Solid-Phase Synthesis of Glycolipids

A Practical Solid-Phase Synthesis of Glycosylphosphatidylinositol Precursors**

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Glycosylphosphatidylinositol (GPI) anchors serve to attach proteins to cellular membranes through a covalent linkage.[1] These glycolipids have been a subject of sustained interest for the last fifteen years.[2] Besides the anchoring function, GPIs are involved in important biological events. They act as a targeting signal to the secretion and insertion of proteins to distinct membrane domains[3] and seem to play a role in transmembrane signaling.[4] GPIs function as a malarial toxin as well[5] and anti-GPI vaccination was shown to prevent malaria in an animal model.[6]

As a general rule, all reported GPI structures present a linear myoinositol containing oligosaccharide backbone. (Figure 1).

While this general structure is conserved, there is a considerable diversity within the GPI anchor family, which is reflected on the nature and the location of branching groups in this pseudo-pentasaccharide skeleton. These branching groups are species-specific carbohydrate side chains, additional phosphoethanolamine units, and variations in the lipid moiety. As for the location, positions 2 and 3 of the proximal mannose unit and positions 2 and 6 of the distal mannose unit seem to invariably constitute the branching points.[1]

Because of their biochemical significance, their biomedical interest, and the challenges involved in their total synthesis, GPIs have received a lot of attention from synthetic chemists, and have been chosen as a target to test methods and strategies in complex oligosaccharide synthesis. .[1–7] Consequently, a number of total syntheses of specific GPIs were reported.[7] However, a general strategy that supports the synthesis of a diversity of GPI structures from a common precursor has not been reported so far.

The development of conditions for the efficient solid-phase synthesis of complex oligosaccharides on automated synthesizers may constitute a key step in deciphering many outstanding questions in Glycobiology.[8] An effective strategy for the synthesis of GPI on solid support that allows the access to a diversity of GPI structures from a common pseudo-pentasaccharide construct is, therefore, highly desirable. With only one exception,[9] all described syntheses of

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[**] This work was supported by the Ministerio de Ciencia y Tecnología (Grant BQU 2002–03734). N.-C. R. thanks the European Union for a fellowship (Grant HRN-CT-2000–00001/Glycotrain).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/anie.200351950

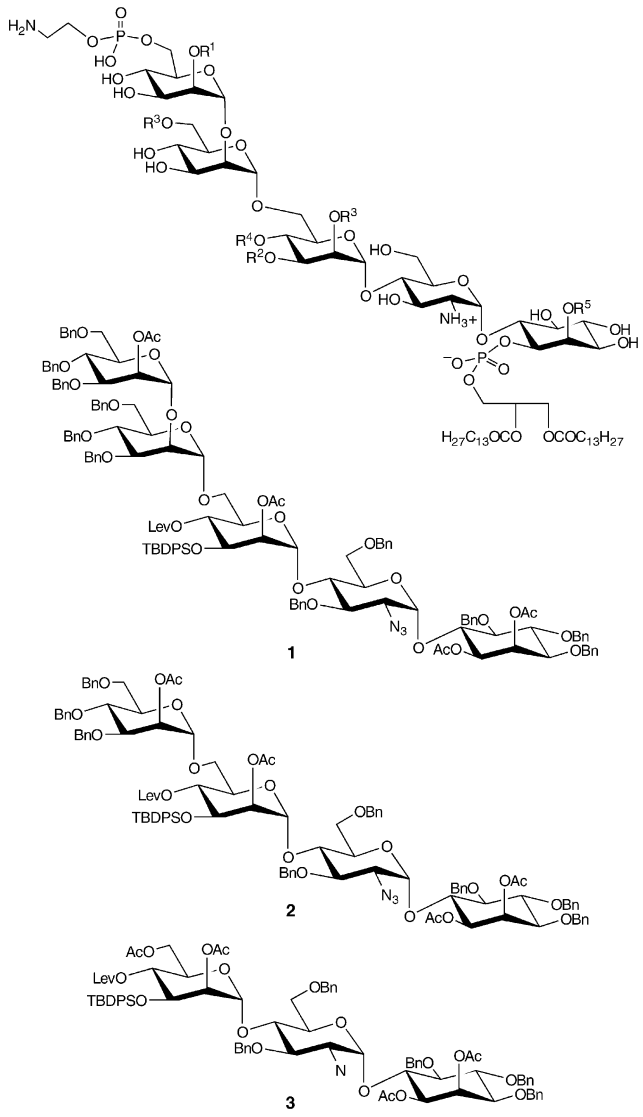


Figure 1. GPI-anchor structures, R^1: Man or not-determined glycan, R^2: Gal_n, GalNAc, GalNAc-Gal-NANA, P-Man, $PEtNH_2$, GlcNAc-Gal, $(GalNAc\text{-}Gal)_9$, GalNAc-Glc, R^3 extra phosphoethanaolamine, R^4: GalNAc, NANA-Gal-GalNAc, R^5: Acyl (not substrate to PI3-Kinase); synthetic GPI-glycan-strucures **1**, **2** and **3**. Man = mannose, Gal = galactose.

