Published in final edited form as:

ChemBiochem. 2010 November 22; 11(17): 2393–2397. doi:10.1002/cbic.201000401.

Gucosamine-6-sulfamate Analogs of Heparan Sulfate as Inhibitors of Endosulfatases

Mathias Schelwies^a, Diana Brinson^b, Shuhei Otsuki^b, Young-Hoon Hong^b, Martin K. Lotz^b, Chi-Huey Wong^{*,a}, and Sarah R. Hanson^{*,a}

^aDr. M. Schelwies, Prof. Dr. C.-H. Wong, Dr. S. R. Hanson Department of Chemistry and Skaggs Institute for Chemical Biology The Scripps Research Institute 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

^bD. Brinson, Dr. Y.-H. Hong, Dr. S. Otsuki, Prof. Dr. M. K. Lotz, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

Abstract

Keeping Sulfate. The extracellular endosulfatases, which modulate signalling pathways by removing sulfate groups from heparan, can be inhibited by replacing the 6-sulfate destined for cleavage with an inhibitory sulfamate motif, as demonstrated by simple glucosamine-6-sulfamate analogs of heparan sulfate.

Keywords

extracellular endosulfatases; heparan sulfate; inhibitor; inflammation; sulfatase

Many signalling pathways are modulated by the specific sulfation patterns displayed on heparan sulfate (HS), the most abundant polysaccharide component of the extracellular matrix (ECM).^[1] The sulfation state of HS is controlled either biosynthetically, by a suite of sulfotransferases that install sulfates in the Golgi; or dynamically in the ECM, by the extracellular HS endosulfatases (Sulfs), Sulf-1 and Sulf-2.^[2, 3] By cleaving 6-*O*-sulfates from glucosamine (GlcN) residues within the HS polymer (Figure 1A), Sulf-1 and Sulf-2 are central players in regulating HS 6-sulfate dependent signalling pathways. This includes documented roles in promoting cascades, by releasing HS 6-sulfate bound cytokines and morphogenes to interact with cognate receptors; as well as in attenuating signal transduction, by breaking down signalling complexes dependent on HS 6-*O*-sulfate co-receptors.^[4] Increasingly, aberrant Sulf-1 and Sulf-2 overexpression and activity are being linked to diseases such as cancer^[5–8] and inflammation,^[9, 10] making them promising therapeutic targets.

Fax: (+1) 858-784-2409, shanson@scripps.edu.

Supporting information for this article is available on the WWW under <http://www.chembiochem.org>

The objective herein was to derive small molecule inhibitors of the human extracellular HS endosulfatases (HSulfs), HSulf-1 and HSulf-2. Using sulfamate inhibition motifs as a starting point, the HSulfs were first verified to be inhibited by the general phenyl sulfamates **1** and **2**, before the first generation of more potent and specific inhibitors (**3** and **4**) were synthesized, wherein sulfamate-based sulfate surrogates were introduced into analogs of the endogenous HS substrate (Figure 1). These HS-based GlcN-6-sulfamates inhibit HSulf-1 and HSulf-2 (Figure 2), representing the first example of carbohydrate-based sulfamate inhibitors of sulfatases.

Reports describing chemical inhibitors of Sulfs are limited because they have only recently emerged as targets of therapeutic interest. There is, however, detailed precedent for sulfatase inhibition in the literature. For steroid sulfatase (STS), a target in hormone-dependent cancers, efficient inhibitors were procured by replacing the substrate's sulfate moiety to that of an inhibitory sulfate surrogate.^[11–13] Steroid structures equipped with an aryl sulfamate were very potent, leading to the clinically viable candidate 3-O-estrone sulfamate, or EMATE; and many examples followed, including non-steroidal sulfamate scaffolds.^[14] In addition, phenyl sulfamates and cyclic sulfamates are active against several other sulfatases of bacterial and eukaryotic origin, although few other human enzymes have been tested.^[15–17] That the sulfamate pharmacophore for sulfatases is general seems reasonable in light of this collective inhibition and also in the context of the highly conserved structure and mechanism of the sulfatase family.^[3, 18] The sulfatase catalytic pocket is superimposable among the solved structures^[19], containing 10 conserved residues and a divalent calcium, and displaying a compact design, ostensibly optimized for the recognition, accommodation, and cleavage of the sulfate moiety. Thus, despite having high specificity for their biological substrates, the binding of which occurs outside the catalytic pocket, sulfatases also possess the ability to generally hydrolyze small aryl sulfates, and, likewise, are susceptible to broad-ranged inhibition by aryl sulfamates.

