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SYNTHETIC STUDY ON GLYCOPHOSPHATIDYL INOSITOL (GPI) ANCHOR OF *TRYPANOSOMA BRUCEI*: GLYCOHEPTAOSYL CORE[1)]

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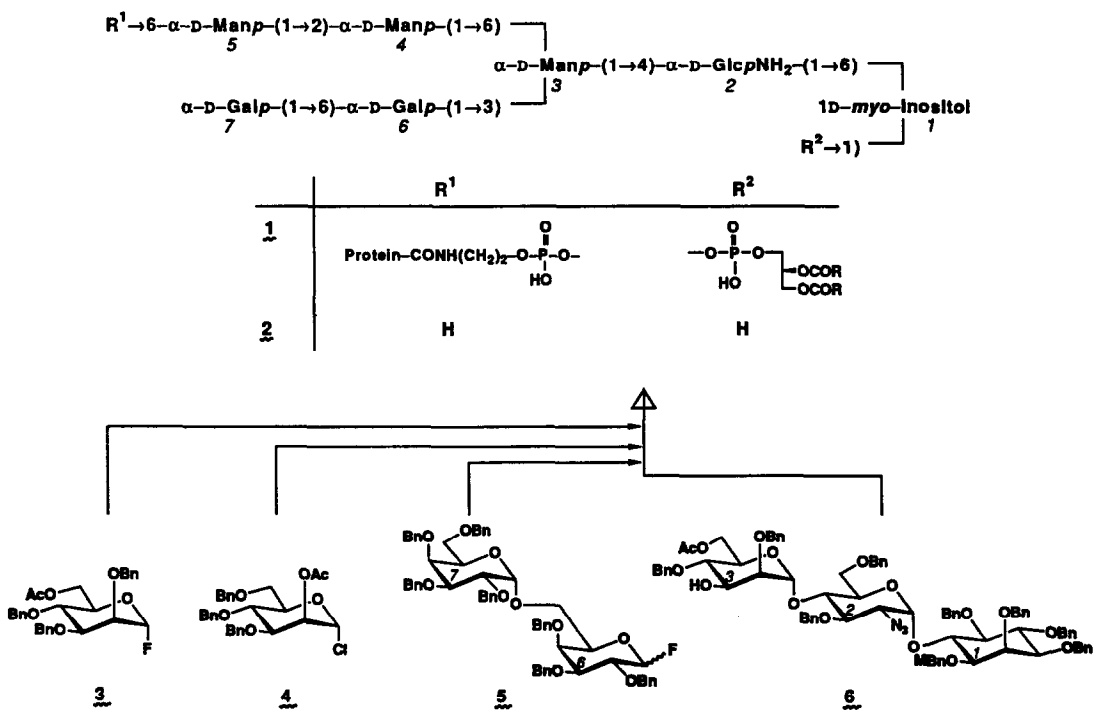
Abstract: A stereocontrolled approach to the synthesis of glycoheptaosyl core of glycophosphatidyl inositol (GPI) anchor of *Trypanosoma brucei* is described for the first time.

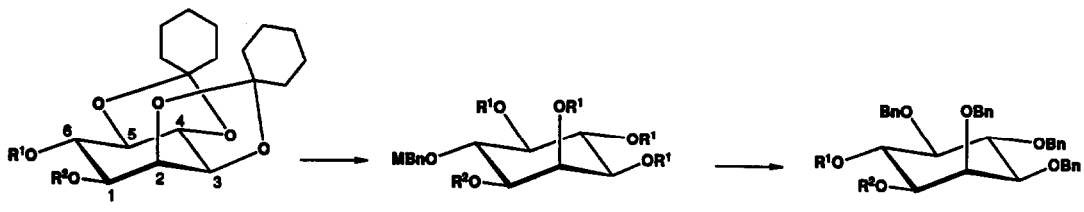
In 1988, chemical structures of the major species of glycophosphatidyl inositol (GPI) anchor present on a variant surface glycoprotein (VSG) of the parasitic protozoan *Trypanosoma brucei* were determined[2]. A typical structure may be depicted as **1**. Subsequently, was reported[3] the structure for GPI anchor of rat brain Thy-1 glycoprotein which has a common backbone structure with the anchor of VSG but shows significant differences in the branching pattern of the glycans. Involvement of these GPI molecules not only in the anchoring of proteins to membranes but also in transmembrane signaling function of insulin was recently reported[4]. We discuss here a stereocontrolled synthesis of glycoheptaosyl core **2** of the GPI anchor **1**. It is to be noted that in close connection with our approach an elegant synthesis of a glycopentaosyl part of **2** was recently described by Fraser-Reid and co-workers[5].



