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Synthetic glycosylphosphatidylinositol (GPI) anchors: how these complex molecules have been made†

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Abstract

Glycosylphosphatidylinositols (GPIs) are a class of natural glycosylphospholipids that anchor proteins, glycoproteins and lipophosphoglycans to the membrane of eukaryotic cells. GPI anchors are widely present in parasitic protozoa, where GPI-anchored mucins and phosphoglycans are abundant and form a dense protective layer (glycocalyx) on the surface of the parasites. This type of anchor appears to be present in these organisms with a much higher frequency than in higher eukaryotes. Since the first full assignment of a GPI structure in 1988, more than 50 glycosylphosphatidylinositols have been structurally characterised. The functions of GPI anchors (in addition to the clear one of linking the above biopolymers to membranes) have been extensively discussed. The high lateral mobility of GPIs and GPI-anchored polymers seems to actively facilitate the selective release of molecules from the cell surface and the exchange of membrane proteins between cells. There is also evidence that GPIs and/or their metabolites can act as secondary messengers, modulating biological events including insulin production, insulin-mediated signal transduction, cellular proliferation and cell–cell recognition. Their discovered role as mediators of regulatory processes makes the chemical preparation of these compounds and their analogues of great interest. This comprehensive review highlights the progress in the chemical synthesis of GPI anchors and related glycoconjugate structures from protozoan parasites, yeast and mammals in the last two decades. The synthesis of a structurally related prokaryotic glycoconjugate of *Mycobacterium tuberculosis* is also discussed.

1 Introduction

Glycosylphosphatidylinositols (GPIs) are a class of natural glycosylphospholipids that anchor proteins and glycoproteins (*via* their C-terminus) as well as lipophosphoglycans (*via* the reducing end of the chain) to the membrane of eukaryotic cells. GPI anchors are widely present in parasitic protozoa, which are the most diverse and amongst the most ancient group of organisms in the eukaryotic kingdom.[1] This type of anchor is not unique to the protozoa, but it appears to be present in these organisms with a much higher frequency than in higher eukaryotes.[2] Although some of the plasma membrane proteins of the parasites use transmembrane polypeptide anchors, most of their major cell-surface macromolecules are GPI-anchored.

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The surface of protozoan parasites is covered with various glycoconjugates: glycoinositolphospholipids (GIPLs) and GPI-anchored glycoproteins (mucins) as well as GPI-anchored lipophosphoglycans (LPGs).[2] The tremendous diversity of the glycoconjugates has implicated their critical importance in the life cycle of these organisms, often determining parasite survival and infectivity. The survival strategies of many protozoan parasites (*Trypanosoma, Leishmania*) involve the formation of an elaborate and dense cell-surface glycocalyx composed of diverse stage-specific glycoconjugates that form a protective barrier.[3,4] The GIPLs form a dense layer on the surface of the parasite, with the GPI-anchored mucins and lipophosphoglycans projecting out and upwards from this layer.[5]

Historically, the first structural identification of a GPI anchor was that of *Trypanosoma brucei* variant surface glycoprotein (published in 1988 by Ferguson *et al.*[6]), followed by the structure of a GPI anchor of rat brain Thy-1 glycoprotein (published by Homans *et al.*[7] in the same year). Since then, more than 50 GPI anchors have been structurally characterised, all having a common core structure of Manα1→4GlcNH$_2$α1→6-*myo*-Ino1-OPO$_3$-lipid.[8]

Two classes of GPI anchors have been identified (Fig. 1). Type I anchors have the above core structure and are usually found in protozoan parasites. These GPIs attach non-protein-bound glycoconjugates such as GIPLs and LPGs onto parasite cell membranes. Amongst the most complex structures is the lipophosphoglycan from *Leishmania* parasites, which is a predominant cell-surface glycoconjugate of *Leishmania* promastigotes.

Type II anchors contain the expanded core structure, H$_2$NC$_2$H$_4$OPO$_3$–6Manα1→2Manα1→6Manα1→4GlcNH$_2$α1→6-*myo*-Ino1–OPO$_3$–lipid, and are found in mammals and lower eukaryotes including protozoan parasites and yeasts.[2] Type II GPIs attach proteins or glycoproteins through their C-termini to the ethanolamine phosphate group at the non-reducing end of the glycan core. Many of type II GPIs diverge from their basic core structure and contain one or more species-specific side chains[9] linked to specific positions of the core shown in Fig. 1. The side chains can comprise mono- or oligosaccharides, ethanolamine phosphate (specific for higher eukaryotes), 2-aminoethylphosphonate (specific for *Trypanosoma cruzi* GPIs) or an additional fatty acid residue. Another modification site is the lipid moiety, where various structures (diacylglycerol, acylalkylglycerol, *lyso*-alkylglycerol and ceramide) can be found.

The functions of GPI anchors (in addition to the clear one of linking the above biopolymers to membranes as well as their special importance on the parasitic cell-surface) have been extensively discussed.[4,9–11] The high lateral mobility of GPIs and GPI-anchored polymers seems to actively facilitate the selective release of molecules from the cell surface and the exchange of membrane proteins between cells. There is also evidence that GPIs and/or metabolites of them can act as secondary messengers, modulating biological events including insulin production, insulin-mediated signal transduction, cellular proliferation and cell–cell recognition. Their discovered role as mediators of regulatory processes makes the chemical preparation of the compounds and their analogues of great interest. To date, two review papers describing the chemical synthesis of GPI-related derivatives have been published: the first by Gigg and Gigg in 1997[12] and the second by Guo and Bishop in 2004.[13] Here, we discuss various methodologies and specific features for the chemical

preparation of glycosylphosphatidylinositol anchors and related glyconjugates. We start our discourse from syntheses of GPIs from the lower eukaryotes including protozoan parasites and yeast, followed by the preparation of GPIs from mammals. The approaches developed for GPI syntheses have also been used for the synthesis of structurally related prokaryotic glycoconjugates, phosphatidylinositol mannosides of *Mycobacterium tuberculosis*, which are discussed in Section 11.

2 Chemical synthesis of glycosylphosphatidylinositols: general notes

GPIs are among the most complex classes of natural products, as they combine lipids, carbohydrates, *myo*-inositol and phosphate groups. Their structural complexity and recently discovered biological importance have inspired widespread chemical interest, and a number of synthetic approaches towards various GPIs (yeast, rat brain Thy-1, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Plasmodium falciparum*, *Toxoplasma gondii*, *Leishmania* and sperm CD52) have been reported. Several problems are faced by those conducting GPI synthesis, such as synthesis of protected and optically pure *myo*-inositol derivatives, the stereoselective construction of the glycan core and the regioselective introduction of the side chains and phosphate moieties.

Positioning of side groups complicates the protecting group strategy. Positions on the glycan core have to be differentiated using orthogonal protecting groups that enable regioselective inclusion of the side groups (such as phosphoethanolamine and phospholipid) at later stages in the synthesis. Branching sugar chains also complicate the protecting group strategy of the main glycan backbone assembly and add a further problem of their (*i.e.* the branching sugars) stereoselective introduction. This can be affected by the nature of protecting groups on both the glycosyl donor and glycosyl acceptor and by the steric hindrance caused by other sugar residues or side groups in the oligosaccharide. If the right glycosidic linkage (α or β) is not obtained, this can result in a complete change to the synthetic strategy. In general, the stereochemistry of the glycosylation can be resolved by ingenious use of protecting groups as well as by varying glycosylation methods and conditions (*e.g.*, experimentation with different promoters and solvents).

GPI structures are generally synthesized by linear or convergent means. The linear approach can be used to build the oligosaccharide from individual monosaccharides in a stepwise manner. This strategy relies on the selective activation of a monoglycosyl donor over the growing oligosaccharide chain. The convergent (or blockwise) approach constructs the oligosaccharide from smaller building blocks, which results in a fewer number of protecting group manipulations within the oligosaccharide chain.

Synthesis of the glycan core usually proceeds with the synthesis of an optically pure differentially protected *myo*-inositol derivative followed by the addition of appropriately protected α-linked D-glucosamine. The next stage involves the formation of the remaining glycan core either by linear or convergent means. The glycan core is then decorated with the ethanolamine phosphate and phospholipid moieties before the global deprotection to provide the final GPI structure.

3 Syntheses of a GPI anchor of *Trypanosoma brucei*

3.1 Combined linear-convergent synthesis by the Ogawa group

The first total synthesis of a glycosylphosphatidylinositol was achieved in 1991–1992 by Ogawa and co-workers, who published the synthesis of a GPI anchor of *T. brucei* variant surface glycoprotein.[14,15] As indicated in Scheme 1, the synthetic plan for the preparation of a GPI anchor **1** was to assemble the glycan core **2** and then introduce phosphoethanolamine first, followed by diacylglycerol phosphate through the H-phosphonate precursors **4** and **3**, correspondingly. Glycan core **2** was further disconnected into four smaller building blocks **5–8**. This synthesis of a GPI anchor did not involve the preparation of the pseudodisaccharide (*i.e.*, azidoglucose-inositol) block separately. Instead, the *myo*-inositol acceptor **12** (Scheme 2) was glycosylated with the disaccharide glycosyl fluoride **16** to form the pseudotrisaccharide **5**.

The *myo*-inositol acceptor **12** was constructed (Scheme 2) starting from the previously synthesised racemic dicyclohexylidene derivative **9**.[16] The latter was subjected to regioselective 6-*O*-methoxybenzylation assisted by Bu_2SnO, 1-*O*-allylation, followed by acidic removal of cyclohexylidene protecting groups and per-*O*-benzylation of the hydroxyl groups to afford racemate **10**. This was *O*-deallylated, and treated with (1*S*)-(−)-camphanic acid chloride to afford the corresponding diastereomeric mixture of (1*S*)-(−)-camphanoyl-D,L-*myo*-inositols. Thus, enantiomeric resolution of *myo*-inositol was achieved by addition of a chiral (1*S*)-(−)-camphanate group[17] and then separation of the diastereomeric mixture by silica gel chromatography. Conversion of the chiral *myo*-inositol derivative **11** to the required glycosyl acceptor **12** was readily achieved in 83% yield over 5 steps (*O*-demethoxybenzylation, 6-*O*-vinylation, hydrolysis of the camphanate, introduction of the *p*-methoxybenzyl group at 1-OH and then cleavage of the vinyl ether).

The glycobiosyl fluoride **16** (Scheme 2) was constructed from the methyl thiomannoside donor **15**[18] and azidoglucose acceptor **14** as described in Scheme 2. The acceptor **14** was obtained[19] in 61% overall yield *via* sequential 4,6-*O*-benzylidenation of azidoglucoside **13**, 3-*O*-benzylation and finally regioselective cleavage of benzylidene group. $CuBr_2$–Bu_4NBr–AgOTf-promoted glycosylation of **14** with methyl thiomannoside donor **15**, followed by removal of the anomeric silyl group and conversion of the hemiacetal intermediate to the glycosyl fluoride, gave the glycobiosyl fluoride donor **16** (89%; α:β 2 : 3). Cp_2ZrCl_2–$AgClO_4$ effected coupling of the glycosyl fluoride **16** to the *myo*-inositol acceptor **12**, furnishing the corresponding α-linked pseudotrisaccharide in 73% yield (plus 20% of the β-anomer),[19] in which the two acetyl groups were removed and the 6-hydroxyl of mannose was selectively acetylated to afford pseudotrisaccharide **5** in 95% yield.

The galactobiose synthon **6** was constructed starting from peracetylated galactose **17** as depicted in Scheme 3. Glycosylation of *p*-methoxyphenol under the influence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) with the donor **17**, followed by sequential *O*-deacetylation, selective 6-*O*-tritylation with *p,p′*-dimethoxytrityl chloride (DMTCl), exhaustive *O*-benzylation and finally removal of the DMT group afforded the desired acceptor **18** in 51% overall yield. This was glycosylated with the thiogalactoside

19[20] in presence of $CuBr_2$–Bu_4NBr to afford the corresponding (α1→6)-linked galactobiose, which was further subjected to cerium(IV) ammonium nitrate (CAN)-mediated cleavage of *p*-methoxyphenyl group followed by conversion of the hemiacetal intermediate to the glycosyl fluoride donor **6** in 62% yield (α:β = 2 : 3).

The tricky coupling between the galactobiose **6** and the pseudotrisaccharide **5** (Scheme 3) was achieved under the influence of Cp_2ZrCl_2–$AgClO_4$ in 76% yield, forming preferentially the α-linked product (α:β = 9 : 1). After the removal of the acetyl group the required α-linked pseudopentasaccharide **20** was isolated. The latter was glycosylated with the mannosyl chloride **8** in the presence of a mixture of $HgBr_2$ and $Hg(CN)_2$ as promoter to offer the corresponding branched pseudohexasaccharide in 89% yield, in which the acetyl group was removed to give the acceptor **21**. The branched pseudoheptasaccharide **22** was then obtained by glycosylation of **21** with the mannosyl fluoride **7** using the Cp_2ZrCl_2–$AgClO_4$ mixture as promoter, followed by removal of the acetyl group.

Due to the problems associated with the CAN-assisted removal of the PMB group from C-1 of the *myo*-inositol unit after the introduction of the ethanolamine phosphate at C-6 in mannose-3, the synthesis was completed (Scheme 4) initially by introduction of the phospholipid and then addition of the ethanolamine phosphate moiety. 6-*O*-Chloroacetylation in mannose-3, then TMSOTf-mediated removal of the methoxybenzyl group at C-1 of *myo*-inositol followed by pivaloyl chloride-assisted condensation with the H-phosphonate **3**, afforded the H-phosphonate **23**. The chloroacetyl group of the latter compound was cleaved with thiourea to yield **24**. This was then phosphitylated with *N*-Cbz-protected aminoethyl H-phosphonate **4** to give the corresponding pseudoheptasaccharide (40%; as a mixture of four diastereomers at P atoms), in which the two H-phosphonate moieties were then oxidized with iodine in aqueous pyridine to give the fully protected GPI **25** in a 68% yield. Finally, global deprotection by hydrogenolysis in the presence of $Pd(OH)_2$/C in $CHCl_3$–MeOH–H_2O solution gave the first synthetic GPI anchor in 23% yield.

It was later proved[12,21,22] that the above synthetic study was based on the incorrect stereoisomer of the *myo*-inositol derivative **11** (Scheme 2), which was chosen by the Ogawa group erroneously, and thus provided the wrong stereoisomer of the glycosyl acceptor **12**. Because of this misassignment of the absolute configuration of the *myo*-inositol derivatives used, the final deprotected synthetic GPI anchor appeared to be a stereoisomer (in the *myo*-inositol portion) of the natural *T. brucei* GPI anchor **1**.

In summary, the first total synthesis of a GPI anchor was achieved by employing glycosyl fluoride, glycosyl chloride and thioglycoside derivatives as glycosyl donors, and benzyl groups as a permanent protecting group, as well as the use of H-phosphonate chemistry to introduce two different phosphodiester units.

3.2 Convergent synthesis by the Ley group

Synthesis of the GPI anchor **1** by the Ley group in 1998 appeared to be the first chemical preparation of a real GPI anchor of *T. brucei* variant surface glycoprotein.[23,24] The synthetic strategy relied on the use of (i) butanediacetal (BDA) groups, chloroacetic esters and benzyl

ethers as permanent protecting groups, (ii) selenoglycosides and thioglycosides as glycosyl donors that allowed as few manipulations on the oligosaccharide core as possible, and (iii) a chiral bis(dihydropyran) for enantiomeric resolution of *myo*-inositol. Ley brought together the 1,2-diacetal protecting group studies from his laboratory to simplify the puzzle of putting together an oligosaccharide chain.

As indicated in Scheme 5, the two phosphate substituents in the GPI anchor **1** were retrosynthetically disconnected as phosphoramidites **27** and **28**, leaving corresponding protected glycan core **26** for further simplification. The group followed a convergent route for assembling the pseudoheptasaccharide **26**. Protecting groups may exhibit a distinctive influence on the reactivity of glycosyl donors. The authors successfully used this effect for rapid assembly of the glycan core with just one protecting group manipulation needed. The core was assembled in just six steps from the six building blocks **29–34**, taking advantage from the reactivity-tuning effects caused by protecting groups and the use of appropriate anomeric leaving groups.

The anomeric PhSe group in compounds **31–34** has higher reactivity towards electrophilic activation than the EtS counterpart in **30** due to the greater polarisability of the selenium atom. The selenoglycosides **33** and **34** are more reactive than the selenoglycosides **31** and **32** due to the nature of the ‘arming–disarming’ effects of the protecting groups. The *O*-benzyl ‘arming’ groups in **33** and **34** enhance the reactivity of the donors, whereas the butanediacetal (BDA) and *O*-chloroacetyl groups in acceptors **31** and **32** exhibit deactivating (‘disarming’) effects on their anomeric leaving groups.

Desymmetrisation or chiral enantiomeric resolution of *myo*-inositol molecule remains a problem, despite significant efforts invested towards establishing methods to obtain enantiomerically pure *myo*-inositol derivatives. The Ley group reported a route to both chiral D-*myo*-inositols and L-*myo*-inositols employing a chiral bis(dihydropyran). This method was illustrated by the synthesis of D-*myo*-inositol derivative **43** (Scheme 6). Regioselective transformation of *myo*-inositol **35** to bis(dispoke) acetal **36** was easily achieved on treatment with butane-2,3-dione.[25] The side product **37** was removed by simple recrystallization. It is important that butane-2,3-dione forms 1,2-*trans*-diacetal derivatives (*i.e.*, involving di-equatorial 1,2-diols), contrasting with isopropylidene and benzylidene acetal formation, when 1,2-*cis*-acetals (*i.e.*, involving axial/equatorial 1,2-diols) are the favoured products. Butane-2,3-dione initially adds to the most reactive hydroxyl group in **35** (*i.e.*, the equatorial hydroxyl groups vicinal to the axial 2-hydroxyl) and then cyclizes with a vicinal equatorial OH to form a six-membered ring.

O-Silylation of the 1,6:3,4-bis(diacetal) **36** with TBDPS chloride followed by removal of the BDA groups with aqueous TFA gave tetraol **38**, which was desymmetrised with chiral bis(dihydropyran) **39** yielding **40** as a single diastereoisomer in 81% yield. This product was subsequently *O*-desilylated, per-*O*-benzylated and oxidized to form bis(phenylsulfone) **41**. Exposure of **41** to lithium hexamethyldisilazide removed the dispiroacetal, furnishing the chiral diol **42**, which in turn was selectively allylated on the C-1 hydroxyl group *via* dibutyltin acetal formation to offer the desired *myo*-inositol acceptor **43**.

Building block **47** (Scheme 7) required for the construction of pseudodisacchride **29** was synthesised from the known phthalimide derivative **44**.[26] Sequential 3-*O*-benzylation, reductive benzylidene acetal ring opening and hydrazine hydrate-assisted cleavage of the phthalimido protection transform **44** into 2-amine **45**. Further transformation into 2-azide **46** was accomplished (98%) employing triflic azide (TfN_3) in the presence of DMAP. Initial *O*-silylation of the alcohol **46** with TBSCl followed by bromination gave glycosyl donor **47**, which was coupled with *myo*-inositol acceptor **43** using Lemieux's halide inversion protocol.[27] This afforded only the desired α-linked pseudodisaccharide (65%), which on de-*O*-silylation furnished the pseudodisaccharide acceptor **29**.

Readily available phenyl 1-selenogalactoside **48**[28,29] was converted into two required building blocks **32** and **34** employing standard procedures (Scheme 8). Consecutive 2,3-*O*-protection with butane-2,3-dione (→**49**), 6-*O*-silyation with TBSCl, *O*-chloroacylation (→**50**) and desilylation gave the acceptor **32**. Conventional *O*-benzylation of the same tetraol **48** afforded the galactoside donor **34**. The mannose building blocks **31** and **33** were synthesised from phenyl 1-selenomannoside **51**.[30] BDA protection of the 3,4-diol, followed by 6-*O*-chloroacetylation *via* 6-tributyltin ether formation produced the acceptor **31**. Compound **33** was prepared by selective 6-*O*-silylation of **51**, followed by per-*O*-benzylation.

Synthesis of the central thiomannoside **30** (Scheme 9) required chloroacetate group at the 2-position to allow further anchimeric assistance and differentiation of 3- and 6-OH groups for regioselective glycosylation. The tetraol **52** was silylated at the 6-OH with TBDPSCl, the 2,3-diol was isopropylidene-protected and the 4-OH group was benzylated. Successive desilylation, acidic removal of acetonide and silylation at the 6-position with TBSCl gave the 2,3-diol **53** in 65% overall yield. Selective silylation of the equatorial 3-hydroxyl group with trimethylsilyl chloride (TMSCl) followed by standard introduction of the chloroacetyl group at 2-position and 3-*O*-desilylation furnished the desired middle building block **30**.

With all the building blocks in hand, the protected glycan core **26** was then assembled (Scheme 10). The assembly began with the stereoselective coupling of the fully benzylated donor **34** with the acceptor **32** under the influence of *N*-iodosuccinimide (NIS) and TMSOTf to afford the α-linked disaccharide fragment **54** (71%). The combined deactivating effects of the BDA and the chloroacetyl groups in **32** are crucial in avoiding any homocoupling. The thiomannoside acceptor **30** was then 3-*O*-glycosylated with the galactobioside donor **54** under the influence of methyl triflate (MeOTf) (76%) followed by 6-*O*-desilylation to furnish the requisite trisaccharide alcohol **55**. Glycosylation of the acceptor **55** with mannobioside donor **56** (5 eq.) [prepared, in turn, by coupling of **33** and **31** (87%) in a similar fashion to the formation of compound **54**] under the influence of MeOTf furnished the branched pentasaccharide **57** (75%). Finally, coupling of the pseudodisaccharide acceptor **29** and the pentasaccharide donor **57** was promoted with NIS/TfOH to offer the branched pseudoheptasaccharide core **26** (50%).

The glycan core **26** was further elaborated to the protected GPI anchor **59** (Scheme 11) by making use of phosphoramidite chemistry, which had been successfully applied earlier in syntheses of GPIs from yeast by Schmidt and co-workers[31,32] and rat brain Thy-1 by

Campbell and Fraser-Reid.[33,34] Removal of the TBS group at the 6-position in mannose-3 with aq. HF enabled the introduction of the ethanolamine phosphate moiety *via* 1*H*-tetrazole-assisted phosphitylation with phosphoramidite **27** and subsequent oxidation of the formed phosphite triester with *m*-chloroperbenzoic acid (*m*-CPBA) to afford the phosphotriester **58** (89%). This was subjected to *O*-deallylation with $PdCl_2$ and 1*H*-tetrazole-assisted phospholipidation with diacylglyceryl phosphoramidite **28**, followed by oxidation with *m*-CPBA to furnish the fully protected compound **59** (81%) as a mixture of four diastereoisomers at the P atoms.

A global deprotection sequence involving, first, palladiumcatalysed hydrogenolysis to remove the *O*-benzyl and the *N*-Cbz groups and to reduce the azide to an amino group, followed by *O*-dechloroacetylation and TFA-assisted deacetalisation gave the required GPI anchor **1** in 90% overall yield.

To summarise, Ley and co-workers developed an efficient and highly convergent synthetic strategy of *T. brucei* VSG GPI anchor **1**, which is adaptable to the preparation of other GPI anchors. The strategy is based on (3 + 2 + 2) building block assembly for construction of the glycan core, use of BDA and chloroacetate protecting groups to tune the reactivity of leaving groups in the glycosyl donors, and the use of bis(dihydropyran) to desymmetrise *myo*-inositol.

4 Synthesis of a glycoconjugate of *Toxoplasma gondii* by the Schmidt group

Toxoplasma gondii is an ubiquitous intracellular protozoan parasite. It is a causative agent for congenital infection (toxoplasmosis) and severe and often lethal encephalitis in the course of acquired immunodeficiency syndrome (AIDS). An oligosaccharide-containing small antigen has been illustrated to express immunological characteristics suitable for serological diagnosis of acute toxoplasmosis.[35] The structure of this antigen was identified to be a family of protein-free GPI glycolipids, from which the structures of two core glycans (A and B, not shown here) were elucidated. Immunological studies revealed that only type B GPIs containing a Glcα1→4GalNAc side chain linked to the first Man moiety were recognised by sera from infected humans, suggesting that the unique glucose modification is essential for immunogenicity.[36] In order to understand this phenomenon, synthesis of the type A GPI glycan of *T. gondii* (containing GalNAc side chain instead) **60** (Scheme 12) was required.

A convergent and versatile strategy for the synthesis of phosphorylated *T. gondii* GPI anchor pseudohexasaccharide **60** was developed by Schmidt and co-workers.[37] They used a strategy that relies on late-stage phosphorylation (after the assembly of the glycan core **61**) with *N,N*-diisopropylphosphoramidites **62** and **63**, and the use of appropriate protecting groups (Scheme 12). The Schmidt group syntheses of GPI anchors rely, in general,[31,32,37,38] on the trichloroacetimidate chemistry together with the participating effect of acetic esters at C-2 of the D-mannose glycosyl donors to confer α-selectivity in every glycosylation. In the retrosynthesis of the branched glycan core **61**, the α-glycoside linkage between the central D-mannose and D-glucosamine units is disconnected, affording the building blocks **64** and

65. The tetrasaccharide **65** could be easily prepared from building blocks **67–70**, while the synthesis of the pseudodisaccharide **64** is challenging.

Synthesis of the pseudodisaccharide **64** is based upon the conversion of the chiral *myo*-inositol derivative **71** into the required product (Scheme 13). *O*-Cyclohexylidenation of *myo*-inositol **35**,[16,39] followed by enantiomeric resolution *via* addition of a chiral (−)-menthylformate group and then separation of diastereomers by crystallisation gave the desired D-*myo*-inositol derivative **71**.[31,40] Subsequent consecutive 6-*O*-allylation, replacement of the (−)-menthyloxycarbonyl group with *p*-methoxybenzyl group and finally acid-catalysed cleavage of the cyclohexylidene groups offered the required tetraol **72**. Perbenzylation of OH groups in **72**, followed by 6-*O*-deallylation using Wilkinson's catalyst and acidic treatment, gave the 6-OH *myo*-inositol acceptor **12**. The latter was glycosylated with the trichloroacetimidate donor **66**[41] in the presence of TMSOTf to furnish the desired (α1→6)-linked pseudodisaccharide **73** in 70% yield. The 2-azido group in **66** served as a non-participating group that assists in the formation of the α-glycoside linkage. Compound **73** was subjected to sequential de-*O*-acetylation, 4′,6′-*O*-benzylidenation, 3′-*O*-benzylation and finally reductive ring opening of the benzylidene acetal with $NaBH_4$ in the presence of HCl, to furnish the pseudodisaccharide acceptor **64**.

The branched trisaccharide **81** (Scheme 14) was assembled from three monosaccharide synthons, **68**, **69** and **70**. Mannosyl donor **68** was derived[32] from 1,2-orthoester **79** (available from D-mannose)[42] *via* the 1,2-di-*O*-acetate **80**.[43] Compound **70** was prepared from D-mannose **76** in five steps,[44] while the D-galactosamine donor **69** was made from the 2-azido derivative **74**.[41]

Anomeric *O*-allylation of **76** and subsequent Bu_2SnO-mediated selective 3-*O*-benzylation afforded the desired triol **77** in 61% overall yield. This was treated with *p*-methoxybenzaldehyde dimethylacetal to form 4,6-*O*-arylidene intermediate, which was 2-*O*-benzoylated with benzoyl cyanide. The 4,6-acetal ring was reductively opened using $NaBH_3CN$–TFA, furnishing the 4-OH mannose derivative **70**. The nature of protecting groups in the acceptor **70** provides the desired regio- and stereoselective glycosylation sequence and further transformation into a glycosyl donor, permitting later the α-selective glycosylation of the pseudodisaccharide **64**.

The D-galactosamine donor **69** was derived[45] from the azide **74** *via* silylation of the 1-OH group with thexyldimethylsilyl chloride [TDSCl; (2,3-dimethylbut-2-yl)dimethylsilyl chloride], reduction of the 2-azido group and trichloroacetylation of amine to furnish **75**, which on successive 1-*O*-desilylation and anomeric *O*-trichloroacetimidation offered **69**. The *N*-trichloroacetyl protecting group was introduced in order to ensure high glycosyl donor properties and anchimeric assistance for β-glycoside bond formation. The trichloroacetimidate **69** and the acceptor **70** were coupled under the influence of $BF_3{\cdot}Et_2O$ to afford stereoselectively the corresponding disaccharide (85%), in which the *p*-methoxybenzyl group was removed (→**78**) to enable TMSOTf-promoted coupling with the mannosyl donor **68**, yielding the branched trisaccharide (95%) that was *O*-deacetylated to give the trisaccharide acceptor **81**.

The third mannose building block **67** (Scheme 15) was derived[32] from the orthoester **79**. 6-*O*-Silylation, *O*-benzylation, subsequent acid-catalysed orthoester opening and then *O*-acetylation afforded **82**. Selective anomeric deacetylation with hydrazinium acetate gave the hemiacetal, which was treated with trichloroacetonitrile in presence of DBU to give the trichloroacetimidate donor **67**. TMSOTf-promoted stereoselective glycosylation of the branched trisaccharide acceptor **81** with the donor **67** gave the corresponding tetrasaccharide (92%), in which the benzoic ester on mannose-1 was then replaced with acetate *via* the standard *O*-deacylation–*O*-acetylation procedure (→**83**).

Transformation of the *N*-trichloroacetyl group of the tetrasaccharide **83** into the *N*-acetyl group was carried out by homolytic reduction and removal of the anomeric allyl group under the influence of Wilkinson's catalyst, followed by the reaction of the hemiacetal with trichloroacetonitrile to afford trichloroacetimidate donor **65**. The pseudodisaccharide **64** was then coupled onto the growing chain (→**61**, 74%) to furnish, after *O*-desilylation, the pseudohexasaccharide **84**.

The introduction of phosphate residues onto **84** (Scheme 15) was achieved *via* 1*H*-tetrazole-assisted phosphitylation reactions. First, treatment with the phosphoramidite **62** furnished the phosphite intermediate, which was oxidised to the phosphotriester with *m*-CPBA followed by treatment with Et_3N to remove the cyanoethyl P-protecting group. The following CAN-assisted removal of the *p*-methoxybenzyl group afforded the aminoethyl phosphate **85**. Phosphorylation of **85** with benzylcyanoethyl-*N,N*-diisopropylaminophosphoramidite **63** in a similar fashion, followed by cyanoethyl cleavage with Me_2NH and *O*-deacetylation, furnished the diphosphorylated compound **86**, which by hydrogenolytic *O*-debenzylation afforded the target compound **60** in good yield and purity.

5 Synthesis of a glycoconjugate of *Leishmania* by the Konradsson–Oscarson group

Two Swedish groups, those of Oscarson and Konradsson, jointly reported[46] the synthesis of *Leishmania* lipophosphoglycan core phosphorylated heptasaccharyl *myo*-inositol **87** (Scheme 16) found in cell-surface structures of *Leishmania* parasites. The synthesis was accomplished using a convergent (3 + 2 + 2 + 1) synthetic strategy, in which the introduction of the α-D-glucose anomeric phosphodiester unit (due to its instability towards acidic conditions) was accomplished at a late stage, just before the global deprotection.

The preparation of the trisaccharide **88** and the disaccharide **89** building blocks is shown in Scheme 17. Ethyl 1-thio-β-D-galactopyranoside **92** was 6-*O*-tritylated and then per-*O*-benzylated, followed by acidic *O*-detritylation to yield derivative **94**. Per-*O*-benzylation of **92** and replacement of the ethanethiol group with bromine gave the galactosyl bromide **93**, which was coupled with **94** using the 'halide inversion' method[27] to give the galactobiose disaccharide **95**. The galactofuranose acceptor **98** was prepared from the 5,6-diol **96**.[47] Sequential *O*-acetylation, to preserve the furanose ring, TFA-assisted cleavage of isopropylidene acetal and *O*-acetylation gave **97**. After hydrogenolysis, the acceptor **98** was glycosylated with the thioglycoside donor **95** under the influence of

dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) to yield the trisaccharide **88** (67%).

The mannobiose disaccharide **89** was synthesised[48] starting from mannose derivative **99**.[49] *O*-Benzylation followed by reductive benzylidene ring cleavage gave **100**. This was 6-*O*-benzylated and 3-*O*-desilylated to furnish the acceptor **102**. 6-*O*-Chloroacetylation of **100**, followed by 1-bromination gave the mannosyl bromide **101**, which was coupled with **102** in the presence of AgOTf to give the corresponding disaccharide (79%), from which the TBS group was removed to furnish **89**. This was coupled with trisaccharide donor **88** under the influence of TMSOTf to yield the pentasaccharide **103** (85%).

The *myo*-inositol-containing building block **90** (Scheme 18) was synthesised *via* AgOTf–dicyclopentadienylzirconium dichloride (Cp_2ZrCl_2)-mediated coupling (48%) of glycosyl fluoride **106** (acquired in five steps from ethyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside **104**)[50] to chiral *myo*-inositol derivative **107**,[51,52] followed by consecutive removal of pivaloyl goups (→**108**, 80%), 4,5-*O*-isopropylidene acetal introduction, phosphorylation of the 1-OH and, finally, removal of the allyl group. The pentasaccharide thioglycoside donor **103** was activated with DMTST in ether and coupled to the acceptor **90** to give the corresponding protected pseudoheptasaccharide (75%), from which the chloroacetyl group was removed to give **109**.

The pseudoheptasaccharide **109** was subjected to pivaloyl chloride mediated coupling with the α-D-glucopyranosyl H-phosphonate **91** (prepared by the reaction of the hemiacetal **110** with diphenyl phosphite)[53] and subsequent oxidation with iodine to produce the protected compound **111** (94%). Three-step deprotection, *i.e.*, standard *O*-deacetylation, then *O*-debenzylation by Birch reduction and subsequent acid hydrolysis of the acetals, gave the phosphorylated glycan-*myo*-inositol **87** in 78% yield.

6 Syntheses of GPI anchors and a glycoconjugate of *Plasmodium falciparum*

6.1 Linear synthesis of a GPI anchor by the Fraser-Reid group

A step-by-step synthetic route for preparation of fully lipidated GPI anchor **112** present on the cell surface of the malaria pathogen *P. falciparum* was developed by Fraser-Reid and co-workers.[54,55] The anchor in this case contains an additional lipid acyl (myristoyl) moiety at the axial 2-OH in *myo*-inositol. The synthetic strategy, which was based on the retrosynthetic analysis presented on Scheme 19, relies on the use of *n*-pentenyl orthoesters as glycosyl donors and on a appropriately protected carbohydrate core to allow site-specific deprotection, phosphorylation and acylation at appropriate stages. The strategy exploited a trityl ether and a cyclohexylidene acetal as orthogonal blocking groups, while the benzyl group was chosen for permanent *O*-protection. In the retrosynthesis, the protected glycan core **113** was assembled from D-mannose 1,2-orthoesters **115–118** and 6-*O*-unprotected *myo*-inositol acceptor **119**. Late phosphorylation was achieved by making use of the phosphoramidites **27** and **114**.

The Fraser-Reid group developed[56] a novel methodology for the preparation of optically pure and differentially protected *myo*-inositol acceptor **119** starting from methyl glucoside **120** (Scheme 20). The procedure relied on the discovery[57,58] that enol esters undergo the Ferrier reaction with excellent stereocontrol. First, the tetraol **120** was converted to the 6-OH derivative **121** and the primary hydroxyl group transformed into the aldehyde using Moffat oxidation conditions, followed by enol acetylation to afford the enol acetate **122**. Lewis acid-promoted rearrangement of **122** employing Ferrier conditions[59] furnished the corresponding cyclohexan-5-one **123**, which was further subjected to consecutive reduction, *O*-deacetylation and 1,2-*O*-cyclohexylidenation to furnish the required 6-OH D-*myo*-inositol derivative **119**.

Building blocks **115–118** were synthesised (Scheme 21) from the readily available *n*-pentenyl 1,2-orthoester **124**.[60] Tin-mediated selective double benzylation of the C-6 and C-3 OH-groups in **124**, followed by *p*-methoxybenzylation of the remaining 4-OH, afforded the donor **118**. Conventional 6-*O*-tritylation of **124** followed by acetylation gave **115**, while standard benzylation furnished **116**, and consecutive 6-*O*-silylation and benzylation provided **117**.

Step-by-step assembly of the glycan core **113** (Scheme 22) started from the glycosylation of **119** with the orthoester **118** in the presence of ytterbium(III) triflate and NIS to give the corresponding pseudodisaccharide (98%), in which the 2-benzoate group was replaced with the 2-triflate group to furnish the pseudodisaccharide **125**. Conversion of the mannose-inositol **125** into the azidoglucose-inositol **126** was achieved *via* azide displacement[61] of the 2-triflate, followed by $BF_3{\cdot}Et_2O$-assisted removal of the *p*-methoxybenzyl group. Glycosylation of the acceptor **126** with the orthoester donor **117** under the influence of NIS and $BF_3{\cdot}Et_2O$ (79%), followed by replacement of the 2-benzoate with a 2-*O*-benzyl group and 6-*O*-desilylation furnished the pseudotrisaccharide **127**. This was then coupled with the orthoester **116** to give the corresponding oligomer (99%), which on conventional debenzoylation afforded the requisite pseudotetrasaccharide **128**. The latter was coupled with the donor **115** (75%), followed by subsequent *O*-deacylation and *O*-benzylation to furnish the desired pseudopentasaccharide **113**.

