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Synthesis and biological evaluation of *gem*-diamine 1-*N*-iminosugars related to L-iduronic acid as inhibitors of heparan sulfate 2-*O*-sulfotransferase

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Abstract—A variety of *gem*-diamine 1-*N*-iminosugars related to L-iduronic acid were synthesized and evaluated as inhibitors of heparan sulfate uronyl 2-*O*-sulfotransferase using an in vitro enzyme assay. Two iminosugars containing guanidino groups acted as potent inhibitors of the enzyme.

Heparan sulfate plays important roles in development and normal physiologic processes. Many of their functions depend on specific sequences of sulfated saccharides, which act as recognition motifs to bind and regulate the activities of protein ligands.[1] Heparan sulfate assembles by the copolymerization of *N*-acetyl-D-glucosamine (GlcNAc) and D-glucuronic acid (GlcA). The chain undergoes a series of modifications that include N-deacetylation and N-sulfation of GlcNAc units, epimerization of D-GlcA to L-iduronic acid (D-IdoA), and multiple sulfation reactions at C-6 and C-3 of glucosamine units and C-2 of IdoA and GlcA units.[2] These modifications occur in contiguous sections of the chain, creating binding sites for adhesion proteins, enzymes and enzyme inhibitors, and a variety of growth factors involved in early development.

Altering heparan sulfate biosynthesis could be of therapeutic benefit for treating disorders related to aberrant growth, such as tumor growth and metastasis.[3] To this end, we have synthesized and tested a variety of *gem*-diamine 1-*N*-iminosugars related to L-iduronic acid as inhibitors of heparan sulfate uronyl 2-*O*-sulfotransferase (2OST). 2OST catalyzes sulfate transfer from the sulfate donor, adenosine 3′-phosphate-5′-phosphosulfate (PAPS), to IdoA residues and with lesser efficiency to GlcA.[4] Only a single isoform of the enzyme is known,[5] making it an attractive drug target. In theory, inhibitors might block the action of key growth factors required for tumorigenesis.[3,6]

In this report, a series of *gem*-diamine 1-*N*-iminosugar analogs were prepared that are structurally similar to IdoA (Fig. 1). *gem*-Diamine 1-*N*-iminosugars are cyclic monosaccharides with a nitrogen atom in place of the

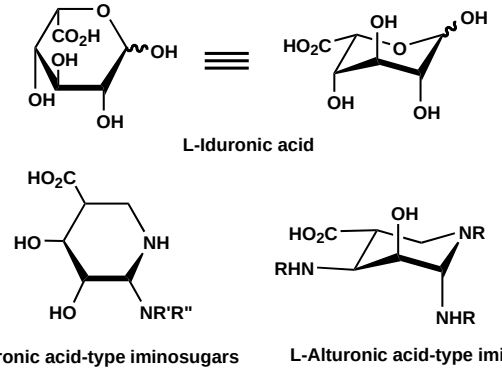

Figure 1. L-Iduronic acid, uronic acid-type *gem*-diamine 1-*N*-iminosugars, and L-alturonic acid-type *gem*-diamine 1-*N*-iminosugars (*R*,*R*′,*R*″ = functional groups).

