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Divergent syntheses of all stereoisomers of phytosphingosine and their use in the construction of a ceramide library

Jeong-Ju Park [a], Ji Hyung Lee [a], Qian Li [b], Kristine Diaz [b], Young-Tae Chang [b,c,*], Sung-Kee Chung [a,*]

[a] Department of Chemistry, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea
[b] Department of Chemistry, New York University, New York, NY 10003, USA
[c] National University of Singapore, Singapore 117543, Singapore

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Dedicated to the memory of the late Professor A. Ian Scott, a mentor, colleague, and friend.

Abstract

Sphingolipids such as ceramide and sphingosine-1-phosphate have recently attracted intense research interests because of their functional roles as signaling molecules in many important physiological processes, such as growth arrest, apoptosis, and inflammatory responses, and cell proliferation, vascular maturation and trafficking of lymphocytes. The well-defined modular structures of ceramides and related glycosylceramides are ideally amenable to library formation for medicinal chemistry investigation. We have developed divergent synthetic routes to all eight phytosphingosine stereoisomers and then proceeded to prepare phytosphingosine-based ceramide library composed of more than 500 compounds.

Keywords: Phytosphingosine; Ceramide; Library; Solid phase acylating reagent; Apoptosis; Inflammatory response

1. Introduction

Sphingolipids have long been recognized as essential structural components of all eukaryotic cell membranes together with glycerolipids and sterols. More recently, however, their functional roles in cellular signaling and physiology, particularly those of ceramides and sphingosine-1-phosphates (S1Ps) as signaling molecules have been appreciated [1]. Ceramides have for many years been known to be involved in the inflammatory responses. Now it is clear that they act as second messengers in the signal transduction pathway triggered by several agents of stress, including oxidative stress, ionizing radiation, and extracellular stimuli such as proinflammatory cytokines and lipopolysaccharide. There are three metabolic pathways available for the production of ceramides: (1) de novo synthesis primarily in the endoplasmic reticulum and mitochondria, (2) hydrolysis of sphingomyelin through the action of sphingomyelinase, which is secreted by cells such as endothelial cells and alveolar macrophages in response to inflammatory stimuli, and (3) acylation of sphingosine [2]. In general, the increase of cellular ceramide level activates NF-κB and activator protein-1, leading to the expression of multiple inflammatory proteins that amplify the inflammatory responses. An increased ceramide level also results in the stimulation of apoptotic signaling pathways, apparently activating caspases and inducing clustering of death receptors in the cell membranes. It is interesting to note that ceramides are pro-apoptotic, whereas the other major sphingolipid, S1P is suppressing apoptosis. The relative level of ceramide and S1P has been proposed to function as an evolutionary conserved rheostat that determines cell fate [1d]. Thus, ceramides play important functional roles in the regulation of

* Corresponding authors. Fax: +82 54 279 3399.
E-mail addresses: chmcyt@nus.edu.sg (Y.-T. Chang), skchung@postech.ac.kr (S.-K. Chung).

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many stress responses, cell cycle arrest, cell differentiation, and apoptosis among others.

Structurally, a ceramide consists of a long-chain sphingoid base, such as sphingosine, sphinganine and phytosphingosine, with an amide-linked fatty acid. Due to the biological relevance, many sphingosine (sphinganine) based ceramide analogues have been synthesized and evaluated [3]. We previously developed highly practical diastereoselective synthetic methods for sphingosine and related compounds [4]. By utilizing these methods we also generated a combinatorial ceramide library of more than 500 ceramide compounds by starting with the four sphingosine stereoisomers and 12 sphingosine analogues, and 33 acyl chain donors [5]. Some biological activities of this library were tested in several cell-based assays for NF-κB activation and the induction of apoptosis [5], and for the inhibitory activity of the production of interleukin-4 in T cells [6]. Phytosphingosine (4-hydroxysphinganine) is one of the major sphingolipid metabolites in human together with sphingosine (sphing-4-enine), and 6-hydroxy-sphingosine [7], and is ubiquitously distributed in many mammalian tissues, fungi, yeast, plants, as well as marine organism [8]. It is a bioactive lipid that has a potential heat stress signaling role in yeast cell [9]. Synthetic and physiological studies of phytosphingosine-based ceramides have not yet been thoroughly investigated, probably because the stereoisomers of phytosphingosine are not readily available [10] (Fig. 1). Although phytosphingosine stereoisomers have been targets of much synthetic efforts [11], the complete set of all stereoisomers of phytosphingosine is not available. Thus, it would be highly desirable to carry out a divergent synthesis of all diastereomers of phytosphingosine, and utilize them in the construction of a phytosphingosine-based ceramide library.

The literature [11] shows three general synthetic approaches to the phytosphingosine stereoisomers. The first two routes rely upon the chiral pool of amino acids and carbohydrates as the source of the required chiral centers, and the third route is based on the chiral amino acid and further creation of the chiral centers by using Sharpless epoxidation and dihydroxylation. For the purpose of the present study, a rapid generation of structural diversity and practical synthesis should be important considerations, such as divergent preparation of all phytosphingosine stereoisomers in good yields and acceptable isomeric ratios and ready structural variations such as incorporating different hydrophobic chain structures and lengths. Nakanishi and his coworkers reported a divergent synthesis of four stereoisomers of D-phytosphingosine from L-serine via Garner's aldehyde. They carried out separate chain elongation on the Garner's aldehyde by the Wittig and Julia olefination methods to obtain the *cis*- and *trans*-products, respectively, and the olefins were dihydroxylated by Sharpless protocol. Our synthetic scheme is based on the divergent and stereoselective synthesis of sphingosines, which were previously developed in our research group [4]. In this method, D-erythro- and L-threo sphingosines are obtained from L-serine and L-erythro- and D-threo-sphingosines from D-serine in excellent yields. We anticipated that epoxidation of the suitably protected sphingosine obtained as above would provide a diastereomeric mixture of the corresponding epoxides, and then each diastereomer would be reduced to yield 'the inside alcohol' on the basis of a literature precedent [12]. Herein, we report the complete synthesis of all phytosphingosine stereoisomers from the sphingosine stereoisomers through epoxidation and reduction. We also report the first phytosphignosine-based ceramide library of more than 500 ceramides by starting out with eight phytosphingosine stereoisomers and 80 acyl chain donors.

