Research paper

Efficient stereoselective synthesis of 2-acetamido-1,2-dideoxyallonojirimycin (DAJNAc) and sp^2-iminosugar conjugates: Novel hexosaminidase inhibitors with discrimination capabilities between the mature and precursor forms of the enzyme

Alex de la Fuente [a], Rocío Rísquez-Cuadro [b], Xavier Verdaguer [a, c],
José M. García Fernández [d], Eiji Nanba [e], Katsumi Higaki [e, **], Carmen Ortiz Mellet [b, ***],
Antoni Riera [a, c, *]

[a] Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac 10, 08028 Barcelona, Spain
[b] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 1203, E-41071 Sevilla, Spain
[c] Departament de Química Orgànica, Universitat de Barcelona, Martí i Franquès, 1, E-08028 Barcelona, Spain
[d] Instituto de Investigaciones Químicas (IIQ), CSIC and Universidad de Sevilla, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain
[e] Division of Functional Genomics, Research Center for Bioscience and Technology, Tottori University, 86 Nishi-cho, Yonago 683-8503, Japan

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ABSTRACT

Due to their capacity to inhibit hexosaminidases, 2-acetamido-1,2-dideoxy-iminosugars have been widely studied as potential therapeutic agents for various diseases. An efficient stereoselective synthesis of 2-acetamido-1,2-dideoxyallonojirimycin (DAJNAc), the most potent inhibitor of human placenta β-N-acetylglucosaminidase (β-hexosaminidase) among the epimeric series, is here described. This novel procedure can be easily scaled up, providing enough material for structural modifications and further biological tests. Thus, two series of sp^2-iminosugar conjugates derived from DAJNAc have been prepared, namely monocyclic DAJNAc-thioureas and bicyclic 2-iminothiazolidines, and their glycosidase inhibitory activity evaluated. The data evidence the utmost importance of developing diversity-oriented synthetic strategies allowing optimization of electrostatic and hydrophobic interactions to achieve high inhibitory potencies and selectivities among isoenzymes. Notably, strong differences in the inhibition potency of the compounds towards β-hexosaminidase from human placenta (mature) or cultured fibroblasts (precursor form) were encountered. The ensemble of data suggests that the ratio between them, and not the inhibition potency towards the placenta enzyme, is a good indication of the chaperoning potential of TaySachs disease-associated mutant hexosaminidase.

1. Introduction

Glycosidases comprise a broad class of enzymes that participate in metabolic processes which are essential for cell life and cell communication, including the catabolism of polysaccharides and glycoconjugates and the trimming of the oligosaccharides present in glycoproteins and glycolipids. Consequently, compounds with selective glycosidase inhibitory or effector activity have strong therapeutic potential in the treatment of pathologies resulting from glycosidase deregulation, including diabetes, cancer, viral and bacterial infection, and lysosomal storage diseases, among others [1–5]. Iminosugars, structural analogs of monosaccharides (glycomimetics) in which the ring oxygen has been replaced by nitrogen, comprise by far the most studied family of compounds towards this end [6–8]. Their ability to mimic the glycosyloxacarbenium cation—a key intermediate in enzymatic glycoside hydrolysis [9]—in their protonated state translates into high binding affinities towards complementary glycosidases. The broad range of potential

* Corresponding author. Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac 10, 08028 Barcelona, Spain.
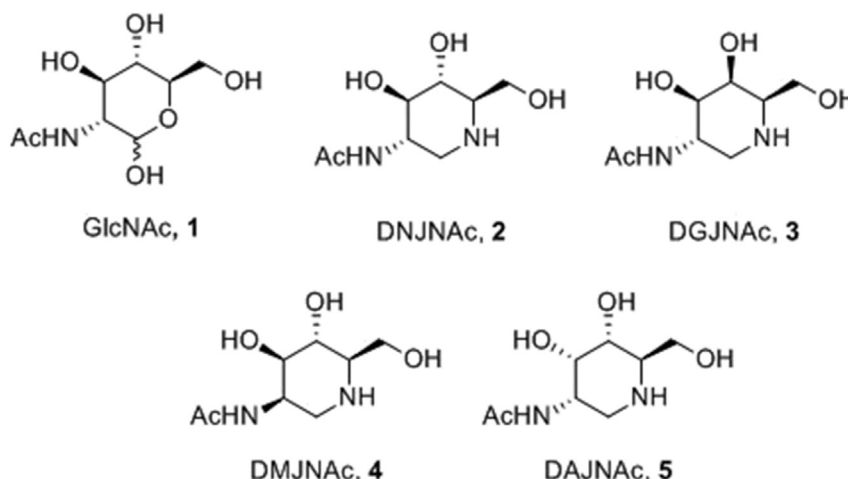** Corresponding author.
*** Corresponding author.
E-mail addresses: kh4060@med.tottori-u.ac.jp (K. Higaki), mellet@us.es (C. Ortiz Mellet), antoni.riera@irbbarcelona.org (A. Riera).
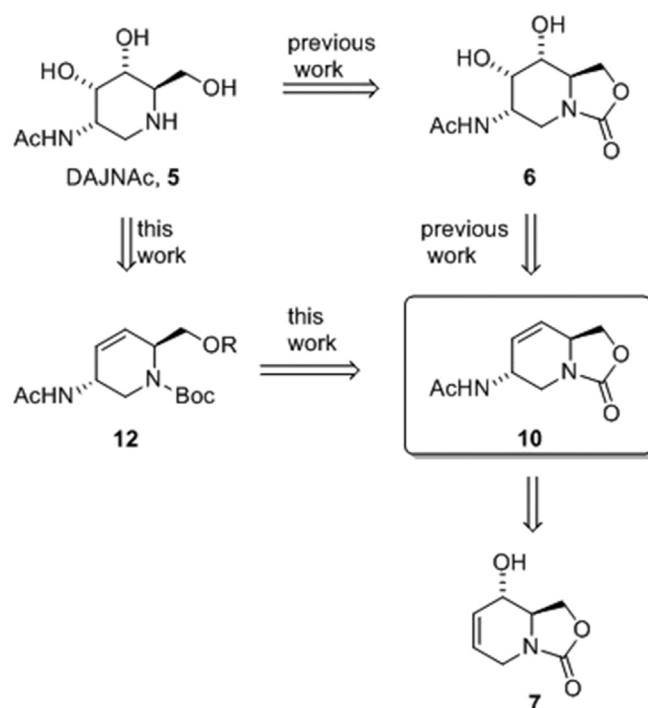
medical applications of iminosugars has been a continuous motivation for synthetic chemists. In this regard, a plethora of analogs has been reported [10−22], some of which are currently on the market, such as *N*-butyl-1-deoxynojirimycin (Zavesca®) for the treatment of type 1 Gaucher disease and miglitol (Glyset®) for the treatment of type II diabetes mellitus.

From the structural point of view, iminosugars share a polyhydroxylated iminoheterocyclic skeleton that differs in the size of the ring, the stereochemical pattern, and the substitution profile [23]. While efficient methods for the introduction of a variety of substituents at the region of the ring nitrogen [24−26] or the pseudoanomeric carbon [27−36] have been reported, replacing hydroxyl groups at non-pseudoanomeric positions by other functionalities such as amide groups, remains a challenge. This might be critical in the design of modulators of particular glycosidase targets. Thus, the dysfunction of hexosaminidases, the glycosidases involved in the hydrolysis of 2-acetamido-2-deoxyglycosides, has been associated with diseases such as osteoarthritis [37,38], Alzheimer [39], *O*-GlcNAcase inhibition [40] and the lysosomal storage diseases Tay-Sachs [41], Sandhoff [42] and Schindler-Kazaki [43]. Not surprisingly, acetamido-iminosugars inhibit these enzymes and have become interesting candidates in the development of pharmacological chaperones for the treatment of some of these conditions [9].

In 1986, Fleet et al. [44,45] described the first synthesis of 2-acetamido-1,2-dideoxy-D-nojirimycin (DNJNAc; **2**), a mimetic of *N*-acetylglucosamine (GlcNAc; **1**) that is a potent and highly specific inhibitor of β-*N*-acetylglucosaminidases (β-GlcNAcases) (Scheme 1). Further studies have proved that modifications on the amide functionality do not improve the inhibitory capacity of this compound [46]. The *galacto* congener DGJNAc (**3**) [47] and derivatives [48] fall in the same inhibition range for β-GlcNAcases and are also potent inhibitors of α-*N*-acetylgalactosaminidase (α-GalNAcase) [43] while the *manno* congener DMJNAc (**4**) shows no hexosaminidase inhibition [49]. A range of other glycomimetic-type inhibitors has been reported, but all of them suffer from lack of selectivity among β-GlcNAcase isoforms and poor drug-like physicochemical properties [50]. The development of novel and specific inhibitors susceptible of systematic chemical modification for structure−activity relationship studies is thus a priority to exploit the potential of function-specific β-GlcNAcase activity modulation in terms of drug design and biological research. Conjugation of acetamido-iminosugar with organic fragments susceptible of imparting amphiphilicity and providing additional interactions

Scheme 2. Retrosynthetic analysis.

with the aglycone site of the enzyme has shown high promise at this respect [51]. Yet, the approach is handicapped by the fact that most synthetic approaches to 2-acetamido iminosugars are based on the chiral pool and often involve long and tedious transformations from carbohydrate precursors [12,52].

We have recently published the first asymmetric synthesis of the *allo*-configured acetamido-iminosugar representative, namely 2-acetamido-1,2-dideoxyallonojirimycin (DAJNAc; **5**), in only six steps from the bicyclic precursor **7** (Scheme 2), which is easily accessible in high enantiomeric purity by Sharpless epoxidation of either penta-1,4-dien-3-ol or penta-2,4-dien-1-ol [53,54]. Preliminary glycosidase inhibition studies indicated that DAJNAc is the strongest competitive inhibitor of human placenta β-GlcNAcase (β-hexosaminidase) among the epimeric series, beating the D-*gluco* and the D-*galacto* stereoisomers **2** and **3** [55]. Using this new

Scheme 1. Structures of *N*-acetylglucosamine (**1**) and the related acetamido-iminosugars (**2**−**5**).

scaffold to generate molecular diversity by conjugation was therefore very appealing. Motivated by the success of sp²-iminosugars analogues of the classical iminosugars as modulators of several lysosomal glycosidases [56–59], we envisioned to selectively modify the endocyclic amine group of **5** into a pseudoamide functionality bearing different appendages. However, the rather modest overall yield (16%) of the initial synthesis prevented the preparation of large amounts of DAJNAc for structural modification and further biological activity studies. Here we report a new methodology to obtain DAJNAc in 7 steps with 41% overall yield. This optimization afforded key intermediates that allowed us to get thiourea and 2-iminothiazolidine sp²-iminosugar conjugates in high yields. Evaluation of their inhibitory abilities showed significant selectivity increases towards human β-hexosaminidase (placenta), with potencies that in the best cases overpassed that of the parent DAJNAc. The new compounds have been also evaluated as potential pharmacological chaperones in fibroblasts harnessing the G269S mutation in the α-subunit of heterodimeric β-hexosaminidase A, the most common disease allele in adult Tay-Sachs patients [60].