GPI structures have been carried out in solution. Because of our long-standing interest in the role of GPI-like molecules in insulin signaling,[10] we have been aiming at an efficient solid-phase step-by-step construction of the GPI pseudo-pentasaccharide backbone. Such a synthesis should permit the introduction of a variety of side chains at the main branching positions by using commercially available linkers and supports, and standard experimental conditions. We have now developed such synthetic procedure, which is reported herein.

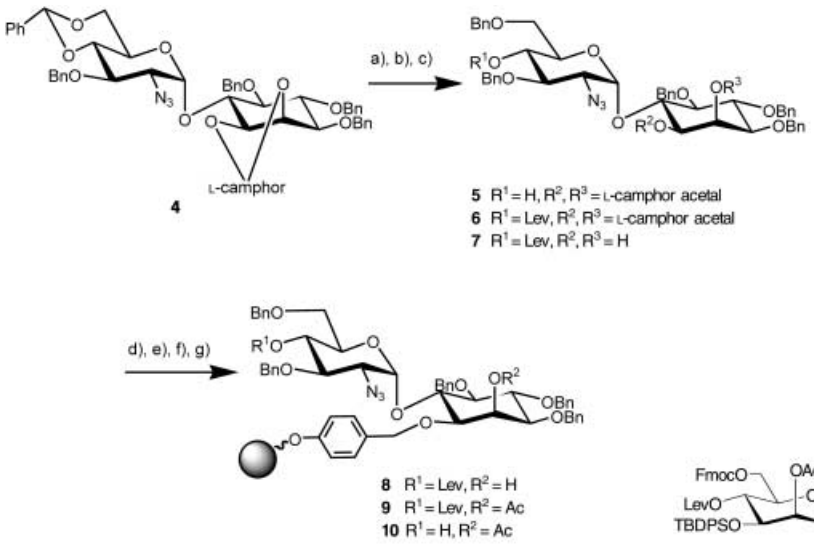
After extensive investigations on the compatibility of all parameters involved such as the type of solid support, linker, attachment strategy, capping procedure, glycosylation method, and protecting group strategy, we chose the following system:

1) A polyethylene glycol (PEG)-grafted polystyrene resin functionalized with a Wang-Cl linker. These flexible PEG grafts provide a solutionlike environment for bound molecules, which results in fast reaction rates and permits the obtainment of high resolution NMR spectra.[11]
2) A Wang-type linker readily cleavable under acidic media but stable to low-temperature-glycosylation conditions with catalytic Lewis acid promoters, and highly stable to basic conditions.[12]
3) A mild experimental procedure for the attachment of the substrate to the solid support through stannylidene activation.
4) *O*-Glycosyl trichloroacetimidates as donors that allow glycosylation under the above conditions.[13]
5) Lev/Fmoc[14] and TBDPS/Lev[15] as orthogonal protecting groups for the construction of the pseudo-pentasaccharide backbone and the establishment of branching points.
6) A fast and traceless cleavage of the target molecule and intermediates.[12]

Under these general conditions pseudo-oligosaccharides **1**, **2** and **3** have been prepared by sequential glycosylation starting from pseudodisaccharide **4**. Compound **4** can be obtained in multigram quantities.[10b,c] The formation of the glucosamine α1→6 myoinositol linkage with complete selectivity is difficult to achieve.[10b,d,e] Therefore, building block **4** was synthesized in solution.

