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PAPER

Bicyclic (*galacto*)nojirimycin analogues as glycosidase inhibitors: Effect of structural modifications in their pharmacological chaperone potential towards β-glucocerebrosidase†

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A molecular-diversity-oriented approach for the preparation of bicyclic sp²-iminosugar glycomimetics related to nojirimycin and galactonojirimycin is reported. The synthetic strategy takes advantage of the ability of endocyclic pseudoamide-type atoms in five-membered cyclic iso(thio)ureas and guanidines to undergo intramolecular nucleophilic addition to the masked carbonyl group of monosaccharides. The stereochemistry of the resulting hemiaminal stereocenter is governed by the anomeric effect, with a large preference for the axial (pseudo-α) orientation. A library of compounds differing in the stereochemistry at the position equivalent to C-4 in monosaccharides (D-*gluco* and D-*galacto*), the heterocyclic core (cyclic isourea, isothiourea or guanidine) and the nature of the exocyclic nitrogen substituent (apolar, polar, linear or branched) has been thus prepared and the glycosidase inhibitory activity evaluated against commercial glycosidases. Compounds bearing lipophilic substituents behaved as potent and very selective inhibitors of β-glucosidases. They further proved to be good competitive inhibitors of the recombinant human β-glucocerebrosidase (imiglucerase) used in enzyme replacement therapy (ERT) for Gaucher disease. The potential of these compounds as pharmacological chaperones was assessed by measuring their ability to inhibit thermal-induced denaturation of the enzyme in comparison with *N*-nonyl-1-deoxynojirimycin (NNDNJ). The results indicated that amphiphilic sp²-iminosugars within this series are more efficient than NNDNJ at stabilizing β-glucocerebrosidase and have a strong potential in pharmacological chaperone (PC) and ERT-PC combined therapies.

Introduction

Sugar mimics bearing endocyclic nitrogen atoms (iminosugars and azasugars), such as deoxynojirimycin (DNJ) **1** and castanospermine **2**, have been shown to behave as potent glycosidase inhibitors (Fig. 1). Given the broad range of biological and pathological processes in which glycosidases are involved, from the catabolism of sugars to the biosynthesis of the complex oligosaccharide chains in glycoproteins and glycolipids, specific inhibitors of these enzymes bear strong potential for the development of new pharmaceuticals[1] *e.g.*, in the treatment of type II diabetes

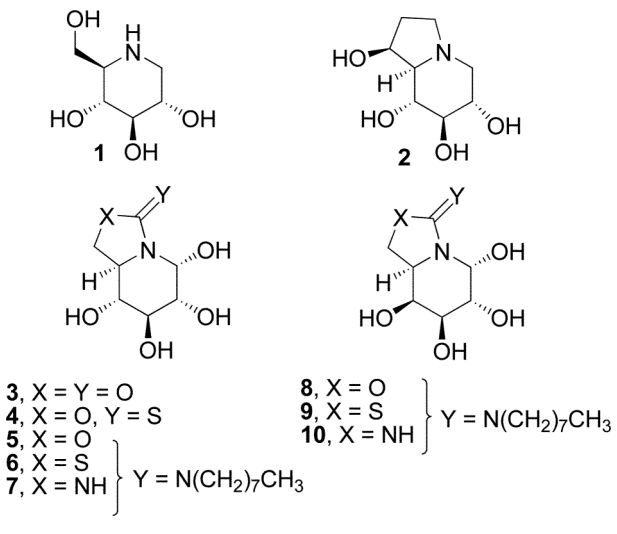

Fig. 1 Structures of DNJ (**1**), castanospermine (**2**), and the bicyclic nojirimycin analogues **3–10**.

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† Electronic supplementary information (ESI) available: Experimental procedures for the preparation of isothiocyanato precursors **16–21**, plots of Imiglucerase activity after thermal denaturation (48 °C) in the presence of increasing concentrations of compounds **5–7**, **9**, **37**, **52**, **55**, Lineweaver–Burk plots for K_i determinations and NMR spectra of all new compounds. See DOI: 10.1039/c1ob05234a

mellitus,[2] viral infections,[3] cancer[4] or hereditary enzyme deficiency diseases.[5]

Despite their promise, neither **1** or **2** nor other members of this family of glycomimetics have realized their full clinical potential. This is largely because of a lack of commercially viable syntheses and difficulties in preparing a comprehensive palette of variant structures. Moreover, in most cases the lack of specificity between different isoenzymes represents an important problem for clinical applications. In order to tackle the problems of enzyme selectivity, several years ago we developed a new family of glycomimetics in which the amine-type nitrogen characteristic of iminosugars was replaced by a pseudoamide-type nitrogen atom with high sp^2-character (sp^2-iminosugars; *e.g.* compounds **3** and **4**).[6] This structural modification profoundly alters the stereoelectronic properties at the pseudoanomeric region, rendering compounds that are chemically, conformationally and configurationally stable in water solution even when incorporating oxygen (hemiaminals), nitrogen (*gem*-diamines) or sulfur substituents (aminothioacetals) at the position equivalent to C-1 in monosaccharides.[7] For instance, the pseudoanomeric hydroxyl group in the cyclic carbamate nojirimycin derivative **3** was found exclusively in the axial orientation, as determined by the generalized anomeric effect, matching the configurational pattern of α-glucopyranosides.[8] Accordingly, it behaved as a potent and selective inhibitor of yeast α-glucosidase (K_i 2.2 μM), being inactive against several β-glucosidases. Notwithstanding, a dramatic shift in the α/β-glucosidase selectivity was observed for cyclic isourea analogues bearing aliphatic substituents at the exocyclic nitrogen atom, *e.g.* **5** (K_i 1.7 μM against almond β-glucosidase; no inhibition of yeast α-glucosidase) even though they likewise existed in the α-like configuration in water solution.[9] Compound **5** was also a potent and selective inhibitor of human lysosomal β-glucosidase (β-glucocerebrosidase, GlcCerase). Most interestingly, it promoted the correct folding in GlcCerase mutants associated with Gaucher disease and their trafficking to the lysosome, being currently under study as pharmacological chaperone for the treatment of this pathology.[10]

X-Ray crystallographic studies showed that isourea **5** is actually present in the β-configuration in the active site of β-glucosidases.[11] This enzyme-dependent induced fit process is promoted by the substituent at the imine functionality, which locates in a hydrophobic pocket at the vicinity of the active site of β-glucosidases resulting in a remarkably high favourable entropic contribution to binding.[11] Interestingly, replacement of the endocyclic oxygen atom by sulfur or nitrogen increased the inhibitory potency for β-glucosidases while preserving the anomeric selectivity (K_i 0.76 and 0.42 μM against almond β-glucosidase for isothiourea **6** and guanidine **7**, respectively).[12] Inversion of the configuration at the position equivalent to C-4 in monosaccharides, thereby shifting from a hydroxylation pattern of structural complementarity to D-glucose to a D-galactose-type profile, further enhanced the inhibitory potential against β-glucosidases (*e.g.*, K_i 0.023 μM against almond β-glucosidase for isothiourea **9** and K_i 0.04 μM against bovine liver β-glucosidase for guanidine **10**). Actually, compounds **6**–**10** also showed chaperone activity in Gaucher fibroblasts with several mutations, with isothiourea derivatives **6** and **9** being the most active candidates followed by guanidine **7**. Contrary to classical sp^3-iminosugars such as **1** or the corresponding *N*-alkyl derivatives, the sp^2-iminosugars were particularly active for mutations leading to neuronopathic types of the disease. Mutation profiling also evidenced differences in their chaperoning activity that were mutation-dependent.[10]

The above commented results illustrate how structural modifications in the skeleton and substitution profile of sp^2-iminosugar glycomimetics can be exploited to finely tune their enzyme inhibition and pharmacological chaperone selectivity and potency even for closely similar mutant enzymes. The implementation of synthetic strategies allowing elaboration of a collection of derivatives for structure–activity relationship (SAR) studies seemed therefore very appealing. Given the promising results obtained with the isothiourea and guanidine-type bicyclic cores both of the D-*gluco* and D-*galacto* isosteric series in pharmacological chaperone therapy approaches, a systematic investigation of the effect of a variety of *N′*-substituents in their biological activity has now been undertaken.

Results and discussion

Synthesis

In principle, the target bicyclic skeleton containing the isothiourea or guanidine functionality **I** (X = S or NH) can be obtained by intramolecular furanose→piperidine rearrangement of 2-aminothiazoline or 2-aminoimidazoline pseudo-*C*-nucleoside precursors (**IIa**), respectively, after liberating the aldehyde functionality (**IIb**) of the monosaccharide. For iminothiazolidine derivatives, the key thiazoline synthetic intermediate (**II**, X = S) can be prepared from vicinal hydroxythiourea precursors **III** following activation of the primary hydroxyl group for nucleophilic displacement by the thiocarbonyl sulfur atom. This retrosynthetic scheme is particularly appealing for generating molecular diversity in an efficient manner since thioureas can be readily prepared by coupling of isothiocyanates and amines, which generally proceed with total chemoselectivity and high yield in the presence of OH functionalities.[13] The corresponding imidazoline derivatives (**II**, X = NH) could be obtained from 5,6-(cyclic isothiouronium) salt intermediates **IV** by nucleophilic displacement of the alkylthio group by amine (Fig. 2).[12]

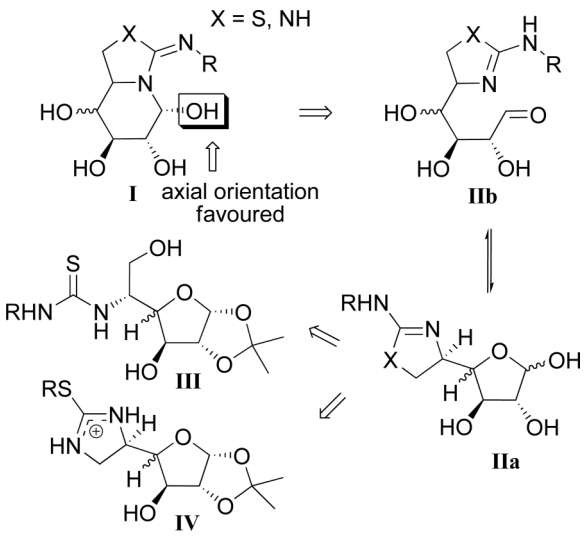


Fig. 2 Retrosynthesis of bicyclic isothiourea and guanidine-type sp^2-iminosugars.

For practical reasons, the already reported 5-azido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-tetrahydropyranyl-α-D-glucofuranose and 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose **11**[14] and **12**[15] were chosen as starting materials (Fig. 3). The corresponding 5-amino-5-deoxy-sugars can be readily generated by catalytic hydrogenation and were used in the subsequent coupling reactions without further purification. For the synthesis of thiourea intermediates **III**, we first selected *n*-butyl isothiocyanate (**13**), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**14**)[16] and phenyl isothiocyanate (**15**) as representative examples of alkyl, glycosyl and aryl substituted electrophile counterparts, respectively. Since a preliminary screening revealed the superiority of alkyl-bearing amphiphilic sp^2-iminosugars as β-glucosidase binders, a broader battery of alkyl isothiocyanate building blocks was incorporated, including branched and linear derivatives bearing different chemical functionalities (**16–21**; Fig. 3).

