Uncovering Biphasic Catalytic Mode of C₅-epimerase in Heparan Sulfate Biosynthesis

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Background: C₅-epimerase converts a glucuronic acid to an iduronic acid residue in the heparan sulfate biosynthetic pathway.

Results: C₅-epimerase displays both “reversible” and “irreversible” catalytic modes.

Conclusion: C₅-epimerase recognizes the saccharide sequence of the substrate to position the iduronic acid.

Significance: The biphasic catalytic mode of C₅-epimerase reveals a unique control mechanism in the biosynthesis of heparan sulfate.

Heparan sulfate (HS), a highly sulfated polysaccharide, is biosynthesized through a pathway involving several enzymes. C₅-epimerase (C₅-epi) is a key enzyme in this pathway. C₅-epi is known for being a two-way catalytic enzyme, displaying a “reversible” catalytic mode by converting a glucuronic acid to an iduronic acid residue, and vice versa. Here, we discovered that C₅-epi can also serve as a one-way catalyst to convert a glucuronic acid to an iduronic acid residue, displaying an “irreversible” catalytic mode. Our data indicated that the reversible or irreversible catalytic mode strictly depends on the saccharide substrate structures. The biphasic mode of C₅-epi offers a novel mechanism to regulate the biosynthesis of HS with the desired biological functions.

Fig. S1). Uniquely distributed sulfo groups and IdoA residues play critical roles in determining the functions of HS (2); however, it remains unclear how to regulate the biosynthesis of these specific sulfated saccharide sequences in vivo.

Understanding the biosynthetic mechanism also aids in developing a chemoenzymatic method to synthesize HS-based drugs. Heparin, a commonly used anticoagulant drug, is a special form of HS with higher levels of sulfation and IdoA. Heparin is currently isolated from animal tissues through a long and poorly regulated supply chain. Worldwide distribution of contaminated heparin in 2007 has raised the concerns over the safety of animal-sourced heparins (3). A cost-effective method to prepare synthetic heparin is desirable. Using HS biosynthetic enzymes, we developed a chemoenzymatic approach to synthesize structurally defined HS and heparin oligosaccharides in high efficiency, which significantly expands the synthetic capability by a purely chemical approach (4, 5).

C₅-epi converts a GlcA unit to an IdoA by forming a putative carbonan intermediate at the C₅-position of a GlcA unit (see Fig. 1A) (6). Only a single isoform of C₅-epi is in human genome, and C₅-epi knock-out mice are neonatal lethal, suggesting its essential physiological roles in vivo (7). C₅-epi reportedly is a two-way catalytic enzyme, performing both the forward and reverse epimerization. Namely, the IdoA unit reverts back to a GlcA unit in the presence of C₅-epi (8). The nature of the reaction renders unique challenges to study the mode of action of C₅-epi. To date, three approaches have been reported to measure the activity of C₅-epi (9–11). Because all these methods utilized structurally heterogeneous polysaccharide substrates and the products were identified by a disaccharide analysis, none of these approaches is capable of locating the GlcA residues participated in the epimerization beyond a disaccharide domain. Therefore, the understanding for the mechanism of action of C₅-epi was limited, especially the effects of neighboring saccharide structures on the action of C₅-epi.

In this article, we report an advanced method to characterize C₅-epi using a series of structurally defined oligosaccharide substrates coupled with tandem mass spectrometry technique. This method permits us to locate the number and position of epimerization at the oligosaccharide levels with molecular pre-
**Modes of Action of Heparan Sulfate C5-epimerase**

**TABLE 1**

| Name   | Structure of substrate and site(s) of epimerization* | Calculated $M_r$ | Measured $M_r$ | $M_r$ after C5-epi treatment in D2O | Reaction mode |
|--------|-----------------------------------------------------|-----------------|----------------|-------------------------------------|---------------|
| Octa-1 | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1154.3          | 1154.5 ± 0.4   | 1156.4 ± 0.3                       | Reversible    |
| Octa-1' | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1154.3          | 1154.5 ± 0.4   | 1156.4 ± 0.3                       | Reversible    |
| Octa-1'' | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1154.3         | 1154.5 ± 0.4   | 1156.4 ± 0.3                       | Reversible    |
| Octa-4 | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1513.2         | 1513.2 ± 0.3   | 1513.1 ± 0.3                       | Reversible    |
| Octa-5 | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1513.2         | 1513.2 ± 0.3   | 1513.1 ± 0.3                       | Reversible    |
| Octa-6 | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1513.2         | 1513.2 ± 0.3   | 1513.1 ± 0.3                       | Reversible    |
| Hexa-2' | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1513.2         | 1513.2 ± 0.3   | 1513.1 ± 0.3                       | Reversible    |
| Deca-8 | GlcA-GlcNS-GlcA-GlcA-GlcA-GlcA-GlcNAc-GlcA-AnMan | 1513.2         | 1513.2 ± 0.3   | 1513.1 ± 0.3                       | Reversible    |

* The EPS sites are in boldface type.

