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Total synthesis of the fully lipidated glycosylphosphatidylinositol (GPI) anchor of malarial parasite *Plasmodium falciparum*

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ABSTRACT

We report a new and convergent strategy for the total synthesis of fully lipidated glycosylphosphatidylinositol (GPI) anchor, the major pro-inflammatory factor of malarial parasite (*Plasmodium falciparum*). The key features of our approach include, the access to the key glucosamine–inositol intermediate by a novel route without a priori resolution of *myo*-inositol, convergent assembly of the tetramannose glycan domain, flexibility for the placement of the three fatty acids in the desired order in the final steps, and the opportunity to construct GPI analogues/mimics to probe the biosynthesis, immunology and cell biology of the GPI anchor pathway in the malaria parasite.

1. Introduction

Malaria affects 600 million people worldwide and kills 2 million annually,[1] mostly children in Africa and Asia, and most of the mortalities are due to the cerebral malaria caused[2] by *Plasmodium falciparum* species. Furthermore, the parasite (*P. falciparum*) has developed resistance against most of the currently used antimalarial drugs and major global vaccine efforts have not succeeded. Severe disease pathology of malarial infection (periodic fever and cerebral malaria) and fatalities have been linked to the unique cell-surface glycosylphosphatidylinositol (GPI) molecules (Fig. 1), the so-called malaria toxins, that are expressed/released by *P. falciparum* during its blood stage life-cycle.[3]

A number of landmark studies have demonstrated that the *P. falciparum* glycosylphosphatidylinositols (Pf-GPIs) trigger a major pro-inflammatory cytokine cascade[4–6] in the host and an anti-GPI antibody was shown to provide protection[7] to the host from the disease. The unique chemical structure of GPI anchor of *P. falciparum* with three fatty acids in the lipid domain (Fig. 1), coupled with its remarkable biosynthetic pathway and immunological mechanism has generated worldwide interest as a potential therapeutic target for anti-disease vaccine and drug design.

Since it is extremely difficult to isolate relevant amounts of homogeneous and pure GPI molecules and their biosynthetic intermediates from the malaria parasite culture, deciphering their biosynthetic assembly and their role in disease pathology and immunology critically depends on the efficient methods for chemical synthesis of Pf-GPIs and the biosynthetic intermediates. Two key structural features that distinguish malarial-GPIs from that of other parasitic species include; the presence of an extra fatty acid at 2-position of the *myo*-inositol residue rendering Pf-GPIs resistant to the host phosphatidylinositol-specific phospholipase C (PI-PLC) mediated hydrolysis, and an additional fourth mannose at the

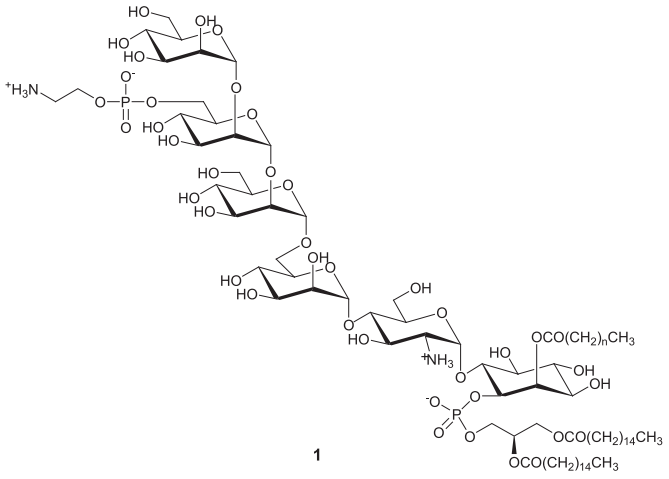

Figure 1. Structure of GPI anchor of *P. falciparum*.

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non-reducing end of the phosphoglycan domain. These features of Pf-GPIs imparting remarkable (nM) pro-inflammatory activity[4–6] (comparable to that of the bacterial lipopolysaccharide) present substantial difficulty for synthesis.

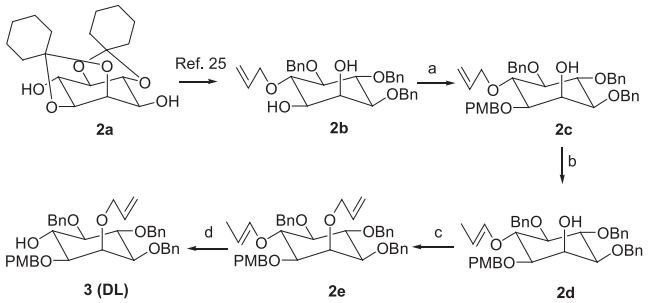
Although a number of approaches have been reported[8–12] by various leading groups for the synthesis of GPI anchors of various parasitic species, including our own approach,[13] the GPI molecules of the malarial parasite present increasing challenges due to the presence of a third fatty acid group at the 2-position of the *myo*-D-inositol residue. For this reason, so far only one total synthesis of fully lipidated Pf-GPI has been reported (Seeberger et al.).[14] In addition to this, a synthesis of a model GPI (lacking fourth mannose and with short-chain fatty acid lipids) has been reported (Fraser-Reid et al.).[15] We have recently reviewed[16] the previous work on synthesis of GPI molecules of various species. Keeping in view the remarkable biological role[4–7] of Pf-GPIs in the malarial biology and immunology, efficient methods for synthesis of Pf-GPIs and their structural and functional mimics are critical for the further advances in this field of glycobiology. We have had continuing interest in the chemical biology of GPI molecules and related phospholycans, including new synthesis,[13,16–18] biosynthesis,[19–22] and cell biology.[23]

2. Results and discussion

We now report a new and convergent total synthesis of the fully lipidated GPI anchor of the malarial parasite *P. falciparum*. The key features of our approach include: (a) access to the glucosamine–inositol intermediate by a novel route without a priori resolution of *myo*-inositol; (b) convergent assembly of the tetramannose glycan; (c) flexibility to place three fatty acids in the desired order in the final steps; (d) possibility to construct [4-deoxy-Man-III]-GPI analogues. Such deoxy-GPI analogues may serve as valuable conformational probes to address a fundamental biological question relevant to the GPI biology: why the proteins are transferred[24] to the terminal primary alcohol (6-OH) of the third mannose residue irrespective to whether the nascent GPI precursor contains either three mannoses as in *Leishmania* and human species or four mannoses as in *Trypanosoma* and *Plasmodium* species. It needs mention here that the GPI molecules isolated from the culture of *P. falciparum* predominantly contain palmitate and stearate (or their combinations) in their lipid domain. We have chosen in the present study to synthesize the target Pf-GPI anchor with the palmitate residues. However, the approach described in this paper is suitable for the placement of other saturated and mixed fatty acids as well.

To address the key issue of the placement of the third fatty acid in the *myo*-inositol residue of the Pf-GPI, we have devised a new way to assemble the optically pure glucosamine–inositol intermediate. Arguably, the most demanding aspect of the GPI synthesis has been to access suitably protected glucosamine–inositol block requiring optically pure protected D-*myo*-inositol acceptor and 2-azido-2-deoxyglucosyl donor. This has mainly been accomplished by previous workers[8–12] either by the a priori resolution of bis-cyclohexylidene-*myo*-inositols by chiral auxiliaries and enzymes or through a multi-step synthesis from D-glucose by the Ferrier reaction.

We addressed this issue differently and instead of resorting to a priori resolution of *myo*-inositol, we reasoned based on structural modeling that if sufficient strain is built through a cyclic benzylidene protecting group, the 2-deoxy-2-azidoglycosyl residue itself could function as an efficient chiral auxiliary, making a number of early resolution steps redundant. This methodology was successfully tested, as reported in our preliminary communication[13] with the glycosylation of racemic 1-*O*-PMB-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol with 2-azido-2-deoxyglycosyl trichloroacetimidate. The product of this reaction on deacylation and 4,6-benzylidenation provided clean separation of two diastereomeric pseudodisaccharides by a simple silica chromatography. This gave us the confidence to extend this approach for the synthesis of 2-azido-2-deoxyglycosyl-(1→6)-*myo*-D-inositol with an additional protecting group at the 2-position of the *myo*-inositol residue for the placement of the third fatty acid in the target Pf-GPI anchor. For this, the first intermediate, racemic 1-*O*-PMB-2-*O*-allyl-3,4,5-tri-*O*-benzyl-(DL)-*myo*-inositol (**3**), was prepared (Scheme 1) from the known[25] 1,2:4,5-bis-cyclohexylidene-(DL)-*myo*-inositol (**2a**). The 3-OH of the starting material **2a** was selectively protected by $BaO/Ba(OH)_2$ mediated benzylation followed by the allylation of the remaining 6-OH. Next three steps, acidic hydrolysis of the 4,5-cyclohexylidene *trans*-ketal (*p*-TSA, CH_2Cl_2/CH_3OH, 3:1, rt), benzylation, and hydrolysis of the 1,2 *cis*-ketal (*p*-TSA, 50 °C) were carried out as described by Brimacombe et al.[25] to afford compound **2b**.

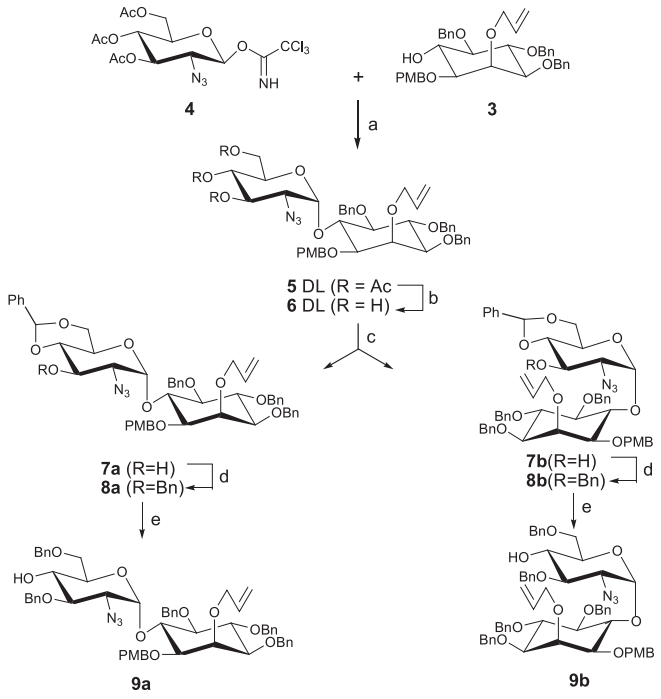


Scheme 1. (a) Bu_2SnO, CsF, KI, MeOH, PMBCl, DMF, rt, 24 h, 94%; (b) *t*-BuOK, DMSO, 80 °C, 3 h, 85%; (c) Allyl Br, NaH, DMF, 3 h, 90%; (d) acetone/1 N HCl (9:1), 50 °C, 0.5 h, 83%.

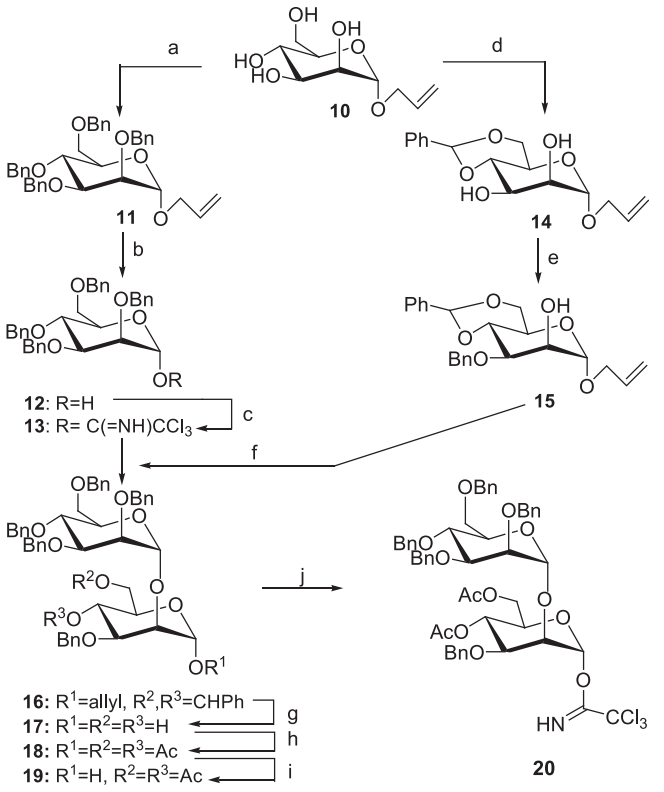
The 1-OH of **2b** was selectively alkylated with PMB group using dibutyltin chemistry to get **2c** in excellent yield. Next step involved the 'half' deprotection of an allyl group to a vinyl group under basic condition (**2c**→**2d**) enabling the placement of an additional allyl group at the 2-OH (**2d**→**2e**); this allyl group would eventually be unmasked in the final steps of the synthesis to install the critical third fatty acid characteristic of the Pf-GPI anchor. The removal of the vinyl from the 6-OH of **2e** under mild acidic condition provided the desired *myo*-(DL)-inositol building block **3** in racemic form.