Mildly acidic removal of the trityl group in **113** (Scheme 23) enabled further *O*-phosphitylation with the phosphoramidite **27** and 1*H*-tetrazole, followed by oxidation to provide the *N*-Cbz-aminoethyl phosphotriester **129**. Removal of the cyclohexylidene protection in **129**, followed by myristoylation of the diol with trimethylorthomyristate,[62] gave the corresponding 1,2-cyclic orthoester, which was opened efficiently and with good regioselectivity employing $Yb(OTf)_3$ to provide 4.7 : 1 ratio of the axial **130** (71%, the required one) and the equatorial **131** (15%, the isomeric) myristic esters.

The axial ester **130** was phospholipidated by making use of the diacylglyceryl phosphoramidite **114**[33] to provide the fully protected GPI **132** (72%), which on global deprotection by hydrogenolysis (first in an organic solvent followed by addition of water) gave the desired GPI anchor **112** in 87% yield.

6.2 Convergent synthesis of a GPI anchor by the Seeberger group

Seeberger and co-workers[63] synthesized another GPI anchor **133** (Scheme 24) of *P. falciparum* with a structure similar to **112**, but with an extra D-mannose residue (α1→2)-linked to mannose-3 and differing in the *myo*-inositol fatty acyl moiety.

The glycan core **134** was assembled using a convergent (4 + 2) synthetic strategy *via* coupling of the tetramannose trichloroacetimidate **137** and the pseudodisaccharide **136**. The strategy exploited orthogonal protecting groups as PMB ether for the C-2 inositol acylation site, a TIPS ether for the ethanolamine phosphate in mannose-3, and an allyl ether for the phospholipid at C-1 of *myo*-inositol. Late phosphorylation was achieved by making use of the H-phosphonate derivatives **4** and **135**.

The pseudodisaccharide **136** (Scheme 25) was constructed starting from compound **140**,[56,64] used earlier by the Fraser-Reid group. Deacetylation of **140** and subsequent Bu_2SnO-assisted regioselective 1-*O*-allylation and 2-*O*-methoxybenzylation gave the *myo*-inositol acceptor **141**. This was glycosylated with the known trichloroacetimidate **66**[41,65] to give the corresponding pseudodisaccharide as inseparable anomeric mixture (α:β = 4 : 1), which was *O*-deacetylated to provide triol **142** (89%). Installation of 4′,6′-benzylidene acetal followed by 3′-*O*-benzylation and reductive opening of the 4′,6′-*O*-benzylidene protection with $NaBH_3CN$–HCl gave the pseudodisaccharide, while the separation of the α- (**136**) and β-anomers became possible.

The tetrasaccharide block **137**[66,67] was formed (Scheme 26) by making use of trichloroacetimidate donors employing a similar approach to Schmidt (Section 4) towards the construction of (α1→2)-linked mannose residues. The mannose building blocks **68**, **138** and **139**[64,68,69] were derived from the orthoesters **143**, **144** and **79**, respectively.

Construction of the tetramannoside **137** started with the TMSOTf-catalysed coupling of acceptor **138** with the donor **68** (91%), followed by removal of the acetic ester in presence of benzoate by acidic methanolysis[74] to furnish the disaccharide **145**. The latter was further elongated to the tetrasaccharide **147** employing iterative glycosylations with donors **139** [93%, followed by deacetylation with $Mg(OMe)_2$ to give **146**] and **68** (80%). Late removal of the anomeric allyl group and introduction of the trichloroacetimidate gave the tetrasaccharide glycosyl donor **137**.

Stereoselective TMSOTf-catalysed coupling of **137** and the pseudodisaccharide acceptor **136** (Scheme 27) gave the corresponding pseudohexasaccharide (94%; the 2-*O*-benzoyl group in the donor **137** assisted in the α-selectivity), in which the *O*-acetyl and -benzoyl groups were replaced with *O*-benzyl to yield the protected glycan core **134**. Oxidative cleavage of the PMB protecting group enabled the introduction of the palmitoyl moiety at O-2 of *myo*-inositol using DCC-assisted acylation. Subsequent removal of the allyl group and introduction of the phospholipid by pivaloyl chloride assisted phosphitylation with the H-phosphonate **135** followed by oxidation gave the phosphodiester **148** (72%). Finally, after removal of the silyl ether in mannose-3 with the help of $Sc(OTf)_3$, the ethanolamine phosphate was installed in a similar manner using the H-phosphonate **4** to provide the fully

protected GPI **149** (94%). Global deprotection was then achieved by hydrogenolysis over Pearlman's catalyst to give the GPI anchor **133** in 94% yield.

6.3 Solid-phase synthesis of pseudohexasaccharide malarial toxin by the Seeberger group

The group has also synthesized the phosphorylated pseudohexasaccharide **150** (Scheme 28) of the GPI anchor of *P. falciparum*, which they termed a 'pseudohexasaccharide malarial toxin',[68] employing automated solid-phase synthesis for the preparation of the tetramannoside **152**. The glycan core **151** was assembled utilising a (4 + 2) strategy *via* the coupling of a tetramannose trichloracetimidate made from **152** and the pseudodisaccharide **153**. Late introduction of the phosphate groups was achieved using methyl dichlorophosphate and the phosphoramidite **62**.

The tetramannoside **152** was assembled on the solid phase by making use of trichloroacetimidate mannosyl donors **68**, **139**, **154** and **155** and octenediol-functionalized Merrifield resin **156** (Scheme 29) as the first polymer-bound acceptor. Each chain elongation cycle relied on double glycosylations (using catalytic TMSOTf) to secure high coupling efficiencies followed by removal of the acetic ester with NaOMe. Three chain elongations of the acceptor **156** using, consecutively, the donors **154**, **68** and **139** provided the polymer-bound acceptor **157**, which on further glycosylation with the donor **155**, followed by cleavage of the octendiol linker with Grubbs' catalyst[70] in an atmosphere of ethylene, afforded the *n*-pentenyl tetrasaccharide **152**.

The aglycon moiety in **152** was then hydrolysed with NBS, and the hemiacetal was converted to the corresponding glycosyl trichloracetimidate prior to coupling with the pseudodisaccharide **153**, thus forming the glycan core **151** (32%). Further protecting group remodelling released the 1,2-diol in the *myo*-inositol moiety, which was cyclophosphorylated with $MeOP(O)Cl_2$, followed by *O*-demethylation with aqueous HCl. Subsequent standard *O*-desilylation on mannose-3 provided the glycan cyclophosphate **158** (70%). Final phosphorylation was achieved *via* 1*H*-tetrazole-catalysed coupling with the phosphoramidite **62** followed by oxidation with *tert*-BuOOH to give the protected compound **159** (84%). Cleavage of the cyanoethyl ester with DBU followed by Birch reduction for global deprotection gave the required phosphoglycan **150**. The chemically synthesised compound **150** was then bound to a protein carrier, and the conjugate was tested as a malarial toxin glycovaccine in mice, which appeared to be substantially protected from death caused by malaria parasites.[69]

7 Syntheses of GPI anchors and a glycoconjugate of *Trypanosoma cruzi*

7.1 Convergent syntheses of GPI anchors by the Nikolaev group

The protozoan parasite *Trypanosoma cruzi* is a causative agent of Chagas' disease, which is widespread in South and Central America. Throughout the life cycle, *T. cruzi* produce both common and stage-specific GPI-anchored cell-surface macromolecules.[5] Local release of GPI-anchored mucins by the bloodstream trypomastigote stage of the parasite is believed to be responsible for development of parasite-elicited inflammation causing cardiac and other pathologies associated with acute and chronic phases of Chagas' disease. It has been

discovered[71] that the purified GPI fraction of *T. cruzi* trypomastigote mucins (trypomastigote GPI or tGPI) has extraordinary pro-inflammatory activities, comparable to those of bacterial lipopolysaccharide. The extreme biological activity was reportedly associated with the presence of unsaturated fatty acids in the *sn*-2 position of the alkylacylglycerophosphate moiety (structures **160a** and **160b** in Scheme 30). The content of fatty acid components in the biologically active tGPI anchor fraction was found to be: oleic acid (C18:1, 31%), linoleic acid (C18:2, 21%) and palmitic acid (C16:0, 37%). Nikolaev and co-workers recently reported chemical syntheses of *T. cruzi* tGPIs bearing oleic (**160a**) and linoleic (**160b**) acid moieties.

There are two major structural features distinguishing compounds **160a** and **160b** from the GPIs synthesised previously: (i) the presence of unsaturated fatty acids in the lipid moiety instead of saturated ones and (ii) the presence of a 2-aminoethylphosphonate at C-6 of the D-glucosamine moiety, which is a parasite-specific substituent for *T. cruzi* only. Since the presence of double bonds was not compatible with the use of benzyl ethers (widely used before) as permanent *O*-protecting groups, novel strategies were developed. *Strategy A* was designed to use mildbase-labile (esters) and acid-labile (acetals and *N*-Boc) permanent protecting groups, whereas *Strategy B* required acid-labile (diacetals, acetals and *N*-Boc) and fluoride-labile (primary and secondary TBS ethers) groups for this purpose. *Strategy C* was designed for acid-labile (diacetals, acetals, PMB ethers and *N*-Boc) permanent protecting groups only, and was used for the preparation of *T. cruzi* tGPI bearing a palmitic acid moiety (**160c**).

Synthetic strategy A. [72,75]**—**The C-phosphonate and phosphate linkers in the GPI anchors **160a** and **160b** (Scheme 30) were retrosynthetically disconnected sequentially as phosphonodichloridate **162**, ethanolamine H-phosphonate **163** and acylalkylglycerol H-phosphonates **164a** and **164b**, leaving the corresponding glycan core **161** for further simplification. The core was assembled in a (4 + 2) manner from benzoylated mannotetraose building block **166** and acetal-protected pseudodisaccharide **165**. Various silyl ethers were employed as orthogonal blocking groups for C-6 of D-glucosamine (TES), C-6 of D-mannose-3 (primary TBS) and C-1 of *myo*-inositol (secondary TBS) to ensure further introduction of the P-containing esters. The tetramannoside **166**, in turn, was assembled stepwise from four building blocks **167–170** in an upstream manner.

The acceptor **167** and the donor **170** were prepared from D-mannose in three basic steps (for each). The trichloroacetimidate donors **168** and **169** were again derived (Scheme 31) from D-mannose *via* the 1,2-orthoester **171**.[73] Successive *O*-deacetylation, 6-*O*-silylation, *O*-benzoylation and TFA-catalysed opening of the orthoester ring gave the hemiacetal **172**, which was *O*-acetylated at the anomeric position, *O*-desilylated and then 6-*O*-benzylated with benzyl trichloroacetimidate to give **173**. Anomeric *O*-deacetylation of **173**, followed by treatment with Cl_3CCN in the presence of Cs_2CO_3, furnished the trichloroacetimidate donor **169**.

Replacement of the acetyl groups with benzoates in the orthoester **171**, followed by opening of the orthoester ring and reaction with Cl_3CCN in the presence of Cs_2CO_3, offered the α-trichloroacetimidate donor **168**. Coupling of **168** with the acceptor **167** under the influence

of TMSOTf (Scheme 31), followed by selective removal of the lone acetyl group in the presence of benzoates with HCl in methanol–dichloromethane,[74] gave the disaccharide acceptor **174**, which on glycosylation with the trichloroacetimidate donor **169** and *O*-deacetylation produced the trisaccharide **175**. One more glycosylation of **175** with **170**, followed by replacement of the lone benzyl ether with the TBS protecting group, gave the tetrasaccharide **176**, which was subjected to anomeric debenzoylation with ethylenediamine and trichloroacetimidation to furnish the tetrasaccharide building block **166**.

Synthesis of the novel pseudodisaccharide acceptor **165** (Scheme 32) was developed starting from glycosylation of the diastereomeric mixture of *myo*-inositol derivatives **177** and **71** (prepared first by Mayer and Schmidt)[40] with the known glycosyl donor **66**.[41] This produced the required pseudodisaccharide **179** (D-D) in 64% yield, which appeared to be separable from the diastereoisomer **178** (D-L) (30%) by standard flash column chromatography. Manipulation of the protecting group pattern in **179** was achieved *via* consecutive mild selective de-*O*-acetylation in the presence of (−)-menthyl carbonate, 4′,6′-orthoesterification and introduction of the acid-labile trimethylsilylethoxymethyl (SEM) group at 3′-OH to give fully protected azidoglucose-inositol **180**. Replacement of the (−)-menthylcarbonate with the TBS group, then mildly acidic ring opening of the 4′,6′-orthoester (thus forming a mixture of the 4′- and 6′-benzoates), followed by standard de-*O*-benzoylation and selective 6′-*O*-silylation with Et_3SiCl, furnished compound **165**.

With both building blocks in hand, the assembly of the GPI glycan backbone was only one step away (Scheme 33). Glycosylation of the pseudodisaccharide acceptor **165** with the tetrasaccharide trichloroacetimidate donor **166** in the presence of the TMSOTf catalyst gave the pseudohexasaccharide **161** (71%). Selective cleavage of the TES ether with TBAF produced the corresponding 6′-OH derivative (85%). 1*H*-Tetrazole-assisted esterification of the 6′-OH group with phosphonodichloridate **162**, followed by methanolysis, gave the corresponding phosphonic diester (79%), which was subjected to successive reduction of the two azido groups employing Staudinger conditions and *N*-protection with Boc anhydride to afford **181**. Selective removal of the primary TBS group, phosphitylation with the H-phosphonate **163**, and subsequent oxidation with iodine gave the phosphate-phosphonate block **182** (88%).

Cleavage of the secondary TBS group in the *myo*-inositol moiety enabled the final addition of the phospholipid *via* coupling with the corresponding acylalkylglycerol H-phosphonate (**164a** or **164b**) to afford fully protected GPIs (85–95%), which were demethylated at the aminoethylphosphonate moiety to furnish **183a** and **183b**. Global deprotection involved first controlled *O*-debenzoylation, which provided corresponding debenzoylated GPIs (38–40%) isolated by flash column chromatography. Mild base treatment in polar solvent cleaved the benzoic esters preferentially, and left the fatty ester of the lipid mostly intact, probably because of micelle formation. Final removal of the acid-labile acetal and *N*-Boc protections with aqueous TFA gave the required products **160a** and **160b**.

Synthetic strategy B.[75]—In the second approach, the pseudohexasaccharide core **184** (Scheme 34) was assembled by (3 + 3) coupling of derivatives **185** and **186**. These building blocks were designed utilising acid- and fluoride-labile permanent protecting groups, whilst

also using orthogonal protecting groups (TES ether at C-6 of D-glucosamine, phenoxyacetate at C-6 of D-mannose-3 and benzoic ester at C-1 of *myo*-inositol) required to achieve further 'P-decoration' at a later stage. The pseudotrisaccharide **186** was formed from the pseudodisaccharide **190** and the thiomannoside donor **191**. The trimannoside **185** was made up of three mannosyl derivatives **187–189**.

The trimannoside **185** was constructed (Scheme 35) by starting with coupling of the trichloroacetimidate **188** with the acceptor **189** (83%), followed by *O*-debenzyolation and 6-*O*-phenoxyacetylation to furnish the disaccharide acceptor **192**, which on glycosylation with the thioglycoside **187** in the presence of MeOTf gave the trimannoside **185** (65%). The pseudotrisaccharide block **186** was assembled from the thiomannoside donor **191** and the azidoglucose-inositol derivative **190**.

Compound **190**, in turn, was made from the pseudodisaccharide **179** by manipulation of the protecting group pattern, as shown in Scheme 35. It was also discovered that more efficient separation of the diastereomers **178** and **179** (Scheme 32) could be achieved after addition of tetraisopropyldisiloxane (TIPDS) 4′,6′-*O*-protection.

Replacement of the (−)-menthyl carbonate with benzoate in TIPDS-protected derivative **193**, followed by TIPDS cleavage (with TBAF) and selective 6′-*O*-silylation with TESCl furnished **190**. Methyl triflate-promoted glycosylation of the pseudodisaccharide acceptor **190** with **191** in the presence of 2,6-di-*tert*-butyl-4-methylpyridine to avoid cleavage of the acid-labile groups, followed by removal of the phenoxyacetate (with $MeNH_2$) gave the pseudotrisaccharide **186** (71% over two steps). The latter was coupled to the trimannoside **185** under the conditions described above to furnish the pseudohexasaccharide **184** (75%).

Selective removal of the TES ether in the azidoglucose moiety in **184** (Scheme 36), followed by introduction of the azidoethylphosphonate with **162** (77%) and conversion of the azido groups into Boc-protected amines (as performed in strategy A) gave the phosphonic diester **194**. Cleavage of the phenoxyacetate, followed by introduction of the *N*-Boc-protected ethanolamine phosphate by the use of the H-phosphonate **163**, gave the phosphate-phosphonate block **195** (80%). Removal of the benzoate in the *myo*-inositol moiety of **195** enabled the final addition of the phospholipid (using the H-phosphonate **164a**, 65%), followed by demethylation (with thiophenol) to give the protected GPI anchor **196**.

Total deprotection involved removal of the silyl ethers with $3HF{\cdot}Et_3N$ and cleavage of the acid-labile acetals and *N*-Boc groups with aqueous TFA to give the required product **160a**.

Synthetic strategy C.[76]—In the third approach, the glycan core **197** (Scheme 37) was retrosynthetically broken down into three major building blocks: the mannobiosides **198** and **199** and the pseudodisaccharide **190**. The disaccharides **198** and **199** were further disconnected to the mannosyl derivatives **200–203**. These building blocks were designed in such a way that only acid-labile protecting groups – such as cyclohexylidene and SEM acetals, butanediacetal (BDA), *p*-methoxybenzyl ethers and primary TBS ethers, which are known to be stable towards miscellaneous reaction conditions – were used for permanent OH-protection. Their simultaneous removal in the final deprotection step was therefore

anticipated. The orthogonal protecting groups required to execute further ‘P-decoration’ of the glycan core **197** and to prepare the GPI anchor **160c** were identical to those used in strategy B.

The derivatives **200–203** derived from BDA-protected thiomannoside **204** (Scheme 38) (prepared, in turn, by selective incorporation of the BDA motif at the 3,4-vicinal diol).[77] Selective 6-*O*-silylation of **204** and subsequent benzylation with *p*-methoxybenzyl chloride and desilylation gave **203**. 2,6-Di-*O*-methoxybenzylation of **204**, followed by subsequent NIS–TfOH-mediated hydrolysis of the ethanethiol group and treatment with Cl_3CCN in the presence of DBU, furnished the trichloroacetimidate **200**.

Coupling of the donor **200** with acceptor **201** (prepared by selective 6-*O*-phenoxyacetylaton of **204**) under the influence of TMSOTf gave the corresponding disaccharide (88%), which upon hydrolysis of the ethanethiol moiety and installation of the trichloroacetimidate gave the disaccharide glycosyl donor **198**. The dimannoside building block **199** was assembled by TMSOTf-activated glycosylation of **203** with the trichloroacetimidate **202**, prepared from **204** by successive 6-*O*-silylation, 2-*O*-phenoxyacetylation, anomeric deprotection and trichloroacetimidation. The protected mannobiose **199** intermediate was ideally suited to further glycan chain extension, either upstream or downstream.

For upstream extension, a portion of the compound was transformed into the trichloroaceimidate donor **205**. This was used for TMSOTf-promoted glycosylation of the pseudodisaccharide **190** (Scheme 39) to give the corresponding pseudotetrasaccharide (87%), in which the phenoxyacetyl group was removed to yield the acceptor **208**. This was further glycosylated with the disaccharide donor **198** in the presence of TMSOTf to give the glycan core **197** (88%), from which the TES group was removed with TBAF to yield the pseudohexasaccharide **209**. A second portion of **199** was converted (Scheme 38) to the acceptor **206** (required for downstream chain assembly) by removal of the phenoxyacetyl group. Coupling of **206** and the donor **198** (93%), followed by anomeric deprotection and trichloroacetimidation of the hemiacetal, afforded the tetrasaccharide trichloroacetimidate **207**. The latter was coupled (TMSOTf) with the acceptor **190** (→**197**, 92%), followed by selective TES removal to afford the pseudohexasaccharide **209**.

Successive ‘P-decoration’ of the glycan core (Scheme 40) was achieved as described above in strategy B. First, 1*H*-tetrazole-assisted phosphonylation of the alcohol function in **209** with phosphonodichloridate **162** followed by methanolysis afforded a phosphonic diester (60%), in which the azido groups were reduced with PPh_3 and *N*-protected with Boc-anhydride to afford the phosphonylated block **210**.

Removal of the PAc group in **210** and subsequent pivaloyl chloride assisted phosphitylation with the H-phosphonate **163**, followed by oxidation of the intermediate with iodine, gave the phosphate-phosphonate block **211** (79%). Cleavage of the benzoic ester in the *myo*-inositol moiety enabled successful phospholipidation (86%) using the palmitoylhexadecylglycerol H-phosphonate **164c**. The *O*-methyl group in the aminoethylphosphonate moiety was removed with thiophenol to afford **212** (85%). Global deprotection of **212** was successfully carried out in one step with 90% aqueous TFA to provide **160c** in almost quantitative yield.

7.2 Convergent synthesis of a GPI anchor by the Vishwakarma group—

Vishwakarma and co-workers reported[78] a downstream (2 + 2 + 2) approach to synthesise GPI anchor **213** of the *T. cruzi* IG7 antigen (Scheme 41) *via* the efficient construction of the glycan core **214** from the azidoglucose-inositol **64** and mannotetraose **216** intermediates. The tetramannoside **216** was retrosynthetically broken down into two disaccharide building blocks, **217** and **218**, which were further disconnected to monosaccharide derivatives **116, 155, 219** and **221** (Scheme 42).

The assembly of the tetramannoside **216** (Scheme 42) began by coupling of the acceptor **219** with the trichloroacetimidate **155** to afford a disaccharide intermediate (81%); this was subjected to the anomeric *O*-allyl and the 4,6-*O*-benzylidene removal followed by *O*-acetylation to afford the disaccharide **220**. Anomeric deacetylation followed by Schmidt activation (CCl_3CN, DBU) provided the required glycosyl donor **217**. Glycosylation of the acceptor **221** with the orthoester donor **116** in the presence of TESOTf–NIS (72%) followed by removal of the *O*-benzoyl group from position-2 gave the mannobioside **218**. The latter was coupled with the donor **217** (69%), followed by consecutive anomeric deallylation and trichloroacetimidation to give the tetramannoside donor **216**. TMSOTf-activated glycosylation of the pseudodisaccharide **64**[37] with compound **216** afforded the corresponding pseudohexasaccharide intermediate (60%).

Further standard *O*-deacetylation prior to selective silylation of the primary 6′′′′-OH and conventional benzylation of the 4′′′′-OH provided the glycan core **214**. Sequential 6′′′′-*O*-desilylation (TBAF), 1*H*-tetrazole-assisted phosphitylation with *N*-Cbz-aminoethyl phosphoramidite **27** and oxidation with *m*-CPBA gave the phosphotriester **222** (60%). The PMB group was removed from position-1 of the *myo*-inositol moiety with CAN, followed by phospholipidation with 1-*O*-stearyl-2-*O*-stearoylglycerol H-phosphonate **215** to afford the protected conjugate **223** (55%), which on global deprotection by hydrogenolysis over the $Pd(OH)_2/C$ gave the target GPI anchor **213**.

7.3 Synthesis of phosphorylated glycoconjugate by the Konradsson group

Hederos and Konradsson developed[79,80] a convergent methodology for the preparation of the phosphorylated heptasaccharyl *myo*-inositol **224** (Scheme 43) found of the *T. cruzi* lipopeptidophosphoglycan.[81] This glycan is attached to a ceramide lipid anchor and constitutes a major cell membrane component in the proliferative epimastigote stage of *T. cruzi* found in the insect vector.

Structurally, the phosphoglycan **224** consists of the basic GPI sequence of the Man_4GlcNH_2Ino core with an extra 2-aminoethylphosphonic ester at C-6 of D-glucosamine, and two β-D-galactofuranose branches linked to the mannose-3 and mannose-4 residues. The synthetic plan relied on a (4 + 2 + 2) downstream assembly strategy and the use of tetrasaccharide **225** and disaccharide **226** building blocks, whereas the phosphonatephosphate-decorated pseudodisaccharide **227** was introduced just before the global deprotection steps. The tetrasaccharide block **225** was further disconnected into two smaller Gal*f*-Man building blocks **228** and **229**.

Construction of the branched hexasaccharide **236** (Scheme 44) began with regioselective glycosylation of the diol **233**[82] with the galactofuranose donor **232**.[83] The 2-*O*-acetyl participating group in the donor assists the reaction toward the formation of the target (β1→3)-linked disaccharide **229**. The acetyl groups in compound **229** were then replaced with benzyl ethers prior to bromination to generate the glycosyl bromide donor **228**.

Compound **228** was coupled with the acceptor **229** using AgOTf as promoter to give the corresponding tetrasaccharide (60%), in which the anomeric ethanethiol group was then replaced with the trichloroacetimidate group to furnish the tetrasaccharide donor **225**. This was used in the glycosylation of the disaccharide **226** (prepared by condensation of the glycosyl bromide **234**[84] with the mannose acceptor **235**[85] followed by 2′-*O*-debenzoylation) using TMSOTf as a catalyst to give the branched hexasaccharide **236** (89%).

Glycosylation of chiral D-camphor acetal-protected *myo*-inositol acceptor **107** (Section 5) with the glycosyl sulfoxide donor **237** (Scheme 45) using triflic anhydride (Tf_2O) as promoter gave the pseudodisaccharide **238**. Protecting group manipulation by the removal of pivalic esters and introduction of the 4,5-*O*-isopropylidene acetal gave the required pseudodisaccharide synthon **230**, which was further 1-*O*-phosphorylated (→**239**; 85%) using phosphoramidite chemistry.

The pseudodisaccharide phosphate **239** was *O*-deallylated using an iridium(I) catalyst and then regioselectively 6′-*O*-phosphonylated with the phosphonochloridate **231** to furnish the ‘P-decorated’ glycosyl acceptor **227** (82%). The latter was glycosylated with the branched hexasaccharide donor **236** utilising dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) as a promoter to afford the fully protected pseudooctasaccharide derivative **240** (71%). Global deprotection of **240** started with *O*-deacetylation using Zemplén conditions, was followed by *O*-debenzylation with Na in liquid NH_3 and concluded with removal of the acetals by acid hydrolysis, to provide the desired phosphoglycan **224** in 83% yield.

8 Synthesis of a GPI anchor of the yeast *Saccharomyces cerevisiae* by the Schmidt group

The preparation in 1994 of a GPI anchor **241** (Scheme 46) of the yeast *S. cerevisiae* by the Schmidt group[31,32] was, historically, the second (see Section 3.1) successful total synthesis of a GPI anchor. The lipid moiety of this glycoconjugate is different from the other GPIs in this paper, as in GPI anchors of yeast, ceramide-containing phytosphingosine was found as a lipid instead of glycerolipids.[86,87] With the synthesis of the structure **241**, the Schmidt group established a convergent and versatile synthetic strategy that was later adopted in their syntheses of GPI anchors of *T. gondii*[37] (Section 4) and rat brain Thy-1[38,88] (Section 9.2). The strategy relied on a (4 + 2) retrosynthetic disconnection of the glycan core **242** and making use of trichloroacetimidate chemistry, together with the participating effect of acetic esters at C-2 in D-mannopyranosyl donors to ensure the α-selectivity in every glycosylation step. After the assembly of the glycan core, the *N,N*-diisopropylphosphoramidites **62** and **243** (the synthesis of protected ceramide compound **243** is described in ref. 89) were used

for the introduction of the phosphoethanolamine moiety and the ceramidecontaining phospholipid, respectively. The backbone **242** was further dissected to the pseudodisaccharide block **244** and the tetramannoside **245**.

Synthesis of the pseudodisaccharide **244** (Scheme 47) was based on direct glycosylation of the chiral *myo*-inositol derivative **71** [prepared (see Scheme 13) by enantiomeric resolution of racemic 2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol **9** (Scheme 2)[16,39] *via* selective addition of a chiral (−)-menthyl formate group at O-1 and then crystallisation of the desired product[31,40]] with the 2-azidoglucose donor **66**[41] in the presence of triflic acid to furnish the desired (α1→6)-linked pseudodisaccharide **179** in 86% yield.[89] Compound **179** was subjected to sequential de-*O*-acetylation, 6-*O*-benzoylation with benzoyl cyanide and 3-*O*-benzylation using benzyl bromide in the presence of Ag_2O, to give the pseudodisaccharide acceptor **244**.

The target α-linked tetramannoside **245** was assembled from the acceptor **246** and glycosyl donors **67** and **68** (Scheme 46). The glycosyl donors were readily available from D-mannose 1,2-orthoester **79** (see Section 4), while compound **246** was formed (Scheme 48) by the TMSOTf-catalysed coupling of the donor **67** with allyl alcohol, followed by sequential (and conventional) 2-*O*-deacetylation, 2-*O*-allylation and removal of the 6-*O*-silyl group. TMSOTf-promoted glycosylation of **246** with the glycosyl donor **68** (86%), followed by *O*-deacetylation and glycosylation with the trichloroacetimidate **67**, gave the corresponding trisaccharide (92%), which on *O*-deacetylation gave the trimannoside acceptor **247**.

The tetrasaccharide glycosyl donor **245** was easily available by the following reaction sequence: glycosylation of **247** with the donor **68** (91%), removal of the allyl group at O-1 and O-2 of mannose-1 with Wilkinson's catalyst, exhaustive *O*-acetylation and regioselective anomeric *O*-deacetylation (with ammonium carbonate) to enable installation of the trichloroacetimidate with trichloroacetonitrile in the presence of DBU. This was then coupled with the pseudodisaccharide **244** in the presence of TMSOTf to form stereoselectively the corresponding pseudohexasaccharide in 91% yield. Subsequent cleavage of all the acetic and benzoic esters with KCN in methanol and benzylation of the free OH-groups, followed by replacement of the 1-*O*-(−)-menthyloxycarbonate group (cleaved with K_2CO_3 in methanol) in the *myo*-inositol moiety with *O*-acetate afforded the target pseudohexasaccharide glycan core **242** ready for the installation of the phosphate residues.

First, 6‴-*O*-desilylation of **242** with TBAF and 1*H*-tetrazole-activated phosphitylation with the phosphoramidite **62**, followed by oxidation with *tert*-BuOOH, gave the corresponding protected ethanolamine phosphate derivative (81%). Simultaneous removal of the 1-*O*-acetate in the *myo*-inositol unit and the cyanoethyl P-protecting group was achieved (96%) with methanolic NaOMe prior to the final phospholipidation *via* 1*H*-tetrazole-catalysed coupling with the ceramide-containing phosphoramidite **243**. Oxidation and P-deprotection (with Me_2NH) then afforded the protected GPI compound **248** (59%). Global deprotection was achieved in two steps: first, acid-catalysed cleavage of the acetal protecting groups with 1,2-ethanediol (63%), followed by hydrogenolysis of the *O*-benzyl groups, the *N*-Cbz group and the azide to give the GPI anchor **241** (70%).

9 Syntheses of a GPI anchor of the rat brain Thy-1 glycoprotein

9.1 Convergent synthesis by the Fraser-Reid group

Before their elegant synthetic approach to the highly lipidated GPI anchor of *P. falciparum* was published[54,55] in 2004 (Section 6.1), Fraser-Reid and co-workers reported[34,90] in 1995 the synthesis of the highly phosphorylated GPI membrane anchor of the rat brain Thy-1 glycoprotein (which appeared to be the third total synthesis of a GPI anchor). Structurally, the rat brain Thy-1 anchor (compound **249**, Scheme 49) has a basic type II GPI core (see Fig. 1) with extra 2-acetamido-2-deoxy-β-D-galactose and ethanolamine phosphate moieties both linked to mannose-1 and an extra α-D-mannose residue linked to mannose-3. The assembly of the GPI branched backbone **250** involved a (3 + 4) coupling of the trimannoside **252** and the pseudotetrasaccharide **253**, which, in turn, was assembled from the GalNAc-Man block **254** and the pseudodisaccharide **29**. Phosphoramidite chemistry and the synthons **27** and **251** were used for the later phosphorylation steps.

In contrast to their synthesis of *P. falciparum* GPI anchor that relied heavily on the use of *n*-pentenyl orthoester glycosyl donors, this strategy made use of a combination of *n*-pentenyl glycoside, glycosyl bromides and trichloroacetimidate donors. Construction of the trimannoside **252** (Scheme 50) commenced[91] with coupling of the glycosyl bromide **255** and the pentenyl glycoside acceptor **256** in the presence of AgOTf (89%) to furnish (after NaOMe-catalysed removal of benzoate) the dimannoside **257**. AgOTf-assisted glycosylation of compound **257** with the glycosyl bromide **234** gave the corresponding trimannoside (74%), in which the benzoate was replaced with a benzyl group (→**258**) and the silyl group was replaced with a chloroacetyl group to give **252** (this was to enable selective introduction of ethanolamine phosphate further along the synthetic process).

Assembly of the pseudotetrasaccharide **253** (Scheme 51) was split into two: assembly of the pseudodisaccharide **29**, and coupling of this to the GalNAc-Man disaccharide donor **254**. The latter compound was prepared[90] *via* TMSOTf-catalysed glycosylation of the pentenyl mannoside acceptor **260** with the 2-phthalimidogalactosyl trichloroacetimidate donor **259** (79%; the 2-phthalimido group secured formation of the β-linkage only), followed by replacement of the *N*-phthalyl group with an *N*-acetyl group and successive introduction of the orthogonal 6-*O*-chloroacetyl (to enable further chain elongation to form glycan core **250**) and 2-*O*-acetyl (to enable further introduction of the second ethanolamine phosphate) protecting groups in D-mannose.

Synthesis of the pseudodisaccharide **29** involved $AgClO_4$-promoted reaction of the *myo*-inositol acceptor **43**[34] with the glycosyl bromide **261**,[92] followed by standard *O*-deacetylation to give the corresponding α-linked pseudodisaccharide (63%; 22% of the β-linked isomer was also isolated), in which the 6″-OH group was then selectively benzylated. The selective 6″-*O*-benzylation (69% overall yield) was achieved through a reaction sequence that involved selective 6″-*O*-acetylation, 4″-*O*-tetrahydopyranylation, 6″-*O*-deacetylation, 6″-*O*-benzylation and, finally, cleavage of the acetal group by mildly acidic methanolysis. Thus prepared compound **29** was glycosylated with the disaccharide *n*-pentenyl donor **254** using NIS–TESOTf as an activator to afford a pseudotetrasaccharide (66%), which on thiourea-assisted removal of the chloroacetate gave the tetrasaccharide

glycosyl acceptor **253**, ready for glycosylation with the *n*-pentenyl trimannoside **252** to give the glycan core **250** (39%).[90,91] The latter compound provided (Scheme 51) the branched pseudoheptasaccharide **262** once the chloroacetyl group in mannose-3 was removed.

The ethanolamine phosphates were sequentially introduced on the glycan core (Scheme 52) at O-6 of mannose-3 (89% yield) and then at O-2 of mannose-1 (75% yield) by making use of the phosphoramidite **27** and 1*H*-tetrazole followed by oxidation with *m*-CPBA to give the fully protected diphosphate **263**, consisting of four diastereomers at P atoms. 1-*O*-Deallylation and subsequent 1*H*-tetrazole-mediated *O*-phospholipidation with the phosphoramidite **251** and oxidation (75%) gave the totally protected GPI anchor **264**. This was deprotected in one step by hydrogenolysis over palladium catalyst to give the desired GPI anchor **249** in 75% yield.

9.2 Convergent synthesis by the Schmidt group

The Schmidt group has also contributed[38,88] to the total synthesis of the rat brain Thy-1 GPI anchor **249** (Scheme 53), along with its non-lipidated water-soluble glycoconjugate analogue **279** (Scheme 55). The work followed on from the group's two previous GPI syntheses [of *S. cerevisiae*[31,32] (Section 8) and *T. gondii*[37] (Section 4)] and relied on (i) use of trichloroacetimidate and phosphoramidite chemistry, and (ii) the combination of a convergent design with a sequential glycosylation strategy. The main difference in the GPIs structure of rat brain Thy-1 and *T. gondii* is that the former contains an extra D-mannose (α1→2)-linked to mannose-3 and an extra ethanolamine phosphate group at C-2 of mannose-1. This resulted in the design of the GalNAc-Man fragment being altered so that the ethanolamine phosphate side group could be added at a later stage. The assembly of the GPI branched spine **265** (Scheme 53) involved a (5 + 2) coupling of the pentasaccharide trichloroacetimidate **268** and the pseudodisaccharide **269**. The glycan **268** was, in turn, further disconnected to monosaccharide donors **67–69** and acceptors **270** and **271** for stepwise chain elongation. In contrast to the synthesis of **249** by the Fraser-Reid group (see Section 9.1), three different P-cyanoethyl-protected synthons, **62, 266** and **267**, were used for the phosphorylation steps.

