Bioorganic & Medicinal Chemistry 20 (2012) 4540–4548

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

RCAI-84, 91, and 105-108, ureido and thioureido analogs of KRN7000: Their synthesis and bioactivity for mouse lymphocytes to produce Th1-biased cytokines ☆

Takuya Tashiro [a], Tomokuni Shigeura [b], Hiroshi Watarai [b], Masaru Taniguchi [b], Kenji Mori [a,*]

[a] Glycosphingolipid Synthesis Group, Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan
[b] Laboratory for Immune Regulation, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Suehiro-cho 1-7-22, Tsurumi-ku, Yokohama-shi, Kanagawa 230-0045, Japan

ARTICLE INFO

Article history:
Received 16 April 2012
Revised 7 May 2012
Accepted 8 May 2012
Available online 7 June 2012

Keywords:
CD1d
Glycosphingolipid
KRN7000
NKT cell
Ureide

ABSTRACT

RCAI-84, 91, and 105-108 (**1–6**), the analogs of KRN7000 (**A**) with a ureido or a thioureido linkage instead of a carboxamido bond, were synthesized to examine their immunostimulatory activity against mouse lymphocytes. According to their bioassay, the ureido analog of KRN7000 [RCAI-105 (**1**)] and its 6′-*O*-methylated derivative [RCAI-106 (**4**)] induced a large amount of IFN-γ in mice in vivo. The hexadecyl ureido analog [RCAI-84 (**2**)] was comparable to KRN7000 in its bioactivity. The octylureido [RCAI-107 (**3**)], 5-phenylpentylureido [RCAI-108 (**5**)], and thioureido [RCAI-91 (**6**)] analogs were almost inactive.

1. Introduction

KRN7000 (α-GalCer, **A**), developed by researchers at KIRIN Brewery Co. in 1995, is an immunostimulant agent that induces antitumor activity in vivo in mice and humans.[2] It binds to a CD1d protein, an MHC class I-like glycolipid presentation protein, on the surface of the antigen presenting cells.[3] The resulting CD1d-**A** complex is recognized by the invariant (mouse: Vα14-Jα18, human: Vα24-Jα18) T cell receptor (TCR) of natural killer T (NKT) cells and activates them to release helper T(Th)1 and/or Th2 cytokines.[4] Th1 cytokines such as interferon(IFN)-γ mediate protective immune functions like tumor rejection, whereas Th2 cytokines such as interleukin(IL)-4 and IL-10 mediate regulatory immune functions to ameliorate autoimmune diseases. If there were a novel glycolipid, which can induce NKT cells to produce cytokines with directed Th1/Th2 balance, it will be a promising drug candidate against many types of diseases.[5] Unfortunately, KRN7000 (**A**) induces both Th1 and Th2 cytokines in large quantities at the same time by a single injection.[4,6] Accordingly, many groups are trying to develop new analogs more effective than **A** which induce NKT cells to produce only Th1 (or Th2) type immune responses (see reviews[7]).

The structure **A** contains three parts which can be modified: (i) the galactose-ceramide linkage, (ii) the galactose part, and (iii) the lipid chains. Representative analogs belonging to category (iii) are shown in Figure 1. In 2001, Miyamoto et al. found that OCH (**B**) caused NKT cells to produce predominantly IL-4 in mice in vivo.[8] Baek et al. reported their KBC-007 (**C**) induced enhanced and biased IL-4 production in vitro (using splenocytes from mice).[9] On the other hand, an aromatic octanamide analog C8PhF (**D**) exhibited a potent Th1-biased cytokine response in vitro (using peripheral mononuclear cell population from humans).[10] Isosteric replacement of 2-amido moiety of **A** with a 1,2,3-triazole (**E**),[11] an ester (RCAI-80, **F**),[12] a sulfonamido (RCAI-34, **G**),[13] a carbamate (RCAI-41, **H**)[1] or an α,α-difluorocarboxamido group (**I**)[14] was reported, and led to the analogs of Th2-type.

According to the X-ray crystallographic analyses of the binary complexes of mouse and human CD1d-**A** reported in 2005, the amide H of **A** makes a hydrogen-bonding interaction with Thr156 of mouse CD1d (human: Thr154).[15,16] In addition, the order of pK_a values of amide H is carboxamide > sulfonamide ≈ α,α-difluorocarboxamide [for example, acetamide: pK_a = 25.5; methanesulfonamide: pK_a = 17.5 (in DMSO, 25 °C)].[17] We thought that the analog which had an amide NH with a low pK_a value would induce the Th2-biased cytokine production. If so, an analog possessing a less acidic amide NH than that of **A** should induce Th1-type immune response. Based on this speculation, we synthesized ureido analogs **1**–**5** (RCAI-84 and 105–108, Scheme 1) and investigated

☆ Synthesis of sphingosine relatives, Part 34. For Part 33, see Ref. 1.

* Corresponding author. Tel.: +81 3 3816 6889; fax: +81 48 467 9381.
E-mail address: kjk-mori@arion.ocn.ne.jp (K. Mori).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.073

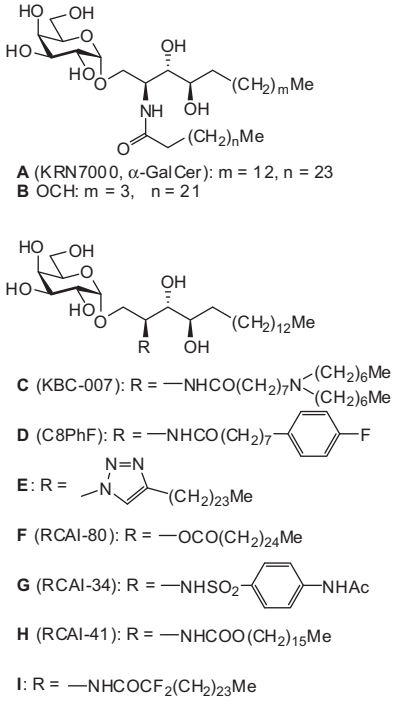


Figure 1. Structures of KRN7000 (**A**) and its typical analogs developed by modification of the lipid chains of **A**.

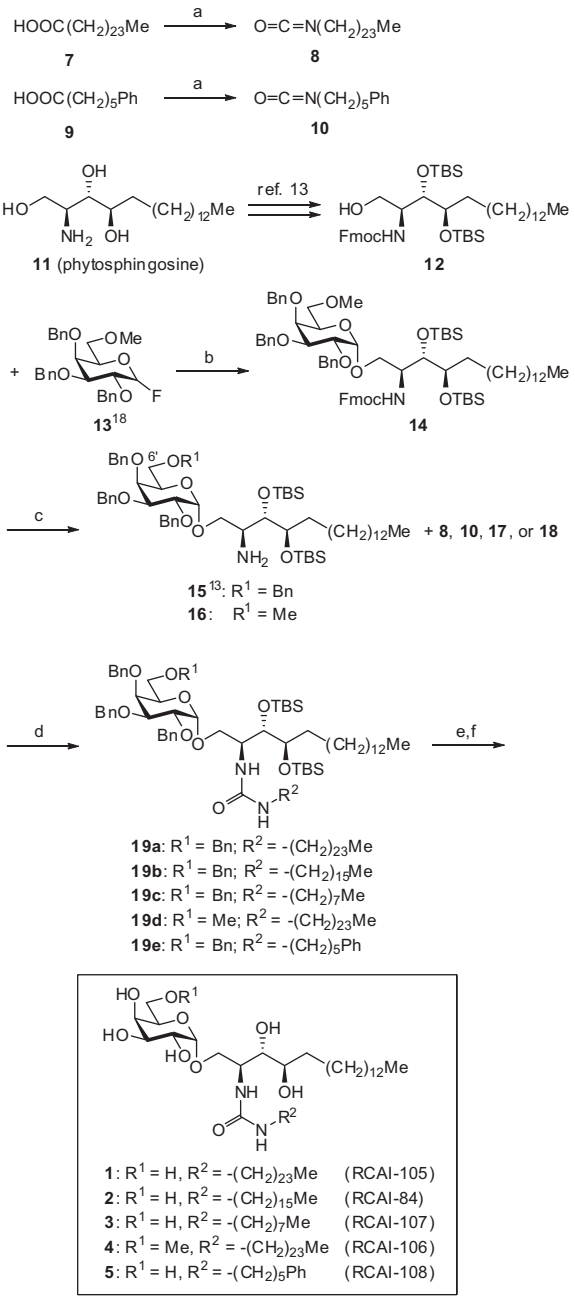
their bioactivity. Because it is known that the pK_a value of ureide H [for example, urea: pK_a = 26.95 (in DMSO, 25 °C)] is higher than that of carboxamide H.[17]

In the event, we discovered that the ureido analog RCAI-105 (**1**), which has a ureido linkage instead of the carboxamido group of **A**, induced potent Th1-biased cytokine production in mice in vivo. In addition, it was also found that the modification of the 6′-hydroxy group of the galactose part of **1** was allowed, although it was accompanied by a slight decline of its immunostimulatory activity. Indeed, a 6′-*O*-methylated derivative of **1** (RCAI-106, **4**) showed stronger Th1-type bioactivity than **A**, although weaker than **1**. We then synthesized RCAI-108 (**5**) by introducing a phenyl group at the end of the alkyl chain of the ureido group of **1**. The bioactivity of **5**, however, was not so remarkable. It should also be added that the thioureido analog (RCAI-91, **6**) was almost inactive in mice in vivo.

