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Synthesis and Evaluation of Sphinganine Analogues of KRN7000 and OCH

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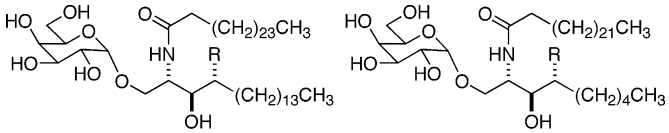

The phytosphingosine-containing α-galactosylceramides (α-GalCers), KRN7000 and OCH, have been shown to activate NKT cells via interaction with CD1d, a member of the CD1 family of antigen presenting proteins. Evidence from KRN7000 stimulation of NKT cells suggests that α-GalCers may have applications in the treatment or prevention of a range of viral, bacterial, and autoimmune conditions. Moreover, OCH, a truncated analogue of KRN7000, appears to induce a T_H2 bias, which could have implications for the treatment of autoimmune and inflammatory conditions. We have prepared the direct sphinganine-containing analogues of KRN7000 and OCH, **1** and **2**, and found them to be comparable in activity to the parent compounds in inducing the release of IL-2, IL-4, and IFNγ. In addition, compound **2** leads to a cytokine bias similar to that seen with OCH. This is significant because sphinganines are more easily accessed than phytosphingosines, which should facilitate SAR studies.

Introduction

Studies have identified the CD1 family of proteins as novel, antigen-presenting molecules encoded by genes located outside of those coding for major histocompatibility complexes.[1] CD1d is a member of the CD1 family that has been shown to be an antigen presenting protein for the T cell receptors (TCR) of natural killer (NK) T cells.[2−4] CD1d activated NKT cells have been demonstrated to play a role in a variety of immune responses. The α-galactosylceramide (α-GalCer), KRN7000, has proved to be an invaluable tool for enhancing understanding of the role of CD1d antigen presentation and NKT cell function. Activation of NKT cells in vivo by administering KRN7000 to mice leads to the production of several cytokines, including IFNγ and IL-4. KRN7000 stimulation has provided evidence suggesting that NKT cell-mediated pathways may be used to inhibit hepatitis B virus replication[5,6] or protect against diabetes,[7−9] malaria,[10−12] and tuberculosis.[12] A truncated analogue of

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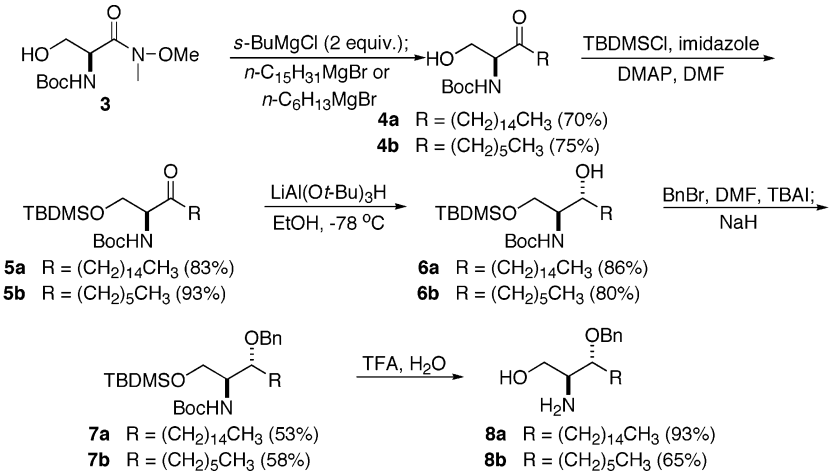
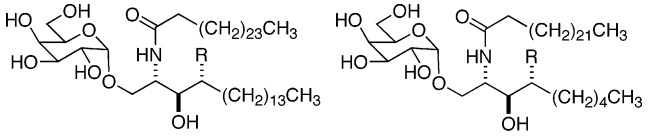
SCHEME 1



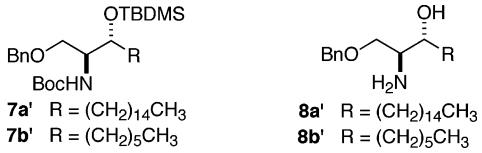
KRN7000, OCH, was found to selectively induce IL-4, as opposed to IFNγ, and to offer protection in mice against experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis.[13] More recently, OCH has been shown to offer protection against diabetes in NOD mice[14] and against collagen induced arthritis.[15] These results illustrate the importance of the CD1d pathway of immunomodulation, and they also demonstrate the potential for using structural analogues of KRN7000 to modulate the responses of NKT cells in potentially useful ways.

To further elucidate the role of CD1d antigen presentation and NKT cell responses, access to glycosyl ceramide tools is critical. In addition, the development of more extensive libraries of α-GalCer structural variants would be of great value in searching for compounds with optimal bioactivities for a range of applications. Both KRN7000 and OCH have a phytosphingosine (aminotriol) sphingoid base moiety. The preparation of phytosphingosines is not trivial.[16] In contrast, sphinganines (aminodiols) are generally more readily prepared.[17] If sphinganine-containing α-GalCers were shown to mirror the potency and types of activation seen with phytosphingosine-containing compounds, access to biological tools and targeted structure−activity studies would be more straightforward. In this paper we report the preparation and initial biological evaluation of the direct sphinganine analogues **1** and **2** of KRN7000 and OCH, respectively.



Results and Discussion

We have recently reported straightforward access to sphinganines and C3 protected sphinganines using serine-derived Weinreb amide **3** as a template.[18] Thus, the sphingoid bases **8a** and **8b** required for the synthesis of α-GalCers **1** and **2** were prepared as shown in Scheme 1. Double deprotonation of **3** with the sacrificial base *sec*-butylmagnesium chloride under conditions previously described,[18,19] followed by addition of *n*-pentadecylmagnesium bromide or *n*-hexylmagnesium bromide (prepared from the corresponding alkylbromides), provided aminohydroxyketone **4a** or **4b**, respectively. Silyl protection of **4** gave ketones **5**. Diastereoselective reduction of **5** with lithium tri-*tert*-butoxyaluminum hydride under conditions described by Hoffman[20] produced *anti*-alcohols **6** in excellent yields. Benzyl protection of the secondary alcohol, followed by cleavage of both the Boc and silyl protecting groups with trifluoroacetic acid (TFA), gave the required protected sphinganines **8a** and **8b**.



The modest yield in the benzylation of **6** was found to be a result of migration of the TBDMS moiety (∼30% migration) to the secondary alcohol (giving **7′**) under the reaction conditions. Although **7a** could be separated with difficulty from **7a′** (and **8a** could be readily separated from **8a′**), **7b** and **7b′** were essentially inseparable, as were **8b** and **8b′**. However, following acylation (Scheme 3), pure **11b** was easily isolated. The yield quoted for **7b** is based on the ^{1}H NMR ratios of **7b/7b′** (2:1) and the combined isolated yield (86%). We have found that the unwanted migration of the silyl group can be circum-

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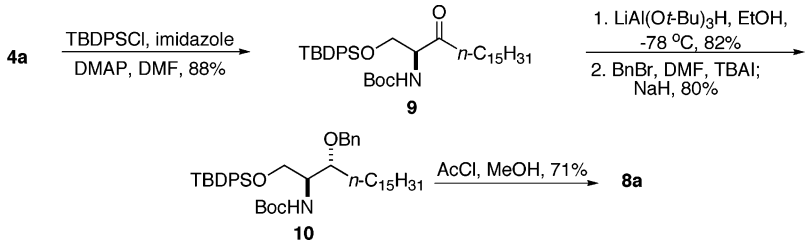

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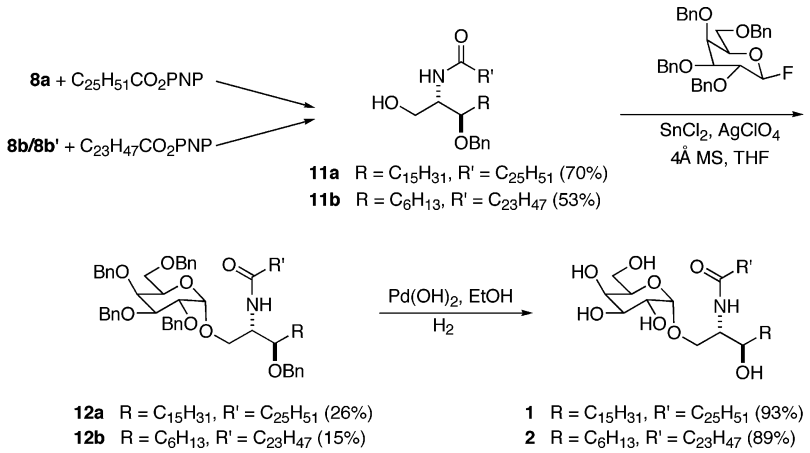

vented by employing TBDPSCl, rather than TBDMSCl, as illustrated for the conversion of **4a** to **8a** in Scheme 2. Thus, after protection of **4** with a TBDPS group, reduction and benzylation of **9** proceeded in a straightforward manner. No significant silyl migration was observed in the benzylation step. One-step cleavage of the silyl- and Boc-protecting groups of **10** was achieved by using in situ generation of HCl.

Sphinganines **8** were converted to α-GalCer analogues **1** and **2** as shown in Scheme 3. Compound **8a** was acylated with the *p*-nitrophenyl (PNP) ester of hexacosanoic acid, and **8b** was similarly reacted with the corresponding ester of tetracosanoic acid. The resulting ceramides **11a** and **11b**, respectively, were glycosylated with use of β-tetrabenzylgalactosyl fluoride in the presence of silver perchlorate and tin(II) chloride. Although the α-selectivity was high, the yields were low. Global deprotection of **12a** and **12b** provided analogues **1** and **2**, respectively.

