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Synthesis and glycosidase inhibitory activity of isourea-type bicyclic sp^2-iminosugars related to galactonojirimycin and allonojirimycin

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ABSTRACT

A series of sp^2-iminosugars featuring a fused piperidine–isourea bicyclic core and hydroxylation profiles of stereochemical complementarity with the 'classical' iminosugars galactonojirimycin and allonojirimycin have been prepared and their inhibitory activity evaluated against a panel of commercial glycosidases. The synthetic methodology involves 2-aminooxazoline pseudo-C-nucleosides, accessible from *vic*-hydroxycarbodiimide precursors, as key intermediates and is compatible with molecular diversity-oriented strategies. Alkyl, aryl and glycosyl substituents have been incorporated in order to assess the potential of non-glyconic interactions to modulate the enzyme selectivity. All the galactonojirimycin derivatives behaved as potent competitive inhibitors of β-glucosidases. The inhibition potency was higher for aliphatic substituents (in the nM range), but the highest selectivity within β-glucosidase isoenzymes was achieved for a *N*′-glucopyranosyl pseudodisaccharide analogue. Going from D-*galacto* to D-*allo* configuration further increased enzyme selectivity, but strongly penalized the inhibition potency.

1. Introduction

sp^2-Iminosugars are carbohydrate mimics[1] characterized by the presence of a pseudoamide-type (e.g., (thio)urea, guanidine, (thio) carbamate, or iso(thio)urea) nitrogen atom at the position equivalent to that of the endocyclic oxygen in monosaccharides.[2] In these compounds the anomeric effect is exacerbated, which confers chemical and conformational stability to reducing derivatives as well as to the corresponding *O*, *S*- and *N*-pseudoglycosides.[3] This is remarkably different to the situation encountered in classical iminosugars, such as nojirimycin (NJ, **1**) or galactonojirimycin (GNJ, **2**) due to the lability of acetal functions involving nitrogen (Fig. 1).[4] sp^2-Iminosugars related to the piperidine,[5] indolizidine,[6] pyrrolidine,[7] pirrolizidine[7] and nor-tropane[8] (calystegine) polyhydroxylated alkaloids have already been reported. Notably, several representatives exhibited potent glycosidase inhibitory activity and higher enzyme selectivities as compared with the parent iminosugars. Given the multitude of roles of glycosidases in biological and pathological processes, selective inhibitors of these enzymes bear strong potential as chemotherapeutic agents, e.g., in the treatment of type II diabetes mellitus,[9] viral infections,[10] cancer[11] or lysosomal storage diseases.[12]

Among sp^2-iminosugars, bicyclic isourea derivatives have proven particularly interesting.[13] This structural feature is present in the natural α,α′-trehalose mimic trehazoline (**3**), a nanomolar inhibitor of trehalase.[14] Trehazoline binding to the active site of trehalase benefits from simultaneous glyconic and non-glyconic interactions, which has been further exploited in the design of potent analogues.[15] By closing an isourea-type ring between *N*-5 and *O*-6 in nojirimycin, the incorporation of a battery of substituents become feasible, which likewise provide an opportunity for non-glyconic contributions to enzyme binding.[13,16] As a proof of concept, the bicyclic (*N*-5,*O*-6)-*N*′-(*n*-octylimino)-NJ derivative **4** was shown to be a very potent inhibitor of β-glucosidases, including human β-glucocerebrosidase (GCerase), that exhibited total anomeric selectivity.[17] X-ray data demonstrated that the octyl chain of **4** and related sp^2-iminosugar NJ derivatives sits in a hydrophobic pocket at the vicinity of the active site in β-glucosidase while it leads to steric clashes in the case of α-glucosidase, playing thus a crucial role in both the free energy of binding and the enzyme selectivity.[18] The ability of **4** to increase the lysosomal concentration of a range of GCerase mutants though a rescuing mechanism[19] and its facility to cross cell membranes makes it a good candidate as pharmacological chaperone[20] for the treatment of Gaucher disease.[17,21]

The synthesis developed to build the fused piperidine–isourea skeleton (**I**) relies in the spontaneous rearrangement of 2-aminooxazoline pseudo-C-nucleosides (**IIb**) through the transient

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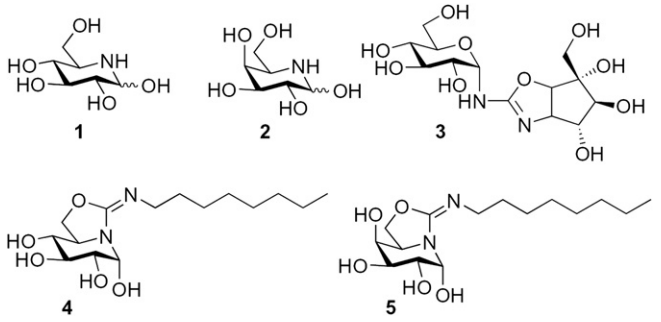


Fig. 1. Structures of NJ (**1**), GNJ (**2**), trehazoline (**3**), and the bicyclic isourea-type sp^2-iminosugars **4** (a NJ analogue) and **5** (a GNJ analogue).

open-chain aldehydo form (**IIa**), and was purposely conceived to allow introducing molecular diversity at a relatively low synthetic cost (Fig. 2). In principle, both the configurational pattern and the nature of the *N*′-substituent can be systematically modified. Preliminary results indicated that the GNJ bicyclic isourea analogue **5** adopted a similar orientation as **4** in the active site of β-glucosidase, being a 50–100-fold more potent inhibitor.[18a]

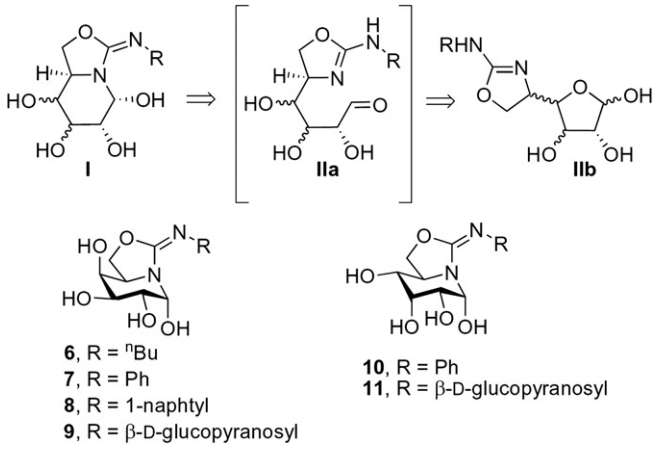


Fig. 2. Retrosynthetic analysis and structures of the new isourea-type bicyclic sp^2-iminosugars prepared and evaluated in this work.

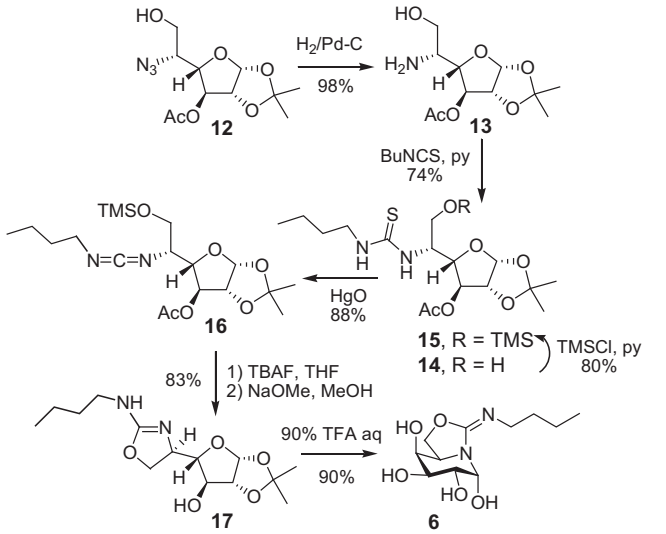
While the above commented kinetic and crystallographic data demonstrate the utmost importance of non-glyconic interactions involving the *N*-substituent in the β-glucosidase inhibitory potency and selectivity of bicyclic sp^2-iminosugars, there is not much information available about the optimal requirements that this substituent must fulfil for optimal binding. Thermodynamic studies by isothermal titration calorimetry (ITC) point to a favourable entropic contribution in the case of the octyl chain in **4** and **5**. On the other hand, X-ray structures point to some unfulfilled potential for aromatic and polar interactions with amino acid residues at the vicinity of the active site. It is also unclear whether or not the observed configurational promiscuity at the position equivalent to C-4 in monosaccharides can also occur at other positions in the six-membered piperidine ring.

A second important conclusion from our pervious work in this field is that evaluation of the sp^2-iminosugar inhibitor towards commercial β-glucosidases from bovine liver and almonds, belonging to the same clan A as human GCrase in the CAZy classification,[22] provides a reliable feed-back for pre-selection of suitable candidates for most costly tests on the non-commercial human lysosomal enzymes as well as in vitro evaluation as pharmacological chaperones. In the light of these considerations, the preparation of a wider series of derivatives differing in the nature of the exocyclic substituent or in the configurational pattern and the study of the influence of such structural modifications in the inhibitory activity was very appealing. Here we report the synthesis of the new isourea-type bicyclic sp^2-iminosugars **6**–**9**, related to GNJ, and the D-allo configured diastereomers **10** and **11** and their evaluation against a panel of representatives commercial glycosidases.