2. Experimentals

2.1. General procedure

Analytical TLC was performed on Merck 60 F254 silica gel plate (0.25 mm thickness) and visualization was done with UV light, and/or by spraying with a 5% solution of phosphomolybdic acid followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (70–230 mesh or 230–400 mesh). Melting points were determined on a Thomas–Hoover apparatus and uncorrected. NMR spectra were recorded on a Bruker AM 300, DPX 300 spectrometer. Tetramethylsilane was used as the internal standard for ^{1}H NMR. High resolution mass spectra (FAB) were determined on a JMS-700 at the Korea Basic Science Center, Daegu, Korea. Optical rotations were measured with a JASCO DIP-360 digital polarimeter.

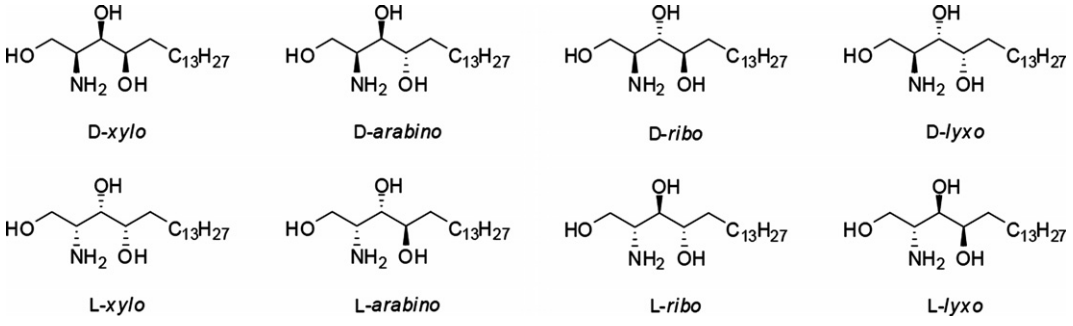


Fig. 1. Structure of eight phytosphingosine stereoisomers.

2.2. (2S,3S,4E)- and (2R,3R,4E)-2-amino-1-(t-butyl-dimethyl-silanyloxy)-octadec-4-en-3-ol [5a and 5b]

To a solution of **3a** [4b] (1.6 g, 2.44 mmol) and $Me_3N{\cdot}BH_3$ (231 mg, 3.17 mmol) in dry CH_2Cl_2 (50 ml) at 0 °C under N_2, TFA (0.75 ml, 9.76 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C and then quenched with saturated aq $NaHCO_3$. It was poured into water and extracted with CH_2Cl_2, and the extract was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **5a** (913 mg, 91%). Similarly, **5b** was prepared from **3b** [4b].

Compound **5a**: $[\alpha]_D^{25}$ +2.6° (*c* 0.99, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.88 (12H, m), 1.25–1.38 (22H, m), 2.11 (2H, m), 2.73 (1H, m), 3.59 (1H, dd, $J = 9.8$, 4.6 Hz), 3.68 (1H, dd, $J = 9.9$, 3.9 Hz), 3.97 (1H, t, $J = 6.2$ Hz), 5.41 (1H, dd, $J = 15.4$, 6.7 Hz), 5.72 (1H, m); ^{13}C NMR ($CDCl_3$) δ −5.3, −5.2, 14.3, 18.4, 22.9, 26.1, 29.4, 29.6, 29.7, 29.8, 29.9, 32.1, 32.6, 56.9, 65.4, 73.1, 130.0, 134.2; HRMS (FAB) *m*/*z* calcd for $C_{24}H_{51}NO_2Si$: $(M+H)^+$ 414.3767; found 414.3767.

Compound **5b**: $[\alpha]_D^{25}$ −2.9° (*c* 1.03, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **3a**.

2.3. (2S,3R,4E)- and (2R,3S,4E)-(2-hydroxy-1-hydroxymethyl-heptadec-3-enyl)-carbamic acid benzyl ester [6a and 6b]

To an ice-cooled solution of **4a** [4b] (700 mg, 2.34 mmol) in THF (15 ml) and H_2O (15 ml), were added $NaHCO_3$ (197 mg, 2.34 mmol) and CbzCl (0.37 ml, 2.57 mmol). After stirring for 1.5 h at rt, the reaction mixture was poured into water and extracted with CH_2Cl_2. The extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **6a** (927 mg, 91.5%). Similarly, **6b** was prepared from **4b** [4b].

Compound **6a**: $[\alpha]_D^{25}$ +8.3° (*c* 1.20, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, $J = 6.3$ Hz), 1.26 (22H, m), 2.03 (2H, m), 3.70 (2H, m), 3.93 (1H, dd, $J = 11.2$, 3.3 Hz), 4.31 (1H, m), 5.10 (2H, s), 5.51 (1H, dd, $J = 14.9$, 6.7 Hz), 5.64 (1H, d, $J = 8.0$ Hz), 5.76 (1H, m), 7.35 (5H, m); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.9, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 32.1, 32.5, 56.0, 62.5, 67.1, 74.7, 128.3, 128.4, 128.8, 128.9, 134.5, 136.5, 156.8; HRMS (FAB) *m*/*z* calcd for $C_{26}H_{43}NO_4$: $(M+H)^+$ 434.3270; found 434.3270.

Compound **6b**: $[\alpha]_D^{25}$ −9.1° (*c* 1.08, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **6a**.

2.4. (2S,3S,4E)- and (2R,3R,4E)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-heptadec-3-enyl]-carbamic acid benzyl ester [7a and 7b]

Compound **5a**: (740 mg, 1.79 mmol) was treated by the same procedure as described for compound **6a** to give compound **7a** (879 mg, 89.6%). Similarly, **7b** was prepared from **5b**.

Compound **7a**: $[\alpha]_D^{25}$ +11.0° (*c* 1.15, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.07 (6H, s), 0.89 (12H, m), 1.26 (22H, m), 2.02 (2H, m), 3.67 (1H, m), 3.83 (2H, s), 4.41 (1H, m), 5.12 (2H, s), 5.47 (1H, dd, $J = 15.4$, 6.3 Hz), 5.75 (1H, m), 7.35 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.3, 22.9, 26.0, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 32.1, 32.5, 55.5, 65.0, 67.0, 73.4, 128.2, 128.3, 128.7, 128.9, 133.9, 136.7, 156.8; HRMS (FAB) *m*/*z* calcd for $C_{32}H_{57}NO_4Si$: $(M+H)^+$ 548.4135; found 548.4135.

