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Convergent Synthesis of a Fully Phosphorylated GPI Anchor of the CD52 Antigen

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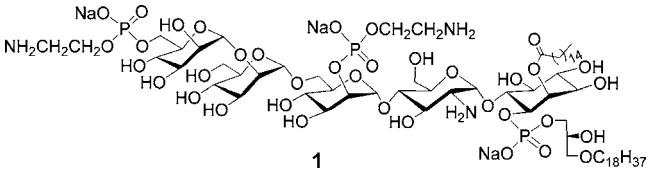
ABSTRACT



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A fully phosphorylated GPI anchor (1) of the CD52 antigen was synthesized by a highly convergent strategy. After a trimannose and a phospholipidated pseudodisaccharide were prepared separately, they were coupled together to form the GPI core, which was then phosphorylated to introduce two phosphoethanolamine moieties in one step to afford CD52 GPI in its fully protected form. Finally, global deprotection of the product resulted in 1.

Glycosylphosphatidylinositol (GPI) anchors are a class of natural glycolipids which are expressed by all eukaryotic cells.[1] These compounds have many important biological functions, of which anchoring proteins and glycoproteins to cell membranes is the most obvious.[1−5] GPIs share a remarkably conserved core structure, which is composed of a tetrasaccharide, a phosphoethanolamine group and an inositol residue at the nonreducing and reducing ends of the glycan, respectively, and a phosphatidyl moiety that is attached to the inositol ring. Owing to the interesting and complex structures and important biological functions of GPIs and related molecules, their chemical synthesis has attracted great attention in the past decade.[6,7]

Recently, we became especially interested in the CD52 antigen, a GPI-anchored glycopeptide antigen involved in the human reproduction and human immune recognition processes.[8−11] Because of its simple structure and evident bioactivity, the CD52 antigen can be a useful model for studying the functions of GPI anchors. In this regard, homogeneous GPIs and GPI-anchored molecules, which are difficult to obtain from nature, are critical.

This paper reports the chemical synthesis of a fully phosphoryated GPI anchor **1** of CD52 (Scheme 1) having a long acyl chain attached to the inositol 2-*O*-position.

As shown in Scheme 1, our overall synthetic plan was to first assemble the phospholipidated core (**2**) and then introduce the two phosphoethanolamine moieties through two-step phosphorylation. Because the α-glycosylation of mannose is relatively easy, but not that of glucosamine, a logical and convergent design for the assembly of the GPI

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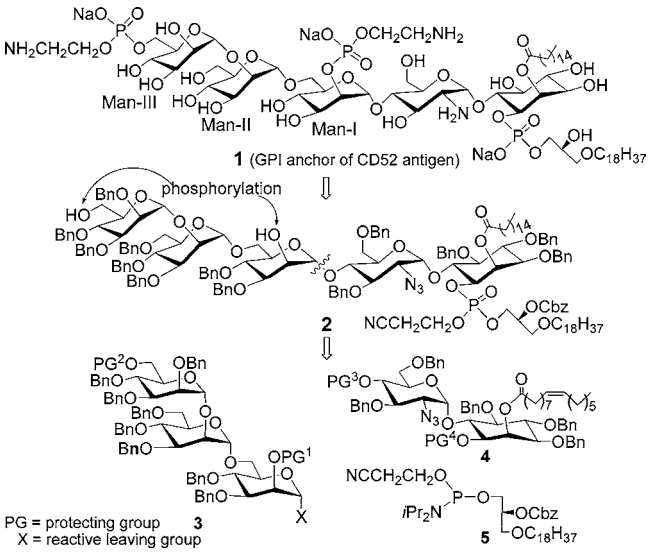
Scheme 1. Retrosynthesis of Target GPI Anchor **1**

