

Focus on the Controversial Activation of Human iNKT Cells by 4-Deoxy Analogue of KRN7000

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4-Deoxy- α -GalCer analogues are considered weaker agonists than KRN7000 for the stimulation of human iNKT cells, but this remains strongly debated. In this work, we described a strategy toward 4-deoxy- α -GalCers with, as a key step, a metathesis reaction allowing sphingosine modifications from a single ethylenic α -galactoside precursor. The 4-deoxy-KRN7000 derivative **2**, described here, induced potent cytokinic responses, comparable to those of KRN7000, both from human iNKT cells in vitro and from their murine counterpart in vivo.

Introduction

Natural killer T cells (NKT⁴) are a subset of T cells, which play a major role at regulating immune responses.¹ A major subpopulation of the CD1d restricted T lymphocytes, called iNKT cells, can elicit a proinflammatory response through the secretion of T helper 1 (Th1) cytokines (IFN- γ , TNF- α) or an immunomodulatory response when releasing Th2 cytokines (IL-4, IL-10). Disruption of the balance between Th1 and Th2 cytokines is involved in a broad range of diseases, and therapeutic strategies could imply its restoration through in vivo modulation of NKT cells.² iNKT cells stimulation is mediated by the formation of a ternary complex between the semi-invariant T cell receptor (V α 24J α 18 human TCR and V α 14J α 18 for mouse) at the surface of the lymphocyte and the CD1d membrane protein of antigen presenting cells (APCs), complexed with a glycolipid.³ Surprisingly, while the first sugar unit of all mammalian glycolipids is in β -anomeric position, the first active glycolipid antigens (e.g., agelasphin 9b), isolated from the marine sponge *Agelas mauritanus*, were identified as α -galactosylceramides (α -GalCers) by the Kirin Brewery Company in 1993.⁴ Recently, various α -glucuronylceramides (α -GluCers, GSL-1) and *iso*-globotriose (iGb3-Gal α 3Gal β 4Glc β -Cer) have also been shown to stimulate both human and mouse NKT cells.⁵ Because of their strong

therapeutic potential, extensive structure–activity relationship (SAR) studies have been conducted to optimize α -GalCers activity. Initial studies by Koezuka and co-workers focused on the ceramide portion of the molecule and led to the synthetic 2'-deoxy-GalCer analogue (KRN7000, **1**) as a potential candidate for clinical application (Chart 1).⁶ Advances in the elucidation of SARs for a number of synthetic KRN7000 analogues have been widely reviewed in recent update publications.⁷ We focused here on 4-deoxy-GalCer analogues of KRN7000. Those latter derivatives, devoid of 4-OH group, failed to stimulate human iNKT lymphocytes when presented by CD1d-transfected Hela cells.⁸ However, subsequent data obtained from mouse iNKT cells indicated that 4-deoxy- α -GalCer was an active agonist, albeit weaker, than parent KRN7000.⁹ Crystal structure of the ternary complex of human NKT TCR with CD1d and KRN7000 highlighted the involvement of the 3 and 4 hydroxyl groups on the phytosphingosine scaffold through concomitant hydrogen bonding with CD1d Asp80 residue and an additional H-bond between the donating Arg95 of the CDR3 α -loop and the 3-OH group of the glycolipidic ligand.¹⁰ Nevertheless, these studies did not identify a specific interaction between the 4-OH group and the hCD1d binding domains. This contradicts the assumption, postulated for now a decade, that this position could be responsible for a loss of activity in the human context. A comparison between human and mouse empty CD1d crystal structures confirmed that the A' and C' hydrophobic binding pockets were superimposable in size and shape.¹¹ From these observations, it was admitted that α -GalCer should adopt a similar conformation in both CD1d molecules. Thus, even if a somewhat more potent activation of mouse NKT cells by KRN7000, versus its 4-deoxy analogue, has been attributed to an increased stability of the ternary complex, the structural information available to date does not fit well with the loss of IFN- γ and IL-4 stimulation with 4-deoxy α -GalCers mentioned in the human context. In the