In identifying KRN7000 as a lead clinical candidate several related sphinganine analogues were prepared and assayed.[21,22] Although the sphinganine-containing compounds showed tumor inhibitory properties and comparable abilities to stimulate mouse spleen cells, they were not as potent as the aminotriols. More recently, two of the Kirin aminodiols (C24 acyl/C18 sphinganine and C14 acyl/C16 sphinganine) were assayed for their ability to induce the release of IL-2 in mouse $V_\alpha14^+$ NKT cell hybridomas,[23] a response more directly related to CD1d-mediated effects. The longer chain sphinganine-containing α-GalCer elicited a greater level of response than the shorter analogue, but not as great a response as KRN7000, consistent with the trend seen in evaluation of T cell proliferation. However, the direct analogue **1** of KRN7000 was not evaluated.[24] Moreover, in efforts to gain an understanding of the role of CD1d in immune responses, it is also appropriate to evaluate other markers related to NKT-mediated responses. Since KRN7000 is the α-GalCer used most widely in studies related to CD1d, and since OCH has been shown to display both similarities and differences compared to KRN7000, we decided to compare the sphinganine analogues **1** and **2** of KRN7000 and OCH for their ability to induce the release of IL-2, IL-4, and IFNγ. These cytokines are well-known products of activated NKT cells and are frequently used in evaluating α-GalCers.

KRN7000 was available to us, and OCH[25] was prepared by using a combination of literature procedures, as shown in Scheme 4. Garner's aldehyde **13**, prepared in three steps as described by Taylor and co-workers,[26] was converted by minor modifications of the procedure of Imashiro et al.[27] to *ribo*-phytosphingosine **14**. Acylation gave OCH ceramide **15**. Following an approach similar to that used by Savage and co-workers in preparing a biotinylated KRN7000 from its ceramide,[28] global protection of **15**, followed by selective deprotection and glyco-

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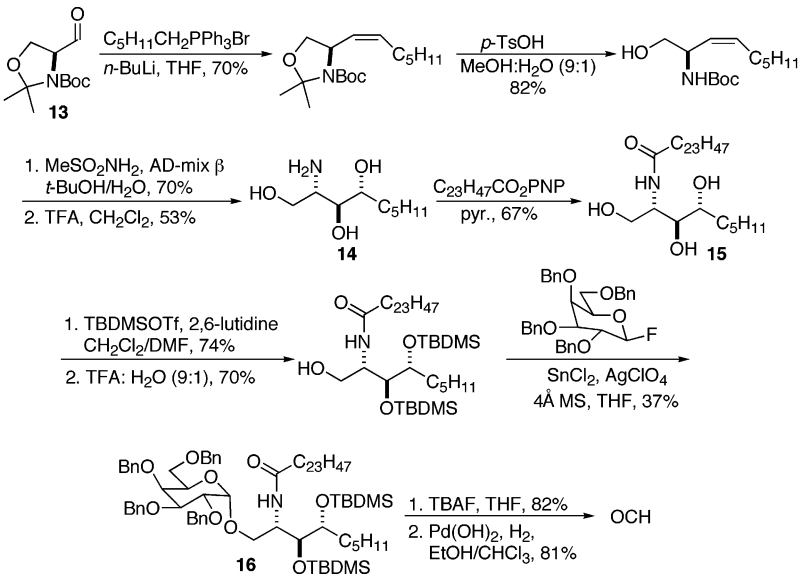
SCHEME 4



sylation gave protected OCH **16**. Conversion to OCH was achieved by a two-stage deprotection.

IL-2 production is typically measured to assess the relative abilities of preparations of α-GalCers to stimulate immortalized NKT cells (NKT cell hybridomas). The relative potencies of KRN7000, OCH, **1**, and **2** were measured with use of the $V_\alpha14^+$ iNKT hybridoma DN3A4-1.2 and three different CD1d-expressing antigen presenting cells: A20.mCD1d (a B cell lymphoma), RMA-S.mCD1d (a lymphoma), and JAWS II (a myeloid/dendritic cell line). IL-2 production was measured by ELISA from co-cultured supernatants of the hybridoma and one of the three antigen presenting cells previously pulsed with varying amounts of lipid. The relative potencies of the lipids (based on reciprocal effective concentrations for half-maximal response, $1/EC_{50}$) in the presence of the RMA-S and JAWS II antigen presenting cells (APC's) showed the sphinganine analogues **1** and **2** to be essentially as potent as the parent phytosphingosine-containing α-GalCers, KRN7000 and OCH (Figure 1). Moreover, the shorter chain analogue **2**, like the corresponding OCH, was slightly less effective at eliciting IL-2 release than KRN7000. Similar results were obtained by using A20.CD1d APCs, with all of the compounds showing potent stimulation of NKT cells, even in the 100 nM range (see the Supporting Information). Thus, with respect to this assay for NKT cell activation, the more easily accessible sphinganine-containing α-GalCers mirrored the activities of the corresponding phytosphingosine-containing compounds.

These results are interesting in light of recently published structural studies.[29,30] Zajonc et al. recently reported a crystal structure of mouse CD1d in complex with a variant of KRN7000 shortened in the acyl chain. Crucial H-bonding interactions were seen between CD1d residue Arg79 and the 3′-OH of the phytosphingosine,

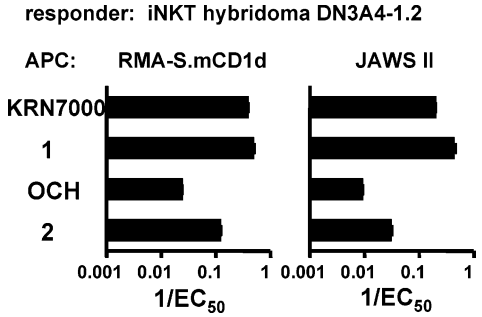


FIGURE 1. Relative potencies of KRN7000, OCH, **1**, and **2** in two CD1d-restricted antigen presentation systems. IL-2 production was measured from co-cultured supernatants of the $V_\alpha14^+$ iNKT hybridoma DN3A4-1.2 and one of two CD1d antigen-presenting cells (RMA-S.mCD1d or JAWS II) previously pulsed with varying amounts of lipid. IL-2 levels were fitted to 4-parameter dose/response curves. Relative potencies of each lipid (reciprocal effective concentrations in nM for half-maximal response, $1/EC_{50}$) are displayed for RMA-S.mCD1d and JAWS II.

and residue Asp80 showed H-bonding with both the 3′- and 4′-OH's of the phytosphingosine. However, given the similar responses of NKT cell hybridoma DN3A4-1.2 to sphinganine analogues **1** and **2** compared to their phytosphingosine counterparts, it is evident that the interaction with the 4′-OH is not crucial to activity. It is noteworthy that an analogue lacking both the 3′- and 4′-OH's on the sphingoid base has been shown to be inactive.[23]

Immortalized T cell hybridomas tend to produce IL-2, regardless of the cytokines that were produced by the parent cell they were derived from. Therefore, assay of the activation of T cell hybridomas, including those derived from NKT cells, is generally restricted to measurement of IL-2 secretion. By contrast, primary NKT cells from mice and humans tend to produce IL-4 and IFNγ when stimulated, plus a variety of other cytokines and chemokines that have been less well studied. T cell cytokine responses have been categorized into two broad groups. T_H1 responses, typified by the release of IFNγ and several other cytokines, are important for fighting

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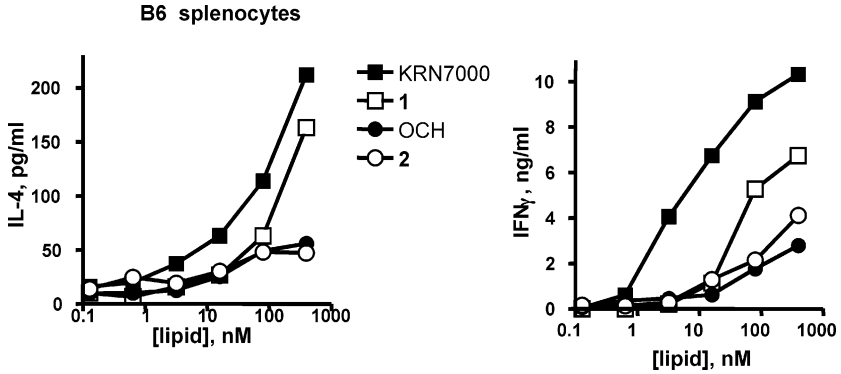

FIGURE 2. C57BL/6 splenocytes were incubated with varying doses of lipid for 48 h. Supernatant levels of IL-4 and IFNγ were measured by ELISA. Means of duplicate wells are shown; SEM were typically <10% of the mean.

intracellular infections by bacteria and viruses, and overexuberant T_H1 responses are typical of several autoimmune diseases. By contrast, the secretion of IL-4 characterizes T_H2 responses. Although excessive T_H2 responses can cause allergy, they are important for stimulating humoral immune responses and for the elimination of several protozoan parasites. OCH, truncated in both the acyl chain and sphingoid base in comparison to KRN7000, generated intense interest because, although it was not as potent as KRN7000 in inducing NKT cell responses, it consistently induced a T_H2 bias of NKT cell cytokine responses, as reflected in the proportionately larger production of IL-4 in comparison to IFNγ.[13] In the phytosphingosine series, recent results appear to demonstrate that shortening of either the acyl or the sphingoid base chains induces a T_H2 bias.[31,32] This suggests that OCH or related compounds (i.e., those that elicit an increased ratio of IL-4 to IFNγ secretion) might be potential chemotherapeutic agents for autoimmune or inflammatory conditions mediated by T_H1 cytokines, as T_H2 cytokines can inhibit production of T_H1 cytokines and in some cases reverse their effects. Moreover, ready access to compounds that further probe this hypothesis would be timely. To examine the effects of the sphinganine compounds on the production of cytokines by NKT cells, we measured the levels of IL-4 and IFNγ in supernatants of mouse splenocytes cultured in the presence of the various α-GalCer analogues. Sphinganine analogues **1** and **2**, although not as potent, paralleled the parent phytosphingosines in their production of IL-4 and IFNγ (Figure 2). There was an indication of a T_H2 bias of both OCH and **2** in comparison to the longer chain variants. For example, at the 100 nm dose, the ratio of IL-4 to IFNγ production with OCH or **2** was approximately twice that observed for KRN7000 or **1** (Figure 2). This was consistent with the similar degree of T_H2 cytokine bias that has been seen in vitro in other studies.[13,31,32] It should be noted that previous studies of the T_H2 skewing effect of some α-GalCer analogues have shown that this effect tends to be more pronounced in vivo when analyzed by injection of compounds into mice and measurement of serum cytokine levels.[32,33]

In summary, the results reported here support the utility of sphinganine-containing α-GalCers for examining the role of α-GalCer analogues in modulating immune response. The sphinganine-containing compounds are more readily prepared. Although not quite as potent as the corresponding phytosphingosine-containing α-GalCers in some assays, such as the in vitro splenocyted stimulation assay, **1** and **2** mirror these compounds in the cytokine responses elicited.

