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Synthesis of octyl *O*- and *S*-glycosides related to the GPI anchor of *Trypanosoma brucei* and their in vitro galactosylation by trypanosomal α-galactosyltransferases

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Abstract

Octyl *O*- and *S*-glycosides of mono- to tri-saccharides related to the core structure α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-α-D-Man*p* of the GPI anchor of *Trypanosoma brucei* have been prepared via regioselective protodesilylation and glycodesilylation of octyl *O*- and *S*-glycosides of 2-*O*-benzoyl-4,6-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside. The synthetic saccharides have been used as substrates for enzymatic α-galactosylation with membrane fractions of bloodstream forms of *T. brucei* strain 427 variants MITat 1.4, MITat 1.2, and MITat 1.5, respectively. © 1996 Elsevier Science Ltd.

Keywords: GPI anchor; *Trypanosoma brucei*; Galactosyltransferase; Glycodesilylation

1. Introduction

Membrane attachment of eukaryotic membrane proteins occurs either by membrane-spanning hydrophobic peptide domains or by a glycosyl-phosphatidylinositol (GPI) anchor, which is covalently linked to the C-terminus of the protein. The latter was first described ten years ago as the membrane anchor of variant surface glycoprotein (VSG)

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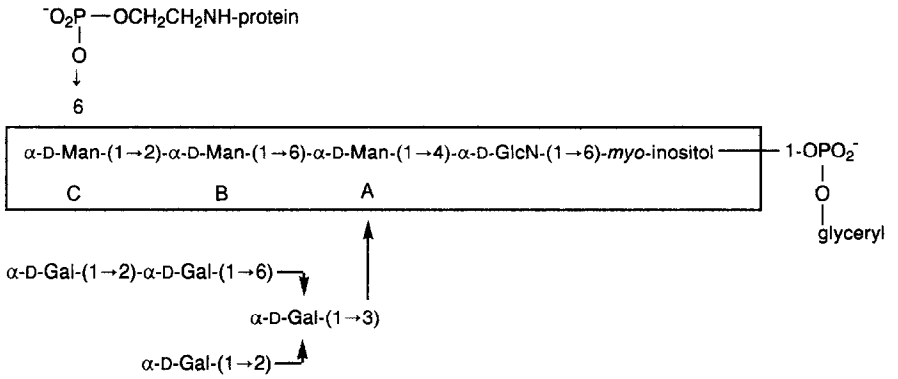


Fig. 1. Structure of the GPI anchor of *Trypanosoma brucei* VSG.

[1], a surface protein, which forms a protective barrier around the bloodstream forms of the protozoan parasite *Trypanosoma brucei*. Since then, GPI anchored proteins have been detected in all classes of eukaryotes [2]. VSG accounts for about 10% of the total trypanosomal proteins and is thus perfectly suitable for investigating the GPI structure in detail. Indeed VSG was the first protein of which the GPI structure was completely elucidated. However, a clear and defined biological function of the GPI anchor has still to be elucidated, but it is noteworthy that GPI anchored proteins are involved in important processes of cell–cell interaction, cell adhesion, and immunoresponse [3].

Further studies of GPI structures obtained from protozoa (such as *T. brucei*-VSG [2,4] and *T. crucei*-1G7 [4,5]), fish (such as Torpedo-acetylcholinesterase (AchE) [6]), and mammalian sources (such as rat brain Thy-1 [7] and human AchE [8]) revealed that all GPI anchors contain a highly conserved pentasaccharide core structure (Fig. 1). In the case of the VSG GPI anchor, this core structure is, depending on the trypanosomal clone investigated, galactosylated differently. These variable galactose side-chains may be important for the integrity of the VSG coat in order to serve as a molecular diffusion barrier. Since several hundred different VSG variants are encoded within the trypanosomal genome which will be sequentially expressed during a persisting infection (antigenic variation), galactose residues could perform space-filling functions in order to compensate for differences in the three-dimensional VSG structures [9]. Thus the respective galactosyltransferases are of fundamental interest as possible targets for a new antitrypanosomal strategy.

In the present study, we used, unless otherwise stated, variant MITat 1.4 of *T. brucei* strain 427 [10]. In this case, the core structure of the GPI anchor is modified by a diantennary α-D-galactosyl tetrasaccharide, α-(1 → 3)-linked with the mannosyl residue A (Fig. 1). The enzymes involved in the biosynthetic pathway are still unknown, but it was reported recently that octyl 1-thio-mannopyranosides may serve as suitable substrates for trypanosomal α-galactosyltransferases [11]. Similarly, octyl *O*-glycosides have been shown to exhibit sufficient hydrophobicity to act as substrates for glycosyltransferases [12].

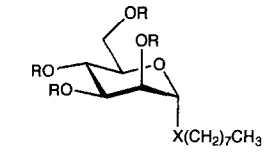
Here the synthesis of a series of mono- to tri-saccharide octyl *O*- and *S*-glycosides related to the core structure ABC (Fig. 1) is described. Additionally, the suitability of these glycosides for serving as synthetic substrates for α-galactosyltransferases in membrane fractions from *T. brucei* has been determined.

2. Results and discussion

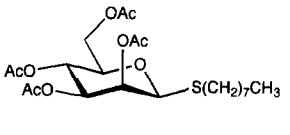
Synthesis of acceptors.—In recent years, several syntheses of the complete structure of the GPI anchor of *T. brucei* have been published [13–15]. Furthermore, a flexible synthesis of the GPI anchor of *Saccharomyces cerevisiae* was described [16] whilst the present work was underway. Since we intended to prepare mono- to tri-saccharide fragments of the core structure of the trypanosomal GPI anchor in order to demonstrate the possible enzymatic galactosylation step (i.e. at site A in Fig. 1), we used the glycodesilylation protocol [17] for the construction of the desired saccharides. Therefore, octyl *O*- and *S*-mannopyranosides that were shown to function as substrates for *T. brucei* α-galactosyltransferase [11] were needed. Furthermore, octyl 4,6-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside derivatives were required for this approach. To this end, D-mannose pentaacetate was converted with 1-octanol in the presence of $SnCl_4$ [18] into octyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside **1a** (48%), deacetylation of which gave octyl α-D-mannopyranoside **2a**. Silylation of **2a** with 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane afforded compound **3a** which was selectively monobenzoylated without further purification to give the 2-*O*-benzoyl-4,6-*O*-siloxane protected derivative **4a** in 45% yield over 3 steps. Due to the steric hindrance [17] of HO-3 in **3a**, HO-2 was benzoylated preferentially. However, a small amount of the corresponding 2,3-di-*O*-benzoyl derivative **5** was obtained as well. Since it was originally planned to use compound **5** as the glycosyl acceptor for the elongation of the sugar chain at O-6 by a glycodesilylation reaction, the same compound was also prepared from the monobenzoate **4a** by treatment with benzoyl bromide [17,19]. Alternatively, condensation of the imidate **6** [17] with 1-octanol afforded **5** in 89% yield. Finally, compounds **4a** and **5** were selectively converted into the mannosyl acceptors **7a** (84%) and **8** (70%) by protodesilylation with HF in pyridine [17]. In a similar sequence, the corresponding octyl 1-thio-α-D-mannopyranoside derivatives **1b**, **2b**, **3b**, **4b**, and **7b** were prepared. Here, the initial condensation of D-mannose pentaacetate with 1-octanethiol in the presence of boron trifluoride diethyletherate gave a small amount of the corresponding β-D-mannopyranoside **1b′** (6%) along with the desired α-anomer **1b** (52%).

