concentration was 5×10^{-4} mol/L, and the temperature was maintained within 0.1 K. Chromatographic separations were carried out on a Hypersil ODS column (4 × 250 mm, 5 μm), using an acetic acid buffer (pH 4.2) containing 0.1 mol/L of ammonium chloride as eluant. The concentrations were assumed to be proportional to the integrated areas of the UV signals, since the structure of the base moiety was not changed by the reactions followed.

Calculation of Rate Constants. The first-order rate constants indicated in Scheme I were obtained by simulating the time-dependent product distributions with the aid of a numerical intergration method based on the Runge-Kutta algorithm.[22] Equations 1-6 were used to describe the reaction system. Here A stands for **2** (or **3**), B for **4a** (or **5a**), C for **4b** (or **5b**), and D for **4c** (or **5c**). $(\chi_B/\chi_C)_{init}$ denotes the ratio of the mole fractions of B and C during the early stage of the hydrolysis of A, and $(\chi_B/\chi_C)_{eq}$ denotes the same ratio after equilibration of B and C. The method of least-squares was applied to fit the experimental data.

$$d\chi_A/dt = -(k_1 + k_2)\chi_A + k_{-1}\chi_B + k_{-2}\chi_C \quad (1)$$

$$d\chi_B/dt = k_1\chi_A - (k_{-1} + k_3 + k_4)\chi_B + k_{-3}\chi_C \quad (2)$$

$$d\chi_C/dt = k_2\chi_A + k_3\chi_B - (k_{-2} + k_{-3} + k_5)\chi_C \quad (3)$$

$$d\chi_D/dt = k_4\chi_B + k_5\chi_C \quad (4)$$

$$k_1/k_2 = (\chi_B/\chi_C)_{init} \quad (5)$$

$$k_{-3}/k_3 = (\chi_B/\chi_C)_{eq} \quad (6)$$

Registry No. **1a**, 606-02-0; **2**, 141635-70-3; **3**, 87215-04-1; **4a**, 141635-71-4; **4b**, 141635-72-5; **4c**, 31448-54-1; **5a**, 87215-02-9; **5b**, 87215-03-0; **5c**, 80541-15-7.

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Enzyme-Catalyzed Glycosylation of Peptides Using a Synthetic Lipid Disaccharide Substrate

Jung Lee and James K. Coward*

Interdepartmental Program in Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

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A lipid disaccharide, consisting of chitobiose linked to dolichol via an α-1-pyrophosphate, has been synthesized for use as a substrate in the enzyme-catalyzed glycosylation of peptides. For the purpose of confirming the structure of the reaction product, the expected glycopeptide was synthesized via an unambiguous, convergent method. Chromatographic and spectral comparison of the synthetic vs biosynthetic glycopeptides showed that they were identical. Thus, glycosylation of synthetic peptides by a synthetically accessible lipid disaccharide can be effected using oligosaccharyltransferase isolated from yeast.

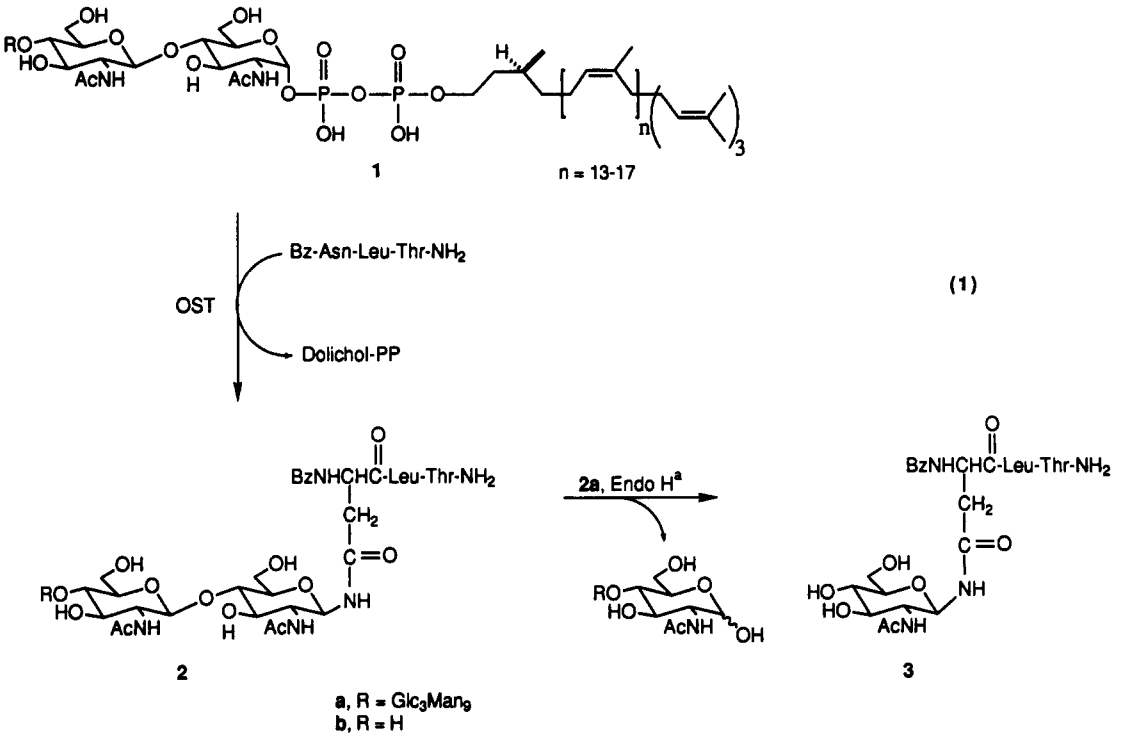
Introduction

A key reaction in the biosynthesis of N-linked glycoproteins involves the coupling of a growing peptide to a lipid-linked oligosaccharide.[1] This is a cotranslational process, catalyzed by the enzyme, dolichyl-diphosphooligosaccharide-protein glycotransferase (EC 2.4.1.119), commonly referred to as oligosaccharyltransferase (OST).[2] As shown in Figure 1, biosynthesis of the lipid-linked oligosaccharide substrate involves a series of glycosyl transfer reactions in which the sugar donor is either a nucleotide sugar or a dolicyl sugar.[3] The entire process occurs in the rough endoplasmic reticulum and thus involves a series of reactions which are catalyzed by membrane-bound enzymes.[4] The intact lipidoligosaccharide (LOS, **1a**) containing the so-called "core" oligosaccharide is shown in Figure 2. The standard method used to isolate lipid-linked substrates for studying the OST-catalyzed reaction (eq 1) involves microsomal preparations in which a specific radioactive nucleotide sugar is added in order to obtain radioactive lipidoligosaccharides labeled at a specific sugar residue. Using full-length biosynthetic **1a**, we have previously shown that only isomer **3** is obtained following enzyme-catalyzed hydrolysis of the GlcNAc-GlcNAc bond of the primary glycopeptide product **2a**, isolated from the yeast OST-catalyzed reaction (eq 1).[5] However, preparation of LOS is a cumbersome process, the yields are low, and the isolated biosynthetic LOS is unstable over periods of several months, even at -80 °C (J. Lee, R. S. Clark, and J. K. Coward, unpublished results).

For the purposes of carrying out mechanistic studies on the reaction catalyzed by OST, we required a lipid-linked oligosaccharide which would be amenable to total chemical synthesis by which we could ultimately introduce selected isotopic probes. Previous work with truncated biosynthetic lipid-linked oligosaccharides[6] or with yeast mutants unable to carry out specific steps in the biosynthetic pathway[7] have revealed that a variety of lipid-linked oligosaccharides are able to act as substrates for the OST-catalyzed reaction. Although these studies provide good precedent for the use of shorter lipid oligosaccharides as glycosyl donors in the OST-catalyzed reaction, the quantities of materials

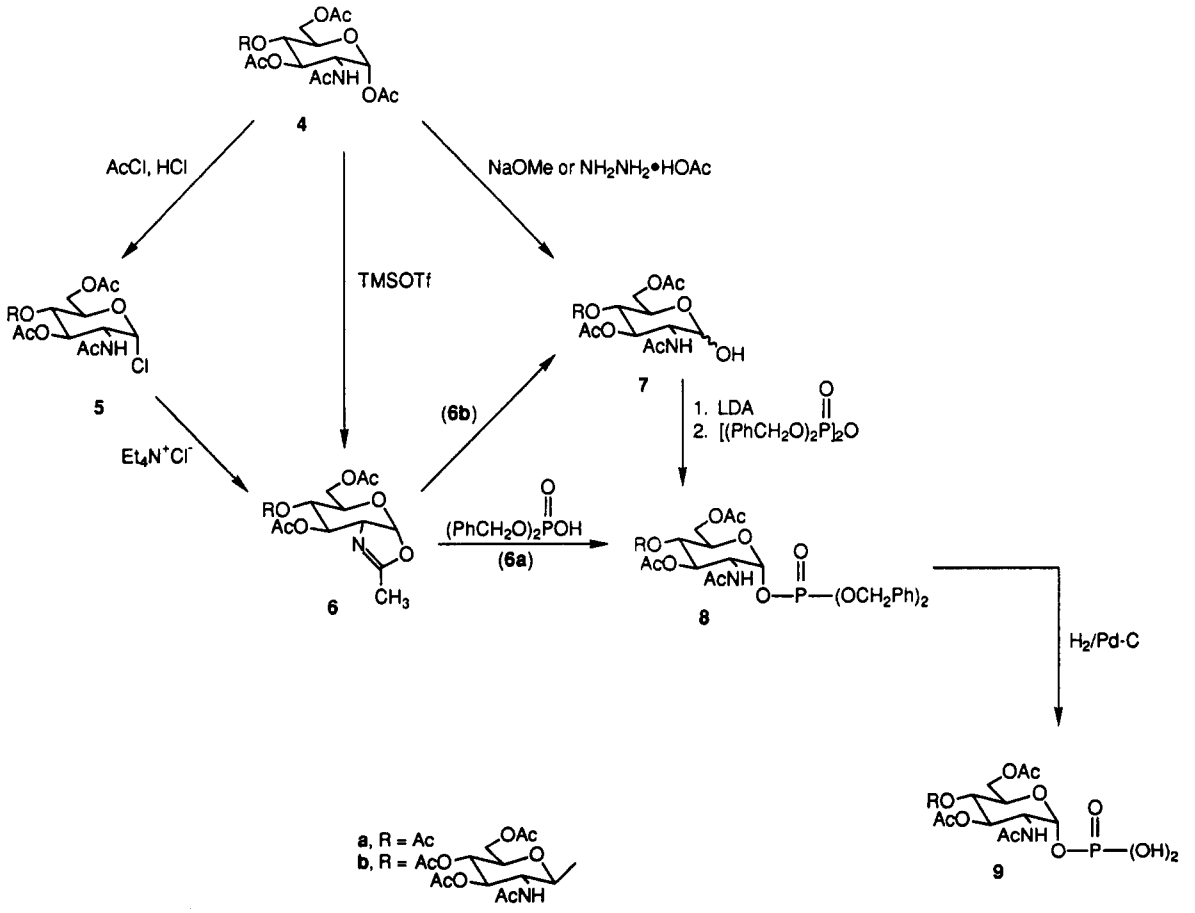
(1) Glabe, C. G.; Hanover, J. A.; Lennarz, W. J. *J. Biol. Chem.* **1980**, *255*, 9236.
(2) Kaplan, H. A.; Welply, J. K.; Lennarz, W. J. *Biochim. Biophys. Acta* **1987**, *906*, 161.
(3) Kennedy, J. F. *Carbohydrate Chemistry*; Clarendon Press: Oxford, 1988; p 282.
(4) Lennarz, W. J. *Biochemistry* **1987**, *26*, 7205.
(5) Clark, R. J.; Banerjee, S.; Coward, J. K. *J. Org. Chem.* **1990**, *55*, 6275.
(6) (a) Bause, E.; Lehle, L. *Biochim. Biophys. Acta* **1984**, *799*, 246. (b) Lehle, L.; Tanner, W. *Eur. J. Biochem.* **1978**, *83*, 563.
(7) Huffaker, T. C.; Robbins, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7466.