Experimental Section

[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]-carbamic Acid *tert*-Butyl Ester (3). Boc-L-serine (8.00 g, 39.0 mmol) was dissolved in dry CH_2Cl_2 (153 mL) and the solution cooled to −15 °C under N_2. *N*,*O*-Dimethylhydroxylamine hydrochloride (3.92 g, 40.0 mmol) was added, followed by *N*-methylmorpholine (4.42 mL, 40.2 mmol). After 5 min 1-(3-methylaminopropyl-3-ethylcarbodiiimide hyrdrochloride (7.70 g, 40.2 mmol) was added in five portions over 30 min. After being stirred for 1 h at −15 °C, the reaction was quenched with HCl (1 M, 25 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, washed with saturated $NaHCO_3$ (23 mL) and H_2O (23 mL), dried ($MgSO_4$), and concentrated to provide **3** as a white solid (8.33 g, 86%):[34] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (br s, 1H), 4.95 (br s, 1H), 3.82−3.78 (m, 5H), 3.23 (s, 3H), 2.90 (br s, 1H), 1.43 (s, 9H).

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (4a). [2-Hydroxy-1-(methoxymethylcarbamoyl)-ethyl]carbamic acid *tert*-butyl ester (**3**) (1.6 g, 6.5 mmol) was dissolved in dry THF (13 mL) under N_2. The resulting solution was cooled to −15 °C and *s*-BuMgCl (2.0 M in Et_2O, 6.5 mL, 13 mmol) was added dropwise, affording a clear solution. After 5 min, pentadecylmagnesium bromide (0.42 M in THF, 20 mL, 8.4 mmol) was added at −15 °C. The resulting solution was allowed to warm to room temperature and stirred overnight. The mixture was cooled to −15 °C, and HCl (1 M, 15 mL) was added, followed by EtOAc (15 mL). The two layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded **4a** as a white solid (1.8 g, 70%): mp 48−50 °C; $[\alpha]^{25}_D$ +28.0 (*c* 1.0, $CHCl_3$); IR (KBr) 3420, 2923, 2853, 1710, 1169 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48 (br s, 1H), 4.26 (m,

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1H), 3.94 (m, 2H), 2.63 (br s, 1H), 2.55 (m, 2H), 1.57 (m, 2H), 1.45 (s, 9H), 1.27−1.22 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 164.0, 80.3, 70.1, 68.9, 63.3, 61.6, 52.3, 49.8, 39.9, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.3, 23.5, 22.7, 14.1; MS (EI) *m/z* 327 (M^+ − *t*-Bu-CH_3), 281, 239, 86, 57 (100). Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 69.12; H, 11.06; N, 3.60.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxynonan-3-one (4b). [2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]-carbamic acid *tert*-butyl ester (**3**) (4.3 g, 17.5 mmol) was dissolved in dry THF (60 mL) under N_2. The resulting solution was cooled to −15 °C and *s*-BuMgCl (2.0 M in Et_2O, 17.5 mL, 35.0 mmol) was added dropwise. After 10 min hexylmagnesium bromide (0.65 M in THF, 35.0 mL, 22.8 mmol) was added dropwise at −15 °C. The resulting solution was allowed to warm to room temperature and was stirred for 4 h. The gray solution was cooled to −15 °C and saturated aqueous NH_4Cl (40 mL) was added slowly, in one portion, followed by EtOAc (75 mL) and H_2O (75 mL). The aqueous layer was extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with H_2O (2 × 75 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 90:10) provided **4b** as a pale yellow oil (3.6 g, 75%): $[\alpha]^{25}_D$ +36.3 (*c* 0.5, $CHCl_3$); IR (neat) 3422, 2931, 1709, 1500 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.67 (d, J = 5.6 Hz, 1H), 4.32 (br s, 1H), 3.94 (dd, J = 11.2, 3.2 Hz, 1H), 3.88 (dd, J = 11.2, 3.6 Hz, 1H), 2.81 (br s, 1H), 2.55 (m, 2H), 1.58 (m, 2H), 1.44 (s, 9H), 1.34−1.21 (m, 6H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.3, 156.2, 80.4, 63.3, 61.8, 40.1, 31.7, 29.0, 28.5, 23.6, 22.6, 14.2; MS (EI) *m/z* 200 (M^+ − C_5H_{10}), 160, 113, 104, 60, 57 (100). Anal. Calcd for $C_{14}H_{27}NO_4$: C, 61.51; H, 9.96; N, 5.12. Found: C, 61.70; H, 9.67; N, 5.01.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-one (5a). A catalytic amount of DMAP was added to a solution of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (**4a**) (6.00 g, 15.0 mmol) and imidazole (3.07 g, 45.1 mmol) in dry DMF (17 mL) under N_2. After 20 min TBDMSCl (2.71 g, 18.0 mmol) was added, and the reaction was stirred overnight. The reaction mixture was diluted with saturated aqueous NH_4Cl (39 mL) and the aqueous layer extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, washed with H_2O (30 mL) and brine (30 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2 to 95:5) provided **5a** as a colorless oil (6.42 g, 83%): $[\alpha]^{25}_D$ +36.6 (*c* 1.0, $CHCl_3$); IR (neat) 3432, 2926, 2855, 1712, 1491, 1470 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (d, J = 7.0 Hz, 1H), 4.26 (t, J = 3.3 Hz, 1H), 4.05 (d, J = 10.3 Hz, 1H), 3.82 (dd, J = 10.2, 3.6 Hz, 1H), 2.62−2.43 (m, 2H), 1.58 (m, 2H), 1.45 (s, 9H), 1.26 (br s, 24H), 0.88 (t, J = 5.7 Hz, 3H), 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0, 155.5, 79.8, 63.6, 61.3, 40.3, 32.1, 29.8, 29.8, 29.8, 29.6, 29.6, 29.5, 29.4, 28.5, 25.9, 23.5, 22.9, 18.3, 14.3, −5.4. Anal. Calcd for $C_{29}H_{59}NO_4Si$: C, 67.78; H, 11.57; N, 2.73. Found: C, 67.39; H, 11.18; N, 3.08.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxynonan-3-one (5b). A catalytic amount of DMAP was added to a solution of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-hydroxynonan-3-one (**4b**) (3.2 g, 11.5 mmol) and imidazole (2.4 g, 34.6 mmol) in dry DMF (12 mL) under N_2. After 10 min TBDMSCl (2.1 g, 13.9 mmol) was added in one portion and the mixture was stirred for 24 h. The mixture was slowly diluted with saturated NH_4Cl (30 mL), and the solution was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layers were washed with H_2O (75 mL) and brine (75 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 100:0 to 98:2) provided **5b** as a colorless oil (4.2 g, 93%): $[\alpha]^{25}_D$ +33.0 (*c* 0.5, $CHCl_3$); IR ($CDCl_3$) 3433, 2930, 1712, 1492 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (d, J = 6.8 Hz, 1H), 4.25 (m, 1H), 4.03 (dd, J = 10.4, 2.8 Hz, 1H), 3.80 (dd, J = 10.4, 4.0 Hz, 1H), 2.51 (m, 2H), 1.58 (m, 2H), 1.43 (s, 9H), 1.30−1.25 (m, 6H), 0.87−0.84 (m, 12H), 0.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 155.5, 79.8, 63.6, 61.4, 40.3, 31.7, 29.0, 28.5, 25.9, 23.5, 22.6, 18.3, 14.2, −5.4; MS (EI) *m/z* 314 (M^+ − C_5H_{10}), 274, 218, 174, 73, 57 (100). Anal. Calcd for $C_{20}H_{41}NO_4Si$: C, 61.97; H, 10.66; N, 3.61. Found: C, 62.24; H, 10.27; N, 3.77.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-ol (6a). LiAl(O*t*-Bu)$_3$H (19.0 g, 74.8 mmol) was added to dry EtOH (105 mL) at −78 °C under N_2. Then, a solution of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)-amino-1-*tert*-butyldimethylsilanyloxyoctadecane-3-one (**5a**) (6.40 g, 12.5 mmol) in dry EtOH (105 mL) was added dropwise. After being stirred for 6 h at −78 °C the reaction mixture was diluted with CH_2Cl_2 (30 mL), and 10% citric acid (310 mL) was added. This mixture was stirred for 1.5 h at room temperature then extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2 to 95:5) afforded **6a** as a colorless oil (5.52 g, 86%): $[\alpha]^{25}_D$ +21.3 (*c* 1.0, $CHCl_3$); IR (neat) 3448, 2925, 2854, 1698, 1499, 1468 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (d, J = 6.6 Hz, 1H), 3.97 (dd, J = 10.6, 2.8 Hz, 1H), 3.83 (d, J = 9.3 Hz, 1H), 3.65 (m, 1H), 3.51 (br s, 1H), 3.01 (br s, 1H), 1.53 (br s, 4H), 1.46 (s, 9H), 1.26 (m, 24H), 0.92 (s, 9H), 0.89 (t, J = 7.7 Hz, 3H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 79.6, 74.5, 63.7, 54.1, 35.1, 32.1, 29.9, 29.8, 29.8, 29.6, 28.6, 26.2, 26.0, 22.9, 18.3, 14.3, −5.4, −5.4. Anal. Calcd for $C_{29}H_{61}NO_4$: C, 67.52; H, 11.92; N, 2.72. Found: C, 67.89; H, 12.17; N, 3.07.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxynonan-3-ol (6b). LiAl(O*t*-Bu)$_3$H (23.2 g, 91.3 mmol) was added in six portions over 15 min to a solution of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxynonan-3-one (**5b**) (7.10 g, 18.3 mmol) in dry EtOH (300 mL) at −78 °C under N_2. After being stirred for 5 h, the mixture was quenched slowly with aqueous citric acid (10%, 100 mL). The mixture was warmed to room temperature and allowed to stir for 1.5 h. The mixture was then diluted with H_2O (1 L) and CH_2Cl_2 (400 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with H_2O (3 × 200 mL) and brine (200 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) gave **6b** as a colorless oil (5.7 g, 80%): $[\alpha]^{25}_D$ +34.5 (*c* 0.5, $CHCl_3$); IR ($CDCl_3$) 3448, 2930, 1697, 1501 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (d, J = 7.2 Hz, 1H), 3.97 (dd, J = 10.4, 2.4 Hz, 1H), 3.83 (d, J = 9.6 Hz, 1H), 3.66 (m, 1H), 3.51 (br s, 1H), 3.02 (br s, 1H), 1.54 (br s, 2H), 1.46 (s, 9H), 1.32−1.30 (m, 8H), 0.94−0.87 (m, 12H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 79.5, 74.4, 63.7, 54.1, 35.1, 32.0, 29.5, 28.6, 26.1, 26.0, 22.8, 18.3, 14.3, −5.4, −5.4; MS (EI) *m/z* 316, 276 (M^+ − $C_7H_{14}O$), 218, 174, 144, 116, 75, 57 (100). Anal. Calcd for $C_{20}H_{43}NO_4Si$: C, 61.65; H, 11.12; N, 3.59. Found: C, 61.67; H, 10.79; N, 3.78.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxyoctadecane (7a). (2*S*,-3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-ol (**6a**) (5.40 g, 10.5 mmol) was dissolved in dry DMF (35 mL), and the solution was cooled to 0 °C and stirred under N_2. Tetrabutylammonium iodide (6.17 g, 16.7 mmol) was added, followed by NaH (60% in mineral oil, 0.50 g, 20.9 mmol). After 10 min benzylbromide (2.80 g, 16.7 mmol) was added dropwise via syringe. After the addition the cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (90 mL), and the mixture was extracted with Et_2O (4 × 100 mL). The combined organic extracts were washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1 to 97:3) afforded **7a** as a clear oil (3.3 g, 53%): $[\alpha]^{25}_D$ +5.66 (*c* 1.0, $CHCl_3$); IR (neat) 3450, 3031, 2925, 2854, 1720, 1497 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.65 (br s, 1H), 4.49 (s, 2H), 3.79 (m, 2H), 3.58 (m, 1H), 3.47 (br s, 1H), 1.49 (m, 2H), 1.39 (s, 9H), 1.21 (br s, 26H), 0.85 (s, 9H), 0.82 (t, J = 4.5 Hz, 3H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 138.9, 128.8, 128.3, 127.8, 79.2, 78.6, 72.6, 62.2, 53.8, 32.1, 30.7, 30.1, 29.9, 29.9, 29.8, 29.8, 29.6, 28.6, 25.5, 25.1, 22.9, 18.4, 18.3, 14.3, −4.3, −5.2. Anal. Calcd for $C_{36}H_{67}NO_4Si$: C, 71.35; H, 11.14; N, 2.31. Found: C, 71.21; H, 11.22; N, 2.44.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxynonane (7b). (2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxynonan-3-ol (**6b**) (0.65 g, 1.66 mmol) was dissolved in dry DMF (7 mL), and the solution was cooled to 0 °C under N_2. Tetrabutylammonium iodide (0.92 g, 2.49 mmol) was added at once, followed by the dropwise addition of benzylbromide (0.29 mL, 2.59 mmol). NaH (60% in mineral oil, 0.09 g, 2.32 mmol) was then added. The cloudy mixture was stirred at 0 °C for 20 min and then allowed to warm to room temperature and stirred for 2 h. The pale yellow solution was diluted with saturated aqueous NH_4Cl (90 mL), and the mixture was extracted with Et_2O (4 × 100 mL). The combined organic extracts were washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 100:0 to 98:2) provided a pale yellow oil that was an inseparable mixture of regioisomers **7b** and **7b′** (2:1 based on ^{1}H NMR) (0.68 g, 86%). **7b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.28 (m, 5H), 4.71 (br s, 1H), 4.55 (s, 2H), 3.84 (br s, 2H), 3.64 (br s, 1H), 3.53 (br s, 1H), 1.44 (s, 9H), 1.27 (br s, 10H), 1.04−0.67 (m, 12H), 0.06 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8, 138.9, 128.5, 128.0, 127.7, 79.2, 78.6, 72.3, 62.2, 53.8, 32.0, 30.7, 29.7, 28.6, 26.1, 25.4, 22.8, 18.4, 14.3, −5.20, −5.31.