When compound **5** was treated with 2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl fluoride **9**, prepared in 81% yield from 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (benzobromomannose) with KHF_2 [20], and boron trifluoride diethyletherate as the catalyst, a clean glycodesilylation at O-6 of the acceptor occurred. The crude intermediate disaccharide was directly desilylated (tetrabutyl ammonium fluoride in THF) to give the α-(1 → 6)-linked disaccharide octyl glycoside **10a** (72%). The same compound was obtained in 81% yield by a similar sequence, involving silver trifluoromethanesulfonate-promoted glycosylation of compound **8** with benzobromomannose

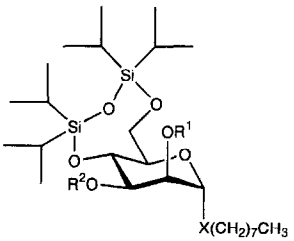
followed by fluoride-catalysed desilylation. Final deblocking of compound **10a** gave the desired disaccharide octyl glycoside **11a** in an almost quantitative yield. For the synthesis of the corresponding octyl 1-thio-mannoside disaccharide, compound **7b** was first condensed with benzobromomannose in the presence of silver trifluoromethanesulfonate, followed by fluoride-catalysed desilylation to give disaccharide **10b** (58%). As has previously been observed in similar cases [17], no mannosylation of O-3 in **7b** occurred. Deblocking of compound **10b** then afforded the free 1-thio-disaccharide **11b** in 86% yield.



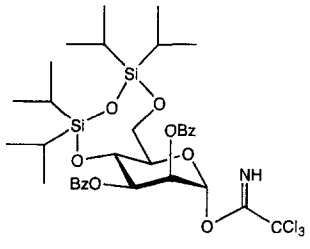
1a R = Ac X = O
1b R = Ac X = S
2a R = H X = O
2b R = H X = S



1b'



3a $R^1 = R^2 = H$ X = O
3b $R^1 = R^2 = H$ X = S
4a R^1 = Bz R^2 = H X = O
4b R^1 = Bz R^2 = H X = S
5 $R^1 = R^2$ = Bz X = O



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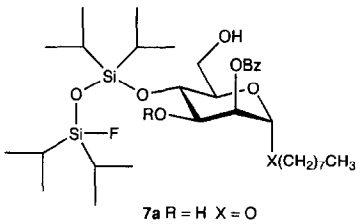
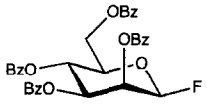


7a R = H X = O
7b R = H X = S
8 R = Bz X = O



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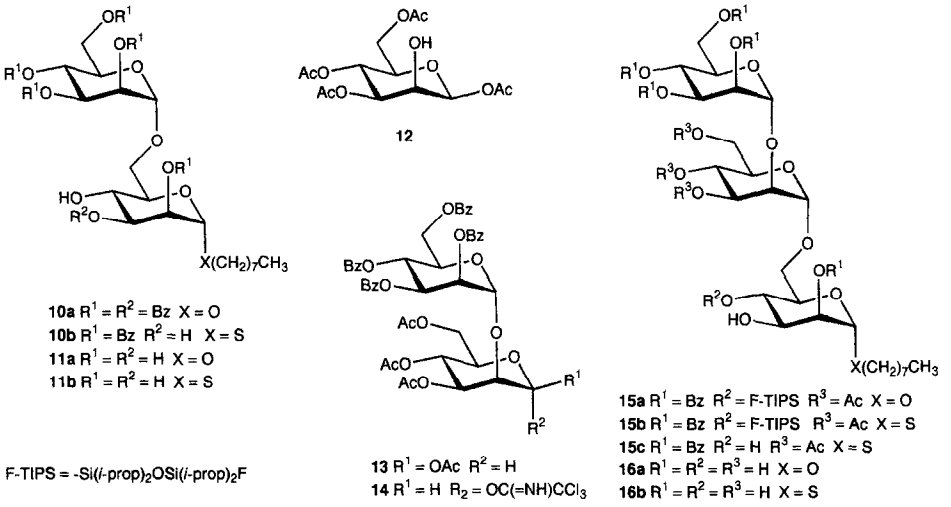
For the construction of the corresponding trisaccharides, a blockwise synthesis was applied. First, benzobromomannose was condensed with 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose [21] **12** to give the α-(1 → 2)-linked disaccharide block **13** (86%). Benzobromomannose was chosen as the glycosyl donor because glycosylation of **12** with acetobromomannose was described as proceeding only in low yield [22]. Then, compound **13** was converted into the disaccharide donor **14** (72%) by sequential treatment with hydrazine acetate [23] followed by trichloroacetonitrile and K_2CO_3. Trimethylsilyl trifluoromethanesulfonate-catalysed condensation of donor **14** with octyl *O*-mannoside **7a** proceeded in a clean reaction and afforded trisaccharide **15a** in 81% yield. Sequential removal of the fluorodisiloxane residue at O-4 of the intermediate with tetrabutyl ammonium fluoride and deacylation gave trisaccharide octyl *O*-glycoside **16a** (77%). Similarly, octyl *S*-mannoside **7b** was condensed with donor **14** and furnished the blocked trisaccharide octyl *S*-glycoside **15b** in 75% yield. Fluoride-catalysed desilyla-

tion then afforded compound **15c** (73%) which was finally deacylated to give the desired trisaccharide **16b** (95%).



All six saccharides **2a**, **2b**, **11a**, **11b**, **16a**, and **16b** were used for the enzymatic galactosylation by a *T. brucei* α-galactosyltransferase as described below.

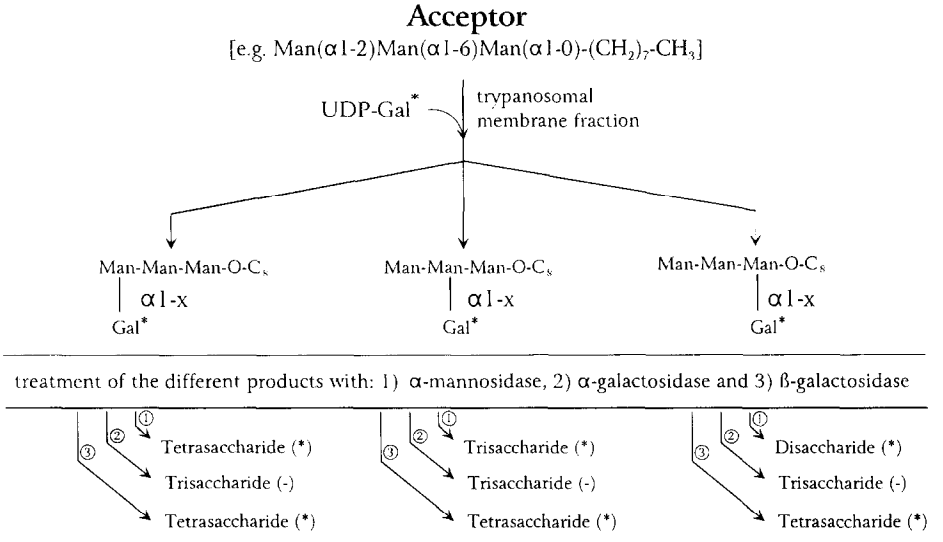
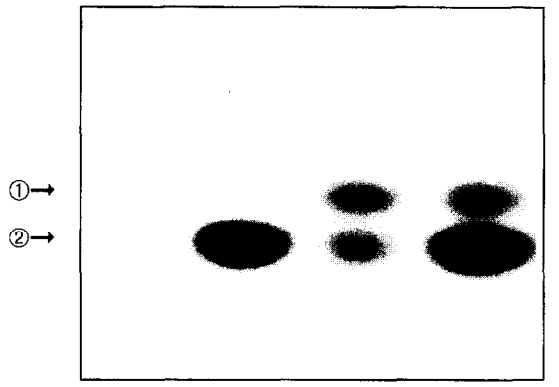


Fig. 2. As described, the trypanosomal membrane fraction, containing galactosyltransferases, was incubated with different substrates in the presence of radiolabelled UDP-Gal. The graph shows the expected products, if the acceptor **16a** is used. The products are treated with α-mannosidase and the galactosidases and applied to TLC. Analysis of the resulting fluorography reveals which products have been formed in the initial reaction. The shown scheme can easily be applied to all acceptors used in this study (compare Fig. 4, lanes 6–10). The asterisk indicates radiolabelled products.