Compound **7b**: $[\alpha]_D^{25}$ −11.6° (*c* 0.81, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **7a**.

2.5. (2S,3R,4E)- and (2R,3S,4E)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-heptadec-3-enyl]-carbamic acid benzyl ester [8a and 8b]

To a solution of **6a** (900 mg, 2.08 mmol) in dry CH_2Cl_2 (40 ml) at 0 °C, was added TBSCl (627 mg, 4.16 mmol) and imidazole (566 mg, 8.32 mmol). After stirring for 12 h at rt, the reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel to give compound **8a** (994 mg, 87.2%). Similarly, **8b** was prepared from **6b**.

Compound **8a**: $[\alpha]_D^{25}$ +11.2° (*c* 1.06, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.06 (6H, s), 0.88 (12H, m), 1.26 (22H, m), 2.14 (2H, m), 3.66 (1H, m), 3.76 (1H, m), 3.98 (1H, dd, $J = 10.3$, 2.5 Hz), 4.23 (1H, m), 5.12 (2H, s), 5.49 (1H, d, $J = 6.8$ Hz), 5.54 (1H, d, $J = 5.7$ Hz), 5.77 (1H, m), 7.36 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, −5.5, 14.3, 18.3, 22.9, 26.0, 29.3, 29.4, 29.6, 29.7, 29.8, 29.9, 32.1, 32.5, 55.1, 63.6, 67.0, 74.6, 128.2, 128.3, 128.7, 129.4, 133.6, 136.7, 156.4; HRMS (FAB) *m*/*z* calcd for $C_{32}H_{57}NO_4Si$: $(M+H)^+$ 548.4135; found 548.4128.

Compound **8b**: $[\alpha]_D^{25}$ −11.9° (*c* 0.81, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **8a**.

2.6. Epoxidation of Compound 7a

To a solution of **7a** (850 mg, 1.55 mmol) in dry CH_2Cl_2 (30 ml) at 0 °C, were added $NaHCO_3$ (326 mg, 3.88 mmol) and then *m*-CPBA (670 mg, 3.88 mmol). After stirring for 20 h at rt, the reaction mixture was quenched with Na_2SO_3 and the resulting mixture was stirred for 30 min. The reaction mixture was extracted with EtOAc and the extract was washed with satd aq $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), concentrated, and chromatographed on silica gel to give compound **9a** (480 mg, 54.9%) and **10a** (324 mg, 37.1%). Similarly, **9b** and **10b** were prepared from **7b**.

2.7. (2S,3R,4S,5S)- and (2R,3S,4R,5R)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-2-(3-tridecyl-oxiranyl)- ethyl]-carbamic acid benzyl ester [9a and 9b]

Compound **9a**: $[\alpha]_D^{25}$ +12.3° (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.06 (6H, m), 0.88 (12H, m), 1.26 (20H, m), 1.40 (2H, m), 1.52 (2H, m), 2.86 (2H, m), 3.79 (4H, m),

5.12 (2H, s), 5.38 (1H, d, $J = 6.7$ Hz), 7.34 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.4, 22.9, 26.0, 26.1, 29.5, 29.6, 29.7, 29.8, 29.9, 31.7, 32.1, 54.4, 56.3, 59.6, 63.9, 67.1, 71.3, 128.2, 128.3, 128.7, 136.6, 156.5; HRMS (FAB) *m/z* calcd for $C_{32}H_{57}NO_5Si$: $(M+H)^+$ 564.4084; found 564.4084.

Compound **9b**: $[\alpha]_D^{25}$ −13.6° (*c* 0.94, $CHCl_3$); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **9a**.

2.8. (2S,3R,4R,5R)- and (2R,3S,4S,5S)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-2-(3-tridecyl-oxiranyl)-ethyl]-carbamic acid benzyl ester [10a and 10b]

Compound **10a**: $[\alpha]_D^{25}$ +11.7° (*c* 1.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.07 (6H, m), 0.89 (12H, m), 1.26 (22H, m), 1.48 (2H, m), 2.80 (1H, m), 2.99 (1H, m), 3.82 (2H, m), 3.89 (1H, m), 3.98 (1H, m), 5.11 (2H, dd, $J =$ 17.8, 12.1 Hz), 5.32 (1H, d, $J = 8.6$ Hz), 7.36 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.3, 22.9, 26.0, 29.5, 29.7, 29.7, 29.8, 29.9, 31.8, 32.1, 53.1, 56.4, 58.6, 64.6, 67.1, 70.2, 128.3, 128.4, 128.7, 136.5, 156.4; HRMS (FAB) *m/z* calcd for $C_{32}H_{57}NO_5Si$: $(M+H)^+$ 564.4084; found 564.4081.

Compound **10b**: $[\alpha]_D^{25}$ −12.1° (*c* 2.15, $CHCl_3$); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **10a**.

2.9. Epoxidation of Compound 8a

Method A. To a solution of **8a** (300 mg, 0.548 mmol) in dry CH_2Cl_2 (10 ml) at 0 °C, were added $NaHCO_3$ (116 mg, 1.38 mmol) and then *m*-CPBA (238 mg, 1.38 mmol). After stirring for 20 h at rt, the reaction mixture was quenched with Na_2SO_3, and the resulting mixture was stirred for 30 m. The reaction mixture was extracted with EtOAc, and the extract was washed with satd aq $NaHCO_3$ and brine. The organic phase was dried ($MgSO_4$), concentrated, and chromatographed on silica gel to give compound **11a** (58 mg, 18.8%) and **12a** (223 mg, 72.2%).

Method B. To **8a** (640 mg, 1.17 mmol) at 0 °C, a solution of dimethyldioxirane (DMD) in acetone (0.03–0.05 M, 100 ml) was added. After stirring for 28 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **11a** (209 mg, 31.7%) and **12a** (325 mg, 49.2%). Similarly, **11b** and **12b** were prepared from **8b**.