(2*S*,3*R*)-2-Amino-3-benzyloxyoctadecan-1-ol (8a). Trifluoroacetic acid and H_2O (19:1, 20 mL) were added to (2*S*,3*R*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxyoctadecane (**7a**) (4.50 g, 7.60 mmol), and the solution was stirred under N_2 for 2 h. Saturated $NaHCO_3$ was added dropwise to pH ∼8.0; then, the solution was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic extracts were washed with H_2O (100 mL), dried ($MgSO_4$), and concentrated to give **8a** as a pale yellow oil (2.74 g, 93%): $[\alpha]^{25}_D$ −8.90 (*c* 1.0, $CHCl_3$); IR (neat) 3351, 3285, 2916, 1647, 1602, 1469 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 5H), 4.50 (m, 2H), 3.69 (m, 3H), 3.01 (br s, 1H), 2.47 (br s, 3H), 1.35 (m, 2H), 1.20 (m, 26H), 0.82 (t, J = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 128.7, 128.0, 128.0, 82.5, 72.5, 63.7, 54.5, 32.1, 30.7, 30.1, 29.9, 29.9, 29.8, 29.8, 29.6, 25.6, 22.9, 14.3; MS (EI) *m/z* 250, 221, 168, 112, 91(100), 84; HRMS (FAB) calcd for $C_{25}H_{46}NO_2$ (M^+ + H) 392.3529, found 392.3510.

