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Synthesis of Iminosugar-Containing KRN7000 Analogues

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Three new iminosugar-containing KRN7000 (also referred to as *α*-GalCer) analogues were designed and synthesized. In the design, the galactose moiety of KRN7000 was replaced by iminosugars and the iminosugar structures were connected with ceramide in different manners with a *C*-glycosidic bond instead of the *O*-glycosidic bond. To our knowledge, this is the first report in which iminosugars are incorporated in KRN7000 structure modifications. The synthetic compounds were evaluated for their ability to stimulate cytokine release. The results may benefit better understanding of structure-activity relationships and facilitate future design of more KRN7000 derivatives.

Keywords KRN7000 analogue, glycolipid, *α*-GalCer, iminosugar, synthesis

Introduction

KRN7000 (Figure 1), a synthetic *α*-galactosylceramide (*α*-GalCer) derived from structural modifications of the marine natural product named Agelasphin-9b, has a strong ability to stimulate natural killer T (NKT) cells, in a CD1d-restricted and T cell antigen receptor (TCR)-mediated manner.[1,2] KRN7000 binds to CD1d molecule and after being presented to NKT cells by CD1d, stimulates rapid production of Th1 and Th2 cytokines by NKT cells. However, the therapeutic applications of KRN7000 were limited by the antagonizing effect between Th1 and Th2 cytokines. Therefore, it is essential to develop more effective analogues that can produce polarizing cytokine release profile towards either Th1 or Th2, by structural modifications or derivations based on KRN7000.[3,4]

A large number of KRN7000 analogues and related compounds have been synthesized and their activity towards NKT cell-activation has been tested.[3-10] Modifications of KRN7000 are roughly based on three modifiable parts: sugar head moiety, ceramide part, and sugar-ceramide linkage. Sugar moiety modification studies suggested that: (1) *α*-glycosidic bond is essential for NKT cell-stimulation activity; (2) minor changes on sugar head can result in activity loss except that C-6 position can tolerate small substitutions.[11-15] Regarding the sugar-ceramide linkage modifications, a *C*-galactoside analogue of KRN7000, *α*-*C*-GalCer, induced a potent Th1-type response in mice *in vivo*, due to the stability of *C*-glycosidic bond which can resist enzymatic degradation at low pH.[16-18] Interestingly, replacement of galactose ring oxygen by carbon (carba-*α*-*D*-galactopyranosyl analogue of KRN7000)[19] or sulfur (5-thio-*α*-*D*-galactopyranosyl analogue of KRN7000)[20] remarkably induced Th1-biased cytokine production *in vivo* as well. Although many studies have been performed regarding KRN7000 modifications, accumulation of structure-activity-relationship information is still essential to decipher the critical structural factors of KRN7000 that induced biased cytokine release profiles.

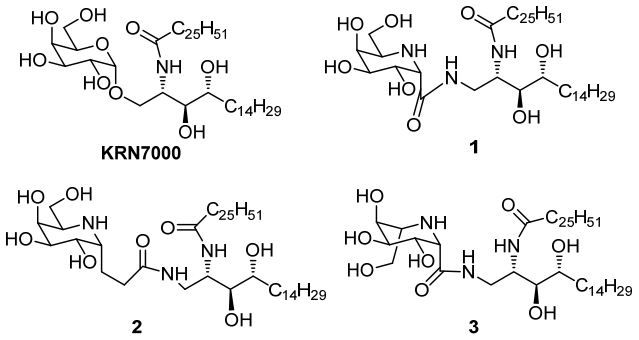

Figure 1 The structures of KRN7000 and its designed analogues.

Iminosugars are carbohydrate analogues in which the ring oxygen is replaced by nitrogen. Iminosugars are frequently found to be inhibitors of glycosidases that are involved in important biological systems.[21-23] Iminosugar-based compounds are promising medicine candidates against various diseases such as tumor metastasis, diabetes, viral infection, and lysosomal storage disorders.[23-25] Our group discovered that certain iminosugar derivatives possess immunosuppressive activities while

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In Memory of Professor Enze Min.

minor structure changes lead to immunostimulating activities, showing the potential as the lead compounds with various biological activities.[26-28] Two castanospermine (an iminosugar) analogues also showed up-regulation of the Th1/Th2 cytokine ratio, with apparent bias towards Th1 cytokines.[29] Based on these observations, and considering that very few examples for iminosugar analogues of GalCer have been reported,[30] it is possible to create new iminosugar-containing compounds and explore their biological activities. Therefore, three KRN7000 analogues were designed, in which the galactose moiety was replaced by iminosugars and the iminosugars were connected with ceramide in different manners (Figure 1, compounds **1**—**3**). Due to the stability of *α*-*C*-GalCer and its potent Th1-biased responses, all the three structures adopted *C*-glycosidic bonds instead of *O*-glycosidic bonds. Also the linker between galactose and phytosphingosine was elongated by different number of carbons with the aim of studying how linker elongation affects the bioactivity of *α*-GalCer.

Experimental

General

Air- and/or moisture-sensitive reactions were carried out under protection of nitrogen and standard syringe/septa techniques. All chemicals were purchased as reagent grade and used without further purification. Dichloromethane (CH_2Cl_2) was distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. dimethylformamide (DMF) was stirred with CaH_2 and distilled over sodium/benzophenone. Tetrahydrofuran (THF) was distilled over sodium/benzophenone. Analytical TLC was performed on silica gel 60-F_{254} precoated on aluminum plates (E. Merck), with detection by UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 35 ℃ (water bath). Organic solutions of crude products were dried over anhydrous Na_2SO_4. Column chromatography was performed employing silica gel (200—300 mesh). ^{1}H NMR spectra were recorded on a JEOL AL-300, Varian INOVA-500 or Avance DRX Bruker-400 spectrometers at 25 ℃. Chemical shifts (in parts per million) were referenced to tetramethylsilane (TMS, δ 0) in deuterated chloroform. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with $CDCl_3$ (δ 77.00) or CD_3OD (δ 49.00). Mass spectra were recorded using a PE SCLEX QSTAR spectrometer. High-resolution mass spectrometry (HRMS) was performed on a Bruker APEX IV. Elemental analysis data were recorded on a Vario EL-III element analyzer.

Benzyl(2*R*,3*S*,4*R*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(3-methoxy-3-oxoprop-1-en-1-yl)piperidine-1-carboxylate (8)

To a solution of **6**[26] (142.2 mg, 0.208 mmol) in 8 mL of dry THF was added Ph_3P=CHCOOMe (142.5 mg, 0.415 mmol) at room temperature under nitrogen. The reaction mixture was stirred overnight at room temperature and then evaporated *in vacuo*. The residue was purified by column chromatography with petroleum ether/EtOAc (5∶1 to 3∶1) to give the desired product **8** as colorless oil (147.6 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31—7.19 (m, 25H), 6.00 (d, J=16.2 Hz, 1H), 5.20—5.06 (m, 2H), 4.90—4.67 (m, 6H), 4.50—4.20 (m, 2H), 4.07—4.00 (m, 3H), 3.73 (s, 3H), 3.49—3.46 (m, 2H). MS (*m*/*z*): calcd for $C_{46}H_{47}NO_8$: 741; found 742 $[M+H]^+$. Anal. calcd for $C_{21}H_{41}N_3O_3$ (%): C 74.47, H 6.39, N 1.89; found C 74.52, H 6.35, N 1.86.