2.10. (2S,3S,4S,5S)- and (2R,3R,4R,5R)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-2-(3-tridecyl-oxiranyl)-ethyl]-carbamic acid benzyl ester [11a and 11b]

Compound **11a**: $[\alpha]_D^{25}$ +8.7° (*c* 0.94, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.07 (6H, m), 0.88 (12H, m), 1.25 (20H, m), 1.43 (2H, m), 1.62 (2H, m), 2.83 (1H, m), 2.98 (1H, m), 3.70 (1H, m), 3.78 (2H, m), 4.02 (1H, m), 5.11 (2H, s), 5.40 (1H, d, $J = 7.8$ Hz), 7.34 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.4, 22.9, 26.0, 26.2, 29.6, 29.7, 29.8, 29.9, 31.8, 32.1, 54.2, 56.5, 58.7, 63.4, 67.1, 71.3, 128.3, 128.4, 128.7, 136.6, 156.4; HRMS (FAB) *m/z* calcd for $C_{32}H_{57}NO_5Si$: $(M+H)^+$ 564.4084; found 564.4088.

Compound **11b**: $[\alpha]_D^{25}$ −8.1° (*c* 0.98, $CHCl_3$); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **11a**.

2.11. (2S,3S,4R,5R)- and (2R,3R,4S,5S)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2-hydroxy-2-(3-tridecyl-oxiranyl)-ethyl]-carbamic acid benzyl ester [12a and 12b]

Compound **12a**: $[\alpha]_D^{25}$ +23.4° (*c* 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.07 (6H, m), 0.88 (12H, m), 1.27 (20H, m), 1.42 (2H, m), 1.51 (2H, m), 2.93 (2H, m), 3.68 (1H, m), 3.77 (2H, m), 4.06 (1H, m), 5.12 (2H, s), 5.43 (1H, d, $J = 8.7$ Hz), 7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.4, 22.9, 26.0, 26.1, 29.5, 29.6, 29.7, 29.9, 31.7, 32.1, 54.5, 56.2, 59.4, 63.0, 67.1, 71.3, 128.2, 128.3, 128.7, 136.6, 156.3; HRMS (FAB) *m/z* calcd for $C_{32}H_{57}NO_5Si$: $(M+H)^+$ 564.4084; found 564.4081.

Compound **12b**: $[\alpha]_D^{25}$ −24.9° (*c* 0.96, $CHCl_3$); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **12a**.

2.12. (2S,3R,4R)- and (2R,3S,4S)-[1-(t-butyl-dimethyl-silanyloxymethyl)-2,3-dihydroxy-heptadecyl]-carbamic acid benzyl ester [13a and 13b]

Method A. To a solution of **9a** (164 mg, 0.291 mmol) in dry toluene (5 ml) at −78 °C under N_2, DIBAL (1.5 M in toluene; 1.4 ml, 2.03 mmol) was added dropwise. The reaction mixture was warmed gradually to −10 °C with stirring over 8 h. To the solution were added satd aq Rochelle's salt and Et_2O, and the resulting slurry was stirred at rt until two clear layers separated. The reaction mixture was extracted with Et_2O and the extract was washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **13a** (64.7 mg, 39.3%).

Method B. To a solution of **9a** (280 mg, 0.497 mmol) in dry toluene (7 ml), was added $Ti(O^iPr)_4$ (0.3 ml, 1.0 mmol). After stirring for 10 min at rt, $LiBH_4$ (87 mg, 4.0 mmol) was added at 0 °C. The reaction mixture was stirred for 14 h at rt. To the mixture were added aq 5% H_2SO_4 and Et_2O, and the resulting solution was stirred at rt until two clear layers separated. The mixture was extracted with EtOAc, and the extract was washed with water and brine, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel to give compound **13a** (144 mg, 51.2%). Similarly, **13b** was prepared from **9b**.

Compound **13a**: $[\alpha]_D^{25}$ +12.7° (*c* 0.77, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.08 (6H, m), 0.89 (12H, m), 1.26 (22H, m), 1.45 (2H, m), 3.59 (1H, m), 3.71 (1H, m), 3.81 (3H, m), 5.12 (2H, s), 5.42 (1H, d, $J = 8.9$ Hz), 7.36 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.4, 14.3, 18.3, 22.9, 25.6, 26.0, 29.6, 29.8, 29.9, 32.1, 33.3, 52.9, 64.8, 67.2, 71.8, 74.7, 128.3, 128.4, 128.8, 136.6, 156.6; HRMS (FAB) *m/z* calcd for $C_{32}H_{59}NO_5Si$: $(M+H)^+$ 566.4241; found 566.4241.

Compound **13b**: $[\alpha]_D^{25}$ −12.5° (*c* 0.49, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **13a**.

2.13. (2S,3R,4S)- and (2R,3S,4R)-[1-(t-butyl-dimethylsilanyloxymethyl)-2,3-dihydroxy-heptadecyl]-carbamic acid benzyl ester [14a and 14b]

Compound **10a** (400 mg, 0.709 mmol) was treated by the same procedure as described for compound **13a** (Method A) to give compound **14a** (285 mg, 71%). Similarly, **14b** was prepared from **10b**.

Compound **14a**: $[\alpha]_D^{25}$ −8.8° (*c* 0.86, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.09 (6H, m), 0.89 (12H, m), 1.26 (22H, m), 1.40 (1H, m), 1.74 (1H, m), 3.32 (1H, t, $J = 7.5$ Hz), 3.66 (1H, d, $J = 8.3$ Hz), 3.95 (2H, d, $J = 2.5$ Hz), 4.07 (1H, d, $J = 8.9$ Hz), 5.14 (2H, dd, $J = 14.3$, 12.1 Hz), 5.45 (1H, d, $J = 9.1$ Hz), 7.35 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.5, 14.3, 18.3, 22.9, 26.0, 29.6, 29.8, 29.9, 30.0, 32.1, 33.0, 50.7, 65.7, 67.3, 67.5, 67.6, 71.3, 128.4, 128.5, 128.8, 136.4, 157.7; HRMS (FAB) *m/z* calcd for $C_{32}H_{59}NO_5Si$: $(M+H)^+$ 566.4241; found 566.4239.

Compound **14b**: $[\alpha]_D^{25}$ +7.8° (*c* 0.81, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **14a**.

2.14. (2S,3S,4R)- and (2R,3R,4S)-[1-(t-butyl-dimethylsilanyloxymethyl)-2,3-dihydroxy-heptadecyl]-carbamic acid benzyl ester [15a and 15b]

Compound **11a** (210 mg, 0.372 mmol) was treated by the same procedure as described for compound **13a** (Method B) to give compound **15a** (8 mg, 40.4%). Similarly, **15b** was prepared from **11b**.

