

Galacto-Configured Aminocyclitol Phytoceramides Are Potent in Vivo Invariant Natural Killer T Cell Stimulators

Youssef Harrak,[†] Carolina M. Barra,[§] Antonio Delgado,^{†,‡} A. Raúl Castaño,^{*,§} and Amadeu Llebaria^{*,†}

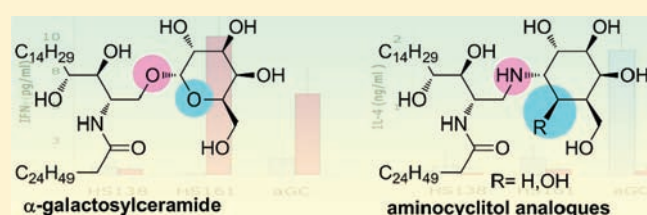
[†]Research Unit on BioActive Molecules (RUBAM), Departament de Química Biomèdica, Institut de Química Avançada de Catalunya (IQAC-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

[‡]Unitat de Química Farmacèutica (Unitat Associada al CSIC), Facultat de Farmàcia, Universitat de Barcelona (UB), Avgda. Joan XXIII, s/n, 08028 Barcelona, Spain

[§]Grupo de Inmunología Molecular, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Bellaterra 08193 Cerdanyola del Vallès, Barcelona, Spain

S Supporting Information

ABSTRACT: A new class of α -galactosylceramide (α GC) nonglycosidic analogues bearing galacto-configured aminocyclitols as sugar surrogates have been obtained. The aminocyclitols having a hydroxyl substitution pattern similar to an α -galactoside is efficiently obtained by a sequence involving Evans aldol reaction and ring-closing metathesis with a Grubbs catalyst to give a key intermediate cyclohexene, which has been converted in galacto-aminocyclohexanes that are linked through a secondary amine to a phytoceramide lipid having a cerotyl *N*-acyl group. Natural Killer T (NKT) cellular assays have resulted in the identification of an active compound, HS161, which has been found to promote NKT cell expansion in vitro in a similar fashion but more weakly than α GC. This compound stimulates the release of Interferon- γ (IFN γ) and Interleukin-4 (IL-4) in iNKT cell culture but with lower potency than α GC. The activation of Invariant Natural Killer T (iNKT) cells by this compound has been confirmed in flow cytometry experiments. Remarkably, when tested in mice, HS161 selectively induces a very strong production of IFN- γ indicative of a potent Th1 cytokine profile. Overall, these data confirm the agonist activity of α GC lipid analogues having charged amino-substituted polar heads and their capacity to modulate the response arising from iNKT cell activation in vivo.



Invariant Natural Killer T (iNKT) cells are a distinct lymphocyte class that regulates a broad range of immune responses.¹ They recognize glycolipid antigens presented by the MHC class I-like protein CD1d.² iNKT cells are involved in the regulation of autoimmunity and³ the response to tumors⁴ or microbial infections.⁵ The glycolipid α -galactosylceramide (α GC) is the prototypical lipid antigen presented by CD1d that stimulates the invariant T cell receptor (TCR) expressed by iNKT cells.⁶ However, the exceptional potency of α GC on iNKT cell stimulation is associated to different side effects, arising from the simultaneous production of Th1 and Th2 type cytokines, which have opposite cell polarizing functions, followed by an unresponsive phase before recovering homeostatic equilibrium.⁷ This has prompted the design and synthesis of α GC analogues to improve its biological properties and modulate the strong induced response.^{8,9} In this context, we have previously described the in vitro activity of aminocyclitol phytoceramides as iNKT cell activators.¹⁰ These nonglycosidic α GC mimetics bear a polyhydroxylated aminocyclohexane as a galactose surrogate, which is linked through a secondary amine to a phytoceramide lipid (Chart 1).