Benzyl (2*R*,3*S*,4*R*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(3-hydroxy-3-oxoprop-1-en-1-yl)piperidine-1-carboxylate (9)

A mixture of **8** (147.6 mg, 0.199 mmol) and 2 mol/L NaOH (3.0 mL, 6.0 mmol) in MeOH (3.0 mL) was stirred at 40 ℃ for 4 h. The mixture was cooled down to room temperature, the H^+ resin was added to the reaction mixture to neutralize the solution to pH=7. The mixture was filtered, the filtrate was concentrated. The residue was dissolved in EtOAc, the solution was washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give compound **9** which was directly used for the next step reaction.

(2*S*)-2-Azido-2-((4*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethan-1-ol (13)

To a stirred solution of **12**[31] (0.80 g, 2.33 mmol) in 10 mL of DMP, catalytic amount of TsOH (19.8 mg, 5%) was added at 0 ℃ under nitrogen. Then the reaction was allowed to continue at room temperature for 1.5 h under nitrogen. TLC test showed the reaction was complete. The reaction was quenched by adding MeOH (15 mL) and stirred for another 1 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography with petroleum ether/EtOAc (8∶1 to 5∶1) to afford **13** as white foams (0.55g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.20—4.16 (m, 1H), 4.02—3.95 (m, 2H), 3.89—3.85 (m, 1H), 3.47 (m, 1H), 2.10 (br, 1H), 1.62—1.53 (m, 2H), 1.43 (s, 3H), 1.34 (s, 3H), 1.40—1.26 (m, 24H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 108.42, 77.73, 76.69, 63.96, 61.16, 31.91, 29.66, 29.57, 29.52, 29.38, 29.34, 28.01, 26.51, 25.54, 22.68, 14.10. MS (*m*/*z*): calcd for $C_{21}H_{41}N_3O_3$: 383; found 356 $[M+H-N_2]^+$, 401 $[M+NH_4]^+$. Anal. calcd for $C_{21}H_{41}N_3O_3$ (%): C 65.76, H 10.77, N 10.96; found C 66.02, H 10.63, N 10.91.

2-((2*S*)-2-Azido-2-((4*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethyl)isoindoline-1,3-dione (14)

To a solution of **13** (123.3 mg, 0.321 mmol) in dry toluene under nitrogen was added Ph_3P (168.4 mg, 0.642 mmol), phthalimide (94.6 mg, 0.642 mmol) and DEAD (106 μL, 0.642 mmol). The reaction mixture was stirred at room temperature under nitrogen for 1.5 h. The solvent was removed *in vacuo*. The residue was

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purified by column chromatography with petroleum ether/EtOAc (10∶1) to provide **14** as white foams (157.5 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.88 (dd, J=5.4, 3.0 Hz, 2H), 7.75 (dd, J=5.4, 3.3 Hz, 2H), 4.20 (dd, J=13.2, 5.4 Hz, 1H), 4.09−3.94 (m, 3H), 3.77 (td, J=9.0, 4.2 Hz, 1H), 1.64−1.54 (m, 3H), 1.50 (s, 3H), 1.35 (s, 3H), 1.38−1.21 (m, 23H), 0.87 (t, J=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.22, 134.16, 131.87, 123.43, 108.65, 77.84, 58.60, 39.59, 31.89, 29.65, 29.55, 29.50, 29.33, 29.11, 27.81, 26.57, 25.54, 22.66, 14.10. MS (*m*/*z*) calcd for $C_{29}H_{44}N_4O_4$: 512.3; found 530.2 $[M+NH_4]^+$. Anal. calcd for $C_{29}H_{44}N_4O_4$ (%): C 67.94, H 8.65, N 10.93; found C 68.07, H 8.54, N 10.83.

(2*S*,3*S*,4*R*)-2-Azido-3,4-isopropylidene ketal-octadecane-1-amine (15)

A mixture of **14** (166.0 mg, 0.324 mmol) and NH_2NH_2•H_2O (3.0 mL) in MeOH (6.0 mL) was heated at 70 ℃ for 0.5 h. The mixture was cooled down to room temperature. The reaction mixture was concentrated, and the residue was dissolved in EtOAc. The solution was washed with sat. brine, dried over Na_2SO_4, and concentrated to dryness, yielding **15** which was directly used for the next step reaction.

Benzyl (2*S*,3*S*,4*S*,5*S*,6*R*)-2-(((2*S*)-2-azido-2-((4*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethyl)-carbamoyl)-3,4,5-tris(benzyloxy)-6-((benzyloxy)-methyl)piperidine-1-carboxylate (16)

To a mixture of **7**[26] (150 mg, 0.214 mmol) and **15** (91.3 mg, 0.238 mmol) in dry THF, HBTU (99.2 mg, 0.262 mmol) and DIEA (91.4 μL, 0.523 mmol) were added under nitrogen. The reaction mixture was stirred overnight at room temperature under nitrogen. TLC detection showed the reaction was complete, then the solvent was removed *in vacuo*, and the residue was purified by column chromatography with petroleum ether/EtOAc (15∶1 to 6∶1) to afford **16** as colorless oil (207.5 mg, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.29−7.20 (m, 25H), 5.09 (d, J=11.5 Hz, 1H), 4.88 (d, J=12.0 Hz, 2H), 4.76−4.65 (m, 6H), 4.52−4.31 (m, 3H), 4.25 (dd, J=7.5, 4.5 Hz, 1H), 4.11−4.03 (m, 3H), 3.98−3.80 (m, 2H), 3.76 (d, J=5.0 Hz, 1H), 3.68 (dd, J=8.5, 5.5 Hz, 1H), 3.47 (brs, 1H), 3.28−2.98 (m, 1H), 1.56−1.48 (m, 3H), 1.40 (s, 3H), 1.34−1.22 (m, 26H), 1.25 (s, 3H), 0.85 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.04, 138.56, 138.35, 138.20, 137.72, 136.11, 128.44, 128.39, 128.31, 128.21, 127.97, 127.91, 127.57, 127.48, 108.38, 79.97, 77.63, 77.11, 76.12, 74.17, 73.38, 73.08, 72.92, 67.68, 59.92, 56.64, 41.18, 31.91, 29.69, 29.65, 29.60, 29.34, 29.28, 27.96, 26.56, 25.52, 22.68, 22.37, 16.52, 14.11. MS (*m*/*z*): calcd for $C_{64}H_{83}N_5O_9$: 1065.6; found 1066.4 $[M+H]^+$, 1089.3 $[M+Na]^+$. Anal. calcd for $C_{64}H_{83}N_5O_9$ (%): C 72.08, H 7.85, N 6.57; found C 71.97, H 7.72, N 6.52.