Taking into account the observations in sulfatase inhibition, it was envisaged that the introduction of sulfamate-based motifs in place of sulfates groups in the context of the HS substrate would result in potent and specific inhibitors of the Sulfs. Biochemically, Sulf-1 and Sulf-2 are redundant, showing a neutral pH optimum and endolytic activity with GlcN-6-*O*-sulfate groups within highly sulfated HS domain, with particular preference for the trisulfated disaccharide unit, ioduronic acid 2-sulfate linked to *N*-sulfated glucosamine 6-sulfate (IdoA2S-GlcNS6S, Figure 1A).^[20–23] Access to different 6-sulfate groups within HS may be provided by unique hydrophilic domains, which are thought to allow the extracellular sulfatases to navigate and bind to different HS regions.^[24, 25] Because HS is a notoriously complicated molecule replete with synthetic challenges, preliminary proof-of-concept studies began with the design of inhibitors based on the smallest and most relevant HS substructure, the α -*O*-GlcN unit from which the enzymes cleave 6-sulfate. As such, GlcN analogs **3a**, **3b**, **4a**, and **4b** with 6-sulfamate-based inhibition motifs were targeted, as depicted in Figure 1C. Two inhibitory sulfate surrogates were chosen based on sulfamate (a-series compounds) and *N,N*-dimethyl sulfamate (b-series compounds). These two surrogates can potentially alter the nature of inhibition, as sulfamates frequently inactivate sulfatases in a mechanism-based fashion, whereas *N*-alkylated sulfamates are competitive, but often at the price of decreased potency from the increased steric bulk of the alkyl groups.^[14]

To begin probing inhibition of the endosulfatases, it was first verified that the known sulfamate inhibition motif applies. Linear sulfamate **1** and phenyl cyclic sulfamate **2** were previously synthesized.^[15] Activity testing was carried out on recombinant HSulf-1 and HSulf-2 protein purified from the conditioned media of human embryonic kidney cells using FreeStyle 293F cells (see Methods and Supporting Information, Figure S1). Biochemical activity was evaluated by a fluorescent arylsulfatase (ARS) assay, using the surrogate

substrate 4-methyumbelliferone sulfate (4MUS). Consistent with the idea of the general sulfamate pharmacophore, Sulf-1 and Sulf-2 were inhibited in the presence of the two general sulfamate inhibitors, **1** and **2** around 30 and 50%, respectively, of control activity at 5mM inhibitor (Figure 2A). Interestingly, the cyclic inhibitor **2** is slightly more active for both Sulf-1 and Sulf-2 than the free sulfamate motif **1**, in contrast to our previous findings with the arylsulfatase from *Pseudomonas aeruginosa* (PARS), a common model for human arylsulfatases like STS.^[15] Promising activity was also seen in vivo with **2**, where an important proinflammatory response in arthritis linked to increased Sulf activity, was inhibited (i.e., the stimulation of interleukin (IL)-6 production by IL-1 β in fibroblast-like synoviocytes was inhibited; Figure 2C).^{[9] [26]}

Encouraged by the general sulfamate inhibition and promising in vivo data, more potent and specific inhibitors based on HS GlcN sulfamates were targeted. Synthesis of sulfamates **3a** and **3b** was accomplished starting from GlcN hydrochloride, which was fully protected as building block **5** (Ref A).^[27] A Selective acetal opening with BH₃/Bu₂BOTf gave 6-deprotected azide **6**, which was converted into the N-sulfated inhibitors **3a** and **3b** in 4 steps (Scheme 1). Similarly, N-acetylglucosamine (GlcNAc)-based **4a** and **4b** were accessed from GlcNAc, which was fully protected as building block **10** (Ref B),^[28] followed by sulfamoylation and deprotection. As a control to probe the effect of the sulfamate moiety, **11**^[29] was prepared by deprotecting **10**.

