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Phytosphingosines — A Facile Synthesis and Spectroscopic Protocol for Configurational Assignment

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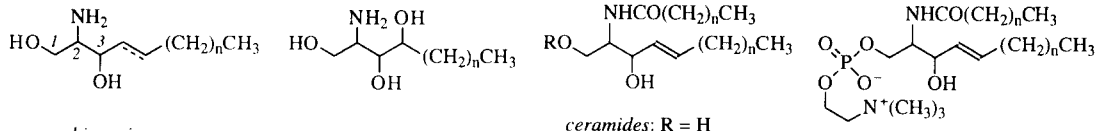
Abstract: A facile synthesis of four of the eight configurational isomers of phytosphingosine has been performed by employing Wittig and Julia olefination followed by Sharpless dihydroxylation. The set of these four stereoisomers served as model compounds for developing general chemical/CD/NMR protocols for assigning relative and absolute configurations of all phytosphingosine isomers. The procedure is based on a two-step derivatization to 2-*N*-naphthimide-1,3,4-*O*-trinaphthoate derivatives which gives rise to unique exciton coupled circular dichroic and ^{1}H-NMR spectra in selected solvents. The spectra can be used as reference data for assignment of relative as well as absolute configurations of phytosphingosine isomers and congeners at the low-nanomole level.

Keywords: phytosphingosine; sphingolipids; circular dichroism; relative and absolute configurations

Introduction

Sphingolipids, namely sphingomyelins, gangliosides, cerebrosides and other complex cellular lipid homologues which have long-chain bases as the backbone, i.e., sphingosines, dihydrosphingosines, phytosphingosines, are important membrane components. They function as endogenous media for cell recognition and cell regulation.[1,2] The free long-chain bases are intermediates in the biosynthetic and degradative pathways of sphingolipids. Over 300 sphingolipids have been described in mammalian tissues and cell types. Breakdown products of sphingolipids such as ceramide, sphingosine and sphingosine-1-phosphate are implicated in cell regulation and exhibit a wide variety of activities related to signal transduction.[1,3] They play important roles as agonists or second messengers involved in the modulation of receptor function, inhibition of platelet and neutrophil activation, growth factor action, phorbol ester-induced responses, and kinase C transduction.[1,2] The findings that diastereomers of ceramide, sphingosine and dihydrosphingosine exhibit different activities and metabolisms suggested the subtle



sphingosines
dihydrosphingosines

phytosphingosines

ceramides: R = H
cerebrosides : R = sugar
gangliosides : R = sugar with sialic acid residues

sphingomyelins

Representative sphingolipids

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biostereoselectivities of sphingolipids.[4-7] It follows that microscale analysis including stereochemical assignment is indispensable for biological investigations of sphingolipids and their breakdown products, particularly in clinical studies where only minuscule quantities are involved. However, no general method was available for configurational assignments except for measurements of optical rotation, which not only requires substantial amount of sample but is also frequently not conclusive. We recently developed a two-step CD microscale derivatization protocol to determine relative and absolute configurations of sphingosines and dihydrosphingosines containing two stereogenic centers at C-2 and C-3.[8,9] This method was successfully employed to determine the absolute stereochemistry of several naturally occurring sphingosine derivatives;[10,11] however, it cannot be applied directly to phytosphingosines that contain an additional stereogenic center at C-4.

Phytosphingosines are found in various glycosphingolipids such as cerebrosides and gangliosides.[12] The first phytosphingosine was isolated from the mushroom *Amanita muscaria* in 1911 by Zellner[13] as a nitrogen-containing "fungus cerebrin" and subsequently from yeast,[14] fungi,[15] and plants.[16] Structure of the "cerebrin base" was established as 2-amino-1,3,4-trihydroxyoctadecane in 1952;[15] its absolute configuration was assigned as D-(+)-*ribo* through degradative studies by Carter in 1963,[17] and through chemical correlation with synthetic and natural D-*erythro*-sphingosine in 1965.[18,19] Because of its plant origin and structural similarity to sphingosine, the term "phytosphingosine" was proposed for this base.[16] However, it has since become clear that phytosphingosines are not only present in plants, but also occur in marine organisms[12,20] and in mammalian tissues, such as kidney,[21] liver,[22] uterus,[23] intestine,[24] skin,[25] blood plasma,[26] and thymocytes.[27] In view of its biological significance, several syntheses of phytosphingosines, both the racemic and optically active forms, have been described. The first synthesis of all four racemic modifications were achieved in 1970,[28] while the optically active D-*ribo*-, D-*arabino*- D-*xylo*-, and D-*lyxo*-phytosphingosines were synthesized in 1990.[29] Physical data including NMR and $[\alpha]_D$ values of acetates have been used to identify the stereochemistry of unknown phytosphingosine and congeners.[30-32] Although convenient, the NMR/$[\alpha]_D$ protocol requires a certain amount of sample; furthermore, as mentioned above, $[\alpha]_D$ values are not only less sensitive than other analytical data[33] but also do not necessarily reflect sufficient structural and stereochemical differences.[34]

In this paper, we report a convenient synthesis of four of eight phytosphingosines, and a chemical/spectroscopic method for distinguishing the eight phytosphingosines: a two-step derivatization leading to bichromophoric derivatives, ^{1}H-NMR data for assigning relative configurations, and CD data for determining the relative and absolute configurations are also described. These NMR and CD data serve as references for determination of structures of unknown phytosphingosines and congeners. Furthermore, it is to be noted that the CD protocol can be performed at microgram or low-nanomole levels.

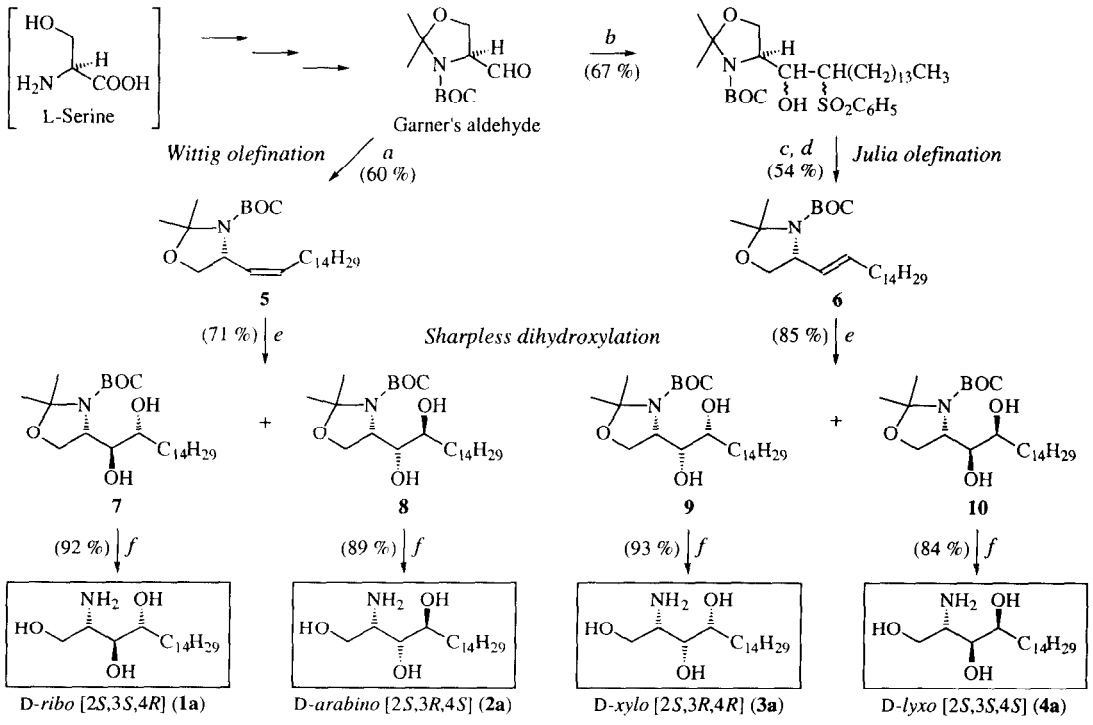
Results and Discussion

Synthesis of 4 isomers of phytosphingosines

In view of their biological significance, several syntheses of optically active phytosphingosines have been reported. For example, in the case of D-*ribo*-phytosphingosine, several chiral precursors, such as D-glucosamine, D-galactose, D-mannitol, D-threose, D-arabitol, D-xylose, (*S*)-malic acid, L-ascorbic acid, D-galactamic acid, and L-serine[29,35-37] have been employed as starting materials. The asymmetric synthesis was first reported in 1988,[38] and then followed by others. However, most synthetic protocols require a large number of steps or specific techniques, and do not yield all four of the eight isomers.

The synthetic route from L-serine, which is considered to be the biosynthetic precursor of sphingosine bases,[1,2] is shown in Scheme 1. This synthetic route yields the naturally occurring D-*ribo*-[2S,3S,4R] (**1a**), and D-*arabino*-

[2S,3R,4S] (**2a**), D-*xylo*-[2S,3R,4R] (**3a**), and D-*lyxo*-[2S,3S,4S] (**4a**) phytosphingosines. L-Serine was converted into Garner's aldehyde,[39] an *N*-protected cyclic serinal oxazolidine aldehyde, which is one of the most convenient chiral building blocks in the synthesis of bioactive compounds and which can be prepared without significant racemization.[40] Garner's aldehyde was then coupled to C_{15} long-chain Wittig phosphonium salt using *n*-BuLi in THF (–75°C) to give Z-olefin (**5**) as the major product (*Z*:*E* = ca. 95:5).[37] Garner's aldehyde was also coupled to a C_{15} phenylsulfone with *n*-BuLi in THF (–75°C); the resulting diastereomeric mixture was then acetylated and submitted to reductive elimination via Julia olefination[41] using sodium amalgam in MeOH–$NaHPO_4$ (–20°C)[42] to afford *E*-olefin (**6**) as major product (*E*:*Z* = ca. 9:1). Enantiomeric purities of olefins **5** (95 % ee) and **6** (94 % ee) were estimated by conversions to corresponding MTPA amides after deprotection. Note that the C_{15} phosphonium salt and phenylsulfone can both be readily prepared from the commercially available 1-bromopentadecane. Sharpless dihydroxylation[43] of the optically active *Z*-olefin using AD-mix-β gave a ca. 1:1 mixture of 2S,3S,4R (**7**) and 2S,3R,4S (**8**) dihydroxylated isomers, while that of the *E*-olefin using AD-mix-β gave a ca. 6:4 mixture of 2S,3R,4R (**9**) and 2S,3S,4S (**10**) isomers, respectively. The dihydroxylated compounds were separated by silica gel MPLC and deprotected with TFA–H_2O (20:1), to yield the four diastereomers, D-*ribo*- (**1a**), D-*arabino*- (**2a**), D-*xylo*- (**3a**) and D-*lyxo*- (**4a**) C_{18}-phytosphingosines. Their relative and absolute configurations were confirmed by comparison of optical rotation values[37,44-46] and NMR data of their acetates with those of authentic samples.[29]



