First Synthesis of a Fully Phosphorylated GPI Membrane Anchor: Rat Brain Thy-1†

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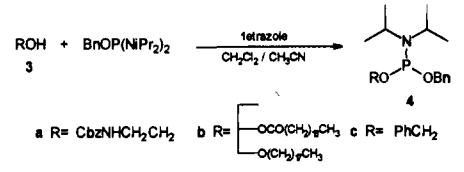
Received February 24, 1995

Glycophosphatidylinositol (GPI) membrane anchors constitute a class of glycolipids that covalently link certain proteins to cell and virion surfaces.[2,3] A boost in their chemistry occurred in 1988 when Ferguson et al. reported the first covalent structure of a member of the family, the variant surface glycoprotein of *Trypanosoma brucei*.[4] This landmark achievement has become a prototype for other membrane anchors, and as a result of these investigations the general structural pattern depicted in Figure 1 has begun to emerge.[5] Thus the highlighted pentasaccharide core is conserved throughout the evolutionary scale from bacteria to mammals. The latter is exemplified in the rat brain Thy-1 glycoprotein anchor **2** (Scheme 1) with two ethanolamines, one free while the other links the anchor to cysteine, the carboxy terminal residue of the protein.[6]

The biological function of these glycoprotein anchors is in need of clarification, and in this context, laboratory syntheses will be of great help in providing anchors, in whole or in part, or analogues thereof. In this manuscript we report the first total synthesis of a membrane anchor with particular emphasis on crucial end game strategies for installing the essential phosphodiester residues. We focus on two variations, the lipidated structure **2b** and its nonlipidated analog **2c** (Scheme 1).

The phosphitylation/oxidation protocol for phosphorylation was adopted because it had served well in previous work in our laboratory.[7] The required phosphoramidite reagents, **4a**−**c**, were synthesized from (benzyloxy)bis(diisopropylamino)-phosphine and the corresponding alcohols **3a**−**c**, respectively.[7a]



We began with the heptasaccharide **5a** (Scheme 1), whose assembly by *n*-pentenyl glycoside strategy was reported recently,[8] the locations for future phosphorylations, A, B, and C, being differentially protected. Site A would be manipulated first in order to take advantage of chemospecific removal of the chloroacetyl residue.[9] The acetate and allyl groups at B and C are orthogonal and, hence, could be removed in any order; however, for target **2b**, prior cleavage of the acetate was indicated, since once the phospholipid moiety was attached at site C, deacetylation at site B in the presence of the glyceryl ester would be precluded.

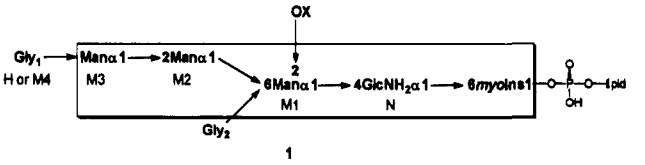


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Figure 1. Generalized structure of glycosylphosphatidylinositol anchor.

