Full Paper

Synthesis and Biological Evaluation of Antifungal Activities of Novel 1,2-*trans* Glycosphingolipids

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The synthesis and *in-vitro* biological evaluation of antifungal activities of a series of 1,2-*trans* glycosphingolipids (GSL) against *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* were described. The preliminary study indicated that the sort of sugar moiety of GSLs affected their antifungal activities and selectivities towards the three *Candida* species, and the permeability might not be the sole parameter affecting their antifungal properties as presumed before, which would bring new clues to understand the antifungal profile for these types of compounds.

Keywords: Antifungal / *Candida* / Glycosylation / Glycosphingolipids

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Introduction

The rising incidence of infections caused by antibiotic-resistant fungi has become a major concern for clinicians and the public health system. *Candida* species are among the most common causes of fungal infections ranging from non-life-threatening mucocutaneous illnesses to disseminated mycoses that affect blood and organs [1]. Studies have shown that *Candida albicans* can become transiently resistant to azole drugs rapidly after exposure to the antifungal drug fluconazole [2]. These medical azoles are first-line antifungals for the treatment of human submucosal and invasive mycoses. Therefore, the availability of new classes of antifungal agents enhances opportunities to effectively combat infections that are notoriously difficult to treat.

Glycosphingolipids (GSLs) are ubiquitous components of the cellular membranes of all eukaryotic cells. They are glycolipids composed of a long-chain amino alcohol, known as a sphingoid base, with a fatty acid residue linked to its amino group (the resulting amide is called ceramide), and a carbohydrate chain attached to the primary hydroxyl group of the ceramide. The most frequently occurring sphingoid bases in GSLs that contain a C4–C5 double bond in the *trans*-D-erythro configuration are called sphingosines (see **T2**, Fig. 1). Less frequent are sphinganines (dihydrosphingosine, DHS, Fig. 1), which lack the double bond, and phytosphingosine (PHS), which carries an additional hydroxyl group on C4 (see **T4**, Fig. 1).

The scientific interest in GSLs has increased on account of their broad biological activities [3]. However, only scattered data on the antifungal activities of GSLs has been reported in the literature. To the best of our knowledge, all of these studies were focused on one specific type of GSL called ''two-headed glycosphingolipids'' [4]. Among them a few compounds were discovered to possess moderate antifungal activities, whereas removal of the *O*-glycosyl group from the above compounds enhanced the activities [4]. The presence of sugar moiety was presumed to reduce the membrane permeability and thus depressed the activities. Other studies also demonstrated that the long-chain sphingoid bases themselves (sphingosine, PHS, and DHS) or their ceramide form, rather than the glycosylated ones, can inhibit the growth of several fungal species efficiently [5].

It has been widely known that the sugar is often the critical determination of the key biological activity in many compounds. With our continuous interest in the effect of sugar attachments to various types of aglycones on the biological activities [6] and in order to search for potential new antimicroorganism agents [6], we decided to investigate the effect of the sort of sugar moiety of GSL on their fungal growth

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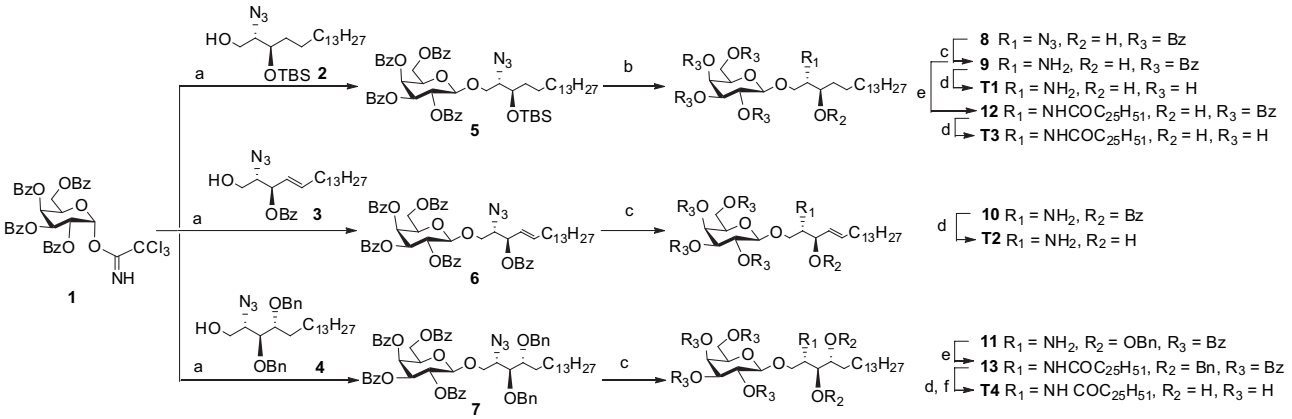


Conditions: a. donor **1**, TMSOTf, CH_2Cl_2, 92% for **5**, 92% for **6**, 87% for **7**; b. TBAF, THF, 91%; c. PPh_3, THF/H_2O; d. $NaOCH_3$, CH_3OH/CH_2Cl_2, 87% for **T1** over 2 steps, 73% for **T2** over 2 steps, 92% for **T3**; e. hexacosanoic acid, HOBt, EDC/HCl, DIPEA, 88% for **12** over 2 steps, 73% for **13** over 2 steps; f. Pd/C-H_2, MeOH/EtOAc, 76% over 2 steps.

Scheme 1. Synthesis of **T1–T4**.

inhibitory activities. Here, we would like to report the synthesis and *in-vitro* biological evaluation of antifungal activities of a series of glycosylated sphingolipids against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. The results obtained here will render new clues to the understanding of the antimicroorganism profile for these types of compounds.

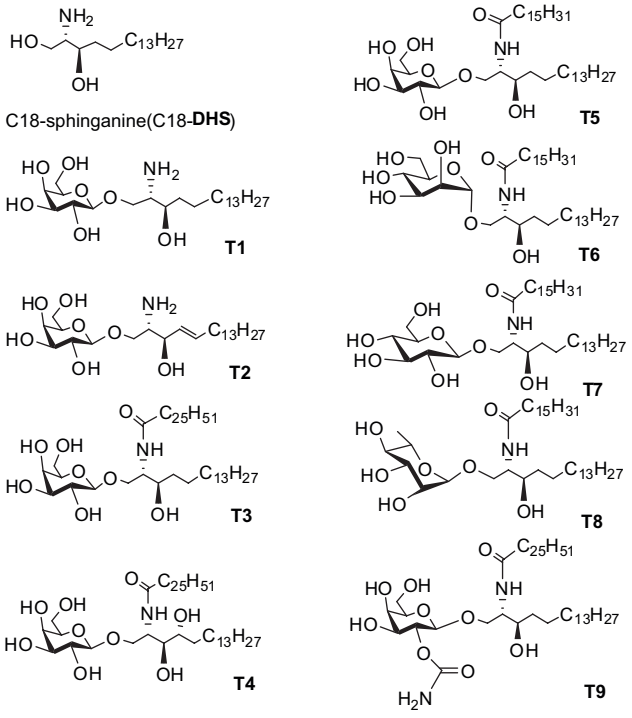


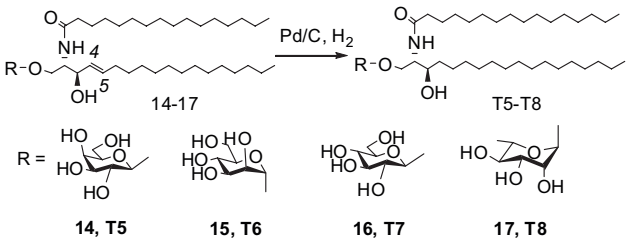
Figure 1. Structures of glycosphingolipids and sphinganine.