2. Results and discussion

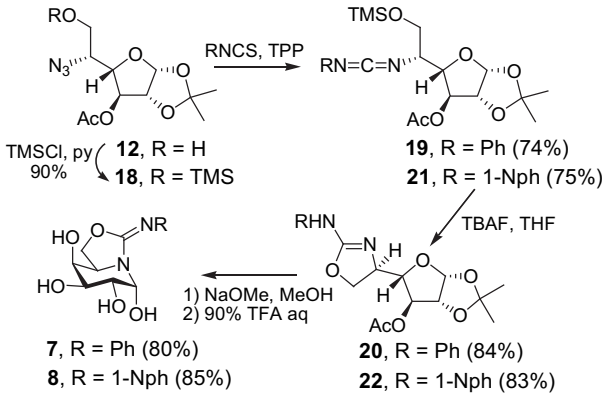
2.1. Synthesis

Structures **6**–**9** were selected to check the influence of the nature (aromatic, aliphatic, polar) of the exocyclic substituent in the glycosidase inhibitory activity. The synthesis of the requested aminooxazoline precursors started from the pivotal *vic*-azidoalcohol **12**,[18a] derived from 1,2-*O*-isopropylidene-α-D-galactofuranose, and involved its transformation into 2-hydroxycarbodiimide intermediates, which were expected to undergo spontaneous intramolecular ring closing by nucleophilic addition of the primary hydroxyl to the heterocummulene group. Two different strategies were considered for the key azide→carbodiimide transformation,[23] namely (i) reduction to the corresponding aminoalcohol (**13**) and conversion into a thiourea derivative that can be further desulfurated, or (ii) tandem Staudiger–aza-wittig-type reaction[24] with triphenylphosphine and an isothiocyanate. The later reaction has been shown to proceed through a transient phosphazide, rather than an iminiphosphorane, when both reagents are added simultaneously to the reaction mixture containing the azide substrate, affording directly the target carbodiimide.[25] Preliminary experiments evidenced that the efficiency of each route was dependent on the nature of the isothiocyanate reagent, the first one being preferably for the less reactive aliphatic isothiocyanates and the second one in the case of aromatic derivatives. The respective reaction sequences leading to compounds **6**–**8** are depicted in Schemes 1 and 2.



Scheme 1. Synthesis of the isourea-type bicyclic sp^2-iminosugar **6**.

Coupling of **13** and *n*-butyl isothiocyanate afforded the thiourea adduct **14** with total chemoselectivity in 74% yield. Protection of the hydroxyl group as the corresponding trimethylsilyl ether (→**15**) and subsequent desulfuration with mercury(II) oxide led to carbodiimide **16**. Sequential fluorolysis of the silyl ether function and deacetylation gave the 2-butylaminooxazoline derivative **17** that, after trifluoroacetic acid-catalyzed hydrolysis of the acetal protecting group and neutralization of the reaction mixture,

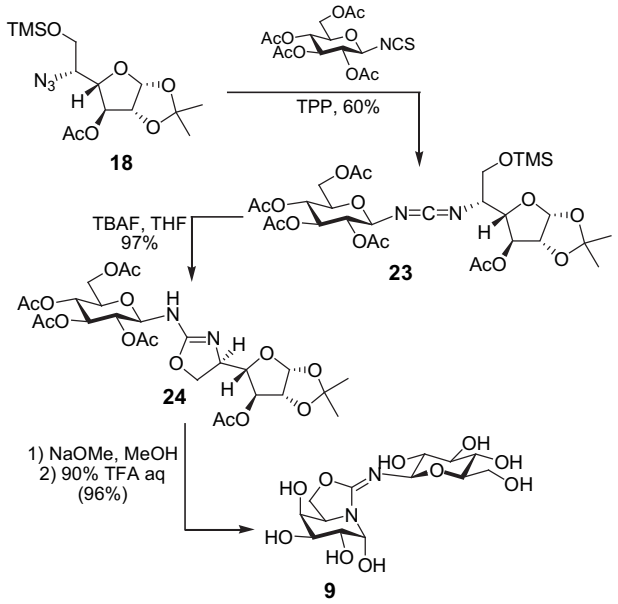


Scheme 2. Synthesis of the isourea-type bicyclic sp^2-iminosugars **7** and **8**.

underwent spontaneous furanose→piperidine rearrangement to yield the target bicyclic sp^2-iminosugar **6** (Scheme 1).

For the synthesis of the 5-*N*,6-*O*-(*N*′-phenyl and *N*′-1-naphtyliminomethylidene)GNJ derivatives **7** and **8**, compound **12** was first transformed into the 6-*O*-trimethylsilyl derivative **18**. Reaction of **18** with triphenylphosphine in the presence of phenyl or 1-naphtyl isothiocyanate afforded the carbodiimide adducts **19** and **21** in 74 and 75% yield, respectively. Further removal of the silyl ether group (→**20** and **22**) and final standard deprotection of the alcohol functions proceeded as previously to give the fully unprotected glycomimetics **7** and **8** (Scheme 2).

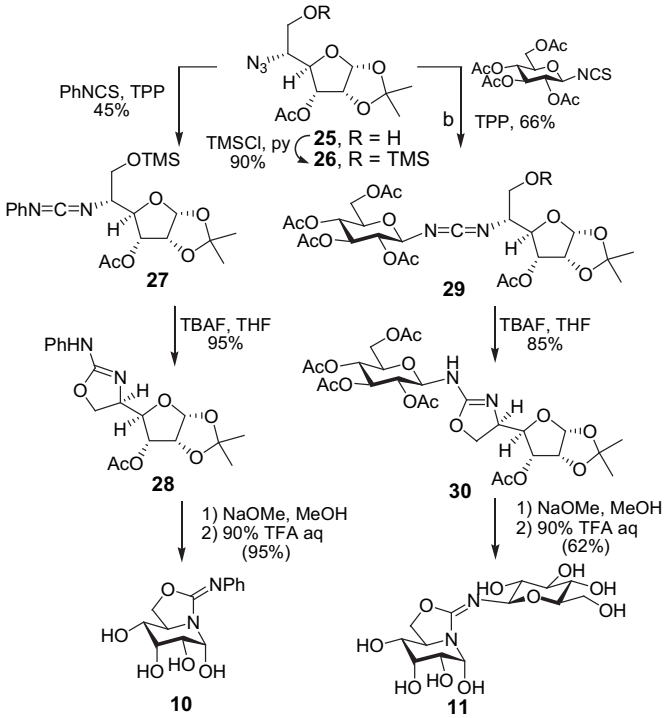
The synthesis of the pseudodisaccharide derivative **9** was motivated by the previous observation that glycosyl substituents can modulate the inhibitory activity of sp^2-iminosugar glycomimetics towards different isoenzymes, imparting selectivity.[26] Similarly to aromatic isothiocyanates, glycopyranosyl isothiocyanates are activated towards nucleophilic addition due to the global electron withdrawing effect of the glycosyl substituent.[27] Direct transformation of azide **18** into the β-(1→5) carbodiimide-linked *gluco*–*galacto* pseudodisaccharide derivative **23** was thus performed in 60% yield by Staudinger–aza-Wittig-type reaction with triphenylphosphine and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate.[27] Oxazoline ring closing (→**24**) and final rearrangement to the bicyclic sp^2-iminosugar **9** parallelled the conditions and yields of the previous syntheses (Scheme 3).



Scheme 3. Synthesis of the isourea-type bicyclic sp^2-iminosugar **9**.

It is known that β-glucosidases are often not very sensitive to the configuration at position C-4 in glycopyranosides or the equivalent position in iminosugar glycomimetics. For instance, the enzyme from bovine liver can hydrolyse β-glucopyranosides and β-galactopyranosides at essentially the same rate.[28] The existence of additional non-glyconic interactions can alter the relative binding affinity of epimers, as seen by the 50-fold lower K_i value observed for **5** as compared to **4** for this enzyme.[18a] We were also interested to investigate whether or not non-glyconic interactions could drive the binding of sp^2-iminosugars that do not match the normally accepted hydroxylation patterns of the enzyme. The presence of a hydroxyl group at C-2 in the right configuration is known to be essential for strong binding,[29] but the possibility for the enzyme accepting epimers at C-3 cannot be discarded. The D-*allo* configured derivatives **10** and **11**, with phenyl and β-D-glucopyranosyl exocyclic substituents, were prepared for this purpose. To the best of our knowledge, no activity data have been reported for allonojirimycin,[30] the C-3 epimer of compound **1**, although the corresponding 1-deoxy and 1-homo derivative have been shown to keep some residual activity towards several glucosidases and galactosidases.[31] Comparison of the inhibition data for **10** and **11** with those for the D-*galacto* analogues **7** and **9**, bearing identical *N*-substituents, should allow estimating the effect of the configurational change in the inhibitory properties. The results (see Section 2.2 hereinafter) did not recommend the synthesis of a wider series of derivatives in this case.

