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CHEMICAL SYNTHESIS OF GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORS

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Abstract

Many eukaryotic cell-surface proteins and glycoproteins are anchored to the plasma membrane by glycosylphosphatidylinositols (GPIs), a family of glycolipids that are post-translationally attached to proteins at their C-termini. GPIs and GPI-anchored proteins play important roles in many biological and pathological events, such as cell recognition and adhesion, signal transduction, host defense, and acting as receptors for viruses and toxins. Chemical synthesis of structurally defined GPI anchors and GPI derivatives is a necessary step toward understanding the properties and functions of these molecules in biological systems and exploring their potential therapeutic applications. In the first part of this comprehensive article on the chemical synthesis of GPIs, classic syntheses of naturally occurring GPI anchors from protozoan parasites, yeast, and mammals are covered. The second part of the article focuses on recent diversity-oriented strategies for the synthesis of GPI anchors containing unsaturated lipids, “click chemistry” tags, and highly branched and modified structures.

Keywords

carbohydrate; inositol; glycolipid; glycosylphosphatidylinositol (GPI) anchor; synthesis

I. Introduction

The attachment of membrane proteins and glycoproteins to the cell surface by glycosylphosphatidylinositol (GPI) anchors, a structurally diverse class of glycolipids, is ubiquitous in eukaryotic species.^{1–4} To date, more than 250 eukaryotic membrane proteins have been established as GPI-anchored,⁵ and genomic investigations have suggested that as many as 0.5–1% of all eukaryotic proteins may be anchored to the cell surface by GPIs.⁶ The GPIs and GPI-anchored molecules play vital roles in numerous biological and pathological processes, such as cell recognition and adhesion,⁷ signal transduction,^{8,9} pathogenic infections,^{10,11} enzymatic reactions on the cell surface,¹² and functioning as cellular markers.⁴

The discovery of GPI anchorage as a unique mode of membrane-protein binding spanned the 1970s and 1980s¹ and culminated in the full characterization of the *Trypanosomabrucei* variant surface glycoprotein GPI anchor in 1988 by Ferguson and co-workers.¹³ Since then, numerous GPIs have been identified and characterized, and a highly conserved core structure has been established: H₂N-(CH₂)₂-(P)-6- α -Man-(1 $\rightarrow$ 2)- α -Man-(1 $\rightarrow$ 6)- α -Man-(1 $\rightarrow$ 4)- α -GlcN-(1 $\rightarrow$ 6)-*myo*-inositol-1-(P)-glycerolipid (Fig. 1). The protein C-terminus is covalently linked to the non-reducing end of the GPI core glycan through a phosphoethanolamine bridge, and the entire structure is anchored to the cell surface by insertion of the phosphatidylinositol (PI) fatty acid chains into the membrane bilayer. The tetrasaccharide core, whose function remains obscure, exhibits considerable structural diversity amongst known GPIs, primarily in the form of additional carbohydrate and phosphoethanolamine units linked to various positions. For example, mannose and galactose

mono/oligomers are commonly appended to the O-2 position of Man-III and the O-3/4 positions of Man-I, respectively, while the O-2 position of Man-I frequently carries a phosphoethanolamine group. Additionally, the PI moiety may be palmitoylated (hexadecanoylated) at the inositol O-2 position, while its phosphoglycerolipid can undergo fatty acid remodeling,¹⁴ leading to modified lipids that can be acyl- or alkyl-linked or exhibit various chain lengths and unsaturation patterns.

Despite impressive advances in elucidating the biosynthesis, structure, and function of GPI anchors and GPI-anchored proteins,¹⁵ many aspects of these molecules remain poorly understood. For example, the significance of structural complexity and diversity of GPI anchors, particularly in the conserved glycan core, is essentially unexplained and prompts studies on structure–activity relationships in various contexts. The use of GPI partial structures in the development of antimalarial vaccines^{16,17} suggests GPIs have therapeutic value, but new technologies are needed to explore their potential more fully. Imaging of GPI anchors *in vivo* could provide insight into their expression, distribution, and endocytosis.¹⁸ Another emerging area of research is “GPIomics,” which seeks to develop proteomic tools for identifying proteins carrying the GPI anchor as a post-translational modification.¹⁹

To accelerate progress in these and other areas of GPI research, homogeneous and structurally defined GPIs and GPI derivatives must be accessible in sufficient purity and quantity, a requirement that can be addressed by chemical synthesis. GPIs are among the most complex of natural products, as they incorporate carbohydrate, lipid, phosphate, and inositol groups, the combination of which makes their synthesis very challenging. Difficult tasks in GPI synthesis include the preparation of enantiomerically pure and properly discriminated inositol derivatives, stereoselective formation of glycosidic bonds, and regiocontrolled introduction of side chains. A general convergent approach for GPI assembly is shown in Scheme 1, where the target GPI anchor **I** is typically accessed from a fully protected intermediate **II** bearing orthogonal protecting groups at sites for late-stage introduction of the phosphoglycerolipid and phosphoethanolamine groups. In most cases, structure **II** is disconnected at the glucosamine–mannose glycosidic bond, leading back to trimannose and pseudodisaccharide fragments **III** and **IV**, which are in turn generated from their corresponding monomeric subunits.

In this survey, the field of GPI-anchor synthesis is covered chronologically, starting with the first synthesis of a GPI anchor by the Ogawa group in 1991. In the ensuing two decades, numerous impressive total syntheses of naturally occurring GPI anchors were completed by various research groups, and these works are discussed in the first section, “Classic Approaches to GPI Anchor Synthesis.” Subsequently, more effort has been focused on the development of novel synthetic strategies that have emphasized flexibility, which has enabled access to GPI anchors containing biologically important unsaturated lipids, versatile “click chemistry” tags, and branched structures. These syntheses are covered in the second section, “Diversity-Oriented Approaches to GPI Anchor Synthesis.” It is expected that this article will have some overlap with previous reviews on the subject,^{20–22} but will offer coverage of new material as well as a contemporary and unique perspective.

To keep this article within reasonable constraints, detailed discussion of research involving GPI partial structures has been omitted. Notable works in this category include syntheses of non-lipidated GPIs by Martín-Lomas²³ and Schmidt,²⁴ lipophosphoglycans (LPGs) by Konradsson and Oscarson,^{25,26} GPI-based antimalarial candidates by Seeberger,^{17,27,28} and GPI–peptide/glycopeptide constructs by Seeberger²⁹ and Guo.^{30,31} Also excluded from this discussion is recent work from the Bertozzi group using GFP-modified GPI partial structures to explore their behavior in live cells^{18,32} and work from the Guo laboratory focused on chemoenzymatic ligation of GPI analogues and proteins.^{33–35}

II. Classic Approaches To GPI Synthesis

1. Synthesis of a *T. brucei* GPI Anchor by the Ogawa Group (1991)

The Ogawa group reported the chemical synthesis of a *T. brucei* GPI anchor in 1991,^{36,37} three years after Ferguson and co-workers published its complete structural determination. Ogawa's reports, which were preceded by synthetic studies on GPI-related pseudooligosaccharides in the Fraser-Reid,³⁸ Ogawa,³⁹ and van Boom⁴⁰ laboratories, marked the first total synthesis of a GPI anchor. In accordance with the natural structure of the *T. brucei* GPI anchor, an additional α -Gal-(1 $\rightarrow$ 6)- α -Gal-(1 $\rightarrow$ 3) component located at Man-I was incorporated into the GPI core structure.

Scheme 2 depicts the retrosynthesis of *T. brucei* GPI anchor **1**. While most modern GPI syntheses involve a convergent assembly featuring formation of the mannose–glucosamine linkage as the key and final glycosidic-bond formation (see Scheme 1), Ogawa's pioneering effort relied on a different approach. The assembly strategy had both linear and convergent components, as benzyl-protected GPI precursor **2**, which contained orthogonal protection for late-stage phosphorylation with *H*-phosphonates **3** and **4**, was disconnected to give digalactosyl fluoride **5**, mannosyl halide donors **6** and **7**, and pseudotrisaccharide **8**. The latter compound was further disconnected into the monosaccharide and inositol building blocks **9–11**. For stereoselective glycosylation reactions, the Ogawa group primarily employed the Suzuki method, using glycosyl fluoride donors,⁴¹ while also making use of Lemieux's halide ion-catalyzed glycosylation⁴² and the classical Koenigs–Knorr reaction.⁴³ The *H*-phosphonate method⁴⁴ was used to introduce the phosphoglycerolipid and phosphoethanolamine groups.

The preparation of digalactosyl fluoride **5** is shown in Scheme 3. Penta-*O*-acetyl- α -D-galactopyranose (**12**) underwent treatment with *p*-methoxyphenol in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloroethane (DCE) to give a *p*-methoxyphenyl (PMP) glycoside, which was subsequently deacetylated and selectively protected at O-6 with 4,4'-dimethoxytrityl chloride (DMTrtCl) to provide **13**. After the remaining hydroxyl groups had been protected by benzyl ether groups, acid-catalyzed methanolysis of the DMTrt group was performed to give the glycosyl acceptor **14**. This compound underwent coupling with the previously described 1-thiogalactoside **15**⁴⁵ under Lemieux conditions,⁴² namely α -stereoselective halide ion-catalyzed glycosidation of the *in situ*-generated glycosyl bromide. This procedure generated α -galactoside **16** in 67% yield (the β -anomer was isolated in 10% yield), and subsequent conversion into glycosyl fluoride **5** was accomplished by oxidative hydrolysis of the anomeric PMP group with ceric ammonium nitrate (CAN), followed by treatment with diethylaminosulfur trifluoride (DAST).

The main fragment in Ogawa's synthesis, pseudotrisaccharide **8**, was synthesized from building blocks **9–11** (Scheme 4). The mannosyl thioglycoside **9** was obtained from compound **18**⁴⁶ through acetylation, anomeric acetolysis, and finally treatment with methyl tributyltin sulfide in the presence of tin (IV) chloride. The azidoglucose acceptor **10** was synthesized from triol **19**,⁴⁷ which required acid-catalyzed 4,6-benzylidenation with PhCH(OMe)₂, benzylation at O-3, and regioselective benzylidene ring-opening with BH₃·NMe₃ in the presence of AlCl₃.⁴⁸

Preparation of the enantiomerically pure inositol derivative **11** was lengthy. The synthesis began from racemic 2,3;4,5-di-*O*-cyclohexylidene-*myo*-inositol ($\pm$)-**20**,⁴⁹ Garegg's inositol derivative, a common starting point for inositol synthesis. Stannylene acetal-directed *p*-methoxybenzylation of ($\pm$)-**20** favored the O-6 position over the O-1 position (ratio = 3:1), leaving the latter site free for protection with an allyl group. After exchanging the

cyclohexylidene acetals for benzyl groups to give ($\pm$)-**21**, the allyl group at O-1 was removed, exposing this position for reaction with the chiral resolving reagent (1*S*)-(-)-camphanic chloride, which generated separable diastereomeric inositol derivatives. At this stage, the absolute structure of the separated inositols was assigned by conversion into the previously characterized compound 2,3,4,5-penta-*O*-benzyl-*myo*-inositol. Unfortunately, the specific rotations for the D and L enantiomers of this compound were incorrectly reported in 1987,⁵⁰ an error whose correction in 1991⁵¹ was undetected by the Ogawa group prior to publication of their work.^{52,53} Consequently, the wrong inositol enantiomer was chosen, which led to the total synthesis of an unnatural stereoisomer of the *T. brucei* GPI anchor, a feat made no less spectacular by this minor mishap.

Moving forward, the inositol intermediate was converted into **11** through a series of manipulations at the 1 and 6 positions. After CAN-mediated oxidative cleavage of the 6-*O*-PMB group, four steps were performed consecutively without chromatographic purification, including acid-catalyzed reprotection with a 1-ethoxyethyl ether (EE) group, exchange of the (-)-CAM group for a PMB group at O-1, and finally methanolysis of the 6-EE group by use of AcOH/MeOH.

The assembly of pseudotrisaccharide **8** began by the coupling of compounds **9** and **10** (Scheme 4). The glycosylation occurred efficiently under the modified Lemieux conditions previously developed by Ogawa, specifically the use of AgOTf as an additional promoter in the presence of CuBr₂/Bu₄NBr to improve reactivity with poor glycosyl acceptors such as **10**.⁵⁴ The α -disaccharide was generated in 90% yield and subsequently converted into glycosyl fluoride **23** by tetrabutylammonium fluoride (TBAF)-mediated anomeric desilylation followed by treatment of intermediate hemiacetal **22** with DAST. Compound **23**, bearing a non-participating 2-azido group adjacent to the leaving group, then underwent glycosylation with the inositol acceptor **11** by Suzuki's method employing Cp₂ZrCl₂ and AgClO₄⁴¹ to form the crucial 1,2-*cis* glycosidic bond between the glucosamine and inositol components. The α anomer **24** was obtained in 73% yield, whereas the amount of the β anomer was minor (α : β ratio = 3.7:1), probably because of solvent participation from diethyl ether.⁵⁵ To expose the O-3 position of Man-I for addition of the digalactose moiety, compound **24** was deacetylated at the O-2 and O-6 positions of Man-I with NaOMe/MeOH and selectively re-acetylated at the O-6 position of Man-I to give **8**.

Pseudotrisaccharide **8** was carried forward to the fully protected GPI precursor **2** as shown in Scheme 5. First, the digalactosyl group was installed at O-3 of Man-I by reaction of **8** with the digalactosyl fluoride **5** in the presence of Cp₂ZrCl₂/AgClO₄ in diethyl ether. An α , β mixture was obtained in 76% yield (α : β ratio = 9:1), and separation of the two anomers was performed following deacetylation at O-6 of Man-I with NaOMe in MeOH to give **25** (68% over two steps). During the glycosylation, pseudotetrasaccharide **26** and 1,6-anhydro sugar **27** were also formed (Scheme 6), suggesting that a portion of glycosyl fluoride **5** underwent decomposition via ring flip, cleavage of the galactose at O-6, and intramolecular attack of the liberated primary hydroxyl group on the oxocarbenium cation. Compound **25**, bearing a primary hydroxyl group at O-6 of Man-I, was coupled with mannosyl chloride **6**⁵⁶ in the presence of mercury(II) bromide and mercury(II) cyanide to generate the branched pseudohexasaccharide **28** with excellent α stereoselectivity (89% yield). Deacetylation of **28** gave compound **29**, which reacted with mannosyl fluoride **7** (generated from its corresponding anomeric acetate⁵⁷ by deacetylation and treatment with DAST) in the presence of Cp₂ZrCl₂/AgClO₄ in diethyl ether to provide pseudoheptasaccharide **2** in 93% yield. Because of protecting-group complications, the acetyl group at O-6 on Man-III was exchanged for a chloroacetyl (CA) group, followed by removal of the PMB group at O-1 of the inositol with TMSOTf to give compound **30**, which was set up for both late-stage phosphorylation events.

First, the *H*-phosphonate method⁴⁴ was used to install a diacylglycerophosphate moiety to the O-1 position of inositol by reaction of **30** with *H*-phosphonate **3** and pivaloyl chloride (PivCl) in pyridine, affording intermediate **31** in 64% yield. Following removal of the 6-chloroacetyl group on Man-III with thiourea, this site was then allowed to react with *H*-phosphonate **4** in the presence of PivCl in 40% yield. After the intermediate had been oxidized with aqueous iodine, product **33** was subjected to global deprotection by hydrogenolysis, using Pd(OH)₂/C as catalyst, which afforded the target GPI anchor **1** in 23% yield.

In summary, the Ogawa group accomplished the first synthesis of a GPI anchor by using a combined linear–convergent strategy in concert with classical methods for formation of glycosidic bonds together with *H*-phosphonate chemistry. Although it was an impressive starting point for GPI synthesis, future improvements were called for in the areas of assembly sequence, inositol preparation, orthogonal protecting-group selection, global deprotection efficiency, and usage of more environmentally acceptable reagents.