2. Results and discussion

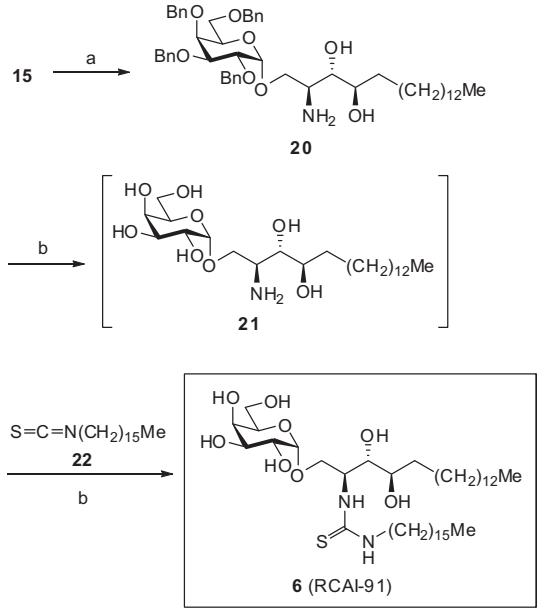
2.1. Synthesis of ureido and thioureido analogs

We prepared five ureido analogs as summarized in Scheme 1. To investigate the influence of the chain length versus immunostimulatory activity, RCAI-105 (**1**), 84 (**2**), and 107 (**3**), which have C_{24}, C_{16}, and C_8 linear alkylureido groups, respectively, were synthesized. As we have found that 6′-*O*-methylated analog (RCAI-61) of **A** induces the potent IFN-γ production against mouse lymphocytes,[18] we synthesized RCAI-106 (**4**) possessing both the 6′-*O*-methyl group and the ureido linkage. Furthermore, RCAI-108 (**5**) which has a 5-phenylpentylureido group was also synthesized, because Wong and his co-workers reported that the introduction of an aromatic group at the end of the acyl chain (**D** in Fig. 1 is one of their potent analogs) led to potent Th1-biased cytokine inducers by making a stable CD1d/ligand complex due to π–π stacking or some additional interaction.[10,19] In addition, a thioureido analog RCAI-91 (**6**, Scheme 2) was also synthesized and its bioactivity was studied.



Scheme 1. Synthesis of ureido analogs **1**–**5**. Reagents and conditions: (a) (i) $(COCl)_2$, benzene, 60 °C; (ii) aq NaN_3, THF, DMF, 0 °C; (iii) benzene, 75 °C (91% for **8**, 87% for **10**); (b) $AgClO_4$, $SnCl_2$, MS 4A, THF, −18 to 15 °C (75%); (c) piperidine, DMF, rt (98%); (d) **8**, **10**, hexadecyl isocyanate (**17**), or octyl isocyanate (**18**), $CHCl_3$, rt (65–97%) (e) TBAF, THF, rt (92%–quant.); (f) H_2, 20% $Pd(OH)_2$-C, EtOH, $CHCl_3$, rt (72–88%).

At first, two isocyanates **8** and **10** were prepared by Curtius rearrangement reaction from pentacosanoic acid **7** and 6-phenylhexanoic acid **9**, respectively, via their acyl chloride and acyl azide.[20] A 6′-*O*-benzylated amine **15** was synthesized from an alcohol **12**, which was readily prepared from commercially available phytosphingosine (**11**) in three steps according to our previous report.[13] The 6′-*O*-methylated amine **16** was obtained by galactosylation of **12** with the sugar donor **13**, which could be prepared from methyl α-D-galactopyranoside,[18] under Mukaiyama's conditions to give **14** (75%).[21] Subsequent treatment of **14** with piperidine for deprotection of (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) group gave **16** in 98% yield.[22] The amine **15** reacted with the isocyanate **8** to give **19a** (97% yield). Protective groups of



Scheme 2. Synthesis of RCAI-91 (**6**). Reagents and conditions: (a) TBAF, THF, rt (98%). (b) H_2, 20% $Pd(OH)_2$-C, EtOH, $CHCl_3$, rt. (c) EtOH, $CHCl_3$, rt (44%, two steps).

19a, *tert*-butyldimethylsilyl (TBS) and benzyl groups, were successively removed by treatment with tetrabutylammonium fluoride (TBAF) followed by hydrogenolysis, to furnish RCAI-105 (**1**) in 73% yield in two steps.[23] In the same manner, RCAI-84 (**2**), 107 (**3**), and 108 (**5**) were synthesized from **15** by using commercially available hexadecyl and octyl isocyanates (**17** and **18**), and freshly prepared **10** in 74%, 48%, and 51% yield in three steps, respectively.[23] Similarly, RCAI-106 (**4**) was obtained from the amine **16** and the isocyanate **8** in 79% yield in three steps.[23]

The thioureido analog RCAI-91 (**6**) was synthesized as shown in Scheme 2 from an amine **20**, which was prepared from **15** by deprotection of two TBS groups (98%). Hydrogenolysis of **20** gave **21**, which was then treated with commercially available hexadecyl isothiocyanate **22** to furnish **6** in 44% yield in two steps.[23]

2.2. Results of bioassay

Figures 2 and 3 show the results of bioassay.[24] To investigate the ability of **1**–**5** to induce cytokine production by mouse lymphocytes in vivo, the concentrations of cytokines in sera of mice were monitored after intravenous (iv) administration of KRN7000 (**A**) or synthesized ureido analogs (**1**–**5**) as phosphate buffered saline (PBS) solutions into C57BL/6J mice.[25] The sera samples were collected at 3, 6, 12, 24, 36, 48, and 60 h. The measurement of cytokine concentrations were performed by ELISA system (Thermo Fisher Scientific K.K.) for IFN-γ (A and B, in Figure 2) or cytometric bead array (CBA) system (BD Biosciences) for IL-4 (C and D).

As can be seen, RCAI-105 (**1**) induced potent IFN-γ production (peaked at 24 h). It also induced almost the same level of IL-4 production as observed with **A**. These results mean that **1** is regarded as a potent Th1-biased cytokine inducer. Additionally, truncation of the alkyl chain of the ureido group from C_{24} to C_{16} (RCAI-84, **2**) or C_8 (RCAI-107, **3**) caused drastic decrease of immunostimulatory activity. RCAI-84 (**2**), which has a C_{18} phytosphingosine chain and the ureido linkage with a C_{16}-linear alkyl chain, induced almost the same amount of cytokines as those observed with **A**, whereas RCAI-53, one of the truncated analog of **A** possessing the C_{18} phytosphingosine chain and octadecanamido group, induced lower amount of cytokines (the peak concentration of IFN-γ and IL-4 were 10% and 40%, respectively, relative to those secreted by

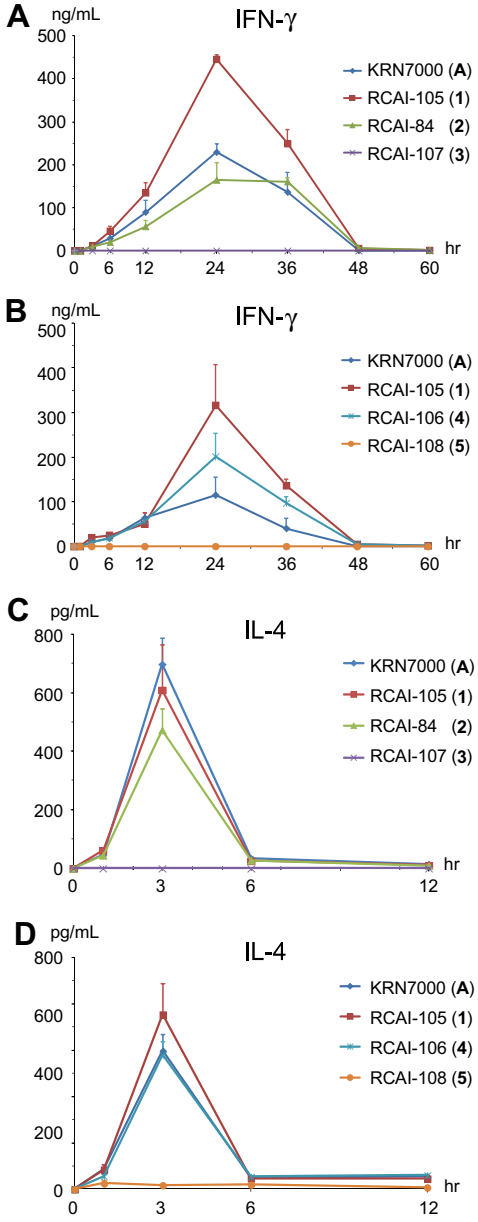


Figure 2. Cytokine production after injection of synthetic analogs in mice in vivo (2 μg/mouse, iv). Serum concentrations of IFN-γ(A, B) and IL-4 (C, D) were measured by ELISA and CBA, respectively, at the indicated time points. Data are means ± standard deviation (SD) from 3 mice, and repeated 3 times with similar results.

A).[1] Since RCAI-105 (**1**) was more potent than KRN7000 (**A**), the ureido group seems to increase the stability of the complex with CD1d. In the case of RCAI-107 (**3**), the alkyl chain of the ureido part was too short to make the CD1d/ligand complex stable, so **3** was not able to stimulate NKT cells enough, and was almost inactive.

RCAI-106 (**4**), 6′-*O*-methylated derivative of **1**, also induced a large amount of IFN-γ and a similar extent of IL-4 production. As shown in Figure 3, **1** and **4** induced increased IL-12p70 production at 3, 6, and 12 h after administration in comparison to **A**. Therefore, it was found that the potent IFN-γ production induced by **1** and **4** observed in Fig. 2 (peaked at 24 h) might be related to the remarkably increased production of IL-12p70 (peaked at 6 h).[26]

As we reported in the case of RCAI-61, methylation of the 6′-hydroxy group of **A** enhances IFN-γ production.[18] Although RCAI-106 (**4**) also induced larger amounts of IFN-γ than **A**, its bioactivity was

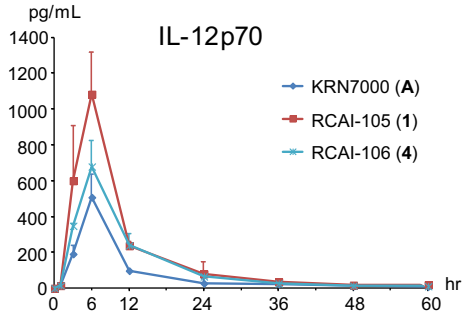


Figure 3. IL-12p70 production after injection of synthetic analogs in mice in vivo (2 μg/mouse, iv). Serum concentrations of IL-12p70 were measured by CBA at the indicated time points. Data are means ± SD from 3 mice, and repeated 3 times with similar results.