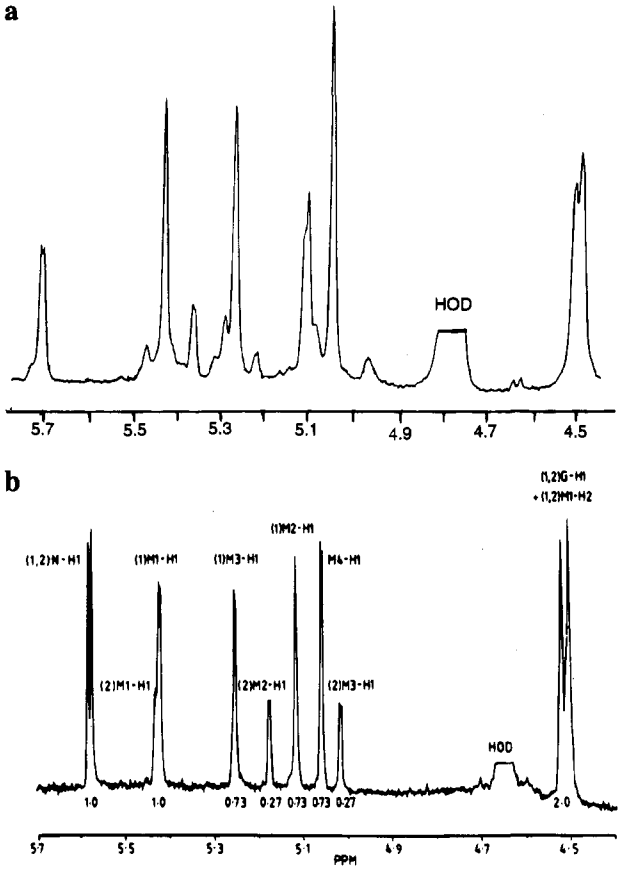

Figure 2. Partial ^{1}H NMR spectra of (a) synthetic **2c** and (b) isolated Thy-1 GPI glycan, presumably **2d**.[7] Spectrum b reveals the presence of a mixture of two compounds as discussed by Homans *et al.*[7] It is postulated that the second, minor compound is different from **2d** in that one mannose residue (M4) is missing. It is unclear whether it arises from nature or from the conditions used for purification or characterization.

Each stage of the transformations at site A (Scheme 1), i.e., dechloroacetylation (**5a** → **5b**), phosphitylation, and oxidation, could be monitored by TLC,[10] affording **5c** as 3:2 mixture of phosphate diastereomers. The latter was resolved on analytical HPLC, but no attempt was made to separate the isomers preparatively.

Deacetylation at site B, **5c** → **5d**, was sluggish, suggestive of severe steric hindrance. However, the reaction of **5d** with phosphoramidite **4a** proceeded smoothly, leading to bis(phosphodiester) **6a** in 79% yield after oxidation. Four phosphate diastereomers were evident (^{31}P NMR), the eight expected signals appearing as six distinct and two overlapping (data not shown).

† Dedicated to Professor R. U. Lemieux on the occasion of his 75th birthday.

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(9) Thiourea method: Naruto, N.; Ohno, K.; Naruse, N.; Takeuchi, H. *Tetrahedron Lett.* **1979**, 251. Hydrazine dithiocarbonate method: van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *24*, 3775.

(10) The intermediate phosphite in each one-pot phosphitylation/oxidation reaction migrated considerably higher than the starting alcohol (70% hexane: 30% EtOAc:1% Et_3N); however, upon *in situ* oxidation the resulting phosphate migrated lower than the starting alcohol, thus facilitating monitoring of the progress of the reaction.

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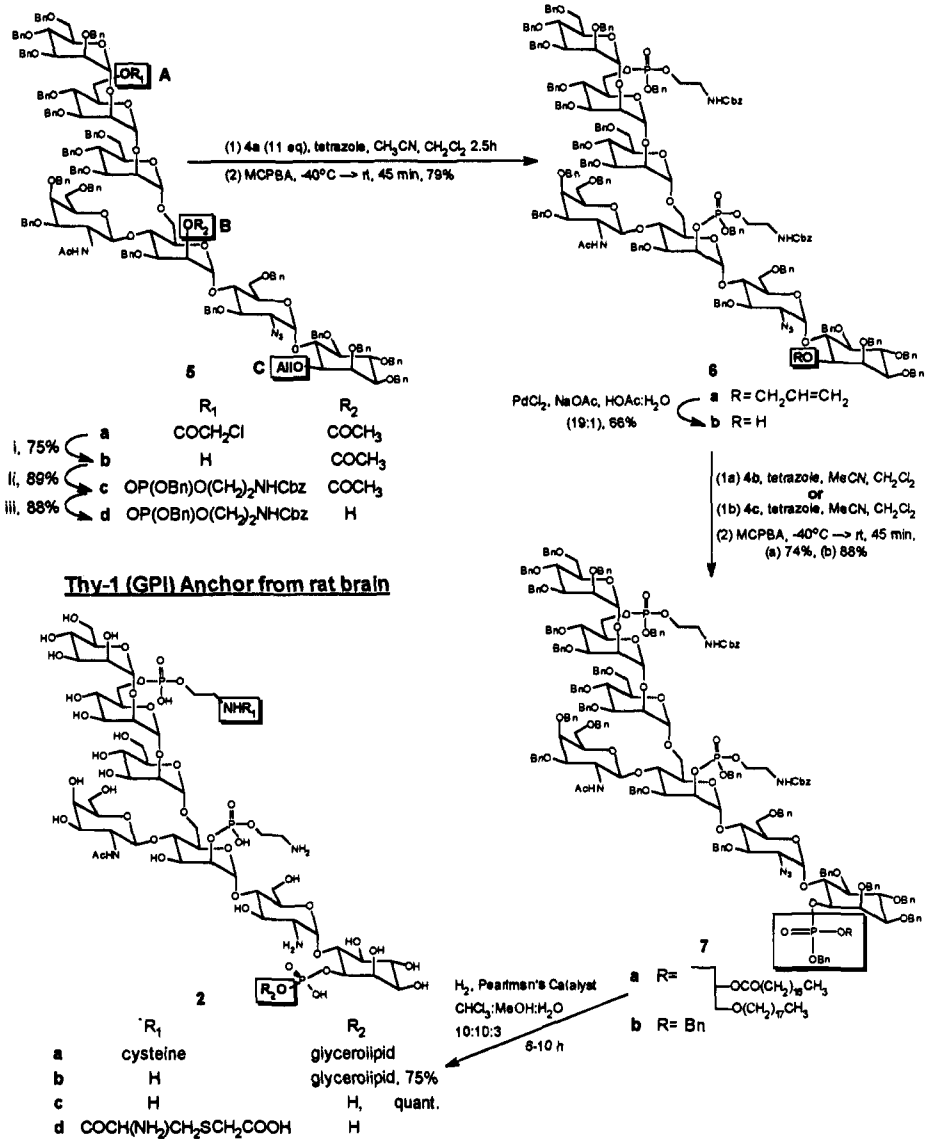
Scheme 1[a]