The required pentasaccharide donor **268** was formed starting from the D-mannose derivative **272** (Scheme 54), which was readily available from allyl 3-*O*-benzyl-α-D-mannopyranoside **77** (Scheme 14) in two steps.[37,38] Compound **272** was converted to glycosyl acceptors **270** and **271**, each of them to be further glycosylated with the galactosamine trichloroacetimidate **69**. $BF_3{\cdot}Et_2O$-promoted glycosylation of either of the 1,2-di-*O*-allyl intermediates furnished the corresponding disaccharides, which were further converted to the same glycosyl acceptor **273** after removal of the 6-*O*-protecting group, either by CAN-mediated oxidative cleavage of the PMB ether for the compound derived from **271**, or $MeNH_2$-assisted removal of the *O*-chloroacetyl group for the disaccharide prepared from **270**. It was observed that using the 6-chloroacetate **270** instead of the 6-*O*-PMB protected **271** caused a dramatic increase of the overall yield and reduced the preparation time of **273**. Construction of the branched pentasaccharide **274** proceeded sequentially and almost exactly as previously reported[37] for the branched tetrasaccharide block **83** in the *T. gondii* GPI synthesis (Section 4). Glycosylation of the disaccharide **273** with the trichloroacetimidate **68** under TMSOTf

catalysis (95%), removal of the 2-*O*-acetyl group, followed by glycosylation with the donor **67** (96%), and again 2-*O*-deacetylation and α-manosylation with **68** (92%) led to the required branched pentasaccharide **274**. All the mannosylation reactions were stereospecific due to the anchimeric effect of the 2-*O*-acetyl group, and very high-yielding due to the 'arming' effect of the *O*-benzyl protecting groups in the glycosyl donors.

After the formation of compound **274**, the *N*-trichloroacetyl group was reduced with Bu_3SnH and AIBN to the *N*-acetyl group, the 2-*O*-acetyl group in mannose-4 was replaced with an *O*-benzyl group, and the 1,2-di-*O*-allyl grouping in mannose-1 was cleaved using Wilkinson's catalyst. After phenoxyacetylation of the 1,2-diol and anomeric deprotection, the trichloroacetimidate was installed at the anomeric position to afford the pentasaccharide donor **268** in good overall yield. 4-*O*-Phenoxyacetylation of the pseudodisaccharide **64** (used previously in the synthesis of *T. gondii* GPI) followed by protecting group remodelling gave first the fully protected derivative **275**, which was subjected to successive removal of the phenoxyacetyl group with $MeNH_2$, reduction of the azido group, and finally selective *N*-Boc protection of the free amino group to afford the pseudodisaccharide acceptor **269**.

Glycosylation of compound **269** with the pentasaccharide donor **268** (Scheme 54) in the presence of TMSOTf afforded the branched glycan **265** (74%). The 6-*O*-TBDPS group in mannose-3 was cleaved with TBAF–AcOH prior to selective installation of the first ethanolamine phosphate. This was achieved by making use of 1*H*-tetrazole-catalysed phosphitylation with the phosphoramidite **62**, followed by oxidation with *m*-CPBA and subsequent removal (in one step) of the 2-*O*-phenoxyacetate in mannose-1 and the cyanoethyl P-protecting group with $MeNH_2$ to give the phosphodiester **276** (74%) ready for the next phosphorylation step.

The second ethanolamine phosphate was attached in a similar manner (Scheme 55) using the phosphoramidite **266** and *tert*-BuOOH for the oxidation step. Stepwise removal of the P-cyanoethyl group (with $MeNH_2$; 78% over three steps) and the 1-*O*-benzoyl group (with NaOMe) provided the pseudoheptasaccharide diphosphate **277**. This was first phosphorylated with dibenzyl-*N,N*-diisopropylaminophosphoramidite (in a similar fashion) to furnish the protected triphosphoglycan **278** (90%), which on hydrogenolytic *O*-debenzylation, followed by cleavage of *N*-Boc groups with aqueous TFA, afforded the non-lipidated water-soluble glycoconjugate **279**.

Final phospholipidation of **277** was performed *via* 1*H*-tetrazole-catalysed coupling with the phosphoramidite **267**, followed by oxidation (*tert*-BuOOH) and P-deprotection (Me_2NH) to form the protected GPI compound **280** (85%). Global deprotection was completed in two steps: removal of the *N*-Boc protecting groups with TFA and Et_3SnH as a scavenger (90%), followed by hydrogenolysis of the *O*-benzyl groups and the *N*-Cbz group over Pearlman's catalyst to give the GPI anchor **249** (60%).

10 Syntheses of GPI anchors and related glycopeptide conjugates of sperm CD52 glycopeptide antigen by the Guo group

10.1 Synthesis of a GPI anchor containing three phosphate residues

Guo and co-workers have been interested in the chemical preparation of the CD52 antigen – a GPI-anchored glycopeptide antigen involved in the human reproduction and human immune recognition processes.[93] The Guo group's most recent achievement was the first synthesis of a highly phosphorylated and highly lipidated GPI anchor (compound **281**, Scheme 56) bearing a phospholipid, two ethanolamine phosphate moieties and having a bulky acyl (palmitoyl) at the *myo*-inositol O-2 position that makes the inositol moiety more crowded.[94,95] Earlier synthetic work by the Fraser-Reid group[96] encountered acyl migration and steric hindrance for the phospholipid introduction at O-1 whilst working with a 2-*O*-acyl group in the *myo*-inositol moiety. In contrast to the majority of synthetic plans of GPI structures that have been already discussed, the Guo group's synthetic design[94,95] relied on the introduction of the phospholipid and lipid moieties in *myo*-inositol at an early stage and further introduction of the phosphoethanolamine functions (*via* the phosphoramidite **27**) once the acylated and phospholipidated core **282** was constructed.

The glycan core **282** was retrosynthetically broken down into the trimannoside **283** and the acylated/phospholipidated pseudodisaccharide **284**. The trisaccharide was further disconnected to simple monosaccharide derivatives **288–290**, while the complex synthon **284** was built up from the differentially protected pseudodisaccharide **285**, the phosphoramidite **287** and palmitoleic acid **286**. The unsaturated fatty acid was introduced because, before the global deprotection, the double bond could be modified (*e.g.*, oxidized) to produce some reactive intermediates[97] that are useful for the preparation of various GPI intermediates. Alternatively, the reductive debenzylation at the final step could readily convert the 2-*O*-palmitoleoyl group to the palmitic ester, the natural acyl chain of the CD52 anchor.

As the target GPI **281** has three positions on the *myo*-inositol ring (O-1, O-2 and O-6) to be differentiated, a considerable amount of research was performed towards the synthesis of suitable *myo*-inositol derivatives. This resulted in an effective synthetic procedure for a novel differentially protected synthon **294** (Scheme 57).[98,99] Enantiomeric resolution of the racemic 2,3:4,5-di-*O*-cyclohexylidene-(±)-*myo*-inositol **9**[16,39] was achieved using the literature procedure[100,101] *via* enantioselective enzymatic degradation of its 6-butyric ester (±)-*myo*-inositol **291**. When the racemate (±)-**291** was digested with porcine pancreatic lipase or cholesterol esterase, only one enantiomer (+)-**291** was deacylated, providing the optically pure diol (−)-**9** that was readily separable from the undigested 6-butyrate (−)-**291**. Subsequent basic deacylation of (−)-**291** gave the optically pure enantiomer (+)-**9**. As the O-1 and O-6 positions have already been discriminated from the others in the diol (+)-**9**, it was transformed to 1,6-*O*-differentially protected/2-*O*-deprotected synthon **294** *via* a 6-step sequence of regioselective protections and deprotections in 32% overall yield. Because the *myo*-inositol molecule is symmetric and the chiral property of compound **9** was created by derivatisation, the authors accomplished the preparation of the same synthetic target **294**

from the other enantiomer (−)-**9** as well (in 42% overall yield) using a reverse 6-step sequence of synthetic transformations.[98,99]

The O-2 position in compound **294** was further acetylated (Scheme 58), followed by cleavage of the 6-allyl ether to furnish the glycosyl acceptor **295**. The pseudodisaccharide **285** was prepared[94,95] *via* Bu_4NBr-promoted glycosylation of the acceptor **295** with the known[24] 2-azido-α-D-glucosyl bromide **47** (56%) and subsequent deacetylation. 2-*O*-Acylation of the derivative **285** with palmitoleic acid **286** in presence of DCC (85%), followed by oxidative removal of the 1-*O*-PMB group and then 1*H*-tetrazolecatalysed phosphorylation with the lipidated phosphoramidite **287** (51%), gave the corresponding acylated/phospholipidated pseudodisaccharide, which afforded the target synthon **284** after 4′-*O*-desilylation as a diastereomeric mixture at the P atom.

The trimannoside building block **283** (Scheme 59), the preparation of which was not described in detail by the authors,[94,95] was constructed from monosaccharide compounds **288–290** according to a procedure developed earlier for a similar structure **296**[99] (see Section 10.2). Glycosylation of the pseudodisaccharide acceptor **284** using the thioglycoside donor **283** and DMTST or methyl triflate as promoter failed to form the desired product, while the use of *N*-iodosuccinimide and silver triflate (NIS–AgOTf) would influence the double bond in the palmitoleoyl moiety. Therefore, compound **283** transformed into the trisaccharide trichloroacetimidate **297** *via* subsequent NIS–TfOH-assisted oxidative hydrolysis of the thioglycoside and then *O*-trichloroacetimidation of the formed hemiacetal.[94] TMSOTf-catalysed glycosylation of the pseudodisaccharide **284** with the donor **297**, followed by $BF_3{\cdot}Et_2O$-mediated simultaneous removal of the TBS and PMB ethers, gave the pseudopentasaccharide 2″,6‴-diol **298** (35%) as two separable diastereomers at the P atom.

One-step double phosphitylation of the diol **298** (of one isomer only) employing the *N*-Cbz-aminoethanol phosphoramidite **27** in the presence of 1*H*-tetrazole, followed by oxidation with *tert*-BuOOH gave the fully protected GPI anchor **299** (50%). Removal of the cyanoethyl P-protecting group with DBU (78%) and global deprotection *via* Pd-catalysed hydrogenolysis (82%) gave the desired fully phosphorylated and lipidated GPI anchor **281**.

10.2 Synthesis of a GPI anchor containing two phosphate residues

This convergent and versatile synthesis of the fully functionalized GPI anchor **281** was accomplished as a result of the experience gained by the authors from their previous trials. Earlier attempts by the Guo group[102] to adopt the traditional approach for the synthesis of the CD52 GPI anchor **300** (Scheme 60), by introducing the phospholipid at O-1 position at a late stage once the glycan core was assembled, seemed not to work. Compared to the structure **281** (Scheme 56), the GPI anchor **300** lacks the 2″-ethanolamine phosphate moiety. The initial synthetic strategy for compound **300** involved the final introduction of the 6‴-phosphoethanolamine function (*via* the *N*-Fmoc-protected phosphoramidite **302**) once the 2-*O*-acylated-1-*O*-phospholipidated core **301** was constructed. It also relied[102] on a traditional (3 + 2) assembly of a non-phosphorylated glycan core first, using the trimannoside **296** and the pseudodisaccharide **303**, followed by phospholipidation with the

O-Cbz-protected phosphoramidite **287** or its *O*-Bn-protected analogue **304**. The trisaccharide **296** was further disconnected to simple monosaccharide derivatives **288, 289** and **235**, while the synthon **303** was built up from the 2-azidoglucose donor **106** and the 2-*O*-palmitoylated *myo*-inositol acceptor **305**.

The trimannoside building block **296** was constructed[99] from two glycosyl halide donors **288** and **289** and a glycosyl acceptor **235**, as depicted in Scheme 61. The glycosyl chloride **289** was obtained from the D-mannose 1,2-orthoester **171** following a reported procedure,[103] while the glycosyl bromide **288** was formed from methyl α-D-mannoside **306** by successive perbenzylation, acetolysis (→**307**) and 1-bromination with HBr in acetic acid.

Treatment of the 1,6-diacetate **307** with ethanethiol and $BF_3{\cdot}Et_2O$ gave the corresponding thioglycoside, which provided the acceptor **235** upon conventional deacetylation. Coupling of the chloride **289** and the thiomannoside **235** in the presence of AgOTf as a promoter furnished, after basic deacetylation, the dimannoside acceptor **308**. Subsequent AgOTf-activated glycosylation with the glycosyl bromide **288** gave the matching trimannoside (53%), in which the 6″-*O*-acetyl group was then replaced with the TBS ether to afford the required synthon **296**.

The pseudodisaccharide synthon **303** (Scheme 62) was synthesised[98,99] by coupling of the 2-*O*-lipidated *myo*-inositol derivative **305** with the 2-azido-D-glucosyl fluoride **106**. The acceptor **305** was prepared by 2-*O*-acylation of the key *myo*-inositol synthon **294** (Scheme 57) with palmitic acid in presence of DCC and DMAP (>99%), followed by cleavage of the 6-allyl ether with $PdCl_2$ and acetic acid. The donor **106** was derived from the 1,6-anhydro compound **309**, obtained from the commercially available D-glucal according to a literature procedure.[104,105] Five-membered ring-opening acetolysis of **309** provided the 1,6-diacetate **310**. Subsequent anomeric *O*-deacetylation with $BnNH_2$ and fluorination with DAST gave the glycosyl fluoride **311**, which on replacement of the 6-*O*-acetyl with benzyl ether provided the target **106** in 75% yield. This was used in a glycosylation (promoted by AgOTf–hafnocene dichloride) of the acceptor **305** to give the α-linked pseudodisaccharide **303** (40%) along with its β-linked isomer (30%). Compound **303** was 4′-*O*-deallylated with $PdCl_2$ and acetic acid to furnish the 2-*O*-lipidated pseudodisaccharide acceptor **312** (99%). It was found that the removal of the 4′-*O*-allyl group allowed better separation of **312** from its β-anomer.

Coupling of the thioglycoside donor **296** and the acceptor **312** in the presence of NIS–TfOH afforded the protected glycan core **313** (Scheme 63) in excellent yield.[99] Further oxidative cleavage of the PMB ether at O-1 gave the pseudopentasaccharide **314**. The phospholipidation reaction of **314** with the phosphoramidite **304** was performed under the established conditions[102] in the presence of 1*H*-tetrazole, followed by oxidation with *m*-CPBA. Very much to the authors' surprise, a cyclic phosphoramidate **315** was isolated as a major product (83%, as a diastereomeric mixture at the P atom), thus indicating the involvement of the azido group in the reaction. They proposed that this unwanted phenomenon was due to participation of the azido group in an intramolecular Staudinger-type reaction, which seemed to be possible in the adopted conformation (of the intermediate

phosphite triester at O-1) forced by the steric interactions between the trimannoside segment and the long lipid chain at O-2.

The expectation that exclusion of the azido group involvement might solve the problem proved to be impractical. Reduction to amino group, which was then protected with *N*-Cbz functionality (→**316**), did not help to add the phospholipid. The pseudopentasaccharide **316** appeared to be totally unreactive towards the lipidated phosphoramidite **304**, probably due to steric hindrance around the 1-OH group. This assumption was confirmed by the quantitative yield of the phosphite triester **317** obtained from phosphitylation of **316** under the same conditions with the less bulky phosphoramidite reagent $(BnO)_2PN(iso\text{-}Pr)_2$.

Trying to circumvent this problem, a novel synthetic strategy for the GPI anchor **300** was developed (Scheme 64). The acylated/phospholipidated glycan core **301** (Scheme 60) was now assembled[102] *via* the phospholipidation of the pseudodisaccharide, and later addition of the trimannoside block. To this end, the acylated pseudodisaccharide synthon **303** was subjected to TFA-assisted removal of the 1-*O*-PMB group followed by 1*H*-tetrazole-catalysed reaction with the lipid phosphoramidite **287** and oxidation (68%). Further cleavage of the 4′-*O*-allyl group afforded the acylated/phospholipidated synthon **318** (as a diastereomeric mixture at P). NIS–TfOH-assisted glycosylation of the pseudodisaccharide **318** with the trimannoside donor **296** (→**301**) and subsequent $BF_3{\cdot}Et_2O$-mediated removal of the 6‴-*O*-TBS group gave the pseudopentasaccharide **319** (52%) as two separable diastereomers at the P atom. Final phosphorylation employing the *N*-Fmoc-aminoethanol phosphoramidite **302** in the presence of 1*H*-tetrazole, followed by oxidation with *tert*-BuOOH, gave the fully protected GPI anchor **320** (74%).

Two-step deprotection involved removal of the cyanoethyl P-protecting and the Fmoc N-protecting groups with DBU (87%), and then global deprotection *via* Pd-catalysed hydrogenolysis (84%) to furnish the desired twice-phosphorylated and twice-lipidated GPI anchor **300**.

10.3 Syntheses of CD52 glycopeptide conjugates

In order to benefit from the pseudopentasaccharide structure **313**, which appeared to be unsuitable for the phospholipidation (see Scheme 63), the Guo group made use of it for the first syntheses of CD52 GPI-related glycopeptide conjugates.[99,106] First, they synthesised the GPI core-anchored peptide **325** (Scheme 65), in which the amino group of the ethanolamine phosphate is acylated with a Pro-Ser dipeptide.[99]

After removal of the 6‴-*O*-TBS group in **313** with TBAF, attempted phosphorylation of **321** with the *N*-Fmoc-aminoethanol phosphoramidite **302** in the presence of 1*H*-tetrazole led to a slow and incomplete reaction, yielding, after oxidation, only 15% of the fully protected derivative **322**. Once the azido group in **321** was reduced under Staudinger conditions (PEt_3, MeOH) and transformed to the *N*-Cbz-protected amino group, the phosphorylation reaction with the same reagents was effective and produced the phosphotriester **323** (84%) as a diastereomeric mixture at the P atom. After mildly basic removal of the *N*-Fmoc group, the GPI fragment having a free amino group was coupled with the dipeptide Fmoc-Pro-Ser **324** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-

hydroxybenzotriazole (HOBt) as condensing reagents to give the corresponding *O,N*-protected conjugate (60%). Two-step deprotection – cleavage of the *N*-Fmoc group with Me_2NH (85%), and then global Pd-catalysed hydrogenolysis (91%) – furnished the desired GPI core-anchored dipeptide **325**.

The phosphorylated/lipidated glycan core **323** was also elaborated in the synthesis of the complex GPI-anchored glycopeptide[106] containing native CD52 dodecapeptide with one *N*-glycosylation site bearing a $Man_3FucGlcNAc_2$ glycoform (structure **332**, Scheme 67). The *O,N*-protected glycopeptide synthon **326** (Scheme 66) was prepared first, starting from the *N*-glycan **327** with an azido group attached at C-1.[107] After selective reduction to a glycosyl amine (using Lindlar catalyst), the latter was coupled to the allyl aspartate side-chain-activated ester **328**, thus forming the *N*-glycosyl asparagine block **329** (82%). Cleavage of the allyl ester with $Pd(PPh_3)_4$ gave the asparagine derivative **330** ready for the glycopeptide assembly. The nonapeptide fragment (starting from the carboxy terminus) of the synthon **326** was assembled using *N*-Fmoc amino acids and conventional automatic solid-phase synthesis on the 2-chlorotrityl resin, which contained a highly acid-sensitive 2-chlorotrityl linker. Next, the *N*-glycosyl asparagine **330** was introduced manually using DCC and HOBt as condensing reagents, followed by *N*-deprotection (with piperidine) and manual introduction of the two final amino acids. The protected glycopeptide **326** was then freed from the resin by treatment with a mixture of AcOH, trifluoroethanol and CH_2Cl_2 (1 : 1 : 8).

Before the conjugation of the structure **326** to the GPI core (Scheme 67), the phosphorylated/lipidated glycan **323** was deprotected at O-1 by acidic treatment prior to phosphorylation by making use of the phosphoramidite chemistry (57%). DBU-assisted removal of the *N*-Fmoc group afforded the protected 1,6″″-diphosphate **331** having a free amino group ready for the attachment of the glycopeptide **326**. Amide bond formation was performed using EDC and HOBt as condensing reagents (as for the preparation of the glycodipeptide **325**) to furnish the corresponding fully protected glycopeptide conjugate (70%). Two-step global deprotection was accomplished by catalytic hydrogenolysis to remove the benzyl ethers and esters as well as the *N*-Cbz group, followed by acidic removal of the peptide protecting groups, and provided the desired sperm CD52 GPI-anchored glycopeptide **332** (85%).

11 Synthesis of a phosphatidylinositol mannoside of *Mycobacterium tuberculosis* by the Seeberger group

Phosphatidylinositol mannosides (PIMs) are a major class of antigenic glycolipids found in the cell wall of the prokaryote *M. tuberculosis*, one of the most effective human pathogens and a causative agent of tuberculosis. PIMs contain a phosphatidyl *myo*-inositol anchor that is extended by mannosyltransferases to phosphatidylinositol oligomannosides of various lengths. The phosphatidylinositol tetramannoside (PIM_4) is postulated to be a key biosynthetic intermediate and a precursor for the lipoarabinomannan, which is one of the major polymeric components of the bacterium glycocalyx.[108,109] PIMs are also present in the mycobacterial cell wall, mostly in the form of PIM_2 and PIM_6, and elicit a variety of

immune responces.[110,111] Seeberger and co-workers recently reported[64] the total synthesis of the PIM_6 molecule, phosphatidylinositol mannoside **333** (Scheme 68).

The PIM_6 structure **333** has a *myo*-inositol moiety glycosylated at O-6 with a mannopentaose glycan and at O-2 with 6-*O*-palmitoyl-α-D-mannopyranose. Palmitic acid is also present in the glycerophospholipid at the *sn*-2 position, while the primary position is acylated with chiral tuberculostearic acid [(*R*)-10-methyloctadecanoic acid], which was found to be the basic cell wall constituent of mycobacteria.[112,113] The strategy adopted for the synthesis was similar to the one used by the group in their preparation of a GPI anchor of *Plasmodium falciparum*[63,66,69] (Section 6.2) and relied on a (4 + 3) glycosylation of the branched pseudotrisaccharide **337** with the mannotetraose donor **336** to form (after protecting group remodelling) the glycan backbone **334**, which was subsequently lipidated with palmitic acid and phospholipidated with the H-phosphonate **335**. The building block **337** was further disconnected to the monosaccharide donor **338** and the pseudodisaccharide **339**, while the tetrasaccharide **336** was prepared from the smaller synthons **68** and **145**.

The assembly of the pseudotrisaccharide **337** (Scheme 69) commenced with the synthetic elaboration of enantiopure *myo*-inositol derivative **140** that was readily derived from methyl α-D-glucoside and used earlier by the authors[64] and by the Fraser-Reid group.[56] Protection of the 2,6-diol in **140** as the 1-ethoxyethyl ethers (EE), followed by consecutive 2-*O*-deacetylation, 2-*O*-allylation, acid hydrolysis of the two EE groups and regioselective reaction with 2-naphthylmethyl bromide (NAPBr) for orthogonal 2-*O*-protection, afforded compound **341**. The 6-*O*-TIPS-protected glycosyl donor **338** was prepared from the β-D-mannose 1,2-orthobenzoic ester **340** (*via* the intermediate **144**) in a manner similar to the preparation of the donors **68** and **139** (Scheme 26). TMSOTf-promoted coupling of **341** and **338** afforded the corresponding pseudodisaccharide with complete α-selectivity. Replacement of 6′-*O*-TIPS ether with the levulinoyl (Lev) group and DDQ-mediated removal of the 2-*O*-NAP protection furnished **339** ready for the subsequent 2-*O*-mannosylation. Glycosylation of **339** with the trichloroacetimidate **338** prior to the removal of the levulinic ester gave the pseudotrisaccharide **337** ready for coupling with the tetramannoside **336**.

The fragment **336** was assembled (Scheme 70) by the route prescribed for construction of the tetramannoside **137**[63] (Scheme 26) with a minor modification. TMSOTf-assisted elongation of the disaccharide **145**[63,64] with the donor **68**, followed by mildly acidic methanolysis[74] to cleave the 2′-*O*-acetyl group, gave the trimannoside **342** (74%), which was extended to the corresponding tetramannoside (83%) using iterative mannosylation with the same donor. Subsequent replacement of the anomeric *O*-allyl group with the trichloroacetimidoyl group provided the desired mannotetraose donor **336**.

TMSOTf-catalysed combination of **336** with **337** (91%) and conventional replacement of the acetic and benzoic esters with *O*-benzyl groups furnished branched pseudoheptasaccharide **334**. Mildly acidic methanolytic cleavage of TIPS, esterification of the generated OH-group with palmitic acid and DCC, followed by removal of the 1-*O*-allyl group using a cationic cyclopentadienyl ruthenium(II) complex with quinaldic acid[114] and pivaloyl chloride assisted phospholipidation with the H-phosphonate **335**, afforded the fully protected

glycoconjugate **343** (81%). Global *O*-debenzylation was then accomplished by hydrogenolysis over palladium catalyst to furnish the target phosphatidylinositol mannoside **333** in 76% yield.

12 Future advances

Despite the appreciable progress in the chemical synthesis of GPI anchors, there are still several unresolved issues that prevent these complex molecules from being routinely synthesised and, therefore, require special attention and experience:

1. Additional side chains in GPIs, or positional alteration in the side chains, results in total change to the whole plan, particularly in the selection of orthogonal protecting groups. Thus, advanced strategies in oligosaccharide synthesis[115] could play an increasingly important role in the design and preparation of the compounds.
2. All the utilized synthetic plans are time-consuming; therefore, reduction of the number of steps is highly desirable. This is achievable by adopting and developing one-pot strategies[116] that enable the construction of the glycan core by means of sequential multiple glycosylations under catalytic activation.
3. Installation and removal of protecting groups are the most time-consuming aspects of GPI glycan core synthesis. Minimizing their use by employing the ‘glycosyl donor–glycosyl acceptor match’ concept is a valuable goal (*e.g.*, see Section 3.2), since there is no need to protect a hydroxyl that does not match the specific donor used.
4. A combined chemo-enzymatic approach to GPI anchors would be an interesting challenge. This would probably require chemical preparation of a pseudodisaccharide (or a pseudotrisaccharide), followed by further chain elongation with the help of recombinant glycosyltransferases, phospholipases, phosphoryltransferases and acyltransferases.

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Biography

Andrei Nikolaev graduated from Lomonosov Moscow State University (Moscow) with an MSc, and obtained his PhD in the synthetic chemistry of saccharides under the legendary

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Nikolay Kochetkov at the Zelinsky Institute of Organic Chemistry (Moscow). This experience, and his postdoctoral studies with Klaus Jann at the Max-Planck-Institute of Immunobiology (Freiburg), crystallised his interest in the chemical preparation of biologically important carbohydrates, to be used as tools for biosynthetic, biochemical and immunological studies, and for the preparation of carbohydrate synthetic vaccines. In 1995 he was appointed as a Lecturer at Dundee University (UK), where he is currently a Reader. In 2007 he was distinguished with the Royal Society of Chemistry Medal and Award in Carbohydrate Chemistry “for the synthesis of complex carbohydrates of biological importance, enabling the study of their operation in Nature”.

Nawaf Al-Maharik received his MSc in chemistry from the Technische Hochschule Leuna-Merseburg (Germany). He obtained his PhD in synthetic chemistry of isoflavonoids under Tapio Hase and Kristtiina Wähälä at Helsinki University (Finland). He subsequently joined the group of Lars Engman at Uppsala University (Sweden), and in 2001 the group of Nigel Botting at St Andrews University (UK) to work on synthesis of ^{13}C-labelled polyphenols. In 2006 he joined Andrei Nikolaev’s group at Dundee University to work towards the chemical preparation GPI anchors and other biologically important carbohydrates. In 2010 he joined David O’Hagan’s group in St Andrews to work on the synthesis of diastereoisomeric multi-vicinal fluoroalkanes. His research interests include the chemistry of natural products, tellurium- and fluorinecontaining compounds, ^{18}F-labelled radiopharmaceuticals and carbohydrate chemistry.

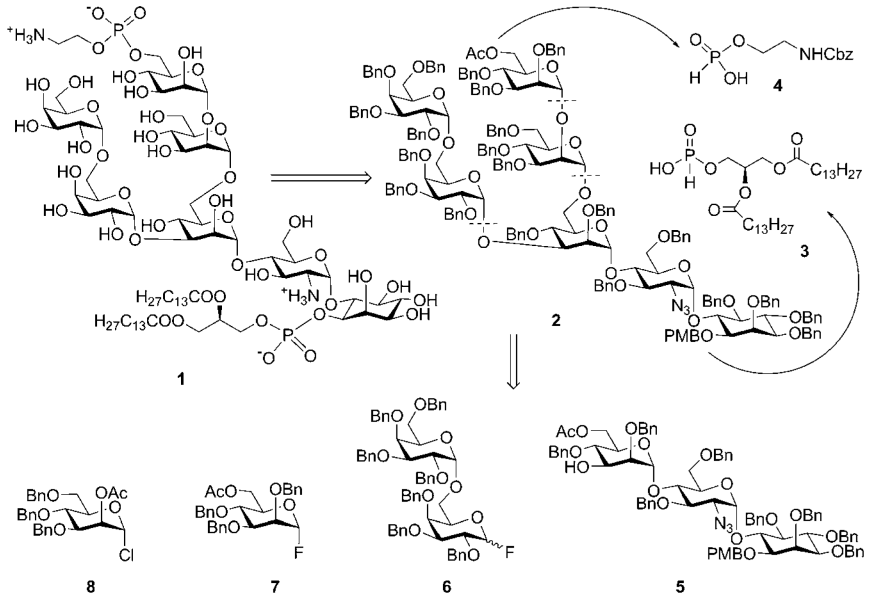
14 References

1. Sogin ML, Gunderson JH, Elwood HJ, Alonso RA, Peattie DA. Science. 1989; 243:75–77. [PubMed: 2911720]
2. McConville MJ, Ferguson MAJ. Biochem. J. 1993; 294:305–324. [PubMed: 8373346]
3. Ferguson MAJ. Philos. Trans. R. Soc. London, Ser. B. 1997; 352:1295–1302. [PubMed: 9355120]
4. Ferguson MAJ. J. Cell Sci. 1999; 112:2799–2809. [PubMed: 10444375]
5. Almeida IC, Gazzinelli RT. J. Leukoc. Biol. 2001; 70:467–477. [PubMed: 11590183]
6. Ferguson MAJ, Homans SW, Dwek RA, Rademacher TW. Science. 1988; 239:753–759. [PubMed: 3340856]
7. Homans SW, Ferguson MAJ, Dwek RA, Rademacher TW, Anand R, Williams AF. Nature. 1988; 333:269–272. [PubMed: 2897081]
8. Ferguson MAJ, Williams AF. Annu. Rev. Biochem. 1988; 57:285–320. [PubMed: 3052274]
9. McConville, MJ., Ralton, JE. Glycopeptides and Related Compounds. Large, DG., Warren, CD., editors. Marcel Dekker, Inc.; New York: 1997. p. 393-425.
10. Varma R, Mayor S. Nature. 1998; 394:798–801. [PubMed: 9723621]
11. McConville MJ, Menon AK. Mol. Membr. Biol. 2000; 17:1–16. [PubMed: 10824734]

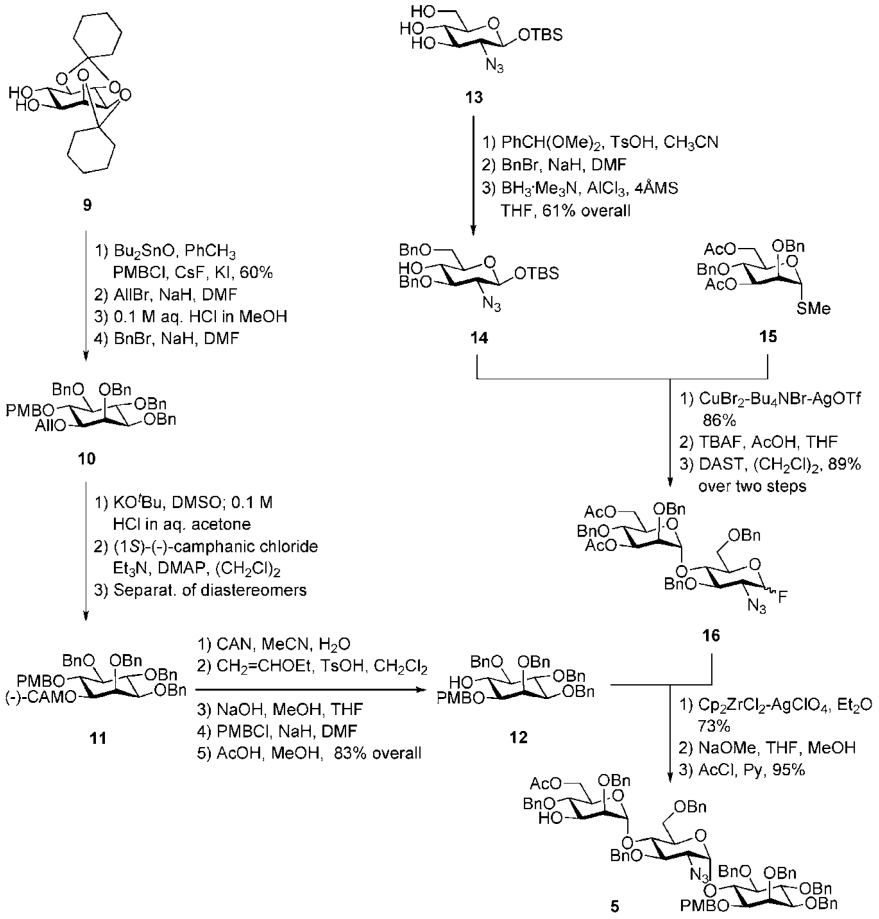
12. Gigg, R., Gigg, J. Glycopeptides and Related Compounds. Large, DG., Warren, CD., editors. Marcel Dekker, Inc.; New York: 1997. p. 327-392.
13. Guo Z, Bishop L. Eur. J. Org. Chem. 2004:3585–3596.
14. Murakata C, Ogawa T. Tetrahedron Lett. 1991; 32:671–674.
15. Murakata C, Ogawa T. Carbohydr. Res. 1992; 235:95–114. [PubMed: 1473115]
16. Garegg PJ, Iversen T, Johansson R, Lindberg B. Carbohydr. Res. 1984; 130:322–326.
17. Murakata C, Ogawa T. Tetrahedron Lett. 1990; 31:2439–2442.
18. Ogawa T, Sasajima K. Tetrahedron. 1981; 37:2787–2792.
19. Murakata C, Ogawa T. Carbohydr. Res. 1992; 234:75–91. [PubMed: 1468082]
20. Koike K, Sugimoto M, Sato S, Ito Y, Nakabara Y, Ogawa T. Carbohydr. Res. 1987; 163:189–208. [PubMed: 3621236]
21. Cottaz S, Brimacombe JS, Ferguson MAJ. J. Chem. Soc., Perkin Trans. 1. 1993:2945–2951.
22. Ogawa T. Chem. Soc. Rev. 1994; 23:397–407.
23. Baeschlin DK, Chaperon AR, Charbonneau V, Green LG, Ley SV, Lücking U, Walther E. Angew. Chem., Int. Ed. 1998; 37:3423–3428.
24. Baeschlin DK, Chaperon AR, Green LG, Hahn MG, Ince SJ, Ley SV. Chem. Eur. J. 2000; 6:172–186. [PubMed: 10747402]
25. Riley AM, Jenkins DJ, Potter BVL. Carbohydr. Res. 1998; 314:277–281.
26. Nicolaou KC, Caulfield TJ, Kataoka H, Stylianides NA. J. Am. Chem. Soc. 1990; 112:3693–3695.
27. Lemieux RU, Hendriks KB, Stick RV, James K. J. Am. Chem. Soc. 1975; 97:4056–4062.
28. Zuurmond HM, van der Meer PH, van der Klein PAM, van der Marel GA, van Boom JH. J. Carbohydr. Chem. 1993; 12:1091–1103.
29. Mallet A, Mallet JM, Sinay P. Tetrahedron: Asymmetry. 1994; 5:2593–2608.
30. Grice P, Ley SV, Pietruszka J, Osborn HMI, Priepke HWM, Warriner SL. Chem. Eur. J. 1997; 3:431–440.
31. Mayer TG, Kratzer B, Schmidt RR. Angew. Chem., Int. Ed. Engl. 1994; 33:2177–2181.
32. Mayer TG, Schmidt RR. Eur. J. Org. Chem. 1999:1153–1165.
33. Campbell AS, Fraser-Reid B. Bioorg. Med. Chem. 1994; 2:1209–1219. [PubMed: 7757418]
34. Campbell AS, Fraser-Reid B. J. Am. Chem. Soc. 1995; 117:10387–10388.
35. Striepen B, Zinecker CF, Damm JBL, Melgers PAT, Gerwig GJ, Koolen M, Vliegenthart JFG, Dubremetz J-F, Schwarz RT. J. Mol. Biol. 1997; 266:797–813. [PubMed: 9102470]
36. Striepen B, Dubremetz J-F, Schwarz RT. Biochemistry. 1999; 38:1478–1487. [PubMed: 9931013]
37. Pekari K, Tailler D, Weingart R, Schmidt RR. J. Org. Chem. 2001; 66:7432–7442. [PubMed: 11681958]
38. Pekari K, Schmidt RR. J. Org. Chem. 2003; 68:1295–1308. [PubMed: 12585868]
39. Massy DJR, Wyss P. Helv. Chim. Acta. 1990; 73:1037–1057.
40. Mayer TG, Schmidt RR. Liebigs Ann./Recl. 1997:859–863.
41. Grundler G, Schmidt RR. Liebigs Ann. Chem. 1984:1826–1847.
42. Kaur KJ, Hindsgaul O. Carbohydr. Res. 1992; 226:219–231. [PubMed: 1535553]
43. Ponpipom MM. Carbohydr. Res. 1977; 59:311–317.
44. Goebel M, Nothofer H-G, Ross G, Ugi I. Tetrahedron. 1997; 53:3123–3134.
45. Tailler D, Ferrieres V, Pekari K, Schmidt RR. Tetrahedron Lett. 1999; 40:679–682.
46. Ruda K, Lindberg J, Garegg PJ, Oscarson S, Konradsson P. J. Am. Chem. Soc. 2000; 122:11067–11072.
47. Morris PE, Kiely DE. J. Org. Chem. 1987; 52:1149–1152.
48. Ruda K, Lindberg J, Garegg PJ, Oscarson S, Konradsson P. Tetrahedron. 2000; 56:3969–3975.
49. Garegg PJ, Olsson L, Oscarson SJ. J. Carbohydr. Chem. 1993; 12:955–967.
50. Buskas T, Garegg PJ, Konradsson P, Maloisel J-L. Tetrahedron: Asymmetry. 1994; 5:2187–2194.
51. Bruzik KS, Tsai MD. J. Am. Chem. Soc. 1992; 114:6361–6374.
52. Garegg PJ, Konradsson P, Oscarson S, Ruda K. Tetrahedron. 1997; 53:17727–17734.