The inhibition profile of the HSulfs against the panel of GlcN sulfamate compounds and controls is shown in Figure 2A. As expected, the GlcN-6-sulfamates are inhibitors of HSulf-1 and HSulf-2, and represent the first example carbohydrate-based sulfamate inhibitors of sulfatases. Analogs **3a** and **3b**, which bear the closest resemblance to the biological HS substrate were the most potent inhibitors with respective IC₅₀ values of 95 and 180 μ M, against HSulf-1, and 130 and 240 μ M against HSulf-2 (IC₅₀ curves in Supporting Information, Figure S2). This represents between one to two orders of magnitude improvement in inhibition of the HSulfs over that generally elicited by **1** and **2**. Importantly, the GlcN-6-sulfamates are also more selective for the HSulfs than the general compounds **1** and **2**. As shown in Figure 2B, at 500 μ M inhibitor, type **3** GlcN-6-sulfamate analogs significantly reduce the biochemical activity of HSulf-1 and HSulf-2, while PARS is unaffected. Conversely, general inhibitors **1** and **2**, potently knock down PARS activity, while Sulf-1 and Sulf-2 remain largely active (Figure 2B).

The GlcN analogs in the panel reveal important information about the structural requirements for Sulf-1 and Sulf-2 inhibition. The most striking trend is the gain in incremental inhibition seen by the addition of sulfamate surrogate at position six (**11** $\rightarrow$ **4a** and **4b**) and then sulfate at position 2 of the GlcN unit (**4a** $\rightarrow$ **3a** and **4b** $\rightarrow$ **3b**). Adding the 2-sulfate over the free amine results in at least a 7-fold increase in inhibition (**8a** $\rightarrow$ **9a** and **8b** $\rightarrow$ **9b**). The effect of N-acetylation vs. N-sulfation is even greater, producing a 20- to 50-fold increase in potency, as demonstrated by compounds of type **4**, which are near 50% inhibition at 5 mM compared to compounds of type **3**, which all have IC₅₀ values below 250 μ M (Figure 2A). That the inhibition trends for HSulf-1 and HSulf-2 are very similar is not surprising, since both have been reported to have nearly identical biochemical activity and substrate preferences, wherein the HS sulfates at position 2- and 6- on GlcN are important structural features.^[20–23]

In line with their HS specificity, inhibition of the endosulfatases appears to be the most sensitive to the carbohydrate backbone structure, and less affected by the nature of the sulfate surrogate. As shown in Figure 2A, similar inhibition profiles against the HSulfs are apparent for inhibitors containing the free sulfamate (a-series) and *N,N*-dimethyl (b-series) at the 6-position. Despite the extra steric bulk of *N*-alkyl groups in the latter series, little

difference is seen between compounds of type **4**, while only around a 2-fold increase in the IC₅₀ value was measured for **3b** versus **3a**. Taken together with the general inhibition data (i.e., **1** versus the more bulky **2**) it seems the sulfate binding pocket of the endosulfatases may not be as sterically encumbered as other arylsulfatases. It may be that a more permissive sulfate binding pocket is necessary for the traversal and endolytic cleavage of sulfates displayed on HS, a process that is unique to the extracellular Sulf. While mechanism-based inhibition has been documented in the case of linear and cyclic aryl sulfamates,^[14–17] the nature of sulfamate inhibition with the Sulf is still under investigation. Under the reported assay conditions, control and inhibition activity after 10 minutes of pre-incubation with GlcN compounds of type **3** and **4** is the same as that at time zero, suggesting reversible inhibition without significant inactivation (see Supporting Information, Figure S3). Irreversible inhibition, if any, may still arise after longer incubation periods and it would likely be accelerated by tighter binding HS-like scaffolds, which is the subject of future work.

Sulf-1 and Sulf-2 are involved in complex signalling pathways with high therapeutic potential. Specific Sulf inhibitors are important tools to better define the signalling roles of the Sulf and to validate them as therapeutic targets. For example, Sulf-1 and Sulf-2 are involved in cartilage development and homeostasis, but are overexpressed in arthritis where they may contribute to the disease process.^[9, 10, 30] When Sulf-1 and Sulf-2 are knocked down in synovial fibroblasts by SiRNA, there is a reduction in the pro-inflammatory cytokine IL-6 (manuscript in preparation). The IL-6 proinflammatory response was also reduced by **2**, and also with the Sulf-directed inhibitors **4a** and **4b** (Figure 2C). These results support that endosulfatases are involved in inflammation and that the inflammatory IL-6 response can be attenuated through Sulf inhibition, representing a promising avenue for the treatment of inflammation in arthritis. Studies on the inhibition of other Sulf-related signalling pathways by the more potent analogs **3a** and **3b** is currently underway.