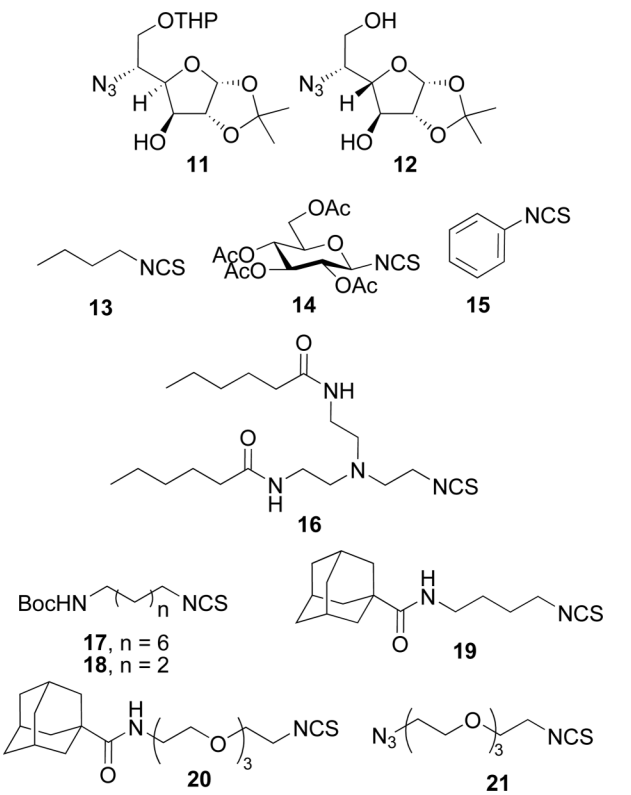
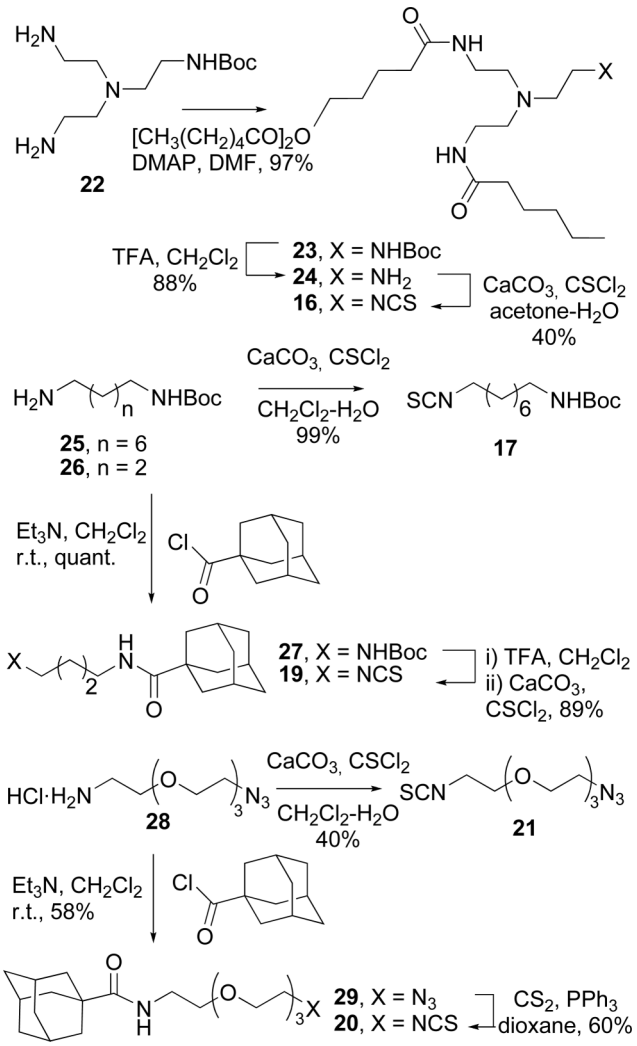


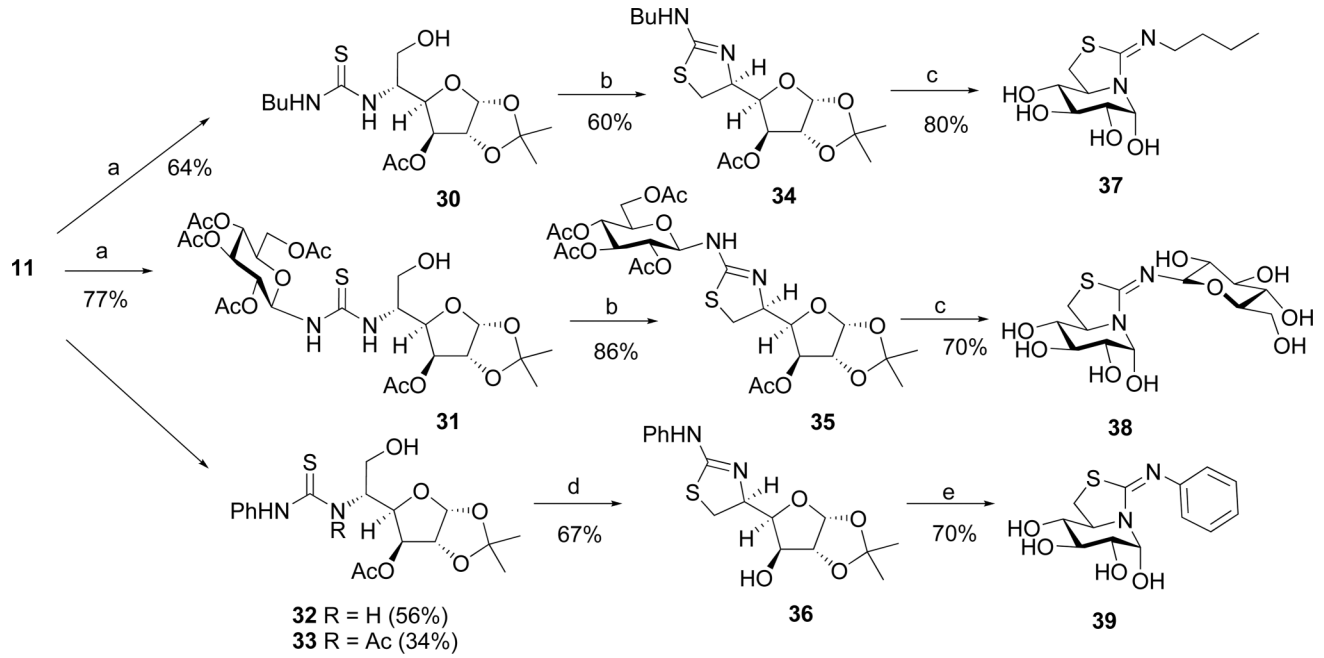
Fig. 3 Building block precursors for the synthesis of bicyclic isothiourea–type sp^2-iminosugars.

The selected isothiocyanate precursors were commercially available (**13**, **15** and **18**) or were obtained in a limited number of steps by isothiocyanation of the corresponding amines. Thus, the glucopyranosyl derivative **14** was prepared from D-glucose as reported in the literature.[16] The branched isothiocyanate **16** was synthesized from [bis(2-aminoethyl)-2-(*tert*-butoxycarbonylamino)ethyl]amine (**22**)[17] by *N*-hexanoylation of the free primary amino groups (→**23**), subsequent deprotection of the carbamate group (→**24**) and isothiocyanation of the generated primary amine by treatment with thiophosgene. Compound **17** was obtained in good yield by isothiocyanation of the corresponding amine **25**[18] using thiophosgene. The preparation of 4-(1-adamantylcarboxamido)butyl isothiocyanate (**19**) was carried out using a two-step process that involved condensation of *N*-(*tert*-butoxycarbonyl)butanediamine (**26**) and adamantane-1-carbonyl chloride (→**27**) followed by carbamate hydrolysis and isothiocyanation. A similar strategy was followed for the preparation of compound **20**, incorporating an oligoethylene segment, from 11-azido-3,6,9-trioxaundecylamine (**28**)[19] but in this case the azido group in the amine adduct **29** was directly transformed into isothiocyanate by reaction with triphenylphosphine–carbon disulfide.[20] Finally, the azidoalkyl isothiocyanate **21** was obtained from **28** (Scheme 1) following isothiocyanation with thiophosgene.



Scheme 1 Synthesis of the isothiocyanate precursors **16**, **17**, and **19–21**.

The synthesis of the 5-*N*,6-*S*-(*N*′-butyl-, *N*′-β-D-glucopyranosyl- and *N*′-phenyl-iminomethylidene)-6-thionojirimycin derivatives starts with reduction of the azido group in **11** by catalytic hydrogenation, coupling of the resulting amine with the corresponding isothiocyanate (**13–15**), acetylation and final hydrolysis of the tetrahydropyranyl group to afford the *N*′-thiourea adducts **30–32** (Scheme 2). Temporary protection of the secondary hydroxyl as the corresponding acetate was considered advantageous in order to prevent regioselectivity problems



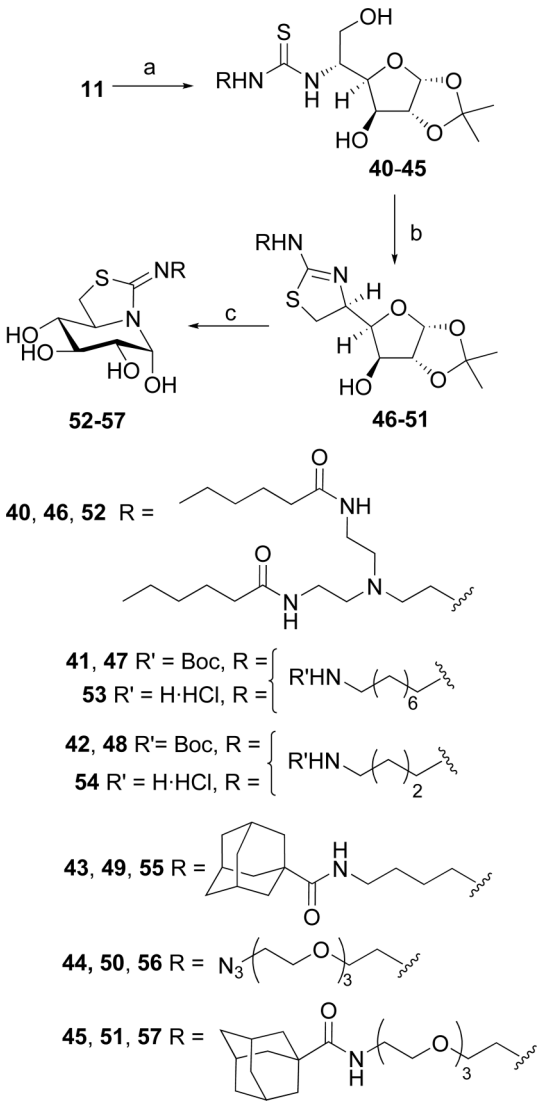
Scheme 2 Reagents and conditions: (a) 1. H_2, Pd/C 10%, MeOH, r.t., 1 h. 2. **13–15**, Et_3N, pyridine, r.t., 18 h. 3. (1 : 1) Ac_2O–pyridine, −15 °C, 5 h. 4. *p*-TsOH, CH_2Cl_2–MeOH (1 : 1), r.t., 2 h; (b) MsCl, pyridine, −20 °C → 10 °C, 7 h; (c) 1. NaOMe, MeOH, r.t., 30 min, solid CO_2. 2. 90% TFA–H_2O, r.t., 30 min. 3. Amberlite IRA 68 (OH^-); (d) 1. MsCl, pyridine, −20 °C → 10 °C, 7 h. 2. NaOMe, MeOH, r.t., 30 min, solid CO_2; (e) 1. 90% TFA–H_2O, r.t., 30 min. 2. Amberlite IRA 68 (OH^-).

during the subsequent thiazoline ring formation. In the case of the *N′*-phenylthioureido derivative concomitant *N*-acetylation occurred,[21] leading to a 2 : 1 mixture of **32** and **33**. This is not detrimental for the overall yield of the reaction sequence as discussed hereinafter. Attempts to activate the primary hydroxyl as leaving group by trifluoromethanesulfonylation (triflation)[22] in **30–33** were unsuccessful. Nevertheless, treatment with mesyl chloride and pyridine at low temperature proceeded with spontaneous nucleophilic displacement of the transient mesylate by the vicinal thiocarbonyl sulfur atom to afford the requested 2-aminothiazolines with total selectivity in good yield (60–86%). The thiourea sulfur is reported to react faster than the nitrogen atoms in nucleophilic displacements, which is consistent with the obtained result.[23] In the case of the *N′*-butyl and *N′*-β-D-glucopyranosyl derivatives the 3-*O*-acetyl derivatives **34** and **35** were thus characterized, whereas in the case of the of *N′*-phenylthioureas **32** and **33** the cyclization product was subjected to subsequent deacetylation to yield the 2-aminothiazoline **36** as the only reaction product (67%). Further removal of the protecting groups afforded the target bicyclic nojirimycin derivatives **37–39** in 70–80% yield.

