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A Synthetic Acceptor Substrate for *Trypanosoma brucei* UDP-Gal : GPI Anchor Side-Chain α-Galactosyltransferases

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Abstract: A synthetic analogue of a trisaccharide fragment of the *Trypanosoma brucei* Variant Surface Glycoprotein (VSG) GlycosylPhosphatidylInositiol (GPI) anchor, Gal-α-1,3(Man-α-1,6)-Man-α-*O*-octyl (**1**), serves as a substrate for two *T. brucei* α-galactosyltransferases. The principle tetrasaccharide product derived from (**1**) contains a Gal-α-1,2-Gal linkage. © 1998 Elsevier Science Ltd. All rights reserved.

GlycosylPhosphatidylInositol (GPI) membrane-anchors for proteins are widespread in Nature where they serve as a functional alternative to a *trans*-membrane peptide sequence in the attachment of proteins to the cell surface.[1] Whilst the GPI core structure is conserved throughout biology, a variety of protein-specific modifications to the core have been reported.[1] In the case of the Variant Surface Glycoprotein (VSG) of *Trypanosoma brucei*, the causative agent of African sleeping sickness, a number of α- and β-galactose units are attached to the core;[2,3] the precise nature of the modifications is variant-dependent (Figure 1).[2,3]

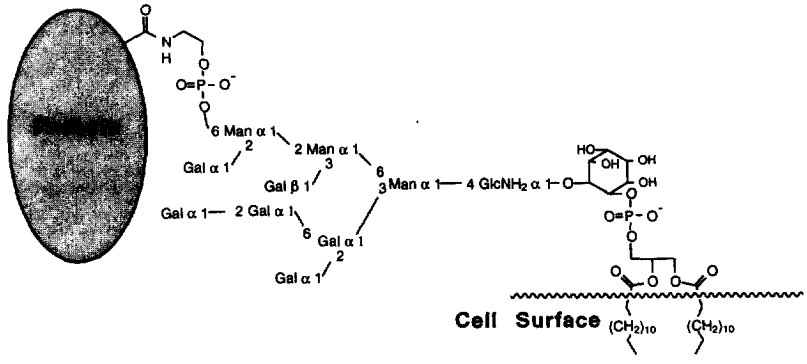

Figure 1 : The GPI Anchor of *Trypanosoma brucei* Variant Surface Glycoprotein

The surface glycoprotein coat is the principle mechanism employed by the parasite to avoid destruction by the infected host.[4] The branched oligo-galactose side-chain of the VSG anchor is thought to be important for maintaining optimal arrangement of VSG molecules on the parasite cell surface, thus permitting uptake of small nutrient molecules whilst preventing recognition of parasite cell membrane components by host defence proteins.[5] Molecules that interfere with formation of this oligo-galactose structure might therefore be expected to

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render the parasite sensitive to the host immune system. Since these branched galactose structures are specific to this parasite, enzymes involved in their formation might be suitable targets for the development of novel anti-parasitic agents.

We[6] and others[7] have shown that synthetic analogues of fragments of the GPI anchor core, namely Man-α-1,6-Man-α-*O*-octyl and the corresponding thiooctyl glycoside, can serve as substrates for *T. brucei* α-1,3-galactosyltransferase. Here we report an extension of these studies aimed at investigating the glycosylation events involved in formation of the branched oligo-galactose side chain of the VSG anchor (Figure 2).[8] Herein we describe the synthesis of an octyl glycoside of a GPI anchor fragment, Gal-α-1,3(Man-α-1,6)-Man-α-*O*-octyl (**1**), and assessment of its' ability to serve as a substrate for *T. brucei* α-galactosyltransferases.