The results herein show that aryl sulfamates generally inhibit human Sulf-1 and Sulf-2 and that replacement of sulfates with sulfamate surrogates in the context of their native substrate heparan-6-sulfate leads to more potent and specific inhibition. The HSulf is tolerant of *N*-alkylated sulfamate surrogates, which may allow the mechanism of future inhibitors to be tuned for different applications. The potency of the HS-based GlcN inhibitors follow the known trends of substrate preference for the extracellular HS endosulfatases (Figure 1A), with the 6-sulfamate surrogate and *N*-sulfate being the most dominant factors. It also follows that inhibitors with even greater potency can be obtained by making HS-sulfamate structures of increasing HS-character. It may even be possible to create specific-sulfamate bearing HS epitopes to selectively address a detrimental signalling pathway, while preserving others important for healthy cellular activity. As demonstrated with the pro-inflammatory IL-6 response, the reported sulfamate inhibitors are already useful tools for probing the role of the extracellular sulfatases in signalling and disease.

Experimental Methods

Synthesis

See Scheme 1 and Supporting Information for detailed synthetic routes and compound characterization.

Sulfatase proteins

Full-length human Sulf-1 and Sulf-2 and catalytically inactive Δ CC analogs in the pcDNA3.1 vector containing a C-terminal hexa histidine tag (clones created and gifted from Dr. Steven Rosen)^[20] were overexpressed in human embryonic kidney (HEK) 293-F cells

using the FreeStyle 293-F suspension cell system. Briefly, 293-F cells (1.0×10^6 cell/mL) were transiently transfected ($2\mu\text{g}$ of DNA/mL precomplexed 1:1 with 293Fectin) for 4 days. Conditioned supernatant was then separated from cells by centrifugation and supplemented with TBST (20 mM Tris, 250 mM NaCl, 0.025% Tween20, pH 8.0). Hexahistidine tagged protein was immobilized using nickel-affinity resin (SuperFlow Ni-NTA agarose, Qiagen; $25\mu\text{L}$ resin/ 50mL of supernatant), washed three times with TBST (10 bead volumes), and eluted with TBST plus imidazole (200 mM, 2 bead volumes). Eluted protein was desalted in HBST (50 mM HEPES, 250 mM NaCl, 0.025% Tween, 20; pH 7.5). Protein concentrations were determined by absorbance at 280 nm using the molar extinction coefficient. PARS protein was prepared as described previously.^[15]

4-Methumbelliferone sulfate (4MUS) assay

Arylsulfatase activity was monitored by the hydrolysis 4-MUS to fluorescent methyl umbelliferone (4MU, λ_{EX} 360 nm, λ_{EM} 460 nm). Assays were performed in opaque black 96-well half-area plates (Nunc) and analyzed on a fluorescence plate reader (Fusion, Perkin Elmer). Activity was measured over 30 minutes using 5 ng/ μL Sulf-1 or Sulf-2 and 15 mM 4-MUS in assay buffer (50 mM HEPES, 2.5 mM CaCl_2 , 250 mM NaCl, 0.025% Tween20; pH 7.5) with or without inhibitor. Rates for enzymatic activity were determined by fitting the fluorescence increase overtime by linear regression. Inhibition values are reported as percentages of the uninhibited control values: % Activity = [rate with inhibitor/ rate control * 100]; and, % Inhibition = [100 - % Activity]. All values represent an average of a minimum of triplicate measurements. Half maximal biochemical activity values, or IC_{50} values, were determined using a 4-parameter dose-response curve in Prism software (GraphPAD), see Supporting Information. Catalytically inactive Sulf-1 and Sulf-2 ΔCC analogs were used as controls.

