N-Diphenylmethylene-Protected Glycosyl Acceptors. Selective β-O-Glycosylation to Form Lactosyl-*threo*-Ceramides[1]

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A series of *N*-diphenylmethylene-protected sphingosine derivatives was synthesized (e.g **3a,b** and **9a,b**). These compounds are efficient glycosyl acceptors and were shown to undergo highly β-selective glycosylation using a modification of the Koenigs–Knorr reaction previously used in this laboratory for the synthesis of O-linked glycopeptides (none of the α-anomers were detected by either ^1H or ^{13}C NMR).[1] The *N*-diphenylmethylene (Schiff base) protection imparts a favorable intramolecular hydrogen-bonding pattern ($Ph_2C{=}N{:}{\rightarrow}H{-}O{:}$). This intramolecular hydrogen-bonding enhances the nucleophilicity of the glycosyl acceptor relative to glycosyl acceptors with more conventional N-protection (*i.e.* Cbz, Boc, acyl *etc.*). The enhanced nucleophilicity allowed the glycosylation to be carried out under mild conditions (AgOTfl, CH_2Cl_2, rt overnight), and provided the corresponding β-glycosphingolipids **4a–c** and **11a–d** in excellent chemical yield (approximately 70%). After glycosylation, selective acid-catalyzed hydrolysis of the Schiff base protecting group was accomplished without cleavage of the glycosidic bond or the carbohydrate acetate protection. N-Acylation with palmitoyl chloride, followed by Zemplèn deacetylation (cat. NaOMe in MeOH), provided the two *threo*-β-lactosylceramide analogues **7a** and **7b**. These analogues possess the unnatural *threo*-configuration in the ceramide moiety and may prove useful in studies of the biosynthesis and cell surface composition of more complex glycosphingolipids.

Introduction

Glycosphingolipids are cell membrane constituents composed of various oligosaccharides bound to ceramide via a glycosidic bond. Several classes of glycosphingolipids with different carbohydrate core structures have been characterized.[2] Substitution of these core structures gives rise to glycosphingolipids with a high degree of variation in the carbohydrate moiety. In recent years a great deal of research has been directed toward better understanding of the biological roles these naturally occurring compounds play. As a result, glycosphingolipids have been found to play important roles in a host of biological functions.[2a] For example, glycosphingolipids have been shown to be involved in such processes as cell growth and differentiation cell–cell recognition and adhesion, oncogenesis, molecular recognition, and neuronal repair.[2a,3] Glycosphingolipids are also known to play a role in various lipid storage diseases.[4]

In spite of the wide range of biological functions exhibited by glycosphingolipids, they are actually relatively scarce and difficult to obtain in homogeneous form from biological sources.[5] Because of this scarcity and the difficulty of isolation, the synthesis of isomerically pure glycosphingolipids is necessary in order to further explore the functions of these compounds in biological systems.[5] In addition, synthesis of analogues of glycosphingolipids is also necessary. Interest in analogues stems from their unique biological activities relative to the naturally occurring compounds. Several classes of glycosphingolipid analogues have proven to be potent enzyme inhibitors.[6a,d] For example, Hasegawa et al. have synthesized several ganglioside analogues containing α-thioglycosides of sialic acid. These compounds were found to be potent inhibitors of sialidase activities of different subtypes of influenza viruses.[6a] Radin et al. have reported that D-*threo*-(decanoylamino)-3-morpholino-1-phenyl-1-propanol (*threo*-PDMP or *ceredase*) acts as a glucosyltransferase inhibitor.[6d] *threo*-PDMP has been shown to decrease cellular glycosphingolipid content in a variety of systems.[7]

Recently we reported a mild and stereoselective glycosylation of *N*-diphenylmethylene-protected serine and threonine esters (Scheme I).[1] This method capitalized on

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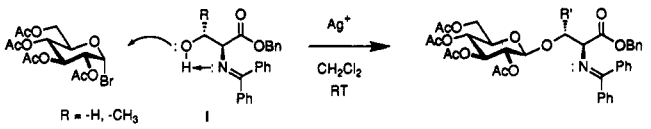
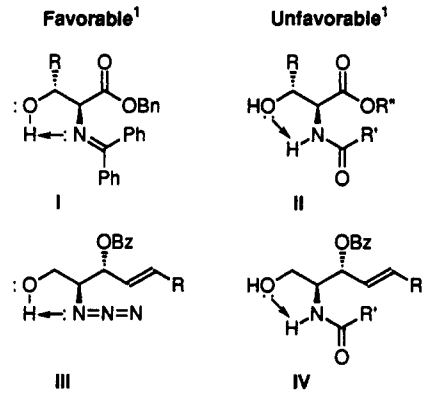
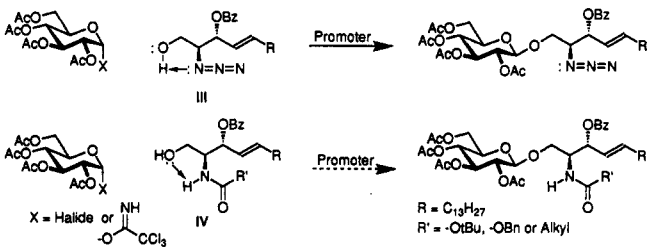
Scheme I