**FIGURE 1.** The reaction catalyzed by C5-epi and the schematic presentation for determining the activity of C5-epi. A shows the reaction catalyzed by C5-epi. A trisaccharide segment of polysaccharide is shown. C5-epi removes the proton from C5 of the GlcA residue to form a putative carbanion intermediate. An H2O molecule then reacts with the carbanion to form an IdoA residue. Conversely, C5-epi can catalyze the reverse reaction, namely to convert an IdoA residue irreversibly, displaying an irreversible catalytic unit increase in $M_r$.

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**EXPERIMENTAL PROCEDURES**

**Expression and Purification of C5-epi—Recombinant C5-epi** was expressed and purified as described previously (10). Briefly, a fusion protein of maltose-binding protein and the human C5-epi catalytic domain (Glu53–Asn617) was constructed in pMal c2x vector (New England BioLabs). The expression was carried out in Origami-B DE3 cells (Novagen), which contained pGro7 (Takara, Japan) plasmid expressing chaperonin proteins. The expression was measured by coupling the reaction of C5-epi and 2-OST followed by a disaccharide analysis (12). In this assay, C5-epi (1.5 μg) was incubated with 2 μg of N-sulfo heparosan in the buffer containing 50 mM MES (pH 7.0), Triton X-100, and 1 mM CaCl2 at 37 °C for 30 min. To the reaction mixture, 0.6 μg of 2-OST and 3'-phosphoadenosine 5'-phosphosulfate ([35S]PAPS) (5–7 × 10⁵ cpm) were added. The reaction was incubated at 37 °C for two additional hours. The [35S]-labeled polysaccharide was purified by a DEAE column and was then subjected to nitrous acid degradation (at pH 1.5) to yield the disaccharides. The identities of the resultant disaccharides were determined by reverse-phase ion pairing HPLC using a C18 column as described previously (13). As a fully active C5-epi, >90% of resultant disaccharides from the degraded polysaccharide have a structure of IdoA2S-AnMan, where AnMan represents 2,5-anhydromannitol.
Preparation of Oligosaccharide Substrates—A total of eight oligosaccharides, differing in the size of the oligosaccharides and the distribution of N-sulfo groups, were prepared in this study. The preparation of the substrates followed essentially the same procedures described in our previous work (14–16). The synthesis initiated from a disaccharide of GlcA-AnMan, which was prepared from nitrous acid, degraded heparosan. The elongation from disaccharide to the desired size of oligosaccharide substrates was completed by KfiA (N-acetyl glucosaminyl transferase of E. coli K5 strain) and PmHS2 (heparosan synthase 2 from Pasteurella multocida). In a typical elongation reaction from the disaccharide to tetrasaccharide, 6 mg of GlcA-AnMan was incubated with 18 μmol of UDP-GlcNTFA (UDP-N-trifluoroacetyl glucosamine) and 2 mg of KfiA in 40 ml buffer containing 25 mM Tris-HCl, pH 7.2, 10 mM MgCl₂ at room temperature overnight. Upon the complete consumption of UDP-GlcNTFA, 2 mg of PmHS2, and 27 μmol of UDP-GlcUA were added into the reaction mixture and allowed to incubate overnight at room temperature. The product was purified by a BioGel P-2 column (0.75 × 1100 cm), which was equilibrated with 0.1 M ammonium bicarbonate at a flow rate of 4 ml/h. The product fraction was located by electrospray ionization mass spectrometry (ESI-MS) analysis. For the additional elongation reaction to the hexa-, octa-, and decasaccharides, the conditions were identical essentially to the above, whereas the reaction volumes were adjusted to the smaller sizes depending on the amount of substrates. The conversion of GlcNTFA to GlcNS was performed under an alkaline condition to remove the trifluoroacetyl group followed by N-sulfation using N-sulfotransferase and PAPS. We completed the synthesis of milligrams of each oligosaccharide substrates. The structures of the oligosaccharides were confirmed by ESI-MS analysis.

