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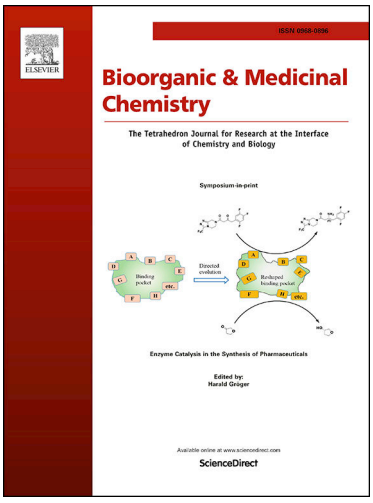

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Synthesis and Biological Activities of Amino Acids Functionalized α-GalCer Analogues

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Abstract

Invariant natural killer T-cells (iNKT-cells) are promising targets for manipulating the immune system, which can rapidly release a large amount of Th1 and Th2 cytokines upon the engagement of their T cell receptor with glycolipid antigens presented by CD1d. In this paper, we wish to report a novel series of α-GalCer analogues which were synthesized by incorporation of L-amino acid methyl esters in the C-6' position of glycolipid. The evaluation of these synthetic analogues for their capacities to stimulate iNKT-cells into producing Th1 and Th2 cytokines both *in vitro* and *in vivo* indicated that they were potent CD1d ligands and could stimulate murine spleen cells into a higher release of the Th1 cytokine IFN-γ *in vitro*. *In vivo*, Gly-α-GalCer (**1**) and Lys-α-GalCer (**3**) showed more Th1-biased responses than α-GalCer, especially analogue **3** showed the highest selectivity for IFN-γ production (IFN-γ/IL-4 = 5.32) compared with α-GalCer (IFN-γ/IL-4 = 2.5) *in vivo*. These novel α-GalCer analogues might be used as efficient X-ray crystallographic probes to reveal the relationship between glycolipids

and CD1d proteins in α-GalCer/CD1d complexes and pave the way for developing new potent immunostimulating agents.

Keywords

α-GalCer, iNKT cells; Th1 response; amino acids functionalized α-GalCers.

1. Introduction

Regulation of iNKT-cells by glycolipids is a promising approach to treat cancer and some autoimmune diseases.[1,2] When the iNKT-cells are activated by glycolipid antigens, they could rapidly secrete cytokines which could be classified as proinflammatory T-helper 1 (Th1) type (e.g., IFN-γ, TNF α, IF-2) and immunomodulatory T-helper 2 (Th2) type (e.g., IL-4, IL-10).[3-6] However, these two kinds of cytokines have opposing roles *in vivo*. The Th1 is involved in antitumor and anti-infection effects, whereas the Th2 is suited to control the development of autoimmune diseases. The negative cross-regulation of Th1 and Th2 cytokines and high levels of both cytokines release are considered as a major limitation of glycolipids for clinical applications.[7-13] Consequently, a major challenge for glycolipid-based therapies is the search for glycolipid antigens which can selectively control the release of Th1 or Th2 cytokines.

Since the first synthetic α-galactosylceramide (α-GalCer, KRN7000, Figure 1) was developed from systematic structure-activity relationship studies,[14] various derivatives of α-GalCer modifying on sugar part, ceramide moiety, galactose-ceramide linkage, or the incorporation of unsaturation in the acyl chain, have been synthesized to improve the cytokine production activity and selectivity.[15-23] For example, α-GalCer analogues with *C*-modified glycosidic linkages (α-C-GalCer) generated a higher Th1-biased response and had been applied to the murine model for melanoma metastasis.[24] Nevertheless, analogues containing truncated acyl and sphingosine chains (OCH),[25] multiple cis-double bonds in the acyl chain (C20:2)[26] or an amide group in the shorter fatty-acylchain[27] potently induced a Th2-biased cytokine response. Up to now, the

approximate immunological mechanism of α-GalCer is that by binding with the CD1d protein, the binary complex is recognized by the T cell receptor (TCR) on iNKT-cells to form the bioactive ternary complex. And then it stimulates iNKT cells to release Th1 and/or Th2 cytokines.[28] The stability of the CD1d/glycolipid/TCR complex is an important contributing factor to the proportion of Th1/Th2 cytokine production.[29-32] The crystal structure of CD1d/α-GalCer/TCR complex[33,34] shows that the 2, 3 and 4-OH of the galactose unit form hydrogen bonds with TCR or CD1d while the 6-OH shows no obvious interactions with TCR and CD1d, implying that chemical modifications at this position are feasible. Savage' group first reported that acetamide group[35] (PBS-57), different amide-linked fluorophores or biotin[36] appended at the C6' position of the sugar could also activate iNKT-cells and participate in CD1d and TCR interactions, which could be used in studying glycosphingolipid transportation, but had no obvious Th1/Th2-biased selectivity. Among the more recent reports, [37-44] the sugar modified by C6'-substituted methyl or COOH showed minor cytokine secretion *in vivo* in mice and resulted in no polarization.[37] α-GalCer analogues modified by aryl amides, carbamates and ureas at 6'-position α-GalCer analogues revealed a strongly biased Th1 response 3-12 times higher than α-GalCer[39]. However, acyl chain with length 12-13 (carbon atoms) at C6' of α-GalCer or the 6'-amido group linked to an ethylene glycol chain may have stronger ability to trigger Th2 immune response.[42] Moreover, literature search revealed that the presence of a triazole ring at position C6'[43,44] sufficed to cause superior antigenic activity than α-GalCer. C6'-triazole-substituted α-GalCer analogues could induce both IL-4 and IFN-γ secretion *in vivo*, and elicited a small Th2 cytokine-biased response. The 1,4-disubstituted triazole has a large dipole moment and is capable of participating in hydrogen bond formation, dipole-dipole and π-stacking interactions. And a triazole moiety can act as H-bonding acceptor and form extra hydrogen bond between compound and CD1d.[44] These examples demonstrate that chemical modifications at C6' position are tolerated, but the relationship between the structural changes of C6'-site substitution and cytokine release is not particularly clear.

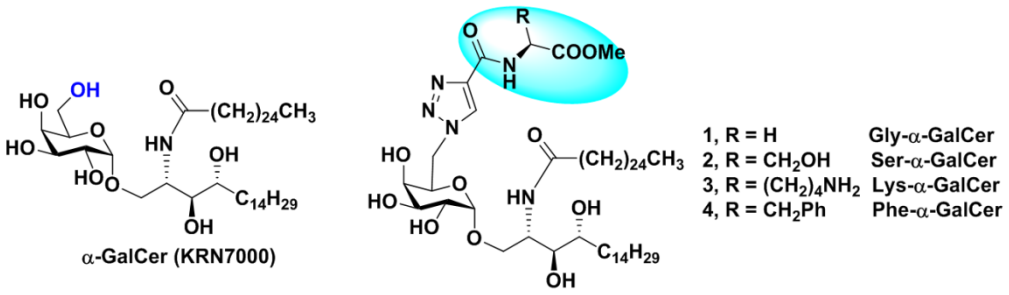


Figure 1. The structure of α-GalCer and new designed analogues of α-GalCer.

Amino acids play significant roles in living organisms. Furthermore, in modern medicinal chemistry, amino acids are indispensable components and become increasingly prominent in new drugs discovery.[45] For example, the oral bioavailability of 5'-amino acid ester prodrugs of Decitabine (DAC) was greater than that of DAC.[46] The phenylalanine-based organometallic complexes show anti-lung cancer and anti-angiogenic activities by using L-amino acid transport system.[47]

The well-known crystal structure of the CD1d/α-GalCer/TCR complex indicated that galactose part of α-GalCer was recognized by the T cell receptor (TCR) to form a stable complex and the 6-OH group of galactose in the crystal structure of CD1d/α-GalCer/TCR complex showed no obvious interactions with TCR.[48] Structural analyses of CD1d and TCR indicated that there were hydrophilic residues ASP-29, ASN-30, ASP-51, GLN-52, LYS-68 and LYS-71 in the binding site of TCR from the crystal structure of CD1d/α-GalCer/TCR complex. Therefore, the hydrophilic substitutes introduced at C6' of α-GalCer may form hydrogen bond interactions or salt-bridge interaction with these residues, The introduced aryl groups may form π-cation, π-anion interaction with these hydrophilic residues as well. Based on these analyses, several glycolipids with different amino acid methyl esters were designed by introducing hydrophilic groups at C6' of α-GalCer with triazole as a linker (structures refer to Fig. 1), expecting to develop new glycolipid antigens and explore the influence of amino acids on Th1/Th2 cytokines selectivity. Preliminary biological evaluation of these α-GalCer derivatives containing different C6'-substituted triazole-moieties were also carried out both *in vivo* and *in vitro*.

2. Results and Discussion

2.1. Computational modeling and activity predication

To predict the binding ability of newly designed α-GalCer analogues with CD1d and TCR, a docking study was performed with the Surflex-Dock program in SYBYL-X software (Tripos Inc., St. Louis, MO, USA). As shown in Table S1, the TotalScore of the newly designed α-GalCer analogues **1-4** was higher than α-GalCer, which means these newly designed glycolipids had stronger CD1d, TCR affinities than α-GalCer. Detailed structure analyses indicated that the binding interaction of newly designed glycolipids was very similar to α-GalCer (Fig. 2). For Gly-α-GalCer (**1**), there was hydrophilic interaction between MET-69 and the newly introduced groups. For Ser-α-GalCer (**2**), the newly introduced hydroxyl formed a hydrogen bond with GLN-52 of TCR and enhanced their TCR affinity. For Lys-α-GalCer (**3**), it was hoped that the ammonium would form hydrogen bonds with polar residues in TCR. Unexpectedly it formed a hydrogen bond with the carbonyl of LYS-65 of CD1d and the methyl formed π…H-C interaction with aryl residue HIS-68 of CD1d, which strengthened their CD1d binding interaction. Similar to Lys-α-GalCer, there was π…H-C interaction between the newly introduced group in Phe-α-GalCer (**4**) and HIS-68 and MET-69 of CD1d.