Scheme II



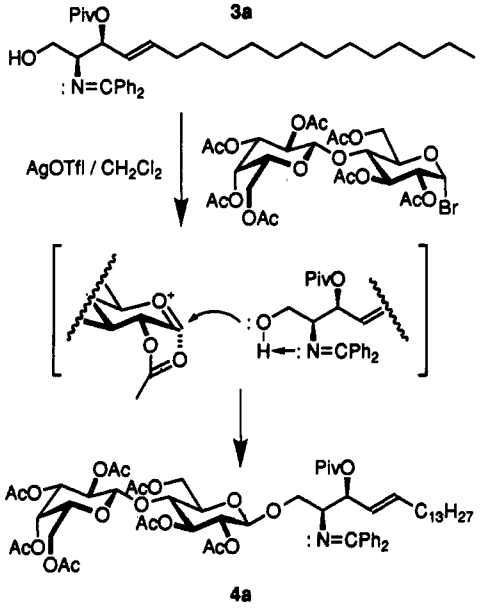
Scheme III



the enhanced nucleophilicity of the glycosyl acceptor relative to the *N*-acyl-protected glycosyl acceptors traditionally used in *O*-glycopeptide synthesis (Fmoc, Cbz, etc.).[1] Chemical yields were excellent (81–90%) and the β-glycosides were formed stereospecifically. The success of this method was attributated to the existence of a favorable hydrogen-bonding pattern in the *N*-diphenylmethylene-protected glycosyl acceptors (Scheme II, compounds **I** and **II**). Earlier work reported from this laboratory confirms the hypothesis that *N*-acyl-protected glycosyl acceptors undergo unfavorable intramolecular hydrogen-bonding, while Schiff base-protected glycosyl acceptors experience enhanced nucleophilicity due to a favorable hydrogen-bonding pattern.[1]

A similar trend exists in the reactivity of glycosyl acceptors used for glycosphingolipid synthesis (Scheme II, compounds **III** and **IV**).[8,9] Synthetic methods for glycosphingolipids have recently been reviewed.[10] Two of the methods which have been commonly used for glycosphingolipid synthesis employ either *N*-acyl-protected sphingosine derivatives (**IV**)[8] or 2-azido sphingosines (**III**)[9] as glycosyl acceptors (Scheme III). The yields for the latter case are usually quite good,[9] while those for the former are usually poorer.[8] Because of the poor reactivity exhibited by the *N*-acyl-protected glycosyl acceptors, harsh reaction conditions (reflux) are often required to effect bond formation. As a result, chemical yields suffer as well as the α- vs β-selectivity.[1]

Scheme IV



We reasoned that the difference in reactivity between 2-azido-(**III**) and *N*-acyl-protected sphingosines (**IV**) was indeed due to a difference in hydrogen-bonding. Since we have already demonstrated that *N*-diphenylmethylene-protected serine and threonine esters (**I** and **Ia**) undergo β-selective glycosylation to form O-linked glycopeptides, it seemed likely that *N*-diphenylmethylene-protected sphingosine derivatives would also undergo favorable hydrogen-bonding and thus be ideal substrates for AgOTf-catalyzed synthesis of glycosphingolipids (Scheme IV). Our recently reported method[11] for stereoselective synthesis of *N*-diphenylmethylene-protected *threo*-2-amino alcohols and *threo*-sphingosines provided easy access to glycosyl acceptors such as **3a** and **3b**. We now wish to report that *N*-diphenylmethylene-protected *threo*-sphingosines may be coupled to various glycosyl donors using the AgOTfl-catalyzed conditions we initially developed for O-linked glycopeptides. This method provides β-lactosyl-*threo*-ceramides **7a** and **7b** stereoselectively and in good chemical yield. These β-lactosyl-*threo*-ceramides are likely to possess unique biological activities relative to the *erythro* isomers and may prove useful in studies of the biosynthesis and cell surface composition of more complex glycosphingolipids.[12]

Discussion

In order to accomplish the chemistry outlined in Scheme II a suitably derivatized *N*-diphenylmethylene-protected

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(10) Gigg, J.; Gigg, R. *Topics in Current Chemistry*; Springer-Verlag: Berlin, Heidelberg, 1990; Vol. *154*, pp 77–139.