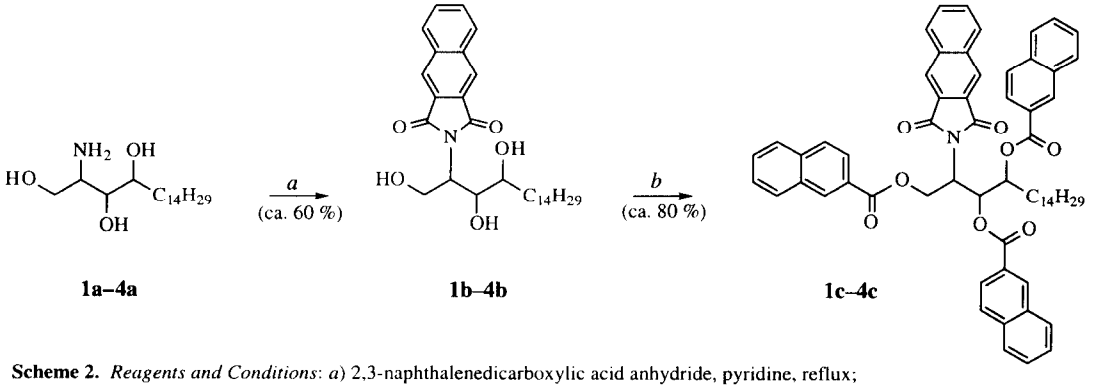
Scheme 1. *Reagents and Conditions*: *a*) $[Ph_3PCH(CH_2)_{13}CH_3]^+Br^-$, *n*-BuLi, THF, –75°C; *b*) $CH_3(CH_2)_{14}SO_2C_6H_5$, *n*-BuLi, THF, –75°C; *c*) Ac_2O, pyridine, CH_2Cl_2, rt; *d*) Na(Hg), $NaHPO_4$, MeOH, –20°C; *e*) AD-mix-α or β, methanesulfonamide, *t*-BuOH–H_2O = 1:1, rt; *f*) TFA–H_2O = 20:1, rt.

Two-step bichromophoric derivatization of phytosphingosines and their CD spectra

The circular dichroic exciton chirality method[47,48] is a microscale procedure for determining the absolute configuration and conformations of compounds containing two or more functional groups such as hydroxyl, amino and carboxyl groups which are converted into a variety of chromophores. The electric transition moments of these chromophores can couple through space to give bisignate CD curves that reflect their absolute sense of twist, and hence the absolute configurations of the stereogenic centers involved. In the case of three or more identical or different interacting chromophores, the exciton-split CD curve can be approximated by addition of all pair-wise contributions. While in general the application of exciton chirality to conformationally rigid molecules is straightforward and in fact non-empirical, the application to flexible compounds is more complicated since several conformers with different interchromophoric orientations often co-exist in solution. It is therefore not surprising that absolute configurational analysis of acyclic polyols and aminopolyols with multiple stereogenic centers including phytosphingosines is far more challenging and requires specific approaches. Our recent studies have described various applications of the so-called bichromophoric exciton chirality method, in which different exciton chromophores are selectively introduced into different types of functional groups.[49-52] The method turns out to be especially suited for acyclic compounds since it gives rise to unique "fingerprint" CD curves that reflect not only the absolute configutrations of the multiple stereogenic centers, but also the conformational population in solution. Thus, the selection of suitable chromophores and solvents for CD measurements is crucial in order to obtain highly characteristic CD curves. The latter can serve as reference data for identification and stereochemical analysis of biologically important molecules at the microscale level.

It was thought that the selective derivatization of the 2-amino-1,3,4-moiety with two or three types of chromophores could give rise to CD curves differentiating all eight phytosphingosines. For example, introduction of an amino-specific chromophore at C-2, followed by selective derivatization of the C-1 primary hydroxyl with a bulky chromophore and subsequent chromophoric acylation of the C-3 and C-4 secondary hydroxyls could yield "fingerprint" type CD curves. However, such a 3-step derivatization would be a drawback in microscale analysis. A recent two-step bichromophoric approach gave rise to a sub-micromole scale characterization of eight isomers of sphingosines and dihydrosphingosines.[9] In this protocol, the 2-amino group was converted into the fluorescent and stable naphthimide chromophore, while the remaining hydroxyls were converted into their naphthoates. Since the phytosphingosines have an extra 4-hydroxyl in *erythro* and *threo* relation to the 3-hydroxyl, an additional 4-naphthoate group might lead to a further set of characteristic CD curves. Indeed this turned out to be the case.

The two-step derivatization sequence is shown in Scheme 2. In the first step, the synthetic phytosphingosines



1a–4a **1b–4b** **1c–4c**

Scheme 2. *Reagents and Conditions*: *a*) 2,3-naphthalenedicarboxylic acid anhydride, pyridine, reflux; *b*) 2-naphthoylimidazole, DBU, acetonitrile, rt.

(**1a–4a**) were converted into *N*-naphthimide derivatives. Although the yield of this step was ca. 60 % against 80 % in the case of sphingosines and dihydrosphingosines,[9] the product appeared as a single spot on TLC except for the unreacted reagent with a higher R_f value and polar byproducts at the origin. The *N*-naphthimide derivatives (**1b–4b**) were then esterified to give pernaphthoate derivatives **1c–4c** as a single product in ca. 80 % yield.

The UV and CD of fully derivatized phytosphingosines (**1c–4c**) in two different solvents, the polar acetonitrile and nonpolar methylcyclohexane, are depicted in Figure 1. The CD of L-series of phytosphingosine derivatives should exhibit mirror image spectra of those of the D-series. The UV ε-values and $\varepsilon_{240}/\varepsilon_{260}$ ratio of naphthoate/naphthimide bands, shown in Table 1, serve as a check for the full derivatization and the purity.

Surprisingly, the resulting CD curves from summation of positive and negative naphthoate/naphthoate (ca. 230/240 nm) and naphthimide/naphthoate (ca. 235/260 nm) exciton couplets of all conformers present in solution were simpler in acetonitrile (Figure 1, solid line) than in methylcyclohexane (dotted line). In acetonitrile only two types of CD curves were observed: (i) negative CD couplets of **1c** and **3c** with a 1.35 and 4.89 ratio of Cotton effect

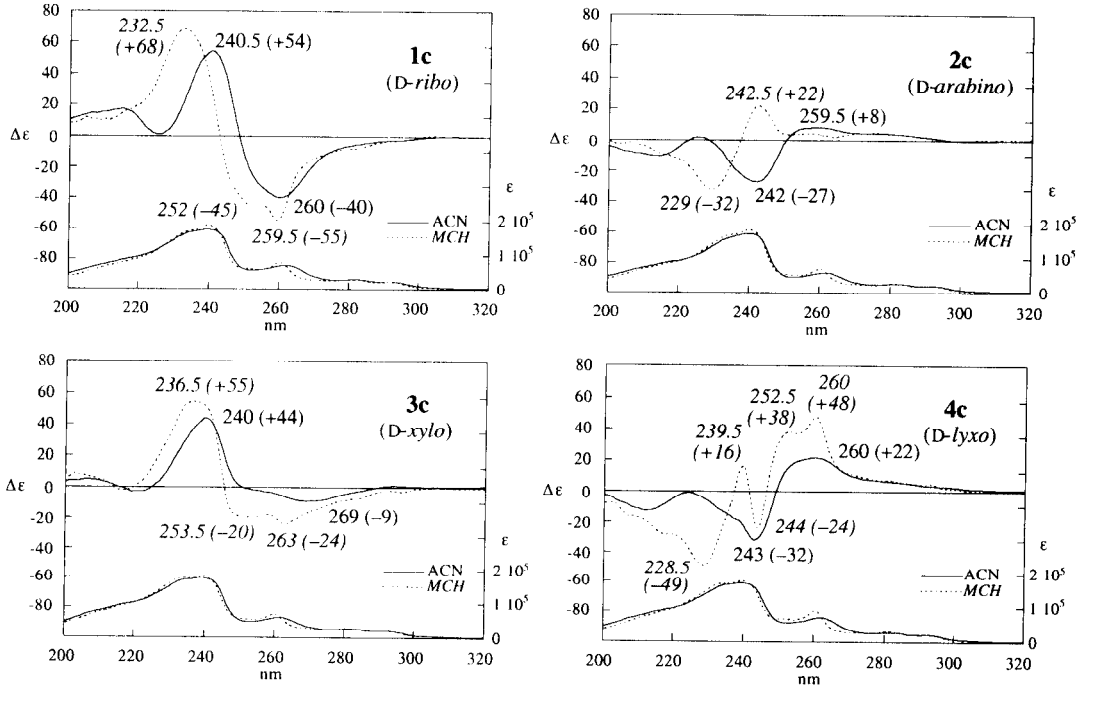


Figure 1. CD and UV spectra of D-series phytosphingosine *N*-naphthimide-*O*-trinaphthoate derivatives (**1c–4c**), in acetonitrile (ACN; solid line), and in methylcyclohexane (*MCH*; dotted line, Italic letters).

Table 1. ε-Values and $\varepsilon_{240}/\varepsilon_{260}$ ratios of naphthoate–naphthimide derivatives **1c**, **2c**, **3c** and **4c**.