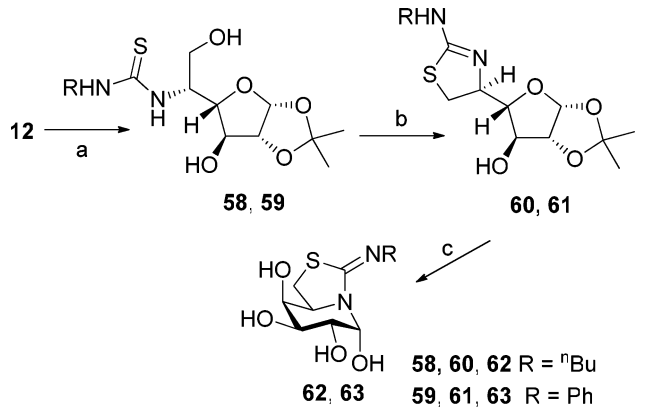
The synthesis of the 5-*N*,6-*S*-(*N′*-iminomethylidene)-6-thionojirimycin derivatives **52–57** followed a similar approach, starting from azide **11** as a common intermediate. Protection of the secondary hydroxyl group OH-3 in the thiourea derivatives **40–45** was found to be not necessary since the mesyl-promoted cyclization proceeded with total regioselectivity to give the desired thiazolines **46–51** (Scheme 3). This general synthetic strategy was next applied to the preparation of the bicyclic galactonojirimycin derivatives **62** and **63**, the C-4 epimers of compounds **37** and **39** (Scheme 4). 5-Azido-5-deoxy-1,2-*O*-isopropylidene-β-D-galactofuranose (**12**), available from commercial α-D-galactose,[15] was used as starting material for accessing the corresponding thioureas **58** and **59**, which were subjected to cyclization (→**60** and **61**) and final pyranose→sp^2-iminosugar rearrangement.

5-Deoxy-5-guanidinosugars can be accessed from the corresponding 5-azido-5-deoxysugars *via* carbodiimide intermediates;[24] however, their use as precursors of cyclic guanidines *via* nucleophilic displacement of a vicinal sulfonate ester is not appropriate due to the high basicity and low nucleophilicity of the guanidine functionality. An alternative strategy was thus developed for the preparation of 6-amino-6-deoxy-5,6-di-*N′*-(*N′*-iminomethylidene)nojirimycin (**70**, **71**) and galactonojirimycin derivatives (**72**, **73**) starting from the 5,6-(cyclic thiourea) intermediates **64** and **65**.[10,12] Transformation of the thioureas into *S*-methyl isothiouronium salts followed by reaction with *n*-butylamine or benzylamine afforded the corresponding cyclic guanidines **66–69** in good yields, which were characterized as the corresponding hydrochlorides. Deprotection of the acetal functionality and treatment with sodium hydroxide gave the target sp^2-iminosugar glycomimetics **70–73**, likewise characterized as the corresponding hydrochloride salts (Scheme 5).

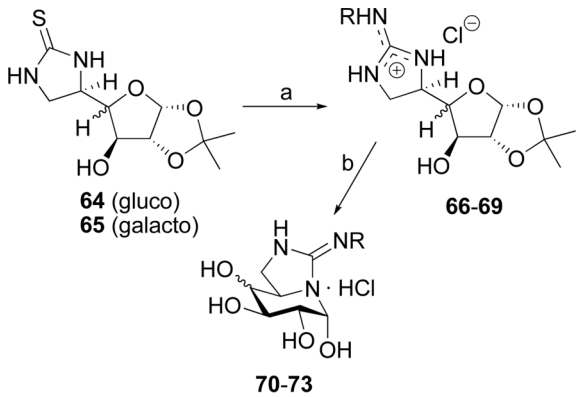
The structure and purity of all the prepared compounds were confirmed by spectroscopic and analytical techniques. The ^{13}C NMR spectra of the 2-aminothiazoline derivatives (**34–36**, **46–51**, **60**, **61**) showed a characteristic signal at 166.2–161.3 ppm corresponding to the double bonded carbon and a high field shift of the C-6 resonance (37.9–33.3 ppm) with respect to the thiourea precursors (**30–33**, **40–45**, **58** and **59**), confirming the replacement of oxygen by sulfur at this position. In the case of the 2-aminoimidazoline derivatives (**66–69**) a signal at 159.9–155.9 ppm confirmed the presence of the imino functionality.



Scheme 3 Reagents and conditions: (a) 1. H_2, Pd/C 10%, MeOH, r.t., 1 h. 2. **16–21**, Et_3N, pyridine, r.t., 18 h. 3. *p*-TsOH, CH_2Cl_2–MeOH (1 : 1), r.t., 2 h, 47–85%; (b) MsCl, pyridine, –20 °C → 10 °C, 7 h, 46–78%; (c) 1. 90% TFA-H_2O, r.t., 30 min. 2. Amberlite IRA 68 (OH^-), 82–99%.



Scheme 4 Reagents and conditions: (a) 1. H_2, Pd/C 10%, MeOH, r.t., 1 h. 2. **13** or **15**, Et_3N, pyridine, r.t., 18 h, 69–72%; (b) MsCl, pyridine, –20 °C → 10 °C, 7 h, 52–60%; (c) 1. 90% TFA–H_2O, r.t., 30 min. 2. Amberlite IRA 68 (OH^-), 60–86%.



Scheme 5 Reagents and conditions: (a) 1. MeI, MeOH, 70 °C, 2 h, 90%. 2. $nBuNH_2$ or $PhCH_2NH_2$, DMF, 70 °C, 18 h, 55–76%; (b) 1. 90% TFA–H_2O, 0 °C, 1 h. 2. NaOH 0.1 N, 60–80%.

The ^1H and ^{13}C NMR spectra of the sp^2-iminosugars (**37–39**, **52–57**, **62**, **63**, **70–73**) registered in D_2O solution confirmed their bicyclic character. Thus, the high field resonance of the pseudoanomeric carbon (C-1) resonance (76.7–74.9 ppm) was in agreement with the presence of the hemiaminal functionality, whereas the vicinal $^3J_{H,H}$ values about the six-membered ring were indicative of the $^4C_1$ chair conformation with the pseudoanomeric groups in axial (α-like) orientation ($J_{1,2}$ = 3.2–4.0 Hz) fitting the anomeric effect, a distinctive feature of sp^2-iminosugars. In the case of compounds with aliphatic substituents, the presence of the β-like anomer in the solution was detected by NMR, but with a large preference (>95%) for the α-like diastereomer.

Evaluation of the glycosidase inhibitory activity

All the new sp^2-iminosugars synthesised were first tested as inhibitors against a series of commercial glycosidases including α-glucosidase (yeast), β-glucosidase (almonds), β-glucosidase (bovine liver, cytosolic), α-mannosidase (Jack bean), β-mannosidase (*Helix pomatia*), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), naringinase (β-glucosidase/α-L-rhamnosidase; *Penicillium decumbens*),[25] α-galactosidase (green coffee beans), isomaltase (yeast), and α-L-fucosidase (pig kidney). The corresponding inhibition constants (K_i) are collected in Tables 1 (for bicyclic nojirimycin derivatives) and 2 (for bicyclic galactonojirimycin derivatives). The corresponding data for the previously synthesized derivatives **5–10** have been also included for comparative purposes.

In agreement with previous results on related *N*-substituted iminomethylydene derivatives,[9] no or weak inhibition of the glycosidases acting on α-glucopyranosides, namely yeast α-glucosidase, isomaltase or amyloglucosidase, was observed in all cases in spite of the matching configuration at the (pseudo)anomeric centre. It must be noted, however, that except compounds **38** and **52** all compounds with D-*gluco* configurational pattern were weak to moderate inhibitors of pig kidney trehalase, an enzyme known to be strongly inhibited by isourea and isothiourea derivatives (Table 1).[26] The isothiourea-type nojirimycin derivatives also

Table 1 K_i values (μM) for the bicyclic nojirimycin derivatives **5–7**, **37–39**, **52–57**, **70** and **71** against a panel of glycosidases[a]

Enzyme	**5**	**6**	**7**	**37**	**38**	**39**	**52**	**53**	**54**	**55**	**56**	**57**	**70**	**71**
α-glucosidase (baker yeast)	168	n.i.[b]	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	292	n.i.	n.i.	n.i.	n.i.
β-glucosidase (almond)	1.9	0.76	0.42	24	654	n.i	19	13	13	0.45	28	33	144	23
β-gluco/β-galactosidase (bovine liver)	2.7	3.7	35	58	251	383	24	9.3	256	68	165	126	508	85
trehalase (pig kidney)	182	13.4	40	20.1	n.i.	226	n.i.	24	116	103	433	398	87	154
naringinase (*Penicillium decumbens*)	n.d.[c]	0.23	0.18	1.1	110	14	1.3	0.23	1.8	0.10	0.35	0.67	1.9	0.68
α-L-fucosidase (pig kidney)	n.d.	18	260	6.2	48	2.4	151	2.6	10.8	7.1	17.6	4.4	514	529
Imiglucerase (Cerezyme®, Genzyme)[d]	3.44	0.69	3.8	24.1	n.d.	n.d.	5.9	n.d.	n.d.	5.5	173	22.3	101	150

[a] Inhibition was competitive in all cases. No inhibition was observed for any compound at 2 mM concentration on green coffee bean α-galactosidase, *Aspergillus niger* amyloglucosidase, Jack bean α-mannosidase or *Helix pomatia* β-mannosidase. [b] n.i., no inhibition observed at 2 mM concentration of the inhibitor. [c] n.d., not determined. [d] IC_{50} values [μM].

inhibited pig kidney α-L-fucosidase in the low micromolar range (Table 1).