One of these aminocyclitol phytoceramides, HS44, activated iNKT cells despite that it better matches an α -gluco than α -galacto configuration because of the equatorial orientation of 4'OH, a change that would not influence ligand binding to CD1d but is

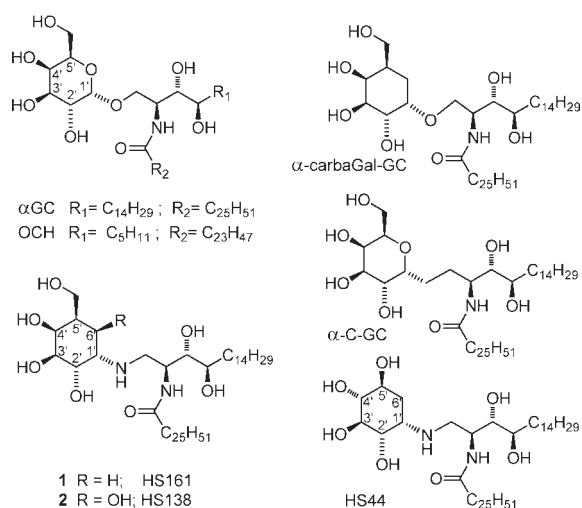
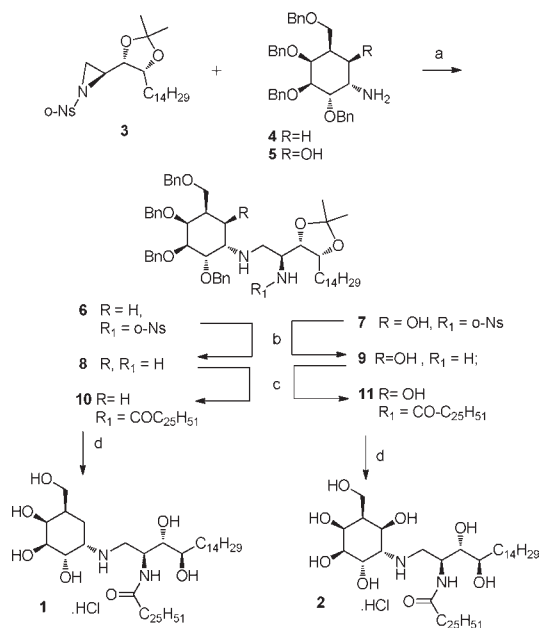
relevant for TCR recognition. Moreover, the galactose hydroxymethyl group, not involved in α GC CD1d binding and TCR recognition, is replaced in HS44 by a hydroxyl group. The in vitro activity found for HS44 is in accordance with the iNKT activity of the structurally related α -glucosylphytoceramide,⁶ which is an agonist weaker than α GC. This interaction mode suggested the design of the new aminocyclitol derivatives **1** and **2** (also designed as HS161 and HS138, respectively, see Chart 1) having substituents to better mimic galactose and specially address the important role of 4'OH in α GC recognition by a TCR protein and therefore with potentially higher activity on NKT stimulation. Other related compounds resulting from the substitution of glycosidic oxygen atoms by carbon are known, such as α -carbaGal-GC¹¹ or α -C-GC¹² and found to strongly stimulate iNKT cells exhibiting biological profiles different from α GC. In this article, we describe the synthesis of **1** and **2** and the results obtained in their biological assays.

RESULTS

Synthesis of Aminocyclitol Compounds. The cyclitol phytoceramides **1** and **2** were obtained by the regioselective opening of aziridine **3** with cyclitol amines **4** or **5**, using a method

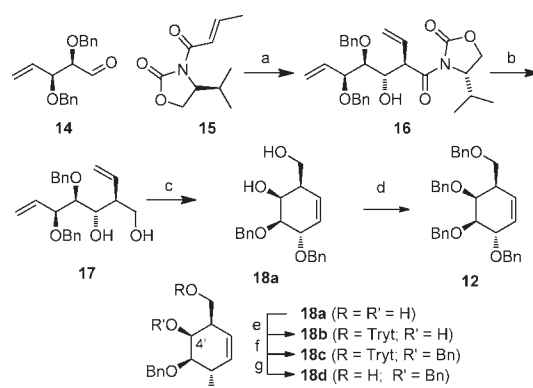
Received: March 23, 2011

Published: July 05, 2011

Chart 1. Aminocyclitol and Related Glycolipid Mimetics of α -GalactosylceramideScheme 1. Synthesis of α GC Analogues 1 and 2^a

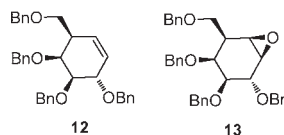
^a Reagents and conditions: (a) MeCN, reflux; 6, 81%; 7, 83%; (b) PhSH, Cs₂CO₃, MeCN, 25 °C; 8, 74%; 9, 83%; (c) EDC, cerotic acid, THF, reflux; 10, 71%; 11, 79%; (d) H₂, Pd/C, MeOH, HCl (cat.) 25 °C; 1, 81%; 2, 85%.