Compound **15a**: $[\alpha]_D^{25}$ +18.7° (*c* 0.66, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.09 (6H, m), 0.89 (12H, m), 1.26 (22H, m), 1.57 (1H, m), 1.73 (1H, m), 3.59–3.63 (2H, m), 3.77 (1H, m), 3.91–3.95 (2H, m), 5.10 (2H, dd, $J = 14.4$, 12.1 Hz), 5.45 (1H, d, $J = 8.3$ Hz), 7.36 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, 14.4, 18.3, 22.9, 26.0, 29.6, 29.8, 29.9, 30.0, 32.1, 33.6, 52.2, 62.8, 67.1, 73.4, 76.1, 128.3, 128.4, 128.8, 136.6, 156.2; HRMS (FAB) *m/z* calcd for $C_{32}H_{59}NO_5Si$: $(M+H)^+$ 566.4241; found 566.4238.

Compound **15b**: $[\alpha]_D^{25}$ −18.2° (*c* 0.52, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **15a**.

2.15. (2S,3S,4S)- and (2R,3R,4R)-[1-(t-butyl-dimethylsilanyloxymethyl)-2,3-dihydroxy-heptadecyl]-carbamic acid benzyl ester [16a and 16b]

Compound **12a** (470 mg, 0.834 mmol) was treated by the same procedure as described for compound **13a** (Method B) to give compound **16a** (315 mg, 66.7%). Similarly, **16b** was prepared from **12b**.

Compound **16a**: $[\alpha]_D^{25}$ +12.6° (*c* 0.69, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.09 (6H, m), 0.89 (12H, m), 1.27 (22H, m), 1.44 (1H, m), 1.61 (1H, m), 3.39 (1H, m), 3.52 (1H, m), 3.59 (1H, m), 3.73 (1H, dd, $J = 10.0$, 3.9 Hz), 4.06 (1H, dd, $J = 10.0$, 1.8 Hz), 5.14 (2H, dd, $J = 21.6$, 12.1 Hz), 5.43 (1H, d, $J = 8.4$ Hz), 7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ −5.3, 14.3, 18.5, 22.9, 26.0, 26.4, 29.6, 29.8, 29.9, 32.1, 33.1, 54.4, 62.0, 67.5, 70.0, 72.4, 128.4, 128.6, 128.8, 136.4, 157.7; HRMS (FAB) *m/z* calcd for $C_{32}H_{59}NO_5Si$: $(M+H)^+$ 566.4241; found 566.4247.

Compound **16b**. $[\alpha]_D^{25}$ −13.7° (*c* 1.20, $CHCl_3$); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **16a**.

2.16. (2S,3R,4R)-D- and (2R,3S,4S)-L-xylo-phytosphingosine [17a and 17b]

To a solution of **13a** (124 mg, 0.219 mmol) in dry THF (5 ml), was added Pd/C (10% on carbon, 30 mg). The resulting mixture was stirred under H_2 (1 atm) for 12 h. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure. The residue was dissolved in dry THF (3 ml), and TBAF (1.0 M in THF, 0.44 ml, 0.44 mmol) was added dropwise at rt. After stirring for 2 h, the reaction mixture was evaporated under reduced pressure. The residue was chromatographed on silica gel (eluent: $CHCl_3$–MeOH–NH_4OH, 40:10:1) to give compound **17a** (51 mg, 73.4%). Similarly, **17b** was prepared from **13b**.

Compound **17a**: mp 99 °C (lit. [11i] mp 98–99 °C); $[\alpha]_D^{25}$ +3.9° (*c* 0.81, pyridine) (lit. [11i] $[\alpha]_D^{25}$ +11.8° (*c* 0.45, pyridine)); ^1H NMR (pyridine-d_5) δ 0.88 (3H, t, $J = 6.7$ Hz), 1.17–1.41 (22H, m), 1.53 (1H, m), 1.78 (1H, m), 1.91 (1H, m), 1.99 (1H, m), 3.46 (1H, dt, $J = 6.2$, 2.7 Hz), 4.03–4.09 (2H, m), 4.13–4.20 (2H, m); ^{13}C NMR (pyridine-d_5) δ 14.7, 23.4, 27.0, 30.1, 30.3, 30.4, 30.5, 30.7, 32.6, 35.2, 57.7, 66.4, 73.3, 75.2; HRMS (FAB) *m/z* calcd for $C_{18}H_{39}NO_3$: $(M+H)^+$ 318.3008; found 318.3008.

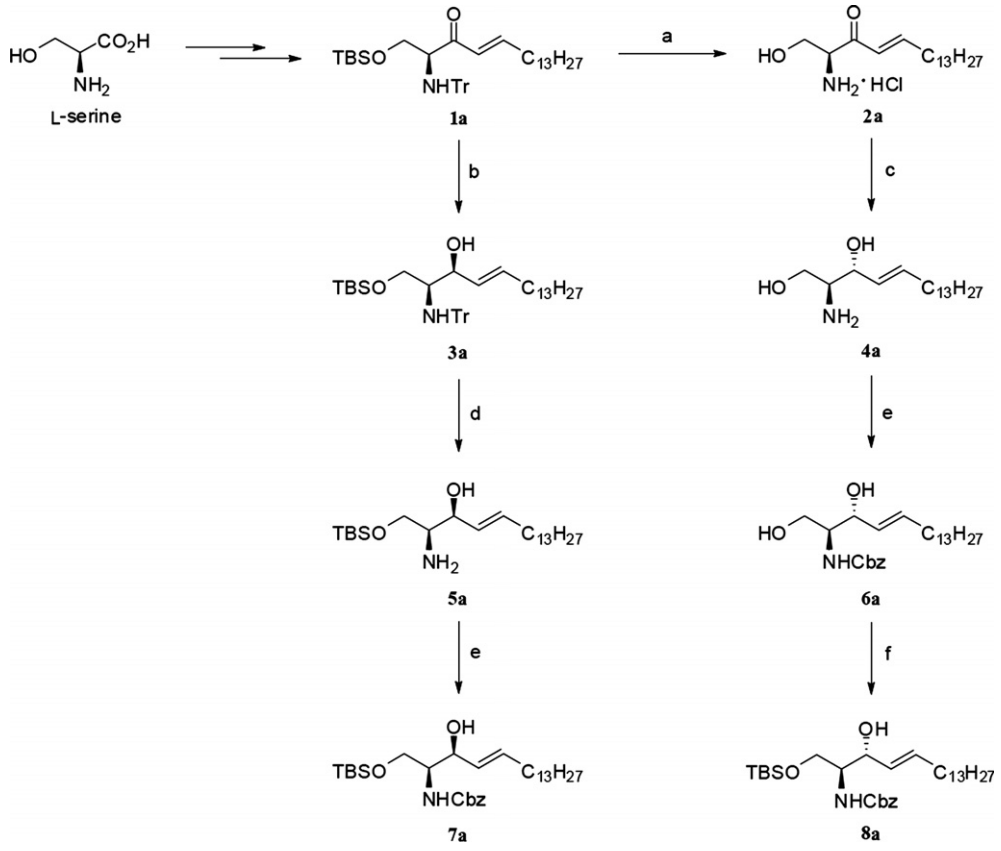
Compound **17b**: mp 101 °C; $[\alpha]_D^{25}$ −2.6° (*c* 0.44, pyridine); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **17a**.