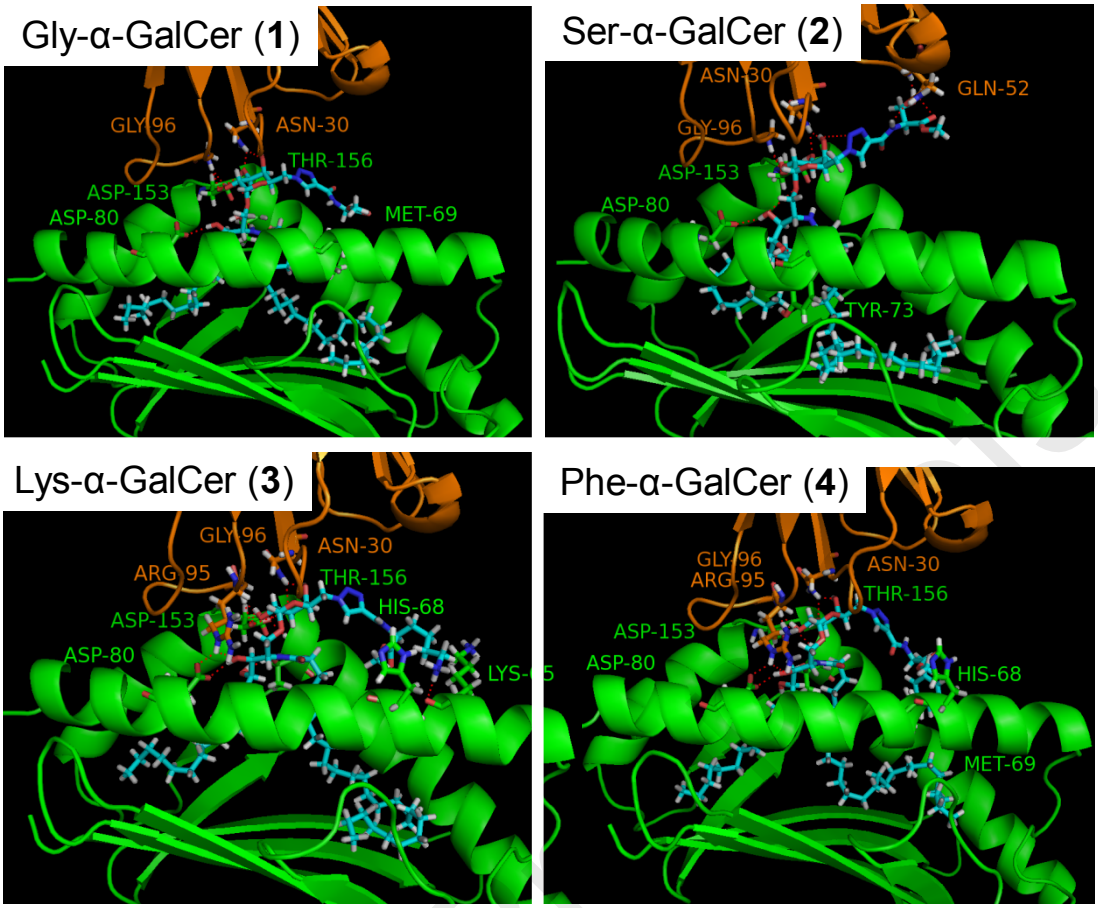
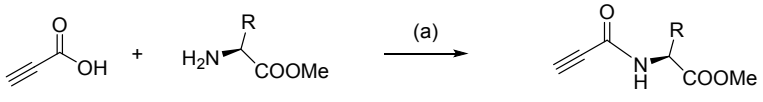


Figure 2. Structure analyses of ternary complexes of newly designed glycolipids (shown in cyan) within the mCD1d (shown in green) and TCR (shown in orange). Hydrogen bond interactions between ligand and residues around it were labeled in red dot line and the carbon skeleton of the ligands was shown in blue.

2.2. Chemistry

We synthesized the desired glycolipid analogues starting from the synthesis of propiolic acid amides **7** in Scheme 1. Propiolic acid **5** condensed with different L-amino-acid methyl esters **6** in the presence of DCC and DIPEA[49] to afford propiolic acid amides **7a-d** with good yields.



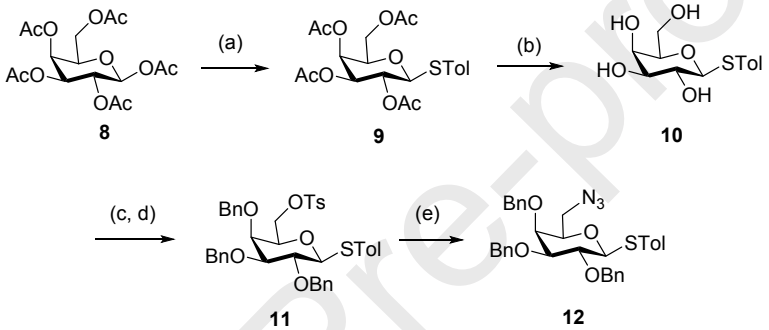
5

6a, R = H
6b, R = CH_2OH
6c, R = $(CH_2)_4NHCBz$
6d, R = CH_2Ph

7a, R = H
7b, R = CH_2OH
7c, R = $(CH_2)_4NHCBz$
7d, R = CH_2Ph

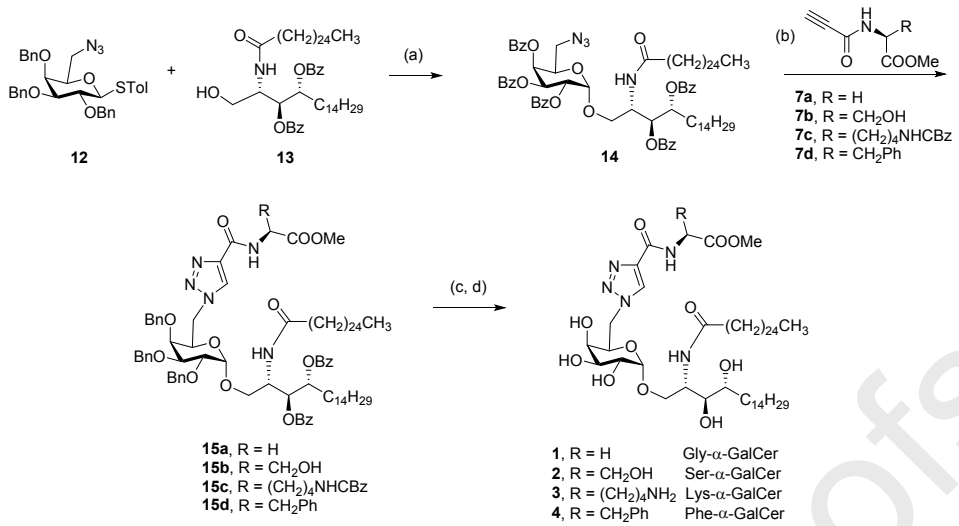
Scheme 1. Reagents and conditions: (a) DCC, DIPEA, CH_2Cl_2, 0 °C-r.t.; 82% for **7a**, 79% for **7b**, 75% for **7c**, 80% for **7d**. DCC, dicyclohexylcarbodiimide; DIPEA, N,N-diisopropylethylamine.

Synthesis of glycosyl donor **12** starting from the known 1,2,3,4,6-penta-*O*-acetyl-D-galactose **8** was shown in Scheme **2**. The galactose pentaacetate and *p*-thiocresol were catalyzed by boron trifluoride diethyl etherate to afford thio-β-D-galactopyranoside **9** in a good yield. Subsequent deacetylation under Zemplén conditions yielded **10** quantitatively. Treatment of thiogalactoside **10** with *p*-toluenesulfonyl chloride[50] and then benzyl bromide yielded compound **11** in two steps with a total yield of 69%. Then the compound **11** reacted with sodium azide in DMF [51] to afford the donor **12** in excellent yield.



Scheme 2. Reagents and conditions: (a) *p*-Thiocresol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C-r.t., 69%; (b) NaOMe, MeOH, r.t., quantitative; (c) TsCl, Py., r.t.; (d) NaH, BnBr, DMF, r.t., 69% for 2 steps; (e) NaN_3, DMF, 65 °C, 90%. TsCl, 4-toluene sulfonyl chloride; Py., pyridine; BnBr, benzyl bromide; DMF, N,N-dimethylformamide.

With glycosyl donor **12** and our sphingosine acceptor **13** [52] in hand, amino acids functionalized α-GalCer analogues could be prepared in Scheme 3. Firstly, the coupling of C6' azido galactose donor **12** with acceptor **13** was carried out successfully in the presence of NIS/TfOH to provide compound **14** with 65% yield with α-configuration as a major product. [53] Then, the reaction of azide **14** and propiolic acid amides **7** in the presence of $CuSO_4$ and sodium ascorbate provided the desired C6'-triazole-substituted α-GalCer analogues **15a-15d** in excellent yields. Final debenzylation by catalytic hydrogenolysis and debenzoylation with NaOMe in MeOH afforded the desired analogues **1-4** in approximate 88-93% yields.



Scheme 3. Reagents and conditions: (a) NIS, TfOH, CH_2Cl_2, 0°C-r.t., 65%; (b) **7**, $CuSO_4$, sodium ascorbate, THF:H_2O = 1:1, 65 °C, 88% for **15a**, 83% for **15b**, 85%; for **15c**, 86% for **15d**; (c) $Pd(OH)_2$, H_2, r.t., (d) NaOMe, MeOH, r.t., 91% for **1**, 93% for **2**, 92% for **3**, 91% for **4** (over 2 steps). NIS, N-iodosuccinimide; TfOH, trifluoromethanesulfonic acid.

2.3. Biological evaluation

2.3.1. Cytotoxicity and cell reproductive activity assay

The α-GalCer/CD1d binary complex formed by α-GalCer and CD1d protein interacts with T cell receptor (TCR) of iNKT cells to activate immune responses.[28] This activity produces a large number of Th1 or Th2 cytokines release. Before the cytokine production assays, the MTT assay was carried out to evaluate the cytotoxicity and cell reproductive activity of these amino acids functionalized α-GalCer analogues on murine splenocytes.[54] Cell viability was examined after splenocytes treated with these novel glycolipid analogues for 48 h at concentrations of 1, 0.1, and 0.01 μM, respectively. As the results illustrate in Figure 3, all of the four glycolipid analogues were found no cytotoxicity to the splenocytes at experimental concentration. The splenocyte cells proliferation increased in a dose-dependent manner, and at the concentration of 1 μM, these compounds all revealed acceptable cell proliferative activity.

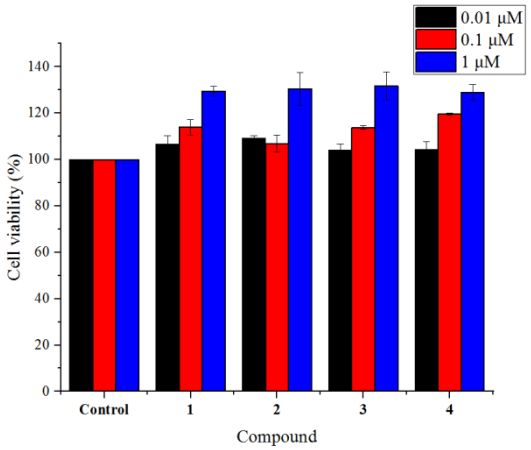


Figure 3. Effect of analogues **1-4** on splenocyte toxicity and proliferation. Splenocytes were treated with analogues **1-4** for 48 h, and proliferation of splenocytes was estimated with the MTT method.

