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Synthesis of Uronic Acid 1-Azasugars as Putative Inhibitors of α -Iduronidase, β -Glucuronidase and Heparanase**

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1-Azasugar analogues of L-iduronic acid (L-IdoA) and D-glucuronic acid (D-GlcA) and their corresponding enantiomers have been synthesized as potential pharmacological chaperones for mucopolysaccharidosis I (MPS I), a lysosomal storage disease caused by mutations in the gene encoding α -iduronidase (IDUA). The compounds were efficiently synthesized in nine or ten steps from D- or L-arabinose, and the structures were confirmed by X-ray crystallographic analysis of key intermedi-

ates. All compounds were inactive against IDUA, although L-IdoA-configured **8** moderately inhibited β -glucuronidase (β -GLU). The D-GlcA-configured **9** was a potent inhibitor of β -GLU and a moderate inhibitor of the endo- β -glucuronidase heparanase. Co-crystallization of **9** with heparanase revealed that the endocyclic nitrogen of **9** forms close interactions with both the catalytic acid and catalytic nucleophile.

Introduction

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease caused by mutations in the gene encoding α -iduronidase (IDUA),^[1] a glycosidase that cleaves terminal, nonreducing L-

iduronic acid (L-IdoA) residues in the glycosaminoglycans heparan sulfate (HS) and dermatan sulfate (DS). In MPS I, low levels of lysosomal IDUA activity cause HS and DS to accumulate in cells, resulting in progressive disease in multiple tissues and organs, including the brain. The more severe forms of MPS I result in mental retardation and premature death, usually within the first decade of life. There are two available treatments for MPS I: i) intravenously delivered enzyme replacement therapy using recombinant human IDUA,^[2] and ii) hematopoietic stem cell transplantation to enable production of IDUA from healthy transplanted cells, however, both have substantial limitations. For example, the replacement enzyme cannot cross the blood-brain barrier (BBB) and thus has no effect on neurological symptoms, whereas hematopoietic stem cell transplantation has substantial morbidity and mortality risks. In addition, both treatments are extremely expensive. The development of small molecule drugs that can cross the BBB and provide relief of neurological symptoms for MPS I is therefore desirable.

Small-molecule inhibitors are currently being explored as treatments for lysosomal storage diseases. For instance, inhibitors of enzymes involved in the biosynthesis of the accumulated substrates have been employed in substrate reduction therapy. Recently, the organoselenium drug candidate ebselen (2-phenyl-1,2-benzisoxaselenazol-3(2H)-one) was investigated as a potential substrate reduction therapy for MPS I.^[3] Ebselen reduced glycosaminoglycan accumulation in MPS I cells via inhibition of L-IdoA biosynthesis. However, it failed to reduce glycosaminoglycan accumulation in a MPS I mouse model. Another common small molecule approach for the treatment of lysosomal storage diseases is pharmacological chaperone therapy (PCT). In PCT, a chaperone molecule, typically an active site-directed inhibitor, can bind and stabilize the mutant enzyme to prevent its degradation and improve trafficking to the lysosome.^[4] Once in the low pH environment of the lysosome, the chaperone dissociates resulting in increased

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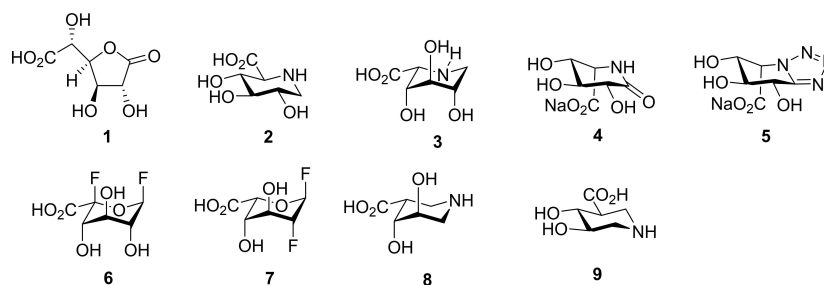