2. Synthesis of a Yeast GPI Anchor by the Schmidt Group (1994)

In 1994, Schmidt and co-workers reported the total synthesis of a GPI anchor from yeast (*Saccharomyces cerevisia*).^{58,59} This anchor contains a ceramide lipid region rather than the glycerolipid commonly found in other organisms. Synthesis of the target GPI **34** made use of a fully convergent strategy (Scheme 7). As with Ogawa, Schmidt's laboratory performed two late-stage phosphorylations on a benzyl-protected GPI intermediate (**35**) to install the phosphoethanolamine and ceramide components. For this purpose, the phosphoramidite method,⁶⁰ employing building blocks **36** and **37**, was used as an alternative to the *H*-phosphonate method, allowing incorporation of fully protected phosphate groups. Compound **35** was formed in a key glycosylation between the tetramannosyl trichloroacetimidate **38** and pseudodisaccharide **39**. The former compound **38** was built up from mannose derivatives **40–42**, while the latter was synthesized from inositol acceptor **43** and azidoglucosyl trichloroacetimidate **44**. Schmidt's own powerful method,⁶¹ employing glycosyl trichloroacetimidate donors, was exclusively used for glycosidic-bond formation, and acyl protecting groups at O-2 on the glycosyl donors aided the α -stereoselective formation of glycosidic bonds.

For synthesis of the tetramannosyl donor **38**, all required building blocks (**40–42**) were accessed from the known orthoester **45** (Scheme 8).⁵⁶ En route to the Man-I monomer **40**, orthoester **45** was treated with *tert*-butylchlorodiphenylsilane in the presence of imidazole to selectively protect O-6, leaving the remaining sites for protection with benzyl groups to give the 3,4-diether **46**. Acid-catalyzed hydrolysis of the 1,2-orthoester was followed by acetylation and regioselective hydrolysis of the anomeric acetate with (NH₄)₂CO₃. Subsequent treatment of the hemiacetal intermediate with trichloroacetonitrile and the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the Man-III glycosyl donor **42**. This compound was also elaborated to Man-I derivative **40** in four steps, including installation of allyl groups at O-1 and O-2 followed by 6-desilylation with TBAF/AcOH. The Man-II donor **41** was accessed from orthoester **45** by successive per-*O*-benzylolation, orthoester hydrolysis, acetylation, selective anomeric deacetylation, and trichloroacetimidation, as just described for compound **42**.

Coupling of the Man-I acceptor **40** and Man-II trichloroacetimidate donor **41** in the presence of catalytic TMSOTf in Et₂O provided dimannoside **47** in 84% yield with complete α -stereoselectivity. Following removal of the temporary stereodirecting Man-II 2-*O*-acetyl group with NaOMe/MeOH, the acceptor **48** was allowed to react with Man-III trichloroacetimidate donor **42** under similar conditions, affording trimannoside **49** in 92%

yield, again with complete α -stereoselectivity through neighboring-group participation. A final iteration of this process, using building block **41**, installed the Man-IV unit in 91% yield. Conversion of the allyl tetramannoside **50** into trichloroacetimidate **38** required four steps, including double deallylation with Wilkinson's catalyst, acetylation of the intermediate diol, regioselective anomeric deacetylation with $(\text{NH}_4)_2\text{CO}_3$, and treatment with trichloroacetonitrile and DBU. This sequence served to activate the anomeric center as a trichloroacetimidate and install a participating acetyl group at the O-2 position of Man-I to facilitate α -stereoselectivity in the key glycosylation.

The synthesis of pseudodisaccharide **39** featured efficient preparation of the inositol derivative **43**, followed by a highly stereoselective glycosylation reaction with the azidoglucosyl donor **44** (Scheme 9). Synthesis of **43** was accomplished by enantiomeric resolution of inositol diketal $(\pm)$ -**20**,⁴⁹ which underwent treatment with bis(tributyltin)oxide followed by reaction with the chiral reagent $(-)$ -menthyl chloroformate $[(-)\text{-MntOCOCI}]$ to give the diastereomeric carbonates **43** and **51**,⁶² which were separated by recrystallization. Compound **43** was directly glycosylated by the azidoglucosyl trichloroacetimidate **44**⁶³ in the presence of TMSOTf to give exclusively the α stereoisomer **52** in 85% yield. The authors attributed the exceptional stereoselectivity observed in this coupling to an appropriate choice of protecting groups and reaction optimization, which still remains a challenging task for forming the 1,2-*cis* glycosidic bond between azidoglucosyl donors and inositol acceptors.

To expose the O-4 of the azidoglucose unit for the key glycosylation, compound **52** was converted into pseudodisaccharide **39** in three steps, each featuring crucial regiocontrol. First of all, selective deacetylation with NaOMe/MeOH formed an intermediate triol while leaving the $(-)$ -menthyl carbonate intact. The O-6 position of the azidoglucose component was then selectively benzoylated with BzCN in the presence of triethylamine at -70°C . Finally, selective 3-*O*-benzylation with BnBr promoted by Ag_2O gave the pseudodisaccharide **39** in 53% yield over three steps. Overall, the highly economical synthesis of this key intermediate constituted an impressive demonstration of regio- and stereocontrol.

Completion of the synthesis of yeast GPI anchor **34** is shown in Scheme 10. The key glycosylation reaction between tetramannosyl donor **38** and pseudodisaccharide acceptor **39** was accomplished using catalytic TMSOTf in diethyl ether, which afforded the desired α -pseudohexasaccharide intermediate in an excellent 91% yield. Unfortunately, several protecting-group changes were required to access GPI intermediate **35** prior to the two phosphorylation events, including removal of all acyl groups with KCN/MeOH (45%) and reprotection of the exposed positions with benzyl groups, as well as replacement of the 1-*O*- $(-)$ -menthyl carbonate group of the inositol by an acetyl group. These late-stage protecting-group manipulations, which are best avoided on complex intermediates, were the price to be paid for a short pseudodisaccharide synthesis (Scheme 9), where appropriate protection steps were postponed.

Compound **35** was made ready for installation of the phosphoethanolamine group after TBAF-mediated removal of the 6-*O*-TBDPS group on Man-III. Formation of the phosphotriester was achieved by 1*H*-tetrazole-promoted phosphitylation with phosphoramidite **36**, followed by *in situ* oxidation with *t*-BuOOH. Subsequent treatment with NaOMe/MeOH cleaved both the cyanoethoxyl and the inositol 1-*O*-acetyl groups to give phosphodiester **54**, which was poised for attachment of the ceramide moiety. Ceramide phosphoramidite **37**, which was prepared readily from a previously reported azido lipid,⁶⁴ reacted with intermediate **54** in the presence of 1*H*-tetrazole, and sequential *in situ* oxidation with *t*-BuOOH, and cyanoethoxyl removal with dimethylamine afforded GPI intermediate

55. Two steps were required for global deprotection, including acid-catalyzed alcoholysis of the acetal protecting groups (63%) and Pd-catalyzed reductive removal of the benzyl/benzyloxycarbonyl groups and reduction of the azido group (70%) to give the target GPI anchor **34**.

Schmidt's total synthesis of yeast GPI anchor **34** was highlighted by incorporation of a ceramide phospholipid, showcasing the trichloroacetimidate "Schmidt" glycosylation method, and perhaps most impressively synthesizing pseudodisaccharide **39** with high efficiency and superb control of regio- and stereoselectivity. Furthermore, global removal of the benzyl groups using Pd-catalyzed hydrogenolysis featured an improved yield (70%) as compared with Ogawa's result in the first total synthesis of a GPI anchor (23%). The versatility of the strategy was further demonstrated by Schmidt's use of building blocks from this synthesis in subsequent syntheses of GPIs from *Toxoplasma gondii*²⁴ and rat brain Thy-1 (for the latter, see Section II-5).

3. Synthesis of a Rat Brain Thy-1 GPI Anchor by the Fraser-Reid Group (1995)

The Fraser-Reid group published several synthetic studies on GPI partial structures^{38,65–69} prior to the 1995 communication by Campbell and Fraser-Reid describing the synthesis of a rat brain Thy-1 GPI anchor, which constituted the first total synthesis of a mammalian GPI and the third total synthesis in general of a GPI.⁷⁰ The Thy-1 GPI glycan contains an additional phosphoethanolamine group at the O-2 position of Man-I (a common modification in mammalian GPIs), as well as GalNAc and Man components attached to the O-4 position of Man-I and the O-2 position of Man-III, respectively. These modifications, while numerous, were all effectively incorporated into the "fully phosphorylated" target molecule by Fraser-Reid.

A convergent approach was used to synthesize the Thy-1 GPI (compound **56**), in which a [3 + 2 + 2] block strategy was applied (Scheme 11). Because of the additional phosphoethanolamine group present in the target, the benzyl-protected intermediate **57** would undergo three sequential phosphorylation reactions at sites bearing appropriate orthogonal protecting-groups. As with Schmidt, Fraser-Reid used the phosphoramidite method^{60,66} with precursors **58** and **59** to install the phosphoethanolamine and phosphoglycerolipid groups. Compound **57** was synthesized from trimannoside **60**, disaccharide **61**, and pseudodisaccharide **62**, which were each accessed from their corresponding sugar and inositol monomers **63–69**. A combination of glycosylation methods were used for stereoselective formation of glycosidic bonds, including the Koenigs–Knorr method,⁴³ Schmidt's trichloroacetimidate method,⁷¹ and Fraser-Reid's own method employing the versatile *n*-pentenyl glycosides (NPGs).⁷²

The synthesis of *n*-pentenyl trimannoside **60** relied on sequential Koenigs–Knorr glycosylations using mannose monomers **63–65**, which were all prepared from the *n*-pentenyl orthoester⁷³ **70**⁶⁸ (Scheme 12). This intermediate allowed for ready conversion into 2-*O*-benzoyl-protected donors, thus facilitating α -stereoselective glycosylations and flexibility with regard to choice of leaving group at the anomeric position. The Man-II component **63** was synthesized from **70** by benzylation, acid-catalyzed rearrangement of the *n*-pentenyl orthoester to the corresponding *n*-pentenyl glycoside, and then removal of the newly formed 2-*O*-benzoyl group with NaOMe. Compound **70** was converted into the Man-III component **64** by 6-*O*-silylation with TBDPSCl/imidazole, benzylation, and treatment with bromine. The Man-IV donor **65**, also a glycosyl bromide, was readily generated from **70** by benzylation and treatment with bromine.

The Man-II acceptor **63** and Man-III donor **64** were coupled in the presence of promoter AgOTf, resulting in the formation of α -dimannoside **73** in 89% yield. Removal of the temporary stereodirecting 2-*O*-benzoyl group was accomplished with NaOMe to afford alcohol **74**, which underwent α -stereoselective glycosylation with the Man-IV glycosyl bromide **65**, again under the influence of AgOTf. The product **75** was isolated in 74% yield, and subsequently underwent two protecting-group exchanges to provide the *n*-pentenyl trimannoside **60**. First, the stereodirecting 2-*O*-benzoyl group of Man-IV was replaced by a benzyl group, and then the 6-*O*-TBDPS group of Man-III was exchanged for a chloroacetyl group by TBAF-mediated desilylation followed by treatment with chloroacetic anhydride in the presence of triethylamine.

The β -GalNAc-Man disaccharide **61** was prepared from the *n*-pentenyl mannoside **77**⁷⁴ and tetraacetate **79**,⁷⁵ as shown in Scheme 13. Compound **77**, constituting the Man-I building block, was treated with PhCH(OMe)₂ in the presence of pyridinium *p*-toluenesulfonate (PPTS) to generate the 4,6-benzylidene acetal. Stannylene acetal-directed, regioselective 3-*O*-benzylation was followed by acetylation to give compound **78**, which was converted in to the Man-I acceptor **66** by acid-promoted hydrolysis of the benzylidene group and selective acetylation at O-6. The 2-phthalimido galactosyl trichloroacetimidate **67** was accessed from 2-deoxy-2-phthalimido- β -D-galactose tetraacetate (**79**) by successive TMSOTf-promoted glycosidation with *p*-methoxyphenol, deacetylation, benzylation, oxidative hydrolysis of the anomeric PMP group, and finally reaction with trichloroacetonitrile and DBU.

The TMSOTf-catalyzed Schmidt glycosylation of **66** by **67** was completely β -stereoselective because of the participating effect of the 2-phthalimido group. The β -disaccharide product **81** was isolated in 79% yield, and afterwards the temporary 2-phthalimido group was replaced by a 2-acetamido group via sequential aminolysis and selective *N*-acetylation. Differentiation of the two free hydroxyl groups on Man-I was accomplished by selective O-6 chloroacetylation followed by acetylation at O-2, affording building block **61**.

Fraser-Reid's synthesis of the pseudodisaccharide fragment began with Garegg's indispensable inositol derivative ($\pm$)-**20**⁴⁹ (Scheme 14), as did the previous GPI syntheses by Ogawa and Schmidt. The route was initiated by stannylene-mediated *p*-methoxybenzylation at O-6, which was followed by esterification of the free O-1 position by the chiral resolution reagent (1*S*)-(-)-camphanic chloride.⁷⁶ After separation, the correct diastereomer was subjected to saponification with LiOH followed by allylation to provide the optically active **82**. After the cyclohexylidene acetals had been replaced by benzyl groups, CAN-mediated cleavage of the PMB group at O-6 furnished the inositol acceptor **68**. To decrease loss of material, Fraser-Reid also developed a route to convert the 1-*p*-methoxybenzylated inositol regioisomer, generated from ($\pm$)-**20** in the first step, into compound **68** (not shown here).⁶⁹ The 2-azido glucosyl bromide **69** was synthesized from 1,6-anhydro-2,3,4-tri-*O*-benzyl- β -D-mannopyranose (**83**) via intermediate **84** (a route developed by Hori and co-workers⁷⁷), which after trifluoroacetic acid (TFA)-promoted ring opening/acetylation was treated with TiBr₄ to afford **69**.

The Koenigs-Knorr-type glycosylation between inositol acceptor **68** and azido glucosyl bromide **69** proceeded with acceptable α -stereoselectivity when using AgClO₄ in diethyl ether. The inseparable α,β mixture **85**, obtained in 85% yield ($\alpha:\beta = 3:1$), was subjected to deacetylation at O-4 and O-6, after which the preponderant α -pseudodisaccharide diol **86** was separated out. The remaining task of selective benzylation at O-6 turned out to be quite problematic, as the authors were forced to perform five successive steps, namely selective 6-*O*-acetylation, 4-*O*-tetrahydropyranylation (THP), deacetylation, 6-*O*-benzylation, and acid-promoted methanolysis of the THP group. This sequence blemished an otherwise efficient synthesis of pseudodisaccharide fragment **62**.

Assembly of the pseudoheptasaccharide from subunits **60–62** using the [3 + 2 + 2] block approach was accomplished with *n*-pentenyl glycoside chemistry (Scheme 15). The *n*-pentenyl disaccharide **61** was chemoselectively activated by *N*-iodosuccinimide (NIS) and triethylsilyl trifluoromethanesulfonate (TESOTf), and subsequent reaction with pseudodisaccharide acceptor **62** gave the α -pseudotetrasaccharide **87** as the sole isomer in 55% yield. The remainder of the mass balance was attributed to triethylsilylation of the acceptor hydroxyl group. To prepare for chain extension of the glycan, the 6-chloroacetyl group on Man-I of **87** was removed with thiourea to give **88**, which reacted with *n*-pentenyl trimannoside **60** in the presence of NIS/TESOTf to afford α -pseudoheptasaccharide **57** in moderate (32%) yield [39% based on recovered starting material (BRSM)]. Again, triethylsilylation of the acceptor hydroxyl group accounted for the low yield, although the silylated product could be recovered and transformed back into **88** by treatment with TBAF. This recycling effort would not have been possible without the earlier protecting-group exchange at O-6 of Man-III from TBDPS to chloroacetyl (Scheme 12), which on first glance may have seemed unnecessary. Also of note, Fraser-Reid took advantage of the ability of *n*-pentenyl glycosides to be selectively activated with NIS/TESOTf in the presence of allyl protecting groups in this sequence.