2.3.2. Cytokine production assay

To determine the immunostimulatory activity of the new synthetic analogues **1-4**, the *in vitro* assays were first manipulated using the primary culture of murine spleen cells. [55] Three concentrations (0.01-1 μM) of analogues were incubated with freshly prepared mouse splenocytes and the level of cytokine productions was evaluated by ELISA kits after incubation for 48 hours. α-GalCer was used as a positive control in this assay. As shown in Figure 4, cytokine estimation revealed that all of the four analogues exhibited iNKT-cell stimulating activity by concentration-dependent increases and maximized at 1 μM, just like α-GalCer. [54] It showed the efficacy to release IFN-γ and IF-4 that was comparable to the standard α-GalCer. As can be seen from Figure 4A, Gly-α-GalCer (**1**) presented a similar ability to induce the release of IFN-γ comparing to α-GalCer. Compounds **2**, **3** and **4**, with serine, lysine and phenylalanine methyl ester substitution respectively, were found to produce more significant IFN-γ cytokine levels at all three concentrations. In comparison with the IFN-γ production, the levels of IL-4 cytokine secretion induced by **1**, **3** and **4** were inferior to those observed for α-GalCer (Figure 4B). However, Ser-α-GalCer (**2**) showed a similar tendency and ability to induce the release of IL-4 in mouse splenocytes. Lys-α-GalCer (**3**) induced only a half of the amount of IL-4 secretion induced by α-GalCer. Further analysis revealed that Lys-α-GalCer (**3**) exhibited a notable increase in IFN-γ and greatly decrease in IL-4 at all three

concentrations compared to that of α-GalCer. On the other hand, the significant compress of the Th2 cytokine secretion made these novel synthetic glycolipid analogues biased toward Th1 cytokine production. The relative rates of cytokine secretion levels at 0.01 μM concentration were shown in Figure 4C. The IFN-γ/IL-4 ratios of α-GalCer analogues **1-4** were 2.1, 1.7, 4.9 and 3.3, respectively. These results clearly demonstrated that all of the synthetic analogues had significant effects on forming the bioactive ternary complex with CD1d and TCR on iNKT cells, and induce signaling cascades for cytokine production *in vitro*.

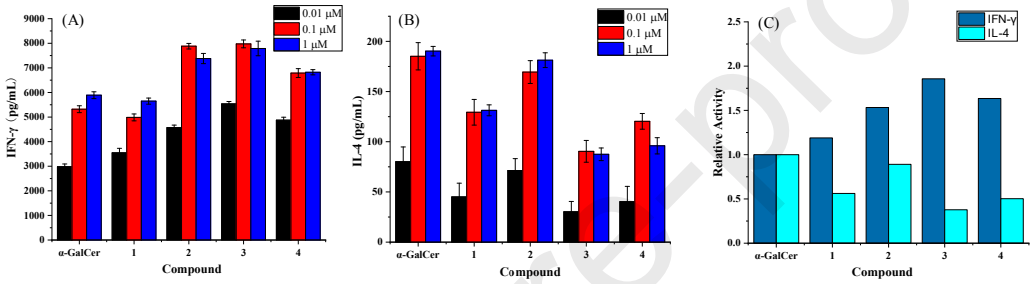


Figure 4. Cytokine release from C57BL/6 murine splenocytes stimulated with compound **1-4**. IFN-γ and IL-4 concentrations were measured after 48 h treatment. The secretion amount (pg/mL) of IFN-γ (A) and IL-4 (B) are summed up. (C) The relative proportion of cytokine production of α-GalCer and its analogues at 0.01 μM concentration is summed up.

In addition, to further examine the immunostimulatory activity and Th1/Th2 selectivity of the synthetic α-GalCer analogues **1-4** *in vivo*, the serum levels of IFN-γ and IL-4 were measured by ELISA kits at several time points in C57BL/6 mice following intraperitoneal injection. Similar to previous reports,[25,52] after α-GalCer was injected, the secretion amount of IL-4 peaked at 3 h and IFN-γ production had a delayed but elevated release in C57BL/6 mice from 6 to 24 h. As shown in Figure 5A, all the four glycolipids could induce Th1 and Th2 cytokine secretion *in vivo* effectively. Compound **1**, **2** and **4** induced kinetic release of cytokines in agreement with α-GalCer with a short period of IL-4 peaked at 3 h, followed by high IFN-γ production after a long period (6-24 h) of time. It is interesting to note that compound **3** stimulated the release of IL-4 peaked at 6 h. The injection of nonpolar amino acid substituted α-GalCer

analogues **1** and **4** produced levels of the two kinds of cytokine that were similar to those elicited by α-GalCer, which demonstrated that compound **1** and **4** had equally powerful abilities to stimulate iNKT cells. Serine substituted analogue Ser-α-GalCer (**2**) induced noticeably increased production of both IFN-γ and IL-4. Interestingly, Lys-α-GalCer (**3**) showed greatly reduced production of IL-4 while the production of IFN-γ still remained relatively high (3886 pg/mL), compared with α-GalCer (2925 pg/mL). In Figure 5B, it can be seen more clearly that compound **1** and compound **4** appeared to induce comparable IFN-γ levels as α-GalCer at 24 hours after injection. Compound **2** (4755 pg/mL) and compound **3** revealed more effective IFN-γ release concerning α-GalCer. The levels of Th2 cytokine IL-4 induced by compound **2** was apparently higher than α-GalCer. While compound **3** revealed IL-4 secretion at a low level during the mice testing *in vivo*. The ratio of IFN-γ/IL-4 at the maximum value was used to estimate Th1/Th2 selectivity of the synthetic α-GalCer analogues **1-4** (Figure 5C). It's worth noting that compound **3** contained a higher ratio of IFN-γ/IL-4 (5.6/1) compared to α-GalCer (2.5/1), while compound **2** contained a low ratio of IFN-γ/IL-4 (1.8/1). Meanwhile, the area under curve (AUC) ratio of IFN-γ/IL-4 cytokine release for α-GalCer is 13.2/1, which is consistent with the previous report. Lys-α-GalCer (**3**) showed high AUC ratio (23.6/1), suggesting compound **3** resulted in remarkable Th1-biased response (see Supporting Information Figure S33). Thus, the addition of an amino acid methyl esters moiety at the 6'-position of the galactose did indeed affect the cytokine secretions and alkaline amino acid lysine led to a marked functional Th1 polarization *in vivo*.

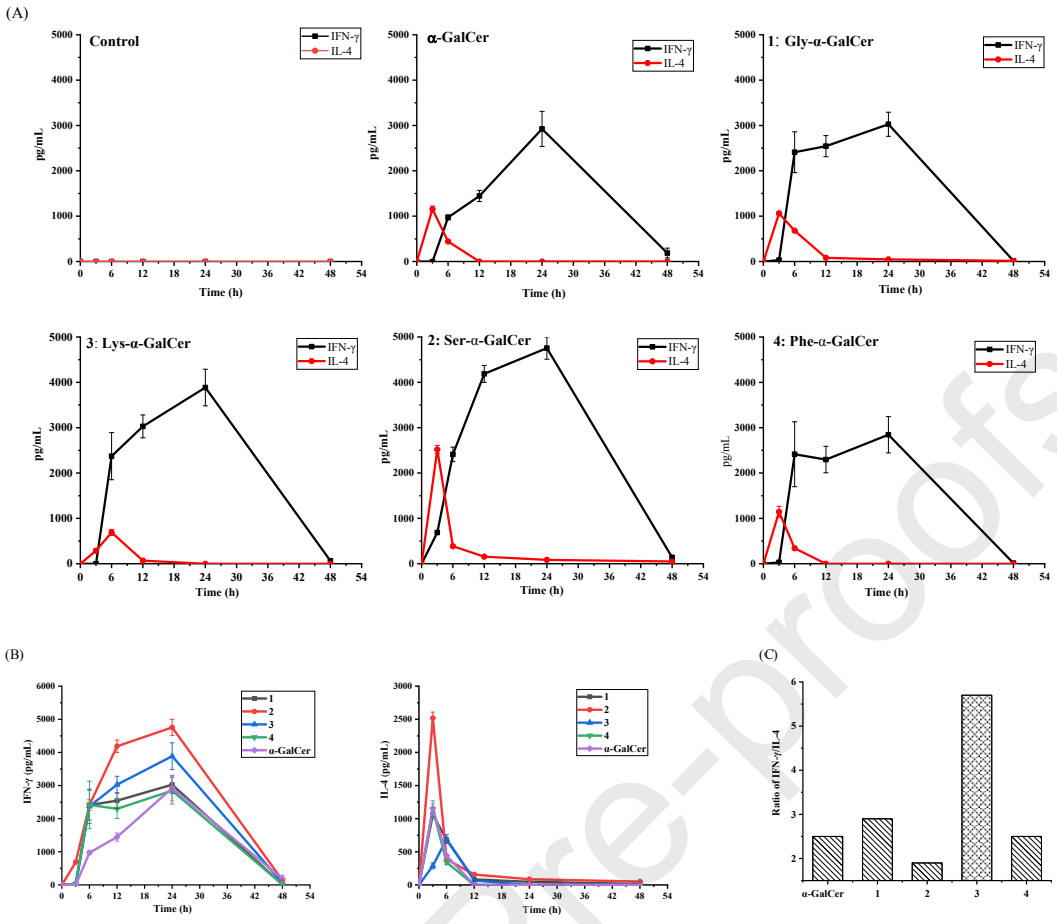


Figure 5. Biological assay of α-GalCer and its analogues *in vivo*. (A, B) IFN-γ and IL-4 secretion *in vivo* after intraperitoneal injection of α-GalCer or its analogues **1-4** (100 μg/kg) in C57BL/6 mice. Each group includes 3 mice and their serum samples were tested at the indicated time individually. (C) The peak production ratio of IFN-γ/IL-4 for α-GalCer and analogues **1-4**.