Figure 1. Previously reported inhibitors of IDUA (1–7) and putative 1-azasugar inhibitor **8** and β -glucuronidase inhibitor **9**.

enzyme activity. Chaperones may also enhance the efficacy of enzyme replacement therapy by protecting the recombinant wild-type enzyme against misfolding and degradation within blood, that is, by acting as an enzyme stabilizer.^[5] There has been one recent report of L-IdoA-triazole conjugates that stabilize IDUA and protect the enzyme from thermal denaturation.^[6] However, reduction of stored HS/DS in MPS I cells was not demonstrated.

Given the above results and that compounds showing promising chaperone activity have been reported for several other MPS disorders,^[7] we set out to identify putative inhibitors of IDUA as potential chaperones for MPS I. There have only been a few inhibitors reported for IDUA (Figure 1). The idarolactone **1** was the first reported IDUA inhibitor (IC_{50} = 40 μ M),^[8] while the D-glucuronic acid (D-GlcA) analogue of 1-deoxynojirimycin, iminosugar **2**, itself only a modest inhibitor of β -glucuronidase (β -GLU, K_i = 80 μ M), was also a weak inhibitor of IDUA.^[9] Interestingly, the L-IdoA-configured iminosugar **3** also binds IDUA with only weak affinity (IC_{50} = ~3 mM).^[10] Lactam **4** is a modest inhibitor (K_i = 94 μ M) whereas tetrazole **5** is only a weak inhibitor (K_i = 1.3 mM).^[11] The fluorinated L-IdoA derivatives **6** and **7**, designed as reagents to trap the glycosyl-enzyme intermediate, turnover very quickly and give apparent K_i values of 1.2 and 4.6 μ M, respectively.^[12]

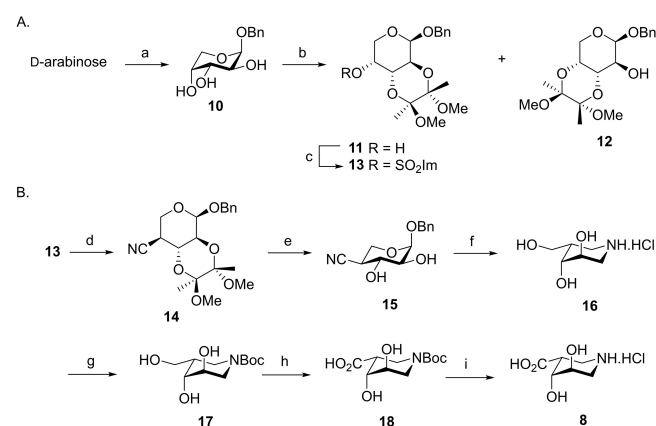
All the previously reported inhibitors 1–7 display relatively weak affinity for IDUA. 1-Azasugars (or 1-N-iminosugars), sugar analogues consisting of a monosaccharide in which the anomeric carbon has been replaced by nitrogen, are often very potent [nM] glycosidase inhibitors, as exemplified by isofagomine,^[13] and many display activity as pharmacological chaperones.^[14] Therefore, we sought to determine whether the L-IdoA-configured 1-azasugar **8**, its D-GlcA-configured isomer **9**, and their corresponding enantiomers would be more potent IDUA inhibitors and therefore candidate pharmacological chaperones for MPS I. Herein we describe the synthesis of these uronic acid 1-azasugars and their evaluation as inhibitors of IDUA as well as the related HS-degrading enzymes β -glucuronidase and heparanase. Inhibitors of β -glucuronidase are of interest as potential pharmacological chaperones for the related lysosomal storage disease MPS VII^[15] and indeed for many other therapeutic applications.^[16] The endo- β -glucuronidase heparanase is implicated in a broad range of diseases such as cancer, inflammation and viral infections,^[17] with several inhibitors currently in clinical trials for cancer.^[18]