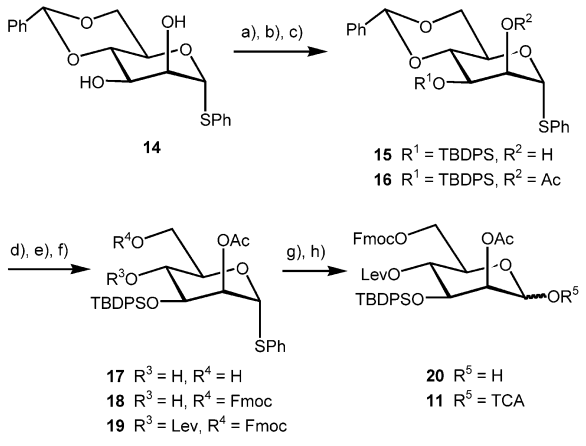
After attempting different strategies, we decided to attach the pseudo-disaccharide glycosyl acceptor to the solid support through the myoinositol moiety. The scarce differences of reactivity among the myoinositol hydroxyl groups may complicate the anchoring process, but results of previous experiments prompted us to use this strategy instead of an alternative attachment through C6-OH of the D-glucosamine unit. Thus, compound **4** was transformed into diol **7** by the regioselective opening of the benzylidene acetal (→**5**), addition of the levulinate group (→**6**) and removal of the L-camphor ketal (→**7**). The attachment of **7** to the solid support was performed by stannylidene acetal-mediated activation of the diol system followed by the reaction with the linker functionalized resin (→**8**) (Scheme 1); the loading was 0.31 mmol g^{-1}. Unreacted linker sites were capped by quenching with methanol or by transforming the hydrolized benzyl chloride functionality into the corresponding trichloroacetimidate and subsequent methylation with methanol in the presence of BF_3OEt_2.[16] Acetylation (→**9**) and subsequent removal of the levuline group afforded **10**. ^1H NMR analysis of a cleaved sample showed a 2:1 C1-OH/C2-OH regioselectivity for the attachment process. This is of no consequence for the solid phase synthesis of the oligosaccharide skeleton herein described but has to be taken into account for the further solution phase installation of the lipid moiety.

Mannopyranosyl trichloroacetimidates **11**, **12**[17] and **13**[18a,b,c] have been sequentially used in the step-by-step construction of the orthogonally protected pseudo-oligosaccharide backbone **1**. The highly functionalized glycosyl donor **11** was designed as to permit selective deprotection at C3, C4, and C6 for further attachment of branching groups. It contains a combination of acetyl, silyl, levulinoyl and Fmoc orthogonal protecting groups[14,15] and could be obtained in a



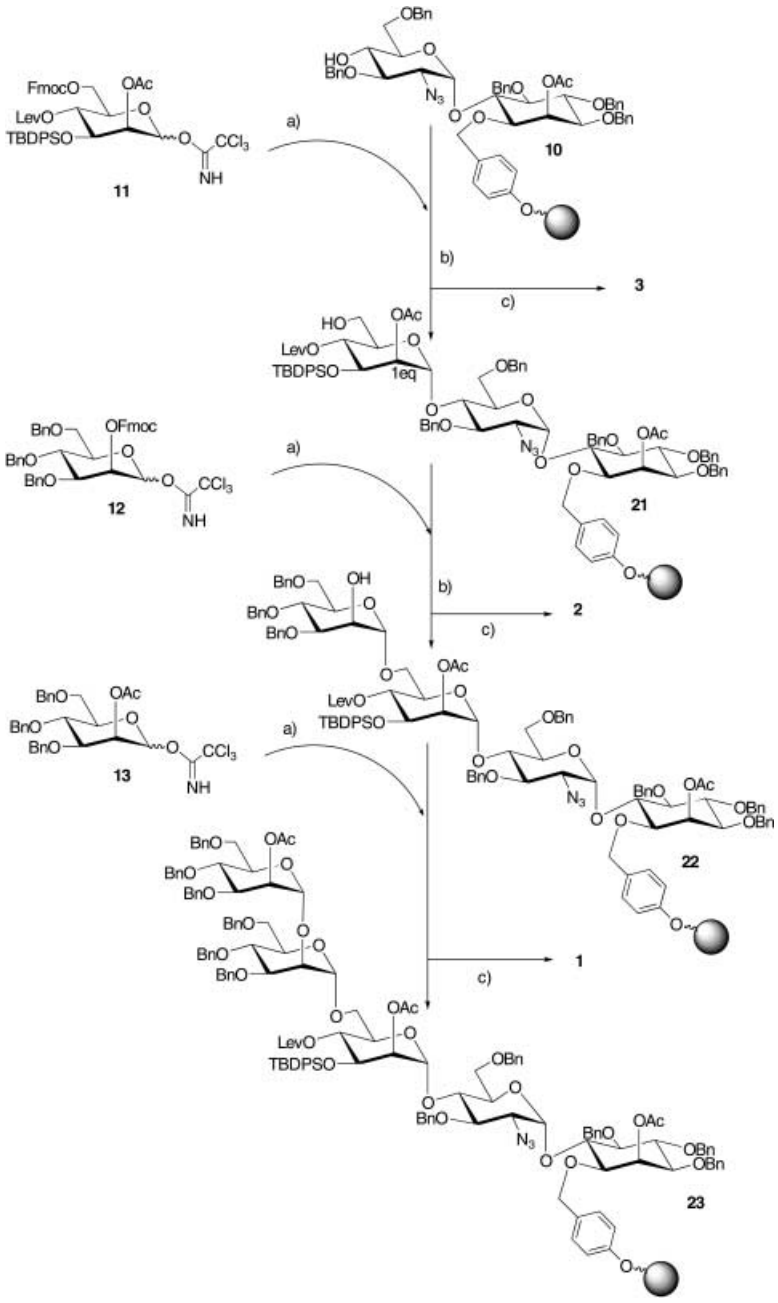
Scheme 1. Preparation and attachment of pseudodisaccharide **5** to resin. a) $NaCNBH_3$, HCl (1 M sol. in Et_2O), THF, 10 min, 81%; b) LevOH, DCC, DMAP, CH_2Cl_2, 1 h, 95%; c) TFA 20% in $CHCl_3$, 80%; d) Bu_2SnO, MeOH, reflux, 3 h; e) resin Argo-Gel-Wang-Cl, [21] (0.43 mmol g^{-1}), TBAI, CsF, MeCN, 70–80%, 1-OH/2-OH 1:2; f) Ac_2O, py, DMAP, CH_2Cl_2, 1.5 h, quant.; g) N_2H_4:AcOH, CH_2Cl_2, 20 h, quant. Lev = $RCOCH_2CH_2COCH_3$ DCC = dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, TFA = trifluoroacetic acid, TBAI = tetrabutylammonium iodide, py = pyridine.

