonium) salt (47)$^{111,112}$

α-D-Galactofuranosyl bis-(triethylammonium) phosphate (46, 70 mg, 0.27 mmol) was dried by coevaporating with anhydrous pyridine (1 mL) a few times. To this residue was added commercially available UMP-morpholidate (266 mg, 0.385 mmol, SIGMA) in anhydrous pyridine (1 mL), and the solution was evaporated again to dryness under vacuum. This was followed by the addition of 1H-tetrazole (35 mg, 0.49 mmol) in anhydrous pyridine (1.75 mL). The resulting solution was stirred at room temperature for 40 h. Removal of the solvent under vacuum gave a solid residue which was filtered quickly through a silica column (75% MeOH-CH$_2$Cl$_2$, 1% Et$_3$N). The desired fractions were concentrated and resuspended in water and loaded onto a Sephadex G-15 in water (5 cm x 1 cm); flow rate 1.5 ml/min. Water was used as the eluant and the desired fractions (detection by UV) were pooled and lyophilized. The residue was redissolved in water and the compound further purified by HPLC using C$_{18}$ column (Phenomenex, 250 x 10 mm). The eluant was 1.5% acetonitrile in 50 mM triethylammonium acetate buffer, pH 6.8, flow rate 2.5 mL/min. The retention time of compound 47 in these conditions was 17.1 min; $R_t$ (UDP-Galp) 13.0 min. Using analytical C-18 column,
elution with 50 mM triethylammonium acetate buffer, pH 6.8 containing 1.5% acetonitrile, 262 nm, 1 mL/min; Rf (UDP-Galp) 6.8 min and Rf (UDP-α-Galf) 8.2 min. Using PA-100 column, elution with 75 mM KH₂PO₄, pH 4.5, 262 nm, 1 mL/min; Rf (UDP-Galp) 23.5 min; Rf (UDP-α-Galp) 34.1 min. TLC (isopropanol: 1M ammonium acetate 2:1 v/v); Rf (UDP-Galp) 0.4; Rf (UDP-α-Galp) 0.46; ¹H NMR (D₂O, 300 MHz) δ 7.80 (d, J = 5.1 Hz, 1H), 5.86-5.78 (m, 2H), 5.48 (s, 1H), 4.22 (s, 2H), 4.16-3.96 (m, 5H), 3.71-3.46 (m, 4H), 3.05 (q, J = 7.5 Hz, 12H), 1.28 (t, J = 7.2 Hz, 18H); ESMS m/z 565.37 (M-H), m/z 687.34 (M+Na-H).

2.4.1.2 Synthesis of UDP-β-galactofuranose
(Scheme-21 of Results and Discussion)

2,3,5,6-Tetra-O-benzoyl-β-D-galactofuranosyl bis-(triethylammonium) phosphate (48)

To a stirred solution of acetyl O-2,3,5,6-tetra-O-benzoyl-D-galactofuranoside (44, 250 mg, 0.4 mmol) in anhydrous CH₂Cl₂ (2 mL) at 0 °C, 33% HBr-AcOH (0.625 mL) was added dropwise. The reaction mixture was stirred for 3.5 h. TLC analysis showed the complete conversion of compound 44 to its bromide derivative. The reaction mixture was diluted with CH₂Cl₂, and washed with ice cold water and saturated NaHCO₃ solution. The CH₂Cl₂ layers were dried (anhydrous Na₂SO₄) and concentrated. The residue was dried thoroughly with anhydrous toluene and was resuspended in anhydrous DMF (1.5 mL) with freshly activated 4 Å molecular sieves. To this solution, phosphoric acid (200 mg, 2 mmol, pre-dried with toluene) was added. The reaction was stirred for 1.5 h after which TLC confirmed consumption of the bromide. The reaction was quenched with Et₃N, filtered through celite and filtrate dried under high vacuum. Residue was purified using silica column chromatography (50% MeOH-CH₂Cl₂) to yield pure compound 48 (100.5 mg, 45%) Rf 0.3 (15% MeOH-CH₂Cl₂); ¹H NMR (CDCl₃, 300 MHz) δ 8.05-6.94 (m, 20H), 6.03 (s, 1H), 5.69-5.45 (m, 2H), 4.87-4.57 (m, 2H), 3.75-3.35 (m, 2H), 3.02 (q, J = 7.5 Hz, 12H), 1.30 (t, J = 7.2 Hz, 18H); ¹³C NMR (CDCl₃, 75 MHz) δ 165.99-165.37, 133.12-132.56, 129.84-128.08, 102.05 (d, J = 0.55 Hz), 82.43 (d, J
= 7.5 Hz), 76.91, 75.26, 70.31, 63.87, 45.61, 8.36; ESMS (negative ion) m/z 675.21 (M-H)^-.

