The First Synthesis of a Thioglycoside Analogue of the Immunostimulant KRN7000

ORGANIC

Ravindra T. Dere and Xiangming Zhu*

Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland

xiangming.zhu@ucd.ie

Received August 21, 2008

ABSTRACT



The first total synthesis of a thioglycoside analogue of KRN7000, a potential immunostimulant, is described. Two key intermediates are α -galactosyl thiol 4 and phytosphingosine derivative 5, which were both prepared from D-galactose.

Glycosphingolipids have been a target of increasing interest in immunostimulant research since the discovery of the therapeutic potential of α -galactosylceramides.¹ To date, very few compounds have been found that specifically stimulate natural killer T (NKT) cells, the unique lymphocytes defined by their coexpression of surface marker associated with NK cells along with a T cell antigen receptor (TCR). The bestcharacterized is the glycolipid, α -galactosylceramide, which stimulates the production of large amounts of cytokines, such as interferon IFN- γ and interleukin IL-4, by these cells within 2 h of injection.^{2,3} These cytokines are recognized subsequently by other cells of the immune system and may have a widespread influence on immune responses, including protection against autoimmune diseases, the host response to parasites and bacteria, and antitumor responses. Very recently, several excellent reviews have been published describing the immunostimulatory role of glycolipids and the potential for use of NKT cell responses for intervention in human diseases.⁴ Molecules belonging to the α -galactosylceramide family may thus evolve into useful anticancer drugs or immunomodulatory drugs.

The first isolation of α -galactosylceramides was reported by a research group at Kirin Pharmaceuticals in 1993.⁵ The glycolipid that they named agelasphins⁶ (Figure 1) demonstrated potent antitumor and immunostimulatory activities. Further experiments also showed that these molecules were potent in vivo active agents against the murine B16 melanoma. Encouraged by these results, various derivatives were synthesized,^{1a} culminating in the production of KRN7000.⁷ The biological action of KRN7000 comprises several consecutive steps. First, the glycolipid is bound to

For some examples, see: (a) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. J. Med. Chem. 1995, 38, 2176–2187. (b) Vankar, Y. D.; Schmidt, R. R. Chem. Soc. Rev. 2000, 29, 201–216. (c) Schmieg, J.; Yang, G.; Franck, R. W.; Tsuji, M. J. Exp. Med. 2003, 198, 1631–1641. (d) Wu, D.; Xing, G.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plettenburg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C. H. Proc. Natl. Acad. Sci. U.S.A. 2005, 102, 1351–1356. (e) Kinjo, Y.; Wu, D.; Kim, G.; Xing, G.; Poles, M. A.; Ho, D. D.; Tsuji, M.; Kawahara, K.; Wong, C. H.; Kronenberg, M. Nature 2005, 434, 520–525.
 (f) Chen, W.; Xia, C.; Wang, J.; Thapa, P.; Li, Y.; Nadas, J.; Zhang, W.; Zhou, D.; Wang, P. G. J. Org. Chem. 2007, 72, 9914–9923.

⁽²⁾ 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.

⁽³⁾ Stetson, D. B.; Mohrs, M.; Reinhardt, R. L.; Baron, J. L.; Wang, Z. E.; Gapin, L.; Kronenburg, M.; Locksley, R. M. J. Exp. Med. 2003, 198, 1069–1076.

^{(4) (}a) Yu, K. O. A.; Porcelli, S. A. *Immunol. Lett.* 2005, 100, 42–55.
(b) Savage, P. B.; Teyton, L.; Bendelac, A. *Chem. Soc. Rev.* 2006, 35, 771–779.
(c) Wu, D.; Fujio, M.; Wong, C. H. *Bioorg. Med. Chem.* 2008, 16, 1073–1083.

⁽⁵⁾ Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591–5592.

⁽⁶⁾ Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, 50, 2771–2784.



Figure 1. Structures of agelasphins (1), KRN7000 (2), and S-glycoside analogue (3).

the CD1d protein on antigen-presenting cells. The KRN7000/ CD1d complex is then recognized by the TCR on NKT cells, stimulating rapid T helper type 1 (T_H1) and type 2 (T_H2) cytokine production. Release of T_H1-biased cytokines, like IFN- γ , is believed to be responsible for the antitumor, antiviral, and antibacterial effects of α -galactosylceramide, while T_H2-biased cytokines, like IL-4, can attenuate proinflammatory responses, and thereby prevent the onset of some autoimmune diseases.