Derivative	acetonitrile			methylcyclohexane		
	ε (nm)		$\varepsilon_{240}/\varepsilon_{260}$	ε (nm)		$\varepsilon_{240}/\varepsilon_{260}$
D-*ribo*, **1c**	175,000 (239.5),	70,500 (262)	2.48	188,000 (240),	81,000 (260)	2.32
D-*arabino*, **2c**	173,500 (239.5),	59,000 (261.5)	2.94	185,000 (240),	70,000 (260)	2.64
D-*xylo*, **3c**	176,500 (239.5),	59,000 (261.5)	2.99	182,000 (240),	68,500 (260)	2.66
D-*lyxo*, **4c**	173,000 (239),	71,000 (262)	2.44	179,000 (240),	89,500 (260)	2.00

(CE) Δε values at 240 nm and 260 nm, respectively; and (ii) the positive couplets of **2c** and **4c** with corresponding ratio 3.38 and 1.45. It is obvious that these ratios are not very different and would not allow discrimination of the mirror image spectra of **1c** (D-*ribo*) and **3c** (D-*xylo*) derivatives, i.e., L-*ribo* and L-*xylo*, derivatives from **4c** (D-*lyxo*) and **2c** (D-*arabino*) derivatives. In contrast, in methylcyclohexane all four derivatives **1c–4c** exhibit characteristic CD curves differing in overall shape and intensity. Such pronounced solvent dependent CD changes were also noted in the curves of other acyclic derivatives with multiple stereogenic centers.[8,53,54] Thus, as in the case discussed above, the CD measurements in polar and non-polar solvents are expected to provide better "fingerprint" data for structural analysis. It is worth mentioning that since the change of solvent alters the conformational population, and hence the sign and intensity of exciton coupled interactions contributing to overall CD, some CD Cotton effects become more conspicuous while others almost vanish. In methylcyclohexane, the CD curve of **2c** does not show any contribution of the naphthimide group, but exhibits only naphthoate/naphthoate homo exciton couplet with a positive CE at 242.5 nm (Δε +22) and a negative CE at 229 nm (Δε −32). Moreover, **4c** exhibited a surprisingly unique CD curve; its complex CD shows a positive first CE at 260 nm (Δε +48), a negative second CE at 244 (Δε −24), a positive third CE at 239.5 nm (Δε +16), and a negative fourth CE at 229 (Δε −49). This spectral shape is completely different from others. The observed changes from acetonitrile to methylcyclohexane in the overall CD spectra of **1c–4c**, although more significant than in other cases, are not surprising. These changes reflect the conformational complexity of flexible phytosphingosine system with three stereogenic centers and long aliphatic side chain, which presumably undergoes large conformational changes in polar acetonitrile and in nonpolar methylcyclohexane. Importantly, none of the mirror image spectra resemble the four spectra in methylcyclohexane. In summary, the CD reference curves in methylcyclohexane can allow for non-ambiguous configurational assignments of all eight phytosphingosines derivatives, **1c–4c** and the their enantiomers. In addition, corroborative evidence for relative configurational assignments can be provided by ^{1}H-NMR spectra of these derivatives (see below).

Table 2. Selected ^{1}H-NMR chemical shifts (δ/ppm in bold, multiplicity, *J*/Hz) for phytosphingosine *N*-naphthimide-*O*-trinaphthoate derivatives measured in three different solvents.

Solvent	assign.	D-*ribo*, **1c**	D-*arabino*, **2c**	D-*xylo*, **3c**	D-*lyxo*, **4c**
$CDCl_3$	1-H_a	**5.03**, dd, 4.7, 11.5	**5.11**, dd, 5.9, 11.5	**5.09**, dd, 5.6, 11.5	**4.79**, dd, 4.0, 11.6
	1-H_b	**5.11**, dd, 8.9, 11.5	**5.17**, dd, 8.1, 11.5	**5.16**, dd, 8.1, 11.5	**4.97**, dd, 8.7, 11.6
	2-H	**5.41**, ddd, 4.7, 8.4, 8.9	**5.38**, ddd, 5.9, 6.8, 8.1	**5.36**, ddd, 5.6, 7.3, 8.1	**5.26**, ddd, 4.0, 8.7, 9.8
	3-H	**6.45**, dd, 4.2, 8.4	**6.24**, dd, 4.2, 6.8	**6.29**, dd, 2.9, 7.3	**6.66**, dd, 2.1, 9.8
	4-H	**5.63**, ddd, 4.2, 4.2, 8.5	**5.73**, ddd, 4.2, 4.2, 8.7	**5.66**, ddd, 2.9, 6.5, 6.5	**5.58**, ddd, 2.1, 7.0, 7.0
C_6D_6	1-H_a	**5.41**, dd, 4.8, 11.6	**5.32**, dd, 5.5, 11.4	**5.42**, dd, 5.7, 11.6	**5.08**, dd, 3.7, 11.7
	1-H_b	**5.48**, dd, 8.5, 11.6	**5.44**, dd, 8.5, 11.4	**5.49**, dd, 7.9, 11.6	**5.33**, dd, 8.3, 11.7
	2-H	**5.93**, ddd, 4.8, 8.5, 8.5	**5.88**, ddd, 5.5, 6.5, 8.5	**5.89**, ddd, 5.7, 7.4, 7.9	**5.80**, ddd, 3.7, 8.3, 9.9
	3-H	**7.0**,[a] dd, 4.4, 8.5	**6.73**, dd, 4.3, 6.5	**6.70**, dd, 3.0, 7.4	**7.0**,[a] dd, 2.0, 9.9
	4-H	**6.17**, ddd, 5.1, 6.2, 6.2	**6.20**, ddd, 4.3, 4.3, 9.2	**6.01**, ddd, 3.0, 5.8, 7.6	**6.16**, ddd, 2.0, 6.4, 7.9
CD_3CN	1-H_a	**4.96**, dd, 7.7, 11.5	**5.07**, dd, 7.8, 11.4	**5.05**, dd, 7.9, 11.4	**4.83**, dd, 5.2, 11.6
	1-H_b	**5.00**, dd, 5.4, 11.5	**5.15**, dd, 6.2, 11.4	**5.10**, dd, 6.1, 11.4	**4.88**, dd, 7.5, 11.6
	2-H	**5.35**, ddd, 5.4, 7.7, 9.0	**5.38**, ddd, 6.2, 6.4, 7.8	**5.32**, ddd, 6.1, 6.4, 7.9	**5.27**, ddd, 5.2, 7.5, 9.8
	3-H	**6.46**, dd, 4.4, 9.0	**6.19**, dd, 3.9, 6.4	**6.21**, dd, 2.7, 6.4	**6.60**, dd, 2.5, 9.8
	4-H	**5.59**, ddd, 4.4, 4.4, 8.9	**5.69**, ddd, 3.9, 3.9, 9.0	**5.67**, ddd, 2.7, 6.8, 6.8	**5.52**, ddd, 2.5, 5.4, 8.1

[a] Signals bearing this superscript were overlapped on aromatic region proton signals so that coupling constants were estimated from their relationships.

Sub-micromole scale derivatization for NMR measurement

Komori et al.[29] have reported the NMR and optical rotation data of the four phytosphingosine acetates, which can be used to assign the configurations of new phytosphingosine analogs isolated from natural sources.[30,31,55] This NMR/$[\alpha]_D$ approach is convenient and rapid. However, the acetylated phytosphingosines are devoid of specific UV absorption for detection of small amounts of sample. Furthermore, measurements of optical rotation need larger amounts of sample than CD or even ^{1}H-NMR require,[33] and the $[\alpha]_D$ value itself does not fully reflect structural and stereochemical details.[34] The two-step derivatization of phytosphingosines, i.e., *N*-naphthimidation followed by *O*-trinaphthoylation yielding derivatives **1c–4c** with intense UV absorption and fluorescence emission, greatly facilitates handling of minute quantities. A comparison of the ^{1}H-NMR spectra in deuterated chloroform, acetonitrile and benzene were performed in order to select the solvent of choice for microscale differentiation of the four diastereomers by NMR (Table 2). Only three of the four diastereomers could be distinguished in chloroform and acetonitrile by their chemical shifts; however, in benzene all four could be readily differentiated (Figure 2a). Furthermore, all four diastereomers could be distinguished from their coupling constants (Figure 3). Isomers with 2,3-*erythro* configuration, i.e., D-*ribo* and D-*lyxo* isomers, have $J_{2,3}$ values > 8 Hz (8.4–9.9 Hz) corresponding to an *anti* relationship between 2-H/3-H. In contrast, the 2,3-*threo* configuration, i.e., D-*arabino* and D-*xylo* isomers, have $J_{2,3}$ < 8 Hz (6.4–7.4 Hz) indicating that the protons adopt a predominantly *gauche* relationship. In the case of 3,4-*erythro* isomers, D-*ribo* and D-*arabino*, and 3,4-*threo* isomers, D-*xylo* and D-*lyxo*, the $J_{3,4}$ values are 3.9–4.8 Hz (> 3.5 Hz), and 2.0–3.0 Hz (< 3.5 Hz), respectively. Thus, these differences in the four diastereomers in chemical shifts in benzene and coupling constants can be utilized for confirming the relative configurations of phytosphingosines. A

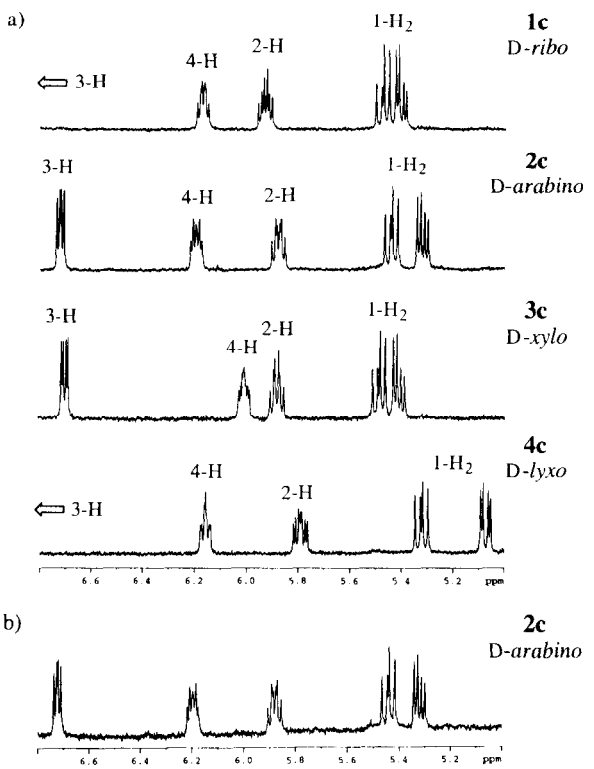


Figure 2. a) Selected ^{1}H-NMR spectral expansions of phytosphingosine *N*-naphthimide-*O*-trinaphthoate derivatives (**1c–4c**) measured in benzene-d_6. b) **2c** obtained by a two-step sub-micromole scale derivatization of **2a**.