Most interestingly, all the *N′*-alkyl-substituted iminothiazolidine derivatives (**6**, **37**, **52–57**) behaved as micromolar to nanomolar inhibitors of the β-glucosidases from almonds and bovine liver as well as of naringinase from *Penicillium decumbens*, an enzyme having β-glucosidase (E.C. 3.2.1.21) and α-L-rhamnosidase (E.C. 3.2.1.40) activities, in spite of the mismatching configuration at the pseudoanomeric centre. Compounds exhibiting strong inhibition for the two first β-glucosidases have been shown previously to be good candidates as pharmacological chaperones for the treatment of Gaucher disease. Therefore, this assay was used to select compounds for further evaluation against human β-glucocerebrosidase. The inhibition potency seems to be related to the hydrophobicity of the *N′*-substituent. Thus, incorporation of a terminal protonatable amino group (*i.e.*, **53** and **54**) resulted in a significant increase in the corresponding K_i values (2.5 and 4.4-fold in the case of the bovine liver enzyme) as compared with the parent *N′*-alkyl derivative (*i.e.*, **6** and **37**). Similarly, compounds **56** and **57**, incorporating a triethylene glycol moiety, were one to two orders of magnitude weaker inhibitors than compounds having hydrophobic substituents. This substantially worse inhibition might have its origin in the high desolvation energy of the polyethylene glycol-based arm, an effect that increases with length.[27]

The mammalian β-glucosidase seems to be more sensitive to modifications at the exocyclic nitrogen substituent of the inhibitor. Thus, the adamantylcarboxamidobutyl derivative **55** was up to 1.7-fold a more potent inhibitor that the *N′*-octyl derivative **6** against almond β-glucosidase but 18.4-fold weaker against bovine liver β-glucosidase. Replacement of the octyl chain (*i.e.*, in **7**) into butyl and phenyl (*i.e.*, in **70** and **71**, respectively) was also strongly detrimental for the inhibitory activity in the cyclic guanidine-type bicyclic nojirimycin series.

Comparison of the inhibition data for D-*gluco* (Table 1) and D-*galacto*-configured compounds (Table 2) indicated that the later tend to be more potent inhibitors against the β-glucosidases from almonds and bovine liver (*i.e.*, **7**, **37**, **39**, and **70** *versus* **10**, **62**, **63**, and **72**, respectively). It is known that iminosugars with hydroxylation patterns of stereochemical complementarity to D-galactose are often moderate to good inhibitors of family 1 β-glucosidases,[28] which are not very selective enzymes regarding the configuration at C-4 in the substrates.

Compounds **5–10**, **37**, **52**, **55–57**, **62**, **70**, and **71** were further evaluated as inhibitors of the recombinant GlcCerase (imiglucerase, Cerezyme® from Genzyme) (Tables 1 and 2). Activity data reflect a clear dependence of GlcCerase inhibition on the nature of the exocyclic substituent. In agreement with the correlation between amphiphilicity and inhibitory activity observed in other glycomimetic families,[29] the inhibitory potency decreased when decreasing the hydrophobicity of the *N′*-substituent, irrespective of the nature of the endocyclic heteroatom at the five-membered ring. Thus, the presence of polar groups (*e.g.* **56** and **57** *versus* **55**) or shortening the aliphatic alkyl chain (*i.e.* **37**, **62** and **70** *versus* **6**, **9** and **7**, respectively) involved a significant diminution of the inhibitory activity.

Evaluation of the potential for chaperoning activity

Enzyme stabilization under thermal denaturation conditions was used as an indication of the potential of the target compounds to behave as pharmacological chaperones.[30] Recovery of GlcCerase activity after heating at 48 °C was measured in the absence and in the presence of increasing concentrations (50, 100 and 150 μM)

Table 2 K_i values (μM) for **8**, **9**, **10**, **62**, **63**, **72**, and **73** against a panel of glycosidases[a]

Enzyme	**8**	**9**	**10**	**62**	**63**	**72**	**73**
β-glucosidase (almond)	0.019	0.023	0.16	0.49	69	5.7	n.i.
α-galactosidase (green coffee bean)	n.i.[b]	n.i.	n.i.	458	n.i.	n.i.	n.i.
β-gluco/β-galactosidase (bovine liver)	0.052	0.042	0.04	1.2	55	2.5	n.i.
naringinase (*Penicillium decumbens*)	n.d.[c]	37	44	144	n.i.	n.d.	63
α-L-fucosidase (pig kidney)	n.d.	11	n.d.	n.i.	n.i.	n.i.	n.d.
Imiglucerase (Cerezyme®, Genzyme)[d]	10.6	4.0	0.9	83	n.d.	n.d.	n.d.

[a] Inhibition was competitive in all cases. No inhibition was observed for any compound at 2 mM concentration on baker's yeast α-glucosidase, pig kidney trehalase, yeast isomaltase, Jack bean α-mannosidase or *Helix pomatia* β-mannosidase. [b] n.i., no inhibition observed at 2 mM concentration of the inhibitor. [c] n.d., not determined. [d] IC_{50} values [μM].

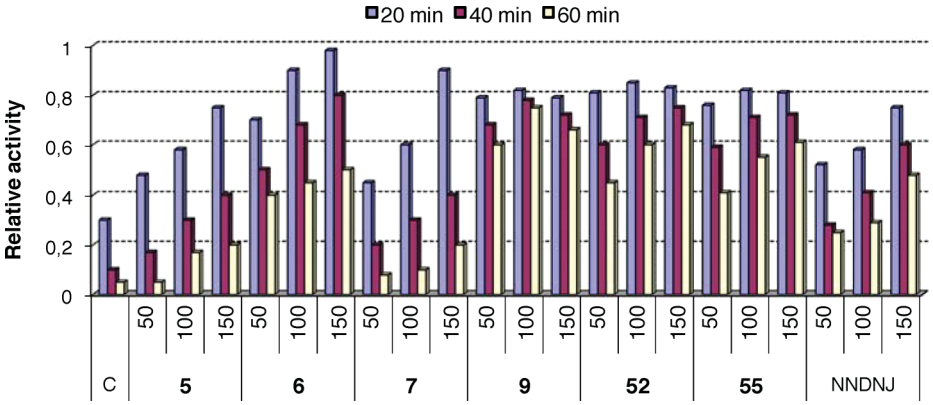


Fig. 4 Relative enzymatic activity after thermal denaturation (48 °C) for 20 min, 40 min, and 60 min at the indicated inhibitor concentrations (μM) compared to the corresponding assay at 37 °C. Data for control (C) are obtained as above except that no inhibitor is present.

of the selected sp²-iminosugars at different incubation times (Fig. 4). Untreated GlcCerase lost most of its activity after 1 h of denaturation under these conditions (less than 10% activity remained). Compounds **5**, **6**, **7**, **9**, **52**, and **55** exhibited a remarkable stabilization effect, whereas compounds **8**, **37**, **62**, **10**, **56**, **57**, **71** did not protect the enzyme against thermal inactivation. Accordingly, this protective effect increased gradually with the lipophilicity.

The stabilization activity of the sp²-iminosugars was challenged against that of *N*-(*n*-nonyl)deoxynojirimycin (NNDNJ),[29] an amphiphilic iminosugar currently under investigation as pharmacological chaperone for Gaucher disease. Compound **5** behaved similarly to NNDNJ, whereas compounds **6**, **7**, **9**, **52**, and **55** were significantly more efficient. For example, the recombinant enzyme incubated with 50 μM **6** retained over 40% of the initial activity after 1 h, which is eight-fold higher than the untreated enzyme and about twice as compared with 50 μM NNDNJ-treated enzyme.[32] The protective effect was strongly dose-dependent for the D-*gluco* configured derivatives bearing the *n*-octyl substituent (**5**–**7**), a feature also observed for NNDNJ, but was almost dose-independent in the 50–150 μM range in the case of the branched derivative **52**, the adamantane derivative **55** and, especially, for the bicyclic galactonojirimycin derivative **9**. This might reflect that those compounds have already reached the maximum stabilizing activity at 50 μM, about 80% imiglucerase retained activity after 20 min at 48 °C. Although this compares unfavourably with **6** or **7** (about 100 and 90% retained activity at 150 μM M after 20 min, respectively), it should be noted that **9**, **52** and **55** are much more efficient at protecting the enzyme over longer period of times (*e.g.*, 60% activity after 60 min in the presence of 50 μM of **9** or 70% after 60 min in the presence of 150 μM of **52**; to be compared with only 50% for **6** or NNDNJ after 60 min at 150 μM),[30] which makes them very attractive candidates for further biological studies.

It is worth noting that the ensemble of results above discussed evidences that the inhibitory activity is not necessarily correlated with the enzyme stabilization effect. Thus, compounds **5**–**7** and **8**–**10** very similarly inhibited imiglucerase (Tables 1 and 2) but behaved quite differently in the thermal-denaturation protection assay, with compounds **8** and **10** being actually inactive.

Conclusions

In summary, a general strategy for the preparation of bicyclic nojirimycin and galactonojirimycin related sp²-iminosugars analogues adapted to molecular diversity-oriented schemes has been disclosed and applied to the synthesis of a library of glycomimetics differing in C-4 configuration (D-*gluco* and D-*galacto*), the structure of the five-membered ring (cyclic isourea, isothiourea or guanidine) and the nature of the *N′*-substituent (apolar, polar, linear or branched). Glycosyl hydrolase inhibition studies evidenced a remarkable influence of the nature of the exocyclic substituent on the potency and selectivity of binding towards a range of commercial glycosidases, although selectivity towards β-glucosidases was generally observed. Amphiphilic derivatives bearing hydrophobic *N′*-substituents were much more potent than derivatives bearing polar substituents, which also correlated with potent inhibition of the recombinant human β-glucocerebrosidase (imiglucerase) used in enzyme replacement therapy for Gaucher disease. We have further tested the ability of these compounds to increase imiglucerase stability against thermal denaturation, as an *in vitro* assessment of their potential as pharmacological chaperones. Analysis of the results in comparison with data for the iminosugar derivative NNDNJ evidenced the higher chaperoning potency of sp²-iminosugars and stresses their potential in pharmacological chaperone therapy and combined therapy strategies.

Experimental

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), 400 (100.6), and 500 (125.7) MHz using Bruker DMX300, DRX400, 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 30F-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. 5-Azido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-tetrahydropyranyl-α-D-glucofuranose **11** was prepared from commercial 6,3-D-glucuronolactone following the procedure already reported.[14] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**14**) was synthesized from the corresponding per *O*-acetyl glucopyranosyl bromide following the procedure of Camarasa *et al.*[16] 5-Azido-5-deoxy-1,2-di-*O*-isopropylidene-α-D-galactofuranose **12** was obtained from D-galactose using the reported route.[15] The cyclic thiourea α-D-glucofuranose derivative **64** was prepared from commercial 6,3-D-glucuronolactone following the procedure already reported.[12] The cyclic thiourea α-D-galactofuranose derivative **65** was synthesized from 5-azido-3-*O*-benzoyl-5-deoxy-α-D-galactofuranose following the reported route.[10]

General procedure for the inhibition assay against the commercial enzymes

Inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-glucosidase 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 **37–39**, **52–57**, **62**, **63**, and **70–73**. 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 or a solution of GLC-Trinder (Sigma, for trehalase). 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 Electronic Supplementary Information.†

Imiglucerase inhibition assay

In vitro activity was determined with 4 mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (pH 5.2). Enzyme solutions (25 μL from a stock solution containing 0.1 mg of protein/mL) in the presence of 0.2% (w/v) sodium taurocholate and 0.1% (v/v) TritonX-100 in McIlvaine buffer (pH 5.2) were incubated at 37 °C without (control) or with inhibitor during 30 min, and after addition of corresponding substrate solution (60 μL), incubations were maintained at 37 °C for 10 min. Enzymatic reactions were stopped by the addition of 150 μL of 100 mm glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferone formed was determined with a 1420 VICTOR² Multilabel Counter (Wallac) fluorometer at 355 nm (excitation) and 460 nm (emission). IC_{50} values were determined by plotting percent activity *versus* log [*I*], using at least five different inhibitor concentrations.