Results and discussion

Several typical sugar heads, such as **D**-galactopyranosyl, D-mannopyranosyl, D-glucopyranosyl, L-rhamnopyranosyl as well as a 2-*O*-carbamoyl modified D-galactopyranosyl were introduced to the primary hydroxyl group of sphingoid bases or ceramides (**T1–T9**, Fig. 1). It has been previously demonstrated that the optimal chain length required for antifungal activities of sphingoid bases against *C. glabrata* lies in the C7–C18 range [4]. In addition, C18-sphinganine (C18-DHS) was reported to have excellent fungicidal effect towards *C. albicans* (MFC 5 μg/mL) [5]. In this respect, C18-DHS was chosen as the positive control for comparison in our testing and in all of the designed GSLs 18 is the chain length of selected sphingoid bases.

Compounds **T1–T4** were synthesized following an optimized modular synthetic approach developed in our previous work (Scheme 1) [7]. With TBS protected azido-sphinganine **2** [8], benzoyl protected azido-sphingosine **3** [9], and benzyl protected azido-phytosphingosine **4** [10] in hand, perbenzoylated galactopyranosyl trichloroacetimidate **1** [7] was employed as glycosyl donor to carry out the glycosylations catalyzed by TMSOTf efficiently and form azido-GSLs **5**, **6** [7] and **7**, respectively. Removal of the protecting TBS group on **5** produced compound **8**. Staudinger reductions of the azido groups in **8**, **6** and **7** led to amino intermediates **9**, **10** and **11**, respectively. **9** and **10** were then de-esterified to furnish desired compounds **T1** and **T2**. In the meantime, intermediates **9** and **11** were coupled with hexacosanoic acid in the presence of HOBt, EDC/HCl to provide **12** and **13**, which were followed by global deprotection to give galactosyl ceramides **T3** and **T4**, respectively.

1,2-*trans* D-Galactopyranosyl, D-mannopyranosyl, D-glucopyranosyl, and L-rhamnopyranosyl ceramides **T5–T8**, which all carry a sphingoid base of DHS, were synthesized from



Scheme 2. Synthesis of **T5–T8**.

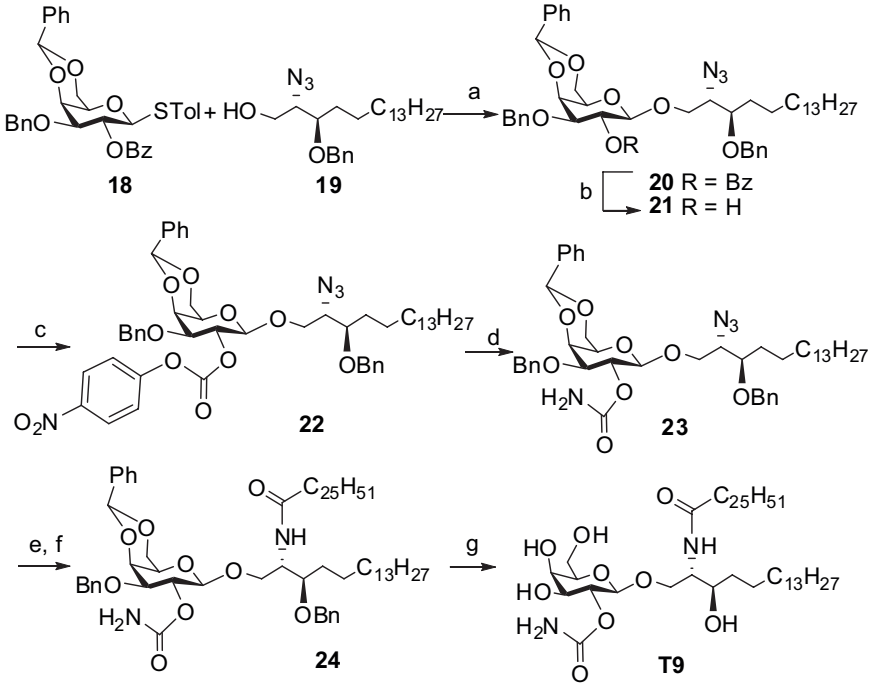
their corresponding sphingosine containing forms **14–17** respectively, by hydrogenation of the C4-C5 double bond (Scheme 2). The synthesis of compounds **14–17** has been reported by us earlier [7].

The synthesis of 2′-*O*-carbamoyl galactosyl ceramide **T9** was performed as shown in Scheme 3. With the known thiogalactoside **18** and monobenzyl protected azido-sphinganine **19** [11] in hand, the coupling reaction was performed under the promotion of NIS/AgOTf to provide the desired glycoside **20**. The 2′-benzoate group of **20** was then transformed to a free OH, on which the carbamoyl group was introduced by reaction with $ClCO_2C_6H_4NO_2$ in the presence of DMAP in pyridine, and followed by an ammonolysis reaction. After the deprotection of **24**, target **T9** was provided.

Target compounds **T1–T9** were evaluated for their *in-vitro* antifungal activities against *C. albicans* (EU583481), *C. parapsilosis* (EF198017), and *C. tropicalis* (EU651853) using serial fourfold dilutions technique in triplicate [12]. The minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give 80% inhibition of fungal growth, and MICs of the target compounds along with C18-DHS for comparison are presented in Table 1.

Compared with the aglycone C18-DHS, galactosyl sphinganine **T1** and galactosyl sphingosine **T2** showed slightly less activity against *C. albicans* and *C. tropicalis* (MIC 43.1 and 43.3 μM) and retained the moderate inhibitory activities against *C. parasilosis* (MIC 173 μM). These results were in accordance with those previously reported by Nicholas *et al*. that glycosyl group could reduce the antifungal activities [4].