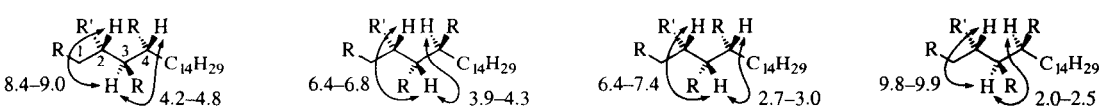


Figure 3. $J_{2,3}$ and $J_{3,4}$ values (in Hz) for phytosphingosine *N*-naphthimide-*O*-trinaphthoate derivatives.

two-step sub-micromole scale derivatization of a phytosphingosine to its *N*-naphthimide-*O*-trinaphthoate for NMR measurements was carried out with 46 μg of D-*arabino* phytosphingosine (**2a**; 0.145 μmol) to give 59.8 μg (0.0623 μmol) of the final derivative (**2c**) after prep-TLC. The ^{1}H-NMR of the entire sample after dissolving in ca. 0.6 mL deuterated benzene (Figure 2b) clearly demonstrates its identity as **2c**. At this sub-micromole scale, the assignment of the relative and absolute configurations by CD described above is readily achieved (Figure 3, solid curve a).

Low-nanomole scale derivatization for CD measurement

Although it is possible to distinguish four diastereomeric phytosphingosine *N*-naphthimide-*O*-trinaphthoate derivatives by the sub-micromole scale NMR analysis, further scale down is needed in order to analyze the minuscule constituents of unknown phytosphingosines. In a previous report,[9] we described a picomole scale HPLC analysis for the determination of relative and absolute configurations of sphingosines and dihydrosphingosines, where *N*-naphthimide derivatives were analyzed by normal phase HPLC followed by chiral HPLC. However, in the case of phytosphingosines with a total of eight stereoisomers, a satisfactory base-line separation could not be achieved between the four synthetic diastereomers under various HPLC conditions (data not shown). Thus the HPLC protocol is not suited for applications in native phytosphingosine congeners since the difference in aliphatic chain lengths would lead to ambiguous characterization. It follows that CD is the protocol of choice for micro-scale determination of configurations. Preliminary experiments suggested that the CD curve of **2c**, with the smallest amplitude (A = 54 in methylcyclohexane) among the four isomers, could be clearly recognized at a concentration around 1–0.5 μM (1–0.5 nmole in 1 mL). D-*Arabino* phytosphingosine **2a** (1 μg, 3 nmole) was used to carry out a low-nanomole level derivatization and CD measurement as a model for newly isolated unknown phytosphingosines. The naphthimidation step was performed in a melting point capillary tube using a melting point apparatus.[9] After purification by TLC, the product was subjected to naphthoylation in a small test tube. The TLC purified reaction product was dissolved in 1 mL methylcyclohexane for subsequent UV and CD measurements. The dotted curve b (c = ca 6 E–7 M) in Figure 4 shows that the CD of **2c** derivatized at the low-nanomole level is in excellent agreement with the measurement of sub-micromole scale derivatization (solid curve a, c = 6.2 E–6 M).

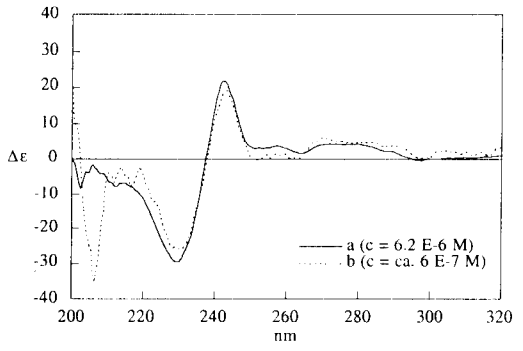


Figure 4. CD of **2c** obtained from **2a** by sub-micromole scale derivatization (a, solid line) and low-nanomole scale (b, dotted line) of **2a**, in methylcyclohexane.

Conclusion

A short and general synthesis of the four phytosphingosine diastereomers utilizing Wittig and Julia olefinations followed by Sharpless dihydroxylation reaction is described. Readily available L-serine and 1-bromopentadecane were used as starting materials for preparing D-*ribo* (**1a**), D-*arabino* (**2a**), D-*xylo* (**3a**), and D-*lyxo* (**4a**) phytosphingosines. D-Serine, instead of L-serine, would yield the L-series of phytosphingosines. A two-step derivatization of synthetic phytosphingosines yields *N*-naphthimide-*O*-trinaphthoate derivatives in moderate total yields of ca. 50 %. CD spectra of these four diastereomeric phytosphingosines derivatives (**1c–4c**) measured in methylcyclohexane give rise to four unique CD curves, the mirror images of which would represent the

corresponding four enantiomers. The four phytosphingosine derivatives (**1c–4c**) are also suited for assigning the relative configurations by ^{1}H-NMR (measured in benzene-d_6). The intense UV absorption and fluorescence emission of the *N*-naphthimide-*O*-trinaphthoates facilitate the sub-micromole two-step derivatization leading to determinations of the relative configuration by NMR and the relative and absolute configurations by CD. The protocol described above represents a low-nanomole scale determination of configurations of unknown phytosphingosine congeners by CD. Although C_{18} was the chain length of synthetic phytosphingosines used as reference, the effect of chain length on the shape of characteristic CD curves (Figure 1) and NMR (Figure 2) should be minimal.

Experimental

General methods

TLC plates used for analysis and preparative were Silica gel 60 F_{254} (250 μm; E. Merck) and Silica Gel GF Preparative Uniplates (500 μm, Analtech), respectively. ICN silica gel (32–63 mesh) was employed for flash chromatography. Medium pressure Liquid Chromatography (MPLC) was performed on an ULTRA PACK SI-40B silica gel pre-packed column (26 × 300 mm; Yamazen, Co., Japan). NMR spectra were recorded on a Bruker DMX 400 instrument and performed in $CDCl_3$ or in benzene-d_6. Chemical shifts (δ) are reported in ppm downfield from internal TMS and coupling constants (*J*) in Hz. CI mass spectra were measured on a NERMAG R10-10 spectrometer with NH_3 and CH_4 as ionizing gases for CI mass. ESI mass spectra were measured on a JEOL LCmate mass spectrometer. Low- and high-resolution FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometer using a glycerol matrix and Xe ionizing gas. UV spectra were measured on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer. CD spectra were measured by JASCO J-720 spectropolarimeter. The following parameters were employed: band width 1.0 nm; auto slit width; sensitivity 20 mdeg; response 2 sec; start wave length 400 nm; end wave length 200 nm; scan speed 100 nm/min; step resolution 0.5 nm; accumulation 8 (low-nanomole scale measurement was done by accumulation 32).

Preparation of *Z*-olefin (5)

To a suspension of pentadecylphosphonium bromide (8.2 g, 14.8 mmol; prepared from 1-bromopentadecane and triphenylphosphine, refluxed in toluene for 5 days, 98 % yield) in THF (50 mL) was added dropwise *n*-BuLi (1.6 M in hexane; 7.4 mL, 11.9 mmol) at –75°C under argon atmosphere. The solution was gradually warmed to rt and stirred for additional 1 h. To this solution, which was cooled down to –75°C again, Garner's aldehyde (2.5 g, 10.9 mmol) in THF (5 mL) was added dropwise over 30 min. The reaction mixture was then gradually warmed to rt and stirred for additional 2 h. The reaction was quenched by addition of saturated aqueous NH_4Cl (20 mL) and extracted with ether. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was separated by flush chromatography (hexane–ethyl acetate = 95:5 to 9:1) to give the olefin mixture (ca. 2.9 g, 63 % yield; *Z*:*E* = ca. 95:5, estimated by ^{1}H-NMR) as an oil. MPLC purification (hexane–ethyl acetate = 95:5 to 9:1) of this olefin mixture gave pure Z-olefin (**5**) as a colorless oil.

5: A colorless oil; $[\alpha]^{21}_D$ +50.7 (*c* 0.875, $CHCl_3$) [lit.[37] $[\alpha]^{26}_D$ +53.5 (*c* 1.0, $CHCl_3$)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.20–1.40 (br s, 24H), 1.44 (s, 9H), 1.52 (br s, 3H), 1.59 (br s, 3H), 1.90–2.25 (br m, 2H), 3.64 (dd, *J* = 3.3, 8.6 Hz, 1H), 4.05 (dd, *J* = 6.3, 8.6 Hz, 1H), 4.50–4.80 (br m, 1H), 5.35–5.45 (br m, 1H), 5.35–5.55 (br s, 1H); MS (CI, CH_4) *m/z* 424 $[M+H]^+$; HRMS (FAB) *m/z* found 424.3807, calcd for $C_{26}H_{50}NO_3$ 424.3791 $[M+H]^+$.

Preparation of *E*-olefin (6)

Pentadecyl phenyl sulfone (710 mg, 2.0 mmol; prepared from 1-bromopentadecane and thiophenol by way of pentadecyl phenyl sulfide, 95 % yield in two steps) was dissolved in THF (25 mL) and stirred at –75°C under argon atmosphere. *n*-BuLi (0.9 M in hexane; ca. 0.1 mL) was slowly added to the solution until a yellow color persisted. Additional *n*-BuLi (0.9 M in hexane; 2.0 mL, 1.82 mmol) was then added dropwise, resulting in a clear yellow solution. Garner's aldehyde (377 mg, 1.65 mmol) in THF (4 mL)

was added dropwise to the cooled sulfone solution over 10 min until the yellow color disappeared. After 20 min, the reaction was quenched by addition of saturated aqueous NH_4Cl (10 mL) and extracted with ether. The combined organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was separated by flush chromatography (hexane–ethyl acetate = 95:5 to 9:1, then 8:2) to give hydroxy sulfone (643 mg, 67 % yield) as a mixture of diastereomers. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.8–1.0 (m, 3H), 1.0–2.0 (m, 39H), 3.0–5.2 (m, 6H), 7.5–7.7 (m, 3H), 7.8–8.0 (m, 2H); MS (CI, NH_3) m/z 599 $[M+NH_3]^+$, 582 $[M+H]^+$; MS (FAB, + KI) m/z 621 $[M+K]^+$.