Pseudoheptasaccharide **57** was elaborated to the target GPI (**56**) via three phosphorylation events and global deprotection. Phosphoramidite chemistry optimized for GPI synthesis by Fraser-Reid⁶⁶ was used to form the appropriate phosphotriester bonds efficiently. For the phosphoethanolamine group on Man-III, dechloroacetylation of **57** was followed by 1*H*-tetrazole-promoted phosphitylation with **58** and *in situ* oxidation with *m*-chloroperoxybenzoic acid (*m*-CPBA). After deacetylation of the O-2 position of Man-I, the same conditions were used to install a phosphoethanolamine group at this site. Next, compound **90** was subjected to Pd-mediated removal of the inositol O-1 allyl group to give **91**, which was coupled with phospholipid precursor **59**, again using the phosphoramidite method. All three phosphorylation events proceeded smoothly and in high yield (75–90%). Finally, hydrogenolytic global deprotection with Pd(OH)₂/C and H₂ provided the fully phosphorylated Thy-1 GPI **56** in 75% yield.

The in-depth synthetic studies on GPI synthesis between 1989 and 1995 by the Fraser-Reid group culminated in the first total synthesis of a mammalian GPI anchor, namely Thy-1 GPI (**56**). Through careful choice of assembly strategy and orthogonal protecting groups, the structural modifications present in the natural compound were successfully incorporated into the target molecule, including two sugar groups and an additional phosphoethanolamine group. Fraser-Reid's *n*-pentenyl glycoside chemistry was used prominently, both in the preparation of mannose building-blocks and in the final two stereoselective glycosylations to join fragments **60–62**. Where necessary, the glycosylation methods of Koenigs–Knorr and Schmidt were also used for stereoselective formation of glycosidic bonds.

4. Synthesis of a *T. brucei* GPI Anchor by the Ley Group (1998)

In contrast to the combined linear–convergent synthesis of the *T. brucei* GPI anchor **1** reported by Ogawa,³⁶ the Ley group used a highly convergent route (Scheme 16).^{78,79} The target molecule **1** was accessed from a fully protected pseudoheptasaccharide **93** via sequential phosphorylation reactions with **58** and **94** using the phosphoramidite method.⁶⁰ In turn, compound **93** was formed in a key glycosylation by two highly elaborated building-blocks, namely pentasaccharide donor **95** and pseudodisaccharide **96**. To synthesize these intermediates, the Ley group made elegant use of their bis(dihydropyran) chemistry for selective protection and desymmetrization procedures.^{80–82} Rapid preparation of pentasaccharide **95** from building blocks **99–103** (via **97** and **98**) relied on tuning of the reactivity of glycosyl donors using judicious protecting-group choices,⁸³ as well as by

taking advantage of selenoglycoside and thioglycoside orthogonality.⁸⁴ Pseudodisaccharide **96** was generated from azido glucosyl bromide **104** and inositol acceptor **68**, the latter of which was made chiral by using bis(dihydropyran)-based desymmetrization.

Building blocks **99–103** were generated from seleno- and thio-glycosides **105–107** (Scheme 17). The Man-I building-block **99** was obtained from thioglycoside **105**⁸⁵ in seven steps. Selective installation of a TBS group at O-6 was followed by *cis*-acetonation to protect the O-2 and O-3 positions, which readied O-4 for benzylation. After deacetonation, intermediate **108** underwent trimethylsilylation at O-3, chloroacetylation at O-2, and aqueous HF-mediated desilylation to provide compound **99**. The selenogalactoside **106**⁸⁶ was used as a common starting point for the preparation of compounds **100** and **101**. Thus, compound **106** was subjected to 2,3-butanediactal (BDA) protection with butane-2,3-dione/CSA, selective silylation at O-6 with TBSCl, chloroacetylation of the free axial OH group at O-4, and removal of the TBS group with aqueous HF to give the glycosyl acceptor **100**. Alternatively, compound **106** was readily perbenzylated to give glycosyl donor **101**. Finally, selenomannoside **107**⁸⁷ was readily converted into the Man-II and Man-III building blocks **102** and **103**, the former being accessed by 3,4-BDA protection followed by tin-mediated chloroacetylation at O-6, and the latter by silylation at O-6 with TBSCl followed by benzylation.

With careful use of “armed” and “disarmed” glycosyl donors, as introduced by Fraser-Reid,⁸³ as well as by using selectively activatable selenoglycosides and thioglycosides,⁸⁴ the preparation of pentasaccharide **95** remarkably took only five steps from building blocks **99–103** (Scheme 18). First, the glycosyl acceptor **100**, whose anomeric selenoacetal group was disarmed by virtue of the deactivating BDA⁸¹ and chloroacetyl groups, was coupled with the benzyl-protected, armed glycosyl donor **101** in the presence of NIS/TMSOTf. The α -digalactoside **111** was obtained in 71% yield, while the β anomer was isolated in 15% yield, and no homocoupling product was observed. Subsequent reaction of **111** with the Man-I thioglycoside acceptor **99** was facilitated by chemoselective activation of the selenoacetal with MeOTf,⁸⁸ affording the α -trisaccharide **112** in 75% yield. The α -dimannose fragment **98** was generated in 87% yield by selective coupling of selenoglycosides **102** (disarmed) and **103** (armed) in the presence of NIS/TMSOTf. Finally, compound **98** was combined with **97** (generated from **112** by desilylation), again relying on the higher reactivity of selenoglycosides with MeOTf as compared to thioglycosides (product generated in 75% yield). A large excess of the donor and high concentrations of reactants were used to minimize formation of an anhydro sugar resulting from intramolecular glycosidation of **97** through attack from O-6. Overall, the α -pentasaccharide **95** was efficiently prepared from compounds **99–103** by employing strategies to control anomeric reactivity.

The synthesis of pseudodisaccharide **62** shown in Scheme 19 was highlighted by Ley's efficient preparation of inositol derivative **68** using bis(dihydropyran)-mediated desymmetrization. The known symmetrical tetraol **113**⁸⁹ reacted with the chiral bis(dihydropyran) **114** in the presence of CSA to form dispiroketal **115** (ee 98%) in 88% yield. Positions 2–5 were masked with benzyl groups by deacylation and perbenzylation. The dispiroketal group was removed in a two-step process involving oxidation with *m*-CPBA followed by treatment with LiN(TMS)₂. The inositol derivative **68** was then formed by stannylene acetal-directed regioselective allylation at the O-1 position. The azido glucosyl bromide **104** was prepared by conventional methods beginning with thioglycoside **116**,⁹⁰ which underwent benzylation at O-3, regioselective cleavage of the benzyldiene ring with Et₃SiH/TFA, and exchange of the phthalimido group by an azido group using the diazo-transfer chemistry developed by Vasella and co-workers.⁹¹ This four-step procedure generated compound **117**, which was silylated at O-4 with TBSCl/KHMDS and then treated

with bromine to give the glycosyl bromide **104**. Lemieux conditions⁴² were employed for α -stereoselective joining of inositol acceptor **68** and glycosyl bromide **104**, which provided compound **96** in 65% yield. Removal of the TBS group at O-4 was accomplished by treatment with TBAF, giving the pseudodisaccharide acceptor **62**.

Completion of the synthesis of *T. brucei* GPI anchor **1** by the Ley group is shown in Scheme 20. The key glycosylation between the pentasaccharide ethylthio donor **95** and pseudodisaccharide **62** was promoted by NIS/TMSOTf and generated the α -pseudoheptasaccharide **93** in 51% yield. Installation of the phosphoethanolamine group was performed by treatment with aqueous HF to expose the O-6 position of Man-III, followed by reaction at this site with the phosphoramidite **58** and *in situ* oxidation with *m*-CPBA. Intermediate **119** underwent Pd-mediated deallylation to deprotect the O-1 position of the inositol, which was followed by phospholipidation with **94** [synthesized using a bis(dihydropyran)-controlled desymmetrization of glycerol], again using phosphoramidite chemistry, to provide compound **121**. The global deprotection required three sets of conditions, including Pd-catalyzed hydrogenolysis, thiourea-mediated dechloroacetylation, and finally TFA-promoted hydrolysis of the BDA groups. This sequence generated the target GPI **1** in an impressive 90% yield over the final three steps.

In summary, Ley and co-workers successfully completed the fourth total synthesis of a GPI anchor, and this was the second effort aimed at the GPI (**1**) of *T. brucei*, following Ogawa's seminal work. This synthesis served as a proving ground for methods developed in the Ley laboratory, namely the usage of bis(dihydropyrans) as reactivity-tuning BDA protecting-groups and desymmetrizing chiral reagents. These chemistries were elegantly integrated with strategies for selective activation of the glycosyl donor, specifically Fraser-Reid's armed/disarmed concept and orthogonally activatable anomeric groups (selenoglycosides and thioglycosides), culminating in a highly efficient convergent synthesis of the target molecule.

5. Synthesis of a Rat Brain Thy-1 GPI Anchor by the Schmidt Group (1999, 2003)

Following their 1994 synthesis of a yeast GPI anchor,⁵⁸ the Schmidt group reported a synthesis of the Thy-1 GPI anchor (**56**) of rat brain in 1999,⁹² a target that had previously yielded to total synthesis by Fraser-Reid in 1995 (see Section II-3).⁷⁰ Unsurprisingly, Schmidt's assembly strategy closely resembled that of Fraser-Reid, particularly in the synthetic end-game. We forgo here a detailed discussion of this 1999 report, instead opting to cover a 2003 paper by Schmidt in which the Thy-1 GPI anchor was synthesized by a similar but modified strategy; it emphasized future couplings with peptides/glycopeptides at the phosphoethanolamine group of Man-III,⁹³ a strategic decision that foreshadowed looming advances in GPI-protein synthetic methods. In terms of synthetic strategy, this work was also closely tied to a 2001 report by Schmidt disclosing the synthesis of a partial structure of a non-lipidated GPI from the parasitic protozoan *T. gondii*.²⁴

The retrosynthetic analysis from Schmidt's 2003 synthesis of the Thy-1 GPI anchor is shown in Scheme 21. The precursor to the target molecule, the benzyl-protected pseudoheptasaccharide **122**, differed only slightly from the corresponding intermediate in Fraser-Reid's synthesis of the Thy-1 GPI (compound **57**, Scheme 11). However, late-stage phosphorylation events were designed to install orthogonally protected phosphoethanolamine groups (using the phosphoramidites **36** and **123**), which would permit selective amino group deprotection and therefore the opportunity for future peptide/glycopeptide conjugation at Man-III. Intermediates **125** and **126** were employed in a highly convergent assembly strategy, for which the Schmidt group capitalized on the versatility of their previous GPI syntheses by employing some of the same building blocks. Naturally, the

Schmidt trichloroacetimidate method⁶³ was used for stereoselective formation of glycosidic bonds.

The synthesis of pentasaccharide donor **125** was accomplished by stepwise elongation of the β Gal-Man disaccharide **127** with mannosyl trichloroacetimidates **41** and **42** (Scheme 22). In turn, disaccharide **127** was prepared from monosaccharides **129** and **131**. First, the allyl mannoside **128**⁹⁴ was converted into Man-I derivative **129** via stannylene-mediated benzylation at O-3, *p*-methoxybenzylidenation at O-4 and O-6, allylation at O-2, and regioselective reductive cleavage of the acetal ring with NaBH₃CN/TFA. Preparation of the galactosyl trichloroacetimidate **131**, which contained a trichloroacetamido (TCA) group to ensure β -selective glycosylation while also acting as a latent acetamido group, was initiated from the known azido galactose derivative **130**.⁷¹ The anomeric position of **130** was temporarily masked with a hexyldimethylsilyl (TDS) group, followed by exchange of the azide group for a trichloroacetamido group. Conversion into the glycosyl donor **131** was accomplished by anomeric desilylation with TBAF, followed by treatment with trichloroacetonitrile and DBU. Coupling of Man-I acceptor **129** and trichloroacetamido galactosyl donor **131** in the presence of boron trifluoride etherate afforded exclusively the β -linked disaccharide **132**, which was treated with CAN to oxidatively cleave the PMB group to give **127**.

Starting from protected disaccharide **127**, consecutive mannosylations using donors **41** and **42** were effected under standard Schmidt glycosylation conditions to extend the glycan chain. Each of the three glycosylations was high yielding (92–96%) and highly α -stereoselective because of neighboring-group participation. The pentasaccharide **137** generated through this process required several manipulations to obtain donor **125**, starting with radical reduction of the trichloroacetamido group to form an acetamido group, followed by deacetylation–benzylation of the O-2 position of Man-III. The Man-I component was then adjusted via double deallylation with Wilkinson's catalyst, followed by diacylation with phenoxyacetyl (PA) chloride. Selective anomeric deacylation with (NH₄)₂CO₃ generated an intermediate hemiacetal that was treated with trichloroacetonitrile and DBU to provide the donor compound **125**.

Compound **126** differed from the pseudodisaccharide building-blocks in previous GPI syntheses because *N*-*tert*-butyloxycarbonyl (Boc) protection was used rather than the traditional azido group. This was necessary to permit late-stage selective deprotection of the Man-III phosphoethanolamine (Cbz-protected) for peptide conjugation. The preparation of **126** began from inositol derivative **11**, which Schmidt generated from the optically active inositol **43** in a few straightforward steps (Scheme 23). Allylation at O-6 was assisted by Ag₂O rather than basic conditions, so as not to affect the carbonate bond. After replacement of the 1-*O*-menthyl carbonate with a PMB group and the cyclohexylidene acetals with benzyl groups, the allyl group at O-6 was removed with Wilkinson's catalyst to give **11**.

Inositol acceptor **11** was then coupled with azido glucosyl trichloroacetimidate **44** in the presence of TMSOTf, which afforded pseudodisaccharide **139** in 70% yield. Numerous protecting-group manipulations were required at this point to prepare the azido glucose unit for the remainder of the synthesis. First, deacetylation with NaOMe gave an intermediate triol that was masked with a 4,6-*O*-benzylidene ring to give the pseudodisaccharide derivative **140**, which was converted into compound **141** by benzylation at O-3, regioselective benzylidene cleavage, and phenoxyacetylation at O-4 with PACl in pyridine. Exchange of the inositol 1-*O*-PMB group for a benzoyl group, and dephenoxyacetylation with MeNH₂ preceded the crucial amino-protection swap, which was accomplished by propanedithiol-assisted azide reduction and treatment with Boc anhydride. The lengthy route to **126** dampened the overall efficiency of this GPI synthesis.

Completion of the Thy-1 GPI anchor synthesis by Schmidt is shown in Scheme 24. The key glycosylation between pentasaccharide donor **125** and pseudodisaccharide acceptor **126** under promotion by TMSOTf afforded the expected α -pseudoheptasaccharide **122** in 74% yield. The TBDPS group on O-6 of Man-III was removed with TBAF, making intermediate **142** ready for phosphorylation with compound **36** using the phosphoramidite method. *In situ* cleavage of the PA group at O-2 of Man I and cyanoethoxyl groups with MeNH₂ generated alcohol **143**, which underwent installation of the second phosphoethanolamine group by reaction with **123**, again using phosphoramidite chemistry. Phospholipidation of GPI intermediate **144** was accomplished by 1-*O*-debenzoylation of the inositol, followed by reaction with phosphoramidite **124**. Concomitant *in situ* oxidation and aminolysis (to remove the final cyanoethoxyl group) afforded the partially deprotected GPI derivative **146**. At this stage, the authors demonstrated the feature of orthogonal amino-group protection by subjecting compound **146** to Pd-catalyzed hydrogenolysis to generate GPI **147** (75%), which contained a single free amino group at the Man-III phosphoethanolamine component for future coupling with peptides/glycopeptides. In addition, intermediate **146** was subjected to a two-step global deprotection, including acidic and reductive conditions, resulting in formation of the rat-brain Thy-1 GPI anchor **56** in 54% yield over the final two steps.