-	+	+	+	acceptor
-	+	-	-	α-Gal
-	-	+	-	β-Gal
+	+	+	+	UDP-Gal*
+	+	+	+	MF

Fig. 3. Product analysis of the acceptor substrate **2a**. Analysis was performed by TLC and fluorography of the HPTLC plates. At least two different radiolabelled disaccharide products appear, which are either α-galactosidase (α-Gal) sensitive (1) or β-galactosidase (β-Gal) sensitive (2). As shown in lane 3, β-galactosidase treatment removes about 90% of the radiolabelled product. UDP-Gal*, Uridine diphospho-D-[U-^{14}C]galactose; MF, trypanosomal membrane fraction; the asterisk indicates radiolabelled products.

Enzymatic α-galactosylation.—Using trypanosomal membrane fractions prepared from MITat 1.4 and radiolabelled UDP-galactose, we have been able to show that the synthetic substrates **2a**, **11a**, **16a**, and **16b** were all galactosylated in vitro (Fig. 2). In accordance with results reported earlier for **2b** [11], **2a** resulted in 90% β-galactosylation and 10% α-galactosylation (Fig. 3). The different trisaccharides used in this study gave the following results: **16a** led to at least three different tetrasaccharides (Fig. 4), which were all sensitive to coffee bean α-galactosidase digestion but resistant to bovine testes β-galactosidase. Digestion with α-mannosidase resulted in radioactive labelled disaccharide (M_2), trisaccharide (M_3) and tetrasaccharide (M_4) bands on HPTLC plates (Fig. 4). As judged from these results, the following structures are most likely: two branched tetrasaccharide structures: (1) α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-[α-D-Gal*p*-(1 → x)]-α-D-Man*p*-1-O-$(CH_2)_7$-CH_3, leading to a labelled disaccharide, and (2) α-D-Man*p*-(1 → 2)-[α-D-Gal*p*-(1 → x)]-α-D-Man*p*-(1 → 6)-α-D-Man*p*-1-O-$(CH_2)_7$-CH_3, leading to a labelled trisaccharide; and the terminally galactosylated structure α-D-Gal*p*-(1 → x)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-α-D-Man*p*-1-O-$(CH_2)_7$-CH_3, which is not susceptible to α-mannosidase. In contrast, 1-thio-glycoside **16b** resulted in a totally different product pattern and the total incorporation of radioactivity was about two times higher. The higher incorporation rate might be due to the fact that **16b** always led to a band in the disaccharide region of the HPTLC (M'_2 in Fig. 4). Considering the fact that the acceptor **16b** was pure as judged by NMR and HPTLC analysis, we assume that α-mannosidase activity in the membrane fraction degrades **16b** to the monosaccharide **2b** which is readily galactosylated thereafter.

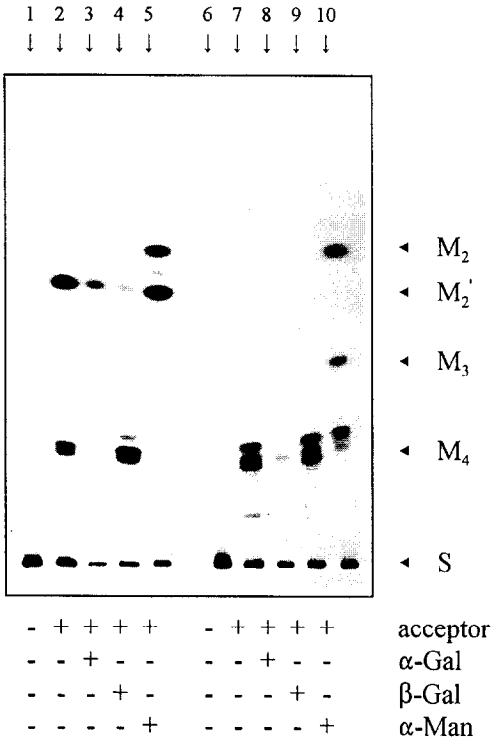


Fig. 4. Product analysis of the acceptor substrates **16b** (lanes 1–5) and **16a** (lanes 6–10). Fluorography of HPTLC plates.

In a second set of experiments, we used the disaccharides **11a** and **11b** in concentrations ranging from 0.5 mM to 10 mM as acceptors for galactosylation (Fig. 5). All products were sensitive to α-galactosidase and α-mannosidase digestion. Acceptor **11a**, however, gave rise to at least two products. Using the latter, galactosylation of both the branched and the terminal products increased with increasing acceptor concentration (data not shown), whereas with the sulfur-containing disaccharide **11b** galactosylation of the branched product reached a maximum at a concentration of 2 mM.

All experiments described so far were performed with membrane fractions isolated from the trypanosome clone MITat 1.4. This variant has been chosen because the GPI membrane anchor of the respective VSG variant contains a di-branched galactosyl side-chain. Galactosylation of the invariant GPI core structure is, however, variable. We have thus used membrane fractions of trypanosome variants which express a completely different galactosylation pattern of their respective VSG membrane anchors, e.g. MITat 1.2 and MITat 1.5 [1]. Galactosylation of **11b**, however, was virtually identical in all three clones (Fig. 6). These results show that the responsible galactosyltransferases are always present in bloodstream forms of *T. brucei* and that the expression of a different VSG

[1] A. Mehlert and M.A.J. Ferguson, personal communication.

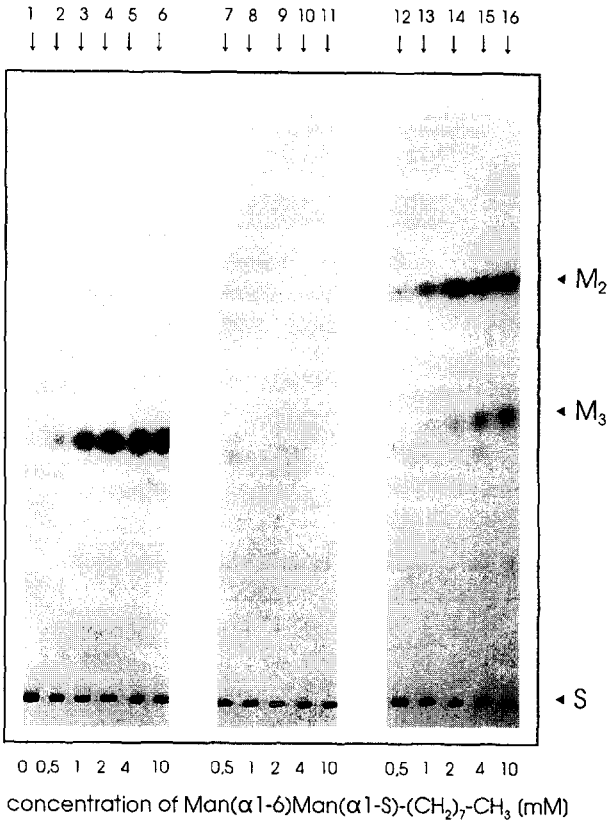


Fig. 5. Galactosylation of **11b** depending on different concentrations of the acceptor substrate. Lanes 1–6: mock treatment; lanes 7–11: α-galactosidase digestion; lanes 12–16: α-mannosidase digestion.