Results and Discussion

Synthesis

The L-IdoA-configured 1-azasugar **8** has been reported previously;^[19] however, it was obtained as a by-product in 8% isolated yield during the synthesis of the D-GlcA 1-azasugar **9** and it was not tested for inhibition of IDUA. Inspired by the work of Stick^[20] and Bols,^[21] we envisaged a route to **8** from readily available and inexpensive D-arabinose involving introduction of a nitrile group with inversion of stereochemistry at C-4 (Scheme 1). This route was particularly attractive because a double inversion at C-4 would also allow access to D-GlcA configured **9**. Given that **8** is a modest inhibitor of β -glucuronidase (IC_{50} = 1.3 μ M)^[19b] it seemed worthwhile to also evaluate the inhibitory potency of **9** against IDUA. Furthermore, enantiomeric glycosidase inhibitors can also display pharmacological chaperone activity.^[22] Therefore, subjecting inexpensive L-arabinose to the same chemistry should deliver the corresponding enantiomers of both **8** and **9**.

The synthesis of **8** began with the Fischer glycosidation of D-arabinose to give the known^[23] benzyl glycoside **10** in



Scheme 1. a) BnOH, AcCl, RT, 24 h, 86%; b) butan-2,3-dione, (MeO)₃CH, CSA, RT, 72 h, 56%; c) NaH, *N,N*-sulfuryldiimidazole, DMF, –45 °C, 45 min; d) KCN, 18-crown-6, DMF, RT, 2 h, 67%, 2 steps; e) TFA/H₂O (9:1), RT, 10 min, 97%; f) H₂, Pd(OH)₂/C, conc. HCl, MeOH, RT, 24 h; g) (Boc)₂O, NaHCO₃, MeOH, RT, 24 h, 74%, 2 steps; h) TEMPO, MeCN, NaH₂PO₄ (aq), 80% (w/w) NaClO₂, NaOCl (aq), 35 °C, 24 h, 23% (59% based on recovered s.m.); i) 0.1 M HCl, evaporation, 100%.

excellent yield (86%). The C-2 and C-3 hydroxy groups were then protected as the butan-2,3-bisacetal (BBA) by treatment with trimethylorthoformate and butan-2,3-dione catalysed by (\pm)-10-camphorsulfonic acid to give the alcohol **11** in 56% yield after purification by recrystallization. The yield was lower than anticipated^[24] due to the formation of a higher R_f by-product (TLC), isolated by flash chromatography from the mother liquors in 12% yield. The by-product was identified as the isomeric 3,4-acetal **12** by NMR spectroscopy and the structure confirmed by single crystal X-ray diffraction analysis (see Figure S1 in the Supporting Information). Although the yield was diminished, compound **12** could be recycled to triol **10** by hydrolysis with TFA/H₂O (9:1). The C-4 alcohol of **11** was next converted into the imidazylate **13** by treatment with sodium hydride and *N,N*-sulfuryldiimidazole at -45°C , and used in the next step without further purification. The crude imidazylate was treated with KCN in the presence of 18-crown-6 to give the nitrile **14** as a white solid in 67% yield (two steps). The BBA protecting group was subsequently removed by stirring with TFA/H₂O (9:1) for 10 minutes to give diol **15** in quantitative yield. Compound **15** was then hydrogenated with Pd(OH)₂/C as catalyst in the presence of conc. HCl for 24 hours and monitored by TLC using the ninhydrin stain. These conditions effected the hydrogenolysis of the benzyl group, reduction of the nitrile to the amine and subsequent reductive amination to produce 1-azasugar **16** as the hydrochloride salt,^[19b] used in the next step without further purification. Compound **16** was then treated with (Boc)₂O and NaHCO₃ to give the Boc-protected 1-azasugar **17** as a white solid in 74% yield (two steps). High-resolution mass spectral data and the ¹H NMR spectrum of **17** were consistent with the structure, but the latter suffered from broadening due to the presence of the Boc group. The structure, however, was confirmed by single crystal X-ray diffraction analysis (Figure 2).

