Design and Synthesis of 1-O- and 6′-C-Modified Heparan Sulfate Trisaccharides as Human Endo-6-O-Sulfatase 1 Inhibitors

Kuei-Yao Tseng1,2, Zheng-Hao Tzeng2, Ting-Jen Rachel Cheng2, Pi-Hui Liang1,2* and Shang-Cheng Hung2,3,4*

1School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan, 2Genomics Research Center, Academia Sinica, Taipei, Taiwan, 3Department of Applied Science, National Taitung University, Taitung, Taiwan, 4Department of Chemistry, National Cheng Kung University, Tainan, Taiwan

The extracellular human endo-6-O-sulfatases (Sulf-1 and Sulf-2) are responsible for the endolytic cleavage of the 6-sulfate groups from the internal D-glucosamine residues in the highly sulfated subdomains of heparan sulfate proteoglycans. A trisaccharide sulfate, IdoA2OS-GlcNS6OS-IdoA2OS, was identified as the minimal size of substrate for Sulf-1. In order to study the complex structure with Sulf-1 for developing potential drugs, two trisaccharide analogs, IdoA2OS-GlcNS6OSO2NH2-IdoA2OS-OMe and IdoA2OS-GlcNS6NS-IdoA2OS-OMe, were rationally designed and synthesized as the Sulf-1 inhibitors with IC50 values at 0.27 and 4.6 μM, respectively.

Keywords: heparan sulfate, glycosaminoglycans, endo-6-O-sulfatases, carbohydrate chemistry, inhibitors

INTRODUCTION

Heparan sulfate (HS) proteoglycans, which are ubiquitously distributed on the cell surface and in the extracellular matrix and basement membrane, play significant roles in adhesion, recognition, and signal transduction events (Vives et al., 2014). HS is a polyanionic polysaccharide belonging to the glycosaminoglycan (GAG) families, covalently bound to a core protein with a tetrasaccharide linkage region (El Masri et al., 2020). The HS backbone is composed of an alternative disaccharide repeating unit with all 1→4-linkages of N-acetyl-α-D-glucosamine (GlcNAc) and β-D-glucuronic acid (GlcA)/α-L-iduronic acid (IdoA). The acetyl group of GlcNAc could possibly be hydrolyzed to yield the amino group followed by N-sulfation, and/or the 2-O position of the uronic acid and/or the 3-O and/or 6-O positions of GlcN could undergo sulfation through a series of enzymatic modifications (Kreuger and Kjellén, 2012). The highly sulfated regions of HS, which were characterized as S-domains, are involved in binding with various proteins, such as fibroblast growth factors, transforming growth factor-β, Wnt, and bone morphogenetic protein (Li and Kusche-Gullberg, 2016). Changes in the HS sulfation patterns may cause dissociations with these proteins, resulting in up or downregulation of the corresponding signal transduction factors. Human endo-O-sulfatases 1 and 2 (Sulf-1 and Sulf-2) are two isofoms of the extracellular endo-6-O-sulfatases responsible for the hydrolysis of the sulfate groups at the 6-O positions of the internal D-glucosamine residues (Justo et al., 2022). Both enzymes modulate the sulfation patterns and regulate the HS-protein interactions (Hanson et al., 2004; El Masri et al., 2017). Numerous diseases have been proved to be related to the overexpression of Sulf-1 and Sulf-2, including gastric and pancreatic cancers, invasive breast
cancer, lung adenocarcinoma, and osteoarthritis (OA) (Lai et al., 2003; Viviano et al., 2004; Otsuki et al., 2008; Otsuki et al., 2010; Rosen and Lemjabbar-Alaoui, 2010; Hur et al., 2012; Chanalaris et al., 2019; Lin et al., 2020; Severmann et al., 2020; Shamdani et al., 2020; Chivu-Economescu et al., 2022). Thus, the development of effective inhibitors may provide detailed information on their complex structures with Sulfs at the molecular level and offer an opportunity for structure-activity relationship for new drug discovery.