0022-3263/92/1957-4126$03.00/0



[a]Endo H = *endo*-β-*N*-acetylglucosaminidase H, EC 3.2.1.96

Scheme I



available from biosynthesis[6] are quite limited. In this paper, we describe the chemical synthesis of a lipid disaccharide (LDS, **1b**) in which chitobiose is linked to dolichol via an α-1-pyrophosphate bridge and show that it is a substrate for OST (eq 1). We also describe the synthesis of glycopeptide **2b** which we anticipated would be the product of OST-catalyzed glycosylation of the tripeptide, Bz-Asn-Leu-Thr-NH_2, with **1b** (eq 1) and have verified that to be the case. In addition to providing an unambiguous structural proof of the glycosylation product formed when using the truncated substrate LDS, the synthesis of **2b** allows for its future use in steady state

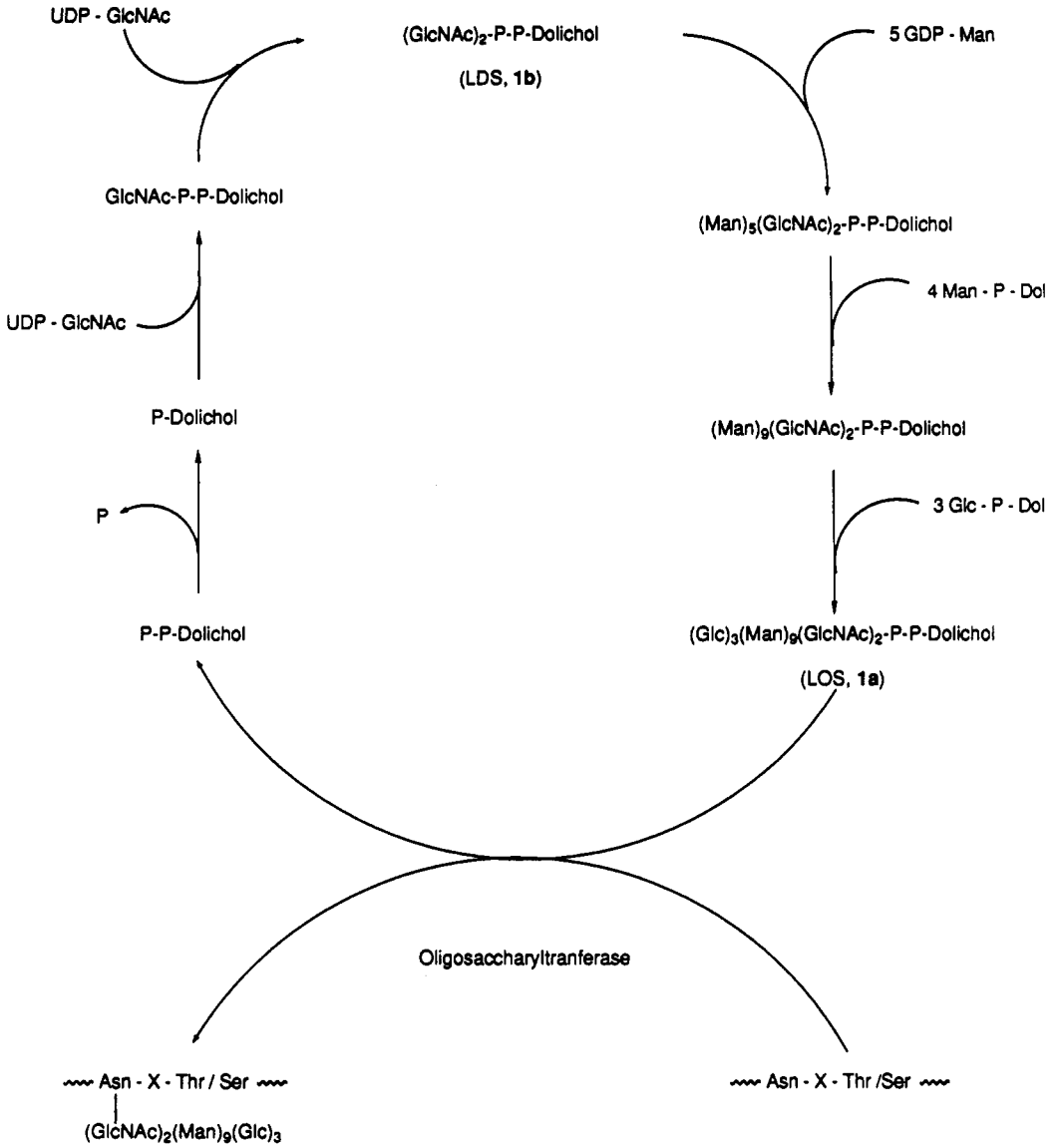
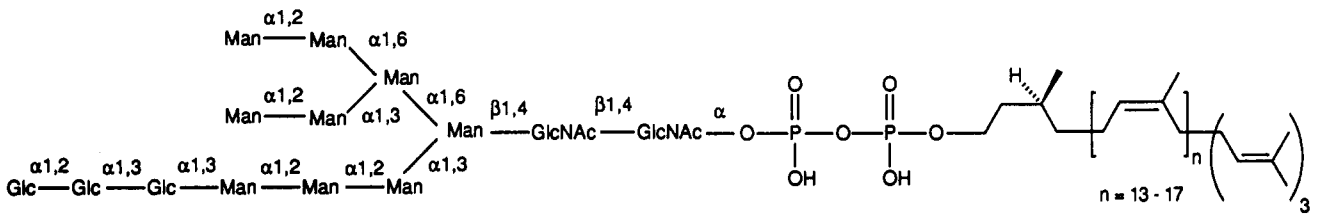


Figure 1. Biosynthesis of lipidoligosaccharides and the use of LOS (**1a**) as a substrate for the OST-catalyzed glycosylation of proteins.



1a

Figure 2. Oligosaccharide linkages in LOS (**1a**).

kinetic studies involving possible product inhibition.

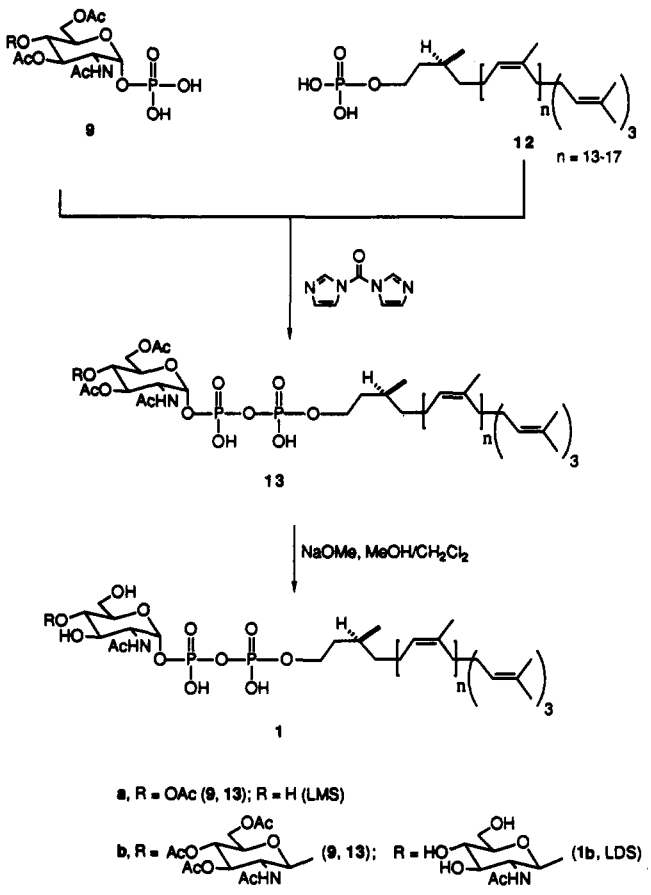
Results and Discussion

A chemical synthesis of lipid-linked oligosaccharides has been reported previously by Warren, Jeanloz, and coworkers.[8,9] In general, their approach involved the coupling of two phosphates: the sugar phosphate **9** (Scheme I) and lipid phosphate **12**, as depicted in Scheme II. Although our initial plan was to synthesize LDS as reported by Warren et al.[9] for use in our biochemical studies, we have investigated alternate approaches to several steps in order to improve their synthesis. In terms of the basic strategy, we decided to use a similar methodology: syntheses of two phosphates and coupling the two phosphates with a suitable coupling agent.

