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Synthesis of the Glycosyl Phosphatidyl Inositol Anchor of Rat Brain Thy-1[1]

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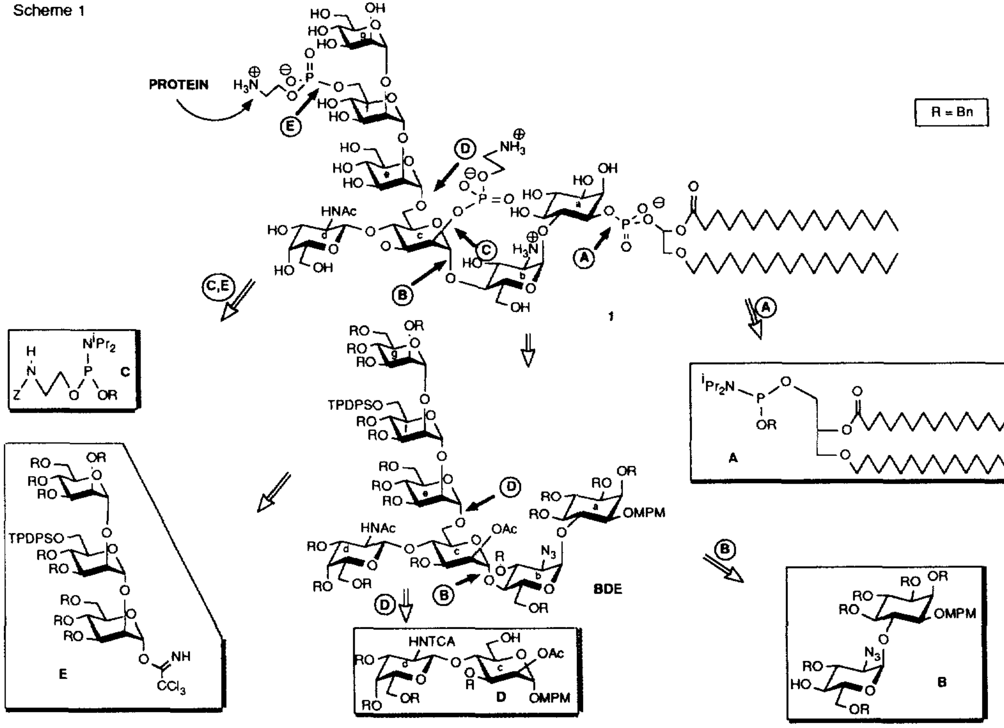
Abstract: Disintegration of the target molecule **1** into building blocks **A-E** was performed. For **D**, an efficient synthesis of mannose derivative **5**, representing mannose residue c in the target molecule, could be performed; **5** permits the required regioselective access to *C*-1, 2-*O*, 4-*O*, and 6-*O*. Reaction of **5** with galactosamine donor **3** led to **D** in high yield. **E** could be readily prepared from known mannosyl donors **7** and **9**. Combination of **A-E** led to the fully *O*-benzyl protected target molecule **14** in only eleven highyielding steps, thus exhibiting the efficiency of this convergent strategy, which provides target molecule **1**. © 1999 Elsevier Science Ltd. All rights reserved.

Glycosylphosphatidylinositols (GPIs) are a class of naturally occurring glycophospholipids that anchor the C-termini of various proteins and glycoproteins to the membrane of eucaryotic cells.[2,3] Full structural assignment was first reported by Ferguson et al. in 1988.[4,5] Since then various GPI anchors have been characterized, thus leading to a core structure which consists of a 6-*O*-aminoethylphosphoryl-Manα(1-2)Manα(1-6)GlcNH$_2\alpha$(1-6)-