The hydroxy sulfone was dissolved in methylene chloride (2 mL) and pyridine (1 mL), and acetic anhydride (1 mL, 10.6 mmol) was added. The resulting mixture was stirred at rt, overnight. The reaction mixture was concentrated, and the residue was passed through flash chromatography (hexane–ethyl acetate = 95:5 to 9:1) to give acetylated sulfone (654 mg, 95 % yield) as a mixture of diastereomers. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.8–1.0 (m, 3H), 1.0–2.0 (m, 39H), 2.0–2.2 (m, 3H) 3.0–5.7 (m, 6H), 7.5–7.7 (m, 3H), 7.8–8.1 (m, 2H); MS (FAB, + KI) m/z 663 $[M+K]^+$.

The product from above was dissolved in methanol (25 mL), and $NaHPO_4$ (2.5 g) was added to them. The solution was cooled to 20°C, and 10 % Na(Hg) (1.43 g, 6.2 mmol) was added. The resulting mixture was stirred at –20°C for 3 h after which a second portion of Na(Hg) (0.87 g, 3.8 mmol) was added and stirring at –20°C was continued for 3 h. The reaction mixture was treated with saturated aqueous NH_4Cl (20 mL) and extracted with ethyl acetate. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was separated by flash chromatography (hexane–ethyl acetate = 95:5 to 9:1) to give an olefin mixture (282 mg, 63.5 % yield; *Z*:*E* = ca. 9:1, estimated by ^{1}H-NMR) as an oil. MPLC purification (hexane–ethyl acetate = 95:5 to 9:1) of this olefin mixture gave a pure E-olefin (**6**) as a white oily solid.

6: A white oily solid; $[\alpha]^{21}_D$ –5.7 (c 0.96, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.20–1.40 (m, 24H), 1.44 (s, 9H), 1.50 (s, 3H), 1.60 (br s, 3H), 2.02 (m, 2H), 3.71 (dd, *J* = 2.1, 8.7 Hz, 1H), 4.01 (dd, *J* = 6.1, 8.7 Hz, 1H), 4.15–4.45 (br m, 1H), 5.41 (br dd, *J* = 7.7, 15.2 Hz, 1H), 5.45–5.75 (br s, 1H); MS (CI, NH_3) *m/z* 424 $[M+H]^+$; HRMS (FAB) *m/z* found 424.3806, calcd for $C_{26}H_{50}NO_3$ 424.3791 $[M+H]^+$.

Preparation of MTPA amides of olefins 5 and 6

Z-Olefin **5** (27.3 mg, 64.5 μmol) was deprotected by TFA–H_2O (20:1) solution (0.5 mL) for 30 min. The reaction mixture was diluted with methylene chloride (2 mL), neutralized with saturated aqueous $NaHCO_3$, and extracted with methylene chloride. The extract was dried over Na_2SO_4 and concentrated to give an oily solid. To a solution of the product from above (3.0 mg, ca. 10 μmol), (*R*)-(–)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (2.9 mg, 12.4 μmol) and benzotriaol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP reagent; 7.7 mg, 17.4 μmol) in THF (0.5 mL) was added *N*,*N*-diisopropylethylamine (1 drop). The reaction mixture was stirred for 1h at rt, and then concentrated. The residue was passed through a mini-flash chromatography using pasteur pipette (hexane–ethyl acetate = 8:2 to 7:3) to give (*R*)-MTPA amide of **5** (2.9 mg, 55 % yield in two steps; 95 % de, determined by ^{1}H-NMR). By using (*S*)-(–)-α-methoxy-α-(trifluoromethyl)phenylacetic acid in the same procedure, (*S*)-MTPA amide of **5** (94 % de) was obtained.

(*R*)-MTPA amide of **5**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.20–1.40 (br s, 24H), 2.12 (m, 2H), 3.46 (s, 3H), 3.65–3.80 (m, 2H), 4.86 (m, 1H), 5.36 (m, 1H), 5.66 (m, 1H), 6.98 (br d, *J* = 7.1 Hz, 7.37–7.48 (m, 3H), 7.50–7.60 (m, 2H); MS (ESI) *m/z* 500 $[M+H]^+$.

(*S*)-MTPA amide of **5**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.7 Hz, 3H), 1.15–1.45 (br s, 24H), 2.10–2.25 (br m, 2H), 3.40 (s, 3H), 3.60–3.75 (m, 2H), 4.87 (m, 1H), 5.36 (m, 1H), 5.70 (m, 1H), 6.99 (br d, *J* = 7.2 Hz), 7.38–7.48 (m, 3H), 7.49–7.58 (m, 2H); MS (ESI) *m/z* 500 $[M+H]^+$.

E-Olefin **6** was also converted into the (*R*)- and (*S*)-MTPA amides by the same procedure.

(*R*)-MTPA amide of **6** (93 % de): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.91 (t, *J* = 6.8 Hz, 3H), 1.15–1.45 (br s, 24H), 1.96–2.07 (m, 2H), 3.49 (s, 3H), 3.69–3.80 (m, 2H), 4.59 (br m, 1H), 5.42 (dd, *J* = 5.9, 15.5 Hz, 1H), 5.62 (m, 1H), 7.04 (d, *J* = 7.8 Hz), 7.38–7.50 (m, 3H), 7.50–7.60 (m, 2H); MS (ESI) *m/z* 500 $[M+H]^+$.

(*S*)-MTPA amide of **6** (94 % de): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.91 (t, *J* = 6.8 Hz, 3H), 1.16–1.49 (br s, 24H), 2.03–2.12 (m, 2H), 3.43 (s, 3H), 3.66–3.77 (m, 2H), 4.61 (br m, 1H), 5.46 (dd, *J* = 6.1, 15.5 Hz, 1H), 5.77 (m, 1H), 7.13 (d, *J* = 7.7 Hz), 7.39–7.49 (m, 3H), 7.52–7.61 (m, 2H); MS (ESI) *m/z* 500 $[M+H]^+$.

Dihydroxylation of olefins 5 and 6

To a solution of AD-mix-β (1.4 g) and methansulfonamide (98 mg, 1.01 mmol) in *t*-BuOH–H_2O (1:1, 10 mL) was added a

solution of **5** (428 mg, 1.01 mmol) in the same solvent system (2 mL) at 0°C. The reaction mixture was stirred at 0°C for 10 h, and then at rt for 2 days, upon which the reaction mixture was quenched by addition of Na_2SO_3 (2 g), and extracted with methylene chloride. The organic extract was washed with 1M NaOH and brine, dried over Na_2SO_4, and concentrated. The residue was separated by flash chromatography (hexane–ethyl acetate = 95:5 to 9:1, then 8:2) to give a diol mixture (325 mg, 71 % yield; **7**:**8** = ca. 1:1, estimated by ^{1}H-NMR) and recovered olefin **5** (118 mg). MPLC separation (hexane–ethyl acetate = 9:1 to 8:2, then 7:3) of the diol mixture gave pure diols **7** and **8**.

7: A white oily solid; $[\alpha]^{21}_D$ –2.9 (*c* 1.485, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.7 Hz, 3H), 1.15–1.75 (m, 41H), 3.31 (br s, 2H), 3.62 (br s, 2H), 4.02 (br m, 1H), 4.18 (br s, 2H); MS (CI, CH_4) *m/z* 458 $[M+H]^+$; HRMS (FAB) *m/z* found 458.3849, calcd for $C_{26}H_{52}NO_5$ 458.3845 $[M+H]^+$.

8: A colorless oil; $[\alpha]^{22}_D$ –29.5 (*c* 1.055, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.7 Hz, 3H), 1.15–1.80 (m, 41H), 2.78 (br d, *J* = 3.7 Hz, 1H), 3.29 (br s, 1H), 3.46 (br m, 1H), 4.01 (d, *J* = 9.2 Hz, 1H), 4.11 (dd, *J* = 6.5, 9.2 Hz, 1H), 4.37 (br m, 2H); MS (CI, CH_4) *m/z* 458 $[M+H]^+$; HRMS (FAB) *m/z* found 458.3849, calcd for $C_{26}H_{52}NO_5$ 458.3845 $[M+H]^+$.

E-Olefin **6** was also dihydroxylated by AD-mix-β to give **9** and **10** (85 % yield; **9**:**10** = ca. 6:4, estimated by ^{1}H-NMR) by the same procedure.

9: A colorless oil; $[\alpha]^{23}_D$ –25.9 (*c* 1.145, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.15–1.73 (br s, 41H), 3.48 (br s, 1H), 3.62 (br m, 2H), 3.90 (br s, 1H), 4.00 (br s, 2H), 4.26 (br s, 1H); MS (CI, NH_3) *m/z* 475 $[M+NH_4]^+$, 458 $[M+H]^+$; HRMS (FAB) *m/z* found 458.3849, calcd for $C_{26}H_{52}NO_5$ 458.3845 $[M+H]^+$.

10: A white oily solid; $[\alpha]^{23}_D$ –2.5 (*c* 0.65, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J* = 6.9 Hz, 3H), 1.15–1.75 (m, 41H), 2.0–2.6 (br s, 1H), 3.29 (d, *J* = 9.5 Hz, 1H), 3.51 (t like, *J* = 6.7 Hz, 1H), 3.83 (dd, *J* = 5.1, 9.5 Hz, 1H), 4.00 (dd, *J* = 5.1, 9.0 Hz, 1H), 4.16 (d, *J* = 9.0 Hz, 1H), 4.0–4.6 (br s, 1H); MS (CI, CH_4) *m/z* 475 $[M+NH_4]^+$, 458 $[M+H]^+$; HRMS (FAB) *m/z* found 458.3849, calcd for $C_{26}H_{52}NO_5$ 458.3845 $[M+H]^+$.