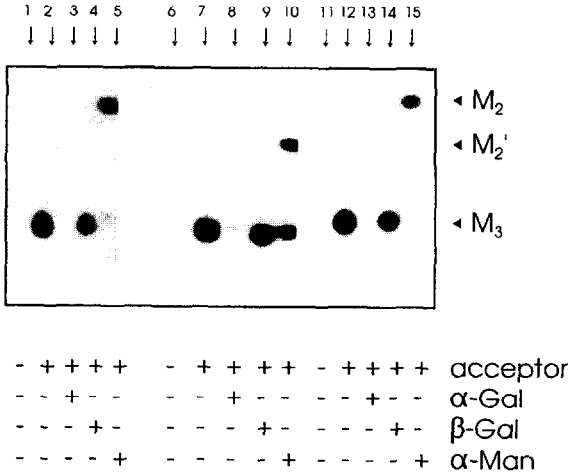


Fig. 6. Galactosylation pattern of **11b** using membrane fractions from either MITat 1.5 (lanes 1–5), MITat 1.4 (lanes 6–10) or MITat 1.2 (lanes 11–15). M_2 and M'_2 mark disaccharide bands with identical R_f values.

variant does not influence the expression of the galactosyltransferases regardless of the final galactosylation pattern. This explanation would support the hypothesis that galactosyltransferases of *T. brucei* express a proofreading function on VSG since the distinctive galactosylation pattern is responsible for the correct orientation of VSG in the membrane which allows a dense packaging and formation of the protective surface coat.

3. Experimental

General methods.—NMR data were extracted from spectra measured in solutions of $CDCl_3$ for blocked compounds (with Me_4Si as an internal standard) and of D_2O for deblocked compounds (with MeOH as an internal standard) at 25 °C with a Bruker AC 250F spectrometer. Proton-signal assignments were made by first order analysis of the spectra. Of the two magnetically non-equivalent geminal protons at C-6 the one resonating at lower field was referred to as H-6a and the one resonating at higher field as H-6b. ^{13}C-assignments were made by mutual comparison of the spectra, by DEPT spectra and by comparison with spectra of related compounds. Optical rotations were measured at 25 °C with a Perkin–Elmer automatic polarimeter, Model 241. Melting points were measured with a Büchi apparatus, Model SMP-20. Thin-layer chromatography (TLC) was performed on precoated plastic sheets, Polygram SIL UV_{254}, 40 × 80 mm (Macherey–Nagel) using appropriately adjusted mixtures of CCl_4–acetone for developing. Detection was effected with UV light, where applicable, by I_2, and by charring with 5% H_2SO_4 in ethanol. Preparative chromatography was performed by elution from columns of Silica Gel 60 (E. Merck) using appropriately adjusted mixtures of CCl_4–acetone. Solutions in organic solvents were dried with anhydrous Na_2SO_4, and concentrated at 2 kPa, ≤ 40 °C.

*Octyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranoside* (**1a**).—$SnCl_4$ (3.4 mL, 29 mmol) was added at 0 °C to a stirred mixture of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose [24] (10.6 g, 29 mmol), 1-octanol (5.5 mL, 35 mmol), and molecular sieves 4 Å (11 g) in CH_2Cl_2 (100 mL). The mixture was allowed to warm up to room temperature and stirred for 17 h. It was finally washed with cold aq HCl (3 × 100 mL) and saturated aq $NaHCO_3$, then concentrated. Column chromatography (5:1 CCl_4–acetone) of the residue afforded **1a** (7.38 g, 48%); identical to [12].

*Octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-*D*-mannopyranoside* (**1b**) *and octyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-mannopyranoside* (**1b′**).—BF_3 diethyletherate (8.2 mL, 65.0 mmol) was added at room temperature to a stirred solution of 1,2,3,4,6-tetra-*O*-acetyl-D-mannopyranose (25.23 g, 64.6 mmol) and 1-octyl mercaptane (11.3 mL, 65.0 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred for 24 h, washed with aq $NaHCO_3$, and concentrated. Column chromatography (5:1 CCl_4–acetone) of the residue afforded first **1b** (16.06 g, 52%), mp 61–62 °C (n-hexane); $[\alpha]_D$ +93.0° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.34–5.24 (m, 3 H, H-2,3,4), 5.27 (bd, 1 H, H-1), 4.40–3.36 (m, 1 H, H-5), 4.33 (dd, 1 H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ −11.8 Hz, H-6a), 4.08 (dd, 1 H, $J_{5,6b}$ 1.8 Hz, H-6b), 2.71–2.51 (m, 2 H, SCH_2); ^{13}C NMR ($CDCl_3$): δ 82.5 (C-1), 71.2 (C-2), 69.5 (C-3), 68.9 (C-5), 66.3 (C-4), 62.4 (C-6). Anal. Calcd for $C_{22}H_{36}O_9S$: C, 55.44; H, 7.61; S, 6.73. Found: C, 55.22; H, 7.75; S, 6.54.

Eluted next was **1b'** (1.70 g, 6%), mp 109 °C (n-hexane); $[\alpha]_D$ −59.9° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.52 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.4 Hz, H-2), 5.27 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.08 (dd, 1 H, H-3), 4.78 (d, 1 H, H-1), 4.28 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ −12.3 Hz, H-6a), 4.13 (dd, 1 H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.71 (ddd, 1 H, H-5), 2.71 (t, 2 H, *J* 7.3 Hz, SCH_2); ^{13}C NMR ($CDCl_3$): δ 82.9 (C-1), 76.5, 71.9, 70.5, and 65.9 (C-2,3,4,5), 62.8 (C-6). Anal. Calcd for $C_{22}H_{36}O_9S$: C, 55.44; H, 7.61; S, 6.73. Found: C, 55.51; H, 7.78; S, 6.42.

Octyl α-D-mannopyranoside (**2a**).—NaOMe (0.1 mL, 1 M in MeOH) was added at room temperature to a solution of **1a** (4.69 g, 10.2 mmol) in MeOH (50 mL) and stirred for 16 h. Neutralisation of the solution with ion-exchange resin (Dowex 1X8, H^+) and concentration furnished **2a** (2.20 g) which was used without further purification for the next step.

Octyl 1-thio-α-D-mannopyranoside (**2b**).—Treatment of **1b** (11.13 g, 23.3 mmol) as described for compound **2a** afforded **2b** (7.15 g, 99%); $[\alpha]_D$ +142.6° (*c* 0.4, Me_2SO); ^{13}C NMR (Me_2SO-d_6): δ 86.4 (C-1), 74.8 (C-5), 73.8 (C-3), 73.2 (C-2), 68.8 (C-4), 62.7 (C-6). Anal. Calcd for $C_{14}H_{28}O_5S$: C, 54.52; H, 9.15; S, 10.40. Found: C, 54.24; H, 9.34; S, 9.99.