Thermal stabilization assay

Following a modification of a reported method,[31] Imiglucerase aliquots (48 μL, 2 mg ml^{-1}) were incubated at pH 7.4 with 0 (control), 150, 100, or 150 μm test compound at 48 °C. Subsequently, 150 μL of 0.1 m acetate–phosphate buffer (pH 5.0) and 100 μL of substrate (4 mM 4-methyllumbelliferyl β-D-glucoside) in McIlvaine buffer (pH 5.2) were added at different times and incubated for 10 min at 37 °C, in the presence of 0.1% Triton X-100 and 0.2% taurodeoxycholic acid. Then an amount of 300 μL of glycine/NaOH buffer (100 mM, pH 10.6) was added and liberated 4-methylumbelliferone was measured. Enzyme activity was reported relative to that of the enzyme at 37 °C.

General procedure for the synthesis of 5-thioureido-α-D-glucofuranose derivatives 30–33 and 40–45. A solution of 5-azido-5-desoxy-1,2-*O*-isopropylidene-6-*O*-tetrahydropyranyl-α-D-glucofuranose[14] **11** (300 mg, 0.91 mmol) in MeOH (5 mL) was hydrogenated at atmospheric pressure for 1 h using 10% Pd/C (90 mg) as catalyst. The suspension was filtered through Celite and concentrated. To a solution of the resulting residue in pyridine (6 mL), Et_3N (0.71 mL, 5.1 mmol, 5.6 eq) and the corresponding isothiocyanate (1.1 mmol, 1.2 eq) were added and the mixture was stirred at room temperature for 18 h. In the case of **30–33** the reaction mixture was cooled at –15 °C, Ac_2O (3 mL) was added dropwise, and the mixture was further stirred for 5 h. Then, the solvent was removed under reduced pressure and the resulting residue coevaporated several times with toluene. The crude product was dissolved in CH_2Cl_2–MeOH (1 : 1, 12 mL) and *p*-toluenesulfonic acid (50 mg, 0.26 mmol, 0.3 eq) was added. The reaction mixture was stirred for 2 h at room temperature, and then diluted with CH_2Cl_2 (8 mL), washed with saturated aqueous $NaHCO_3$ (2 × 8 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography using the eluent indicated in each case.

3-*O*-Acetyl-5-(*N*′-butylthioureido)-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (30). Column chromatography, eluent 50 : 1 CH_2Cl_2–MeOH. Yield: 220 mg (64%). $[\alpha]_D$ –24.7 (*c* 1.0, CH_2Cl_2). R_f 0.32 (20 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 2929, 1747, 1375, 1070 cm^{-1}. UV (CH_2Cl_2) 251 nm (ε_{mM} 15.1). ^1H NMR (500 MHz, $CDCl_3$, 323 K) δ 6.26 (bs, 1 H, N′H), 6.16 (bd, 1 H, $J_{5,NH}$ = 7.9 Hz, NH), 5.88 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.28 (d, 1 H, $J_{3,4}$ = 3.1 Hz, H-3), 4.79 (m, 1 H, H-5), 4.46 (d, 1 H, H-2), 4.44 (dd, 1 H, $J_{4,5}$ = 7.9 Hz, H-4), 3.91 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.5 Hz, H-6a), 3.82 (dd, 1 H, $J_{5,6b}$ = 3.3 Hz, H-6b), 3.33 (m,

2 H, CH_2N), 2.11 (s, 3 H, MeCO), 1.54 (m, 2 H, CH_2CH_2N), 1.48, 1.28 (2 s, 6 H, CMe_2), 1.36 (m, 2 H, CH_2CH_3), 0.92 (t, 3 H, $^3J_{H,H}$ = 7.4 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 182.1 (CS), 169.9 (CO), 112.3 (CMe_2), 104.8 (C-1), 83.4 (C-2), 78.4 (C-4), 75.7 (C-3), 62.7 (C-6), 53.8 (C-5), 43.9 (CH_2N), 30.9 (CH_2CH_2N), 26.6, 26.1 (CMe_2), 21.3 (*Me*CO), 20.0 (CH_2CH_3), 13.6 (CH_3). ESIMS: *m/z* 375 $[M - H]^+$. Anal. Calcd for $C_{16}H_{28}N_2O_6S$: C, 51.05; H, 7.50; N, 7.44. Found: C, 50.88; H, 7.36; N, 7.33%.

3-*O*-Acetyl-5-deoxy-1,2-*O*-isopropylidene-5-[*N*′-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)thioureido]-α-D-glucofuranose (31). Column chromatography, eluent 1 : 1 → 2 : 1 EtOAc–petroleum ether. Yield: 456 mg (77%). $[\alpha]_D$ −6.9 (*c* 1.0, CH_2Cl_2). R_f 0.46 (2 : 1 EtOAc-petroleum ether). UV (CH_2Cl_2) 255 nm (ε_{mM} 12.6). IR (KBr) ν_{max} 2942, 1749, 1375, 1038 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 6.84 (d, 1 H, $J_{NH,5}$ = 8.7 Hz, NH), 6.76 (bd, 1 H, N′H), 5.93 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.55 (t, 1 H, $J_{1',2'} = J_{1',N'H}$ = 9.4 Hz, H-1′), 5.34 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.4 Hz, H-3′), 5.29 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-3), 5.07 (t, 1 H, $J_{4',5'}$ = 9.4 Hz, H-4′), 5.00 (t, 1 H, H-2′), 4.78 (m, 1 H, H-5), 4.50 (d, 1 H, H-2), 4.46 (dd, 1 H, $J_{4,5}$ = 7.8 Hz, H-4), 4.27 (dd, 1 H, $J_{6'a,6'b}$ = 12.4 Hz, $J_{5',6'a}$ = 4.7 Hz, H-6′a), 4.15 (dd, 1 H, $J_{5',6'b}$ = 5.2 Hz, H-6′b), 3.98 (dd, 1 H, $J_{6a,6b}$ = 11.4 Hz, $J_{5,6a}$ = 2.9 Hz, H-6a), 3.85 (dd, 1 H, $J_{5,6b}$ = 2.9 Hz, H-6b), 3.84 (m, 1 H, H-5′), 2.15, 2.09, 2.08, 2.04, 2.03 (s, 15 H, MeCO), 1.51, 1.32 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 184.0 (CS), 171.4, 170.7, 169.9, 169.8, 169.5 (Me*C*O), 112.3 (CMe_2), 104.7 (C-1), 83.4 (C-2), 82.6 (C-1′), 77.9 (C-4), 75.7 (C-3), 73.3 (C-3′), 73.0 (C-5′), 71.0 (C-2′), 68.5 (C-4′), 62.1 (C-6), 61.9 (C-6′), 53.8 (C-5), 26.6, 26.0 (CMe_2), 21.2, 20.7, 20.6, 20.5 (*Me*CO). FABMS: *m/z* 674 (100, $[M + Na]^+$), 652 (15, $[M + H]^+$). Anal. Calcd for $C_{26}H_{38}N_2O_{15}S$: C, 47.99; H, 5.89; N, 4.31. Found: C, 47.84; H, 5.66; N, 4.24%.

3-*O*-Acetyl-5-deoxy-1,2-*O*-isopropylidene-5-(*N*′-phenylthioureido)-α-D-glucofuranose (32). Column chromatography, eluent 100 : 1 → 30 : 1 CH_2Cl_2–MeOH. Yield: 202 mg (56%). $[\alpha]_D$ −51.0 (*c* 1.0, CH_2Cl_2). R_f 0.27 (20 : 1 CH_2Cl_2–MeOH). UV (CH_2Cl_2) 268 nm (ε_{mM} 13.3). IR (KBr) ν_{max} 3052, 1741, 1524, 1370, 1076 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 7.97 (bs, 1 H, N′H), 7.42–7.22 (m, 5 H, Ph), 6.54 (d, 1 H, $J_{5,NH}$ = 9.0 Hz, NH), 5.88 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.25 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-3), 4.94 (m, 1 H, H-5), 4.45 (d, 1 H, H-2), 4.38 (dd, 1 H, $J_{4,5}$ = 7.8 Hz, H-4), 3.90 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.1 Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6b}$ = 3.4 Hz, H-6b), 2.02 (s, 3 H, MeCO), 1.46, 1.27 (2 s, 6 H, CMe_2). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 180.7 (CS), 169.8 (CO), 136.0–125.2 (Ph), 112.4 (CMe_2), 104.6 (C-1), 83.3 (C-2), 78.0 (C-4), 75.7 (C-3), 62.3 (C-6), 54.5 (C-5), 26.7, 26.1 (CMe_2), 21.3 (*Me*CO). FABMS: *m/z* 419 (75, $[M + Na]^+$), 397 (100, $[M + H]^+$). Anal. Calcd for $C_{18}H_{24}N_2O_6S$: C, 54.53; H, 6.10; N, 7.07. Found: C, 54.36; H, 5.99; N, 6.88%.

3-*O*-Acetyl-5-(*N*′-acetyl-*N*′-phenylthioureido)-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (33). Column chromatography, eluent 100 : 1 → 30 : 1 CH_2Cl_2–MeOH. Yield: 136 mg (34%). $[\alpha]_D$ +37.4 (*c* 1.1, CH_2Cl_2). R_f 0.53 (20 : 1 CH_2Cl_2–MeOH). UV (CH_2Cl_2) 277 nm (ε_{mM} 9.1), 230 nm (ε_{mM} 10.3). IR (KBr) ν_{max} 3479, 3090, 2935, 1747, 1677, 1523, 1370, 1217, 1070 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 7.46–7.20 (m, 5 H, Ph), 5.93 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.42 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-3), 4.96 (m, 1 H, H-5), 4.57 (dd, 1 H, $J_{4,5}$ = 9.1 Hz, H-4), 4.49 (d, 1 H, H-2), 3.96 (m, 2 H, H-6a, H-6b), 2.07 (s, 3 H, MeCO), 1.92 (s, 3 H, *Me*CONH), 1.53, 1.30 (2 s, 6 H, CMe_2). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 184.6 (CS), 175.1 (CO amide), 169.8 (CO ester), 142.3–128.9 (Ph), 112.5 (CMe_2), 105.0 (C-1), 83.5 (C-2), 77.9 (C-4), 75.3 (C-3), 62.1 (C-6), 55.5 (C-5), 28.0 (*Me*CONH), 26.8, 26.2 (CMe_2), 21.6 (*Me*CO). FABMS: *m/z* 461 (15, $[M + Na]^+$), 439 (30, $[M + H]^+$). FABMSHR *m/z* 461.136042. Calcd for $C_{20}H_{26}N_2O_7NaS$: 461.135843.

