Synthesis and biological activity of α-glucosyl C24:0 and C20:2 ceramides

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α-Glucosyl ceramides 4 and 5 have been synthesised and evaluated for their ability to stimulate the activation and expansion of human iNKT cells. The key challenge in the synthesis of both target molecules was the stereoselective synthesis of the α-glycosidic linkage. Of the methods examined, glycosylation using per-TMS-protected glucosyl iodide 16 was completely α-selective and provided gram quantities of amine 11, from which α-glucosyl ceramides 4 and 5 were obtained by N-acylation. α-GlcCer 4, containing a C24 saturated acyl chain, stimulated a marked proliferation and expansion of human circulating iNKT cells in short-term cultures. α-GlcCer 5, which contains a C20 11,14-cis-diene acyl chain (C20:2), induced extremely similar levels of iNKT cell activation and expansion.

CD1d is a non-polymorphic glycoprotein expressed on the surface of antigen-presenting cells (APCs). It is specifically associated with presenting lipid antigens that activate the distinctive class of T cells known as invariant Natural Killer T (iNKT) cells. iNKT cells display characteristics of both T cells and NK cells and play a crucial role in diverse immune responses and other pathologic conditions.1–4 When the synthetic glycolipid α-galactosyl ceramide (α-GalCer),5 also known as KRN7000 (1, Fig. 1), is bound to CD1d and presented to T cell receptors (TCRs) on the surface of iNKT cells, the latter are activated to release diverse cytokines, including both Th1 and Th2 cytokines.5–8 Similar results are obtained with the more readily obtained C24:0 analogue (2, Fig. 1).9,10 It is believed that the release of Th1 cytokines may contribute to antitumour and antimicrobial functions, whilst the secretion of Th2 cytokines may help alleviate autoimmunity diseases such as multiple sclerosis and arthritis.14 The opposing effects induced by Th1 and Th2 cytokines have complicated efforts to develop KRN7000 as a therapeutic agent, since it induces high levels of both types of cytokine and therefore may induce mixed and unpredictible biological effects.16 Switching the C26:0 acyl chain of KRN7000 for a C20 11,14-cis-diene acyl chain modifies the outcome of iNKT cell activation and potently induces a Th2-biased cytokine response.9 This C20:2 analogue (3, Fig. 1) also exhibits less stringent requirements for loading on to CD1d.10 Although extensive studies have examined the impact on the iNKT cell-stimulating activities of modifications to the fatty acyl...
Since targets 4 and 5 differ only in their acyl chain substitution, we elected to pursue a synthetic strategy that would allow the introduction of this point of diversity in the final step. We therefore examined several routes to amine 11 from which both glucosyl ceramide targets would then be accessed through chemoselective acylation of the amino residue. The key challenge in a synthesis of amine 11 is to form the glycosyd linkage with high α-selectivity. To this end, we first opted to employ a stereospecific glycosylation method developed by Bols (Scheme 1).19 This method involves the use of a silyl tether to attach the acceptor temporarily to the 2-position of the glucosyl donor prior to the key glycosylation step. Glycosylation proceeds with 1,2-syn specificity, owing to the formation of a five-membered silylacetal intermediate, which in the case of glucosyl donors, ensures the formation of the α-glycoside product. Thioglucoside 7, synthesised in three steps from D-glucal,20 was reacted with a fivefold excess of dichlorodimethylsilane. This reaction afforded a silyl chloride intermediate, which, after removal of the excess dichlorosilane reagent under reduced pressure, reacted with known alcohol 821 to form mixed silyl acetal 9, our glycosylation precursor, in modest yield. Treatment of silyl acetal 9 with N-iodosuccinimide (NIS) furnished the desired glucoside 10 as a single diastereoisomer, albeit in modest yield. Hydrogenolysis of the benzyl groups and reduction of the azide in 10 using Pd(OH)$_2$ as the catalyst22 provided our acylation precursor, amine 11 in 57% yield (Scheme 1).

Although this synthetic approach allowed a completely stereoselective route to our target amine 11, a number of steps in the sequence suffered from poor yields, which hindered access to significant quantities of material. We therefore examined other glycosylation methods. Kobayashi has described a stereoselective route to our target amine 11 by reacting an O-deprotected glucoside with perbenzylated glucosyl fluoride 12, which proved difficult to separate (Scheme 2).