There are two widely used strategies for phosphorylating a carbohydrate at the anomeric hydroxyl group. As shown in Scheme I, we have investigated both approaches to the synthesis of the fully blocked sugar phosphate **8** and the desired monoester **9**. Introduction of a latent electrophilic moiety such as an oxazoline (e.g., **6**) renders the sugar susceptible to acid-mediated phosphorylation by phosphoric acid diesters. The synthesis of this type of latent

(8) Warren, C. D.; Jeanloz, R. W. *Methods Enzymol.* **1978**, *50*, 122 and references therein.
(9) Warren, C. D.; Herscovics, A.; Jeanloz, R. W. *Carbohydr. Res.* **1978**, *61*, 181.

Scheme II



electrophilic sugar was pioneered by Khorlin and coworkers.[10] They synthesized 2-methyl-[4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3,6-di-*O*-acetyl-α-D-glucopyranoso][2,1-*d*]oxazoline (**6b**) from the α-chloro sugar **5b** using AgCl. Subsequent phosphorolytic opening of the oxazoline sugar provides the desired sugar phosphate ester 8. Alternatively, phosphorylation of sugar hydroxyl groups has been used in the synthesis of lipid A and several analogs[11,12] and would appear to be a viable route to 8. One of the problems encountered using this methodology involves the synthesis of a protected sugar having only one free hydroxyl group at the anomeric carbon. Many methods have been reported[13] in which fully protected monosaccharides were selectively deprotected at the anomeric position by using reagents such as organic tin oxides, hydrazine acetate, potassium hydroxide, potassium cyanide, sodium methoxide, benzylamine, and tributyltin methoxide. We have investigated the reactions discussed above, using first peracetylated *N*-acetylglucosamine (**4a**) and then peracetylated chitobiose (**4b**) in order to optimize the reaction conditions on the more accessible monosaccharide prior to proceeding to the disaccharide.

Warren et al.[8] synthesized small quantities (ca. 15 mg) of the second phosphate monoester, **12**, by reacting dolichol with *o*-phenylene phosphorochloridate followed by oxidative cleavage of the resulting unsymmetric dolichyl *o*-hydroxyphenyl ester. The dolichol used in their studies was isolated from rat liver and thus available in limited supply. Recently, a paper by Imperiali and Zimmerman[14] described the chemical synthesis of (*S*)-dolichols by an enantioselective hydrogenation of the terminal allylic alcohol of polyprenols isolated from the leaves of *Ginkgo biloba* trees. This is a major advance and allows for the routine synthesis of larger quantities (0.5–1.0 g) of **12**.

Starting with the monosaccharide **4a**, we found that the most satisfactory method for the synthesis of oxazoline **6a** was via the α-chloro sugar **5a** as previously described.[15] Although this two-step procedure was also effective in converting the disaccharide **4b** to **6b**, a more effective one-step procedure using TMSOTf[16] resulted in a 91% yield of **6b**. The synthesis of **9a** from **6a** via **8a** proceeded in good yield but the corresponding conversion of **6b** to **9b** gave a poor yield.[17] This is similar to the low yield (20% overall) reported by Warren et al.[9] in their synthesis of **9b**. In our hands, attempted phosphorylation of **6b** gave predominantly 1-O-deacetylated chitobiose (**7b**), despite the use of anhydrous reaction conditions as recommended by Warren et al.[16a] The alternative route, namely, phosphorylation of an oxyanion derived from the protected reducing sugar **7**, proved to be much more satisfactory. Thus, selective deacetylation at C-1 was best accomplished by hydrazinolysis.[13a] Subsequent phosphorylation of **7** was achieved using LDA and tetrabenzyl pyrophosphate to give the unstable phosphotriester 8, which was converted to the monoester **9** by hydrogenolysis of the benzyl esters. Conversion of **7** to **9** via 8 was effected in yields of 72% (**a** series) and 70% (**b** series). Thus, our results indicate that the preferred synthesis of the key intermediate **9b** is via the reducing sugar **7b**.

Synthesis of reasonable quantities of the second key intermediate, **12**, required for the synthesis of **1** (Scheme II) was made feasible by the recent description of a stereoselective synthesis of (*S*)-dolichol from polyprenols.[14] As described in the supplementary material, we have used this method to prepare (*S*)-dolichol in 97% yield, following which phosphorylation with $POCl_3$[18] led to **12** in 76% yield. Carbonyldiimidazole-mediated coupling of the two phosphomonoesters **9** and **12** was carried out using the method of Hoard and Ott[19] in 66% crude yield in both **a** and **b** series. Some unreacted dolichyl phosphate (**12**) could not be separated from the desired product **13** by ion-exchange chromatography. Further purification by preparative TLC gave **13b** in 22% overall yield, following which deacetylation (NaOMe/MeOH) gave **1** in quantitative yield. It

(10) (a) Khorlin, A. J.; Shulman, M. L.; Zurabyan, S. E.; Privalova, I. M.; Kopeacivh, Y. L. *Izv. Akad. Nauk. SSSR, Ser. Khim.* **1968**, *227*, 2094. (b) Khorlin, A. Y.; Zurabyan, S. E.; Antonenko, T. S. *Tetrahedron Lett.* **1970**, 4803.

(11) (a) Inage, M.; Chaki, H.; Moto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T.; *Tetrahedron Lett.* **1983**, *24*, 2011. (b) Inage, M.; Chaki, H.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1981**, *22*, 2281. (c) Imoto, M.; Yoshimura, M.; Yamamoto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 2667. (d) Imoto, M.; Kusumoto, S.; Shiba, T.; Rietschel, E. T.; Galanos, C.; Ludertiz, O. *Tetrahedron Lett.* **1985**, *26*, 907. (e) Kusumoto, S.; Yoshimura, H.; Imoto, M.; Shimamoto, T.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 909. (f) Zahringer, U.; Lindner, B.; Seydel, U.; Rietschel, E. T.; Naoki, H.; Unger, F. M.; Imoto, M.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 632.

(12) Szabo, P. *J. Chem. Soc., Perkin Trans. I.* **1989**, 919.

(13) (a) Excoffier, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39*, 368. (b) Itoh, T.; Takamura, H.; Watanabe, K.; Araki, Y.; Ishido, Y. *Carbohydr. Res.* **1986**, *156*, 241. (c) Watanabe, K.; Itoh, K.; Araki, Y.; Ishido, Y. *Carbohydr. Res.* **1986**, *154*, 165. (d) Salat, C.; Agnesseti, A.; Caniato, M. B. Spanish Pat. 430,630, 1976. (e) Nudelman, A.; Herzig, J.; Gottlieb, H. E.; Kainan, E.; Sterling, J. *Carbohydr. Res.* **1987**, *162*, 145.

(14) Imperiali, B.; Zimmerman, J. W. *Tetrahedron Lett.* **1988**, *29*, 5343.

(15) Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4063.

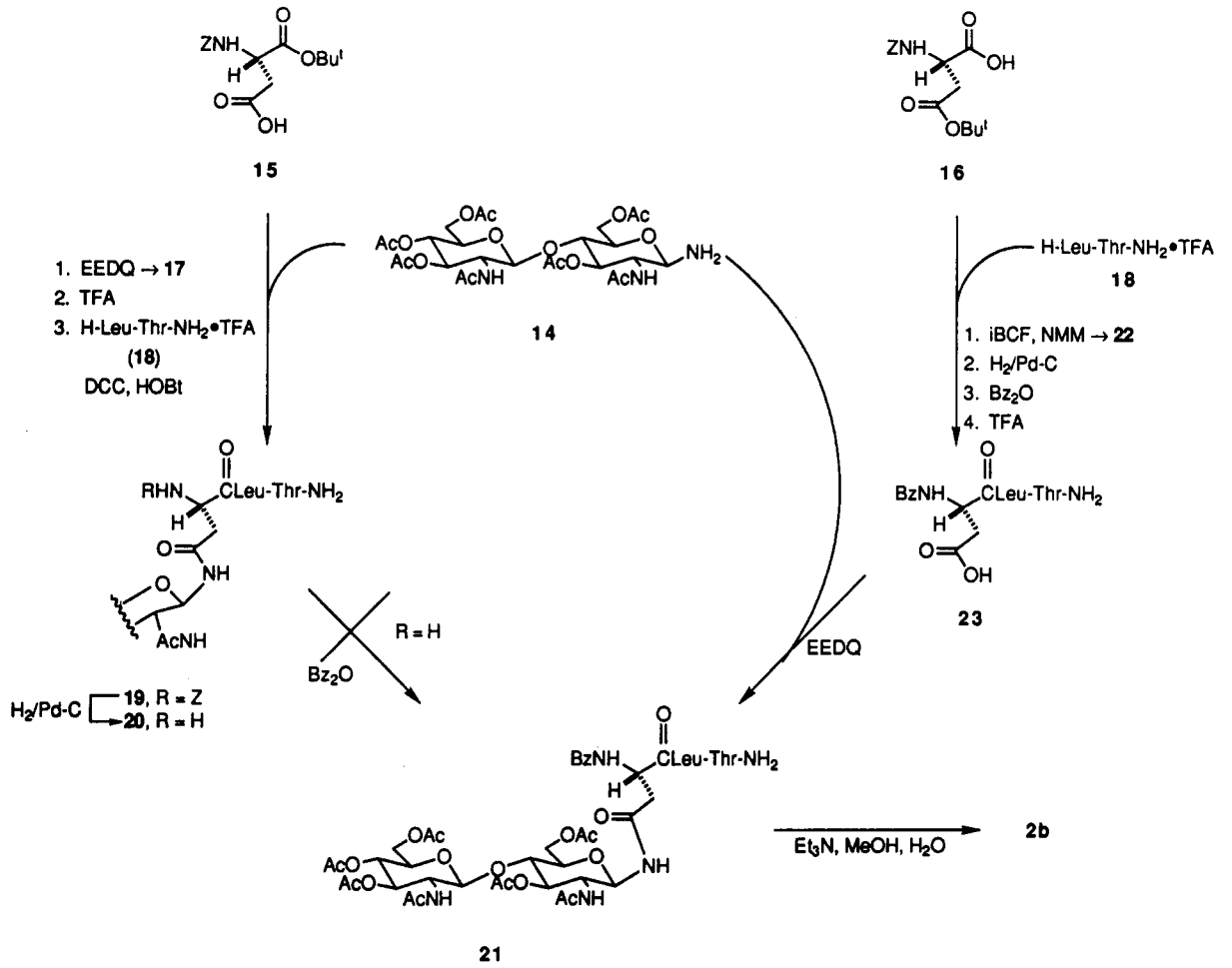
(16) (a) Warren, C. D.; Milat, M.-L.; Augé, C.; Jeanloz, R. W. *Carbohydr. Res.* **1984**, *126*, 61. (b) Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1986**, *150*, C7.