Selective oxidation of the primary alcohol of **17** to the carboxylic acid with TEMPO and BAIB^[25] or NaOCl^[26] as co-oxidants proved problematic and difficult to monitor by TLC, with multiple products evident. However, the method of Zhao and co-workers^[27] using stoichiometric sodium chlorite (NaClO₂) with catalytic TEMPO and NaOCl was successful, although some chlorinated by-products were observed. The resulting crude carboxylic acid **18** was purified by flash chromatography followed by ion exchange (Amberlite IRA 400, OH⁻ form). Elution of the product with 1 M HCl (aq) resulted in simultaneous cleavage of the Boc group to give the target 1-azasugar **8** as the hydrochloride salt in 22% yield over two steps.

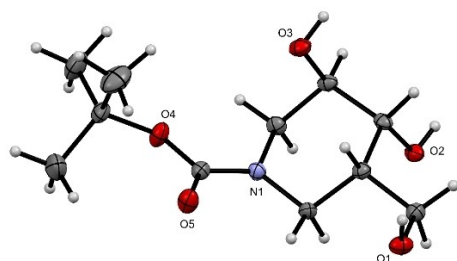
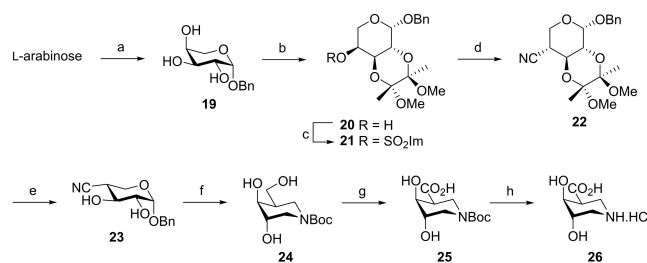


Figure 2. X-ray crystal structure of the Boc-protected 1-azasugar **17**.

Subsequently, the oxidation step was optimized by following the procedure of Del Valle,^[28] consisting of TEMPO, NaClO₂ and dilute NaOCl in a NaH₂PO₄ buffered (pH 6.6) solution. Although the starting material was not completely consumed, the reaction was very clean by TLC with no sign of chlorinated by-products. The product **18** was thus obtained in a yield of 23% (59% based on recovered starting material) following purification by flash chromatography. The final deprotection was then carried out by co-evaporating **18** with 0.1 M HCl and HPLC grade water to quantitatively produce **8** as the HCl salt,^[19a] which was fully characterized by ¹H and ¹³C NMR spectroscopy and HRMS.

To synthesize the enantiomer of **8**, the synthetic route was repeated starting from L-arabinose (Scheme 2) to give the desired 1-azasugar **26** in a similar overall yield. Thus, L-arabinose was transformed into the benzyl glycoside **19**^[29] and thence the BBA-protected alcohol **20**.^[29] Once again, the 3,4-BBA by-product was formed which could be recycled to the starting material by hydrolysis with aqueous TFA. The alcohol was then converted into the imidazylate **21** followed by displacement with KCN and hydrolysis to give nitrile **23**. Hydrogenation followed by treatment with (Boc)₂O gave the Boc-protected 1-azasugar **24**,^[30] which was characterized by single crystal X-ray diffraction analysis (Figure S2). Selective oxidation of the primary alcohol using the modified Zhao conditions gave the carboxylic acid **25** in 31% yield (65% based on recovered starting material). Finally, removal of the Boc protecting group with dilute aqueous HCl gave the desired 1-azasugar **26** as the HCl salt, which was fully characterized by ¹H and ¹³C NMR spectroscopy and HRMS.