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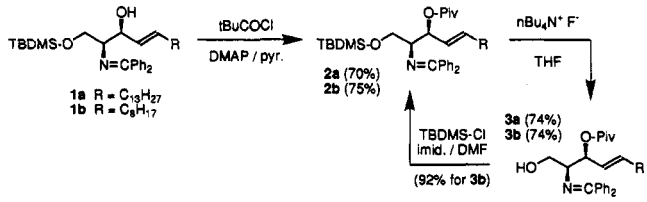
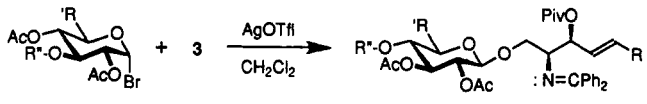
(12) Due to their *threo*-ceramide configuration, compounds **7a** and **7b** are predicted to act as glycosyl transferase inhibitors via a mechanism similar to *threo*-PDMP.[7a-c] This hypothesis is presently being tested at the Arizona Cancer Center. The search for effective glycosyltransferase inhibitors is at an early stage, and since little is known about the functional groups in the active sites of these enzymes, designing efficient inhibitors must be done empirically. See: (a) Kahn, S. H.; Matta, K. L. In *Glyconjugates*; Allen, H. J., Kisailus, E. C., Eds.; Marcel Dekker, Inc.: New York, Basel, Hong Kong, 1992; pp 361–378. (b) Vunnam, R. R.; Radin, N. S. *Chem. Phys. Lipids* **1980**, *26*, 265.

Scheme V



Scheme VI

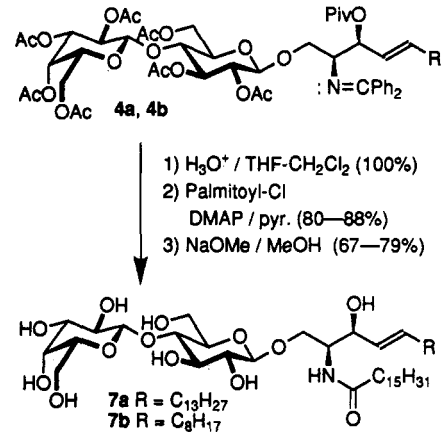


St. Mat.	R	R'	R"	Glycoside	Yield
3a	$C_{13}H_{27}$	CH_2OAc	$(AcO)_4$-Gal-β	**4a**	72%
3b	C_8H_{17}	CH_2OAc	$(AcO)_4$-Gal-β	**4b**	71%
3b	C_8H_{17}	CH_2OAc	Ac	**4c**	69%

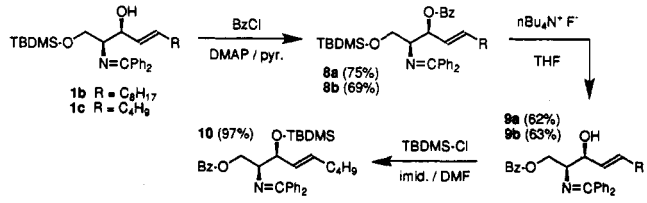
threo-sphingosine was needed (Scheme V). When compounds **1a** and **1b** were treated with pivaloyl chloride and DMAP in refluxing pyridine, compounds **2a** and **2b** were obtained in approximately 70% yield. The TBDMS protection could be cleaved using standard conditions (TBAF in THF) to provide compounds **3a** and **3b** in good yield.[13] Since acyl migration is a problem often associated with TBAF cleavage of silyl ethers in the presence of *O*-acyl protecting groups, compound **3b** was resilylated to determine that the bulky pivalate had not migrated. Comparison of both the ^1H and ^{13}C NMR spectra of the resilylated product revealed that it was identical to compound **2b**. Thus no migration had occurred (Scheme V). When compounds **3a** and **3b** were treated with various glycosyl bromides using the AgOTfl method previously reported for O-linked glycopeptides,[1] β-glycosphingolipids **4a–c** were obtained (Scheme VI). The chemical yields for **4a–c** were good (approximately 70%) and the stereoselectivity was excellent (α-anomers could not be detected in the crude reaction mixtures by either ^1H or ^{13}C NMR spectroscopy).

The excellent β-stereoselectivity observed in the glycosylation is due to the fact that very mild reaction conditions can be used for the glycosylation. The α-glycosides are generally more stable due to the anomeric effect and are thus the thermodynamically-favored reaction products. This, in turn, is most probably due to the increased nucleophilicity of the structures **I** and **III** relative to the "unfavorable" structures **II** and **IV** (Scheme II). In these unfavorable structures, the hydroxyl lone pairs are less available to act as nucleophiles due to their involvement in an intramolecular hydrogen bond (HO:→H–NC=O). In the case of the imino alcohols **I** and the Schiff base-protected β-hydroxy-α-amino esters **II**, the imine lone pair may be regarded as an internal base which is poised in a geometrically favorable position[17] so that it can remove the incipient proton in any nucleophilic reactions involving the hydroxyl (C=N:→H–O$^+$). It is not clear at this point

Scheme VII



Scheme VIII



if Ag^+ is chelated between the imine and hydroxyl during the Koenigs–Knorr reaction, but there is some circumstantial evidence for this: Base-catalyzed O-alkylation of α-imino-β-hydroxy esters **I** with BnBr has proven to be quite difficult. Whereas, the Ag^+-catalyzed alkylation of the same substrates proceeded without difficulty.[18]

The Schiff base protecting groups of compounds **4a** and **4b** were easily removed using 10% TFA in THF/CH_2Cl_2 (1:1). This hydrolysis was rapid (approximately 4 h) and provided compound **5** in quantitative yield without affecting the glycosidic bond (Scheme VII). The resulting mixture of free amine and benzophenone could be separated via flash chromatography to provide pure **5** (from **4b**). Alternatively, the resulting mixture could be directly subjected to the N-acylation chemistry (palmitoyl chloride, DMAP, pyridine) and the benzophenone separated later, as was done in the synthesis of **6a**. Compounds **6a** and **6b** were obtained in 88 and 80% yields, respectively, after acylation with palmitoyl chloride in pyridine (Scheme VII). The acetate and pivalate protecting groups were removed using standard Zemplen-deacetylation methodology (NaOMe/anhydrous MeOH/50 °C/19 h) to provide compounds **7a** and **7b** in 78 and 67% yields, respectively.