(17) Heidlas, J. E.; Lees, W. J.; Pale, P.; Whitesides, G. M. *J. Org. Chem.* **1992**, *57*, 146. The authors report similar success in the conversion of **6a** to **9a**, isolated as the cyclohexylamine salt, but no attempt was made to synthesize **9b** from **6b**.

(18) Danilov, L. L.; Chojnacki, T. *FEBS Lett.* **1981**, *131*, 310.

(19) (a) Hoard, D.; Ott, D. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785. (b) Johnson, T. B.; Coward, J. K. *J. Org. Chem.* **1987**, *52*, 1771.

Scheme III



is of interest to note that the ^{31}P-NMR spectrum of **13a** in $CDCl_3$ showed a pair of broad singlets (−9.90, −11.46 ppm), as expected for a pyrophosphate diester.[20] In contrast, the ^{31}P-NMR spectrum of **13b** in $CDCl_3$ showed a rather broad peak centered at −13 ppm. Only by dilution of the sample in MeOD ($CDCl_3$/MeOD, 2:1) was the expected pair of broad singlets (−10.80, −13.31 ppm) observed. Broadening of ^{31}P-NMR peaks has been previously associated with the formation of micelles.[20] It is somewhat surprising that this apparent formation of micelles should occur only in the case of the disaccharide-containing molecule **13b** and not in the case of the monosaccharide-containing molecule **13a**.

Synthesis of the Glycopeptide *N*-Bz-Asn-(GlcNAc)$_2$-Leu-Thr-NH$_2$. For the structure of the product isolated from the yeast OST-catalyzed reaction (eq 1) between LDS (**1b**) and the tripeptide Bz-Asn-Leu-Thr-NH_2 to be verified, a chemical synthesis of the anticipated glycopeptide **2b** was initiated. The usual method for synthesizing N-linked glycopeptides involves the coupling of an amino sugar to a protected aspartic acid and subsequent elongation of the glycoamino acid with amino acids.[21] While this linear synthesis of glycopeptides has been the traditional approach, we also investigated a convergent synthesis (Scheme III). During the course of this study, Anisfeld and Lansbury[22] reported a similar convergent synthesis of a glycopeptide.

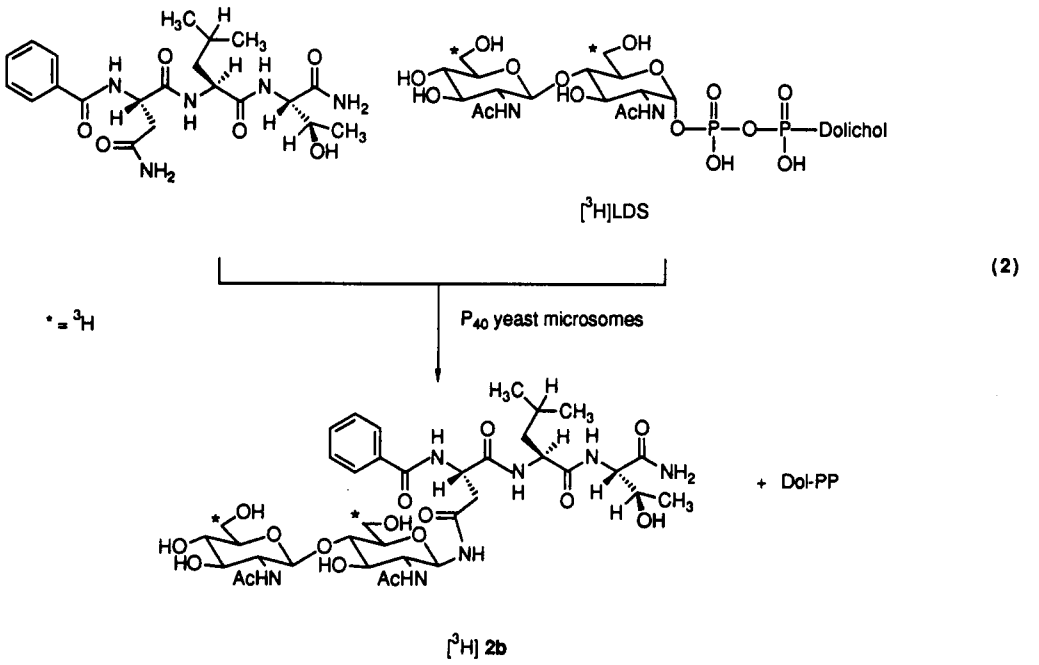
The Linear Approach. Using ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ), the amino sugar **14** was coupled with *N*-Cbz-L-aspartic acid *α-tert*-butyl ester (**15**) to give a 68% yield of the fully blocked glycosylasparagine **17**. Removal of the *α-tert*-butyl ester followed by DCC-mediated coupling with H-Leu-Thr-NH_2 (**18**) led to the blocked glycopeptide **19**, which was converted to **20** in 32% overall yield from **17**. Completion of the synthesis of **21** by the linear approach was not possible because all attempts to benzoylate the N-terminus of **20** were unsuccessful. In contrast, benzoylation of the glycopeptide containing only one GlcNAc, H-Asn(GlcNAc-$(OAc)_3$)-Leu-Thr-NH_2, proceeded smoothly in previous work reported from this laboratory.[5] The difficulties in effecting acylation of the larger disaccharide-containing glycopeptide **20** may be due to a steric shielding of the terminal amino group by the large, conformationally mobile carbohydrate moiety.

The Convergent Synthesis. The readily available *N*-Cbz-L-aspartic acid *β-tert*-butyl ester (**16**) was first coupled with Leu-Thr-NH_2 (**18**) using isobutyl chloroformate to give the fully blocked tripeptide **22** in 60% yield. Following removal of the Cbz group by hydrogenolysis, benzoylation of the free N-terminus and removal of the *tert*-butyl ester gave **23** in 77% overall yield from **22**. The coupling of benzoylated tripeptide **23** and peracetylated chitobiosylamine **14** was accomplished using EEDQ to give the fully blocked glycopeptide *N*-Bz-Asn-((OAc)$_5$GlcNAc$_2$)-Leu-Thr-NH_2 (**21**) in 75% yield. Two

(20) Gorenstein, D. G. *Phosphorus-31 NMR*; Academic Press: New York, 1984.

(21) (a) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 294. (b) Kunz, H.; Dombo, B. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711. (c) Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 883. (d) Garg, H. G.; Jeanloz, R. W. *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press: New York, 1985; p 135.

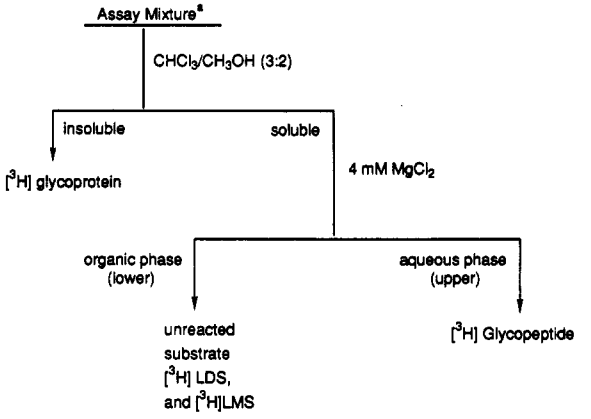
(22) Anisfeld, S. T.; Lansbury, P. T., Jr. *J. Org. Chem.* **1990**, *55*, 5560.



different methods were compared for removing the acetyl protecting groups of **21**. Use of the procedure described by Kunz[21a] (MeOH saturated with NH_3) gave the desired product, but only in low yield along with many byproducts. Deacetylation was best accomplished using a procedure ($H_2O/Et_3N/MeOH$) previously reported from our laboratory[5] to give the desired glycopeptide **2b**. However, this final deblocking was not without problems as the conditions previously employed[5] to O-deacetylate a GlcNAc-$(OAc)_3$-containing glycopeptide led to incomplete hydrolysis of **21**. Again it is interesting to note that conformational mobility of the large disaccharide moiety of **21** may result in a shielding of certain portions of the molecule when compared to the monosaccharide-containing material. Similar differences in the reactivity of the N-terminal amine of **20** vs the corresponding monosaccharide-containing compound were noted in the attempted linear synthesis of **2b** discussed above. Purification of the crude product was accomplished by semi-preparative HPLC to give the desired glycopeptide, **2b**. The incomplete hydrolysis product, obtained in pure form by this HPLC procedure, was shown to be a mono-O-acetyl derivative of **2b** on the basis of mass spectral data.

Biochemical Studies Using Synthetic LDS (1b) and Glycopeptide (2b). Having synthesized the putative glycopeptide product of the OST reaction, we wished to establish that **2b** is, in fact, the product of this enzyme-catalyzed glycosylation reaction. Using the isolated [^{3}H]LDS prepared as described in the supplementary material, the reaction shown in eq 2 was assayed according to Sharma and co-workers.[23] Separation of the enzyme-catalyzed product ([^{3}H]glycopeptide) from [^{3}H]LDS, [^{3}H]lipidmonosaccharide (LMS), and [^{3}H]glycoprotein (from endogenous protein in the crude microsomal OST preparation) was performed on the basis of differences in their solubilities as shown in Scheme IV. [^{3}H]Glycopeptide, [^{3}H]LDS, and [^{3}H]LMS were recovered in the $CHCl_3$/MeOH (3:2)-soluble fraction of the assay mixture. [^{3}H]Glycopeptide was separated further from [^{3}H]LDS and [^{3}H]LMS by washing the soluble fraction with 4 mM $MgCl_2$. The upper aqueous layer contained the desired

Scheme IV. Separation of Products of the OST-Catalyzed Glycosylation Reaction



[a] [^{3}H]LDS, [^{3}H]LMS, Bz-Asn-Leu-Thr-NH_2, P_{40} containing OST and other microsomal proteins.