Schmidt and co-workers' 2003 synthesis of the rat-brain Thy-1 GPI anchor, the second time they accomplished this feat, combined elements from their previous work in the field, including syntheses of GPI anchors (or related glycoconjugates) from yeast (1994), Thy-1 (1999), and *T. gondii* (2001). Thoughtfully-designed building blocks were reused throughout these projects, a testament to their versatility. The syntheses of these GPIs relied on convergent assembly tactics in combination with expert use of glycosyl trichloroacetimidate and phosphoramidite chemistries for key bond-forming events. In the 2003 Thy-1 GPI synthesis, a strategy for orthogonal amino-group protection, which would permit future peptide/glycopeptide conjugation at Man-III, was demonstrated, although this feature was not applied. The Guo group was the first team to report the chemical synthesis of GPI-anchored peptides³⁰ and glycopeptides,³¹ in 2003 and 2004, respectively (see Scheme 31), but these constructs did not contain GPI anchors of natural structures.

6. Synthesis of a Human Sperm CD52 Antigen GPI Anchor Containing an Acylated Inositol by the Guo Group (2003)

The second mammalian GPI anchor to be chemically synthesized was that of the human sperm CD52 antigen by the Guo group in 2003.⁹⁵ Related synthetic studies by the Guo group included the total synthesis of CD52 GPI-peptide/glycopeptide conjugates bearing GPI partial structures (see Scheme 31).^{30,31} In addition to the intriguing and therapeutically relevant biological activities^{96,97} of the sperm and lymphocyte CD52 antigens, these GPI-anchored glycopeptides exhibit a short peptide chain (12 amino acids) and interesting GPI structures,^{98,99} making them an ideal model for synthetic studies on GPIs and GPI-anchored molecules. Notably, the 2003 report by Xue and Guo was the first synthesis of a GPI anchor of the CD52 antigen and also the first of a GPI containing an acylated inositol, which is a structural feature present in many GPIs.¹ Acylation at O-2 of the inositol is important during GPI biosynthesis, although it is often deleted after biosynthesis is accomplished, and it also confers resistance of GPIs to cleavage by bacterial PI phospholipase C.^{2,100} To develop a synthetic strategy to access these GPIs for structural and biological studies, the inositol-acylated GPI **148** was targeted for synthesis.

A convergent strategy was used for synthesizing **148** (Scheme 25), whose fully protected precursor **149** would undergo late-stage installation of phosphoethanolamine by phosphoramidite **150**, which exhibited orthogonal protection of the amino group to permit future peptide/glycopeptide coupling. Uniquely, rather than perform phospholipidation at a

late stage as in other reported GPI syntheses, this component was attached to the pseudodisaccharide fragment prior to the key glycosylation reaction. This decision was made because of the observation of an undesired cyclophosphitamidation reaction during the attempted phospholipidation of inositol-acylated pseudopentasaccharide **158**, which in fact generated compound **161** (Scheme 26). Because such reactivity was not observed by other research groups, or on pseudodisaccharide model-systems,¹⁰¹ it was proposed that the coexistence of the 2-*O*-acyl group on the inositol along with the trimannose moiety forced the GPI intermediate **159** into a conformation that favored the intramolecular redox cyclization reaction that was observed. Therefore, the key coupling to give **149** would take place between the trimannose thioglycoside **151** and phospholipidated pseudodisaccharide **152**. The former intermediate was built up from the monosaccharide mannose derivatives **153**, **6**, and **154**, while the latter was synthesized from the inositol derivative **155**, the azido glucosyl fluoride **156**, and phosphoramidite **157**. Glycosyl halides were primarily used as sugar donors, and the phosphoramidite method was used for phosphotriester formation.

The synthesis of trimannose thioglycoside **151** was straightforward and relied on the orthogonal relationship of glycosyl halides and thioglycosides (Scheme 27). The thioglycoside (**153**) of Man-I was obtained from methyl α -D-mannopyranoside (**162**). Benzoylation and sulfuric acid-promoted acetolysis of **162** gave diacetate **163**, which on BF₃-promoted glycosylation with ethanethiol and subsequent deacetylation at O-6 provided **153**. Koenigs–Knorr glycosylation of the Man-I acceptor **153** by the Man-II glycosyl chloride **6**⁵⁶ in the presence of AgOTf provided α -dimannoside **164** in 82% yield with complete stereoselectivity. Removal of the temporary stereodirecting 2-*O*-acetyl group on Man-II generated **165**, which was further glycosylated by glycosyl bromide **154** (also formed from **163** by treatment with HBr/AcOH), under the influence of AgOTf, to give the α -trimannoside product **166** in moderate (53%) yield. Finally, the 6-*O*-acetyl group on Man-III was exchanged for a TBS group to furnish **151**.

Motivated by the typical 50% material loss when resolving inositol enantiomers, Guo devised an inositol preparation based largely on tin-mediated chemistry for utilizing both enantiomers, namely (+)-**20** and (–)-**20**, to improve efficiency (Scheme 28). Compound (+)-**20** underwent stannylene acetal-directed allylation at O-6, leaving the O-1 position free for *p*-methoxybenzylation. Next, the less-stable *trans*-cyclohexylidene ring was selectively methanolized under acidic conditions, freeing the O-4 and O-5 positions for benzylation. The product from this sequence (**167**) was treated under acidic conditions for a prolonged period to cleave the more stable *cis*-cyclohexylidene ring, thus generating a diol whose O-3 position was selectively benzylated by using tin chemistry. This route gave the inositol derivative **168**, which was also accessed from the enantiomer (–)-**20** by using the opposite sequence. Dibenzoylation of (–)-**20** was followed by removal of the *trans*-cyclohexylidene group, selective allylation at O-6, and benzylation at O-5. The product **169** converged to the common intermediate **168** by cleavage of the *cis*-cyclohexylidene substituent and selective *p*-methoxybenzylation at O-1. Transformation of **168** into the correct derivative involved esterification of the free axial alcohol with hexadecanoic (palmitic) acid in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), followed by Pd-mediated deallylation to give the 2-*O*-acylated inositol **155**.

Synthesis of the phospholipidated pseudodisaccharide **152** was completed as shown in Scheme 29. The azido glucosyl fluoride building block **156** was prepared from the known anhydro sugar **170**.¹⁰² First, acetolysis of **170** gave the diacetate **171**, whose anomeric acetate was exchanged for fluoride through selective deacetylation and treatment with DAST. Deacetylation at O-6 followed by benzylation gave donor **156**, which underwent coupling with **155** in the presence of Cp₂HfCl₂/AgOTf.⁴¹ An anomeric mixture was

generated in 70% yield ($\alpha:\beta = 4:3$), from which the desired α -pseudodisaccharide **172** was isolated in 40% yield. Oxidative hydrolysis of the PMB group gave compound **173**, which was then phosphorylated with phosphoramidite **157** in the presence of 1*H*-tetrazole. *In situ* oxidation occurred on treatment with *t*-BuOOH, and subsequent Pd-mediated deallylation afforded the pseudodisaccharide phosphotriester **152**. Notably, no migration of the 2-*O*-hexadecanoyl group on the inositol was observed during removal of the PMB group or phosphorylation.

Owing to the early installation of the alkylglycerophosphate moiety, the synthetic end-game was considerably shorter than GPI syntheses previously reported (Scheme 30). The key coupling of trimannose thioglycoside **151** and pseudodisaccharide acceptor **152** was carried out with NIS/TfOH in CH₂Cl₂, with the addition of Et₂O to improve α -stereoselectivity. Desilylation at O-6 of Man-III with boron trifluoride etherate in the same vessel afforded the desired pseudopentasaccharide intermediate **149** in 52% yield over two steps. Facile installation of the phosphoethanolamine group by using phosphoramidite chemistry generated the fully protected GPI derivative **174**. An orthogonal 9-fluorenylmethoxycarbonyl (Fmoc) group was used to block the phosphoethanolamine group, a feature designed to allow future coupling with peptides/glycopeptides (see Scheme 31 for related work). In this case, the Fmoc group was selectively removed with DBU in 87% yield, and subsequent hydrogenolytic removal of the remaining protecting groups provided the target GPI **148** in 84% yield.

Xue and Guo's first total synthesis of a GPI anchor of sperm CD52 antigen was founded on well-established synthetic methods, such as the Koenigs–Knorr and Suzuki glycosylations to form glycosidic bonds, and phosphoramidite chemistry to form phosphotriester bonds. However, problems arising from the incorporation of a 2-*O*-acylated inositol group necessitated the development of a novel assembly strategy involving early installation of the phospholipid. Other noteworthy aspects of the synthesis were (i) an efficient preparation of inositol **168** that employed both enantiomers of the starting material, and (ii) orthogonal protection of the Man-III phosphoethanolamine group for future peptide coupling. Concerning the latter feature, the Guo group published closely related chemical syntheses of GPI-peptide/glycopeptide conjugates **175** and **176** in 2003 and 2004 (Scheme 31).^{30,31} Since they contained only GPI partial structures (without the glycerolipid moiety), they are not discussed in detail here. Current research efforts by the Guo laboratory concerning the synthesis of GPI-anchored peptides/proteins are focused on developing chemoenzymatic methods,^{33–35} which likewise are not covered here.

7. Synthesis of a *P. falciparum* GPI Anchor Containing an Acylated Inositol by the Fraser-Reid Group (2005)

The Fraser-Reid group explored the consequences of incorporating a 2-*O*-acylated inositol into GPIs in parallel with, but independent of, the Guo group. A brief 2004 communication by Jayaprakash and Fraser-Reid¹⁰³ describing the synthesis of a *P. falciparum* GPI “prototype” (bearing unnatural truncated lipids) was followed by a comprehensive 2005 report¹⁰⁴ that included a fully lipidated target GPI anchor. The broader implications of this work lie in the fact that some protozoan parasites, including the malaria-causing agent *P. falciparum*, use free GPI anchors and GPI-anchored proteins to modulate the immune system of the host. As a result, the development of synthetic GPI-based constructs as anti-parasitic vaccines has emerged as a promising area of research.¹⁰⁵

Fraser-Reid's group set out to synthesize the GPI anchor **177** of *P. falciparum* bearing an acylated inositol (Scheme 32). In contrast to Guo's early-stage phospholipidation to avoid a side reaction, Fraser-Reid disconnected the target to pseudopentasaccharide **178**, which after

introduction of phosphoethanolamine would undergo late-stage acylation at O-2 of the inositol and phospholipidation at O-1 via an intermediate orthoester. The preparation of **178** made use of a linear assembly strategy involving consecutive mannosylations of pseudodisaccharide **181** with *n*-pentenyl orthoester (NPOE)⁷³ building blocks **182**, **72**, and **183**. A novel strategy was developed to enhance the α -stereoselectivity in the formation of pseudodisaccharide **181**. Rather than employing an azido glucosyl donor, which often gives low stereoselectivity, the authors used the mannosyl NPOE **184**, which enhanced stereoselective formation of the glycosidic bond while acting as a latent azido glucose unit via inversion at the 2-position with an azide nucleophile. The synthesis of **177** was thus centered on the versatility of NPOEs as building blocks.

The mannosyl NPOEs **182–184** were prepared from the readily available precursor **70**⁶⁸ (Scheme 33). The NPOE **182** of Man-I was obtained by silylation at O-6 followed by benzylation. The Man-III diacetate **183** was generated in a single flask from precursor **70** by sequential treatment with TrtCl and Ac_2O . The latent azido glucosyl building-block **184**, at this stage in the form of a mannosyl NPOE, was synthesized by tin-mediated selective dibenylation at O-3 and O-6, leaving the O-4 position available for *p*-methoxybenzylation.

Pseudodisaccharide **181** was prepared according to Scheme 34. De novo synthesis of the inositol building-block **185** from methyl α -D-glucopyranoside (**186**) was performed by using a modified version of Bender's method.¹⁰⁶ After elaboration of the glucoside **186** to the inositol derivative **190**, a sequence highlighted by stereocontrolled Hg(II) -mediated Ferrier rearrangement and ketone reduction, conversion into compound **185** was accomplished by deacetylation and *cis*-cyclohexylidenation. Stereoselective glycosylation of the inositol acceptor **185** by the mannosyl NPOE **184** under activation by NIS/Yb(OTf)_3 in CH_2Cl_2 afforded the desired pseudodisaccharide **191** in an impressive 98% yield. Transformation into the desired azido glucose (2-equatorial) configuration was accomplished by debenzoylation, triflation at O-2, and azide displacement by DeShong's procedure ($\text{TMSN}_3/\text{TBAF}$),¹⁰⁷ although the desired product **193** was obtained in only 39% yield. Nevertheless, the concept introduced by Fraser-Reid is an attractive one, given first the notoriously low stereoselectivity of azido glucose–inositol couplings, and second the prospect that azide displacement might be optimized. Finally, pseudodisaccharide acceptor **181** was obtained by treatment of **193** with boron trifluoride etherate to cleave the PMB group.

Stepwise elongation of the pseudodisaccharide by mannose building blocks took advantage of the propensity of NPOE donors to form α -configured products (Scheme 35). Thus, acceptor **181** reacted with the NPOE **182** of Man-I in the presence of $\text{NIS/BF}_3\cdot\text{OEt}_2$, providing α -pseudotrisaccharide **194** in 79% yield. Protecting-group manipulations of the Man-I unit furnished compound **195**, which had a free O-6 position, and this was linked to Man-II **72** under the same activation conditions to form the α -pseudotetrasaccharide **196** in excellent yield (99%). Debzoylation at O-2 of the Man-II component gave alcohol **197**, which was elongated with the NPOE **183** of Man-III, again activated by $\text{NIS/BF}_3\cdot\text{OEt}_2$, to generate the all- α -linked pseudopentasaccharide **198** in 75% yield. The acyl protecting-groups on Man-III were replaced by benzyl groups, and subsequent removal of the O-6 trityl group of Man-III by mild acid gave intermediate **178**, which was ready for the three phosphorylation reactions.

Completion of the GPI synthesis is shown in Scheme 36. Introduction of the phosphoethanolamine group to the Man-III O-6 position of pseudopentasaccharide **178** using the phosphoramidite method was successful. Cleavage of the 1,2-cyclohexylidene acetal group from the inositol was accomplished with CSA/ethylene glycol to give diol **199**. Model studies by Fraser-Reid predicted that introduction of an acyl group at the axial O-2 position of **199** using manipulations of protecting groups would be precarious, and so an

alternative approach involving intermediate cyclic orthoesters was pursued. The intermediate diol **199** was therefore allowed to react with the trimethyl orthoester **179** in the presence of CSA to give the intermediate GPI-orthoester **200**, which underwent rearrangement with Yb(OTf)₃ to provide a 2:1 mixture of regioisomers in favor of the desired 2-*O*-acylated product **201** (isolated in 50% yield). Despite Guo's observation that attempted late-stage phospholipidation was thwarted by the coexistence of 2-*O*-acylated inositol and the trimannose moiety (see Scheme 26), Fraser-Reid encountered no problems in converting compound **201** into **202** (60–70%) using phosphoramidite chemistry, suggesting that minor structural differences in the substrate and/or phosphoramidite reagent can impact the reaction outcome. Global deprotection by Pd-catalyzed hydrogenolysis, although challenging because of unanticipated solubility issues, was eventually completed to deliver the target GPI anchor **177**.

In summary, the Fraser-Reid group accomplished the second total synthesis of an inositol-acylated GPI anchor using a linear-assembly strategy in concert with NPOE chemistry. Four mannosyl NPOEs, all accessed from a single synthetic precursor (**70**), were used for stereoselective glycosidic-bond formation, including the glucosamine–inositol linkage. In the latter instance, a mannose-configured NPOE donor was employed to facilitate the typically difficult α -glycosylation of inositol, and after serving this purpose was converted into an azido glucose moiety by invertive introduction of an azido group at the 2-position. Also contributing to the overall efficiency of the synthesis was the use of Bender's de novo inositol synthesis, which obviated the need for cumbersome enantiomeric resolution protocols. In the final synthetic sequence, an inositol 1,2-orthoester intermediate was used to attach regioselectively the correct (phospho)lipids to the GPI.