[a] (i) Thiourea, $NaHCO_3$, MeOH, CH_3Cl, 60 °C, 5.5 h; (ii) (a) **4a** (4 equiv), tetrazole, CH_3CN, CH_2Cl_2, 3 h; (b) MCPBA −40 °C → room temperature, 45 min; (iii) NaOMe, MeOH, THF, room temperature, 12 h.

Deallylation at site C of **6a** proceeded smoothly by use of $PdCl_2$ in acetate buffer to give alcohol **6b** in the encouraging yield of 66%. A portion of this material was phospholipidated with reagent **4b**. Product **7a** (whose eight isomers were not all resolvable by HPLC) was hydrogenolyzed over $Pd(OH)_2$[11] for 10 h, affording material that gave positive tests for sugar[12] and free amino[13] groups. Purification was effected by hydrophobic chromatography on octyl-Sepharose as described by Ferguson *et al.* for a similar GPI anchor,[14] followed by gel filtration on Sephadex G-25[15] to give **2b**, the first of the desired targets.

Another portion of alcohol **6b** was phosphorylated *via* **4c** to give the fully protected Thy-1 GPI glycan **7b** in excellent yield. Consistent with the last operation, the number of stereoisomers did not increase, since the newly introduced phosphodiester is prochiral.

Hydrogenolysis of **7b** for 6 h gave glycan **2c**, which was recovered in nearly quantitative yield after gel filtration chromatography on Sephadex G-25. Establishing the product **2c** as the rat brain Thy-1 glycan is not easily done, because the structure characterized by Ferguson and co-workers was presumably **2d**,[16] differing from our **2c** by the presence of the carboxymethyl cysteine substituent at one of the ethanolamines. The anomeric regions of the ^1H NMR spectra of **2d** and **2c** overlap very well (Figure 2), thus supporting the initial structural assignment. It is noteworthy that no ^{31}P NMR data has been published for the natural material, which complicates further the comparison of **2c** and **2d**.

The methodology reported above should be applicable for the synthesis of analogs, which will facilitate investigations of the biological roles of GPI anchors. Efforts in this direction are underway and will be reported in due course.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM 41071).

Supporting Information Available: Experimental details of the preparation of and key MR data for compounds **5b−d**, **6a,b**, **7a,b**, and **2b,c** (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(16) It was not possible to definitively assign which of the two ethanolamine residues was cysteinylated.[6] The assignment shown in **2a** is based on analogy with other GPI anchors.[5]