3. Conclusions

In summary, a series of L-amino-acid methyl ester-substituted C6'-triazolyl α-GalCer analogues have been designed by computational modeling and successfully synthesized using a copper-catalyzed azide-alkyne cycloaddition and glycosylation coupling reactions with acceptable total yields. Cytotoxicity and cell reproductive activity assay displayed that all of the four glycolipid analogues had no cytotoxicity to the splenocytes at experimental concentrations. What is surprising is that they have acceptable splenocyte cell proliferative activities. The immunostimulatory activities of α-GalCer and the new synthetic analogues **1-4** were investigated both *in vitro* and *in*

vivo. Initial *in vitro* experiments showed that all of the synthetic analogues exhibited effective iNKT-cell stimulation and Lys-α-GalCer (**3**) revealed a notably higher IFN-γ/IL-4 ratio (4.9) at all three concentrations compared to that of α-GalCer. *In vivo*, compound **1** and **4** exhibited stimulating abilities similar to α-GalCer and compound **2** was more potent than α-GalCer in producing both IFN-γ and IL-4. Compound **3** demonstrated excellent enlargement of IFN-γ secretion and reduction of IL-4 secretion, proving that compound **3** is a fantastic immunostimulant for Th1-biased response, consistent with previous *in vitro* experiments. These biological results suggest that modification at the C6' position with different L-amino-acid methyl esters can have a significant effect on cytokine bias, which may pave the way for developing new potent immunostimulating agents. Future work will involve producing more amino acid or short-chain polypeptides modified glycolipids at C-6' for understanding of the cytokine profile release bias and the relationship between glycolipids and CD1d proteins.

4. Experimental Section

4.1. Chemistry and synthesis

All chemicals were purchased from commercial sources and used without further treatment unless otherwise stated. All moisture sensitive reactions were carried out in a flame dried flask under a nitrogen atmosphere. Anhydrous solvents were purified according to standard procedures before use. Flash chromatography was carried out on SiO_2 (silica gel 200-300 mesh). All reactions were monitored by TLC with GF254 silica gel coated plates and/or by charring following immersion in a 10% ethanolic solution of sulfuric acid. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance/600 (^{1}H: 600 MHz, ^{13}C: 150 MHz at 25 ºC) or Bruker Avance/400 (1H: 400 MHz, ^{13}C: 100 MHz at 25 ºC) and TMS as an internal standard. Chemical shifts of ^{1}H NMR spectra were given in ppm relative to a TMS internal standard to the residual solvent peak (abbreviation in spectra: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad). All high-resolution mass spectra (HRMS) were measured on a mass spectrometer by

using electrospray ionization (ESI-oa-TOF), and the purity of all samples used for HRMS (>95%) was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopic analysis.

4.1.1. General procedure for the synthesis of compounds 7a-7d

To a solution of propiolic acid **5** (100.0 mg, 1.4 mmol) in dry CH_2Cl_2 (5 mL) was added molecular sieve (4 Å) under an inert atmosphere at 0 °C. The reaction mixture was stirred 30 minutes at 0 °C. In another flask, the corresponding methyl ester hydrochloride **6a-6d** (1.6 mmol) and DCC (309.2 mg, 1.5 mmol) were dissolved in CH_2Cl_2 (2 mL). Then DIPEA (193.9 mg, 1.5 mmol) was added. This solution was added slowly to the propiolic acid-containing mixture. The reaction was stirred overnight while the ice bath was allowed to warm to room temperature. After the completion of the reaction, the mixture was filtered, washed with CH_2Cl_2 and the solvent was evaporated. The residue was purified by a short flash silica gel column chromatography (petroleum ether /EtOAc = 3/2) to give **7a-7d**.

Methyl propioloylglycinate **7a**. This product was obtained as a slight yellow liquid in 82% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.60 (s, 1H), 4.08 (d, J = 4.8 Hz, 2H), 3.77 (s, 3H), 2.86 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.44, 152.07, 76.67, 74.31, 52.63, 41.34. HRMS (ESI), m/z calcd. for $C_6H_8NO_3$ ($[M+H]^+$) 142.0499, found: 142.0497.

Methyl propioloyl-*L*-serinate **7b**. This product was obtained as a slight yellow liquid in 79% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.99 (d, J = 6.0 Hz, 1H), 4.72-4.67 (m, 1H), 4.03 (ddd, J = 11.3, 6.0, 3.5 Hz, 1H), 3.94 (ddd, J = 11.3, 5.8, 3.4 Hz, 1H), 3.80 (s, 3H), 2.91 (s, 1H), 2.69 (t, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.08, 152.11, 76.60, 74.68, 62.64, 54.71, 53.01. HRMS (ESI), m/z calcd. for $C_7H_{10}NO_4$ ($[M+H]^+$) 172.0604, found: 172.0608.

Methyl N^6-((benzyloxy)carbonyl)-N^2-propioloyl-*L*-lysinate **7c**. This product was

obtained as a slight yellow liquid in 75% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.28 (m, 5H), 6.60 (d, J = 7.2 Hz, 1H), 5.10 (s, 2H), 4.79 (s, 1H), 4.61 (dd, J = 12.6, 7.6 Hz, 1H), 3.76 (s, 3H), 3.21-3.17 (m, 2H), 2.82 (s, 1H), 1.98-1.83 (m, 1H), 1.83-1.68 (m, 1H), 1.52 (m, 2H), 1.46-1.34 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.85, 156.55, 151.87, 136.31, 128.28, 127.86, 127.81, 74.20, 66.35, 52.38, 52.16, 40.03, 30.97, 29.10, 22.02. HRMS (ESI), m/z calcd. for $C_{18}H_{22}N_2NaO_5$ ($[M+Na]^+$) 369.1421, found: 369.1426.

Methyl propioloyl-*L*-phenylalaninate **7d**. This product was obtained as a slight yellow liquid in 80% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (t, J = 7.2 Hz, 2H), 7.25 (d, J = 7.2 Hz, 1H), 7.11 (d, J = 7.2 Hz, 2H), 6.68 (d, J = 7.8 Hz, 1H), 4.90-4.86 (m, 1H), 3.71 (s, 1H), 3.16 (dd, J = 14.1, 5.7 Hz, 1H), 3.09 (dd, J = 14.0, 6.0 Hz, 1H), 2.83 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.96, 151.36, 135.22, 129.18, 128.62, 127.25, 74.02, 53.41, 52.48, 37.50. HRMS (ESI), m/z calcd. for $C_{13}H_{14}NO_3$ ($[M+H]^+$) 232.0968, found: 232.0971.

4.1.2. *p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-α-D-glactopyranoside 9. [50]

To a solution of 1,2,3,4,6-penta-O-acetyl-D-galactose **8** (10.46 g, 26.8 mmol) in dry CH_2Cl_2 (20 mL) at 0 ºC were added *p*-thiocresol (3.99 g, 32.2 mmol) and $BF_3{\cdot}Et_2O$ (6.8 mL, 53.6 mmol). The reaction mixture was allowed to warm to r.t. and stirred overnight. After the completion of the reaction, the reaction mixture was quenched with saturated aqueous sodium bicarbonate sequentially. The organic layer was washed sequentially with water and brine, then was dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether /EtOAc = 4/1) to afford product **9** (8.43 g, 69%) as a white powder. Mp 98-103 ºC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, J = 7.6 Hz, 2H), 7.12 (d, J = 7.6 Hz, 2H), 5.40 (s, 1H), 5.21 (t, J = 9.6 Hz, 1H), 5.03 (d, J = 9.6 Hz, 1H), 4.64 (d, J = 9.6 Hz, 1H), 4.24-4.15 (m, 1H), 4.14-4.05 (m, 1H), 3.91 (t, J = 6.2 Hz, 1H), 2.34 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). HRMS (ESI), m/z calcd. for $C_{21}H_{27}O_9S$ ($[M+H]^+$) 455.1370, found: 455.1372.

4.1.3. *p*-Tolyl α-D-glactopyranoside 10. [50]

To a solution of compound **9** (7.99 g, 17.6 mmol) in MeOH (80 mL) was added 1M solution of sodium methoxide in MeOH until pH 9-10 was reached. The reaction mixture was stirred at r.t. (the whole process was closely monitored by TLC). After the completion of the reaction, amberlite IR-120 (H^+) was added to neutralize the mixture. The exchange resin was then filtered off and the solvent was removed in vacuo gave the crude residue **10** (5.04 g, quant) as a white solid. Mp 140-143 ºC. ^{1}H NMR (600 MHz, MeOD) δ 7.47 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 4.52 (d, J = 9.6 Hz, 1H), 3.90 (d, J = 3.2 Hz, 1H), 3.78-3.70 (m, 2H), 3.59 (t, J = 9.4 Hz, 1H), 3.55 (t, J = 6.0 Hz, 1H), 3.50 (dd, J = 9.2, 3.3 Hz, 1H), 2.32 (s, 3H). HRMS (ESI), m/z calcd. for $C_{13}H_{19}O_5S$ ($[M+H]^+$) 287.0948, found: 287.0952.

4.1.4. *p*-Tolyl 2,3,4-tri-*O*-benzyl-6-*O*-tosyl-α-D-glactopyranoside 11.

To a solution of compound **10** (2.86 g, 10 mmol) in anhydrous pyridine (10 mL) was added TsCl (3.81 g, 20 mmol) at 0 °C.[50] The mixture was allowed to reach room temperature and was stirred overnight. After the completion of the reaction, the solvent was removed in vacuo to give a crude residue as an oil. To a solution of the crude product from the previous step in DMF (10 mL) was added sodium hydride (2.40 g, 60%, 60 mmol) at 0 ºC. After 5 min, BnBr (6.0 mL, 50 mmol) was added. The reaction mixture was allowed to warm to r.t. and stirred for 6 h, then the mixture was quenched by addition of MeOH (40 mL). The reaction mixture was then concentrated in vacuo (co-evaporation with toluene) and the residue was dissolved in CH_2Cl_2. The resulting solution was washed with brine, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether /EtOAc = 10/1) to afford product **11** (5.12 g, 69%) as a white solid. Mp 105-109 ºC. ^{1}H

NMR (600 MHz, $CDCl_3$) δ 7.74 (d, J = 8.4 Hz, 2H), 7.37 (t, J = 7.4 Hz, 4H), 7.34-7.27 (m, 13H), 7.20 (dd, J = 7.0, 2.2 Hz, 2H), 6.98 (d, J = 8.0 Hz, 2H), 4.94 (d, J = 11.2 Hz, 1H), 4.78-4.68 (m, 4H), 4.50 (dd, J = 21.0, 10.4 Hz, 2H), 4.12 (dd, J = 9.8, 6.1 Hz, 1H), 4.06 (dd, J = 9.8, 6.7 Hz, 1H), 3.90 (d, J = 2.4 Hz, 1H), 3.83 (t, J = 9.4 Hz, 1H), 3.63 (t, J = 6.4 Hz, 1H), 3.57 (dd, J = 9.2, 2.6 Hz, 1H), 2.40 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.15, 138.30, 138.07, 137.51, 132.47, 132.29, 130.20, 129.82, 129.67, 128.57, 128.43, 128.35, 128.91, 128.08, 127.89, 127.86, 127.66, 88.04, 83.84, 77.01, 75.68, 75.47, 74.42, 73.04, 68.00, 21.72, 21.20. HRMS (ESI), m/z calcd. for $C_{41}H_{42}NaO_7S_2$ ($[M+Na]^+$) 733.2264, found: 733.2265.