Keywords: Synthesis; Iminosugars; Inhibitors; Glycosyltransferase; Heparan sulfate.
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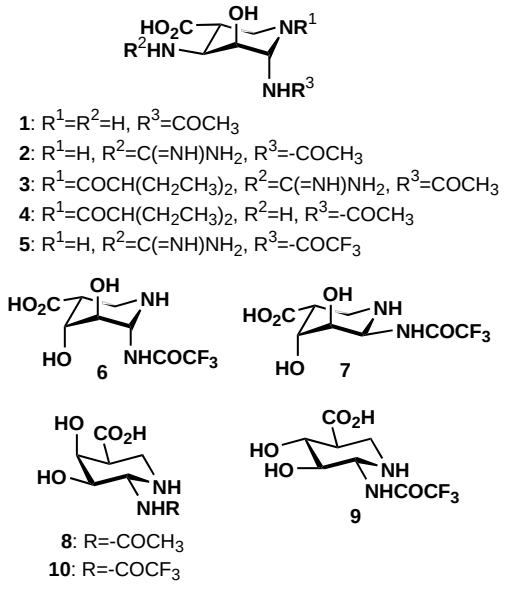
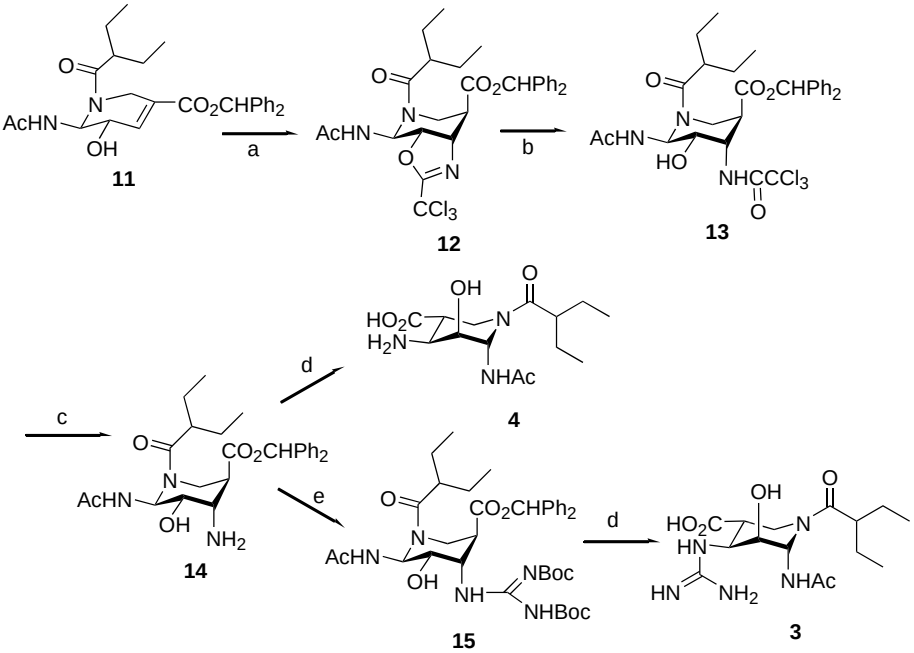


Scheme 1. L- and D-Uronic acid-type *gem*-diamine 1-*N*-iminosugars.

anomeric carbon.[7] Two of the compounds containing positively charged guanidinium groups act as inhibitors of 2OST. This directed approach complements more general high throughput screening methods of chemical libraries directed at inhibitors of other sulfotransferases.[8]

1-*N*-Iminosugars of **1**, **2**, and **5**,[7] **6**, **7**, and **9**[9] and **10**[10] were prepared by methods previously reported (Scheme 1). Natural siastatin B (**8**) was obtained from *Streptomyces* culture.[11] The synthetic method for 1-*N*-iminosugars **3** and **4** is outlined in Scheme 2.[12] Synthesis of **3** and **4** involved an intramolecular Michael addition of *O*-imidate to α,β-unsaturated ester **11** through *cis*-oxiamination.[13] Compound **11** was easily obtainable from siastatin B (**8**) and smoothly underwent *cis*-oxiamination by treatment with trichloroacetonitrile to give the desired oxazoline **12**. Hydrolysis of the oxazoline ring of **12** was achieved by treatment with *p*-toluenesulfonic acid in a mixture of pyridine and water to afford the trichloroacetamide **13** in 77% yield. Reductive cleavage of the trichloroacetyl group with sodium borohydride gave the amine **14** in 57% yield. Compound **14** can be then utilized for guanidine formation by use of *N*,*N*′-bis(-*tert*-butoxycarbonyl)thiourea in the presence of mercuric chloride. The reaction efficiently proceeded to afford the bis-Boc-protected guanidine **15** in 95% yield. Compound **15** was transformed into **3** by removal of the protecting groups with hydrogen chloride in dioxane in 90% yield. Compound **4** was also obtained by removal of the protecting groups from amine **14** with hydrogen chloride in dioxane in 93% yield.

