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A TOTAL SYNTHESIS OF GPI ANCHOR OF *TRYPANOSOMA BRUCEI*[1)]

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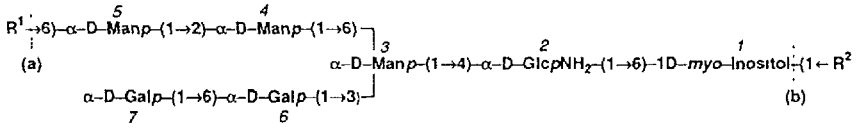
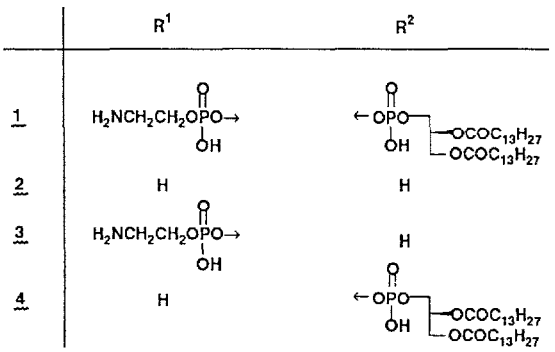
Abstract: A first total synthesis of GPI anchor of *Trypanosoma brucei* is achieved by employing H-phosphonate strategy to introduce two phosphodiester functions into the glycoheptaosyl core intermediate.

In order to develop an efficient synthetic route to the glycophosphatidylinositol (GPI) anchor **1**[2] we have described stereocontrolled approaches to both glycoheptaosyl core[3] **2** and glycobiosyl phosphatidylinositol[1] part structure of **1**. As part of our project on synthetic studies on GPI anchors, we describe here a first total synthesis of GPI anchor **1** of *Trypanosoma brucei*.

Strategic bond disconnection of **1** may be designed either at the dotted line (a) or (b) in order to introduce regioselectively two different kinds of phosphodiester functions to the two hydroxyl groups at C-6[5] and C-1[1] of the glycoheptaosyl core structure **2**. In harmony with this retrosynthetic consideration, we have already reported[3] the synthesis of a properly protected glycoheptaose derivative **5**.





To start with, we planned to introduce a phosphodiester function at C-6[5] hydroxyl group of **6** that was quantitatively obtained (NaOMe in 2:1 MeOH-THF) from acetate **5**. Conversion of **6** into **10** was efficiently achieved by use of phosphitylation[5] approach. In the first step, N-benzyloxycarbonyl-2-aminoethanol was treated with bifunctional phosphitylating reagent chloro-2-cyanoethoxy-N,N-diisopropylaminophosphine[6] in the presence of iPr_2NEt in CH_2Cl_2 for 30 min at 20° to afford after SiO_2 chromatography monofunctional phosphitylating agent **9**[4] in 88% yield. Subsequent tetrazole mediated coupling between **6** and **9** in 5:1 $(ClCH_2)_2$-CH_3CN for 40 min at 20° gave the intermediate phosphite triester that was oxidized *in situ* with m-$ClPhCO_3H$[7] for 20 min at 0° to afford **10**[4] as a diastereomeric mixture in 97% yield. The structure of **10** was

firmly confirmed by conversion into free phosphodiester **3**[4] via **11**[4] in two steps in 85% overall yield, (1) DBU in CH_2Cl_2 for 2h at 20°; (2) $Pd(OH)_2/C$ and H_2 in 5:5:1 MeOH-THF-H_2O →1.1:1 MeOH-THF-H_2O for 6h at 20°. However, attempted removal of methoxybenzyl group at O-1[1] of **10** by either $(NH_4)_2Ce(NO_3)_6$[8] or TMSOTf[9] failed in our hands to give the desired key intermediate **12**

