European Journal of Medicinal Chemistry 134 (2017) 43–51

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and biological evaluation of neoglycosphingolipids

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ARTICLE INFO

Article history:
Received 3 January 2017
Received in revised form
1 April 2017
Accepted 3 April 2017
Available online 5 April 2017

Keywords:
Neoglycosphingolipids
Stereoselective
One-step synthesis
MMP-9
Anti-tumor agent

ABSTRACT

Various neoglycosphingolipids were efficiently synthesized in a one-step reaction by the coupling of free sugars with an *N*-alkylaminooxy-functionalized ceramide analogue. The bioactivity studies demonstrated that most of these compounds could upregulate the expression of matrix metalloproteinase-9 (MMP-9, extracellular matrix proteins associated with tumor migration) in murine melanoma B16 cells in a similar manner to the natural ganglioside monosialodihexosylganglioside (GM3), which highlights the potential use of these neoglycosphingolipids as inhibitors of tumor migration.

1. Introduction

Glycosphingolipids (GSLs) are ubiquitous membrane constituents of the outer leaflet of plasma membranes in all mammalian cells [1], and are believed to play critical roles in a variety of biological events, including cell recognition, signal transduction, growth, differentiation, immune response, progress of malignancy and the development and functioning of the nervous system [2–4]. Recent evidence suggests that GSLs may be useful for treating cancer, Parkinson's disease, malaria, auto-immune diseases, neurodegenerative disease, amyotrophic lateral sclerosis, diabetes, and also serve as vaccines [5–14]. The broad spectrum of biological and pharmacological activities of GSLs and the challenges of isolating them from nature have driven chemists to research methods for their syntheses.

GSLs are amphipathic molecules consisting of a ceramide lipid moiety, and a hydrophilic carbohydrate chain, which makes them more difficult to synthesize. Many efforts have been made to solve the problem based on chemical and chemo-enzymatic strategies [15–17]. However, the chemical syntheses involve multiple protecting/deprotecting group manipulations and harsh glycosylation conditions and generally suffer from poor product yield and limit large-scale chemical synthesis. Although enzymatic syntheses can be used to obtain pure product with specific glycoside linkages and without protection/deprotection manipulation, the high substrate specificity of enzymes and the divergent solvent requirements for hydrophilic sugar donors and hydrophobic acceptors hinder the application of this method [18].

Based on the central premise of medicinal chemistry that structurally similar molecules have similar bioactivities [19], a great deal of effort has been devoted to synthesize analogues of known active drugs to develop new drug candidates [20]. Some studies have shown that neoglycoconjugates even have a number of advantages over their native counterparts as therapeutic agents, such as higher stability toward endogenous hydrolytic enzymes, better bioavailability, and lower clearance rates [21]. Since GSLs are difficult to synthesize, and structurally similar molecules often have similar bioactivities, chemists and pharmacologists have turned their attention to preparing and investigating GSL analogues.

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http://dx.doi.org/10.1016/j.ejmech.2017.04.006
0223-5234/

Recently, several studies on the synthesis of GSLs analogues have been reported [22–28]. However, protecting/deprotecting manipulations of the carbohydrate units is still generally required, and some approaches even yielded an epimeric mixture. Apparently, the limited availability of large quantities of defined GSLs and GSLs analogues is still the main obstacle to exploit the application of these compounds.

Nishimura and co-workers [28] reported a strategy to synthesize a lactosylceramide analogue by using unprotected lactose and *N*-methoxyamino-functionalized ceramide in a one-step reaction. Herein, we expanded on their strategy to synthesize various ganglioside monosialodihexosylganglioside (GM3) analogues by direct coupling of free sugars and the lipid moiety by employing an *N*-alkylaminooxy linker. Previous reports have proven that the stereoselective glycosylation of *N*-alkylaminooxy or *N*-methoxyamine chains with unprotected reducing sugars is not only useful for the rapid synthesis of *N*(OMe)-linked disaccharide [29] and neoglycopeptides [30–35], but also efficient for glycan microarray fabrication [36–41]. The *N*H(Me)-*O*-linker was used to construct a series of *N*-glycosidic analogues of GSLs including a sialic acid-containing GM3 analogue in mild acidic conditions with high stereoselectivity and high yield to facilitate GSLs synthesis. The strategy was also demonstrated to be efficient for large-scale preparation of neoglycosphingolipids. Furthermore, the regulation activity of the synthesized neoglycosphingolipids to matrix metalloproteinase-9 (MMP-9) was evaluated.