Scheme 1 (MBn = 4-MeO-Bn)

Our synthetic strategy for the target molecule **2** is mainly based on the proper choice of the protective groups that should be in harmony with an overall strategy aiming at the total

synthesis of **1**. Therefore the hydroxyl groups of the immediate precursor **2** of **2** were protected so that site-selective phosphorylation at O-1^1 and O-6^5 should be possible in priniciple. Structure of the key glycotriosyl acceptor **6** was designed in order to elongate three different chains at O-1^1, O-3^3, and O-6^3. Addition of the mannobiosyl chain at O-3^6 of **6**, may be achieved by use of two mannosyl donors **3** and **4**, while chain elongation at O-3^3 may be examined by use of a galactobiosyl donor **5**.



	R^1	R^2
7	H	H
8	MBn	H
9	H	MBn

	R^1	R^2
10	Bn	H
11	Bn	CAM

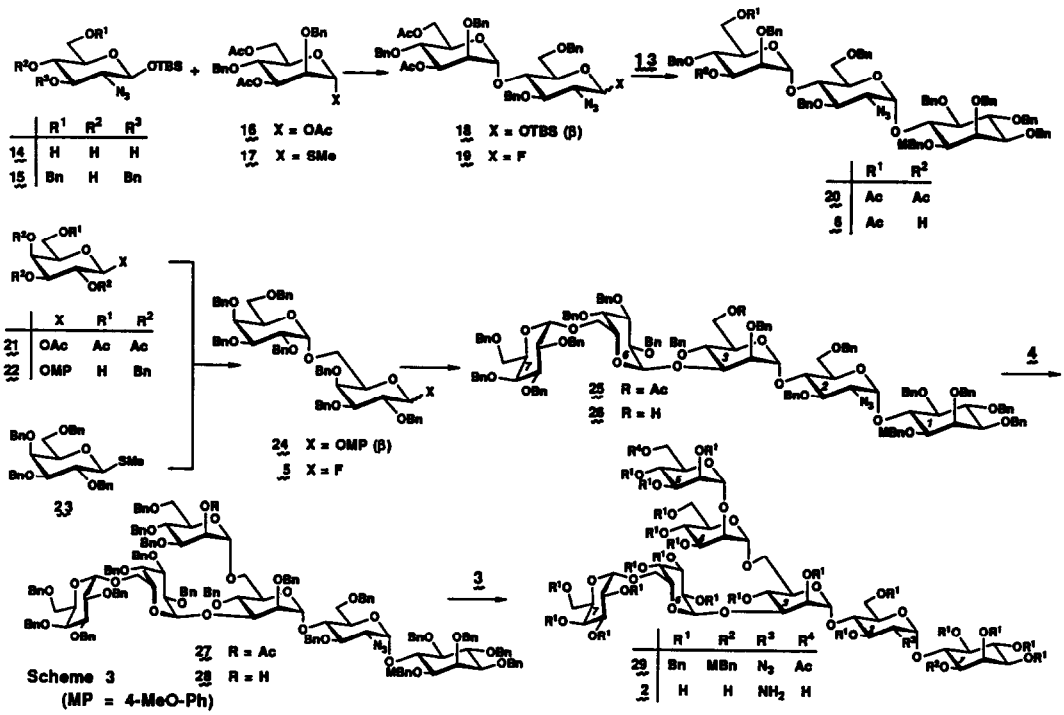
	R^1	R^2
12	H	CAM
13	H	MBn

Scheme 2 (CAM = (1*S*)-(-)-camphanoyl)