Scheme 1



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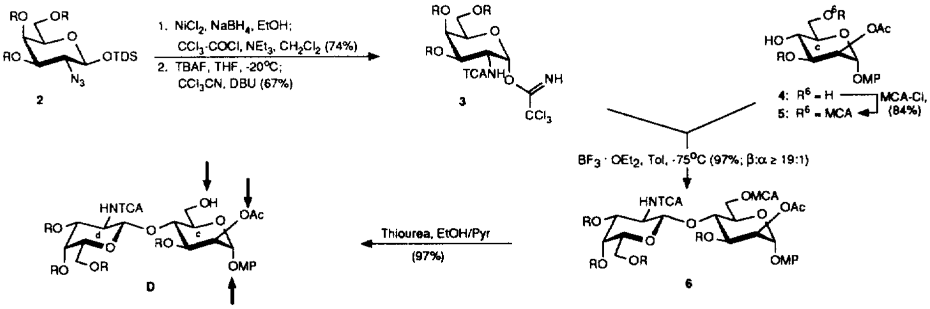
D-*myo*-inositol residue. The occurrence of additional carbohydrate residues or ethanolamine phosphate side chains is species specific.[2,3] There is also a variation in the membrane anchoring lipid structures, which depend on species and on cell-type. The role of GPI-anchored proteins as enzymes, in interactions with bioactive factors, and in cell-cell recognition has been extensively discussed.[2,3,6] Moreover, there is evidence that metabolites derived from GPI anchors or structurally related compounds are mediators of regulatory processes.[6,7] Therefore, the GPI anchors themselves and partial structures thereof are gaining in significance . Thus, the challenging chemical synthesis of structurally homogeneous GPI anchors and derivatives is an important objective in order to perform biological studies.

For the total synthesis of GPI anchors, a combination of lipid, phosphate, and oligosaccharide chemistry is required. This has been successfully carried out for a ceramide containing GPI anchor of yeast[8,9] and for the acylglycerol containing GPI anchors of *Trypanosoma brucei*[10] and of rat brain Thy-1.[11,12] We report here on a total synthesis of rat brain Thy-1 GPI anchor (**1**, Scheme 1). The strategy is highly convergent and partly building blocks of the yeast GPI anchor total synthesis could be employed, thus exhibiting the versatility of this approach. The target molecule **1** was disconnected at positions Ⓐ - Ⓔ yielding building blocks **A**-**E**. **A**[11,13] and **C**[8,10] have been previously prepared. **B** and **E** are composed of monosaccharide building blocks which are also known from previous work.[8,9] For **D** a mannose derivative (mannose residue c in **1**) is required which permits regioselective access to *C*-1, 2-*O*, 4-*O*, and 6-*O*; additionally, a highly reactive galactosamine donor is required. Finally, construction of heptasaccharide **BDE** (Scheme 1) and regioselective linkage of **A** and **C** accommodating consecutive attachment of two aminoethylphosphate residues has to be accomplished. Our aim is to demonstrate the efficiency of this strategy with respect to the synthesis of the individual building blocks and their coupling in order to provide target molecule **1**.