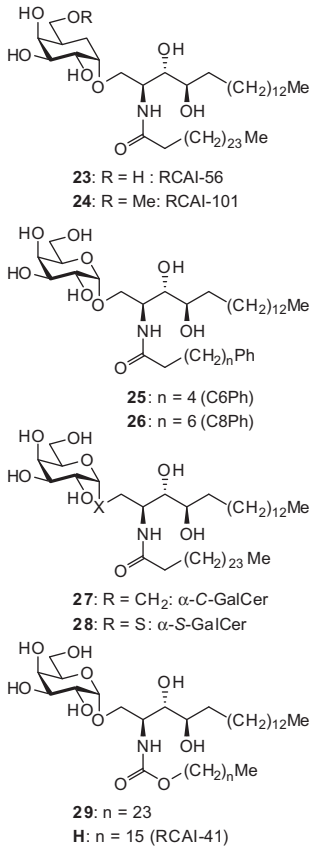


Figure 4. Structures of RCAI-56 (**23**) and 101 (**24**), C6Ph (**25**), C8Ph (**26**), α-*C*-GalCer (**27**), α-*S*-GalCer (**28**), and Wojno's carbamate (**29**).

weaker than that of **1**. The similar tendency was observed by us in another case, too.[27] A carba-α-D-galactosyl analog of **A** (RCAI-56, **23** in Fig. 4) induced a large quantity of the Th1-biased cytokine production. Its 6′-*O*-methylated derivative (RCAI-101, **24**) also showed strong bioactivity, but it induced only half the amount of IFN-γ production in comparison to **23**. The *M*log*P* values of these glycolipids were calculated as 4.64 and 4.80 for **23** and **24**, respectively, whereas that of **A** was 4.16. On the other hand, *M*log*P* of **1** and **4** were 4.10 and 4.25.[28] Therefore, we concluded that methylation of the 6′-hydroxy group enhanced the hydrophobicity of the analog which caused the depression of its bioactivity.

Wong and his co-workers reported that both 6-phenylhexanamido and 8-phenyloctanamido analog [C6Ph (**25**) and C8Ph (**26**), Fig. 4] induced the potent Th1-biased cytokine production in humans in vitro.[19] The spatial location between the ureido carbonyl and the terminal phenyl groups of RCAI-108 is between the amide carbonyl distances from the phenyl groups in **25** and **26**. Hence, we expected **5** to show Th1-type immunostimulatory activity. Until now, many analogs have been prepared to improve bioactivity by installing a phenyl group at the end of the acyl chain.[29,30] In contrast to those reports, however, **5** induced only very weak cytokine secretion. A similar observation was reported by Van Calenbergh and his co-workers.[31] It has been known that α-D-fucosyl analog of **A** induces the enhanced IFN-γ production in mice in vivo relative to **A**.[18] The Van Calenbergh α-D-fucosyl analog with a C6Ph (**25**)-type acyl chain showed very weak bioactivity. Perhaps, our modification of amide to ureido alters the binding conformation of CD1d/ligand complex and abolishes the stabilizing effect in the complex induced by the aromatic group, leading to decrease in its bioactivity. This change in binding conformation might also be responsible for the observed better Th1-bias of **1** and **4**.

It is known that the pK_a value of the thioureide H is lower than that of carboxamide H [for example, thiourea: pK_a = 21.1 (in DMSO, 25 °C)].[17] Therefore, we anticipated that the thioureido analog (RCAI-91, **6**) would induce Th2-type cytokine production. However, the analog **6** was almost inactive in mice in vivo (see Supplementary data). Because the atomic radius of S is larger than that of O, **6** might not be able to make a stable complex with CD1d, and therefore showed only very weak bioactivity. Indeed, **A** and its *C*-glycoside analog, α-*C*-GalCer (**27**), are the most potent immunostimulants of this class,[32] while the corresponding *S*-glycoside [α-*S*-GalCer (**28**)] was almost inactive in mice in vivo.[33–35]

2.3. The relationship between pK_a value of amide H and Th1/Th2 bias of immune response

According to the X-ray structure of the binary mouse and human CD1d-**A** complex, the amide H of **A** makes a hydrogen-bonding interaction with Thr154 of human CD1d (mouse: Thr156).[15,16] In addition, based on their molecular dynamics simulation study, Hénon et al. postulated that Thr154 acts as an H-bond acceptor with the amide group of **A**, and this amide H-Thr154 hydrogen bond is one of the key interactions to set the galactose head group of **A** at the proper position for making CD1d–glycolipid complex stable.[36]

We focused on the relationship between the acidity of the amide H and Th1/Th2 balances of immune response, and synthesized the ureido analogs, of which the pK_a value of the ureide H is higher than that of the carboxamide H as mentioned in the Introduction. From the results of bioassay, IFN-γ/IL-4 ratios at the peak time of each cytokine induced by KRN7000 (**A**) and RCAI-105 (**1**) were calculated as 3.3–3.9 × 10^2 and 7.3–8.4 × 10^2, respectively. Therefore, it is clear that **1** induced a somewhat more selective Th1-biased cytokine production than what observed for **A**. On the other hand, Linclau and his co-workers reported the synthesis of the analog **I** (Fig. 1), and its Th2-type bioactivity (5 μg/mouse, the peak concentrations of IFN-γ and IL-4 were 50% and 100%, respectively, of those induced by **A**) in mice in vivo (intraperitoneal dosing).[14] Due to the presence of two electronegative fluorine atoms at the α-position to the carbonyl group, the pK_a value [trifluoroacetamide: pK_a = 17.2; monofluoroacetamide: pK_a = 22.3 (in DMSO, 25 °C)][17,37] of the amide H of analog **I** is lower than that [acetamide: pK_a = 25.5 (in DMSO, 25 °C)][17] of **A**. From these considerations, the analogs possessing the more basic amide H have a tendency to induce the larger amount of IFN-γ, whereas the analogs having the more acidic amide H show a trend toward more Th2-type. Further studies to clarify the relationship between pK_a of the amide H and Th1/Th2 bias of bioactivity are in progress by means of computational docking studies, and the results will be reported in due course.

Very recently Wojno et al. reported independently the immunostimulant activity of a ureido analogs of KRN7000.[38] They also found a thioamido analog of KRN7000 as bioactive, although they did not prepare any thioureido analog.[38] A carbamate analog **29** (n = 23) synthesized by them was as active as their ureido analog to induce IFN-γ production.[38] Our carbamate analog **H** (n = 15, RCAI-41) with a shorter alkyl group showed Th2-biased cytokine production.[1] Accordingly, even among carbamates [pK_a = 24.6 (in DMSO, 25 °C)][17] the length of the alkyl chain causes different bias in cytokine production.

3. Conclusion

We synthesized RCAI-84, 91, and 105-108, the ureido and thioureido analogs of KRN7000. Among them, RCAI-105 (**1**, tetracosylureido analog) and RCAI-106 (**4**, 6′-*O*-methylated derivative of **1**) were found to be the remarkably potent inducers of Th1-biased cytokine production in mice in vivo. In addition, RCAI-84 (**2**) showed almost the same activity as that of KRN7000. The former two ureido analogs are readily synthesized from tetracosyl isocyanate (**8**), which is easily prepared from pentacosanoic acid (**7**) by Curtius rearrangement reaction. Due to their availability, RAI-105 (**1**) and 106 (**4**) might be promising anticancer drug candidates.

Additionally, it was found that an analog possessing the amide H with the larger pK_a value like RCAI-105 (**1**) induced the larger IFN-γ production compared to the analog with a carboxamide group.

4. Experimental

4.1. Chemistry

4.1.1. General

Refractive indices (n_D) were measured on an Atago 1T refractometer. Melting points were recorded using a Yanaco MP-S3 melting point measuring apparatus and are uncorrected. Optical rotation values were measured on a Jasco P-1010 polarimeter. IR spectra were measured on a Jasco FT/IR-460 plus spectrometer. ^{1}H NMR spectra (TMS at δ = 0.00, $CHCl_3$ at δ = 7.26, or pyridine at δ = 7.55 as the internal standards) and ^{13}C NMR spectra (pyridine at δ = 135.5 as the internal standard) were recorded on a Varian VNMRS-500 (500 MHz) spectrometer. High resolution mass spectrometry (HRMS) was performed on a Jeol JMS-100LC or a Varian QFT-7 Fourier transform ion cyclotron resonance (FT ICR) mass spectrometers [electrospray ionization (ESI)]. Column chromatography was performed by using Merck Kieselgel 60 Art 1.07734.

4.1.2. Preparation of isocyanates

4.1.2.1. Tetracosyl isocyanate 8. To a stirred solution of pentacosanoic acid (**7**, 204 mg, 0.533 mmol) in dry benzene (5 mL) at 60 °C, oxalyl chloride (460 μL, 5.36 mmol) was added. The mixture was stirred at 60 °C for 2 h, and then concentrated in vacuo. The obtained crude acyl chloride was diluted with dry THF (5 mL) and *N*,*N*-dimethylformamide (DMF, 2 mL), and cooled to 0 °C. To this mixture, an aqueous solution of sodium azide (69 mg, 1.06 mmol) in distilled water (1.0 mL) was added dropwise at 0 °C, and the resulting mixture was vigorously stirred at 0 °C for 1 h. The mixture was then diluted with benzene and poured into water. The separated organic phase was washed with water and brine, dried with Na_2SO_4, and filtered. The filtrate was stirred at 75 °C for 90 min, and then concentrated in vacuo. The residue was diluted with hexane, filtered through a bed of Celite, and concentrated in vacuo to give **8** (185 mg, 91%) as a colorless solid. ν_{max} (KBr): 2335 (s, N=C=O) cm^{-1}. The obtained isocyanate was immediately used in the next step without further purification.

4.1.2.2. 5-Phenylpentyl isocyanate 10. In the same manner as described above, commercially available 6-phenylhexanoic acid (**9**, 237 mg, 1.23 mmol) was converted to **10** (204 mg, 87%) as a colorless oil. ν_{max} (film): 2380 (s, N=C=O), 1600 (w), 1495 (w), 750 (br m), 700 (s) cm^{-1}. The obtained isocyanate was immediately used in the next step without further purification.