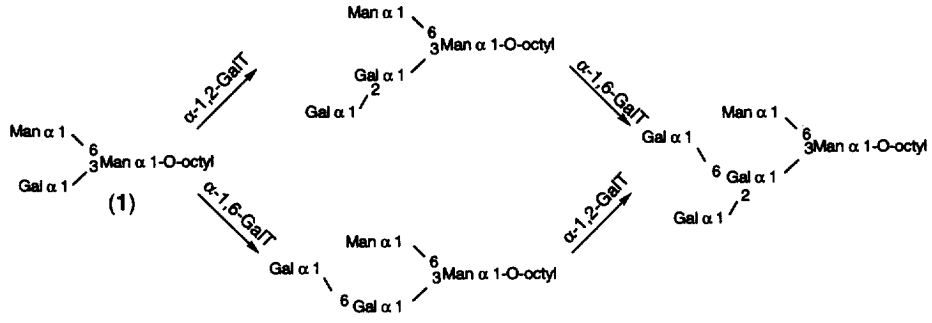
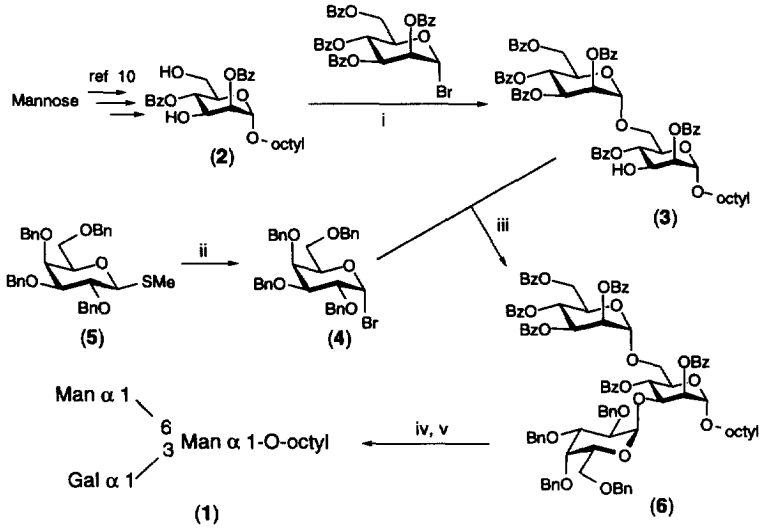


Figure 2 : Possible Biosynthetic Transformations of GPI Anchor Fragment (1)

Substrate synthesis:- Prospective α-galactosyltransferase substrate (**1**) was prepared as described in Figure 3; the corresponding methyl glycoside has previously been reported by Garegg and co-workers.[9] Diol (**2**)[10] was selectively 6-*O*-mannosylated with benzobromomannose to give disaccharide intermediate (**3**). Galactosylation of (**3**) with glycosyl halide (**4**) [prepared from thioglycoside (**5**) by treatment with IBr[11]] gave protected trisaccharide (**6**) as a separable 1:1 α /β mixture in a combined yield of 75% (based on recovered acceptor).[12] Standard deprotection of (**6**) and purification on a Bio-gel P-4 column then gave the desired trisaccharide (**1**) as an amorphous solid.[13]

Biological evaluation:- Compound (**1**) was incubated with washed trypanosome membranes in the presence of UDP-[^{3}H]Gal and radiolabelled products were extracted and characterised by a combination of TLC, *exo*-glycosidase digestion and mass spectrometry.[14,15] These experiments confirmed the generation of two radiolabelled products (approx. 9:1 ratio) with very similar TLC mobilities, consistent with the formation of a pair of isomeric tetrasaccharides.[16] Analysis of the products by electrospray mass spectrometry in negative ion mode revealed a pseudomolecular ion at *m/z* 777, consistent with the expected mass (778) of a Hex_4-*O*-octyl product; this was further supported by the *m/z* 777 CID daughter ion spectrum. The specificity of the glycosylation process was confirmed by the observation that the tetrasaccharide products derived from (**1**) were sensitive to digestion with jack bean α-mannosidase (producing a labelled trisaccharide product), and that the Man-α-1,3(Man-α-1,6)-Man-α-*O*-octyl[10] stereoisomer of (**1**) was not a substrate for the *T. brucei* α-galactosyltransferases. Further analysis of the products derived from (**1**) showed them to be insensitive to bovine testes β-galactosidase, but radiolabel was released as free galactose on treatment with coffee bean α-galactosidase, thus indicating the formation of α-galactosyl linkages. These results are consistent with trisaccharide (**1**) acting as an acceptor substrate for two *T. brucei* α-galactosyltransferases, with the newly

formed α-galactosyl linkages being to the galactose moiety of (**1**).



i. $Hg(CN)_2$, CH_2Cl_2 (87%); ii.IBr, CH_2Cl_2 (72%); iii. AgOTf, CH_2Cl_2 (α/β mixture; 75%); iv. H_2, Pd-C, AcOH/EtOH (90%); v. NaOMe, MeOH (90%).

