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Synthesis of tritium labeled KRN7000

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Abstract—The synthesis of a multiple tritiated analog of KRN7000 (α-galactosyl ceramide) is described, enabling further studies to quantitate the affinity of KRN7000 for its receptor and to study its pharmacokinetic properties.

Presentation of antigens to T cells is a key feature of cell-mediated immunity. Whereas peptide antigens are presented by major histocompatibility complexes (MHC) class I or class II, lipid antigens are presented by CD1 complexes,[1] heterodimeric protein complexes with a high degree of structural analogy to MHC complexes. The CD1 family is expressed on dendritic cells, B-cells and macrophages and comprises five members, CD1a–e, each of which have been shown to bind and present different lipids. Lipid antigen presentation by members of the CD1 family has recently gained interest and holds potential for the development of novel lipid-based immunotherapeutics. Despite the recent gain in interest, few ligands have been described.

KRN7000, also known as α-galactosyl ceramide or α-GalCer (**1**),[2] is a synthetic glycolipid originally described as an anti-tumor agent. It was shown to act as a ligand for CD1d[3] with the ability to activate natural killer T (NKT) cells. α-GalCer, is a simplified analog of a glycolipid extracted from the marine sponge *Agelas mauritianus*[4] and is constructed from phytosphingosine,

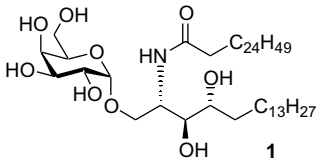

Figure 1. Structure of KRN7000 (α-galactosyl ceramide) **1**.

D-galactose and cerotic acid (hexacosanoic acid) (Fig. 1). Binding of the linear hydrocarbon moieties, into two deep hydrophobic binding grooves aligned by two α-helices in the CD1d heavy chain,[5] results in activation of NKT cells through interaction of glycolipid-loaded CD1d and the appropriate T-cell receptor.

Although α-GalCer has enabled the immunological community to extensively study the role of CD1d in cell-mediated immunity, it has been difficult to study α-GalCer pharmacokinetics or to quantitate the extent of interaction between α-GalCer and CD1d. The binding half-life of the interaction of α-GalCer with CD1d has indirectly been determined by different means, and is estimated to range anywhere from 6 min to 24 h. Several groups have reported on α-GalCer derivatives that allow tracking of the glycolipid, none of them using radiolabelling. α-GalCer has been derivatized by labeling with fluorescent tags or with biotin.[6] In most of these cases the tracer molecule has been incorporated in the acyl side chain, that binds into one of the two CD1d lipid binding grooves.

Since chemical modifications of α-GalCer are likely to alter its binding properties and thus limit their use, it was decided to track α-GalCer by the incorporation of

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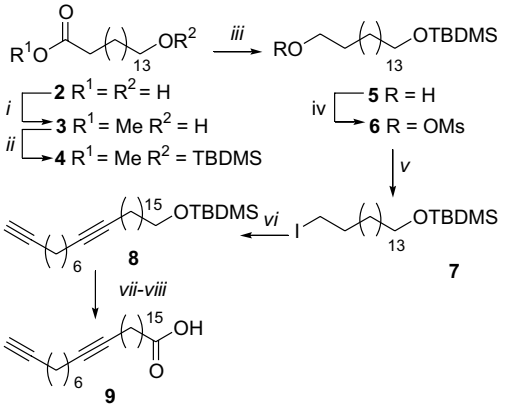
radio nuclides. The advantage of radiolabeling is several-fold: it allows for both qualitative analysis and for quantitation. We decided to construct the radioactive derivative in such a way that the dimensions and elemental composition are very similar to the unmodified counterpart.