**β-D-galactofuranosyl bis-(triethylammonium) phosphate (49)**

2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl bis-(triethylammonium) phosphate (48, 90 mg, 0.133 mmol) was dissolved in methanol:water:triethylamine (5:2:1; v/v/v; 5 mL) and stirred at room temperature for 72 h, after which TLC and ESMS analysis showed that all the benzoyl groups had been removed. The solvent was removed under vacuum and residue dissolved in water and loaded onto a Sephadex LH-20 column (5.7 x 1 cm). Elution was done with water. The fractions containing the compound (as judged by TLC) were pooled and lyophilized to give pure compound 49 (35 mg, 92%) Rf 0.22 (5:3:1 v/v/v EtOH:ammonia:water); \(^1\)H NMR (D\(_2\)O, 300 MHz) \(\delta\) 5.32 (d, \(J = 5.1\) Hz, 1H), 4.02-3.87 (m, 3H), 3.70-3.44 (m, 4H), 3.02 (q, \(J = 7.5\) Hz, 12H), 1.30 (t, \(J = 7.2\) Hz, 18H); \(^13\)C NMR (D\(_2\)O, 75 MHz) \(\delta\) 102.69 (d, \(J = 4.15\) Hz), 83.47, 81.82 (d, \(J = 7.61\) Hz), 76.71, 70.92, 62.56, 46.57, 8.17; \(^31\)P NMR (D\(_2\)O) \(\delta\) 0.32; ESMS (negative ion) m/z 257.99 (M-H)^-.

**Uridine 5-(β-D-galactofuranosyl diphosphate) bis-(triethylammonium) salt (50)**

β-D-Galactofuranosyl bis-(triethylammonium) phosphate (49, 30 mg, 0.25 mmol) was dried by coevaporating with anhydrous pyridine (1 mL) a few times. To this residue was added commercially available UMP-morpholidate (152 mg, 0.22 mmol, SIGMA) in anhydrous pyridine (1 mL), and the solution was evaporated again to dryness under vacuum. This was followed by the addition of 1H-tetrazole (20 mg, 0.28 mmol) in anhydrous pyridine (1 mL). The resulting solution was stirred at room temperature for 40 h. Removal of the solvent under vacuum gave a solid residue, which was applied to a preparative TLC plate (ethanol:water:ammonia; 5:1:3). The desired band was scraped and eluted from silica using the same solvent. The fractions were concentrated to give partially purified 50 (17.2 mg). The residue was redissolved in water and the compound further purified by HPLC using C\(_{18}\) column (Phenomenex, 250 x 10 mm). The eluant was 1.5% acetonitrile in 50 mM triethylammonium acetate buffer, pH 6.8, flow rate 2.5 mL/min. The retention time...
of compound 50 in these conditions was 14.8 min. Using analytical C-18 column, elution with 50 mM triethylammonium acetate buffer, pH 6.8 containing 1.5% acetonitrile, 262 nm, 1 mL/min; Rf (UDP-α-Gal) 8.2 min; Rf (UDP-β-Gal) 7.1 min. 