developed to obtain 1-amino-1-deoxyphytosphingosine derivatives,¹³ and employed previously in the preparation of HS44.¹⁰ The reaction of 3 with aminocyclitols 4 and 5 was straightforward with exclusive attack at the less substituted aziridine carbon atom to give 6 and 7, respectively, in good yields. Desulfonation to diamines 8 and 9 and selective N-acylation at the primary amine with cerotic acid gave the hydroxyl-protected products 10 and 11 (Scheme 1), which were transformed in the final aminocyclitol lipids 1 and 2 after the simultaneous removal of acetal and benzyl protecting groups by catalytic hydrogenolysis in acidic media.

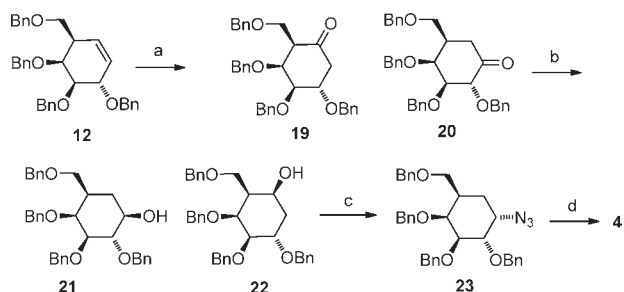
Scheme 2. Synthesis of Key Cyclohexene Intermediates for Aminocyclitols^a

^a Reagents and conditions: (a) Bu₂BOTf, Et₃N, 83%; (b) 2 M LiBH₄, Et₂O, 95%; (c) Grubbs' second-generation catalyst, CH₂Cl₂, 91%; (d) NaH, BnBr, DMF, 94%; (e) Pyr-trityl BF₄, CH₃CN; (f) NaH, BnBr, DMF; (g) *p*-TsOH, MeOH (89% combined e–g).

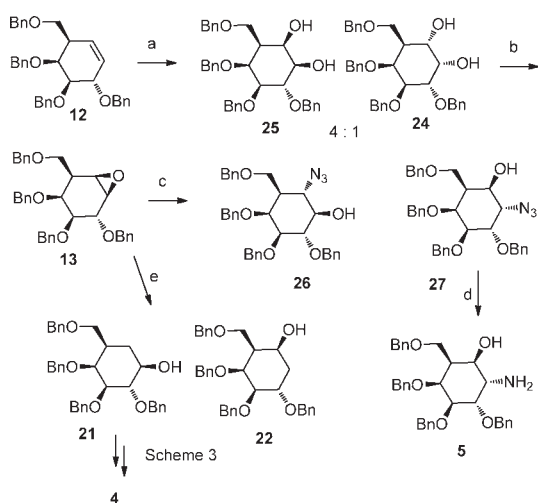
The plan for the synthesis of the necessary aminocyclitols 4 and 5 was to obtain them from key intermediates cyclohexene 12 and its related epoxide 13.



As depicted in Scheme 2, cyclohexene 12 was efficiently obtained in four steps involving an aldol reaction and ring-closing metathesis (RCM) as strategic steps. We started from aldehyde 14, obtained from D-xylose,¹⁴ which was treated with Evans' oxazolidinone 15,¹⁵ following conditions similar to those reported in the literature,^{16,17} to give the *anti*-aldol adduct 16 as the only isolated compound in high yield. Reduction of the chiral auxiliary amide gave the corresponding hydroxymethyl derivative 17, having all the substituents with the required stereochemistry for the galacto carbasugar synthesis. RCM with second-generation Grubbs catalyst^{18,19} gave cyclohexene 18a, which was benzylated to give the key intermediate 12. The epoxidation of compound 12 under several conditions (*m*-CPBA/CH₂Cl₂; *m*-CPBA/NaH₂PO₄/THF; or Caro's salt in K₂CO₃/acetone) was unsuccessful, recovering the starting material unaltered. When these conditions were applied to diol 18a, starting material was consumed, but only trace amounts of the desired epoxide were observed, together with unidentified degradation products. On the other hand, *m*-CPBA epoxidation of tri-*O*-benzyl derivative 18d, arising from transient trityl protection of the primary hydroxyl group in 18a, benzoylation of the secondary hydroxyl group of 18b, and final trityl removal from 18c (Scheme 2), was also fruitless since the starting material was recovered in all cases. Apparently, the presence of an axial substituent at the 4' position (see Scheme 2 or Chart 1 for numbering) affects the reactivity of cyclohexenes 18d and 12 toward epoxidation. Moreover, the stability of the expected epoxides formed from 18a under the above reaction conditions differs also from that observed in gluco-related systems (epimeric with 18a at the 4' position), whose epoxidation has been reported to take place without incident.¹⁴