Octyl 4,6-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside (**3a**).—A solution of 1,1,3,3-tetraisopropyl-1,3-dichloro-1,3-disiloxane ($TIPSCl_2$) [25] (2.61 g, 8.3 mmol) in CH_2Cl_2 (5 mL) was added at 0 °C to a solution of crude **2a** (2.20 g, 7.5 mmol) and imidazole (2.25 g, 33.1 mmol) in DMF (25 mL). The mixture was stirred at room temperature for 15 min, diluted with water and extracted with CH_2Cl_2. The combined organic extracts were washed with aq $NaHCO_3$. Concentration of the solution afforded crude **3a** (3.38 g) which was used without further purification for the next step.

Octyl 4,6-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-1-thio-α-D-mannopyranoside (**3b**).—Treatment of **2b** (3.08 g, 10.0 mmol) with $TIPSCl_2$ (3.22 g, 10.2 mmol) and imidazole (2.79 g, 41.0 mmol) in DMF (20 mL) as described for the preparation of compound **3a** afforded crude **3b** (5.51 g) that was used without further purification in the next step.

Octyl 2-O-benzoyl-4,6-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside (**4a**) *and octyl 2,3-di-O-benzoyl-4,6-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-α-D-mannopyranoside* (**5**).—(A) Benzoyl chloride (1.33 g, 9.5 mmol) was added at 0 °C to a solution of crude **3a** (3.38 g, 6.3 mmol) in pyridine (50 mL) and the mixture was stirred at room temperature for 24 h. The mixture was diluted with water and extracted with CH_2Cl_2. The combined organic extracts were washed with aq HCl and $NaHCO_3$ and concentrated. Column chromatography (10:1 CCl_4–acetone) of the residue afforded first **5** (1.36 g, 18% with respect to **1a**); $[\alpha]_D$ −53.0° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.72 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.8 Hz, H-3), 5.59 (dd, 1 H, $J_{1,2}$ 1.5 Hz, H-2), 4.99 (d, 1 H, H-1), 4.61 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 4.26 (bd, 1 H, $J_{6a,6b}$ −12.6 Hz, H-6a), 3.98 (bd, 1 H, H-6b), 3.80 (bd, 1 H, H-5), 3.71 and 3.47 (2 dt, each 1 H, OCH_2); ^{13}C NMR ($CDCl_3$): δ 98.2 (C-1), 73.1 and 72.4 (C-3,5), 71.2 (C-2), 68.2 (OCH_2), 65.0 (C-4), 60.9 (C-6). Anal. Calcd for $C_{40}H_{62}O_9Si_2$: C, 64.65; H, 8.41. Found: C, 64.86; H, 8.53.

Eluted next was **4a** (2.90 g, 45% with respect to **1a**); $[\alpha]_D$ −2.8° (*c* 0.7, $CHCl_3$); ^1H

NMR ($CDCl_3$): δ 5.41 (dd, 1 H, $J_{2,3}$ 3.3, $J_{1,2}$ 1.5 Hz, H-2), 4.95 (d, 1 H, H-1), 4.29 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 4.20 (dd, 1 H, $J_{5,6a}$ 1.9, $J_{6a,6b}$ −12.7 Hz, H-6a), 4.16 (dd, 1 H, H-3), 3.92 (dd, 1 H, $J_{5,6b}$ 1.0 Hz, H-6b), 3.60 (bd, 1 H, H-5), 3.67 and 3.43 (2 dt, each 1 H, OCH_2); ^{13}C NMR ($CDCl_3$): δ 98.1 (C-1), 73.1 and 72.9 (C-3,5), 70.7 (C-2), 68.0 (OCH_2), 67.1 (C-4), 60.9 (C-6). Anal. Calcd for $C_{33}H_{58}O_8Si_2$: C, 62.03; H, 9.15. Found: C, 61.95; H, 9.24.

(B) Benzoyl bromide (0.57 g, 3.1 mmol) was added at room temperature to a solution of crude **3a** (1.31 g, 2.1 mmol) and the mixture was stirred at 60 °C for 5 h. Work-up as described under (A) afforded **5** (1.04 g, 68%).

(C) Trimethylsilyl trifluoromethanesulfonate (5 μL) was added at −30 °C to a solution of **6** [17] (421.6 mg, 0.54 mmol) and 1-octanol (651.2 mg, 5.0 mmol) in CH_2Cl_2 (5 mL). The solution was stirred for 0.5 h, neutralised by addition of pyridine and concentrated. Column chromatography (10:1 CCl_4–acetone) of the residue afforded **5** (321.0 mg, 89%).

*Octyl 2-O-benzoyl-4,6-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-1,3-diyl)-1-thio-α-*D*-mannopyranoside* (**4b**).—Treatment of crude **3b** (5.51 g, 10.0 mmol) with benzoyl chloride (1.70 g, 12.0 mmol) in pyridine (20 mL) as described for the preparation of **4a** afforded **4b** (5.66 g, 86% with respect to **2a**); $[\alpha]_D$ +38.9° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.52 (dd, 1 H, $J_{2,3}$ 3.3, $J_{1,2}$ 1.0 Hz, H-2), 4.44 (d, 1 H, H-1), 4.34 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 4.22 (dd, 1 H, $J_{5,6a}$ 1.9, $J_{6a,6b}$ −12.6 Hz, H-6a), 4.16–4.09 (m, 1 H, H-5), 4.00 (bd, 1 H, H-3), 3.90 (bd, 1 H, H-6b), 2.71–2.50 (m, 2 H, SCH_2); ^{13}C NMR ($CDCl_3$): δ 83.4 (C-1), 74.7 (C-3), 73.4 (C-5), 71.2 (C-2), 67.8 (C-4), 61.1 (C-6). Anal. Calcd for $C_{33}H_{58}O_7SSi_2$: C, 60.51; H, 8.92. Found: C, 60.25; H, 9.09.

*Octyl 2-O-benzoyl-4-O-(1-fluoro-1,1,3,3-tetraisopropyl-1,3-disiloxane-3-yl)-α-*D*-mannopyranoside* (**7a**).—HF–pyridine (70%, 1.0 mL) was added at room temperature to a solution of **4a** (2.43 g, 3.8 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred for 10 min. The suspension was washed with aq $NaHCO_3$ and concentrated. Column chromatography (10:1 CCl_4–acetone) of the residue afforded **7a** (2.10 g, 84%); $[\alpha]_D$ +4.8° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.33 (dd, 1 H, $J_{2,3}$ 3.3, $J_{1,2}$ 1.5 Hz, H-2), 4.93 (d, 1 H, H-1), 4.21 (t, 1 H, $J_{3,4} = J_{4,5} = 9.2$ Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 97.5 (C-1), 74.7 (C-3), 73.3 (C-5), 70.5 and 70.0 (C-2,4), 68.2 (OCH_2), 62.1 (C-6). Anal. Calcd for $C_{33}H_{59}FO_8Si_2$: C, 60.15; H, 9.02. Found: C, 60.25; H, 9.12.