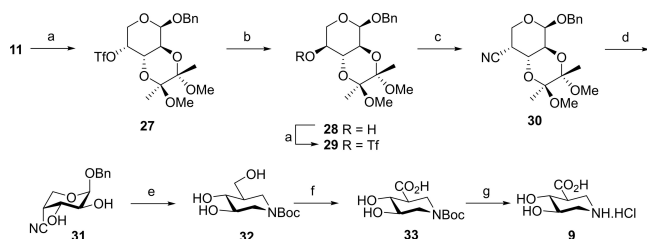
As discussed above, it was envisaged that a modification of the above synthetic routes to include a double inversion of the configuration at C-4 of alcohols **11** and **20** would lead to the D-GlcA configured 1-azasugar **9** and its enantiomer **40**. Thus, imidazylate **13** was treated with sodium nitrite in anhydrous DMF to effect a Latrell-Dax inversion to produce alcohol **28** in 64% yield over two steps. Inversion of configuration at C-4 was confirmed by ¹H NMR spectroscopy ($J_{3,4} = 10$ Hz). The use of the corresponding triflate **27**,^[31] readily obtained from alcohol **11**, gave an improved two-step yield of 74% for the inversion



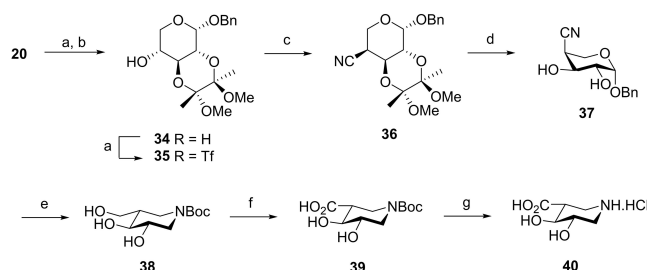
Scheme 2. a) BnOH, AcCl, RT, 24 h, 88%; b) butan-2,3-dione, (MeO)₃CH, CSA, RT, 72 h, 50%; c) NaH, *N,N*-sulfuryldiimidazole, DMF, -45°C , 45 min; d) KCN, 18-crown-6, DMF, RT, 2 h, 66%, 2 steps; e) TFA/H₂O (9:1), RT, 10 min, 97%; f) i) H₂, Pd(OH)₂/C, conc. HCl, MeOH, RT, 8 h; ii) (Boc)₂O, NaHCO₃, MeOH, RT, 11 h, 42%, 2 steps; g) TEMPO, MeCN, NaH₂PO₄ (aq), 80% (w/w) NaClO₂, NaOCl (aq), 35 $^\circ\text{C}$, 24 h, 31% (65% based on recovered s.m.); h) 0.1 M HCl, evaporation, 95%.

(Scheme 3). Alcohol **28** was then converted into the triflate **29** for the subsequent displacement with KCN, giving the nitrile **30**^[31] in good yield (76%, two steps). Hydrolytic removal of the BBA protecting group to give diol **31**^[31] (97%) was followed by hydrogenation and treatment with (Boc)₂O and NaHCO₃ to give the known^[32] Boc-protected isofagomine **32** in 37% overall yield. Selective TEMPO-mediated oxidation of the primary alcohol then gave the carboxylic acid **33** in 30% yield (85% based on recovered starting material). Finally, co-evaporation with 0.1 M HCl (aq) effected the quantitative removal of the Boc group to give the 1-azasugar **9** as the HCl salt.^[19]

For the synthesis of the enantiomer **40**, alcohol **20** was treated with triflic anhydride and pyridine and the resulting crude triflate was then subjected to a Latrell-Dax inversion to produce alcohol **34** in 66% yield over two steps (Scheme 4). Alcohol **34** was then converted into the triflate **35** and displaced with KCN to give the nitrile **36** in good yield (83%, two steps). Hydrolytic removal of the BBA protecting group to give diol **37** (87%) was followed by hydrogenation and treatment with (Boc)₂O and NaHCO₃ to give the Boc-protected 1-azasugar **38** in 40% overall yield. The structure of **38** was confirmed by single crystal X-ray diffraction analysis (Figure S3). Selective TEMPO-mediated oxidation of the primary alcohol then gave the carboxylic acid **39** in 32% yield (62% based on recovered starting material). Finally, co-evaporation with 0.1 M HCl (aq) effected the quantitative removal of the Boc group to