(2*S*,3*R*)-2-Amino-3-benzyloxynonan-1-ol (8b). TFA (11.4 mL) and H_2O (0.6 mL) were added to a mixture of (2*S*,3*R*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxynonane (**7b**) and its regioisomer **7b′**. The yellow solution was stirred for 2 h. Most of the TFA was evaporated at 30 °C under reduced pressure. Residual TFA was neutralized with aqueous NaOH (7.5% w/v, 75 mL). The mixture was extracted with EtOAc (4 × 60 mL). The combined organic extracts were washed with NaOH (7.5%, 2 × 50 mL), dried ($MgSO_4$), and concentrated. Purification on silica gel (CH_2Cl_2/MeOH 95:5) provided an inseparable mixture of regioisomers **8b** and **8b′** as a light yellow oil (0.42 g, 65%). The mixture was used directly in the synthesis of **11b**.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-one (9). DMAP (0.23 g, 0.18 mmol) was added to a stirred solution under N_2 of (2*S*)-2-(*N*-*tert*-butoxycarbonylamino)-1-hydroxyoctadecan-3-one (**4a**) (3.7 g, 9.2 mmol) and imidazole (1.9 g, 28 mmol) in dry DMF (25 mL). TBDPSCl (3.1 g, 11 mmol) was then added dropwise, and the reaction mixture was stirred overnight, then diluted with EtOAc (400 mL). The solution was washed with H_2O (6 × 75 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography on silica gel (petroleum ether/EtOAc 90:10) afforded **9** as a clear oil (5.1 g, 88%): $[\alpha]^{25}_D$ +32.4 (*c* 2.28, $CHCl_3$); IR (neat) 3433, 3072, 3050, 2925, 2855, 1712, 1499, 1172, 1113 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (m, 4H), 7.39 (m, 6H), 5.53 (d, J = 7.7 Hz, 1H), 4.33 (m, 1H), 4.04 (dd, J = 10.6, 3.1 Hz, 1H), 3.90 (dd, J = 10.9, 3.8 Hz, 1H), 2.51 (m, 2H), 1.58 (m, 2H), 1.44 (s, 9H), 1.26 (m, 24H), 1.03 (s, 9H), 0.89 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.4, 155.3, 135.5, 132.8, 129.9, 129.9, 127.8, 79.5, 64.2, 61.1, 60.3, 41.3, 40.0, 31.9, 29.7, 29.6, 29.6, 29.4, 29.4, 29.3, 29.2, 29.0, 28.3, 27.6, 26.7, 26.2, 23.3, 22.7, 22.6, 20.9, 20.4, 19.4, 19.2, 18.7, 14.3; HRMS (FAB) calcd for $C_{39}H_{64}NO_4Si$ (M^+ + H) 638.4605, found 638.4600. Anal. Calcd for $C_{39}H_{63}NO_4Si$: C, 73.42; H, 9.95; N, 2.20. Found: C, 73.16; H, 9.58; N, 2.31.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-ol. Dry EtOH (17 mL) was cooled to −78 °C and stirred under N_2 for 20 min. LiAl(O-*t*-Bu)$_3$H (3.1 g, 12.4 mmol) was added at −78 °C and stirred for 20 min. (2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-one (**9**) (1.32 g, 2.1 mmol) in dry EtOH (17 mL) was added dropwise to the stirred solution. The temperature was maintained at −78 °C, and the mixture was stirred for 6 h. Aqueous citric acid (10%, 50 mL) was added, and the reaction mixture was allowed to warm to room temperature over 1.5 h. The cloudy suspension was extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were combined, washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded (2*S*,3*R*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-ol as a clear oil (1.1 g, 82%): $[\alpha]^{25}_D$ +17.4 (*c* 1.35, $CHCl_3$); IR (neat) 3447, 3071, 3050, 2925, 1698, 1499, 1172, 1113 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (m, 4H), 7.41 (m, 6H), 5.30 (d, J = 8.1 Hz, 1H), 3.93 (m, 1H), 3.90 (m, 1H), 3.67 (m, 1H), 3.57 (br s, 1H), 2.88 (d, J = 8.1 Hz, 2H), 1.44−1.06 (m, 45H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 135.8, 132.8, 132.7, 130.2, 130.1, 128.1, 128.0, 79.6, 74.0, 64.4, 54.7, 34.7, 32.1, 31.1, 29.8, 29.6, 28.6, 27.1, 26.1, 22.9, 19.4, 14.3; HRMS (FAB) calcd for $C_{39}H_{66}NO_4Si$ (M^+ + H) *m/z* 640.4761, found 640.4738. Anal. Calcd for $C_{39}H_{65}NO_4Si$: C, 73.19; H, 10.24; N, 2.19. Found: C, 73.30; H, 10.17; N, 2.36.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (10). (2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxyoctadecan-3-ol (1.33 g, 2.1 mmol) was dissolved in dry DMF under N_2 (10 mL), and tetrabutylammonium iodide (1.15 g, 3.1 mmol) and benzyl bromide (0.53 g, 3.1 mmol) were added. The reaction mixture was then cooled to 0 °C, and NaH (60% in mineral oil, 62 mg, 2.6 mmol) was added in two portions. The reaction mixture was stirred for 45 min at 0 °C and then at room temperature for 45 min. EtOAc (250 mL) was added, and the solution was washed with saturated aqueous NH_4Cl (50 mL), H_2O (4 × 50 mL), and brine (50 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1) afforded **10** as a clear oil (1.6 g, 80%): $[\alpha]^{25}_D$ +8.47 (*c* 3.05, $CHCl_3$); IR (neat) 3450, 3070, 3048, 3030, 2925, 2854, 1719, 1497, 1365, 1172, 1112 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (m, 4H), 7.63−7.27 (m, 11H), 4.71 (d, J = 8.0 Hz, 1H), 4.54 (d, J = 11.3 Hz, 1H), 4.49 (d, J = 11.3 Hz, 1H), 3.86 (m, 2H), 3.72 (dd, J = 9.0, 4.1 Hz, 1H), 3.58 (m, 1H), 1.42 (s, 9H), 1.42 (s, 2H), 1.26 (br s, 26H), 1.05 (s, 9H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 138.8, 135.9, 133.5, 130.0, 128.5, 128.0, 127.7, 79.3, 79.1, 72.3, 63.1, 53.7, 32.2, 30.7, 30.1, 29.9, 29.9, 29.8, 29.6, 28.6, 27.1, 25.6, 22.9, 19.5, 14.4; HRMS (FAB) calcd for $C_{46}H_{72}NO_4Si$ (M^+ + H) 730.5231, found 730.5246.

(2*S*,3*R*)-2-Amino-3-benzyloxyoctadecan-1-ol (8a). Acetyl chloride (6.35 mL, 73.3 mmol) was added to MeOH (63 mL), and the solution was allowed to cool to room temperature under N_2. (2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (**10**) (1.6 g, 2.12

mmol) dissolved in dry Et_2O (63 mL) was added, and the solution was stirred for 2 d at room temperature. The reaction mixture was concentrated then taken up in CH_2Cl_2 (10 mL), and saturated $NaHCO_3$ (20 mL) was carefully added, followed by NaOH (1 M, 10 mL). The solution was stirred for 1 h. The solution was then diluted with CH_2Cl_2 (100 mL), and the two layers were separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 90:10) afforded **8a** as a pale yellow oil (0.59 g, 71%) giving the same spectral characteristics as described above.

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-hexacosanoylamino)octadecan-1-ol (11a). *p*-Nitrophenyl hexacosanoate (1.26 g, 2.43 mmol) was added to a solution under N_2 of (2*S*,3*R*)-2-amino-3-benzyloxyoctadecan-1-ol (**8a**) (0.80 g, 2.03 mmol) in dry pyridine (40 mL). The reaction was left to stir for 18 h. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 90:10 to 70:30) to provide **11a** as a white solid (1.11 g, 70%): mp 85−87 °C; $[\alpha]^{25}_D$ −24.6 (*c* 1.0, $CHCl_3$); IR (KBr) 3317, 3033, 2919, 2849, 1638, 1549 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.09 (d, J = 7.2 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.36 (d, J = 11.6 Hz, 1H), 3.94 (m, 2H), 3.63 (br s, 1H), 3.55 (d, J = 9.1 Hz, 1H), 2.04 (m, 2H), 1.68 (m, 1H), 1.53 (m, 3H), 1.22 (br s, 70H), 0.85 (t, J = 5.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 138.2, 128.9, 128.3, 128.1, 82.2, 73.0, 62.4, 52.3, 36.9, 32.1, 31.6, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 25.9, 25.9, 22.9, 14.3. Anal. Calcd for $C_{51}H_{95}NO_3$: C, 79.52; H, 12.43; N, 1.82. Found: C, 79.54; H, 12.31; N, 1.87.

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-tetracosanoylamino)-1-nonanol (11b). (2*S*,3*R*)-2-Amino-3-benzyloxynonan-1-ol (**8b**) (0.22 g, 0.82 mmol) was dissolved in dry pyridine (5 mL) under N_2. *p*-Nitrophenyl tetracosanoate (0.48 g, 0.98 mmol) was added in one portion. An additional portion of dry pyridine (2 mL) was added to aid in dissolution. After being stirred for 24 h, the mixture was diluted with EtOAc (50 mL) and washed with HCl (1 M, 3 × 50 mL), NaOH (7.5% w/v, 3 × 75 mL), and H_2O (2 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification on silica gel (petroleum ether/EtOAc 70:30 to 60:40) provided **11b** as a white solid (0.27 g, 53%): mp 87.0−88.5 °C; $[\alpha]^{25}_D$ −28.4 (*c* 0.5, $CHCl_3$); IR (KBr) 3439, 2928, 2842, 1633 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43−7.28 (m, 5H), 6.13 (d, J = 4 Hz, 1H), 4.65 (d, J = 12 Hz, 1H), 4.40 (d, J = 12 Hz, 1H), 3.98 (br s, 2H), 3.74−3.51 (m, 2H), 3.18 (br s, 1H), 2.18−1.98 (m, 2H), 1.77−0.78 (m, 58H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 138.2, 128.9, 128.3, 128.1, 82.2, 73.0, 62.4, 52.3, 37.0, 32.1, 32.0, 31.6, 30.0, 29.9, 29.7, 29.6, 29.6, 29.5, 25.9, 22.9, 22.8, 14.3, 14.3; HRMS (FAB) calcd for $C_{40}H_{74}NO_3$ (M^+ + H) 616.5669, found 616.5647.

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-hexacosanoylamino)-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane (12a). Tin(II) chloride (0.2 g, 1.05 mmol), silver perchlorate (0.22 g, 1.06 mmol), and freshly ground 4Å molecular sieves (1.62 g) were added to a solution under N_2 of (2*S*,3*R*)-3-benzyloxy-2-(*N*-hexacosanoylamino)-1-octadecanol **(11a)** (0.27 g, 0.35 mmol) in dry THF (6.5 mL), and the mixture was stirred for 30 min and then cooled to −10 °C. A solution of β-benzylgalactosyl fluoride (0.29 g, 0.54 mmol) in dry THF (6.5 mL) was added. The reaction mixture was then warmed gradually to room temperature and stirred for 2 h. The reaction mixture was filtered through Celite and the filter cake washed with CH_2Cl_2. The filtrate was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc 95:5 to 85:15) to provide **12a** as a white solid (0.11 g, 26%): mp 71−73 °C; $[\alpha]^{25}_D$ +18.5 (*c* 1.0, $CHCl_3$); IR (KBr) 3310, 3030, 2925, 2850, 1640, 1542 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 25H), 6.05 (d, J = 8.8 Hz, 1H), 4.94−4.36 (m, 10H), 4.17 (br s, 1H), 4.03 (dd, J = 10.1, 2.8 Hz, 1H), 3.98−3.87 (m, 4H), 3.68 (dd, J = 11.0, 3.7 Hz, 1H), 3.59 (dd, J = 11.0, 6.0 Hz, 1H), 3.52 (dd, J = 9.3, 6.8 Hz, 2H), 3.38 (dd, J = 9.3, 5.9 Hz, 1H), 1.99 (m, 2H), 1.52 (br s, 4H), 1.26 (br s, 70H), 0.89 (t, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 138.9, 138.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.9, 127.7, 127.7, 99.7, 79.0, 78.4, 75.1, 74.9, 73.7, 73.6, 73.2, 72.2, 70.2, 37.0, 32.1, 30.2, 29.9, 29.9, 29.9, 29.8, 29.7, 29.6, 25.9, 25.2, 22.9, 14.3. Anal. Calcd for $C_{85}H_{129}NO_8$: C, 78.96; H, 10.06; N, 1.08. Found: C, 78.78; H, 9.71; N, 1.36.