[^{3}H]glycopeptide and the lower organic layer contained the unreacted radioactive material. The aqueous phase was concentrated in vacuo at ambient temperature using a Speed Vac vacuum centrifuge. The dry residue was redissolved in 0.1 N HOAc and analyzed by gel filtration using a Bio-Gel P-4 column to obtain evidence that the ^{3}H-labeled product in the aqueous layer was the anticipated [^{3}H]glycopeptide, Bz-Asn([^{3}H]GlcNAc)$_2$-Leu-Thr-NH_2. The carbohydrates $(GlcNAc)_2$, GlcNAc, and mannose, in addition to the GlcNAc-containing glycopeptide *N*-Benzoyl-Asn(GlcNAc)-Leu-Thr-NH_2,[5] are cleanly resolved on this column, and the results (not shown) indicate that the ^{3}H-labeled product elutes earlier (i.e., is larger) than any of the carbohydrates just mentioned or the GlcNAc-containing glycopeptide. In addition, the ^{3}H-labeled product was analyzed by HPLC along with the synthetic peptide substrate Bz-Asn-Leu-Thr-NH_2 and the synthetic glycopeptide **2b**. The result of the HPLC analysis is shown in Figure 3 and provides additional evidence that **2b** is the product of the OST-catalyzed reaction shown in eq 2. Finally, use of chemically synthesized LDS (**1b**) as a substrate has permitted the isolation of sufficient quantities of glycopeptide to allow for the acquisition of ^{1}H-NMR spectra at 500 MHz. These spectra of biosyn-

(23) Sharma, C. B.; Lehle, L.; Tanner, W. *Eur. J. Biochem.* 1981, *116*, 101.

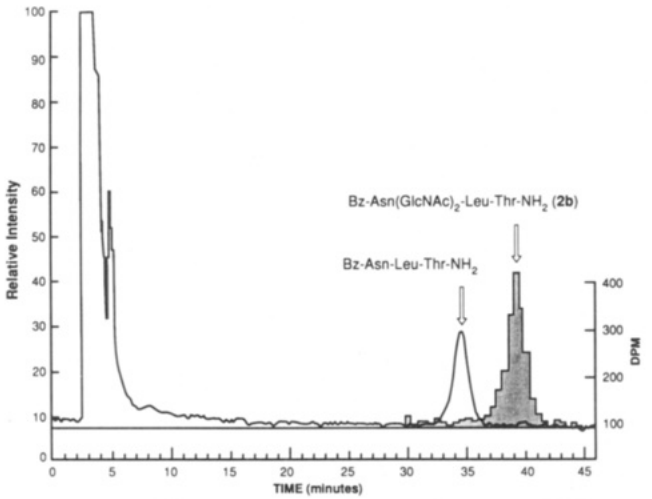


Figure 3. HPLC analysis of product resulting from OST-catalyzed glycosylation of Bz-Asn-Leu-Thr-NH_2 by [^{3}H]-LDS. See supplementary material for details on product isolation. Retention times of the peptide substrate Bz-Asn-Leu-Thr-NH_2 and expected glycopeptide product **2b**, established in separate HPLC analyses, are shown for comparative purposes. Peaks in the chromatograph were detected by absorbance at 254 nm (solid line) or radioactivity (speckled). Column: Vydac C_{18} reversed phase (4.3 × 250 mm). Eluent: CH_3OH:H_2O (25:75). Flow rate: 1.2 mL/min.

thetic glycopeptide are identical to spectra of synthetic **2b** (see supplementary material). Additional NMR data will be reported separately in a paper describing the use of **1b** and stereospecifically deuteriated tripeptides as mechanistic probes of the OST-catalyzed reaction (J. Lee and J. K. Coward, manuscript in preparation).

In conclusion, we have shown that synthetic LDS (**1b**) can be substituted for LOS (**1a**) as a substrate in the reaction catalyzed by OST. The nature of glycopeptide product **2b** has been determined by comparison of the biosynthetic material with chemically synthesized **2b**. With these synthetic substrates and products in hand, we are now able to proceed with more detailed mechanistic studies of the unusual reaction catalyzed by OST. In a recent study comparing acceptor peptide solution conformation and OST substrate activity, Imperiali and Shannon[24] used a semisynthetic [^{3}H]LDS for routine assay of OST activity, thus providing additional evidence that LDS is a useful saccharyl donor in OST-catalyzed glycosylation of peptides.

Experimental Section

General. ^{1}H NMR chemical shifts are reported in ppm downfield from a tetramethylsilane (TMS) internal or external standard. ^{13}C NMR chemical shifts are reported in ppm downfield from TMS. ^{31}P NMR chemical shifts are reported in ppm relative to phosphoric acid external standard. Melting points are uncorrected. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Most reagents and starting materials were obtained from commercial suppliers and were used without further purification. Ethereal solvents were distilled from sodium benzophenone ketyl under Ar. Dichloromethane, acetonitrile, triethylamine, toluene, ethyl acetate, chloroform, *tert*-butyl alcohol, and methanol were distilled from CaH_2 under Ar. GDP-[^{3}H]Man and UDP-[^{3}H]GlcNAc were purchased from DuPont NEN. Bio-Gel P-4 (−400 mesh) was purchased from Bio-Rad. Analytical HPLC analyses were performed on an Altex liquid chromatography system (Vydac column, 4.6 mm × 25 cm, 5 μm C_{18}, 1.2 mL/min, 254 nm) or a Rainin liquid chromatography system (Dynamax 60A column, 4.6 mm × 25 cm, 5 μm C_{18}, 1.2 mL/min, 254 nm). Preparatory HPLC purification was performed on a Rainin liquid chromatography system (Dynamax 60A column, 10 mm × 25 cm, 5 μm C_{18}, 5.64 mL/min, 254 nm). Compounds **4a**,[25] **4b**,[26] and **14**[27] were synthesized as described in the literature with minor modifications.[28] Tetrabenzyl pyrophosphate was prepared according to Khorana and Todd.[29] Polyisoprenyl acetates were isolated from leaves of *Ginkgo biloba* as described previously.[30] As described in the supplementary material, hydrolysis to the free polyprenols **10** followed by stereospecific reduction of the allylic alcohol and phosphorylation gave **11**[14] and **12**.[18] Bz-Asn-Leu-Thr-NH_2 was prepared from H-Leu-Thr-NH_2·TFA (**18**) as previously described.[5]

2-Methyl-[4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoso][2,1-*d*]-2-oxazoline (6b). A. A solution containing 675 mg (1 mmol) of octaacetyl chitobiose (**4b**) and 4 mL of acetyl chloride was saturated with dry HCl at −10 °C. After sealing the flask, the solution was allowed to stir for 20 h at ambient temperature. At the end of 20 h, 67 mL of CH_2Cl_2 was added and the mixed solution was poured onto a mixture of H_2O (13.5 mL) and ice (54 g). The organic solution was removed, washed with saturated solutions of $NaHCO_3$ and NaCl, dried over Na_2SO_4, and then evaporated under reduced pressure to obtain 440 mg (72%) of the α-chloro sugar **5b**. The crude α-chloro sugar was not purified further. The solid was redissolved in 16 mL of dry CH_3CN and to the solution was added tetraethylammonium chloride (134 mg, 0.810 mmol) and $NaHCO_3$ (68 mg, 0.810 mmol). The solution was then allowed to stir for 3 h at ambient temperature. At the end of 3 h, the solvent was removed in vacuo and to the dry residue was added 20 mL of EtOAc. The organic solution was washed with an ice-cold saturated solution of $NaHCO_3$ and NaCl, dried over Na_2SO_4, and evaporated under reduced pressure to obtain 370 mg (60% overall from **4b**) of the crude product. In a smaller scale reaction, the crude product was purified by filtering-column chromatography with Florisil (EtOAc/hexanes, gradient 30:70 → 100:0) to obtain **6b** as white needles: mp 165–167 °C (lit.[10a] mp 189–190 °C); IR (neat) 3440, 1750, 1673, 1558 cm^{-1}; ^{1}H NMR data are very similar to those reported for the product obtained by method B; HR mass spectrum calcd for $C_{26}H_{55}O_{14}$ (MH^+) *m/e* 617.2194, obsd *m/e* 617.2189.

B. A mixture of octaacetyl chitobiose (**4b**, 200 mg, 0.296 mmol) in 1,2-dichloroethane (3 mL) was treated with trimethylsilyl trifluoromethanesulfonate (60 μL, 0.312 mmol) under Ar. The solution was allowed to stir for 5 h at 50 °C. The reaction was stopped by adding 130 μL of Et_3N and allowing the solution to stir for 10 min. The desired product was purified directly by filtering-column chromatography on Florisil (EtOAc, 1% Et_3N) to obtain 170 mg (91%) of the pure oxazoline **6b** as white needles as described in method A: ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.86–2.05 (4 s, 21 H), 3.41 (br s, 1 H), 3.52 (br d, 1 H), 3.71 (br d, 1 H), 3.85 (dd, 1 H), 4.06 (m, 3 H), 4.20 (br d, 2 H), 4.74 (d, 1 H, *J* = 8.4 Hz), 5.00 (t, 1 H), 5.17 (t, 1 H), 5.59 (s, 1 H), 5.85 (d, 1 H, *J* = 7.2 Hz), 6.25 (d, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.9, 170.5, 170.5, 170.3, 169.2, 169.1, 166.5, 102.1, 98.9, 77.5, 77.4, 77.0, 76.7, 72.6, 71.7, 70.4, 68.4, 67.6, 64.8, 63.1, 62.0, 54.3, 22.9, 20.8, 20.6, 20.5, 20.5, 20.4, 13.8.

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (7b). A. To a suspension of NaOMe (54 mg, 1 mmol) in 10 mL of dry THF was added octaacetyl chitobiose (**4b**, 338 mg, 0.5 mmol) at −10 °C. The reaction mixture was allowed to stir for 30 min at −10 °C. After this time, an additional 54 mg of NaOMe was added and the mixture was allowed to stir for 24 h at ambient temperature. The reaction was stopped by adding 200 μL of glacial HOAc and then stirring the solution for 10 min. The mixture was evaporated under reduced pressure and the residue was redissolved in 20 mL of $CHCl_3$. The organic

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solution was then washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and evaporated under reduced pressure to obtain 380 mg of the crude product. The crude product was purified by filtering-column chromatography on silica gel ($CHCl_3$/MeOH, 10:1, R_f = 0.38) to give 83 mg (26%) of **7b** as a crystalline solid: mp 191 °C dec; IR (KBr) 3300 (OH, intramolecular H bonding), 1780, 1240, 1100 cm^{-1}; ^1H NMR (360 MHz, DMSO) δ 1.76–2.05 (m, 21 H), 3.57 (q, 1 H), 3.70 (t, 1 H), 3.80 (d, 1 H), 4.02 (m, 3 H), 4.12 (dd, 1 H), 4.30 (m, 2 H), 4.66 (d, 1 H, J = 7.8 Hz), 4.81 (t, 1 H), 4.89 (s, 1 H), 5.11 (m, 2 H), 7.02 (br s, 1 H), 7.67 (br d, 1 H), 7.99 (br d, 1 H); ^{13}C NMR (90 MHz, DMSO) δ 169.9, 169.9, 169.5, 169.4, 169.4, 169.2, 169.1, 100.2, 90.6, 76.2, 72.3, 70.9, 70.4, 68.3, 67.5, 62.5, 61.6, 53,8, 51.6, 22.4, 22.2, 20.6, 20.4, 20.3, 20.2, 20.2; mass spectrum (FAB) m/e (rel intensity), 635 (M^+, 65).