Preparation of Udp-GlcNTFA was started from glucosamine (Sigma), which was first converted to GlcNTFA by

FIGURE 2. MS and MS/MS analysis of Octa-1 treated with D₂O buffer with and without C₅-epi. Octa-1 was incubated with D₂O buffer with and without C₅-epi. The products were then subjected to MS and MS/MS analysis. A showed the ESI-MS spectrum of D-labeled epi-Octa-1, which was prepared by incubating Octa-1 with D₂O-exchanged C₅-epi in D₂O buffer. B showed the ESI-MS spectrum of Octa-1 that was treated D₂O buffer without C₅-epi. C showed the MS/MS spectrum of D-labeled epi-Octa-1. Precursor ion selection was at [M – 3H]³⁺, m/z 517.90. D showed the MS/MS spectrum of Octa-1 that was treated with D₂O buffer only. Precursor ion selection was at [M – 3H]³⁺, m/z 517.30. The fragmentation pattern of MS/MS analysis is depicted on the top of C and D. Both the fragment B₆ (m/z value of 505.39) and C₆ (m/z value of 615.88) of D-labeled epi-Octa-1 are ~2 units higher than those of Octa-1 (the m/z value of 504.37 and 614.86), suggesting that the D-labeled residues are located at residue C and E in D-labeled epi-Octa-1. The product ions in the MS/MS data were labeled according to Domon-Costello nomenclature (25).
reacting with S-ethyl trifluorothioacetate (Sigma-Aldrich) followed the protocol described previously (16). The resultant GlcNTFA was converted to GlcNTFA-1-phosphate using N-Acetylhexosamine 1-kinase (17). The plasmid expressing N-Acetylhexosamine 1-kinase was a generous gift from Professor Peng George Wang (Georgia State University, Atlanta, GA), and the expression of the enzyme was carried out in E. coli as reported (17). The UDP-GlcNTFA synthesis was completed by transforming GlcNTFA-1-phosphate using glucosamine-1-phosphate acetyltransferase/N-Acetylglucosamine-1-phosphate uridylyltransferase as described (16). The protocols for the expression of other HS biosynthetic enzymes and the preparation of PAPS were described elsewhere (16, 18). Additional methods are presented under supplemental “Methods.”

RESULTS AND DISCUSSION

For the current work, eight structurally defined substrates were synthesized using a chemoenzymatic approach (Table 1)(4). An MS-based method to monitor the reaction was devised, which allowed us to pinpoint the epimerization site (EPS) and to examine the reversibility of C5-epi. A substrate is incubated with C5-epi and D2O, leading to an epimerized product with deuterium incorporated. One unit of increase in molecular weight (M_r) for epi-oligosaccharide suggests that one GlcA residue is converted. Using a tandem MS technique, we located the residue that carries the deuterium in the epi-oligosaccharide, thus identifying the EPS site. The reversibility of the epimerization is studied by incubating the deuterated epi-oligosaccharide with C5-epi in H2O followed by MS analysis (Fig. 1B).

The reversibility of C5-epi was confirmed using Octa-1 as a substrate, which has a structure of GlcA-GlcNS-GlcA-GlcNS-GlcA-GlcNAc-GlcA-AnMan (Fig. 2). The M_r of epi-Octa-1 was increased to 1556.4 ± 0.4 from 1554.5 ± 0.3 (Table 1 and Fig. 2) when it was treated with C5-epi in the presence of D2O. Furthermore, the incubation of epi-Octa-1 with C5-epi in H2O resulted in the decrease of its M_r by 1.9 units (from 1556.4 ± 0.4...
Our data suggest that two GlcA units in Octa-1 were susceptible to C5-epi modification, and both EPS sites were reversible. These results were consistent with the previous report by Lindahl and co-workers (8).