2. Results and discussion

2.1. Synthesis

In our previous synthesis, DAJNAc **5** was obtained after hydrolysis of the bicyclic carbamate **6** (40% yield), which in turn was prepared by dihydroxylation of the key intermediate **10** using *N*-methylmorpholine-*N*-oxide (NMO) and catalytic amounts of $K_2OsO_4 \cdot 2H_2O$ in acetone/water (Scheme 2). In accordance with the widely studied directing effect of allylic amides [61–63], we had anticipated that the dihydroxylation reaction would proceed preferentially from the same face of the acetamido substituent (α-face). However, the dihydroxylation of **10** into **6** took place with modest diastereoselectivity (4:1 ratio *allo*:*galacto*) and yield (66%, Scheme 1), thus representing a bottleneck for scaling-up purposes. We conceived that increasing the steric hindrance at the opposite β-face of the six-membered ring, by opening the planar oxazolidinone ring and introducing appropriate substituents at the primary oxygen and the nitrogen atom, would further improve the stereoselectivity of the reaction in favor of the desired *allo* derivative. Indeed, preliminary calculations suggested that the carbonyl oxygen of an acyclic carbamate group at the endocyclic nitrogen (as in **12b**) would be suitably disposed to form an intramolecular seven-membered hydrogen bond with the acetamide proton, thus favoring the ^1S_N skew-boat conformation over the sofa conformation, present in the bicyclic carbamate **10** (Fig. 1). Consequently, the primary carbon C6, vicinal to the double bond, would be shifted from a pseudoequatorial to a pseudoaxial orientation, thereby increasing the steric shield on the β-face of the molecule. Such an increase may, in turn, improve the diastereoselectivity of the dihydroxylation reaction. In order to confirm this hypothesis, we explored an alternative synthetic route involving dihydroxylation of monocyclic enamides **12**, which were obtained from **10** after hydrolysis of the cyclic carbamate functionality (Scheme 2).

The known intermediate **7** was easily prepared in multi-gram scale from penta-2,4-dien-1-ol [53,54]. Bicyclic acetamide **10** was obtained as a single diastereomer in 3 steps following the previously described methodology [55]. After careful optimization of the reaction conditions, the 2-oxazolidinone ring was hydrolyzed with NaOH 6 M at reflux, and the resulting free amine was protected as the corresponding *tert*-butylcarbamate to afford the *N*-Boc-protected derivative **11** (80% overall yield). The primary hydroxyl was next either silylated by treatment with TBSCl/imidazole or acetylated with Ac_2O/pyridine to give **12a** or **12b** in 75% and 86% yield,
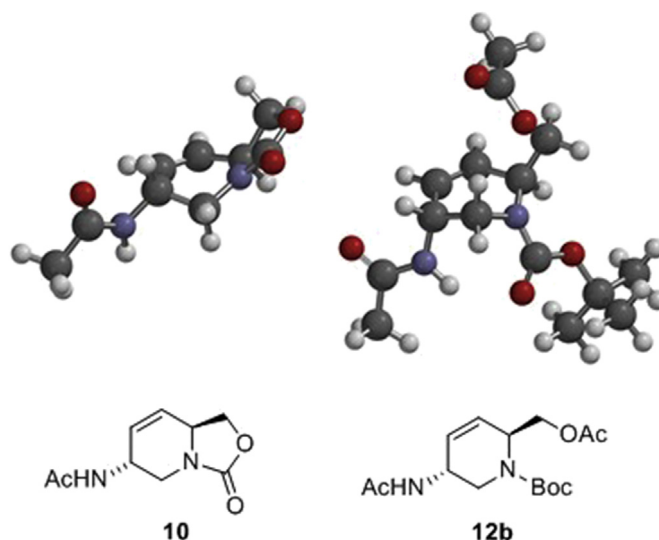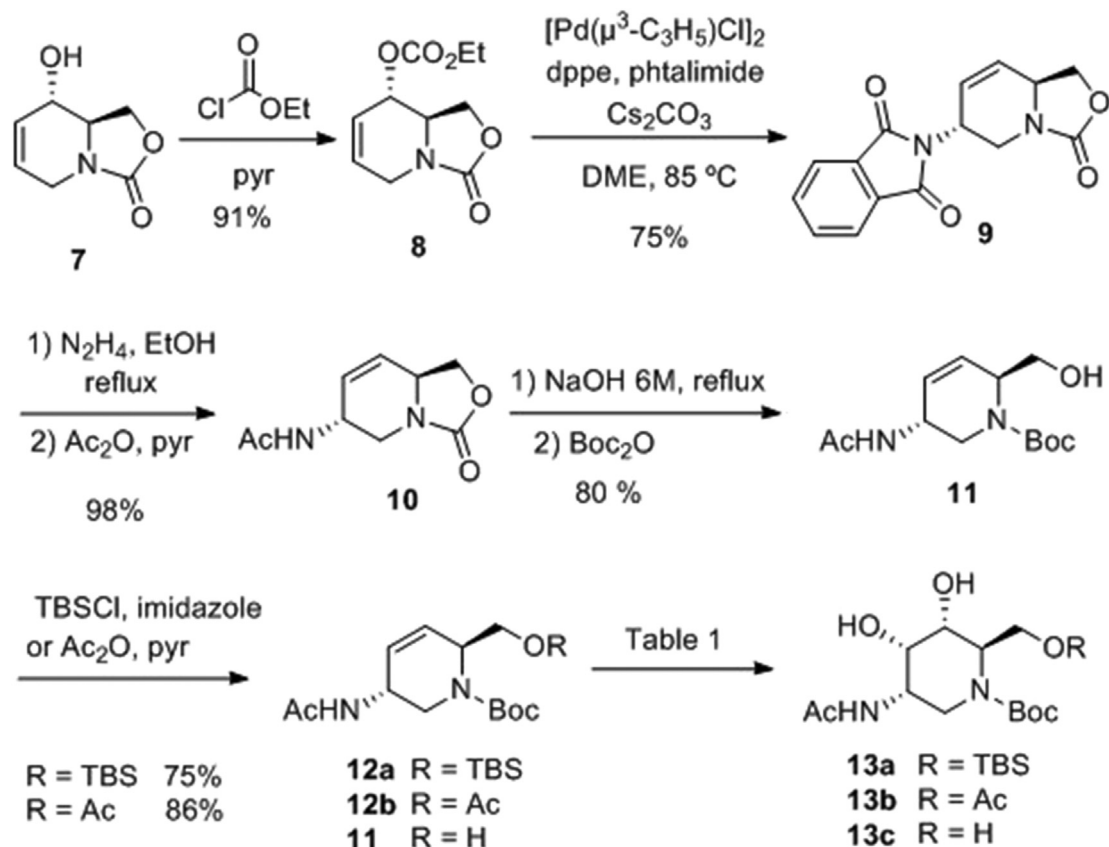

Fig. 1. Optimized structures calculated at DFT level.

respectively (Scheme 3).

The results for the dihydroxylation of the new *N*-Boc-protected enamides using a variety of conditions are shown in Table 1. The silyl ether derivative **12a** showed remarkable selectivity toward the desired *syn* diastereomer even under non-asymmetric conditions, although with moderate yields (Table 1, entries 1 and 2). Sharpless asymmetric dihydroxylation (AD) conditions using dihydroquinidine (DHQD) or dihydroquinine (DHQ) derivatives based on phthalazine (Phal) or pyrimidine (Pyr) afforded similar results even when working with the *miss-matched* chiral ligand (Table 1, entries 3 and 4). The fourth generation Sharpless ligands based on anthraquinone (AQN) [64] were also explored, showing a slightly lower *allo/galacto* ratio for the *miss-matched* case (Table 1, entries 5 and 6). These findings suggested that the observed stereoselectivity was due to the interplay of the directing effect of the acetamide and the steric hindrance of the substituent at C6. Excellent diastereoselectivities were obtained in the more favorable conditions. However, the reaction times, yields, and stability of TBS-compounds were not considered convenient for high-scale preparations. Of note, dihydroxylation of the more stable acetate **12b** using $K_2OsO_4 \cdot 2H_2O$/NMO gave an excellent yield and diastereoisomeric ratio in a much shorter reaction time. Sharpless conditions improved the diastereomeric ratio but at the expense of a dramatic decrease in yield (entries 7–8). Finally, dihydroxylation of compound **11**, bearing a free hydroxymethyl substituent, with $K_2OsO_4 \cdot 2H_2O$/NMO evidenced a significant decrease in the diastereoselectivity as compared with TBS or the acetylated counterparts **12a** and **12b** (entry 9). The yield was hindered in this case by difficulties in isolating the highly polar triol **13c**.

The *allo* stereochemistry of the major diastereomer **13b** was confirmed by NMR. As a diagnostic tool, 1D-NOESY analysis showed positive NOE contacts between H2–H3 and H3–H4 Furthermore, coupling constants between protons H2 and H3 suggest an equatorial-axial coupling, thus corroborating the *allo* configuration and the *syn* orientation of the dihydroxylation reaction (Fig. 2).

Consistent with our conformational analysis-based prediction, comparison of the stereochemical outcome of the dihydroxylation reaction for compounds **11**, **12a** and **12b** illustrated the influence of the bulkiness of the C6 substituent on the outcome of the reaction. However, the role of the acetamide functionality as a stereo-directing group remained to be checked. According to the work of Donohoe et al. [65,66], enamides coordinate to the OsO₄-alkene

Scheme 3. Synthesis of diols **13a**–**c**.

adduct under dihydroxylation reaction conditions, favoring the *syn* diastereomer. *N*-Alkylation of the acetamido group should lead to a loss of its coordination ability and therefore a drop in reactivity and stereoselectivity. In order to confirm this point, enamide **10** was benzylated with BnBr/NaH, the oxazolidone ring was subsequently opened (NaOH 6 M/reflux), and the free amino group generated was transformed into the corresponding *N*-Boc-protected

derivative **14** (46% yield overall). Acetylation of the primary alcohol with Ac$_2$O/pyr afforded acetate **15** (84% yield), a structural analog of **12a** but with the coordination capacity of the amide diminished. Dihydroxylation of **15** using K$_2$OsO$_4$·2H$_2$O/NMO with 10%, 30% and up to equimolar catalyst loadings did not afford the desired product, thereby demonstrating the key role of the acetamido group in both progress of the reaction and diastereoselectivity (Scheme 4).

Finally, *N*-Boc acetamide **13b** was de-*O*-acetylated by treatment with NH$_3$/MeOH, and the carbamate group was hydrolyzed with HCl/MeOH to give the target acetamido-iminosugar glycomimetic DAJNAc **5** as the corresponding hydrochloride salt in 91% yield (Scheme 5). The overall yield of the complete sequence (seven steps) from carbamate **7** was 41%.

