A Ready, Convergent Synthesis of the Heptasaccharide GPI Membrane Anchor of Rat Brain Thy-1 Glycoprotein[1]

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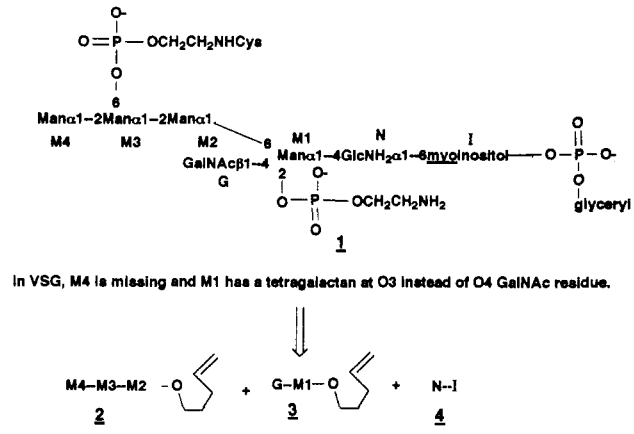
That certain proteins are covalently attached to cell surfaces *via* glycosylphosphatidylinositol (GPI) anchors was first established eight years ago by Ferguson and co-workers for the variant surface glycoprotein (VSG) of the parasitic protozoan *Trypanosoma brucei*.[3] Since then over 100 GPI-anchored proteins have been identified in eukaryots[4] with diverse functions, including adhesion molecules, hydrolases, receptors, and transmembrane signal inducers.[4,5] Common structural features in the complex oligosaccharide anchoring domain, indicative of a high degree of evolutionary conservation, would seem to suggest functional interrelationships.[6] However, Ferguson has cautioned that " 'x does y rules' may be hard to realize".[4] Furthermore, a structure/activity debate may be premature since complete structural details[7] are available for only two GPI anchors, VSG[3] and the rat brain Thy-1 glycoprotein,[8] the latter being of special interest since it was the first to be isolated from a mammalian source. In this communication we describe the first synthesis of the complete glycan moiety of Thy-1, by a route which is convergent, provides the material in multimilligram amounts, and makes provisions for attaching the protein components.

Although the structures of the Thy-1 (**1**) and VSG anchors (Scheme I) may seem similar biologically, their laboratory syntheses[9,10] require vastly different strategies. We envisaged a triply convergent approach based on subunits **2** and **3** in the form of *n*-pentenyl glycosides (NPGs)[11] and the pseudodisaccharide **4**.

The retrosynthetic plan in Scheme I gives subunits **2**, **3**, and **4**. The trisaccharide **2** presented an opportunity to explore the advantages of the pentenyl activating group for reducing the numbers of "starting materials" required for oligosaccharide syntheses. As described in a recent report from this laboratory, the ability to readily protect and deprotect pentenyl double bonds enabled the preparation of a nonamannan from only two starting materials.[12] In this communication we focus attention on the divergent pathways followed by pentenyl ortho esters upon

Scheme I



treatment with different electrophiles (Scheme II). For example, reaction of **5a**[11] with a protic acid induces transfer of the pentenyloxy group to the anomeric center of **10a**.[13] However, when titrated with bromine, pentenyl ortho esters yield glycosyl bromides such as **9**.[11] These reaction pathways can be rationalized by invoking the protonated intermediate **7** and the furanylium ion **6**, both of which proceed to the dioxolenium ion **8**, which then reacts with the best nucleophile available to give **9** or **10**. Coupling of the former is best carried out *in situ* after discharging excess bromine with a stream of argon. In this way, **9b** and **10b** furnished disaccharide **11a** in 89% yield and, in turn, acceptor **11b** reacted with **9a** to give trisaccharide **12a**. The protecting groups of the latter were then changed to those in **12b** to provide for future phosphorylations.

With respect to the disaccharide segment **3** (Scheme I), the galactosyl donor is disarmed by virtue of the phthalimide residue, and hence the armed/disarmed strategy employing two NPGs[11] would be problematic. Hence a different glycosyl donor, the trichloroacetimidate,[14] was chosen since it had been tested in a comparable coupling.[15] Accordingly, compound **13**[16] was coupled with **14**, obtained from *n*-pentenyl mannopyranoside[11] by standard transformations,[17] to give disaccharide **15a** (Scheme III). Cleavage of the phthalimide[18] and selective acylation then led to **15b**.

Synthetic routes to variously protected forms of the NI pseudodisaccharide **4** (Scheme I) have emerged from this[9] and other[10,19] laboratories, and so there were several options that could be retooled to fit our present needs. A critical factor in our choice was the desire to protect the future site of inositol phosphorylation with a functionality that would be refractory to the conditions of NPG activation[11] (*vide infra*). An allyl group fit this need, and hence the known[20] derivative **17** was chosen for coupling