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-tetracosanoylamino)-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonane (12b). (2*S*,-3*R*)-3-Benzyloxy-2-(*N*-tetracosanoylamino)-1-nonanol (**11b**) (0.27 g, 0.44 mmol) was dissolved in dry THF (6.5 mL) and stirred under N_2. Freshly ground molecular sieves (4Å, 1.64 g), silver perchlorate (0.27 g, 1.31 mmol), and tin(II) chloride (0.25 g, 1.31 mmol) were added successively to give a mixture that was stirred for 30 min. The mixture was cooled to −15 °C, and a solution of β-tetrabenzylgalactosyl fluoride (0.35 g, 0.66 mmol), in dry THF (6.5 mL), was added dropwise. After 10 min, the mixture was allowed to warm to room temperature and was stirred for 2 h. The mixture was filtered through Celite and the filter cake washed with Et_2O. The filtrate was washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5 to 90:10) provided **12b** as a white solid (0.11 g, 15%): $[\alpha]^{25}_D$ +21.1 (*c* 0.5, $CHCl_3$); IR (NaCl) 3321, 2919, 2850, 1638 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38−7.25 (m, 25H), 6.07 (d, J = 8 Hz, 1H), 4.92 (d, J = 12 Hz, 1H), 4.82−4.35 (m, 10H), 4.16−3.88 (m, 7H), 3.68−3.34 (m, 4H), 2.00 (m, 2H), 1.51−1.22 (m, 52H), 0.88−0.83 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 138.9, 138.9, 138.7, 138.7, 137.9, 128.6, 128.6, 128.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 99.8, 79.0, 78.4, 75.1, 74.9, 73.7, 73.6, 73.2, 72.2, 70.2, 69.6, 69.3, 51.7, 37.0, 32.1, 32.0, 30.9, 29.9, 29.9, 29.8, 29.7, 29.6, 29.6, 25.9, 25.1, 22.9, 22.8, 14.3, 14.3; HRMS (FAB) calcd for $C_{74}H_{108}NO_8$ (M^+ + H) 1138.8075, found 1138.8070.

(2*S*,3*R*)-1-(α-D-Galactopyranosyl)-2-hexacosanoylaminooctadecan-3-ol (1). $Pd(OH)_2$ (20% on carbon, 123 mg) was added to a stirred solution of (2*S*,3*R*)-3-benzyloxy-2-(*N*-hexacosanoylamino)-1-(2,3,4,6-tetra-*O*-benzyl-α-d-galactopyranosyl)octadecane (**12a**) (30 mg, 0.025 mmol) in ethanol (1.6 mL) and $CHCl_3$ (0.4 mL). The mixture was placed under a H_2 atmosphere, and stirring was continued vigorously for 24 h. The mixture was filtered through Celite, and the filter cake was washed with $CHCl_3$ and MeOH. The filtrate was evaporated, and purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5 to 90:10) provided **1** as a white solid (17.5 mg, 93%): mp 168−170 °C; $[\alpha]^{24}_D$ +39.8 (*c* 0.5, pyridine); ^1H NMR (400 MHz, pyr-d_5) δ 8.54 (d, J = 8.7 Hz, 1H), 5.48 (br s, 5H), 5.08 (br s, 2H), 4.68 (br s, 1H), 4.57 (dd, J = 6.7, 2.4 Hz, 2H), 4.52 (m, 4H), 4.48 (m, 1H), 4.44 (br s, 1H), 2.51 (m, 2H), 1.87 (m, 4H), 1.47 (br s, 70H), 0.90 (t, J = 4.9 Hz, 6H); ^{13}C NMR (100 MHz, pyr-d_5) δ 173.8, 102.5, 73.5, 72.3, 72.0, 71.4, 70.9, 70.1, 63.1, 55.3, 37.2, 35.5, 32.5, 32.5, 30.6, 30.5, 30.4, 30.3, 30.3, 30.2, 30.1, 30.0, 30.0, 27.0, 26.8, 23.3, 14.7; HRMS (FAB) calcd for $C_{50}H_{100}NO_8$ (M^+ + H) 842.7449, found 842.7464.

(2*S*,3*R*)-1-(α-D-Galactopyranosyl)-2-tetracosanoylaminononan-3-ol (2). (2*S*,3*R*)-3-Benzyloxy-2-(*N*-tetracosanoylamino)-1-(2,3,4,6-tetra-*O*-benzyl-α-d-galactopyranosyl)nonane (**12b**) (0.08 g, 0.07 mmol) was dissolved in EtOH (4 mL) and $CHCl_3$ (1 mL). $Pd(OH)_2$ (20% on carbon, 0.32 g) was added in one portion, and the mixture was stirred under H_2. After 24 h, the mixture was filtered through Celite and the filter cake washed with a combination of EtOH and $CHCl_3$. The filtrate was concentrated, and the residue was purified on silica gel (CH_2Cl_2/MeOH 95:5 to 91.5:8.5) to provide a white solid (0.04 g, 89%): IR (KBr) 3347, 2919, 2850, 1678 cm^{-1}; ^1H NMR (400 MHz, pyr-d_5) δ 8.52 (d, J = 8 Hz, 1H), 6.54 (br s, 2H), 6.45 (br s, 1H), 6.35 (br s, 1H), 6.14 (d, J = 4 Hz, 1H), 5.48 (d, J = 4 Hz, 1H), 4.76−4.25 (m, 9H), 3.65 (s, 1H), 2.50 (t, J = 4 Hz, 2H), 1.87−0.81 (m, 58H); ^{13}C NMR (100 MHz, pyr-d_5) δ 173.8, 102.5, 73.5, 72.3, 72.0, 71.4, 70.9, 70.0, 63.1, 55.3, 37.2, 35.5,

32.5, 30.4, 30.3, 30.2, 30.1, 30.0, 26.9, 26.8, 23.3, 23.3, 14.6; HRMS (FAB) calcd for $C_{39}H_{77}NO_8$ ($M^+ + H$) 688.5727, found 688.5717.

(4*R*,1′*Z*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-(1′-heptenyl)oxazolidine. *n*-BuLi (2.5 M in hexane, 14.4 mL, 36.0 mmol) was added to freshly prepared hexylphosphonium bromide (16.4 g, 38.4 mmol) in dry THF (250 mL) at −78 °C under N_2. The resulting orange solution was allowed to warm to 0 °C and stirred for 30 min. The solution was then cooled to −78 °C, and Garner's aldehyde (**13**) (5.5 g, 24 mmol) in dry THF (24 mL) was added dropwise. After being stirred for 2 h at room temperature, the reaction was diluted with saturated aqueous NH_4Cl (30 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1 to 98:2) provided (4*R*,1′*Z*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-(1′-heptenyl)oxazolidine as a pale yellow oil (5.0 g, 70%): $[\alpha]^{25}_D$ +75.5 (*c* 1.0, $CHCl_3$); IR (neat) 2979, 2930, 2862, 1700, 1457, 1385 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.46−5.39 (m, 2H), 4.70−4.65 (m, 1H), 4.06 (dd, J = 8.5, 6.5 Hz, 1H), 3.65 (dd, J = 8.5, 3.0 Hz, 1H), 2.09 (br s, 2H), 1.60−1.26 (m, 21H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.2, 132.0, 131.0, 79.9, 69.3, 54.8, 31.7, 29.9, 29.6, 28.7, 27.7, 22.8, 14.3; MS (EI) *m/z* 226 ($M^+ - C_5H_{11}$), 67, 57 (100). Anal. Calcd for $C_{17}H_{31}NO_3$: C, 68.65; H, 10.51; N, 4.71. Found C, 68.55; H, 10.48; N, 4.64.

(2*R*,3*Z*)-2-(*tert*-Butoxycarbonyl)amino-3-nonen-1-ol. *p*-TsOH (0.44 g, 2.31 mmol) was added to a stirred solution under N_2 of (4*R*,1′*Z*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-(1′-heptenyl)oxazolidine (4.40 g, 14.8 mmol) in MeOH:H_2O (73 mL, 9:1) and the reaction was stirred for 48 h. The reaction mixture was concentrated under high vacuum to provide a white solid, which was then dissolved in CH_2Cl_2 (100 mL). The resulting solution was washed with brine (30 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 85:15 to 70:30) afforded (2*R*,3*Z*)-2-(*tert*-butoxycarbonyl)amino-3-nonen-1-ol as a white solid (3.1 g, 82%): mp 98−100 °C; $[\alpha]^{25}_D$ +27.9 (*c* 1.0, $CHCl_3$); IR (KBr) 3376, 2948, 1686, 1530 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.59 (dd, J = 17.5, 7.5 Hz, 1H), 5.27 (t, J = 10.0 Hz, 1H), 4.80 (br s, 1H), 4.49 (br s, 1H), 3.59 (m, 2H), 2.90 (br s, 1H), 2.13 (m, 2H), 1.46 (s, 9H), 1.35 (m, 6H), 0.89 (t, J = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 135.0, 126.3, 80.1, 66.8, 50.9, 31.7, 29.4, 28.6, 28.2, 22.8, 14.3; MS (EI) *m/z* 226 ($M^+ - CH_2OH$), 170 (100), 126, 57. Anal. Calcd for $C_{14}H_{27}NO_3$: C, 65.33; H, 10.57; N, 5.55. Found: C, 65.24; H, 10.20; N, 5.67.