We therefore turned our attention to the use of glucosyl iodides,23 specifically per-TMS-protected glucosyl iodide 16, as an alternative donor. Du et al. have shown that the corresponding galactosyl iodide provides excellent levels of α-selectivity with a variety of alcohol acceptors.24 The reaction conditions for this glycosylation are also extremely mild and the silyl protecting groups are easily removed using an acid work-up. We reasoned that the use of phytosphingosine acceptor 17,27 in which the internal 1,2-diol is protected as an acetal, would deliver the completely O-deprotected glucoside 18 upon acid work-up. To this end, 1,2,3,4,6-penta-O-trimethylsilyl glucose 15, which is commercially available or can be readily synthesised on large scale by treating glucose with a mixture of TMSCl and hexamethyldisilazane (HMDS) in pyridine,28 was converted to glycosyl iodide 16 by treatment with TMSI in CH$_2$Cl$_2$ (Scheme 3). Adding a solution of crude 16 to a solution of alcohol 17,27 Bu$_4$N, Hünig’s base and 4 Å molecular sieves in CH$_2$Cl$_2$ successfully effected glycosylation. Treating the initially formed glucoside product with p-toluenesulfonic acid (pTSA) in methanol provided the fully O-deprotected glucoside 18 as a single anomer. Although the yield for this three-step process was a modest 45%, we now had very rapid access to our target molecules. A final Staudinger reduction of azide 18 delivered our requisite amine 11 in quantitative yield (Scheme 3).29 This reaction sequence is short and scalable and proved to be particularly effective for accessing multigram quantities of amine 11.

Scheme 1. Reagents: (a) Me$_2$SiCl$_2$, pyridine, toluene; (b) acceptor 8, pyridine, toluene, 58% over two steps; (c) NIS, MeNO$_2$, 47%; (d) H$_2$, Pd(OH)$_2$, CHCl$_3$/MeOH (1:1), 57%.

Scheme 2. Reagents: (a) From 12: CBr$_4$, PPh$_3$, CH$_2$Cl$_2$, then 8, *Bu$_4$NBr, tetramethyl urea, 67% (α:β ratio: 1:1); (b) from 13: 8, SnCl$_4$, AgIO$_4$, THF, 41% (α:β ratio: 5:1).

Scheme 3. Reagents: (a) TMSI, CH$_2$Cl$_2$; (b) 17, *Bu$_4$N, Pr$_2$NEt, 4 Å molecular sieves, CH$_2$Cl$_2$; then pTSA, MeOH, 45% from 15; (c) PMe$_3$, wet THF, quant.; (d) tetracosanoyl chloride, THF/8 M NaOAc, 68%; (e) 11,14-eicosadienoyl chloride, THF/8 M NaOAc, 66%.
acylation reactions were accomplished by adding either tetracosa-
noyl chloride or 11,14-eicosadienoyl chloride (formed from the
corresponding carboxylic acids using oxalyl chloride) in THF to
amine 11 in a vigorously stirred biphasic mixture of THF and 8 M
NaOAc solution. Both reactions provided the desired amide prod-
ucts 4 and 5 in good yields (Scheme 3).30,31

To assess the biological activity of the α-glucosyl ceramides 4 and
5 and compare these to KRN7000 1 and the α-galactosyl cera-
mide analogues 2 (C24:0) and 3 (C20:2), we assessed the ability of
each compound to induce the expansion of iNKT cells in samples of
human peripheral blood mononuclear cells (PBMC) during an
eight-day in vitro culture.32 The results showed that both the per-
centages and absolute numbers of iNKT cells in the cultures were
markedly increased to similar levels by stimulation with both of
the α-GlcCer analogues 4 and 5 (Fig. 3). The level of iNKT cell
expansion, at least with a relatively high concentration of the glyco-
lipids (250 nM), was comparable for both of the N-acyl variants
of α-GlcCer and very similar to levels obtained with the related
α-GalCer analogues (2 (C24:0) and 3 (C20:2)) and with the proto-
typical iNKT cell activator KRN7000 (1 (C26:0)). Representative
profiles obtained by flow cytometry of cultures from one normal
blood donor are shown in Figure 3A. This analysis was carried
out with PBMC from four separate donors (Fig. 3B). Although differ-
ences were observed for the levels of iNKT cell expansion between
different donors, all donors responded well to the two α-GalCer
analogues. In all cases, these responses were similar to those gen-
erated by the analogous α-GalCer compounds.

Figure 3. Ex vivo expansion of human iNKT cells by α-GlcCer and α-GalCer
analogues. Peripheral blood mononuclear cells (PBMC) from four different donors
were stimulated with the indicated glycolipids at a concentration of 250 nM in the
presence of low levels of exogenous IL-2 and IL-7. At day 8, cultures were harvested
and analysed by flow cytometry using monoclonal antibodies specific for CD3 and
for the invariant TCRα chain expressed by iNKT cells (6B11). (A) Dot plots showing
relative levels of CD3+ 6B11+ iNKT cells are shown for one representative donor.
Numbers in upper right quadrant indicate percentages of total lymphocytes that are
iNKT cells. (B) Absolute numbers of iNKT cells in the cultures were determined by
flow cytometry using fluorescent counting beads, and the values of iNKT cell fold
expansion were determined by dividing by the input number of iNKT cells.