8. Synthesis of a *P. falciparum* GPI Anchor Containing an Acylated Inositol by the Seeberger Group (2005)

The Seeberger laboratory became involved in the study of GPI anchors in the early 2000s, when they used chemical synthesis to construct a GPI-based anti-toxin vaccine against malaria, which was effective in a rodent model of the disease.¹⁰⁸ An intriguing aspect of this project was the application of automated solid-phase oligosaccharide synthesis for rapid generation of large quantities of GPI intermediates, which could be elaborated to conjugate vaccines.¹⁷ These exciting developments offered further support to the notion that synthetic GPIs—indeed, carbohydrates in general—can be harnessed to develop novel therapeutic approaches.¹⁰⁵ Seeberger expanded on this early work in 2005 with the solution-phase total synthesis of a fully lipidated GPI anchor of *P. falciparum*.

The inositol-acylated GPI anchor **203** was almost identical in structure to that synthesized by Fraser-Reid (compound **177**, see Scheme 32), but it contained the additional mannose unit present in natural GPIs from *P. falciparum* (Scheme 37). The target compound was accessed from the GPI pseudohexasaccharide **204**, which had orthogonal protecting-groups, including PMB, allyl, and triisopropylsilyl (TIPS), for sequential installation of hexadecanoyl, phospholipid, and phosphoethanolamine groups. For the two phosphorylation events, the *H*-phosphonate method⁴⁴ was used. Intermediate **204** was formed convergently from the tetramannosyl trichloroacetimidate **207** and pseudodisaccharide alcohol **208**, which were both prepared in a straightforward manner using the building blocks **41**, **44**, and **209–211**. Schmidt's trichloroacetimidate method,⁶³ in combination with 2-*O*-acyl protecting groups, was used for the stereocontrolled formation of glycosidic bonds.

Synthesis of the tetramannose fragment **207** involved extension of the Man-I acceptor **209** using mannosyl trichloroacetimidate donors (Scheme 38). Compound **209** was glycosylated by imide **41** in the presence of TMSOTf, generating dimannoside **212**, which then

underwent selective deacetylation by treatment with AcCl/MeOH to give product **213** in 88% yield over two steps. This process was repeated twice more, first using the TIPS-protected Man-III donor **210** followed by reuse of donor **41** to install the Man-IV group, which afforded tetramannoside **216** (58%, three steps). Conversion of the anomeric allyl group into trichloroacetimidate **207** was accomplished by Pd-mediated deallylation and subsequent treatment with trichloroacetonitrile and DBU.

The pseudodisaccharide fragment was efficiently prepared starting from the known inositol diol **217** generated from compound **190** by deacetylation/allylation (Scheme 39).^{106,109} Selective protection at O-2 of **217** with a PMB group allowed for late-stage hexadecanoylation of this site. The union of the resulting product **211** and the azido glucosyl trichloroacetimidate **44**⁶³ under standard activation conditions yielded the α,β mixture **218** in 89% yield with good stereoselectivity ($\alpha:\beta = 4:1$). This inseparable mixture was carried forward by deacetylation to give a triol, which underwent successive 4,6-*O*-benzylidenation, benzylation, and regioselective benzylidene cleavage to afford the α anomer **208**, which was separated from the corresponding β anomer at this stage.

The key glycosylation between tetramannosyl trichloroacetimidate **207** and pseudodisaccharide alcohol **208** was performed in the presence of TMSOTf, while the 2-*O*-benzoyl group of the donor ensured α -stereoselectivity through neighboring-group participation (Scheme 40). The reaction generated pseudoexasaccharide **204** in 94% yield, and the subsequent transformation of this to the intermediate **220** required deacylation followed by benzylation. The fully protected GPI intermediate **220** could be obtained in nearly gram quantities, an important accomplishment considering the large amount of material required for generating and testing antimalarial vaccine candidates.

The remaining steps centered on lipidation and phosphorylation events. After oxidative removal of the PMB group at O-2 of the inositol, the axial hydroxyl group thus exposed was esterified with hexadecanoic acid in the presence of DCC and DMAP to give **221**. Subsequent removal of the neighboring allyl group at O-1 of the inositol to furnish compound **222** was successfully performed by using an excess of PdCl₂ without acyl migration or decomposition. This outcome was in contrast to Fraser-Reid's previously reported difficulties in a nearly identical system. The phospholipidation step was performed by using *H*-phosphonate **205** in the presence of PivCl and pyridine, and this was followed by iodine-mediated oxidation, to give **223** without any side reactions involving the azido group. After the 6-*O*-TIPS group on Man-III had been hydrolyzed in the presence of Sc(OTf)₃ to give compound **224**, the *H*-phosphonate method was again used to install the phosphoethanolamine group. Final deprotection of intermediate **225** was effected by Pd-catalyzed hydrogenolysis in a mixture of solvents, which provided the target GPI **203** in excellent (94%) yield.

Seeberger's synthesis of the *P. falciparum* GPI anchor **203** bearing an acylated inositol group featured a logical and straightforward convergent assembly strategy based on previous GPI syntheses. Proven methods were used for the formation of glycosidic and phosphodiester bonds, while difficulties encountered by the Guo and Fraser-Reid groups in the late-stage lipidation and phosphorylation steps were avoided. Impressively, gram-scale quantities of complex pseudoexasaccharide intermediates were obtained on scale-up, a critical point given the group's development of GPI-based antimalarial vaccines. Subsequent advances in this area by the Seeberger group include the 2008 development of synthetic GPI glycan arrays to characterize malaria-induced antibody responses,^{110,111} which should aid in the design of improved vaccine candidates. Also reported by the Seeberger laboratory in 2008 was a project describing the semisynthesis of a GPI-anchored prion-protein construct, which hinged on the use of native chemical ligation to couple the GPI and protein

fragments.²⁹ As these research efforts involved partial GPI structures (missing the phosphoglycerolipid), they are not covered in detail here, but the target GPI--prion conjugate **226** is shown in Scheme 41.

9. Synthesis of a *T. cruzi* GPI Anchor by the Vishwakarma Group (2005)

In 2000, Ferguson and co-workers discovered that GPIs from *T. cruzi*, the causative agent of Chagas' disease, have potent proinflammatory activity. This leads to various pathologies and, interestingly, was attributed to unsaturated fatty acids at the *sn*2 position of the GPI acylalkylglycerophosphate.¹¹² In 2005, the Vishwakarma group reported the first synthesis of a *T. cruzi* GPI anchor,¹¹³ although the authors targeted a GPI containing saturated lipids that was more synthetically accessible and would be compatible with global benzyl-group protection (for the synthesis of *T. cruzi* GPIs bearing naturally occurring unsaturated lipids and a 2-aminoethyl phosphonate group, see Nikolaev's work in section III-1).

Vishwakarma's chemical synthesis of the GPI anchor **227** adopted a convergent strategy resembling previous syntheses by other groups (Scheme 42). The key intermediate **228** was obtained from compounds **230** and **231**, the former of which was constructed in a [2 + 2] fashion using disaccharides **232** and **233** rather than the typical linear elongation of a Man-I building block. Notably, pseudodisaccharide **231** was prepared from the racemic inositol derivative **11** and azido glucosyl trichloroacetimidate **44**, with the latter being conveniently used as a chiral auxiliary for inositol resolution, eliminating the need for lengthy routes for resolution used in previous GPI syntheses. Glycosyl NPOE⁷³ and trichloroacetimidate donors⁶³ were used for glycosylations, while both phosphoramidite⁶⁰ and *H*-phosphonate⁴⁴ methods were used for phosphorylations.

The [2 + 2] construction of tetramannosyl donor **230** from dimannosides **232** and **233** is shown in Scheme 43. Allyl α -D-mannopyranoside (**128**) was used as a common starting material for three of the four mannose building blocks. En route to **232**, compound **128** was advanced by tritylation at O-6, perbenzylation at the O-2, 3, and 4 positions, and detritylation to give **234**. Subsequent α -stereoselective glycosylation of **234** by the mannosyl NPOE **72** in the presence of NIS and TESOTf followed by debenzoylation furnished compound **232** (92%, two steps). The other dimannoside **233** was accessed from monomers **235** and **236**, each of which arose from allyl mannoside **128**, the first by successive benzylation, deallylation, and 1-*O*-trichloroacetimidation and the second by sequential 4,6-*O*-benzylidenation and selective benzylation at O-3. Compounds **235** and **236** were then joined via Schmidt glycosylation in 81% yield. A series of subsequent protecting-group manipulations were used to generate **233**, including concomitant deallylation/benzylidene hydrolysis with treatment with *t*-Bu OK then HCl, peracetylation, selective anomeric deacetylation with MeNH₂, and finally treatment with DBU and trichloroacetonitrile. The [2 + 2] coupling of **232** and **233** under activation by TMSOTf proceeded in moderate (65%) yield, perhaps because of the lack of a participating 2-*O*-acyl group on the donor. Subsequent transformation into the tetramannosyl donor **230** was completed by Pd-mediated anomeric deallylation and treatment with DBU and trichloroacetonitrile.

In the preparation of pseudodisaccharide **231** (Scheme 44), the azido glucosyl group was used as an efficient chiral auxiliary, and this eliminated the yield-depleting techniques for inositol resolution that are traditionally used. The racemic mixture of inositols ($\pm$)-**11** was treated with the azido glucosyl trichloroacetimidate **44** in the presence of TMSOTf to generate a 1:1 mixture of diastereomers **237** and **238**. The high α -stereoselectivity achieved using donor **44**, as previously reported by Schmidt and other groups, prevented the formation of a complex mixture of products. As **237** and **238** were inseparable at this stage, they were subjected to deacetylation followed by 4,6-*O*-benzylidenation, which produced

the separable diastereomers **239** and **240**. The desired isomer **239** was carried forward to compound **231** by benzylation and regioselective benzylidene cleavage. This approach for resolving inositol enantiomers was efficient, and should be useful in future GPI syntheses.

Vishwakarma's completion of the synthesis of GPI anchor **227** (Scheme 45) commenced with the coupling of **230** and **231**, promoted by TMSOTf, to afford the α -linked pseudohexasaccharide **228** in 70% yield. The product was designed to permit orthogonal protection of the O-4 and O-6 positions of Man-III to allow first the installation of phosphoethanolamine, and second the synthesis of 4-deoxy Man-III GPI analogues for biological studies. In this synthesis, the Man-III moiety was subjected to deacetylation, 6-*O*-silylation, 4-*O*-benzylation, and finally desilylation. This lengthy sequence furnished intermediate **241**, which was then treated with phosphoramidite **58** in the presence of 1*H*-tetrazole, followed by *in situ* oxidation with *m*-CPBA, to install the phosphoethanolamine group. Phospholipidation of the inositol was accomplished by CAN-mediated oxidative cleavage of the PMB group, followed by coupling with building block **229** using the *H*-phosphonate method. Global deprotection by hydrogenolysis over Pd(OH)₂/C provided the target GPI **227** in 73% yield.

Vishwakarma and co-workers thus accomplished the first chemical synthesis of a *T. cruzi* GPI anchor. Overall, standard building blocks, assembly strategies, and carbohydrate synthetic methods were used to access the target. Of note was the efficient preparation of the pseudodisaccharide, which was highlighted by inositol resolution using the azido glucosyl group as a chiral auxiliary.

10. Synthesis of a Fully Phosphorylated and Lipidated Human Sperm CD52 Antigen GPI Anchor by the Guo group (2007)

In 2007, Wu and Guo reported the total synthesis of a fully phosphorylated and lipidated GPI anchor of the human sperm CD52 antigen,^{114,115} which followed their 2003 synthesis of a related CD52 antigen GPI that contained an acylated inositol but lacked the phosphoethanolamine group at O-2 of Man-I of the natural product (see section II-6). Thus, the target molecule **245**, bearing an additional phosphoethanolamine unit, was synthesized by a similar convergent strategy (Scheme 46). To install both phosphoethanolamine groups, a late-stage double phosphorylation using phosphoramidite **58** was performed on the intermediate GPI diol **246**, which in turn was accessed from trimannose thioglycoside **247** and phospholipidated pseudodisaccharide **248**. The latter compound was obtained by consecutive glycosylation, lipidation, and phospholipidation of inositol **249** by building blocks **104**, **250**, and **251**. For acylation of the inositol, the unsaturated hexadecane-9-enoyl group was used to act as either a latent aldehyde (on oxidative cleavage of the C=C bond) for conjugation chemistry, or a latent hexadecanoyl group (on reductive global deprotection).

The synthesis of phospholipidated pseudodisaccharide **248** is shown in Scheme 47. The required inositol derivative **249** was readily accessed from the previously reported compound **168** by acetylation followed by deallylation. Reminiscent of a previous route by Ley (see Scheme 19), glycosylation of **249** by the azido glucosyl bromide **104** under Lemieux conditions⁴² generated pseudodisaccharide **252** in moderate (56%) yield but with complete α -stereoselectivity. Deacetylation at O-2 of the inositol was followed by esterification with hexadec-9-enoic acid in the presence of DCC/DMAP to give **254**, which then underwent removal of the PMB group with CAN. Phospholipidation of **255** using phosphoramidite **251** in the presence of 1*H*-tetrazole followed by *t*-BuOOH-mediated oxidation provided an intermediate phosphotriester, which was subjected to desilylation with boron trifluoride etherate to give **248**. Phospholipidation was performed at this stage rather

than later, so as to prevent the cyclophosphitamidation side-reaction previously discussed (see Scheme 26).

Completion of the target molecule is shown in Scheme 48. Prior to the key glycosylation, the trimannose thioglycoside **247** (synthesized according to Guo's previous route, see Scheme 27) was converted into trichloroacetimidate **256** via NIS-mediated hydrolysis of the thioacetal followed by reaction with DBU and trichloroacetonitrile. The union of trichloroacetimidate **256** and pseudodisaccharide **248** under Schmidt glycosylation conditions formed the desired intermediate, which was immediately treated with boron trifluoride etherate to remove simultaneously the TBS and PMB groups. This sequence provided the α -pseudopentasaccharide diol **246** in moderate (40%) yield over two steps. Double phosphorylation of **246** using phosphoramidite **58**, a difficult task, proceeded in 50% yield to give the fully protected GPI **257**. Global deprotection was accomplished by treatment with DBU to remove the cyanoethoxyl group, followed by debenzoylation using Pd-catalyzed hydrogenolysis to afford the target GPI **245** in 82% yield.

In summary, the synthesis of **245**, a fully phosphorylated and lipidated GPI, was guided by Guo's 2003 synthesis of a CD52 antigen GPI while using an orthogonal protection strategy that enabled late-stage double phosphorylation. The target molecule and related GPI derivatives were used to investigate their relative abilities to bind CAMP factor, a pore-forming toxin secreted by *Streptococcus agalacticae* that is thought to utilize GPI anchors as its binding partners.¹¹⁵ Initial results suggested that both the intact GPI anchor **245**, and its corresponding truncated phospholipidated pseudodisaccharide, bound CAMP factor with similarly strong affinities. In general, such studies using well-characterized synthetic GPIs and GPI derivatives should prove instrumental in elucidating GPI structure–activity relationships in various contexts.

III. Diversity-Oriented Approaches to GPI Synthesis

The so-called classic GPI syntheses thus far detailed here were accomplished by strategies having limited potential for structural diversity, and this can be attributed primarily to the global protection tactics used. Traditionally, carbohydrate chemists have used benzyl groups for permanent protection of hydroxyl groups because of their ease of installation, stability under a broad range of reaction conditions, numerous choices for orthogonal protection, and mild conditions for global deprotection. However, the latter feature, deprotection via Pd-catalyzed hydrogenolysis, constitutes a considerable synthetic shortcoming given its incompatibility with many important functional groups. In this category are alkenes, alkynes, azides, thiols, thioethers, and other potentially reducible or catalyst-poisoning functionalities. Therefore, global protection by benzyl groups in GPI synthesis precludes the incorporation of the biologically important unsaturated fatty acids and indispensable functional handles such as “click” chemistry, imaging, and affinity-purification tags. Furthermore, the natural diversity of GPIs arising from core structure modifications, such as additional glycans, phosphoethanolamine groups, and variation in lipids (see Figure 1), has prompted the development of newer general methods for efficient synthesis of a range of GPIs from common building-blocks. Research at the recent forefront of GPI synthesis has been focused on developing diversity-oriented synthetic strategies to address these drawbacks. Progress in this area by the Guo, Nikolaev, and Seeberger groups is discussed in this section.