B. To 1.0 g (1.48 mmol) of octaacetyl chitobiose (**4b**) were added 16 mL of dry DMF and 164 mg (1.78 mmol) of hydrazine acetate under N_2. The reaction solution was then allowed to stir at ambient temperature. The reaction was monitored by TLC ($CHCl_3$/MeOH, 5:1) and after 42 min, a small amount of unreacted **4b** remained. Therefore additional hydrazine acetate (50 mg) was added in two portions. At the end of 70 min total reaction time, the reaction solution was diluted with 50 mL of EtOAc and the diluted solution was washed with 2 × 50 mL of a saturated solution of NaCl, dried over Na_2SO_4, and evaporated in vacuo to obtain 755 mg (80%) of a yellowish residue with chromatographic properties similar to the product obtained in method A. The crude residue was sufficiently pure for use in further transformations.

2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-Phosphate (9b). A solution of LDA (1.10 mL, 0.66 mmol) in dry THF was added to a solution of the 1-O-deacetylated peracetyl chitobiose (**7b**, 300 mg, 0.47 mmol) in dry THF (10.0 mL) at −68 °C. After the solution was allowed to stir for 15 min, a solution of tetrabenzyl pyrophosphate (315 mg, 0.59 mmol) in dry THF (6.0 mL) was added. After warming the reaction solution slowly to 0 °C, it was allowed to stir 1 h at this temperature. The reaction solution was then diluted with 40 mL of Et_2O. The organic phase was washed successively with cold saturated solutions of $NaHCO_3$ and NaCl and dried with Na_2SO_4. After removing the solvent under reduced pressure, the crude residue (**8b**) was redissolved in 20 mL of EtOH. The solution was hydrogenated in the presence of Pd–C for 3 h. At the end of 3 h the catalyst was filtered. The pH of the filtrate was adjusted to 6.0 with pyridine. The solvent was removed again under reduced pressure to give 240 mg (70%) of a white solid. This crude product was sufficiently pure for use in further transformations. In a smaller scale reaction, the crude product was converted to the pyridinium salt, dissolved in a minimum amount of distilled H_2O, and purified using DEAE Cellulose-column chromatography (DE-53, acetate form, 1 × 20 cm, a gradient of 0 to 0.1 M NH_4HCO_3 in distilled H_2O). The desired fractions were combined and lyophilized to obtain **9** as a white powder: mp 177–178 °C (lit.[9] mp 228–229 °C); ^1H NMR (360 MHz, D_2O) δ 1.98–2.18 (7 s, 21 H), 3.85 (dd, 1 H, J = 8.6 Hz, 10.4 Hz), 4.01 (m, 1 H), 4.10 (m, 3 H), 4.21–4.28 (m, 2 H), 4.48 (dd, 1 H, J = 3.5 Hz, 12.7 Hz), 4.55 (d, 1 H, J = 12.3 Hz), 4.84 (d, 1 H, J = 9.9 Hz), 5.05 (t, 1 H, J = 9.6 Hz, 9.6 Hz), 5.22–5.33 (m, 2 H), 5.44 (dd, 1 H, J = 3.4 Hz, 7.0 Hz); ^{13}C NMR (90 MHz, MeOD) δ 173.6, 173.5, 172.6, 172.3, 171.8, 171.3, 101.7, 95.1 (d, J_{CP} = 6.2 Hz), 77.0, 73.6, 73.0, 72.8, 70.8, 70.1, 63.3, 63.2, 56.6, 53.3 (d, J_{CP} = 7.6 Hz), 22.9, 22.6, 21.1, 20.8, 20.7, 20.6, 20.5; ^{31}P NMR (146 MHz, D_2O) δ −0.81; mass spectrum (FAB), m/e (rel intensity) 713 (M^-, 100). Anal. Calcd for $C_{26}H_{39}N_2O_{19}P \cdot 1.5H_2O$: C, 42.11; H, 5.71; N, 3.78. Found: C 42.03; H 5.40; N, 3.89.

P^1-Dolichyl P^2-[2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl] Diphosphate (13b). A. Preparation of Tri-*n*-butylammonium [2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl] Phosphate. A solution containing 100 mg (0.14 mmol) of peracetylated chitobiose 1-α-phosphate (**9b**), 5.0 mL of dry MeOH, and 1.0 mL of pyridine was concentrated to an oil and the oil was redissolved in 5.0 mL of dry MeOH. To the solution, was added 89 μL (69 mg, 0.37 mmol) of n-Bu_3N. After addition of 1.2 mL of distilled H_2O, the excess n-Bu_3N was removed by three extractions with hexane (3 × 3 mL). The aqueous layer was concentrated in vacuo and the residual H_2O was removed by coevaporation with toluene (3 × 3 mL). The product was obtained as an oil.

B. Preparation of Tri-*n*-butylammonium Dolichyl Phosphate. A solution containing 178 mg (0.15 mmol) of dolichyl phosphate (**12**) 4 mL of $CHCl_3$/MeOH (2:1), and 1.0 mL of pyridine was concentrated to an oil, which was redissolved in 5.0 mL of $CHCl_3$/MeOH (2:1). To the resulting solution was added 82 μL (64 mg, 0.34 mmol) of n-Bu_3N. The solvents were then removed in vacuo to obtain a clear oil.

C. Coupling Reaction and Purification. To a solution of the tri-n-butylammonium peracetylated chitobiose 1-α-phosphate (0.14 mmol) in 4.0 mL of dry DMF was added via syringe a solution of 122 mg (0.75 mmol) of carbonyldiimidazole in 3.0 mL of dry DMF. The reaction solution was allowed to stir for 4 h at ambient temperature. The excess carbonyldiimidazole was consumed by the addition of 50 μL (1.3 mmol) of dry MeOH. After the solution was allowed to stir for 30 min, a solution of tri-n-butylammonium dolichyl phosphate (ca. 0.15 mmol) in 4.0 mL of dry CH_2Cl_2 was then added and the reaction solution was allowed to stir for 48 h at ambient temperature. The solvents were then removed under reduced pressure. The oily residue was redissolved in 4.0 mL of $CHCl_3$/MeOH (2:1) and loaded by gravity on a 1 × 15 cm column of DEAE Cellulose (DE-53, acetate form, pre-equilibrated with $CHCl_3$/MeOH (2:1)) at ambient temperature. After the loaded column was washed with 3 column volumes of $CHCl_3$/MeOH (2:1), the product was eluted with a linear gradient of 0 to 0.1 M NH_4OAc solution in $CHCl_3$/MeOH (2:1, total volume = 200 mL). Fractions (4 mL) were analyzed by TLC, and appropriate fractions were pooled and concentrated to obtain 105 mg (66%) of the desired peracetylated lipid disaccharide **13b** as a clear oil, which was contaminated with dolichyl phosphate (**12**). The crude peracetylated lipid disaccharide was further purified by preparatory TLC to remove **12** and obtain 34 mg (22%) of pure **13b** as a clear oil; 25 mg of the contaminated product was recovered: R_f ($CHCl_3$/MeOH/H_2O, 60:25:4) = 0.48; ^1H NMR (360 MHz, $CDCl_3$) δ 0.85 (d, 3 H), 1.27 (br s, 4 H), 1.61 (br s, ca. 9 H), 1.69 (br s, ca. 30 H), 2.0 (br s, ca. 64 H), 3.40 (br s, 1 H), 3.70 (br s, 1 H), 4.05 (m, 2 H), 4.50 (m, 10 H), 5.10 (m, ca. 16 H), 7.50 (br s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.4, 169.6, 140.7, 135.0, 134.9, 134.8, 134.7, 134.6, 130.8, 128.0, 124.9, 124.7, 124.1, 124.0, 123.9, 72.0, 71.3, 68.4, 61.7, 39.4, 37.1, 36.7, 31.9, 31.6, 29.3, 29.0, 26.4, 26.3, 26.1, 26.0, 25.1, 24.8, 22.9, 22.1, 20.2, 20.0, 19.2, 18.6, 17.1, 15.5; ^{31}P NMR (202 MHz, $CDCl_3$/MeOD, 2:1) δ −10.80, −13.31.[31]

P^1-Dolichyl P^2-[2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranosyl] Diphosphate (LDS, 1b). Peracetylated lipid disaccharide **13b** (20 mg, 0.012 mmol) was dissolved in 5.0 mL of CH_2Cl_2 under N_2. To the solution was added 8 mg of NaOMe in 1 mL of MeOH (1% NaOMe in MeOH). The reaction solution was allowed to stir for 30 min at ambient temperature. At the end of 30 min, excess cation exchange resin (Dowex 50X8, pyridinium form) was added, and the mixture was allowed to stir 10 min. The resin was filtered and washed with $CHCl_3$/MeOH (2:1). The combined filtrate was evaporated under reduced pressure to obtain 17 mg (99% yield) of **1b** as a clear oil: R_f ($CHCl_3$/MeOH/H_2O, 60:25:4) = 0.13; ^1H NMR (360 MHz, $CDCl_3$/MeOD, 2:1) δ 0.83 (m, 6 H), 1.09 (br s, 2 H), 1.36 (br s, 4 H), 1.52 (d, 21 H), 1.60 (s, ca. 76 H), 1.96 (s, ca. 127 H), 3.40–3.70 (m, 6 H), 3.80 (br s, 2 H), 3.95–4.05 (br s, 3 H), 4.44 (s, ca. 41 H), 5.05 (s, ca. 29 H), 8.01 (br s, 6 H), 8.51 (br s, 3 H); ^{13}C NMR (90 MHz, $CDCl_3$/MeOD, 2:1) δ 173.0, 146.0, 141.6, 134.7, 127.1, 124.6, 123.8, 79.8, 76.2, 74.4, 70.3, 65.6, 64.5, 61.0, 59.9, 55.6, 39.2, 36.9, 31.8, 31.5, 29.1, 28.8, 26.0, 24.9, 24.7, 22.8, 22.0, 18.4, 17.5, 15.3.[31]

N^4-[2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-N^2-Cbz-L-aspartic Acid α-*tert*-Butyl Ester

(31) All attempts to obtain either nominal or high resolution mass spectra of **13b** or **1b** failed. In contrast, coupling of **9a** and **12** led to the peracetylated lipidmonosaccharide **13a**, for which an acceptable mass spectrum was obtained. Combustion analysis for these compounds is of little value since the lipid side chain is a mixture of isoprene oligomers, i.e., Scheme II, **13** or **1** (n = 13–17).