2.17. (2S,3R,4S)-D- and (2R,3S,4R)-L- arabino-phytosphingosine [18a and 18b]

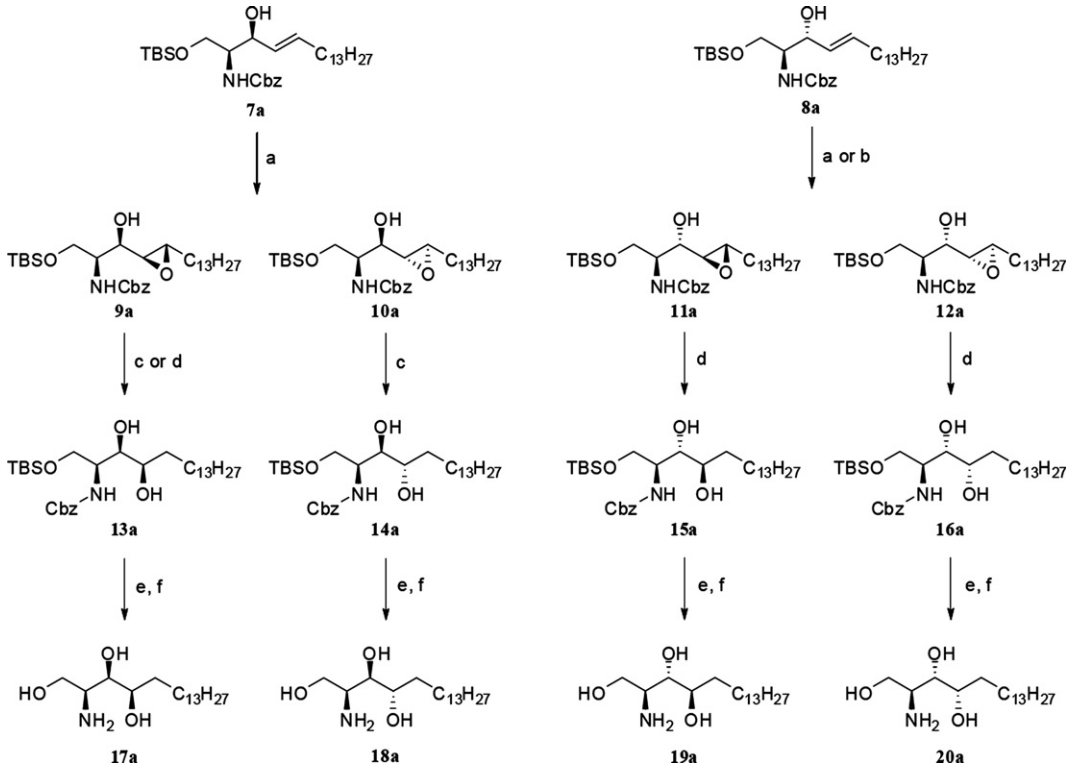
Compound **14a** (210 mg, 0.371 mmol) was treated by the same procedure as described for compound **17a** to give compound **18a** (82 mg, 69.6%). Similarly, **18b** was prepared from **14b**.

Compound **18a**: mp 96 °C (lit. [11a] mp 86 °C); $[\alpha]_D^{25}$ −4.3° (*c* 0.50, pyridine) (lit. [11a] $[\alpha]_D^{25}$ −3.7° (*c* 1.0, pyridine), lit. [11b] $[\alpha]_D^{25}$ −4.5° (*c* 0.58, pyridine)); ^1H NMR (pyridine-d_5) δ 0.88 (3H, t, $J = 6.7$ Hz), 1.20–1.45 (22H, m), 1.65 (1H, m), 1.88 (2H, m), 2.10 (1H, m), 3.85 (1H, t-like, $J = 5.6$ Hz), 4.08–4.15 (2H, m), 4.21–4.29 (2H, m); ^{13}C NMR (pyridine-d_5) δ 14.7, 23.3, 27.0, 30.0, 30.3, 30.4, 30.5, 30.7, 32.5, 35.7, 54.9, 66.4, 74.3, 74.5; HRMS (FAB) *m/z* calcd for $C_{18}H_{39}NO_3$: $(M+H)^+$ 318.3008; found 318.3012.

Compound **18b**: mp 93 °C; $[\alpha]_D^{25}$ +5.1° (*c* 0.51, pyridine); identical ^1H NMR, ^{13}C NMR and FABMS data to those of **18a**.



Scheme 1. Reagents and conditions: (a) 2 N HCl, THF–MeOH, reflux, 78%; (b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 92%; (c) $Zn(BH_4)_2$, THF, −78 °C, 80%; (d) $Me_3N{\cdot}BH_3$, TFA, CH_2Cl_2, 0 °C, 91%; (e) CbzCl, $NaHCO_3$, THF–H_2O, rt, 90–92%; (f) TBSCl, imidazole, CH_2Cl_2, rt, 87%.



Scheme 2. Reagents and conditions: (a) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 91–92%; (b) DMD, acetone, rt, 81%; (c) DIBAL, toluene, −78 °C, 39–71%; (d) $LiBH_4$, $Ti(O^iPr)_4$, toluene, rt, 40–67%; (e) Pd/C, H_2, THF, rt; (f) TBAF, THF, rt, 70 ~ 73% over for two steps.

2.18. (2S,3S,4R)-D- and (2R,3R,4S)-L-ribo-phytosphingosine [**19a** and **19b**]

Compound **15a** (70 mg, 0.124 mmol) was treated by the same procedure as described for compound **17a** to give compound **19a** (28 mg, 71.1%). Similarly, **19b** was prepared from **15b**.