The key intermediate **6** was prepared in a following way. Treatment of *myo*-inositol derivative **7**[6] with Bu_2SnO and CsF-MeOBnCl-KI[7] afforded an 81% yield of a mixture of **8**[7] and **9**[8] in a ratio of 3:1. A major product **8** was converted via **10**[8], in 5 steps into a mixture of **11** and the diastereoisomer, from which **11**[8] was isolated by chromatography (*1* CH_2=$CHCH_2Br$, NaH, in DMF, *2* 0.1*M* HCl in MeOH, *3* BnBr, NaH in DMF, *4* KO^{t}Bu in DMSO, then 0.1*M* HCl in aq. Me_2CO[9], *5* (1S)-(-)-camphanic acid chloride[10], Et_3N, DMAP in $(ClCH_2)_2$, then SiO_2 in Et_2O-CH_2Cl_2, 32% overall). Conversion of **11** into a glycosyl acceptor **13**[8] was achieved via **12**[8] in 5 steps (*1* CAN in 4:1 CH_3CN-H_2O[11], *2* CH_2=CHOEt, TsOH•H_2O in CH_2Cl_2, *3* NaOH in MeOH-THF, *4* MeOBnCl[12], NaH in DMF, *5* AcOH-MeOH, 83% overall). Absolute configuration of **13** was confirmed by conversion into the known 2,3,4,5,6-penta-O-benzyl-1D-*myo*-inositol[10,13] in 2 steps {*1* BnBr, NaH in DMF, *2* CAN in 10:1 CH_3CN-H_2O, 84% overall, $[\alpha]_D$ +10.6° (c 0.7, $CHCl_3$)}.

A glycobiosyl fluoride **19** was prepared from two monosaccharide derivatives **14**[14] and **16**[15]. Conversion of **14** into a glycosyl acceptor **15**[14] was achieved in 3 steps (*1* $PhCH(OMe)_2$, TsOH•H_2O in CH_3CN, *2* BnBr, NaH in DMF, *3* BH_3NMe_3, $AlCl_3$, powdered molecular sieves 4A (MS4A) in THF[16], 61% overall). Copper(II) bromide-Bu_4NBr-AgOTf promoted glycosylation[17] of **15** with **17**[8], readily obtainable in 86% from **16**[15] by treatment with Bu_3SnSMe[18] and $SnCl_4$ in $(ClCH_2)_2$, gave 86% of **18** which was converted into **19** in 2 steps (*1* Bu_4NF in AcOH-THF[19], *2* DAST in $(ClCH_2)_2$[20], 96% overall). Crucial coupling of **19** with **13** in the presence of Cp_2ZrCl_2-$AgClO_4$[21] in Et_2O gave **20**[8] and the β-epimer in 73 and 20%, respectively. Conversion of **20** into a key intermediate **6**[8] was performed in 2 steps (*1* NaOMe in THF-MeOH, *2* AcCl in Py, 95% overall).

Copper(II) bromide-Bu_4NBr promoted glycosylation of a primary alcohol **22**[8] obtainable from **21** in 5 steps (*1* 4-MeOPhOH, TMSOTf in CH_2Cl_2, *2* NaOMe in MeOH, *3* 4,4'-$(MeO)_2TrCl$ in Py, *4* BnBr, NaH in DMF, *5* TsOH•H_2O in MeOH, 51% overall) with **23**[22] afforded **24**[8] and the β-(1→6) isomer in 68 and 10% yield, respectively. Conversion of **24** into fluoride **5** (α:β=2:3) was performed in 2 steps (*1* CAN in 5:6:3 toluene-CH_3CN-H_2O, *2* DAST in $(ClCH_2)_2$, 64% overall).



Scheme 3 (MP = 4-MeO-Ph)

Crucial α-stereoselective glycosylation at O-3[3] of **6** with **5** was achieved in the presence of Cp_2ZrCl_2-$AgClO_4$ in Et_2O to give 69% of **25** along with 7% of the β-epimer at C-1[6]. Glycopentaoside **25** was converted in a well established manner into **28** via **26** in 3 steps (*1* NaOMe in THF-MeOH, *2* $HgBr_2$-$Hg(CN)_2$-MS4A, 4[23] in CH_2Cl_2, *3* NaOMe in THF-MeOH, 75% overall).