We tested 2-*O*-desulfated heparin (2-DSH) an acceptor substrate using assay conditions described previously.[14] The assays were proportional to time up to 30 min at 37 °C and showed conventional saturation kinetics with 2-*O*-desulfated heparin ($K_m \sim 4$ μM, 80 μg/ml) as substrate. In each assay, the iminosugar inhibitor was added at 1 mM (~250 times K_m for 2-*O*-desulfated heparin). Assays of *gem*-diamine 1-*N*-iminosugars **1**–**10** revealed that **2** and **3** inhibited sulfate transfer by 76% and 84%, respectively (Fig. 2A). A dose–response curve showed that **2** and **3** caused 80% inhibition at the lowest concentration tested (25 μM) (Figs. 2B and C). In contrast to these results, addition of **2** or **3** to cultured Chinese hamster ovary cells did not affect sulfation of heparan sulfate in vivo. The lack of activity most likely



Scheme 2. Synthesis of iminosugars **3** and **4**. Reagents and conditions: (a) CCl_3CN, DBU, CH_2Cl_2, rt, 30 min, 64%; (b) *p*-TsOH, Py/H_2O, 80 °C, 2 h, 77%; (c) $NaBH_4$, EtOH, rt, 2 h, 57%; (d) 4 M HCl/dioxane, rt, 12 h, 90–93%; (e) $(BocNH)_2CS$, $HgCl_2$, Et_3N, DMF, rt, 2 h, 95%.

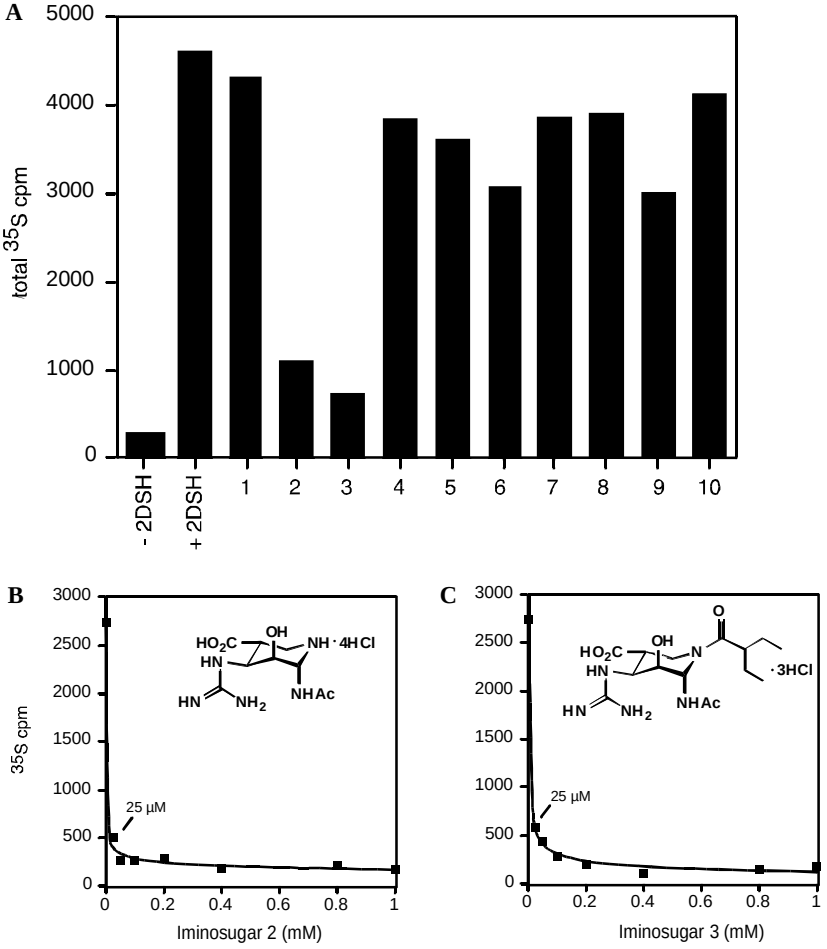


Figure 2. (A) *gem*-Diamine 1-*N*-iminosugars (**1**–**10**) screened as inhibitors of HS 2-*O*-ST. Iminosugar **2** (B) and iminosugar **3** (C) were mixed at various concentrations with acceptor substrate, 2-*O*-desulfated heparin (2DSH).

reflects poor uptake by cells due to the positively charged guanidine moiety.