(2*S*,3*S*,4*R*)-2-(*tert*-Butoxycarbonyl)amino-1,3,4-nonanetriol. (2*R*,3*Z*)-2-(*tert*-Butoxycarbonyl)amino-3-nonen-1-ol (1.50 g, 5.84 mmol) was dissolved in *t*-BuOH:H_2O (58 mL, 1:1) under N_2 and methanesulfonamide (0.56 g, 5.84 mmol) added. The reaction mixture was cooled to 0 °C, and AD-mix-β (8.17 g) was added. The resulting mixture was stirred at 0 °C for 36 h and at room temperature for 8 h, then quenched with solid Na_2SO_3 (8.8 g) and extracted with EtOAc (3 × 50 mL). The organic extracts were washed with NaOH (1 M, 30 mL), H_2O (30 mL), and brine (30 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 50:50 to 30:70) provided (2*S*,3*S*,4*R*)-2-(*tert*-butoxycarbonyl)amino-1,3,4-nonanetriol as a white solid (1.2 g, 70%): mp 103−105 °C; $[\alpha]^{25}_D$ +8.32 (*c* 1.0, $CHCl_3$); IR (KBr) 3363, 2922, 1674, 1544 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.55 (d, J = 8.0 Hz, 1H), 4.20 (br s, 1H), 3.94 (d, J = 5.0 Hz, 1H), 3.83 (m, 3H), 3.71−3.64 (m, 4H), 1.65 (br s, 1H), 1.43 (s, 9H), 1.31 (br s, 6H), 0.87 (t, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.6, 80.3, 76.1, 73.2, 62.0, 52.9, 33.0, 32.0, 28.6, 25.8, 22.8, 14.2; MS (EI) *m/z* 207, 187, 117, 86 (100), 57. Anal. Calcd for $C_{14}H_{29}NO_5$: C, 57.71; H, 10.03; N, 4.81. Found: C, 57.57; H, 9.74; N, 4.83.

(2*S*,3*S*,4*R*)-2-Amino-1,3,4-nonanetriol (14). A solution of TFA:CH_2Cl_2 (15 mL, 1:1) was added to a solution under N_2 of (2*S*,3*S*,4*R*)-2-(*tert*-butoxycarbonyl)amino]-1,3,4-nonanetriol (0.63 g, 2.15 mmol) in CH_2Cl_2 (37 mL). The solution was stirred for 30 min then neutralized with saturated aqueous $NaHCO_3$ (270 mL) to pH ∼8.0. The resulting solution was concentrated under high vacuum to provide a white solid. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 90:10 to 85:15) provided **14** as a pale yellow oil (0.22 g, 53%): IR (neat) 3115, 1683, 1443, 1206, 1139 cm^{-1}; ^1H NMR (500 MHz, pyr-d_5) δ 4.66 (dd, J = 11.5, 3.5 Hz, 1H), 4.60−4.56 (m, 1H), 4.50−4.44 (m, 2H), 4.10 (t, J = 6.5 Hz, 1H), 3.64 (br s, 4H), 2.20 (t, J = 3.0 Hz, 1H), 1.76 (m, 2H), 1.58 (m, 1H), 1.32 (m, 5H), 0.89 (t, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, pyr-d_5) δ 73.8, 73.0, 59.3, 56.5, 35.3, 32.3, 25.7, 23.0, 14.3; HRMS (FAB) calcd for $C_9H_{22}NO_3$ ($M^+ + H$) 192.1600, found 192.1601.

(2*S*,3*S*,4*R*)-2-Tetracosanoylamino-1,3,4-nonanetriol (15). *p*-Nitrophenyl tetracosanoate (0.63 g, 1.2 mmol) was added to a solution of (2*S*,3*S*,4*R*)-2-amino-1,3,4-nonanetriol (**14**) (0.20 g, 1.0 mmol) in dry pyridine (20 mL). The reaction was stirred for 18 h, concentrated, and purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2 to 95:5) to provide **15** as a white solid (0.40 g, 67%): mp 117−119 °C; $[\alpha]^{25}_D$ −6.51 (*c* 1.0, pyr); IR (KBr) 3350, 2908, 1648, 1543, 1453 cm^{-1}; ^1H NMR (500 MHz, pyr-d_5) δ 7.93 (d, J = 8.5 Hz, 1H), 5.15 (br s, 3H), 4.80 (m, 1H), 4.34 (dd, J = 11.0, 4.5 Hz, 1H), 4.27 (dd, J = 10.5, 5.0 Hz, 1H), 4.15 (m, 1H), 4.10 (m, 1H), 2.41 (t, J = 7.5 Hz, 2H), 2.12 (m, 1H), 1.82 (m, 4H), 1.64 (br s, 1H), 1.40 (m, 44H), 1.02 (t, J = 6.0 Hz, 3H), 0.99 (t, J = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, pyr-d_5) δ 173.8, 77.2, 73.5, 62.6, 54.2, 37.3, 34.4, 32.9, 32.6, 30.5, 30.4, 30.3, 30.3, 30.2, 30.1, 26.9, 26.7, 23.5, 23.4, 14.7, 14.7; HRMS (FAB) calcd for $C_{33}H_{68}NO_4$ ($M^+ + H$) 542.5148, found 542.5139.

(2*S*,3*S*,4*R*)-1,3,4-Tris(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylaminononane. A solution of (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1,3,4-nonanetriol (**15**) (0.19 g, 0.32 mmol) and 2,6-lutidine (0.34 mL, 2.90 mmol) in dry CH_2Cl_2:DMF (4.5 mL, 1:2) was cooled to 0 °C and TBDMSOTf (0.6 mL, 2.6 mmol) was added dropwise under N_2. After 10 min, the cooling bath was removed, and the reaction was stirred at room temperature for 5 h. The reaction mixture was quenched with MeOH, poured into H_2O (10 mL), and extracted with Et_2O (3 × 30 mL). The organic extracts were washed with H_2O (30 mL), saturated aqueous $NaHCO_3$ (30 mL), and brine (30 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 99:1 to 97:3) provided (2*S*,3*S*,4*R*)-1,3,4-tris(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylaminononane as a clear oil (0.32 g, 74%): $[\alpha]^{25}_D$ +1.50 (*c* 1.0, $CHCl_3$); IR (neat) 3440, 3341, 2925, 2854, 1687, 1495, 1469 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (d, J = 8.5 Hz, 1H), 3.95 (m, 1H), 3.88 (dd, J = 10.0, 4.0 Hz, 1H), 3.83 (d, J = 7.0 Hz, 1H), 3.69 (dd, J = 7.5, 4.0 Hz, 1H), 3.64 (dd, J = 10.0, 4.0 Hz, 1H), 2.15 (t, J = 7.5 Hz, 2H), 1.60 (m, 2H), 1.56−1.39 (m, 3H), 1.30−1.26 (m, 45H), 0.94−0.89 (m, 33H), 0.14−0.02 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 75.6, 61.6, 52.8, 37.4, 32.4, 32.4, 32.2, 29.9, 29.9, 29.9, 29.7, 29.7, 29.6, 29.6, 26.3, 26.3, 26.3, 26.1, 26.0, 22.9, 22.8, 18.6, 18.5, 18.4, 14.3, 14.3, −3.28, −3.59, −4.40, −4.96, −4.99, −5.34. Anal. Calcd for $C_{51}H_{109}NO_4Si_3$: C, 69.24; H, 12.42; N, 1.58. Found: C, 68.89; H, 12.08; N, 1.57.

(2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylamino-1-nonanol. A solution of (2*S*,3*S*,4*R*)-1,3,4-tris(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylaminononane (0.30 g, 0.29 mmol) in dry THF (3.5 mL) was cooled to −10 °C, and a solution of TFA:H_2O (1.3 mL, 9:1) was added dropwise under N_2. The reaction mixture was gradually warmed to 10 °C and stirred for 2.5 h, then quenched with NaOH (1 M, 1 mL). The reaction mixture was diluted with Et_2O (30 mL), washed with H_2O (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5 to 85:15) provided (2*S*,3*S*,4*R*)-3,4-bis(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylamino-1-nonanol as a clear oil (0.19 g, 70%): $[\alpha]^{25}_D$ −10.4 (*c* 1.0, $CHCl_3$);

IR (neat) 3298, 2925, 2854, 1649, 1511, 1465, 1254 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.25 (d, J = 7.5 Hz, 1H), 4.22 (dd, J = 11.5, 2.5 Hz, 1H), 4.07 (m, 1H), 3.92 (t, J = 2.5 Hz, 1H), 3.77 (ddd, J = 15.0, 6.0, 2.5 Hz, 1H), 3.60 (br s, 1H), 3.15 (br s, 1H), 2.19 (t, J = 7.0 Hz, 2H), 1.30−1.26 (m, 50H), 0.89−0.83 (m, 24 H), 0.12−0.09 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 76.7, 63.9, 51.5, 37.2, 34.6, 32.2, 32.2, 30.3, 30.0, 29.9, 29.9, 29.7, 29.6, 29.6, 26.3, 26.3, 26.2, 25.9, 25.8, 22.9, 22.8, 18.4, 18.4, 14.4, 14.3, −3.51, −3.81, −4.28, −4.65. Anal. Calcd for $C_{45}H_{95}NO_4Si_2$: C, 70.15; H, 12.43; N, 1.82. Found: C, 69.82; H, 12.04; N, 1.74.