Scheme 3. a) Tf₂O, Py, CH₂Cl₂, -20 °C, 30 min; b) NaNO₂, DMF, RT, 18 h, 74%, 2 steps; c) KCN, 18-crown-6, DMF, RT, 2 h, 76%, 2 steps; d) TFA/H₂O (9:1), RT, 10 min, 97%; e) i: H₂, Pd(OH)₂/C, conc. HCl, MeOH, RT, 3 d; ii: (Boc)₂O, NaHCO₃, MeOH, RT, 21 h, 37%, 2 steps; f) TEMPO, MeCN, NaH₂PO₄ (aq), 80% (w/w) NaClO₂, NaOCl (aq), 35 °C, 24 h, 30% (85% based on recovered s.m.); g) 0.1 M HCl, evaporation, 100%.



Scheme 4. a) Tf₂O, Py, CH₂Cl₂, -20 °C, 30 min; b) NaNO₂, DMF, RT, 20 h, 66%, 2 steps; c) KCN, 18-crown-6, DMF, RT, 24 h, 83%, 2 steps; d) TFA/H₂O (9:1), RT, 10 min, 87%; e) i: H₂, Pd(OH)₂/C, conc. HCl, MeOH, RT, 3 d; ii: (Boc)₂O, NaHCO₃, MeOH, RT, 12 h, 40%, 2 steps; f) TEMPO, MeCN, NaH₂PO₄ (aq), 80% (w/w) NaClO₂, NaOCl (aq), 35 °C, 24 h, 32% (62% based on recovered s.m.); g) 0.1 M HCl, evaporation, 100%.

give the 1-azasugar **40** as the HCl salt, which was fully characterized by ¹H and ¹³C NMR spectroscopy and HRMS.

Enzyme inhibition studies

With 1-azasugars **8**, **9**, **26** and **40** in hand, the compounds were tested against both human IDUA and β-glucuronidase to determine their respective K_i values (see Table 1

1 > and the Supporting Information). Inhibitors of the latter enzyme are of interest as potential pharmacological chaperones for the related lysosomal storage disease MPS VII^[15] and indeed for many other therapeutic applications.^[16] Unfortunately, none of the compounds showed any inhibition of human IDUA up to 1200 μM. While compounds **9**, **26** and **40** were not expected to be potent IDUA inhibitors, lack of inhibitory activity for L-IdoA-configured compound **8** was somewhat surprising. It has been postulated from previous enzyme kinetic^[12] and X-ray crystallographic studies^[10] that IDUA hydrolyses its substrate through a mechanism involving a covalent-glycosyl enzyme intermediate that adopts an uncommon ^{2,5}B conformation. The ¹H NMR spectrum of **8** is consistent with a conformation similar to ¹C₄ rather than ^{2,5}B as required to bind to the catalytic site. Therefore, a possible explanation for the lack of activity for **8** is that it is a poor mimic of, or is unable to adopt, the required active conformation. Interestingly, **8** weakly inhibited human β-glucuronidase (IC₅₀ = 399 μM) although with even less potency than previously reported against the bovine liver enzyme (IC₅₀ = 1.3 μM).^[19a] The D-GlcA-configured **9** strongly inhibited human β-glucuronidase (K_i = 370 nM), again with less potency than for the bovine liver enzyme (K_i = 79 nM),^[19a] but did not inhibit IDUA. Both enantiomeric 1-azasugars **26** and **40** failed to inhibit either enzyme.