Table 1
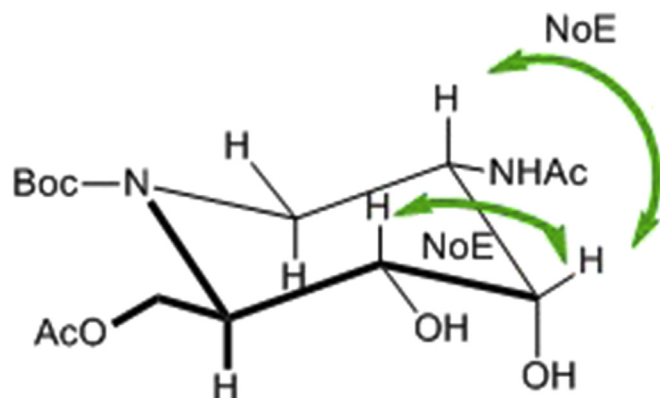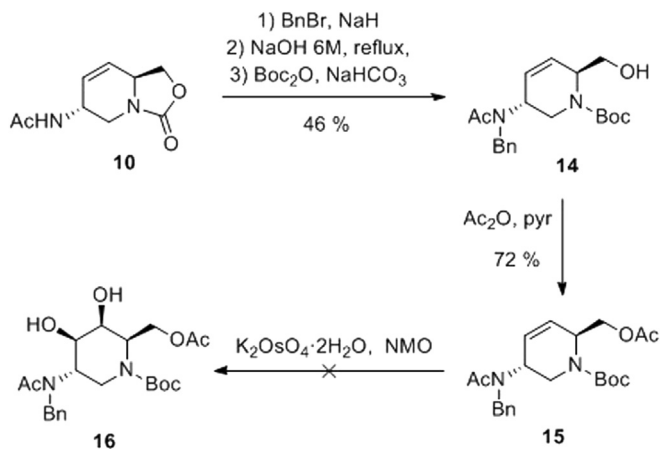Dihydroxylation of **11**, **12a** and **12b**.

	Substrate	Conditions[a]	t/day	d.r.[b]	Product	Yield/%
1	**12a**	OsO$_4$/NMO	2.5	>30: 1[c]	**13a**	65
2	**12a**	K$_2$OsO$_4$·2H$_2$O/NMO	2.5	26: 1	**13a**	74
3	**12a**	AD with (DHQD)$_2$Phal	4	>30: 1[c]	**13a**	56
4	**12a**	AD with (DHQ)$_2$Pyr	2	25: 1	**13a**	79
5	**12a**	AD with (DHQD)$_2$AQN	2	>30: 1[c]	**13a**	46
6	**12a**	AD with (DHQ)$_2$AQN	2	8: 1	**13a**	48
7	**12b**	K$_2$OsO$_4$·2H$_2$O/NMO	1	11: 1	**13b**	97
8	**12b**	AD with (DHQD)$_2$Phal	1	17: 1	**13b**	51
9	**11**	K$_2$OsO$_4$·2H$_2$O/NMO	1	7: 1	**13c**	48

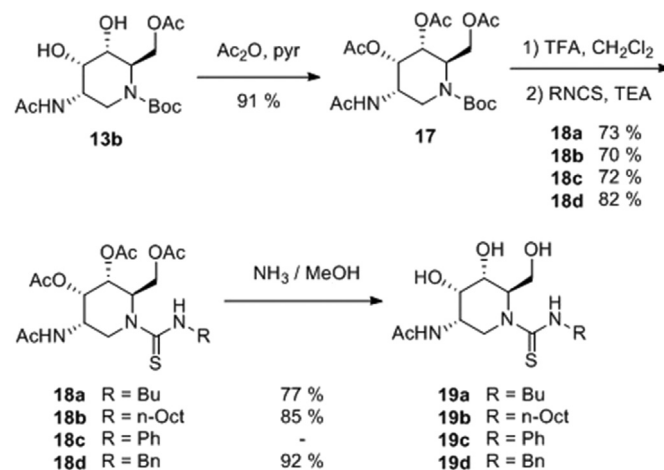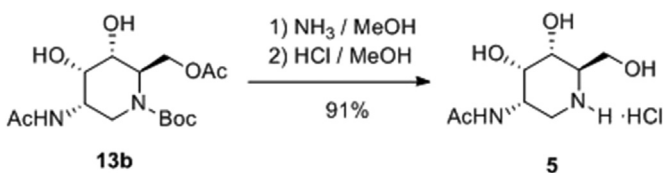[a] AD: Sharpless Asymmetric Dihydroxylation.
[b] Diastereomeric ratio determined by ^{1}H NMR.
[c] Only one diastereomer was observed by ^{1}H NMR.

Fig. 2. NOESY analysis of **13b**.

Scheme 4. Checking the effect of the acetamide group in the dihydroxylation.

Scheme 5. Final deprotection steps to provide DAJNAc·HCl.

Scheme 6. Synthesis of *N*-thioureido derivatives of DAJNAc.

Diols **13a** and **13b** were highly valuable intermediates for the synthesis of DAJNAc conjugates by derivatization of the endocyclic amine. *N*-alkylation has been extensively explored in the case of DNJNAc and DGJNAc **2** and **3**, but although significant increases in the inhibition potency against β-GlcNAcase have been achieved for some derivatives, the approach was generally inefficient to improve the selectivity among enzyme isoforms [50]. Interestingly, non-iminosugar glycomimetics incorporating five-membered heterocycles containing sulfur and nitrogen (thiazoline, thiadiazol) [67–69] have shown much higher enzyme discrimination capabilities. In order to access DAJNAc derivatives with this structural motif, condensation of the six-membered ring of **5** with a 2-iminothiazolidine fragment was considered. On the one hand, such transformation modifies the hybridation character of the N-atom from sp³ to sp², imposing a distortion of the chair conformation that better mimics the transition state of enzymatic glycosyl hydrolysis. On the second hand, the new exocyclic nitrogen is well-suited to bear substituents that can provide further interactions with the enzyme.
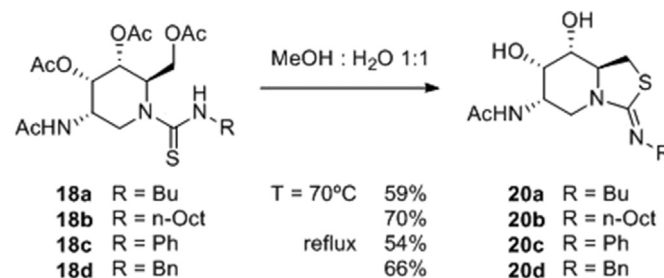
The synthesis of bicyclic DAJNAc-thiazolidine conjugates started by the protection of the secondary hydroxyls in **13b** by treatment with Ac₂O/pyridine to give **17** in 91% yield. Deprotection of the *N*-Boc was carried out with TFA and the resulting secondary amine was treated *in situ* with n-butyl, *n*-octyl, phenyl or benzyl isothiocyanate to give thioureas **18a**–**d** in good yields. Deprotection of the acetyl groups in the adducts using sat. NH₃/MeOH gave the fully unprotected *N*-thioureido derivatives **19a-d** in excellent yields except for **19c**, which could not be obtained. In this particular case the reaction crude showed many products. Among them we could identify the oxazolidine-2-thione resulting from the attack of the primary alcohol to the thiourea. The better leaving group capability of the aniline versus alkylamines would explain the different behavior of **18c** (Scheme 6). On the other hand, treatment of the *N'*-butyl and *N'*-octyl derivatives **18a** and **18b** with 1:1 MeOH/H₂O at 70 °C generated the corresponding 2-iminothiazolidines **20a** and

20b in good yields. Intramolecular attack of the thiocarbonyl sulfur at the C6 position with concomitant transesterification of the secondary acetates accounts for this transformation. The *N'*-phenyl and *N'*-benzyl thioureas **18c** and **18d** required heating at reflux 1:1 MeOH/H₂O to afford the corresponding bicyclic 2-iminothiazolidines derivatives **20c** and **20d** in moderate yields (Scheme 7).

2.2. Glycosidase inhibitory activity

Evaluation of the glycosidase inhibitory activity of **5** confirmed the total selectivity towards hexosaminidases among a panel that included β-glucosidases (almonds and bovine liver), α-glucosidase (yeast), α-mannosidase (jack bean), β-mannosidase (*Helix pomatia*), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), α-rhamnosidase (naringinasa; *Penicillium decumbens*), α-galactosidase (green coffee), β-galactosidase (*Escherichia coli*), or isomaltase (yeast). In addition to low μM inhibition of β-*N*-acetylglucosaminidases from different sources (human placenta, K_i 5.6 μM; bovine kidney, K_i 2.6 μM; jack bean, K_i 2.6 μM), DAJNAc was also found to inhibit the β-*N*-acetylgalactosaminidase activity of the enzyme from jack bean at its optimal pH [70] (K_i 46 μM).

The structure information of β-*N*-acetylglucosaminidase [51,69,71] reveals that the vicinity of the enzyme active site contains multiple negative charges. This feature has guided the design of inhibitors towards compounds containing protonable groups in the structure and is the main reason why *N*-alkylation strategies, keeping the sp³-hybridized character of the endocyclic nitrogen, have been privileged by other authors in the case of DNJNAc and DGJNAc conjugates [48]. Yet, interactions of lipophilic moieties

Scheme 7. Synthesis of 2-iminothiazolidine derivatives of DAJNAc.

with a neighboring hydrophobic pocket have been found instrumental both for glycomimetic conjugates and non-carbohydrate based inhibitors discovered by high throughput screening. We thus decided to include thioureas **19a**, **19b** and **19d** in our study. Whereas a strong decrease in the inhibition potency was observed for the N'-butyl and N'-octyl conjugates, the N'-benzylthiourea adduct was almost as potent as the parent iminosugar **5** (K_i 8.6 μM; Table 2). Most interestingly, the selectivity between the human and plant enzyme shifted from 1:2.2 to 1.6:1. To the best of our knowledge, this is the first neutral inhibitor of β-GlcNAcase reported up to date, expanding the current portfolio of potential drug candidates.

The basic character is restored in the bicyclic 2-iminothiazolidine sp^2-iminosugar conjugates, translating into a dramatic increase in the inhibitory potency, between two and three orders of magnitude, for the N'-alkyl derivatives **20a** and **20b** (K_i values 4.9 and 0.6 μM against the human placenta enzyme, respectively; Table 2) as compared with the thiourea progenitors **19a** and **19b**. The inhibition potency enhancement was less significant for the N'-benzyl analogue **20d** (K_i 4.9 μM), whereas the aromatic iminothiazolidine **20c** was about 5-fold a weaker inhibitor. Remarkably, except for **20c**, all the new bicyclic sp^2-iminosugar conjugates behaved as more potent inhibitors of human β-GlcNAcase than the parent monocyclic iminosugar DAJNAc **5**, up to almost 10-fold in the case of **20b**. Moreover, contrary to **5**, they also exhibited a marked selectivity towards the human enzyme relative to the plant enzyme.
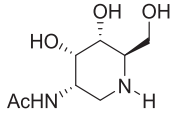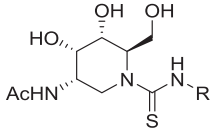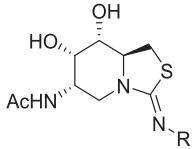
Unexpectedly, the inhibition trend of the new DNJNAc thioureas and iminothiazolidines towards total β-hexosaminidase (A and B isoforms) and β-hexosaminidase A, the isoform dysfunctional in Tay-Sachs patients, in wild type fibroblast lysates was totally different from that encountered in the placenta enzyme. Thus, only the N'-octyl and N'-phenyliminothiazolidines **20b** and **20c** exhibited submillimolar IC$_{50}$ values in the two assays (315 μM against total hexosaminase, 100 μM against hexosaminisadase A for both compounds). The N'-octylthiourea derivative **19b** additionally behaved as a modest inhibitor of hexosaminisadase A (IC$_{50}$ 350 μM). To the best of our knowledge, such a sharp difference in the inhibitory activity towards lysosomal β-hexosaminidases from different tissues has never been reported before. A plausible explanation is that the DAJNAc derivatives prepared in this work are highly sensitive to structural differences between the mature and precursor form of the enzyme. Thus, β-hexosaminidase from human placenta is a mature form of the lysosomal enzyme, whereas the precursor form has been reported to be present in the extracellular medium in which cultured fibroblasts were grown [72].