4.1.3. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-(9*H*-fluorenylmethoxycarbamido)octadecane 14

To a stirred solution of **12**[13] (2.91 g, 3.79 mmol) in dry THF (80 mL), tin(II) chloride (2.48 g, 13.1 mmol), silver(I) perchlorate (2.75 g, 13.3 mmol) and powdered MS 4A (20.2 g) were added under argon. After stirring at rt for 2 h, the mixture was cooled to −18 °C. To this mixture, a solution of **13**[18] (2.02 g, 4.33 mmol) in dry THF (30 mL) was added at −18 °C. The reaction mixture was gradually warmed up to 15 °C with stirring over 5 h. The mixture was then diluted with ether, and the resulting mixture was filtered through a bed of Celite. The filtrate was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with K_2CO_3, and concentrated in vacuo. The residue was purified by silica gel column chromatography (80 g, hexane/EtOAc = 50:3) to give **14** (1.97 g, 75%) as a colorless oil, n_D^{21} 1.5187; $[\alpha]_D^{21}$ +21.4 (c 1.01, $CHCl_3$); ν_{max} (film): 3340 (m, NH), 1725 (s, CO), 1605 (w), 1540 (br s), 1500 (m), 1255 (br s, *t*-Bu, Si–Me), 1100 (br s, C–O), 1055 (br s, C–O), 840 (s), 755 (s), 740 (s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.72 (2H, t, J = 7.5 Hz), 7.58 (2H, br t, J = 8.0 Hz), 7.36–7.22 (19H, m), 5.69 (1H, d, J = 7.0 Hz),4.95 (1H, d, J = 12 Hz), 4.803 (1H, d, J = 4.0 Hz), 4.797 (1H, d, J = 12 Hz), 4.76 (1H, d, J = 12 Hz), 4.70 (1H, d, J = 12 Hz), 4.65 (1H, d, J = 12 Hz), 4.59 (1H, d, J = 12 Hz), 4.35 (2H, d, J = 7.0 Hz), 4.18 (1H, t, J = 7.0 Hz), 4.03 (1H, dd, J = 9.5, 3.5 Hz), 3.97 (1H, dd, J = 12, 8.5 Hz), 3.91–3.82 (6H, m), 3.71 (1H, br t, J = 5.0 Hz), 3.41 (1H, dd, J = 9.0, 6.5 Hz), 3.32 (1H, dd, J = 9.0, 6.0 Hz), 3.19 (3H, s), 1.52–1.46 (2H, m), 1.42–1.34 (1H, m), 1.34–1.18 (23H, m), 0.90 (9H, s), 0.89 (9H, s), 0.88 (3H, t, J = 7.0 Hz), 0.07 (3H, s), 0.05 (3H, s), 0.04 (3H, s), 0.03 (3H, s) ppm; HRMS (ESI+) m/z calcd for $C_{73}H_{107}NO_{10}Si_2Na$ [M+Na]$^+$ 1236.7326, found 1236.7326.

4.1.4. (2*S*,3*S*,4*R*)-2-Amino-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-octadecane 16

To a stirred solution of **14** (1.92 g, 1.58 mmol) in DMF (50 mL), piperidine (10 mL) was added at rt. After stirring at rt for 5 h, the mixture was diluted with EtOAc. The organic phase was then washed with water and brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on silica gel (40 g, hexane/EtOAc = 7:1) to give **16** (1.53 g, 98%) as a colorless oil, n_D^{21} 1.5062; $[\alpha]_D^{22}$ +31.5 (c 1.04, $CHCl_3$); ν_{max} (film): 3380 (w, NH), 3320 (w, NH), 1605 (w), 1585 (w), 1495 (m), 1255 (br s, *t*-Bu, Si–Me), 1100 (br s, C–O), 1060 (br s, C–O), 840 (br s), 780 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.23 (15H, m), 4.95 (1H, d, J = 12 Hz), 4.88 (1H, d, J = 4.0 Hz), 4.81 (1H, d, J = 12 Hz), 4.79 (1H, d, J = 12 Hz), 4.74 (1H, d, J = 12 Hz), 4.68 (1H, d, J = 12 Hz), 4.61 (1H, d, J = 12 Hz), 4.05 (1H, dd, J = 10, 4.0 Hz), 3.98 (1H, dd, J = 12, 2.0 Hz), 3.98–3.94 (1H, m), 3.94 (1H, dd, J = 10, 3.0 Hz), 3.90 (1H, br t, J = 6.5 Hz), 3.77–3.73 (1H, m), 3.50 (1H, br d, J = 6.5 Hz), 3.44 (1H, dd, J = 9.0, 7.5 Hz), 3.36 (1H, dd, J = 9.0, 5.5 Hz), 3.25 (3H, s), 3.21 (1H, br t, J = 9.0 Hz), 3.12–3.07 (1H, m), 1.60–1.45 (2H, m), 1.43–1.18 (24H, m), 0.894 (9H, s), 0.885 (9H, s), 0.88 (3H, t, J = 7.0 Hz), 0.079 (3H, s), 0.077 (3H, s), 0.07 (3H, s), 0.06 (3H, s) ppm; HRMS (ESI+) m/z calcd for $C_{58}H_{98}NO_8Si_2$ [M+H]$^+$ 992.6825, found 992.6825.

4.1.5. Preparation of ureides

4.1.5.1. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-tetracosylureido)octadecane-1,3,4-triol 19a. To a stirred solution of **15**[13] (211 mg, 0.197 mmol) in $CHCl_3$ (3 mL), a solution of isocyanate **8** (187 mg, 0.493 mmol) in $CHCl_3$ (2 mL) was added at 0 °C. After stirring at rt for 16 h, the mixture was poured into water, and extracted with EtOAc. The separated organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (20 g, hexane/EtOAc = 10:1) to give **19a** (276 mg, 97%) as a colorless oil, n_D^{25} 1.5010; $[\alpha]_D^{26}$ +26.1 (*c* 1.33, $CHCl_3$); ν_{max} (film): 3380 (m, NH), 1680 (m, CO), 1660 (m, CO), 1540 (m), 1255 (m, *t*-Bu, Si–Me), 1100 (br s, C–O), 1060 (br s, C–O), 835 (s), 755 (br s), 700 (m) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.23 (20H, m), 4.93 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 6.5 Hz), 4.83 (1H, d, *J* = 4.0 Hz), 4.80 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.46 (1H, d, *J* = 12 Hz), 4.43 (1H, d, *J* = 12 Hz), 4.34 (1H, br s), 4.04 (1H, dd, *J* = 9.5, 3.5 Hz), 3.97 (1H, t, *J* = 6.0 Hz), 3.95 (1H, dd, *J* = 11, 6.5 Hz), 3.90 (1H, dd, *J* = 11, 3.5 Hz), 3.89 (1H, s), 3.87 (1H, dd, *J* = 6.0, 1.5 Hz), 3.81 (1H, dd, *J* = 11, 3.5 Hz), 3.81–3.75 (1H, m), 3.72 (1H, dt, *J* = 6.0, 1.5 Hz), 3.57 (1H, dd, *J* = 9.5, 6.5 Hz), 3.38 (1H, dd, *J* = 9.5, 6.0 Hz), 2.98–2.87 (2H, m), 1.69 (2H, br s), 1.50 (2H, br q, *J* = 7.0 Hz), 1.44–1.16 (66H, m), 0.90 (9H, s), 0.884 (9H, s), 0.879 (6H, t, *J* = 7.0 Hz), 0.08 (3H, s), 0.06 (3H, s), 0.05 (3H, s), 0.03 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{89}H_{151}N_2O_9Si_2$ $[M+H]^+$ 1448.0953, found 1448.0943.

4.1.5.2. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-hexadecylureido)octadecane-1,2,3-triol 19b. In the same manner as described above, **15** (118 mmol, 0.110 mmol) was reacted with a commercially available hexadecyl isocyanate **17** (69 μL, 0.22 mmol) to give **19b** (133 mg, 90%) as a colorless oil, n_D^{25} 1.5061; $[\alpha]_D^{26}$ +26.8 (*c* 1.04, $CHCl_3$); ν_{max} (film): 3380 (m, NH), 1685 (m, CO), 1660 (m, CO), 1540 (br m), 1250 (m, *t*-Bu, Si–Me), 1100 (br s, C–O), 1080 (br s, C–O), 835 (s), 775 (s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.24 (20H, m), 4.93 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 6.5 Hz), 4.83 (1H, d, *J* = 3.5 Hz), 4.80 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 12 Hz), 4.56 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.43 (1H, d, *J* = 12 Hz), 4.33 (1H, br s), 4.04 (1H, dd, *J* = 9.5, 3.5 Hz), 3.99–3.92 (2H, m), 3.92–3.88 (1H, m), 3.89 (1H, s), 3.87 (1H, dd, *J* = 6.0, 2.0 Hz), 3.83–3.74 (2H, m), 3.74–3.70 (1H, m), 3.57 (1H, dd, *J* = 9.5, 6.0 Hz), 3.38 (1H, dd, *J* = 9.5, 6.0 Hz), 2.97–2.88 (2H, m), 1.54–1.46 (2H, m), 1.44–1.16 (52H, m), 0.90 (9H, s), 0.89 (9H, s), 0.88 (6H, t, *J* = 7.0 Hz), 0.08 (3H, s), 0.06 (3H, s), 0.05 (3H, s), 0.03 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{81}H_{135}N_2O_9Si_2$ $[M+H]^+$ 1335.9701, found 1335.9699.

4.1.5.3. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-octylureido)octadecane-1,3,4-triol 19c. In the same manner as described above, **15** (205 mmol, 0.192 mmol) was reacted with a commercially available octyl isocyanate **18** (102 μL, 0.578 mmol) to give **19c** (170 mg, 72%) as a colorless oil, n_D^{26} 1.5092; $[\alpha]_D^{24}$ +28.4 (*c* 0.86, $CHCl_3$); ν_{max} (film): 3380 (m, NH), 1680 (m, CO), 1660 (m), 1540 (br m), 1495 (w), 1255 (m, *t*-Bu, Si–Me), 1100 (br s, C–O), 1060 (br s, C–O), 835 (s), 780 (m), 750 (m), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.45–7.24 (20H, m), 4.93 (1H, d, *J* = 12 Hz), 4.88 (1H, d, *J* = 6.5 Hz), 4.83 (1H, d, *J* = 4.0 Hz), 4.80 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.63 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.46 (1H, d, *J* = 12 Hz), 4.43 (1H, d, *J* = 12 Hz), 4.32 (1H, br s), 4.04 (1H, dd, *J* = 9.5, 3.5 Hz), 3.99–3.92 (2H, m), 3.92–3.88 (1H, m), 3.89 (1H, s), 3.86 (1H, dd, *J* = 6.0, 2.0 Hz), 3.81 (1H, dd, *J* = 11, 3.5 Hz), 3.77 (1H, dd, *J* = 6.0, 3.5 Hz), 3.73–3.70 (1H, m), 3.57 (1H, dd, *J* = 9.5, 7.0 Hz), 3.38 (1H, dd, *J* = 9.5, 6.0 Hz), 2.97–2.88 (2H, m), 1.52–1.47 (2H, m), 1.44–1.16 (36H, m), 0.90 (9H, s), 0.888 (9H, s), 0.879 (3H, t, *J* = 7.0 Hz), 0.87 (3H, t, *J* = 7.0 Hz), 0.08 (3H, s), 0.06 (3H, s), 0.05 (3H, s), 0.03 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{73}H_{119}N_2O_9Si_2$ $[M+H]^+$ 1223.8449, found 1223.8444.