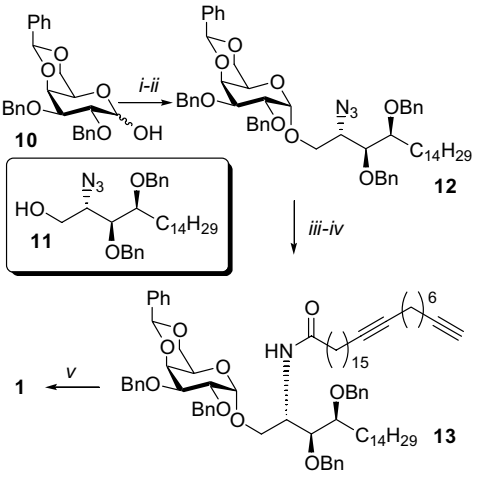
It was envisaged that tritium (T, ^3H) could be incorporated by catalytic reduction of unsaturations using tritium gas, the most obvious site for the introduction of tritium atoms being the fatty acyl chain of **1**. This chain contains neither heteroatoms nor chiral centers, potentially sensitive to the effects of radioactive decay, unlike the carbohydrate and the phytosphingosine moieties. The introduction of tritium into the fatty acid requires an unsaturated analog of cerotic acid. Bisacetylene fatty acid **9** was constructed according to Scheme 1. The synthesis was initiated from commercially available 16-hydroxypalmitic acid (**2**), which was converted into the methyl ester **3** under acidic conditions.

Protection of the free hydroxyl group as a silyl ether (TBDMS) followed by reduction with $LiAlH_4$ yielded alcohol **5**. Treatment of this alcohol with methanesulfonyl chloride yielded mesylate **6**, which was converted into iodide **7** under Finkelstein conditions in 82% overall yield. Elongation of the iodide with 1,9-decadiyne afforded compound **8**. Desilylation (TBAF) followed by Jones oxidation yielded bisacetylene fatty acid **9** in 48% (two steps).

Having the fatty acid **9** in hand, stable intermediate **13** was synthesized according to Scheme 2. Known D-galactose derivative **10**[7] was coupled to the azido-phytosphingosine derivative **11**[8] using Schmidt's trichloroacetimidate method.[9] NMR analysis indicated that, in accordance with analogous glycosylations using the benzylidene protected galactose donor, only the α isomer **12** was formed.[10,11] Staudinger reduction using Me_3P in THF yielded a labile amine, which was immediately condensed with bisacetylene fatty acid **9** to afford compound **13**.[12]



Scheme 1. Reagents and conditions: (i) AcCl, MeOH quant.; (ii) TBDMSCl, Imidazole, DMF; (iii) $LiAlH_4$, THF; (iv) MsCl, TEA, CH_2Cl_2; (v) NaI, Acetone, Δ, 82% four steps; (vi) 1,9-decadiyne, *n*-BuLi, THF, DMPU, 32%; (vii) TBAF, THF; (viii) Jones' reagent, acetone (48%, two steps).

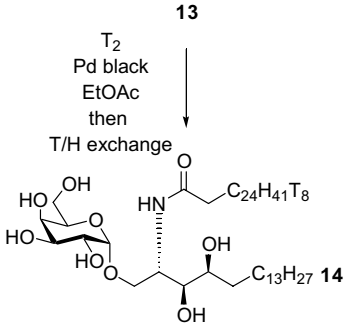


Scheme 2. Reagents and conditions: (i) Cl_3CN, DBU, CH_2Cl_2, 81%; (ii) compound **11**, $BF_3{\cdot}OEt_2$, THF, Et_2O, powdered molecular sieves 4 Å, −20 °C, 70%; (iii) Me_3P, THF, 0 °C → rt; (iv) compound **9** PyBOP, DIPEA, CH_2Cl_2, 65%, two steps; (v) H_2 (1 atm), Pd black, EtOAc, rt quant.

Glycolipid intermediate **13** was transformed into the tritiated α-GalCer (**14**) by catalytic reduction with tritium gas over palladium black in ethyl acetate followed by alcohol T/H exchange and silica gel chromatography (5–10% MeOH in CH_2Cl_2).

This transformation not only achieves the simultaneous incorporation of eight tritium nuclei in the acyl side chain, but also conveniently removes all the protective groups (Scheme 3) in the same transformation. The reduction was carried out using palladium black in ethyl acetate since hydrogenation trials using different solvents and catalysts (palladium carbon formulations, alcoholic solvents, addition of carboxylic acids) had yielded impure, partially decomposed products.