In our previous study, a variety of HS oligosaccharides with different chain lengths and N- and O-sulfation patterns was screened for the substrate specificity of Sulf-1, and a trisaccharide sulfate, IdoA2OS-GlcNS6OS-IdoA2OS (Figure 1), was identified as the minimal size of substrate for Sulf-1 (Chiu et al., 2020). A substrate analog 2 with the sulfonamide at the 6′-O position of GlcNS and a 5-amino-1- n-pentyl group at the 1-O position of IdoA had been developed as an inhibitor of Sulf-1. Although compound 2 could be immobilized on the chip of Surface Plasmon Resonance (SPR) for measurement of the dissociation constant (K_D) for measurement of the dissociation constant (K_D) and the 6′-O-sulfonamide inhibitor 5 with the methyl group at 1-O.

RESULTS AND DISCUSSION

Synthesis of the Sulf-1 substrate 4. The backbone assembly of the target trisaccharide 4 is approached through a [1 + 2] strategy. The preparation of the disaccharide acceptor 13 is illustrated in Scheme 1. A coupling of the D-glucosamine-derived thioglycoside 7 with the 1,6-anhydro-β-L-idopyranosyl 4-alcohol 8 was carried out to yield the desired α-disaccharide 9 (71%), according to our previous report (Zulueta et al., 2012). Copper (II) trifluoromethanesulfonate-catalyzed acetylation of compound 9 to open the 1,6-anhydro ring in acetic anhydride furnished the 1,6-diacetate 10 (88%), which underwent anomic acetoxy replacement via a combination of trimethylsilyl p-toluenyl thioether (TMSSTol) and zinc iodide (ZnI2) to give the corresponding thioglycoside 11 in 76% yield. Treatment of the disaccharide donor 11 with methanol in the presence of N-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) as the activators afforded the expected α-methyl disaccharide 12 (60%) because of neighboring group participation of the 2-O-benzoyl group. The stereochemistry was determined through a series of NMR spectral analyses, indicating the correlation of W-coupling between 1-H and 3-H in the 2D spectrum (Supplementary Material, Supplementary Pages S19–S22). Removal of the 2-naphthylmethyl (2-NAP) group at the 4′-O position of compound 12 using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) provided the desired 4′-alcohol 13 in 79% yield.

With the glycosyl acceptor 13 in hand, the total synthesis of substrate 4 is depicted in Scheme 2. Benzylation of the 4-alcohol 8 (Ag2O, BnBr) led to the ether 14 (94%), which was opened under acetolysis conditions to generate the 1,6-diacetate 15 (87%). Anomeric substitution of compound 15 promoted by ZnI2 and TMSSTol was carried out, and the thioglycoside 16 was obtained in a 74% yield (Hu et al., 2011). Highly stereoselective α-glycosylation of the donor 16 with the acceptor 13 using NIS/ TiOH delivered the desired trisaccharide 17 (71%), and all acyl groups in 17 were removed under Zemplén transesterification.

**FIGURE 1** Structures of Sulf-1’s trisaccharide unit 1 and inhibitor 2 with a 5-amino-1-n-pentyl linker, the anticoagulant Fondaparinux 3, and the proposed Sulf-1’s substrate 4 and inhibitors of the 6′-O-sulfonamide 5 and the 6′-N-sulfate 6 with the methyl group at 1-O.
SCHEME 1 | Synthesis of the disaccharide acceptor 13. Reagents and conditions: (A) NIS, TiOCl, CH2Cl2, 3 Å MS, −78°C to −20°C, 4 h, 71%; (B) CuOTf2, Ac2O, RT, 1 h, 88%; (C) TMSStol, ZnI2, CH2Cl2, 0°C to RT, 3 h, 76%; (D) NIS, TiOCl, MeOH, CH2Cl2, 3 Å MS, −50°C, 2 h, 60%; (E) DDQ, CH2Cl2/H2O = 19/1, RT, 3 h, 79%.