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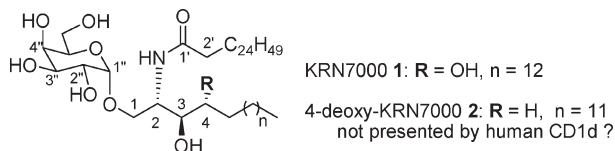
^a Abbreviations: IL, interleukin; IFN- γ , interferon γ ; TNF- α , tumor necrosis factor α ; NKT, natural killer T; hiNKT, human iNKT; Th, T helper; APC, antigen presenting cell; TCR, T cell receptor; CD, cluster of differentiation; hCD1d, human CD1d; SAR, structure–activity relationship; ip, intraperitoneal; α -GalCer, α -galactosylceramide; α -GluCer, α -glucuronylceramide; iGb3, *iso*-globotriose; DMAP, *N,N*-dimethylaminopyridine; Boc, *tert*-butoxycarbonyl; DMEM, Dulbecco's modified Eagle's medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide; FCS, fetal calf serum; ELISA, enzyme-linked immunosorbent assay.

present work, we propose to investigate the in vitro activity on human iNKT cells, in complement to the in vivo activity in mice, of the 2',4-dideoxy- α -galactosylceramide **2**.

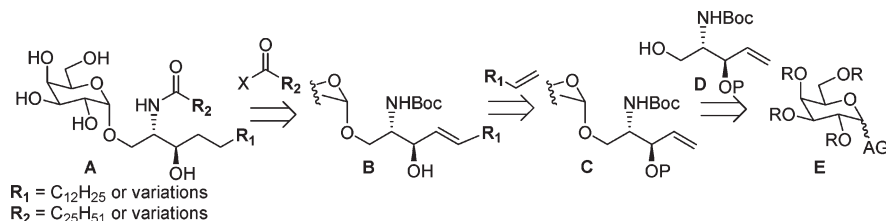
Chemistry. To reinvestigate the ambiguous influence of the 4-OH group in CD1d recognition inducing human iNKT cell stimulation, we first focused our efforts on developing an original and efficient access toward a variety of 2',4-dideoxy- α -GalCer derivatives **A** (Scheme 1).^{12,13} Until now, all the strategies developed to synthesize deoxy α -GalCers analogues suffered from the constraint of building the variable sphinganine fragments from a specific chiral pool at an early step of the synthesis for each proposed variation.¹⁴ We deal with the possibility of introducing both the sphingosine and acyl chains of the glycosylceramide in the last few steps of the synthesis. Thus, as a key step of our retrosynthetic approach (Scheme 1), the sphingosine fragment would be completed using an olefin cross metathesis process from a same precursor **C** to provide a variety of α -galactosylsphingosines **B**. This original procedure widens the access to a large range of 4-deoxy- α -GalCer analogues **A**, from a galactosyl donor **E** and the ethylenic aminodiol **D**.

The required acceptor **D** (P = TBDPS, compound **3**) was prepared through diastereoselective aldol reaction with slight modifications of the procedure described in the literature¹⁵ from commercially available glycinethylester hydrochloride and (+)-(1*R*,2*R*,5*R*)-2-hydroxy-3-pinanone in 36% yield over six steps.¹³ The targeted 4-deoxy-Galcer analogue