53. Jankowska J, Sobkowski M, Stawinski J, Kraszewski A. Tetrahedron Lett. 1994; 35:3355–3358.
54. Lu J, Jayaprakash KN, Fraser-Reid B. Tetrahedron Lett. 2004; 45:879–882.
55. Lu J, Jayaprakash KN, Schlueter U, Fraser-Reid B. J. Am. Chem. Soc. 2004; 126:7540–7547. [PubMed: 15198601]
56. Jia ZJ, Olsson L, Fraser-Reid B. J. Chem. Soc., Perkin Trans. 1. 1998:631–632.
57. Bender SL, Budhu RJ. J. Am. Chem. Soc. 1991; 113:9883–9885.
58. Estevez VA, Prestwich GD. J. Am. Chem. Soc. 1991; 113:9885–9887.
59. Ferrier RJ, Middleton S. Chem. Rev. 1993; 93:2779–2831.
60. Mach M, Schlueter U, Mathew F, Fraser-Reid B, Hazen KC. Tetrahedron. 2002; 58:7345–7354.
61. Soli ED, Manoso AE, Patterson MC, DeShong P, Favor DA, Hirschmann R, Smith ABI. J. Org. Chem. 1999; 64:3171–3177. [PubMed: 11674417]
62. Presova M, Smrt J. Collect. Czech. Chem. Commun. 1989; 54:487–497.
63. Liu X, Kwon Y-U, Seeberger PH. J. Am. Chem. Soc. 2005; 127:5004–5005. [PubMed: 15810819]
64. Liu X, Stocker BL, Seeberger PH. J. Am. Chem. Soc. 2006; 128:3638–3648. [PubMed: 16536536]
65. Orgueira HA, Bartolozzi A, Schell P, Litjens REJN, Palmacci ER, Seeberger PH. Chem. Eur. J. 2003; 9:140–169. [PubMed: 12506372]
66. Seeberger PH, Soucy RL, Kwon Y-U, Snyder DA, Kanemitsu T. Chem. Commun. 2004:1706–1707.
67. Kwon Y-U, Soucy RL, Snyder DA, Seeberger PH. Chem. Eur. J. 2005; 11:2493–2504. [PubMed: 15729674]
68. Hewitt MC, Snyder DA, Seeberger PH. J. Am. Chem. Soc. 2002; 124:13434–13436. [PubMed: 12418894]
69. Schofield L, Hewitt MC, Evans K, Siomos M-A, Seeberger PH. Nature. 2002; 418:785–789. [PubMed: 12181569]
70. Schwab P, Grubbs RH, Ziller JW. J. Am. Chem. Soc. 1996; 118:100–110.
71. Almeida IC, Camargo MM, Procopio DO, Silva LS, Mehlert A, Travassos LR, Gazzinelli RT, Ferguson MAJ. EMBO J. 2000; 19:1476–1485. [PubMed: 10747016]
72. Yashunsky DV, Borodkin VS, Ferguson MAJ, Nikolaev AV. Angew. Chem., Int. Ed. 2006; 45:468–474.
73. Dais P, Shing TKM, Perlin AS. Carbohydr. Res. 1983; 122:305–313.
74. Byramova NE, Ovchinnikov MV, Backinowsky LV, Kochetkov NK. Carbohydr. Res. 1983; 124:c8–c11.
75. Yashunsky, DV., Borodkin, VS., McGivern, PG., Ferguson, MAJ., Nikolaev, AV. Frontiers in Modern Carbohydrate Chemistry. Demchenko, AV., editor. American Chemical Society; Washington, DC: 2007. p. 285-306.
76. Al-Maharik, N., Nikolaev, AV. 2008. unpublished results
77. Baeschlin DK, Green LG, Hahn MG, Hinzen B, Ince SJ, Ley SV. Tetrahedron: Asymmetry. 2000; 11:173–197.
78. Ali A, Gowda DC, Vishwakarma RA. Chem. Commun. 2005:519–521.
79. Hederos M, Konradsson P. J. Org. Chem. 2005; 70:7196–7207. [PubMed: 16122238]
80. Hederos M, Konradsson P. J. Am. Chem. Soc. 2006; 128:3414–3419. [PubMed: 16522122]
81. de Lederkremer RM, Lima C, Ramirez MI, Ferguson MAJ, Homans SW, Thomas-Oates J. J. Biol. Chem. 1991; 266:23670–23675. [PubMed: 1748644]
82. Sarbajna S, Misra AK, Roy N. Synth. Commun. 1998; 28:2559–2570.
83. Lerner LM. Carbohydr. Res. 1996; 282:189–192.
84. Elie CJJ, Verduyn R, Dreef CE, Brounts DM, van der Marel GA, van Boom JH. Tetrahedron. 1990; 46:8243–8254.
85. Ottosson H. Carbohydr. Res. 1990; 197:101–107.
86. Conzelmann A, Puoti A, Lester RL, Desponds C. EMBO J. 1992; 11:457–466. [PubMed: 1531630]

87. Fankhauser C, Homans SW, Thomas-Oates JE, McConville MJ, Desponds C, Conzelmann A, Ferguson MAJ. J. Biol. Chem. 1993; 268:26365–26374. [PubMed: 8253761]
88. Tailler D, Ferrieres V, Pekari K, Schmidt RR. Tetrahedron Lett. 1999; 40:679–682.
89. Kratzer B, Mayer TG, Schmidt RR. Eur. J. Org. Chem. 1998:291–298.
90. Madsen R, Udodong UE, Roberts C, Mootoo DR, Konradsson P, Fraser-Reid B. J. Am. Chem. Soc. 1995; 117:1554–1565.
91. Udodong UE, Madsen R, Roberts C, Fraser-Reid B. J. Am. Chem. Soc. 1993; 115:7886–7887.
92. Hori H, Nishida Y, Ohrui H, Meguro H. J. Org. Chem. 1989; 54:1346–1353.
93. Schroter S, Derr P, Conradt HS, Nimtz M, Hale G, Kirchoff C. J. Biol. Chem. 1999; 274:29862–29873. [PubMed: 10514467]
94. Wu X, Guo Z. Org. Lett. 2007; 9:4311–4313. [PubMed: 17877362]
95. Wu X, Shen Z, Zeng X, Lang S, Palmer M, Guo Z. Carbohydr. Res. 2008; 343:1718–1729. [PubMed: 18439573]
96. Schlueter U, Lu J, Fraser-Reid B. Org. Lett. 2003; 5:255–257. [PubMed: 12556165]
97. Xue J, Pan Y, Guo Z. Tetrahedron Lett. 2002; 43:1599–1602.
98. Xue J, Guo Z. Bioorg. Med. Chem. Lett. 2002; 12:2015–2018. [PubMed: 12113831]
99. Xue J, Shao N, Guo Z. J. Org. Chem. 2003; 68:4020–4029. [PubMed: 12737586]
100. Gou D, Liu Y, Chen C. Carbohydr. Res. 1992; 234:51–64.
101. Lu P, Gou D, Shieh W, Chen C. Biochemistry. 1994; 33:11586–11587. [PubMed: 7918372]
102. Xue J, Guo Z. J. Am. Chem. Soc. 2003; 125:16334–16339. [PubMed: 14692775]
103. Garegg PJ, Maron L. Acta Chem. Scand., Ser. B. 1979; B33:39–41.
104. Tailler D, Jacquinet J-C, Noirot A-M, Beau J-M. J. Chem. Soc., Perkin Trans. 1. 1992:3163–3165.
105. Xue J, Guo Z. Tetrahedron Lett. 2001; 42:6487–6489.
106. Shao N, Xue J, Guo Z. Angew. Chem., Int. Ed. 2004; 43:1569–1573.
107. Guo Z, Nakahara Y, Nakahara Y, Ogawa T. Bioorg. Med. Chem. 1997; 5:1917–1924. [PubMed: 9370036]
108. Nigou M, Gilleron M, Puzo G. Biochimie. 2003; 85:153–166. [PubMed: 12765785]
109. Morita YS, Patterson JH, Billman-Jacobe H, McConville MJ. Biochem. J. 2004; 378:589–597. [PubMed: 14627436]
110. Gilleron M, Ronet C, Mempel M, Monsarrat B, Gachelin G, Puzo G. J. Biol. Chem. 2001; 276:34896–34904. [PubMed: 11441009]
111. Gilleron M, Quesniaux VFJ, Puzo G. J. Biol. Chem. 2003; 278:29880–29889. [PubMed: 12775723]
112. Anderson RJ, Chargaff E. J. Biol. Chem. 1929; 85:77–88.
113. Spielman MA. J. Biol. Chem. 1934; 106:87–96.
114. Tanaka S, Saburi H, Ishibashi Y, Kitamura M. Org. Lett. 2004; 6:1873–1875. [PubMed: 15151436]
115. Huang, X., Wang, Z. Comprehensive glycoscience. From chemistry to system biology. Kamerling, JP.Boons, G-J.Lee, YC.Suzuki, A.Taniguchi, N., Voragen, AGJ., editors. Vol. Vol. 1. Elsevier; Amsterdam: 2007. p. 379-412.
116. Parameswar, AR., Demchenko, AV. Progress in the synthesis of complex carbohydrate chains of plant and microbial polysaccharides. Nifantiev, NE., editor. Transworld Research Network; Kerala, India: 2009. p. 465-490.



Scheme 1.
Retrosynthesis of a GPI anchor of *T. brucei* (the Ogawa group).



Scheme 2.

myo-Inositol enantiomeric resolution and the synthesis of a pseudotrisaccharide derivative (the Ogawa group).

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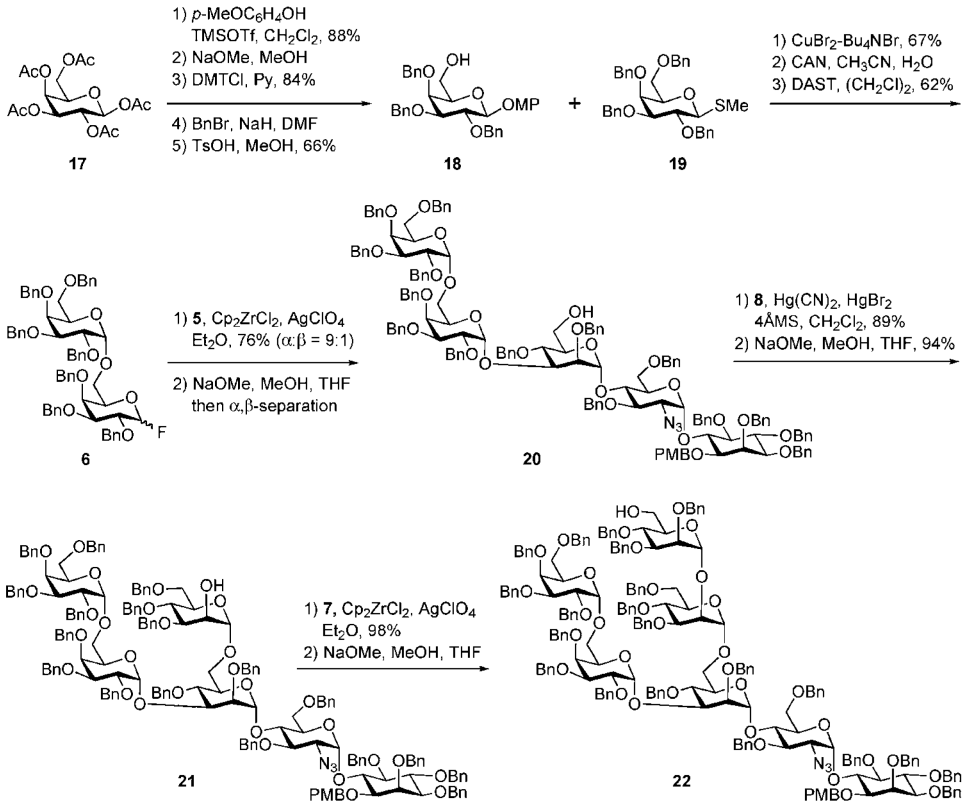

Scheme 3.
GPI branched glycan core assembly (the Ogawa group).

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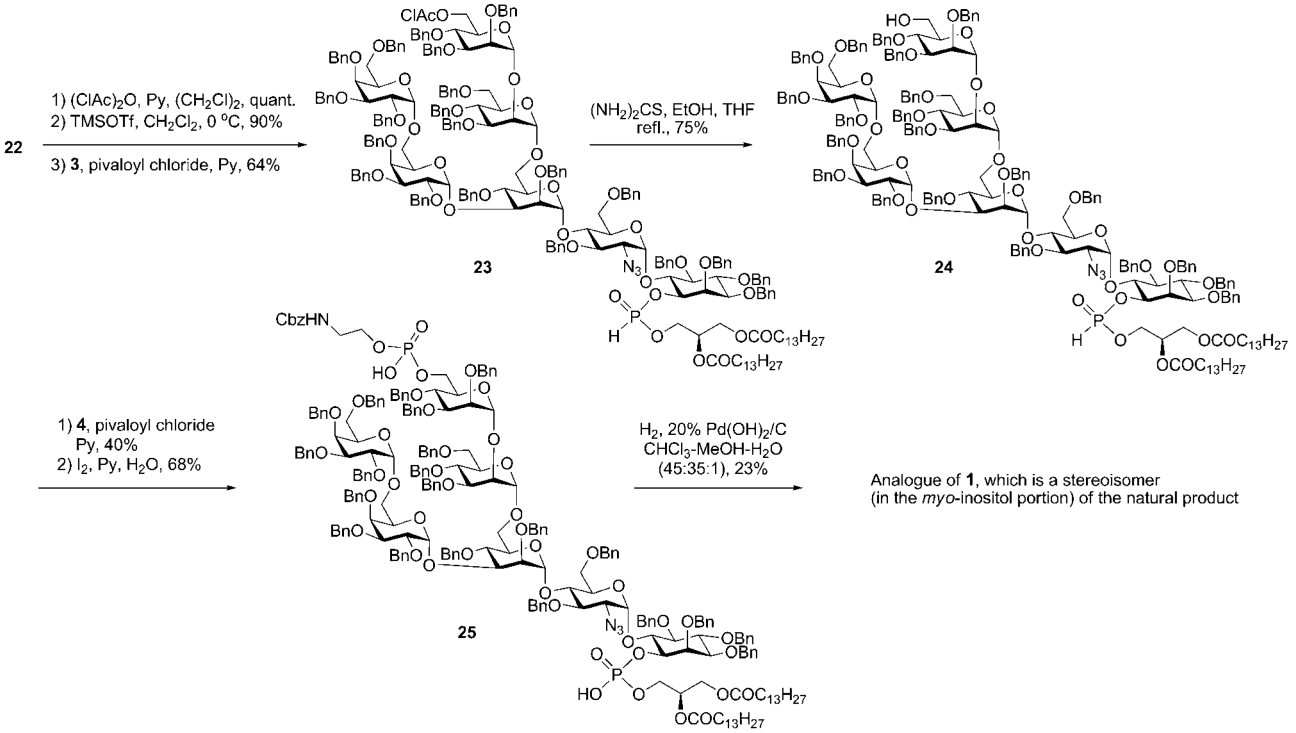

Scheme 4.

Successive phosphorylation steps and global deprotection (the Ogawa group).

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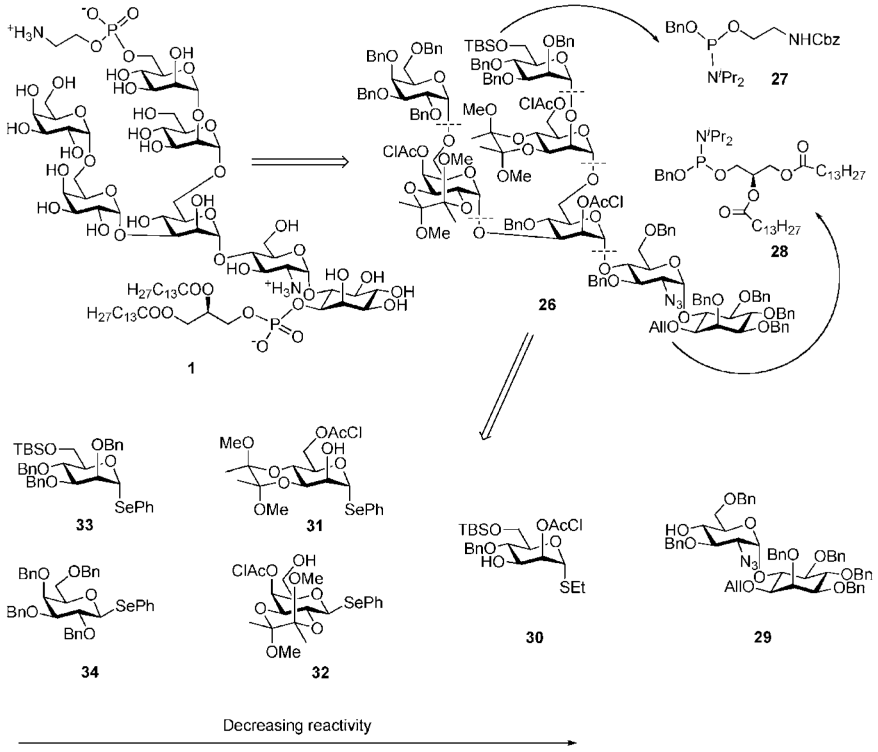

Scheme 5.
Retrosynthesis of a GPI anchor of *T. brucei* (the Ley group).

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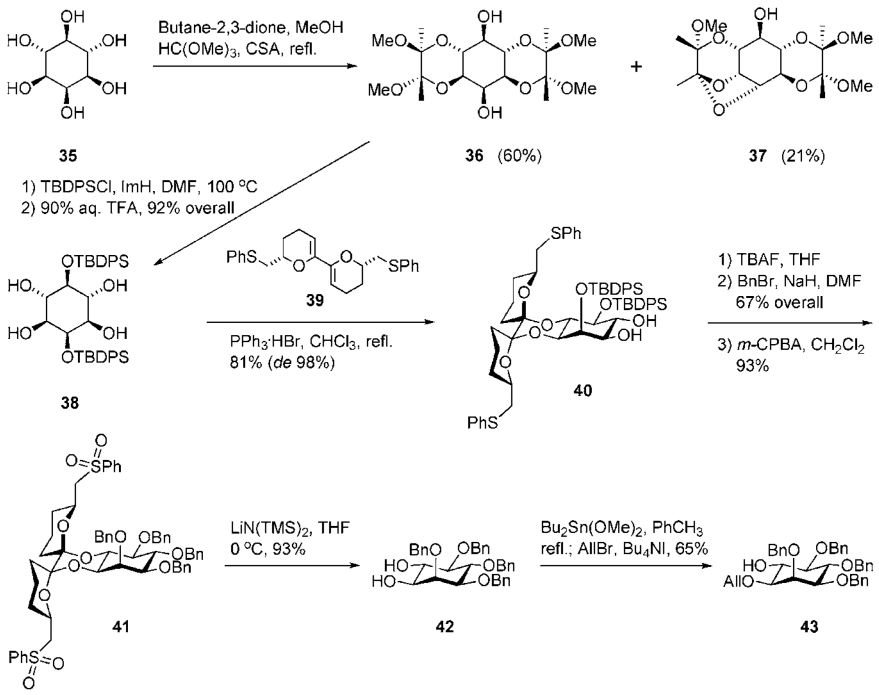

Scheme 6.

myo-Inositol chiral desymmetrisation (the Ley group).

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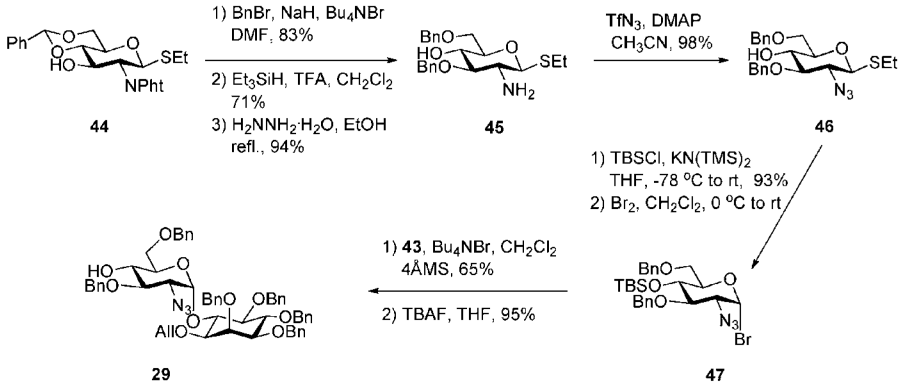

Scheme 7.

Synthesis of a pseudodisaccharide derivative (the Ley group).

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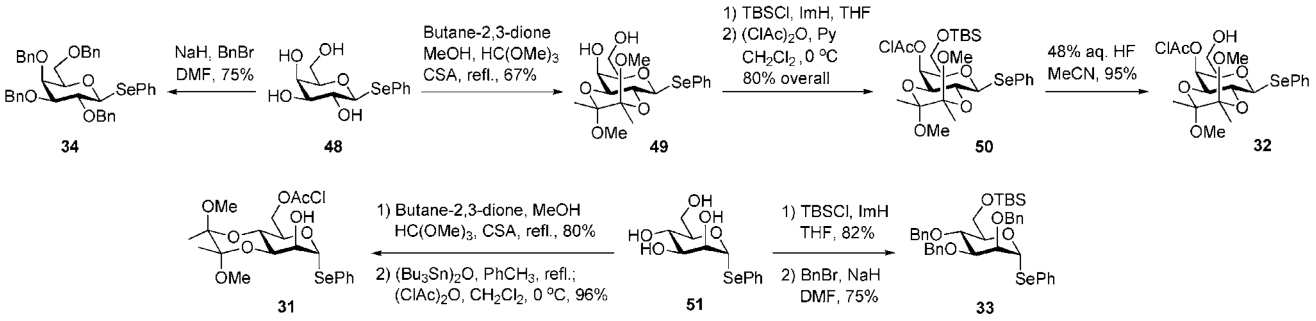

Scheme 8.

Synthesis of D-galactose and D-mannose building blocks (the Ley group).

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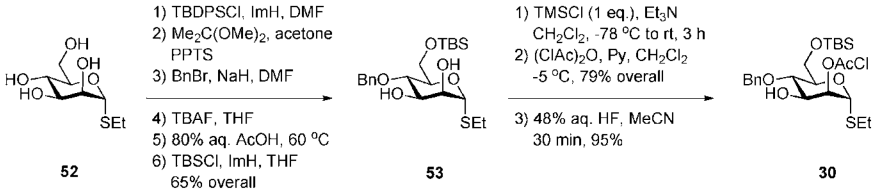

Scheme 9.

Synthesis of a central D-mannose building block (the Ley group).

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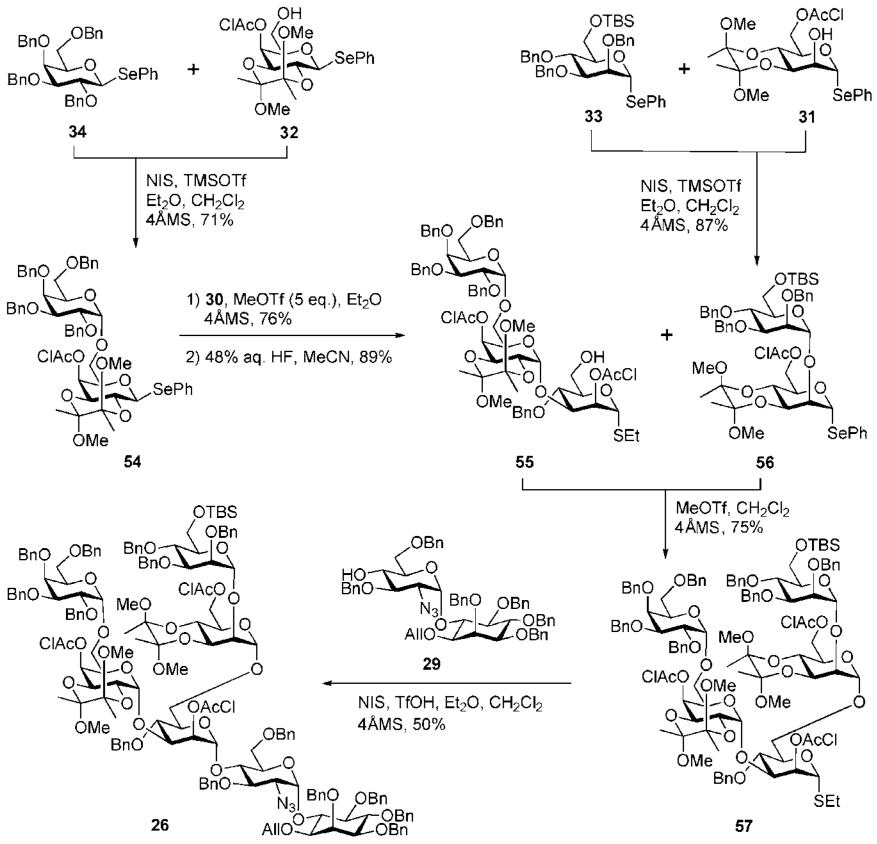

Scheme 10.
GPI branched glycan core assembly (the Ley group).

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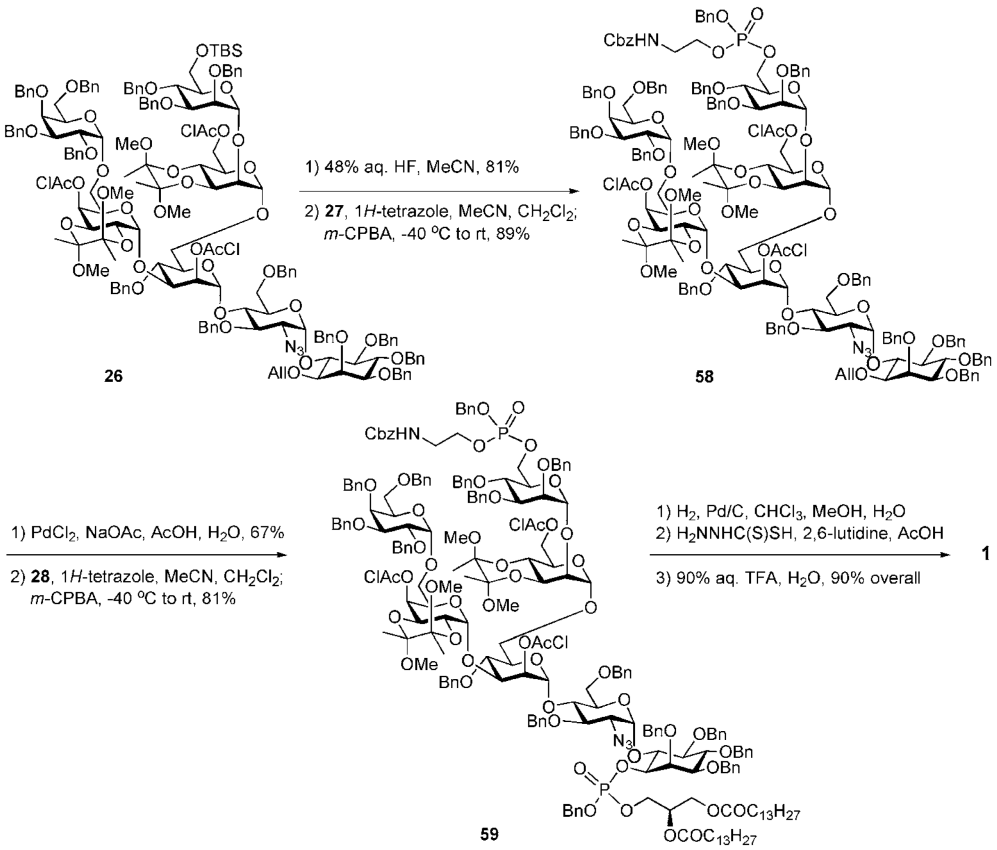

Scheme 11.

Successive phosphorylation steps and global deprotection (the Ley group).

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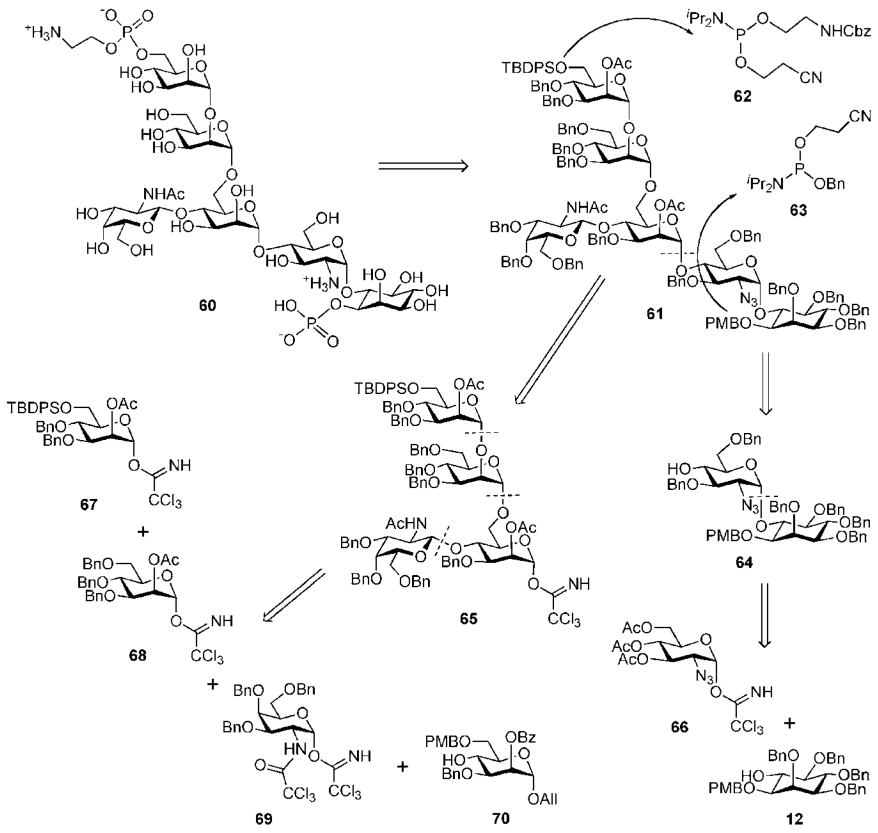

Scheme 12.
Retrosynthesis of a GPI-related glycoconjugate of *T. gondii* (the Schmidt group).

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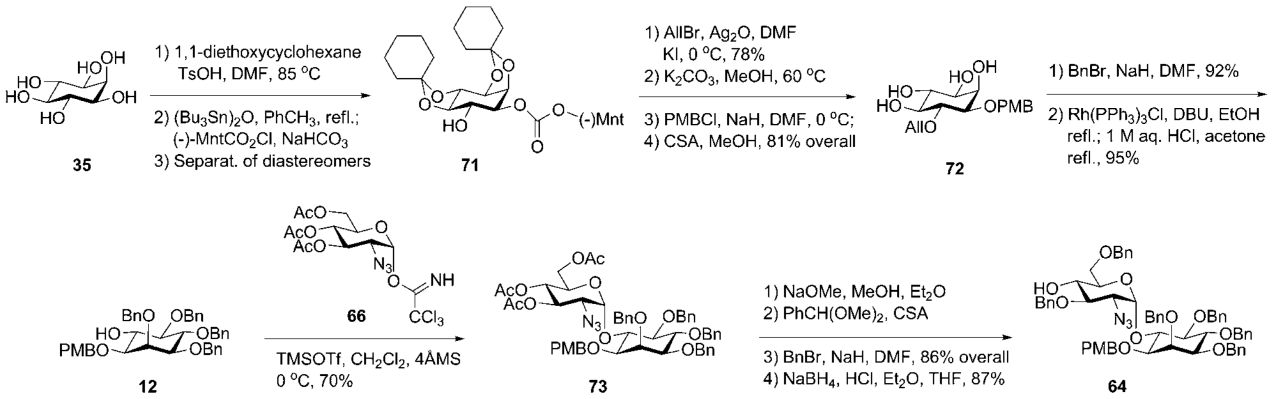

Scheme 13.

myo-Inositol enantiomeric resolution and the synthesis of a pseudodisaccharide derivative (the Schmidt group).

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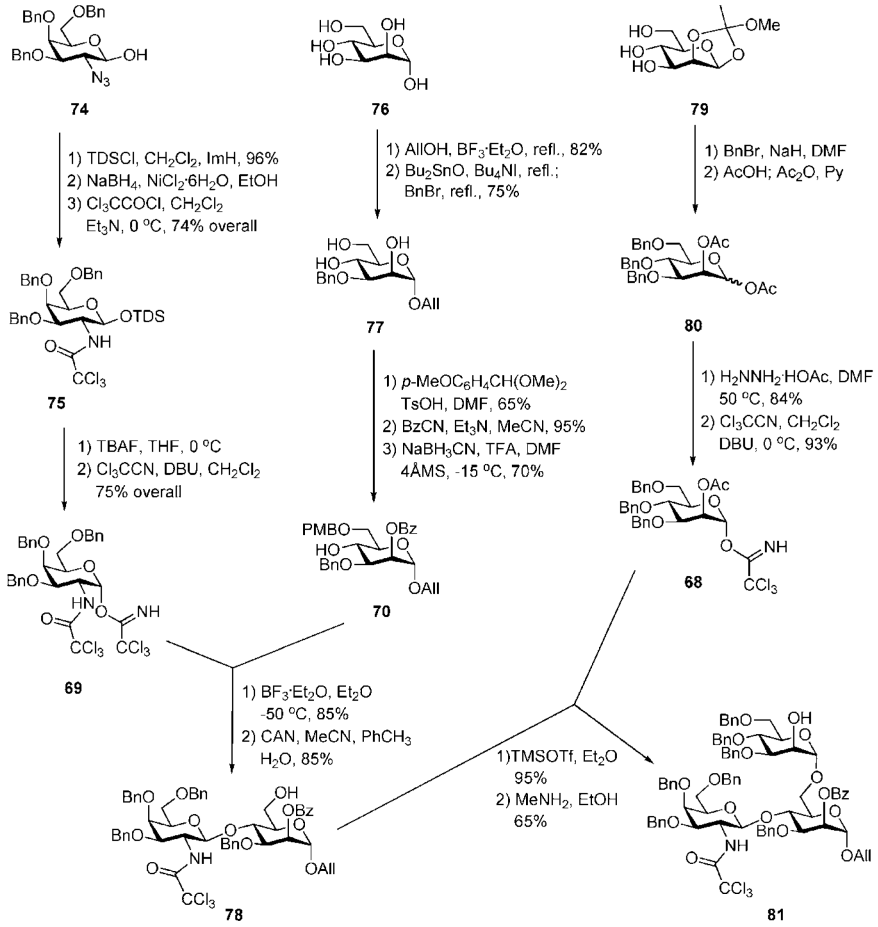

Scheme 14.

The synthesis of monosaccharide building blocks and assembly of a branched trisaccharide derivative (the Schmidt group).

