Synthesis of a Novel Acceptor Substrate for a Mannosyl Transferase

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Two novel analogues of the mannosyl transferase acceptor substrate $(GlcNAc)_2$-pyrophosphate-dolichyl **2** have been prepared in which dolichyl is replaced by phytanyl **4** and lauryl **5**; both **4** and **5** were synthesised using readily available chitin as the disaccharide precursor.

The oligosaccharide side chains of glycoproteins are well known to control both the biodistribution and the biological properties of the parent protein or peptide.[1] Common to most glycoproteins containing asparagine linked oligosaccharides is the pentasaccharide core structure of **1**.

In view of the inherent problems associated with the chemical synthesis[2] of the pentasaccharide core, we have initiated a programme whose aim is to prepare it by a chemoenzymatic approach. The use of glycosyl transferases for the synthesis of oligosaccharides is currently being explored since it offers an efficient method for controlling both the regio- and the stereo-selectivity of glycoside bond formation.[3] However, the mannosyl transferases involved in the assembly of the core of **1** have not been used to any significant extent owing to the complexity of the substrates involved (*vide infra*).

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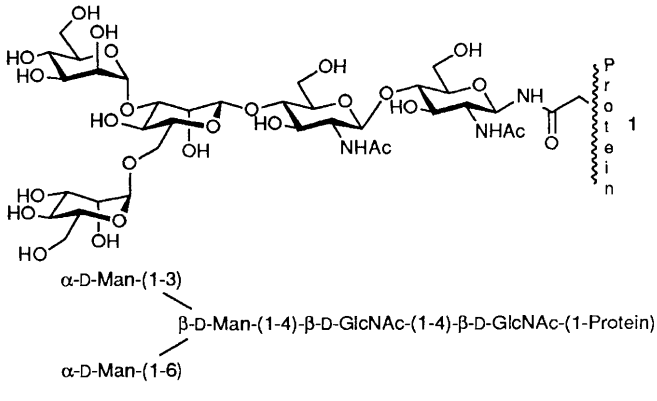
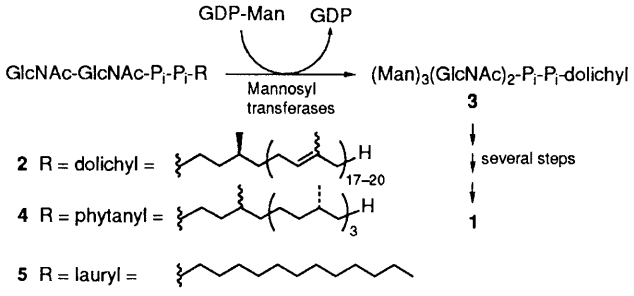
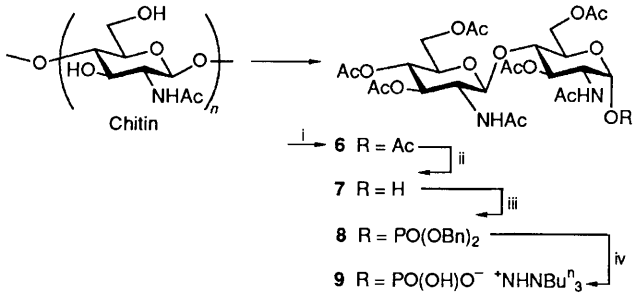
The biosynthesis of the pentasaccharide starts at the reducing end by sequential attachment of two *N*-acetylglucosamine residues to the polyisoprenoid dolichol through a pyrophosphate linkage **2** (Scheme 1). Subsequent mannosyl