Interestingly, C5-epi displayed an irreversible reaction mode when a different substrate (Octa-3) was used, which has the structure of GlcA-GlcNAc-GlcA-GlcNS-GlcA-GlcNS-GlcA-AnMan. Incubation of Octa-3 with C5-epi in D2O resulted in D-labeled epi-Octa-3. D-labeled epi-Octa-3 exhibited the Mr of 1555.4 ± 0.4 (Fig. 3A) with an increase of 0.9 units (Fig. 3B), suggesting that a single GlcA residue was converted to IdoA. The Mr of epi-Octa-3 remained unchanged after incubation with C5-epi, suggesting that C5-epi was unable to reverse the reaction. To further confirm the structure of D-labeled epi-Octa-3, a series of experiments were conducted. Tandem MS analysis was employed to prove that the D-labeled residue is located at residue E. For example, the fragment Y3 of epi-Octa-3 (m/z value of 499.23 for the doubly charged ion) is ~1.3 units higher than that of Octa-3 (m/z value of 498.57) (Fig. 3A, C and D). NMR analyses of epi-Octa-3 confirmed the presence of IdoA at residue E (supplemental Table S1). The presence of an IdoA in epi-Octa-3 was also demonstrated after modifying epi-Octa-3 with 2-O-sulfotransferase followed by a disaccharide analysis (supplemental data). High resolution ESI-MS analysis of D-labeled epi-Octa-3 showed a signal at a mass/charge ratio of 517.1121, consistent with [M – 3H]3− of the anticipated structure (calculated mass/charge ratio, 517.1040).

Subjecting additional oligosaccharides to the analysis revealed a relationship between the reaction mode of C5-epi and the structures of substrates (Table 1). C5-epi appears to recognize the flanking saccharide sequence at the nonreducing end of the EPS residue. Oligosaccharides containing a nonreducing end GlcNS residue immediately adjacent to the EPS residue are reactive to C5-epi. In contrast, when the GlcNS is replaced with GlcNAc (e.g. Octa-5), the oligosaccharide is no longer a substrate of C5-epi, proving the critical role of GlcNS at the nonreducing end of the EPS (8). Whether C5-epi exhibits an irreversible or a reversible mode depends on the residue at the mode of reaction recognition site (MRRS) that is three residues away from the EPS site toward the nonreducing end (Fig. 4A). If a GlcNAc is present at the MRRS site, C5-epi displays an irreversible reaction mode (as for Octa-3 and Octa-4). If a GlcNS or a GlcNH2 residue is present at the MRRS site (e.g. Octa-1, Octa-2, and Octa-6) or the MRRS site is unoccupied (i.e. Hexa-7 or the second EPS site (EPS2) for Octa-1 and -2), C5-epi displays a reversible reaction mode (Fig. 4A).

To further strengthen our conclusion, we synthesized a decasaccharide substrate (Deca-8), permitting C5-epi to display...
mixed reaction modes (Fig. 4B). Here, two EPS sites were constructed at the residue 0 (EPS1) and residue −2 (EPS2), respectively. Two MRRS sites were also introduced: a GlcNS and a GlcNAc residue were placed at residue −3 and residue −5, which should make a reversible EPS1 site and an irreversible EPS2 site, respectively. Indeed, our data confirmed that EPS1 is a reversible site, whereas EPS2 is irreversible (supplemental Fig. S5).

Lastly, we tested the role of irreversible reaction mode of C$_5$-epi in contributing to the biosynthesis of HS that binds to antithrombin (AT). HS or heparin forms a 1:1 complex with AT, which deactivates the activities of factor Xa and IIa in the blood coagulation cascade, to exhibit the anticoagulant activity. Anticoagulant HS and heparin isolated from natural sources have an AT-binding pentasaccharide with a structure of GtCNAc6S-GlCAGlCN3S±6S-IdoA2S-GlCN5S6S- (where GlcNS+6S represents N-sulfo glucosamine 3-O-sulfate with or without 6-O-sulfate) (19, 20). The IdoA2S residue in the pentasaccharide domain is known to be essential for high AT-binding affinity (21).

The uncovered irreversible reaction mode of C$_5$-epi provides insight for the natural selection for a GlcNAc6S (not a GlcNS6S) residue as part of the AT-binding site for its role in positioning the IdoA residue. The GlcNAc residue is expected to serve as a MRRS site to direct C$_5$-epi to synthesize an irreversible IdoA residue in the AT-binding pentasaccharide site, thus increasing the efficiency for the biosynthesis of AT-binding HS. To prove this assertion, the synthesis of AT-binding decasaccharides was completed using three decasaccharide substrates, including Deca-9, Deca-10, and Deca-11 (supplemental Fig. S6). Deca-9 had no IdoA residue; Deca-10 contained two reversible IdoA residues; and Deca-11 had one irreversible IdoA residue at EPS2 site and one reversible IdoA residue at EPS1 site. The irreversible IdoA residue at EPS2 site in Deca-11 was achieved by exposing Deca-10 to C$_5$-epi modification. These decasaccharides were modified by O-sulfotransferases to produce O-sulfated decasaccharides and were then fractionated by AT affinity columns to determine the amount of AT-binding site in the products. The results revealed that 40% O-sulfated Deca-11 bound to the AT affinity column, whereas the amount of the AT-binding portion for Deca-10 was determined to be 16%, a significant decrease in comparing to that of Deca-11 (Fig. 5). As expected, only 4% of O-sulfated Deca-8 bound to AT affinity column because it lacked an IdoA residue. Taken together, our data suggest that an irreversible IdoA residue at EPS2 site enhances the biosynthesis of AT-binding site.