4.1.5. *p*-Tolyl 2,3,4-tri-*O*-benzyl-6-azide-α-D-glactopyranoside 12.

To a solution of compound **11** (2.99 g, 4.22 mmol) in dry DMF (5 mL) was added sodium azide (1.37 g, 5.5 mmol) at 65 ^{o}C overnight. [51] Then the solvent was removed in vacuo and the residue was dissolved in EtOAc. The resulting solution was washed with brine, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether /EtOAc = 10/1) to afford product **12** (2.23 g, 90%) as a white solid. Mp 113-118 ºC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52-7.00 (m, 19H), 5.03 (d, J = 11.4 Hz, 1H), 4.86 (d, J = 10.2 Hz, 1H), 4.82-4.72 (m, 3H), 4.62-4.57 (m, 2H), 3.89 (t, J = 9.4 Hz, 1H), 3.80 (s, 1H), 3.61 (dd, J = 12.8, 7.4 Hz, 1H), 3.45 (t, J = 6.2 Hz, 1H), 3.17 (dd, J = 12.3, 5.7 Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.41, 138.22, 137.73, 132.78, 130.01, 129.75, 128.64, 128.50, 128.47, 128.45, 128.22, 127.96, 127.92, 127.89, 127.76, 88.61, 84.28, 77.43, 75.83, 74.47, 73.77, 73.28, 51.41, 21.28. HRMS (ESI), m/z calcd. for $C_{34}H_{35}N_3NaO_4S$ ($[M+Na]^+$) 604.2240, found: 604.2240.

4.1.6. (2S,3S,4R)-2-(*N*-hexacosanoylamino)-3,4-di-*O*-benzoyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-azide-α-D-galactopyranosyl)-1,3,4-octadecanetriol 14.

To a solution of donor **12** (145.3 mg, 0.25 mmol) and acceptor **13** (180.9 mg, 0.2 mmol) in dry CH_2Cl_2 (4 mL) was added freshly dried 4Å molecular sieves (20 mg) under nitrogen, then the mixture was stirred at 0 °C for 1 h. After addition of NIS (54 mg, 0.24 mmol) and TfOH (10.2 mL, 0.02 mmol) at 0 °C, the reaction mixture was continuously stirred at 0 °C until the donor was completely consumed on TLC analysis. The mixture was filtered, washed with CH_2Cl_2, and the combined filtrate and washings were washed with saturated Na_2CO_3 solution, saturated $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether /EtOAc = 10/1) to afford product **14** (176.9 mg, 65%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, J = 7.8 Hz, 2H), 7.94 (d, J = 7.8 Hz, 2H), 7.59 (t, J = 7.2 Hz, 1H), 7.53 (t, J = 7.2 Hz, 1H), 7.45 (t, J = 7.2 Hz, 2H), 7.41-7.23 (m, 13H), 7.20-7.15 (m, 3H), 6.98 (d, J = 9.0 Hz, 1H), 5.68 (d, J = 8.8 Hz, 1H), 5.36 (s, 1H), 4.95 (d, J = 11.4 Hz, 1H), 4.78 (d, J = 11.4 Hz, 2H), 4.72-4.52 (m, 5H), 3.98 (dd, J = 10.2, 3.0 Hz, 1H), 3.87-3.76 (m, 4H), 3.67 (d, J = 10.2 Hz, 1H), 3.51 (dd, J = 12.0, 8.4 Hz, 1H), 3.01 (dd, J = 12.3, 4.5 Hz, 1H), 2.20 (t, J = 7.2 Hz, 2H), 2.09-2.06 (m, 2H), 1.91-1.88 (m, 2H), 1.33-1.16 (m, 68H), 0.88 (t, J = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.06, 166.29, 165.37, 138.53, 138.00, 133.30, 132.92, 130.07, 129.79, 129.74, 128.53, 128.45, 128.38, 128.36, 128.30, 128.11, 127.91, 127.71, 127.61, 99.19, 78.36, 76.45, 74.90, 74.60, 73.92, 73.48, 73.28, 72.13, 70.21, 68.70, 51.29, 48.47, 36.76, 31.91, 29.72, 29.68, 29.64, 29.60, 29.56, 29.53, 29.46, 29.37, 29.34, 28.06, 25.77, 25.65, 22.67, 14.10. HRMS (ESI), m/z calcd. for $C_{85}H_{125}N_4O_{10}$ ($[M+H]^+$) 1361.9390, found:1361.9383.

4.1.7. General procedure for the synthesis of compounds 15a-15d

To a mixture of compound **14** (204.1 mg, 0.15 mmol) and the corresponding compounds **7a-7d** (0.20 mmol) in H_2O-THF (1:1, 5 mL) were added sodium ascorbate (30 mg, 0.15 mmol) and $CuSO_4 \cdot 5H_2O$ (19 mg, 0.075 mmol). The reaction mixture was stirred vigorously in dark at 65 °C for 4 h. After removal of THF under reduced pressure, water (20 mL) was added, and the product was extracted with EtOAc. The combined

organic layers were dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified by a flash silica gel column chromatography (petroleum ether /EtOAc = 1:4) to give **15a-15d** as a slight yellow oil.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-3,4-di-*O*-benzoyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-(4-((*N*-methyl glycinate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4- octadecanetriol 15a.

This product was obtained as a slight yellow liquid in 88% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (s, 1H), 8.03 (d, J = 7.2 Hz, 2H), 7.91 (d, J = 7.2 Hz, 2H), 7.65-7.55 (m, 2H), 7.51 (t, J = 7.2 Hz, 1H), 7.46 (t, J = 7.2 Hz, 2H), 7.37-7.34 (m, 6H), 7.32-7.29 (m, 4H), 7.25 (d, J = 6.6 Hz, 2H), 7.19-7.16 (m, 3H), 6.67 (d, J = 9.0 Hz, 1H), 5.62 (d, J = 9.0 Hz, 1H), 5.30 (s, 1H), 4.96 (d, J = 11.0 Hz, 1H), 4.75 (d, J = 12.0 Hz, 2H), 4.65 (t, J = 11.4 Hz, 2H), 4.59-4.53 (m, 3H), 4.35 (d, J = 5.9 Hz, 2H), 4.20 (t, J = 4.8 Hz, 2H), 4.15 (t, J = 12 Hz, 1H), 3.98 (d, J = 9.6 Hz, 1H), 3.81(d, J = 10.0 Hz, 1H), 3.77 (s, 3H), 3.68 (s, 1H), 3.56 (d, J = 10.8 Hz, 1H), 3.34 (d, J = 10.8 Hz, 1H), 2.20 (t, J = 7.2 Hz, 2H), 1.88-1.85 (m, 2H), 1.65-1.62 (m, 2H), 1.25-1.19 (m, 68H), 0.87 (t, J = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.03, 169.85, 166.53, 165.50, 160.19, 142.53, 138.43, 138.01, 137.90, 133.42, 133.06, 129.91, 129.75, 129.69, 128.67, 128.54, 128.45, 128.37, 128.34, 128.20, 128.12, 127.77, 127.71, 127.66, 126.86, 121.59, 99.13, 78.32, 77.29, 77.08, 76.87, 76.16, 74.70, 74.45, 74.02, 73.64, 73.36, 72.41, 69.83, 68.61, 52.38, 50.77, 48.42, 40.89, 36.74, 31.95, 29.77, 29.73, 29.68, 29.65, 29.61, 29.58, 29.52, 29.39, 28.18, 25.74, 25.64, 22.72, 14.15. HRMS (ESI), m/z calcd. for $C_{91}H_{132}N_5O_{13}$ ($[M+H]^+$) 1502.9816, found: 1502.9832.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-3,4-di-*O*-benzoyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-(4-((*N*-methyl *L*-serinate)carbonyl)-1*H*-1,2,3-triazolyl)-1,3,4-octadecanetriol 15b.

This product was obtained as a slight yellow liquid in 83% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, J = 7.8 Hz, 2H), 8.02 (s, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.93 (d, J =

7.8 Hz, 2H), 7.58 (t, *J* = 7.2 Hz, 1H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 2H), 7.40-7.36 (m, 6H), 7.35-7.30 (m, 5H), 7.28-7.25 (m, 1H), 7.18-7.14 (m, 3H), 7.10 (t, *J* = 7.2 Hz, 2H), 6.70 (d, *J* = 9.0 Hz, 1H), 5.60 (d, *J* = 9.6 Hz, 1H), 5.22 (d, *J* = 9.6 Hz, 1H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.91-4.82 (m, 1H), 4.77 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.61 (d, *J* = 11.6 Hz, 2H), 4.57 (d, *J* = 12.0 Hz, 2H), 4.46 (d, *J* = 11.8 Hz, 1H), 4.40 (t, *J* = 9.2 Hz, 1H), 4.29 (dd, *J* = 13.8, 9.2 Hz, 1H), 4.20 (d, *J* = 13.6 Hz, 1H), 4.06 (s, 2H), 3.92 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.82 (s, 3H),3.79-3.77 (m, 1H), 3.40 (d, *J* = 10.2 Hz, 1H), 2.98 (d, *J* = 9.6 Hz, 1H), 2.31-2.28 (m, 1H), 2.16-2.13 (m, 1H), 1.81-1.76 (m, 2H), 1.63 (s, 2H), 1.39-1.07 (m, 68H), 0.88 (t, *J* = 5.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.44, 170.53, 166.65, 165.22, 159.62, 142.55, 138.39, 137.87, 137.65, 133.34, 133.06, 129.83, 129.71, 129.58, 128.67, 128.57, 128.55, 128.42, 128.32, 128.19, 128.03, 127.65, 126.48, 98.11, 78.18, 77.21, 77.00, 76.79, 75.58, 74.46, 74.39, 74.04, 73.62, 72.92, 71.78, 70.52, 67.80, 62.91, 54.66, 52.59, 51.74, 47.75, 36.62, 31.88, 29.70, 29.65, 29.61, 29.58, 29.52, 29.48, 29.41, 29.32, 29.27, 28.03, 25.60, 25.55, 22.64, 14.07. HRMS (ESI), m/z calcd. for $C_{92}H_{133}N_5NaO_{14}$ ($[M+Na]^+$) 1554.9741, found:1554.9778.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-3,4-di-*O*-benzoyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-(4-((*N*-methyl N^6-((benzyloxy)carbonyl)-*L*-lysinate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4-octadecanetriol 15c.