Interestingly, while **T1** was acylated by a C24 or C16 fatty acid at the sphingoid base (**T3** or **T5**), they were completely inactive as growth inhibitors. However, replacements of the D-galactosyl residue of inactive compound **T5** by several other glycosyl residues, like D-mannosyl (**T6**), D-glucosyl (**T7**) and L-rhamnosyl (**T8**) were all shown to get enhanced inhibitory activities (MIC 5–80 μg/mL). Within them, the best inhibitory activity was displayed by **T7**, which was 2-fold more active



Conditions: a. NIS, AgOTf, CH_2Cl_2, 86%; b. $NaOCH_3$, CH_3OH/CH_2Cl_2, 92%; c. *p*-nitrophenyl chloroformate, DMAP, pyridine; d. NH_3, THF, 82% over 2 steps; e. PPh_3, THF/H_2O; f. hexacosanoyl acid, HOBt, EDC/HCl, DIPEA, 64% over 2 steps; g. Pd/C-H_2, MeOH/EtOAc, 64%.

Scheme 3. Synthesis of **T9**.

Table 1. *In-vitro* antifungal activities (MIC).

	C. albicans		*C. parasilosis*		*C. tropicalis*	
	μg/mL	μM	μg/mL	μM	μg/mL	μM
C18-DHS	5	16.5	80	265	5	16.5
T1	20	43.1	80	173	20	43.1
T2	20	43.3	80	173	20	43.3
T3	>320	>393	>320	>393	>320	>393
T4	20	24.1	>320	>385	>320	>385
T5	>320	>456	>320	>456	>320	>456
T6	80	114	80	114	80	114
T7	80	114	20	28.5	5	7.1
T8	20	29.1	80	117	80	117
T9	5	5.8	20	23.3	5	5.8

than native C18-DHS against *C. tropicalis* (MIC 7.1 vs. 16.5 μM). The different fungistatic activities between **T5** and **T6–T8** implied that the permeability might not be the sole parameter affecting their antifungal properties as presumed before [4a]. Additionally, some of the GSLs also showed good selectivities against the selected microbial panel. For example, glucosyl ceramide **T7** was 4-fold more effective against *C. parasilosis* than *C. albicans* (MIC 20 vs. 80 μg/mL), and 16-fold more effective against *C. tropicalis* (MIC 5 μg/mL). Rhamnosyl ceramide **T8** showed 4-fold more inhibition against *C. albicans* than the other two strains (MIC 20 vs. 80 μg/mL). These results clearly showed the sort of sugar moiety of GSLs played an important role on their antifungal activities.

Another interesting result was observed that when a carbamoyl group was introduced to the sugar part of inactive compound **T3**, compound **T9** displayed strong antifungal activities against all the three testing organisms with MIC values of 5.8 μM for *C. albicans*, 23.3 μM for *C. parasilosis*, and 5.8 μM for *C. tropicalis*. Thus, **T9** was a potent lead compound to be further optimized as antifungal agent and the study is currently on the way. The detailed mechanism and its further application as fungal growth inhibitors are under investigation.

The investigation also showed that the subtle structure of sphingoid bases of GSLs affected the antifungal activities. For example, **T4** showed commendable fungistatic activity as compared to **T3** against *C. albicans* (MIC 24.1 vs. >393 μM), indicating that an additional hydroxyl group at position 4 of the sphingoid base can increase the antifungal activity, which were consistent with those reported data [5] that PHS is more effective than DHS on their antifungal activities in some cases. Additionally, it has long been known that amino groups could benefit the antibacterial activity in many antibiotics [13]. In our study, we observed that GSLs with a primary amine (**T1** and **T2**) both had better antifungal activities (MIC 20–80 μg/mL) compared to those without free amine in general.

Conclusion

In conclusion, we have conducted the synthesis and *in-vitro* biological evaluation of antifungal activities of a series of 1,2-*trans* glycosphingolipids against *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. The preliminary study indicated that the sort of sugar moiety of the tested GSLs played an important role in their antifungal activities, and the permeability might not be the sole parameter affecting their antifungal properties as presumed before. Additionally, a carbamoyl group modified GSL (**T9**) displayed strong antifungal activities against all the three tested organisms, which would be a potent lead compound to be further optimized. All the results obtained here would bring new clues to understand the antifungal profile for these types of compounds.

Experimental

General

All commercial reagents and solvents were used as received without further purification unless specified. Reaction solvents were distilled from CaH_2 for dichloromethane and from sodium metal and benzophenone for tetrahydrofuran. Flash column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). ^{1}H-NMR and ^{13}C-NMR spectra were taken on a JEOL JNM-ECP 600 spectrometer (Jeol, Japan) with tetramethylsilane (TMS) as an internal standard at room temperature, except for the ^{13}C-NMR spectra of compounds **T5–T8**, which were taken on a Bruker Avance 400 MHz spectrometer at 60°C. Mass spectra were obtained on a Waters Q-TOF micro mass spectrometer (Waters).

*General procedure for the synthesis of glycosides **5**, **6**, and **7***

To a stirred solution of perbenzoylated glycosyl trichloroacetimidate donor **1** (1.3 mmol) and acceptor **2**, **3**, or **4** (1.0 mmol) in 20 mL of dry CH_2Cl_2 was added powdered 4-Å molecular sieves (0.2 g). The mixture was stirred for 30 min at −30°C and then TMSOTf (0.2 mmol) was added to the stirred mixture. The reaction mixture was stirred at −30°C for additional 30 min and

then warmed to room temperature and stirred until TLC revealed full conversion of acceptor. Et_3N was added to quench the reaction. The solid was then filtered off, and the filtrate was concentrated under vacuum. The syrupy residue was purified by silica gel flash chromatography, eluted with EtOAc and hexane to give glycosides **5** (92%), **6** (92%), or **7** (87%) as a colorless oil.

*(2S,3R)-2′,3′,4′,6′-Tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-azido-3-O-(tert-butyldimethylsilyl)-octadecan-1,3-diol (**5**)*

^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 8.09–7.20 (20H, Ar-H), 6.00 (dd, 1H, J = 3.6, 1.2 Hz, C4′-H), 5.82 (dd, 1H, J = 10.2, 7.8 Hz, C2′-H), 5.61 (dd, 1H, J = 10.2, 3.6 Hz, C3′-H), 4.87 (d, 1H, J = 7.8 Hz, C1′-H), 4.67 (dd, 1H, J = 11.4, 7.2 Hz, C6′-Ha), 4.43 (dd, 1H, J = 11.4, 5.6 Hz, C6′-Hb), 4.34 (dd, 1H, J = 6.6, 1.2 Hz, C5′-H), 4.07 (dd, 1H, J = 10.2, 6.0 Hz, C1-Ha), 3.78 (dd, 1H, J = 9.0, 4.8 Hz, C2-H), 3.68 (dd, 1H, J = 10.2, 4.8 Hz, C1-Hb), 3.57 (dd, 1H, J = 9.0, 5.4 Hz, C3-H), 1.57 (s-like, 2H, C4-H), 1.31–1.19 (m), 0.88–0.84 (m); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C 166.2, 165.8, 165.7, 165.3, 133.8, 133.5, 130.2, 130.0, 128.8, 128.7, 128.6, 128.5, 128.4, 101.6, 71.9, 71.7, 71.6, 69.8, 68.9, 68.2, 64.7, 62.1, 32.2, 32.1, 29.9–29.6, 26.0, 22.9, 14.3; HRMS-ESI: *m/z* calcd. for $C_{58}H_{77}N_3NaO_{11}Si$ $[M+Na]^+$: 1042.5220; Found 1042.5237.