Scheme 3. Synthesis of Aminocyclitol 4 from 12^a

^a Reagents and conditions: (a) (1) BH_3 , THF; then H_2O_2 , aq. NaOH, 0 to 25 °C; (2) IBX, DMSO, 25 °C, 75% (2 steps). (b) $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, NaBH_4 , MeOH, -50 °C, chromatography; **21**, 53%; **22**, 27%. (c) From **21**, (1) MsCl , Et_3N , CH_2Cl_2 , 0 to 25 °C; (2) NaN_3 , DMF, 90 °C; 75% (2 steps). (d) LiAlH_4 , THF 0 to 25 °C, 93%.

Scheme 4. Synthesis of Epoxide 13 and Aminocyclitol 5 from 12^a

^a Reagents and conditions: (a) OsO_4 , NMNO, 25 °C, acetone/ H_2O 95%; (b) (1) $\text{BrCOC}(\text{CH}_3)_2\text{OAc}$; (2) K_2CO_3 , MeOH, 0 to 25 °C 91%; (c) NaN_3 , $\text{EtOCH}_2\text{CH}_2\text{OH}$, 150 °C, 93%, **26/27** 1:1; or NaN_3 , LiClO_4 , MeCN, reflux, 89%, **26/27** 1:4; (d) from **27**, LiAlH_4 , THF 0 to 25 °C, 93%; (e) LiAlH_4 , THF, 50 °C, **21**, 20% **22**, 43%; or LiHBET_3 , THF -15 °C; **21**, 55% **22**, 37%.

For the synthesis of aminocyclitol **4**, we tried a route not requiring epoxide **13** (Scheme 3). The hydroboration–oxidation of **12** gave a mixture of four alcohols that were directly oxidized to an inseparable mixture of cyclohexanones **19** and **20**, which were reduced with Luche reagent to a 2:1 mixture of β -alcohols **21** and **22** that were isolated after chromatography. The hydroboration of cyclohexenes **18a–d** did not improve the results obtained with **12**. Cyclohexanol **21** was transformed after conventional mesylation and azidation in the epimeric azide **23**, which was converted to the desired amine **4** by LiAlH_4 reduction.

For the synthesis of aminocyclitol **5**, we needed epoxide **13** or a cyclohexane compound with an equivalent substitution pattern. Due to the difficulties found in the direct formation of epoxide **13** from alkenes **12** or **18a,d**, we resorted to the osmium-promoted dihydroxylation of cyclohexene **12**, which proceeded in good yield to give a 4:1 mixture of diastereomeric tetra-*O*-benzyl-*cis*-1,2-diols **24** and **25** (Scheme 4) that were separated by chromatography.

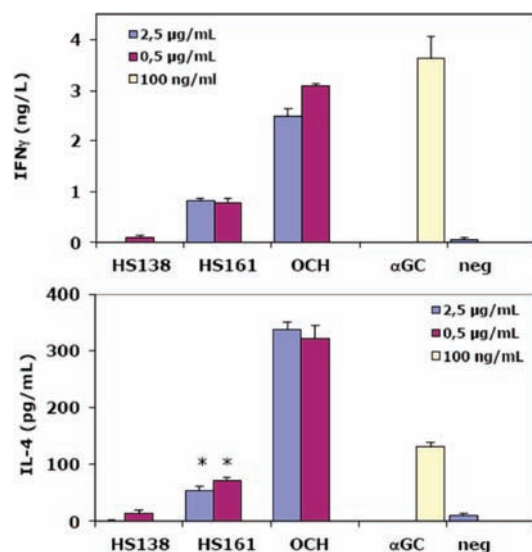


Figure 1. In vitro cytokine induction in cultured splenocytes. IFN γ and IL-4 present in the culture supernatants at day 4 after compound incubation was measured by ELISA. * Denotes statistical significance of low cytokine levels.