Benzyl (2*S*,3*S*,4*S*,5*S*,6*R*)-2-(((2*S*,3*S*,4*R*)-2-azido-3,4-dihydroxyoctadecyl)carbamoyl)-3,4,5-tris(benzyl-oxy)-6-((benzyloxy)methyl)piperidine-1-carboxylate (17)

To compound **16** (174.4 mg, 0.164 mmol) in 7 mL of MeOH/THF (V∶V=1∶1) was added 0.2 mL of concentrated HCl, and the reaction mixture was stirred overnight at room temperature. TLC test showed that the reaction was complete. The solvent was removed *in vacuo*, and the residue was purified using column chromatography with petroleum ether/EtOAc (2∶1) to afford **17** as colorless oil (158.9 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37−7.19 (m, 25H), 5.08 (d, J=12.0 Hz, 1H), 4.90 (d, J=12.0 Hz, 1H), 4.74−4.60 (m, 6H), 4.48−4.28 (m, 3H), 4.24−4.18 (m, 1H), 4.10−4.02 (m, 2H), 3.94−3.83 (m, 3H), 3.69−3.65 (m, 2H), 3.50 (brs, 1H), 3.43 (brs, 1H), 3.23 (brs, 1H), 2.38 (d, J=6.5 Hz, 1H), 1.74 (d, J=2.5 Hz, 1H), 1.54−1.40 (m, 3H), 1.31−1.26 (m, 23H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.79, 138.37, 138.12, 138.00, 137.45, 136.02, 128.49, 128.41, 128.36, 128.33, 128.25, 128.05, 127.99, 127.70, 127.66, 127.61, 127.54, 78.95, 75.71, 74.22, 73.77, 73.34, 72.96, 72.86, 71.95, 67.80, 67.32, 62.44, 56.71, 56.58, 39.91, 31.90, 31.53, 29.67, 29.34, 25.85, 22.67, 14.10. MS (*m*/*z*): calcd for $C_{61}H_{79}N_5O_9$: 1025.6; found 1026.3 $[M+H]^+$, 1048.3 $[M+Na]^+$. Anal. calcd for $C_{61}H_{79}N_5O_9$ (%): C 71.39, H 7.76, N 6.82; found C 71.51, H 7.93, N 6.80.

Benzyl (2*R*,3*S*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(((2*S*,3*S*,4*R*)-2-hexacosan-amido-3,4-dihydroxyoctadecyl)carbamoyl)piperi-dine-1-carboxylate (18)

To a solution of **17** (37.6 mg, 0.0366 mmol) in 7 mL of MeOH were added $NiCl_2$•$6H_2O$ (17.4 mg, 0.0733 mmol) and $NaBH_4$ (6.8 mg, 0.183 mmol) at 0 ℃. The reaction mixture was allowed to warm up to room temperature and stirred overnight. A few drops of ammonium hydroxide were added to the reaction mixture and the solvent was removed *in vacuo*. The residue was re-dissolved in EtOAc, and washed by saturated $NaHCO_3$ and NaCl solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was directly used for the next step. The crude product was dried by vacuum and dissolved in 5 mL of dry THF. $C_{25}H_{51}COOH$ (29.0 mg, 0.0733 mmol), HBTU (27.7 mg, 0.0733 mmol) and DIEA (19.2 μL, 0.110 mmol) were added under nitrogen, and the reaction mixture was stirred overnight at room temperature. TLC test showed the reaction was complete. The solvent was removed *in vacuo*. Column chromatography with petroleum/EtOAc (1∶1) provided **18** as colorless oil (47.4 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.31−7.20 (m, 25H), 6.78 (brs, 1H), 6.44 (brs, 1H), 5.10 (d, J=12.5 Hz, 1H), 4.90 (d, J=12.5 Hz, 1H), 4.66−4.62 (m, 3H), 4.56 (d, J=11.5 Hz, 2H), 4.52−4.38 (m, 5H), 4.18 (dd, J=6.0, 3.5 Hz, 1H), 4.00 (brs, 3H), 3.87 (brs, 1H), 3.76 (dd, J=6.5, 3.5 Hz, 1H), 3.65 (brs, 1H), 3.49

(brs, 1H), 3.44 (brs, 1H), 3.30－3.25 (m, 1H), 2.35 (brs, 1H), 2.07－1.97 (m, 2H), 1.57－1.51 (m, 3H), 1.50－1.44 (m, 1H), 1.40－1.18 (m, 68H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.85, 170.71, 137.96, 137.74, 135.91, 128.49, 128.41, 128.36, 128.15, 127.93, 127.85, 127.72, 127.60, 75.24, 74.35, 73.50, 73.15, 73.00, 72.77, 67.73, 66.68, 56.47, 52.14, 39.95, 36.68, 32.37, 31.92, 29.70, 29.56, 29.43, 29.36, 25.95, 25.62, 22.69, 14.13. HRMS: calcd for $C_{87}H_{132}N_3O_{10}$ $[M+H]^+$ 1378.9907, found 1378.9975; calcd for $C_{87}H_{131}N_3O_{10}Na$ $[M+Na]^+$ 1400.9727, found 1400.9764.

(2*S*,3*S*,4*S*,5*S*,6*R*)-*N*-((2*S*,3*S*,4*R*)-2-Hexacosanamido-3,4-dihydroxyoctadecyl)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidine-2-carboxamide (1)

To a solution of **18** (20.0 mg, 0.0145 mmol) in 6 mL of THF/H_2O (V∶V=2∶1) was added concentrated HCl (0.6 mL), catalytic amount of Pd/C, and the mixture was stirred for 3 d under H_2 atmosphere. The reaction mixture was filtered and the filtrate was concentrated, and the residue was purified by column chromatography with CH_2Cl_2/MeOH (15∶1 to 3∶1) to afford **1** as white foams (12.8 mg, 100%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ: 4.16 (dd, J=10.0, 6.0 Hz, 1H), 4.00 (d, J=6.0 Hz, 1H), 3.86 (s, 1H), 3.74－3.70 (m, 2H), 3.63 (dd, J=13.5, 3.5 Hz, 1H), 3.57－3.51 (m, 3H), 3.39 (d, J=9.5 Hz, 1H), 2.72 (brs, 1H), 2.28－2.18 (m, 2 H), 1.61－1.57 (m, 4H), 1.42－1.40 (m, 2H), 1.31－1.27 (m, 67H), 0.89 (t, J=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD) δ: 175.24, 75.41, 72.22, 71.21, 69.51, 68.96, 62.65, 57.66, 56.08, 53.72, 50.02, 30.01, 29.93, 29.78, 29.71, 29.60, 26.21, 22.91, 14.20. HRMS: calcd for $C_{51}H_{102}N_3O_8$ $[M+H]^+$ 884.7661, found 884.7681.