\[ \text{1H NMR (D}_2\text{O, 300 MHz)} \delta 7.8 (d, J = 5.1 \text{ Hz}, 1\text{H}), 5.86-5.70 (m, 2\text{H}), 5.45 (s, 1\text{H}), 4.23 (s, 2\text{H}), 4.16-3.89 (m, 5\text{H}), 3.71-3.45 (m, 4\text{H}), 3.18 (q, J = 7.5 \text{ Hz}, 12\text{H}), 1.28 (t, J = 7.2 \text{ Hz}, 18\text{H}); \text{ESMS (negative ion) } m/z 565.83 (M-H)^- \]

2.4.2 Synthesis of exogenous acceptors

(Scheme-22 and 23 of Results and Discussion)

2,4,6-Tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl) mannopyranose (51)

To a solution of benzyl 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl) mannopyranoside (34, 600 mg, 0.83 mmol) in ethyl acetate (12 mL), sodium bromate solution (0.372 g in 8.2 mL water) was added and the biphasic solution was stirred vigorously at room temperature. Sodium disulphite solution (0.43 g in 16.4 mL water) was added dropwise to this solution. The reaction was stirred at room temperature for 7 h, TLC after which showed 70% reaction completion. The reaction was quenched with saturated sodium thiosulphate solution and extracted with ethyl acetate. The ethyl acetate layers were dried and concentrated. The residue was purified using silica column chromatography (60% ethyl acetate in hexane) to give pure compound 51 (310 mg, 60%). Rf 0.2 (60% ethyl acetate in hexane); \[ \text{1H NMR (CDCl}_3, 300 \text{ MHz)} \delta 5.37-5.21 (m, 5\text{H}), 5.01 (d, J = 2.4 \text{ Hz}, 1\text{H}), 4.32-4.20 (m, 9\text{H}), 2.22-1.97 (7 \times s, 21\text{H}); \text{13C NMR (CDCl}_3, 75 \text{ MHz)} \delta 170.79, 170.74, 170.43, 169.92, 169.87, 169.80, 169.51, 98.68, 91.99, 74.07, 71.36, 69.95, 68.96, 68.24, 68.17, 67.77, 66.04, 62.60, 62.32, 20.72-20.48, \text{ESMS } m/z 669.23(M+Na)^+ \]
2,4,6-Tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl) mannopyranosyl trichloroacetimidate (52)

2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl) mannopyranose (51, 150 mg, 0.3 mmol) was dissolved in anhydrous CH\(_2\)Cl\(_2\) (1.4 mL). The solution was cooled to 0 °C and trichloroacetonitrile (321 \(\mu\)L) and DBU (22 \(\mu\)L) were added sequentially. The reaction mixture was stirred at 0 °C for 4 h, after which TLC showed reaction completion. The solution was filtered through celite and concentrated. The residue was purified using silica column chromatography (45% ethyl acetate in hexane) to give pure compound 52 (150 mg, 82%) \(R_f\) 0.28 (60% ethyl acetate in hexane).

Decenyl 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl) mannopyranoside (53)

2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl) mannopyranosyl trichloroacetimidate (52, 130 mg, 0.167 mmol) and 9-dec-1-enol (59 \(\mu\)L, 0.334 mmol, Aldrich) were taken in anhydrous diethyl ether (3 mL). The solution was stirred with freshly activated 4 Å molecular sieves under Ar atmosphere at room temperature for 10 min. To this stirred solution, TMSOTf (13 \(\mu\)L, 0.33M solution in diethyl ether) was added dropwise and reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with solid NaHCO\(_3\) and filtered through celite. The filtrate was concentrated and residue purified using silica column chromatography (25% ethyl acetate in hexane) to give pure compound 53 (58 mg, 45%). \(R_f\) 0.35 (50% ethyl acetate in hexane); \(^1\)H NMR (CDCl\(_3\), 300 MHz) \(\delta\) 5.87-5.73 (m, 1H), 5.28 (m, 4H), 5.03-4.89 (m, 4H), 4.80 (s, 1H), 4.32-4.00 (m, 8H), 3.90-3.80 (m, 1H), 3.69-3.59 (m, 1H), 3.48-3.37 (m, 1H), 2.19 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.60-1.51 (m, 2H), 1.40-1.21 (m, 12H); ESMS \(m/z\) 797.42 (M+Na)