Lysosomal hexosaminidase hydrolyze terminal N-acetyl-β-D-glucosamine and N-acetyl-β-D-galactosamine residues. Consequently, the contacts of the mismatching *allo*-configured DAJNAc glycomimetics with amino acids at the catalytic site will likely be poor, the stability of the complex with the enzyme relying much on aglycone interactions. The peripheral enzyme regions involved in such aglycone interactions are expected to be more sensitive to structural differences between the mature and precursor forms of the enzyme as compared to the catalytic site, which provides a rational for the observed discrepancies in the inhibitory activities when going from the placenta enzyme to fibroblast lysates.

The mature β-hexosaminidase from human placenta has been previously used in high throughput screenings to identify inhibitors that could act as pharmacological chaperones against disease-associated hexosaminidase mutants [42]. However, an ideal chaperone candidate should bind to the precursor form of the mutant enzyme (hexosaminidase A in the case of Tay-Sachs disease) at the endoplasmic reticulum, restore trafficking and dissociate from the mature hexosaminidase at the lysosome. In our case, the 2-iminothiazolidines **20b** and **20c** inhibited hexosaminidase A in fibroblasts (precursor form) with IC$_{50}$ of 100 μM but were much stronger ligands of the mature placenta enzyme (IC$_{50}$ 0.6 and 27 μM, respectively), which will likely translate into an unfavorable chaperone-inhibitor balance. Accordingly, a very modest hexosaminidase activity enhancement was observed for **20c** (1.2-fold at 2 μM concentration) in fibroblasts heterozygous for the p.G269S/c.1278insTACT mutations from a Tay-Sachs patient with the adult-onset form of the disease, whereas **20b** was inactive in the 0.2–20 μM concentration range. Interestingly, the thiourea derivative **19b** induced a 30% hexosaminidase activity enhancement at only 0.2 μM concentration in the same cell line, in spite of its much weaker fibroblast hexosaminidase A affinity (350 μM). The ensemble of data strongly suggest that the ratio between the inhibitory potencies against the placenta and fibroblast hexosaminidase provides a much accurate indication for the selection of pharmacological chaperone candidates for Tay-Sachs disease than the classical screening using only the placenta enzyme. Using inhibition of the mature form of the enzyme as a selection tool bear the risk to privilege strong inhibitors over good chaperones and might be at the origin of some disappointing results in clinical trials [73]. Even though the hexosaminidase activity enhancement achieved in vitro with the best compound identified in this work, **19b**, is far from other examples reported in the literature [41,74,75], the fact that it binds much weakly to the mature as compared to the precursor enzyme is unprecedented and open the door to optimization strategies.

Table 2
Inhibition constants (K_i, μM) against commercial β-N-acetylglucosaminidases for DAJNAc (**5**), **19a**–**d** and **20a**–**d** determined from thee slope of Lineweaver–Burk plots and double reciprocal analysis[a].

Enzyme origin	**5**	**19a**, R = Bu	**19b**, R = n-Oct	**19d**, R = Bn	**20a**, R = Bu	**20b**, R = n-Oct	**20c**, R = Ph	**20d**, R = Bn
Human placenta	5.6 ± 0.3	118 ± 5	909 ± 5	8.6 ± 0.3	4.9 ± 0.3	0.60 ± 0.05	27 ± 1	4.9 ± 0.4
Bovine kidney	2.6 ± 0.2	88 ± 3	n.i.[b]	5.9 ± 0.2	2.9 ± 0.2	0.65 ± 0.05	38 ± 2	11 ± 0.5
Jack bean	2.6 ± 0.2	75 ± 2	363 ± 5	13.4 ± 0.5	24 ± 1	2.3 ± 0.1	20 ± 1	9.5 ± 0.5

[a] No significant inhibition was detected against β-glucosidases (almonds and bovine liver), α-glucosidase (yeast), α-mannosidase (jack bean), β-mannosidase (*Helix pomatia*), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), α-rhamnosidase (naringinasa; *Penicillium decumbens*), α-galactosidase (green coffee), β-galactosidase (*E. coli*), or isomaltase (yeast).
[b] No inhibition detected a 2 mM concentration.

3. Conclusions

In summary, we have devised a new synthetic route for DAJNAc **5** that increases the yield from 16 to 41% and the diastereoselectivity of the key dihydroxylation step from 4:1 to 11:1 as compared with the previously reported preparation. This achievement paves the way for the preparation of large amounts of **5**, currently the best inhibitor of placenta human β-GlcNAcase among the acetamido-iminosugar analogues of the natural monosaccharide **1**. Moreover, the orthogonally protected intermediate **13b** has been used for the implementation of molecular diversity-oriented schemes leading to sp²-iminosugar conjugates with a monocyclic glycone moiety, namely the thiourea derivatives **19a**, **19b** and **19d**, or with a bicyclic skeleton, namely the 2-iminothiazolidines **20a**–**d**. Evaluation of the inhibitory properties of the new compounds against hexosaminidase from human placenta and cultured fibroblasts unveiled remarkable disparities that could be ascribed to differences in the efficiency of aglycone interactions with the mature and precursor forms of the enzyme, which has been demonstrated to be critical for the identification of pharmacological chaperone candidates for Tay-Sachs disease. It is worth mentioning that β-hexosaminidase is also the dominant glycosaminoglycan-degrading glycosidase released by chondrocytes into the extracellular milieu [76]. Inhibition of the mature form of this enzyme can indeed prevent or even reverse cartilage matrix degradation, representing a new avenue in the development of therapeutic treatments for osteoarthritis [38,76,77]. Altogether, the results support that modifications of the DAJNAc core is a promising strategy for the development of therapeutic agents against β-GlcNAcase-related diseases.

4. Experimental

4.1. Theoretical calculations

The structures were fully optimized using DFT at the level RB3LYP/6-31G** as implemented in Spartan'10 package of programs [79].

4.2. Chemistry

4.2.1. General remarks

Non-aqueous reactions were carried out under nitrogen atmosphere. Dry dichloromethane was obtained using a Solvent Purification System (SPS). Other commercially available reagents and solvents were used with no further purification. All reactions were monitored by TLC analysis using Merck 60 F_{254} silica gel on aluminum sheets. Silica gel chromatography was performed by using 35–70 mm silica or an automated chromatography system (Combiflash®, Teledyne Isco) with hexanes/ethyl acetate gradients as eluent unless noted otherwise.

NMR spectra were recorded at room temperature on a Varian Mercury 400. ¹H and ¹³C NMR spectra were referenced to the residual peaks of the deuterated solvent. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad signal. The chemical shifts (δ) are expressed in ppm and the coupling constants (J) in hertz (Hz).

IR spectra were recorded in a Thermo Nicolet Nexus FT-IR apparatus, either by preparing a KBr disk or by depositing a film of the product on a NaCl plate. Absorptions are given in wavenumbers (cm⁻¹).

Melting points were recorded in a Büchi M-540 apparatus without recrystallization of the final solids.

Optical rotations were measured at room temperature (25 °C) using a Jasco P-2000 iRM-800 polarimeter (589 nm). Concentration (g/100 mL) and solvent are shown in brackets.

High Resolution Mass Spectra were recorded in a LTQ-FT Ultra (Thermo Scientific) using the Nanoelectrospray technique.

Elemental analyses were done in an EA-1108 CE Instrument (Thermo Fisher).

Compounds **8**, **9** and **10** were obtained following the previously described methodology [55].

4.2.2. (2S,5R)-tert-Butyl-5-acetamido-2-(hydroxymethyl)-5,6-dihydropyridine-1(2H)-carboxylate (11)

NaOH 6 M (2.72 mL, 16.3 mmol) was added to a solution of **10** (320 mg, 1.63 mmol) in acetone:water 10:1 (25 mL), and the mixture was heated at reflux for 7 h. After cooling to rt, the crude product was neutralized by adding HCl(c) until pH 8, and solvents were removed *in vacuo* to give a white solid, which was redissolved in EtOAc:aqueous saturated NaHCO₃ 3:1 (20 mL). Boc₂O (791 mg, 3.58 mmol) was added, and the mixture was heated at reflux for 16 h. The mixture was extracted with EtOAc (3 × 15 mL), washed with water (1x 20 mL), dried over MgSO₄, and purified in silica gel using CH₂Cl₂/methanol, increasing the polarity ratio from 0 to 15% MeOH to give **11** (361 mg, 80%) as a white solid.

[α]²⁰_D = −115.5° (c = 0.22, CHCl₃). Mp: 65−68 °C. ¹H NMR (400 MHz, CDCl₃, δ/ppm): 6.08−5.87 (m, 2H), 5.68 (br, 1H), 4.66 (br, 1H), 4.33 (m, 1H), 4.22 (d, J = 14.0 Hz, 1H), 3.70 (m, 2H), 3.13 (dd, J = 14.0, 2.5 Hz, 1H), 1.96 (s, 3H), 1.47 (s, 9H). ¹³C NMR (100 MHz, CDCl₃, δ/ppm): 169.5 (CO), 156.3 (CO), 129.7 (CH), 126.4 (CH), 80.6 (C), 63.5 (CH₂), 53.9 (CH), 43.2 (CH) 43.2 (CH₂), 28.4 (CH₃), 23.1 (CH₃). IR (film, ν_max/cm⁻¹): 3288, 2968, 2917, 1675, 1653, 1422, 1366, 1173, 1133. HRMS (ES): Calcd. for C₁₃H₂₂N₂O₄: 271.16523, found 271.16505. EA: Calcd. for C₁₃H₂₂N₂O₄·²/₃ H₂O: C, 55.30%; H, 8.33%; N, 9.92%; found C, 55.25%; H, 8.01%; N, 10.07%.

4.2.3. tert-Butyl-(3R,6S)-3-acetamido-6-(((tert-butyldimethylsilyl)oxy) methyl)-3,6-dihydropyridine-1(2H)-carboxylate (12a)

Compound **11** (70 mg, 0.26 mmol), TBSCl (61 mg, 0.39 mmol), and imidazole (35 mg, 0.52 mmol) were placed in a 25-mL round bottom flask and dissolved in CH₂Cl₂ (5 mL). The reaction was then stirred at rt for 2 h. Solvents were removed *in vacuo*, and the crude product was purified in SiO₂·TEA (2.5% v/v) using hexane/ethyl acetate 30:70 to obtain **12a** (75 mg, 75%) as a colorless oil.

[α]²⁰_D = −150.3° (c = 0.11, CHCl₃). ¹H NMR (400 MHz, CDCl₃, δ/ppm): 5.95 (m, 2H), 4.52 (br, 1H), 4.27 (m, 1H), 4.18 (d, J = 13.5 Hz, 1H), 3.68 (m, 2H), 3.18 (m, 1H), 1.94 (s, 3H), 1.44 (s, 9H), 0.86 (s, 9H), 0.02 (s, 6H). ¹³C NMR (100 MHz, CDCl₃, δ/ppm): 169.3 (CO), 155.4 (CO), 154.9* (CO), 131.4 (CH), 129.6* (CH), 126.0 (CH), 125.4* (CH), 80.1 (C), 79.7* (C), 63.6 (CH₂), 53.9 (CH), 52.9* (CH), 44.1 (CH₂), 43.3 (CH), 28.4 (CH₃), 25.8 (CH₃), 23.1 (CH₃), 18.1 (C), −5.6 (CH₃) (*Rotamer signal). IR (film, ν_max/cm⁻¹): 2962, 2929, 1693, 1656, 1421, 1365, 1133, 1104. HRMS (ES): Calcd. for C₁₉H₃₆N₂O₄Si: 385.25171, found 385.25145.

