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Design and synthesis of a novel glycosphingolipid derived from polyhydroxy 2-pyrrolidinone and phytoceramide appended by a 1,2,3-triazole linker

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ABSTRACT

Synthetic analogs of glycosphingolipids (GSLs) have been demonstrated as potential therapeutic interventions in certain patho-physiological conditions. This article reviews reports of various bioactive synthetic GSLs, emanated from the Bittman laboratory. KRN7000, a synthetic GSL which is a α-galactosylceramide (α-GalCer) is a potent immunomodulatory agent. Bittman et al. reported several modifications of *C*-glycosides of KRN7000 with an eye towards achieving selective cytokine response during iNKT cell activation. However, GSLs with azasugar variants were not explored which inspired us to derive a polyhydroxy 2-pyrrolidinone azasugar from D-galactose and append to the phytoceramide via a 1,2,3-triazole linker to afford GSL analog **12**. This novel GSL analog **12** may be used to explore the immunomodulatory activity, and other biological activities against targets involving iminosugar or azasugar based therapeutics.

1. Introduction

Glycosphingolipids (GSLs) present in the outer leaflet of the plasma membrane are the primary attachment sites for various pathogens (Varki, 1993; Fantini et al., 2001). Bittman et al. designed and synthesized various GSL analogs for therapeutic intervention in certain patho-physiological conditions (Bittman, 2004, 2009). A fluorescent tagged BODIPY™-lactosylceramide (LacCer) GSL analog was used to demonstrate that caveolar endocytosis, a mechanism for uptake of certain pathogens, is stimulated by exogenous GSLs (Singh et al., 2003). To study the effect of stereochemistry of the sphingosine backbone, BODIPY™-tagged natural (D-erythro) and non-natural (L-threo) LacCer analogs were examined in the endocytosis and the results indicated that the natural analog is internalized via caveolae and the non-natural analog is excluded from uptake via caveolae (Liu and Bittman, 2006; Singh et al., 2006). Furthermore, β-D-lactosyl-*N*-octanoyl-L-threo-sphingosine with non-natural stereochemistry was shown to selectively inhibit caveolar endocytosis and SV40 virus infection, block lipid rafts in the plasma membrane, and inhibit β1-integrin activation and downstream signaling (Singh et al., 2007). In a related family of GSLs, a structure–activity derived synthetic GSL analog of a naturally occurring agelasphin with phytoceramide known as KRN7000 (α-GalCer) activate iNKT cells which in turn results in release of cytokines that are potent immunomodulatory agents (For reviews, see: Wu et al., 2008; Banchet-Cadeddu et al., 2011). The *C*-glycoside of KRN7000 (α-*C*-GalCer) was found to be 100 times more potent in a melanoma challenge assay on C57BL/6 mice than *O*-glycoside (Yang et al., 2004). Ever since it was discovered that α-*C*-GalCer and related *C*-glycoside ligands promote biased cytokine response that is essential for therapeutic potential (Patel et al., 2011), a series of *C*-glycosides were designed and synthesized by Bittman et al. The design of these *C*-glycosides involved structural variations essential in understanding activation of iNKT cells and its subsequent selective cytokine response. The first analog of α-*C*-GalCer reported was a truncated non-isosteric α-*C*-glycoside where the anomeric carbon is directly appended to the C1 of phytoceramide that promoted higher IFN-γ cytokine production, displaying its potential as an adjuvant for immunotherapy (Lu et al., 2006). The nonisosteric α-*C*-glycoside analog was further synthesized in an alternative approach to confirm the stereochemistry in the previous synthesis (Liu et al.,

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2011). α-*C*-Glycoside analogs of KRN-7000 with variations in the linker area viz *E*-alkene, acetylene, and exo-methylene were prepared in a stereocontrolled synthesis to promote biased cytokine responses (Liu and Bittman, 2012; Liu et al., 2010, 2012). Finally, KRN7000 bearing different alkyl chains at meta-or para-positions of phenyl group that is embedded within the amide chain were synthesized. The biological results of the latter compounds indicated that meta- and para-substituted compounds displayed increased Th2 response and IFN-γ secretion, respectively, compared to the analog bearing phenyl group at the amide chain terminus (Baek et al., 2013). Inspired by synthetic GSL analogs reported by Bittman et al. (vide supra), we report herein a novel GSL analog **12** (Fig. 1) with a polyhydroxy 2-pyrrolidinone and phytoceramide linked via a 1,2,3-triazole. Among the *C*-glycoside analogs with linkers reported by Bittman et al. the exo-methylene analog served as an agonist for both human and mouse iNKT cells, perhaps due to flexibility in the latter compound allowing the sugar to rotate along the methylene unit in the linker (Fig. 1). For the same reason, our design involves the attachment of the sugar lactam via methylene to the triazole linker produced by "click chemistry" which is widely employed in appending biomolecules. Structural variants and mimics of KRN7000 with a presence of triazole moiety at different positions (Fig. 1) afforded selective cytokine responses (Jervis et al., 2012; Lee et al., 2007; Perez-Labrada et al., 2014). Recently, we have reported for the first time synthesis of a novel GSL analog which is an aza-variant of β-*C*-GalCer where the ring oxygen is replaced with nitrogen that can be further functionalized for introducing diversity (Gorantla and Lankalapalli, 2014). The rationale for introducing an azasugar was due to their ability to exhibit a broad range of biological activities (For selected reviews, see: Alonzi and Butters, 2011; Martin, 2007; Nash, 2004; Dwek et al., 2002; Hausler et al., 2000; Horne et al., 2011; Wrodnigg et al., 2008). To further prepare more GSL analogs with different azasugars, we propose herein the azasugar moiety as a polyhydroxy 2-pyrrolidinone that was derived from D;1;-galactose. Finally, the present design offers a new entry to the repertoire of GSL analogs of therapeutic significance with azasugar.