(2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonane (16). Tin(II) chloride (0.11 g, 0.58 mmol), silver perchlorate (0.12 g, 0.59 mmol), and freshly ground 4Å molecular sieves (0.90 g) were added successively to a solution under N_2 of (2*S*,3*S*,4*R*)-3,4-bis(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylamino-1-nonanol (0.18 g, 0.20 mmol) in dry THF (3.6 mL). The reaction mixture was stirred for 30 min, then cooled to −10 °C, and a solution of β-tetrabenzylgalactosyl fluoride (0.16 g, 0.30 mmol) in dry THF (3.6 mL) was added. The reaction mixture was warmed gradually to room temperature and stirred for 2 h. The reaction mixture was then filtered through Celite, and the filter cake was washed with CH_2Cl_2. The filtrate was washed with brine (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5 to 85:15) provided **16** as a clear oil (0.10 g, 37%): $[\alpha]^{25}_D$ +5.80 (*c* 1.0, $CHCl_3$); IR (neat) 3031, 2924, 2853, 1681, 1496, 1462 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30−7.23 (m, 20H), 6.00 (d, J = 7.5 Hz, 1H), 4.93 (d, J = 11.5 Hz, 1H), 4.84 (d, J = 3.5 Hz, 1H), 4.79 (m, 2H), 4.73−4.65 (m, 2H), 4.56 (d, J = 11.5 Hz, 1H), 4.49 (d, J = 12.0 Hz, 1H), 4.40 (d, J = 12.0 Hz, 1H), 4.08 (m, 1H), 4.04 (dd, J = 10.0, 3.5 Hz, 1H), 3.97−3.86 (m, 6H), 3.66 (m, 1H), 3.50 (m, 2H), 2.01 (t, J = 7.5 Hz, 2H), 1.30−1.25 (m, 50H), 0.91−0.86 (m, 24H), 0.08−0.03 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 139.0, 138.9, 138.9, 138.1, 128.7, 128.6, 128.6, 128.5, 128.4, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 100.6, 79.3, 77.9, 76.8, 76.0, 75.2, 75.0, 73.8, 73.7, 73.2, 70.1, 69.9, 69.0, 52.0, 37.0, 33.4, 32.4, 32.2, 30.0, 30.0, 29.9, 29.8, 29.7, 29.6, 26.4, 26.4, 26.0, 25.9, 23.0, 22.9, 18.6, 18.4, 14.4, 14.4, −3.39, −3.57, −4.38, −4.68. Anal. Calcd for $C_{79}H_{129}NO_9Si_2$: C, 73.38; H, 10.06; N, 1.08. Found: C, 72.99; H, 9.68; N, 1.08.

(2*S*,3*S*,4*R*)-2-Tetracosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonan-3,4-diol. (2*S*,3*S*,4*R*)-3,4-Bis(*tert*-butyldimethylsilanyloxy)-2-tetracosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonane (**16**) (0.10 g, 0.07 mmol) was dissolved under N_2 in THF (4 mL), and the solution was cooled to 0 °C. TBAF (1.0 M in THF, 0.28 mL, 0.28 mmol) was added, and the cooling bath was removed. The reaction mixture was stirred for 2 h at room temperature then diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20 to 70:30) provided (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonan-3,4-diol as a clear oil (0.070 g, 82%): $[\alpha]^{25}_D$ +32.7 (*c* 1.0, $CHCl_3$); IR (neat) 3319, 2918, 2850, 1637, 1543, 1468 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.55−7.26 (m, 20H), 6.37 (d, J = 8.5 Hz, 1H), 4.94−4.85 (m, 3H), 4.77 (m, 2H), 4.68 (d, J = 11.5 Hz, 1H), 4.57 (d, J = 11.5 Hz, 1H), 4.48 (d, J = 11.5 Hz, 1H), 4.40 (d, J = 11.5 Hz, 1H), 4.22 (m, 1H), 4.06 (dd, J = 10.0, 4.0 Hz, 1H), 3.97 (br s, 1H), 3.93−3.86 (m, 4H), 3.80 (d, J = 8.5 Hz, 1H), 3.53−3.45 (m, 4H), 2.14−2.10 (m, 4H), 1.27 (br s, 50H), 0.91−0.84 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 138.7, 138.6, 138.1, 137.8, 128.7, 128.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.7, 99.5, 79.6, 76.5, 76.3, 75.0, 74.7, 74.5, 73.9, 73.5, 73.0, 70.3, 70.2, 69.2, 49.8, 37.0, 33.4, 32.2, 32.1, 29.9, 29.9, 29.8, 29.6, 29.6, 29.5, 25.9, 25.8, 22.9, 22.9, 14.3, 14.3; HRMS (FAB) calcd for $C_{67}H_{102}NO_9$ (M^+ + H) 1065.7555, found 1065.7513.

(2*S*,3*S*,4*R*)-1-(α-D-Galactopyranosyl)-2-tetracosanoylaminononan-3,4-diol (OCH). $Pd(OH)_2$ (20% on carbon, 224 mg) was added to a vigorously stirred solution of (2*S*,3*S*,4*R*)-2-tetracosanoylamino-1-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)nonan-3,4-diol (47 mg, 0.045 mmol) in EtOH (3.0 mL) and $CHCl_3$ (0.75 mL). The mixture was placed under H_2, and stirring was continued for 24 h. The mixture was then filtered through Celite, and the filter cake was rinsed with $CHCl_3$ and MeOH. The filtrate was concentrated, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5 to 85:15) to provide OCH[31] as a white solid (25.0 mg, 81%): mp 125−127 °C; $[\alpha]^{25}_D$ +37.1 (*c* 1.0, pyr); IR (KBr) 3428, 2934, 2856, 1648, 1544, 1466 cm^{-1}; ^1H NMR (500 MHz, pyr-d_5) δ 8.44 (d, J = 9.0 Hz, 1H), 5.51 (d, J = 4.5 Hz, 2H), 5.26 (m, 2H), 4.68 (m, 2H), 4.55 (d, J = 3.5 Hz, 1H), 4.51 (t, J = 6.0 Hz, 1H), 4.40 (m, 4H), 4.30 (m, 2H), 3.64 (d, J = 3.0 Hz, 1H), 3.44 (br s, 1H), 2.47 (t, J = 7.0 Hz, 2H), 2.26 (m, 1H), 1.91−1.77 (m, 4H), 1.74 (br s, 1H), 1.64 (m, 1H), 1.32−1.23 (m, 44H), 0.88 (t, J = 7.0 Hz, 3H), 0.82 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, pyr-d_5) δ 173.7, 102.0, 77.3, 73.5, 73.0, 72.1, 71.5, 71.3, 71.3, 70.8, 69.1, 63.2, 51.9, 37.3, 34.8, 32.9, 32.6, 32.4, 30.5, 30.5, 30.4, 30.3, 30.3, 30.2, 30.1, 27.6, 26.9, 26.6, 23.5, 23.4, 14.8, 14.7; HRMS (FAB) calcd for $C_{39}H_{78}NO_9$ (M^+ + H) 704.5677, found 704.5662.

Antigen Presentation Assays. Bulk splenocytes were isolated from normal, 8−12 week old female C57BL/6 mice (Jackson Labs). Cells were plated at 300 000 per well in 96-well flat-bottom tissue culture plates in 200 μL complete medium: RPMI-1640 supplemented with 10 mM HEPES, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 55 μM 2-mercaptoethanol, 100 U/mL of penicillin and 100 μg/mL of streptomycin (Gibco), and 10% heat-inactivated fetal calf serum (Gemini). Cultures contained varying doses of glycolipids, diluted from 100 μM stocks in 100% DMSO. After 48 h in a 37 °C, 5% CO_2 humidified incubator, cell-free supernatants were collected and assayed for mouse IL-4 and IFNγ content, using standard sandwich ELISA. Capture and biotinylated antibodies were obtained from BD Pharmingen, cytokine standards from Peprotech, streptavidin-horseradish peroxidase from Zymed, and TMB-turbo substrate from Pierce. Absorbance at 450 nm was monitored with a microplate reader (Titertek).

Alternatively, lipid presentation by CD1d was tested by using a hybridoma-based assay. The CD1d-transfected RMA-S.mCD1d clone was provided by Dr. S. Behar.[35] The V_α-14^+ iNKT hybridoma DN3A4-1.2 has been described previously.[36] Both clones were maintained in complete RPMI medium as above. The $CD1d^+$ mouse dendritic cell line JAWS II was obtained from the American Type Culture Collection and maintained in MEMα medium with ribonucleosides, deoxyribonucleosides, 4 mM L-glutamine, 1 mM sodium pyruvate (Gibco), 20% heat-inactivated fetal calf serum, and 5 ng/mL mouse GM-CSF (Peprotech). Antigen presenting cells were plated at 50 000 per well on 96-well flat bottom plates in complete RPMI medium with varying amounts of glycolipids for 6 h at 37 °C. The cells were then washed three times with PBS (430*g*, 3 min). These were then fixed with 0.05% glutaraldehyde in PBS (grade I, Sigma) to prevent further antigen processing or loading, followed by quenching with 0.2 M L-lysine (pH 7.4) for 2 min and washes in complete medium. Fifty thousand iNKT hybridoma cells were then added in 100 μL complete medium for 12 h. Cell-free supernatants were collected and tested for the presence of IL-2 by ELISA. Antibodies were obtained from BD Pharmingen, mouse IL-2

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cytokine standard from R&D Systems, streptavidin-alkaline phosphatase from Zymed, and 4-nitrophenyl phosphate substrate from Sigma. Absorbance at 405 nm was monitored with a microplate reader (Titertek). IL-2 versus logarithm of lipid concentration plots and classical 4-parameter dose/response curves were drawn with Prism 4.02 (GraphPad). Relative potencies of the individual lipids were estimated by the reciprocals of the effective concentrations at 50% maximal response ($1/EC_{50}$).

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Supporting Information Available: General experimental information and copies of high-resolution ^{13}C NMR spectra for those new compounds for which elemental analyses are not reported; copies of high-resolution ^{1}H NMR spectra for OCH and analogues **1** and **2**; and results for hydbridoma assay with A20.CD1d APC's. This material is available free of charge via the Internet at http://pubs.acs.org.

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