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(2) (a) Present address for U.E.U.: Eli Lilly Corporation, Indianapolis, IN; (b) R.M. thanks the Danish Technical Research Council for a P.D. Fellowship; (c) C.R. is the recipient of an NIH Award for Underrepresented Minorities.
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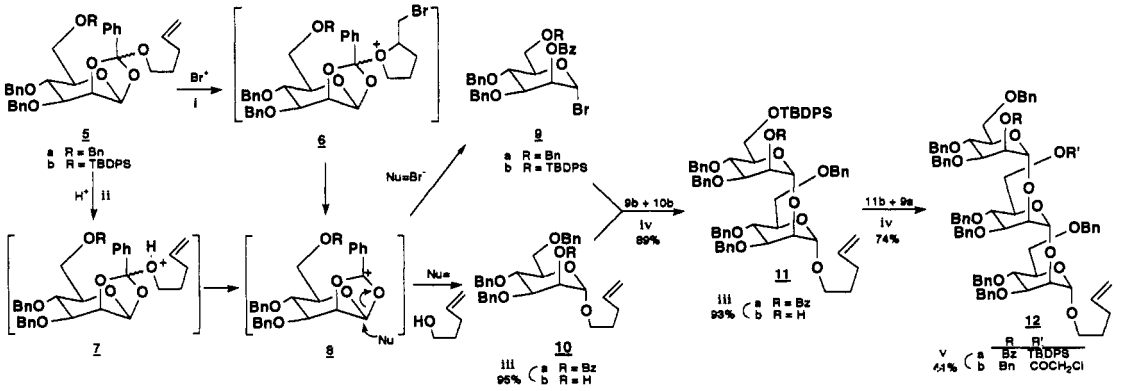
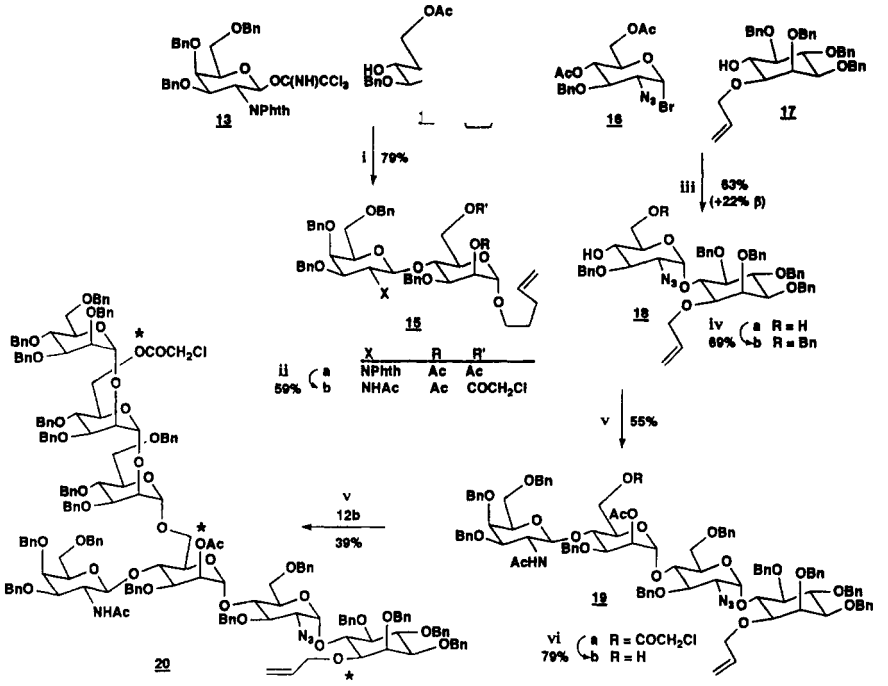
Scheme II[a]



[a] (i) Br_2, 0 °C; (ii) TsOH, CH_2Cl_2, 60 °C; (iii) NaOMe; (iv) AgOTf, 4 Å, CH_2Cl_2, −30 °C, 30 min; (v) NaOMe, then BnBr, then Bu_4NF/THF, then $(ClCH_2CO)_2O$.

Scheme III[a]



[a] (i) TMSOTf, 4 Å, toluene, −20 °C, 2 h; (ii) $MeNH_2$, EtOH, 70 °C, 35 h, then Ac_2O, then $(ClCH_2CO)_2O$ (1.1 equiv), Et_3N, then Ac_2O; (iii) $AgClO_4$, Et_2O, −35 °C → rt, 30 min, then NaOMe; (iv) Ac_2O (1.2 equiv), then DHP, PPTS, CH_2Cl_2, 40 °C, 15 h, then NaOMe, then BnBr, then PPTS/MeOH, CH_2Cl_2, 60 °C, 2 h; (v) NIS, Et_3SiOTf, CH_2Cl_2, rt, 15 min; (vi) thiourea. * represents sites for future couplings.

with the glycosyl bromide **16**,[21] the product **18a** being obtained in 63% yield.

Assembly of the heptasaccharide was now undertaken. Coupling of acceptor **18b** and donor **15b** in 1.2:1 ratio[22] was complete within 15 min to give tetrasaccharide **19a** in 55% yield based on **15b**, substantial amounts of acceptor **18b** also being recovered.[23] Dechloroacetylation then readied **19b** for coupling to trisaccharide**12b**. For this task, compounds **12b** (250 mg) and **19b** (200 mg) were used in 1.5:1 ratio, affording 115 mg of heptasaccharide **20** (39% yield based on recovered acceptor **19b**).

The sites for future couplings in **20** have been provided with temporary protecting groups that can be removed selectively. These tasks are now being carried out as a prelude to biological testing.

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Supplementary Material Available: Listings of experimental procedures for the preparation of all key compounds and their NMR data (16 pages). Ordering information is given on any current masthead page.

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(22) The unusual use of the acceptor **18b** in excess was due to the observation that the material was highly prone to silylation by the NIS/Et_3SiOTf promoter.

(23) The recovered silylated glycosyl acceptor was treated with tetra-*n*-butylammonium fluoride in THF to regenerate the corresponding alcohol.