Ac, acetyl; Bn, benzyl; Bz, benzoyl; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; 2-NAP, 2-naphthylmethyl; NIS, N-iodosuccinimide; PBB, p-bromobenzyl; TBDPS, tert-butyldiphenylsilyl; Tl, trifluoromethanesulfonyl; TMS, trimethylsilyl; Tol, 4-methylphenyl.

SCHEME 2 | Synthesis of the Sulf-1 substrate 4. Reagents and conditions: (A) BnBr, Ag2O, CH2Cl2, 3 Å MS, RT, 48 h, 94%; (B) CuOTf2, Ac2O, RT, 1 h, 87%; (C) TMSStol, ZnI2, CH2Cl2, 0°C to RT, 3 h, 74%; (D) NIS, TiOCl, CH2Cl2, 3 Å MS, −40°C, 2 h, 71%; (E) NaOMe, CH2Cl2/MEOH = 1/1, 0°C to RT, 24 h, 86%; (F) TEMPO, BAIB, CH2Cl2/H2O = 2/1, RT, 6 h, 76%; (G) HF•pyridine, THF, 0°C to rt, 24 h, 78%; (H) 1 M LiOH, THF, RT, 1 h, 90%; (I) SO3•Et3N, DMF, 65°C, 24 h, 77%; (J) Pt(OH)2/C, H2(g), phosphate buffer (pH = 7.0), MeOH, RT, 48 h, 90%; (K) SO3•pyridine, 1 M NaOH, H2O, RT, 48 h, 77%. BAIB, [bis(acetoxy)iodo]benzene; DMF, N,N-dimethylformamide; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; THF, tetrahydrofuran.
conditions to give the tetraol 18 (86%). Oxidation of 18 with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and bis(acetoxylimidobenzene (BAIB) furnished the dilactone 19 (76%), which was subjected upon cleavage of the tert-butyldiphenylsilyl (TBDDS) group with HF•pyridine complex at the 6′-O position yielding the 6′-alcohol 20 (78%). Opening of two lactone rings in compound 20 employing 1 M lithium hydroxide aqueous solution provided the 2,6′,2″-triol 21 (90%), which could be sulfonated with SO3•Et3N complex to afford the corresponding 2,6′,2″-tri-O-sulfate 22 (77%). Hydrogenolysis of compound 22 with Pd(OH)2/C and H2 in neutral buffer solution, allowing removal of the 1,3″,4″-tri-O-benzyl and 3′,4′-O-p-bromobenzyl groups and reducing the 2′,C-azido group to the 2′-C-amino group, led to the desired product 23 (90%). N-Sulfonation of the amine 23 with SO3•pyridine complex at ca. pH 9.5, controlled by the addition of 1 M NaOH aqueous solution, furnished a crude mixture, which was purified through sequential Sephadex G10 size-exclusion column chromatography and Dowex 50WX8-Na+ ion-exchange column chromatography to yield target molecule 4 (Na+ salt, 77%). The structure of compound 4 was characterized through analyses of its 1D and 2D NMR spectra (Supplementary Material, Supplementary Pages S45–S49). The molecular weight of 4 (M+6Na+H+), calculated for C19H26N2O2S4Na6 (997.8854, found 997.8815) was further confirmed by the high-resolution electrospray ionization mass spectrum (Supplementary Material, Supplementary Page S50).