The synthesis of **10** and **11** was accomplished in four steps from the previously reported 3-*O*-acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-allofuranose **25**,[6c] after protection of the primary hydroxyl group as the trimethylsilyl ether **26**. Condensation with phenyl or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate provided the corresponding carbodiimides **27** and **29**, respectively. Further transformation into the oxazoline derivatives **28** and **30** and the desired bicyclic isoureas proceeded smoothly following a parallel reaction sequence to that above discussed for GNJ derivatives (Scheme 4).



Scheme 4. Synthesis of the isourea-type bicyclic sp^2-iminosugars **10** and **11**.

The ^{1}H and ^{13}C NMR spectra of the new sp^2-iminosugars confirmed their bicyclic character. Thus, the high field resonance of the pseudoanomeric carbon (C-1) (78.3–76.2 ppm) was in agreement with the presence of the hemiaminal functionality. The vicinal $^3J_{H,H}$ values about the six-membered ring agreed with the D-*galacto* (**6**–**9**) and D-allo (**10**, **11**) configurational patterns in the $^4C_1$ chair conformation. In all cases, the α-anomer with the pseudoanomeric hydroxyl group in the axial orientation fitting the anomeric effect, was by far the major species in D_2O solution (>95%). Minor proportions of the β-anomer were detected in some cases in the ^{1}H NMR spectra (see Supplementary data). Purity was further confirmed by combustion analysis.

2.2. Glycosidase inhibitory activity

The inhibitory activity of compounds **6**–**11** was evaluated against a series of glycosidases including α-glucosidase (yeast), β-glucosidase (almonds), β-glucosidase/β-galactosidase (bovine liver, cytosolic), α-mannosidase (Jack bean), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), α-galactosidase (green coffee beans and *A. niger*) and isomaltase (yeast). The corresponding inhibition constants (K_i) are collected in Table 1. Data for the previously synthesized derivatives **4** and **5** have been also included for comparative purposes.

Table 1
K_i values (μM) for **4**–**11** against a panel of glycosidases[a]

Enzyme	4	5	6	7	8	9	10	11
α-Glucosidase (baker's yeast)	168	n.i.[b]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
β-Glucosidase (almond)	1.9	0.019	0.073	5	1.5	33	346	n.i.
α-Galactosidase (green coffee bean)	n.i.	n.i.	288	249	383	308	n.i.	n.i
β-Gluco/β-galactosidase (bovine liver)	2.7	0.052	0.2	13	5.2	956	n.i.	933
Trehalase (pig kigney)	182	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	320
Amyloglucosidase (*A. niger*)	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	609

[a] Inhibition was competitive in all cases. No inhibition was observed for any compound at 2 mM concentration on Jack bean α-mannosidase, *A. niger* α-galactosidase and baker's yeast isomaltase.
[b] n.i., no inhibition observed at 2 mM concentration of the inhibitor.

The bicyclic GNJ derivatives **6**–**9** strongly inhibited β-glucosidase, did no inhibit α-glucosidase at concentrations up to 2 mM, in agreement that previously observed for compound **5**, and showed very weak inhibitory activity towards α-galactosidase (K_i in the range 249–383 μM). The influence of the nature of the *N*′-substituent in enzyme selectivity was evident when comparing the β-glucosidases from almonds and bovine liver (cytosolic). Aliphatic substituents (**5** and **6**) afforded the most potent inhibitors of these enzymes, followed by the aromatic derivatives (**7** and **8**). In all cases the selectivity ratio between these two isoenzymes was in the range 2.6–3.5. In sharp contrast, the pseudodisaccharide derivative **9** was a very selective inhibitor of the almonds enzyme (selectivity ration ca. 30), which is remarkable considering that both β-glucosidases belong to the same glycosyl hydrolase GH1 family in the CaZy classification.[22]

Changing the hydroxylation configurational profile from D-*galacto* to D-allo had a dramatic effect in the enzyme binding abilities. Thus, the *N*′-phenyl derivative **10** was a totally selective but very weak inhibitor of almonds β-glucosidase (K_i 346 μM, that is, ca. 70-fold weaker as compared with the galactonojirimycin analogue **7**). The pseudosaccharide derivative **11** completely lost the activity against this enzyme. Although it has been previously suggested that there might be, in the active site of β-glucosidase, an extra hydrogen-bond acceptor positioned in the vicinity of the 3-position somewhere beneath the sugar ring,[32] this interaction does not seem to be efficient enough in the case of the bicyclic sp^2-iminosugars considered in this work.

3. Conclusions

The synthesis of 2-aminooxazoline pseudo-C-nucleosides via transient *vic*-hydroxycarbodiimides and their rearrangement into fused piperidine–isourea bicyclic systems has been implemented to access a small collection of six sp^2-iminosugar glycomimetics. The efficiency of the reaction sequence and the possibility of using different monosaccharides as starting materials make the approach very well-suited for structure–activity relationship studies where both the configurational pattern and the nature of pseudoaglyconic substituents can be systematically profiled. Both features contribute decisively to glycosidase inhibition potency and selectivity. Although in this particular case the new derivatives synthesized did not improve the inhibition results against β-glucosidases previously attained with the lead compound **5**, discouraging further biological tests, the body of results here collected illustrates how the configurational, anomeric and isoenzyme selectivities are highly sensitive to structural modifications. The possibility to optimize the inhibition properties by acting on the nature of non-glyconic substituents in combination with the systematic evaluation of the products against a panel of commercial glycosidases with structural resemblance to human enzymes is particularly interesting in terms of time-saving and pre-selection of candidates for specific targets. Application of this strategy to the control of therapeutically relevant enzymes is currently pursued in our laboratories.

4. Experimental section

4.1. General methods

Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured with a JASCO P-2000 polarimeter, using a sodium lamp (λ=589 nm) at 22 °C in 1 cm or 1 dm tubes. IR spectra were recorded on a JASCO FTIR-410 instrument. UV spectra were recorded on Philips PU-8710 instrument, unit for ε values: mM^{-1} cm^{-1}. NMR experiments were performed at 300 (75.5), and 500 (125.7) MHz using Bruker DMX300, and DRX500. 1-D TOCSY as well as 2-D COSY and HMQC experiments were carried out to assist in signal assignment. In the FABMS spectra, the primary beam consisted of Xe atoms with maximum energy of 8 keV. The samples were dissolved in *m*-nitrobenzyl alcohol or thioglycerol as the matrices and the positive ions were separated and accelerated over a potential of 7 keV. NaI was added as cationizing agent. Thin-layer chromatography was performed on E. Merck precoated TLC plates, silica gel 30 F_{245}, with visualization by UV light and by charring with 10% H_2SO_4 or 0.2% w/v cerium (IV) sulfate–5% ammonium molybdate in 2 M H_2SO_4 or 0.1% ninhydrin in EtOH. Column chromatography was performed on Chromagel (SDS silica 60 AC.C 70–200 μm). Elemental analyses were performed at the Servicio de Microanálisis del Instituto de Investigaciones Químicas de Sevilla, Spain. 3-*O*-Acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose (**12**) was prepared from D-galactose using the reported route.[6c] 3-*O*-Acetyl-5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose (**13**) was synthesized by catalytic hydrogenation of azide **12** following the procedure already reported.[18a] 3-*O*-Acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-allofuranose **25** was synthesized from 3-*O*-acetyl-1,2-*O*-isopropylidene-6-*O*-trityl-α-D-allofuranose using the previously described route.[6c]

4.2. General procedure for the inhibition assays

Inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of the

glycosidases against the respective *o*- (for β-gluco/β-galactosidase from bovine liver) or *p*-nitrophenyl α- or β-D-glycopyranoside (for other glycosidases) or α,α′-trehalose (for trehalase) in the presence of compounds **4**–**11**. Each assay was performed in phosphate buffer or phosphate–citrate buffer (for α- or β-mannosidase and amyloglucosidase) at the optimal pH for the enzymes. The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. The mixture was incubated for 10–30 min at 37 °C or 55 °C (for amyloglucosidase) and the reaction was quenched by addition of 1 M Na_2CO_3. For trehalase the reaction was stopped by placing the mixture over boiling water for 3 min, cooled in ice/water and centrifuged at 12,000 rpm for 5 min for remove the denatured protein. The concentration of D-glucose in the supernatant was determined by the glucose oxidase–peroxidase method (Glucose Trinder 100, from Sigma). Reaction times were appropriate to obtain 10–20% conversion of the substrate in order to achieve linear rates. The absorbance of the resulting mixture was determined at 405 nm or 505 nm (for trehalase). Approximate values of K_i were determined using a fixed concentration of substrate (around the K_M value for the different glycosidases) and various concentrations of inhibitor. Full K_i determinations and enzyme inhibition mode were determined from the slope of Lineweaver–Burk plots and double reciprocal analysis. Representative examples of the Lineweaver–Burk plots, with typical profile for competitive inhibition mode, are shown in the Supplementary data.