In vivo inhibitor testing

Fibroblast-like synoviocytes were isolated from synovial tissue from patients with rheumatoid arthritis or osteoarthritis removed during total joint replacement surgery as described.^[31] Cells at passage 3 to 7 were stimulated with IL-1 β in the absence or presence of Sulfatase inhibitors for 24 hours when culture supernatants were collected for IL-6 ELISA. Half-area ELISA plates (Costar, Cambridge, MA) were coated with $25\mu\text{L}$ of a $2\mu\text{g/mL}$ monoclonal mouse antihuman IL-6 capture antibody (R & D Systems) for 4 hours and blocked with 1 % BSA. Cell-free culture supernatants or human recombinant IL-6 standard ($25\mu\text{L}$) was added to the plates and incubated for 2 hours followed by the addition of $1\mu\text{g}$ biotinylated goat antihuman IL-6 detection antibody (R & D Systems) for 1 hour. Biotin was detected with 0.16 ng/mL of poly-horseradish peroxidase-labeled streptavidin (Accurate Chemical, Westbury, NY) and tetra-methylbenzidine (TBM) plus hydrogen peroxide substrate (Kirkegaard & Perry, Gaithersburg, MD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants A1072155-03 (C.-H.W.) and AG07996 (M.K.L.) and by a Feodor Lynen Research Fellowship of the Alexander von Humboldt-Foundation (M.S.).

References

1. Esko JD, Selleck SB. Annu. Rev. Biochem. 2002; 71:435. [PubMed: 12045103]

2. Chapman E, Best MD, Hanson SR, Wong CH. *Angew. Chem. Int. Ed. Engl.* 2004; 43:3526. [PubMed: 15293241]
3. Hanson SR, Best MD, Wong CH. *Angew. Chem. Int. Ed. Engl.* 2004; 43:5736. [PubMed: 15493058]
4. Parker RB, Kohler JJ. *ACS Chem. Biol.* 2009; 5:35. [PubMed: 19968325]
5. Abiatari I, Kleeff J, Li J, Felix K, Bachler MW, Friess H. *J. Clin. Path.* 2006; 59:1052. [PubMed: 16603650]
6. Nawroth R, van Zante A, Cervantes S, McManus M, Hebrok M, Rosen SD. *PLoS ONE.* 2007; 2:e392. [PubMed: 17460759]
7. Lai J-P, Thompson JR, Sandhu DS, Roberts LR. *Future Oncol.* 2008; 4:803. [PubMed: 19086847]
8. Lai J-P, Sandhu DS, Yu C, Han T, Moser CD, Jackson KK, Guerrero RB, Aderca I, Isomoto H, Garrity-Park MM, Zou H, Shire AM, Nagorney DM, Sanderson SO, Adjei AA, Lee J-S, Thorgeirsson SS, Roberts LR. *Hepatology.* 2008; 47:1211. [PubMed: 18318435]
9. Otsuki S, Taniguchi N, Grogan SP, D'Lima D, Kinoshita M, Lotz M. *Arthritis Res. Ther.* 2008; 10:R61. [PubMed: 18507859]
10. Otsuki S, Hanson SR, Miyaki S, Grogan SP, Kinoshita M, Asahara H, Wong C-H, Lotz MK. *Proc. Natl. Acad. Sci. U.S.A.* 2010
11. Ahmed S, Owen CP, James K, Sampson L, Patel CK. *Curr. Med. Chem.* 2002; 9:263. [PubMed: 11860358]
12. Nussbaumer P, Billich A. *Med. Res. Rev.* 2004; 24:529. [PubMed: 15170594]
13. Reed MJ, Purohit A, Woo LWL, Newman SP, Potter BVL. *Endocr. Rev.* 2005; 26:171. [PubMed: 15561802]
14. Woo LWL, Howarth NM, Purohit A, Hejaz HAM, Reed MJ, Potter BVL. *J. Med. Chem.* 1998; 41:1068. [PubMed: 9544207]
15. Hanson SR, Whalen LJ, Wong C-H. *Bioorg. Med. Chem.* 2006; 14:8386. [PubMed: 17045481]
16. Bojarová P, Denehy E, Walker I, Loft K, Souza DPD, Woo LWL, Potter BVL, McConville MJ, Williams SJ. *ChemBioChem.* 2008; 9:613. [PubMed: 18288656]
17. Bojarová P, Williams SJ. *Bioorg. Med. Chem. Lett.* 2009; 19:477. [PubMed: 19058962]
18. Bojarová P, Williams SJ. *Curr. Opin. Chem. Biol.* 2008; 12:573. [PubMed: 18625336]
19. Ghosh D. *Cell Mol Life Sci.* 2007; 64:2013. [PubMed: 17558559]
20. Morimoto-Tomita M, Uchimura K, Werb Z, Hemmerich S, Rosen SD. *J. Biol. Chem.* 2002; 277:49175. [PubMed: 12368295]
21. Ai X, Do A-T, Kusche-Gullberg M, Lindahl U, Lu K, Emerson CP Jr. *J. Biol. Chem.* 2006; 281:4969. [PubMed: 16377625]
22. Holst CR, Bou-Reslan H, Gore BB, Wong K, Grant D, Chalasani S, Carano RA, Frantz GD, Tessier-Lavigne M, Bolon B, French DM, Ashkenazi A. *PLoS ONE.* 2007; 2:e575. [PubMed: 17593974]
23. Lamanna WC, Baldwin RJ, Padva M, Kalus I, Ten Dam G, van Kuppevelt TH, Gallagher JT, von Figura K, Dierks T, Merry CL. *Biochem. J.* 2006; 400:63. [PubMed: 16901266]
24. Tang R, Rosen SD. *J. Biol. Chem.* 2009; 284:21505. [PubMed: 19520866]
25. Frese M-A, Milz F, Dick M, Lamanna WC, Dierks T. *J. Biol. Chem.* 2009; 284:28033. [PubMed: 19666466]
26. Bartok B, Firestein GS. *Immunological Reviews.* 233
27. Zhou Y, Lin F, Chen J, Yu B. *Carb. Res.* 2006:1619.
28. Falcone-Hindley ML, Davis JT. *J. Org. Chem.* 1998:5555.
29. Gao F, Yan X, Shakya T, Baettig OM, Ait-Mohand-Brunet S, Berghuis AM, Wright GD, Auclair K. *J. Med. Chem.* 2006; 49:5273. [PubMed: 16913716]
30. Ratzka A, Kalus I, Moser M, Dierks T, Mundlos S, Vortkamp A. *Develop. Dynam.* 2008; 237:339.
31. Guerne PA, Zuraw BL, Vaughan JH, Carson DA, Lotz M. *The Journal of Clinical Investigation.* 1989; 83:585. [PubMed: 2464001]

