Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 14, pp. 1715-1718, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00306-X

GPI MEMBRANE ANCHORS: SYNTHESIS AND FUNCTIONAL EVALUATION OF AMINOGLUCOSYL PHOSPHATIDYLINOSITOL CORE ANALOGS

Jianhua Ye,[†] William T. Doerrler,[#] Mark A. Lehrman,[#]
and J. R. Falck[†#*]

Departments of Biochemistry[†] and Pharmacology[#]
University of Texas Southwestern Medical Center
Dallas, Texas 75235

Abstract: Analogs of GPI membrane anchor precursors were prepared from chiral inositol **1** and evaluated as substrates in rodent and trypanosomal cell-free incubates. Di-octanoyl GlcNα-PI **5b** was an efficient mannose acceptor whereas di-palmitoyl GlcNα-PI **5a** was unexpectedly refractory. Di-octanoyl β-anomer **8** was mannosylated only under conditions that permitted acylation of the inositol residue.

Virtually all eukaryotic cells utilize glycosyl phosphatidylinositides (GPIs) to target[1] maturing cell-surface proteins to the outer leaflet of the plasma membrane and to anchor them therein.[2] Other forms of this glycolipid have been implicated in cellular signal transduction.[3] They are added to proteins *en bloc* posttranslationally[4] and typically consist of a highly conserved 2-amino-2-deoxyglucosyl α-L-phosphatidyl-**D**-*myo*-inositol (GlcNα-PI) core attached glycosidically to a mannose-rich oligosaccharide domain which in turn is bound to the polypeptide via a phosphoethanolamine linker (Fig. 1).[5]

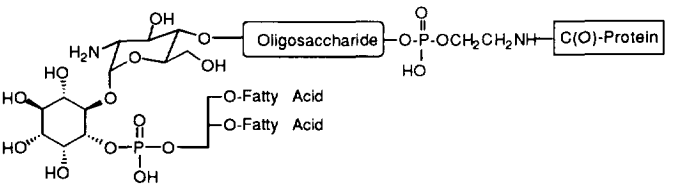
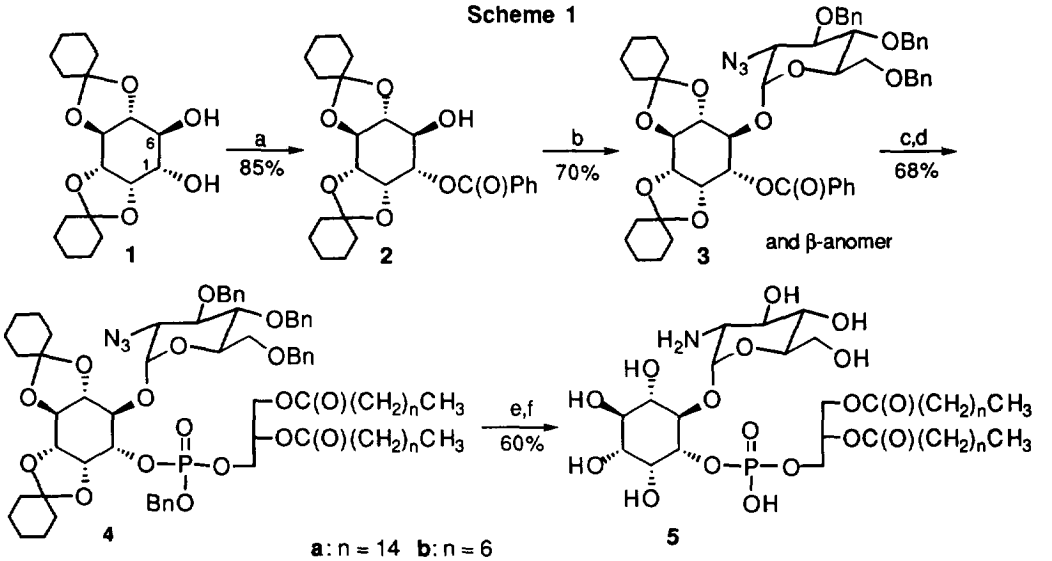


Figure 1. Diagrammatic Representation of GPI Membrane Anchor

Palmitoylation of the inositol and O-mannosylation of the glucosamine at C(4) constitute key steps in the biosynthesis of GPI anchors. The former has been reported to be acyl-CoA dependent in yeast,[6] but acyl-CoA independent in rodents.[7] Available data also suggest palmitoylation precedes mannosylation in yeast and rodents, but the order may be reversed in trypanosomes.[6,8] The specificities of the various transferases, their species/tissue dependency, and the role of the acyl modification are incompletely understood, but nevertheless, have attracted considerable attention since divergent biochemical pathways between organisms could be exploited in differential therapies, especially against parasites such as *T. brucei*.[9] To expedite these investigations, we describe herein a concise, asymmetric synthesis[10] of the isomeric GlcN-PIs **5** and **8** along with an evaluation of their ability to prime the GPI pathway in trypanosome and rodent cell-free systems.[11]

