Glycolipid Precursors for the Membrane Anchor of *Trypanosoma brucei* Variant Surface Glycoproteins

I. CAN STRUCTURE OF THE PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C SENSITIVE AND RESISTANT GLYCOLIPIDS^*

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A number of eukaryotic surface glycoproteins, including the variant surface glycoproteins of *Trypanosoma brucei*, are synthesized with a carboxyl-terminal hydrophobic peptide extension that is cleaved and replaced by a complex glycosyl-phosphatidylinositol (GPI) membrane anchor within 1–5 min of the completion of polypeptide synthesis. The rapidity of this carboxyl-terminal modification suggests the existence of a prefabricated precursor glycolipid that can be transferred en bloc to the polypeptide. We have reported the purification and partial characterization of a candidate precursor glycolipid (P2) and of a compositionally similar glycolipid (P3) from *T. brucei* (Menon, A. K., Mayor, S., Ferguson, M. A. J., Duszenko, M., and Cross, G. A. M. (1988) *J. Biol. Chem.* 263, 1970–1977). The primary structure of the glycan portions of P2 and P3 have now been analyzed by a combination of selective chemical fragmentation and enzymatic glycan sequencing at the subnanomolar level. The glycans were generated by deamination, NaB₃H₄ reduction, and dephosphorylation of glycolipids purified from different trypanosome variants. Glycan fragments derived from biosynthetically labeled glycolipids were also analyzed. The cumulative data strongly suggest that P2 and P3 contain ethanolamine-phosphate-Man₁-2Man₁-6Man₁-4GlcN linked glycosidically to an inositol residue, as do all the GPI anchors that have been structurally characterized. The structural similarities suggest that GPI membrane anchors are derived from common precursor glycolipids that become variably modified during or after addition to newly synthesized proteins.

The covalent linkage of a glycosylinositol phospholipid to the carboxyl-terminal amino acid of many eukaryotic cell surface glycoproteins provides the sole means of membrane attachment (Cross, 1987; Low, 1987; Low and Saltiel, 1988; Ferguson and Williams, 1988), and in many cases a fraction of these proteins can be released from the membranes by treatment with a phosphatidylinositol-specific phospholipase C (PI-PLC).^1^ Chemical structures of the glycosylphosphatidylinositol phospholipid (GPI) membrane anchors of a trypanosome variant surface glycoprotein (VSG) (Ferguson et al., 1988), rat brain Thy-1 (Homans et al., 1988), and human erythrocyte acetylcholine esterase (Roberts et al., 1988a; Roberts et al., 1988b) are known. These GPI anchors have a common backbone structure of ethanolamine-phosphate-Man₁-2Man₁-6GlcN linked to an inositol phosphate. The carboxyl terminus of the mature protein is attached to the glycolipid via an amide linkage to ethanolamine. The GPI membrane anchors of decay accelerating factor from human erythrocytes (Medof et al., 1986), HeLa cell alkaline phosphatase (Humphries and Low, 1987), and human placental alkaline phosphatase (Howard et al., 1987; Ogata et al., 1988) are not completely characterized, but current data suggest similar backbone structures.

The different anchors appear to have both protein and cell-type specific modifications that are manifest as "decorations" branching from the core backbone structure and variations in the composition and linkage of fatty acids. For example, a variable α-mannose (attached to the mannose residue linked to ethanolamine phosphate) found in rat brain Thy-1 (Homans et al., 1988) is absent from rat thymocyte Thy-1 (Tse et al., 1985). Both the Thy-1 anchors, however, have the same asparagine residue adjacent to glucosamine (Homans et al., 1988), a feature absent from the glycolipid anchors of trypanosome VSGs which have, instead, a variable number of α-galactose residues attached to the mannose adjacent to the glucosamine. The extra ethanolamine phosphate may be an otherwise common feature of protein glycolipid anchors, since ethanolamine has been detected at a stoichiometry of about 2 in human erythrocyte acetylcholine esterase, bovine erythrocyte acetylcholine esterase (Haas et al., 1986; Roberts et al., 1987), alkaline phosphatase (Duszenko et al., 1987), and human placental alkaline phosphatase (Howard et al., 1987; Ogata et al., 1988). The different GPI anchors can thus be released from the membranes by treatment with a phosphatidylinositol-specific phospholipase C (PI-PLC).

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The abbreviations used are: PI-PLC, phosphatidylinositol-specific phospholipase C; VSG, variant surface glycophorin; AHA, 2,5-anhydromannitol; GPI, glycosylinositol phospholipid; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry; GL, glucose units; ABO, *A. phoenicis* α-mannosidase I; jBoM, jack bean α-mannosidase; E2°, AChE, human erythrocyte acetylcholine esterase; Inos, inositol; TMS, trimethylsilyl; dansyl, 5-dimethylaminonaphthalene-1-sulfonil.
phosphatase (Ogata et al., 1988), scrapie prion protein (Stahl et al., 1987), squid brain Sgp2 (Williams et al., 1988) and decay accelerating factor (Medof et al., 1986).

cDNA sequence analyses of GPI-anchored proteins suggest that the proteins are synthesized as precursor polypeptides with an amino-terminal signal sequence and a hydrophobic carboxyl-terminal extension that is rapidly replaced by the glycolipid. The addition of the glycolipid anchor occurs within 1 min of completion of protein synthesis for the VSGs of *Trypanosoma brucei* (Bangs et al., 1985; Ferguson et al., 1986) and the neutral cell adhesion molecule, N CAM 120 (He et al., 1988), within 2 min for Thy-1 (Conzelmann et al., 1987) and within 5 min for alkaline phosphatase (Takami et al., 1988). The rapidity of this modification suggested the existence of a prefabricated precursor glycolipid that could be transferred in bloc to the newly synthesized protein, probably in the endoplasmic reticulum. Recent studies using a yeast secretory mutant (sec18) support an endoplasmic reticulum localization for the addition of the GPI anchor (Conzelmann et al., 1988).

