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A Solvent-free Approach to Glycosyl Amides: Towards the Synthesis of α -*N*-Galactosyl Ceramides

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Abstract

A new, simple and efficient method for the synthesis of both α - and β -glycosyl amides using solvent-free conditions is described. This method involves the coupling of glycosyl amines with the *p*-nitrophenol esters of lipids as a key step.

Keywords

glycolipids; glycosyl amides; solvent-free; *i*NKT cells

Invariant Natural Killer T (*i*NKT) cells are a class of lymphocytes that recognize glycolipid antigens presented by CD1d proteins.¹ Upon activation, *i*NKT cells rapidly release a host of cytokines, and murine, as well as human cell line, studies have suggested that their activation may have relevance to the treatment of autoimmune diseases,² cancer,³ tuberculosis,⁴ malaria,⁵ diabetes⁶ and inflammation.⁷

The current understanding of *i*NKT cells has largely been possible because of the discovery of KRN7000 (**1**), a potent glycolipid antigen identified more than a decade ago.⁸ The ability of KRN7000, commonly called α -GalCer, to suppress the growth of liver tumors was ultimately linked to its ability to stimulate *i*NKT cells. KRN7000's lack of broad success in clinical trials is partially attributed to the fact that it induces significant levels of both proinflammatory T helper (Th) 1 and immunoregulatory Th2 cytokines. This can be problematic because the production of Th1 cytokines can offset the immunological impact of Th2 cytokines and vice versa.⁹ Because of this, a large number of glycolipid analogs of KRN7000 have been synthesized with the goal of identifying compounds that provide a biased Th1 or Th2 response. Some of these alterations have included modification of the glycosidic bond.¹⁰

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Dedicated to the memory of Harry Wasserman, an outstanding scholar, teacher and encourager

Supplementary Information

Supplementary data associated with this article can be found, in the online version, at

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X = O, Y = O, α -GalCer, **1** (KRN 7000)

X = O, Y = C, α -C-GalCer, **2**

X = O, Y = S, α -S-GalCer, **3**

X = C, Y = N, aminocyclitol, **4**

X = O, Y = N, α -N-GalCer, **5** (Target Molecule)

The C-galactoside analog¹¹ (α -C-GalCer, **2**) of KRN7000, has the glycosidic bond replaced with a methylene group and thus resists enzymatic degradation and decomposition in a low pH environment. α -C-GalCer elicited a stronger Th1 response in comparison to KRN7000 in mice *in vivo*¹² but was reported to show weak activity against human lymphocytes.¹³ The S-galactoside analogue¹⁴ (α -S-GalCer, **3**) on the other hand did not stimulate iNKT cells either *in vitro* or *in vivo* in mice,¹⁵ but a later study showed that it was bioactive in human cell lines.¹⁶ Recently, it has been reported that galacto-configured aminocyclitol analog **4** is able to stimulate iNKT cells and is a potent Th1 activator *in vivo*.¹⁷ In order to further explore the importance of the glycosidic linkage, we targeted α -N-GalCer (**5**).¹⁸ In the process, an efficient, solvent-free approach to glycosyl amides has been established.

Not unexpectedly, efforts to prepare α -N-GalCer **5** via traditional glycosylations using appropriately protected and functionalized ceramides or sphingoid bases with amino groups at the termini as glycosyl acceptors were unsuccessful. Attempts to alkylate a 1-aminogalactose (a strategy that had been successful for the preparation of α -S-GalCer¹⁴) also failed. Consequently, the approach for target molecule **5** turned to selective formation of glycosyl amides **6** (Figure 1). A tertiary glycosyl amide has been successfully reduced to a glycosyl amine.¹⁹ Even if appropriate reduction conditions cannot be identified, the glycosyl amides could themselves be novel NKT cell antigens.