Finally, Cp_2ZrCl_2 promoted glycosylation of **28** in Et_2O with **3**[8] readily obtainable from corresponding anomeric acetate[24] in 2 steps (*1* NH_2NH_2•AcOH in DMF[25], *2* DAST in $(ClCH_2)_2$, 69% overall) did afford **29**[8] and the β-epimer (98%, α:β=15:1). Deprotection of **29** into **2**[8] was achieved in a conventional way (*1* Pd-C, H_2 in THF-MeOH, *2* NaOMe-MeOH, then Sephadex G25 in H_2O, 94% overall).

In summary, a stereocontrolled approach to the synthesis of core glycoheptaoside **2** of GPI anchor **1** was developed by use of three glycosyl donors **3**, **4**, **5** and a key glycosyl acceptor **6**. Properly protected glycoheptaoside **29** should be regarded as a key intermediate to achieve a total synthesis of **1**.

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Reference and Notes

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8 Physical data for key compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were recorded for solutions in $CHCl_3$ and $CDCl_3$, respectively, at 23°±3°, unless noted otherwise. **2**: $[\alpha]_D$ +2.9° (c 0.07, H_2O); δ_H (D_2O, 60°) 4.97 (d, 3.7 Hz, 1^7), 5.03 (d, 1.4 Hz, 1^5), 5.11 (d, 1.4 Hz, 1^4), 5.19 (d, 4.1 Hz, 1^6), 5.22 (d, 1.6 Hz, 1^3), 5.32 (d, 3.7 Hz, 1^2). **3**: $[\alpha]_D$ +29.2° (c 1.4); δ_H 2.05 (s, Ac), 5.55 (dd, 1.8 and 50.6 Hz, 1); δ_C 106.3 (223 and 182 Hz, 1). **5** (α:β=2:3): δ_H 5.10 (dd, 7.0 and 53.1 Hz, $1^6\beta$), 5.55 (dd, 2.6 and 54.2 Hz, $1^6\alpha$). **6**: $[\alpha]_D$ +69.3° (c 0.6); δ_H 1.92 (s, Ac), 3.66 (s, OMe), 5.27 (d, 1.2 Hz, 1^3), 5.64 (d, 3.7 Hz, 1^2). **8**: δ_H 2.58 (d, 1.2 Hz, O*H*), 3.81 (s, OMe), 4.01 (dt, 3.7 and 1.8 Hz, 1). **9**: δ_H 2.60 (d, 2.1 Hz, O*H*), 3.81 (s, OMe), 4.06 (ddd, 2.1, 7.2, and 9.3 Hz, 6). **10**: δ_H 2.16 (d, 6.4 Hz, O*H*), 3.79 (s, OMe). **11**: $[\alpha]_D$ +9.5° (c 0.6); δ_H 0.91, 1.02 and 1.09 (3s, 3Me), 3.78 (s, OMe), 4.94 (dd, 2.4 and 10.3 Hz, 1). **12**: $[\alpha]_D$ +1.5° (c 0.3); δ_H 0.95, 1.01 and 1.10 (3s, 3Me), 4.91 (dd, 2.6 and 10.2 Hz, 1). **13**: $[\alpha]_D$ +8.8° (c 2.6); δ_H 2.47 (s, O*H*), 3.81 (s, OMe), 4.02 (t, 2.3 Hz, 2). **15**: $[\alpha]_D$ -31.7° (c 