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Sulfated liposaccharides inspired by telomerase inhibitor axinelloside A ☆

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ABSTRACT

Sulfated liposaccharides are known inhibitors of telomerase and here we describe the synthesis of a series of sulfated liposaccharides inspired by the natural product axinelloside A, reported to act as an inhibitor of human telomerase. We established a robust and scalable synthetic route to galactosyl liposaccharides capitalizing on a series of regioselective acylation reactions with 2-decenoic acid and imidazolium sulfate esters.

Introduction

Inhibition of telomerase activity offers potential therapeutic benefits in the treatment of cancer.[1] This assertion is based on the fact that >85% of human cancers express telomerase but this enzyme is not present in most somatic cells.[2] It has been recently reported that sulfated saccharides, specifically axinelloside A,[3] display potent inhibitory activity against human telomerase *in vitro*. While the structural and mechanistic details of how these saccharides interact with the enzyme and which components of the sulfated saccharide are critical for the binding are not known, the sulfated small molecule nonetheless offers a promising strategy to target telomerase.

Telomeres are specialized caps attached at the end of human chromosomes consisting of 1000–2000 non-coding repeats of TTAGGG DNA sequence.[4] The main role of these tandem repeats is to protect DNA from undesired degradation and repair, and a specialized enzyme, telomerase, maintains the integrity of the telomeres. Telomerase is a holoenzyme composed of two units: hTERT (human Telomerase Reverse Transcriptase) and hTR (human Telomerase RNA). The RNA component acts as a template (5′-CUAACCUAA-3′) for the synthesis of telomere repeats which is catalyzed by the reverse-transcriptase subunit. Given the importance of telomerase in maintaining the viability of cancer cells and its potential as a therapeutic target, numerous studies have aimed at the identification of a viable telomerase inhibitor.[5] The recent approaches towards telomerase inhibition focused on inhibition of hTERT and includes small molecules (BIBR1532),[6] anti-sense oligonucleotides (GRN163L),[7–13] immunotherapeutics (hTERT peptides used as epitopes to induce CD4+ and CD8+ cells),[14] and gene therapy (telomelysin[15]). Numerous other approaches focused on stabilizing the G-quadruplex structure, preventing access of telomerase to telomeres (telomestatin,[16] BRACO19[17]), and targeting telomerase-associated complexes (inhibitors of HSP90 such as geldanamycin[18] and curcumin[19]) have been reported as well. Currently, the most promising candidate (GRN163L, Imetelstat) has undergone a total of 12 Phase I/II clinical trials targeting multiple myeloma,[20] myelofibrosis, essential thrombocythemia,[21] and lymphoma (more recently, PII/III in patients with myelodysplastic syndrome). GRN163L is a lipidated oligonucleotide thiophosphate acting as an antagonist of hTERT.

Axinelloside A (**1**), a sulfated oligosaccharide, was isolated from the marine sponge *Axinella infundibula*[3] and the structure of this metabolite was established by extensive 1D/2D NMR and MS studies. The important features of the proposed structure of **1** are: (a) the presence of four fatty acid residues, including three unsaturated (*E*)-2-hexadecenoic esters, (b) *scyllo*-inositol residue (unit A) located at the eastern terminus of axinelloside A,[22] and (c) 19 sulfate groups spread along the chain of the natural product. All but one glycosidic linkages are in a 1,2-*cis* glycosidic configuration, rendering the chemical synthesis of this molecule a formidable task. The inhibitory activity of axinelloside A was evaluated by the Telespot assay[23] and **1** showed an IC_{50} of 2.0 μg/mL with isolated human telomerase. Axinelloside A belongs to a larger group

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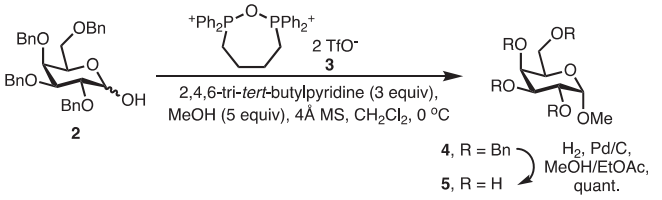
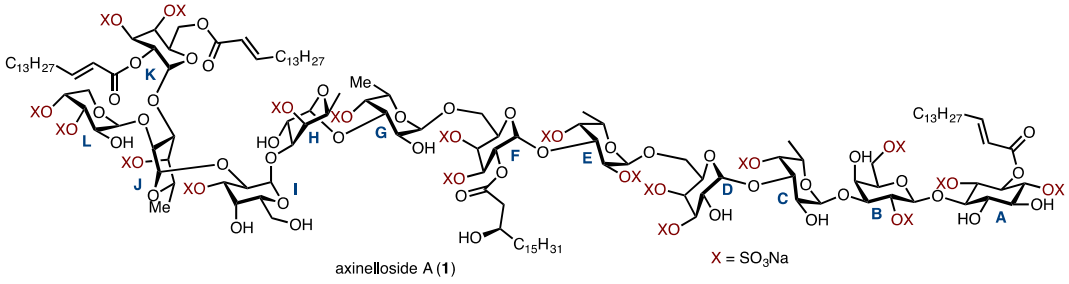
of sulfated natural products that inhibit telomerase. For example, dictyodendrins A-E, which are polycyclic sulfated alkaloids, show appreciable inhibitory activities.[24] A special sub-category of sulfated lipids involves sulfoquinovosyl diacylglycerols (SQDGs) known to inhibit telomerase as well as DNA polymerase.[25] Subsequently these saccharides were found through computational modeling to be also inhibitors of HIV-RT.[26] Recently, Gademann and coworkers tested a series of D-glucose derivatives of 6-sulfonic acid (Na^+ salt) and glycolipid units which proved to be telomerase inhibitors.[25] In addition to the natural product, a focused library of analogs was prepared through chemical synthesis and tested using the Telespot assay and the inhibitory activity of the natural product was improved by the incorporation of a biphenyl acid chain (IC_{50} 11 mM). Taken together, liposaccharides with a varying number of sulfate groups represent a promising class of small molecule telomerase inhibitors, and here we present a synthetic strategy to prepare mono- and disulfate esters of D-galactose lipid derivatives.