5-[*N*′-(*N*,*N*-Bis(2-hexanamidoethyl)aminoethyl)thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (40). Column chromatography, eluent 40 : 1 → 20 : 1 CH_2Cl_2–MeOH. Yield: 204 mg (47%). $[\alpha]_D$ +35.4 (*c* 1.0, CH_2Cl_2). R_f 0.49 (10 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3296, 2956, 1646, 1374, 1074 cm^{-1}. UV (CH_2Cl_2) 241 nm (ε_{mM} 16.0). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 7.82 (bs, 1 H, N′H), 7.35 (d, 1 H, $J_{5,NH}$ = 8.7 Hz, NH), 6.22 (bs, 2 H, NHCO), 5.92 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.17 (bs, 1 H, OH), 4.63 (m, 1 H, H-5), 4.55 (d, 1 H, H-2), 4.17 (d, 1 H, $J_{3,4}$ = 1.8 Hz, H-3), 4.10 (dd, 1 H, $J_{4,5}$ = 10.1 Hz, H-4), 3.99 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.4 Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6b}$ = 3.2 Hz, H-6b), 3.49 (m, 2 H, CH_2NH), 3.49 (m, 4 H, CH_2NHCO), 2.66 (t, 2 H, $^3J_{H,H}$ = 5.1 Hz, CH_2CH_2NH), 2.55 (t, 4 H, $^3J_{H,H}$ = 5.3 Hz, CH_2CONH), 2.18 (t, 4 H, $^3J_{H,H}$ = 7.3 Hz, CH_2CH_2NHCO), 1.61 (m, 4 H, CH_2CH_2CONH), 1.48, 1.31 (2 s, 6 H, CMe_2), 1.29 (m, 8 H, CH_2), 0.88 (t, 6 H, $^3J_{H,H}$ = 6.7 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 182.5 (CS), 174.5 (CO), 111.5 (CMe_2), 105.1 (C-1), 84.6 (C-2), 80.5 (C-4), 73.9 (C-3), 63.0 (C-6), 54.9 (CH_2CH_2NHCO), 53.6 (C-5), 53.4 (CH_2CH_2NH), 42.7 (CH_2NH), 38.0 (CH_2NHCO), 36.7 (CH_2CONH), 31.4 (CH_2), 26.8, 26.1 (CMe_2), 25.3 (CH_2CH_2CONH), 22.3 (CH_2CH_3), 13.8 (CH_3). FABMS: *m/z* 626 (50, $[M + Na]^+$), 604 (35, $[M + H]^+$). Anal. Calcd for $C_{28}H_{53}N_5O_7S$: C, 55.70; H, 8.85; N, 11.60. Found: C, 55.75; H, 8.68; N, 11.52%.

5-[*N*′-(8-*tert*-Butoxycarbonylaminooctyl)thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (41). Column chromatography, eluent 40 : 1 → 30 : 1 CH_2Cl_2–MeOH. Yield: 219 mg (67%). $[\alpha]_D$ +42.6 (*c* 1.0, CH_2Cl_2). R_f 0.20 (20 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3336, 2977, 1683, 1368, 1074 cm^{-1}. UV (CH_2Cl_2) 246 nm (ε_{mM} 14.1). ^{1}H NMR (400 MHz, $CDCl_3$, 313 K) δ 6.58 (bs, 2 H, NH), 5.93 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.07 (bs, 1 H, OH), 4.65 (m, 1 H, H-5), 4.59 (d, 1 H, H-2), 4.20 (s, 1 H, H-3), 4.11 (m, 1 H, H-4, H-6a), 3.78 (dd, 1 H, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6b}$ = 1.7 Hz, H-6b), 3.45 (m, 2 H, CH_2NHCS), 3.20 (bs, 1 H, NH), 3.10 (m, 2 H, CH_2NHCO), 2.07 (bs, 1 H, OH), 1.60 (m, 2 H, CH_2CH_2NHCS), 1.51, 1.33 (2 s, 6 H, CMe_2), 1.48 (m, 2 H, CH_2CH_2NHCO), 1.46 (s, 9 H, CMe_3), 1.33 (m, 8 H, CH_2). ^{13}C NMR (100.6 MHz, $CDCl_3$, 313 K) δ 181.4 (CS), 156.5 (CO), 111.6 (CMe_2), 104.9 (C-1), 84.6 (C-2), 79.7 (C-4), 79.2 (CMe_3), 73.8 (C-3), 62.3 (C-6), 53.7 (C-5), 44.3 (CH_2NHCS), 40.6 (CH_2NHCO), 29.8, 28.8, 28.7, 28.6 (CH_2), 28.4 (CMe_3), 26.7, 26.0 (CMe_2), 26.5, 26.4 (CH_2). FABMS: *m/z* 528 (100, $[M + Na]^+$), 506 (35, $[M + H]^+$). Anal. Calcd for $C_{23}H_{43}N_3O_7S$: C, 54.63; H, 8.57; N, 8.31. Found: C, 54.44; H, 8.42; N, 8.24%.

5-[*N*′-(4-*tert*-Butoxycarbonylaminobutyl)thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (42). Column chromatography, eluent 30 : 1 → 15 : 1 CH_2Cl_2–MeOH. Yield: 204 mg (70%). $[\alpha]_D$ +45.5 (*c* 1.0, CH_2Cl_2). R_f 0.33 (15 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 2933, 1682, 1367, 1075 cm^{-1}. UV (CH_2Cl_2) 247 nm

(ε_{mM} 12.3). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 6.96 (bs, 1 H, N′H), 6.96 (bd, 1 H, $J_{NH,5}$ = 7.8 Hz, NH), 5.92 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.07 (bs, 1 H, OH), 4.82 (bs, 1 H, NH), 4.58 (d, 1 H, H-2), 4.54 (m, 1 H, H-5), 4.19 (d, 1 H, $J_{3,4}$ = 1.9 Hz, H-3), 4.09 (dd, 1 H, $J_{4,5}$ = 9.8 Hz, H-4), 4.03 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.1 Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6b}$ = 3.0 Hz, H-6b), 3.47 (m, 2 H, CH_2NHCS), 3.11 (m, 2 H, CH_2NHCO), 1.60 (m, 4 H, CH_2), 1.49, 1.31 (2 s, 6 H, CMe_2), 1.49 (s, 9 H, CMe_3). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 181.8 (CS), 156.7 (CO), 111.6 (*C*Me_2), 104.9 (C-1), 84.7 (C-2), 79.8 (*C*Me_3), 79.7 (C-4), 73.8 (C-3), 62.4 (C-6), 53.7 (C-5), 44.1 (*C*H_2NHCS), 40.2 (*C*H_2NHCO), 28.4 (C*Me*$_3$), 27.4 (CH_2), 26.7, 26.0 (C*Me*$_2$), 25.7 (CH_2). FABMS: *m*/*z* 472 (100, [M + Na]$^+$), 450 (15, [M + H]$^+$). Anal. Calcd for $C_{19}H_{35}N_3O_7S$: C, 50.76; H, 7.85; N, 9.35, S, 7.13. Found: C, 50.78; H, 7.93; N, 9.28; S, 6.96%.

5-[*N*′-(4-Adamantan-1-ylcarbonylaminobutyl)thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (43). Column chromatography, eluent 1 : 1 acetone–cyclohexane. Yield: 236 mg (71%). $[\alpha]_D$ +29.5 (*c* 1.0, CH_2Cl_2). R_f 0.44 (1 : 1 acetone-cyclohexane). IR (KBr) ν_{max} 2909, 1627, 1374, 1074 cm^{-1}. UV (CH_2Cl_2) 246 nm (ε_{mM} 9.8). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 6.75 (bd, 1 H, $^3J_{NH,H\text{-}5}$ = 8.6 Hz, NH), 5.92 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.91 (bs, 1 H, N′H), 5.29 (bs, 1 H, OH), 4.60 (d, 1 H, H-2), 4.52 (m, 1 H, H-5), 4.18 (s, 1 H, H-3), 4.12 (d, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.02 (dd, 1 H, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 3.3 Hz, H-6a), 3.83 (dd, 1 H, $J_{5,6b}$ = 3.1 Hz, H-6b), 3.48 (m, 2 H, CH_2NHCS), 3.26 (m, 2 H, CH_2NHCO), 2.05 (bs, 3 H, CH), 1.83 (m, 6 H, CCH_2) 1.73 (m, 6 H, CHCH_2), 1.62 (m, 4 H, CH_2), 1.49, 1.26 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 182.1 (CO), 179.3 (CO), 111.6 (*C*Me_2), 105.0 (C-1), 84.7 (C-2), 79.8 (C-4), 73.8 (C-3), 62.6 (C-6), 53.7 (C-5), 44.0 (*C*H_2NHCS), 40.7 (*C*CONH), 39.3 (CH), 38.8 (*C*H_2NHCO), 36.4 (C*C*H_2), 28.1 (CH*C*H_2), 27.3, 26.8 (CH_2), 26.1, 25.3 (C*Me*$_2$). FABMS: *m*/*z* 534 (100, [M + Na]$^+$), 512 (25, [M + H]$^+$). Anal. Calcd for $C_{25}H_{41}N_3O_6S$: C, 58.68; H, 8.08; N, 8.21; S, 6.27. Found: C, 58.84; H, 7.91; N, 8.12, S, 5.94%.

5-[*N*′-(11-Azido-3,6,9-trioxaundecyl)thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (44). Column chromatography, eluent 40 : 1 → 30 : 1 CH_2Cl_2–MeOH. Yield: 150 mg (48%). $[\alpha]_D$ +43.7 (*c* 1.0, CH_2Cl_2). R_f 0.49 (10 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 2932, 2108, 1375, 1075 cm^{-1}. UV (CH_2Cl_2) 246 nm (ε_{mM} 14.4). ^{1}H NMR (500 MHz, $CDCl_3$, 313 K) δ 6.90 (bs, 2 H, NH), 5.94 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.97 (bs, 1 H, OH), 4.58 (d, 1 H, H-2), 4.56 (m, 1 H, H-5), 4.20 (s, 1 H, H-3), 4.10 (d, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.06 (bd, 1 H, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.82(dd, 1 H, $J_{5,6b}$ = 2.4 Hz, H-6b), 3.79 (m, 2 H, CH_2NH), 3.69 (m, 12 H, OCH_2), 3.44 (t, 1 H, $^3J_{H,H}$ = 5.2 Hz, CH_2N_3), 2.94 (bs, 1 H, NH), 2.94 (bs, 1 H, OH), 1.51, 1.33 (2 s, 6 H, CMe_2). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 182.4 (CS), 111.6 (*C*Me_2), 105.0 (C-1), 84.7 (C-2), 79.8 (C-4), 73.7 (C-3), 71.4, 70.6, 70.5, 70.2, 69.9 (OCH_2), 62.4 (C-6), 53.7 (C-5), 50.8 (CH_2N_3), 44.9 (CH_2NH), 42.6 (*C*$H_2CH_2N_3$), 26.7, 26.1 (C*Me*$_2$). FABMS: *m*/*z* 502 (100, [M + Na]$^+$), 480 (45, [M + H]$^+$). Anal. Calcd for $C_{18}H_{33}N_5O_8S$: C, 45.08; H, 6.94; N, 14.60. Found: C, 44.85; H, 6.76; N, 14.46%.