4.2.4. tert-Butyl-(3R,6S)-3-acetamido-6-(acetoxymethyl)-3,6-dihydropyridine-1(2H)-carboxylate (12b)

Pyridine (0.45 mL, 5.34 mmol) and acetic anhydride (0.61 mL, 5.77 mmol) were added to a solution of **11** (578 mg, 2.14 mmol) in 32 mL of CH₂Cl₂. The solution was then stirred at rt for 3 h. Water (10 mL) was added, and the crude product extracted with CH₂Cl₂ (3 × 15 mL), dried over MgSO4, and purified in silica gel using CH₂Cl₂/methanol, increasing the polarity ratio from 0 to 10% MeOH to give **12b** (572 mg, 86%) as a white solid.

[α]²⁰_D = −171.1° (c = 0.35, CHCl₃). Mp: 142−143 °C. ¹H NMR (400 MHz, CDCl₃, δ/ppm): 6.16−5.66 (m, 3H), 4.83 (br, 1H), 4.64* (br, 1H), 4.39 (m, 1H), 4.22 (d, J = 13.5 Hz, 1H), 4.14 (m, 2H), 3.11 (dd, J = 13.5, 3.0 Hz, 1H), 2.06 (s, 3H), 2.04* (s, 3H), 1.96 (s, 3H), 1.46 (s,

9H) (*Rotamer signals). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 170.6 (CO), 169.3 (CO), 154.8 (CO), 129.2 (CH), 127.6 (CH), 126.8* (CH), 80.5 (C), 80.1* (C), 63.3 (CH$_2$), 63.1* (CH$_2$), 51.2 (CH), 50.2* (CH), 43.1 (CH), 42.1 (CH$_2$), 28.3 (CH$_3$), 23.0 (CH$_3$), 20.7 (CH$_3$) (*Rotamer signal). IR (film, ν$_{max}$/cm^{-1}): 2968, 1751, 1693, 1661, 1418, 1367, 1239, 1136. HRMS (ES): Calcd. for C$_{15}$H$_{25}$N$_2$O$_5$: 313.17580, found 313.17587. EA: Anal. Calcd. for C$_{15}$H$_{24}$N$_2$O: C, 57.68%; H, 7.74%; N, 8.97%; found C, 57.77%; H, 7.82%; N, 8.81%.

4.2.5. tert-Butyl-(2R,3R,4S,5S)-5-acetamido-2-(((tert-butyldimethylsilyl)oxy)methyl)-3,4-dihydroxypiperidine-1-carboxylate (13a)

N-Methylmorpholine-N-oxide (43 mg, 0.36 mmol) and K$_2$OsO$_4$·2H$_2$O (6.3 mg, 12%) were added to a solution of **12a** (55 mg, 0.14 mmol) in acetone:water 10:1 (4 mL). The solution was then stirred for 2.5 d at rt. Na$_2$S$_2$O$_3$ (40 mg) and MgSO$_4$ were added, and the reaction was stirred for 1 h and filtered over Celite. Solvents were remove in vacuo, and the crude product was purified in silica gel using CH$_2$Cl$_2$/methanol, increasing the polarity ratio from 0 to 10% MeOH to give **13a** (44 mg, 74%) as a colorless oil with a 26:1 diastereoisomer ratio.

^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 6.92 (br, 1H), 4.42 (t, $J = 6.0$ Hz, 1H), 4.24 (dd, $J = 6.0$, 3.5 Hz 1H), 4.16 (d, $J = 14.0$ Hz, 1H), 4.10 (s, 1H), 3.97 (t, $J = 3.5$ Hz, 1H), 3.72 (m, 2H), 3.13 (dd, $J = 14.0$, 2.5 Hz, 1H), 1.98 (s, 3H), 1.45 (s, 9H), 0.87 (s, 9H), 0.04 (d, $J = 1.7$ Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 171.4 (CO), 156.2 (CO), 80.4 (C), 69.7 (CH), 67.1 (CH), 62.4 (CH$_2$), 58.2 (CH), 48.8 (CH), 44.1 (CH$_2$), 28.3 (CH$_3$), 25.8 (CH$_3$), 23.5 (CH$_3$), 18.1 (C), −5.6 (CH$_3$). IR (film, ν$_{max}$/cm^{-1}): 3373, 2929, 1693, 1653, 1420, 1155, 1103. HRMS (ES): Calcd. for C$_{19}$H$_{39}$N$_2$O$_6$Si: 419.25719, found 419.25686.

4.2.6. tert-Butyl-(2R,3R,4S,5S)-5-acetamido-2-(acetoxymethyl)-3,4-dihydroxypiperidine-1-carboxylate (13b)

4.2.6.1. Method A: non-asymmetric conditions.
N-Methylmorpholine N-oxide (751 mg, 6.21 mmol) and K$_2$OsO$_4$·2H$_2$O (110 mg, 10%) were added to a solution of **12b** (925 mg, 2.96 mmol) in acetone:water 10:1 (27 mL). The solution was then stirred for 1 d at rt. Na$_2$S$_2$O$_3$ (500 mg) and MgSO$_4$ were added, and the reaction was stirred for 1 h and filtered over Celite. Solvents were remove in vacuo, and the crude product was purified in silica gel using CH$_2$Cl$_2$/methanol, increasing the polarity ratio from 0 to 15% MeOH to give **13b** (1.01 g, 97%) as a white solid with a 11:1 diastereoisomer ratio.

4.2.6.2. Method B: sharpless asymmetric dihydroxylation.
(DHQD)$_2$Phal (6.1 mg, 5%), K$_2$OsO$_4$·2H$_2$O (1.4 mg, 2.5%), K$_3$[Fe(CN)$_6$] (153 mg, 0.46 mmol) and K$_2$CO$_3$ (64 mg, 0.46 mmol) were dissolved in water (1.5 mL) and cooled at 0 °C. Methanesulfonamide (15 mg, 0.15 mmol) was added in one portion. After 5 min, a solution of **12b** (48 mg, 0.15 mmol) in ^{t}Bu-OH (1.5 mL) was added. The mixture was vigorously stirred at 0 °C for 20 min, then allowed to warm to rt and stirred for 1 d. Na$_2$S$_2$O$_3$ (150 mg) was added. After 30 min, the crude product was extracted with EtOAc (3 × 5 mL), dried over MgSO$_4$, and purified in silica gel using CH$_2$Cl$_2$/methanol, increasing the polarity ratio from 0 to 15% MeOH to give **13b** (27 mg, 51%) as a white solid with a 17:1 diastereoisomer ratio.

[α]^{20}$_D$ = +13.5° (c = 0.25, CHCl$_3$). Mp: 47–49 °C. ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 7.02 (br, 1H), 4.75 (t, $J = 6.5$ Hz, 1H), 4.27 (m, 1H), 4.20 (d, $J = 14.5$ Hz, 1H), 4.17 (s, 1H), 4.15 (d, $J = 2.5$ Hz, 1H), 3.99 (s, 1H), 3.82 (dd, $J = 4.5$, 3.0 Hz, 1H), 3.04 (dd, $J = 14.5$, 2.5 Hz, 1H), 2.06 (s, 3H), 1.99 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 171.4 (CO), 170.6 (CO), 156.1 (CO), 80.7 (C), 68.9 (CH), 66.6 (CH), 60.7 (CH$_2$), 55.8 (CH), 48.8 (CH), 42.9 (CH$_2$), 28.3 (CH$_3$), 23.4 (CH$_3$), 20.8 (CH$_3$). IR (film, ν$_{max}$/cm^{-1}): 3377, 2968, 1741, 1689, 1431, 1366, 1254, 1146. HRMS (ES): Calcd. for C$_{15}$H$_{27}$N$_2$O$_7$: 347.18128, found 347.18134. EA: Calcd. for C$_{15}$H$_{26}$N$_2$O$_7$·H$_2$O: C, 49.44%; H, 7.75%; N, 7.69%; found C, 49.75%; H, 7.71%; N, 7.74%.

4.2.7. tert-Butyl (2R,3R,4S,5S)-5-acetamido-3,4-dihydroxy-2-(hydroxymethyl)piperidine-1-carboxylate (13c)

N-Methylmorpholine-N-oxide (47 mg, 0.39 mmol) and K$_2$OsO$_4$·2H$_2$O (8.2 mg, 12%) were added to a solution of **11** (50 mg, 0.18 mmol) in acetone:water 10:1 (5 mL). The solution was then stirred for 1 d at rt. Na$_2$S$_2$O$_3$ (45 mg) and MgSO$_4$ were added, and the reaction was stirred for 1 h and filtered over Celite. Solvents were removed in vacuo, and the crude product was purified in silica gel using CH$_2$Cl$_2$/methanol, increasing the polarity ratio from 0 to 20% MeOH to give **13c** (27 mg, 48%) as a white solid and one diastereoisomer.

[α]^{20}$_D$ = +28.6° (c = 0.31, CH$_3$OH). ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 7.74 (d, $J = 7.5$ Hz, 1H), 4.45 (tt, $J = 7.5$, 2.0 Hz, 1H), 4.24 (ddd, $J = 14.0$, 3.0, 2.0 Hz, 1H), 4.02 (m, 2H), 3.84 (dd, $J = 4.5$, 3.0 Hz, 1H), 3.63 (m, 2H), 3.01 (dd, $J = 14.0$, 2.0 Hz, 1H), 1.96 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 173.0 (CO), 158.0 (CO), 81.2 (C), 70.3 (CH), 66.6 (CH), 60.5 (CH, CH$_2$), 51.4 (CH), 43.9 (CH$_2$), 28.6 (CH$_3$), 23.3 (CH$_3$).

IR (film, ν$_{max}$/cm^{-1}): 3367, 2975, 1658, 1425, 1366, 1147. HRMS (ES): Calcd. for C$_{13}$H$_{25}$N$_2$O$_6$: 305.17071, found 305.17062.

4.2.8. tert-Butyl (3R,6S)-3-(N-benzylacetamido)-6-(hydroxymethyl)-3,6-dihydropyridine-1(2H)-carboxylate (14)

BnBr (108 μL, 0.89 mmol) was added to a suspension of NaH (18 mg, 0.71 mmol), tBuONa (3.4 mg, 0.03 mmol) and **10** (70 mg, 0.35 mmol) in 3 mL of DMF, and the mixture was stirred for 4 h at rt. After water (3 mL) addition, the crude product was extracted with CH$_2$Cl$_2$ (3x 5 mL), and solvents were removed in vacuo. The yellow oil was redissolved in MeOH:H$_2$O 9:1 (8 mL), then NaOH 6 M (0.85 mL, 5.09 mmol), was added and the mixture was stirred 16 h at reflux. After allowing the mixture to cool to rt, HCl(c) was added until a pH of 7−8 was achieved. Solvents were removed in vacuo, and the white solid obtained was redissolved in EtOAc:aq NaHCO$_3$ $_{sat}$ (12 mL). Boc$_2$O (247 mg, 1.12 mmol) was added, and the mixture was stirred at reflux for 4 h. The crude product was extracted with EtOAc (3x 5 mL), dried over MgSO$_4$, and purified in silica gel using hexane/ethyl acetate, increasing the polarity ratio from 0 to 100% ethyl acetate to give **14** (65 mg, 48%) as a colorless oil.