*(2S,3R,4E)-2′,3′,4′,6′-Tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-azido-3-benzoyl-4-octadecene-1,3-diol (**6**)*

The physical data are identical with those provided in the literature: Liu, Y. P.; Ding, N.; Xiao, H. L.; Li, Y. X. *J. Carbohydr. Chem.* **2006**, *25*, 471–489.

*(2S,3S,4R)-2′,3′,4′,6′-Tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-azido-3,4-di-O-benzyl-octadecan-1,3,4-triol (**7**)*

^{1}H-NMR(600 MHz, $CDCl_3$) δ_H 8.07–7.20 (30H, Ar-H), 5.97 (dd, 1H, J = 3.6, 1.2 Hz, C4′-H), 5.82 (dd, 1H, J = 10.8, 7.8 Hz, C2′-H), 5.57 (dd, 1H, J = 10.8, 3.6 Hz, C3′-H), 4.79 (d, 1H, J = 7.8 Hz, C1′-H), 4.72–4.57 (5H, 2 PhCH_2O, C5′-H′), 4.40 (dd, 1H, J = 11.4, 7.2 Hz, C6′-Ha), 4.24 (dd, 1H, J = 11.8, 6.6 Hz, C1-Ha), 4.16 (dd, 1H, J = 11.8, 4.2 Hz, C6′-Hb), 3.91 (dd, 1H, J = 10.2, 3.6 Hz, C1-Hb), 3.71–3.69 (m, 1H, C2-H), 3.68–3.61 (m, 2H, C3-H, C4-H), 1.57 (m, 1H, C5-Ha), 1.42 (m, 1H, C5-Hb), 1.31–1.16 (m), 0.88 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C 166.2, 165.7, 165.6, 165.4, 138.6, 138.2, 133.7, 133.5–133.4, 130.1, 130.0, 129.9, 129.6, 129.2–127.9, 101.4, 79.2, 79.0, 74.1, 72.0, 71.8, 71.4, 69.9, 69.4, 68.2, 63.4, 63.2, 62.4, 62.0, 61.7, 32.1, 30.1–29.6, 25.4, 22.9, 14.3; HRMS-ESI: *m/z* calcd. for $C_{66}H_{75}N_3NaO_{12}$ $[M+Na]^+$: 1124.5248; Found 1124.5227.

*Preparation of (2S,3R)-2′,3′,4′,6′-tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-azido- octadecan-1,3-diol (**8**)*

To a solution of **5** (400 mg, 0.4 mmol) in dry THF (25 mL), a solution of TBAF (209 mg, 0.8 mmol) was added at 0°C. After 20 min, the solution was allowed to warm to room temperature and stirred for 90 min. The reaction mixture was treated with water (50 mL) and extracted with EtOAc (3 × 100 mL). The organic phases were combined, dried, and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography eluted with petroleum ether and EtOAc (v/v = 15:1) to give compound **8** as a colorless oil (323.3 mg, 91.4%).

*General procedure for the synthesis of amino intermediates **9**, **10**, **11***

To a solution of compound **8**, **6** or **7** (0.08 mmol) in THF (10 mL) and water (1.0 mL) co-solvent system was added PPh_3 (0.20 mmol). The reaction mixture was heated at 50°C for 12 h until TLC indicated the complete transformation of the starting azide into corresponding amine and then concentrated. The amine residue was used directly to the next step.

*General procedure for the synthesis of glycosyl ceramides **12**, **13***

To a solution of the above crude amino intermediate **9** or **11** and hexacosanoic acid (0.10 mmol) in dried CH_2Cl_2 (10 mL) were added HOBt (0.10 mmol), EDC · HCl (0.10 mmol) and DIPEA (0.12 mmol) at 0°C under Ar. The reaction mixture was stirred at room temperature for 22 h, and then concentrated. The residue was partitioned between CH_2Cl_2/water. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel eluted with CH_3Cl_3 and MeOH (v/v = 20:1) to furnish **12** (88% over 2 steps) or **13** (73% over 2 steps) as a colorless oil.

*(2S,3R)-2′,3′,4′,6′-Tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-docosanoylamino-octadecan-1,3-diol (**12**)*

^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 8.10–7.23 (20H, Ar-H), 6.00 (d, 1H, J = 3.3 Hz, C4′-H), 5.72 (dd, 1H, J = 9.9, 7.7 Hz, C2′-H), 5.67 (dd, 1H, J = 9.9, 3.3 Hz, C3′-H), 4.71 (d, 1H, J = 7.7 Hz, C1′-H), 4.66 (dd, 1H, J = 11.6, 7.1 Hz, C6′-Ha), 4.44 (dd, 1H, J = 11.6, 6.6 Hz, C6′-Hb), 4.32 (t, 1H, J = 6.6 Hz, C5′-H), 4.30 (dd, 1H, J = 10.4, 2.8 Hz, C1-Ha), 3.92 (s-like, 1H, C2-H), 3.72–3.71 (m, 1H, C1-Hb), 3.57 (s-like, C3-H), 1.74–1.68 (m, 2H), 1.61–1.25 (m), 0.88 (t, 6H, J = 6.6 Hz, 2 CH_3); HRMS-ESI: *m/z* calcd. for $C_{78}H_{115}NNaO_{12}$ $[M+Na]^+$: 1280.8317; Found 1280.8310.

*(2S,3S,4R)-2′,3′,4′,6′-Tetra-O-benzoyl-β-D-galactopyranosyl-(1′→1)-2-docosanoylamino-3,4-di-O-benzyl-octadecan-1,3,4-triol (**13**)*

^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 8.03–7.24 (30H, Ar-H), 6.00 (dd, 1H, J = 3.3 Hz, C4′-H), 5.79 (dd, 1H, J = 10.4, 7.7 Hz, C2′-H), 5.66 (dd, 1H, J = 10.4, 3.3 Hz, C3′-H), 5.51 (d, 1H, J = 8.8 Hz, N*H*), 4.86 (d, 1H, J = 11.0 Hz, PhCH*H*O), 4.81 (d, 1H, J = 7.7 Hz, C1′-H), 4.72 (d, 1H, J = 11.0 Hz, PhCH*H*O), 4.63 (d, 1H, J = 11.5 Hz, PhCH*H*O), 4.59 (dd, 1H, J = 11.0, 6.6 Hz, H-6′a), 4.48 (d, 1H, J = 12.1 Hz, PhCH*H*O), 4.44 (dd, 1H, J = 9.9, 3.3 Hz, C1-Ha), 4.41 (dd, 1H,