Iminosugars **2** and **3** both contain a guanidine and an acetamide group at C-4 and C-2, respectively. Iminosugar **3** also contains 1-*N*-2-ethyl-butyramide present in the starting material **1**. Since iminosugar **4** also contained the 1-*N*-2-ethyl-butyramide but lacked activity, this moiety does not appear to be responsible for the inhibitor activity of iminosugar **3**. Iminosugar **5**, which also contains the guanidine moiety at C-4, lacked activity, but the large bulky $-NHCOCF_3$ moiety at C-2 may have blocked binding. The guanidine moiety may bind to the heparin substrate through charge–charge interactions or form a salt bridge with an appropriately positioned carboxylate in the active site of the enzyme. The guanidine moiety is an important feature of many biologically active compounds, for example, 4-guanidino-Neu5Ac-2-en is a potent inhibitor of influenza virus sialidase.[15]

In conclusion, we have identified two *gem*-diamine 1-*N*-iminosugars that inhibit a central sulfotransferase involved in heparan sulfate biosynthesis. To our knowledge, this is the first evidence that *gem*-diamine 1-*N*-iminosugars related to L-iduronic acid act as inhibitors of an enzyme involved in heparan sulfate synthesis. Further modification of **2** and **3** may render them as potential inhibitors of glycosaminoglycan chain biosynthesis in vivo.

Acknowledgments

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References and notes

1. Esko, J. D.; Selleck, S. B. *Annu. Rev. Biochem.* **2002**, *71*, 435.
2. Esko, J. D.; Lindahl, U. *J. Clin. Invest.* **2001**, *108*, 169.
3. Fuster, M. M.; Esko, J. D. *Nat. Rev. Cancer* **2005**, *5*, 526.
4. Rong, J.; Habuchi, H.; Kimata, K.; Lindahl, U.; Kusche-Gullberg, M. *Biochemistry* **2001**, *40*, 5548.
5. Kobayashi, M.; Habuchi, H.; Habuchi, O.; Saito, M.; Kimata, K. *J. Biol. Chem.* **1996**, *271*, 7645.