Decenyl 3-O-(\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-D-mannopyranoside (54)

Decenyl 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl) manno pyranoside (53, 50 mg, 0.06 mmol) was dissolved in methanol (1.5 mL). To this
solution, sodium methoxide (30 μL, saturated solution in methanol) was added and reaction mixture stirred at room temperature for 2 h. Amberlite 1R-120 (10 mg) was added to neutralize the reaction mixture. The solution was filtered and the filtrate concentrated to give a residue which was purified using silica column chromatography (25% MeOH in CH₂Cl₂) to yield compound 54 (21 g, 68%). Rf 0.4 (isopropanol: ammonia: water 7:2:1 v/v/v); ¹H NMR (D₂O, 300 MHz) δ 5.86-5.61 (m, 1H), 5.11-4.79 (m, 3H), 4.04-3.94 (m, 2H), 3.88-3.45 (m, 12H), 2.02-1.90 (m, 2H), 1.60-1.47 (m, 2H), 1.36-1.31 (m, 12H); ¹³C NMR (D₂O, 75 MHz) δ 138.73, 102.21, 99.97, 78.81, 73.15, 72.68, 70.43-69.91, 67.73, 66.52, 65.38, 62.46, 60.92-60.51, 33.62, 29.33-28.77, 25.95; ESMS m/z 503.00 (M+Na)⁺

Benzyi 3-0-(-α-D-mannopyranosyl) mannopyranoside (55)

Benzyi 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl) manno-pyranoside (34, 600 mg, 0.83 mmol) was dissolved in methanol (7 mL). To this solution, sodium methoxide (75 μL, saturated solution in methanol) was added and reaction mixture stirred at room temperature for 2 h. Amberlite 1R-120 (50 mg) was added to neutralize the reaction mixture. The solution was filtered and the filtrate concentrated to give pure compound 55 (280 mg, 78%); ¹H NMR (D₂O, 300 MHz) δ 7.28-7.20 (m, 5H), 4.94 (s, 1H), 4.76 (s, 1H), 4.55 (d, J = 12 Hz, 1H), 4.38 (d, J = 12 Hz, 1H), 3.92 (d, J = 6 Hz, 2H), 3.79-3.47 (m, 11H); ESMS m/z 455.41 (M+Na)⁺
2.4.3 Characterization of enzymes involved in the galactofuranose assembly in LPG biosynthesis.

2.4.3.1 Culture of *L. donovani* promastigotes

As a prerequisite of biosynthetic experiments, *L. donovani* (DD8 strain) promastigotes were grown in the following media.

1. **Medium 199**: This medium was prepared by dissolving one sachet (9.5 g) of powdered Medium-199 (GIBCO) in one liter of Milli Q UF water. To this was added 50 mg of gentamycin sulfate and 5.9 g of HEPES (25 mM). The medium was filter-sterilized using bell filter (0.22 μ, Sterivex-GV; Millipore).

2. **Medium 199 with 10% HI-FBS**: To the above Medium-199, as per the requirement of routine culture, heat inactivated fetal bovine serum (HI-FBS, prepared by heating the sealed bottle of FBS in a water bath at 56 °C for 30 min) was added @ 10%.

3. **dDME medium**: This was prepared from powdered Dulbeccos modified Eagles medium (DMEM) sachet (13.5 g) by dissolving the contents in one liter Milli Q UF water and then adding 50 mg/L biotin, 40 mg/L Tween 80, 5mg/L hemin and 0.5 ml triethanolamine. This modification of DMEM was done according to Orlandi and Turco\(^{36}\) who termed it as dDME. To this was added 50 mg of gentamycin sulfate and 5.9 g of HEPES (25 mM) were added. This modified DMEM was filter-sterilized using bell filter (0.22 μ, Sterivex-GV; Millipore).