2. Experimental

2.1. Materials and analytical procedures

2.1.1. Chemicals

The sources of the chemicals were as follows: Dowex 50 × 8 resin (H^+), 7 N ammonia in methanol, 10% Pd/C, and sodium cyanoborohydride from Sigma–Aldrich, India; D-galactose, benzyl bromide, NaH 60% suspension in mineral oil, Dess–Martin periodinane, anhydrous *N*,*N*-dimethylformamide, and *N*,*N*-diisopropylethylamine from Spectrochem, India; phytosphingosine from TCI, India. 4-Nitrophenyl decanoate was prepared from decanoic acid and 4-nitrophenol using DCC coupling conditions.

2.1.2. General methods

The solvents were dried as follows: CH_2Cl_2 and CH_3CN were distilled over calcium hydride, and MeOH was dried over magnesium turnings. All reactions were carried out under nitrogen atmosphere using oven-dried glassware. Silica gel 60 F_{254} aluminum TLC plates were used to monitor the reactions with short-wavelength ultraviolet light, iodine staining, and by charring the TLC plate after spraying with 15% sulfuric acid to visualize the spots. Column chromatography was performed on silica gel 120–200 and 230–400 mesh. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively. Chemical shifts are given in parts per million and coupling constants in hertz. HR-ESI-MS analysis was performed on a Thermo Scientific Exactive mass spectrometer with ions given in *m*/*z*.

2.2. Synthesis

2.2.1. Methyl-D-galactofuranoside (*1*) and methyl-D-galactopyranoside (*1a*)

To a solution of D-galactose (5 g, 27.7 mmol, 1 equiv) in MeOH (80 mL) was added Dowex H^+ resin (2 g) and the resulting mixture was heated at reflux. After 3 h, the reaction mixture was allowed to attain room temperature, filtered through cotton plug and washed with methanol (50 mL), concentrated, and dried under vacuum for 4 h which resulted in a colorless sticky mixture of compounds **1** and **1a** (5.37 g). The crude mixture was directly used for the next step without further purification.

2.2.2. 2,3,5,6-Tetra-O-benzyl-methyl-D-galactofuranoside (*2*) and 2,3,4,6-tetra-O-benzyl-methyl-D-galactopyranoside (*2a*)

To a solution of the mixture of isomers **1** and **1a** (5.37 g) in DMF (150 mL) 60% NaH was added (8.84 g, 222.1 mmol, 8 equiv) at 0 °C and stirred for 30 min under nitrogen atmosphere. BnBr (26.4 mL, 222.1 mmol, 8 equiv) was added slowly at 0 °C and the resulting mixture was continuously stirred overnight at room temperature. The reaction mixture was quenched by adding cold water (100 mL) drop wise at 0 °C and extracted with EtOAc (2 × 250 mL), the extracts were dried over anhydrous Na_2SO_4 and concentrated. Purification by flash chromatography using hexane/EtOAc 95:5 to 90:10 afforded perbenzylated isomers **2** and **2a** (7.10 g) as a

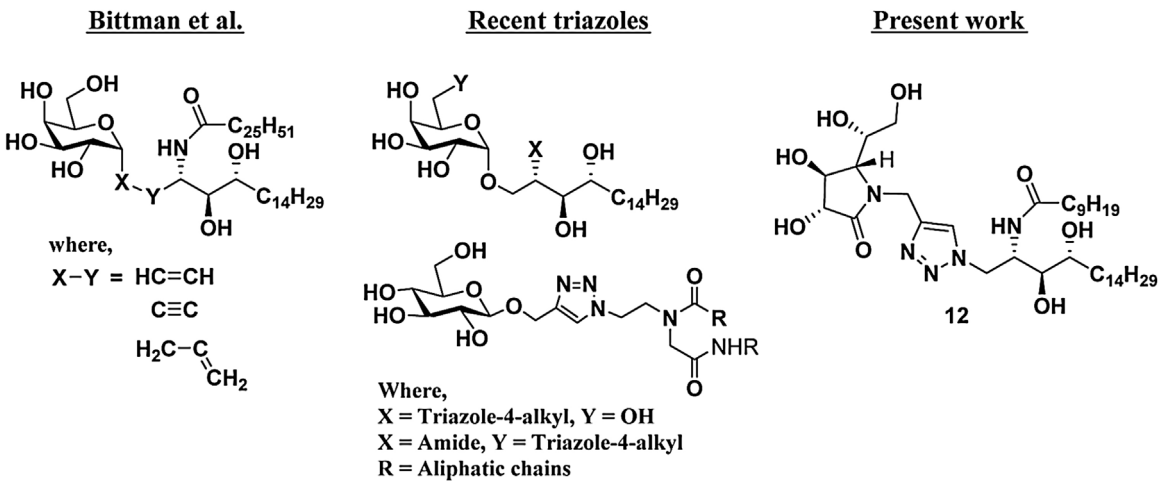

Fig. 1. Design of the novel GSL analog: 2-pyrrolidinone-triazole-phytoceramide (**12**).