0.4); δ_H 0.16 (s, $SiMe_2$), 0.94 (s, tBu), 3.64 (dt, 2.2 and 9.5 Hz, 4), 4.53 (d, 7.6 Hz, 1). **17**: $[\alpha]_D$ +53.1° (c 0.4); δ_H 1.97, 2.06, 2.12 (3s, 3Me), 5.18 (dd, 3.4 and 9.5 Hz, 3), 5.24 (d, 1.5 Hz, 1); δ_C 83.0 (167 Hz, 1). **18**: $[\alpha]_D$ +5.4° (c 0.4); δ_H 0.17 and 0.18 (2s, $SiMe_2$), 0.94 (s, tBu), 1.96 and 1.98 (2s, 2Ac), 4.55 (d, 6.1 Hz, 1^2), 5.21 (dd, 2.9 and 8.1 Hz, 3^3), 5.24 (d, 2.4 Hz, 1^3); δ_C 97.2 (162 Hz, 1^2), 99.8 (167 Hz, 1^3). **19** (α:β=1:2): δ_H 5.11 (dd, 7.0 and 52.4 Hz, $1^2\beta$), 5.68 (dd, 2.2 and 52.8 Hz, $1^2\alpha$). **20**: $[\alpha]_D$ +49.2° (c 0.6); δ_H 1.92 and 1.96 (2s, 2Ac), 3.62 (s, OMe), 5.19 (d, 2.1 Hz, 1^3), 5.29 (dd, 3.1 and 9.5 Hz, 3^3), 5.60 (d, 3.7 Hz, 1^2); δ_C 97.6 (177 Hz, 1^2), 100.3 (172 Hz, 1^3). **22**: $[\alpha]_D$ -24.8° (c 0.2); δ_H 3.76 (s, OMe), 4.88 (d, 8.1 Hz, 1). **24**: $[\alpha]_D$ +17.7° (c 0.4); m.p. 126-127° (iPr_2O); δ_H 3.67 (s, OMe), 4.73 (d, 3.3 Hz, 1^7), 4.82 (d, 7.7 Hz, 1^6); δ_C 98.2 (170 Hz, 1^7), 103.1 (161 Hz, 1^6). **25**: $[\alpha]_D$ +72.2° (c 0.5); δ_H 1.82 (s, Ac), 3.18 (dd, 3.7 and 10.1 Hz, 2^2), 3.48 (s, OMe), 5.24 (s, 1^3), 5.63 (d, 3.7 Hz, 1^2); δ_C 97.6 (176 Hz, 1^2), 98.9 (170 Hz, 1^7), 99.4 (170 Hz, 1^6), 99.9 (166 Hz, 1^3). **26**: $[\alpha]_D$ +63.0° (c 0.4); δ_H 3.47 (s, OMe), 5.28 (bs, 1^3), 5.58 (d, 3.7 Hz, 1^2). **27**: $[\alpha]_D$ +73.2° (c 0.6); δ_H 2.05 (s, Ac), 3.45 (s, OMe), 5.24 (1.1 Hz, 1^3), 5.32 (d, 1.5 Hz, 1^4), 5.38 (d, 1.8 Hz, 1^6), 5.39 (dd, 1.8 and 3.3 Hz, 2^4), 5.61 (d, 3.7 Hz, 1^2); δ_C 97.7 (176 Hz, 1^2), 98.2 (171 Hz, 1^7), 98.5 (172 Hz, 1^4), 99.4 (169 Hz, 1^6), 100.2 (1^3). **28**: $[\alpha]_D$ +72.9° (c 0.6); δ_H 3.45 (s, OMe), 5.31 (d, 1.8 Hz, 1^4), 5.63 (d, 3.7 Hz, 1^2); δ_C 97.7 (177 Hz, 1^2), 98.5, 99.3 and 99.8 (~169 Hz, $1^{4,6,7}$), 100.1 (163 Hz, 1^3). **29**: $[\alpha]_D$ +51.0° (c 0.3); δ_H (C_6D_6, 60°) 1.76 (s, Ac), 3.40 (s, OMe), 5.03, and 5.38 (2d, 3.7 Hz, $1^{6,7}$), 5.19, 5.23 and 5.78 (3d, 1.8 Hz, $1^{3,4,6}$), 5.87 (d, 3.7 Hz, 1^2); δ_C 97.6 (175 Hz, 1^2), 98.5, 99.2, 99.4 and 99.5 (~170 Hz, $1^{4,5,6,7}$), 100.1 (1^3).
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