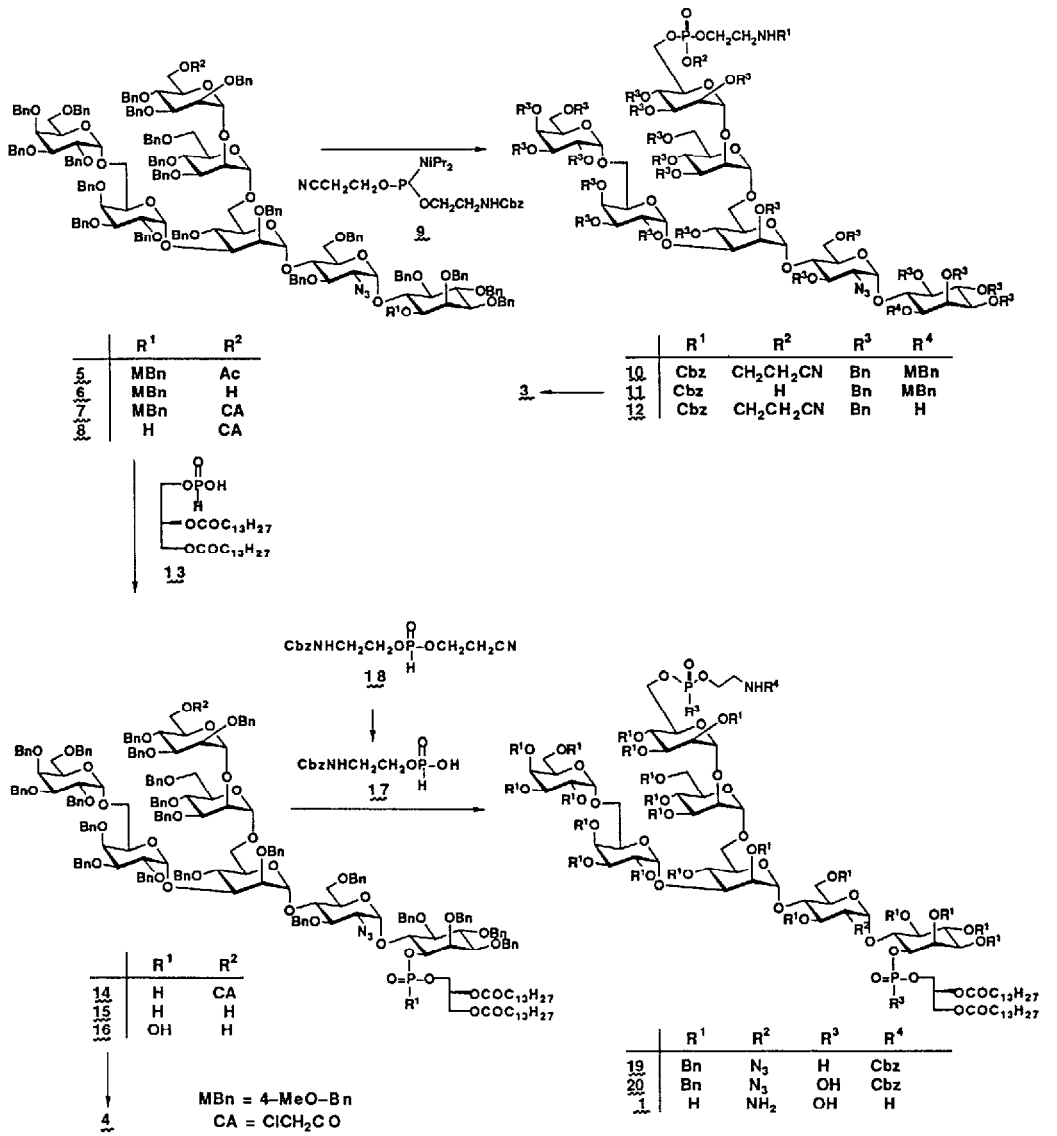
According to the difficulty met with for the conversion of **10** into **12**, an alternative strategy was now pursued. The order of the introduction of phosphodiester functions at O-1[1] and O-6[5] of the glycoheptaosyl core **5** was to be reversed. First, acetyl group at O-6[5] of **5** was exchanged with monochloroacetyl group to secure in later stage of the synthesis a facile selective removal of O-6[5] protective group in the presence of diacyl functions of glycerol moiety Treatment of **6** with $(ClCH_2CO)_2O$[10] in the presence of 2 equivalents of pyridine in $(CH_2Cl)_2$ gave a quantitative yield of **7**[4] that was further converted into alcohol **8**[4] in 90% yield by treatment with 1 5 equivalents of TMSOTf[9] in CH_2Cl_2 for 20 min at 0°.

Formation of the phosphodiester linkage between **8** and 1,2-diacyl-sn-glycerol was executed by use of the H-phosphonate approach[11] that had been shown highly efficient in the case of a model reaction[1] using a glycobiosyl-*myo*-inositol derivative. Coupling of **8** with H-phosphonate **13**[1] in the presence of pivaloyl chloride in pyridine for 1 5 h at 20° gave a 64% yield of **14**[4], that was completely deblocked in 3 steps via **15**[4] and **16**[4] to afford **4**[4] in 59% overall yield, (*1*) $(NH_2)_2CS$[12] in 1·1 EtOH-THF, reflux for 5h; (*2*) I_2 in 50:1 pyridine-water; (*3*) $Pd(OH)_2/C$ and H_2 in 45.35:1 $CHCl_3$-MeOH-H_2O for 4.5 h at 20°