The selective synthesis of α -glycosyl amides, particularly with substituents (other than carbon chain extensions) alpha to the carbonyl is challenging. Commonly employed approaches to the synthesis of glycosyl amides include acylation of glycosyl amines with carboxylic acids (or the corresponding mixed anhydrides or acyl halides) or acid thiols, but these methods are only efficient for producing β -glycosyl amides.²⁰ A major challenge is that α -glycosyl amines readily convert to the β -anomer *in situ* (see examples in ref 21). There are few reported methods to selectively afford α -glycosyl amides; most use glycosyl azides (with a limited range of substrates) and require moderate to high temperatures.²¹ For example, the Bernardi lab has developed a very nice approach to α -glycosyl amides using traceless Staudinger ligation of perbenzylated α -glycosyl azides with triaryl phosphines.^{21c,d}

Interestingly, relevant to our results below, acetylated α -glycosyl azides gave only β -glycosyl amides. In another example, Theiring *et al.* isolated α/β -amide mixtures when persilylated α - or β -azidosugars were hydrogenolyzed, then reacted with succinic anhydride.^{21f} None of the examples in these or the other manuscripts cited were α -glycosyl amides that had branching at the alpha carbon of the acyl moiety.

Although we recognized that the ready mutarotation of α -amino sugars to the β -anomer and the lack of close precedent for coupling with acyl chains like **8** were not encouraging, the simplicity of direct coupling between glycosyl amines and activated esters was attractive. To this end a coupling reaction between activated esters **8** and glycosyl amine **9**, derived by hydrogenolysis of glycosyl azides **7**, was planned (see Scheme with Table 1).

The synthesis of glycosyl azides **7a–e** followed reported procedures.²² Activated esters **8a–d** were prepared by oxidation²³ of the corresponding phytosphingosine alcohols^{11b,24} with BAIB and subsequent esterification. Esters **8e–g** were prepared from commercially available acids. Initial studies focused on galactosyl azides **7a–c** and phytosphingosine ester **8a**. Reduction of the glycosyl azide to the amine, followed by filtration, concentration, then coupling with the activated ester (Table 1) afforded the glycosyl amides. The reaction between benzyl-protected amine **9a**, derived from α -azide **7a**, and ester **8a** in the presence of catalytic DMAP and base at room temperature gave only β -product **6aa** with low yield. Interestingly, however, the reaction between acetyl-protected azide **7b** and ester **8a** afforded some α -**6ba**. Moreover, an increase in yield of **6ba** was observed when the reaction was performed in the absence of base. When no solvent was used, both the selectivity and yield of **6ba** increased. It was also observed that benzyl-protected sugar **7a** produced only β -glycosyl amide **6aa** under solvent-free conditions, but the yield of the coupling improved. When galactosyl amine **9c**, derived from β -azide **7c**, was reacted with ester **8a** in CH_2Cl_2 , expected β -glycosyl azide **6ca** was isolated in 72% yield. Without solvent this reaction proceeded in higher yield (88%), and the product mixture contained 17% of the α -anomer.

With the straightforward, solvent free process giving good yields and providing a previously unreported class of α -glycosyl amide, we decided to examine the method with other *p*-nitrophenol activated esters and to include glucosyl azides **7d/e** (Table 2). Since our initial goal was to prepare α -N-GalCers, the coupling of galactosyl amine **9b** and other lipid donors **8b–d** was examined. The neat reactions proceeded in moderate yields, but in contrast to the reaction with **8a**, only provided the β -anomers **6bb/6bc/6bd**, respectively. Straight chain activated esters (**8e/f**) gave moderate to good α -selectivities and high yields, and **8g** with amine functionalization in the β -position also coupled with good α -selectivity and moderate yield.

Acetylated glucosyl azides **7d/e** were also examined. The anomeric selectivity and yields in coupling reactions with esters **8a/e** were similar to what was seen with the galactosyl azide partners.

The amide anomers, with the exception of **6bg**, were readily separable. However, initial identification was sometimes complicated by the presence of rotamers. With **6ba** the fact that only the β -isomer was forming was confirmed by reacting ester **8a** with 2,3,4,6-tetra-*O*-

benzyl- β -D-galactosyl amine (the β -anomer of **9a**). α -Amide **6aa** showed rotamers in the ^1H and ^{13}C NMRs in CDCl_3 ; its β -anomer (**6ca**) did not. Although not shown in Table 2, β -amine **9c** reacted with ceramide ester **8c** to give the same rotamer distribution as was seen with the reaction between **9c** and **8b**. It is not uncommon to observe rotamers with glycosyl ceramides (or glycosyl amides). β -Azidoamide **6bd** was isolated as an inseparable diastereomeric mixture (presumably from epimerization at the azidocarbon). In contrast to what was observed for the previously mentioned rotamers, NMR signals (both proton and carbon) for **6bd** did not show any change when its solution was heated in the probe.