This product was obtained as a slight yellow liquid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 7.6 Hz, 2H), 7.99 (s, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.66-7.42 (m, 5H), 7.37-7.28 (m, 18H), 7.26-7.24 (m, 2H), 7.22-7.13 (m, 3H), 6.91 (d, *J* = 9.6 Hz, 1H), 5.64 (d, *J* = 9.2 Hz, 1H), 5.38-5.17 (m, 1H), 5.08-5.05 (m, 3H), 4.97 (d, *J* = 11.2 Hz, 1H), 4.84-4.43 (m, 8H), 4.42-4.24 (m, 2H), 4.13 (t, *J* = 6.2 Hz, 1H), 3.98 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.83 (d, *J* = 9.6 Hz, 1H), 3.76 (br s, 3H), 3.69 (s, 1H), 3.52 (d, *J* = 9.2 Hz, 1H), 3.31 (dd, *J* = 11.0, 2.6 Hz, 1H), 3.18 (dd, *J* = 12.7, 6.4 Hz, 2H), 2.20-2.15 (m, 2H), 2.11-2.05 (m, 2H), 1.62-1.60 (m, 2H), 1.55-1.53 (m, 2H), 1.43-1.40 (m, 2H), 1.41-1.33 (m, 2H), 1.33-1.13 (m, 68H), 0.87 (t, *J* = 6.8 Hz, 6H). ^{13}C NMR (100 MHz,

$CDCl_3$) δ 172.96, 172.19, 166.46, 165.40, 159.70, 156.40, 142.54, 138.35, 137.94, 137.81, 136.69, 133.34, 132.97, 129.87, 129.82, 129.66, 128.58, 128.45, 128.40, 128.36, 128.29, 128.25, 128.08, 128.04, 127.95, 127.92, 127.68, 127.61, 127.55, 126.75, 99.16, 78.21, 77.20, 76.03, 74.64, 74.37, 73.91, 73.55, 73.29, 72.27, 69.78, 68.67, 66.43, 52.32, 51.69, 50.61, 48.38, 40.62, 36.65, 32.07, 31.86, 29.70, 29.68, 29.64, 29.60, 29.57, 29.52, 29.51, 29.43, 29.30, 28.11, 25.65, 25.59, 22.63, 22.57, 14.06. HRMS (ESI), m/z calcd. for $C_{103}H_{147}N_6O_{15}$ ($[M+H]^+$) 1708.0919, found: 1708.0933.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-3,4-di-*O*-benzoyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-(4-((*N*-methyl *L*-phenylalaninate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl) -1,3,4-octadecanetriol 15d.

This product was obtained as a slight yellow liquid in 86% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, 2H), 7.99 (s, 1H), 7.89 (d, J = 7.6 Hz, 2H), 7.59 (t, J = 7.2 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.48 (m, 3H), 7.34-7.32 (m, 8H), 7.31-7.27 (m, 8H), 7.23-7.14 (m, 6H), 7.03 (d, J = 9.4 Hz, 1H), 5.69 (d, J = 9.6 Hz, 1H), 5.30 (s, 1H), 5.02 (dd, J = 13.4, 6.6 Hz, 1H), 4.97 (d, J = 11.4 Hz, 1H), 4.77 (d, J = 10.2 Hz, 2H), 4.71 (d, J = 12.0 Hz, 1H), 4.66 (d, J = 11.6 Hz, 1H), 4.61 (d, J = 11.6 Hz, 1H), 4.53 (d, J = 11.0 Hz, 2H), 4.35-4.30 (m, 2H), 4.16 (t, J = 6.0 Hz, 1H), 4.00 (dd, J = 9.8, 2.4 Hz, 1H), 3.84 (d, J = 10.0 Hz, 1H), 3.69 (d, J = 12.0 Hz, 4H), 3.54 (d, J = 9.6 Hz, 1H), 3.36 (d, J = 9.6 Hz, 1H), 3.21 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.4 Hz, 2H), 1.91-1.89 (m, 2H), 1.71-1.59 (m, 2H), 1.25-1.19 (m, 68H), 0.88 (t, J = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.92, 171.43, 166.51, 165.49, 159.50, 142.44, 138.30, 137.76, 135.82, 133.36, 132.99, 129.83, 129.81, 129.67, 129.55, 129.18, 128.60, 128.58, 128.54, 128.45, 128.36, 128.26, 128.17, 128.04, 127.71, 127.65, 127.63, 127.08, 126.71, 98.88, 78.10, 76.15, 74.63, 74.44, 74.05, 73.56, 73.32, 72.00, 69.61, 68.01, 53.11, 52.20, 50.61, 48.28, 38.26, 36.66, 31.86, 29.69, 29.64, 29.59, 29.57, 29.51, 29.44, 29.34, 29.30, 27.86, 25.71, 25.60, 22.63, 14.06. HRMS (ESI), m/z calcd. for $C_{98}H_{138}N_5O_{13}$ ($[M+H]^+$) 1593.0286, found: 1594.0273.

4.1.8. General procedure for the synthesis of compounds 1-4

To a solution of the corresponding compounds **15a-15d** (0.13 mmol) in EtOAc-MeOH (1:1, 5 mL) was added 10% palladium(II) hydroxide (100 mg) under hydrogen atmosphere at ambient temperature for 8 h. After filtration, the solvent was evaporated to give the crude residue as a colorless oil. To a solution of the crude residue from the previous step in anhydrous methanol (2 mL) was added a 1M solution of sodium methoxide in MeOH until pH 9-10 was reached. The reaction mixture is stirred at r.t. for 4 h. After completion, the reaction was neutralized with amberlite IR-120 (H^+), dissolved with CH_2Cl_2, and filtered. The filtrate was concentrated under reduced pressure to afford **1-4**.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-1-*O*-(6-(4-((*N*-methyl glycinate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4-octadecanetriol 1.

This product was obtained as a white foam solid in 91% yield. Mp 112-115 °C. ^{1}H NMR (600 MHz, MeOD/$CDCl_3$ = 5/1) δ 8.34 (s, 1H), 4.83 (br s, 2H), 4.64 (d, J = 6.0 Hz, 2H), 4.23 (t, J = 6.0 Hz, 1H), 4.15 (d, J = 6.0 Hz, 1H), 4.11-4.06 (m, 1H), 3.86 (s, 1H), 3.80 (d, J = 9.2 Hz, 1H), 3.76 (br s, 4H), 3.58-3.44 (m, 2H), 3.40 (s, 2H), 2.30-2.11 (m, 2H), 1.67-1.46 (m, 4H), 1.30-1.25 (m, 68H), 0.87 (t, J = 6.4 Hz, 6H). ^{13}C NMR (150 MHz, MeOD/$CDCl_3$ = 5/1) δ 175.41, 171.32, 162.51, 143.02, 128.13, 100.48, 75.08, 72.72, 70.78, 70.53, 70.40, 69.54, 67.89, 52.71, 52.14, 51.00, 41.52, 37.13, 32.76, 32.74, 30.59, 30.53, 30.51, 30.44, 30.39, 30.28, 30.18, 30.15, 26.76, 26.69, 23.43, 14.38. HRMS (ESI), m/z calcd. for $C_{56}H_{105}N_5NaO_{11}$ ($[M+Na]^+$) 1046.7703, found:1046.7712.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-1-O-(6-(4-((*N*-methyl *L*-serinate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4-octadecanetriol 2.

This product was obtained as a white foam solid in 93% yield. Mp 121-125 °C. ^{1}H NMR (600 MHz, MeOD/$CDCl_3$ = 5/1) δ 8.35 (d, J = 12.0 Hz, 1H), 4.84-4.21 (m, 3H),

4.65 (s, 2H), 4.36-4.12 (m, 1H), 4.14-4.01 (m, 2H), 4.01-3.91 (m, 1H), 3.88 (d, *J* = 12.6 Hz, 1H), 3.81-3.75 (m, 4H), 3.59-3.36 (m, 4H), 2.32-2.09 (m, 2H), 1.73-1.47 (m, 4H), 1.28-1.15 (m, 68H), 0.87 (s, 6H). ^{13}C NMR (150 MHz, MeOD/$CDCl_3$ = 5/1) δ 175.43, 171.76, 161.86, 142.97, 128.17, 100.41, 75.10, 72.74, 70.79, 70.63, 70.57, 70.43, 69.54, 67.94, 62.71, 55.66, 53.01, 52.31, 51.04, 49.43, 37.12, 32.76, 32.74, 30.60, 30.53, 30.52, 30.46, 30.44, 30.30, 30.18, 30.14, 26.78, 26.71, 23.44, 23.43. HRMS (ESI), m/z calcd. for $C_{57}H_{107}N_5NaO_{12}$ ([M+Na]$^+$)1076.7808, found: 1076.7828.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-1-*O*-(6-(4-((*N*-methyl *L*-lysinate)carbonyl)-1*H*-1,2,3-triazolyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4-octadecanetriol 3.

This product was obtained as a white foam solid in 92% yield. Mp 128-130 ^{o}C. ^{1}H NMR (400 MHz, MeOD) δ 3.83 (s, 1H), 4.83 (d, *J* = 2.6 Hz, 1H), 4.70-4.64 (m, 3H), 4.24 (t, *J* = 6.1 Hz, 1H), 4.06 (d, *J* = 4.8 Hz, 1H), 3.90 (s, 1H), 3.80-3.74 (m, 5H), 3.58-3.46 (m, 3H), 3.40 (dd, *J* = 10.1, 3.2 Hz, 1H), 2.96 (t, *J* = 7.0 Hz, 2H), 2.37-2.15 (m, 2H), 2.10-1.96 (m, 1H), 1.98-1.85 (m, 1H), 1.78-1.72 (m, 2H), 1.66-1.45 (m, 6H), 1.40-1.29 (m, 68H), 0.89 (t, *J* = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, MeOD/$CDCl_3$ = 1/1) δ 174.37, 172.28, 167.45, 160.90, 141.83, 127.35, 99.37, 73.47, 71.97, 69.64, 69.50, 69.39, 68.45, 67.11, 52.20, 51.74, 51.35, 49.78, 39.20, 36.25, 31.80, 31.79, 31.68, 31.03, 29.68, 29.59, 29.51, 29.39, 29.34, 29.31, 29.23, 29.20, 29.11, 26.97, 26.67, 25.83, 22.50, 22.46, 13.60. HRMS (ESI), m/z calcd. for $C_{60}H_{115}N_6O_{11}$ ([M+H]$^+$) 1095.8618, found:1095.8626.