Conclusions—Although the biosynthesis of HS is not a template-driven process, our results support the notion that HS biosynthetic pathway adopts an exquisite way to fine tune the extents of modifications through substrate control (22). Those enzymes involved in modifying the highly sulfated substrates (supplemental Fig. S1), i.e. 3-O-sulfotransferase, are able to distinguish the saccharide sequences with complicated sulfation patterns (23). In contrast, the mechanism used by those enzymes that modify the nonsulfated or low sulfated substrates, including N-deacetylase/N-sulfotransferase and C$_5$-epi, is perceived to be subtle because the polysaccharide substrates have relatively simple repetitive structures. In the initial N-sulfation step, N-deacetylase/N-sulfotransferase introduces the GlcNS residues consecutively, rather than randomly, along the polysaccharide substrate (24). Esko and Selleck (20) hypothesized that the N-sulfation step introduces the codes to direct the extent in the subsequent modification steps. Indeed, our findings demonstrate that C$_5$-epi recognizes the distribution of GlcNS residues in the substrate and displays distinctive catalytic modes, namely translating the N-sulfation code into the positions of GlcA/IdoA residues. Our data clearly show the impact of the irreversible mode of C$_5$-epi on the efficiency for the biosynthesis of anticoagulant HS. Further investigation will unveil the full implication of the biphasic mode possessed by C$_5$-epi in controlling the biosynthesis of HS.