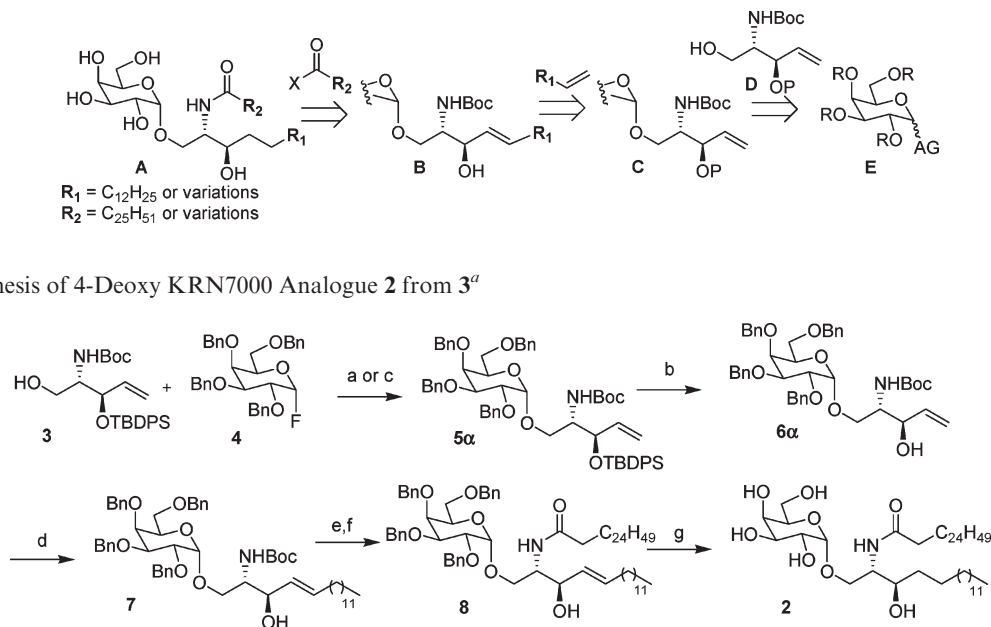
Chart 1. Structure of KRN7000 and Targeted 4-Deoxy- α -GalCer Analogue **2**



Scheme 1. Retrosynthetic Approach to 4-Deoxy KRN7000 Analogues



Scheme 2. Synthesis of 4-Deoxy KRN7000 Analogue **2** from **3**^a



^a Conditions: (a) AgClO₄, SnCl₂, THF/Et₂O, 0 °C, 30 min (**5a** 30% and **5b** 18%); (b) TBAF, THF, RT, 24h, 85% from pure **5a** (c) one-pot procedure: (a), then Boc₂O, Et₃N, DMF, RT, 12h then (b) (**6a** 40% and **6b** 20%, from **3**); (d) Grubbs second generation catalyst 5%, C₁₂H₂₅-CH=CH₂, CH₂Cl₂, reflux, 24h, 77%; (e) HClg, THF, RT, 1h; (f) *para*-nitrophenyl hexacosanoate, Et₃N, cat. DMAP, 44% (over 2 steps); (g) H₂, Pd/C 10%, THF/MeOH, RT, 72h, 67%.

2 was prepared following our original strategy as illustrated in Scheme 2. Because of an unsuitable removal of Boc group during the glycosidic coupling of the α -fluoro-galactopyranosyl donor **4** with the alcohol acceptor **3**, using Mukaiyama procedure,¹⁶ the target glycoside **5a** was in first attempt isolated in a modest 30% yield (Scheme 2). To overcome this side degradation, the crude glycosidic mixture **5a,b** was submitted to another Boc₂O, Et₃N treatment, and the silyl protecting group was removed (85%) subsequently from the allylic alcohols, anticipating a steric crowding of the terminal olefin. This procedure significantly improved the yield of isolated galactoside **6a** to 40% over three steps from the ethylenic intermediate **3**, while the anomer **6b** was present in only 20% yield. Olefin metathesis, in the presence of Grubbs II catalyst,¹⁷ on the free allylic alcohol **6a** was then efficiently performed with tetradecene in refluxing dichloromethane to give **7** in 77% yields. The synthesis was then completed by removal of the *N*-Boc protecting group, acylation of the resulting hydrochloride amine, with 4-nitrophenyl hexacosanoate, in the presence of Et₃N and catalytic DMAP (44% yield over two steps), prior hydrogenation of the intermediate **8**, to furnish the targeted 4-deoxy-KRN7000 analogue **2** in 67% yield.