The racemic *myo*-inositol intermediate **3** was now glycosylated (TMSOTf, CH_2Cl_2, −5 °C) with known[26] glycosyl donor (2-azidoglycosyl trichloroacetimidate, **4**) providing the pseudodisaccharide **5** in 85% yield (Scheme 2) as a diastereoisomeric mixture containing D and L *myo*-inositol residues. The glycosylation at the 6-OH of compound **3** proceeded with excellent α stereoselectivity (α/β ratio of 95:5) as established by detailed ^1H and ^{13}C NMR analysis. The desired α-isomeric product was purified by silica column chromatography to remove 5% impurity of the β-isomer. Moreover, the presence of an allyl group at the 2-OH position did not impact on the anomeric selectivity at 6-OH. The next steps involved removal of three acetyl groups (**5**→**6**) followed by reaction with benzaldehyde dimethylacetal under acid catalysis, which provided diastereomeric mixture of 4,6-cyclic acetals **7a** and **7b**. These diastereoisomers showed excellent chromatographic separation, as observed[13] previously by us in the case of their 2-*O*-benzylated counterparts. The pseudodisaccharides **7a** and **7b** were isolated by silica column and rigorously characterized by detailed ^1H and ^{13}C NMR analysis.

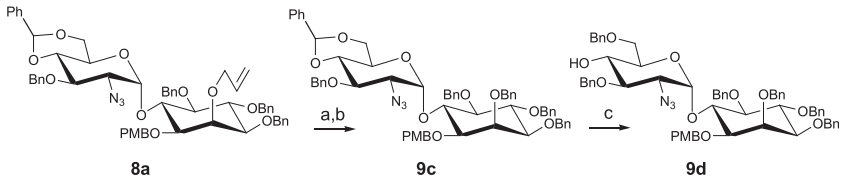
The benzylation of the free hydroxyl at the 3-position of both isomers (**7a**→**8a** and **7b**→**8b**) followed by the regioselective cleavage of 4,6-benzylidene acetal of the corresponding **8a** and **8b** by $NaCNBH_3$ under acid catalysis provided desired 2-azido-2-deoxyglucopyranosyl-(1→6)-*myo*-D-inositol **9a** and its non-natural counterpart 2-azido-2-deoxyglucopyranosyl-(1→6)-*myo*-L-inositol **9b** in excellent yields.



Scheme 2. (a) TMSOTf, CH_2Cl_2, 4 Å MS, −5 °C, 0.5 h, 85%; (b) NaOMe, CH_2Cl_2/CH_3OH (1:3), 2 h, rt, 91%; (c) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 24 h, 79%; (d) BnBr, NaH, DMF, rt, 4 h, 98%; (e) $NaBH_3CN$, HCl/Et_2O, 0 °C, 0.5 h, 94%.



Scheme 4. (a) BnBr, NaH, DMF, 24 h, 83%; (b) *t*-BuOK, DMSO, 80 °C, 2 h, acetone/1 M HCl (9:1), 40 °C, 0.5 h, 95%; (c) CCl_3CN, CH_2Cl_2, DBU, rt, 1.5 h, 98%; (d) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 18 h, 74%; (e) BnBr, NaH, DMF, 0 °C, 65%; (f) TMSOTf, Et_2O, rt, 2 h, 81%; (g) *t*-BuOK, DMSO, 80 °C, 3 h, acetone/1 M HCl (9:1), 55 °C, 2 h, 82%; (h) Ac_2O, pyridine, rt, 24 h, 93%; (i) Me_2NH, CH_3CN, −20 °C, 1 h, 94%; (j) CCl_3CN, CH_2Cl_2, DBU, rt, 1 h, 96%.



Scheme 3. (a) *t*-BuOK, DMSO, 80 °C, 3 h, $HgCl_2$, HgO, acetone/H_2O (3:1), 10 min, rt, 74%; (b) BnBr, NaH, DMF, rt, 3 h, 92%; (c) $NaBH_3CN$, HCl/Et_2O, 0 °C, 30 min, 74%.

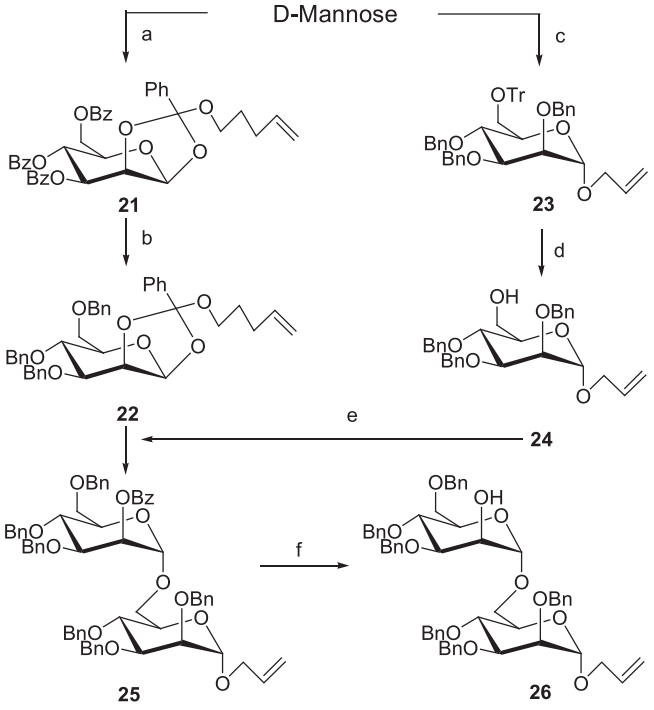
To confirm the absolute stereochemistry of **7a**/**8a**/**9a** series, compound **8a** was transformed (Scheme 3) to a previously reported[27] compound, (2-azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1-6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol (**9c**), by replacing 2-*O*-allyl of **8a** with a benzyl group. Compound **9c** showed all the spectral data and $[\alpha]_D$ value identical to that reported[27] for this compound by an alternative route. Furthermore, compound **9c** on regiospecific opening of the benzylidene ring afforded (2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol (**9d**) with $[\alpha]_D$ matching with the reported[27] compound.

After the efficient access to the optically pure key 2-azido-2-deoxyglucopyranosyl-(1→6)-*myo*-D-inositol **9a** in practical yields, a convergent [2+2] approach for synthesis of tetramannose glycan **29** was designed from two suitably protected mannobiosides, the activated donor **20** and the acceptor **26** (Schemes 4 and 5, respectively). Compound **20** was prepared by the coupling of tetra-*O*-benzyl-α-D-mannosyl trichloroacetimidate[28] (**13**, prepared from known[29] allyl mannopyranoside **10** by perbenzylation, allyl deprotection, and trichloroacetimidation) with allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannoside (**15**, prepared from **10** by 4,6-benzylidenation,[30] and benzylation[27] at 3-OH). The above glycosylation (TMSOTf, Et_2O, rt, 2 h) went smoothly to afford **16** and major α anomer was isolated by silica column chromatography, the stereochemistry of new glycoside bond ascertained by NMR (δ_H 5.41, br s; δ_C 98.23, COSY and HSQC).

The simultaneous removal of anomeric allyl and 4,6-benzylidine groups (*t*-BuOK, DMSO, 80 °C; 1 M HCl/acetone, 1:9 v/v) from **16** provided the triol **17**, and no unwanted deprotection of benzyloxy groups was observed under the acidic conditions (1 M HCl/acetone; 1:9 v/v). The per-acetylation (**17**→**18**), selective removal of anomeric acetyl group by freshly prepared dimethylamine solution in anhyd acetonitrile at −20 °C provided compound **19** with free anomeric hydroxyl group. The glycosyl activation of compound **19** by Schmidt method (CCl_3CN, DBU) provided desired mannobiose donor **20**. Notably, two acetyl groups were placed strategically at 4- and 6-OH to open the avenue for the synthesis of [4-deoxy-Man-III]-GPI probes.

The mannobiose intermediate **26** was synthesized (Scheme 5) by glycosylation of allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**24**) (prepared from D-mannose by anomeric allylation[29] (**10**), selective tritylation at the 6-OH, and perbenzylation followed by the removal of trityl group) with known[31] pentenyl mannose orthoester (**22**).

The glycosylation of **24** with **22** went cleanly using triethylsilyltriflate (TESTOf) and *N*-iodosuccinimide (NIS)-mediated condition, which provided the desired disaccharide **25** in excellent yield and isomeric purity. The stereochemistry of new glycoside bond in **25** was confirmed by NMR (δ_H 5.19, J=1.9 Hz; δ_C 96.83, COSY and



Scheme 5. (a) (i) BzCl, pyridine, rt, 24 h; 30% HBr/AcOH, CH_2Cl_2, 0 °C, 12 h; (ii) 4-pentenol, 2,6-lutidine, CH_2Cl_2, 60 h, 82%; (b) (i) NaOMe, CH_2Cl_2/CH_3OH, 2 h; (ii) BnBr, NaH, DMF, rt, 24 h, 93%; (c) (i) allyl alcohol, $BF_3{\cdot}Et_2O$, 80 °C, 4 h; (ii) TrCl, pyridine, 80 °C, 16 h, 70%; (iii) BnBr, NaH, DMF, 4 h, 89%; (d) *p*-TSA, CH_2Cl_2/CH_3OH, rt, 12 h, 93%; (e) TESOTf, NIS, CH_2Cl_2, 4 Å MS, rt, 0.5 h, 72%; (f) NaOMe, CH_2Cl_2/CH_3OH, rt, 3 h, 92%.

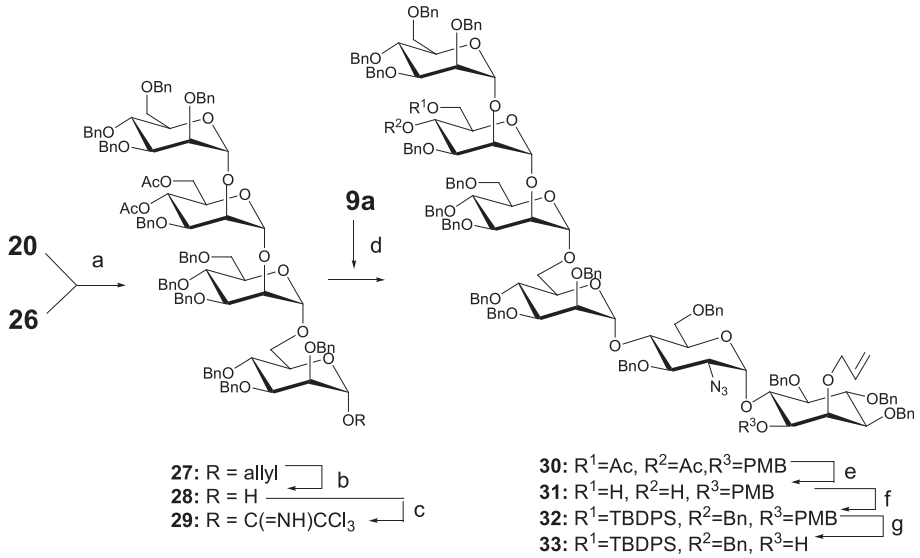
HSQC). Finally, the lone benzoyl group from the 2-position of the upper mannose residue was easily removed (NaOMe/MeOH) to expose free 2-OH in compound **26**.

The glycosylation of the mannobiose donor **20** with the mannobiose acceptor **26** (Scheme 6) required considerable efforts. Various glycosylation conditions (temperature variation, solvents, and catalysts such as $BF_3{\cdot}Et_2O$, triflic acid, TBDMOSTf, etc.) failed to provide the desired product. Finally, the optimum yield (69%) of the desired α-anomer of the tetrasaccharide (**27**) was obtained using TMSOTf at 0 °C in anhyd Et_2O for 0.5 h and freshly activated powdered molecular sieves (4 Å), the stereochemistry of new glycoside bond confirmed by NMR analysis. This was followed by the deprotection of the anomeric allyl group ($PdCl_2$, AcOH) providing compound **28**. The activation of the free anomeric hydroxyl by the Schmidt method afforded the tetramannose trichloroacetimidate donor **29**.