Deprotection of compounds 7, 8, 9, and 10

Diol **7** (98 mg, 0.21 mmol) was treated with TFA–H_2O (20:1) solution (2 mL) for 30 min. The reaction mixture was diluted with methylene chloride (10 mL) and neutralized with saturated aqueous $NaHCO_3$. Resulting white solid was filtered and washed with H_2O to give **1a** (61 mg, 92 %) as a white solid. This solid was lyophilized to give a white powder, and stored below 5°C until further use. Diols **8**, **9**, and **10** were also deprotected by the same procedure to give phytosphingosines **2a**, **3a**, and **4a** as white powders.

1a: White powder; $[\alpha]^{23}_D$ +7.6 (*c* 1.0, pyridine) [lit.[37] $[\alpha]^{26}_D$ +9.5 (*c* 1.0, pyridine), lit.[44] $[\alpha]^{20}_D$ +7.9 (*c* 1.0, pyridine)]; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.84 (t, *J* = 6.8 Hz, 3H), 1.13–1.51 (m, 22H), 1.62–1.78 (m, 1H), 1.83–2.00 (m, 2H), 2.19–2.32 (m, 1H), 3.53 (br s, 1H), 3.98 (br t-like, *J* = 7.1 Hz, 1H), 4.22 (t-like, *J* = 7.9 Hz, 1H), 4.24 (dd, *J* = 5.7, 10.8 Hz, 1H), 4.32 (br dd, *J* = 4.0, 10.8 Hz, 1H), 6.32 (br s, 1H); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25 (br s, 24H), 1.44 (m, 1H), 1.60 (m, 1H), 2.67 (dt-like, *J* = 3.8, 6.7 Hz, 1H), 3.04 (br dd, *J* = 6.4, 12.1 Hz, 1H), 3.3–3.4 (m, 2H), 3.52 (br d, *J* = 11.0 Hz, 1H), 4.35–4.60 (br m, 2H); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ 14.2 (q), 22.9 (t), 26.1 (t), 29.6 (t), 29.9 (t) × 6, 30.1 (t), 30.4 (t), 32.1 (t), 34.7 (t), 57.5 (d), 64.7(t), 74.9 (d), 75.7 (d); MS (CI CH_4) *m/z* 318 $[M+H]^+$; HRMS (FAB) *m/z* found 318.3005, calcd for $C_{18}H_{40}NO_3$ 318.3008 $[M+H]^+$.

2a: White powder; $[\alpha]^{23}_D$ –4.5 (*c* 0.58, pyridine) [lit.[37] $[\alpha]^{26}_D$ –3.7 (*c* 1.0, pyridine), lit.[44] $[\alpha]^{20}_D$ –12.3 (*c* 0.6, pyridine)]; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.84 (t, *J* = 6.8 Hz, 3H), 1.13–1.49 (m, 22H), 1.53–1.70 (m, 1H), 1.77–1.95 (m, 2H), 2.00–2.12 (m, 1H), 3.81 (t-like, *J* = 6.5 Hz, 1H), 4.03–4.13 (m, 2H), 4.16–4.28 (m, 2H), 5.59 (br s, 1H), 6.33 (br s, 1H); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25 (br s, 24H), 1.44 (m, 1H), 1.56 (m, 1H), 2.92 (dt-like, *J* = 1.8, 6.8 Hz, 1H), 3.14 (dd, *J* = 1.9, 7.3 Hz, 1H), 3.25 (br dd, *J* = 7.5, 10.4 Hz, 1H), 3.3–3.4 (m, 2H), 4.3–4.6 (br s, 2H); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ 14.3 (q), 22.9 (t), 26.5 (t), 29.6 (t), 30.0 (t) × 6, 30.1 (t), 30.3 (t), 32.1 (t), 35.2 (t), 54.6 (d), 65.4 (t), 73.7 (d) × 2; MS (CI, CH_4) *m/z* 318 $[M+H]^+$; HRMS (FAB) *m/z* found 318.3018, calcd for $C_{18}H_{40}NO_3$ 318.3008 $[M+H]^+$.

3a: White powder; $[\alpha]^{23}_D$ –7.8 (*c* 0.46, pyridine) [lit.[45] $[\alpha]^{20}_D$ –7.1 (*c* 0.4, pyridine), lit.[46] $[\alpha]^{20}_D$ –6.2 (*c* 1.0, pyridine)]; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.84 (t, *J* = 6.8 Hz, 3H), 1.13–1.44 (m, 22H), 1.48–1.62 (m, 1H), 1.66–1.80 (m, 1H), 1.80–1.91 (m, 1H), 1.93–2.05 (m, 1H), 3.47 (br s, 1H), 3.95–4.09 (m, 2H), 4.09–4.19 (m, 2H); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25 (br s, 24H), 1.38 (br s, 2H), 2.73 (dt-like, *J* = 3.6, 6.3 Hz, 1H), 3.25–3.30 (m, 2H), 3.39 (dd, *J* = 6.1, 10.6 Hz, 1H), 3.47 (m, 1H), 4.3–4.8 (br s, 2H); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ 14.3 (q), 22.9 (t), 26.5 (t), 29.6 (t), 30.0 (t) × 7, 30.2 (t), 32.1 (t), 34.8 (t), 57.3 (d), 65.4 (t), 72.6 (d), 74.6 (d); MS (CI, NH_3) *m/z* 318 $[M+H]^+$; HRMS (FAB) *m/z* found 318.3003, calcd for $C_{18}H_{40}NO_3$ 318.3008 $[M+H]^+$.

4a: White powder; $[\alpha]^{23}_{D}$ +11.9 (*c* 0.89, pyridine); ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.85 (t, *J* = 6.8 Hz, 3H), 1.12–1.45 (m, 22H), 1.49–1.66 (m, 1H), 1.69–1.84 (m, 1H), 1.85–1.96 (m, 1H), 1.96–2.07 (m, 1H), 3.68 (br m, 1H), 4.00 (br d, *J* = 4.8 Hz, 1H), 4.20 (dd, *J* = 6.6, 10.5 Hz, 1H), 4.30 (m, 1H), 4.32 (dd, *J* = 4.4, 10.5 Hz, 1H); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25 (br s, 24H), 1.39 (br s, 2H), 2.81 (dt-like, *J* = 4.2, 7.1 Hz, 1H), 3.14 (m, 1H), 3.3–3.4 (m, 1H), 3.5–3.6 (m, 2H), 4.33 (br s, 1H), 4.55 (br s, 1H); ^{13}C-NMR (pyridine-d_5, 75 MHz) δ 14.3 (q), 22.9 (t), 26.7 (t), 29.6 (t), 30.0 (t) × 6, 30.1 (t), 30.2 (t), 32.1(t), 34.6 (t), 56.6 (d), 64.5 (t), 72.2 (d), 74.7 (d); MS (CI, NH_3) *m/z* 318 $[M+H]^+$; HRMS (FAB) *m/z* found 318.3018, calcd for $C_{18}H_{40}NO_3$ 318.3008 $[M+H]^+$.

Acetylation of phytosphingosines 1a–4a

The phytosphingosines (**1a–4a**; 13–28 mg) were dissolved in pyridine (1 mL), and acetic anhydride (1 mL) was added. The resulting mixture was stirred at rt, overnight. The reaction mixture was concentrated, and the residue was flash chromatographed (hexane–ethyl acetate = 8:2 to 1:1) to give the tetraacetyl derivative (ca. 75–80 % yield) as a white oily solid.

Tetraacetate of **1a**: $[\alpha]^{20}_{D}$ +21.9 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.15–1.75 (m, 26H), 2.03 (s, 3H), 2.05 (s, 6H), 2.08 (s, 3H), 4.00 (dd, *J* = 3.1, 11.7 Hz, 1H), 4.29 (dd, *J* = 4.8, 11.7 Hz, 1H), 4.47 (m, 1H), 4.93 (dt, *J* = 3.2, 9.7 Hz, 1H), 5.10 (dd, *J* = 3.0, 8.3 Hz, 1H), 5.98 (d, *J* = 9.4 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.1 (q), 20.8 (q) × 2, 21.1 (q), 22.7 (t), 23.3 (q), 25.5 (t), 28.2 (t), 29.3 (t), 29.4 (t), 29.5 (t), 29.7 (t) × 6, 31.9 (t), 47.6 (d), 62.8 (t), 72.0 (d), 73.0 (d), 169.7 (s), 170.1 (s), 170.9 (s), 171.2 (s); MS (CI, NH_3) *m/z* 485 $[M+H]^+$.

Tetraacetate of **2a**: $[\alpha]^{21}_{D}$ –21.2 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.15–1.75 (m, 26H), 1.99 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 4.00 (d, *J* = 6.0 Hz, 2H), 4.60 (m, 1H), 5.00 (m, 1H), 5.19 (dd, *J* = 3.3, 6.6 Hz, 1H), 5.62 (d, *J* = 9.6 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.1 (q), 20.7 (q), 20.8 (q), 21.0 (q), 22.7 (t), 23.2 (q), 25.1 (t), 29.4 (t) × 3, 29.5 (t), 29.7 (t) × 5, 30.3 (t), 31.9 (t), 47.0 (d), 63.0 (t), 70.9 (d), 72.0 (d), 169.7 (s), 169.8 (s), 170.3 (s), 170.7 (s); MS (CI, NH_3) *m/z* 485 $[M+H]^+$.

Tetraacetate of **3a**: $[\alpha]^{21}_{D}$ +7.0 (*c* 0.86, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.15–1.70 (m, 26H), 2.02 (s, 3H), 2.06 (s, 6H), 2.09 (s, 3H), 4.00 (dd, *J* = 5.8, 11.3 Hz, 1H), 4.05 (dd, *J* = 6.0, 11.3 Hz, 1H), 4.52 (m, 1H), 5.05 (dd, *J* = 6.5, 12.8 Hz, 1H), 5.16 (dd, *J* = 4.3, 6.5 Hz, 1H), 5.74 (d, *J* = 9.5 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.1 (q), 20.7 (q) × 2, 20.9 (q), 22.7 (t), 23.3 (q), 24.8 (t), 29.3 (t), 29.4 (t) × 2, 29.5 (t), 29.7 (t) × 5, 30.5 (t), 31.9 (t), 48.0 (d), 62.9 (t), 71.9 (d), 72.2 (d), 169.9 (s), 170.2 (s), 170.6 (s) × 2; MS (CI, NH_3) *m/z* 485 $[M+H]^+$.