Enzyme inhibitory potency in vitro does not always correlate with optimal enzyme stabilization in cells.^[34] The chaperone activity of compound **8** was therefore also evaluated in a cell-based assay to determine if the compound could increase cellular (mutant) IDUA activity. Mutant MPS I pP533R/pG51D lysate contains a measurable low IDUA activity, which is approximately 2,000 times less than in WT cell lysate.^[35] Incubation with 180 μM substrate (K_m value) shows that 50% of the reaction velocity is reached compared to the one reached with 2 mM substrate.^[36] MPS I fibroblasts were treated for a total of six days with **8** at a concentration of 0.5 mM (compound changed in fresh medium every second day). The IDUA assay showed no difference between the control and treated cells (see the Supporting Information). A similar experiment after 4 days of treatment showed no difference between control and treated cells (not shown). Altogether, the compound failed to act as a chaperone at 0.5 mM for the two mutated IDUA enzymes expressed in these primary fibroblasts. The ability of the compound to inhibit IDUA activity in lysates from WT cells was also tested. However, compound **8**, added to the lysate at final concentrations of 100 and 200 μM, did not inhibit the activity (see Supporting Information).

We recognized that the uronic acid 1-azasugars we prepared, in particular the D-GlcA-configured **9**, might also have

potential as inhibitors of heparanase, an endo- β -glucuronidase of considerable biomedical importance.^[18] While most carbohydrate-based heparanase inhibitors^[38] require at least a (pseudo)disaccharide for effective inhibition,^[39] some monosaccharide derivatives have been reported as good inhibitors, notably geminal diamine 1-azasugars **41–43** (Figure 3) with IC_{50} values ranging from 29 to 1 μ M.^[37] 1-Azasugar **9** was thus tested against recombinant human heparanase using the fondaparinux assay^[40] and was found to inhibit the enzyme with an IC_{50} value of approximately 250 μ M (estimated $K_i=8.6$ μ M, see the Supporting Information). While the inhibitory potency was relatively modest, X-ray structures (solved to 2.05 Å resolution, Figure 4) of pre-formed heparanase crystals soaked with compound **9** revealed this inhibitor bound in the active site.

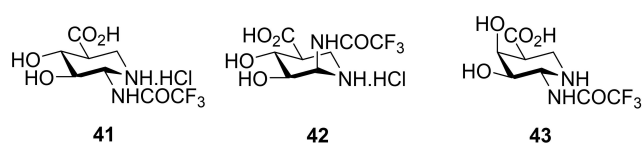


Figure 3. Previously reported heparanase inhibitors **41–43**.^[37]

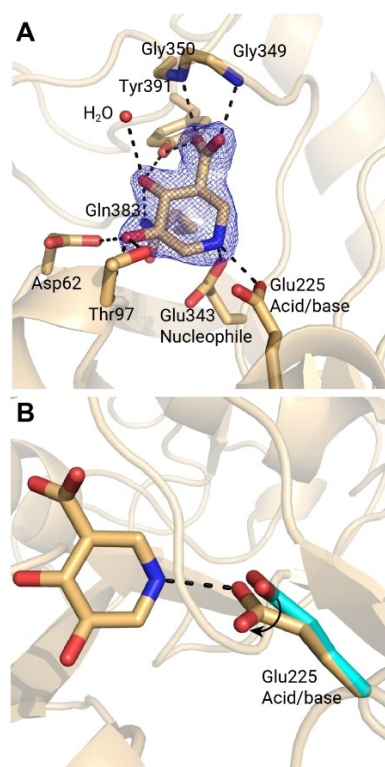


Figure 4. A) Close-up of the active site of human heparanase with the D-glucuronic-acid-configured 1-azasugar **9** bound in the active-site pocket. Key residues in the active site are labelled. Polar interactions within hydrogen bonding distance are labelled with black dashes. The electron density mesh is σ_A -weighted $2F_o - F_c$ densities contoured at 2σ ($0.43e^-/\text{\AA}^3$) around the inhibitor. B) The shift of the catalytic acid/base from its position in an unliganded structure (5E8M, shown in cyan) to the structure bound to inhibitor **9** (shown in light orange). Images were rendered with the program PyMOL.