The novel 3-OH *N*-diphenylmethylene-protected glycosyl acceptors (**9a** and **9b**) were synthesized by substituting the 3-*O*-pivalate protection (Scheme V, compound **2**) with 3-*O*-benzoate (Scheme VIII, compound **8**). When the TBDMS groups of compounds **8a** and **8b** were cleaved with TBAF, the benzoate migrated to the 1-OH. This migration was detected by resilylating compound **9b**. The ^1H and ^{13}C NMR spectra of compound **9b** were consistent with migration. In addition, ^1H and ^{13}C NMR spectra of **9b** and **10** were clearly distinct. When compounds **9a** and **9b** were glycosylated, 3′-β-glycosyl-*threo*-ceramides (**11a–d**) were obtained in good chemical yield and with excellent stereoselectivity (Scheme IX). As with compounds **4a–c**,

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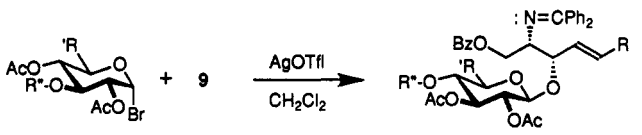
(17) (a) Menger, F. M.; Chow, J. F.; Kaiserman, H.; Vasquez, P. C. *J. Am. Chem. Soc.* **1983**, *105*, 4996. (b) Gandour, R. *Tetrahedron Lett.* **1974**, 295. (c) Scheiner, F. *Acc. Chem. Res.* **1985**, *18*, 174.

(18) Wijayaratne, T.; Polt, R., *unpublished results.*

Scheme IX



St. Mat.	R	R'	R''	Glycoside	Yield
9a	C_8H_{17}	H	Ac	11a	56%
9a	C_8H_{17}	CH_2OAc	Ac	11b	63%
9a	C_8H_{17}	CH_2OAc	$(AcO)_4$-Gal-β	11c	71%
9b	C_4H_9	CH_2OAc	$(AcO)_4$-Gal-β	11d	60%

the α-anomers were not detected by either ^1H or ^{13}C NMR spectroscopy. 2-D ^1H NMR data (COSY-45°) for compounds **4a–c** and **11a–d** were obtained and are reported in Table I. ^{13}C NMR data for compounds **4a–c** and **11a–d** are reported in Table II.

Conclusions

N-Diphenylmethylene-protected sphingosine derivatives provide a useful alternative to the *N*-acyl-protected sphingosine (**IV**)[8] or the 2-azido sphingosine derivatives (**III**)[9] typically employed as glycosyl acceptors in glycosphingolipid synthesis. These more reactive glycosyl acceptors were easily synthesized via an elaboration of our recently reported[11] *threo*-selective method for Schiff base protected β-amino alcohols and undergo highly β-selective glycosylation under very mild conditions (AgOTfl, CH_2Cl_2, rt) to provide novel glycosphingolipid analogues in excellent chemical yield.

Experimental Section

General Methods. All air- and moisture-sensitive reactions were performed under an argon atmosphere in flame-dried reaction flasks using standard Schlenk methodology. All solvents were dried over standard drying agents[14] and freshly distilled prior to use. For flash chromatography,[15] 400–230 mesh silica gel 60 (E. Merck No. 9385) was employed. All compounds described were characterized by ^1H- and ^{13}C-NMR. The ^1H- and ^{13}C-NMR spectra were recorded at 250 and 62.9 MHz, respectively. COSY spectra were obtained at 500 MHz.

Pivaloylation. A solution of **1b** (507 mg, 1.0 mmol) in pyridine (4 mL) was reacted with pivaloyl chloride (4 mmol, 4 equiv) and DMAP (cat.) at 50 °C for 14 h. The reaction was then quenched with $NaHCO_3$, extracted with EtOAc, and dried over K_2CO_3. Pure product (**2b**) was isolated via flash chromatography (435 mg, 0.75 mmol, 75%).

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-1-*O*-(*tert*-butyldimethylsilyl)-3-*O*-pivaloyl-4-octadecene-1,3-diol (2a): oil, 925 mg, 1.4 mmol, 70% yield, chromatography eluent; 5% EtOAc in petroleum ether; $[\alpha]_D = +2.4°$ (c = 1.2, $CHCl_3$). Anal. Calcd for $C_{42}H_{67}NO_3Si$: C, 76.19; H, 10.20. Found: C, 76.01; H, 10.34.