Nevertheless, the efficacy of KRN7000 has been limited because of the reciprocal inhibition exhibited by T_H1 and T_H2 cytokines.⁸ Hence, many efforts have been devoted in the past decade to synthesize KRN7000 analogues with the hope of developing novel lead compounds with better cytokine-inducing selectivities and greater potency as immunostimulatory agents.⁹ A series of structural modifications toward both the galactose residue and the lipid chains of the galactosylceramide have been conducted.^{9,10} In addition, due to the inherent in vivo instability of O-glycosides, Franck and his co-workers synthesized a C-glycoside analogue of KRN7000,¹¹ and examined its biological activities. Surprisingly, the anomeric analogue exhibited a 1000-fold more potent antimalaria activity and a 100-fold more potent antimetastatic activity than KRN7000.1c Compared with KRN7000, the C-glycoside consistently stimulated prolonged

(8) Berkers, C. R.; Ovaa, H. Trends Pharmacol. Sci. 2005, 26, 252-257.

production of the T_H1 cytokines and decreased production of $T_{\rm H}2$ cytokines, which suggests that the C-glycoside analogue may be an excellent therapeutic option for diseases resolved by T_H1-type responses. An underlying cause for the dramatic difference between C- and O-glycosides remains to be clarified. Other different procedures for the synthesis of the C-glycoside analogue of KRN7000 have also been reported.¹² Also, the C-glycoside version of a truncated analogue of KRN7000 termed OCH13a and a truncated nonisosteric α -C-galactosylceramide^{13b} have also been synthesized.

Recently, Cerundolo and his co-workers reported the X-ray crystal structures of KRN7000 bound to human CD1d molecules,¹⁴ which shows that the lipid chains of the glycolipid are buried in the two grooves in CD1d composed largely of hydrophobic amino acids, while the galactose residue is largely exposed for the recognition by the T cell receptor. From the structure, it is not immediately apparent how lipid chain length and sugar moiety influence NKT cell responses. However, several hydrogen bonds identified between human CD1d and KRN7000 at the junction of the two alkyl chains and the polar headgroup did answer theoretically some of the structure-activity data derived from α -galactosylceramide analogues.¹⁴

As part of our ongoing program on the synthesis of catabolically stable glycosides,^{15,16} particularly *S*-linked glycoconjugates,¹⁶ we wish to report here the first total synthesis of a thioglycoside analogue of KRN7000 3 as a potential immunostimulant. Thioglycosides¹⁷ in which the

(11) (a) Franck, R. W.; Tsuji, M. Acc. Chem. Res. 2006, 39, 692-701. (b) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. Angew. Chem., Int. Ed. 2004, 43, 3818-3822. (c) Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. Org. Lett. 2004, 6, 4077-4080. (d) Chen, G.; Chien, M.; Tsuji, M.; Franck, R. W. *ChemBioChem* **2006**, *7*, 1017–1022. (12) Wipf, P.; Pierce, J. G. *Org. Lett.* **2006**, *8*, 3375–3378, and references

cited therein.

(13) (a) Toba, T.; Murata, K.; Yamamura, T.; Miyake, S.; Annoura, H. Tetrahedron Lett. 2005, 46, 5043-5047. (b) Lu, X.; Song, L.; Metelitsa, L. S.; Bittman, R. ChemBioChem 2006, 7, 1750-1756.

(14) 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, 819-826.

(15) Zhu, X.; Jin, Y.; Wickham, J. J. Org. Chem. 2007, 72, 2670-2673. (16) (a) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. Org. Lett. 2004, 6, 1083-1085. (b) Zhu, X.; Schmidt, R. R. Tetrahedron Lett. 2003, 44, 6063-6067. (c) Zhu, X.; Stolz, F.; Schmidt, R. R. J. Org. Chem. 2004, 69, 7367-7370. (d) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. J. Org. Chem. 2003, 68, 5641-5651. (e) Zhu, X.; Haag, T.; Schmidt, R. R. Org. Biomol. Chem. 2004, 2, 31-33.

(17) Driguez, H. Top. Curr. Chem. 1997, 187, 85-116.

⁽⁷⁾ For some recent examples of the synthesis of KRN7000, see: (a) Figueroa-Pérez, S.; Schmidt, R. R. Carbohydr. Res. 2000, 328, 95-102. (b) Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C. H. J. Org. Chem. 2002, 67, 4559–4564. (c) Fan, G. T.; Pan, Y.; Lu, K. C.; Cheng, Y. P.; Lin, W. C.; Lin, S.; Lin, C. H.; Wong, C. H.; Fang, J. M.; Lin, C. C. Tetrahedron 2005, 61, 1855-1862. (d) Risseeuw, M. D. P.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. Tetrahedron Lett. 2006, 47, 3677-3679. (e) Lee, A.; Farrand, K. J.; Dickgreber, N.; Hayman, C. M.; Jürs, S.; Hermans, I. F.; Painter, G. F. Carbohydr. Res. 2006, 341, 2785–2798. (f) Luo, S. Y.; Kulkarni, S. S.; Chou, C. H.; Liao, W. M.; Hung, S. C. J. Org. Chem. 2006, 71, 1226-1229. (g) Tsujimoto, T.; Ito, Y. Tetrahedron Lett. 2007, 48, 5513-5516.