J = 11.0, 6.6 Hz, C6′-Hb), 4.28 (t, 1H, J = 7.1 Hz, C5′-H), 4.18 (m, 1H, H-2), 3.85 (dd, 1H, J = 8.8, 2.2 Hz, C3-H), 3.61 (dd, 1H, J = 9.3, 3.3 Hz, C1-Hb), 3.38 (d-like, 1H, J = 8.8, 2.8 Hz, C4-H), 1.69–1.64 (m, 2H), 1.60 (s, 2H), 1.54 (t, 1H, J = 7.1 Hz), 1.31–1.13 (m), 0.88 (t, 6H, J = 6.8 Hz, 2 CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C 172.5, 166.2, 165.7, 165.6, 138.9, 133.9, 133.8, 133.5, 130.1–127.8, 101.6, 80.1, 78.4, 74.4, 71.6, 71.5, 71.4, 70.7, 69.0, 68.2, 62.1, 49.4, 36.5, 32.1, 29.9–29.4, 26.3, 25.6, 22.9, 14.3; HRMS-ESI: m/z calcd. for $C_{92}H_{127}NNaO_{13}$ $[M+Na]^+$: 1476.9205; Found 1476.9200.

*General procedure for the removal of benzoyl protecting groups (Preparation of **T1–T4**)*

Compound **9**, **10** or **12** was dissolved in MeOH-CH_2Cl_2 (v/v = 2:1, 6 mL), and then NaOMe in MeOH was added until pH = 9. After stirring at room temperature for 12 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered. To the filtrate was added silica gel (100 mg), and the solvent was removed under vacuum. The resulting silica powder was loaded into a silica gel column and purified to form compound **T1** (87% over 2 steps), **T2** (73% over 2 steps) or **T3** (92%) as a white powder.

*(2S,3R)-β-D-Galactopyranosyl-(1′→1)-2-amino-octadecan-1,3-diol (**T1**)*

^{1}H-NMR (600 MHz, methanol-d_4) δ_H 4.18 (d, 1H, J = 7.3 Hz, C1′-H), 3.78–3.76 (m, 3H, C2-H, C1-Ha, C4′-H), 3.72 (dd, 1H, J = 11.5, 7.4 Hz, C6′-Ha), 3.67 (dd, 1H, J = 11.5, 5.0 Hz, C6′-Hb), 3.49–3.48 (m, 1H, C2′-H), 3.46–3.45 (m, 2H, C1-Hb, C5′-H), 3.42 (dd, 1H, J = 9.6,3.2 Hz, C3′-H), 1.96 (s-like, 1H, C3-H), 1.43–1.41 (m, 2H, C4-H), 1.31–1.22 (m, CH_2), 0.86 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C-NMR (150 MHz, methanol-d_4) δ_C 104.9, 78.1, 76.9, 75.0, 72.6, 70.9, 70.4, 62.7, 49.9, 37.5, 36.1, 34.1, 33.2, 30.9, 30.6, 28.8, 23.8, 14.5; HRMS-ESI: m/z calcd. for $C_{24}H_{50}NO_7$ $[M+H]^+$: 464.3587; Found 464.3582.

*(2S,3R,4E)-β-D-Galactopyranosyl-(1′→1)-2-amino-4-octadecene-1,3-diol (**T2**)*

^{1}H-NMR (600 MHz, DMSO-d_6) δ_H 5.54 (dt, 1H, J = 15.2, 7.0 Hz, C5-H), 5.36 (dd, 1H, J = 15.2, 7.4 Hz, C4-H), 4.03 (d, 1H, J = 7.8 Hz, C1′-H), 3.94–3.90 (m, 2H, C1-Ha, H-3), 3.78–3.76 (m, 1H, H-2), 3.63 (d, 1H, J = 3.2 Hz, C4′-H), 3.53 (dd, 1H, J = 11.0, 6.2 Hz, C6′-Ha), 3.47 (dd, 1H, J = 11.0, 5.8 Hz, C6′-Hb), 3.42 (dd, 1H, J = 11.0, 4.0 Hz, C1-Hb), 3.33–3.29 (m, 3H, C2′-H, C5′-H, C3′-H), 1.94–1.92 (m, 2H, CH_2=CH–CH_2), 1.45–1.21 (m, CH_2), 0.88 (t, 3H, J = 6.8 Hz, CH_3); HRMS-ESI: m/z calcd. for $C_{24}H_{48}NO_7$ $[M+H]^+$: 462.3431; Found 462.3425.

*(2S,3R)-β-D-Galactopyranosyl-(1′→1)-2-hexacosanoylamino-octadecan-1,3-diol (**T3**)*

^{1}H-NMR (600 MHz, pyridine-d_5) δ 8.52 (d, 1H, J = 9.1 Hz, N*H*), 4.88 (d, 1H, J = 7.7 Hz, C1′-H), 4.79 (m, 1H, C2-H), 4.60–4.57 (m, 2H, C4′-H, C1-Ha), 4.55–4.52 (m, 1H, C2′-H), 4.50–4.44 (m, 2H, C5′-H, C6′-Ha), 4.19 (dd, 1H, J = 9.2, 3.0 Hz, C3′-H), 4.11–4.09 (m, 1H, C6′-Hb), 4.07 (dd, 1H, J = 11.0, 4.1 Hz, C1-Hb), 3.69–3.66 (m, 1H, C3-H), 3.45 (s, 3H, OCH_3), 2.48 (t, 2H, J = 6.4 Hz, COCH_2), 1.91–1.27 (CH_2), 0.91–0.88 (m, 6H, 2 CH_3); HRMS-ESI: m/z calcd. for $C_{50}H_{99}NNaO_8$ $[M+H]^+$: 864.7263; Found 864.7257.

*(2S,3S,4R)-β-D-Galactopyranosyl-(1′→1)-2-hexacosanoylamino-octadecan-1,3,4-triol (**T4**)*