The reductive tritiation yielded pure $^3H_8$-α-GalCer as judged by analysis of the product formed by autoradiography and thin layer chromatography. Reductive hydrogenation under otherwise identical conditions afforded α-GalCer (**1**), which proved in all aspects identical to α-GalCer, obtained by elongation of **12** with cerotic acid followed by reductive hydrogenation.



Scheme 3. Catalytic reduction of intermediate **13** with $^3H_2$ to afford tritiated ceramide **14**.

Through the construction of an unsaturated analog of the fatty acid cerotic acid it was possible to synthesize protected glycolipid **13**, which provided homogeneous ^{3}H8-α-GalCer upon reduction with tritium gas over palladium black. As a result of the high specific activity (200 Ci/mmol) obtained, $^{3}H_{8}$-α-GalCer (**14**) will enable facile quantitation of interactions with this glycolipid or with glycolipid-loaded CD1d complexes to study in vivo α-GalCer pharmacokinetic properties, on which we will report in due course.

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References and notes

1. For recent reviews on CD1 see: (a) Sugita, M.; Cernadas, M.; Brenner, M. B. *Curr. Opin. Immunol.* **2004**, *16*, 90; (b) Brigl, M.; Brenner, M. B. *Annu. Rev. Immunol.* **2004**, *22*, 817; (c) Watts, C. *Nat. Immunol.* **2004**, *5*, 685; (d) Moody, D. B.; Porcelli, S. A. *Nat. Rev. Immunol.* **2003**, *3*, 11.
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. (a) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591; (b) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771.
5. Zheng, Z.-H.; Castaño, A. R.; Segelke, B. W.; Stura, E. A.; Peterson, P. A.; Wilson, I. A. *Science* **1997**, *277*, 339.
6. (a) Sakai, T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359; (b) Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1999**, *42*, 1836; (c) Vo-Hoang, Y.; Micouin, L.; Ronet, C.; Gachelin, G.; Bonin, M. *Chembiochem* **2003**, *4*, 27; (d) Zhou, X. T.; Forestier, C.; Goff, R. D.; Li, C.; Teyton, L.; Bendelac, A.; Savage, P. B. *Org. Lett.* **2002**, *4*, 1267.
7. Koeman, F. A. W; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron* **1993**, *49*, 5291.
8. Barbieri, L.; Constantino, V.; Fattorusso, E.; Mangoni, A.; Aru, E.; Parapini, S.; Tarameli, D. *Eur. J. Org. Chem.* **2004**, 468.
9. Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731.
10. Figueroa-Pérez, S.; Schmidt, R. R. *Carbohydr. Res.* **2000**, *328*, 95.
11. Plettenburg, O.; Bodmer-Narkevitch, V.; Wong, C.-H. *J. Org. Chem.* **2002**, *67*, 4559.
12. Spectroscopic data for compound **13**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.46–7.28 (m, 25H); 5.93 (d, 1H, $J = 16.5$ Hz NH); 5.51 (s, 1H PhCH); 5.00 (d, 1H, $J = 3.0$ Hz); 4.96–4.53 (m, 13H); 4.27–3.58 (m, 17H); 2.23–1.90 (m, 3H), 1.70–1.30 (m, 52H); 0.94 (m, 6H). ^{13}C NMR ($CDCl_3$ 125 MHz) δ: 173.2; 138.8; 138.7; 138.1; 129.1; 128.7; 128.7; 128.6; 128.4; 128.1; 128.0; 127.8; 126.6; 101.3; 100.0; 80.7; 80.2; 79.8; 77.3; 76.0; 74.7; 74.1; 73.6; 72.2; 72.0; 69.7; 68.5; 63.2; 50.6; 37.0; 32.2; 30.6; 30.1; 30.0; 29.7; 29.6; 29.5; 29.2; 29.2; 28.7; 28.5; 26.1; 26.0; 23.0; 19.0; 18.9; 18.6; 14.4.