Benzyl (3*S*,4*R*,5*S*,6*R*)-2-(3-(((2*S*)-2-azido-2-((4*S*)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethyl)-amino)-3-oxoprop-1-en-1-yl)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidine-1-carboxylate (19)

The synthetic procedure was the same as that described in the synthesis of compound **16**, using **9** (24.8 mg, 0.0341 mmol) and **15** (13.0 mg, 0.0341 mmol), HBTU (25.8 mg, 0.0682 mmol), and DIEA (12.0 μL, 0.0682 mmol) to afford **19** (34.9 mg, 80%) as colorless oil. The product was a mixture of separable *E*- and *Z*-isomers with a ratio of *E*-/*Z*-configuration 5∶1. *E*-isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36－7.18 (m, 25H), 7.08－7.06 (m, 1H), 6.60－6.55 (m, 1H), 5.84－5.79 (m, 1H), 5.30－5.19 (m, 2H), 4.92 (d, J=12.0 Hz, 1H), 4.78 (d, J=10.5 Hz, 1H), 4.66－4.03 (m, 8H), 3.90－3.63 (m, 4H), 3.49－3.46 (m, 1H), 3.25－3.04 (m, 3H), 2.77－2.61 (m, 1H), 1.60－1.26 (m, 32H), 0.88 (t, J=7.2 Hz, 3H). *Z*-Isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33－7.16 (m, 25H), 5.88－5.67 (m, 1H), 5.27－5.05 (m, 3H), 4.78－4.30 (m, 6H), 4.01－3.48 (m, 8H), 3.48－3.02 (m, 3H), 1.58－1.25 (m, 32H), 0.88 (t, J=6.6 Hz, 3H). MS (m/z): calcd for $C_{66}H_{85}N_5O_9$: 1091.6; found 1092.6 $[M+H]^+$. Anal. calcd for $C_{66}H_{85}N_5O_9$ (%): C 72.56, H 7.84, N 6.41; found C 72.41, H 7.76, N 6.28.

Benzyl (3*S*,4*R*,5*S*,6*R*)-2-(3-(((2*S*,3*S*,4*R*)-2-azido-3,4-dihydroxyoctadecyl)amino)-3-oxoprop-1-en-1-yl)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidine-1-carboxylate (20)

Treatment of **19** (62.1 mg, 0.0568 mmol) with concentrated HCl as described in the synthesis of **17**, led to **20** (52.2 mg, 87%) as colorless oil. The product was a mixture of separable *E*- and *Z*-isomers with a ratio of *E*-/*Z*-configuration 5∶1. *E*-Isomer: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34－7.21 (m, 25H), 5.96－5.77 (m, 1H), 5.21 (t, J=6.0 Hz, 1H), 5.08 (d, J=12.3 Hz, 1H), 4.93 (d, J=11.7 Hz, 1H), 4.80－4.16 (m, 10H), 4.10－4.00 (m, 1H), 3.98 (s, 1H), 3.91－3.60 (m, 5H), 2.70－2.43 (m, 1H), 1.83 (s, 1H), 1.51－1.47 (m, 3H), 1.46－1.25 (m, 23H), 0.88 (t, J=6.6 Hz, 3H). *Z*-Isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37－7.05 (m, 25H), 6.82 (s, 1H), 5.84－5.71 (m, 1H), 5.24 (d, J=12.5 Hz, 1H), 5.15 (dd, J=10.5, 2.0 Hz, 1H), 5.08 (d, J=12.0 Hz, 1H), 4.86 (brs, 1H), 4.79 (d, J=8.0 Hz, 1 H), 4.65 (dd, J=12.0, 9.5 Hz, 1H), 4.60－4.44 (m, 3H), 4.35 (dd, J=16.0, 11.5 Hz, 1H), 4.26 (d, J=3.5 Hz, 1H), 4.18 (t, J=11.5 Hz, 1H), 4.03 (dd, J=6.0, 3.0 Hz, 1H), 3.96 (dd, J=6.0, 3.0 Hz, 1H), 3.88－3.86 (m, 1H), 3.82 (d, J=10.5 Hz, 1H), 3.75－3.68 (m, 1H), 3.68－3.52 (m, 1H), 3.44－3.41 (m, 1H), 3.39－3.34 (m, 1H), 3.32－3.28 (m, 1H), 3.25－3.14 (m, 2H), 3.12－3.04 (m, 1H), 2.70－2.61 (m, 1H), 2.33 (dd, J=13.0, 7.5 Hz, 1H), 1.56 (s, 3H), 1.48－1.40 (m, 2H), 1.31－1.26 (m, 22H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.09, 172.75, 155.00, 138.32, 138.11, 137.72, 136.39, 131.75, 128.65, 128.53, 128.42, 128.34, 128.18, 128.03, 127.97, 127.74, 127.65, 127.43, 127.36, 81.13, 74.08, 73.93, 73.16, 72.72, 72.37, 72.24, 71.80, 71.48, 67.90, 67.07, 65.86, 62.47, 56.72, 56.35, 55.79, 52.70, 40.06, 39.88, 36.31, 36.22, 31.92, 31.37, 31.21, 29.69, 29.36, 25.87, 22.68, 14.12. MS (m/z): calcd for $C_{63}H_{81}N_5O_9$: 1051.6, found 1052.4 $[M+H]^+$. Anal. calcd for $C_{63}H_{81}N_5O_9$ (%): C 71.90, H 7.76, N 6.65; found C 71.74, H 7.89, N6.79.

Benzyl (2*R*,3*S*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(3-(((2*S*,3*S*,4*R*)-2-hexacosanamido-3,4-dihydroxyoctadecyl)amino)-3-oxopropyl)piperidine-1-carboxylate (21)

Compound **21** was synthesized by the same procedure as that in the preparation of **18**. Compound **20** (102.1 mg, 0.0970 mmol) was reduced and conjugated with $C_{25}H_{51}COOH$. Following column chromatography compound **21** was obtained (45.0 mg, 33%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.31－7.14 (m, 25H), 6.86 (brs, 1H), 6.27 (brs, 1H), 5.02 (brs, 1H), 4.88 (brs, 1H), 4.69－4.47 (m, 5H), 4.37－4.35 (m, 2H), 4.25－4.18 (m, 2H), 4.12－3.94 (m, 3H), 3.81－3.71 (m, 2H), 3.56 (d, J=10.0 Hz, 2H), 3.45 (brs, 4H), 2.83 (brs, 1H), 2.23 (brs, 1H), 2.14－2.09 (m, 4H), 1.76 (brs, 2H), 1.63－1.56 (m, 3H), 1.50－1.48 (m, 1H), 1.38－1.13 (m, 68H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (125

MHz, $CDCl_3$) δ: 174.67, 174.26, 157.79, 138.59, 138.31, 137.80, 135.85, 130.85, 128.49, 128.42, 128.33, 128.18, 128.05, 127.85, 127.78, 127.63, 127.52, 127.44, 74.74, 74.36, 73.97,73.21, 72.95, 68.14, 67.85, 55.14, 54.02, 52.54, 40.84, 36.74, 32.90, 31.91, 30.34, 29.70, 29.55, 29.42, 29.36, 28.91, 26.01, 25.70, 23.73, 22.97, 22.67, 14.10. HRMS: calcd for $C_{89}H_{136}N_3O_{10}$ $[M+H]^+$ 1407.0220, found 1407.0231.