Ceramide **13** (60 mg, 0.041 mmol) was dissolved in MeOH/CH_2Cl_2 (v/v = 2:1, 6 mL), and then NaOMe in MeOH was added until pH was 9. After stirring at room temperature for 12 h, the solution was neutralized with ion-exchange resin (H^+) and then filtered. To the filtrate was added silica gel (100 mg), and the solvent was removed under vacuum. The resulting silica powder was loaded into a column and purified to form a syrup. To a solution of the above syrup in MeOH (3 mL) and EtOAc (3 mL) was added Pd/C (10% wt% Pd dry basis on carbon), and the mixture was hydrogenated at room temperature for 3 h. The suspension was filtered through Celite pad. The filter cake was rinsed with $CHCl_3$/MeOH (v/v = 5:1, 30 mL). The combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel flash chromatography eluted with $CHCl_3$ and MeOH (v/v = 10:1) to give the product **T4** (25.9 mg, 76%) as a white powder. ^{1}H-NMR (600 MHz, pyridine-d_5) δ_H 8.60 (d, 1H, J = 8.7 Hz, N*H*), 5.18 (bs, 1H, C2-H), 4.91 (d, 1H, J = 7.8 Hz, C1′-H), 4.85 (dd, 1H, J = 10.5, 5.5 Hz, C1-Hb), 4.57 (d, 1H, J = 3.2 Hz, C4′-H), 4.53–4.50 (m, 1H, C2′-H), 4.47 (dd, 1H, J = 10.5, 4.1 Hz, C1-Ha), 4.45–4.40 (m, 3H, C3-H, C6′-Ha,b), 4.26–4.25 (m, 1H, C4-H), 4.17 (dd, 1H, J = 9.7, 3.7 Hz, C3′-H), 4.06 (t, 1H, J = 5.9 Hz, C5′-H), 2.46 (t, 2H, J = 7.7 Hz, COCH_2), 2.20 (s, 1H), 1.97–1.25 (m, 2H, CH_2), 0.90–0.88 (m, 6H, 2 CH_3); ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C 106.4, 101.1, 77.3, 76.1, 75.6, 73.1, 70.8, 70.4, 62.6, 52.4, 37.3, 33.8, 32.4, 30.8–29.8, 27.0, 26.7, 23.2, 14.5; HRMS-ESI: m/z calcd. for $C_{50}H_{99}NNaO_9$ $[M+Na]^+$: 880.7218; Found 880.7212.

*General procedure for the hydrogenation of the compounds **14–17** (preparation of **T5–T8**)*

To a solution of compound **14**, or **15**–**17** (0.1 mmol) in MeOH (3 mL) and EtOAc (3 mL) was added Pd/C (100 mg, 10% wt% Pd dry basis on carbon), and the mixture was hydrogenated at room temperature for 3 h. The suspension was filtered through Celite pad. The filter cake was rinsed with $CHCl_3$/MeOH (v/v = 5:1, 30 mL). The combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel flash chromatography ($CHCl_3$/MeOH, v/v = 10:1) to give the product **T5**, or **T6**–**T8** as a colorless oil.

*(2S,3R)-β-D-Galactopyranosyl (1′→1)-2-(hexadecanoylamido)-octadecane-1,3-diol (**T5**)*

^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 7.60 (d, 1H, J = 9.2 Hz, N*H*), 4.86 (d, 1H, J = 3.6 Hz, 2′-O*H*), 4.72 (d, 1H, J = 5.5 Hz, 4′-O*H*), 4.57 (t, 1H, J = 5.5 Hz, 6′-O*H*), 4.56 (d, 1H, J = 6.4 Hz, 3-O*H*), 4.36 (d, 1H, J = 4.1 Hz, 3′-O*H*), 4.03 (d, 1H, J = 7.4 Hz, C1′-H), 3.96 (dd, 1H, J = 10.1, 4.6 Hz, C1-Ha), 3.75–3.71 (m, 1H, C2-H), 3.62–3.61 (m, 1H, C4′-H), 3.54–3.50 (m, 1H, C6′-Ha), 3.48–3.44 (m, 1H, C6′-Hb), 3.42–3.40 (m, 2H, C1-Hb, C3-H), 3.32–3.25 (m, 3H, C5′-H, C2′-H, C3′-H), 2.11–2.01 (m, 2H, CH_2), 1.54–1.41 (m, 4 H, 2 CH_2), 1.29–1.18 (m, 50 H, 25 CH_2), 0.86–0.84 (m, 6 H, 2 CH_3); ^{13}C-NMR (100 MHz, 60°C, DMSO-d_6) δ_C 171.9, 104.2, 75.1, 73.1, 70.6, 69.5, 69.1, 68.0, 60.4, 53.1, 35.5, 33.4, 31.0, 29.0–28.4, 25.2, 24.8, 21.8, 13.6; HRMS-ESI: m/z calcd. for $C_{40}H_{79}NNaO_8$ $[M+Na]^+$: 724.5703; Found 724.5698.

*(2S,3R)-β-D-Mannopyranosyl (1′→1)-2-(hexadecanoylamido)-octadecane-1,3-diol (**T6**)*

^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 7.56 (d, 1H, J = 9.2 Hz, N*H*), 4.67 (d, 1H, J = 5.0 Hz, 2′-O*H*), 4.65 (d, 1H, J = 4.1 Hz, 3′-O*H*), 4.57 (1H, d, J = 1.0 Hz, C1′-H), 4.56 (d, 1H, J = 6.4 Hz, 3-O*H*), 4.51 (d, 1H, J = 6.4 Hz, 4′-O*H*), 4.38 (t, 1H, J = 5.9 Hz, 6′-O*H*), 3.75–3.71 (m, 1H, C2-H), 3.69–3.67 (m, 1H, C1-Ha), 3.62–3.59 (m, 1H, C6′-Ha), 3.58–3.56 (m, 1H, C3′-H), 3.49–3.43 (m, 2H, C4′-H, C6′-Hb), 3.41–3.36 (m, 3H, H-1b, C3-H, C2′-H), 3.29–3.26 (m, 1H, C5′-H), 2.10–2.00

(m, 2H, CH_2), 1.44–1.40 (m, 2H, CH_2), 1.28–1.18 (m, 52H, 26 CH_2), 0.86–0.84 (m, 3H, 2 CH_3); ^{13}C-NMR (100 MHz, 60°C, DMSO-d_6) δ_C 171.8, 100.5, 73.5, 70.8, 70.2, 69.9, 67.1, 66.5, 61.2, 53.3, 35.4, 33.3, 31.0, 29.0–28.4, 25.2, 24.9, 21.8, 13.6; HRMS-ESI: *m*/*z* calcd. for $C_{40}H_{79}NNaO_8$ $[M+Na]^+$: 724.5703; Found 724.5698.

*(2S,3R)-β-D-Glucopyranosyl (1′→1)-2-(hexadecanoylamido)-octadecane-1,3-diol (**T7**)*

^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 7.59 (d, 1H, *J* = 9.2 Hz, N*H*), 4.99 (d, 1H, *J* = 3.7 Hz, 2′-O*H*), 4.94 (d, 1H, *J* = 5.0 Hz, 3′-O*H*), 4.91 (d, 1H, *J* = 5.5 Hz, 4′-O*H*), 4.56 (d, 1H, *J* = 6.4 Hz, 3-O*H*), 4.51 (t, 1H, *J* = 5.9 Hz, 6′-O*H*), 4.09 (d, 1H, *J* = 7.8 Hz, C1′-H), 3.94 (dd, 1H, *J* = 10.1, 5.0 Hz, C1-Ha), 3.75–3.71 (m, 1H, C2-H), 3.68–3.65 (m, 1H, C6-Ha,), 3.90–3.46 (m, 3H, C3-H, C1-Hb, C6′-Hb), 3.15–3.11 (m, 1H, C3′-H), 3.10–3.07 (m, 1H, C5′-H), 3.04–3.00 (m, 1H, C4′-H), 2.98–2.94 (m, 1H, C2′-H), 2.11–2.01 (m, 2H, CH_2), 1.54–1.50 (m, 2H, CH_2), 1.45–1.41 (m, 2H, CH_2), 1.28–1.20 (m, 50H, 25 CH_2), 0.86–0.84 (m, 6H, 2 CH_3); ^{13}C-NMR (100 MHz, 60°C, DMSO-d_6) δ_C 172.6, 104.2, 77.3, 77.0, 74.0, 70.8, 70.2, 69.8, 61.8, 53.7, 36.1, 34.1, 31.7, 29.6–29.1, 25.8, 25.4, 22.4, 14.2; HRMS-ESI: *m*/*z* calcd. for $C_{40}H_{79}NNaO_8$ $[M+Na]^+$: 724.5703; Found 724.5698.