(17). To a solution of *N*-Cbz-L-aspartic acid α-*tert*-butyl ester (15, 150 mg, 0.47 mmol) in 8 mL of CH_2Cl_2 was added 300 mg (0.47 mmol) of the peracetylated chitobiosylamine 14, 68 μL (0.47 mmol) of Et_3N, and 187 mg (0.75 mmol) of EEDQ at ambient temperature. The reaction mixture was allowed to stir for 2 days at ambient temperature. The precipitate was filtered and the filtrate was evaporated under reduced pressure. The crude product was purified by filtering-column chromatography on silica gel with $CHCl_3$/EtOH (9:1) as the eluent. The product was then extensively washed with Et_2O to remove the unreacted EEDQ and to obtain 303 mg (68% yield) of 17 as a white powder: mp 247–249 °C. In a smaller scale reaction, the following spectral properties were observed: IR (KBr), 3300, 2950, 1740 (C=O ester), 1670 (amide I), 1530 (amide II), 1370, 1230, 1150, 1040 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 1.43 (s, 9 H), 1.88 (s, 3 H), 2.00–2.13 (6 s, 18 H), 2.54 (dd, 1 H, J = 3.7, 17 Hz), 3.02 (m, 2 H), 3.58 (d, 1 H), 3.71 (d, 1 H), 3.86 (t, 1 H), 4.02 (m, 2 H), 4.15 (m, 1 H), 4.40 (m, 2 H), 4.56 (m, 1 H), 4.74 (t, 1 H), 4.94 (t, 1 H), 5.21 (m, 4 H), 5.56 (t, 1 H), 5.98 (d, 1 H, J = 9.14 Hz), 6.97 (d, 1 H), 7.35 (m, 6 H), 7.88 (d, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 172.8, 172.4, 171.9, 171.4, 170.9, 170.4, 170.3, 170.1, 169.5, 156.1, 136.2, 128.5, 128.2, 99.6, 81.6, 80.6, 74.5, 74.3, 72.7, 72.0, 71.1, 68.7, 67.3, 62.1, 61.8, 56.6, 52.7, 51.4, 37.0, 27.9, 23.2, 23.0, 21.0, 20.9, 20.7, 20.5, 20.5; HR mass spectrum calcd for $C_{42}H_{59}N_4O_{20}$ (MH^+) m/e 939.3723, obsd m/e 939.3724.

N^4-[2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-N^2-Cbz-Asn-Leu-Thr-NH_2 (19). A solution containing 97 mg (0.10 mmol) of 17 in 4 mL of TFA/CH_2Cl_2 (1:1) was allowed to stir for 30 min at ambient temperature and the solvents were removed in vacuo. Triturations with Et_2O resulted in 87 mg of white crystals. To 87 mg (0.1 mmol) of the resulting glycoamino acid in 10 mL of dry DMF were added 11 μL (0.1 mmol) of NMM, 27 mg (0.13 mmol) of DCC, and 27 mg (0.2 mmol) of HOBt at −10 °C. After the solution was allowed to stir for 5 min, 35 mg (0.1 mmol) of the TFA salt of Leu-Thr-NH_2 (18) was added. The reaction mixture was allowed to stir for 2 days at ambient temperature. The reaction mixture was filtered and the filtrate was evaporated in vacuo. EtOAc (20 mL) was added to the residue and the insoluble material, which contained the desired product, was isolated by filtration. The crude product was extensively washed with MeOH to obtain 50 mg (41%) of 19 as a white powder: mp 291–293 °C dec; ^1H NMR (360 MHz, DMSO) δ 0.80 (2 d, 6 H), 1.01 (d, 3 H), 1.59 (m, 3 H), 1.75 (s, 6 H), 1.96 (4 s, 15 H), 2.49 (m, 2 H), 3.60 (m, 2 H), 3.70 (t, 1 H), 3.83–4.08 (m, 6 H), 4.30 (m, 3 H), 4.66 (d, 1 H, J = 8.3 Hz), 4.81 (m, 3 H), 4.99 (br s, 4 H), 5.13 (t, 1 H), 5.57 (d, 1 H), 7.08 (d, 2 H), 7.35 (br s, 5 H), 7.60 (d, 1 H), 7.81 (d, 1 H), 7.99 (m, 2 H), 8.35 (d, 1 H); ^{13}C NMR (90 MHz, DMSO) δ 171.9, 171.6, 169.8, 169.4, 169.2, 155.5, 136.8, 128.1, 127.6, 99.9, 78.3, 75.5, 73.5, 72.2, 70.4, 68.3, 66.2, 65.6, 62.4, 61.6, 58.0, 53.8, 52.1, 51.4, 47.4, 33.2, 25.2, 24.3, 23.9, 22.9, 22.4, 21.3, 20.6, 20.2, 19.8; HR mass spectrum calcd for $C_{48}H_{70}O_{22}N_7$ (MH^+) m/e 1096.4574, obsd m/e 1096.4553.

N^4-[2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-Asn-Leu-Thr-NH_2 (20). To 50 mg (0.05 mmol) of the *N*-Cbz-protected peracetyl glycopeptide 19 were added 20 mL of MeOH, 1 mL of HOAc, 4 mL of H_2O, and 20 mg of Pd–C. The reaction mixture was hydrogenated 12 h at 24 psi. The catalyst was filtered and washed with MeOH and H_2O. The combined filtrate was evaporated in vacuo. The dry residue was repeatedly washed with Et_2O to obtain 35 mg (80%) of 20 as a white solid: mp 220–222 °C; R_f (*n*-BuOH/HOAc/H_2O, 4:1:5, upper layer) = 0.17; ^1H NMR (300 MHz, $CDCl_3$/MeOD, 2:1) δ 0.94 (d, 3 H), 0.99 (d, 3 H), 1.18 (d, 3 H), 1.75 (m, 3 H), 1.91–2.11 (7 s, 21 H), 2.55–2.76 (m, 2 H), 3.60–3.81 (m, 6 H), 3.93–4.04 (m, 3 H), 4.37 (br s, 3 H), 4.44 (m, 1 H), 4.51 (d, 1 H), 4.98–5.13 (m, 3 H), 5.32 (t, 1 H); HR mass spectrum calcd for $C_{40}H_{64}N_7O_{20}$ (MH^+) m/e 962.4206, obsd m/e 962.4193.

***N*-Cbz-Asp(β-OBu^t)-Leu-Thr-NH_2 (22).** To a solution of *N*-Cbz-L-aspartic acid β-*tert*-butyl ester (16, 690 mg, 2.13 mmol) in 15 mL of dry THF was added 236 μL (2.15 mmol) of NMM at −10 °C, followed by 285 μL (2.20 mmol) of isobutyl chloroformate. The solution was allowed to stir for 7 min and then a pre-cooled (−10 °C) solution of the TFA salt of Leu-Thr-NH_2 (18, 736 mg, 2.134 mmol) and NMM (236 μL, 2.15 mmol) in 15 mL of dry DMF were added. After the mixture was allowed to stir overnight at ambient temperature, the solvent was evaporated in vacuo. To the dry residue was added 20 mL of a cold 5% citric acid solution. The white precipitates were filtered and washed with H_2O and ether. The crude product (735 mg) was recrystallized from EtOAc to obtain 680 mg (60%) of 22 as white crystals: mp 109–111 °C; R_f (*n*-BuOH/HOAc/H_2O, 4:1:5, upper layer) = 0.65; ^1H NMR (300 MHz, DMSO) δ 0.84 (d, 3 H), 0.88 (d, 3 H), 1.00 (d, 3 H), 1.38 (s, 9 H), 1.50–1.62 (m, 3 H), 2.39–2.70 (m, 2 H), 4.02–4.09 (m, 2 H), 4.28–4.40 (m, 2 H), 4.87 (d, 1 H), 5.04 (d, 2 H), 7.10 (d, 2 H, J = 8.7 Hz), 7.35 (s, 5 H), 7.57 (d, 1 H, J = 8.4 Hz), 7.63 (d, 1 H, J = 8.1 Hz), 8.12 (d, 1 H, J = 7.8 Hz); ^{13}C NMR (90 MHz, DMSO), δ 171.7, 171.6, 170.4, 169.1, 136.8, 128.1, 127.6, 127.4, 80.0, 66.2, 65.4, 57.7, 51.5, 40.3, 38.8, 37.3, 27.5, 24.0, 22.8, 21.4, 19.7. Anal. Calcd for $C_{26}H_{40}N_4O_8 \cdot 0.5H_2O$: C, 57.23; H, 7.76; N, 10.27. Found: C, 57.49; H, 7.54; N, 10.24.