4.3. Synthesis of 3-*O*-acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-trimethylsilyl-α-D-furanoses (18, 26)

To a solution of 3-*O*-acetyl-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose **12**[6c] or 3-*O*-acetyl-5-azido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-trimethylsilyl-α-D-allofuranose **25**[6c] (0.57 mmol) in pyridine (3.7 mL), trimethylsilyl chloride (0.93 mL) and hexamethyldisilazane (1.84 mL) were added and the reaction mixture was stirred at room temperature for 2 h. The solvents were removed, and the residue was extracted with petroleum ether, concentrated and purified by column chromatography using 1:6 EtOAc/petroleum ether as eluent.

*4.3.1. 3-O-Acetyl-5-azido-5-deoxy-1,2-O-isopropylidene-6-O-trimethylsilyl-α-D-galactofuranose (**18**).* Yield: 184 mg (90%). R_f 0.47 (1:6 EtOAc/petroleum ether); $[\alpha]_D$ −48 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2965, 1750, 1375, 1227, 1103 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.19 (d, 1H, $J_{3,4}$=3.0 Hz, H-3), 4.60 (d, 1H, H-2), 4.00 (dd, 1H, $J_{4,5}$=7.4 Hz, H-4), 3.80 (dd, 1H, $J_{6a,6b}$=10.0 Hz, $J_{5,6a}$=4.0 Hz, H-6a), 3.75 (ddd, 1H, $J_{5,6b}$=6.2 Hz, H-5), 3.70 (dd, 1H, H-6b), 2.08 (s, 3H, MeCO), 1.59, 1.33 (2s, 6H, CMe_2), 0.12 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.0 (CO), 113.7 (CMe_2), 105.5 (C-1), 85.0 (C-2), 84.3 (C-4), 77.5 (C-3), 63.4 (C-5), 63.0 (C-6), 27.1, 26.3 (CMe_2), 21.0 (*Me*CO), −0.49 ($SiMe_3$); FABMS: *m*/*z* 382 (100, M+Na^+). Anal. Calcd for $C_{14}H_{25}N_3O_6Si$: C, 46.78; H, 7.01; N, 11.69. Found: C, 46.81; H, 6.77; N, 11.66.

*4.3.2. 3-O-Acetyl-5-azido-5-deoxy-1,2-O-isopropylidene-6-O-trimethylsilyl-α-D-allofuranose (**26**).* Yield: 184 mg (90%). R_f 0.33 (1:6 EtOAc/petroleum); $[\alpha]_D$ −88 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2936, 2109, 1760, 1379, 1251 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 4.83 (dd, 1H, $J_{2,3}$=4.7 Hz, $J_{3,4}$=3.0 Hz, H-3), 4.81 (m, 1H, H-2), 4.20 (dd, 1H, $J_{4,5}$=4.3 Hz, H-4), 3.80 (m, 1H, H-5), 3.64 (m, 2H, H-6a, H-6b), 2.11 (s, 3H, MeCO), 1.54, 1.29 (2s, 6H, CMe_2), 0.13 (s, 9H, $SiMe_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.8 (CO), 113.1 (CMe_2), 103.9 (C-1), 77.4 (C-2), 76.0 (C-4), 72.9 (C-3), 63.7 (C-6), 62.3 (C-5), 27.1, 26.5 (CMe_2), 20.5 (*Me*CO), −0.83 ($SiMe_3$); FABMS: *m*/*z* 382 (100, M+Na^+). Anal. Calcd for $C_{14}H_{25}N_3O_6Si$: C, 46.78; H, 7.01; N, 11.69. Found: C, 46.68; H, 6.65; N, 11.46.

4.4. Preparation of 3-*O*-acetyl-5-(3-butylcarbodiimido)-5-deoxy-1,2-*O*-isopropylidene-6-*O*-trimethylsilyl-α-D-galactofuranose (16)

*4.4.1. 3-O-Acetyl-5-(3-butylthioureido)-5-deoxy-1,2-O-isopropylidene-α-D-galactofuranose (**14**).* A solution of amine **13**[18a] (130 mg, 0.5 mmol) in pyridine (5 mL), Et_3N (0.5 mL, 3.6 mmol) and *n*-butyl isothiocyanate (0.6 mmol) were added and the mixture was stirred at room temperature for 18 h. Then, the solvent was removed under reduced pressure and the resulting residue coevaporated several times with toluene and purified by column chromatography (40:1→20:1 CH_2Cl_2/MeOH) to afford the thioureido derivative **14** (139 mg, 74%). R_f 0.58 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −35 (*c* 1.0, CH_2Cl_2); UV (CH_2Cl_2) 251 nm (ε_{mM} 13.4); IR (KBr) ν_{max} 3362, 2959, 1746, 1553, 1381, 1236, 1094 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 313 K) δ 6.61 (br s, 1H, NH), 6.42 (br s, 1H, NH′), 5.88 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 5.11 (d, 1H, $J_{3,4}$=3.0 Hz, H-3), 4.63 (d, 1H, H-2), 4.34 (m, 1H, H-5), 4.17 (dd, 1H, $J_{4,5}$=6.9 Hz, H-4), 3.91 (dd, 1H, $J_{6a,6b}$=11.8 Hz, $J_{5,6a}$=3.9 Hz, H-6a), 3.83 (br d, 1H, H-6b), 3.43 (m, 2H, CH_2N), 3.02 (br s, 1H, OH), 2.07 (s, 3H, MeCO), 1.55 (m, 2H, CH_2CH_2N), 1.57, 1.31 (2s, 6H, CMe_2), 1.32 (m, 2H, CH_2), 0.90 (t, 3H, $^3J_{H,H}$=7.4 Hz, CH_3); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 182.7 (CS), 170.5 (CO), 113.5 (CMe_2), 105.4 (C-1), 84.6 (C-2, C-4), 77.4 (C-3), 63.1 (C-6), 56.7 (C-5), 44.6 (CH_2N), 30.9 (CH_2), 26.9, 25.8 (CMe_2), 20.7 (*Me*CO), 20.0 (CH_2), 13.7 (CH_3); FABMS: *m*/*z* 399 (50, M+Na^+), 377 (100, M+H^+). Anal. Calcd for $C_{16}H_{28}N_2O_6S$: C, 51.05; H, 7.50; N, 7.44. Found: C, 50.91; H, 7.53; N, 7.35.

*4.4.2. 3-O-Acetyl-5-(3-butylthioureido)-5-deoxy-1,2-O-isopropylidene-6-O-trimethylsilyl-α-D-galactofuranose (**15**).* To a solution of the thioureido derivative **14** (218 mg, 0.58 mmol) in pyridine (5.5 mL), trimethylsilyl chloride (0.94 mL) and hexamethyldisilazane (1.85 mL) were added and the reaction mixture was stirred for 2 h at room temperature. The solvents were removed, and the residue was extracted with petroleum ether, concentrated and purified by column chromatography using 1:2 EtOAc/petroleum ether as eluent. Yield: 208 mg (80%). R_f 0.63 (1:1 EtOAc/petroleum ether); $[\alpha]_D$ −27 (*c* 1.0, CH_2Cl_2); UV (CH_2Cl_2) 251 nm (ε_{mM} 18.8); IR (KBr) ν_{max} 3418, 2957, 1750, 1643, 1541, 1383, 1256, 1094 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.75 (br s, 1H, NH), 6.21 (br s, 1H, NH′), 5.82 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 5.10 (d, 1H, $J_{3,4}$=2.3 Hz, H-3), 4.55 (d, 1H, H-2), 4.12 (m, 2H, H-4, H-5), 3.72 (m, 1H, H-6a), 3.66 (dd, 1H, $J_{6a,6b}$=10.1 Hz, $J_{5,6b}$=5.6 Hz, H-6b), 3.38 (m, 2H, CH_2N), 2.03 (s, 3H, MeCO), 1.52 (m, 2H, CH_2CH_2N), 1.52, 1.25 (2 s, 6H, CMe_2), 1.32 (m, 2H, CH_2), 0.87 (t, 3H, $^3J_{H,H}$=7.3 Hz, CH_3), 0.05 (s, 9H, $SiMe_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 182.6 (CS), 169.9 (CO), 113.4 (CMe_2), 105.2 (C-1), 84.8 (C-2, C-4), 76.9 (C-3), 62.5 (C-6), 56.4 (C-5), 45.1 (CH_2N), 30.9 (CH_2), 26.9, 25.8 (CMe_2), 25.8 (CH_2CH_2N), 20.7 (*Me*CO), 20.0 (CH_2), 13.7 (CH_3), −0.74 ($SiMe_3$); FABMS: *m*/*z* 471 (20, M+Na^+), 449 (100, M+H^+). Anal. Calcd for $C_{19}H_{36}N_2O_6SSi$: C, 50.86; H, 8.09; N, 6.24. Found: C, 50.79; H, 7.78; N, 6.20.