5-[*N*′-(11-Adamantan-1-ylcarbonylamino-3,6,9-trioxaundecyl)-thioureido]-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (45). Column chromatography, eluent 1 : 2 → 1 : 1 acetone–cyclohexane. Yield: 340 mg (85%). $[\alpha]_D$ +30.4 (*c* 1.0, CH_2Cl_2). R_f 0.36 (1 : 1 acetone-cyclohexane). IR (KBr) ν_{max} 2906, 1634, 1350, 1076 cm^{-1}. UV (CH_2Cl_2) 243 nm (ε_{mM} 16.3). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 7.25 (bs, 2 H, NH), 6.17 (bs, 1 H, NH), 5.93 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.63 (m, 1 H, H-5), 4.57 (d, 1 H, H-2), 4.19 (d, 1 H, $J_{3,4}$ = 1.7 Hz, H-3), 4.10 (dd, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.02 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 3.2 Hz, H-6a), 3.82 (dd, 1 H, $J_{5,6b}$ = 3.2 Hz, H-6b), 3.64 (m, 14 H, OCH_2, CH_2NHCS), 3.45 (m, 2 H, CH_2NHCO), 2.06 (bs, 3 H, CH), 1.86 (m, 6 H, CCH_2), 1.74 (m, 6 H, CH_2), 1.49, 1.32 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 182.5 (CS), 178.8 (CO), 111.5 (*C*Me_2), 105.0 (C-1), 84.7 (C-2), 80.0 (C-4), 73.8 (C-3), 70.7, 70.5, 70.3, 70.1, 69.9 (OCH_2), 62.5 (C-6), 53.7 (C-5), 44.9 (*C*H_2NHCS), 40.7 (*C*CONH), 39.3 (*C*H_2NHCO), 39.2 (CH), 36.5 (C*C*H_2), 28.1 (CH_2), 26.8, 26.1 (C*Me*$_2$). FABMS: *m*/*z* 638 (100, [M + Na]$^+$). Anal. Calcd for $C_{29}H_{49}N_3O_9S$: C, 56.56; H, 8.02; N, 6.82. Found: C, 56.45; H, 7.90; N, 6.72%.

General procedure for the synthesis of (4*R*)-4-(L-treofuranos-4′-yl)-2-amino-2-thiazoline derivatives 34–36 and 46–51. To a solution of the corresponding thioureido derivative **30–33** and **40–45** (0.86 mmol) in pyridine (23 mL) at –20 °C under Ar, methanesulfonic chloride (80 μL, 1.03 mmol, 1.2 eq) was added. The reaction mixture was stirred for 7 h and allowed to warm to 10 °C. Then, ice-water (30 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with iced saturated aqueous $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated. In the case of the mixture of thioureido derivatives **32** and **33** the reaction was deacetylated by the Zemplen method. The resulting residue was purified by column chromatography using the eluent indicated in each case.

(4*R*)-4-[(4′*R*)-3′-*O*-Acetyl-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-butylamino-2-thiazoline (34). Column chromatography, eluent 1 : 4 acetone–cyclohexane, recovering 35% of the starting material. Yield: 185 mg (60%). $[\alpha]_D$ –45.4 (*c* 1.0, CH_2Cl_2). R_f 0.76 (1 : 2 acetone–cyclohexane, 2 elutions). IR (KBr) ν_{max} 3383, 2931, 1747, 1624, 1374, 1078 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 5.23 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-3), 4.48 (d, 1 H, H-2), 4.41 (m, 1 H, H-5), 4.13 (dd, 1 H, $J_{4,5}$ = 9.2 Hz, H-4), 3.45 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 7.3 Hz, H-6a), 3.39 (dd, 1 H, $J_{5,6b}$ = 5.1 Hz, H-6b), 3.20 (t, 2 H, $^3J_{H,H}$ = 7.3 Hz, CH_2N), 2.08 (s, 3 H, MeCO), 1.49 (m, 2 H, CH_2CH_2N), 1.48, 1.28 (2 s, 6 H, CMe_2), 1.33 (m, 2 H, CH_2CH_3), 0.87 (t, 3 H, $^3J_{H,H}$ = 7.4 Hz, CH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.8 (CO), 163.0 (CN), 112.1 (*C*Me_2), 104.9 (C-1), 83.5 (C-2), 80.0 (C-4), 76.5 (C-3), 69.9 (C-5), 44.9 (CH_2N), 37.6 (C-6), 31.9 (*C*H_2CH_2N), 26.7, 26.2 (C*Me*$_2$), 21.0 (*Me*CO), 20.0 (*C*H_2CH_3), 13.7 (CH_3). FABMS: *m*/*z* 359 (100, [M + H]$^+$). Anal. Calcd for $C_{16}H_{26}N_2O_5S$: C, 53.61; H, 7.31; N, 7.82. Found: C, 53.56; H, 7.33; N, 7.67%.

(4*R*)-4-[(4′*R*)-3′-*O*-Acetyl-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)amino-2-thiazoline (35). Column chromatography, eluent 1 : 1 EtOAc–petroleum ether. Yield: 468 mg (86%). $[\alpha]_D$ –13.2 (*c* 1.0, CH_2Cl_2). R_f 0.59 (2 : 1 EtOAc-petroleum ether). IR (KBr) ν_{max} 3462, 2942, 1749, 1631, 1374, 1036 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.31 (d, 1 H, $J_{3,4}$ = 3.0 Hz, H-3), 5.30 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3′), 5.07 (t, 1 H, $J_{4',5'}$ = 9.5 Hz, H-4′), 5.02 (d, 1 H, $J_{1',2'}$ = 9.5 Hz, H-1′), 4.93 (t, 1 H, H-2′), 4.49 (d, 1 H, H-2), 4.37 (m, 1 H, H-5), 4.36 (dd, 1 H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{5',6'a}$ = 4.4 Hz, H-6′a), 4.18 (dd, 1 H, $J_{5',6'b}$ = 1.9 Hz, H-6′b), 4.13 (dd,

1 H, $J_{4,5}$ = 4.5 Hz, H-4), 3.76 (ddd, 1 H, H-5′), 3.53 (dd, 1 H, $J_{6a,6b}$ = 11.1 Hz, $J_{5,6a}$ = 7.3 Hz, H-6a), 3.45 (dd, 1 H, $J_{5,6b}$ = 5.0 Hz, H-6b), 2.05, 2.03, 1.99, 1.96, 1.94 (s, 15 H, MeCO), 1.42, 1.23 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 170.8, 170.0, 169.6, 169.5 (Me*C*O), 161.3 (CN), 112.2 (*C*Me$_2$), 105.1 (C-1), 83.5 (C-1′, C-2), 79.9 (C-4), 75.9 (C-3), 73.2 (C-3′), 73.0 (C-5′), 70.7 (C-2′), 69.6 (C-5), 68.2 (C-4′), 61.8 (C-6′), 37.7 (C-6), 26.7, 26.1 (C*Me*$_2$), 21.4, 21.0, 20.7, 20.6 (*Me*CO). FABMS: *m/z* 655 (25, [M + Na]$^+$), 633 (100, [M + H]$^+$). Anal. Calcd for $C_{26}H_{36}N_2O_{14}S$: C, 49.36; H, 5.74; N, 4.43. Found: C, 49.16; H, 5.56; N, 4.36%.

(4*R*)-4-[(4′*R*)-1′,2′-*O*-Isopropylidene-β-L-threofuranos-4′-yl]-2-phenylamino-2-thiazoline (36). Column chromatography, eluent 60 : 1 → 30 : 1 CH_2Cl_2–MeOH. Yield: 257 mg (67%). $[\alpha]_D$ +23.1 (*c* 0.9, MeOH). R_f 0.30 (20 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3324, 2929, 1629, 1588, 1370, 1070 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ 7.34–6.96 (m, 5 H, Ph), 5.89 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.52 (d, 1 H, H-2), 4.43 (m, 1 H, H-5), 4.18 (d, 1 H, $J_{3,4}$ = 2.6 Hz, H-3), 4.08 (dd, 1 H, $J_{4,5}$ = 9.0 Hz, H-4), 3.45 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 7.2 Hz, H-6a), 3.35 (dd, 1 H, $J_{5,6b}$ = 5.3 Hz, H-6b), 1.44, 1.29 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, CD_3OD) δ 162.4 (CN), 144.5–121.0 (Ph), 112.8 (*C*Me$_2$), 106.4 (C-1), 86.8 (C-2), 82.6 (C-4), 75.6 (C-3), 69.5 (C-5), 35.9 (C-6), 27.1, 26.4 (C*Me*$_2$). FABMS: *m/z* 359 (45, [M + Na]$^+$), 337 (100, [M + H]$^+$). Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C, 57.12; H, 5.99; N, 8.33. Found: C, 57.08; H, 5.89; N, 8.27%.

(4*R*)-2-[2′-[*N*,*N*-Bis(2-hexanamidoethyl)aminoethyl]amino]-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (46). Column chromatography, eluent 9 : 1 : 0 → 7 : 1 : 0 → 70 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O, recovering 35% of the starting material. Yield: 344 mg (70%). $[\alpha]_D$ –9.1 (*c* 1.0, CH_2Cl_2). R_f 0.41 (70 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3286, 2953, 1645, 1374, 1070 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (bs, 1 H, NH), 5.96 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.57 (d, 1 H, H-2), 4.45 (m, 1 H, H-5), 4.29 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-3), 4.11 (dd, 1 H, $J_{4,5}$ = 8.0 Hz, H-4), 3.54 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 7.5 Hz, H-6a), 3.42 (dd, 1 H, $J_{5,6b}$ = 5.6 Hz, H-6b), 3.28 (m, 6 H, C*H*$_2$NH, C*H*$_2$NHCO), 2.62 (m, 2 H, C*H*$_2$CH$_2$NH), 2.54 (t, 4 H, $^3J_{H,H}$ = 5.4 Hz, C*H*$_2$CONH), 2.22 (t, 4 H, $^3J_{H,H}$ = 7.4 Hz, C*H*$_2$CH$_2$NHCO), 1.63 (m, 4 H, C*H*$_2$CH$_2$CONH), 1.48, 1.31 (2 s, 6 H, CMe_2), 1.29 (m, 8 H, CH_2), 0.88 (t, 6 H, $^3J_{H,H}$ = 6.8 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3 (CO), 163.0 (CN), 111.5 (*C*Me$_2$), 105.3 (C-1), 85.3 (C-2), 81.7 (C-4), 75.1 (C-3), 68.2 (C-5), 54.6 (*C*H$_2$CH$_2$NHCO), 52.6 (*C*H$_2$CH$_2$NH), 43.1 (CH$_2$NH), 37.6 (*C*H$_2$NHCO), 37.0 (C-6), 36.5 (*C*H$_2$CONH), 31.6 (CH_2), 26.9, 26.2 (C*Me*$_2$), 25.5 (*C*H$_2$CH$_2$CONH), 22.4 (*C*H$_2$CH$_3$), 14.0 (CH_3). FABMS: *m/z* 608 (75, [M + Na]$^+$), 586 (90, [M + H]$^+$). Anal. Calcd for $C_{28}H_{51}N_5O_6S$: C, 57.41; H, 8.78; N, 11.96. Found: C, 57.33; H, 8.71; N, 11.84%.