(2S,3S,4R)-2-(*N*-hexacosanoylamino)-1-*O*-(6-(4-((*N*-methyl *L*-phenylalaninate)carbonyl)-1*H*-1,2,3-triazolyl)-α-D-galactopyranosyl)-1,3,4-octadecanetriol 4.

This product was obtained as a white foam solid in 91% yield. Mp 125-129 ^{o}C. ^{1}H NMR (600 MHz, MeOD/$CDCl_3$ = 5/1) δ 8.29 (s, 1H), 7.26 (t, *J* = 6.9 Hz, 2H), 7.21 (d, *J* = 6.6 Hz, 3H), 4.90 (t, *J* = 6.0 Hz, 2H), 4.83 (d, *J* = 2.8 Hz, 1H), 4.62 (d, *J* = 6.0 Hz,

2H), 4.23 (t, J = 6.0 Hz, 1H), 4.11 (d, J = 4.2 Hz, 1H), 3.84 (s, 1H), 3.80 (dd, J = 9.8, 2.6 Hz, 1H), 3.75 (dd, J = 10.2, 2.6 Hz, 1H), 3.71 (s, 3H), 3.53-3.47 (m, 2H), 3.42 (d, J = 3.0 Hz, 1H), 3.25 (dd, J = 13.4, 5.6 Hz, 1H), 3.17 (dd, J = 13.6, 7.6 Hz, 1H), 2.30-2.13 (m, 2H), 1.59-1.50 (m, 4H), 1.34-1.25 (m, 68H), 0.87 (t, J = 6.6 Hz, 6H). ^{13}C NMR (150 MHz, MeOD/$CDCl_3$ = 5/1) δ 175.35, 172.76, 161.63, 142.80, 137.22, 129.98, 129.34, 128.10, 127.80, 100.45, 74.91, 72.74, 70.71, 70.43, 70.26, 69.50, 67.85, 54.60, 52.81, 52.09, 50.97, 38.41, 37.15, 32.73, 32.72, 30.60, 30.49, 30.42, 30.26, 30.19, 30.15, 30.12, 27.86, 26.75, 26.67, 23.41, 14.38, 14.37. HRMS (ESI), m/z calcd. for $C_{63}H_{112}N_5O_{11}$ ($[M+H]^+$) 1114.8353, found: 1114.8380.

4.2. Splenocyte proliferation assay

Murine spleen cells were collected from female C57BL/6 mice. The mice were sacrificed and the spleens were immediately collected. Then they were made into a single-cell suspension by passing through a 100 μM cell strainer. Red Blood Cell (RBC) was lyzed with RBC Lysis buffer (0.5 M ammonium chloride, 10 mM potassium bicarbonate, and 0.1 mM disodium ethylene diamine tetraacetic acid, pH 7.2) for 5 min at 4 °C. The cells were washed with PBS three times by centrifugation (1000 rpm, 10 ^{o}C, 5 min), and the resulting pellet was resuspended. Splenocytes were seeded into 96-well plates (1×10^5/well) and incubated with indicated doses of synthetic compounds in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin solution. After incubation for 48 h at 37 ^{o}C, 20 μL of MTT (5 mg/mL in PBS) solution was added into each well and incubated for another 4 h. Following, the plates were centrifuged and the supernatant was removed. To each well, 100 μL of a DMSO solution was added to dissolve the formazan crystals and the plate was shaken for 10 min to fully dissolve formazan and homogenize. The optical density of the dissolved formazan crystals was measured at a wavelength of 570 nm. Splenocyte cells cultured with culture media were selected as controls, and their viabilities were defined as 100%. And the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

4.3. *In vitro* cytokine production assay

The *in vitro* production of IFN-γ and IL-4 were examined using murine spleen cells. Splenocytes were seeded into 96-well plates (1×10^5/well) and incubated with indicated doses of α-GalCer and synthetic compounds in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin solution. After incubation for 48 h at 37 °C, cytokine concentrations were determined using ELISA (eBioscience) according to the general procedures.

4.4. *In vivo* cytokine production assay

All mouse experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of the People's Republic of China. Female C57BL/6 (B6) mice weighing 25-28 g, 7 to 8 weeks old, were housed in an animal facility, given free access to water and food under specific pathogen-free conditions. Stock solutions of glycolipids in DMSO at 1 mg/mL were prepared and kept at -20 °C. For research, the stock solution was further diluted with PBS. B6 mice were injected intraperitoneally with 100μg/kg of the glycolipids, and serum samples were obtained at indicated times after injection. The serum concentrations of IFN-γ and IL-4 were assessed by ELISA kit (eBioscience). Every group contains 3 mice and their serum of indicated times was detected individually.

4.5. Computational methods [57]

The X-ray crystal structure of the CD1d and TCR (PDB code: 3HE6) was obtained from the Protein Data Bank (PDB, www.rcsb.org). The initial structure of α-GalCer was extracted from The X-ray crystal structure of 3HE6, while the initial structures of its analogues **1**, **2**, **3** and **4** were built by modifying the structure of α-GalCer with Sybyl package. Structures of protein and ligands were minimized with Powell method and

Tripos force field provided in Sybyl (Termination for protein minimization: Gradient was set to 1 kcal/(mol*Å) or Max Iterations was set to 1000; Termination for ligands minimization: Gradient was set to 0.05 kcal/(mol*Å) or Max Iterations was set to 100). The Gasteiger-Hückel charge was added for both protein and ligands. Protomol was built with default parameters (threshold was set to 0.5 Å, and bloat was set to 10.0 Å), and the Surflex-docking was performed with default input options except for the post-docking minimization flag. The bindings of α-GalCer analogues with CD1d and TCR were estimated using a variety of scoring functions within the Total Score. Pictures of complexes in the article were drawn with PyMol software (https://pymol.org/2/).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/....

References and notes

[1] Shissler SC, Bollino DR, Tiper IV, Bates JP, Derakhshandeh R, Webb TJ. *Immunogenetics*. 2016;68:623-638. https://doi.org/10.1007/s00251-016-0928-8.
[2] Carreno LJ, Saavedra-Avila NA, Porcelli SA. *Clin Transl Immunology*. 2016;5:e69. https://doi.org/10.1038/cti.2016.14.
[3] Hung JT, Huang JR, Yu AL. *J Biomed Sci.* 2017;24:22. https://doi.org/10.1186/s12929-017-0325-0.

[4] Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, Bendelac A. *J Immunol*. 1999;163:4647-4650. http://www.jimmunol.org/content/163/9/4647.
[5] Brossay L, Chioda M, Burdin N, Koezuka Y, Casorati G, Dellabona P, Kronenberg M. *J Exp Med*. 1998;188:1521-1528. https://doi.org/10.1084/jem.188.8.1521.
[6] Crowe NY, Uldrich AP, Kyparissoudis K, Hammond KJL, Hayakawa Y, Sidobre S, Keating R, Kronenberg M, Smyth MJ, Godfrey DI. *J Immunol*. 2003;171: 4020-4027. https://doi.org/10.4049/jimmunol.171.8.4020.
[7] O'Garra A. *Immunity*. 1998;8:275-283. https://doi.org/10.1016/S1074-7613(00)80533-6.
[8] Laurent X, Bertin B, Renault N, Farce A, Speca S, Milhomme O, Millet R, Desreumaux P, Henon E, Chavatte P. *J Med Chem*. 2014;57:5489-5508. https://doi.org/10.1021/jm4010863.
[9] Parekh VV, Wilson MT, Olivares-Villagomez D, Singh AK, Wu L, Wang CR, Joyce S, Van Kaer L. *J Clin Invest*. 2005;115:2572-2583. https://doi.org/10.1172/JCI24762.
[10] Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Kaer LV. *Nat Rev Immunol*. 2004;4:231-237. https://doi.org/10.1038/nri1309.
[11] Uldrich AP, Crowe NY, Kyparissoudis K, Pellicci DG, Zhan Y, Lew AM, Bouillet P, Strasser A, Smyth MJ, Godfrey DI. *J Immunol*. 2005;175:3092-3101. https://doi.org/10.4049/jimmunol.175.5.3092.
[12] Gonzalez-Aseguinolaza G, Kaer LV, Bergmann CC, Wilson JM, Schmieg J, Kronenberg M, Nakayama T, Taniguchi M, Koezuka Y, Tsuji M. *J Exp Med*. 2002;195:617-624. https://doi.org/10.1084/jem.20011889.
[13] Wingender G, Birkholz AM, Sag D, Farber E, Chitale S, Howell AR, Kronenberg M. *J Immunol*. 2015;195:3838-3848. https://doi.org/10.4049/jimmunol.1500203.
[14] Morita M, Motoki K, Akimoto K, Natori T, Sakai T, Sawa E, Yamaji K, Koezuka Y, Kobayashi E, Fukushima H. *J Med Chem*. 1995;38:2176-2187. https://doi.org/10.1021/jm00012a018.
[15] Goff RD, Gao Y, Mattner J, Zhou D, Yin N, Cantu C, Teyton L, Bendelac A, Savage PB. *J Am Chem Soc*. 2004;26:13602-13603. https://doi.org/10.1021/ja045385q.
[16] Chang YJ, Huang JR, Tsai YC, Hung JT, Wu D, Fujio M, Wong CH, Yu AL. *Proc Natl Acad Sci U.S.A.* 2007;104:10299-10304. https://doi.org/10.1073/pnas.0703824104.
[17] Janssens J, Bitra A, Wang J, Decruy T, Venken K, van der Eycken J, Elewaut D, Zajonc DM, van Calenbergh S. *Chem Med Chem*. 2019;14:147-168. https://doi.org/10.1002/cmdc.201800649.
[18] Cui Y, Li Z, Cheng Z, Xia C, Zhang Y. *Chem Res Toxicol.* 2015;28:1209-1215. https://doi.org/10.1021/acs.chemrestox.5b00047.
[19] Lee T, Cho M, Ko SY, Youn HJ, Baek DJ, Cho WJ, Kang CY, Kim S. *J Med Chem*. 2007;50:85-589. https://doi.org/10.1021/jm061243q.
[20] Kim H, Song H, Park JG, Lee DS, Park SB. *ACS Med Chem Lett*. 2019;10:773-779. https://doi.org/10.1021/acsmedchemlett.9b00026.
[21] Kishi J, Inuki S, Hirata N, Kashiwabara E, Yoshidome D, Ichihara O, Fujimoto Y. *Bioorg Med Chem Lett*. 2019;29:970-973. https://doi.org/10.1016/j.bmcl.2019.02.018.