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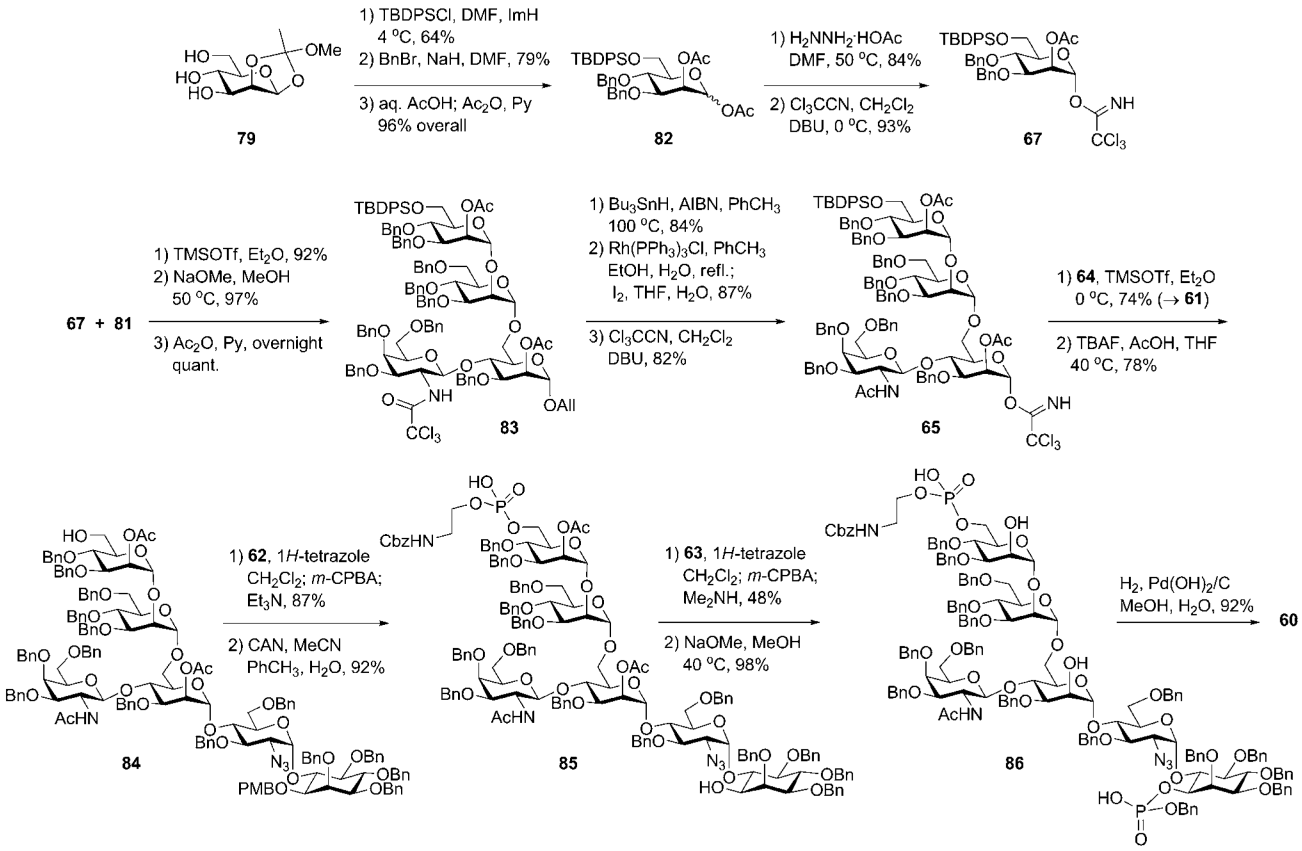

Scheme 15.

T. gondii GPI branched glycan core assembly, successive phosphorylation steps and global deprotection (the Schmidt group).

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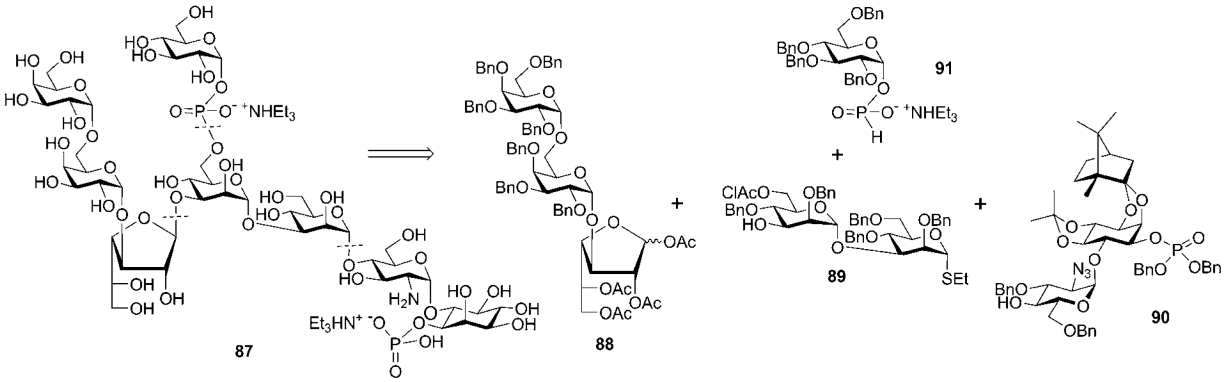
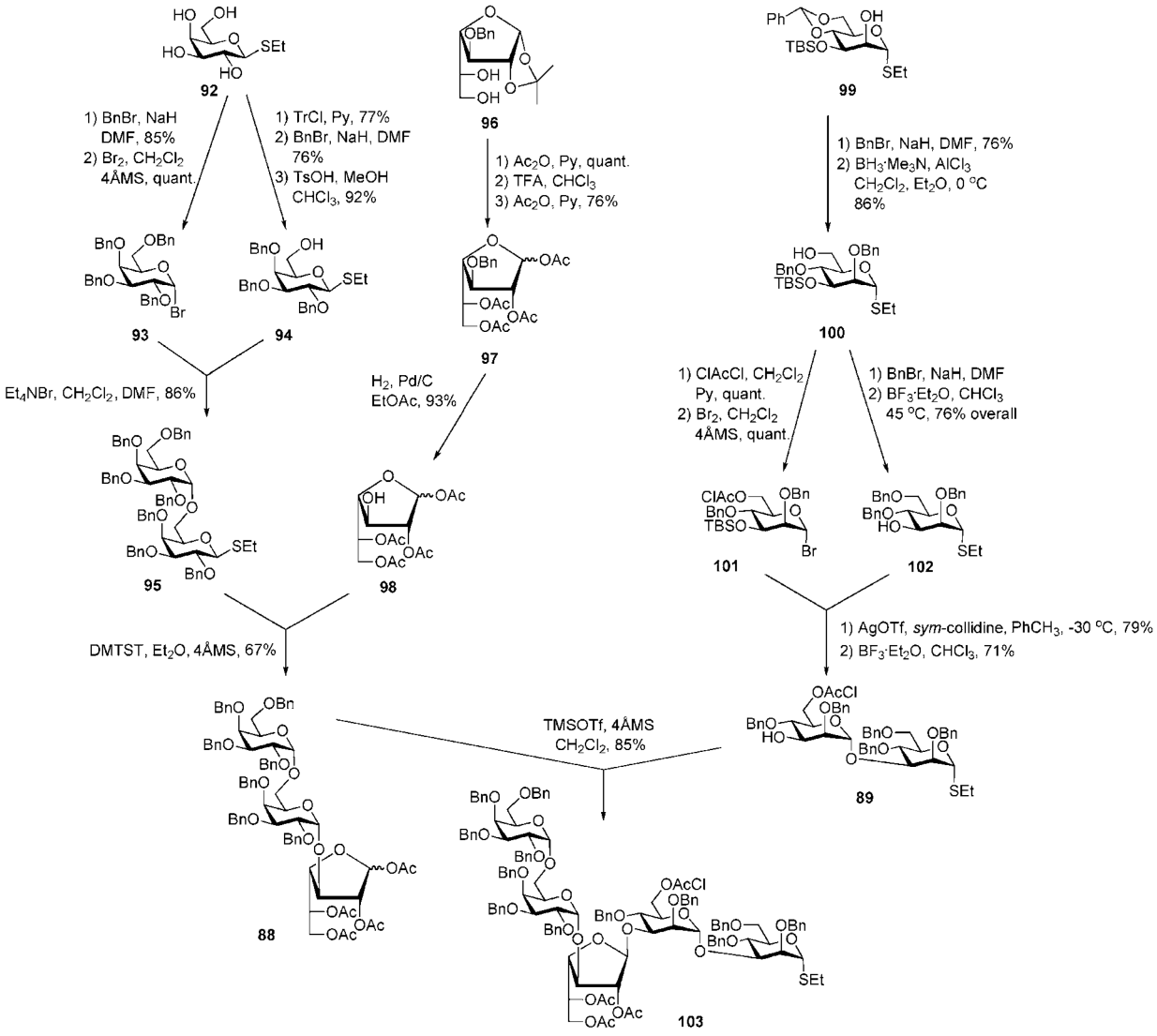


Scheme 16.

Retrosynthesis of a GPI-related glycoconjugate of *Leishmania* (the Konradsson–Oscarson group).



Scheme 17.

The synthesis of monosaccharide building blocks and assembly of a linear pentasaccharide (the Konradsson–Oscarson group).

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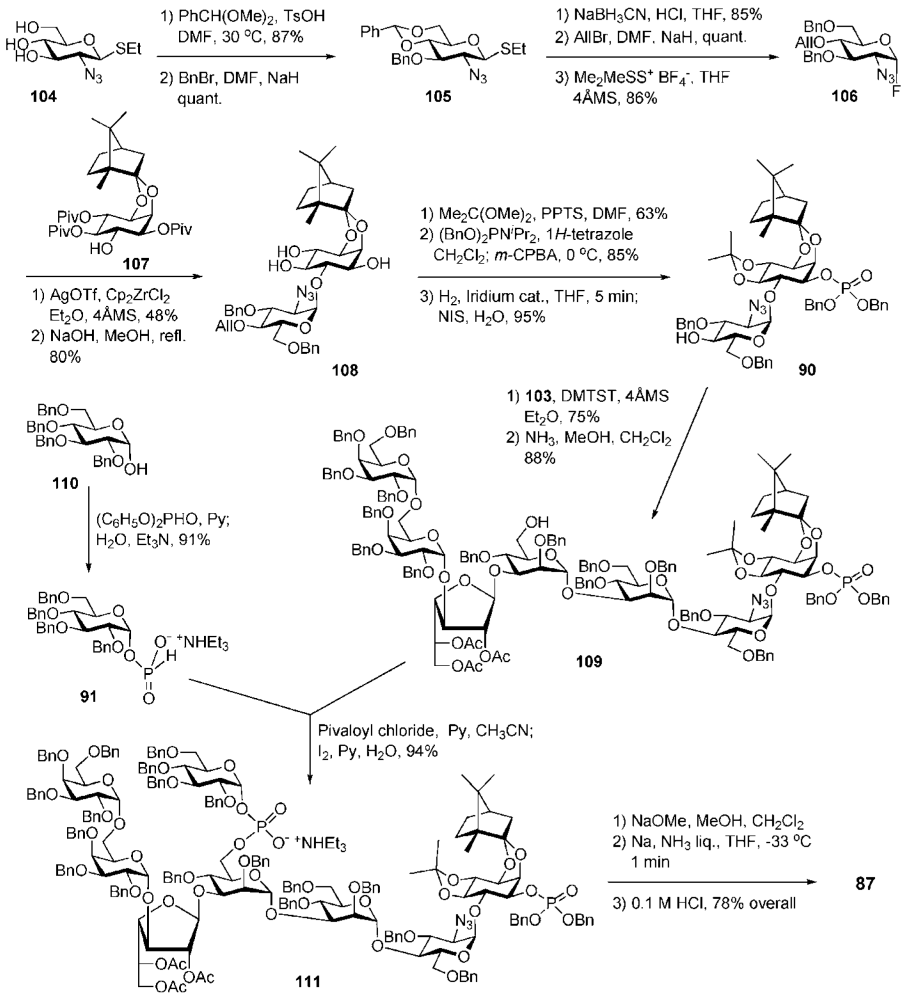

Scheme 18.

GPI glycan core assembly (including phosphorylation steps) and global deprotection (the Konradsson–Oscarson group).

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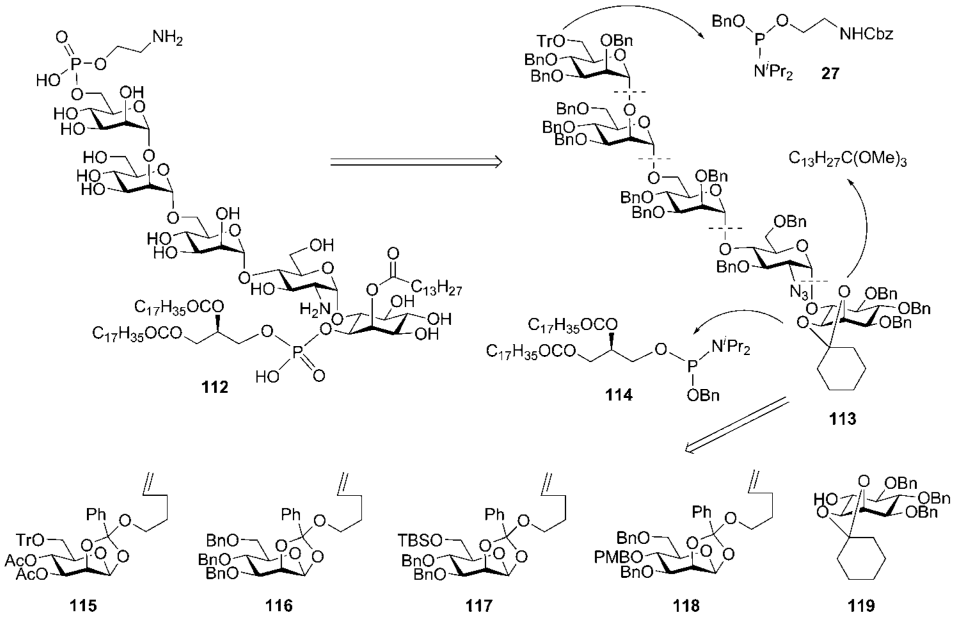
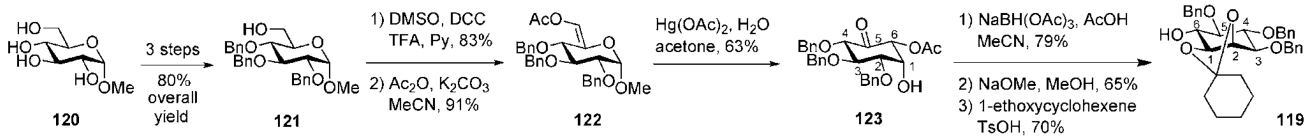


Scheme 19.

Retrosynthesis of a GPI anchor of *P. falciparum* (the Fraser-Reid group).



Scheme 20.

De novo synthesis of an optically pure *myo*-inositol derivative (the Fraser-Reid group).

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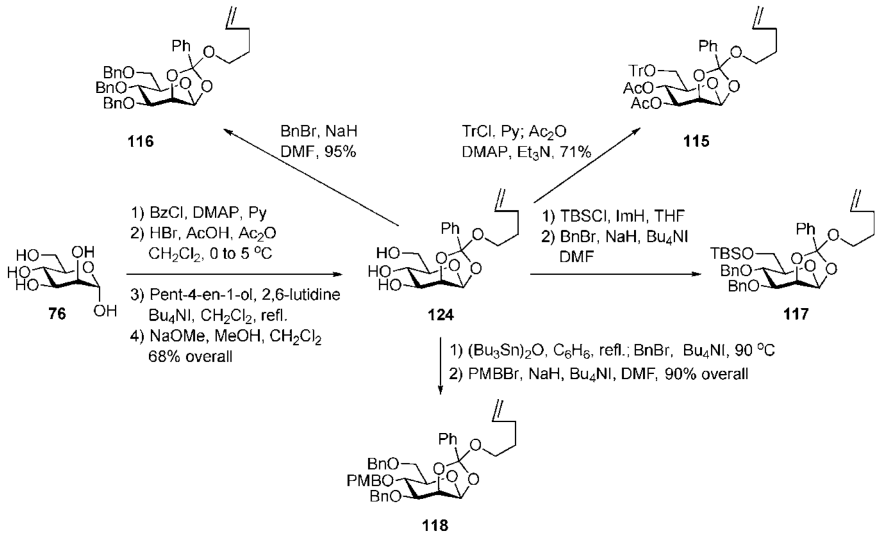
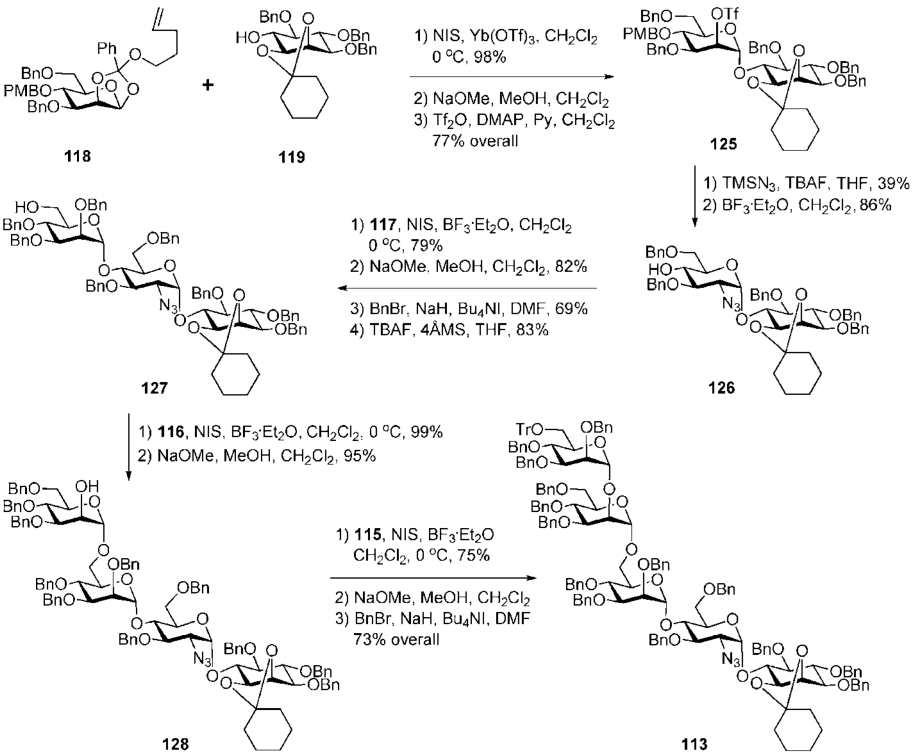


Scheme 21.

Synthesis of monosaccharide building blocks (the Fraser-Reid group).



Scheme 22.
GPI glycan core assembly (the Fraser-Reid group).

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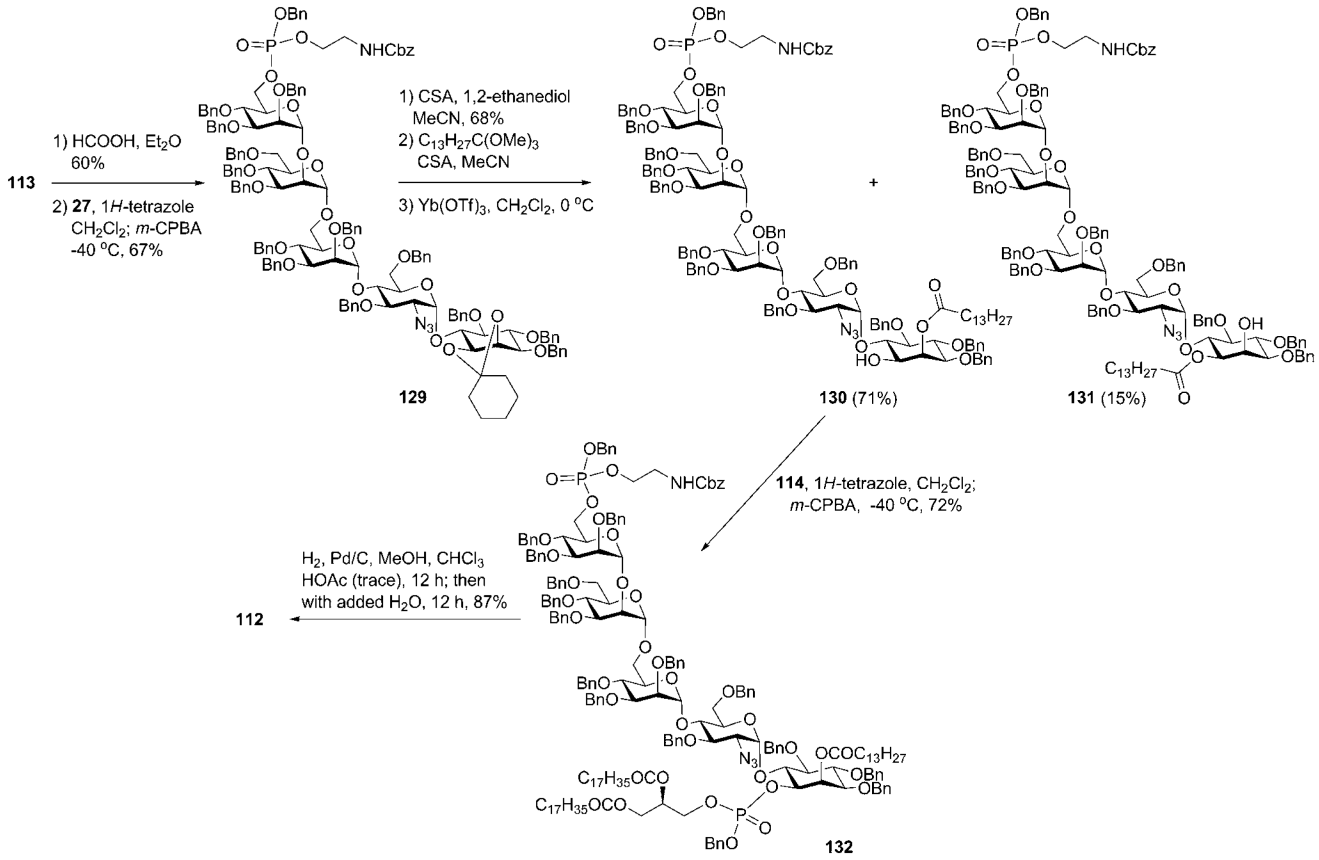

Scheme 23.

Successive phosphorylation and lipidation steps and global deprotection (the Fraser-Reid group).

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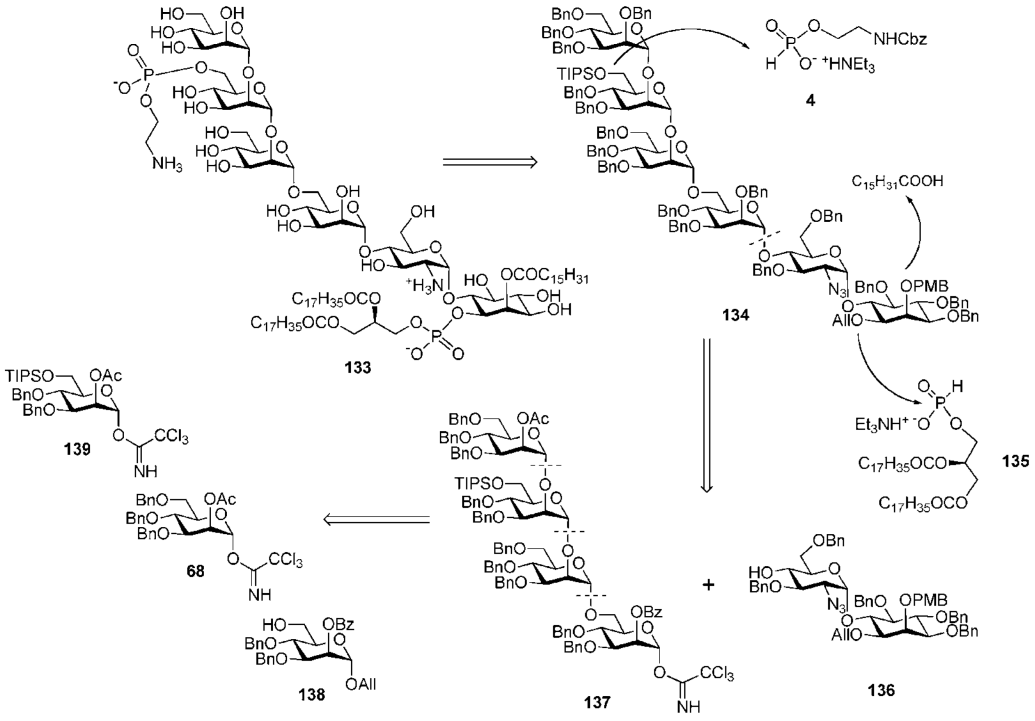
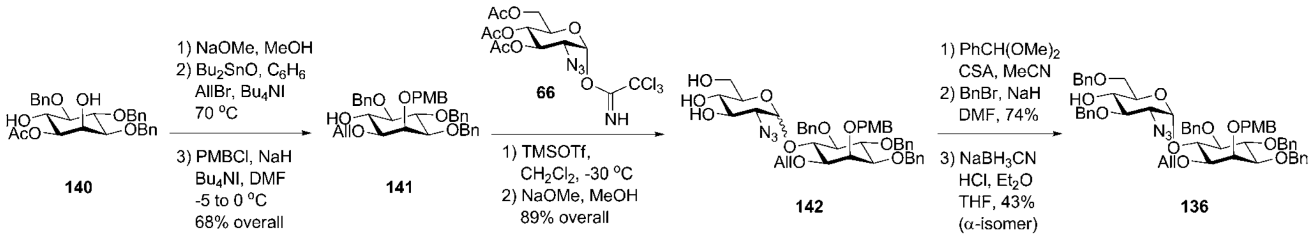


Scheme 24.

Retrosynthesis of a GPI anchor of *P. falciparum* (the Seeberger group).



Scheme 25.

Synthesis of a pseudodisaccharide derivative (the Seeberger group).

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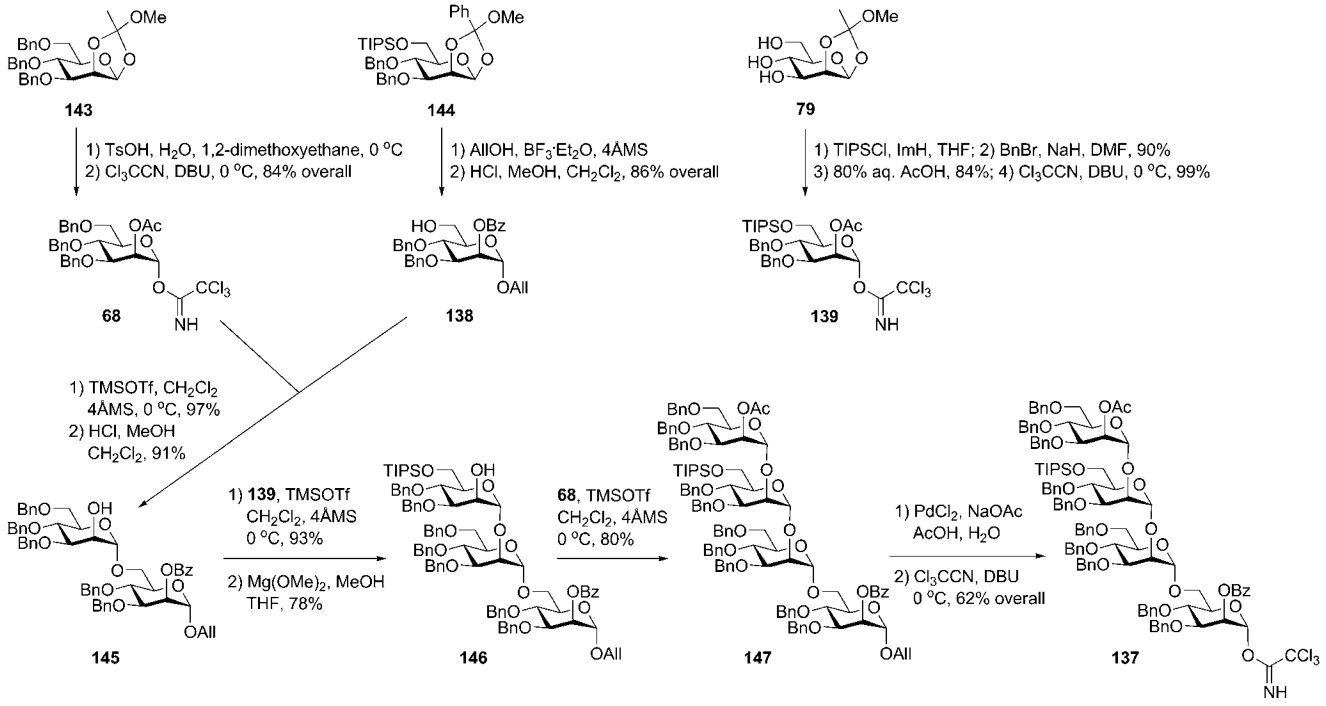

Scheme 26.

Synthesis of monosaccharide building blocks and assembly of a linear tetramannoside (the Seeberger group).

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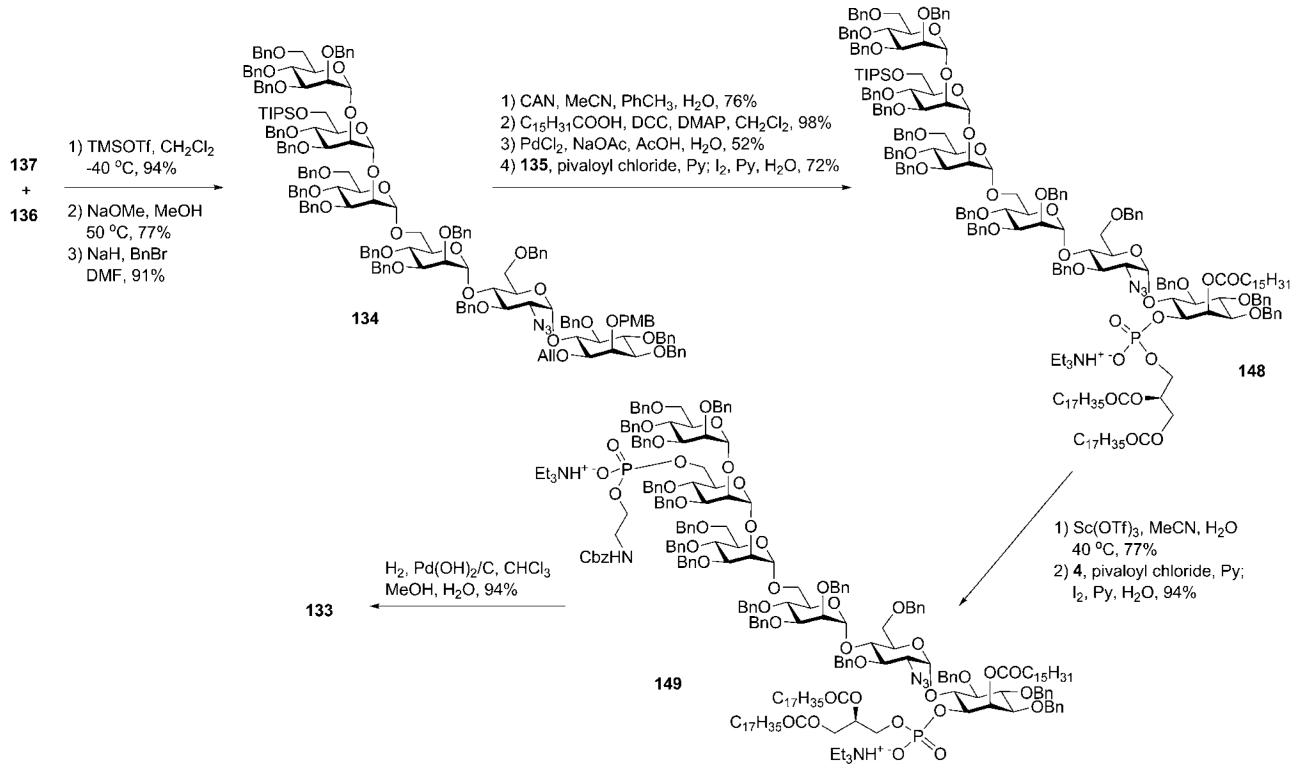

Scheme 27.

GPI glycan core assembly, successive lipidation and phosphorylation steps and global deprotection (the Seeberger group).

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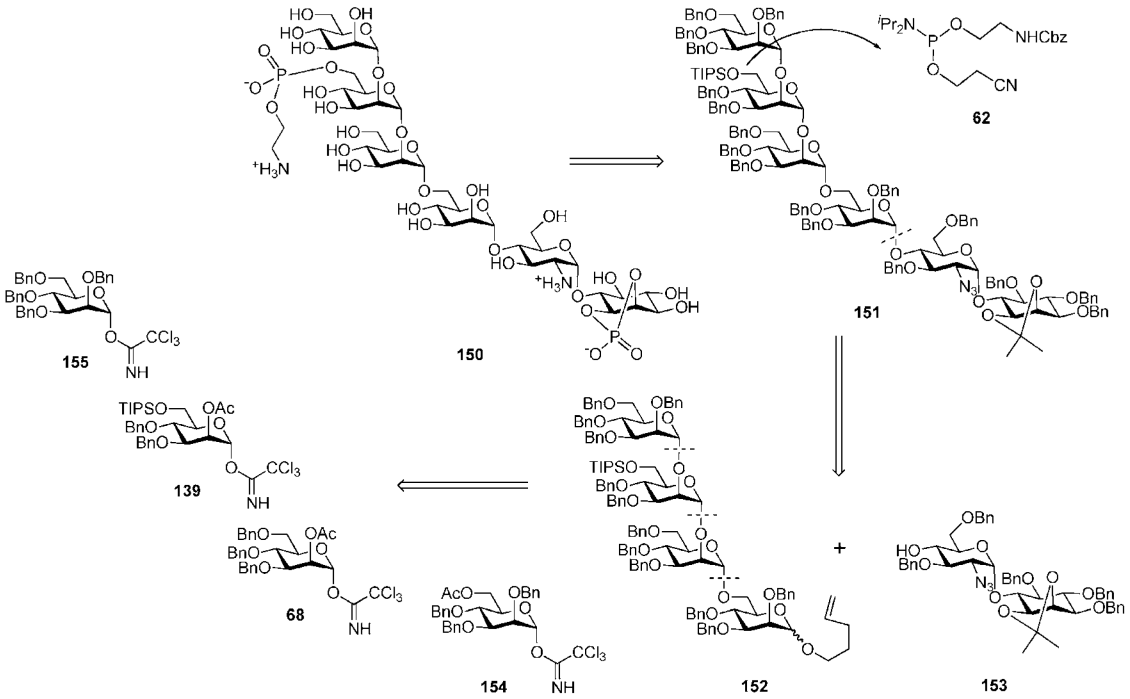
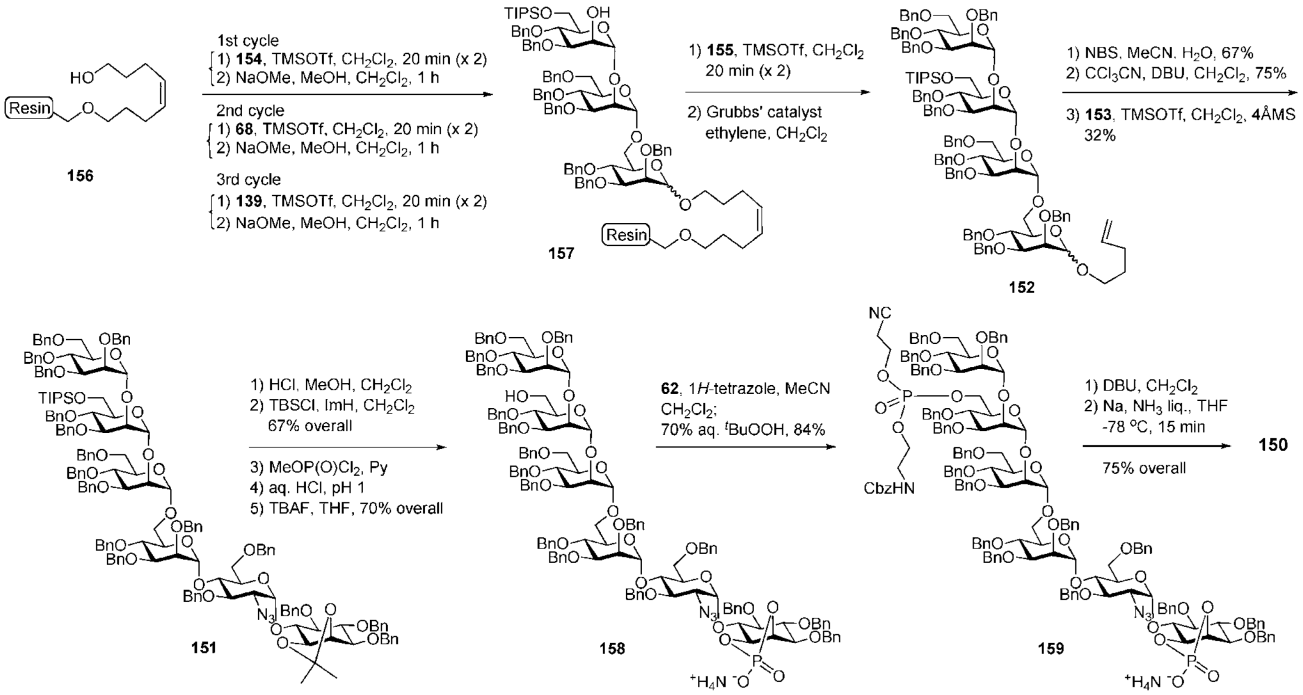


Scheme 28.