4.1.5.4. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-(3-tetradecylureido)octadecane-1,3,4-triol 19d. In the same manner as described above, **16** (153 mg, 0.154 mmol) was reacted with isocyanate **8** (198 mg, 0.522 mmol) to give **19d** (196 mg, 93%) as a colorless oil, n_D^{26} 1.4970; $[\alpha]_D^{23}$ +29.9 (*c* 1.03, $CHCl_3$); ν_{max} (film): 3380 (m, NH), 1690 (m, CO), 1660 (br m, CO), 1550 (br s), 1250 (m, *t*-Bu, Si-Me), 1100 (br s, C–O), 1060 (br s, C–O), 835 (s), 780 (m), 730 (m), 695 (m) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.40–7.26 (15H, m), 5.01–4.80 (1H, m), 4.98 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 12 Hz), 4.82 (1H, d, *J* = 12 Hz), 4.80 (1H, d, *J* = 4.0 Hz), 4.79 (1H, d, *J* = 12 Hz), 4.72 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.60 (1H, d, *J* = 12 Hz), 4.51 (1H, br s), 4.18–4.13 (1H, m), 4.04 (1H, dd, *J* = 10, 3.5 Hz), 3.94–3.91 (1H, m), 3.89 (1H, dd, *J* = 10, 3.0 Hz), 3.83–3.82 (1H, m), 3.74–3.69 (3H, m), 3.61–3.58 (1H, m), 3.57 (1H, dd, *J* = 10, 8.0 Hz), 3.28 (3H, s), 3.20 (1H, dd, *J* = 10, 4.0 Hz), 3.10 (1H, dq, *J* = 14, 7.0 Hz), 2.98 (1H, dq, *J* = 14, 7.0 Hz), 1.51–1.43 (2H, m), 1.43–1.22 (68H, m), 0.90 (9H, s), 0.884 (9H, s), 0.879 (6H, t, *J* = 7.0 Hz), 0.08 (3H, s), 0.05 (3H, s), 0.02 (6H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{83}H_{147}N_2O_9Si_2$ $[M+H]^+$ 1372.0640, found 1372.0642.

4.1.5.5. (2*S*,3*S*,4*R*)-1-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-3,4-bis-*O*-(*tert*-butyldimethylsilyl)-2-[3-(5-phenylpentyl)-ureido]octadecane-1,3,4-triol 19e. In the same manner as described above, **15** (190 mmol, 0.178 mmol) was reacted with isocyanate **10** (101 mg, 0.534 mmol) to give **19e** (146 mg, 65%) as a colorless oil, n_D^{26} 1.5173; $[\alpha]_D^{23}$ +26.7 (*c* 1.10, $CHCl_3$); ν_{max} (film): 3380 (m, NH), 1680 (br s, CO), 1660 (br s, CO), 1605 (w, aromat.), 1540 (br s), 1500 (m, aromat.), 1250 (s, *t*-Bu, Si-Me), 1100 (br s, C–O), 1060 (br s, C–O), 835 (s), 780 (s), 735 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.36–7.24 (22H, m), 7.20–7.09 (3H, m), 4.92 (1H, d, *J* = 12 Hz), 4.89 (1H, d, *J* = 6.5 Hz), 4.82 (1H, d, *J* = 3.5 Hz), 4.79 (2H, d, *J* = 12 Hz), 4.71 (1H, d, *J* = 12 Hz), 4.62 (1H, d, *J* = 12 Hz), 4.55 (1H, d, *J* = 12 Hz), 4.45 (1H, d, *J* = 12 Hz), 4.42 (1H, d, *J* = 12 Hz), 4.33 (1H, br s), 4.04 (1H, dd, *J* = 9.5, 3.5 Hz), 3.97–3.94 (2H, m), 3.91–3.87 (1H, m), 3.88 (1H, s), 3.86 (1H, dd, *J* = 6.0, 2.5 Hz), 3.82–3.74 (2H, m), 3.73–3.70 (1H, m), 3.57 (1H, dd, *J* = 9.5, 7.0 Hz), 3.37 (1H, dd, *J* = 9.5, 6.0 Hz), 2.95–2.86 (2H, m), 2.54 (2H, t, *J* = 7.5 Hz), 1.58–1.47 (4H, m), 1.44–1.20 (28H, m), 0.90 (9H, s), 0.883 (9H, s), 0.879 (3H, t, *J* = 7.0 Hz), 0.08 (3H, s), 0.06 (3H, s), 0.04 (3H, s), 0.03 (3H, s) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{76}H_{117}N_2O_9Si_2$ $[M+H]^+$ 1257.8292, found 1257.8292.

4.1.6. Desilylation

4.1.6.1. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(3-tetracosylureido)octadecane-1,3,4-triol 19a′. To a stirred solution of **19a** (174 mg, 0.120 mmol) in THF (5 mL), a solution of tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 1.2 mL, 1.2 mmol) was added at rt. After stirring at rt for 17 h, the mixture was poured into water, and extracted with EtOAc. The separated organic phase was successively washed with water, a saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (15 g, hexane/EtOAc = 3:2) to give **19a′** (136 mg, 93%) as colorless powder. Mp 71.0–72.5 °C; $[\alpha]_D^{22}$ +23.2 (*c* 1.10, $CHCl_3$); ν_{max} (KBr): 3360 (br s, OH, NH), 1630 (br s,

CO), 1570 (br s), 1110 (br s, C–O), 1040 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.24 (20H, m), 5.32 (1H, d, J = 7.5 Hz), 4.92 (1H, d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.83 (1H, d, J = 4.0 Hz), 4.78 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 12 Hz), 4.48 (1H, d, J = 12 Hz), 4.39 (1H, d, J = 12 Hz), 4.35 (1H, t, J = 5.5 Hz), 4.17 (1H, d, J = 7.0 Hz), 4.04 (1H, dd, J = 10, 4.0 Hz), 3.99–3.92 (4H, m), 3.88–3.83 (2H, m), 3.55–3.49 (2H, m), 3.54 (1H, dd, J = 9.5, 6.5 Hz), 3.45 (1H, dd, J = 9.5, 6.0 Hz), 3.08–2.97 (2H, m), 2.39 (1H, d, J = 5.0 Hz), 1.66–1.58 (1H, m), 1.52–1.44 (1H, m), 1.42–1.20 (68H, m), 0.88 (6H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{77}H_{123}N_2O_9$ $[M+H]^+$ 1219.9223, found 1219.9212.

4.1.6.2. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(3-hexadecylureido)octadecane-1,3,4-triol

19b′. In the same manner as described above, **19b** (145 mg, 0.109 mmol) was converted to **19b′** (113 mg, 93%) as a colorless solid. Mp 64.5–66.0 °C; $[\alpha]_D^{26}$ +30.0 (c 1.20, $CHCl_3$); ν_{max} (KBr): 3370 (br s, OH, NH), 1635 (br s, CO), 1570 (br s), 1495 (w, aromat.), 1110 (br s, C–O), 1040 (br s, C–O), 730 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.25 (20H, m), 5.37 (1H, br d, J = 7.5 Hz), 4.92 (1H, d, J = 12 Hz), 4.84 (1H, d, J = 12 Hz), 4.83 (1H, d, J = 3.5 Hz), 4.78 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 12 Hz), 4.48 (1H, d, J = 12 Hz), 4.44 (1H, br t, J = 5.5 Hz), 4.39 (1H, d, J = 12 Hz), 4.16 (1H, br s), 4.04 (1H, dd, J = 10, 3.5 Hz), 3.99–3.91 (4H, m), 3.88–3.84 (2H, m), 3.56–3.48 (2H, m), 3.54 (1H, dd, J = 9.5, 7.0 Hz), 3.45 (1H, dd, J = 9.5, 6.0 Hz), 3.08–2.98 (2H, m), 2.52 (1H, br s), 1.66–1.58 (1H, m), 1.54–1.44 (1H, m), 1.42–1.20 (52H, m), 0.88 (6H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{69}H_{107}N_2O_9$ $[M+H]^+$ 1107.7971, found 1107.7963.

4.1.6.3. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)-2-(3-octylureido)octadecane-1,3,4-triol 19c′.

In the same manner as described above, **19c** (131 mg, 0.107 mmol) was converted to **19c′** (98 mg, 92%) as a colorless solid. Mp 61.0–63.0 °C; $[\alpha]_D^{22}$ +29.2 (c 1.09, $CHCl_3$); ν_{max} (KBr): 3360 (br s, OH, NH), 1635 (br s, CO), 1565 (br s), 1500 (w, aromat.), 1040 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.25 (20H, m), 5.36 (1H, d, J = 7.5 Hz), 4.92 (1H, d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.83 (1H, d, J = 3.5 Hz), 4.78 (1H, d, J = 12 Hz), 4.73 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 12 Hz), 4.48 (1H, d, J = 12 Hz), 4.41 (1H, t, J = 5.5 Hz), 4.39 (1H, d, J = 12 Hz), 4.20 (1H, d, J = 6.5 Hz), 4.04 (1H, dd, J = 10, 4.0 Hz), 3.99–3.92 (4H, m), 3.88–3.84 (2H, m), 3.55–3.49 (2H, m), 3.54 (1H, dd, J = 9.5, 6.5 Hz), 3.45 (1H, dd, J = 9.5, 6.0 Hz), 3.08–2.98 (2H, m), 2.48 (1H, br s), 1.65–1.58 (1H, m), 1.53–1.44 (1H, m), 1.41–1.21 (36H, m), 0.88 (3H, t, J = 7.0 Hz), 0.87 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{61}H_{91}N_2O_9$ $[M+H]^+$ 995.6719, found 995.6716.