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-1-*O*-(*tert*-butyldimethylsilyl)-3-*O*-pivaloyl-4-tridecene-1,3-diol (2b): oil, 435 mg, 0.75 mmol, 75% yield; chromatography eluent, 5% EtOAc in petroleum ether; $[\alpha]_D = +1.9°$ (c = 1, $CHCl_3$). Anal. Calcd for $C_{37}H_{57}NO_3Si$: C, 75.08; H, 9.71. Found: C, 75.06; H, 9.65.

Benzoylation. A solution of 177 mg (0.39 mmol) of compound **1c** in 0.4 mL of dry pyridine was reacted with a catalytic amount of DMAP and 50 μL of PhCOCl under argon at rt for 16 h. Solvent removal and flash chromatography provided pure product (**8b**): oil, 148 mg, 0.27 mmol, 69% yield; chromatography eluent, 10% EtOAc in petroleum ether).

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-3-*O*-benzoyl-1-*O*-(*tert*-butyldimethylsilyl)-4-tridecene-1,3-diol (8a): oil, 462 mg, 0.75 mmol, 75% yield; chromatography eluent, 10% EtOAc in petroleum ether. Anal. Calcd for $C_{39}H_{53}NO_3Si$: C, 76.55; H, 8.73. Found: C, 76.63; H, 8.79.

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-3-*O*-benzoyl-1-*O*-(*tert*-butyldimethylsilyl)-4-nonene-1,3-diol (8b): oil, 148 mg, 0.267 mmol, 69% yield. Anal. Calcd for $C_{35}H_{45}NO_3Si$: C, 75.63; H, 8.16. Found: C, 75.50; H, 8.26.

Synthesis of Glycosyl Acceptors. An example is provided. All other desilylations were performed in a similar fashion. Yields and chromatography eluents are listed in parentheses. A solution of **2b** (422 mg, 0.73 mmol) in 1 mL of dry THF was reacted with 1.1 equiv of TBAF (800 μL of 1.0 M TBAF in THF) for 2 h at rt. The reaction was passed through a plug of SiO_2 and solvent was removed. Pure product (**3b**) was isolated (74% yield, 259 mg, 0.54 mmol, eluent, 10% EtOAc in petroleum ether).

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-3-*O*-pivaloyl-4-octadecene-1,3-diol (3a): oil, 431 mg, 0.74 mmol, 74% yield, eluent, 10% EtOAc in petroleum ether; $[\alpha]_D = -37.5°$ (c = 1.3, $CHCl_3$). Anal. Calcd for $C_{36}H_{53}NO_3$: C, 78.93; H, 9.75. Found: C, 78.84; H, 9.70.

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-3-*O*-pivaloyl-4-tridecene-1,3-diol (3b): oil, 74% yield, 259 mg, 0.54 mmol; eluent, 10% EtOAc in petroleum ether. Anal. Calcd for $C_{31}H_{43}NO_3$: C, 77.95; H, 9.07. Found: C, 77.87; H, 9.15.

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-1-*O*-benzoyl-4-tridecene-1,3-diol (9a): oil, 212 mg, 0.427 mmol, 62% yield; eluent, 15% EtOAc in petroleum ether. Anal. Calcd for $C_{33}H_{39}NO_3$: C, 79.64; H, 7.90. Found: C, 79.80; H, 7.76.

(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-1-*O*-benzoyl-4-nonene-1,3-diol (9b): oil, 25 mg, 0.06 mmol, 63% yield; eluent, 10% EtOAc in petroleum ether; $[\alpha]_D = +14.5°$ (c = 1.1, $CHCl_3$). Anal. Calcd for $C_{29}H_{31}NO_3$: C, 78.88; H, 7.08. Found: C, 78.89; H, 7.20.

Pivalates. A solution of compound **3b** (69 mg, 0.145 mmol) in 1 mL of dry DMF was reacted with TBDMSCl (45 mg, 0.30 mmol) and imidazole (30 mg, 0.44 mmol) at rt for 20 h before pouring onto EtOAc and extracting with two volumes of 0.1% $NaHCO_3$ and two volumes of saturated $NaHCO_3$. After drying over K_2CO_3, pure product (oil, 92% yield, 77 mg, 0.133 mmol) was obtained after solvent removal and had ^1H and ^{13}C NMR spectra identical to that of **2b**.

Benzoates. A solution of compound **9a** (123 mg, 0.25 mmol) in 1 mL of dry DMF was reacted with TBDMSCl (74 mg, 0.5 mmol, 2 equiv) and imidazole (50 mg, 0.73 mmol, 2.9 equiv) at rt for 48 h and worked up as above to provide pure **10** (oil, 97% yield, 147 mg, 0.241 mmol). Analysis of the ^1H, ^{13}C, and IR spectra clearly showed that migration had occurred (*i.e.* spectra were different from spectra for **8a**).

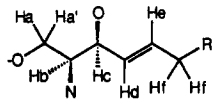
(2*S*,3*S*,4*E*)-2-[*N*-(Diphenylmethylene)amino]-1-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-4-tridecene-1,3-diol (10): oil, 97% yield, 147 mg, 0.241 mmol; $[\alpha]_D = +16.4°$ (c = 1, $CHCl_3$). Anal. Calcd for $C_{39}H_{53}NO_3Si$: C, 76.55; H, 8.73. Found: C, 76.45; H, 8.82.