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REFERENCES
1. Bishop, J. R., Schuksz, M., and Esko, J. D. (2007) Heparan sulfate proteoglycans fine-tune mammalian physiology. Nature 446, 1030–1037
2. Gama, C. I., Tully, S. E., Sotogaku, N., Clark, P. M., Rawat, M., Vaidhehi, N., Goddard, W. A., 3rd, Nishi, A., and Hsieh-Wilson, L. C. (2006) Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. Nat. Chem. Biol. 2, 467–473
3. Liu, H., Zhang, Z., and Linhardt, R. J. (2009) Lessons learned from the
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contamination of heparin. Nat. Prod. Rep. 26, 313–321
4. Xu, Y., Masuko, S., Takieddin, M., Xu, H., Liu, R., Jing, J., Mousa, S. A., Linhardt, R. I., and Liu, J. (2011) Chemoenzymatic synthesis of homogeneous ultralow molecular weight heparins. Science 334, 498–501
5. Wang, Z., Xu, Y., Yang, B., Tiruchinapally, G., Sun, B., Liu, R., Dulaney, S., Liu, J., and Huang, X. (2010) Preactivation-based, one-pot combinatorial synthesis of heparin-like hexasaccharides for the analysis of heparin-protein interactions. Chem. Eur. J. 16, 8365–8375
6. Hagner-McWhirter, A., Hannesson, H. H., Campbell, P., Westley, J., Rodén, L., Lindahl, U., and Li, J. (2008) Biosynthesis of heparin/heparan sulfate: kinetic studies of the glucuronyl C5-epimerase with N-sulfated derivatives of the Escherichia coli K5 capsular polysaccharide as substrates. Glycobiology 10, 159–171
7. Feyereabend, T. B., Li, J. P., Lindahl, U., and Rodewald, H. R. (2006) Heparin sulfate C5-epimerase is essential for heparin biosynthesis in mast cells. Nat. Chem. Biol. 2, 195–196
8. Li, J., Hagner-McWhirter, A., Kjellén, L., Palgi, J., Jalkanen, M., and Lindahl, U. (1997) Biosynthesis of heparin/heparan sulfate: cDNA cloning and expression of $\alpha$-glucuronyl C5-epimerase from bovine lung. J. Biol. Chem. 272, 28158–28163
9. Campbell, P., Hannesson, H. H., Sandbäck, D., Rodén, L., Lindahl, U., and Li, J. P. (1994) Biosynthesis of heparin/heparan sulfate. Purification of the $\alpha$-glucuronyl C5-epimerase from bovine liver. J. Biol. Chem. 269, 26953–26958
10. Li, K., Bethea, H. N., and Liu, J. (2010) Using engineered 2-O-sulfotransferase to determine the activity of heparan sulfate C5-epimerase and its mutants. J. Biol. Chem. 285, 11106–11113
11. Babu, P., Victor, X. V., Nelsen, E., Nguyen, T. K., Raman, K., and Kubera, B. (2011) Hydrogen/deuterium exchange-LC-MS approach to characterize the action of heparan sulfate C5-epimerase. Anal. Bioanal. Chem. 401, 237–244
12. Chen, J., Jones, C. L., and Liu, J. (2007) Using an enzymatic combinatorial approach to identify anticoagulant heparan sulfate structures. Chem. Biol. 14, 986–993
13. Kobayashi, M., Sugumaran, G., Liu, J., Shworak, N. W., Silbert, J. E., and Rosenberg, R. D. (1999) Molecular cloning and characterization of a human uronyl 2-sulfotransferase that sulfates iduronyl and glucuronyl residues in dermatan/chondroitin sulfate. J. Biol. Chem. 274, 10474–10480
14. Bethea, H. N., Xu, D., Liu, J., and Pedersen, L. C. (2008) Redirecting the substrate specificity of heparan sulfate 2-O-sulfotransferase by structurally guided mutagenesis. Proc. Natl. Acad. Sci. U.S.A. 105, 18724–18729
15. Xu, Y., Wang, Z., Liu, R., Bridges, A. S., Huang, X., and Liu, J. (2012) Directing the biological activities of heparan sulfate oligosaccharides using a chemoenzymatic approach. Glycobiology 22, 96–106
16. Liu, R., Xu, Y., Chen, M., Weiwer, M., Zhou, X., Bridges, A. S., DeAngelis, P. L., Zhang, Q., Linhardt, R. J., and Liu, J. (2010) Chemoenzymatic design of heparan sulfate oligosaccharides. J. Biol. Chem. 285, 34240–34249
17. Zhao, G., Guan, W., Cai, L., and Wang, P. G. (2010) Enzymatic route to preparative-scale synthesis of UDP-GlcNAc/GalNAc, their analogues and GDP-fucose. Nat. Protoc. 5, 636–646
18. Zhou, X., Chandarajoti, K., Pham, T. Q., Liu, R., and Liu, J. (2011) Expression of heparan sulfate sulfotransferases in Kluyveromyces lactis and preparation of 3’-phosphoadenosine-5’-phosphosulfate. Glycobiology 21, 771–780
19. Guerrini, M., Guglieri, S., Casu, B., Torri, G., Mourier, P., Boudier, C., and Viskov, C. (2008) Antithrombin-binding octasaccharides and role of extensions of the active pentasaccharide sequence in the specificity and strength of interaction. Evidence for very high affinity induced by an unusual glucuronic acid residue. J. Biol. Chem. 283, 26662–26675
20. Esko, J. D., and Selleck, S. B. (2002) Order out of chaos: Assembly of ligand binding sites in heparan sulfate. Annu. Rev. Biochem. 71, 435–471
21. Das, S. K., Mallet, J. M., Esnault, J., Driguez, P. A., Duchaussay, P., Sizun, P., Herault, J. P., Herbert, J. M., Petitou, M., and Sinay, P. (2001) Synthesis of conformationally locked L-iduronic acid derivatives: Direct evidence for a critical role of the skew-boat 2S0 conformer in the activation of antithrombin by heparin. Chemistry 7, 4821–4834
22. Kreuger, J., Spillmann, D., Li, J. P., and Lindahl, U. (2006) Interactions between heparan sulfate and proteins: The concept of specificity. J. Cell Biol. 174, 323–327
23. Xu, D., Moon, A. F., Song, D., Pedersen, L. C., and Liu, J. (2008) Engineer- ing sulfotransferases to modify heparan sulfate. Nat. Chem. Biol. 4, 200–202
24. Sheng, J., Liu, R., Xu, Y., and Liu, J. (2011) The dominating role of N- deacetylase/N-sulfotransferase 1 in forming domain structures in heparan sulfate. J. Biol. Chem. 286, 19768–19776
25. Domon, B., and Costello, C. E. (1988) A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. Glycoconj. J. 5, 397–409