1. Synthesis of *T. cruzi* GPI Anchors Containing Unsaturated Lipids by the Nikolaev Group (2006)

As already mentioned, Ferguson and co-workers identified GPIs from *T. cruzi* exhibiting potent proinflammatory activity that was thought to be associated with unsaturated fatty

acids in the *sn*2 position of the GPI acylalkylglycerophosphate.¹¹² In an effort to establish rigorous structure activity relationships in this system, the Nikolaev group set out to synthesize *T. cruzi* GPIs containing the unsaturated fatty acids present in the natural structure.¹¹⁶ The GPI anchors **258** and **259** (Scheme 49), bearing the potentially crucial *sn*2-oleoyl [(*Z*)-octadec-9-enoyl] and linoleoyl [*cis,cis*-octadec-9,11-dienoyl] groups, respectively, were targeted for chemical synthesis.

Nikolaev's 2006 publication was the first total synthesis of GPIs containing unsaturated lipids, and accordingly required the development of a novel strategy to obviate the need for reductive conditions employed in global benzyl-group deprotection. Acyl-based permanent protection of hydroxyl groups in the form of benzoic esters was chosen, despite potential compatibility issues with the ester-linked lipid in the acylalkylglycerophosphate moiety. The authors' hypothesis was that, upon deprotection by mild base in a polar solvent, the nascent amphiphilic GPI intermediate may undergo micelle formation, thereby protecting the bond of the fatty ester during selective removal of the benzoic esters. Further increasing the synthetic challenge, the target GPIs had a 2-aminoethylphosphonate group linked to O-6 of the glucosamine component, which is a parasite-specific modification of some GPIs.

The retrosynthesis of GPIs **258** and **259** is shown in Scheme 49. Both targets were accessed from the pseudohexasaccharide **260**, which was poised to undergo a triad of phosphorylation events with building blocks **261**, **262**, and **263/264**, to install the 2-aminoethylphosphonate, phosphoethanolamine, and phosphoglycerolipid components, respectively. Intermediate **260** was assembled convergently from the benzoyl-protected tetramannosyl donor **265** and a derivative of Schmidt's pseudodisaccharide **52**, which in turn were built up from their respective monomers. For all steps involving glycosidic-bond formation, the Schmidt method⁶¹ using glycosyl trichloroacetimidate donors was applied.

The tetramannose fragment **265** was prepared by the stepwise joining of mannose monosaccharides **267–270** (Scheme 50), a process entailing sequential stereoselective α -mannosylations, each aided by neighboring-group participation. The Man-I component (**267**) was coupled with the Man-II component (**268**) in the presence of TMSOTf, providing an α -dimannoside in 94% yield that was subjected to selective deacetylation at O-2 of the Man-II component with HCl/MeOH to give compound **271**. The same two-step sequence was used to extend the glycan chain with the Man-III component (**269**, 96% yield) and expose the O-2 position of the Man-III component. Subsequent reaction of this site with the Man-IV component **270**, again promoted by TMSOTf, afforded fully α -configured tetramannose product **273** in nearly quantitative yield. The O-6 benzyl group on the Man-III precursor was then exchanged for a TBS group via Pd-catalyzed hydrogenolysis, followed by treatment with TBSOTf/Et₃N. Finally, the anomeric benzoyl group of the Man-I precursor was converted into a trichloroacetimidate by regioselective debenzoylation with ethylenediamine followed by treatment with Cl₃CCN/Cs₂CO₃.

For the pseudodisaccharide fragment, the authors converted known compound **52**, previously described by Schmidt (see Scheme 9 for its preparation),⁵⁸ into a suitably protected derivative containing only acid-labile groups whose late-stage removal would not interfere with the unsaturated lipid chains. However, rather than perform the separation of inositol diastereomers **43** and **51** as reported in the literature,^{58,117} it was found that conducting the glycosylation directly on the diastereomeric mixture afforded readily separable pseudodisaccharide products **52** and **275** (Scheme 51), indicating that the azidoglucose component acted as a supplementary chiral auxiliary. Notably, this approach relied on the exceptional α -stereoselectivity observed in the glycosylation reaction previously fine-tuned by Schmidt,⁵⁸ otherwise a complex and possibly inseparable mixture of isomers would have been generated.

From Schmidt's pseudodisaccharide **52**, several protecting-group manipulations were required to obtain **266**. First, the azidoglucose component was deacetylated and then treated with $\text{PhC}(\text{OEt})_3$ under acidic conditions to form a 4,6-orthoester derivative. Subsequent protection of the free O-3 position with an acid-labile 2-trimethylsilylethoxymethyl (SEM) group, using $\text{SEMCl}/i\text{-Pr}_2\text{Net}$, gave compound **276**, which was subjected to cleavage of the inositol 1-*O*-(α)-menthylcarbonate group with NaOMe . Protection of the exposed hydroxyl group with a TBS ether was accomplished by treatment with $\text{TBSOTf}/\text{Et}_3\text{N}$ to give the protected pseudodisaccharide **277**. The final sequence involved removal of the 4,6-orthoester from the azidoglucose, followed by selective triethylsilylation at O-6. This synthetic route from **52**, while somewhat lengthy, was very efficient, providing **266** in 50% yield over seven steps.

Elaboration of the tetramannose and pseudodisaccharide fragments to the target GPI anchors is shown in Scheme 52. The key glycosylation reaction between the tetramannosyl trichloroacetimidate **265** and pseudodisaccharide **266** proceeded smoothly, giving the fully protected α -pseudohexasaccharide **260** in 71% yield. At this stage, three phosphorylation events were required to decorate the GPI with the appropriate structures found in GPIs of *T. cruzi*. First, the unique 2-aminoethylphosphonate group was installed at O-6 of the azidoglucose in three steps, including selective cleavage of the TES ether using AcOH -buffered TBAF, 1*H*-tetrazole-promoted phosphorylation with 2-azidoethylphosphonodichloridate **261** (generated from 2-bromoethylphosphonate in two steps), and methanolysis of the intermediate chlorophosphonate to give **278**. Subsequent Staudinger reduction and treatment with Boc anhydride converted the azido groups into *N*-Boc-protected amino groups. The next transformation involved selective O-6 desilylation of the Man-III TBS group with triethylamine trihydrofluoride, which exposed this position for introduction of the phosphoethanolamine unit by using *H*-phosphonate derivative **262** in the presence of pivaloyl chloride, followed by *in situ* iodine-mediated oxidation to phosphodiester **280**. Subsequent removal of the TBS from O-1 of the inositol under strong conditions, namely TBAF/ AcOH at 55 °C, set the stage for installation of the unsaturated lipid-containing acylalkylglycerophosphate components. Again, the *H*-phosphonate method was successfully employed, resulting in the phospholipidation of **281** with precursors **263** or **264** to introduce oleic [(*Z*)-octadec-9-enoic] or linoleic (*cis,cis*-octadec-9,11-dienoic) acids, respectively, into the GPI.

The global deprotection of fully protected GPI intermediates **282** and **283** required three reactions. First, demethylation of the 2-aminoethylphosphonate group was effected by using PhSH and Et_3N . The second and most critical deprotection step required base-promoted debenzoylation, a process that risked cleavage of the GPI fatty esters. Nikolaev used 0.05 M NaOMe in MeOH , which successfully delivered the products, albeit in moderate (38–40%), yields, most probably a result of poor selectivity. Finally, the purified intermediates were subjected to treatment with aqueous 90% TFA to hydrolyze the remaining acetal and *N*-Boc protecting groups, which proceeded with high efficiency to afford, after purification by reverse-phase chromatography, the target GPIs **258** and **259**.

Overall, use of benzoyl groups as an acyl-based global-protection strategy to access GPIs containing unsaturated lipids was successful, but compatibility issues with acylglycerophosphates render this approach a precarious one for synthesizing functionalized GPIs. Shortly after their 2006 publication, Nikolaev and co-workers adapted their synthetic strategy by using acetal- and silyl-based protection in lieu of acyl-based protection to address this issue.¹¹⁸ In this work, a convergent [3 + 3] strategy was used to synthesize GPI **258** from the trimannose thioglycoside **284** and pseudotrisaccharide **285**, which were both permanently protected with acetal or silyl groups (Scheme 53). Briefly, these two components combined in the presence of methyl triflate and 2,6-di-*tert*-butyl-4-

methylpyridine (DTBMP), with subsequent selective removal of the TES group using TBAF and AcOH to generate α -pseudo-hexasaccharide **286** in 75% yield. The 2-aminoethylphosphonate, phosphoethanolamine, and alkyl oleoylglycerophosphate groups were installed as previously to arrive at the fully protected GPI **290**. Final deprotection was accomplished by a two-step sequence comprising desilylation with triethylamine trihydrofluoride and TFA-promoted acetal hydrolysis. The target oleoyl [(Z)-octadec-9-enoyl] GPI **258** was obtained in enhanced (67%) yield over two steps, although the desilylation step required 18 days to complete.

In summary, Nikolaev's innovative strategies for access to diversely structured GPIs were used for the synthesis of biologically important GPIs of *T. cruzi* containing unsaturated lipids. Both strategies were focused on using alternative protecting groups that would not require Pd-catalyzed hydrogenolysis for their removal. Although acyl-based protection probably led to compatibility issues with the acylalkylglycerophosphate during global deprotection with NaOMe, an improved strategy based on acetal and silyl protection was developed that featured a higher-yielding, but time-consuming, global deprotection. Synthetic GPIs **258** and **259** were subjected to a preliminary biological evaluation, which showed activity similar to that of the naturally occurring GPIs in a Toll-like receptor stimulation-assay.¹¹⁶ More detailed biological studies of these compounds are ongoing.

2. Synthesis of a GPI Anchor Containing Unsaturated Lipid Chains by the Guo group (2010)

In an effort to develop a robust approach for the synthesis GPI anchors bearing unsaturated lipids and other important functionalities, the Guo group focused on a global hydroxyl-protection strategy based on the PMB group, which can be removed under relatively mild acidic conditions, such as 5–10% TFA, or oxidation, as with CAN or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). It was expected that PMB protection would offer a major improvement in functional-group tolerance during the global deprotection, thus allowing access to highly complex and sensitively functionalized GPI anchors.

In 2010, Swarts and Guo provided a proof-of-concept for the PMB-protection strategy by synthesizing a GPI anchor containing unsaturated lipids,¹¹⁹ specifically a dioleoylglycerophosphate moiety. The retrosynthetic analysis, shown in Scheme 54, was guided by previous convergent syntheses from the Guo group. The target GPI anchor **291** was accessed from intermediate **292**, which was formed in a key glycosylation reaction between the trimannosyltrichloroacetimidate donor **294** and pseudodisaccharide **295**. The trimannose fragment, joined together from PMB-protected monomers **296–298**, contained orthogonal silyl ether protecting-groups on the Man-I and Man-III components for late-stage phosphorylation with the phosphoethanolamine precursor **293**. The pseudodisaccharide fragment, already containing the dioleoylglycerophosphate group at O-1 of the inositol, was synthesized from the optically pure inositol **299**, the azido glucosyl trichloroacetimidate **300**, and the phospholipid precursor **301**.

The preparation of trimannoside **294** involved the α -stereoselective coupling of mannose building blocks **296–298**, which were prepared from a common intermediate, mannose pentaacetate **302** (Scheme 55). The Man-I building block, thioglycoside alcohol **296** bearing a free 6-OH group, was obtained from diol **303**¹²⁰ via sequential stannylene acetal-directed regioselective *p*-methoxybenzylation at O-3, allylation at O-2, and regioselective opening of the *p*-methoxybenzylidene ring using DIBAL-H. the Man-II component **297** was prepared from **302** via orthoester **304**,⁵⁶ which after deacetylation and methoxybenzylation was treated with acetic acid to open the orthoester ring, providing a hemiacetal that was converted into the corresponding trichloroacetimidate. Synthesis of mannose derivative **298**

started with a BF_3 -promoted glycosidation of **302** with allyl alcohol. Subsequent deacetylation gave tetraol **305**, which then underwent differentiation of the 6-position by reaction with *p*-methoxytrityl (PMTTrt) chloride and methoxybenzylation of the 2-, 3-, and 4-positions to generate **306**. After exchanging the PMTTrt protecting group for a TBS group, Pd-catalyzed deallylation freed up the anomeric hydroxyl group for transformation into trichloroacetimidate **298** by treatment with trichloroacetonitrile and DBU. In general, the preparation of PMB-protected mannose building-blocks was comparable to the corresponding benzyl-protected variants in terms of both strategy and efficiency.

With monosaccharides **296–298** in hand, the trimannosyl donor **294** was constructed by using the Schmidt glycosylation method.⁶¹ The coupling of Man-I component **296** and Man-II component **297** proceeded well using catalytic TMSOTf in CH_2Cl_2 , and complete α -stereoselectivity was imparted by the participating acetyl group at O-2. Deacetylation with $\text{K}_2\text{CO}_3/\text{MeOH}$ exposed the O-2 position, resulting in the dimannoside alcohol **308** in 66% yield over two steps. Coupling of **308** with the Man-III trichloroacetimidate **298**, also under Schmidt conditions, gave trimannoside **309** in a 5:1 α : β ratio, a figure that was improved to exclusively α (76% yield) upon changing the reaction solvent to diethyl ether. The trimannose thioglycoside **309** was also used as a divergence point for the preparation of other functionalized GPI anchors (see section III-4). In this instance, compound **309** was elaborated to the trichloroacetimidate **294** in four steps, including titanium-mediated deallylation using Cha's protocol¹²¹ and triethylsilylation to change the protecting group at O-2 of the Man-I component, followed by treatment with NIS/AgOTf/2,4,6-tri-*tert*-butylpyrimidine (TTBP) to hydrolyze the anomeric thioacetal, and final treatment with trichloroacetonitrile and DBU.

For the pseudodisaccharide fragment, the PMB-protected inositol derivative **299** was required (Scheme 56). The O-1 and O-6 positions of racemic diol ($\pm$)-**20** were simultaneously protected as allyl ethers, which were unaffected by the subsequent cyclohexylidene-group removal and *p*-methoxybenzylation reactions. Next, deallylation of the bis-allyl ether ($\pm$)-**311** using Cha's protocol¹²¹ provided inositol derivative ($\pm$)-**312** in 85% yield. For differentiation of the PMB-protected diol ($\pm$)-**312** at O-1 and O-6, regioselective stannylene acetal-directed allylation was used to generate compound ($\pm$)-**299** in 72% yield. Enantiomeric resolution of racemate ($\pm$)-**299** was achieved by acylation of the free O-6 position with the chiral reagent (1*S*)-(-)-camphanic chloride to yield HPLC-separable diastereomers. Finally, the desired, purified diastereomer (-)-**313** was subjected to saponification to provide the enantiomerically pure inositol **299** in 44% yield over two steps (maximum yield 50%).

The synthesis of phospholipidated pseudodisaccharide **295** is depicted in Scheme 57. Preparation of the azido glucosyl donor **300** began with the transformation of compound **314**¹²² into the allyl glycoside **315** via BF_3 -promoted glycosidation with All OH, deacetylation, and formation of the 4,6-*p*-methoxybenzylidene acetal. A PMB group was installed at the O-3 position, which was followed by regioselective opening of the *p*-methoxybenzylidene ring using dry HCl and NaBH_3CN to provide compound **316**. After use of TBSOTf to protect the O-4 position to afford compound **317**, the anomeric allyl ether was removed by Ir(I)-catalyzed isomerization to the corresponding vinyl ether and subsequent hydrolysis by Hg(II).¹²³ The resulting hemiacetal was converted into trichloroacetimidate **300** by using trichloroacetonitrile and DBU.