Figure 3: Synthesis of Target Trisaccharide (1)

Linkage analysis[14] of the tetrasaccharide products was conducted by periodate oxidation / $NaBD_4$ reduction / mass spectrometry, as described in reference 6. The principle product from this analysis showed an ion at *m/z* 729, consistent with 5 oxidation sites (4 oxidation sites would be present in the Gal-α-1,3-Gal product, and 6 oxidation sites in the Gal-α-1,6-Gal product or in any product where the Gal residue had been transferred to the reducing terminal Man residue). This result supports the presence of a Gal-α-1,2-Gal or a Gal-α-1,4-Gal linkage in the major product derived from (**1**). Since the Gal-α-1,4-Gal linkage has not previously been reported in any *T. brucei* glycan structure, it is reasonable to assume that the major product of enzymatic glycosylation of compound (**1**) is Gal-α-1,2-Gal-α-1,3(Man-α-1,6)-Man-α-*O*-octyl. That is, Gal-α-1,3(Man-α-1,6)-Man-α-*O*-octyl is a good substrate for the UDP-Gal : GPI side-chain α-1,2-galactosyltransferase. Using this particular synthetic substrate, the minor α-galactosyltransferase activity found in *T. brucei* membranes is thought to be attributable to the UDP-Gal : GPI side-chain α-1,6-galactosyltransferase. At first sight, the order of transferase activities observed (α-1,2 > α-1,6) using the synthetic acceptor is unexpected, given that most VSG GPI anchors are quantitatively α-1,6-galactosylated but not quantitatively α-1,2-galactosylated.[2,3] However, the heterogeneity in VSG GPI α-1,2-galactosylation is most likely due to steric constraints imposed by the VSG polypeptide,[3] which do not apply for the synthetic acceptor, rather than to the absolute levels of α-1,2-galactosyltransferase activity.

Conclusion:

In summary, we have demonstrated that the synthetic GPI anchor fragment Gal-α-1,3(Man-α-1,6)-Man-α-*O*-octyl serves as a substrate for two *T. brucei* α-galactosyltransferases, with the predominant activity associated with α-1,2-galactosylation of the non-reducing galactose unit. The minor activity presumably results in α-1,6-galactosylation of the same galactose unit. Both of these activities are believed to reside in the Golgi appartus of the parasite.[2]

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12. Attempts to perform selective α-galactosylation of alcohol (**3**) with glycosyl bromide (**4**) under halide-assisted glycosylation conditions[9] proved extremely slow. Whilst the more forcing coupling conditions reported here give an anomeric mixture of trisaccharides, sufficient material was obtained to proceed with biochemical studies.
13. Selected data for compound (**1**): $[\alpha]_D$ +38°(*c* 0.2, CH_3OH); δ_H (D_2O): 4.85 (1H, d, <2Hz), 4.92 (1H, d, <2Hz), 5.25 (1H, d, 3.6Hz); δ_C (D_2O): 14.1, 22.7, 26.1, 29.1 (2), 29.2, 31.8, 61.6, 61.9, 66.1, 66.3, 67.4, 68.8, 71.5, 70.0, 70.1, 70.5, 70.8, 71.4, 71.7, 72.1, 73.1, 73.4, 80.0, 100.2, 100.4, 101.6. ES-MS: Calcd. for $[C_{26}H_{48}O_{16}]$: *m/z* 616.7. Found $[M-1]^-$: *m/z* 615.0
14. Further experimental details can be found in : Brown, J. Ph.D. Thesis, 1997, University of Dundee, Dundee, UK. See also reference 6 for similar experimental protocols.
15. Endogenous diacylglycerol-containing glycolipids were removed by treatment with base prior to analysis.
16. It is important to note that the products formed from (**1**) are only present in radiochemical quantities in the assay, and hence at very low concentration. It is conceivable that at higher concentrations the α-Gal-(**1**) adduct would undergo further galactosylation, as expected of a GPI pathway substrate.