Scheme 1.

Esterification of **6** with 2-hexadecenoic acid **7** provided diester **8** in 98% isolated yield. We have also tested alternative methods for the introduction of the unsaturated groups such as double acetylation with acryloyl chloride followed by metathesis with Grubbs II catalyst and 1-pentadecene, but this sequence was significantly less yielding and resulted only in ~20% of the product **8**. The removal of the acetal group required some experimentation and typical conditions (AcOH, THF/H_2O; $TsOH/H_2O$; $TfOH/H_2O$)



Results and discussion

The western fragment of axinelloside A **1** contains a D-galactose unit modified with two identical fatty acid chains at C2 and C6 and two sulfate groups on C3 and C4 (residue K). We postulated that this fragment of axinelloside A may contribute significantly to the inhibitory activity because similar sulfated molecules with lipid groups were reported to act as telomerase inhibitors on their own (mM concentration range).[25] However, this study focused only on sulfonic acids, which may behave similarly to sulfate monoesters but have higher physiological stabilities. Based on this hypothesis, we aimed to prepare the galactose fragment K with two unsaturated acids at positions C2 and C6 before installing two sulfate monoester groups at C3 and C4. This study allows for the evaluation of various conditions for the installation of the ester groups and, more importantly, will establish a robust scheme for the introduction of sulfate groups.

The initial investigations were aimed at evaluating whether the D-galactose residue K can be stereoselectively installed with the correct 1,2-*cis* anomeric configuration. In the context of this question, we elected to prepare the methyl glycoside **4**, which was accessed from the anomeric alcohol **4** and cyclic phosphonium anhydride under previously reported conditions.[27] Thus, alcohol **2** was pre-activated with phosphonium salt **3** formed *in situ* from triflic anhydride and $DPPBO_2$ (1,4-bis(diphenylphosphino)butane dioxide) in the presence of 2,4,6-tri-*tert*-butylpyridine. After 30 min at 0 °C, the nucleophile (MeOH) was added and the reaction was continued for additional 2 h. The methyl glycoside **4** was isolated in 89% and α:β > 95:5. The product of this reaction was then advanced to tetraol **5** by removal of all benzyl groups and conversion into 3,4-dimethyl acetal **6** under the previously reported conditions[28] (Scheme 1).

resulted in decomposition of the substrate. However, we found that In(III) salts[29] were compatible with the sensitive α,β-unsaturated esters and the exposure of **8** to 4 equiv of $InCl_3$[30] resulted in a clean removal of the acetal group without undesired scrambling of the anomeric configuration and degradation of the unsaturated bis-esters.

At this stage, the installation of two sulfate groups was in place.[31] A set of conditions using various stable SO_3 sources known to directly convert alcohols into sulfate monoesters ($SO_3{\cdot}Et_3N$, SO_3-·pyridine, $SO_3{\cdot}DMF$ in MeCN at rt and 40 °C) resulted in decomposition of the material, most likely due to the acidic nature of the reagents. Efforts to buffer the reaction mixture with inorganic bases ($NaHCO_3$, K_2CO_3, K_3PO_4, NaOAc) and soluble pyridine-based additives (pyridine, 2,6-lutidine, 2,4,6-tri-*tert*-butylpyridine) were also unsuccessful, resulting in either no reaction or decomposition. The deterioration of (oligo)saccharide material in reactions with SO_3 sources has been reported because these conditions often require elevated temperatures and eventually lead to a highly acidic environment incompatible with *O*-glycosides. The introduction of two sulfated groups could be accomplished, however, by applying a two step-protocol involving protected imidazolium salt of 2,2,2-trichloroethyl sulfate monoester salt **8** described by Taylor.[32–36] Thus, double esterification of **7** with **8** and 1-methylimidazole at room temperature afforded 42% of D-galactose derivative **9**. The remainder of the material was either a mixture of mono-protected galactose isomers, and addition of excess of **8** did not result in improved yields and only complicated the purification. We have also investigated other nucleophilic additives which could facilitate the esterification of the diol **7** (DMAP, pyridine, 1*H*-imidazole, trialkyl and triaryl phosphines) and we found that without 1-methylimidazole, the transfer of the sulfate group was minimal (<10%) even after extended reaction times (2 days) and heating