Retrosynthesis of a GPI-related glycoconjugate of *P. falciparum* (pseudohexasaccharide malarial toxin) (the Seeberger group).



Scheme 29.

Solid-phase synthesis of a pseudohexasaccharide malarial toxin (the Seeberger group).

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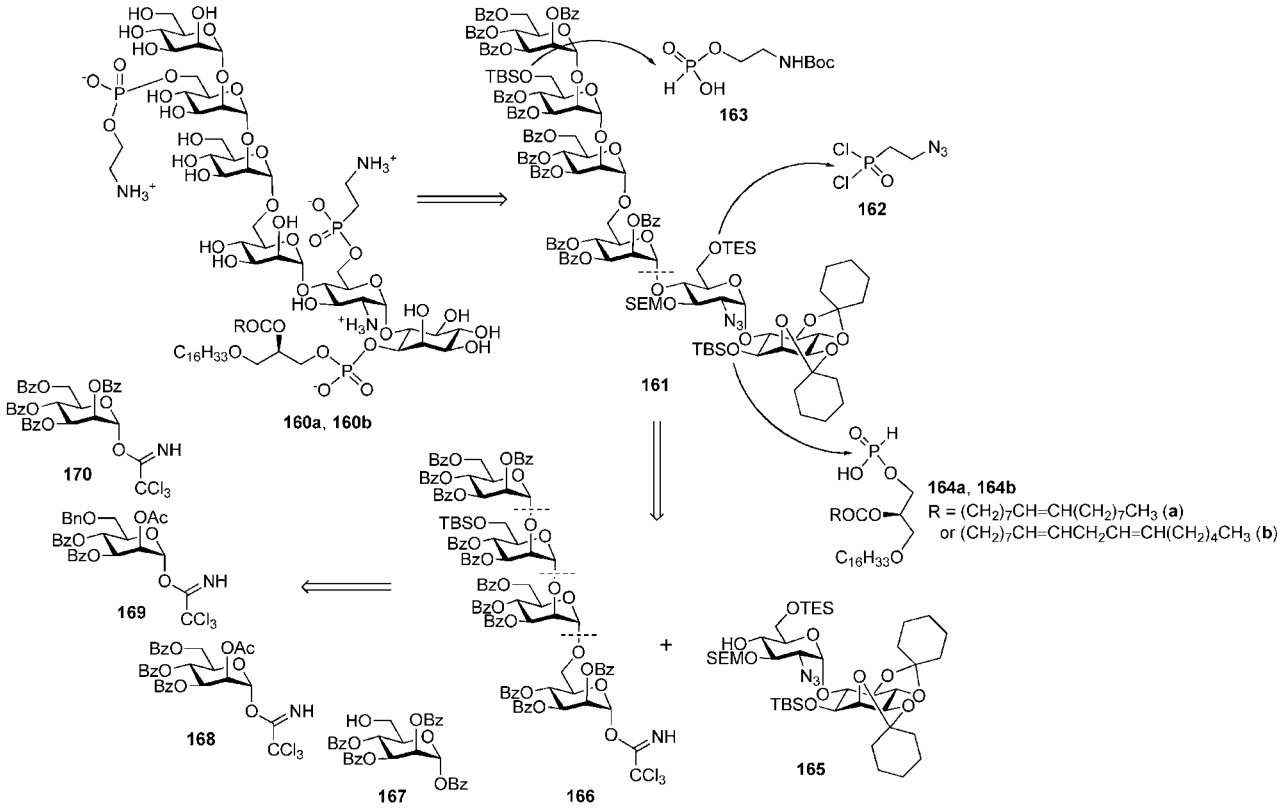

Scheme 30.

Retrosynthesis of GPI anchors of *T. cruzi*; synthetic strategy A (the Nikolaev group).

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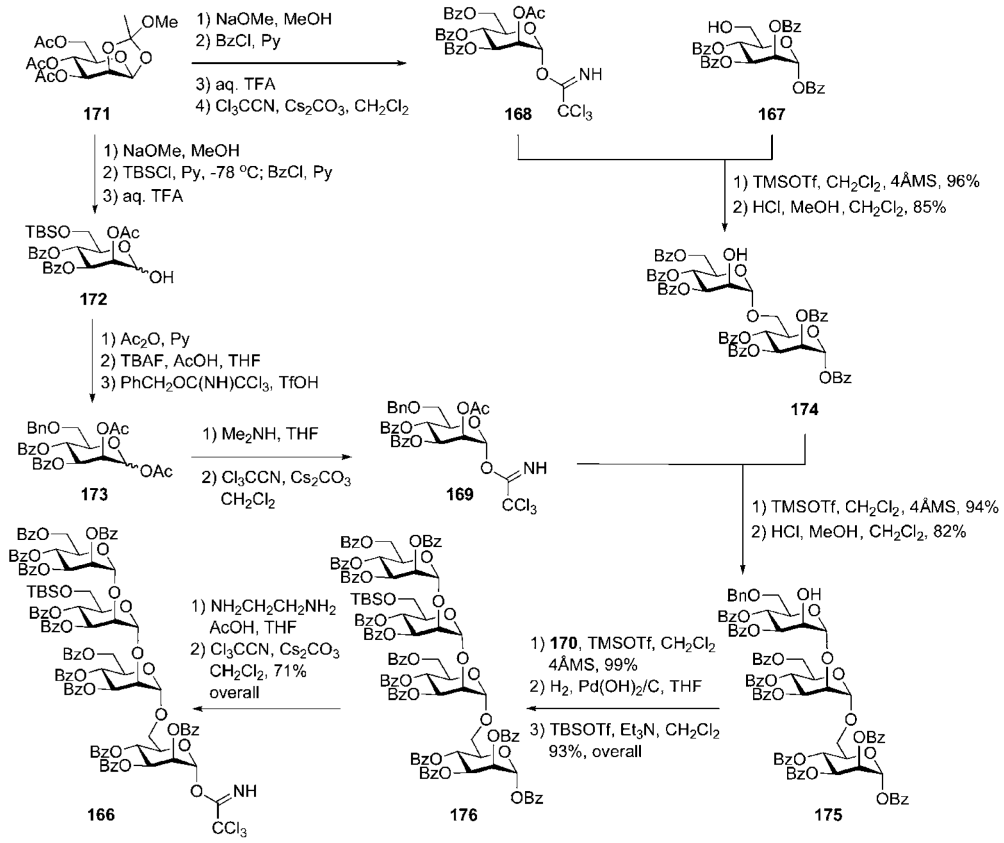

Scheme 31.

Synthesis of D-mannose building blocks and assembly of a linear tetramannoside; synthetic strategy A (the Nikolaev group).

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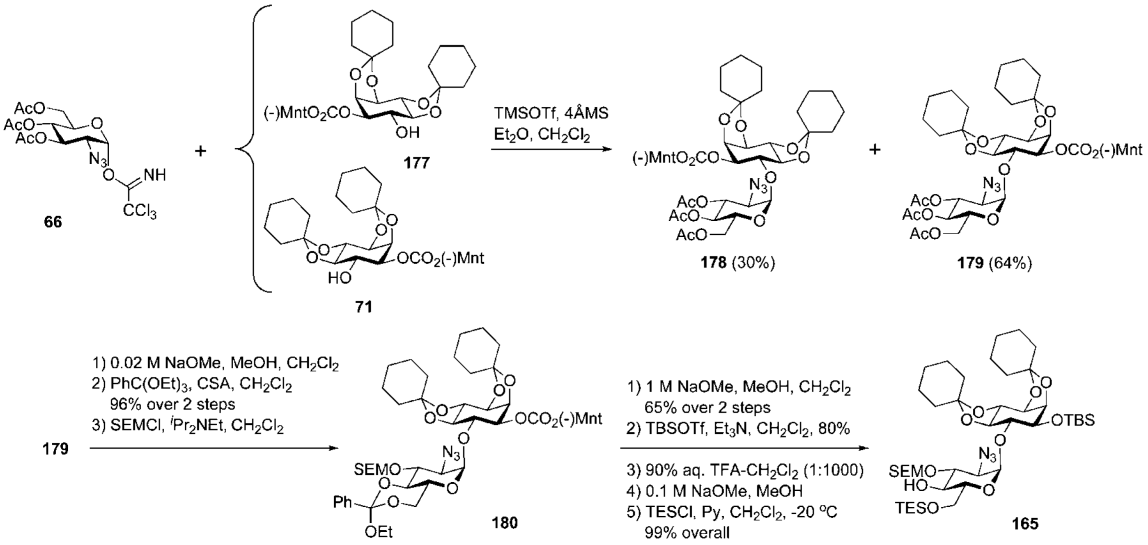

Scheme 32.

Enantiomeric resolution of *myo*-inositol and the synthesis of a pseudodisaccharide derivative (the Nikolaev group; synthetic strategy A).

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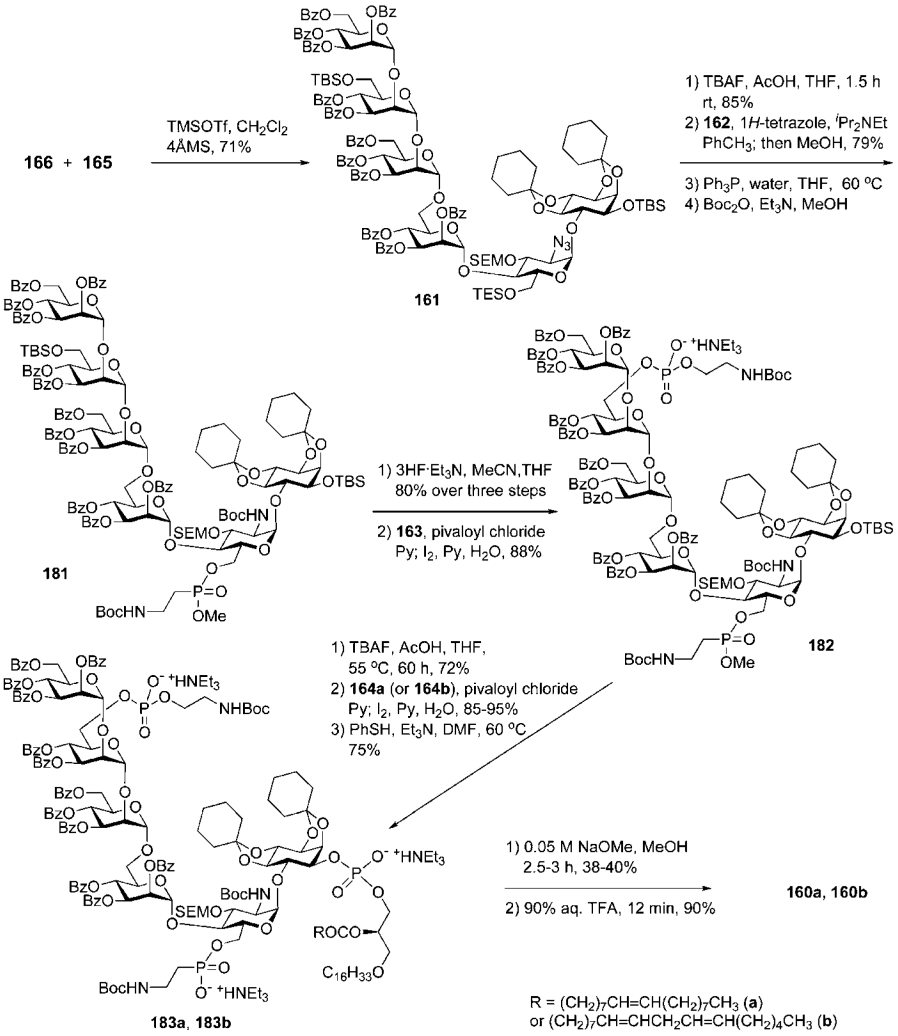

Scheme 33.

GPI glycan core assembly, successive phosphonylation and phosphorylation steps and global deprotection (the Nikolaev group; synthetic strategy A).

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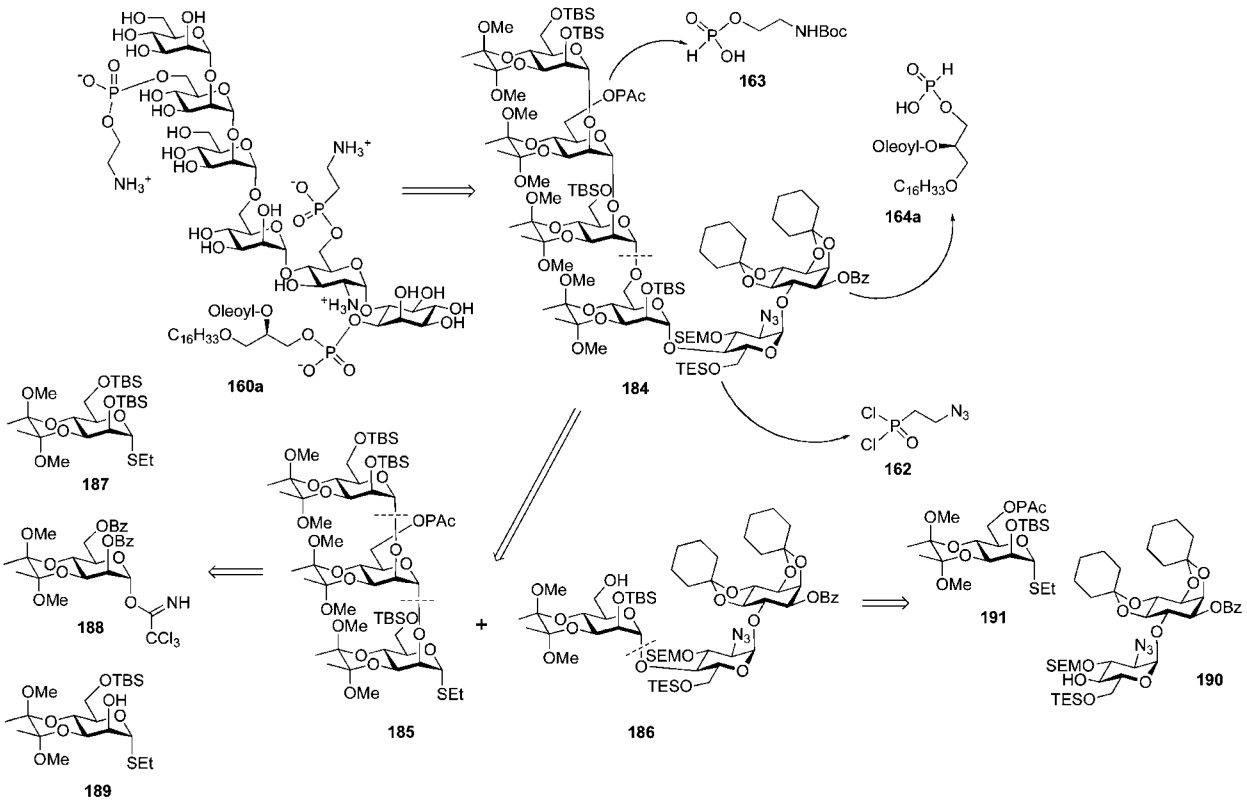

Scheme 34.
Retrosynthesis of GPI anchors of *T. cruzi* (the Nikolaev group; synthetic strategy B).

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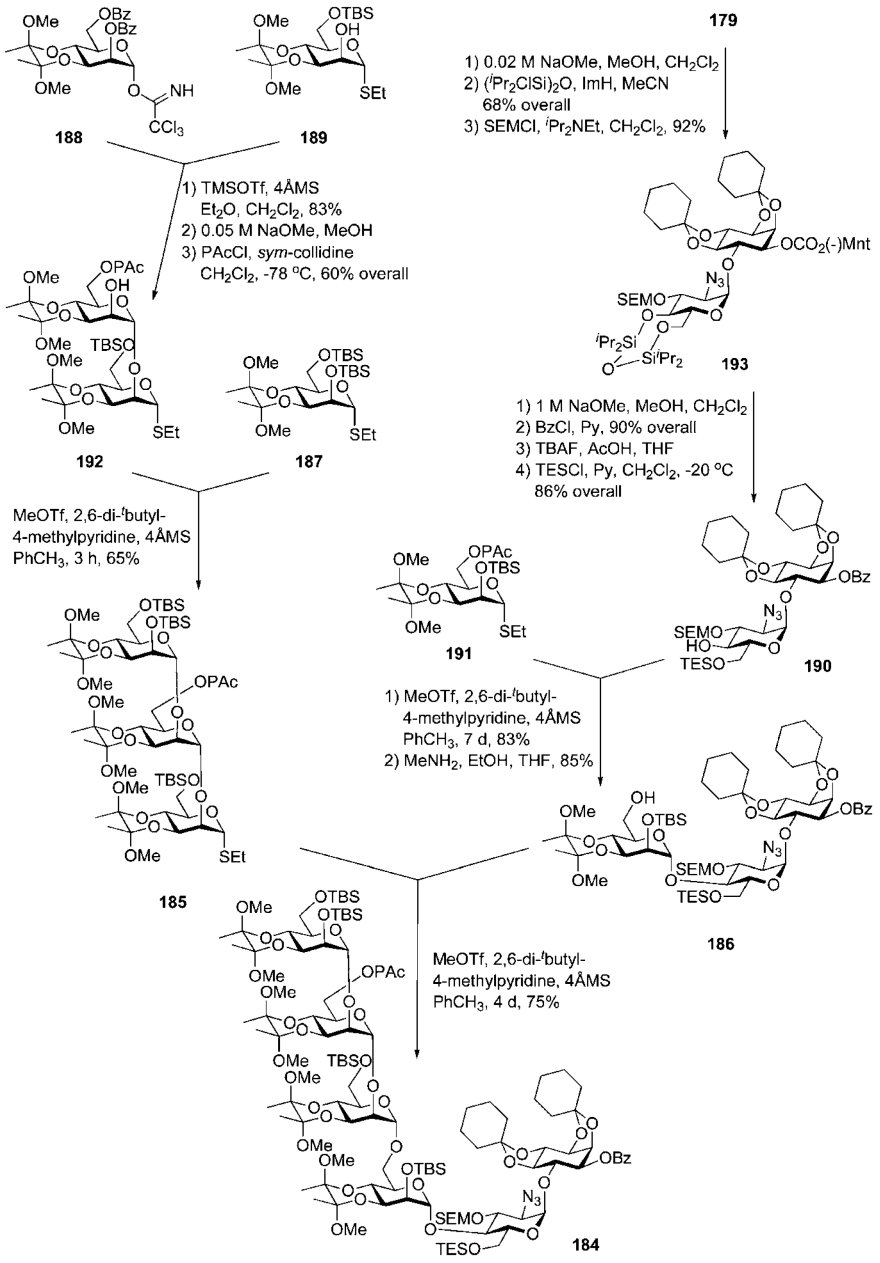

Scheme 35.

Synthesis of a linear trimannoside and a pseudotrisaccharide derivative and GPI glycan core assembly (the Nikolaev group; synthetic strategy B).

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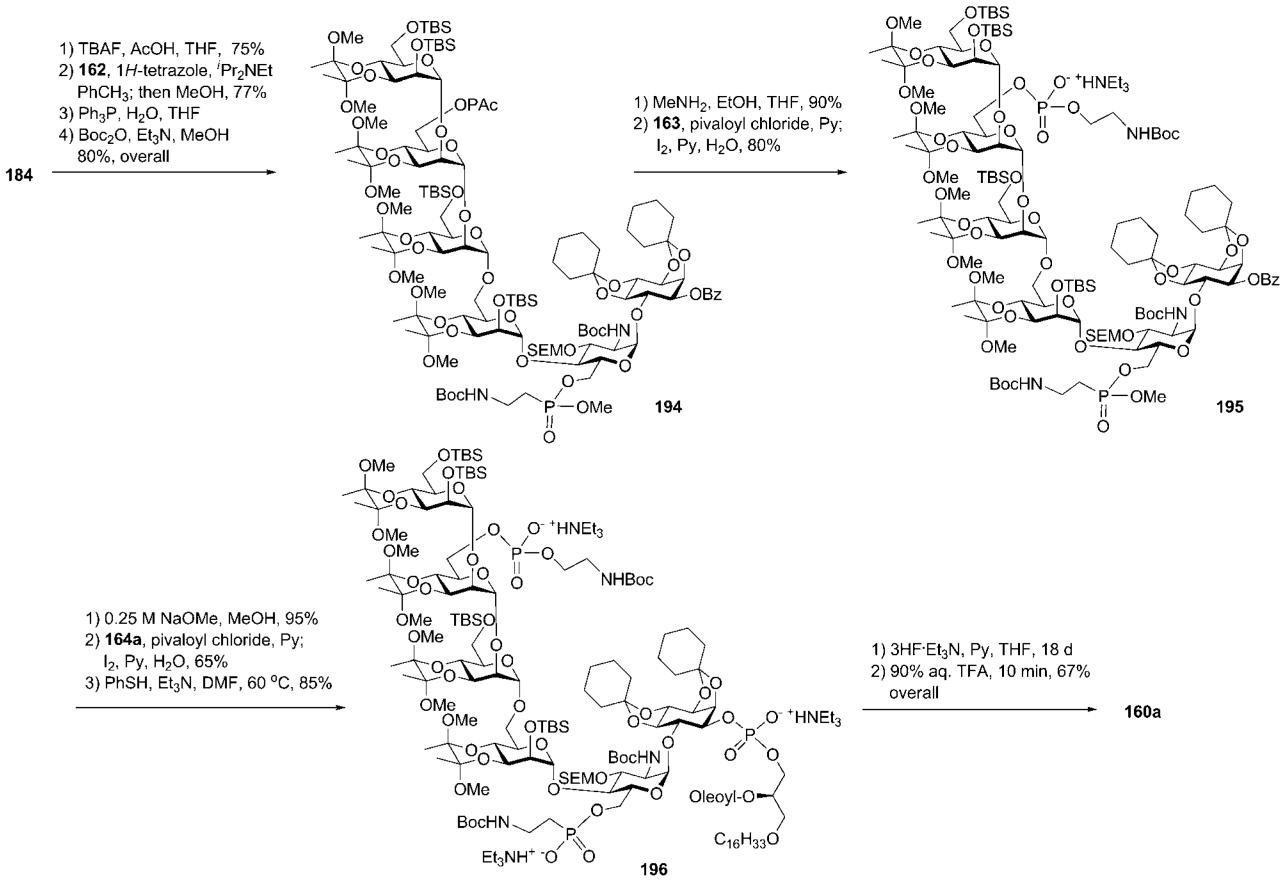

Scheme 36.

Successive phosphonylation and phosphorylation steps and global deprotection (the Nikolaev group; synthetic strategy B).

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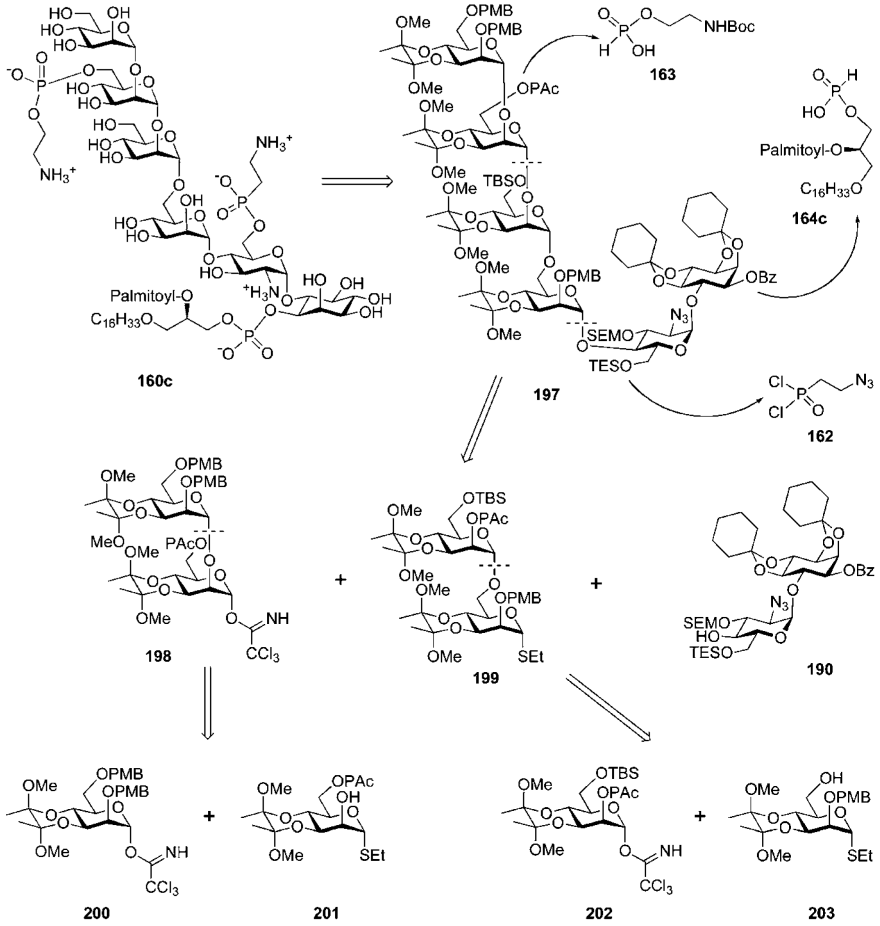
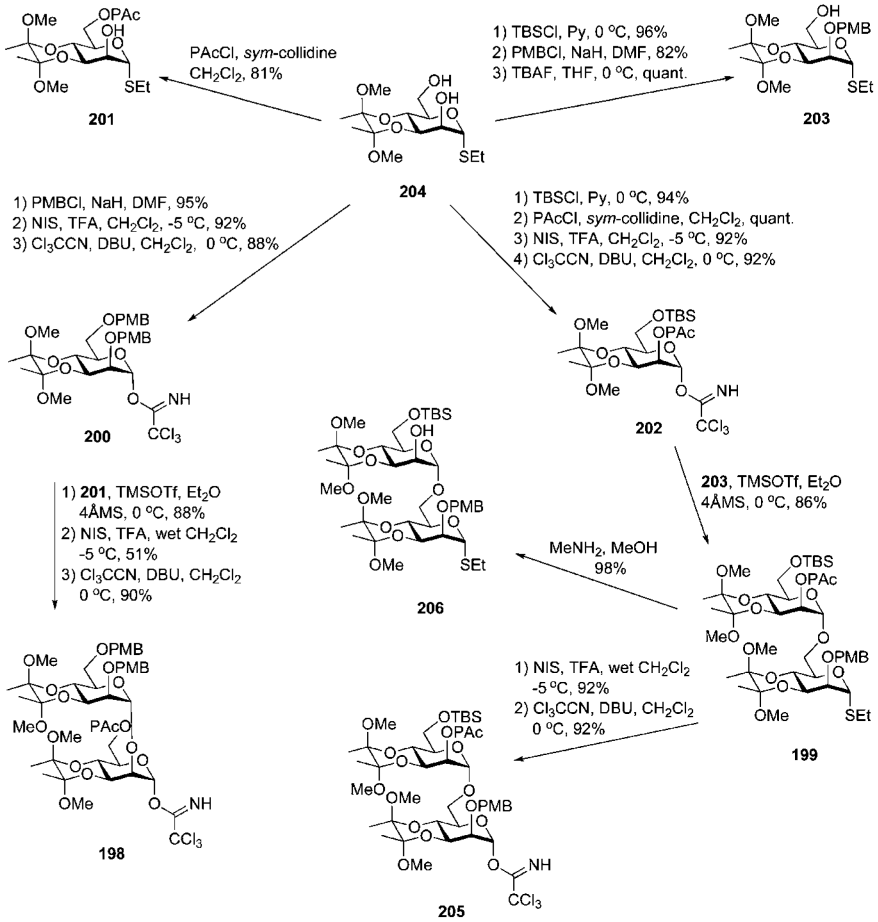


Scheme 37.
Retrosynthesis of a GPI anchor of *T. cruzi* (the Nikolaev group; synthetic strategy C).



Scheme 38.

Synthesis of D-mannose mono- and disaccharide building blocks (the Nikolaev group; synthetic strategy C).

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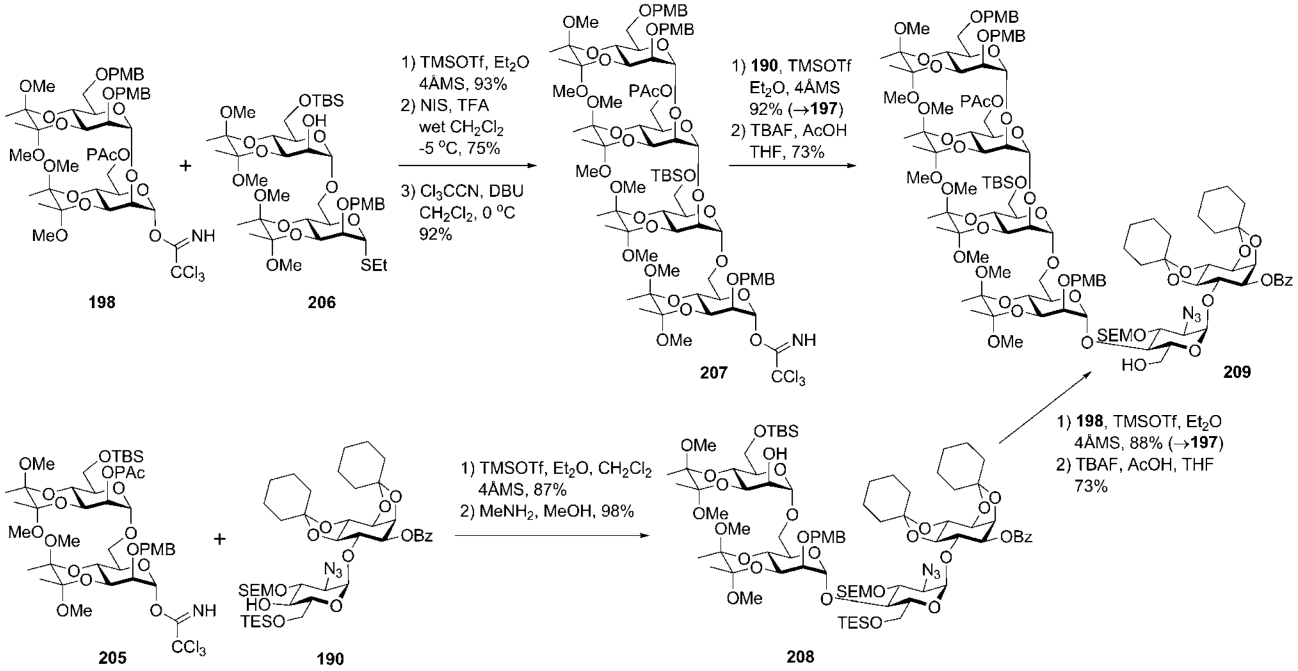

Scheme 39.
GPI glycan core assembly (the Nikolaev group; synthetic strategy C).

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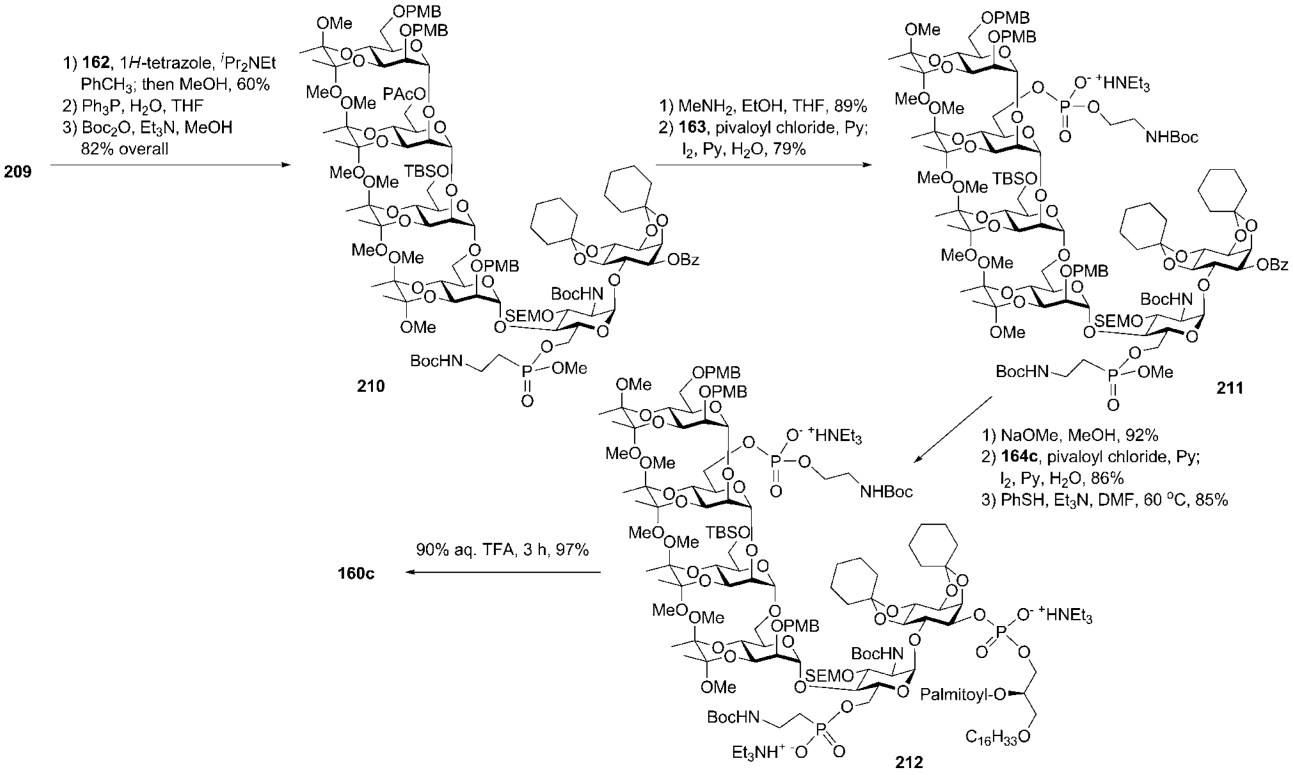

Scheme 40.

Successive phosphonylation and phosphorylation steps and global deprotection (the Nikolaev group; synthetic strategy C).

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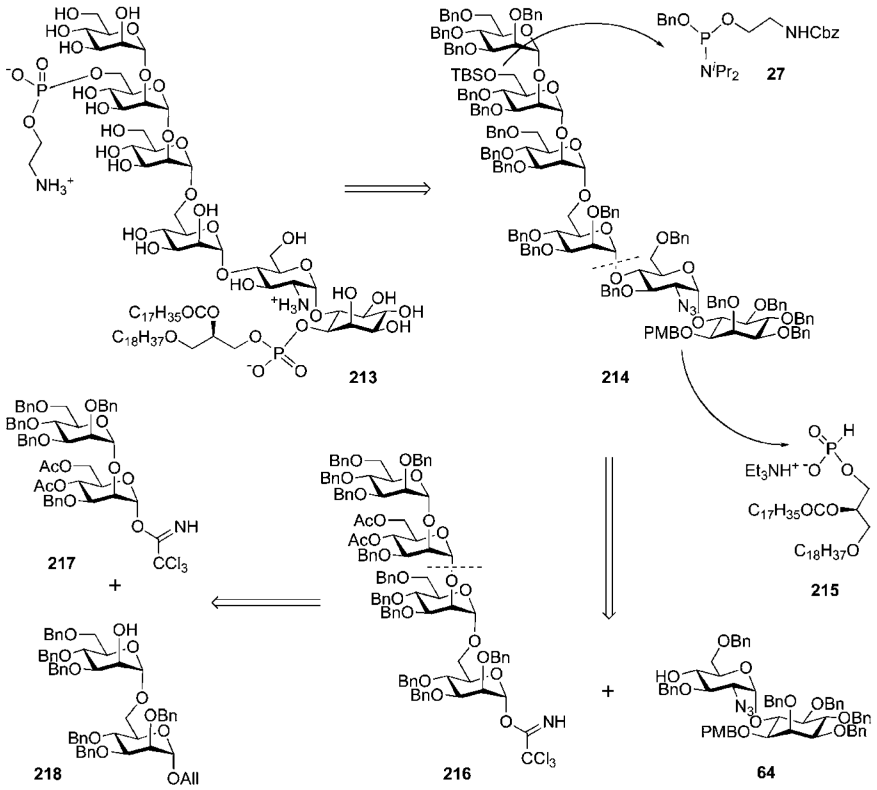

Scheme 41.

Retrosynthesis of a GPI anchor of *T. cruzi* (the Vishwakarma group).