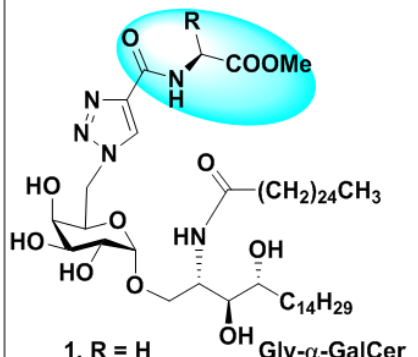
[22] Hwang YS, Yim J, Song H, Park SB. *ACS Med Chem Lett*. 2019;10:720-725. https://doi.org/10.1021/acsmedchemlett.8b00556.
[23] Golten S, Patinec A, Akoumany K, Rocher J, Graton J, Jacquemin D, Le Questel JY, Tessier A, Lebreton J, Blot V, Pipelier M, Douillard JY, Le Pendu J, Linclau B, Dubreuil D. *Eur J Med Chem*. 2019;178:195-213. https://doi.org/10.1016/j.ejmech.2019.05.069.
[24] Schmieg J, Yang G, Franck RW, Tsuji M. *J Exp Med*. 2003;198:1631-1641. https://doi.org/10.1084/jem.20031192.
[25] Miyamoto K, Miyake S, Yamamura T. *Nature*. 2001;413:531-534. https://doi.org/10.1038/35097097.
[26] Yu KOA, Im JS, Molano A, Dutronc Y, Illarionov P A, Forestier C, Fujiwara N, Arias I, Miyake S, Yamamura T, Chang YT, Besra GS, Porcelli SA. *Proc Natl Acad Sci U.S.A*. 2005;102:3383-3388. https://doi.org/10.1073/pnas.0407488102.
[27] Inuki S, Kashiwabara E, Hirata N, Kishi J, Nabika E, Fujimoto Y. *Angew Chem Int Ed*. 2018;57:9655-9659. https://doi.org/10.1002/anie.201802983.
[28] Wu D, Xing G-W, Poles MA, Horowitz A, Kinjo Y, Sullivan B, Bodmer-Narkevitch V, Plettenburg O, Kronenberg M, Tsuji M, Ho DD, Wong CH. *Proc Natl Acad Sci U.S.A.* 2005;102:1351-1356. https://doi.org/10.1073/pnas.0408696102.
[29] Berkers CR, Ovaa H. *Trends Pharmacol Sci*. 2005;26:252-257. https://doi.org/10.1016/j.tips.2005.03.005.
[30] Brutkiewicz RR. *J Immunol*. 2006;177:769-775. https://doi.org/10.4049/jimmunol.177.2.769.
[31] Wu TN, Lin KH, Chang YJ, Huang JR, Cheng JY, Yu AL, Wong CH. *Proc Natl Acad Sci U.S.A*. 2011;108:17275-17280. https://doi.org/10.1073/pnas.1114255108.
[32] Rossjohn J, Pellicci DG, Patel O, Gapin L, Godfrey D I. *Nat Rev Immunol*. 2012;12:845-857. https://doi.org/10.1038/nri3328.
[33] Koch M, Stronge VS, Shepherd D, Gadola SD, Mathew B, Ritter G, Fersht AR, Besra GS, Schmidt RR, Jones EY, Cerundolo V. *Nat Immunol*. 2005;6:819-826. https://doi.org/10.1038/ni1225.
[34] Zajonc DM, Cantu III C, Mattner J, Zhou D, Savage PB, Bendelac A, Wilson IA, Teyton L. *Nat Immunol*. 2005;6:810-818. https://doi.org/10.1038/ni1224.
[35] Liu Y, Goff RD, Zhou D, Mattner J, Sullivan BA, Khurana A, Cantu III C, Ravkov EV, Ibegbu CC, Altman JD, Teyton L, Bendelac A, Savage PB. *J Immunol Methods*. 2006;312:34-39. https://doi.org/10.1016/j.jim.2006.02.009.
[36] Zhou XT, Forestier C, Goff RD, Li C, Teyton L, Bendelac A, Savage PB. *Org Lett*. 2002;4:1267-1270. https://doi.org/10.1021/ol025565+.
[37] Pauwels N, Aspeslagh S, Vanhoenacker G, Sandra K, Yu ED, Zajonc DM, Elewaut D, Linclau B, Calenbergh SV. *Org Biomol Chem*. 2011;9:8413-8421. https://doi.org/10.1039/c1ob06235b.
[38] Tashiro T, Nakagawa R, Shigeura T, Watarai H, Taniguchi M, Mori K. *Bioorg Med Chem*. 2013;21:3066-3079. https://doi.org/10.1016/j.bmc.2013.03.028.
[39] Trappeniers M, Beneden KV, Decruy T, Hillaert U, Linclau B, Elewaut D, Calenbergh SV. *J Am Chem Soc*. 2008;130:16468-16469. https://doi.org/10.1021/ja8064182.

[40] Aspeslagh S, Li Y, Yu ED, Pauwels N, Trappeniers M, Girardi E, Decruy T, Beneden KV, Venken K, Drennan M, Leybaert L, Wang J, Franck RW, Calenbergh SV, Zajonc DM, Elewaut D. *EMBO J*. 2011;30:2294-2305. https://doi.org/10.1038/emboj.2011.145.
[41] Chennamadhavuni D, Saavedra-Avila NA, Carreno LJ, Guberman-Pfeffer MJ, Arora P, Yongqing T, Pryce R, Koay HF, Godfrey DI, Keshipeddy S, Richardson SK, Sundararaj S, Lo JH, Wen X, Gascon JA, Yuan W, Rossjohn J, Le Nours J, Porcelli SA, Howell AR. *Cell Chem Biol*. 2018;25:571-584. https://doi.org/10.1016/j.chembiol.2018.02.009.
[42] Hung JT, Sawant RC, Chen JC, Yen YF, Chen WS, Yu AL, Luo SY. *RSC Adv*. 2014;4:47341-47356. https://doi.org/10.1039/c4ra08602c.
[43] Jervis PJ, Graham LM, Foster EL, Cox LR, Porcelli SA, Besra GS. *Bioorg Med Chem Lett*. 2012;22:4348-4352. https://doi.org/10.1016/j.bmcl.2012.05.009.
[44] Pauwels N, Aspeslagh S, Elewaut D, Calenbergh SV. *Bioorg Med Chem*. 2012;20:7149-7154. https://doi.org/10.1016/j.bmc.2012.09.063.
[45] De la Torre BG, Albericio F. *Molecules*. 2018;23:533-560. https://doi.org/10.3390/molecules23030533.
[46] Tao W, Zhao D, Sun M, Wang Z, Lin B, Bao Y, Li Y, He Z, Sun Y, Sun J. *Int J Pharm*. 2018;541:64-71. https://doi.org/10.1016/j.ijpharm.2018.02.033.
[47] Varol M, Koparal AT, Benkli K, Bostancioglu RB. *Curr Drug Deliv*. 2018;15:1417-1425. https://doi.org/10.2174/1567201815666180727145724.
[48] Borg NA, Wun KS, Kjer-Nielsen L, Wilce1 MCJ, Pellicci DG, Koh R, Besra GS, Bharadwaj M, Godfrey DI, McCluskey J, Rossjohn J. *Nature*. 2007;448:44-49. https://doi.org/10.1038/nature05907.
[49] Burkhart JL, Kazmaier U. *RSC Advances*. 2012;2:3785-3790. https://doi.org/10.1039/c2ra20191g.
[50] Yang B, Yoshida K, Yin Z, Dai H, Kavunja H, El-Dakdouki MH, Sungsuwan S, Dulaney SB, Huang X. *Angew Chem Int Ed*. 2012;51:10185-10189. https://doi.org/10.1002/anie.201205601.
[51] Li C, Sun Y, Zhang J, Zhao Z, Yu G, Guan H. *Carbohydr Res*. 2013;376:15-23. https://doi.org/10.1016/j.carres.2013.02.008.
[52] Bi J. Wang J, Zhou K, Wang Y, Fang M, Du Y. *ACS Med Chem Lett*. 2015;6:476-480. https://doi.org/10.1021/acsmedchemlett.5b00046.
[53] Liu Y, Xu X, Gao Q, Yan S, Li Y, Ding N. *Bioorg Med Chem Lett*. 2017;27:1795-1798. https://doi.org/10.1016/j.bmcl.2017.02.055.
[54]Verma YK, Reddy BS, Pawar MS, Bhunia D, Sampath Kumar HM. *ACS Med Chem Lett*. 2016;7:172-176. https://doi.org/10.1021/acsmedchemlett.5b00340.
[55] Hossain MI, Hanashima S, Nomura T, Lethu S, Tsuchikawa H, Murata M, Kusaka H, Kita S, Maenaka K. *Bioorg Med Chem*. 2016;24:3687-3695. https://doi.org/10.1016/j.bmc.2016.06.007.
[56]He P, Zhao C, Lu J, Zhang Y, Fang M, Du Y. *ACS Med Chem Lett*. 2019;10:221-225. https://doi.org/10.1021/acsmedchemlett.8b00640.
[57] Hu L, Zhao C, Ma J, Jing Y, Du Y. *Bioorg Med Chem Lett*. 2019;29:1357-1362. https://doi.org/10.1016/j.bmcl.2019.03.043.

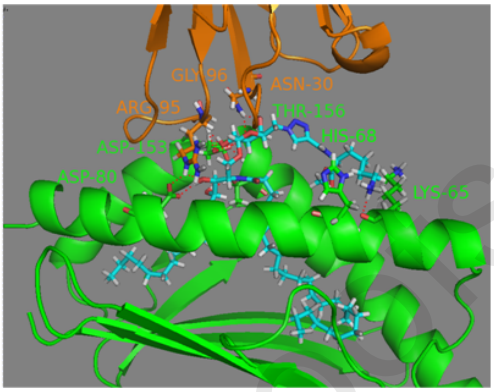
Highlights

- L-amino-acid methyl esters substituted C6'-triazolyl α-GalCer analogues are designed and synthesized efficiently.
- Compared to α-GalCer, amino acids-functionalized α-GalCers exhibits good selectivity for Th1-biased response.
- Compond **3** (Lys-α-GalCer) shows the highest IFN-γ/IL-4 rate and AUC ratio.

Graphical abstract



1, R = H Gly-α-GalCer
2, R = CH_2OH Ser-α-GalCer
3, R = $(CH_2)_4NH_2$ Lys-α-GalCer
4, R = CH_2Ph Phe-α-GalCer



Molecular docking of Lys-α-GalCer (3)

conflicts of interest

The authors declared that they have no conflicts of interest to this work.

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.