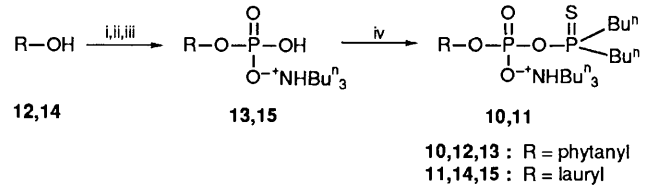




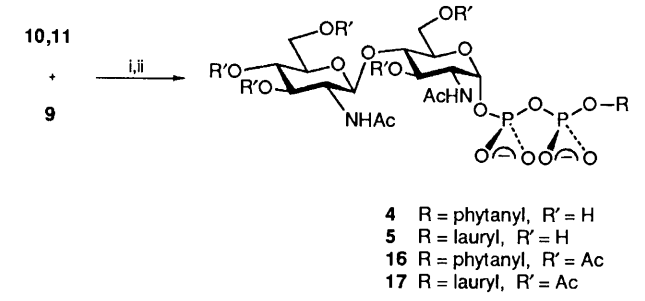
Scheme 1 $-P_i-P_i-$ = pyrophosphate



Scheme 2 *Reagents and conditions*: i, Ac_2O, H_2SO_4; ii, $NH_2NH_2{\cdot}HOAc$ (82%); iii, Bu^nLi (1.1 equiv.), $(PhCH_2O)_2POCl$ (56%); iv, H_2/Pd, NBu^n_3 (1 equiv.) (≥ 95%)



Scheme 3 *Reagents and conditions*: i, $Et_2NP(OBu^t)_2$, 1*H*-tetrazole; ii, *m*-$ClC_6H_4CO_3H$; iii, CF_3CO_2H; NBu^n_3 (1 equiv.); iv, $BrPS(Bu^n)_2$



Scheme 4 *Reagents and conditions*: i, AgOAc, H_2S; ii, NaOMe, MeOH, NH_4Cl

transfer is catalysed by various mannosyl transferases that use GDP-mannose as the donor substrate to give the pentasaccharide **3**. After further modification, transfer of the oligosaccharide from the lipid anchor to the protein occurs resulting in the asparagine linked structure **1**.[4]

A major drawback to employing the enzymatic approach has been the need for dolichyl-linked substrates. Dolichol must be isolated from pig liver where it is present only in low abundance (5 kg of liver yields 100–200 mg of dolichol as a mixture of homologues).[5] Since a possible function of dolichol is to act as a lipophilic membrane anchor, we reasoned that its properties might be effectively mimicked by the more readily available isoprenoid phytanol,[6] or even the simple straight chain lauryl alcohol. In this communication we report an efficient and novel chemical synthesis of the substrates **4** and **5** required to test this theory, and in the following paper[7] we describe the results of assessing these two compounds as acceptor substrates for mannosyl transfer using a crude enzyme preparation.

The dolichyl substrate **2** was previously prepared during the pioneering studies of Jeanloz and Warren.[8] Initially we used their protocol to prepare **4** and **5** but encountered several problems associated with scale-up and reproducibility and therefore devised an alternative route. Chitobiose octaacetate **6** was obtained by controlled acetolysis of chitin[9] and selectively 1-*O*-deacetylated with hydrazine acetate to give **7** (82%) using the same conditions described for monosaccharides.[10] Treatment of **7** with n-butyllithium (1.1 equiv., dry tetrahydrofuran) followed by dibenzyl phosphochloridate[11] (2.5 equiv.) gave the required glycosyl phosphate[12] **8** exclusively as the α-anomer (56%). Catalytic hydrogenolysis of the benzyl protecting groups followed by treatment with tri-*n*-butylamine (1 equiv.) gave the desired phosphate **9** in quantitative yield (Scheme 2).

The corresponding activated phytanyl **10** and lauryl **11** phosphates required for coupling to **9** were prepared as outlined in Scheme 3. Phytol (Sigma, 97% pure) was catalytically hydrogenated with Pt/C to give phytanol **12** in 48% isolated yield as a mixture of epimers at C-3.[6] Conversion of **12** to phytanyl phosphate **13** was achieved in 80% yield using the recently published phosphorylation procedure.[13] Treatment of the phosphate **13** with di-*n*-butylthiophosphoryl bromide (1.1 equiv., tetrahydrafuran, room temperature) yielded the required thiophosphoryl anhydride **10**.[14] A similar sequence of reactions furnished the corresponding lauryl compound **11** from lauryl alcohol **14**. Both **10** and **11** were used directly for the next step without further purification.

Thus individual coupling of **10** and **11** with peracetylated chitobiose phosphate **9** (AgOAc then H_2S)[14] gave, after purification on silica ($CHCl_3$–MeOH, 9:1), the required protected diphosphates **16** (26%) and **17** (40%) respectively. Deprotection of **16** and **17** (7% NaOMe–MeOH in CH_2Cl_2) gave **4** and **5** respectively. Both the phytanyl **4** and lauryl **5** substrates gave spectroscopic data in full accord with their structures.‡

In conclusion we believe that the versatile synthetic route described above provides an efficient method for the preparation of this class of compounds. It is worth noting that related lipid-linked oligosaccharides are known to inhibit glycosyl transferases in bacterial cell wall biosynthesis and hence have potential antibacterial activity.[15] In the following paper we describe the results of testing **4** and **5** as substrates for mannosyl transferases.