4.1.6.4. (2*S*,3*S*,4*R*)-1-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-methyl-α-D-galactopyranosyl)-2-(3-tetracosylureido)octadecane-1,3,4-triol

19d′. In the same manner as described above, **19d** (142 mg, 0.103 mmol) was converted to **19d′** (118 mg, quant.) as colorless powder. Mp 94.5–96.0 °C; $[\alpha]_D^{23}$ +30.1 (c 1.21, $CHCl_3$); ν_{max} (KBr): 3400 (br s, OH, NH), 1630 (br s, CO), 1560 (br s), 1100 (br s, C–O), 1060 (br s, C–O), 720 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.26 (15H, m), 5.36 (1H, d, J = 7.5 Hz), 4.94 (1H, d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.82 (1H, d, J = 4.0 Hz), 4.80 (1H, d, J = 12 Hz), 4.74 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.60 (1H, d, J = 12 Hz), 4.52 (1H, d, J = 5.5 Hz), 4.11 (1H, d, J = 7.0 Hz), 4.04 (1H, dd, J = 10, 3.5 Hz), 4.01–3.94 (2H, m), 3.90–3.81 (4H, m), 3.55–3.50 (2H, m), 3.46 (1H, dd, J = 9.5, 7.0 Hz), 3.30 (1H, dd, J = 9.5, 5.5 Hz), 3.28 (3H, s), 3.08 (2H, br q, J = 6.5 Hz), 2.54 (1H, d, J = 4.0 Hz), 1.66–1.60 (1H, m), 1.53–1.46 (1H, m), 1.42 (2H, quint., J = 6.5 Hz), 1.38–1.22 (68H, m), 0.88 (6H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{71}H_{119}N_2O_9$ $[M+H]^+$ 1143.8910, found 1143.8912.

4.1.6.5. (2*S*,3*S*,4*R*)-2-[3-(5-Phenylpentyl)ureido]-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol

19e′. In the same manner as described above, **19e** (104 mg, 0.0827 mmol) was converted to **19e′** (79 mg, 93%) as a colorless solid. Mp 49.5–51.0 °C; $[\alpha]_D^{23}$ +27.1 (c 0.87, $CHCl_3$); ν_{max} (KBr): 3360 (br s, OH, NH), 1630 (s, CO), 1570 (br s), 1495 (m, aromat.), 1110 (br s, C–O), 1045 (br s, C–O), 740 (br s), 700 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.38–7.24 (22H, m), 7.18–7.13 (3H, m), 5.31 (1H, d, J = 7.5 Hz), 4.92 (1H, d, J = 12 Hz), 4.85 (1H, d, J = 12 Hz), 4.82 (1H, d, J = 3.5 Hz), 4.78 (1H, d, J = 12 Hz), 4.72 (1H, d, J = 12 Hz), 4.66 (1H, d, J = 12 Hz), 4.56 (1H, d, J = 12 Hz), 4.47 (1H, d, J = 12 Hz), 4.38 (1H, d, J = 12 Hz), 4.34 (1H, t, J = 5.5 Hz), 4.17 (1H, br d, J = 5.0 Hz), 4.03 (1H, dd, J = 10, 4.0 Hz), 3.99–3.91 (4H, m), 3.88–3.84 (2H, m), 3.55–3.49 (2H, m), 3.54 (1H, dd, J = 9.5, 6.5 Hz), 3.44 (1H, dd, J = 9.5, 6.0 Hz), 3.08–2.98 (2H, m), 2.57 (2H, t, J = 7.5 Hz), 2.36 (1H, br s), 1.66–1.58 (1H, m), 1.59 (2H, quint., J = 7.5 Hz), 1.53–1.43 (1H, m), 1.40 (2H, quint., J = 7.5 Hz), 1.40–1.21 (26H, m), 0.88 (3H, t, J = 7.0 Hz) ppm; HRMS (ESI+) m/z calcd for $C_{64}H_{89}N_2O_9$ $[M+H]^+$ 1029.6563, found 1029.6560.

4.1.7. Hydrogenolysis

4.1.7.1. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(3-tetracosylureido)octadecane-1,3,4-triol 1 (RCAI-105).

A mixture of **19a′** (105 mg, 0.0861 mmol) and $Pd(OH)_2$-C (20%, wet, 40 mg) in EtOH–$CHCl_3$ (4:1, 10 mL) was stirred at rt under hydrogen atmosphere (balloon) for 13 h. The mixture was then filtered through a bed of Celite, and the filter cake was washed with $CHCl_3$–MeOH (5:1). The combined filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (10 g, $CHCl_3$/MeOH = 50:6) to give **1** (58 mg, 78%) as colorless powder. Mp 170–172 °C; $[\alpha]_D^{26}$ +46.5 (c 0.30, pyridine); ν_{max} (KBr): 3360 (br s, OH, NH), 1635 (br m, CO), 1570 (br m), 1070 (br s, C–O) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 6.79 (1H, t, J = 6.0 Hz), 6.75 (1H, d, J = 9.0 Hz), 6.44–5.90 (6H, m), 5.54 (1H, d, J = 4.0 Hz), 5.09–5.04 (1H, m), 4.60 (1H, dd, J = 9.5, 4.0 Hz), 4.58 (1H, dd, J = 11, 5.0 Hz), 4.48 (1H, br d, J = 4.0 Hz), 4.46–4.36 (3H, m), 4.34 (1H, dd, J = 11, 5.0 Hz), 4.32 (1H, dd, J = 9.5, 3.0 Hz), 4.28–4.23 (2H, m), 3.48 (1H, ddt, J = 13, 7.5, 6.0 Hz), 3.40 (1H, ddt, J = 13, 7.5, 6.0 Hz), 2.28–2.21 (1H, m), 1.91–1.78 (2H, m), 1.68–1.58 (1H, m), 1.55 (2H, quint., J = 7.5 Hz), 1.44–1.16 (64H, m), 0.85 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 159.5, 101.6, 77.4, 73.0, 72.8, 71.6, 70.9, 70.2, 69.7, 62.7, 51.9, 40.6, 34.7, 32.1, 31.0, 30.3, 30.1, 30.01, 30.00, 29.98, 29.96, 29.93, 29.91, 29.89, 29.7, 29.59, 29.58, 27.4, 26.5, 22.9, 14.3 ppm; HRMS (ESI+) m/z calcd for $C_{49}H_{99}N_2O_9$ $[M+H]^+$ 859.7345, found 859.7346.

4.1.7.2. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(3-hexadecylureido)octadecane-1,3,4-triol 2 (RCAI-84).

In the same manner as described above, **19b′** (81 mg, 0.073 mmol) was converted to **2** (48 mg, 88%) as a colorless solid, mp 168–170 °C; $[\alpha]_D^{26}$ +55.7 (c 0.30, pyridine); ν_{max} (KBr): 3320 (br s, OH, NH), 1630 (br s, CO), 1570 (br s), 1070 (br s, C–O), 1030 (br s, C–O) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 6.81 (1H, t, J = 5.5 Hz), 6.79 (1H, d, J = 9.5 Hz), 6.66–5.70 (6H, m), 5.54 (1H, d, J = 4.0 Hz), 5.10–5.05 (1H, m), 4.61 (1H, dd, J = 9.5, 3.5 Hz), 4.59 (1H, dd, J = 11, 4.5 Hz), 4.48 (1H, d, J = 3.0 Hz), 4.47–4.42 (2H, m), 4.41–4.33 (2H, m), 4.37 (1H, dd, J = 11, 6.0 Hz), 4.28–4.23 (2H, m), 3.48 (1H, ddt, J = 13, 7.5, 5.5 Hz), 3.40 (1H, ddt, J = 13, 7.5, 5.5 Hz), 2.28–2.21 (1H, m), 1.92–1.78 (2H, m), 1.66–1.56 (1H, m), 1.55 (2H, quint., J = 7.5 Hz), 1.43–1.15 (48H, m), 0.84 (6H, t, J = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 159.5, 101.6, 77.4, 73.0, 72.8, 71.6, 70.9, 70.2, 69.7, 62.7, 52.0, 40.6, 34.7, 32.1, 31.0, 30.4, 30.1,

30.01, 29.98, 29.92, 29.91, 29.7, 29.6, 27.4, 26.5, 22.9, 14.3 ppm; HRMS (ESI+) *m*/*z* calcd for $C_{41}H_{83}N_2O_9$ $[M+H]^+$ 747.6093, found 747.6091.

4.1.7.3. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(3-octylureido)octadecane-1,3,4-triol 3 (RCAI-107). In the same manner as described above, **19c′** (73 mg, 0.073 mmol) was converted to **3** (34 mg, 72%) as a colorless solid. Mp 150–151 °C; $[\alpha]_D^{22}$ +56.5 (*c* 0.32, pyridine); ν_{max} (KBr): 3360 (br s, OH, NH), 1640 (br s, CO), 1570 (br s), 1070 (br s, C–O) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 7.02 (1H, br s), 6.80 (1H, t, *J* = 6.0 Hz), 6.76 (1H, d, *J* = 9.0 Hz), 6.63 (2H, br s), 6.37 (1H, br s), 6.34 (1H, br s), 6.05 (1H, br s), 5.55 (1H, d, *J* = 4.0 Hz), 5.15–5.06 (1H, m), 4.61 (1H, dd, *J* = 8.0, 4.0 Hz), 4.59 (1H, dd, *J* = 10, 4.5 Hz), 4.49 (1H, d, *J* = 3.0 Hz), 4.47–4.35 (3H, m), 4.37 (1H, dd, *J* = 10, 5.5 Hz), 4.33 (1H, dd, *J* = 10, 3.0 Hz), 4.30–4.22 (2H, m), 3.46 (1H, ddt, *J* = 13, 7.0, 6.0 Hz), 3.39 (1H, ddt, *J* = 13, 7.0, 6.0 Hz), 2.30–2.22 (1H, m), 1.91–1.78 (2H, m), 1.66–1.56 (1H, m), 1.52 (2H, quint., *J* = 7.0 Hz), 1.42–1.08 (32H, m), 0.84 (3H, t, *J* = 7.0 Hz), 0.79 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 159.5, 101.6, 77.4, 73.0, 72.8, 71.6, 70.9, 70.2, 69.7, 62.7, 52.0, 40.6, 34.7, 32.1, 32.0, 31.0, 30.4, 30.1, 30.01, 30.00, 29.97, 29.96, 29.90, 29.59, 29.58, 29.5, 27.3, 26.5, 22.9, 22.8, 14.3, 14.2 ppm; HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{67}N_2O_9$ $[M+H]^+$ 635.4841, found 635.4841.