_N_-((2_S_,3_S_,4_R_)-3,4-Dihydroxy-1-(3-((3_S_,4_R_,5_S_,6_R_)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidin-2-yl)-propanamido)octadecan-2-yl)hexacosanamide (2)

Compound **21** (20.0 mg, 0.0142 mmol) was deprotected and the product was purified as in the preparation of **1**, providing **2** (13.0 mg, 100%) as white foams. ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ: 4.19−4.11 (m, 3H), 3.75−3.71 (m, 1H), 3.68−3.50 (m, 5H), 3.27 (brs, 1H), 3.00 (brs, 1H), 2.76−2.66 (m, 1H), 2.52 (ddd, J= 15.5, 8.0, 5.0 Hz, 1H), 2.33−2.23 (m, 3H), 2.07−1.98 (m, 1H), 1.61−1.55 (m, 4H), 1.45−1.14 (m, 69H), 0.88 (t, J = 7.0 Hz, 6H); ^{13}C NMR (150 MHz, MeOD/$CDCl_3$) δ: 175.90, 175.67, 75.92, 72.61, 70.83, 68.56, 66.33, 51.15, 40.91, 37.15, 33.99, 32.71, 32.70, 30.53, 30.52, 30.48, 30.47, 30.42, 30.40 30.36, 30.26, 30.13, 30.10, 26.76, 26.67, 23.39, 14.37. HRMS: calcd for $C_{53}H_{106}N_3O_8$ $[M+H]^+$ 912.7974, found 912.7993.

Benzyl (2_S_,3_S_,4_S_,5_S_,6_S_)-2-(((2_S_)-2-azido-2-((4_S_)-2,2-dimethyl-5-tetradecyl-1,3-dioxolan-4-yl)ethyl)carbamoyl)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-piperidine-1-carboxylate (22)

The synthetic procedure was the same as that described in the synthesis of compound **16**, using **10**[26] (97.8 mg, 0.140 mmol), **15** (59.5 mg, 0.155 mmol), HBTU (64.6 mg, 0.170 mmol), and DIEA (89.3 μL, 0.511 mmol) to afford **22** (146.7 mg, 99%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36−7.17 (m, 25H), 5.15 (dd, J=19.5, 12.5 Hz, 2H), 4.98 (brs, 1H), 4.79 (brs, 1H), 4.68 (brs, 1H), 4.52 (dd, J=32.0, 11.5 Hz, 5H), 4.39−4.36 (m, 3H), 4.03 (d, J=28.5 Hz, 2H), 3.83 (brs, 1H), 3.61 (s, 3H), 3.40−3.25 (m, 1H), 2.95 (brs, 1H), 2.68 (brs, 1H), 1.49 (brs, 3H), 1.38 (s, 3H), 1.31−1.24 (m, 23H), 1.23 (s, 3H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz,$CDCl_3$) δ: 170.05, 138.30, 138.03, 137.79, 136.32, 128.46, 128.31, 128.30, 128.09, 127.91, 127.66, 127.55, 108.26, 77.58, 74.76, 74.44, 73.39, 72.74, 72.50, 71.74, 68.90, 67.77, 60.41, 59.10, 57.38, 55.29, 40.80, 31.91, 29.68, 29.60, 29.34, 29.18, 27.97, 26.59, 25.54, 22.68, 14.11. MS (_m_/_z_): calcd for $C_{64}H_{83}N_5O_9$: 1065.6; found 1066.4 $[M+H]^+$, 1088.3 $[M+Na]^+$. Anal. calcd for $C_{64}H_{83}N_5O_9$ (%): C 72.08, H 7.85, N 6.57; found C 71.96, H 7.81, N 6.68.

Benzyl (2_S_,3_S_,4_S_,5_S_,6_S_)-2-(((2_S_,3_S_,4_R_)-2-azido-3,4-dihydroxyoctadecyl)carbamoyl)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)piperidine-1-carboxylate (23)

Treatment of **22** (68.8 mg, 0.0645 mmol) with concentrated HCl as described in the synthesis of **17**, led to **23** (60.7 mg, 92%) colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.86−7.53 (m, 1H), 7.33−7.19 (m, 25H), 5.18 (d, J=8.0 Hz, 1H), 5.11 (d, J=11.5 Hz, 1H), 4.90 (brs, 1H), 4.72 (s, 2H), 4.55 (s, 2H), 4.46 (d, J=9.5 Hz, 2H), 4.38 (d, J=12.0 Hz, 2H), 4.32 (t, J=7.5 Hz, 1H), 4.25−4.11 (m, 1H), 3.92 (t, J=3.5 Hz, 2H), 3.58 (d, J=4.5 Hz, 3H), 3.48 (brs, 1H), 3.35 (s, 1H), 3.23 (brs, 1H), 3.00 (brs, 1H), 2.32 (s, 1H), 1.66 (s, 1H), 1.54−1.50 (m, 1H), 1.42 (brs, 2H), 1.32−1.25 (m, 23H), 0.88 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.37, 156.47, 138.16, 137.86, 137.76, 137.47, 136.18, 129.78, 128.53, 128.50, 128.41, 128.34, 128.16, 128.05, 127.99, 127.75, 127.67, 127.63, 74.72, 74.14, 73.08, 72.68, 72.52, 71.82, 71.64, 68.45, 67.87, 62.73, 57.84, 55.04, 39.93, 31.91, 31.48, 29.68, 29.64, 29.34, 25.90, 22.67, 14.11; MS (_m_/_z_): calcd for $C_{61}H_{79}N_5O_9$: 1025.6; found 1026.3 $[M+H]^+$, 1048.2 $[M+Na]^+$. Anal. calcd for $C_{61}H_{79}N_5O_9$ (%): C 71.39, H 7.76, N 6.82; found C 71.14, H 7.86, N 6.70.