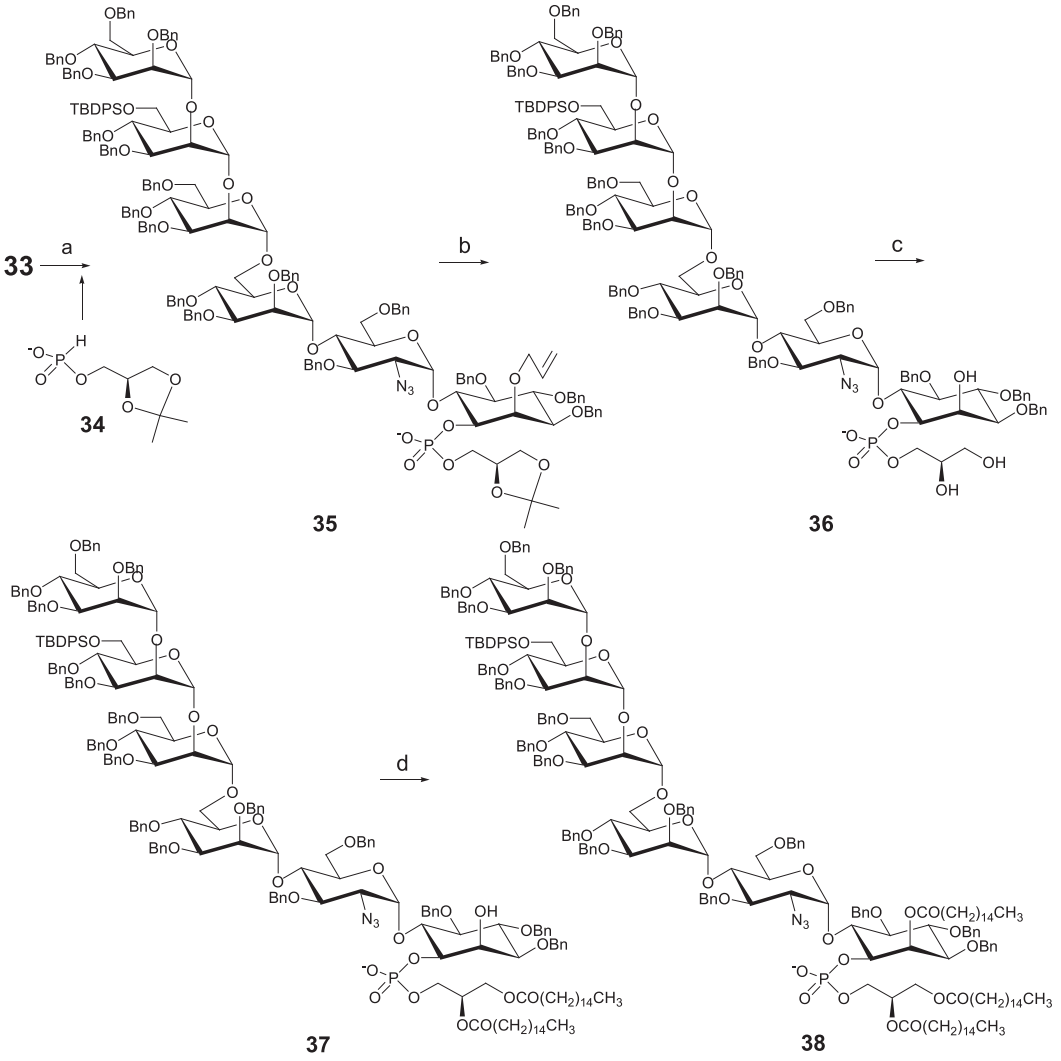
Now, the above tetramannose donor **29** was reacted with 2-azido-2-deoxy glycosyl-(1→6)-*myo*-D-inositol acceptor (**9a**). To our satisfaction, this important glycosylation went smoothly (TMSOTf, CH_2Cl_2, 0 °C) with acceptable stereocontrol (80:20 anomeric ratio of α and β isomers), the desired α isomer pseudohexasaccharide **30** was isolated by silica chromatography and the undesired β-isomer discarded. The stereochemistry of four mannosyl residues (δ_H 5.26, 5.28, 5.37, 5.38) and azidoglycosyl residue (δ_H 5.80, J=3.8 Hz) was determined by NMR analysis. The two acetyl groups from the third mannose residue of **30** were now removed to expose two hydroxyls in compound **31**, which could be easily purified. Selective protection of primary 6-OH with TBDPS group went smoothly (TBDPSCl, imidazole, THF, 5 h, 89%) and the remaining 4-OH was blocked with careful benzylation (BnBr, NaH, DMF, 0 °C, 1 h) to afford pseudohexasaccharide **32** in 97% yield. Now the removal of PMB group from the 1-position of inositol residue by ceric ammonium nitrate provided fully functional pseudohexasaccharide **33**, ready for phosphorylation with the required glycerolipid domain.

Initially we made several attempts at phosphorylation of hexasaccharide-glycan **33** with freshly prepared 1,2-dipalmitoyl-*sn-glycero-H*-phosphonate and 1,2-disteroyl-*sn-glycero-H*-phosphonate and their phophoroamidite counterparts (as reported in all previous syntheses of GPI anchors) but failed to obtain desired phosphorylation at 1-position of the *myo*-inositol residue of acceptor **33**; the reaction being too sluggish and gave very poor yields. Therefore, we changed our approach and decided to attempt a novel method of using simpler and known[32] intermediate, 1,2-isopropylidene-*sn-glycero-H*-phosphonate (**34**), for placing first the phosphoglycerol moiety at the 1-position with the approach that the required fatty acids could be brought at the later stage after simultaneous hydrolysis of 1,2-isopropylidene acetal along with the allyl group from 2-position of the *myo*-inositol residue. Luckily for us, this approach (Scheme 7) worked very nicely and provided us greater flexibility in linking fatty acids.

Accordingly, the glycan **33** was phosphorylated with the freshly prepared 1,2-isopropylidene-*sn-glycero-H*-phosphonate (**34**)[32] to afford compound **35** in excellent yield. The *glycero*-1,2-acetonide and allyl groups were now simultaneously deprotected using palladium



Scheme 6. (a) TMSOTf, Et_2O, 4 Å MS, 0 °C, 0.5 h, 69%; (b) $PdCl_2$, NaOAc, $AcOH/H_2O$, 19:1, 16 h, 91%; (c) CCl_3CN, CH_2Cl_2, DBU, rt, 98%; (d) TMSOTf, Et_2O, 4 Å MS, 0 °C, 0.5 h, 53%; (e) NaOMe, CH_3OH/CH_2Cl_2 (3:1), rt, 1 h, 96%; (f) (i) TBDPSCl, imidazole, THF, 5 h, 89%; (ii) BnBr, NaH, DMF, 0 °C, 1 h, 97%; (g) CAN, $CH_3CN/Tol/H_2O$ (9:5:4), 1.5 h, 77%.



Scheme 7. (a) 1,2-Isopropylidene-*sn*-glycerol-*H*-phosphonate, pivaloyl chloride, pyridine, I_2, pyridine/H_2O (19:1), 94%; (b) $PdCl_2$, NaOAc, AcOH/H_2O, 19:1, 16 h, rt, 88%; (c) $CdCl_2$, palmitic anhydride, DMAP, rt, 18 h, 90%; (d) palmitic acid, DCC, DMAP, rt, 48 h, 80%.

chloride in acetic acid exposing three OH groups in intermediate **36**, and this turned out to be rather clean and robust reaction. Initial attempts to place all the three fatty acids together by standard DCC/DMAP condition failed even after prolonged (5 days) reaction. After attempting several acylation conditions on **36**, we discovered that the activation of charged phosphoryl group as cadmium chloride complex followed by reaction with palmitic anhydride cleanly provided a di-acylated product **37** in very high yield; the idea inspired by the seminal work[33] of Khorana on synthesis of glycerolipid analogues. Having two fatty acids in place at required 1,2-*glycero* positions, the third fatty acid at 2-OH of the inositol residue could now be easily placed by standard DCC/DMAP condition providing fully lipidated GPI (**38**). This selectivity provides an interesting and novel method to synthesize Pf-GPIs with mixed fatty acids, critical for decoding their role in remarkable pro-inflammatory activity.

In the final steps, the TBDPS group was removed from the third mannose of compound **38** (Scheme 8) and the exposed primary OH was phosphorylated with freshly prepared[34] Cbz-NH-ethanolamine-*H*-phosphonate (**40**) in presence of pivaloyl chloride followed by in situ oxidation with iodine to get fully protected Pf-GPI anchor (**41**). Final global deprotection (17 benzyls, 1 Cbz group, and azide reduction) by hydrogenolysis turned out to be most demanding. Eventually it was accomplished by the method reported[14] by Seeberger using hydrogenolysis ($Pd(OH)_2$, DCM/MeOH/H_2O, H_2) with slightly acidic pH, which provided the fully deprotected target malarial GPI anchor (**1**) of *P. falciparum*. The target Pf-GPI anchor was fully characterized by ^1H and ^{31}P NMR and ESMS analysis. The synthetic approach enabled us to initiate chemical and biological studies to decipher the role of the extra fatty acid in pathophysiology of the malaria infection.

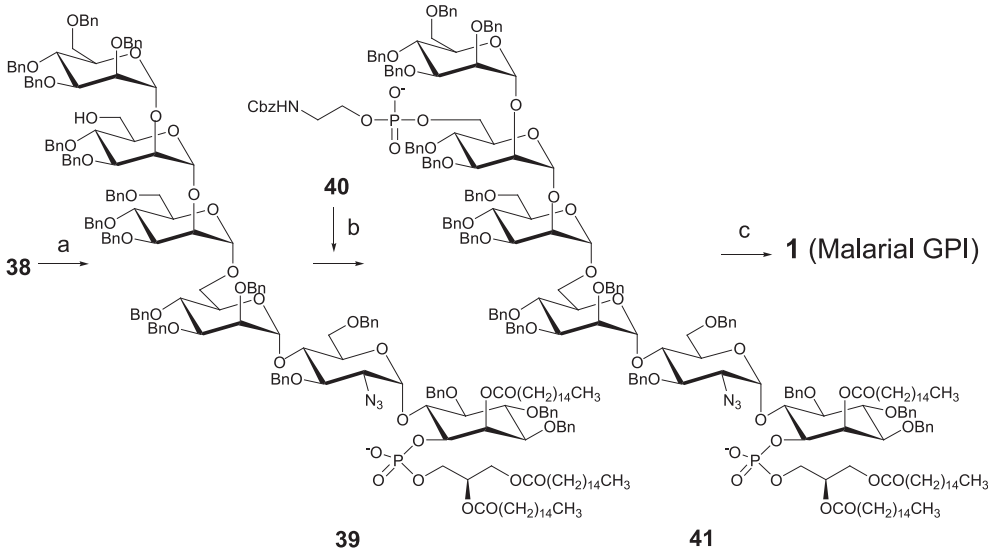
3. Conclusion

We have designed a new total synthesis of the full-length and fully lipidated GPI anchor of malarial parasite (*P. falciparum*). This convergent synthesis provides access to key building blocks and flexibility to bring in three fatty acids in later steps in an efficient manner. Currently, we are exploiting this strategy to synthesize GPI analogues and fluorescent labeled probes to address key issues related to the biosynthetic pathway, cell biology, and membrane biology of this important class of cell-surface glycoconjugates. The immunological studies using this synthetic Pf-GPI will be reported in due course.

4. Experimental

4.1. General procedure

Solvents were purified according to the standard procedures, and reagents used were of highest purity available. All reactions were performed in flame-dried glass apparatus under argon



Scheme 8. (a) 1 M TBAF, THF, rt, 18 h, 85%; (b) Cbz-ethanolamine-*H*-phosphonate **40**, Piv Cl, pyridine, iodine, pyridine/H_2O (95:5), rt, 6 h, 83%; (c) H_2, 20% $Pd(OH)_2$, $CHCl_3$, CH_3OH, H_2O, rt, 18 h, 73%.

atmosphere unless mentioned otherwise. Anhydrous solvents like CH_2Cl_2, Et_2O, THF, CH_3OH, CH_3CN, DMF, pyridine, Et_3N were freshly dried using standard methods. NMR measurements (^1H, ^{13}C, ^{31}P, 2D ^1H–^1H and ^1H–^{13}C COSY, HMQC) were recorded on a 300 MHz spectrometer (Bruker) fitted with pulse-field gradient probe, and trimethylsilane (TMS) or residual resonance of deuterated solvent were used as internal reference. For ^{31}P NMR spectra, phosphoric acid was used as external reference. ^{13}C NMR spectra were broad-band ^1H decoupled or inverse HMQC experiments. Chemical shifts are expressed in parts per million and coupling constants *J* in hertz. Mass spectra (ESI) and high-resolution mass spectra (HRMS) were obtained on quadrupole and LCT-TOF (time of flight) spectrometers, respectively, using acetonitrile/water (1:1) mobile phase. Optical rotations were measured on a digital Perkin–Elmer 241 polarimeter. Analytical TLC was performed on Merck Kieselgel 60 F_{254} plates, and compounds visualized by ammonium molybdate/ceric sulfate developing reagent. Preparative TLC was conducted on Analtech Uniplate silica gel plates (20×20 cm). Silica column chromatography was carried out with silica gel 60 (60–120 mesh) or flash silica gel (230–400 mesh). Analytical and semi-preparative HPLC purification were carried out on a Shimadzu system using RP-18 columns and a photodiode array detector.