β-Selective Glycosylation. The general procedure for glycosylation is illustrated by example. All other glycosylations were carried out in similar fashion. To a stirred suspension of **9a** (206 mg, 0.41 mmol), freshly prepared 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl bromide (209 mg, 0.616 mmol, 1.5 equiv), and flame-dried, powdered 4-Å sieves (400 mg) in 10 mL of CH_2Cl_2 under argon was added solid silver triflate (AgOTfl) (158 mg, 0.616 mmol, 1.5 equiv) in small portions over 20 min. The mixture was protected from light and stirred at room temperature overnight. Silver salts were then removed by filtering through a pad of Celite. After solvent removal, flash chromatography (30% EtOAc in petroleum ether) provided pure **11a** (oil, 56% yield, 173 mg, 0.229 mmol).

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1')-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-3-*O*-pivaloyl-4-octadecene-1,3-diol (4a)**: amorphous white solid, 432 mg, 0.37 mmol, 72% yield; eluent, 40% EtOAc in petroleum ether; $[\alpha]_D = +24.6°$ (c = 0.4, $CHCl_3$). Anal. Calcd for $C_{62}H_{87}NO_{20}$: C, 63.85; H, 7.52. Found: C, 63.70; H, 7.54.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1')-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-3-*O*-pivaloyl-4-tridecene-1,3-diol (4b)**: amorphous white solid, 433 mg, 0.40 mmol, 71%

Table I. ^{1}H-NMR Data for Glycosides (500 MHz, $CDCl_3$)



chemical shifts (ppm)				coupling constants (Hz)				chemical shifts (ppm)							coupling constants (Hz)						
no**	4a	4b	4c	$J_{H,H}$	4a	4b	4c	no**	6a	6b	11a	11b	11c	11d	$J_{H,H}$	6a	6b	11a	11b	11c	11d
H-1	4.55	4.55	4.55	1,2	7.3	7.4	7.9	H-1	4.40	4.41	4.50	4.49	4.47	4.47	1,2	7.9	7.9	6.9	8.0	8.0	8.0
H-2	4.87	4.88	4.95	2,3	8.7	8.8	9.5	H-2	4.87	4.88	4.94	5.13*	4.93	4.93	2,3	9.6	9.6	8.7	nd	9.3	9.2
H-3	5.13	5.13	5.14	3,4	8.8	8.8	9.5	H-3	5.17	5.18	5.08	5.13*	5.10	5.11	3,4	9.4	9.3	8.7	nd	9.3	9.2
H-4	3.78*	3.77*	5.02	4,5	9.6	9.8	9.7	H-4	3.78	3.78	4.94	5.13*	3.78	3.78	4,5	9.4	9.5	9.0	nd	9.4	9.4
H-5	3.5	3.5	3.52	5,6a	2.0	2.1	2.5	H-5	3.58	3.56	3.24	3.58	3.49	3.49	4,5a	–	–	5.2	–	–	–
H-6a	4.34	4.34	3.95	5,6b	5	5	4.6	H-5a	–	–	4.05	–	–	–	5,5a	–	–	11.8	–	–	–
H-6b	4.03	4.01	4.13	6a,6b	12	11.8	12.2	H-6a	4.46	4.46	–	4.03	4.37	4.38	5,6a	2.1	2.0	–	2.2	2	2
H-1′	4.32	4.32	–	1′,2′	7.9	7.9	–	H-6b	4.06	4.07	–	4.23	4.05	4.06	5,6b	4.9	4.9	–	4.7	5.2	5
H-2′	5.07	5.07	–	2′,3′	10.4	10.4	–	H-1′	4.46	4.47	–	–	4.46	4.45	6a,6b	12	12	–	12	11.9	11.8
H-3′	4.90	4.90	–	3′,4′	3.6	3.5	–	H-2′	5.09	5.10	–	–	5.1	5.09	1′,2′	7.9	7.9	–	–	7.9	7.9
H-4′	5.31	5.30	–	4′,5′	1.2	1	–	H-3′	4.94	4.95	–	–	4.93	4.94	2′,3′	10.4	10.4	–	–	10.4	10.4
H-5′	3.78*	3.77*	–	5′,6′	1.6	2.4	–	H-4′	5.36*	5.36*	–	–	5.33	5.33	3′,4′	3.4	3.4	–	–	3.5	3.5
H-6′a	4.06	4.06	–	6′a,6′b	6.9	7	–	H-5′	3.86*	3.86*	–	–	3.84	3.84	4′,5′	nd	nd	–	–	1	1
H-6′b	4.06	4.06	–	a,a′	9.7	9.8	–	H-6′a	4.16	4.16	–	–	4.10	4.10	5′,6′a	6.3	6.4	–	–	6.5	6.5
H-a	3.64	3.64	3.71*	a,b	4.3	4.2	9.5	H-6′b	4.09*	4.08*	–	–	4.06	4.07	5′,6′b	nd	nd	–	–	7.5	7.6
H-a′	3.94	3.94	3.91	a′,b	7.2	7.2	6	H-a	3.44	3.45	4.37*	4.36	4.34*	4.34*	6′a,6′b	11.1	11.2	–	–	11.2	11.2
H-b	3.78*	3.77*	3.71*	b,c	6.8	7	6.6	H-a′	3.86*	3.86*	4.37*	4.36	4.34*	4.34*	a,a′	10	10	nd	nd	nd	nd
H-c	5.37	5.37	5.38	d,c	7.4	7.8	7.4	H-b	4.26*	4.26*	3.94*	4.0*	4.0*	4.0*	a,b	5.8	5.8	nd	nd	nd	nd
H-d	5.26	5.26	5.26	d,e	15.4	15.4	15.4	H-c	5.36*	5.36*	4.27	4.29	4.26	4.26	a′,b	nd	nd	nd	nd	nd	nd
H-e	5.68	5.68	5.66	e,f	7	7	7	H-d	5.36*	5.36*	5.49	5.54	5.51	5.55	b,c	nd	nd	8.4	8.4	8	8.3
								H-e	5.72*	5.69*	5.75	5.77	5.74	5.78	d,c	nd	nd	8.6	8.2	8	8.3
															d,e	15.4	15.4	15.4	15.2	15.3	15.4
															e,f	6.8	6.8	7	7	7	6.8