Coupling of the inositol derivative **299** and azido glucosyl donor **300** was performed in toluene--1,4-dioxane in the presence of TMSOTf to generate the pseudodisaccharide intermediate in 80% yield as an inseparable α , β -mixture (α : β = 2.3:1). Subsequent deallylation of the mixture using the Ir–Hg protocol produced the α -pseudodisaccharide

318, which was readily separable from the accompanying β anomer. Phospholipidation of the inositol at O-1 using phosphoramidite **301**, followed by *in situ* oxidation with *t*-BuOOH installed the dioleoylglycerophosphate moiety to produce compound **319**. Treatment of this intermediate with triethylamine trihydrofluoride cleaved the O-4 TBS group of the azido glucose derivative, which required several days for completion, but cleanly produced the desired pseudodisaccharide **295**.

The final stage of the synthesis centered on union of the trimannose and pseudodisaccharide fragments (Scheme 58). The trimannose trichloroacetimidate **294** reacted smoothly with **295** in the presence of TMSOTf to afford the desired α -pseudopentasaccharide **292** in 64% yield. Overnight treatment of **292** with Et₃N·3HF removed the TBS and TES groups to provide diol **320**. Installation of the phosphoethanolamine unit by treatment with phosphoramidite **293** for a short period (1 h) was selective for the O-6 position of Man-III to give **321**. Alternatively, the diol could be used to access a fully phosphorylated GPI bearing two phosphoethanolamine groups. To obtain the target GPI anchor, compound **291**, a three-step, one-flask protocol was developed to efficiently remove all of the protecting groups from **321** in under 4 hours: first, Zn-mediated reduction of the azide; second, removal of the base-labile Fmoc and cyanoethoxyl groups with DBU; and third, hydrolysis of all PMB ethers with 10% TFA. The target GPI **291** was obtained in 81% yield over the final three steps.

This proof-of-concept synthesis established the PMB-protection strategy as a general approach to the synthesis of functionalized GPIs and potentially other complex functionalized glycoconjugates. Overall, the preparation of PMB-protected monosaccharide and inositol building-blocks was conducted efficiently, and the performance of these intermediates in Schmidt glycosylation reactions was comparable to their benzyl-protected counterparts. The highlight of this convergent synthesis was the three-step, one-flask reaction that afforded the target GPI **291** in 81% yield in under 4 hours. In the next two sections, use of the PMB-protection strategy by the Guo group for access to a complex naturally occurring GPI anchor, as well as “clickable” GPI anchors, is discussed.

3. Synthesis of a Human Lymphocyte CD52 Antigen GPI Anchor Containing a Polyunsaturated Arachidonoyl Lipid by the Guo Group (2011)

The structural diversity of GPI lipids comes about by the “lipid remodeling” process of GPI-anchored proteins.^{14,124–126} GPI biosynthesis begins from cellular phosphatidylinositol, which in mammalian systems typically contains a polyunsaturated arachidonoyl lipid (20:4) at the *sn*-2 position. The arachidonoyl [(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoyl] group can be replaced by other lipids through a series of transformations in the Golgi apparatus, and as a result most mature mammalian GPI-anchored proteins contain only saturated fatty acids. However, several GPI-anchored proteins bearing 2-arachidonoyl phosphatidylinositol have been identified, including a major form of the human lymphocyte CD52 antigen.^{98,127} The effects of unremodeled lipids on the function of lymphocyte CD52 and other GPI-anchored proteins have not been thoroughly explored, although in general it is expected that the structure of GPI lipids has important implications on the localization and function of GPI-APs. For example, a recent study has demonstrated that saturated fatty acids resulting from lipid remodeling are required for the association of mammalian GPI-APs with lipid rafts.¹²⁸

To develop a strategy for accessing GPIs relevant to studying the effects of lipid remodeling and to continue synthetic studies of GPI anchors and the human CD52 antigen (see sections II-6 and II-10), the Guo group targeted the lymphocyte CD52 GPI anchor **322** (Scheme 59) for total synthesis.¹²⁹ The target molecule contained modifications of the GPI core glycan present in the human lymphocyte CD52 antigen, including additional phosphoethanolamine

and mannose groups linked to the O-2 positions of Man-I and Man-III, respectively. Most importantly, compound **322** contained an octadecanoyl-arachidonoyl-glycerophosphate component, which is present in the lymphocyte CD52 antigen and other GPI-anchored proteins that do not undergo lipid remodeling. To achieve the synthetically challenging incorporation of a highly sensitive polyunsaturated arachidonoyl lipid in GPI**322**, the PMB-protection strategy was used.

A convergent approach was used for the construction of GPI **322** that relied on a key glycosylation between PMB-protected coupling partners, namely the tetramannosyl donor **324** and the pseudodisaccharide acceptor **325** (Scheme 59). Silyl ether protecting-groups at the O-2 position of Man-I and O-6 position of Man-III would be selectively removed at a late stage to enable double phosphorylation of these sites with phosphoramidite **293**. The glycosyl donor **324** would arise from sequential α -mannosylations of disaccharide **308** using monomers **326** and **327**. Acceptor **324** could be accessed through phospholipidation of compound **318** with phosphoramidite **328**, which would attach the arachidonoyl-containing phosphoglycerolipid. Guided by previous success, the Schmidt method⁷¹ was used to couple stereoselectively all PMB-protected glycosylation partners. Some PMB-protected building blocks (**308**, **318**) from Guo's 2010 synthesis of a GPI anchor containing unsaturated lipids were also used in this synthesis, showing their versatility.

The preparation of PMB-protected tetramannosyl donor **324** is shown in Scheme 60. To obtain the Man-III building-block **326**, the orthoester **304** was subjected to consecutive deacetylation, silylation at O-6, *p*-methoxybenzylation at O-4 and O-6, acid-promoted regioselective opening of the orthoester ring, and finally treatment of the resulting hemiacetal with trichloroacetonitrile and DBU. The resultant compound **326** was then treated with **308** in the presence of TMSOTf to furnish the α -trimannoside **329** in 72% yield. Deacetylation of **329** gave the acceptor **330**, which was glycosylated by the Man-IV donor **327** to afford tetramannoside **331** in 76% yield, although stereoselectivity for this reaction was low ($\alpha:\beta = 3:2$). At this stage, the allyl group at O-2 of Man-I was exchanged for a TES group using Cha's deallylation protocol,¹²¹ followed by treatment with TESOTf/2,6-lutidine to furnish compound **332**. Transformation of the anomeric thioacetal of **332** to the trichloroacetimidate **324** was accomplished by treatment with NIS/AgOTf/TTBP followed by trichloroacetonitrile and DBU.

Given the documented sensitivity of arachidonoyl-containing compounds to air and light (leading to oxidation of the alkene) and heat and base (leading to rearrangements),^{130–132} care was taken to avoid these conditions in the preparation of phosphoramidite **328** and throughout the completion of the synthesis (Scheme 61). Phospholipidation of the afore-described PMB-protected pseudodisaccharide **318** was performed by using the arachidonoyl-containing phosphoramidite **328** in the presence of 1*H*-tetrazole. *In situ* oxidation with *t*-BuOOH, followed by removal of the TBS group with triethylamine trihydrofluoride, provided compound **325**. The key glycosylation between acceptor **325** and the tetramannosyl donor **324** was performed under conventional Schmidt conditions, leading to the α -pseudoexasaccharide **323** in moderate (39%) yield. Subsequent desilylation to remove the Man-I TES and Man-III TBDPS groups gave diol **333**, which underwent double phosphorylation with phosphoramidite **293** to provide the fully PMB-protected GPI **334**. Application of the previously described three-step, one-flask global-deprotection protocol involving consecutive treatments with Zn/AcOH, DBU, and 10% TFA delivered the target GPI anchor **322** in 90% yield over the final three steps (under 3 hours of reaction time).

Construction of the lymphocyte CD52 GPI anchor **322** constituted the first total synthesis of a GPI anchor bearing unremodeled fatty acids, namely a polyunsaturated arachidonoyl lipid at the *sn*-2 position of the phosphatidylinositol moiety. Inclusion of the oxidation- and

reduction-sensitive arachidonoyl lipid was made possible by utilizing global protection by the PMB group, which featured a mild and rapid three-step, one-flask deprotection protocol that gave the target GPI anchor in 90% yield in under 3 hours. Furthermore, two additional structural modifications in the GPI core present in the lymphocyte CD52 antigen, specifically additional phosphoethanolamine and mannose units, were incorporated into the target molecule, which further demonstrated the applicability of the PMB-protection strategy to the efficient synthesis of highly complex and sensitively functionalized GPIs.

4. Synthesis of “Clickable” GPI Anchors by the Guo Group (2011)

Given the rapid influx of techniques in chemical biology that use “click” chemistry for chemoselective conjugation, particularly the powerful [3+2] cycloaddition reactions between azides and alkynes/cyclooctynes,^{133–136} the arming of GPIs with “click” tags provides a platform for developing novel approaches for exploring their biological properties and therapeutic applications (Fig. 2). For example, “clickable” GPIs could be deployed in studies involving molecular imaging, profiling of GPI-anchored proteins (“GPIomics”), and biophysical characterization of GPIs and GPI-anchored molecules on the surfaces of live cells. However, “click” chemistry tags and other useful functionalities, including many imaging and affinity purification tags, are often incompatible with traditional protecting-group methodologies used in carbohydrate synthesis.

In 2011, Swarts and Guo used the PMB-protection strategy to synthesize “clickable” GPI anchors.¹³⁷ Retrosynthetic analysis (Scheme 62) suggested a convergent–divergent strategy that would optimize efficiency while enabling access to a diverse set of GPIs. Flexibility was emphasized in the synthetic design to accommodate the structural heterogeneity of naturally occurring GPIs, particularly in lipid chains, as well the ability to functionalize GPIs with “click” tags for biological studies. Accordingly, the afore-described trimannose thioglycoside **309** and pseudodisaccharide **318** were used as adaptable, PMB-protected intermediates that would allow for a choice of phosphoglycerolipid structure and functional tag in the target GPI. Using these intermediates, two generations of “clickable” GPIs were synthesized. In this article, synthesis of the second-generation Alkynyl-GPI **335** and Azido-GPI **336**, as well as subsequent chemoselective labeling of these GPIs via “click” chemistry, is discussed.

For both of the target GPI anchors **335** and **336**, a distearoylglycerophosphate group was chosen as the phospholipid (Scheme 63). Toward the synthesis of Alkynyl-GPI **335**, the pseudodisaccharide **318** was phospholipidated by using phosphoramidite **338** under conventional conditions, followed by treatment with triethylamine trihydrofluoride to remove the TBS group, which gave the pseudodisaccharide **339**. En route to Azido-GPI **336**, the azido group of pseudodisaccharide **318** was replaced by an Fmoc group to give compound **337**, which subsequently underwent phospholipidation–desilylation in the same manner to furnish the Fmoc-protected pseudodisaccharide **340**.

Completion of the synthesis of Alkynyl-GPI **335** from trimannoside **309** and pseudodisaccharide **339** is shown in Scheme 64. First, the terminal alkyne “click” tag was installed in the trimannose fragment by consecutive Cha deallylation¹²¹ and propargylation of the O-2 position of Man-I. Following hydrolysis of the anomeric thioacetal using *p*-nitrobenzenesulfonyl chloride (PNBSCI) and AgOTf/TTBP¹³⁸ in wet CH₂Cl₂, the intermediate hemiacetal was converted into trichloroacetimidate **341** by treatment with DBU and trichloroacetonitrile. The key glycosylation reaction of **341** with pseudodisaccharide **339**, promoted by TMSOTf, furnished an intermediate that was immediately subjected to removal of the TBS group on O-6 of Man-III with triethylamine trihydrofluoride to give **342**. This alkyne-modified pseudopentasaccharide was isolated in good yield (82%) over

two steps, and no β anomer was observed. Installation of the phosphoethanolamine group into Man-III using phosphoramidite **293** proceeded well and gave compound **343**, which was finally subjected to the aforementioned three-step, one-flask global deprotection to provide the target Alkynyl-GPI **335** in excellent (93%) yield in under 3 hours (three steps).

The synthesis of Azido-GPI **336**, shown in Scheme 65, followed a similar route. First, the O-2 allyl group on Man-I of compound **309** was exchanged for a 2-azidoethyl group in five steps, including Cha deallylation,¹²¹ base-promoted alkylation using *tert*-butyl bromoacetate, ester reduction with LiAlH_4 , mesylation of the primary hydroxyl group, and finally displacement of the mesylate with NaN_3 .¹³⁹ Transformation into the trichloroacetimidate **344** was accomplished by treatment with NIS/AgOTf/TTBP in wet CH_2Cl_2 , followed by DBU and trichloroacetonitrile. The TMSOTf-promoted coupling of **344** with *N*-Fmoc-protected pseudodisaccharide **340** gave the desired α -pseudopentasaccharide intermediate, which was desilylated with triethylamine trihydrofluoride to generate compound **345** in moderate yield (30%, 88% based on recovered **340**). The remaining steps involved installation of phosphoethanolamine at Man-III as already described to give **346**, followed by global deprotection, which proceeded in two steps (DBU and then 10% TFA) to afford Azido-GPI **336** in 97% yield over the final two steps.

To establish Alkynyl-GPI **335** and Azido-GPI **336** as “clickable” tools for the biological study of GPI anchors, they were coupled via “click” chemistry to imaging and affinity probes (Scheme 66). Alkynyl-GPI **335** was conjugated with Azide-Fluor 488 (**347**), a commercially available regioisomeric mixture, via Cu(I)-catalyzed [3+2] cycloaddition under standard conditions.^{140,141} This Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction led to complete conversion of the starting material into the GPI-Fluor conjugate **348**, a promising tool for visualizing GPI anchors by fluorescence microscopy in cellular contexts. Azido-GPI **336** was effectively coupled via Cu-free, strain-promoted azide-cyclooctyne [3+2] cycloaddition (Cu-free “click”) to BARAC-biotin (**349**), a biarylazacyclooctynone-based affinity probe developed by Jewett et al.¹⁴² This step effected virtually quantitative conversion of the starting material into GPI-Biotin **350**. Conjugation of GPIs to affinity tags such as biotin is a potentially useful strategy for pull-down experiments to probe cell-surface, GPI-anchored proteomics. Alkynyl-GPI **335** and Azido-GPI **336**, which were not accessible by traditional tactics for carbohydrate protection, were synthesized using Guo’s PMB-protection strategy. These “clickable” GPIs were demonstrated to undergo efficient coupling reactions via both CuAAC and Cu-free “click” reactions, and therefore constitute versatile tools for the development of chemical approaches in studying the biology of GPI anchors. In addition, this work further validated the PMB-protection strategy as an alternative global-protection option, most usefully when sensitively functionalized GPI anchors are targeted for synthesis.

5. General Synthetic Strategy for Branched GPI Anchors by the Seeberger Group (2011)

In addition to methods focused on expanding functional-group tolerance, such as the efforts by Nikolaev and Guo already discussed, the development of strategies that address structural diversity in terms of branching sugars and other substituents is also an important goal. As already discussed (see Fig. 1 in the introduction), the conserved core-structure of GPI anchors is often decorated in a species- and cell-type dependent manner, with additional sugars, phosphoethanolamine groups, and lipid chains at various positions. In 2011, the Seeberger group developed a general method for the synthesis of GPI anchors containing, in principle, any type of structural modification that is compatible with global benzyl-group protection.¹⁴³ A general retrosynthetic analysis for this strategy is shown in Scheme 67. It was expected that diverse target GPI anchors could be accessed from orthogonally protected

GPI intermediates containing first of all the desired branching sugars at Man-I and Man-III, and secondly orthogonal protecting-groups at sites for late-stage phospholipidation, O-2 acylation of the inositol, and selective installation of phosphoethanolamine. These orthogonally protected GPI intermediates could be prepared from the interchangeable mannose building-blocks **351–354** and pseudodisaccharides **62** and **208**.