Results and Discussion

First, we assessed NKT cell cytokine responses after 4-deoxy- α -GalCer **2** stimulation in vivo in order to determine precisely the ability of this compound to induce the release of key immunomediators TNF- α , IFN- γ , and IL-4 in mice (A, B, and C of Figure 1, respectively, of Supporting Information). The kinetics of cytokine release were followed at various antigen concentrations (10, 50, 100, and 1000 μ g/kg) at further times after ip injection. TNF- α and IL-4 were detected at their highest level 4 h after ip injection for each concentration, whereas IFN- γ secretion peaked at 18 h after

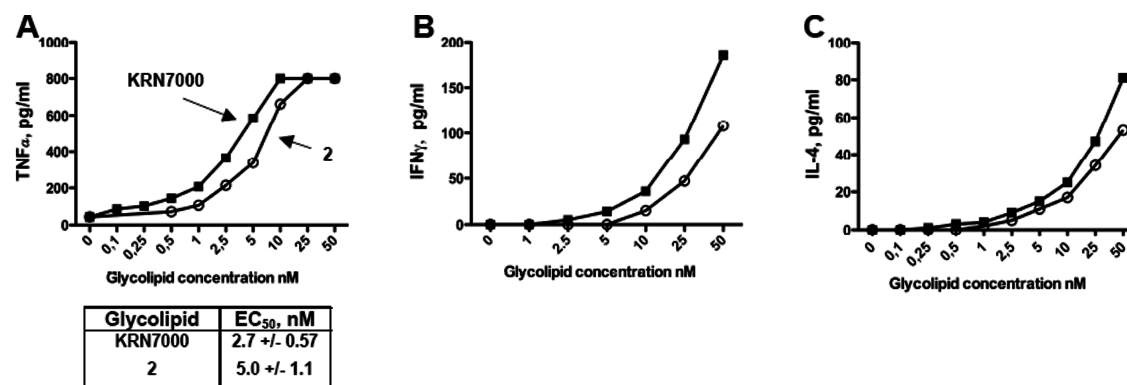


Figure 1. Relative potencies of KRN7000 and 4-deoxy-KRN **2** to stimulate TNF- α (A), IFN- γ (B), and IL-4 (C) releases by a human V α 24 iNKT cell line stimulated for 6 h, by CD1d-transfected Hela cells loaded with increasing concentrations of KRN7000 (—■—) or **2** (—○—). Results are shown as means of triplicates from one representative experiment out of three. The mean half-maximal effective concentrations for eliciting a TNF- α response (EC₅₀) of tested compounds are given in nM \pm SD (Mann–Whitney two-tailed test $p=0.12$). (A). Cytokine release in cell culture supernatants was determined by a cellular assay (TNF- α) or ELISA (IFN- γ , IL-4).

injection. The kinetic profiles appeared identical to those of KRN7000 (100 μ g/kg), and there were no significant differences between the various **2** concentration groups, with the exception of the IFN- γ amount (Figure 1B of Supporting Information), which was higher at 1000 μ g/kg than at 10 μ g/kg of **2** ($P < 0.05$). Thus, we confirmed that the *in vivo* activity of 4-deoxy- α -GalCer **2** did not differ from that of KRN7000 and correlated with previous results of Kronenberg,⁹ showing that in mice both antigens had the same activity in terms of the IFN- γ and IL-2 production at two time points (2 and 14 h).

We further assessed cytokine responses after *in vitro* stimulation by compound **2** of human iNKT cells. When loaded on control cells devoid of CD1d, **2** failed to stimulate the iNKT cell line at concentrations as high as 20 μ M because TNF- α , IFN- γ , or IL-4 productions always remained at background levels (Figure 2 of Supporting Information). By contrast, when loaded on CD1d expressing Hela cells, analogue **2** proved as active as KRN7000 in stimulating TNF- α release because the mean half-maximal effective concentrations (EC₅₀) to elicit a response of each glycolipid were not significantly different (2.7 \pm 0.57 nM vs 5.0 \pm 1.1 nM) (Figure 1). Compound **2** also stimulated a clear production of IFN- γ and IL-4 cytokines from human CD1d-transfected Hela cells, and at each of the concentrations tested, KRN7000 induced only slightly higher responses but not significantly different ($p > 0.05$). Noticeably, **2** proved more difficult to solubilize than KRN7000 and optimal results were obtained following prolonged solubilization times, a couple of hours in DMSO at 37 $^{\circ}$ C, prior to overnight loading onto presenting cells.