‡ *Selected spectroscopic data:* **4**, ^1H NMR (500 MHz, $CDCl_3$–CD_3OD –D_2O, 10:10:3), δ 0.81–1.66 (39H, complex overlapping m, phytanyl), 2.01, 2.02 (2 × 3H, 2 × s, Ac), 3.46–4.00 (14H, complex m, 2 × C-1-H of phytanyl and 12H of C-2′,3′,4′,5′,6′,8′,9′,10′,11′ and 12′ of disaccharide), 4.51 (1H, d, *J* 8.4 Hz, C-7′) and 5.5 (1H, dd, $J_{H\text{-}H}$ 3.2, $J_{H\text{-}P}$ 7.1 Hz, C-1′); ^{31}P NMR [referenced to $(MeO)_3PO$ in H_2O] (101.256 MHz) −13.12 (d, J_{PP} 15 Hz) and −15.74 (d, J_{PP} 15 Hz).

5 ^1H NMR (500 MHz, D_2O) δ 0.8 (3H, t, *J* 7 Hz, lauryl), 1.24–1.80 (18H, broad m, lauryl), 1.59–1.68 (2H, m, C-2-H lauryl), 2.08, 2.10 (2 × 3H, 2 × s, Ac), 3.46–4.04 (14H, complex overlapping m, C-1-H lauryl and C-2′,3′,4′,5′,6′,8′,9′,10′,11′ and 12′ of disaccharide), 4.64 (1H, d, *J* 8.5 Hz, C-7′-H) and 5.36 (1H, dd, $J_{H\text{-}H}$ 3.0, $J_{H\text{-}P}$ 7.7 Hz, C-1′-H); ^{31}P NMR (101.256 MHz) −13.77 (d, J_{PP} 21 Hz) and −16.33 (d, J_{PP} 21 Hz).

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References

1 T. W. Rademacher, R. B. Parekh and R. A. Dwek, *Annu. Rev. Biochem.*, 1988, **57**, 785.
2 T. Ogawa, M. Sugimoto, T. Kitajima, K. K. Sadozai and T. Nukada, *Tetrahedron Lett.*, 1986, **27**, 5739; W. Guenther and H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1050; H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 823.
3 E. J. Toone, E. S. Simon, M. D. Bednarski and G. M. Whitesides, *Tetrahedron*, 1989, **45**, 5365.
4 R. Kornfeld and S. Kornfeld, *Annu. Rev. Biochem.*, 1985, **54**, 631.
5 J. Burgos, F. W. Hemming, J. F. Pennock and R. A. Morton, *Biochem. J.*, 1963, **88**, 470; B. Imperiali and J. W. Zimmerman, *Tetrahedron Lett.*, 1988, **29**, 5343.
6 A. F. Clark and C. L. Villemez, *FEBS Lett.*, 1973, **32**, 84.
7 S. L. Flitsch, J. P. Taylor and N. J. Turner, *J. Chem. Soc., Chem. Commun.*, 1991, following communication.
8 C. D. Warren and R. W. Jeanloz, *Carbohydr. Res.*, 1974, **37**, 252; C. D. Warren, A. Herscovics and R. W. Jeanloz, *Carbohydr. Res.*, 1978, **61**, 181.
9 M. Spinola and R. W. Jeanloz, *J. Biol. Chem.*, 1970, **245**, 4158.
10 G. Excoffier, D. Gognaire and J.-P. Utille, *Carbohydr. Res.*, 1975, **39**, 368.
11 F. R. Atherton, H. T. Honard and A. R. Todd, *J. Chem. Soc. (C)*, 1948, 1106.
12 M. Inage, H. Chaki, S. Kusumoto and T. Shiba, *Chem. Lett.*, 1982, 1281.
13 J. W. Perich and R. B. Johns, *Synthesis*, 1988, 142; D. Coe, S. L. Flitsch, H. Hilpert, M. Liebster, S. M. Roberts and N. J. Turner, *Chem. Ind.*, 1989, 724.
14 K. Furusawa, M. Sekine and T. Hata, *J. Chem. Soc., Perkin Trans. 1*, 1976, 111.
15 S. J. Hecker, M. L. Minich and K. Lackey, *J. Org. Chem.*, 1990, **55**, 4904.