*Octyl 2-O-benzoyl-4-O-(1-fluoro-1,1,3,3-tetraisopropyl-1,3-disiloxane-3-yl)-1-thio-α-*D*-mannopyranoside* (**7b**).—Treatment of **4b** (4.73 g, 7.2 mmol) with HF–pyridine (70%, 1.0 mL) in CH_2Cl_2 (30 mL) as described for **7a** afforded **7b** (3.68 g, 76%); $[\alpha]_D$ +54.3° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.45 (dd, 1 H, $J_{2,3}$ 3.3, $J_{1,2}$ 1.2 Hz, H-2), 5.38 (d, 1 H, H-1), 4.27 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 4.07–3.98 (m, 2 H, H-5,6a), 3.91–3.87 (m, 2 H, H-3,6b); ^{13}C NMR ($CDCl_3$): δ 82.7 (C-1), 74.9 (C-3), 73.3 (C-5), 71.2 (C-2), 70.0 (C-4), 61.9 (C-6). Anal. Calcd for $C_{33}H_{59}FO_7SSi_2$: C, 58.71; H, 8.81. Found: C, 58.45; H, 9.21.

*Octyl 2,3-di-O-benzoyl-4-O-(1-fluoro-1,1,3,3-tetraisopropyl-1,3-disiloxane-3-yl)-α-*D*-mannopyranoside* (**8**).—Treatment of **5** (0.89 g, 1.2 mmol) with HF–pyridine (70%, 0.5 mL) in CH_2Cl_2 (10 mL) as described for **7a** afforded **8** (0.64 g, 70%); $[\alpha]_D$ −46.3° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.62–5.56 (m, 2 H, H-2,3), 4.95 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.57 (t, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 97.5 (C-1),

73.2 (2 C) (C-3,5), 70.7 (C-2), 68.4 (OCH_2), 66.4 (C-4), 61.8 (C-6). Anal. Calcd for $C_{40}H_{63}FO_9Si_2$: C, 62.96; H, 8.32. Found: C, 62.84; H, 8.39.

2,3,4,6-Tetra-O-benzoyl-β-D-mannopyranosyl fluoride (**9**).—A mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide [26] (1.32 g, 2.0 mmol) and KHF_2 (1.56 g, 20.0 mmol) in acetonitrile (20 mL) was refluxed for 16 h and concentrated. The residue was suspended in CH_2Cl_2 and washed with aq $NaHCO_3$. Concentration of the solution and column chromatography (5:1 CCl_4–acetone) of the residue afforded **9** (0.97 g, 81%); $[\alpha]_D$ −82.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.22 (t, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.97–5.86 (m, 2 H, H-2,3), 5.82 (dd, 1 H, $J_{1,2}$ 1.8, $J_{1,F}$ 26.7 Hz, H-1), 4.78 (dd, 1 H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ −12.3 Hz, H-6a), 4.61 (dt, 1 H, $J_{5,6b}$ 3.8 Hz, H-5), 4.49 (dd, 1 H, H-6b); ^{13}C NMR ($CDCl_3$): δ 104.9 (d, $J_{C,F}$ 223.9 Hz, C-1), 71.2 and 69.2 (C-3,5), 68.5 (d, $J_{C,F}$ 39.8 Hz, C-2), 65.7 (C-4), 62.1 (C-6). Anal. Calcd for $C_{34}H_{27}FO_9$: C, 68.22; H, 4.55. Found: C, 68.00; H, 4.52.

Octyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1 → 6)-2,3-di-O-benzoyl-α-D-mannopyranoside (**10a**).—(A) A solution of **9** (230.0 mg, 0.31 mmol), **5** (210.0 mg, 0.35 mmol), and BF_3 diethyletherate (63 μL, 0.5 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 1 h. The mixture was diluted with CH_2Cl_2, washed with aq $NaHCO_3$, and concentrated. The residue (0.31 g) was dissolved in THF (10 mL) and treated with $Bu_4NF \times 3H_2O$ (ca. 10 mg) for 0.5 h at room temperature. Concentration of the solvent and column chromatography (5:1 CCl_4–acetone) of the residue afforded **10a** (240.0 mg, 72%); $[\alpha]_D$ −19.7° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.15 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.0$ Hz, H-4'), 6.00 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 5.85 (dd, 1 H, $J_{1,2}$ 1.6 Hz, H-2), 5.68 (dd, 1 H, $J_{1',2'}$ 1.5, $J_{2',3'}$ 3.4 Hz, H-2'), 5.61 (dd, 1 H, H-3'), 5.29 (d, 1 H, H-1), 5.04 (d, 1 H, H-1'), 4.73 (dd, 1 H, $J_{5,6a}$ 2.6, $J_{6a,6b}$ −12.0 Hz, H-6a), 4.61 (dt, 1 H, H-5), 4.50 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-6b), 4.41 (bdd, 1 H, H-5'), 4.29 (dd, 1 H, $J_{5',6a'}$ 4.4, $J_{6a',6b'}$ −11.3 Hz, H-6a'), 4.08–4.00 (m, 1 H, H-6b'), 4.04 (bt, 1 H, $J_{4,5}$ 10.0 Hz, H-4), 3.84 and 3.55 (2 dt, each 1 H, OCH_2); ^{13}C NMR ($CDCl_3$): δ 97.8 (2 C) (C-1,1'), 68.5 (OCH_2), 66.2 (C-6), 63.0 (C-6'). Anal. Calcd for $C_{62}H_{62}O_{17}$: C, 69.01; H, 5.79. Found: C, 68.84; H, 5.82.

(B) A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide [26] (0.51 g, 0.77 mmol) and *sym*-collidine (68 μL, 0.51 mmol) in CH_2Cl_2 (2 mL) was added at −30 °C to a suspension of **8** (0.55 g, 0.72 mmol), silver trifluoromethanesulfonate (AgOTf, 0.39 g, 1.54 mmol), and molecular sieves 3 Å (0.5 g) in CH_2Cl_2 (10 mL). The mixture was stirred for 0.5 h at −30 °C, neutralised by the addition of *sym*-collidine, and filtered. The filtrate was washed with aq $Na_2S_2O_5$ and concentrated. The residue (0.83 g) was dissolved in THF (20 mL) and treated with $Bu_4NF \times 3H_2O$ as described above. Column chromatography (5:1 CCl_4–acetone) afforded **10a** (0.54 g, 81%).

Octyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1 → 6)-2-O-benzoyl-1-thio-α-D-mannopyranoside (**10b**).—Treatment of a suspension of **7b** (1.51 g, 2.24 mmol), AgOTf (1.28 g, 5.0 mmol), and molecular sieves 3 Å (0.5 g) in CH_2Cl_2 (20 mL) with a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (1.65 g, 2.5 mmol) and *sym*-collidine (239 μL, 1.8 mmol) in CH_2Cl_2 (2.5 mL) at 0 °C as described under (B) for the preparation of **10a** afforded **10b** (1.3 g, 58%); $[\alpha]_D$ +6.7° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.15 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.0$ Hz, H-4'), 5.95 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3'), 5.81 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, H-2'), 5.51 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.5 Hz, H-2),

5.44 (d, 1 H, H-1), 5.22 (d, 1 H, H-1′), 4.73 (dd, 1 H, $J_{5',6a'}$ 3.3, $J_{6a',6b'}$ −11.9 Hz, H-6a′); ^{13}C NMR ($CDCl_3$): δ 97.7 (C-1′), 82.7 (C-1), 66.6 (C-6), 62.9 (C-6′). Anal. Calcd for $C_{55}H_{58}O_{15}S$: C, 66.65; H, 5.90. Found: C, 66.38; H, 5.82.