Conclusions

In conclusion, *in vitro* data indicated that a 4-deoxy analogue can be nearly as active as KRN7000 to stimulate hNKT cells. The removal of an hydroxyl group may have decreased the solubility of the 4-deoxy- α -GalCer analogues, explaining why earlier reports suggested that such compounds had more severely decreased potency. This data paves the route to a novel potent series of immunostimulating agents easier to prepare than KRN7000. Several 4-deoxy analogues of KRN7000 bearing modified sphingosine moieties have been prepared following the exposed strategy, and details of their synthesis and their biological evaluation will be reported elsewhere.

Experimental Section

Chemical shifts are quoted in ppm, and values of coupling constants (J) are given in Hz. The compound tested (**2**) is > 95% pure by elemental analysis.

Preparation of 7. Compound **6a** (327 mg, 0.44 mmol), in dry CH₂Cl₂ (22 mL), was reacted under reflux for 24 h with tetradecene (1.11 mL, 4.41 mmol) and Grubbs II catalyst (18 mg, 0.02 mmol). Purification by flash chromatography on silica gel (petroleum ether/EtOAc 85:15) afforded **7** as a colorless oil (309 mg, 77%); [α]_D²⁵ +31.4 (c 0.7, CHCl₃). ¹H NMR (300 MHz, CDCl₃) δ 7.37–7.28 (m, 20H), 5.66 (dt, $J=15.4$, 6.8, 1H), 5.45 (m, 2H, H₄), 4.96 and 4.58 (AB syst, $J=11.4$, 2H), 4.87 and 4.73 (AB syst, $J=11.8$, 2H), 4.83–4.75 (m, 3H), 4.50 and 4.41 (AB syst, $J=11.8$, 2H), 4.20 (m, 1H, H₃), 4.07 (dd, $J=10.0$, 3.6, 1H), 4.03 (m, 1H), 3.95–3.86 (m, 3H), 3.70 (m, 2H), 3.60–3.49 (m, 3H), 2.01 (m, 2H), 1.48 (s, 9H), 1.29 (s, 20H), 0.91 (t, $J=6.7$, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 155.7, 138.6–137.8, 132.8, 129.5, 128.4–127.5, 98.9, 79.5, 79.3, 75.7, 74.8, 74.5, 74.2, 74.0, 73.6, 72.8, 69.7, 69.3, 68.6, 53.6, 32.4, 31.9, 29.7–29.1, 28.4, 22.7, 14.2. HRMS (ESI+): calcd for C₅₆H₇₇NNaO₉ [M + Na]⁺ 930.5496; found 930.5466.

Preparation of 8. Compound **7** (130 mg, 0.14 mmol) was dissolved in dry THF (15 mL), and HCl gas was bubbled until total consumption of starting material. THF was evaporated to remove excess of HCl, and the crude was dissolved in THF (6 mL) in the presence of 4-nitrophenyl hexacosanoate (74 mg, 0.14 mmol), triethylamine (24 μ L, 0.17 mmol), and a catalytic amount of DMAP. After 16 h, the reaction mixture was diluted with saturated aqueous NaHCO₃ solution (10 mL). The aqueous layer was extracted with Et₂O (2 \times 15 mL). The organic layers were combined, dried over MgSO₄, and concentrated. Flash chromatography on silica gel (petroleum ether/EtOAc 86:14) afforded **8** as white solid (75 mg, 44% over 2 steps); [α]_D²⁵ +27.5 (c 1.2, CHCl₃); mp 80–81 $^{\circ}$ C. ¹H NMR (300 MHz, CDCl₃) δ 7.37–7.24 (m, 20H), 6.45 (d, $J=8.1$, 1H), 5.65 (dt, $J=15.6$, 6.9, 1H), 5.41 (dd, $J=15.6$, 5.4, 1H), 4.91 and 4.55 (AB syst, $J=11.4$, 2H), 4.87 and 4.70 (AB syst, $J=11.7$, 2H), 4.75 (m, 3H), 4.47 and 4.37 (AB syst, $J=11.4$, 2H), 4.14 (m, 1H), 4.03 (dd, $J=10.2$, 3.6, 1H), 4.01 (m, 2H), 3.89–3.82 (m, 4H), 3.69 (dd, $J=10.2$, 3.3, 1H), 3.50 (m, 2H), 2.12 (t, $J=7.5$, 2H), 1.98 (m, 2H), 1.60–1.10 (m, 66H), 0.88 (t, $J=6.3$, 6H). ¹³C NMR (75 MHz, CDCl₃) δ 173.4, 138.4–137.6, 133.0, 129.1, 128.4–127.5, 99.1, 79.2, 75.8, 74.8, 74.4, 74.2, 74.0, 73.6, 72.7, 69.8, 69.1, 68.7, 52.8, 36.7, 32.4, 32.0, 29.7, 29.4, 25.8, 22.4, 14.2. HRMS (ESI+): calcd for C₇₇H₁₁₉NNaO₈ [M + Na]⁺ 1208.8833; found 1208.8866.