6. Guimond, S.; Maccarana, M.; Olwin, B. B.; Lindahl, U.; Rapraeger, A. C. *J. Biol. Chem.* **1993**, *268*, 23906; Maccarana, M.; Casu, B.; Lindahl, U. *J. Biol. Chem.* **1993**, *268*, 23898.
7. Nishimura, Y.; Satoh, T.; Adachi, H.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y. *J. Am. Chem. Soc.* **1996**, *118*, 3051; Nishimura, Y.; Satoh, T.; Adachi, H.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y. *J. Med. Chem.* **1997**, *40*, 2626.
8. Armstrong, J. I.; Portley, A. R.; Chang, Y. T.; Nierengarten, D. M.; Cook, B. N.; Bowman, K. G.; Bishop, A.; Gray, N. S.; Shokat, K. M.; Schultz, P. G.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 1303; Best, M. D.; Brik, A.; Chapman, E.; Lee, L. V.; Cheng, W. C.; Wong, C. H. *Chembiochem* **2004**, *5*, 811.
9. Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 2.
10. Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T.; Tsuruoka, T.; Fukuyasu, H.; Shibahara, S. *J. Antibiot. (Tokyo)* **1994**, *47*, 101; (b) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *Carbohydr. Res.* **1996**, *286*, 173.
11. Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishima, H.; Hamada, M. *J. Antibiot. (Tokyo)* **1974**, *27*, 963.
12. Compound **12**: $[\alpha]_D^{25}$ −59.4 (*c* 0.28, MeOH); a part of ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.23 and 3.54 (total 1H, dt each, J = 13.2 and 4.2 Hz, H-3), 3.35 and 3.36 (total 1H, t each, J = 13.2 Hz, H-2ax), 3.94 and 4.11 (total 1H, dd each, J = 13.2 and 4.2 Hz, H-2eq), 5.12 and 5.39 (total 1H, dd each, J = 9.7 and 4.2 Hz, H-4), 5.26 and 5.69 (total 1H, dd each, J = 9.8 and 2.5 Hz, H-5), 5.69 and 6.08 (total 1H, dd each, J = 5.8 and 2.5 Hz, H-6).
Compound **13**: $[\alpha]_D^{23}$ −27.6 (*c* 0.16, MeOH); a part of ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.99 (1H, t, J = 12.0 Hz, H-2ax), 3.11 (1H, dt, J = 12.0 and 3.4 Hz, H-3), 3.95 (1H, br s, H-5), 4.44 (1H, br m, H-2eq), 4.69 (1H, dt, J = 12.0 and 2.5 Hz, H-4), 4.97 (1H, dd, J = 8.8 and 2.5 Hz, H-6).
Compound **14**: $[\alpha]_D^{23}$ +23.9 (*c* 2.3, MeOH); a part of ^{1}H NMR($CDCl_3$, 400 MHz) δ 2.70 (1H, t, J = 12.0 Hz, H-2ax), 2.84 (1H, dt, J = 12.0 and 3.9 Hz, H-3), 2.80–2.90 (1H, m, –C*H*(CH_2CH_3)$_2$), 3.28 (1H, dd, J = 12.0 and 2.5 Hz, H-4), 3.87 (1H, br s, H-5), 4.06 (1H, br d, J = 12.0 Hz, H-2eq), 4.95 (1H, dd, J = 7.0 and 3.7 Hz, H-6).
Compound **15**: $[\alpha]_D^{23}$ −25.4 (*c* 0.7, MeOH); a part of ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.80–3.00 (2H, m, a part of H-3 and –CH(CH_2CH_3)$_2$), 3.13 (1H, dt, J = 11.7 and 3.9 Hz, H-3), 3.91 (1H, br s, H-5), 4.71 (1H, t, J = 11.0 Hz, H-4), 4.88 (1H, dd, J = 11.7 and 3.9 Hz, H-2), 6.17 (1H, d, J = 8.8 Hz, H-6).
Compound **3** as its hydrochloride: $[\alpha]_D^{25}$ +19.9 (*c* 0.16, H_2O); ^{1}H NMR(D_2O, 400 MHz) δ 0.62 and 0.65 (total 3H, each t, J = 7.3 Hz, –CH($CH_2C\mathit{H}_3$)$_2$), 0.75 and 0.76 (total 3H, each t, J = 7.3 Hz, –CH($CH_2C\mathit{H}_3$)$_2$), 1.3–1.7 (4H, m, –CH($C\mathit{H}_2CH_3$)$_2$), 1.87 and 1.91 (total 3H, s each, NHCO*C*H_3), 2.55–2.75 (3H, m, H-2eq, H-3 and –C*H*(CH_2CH_3)$_2$), 2.96 and 3.28 (total 1H, each t, J = 13.0 Hz, H-2ax), 3.91 (1H, br s, H-5), 3.99 and 4.22 (total 1H, dd each, J = 13.0 and 4.0 Hz, H-4), 5.89 and 6.29 (1H, br s, H-6); ^{13}C NMR (D_2O, 150 MHz) δ 10.71, 10.91, 11.04, 11.12 and 11.22 (–CH($CH_2\mathit{C}H_3$)$_2$), 21.81 and 21.84 (NCO*C*H_3), 25.016, 25.25, 25.38 and 25.42 (–CH($CH_2\mathit{C}H_3$)$_2$), 38.80 and 42.27 (C-2), 42.93 and 44.31 (C-3), 44.05 and 44.22 (–*C*H(CH_2CH_3)$_2$), 50.54 and 50.79 (C-4), 59.92 and 64.04 (C-6), 66.64 and 67.18 (C-5), 156.64 and 156.72 (–NH*C*(=NH)NH_2), 173.81 (N*C*OCH_3 or N*C*OCH(CH_2CH_3)$_2$), 175.68 and 175.74 (N*C*OCH(CH_2CH_3)$_2$) or N*C*OCH_3), 179.39 and 180.28 (CO_2H); HRMS calcd for $C_{15}H_{27}N_5O_5$ (M+Na): 380.19099. Found: 380.18834; Anal. Calcd for $C_{15}H_{27}N_5O_5$·2HCl: C, 41.86; H, 6.79; N, 16.28. Found: C, 42.36; H, 7.03; N, 16.43.