4.2. 6-*O*-Allyl-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-(DL)-*myo*-inositol (2c)

A solution of compound **2b**[25] (5.11 g, 10.4 mmol) and dibutyltin oxide (3.70 g, 15 mmol) in anhyd CH_3OH (280 mL) was heated to reflux for 4 h. Excess of solvent was removed and the residue was dried by repeated evaporation through toluene. The residue was dissolved in anhyd DMF (300 mL) followed by the addition of pre-dried CsF (2.23 g), KI (2.34 g), and PMBCl (1.99 mL, 15.6 mmol). The suspension was vigorously stirred for 24 h at rt. The solvent was removed under reduced pressure and the residue was purified by silica column chromatography (85:15 to 80:20 hexane/EtOAc) to provide **2c** (5.9 g, 94%) as white solid. TLC (hexane/EtOAc, 7:3): R_f=0.32. ^1H NMR (300 MHz, $CDCl_3$): 2.40 (s, 1H), 3.30 (dd, *J*=2.4 and 9.5 Hz, 1H), 3.35 (dd, *J*=2.4 and 9.5 Hz, 1H), 3.38 (t, *J*=9.5 Hz, 1H), 3.81 (s, 3H), 3.82 (dd, 1H), 3.94 (dd, 1H), 4.15 (m, 1H), 4.35 (m, 2H), 4.56–4.90 (m, 8H), 5.14–5.28 (m, 2H), 6.00 (m, 1H), 6.86–6.89 (m, 2H), 7.27–7.40 (m, 17H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.30, 67.70, 67.71, 72.47, 72.70, 74.58, 75.93, 75.97, 79.26, 79.74, 80.89, 81.10, 83.15, 113.85, 113.97, 116.63, 127.59–130.12 (multiple peaks), 135.33, 137.97, 138.70, 138.78, 159.35; MS (positive ion ESMS, M+Na^+) calcd for $C_{38}H_{42}O_7Na$: 633.2828, found 633.2751.

4.3. 6-*O*-Vinyl-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-(DL)-*myo*-inositol (2d)

Compound **2c** (4.42 g, 7.3 mmol) was dissolved in anhyd DMSO (74 ml) and treated with potassium *tert*-butoxide (11.76 g). The reaction mixture was heated at 80 °C after 3 h, poured into ice-cold water, and extracted with EtOAc. The organic layer was washed with brine and water, dried (Na_2SO_4), and concentrated. The residue after silica column purification provided desired vinyl compound **2d** (3.75 g, 85%) as white solid. TLC (hexane/EtOAc, 7:3): R_f=0.34. ^1H NMR (300 MHz, $CDCl_3$): 2.40 (d, 3H), 3.35 (dd, *J*=2.4 and 9.6 Hz, 1H), 3.38 (t, *J*=9.5 Hz, 1H), 3.81 (s, 3H), 3.82 (dd, 1H), 4.05 (dd, 1H), 4.15 (m, 1H), 4.56–4.90 (m, 9H), 6.25 (d, 1H), 6.87 (m, 2H), 7.25–7.36 (m, 17H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.30, 68.03, 72.57, 72.70, 75.82, 75.93, 77.22, 79.44, 80.76, 82.31, 84.08, 98.38, 113.81, 127.56–129.96 (multiple peaks), 137.91, 138.40, 138.80, 147.66, 159.37; MS (positive ion ESMS, M+Na^+) calcd for $C_{38}H_{42}O_7Na$: 633.2828, found 633.2833.

4.4. 2-*O*-Allyl-6-*O*-vinyl-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-(DL)-*myo*-inositol (2e)

Compound **2d** (4.43 g, 7.3 mmol) was dissolved in anhyd DMF (62 mL) and solution was brought to 0 °C and then NaH (0.77 g, 33 mmol) and allyl bromide (0.77 ml) were added. The reaction mixture was stirred for 3 h at rt and brine (500 ml) was added, and extracted with EtOAc (150 ml×3). The organic extract was dried over anhyd Na_2SO_4, concentrated followed by column purification to provide **2e** (4.2 g, 90%) as white solid. ^1H NMR (300 MHz, $CDCl_3$): 2.07 (d, 3H), 3.25 (dd, *J*=2.4 and 9.6 Hz, 1H), 3.33 (t, *J*=9.5 Hz, 1H), 3.83 (s, 3H), 3.90 (br s, 1H), 4.05 (dd, 1H), 4.15 (m, 1H), 4.35 (d, 1H), 4.48 (dd, 1H), 4.56–4.90 (m, 9H), 5.14–5.28 (m, 2H), 5.99 (m, 1H), 6.25 (d, 1H), 6.87 (m, 2H), 7.25–7.36 (m, 17H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.28, 70.88, 72.45, 72.73, 73.39, 74.11, 75.75, 75.80, 80.41, 81.30, 82.88, 84.43, 98.06, 113.73, 116.80, 127.46–130.29, 135.72, 138.28, 138.56, 138.97, 147.77, 159.20 (multiple peaks); MS (positive ion ESMS, M+Na^+) calcd for $C_{41}H_{46}O_7Na$: 673.3141, found 673.3240.

4.5. 2-*O*-Allyl-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-(DL)-*myo*-inositol (3)

Compound **2e** (4.2 g, 6.46 mmol) was dissolved in a solution of 1 M HCl/acetone (1:9, 50 ml) and kept at 50 °C for 30 min, neutralized with triethylamine, and concentrated. The residue was purified by silica column (hexane/EtOAc, 95:05 to 80:20) afforded 3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-(DL)-*myo*-inositol **3** (2.4 g, 83%) as white solid. TLC (hexane/EtOAc, 7:3): R_f=0.34. ^{1}H NMR (300 MHz, $CDCl_3$): 2.59 (s, 1H, OH), 3.15 (dd, *J*=1.8 and 9.8 Hz, 1H, 3-H), 3.35 (dd, *J*=9.8 Hz, 1H, 5-H), 3.37 (dd, *J*=2.0 and 9.6 Hz, 1H, 1-H), 3.79 (s, 3H, OMe), 4.10 (br s, 1H), 4.08 (dd, *J*=9.8 Hz, 1H), 4.14 (m, 1H, 6-H), 4.42–4.92 (m, 10H, CH_2Ph), 5.14–5.28 (m, 2H), 5.99 (m, 1H), 6.84–6.88 (m, 2H), 7.20–7.40 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.31, 71.96, 72.77, 72.86, 73.25, 73.35, 75.34, 75.82, 76.63, 79.66, 80.90, 81.42, 83.42, 113.96, 116.76, 127.32–129.96 (multiple peaks), 135.64, 138.30, 138.85, 138.89, 159.40; MS (positive ion ESMS, M+Na^+) calcd for $C_{38}H_{42}O_7Na$ 633.2828, found 633.2990.

4.6. (3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-(DL)-*myo*-inositol (5)

A mixture of 3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-(DL)-*myo*-inositol acceptor **3** (1.0 g, 1.63 mmol) and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (**4**, 1.1 g, 2.31 mmol, 1.41 equiv, prepared from tri-*O*-acetyl-D-glucal by a reported[11] azidonitration method), and freshly activated powdered 4 Å molecular sieves was dried by azeotropic removal of residual moisture through toluene. The mixture was dissolved in anhyd CH_2Cl_2 (20 mL), stirred under argon at rt for 0.5 h, and then cooled to −5 °C. To the above suspension was added a solution of TMSOTf (1.1 mL, 0.2 M solution in CH_2Cl_2) drop wise and the mixture was stirred further for 0.5 h at −5 °C. After completion, the reaction mixture was neutralized with triethylamine, filtered through Celite, and concentrated. The silica column chromatography (hexane/EtOAc, 3:1) provided the product **5** (1.8 g, 85%, diastereoisomeric mixture) as colorless solid. TLC (hexane/EtOAc, 7:3): R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$): 1.83–2.07 (3×s, 9H, OAc), 3.11–3.15 (m, 1H), 3.39–3.42 (m, 2H), 3.44–3.46 (dd, *J*=9.6 Hz, 1H), 3.47–3.50 (dd, *J*=1.8 and 9.5 Hz, 1H), 3.60–3.63 (m, 2H), 3.82 (s, 3H, OCH_3), 4.06 (s, 1H), 4.13–4.14 (dd, *J*=9.5 Hz, 1H), 4.20–4.33 (m, 2H), 4.40–5.18 (m, 10H), 4.92 (m, 1H), 5.40–5.43 (m, 1H), 5.64 (d, *J*=3.6 Hz, 0.5H), 5.79 (d, *J*=3.6 Hz, 0.5H), 6.00 (m, 1H), 6.84–6.87 (m, 2H, Ph), 7.22–7.43 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.54, 20.58, 20.63, 55.19, 60.89, 61.17, 66.73, 67.99, 68.99, 68.99, 70.57, 71.62, 71.66, 71.84, 72.77, 72.85, 73.61, 73.97, 75.04, 75.23, 75.69, 79.69, 80.74, 81.04, 81.33, 81.96, 83.41, 83.97, 97.35, 97.16, 113.83, 127.72–129.29 (multiple peaks), 138.09–138.81 (multiple peaks), 159.37, 169.48, 169.98, 170.64; MS (ESMS, M+Na^+) calcd for $C_{50}H_{57}O_{14}N_3Na$ 946.3738, found 946.3687.

4.7. (2-Azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-(DL)-*myo*-inositol (6)

The above pseudodisaccharide (**5**, 1.2 g, 1.3 mmol) dissolved in a solvent mixture of anhyd CH_2Cl_2 (10 mL) and CH_3OH (30 mL) and added sodium methoxide (0.8 g, 14.8 mmol) and the reaction mixture was stirred for 2 h at rt. After completion of reaction, it was neutralized with cation exchange resin (Amberlite IR 120H^+), filtered, and concentrated to give (2-azido-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-(DL)-*myo*-inositol (**6**) in 91% yield as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): 3.00 (m, 1H), 3.33–3.42 (m, 2H), 3.44–3.63 (m, 4H), 3.80 (s, 3H, OCH_3), 3.94 (br s, 1H), 4.01–4.14 (m, 1H), 4.20–4.33 (m, 2H), 4.40–5.20 (m, 10H), 4.92 (m, 1H), 5.40–5.43 (m, 1H), 5.64 (d, *J*=3.6 Hz, 0.5H), 5.79 (d, *J*=3.6 Hz, 0.5H), 6.00 (m, 1H), 6.82–6.86 (m, 2H, Ph), 7.23–7.45 (m, 17H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.29, 60.89, 61.61, 66.63, 71.43, 71.89, 72.76, 72.88, 73.51, 73.54, 75.06, 75.46, 75.74, 79.69, 80.69, 81.47, 81.58, 81.91, 97.46, 97.16, 113.83, 116.59, 127.47–129.78 (multiple peaks), 138.09–138.81 (multiple peaks), 159.39; MS (ESMS, M+Na^+) calcd for $C_{44}H_{51}O_{11}N_3Na$ 820.3421, found 820.3395.

4.8. (2-Azido-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (7a) and other diastereoisomer (2-azido-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-L-*myo*-inositol (7b)

This intermediate **6** (1.0 g, 1.25 mmol) was dissolved in anhyd CH_3CN (30 mL) followed by addition of benzaldehyde dimethylacetal (1.5 mL, 9.86 mmol) and camphorsulphonic acid (220 mg, 0.94 mmol). The reaction mixture was stirred for 24 h at rt, neutralized with Et_3N, and concentrated. The residue was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, brine, and H_2O. Organic layer was dried (Na_2SO_4), concentrated, and purified on a flash silica column (hexane/EtOAc, 85:15) provided desired D-inositol isomer **7a** (400 mg, 36%) as white solid. TLC (hexane/EtOAc, 7:3): R_f=0.32; $[\alpha]_D$ +52 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.10–3.14 (dd, *J*=3.9 and 10 Hz, 1H), 3.38 (m, 1H), 3.40–3.46 (m, 4H), 3.82 (s, 3H), 3.96 (br s, 1H), 4.17–4.27 (m, 4H), 4.28 (dd, *J*=9.3 Hz, 1H), 4.45 (br s, 2H), 4.54–4.95 (m, 8H), 5.14–5.28 (m, 2H), 5.41 (s, 1H), 5.75 (d, *J*=3.9 Hz, 1H), 6.00 (m, 1H), 6.88–6.91 (m, 2H), 7.19–7.34 (m, 22H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.21, 61.93, 68.51, 72.72, 72.73, 73.44, 74.98, 76.53, 76.96, 80.63, 81.23, 81.68, 81.84, 81.91, 97.75, 101.85, 113.83, 116.73, 126.38, 126.39–129.68 (multiple peaks), 135.66, 138.16, 138.50, 138.80, 159.34; HRMS (ESMS, M+Na^+) calcd for $C_{51}H_{55}O_{11}N_3Na$ 908.3734, found 908.3790; and the L-inositol isomer **7b** (480 mg, 43%). TLC (hexane/EtOAc, 7:3): R_f=0.39; $[\alpha]_D$ +38 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.15 (dd, *J*=3.9 and 9.8 Hz, 1H), 3.34 (dd, *J*=1.8 and 9.8 Hz, 1H), 3.39 (dd, *J*=2.1 and 9.6 Hz, 1H), 3.45 (t, *J*=9.3 Hz, 1H), 3.71 (dd, *J*=10 Hz, 2H), 3.83 (s, 3H), 3.86 (t, *J*=5 Hz, 1H), 3.94 (br s, 1H), 4.10 (dd, *J*=9.6 Hz, 2H), 4.17–4.28 (m, 2H), 4.33 (t, *J*=9.6 Hz, 1H), 4.53–5.48 (m, 10H), 5.11–5.30 (m, 2H), 5.48 (s, 1H), 5.59 (d, *J*=3.9 Hz, 1H), 6.00 (m, 1H), 6.79–6.82 (d, 2H), 7.25–7.40 (m, 22H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.11, 61.61, 63.00, 68.84, 72.59, 73.36, 74.71, 74.65, 76.53, 76.96, 77.38, 80.47, 81.75, 82.02, 84.26, 97.77, 101.85, 113.90, 116.39, 126.26–129.70 (multiple peaks),135.60, 138.46, 138.59, 159.25; HRMS (ESMS, M+Na^+) calcd for $C_{51}H_{55}O_{11}N_3Na$ 908.3734, found 908.3883.