*4.4.3. 3-O-Acetyl-5-(3-butylcarbodiimido)-5-deoxy-1,2-O-isopropylidene-6-O-trimethylsilyl-α-D-galactofuranose (**16**).* To a solution of the thiourea **15** (0.47 mmol) in CH_2Cl_2/H_2O (1:1, 12.0 mL), HgO (1.36 mmol) was added. The reaction mixture was stirred vigorously at room temperature for 45 min, diluted with CH_2Cl_2 (10 mL), and the organic phase was separated. The aqueous phase was extracted with CH_2Cl_2 (2×10 mL), and the combined organic extracts were dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography using 1:3 EtOAc/petroleum ether as eluent. Yield: 172 mg (88%). R_f 0.60 (1:2 EtOAc/petroleum ether); $[\alpha]_D$ −51 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2954, 2139,

1751, 1377, 1235, 1090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.88 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.28 (d, 1H, $J_{3,4}$=3.3 Hz, H-3), 4.58 (d, 1H, H-2), 3.96 (dd, 1H, $J_{4,5}$=6.7 Hz, H-4), 3.75 (dd, 1H, $J_{6a,6b}$=9.9 Hz, $J_{5,6a}$=4.8 Hz, H-6a), 3.70 (m, 1H, H-5), 3.65 (dd, 1H, $J_{5,6b}$=5.7 Hz, H-6b), 3.26 (m, 2H, CH_2N), 2.09 (s, 3H, MeCO), 1.58 (m, 2H, CH_2CH_2N), 1.41 (m, 2H, CH_2), 1.61, 1.35 (2s, 6H, CMe_2), 0.93 (t, 3H, $J_{H,H}$=7.3 Hz, CH_3), 0.15 (s, 9H, $SiMe_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.7 (CO), 140.6 (NCN), 113.5 (*C*Me_2), 105.1 (C-1), 85.3 (C-2), 83.9 (C-4), 77.3 (C-3), 63.7 (C-6), 59.5 (C-6), 46.3 (CH_2N), 33.1 (CH_2CH_2N), 27.1, 26.4 (C*Me*$_2$), 20.8 (*Me*CO), 20.0 (CH_2), 13.6 (CH_3), −0.61 ($SiMe_3$); FABMS: *m*/*z* 365 (70, $[M+Na-TMS]^+$), 343 (100, $[M+H-TMS]^+$). Anal. Calcd for $C_{19}H_{34}N_2O_6Si$: C, 55.05; H, 8.27; N, 6.76. Found: C, 54.89; H, 8.50; N, 6.72.

4.5. General procedure for the preparation of 3-*O*-acetyl-5-alkyl (aryl, glycopyranosyl)carbodiimido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-trimethylsilyl-α-D-furanoses via aza-Wittig reaction (19, 21, 23, 27 and 29)

A solution of azide **18** or **26** (186 mg, 0.52 mmol) in toluene (3.5 mL) was stirred under Ar for 30 min. Then, the corresponding phenyl, naphtyl, or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (0.62 mmol) and a solution of PPh_3 (0.63 mmol) in toluene (2 mL) were added at room temperature. The reaction mixture was stirred at 80 °C for 2 h and concentrated. The resulting residue was purified by column chromatography using the eluent indicated in each case to afford the carbodiimide derivatives **19**, **21**, **23**, **27** and **29** as amorphous solids.

*4.5.1. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-(3-phenylcarbodiimido)-6-O-trimethylsilyl-α-D-galactofuranose (**19**).* Column chromatography, eluent toluene→1:7 EtOAc/toluene. Yield: 167 mg (74%). R_f 0.50 (1:7 EtOAc/toluene); $[\alpha]_D$ −57 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2968, 2139, 1756, 1389, 1238, 1094 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.15–7.05 (m, 5H, Ph), 5.88 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.25 (dd, 1H, $J_{3,4}$=3.1 Hz, $J_{2,3}$=0.5 Hz, H-3), 4.58 (dd, 1H, H-2), 4.02 (dd, 1H, $J_{4,5}$=7.2 Hz, H-4), 3.96 (ddd, 1H, $J_{5,6b}$=6.3 Hz, $J_{5,6a}$=4.7 Hz, H-5), 3.80 (dd, 1H, $J_{6a,6b}$=10.5 Hz, H-6a), 3.74 (dd, 1H, H-6b), 2.07 (s, 3H, MeCO), 1.57, 1.23 (2s, 6H, CMe_2), 0.07 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.8 (CO), 140.2 (NCN), 137.9–124.1 (Ph), 113.6 (*C*Me_2), 105.3 (C-1), 85.1 (C-2), 83.7 (C-4), 77.4 (C-3), 63.4 (C-6), 60.4 (C-5), 27.1, 26.4 (C*Me*$_2$), 20.8 (*Me*CO), −0.67 ($SiMe_3$); FABMS: *m*/*z* 457 (80, $M+Na^+$), 435 (100, $M+H^+$). Anal. Calcd for $C_{21}H_{30}N_2O_6Si$: C, 58.04; H, 6.96; N, 6.45. Found: C, 57.99; H, 6.91; N, 6.40.

*4.5.2. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-(3-napht-1-ylcarbodiimido)-6-O-trimethylsilyl-α-D-galactofuranose (**21**).* Column chromatography, eluent toluene→1:7 EtOAc/toluene. Yield: 189 mg (75%). R_f 0.46 (1:3 EtOAc/toluene); $[\alpha]_D$ −76 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2943, 2133, 1744, 1585, 1379, 1228, 1116 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 313 K) δ 8.29–6.36 (m, 7H, Ph), 5.91 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.30 (d, 1H, $J_{3,4}$=3.0 Hz, H-3), 4.40 (dd, 1H, H-2), 4.07 (dd, 1H, $J_{4,5}$=7.0 Hz, H-4), 4.04 (ddd, 1H, $J_{5,6b}$=6.0 Hz, $J_{5,6a}$=4.5 Hz, H-5), 3.86 (dd, 1H, $J_{6a,6b}$=10.5 Hz, H-6a), 3.80 (dd, 1H, H-6b), 2.08 (s, 3H, MeCO), 1.66, 1.32 (2s, 6H, CMe_2), −0.06 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 169.8 (CO), 137.5 (NCN), 136.7–120.8 (Ph), 113.7 (*C*Me_2), 105.3 (C-1), 85.2 (C-2), 83.7 (C-4), 76.8 (C-3), 63.5 (C-6), 60.6 (C-5), 27.2, 26.4 (C*Me*$_2$), 20.8 (*Me*CO), −0.64 ($SiMe_3$); FABMS: *m*/*z* 507 (100, $M+Na^+$), 485 (75, $M+H^+$). Anal. Calcd for $C_{25}H_{32}N_2O_6Si$: C, 61.96; H, 6.66; N, 5.78. Found: C, 62.05; H, 6.80; N, 5.71.

*4.5.3. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-[3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)carbodiimido]-6-O-trimethylsilyl-α-D-galactofuranose (**23**).* Column chromatography, eluent 1:1→3:1 Et_2O/petroleum ether. Yield: 215 mg (60%). R_f 0.37 (2:1 Et_2O/petroleum ether); $[\alpha]_D$ −35 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2953, 2143, 1751, 1643, 1375, 1229, 1107 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.85 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 5.18 (d, 1H, $J_{3,4}$=3.5 Hz, H-3), 5.17 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.5 Hz, H-3′), 5.09 (t, 1H, $J_{4',5'}$=9.5 Hz, H-4′), 4.95 (dd, 1H, $J_{1',2'}$=9.5 Hz, H-2′), 4.76 (d, 1H, H-1′), 4.56 (d, 1H, H-2), 4.22 (dd, 1H, $J_{6a',6b'}$=12.3 Hz, $J_{5',6a'}$=4.7 Hz, H-6a′), 4.11 (dd, 1H, $J_{5',6b'}$=2.2 Hz, H-6b′), 3.97 (dd, 1H, $J_{4,5}$=7.0 Hz, H-4), 3.83 (ddd, 1H, $J_{5,6b}$=6.1 Hz, $J_{5,6a}$=5.4 Hz, H-5), 3.73 (m, 1H, H-5′), 3.71 (dd, 1H, $J_{6a,6b}$=10.5 Hz, H-6a), 3.64 (dd, 1H, H-6b), 2.07, 2.06, 2.04, 2.00, 1.98 (5s, 15H, 5MeCO), 1.55, 1.32 (2s, 6H, CMe_2), 0.12 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.7, 170.3, 169.7, 169.3, 169.2 (5 CO), 138.7 (NCN), 113.5 (*C*Me_2), 105.1 (C-1), 85.0 (C-2), 84.9 (C-1′), 83.4 (C-4), 77.2 (C-3), 73.5 (C-5′), 73.1 (C-3′), 72.5 (C-2′), 68.2 (C-4′), 63.1 (C-6), 61.9 (C-6′), 59.6 (C-5), 27.0, 26.3 (C*Me*$_2$), 20.7, 20.6, 20.5, 20.4, 20.3 (*Me*CO), −0.66 ($SiMe_3$); FABMS: *m*/*z* 711 (90, $M+Na^+$), 689 (50, $M+H^+$). Anal. Calcd for $C_{29}H_{44}N_2O_{15}Si$: C, 50.57; H, 6.44; N, 4.07. Found: C, 50.38; H, 6.28; N, 3.91.