* Part of multiplet centered at δ as reported in this table. ** Protons a-e refer to the sphingosine moiety and correspond to carbons 1–5, respectively.

Table II. ^{13}C-NMR Shifts for Glycosides[a]

	4a	4b	4c	6a	6b	11a	11b	11c	11d
C-1	99.71	99.86	100.70	100.61	100.62	100.27	100.06	99.79	99.85
C-2	70.62	70.75	71.21	70.58	70.60	70.68	70.91	70.85	70.91
C-3	71.60	71.71	72.97	70.88	70.88	71.72	73.03	71.38	71.44
C-4	75.94	76.06	68.24	76.00	76.00	68.98	68.26	76.27	76.30
C-5	72.12	72.21	71.65	71.55	71.58	61.95	71.50	72.31	72.38
C-6	61.91	61.98	61.72	61.79	61.80	–	62.00	62.20	62.23
C-1′	100.79	100.96	–	100.99	100.98	–	–	101.03	101.09
C-2′	68.76	68.85	–	68.95	68.98	–	–	68.97	69.03
C-3′	70.24	70.36	–	70.56	70.60	–	–	70.49	70.56
C-4′	66.39	66.44	–	66.50	66.53	–	–	66.47	66.50
C-5′	73.00	73.09	–	72.44	72.44	–	–	73.09	73.15
C-6′	60.57	60.62	–	60.71	60.72	–	–	60.65	60.70
C-a	69.44	69.62	70.11	68.16	68.16	65.63	65.52	65.51	65.59
C-b	64.30	64.03	64.70	51.29	51.31	63.98	63.62	63.68	63.74
C-c	73.87	74.03	74.06	72.52	72.54	83.26	84.68	84.26	84.38
C-d	135.53	135.73	135.75	136.34	136.34	135.70	135.90	135.87	135.91

[a] Published data was useful in making correct assignments.[1,16] C-a to C-d refer to carbons 1–4 (respectively) of the sphingosine moiety.

yield; eluent, 50% EtOAc in petroleum ether; $[\alpha]_D = -1.3°$ (c = 2.8, $CHCl_3$). Anal. Calcd for $C_{57}H_{77}NO_{20}$: C, 62.45; H, 7.08. Found: C, 62.60; H, 7.18.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 1′)-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-3-*O*-pivaloyl-4-tridecene-1,3-diol (4c)**: amorphous white solid, 244 mg, 0.302 mmol, 69% yield; eluent, 30% EtOAc in petroleum ether; $[\alpha]_D = +1.7°$ (c = 0.5, $CHCl_3$). Anal. Calcd for $C_{45}H_{61}NO_{12}$: C, 66.90; H, 7.61. Found: C, 66.75; H, 7.70.

***O*-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl)-(1 → 3)-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-1-*O*-benzoyl-4-tridecene-1,3-diol (11a)**: oil, 173 mg, 0.229 mmol, 56% yield; eluent, 30% EtOAc in petroleum ether; $[a]_D = -29.6°$ (c = 0.72, $CHCl_3$); MS (CI-ammonia) 756 (M + 1), 292 (bp). Anal. Calcd for $C_{44}H_{53}NO_{10}$: C, 69.91; H, 7.07. Found: C, 69.95; H, 7.01.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-1-*O*-benzoyl-4-tridecene-1,3-diol (11b)**: oil, 269 mg, 0.325 mmol, 63% yield; eluent 30% EtOAc in petroleum ether; $[\alpha]_D = -12.5°$ (c = 0.8, $CHCl_3$). Anal. Calcd for $C_{47}H_{57}NO_{12}$: C, 68.18; H, 6.94. Found: C, 68.10; H, 6.97.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-1-*O*-benzoyl-4-tridecene-1,3-diol (11c)**: amorphous white solid, 284 mg, 1.26 mmol, 71% yield; eluent, 50% EtOAc in petroleum ether; $[\alpha]_D = -16.4°$ (c = 0.8, $CHCl_3$). Anal. Calcd for $C_{59}H_{73}NO_{20}$: C, 63.49; H, 6.59. Found: C, 63.50; H, 6.48.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-(2*S*,3*S*,4*E*)-2-[*N*-(diphenylmethylene)amino]-1-*O*-benzoyl-4-nonene-1,3-diol (11d)**: amorphous white solid, 79 mg, 0.074 mmol, 60% yield; eluent, 50% EtOAc in petroleum ether. Anal. Calcd for $C_{55}H_{65}NO_{20}$: C, 62.31; H, 6.18. Found: C, 62.24; H, 6.12.