4.9. (2-Azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (8a)

Compound **7a** (350 mg, 0.39 mmol) was dissolved in anhyd DMF (6 mL) followed by the addition of benzyl bromide (0.16 mL, 1.3 mmol) and NaH (0.12 g, 5 mmol) at 0 °C. After 30 min, the reaction mixture was brought to rt and stirred for 4 h. After completion, the excess of NaH was destroyed by addition of a few drops of methanol. The mixture was diluted with excess of EtOAc, which was washed with saturated solution of $NaHCO_3$, water, dried over Na_2SO_4, and concentrated. Silica column chromatography (hexane/EtOAc, 9:1) afforded **8a** (380 mg, 98%), as colorless syrup. TLC (hexane/EtOAc, 8:2): R_f=0.46; $[\alpha]_D$ +48 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.27 (dd, *J*=3.8 and 10.0 Hz, 1H), 3.39 (dd, *J*=2.2 and 9.8 Hz, 1H), 3.45 (dd, *J*=2.2 and 9.8 Hz, 1H), 3.46 (dd, *J*=9.3 Hz, 1H), 3.63 (m, 2H), 3.82 (s, 3H, OCH_3), 3.96–4.04 (m, 2H), 4.06–4.29

(m, 4H), 4.57–4.96 (m, 10H), 5.15–5.25 (m, 2H), 5.49 (s, 1H), 5.72 (d, *J*=3.8 Hz, 1H), 6.00 (m, 1H), 6.91–6.89 (m, 2H), 7.14 (m, 2H), 7.25–7.37 (m, 25H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): 55.21, 62.25, 62.83, 68.67, 72.70, 73.41, 74.71, 74.92, 75.46, 75.52, 75.56, 76.54, 76.96, 77.38, 80.81, 81.13, 81.84, 82.92, 97.75, 101.15, 113.83, 116.70, 126.16–129.64 (multiple peaks), 135.67, 137.48, 137.85, 137.97, 138.15, 138.59, 159.31; HRMS (ESMS, M+Na^+) calcd for $C_{58}H_{61}O_{11}N_3Na$ 998.4204, found 998.4366.

4.10. (2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (9a)

To a solution of above compound **8a** (350 mg, 3.88 mmol), sodium cyanoborohydride (270 mg, 4 mmol), freshly activated molecular sieves (4 Å) in anhyd THF (14 mL), at 0 °C under argon, was added drop wise a saturated solution of HCl in diethyl ether until pH 1 was reached. After stirring at 0 °C for 30 min, the reaction mixture was neutralized with Et_3N, diluted with EtOAc, and concentrated. The residue was purified on a flash silica column (hexane/EtOAc, 85:15) to provide desired compound **9a** (330 mg, 94%) as a colorless solid. TLC (hexane/EtOAc, 7:3): R_f=0.29; $[\alpha]_D$ +33 (*c* 1.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.17 (dd, *J*=3.8 and 9.9 Hz, 1H), 3.29 (m, 2H), 3.42 (dd, *J*=2.2 and 9.8 Hz, 1H), 3.45 (m, 1H), 3.50 (m, 2H), 3.76 (m, 1H), 3.80 (s, 3H, OCH_3), 3.96 (m, 1H), 4.01 (t, 1H), 4.11 (dd, 1H), 4.28 (m, 1H), 4.29–5.00 (m, 14H), 5.15–5.25 (m, 2H), 5.69 (d, *J*=3.7 Hz, 1H), 6.00 (m, 1H), 6.82–6.88 (m, 2H), 7.23–7.41 (m, 27H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): 55.19, 62.57, 69.00, 69.30, 71.73, 72.63, 73.32, 73.40, 73.64, 74.66, 74.21, 76.51, 76.93, 81.79, 81.83, 81.84, 81.90, 97.24, 113,79, 116.65, 127.52–129.48 (multiple peaks), 135.67, 138.17, 138.33, 138.43, 138.57, 138.83, 159.26; HRMS (ESMS, M+Na^+) calcd for $C_{58}H_{63}O_{11}N_3Na$ 1000.4360, found 1000.4321.

4.11. (2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-L-*myo*-inositol (9b)

An identical method described above for compound **9a** was used for the preparation of diastereoisomeric compound **9b** as white solid; $[\alpha]_D$ +24 (*c* 1.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.15 (dd, *J*=3.8 and 9.9 Hz, 1H), 3.34 (m, 2H), 3.45 (dd, *J*=2.2 and 9.8 Hz, 1H), 3.51 (m, 2H), 3.71 (m, 1H), 3.83 (s, 3H, OCH_3), 3.86 (m, 1H), 4.00 (t, 1H), 4.25 (m, 2H), 4.29–5.00 (m, 14H), 5.15–5.25 (m, 2H), 5.59 (d, *J*=3.9 Hz, 1H), 6.00 (m, 1H), 6.79–6.82 (m, 2H), 7.27–7.40 (m, 27H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): 55.11, 61.61, 68.84, 71.73, 72.59, 73.36, 73.41, 73.64, 74.66, 74.71, 76.53, 76.96, 81.75, 82.02, 84.26, 98.00, 113,90, 116.39, 127.45–128.36 (multiple peaks), 135.60, 138.45–138.83 (multiple peaks), 159.25; HRMS (ESMS, M+Na^+) calcd for $C_{58}H_{63}O_{11}N_3Na$ 1000.4360, found 1000.4350.

4.12. (2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl)-(1→6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol (9c) (for direct correlation of stereochemistry)

Colorless solid. TLC (hexane/EtOAc, 8:2): R_f=0.50; $[\alpha]_D$ +48 (*c* 1.0, $CHCl_3$); reported[27] $[\alpha]_D$ +50 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.24 (dd, *J*=3.8 and 10.0 Hz, 1H, 2b-H), 3.38 (dd, *J*=2.2 and 9.8 Hz, 1H, 1a-H or 3a-H), 3.45 (dd, *J*=2.2 and 9.8 Hz, 1H, 1a-H or 3a-H), 3.46 (dd, *J*=9.3 Hz, 1H), 3.52 (m, 2H), 3.80 (s, 3H, OCH_3), 3.96–4.04 (m, 2H, 2a-H, 3a-H), 4.06–4.29 (m, 4H), 4.48 (s, 2H, CH_2Ph), 4.57–4.96 (m, 10H, CH_2Ph), 5.47 (s, 1H), 5.72 (d, *J*=3.8 Hz, 1H, 1b-H), 6.83–6.89 (m, 2H, Ph), 7.08–7.13 (m, 2H, Ph), 7.17–7.45 (m, 30H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): 55.20, 62.83, 68.67, 71.77, 72.70, 73.50, 74.16, 74.72, 74.99, 75.49, 75.57, 77.36, 80.81, 81.13, 81.84, 82.92, 97.71, 101.14, 113.86, 126.03, 126.15, 127.60, 127.63, 127.80, 127.92, 128.13, 128.17, 128.20, 128.24, 128.31, 128.34, 129.60, 130.00, 137.48, 137.85, 137.97, 138.15, 138.59, 138.81, 159.30; HRMS (ESMS, M+Na^+) calcd for $C_{62}H_{63}O_{11}N_3Na$ 1048.4360, found 1048.4366. The spectral and optical data were identical to that reported for this compound by Schmidt et al.[27] by an alternative route.

4.13. (2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2,3,4,5-tetra-*O*-benzyl-1-*O*-(4-methoxybenzyl)-D-*myo*-inositol (9d)

Colorless solid. TLC (hexane/EtOAc, 5:2): R_f=0.46; $[\alpha]_D$ +33.2 (*c* 1.4, $CHCl_3$); reported[27] $[\alpha]_D$ +34 (*c* 1.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 1.92 (d, *J*=3.7 Hz, 1H, OH), 3.17 (dd, *J*=3.8 and 9.9 Hz, 1H, 2b-H), 3.22 (m, 1H, 6b-H), 3.28 (dd, *J*=3.8 and 10.5 Hz, 1H, 6′b-H), 3.38 (dd, *J*=2.2 and 9.8 Hz, 1H, 3a-H), 3.44 (m, 1H, 5a-H), 3.47 (dd, *J*=2.2 and 9.8 Hz, 1H, 1a-H), 3.68 (m, 1H, 4b-H), 3.76 (m, 1H, 3b-H), 3.80 (s, 3H, OCH_3), 3.95 (m, 1H, 5b-H), 4.01 (dd, *J*=2.2 Hz, 1H, 2a-H), 4.11 (dd, *J*=9.4 Hz, 1H, 4a-H), 4.25 (m, 1H, 6a-H), 4.23–5.04 (m, 14H, CH_2Ph), 5.72 (d, *J*=3.7 Hz, 1H, 1b-H), 6.82–6.88 (m, 2H, Ph), 7.17–7.45 (m, 32H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): 55.20, 62.66, 69.00, 69.30, 71.78, 72.18, 72.64, 73.32, 73.45, 74.16, 74.21, 74.68, 74.76, 75.49, 75.60, 79.27, 80.80, 81.54, 81.84, 81.90, 84.35, 97.30, 113,78, 127.49–129.96 (multiple peaks), 137.92, 138.17, 138.33, 138.43, 138.57, 138.83, 159.28; HRMS (ESMS, M+Na^+) calcd for $C_{62}H_{65}O_{11}N_3Na$ 1050.4517, found 1050.4521.

4.14. Allyl (2,3,4,6-tetra-*O*-benzyl)-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (16)

The known[28] glycosyl donor **13** (2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate, 4.1 g, 6.0 mmol) and the acceptor **15** (allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside, 1.75 g, 4.39 mmol) were dried through anhyd toluene and then dissolved in anhyd diethyl ether (100 mL) under argon. After addition of TMSOTf solution (2.95 mL, 0.1 N in anhyd diethyl ether), the reaction mixture was stirred for 2 h at rt. This was followed by neutralization with Et_3N, concentration, and silica column chromatography (hexane/EtOAc, 7:1) to obtain desired compound **16** (4.4 g, 81%) as a colorless crystalline solid. TLC (hexane/EtOAc, 3:1): R_f=0.36; $[\alpha]_D$ +40 (*c* 1.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 3.70 (m, 2H), 3.75–4.00 (m, 6H), 4.10–4.25 (m, 3H), 4.30 (m, 1H), 4.40 (t, 1H), 4.45–5.00 (m, 11H), 5.15–5.30 (m, 2H), 5.41 (s, 1H), 5.58 (s, 1H), 5.80 (m, 1H), 7.09–7.50 (m, 30H); ^{13}C NMR (75 MHz, $CDCl_3$): 64.05, 67.94, 68.03, 68.69, 68.79, 69.32, 71.60, 72.41, 73.23, 73.34, 73.42, 73.69, 74.07, 74.77, 74.81, 77.55, 78.51, 79.23, 79.50, 98.23, 98.69, 102.03, 117.47, 126.13, 127.20, 127.27, 127.33, 127.48, 127.52, 127.64, 127.68, 127.74, 127.87, 128.00, 128.12, 128.16, 128.19, 128.22, 128.27, 128.32, 128.39, 128.49, 129.12, 133.37, 137.54, 137.75, 138.11, 138.38, 138.41, 138.72; HRMS (ESMS, M+Na^+) calcd for $C_{57}H_{60}O_{11}Na$ 943.4033, found 943.4040.