4. **dDME medium with 10% HI-FBS**: To the above dDME medium, as per the requirement of routine culture, HI-FBS was added @ 10%

*Leishmania donovani (DD8 strain) promastigotes*: In the present study throughout *Leishmania donovani*, DD8 strain, promastigotes (obtained from Prof. K. P. Chang) were used. The cells were expanded in Medium-199 with 10% HI-FBS. \(10^7\) viable parasites were taken in 1ml of complete Medium-199 containing 10% glycerol. These were stored in liquid nitrogen. The revival of these frozen cells was checked by snap-thawing of the contents of one vial at 37 °C and then inoculating 50 ml of medium-199 with the entire contents. A luxuriant growth with healthy viable parasite was observed under microscope.
Routine culture of *L. donovani* promastigotes: Routinely *L. donovani* promastigotes were cultured in either medium-199 (for maintenance of stock) or dDME (for biosynthetic experiments). In a T-125 flask, 50 ml of medium was inoculated with 0.1 ml of previous culture flask containing $10^6$ promastigotes. These flasks were incubated at 23°C in a cooling incubator (CI-12S, REMI). Fresh passaging was done every 4th/5th day in a similar fashion. Random samples from culture flasks, free from any visible contamination and full of all healthy, motile parasites formed the basis of selection of the culture suitable for further use. After culturing, used flasks, pipettes, glassware etc. were decontaminated by immersing them in 5% formaldehyde solution and then discarded. All other routine standard cell-culture practices were observed as per guidelines.

Harvesting and initial processing of promastigotes: Cultured promastigotes were harvested by centrifugation of suspension culture in a Falcon tube (15/50 ml) at 3000 g, 20 min, 4 °C, in a cooling centrifuge (Rota 4R; Plastocraft). The clear spent medium was carefully decanted and pellet resuspended in phosphate buffer saline (PBS, pH 7.2). Centrifugation was again done as earlier and washings collected. Before the third washing, the promastigotes in PBS were counted using a Neubauer Chamber. For this an aliquot was taken and diluted with PBS (normally 10 μl original suspension was mixed with 60 μl PBS) and then formaldehyde was added to this (normally 30 μl to give a final dilution of 1:10). After 10 min of fixing in formaldehyde, 10 μl of this suspension was charged under the coverslip on Neubauer Chamber and counted. Calculations were done by the standard way.

2.4.3.2 Preparation of cell-free system of *L. donovani*

This was done by the using a slightly modified version of a reported procedure. For breaking cells, the cell pellet was suspended in hypotonic buffer (0.1 mM TLCK and 1μg/mL leupeptin) at a cell concentration of $10^9$ cells/mL. The cells were incubated in this hypotonic buffer for 30 min on ice. The cell-suspension was then transferred to a Parr N₂ cavitation bomb. The cells were equilibrated at 1500 psi, 25 min, 4 °C. This was repeated twice. The cell free suspension was collected in a Falcon tube. Centrifugation at 1000g, 7 min, 4 °C (Rota 4R; Plastocraft) removed
the cell debris. Breaking of cells was assessed by a light microscope. The membrane protein was further processed as per the requirement of the experiment. The supernatant (S1) thus obtained contains both the cytosolic and the membrane proteins. S1 was further subjected to ultracentrifugation (1,00,000 g, Beckman L-80, 43,000 rpm, 4 °C, 1h). The supernatant S2 contained the cytosolic proteins and the pellet P2 contained the membrane proteins.

2.4.3.3 **Estimation of protein (using BCA protein estimation kit, Pierce)**
Dilutions (x 5, x 10, x 50, x 100) of the unknown protein sample were made in MQ water. In an ELISA reader plate (96 well plate), 20 μL of the protein sample (designated as Neat) was added in duplicate. 20 μL each of the different diluted samples were also added in duplicates. 20 μL of MQ water was used to give the BLANK readings. 200 μL of the BCA kit working solution (made up mixing Solution A and Solution B in the ratio of 50:1 respectively). The plate was covered with an aluminium foil and incubated at 37 °C for 0.5 h. The plate was cooled to room temperature and OD at 562 nm was noted using an ELISA plate reader. Concentration of the unknown protein solution was found out using the standard BSA curve.