Octyl α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranoside (**11a**).—A solution of **10a** (0.44 g, 0.41 mmol) and a catalytic amount of NaOMe in MeOH (50 mL) was stirred at room temperature for 24 h and the mixture was neutralised as described for compound **2a**. Column chromatography of the residue with water on Bio-Gel P-2 afforded **11a** (192.4 mg, 99%); $[\alpha]_D$ +55.6° (*c* 3.0, H_2O); ^{13}C NMR (D_2O): δ 103.1 (C-1′), 102.6 (C-1), 75.5 (C-5), 74.2 (C-5′), 73.9 and 73.6 (C-3,3′), 73.3 and 72.9 (C-2,2′), 70.6 (OCH_2), 69.5 and 69.1 (C-4,4′), 68.2 (C-6), 63.8 (C-6′). FABMS (positive-ion mode): 455 (MH^+).

Octyl α-D-mannopyranosyl-(1 → 6)-1-thio-α-D-mannopyranoside (**11b**).—A solution of **10b** (0.50 g, 0.5 mmol) and a catalytic amount of NaOMe in MeOH (10 mL) was stirred at room temperature for 24 h and the mixture was neutralised as described for compound **2a**. Column chromatography of the residue with water on Bio-Gel P-2 afforded **11b** (202.0 mg, 86%); $[\alpha]_D$ +78.8° (*c* 0.8, H_2O); ^{13}C NMR (D_2O): δ 100.0 (C-1′), 85.7 (C-1), 73.0 (C-5), 72.4 and 72.3 (C-3,3′), 71.8 (C-5′), 71.1 and 70.4 (C-2,2′), 66.9 (2 C) (C-4,4′), 65.7 (C-6), 61.2 (C-6′). FABMS (positive-ion mode): 471 (MH^+).

(2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl)-(1 → 2)-1,3,4,6-tetra-O-acetyl-β-D-mannopyranose (**13**).—Treatment of a suspension of **12** [27] (0.69 g, 1.98 mmol), AgOTf (1.03 g, 4.0 mmol), and molecular sieves 3 Å (0.5 g) in CH_2Cl_2 (20 mL) with a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (1.32 g, 2.0 mmol) and *sym*-collidine (200 μL, 1.5 mmol) in CH_2Cl_2 (2 mL) at −10 °C as described under (B) for the preparation of **10a**, afforded **13** (1.58 g, 86%); $[\alpha]_D$ −47.5° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.27 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.2$ Hz, H-4′), 6.05 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-3′), 5.89 (bs, 1 H, H-1), 5.77 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, H-2′), 5.49 (t, 1 H, $J_{3,4} = J_{4,5} = 9,7$ Hz, H-4), 5.31 (d, 1 H, H-1′), 5.22 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-3), 4.87 (dt, 1 H, $J_{5',6a'}$ 2.8, $J_{5',6b'}$ 2.4 Hz, H-5′), 4.71 (dd, 1 H, $J_{6a',6b'}$ −12.2 Hz, H-6a′), 4.48–4.31 (m, 3 H, H-2,6a,6b), 4.25 (dd, 1 H, H-6b′), 3.89 (dq, 1 H, H-5); ^{13}C NMR ($CDCl_3$): δ 98.6 (C-1′), 91.1 (C-1), 74.9, 73.3, 72.2, 70.8, 69.3 (2 C), 66.7 and 65.9 (C-2,3, 4,5,2′,3′,4′,5′), 62.5 and 61.9 (C-6,6′). Anal. Calcd for $C_{48}H_{46}O_{19}$: C, 62.20; H, 5.00. Found: C, 61.97; H, 5.21.

(2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**14**).—Hydrazine acetate (0.16 g, 1.78 mmol) was added at 0 °C to a solution of **13** (1.09 g, 1.18 mmol) and the mixture was stirred at room temperature for 15 min. The mixture was diluted with water and extracted with CH_2Cl_2. The combined organic extracts were washed with aq HCl and $NaHCO_3$, and concentrated. A solution of the residue (0.86 g) in CH_2Cl_2 (10 mL) was stirred at room temperature with K_2CO_3 (1.5 g) and trichloroacetonitrile (1.5 mL) for 7 h. Filtration of the mixture, concentration of the filtrate and column chromatography (5:1 CCl_4–acetone) of the residue afforded **14** (0.87 g, 72%); ^{1}H NMR ($CDCl_3$): δ 6.56 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 6.20 (t, 1 H, $J_{3',4'} = J_{4',5'} = 10.1$ Hz, H-4′), 6.02 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.79 (dd, 1 H, H-2′), 5.63 (t, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.45 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-3), 5.36 (d, 1 H, H-1′), 4.78 (dd, 1 H, $J_{5',6a'}$ 1.9, $J_{6a',6b'}$ −12.2 Hz, H-6a′), 4.69–4.63

(m, 1 H, H-5), 4.52 (dd, 1 H, $J_{5',6b'}$ 4.4 Hz, H-6b'), 4.47 (dd, 1 H, H-2), 4.35 (dd, 1 H, $J_{5,6a}$ 4.7, $J_{6a,6b}$ −12.8 Hz, H-6a), 4.27–4.22 (m, 2 H, H-5',6b); ^{13}C NMR ($CDCl_3$): δ 99.2 (C-1'), 95.7 (C-1), 74.5 (C-2), 71.4 (C-2'), 70.5, 70.0, 69.9, and 69.3 (C-3,5,3',5'), 66.6 (C-4'), 65.6 (C-4), 62.6 (C-6'), 61.8 (C-6). Anal. Calcd for $C_{48}H_{44}Cl_3NO_{18}$: C, 56.02; H, 4.31; Cl, 10.33; N, 1.36. Found: C, 59.98; H, 4.21; Cl, 9.92; N, 1.32.

*Octyl (2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 6)-2-*O*-benzoyl-4-*O*-(1-fluoro-1,1,3,3-tetraisopropyl-1,3-disiloxane-3-yl)-α-*D*-mannopyranoside* (**15a**).—A catalytic amount of Me_3SiOTf (10 μL) was added at −30 °C to a solution of **7a** (320 mg, 0.49 mmol) in CH_2Cl_2 (5 mL), followed by a solution of **14** (515 mg, 0.5 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for 15 min, neutralised by addition of pyridine, and concentrated. Column chromatography (5:1 CCl_4–acetone) of the residue afforded **15a** (605 mg, 81%); $[\alpha]_D$ −8.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.16 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 10.1 Hz, H-4''), 5.92 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-3''), 5.64 (dd, 1 H, $J_{1'',2''}$ 1.2 Hz, H-2''), 5.47 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.9 Hz, H-4'), 5.34 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-3'), 5.29 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.2 Hz, H-2), 5.26 (d, 1 H, H-1''), 5.01 (d, 1 H, H-1), 4.72 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'); ^{13}C NMR ($CDCl_3$): δ 99.6 (C-1''), 98.7 (C-1), 97.1 (C-1'), 77.5 (C-2), 73.3 and 72.5 (C-2',2''), 70.5 (2 C), 70.2, 69.9, 69.4, 69.2, and 68.6 (C-3,4,5,3',5',3'',5''), 68.1 (OCH_2), 66.7 and 66.2 (C-4',4''), 65.6 (C-6), 62.3 (2 C) (C-6',6''). Anal. Calcd for $C_{79}H_{101}FO_{25}Si_2$: C, 62.19; H, 6.67. Found: C, 62.02; H, 6.38.