Several advantageous outcomes have emerged from these studies, and the results also show some trends that could provide avenues for enhancing α -selectivity. While coupling glycosyl amines with acid derivatives is not new, the discovery that the efficiency of such couplings can be improved under solvent free conditions is significant. Importantly, the first preparation of sphingoid base-containing α -glycosyl amides (**6ba/6da**) has been achieved, and other α -glycosyl amides have been prepared with moderate to very good α -selectivity and good to excellent yields. It is worth noting that the amides appeared to be configurationally stable ($>$ one year) when stored neat, and there was no obvious anomeric epimerization when the amides were left in solution for several days. This method is attractive because of its simplicity. Using hydrogen, rather than phosphines, to convert the azides to amines means that only filtration and concentration are needed after the first transformation. Better α -selectivity is, however, desirable. There are several observations that suggest this may be achievable. The percentage of α -product (Table 1) was enhanced in the solvent-free conditions, perhaps because mutarotation is faster in solution. The finding that the per-benzylgalactosyl- α -azide provided only β -product, while the acetate protected sugar led to both anomers provides a clear avenue for further exploration—looking at the influence of different protecting groups. While the sugar protecting groups obviously play a role, the fact that the same amino sugar (**7b**) resulted in very different α/β ratios, depending on the ester, supports a critical role for the electrophile. There is some suggestion that both electronic and steric factors in the esters are involved in the outcomes, particularly with the α -substituted esters. With the exception of **8a**, the esters that have α -substitution (**8b–d**) resulted in only β -products. Based on observations related to glycosylation with the corresponding alcohols of **8a–d**, esters **8b–d** would be expected to be more electron rich, thus less electrophilic, than **8a**. Again, it would be straightforward to use alternative protecting groups to test this hypothesis. Also, it should be possible to monitor both the anomeric ratio of unreacted sugar over the course of the reaction and the relative reaction rates.

In conclusion a new method to obtain α -glycosyl amides with moderate to good selectivities and moderate to very good yields has been developed. Further, this approach has delivered the first examples of sphingoid base-linked α -glycosyl amides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Strategy for the synthesis of α -N-GalCer (5)

Chart 1.
Glycosyl azides

Chart 2.
Activated esters

Table 1

Optimization of coupling reaction between **7a–c** and **8a**

Sugar	Ester	Conditions ^a	Yield ^b , ratio ^c (α:β)	Conditions ^a	Yield ^b , ratio ^c (α:β)
7a	8a	DMAP (cat.), Et ₃ N, CH ₂ Cl ₂ DMAP, CH ₂ Cl ₂	58%, β-only 65%, β-only	DMAP, Neat	89%, β-only
7b	8a	DMAP (cat.), Et ₃ N, CH ₂ Cl ₂ DMAP, CH ₂ Cl ₂	56% (1:3) 68% (1:3)	DMAP, Neat	82% (1:2)
7c	8a	DMAP, CH ₂ Cl ₂	72%, β-only	DMAP, Neat	88% (1:5)

^aReactions performed at room temperature for 24 h,^bisolated yields,^cα and β anomers were readily separated; then the ratios were calculated.Ratios could not be deciphered from crude ¹H NMRs.

Table 2

Substrate scope

Sugar	Ester	Yield ^a , ratio ^b (α:β)
7b	8b	60%, β-only
7b	8c	55%, β-only
7b	8d	50%, β-only ^c
7b	8e	70%, (4:1)
7b	8f	89%, (1.4:1)
7b	8g	52%, (5:1) ^d
7d	8a	73%, (1:2)
7d	8e	83%, (2:1)
7e	8a	78%, β-only

^a Isolated yields,^b α and β anomers were separated; then the ratios were calculated,^c an epimeric mixture (~1:1) resulted,^d α and β anomers were not separable and the ratio was determined from ¹H-NMR.