A glycolipid with properties consistent with a role as a VSG anchor precursor was subsequently identified in biosynthetic labeling experiments (Krakow et al., 1986; Menon et al., 1988a). The lipid, referred to as P2 (Menon et al., 1988a), presumably identical to lipid A described by Krakow et al. (1986), could be labeled with \[1^H\]myristic acid, \[1^H\]glucosamine, \[1^H\]ethanolamine, \[1^H\]mannosine, and \[1^P\]. Structural homology between P2 and the VSG glycolipid anchor was established by a variety of chemical and enzymatic treatments, including cleavage by bacterial PI-PLC (Krakow et al., 1986; Menon et al., 1988a). The amino group of the ethanolamine residue in P2 was found to be unsubstituted and chemical analyses of subnanomolar quantities of P2 (purified by thin layer chromatography) confirmed the presence of ethanolamine and glucosamine in a 1:1 molar ratio, as well as the presence of myristic acid and mannose (Menon et al., 1988a). Thin layer chromatography of polar lipid extracts containing P2 revealed another compositionally similar glycolipid, P3 (similar to lipid C; Krakow et al., 1986), apparently differing from P2 only by its somewhat greater hydrophobicity and its insusceptibility to bacterial PI-PLC (Menon et al., 1988a). The reason for the PI-PLC insensitivity of P3 is the subject of the accompanying paper (Mayor et al., 1990).

In this paper we present a detailed analysis of the glycan portions of P2 and P3, purified from three *T. brucei* varients. The primary structure of the glycan portion of these glycolipids has been determined by a combination of selective chemical fragmentation and enzymatic glycan sequencing of subnanomolar amounts of material as well as by analyses of biosynthetically labeled material.

**EXPERIMENTAL PROCEDURES**

**Materials—**NaB\(^3\)H\(_4\) (12 Ci/mmol) was purchased from Du Pont-New England Nuclear. \([1^H]2,5\)-Anhydromannitol (\([1^H]\)AHM) was prepared by deamination and NaB\(^3\)H\(_4\), reduction of glucosamine. \([1^H]2,5\)-Glucosamine hydrochloride (35 Ci/mmol) and \([1^H]\)ethanolamine (29.8 Ci/i mmol) were purchased from Amersham Corp. scyllo-Inositol was purchased from Behring Diagnostics. Ion-exchange resins were partially analytical grade and purchased from Bio-Rad or LKB Biotechnology Inc. Aspergillus phoenicis α-mannosidase I and jack bean α-mannosidase were prepared as previously described (Parekh et al., 1987), unless otherwise mentioned. All solvents were analytical or high performance liquid chromatography (HPLC) grade.

A mixture of partially O-methylated methylmannosides (2,3,4-tri- and 2,3,6-tri-O-methyl-α-D-mannosides) was a gift from Dr. S. J. Turco, University of Kentucky (Hull and Turco, 1985). The mixture was hydrolyzed as described below to generate 2,3,4-tri- and 2,3,6-tri-O methyl mannose. The 2,4-di-, 2,6-di-, and 2,3,4,6-tetra-O-methyl mannose standards were derived from a completely characterized isomer of Man,GlCNAcGlCNac-ol (Vijay and Perdew, 1980), which was a gift from Dr. I. K. Vijay, University of Maryland.

**Trypanosomes—**Trypanosome variants of the Molteno Institute Trypanozoan antigenic types 1.4, 1.5 and 1.2 (clones 117, 118 and 221, respectively) of *T. brucei* strain 427 were purified from infected rat blood as previously described (Cross, 1975).

**Glycolipid Purification—**A mixture of partially 0-methylated methylmannosides (2,3,4-tri- and 2,3,6-tri-O-methyl glucosamine) was a gift from Dr. S. J. Vijay and Perdew, 1980) except that the lysed trypanosomes were centrifuged at 100,000 × g (instead of 45,000 × g). The 100,000 × g pellet was lyophilized and glycolipids extracted as before.

**Inositol Analysis—**Glycolipid samples purified by TLC and corresponding blank regions of the TLC plate were analyzed for inositol NMR, mucin by gas-liquid chromatography (GC-MS) using selected ion monitoring (Smith et al., 1987). Samples were dissolved in methanol/pyridine/water (2:1:1) and aliquots (5 or 10%) were mixed with 20 pmol of scyllo-inositol internal standard, dried, and hydrolyzed in 50 μl of 6 N HCl at 110 °C for 24 h. Following the addition of HCl by radioimmunological determination of I2 decay accelerating factor (Medof et al., 1988). The reason for the PI-PLC insensitivity of P3 was the subsequent deamination efficiency. After drying, the samples were deaminated, reduced, and dephosphorylated following the strategies outlined in Ferguson et al. (1988a). Each sample (~1 nmol) was dissolved in 5 μl of 0.1 M sodium acetate buffer, pH 4.0, and deaminated by the addition of 6 μl of 0.5 M NaNO₂ (freshly prepared) and incubation at 22 °C for 2.5 h. The products were reduced at pH 10.5 by adding 5 μl of 0.4 M boric acid and 10 μl of 12 mM NaB\(^3\)H\(_4\) (12 Ci/mmol) in 0.4 M NaOH. After 1.5 h, excess reducant (10 μl, 1 M NaBD₃) was added and the reduction continued for 1 h. Following deamination with acetic acid, the reaction mixture was desalted by passage through 0.2 ml AG50X12(H₄) and by repeated evaporation from methanol. The resulting radioactive products were subjected to descending paper (Whatman 3MM) chromatography for 12 h with butan-1-ol/ether/water (4:1:1) as the solvent system. The samples were eluted from the origin with water, dried, and dephosphorylated with 50 μl of 50% aqueous HF at 0 °C for 42 h. The acid was neutralized with concentrated LiOH (275 μl), and the supernatant was passed through a 20 ml column of 100(Na) layered over AG50X12(H₄), over AG3X4(OH)₄, over Sephadex QAE-A25(OH)₄, equilibrated and eluted with water. The resulting desalted, labeled neutral products were further purified by high voltage electrophoresis on Whatman 3MM paper in pyridine/acetic acid/water (3:1:9), pH 5.4, at 80 V/cm for 40 min and eluted from the origin with water. The purified products were passed through a tandem column of 0.1 ml each Chelex 100(Na)₄ layered over AG50X12(H₄), over AG3X4(OH)₄, over Sephadex QAE-A25(OH)₄ and filtered through a 0.5-μm Teflon filter.