Benzyl (2_S_,3_S_,4_S_,5_S_,6_S_)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(((2_S_,3_S_,4_R_)-2-hexacosanamido-3,4-dihydroxyoctadecyl)carbamoyl)piperidine-1-carboxylate (24)

Compound **24** was synthesized by the same procedure as the preparation of **18**. Compound **23** (31.8 mg, 0.0310 mmol) was reduced and conjugated with $C_{25}H_{51}COOH$. Following column chromatography compound **24** was obtained (30.3 mg, 71%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.34−7.15 (m, 25H), 6.17 (brs, 2H), 5.14 (s, 2H), 4.83 (d, J=6.5 Hz, 1H), 4.67 (s, 2H), 4.57−4.50 (m, 4H), 4.42 (d, J=12.0 Hz, 2H), 4.35 (t, J=7.5 Hz, 2H), 3.92 (s, 4H), 3.64 (dd, J=10.0, 7.0 Hz, 2H), 3.57 (brs, 1H), 3.38 (s, 2H), 3.23 (dd, J=11.5, 6.0 Hz, 2H), 2.44 (d, J=8.0 Hz, 1H), 1.93 (brs, 3H), 1.74 (s, 1H), 1.51−1.41 (m, 6H), 1.31−1.13 (m, 62H), 0.88 (t, J=7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.58, 172.18, 156.56, 138.10, 137.83, 137.62, 136.07, 128.59, 128.57, 128.43, 128.36, 128.17, 128.10, 127.99, 127.78, 127.70, 127.62, 74.80, 74.25, 74.01, 73.06, 72.77, 72.61, 71.69, 68.80, 67.91, 58.47, 55.35, 51.73, 40.22, 36.56, 32.37, 31.91, 29.70, 29.57, 29.43, 29.35, 26.01, 25.55, 22.67, 14.10. HRMS: calcd for $C_{87}H_{132}N_3O_{10}$ $[M+H]^+$ 1378.9907, found 1378.9975.

(2_S_,3_S_,4_S_,5_S_,6_S_)-_N_-((2_S_,3_S_,4_R_)-2-Hexacosanamido-3,4-dihydroxyoctadecyl)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidine-2-carboxamide (3)

Compound **24** (42.4 mg, 0.0308 mmol) was deprotected and the product was purified as in the synthesis of compound **1**, providing **3** (27.1 mg, 100%) as white foams. ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ: 4.27−4.22 (m, 2H), 4.02−4.00 (m, 3H), 3.97−3.95 (m, 2H), 3.70 (dd, J=14.0, 3.0 Hz, 1H), 3.56−3.53 (m, 1H), 3.50 (t, J=6.0 Hz, 1H), 3.21−3.15 (m, 2H), 2.27 (t, J=7.5 Hz, 2H), 1.70−1.50 (m, 6H), 1.39−1.23 (m, 66H), 0.89 (t, J=7.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ: 175.03, 74.67, 71.22, 69.54, 69.26,

62.48, 55.68, 54.15, 49.42, 39.54, 35.85, 31.33, 29.13, 29.07, 28.93, 28.84, 28.75, 25.32, 22.05, 13.21. HRMS: calcd for $C_{51}H_{102}N_3O_8$ $[M+H]^+$ 884.7661, found 884.7690.

Spleen cell proliferation assay

The splenocytes (8×10^5 cells/well) were plated in 96-well flat-bottom tissue culture plates with synthetic compound (100 ng/mL, 100 μL/well) diluted in 200 μL of medium. After 48 h at 37 ℃, CCK-8 (20 μL) was added to the cultured cell and the colorimetric values were measured by a microplate reader with 600 nm as reference.

In vivo stimulation with synthetic compounds

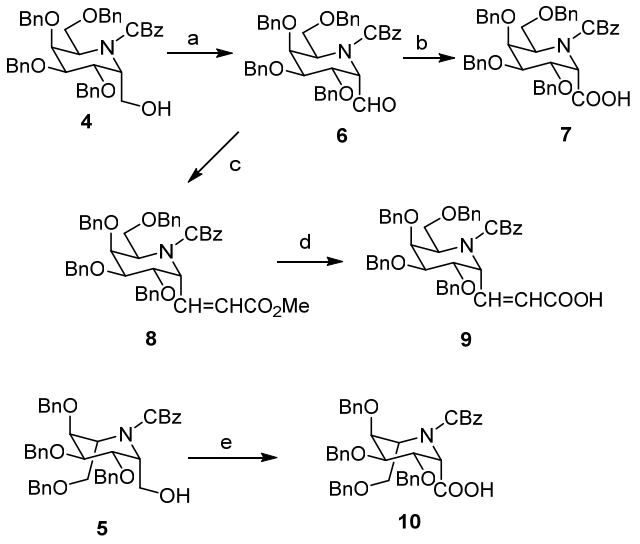
Stock solutions of KRN 7000 and synthetic compounds were prepared in 100% DMSO at a concentration of 1 mg/mL. Before use, the solutions were diluted with phosphate buffered saline (pH 7.4) to obtain a final concentration of 10 μg/mL. Mice were injected intraperitoneally with 5 μg of compound or with diluted DMSO alone. Sera were collected at 2 time points, and the levels of IFN-γ (at 16 h) and IL-4 (at 2 h) were measured by a standard sandwich ELISA using purified capture and biotin-conjugated detection monoclonal antibodies and standards. ELISAs were developed with TMB substrate, followed by evaluation using a microplate reader.

Results and Discussion

The synthesis was divided into three parts: the iminosugar synthesis, the phytosphingosine chain synthesis, and the conjugation. The iminosugar synthesis started from galactose. As shown in Scheme 1, compounds **4** and **5** were prepared according to our previously reported procedures.[26-28] The oxidation of **4** with TEMPO/DAIB led to aldehyde **6**, which was further oxidized by $NaClO_2$ and H_2O_2 to give carboxylic acid **7**. The Witting reaction between **6** and ylide $Ph_3P=CHCO_2Me$ followed by ester hydrolysis afforded compound **9**. Unlike compound **4**, oxidation of **5** with TEMPO/DAIB at room temperature overnight directly provided carboxylic acid **10**.