^{(9) (}a) Sakai, T.; Morita, M.; Matsunaga, N.; Akimoto, K.; Yokoyama, T.; Iijima, H.; Koezuka, Y. Bioorg. Med. Chem. Lett. 1999, 9, 697-702. (b) Goff, R. D.; Gao, Y.; Mattner, J.; Zhou, D.; Yin, N.; Cantu, C., Jr.; Teyton, L.; Bendelac, A.; Savage, P. B. J. Am. Chem. Soc. 2004, 126, 13602–13603. (c) Xing, G. W.; Wu, D.; Poles, M. A.; Horowitz, A.; Tsuji, M.; Ho, D. D.; Wong, C. H. *Bioorg. Med. Chem.* **2005**, *13*, 2907–2916. (d) Fujio, M.; Wu, D.; Garcia-Navarro, R.; Ho, D. D.; Tsuji, M.; Wong, C. H. J. Am. Chem. Soc. 2006, 128, 9022-9023.

^{(10) (}a) Du, W.; Gervay-Hague, J. Org. Lett. 2005, 7, 2063-2065. (b) Ndonye, 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. J. Org. Chem. 2005, 70, 10260-10270. (c) Murata, K.; Toba, T.; Nakanishi, K.; Takahashi, B.; Yamamura, T.; Miyake, S.; Annoura, H. *J. Org. Chem.* **2005**, 70, 2398–2401. (d) Yu, K. O. A.; Im, J. S.; Molano, A.; Dutronc, Y.; Illarionov, P. A.; Forestier, C.; Fujiwara, N.; Arias, I.; Miyake, S.; Yamamura, T.; Chang, Y. T.; Besra, G. S.; Porcelli, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3383–3388. (e) Wu, D.; Zajonc, D. M.; Fujio, M.; Sullivan, B. A.; Kinjo, Y.; Kronenberg, M.; Wilson, I. A.; Wong, C. H. Proc. Natl. Acad. Sci. U.S.A. 2006, 103, 3972-3977. (f) Long, X.; Deng, S.; Mattner, J.; Zang, Z.; Zhou, D.; McNary, N.; Goff, R. D.; Teyton, L.; Bendelac, A.; Savage, P. B. Nat. Chem. Biol. 2007, 3, 559-564. (g) Du, W.; Kulkarni, S. S.; Gervay-Hague, J. Chem. Commun. 2007, 233, 6-2338. (h) Tashiro, T.; Nakagawa, R.; Hirokawa, T.; Inoue, S.; Watarai, H.; Taniguchi, M.; Mori, K. Tetrahedron Lett. 2007, 48, 3343-3347. (i) Trappeniers, M.; Goormans, S.; Beneden, K. V.; Decruy, T.; Linclau, B.; Al-Shamkhani, A.; Elliott, T.; Ottensmeier, C.; Werner, J. M.; Elewaut, D.; Calenbergh, S. V. ChemMedChem 2008, 3, 1061-1070.

glycosidic oxygen atom has been replaced by a sulfur atom are tolerated by most biological systems; moreover, this replacement increases the stability of the sugar-aglycon linkage against enzymatic cleavage as well as chemical degradation. Thus, the target molecule **3** might remain active in the biological systems for a longer period of time. Also, based on the biological results of α -*C*-galactosylceramides,^{11a} the new *S*-glycolipid may have better binding affinity with CD1d, favoring more effective signaling cascades in the immune systems.

Currently, glycosyl thiols are the key building blocks for the construction of thioglycosides,¹⁸ although thioglycosides can also be synthesized conventionally from normal glycosyl donors and the corresponding sulfur-containing acceptors. To a great extent, this nonconventional approach became popular owing to the chemical stability of glycosyl thiols. Unlike sugar hemiacetals, glycosyl thiols are quite stable, and the thioglycosyl anions do not mutarotate even under basic conditions.¹⁶ In other words, the anomeric configuration of a glycosyl thiol can be maintained during the formation of its corresponding thioglycoside products. To circumvent the conventional glycosidation strategy that could suffer from poor α/β selectivity, and to take advantage of the glycosyl thiol chemistry, a synthetic strategy involving the galactosyl thiol 4 and electrophilic lipid building block 5 was envisaged for the construction of the target compound 3, as outlined in Scheme 1. A base-promoted coupling between 4 and 5

Scheme 1. Retrosynthetic Analysis of the Target Molecule 3



was expected to provide the precursor to the *S*-glycolipid **3**, and both **4** and **5** could be derived from D-galactose.