Tetraacetate of **4a**: $[\alpha]^{22}_{D}$ –3.1 (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, *J* = 6.7 Hz, 3H), 1.15–1.60 (m, 26H), 1.96 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 3.96 (dd, *J* = 3.4, 11.7 Hz, 1H), 4.22 (dd, *J* = 4.6, 11.7 Hz, 1H), 4.52 (m, 1H), 5.08 (m, 2H), 5.77 (d, *J* = 9.7 Hz, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 14.1 (q), 20.7 (q), 20.8 (q), 21.0 (q), 22.7 (t), 23.3 (q), 25.2 (t), 29.4 (t) × 2, 29.5 (t) × 2, 29.7 (t) × 5, 30.9 (t), 31.9 (t), 47.4 (d), 63.1 (t), 71.2 (d), 71.8 (d), 169.6 (s), 170.3 (s), 170.6 (s), 170.8 (s); MS (CI, NH_3) *m/z* 485 $[M+H]^+$.

Preparation of *N*-naphthimide derivatives (1b–4b)

D-*ribo*-Phytosphingosine (**1a**; 2.01 mg, 6.3 μmol) and 2,3-naphthalenedicarboxylic acid anhydride (1.70 mg, 8.5 μmol) were placed in a 10 mL round bottomed flask connected to a micro-scale condenser, and was dried in vacuo. After replacement with argon, anhydrous pyridine (200 μL) was added and then refluxed under argon atmosphere overnight. The reaction mixture was concentrated under argon and applied to prep. TLC (10 × 10 cm; *n*-hexane–ethyl acetate = 1:1, developed twice, R_f = ca. 0.35) to give *N*-naphthimide derivative (**1b**; 1.91 mg, 60 % yield). By the same procedure, **2b**, **3b**, and **4b** were also prepared (54–67 % yields).

1b: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.15–1.75 (m, 26H), 3.79 (m, 1H), 4.04 (m, 1H), 4.06 (dd, *J* = 5.7, 12.2 Hz, 1H), 4.22 (dd, *J* = 4.8, 12.2 Hz, 1H), 4.74 (q-like, *J* = 4.8 Hz, 1H), 7.74 (dd, *J* = 3.3, 6.2 Hz, 2H), 8.08 (dd, *J* = 3.3, 6.1, 2H), 8.36 (s, 2H); MS (CI, NH_3) *m/z* 515 $[M+NH_4]^+$, 498 $[M+H]^+$; HRMS (FAB) *m/z* found 498.3218, calcd for $C_{30}H_{44}NO_5$ 498.3219 $[M+H]^+$.

2b: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.15–1.85 (m, 26H), 3.38 (br m, 1H), 3.95 (br s, 1H), 4.14 (dd, *J* = 6.2, 12.4 Hz, 1H), 4.20 (dd, *J* = 6.0, 12.4 Hz, 1H), 4.78 (br s, 1H), 7.74 (dd, *J* = 3.2, 6.4 Hz, 2H), 8.08 (dd, *J* = 3.3, 6.1, 2H), 8.37 (s, 2H); MS (CI, NH_3) *m/z* 515 $[M+NH_4]^+$, 498 $[M+H]^+$; HRMS (FAB) *m/z* found 498.3223, calcd for $C_{30}H_{44}NO_5$ 498.3219 $[M+H]^+$.

3b: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.15–1.75 (m, 26H), 3.66 (br m, 1H), 3.99 (br s, 1H), 4.09 (dd, *J* = 4.3, 12.3 Hz, 1H), 4.15 (dd, *J* = 6.3, 12.3 Hz, 1H), 4.67 (dd, *J* = 6.2, 10.2 Hz, 1H), 7.73 (dd, *J* = 3.3, 6.2 Hz, 2H), 8.07 (dd, *J* = 3.3,

6.1, 2H), 8.36 (s, 2H); MS (CI, NH_3) *m/z* 515 $[M+NH_4]^+$, 498 $[M+H]^+$; HRMS (FAB) *m/z* found 498.3227, calcd for $C_{30}H_{44}NO_5$ 498.3219 $[M+H]^+$.

4b: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, *J* = 6.9 Hz, 3H), 1.15–1.75 (m, 26H), 3.53 (br m, 1H), 4.07 (br d, *J* = 5.7 Hz, 1H), 4.07 (dd, *J* = 6.0, 12.2 Hz, 1H), 4.26 (dd, *J* = 4.3, 12.2 Hz, 1H), 4.55 (q-like, *J* = 6.0 Hz, 1H), 7.74 (dd, *J* = 3.3, 6.2 Hz, 2H), 8.08 (dd, *J* = 3.3, 6.2, 2H), 8.37 (s, 2H); MS (CI, NH_3) *m/z* 515 $[M+NH_4]^+$, 498 $[M+H]^+$; HRMS (FAB) *m/z* found 498.3205, calcd for $C_{30}H_{44}NO_5$ 498.3219 $[M+H]^+$.

Preparation of *N*-naphthimide-*O*-trinaphthoate derivatives (1c–4c)

To a solution of **1b** (0.20 mg, 0.4 μmol) and 2-naphthoylimidazole (1.15 mg, 5.1 μmol) in anhydrous acetonitrile (200 μL), was added DBU (10 % in acetonitrile, 20 μL) and stirred for 3 h. The reaction mixture was concentrated under a stream of argon, and was applied to prep. TLC (10 × 10 cm; *n*-hexane–ethyl acetate = 8:2, developed twice, R_f = ca. 0.40) to give *N*-naphthimide-*O*-trinaphthoate derivative (**1c**; 0.29 mg, 75 % yield). By the same procedure, **2c**, **3c**, and **4c** were also prepared (70–85 % yields).

1c: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.0–1.5 (m, 24H), 1.88–2.02 (m, 2H), 5.03 (dd, *J* = 4.7, 11.5 Hz, 1H), 5.11 (dd, *J* = 8.9, 11.5 Hz, 1H), 5.41 (ddd, *J* = 4.7, 8.4, 8.9 Hz, 1H), 5.63 (ddd, *J* = 4.2, 4.2, 8.5 Hz, 1H), 6.45 (dd, *J* = 4.2, 8.4 Hz, 1H), 7.38–7.57 (m, 5H), 7.61 (ddd, *J* = 1.2, 6.9, 8.1, 1H), 7.64–7.71 (m, 4H), 7.71–7.82 (m, 4H), 7.84 (dd, *J* = 1.7, 8.6 Hz, 1H), 7.87–7.99 (m, 6H), 8.12 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.28 (s, 2H), 8.38 (s, 1H), 8.53 (s, 1H), 8.68 (s, 1H); ^{1}H-NMR (C_6D_6, 400 MHz) δ 0.90 (t, *J* = 6.9 Hz, 3H), 1.0–1.7 (m, 24H), 2.11–2.20 (m, 2H), 5.41 (dd, *J* = 4.8, 11.6 Hz, 1H), 5.48 (dd, *J* = 8.5, 11.6 Hz, 1H), 5.93 (ddd, *J* = 4.8, 8.5, 8.5 Hz, 1H), 6.17 (ddd, *J* = 5.1, 6.2, 6.2 Hz, 1H), 6.97–7.27 (m, 11H), 7.29–7.45 (m, 5H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 7.55 (d, *J* = 9.0 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.87 (s, 2H), 8.12 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.30 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.33 (dd, *J* = 1.7, 8.5 Hz, 1H), 8.64 (s, 1H), 8.91 (s, 1H), 8.93 (s, 1H); ^{1}H-NMR (CD_3CN, 400 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.0–1.5 (m, 24H), 1.95–2.15 (m, 2H), 4.96 (dd, *J* = 7.7, 11.5 Hz, 1H), 5.00 (dd, *J* = 5.4, 11.5 Hz, 1H), 5.35 (ddd, *J* = 5.4, 7.7, 9.0 Hz, 1H), 5.59 (ddd, *J* = 4.4, 4.4, 8.9 Hz, 1H), 6.46 (dd, *J* = 4.4, 9.0 Hz, 1H), 7.45–7.52 (m, 2H), 7.55–7.62 (m, 3H), 7.67 (ddd, *J* = 1.2, 6.9, 8.2, 1H), 7.71–7.88 (m, 9H), 7.89 (dd, *J* = 1.6, 8.6 Hz, 1H), 7.89 (br d, *J* = 8.7 Hz, 3H), 8.04–8.13 (m, 3H), 8.33 (s, 2H), 8.37 (s, 1H), 8.47 (s, 1H), 8.74 (s, 1H); MS (CI, NH_3) *m/z* 977 $[M+NH_4]^+$, 960 $[M+H]^+$; HRMS (FAB, + KI) m/z found 998.4075, calcd for $C_{63}H_{61}NO_8K$ 998.4035 $[M+K]^+$.