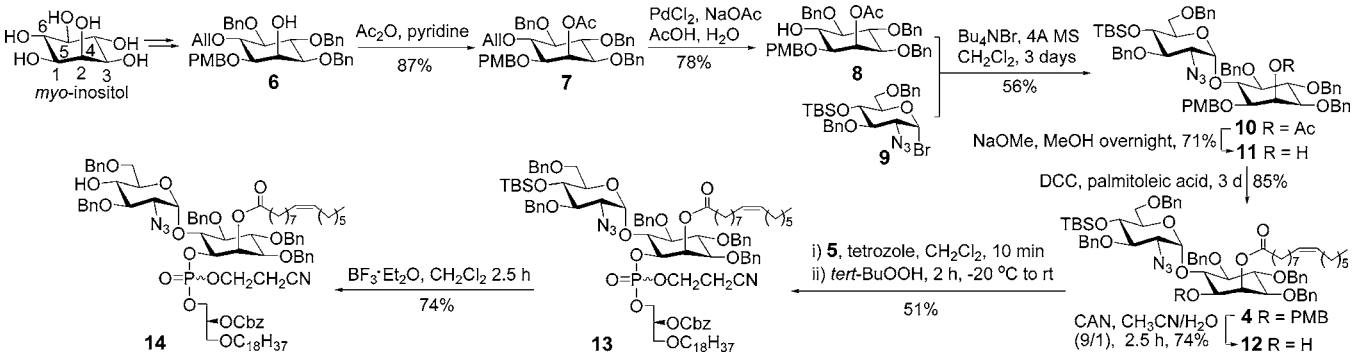


core glycan would be to prepare fragments **3** and **4** first and then couple them together through a relatively easy glycosylation reaction.

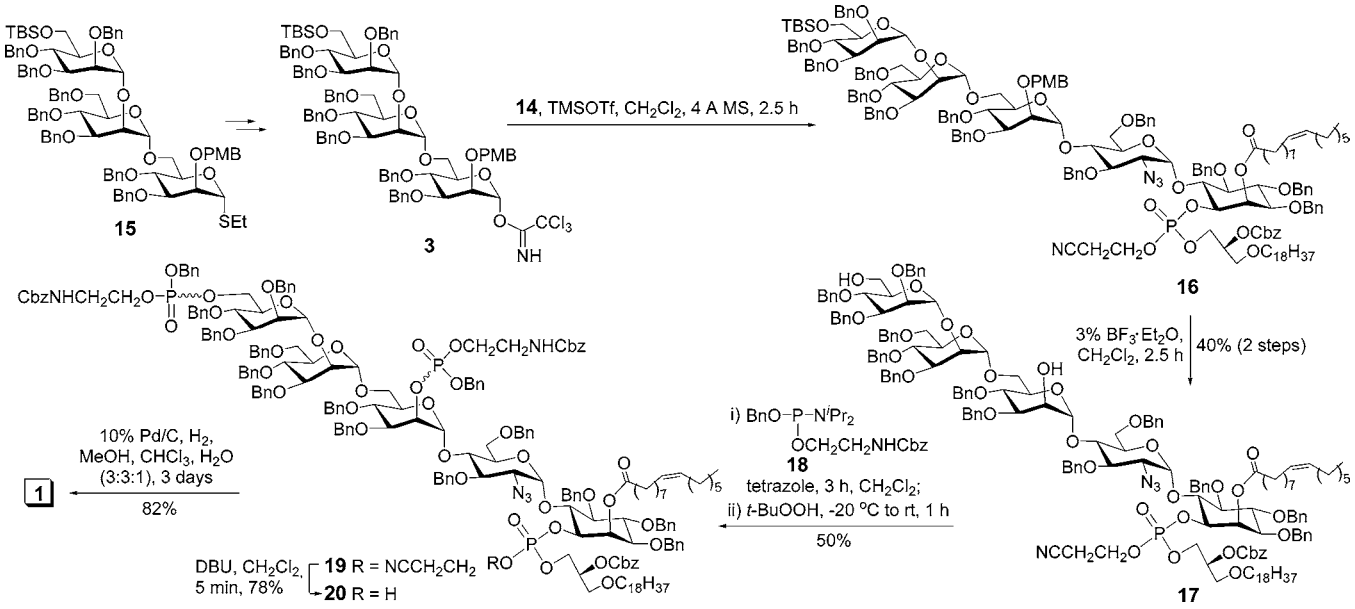
The synthesis of pseudodisaccharide **4**, as well as its phospholipidated derivative **14**, is outlined in Scheme 2. First, an optically pure inositol derivative **6** was prepared according to a reported method.[12] Next, an acetyl group was introduced to temporarily protect the inositol 2-OH, followed by deallylation to afford **8**, which was ready for installation of the glucosamine moiety by glycosylation. To establish reliable conditions to achieve this difficult α-linkage, we have prepared and tested several glucosamine derivatives as potential glycosyl donors, such as glycosyl halides, glycosyl trichloroacetimidate, and thioglycosides of 2-azido-2-deoxyglucose. We found that glycosylation reactions under Lemieux conditions,[13] i.e., using glucosyl bromide **9** as the glycosyl donor and tetrabutylammonium bromide (TBAB) as the promoter, gave the α-glycoside **10** stereospecifically in a good yield (56%). The inositol 2-*O*-position in **10** was then deprotected to expose a free hydroxyl group, to which was introduced a palmitoleoyl group employing dicyclohexylcarbodiimide (DCC) as the condensation reagent. Here, an unsaturated palmitoleoyl chain was introduced because, before global deprotection, its C═C bond could be modified to produce some reactive intermediates, e.g., oxidative cleavage of the double bond to form carbonyl compounds,[14] which are useful for the preparation of various GPI conjugates. On the other hand, during the reductive debenzylation at the final step, the palmitoleoyl group could be readily reduced to form a palmitoyl group—the natural acyl chain of the CD52 GPI anchor. Based on the lessons that we had learned from the synthesis of another CD52 GPI anchor,[15] we decided to introduce the bulky phospholipid moiety to the inositol 1-*O*-position at this stage, because the presence of a large acyl group at the inositol 2-*O*-position might render late-stage phospholipidation impossible. Oxidative removal of the 4-methoxybenzyl (PMB) group on the inositol ring was achieved with ceric ammonium nitrate (CAN), which was followed by phospholipidation employing **5** in a two-step, one-pot procedure to give a diastereomeric mixture (1:1.1) of **13** that was fully characterized by ^{1}H NMR, ^{31}P NMR, and MS. Finally, the *tert*-butyldimethylsilyl (TBS) group in **13** was removed to afford **14**, which was ready to be coupled with the trimannose fragment **3** for the assembly of the final synthetic target.