4.15. 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-α-D-mannopyranoside (17)

The intermediate **16** (2.0 g, 2.19 mmol) dissolved in DMSO (90 mL) was treated with potassium *tert*-butoxide (7 g, 62.5 mmol) and the reaction mixture was stirred at 80 °C for 3 h. After completion of the reaction, ice was added and the mixture extracted with EtOAc. The organic layer was dried with Na_2SO_4 and concentrated to provide thick syrup. This was dissolved in a mixture of acetone/1 M HCl (9:1, 79 mL) and heated at 55 °C for 2 h, the progress of reaction checked by TLC. The reaction mixture was cooled, neutralized with NEt_3, and concentrated. Flash chromatography (hexane/EtOAc, 1:1) provided desired compound **17** (1.4 g, 82%) as colorless foam. TLC (hexane/EtOAc, 3:7): R_f=0.34; $[\alpha]_D$ +35

(*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.58 (m, 2H), 3.75 (m, 5H), 3.85 (m, 3H), 4.00 (br s, 2H), 4.43–4.54 (m, 10H), 5.06 (br s, 1H), 5.34 (d, *J*=1.9 Hz, 1H), 7.12–7.24 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): 62.38, 62.84, 67.34, 67.70, 69.57, 69.73, 71.56, 72.12, 72.32, 72.43, 72.58, 72.67, 73.31, 74.53, 74.87, 75.12, 77.32, 77.65, 79.27, 92.37, 98.70, 127.44–128.31 (multiple peaks), 137.93–138.08 (5 peaks); HRMS (positive ion ESMS, M+Na^+) calcd for $C_{47}H_{52}O_{11}Na$ 815.3407, found 815.3412.

4.16. Acetyl-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4,5-di-*O*-acetyl-α-D-mannopyranoside (18)

A solution of preceding deprotected **17** (1.4 g, 1.78 mmol) in a mixture of pyridine and acetic anhydride (1:1, 6 equiv) was stirred at 0 °C for 15 min and then kept at rt overnight. This was followed by addition of ice and stirring for 30 min, extraction with EtOAc. The organic layer was washed with saturated solution of $NaHCO_3$ and water, dried with Na_2SO_4, and concentrated. Flash chromatography (hexane/EtOAc, 3:1) afforded desired triacetylated compound **18** (1.45 g, 93%), as colorless syrup. TLC (hexane/EtOac, 7:3): R_f=0.58; $[\alpha]_D$ +43 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.84 (s, 3H), 1.96 (s, 3H), 2,03 (s, 3H), 3.75 (m, 1H), 4.10 (dd, *J*=3 and 12 Hz, 1H), 4.15 (dd, *J*=5 Hz, 1H), 4.35 (m, 3H), 4.50–4.60 (m, 6H), 4.70 (t, *J*=11 Hz, 1H), 4.96 (d, *J*=2 Hz, 1H), 5.23 (s, 1H), 5.30 (t, *J*=10 Hz, 1H), 6.13 (d *J*=2 Hz, 1H), 7.25–7.53 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.54, 20.64, 20.71, 61.50, 62.47, 67.71, 69.63, 71.08, 71.93, 72.31, 72.40, 72.46, 72.69, 73.24, 74.01, 74.34, 75.04, 75.49, 75.80, 77.10, 79.49, 90.89, 100.60, 127.40–128.32 (multiple peaks), 138.12–138.38 (5 peaks), 167.99, 168.54, 170.70; HRMS (positive ion ESMS, M+Na^+) calcd for $C_{53}H_{58}O_{14}Na$ 941.3724, found 941.3730.

4.17. 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,5-di-*O*-acetyl-α-D-mannopyranoside (19)

A solution of compound **18** (1.0 g, 1.14 mmol) in a saturated solution of dimethylamine in CH_3CN (55 mL) was stirred at −20 °C for 1 h. The excess of solvent and reagent was carefully removed by evaporation under reduced pressure without heating. Flash chromatography (hexane/EtOAc, 3:1) provided compound **19** (0.9 g, 94%) as colorless foam. TLC (hexane/EtOAc, 7:3): R_f=0.34; $[\alpha]_D$ +47 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.75 (m, 2H), 3.90 (m, 1H), 3.90–4.25 (m, 7H), 4.25–4.56 (m, 10H), 4.75 (t, 1H), 4.90 (d, *J*=1.9 Hz, 1H), 5.08 (d, *J*=1.9 Hz, 1H), 5.27 (t, 1H), 7.10–7.26 (m, 25H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.67, 20.71, 62.67, 62.90, 68.38, 68.71, 69.65, 72.18, 72.27, 72.55, 72.60, 72.67, 72.92, 72.97, 73.27, 74.35, 74.44, 74.03, 75.22, 77.21, 79.66, 92.45, 100.33, 127.37–128.29 (multiple peaks), 138.02–138.30 (5 peaks), 169.47, 170.77; HRMS (positive ion ESMS, M+Na^+) calcd for $C_{51}H_{56}O_{13}Na$ 899.3619, found 899.3625.

4.18. 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,5-di-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (20)

To a solution of compound **19** (800 mg, 0.69 mmol) in anhyd CH_2Cl_2 (10 mL) were added Cl_3CCN (1.7 ml, 17 mmol) and DBU (90 μL) and the reaction mixture was stirred at rt for 1 h. The solvent was removed and the residue was purified by a silica column chromatography (hexane/EtOAc, 3:1) to afford compound **20** (890 mg, 96%) as colorless foam. TLC (hexane/EtOAc, 7:3): R_f=0.50; $[\alpha]_D$ +21 (*c* 1.7, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.76 (m, 2H), 3.93 (m, 1H), 3.95–4.25 (m, 7H), 4.26–4.56 (m, 10H), 4.75 (t, 1H), 5.08 (d, *J*=1.9 Hz, 1H), 5.27 (t, 1H), 6.30 (br s, 1H), 7.10–7.26 (m, 25H), 8.60 (s, 1H, NH); HRMS (ESMS, M+Na^+) calcd for $C_{53}H_{56}O_{13}NCl_3Na$ 1042.2715, found 1042.2720.

4.19. Allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (24)

1-*O*-Allyl-α-D-mannopyranose **10** (24 g, 109 mmol, prepared from D-mannose by reported[29] method) dissolved in anhyd pyridine (100 mL) was treated with trityl chloride (26 g, 93 mmol) and the reaction mixture was stirred overnight at 80 °C. The excess of pyridine was removed by distillation at reduced pressure and residue diluted with EtOAc, washed with saturated solution of $NaHCO_3$, brine and water, dried over Na_2SO_4, and concentrated. Silica column chromatography (CH_2Cl_2/MeOH, 95:5) afforded allyl-6-*O*-trityl-α-D-mannopyranoside (35 g, 70%) as pale yellow syrup, which was dissolved in anhyd DMF (400 mL) followed by the addition of benzyl bromide (58 mL) and NaH (21 g) at 0 °C. After 30 min, the reaction mixture was brought to rt and stirred for 4 h. After completion, the excess of NaH was destroyed by addition of methanol and the solution concentrated. The mixture was diluted with EtOAc, which was washed with saturated solution of $NaHCO_3$, water, dried over Na_2SO_4, and concentrated. Silica column chromatography (hexane/EtOAc, 3:1) afforded allyl-2,3,4-tri-*O*-benzyl-6-*O*-trityl-α-D-mannopyranoside (**23**) (49 g, 89%). This compound (49 g, 67 mmol) dissolved in a solvent mixture of CH_2Cl_2/CH_3OH (720 mL, 1:3) was treated with *p*-toulenesulponic acid (3.6 g) and stirred for 12 h at rt. After completion of the reaction, it was neutralized with Et_3N and concentrated. Silica column chromatography (hexane/EtOAc, 7:3) afforded allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **24** (30 g, 93%) as colorless foam. TLC (hexane/EtOAc, 7:3): R_f=0.25; $[\alpha]_D$ +23 (*c* 2.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.61 (m, 1H), 3.76 (m, 1H), 3.89 (m, 2H), 3.99 (m, 1H), 4.19 (dd, 1H), 4.05 (m, 1H), 4.58–4.80 (m, 6H), 4.80 (d, *J*=1.3 Hz, 1H), 5.19–5.33 (m, 2H), 5.78 (m, 1H), 7.24–7.31 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$): 61.86, 67.65, 72.01, 72.61, 72.78, 74.76, 74.95, 80.10, 97.24, 117.00, 127.43, 127.49, 127.55, 127.73, 127.86, 128.14, 128.23, 133.74, 138.26, 138.48, 138.53; MS (ESMS, M+Na^+) calcd for $C_{30}H_{34}O_6Na$ 513.2253, found 513.2260.

4.20. Allyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzy-L-α-D-mannopyranoside (25)

The known[31] orthoester donor **22** (1.85 g, 3.11 mmol) and mannobiose acceptor **24** (1.55 g, 3.16 mmol) were dissolved in anhyd CH_2Cl_2 (15 mL) and freshly activated 4 Å molecular sieves were added. The solution was stirred at rt for 30 min followed by addition of 0.1 N solution of TESOTf in CH_2Cl_2 (150 μL, 15 mmol) and *N*-iodosuccinimide (525 mg, 2.34 mmol). After 30 min stirring, the solution was neutralized with Et_3N, the solvent was evaporated, and the residue purified by silica column (hexane/EtOAc, 85:15), which provided **25** (2.31 g, 72%) as colorless syrup. TLC (hexane/EtOAc, 7:3): R_f=0.5; $[\alpha]_D$ +27 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.50–4.00 (m, 7H), 4.12–4.25 (m, 4H), 4.50–5.10 (m, 11H), 5.19 (d, *J*=1.9 Hz, 1H), 5.20 (t, 1H), 5.90 (m, 1H), 7.24–8.10 (m, 35H); ^{13}C NMR (75 MHz, $CDCl_3$): 66.68, 67.78, 68.74, 69.00, 71.09, 71.65, 72.06, 72.70, 73.35, 74.23, 74.65, 74.76, 75.03, 75.11, 77.67, 80.30, 96.83, 98.07, 117.45, 127.51–128.35 (multiple peaks), 133.68, 138.49 (multiple peaks), 165.49; HRMS (positive ion ESMS, M+Na^+) calcd for $C_{64}H_{66}O_{12}Na$ 1049.4452, found 1049.4463.

4.21. Allyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (26)

To a solution of compound **25** (2.3 g, 2.24 mmol) in a solvent mixture of CH_3OH/CH_2Cl_2 (20 mL, 7:1) was added sodium methoxide (350 mg, 6.48 mmol). The solution was stirred at rt for 3 h. After completion of the reaction, the solvent was evaporated and the residue was purified by silica column (hexane/EtOAc, 3:1) to get compound **26** (1.9 g, 92%) as colorless foam. TLC (hexane/EtOAc,

7:3): R_f=0.25; $[\alpha]_D$ +30 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.50–3.60 (m, 2H), 3.62–3.70 (m, 2H), 3.75–3.80 (m, 4H), 4.0 (m, 4H), 4.35–4.75 (m, 12H), 5.0 (br s, 1H), 5.10–5.15 (m, 2H), 5.80 (m, 1H), 7.18–7.29 (m, 30H); ^{13}C NMR (75 MHz, $CDCl_3$): 66.24, 67.76, 68.04, 68.87, 71.06, 71.44, 71.62, 72.08, 72.73, 73.32, 74.23, 74.63, 74.89, 75.01, 79.56, 80.20, 96.84, 99.59, 117.39, 127.62–128.29 (multiple peaks), 133.65, 138.46–138.50 (multiple peaks); HRMS (positive ion ESMS, M+Na^+) calcd for $C_{57}H_{62}O_{11}Na$ 945.4190, found 945.4197.

4.22. Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3, 4-tri-*O*-benzyl-α-D-mannopyranoside (27)

The mannobiosyl donor **20** (2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,5-di-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, 1.0 g, 1.08 mmol) and the acceptor **26** (allyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranose, 650 mg, 0.63 mmol) were first dried through co-evaporation with anhyd toluene, further dried for 2 h under high vacuum, and then dissolved in anhyd CH_2Cl_2 (12 mL) and freshly activated molecular sieves (4 Å) were added and the suspension stirred under argon at rt for 30 min. The reaction mixture was cooled to 0 °C and treated with 0.1 N TMSOTf solution (760 μL, 0.76 mmol) and stirred for 30 min. The reaction was neutralized with Et_3N and solvent was evaporated. The residue was purified by silica column (hexane/EtOAc, 85:15) to provide target tetrasaccharide **27** (1.05 g, 69%) as white solid. TLC (hexane/EtOAc, 7:3): R_f=0.68. This glycosylation reaction required strictly anhyd conditions; $[\alpha]_D$ +22 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$); 1.80 (s, 3H), 2.01 (s, 3H), 3.52–4.32 (m, 22H), 4.35–500 (m, 26H), 5.05–5.40 (m, 5H), 5.29 (t, 1H), 5.77–5.87 (m, 1H), 7.16–7.29 (m, 55H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.57, 20.64, 62.87, 66.53, 67.69, 68.47, 68.86, 69.01, 69.13, 71.34, 71.41, 71.74, 72.01, 72.20, 72.24, 72.44, 72.59, 72.99, 73.18, 74.48, 74.61, 74.70, 74.88, 75.37, 75.76, 77.13, 79.03, 79.89, 80.27, 96.87, 99.05, 99.51, 99.81, 117.34, 127.30–128.24 (multiple peaks), 133.57, 137.98–138.76 (multiple peaks), 169.44, 170.69; HRMS (positive ion ESMS, M+Na^+) calcd for $C_{108}H_{116}O_{23}Na$ 1803.7805, found 1803.7811.