**Bio-Gel P4 Chromatography—**Labeled neutral glycan(s) were mixed with 25 μl of a partial acid hydrolysate of dextran (20 mg/ml) and thusly labeled glycoconjugate was subjected to a tandem column (1 mm × 1.5 cm) maintained at a constant temperature of 55 °C and eluted with water at a flow rate of 0.2 ml/min. The eluant was monitored for radioactivity using an LB603 Berthold HPLC radioactivity monitor and by determining the radioactivity in 2.5 min fractions by liquid scintillation counting. The elution positions of the glucosamine standards were determined by using an Erma ERC 7510 refractometer. Analog signals from the monitors were digitized using a Nelson Analytical ADC interface and the digital values collected and analyzed using Hewlett-Packard 8566E computers. Radiochromatograms were smoothed using a Fourier transform routine and sample elution positions were
Glycan Structure—Radiolabeled neutral glycans recovered from Bio-Gel P4 chromatography were divided into three aliquots and treated with A. phoenicis \(\alpha\)-mannosidase I, specific for Manalpha1-3Man alpha 1-6Man linkages (Kobata and Amano, 1987), or treated with jack bean \(\alpha\)-mannosidase, or subjected to acetylation, which selectively cleaves Manalpha1-2Man linkages (Rosenclof and Ballou, 1974). The products of these reactions were analyzed by Bio-Gel P4 chromatography.

Mannosidase digests were carried out in 25 \(\mu\)l of 0.1 M sodium acetate buffer, pH 5.0, under toluene with A. phoenicis \(\alpha\)-mannosidase I (20 mg/ml) for 2 h at room temperature or jack bean \(\alpha\)-mannosidase (30 units/ml) for 16 h at 37 °C. The reactions were stopped by heating to 100 °C, neutralizing the HF reaction mixture with saturated NaOH, and cooling on ice. Three additions of 0.1 M NaHCO\(_3\) solution and cooling on ice. Three additions of 0.1 M NaHCO\(_3\) solution were made at 10-min intervals, and the samples were kept at ambient temperature for an additional 20 min. Samples were deaminated and reduced or N-acetylated. Before deamination and reduction or N-acetylation, the butanol phase was treated with A. phoenicis \(\alpha\)-mannosidase I, specific for Manalpha1-2Man linkages (Kobata and Amano, 1987), or treated with jack bean \(\alpha\)-mannosidase (200 units/ml) for 2 h at 37 °C.

The identity of the radioactivity incorporated into the lipid samples obtained in the butanol fractions of \(\text{[H]}\)Man, \(\text{[H]}\)GlcN, and \(\text{[H]}\) ethanolamine-labeled trypanosomes has been previously shown to be quantitatively \(\text{[H]}\)Man, \(\text{[H]}\)GlcN, and \(\text{[H]}\) ethanolamine, respectively. In the short labeling periods (1-2 h) involved, no significant metabolic conversion of the labels into other components was observed (Menon et al., 1989).

Generation of Biosynthetically Labeled Glycans for HPLC Analysis—Butanol extracts from biosynthetically labeled trypanosomes or purified labeled glycolipids were treated with cold aqueous HF as described above. After 48 h the reaction was terminated by adding the HF reaction mixture to a saturated solution of LiOH (270-300 \(\mu\)l, pre frozen on dry ice), and the pH was adjusted to 4.0. Care was taken to ensure that the pH did not rise above 8.0 during the neutralization process. The LiF precipitate was centrifuged, and the supernatant and a 100-\(\mu\)l wash of the pellet were returned to the original tube. The aqueous solution was adjusted to pH 4.0, extracted twice with an equal volume of water-saturated butanol, and the butanol-rich upper phase and the aqueous-rich lower phase were separated. The aqueous phase was desalted by passing over 0.4 ml of AG3X4A(OH-) resin, and the eluate and washings were pooled and taken for deamination and reduction or N-acetylation. Before deamination and reduction or N-acetylation, the butanol phase was treated with NH\(_3\)/methanol (1:1) for 3-12 h at ambient temperature.

Samples to be \(\text{N}\)-acyetylated were dried and resuspended in 50 \(\mu\)l of saturated NaHCO\(_3\) solution and cooled on ice. Three additions of acetic anhydride (each of 2 \(\mu\)l) were made at 10-min intervals, and the samples were kept at ambient temperature for an additional 20 min. Samples were deaminated and reduced or N-acetylated. Before deamination and reduction or N-acetylation, the butanol phase was treated with NH\(_3\)/methanol (1:1) for 3-12 h at ambient temperature.

Anion Exchange HPLC Analysis of Labeled Glycans—Desalted glycans were analyzed by anion exchange chromatography on a Dionex Basic Chromatography System b (chloroform/methanol/H\(_2\)O, (10:10:2.5)). Radioactivity was detected using a Pertector LB 2842 TLC scanner. Areas corresponding to peaks of radioactivity were scraped, and radiolabeled lipids were extracted by repeated washing of the scrapings with methanol/pyridine/water (2:1:1). The methanol/pyridine/water extracts were either dried down and the residue partitioned between water and butanol, or dried, the residue was flash evaporated with 5% HAc in water, and passed over a mixed-bed ion-exchange column. The pooled eluate and washings was filtered and stored at \(-20 ^\circ\text{C}\) until required for analysis.

Extraction and Purification of Biosynthetically Labeled Glycolipids—All labeling experiments were carried out at 37 °C in a shaking water bath. Trypanosomes were deaminated or desialylated, reduced, and desphosphorylated glycan from VSG 117° (see also Fig. 7D). Soluble form VSGs (20-50 mg) were filtered as described earlier (Cross, 1984) and treated with ice-cold aqueous HF (500 \(\mu\)l 50%) for 60 h at 0 °C. Neutralization process. The LiF precipitate was centrifuged, and the supernatant and a 100-\(\mu\)l wash of the pellet were returned to the original tube. The aqueous solution was adjusted to pH 4.0, extracted twice with an equal volume of water-saturated butanol, and the butanol-rich upper phase and the aqueous-rich lower phase were separated. The aqueous phase was desalted by passing over 0.4 ml of AG3X4A(OH-) resin, and the eluate and washings were pooled and taken for deamination and reduction or N-acetylation. Before deamination and reduction or N-acetylation, the butanol phase was treated with NH\(_3\)/methanol (1:1) for 3-12 h at ambient temperature.

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ethanolamine-labeled P2 and P3 were dried in 200-μl glass micro-dispenser tubes. Samples were dansylated either prior to hydrolysis or after hydrolysis (6 M HCl, 8-10 h, 110 °C) and analyzed by thin layer chromatography as described earlier (Menon et al., 1988a).