*(2S,3R)-α-D-Rhamnoyranosyl (1′→1)-2-(hexadecanoylamido)-octadecane-1,3-diol (**T8**)*

^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 7.35 (d, 1H, *J* = 8.7 Hz, N*H*), 4.51 (brs, 1H, C1′-H), 4.47 (d, 1H, *J* = 4.6 Hz, 4′-O*H*), 4.43 (d, 1H, *J* = 4.1 Hz, 2′-O*H*), 4.33 (d, 1H, *J* = 5.9 Hz, 3-O*H*), 4.19 (d, 1H, *J* = 5.9 Hz, 3′-O*H*), 3.79–3.77 (m, 1H, C2-H), 3.60–3.57 (m, 2H, C2′-H, C1-Ha), 3.48–3.38 (m, 4H, C1-Ha, C2′-H, C3′-H, C5′-H), 3.17–3.15 (m, 1H, C4′-H), 2.12–2.02 (m, 1H, CH_2), 1.51–1.46 (m, 2H, CH_2), 1.42–1.39 (m, 2 H, CH_2), 1.29–1.19 (m, 50 H, 25 CH_2), 1.13 (d, 3H, *J* = 6.0 Hz, C6′-H), 0.87–0.84 (m, 6H, 2 CH_3); ^{13}C-NMR (100 MHz, 60°C, DMSO-d_6) δ_C 171.7, 99.5, 72.0, 70.7, 70.3, 70.1, 68.1, 66.0, 52.5, 35.4, 33.5, 31.0, 29.0–28.4, 25.2, 24.8, 21.8, 17.6, 13.6; HRMS-ESI: *m*/*z* calcd. for $C_{40}H_{79}NNaO_7$ $[M+Na]^+$: 724.5754; Found 708.5749.

*Preparation of (2S,3R)-2′-O-benzoyl-3′-O-benzyl-4′,6′-O-benzylidene-β-D-galactopyranosyl)-(1′→1)-2-azido-3-O-benzyl-octadecan-1,3-diol (**20**)*

To a mixture of glycosyl donor **18** (800 mg, 1.4 mmol), acceptor **19** (0.5 g, 1.2 mmol) and 4-Å MS (200 mg) in CH_2Cl_2 (30 mL) were added NIS (316.4 mg, 1.4 mmol) and AgOTf (30.8 mg, 0.1 mmol) at −20°C under Ar. The reaction mixture was stirred for 60 min at −20°C and then warmed to 0°C. After stirring for 30 min at 0°C the reaction mixture was concentrated. The residue was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography eluted with petroleum ether and EtOAc (v/v = 7:1) to give the desired product **20** as a colorless oil (840 mg, 86.1%).

^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 8.03–7.20 (20H, Ar-H), 5.72 (dd, 1H, *J* = 10.1, 8.4 Hz, C2′-H), 5.53 (s, 1H, PhC*H*), 4.71–4.38 (4H, 2 PhCH_2O), 4.60 (d, 1H, *J* = 7.8 Hz, C1′-H), 4.26 (dd, 1H, *J* = 11.8, 1.4 Hz, C6′-Ha), 4.21 (d, 1H, *J* = 3.6 Hz, C4′-H), 4.12 (dd, 1H, *J* = 10.8, 6.6 Hz, C1-Ha), 4.04 (dd, 1H, *J* = 11.9, 2.3 Hz, C6′-Hb), 3.76 (dd, 1H, *J* = 9.2, 4.8 Hz, C2-H), 3.72 (dd, 1H, *J* = 10.8, 4.2 Hz, C1-Hb), 3.70 (dd, 1H, *J* = 10.1, 3.2 Hz, C3′-H), 3.62 (dd, 2H, *J* = 9.2, 4.8 Hz, C3-H), 3.26 (s-like, 1H, C5′-H), 1.85–1.83 (m, 2H, H-4), 1.32–1.13 (m), 0.88 (t, 3H, *J* = 7.4 Hz, CH_3); HRMS-ESI: *m*/*z* calcd. for $C_{52}H_{67}N_3NaO_8$ $[M+Na]^+$: 884.4826; Found 884.4820.

*Preparation of (2S,3R)-3′-O-benzyl-4′,6′-O-benzylidene-β-D-galactopyranosyl)-(1′→1)-2-azido-3-O-benzyl-octadecan-1,3-diol (**21**)*

Compound **20** (500 mg, 0.6 mmol) was dissolved in 10 mL dry MeOH and 10 mL dry CH_2Cl_2. NaOMe (99%, 180 mg) was added to the mixture and stirred for 20 h. After almost all starting material was consumed, Amberlyst 15 (H^+) was added to neutralize the NaOMe, and the mixture was then diluted with MeOH, and the exchange resin was filtered off. The resin was washed thoroughly and the filtrate was concentrated. The residue was purified by silica gel flash chromatography eluted with petroleum ether and EtOAc (v/v = 7:1) to give the desired product **21** (404.4 mg, 92.0%) as a foamy solid.

^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 7.52–7.23 (15H, Ar-H), 5.46 (s, 1H, PhC*H*), 4.77 (m, 2H, PhCH_2O), 4.66 (d, 1H, *J* = 11.5 Hz, PhC*H*H), 4.54 (d, 1H, *J* = 11.5 Hz, PhC*H*H), 4.37 (d, 1H, *J* = 7.8 Hz, C1′-H), 4.29 (d, 1H, *J* = 12.1 Hz, C6′-Ha), 4.14–4.11 (m, 2H, C4′-H′, C1-Ha), 4.04–4.00 (m, 2H, C2′-H, C6′-Hb), 3.79 (dd, 1H, *J* = 10.4, 3.8 Hz, C1-Hb), 3.72 (m, 1H, C2-H), 3.61 (m, 1H, C3-H), 3.50 (dd, 1H, *J* = 9.4, 3.3 Hz, C3′-H), 3.36 (s-like, 1H, C5′-H), 1.62–1.53 (m, 2H, H-4), 1.45–1.44 (m, 2H, H-5), 1.26 (s-like, CH_2), 0.88 (t, 3H, *J* = 7.1 Hz, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C 138.4, 138.3, 138.0, 129.1, 128.6, 128.5, 128.3, 128.2, 128.0, 127.8, 126.6, 103.3, 101.4, 79.2, 78.3, 73.4, 72.7, 71.8, 70.2, 69.3, 68.9, 67.0, 63.5, 32.1, 31.1, 29.9–29.5, 25.3, 22.9, 14.3; HRMS-ESI: *m*/*z* calcd. for $C_{45}H_{63}N_3NaO_7$ $[M+Na]^+$: 780.4564; Found 780.4558.