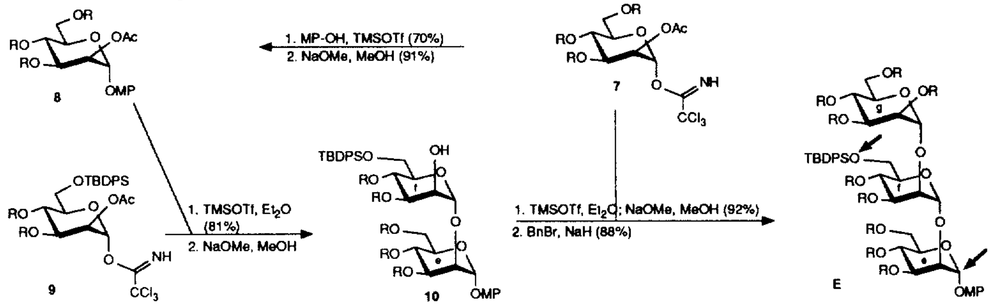
For the synthesis of disaccharide building block **D**, the required galactosamine donor could be readily obtained from known 2-azido-galactose derivative **2**[14] (Scheme 2). In order to generate high glycosyl donor properties, the azido group was transformed into the trichloroacetyl (TCA)-amino group via azide reduction with $NiCl_2/NaBH_4$[16] and then acylation with trichloroacetyl chloride in the presence of triethylamine; ensuing removal of the thexyldimethylsilyl(TDS) group with tetrabutylammonium fluoride (TBAF) in THF at -20 °C and then treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7ene (DBU) gave donor **3** in good overall yield. The variably functionalized mannose residue c was constructed from 4-methoxyphenyl mannopyranoside **4** which can be readily obtained from per-*O*-acetylmannose.[15] Selective 6-*O*-protection with monochloroacetyl(MCA) chloride in the presence of imidazole gave 4-*O*-unprotected mannoside **5**, which exhibited the required properties demanded above: i.e. regioselective access to *C*-1, 2-*O*, 4-*O*, and 6-*O*. Reaction of **5** with donor **3** in the presence of $BF_3 \cdot OEt_2$ as catalyst at -75 °C in toluene afforded the β(1-4)-linked disaccharide in almost quantitative yield; only traces of the α-isomer were found, which could be readily separated. Ensuing treatment of **6** with thiourea in EtOH/pyridine gave desired 6c-*O*-unprotected building block **D** which still permits selective access to 6c-*O*, *C*-1c, and 2c-*O* (see arrows).

Scheme 2 (R = Bn)



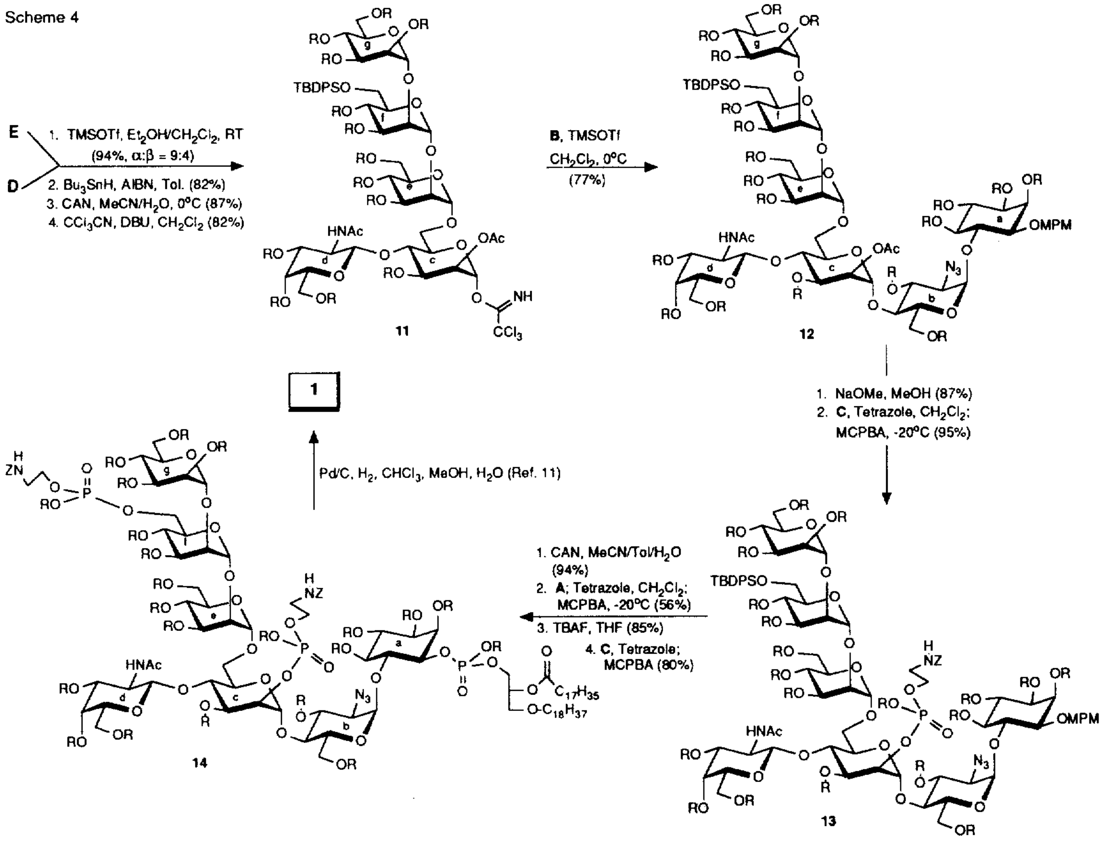
For the synthesis of trimannosyl building block **E**, known mannosyl donor **7**[8,9] (Scheme 3) was transformed into acceptor **8** via glycosylation of methoxyphenol and ensuing removal of the 2-*O*-acetyl group under Zemplén conditions.[17] Glycosylation of **8** with known mannosyl donor **9**[8,9] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst gave the α(1-2)-linked disaccharide in high yield, which on removal of the 2f-*O*-acetyl group afforded acceptor **10**. Treatment of **10** with donor **7** in the presence of TMSOTf as catalyst afforded the α-linked trisaccharide in high yield. Cleavage of the 2g-*O*-acetyl group and then benzylation led to the 2g-*O*-benzyl protected derivative. Oxidative cleavage of the MP group with ceric ammonium nitrate (CAN) as oxidizing agent and then treatment with trichloroacetonitrile/DBU furnished the desired building block **E** in very high overall yield.