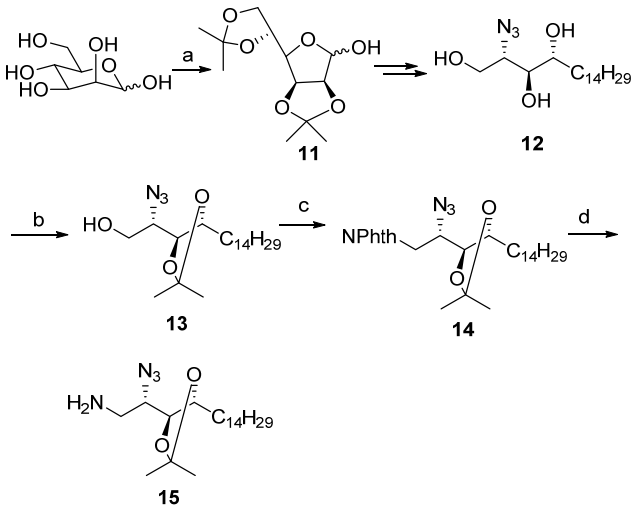
The synthetic route of phytosphingosine chain began with mannose. As shown in Scheme 2, following the method developed previously,[31] 2,3;5,6-di-*O*-*iso*-propylidene-*D*-mannofuranose (**11**) was converted to sphingosine **12** through the seven-step manipulation in a total yield of 69%. Protection of **12** with 2,2-dimethoxypropane (DMP) in the presence of a catalytic amount of TsOH gave isopropylidene ketal protected compound **13**. This step could lead to the side product in which 1,3-hydroxyl groups were protected instead. The side product could be converted to **12** after acidic deprotection and then be reused. The Mitsunobu reaction on the terminal hydroxyl group in **13**, which was followed by the hydrazine deprotection of phthalimide **14**, afforded amine **15**.

Scheme 1 Synthetic pathway of iminosugars



Regents and conditions: (a) TEMPO, DAIB, CH_2Cl_2, r.t., 99%; (b) $NaClO_2$ (aq.), NaH_2PO_4 (aq.), 30% H_2O_2, r.t., 83%; (c) $Ph_3P=CHCO_2Me$, THF, r.t., 96%; (d) 2 mol/L NaOH (aq.), MeOH, 40 ℃, quantitative; (e) TEMPO, DAIB, CH_2Cl_2, r.t. 94%

Scheme 2 Synthetic pathway of phytosphingosine chain



Reagents and conditions: (a) acetone, H_2SO_4, r.t., then NaOH (aq.), 86%; (b) TsOH, DMP, 62%; (c) Phthalimide, DEAD, Ph_3P, toluene, 96%; (d) $NH_2NH_2\cdot H_2O$, MeOH, 70 ℃, quantitative

The target compounds **1**—**3** were assembled via the coupling reactions between sugar heads and sphingosine chain, which was followed by lipid chain (hexacosanoic acid) conjugation (Scheme 3). The carboxylic acid on sugar heads was conjugated to the terminal amine present in sphingosine **15** to form the corresponding amide compounds. The isopropylidene protective group was removed by concentrated hydrochloric acid using MeOH/THF as solvent. It was found that the removal of isopropylidene ketal is necessary for the next-step azide reduction. Azide reduction was not successful in the

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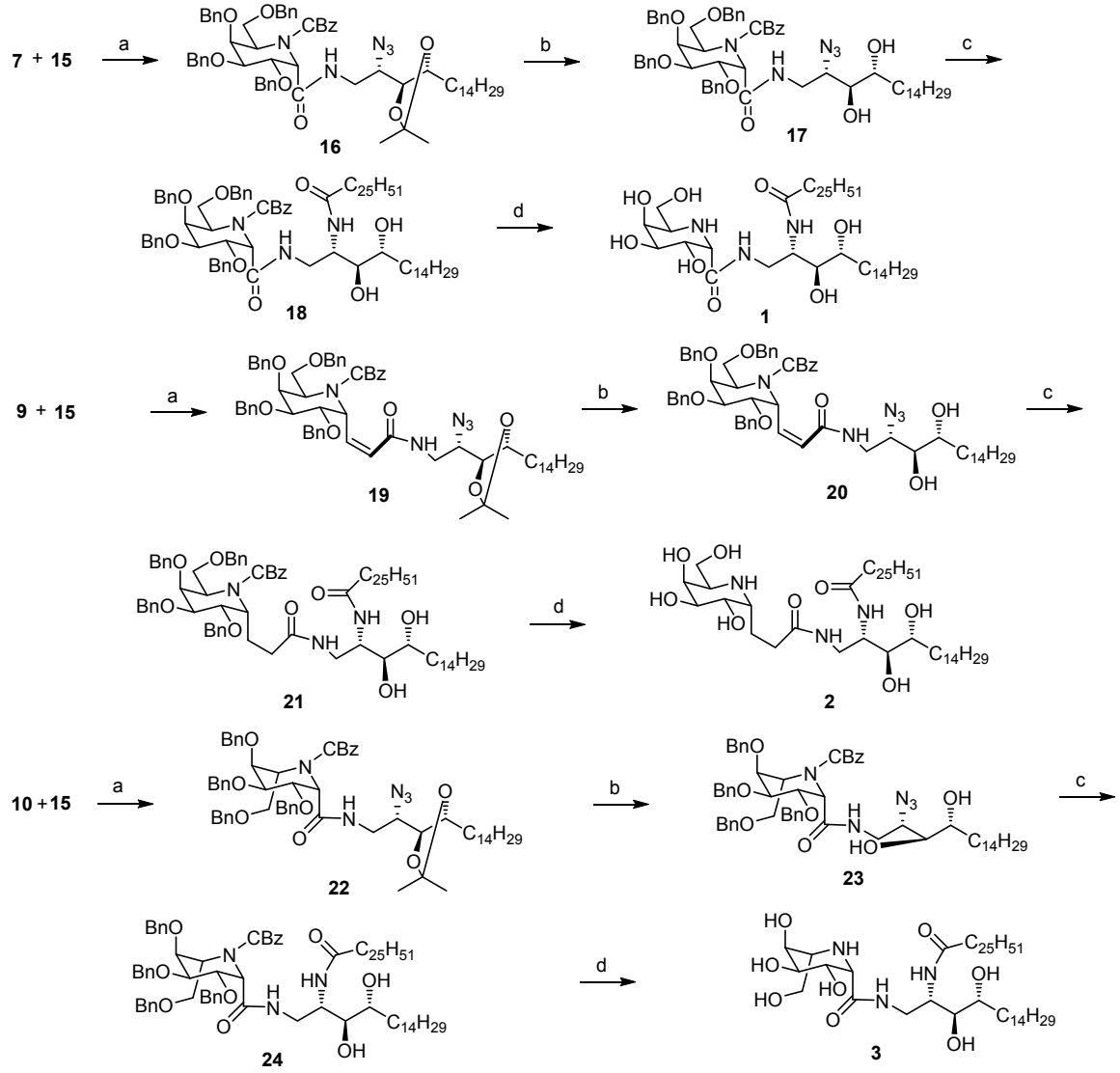
Scheme 3 Synthesis of final compounds **1**—**3**



Reagents and conditions: (a) HBTU, DIEA, THF, 91% for **16**, 80% for **19**, 99% for **22**; (b) HCl, MeOH/THF, 95% for **17**, 87% for **20**, 92% for **23**; (c) i) $NaBH_4$, $NiCl_2$•$6H_2O$, MeOH; ii) $C_{25}H_{51}COOH$, HBTU, DIEA, THF; 94% for **18**, 33% for **21**, 71% for **24**; (d) Pd/C, H_2, THF/H_2O/HCl, quantitative

presence of isopropylidene ketal probably due to steric hindrance. After removal of the isopropylidene group, the azido group on sphingosine chain was reduced by $NaBH_4$ and $NiCl_2$•$6H_2O$ to yield the crude product which was conjugated to hexacosanoic acid through an amide bond. In this step, if the reaction time was elongated to overnight, the double bond in **20** (as a mixture of *Z*- and *E*-configurations) was also reduced (as in **21**). Finally, the benzyl (Bn) groups and the benzyloxycarbonyl (Cbz) group were removed smoothly over catalytic hydrogenolysis to afford the final products **1**—**3**.