To demonstrate the applicability of this general method, Seeberger conducted the total synthesis of *T. gondii* GPI anchor **355**, which contains a core modification of an α -Glc-(1 $\rightarrow$ 4)- β -Gal-(1 $\rightarrow$ 4) disaccharide at Man-I. The target GPI (**355**) was first disconnected to the orthogonally protected GPI intermediate **356**, which was properly set up to undergo late-stage introduction of the phospholipid and phosphoethanolamine groups by the *H*-phosphonate method.⁴⁴ The preparation of **356** centered on Man-I building block **354**, which possessed the appropriate array of orthogonal protecting-groups to allow the following [3 + 2 + 2] assembly sequence: first elongation at the O-3 position with donors **357** and **358** to give a trisaccharide; next [3 + 2] coupling with donor **351**; followed by subsequent [5 + 2] coupling with pseudodisaccharide **62**; and finally optional installation of a phosphoethanolamine group at the O-2 position of Man-I (not performed in this synthesis). For the glycosylation steps, a combination of glycosyl phosphates¹⁴⁴ and trichloroacetimidates⁷¹ were used as donors.

Preparation of the central Man-I derivative **354** and its elaboration to trisaccharide fragment **365** is shown in Scheme 69. En route to compound **354**, the previously reported allyl mannoside **359**¹⁴⁵ was protected at O-4 by the oxidatively cleavable 2-naphthylmethyl (Nap) group, which was followed by acid-catalyzed deacetonation and selective tin-mediated benzylation at O-3 to give compound **360**. Subsequent conversion into **354** was accomplished by diisopropylcarbodiimide (DIC)/DMAP-promoted levulinoylation (Lev) at O-2, followed by oxidative cleavage of the Nap group at O-4 with DDQ. Alternatively, this sequence could be modified to expose the O-3 position to obtain the Man-I derivative **353**, which would be useful for the synthesis of GPIs of *T. brucei* containing additional sugars at this location.

The galactosyl phosphate derivative **357**, the coupling partner of Man-I derivative **354**, was synthesized from the previously reported 2-azido selenoglycoside **361**¹⁴⁶ in four steps, including regioselective benzylidene cleavage, naphthylmethylation at O-4, Staudinger reduction followed by N-trichloroacetylation, and finally formation of the glycosyl phosphate by treatment with NIS/dibutyl phosphate. The Man-I acceptor **354** and galactosyl phosphate donor **357** combined in the presence of TMSOTf to give the β disaccharide **362**, which was treated with DDQ to remove the Nap group, furnishing compound **363** quantitatively over two steps. Subsequent glycosylation of **363** with the glucosyl phosphate donor **358** in the presence of TBSOTf and thiophene (a reported α -enhancing additive),¹⁴⁷ afforded **364** (82% yield, α : β = 6:1), which underwent desilylation by HF·pyridine to generate the central trisaccharide **365**.

Scheme 70 depicts the [3 + 2 + 2] assembly of building blocks **365**, **351**, and **62** to generate the orthogonally protected pseudoheptasaccharide intermediate **356**. TBSOTf and added thiophene were again used to promote α -stereoselective glycosylation between the dimannosyl donor **351** and the acceptor **365**, resulting in the formation of pentasaccharide **366** in 69% yield. Reductive conversion of the trichloroacetamido group to an acetamido group gave compound **367**, which was converted into the trichloroacetimidate donor **368** by deallylation using the Ir–Hg protocol¹²³ and subsequent treatment with trichloroacetonitrile and DBU. Reaction of this donor with the pseudodisaccharide **62** in the presence of catalytic TMSOTf forged the key glycosidic bond, delivering compound **356** in 71% yield. The participating Lev group at O-2 ensured α -stereoselectivity in this crucial coupling reaction.

At this stage, compound **356** was subjected to Ir–Hg deallylation to expose O-1 of the inositol, which was phospholipidated by using *H*-phosphonate **205** and iodine-mediated oxidation to give intermediate **369** (Scheme 71). Acid-promoted removal of the TIPS group at O-6 of Man-III prepared the intermediate for the second phosphorylation, which was accomplished by using the *H*-phosphonate **4** to afford **370**. Hydrazinolysis of the O-2 Lev group on Man-I deprotected the 2-position of Man-I, which had the potential to undergo further phosphorylation at this site if desired. In this synthesis, intermediate **370** was taken forward to global deprotection by hydrogenolysis to provide the target GPI **355** in 62% yield.

In this work, the Seeberger group synthesized the *T. gondii* GPI anchor **355**, which carried a branching disaccharide at Man-I, using a [3 + 2 + 2] assembly strategy. The most important aspect of this strategy is its potential applicability to the synthesis of virtually any type of GPI anchor bearing common modifications, including acyl groups on O-2 of the inositol, phosphoethanolamine on O-2 of Man-I, sugars on O-3 or O-4 of Man-I, and a sugar on O-2 of Man-III. This flexibility is a result of carefully designed building-blocks, in particular the central Man-I components **353** and **354**, and a well-optimized assembly sequence. In addition, synthetic **355** was used in combination with Seeberger's GPI microarray technology¹¹⁰ to confirm that it was indeed the immunogenic "low-molecular-weight antigen" of *T. gondii* responsible for antibody production in toxoplasmosis patients.

IV. Conclusions and Outlook

Starting with Ogawa's seminal work in the early 1990s, the field of chemical synthesis of GPI anchors has enjoyed marked success in melding the knowledge and methods of carbohydrate, inositol, lipid, and phosphate chemistry into a reliable framework for constructing these complex natural products. Numerous naturally occurring GPI anchors from protozoan parasites, yeast, and mammals have been synthesized, and through this many new synthetic methods have been developed and tested. Currently, the approach to GPI synthesis has shifted toward developing novel diversity-oriented strategies that have enabled access to new classes of molecules, including GPI anchors bearing the biologically important unsaturated lipids, indispensable functionalities such as "click" chemistry tags, and highly branched and modified core structures.

In the future, a combination of such diversity-oriented strategies, with the goal of a truly general method for the synthesis of any type of target GPI anchor, would be a highly valuable contribution. Another goal of great importance is improvement of the efficiency by limiting the number of steps in a synthesis, which, taking into account preparation of the monomers, can become prohibitively large. In this context, solid-phase synthesis, one-flask oligosaccharide synthesis, and proper reactivity tuning of glycosyl acceptors and donors will all contribute toward meeting this goal. Synthetic GPIs and GPI derivatives have the potential to play vital roles in deducing structure–activity relationships of these molecules in various contexts, exploring the scope of GPI anchorage as a post-translational modification, probing therapeutic applications, and in general gaining insight into their fundamental biological functions.

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ABBREVIATIONS

Ac	acetyl
All	allyl
BDA	butanediacetal
Bn	benzyl
Boc	<i>tert</i> -butyloxycarbonyl
BRSM	based on recovered starting material
Bz	benzoyl
CAM	camphanoyl
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CA	chloroacetyl
“Click” chemistry	[3 + 2] azide—alkyne cycloaddition
CSA	camphorsulfonic acid
CuAAC	Cu-catalyzed azide—alkyne cycloaddition
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCE	dichloroethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	diisobutylaluminum hydride
DIC	<i>N,N'</i> -diisopropylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMTrt	4,4'-dimethoxytrityl
DTBMP	2,6-di- <i>tert</i> -butyl-4-methylpyridine
EE	1-ethoxyethyl ether
Fmoc	9-fluorenylmethoxycarbonyl
Gal	galactose
GalNAc	<i>N</i> -acetylgalactosamine
GFP	green fluorescent protein
Glc	glucose
GlcN	glucosamine
GPI	glycosylphosphatidylinositol
KHMDS	potassium hexamethyldisilazide
Lev	levulinoyl

LPG	lipophosphoglycan
Man	mannose
<i>m</i>-CPBA	<i>m</i> -chloroperoxybenzoic acid
Mnt	menthyl
MS	molecular sieves
NAP	2-naphthylmethyl
NIS	<i>N</i> -iodosuccinimide
NPG	<i>n</i> -pentenyl glycoside
NPOE	<i>n</i> -pentenyl orthoester
<i>p</i>-TsOH	<i>p</i> -toluenesulfonic acid
PA	phenoxyacetyl
PBS	phosphate-buffered saline
PG	protecting group
PI	phosphatidylinositol
PIV	pivaloyl
PMB	<i>p</i> -methoxybenzyl
PMP	<i>p</i> -methoxyphenyl
PMTrt	<i>p</i> -methoxytrityl
PNBSCI	<i>p</i> -nitrobenzenesulfonyl chloride
PPTS	pyridinium <i>p</i> -toluenesulfonate
pyr	pyridine
SEM	2-trimethylsilylethoxymethyl
TBAF	tetrabutylammonium fluoride
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
TCA	trichloroacetyl
TDS	hexyldimethylsilyl
TES	triethylsilyl
Tf	trifluoromethanesulfonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
TIPS	triisopropylsilyl
TMS	trimethylsilyl
tol	toluene
TTBP	2,4,6- <i>tri</i> - <i>tert</i> -butylpyrimidine

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FIG. 1.
GPI structure and anchoring function.

Fig. 2.
Probing GPI anchors and GPI-anchored molecules with “click” chemistry.

Scheme 1.

General retrosynthesis of a GPI anchor. PG = protecting group, PG' = orthogonal protecting group, X = activatable leaving group, X' = orthogonally activatable leaving group.

Scheme 2.

Retrosynthesis of *T. brucei* GPI anchor 1 by the Ogawa group.

Scheme 3.

Synthesis of digalactosyl fluoride **5** by the Ogawa group.

Scheme 4.

Synthesis of building blocks **9**–**11** and their elaboration to **8** by the Ogawa group.

Scheme 5.

Completion of *T. brucei* GPI anchor **1** by the Ogawa group.

Scheme 6.

Generation of pseudotetrasaccharide **26** and anhydro sugar **27** in a side reaction during the coupling of **5** and **8** by the Ogawa group.

Scheme 7. Retrosynthesis of yeast GPI anchor **34** by the Schmidt group.

Scheme 8.

Synthesis of building blocks **40–42** and their elaboration to tetramannosyl donor **38** by the Schmidt group.

Scheme 9.
Synthesis of pseudodisaccharide **37** by Schmidt.

Scheme 10. Completion of yeast GPI anchor **34** by the Schmidt group.

Scheme 11. Retrosynthesis of Thy-1 GPI anchor **56** by the Fraser-Reid group.

Scheme 12.

Synthesis of *n*-pentenyl trimannoside **60** by the Fraser-Reid group.

Scheme 13.

Synthesis of β -GalNAc-Man disaccharide **61** by the Fraser-Reid group.

Scheme 14.

Synthesis of pseudodisaccharide **62** by the Fraser-Reid group.

Scheme 15. Completion of mammalian Thy-1 GPI anchor **56** by the Fraser-Reid group.

Scheme 16. Retrosynthesis of *T. brucei* GPI anchor **1** by the Ley group.

Scheme 17.

Synthesis of building blocks **99–103** by the Ley group.

Scheme 18.
Synthesis of pentasaccharide **95** by the Ley group.

Scheme 19.

Synthesis of pseudodisaccharide **62** by the Ley group.

Scheme 20. Completion of *T. brucei* GPI anchor **1** by the Ley group.

Scheme 21.
Retrosynthesis of Thy-1 GPI anchor **56** by the Schmidt group (2003).

Scheme 22.
Synthesis of pentasaccharide **125** by the Schmidt group.

Scheme 23.

Synthesis of pseudodisaccharide **126** by the Schmidt group.

Scheme 24.
Completion of Thy-1 GPI anchor **56** by the Schmidt group (2003).

Scheme 25.

Retrosynthesis of GPI anchor **148** of the sperm CD52 antigen by the Guo group.

Scheme 26.

Unexpected cyclophosphitamidation reaction of the inositol-acylated GPI intermediate **159** (Guo group).

Scheme 27.

Synthesis of trimannose thioglycoside **151** by the Guo group.

Scheme 28.

Synthesis of inositol derivative **155** by the Guo group.

Scheme 29.

Synthesis of phospholipidated pseudodisaccharide **152** by the Guo group.

Scheme 30.

Completion of GPI anchor **148** of human CD52 bearing an acylated inositol by the Guo group.

Scheme 31.

GPI-peptide/glycopeptide conjugates **175** and **176** chemically prepared by the Guo group.

Scheme 32.

Retrosynthesis of the GPI anchor **177** of *P. falciparum* by the Fraser-Reid group.

Scheme 33. Synthesis of mannosyl NPOEs **182–184** by the Fraser-Reid group.

Scheme 34.

Synthesis of pseudodisaccharide **181** by the Fraser-Reid group.

Scheme 35.

Construction of pseudopentasaccharide **178** by the Fraser-Reid group.

Scheme 36.

Completion of the target GPI **177** by the Fraser-Reid group.

Scheme 37.
Retrosynthesis of *P. falciparum* GPI anchor **203** by the Seeberger group.

Scheme 38.

Synthesis of tetramannosyl donor **207** by the Seeberger group.

Scheme 39.

Synthesis of pseudodisaccharide acceptor **208** by the Seeberger group.

Scheme 40. Completion of GPI anchor **203** of *P. falciparum* by the Seeberger group.

Scheme 41.

GPI-prion conjugate **226** synthesized by the Seeberger group.

Scheme 42.

Retrosynthesis of *T. cruzi* GPI anchor **227** by the Vishwakarma group.

Scheme 43.

Synthesis of tetramannosyl donor **230** by the Vishwakarma group.

Scheme 44.

Synthesis of pseudodisaccharide **231** by the Vishwakarma group.

Scheme 45. Completion of *T. cruzi* GPI anchor **227** by the Vishwakarma group.

Scheme 46.

Retrosynthesis of fully phosphorylated and lipidated human sperm CD52 antigen GPI anchor **245** by the Guo group.

Scheme 47.

Synthesis of pseudodisaccharide **248** by the Guo group.

Scheme 48.

Completion of fully phosphorylated and lipidated human sperm CD52 antigen GPI anchor **245** by the Guo group.

Scheme 49.

Retrosynthesis of *T. cruzi* GPIs **258** and **259** by the Nikolaev group.

Scheme 50.
Synthesis of tetramannosyl donor **265** by the Nikolaev group.

Scheme 51.

Synthesis of pseudodisaccharide **266** by the Nikolaev group.

Scheme 52.

Completion of *T. cruzi* GPI anchors **258** and **259** by the Nikolaev group using acyl-based global protection.

Scheme 53.

Completion of *T. cruzi* GPI anchor **258** by the Nikolaev group using acetal- and silyl-based global protection.

Scheme 54.

Retrosynthetic analysis of a GPI anchor bearing unsaturated lipid chains by the Guo group using the PMB protection strategy.

Scheme 55.

Synthesis of PMB-protected mannose derivatives, and elaboration to the trimannosyl donor **294** by the Guo group.

Scheme 56.

Synthesis of inositol derivative **299** by the Guo group.

Scheme 57.

Synthesis of pseudodisaccharide **295** by the Guo group.

Scheme 58.

Completion of a GPI anchor bearing unsaturated lipid chains (**291**) using the PMB protection strategy by the Guo group.

Scheme 59.

Retrosynthetic analysis of human lymphocyte CD52 antigen GPI anchor **322** containing a polyunsaturated arachidonoyl lipid by the Guo group.

Scheme 60.

Synthesis of PMB-protected tetramannosyl donor **324** by the Guo group.

Scheme 61.

Completion of the human lymphocyte CD52 antigen GPI anchor **322** by the Guo group.

Scheme 62.

Convergent-divergent strategy for the synthesis of "clickable" GPI anchors by the Guo group.

Scheme 63.

Synthesis of pseudodisaccharides **339** and **340** by the Guo group.

Scheme 64.

Completion of “clickable” alkynyl-GPI **335** by the Guo group.

Scheme 65. Completion of “clickable” azido-GPI **336** by the Guo group.

Scheme 66.

“Click” reactions of alkynyl-GPI 335 and azido-GPI 336 by the Guo group.

Scheme 67.

General retrosynthesis for branched GPI anchors by the Seeberger group.

Scheme 68.

Retrosynthetic analysis of *T. gondii* GPI anchor **355**, using the general method for branched GPI synthesis by the Seeberger group.

Scheme 69.

Synthesis of central trisaccharide **365** by the Seeberger group.

Scheme 70.

Synthesis of orthogonally protected GPI intermediate **356** by the Seeberger group.

Scheme 71.

Completion of synthesis of the *T. gondii* GPI anchor **355**, using the general method for branched GPI synthesis by the Seeberger group.