***N*-Benzoyl-Asp(β-OBu^t)-Leu-Thr-NH_2.** A solution of *N*-Cbz-L-aspartic acid β-*tert*-butyl ester-Leu-Thr-NH_2 (22, 660 mg, 1.23 mmol) in 50 mL of MeOH was hydrogenated overnight with 100 mg of Pd–C at 24 psi. The catalyst was then removed by filtration and the filtrate was evaporated under reduced pressure to obtain a clear oil. Triturations with Et_2O and hexane resulted in 490 mg of white crystals. To 480 mg (1.19 mmol) of the crude free amine in 15 mL of dioxane was added 202 μL (1.45 mmol) of Et_3N at ambient temperature. After the reaction solution was allowed to stir 5 min, 402 mg (1.79 mmol) of benzoic anhydride was added at once and the reaction solution was allowed to stir for an additional 6 h at ambient temperature, after which time the solvent was removed in vacuo to obtain an oil. Triturations with Et_2O resulted in 550 mg (91%) of the crude product as white crystals. The crude product was recrystallized from CH_3CN to obtain 520 mg (86%) of the desired product as white crystals: mp 181–183 °C; R_f (*n*-BuOH, HOAc, H_2O, 4:1:5, upper layer) = 0.73; ^1H NMR (300 MHz, DMSO) δ 0.82 (d, 3 H), 0.86 (d, 3 H), 1.00 (d, 3 H), 1.51 (m, 2 H), 1.61 (m, 1 H), 2.67–2.85 (m, 2 H), 4.07 (m, 2 H), 4.33 (m, 1 H), 4.81–4.91 (m, 2 H), 7.10 (d, 2 H, J = 8.5 Hz), 7.46–7.87 (m, 5 H), 7.62 (d, 1 H, J = 8.6 Hz), 8.13 (d, 1 H, J = 7.9 Hz), 8.74 (d, 1 H, J = 8.1 Hz); ^{13}C NMR (90 MHz, DMSO) δ 171.8, 171.7, 170.4, 169.3, 166.4, 134.0, 131.2, 128.0, 127.3, 80.0, 66.3, 57.8, 51.6, 50.3, 40.3, 37.0, 27.6, 24.1, 22.9, 21.5, 19.8; HR mass spectrum calcd for $C_{25}H_{39}N_4O_7$ (MH^+) m/e 507.2818, obsd m/e 507.2809.

***N*-Benzoyl-Asp-Leu-Thr-NH_2 (23).** A solution containing 480 mg (0.95 mmol) of *N*-benzoyl-Asp(β-OBu^t)-Leu-Thr-NH_2 in 20 mL of TFA/CH_2Cl_2 (1:1) was allowed to stir for 50 min at ambient temperature. At the end of 50 min the solvents were removed in vacuo and the obtained oil was triturated with Et_2O to obtain 404 mg (95%) of the crude product. The crude residue was then extensively washed with EtOAc to obtain 383 mg (90%) of the desired product 23 as white crystals: mp 167–169 °C; R_f (*n*-BuOH/HOAc/H_2O, 4:1:5, upper layer) = 0.58; ^1H NMR (360 MHz, DMSO) δ 0.82 (d, 3 H), 0.86 (d, 3 H), 1.00 (d, 3 H), 1.51 (m, 2 H), 1.60 (m, 1 H), 2.67–2.84 (m, 2 H), 4.06 (m, 2 H), 4.30 (m, 1 H), 4.79 (m, 1 H), 7.09 (d, 2 H, J = 7.5 Hz), 7.45–7.87 (m, 6 H), 8.12 (d, 1 H, J = 7.8 Hz), 8.70 (d, 1 H); ^{13}C NMR (DMSO) δ 172.0, 171.8, 170.8 166.6, 134.0, 131.3, 128.1, 127.4, 66.3, 57.9, 51.7, 50.4, 40.4, 35.6, 24.1, 23.0, 21.5, 19.8. Anal. Calcd for $C_{21}H_{30}N_4O_7 \cdot H_2O$: C, 53.84; H, 6.88; N, 11.96. Found: C, 53.94; H, 6.72; N, 11.66.

N^4-[2-Acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-N^2-benzoyl-Asn-Leu-Thr-NH_2 (21). To 178 mg (0.4 mmol) of *N*-benzoyl-Asp-Leu-Thr-NH_2 (23) and 250 mg (0.4 mmol) of the peracetylated chitobiosylamine (14) in 20 mL of dry DMF was added 156 mg (0.63 mmol) of EEDQ at ambient temperature. The reaction solution was allowed to stir for 6 h at ambient temperature, after which time, the solvent was removed in vacuo to obtain a gelatinous material. The crude material was triturated with Et_2O and $CHCl_3$ to obtain 432 mg of a white powder, which was recrystallized from CH_3CN to give 334 mg (79%) of 21 as a white solid: mp 271–273 °C; R_f (*n*-BuOH/HOAc/H_2O, 4:1:5, upper layer) = 0.60; ^1H NMR (500 MHz, DMSO) δ 0.81 (d, 3 H), 0.85 (d, 3 H), 1.00 (d, 3 H), 1.50 (m, 2 H), 1.57 (m, 1 H), 1.62 (s, 3 H), 1.74 (s, 3 H), 1.90–2.03 (m, 15 H), 2.56–2.77 (m, 2 H), 3.54–4.06 (m, 9 H), 4.28 (m, 3 H), 4.65 (d, 1 H, J = 8.3 Hz), 4.79 (m, 2 H), 4.87 (d, 1 H, J = 5.1 Hz), 4.94 (t,

1 H), 5.06 (t, 1 H), 5.13 (t, 1 H), 7.10 (d, 2 H, J = 9.5 Hz), 7.77 (m, 7 H), 7.99 (d, 1 H, J = 9.0 Hz), 8.12 (d, 1 H, J = 8.0 Hz), 8.51 (d, 1 H, J = 9.0 Hz), 8.57 (d, 1 H, J = 7.5 Hz); ^{13}C NMR (90 MHz, DMSO) δ 171.9, 171.7, 170.8, 170.0, 169.8, 169.4, 169.2, 169.2, 169.0, 169.0, 166.3, 133.9, 131.1, 127.9, 127.2, 99.9, 77.7, 75.4, 74.0, 73.4, 72.2, 70.4, 68.4, 66.2, 62.4, 61.6, 58.0, 53.8, 52.3, 51.6, 50.1, 40.3, 36.5, 24.0, 22.8, 22.3, 21.4, 20.5, 20.3, 20.2, 20.1, 19.7; HR mass spectrum calcd for $C_{48}H_{67}N_7O_{21}$ (MH^+) m/e 1066.4468, obsd m/e 1066.4438. Anal. Calcd for $C_{47}H_{67}O_{21}N_7 \cdot H_2O$: C, 52.07; H, 6.42; N, 9.04. Found: C, 52.09 H, 6.38; N, 9.20.

N^4-[2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-N^2-benzoyl-Asn-Leu-Thr-NH_2 (2b). To 135 mg (0.13 mmol) of the peracetylated glycopeptide **21** were added 27 mL of MeOH, 1 mL of H_2O, and 424 μL of Et_3N. The reaction mixture was allowed to stir for 8 h at ambient temperature, after which time the reaction mixture became clear. The solvents were removed in vacuo to obtain 100 mg (93%) of a white residue. The desired product was purified by preparative HPLC to obtain 30 mg (28%) of **2b** as white crystals: mp 272–274 °C; R_f (n-BuOH/HOAc/H_2O, 4:1:5, upper layer) = 0.26; ^{1}H NMR (500 MHz, DMSO) δ 0.81 (d, 3 H), 0.85 (d, 3 H), 1.00 (d, 3 H), 1.50 (m, 2 H), 1.57 (m, 1 H), 2.56–2.77 (m, 2 H), 3.49–4.07 (m, 9 H), 4.29 (m, 3 H), 4.65 (d, 1 H, J = 11.0 Hz), 4.88 (m, 4 H), 5.09 (m, 2 H), 7.10 (d, 2 H), 7.77 (m, 7 H), 7.99 (d, 1 H), 8.12 (d, 1 H), 8.51 (d, 1 H), 8.57 (d, 1 H); ^{13}C NMR (90 MHz, DMSO) δ 172.0, 171.8, 170.9, 170.1, 169.7, 169.1, 166.3, 133.9, 131.3, 128.1, 127.4, 102.0, 81.3, 78.6, 76.8, 76.5, 73.9, 72.7, 70.6, 66.2, 61.0, 59.9, 58.1, 55.5, 53.7, 51.6, 50.1, 40.4, 36.7, 24.0, 22.9, 22.6, 21.4, 19.9, 16.4; HR mass spectrum calcd for $C_{38}H_{57}N_7O_{16}$ (MH^+) m/e 856.3940, obsd m/e 856.3907.

Enzyme-Catalyzed Synthesis of 2b: Glycosylation of BzAsnLeuThrNH$_2$ by 1b. Chemically synthesized LDS (**1b**) was dissolved in $CHCl_3$/MeOH (2:1) at a concentration of 2 mg/mL (ca. 660 nM). To each of 15 reaction tubes was added 100 μL of the LDS solution, and the solution was evaporated to dryness using a Speed Vac vacuum centrifuge. The LDS was redissolved in a 150-μL solution containing 50 mM Tris/HCl (pH 7.5), 1% (w/v) Triton-X-100, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 5 mM DTT, 5.0% DMSO (containing 7.2 mM of BzAsnLeuThrNH$_2$), and 3 mg of P_{40} yeast microsome.[5] Each reaction mixture was incubated for 4 h at ambient temperature with vigorous shaking (250 rpm). After this time, the reactions were terminated by the addition of 3 mL of $CHCl_3$/MeOH (3:2), followed by a 30-s sonication, and the sonicated reaction mixtures were incubated on ice for 30 min. The soluble portion from each reaction was then separated from the precipitated protein by centrifugation for 15 min at 2000g and the supernatant was extracted with 0.4 mL of a 4 mM $MgCl_2$ solution. After thorough mixing, two layers were separated by centrifugation for 15 min at 2000g. The upper phase, containing water-soluble glycopeptide, was carefully removed from each of the 15 reaction extracts and combined into five portions which were concentrated using a Speed Vac. Each of the five residues, dissolved in 100 μL of 35% MeOH, was purified by HPLC (Altex liquid chromatography, Vydac column, 4.6 mm × 25 cm, C_{18}, 1.2 mL/min, 254 nm) with the 25% MeOH isocratic condition. The glycopeptide-containing fractions were combined and concentrated to dryness using a Speed Vac. The dry residue was redissolved in 500 μL of D_2O/MeOD (2:1) and the ^{1}H NMR spectrum of the glycopeptide was obtained using a GE GN500 MHz spectrometer (see supplementary material).

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Supplementary Material Available: Procedures for the synthesis of **10**, **11**, and **12** with complete spectral data, procedure for isolation and analysis of ^{3}H-labeled lipid disaccharide from yeast microsomes and OST assay using LDS (biosynthetic (^{3}H) or **1b**) as the donor substrate, and ^{1}H NMR spectra (500 MHz) of synthetic glycopeptide (**2b**) vs glycopeptide isolated from OST-catalyzed glycosylation of BzAsnLeuThrNH$_2$ by **1** (9 pages). This material is contained in many libraries on microfiche, immediately following this article in the microfilm version of the journal, or can be ordered from the ACS; see any current masthead page for ordering information.