The major *cis*-diol **25** was converted to epoxide **13** by a two-step sequence involving the reaction with excess 2-acetoxyisobutyryl bromide (Mattocks–Moffat reagent)^{20–22} to give a mixture of regioisomeric *trans*-cyclohexane bromoacetates, which were not isolated but directly converted into tetra-benzylated epoxide **13** after $\text{K}_2\text{CO}_3/\text{MeOH}$ treatment. This stable epoxide was shown to be a convenient precursor for aminocyclitols **4** and **5** (Scheme 4). On one hand, it was transformed to cyclohexanol **21**, a precursor of **4** by reductive opening. Thus, treatment of epoxide **13** with LiAlH_4 afforded a 1:2 mixture of the previously obtained alcohols **21** and **22**, confirming the same stereochemistry on the new hydroxyl groups introduced in compound **12** by the hydroboration and hydroxylation routes. When LiHEt_3B was used as the hydride source, a 3:2 mixture of **21** and **22** was obtained favoring the desired regioisomer. On the other hand, the galacto-configured epoxide **13** was transformed in aminocyclitol **5** by nucleophilic opening with sodium azide. Unexpectedly, the epoxide **13** was rather unreactive. After trying different conditions, it was found that NH_4Cl activation with heating at reflux in ethoxyethanol gave excellent yields of a 1:1 mixture of azidoalcohols **26** and **27**, which could be separated by chromatography. We later discovered that the use of LiClO_4 in acetonitrile at reflux in this reaction favored the formation of the desired regioisomer **27** over **26** (4:1) in good yields. Finally, the reduction of the azido group in **27** afforded aminocyclitol **5**.

Biological Activity. The biological activity of the aminocyclitols **1** (HS161) and **2** (HS138) as iNKT agonists was assayed in vitro by measuring the production of Th1 and Th2 prototypic cytokines, Interferon- γ (IFN γ) and Interleukin-4 (IL-4) of spleen cultures. As depicted in Figure 1, HS161 induced IFN γ production, as measured at day 4 of culture. The amount of IFN γ averaged 1/3 of the amount produced by αGC -stimulated cultures, at concentrations in which it reaches the plateau of maximum IFN γ production (low micromolar range) and closer to OCH, a Th2 agonist analogue of αGC with a shorter sphingosine chain (see Chart 1).²³ We used αGC at 100 ng/mL because at this concentration it reaches the maximum of IFN γ production, and the use of higher concentrations has

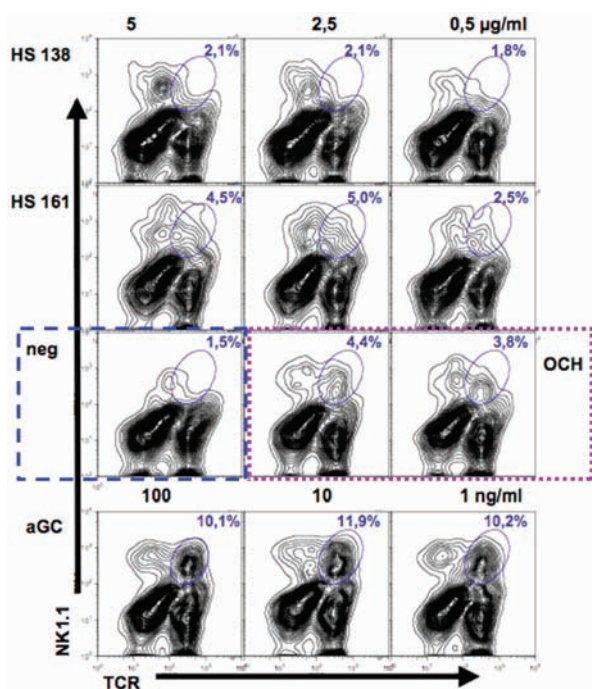


Figure 2. Proliferation of NKT cells after compound activation. Flow cytometry analysis of splenocyte cultures incubated for 6 days with the different compounds at indicated concentrations is shown. NKT percentages (double positive cells) were calculated among electronically gated live lymphocytes.

detrimental effects in cell cultures, including high cell mortality and consequent variability in results. In contrast to HS161, the structurally related aminocyclitol phytoceramide HS138 did not induce a significant production of $\text{IFN}\gamma$.