Trimannose **15** was prepared according to a procedure developed for similar structures,[16] but the 2-*O*-position of mannose-I was protected by a PMB group. Glycosylation of **14** using **15** as glycosyl donor and *N*-iodosuccinamide and silver triflate (NIS/AgOTf) as promoter would affect the double bond in the palmitoleoyl group, whereas the reaction of **14** and **15** with dimethyl(methylthio)sulfonium triflate (DMTST) or methyl triflate (MeOTf) as promoter could not yield the desired product either. Therefore, **15** was transformed into **3** following oxidative hydrolysis of the reducing end by reaction with NIS/TfOH and water and then trichloroacetimidation of resultant hemiacetal. Glycosylation of **14** using a large excess of **3** (3 equiv) with trimethylsilyl triflate (TMSOTf) as promoter went well, but the isolation of **16** was complicated by the hydrolytic products derived from **3**. Therefore, after **16** was purified briefly on a silica gel column, it was directly subjected to treatment by $BF_3{\cdot}Et_2O$ in CH_2Cl_2 to remove the TBS and PMB groups and give **17**, of which the two diastereomers could be separated through careful silica gel column chromatography. Both

Scheme 2. Synthesis of the Phospholipidated Pseudodisaccharide Segment



Scheme 3. Final Assembly of the Target GPI Anchor **1**



stereoisomers were individually characterized by ^{1}H and ^{31}P NMR and MS. Then, one isomer, **17b**, was phosphorylated employing **18** by the two-step, one-pot procedure described above to introduce two phosphoethanolamine groups simultaneously, giving fully protected GPI anchor **19**. Global deprotection of **19** was realized in two steps. First, the 2-cyanoethyl group protecting the phospholipid moiety was removed by DBU treatment for a short period (ca. 5 min) to give **20**, and the product was fully characterized by NMR and MS. The NMR signals of the anomeric protons and carbons (500 and 125 MHz, respectively, in $CDCl_3$), which were clearly identifiable in the HMQC spectrum, suggested α-configurations of all glycosidic bonds. Finally, the benzyl and benzyloxycarbonyl (CBz) groups were removed by hydrogenolysis under an H_2 atmosphere using 10% Pd/C as the catalyst to eventually afford CD52 GPI **1**. The ^{1}H NMR spectra (500 MHz) of **1** obtained in a mixture of CD_3OD, $CDCl_3$, and D_2O (3:3:1) showed no aromatic ^{1}H signals, suggesting a complete removal of all benzyl and CBz groups. This conclusion, as well as the structure of the final product, was confirmed by ^{13}C NMR (125 MHz) and by MS spectrum which shows the highest *m*/*z* peak at 893 [M + $2H^+$]. The MS result further indicates that **1** was obtained as a sodium salt and that its free amino groups were easily protonated to give rise to the observed *m*/*z* peak at the positive ionization mode of MALDI MS.

In summary, a highly convergent synthesis of the fully functionalized GPI anchor (**1**) of the CD52 antigen has been described in this paper. Our collaborator, Professor Xiangqun Zeng at Oakland University, has shown that the synthetic GPI anchor **1** could interact strongly with GPI-binding proteins. Our two laboratories are currently studying the biological activities of **1** in detail, and the results will be reported in due course.

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Supporting Information Available: Experimental and the NMR and MS spectra of **1**, **4**, **6**−**14**, **17**, **19**, and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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