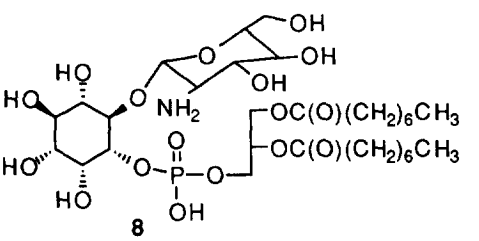
Chiral diol **1**, obtained in three steps from commercial *myo*-inositol according to Vacca[12] and Chen,[13] was regioselectively benzoylated via the corresponding dibutylstannylene to give **2**[14] (Scheme 1), whose structure was

Scheme 1



[a]Bu_2SnO, $PhCH_3$, Dean-Stark, 110°C, 4 h; PhC(O)Cl, 0° to 23°C over 2 h. [b]Imidate **6**, TMSOTf (8 mole %), 4Å mol. sieves, CH_2Cl_2, -40°C, 0.5 h. [c]NaOMe, MeOH/THF, 0°C, 0.5 h. [d]Phosphoramidite **7**, 1 -tetrazole, CH_2Cl_2, 0° to 23°C over 2 h; 3-ClC_6H CO_3H, -78°C, 0.5 h. [e]10% Pd/C, 1 atm H_2, MeOH, 23°C, 18 h. [f]AcOH/H_2O (98:2), 80°C, 2 h.

unambiguously confirmed by ^1H NMR and decoupling analysis.[15] In contrast to an earlier report[16] of predominate acylation of the C(6)-alcohol under similar conditions, none of the regioisomeric benzoate was detected. Trimethylsilyl triflate assisted[17] glycosylation of the remaining alcohol in **2** using 2-azido-3,4,6-tri-O-benzyl-2-deoxy-**D**-glucopyranosyl β-trichloroacetimidate[17] (**6**) generated α-glucoside **3** and its β-anomer as a chromatographically separable mixture (~1:1) in moderate yield; TLC (SiO_2), hexane/Et_2O 7:3, R_f~0.44 and 0.51 for **3** and the β-anomer, respectively. No attempt was made to optimize the product distribution since it was of interest to evaluate transferase specificity using both anomers. Methanolysis of **3** followed by condensation with freshly prepared 1,2-di-O-palmitoyl-*sn*-glycerobenzyl(N,N-diisopropylanmino)phosphoramidite[18] (**7a**) and peracid oxidation gave rise to the fully protected GlcNα-PI **4a**. Two-step deprotection of **4a** by catalytic benzyl ether hydrogenolysis/azide reduction and mild acidic hydrolysis of the cyclohexylidenes liberated GlcNα-PI **5a**. Repetition of the above sequence using di-O-octanoyl phosphoramidite[18] **7b** afforded the truncated GlcNα-PI **5b** in comparable overall yield. Likewise, analog **8** was made from the β-anomer of **3**.



Lipids **5a**, **5b**, and **8** were compared as acceptors for the enzymatic transfer of mannose from mannosyl--P-dolichol (Fig. 2). Lipid **5b** was found to be an efficient acceptor for the mannosyltransferases in membranes from both Chinese hamster ovary cells and *Trypanosoma brucei*. **5b** was converted to mannose-GlcNα-PI(C8) by the *Trypanosoma brucei* enzyme whereas the hamster enzyme converted it to mannose-GlcNα-acylPI(C8) and mannose-GlcNα-PI(C8). However, the former required the addition of palmitoyl-CoA. Unexpectedly, lipid **5a** was not an acceptor substrate for the hamster mannosyltransferase (data not shown) and worked very inefficiently with the enzyme in *T. brucei* membranes, even though the acyl groups on **5a** more closely resembled those of natural GPIs than those of **5b**. This could be due to the enhanced water-solubility of **5b** resulting in more efficient transfer from the aqueous phase of the assay to the membranes. Analog **8**, which differs from **5b** by the presence of a β-linked GlcN, was mannosylated only under conditions that permitted acylation of the inositol residue. **8** was converted into mannose-GlcNβ-acyl-PI(C8) by hamster membranes in the presence of palmitoyl-CoA, but failed to act as an acceptor in the absence of palmitoyl-CoA. Similarly, *T. brucei* membranes, which are unable to acylate the GlcN-PI intermediate,[19] failed to mannosylate **8** in the absence or presence of palmitoyl--CoA. Additional characterization of the acyl- and mannosyltransferases using these analogs will be published elsewhere.[20]