2c: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, *J* = 6.9 Hz, 3H), 1.1–1.4 (m, 22H), 1.4–1.5 (m, 2H), 2.00–2.15 (m, 2H), 5.11 (dd, *J* = 5.9, 11.5 Hz, 1H), 5.17 (dd, *J* = 8.1, 11.5 Hz, 1H), 5.38 (ddd, *J* = 5.9, 6.8, 8.1 Hz, 1H), 5.73 (ddd, *J* = 4.2, 4.2, 8.7 Hz, 1H), 6.24 (dd, *J* = 4.2, 6.8 Hz, 1H), 7.38 (ddd, *J* = 1.0, 7.0, 8.0 Hz, 1H), 7.44–7.59 (m, 5H), 7.61–7.70 (m, 4H), 7.72–7.88 (m, 7H), 7.88–7.98 (m, 4H), 8.04 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.22 (s, 2H), 8.44 (s, 1H), 8.51 (s, 1H), 8.60 (s, 1H); ^{1}H-NMR (C_6D_6, 400 MHz) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.05–1.45 (m, 22H), 1.45–1.70 (m, 2H), 2.08–2.20 (m, 1H), 2.25–2.38 (m, 1H), 5.32 (dd, *J* = 5.5, 11.4 Hz, 1H), 5.44 (dd, *J* = 8.5, 11.4 Hz, 1H), 5.88 (ddd, *J* = 5.5, 6.5, 8.5 Hz, 1H), 6.20 (ddd, *J* = 4.3, 4.3, 9.2 Hz, 1H), 6.73 (dd, *J* = 4.3, 6.5 Hz, 1H), 6.92–7.2 (m, 10H), 7.31–7.42 (m, 6H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.80 (s, 2H), 8.17 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.20 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.41 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.71 (s, 1H), 8.74 (s, 1H), 8.97 (s, 1H); 1H-NMR (CD_3CN, 400 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.1–1.4 (m, 22H), 1.4–1.6 (m, 2H), 2.0–2.3 (m, 2H), 5.07 (dd, *J* = 7.8, 11.4 Hz, 1H), 5.15 (dd, *J* = 6.2, 11.4 Hz, 1H), 5.38 (ddd, *J* = 6.2, 6.4, 7.8 Hz, 1H), 5.69 (ddd, *J* = 3.9, 3.9, 9.0 Hz, 1H), 6.19 (dd, *J* = 3.9, 6.4 Hz, 1H), 7.43 (ddd, *J* = 1.0, 7.0, 8.1 Hz, 1H), 7.52–7.60 (m, 3H), 7.60–7.79 (m, 6H), 7.79–8.06 (m, 12H), 8.26 (s, 2H), 8.41 (s, 1H), 8.56 (s, 1H), 8.58 (s, 1H); MS (CI, NH_3) *m/z* 977 $[M+NH_4]^+$, 960 $[M+H]^+$; HRMS (FAB, + KI) *m/z* found 998.4075, calcd for $C_{63}H_{61}NO_8K$ 998.4035 $[M+K]^+$.

3c: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.1–1.4 (m, 22H), 1.45–1.55 (m, 2H), 1.85–2.00 (m, 2H), 5.09 (dd, *J* = 5.6, 11.5 Hz, 1H), 5.16 (dd, *J* = 8.1, 11.5 Hz, 1H), 5.36 (ddd, *J* = 5.6, 7.3, 8.1 Hz, 1H), 5.66 (ddd, *J* = 2.9, 6.5, 6.5 Hz, 1H), 6.29 (dd, *J* = 2.9, 7.3 Hz, 1H), 7.42 (ddd, *J* = 1.2, 6.9, 8.2 Hz, 1H), 7.45–7.62 (m, 7H), 7.67–7.91 (m, 12H), 8.01 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.04 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.14 (s, 2H), 8.40 (s, 1H), 8.54 (s, 1H), 8.61 (s, 1H); ^{1}H-NMR (C_6D_6, 400 MHz) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.05–1.75 (m, 24H), 1.95–2.20 (m, 2H), 5.42 (dd, *J* = 5.7, 11.6 Hz, 1H), 5.49 (dd, *J* = 7.9, 11.6 Hz, 1H), 5.89 (ddd, *J* = 5.7, 7.4, 7.9 Hz, 1H), 6.01 (ddd, *J* = 3.0, 5.8, 7.6 Hz, 1H), 6.70 (dd, *J* = 3.0, 7.4 Hz, 1H), 6.98–7.04 (m, 2H), 7.07–7.2 (m, 8H), 7.27–7.39 (m, 6H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 2H), 7.76 (s, 2H), 8.07 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.33 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.38 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.60 (s, 1H), 8.85 (s, 1H), 8.94 (s, 1H); ^{1}H-NMR (CD_3CN, 400 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.1–1.4 (m, 22H), 1.45–1.55 (m, 2H), 1.85–2.0 (m, 2H), 5.05 (dd, *J* = 7.9, 11.4 Hz, 1H), 5.10 (dd, *J* = 6.1, 11.4 Hz, 1H), 5.32 (ddd, *J* = 6.1, 6.4, 7.9 Hz, 1H), 5.67 (ddd, *J* = 2.7, 6.8, 6.8 Hz, 1H), 6.21 (dd, *J* = 2.7, 6.4 Hz, 1H), 7.46–7.68 (m, 8H), 7.78–8.00 (m, 13H), 8.05 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.11 (s, 2H), 8.43 (s, 1H), 8.50 (s, 1H), 8.64 (s, 1H); MS (CI, NH_3) *m/z* 977

$[M+NH_4]^+$, 960 $[M+H]^+$; HRMS (FAB, + KI) *m/z* found 998.4069, calcd for $C_{63}H_{61}NO_8K$ 998.4035 $[M+K]^+$.

4c: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, *J* = 6.9 Hz, 3H), 1.05–1.45 (m, 24H), 1.80–1.88 (m, 2H), 4.79 (dd, *J* = 4.0, 11.6 Hz, 1H), 4.97 (dd, *J* = 8.7, 11.6 Hz, 1H), 5.26 (ddd, *J* = 4.0, 8.7, 9.8 Hz, 1H), 5.58 (ddd, *J* = 2.1, 7.0, 7.0 Hz, 1H), 6.66 (dd, *J* = 2.1, 9.8 Hz, 1H), 7.40 (ddd, *J* = 1.1, 6.9, 7.9 Hz, 1H), 7.46–7.53 (m, 2H), 7.55–7.76 (m, 8H), 7.82 (dd, *J* = 1.6, 8.6 Hz, 1H), 7.84–8.02 (m, 9H), 8.24 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.29 (s, 2H), 8.37 (s, 1H), 8.54 (s, 1H), 8.81 (s, 1H); ^{1}H-NMR (C_6D_6, 400 MHz) δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.05–1.65 (m, 24H), 2.00–2.15 (m, 2H), 5.08 (dd, *J* = 3.7, 11.7 Hz, 1H), 5.33 (dd, *J* = 8.3, 11.7 Hz, 1H), 5.80 (ddd, *J* = 3.7, 8.3, 9.9 Hz, 1H), 6.16 (ddd, *J* = 2.0, 6.4, 7.9 Hz, 1H), 6.97–7.28 (m, 10H), 7.33 (br d, *J* = 9.0 Hz, 2H), 7.37 (dd, *J* = 2.1, 9.9 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.53 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.94 (s, 2H), 8.12 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.42 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.49 (dd, *J* = 1.7, 8.6 Hz, 1H), 8.65 (s, 1H), 8.91 (s, 1H), 9.07 (s, 1H); ^{1}H-NMR (CD_3CN, 400 MHz) δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.05–1.50 (m, 24H), 1.85–1.95 (m, 2H), 4.3 (dd, *J* = 5.2, 11.6 Hz, 1H), 4.88 (dd, *J* = 7.5, 11.6 Hz, 1H), 5.27 (ddd, *J* = 5.2, 7.5, 9.8 Hz, 1H), 5.52 (ddd, *J* = 2.5, 5.4, 8.1 Hz, 1H), 6.60 (dd, *J* = 2.5, 9.8 Hz, 1H), 7.45–7.54 (m, 2H), 7.54–7.67 (m, 3H), 7.69 (ddd, *J* = 1.2, 6.9, 8.1 Hz, 1H), 7.72–7.94 (m, 9H), 7.96–8.13 (m, 6H), 8.23 (dd, *J* = 1.6, 8.6 Hz, 1H), 8.28 (s, 2H), 8.37 (s, 1H), 8.51 (s, 1H), 8.87 (s, 1H); MS (CI, NH_3) *m/z* 977 $[M+NH_4]^+$, 960 $[M+H]^+$; HRMS (FAB, + KI) *m/z* found 998.4053, calcd for $C_{63}H_{61}NO_8K$ 998.4035 $[M+K]^+$.

Sub-micromole scale two-step derivatization and NMR measurement

D-*arabino*-Phytosphingosine (**2a**; 46.0 μg, 0.145 μmol) and 2,3-naphthalenedicarboxylic acid anhydride (37.3 μg, 0.188 μmol) were placed in a 5 mL round bottomed flask with a micro-scale condenser and dried in vacuo. After replacement with argon, anhydrous pyridine (100 μL) was added and then refluxed under argon atmosphere overnight. The reaction mixture was concentrated under argon and submitted to prep. TLC (5 × 10 cm; *n*-hexane–ethyl acetate = 1:1, developed twice, R_f = ca. 0.35) to give **2b** (40.9 μg, 56.7 % yield, calculated from UV absorption ε_{262} = 61,000 in MCH). The entire product and 2-naphthoylimidazole (905.6 μg, 4.08 μmol) were placed in a 2 mL vial, and anhydrous acetonitrile (100 μL) and DBU (10 % in acetonitrile, 10 μL) was added and stirred for 3 h. The reaction mixture was concentrated under argon and applied to prep. TLC (5 × 10 cm; *n*-hexane–ethyl acetate = 8:2, developed twice, R_f = ca. 0.40) to **2c** (59.8 μg, 75.8 % yield, calculated from UV absorption ε_{241} = 185,000 in MCH). The final product was dissolved in benzene-d_6 (0.6 mL) and transferred into a standard 5-mm NMR tube, and the 1D ^{1}H-NMR spectrum was taken by using of a standard NMR probe accumulated for ca. 10 h (12800 scans) on 400 MHz NMR spectrometer.

Low-nanomole scale two-step derivatization and CD measurement

Into a melting point capillary tube (90 × 1.5–1.8 mm o.d.), a solution (3 μL of pyridine) of D-*arabino*-phytosphingosine (**2a**; 1.0 μg, 3 nmol) and 2,3-naphthalenedicarboxylic acid anhydride (0.65 μg, 3.3 nmol) were transferred, and the tube was put in the melting point apparatus set at 110–115 °C for 10 h while the solvent was slowly evaporated.[9] The reaction product was purified by anal. TLC (20 × 50 mm; *n*-hexane–ethyl acetate = 1:1, developed twice). The product (**2b**) and excess of 2-naphthoylimidazole (300–500 nmol) were put in a test tube (10 × 75 mm) and pumped to drying up for a while. After replacement with argon, 1 % DBU (in acetonitrile) solution (25 μL) was added and mixed for 3h. The reaction mixture was concentrated under argon and applied to anal. TLC (20 × 50 mm; *n*-hexane–ethyl acetate = 8:2, developed twice). The final product (**2c**; ca. 15–20 % yield from **2a**) was dissolved in 1 mL of methylcyclohexane and the CD was measured (accumulation = 32).

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