[α]^{20}$_D$ = −169.7° (c = 0.18, CH$_3$OH). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 7.32 (t, $J = 7.5$ Hz, 2H), 7.24 (t, $J = 7.5$ Hz, 1H), 7.10 (d, $J = 7.5$ Hz, 2H), 5.91 (dd, $J = 10.5$, 4.0 Hz, 1H), 5.79 (br, 1H), 5.09 (br, 1H), 4.64 (br, 1H), 4.53 (d, $J = 17.5$ Hz, 1H), 4.46 (d, $J = 17.5$ Hz, 1H), 4.31 (d, $J = 14.5$ Hz, 1H), 3.68 (m, 2H), 3.23 (dd, $J = 14.5$, 4.0 Hz, 1H), 2.00 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 171.9 (CO), 138.4 (C), 131.0 (CH), 128.8 (CH), 127.1 (CH), 125.6 (CH), 125.4 (CH), 80.6 (C), 63.6 (CH$_2$), 54.0 (CH), 48.9 (CH$_2$), 47.8 (CH), 43.6 (CH$_2$), 28.4 (CH$_3$), 22.4 (CH$_3$). IR (film, ν$_{max}$/cm^{-1}): 3404, 1691, 1648, 1450, 1365, 1170, 1027. HRMS (ES): Calcd. for C$_{20}$H$_{29}$N$_2$O$_4$: 361.2122, found 361.2122.

4.2.9. tert-Butyl (3R,6S)-6-(acetoxymethyl)-3-(N-benzylacetamido)-3,6-dihydropyridine-1(2H)-carboxylate (15)

Pyridine (37 μL, 0.44 mmol) and Ac$_2$O (40 μL, 0.38 mmol) were added to a solution of **14** (63 mg, 0.17 mmol) and DMAP (8 mg, 0.07 mmol) in CH$_2$Cl$_2$ (4 mL). The solution was allowed to stir overnight. Then water (2 mL) was added, and the crude product was extracted with CH$_2$Cl$_2$ (3 × 5 mL), dried over MgSO$_4$, and purified in silica gel using hexane/ethyl acetate, increasing the polarity ratio from 0 to 100% ethyl acetate to give **15** (51 mg, 72%) as a colorless oil.

[α]^{20}$_D$ = −177.6° (c = 0.2, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 7.31 (t, $J = 7.5$ Hz, 2H), 7.24 (t, $J = 7.5$ Hz, 1H), 7.09 (d,

J = 7.5 Hz, 2H), 5.86 (m, 2H), 5.08 (br, 1H), 4.83 (br, 1H), 4.52 (d, J = 18.0 Hz, 1H), 4.43 (d, J = 18.0 Hz, 1H), 4.30 (m, 1H), 4.13 (s, 2H), 3.22 (dd, J = 14.5, 4.0 Hz, 1H), 2.04 (s, 3H), 2.00 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 172.0 (CO), 170.7 (CO), 154.5 (CO), 138.4 (C), 130.8 (CH), 129.8* (CH), 128.7 (CH), 127.1 (CH), 126.3 (CH), 126.0* (CH), 125.4 (CH), 80.1 (C), 63.3 (CH2), 51.0 (CH), 50.3* (CH), 49.0 (CH$_2$), 48.5* (CH$_2$), 47.8 (CH), 46.6* (CH), 43.7 (CH$_2$), 42.8* (CH$_2$), 28.4 (CH$_3$), 22.4 (CH$_3$), 20.8 (CH$_3$). (* Rotamer signals). IR (film, ν_{max}/cm^{-1}): 2975, 1744, 1694, 1649, 1417, 1365, 1237, 1171. HRMS (ES): Calcd. for C$_{22}$H$_{31}$N$_2$O$_5$: 403.2227, found 403.2228.

4.2.10. N-((3S,4S,5R,6R)-4,5-Dihydroxy-6-(hydroxymethyl) piperidin-3-yl)acetamide hydrochloride (DAJNAc·HCl, 5)

Compound 13b (39 mg, 0.11 mmol) was dissolved in a saturated methanol solution of NH$_3$ (2 mL) and stirred for 2 h. Solvent was removed *in vacuo*, and the crude product was redissolved in HCl 1.25 M MeOH solution (2.5 mL) and stirred for 3 h. Solvent was removed *in vacuo*, and the crude product was purified in silica gel using CH$_2$Cl$_2$/methanol/NH$_3$, increasing polarity ratio from 99:0:1 to 85:13:2 to give 5 (21 mg, 91%) as a white solid.

[α]^{20}$_D$ = +3.0° (c = 0.13, CH$_3$OH). Mp: 45–48 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 4.21 (dt, J = 9.0, 2.0 Hz, 1H), 4.00 (t, J = 2.0 Hz, 1H), 3.91 (dd, J = 12.0, 3.0 Hz, 1H), 3.85 (dd, J = 12.0, 5.0 Hz, 1H), 3.75 (dd, J = 10.5, 3.0 Hz, 1H), 3.29 (m, J = 5.0 Hz, 1H), 3.13 (s, 1H), 3.11 (s, 1H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 173.4 (CO), 69.7 (CH), 67.4 (CH), 59.0 (CH$_2$), 56.7 (CH), 47.8 (CH), 41.5 (CH$_2$), 22.4 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3308, 2936, 1652, 1546, 1533, 1367, 1079, 1034. HRMS (ES): Calcd. For C$_8$H$_{17}$N$_2$O$_4$: 205.11828, found 205.11832.

4.2.11. (2R,3R,4S,5S)-5-Acetamido-2-(acetoxymethyl)-1-(tert-butoxycarbonyl)piperidine-3,4-diyl diacetate (17)

To a solution of compound 13b (380 mg, 1.1 mmol) in CH$_2$Cl$_2$ (13 mL) was added pyridine (0.27 mL, 3.3 mmol) and acetic anhydride (0.28 mL, 2.4 mmol) dropwise. The solution was allowed to stir for 16 h. Solvents were removed *in vacuo* and the crude was purified by chromatography on silica gel using CH$_2$Cl$_2$/methanol increasing polarity ratio from 0 to 10% to give 17 (432 mg, 91%) as a white sticky foam.

[α]^{20}$_D$ = +14.1 (c = 0.35, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 6.33 (br, 1H), 5.25 (m, 1H), 5.14 (dd, J = 4.5, 3.5 Hz, 1H), 4.75 (br, 1H), 4.39 (s, 1H), 4.31 (m, 2H), 4.18 (dd, J = 11.5, 6.0 Hz, 1H), 3.15 (dd, J = 14.5, 2.0 Hz, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 170.2 (CO), 169.5 (CO), 169.4 (CO), 168.7 (CO), 155.3 (CO), 80.9 (C), 68.9 (CH), 66.0 (CH), 60.6 (CH$_2$), 53.9 (CH), 46.2 (CH), 43.2 (CH$_2$), 28.2 (CH$_3$), 23.4 (CH$_3$), 20.9 (CH$_3$), 20.6 (CH$_3$), 20.6 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 2975, 1747, 1691, 1412, 1368, 1228, 1152, 1059. HRMS (ES): Calcd. for C$_{19}$H$_{31}$N$_2$O$_9$: 431.20241, found 431.20283.

4.2.12. (2R,3R,4S,5S)-5-Acetamido-2-(acetoxymethyl)-1-(butylcarbamothioyl)piperidine-3,4-diyl diacetate (18a)

To a solution of 17 (63 mg, 0.15 mmol) in CH$_2$Cl$_2$ (3 mL) was added TFA (0.35 mL) and the solution was stirred at rt for 1 h. Solvent was removed *in vacuo*. The crude was dissolved in CH$_2$Cl$_2$ (4 mL) and TEA (164 μL, 1.17 mmol) and butyl isothiocyanate (54 μL, 0.44 mmol) were added. The solution was stirred at reflux for 4 h. After removal of solvents, the crude was purified in silica gel using hexane/ethyl acetate increasing polarity ratio from 0 to 100% ethyl acetate to give 18a (47 mg, 73%) as a colorless oil.

[α]^{20}$_D$ = −74.6 (c = 0.25, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 6.65 (d, J = 7.5 Hz, 1H), 6.39 (d, J = 4.5 Hz, 1H), 5.35 (br, 1H), 5.25 (m, 2H), 4.89 (d, J = 15.0 Hz, 1H), 4.43 (dd, J = 11.5, 7.0 Hz, 2H), 4.19 (dd, J = 11.5, 7.0 Hz, 1H), 3.64 (m, 2H), 3.30 (dd, J = 15.0, 3.0 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.61 (m, 2H),

1.39 (m, 2H), 0.96 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 185.4 (CS), 170.9 (CO), 170.3 (CO), 169.6 (CO), 169.2 (CO), 68.8 (CH), 66.4 (CH), 61.4 (CH$_2$), 57.7 (CH), 47.0 (CH), 46.5 (CH$_2$), 45.5 (CH$_2$), 30.9 (CH$_2$), 23.4 (CH$_3$), 20.9 (CH$_3$), 20.7 (CH$_3$), 20.6 (CH$_3$), 20.0 (CH$_2$), 13.7 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3327, 2957, 1747, 1666, 1530, 1369, 1222, 1058. HRMS (ES): calcd. for C$_{19}$H$_{31}$N$_3$O$_7$S: 446.19555, found 446.19593.

4.2.13. (2R,3R,4S,5S)-5-Acetamido-2-(acetoxymethyl)-1-(octylcarbamothioyl)piperidine-3,4-diyl diacetate (18b)

To a solution of 17 (110 mg, 0.26 mmol) in CH$_2$Cl$_2$ (4 mL) was added TFA (0.61 mL) and the solution was stirred at rt for 2 h. Solvent was removed *in vacuo*. The crude was dissolved in CH$_2$Cl$_2$ (5 mL) and TEA (213 μL, 1.53 mmol) and octyl isothiocyanate (128 μL, 0.64 mmol) were added. The solution was stirred at reflux for 4 h. After removal of solvents, the crude was purified in silica gel using hexane/ethyl acetate increasing polarity ratio from 0 to 100% ethyl acetate to give 18b (90 mg, 70%) as a colorless oil.

[α]^{20}$_D$ = −66.4 (c = 0.11, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 6.64 (d, J = 7.5 Hz, 1H), 6.36 (s, 1H), 5.34 (br, 1H), 5.25 (m, 2H), 4.89 (d, J = 15.0 Hz, 1H), 4.43 (dd, J = 12.0, 7.0 Hz, 2H), 4.19 (dd, J = 12.0, 7.0 Hz, 1H), 3.62 (m, 2H), 3.29 (dd, J = 15.0, 3.0 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.61 (m, 2H), 1.30 (m, 10H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 185.4 (CS), 170.9 (CO), 170.3 (CO), 169.6 (CO), 169.2 (CO), 68.8 (CH), 66.4 (CH), 61.4 (CH2), 57.8 (CH), 47.0 (CH), 46.9 (CH$_2$), 45.5 (CH$_2$), 31.7 (CH$_2$), 29.2 (CH$_2$), 29.1 (CH$_2$), 28.9 (CH$_2$), 26.9 (CH$_2$), 23.4 (CH$_3$), 22.6 (CH$_2$), 20.9 (CH$_3$), 20.7 (CH$_3$), 20.6 (CH$_3$), 14.0 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3359, 2917, 1747, 1661, 1529, 1368, 1229, 1053. HRMS (ES): Calcd. for C$_{23}$H$_{39}$N$_3$O$_7$S: 502.2581, found 502.2586.