Preparation of 2. Compound **8** (64 mg, 54 μ mol) was dissolved in MeOH (5 mL) and THF (2.5 mL) in the presence of palladium

on activated carbon (64 mg). The mixture was stirred under H₂ for 3 days, and the mixture was filtered through celite washed with hot MeOH and CHCl₃. The filtrate was concentrated, and the residue was purified on silica gel (CHCl₃/MeOH 100:0 to 95:5) to provide **2** as a white solid (30 mg, 67%); $[\alpha]_D^{25} +32.7$ (c 1.0, pyridine); mp 170–171 °C. ¹H NMR (300 MHz, pyridine) δ 8.56 (d, *J* = 8.7, 1H), 5.46 (d, *J* = 3.9, 1H), 5.10 (m, 5H), 4.74 (m, 1H), 4.65 (dd, *J* = 9.9, 3.9, 1H), 4.57–4.29 (m, 8H), 2.48 (t, *J* = 7.2, 2H), 1.95–1.82 (m, 6H), 1.26 (s, 66H), 0.87 (t, *J* = 6.3, 6H). ¹³C NMR (75 MHz, pyridine) δ 173.4, 102.1, 73.1, 71.9, 71.6, 71.0, 70.5, 69.6, 62.7, 54.9, 36.8, 35.1, 32.1, 30.0, 29.6, 26.6, 26.4, 22.9, 14.3. HRMS (ESI+): calcd for C₄₉H₉₇NNaO₈ [M + Na]⁺ 850.7112; found 850.7154. Anal. (C₄₉H₉₇NO₈·H₂O) Calcd: C, 69.54; H, 11.79; N, 1.66. Found: C, 69.45; H, 11.86; N, 1.74.