This structure revealed that aza-sugar **9** was, as expected, positioned in the -1 subsite (subsite nomenclature defined in ref. [41]) of the active site cleft. When the complex with **9** is compared to an unliganded structure of heparanase (PDB ID: 5E8M) it is apparent that the active site amino acids have a nearly identical positioning (RMSD of 0.25 Å, for an 8 Å sphere around the active site). There is, however, a small shift in the position of the acid/base residue (Glu225) such that one of the carboxylate oxygens is shifted 0.5 Å towards to the endocyclic nitrogen of inhibitor **9**, to be within (2.6 Å) of this nitrogen. Inhibitor **9** forms 11 hydrogen bond interactions with the heparanase active site, including those with the acid base. The nucleophile also forms a hydrogen bond with the endocyclic nitrogen of **9**, an interaction that is also observed for other isofagamine derivatives bound to retaining glycoside hydrolases.^[42] The hydrogen bonding network observed with **9**, is similar to that seen when heparanase engages its natural substrates^[43] or cyclophellitol derived inhibitors.^[39] However, the lack of a 2-OH in **9**, results in the loss of a hydrogen bonding interaction with residue Asn224 that is observed for natural substrates and other inhibitors.^[39,43]

Inhibitor **9** is present in a 4C_1 conformation (ϕ, θ : 320.6°, 3.5°) within the active site. This conformation mimics that of the glycosyl intermediate, as the catalytic itinerary of HPSE is proposed to consist of a transition from 1S_3 (Michaelis complex) $\rightarrow [{}^4H_3]^+$ (transition state) $\rightarrow {}^4C_1$ (glycosyl intermediate).^[44] This conformation is also observed for cyclophellitol type inhibitors that are covalently linked to the nucleophile.^[39] Other 1-azasugar derivatives have also been observed in a 4C_1 conformation when bound to a cellulase^[42a] or lichenase,^[42b] representing the conformation expected for the glycosyl-enzyme intermediate in both these cases. The conformation of **9** bound in the active site of HPSE suggests that its inhibition is not due to conformational mimicry of the transition state, but rather through close interaction of the endocyclic nitrogen with the catalytic carboxylate pair.

Conclusions

In conclusion, we have synthesized uronic acid 1-azasugars with L-IdoA and D-GlcA configurations, as well as their corresponding enantiomers, as putative inhibitors of IDUA and β -glucuronidase for evaluation as potential pharmacological chaperones for MPS I (and MPS VII). The syntheses were short and efficient, requiring only nine or ten steps, respectively, from D-arabinose, or L-arabinose for the enantiomers, and key intermediates were characterized by X-ray crystallography. The compounds were tested for their ability to inhibit these enzymes as well as heparanase. None of the compounds inhibited IDUA, including the L-IdoA-configured **8**, although this was a moderate inhibitor of β -glucuronidase. Compound **8** also failed to show any chaperone activity in cell-based assays. On the other hand, D-GlcA-configured **9** was a potent inhibitor of human β -glucuronidase and warrants further investigation as a potential pharmacological chaperone for MPS VII. Compound **9** was also a moderate inhibitor of heparanase, an endo- β -glucuronidase

that is strongly implicated in many diseases including cancer, inflammation and viral infections. X-ray crystallographic analysis of heparanase with **9** bound in the active site revealed a close association of the endocyclic nitrogen with not only the acid/base residue, but also the catalytic nucleophile, a design motif that could be exploited in the future generation of heparanase inhibitors.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: azasugars · iduronidases · glucuronidases · heparanases · inhibitors · MPS I

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