(A) The Sulf substrate is HS. HS is a long polymer of repeating dissaccharide units—comprised of one unit of a uronic acid (U, either GlcA or IdoA) and one hexose (H, GlcN)—made polydisperse by varying degrees of linkage, epimerization, N-acetylation, and N- and O-sulfation. The arrow indicates the preferred site of 6-*O*-sulfate cleavage catalyzed by the Sulfs at IdoA2S-GlcN6S. (B) General sulfamate inhibition motifs of sulfatases based on linear and cyclic phenyl sulfamates, **1** and **2**. (C) Proposed inhibitors of Sulfs based on the HS analog α -*O*-GlcN equipped with inhibitory sulfamate surrogates at the 6-position (GlcN-6-sulfamates).

Figure 2. Inhibition of the human extracellular HS endosulfatases by sulfamates

(A) Biochemical evaluation of inhibitors against Sulf-1 and Sulf-2 using 4-MUS assay.

Bars: percent inhibition in the presence of 5 mM inhibitor relative to uninhibited controls

(*3a and 3b show full inhibition at 5mM, and bars are labelled with half maximal

biochemical activity, or IC₅₀, values). (B) Selectivity of sulfamate inhibitors. GlcN-6-

sulfamates 3a and 3b are more selective for the Sulfs than PARS and are more potent than

general inhibitors 1 and 2 (Bars: percent activity in the presence of 500μM inhibitor relative

to uninhibited control). (C) Anti-inflammatory activity of Inhibitors. Production of IL-6 by

IL-1β stimulation is a proinflammatory response in arthritis. Data show this response is

inhibited by up to 50% by sulfamate-based sulfatase inhibitors (Bars: percent the IL-6

response is attenuated in the presence of 200 μ M inhibitor relative to uninhibited control in IL-1 β stimulated fibroblast-like synoviocytes).

Scheme 1. Synthesis of heparan analogs

Reagents and conditions: a) BH₃, Bu₂BOTf, THF, 0°C, 2 h; b) NaH, ClSO₂NR₂, THF, 0°C; c) H₂, Pd/C, MeOH, RT; d) PySO₃, pyridine, RT, 12 h, then DOWEX-IR Na⁺-form; e) H₂, Pd/C, EtOH, RT.