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Supporting Information Available: Experimental procedure **6a**, biological methods; in vivo secretion of TNF- α , IFN- γ , and IL-4 in mice after ip injection of KRN7000 and **2** at different concentrations; negative control with untransfected Hela cells; complete refs for 2, 3, 5, 9, 10, 12, 14. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Bendelac, A.; Savage, P. B.; Teyton, L. The biology of NKT cells. *Annu. Rev. Immunol.* **2007**, *25*, 297–336.
- (2) Yu, K. O. A.; Porcelli, S. A. The diverse functions of CD1d-restricted NKT cells and their potential for immunotherapy. *Immunol. Lett.* **2005**, *100*, 42–55.
- (3) Moody, D. B. How a T cell sees sugar. *Nature* **2007**, *448*, 36–37.
- (4) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. Agelasphins, novel antitumor and immunostimulatory cerebroside from the marine sponge *Agelas mauritanus*. *Tetrahedron* **1994**, *50*, 2771–2784.
- (5) (a) Mattner, J.; DeBord, K. L.; Ismail, N.; Goff, R. D.; Cantu, C. III; Zhou, D.; Saint-Mezard, P.; Wang, V.; Gao, Y.; Yin, N.; Hoebe, K.; Schneewind, O.; Walker, D.; Beutler, B.; Teyton, L.; Savage, P. B.; Bendelac, A. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **2005**, *434*, 525–529. (b) Zhou, D. P.; Mattner, J.; Cantu, C.; Schrantz, N.; Yin, N.; Gao, Y.; Sagiv, Y.; Hudspeth, K.; Wu, Y. P.; Yamashita, T.; Teneberg, S.; Wang, D. C.; Proia, R. L.; Levery, S. B.; Savage, P. B.; Teyton, L.; Bendelac, A. Lysosomal glycosphingolipid recognition by NKT cells. *Science* **2004**, *306*, 1786–1789.
- (6) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. Structure-activity relationship of α -galactosylceramides against B16-bearing mice. *J. Med. Chem.* **1995**, *38*, 2176–2187.
- (7) (a) Savage, P. B.; Teyton, L.; Bendelac, A. Glycolipids for natural killer T cells. *Chem. Soc. Rev.* **2006**, *35*, 771–779. (b) Wu, D.; Fujio, M.; Wong, C.-H. Glycolipids as immunostimulating agents. *Bioorg. Med. Chem.* **2008**, *16*, 1073–1083.
- (8) Brossay, L.; Naidenko, O.; Burdin, N.; Matsuda, J.; Sakai, T.; Kronenberg, M. Cutting edge: structural requirements for galactosylceramide recognition by CD1-restricted NK T cells. *J. Immunol.* **1998**, *161*, 5124–5128.
- (9) Sidobre, S.; Hammond, K. J. L.; Benazet-Sidobre, L.; Maltsev, S. D.; Richardson, S. K.; Ndonge, R. M.; Howell, A. R.; Sakai, T.; Besra, G. S.; Porcelli, S. A.; Kronenberg, M. The T cell antigen receptor expressed by V α 14i NKT cells has a unique mode of glycosphingolipid antigen recognition. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12254–12259.
- (10) Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellici, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. *Nature* **2007**, *448*, 44–49.
- (11) Godfrey, D. I.; McCluskey, J.; Rossjohn, J. CD1d antigen presentation: treats for NKT cells. *Nat. Immunol.* **2005**, *6*, 754–756.
- (12) Ndonge, R. M.; Izmirian, D. P.; Dunn, M. F.; Yu, K. O. A.; Porcelli, S. A.; Khurana, A.; Kronenberg, M.; Richardson, S. K.; Howell, A. R. Synthesis and evaluation of sphinganine analogues of KRN7000 and OCH. *J. Org. Chem.* **2005**, *70*, 10260–10270.
- (13) Dubreuil, D.; Pipelier, M.; Micouin, L.; Lacône, V.; Bonneville, M.; Le Pendu, J. α -Galactoceramides analogs, their methods of manufacture, intermediate compounds useful in these methods and pharmaceutical compositions containing them. Patent PCT/IB2007/004270, 2007; PCT/IB2006/0039292006.
- (14) See Supporting Information for references on the synthesis of sphinganine fragments from chiral pool.
- (15) (a) Vo-hoang, Y.; Micouin, L.; Ronet, C.; Gachelin, G.; Bonin, M. Total enantioselective synthesis and in vivo biological evaluation of a novel fluorescent BODIPY α -galactosylceramide. *ChemBioChem* **2003**, *4*, 27–33. (b) Cai, Y.; Ling, C.-C.; Bundle, D. R. A general, efficient and stereospecific route to sphingosine, sphinganine, phyto-sphingosines and their analogs. *Org. Biomol. Chem.* **2006**, *4*, 1140–1146.
- (16) Mukaiyama, T.; Murai, Y.; Shoda, S. An efficient method for glucosylation of hydroxy compounds using glucopyranosyl fluoride. *Chem. Lett.* **1981**, 431–432.
- (17) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. Synthesis and activity of a new generation of ruthenium-based olefin metathesis catalysts coordinated with 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligands. *Org. Lett.* **1999**, *1*, 953–956.