*4.5.4. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-(3-phenylcarbodiimido)-6-O-trimethylsilyl-α-D-allofuranose (**27**).* Column chromatography, eluent toluene→1:7 EtOAc/toluene. Yield: 102 mg (45%). R_f 0.51 (1:7 EtOAc/toluene); $[\alpha]_D$ +50 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2962, 2134, 1752, 1386, 1251, 1099 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.07 (m, 5H, Ph), 5.80 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 4.92 (dd, 1H, $J_{3,4}$=8.4 Hz, $J_{2,3}$=4.9 Hz, H-3), 4.84 (dd, 1H, H-2), 4.02 (ddd, 1H, $J_{4,5}$=5.4 Hz, $J_{4,6b}$=1.8 Hz, H-4), 3.83 (dd, 1H, $J_{6a,6b}$=8.5 Hz, $J_{5,6a}$=3.9 Hz, H-6a), 3.82 (ddd, 1H, $J_{5,6b}$=8.5 Hz, H-5), 3.67 (td, 1H, H-6b), 2.14 (s, 3H, MeCO), 1.55, 1.34 (2s, 6H, CMe_2), 0.06 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.9 (CO), 139.7 (NCN), 137.8–123.8 (Ph), 113.0 (*C*Me_2), 104.0 (C-1), 77.6 (C-2), 76.6 (C-4), 73.2 (C-3), 62.3 (C-6), 61.1 (C-5), 26.6, 26.5 (C*Me*$_2$), 20.5 (*Me*CO), −0.84 ($SiMe_3$); FABMS: *m*/*z* 435 (90, $M+H^+$). Anal. Calcd for $C_{21}H_{30}N_2O_6Si$: C, 58.04; H, 6.96; N, 6.45. Found: C, 57.92; H, 6.77; N, 6.36.

*4.5.5. 3-O-Acetyl-5-deoxy-1,2-O-isopropylidene-5-[3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)carbodiimido]-6-O-trimethylsilyl-α-D-allofuranose (**29**).* Column chromatography, eluent toluene→1:5 EtOAc/toluene. Yield: 236 mg (66%). R_f (1:2 EtOAc/toluene) 0.42; $[\alpha]_D$ +51 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2943, 2149, 1760, 1371, 1236, 1045 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.18 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.5 Hz, H-3′), 5.10 (t, 1H, $J_{4',5'}$=9.5 Hz, H-4′), 4.94 (dd, 1H, $J_{1',2'}$=8.8 Hz, H-2′), 4.84 (dd, 1H, $J_{3,4}$=8.2 Hz, $J_{2,3}$=5.1 Hz, H-3), 4.81 (d, 1H, H-2), 4.71 (d, 1H, H-1′), 4.24 (dd, 1H, $J_{6a',6b'}$=12.4 Hz, $J_{5',6a'}$=4.9 Hz, H-6a′), 4.17 (dd, 1H, $J_{4,5}$=4.5 Hz, H-4), 4.13 (dd, 1H, $J_{5',6b'}$=1.8 Hz, H-6b′), 3.80 (m, 2H, H-5, H-5′), 3.73 (dd, 1H, $J_{6a,6b}$=10.2 Hz, $J_{5,6a}$=4.8 Hz, H-6a), 3.54 (dd, 1H, $J_{5,6b}$=7.2 Hz, H-6b), 2.12, 2.08, 2.07, 2.02, 1.99 (5 s, 15H, 5MeCO), 1.53, 1.33 (2 s, 6H, CMe_2), 0.14 (s, 9H, $SiMe_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.5, 170.1, 169.8, 169.7, 169.2 (5 CO), 137.8 (NCN), 112.9 (*C*Me_2), 104.0 (C-1), 84.7 (C-1′), 77.5 (C-2), 76.6 (C-4), 73.6 (C-5′), 72.8 (C-3′), 72.7 (C-3), 72.5 (C-2′), 67.9 (C-4′), 62.0 (C-6), 61.7 (C-6′), 60.1 (C-5), 26.6, 26.5 (C*Me*$_2$), 20.8, 20.7, 20.6, 20.5, 20.4 (*Me*CO), −0.67 ($SiMe_3$); FABMS: *m*/*z* 711 (90, $M+Na^+$). Anal. Calcd for $C_{29}H_{44}N_2O_{15}Si$: C, 50.57; H, 6.44; N, 4.07. Found: C, 50.45; H, 6.37; N, 3.95.

4.6. General procedure for the preparation of 2-alkyl(aryl, glycopyranosyl)amino-4-(tetrafuranos-4′-yl)-2-oxazolines (17, 20, 22, 24, 28 and 30)

To a solution of the corresponding carbodiimido derivative **16**, **19**, **21**, **23**, **27** and **29** (0.34 mmol) in THF (6.3 mL) at 0 °C under Ar, TBAF (1 M in THF, 0.35 mL) was added. In the case of **24** and **30**, the reaction mixture was adjusted at pH 7 using glacial AcOH. The solution was stirred at 0 °C for 25 min, then diluted with Et_2O (6 mL), washed with water or iced saturated aqueous $NaHCO_3$

(2×3 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by column chromatography using the eluent indicated in each case to obtain the corresponding isoureas. In the case of **16**, the resulting residue was directly Zemplén O-deacetylation affording the compound **17** further purification by column chromatography.

*4.6.1. (4S)-2-Butylamino-4-[(4′S)-1′,2′-O-isopropylidene-β-L-threofuranos-4′-yl]-2-oxazoline (**17**).* Column chromatography, eluent 45:5:3 EtOAc/EtOH/H_2O. Yield: 85 mg (83%). R_f 0.26 (45:5:3 EtOAc/EtOH/H_2O); $[\alpha]_D$ −42 (*c* 0.99, CH_2Cl_2); IR (KBr) ν_{max} 3433, 2959, 1740, 1559, 1396, 1261, 1092 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 4.64 (t, 1H, $J_{5,6a}$=$J_{6a,6b}$=8.8 Hz, H-6a), 4.50 (dd, 1H, $J_{2,3}$=1.2 Hz, H-2), 4.46 (dd, 1H, $J_{5,6b}$=5.7 Hz, H-6b), 4.36 (ddd, 1H, $J_{4,5}$=6.8 Hz, H-5), 4.04 (dd, 1H, $J_{3,4}$=4.3 Hz, H-3), 3.77 (dd, 1H, H-4), 3.21 (t, 2H, $^3J_{H,H}$=7.0 Hz, CH_2N), 1.52 (m, 2H, CH_2CH_2N), 1.33 (m, 2H, CH_3), 1.48, 1.26 (2s, 6H, CMe_2), 0.92 (t, 3H, $^3J_{H,H}$=7.0 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD) δ 163.9 (CN), 114.5 (CMe_2), 106.4 (C-1), 89.2 (C-2), 88.4 (C-4), 76.3 (C-3), 72.4 (C-6), 61.1 (C-5), 43.5 (CH_2N), 32.4 (CH_2CH_2N), 27.8, 27.7 (CMe_2), 20.8 (CH_2), 13.9 (CH_3); FABMS: *m*/*z* 301 (100, M+H^+). Anal. Calcd for $C_{14}H_{24}N_2O_5$: C, 55.98; H, 8.05; N, 9.33. Found: C, 56.27; H, 8.01; N, 9.44.

*4.6.2. (4S)-4-[(4′S)-3′-O-Acetyl-1′,2′-O-isopropylidene-β-L-threofuranos-4′-yl]-2-phenylamino-2-oxazoline (**20**).* Column chromatography, eluent 100:1→50:1 CH_2Cl_2/MeOH. Yield: 103 mg (84%). R_f 0.42 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −15 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 3433, 2993, 1746, 1555, 1383, 1233, 1101 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.24–6.95 (m, 5H, Ph), 5.85 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 5.75 (br s, 1H, NH), 5.05 (br s, 1H, H-3), 4.57 (d, 1H, H-2), 4.38 (m, 3H, H-5, H-6a, H-6b), 3.99 (dd, 1H, $J_{4,5}$=6.0 Hz, $J_{3,4}$=3.5 Hz, H-4), 2.02 (s, 3H, MeCO), 1.50, 1.24 (2s, 6H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.8 (CO), 157.2 (CN), 128.9–119.5 (Ph), 113.8 (CMe_2), 105.1 (C-1), 85.3 (C-2), 85.0 (C-4), 76.6 (C-3), 68.6 (C-6), 61.9 (C-5), 27.2, 26.3 (CMe_2), 20.8 (*Me*CO); FABMS: *m*/*z* 385 (80, M+Na^+), 363 (100, M+H^+). Anal. Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.47; H, 6.01; N, 7.64.