On the contrary, production of prototypic Th2 cytokine IL-4 was extremely weak, although statistically significant, after HS161 stimulation (Figure 1). OCH induced more than 5-fold higher levels of IL-4 than HS161 at equal concentrations. Stimulation with a suboptimal concentration of αGC also induced higher levels of IL-4, corroborating the weaker recognition of HS161 by iNKT cells. Again, as was the case for $\text{IFN}\gamma$, HS138 did not induce significant production of IL-4 above background levels.

The *in vitro* data strongly suggest that the HS161 compound was recognized by iNKT cells inducing their activation in a way that seems to be more biased toward the Th1 pathway, while HS138 was not recognized and could not activate iNKT responses in a meaningful manner. To confirm that the observed production of cytokines is due to the recognition of analogues by NKT cells, the splenocyte cultures were analyzed by flow cytometry to detect the presence of NKT cells. As shown in Figure 2, HS161 induced the expansion of double positive NKT cells, up to three times relative to negative controls, at the highest concentrations tested. This activation is similar to the weaker proliferation induced by OCH and weaker than that induced by αGC at concentrations at the plateau level. As in the cytokine assays, HS138 increased the number of iNKT cells, but so weakly that it is not statistically significant. This result is in accordance with a complementary study performed with this compound, which showed that it is a relatively weak iNKT agonist when compared to αGC and $\alpha\text{-C-GC}$.²⁴ In this study, using more

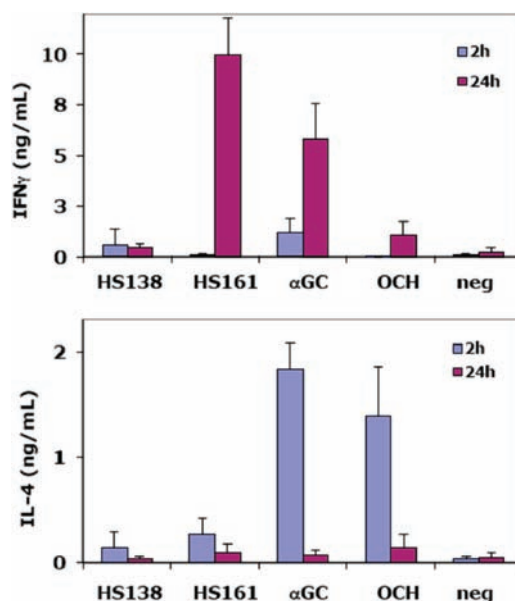


Figure 3. *In vivo* cytokine induction in mice. Amounts of 1 μg of aminocyclitols and 100 ng of αGC and OCH were *i.p.* injected into mice, and $\text{IFN}\gamma$ and IL-4 in serum were measured after 2 and 24 h.

sensitive techniques, HS138 induced iNKT specific proliferation with a much lower potency than αGC and $\text{IFN}\gamma$, and IL-4 induction was some 30-fold lower. Overall, these analyses conclusively show that HS161 is recognized by iNKT cells, inducing their activation, proliferation, and production of cytokines in a way similar to the prototypic CD1d ligand αGC , although with less efficiency and lower potency, while HS138 induces a much weaker response.

To more meaningfully address the biological activity of these analogues and their physiological consequences, we resorted to *in vivo* studies. We intraperitoneally administered 1 μg of the aminocyclitol analogues and measured the levels of $\text{IFN}\gamma$ and IL-4 in serum at two time points, 2 and 24 h, the peaks of maximum production of these cytokines. As controls, we injected mice with a dose of 100 ng of αGC or OCH, an amount that gives a response close to the maximum attainable (less than 50% difference) but is more useful for comparison of the *in vivo* potential of the compounds.

Surprisingly, HS161 induced a very strong production of $\text{IFN}\gamma$ upon *in vivo* administration, as detected in the serum of treated animals 24 h later (Figure 3). This is up to two times higher than the amount induced by the suboptimal dose of 100 ng of αGC . This stimulatory potential is higher than anticipated from the *in vitro* assays, and it may be related to the expected prolonged *in vivo* half-life of the compound. Similarly to splenocyte cultures, HS138 was essentially unable to induce $\text{IFN}\gamma$ production, except for marginal levels just above background.