The synthetic compounds **1**—**3** were tested for their NKT cell-stimulation activity using KRN7000 as a positive control. It turned out that the analogues induced no detectable cytokine or proliferative response.

The X-ray crystallographic analysis of CD1d-α-GalCer complex showed that the two lipid chains of α-GalCer are inserted into two hydrophobic pockets of CD1d while the sugar head is extended above the lipid-binding groove surface for recognition by the TCR of NKT cells.[32-34] The crystal structure of ternary human CD1d-αGalCer-TCR complex revealed that the galactose ring is sandwiched between CD1d and NKT TCR.[35] TCR displays a rigid "lock and key" type interaction with the glycolipid. TCR interacts with not only the 2', 3', and 4' hydroxyl groups of galactose ring but also the 3-hydroxyl group of the sphingosine chain. One reasonable explanation to our bioassay results is that the elongated sphingosine chain in compounds **1**—**3** could probably break the rigid spatial relationship between the sugar head and the ceramide moiety, thus interrupting the interactions of α-GalCer with NKT and CD1d. Concurring with our speculation, Tashiro and Franck *et al*. respectively discovered that the elongated

C- and *O*-glycoside analogues of KRN7000 with an extra methylene unit (CH_2) between the sugar and ceramide showed no bioactivity at all.[5,36]

Conclusions

In summary, three novel KRN7000 analogues, in which the ring oxygen of the galactopyranose residue is replaced by the nitrogen atom along with the variation on the ceramide, were designed and synthesized. These synthetic compounds were evaluated for their ability to stimulate cytokine release. Although the compounds displayed no activity on inducing the cytokine release, the results may benefit design of the next generation KRN7000 analogues. Since iminosugars have shown many applications in drug discovery, the disclosed iminosugar-substitution strategy may facilitate the preparation of more KRN7000 derivatives with biological importance.

Acknowledgement

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References

[1] Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626.

[2] Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176.

[3] Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771.

[4] Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073.

[5] Tashiro, T. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1055.

[6] Chang, Y. J.; Huang, J. R.; Tsai, Y. C.; Hung, J. T.; Wu, D.; Fujio, M.; Wong, C.-H.; Yu, A. L. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10299.

[7] Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem.*, *Int. Ed.* **2004**, *43*, 3818.

[8] Dere, R. T.; Zhu, X. *Org. Lett.* **2008**, *10*, 4641.

[9] Sun, M.; Wang, Y.; Ye, X.-S. *Tetrahedron* **2013**, *69*, 7438.

[10] Feng, J.; Gao, F.; Peng, S.; Chen, H.; Li, X. *Chin. J. Org. Chem.* **2015**, *35*, 997.

[11] Kobayashi, E.; Motoki, K.; Yamaguchi, Y.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem.* **1996**, *4*, 615.

[12] Motoki, K.; Morita, M.; Kobayashi, E.; Uchida, T.; Akimoto, K.; Fukushima, H.; Koezuka, Y. *Biol. Pharm. Bull.* **1995**, *18*, 1487.

[13] Uchimura, A.; Shimizu, T.; Nakajima, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1997**, *5*, 1447.

[14] Uchimura, A.; Shimizu, T.; Morita, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2245.

[15] Liu, Y.; Goff, R. D.; Zhou, D.; Mattner, J.; Sullivan, B. A.; Khurana, A.; Cantu III, C.; Ravkov, E. V.; Ibegbu, C. C.; Altman, J. D.; Teyton, L.; Bendelac, A.; Savage, P. B. *J. Immunol. Methods* **2006**, *312*, 34.

[16] Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631.

[17] Patel, O.; Cameron, G.; Pellicci, D. G.; Liu, Z.; Byun, H. S.; Beddoe, T.; McCluskey, J.; Franck, R. W.; Castano, A. R.; Harrak, Y.; Llebaria, A.; Bittman, R.; Porcelli, S. A.; Godfrey, D. I.; Rossjohn, J. *J. Immunol.* **2011**, *187*, 4705.

[18] Sullivan, B. A.; Nagarajan, N. A.; Wingender, G.; Wang, J.; Scott, I.; Tsuji, M.; Franck, R. W.; Porcelli, S. A.; Zajonc, D. M.; Kronenberg, M. *J. Immunol.* **2010**, *184*, 141.

[19] Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2009**, *17*, 6360.

[20] Bi, J.; Wang, J.; Zhou, K.; Wang, Y.; Fang, M.; Du, Y. *ACS Med. Chem. Lett.* **2015**, *6*, 476.

[21] Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.

[22] Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515.

[23] Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. *Drug Discov. Today* **2011**, *16*, 107.

[24] Durantel, D.; Branza-Nichita, N.; Carrouee-Durantel, S.; Butters, T. D.; Dwek, R. A.; Zitzmann, N. *J. Virol.* **2001**, *75*, 8987.

[25] Sawkar, A. R.; Cheng, W. C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 15428.

[26] Ye, X.-S.; Sun, F.; Liu, M.; Li, Q.; Wang, Y.; Zhang, G.; Zhang, L.-H.; Zhang, X.-L. *J. Med. Chem.* **2005**, *48*, 3688.

[27] Zhang, L.; Sun, F.; Wang, Q.; Zhou, J.; Zhang, L.-H.; Zhang, X.-L.; Ye, X.-S. *Chem. Med. Chem.* **2009**, *4*, 756.

[28] Zhang, L.; Sun, F.; Li, Y.; Sun, X.; Liu, X.; Huang, Y.; Zhang, L.-H.; Ye, X.-S.; Xiao, J. *Chem. Med. Chem.* **2007**, *2*, 1594.

[29] Vyavahare, V. P.; Chakraborty, C.; Maity, B.; Chattopadhyay, S.; Puranik, V. G.; Dhavale, D. D. *J. Med. Chem.* **2007**, *50*, 5519.

[30] Gorantla, J. N.; Lankalapalli, R. S. *J. Org. Chem.* **2014**, *79*, 5193.

[31] Chiu, H. Y.; Tzou, D. L.; Patkar, L. N.; Lin, C. C. *J. Org. Chem.* **2003**, *68*, 5788.

[32] Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nat. Immunol.* **2005**, *6*, 754.

[33] Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819.

[34] Zajonc, D. M.; Cantu III, C.; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810.

[35] Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44.

[36] Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077.

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