Compound **4** as its hydrochloride: $[\alpha]_D^{25}$ +24.5 (*c* 0.56, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 0.78 and 0.82 (total 3H, each t, J = 7.5 Hz, –CH($CH_2C\mathit{H}_3$)$_2$), 0.91 and 0.92 (total 3H, each t, J = 7.5 Hz, –CH($CH_2C\mathit{H}_3$)$_2$), 1.5–1.7 (4H, m, –CH($C\mathit{H}_2CH_3$)$_2$), 2.04 and 0.07 (total 3H, each s, NHCO*C*H_3), 2.85 and 2.91 (total 2H, quintet, J = 6.6 Hz, –C*H*(CH_2CH_3)$_2$), 3.00–3.08 (m, a part of H-3 and a part of H-2ax), 3.13 (dt, J = 4.8 and 12 Hz, a part of H-3), 3.48 (dd, J = 12 and 15 Hz, a part of H-2ax), 3.50–4.20 (total 1H, m, H-4), 4.21 and 4.22 (total 1H, each t, J = 3 Hz, H-5), 4.55 (dd, J = 15 and 3.6 Hz, a part of H-2eq), 4.92 (br d, J = 12 Hz, a part of H-2eq) and 6.08 and 6.51(total 1H, each d, J = 2.4 Hz, H-6); ^{13}C NMR (D_2O, 150 MHz) δ 10.73, 11.09 and 11.18 (–CH($CH_2\mathit{C}H_3$)$_2$), 21.79 (NCO*C*H_3), 25.11, 25.43 and 25.47 (–CH($C\mathit{H}_2CH_3$)$_2$), 37.78 and 41.32 (C-2), 39.06 and 40.00 (C-3), 44.25 and 44.37 (–*C*H(CH_2CH_3)$_2$), 49.00 and 49.14 (C-4), 59.35 and 63.44 (C-6), 65.18 and 65.75 (C-5), 173.16, 173.69, 173.75 and 173.84 (N*C*OCH_3 and N*C*OCH(CH_2CH_3)$_2$), 179.64 and 180.33 (CO_2H); HRMS calcd for $C_{14}H_{25}N_3O_5$ (M+Na): 338.16919. Found: 338.16773; Anal. Calcd for $C_{14}H_{25}N_3O_5$HCl: C, 47.79; H, 7.45; N, 11.94. Found: C, 48.01; H, 7.27; N, 12.15.
13. Shitara, E.; Nishimura, Y.; Nerome, K.; Hiramoto, Y.; Takeuchi, T. *Org. Lett.* **2000**, *2*, 3837.
14. 2-*O*-Sulfotransferase assays: A sequence encoding Chinese hamster ovary (CHO) heparan sulfate 2OST amino acids 28–353 (lacking the presumptive transmembrane domain at the amino terminus) was cloned into pRK5F10PROTA (Glycomed, Inc.). This plasmid was designed to express a secreted fusion protein containing protein A and 2OST. COS-7 cells were transiently transfected with pRK5F10PROTA-2-*O*-ST using LipofectAMINE (Invitrogen) according to the manufacturer's instructions. After 72 h of incubation, the fusion protein was recovered from the cell culture supernatant by affinity chromatography using IgG-agarose beads. The conditioned media and beads were mixed end-over-end overnight at 4 °C and centrifuged for 5 min, and the supernatant was aspirated. The beads were washed twice with 10 ml of 20% (v/v) glycerol in 50 mM Tris–HCl, pH 7.4, and resuspended in the same buffer containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A) to achieve a 50% (v/v) slurry. The immobilized enzyme was stable at 4 °C for at least 4 months. The HS 2-*O*-ST activity was assayed as described.[16] Briefly, the assay (25 μl) contained: 50 mM MES (pH 6.5), 1% TX-100, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 5 mM $CaCl_2$, 87.5 μM NaF, ~0.1 μCi [^{35}S]PAPS, 1 μg 2-*O*-desulfated heparin as an acceptor substrate and 5 μl protein A bead slurry (50%) containing immobilized enzyme. The reaction was incubated for 30 min at 37 °C with occasional mixing and stopped by adding 475 μl of 0.1 M EDTA (pH 7.4) containing 0.25 mg heparin. The ^{35}S-labeled products were separated from unreacted [^{35}S]PAPS by anion exchange chromatography on 0.25-ml columns of DEAE–Sephacel packed in disposable polypropylene tips as described.[17] The column was washed with 15 ml of 0.25 M NaCl, 20 mM sodium acetate (pH 6.0) and eluted with 2.5 ml of a 1 M NaCl, 20 mM sodium acetate (pH 6.0). An aliquot (1 ml) was counted by liquid scintillation (Ultima Gold X-R, Packard BioScience). *Penicillium chrysogenum* APS kinase was a kind gift from Dr. Irwin Segel, University of California, and was used to prepare [^{35}S]PAPS as described.[18]
15. Lednicer, D.; Mitscher, L. A. *The Organic Chemistry of Drug Synthesis*; Wiley: New York, 1997; von Itzstein, M.;

Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W., et al. *Nature* **1993**, *363*, 418.
16. Bai, X.; Esko, J. D. *J. Biol. Chem.* **1996**, *271*, 17711.
17. Bame, K. J.; Esko, J. D. *J. Biol. Chem.* **1989**, *264*, 8059.
18. MacRae, I.; Segel, I. H. *Arch. Biochem. Biophys.* **1997**, *337*, 17.