straightforward manner (Scheme 2). Stannylidene acetal mediated silylation of thioglycoside **14**[19] gave the 3-*O*-silyl derivative **15**. Conventional acetylation afforded **16**. Cleavage of the benzylidene acetal in **16** yielded diol **17** that was selectively protected as a 6-Fmoc ester to give **18**. The levulinic ester group was then installed (→**19**). Mild hydrolysis gave **20** that was activated as trichloroacetimidate **11** (Scheme 3).[20]



Scheme 2. Synthesis of key mannose donor **7**. a) Bu_2SnO, MeOH, reflux, 1 h; b) TBDPSCl, DMF, TBAI, 16 h, 71%; c) Ac_2O, py, DMAP, 2 h, 95%; d) BF_3OEt_2, EtSH, CH_2Cl_2, 92%; e) FmocCl, MeCN, py, DMAP, 1 h, 85%; f) LevOH, DCC, DMAP, 1.5 h, 85%; g) NBS, acetone, H_2O, −20 °C, 2 h, 90%; h) Cl_3CCN, NaH, 40 min, 79%. TBDPS = *tert*-butyldiphenylsilyl, DMF = dimethylformamide, Fmoc = 9-fuorenylmethoxycarbonyl, NBS = *N*-bromosuccinimide.

The synthesis of the pseudopentasaccharide skeleton started with the glycosylation of acceptor **10** with donor **11** in the presence of TMSOTf to afford the corresponding resin-bound trisaccharide. A preparative sample was cleaved from the resin, submitted to treatment with triethylamine to release the Fmoc group, and acetylated to give pseudotrisaccharide **3** that was fully characterized. Glycosyl acceptor **21** was obtained after hydrolysis of the Fmoc group and then glycosylated with glycosyl donor **12**[17] in the previously established conditions. Again a preparative



Scheme 3. Assembly of glycan structure on solid support a) TMSOTf (0.1 equiv), CH_2Cl_2, −20 °C, 2 h; b) Et_3N, CH_2Cl_2, room temperature, 4 h; c) 1. TFA 10% in $CHCl_3$, room temperature, 2 h, 2. Ac_2O, py, DMAP, 0 °C, 2 h. TMS = Trimetylsilyl, Tf = trifluoromethanesulfonate.

sample was cleaved and acetylated to give pseudotetrasaccharide **2** the ^{1}H NMR spectrum of which was fully assigned. The final assembly was performed by generating the glycosyl acceptor as above and performing a further glycosylation step with donor **13**[18a–c] to afford **23**. Compound **1** was obtained after releasing from the resin and acetylation. The overall yield for the target structure **1** was 20% after a ten steps synthesis with an average 85% per step.

The above results describe a practical solid phase strategy for the assembly of GPI precursor structures that combines a solution like environment and a fully orthogonal protecting-group pattern with the efficiency of the trichloroacetimidate method for stereoselective glycosylation. The use of a PG-grafted resin permits high resin loadings without affecting the performance of the coupling reactions. The attachment of the pseudodisaccharide unit to the resin using a mild stannylene activation constitutes a convenient and original procedure for highly functionalized derivatives. The biosynthetically inspired linear construction of the GPI backbone permits to access to GPI-like intermediates from the same precursor. The described system allows for the construction of a diversity of structures in a combinatorial manner and for the generation of a small library of GPI precursors.

Received: May 22, 2003
Revised: July 1, 2003 [Z51950]

Keywords: carbohydrates · glycolipids · glycosylation · protecting groups · solid-phase synthesis

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