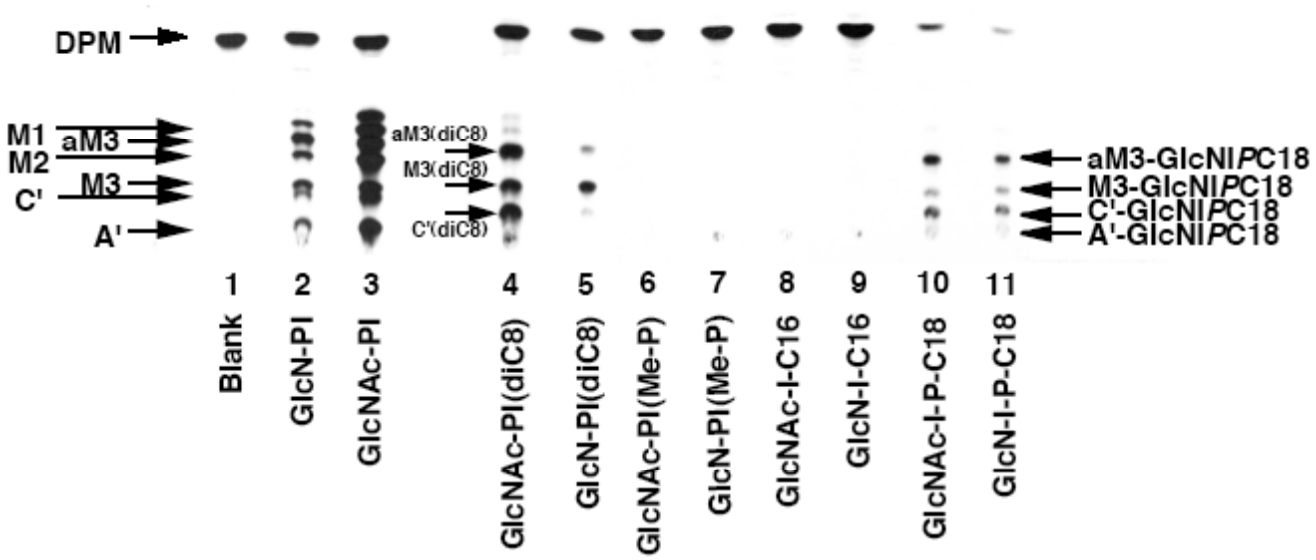

Fig. S1. Uncharged GlcN-PI analogues are not recognised by *T.brucei* GPI pathway enzymes. The modified (Smith *et al*. (1996) *J Biol Chem* **271**: 6477-6482) *T.brucei* cell-free system was labeled with GDP-[^{3}H]Man in the absence of any additive (negative control, lane 1) and with added diplamitoyl GlcN-PI and diplamitoyl GlcNAc-PI (positive controls, lanes 2 and 3). The identities of the labeled products, identified by HPTLC and fluorogaphy, are shown on the left (see legend to Fig. 1). The cell-free system was also labeled in the presence of dioctanoyl GlcN-PI and dioctanoyl GlcNAc-PI (lanes 4 and 5) to demonstrate that reduction of the lipid chain length from C32 to C16 does not abolish substrate recognition. Similarly, analogues containing a single C18 octadecyl group (in the form of inositol-phosphate-octadecanol, (I*P*C18)) in place of diacylglycerol are recognized and processed by the GPI pathway (lanes 10 and 11); the identities of the labeled products are shown on the right. By contrast, analogues lacking a phosphodiester component, like GlcN1-6*myo*-inositol1-O-hexadecanol (GlcN-I-C16) and its N-acetylated derivative (lanes 8 and 9), or that contain a neutral methylphosphonate instead of a phosphodiester component in the PI moiety, like GlcN1-6*myo*-

inositol1-O-PO(-CH_3)-O-octadecanol (GlcN-PI(Me-P)) and its N-acetyl derivative (lanes 6 and 7), are not processed by the GPI pathway. These data suggest that the negatively charged phosphodiester component of the PI moiety is essential for substrate recognition.

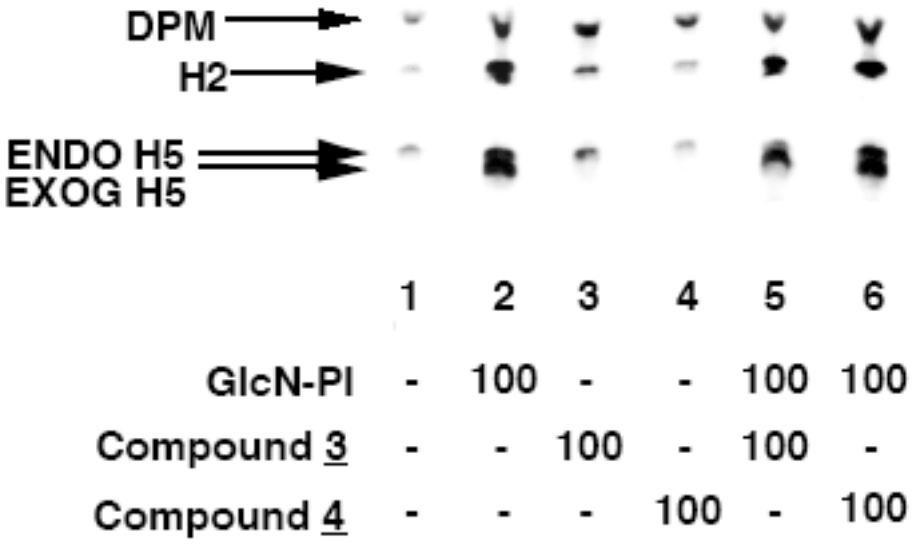

Fig. S2. Compounds **3** and **4** are neither substrates nor inhibitors of GPI biosynthesis in the HeLa cell-free system.

The HeLa cell-free system was labeled with GDP-[^{3}H]Man alone (lane 1) or in the presence of 100 μM GlcN-PI (lane 2). Analysis of the glycolipid products by HPTLC and fluorography reveal the formation of endogenous glycolipid H5 (ENDOG H5, lane 1) and additional products, H2 and EXOG H5, derived from the exogenously added GlcN-PI (lane 2). Lanes 3 and 4 show that no additional products are made in the presence of 100 μM compounds **3** and **4**, indicating that they are not substrates for the HeLa cell GPI pathway. Neither do these compounds affect the processing of GlcN-PI (lanes 5 and 6), indicating that they are not inhibitors of GPI biosynthesis. H2 is ManGlcN-(acyl)PI and H5 is EtN*P*-ManGlcN-(acyl)PI.