4.1.7.4. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-Methyl-α-D-galactopyranosyl)-2-(3-tetracosylureido)octadecane-1,3,4-triol 4 (RCAI-106). In the same manner as described above, **19d′** (83 mg, 0.0724 mmol) was converted to **4** (54 mg, 85%) as colorless powder. Mp 99–102 °C; $[\alpha]_D^{25}$ +50.2 (*c* 0.29, pyridine); ν_{max} (KBr): 3340 (br s, OH, NH), 1640 (br s, CO), 1570 (br s), 1070 (br s, C–O), 1035 (br s, C–O) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 7.04 (1H, br s), 6.77 (1H, t, *J* = 5.5 Hz), 6.68 (1H, d, *J* = 9.0 Hz), 6.63 (1H, br s), 6.29 (2H, br s), 6.01 (1H, d, *J* = 5.5 Hz), 5.51 (1H, d, *J* = 4.0 Hz), 5.09 (1H, dq, *J* = 8.5, 4.0 Hz), 4.60 (1H, dd, *J* = 11, 5.5 Hz), 4.57 (1H, dd, *J* = 10, 4.0 Hz), 4.42–4.36 (2H, m), 4.33–4.21 (4H, m), 3.95 (1H, dd, *J* = 10, 5.5 Hz), 3.90 (1H, dd, *J* = 10, 7.0 Hz), 3.48 (1H, ddt, *J* = 13, 7.0, 5.5 Hz), 3.40 (1H, ddt, *J* = 13, 7.0, 5.5 Hz), 3.32 (3H, s), 2.29–2.21 (1H, m), 1.92–1.78 (2H, m), 1.67–1.58 (1H, m), 1.56 (2H, quint., *J* = 7.0 Hz), 1.44–1.17 (64H, m), 0.84 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 159.3, 101.5, 77.3, 72.9, 72.8, 71.3, 70.7, 70.6, 70.0, 69.6, 58.8, 51.8, 40.6, 34.8, 32.1, 31.0, 30.3, 30.1, 30.01, 30.00, 29.98, 29.96, 29.94, 29.93, 29.91, 29.90, 29.7, 29.60, 29.59, 27.4, 26.4, 22.9, 14.3 ppm; HRMS (ESI+) *m*/*z* calcd for $C_{50}H_{101}N_2O_9$ $[M+H]^+$ 873.7502, found 873.7503.

4.1.7.5. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-[3-(5-phenylpentyl)ureido]octadecane-1,3,4-triol 5 (RCAI-108). In the same manner as described above, **19e′** (57 mg, 0.055 mmol) was converted to **5** (31 mg, 84%) as a colorless solid, mp 124–125 °C; $[\alpha]_D^{25}$ +58.4 (*c* 0.31, pyridine): ν_{max} (KBr): 3360 (br s, OH, NH), 1640 (br s, CO), 1570 (br s), 1070 (br s, C–O), 700 (m) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 7.29 (2H, ddt, *J* = 7.5, 1.5, 1.0 Hz), 7.21–7.14 (3H, m), 7.02 (1H, br s), 6.80 (1H, t, *J* = 5.5 Hz), 6.75 (1H, d, *J* = 9.0 Hz), 6.34 (2H, br s), 6.37 (1H, br s), 6.34 (1H, br s), 6.07 (1H, br s), 5.56 (1H, d, *J* = 3.5 Hz), 5.11–5.06 (1H, m), 4.62 (1H, dd, *J* = 10, 4.0 Hz), 4.60 (1H, dd, *J* = 10, 5.0 Hz), 4.49 (1H, d, *J* = 2.0), 4.47–4.36 (3H, m), 4.38 (1H, dd, *J* = 10, 6.0 Hz), 4.33 (1H, dd, *J* = 10, 4.0 Hz), 4.30–4.22 (2H, m), 3.42 (1H, ddt, *J* = 13, 7.0, 5.5 Hz), 3.36 (1H, ddt, *J* = 13, 7.0, 5.5 Hz), 2.45 (2H, t, *J* = 7.0 Hz), 2.30–2.22 (1H, m), 1.91–1.78 (2H, m), 1.66–1.56 (1H, m), 1.52 (2H, quint., *J* = 7.0 Hz), 1.47 (2H, quint., *J* = 7.0 Hz), 1.42–1.17 (24H, m), 0.84 (3H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 159.4, 143.0, 128.8, 128.6, 126.0, 101.6, 77.4, 73.0, 72.7, 71.5, 70.9, 70.2, 69.6, 62.6, 51.9, 40.4, 36.0, 34.7, 32.1, 31.5, 30.8, 30.3, 30.1, 29.99, 29.98, 29.95, 29.94, 29.88, 29.6, 26.9, 26.4, 22.9, 14.2 ppm; HRMS (ESI+) *m*/*z* calcd for $C_{36}H_{65}N_2O_9$ $[M+H]^+$ 669.4685, found 669.4685.

4.1.8. (2*S*,3*S*,4*R*)-2-Amino-1-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)octadecane-1,3,4-triol 20

In the same manner as described previously for the conversion of **19a** to **19a′**, **20** (311 mg, 98%) was synthesized from **15** (402 mg, 0.376 mmol) as a colorless solid. Mp 45–48 °C; $[\alpha]_D^{22}$ +33.4 (*c* 1.01, $CHCl_3$); ν_{max} (KBr): 3280 (br s, OH, NH), 1600 (br m), 1500 (m), 1100 (br s, C–O), 1050 (br s, C–O), 740 (br s), 695 (s) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 7.39–7.22 (20H, m), 4.92 (1H, d, *J* = 12 Hz), 4.86 (1H, d, *J* = 12 Hz), 4.79 (1H, d, *J* = 3.5 Hz), 4.78 (1H, d, *J* = 12 Hz), 4.74 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.56 (1H, d, *J* = 12 Hz), 4.47 (1H, d, *J* = 12 Hz), 4.40 (1H, d, *J* = 12 Hz), 4.04 (1H, dd, *J* = 10, 3.5 Hz), 3.96 (1H, br d, *J* = 3.0 Hz), 3.93 (1H, br t, *J* = 6.5 Hz), 3.90 (1H, dd, *J* = 10, 3.0 Hz), 3.85 (1H, dd, *J* = 10, 3.5 Hz), 3.61 (1H, dd, *J* = 10, 4.5 Hz), 3.57 (1H, dt, *J* = 8.5, 2.5 Hz), 3.52 (1H, dd, *J* = 9.5, 6.0 Hz), 3.49 (1H, dd, *J* = 9.5, 7.0 Hz), 3.35 (1H, br t, *J* = 7.0 Hz), 3.14–3.10 (1H, m), 2.54 (3H, br s), 1.69–1.63 (1H, m), 1.54–1.46 (1H, m), 1.38–1.18 (24H, m), 0.88 (3H, t, *J* = 7.0 Hz) ppm; HRMS (ESI+) *m*/*z* calcd for $C_{52}H_{74}NO_8$ $[M+H]^+$ 840.5409, found 840.5404.

4.1.9. (2*S*,3*S*,4*R*)-1-*O*-(α-D-Galactopyranosyl)-2-(3-hexadecylthioureido)octadecane-1,3,4-triol 6 (RCAI-91)

In the same manner as described previously for the conversion of **19a′** to **1**, crude **21** (65 mg) was synthesized from **20** (82 mg, 0.098 mmol) as a colorless solid. The obtained crude **21** was immediately used in the next step without further purification.

To a stirred and cooled solution of **21** (65 mg) in EtOH–$CHCl_3$ (2:1, 6 mL), hexadecyl isothiocyanate (**22**, 94 μL, 0.29 mmol) and Et_3N (41 μL, 0.30 mmol) were added at 0 °C. After stirring at room temperature for 18 h, the mixture was concentrated in vacuo. The residual solid was successively washed with water, and water–MeOH (2:1), and then dried. The resulting solid was purified by column chromatography on silica gel (7 g, $CHCl_3$/MeOH = 10:1) to give **6** (33 mg, 44%, two steps) as a colorless solid, Mp 153–156 °C; $[\alpha]_D^{26}$ +44.1 (*c* 0.32, pyridine); ν_{max} (KBr): 3300 (br s, OH), 3120 (w, NH), 1560 (br s), 1345 (br m), 1150 (m), 1065 (br s, C–O), 1035 (br s, C–O) cm^{-1}; δ_H (500 MHz, pyridine-d_5): 8.47 (1H, br s), 7.93 (1H, br s), 7.28 (1H, br s), 6.57 (1H, br s), 6.29 (1H, br s), 6.16 (1H, br s), 5.84 (1H, br s), 5.73 (1H, br s), 4.66 (1H, dd, *J* = 11, 5.5 Hz), 4.62 (1H, dd, *J* = 11, 3.5 Hz), 4.54–4.46 (2H, m), 4.44–4.39 (1H, m), 4.39–4.34 (2H, m), 4.34–4.28 (5H, m), 3.92–3.70 (2H, m), 2.30–2.21 (1H, m), 1.91–1.77 (2H, m), 1.67–1.57 (3H, m), 1.41–1.14 (48H, m), 0.84 (6H, t, *J* = 7.0 Hz) ppm; δ_C (126 MHz, pyridine-d_5): 183.8, 101.3, 76.6, 73.0, 72.4, 71.5, 70.9, 70.1, 68.5, 62.6, 55.6, 44.9, 34.7, 32.1, 30.3, 30.1, 29.97, 29.95, 29.92, 29.88, 29.85, 29.84, 29.7, 29.64, 29.57, 27.4, 26.3, 22.9, 14.2 ppm; HRMS (ESI+) *m*/*z* calcd for $C_{41}H_{83}N_2O_8S$ $[M+H]^+$ 763.5865, found 763.5860.