*Preparation of (2S,3R)-3-O-benzyl-4,6-O-benzylidene-2-O-carbamoyl-β-D-galactopyranosyl-(1′→1)-2-azido-3-O-benzyl-octadecan-1,3-diol (**23**)*

A solution of compound **21** (76 mg, 0.10 mmol) in Py (10 mL) in the presence of DMAP (43 mg, 0.30 mmol) was treated with *p*-nitrophenyl chloroformate (121.3 mg, 0.60 mmol). The reaction mixture was heated at 40°C for 2 h and concentrated. To a solution of the crude product in dried THF (10 mL) was added NH_3 at 0°C. The reaction mixture was stirred for 6 h, and then concentrated. The residue was partitioned between CH_2Cl_2/water. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$ and MeOH (v/v = 40:1) to furnish **23** (65.9 mg, 82.1%) as a white solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H 7.52–7.23 (15H, Ar*H*), 5.48 (s, 1H, PhC*H*), 5.22 (dd, 1H, *J* = 10.4, 8.3 Hz, C2′-H), 4.72–4.66 (m, 3H, PhCH_2), 4.53–4.51 (m, 2H, C1′-H, PhC*H*H), 4.29 (dd, 1H, *J* = 12.6, 1.6 Hz, C6′-Ha), 4.17 (d, 1H, *J* = 3.3 Hz, C4′-H), 4.09 (dd, 1H, *J* = 10.4, 1.3 Hz, C3′-H), 4.02 (dd, 1H, *J* = 12.1, 1.7 Hz, C6′-Hb), 3.73 (dd, 1H, *J* = 11.0, 4.4 Hz, C1-Ha), 3.66 (m, 1H, C2-H), 3.61 (m, 2H, H-1b, C3-H), 3.37 (s-like, 1H, C5′-H), 1.58 (m, 2H, C4-H), 1.45–1.44 (m, 2H, C5-H), 1.26 (s-like, CH_2), 0.88 (t, 3H, *J* = 7.1 Hz, CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C 155.6, 138.3, 129.1–126.6, 101.4, 101.1, 78.3, 73.6, 72.9, 71.4, 71.3, 69.2, 67.7, 66.9, 63.4, 32.1, 31.0, 29.9, 29.6, 25.4, 22.9, 14.3; HRMS-ESI: *m*/*z* calcd. for $C_{46}H_{65}N_4O_8$ $[M+H]^+$: 801.4802; Found 801.4797.

*Preparation of (2S,3R)-β-D-galactopyranosyl-(1′→1)-hexacosanoylamino-octadecan-1,3-diol (**T9**)*

PPh_3 (0.15 mol) and water (0.01 mol) were added to a stirred solution of azide compounds **23** (60.0 mg, 0.075 mmol) in THF (6 mL), and the mixture was then heated at 45°C until TLC

indicated the complete transformation of the starting azide into corresponding amine. After rotary evaporation, the amine residue was redissolved in dry CH_2Cl_2 (6 mL), and Et_3N (0.01 mmol), EDC · HCl (0.09 mmol), HOBt (0.09 mmol), and fatty acid (0.083 mmol) were added. The reaction mixture was stirred at room temperature for 10 h. The solution was concentrated under vacuum, and the syrupy residue was purified by column chromatography on silica gel eluted with petroleum ether and EtOAc (v/v = 5:1). To a solution of the above purified syrupy residue in MeOH (3 mL) and EtOAc (3 mL) was added Pd/C (50 mg, 10% wt% Pd dry basis on carbon), and the mixture was hydrogenated at room temperature for 3 h. The suspension was filtered through Celite pad. The filter cake was rinsed with $CHCl_3$/MeOH (5:1, 30 mL). The combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel flash chromatography eluted with $CHCl_3$ and MeOH (v/v = 10:1) to give the product **T9** (41.1 mg, 64%) as a light yellow powder.

^{1}H-NMR(600 MHz, pyridine-d_5) δ_H 8.06 (d, 1H, J = 7.3 Hz, N*H*), 7.87 (m, 2H, NH_2), 4.91 (d, 1H, J = 7.8 Hz, C1′-H), 4.76 (m, 1H, C2-H), 4.66 (m, 1H, C1-Ha), 4.58 (m, 1H, C4′-H), 4.41–4.40 (m, 2H, C5′-H, C6′-Ha), 4.38–4.36 (m, 3H, C2′-H, C1-Hb, C3-H), 4.21–4.17 (m, 3H, C6′-Hb, C4-H), 4.05 (m, 1H, C3′-H), 2.52 (t, 2H, J = 7.3 Hz, COCH_2), 1.97–1.28 (CH_2), 0.88 (m, 6H, 3 CH_3); ^{13}C-NMR (150 MHz, pyridine-d_5) δ_C 131.3, 129.2, 102.7, 76.9, 74.2, 71.7, 70.8, 79.9, 65.4, 45.9, 44.3, 36.7, 35.0, 31.9, 31.2, 30.7–29.4, 27.8, 22.7, 14.1; HRMS-ESI: *m/z* calcd. for $C_{51}H_{101}N_2O_9$ $[M+Na]^+$: 885.7507; Found 885.7502.

In-vitro antifungal activity evaluation by MIC assay

Compounds C18-DHS and **T1–T9** were evaluated for their *in-vitro* antifungal activity against *Candida albicans* (EU583481), *Candida parapsilosis* (EF198017), and *Candida tropicalis* (EU651853). In this process minimum inhibitory concentration (MIC) of tested compounds were determined by using a modified micro-broth dilution assay following the guidelines of Clinical and Laboratory Standard (NCCLS) described elsewhere. The test was performed by using sterile disposal 96-well polystyrene microtiter plates in RPMI 1640 medium buffered with MOPS (3-[*N*-morpholino]propane sulfonic acid) (Sigma Chemical Co., USA). The solutions of tested compounds were prepared by a serial fourfold dilutions of the product diluted in assay medium to get the test concentration (320–1.25 μg/mL). Initial inocula of fungal strains were maintained at 1–5 × 10^3 cells/mL. These plates were incubated in a moist chamber at 35°C and absorbance at 630 nm was recorded on Versa Max micro-plate reader (Molecular devices, Sunnyvale, USA) after 24, 72 and 168 h. MIC was defined as the lowest concentration of antifungal agent that inhibits 80% development of growth reflected by OD value. Experiments were conducted in triplicate.

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