2.4.3.4 **UDP-galactopyranose mutase assay**
In an eppendorf, UDP-Galp (15 μL from 1 mg/mL solution, 25 nmol) or UDP-Galf (1.2 μL from 1mg/mL solution, 1 nmol), ATP (5 μL, 1 mM, always freshly prepared), NADH (8 μL, 100 mM, freshly prepared), FAD (5 μL, 0.2 mM) were mixed. To this 100 mM MOPS buffer, pH 7.6 (50 μL) was added. The reaction was initiated by adding the protein solution (17 μL, 25 μg, whole cell lysate). The total volume of the reaction mixture was 100 μL (in case of UDP-Galf addition, the volume made up by addition of 13.8 μL of MQ water). The reaction was incubated at 30 °C for 1h. The reaction was quenched by placing the tubes on ice. The reaction mixture was centrifuged at 14000g, 25 min, 4 °C in a microcentrifuge (Eppendorf, Centrifuge 5415 R).
For HPLC detection:
The supernatant was passed through 0.45 μ syringe filters. The filtrate was injected into either (1) C-18 HPLC column, elution with 50 mM triethylammonium acetate buffer, pH 6.8 containing 1.5% acetonitrile, 262 nm, 1 mL/min or (2) PA-100 Dionex HPLC column, elution with 75 mM KH₂PO₄, pH 4.5, 262 nm, 1 mL/min.
For radioactive analysis (in cases where UDP-Galp was used as the substrate and 1 μCi of UDP-³²H-Galp was also incorporated in the assay). The supernatant was dried under a stream of nitrogen and resuspended in 10 μL of MQ water. The solution was now spotted onto a TLC. The solvent system used was isopropanol: 1 M ammonium acetate (2:1). The plate was scanned using a radioactive scanner (BIOSCAN, AR-2000).

2.4.3.5 Galactofuranosyl transferase assay

Standardization of elution of Dec-1-enyl 3-O-(α-D-mannopyranosyl)-α-D-mannopyranoside (Compound 54) from C-18 Sep Pak Cartridges: C-18 Rainin Sep-Pak cartridges were washed with 80% propanol followed by extensive washing with 0.1 M ammonium acetate. 1 mg of compound 54 was suspended in 0.5 mL of 0.1 M ammonium acetate and loaded onto the cartridge. The first 0.5 mL that eluted out first was reloaded so as to ensure maximum binding. The column was washed with 0.1 M ammonium acetate (in 0.5 mL, 0.6 mL, 1.0 mL, 1.0 mL, 1.0 mL fractions). Then the column was eluted with 60% propanol (in 0.5 mL, 1.0 mL, 1.0 mL, 0.5 mL fractions). TLC and NMR analysis showed that compound 54 eluted out in the first 1.0 mL fraction of 60% propanol. There was 80% recovery of the compound. The fraction was concentrated and reloaded on a previously activated cartridge and a similar pattern was reproduced.

Galactofuranosyl transferase assay: In an eppendorf, UDP-Galp (12 μg, effectively 200 μM in assay), UDP-³²H-Galp (1 μCi) and synthetic acceptor, compound 54 (30 μg, effectively 600 μM in assay) were mixed and dried under a stream of nitrogen. To the residue was added 25 μL of 100 mM HEPES-NaOH buffer, pH 7.6 containing 100 mM NADH, 10 mM MgCl₂, 20 mM MnCl₂, 2 mM ATP, 0.2 mM
TLCK. The reaction was initiated by adding protein solution (25 μL, 37.5 μg, whole cell lysate). The reaction mixture was incubated at 37 °C for 15 min and quenched by placing the tubes in ice. The reaction was centrifuged at 5000 g for 15 min at 4 °C. The supernatant was dried under a stream of nitrogen and the residue resuspended in 0.5 mL 0.1 M ammonium acetate. This was loaded onto C-18 Sep Pak cartridge and eluted as described earlier. 10 μL of each fraction was added to 5 mL Cocktail-W and was counted using scintillation counter.