Having the designed key intermediate **15** synthesized, formation of the phosphodiester function between the 2-aminoethanol derivative and **15** was now examined It was to be noted that application of the phosphitylating reagent **9** in this specific case failed to give the desired phosphite intermediate. However, H-phosphonate approach was found to be highly efficient. Treatment of N-benzyloxycarbonyl-2-aminoethanol with a bifunctional phosphitylating reagent $NCCH_2CH_2OP(NiPr_2)_2$[13] in the presence of 1H-tetrazole in CH_3CN for 20min at 20° gave diakloxy N,N-diisopropylphosphine intermediate that was *in situ* treated with H_2O and 1H-tetrazole for 30 min at 20° to afford **18**[4] in 59% yield after SiO_2 chromatography in 97:3 $CHCl_3$-MeOH. Unstable H-phosphonate **17**[4] was obtained by treatment of **18** with DBU in CH_2Cl_2 for 16h at 20° in 38% yield after SiO_2 chromatography in 7.3 $CHCl_3$-MeOH and was used immediately for the next step

Crucial coupling between **15** and **17** was executed in the presence of pivaloyl chloride in pyridine for 1 5 h at 20°. Purification of the product by Bio-Beads SX-2 in 3:1 EtOAc-toluene and subsequent preparative SiO_2 tlc in 9:1 toluene-acetone afforded a 40% yield of the desired **19**[4] as a mixture of four diastereoisomers. Oxidation of **19** with I_2 in 50:1 pyridine-H_2O for 2.5 h at 20° gave **20**[4] in 68% yield. Complete deprotection of **20** by hydrogenolysis in the presence of 20% $Pd(OH)_2/C$ in 45:35.1 $CHCl_3$-MeOH-H_2O for 3.5 h at 20° gave the product which was purified by treatment with Amberlite IRC50(Na^+) and then by Sephadex G-25 in H_2O to afford the target molecule **1**[4] in 23% yield.

In conclusion, a first total synthesis of GPI anchor **1** of *Trypanosoma brucei* was achieved by employing highly stereocontrolled glycosylation methods to construct a key glycoheptaosyl core derivative **5**[3] with proper protective groups on 23 hydroxy groups and then by taking advantage of a highly efficient H-phosphonate strategy for the formation of two phosphodiester functions. Obviously, the synthetic strategy developed in this paper should be applicable to the synthesis of other groups of GPI anchors and their analogues of biological significance.



	R^1	R^2
5	MBn	Ac
6	MBn	H
7	MBn	CA
8	H	CA

	R^1	R^2	R^3	R^4
10	Cbz	CH_2CH_2CN	Bn	MBn
11	Cbz	H	Bn	MBn
12	Cbz	CH_2CH_2CN	Bn	H

	R^1	R^2
14	H	CA
15	H	H
16	OH	H

	R^1	R^2	R^3	R^4
19	Bn	N_3	H	Cbz
20	Bn	N_3	OH	Cbz
1	H	NH_2	OH	H

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Reference and Notes

1 Part 78 in the series "Synthetic Studies on Cell-Surface Glycans". For part 77, see C. Murakata and T Ogawa, *Tetrahedron Lett.*, in press.

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4 All new compounds were characterized by TLC and NMR(500 MHz) as well as by combustion