4.2.14. (2R,3R,4S,5S)-5-Acetamido-2-(acetoxymethyl)-1-(phenylcarbamothioyl)piperidine-3,4-diyl diacetate (18c)

To a solution of 17 (120 mg, 0.28 mmol) in CH$_2$Cl$_2$ (5 mL) was added TFA (0.65 mL) and the solution was stirred at rt for 2 h. Solvent was removed *in vacuo*. The crude was dissolved in CH$_2$Cl$_2$ (5 mL) and TEA (186 μL, 1.36 mmol) and phenyl isothiocyanate (103 μL, 0.83 mmol) were added. The solution was stirred at reflux for 4 h. After removal of solvents, the crude was purified in silica gel using hexane/ethyl acetate increasing polarity ratio from 0 to 100% ethyl acetate to give 18c (92 mg, 72%) as a colorless oil.

[α]^{20}$_D$ = −41.4 (c = 0.6, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 8.26 (s, 1H), 7.40−7.28 (m, 4H), 7.20 (dt, J = 7.5, 1.5 Hz, 1H), 6.76 (d, J = 7.0 Hz, 1H), 5.62 (br, 1H), 5.29 (m, 2H), 4.90 (m, 1H), 4.45 (m, 2H), 4.29 (dd, J = 12.0, 6.0 Hz, 1H), 3.37 (dd, J = 15.0, 3.0 Hz, 1H), 2.15 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 185.9 (CS), 170.6 (CO), 170.3 (CO), 169.5 (CO), 169.1 (CO), 139.6 (C), 128.8 (CH), 125.8 (CH), 124.6 (CH), 69.0 (CH), 66.2 (CH), 61.5 (CH$_2$), 58.3 (CH), 47.3 (CH), 46.3 (CH$_2$), 23.4 (CH$_3$), 20.9 (CH$_3$), 20.7 (CH$_3$), 20.6 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3346, 1747, 1665, 1523, 1369, 1230, 1060. HRMS (ES): Calcd. for C$_{21}$H$_{28}$N$_3$O$_7$S: 466.1642, found 466.1648.

4.2.15. (2R,3R,4S,5S)-5-Acetamido-2-(acetoxymethyl)-1-(benzylcarbamothioyl)piperidine-3,4-diyl diacetate (18d)

To a solution of 17 (96 mg, 0.22 mmol) in CH$_2$Cl$_2$ (5 mL) was added TFA (0.5 mL) and the solution was stirred at rt for 1 h. Solvent was removed *in vacuo*. The crude was dissolved in CH$_2$Cl$_2$ (5 mL) and TEA (186 μL, 1.36 mmol) and benzyl isothiocyanate (90 μL, 0.67 mmol) were added. The solution was stirred at reflux for 5 h. After removal of solvents, the crude was purified in silica gel using hexane/ethyl acetate increasing polarity ratio from 0 to 100% ethyl acetate to give 18d (88 mg, 82%) as a colorless oil.

[α]^{20}$_D$ = −61.8 (c = 0.25, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$, δ/ppm): 7.39−7.28 (m, 5H), 6.63 (m, 2H), 5.46 (br, 1H), 5.24 (m, 2H),

4.98 (dd, J = 14.5, 5.5 Hz, 1H), 4.79 (dd, J = 14.5, 5.0 Hz, 2H), 4.42 (m, 2H), 4.19 (dd, J = 12.0, 6.0 Hz, 1H), 3.33 (dd, J = 15.0, 3.0 Hz, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.89 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$, δ/ppm): 185.8 (CS), 170.6 (CO), 170.3 (CO), 169.5 (CO), 169.2 (CO), 137.6 (C), 128.7 (CH), 127.9 (CH), 127.8 (CH), 68.9 (CH), 66.4 (CH), 61.4 (CH$_2$), 58.3 (CH), 50.4 (CH$_2$), 47.1 (CH), 45.5 (CH$_2$), 23.3 (CH$_3$), 20.9 (CH$_3$), 20.6 (CH$_3$), 20.6 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3308, 1748, 1668, 1532, 1370, 1226, 1059, 1027. HRMS (ES): Calcd. for C$_{22}$H$_{29}$N$_3$O$_7$SNa: 502.1618, found 502.1621.

4.2.16. N-((3S,4S,5R,6R)-1-(Butylcarbamothioyl)-4,5-dihydroxy-6-(hydroxymethyl)piperidin-3-yl)acetamide (19a)

Compound **18a** (47 mg, 0.11 mmol) was dissolved in a saturated solution of NH$_3$ in MeOH (3.5 mL) and stirred at rt for 20 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH$_2$Cl$_2$/methanol increasing polarity ratio from 0 to 15% MeOH to give **19a** (26 mg, 77%) as a white solid.

[α]$^{20}_D$ = +2.0 (c = 0.21, CH$_3$OH). Mp: 57–58 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 5.03 (d, J = 14.0 Hz, 1H), 4.13 (m, 1H), 3.99 (dt, J = 3.0, 1.0 Hz, 1H), 3.88 (dd, J = 5.0, 3.0 Hz, 1H), 3.74 (m, 2H), 3.64 (m, 1H), 3.55 (m, 1H), 3.20 (dd, J = 14.0, 3.0 Hz, 1H), 1.96 (s, 3H), 1.58 (m, 2H), 1.38 (m, 2H), 0.95 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 186.8 (CS), 173.5 (CO), 70.6 (CH), 67.2 (CH), 65.3 (CH), 60.7 (CH$_2$), 51.3 (CH), 47.5 (CH$_2$), 46.9 (CH$_2$), 32.3 (CH$_2$), 23.3 (CH$_3$), 21.1 (CH$_2$), 14.2 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3341, 2923, 1652, 1538, 1360, 1328, 1085, 1008. HRMS (ES): calcd. For C$_{13}$H$_{25}$N$_3$O$_4$S: 320.1639, found 320.1640. EA: Anal. Calcd. For C$_{13}$H$_{25}$N$_3$O$_4$S·H$_2$O: C, 46.27%; H, 8.07%; N, 12.45%; found C, 46.47%; H, 8.25%; N, 12.12%.

4.2.17. N-((3S,4S,5R,6R)-4,5-Dihydroxy-6-(hydroxymethyl)-1-(octylcarbamothioyl)piperidin-3-yl)acetamide (19b)

Compound **18b** (72 mg, 0.14 mmol) was dissolved in a saturated solution of NH$_3$ in MeOH (4 mL) and stirred at rt for 20 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH$_2$Cl$_2$/methanol increasing polarity ratio from 0 to 20% MeOH to give **19b** (46 mg, 85%) as a white solid.

[α]$^{20}_D$ = +3.3 (c = 0.25, CH$_3$OH). Mp: 84–86 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 5.03 (d, J = 14.5 Hz, 1H), 4.15 (m, 1H), 3.99 (td, J = 3.0, 1.0 Hz, 1H), 3.88 (dd, J = 4.5, 3.0 Hz, 1H), 3.74 (m, 2H), 3.62 (m, 1H), 3.52 (m, 1H), 3.20 (dd, J = 14.5, 2.5 Hz, 1H), 1.60 (m, 2H), 1.33 (m, 10H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 186.7 (CS), 173.4 (CO), 70.6 (CH), 67.2 (CH), 65.3 (CH), 60.7 (CH$_2$), 51.3 (CH), 47.5 (CH$_2$), 47.2 (CH$_2$), 33.0 (CH$_2$), 30.5 (CH$_2$), 30.4 (CH$_2$), 30.1 (CH$_2$), 28.0 (CH$_2$), 23.7 (CH$_2$), 23.4 (CH$_3$), 14.4 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3346, 2924, 1657, 1636, 1535, 1367, 1085. HRMS (ES): Calcd. For C$_{17}$H$_{33}$N$_3$O$_4$S: 376.2265, found 376.2264. EA: Anal. Calcd. For C$_{17}$H$_{33}$N$_3$O$_4$S: C, 54.37%; H, 8.86%; N, 11.19%; found C, 54.05%; H, 8.88%; N, 11.23%.

4.2.18. N-((3S,4S,5R,6R)-1-(Benzylcarbamothioyl)-4,5-dihydroxy-6-(hydroxymethyl)piperidin-3-yl)acetamide (19d)

Compound **18d** (88 mg, 0.18 mmol) was dissolved in a saturated solution of NH$_3$ in MeOH (3.5 mL) and stirred at rt for 20 h. Solvent was removed *in vacuo* to give **19d** (92 mg, 92%) as a white solid without extra purification.

[α]$^{20}_D$ = +11.6 (c = 0.22, CH$_3$OH). Mp: 164–165 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 7.42–7.15 (m, 5H), 5.09 (d, J = 14.0 Hz, 1H), 4.93 (d, J = 15.0 Hz, 2H), 4.79 (d, J = 15.0 Hz, 1H), 4.15 (m, 1H), 4.01 (dt, J = 3.0, 2.0 Hz, 1H), 3.90 (dd, J = 5.0, 3.0 Hz, 1H), 3.75 (m, 2H), 3.25 (dd, J = 14.0, 2.0 Hz, 1H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 187.3 (CS), 173.5 (CO), 140.3 (C), 129.4 (CH), 128.4 (CH), 128.0 (CH), 70.6 (CH), 67.2 (CH), 65.4 (CH), 60.6 (CH$_2$), 51.4 (CH), 50.5 (CH$_2$), 47.7 (CH$_2$), 23.3 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3333, 1661, 1636, 1553, 1531, 1373, 1085, 1002. HRMS (ES): calcd. For

C$_{16}$H$_{24}$N$_3$O$_4$S: 354.1482, found 354.1481. EA: Anal. Calcd. For C$_{16}$H$_{23}$N$_3$O$_4$S·^{2}/$_3$H$_2$O: C, 52.59%; H, 6.71%; N, 11.50%; found C, 52.56%; H, 7.06%; N, 11.27%.

4.2.19. N-((6S,7S,8R,8aS,Z)-3-(Butylimino)-7,8-dihydroxyhexahydro-3H-thiazolo[3,4-a]pyridin-6-yl)acetamide (20a)

Compound **18a** (40 mg, 0.10 mmol) was dissolved in MeOH:H$_2$O 1:1 mixture (4 mL) and stirred at 70 °C for 24 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH$_2$Cl$_2$/methanol/NH$_4$OH increasing polarity ratio from 99:1:1 to 80:18:2 to give **20a** (16 mg, 59%) as a white solid.