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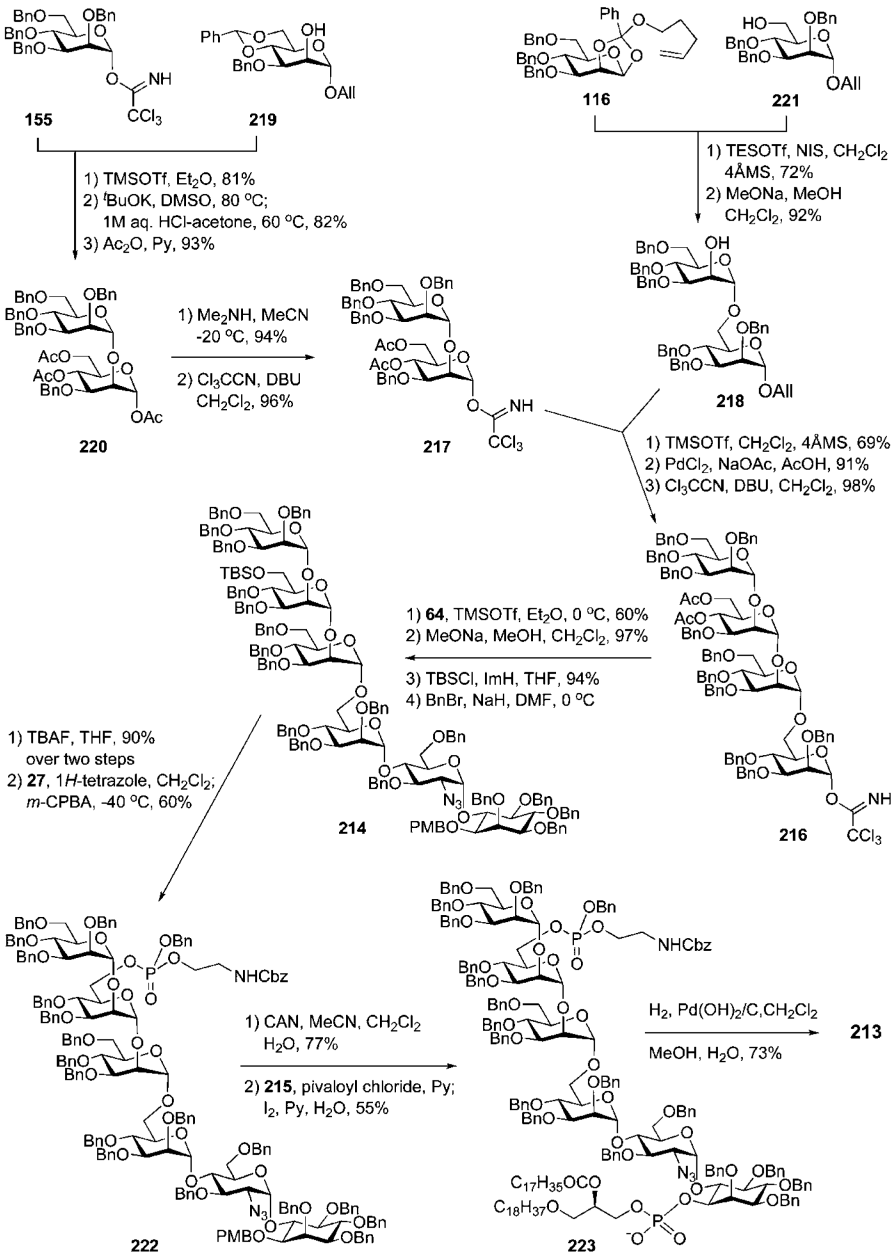

Scheme 42.

GPI glycan core assembly, successive phosphorylation steps and global deprotection (the Vishwakarma group).

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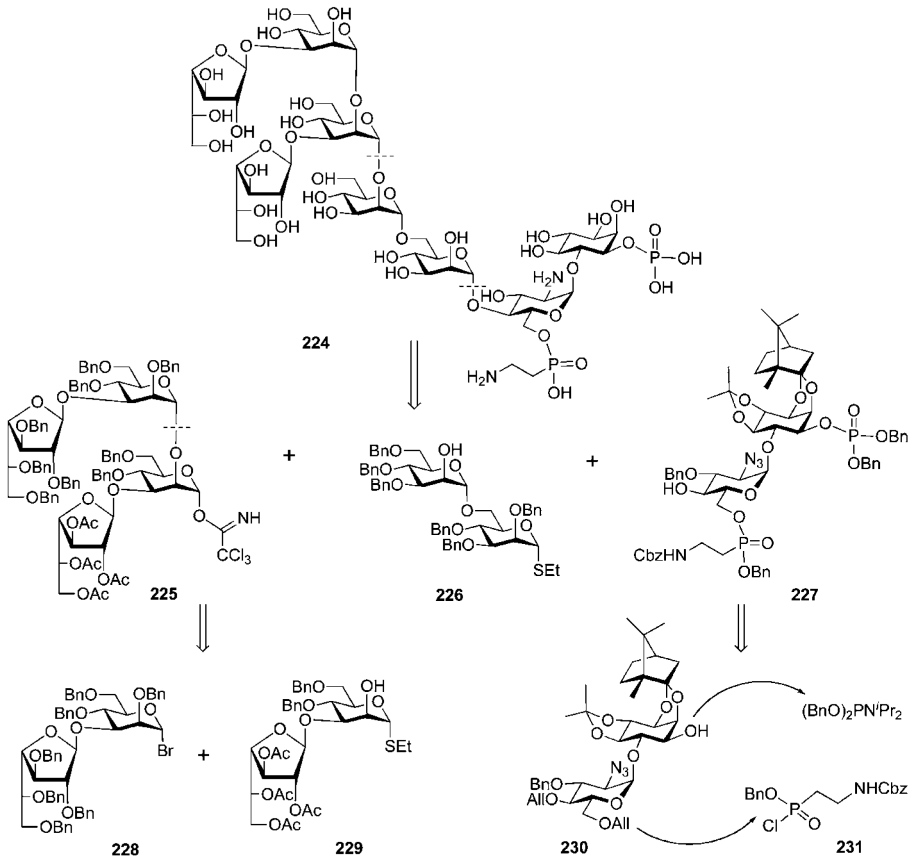
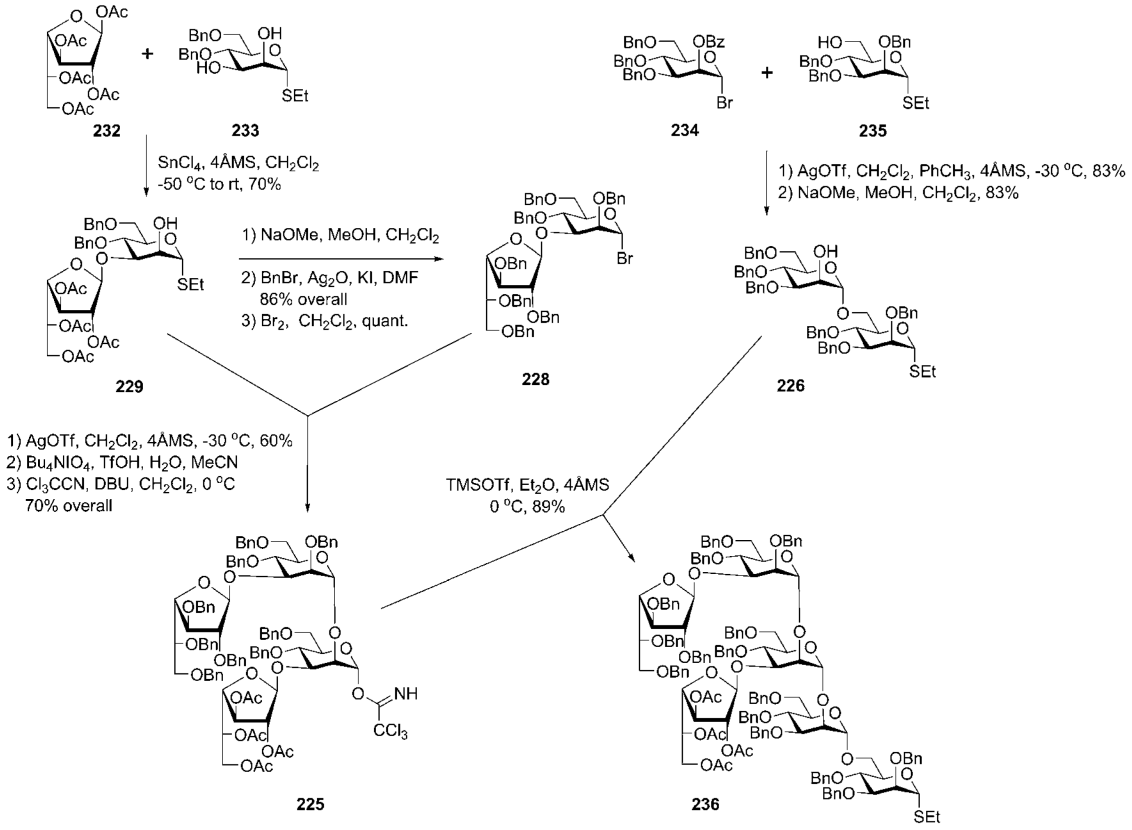


Scheme 43.

Retrosynthesis of a GPI-related glycoconjugate of *T. cruzi* (the Konradsson group).



Scheme 44.

Synthesis of mono- and disaccharide building blocks and assembly of a branched hexasaccharide (the Konradsson group).

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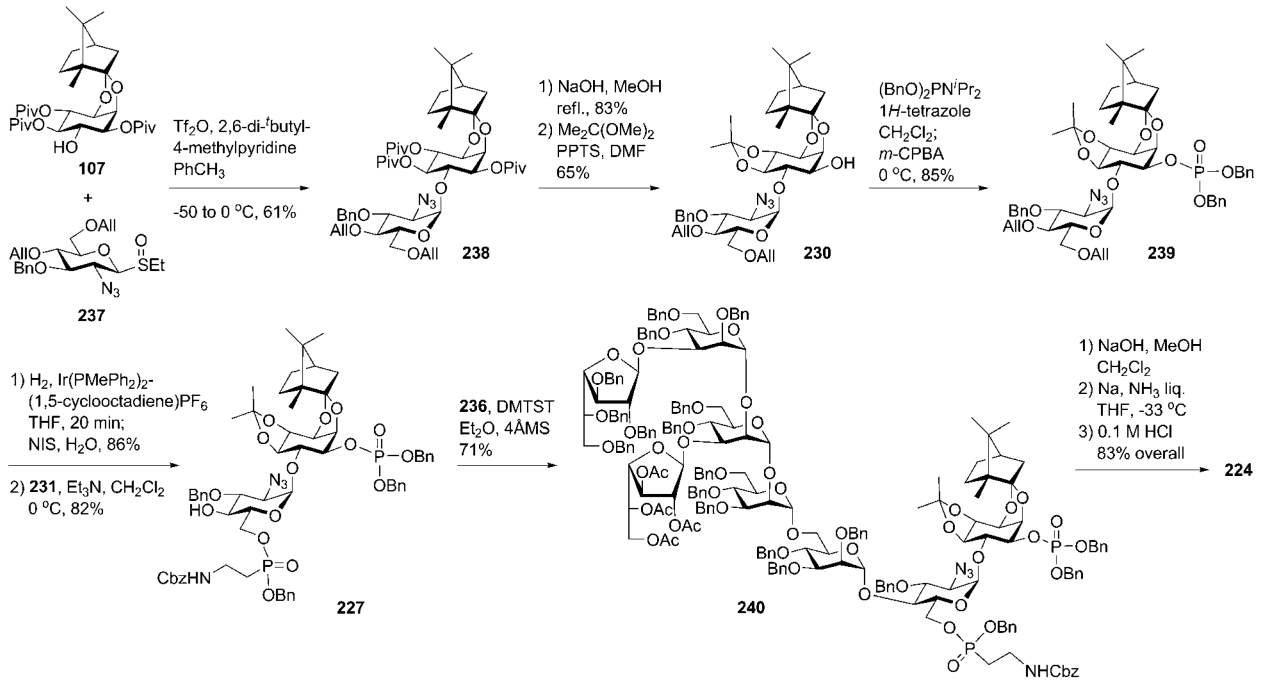

Scheme 45.

GPI glycan core assembly (including successive phosphorylation and phosphonylation steps) and global deprotection (the Konradsson group).

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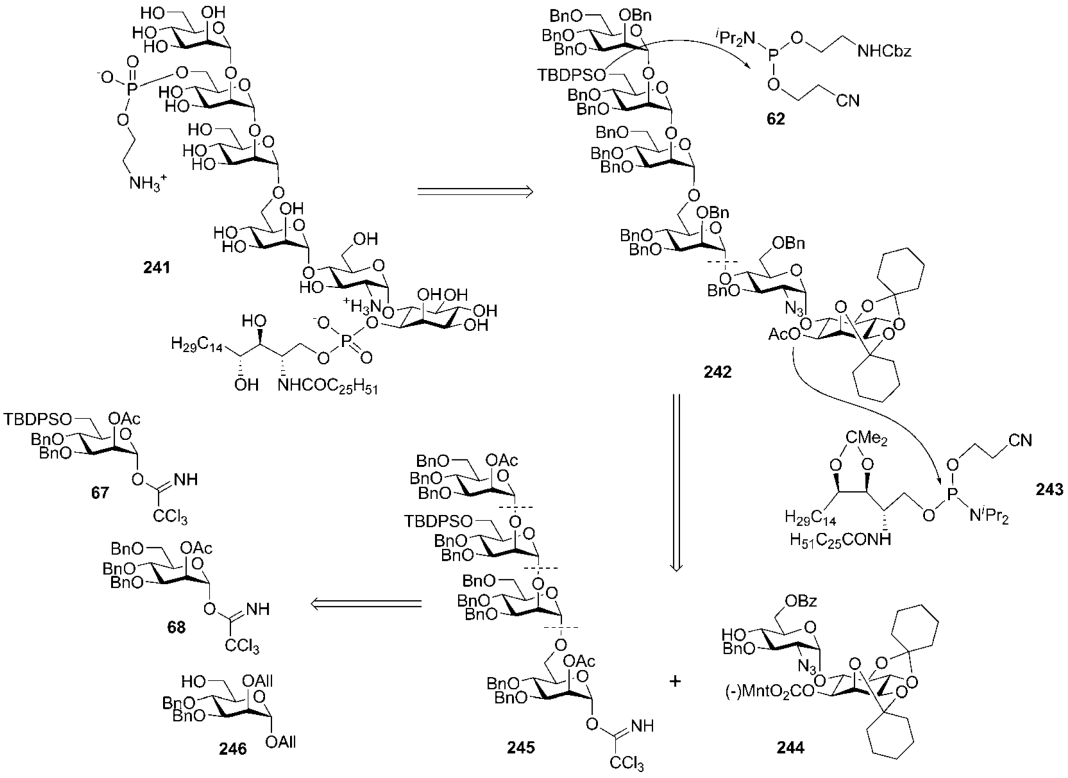
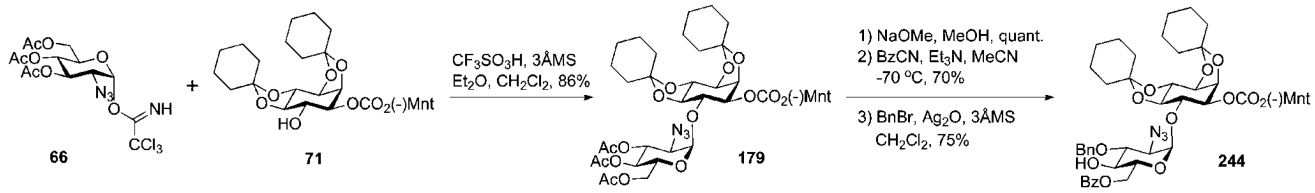


Scheme 46.

Retrosynthesis of a GPI anchor of the yeast *Saccharomyces cerevisiae* (the Schmidt group).



Scheme 47.

The synthesis of a pseudodisaccharide derivative (the Schmidt group).

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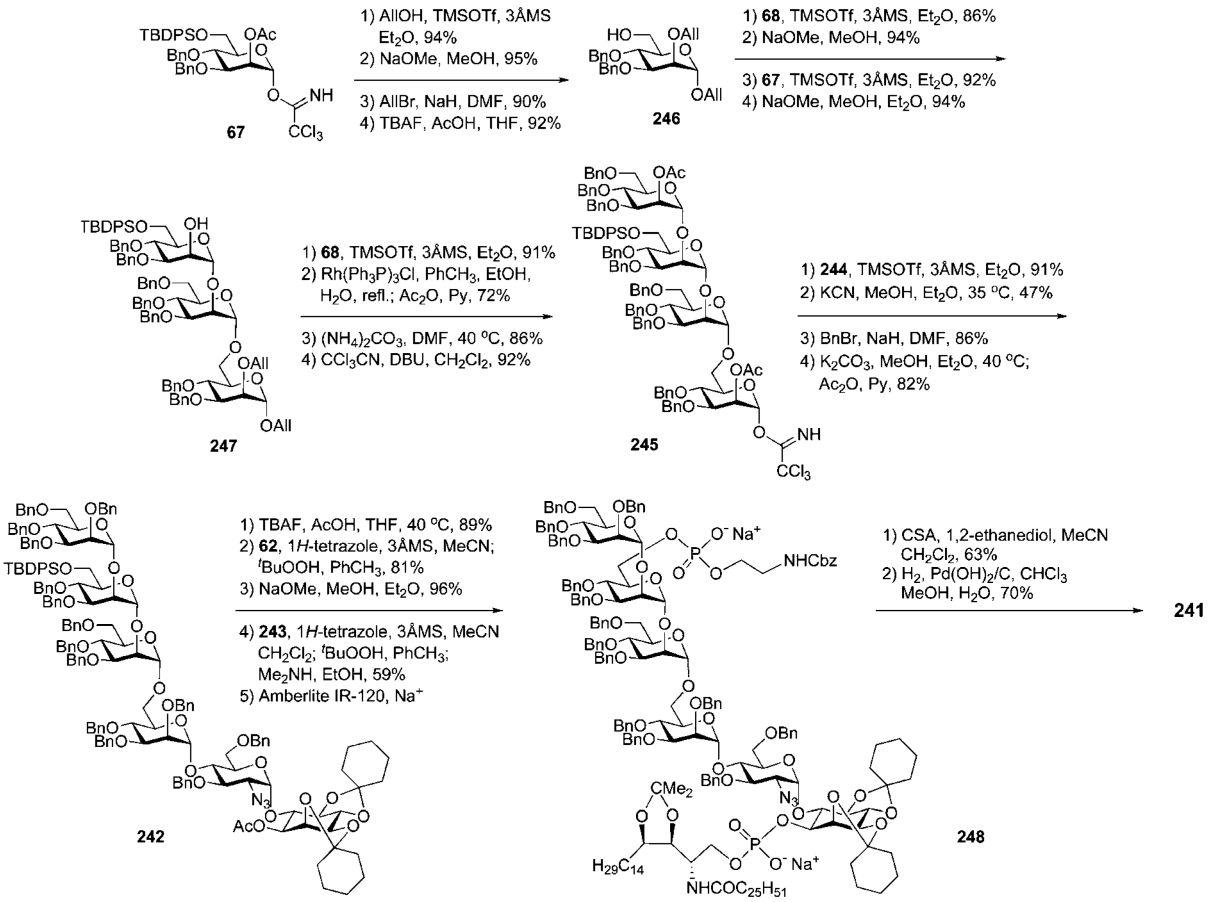

Scheme 48.

GPI glycan core assembly, successive phosphorylation steps and global deprotection (the Schmidt group).

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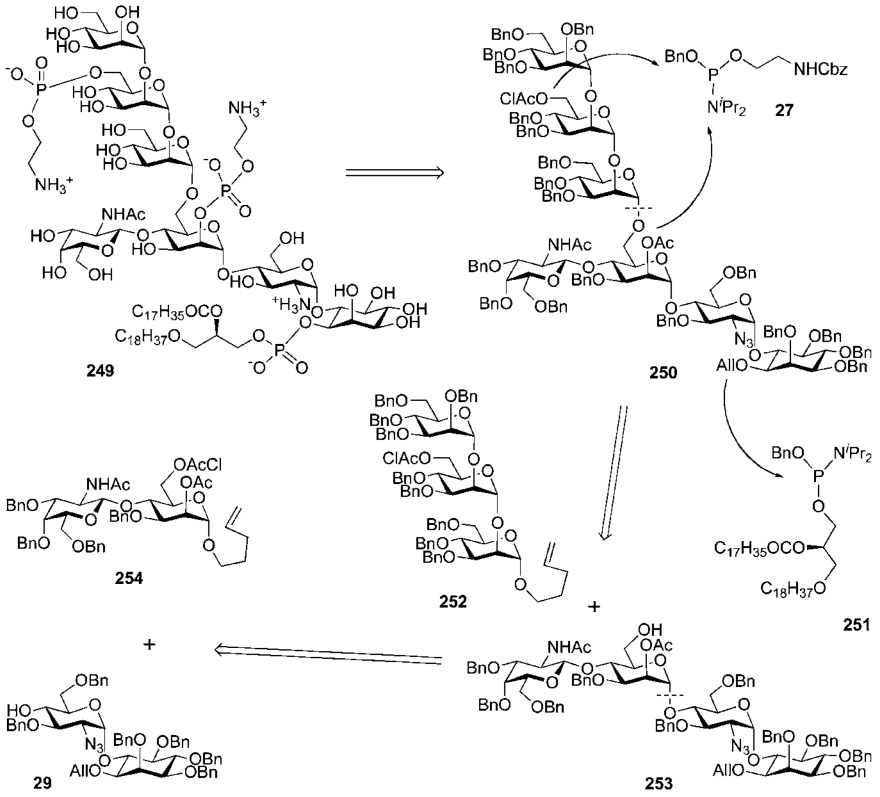
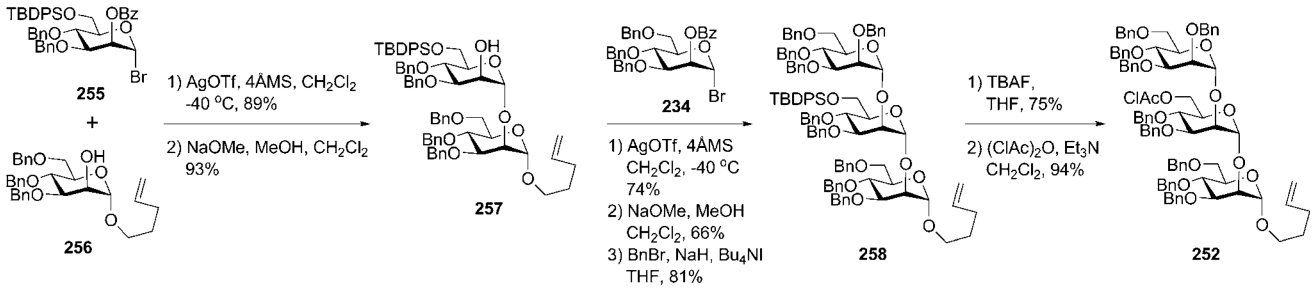


Scheme 49.

Retrosynthesis of a GPI anchor of rat brain Thy-1 glycoprotein (the Fraser-Reid group).



Scheme 50.

Synthesis of a linear trimannoside (the Fraser-Reid group).

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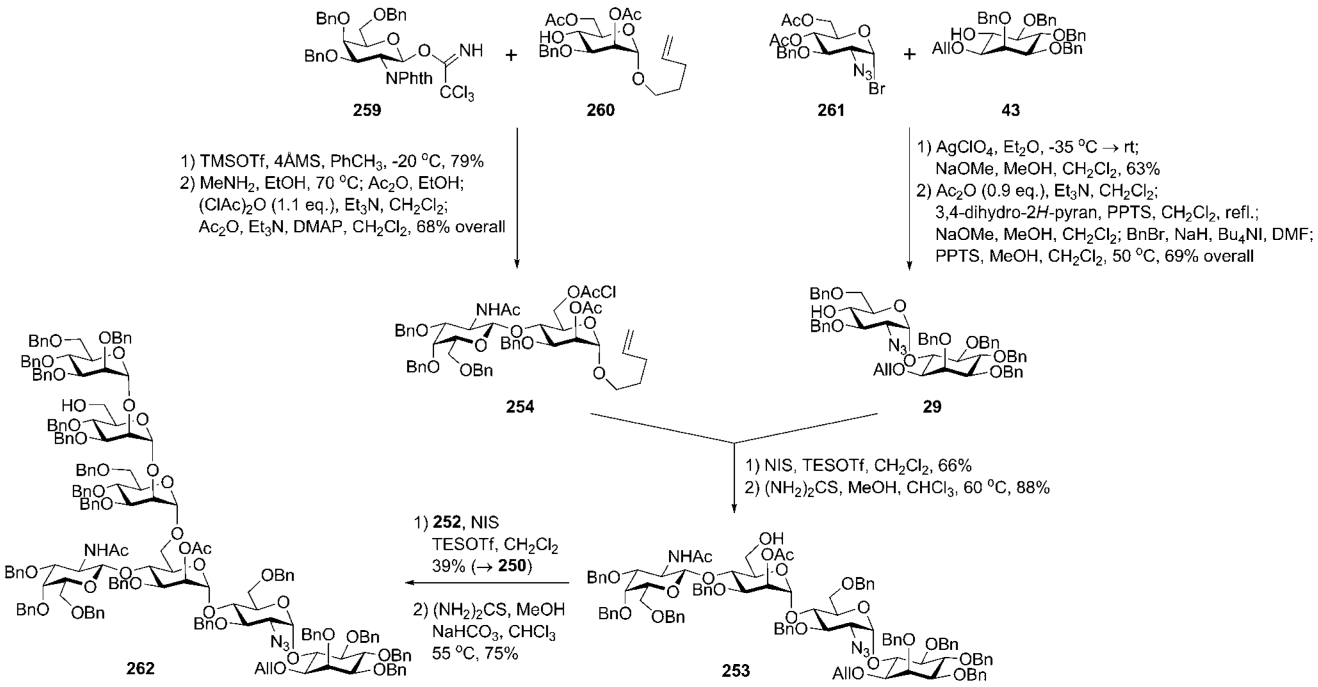

Scheme 51.

GPI branched glycan core assembly (the Fraser-Reid group).

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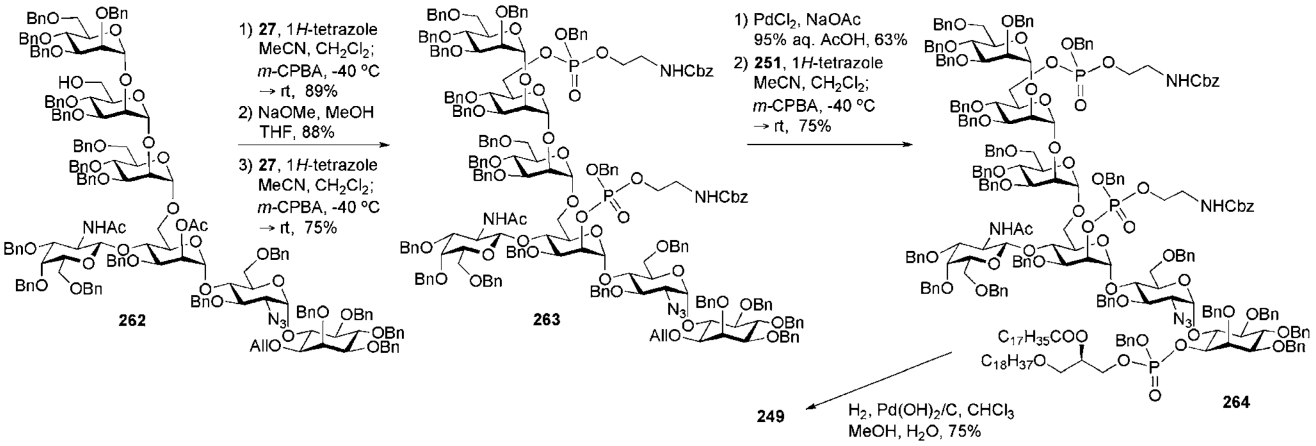

Scheme 52.

Successive phosphorylation steps and global deprotection (the Fraser-Reid group).

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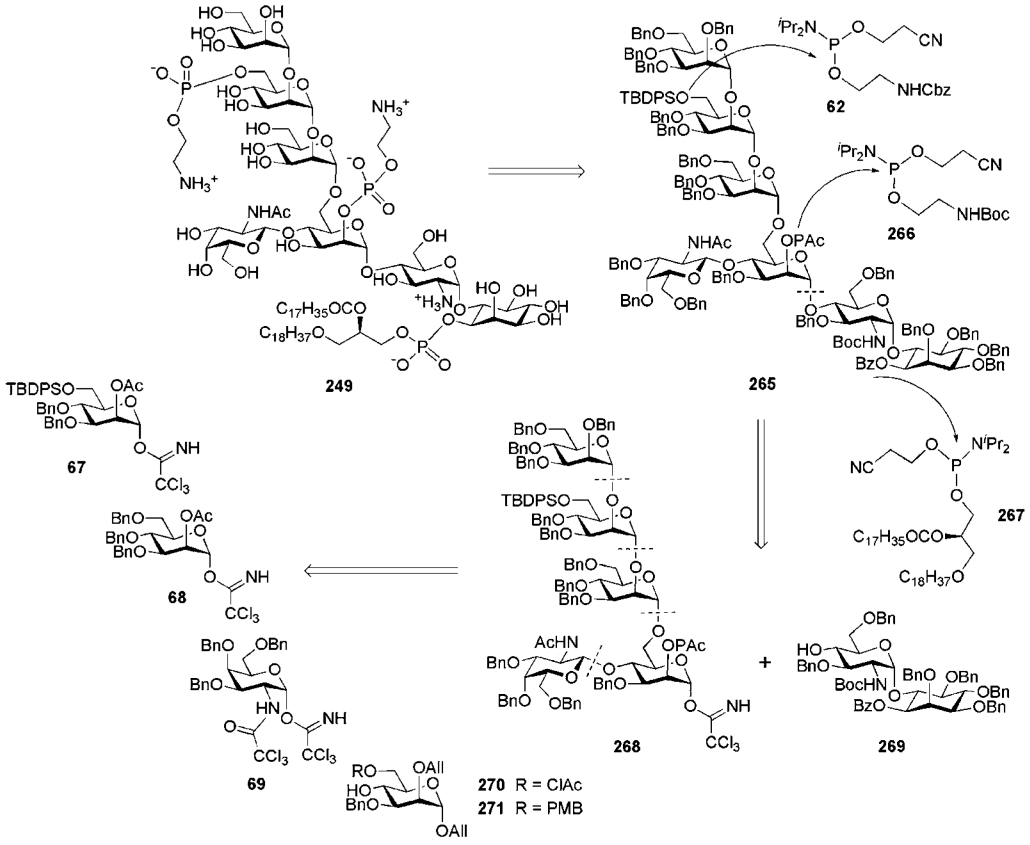

Scheme 53.
Retrosynthesis of a GPI anchor of rat brain Thy-1 glycoprotein (the Schmidt group).

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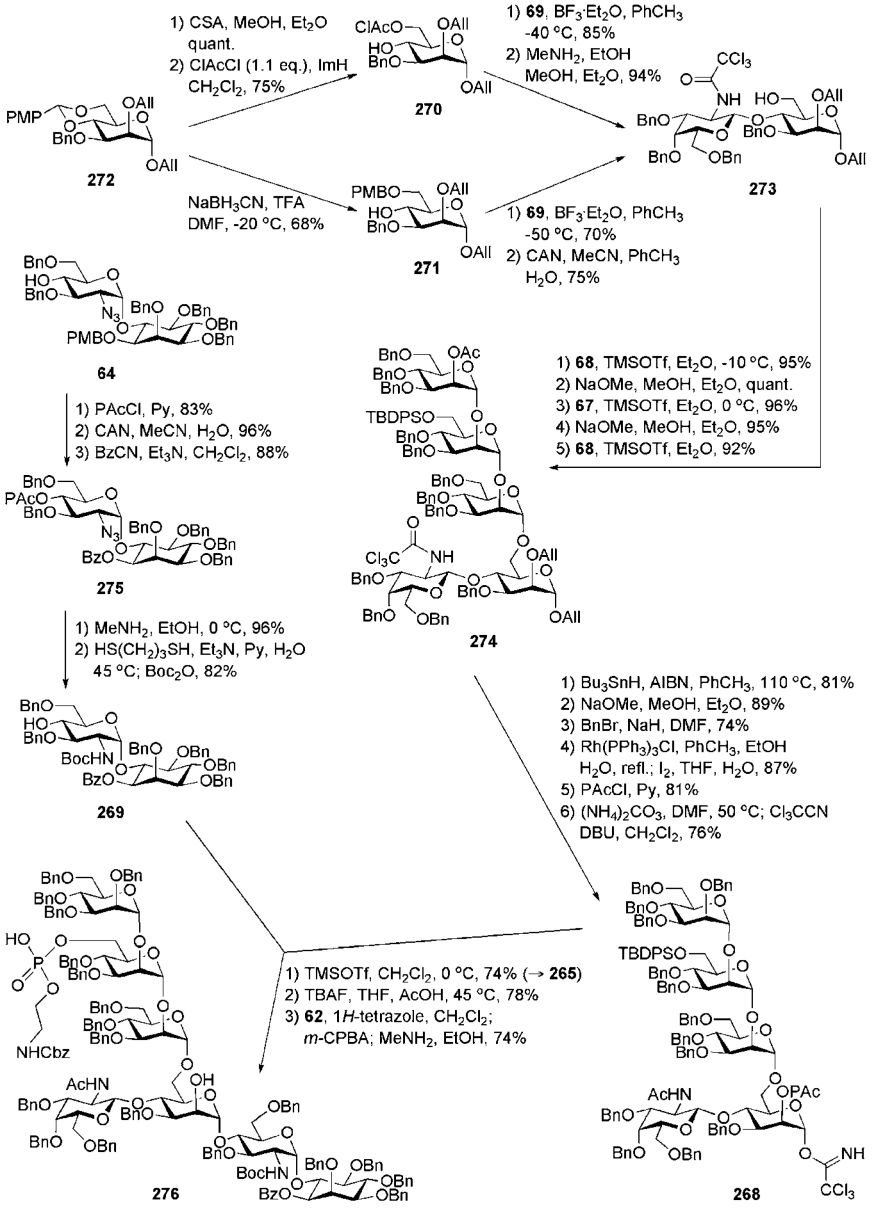

Scheme 54.

GPI branched glycan core assembly and the first phosphorylation step (the Schmidt group).

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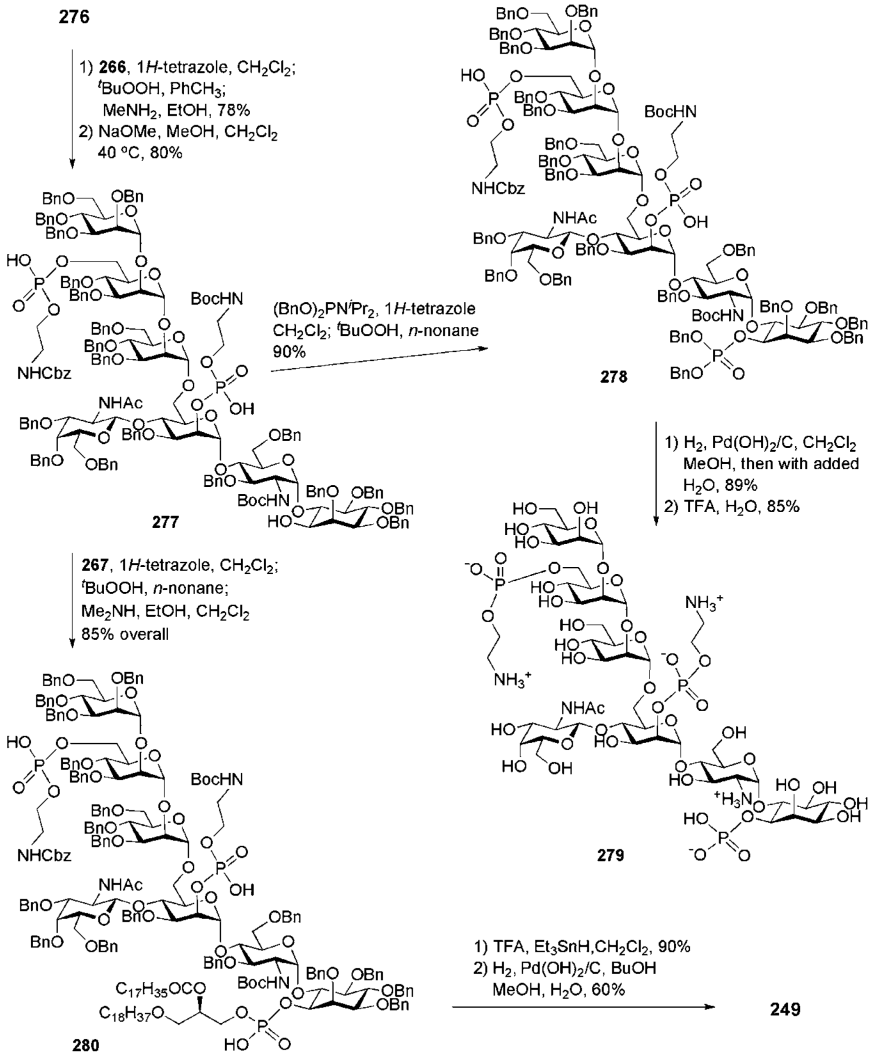

Scheme 55.