**Synthesis of the 6′-O-sulfonamide trisaccharide inhibitor 5.** Scheme 3 describes the synthesis of HS trisaccharide analog 5 containing the 6′-O-sulfonamide as the Sulf-1 inhibitor. Starting from the common trisaccharide intermediate 20, the 6′-hydroxy group was treated with N-benzyl sulfamoyl chloride and pyridine to give the N-benzyl-6′-O-sulfonamide derivative 24 (78%). It should be noted that the reaction needed to be quenched in 15 min since the NH-proton could be removed by the base and further coupled with N-benzyl sulfamoyl chloride to yield the unwanted side product when the reaction time is prolonged. The lactone rings of compound 24 were opened under basic conditions to provide the 2,2″-diol 25 (90%), which was converted into the corresponding di-O-sulfate 26 (70%) via sulfonation at the 2-O and 2″-O positions. Global deprotection of 26 under hydrogenolysis conditions led to the product 27 (73%), which underwent N-sulfonation to furnish the desired 6′-O-sulfonamide inhibitor 5 (Na+ salt) in 75% yield after consecutive purification through the size-exclusion column and ion-exchange column. The structure of compound 5 was determined via the 1D and 2D NMR spectral analyses (Supplementary Material, Supplementary Pages S57–S61). By using the trisaccharide 5 as a representative example, the assignments of all protons, including the splitting patterns and coupling constants, were examined by the 1D-Total Correlation Spectroscopy (1D-TOCSY) experiments in detail. As indicated in Figure 2, three isolated spectroscopic patterns were generated by 1H connectivity through δ-coupling after the excitation of selective 1H nuclei at a given frequency (MacKinnon et al., 2016). The complicated proton NMR patterns of compound 5 could be resolved in three individual pyranosyl rings (purple one: non-reducing end L-iduronic acid sugar unit; blue one: D-glucosamine unit; red one: reducing end L-iduronic acid sugar unit) and characterized by combination with basic 1D and 2D NMR

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Starting from the 2D-HMBC spectrum, the correlation of $^{3}$J$_{C-H}$ coupling between the anomeric carbon of the reducing end L-iduronic acid and the methyl protons at 1-O was identified. Two sets of $^{3}$J$_{C-H}$ coupling correlations between the anomeric carbon of the L-iduronic acid and H-5 were also recognized. All of the anomeric hydrogen atoms could be
60% yield. Such harsh conditions, at 100°C for 24 h, were needed for the reaction of 6 to successfully transform the 6′-hydroxy group into the 6′-position, the interactions with Sulf-1 were different. These results have revealed that the sulfonamide group could be an appropriate moiety to inhibit Sulf-1 activity (Hanson et al., 2004). The N-sulfate moiety, albeit with lower inhibition, provided alternative considerations for the design of new inhibitors.

CONCLUSION

The total syntheses of the 1-O- and 6′-C-modified HS trisaccharides 4, 5, and 6 via the 6′-alcohol 20 as the common intermediate have been successfully developed. Both bicyclo[2.2.2]-lactone rings in 20 block the 2,2″-dihydroxy groups, eliminating two protecting groups and allowing the functional group transformation at the 6′-hydroxy group. A straightforward four-step transformation, including lactone opening, O-sulfonation, hydrogenolysis, and N-sulfonation, has efficiently yielded the desired products 4, 5, and 6. The 1-O-methyl-modified trisaccharide 4 has been identified as the Sulf-1 substrate that can be used to test the enzyme activity. In comparison with compound 2, the 1-O-methyl-modified 6′-O-sulfonamide trisaccharide 5 exhibits similar inhibitory property (IC50 = 0.27 μM) that is an appropriate molecule to complex with Sulf-1 for further 3D structural studies by X-ray single crystal diffraction technique. Compound 5 is also a potent candidate for new drug discovery against diseases related to Sulf-1 overexpression. The 6′-N-sulfate trisaccharide 6, which is not a Sulf-1 substrate, has been characterized as an inhibitor with IC50 = 4.6 μM for Sulf-1. The effectiveness of 5 to inhibit Sulf-1’s activity is 17 times better than 6.
DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

K-YT and Z-HT performed the experimental work and analyzed the spectral data. T-JC obtained Sulf-1 and conducted the IC_{50} measurements. S-CH, P-HL, and K-YT wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2022.947475/full#supplementary-material