Scheme 3



Glycosylation of acceptor **D** with trimannosyl donor **E** (Scheme 4) turned out to be a critical reaction because the expected high α-selectivity, typical for mannosyl donors, was not observed. This problem could be essentially overcome by carrying out the reaction in a dichloromethane/ether mixture at room temperature, thus leading to a high pentasaccharide yield with an acceptable α-selectivity (65% α-product was isolated). The

Scheme 4



transformation of the trichloroacetyl into the acetyl group was readily performed with tributyltin hydride in the presence of azoisobutyronitrile (AIBN).[18] Oxidative cleavage of the MP group as described above and then treatment with trichloroacetonitrile/DBU gave trichloroacetimidate **11** as pentaosyl donor. Reaction of **11** with building block **B** in the presence of TMSOTf as catalyst afforded exclusively α(1-4)-linkage between residues c and b providing heptasaccharide **12** (= **BDE** in Scheme 1) in high yield. Removal of the 2c-*O*-acetyl group and then reaction with phosphitamidite **C**[8,10] in the presence of tetrazole and ensuing oxidation with monochloroperbenzoic acid (MCPBA) led to attachment of the first aminoethyl phosphate residue at 2c-*O*, thus furnishing intermediate **13**. As next step, the MPM group of the inositol residue was removed under oxidative cleavage with CAN. Ensuing treatment with building block **A**[11,13] led under standard conditions to the attachment of the glycerol phosphate residue. Then, the 6f-*O*-TBDPS group was removed with TBAF in THF; ensuing treatment with **C** as described above led to attachment of the second aminoethylphosphate residue affording compound **14** which respresents the fully protected target molecule. **14** was already obtained via a different route,[11] thus the structure could be verified by comparison with the reported data.[19] Hydrogenolysis of all *O*-benzyl groups led, as described,[11] to target molecule **1**.[5]

In conclusion, the required monosaccharide derivatives **2**, **5**, **7**, and **9** are readily available in large scale; they lead to di- and trisaccharide building blocks **D** and **E**, respectively, in an efficient manner. Their combination with known building block **B** furnishes the desired heptasaccharide moiety in just five steps. Only seven additional steps are required in order to regioselectively and consecutively attach two **C** residues and the **A** residue and finally to liberate the target molecule. Thus, the efficiency and versatility of the designed strategy could be demonstrated, which also should permit regioselective attachment of a peptide or a protein molecule at one of the aminoethylphosphate residues.

References and Notes

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