4.23. 2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranose (28)

To a solution of tetrasaccharide **27** (425 mg, 0.238 mmol) in AcOH/H_2O (11 ml, 19:1) were added $PdCl_2$ (400 mg, 2.25 mmol) and anhyd NaOAc (400 mg, 4.87 mmol). The reaction mixture was stirred at rt for 16 h under argon atmosphere. After completion of the reaction, it was diluted with EtOAc, organic layer washed with saturated $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated. The residue was purified by silica chromatography (hexane/EtOAc, 7:3) to provide anomeric deprotected tetrasaccharide **28** (380 mg, 91%) as colorless foam. TLC (hexane/EtOAc, 6:4): R_f=0.3; $[\alpha]_D$ +21 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.79 (s, 3H), 2.00 (s, 3H), 3.30–4.25 (m, 22H), 4.30–4.90 (m, 24H), 4.96 (d, 1H), 5.05 (d, 1H), 5.09 (d, 1H), 5.16 (d, 1H), 5.28 (t, 1H), 7.08–7.29 (m, 55H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.62, 20.76, 62.99, 68.12, 68.65, 68.87, 69.04, 69.17, 69.50, 71.34, 71.50, 71.55, 71.71, 72.01, 72.24, 72.37, 72.46, 72.62, 73.11, 73.20, 73.37, 74.02, 74.26, 74.52, 74.64, 74.78, 74.82, 74.90, 75.00, 75.09, 75.86, 75.99, 77.13, 78.59, 78.98, 79.86, 92.46, 99.09, 99.28, 99.89, 127.53–128.27 (multiple peaks), 138.24–138.41 (multiple peaks), 169.51, 170.96; HRMS (positive ion ESMS, M+Na^+) calcd for $C_{105}H_{112}O_{23}Na$ 1763.7492, found 1763.7503.

4.24. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)trichloroacetimidate (29)

Compound **28** (300 mg, 0.172 mmol) was dissolved in anhyd CH_2Cl_2 (5 mL) followed by addition of CCl_3CN (0.51 mL, 5.1 mmol) and DBU (150 μL) and the reaction mixture was stirred at rt for 1.5 h. After completion of the reaction, the solvent was removed under reduced pressure and the residue was purified on a silica column (hexane/EtOAc, 3:1) to give desired trichloroacetimidate donor **29** (320 mg, 98%) as colorless foam. TLC (hexane/EtOAc, 3:1): R_f=0.53; $[\alpha]_D$ +14 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.79 (s, 3H), 2.00 (s, 3H), 3.30–4.25 (m, 22H), 4.30–4.90 (m, 24H), 5.06 (d, 2H), 5.16 (d, 1H), 5.28 (t, 1H), 6.32 (br s, 1H), 7.08–7.29 (m, 55H), 8.61 (s, 1H, NH); MS (ESMS, M−C_2NCl_3+H^+) calcd for $C_{105}H_{112}O_{23}$ 1740.7594, found 1740.7599.

4.25. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (30)

The above tetramannose trichloroacetimidate donor **29** (421 mg, 0.22 mmol, 1.4 equiv) and the glucosamine–inositol acceptor **9a** (2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-(3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol) (162 mg, 0.16 mmol) were dried by evaporation through anhyd toluene and then dissolved in anhyd diethyl ether (15 mL) and freshly activated molecular sieves (4 Å) under argon and cooled to 0 °C. To this was added a solution of 0.1 N TMSOTf (320 μL, 0.36 mmol) and reaction mixture was stirred for 30 min. After completion of reaction, it was neutralized with Et_3N and solvent evaporated, and residue purified by a flash silica column (hexane/EtOAc, 85:15) to provide desired pseudohexasaccharide **30** (320 mg, 53%) as viscous substance. TLC (hexane/EtOAc, 7:3): R_f=0.27; $[\alpha]_D$ +27 (*c* 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.73 (s, 3H), 1.86 (s, 3H), 3.08–3.15 (m, 2H), 3.18–3.25 (m, 2H), 3.24–3.41 (m, 6H), 3.41–3.56 (m, 4H), 3.71–4.01 (m, 24H), 3.82 (s, 3H), 4.33–5.08 (m, 34H), 5.14 (m, 2H), 5.26 (br s, 1H), 5.28 (br s, 1H), 5.37 (br s, 1H), 5.38 (br s, 1H), 5.80 (d, *J*=3.8 Hz, 1H), 5.90 (m, 1H), 6.89 (d, 2H), 7.07–7.39 (m, 82H); ^{13}C NMR (75 MHz, $CDCl_3$): 20.57, 22.52, 55.19, 62.03, 62.76, 62.93, 65.73, 66.53, 68.48, 68.51, 68.84, 68.98, 69.15, 69.18, 69.58, 71.47, 71.64, 71.95, 72.09, 72.19, 72.45, 72.63, 73.00, 73.09, 73.18, 73.41, 74.16, 74.48, 74.59, 74.76, 74.93, 75.70, 76.48, 77.14, 79.02, 79.92, 80.01, 80.85, 81.45, 81.73, 81.81, 97.19, 99.21, 99.32, 99.78, 100.27, 113.84, 116.66, 127.18–128.34 (multiple peaks), 129.44, 135.63, 137.75, 137.95, 138.02–138.84 (multiple peaks), 159.30, 169.41, 170.68; MS (positive ion ESMS, M+Na^+) calcd for $C_{163}H_{173}O_{33}N_3Na$ 2723.1849, found 2723.1930.

4.26. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (31)

The above pseudohexasaccharide **30** (220 mg, 0.08 mmol) was dissolved in a solvent mixture of CH_3OH/CH_2Cl_2 (3:1, 12 mL) followed by addition of sodium methoxide (250 mg, 4.62 mmol). The reaction mixture was stirred at rt for 1 h, solvent was evaporated under reduced pressure, and the residue purified by a silica column (hexane/EtOAc, 65:35) to obtain the deacetylated pseudohexasaccharide **31**

(205 mg, 96%) as colorless solid; $[\alpha]_D$ +28 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 3.08–3.16 (m, 2H), 3.18–3.26 (m, 2H), 3.27–3.41 (m, 6H), 3.41–3.56 (m, 4H), 3.71–4.01 (m, 24H), 3.86 (s, 3H), 4.25–5.10 (m, 34H), 5.14 (m, 2H), 5.26 (br s, 1H), 5.28 (br s, 1H), 5.37 (br s, 1H), 5.38 (br s, 1H), 5.80 (d, *J*=3.8 Hz, 1H), 5.90 (m, 1H), 6.90 (d, 2H), 7.10–7.30 (m, 82H); ^{13}C NMR (75 MHz, $CDCl_3$): 55.20, 62.03, 62.74, 62.87, 66.04, 66.61, 68.48, 68.51, 68.84, 68.98, 69.08, 69.37, 69.66, 71.64, 71.72, 71.81, 71.90, 72.01, 72.08, 72.65, 72.78, 73.17, 73.26, 73.42, 73.41, 74.16, 74.52, 74.66, 74.79, 74.94, 75.47, 75.66, 76.55, 76.97, 78.07, 78.57, 79.29, 79.61, 79.91, 80.64, 80.48, 81.77, 81.78, 81.81, 97.22, 97.63, 98.81, 99.26, 99.78, 100.38, 113.84, 116.71, 127.29–128.24 (multiple peaks), 129.43, 135.66, 137.78, 138.00, 138.03, 138.24 (multiple peaks), 138.37, 138.42, 128.47, 138.55, 138.68, 138.84, 159.30; MS (positive ion ESMS, M+Na^+) calcd for $C_{159}H_{169}O_{31}N_3Na$ 2639.1638, found 2639.1710.

4.27. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-2-*O*-allyl-D-*myo*-inositol (32)

The above compound (75 mg, 28 μmol) was dissolved in anhyd THF (1 mL) followed by the addition of imidazole (25 mg, 0.36 mmol) and *tert*-butyldiphenylsilyl chloride (TBDPSCl, 60 μL, 0.23 mmol). The reaction mixture was stirred for 5 h, diluted with EtOAc, organic layer washed with saturated aqueous solution of $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated. The residue was purified through a small silica column to provide selectively mono-silylated compound (72 mg, 89%) as a colorless solid, which was dissolved in anhyd DMF (2 mL) followed by addition of benzyl bromide (70 μL, 0.5 mmol) and sodium hydride (5 mg, 0.12 mmol). The reaction mixture was stirred at 0 °C for 1 h, quenched with methanol, diluted with EtOAc, organic layer washed with brine and H_2O, dried over Na_2SO_4, and concentrated. Flash silica column chromatography led to isolation of compound **32** (73 mg, 97%) as a colorless solid. TLC (hexane/EtOAc, 7:3): R_f=0.43; $[\alpha]_D$ +27 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.04 (s, 9H, TBDPS), 3.08–3.16 (m, 2H), 3.18–3.26 (m, 2H), 3.27–3.41 (m, 6H), 3.41–3.56 (m, 4H), 3.71–4.01 (m, 22H), 3.74 (s, 3H), 4.10–4.95 (m, 36H), 4.97 (br s, 1H), 5.14 (m, 2H), 5.17 (br s, 1H), 5.29 (br s, 1H), 5.42 (br s, 1H), 5.72 (d, *J*=3.6 Hz, 1H), 6.00 (m, 1H), 6.80–6.82 (d, 2H), 7.04–7.26 (m, 87H), 7.65–7.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): 19.26, 29.24, 55.17, 62.01, 62.89, 64.95, 68.61, 68.83, 69.11, 69.53, 71.55, 71.66, 71.78, 71.90, 72.05, 72.20, 72.65, 72.98, 73.05, 73.20, 74.13, 74.60, 74.75, 74.86, 75.01, 75.62, 76.18, 77.11, 77.81, 79.81, 79.53, 79.68, 79.91, 80.83, 81.52, 81.79, 81.89, 97.20, 98.29, 99.00, 99.17, 100.25, 113.89, 166.66, 127.01–128.15 (multiple peaks), 133.02, 135.55, 135.47, 137.75–138.89 (multiple peaks), 159.28; MS (positive ion ESMS, M+Na^+) calcd for $C_{182}H_{193}O_{31}N_3SiNa$ 2967.3285, found 2967.3221.

4.28. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-2-*O*-allyl-D-*myo*-inositol (33)

The above pseudohexasaccharide **32** (70 mg, 23 μmol) was dissolved in CH_3CN/toluene/water (2 mL, 9:5:4) and cooled to 0 °C. Ceric ammonium nitrate (CAN) (65 mg, 110 μmol) was added and stirred for 30 min at same temperature and at rt for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution. The aqueous phase was further washed with CH_2Cl_2 and the combined organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was subjected to flash silica column (hexane/EtOAc, 85:15) to afford **33** (52 mg, 77%) as white foam. TLC (hexane/EtOAc, 70:30): R_f=0.31; $[\alpha]_D$ +35 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.04 (s, 9H, TBDPS), 3.15–3.85 (m, 36H), 3.96–4.88 (m, 36H), 4.94 (s, 1H), 5.14 (m, 2H), 5.17 (s, 2H), 5.29 (s, 1H), 5.71 (d, *J*=3.6 Hz, 1H), 6.00 (m, 1H), 7.05–7.26 (m, 91H), 7.65–7.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): 19.26, 26.80, 62.97, 63.90, 66.36, 68.44, 68.33, 69.01, 69.25, 69.64, 70.26, 71.67, 71.95, 72.10, 72.13, 72.55, 72.92, 72.97, 73.03, 73.24, 73.81, 74.06, 74.35, 74.66, 74.82, 74.97, 75.63, 75.50, 76.92, 77.07, 79.33, 79.75, 79.95, 80.45, 80.79, 81.26, 81.66, 81.86, 97.33, 98.71, 99.04, 99.31, 99.64, 99.92, 117.07, 127.13–128.33 (multiple peaks), 129.44, 138.54–138.83 (multiple peaks); MS (positive ion ESMS, M+Na^+) calcd for $C_{174}H_{185}O_{30}N_3SiNa$ 2847.2710, found 2847.2880.