Mannosidase Digestion of Deaminated Reduced Glycans from P2 and P3—TLC purified and [3H]GlcN-labeled P2 and P3 were deaminated in 400 μl of deamination buffer containing 0.1% Nonidet P-40. The reaction was terminated after 4.8 h by adding 200 μl of 0.4 M H3BO3 followed by 130 μl of 1 M NaOH. The samples were reduced by adding 100 μl of 2 M NaBH4 in 0.3 M NaOH, desalted, after destroying the excess NaBH4 with 20 μl of HAc, by passing over a 1-ml column of AG50WX12(2+), and the eluate and washings were pooled and dried. The residue was flash evaporated with 10% HAc in ethanolamine-labeled P2 and P3 were dried in 200-μl glass micro-dispenser tubes. Samples were dansylated either prior to hydrolysis or after hydrolysis (2 x 200 μl) and then with toluene (2 x 20 μl) and an aliquot was analyzed by Dionex HPLC chromatography. The residue was resuspended in 20 μl of 0.2 M NaAc, pH 5.0, containing jack bean α-mannosidase (60 units/ml, Boehringer Mannheim) and incubated for 12-16 h at 37 °C before or after treatment with cold aqueous HF (36 h, 0 °C). The mannosidase digestion was terminated by heating the reaction at 100 °C for 1 min, and the digest was passed over a 0.2-ml column of AG50WX12(2+). The column eluate and washings were pooled, filtered, and analyzed by Dionex HPLC chromatography using gradient program b. Samples that were digested by mannosidase before HF treatment were subsequently dephosphorylated, processed, and analyzed as described above.

Methylation—[3H]Man-labeled dephosphorylated, deaminated-reduced glycans (10,000 cpm) were prepared from [3H]Man-labeled P2 and P3 and permethylated at ambient temperature according to the procedure of Ciucanu and Kerek (1984). Glycan samples were dissolved in 50 μl of dimethyl sulfoxide in 300 μl Reacti-Vials (Pierce Chemical Co.) equipped with magnetic stirrers and continuously stirred during the methylation procedure. A suspension (50 μl) of finely powdered NaOH (120 mg/ml) in dimethyl sulfoxide was added to each vial followed by three additions each of 10 μl of CH3I after 5, 15, and 20 min. 10 min after the final addition the reaction was terminated by adding 200 μl of sodium thiosulfate (100 mg/ml) and transferred to another tube, and the Reacti-Vial was rinsed with 600 μl of sodium thiosulfate and 5 x 200 μl of chloroform. The reaction mixture and washes were pooled. The chloroform phase was separated, washed with water (5 x 1 ml), and dried. The residue containing the permethylated glycans was hydrolyzed for 4 h at 120 °C in 200 μl of 9 M trifluoroacetic acid (Pierce Chemical Co.). The hydrolysate was dried and the residual acid flash-evaporated with 3 x 100 μl of MeOH. The samples were resuspended in 50% aqueous MeOH and analyzed on TLC (Kieselgel-60 TLC plates, solvent system: benzene/acetone/water/30% NH3 (50:200:3:1.5)) as described by Li et al. (1978) and Vijay et al. (1980).

RESULTS
We have previously identified three major [3H]GlcN- and [3H]Man-labeled lipid species P1, P2, and P3, in polar solvent extracts of trypanosomes biosynthetically labeled with [3H]Glc or [3H]Man (Menon et al., 1988a). All three lipids partition quantitatively into the butanol phase obtained from chloroform/methanol/water (10:10:3, v/v/v) extracts of trypanosomes biosynthetically labeled with [3H]GlcN in the absence (A) or presence (B) of 400 ng/ml tunicamycin. The glycolipids were extracted and the butanol-rich phase containing the polar glycolipids was chromatographed on silica high performance thin layer chromatography plates using solvent system a.

Glycan Structure

Aqueous HF Dephosphorylation—The glycolipid anchor of membrane form VSG contains two phosphodiester linkages, one between the glycan core and diacylglycerol and the other connecting the glycan to the VSG polypeptide via ethanolamine. Both bonds can be selectively cleaved by aqueous HF, resulting in the release of the intact glycan, ethanolamine, and diacylglycerol (Ferguson et al., 1988).

Glycolipid samples were incubated at 0 °C with aqueous HF for different periods of time to investigate the susceptibilities of the different phosphodiester linkages in the two molecules and to establish conditions for complete dephosphorylation. The ethanolamine-glycolipid linkage in P2 and P3 was quantitatively cleaved by aqueous HF within 24 h (Fig. 2, A and C).

HF cleavage of the phosphodiester bond linking diacylglycerol to the rest of the molecule was monitored by following the release of water-soluble radioactivity from [3H]GlcN-labeled lipids (Fig. 2). About 80% of the radioactivity in the [3H]GlcN-labeled P2 sample was rendered water-soluble after HF treatment for 60 h. Analysis of the material remaining in the butanol phase (<20% of the starting material) suggested that it was the partially dephosphorylated product (P2 minus ethanolamine phosphate), since it migrated ahead of the starting material on silica high performance-thin layer chromatography (compare Fig. 3, C and D) and, as observed in a separate experiment with [3H]myristic acid-labeled P2, retained the [3H]myristic acid label (data not shown).
completely water soluble by treatment with NH₄OH/methanol prior to either deamination and reduction, or N-acetylation. Analytical deaminated, and reduced glycan from [3H]Man-labeled P2 obtained on Dionex HPLC analysis of the dephosphorylated, phosphate). The slower species, migrating only slightly ahead of the starting material, was not labeled with [3H]myristic acid and was assumed to be the partially dephosphorylated product (P3 minus ethanolamine phosphate). The slower species, migrating only slightly ahead of the starting material, was not labeled with [3H]myristic acid (data not shown) and was assumed to be the completely dephosphorylated product (P3 minus ethanolamine phosphate and minus the phosphodiester-linked diglyceride moiety). These data suggest that the completely dephosphorylated P3 glycan contains a hydrophobic modification that cannot be removed by HF treatment. This hydrophobic modification can be removed by base treatment (Mayor et al., 1990).