analysis. Physical data for key compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C,P}$ were recorded for solutions in $CHCl_3$ and $CDCl_3$, respectively, at 23°±3°, unless noted otherwise **1** R_F 0.68 in 3:2:2 1 H_2O-EtOH-BuOH-28%NH_4OH, δ_H (49:1, DMSOd$_6$-D_2O, 60°) 5 266 (bs, 1^3), 5 091 (m, 2^8), 5.019 (bs, 1^2), 4.987 (bs, 1^4), 4.902 (d, 3 3 Hz, 1^6), 4.826 (s, 1^5), 4.648 (bs, 1^7), 4.312 (dd, 3.1 and 12.3 Hz, 1^8), 4.095 (dd, 7.1 and 11.9 Hz, $1'^8$), 1.245 (m, CH_2 x 20), 0.856 (t, 7 0 Hz, CH_2CH_3 x 2); δ_P (49:1 DMSOd$_6$-D_2O) -0.818 and -0 018. **3** $[\alpha]_D$ +90.3° (c 0.3, H_2O); δ_H 5.385 (d, 3.7 Hz, 1^2), 5.300 (d, 2.3 Hz, 1^3), 5 199 (d, 4 0 Hz, 1^6), 5.094 (d, 1.3 Hz, 1^4), 5.039 (d, 1.5 Hz, 1^5), 4.973 (d, 3.7 Hz, 1^7); δ_P (D_2O) 1.092. **4**: δ_H (49.1 DMSOd$_6$-D_2O, 60°) 5.235 (s, 1^3), 5.086 (m, 2^8), 4.942 (d, 4 0 Hz, 1^2), 4 903 (d, 3.9 Hz, 1^6), 4.893 (d, 1 5 Hz, 1^4), 4 855 (s, 1^5), 4.646 (bs, 1^7), 4 313 (dd, 3.1 and 11.9 Hz, 1^8), 4.096 (dd, 7 3 and 12 1 Hz, $1'^8$), 0.856 (t, 7.0 Hz, CH_2CH_3 x 2) δ_P (DMSOd$_6$) -0.169 **6**: $[\alpha]_D$ +56.7° (c 1.5); δ_H 5.615 (d, 3.7 Hz, 1^2), 5.369 (d, 2.1 Hz, H-1), 3.450 (OMe). **7**. $[\alpha]_D$ +63.8° (c 3.8); δ_H (C_6D_6, 60°) 5.874 (d, 3.7 Hz, 1^2), 5 781 (d, 1 8 Hz, 1^3), 5.388 (d, 3.4 Hz, H-1), 3 402 (OMe). **8**. $[\alpha]_D$ +56.1° (c 2.2), δ_H (C_6D_6, 60°) 5 574 (d, 2 4 Hz, 1^3), 5 440 (d, 4 3 Hz, 1^2), 5.201 (d, 1.8 Hz, H-1), 5.160 (d, 1 2 Hz, H-1), 3.605 (s, $COCH_2Cl$). **9**. δ_H (90 MHz) 7.34 (s, C_6H_5), 5 16 (s, CH_2Ph), 1.18 (d, 6.8 Hz, $2CHCH_3$), 1.17 (d, 6.8 Hz, $2CHCH_3$). **10**: δ_H (C_6D_6, 60°) 5 902 (d, 3 7 Hz, 1^2), 5 829 and 5 818 (2d, 2.0 Hz, H-1 x 2), 5.379 (d, 3.4 Hz, H-1), 3 417 and 3.416 (2s, OMe); δ_P (C_6D_6) -0.136 and -0.054 **11**: $[\alpha]_D$ +53.1° (c 1.4); δ_H (C_6D_6, 60°) 5 902 (bs, H-1 x 2), 5 470, 5.445, 5.388 and 5.350 (4bs, H-1 x 4), 3.409 (OMe); δ_P (C_6D_6) 0.531. **14** δ_H 7.6-7.0 (m, Ph x 21), 5.606 (d, 0.65H, 3.7 Hz, 1^2), 5.546 (d, 0 35 H, 3.4 Hz, 1^2), 0.876 (t, 7.0 Hz, CH_2CH_3 x 2); δ_P 8.629 (d, 708 Hz). **15**: δ_H 7 5-7 0 (m, Ph x 21), 5.597 (d, 0 65 H, 3.4 Hz, 1^2), 5.541 (d, 0 35 H, 3.4 Hz, 1^2), 0.875 (t, 7 0 Hz, CH_2CH_3 x 2); δ_P 8.910 (d, 728 Hz) and 8.575 (d, 709 Hz) in a ratio of 1·2. **16** $[\alpha]_D$ +44 5° (c 1.1); δ_H (C_6D_6-D_2O, 60°) 7.6-7.0 (m, Ph x 21), 5.828 (d, 3.4 Hz, 1^2), 5.363 and 5 316 (2s, H-1 x 2), 0.911 (t, 6.7 Hz, CH_2CH_3 x 2); δ_P (C_6D_6) 0.178. **17**: δ_H (90 MHz) 7.33 (s, C_6H_5), 5.10 (s, CH_2Ph), 4.08 (dt, 9 0 and 4.8 Hz, CH_2CH_2OP), 3.43 (t, 5.0 Hz, CH_2NH). **18**· δ_H 7 38-7.31 (m, C_6H_5), 6.894 (d, 719 Hz, PH), 5.119 (s, CH_2Ph); δ_P 13.15 (d, 719 Hz). **19**: δ_H (C_6D_6-D_2O, 60°) 7.6-7.0 (m, Ph x 22), 0 909 (t, 7.0 Hz, CH_2CH_3 x 2); δ_P (C_6D_6-D_2O) 10 52 (d, 706 Hz), 9.12 (d, 714 Hz), 8.78 (d, 708 Hz) and 8.75 (d, 708 Hz). **20**· $[\alpha]_D$ +42.6° (c 0.3); δ_P ($CDCl_3$) 0 305 and -0.646.

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