The final phosphorylation steps and global deprotection (the Schmidt group).

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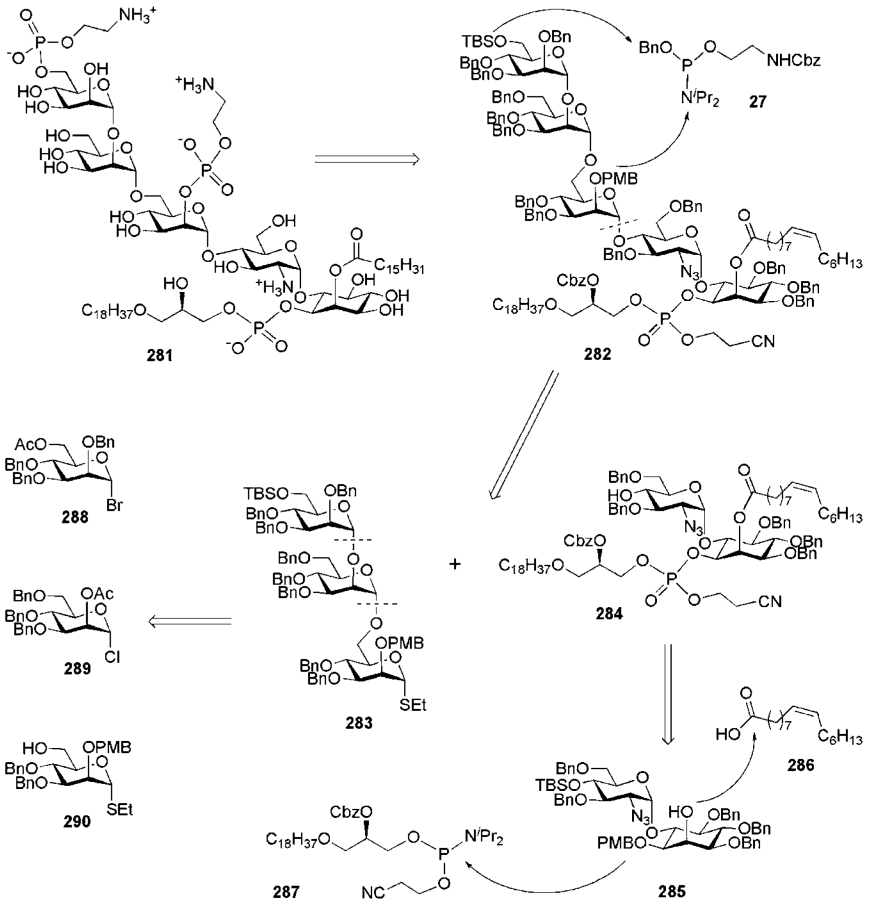

Scheme 56.

Retrosynthesis of the CD52 antigen GPI anchor containing three phosphate residues (the Guo group).

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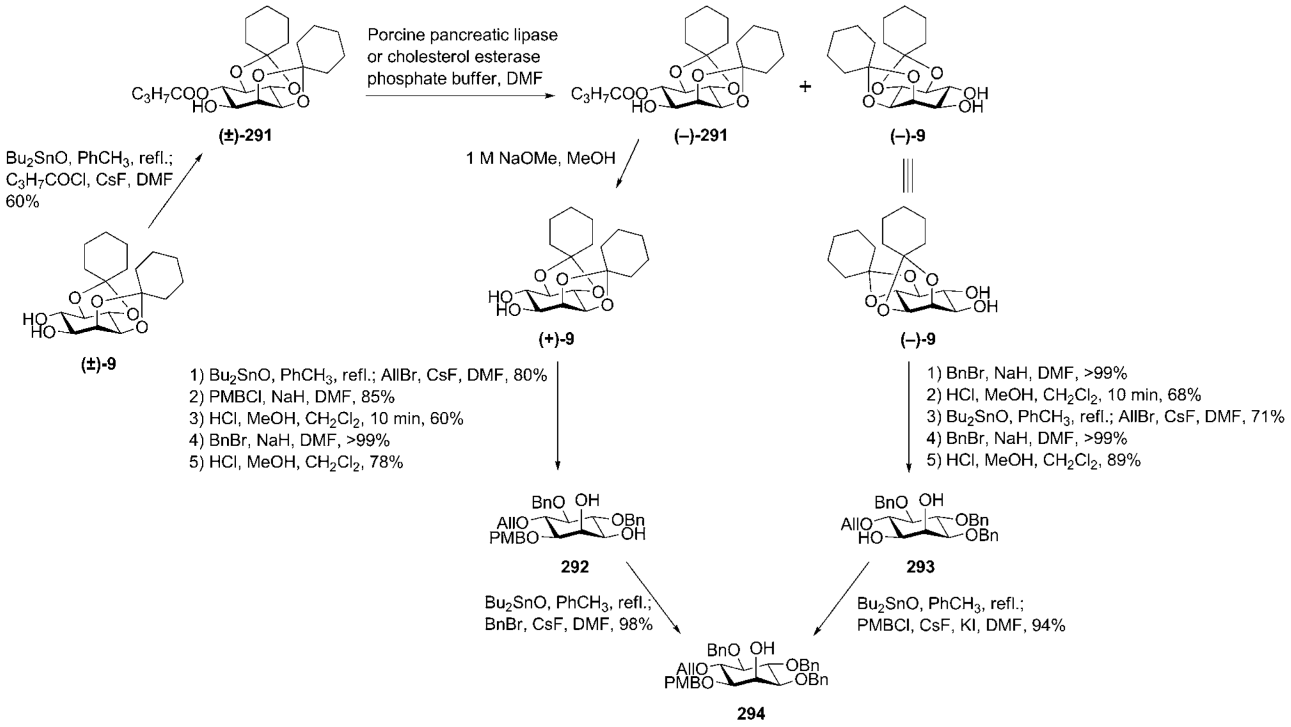

Scheme 57.

myo-Inositol enantiomeric resolution (the Guo group).

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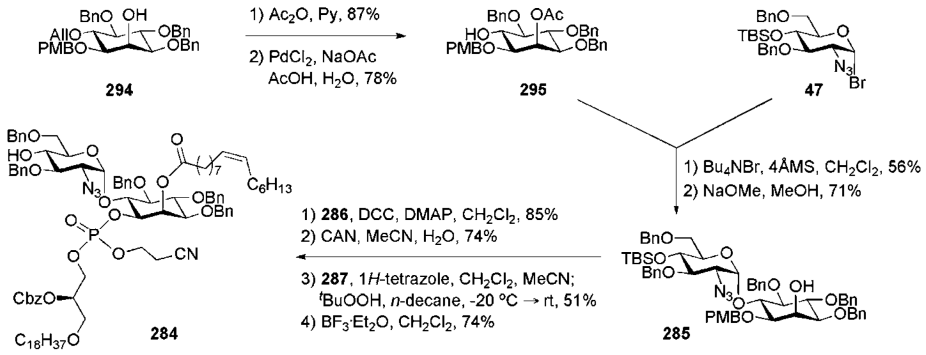
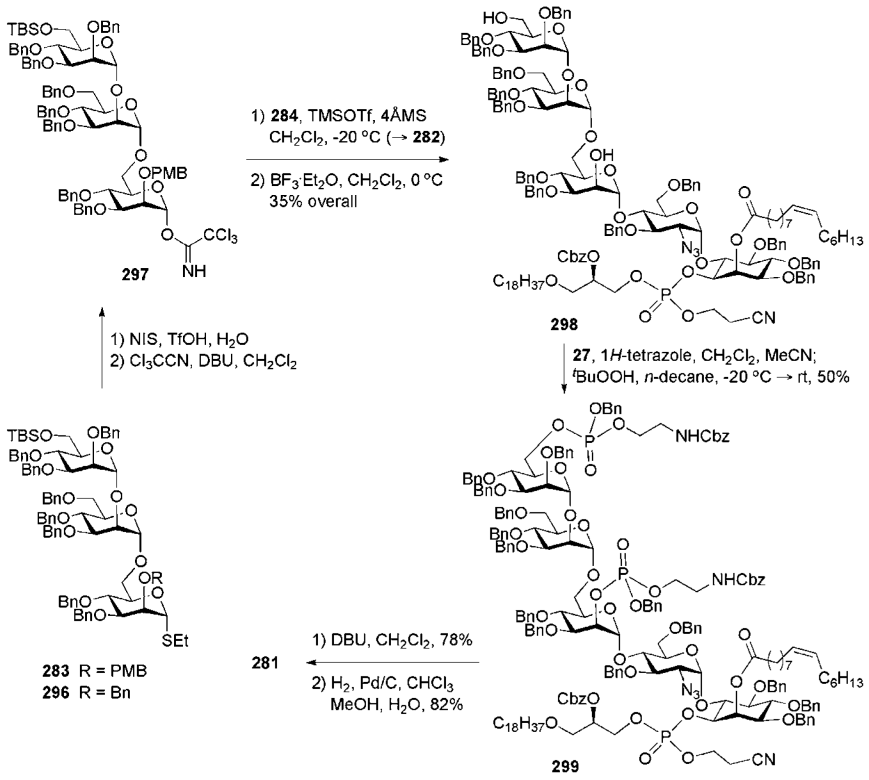


Scheme 58.

The synthesis of a pseudodisaccharide derivative and successive lipidation and phosphorylation steps (the Guo group).



Scheme 59.

GPI glycan core assembly, the final double phosphorylation step and global deprotection (the Guo group).

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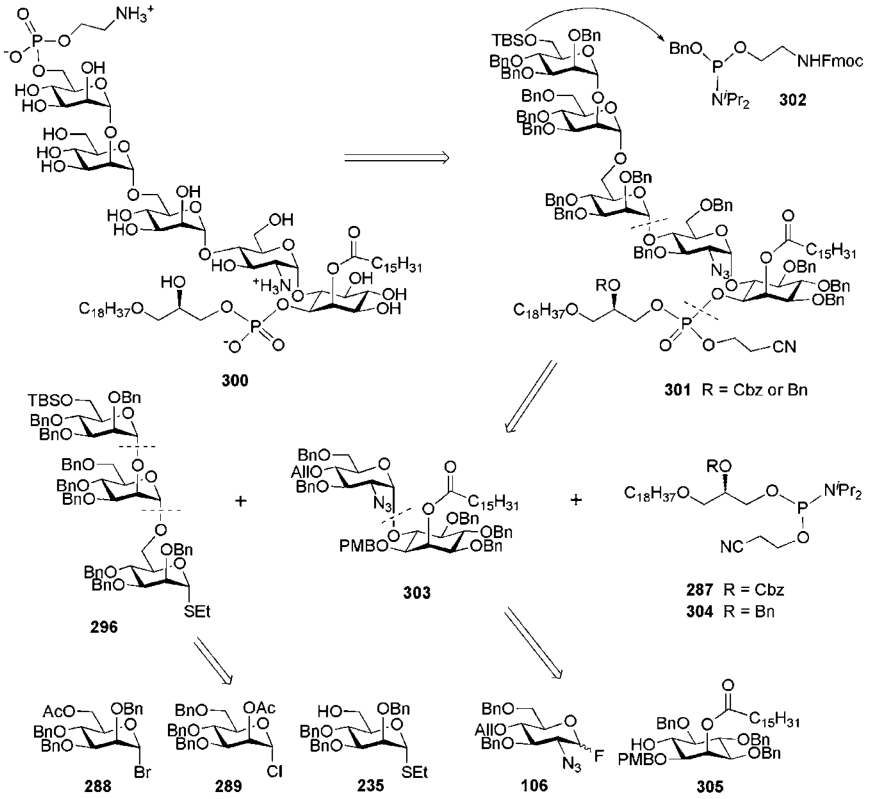

Scheme 60.

Retrosynthesis of the CD52 antigen GPI anchor containing two phosphate residues (the Guo group).

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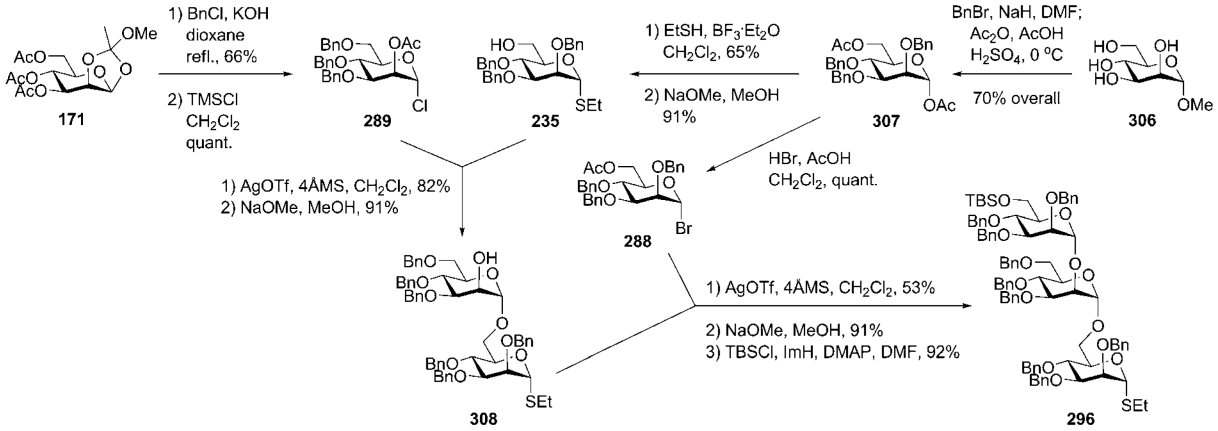

Scheme 61.

The synthesis of a linear trimannoside (the Guo group).

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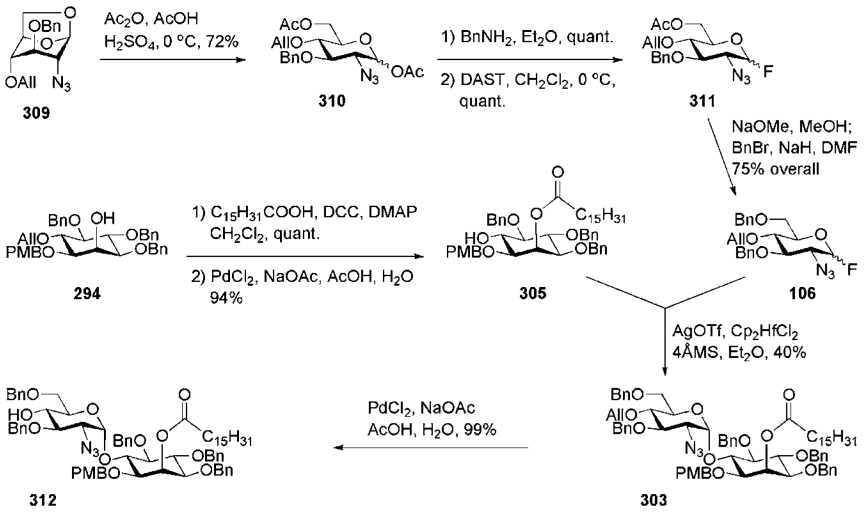

Scheme 62.

The synthesis of lipidated pseudodisaccharide derivative including prior lipidation of *myo*-inositol (the Guo group).

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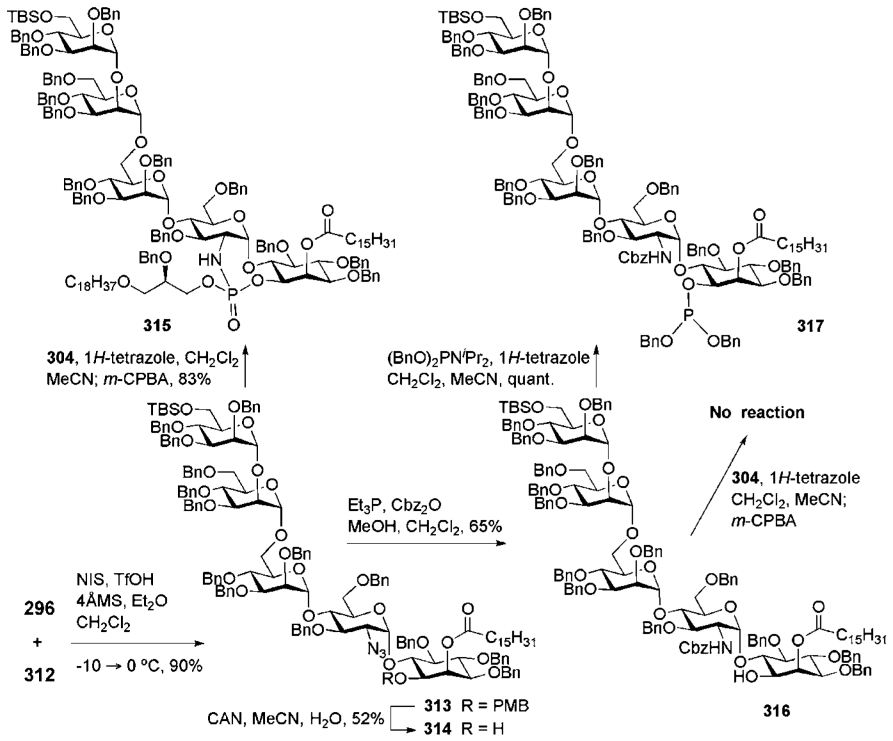

Scheme 63.

GPI glycan core assembly and attempted phosphorylation steps (the Guo group).

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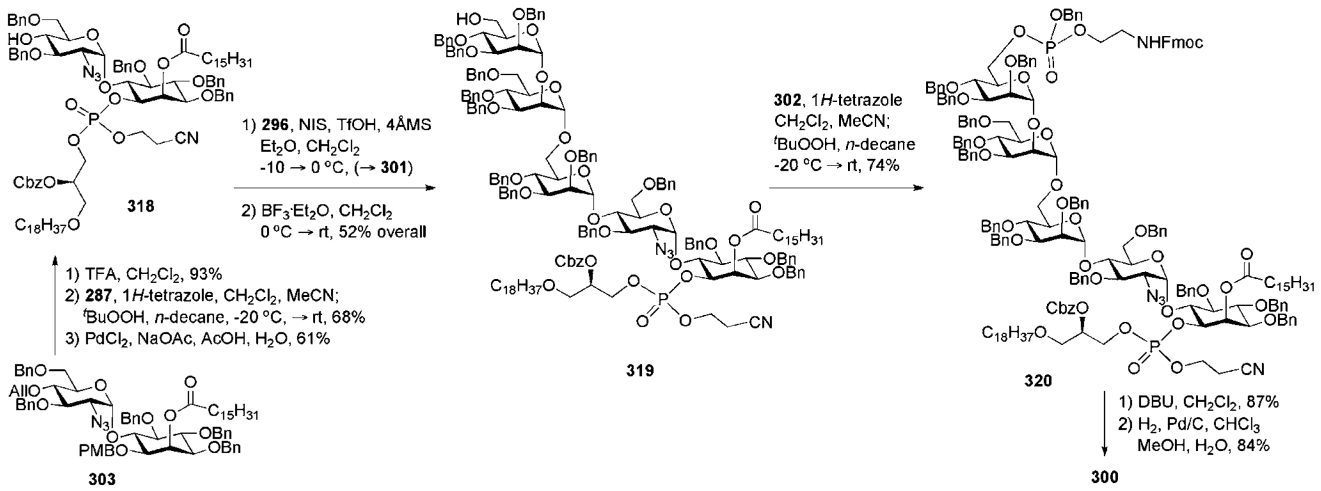

Scheme 64.

The synthesis of lipidated and phosphorylated pseudodisaccharide derivative, GPI glycan core assembly, the final phosphorylation step and global deprotection (the Guo group).

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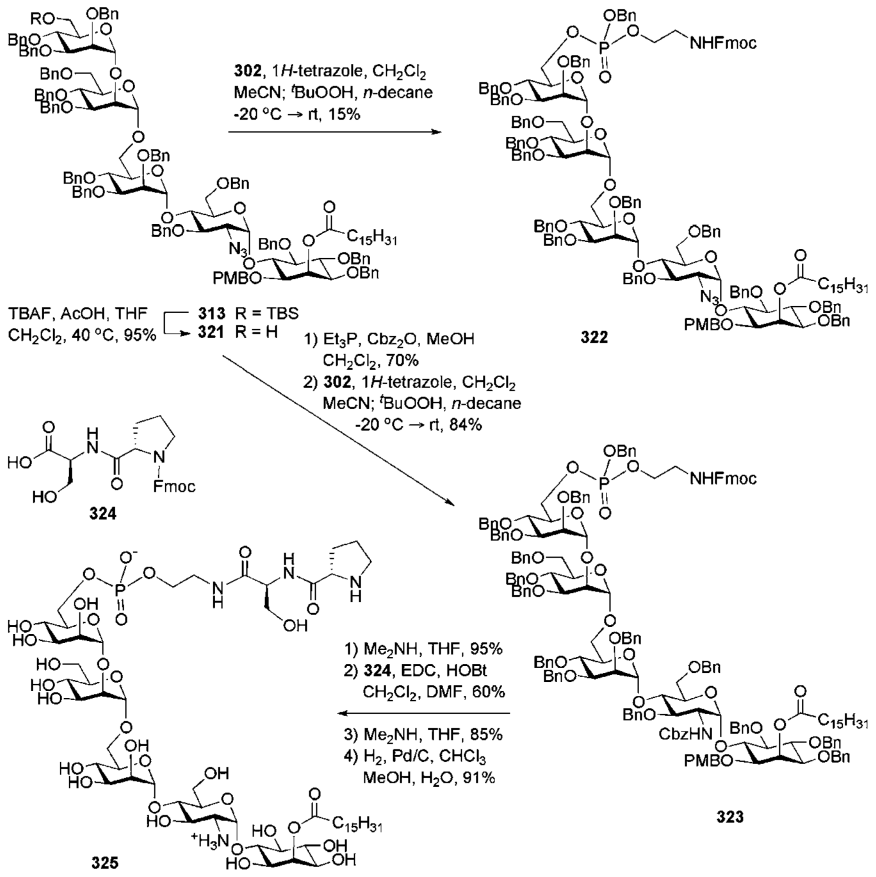

Scheme 65.

The synthesis of a GPI core-anchored dipeptide conjugate (the Guo group).

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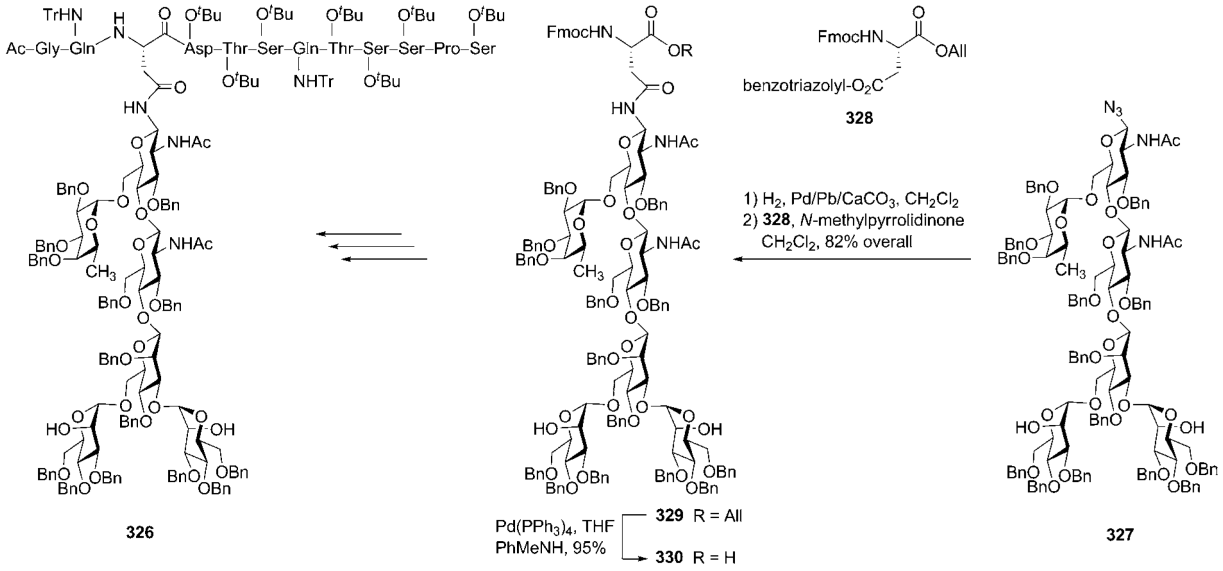

Scheme 66.

Synthesis of the CD52 specific *N*-linked glycopeptide conjugate (the Guo group).

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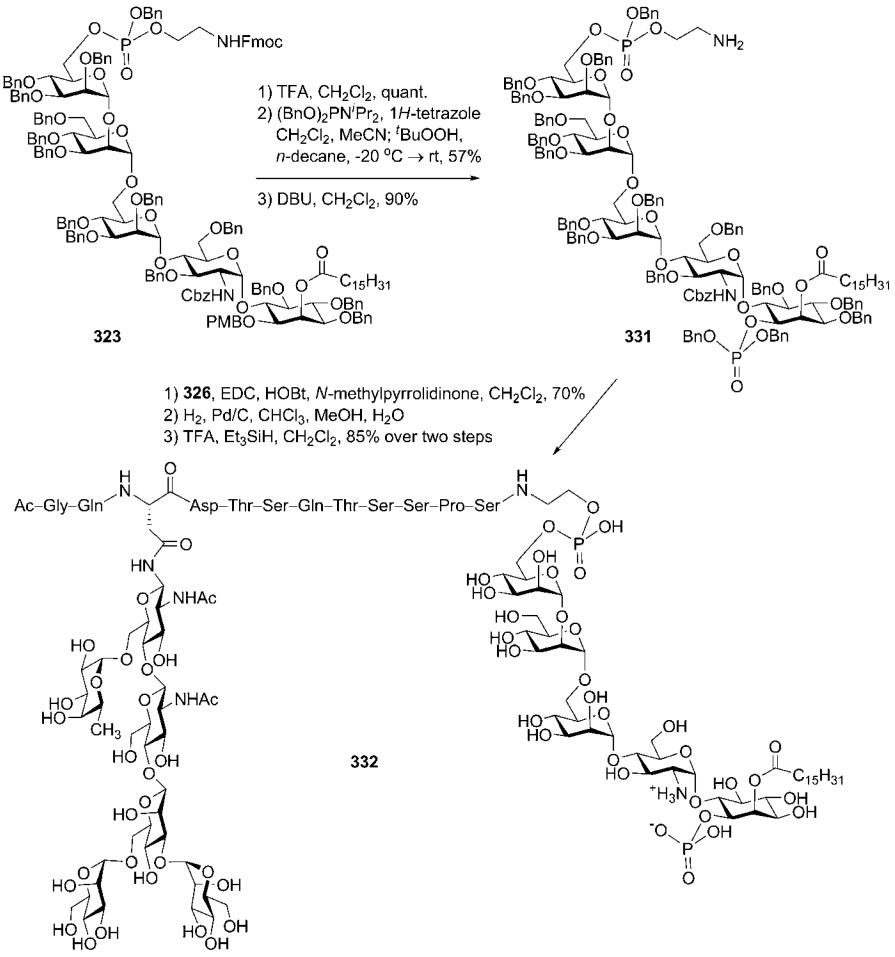

Scheme 67.

Synthesis of the CD52 antigen GPI core-anchored glycopeptide conjugate (the Guo group).

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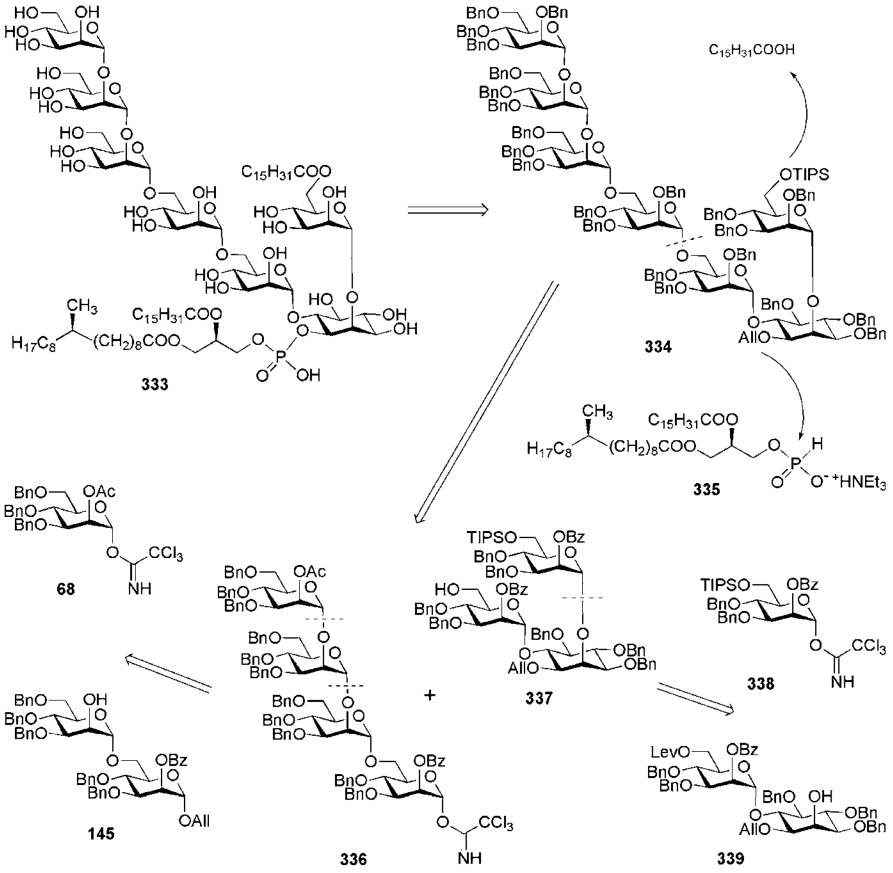

Scheme 68.

Retrosynthesis of a phosphatidylinositol mannoside (PIM_6) of *M. tuberculosis* (the Seeberger group).

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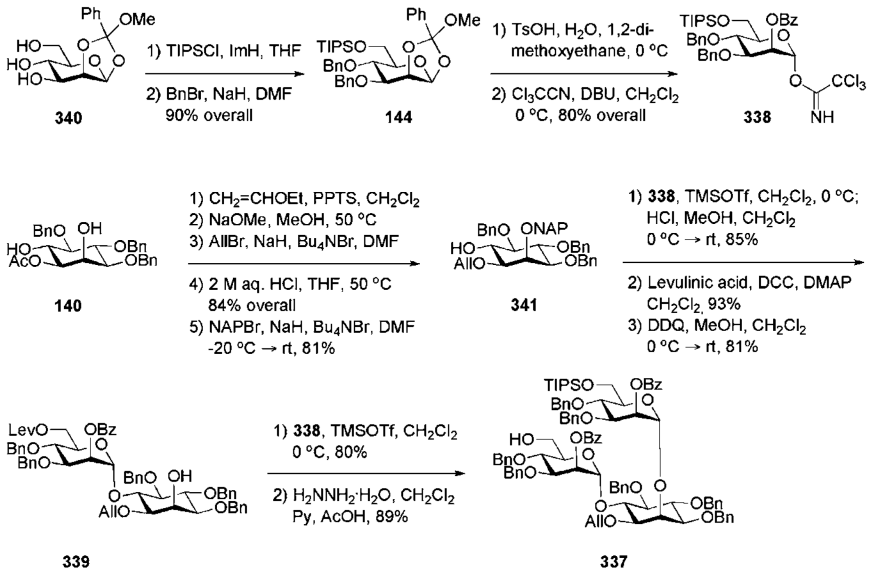

Scheme 69.

The synthesis of a branched pseudotrisaccharide derivative (the Seeberger group).

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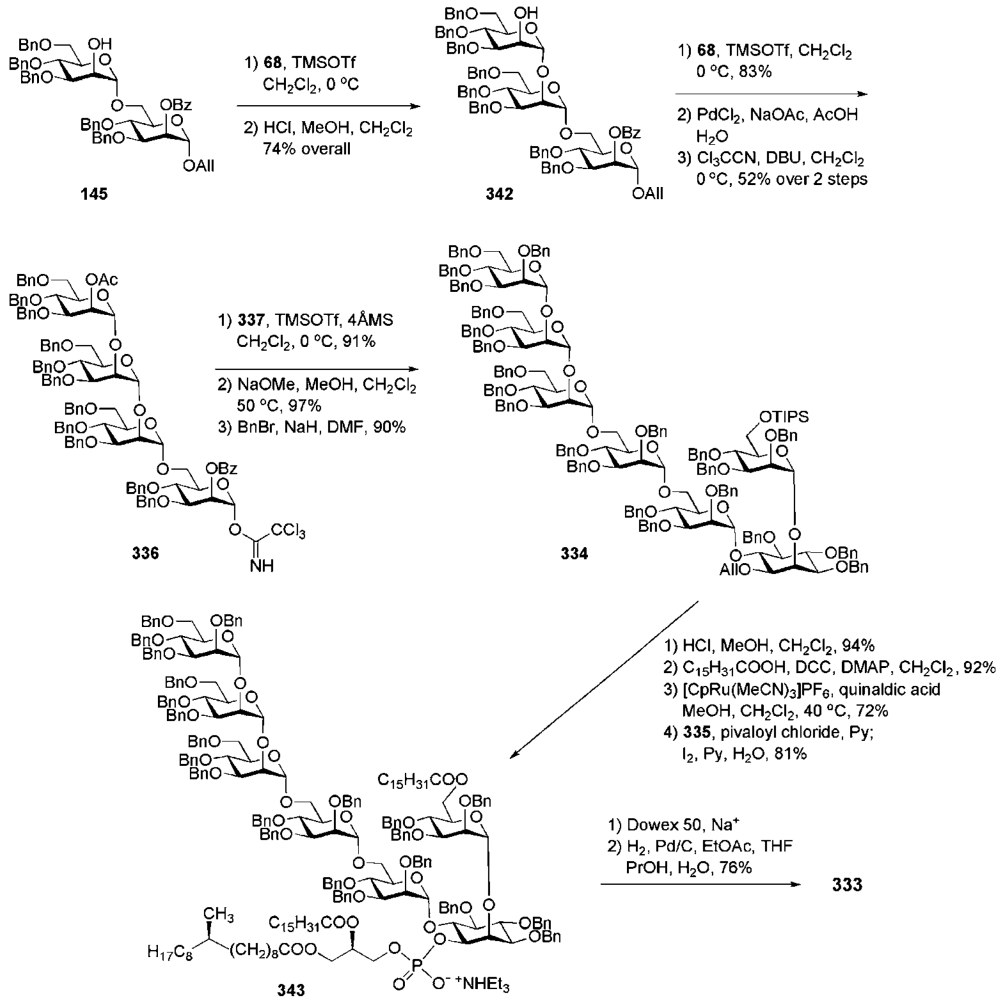

Scheme 70.

PIM_6 glycan core assembly, successive lipidation and phosphorylation steps and global deprotection (the Seeberger group).

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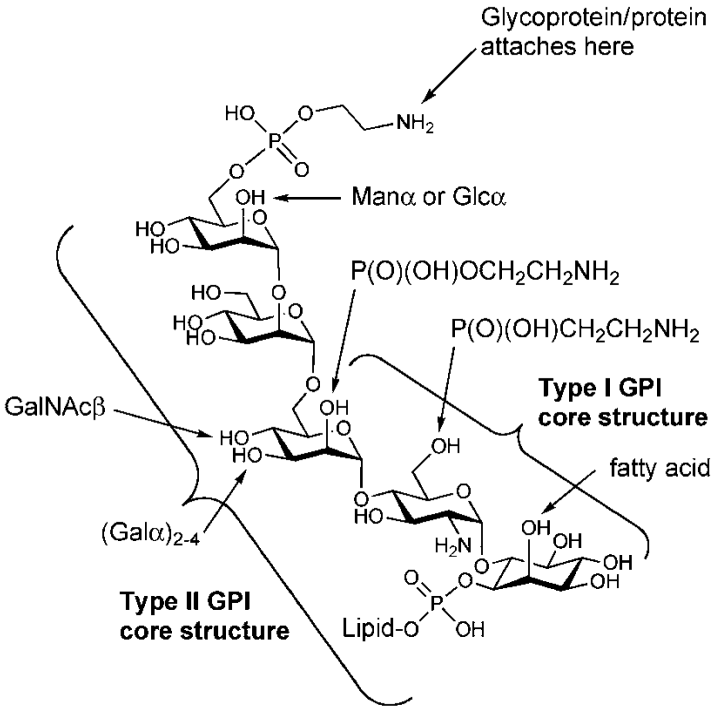

Fig. 1.
Structure of glycosylphosphatidylinositol (GPI) anchors.