Analysis of Glycans from Biosynthetically Labeled Glycolipids—After deamination and reduction, the dephosphorylated water-soluble material from a total butanol extract of [3H]GlcN-labeled trypanosomes was analyzed by anion exchange chromatography (Fig. 4). The major peak obtained cochromatographed with the Man₃AHM neutral glycan standard. The tunicamycin sensitivity of the peaks with retention times > 20 min, which did not coelute with the partially dephosphorylated product (P3 minus ethanolamine phosphate), are likely to be derived from lipid-linked oligosaccharides involved in N-linked glycan biosynthesis.

The analyses were repeated with TLC-purified lipids. The deaminated and reduced water-soluble material obtained from dephosphorylated [3H]GlcN-labeled P2, chromatographed as a single peak on Dionex HPLC corresponding to the Man₃AHM neutral glycan standard (data not shown). Identical results were obtained on Dionex HPLC analysis of the dephosphorylated, deaminated, and reduced glycan from [3H]Man-labeled P2 (Fig. 9B). Analysis of [3H]GlcN-labeled P1 produced a single peak at 21.5 min corresponding to the major tunicamycin-sensitive peak shown in Fig. 4A."

Dephosphorylation of [3H]GlcN-labeled P3 generated a butanol-soluble "hydrophobic glycan." This was made completely water soluble by treatment with NH₄OH/methanol prior to either deamination and reduction, or N-acetylation. Analytical deaminated-reduced material by Dionex HPLC produced a single peak, cochromatographing with Man₃AHM (Fig. 5A). Identical results were obtained with the glycan from [3H]Man-labeled P3 (Fig. 9D). The N-acetylated material chromatographed as two peaks (Fig. 5B), one corresponding to the neutral glycan Man₃GlcNAcInos (generated by N'-acetylation of the glycan portion of the dephosphorylated glycolipid anchor of VSG 110) (Fig. 5, B and C), and a second peak at longer retention time (~36 min), which could be removed by passing the N-acetylated reaction mixture over an anion exchange column prior to HPLC analysis (compare Fig. 5, B and C). This second peak is probably derived from the partially dephosphorylated P3 molecule (P3 minus ethanolamine phosphate) identified in the TLC analysis of dephosphorylated [3H]GlcN-labeled P3 (Fig. 3B) and hence still carries a negative charge and the glycerol moiety.

**Sequence Analysis of the Neutral Glycans Derived from P2 and P3—GC-MS analysis of TLC purified P2 and P3 samples identified myo-inositol in both glycolipids. 904 pmol of inositol were present in a sample of P2 purified from 10¹⁴ trypanosomes (variant 118), and 150 pmol of inositol were present in a sample of P3 purified from a similar number of cells (variant 291). Assuming a stoichiometry of 1 mol of inositol/mol of glycolipid, the analyses represent recoveries corresponding to ~10⁻⁴ and ~10⁻⁵ molecules/trypanosome of P2 and P3, respectively. These figures are consistent with our previous estimates based on ethanolamine analysis and biosynthetic labeling experiments (Menon et al., 1986a).

The analysis scheme used to determine the sequence of the glycans derived from the glycolipids (Fig. 6) was derived from the approach used to determine the structures of the glycan portions of the glycolipid membrane anchors of a trypanosome variant surface glycoprotein (Ferguson et al., 1988) and Thy-1 (Homans et al., 1988). Chemically radiolabeled, neutral core
glycans were generated by base treatment, nitrous acid deamination, NaBH₄ reduction and dephosphorylation of glycolipid samples, and chromatographed on high-resolution Bio-Gel P4 gel filtration columns. The radiochromatograms of the neutral glycans derived from the P2 glycolipid (variant clone 117) and from sVSG 118 showed a single neutral glycan species with a specific activity of ~0.3 Ci/mmol, which chromatographed at 4.2 GU (Fig. 7). This is the same size as the authentic Man₆GlcNAcɪnọ, where n = 3, 4 (indicated as 3', 4' in panels B and C) correspond to the major glycan species derived from the variably galactosylated VSG 117 and MsGlcNAcɪnọ to the glycan derived from the non-galactosylated VSG 118.

Control experiments were performed to investigate the possible nonspecific destruction of glycosidic linkages during the cold aqueous HF dephosphorylation procedure. The deaminated and reduced glycan portion from the glycolipid membrane anchor of VSG 117 (dAR-gp, Ferguson et al., 1988) contains two Galα₁-2Gal bonds, which are known to be relatively acid labile (Ferguson et al., 1985). A sample of dAR-gp was treated with HF (50% aqueous HF, 65 h, 0 °C), neutralized, deailiated, and reduced with NaBH₄. The resulting reduced glycans were desalted, methanolyzed, converted to their
A. phoenicis cy-mannosidase I digestion of acetylation or jack bean α-mannosidase digestion, and the fragments generated were rechromatographed on Bio-Gel P4 (B, C, and D, respectively). The numbers at the top of the figure refer to the elution positions of glucose oligomer internal standards.

Neutral glycans (4.2 GU, filled circles, Fig. 8A) from P2(118), P2(221), and P3 (mixture of 117, 118, and 221). Radioactivity (vertical axis) was measured in an aliquot (0-50 μl) of 0.5 ml column fractions and plotted against fraction number. In each case the deaminated, NaBH₄-reduced and dephosphorylated neutral glycan (4.2 GU peak material (filled circles, A)) was subjected to A. phoenicis α-mannosidase I digestion of acetylation or jack bean α-mannosidase digestion, and the fragments generated were rechromatographed on Bio-Gel P4 (B, C, and D, respectively). The numbers at the top of the figure refer to the elution positions of glucose oligomer internal standards.

trimethylsilyl derivatives and analyzed by GC-MS as described earlier (Ferguson et al., 1985). Only a trace amount of galactitol and barely detectable amounts of mannitol were produced when compared with the untreated sample (data not shown), indicating that all of the glycosidic bonds in the mature GPI glycans were stable to prolonged HF treatment. Therefore, it appears that the HF dephosphorylation method preserves the integrity of the core glycans.

The column elution profiles (obtained by liquid scintillation counting of fractions collected) for the glycans derived from P2(118), P2(221), and P3 (mixture of 117, 118, and 221). Radioactivity (vertical axis) was measured in an aliquot (0-50 μl) of 0.5 ml column fractions and plotted against fraction number. In each case the deaminated, NaBH₄-reduced and dephosphorylated neutral glycan (4.2 GU peak material (filled circles, A)) was subjected to A. phoenicis α-mannosidase I digestion of acetylation or jack bean α-mannosidase digestion, and the fragments generated were rechromatographed on Bio-Gel P4 (B, C, and D, respectively). The numbers at the top of the figure refer to the elution positions of glucose oligomer internal standards.