In opposition, IL-4 induction *in vivo* was weaker than expected (Figure 3). Basically, a very little amount of IL-4 was detected in the serum after HS161 administration. In this case, OCH was as strong an inducer of cytokine production as αGC at a suboptimal dose, as expected from its known propensity to bias Th2 response by iNKT cells.²³ Again, treatment with HS138 produced amounts of IL-4 barely over background levels with no statistical relevance at 2 h, and essentially no IL-4 in serum was found at 24 h in any treatment.

DISCUSSION

In the search for new agonists of iNKT cells that may be useful for immunotherapeutic approaches, including the possibility of obtaining new vaccine adjuvants, we resorted to modifying the polar head structure of the prototypical agonist α GC by using aminocyclitols as galactose replacements. As pointed out before, the structural modifications introduced in **1** and **2** are expected to be differentially recognized by iNKT cells, to induce their activation in a more controllable and specific way, and to increase the relatively weak activation of HS44. These new sugar mimetics with α -galacto configuration could be obtained by an interesting synthetic sequence involving Evans aldol chemistry coupled with ring-closing metathesis to give cyclohexene **12** that was transformed into the final amines **4** and **5** by conventional reactions. The coupling of these aminocyclitols to the phytosphingosine aziridine **3**, deprotection reactions, and N-acylation allowed us to obtain the desired α GC glycolipid mimetics.

The experiments here described show that HS161 is a very efficient activator of Th1 response, as deduced from the high level of IFN γ in the serum of treated animals, compared with the very weak IL-4 induction. Although it could not be deduced from its in vitro results, the strong HS161 in vivo activity was actually awaited in the design of the aminocyclitol phytoceramides, which pursued ligands capable of activating iNKT cells but resistant to the degradative action of glycosidases and, hence, with increased bioavailability in vivo. HS161 is recognized by iNKT, and its presumed stability would allow it to activate iNKT cells in a more sustained manner, thus becoming a strong Th1 inducer, in a mechanism similar to that proposed for the analogue α -C-GC.²⁵ Interestingly, the ether analogue α -carbaGal-GC is also a Th1 biased iNKT cell agonist with increased metabolic stability over α GC.²⁶ Thus, HS161 has an efficacy as high as α GC as an inducer of Th1 response but does not activate Th2 cytokines, so avoiding one of the main problems in the therapeutic application of α GC, the simultaneous and potent induction of contradictory responses. In addition, the lower potency of HS161 suggests that induction of energy that follows excessive NKT activation, the other main pitfall of α GC biological functionality, could be much lower, if not totally avoided, adding to the advantageous possibilities of HS161 as an immunotherapeutic tool.

In contrast, HS138 is weakly recognized by iNKT, either in vitro or in vivo. This compound incorporates an additional hydroxyl group (6'OH) in the cyclitol ring with respect to HS161, in place of the pyranose oxygen in the original galactose. We previously showed that this extra substitution in the weak activator HS44 significantly diminished the recognition by iNKT, probably due to a distorting interaction with V α Pro28 of the TCR. Despite having increased the capacity of the polar head to be recognized by using a galactocyclitol, the introduction of a 6'OH still seems to preclude TCR interaction and therefore the activation of iNKT cells in a very relevant manner. So, relatively minor structural alterations at this position are not well tolerated. The weak activity of HS138 is in accordance with Tashiro et al.,¹¹ who reported that a similar modification in α -carbaGal-GC analogues abolished its agonist iNKT activity.

CONCLUSIONS

In summary, the synthesis of phytoceramide compounds structurally related to α GC having aminocyclitols as galactose mimetics resulted in the identification of HS161, a new non-glycosidic NKT agonist that improves the capacity to activate

these important regulatory T cells by aminocyclitol analogues of α GC and that becomes a potent Th1 activator when used in vivo, despite its lower intrinsic capacity to be recognized by NKT cells. A more complete assessment of the biological properties of HS161 is guaranteed.

ASSOCIATED CONTENT

S Supporting Information. Experimental procedures for the synthesis and biological testing of the compounds, characterization data, and NMR spectra of molecules. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

amadeu.llebaria@iqac.csic.es; raul.castano@uab.cat.