Our synthesis started with the preparation of thiol **4**.¹⁹ 1,6-Anhydrosugar **7** was first prepared from D-galactose by literature procedures²⁰ and then selectively ring-opened with commercially available bis(trimethylsilyl)sulfide following our own procedure¹⁹ to give the desired α -galactosyl thiol **4** in 88% yield (Scheme 2). The anomeric configuration of

Scheme 2. Synthesis of Galactosyl Thiol 4



4 was readily determined from the coupling constant: ${}^{3}J_{\text{H1}-\text{H2}} = 4.5 \text{ Hz}$, whereas analogous β -glycosyl thiols usually have ${}^{3}J_{\text{H1}-\text{H2}} = 7-10 \text{ Hz}$. This ring-opening procedure is a significant advance in glycosyl thiol chemistry because there have not been any reports on direct stereoselective preparation of α -glycosyl thiols prior to our work. Furthermore, **4** was produced exclusively as α -anomer, which made the purification very simple and straightforward.

In the meantime, phytosphingosine derivative **8** (Scheme 3) was also synthesized from D-galactose following Schmidt's procedure,^{7a} and then subjected to the normal isopropylidenation conditions (2,2-dimethoxypropane/*p*-TsOH) to give the intermediate 9^{21} in 80% yield. Compound 9 was subsequently mesylated with methanesulfonyl chloride in pyridine to afford an 87% yield of compound 10, which was converted into the iodide **5** in excellent yield by treatment with LiI in DMF.

With the requisite building blocks **4** and **5** in hand, the task that now confronted us was to find suitable conditions to achieve their ligation. Recently, *S*-linked glycopeptides have been successfully assembled from glycosyl thiols and β -bromoalanine-containing peptides under phase transfer conditions.²² Notably, in this work other nucleophilic functional groups present on both sugars and peptides, such as hydroxyl groups, did not participate in the ligation due to the highly nucleophilic thiolate group and the extremely mild conditions. On the basis of this work, coupling between thiol **4** and iodide **5** was performed in the presence of tetrabuty-lammonium hydrogen sulfate (TBAHS) in ethyl acetate and an aqueous solution of NaHCO₃ at pH 8.5, i.e. phase transfer conditions, as shown in Scheme 3. As expected, the desired product **11** was obtained in very good yield (73%) after SiO₂

⁽¹⁸⁾ Zhu, X. Tetrahedron Lett. 2006, 47, 7935–7938, and references cited therein.

⁽¹⁹⁾ Dere, R. T.; Wang, Y.; Zhu, X. Org. Biomol. Chem. 2008, 6, 2061–2063.

⁽²⁰⁾ Åberg, P. M.; Ernst, B. Acta Chem. Scand. 1994, 48, 228-233.

⁽²¹⁾ Schmidt, R. R.; Maier, T. *Carbohydr. Res.* **1988**, *174*, 169–179.

⁽²²⁾ Zhu, X.; Schmidt, R. R. Chem. Eur. J. 2004, 10, 875-887.

Scheme 3. Synthesis of α -S-Galactosylceramide 3



flash chromatography, and the 6-OH group of **4** also remained inert under the conditions.

The conversion of azide 11 into the corresponding amine was effected by Staudinger reduction, using a 1 M solution of trimethylphosphine in THF,9c and the amine was used directly in the acylation reaction without further purification. Coupling of the amine with hexacosanoic acid in the presence of 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) afforded the desired compound 12 in 84% overall yield. Subsequently, deprotection of 12 to convert into the target compound 3 was achieved in two steps: removal of the isopropylidene protecting group was first conducted by treatment with 4 M HCl in dioxane to give rise to the partially protected S-glycolipid 13 in 68% yield; 13 was then subjected to Birch reduction to furnish the α -S-galactosylceramide 3 in 63% yield. The synthetic sample was purified by flash chromatography on silica gel (eluant: CHCl₃/MeOH 10:1), and characterized by NMR and HR-ESIMS

In conclusion, we have developed a convenient and efficient protocol for the synthesis of α -S-galactosylceramide **3** involving 14 steps starting from D-galactose in 3% overall

yield, and to the best of our knowledge, this is the first total synthesis of a thioglycoside analogue of the immunostimulant KRN7000. An advantage of this protocol is that the anomeric stereochemistry was introduced at an early stage in the synthesis by use of a glycosyl thiol building block. In view of the enhanced catabolic stability of *S*-linked glycoconjugates, the resultant structure of **3** is of particular interest for studying its biological properties, and testing of **3** as immunostimulant is in progress.

Acknowledgment. This work was supported by Research Frontier Program of Science Foundation Ireland. We thank Johannes Klein (School of Chemsitry and Chemical Biology, University College Dublin) for help with the Birch reduction.

Supporting Information Available: Experimental procedures and ¹H and ¹³C data for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8019555