4.29. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-(3,4,5-tri-*O*-benzyl-1-*O*-diisopropylidene-*sn*-glyceryl-phosphonato)-2-*O*-allyl-D-*myo*-inositol (35)

The above compound **33** (32 mg, 11 μmol) and freshly prepared[32] 1,2-isopropylidene-*sn*-glyceryl-*H*-phosphonate (**34**, 12 mg, 60 μmol) were dried by evaporation with anhyd pyridine three times and dried under high vacuum for 2 h. The mixture was dissolved in anhyd pyridine (1.0 mL). This was followed by addition of pivaloyl chloride (11 μL, 91 μmol). After stirring at rt for 30 min, the reaction mixture was treated with iodine (6 mg, 23 μmol, 2 equiv) in a mixture of pyridine/water (19:1, 0.1 ml), was added to oxidize P(III) to P(V) and further stirred for 30 min. The reaction mixture was diluted with $CHCl_3$ and organic layer washed with 5% sodium bisulfite solution to remove the excess of iodine. The organic layer was dried over Na_2SO_4, concentrated, and purified on a flash column using 2% MeOH/CH_2Cl_2 (with 0.1% triethylamine) solvent system, providing the desired compound **35** (32 mg, 94%) as a syrup. TLC (DCM/MeOH, 9.5:0.5): R_f=0.35; $[\alpha]_D$ +24 (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.02 (s, 9H, TBDPS), 137 (s, 3H), 1.41 (s, 3H), 3.05–3.16 (m, 2H), 3.18–3.26 (m, 2H), 3.27–3.41 (m, 6H), 3.41–3.56 (m, 4H), 3.71–4.02 (m, 26H), 3.96–4.88 (m, 37H), 4.95 (s, 1H), 5.18 (s, 2H), 5.29 (s, 1H), 5.71 (d, *J*=3.6 Hz, 1H), 7.05–7.26 (m, 91H), 7.65–7.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): 19.24, 26.63, 62.92, 63.91, 66.36, 68.45, 68.33, 69.05, 69.26, 69.64, 70.27, 71.70, 71.95, 72.11, 72.13, 72.56, 72.92, 72.98, 73.03, 73.24, 73.82, 74.06, 74.35, 74.66, 74.82, 74.97, 75.64, 75.51, 76.94, 77.09, 79.34, 79.75, 79.95, 80.51, 80.79, 81.27, 81.67, 81.86, 97.34, 98.71, 99.05, 99.31, 99.65, 99.92, 117.09, 127.12–128.33 (multiple peaks), 129.45, 138.54–138.83 (multiple peaks); MS (negative ion ESMS, M−H)$^-$ calcd for $C_{180}H_{195}O_{35}N_3PSi$ 3017.3078, found 3017.3144.

4.30. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-(3,4,5-tri-*O*-benzyl-1-*O*-*sn*-glyceryl-phosphonato)-D-*myo*-inositol (36)

Pseudohexasaccharide **35** (27 mg, 8.9 μmol), anhyd NaOAc (75 mg, 0.91 mmol), and $PdCl_2$ (65 mg, 0.36 mmol) were dissolved in a mixture of AcOH/H_2O (19:1, 2 mL) with the aid of sonication. The reaction mixture was stirred at rt for 16 h under argon atmosphere. After completion of the reaction, it was quenched carefully by addition of saturated $NaHCO_3$ solution and solid Na_2CO_3. The aqueous

layer was extracted with EtOAc (3×15 mL) and the combined organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash silica chromatography (DCM/MeOH, 95:5) afforded compound **36** (23 mg, 88%) as a syrup. TLC (DCM/MeOH, 9:1): R_f=0.3; $[\alpha]_D$ +33 (*c* 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 1.14 (s, 9H, TBDPS), 3.05–3.16 (m, 2H), 3.18–3.26 (m, 2H), 3.27–3.41 (m, 6H), 3.41–3.56 (m, 4H), 3.71–4.02 (m, 26H), 3.96–4.88 (m, 37H), 4.95 (s, 1H), 5.18 (s, 2H), 5.29 (s, 1H), 5.71 (d, *J*=3.6 Hz, 1H), 7.05–7.26 (m, 91H), 7.65–7.73 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): 19.25, 26.63, 62.92, 63.91, 66.37, 68.45, 68.33, 69.05, 69.26, 69.64, 70.27, 71.70, 71.95, 72.11, 72.13, 72.56, 72.92, 72.98, 73.04, 73.24, 73.82, 74.06, 74.35, 74.66, 74.82, 74.97, 75.64, 75.51, 76.94, 77.09, 79.35, 79.75, 79.95, 80.51, 80.79, 81.27, 81.67, 81.86, 97.34, 98.71, 99.05, 99.31, 99.66, 99.92, 117.09, 127.12–128.33 (multiple peaks), 129.45, 138.54–138.83 (multiple peaks); MS (negative ion ESMS, M−H)$^-$ calcd for $C_{174}H_{188}O_{35}N_3PSi$ 2937.2452, found 2937.2354.

4.31. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-2-*O*-hexadecanoyl-1-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glyceryl-phosphonato)-D-*myo*-inositol (38)

The previous compound **36** (20 mg, 6.8 μmol) was dissolved in anhyd CH_2Cl_2 (2 ml) and $CdCl_2\cdot H_2O$ was added[33] to it and mixture stirred for 30 min at rt. The solvent was removed and the residue was dried repeatedly through anhyd toluene (10 mg). To this was added anhyd CH_2Cl_2 (1.5 ml) followed by freshly prepared palmitic anhydride (40 mg) and dimethylaminopyridine (5 mg). The reaction mixture was stirred at rt for 18 h under dark condition. After completion of the reaction, solvent was removed and the residue was purified by silica column chromatography (3–5% CH_3OH in CH_2Cl_2 with 0.1% Et_3N) to provide dipalmitoylated intermediate **37** (21 mg). This intermediate (20 mg, 6.8 μmol) dissolved in CH_2Cl_2 (1.5 mL) was treated with palmitic acid (20 mg, 78 μmol) and dicyclohexylcarbodiimide (DCC) (20 mg, 76 μmol) and catalytic amount of DMAP. The reaction mixture was stirred for 48 h at rt. After completion of the reaction, CH_2Cl_2 was removed and the residue was subjected to flash column chromatography (3–5% CH_3OH in CH_2Cl_2 with 0.1% Et_3N) to provide **38** (23 mg, 80%) as white foam. TLC (DCM/MeOH, 95:5): R_f=0.65; $[\alpha]_D$ +31 (*c* 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 0.83 (t, 9H, $3CH_3$ of fatty acids), 1.14 (s, 9H, TBDPS), 1.13–1.27 (m, 72H, $36CH_2$ of fatty acids), 1.45–1.53 (m, 6H), 2.20–2.26 (m, 6H), 3.08–3.59 (m, 12H), 3.60–4.30 (m, 30H), 4.34–4.56 (m, 22H), 4.64 (s, 1H), 4.72–4.86 (m, 10H), 5.12 (m, 2H), 5.15 (d, 1H), 5.24 (d, 1H), 5.25 (m, 1H), 5.85 (br s, 1H), 6.00 (br s, 1H), 7.00–7.38 (m, 85H), 7.65–7.73 (m, 4H); ^{31}P NMR (125 MHz): −0.75; MS (negative ion ESMS), $[M-H]^-$ calcd for $C_{222}H_{277}O_{38}N_3PSi$ 3651.9342, found 3651.9399.

4.32. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-D-mannopyranosyl)-(12)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-2-*O*-hexadecanoyl-1-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glyceryl-phosphonato)-D-*myo*-inositol (39)

Compound **38** (15 mg, 4.1 μmol) was dissolved in 0.4 ml of a 1 M solution of tetrabutylammonium fluoride (TBAF) in anhyd THF and stirred at rt for 18 h. After completion of reaction, solvent was quickly removed under vacuum and the viscous yellow mass was purified through a flash silica column (CH_2Cl_2/CH_3OH, 98:2) affording the desired compound **39** (12 mg, 85%) as colorless solid. TLC (CH_2Cl_2/CH_3OH, 95:5): R_f=0.46; $[\alpha]_D$ +29 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): 0.88 (t, 9H, $3CH_3$ of fatty acids), 1.10–1.27 (m, 72H, $36CH_2$ of fatty acids), 1.55–1.63 (m, 6H), 2.22–2.46 (m, 6H), 3.08–3.45 (m, 12H), 3.60–4.30 (m, 30H), 4.34–4.56 (m, 22H), 4.64 (s, 1H), 4.72–4.86 (m, 10H), 5.08 (d, 1H), 5.20 (m, 2H), 5.24 (d, 1H), 5.55 (m, 1H), 5.85 (br s, 1H), 6.00 (br s, 1H), 7.00–7.38 (m, 85H); ^{31}P NMR (125 MHz): −0.74; MS (negative ion ESMS, M−H)$^-$ calcd for $C_{206}H_{259}O_{38}N_3P$ 3413.8164, found 3413.9300.

4.33. (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl)-amino-ethylphosphonato)-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-2-*O*-hexadecanoyl-1-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glyceryl-phosphonato)-D-*myo*-inositol (41, fully protected GPI anchor)

Compound **39** (20 mg, 5.86 μmol) and freshly prepared[34] Cbz-ethanolamine-*H*-phosphonate (**40**, 24 mg, 92 μmol) were co-evaporated with anhyd pyridine (3×1 mL) and dried under high vacuum for 2 h. The mixture was dissolved in anhyd pyridine (0.5 mL). This was followed by addition of pivaloyl chloride (16 μL, 120 μmol). The reaction mixture was stirred for 6 h. After stirring at rt for 6 h, the reaction mixture was treated with iodine (14 mg, 54 μmol, 2 equiv) in a mixture of pyridine/water (19:1, 0.1 ml), was added to oxidize P(III) to P(V) and further stirred for 6 h at rt to ensure the complete oxidation. The reaction mixture was diluted with $CHCl_3$ and organic layer washed with 5% sodium bisulfite solution to remove the excess of iodine. The aqueous layer was further washed with $CHCl_3$ and the combined organic layer was washed with TEAB buffer and dried over Na_2SO_4, concentrated, and purified on a flash column using 15% MeOH/CH_2Cl_2 (with 0.1% triethylamine) solvent system, afforded the desired compound **41** (18 mg, 83%) as white foam. TLC (CH_2Cl_2/CH_3OH, 9:1): R_f=0.2; $[\alpha]_D$ +27 (*c*=1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$-CD_3OD): 0. 87 (t, 9H, $3CH_3$ of fatty acids), 1.10–1.40 (m, 78H, $36CH_2$ of fatty acids and TEA), 1.50–1.59 (m, 6H), 2.10–2.31 (m, 6H), 3.17 (m, 2H), 3.27–4.00 (m, 34H), 4.05–5.00 (m, 47H), 5.12 (d, 1H), 5.21 (s, 1H), 5.23 (s, 1H), 5.28 (m, 1H), 5.80 (d, 1H), 5.92 (m, 1H), 7.00–7.40 (m, 90H); ^{31}P NMR (125 MHz): −1.70, 0.80; MS (negative ion ESMS, M−2H)$^-$ calcd for $C_{216}H_{270}O_{43}N_4P_2$ 3669.86, found 3669.8550.

4.34. (α-D-Mannopyranosyl)-(1→2)-(6-*O*-(aminoethyl-phosphonato)-α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-*O*-(2-hexadecanoyl-1-*O*-octadecyl-*sn*-glyceryl-phosphonato)-D-*myo*-inositol (1)

The protected and lipidated GPI anchor **41** (12 mg, 4.8 μmol) and 20% $Pd(OH)_2$ (25 mg) were dissolved in a solvent mixture of CH_3OH (1 mL), CH_2Cl_2 (1 mL), and H_2O (0.05 mL) and a trace amount of formic acid (10 μl). The residual and dissolved air from the flask was removed by repeated evacuations by suction (three freeze–thaw–pump cycles) and flushed with hydrogen gas. The reaction mixture was then stirred under positive hydrogen atmosphere for 24 h at rt. At this stage, another lot of CH_3OH/CH_2Cl_2/H_2O (1:1:0.3) (1 mL) and $Pd(OH)_2$ (15 mg) were added and stirring continued under hydrogen pressure for additional 24 h. After the completion of reaction, the mixture was filtered through a small Celite pad, which was washed several times with the solvent mixture, and concentrated under reduced pressure. The residue was washed with anhyd CH_2Cl_2 to remove non-polar impurities. This provided the desired fully lipidated malarial GPI anchor **1** (8.2 mg, 73%) as colorless solid. TLC (CH_2Cl_2/CH_3OH/0.25% KCl; 5:4:1): R_f=0.2. ^1H NMR (300 MHz, CD_3OD/$CDCl_3$/D_2O): 0.81–1.00 (m, 9H), 1.16–1.35 (m, 72H),

1.43–1.60 (m, 6H), 1.63–1.71 (m, 6H), 2.37–4.28 (m, 47H), 4.88 (m, 2H), 5.00 (br s, 1H), 5.20 (br s, 1H), 5.32 (br s, 1H), 5.40 (br s, 1H); ^{31}P NMR (125 MHz): −1.80, 0.25; MS (ESMS, M−H) calcd for $C_{89}H_{165}O_{41}N_2P_2$ 1980.0363, found 1980.1244.

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Supplementary data

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