Compound **19a**: mp 102 °C (lit. [11a] mp 103 °C, lit. [11c] mp 103 °C); $[\alpha]_D^{25}$ +7.4° (*c* 0.50, pyridine) (lit. [11a] $[\alpha]_D^{25}$ +9.5° (*c* 1.0, pyridine), lit. [11c] $[\alpha]_D^{25}$ +7.9° (*c* 1.0, pyridine)); ^{1}H NMR (pyridine-d_5) δ 0.88 (3H, t, $J = 6.7$ Hz), 1.20–1.46 (22H, m), 1.73 (1H, m), 1.92 (2H, m), 2.29 (1H, m), 3.54 (1H, br s), 4.00 (1H, t, $J = 7.2$ Hz), 4.22–4.38 (3H, m); ^{13}C NMR (pyridine-d_5) δ 14.7, 23.4, 26.6, 30.1, 30.3, 30.4, 30.6, 30.9, 32.6, 35.2, 58.1, 65.6, 75.6, 76.3; HRMS (FAB) *m*/*z* calcd for $C_{18}H_{39}NO_3$: $(M+H)^+$ 318.3008; found 318.3008.

Compound **19b**: mp 94 °C; $[\alpha]_D^{25}$ −7.9° (*c* 0.64, pyridine); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **19a**.

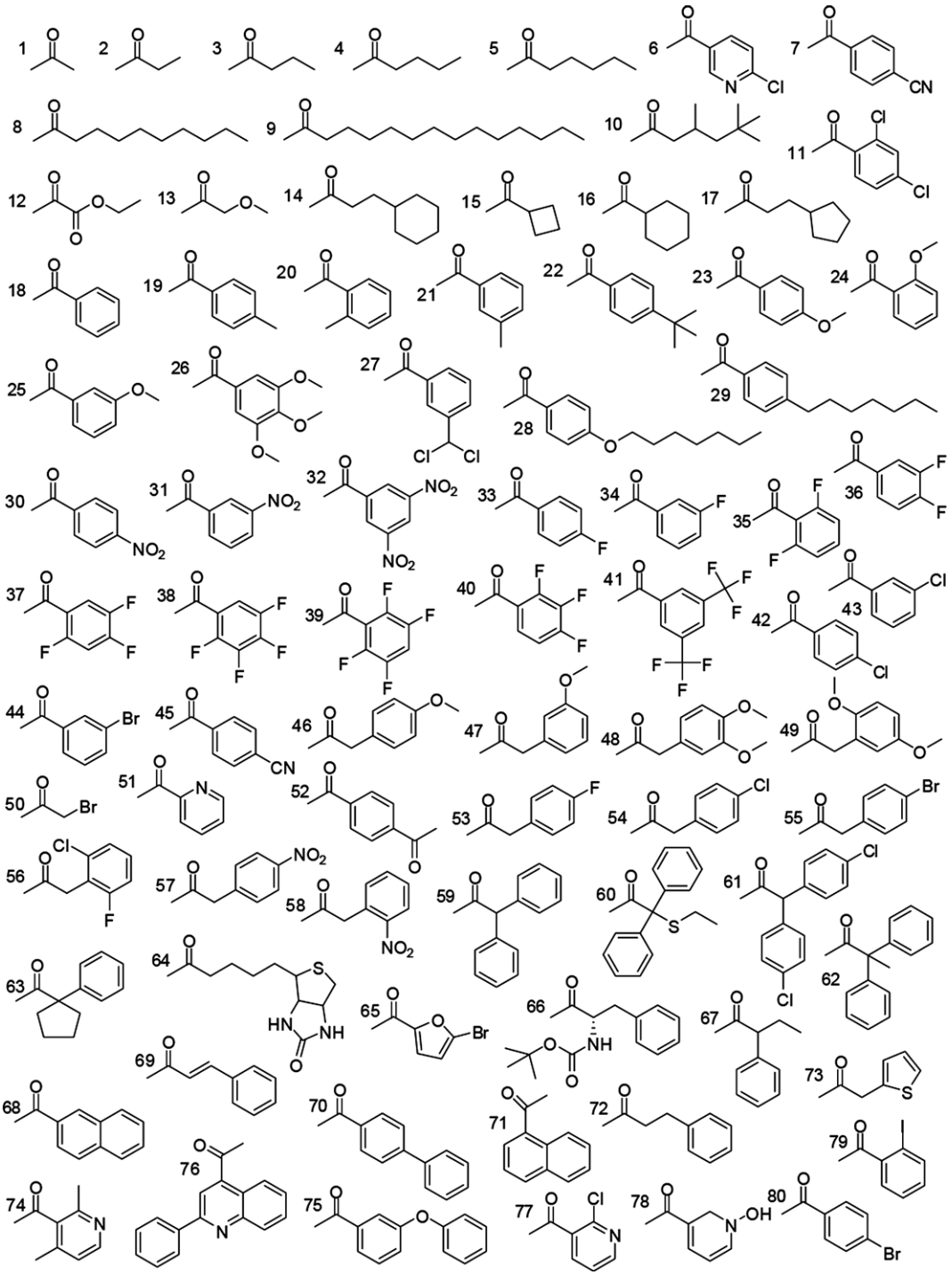


Fig. 2. The acyl chain structures used in the phytosphingosine-based ceramide library.

2.19. (2S,3S,4S)-D- and (2R,3R,4R)-L-lyxo-phytosphingosine [20a and 20b]

Compound **16a** (270 mg, 0.477 mmol) was treated by the same procedure as described for compound **17a** to give compound **20a** (109 mg, 71.9%). Similarly, **20b** was prepared from **16b**.

Compound **20a**: mp 92 °C (lit. [11f] mp 104.8–106.0 °C, lit. [11g] mp 92–95 °C); $[\alpha]_D^{25}$ −9.5° (*c* 0.48, pyridine) (lit. [11f] $[\alpha]_D^{25}$ −7.4° (*c* 0.9, pyridine), lit. [11g] $[\alpha]_D^{25}$ −2.6° (*c* 0.21, pyridine)); ^{1}H NMR (pyridine-d_5) δ 0.88 (3H, t, $J = 6.7$ Hz), 1.22–1.46 (22H, m), 1.59 (1H, m), 1.78 (1H, m), 1.87–2.11 (2H, m), 3.6 (1H, m), 4.00 (1H, dd, $J = 6.8$, 2.2 Hz), 4.20 (1H, dd, $J = 10.4$, 6.6 Hz), 4.30–4.37 (2H, m); ^{13}C NMR (pyridine-d_5) δ 14.7, 23.3, 27.2, 30.0, 30.3, 30.4, 30.5, 30.7, 32.5, 35.0, 57.0, 65.7, 72.8, 75.6; HRMS (FAB) *m*/*z* calcd for $C_{18}H_{39}NO_3$: $(M+H)^+$ 318.3008; found 318.3008.