*4.6.3. (4S)-4-[(4′S)-3′-O-Acetyl-1′,2′-O-isopropylidene-β-L-threofuranos-4′-yl]-2-napht-1-ylamino-2-oxazoline (**22**).* Column chromatography, eluent 2:1 EtOAc/petroleum ether. Yield: 116 mg (83%). R_f 0.57 (2:1 EtOAc/petroleum ether); $[\alpha]_D$ −29 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 3420, 3055, 2928, 1752, 1577, 1379, 1236, 1100 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.76–7.02 (m, 7H, Ph), 5.90 (br s, 1H, H-1), 5.85 (br s, 1H, NH), 4.97 (br s, 1H, H-3), 4.60 (br s, 1H, H-2), 4.37 (m, 3H, H-5, H-6a, H-6b), 4.03 (m, 1H), 2.02 (s, 3H, MeCO), 1.48, 1.34 (2s, 6H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.7 (CO), 155.5 (CN), 134.4–122.8 (Ph), 113.2 (CMe_2), 105.6 (C-1), 86.6 (C-2, C-4), 76.9 (C-3), 68.7 (C-6), 58.2 (C-5), 26.8, 25.8 (CMe_2), 20.8 (*Me*CO); FABMS: *m*/*z* 435 (30, M+Na^+), 413 (100, M+H^+). Anal. Calcd for $C_{22}H_{24}N_2O_6$: C, 64.07; H, 5.86; N, 6.79. Found: C, 64.09; H, 5.94; N, 6.73.

*4.6.4. (4S)-4-[(4′S)-3′-O-Acetyl-1′,2′-O-isopropylidene-β-L-threofuranos-4′-yl]-2-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino-2-oxazoline (**24**).* Column chromatography, eluent 100:1→20:1 CH_2Cl_2/MeOH. Yield: 203 mg (97%). R_f 0.40 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −25 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 3418, 2938, 1750, 1543, 1373, 1231, 1038 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.88 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 5.24 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.5 Hz, H-3′), 5.07 (t, 1H, $J_{4',5'}$=9.5 Hz, H-4′), 4.97 (d, 1H, $J_{1',2'}$=9.5 Hz, H-1′), 4.96 (br s, 1H, H-3), 4.89 (t, 1H, H-2′), 4.56 (d, 1H, H-2), 4.33 (m, 3H, H-5, H-6a, H-6b), 4.26 (dd, 1H, $J_{6a',6b'}$=12.3 Hz, $J_{5',6a'}$=3.8 Hz, H-6a′), 4.08 (dd, 1H, $J_{5',6b'}$=2.6 Hz, H-6b′), 3.93 (dd, 1H, $J_{4,5}$=6.2 Hz, $J_{3,4}$=3.1 Hz, H-4), 3.78 (ddd, 1H, H-5′), 2.07–1.99 (5s, 15H, 5MeCO), 1.55, 1.32 (2s, 6H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.9–169.7 (5 CO), 159.6 (CN), 113.8 (CMe_2), 105.4 (C-1), 86.7 (C-2), 85.2 (C-1′), 83.1 (C-4), 76.9 (C-3), 73.2 (C-3′, C-5′), 71.2 (C-6), 69.7 (C-2′), 68.5 (C-4′), 64.2 (C-5), 62.0 (C-6′), 27.2, 26.5 (CMe_2), 20.9–20.8 (*Me*CO); FABMS: *m*/*z* 639 (100, M+Na^+), 617 (90, M+H^+). Anal. Calcd for $C_{26}H_{36}N_2O_{15}$: C, 50.65; H, 5.88; N, 4.54. Found: C, 50.65; H, 5.92; N, 4.42.

*4.6.5. (4R)-4-[(4′R)-3′-O-Acetyl-1′,2′-O-isopropylidene-α-D-erythrofuranos-4′-yl]-2-phenylamino-2-oxazoline (**28**).* Column chromatography, eluent 100:1→50:1 CH_2Cl_2/MeOH. Yield: 117 mg (95%). R_f 0.55 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +83 (*c* 1.0, CH_2Cl_2); IR (KBr) ν_{max} 2968, 1744, 1553, 1379, 1251, 1093 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.36–6.97 (m, 5H, Ph), 5.81 (d, 1H, $J_{1,2}$=2.4 Hz, H-1), 4.82 (br s, 2H, H-2, H-3), 4.37 (t, 1H, $J_{5,6a}$=$J_{6a,6b}$=8.4 Hz, H-6a), 4.28 (m, 2H, H-5, H-6b), 4.15 (dd, 1H, $J_{3,4}$=7.8 Hz, $J_{4,5}$=5.4 Hz, H-4), 2.11 (s, 3H, MeCO), 1.56, 1.31 (2s, 6H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.5 (CO), 158.0 (CN), 129.1–118.8 (Ph), 113.4 (CMe_2), 104.3 (C-1), 79.5 (C-4), 78.0 (C-2), 74.1 (C-3), 69.3 (C-6), 66.0 (C-5), 26.9, 26.8 (CMe_2), 21.0 (*Me*CO); FABMS: *m*/*z* 385 (80, M+Na^+), 363 (100, M+H^+). Anal. Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.52; H, 5.95; N, 7.52.

*4.6.6. (4R)-4-[(4′R)-3′-O-Acetyl-1′,2′-O-isopropylidene-α-D-erythrofuranos-4′-yl]-2-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino-2-oxazoline (**30**).* Column chromatography, eluent 100:1→20:1 CH_2Cl_2/MeOH. Yield: 178 mg (85%). R_f 0.33 (20:1 CH_2Cl_2/MeOH); $[\alpha]_D$ +42 (*c* 0.98, CH_2Cl_2); IR (KBr) ν_{max} 3420, 2990, 1750, 1540, 1374, 1239, 1040 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.79 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 5.24 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=9.5 Hz, H-3′), 5.06 (t, 1H, $J_{4',5'}$=9.5 Hz, H-4′), 4.95 (d, 1H, $J_{1',2'}$=9.5 Hz, H-1′), 4.91 (t, 1H, H-2′), 4.77 (t, 1H, $J_{3,4}$=$J_{4,5}$=4.3 Hz, H-4), 4.67 (dd, 1H, $J_{2,3}$=8.8 Hz H-3), 4.35 (dd, 1H, $J_{6a,6b}$=13.0 Hz, $J_{5,6a}$=6.7 Hz, H-6a), 4.29 (dd, 1H, $J_{6a',6b'}$=12.4 Hz, $J_{5',6a'}$=4.3 Hz, H-6a′), 4.23 (m, 1H, H-5), 4.20 (dd, 1H, $J_{5,6b}$=6.7 Hz, H-6b), 4.14 (dd, 1H, H-2), 4.08 (dd, 1H, $J_{5',6b'}$=2.2 Hz, H-6b′), 3.76 (ddd, 1H, H-5′), 2.10–1.99 (5s, 15H, 5MeCO), 1.50, 1.29 (2s, 6H, CMe_2); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.9–169.7 (5 CO), 160.1 (CN), 113.3 (CMe_2), 104.2 (C-1), 82.8 (C-1′), 79.0 (C-2), 77.8 (C-4), 73.3 (C-5′), 73.2 (C-3), 73.1 (C-3′), 70.8 (C-2′), 69.6 (C-6), 68.5 (C-4′), 64.8 (C-5), 61,9 (C-6′), 26.8, 26.7 (CMe_2), 20.9–20.8 (*Me*CO); FABMS: *m*/*z* 639 (40, M+Na^+), 617 (100, M+H^+). Anal. Calcd for $C_{26}H_{36}N_2O_{15}$: C, 50.65; H, 5.88; N, 4.54. Found: C, 50.32; H, 5.65; N, 4.36.

4.7. General procedure for the preparation of 5-*N*,6-*O*-(*N*′-alkyl(aryl, glycopyranosyl)iminomethylidene)nojirimycin derivatives (6–11)

To a solution of the corresponding 2-amino-2-oxazoline precursors **20**, **22**, **24**, **28** and **30** (0.28 mmol) in dry MeOH (2 mL), methanolic NaMeO (1 M, 0.1 equiv per mol of acetate) was added. The reaction mixture was stirred at room temperature for 30 min, then neutralized with solid CO_2 and concentrated. The resulting residue and the corresponding desacetylated derivative **17** was treated with TFA/H_2O (9:1, 2.0 mL) for 15 min, concentrated under reduced pressure, coevaporated several times with water, neutralized with Amberlite IRA-68 (OH^-) ion-exchange resin and subjected to column chromatography with the eluent indicated in each case to obtain the isoureas **6**–**11**.

*4.7.1. 5-N,6-O-(N′-Butyliminomethylidene)galactonojirimycin (**6**).* Column chromatography, eluent 10:1 MeCN/H_2O. Yield: 65 mg (90%). R_f 0.41 (10:1:1 MeCN/H_2O/NH_4OH); $[\alpha]_D$ −4.1 (*c* 1.0, H_2O); ^1H NMR (500 MHz, D_2O) δ 5.52 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.82 (t, 1H, $J_{5,6a}$=$J_{6a,6b}$=9.0 Hz, H-6a), 4.69 (dd, 1H, $J_{5,6b}$=7.0 Hz, H-6b), 4.49 (ddd, 1H, $J_{4,5}$=2.5 Hz, H-5), 4.02 (t, 1H, $J_{3,4}$=2.5 Hz, H-4), 3.87 (dd, 1H, $J_{2,3}$=10.5, H-3), 3.77 (dd, 1H, H-2), 3.30 (t, 2H, $^3J_{H,H}$=7.0 Hz, CH_2N), 1.48 (m, 2H, CH_2CH_2N), 1.25 (m, 2H, CH_2), 0.81 (t, 3H,

$^{3}J_{H,H}$=7.0 Hz, CH_3); ^{13}C NMR (125.7 MHz, D_2O) δ 158.5 (CN), 74.9 (C-1), 70.6 (C-6), 68.8 (C-3), 68.0 (C-4), 67.3 (C-2), 56.2 (C-5), 42.3 (CH_2N), 30.3 (CH_2CH_2N), 19.1 (CH_2), 12.8 (CH_3); FABMS: *m*/*z* 283 (20, M+Na^+), 261 (100, M+H^+). Anal. Calcd for $C_{11}H_{20}N_2O_5$: C, 50.78; H, 7.75; N, 10.77. Found: C, 50.66; H, 8.04; N, 10.71.