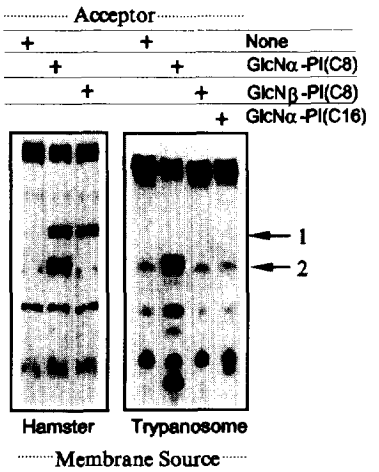


Figure 2. Incubation of GlcN-PI isomers with microsomally-associated mannosyltransferases from Chinese hamster ovary cells and *T. brucei*. Lipids **5a**, **5b**, and **8** were dissolved in 0.03% (w/v) Triton X-100 which was subsequently diluted four-fold into a 0.1 mL reaction volume containing (final concentrations) 50 mM Tris-Cl (pH 7.4), 5mM $MgCl_2$, 5mM $MnCl_2$, 1 mM 5'-AMP, 0.26 μM GDP-[^{3}H]mannose (15 Ci/mmol; American Radiolabeled Chemicals), 2 μM palmitoyl-CoA, and 50 μg of hamster membrane protein. Trypanosomal membranes were assayed similarly, except that assays lacked Tris buffer and palmitoyl-CoA and included 50 mM Na-HEPES (pH 7.4) and 5 mM KCl. Reactions were incubated at 37 ^{0}C for 20 min at which time they were chilled on ice and extracted twice with 0.2 mL water-saturated *n*-butanol. Pooled *n*-butanol extracts were backwashed once with 0.1 mL *n*-butanol-saturated water and then dried under a stream of nitrogen. Lipids were dissolved in 30 μL $CHCl_3$:MeOH (2:1) and applied to a pre-activated Whatman Silica Gel 60 TLC plate. TLC plates were developed in $CHCl_3$:MeOH:0.25% KCl (55:45:10), sprayed with fluor,[21] and exposed to X-ray film (Kodak) for 2-4 days. Solvent flow was from bottom to top. The positions of [^{3}H]mannose-GlcN-acylPI(C8) and [^{3}H]mannose-GlcN-PI(C8) are shown by the arrows labeled 1 and 2, respectively.

Acknowledgment: Supported financially by the Robert A. Welch Foundation (I-782 and I-1168) and the NIH (GM31278 and GM38545).

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15. ^{1}H NMR (250 MHz, $CDCl_3$) of **2**: δ 1.26-1.38 (m, 4 H), 1.58-1.66 (m, 16 H), 2.57 (d, $J \approx 3.4$ Hz, 1 H), 3.57 (dd, $J \approx 8.5$, 10.6 Hz, 1 H), 4.05 (dd, $J \approx 7.7$, 10.6 Hz, 1 H), 4.19 (ddd, $J \approx 3.4$, 3.7, 8.8 Hz, 1 H), 4.43 (t, $J \approx 7.7$ Hz, 1 H), 4.64 (dd, $J \approx 4.1$, 6.5 Hz, 1 H), 5.25 (dd, $J \approx 3.7$, 4.1 Hz, 1 H), 7.42-7.49 (m, 2 H), 7.55-7.59 (m, 1 H), 8.05-8.08 (m, 2 H); ^{13}C NMR (62 MHz, $CDCl_3$/CD_3OD) of **5**: δ 12.28, 21.49, 23.77, 27.93, 30.66, 32.70, 32.87, 53.50, 59.75, 61.42, 62.63, 62.80, 69.15, 69.40, 69.52, 69.62, 70.40, 71.11, 71.48, 71.95, 72.61, 75.94, 76.05, 76.80, 76.89, 94.78, 172.50, 172.80. ^{1}H NMR ($CDCl_3$) of **4**: δ 0.85 (t, $J \approx 7$ Hz, 6 H), 1.18-1.42 (m, 28 H), 1.42-1.78 (m, 12 H), 2.31 (t, $J \approx$ 6.8 Hz, 4 H), 3.28 (ddd, $J \approx 3.5$, 10.1, 20.5 Hz, 0.5 H), 3.43-3.57 (m, 0.5 H), 3.63-3.74 (m, 3 H), 3.77-3.82 (m, 3 H), 3.90-4.19 (m, 5 H), 4.22-4.40 (m, 6 H), 4.42-4.75 (m, 4 H), 4.78 (d, $J \approx 10.8$ Hz, 1 H), 4.85 (s, 2 H), 5.11-5.27 (m, 3 H), 7.15-7.42 (m, 20 H); ^{13}C NMR ($CDCl_3$) δ 14.01, 22.54, 23.45, 23.62, 23.75, 23.86, 24.76, 24.83, 24.88, 24.99, 28.86, 28.97, 29.01, 31.59, 33.92, 34.01, 34.23, 61.44, 61.52, 61.99, 63.02, 65.65, 68.12, 69.20, 69.30, 69.56, 69.64, 69.77, 70.89, 72.04, 73.42, 74.93, 75.30, 76.14, 76.25, 76.78, 78.12, 80.01, 80.09, 96.82, 112.25, 113.27, 127.61, 127.73, 127.78, 127.90, 128.05, 128.30, 128.37, 128.63, 128.68, 135.21, 137.90, 137.94, 138.02, 172.68, 172.76.
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(*Received in USA* 15 *May* 1996; *accepted* 24 *June* 1996)