4.2. Pharmacology

4.2.1. Mice

C57BL/6J mice were purchased from Charles River Japan, Inc. or Clea Japan, Inc. Mice were kept under specific pathogen-free conditions and used at 8 weeks of age.[24]

4.2.2. Preparation of solutions of glycolipids

KRN7000 (**A**) and synthesized glycolipidses (**1-6**) (1.0 mg) were dissolved in dimethyl sulfoxide (DMSO, 1.0 mL) at 80 °C.[25] After 30 min at 80 °C, the solutions were cooled to rt, and diluted to 200 μg/mL with Dulbecco's phosphate-buffered saline (PBS, Invitrogen™) containing 0.5% Tween20 (polymethylene sorbitan monolaurate). The obtained solutions were diluted to 10 μg/mL with PBS (Invitrogen™) in just before injection into mice.

4.2.3. In vivo experiment

Each glycolipid solution (10 μg/mL, 200 μL) was administered intravenously. Peripheral blood was collected from the retro-orbital plexus of mice at 3, 6, 12, 24, 36, 48, and 60 h using heparin-coated capillary tubes (Hirschmann Laborgeräte GmbH & Co. KG), and plasma was prepared.

4.2.4. Cytokine measurement

The cytokine concentrations in plasma were measured by mouse IFN-γ ELISA kit (Thermo Fisher Scientific K.K.) for IFN-γ, and cytometric bead array (CBA) system (BD Bioscience) for IL-4 and IL-12p70 according to the manufacturer's protocol.

Acknowledgments

We thank Dr. R. Nakagawa (University of Yamanashi) and Mrs S. Suzuki (née Inoue, RIKEN RCAI) for their preliminary contribution. Our thanks are due to Drs. T. Nakamura and Y. Hongo (RIKEN) for HRMS analyses. We are grateful to Dr. M. Shiozaki (RIKEN RCAI), for his helpful comments. We thank one of the reviewers for bringing ref.38 to our attention. This work was partly supported by Mizutani Foundation for Glycoscience.

Supplementary data

Supplementary data (results of bioassay of RCAI-91 (**6**) and ^{1}H- and ^{13}C-NMR spectra of synthesized analogs (**1**–**6**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.073.

References and notes

1. Tashiro, T.; Ishii, Y.; Shigeura, T.; Nakagawa, R.; Watarai, H.; Taniguchi, M.; Mori, K. *Med. Chem. Commun.* **2011**, *2*, 620.
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. 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.
4. Taniguchi, M.; Harada, M.; Kojo, S.; Nakayama, T.; Wakao, H. *Annu. Rev. Immunol.* **2003**, *21*, 483.
5. Godfrey, D. I.; Kronenberg, M. *J. Clin. Invest.* **2004**, *114*, 1379.
6. Kronenberg, M. *Annu. Rev. Immunol.* **2005**, *23*, 877.
7. (a) Franck, R. W. *C.R. Chimie* **2012**, *15*, 46; (b) Banchet-Cadeddu, A.; Hénon, E.; Dauchez, M.; Renault, J.-H.; Monneaux, F.; Haudrechy, A. *Org. Biomol. Chem.* **2011**, *9*, 3080; (c) Mori, K.; Tashiro, T. *Heterocycles* **2011**, *83*, 951; (d) Murphy, N.; Zhu, X.; Schmidt, R. R. *Carbohydr. Chem.* **2010**, *36*, 64; (e) Wu, D.; Fujio, M.; Wong, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 1073; (f) Franck, R. W.; Tsuji, M. *Acc. Chem. Res.* **2006**, *39*, 692; (g) Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* **2006**, *35*, 771; (h) Berkers, C. R.; Ovaa, H. *Trends Pharm. Sci.* **2005**, *26*, 252.
8. Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531.
9. Baek, D. J.; Lee, Y.-S.; Lim, C.; Lee, D.; Lee, T.; Lee, J.-Y.; Lee, K.-A.; Cho, W.-J.; Kang, C.-Y.; Kim, S. *Chem. Asian J.* **2010**, *5*, 1560.
10. (a) Wu, T.-N.; Lin, K.-H.; Chang, Y.-J.; Huang, J.-R.; Cheng, J.-Y.; Yu, A. L.; Wong, C.-H. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 17275; (b) Li, X.; Fujio, M.; Imamura, M.; Wu, D.; Vasan, S.; Wong, C.-H.; Ho, D. D.; Tsuji, M. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13010.
11. Lee, T.; Cho, M.; Ko, S.-Y.; Youn, H.-J.; Baek, D. J.; Cho, W.-J.; Kang, C.-Y.; Kim, S. *J. Med. Chem.* **2007**, *50*, 585.
12. Shiozaki, M.; Tashiro, T.; Koshino, H.; Nakagawa, R.; Inoue, S.; Shigeura, T.; Watarai, H.; Taniguchi, M.; Mori, K. *Carbohydr. Res.* **2010**, *345*, 1663.
13. Tashiro, T.; Hongo, N.; Nakagawa, R.; Seino, K.; Watarai, H.; Ishii, Y.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2008**, *16*, 8896.
14. Leung, L.; Tomassi, C.; Van Beneden, K.; Decruy, T.; Trappeniers, M.; Elewaut, D.; Gao, Y.; Elliott, T.; Al-Shamkhani, A.; Ottensmeier, C.; Werner, J. M.; Williams, A.; Van Calenbergh, S.; Linclau, B. *ChemMedChem* **2009**, *4*, 329.
15. Zajonc, D. M.; Cantu, C., III; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810.
16. 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.
17. Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456.
18. Tashiro, T.; Nakagawa, R.; Inoue, S.; Shiozaki, M.; Watarai, H.; Taniguchi, M.; Mori, K. *Tetrahedron Lett.* **2008**, *49*, 6827.
19. (a) Fujio, M.; Wu, D.; Garcia-Navarro, R.; Ho, D. D.; Tsuji, M.; Wong, C.-H. *J.Am. Chem. Soc.* **2006**, *128*, 9022; (b) Liang, P.-H.; Imamura, M.; Li, X.; Wu, D.; Fujio, M.; Guy, R. T.; Wu, B.-C.; Tsuji, M.; Wong, C.-H. J. *Am. Chem. Soc.* **2008**, *130*, 12348.
20. Smith, P. A. *Org. Reactions* **1946**, *3*, 337.
21. (a) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, *10*, 431; (b) Takikawa, H.; Muto, S.; Mori, K. *Tetrahedron* **1998**, *54*, 3141.
22. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
23. ^{1}H and ^{13}C NMR spectra of RCAI-105 (**1**), 84 (**2**), 107 (**3**), 106 (**4**), 108 (**5**), and 91 (**6**) are included in Supplementary data.
24. All biological experiments were in accordance with protocols approved by RIKEN Animal Care and Use Committee.
25. KRN7000 (**A**) was purchased from Funakoshi Co. (Japan).
26. (a) Tomura, M.; Yu, W.-G.; Ahn, H.-J.; Yamashita, M.; Yang, Y.-F.; Ono, S.; Hamaoka, T.; Kawano, T.; Taniguchi, M.; Koezuka, Y.; Fujiwara, H. J. *Immunol.* **1999**, *163*, 93; (b) Kitamura, H.; Iwakabe, K.; Yahata, T.; Nishimura, S.; Ohta, A.; Ohmi, Y.; Sato, M.; Takeda, K.; Okumura, K.; Kaer, L. V.; Kawano, T.; Taniguchi, M.; Nishimura, T. *J.Exp. Med.* **1999**, *189*, 1121.
27. Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2009**, *17*, 6360.
28. *MlogP* (Moriguchi's estimation of logP: Moriguchi, I.; Hirono, S.; Liu, Q.; Nakagome, I.; Matsushita, Y. *Chem. Pharm. Bull. 1992, 40,* 127) values were calculated by MedChem Designer™ ver. 1.0 (Simulations Plus, Inc.).
29. Li, X.; Chen, G.; Garcia-Navarro, R.; Franck, R. W.; Tsuji, M. *Immunology* **2009**, *127*, 216.
30. Park, J.-J.; Lee, J. H.; Seo, K.-C.; Bricard, G.; Venkataswamy, M. M.; Porcelli, S. A.; Chung, S.-K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 814.
31. Pauwels, N.; Aspeslagh, S.; Vanhoenacker, G.; Sandra, K.; Yu, E. D.; Zajonc, D. M.; Elewaut, D.; Linclau, B.; Van Calenbergh, S. *Org. Biomol. Chem.* **2011**, *9*, 8413.
32. (a) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. J. *Exp. Med.* **2003**, *198*, 1631; (b) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem. Int. Ed.* **2004**, *43*, 3818.
33. Dere, R. T.; Zhu, X. *Org. Lett.* **2008**, *10*, 4641.
34. Blauvelt, M. L.; Khalili, M.; Jaung, Weonjoo; Paulsen, J.; Anderson, A. C.; Wilson, S. B.; Howell, A. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6374.
35. Recently, Hogan et al. reported that α-S-GalCer showed potent immunostimulatory activity in humans in vitro. Hogan, A. E.; O'Reilly, V.; Dunne, M. R.; Dere, R. T.; Zeng, S. G.; O'Brien, C.; Amu, S.; Fallon, P. G.; Exley, M. A.; O'Farrelly, C.; Zhu, X.; Doherty, D. G. *Clin. Immunol.* **2011**, *140*, 196.
36. Hénon, E.; Dauchez, M.; Haudrechy, A.; Banchet, A. *Tetrahedron* **2008**, *64*, 9480.
37. Bordwell, F. G.; Fried, H. E.; Hughes, D. L.; Lynch, T.-Y.; Satish, A. V.; Whang, Y. E. *J. Org. Chem.* **1990**, *55*, 3330.
38. Wojno, J.; Jukes, J.-P.; Ghadbane, H.; Shepherd, D.; Besra, G. S.; Cerundolo, V.; Cox, L. R. *ACS Chem. Biol.* **2012**, *7*, 847.