Neutral glycans (4.2 GU, filled circles, Fig. 8A) from P2 (118), P2 (221), and P3 (117, 118, and 221) were subjected to further analysis. On treatment with A. phoenicis α-mannosidase I, the 4.2-GU material from each sample was completely digested to a 3.2 GU product (Fig. 8B), consistent with the removal of a single terminal mannose residue in a α1-2 linkage to Man₃AHM.

Acetolysis of the 4.2-GU neutral glycan from each lipid sample generated Bio-Gel P4 profiles (Fig. 8C) consistent with the presence of a Man₃-6Man linkage between the second and third mannose residues. The major product in each case (Fig. 8C) was a 2.4-GU molecule corresponding exactly to Man₃AHM. The two other species at 4.2 and 1.7 GU correspond to the starting material and AHM (see below), respectively. Profiles identical to those shown in C were obtained on acetolysis of the defined Man₃AHM derived from sVSG 118 (data not shown).

The observation of under- and over-digestion products in acetolysis studies is by no means unusual (see Natsuka et al., 1987, 1988), and the partial acetolysis conditions employed in these reactions were chosen to exploit the relative selectivity of acetolysis for the Man₃-6Man bond (Rosenfeld and Bal-lou, 1974). The conditions were optimized using the defined Man₃AHM glycan structure as follows. The reaction period was chosen so that a small portion of the starting material (Man₃AHM, 4.2 GU) remained behind to show that no partial product at 3.2 GU (i.e. minus one mannose residue) was present. Also, conditions where all the 4.2-GU material disappeared were not used since, under these conditions, 2,5-anhydromannitol (1.7 GU) was the major product. The appearance of 2,5-anhydromannitol suggests that the Man₃-4(2,5-anhydromannitol) glycosidic bond is relatively labile to acetolysis compared with other glycosidic bonds. This is not surprising since, the Man₃-4(2,5-anhydromannitol) linkage in Man₃AHM is an artificial and novel linkage whose susceptibility to acetolysis was previously unknown.

Digestion with jack bean α-mannosidase produced a characteristic 1.7-GU species (Fig. 8D) corresponding to the elution position of authentic 2,5-anhydromannitol. The 1.7 GU peak, in all cases, quantitatively cochromatographed with authentic 2,5-anhydromannitol on TLC and Dionex HPLC (using elution program a and b) analyses (data not shown).

These results strongly suggest that the sequence of the neutral glycan from P2 and P3 is Man₃-2-Man₃-6Man₃-6Man₃-6Man₃-6Man₃-6Man₃ (2,5-anhydromannitol) regardless of the trypanosome variant from which the glycolipids were obtained.

Methylation Analysis—TLC analyses of the O-methylated mannose derivatives obtained after methylation and hydrolysis of [3H]Man-labeled, dephosphorylated, deaminated, and reduced glycans from P2 and P3 (Fig. 9, B and D) showed identical profiles consisting of three distinct partially methylated mannose species (Fig. 9, A and C). The three products in each case, 2,3,4-tri, 3,4,6-tri-, and 2,3,4,6-tetra-O-methyl mannose, were derived from 6 O substituted, 2 O substituted, and terminal mannose residues, respectively, in agreement with the Man₃-2-Man₃-6Man₃ sequence determined by enzymatic and chemical sequencing as described above. The nonunitary ratio of radioactivity observed for the three methylated mannose derivatives does not necessarily reflect the stoichiometry of the differently substituted mannose residues present in the neutral glycan since the incorporation of [3H] Man into the lipids P2 and P3 had not achieved a steady state level at the time of extraction of the lipids. Attempts to biosynthetically label trypanosomes for longer lengths of time have proved difficult, and hence methylation analyses on steady state-labeled lipids were not carried out. Thus, these results only provide a qualitative analysis of the differently substituted mannose residues present in the neutral glycans.

4 S. Mayor and A. K. Menon, unpublished observations.
Glycan Structure of Glycolipid Anchor Precursors

Site of Ethanolamine Phosphate Linkage—Jack bean α-mannosidase requires an unsubstituted terminal mannose residue for activity but has little or no specificity for the aglycon to which the mannose residue is linked (Li and Li, 1972). The substrate specificity of jack bean α-mannosidase was exploited in experiments designed to determine the site of attachment of ethanolamine phosphate to the P2 and P3 glycan (Ferguson et al., 1988; Homans et al., 1988). The results presented in this paper show that the P2 and P3 glycan residues to give 2,5-anhydro-1-O-mannitol (Fig. 10, C and F). Jack bean α-mannosidase digestion results in the removal of all mannose residues except for their poor solubility in the reaction mixture. These results are similar to those described previously with [3H]ethanolamine-labeled P2 (Menon et al., 1988a) and suggest that P3, like P2, contains ethanolamine with an unsubstituted amino group.

Dansylation Analysis—When [3H]ethanolamine-labeled P3 (7500 cpm) and [3H]ethanolamine-labeled P2 (6000 cpm) were dansylated and hydrolyzed and analyzed by TLC about 65% of the radioactivity chromatographed as dansyl-[3H]ethanolamine. Hydrolysis prior to dansylation resulted in greater dansylation efficiencies (≥85%). The lower efficiency of dansylation of [3H]ethanolamine obtained with the intact lipids compared with that with the hydrolyzed lipids is probably due to their poor solubility in the reaction mixture. These results are similar to those described previously with [3H]ethanolamine-labeled P2 (Menon et al., 1988a) and suggest that P3, like P2, contains ethanolamine with an unsubstituted amino group.