ACKNOWLEDGMENT

This work was supported by MICINN (Project CTQ2008–01426/BQU), Fondos Feder (EU), Generalitat de Catalunya (2005SGR01063), UAB (PRP2007–06), and CSIC (200480E561). The authors thank E. Dalmau for HRMS analysis and Dr. M. Egido-Gabas and Dr. Amaya Castro for analytical support. Y.H. thanks MICINN for a Juan de la Cierva fellowship.

REFERENCES

- (1) Bendelac, A.; Savage, P. B.; Teyton, L. *Annu. Rev. Immunol.* **2007**, *25*, 297–336.
- (2) Matsuda, J. L.; Malleveay, T.; Scott-Browne, J.; Gapin, L. *Curr. Opin. Immunol.* **2008**, *20*, 358–368.
- (3) Ronchi, F.; Falcone, M. *Front. Biosci.* **2008**, *13*, 4827–4837.
- (4) Molling, J. W.; Moreno, M.; van der Vliet, H. J.; van den Eertwegh, A. J.; Scheper, R. J.; von Blomberg, B. M.; Bontkes, H. J. *Clin. Immunol.* **2008**, *129*, 182–194.
- (5) Brigl, M.; Brenner, M. B. *Semin. Immunol.* **2010**, *22*, 79–86.
- (6) 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*, 1626–1629.
- (7) Parekh, V. V.; Wilson, M. T.; Olivares-Villagomez, D.; Singh, A. K.; Wu, L.; Wang, C. R.; Joyce, S.; Van Kaer, L. *J. Clin. Invest.* **2005**, *115*, 2572–2583.
- (8) Wu, D.; Fujio, M.; Wong, C. H. *Bioorg. Med. Chem.* **2008**, *16*, 1073–1083.
- (9) Wun, K. S.; Borg, N. A.; Kjer-Nielsen, L.; Beddoe, T.; Koh, R.; Richardson, S. K.; Thakur, M.; Howell, A. R.; Scott-Browne, J. P.; Gapin, L.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *J. Exp. Med.* **2008**, *205*, 939–949.
- (10) Harrak, Y.; Barra, C. M.; Bedia, C.; Delgado, A.; Castano, A. R.; Llebaria, A. *ChemMedChem* **2009**, *4*, 1608–1613.
- (11) Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. *Bioorg. Med. Chem.* **2009**, *17*, 6360–6373.
- (12) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818–3822.
- (13) Harrak, Y.; Llebaria, A.; Delgado, A. *Eur. J. Org. Chem.* **2008**, 4647–4654.
- (14) Hansen, F. G.; Bundgaard, E.; Madsen, R. *J. Org. Chem.* **2005**, *70*, 10139–10142.
- (15) Evans, D. A.; Sjogren, E. B.; Bartroli, J.; Dow, R. L. *Tetrahedron Lett.* **1986**, *27*, 4957–4960.
- (16) Fleming, K. N.; Taylor, R. E. *Angew. Chem., Int. Ed.* **2004**, *43*, 1728–1730.
- (17) Taylor, R. E.; Hearn, B. R.; Ciavarrri, J. P. *Org. Lett.* **2002**, *4*, 2953–2955.

- (18) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953–956.
- (19) Plumet, J. *Mini Rev. Org.Chem.* **2007**, 201–216.
- (20) Mattocks, A. R. *J. Chem. Soc.* **1964**, 4840–4845.
- (21) Mattocks, A. R. *J. Chem. Soc.* **1964**, 1918–1930.
- (22) Russell, A. F.; Greenberg, S.; Moffatt, J. G. *J. Am. Chem. Soc.* **1973**, *95*, 4025–4030.
- (23) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531–534.
- (24) Patel, O.; Cameron, G.; Pellicci, D. G.; Liu, Z.; Byun, H.-S.; Franck, R. W.; Castaño, A. R.; Llebaria, A.; Bittman, R.; Porcelli, S. A.; Godfrey, D. I.; Rossjohn, J., submitted.
- (25) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. *J. Exp. Med.* **2003**, *198*, 1631–1641.
- (26) Tashiro, T.; Sekine-Kondo, E.; Shigeura, T.; Nakagawa, R.; Inoue, S.; Omori-Miyake, M.; Chiba, T.; Hongo, N.; Fujii, S.; Shimizu, K.; Yoshiga, Y.; Sumida, T.; Mori, K.; Watarai, H.; Taniguchi, M. *Int. Immunol.* **2010**, *22*, 319–328.