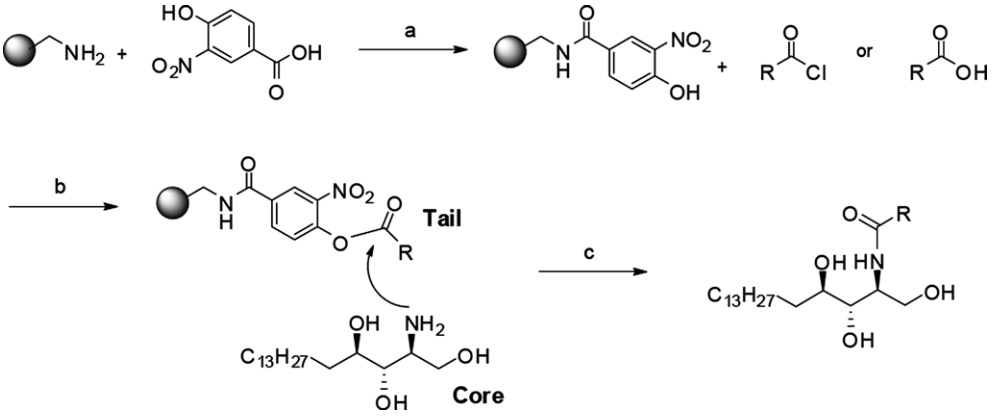
Compound **20b**: mp 96 °C (lit. [11g] mp 95–96 °C); $[\alpha]_D^{25}$ +8.1° (*c* 0.54, pyridine) (lit. [11h] $[\alpha]_D^{25}$ +7.7° (*c* 1.4, pyridine)); identical ^{1}H NMR, ^{13}C NMR and FABMS data to those of **20a**.

3. Results and discussion

We first prepared all eight phytosphingosine stereoisomers in a divergent fashion, and then proceeded to construct the ceramide library. Our synthesis began with four stereoisomers of sphingosine (*N*,*O*-diprotected L-*threo*-sphingosine **3a**, D-*erythro*-sphingosine **4a** from L-serine, and *N*,*O*-diprotected D-*threo*-sphingosine **3b**, L-*erythro*-sphingosine **4b** from D-serine), that were prepared by the practical method previously developed in our laboratory [4]. Removal of the *N*-trityl group of compound **3a** with $Me_3N{\cdot}BH_3$ and TFA in CH_2Cl_2 at 0 °C produced free amine **5a** in 91% yield. *N*-Cbz protection of **5a** with CbzCl and sodium bicarbonate in aqueous THF gave the *N*-Cbz and *O*-TBS protected L-*threo*-sphingosine **7a**. In the same manner as described for **5a**, *N*-Cbz protected **6a** was synthesized from D-*erythro*-sphingosine **4a**. The *O*-TBS protection of **6a** with TBSCl and imidazole in CH_2Cl_2 yielded *N*-Cbz and *O*-TBS protected D-*erythro*-sphingosine **8a** in 87% yield (Scheme 1).

Epoxidation of the *syn*-sphingosine derivative **7a** and *anti*-sphingosine derivative **8a** with *m*-CPBA or dimethyldioxirane (DMD) provided in variable yields the diastereomeric mixture of the corresponding epoxides (**9a**, **10a** and **11a**, **12a**), respectively. After separation, each diastereomer was reduced with DIBAL or $LiBH_4$ to provide the 'inside alcohols' (**13a**, **14a**, and **15a**, **16a**), which were deprotected to the corresponding phytosphingosines (**17a**, **18a**, and **19a**, **20a**, in Scheme 2). In these epoxidation followed by regioselective reduction sequence, the pre-existing stereocenters were found to exert substantial influences in their interaction with the reagents, and these observations leaves some room for further improvement on the selectivity control. By employing the identical procedures on the D-serine derived D-*threo*-sphingosine **3b**, L-*erythro*-sphingosine **4b**, the corresponding phytosphingosine stereoisomers (**17b**, **18b** and **19b**, **20b**) were similarly synthesized.

For the construction of the ceramide library, the "core" structure (phytosphingosine stereoisomer) was derivatized in solution with a series of the solid-phase acylating reagents carrying the "tail" part. Previously we optimized the reaction conditions for preparing the combinatorial ceramide library based on sphingosine backbone [5,13]. The nitrophenol resin, prepared by a literature procedure, was suspended in 1-methyl-2-pyrrolidinone and pyridine, and activated esters were prepared by reacting with either the acyl halide in pyridine or the isopropylcarbodiimide-activated acid. The use of a nitrophenol ester on the solid support in THF generally yielded very pure products after simple filtration, without much of phytosphingosine or by-products containing the *O*-acylated. In this way, we have constructed a library of more than 500 ceramide compounds by combining the eight phytosphingosine stereoisomers and 80 acyl chains (Fig. 2 and Scheme 3). Completion of the reaction was easily confirmed by the negative ninhydrin staining and thin-layer chromatography with phosphomolybdic acid staining. The identity of the products



Scheme 3. Reagents and conditions: (a) DIC/HOBt, DMF, rt, overnight; (b) NMP, pyridine, rt or DIC/HOBt, DMF, rt, overnight; (c) DMSO, rt, overnight.

was confirmed by LC–ESI mass spectrometry. While the great majority of the cases provided a single pure product, a few reaction products were not pure enough, perhaps due to a low reactivity of the tail groups. Details of the characterization of the products are described in supporting information. The constructed ceramide library is currently being tested in a variety of existing (the NF-κB activation and the induction of apoptosis [5], and for the inhibitory activity of the production of interleukin-4 in T cells [6]) and new bioassay systems (inhibition of signal transducers and activators of transcription: STATs, and for the activity of hypoxia-induced VEGF and HRE promoters), and these results will be reported in due courses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2007.12.004.

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