[α]$^{20}_D$ = +16.4 (c = 0.11, CH$_3$OH). Mp: 188–190 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 3.95 (m, 2H), 3.76 (m, 1H), 3.61 (m, 1H), 3.45 (dd, J = 10.0, 2.5 Hz, 1H), 3.38 (dd, J = 10.5, 6.5 Hz, 1H), 3.15 (m, 2H), 3.06 (dd, J = 10.5, 7.5 Hz, 1H), 2.89 (m, 1H), 1.98 (s, 3H), 1.52 (m, 2H), 1.36 (m, 2H), 0.93 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 172.9 (CO), 162.7 (CN), 72.6 (CH), 71.5 (CH), 61.3 (CH), 55.6 (CH$_2$), 49.0 (CH), 43.1 (CH$_2$), 34.3 (CH$_2$), 31.2 (CH$_2$), 22.6 (CH$_3$), 21.5 (CH$_2$), 14.3 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3319, 2925, 1621, 1547, 1373, 1056. HRMS (ES): Calcd. for C$_{13}$H$_{24}$N$_3$O$_3$S: 302.1533, found 302.1532.

4.2.20. N-((6S,7S,8R,8aS,Z)-3-(Butylimino)-7,8-dihydroxyhexahydro-3H-thiazolo[3,4-a]pyridin-6-yl)acetamide (20b)

Compound **18b** (54 mg, 0.11 mmol) was dissolved in MeOH:H$_2$O 1:1 mixture (4 mL) and stirred at 70 °C for 24 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH$_2$Cl$_2$/methanol/NH$_4$OH increasing polarity ratio from 99:1:1 to 80:18:2 to give **20b** (27 mg, 70%) as a white solid.

[α]$^{20}_D$ = +11.5 (c = 0.13, CH$_3$OH). Mp: 180–181 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 3.95 (m, 2H), 3.76 (m, 1H), 3.62 (m, 1H), 3.46 (dd, J = 9.5, 2.5 Hz, 1H), 3.39 (dd, J = 10.5, 6.5 Hz, 1H), 3.15 (m, 2H), 3.07 (dd, J = 11.0, 7.5 Hz, 1H), 2.90 (dd, J = 12.0, 11.0 Hz, 1H), 1.98 (s, 3H), 1.55 (m, 2H), 1.30 (m, 10H), 0.90 (m, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 172.9 (CO), 162.9 (CN), 72.6 (CH), 71.5 (CH), 61.4 (CH), 55.7 (CH$_2$), 49.0 (CH), 43.1 (CH$_2$), 33.0 (CH$_2$), 32.0 (CH$_2$), 31.2 (CH$_2$), 30.5 (CH$_2$), 30.4 (CH$_2$), 28.3 (CH$_2$), 23.7 (CH$_2$), 22.6 (CH$_3$), 14.5 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3315, 2923, 1621, 1546, 1443, 1303, 1226, 1057. HRMS (ES): calcd. For C$_{17}$H$_{32}$N$_3$O$_3$S: 358.2159, found 358.2158. EA: Anal. Calcd. For C$_{17}$H$_{31}$N$_3$O$_3$S·H$_2$O: C, 54.37%; H, 8.86%; N, 11.19%; found C, 54.76%; H, 9.23%; N, 8.18%.

4.2.21. N-((6S,7S,8R,8aS,Z)-7,8-Dihydroxy-3-(phenylimino)hexahydro-3H-thiazolo[3,4-a]pyridin-6-yl)acetamide (20c)

Compound **18c** (59 mg, 0.13 mmol) was dissolved in MeOH:H$_2$O 1:1 mixture (5 mL) and stirred at reflux for 24 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH$_2$Cl$_2$/methanol/NH$_4$OH increasing polarity ratio from 99:1:1 to 80:18:2 to give **20c** (22 mg, 54%) as a white solid.

[α]$^{20}_D$ = −11.7 (c = 0.16, CH$_3$OH). Mp: 229–231 °C. ^{1}H NMR (400 MHz, CD$_3$OD, δ/ppm): 7.24 (m, 2H), 7.03 (m, 1H), 6.88 (m, 2H), 4.05 (m, 2H), 3.96 (dd, J = 12.0, 5.0 Hz, 1H), 3.77 (m, 1H), 3.53 (dd, J = 10.0, 2.5 Hz, 1H), 3.36 (dd, J = 10.0, 6.5 Hz, 1H), 3.05 (m, 2H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, CD$_3$OD, δ/ppm): 172.9 (CO), 163.0 (CN), 152.8 (C), 129.8 (CH), 124.5 (CH), 123.3 (CH), 72.7 (CH), 71.5 (CH), 61.4 (CH), 49.0 (CH), 43.0 (CH$_2$), 31.3 (CH$_2$), 22.6 (CH$_3$). IR (film, ν_{max}/cm^{-1}): 3304, 1616, 1586, 1375, 1250, 1137, 762. HRMS (ES): Calcd. For C$_{15}$H$_{20}$N$_3$O$_3$S: 322.12199, found 322.12175.

4.2.22. N-((6S,7S,8R,8aS,Z)-3-(Benzylimino)-7,8-dihydroxyhexahydro-3H-thiazolo[3,4-a]pyridin-6-yl)acetamide (20d)

Compound **18d** (56 mg, 0.12 mmol) was dissolved in MeOH:H$_2$O

1:1 mixture (5 mL) and stirred at reflux for 24 h. Solvent was removed *in vacuo*. The crude was purified by chromatography in silica gel using CH_2Cl_2/methanol/NH_4OH increasing polarity ratio from 99:1:1 to 90:9:1 to give **20d** (26 mg, 66%) as a white solid.

$[\alpha]^{20}_D = +6.8$ (c = 0.2, CH_3OH). Mp: 190–192 °C. ^1H NMR (400 MHz, CD_3OD, δ/ppm): 7.28 (m, 4H), 7.20 (m, 1H), 4.41 (d, $J = 15.0$ Hz, 1H), 4.36 (d, $J = 15.0$ Hz, 1H), 3.99 (m, 2H), 3.87 (m, 1H), 3.72 (m, 1H), 3.49 (dd, $J = 9.5, 2.5$ Hz, 1H), 3.44 (dd, $J = 11.0, 6.5$ Hz, 1H), 3.14 (dd, $J = 11.0, 7.5$ Hz, 1H), 2.98 (m, 1H), 1.97 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD, δ/ppm): 172.9 (CO), 164.4 (CN), 141.3 (C), 129.3 (CH), 128.5 (CH), 127.8 (CH), 72.5 (CH), 71.5 (CH), 61.7 (CH), 58.7 (CH_2), 49.0 (CH), 43.1 (CH2), 31.4 (CH_2), 22.6 (CH_3). IR (film, ν_{max}/cm^{-1}): 3327, 1612, 1540, 1450, 1380, 1213, 1046, 752. HRMS (ES): Calcd. For $C_{16}H_{22}N_3O_3S$: 336.13764, found 336.13739. EA: Anal. Calcd. For $C_{16}H_{21}N_3O_3S$: C, 57.29%; H, 6.31%; N, 12.53%; found C, 57.73%; H, 6.38%; N, 12.21%.

4.3. General procedures for inhibition and chaperone assays

4.3.1. Kinetic studies with commercial glycosidases

The glycosidases α-glucosidase (from yeast), amyloglucosidase (from *A. niger*), isomaltase (from yeast), β-glucosidases (from almond and bovine liver), naringinase (*Penicillium decumbes*), α-galactosidase (from green coffee beans), β-galactosidase (from *E. coli*), α-mannosidase (from jack bean), β-mannosidase (from *H. pomatia*), β-*N*-acetylglucosaminidases (from human placenta, bovine kidney and jack bean) used in the inhibition studies, as well as the corresponding *o*- or *p*-nitrophenyl glycoside substrates, were purchased from Sigma Chemical Co. Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-galactosidases) or *p*-nitrophenyl α- or β-D-glycopyranoside (for α-glucosidases, β-glucosidases, α-galactosidases, α-mannosidases and β-mannosidases) or *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide/galactosaminide (for hexosaminidases), in the presence of DAJNAc. Each assay was performed in phosphate-citrate (for α or β-mannosidase, amyloglucosidase or β-*N*-acetylglucosaminidase at pH 5.5 or 3.5) or in phosphate buffer (at pH 7.3 or 6.8 for the other glycosidases) at the optimal pH for each enzyme. The K_m values for the different glycosidases used in the tests and the corresponding working pHs are listed herein: α-glucosidase (yeast), $K_m = 0.35$ mM (pH 6.8); amyloglucosidase (*A. niger*), $K_m = 3.0$ mM (pH 5.5); isomaltase (from yeast), $K_m = 1.0$ mM (pH 6.8); β-glucosidase (almond), $Km = 3.5$ mM (pH 7.3); β-glucosidase (bovine liver), $K_m = 1.0$ mM (pH 7.3); naringinase (*P. decumbes*), $K_m = 2.7$ mM (pH 6.8); α-galactosidase (coffee beans), $K_m = 2.0$ mM (pH 6.8); β-galactosidase (from *E. coli*), $K_m = 0.12$ mM (pH 7.3); α-mannosidase (jack bean), $K_m = 2.0$ mM (pH 5.5); β-mannosidase (*H. pomatia*), $K_m = 0.6$ mM (pH 5.5); β-*N*-acetylglucosaminidase (from human placenta), $K_m = 0.34$ mM (pH 5.5); β-*N*-acetylglucosaminidase (from bovine kidney), $K_m = 0.48$ mM (pH 5.5); β-*N*-acetylglucosaminidase (from jack bean), $K_m = 0.49$ mM (pH 5.5). The latter enzyme was also assayed for its β-*N*-acetylgalactosaminidase activity; $K_m = 0.31$ mM (pH 3.5). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10–30 min at 37 °C (or 55 °C for amyloglucosidase), the reaction was quenched by addition of 1 M Na_2CO_3. The absorbance of the resulting mixture was determined at 405 nm. The K_i value and enzyme inhibition mode were determined from the slope of Lineweaver–Burk plots and double reciprocal analysis using a Microsoft Office Excel 2007 program. Inhibition mode of DAJNAc was competitive against human placenta β-*N*-acetylglucosaminidase and non-competitive against bovine kidney and jack bean β-*N*-acetylglucosaminidase.

4.3.2. Hexosaminidase assay and measurement of inhibition activities in vitro

The fluorogenic 4-methyumbellyferone-conjugated substrates, 4-MU-(2-acetamido-2-deoxy)-β-D-glucopyranoside for total hexosaminidase and 4-MU-N-acetyl-β-D-glucosaminide-6-sulfate sodium salt for hexosaminidase A, were purchased from Sigma Japan (Tokyo, Japan) and Slater & Frith Ltd (Norwich, UK) respectively. For measurement of enzyme activities, cell lysates in 0.1% Triton X-100 in distilled water were mixed with 4-MU substrate solution and was incubated at 37 °C. The reaction terminated with 0.2 M glycin-NaOH (pH 10.7) and the librated 4-MU was measured with a fluorescence plate reader (ex. 340 nm and em. 460 nm; Infinite F500, TECAN Japan, Kawasaki, Japan). For measurement of inhibition activities, cell lysates from cultured human normal skin fibroblasts were mixed with 4-MU substrates and compounds and incubate at 37 °C. The activities were measured as described above.

4.3.3. Cell culture and chaperone treatment

Human skin fibroblasts from unaffected subject were cultured as described [58,78]. Human fibroblasts from Tay-Sachs disease patient with p.G269S/c.1278insTACT *HEXA* mutations were obtained from Coriell Cell Repositories (Camden, NJ). For measurement of chaperone effects, cells were cultured in the medium with or without compounds for 4 days and then the cell lysates were subjected for the lysosomal enzyme assay as described above. The enzyme activities were normalized with protein concentrations measured by protein assay rapid kit (Wako, Tokyo, Japan).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.10.038.

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