*Octyl (2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 6)-2-*O*-benzoyl-4-*O*-(1-fluoro-1,1,3,3-tetraisopropyl-1,3-disiloxane-3-yl)-1-thio-α-*D*-mannopyranoside* (**15b**).—Treatment of **7b** (533.6 mg, 0.79 mmol) and **14** (1.13 g, 1.1 mmol) in CH_2Cl_2 (10 mL) with Me_3SiOTf (15 μL) as described for the preparation of **15a** afforded **15b** (920 mg, 75%); $[\alpha]_D$ +11.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.15 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 10.0 Hz, H-4''), 5.90 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-3''), 5.63 (dd, 1 H, $J_{1'',2''}$ 1.8 Hz, H-2''), 5.49 (d, 1 H, H-1''), 5.47 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.9 Hz, H-4'), 5.41 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.3 Hz, H-2), 5.31 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3'), 5.20 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1'), 4.73 (d, 1 H, H-1); ^{13}C NMR ($CDCl_3$): δ 99.6 (C-1''), 98.7 (C-1'), 82.3 (C-1), 77.4 (C-2'), 74.8 and 73.0 (C-2,2''), 71.2, 70.5, 70.3 (2 C), 69.4 (2 C), and 68.6 (C-3,4,5,3',5',3'',5''), 66.7 and 66.1 (C-4',4''), 65.8 (C-6), 62.2 (2 C) (C-6',6''). Anal. Calcd for $C_{79}H_{101}FO_{24}SSi_2$: C, 61.53; H, 6.60. Found: C, 61.47; H, 6.65.

*Octyl (2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 6)-2-*O*-benzoyl-1-thio-α-*D*-mannopyranoside* (**15c**).—A catalytic amount of $Bu_4NF \times 3H_2O$ (ca. 15 mg) was added to a solution of **15b** (880 mg, 0.57 mmol) in THF (22 mL) and stirred at room temperature for 20 min. Concentration of the solution and column chromatography (5:1 CCl_4–acetone) of the residue afforded **15c** (540 mg, 73%); $[\alpha]_D$ +1.5° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.12 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 10.0 Hz, H-4''), 5.96 (dd, 1 H, $J_{2'',3''}$ 3.2 Hz, H-3''), 5.69 (dd, 1 H, $J_{1'',2''}$ 1.9 Hz, H-2''), 5.43 (d, 1 H, H-1''), 5.44 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.7 Hz, H-4'), 5.43 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.9 Hz, H-2), 5.42 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-3'), 5.18 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.99 (d, 1 H, H-1); ^{13}C NMR ($CDCl_3$): δ 99.4 (C-1''), 98.2 (C-1'), 82.8 (C-1), 77.5 (C-2), 71.7 and 71.3 (C-2',2''), 70.6, 70.2, 69.4 (2 C), 69.3, 68.6, and 68.0 (C-3,4,5,3',5',3'',5''), 67.0 and 66.5 (C-4',4''), 65.7 (C-6), 62.6 and 62.4 (C-6',6''). Anal. Calcd for $C_{67}H_{74}O_{23}S$: C, 62.90; H, 5.83. Found: C, 62.66; H, 5.80.

Octyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranoside (**16a**).—A catalytic amount of $Bu_4NF \times 3H_2O$ (ca. 10 mg) was added at room temperature to a solution of **15a** (490 mg, 0.32 mmol) in THF (10 mL) and the mixture was stirred for 1 h. The solution was diluted with CH_2Cl_2, washed with aq $NaHCO_3$, and concentrated. The residue was dissolved in MeOH (20 mL) containing a catalytic amount of NaOMe and stirred for 24 h at room temperature. Neutralisation of the solution with ion-exchange resin (Dowex 1X8, H^+), concentration, and column chromatography of the residue with water on Bio-Gel P-2 afforded **16a** (151.3 mg, 77%); $[\alpha]_D$ +47.7° (*c* 0.3, H_2O); ^{13}C NMR (D_2O): δ 102.7 (C-1″), 100.5 (C-1), 98.6 (C-1′), 79.1 (C-2′), 73.5 and 73.0 (C-2,2″), 71.6 (2 C), 70.7 (3 C), and 70.3 (C-3,5,3′,5′,3″,5″), 68.1 (OCH_2), 67.1 (2 C) (C-4,4′), 66.8 (C-6), 65.9 (C-4″), 61.5 (2 C) (C-6′,6″). FABMS (positive-ion mode): 617 (MH^+).

Octyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-1-thio-α-D-mannopyranoside (**16b**).—Treatment of **15c** (218 mg, 0.17 mmol) with a catalytic amount of NaOMe in MeOH (20 mL) as described for the preparation of **11b** afforded **16b** (101.8 mg, 95%); $[\alpha]_D$ +100.9° (*c* 0.2, H_2O); ^{13}C NMR (D_2O): δ 102.6 (C-1″), 98.5 (C-1′), 85.6 (C-1), 79.1 (C-2′), 73.5 and 73.0 (C-2,2″), 72.4, 72.1, 71.8, 70.7 (2 C), and 70.3 (C-3,5,3′,5′,3″,5″), 67.0 (3 C) (C-4,4′,4″), 65.9 (C-6), 61.4 and 61.2 (C-6′,6″). FABMS (positive-ion mode): 633 (MH^+).

Preparation of trypanosome membrane fractions.—Bloodstream forms of *Trypanosoma brucei* strain 427 variant MITat 1.4, MITat 1.2, and MITat 1.5 were isolated from infected rats as described previously [10]. Trypanosomes (5×10^9 cells/mL) were lysed for 5 min at 37 °C in 10 mM PIPES buffer (pH 6.5), containing 1 mM dithiothreitol and protease inhibitors (chymostatin, leupeptin, and pepstatin; 1 μM each). The lysate was centrifuged (14,000 *g*; 10 min, 4 °C) and the pellet (membrane fraction) finally resuspended in 50 mM PIPES buffer (pH 6.5), containing 15 mM $MnCl_2$, 1 mM dithiothreitol, 1 mM ATP, and protease inhibitors as before.

Galactosyltransferase assay.—Galactosyltransferase activity was assayed in a total volume of 50 μL 50 mM PIPES buffer (see above), containing up to 40 μL membrane fraction, 0.025% Triton X-100, 50 nCi UDP-[U-^{14}C]galactose (1.8 μM), and a sugar acceptor in a range between 0–10 mM. The mixtures were incubated for 90 min at 35 °C. The reaction was stopped by adding 50 μL of icecold acetone and the denatured protein was removed by centrifugation (14,000 *g*; 5 min, 4 °C). The supernatants (100 μL) were diluted with 1 mL HOAc (100 mM) and loaded onto 100 mg ISOLUTE C18 (endcapped) solid phase extraction columns (ICT, Bad Homburg) equilibrated with 3 mL MeOH and 5 mL 100 mM HOAc. Following 5 washes with 1 mL 100 mM HOAc each, hydrophobic sugars were eluted with 3 × 250 μL MeOH. Aliquots of the combined eluates (650 μL) were dried using a speed vac evaporator and used for glycosidase digestions or directly applied to aluminium backed 20 cm × 20 cm Silica Gel 60 HPTLC plates (E. Merck, Darmstadt). The remaining eluate (100 μL) was assayed for radioactivity, using Ultimagold liquid scintillation cocktail (Packard, Frankfurt).

Exoglycosidase digestion.—Galactosylated products were dried and digested as described earlier [11]. The digested samples were prepared for HPTLC analysis as described above.

HPTLC.—Dried samples were dissolved in 4 μL 1:1 MeOH–water, applied to HPTLC plates, and treated as described earlier [11]. Standard sugar derivatives, 5 μg each of **11a** and **16a**, were always run along with the samples.

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