Hydrolysis of Schiff Bases. The general procedure for quantitative cleavage of the Schiff base is provided in the example. To a solution of **4b** (100 mg, 0.09 mmol) in 2 mL of THF/CH_2Cl_2 (1:1) were added 200 μL of CF_3CO_2H and 5 drops of H_2O. The reaction stirred at rt until TLC indicated cleavage was complete (4 h). The reaction was quenched by pouring into saturated $NaHCO_3$. The aqueous layer was extracted with several volumes of CH_2Cl_2 and dried over K_2CO_3. Flash chromatography provided

pure **5**: oil, 86 mg, 0.092 mmol, 100% yield; eluent, 90% EtOAc in hexanes.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1′)-(2*S*,3*S*,4*E*)-2-amino-3-*O*-pivaloyl-4tridecene-1,3-diol (5)**: oil, 86 mg, 0.092 mmol, 100% yield; eluent, 90% EtOAc in hexanes; $[\alpha]_D = -4.5°$ (c = 3.5, $CHCl_3$).

N-Acylation. The general procedure for acylation of the amino group is given. To a stirred solution of azeotropically dried compound **5** (86 mg, 0.09 mmol) and DMAP (cat.) in 1 mL of pyridine (dried over CaH_2) was added palmityl chloride (32 mg, 0.12 mmol, 1.3 equiv). Stirring was continued for 12 h at rt until TLC revealed complete consumption of starting material. The reaction was quenched by pouring onto $Et_2O/NaHCO_3$ (saturated). Normal workup and flash chromatography provided pure **6b**: oil, 86 mg, 0.07 mmol, 80%; eluent, 50% EtOAc in hexanes.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1)-(2*S*,3*S*,4*E*)-2-[*N*-(hexadecanoyl)amino]-3-*O*-pivaloyl-4-octadecene-1,3-diol (6a)**: oil, 94 mg, 0.076 mmol, 88% yield; eluent, 50% EtOAc in hexanes; $[\alpha]_D = -1.6°$ (c = 3, $CHCl_3$). Anal. Calcd for $C_{65}H_{109}NO_{21}$: C, 62.93; H, 8.86. Found: C, 62.85; H, 8.86.

***O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→1′)-(2*S*,3*S*,4*E*)-2-[*N*-(hexadecanoyl)amino]-3-*O*-pivaloyl-4-tridecene-1,3-diol (6b)**: oil, 86 mg, 0.073 mmol, 80% yield, chromatography eluent, 50% EtOAc in hexanes; $[\alpha]_D = -2.3°$ (c = 2.6, $CHCl_3$). Anal. Calcd for $C_{60}H_{99}NO_{21}$: C, 61.57; H, 8.53. Found: C, 61.52; H, 8.39.

Zemplèn Deacetylation. The general procedure for Zemplen deacetylation is provided. A solution of **6b** (68 mg, 0.058 mmol) and NaOMe (8.5 mg, 0.16 mmol, 2.7 equiv) in anhydrous MeOH (1 mL) was refluxed at 50 °C for 19 h. Flash chromatography provided 30.4 mg of pure **7b**: oil, 30.4 mg, 0.038 mmol, 66% yield; eluent, 30% MeOH in CH_2Cl_2.

***O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→1′)-(2*S*,3*S*,4*E*)-2-[*N*-(hexadecanoyl)amino]-4-octadecene-1,3-diol (7a)**: white amorphous solid, 32 mg, 0.049 mmol, 78% yield; eluent, 25% MeOH in CH_2Cl_2; $[\alpha]_D = -23.4°$ (c = 0.24, pyridine); MS (CI) 862 (M + 1), 264 (bp). Anal. Calcd for $C_{46}H_{87}NO_{13}$: C, 64.08; H, 10.17. Found: C, 64.12; H, 10.27.

***O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→1′)-(2*S*,3*S*,4*E*)-2-[*N*-(hexadecanoyl)amino]-4-tridecene-1,3-diol (7b)**: white amorphous solid, 31 mg, 0.039 mmol, 67% yield; eluent, 30% MeOH in CH_2Cl_2; $[\alpha]_D = -58.9°$ (c = 0.1, pyridine); MS (CI) 792 (M + 1), 194 (bp). Anal. Calcd for $C_{41}H_{77}NO_{13}$: C, 62.17; H, 9.80. Found: C, 62.24; H, 9.75.

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Supplementary Material Available: Additional characterization data for each compound (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.