The strong biological activity of the α-GlcCer compounds was
consistent with findings from the initial study that described the
reactivity of CD1d-restricted iNKT cells to synthetic glycosylcer-
amides.5 This showed an α-GlcCer with a C26 saturated acyl group
to be stimulatory for mouse iNKT cells, with a level of activity only
slightly less than that of KRN7000 (1). Our analysis confirms the
activity of α-GlcCer compounds as ligands for human iNKT cells.
It is also notable that we observed human iNKT cell activation
and expansion for an α-GlcCer with a shorter acyl chain containing
unsaturations (5). Previous work with analogues of α-GalCer con-
taining C20:2 or other unsaturated fatty acyl groups revealed a
marked tendency for these to bias iNKT cell-dependent cytokine
responses in mice to give preferential secretion of Th2 cytokines
such as IL-4 and IL-13.3 This Th2 cytokine bias has been associated
with therapeutic benefits in a variety of mouse models of autoim-
mune and inflammatory diseases, indicating potential therapeutic
applications for such glycolipids in human diseases.17 It will thus
be important to determine whether compound 5 or other α-GlcCer
analogues bearing an unsaturated acyl chain also show an ability
to induce Th2-biased cytokine responses, which is a focus for fu-
ture studies.

In summary, we have developed an efficient route to α-glucosyl
ceramides that provided two biologically active ligands 4 and 5 for
stimulation of human iNKT cell responses. Of the range of glycosyl-
ation methods that were investigated for accessing the target
target molecules with high levels of stereoelectricity, the use of per-
TMS-protected glucosyl iodide 16 as the donor is the most attrac-
tive, reacting with acceptor 17 to provide a single α-glycoside
product. This glycosylation reaction is also scalable and with an
acidic work-up effecting global deprotection, followed by Staude-
inger reduction of the azide, allows rapid access to advanced
intermediate 11, which can now be used to provide a broad range
of α-GlcCer compounds with different acyl chains. Compounds
produced using this approach will assist in expanding the current
understanding of the structure–activity relationships for glycolipid
activators of iNKT cells, which is of central importance to the fur-
ther development of this class of compounds as clinically useful
immunomodulators.

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Supplementary data

Supplementary data associated with this article can be found, in

References and notes


30. Data for s-gCer 4. Mp 151–152 °C; [α]D 0.21 (10% MeOH in CHCl3) [29] 247.3 (1/2 0.50, CHCl3/MeOH (1:1)); vmax (film) cm−1 3380 br s (OH), 2975 s, 1623 m (C=O), 1463 s, 1143 s, 1071 m, 1033 m, 798 s, 718 m; δH (500 MHz, CDCl3/CD3OD (2:1)) 0.88 (6H, t, J = 6.7, 2- terminal CH3), 1.20–1.44 (48H, stack, alkyl chain), 1.51–1.69 (4H, stack, alkyl chain), 2.19 (app. t, J = 7.6, CH3CONH), 3.31–3.37 (1H, m, 4- terminal CH3), 3.75–3.83 (7H, stack, 5-H, 4’,-4’H), 3.82 (1H, app. t, J = 9.5–9.3, 3’-H), 3.62 (2H, stack, 6- H1, 1’-H1), 3.79 (1H, dd, dJ = 12.0, 2.5, 6.86) (1H, dd, dJ = 10.5, 4.5, 1’-H4), 4.17 (3H, app. q, J = 4.5, 2’-H), 4.85 (1H, d, dJ = 3.5, 4’-H), CONH resonance not observed. δC (125 MHz, CHCl3/CD3OD (3:1)) 13.5 (CH2, 2- terminal CH2), 22.2, 25.5, 28.9, 29.0, 29.2, 29.27, 29.30, 29.34, 31.5, 32.0, 36.0 (CH3, alkyl chain, some overlapping resonances), 50.1 (CH2-C6), 61.2 (CH2-C6), 66.8 (CH3-C1, 69.9 (CH-C4), 71.6 (CH-C2), overlapping resonances). J.C. Chem. Soc., Chem. Commun. 1992, 913.


32. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll–Hypaque overlapping resonances), 50.1 (CH2-C6), 61.2 (CH2-C6), 66.8 (CH3-C1), 69.9 (CH-C4), 71.6 (CH-C2), overlapping resonances). J.C. Chem. Soc., Chem. Commun. 1992, 913.