2. Results and discussion

2.1. Synthesis and characterization of neoglycosphingolipids

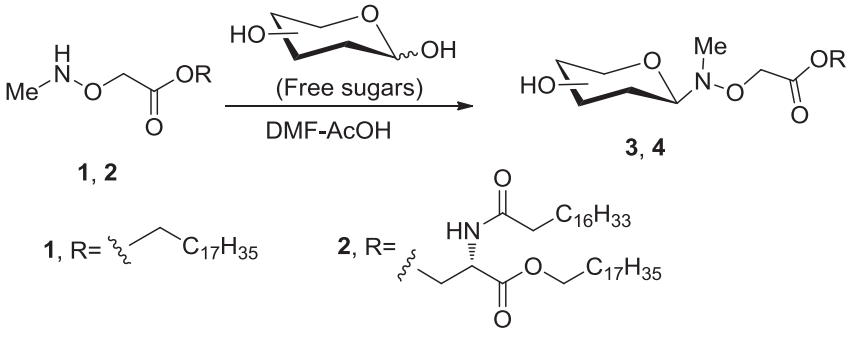
The design of our approach for the rapid stereoselective synthesis of neoglycosphingolipids was based on the following three considerations. First, the methylated Aoa ($MeNHOCH_2COOH$) residue constructed at the terminal of the lipid moiety was used as the linker between the free sugars and the lipid moiety (Scheme 1). As described in previous reports, coupling of an alkyl *N*, *O*-disubstituted hydroxylamine of methylated Aoa with the hemiacetal of the sugar generates glycoconjugates in closed-ring form, and mainly as β configuration [28–30,35]. However, the secondary hydroxylamine, non-methylated Aoa, also generates products as an equilibrium between the open- and closed-ring form [42–45]. Second, the coupling reaction was carried out in DMF-AcOH, which is a mild acidic and anhydrous solvent system. It is much simpler to manipulate compared with NaOAc buffer [30,31,33] and $CHCl_3$-MeOH-H_2O-AcOH [28] as previously described. Finally, to test the efficiency of our method, we focused our initial efforts on coupling free sugars with the structurally simple sphingosine analogue **1** to determinate the optimal reaction conditions, including the best pH and the most appropriate temperature, and then, neoglycosphingolipid synthesis using the ceramide analogue **2** was performed based on the optimized conditions.

Sphingosine mimic **1** and ceramide analogue **2** were synthesized simply from BocAoa **5** [46] in three steps and from Z-L-Serine in five steps, respectively, as depicted in Scheme 2. Since the Boc-protected amine is acid sensitive, esterification of **5** with octadecanoic acid required non-acidic conditions and the reaction was performed using the WSC/DMAP procedure in DCM, giving ester **6** in 90% yield. The secondary amino group in **6** was then methylated using methyl iodide and sodium hydride to give the tertiary amine **7** in 95% yield. Removal of the *N*-Boc protecting group found in **7** in the presence of hydrogen chloride gas in EtOAc gave the desired sphingosine analogue **1** in nearly quantitative yield (98%).

Treatment of the commercially available Z-L-Ser with stearyl alcohol in DCM presaturated with anhydrous HCl produced ester **8** in a yield of 70%. The major by-product was generated by self-esterification of Z-L-Ser, which can be reduced by dripping ester **8** into a stearyl alcohol solution. Pd/C-catalyzed hydrogenolysis to remove the Cbz group proceeded smoothly to generate the amine **9** in 92% yield, which was then subjected to amidation with octadecanoic acid to afford the key intermediate **10** in acceptable yield (71%). The second esterification between **10** and the disubstituted Aoa **11** [47], was carried out under similar conditions to those employed in the synthesis of **6** to furnish **12** in excellent yield (91%). Selective removal of the Boc protecting group using the same conditions as those described above provided the ceramide analogue **2** in high yield (96%).

With sphingosine mimic **1** and ceramide analogue **2** in hand, we formally assembled the neoglycosphingolipids. Based on the last consideration mentioned above, the initial focus of our work was condensation of the free sugars with the sphingosine mimic **1**. Model reactions were first carried out to study the stereoselectivity glycosylation of D-(+)-glucose (3.0 eq.) with sphingosine mimic **1**. The reaction was performed at pH values varied from 1.8 to 5.2, and temperatures varied from 35 °C to 65 °C. As shown in Table 1, the highest yield (91%) of product **3a** was achieved at pH 3.7 and 45 °C overnight. The structure of **3a** was unambiguously determined by its 1D and 2D NMR spectra and HR-ESIMS spectral analyses. The anomeric ^1H-^{13}C correlation of **3a** was observed at 3.97 ppm and 94.74 ppm (as indicated in the HSQC NMR of **3a** by an arrow in the supporting information), and a doublet at 3.97 ppm ($J_{1,2} = 7.1$ Hz) in the ^1H NMR spectrum, clearly demonstrating the pure β-configuration of **3a**. No trace of α-isomer was isolated in our experiment.

Encouraged by these preliminary results, we next turned our attention to glycosylation of other different free carbohydrates with sphingosine mimic **1** and ceramide analogue **2** by employing the



Scheme 1. One-step stereoselective glycosylation of sphingosine mimic **1** and ceramide analogue **2** with free sugars.