DISCUSSION

The results presented in this paper show that the P2 and P3 glycolipids from different trypanosome variants have the same carbohydrate structure. The structure suggested by the data is ethanolamine-phosphate-Manα1-2Manα1-6Manα1-GlcN, identical to the core backbone structure found in the glycolipid anchors of VSG and Thy-1 (Ferguson et al., 1988; Homans et al., 1988). Deaminated-reduced and dephosphorylated glycans from [3H]GlcN-labeled P2 and P3 cochromatographed as above. M,AHM and M,AHM refer to the elution positions of the fragments (2.4 and 3.2 GU, respectively), generated as outlined in the scheme shown in Fig. 6, from the M,AHM neutral glycan (4.2 GU) standard. Radioactivity and neutral glycans were detected as described in Fig. 4.

\[3\text{H}]\text{GlcN-labeled P2 and P3 were deaminated with nitrous acid, and the resulting water soluble, \(\text{[3H]}\text{GlcN-labeled, dephosphorylated, deaminated, and reduced glycan (10,000 cpm) were deaminated, reduced, and dephosphorylated prior to HF treatment, and the appearance of products (Man}_{2}\text{AHM, Man}_{2}\text{AHM, or 2,5-anhydro-1-O-methyl mannitol) other than Man}_{2}\text{AHM. These data strongly suggest that ethanolamine phosphate is linked to the terminal mannose in a linear arrangement of mannose residues in both P2 and P3, analogous to the position of ethanolamine phosphate in the glycolipid membrane anchor of membrane from VSG (Ferguson et al., 1988), Thy-1 (Homans et al., 1988), and human erythrocyte acetylcholine esterase (Roberts et al., 1988b).}\]

![Fig. 9. Methylation analysis of neutral glycans from [3H]Man-labeled P2 and P3.](http://www.jbc.org/)

![Fig. 10. Mannosidase digestion of deaminated-reduced glycans from [3H]GlcN-labeled P2 and P3: site of ethanolamine phosphate linkage.](http://www.jbc.org/)
oographed with the Manα1-2Manα1-6Manα1-4AHM glycan standard, and dephosphorylated, N-acetylated glycans from [3H]GlcN-labeled P2 and P3 cochromatographed with the Manα1-2Manα1-6Manα1-4GlcNAcα1-6lmos glycan standard when analyzed by Dionex HPLC. Even though cochromatography on the highly “structure sensitive” anion exchange Dionex HPLC cannot be taken as total proof of structure, it suggests that the dephosphorylated glycans from P2 and P3 have structures identical to the dephosphorylated glycan core of the VSG glycolipid anchor. To obtain additional support for these data, the dephosphorylated, deaminated, and reduced glycans from [3H]Man-labeled P2 and P3 were methylated and hydrolyzed, and the hydrolysate was analyzed by thin layer chromatography. Three distinct methylated derivatives were resolved, consistent with the glycan sequence determined by the enzymatic/chemical sequence analyses.

The stoichiometry of ethanolamine and glucosamine in P3 is 1:1 (Menon et al., 1988a) and dansylation analyses of [3H] ethanolamine-labeled P2 and P3 showed that the ethanolamine amino group was unsubstituted in both lipids. The data from jack bean α-mannosidase digestion of the deaminated-reduced glycans from both lipids suggest that the ethanolamine-phosphate group is attached to the terminal mannose residue of a linear arrangement of the 3 mannose residues and, given the striking identity between the glycolipid structure and the VSG anchor, it is quite likely that the ethanolamine-phosphate group is attached to the 6-position of the terminal mannose residue.

Methylation analysis of [3H]Man labeled neutral glycans from P2 and P3 and the data from jack bean α-mannosidase digestion of the deaminated-reduced glycans from [3H]GlcN-labeled P2 and P3 provide no evidence for an alternate arrangement of the mannose residues such as the branched mannose structure as proposed by Schmitz et al., (1987) for the glycolipid anchor of a VSG variant, VSG 121. Given the sensitivity of the analyses, alternate arrangements of the mannose residues would have been detected if they were present at ≥10% of the amount of the linear structure. The limiting amounts of available material prohibit further confirmation of these results by conventional methylation/GC-MS or NMR analyses.

The single structural difference between P2 and P3 has been identified as an ester-linked fatty acid(s) on the P3 mannose residues. The single structural difference between P2 and P3 has been described (Menon et al., 1988b; Masterson et al., 1989). The two types of structures have been differentiated and characterized on the basis of their hydrophobicity after dephosphorylation. The glycolipid membrane anchors of the VSGs from the three subclasses of T. brucei, classified on the basis of carbohydrate-terminal amino acid of the mature protein (Holder, 1985), have a common backbone structure that is variably galactosylated depending on the subclass to which they belong (Holder, 1985; Ferguson et al., 1988). The galactose residues are arranged in a variably branched structure attached to the mannose residue immediately adjacent to the glucosamine (Ferguson et al., 1988). In general, class I VSGs (e.g. VSG 117) contain 2-4 mol of galactose/mol of glycolipid, class II VSGs (e.g. VSG 221) contain 8 mol of galactose/mol of glycolipid, and the only known example of class III VSGs, VSG 118, contains no galactose in its glycolipid (Holder, 1985). Galactose addition could conceivably occur in at least two steps, with the core galactose residues (Ferguson et al., 1988) added just before, or soon after glycolipid addition, and the terminal galactose residues added later in the secretory pathway. Recent data on the processing kinetics of the carboxyl-terminal glycopeptide from a class I VSG (ILTat 1.2) (Bangs et al. 1988) are consistent with this two-step hypothesis.

The common, variant-independent structure for P2 and P3 that we have presented in this paper strongly supports our previous proposal (Menon et al., 1988a) that the VSG glycolipid membrane anchor is prefabricated as a non-galactosylated glycolipid. However, minor amounts of tunicamycin-insensitive [3H]GlcN-labeled glycolipids with dephosphorylated, deaminated, and reduced glycan moieties larger than Manα1AHM and with retention times corresponding to galactosylated (Galα1,3) Manα1AHM have been observed, consistent with the possibility that a small fraction of the P2/P3 pool is galactosylated. Thus, it is possible that a pre-galactosylated glycolipid is transferred to VSG and further processed to generate the heterogeneous branched structure observed on the mature protein. Galactosylation may enhance the efficiency of transfer of the glycolipid to VSGs, similar to the effect of glucosylation on the transfer of the high mannose oligosaccharide, Manα1-4GlcNAc, from the dolichol-linked precursor to proteins in mammalian systems (Turco et al., 1977; Robbins et al., 1977).