*4.7.2. 5-N,6-O-(N′-Phenyliminomethylidene)galactonojirimycin (**7**).* Column chromatography, eluent 20:1→4:1 CH_2Cl_2/MeOH. Yield: 63 mg (80%). R_f 0.45 (4:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −5.2 (*c* 0.58, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 7.23–6.91 (m, 5H, Ph), 5.44 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.36 (t, 1H, $J_{5,6a}$=$J_{6a,6b}$=8.3 Hz, H-6a), 4.20 (t, 1H, $J_{5,6b}$=7.8 Hz, H-6b), 4.11 (m, 1H, H-5), 3.93 (m, 1H, H-4), 3.82 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{3,4}$=2.6 Hz, H-3), 3.77 (dd, 1H, H-2); ^{13}C NMR (125.7 MHz, D_2O) δ 159.5 (CN), 146.1–123.4 (Ph), 78.3 (C-1), 69.5 (C-3), 68.2 (C-4), 67.7 (C-2), 66.4 (C-6), 53.6 (C-5); FABMS: *m*/*z* 303 (60, M+Na^+), 281 (30, M+H^+). Anal. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 9.99. Found: C, 55.74; H, 5.64; N, 9.87.

*4.7.3. 5-N,6-O-(N′-Napht-1-yliminomethylidene)galactonojirimycin (**8**).* Column chromatography, eluent 45:5:3 EtOAc/EtOH/H_2O. Yield: 63 mg (85%). R_f 0.36 (45:5:3 EtOAc/EtOH/H_2O); $[\alpha]_D$ −16.6 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, CD_3OD, 313 K) δ 8.07–7.10 (m, 7H, Ph), 5.74 (br s, 1H, H-1), 4.39 (d, 2H, $J_{5,6}$=7.3 Hz, H-6), 4.25 (m, 1H, H-5), 3.95 (dd, 1H, $J_{2,3}$=9.5 Hz, $J_{1,2}$=3.5 Hz, H-2), 3.92 (m, 1H, H-4), 3.88 (dd, 1H, $J_{3,4}$=2.8 Hz, H-3); ^{13}C NMR (125.7 MHz, D_2O, 313 K) δ 158.5 (CN), 134.2–121.5 (Ph), 76.0 (C-1), 69.1 (C-3), 68.5 (C-4),68.4 (C-6), 67.8 (C-2), 57.1 (C-5); FABMS: *m*/*z* 353 (50+Na^+), 331 (100, M+H^+). Anal. Calcd for $C_{17}H_{18}N_2O_5$: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.65; H, 5.44; N, 8.46.

*4.7.4. 5-N,6-O-(N′-β-D-Glucopyranosyliminomethylidene)galactonojirimycin (**9**).* Column chromatography, eluent 4:1 MeCN/H_2O. Yield: 98 mg (96%). R_f 0.37 (6:3:1 MeCN/H_2O/NH_4OH); $[\alpha]_D$ −6.0 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.46 (d, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.72 (d, 1H, $J_{1′,2′}$=9.0 Hz, H-1′), 4.54 (t, 1H, $J_{6a,6b}$=$J_{5,6a}$=7.7 Hz, H-6a), 4.39 (t, 1H, $J_{5,6b}$=7.7 Hz, H-6b), 4.22 (m, 1H, H-5), 3.94 (m, 1H, H-4), 3.79 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{3,4}$=2.7 Hz, H-3), 3.75 (m, 1H, H-2), 3.73 (dd, 1H, $J_{6a′,6b′}$=12.4 Hz, $J_{5′,6a′}$=2.5 Hz, H-6a′), 3.60 (dd, 1H, $J_{5′,6b′}$=4.9 Hz, H-6b′), 3.38 (t, 1H, $J_{2′,3′}$=$J_{3′,4′}$=9.0 Hz, H-3′), 3.37 (m, 1H, H-5′), 3.31 (t, 1H, $J_{4′,5′}$=9.0 Hz, H-4′), 3.19 (t, 1H, H-2′); ^{13}C NMR (125.7 MHz, D_2O) δ 157.8 (CN), 85.5 (C-1′), 77.5 (C-5′), 76.1 (C-3′), 74.7 (C-1), 73.8 (C-2′), 69.4 (C-4′), 69.2 (C-3), 68.2 (C-4), 68.1 (C-6), 67.5 (C-2), 60.6 (C-6′), 54.5 (C-5); FABMS: *m*/*z* 389 (100, M+Na^+), 367 (35, M+H^+). Anal. Calcd for $C_{13}H_{22}N_2O_{10}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.29; H, 5.77; N, 7.39.

*4.7.5. 5-N,6-O-(N′-Phenyliminomethylidene)allonojirimycin (**10**).* Column chromatography, eluent 10:1→4:1 CH_2Cl_2/MeOH. Yield: 74 mg (95%). $[\alpha]_D$ −46.1 (*c* 0.78, H_2O). R_f 0.50 (4:1 CH_2Cl_2/MeOH); ^{1}H NMR (500 MHz, D_2O) δ 7.30–6.99 (m, 5H, Ph), 5.38 (br s, 1H, $J_{1,2}$=4.0 Hz, H-1), 4.59 (t, 1H, $J_{5,6a}$=$J_{6a,6b}$=8.1 Hz, H-6a), 4.20 (t, 1H, $J_{5,6b}$=8.8 Hz, H-6b), 4.11 (t, 1H, $J_{2,3}$=$J_{3,4}$=2.9 Hz, H-3), 4.09 (dt, 1H, $J_{4,5}$=9.9 Hz, H-5), 3.77 (br s, 1H, H-2), 3.66 (dd, 1H, H-4); ^{13}C NMR (125.7 MHz, D_2O) δ 155.7 (CN), 146.2–124.5 (Ph), 76.2 (C-1), 73.6 (C-3), 71.6 (C-4), 71.3 (C-6), 68.5 (C-2), 51.3 (C-5); FABMS: *m*/*z* 303 (100, M+Na^+). Anal. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.75; N, 9.99. Found: C, 55.77; H, 5.49; N, 9.81.

*4.7.6. 5-N,6-O-(N′-β-D-Glucopyranosyliminomethylidene)allonojirimycin (**11**).* Column chromatography, eluent 10:1→2:1 MeCN/H_2O. Yield: 63 mg (62%). R_f 0.28 (6:3:1 MeCN/H_2O/NH_4OH); $[\alpha]_D$ −11.9 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 5.28 (d, 1H, $J_{1,2}$=4.3 Hz, H-1), 4.72 (d, 1H, $J_{1′,2′}$=9.0 Hz, H-1′), 4.59 (t, 1H, $J_{6a,6b}$=$J_{5,6a}$=8.0 Hz, H-6a), 4.19 (t, 1H, $J_{5,6b}$=8.0 Hz, H-6b), 4.04 (t, 1H, $J_{2,3}$=$J_{3,4}$=2.8 Hz, H-3), 3.40 (m, 1H, H-5), 3.75 (dd, 1H, $J_{6a′,6b′}$=12.3 Hz, $J_{5′,6a′}$=1.8 Hz, H-6a′), 3.69 (dd, 1H, H-2), 3.60 (dd, 1H, $J_{4,5}$=8.4 Hz, $J_{3,4}$=2.8 Hz, H-4), 3.58 (dd, 1H, $J_{5′,6b′}$=1.7 Hz, H-6b′), 3.39 (t, 1H, $J_{2′,3′}$=$J_{3′,4′}$=9.0 Hz, H-3′), 3.36 (m, 1H, H-5′), 3.33 (t, 1H, $J_{4′,5′}$=9.0 Hz, H-4′), 3.11 (t, 1H, H-2′); ^{13}C NMR (125.7 MHz, D_2O) δ 157.2 (CN), 86.5 (C-1′), 77.4 (C-5′), 76.3 (C-3′), 74.9 (C-2′), 74.5 (C-1), 72.5 (C-3), 70.2 (C-6), 69.7 (C-4′), 69.6 (C-4), 67.4 (C-2), 60.8 (C-6′), 50.2 (C-5); FABMS: *m*/*z* 389 (50, M+Na^+), 367 (30, M+H^+). Anal. Calcd for $C_{13}H_{22}N_2O_{10}$: C, 42.62; H, 6.05; N, 7.65. Found: C, 42.34; H, 6.01; N, 7.56.

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Supplementary data

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References and notes

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