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REFERENCES
Bangs, J. D., Hered, D., Krakow, J. L., Hart, G. W., and Englund, P. T. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3207-3211
Bangs, J. D., Doering, T. L., Englund, P. T., and Hart, G. W. (1988) J. Biol. Chem. 263, 17697-17705
Ciucanu, I., and Kerek, F. (1984) Carbohydr. Res. 131, 209-217
Conzelmann, A., Episazzi, A., and Bron, C. (1987) Biochem. J. 246, 605-610
Conzelmann, A., Riezman, H., Desponds, C., and Bron, C. (1988) EMBO J. 7, 2235-2240
Cross, G. A. M. (1975) Parasitology 71, 393-417
Cross, G. A. M. (1984) J. Cell. Biochem. 24, 79-90
Cross, G. A. M. (1987) Cell 48, 179-191
Ferguson, M. A. J., and Williams, A. F. (1988) Annu. Rev. Biochem. 57, 255-300
Ferguson, M. A. J., Low, M. G., and Cross, G. A. M. (1985) J. Biol. Chem. 260, 14547-14555
Ferguson, M. A. J., Duszenko, M., Lamont, G. S., Overath, P., and Cross, G. A. M. (1986) J. Biol. Chem. 261, 356-362
Ferguson, M. A. J., Homans, S. W., Dwek, R. A., and Rademacher, T. W. (1988) Science 239, 753-759
Fernandes, D. L. (1986) D. Phil. Thesis, University of Oxford

6 Menon, A. K., Schwartz, R. T., Mayor, S., and Cross, G. A. M. (1986) J. Biol. Chem., in press.
Haas, R., Brandt, P. T., Knight, J., and Rosenberry, T. L. (1986) Biochemistry 25, 3098–3105
Hardy, M. R., and Townsend, R. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3289–3293
Hansen, S. A. (1975) J. Chromatogr. 107, 224–226
He, H.-T., Finne, J., and Goridis, C. (1987) J. Cell Biol. 105, 2489–2500
Holder, A. A. (1985) Curr. Top. Microbiol. Immunol. 117, 57–74
Homans, S. W., Ferguson, M. A. J., Dwek, R. A., and Rademacher, T. W. (1983) Nature 303, 269–272
Howard, A. D., Berger, J., Gerber, L., Famillettis, P., and Udenfriend, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6055–6059
Hull, S. R., and Turco, S. J. (1985) Anal. Biochem. 151, 554–560
Jemmersen, R., and Low, M. G. (1997) Biochemistry 26, 5703–5709
Kobata, A., and Amano, J. (1987) Methods Enzymol. 138, 779–785
Karak, J. L., Hered, D., Bangs, J. D., Hart, G. W., and Englund, P. T. (1986) J. Biol. Chem. 261, 12147–12153
Li, Y.-T., and Li, S.-C. (1972) Methods Enzymol. 16, 996–997
Low, M. G. (1987) Biochem. J. 244, 1–13
Low, M. G., and Saltiel, A. (1988) Science 239, 268–275
Masterson, W. J., Doering, T. L., Hart, G. W., and Englund, P. T. (1989) Cell 56, 793–800
Mayor, A. K., and Cross, G. A. M. (1990) J. Biol. Chem. 265, 6174–6181
Medof, M. E., Walter, E. L., Haas, R., and Rosenberry, T. L. (1986) Biochemistry 25, 6740–6747
Menon, A. K., Mayor, S., Ferguson, M. A. J., Duszenko, M., and Cross, G. A. M. (1988a) J. Biol. Chem. 263, 1970–1977
Menon, A. K., Schwarz, R. T., Mayor, S., and Cross, G. A. M. (1988b) Biochem. Soc. Trans. Lond. 16, 996–997
Natsuka, S., Hase, S., and Ikenaka, T. (1987) Anal. Biochem. 167, 154–159
Natsuka, S., Masaru, H., Hase, S., Ito, H., Ueda, T., Kato, K., and Ikenaka, T. (1988) J. Biochem. (Tokyo) 103, 986–991
Ogata, S., Hayashi, Y., Takami, N., and Ikehara, Y. (1988) J. Biol. Chem. 263, 10489–10494
Parekh, R. B., Tse, A. G. D., Dwek, R. A., Williams, A. F., and Rademacher, T. W. (1987) EMBO J. 6, 1933–1944
Robbins, P. W., Hubbard, S. C., Turco, S. J., and Wirth, D. F. (1977) Cell 12, 893–900
Roberts, W. L., Kim, B. H., and Rosenberry, T. L. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7817–7821
Roberts, W. L., Myher, J. J., Kuksis, A., Low, M. G., and Rosenberry, T. L. (1989a) J. Biol. Chem. 263, 10706–10715
Roberts, W. L., Santikarn, S., Reinhold, V. N., and Rosenberry, T. L. (1989b) J. Biol. Chem. 265, 18776–18784
Rosenthal, L., and Ballou, C. E. (1974) Carbohydr. Res. 32, 287–298
Schmitz, B., Klein, R. A., Duncan, I. A., Egge, H., Gunawan, J., Petere-Katalinic, J., Dabrowski, V., and Dabrowski, J. (1987) Biochem. Biophys. Res. Commun. 146, 1059–1063
Smith, R., Braun, P. E., Ferguson, M. A. J., Low, M. G., and Sherman, W. R. (1987) Biochem. J. 248, 285–288
Stahl, N., Borchelt, D. R., Hsieh, K., and Prusiner, S. B. (1987) Cell 51, 229–240
Takami, N., Ogata, S., Oda, K., Misumi, Y., and Ikehara, Y. (1988) J. Biol. Chem. 263, 3016–3021
Tse, A. G. D., Barclay, A. N., Watts, A., and Williams, A. F. (1985) Science 228, 1933–1944
Turco, S. J., Stetson, B., and Robbins, P. W. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4411–4414
Vijay, I. K., and Perdew, G. H. (1980) J. Biol. Chem. 255, 11221–11226
Vijay, I. K., Perdew, G. H., and Lewis, D. E. (1980) J. Biol. Chem. 255, 11220–11229
Williams, A. P., Tse, A. G.-D., and Gagnon, J. (1988) Immunogenetics 27, 265–272
Yamashita, K., Mitsuishi, T., and Kobata, A. (1982) Methods Enzymol. 83, 105–126
Glycolipid precursors for the membrane anchor of Trypanosoma brucei variant surface glycoproteins. I. Can structure of the phosphatidylinositol-specific phospholipase C sensitive and resistant glycolipids.
S Mayor, A K Menon, G A Cross, M A Ferguson, R A Dwek and T W Rademacher
J. Biol. Chem. 1990, 265:6164-6173.

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