(4*R*)-2-(8-*tert*-Butoxycarbonylamino)octylamino-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (47). Column chromatography, eluent 30 : 1 → 20 : 1 CH_2Cl_2–MeOH, recovering 20% of the starting material. Yield: 319 mg (76%). $[\alpha]_D$ –9.0 (*c* 1.0, CH_2Cl_2). R_f 0.47 (10 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3356, 2971, 1697, 1367, 1076 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.99 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.59 (d, 1 H, H-2), 4.58 (bs, 1 H, NH), 4.45 (m, 1 H, H-5), 4.28 (d, 1 H, $J_{3,4}$ = 2.5 Hz, H-3), 4.12 (dd, 1 H, $J_{4,5}$ = 8.1 Hz, H-4), 3.78 (bs, 1 H, OH), 3.56 (dd, 1 H, $J_{6a,6b}$ = 10.9 Hz, $J_{5,6a}$ = 7.4 Hz, H-6a), 3.41 (dd, 1 H, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.25 (m, 2 H, C*H*$_2$N), 3.13 (m, 2 H, C*H*$_2$NHCO), 1.52, 1.35 (2 s, 6 H, CMe_2), 1.56 (m, 2 H, C*H*$_2$CH$_2$N), 1.47 (s, 9 H, CMe_3), 1.49 (m, 2 H, C*H*$_2$CH$_2$NHCO), 1.46 (s, 9 H, CMe_3), 1.33 (m, 8 H, CH_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.3 (CN), 156.0 (CO), 111.5 (*C*Me$_2$), 105.2 (C-1), 85.2 (C-2), 81.9 (C-4), 79.0 (*C*Me$_3$), 75.3 (C-3), 70.5 (C-5), 45.3 (CH$_2$N), 40.6 (*C*H$_2$NHCO), 37.7 (C-6), 30.0, 29.7, 29.0 (CH_2), 28.4 (C*Me*$_3$), 26.9, 26.2 (C*Me*$_2$), 26.7 (CH_2). FABMS: *m/z* 510 (10, [M + Na]$^+$), 488 (100, [M + H]$^+$). Anal. Calcd for $C_{23}H_{41}N_3O_6S$: C, 56.65; H, 8.47; N, 8.62. Found: C, 56.25; H, 8.34; N, 8.45%.

(4*R*)-2-(4-*tert*-Butoxycarbonylamino)butylamino-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (48). Column chromatography, eluent 20 : 1 → 10 : 1 CH_2Cl_2–MeOH. Yield: 291 mg (78%). $[\alpha]_D$ –7.3 (*c* 1.0, CH_2Cl_2). R_f 0.46 (7 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3372, 2934, 1696, 1367, 1166 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.79 (bs, 1 H, NH), 4.54 (d, 1 H, H-2), 4.42 (m, 1 H, H-5), 4.25 (bs, 2 H, OH, NH), 4.23 (d, 1 H, $J_{3,4}$ = 2.5 Hz, H-3), 4.03 (dd, 1 H, $J_{4,5}$ = 8.4 Hz, H-4), 3.51 (dd, 1 H, $J_{6a,6b}$ = 10.9 Hz, $J_{5,6a}$ = 7.4 Hz, H-6a), 3.39 (dd, 1 H, $J_{5,6b}$ = 4.9 Hz, H-6b), 3.24 (m, 2 H, C*H*$_2$N), 3.11 (m, 2 H, C*H*$_2$NHCO), 1.53 (m, 4 H, CH_2), 1.48, 1.30 (2 s, 6 H, CMe_2), 1.33 (s, 9 H, CMe_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.6 (CN), 156.1 (CO), 111.5 (*C*Me$_2$), 105.1 (C-1), 85.2 (C-2), 81.6 (C-4), 79.3 (*C*Me$_3$), 74.9 (C-3), 69.8 (C-5), 44.9 (CH$_2$N), 40.0 (*C*H$_2$NHCO), 37.4 (C-6), 28.4 (C*Me*$_3$), 27.3, 26.8 (CH_2), 26.8, 26.1 (C*Me*$_2$). FABMS: *m/z* 454 (50, [M + Na]$^+$), 432 (100, [M + H]$^+$). Anal. Calcd for $C_{19}H_{33}N_3O_6S$: C, 52.88; H, 7.71; N, 9.74; S, 7.43. Found: C, 52.66; H, 7.65; N, 9.48; S, 7.19%.

(4*R*)-2-(4-Adamantan-1-ylcarbonylamino)butylamino-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (49). Column chromatography, eluent 20 : 1 → 15 : 1 → 7 : 1 CH_2Cl_2–MeOH. Yield: 315 mg (74%). $[\alpha]_D$ –6.0 (*c* 1.0, CH_2Cl_2). R_f 0.45 (7 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3344, 2907, 1620, 1373, 1075 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.86 (t, 1 H, $^3J_{NH,H}$ = 5.5 Hz, NH), 4.52 (d, 1 H, H-2), 4.42 (m, 1 H, H-5), 4.24 (d, 1 H, $J_{3,4}$ = 2.5 Hz, H-3), 4.05 (dd, 1 H, $J_{4,5}$ = 8.5 Hz, H-4), 3.51 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 7.4 Hz, H-6a), 3.39 (dd, 1 H, $J_{5,6b}$ = 5.1 Hz, H-6b), 3.25 (m, 4 H, C*H*$_2$N, C*H*$_2$NHCO), 2.02 (bs, 3 H, CH), 1.82 (m, 6 H, CCH_2) 1.69(m, 6 H, CHC*H*$_2$), 1.54 (m, 4 H, CH_2), 1.47, 1.29 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.4 (CO), 165.0 (CN), 111.5 (*C*Me$_2$), 105.2 (C-1), 85.3 (C-2), 81.6 (C-4), 74.9 (C-3), 69.2 (C-5), 45.1 (CH$_2$N), 40.6 (*C*CONH), 39.3 (CH), 38.7 (*C*H$_2$NHCO), 37.2 (C-6), 36.5 (C*C*H$_2$), 28.1 (CH*C*H$_2$), 27.1, 26.7 (CH_2), 26.9, 26.2 (C*Me*$_2$). FABMS: *m/z* 516 (90, [M + Na]$^+$), 494 (100, [M + H]$^+$). Anal. Calcd for $C_{25}H_{39}N_3O_5S$: C, 60.82; H, 7.96; N, 8.51; S, 6.50. Found: C, 60.63; H, 7.79; N, 8.37, S, 6.29%.

(4*R*)-2-(11-Azido-3,6,9-trioxaundecyl)amino-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (50). Column chromatography, eluent 1 : 1 → 2 : 1 acetone–cyclohexane. Yield: 279 mg (70%). $[\alpha]_D$ –7.6 (*c* 1.0, CH_2Cl_2). R_f 0.26 (1 : 1 acetone–cyclohexane). IR (KBr) ν_{max} 3379, 2930, 2107, 1620, 1075 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.97 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.57 (d, 1 H, H-2), 4.43 (m, 1 H, H-5), 4.25 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-3), 4.08 (dd, 1 H, $J_{4,5}$ = 8.2 Hz, H-4), 3.65 (m, 12 H, OCH_2), 3.54 (dd, 1 H, $J_{6a,6b}$ = 10.9 Hz, $J_{5,6a}$ = 7.4 Hz, H-6a), 3.42(m, 5 H, H-6b,

CH_2N_3, CH_2N), 1.50, 1.29 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 163.7 (CN), 111.4 (CMe_2), 105.2 (C-1), 85.1 (C-2), 82.0 (C-4), 75.4 (C-3), 71.4, 71.1 (OCH_2), 70.7 (C-5), 70.6, 70.0, 69.6 (OCH_2), 50.7 (CH_2N_3), 44.6 (CH_2N), 42.7 ($CH_2CH_2N_3$), 37.9 (C-6), 26.9, 26.2 (CMe_2). FABMS: m/z 484 (65, $[M + Na]^+$), 462 (80, $[M + H]^+$). Anal. Calcd for $C_{18}H_{31}N_5O_7S$: C, 46.84; H, 6.77; N, 15.17. Found: C, 46.65; H, 6.48; N, 14.99%.

(4*R*)-2-(11-Adamantan-1-ylcarbonylamino-3,6,9-trioxaundecyl)amino-4-[(4′*R*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (51). Column chromatography, eluent 20 : 1 → 7 : 1 CH_2Cl_2–MeOH. Yield: 385 mg (46%). $[\alpha]_D$ −9.5 (*c* 1.0, CH_2Cl_2). R_f 0.55 (7 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3356, 2905, 1623, 1375, 1075 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 6.14 (bs, 1 H, NH), 5.95 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.56 (d, 1 H, H-2), 4.43 (m, 1 H, H-5), 4.25 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-3), 4.09 (dd, 1 H, $J_{4,5}$ = 8.1 Hz, H-4), 3.63 (m, 12 H, OCH_2), 3.57 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 5.3 Hz, H-6a), 3.46 (m, 1 H, H-6b), 3.42 (m, 4 H, CH_2N, CH_2NHCO), 2.02 (bs, 3 H, CH), 1.84 (m, 6 H, CCH_2), 1.70 (m, 6 H, CH_2), 1.48, 1.31 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.0 (CO), 163.9 (CN), 111.3 (CMe_2), 105.1 (C-1), 85.0 (C-2), 81.8 (C-4), 75.2 (C-3), 70.4, 70.3, 70.2, 70.0, 69.8 (OCH_2), 69.2 (C-5), 44.6 (CH_2N), 40.4 (CCONH), 39.0 (CH), 38.9 (CH_2NHCO), 37.5 (C-6), 36.4 (CCH_2), 28.0 (CH_2), 26.7, 26.0 (CMe_2). FABMS: m/z 620 (100, $[M + Na]^+$), 598 (20, $[M + H]^+$). Anal. Calcd for $C_{29}H_{47}N_3O_8S$: C, 58.27; H, 7.93; N, 7.03. Found: C, 57.98; H, 7.77; N, 6.81%.

General procedure for the synthesis of 5-*N*,6-*S*-(*N*′-iminomethylidene)-6-thionojirimycin derivatives 37–39 and 52–57. To a solution of the corresponding 2-amino-2-thiazoline precursor **34** and **35** (0.39 mmol) in dry MeOH (3.5 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 deacetylated product was treated with TFA-H_2O (9 : 1, 2.4 mL) for 30 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 isothioureas **16** and **17**. An analogous TFA-catalyzed reaction starting from **36** afforded the phenyl derivative **18**. The corresponding 2-amino-2-thiazoline precursor **46–51** (0.13 mmol), after treatment with TFA-H_2O (9 : 1, 1 mL) for 30 min at 0 °C followed by concentration under reduced pressure, coevaporation several times with water, neutralization with Amberlite IRA-68 (OH-) ion-exchange resin and column chromatography with the eluent indicated in each case afforded the corresponding isothioureas **52–57**. Compounds **53** and **54** were obtained as the corresponding hydrochloride salts by freeze-drying from a solution of hydrochloric acid (pH 5).

5-*N*,6-*S*-(*N*′-Butyliminomethylidene)-6-thionojirimycin (37). Column chromatography, eluent 70 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 86 mg (80%). $[\alpha]_D$ −22.9 (*c* 0.7, H_2O). R_f 0.26 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). ^1H NMR (500 MHz, D_2O) δ 5.52 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.75 (td, 1 H, $J_{4,5} = J_{5,6b}$ = 9.5 Hz, $J_{5,6a}$ = 6.4 Hz, H-5), 3.71 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.55 (dd, 1 H, H-2), 3.45 (dd, 1 H, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.40 (t, 1 H, H-4), 3.18 (m, 2 H, CH_2N), 3.07 (dd, 1 H, H-6b), 1.52 (m, 2 H, CH_2CH_2N), 1.30 (m, 2 H, CH_2CH_3), 0.87 (t, 3 H, $^3J_{H,H}$ = 7.4 Hz, CH_3). ^{13}C NMR (125.7 MHz, D_2O) δ 162.9 (CN), 76.0 (C-1), 74.3 (C-4), 72.8 (C-3), 71.4 (C-2), 59.8 (C-5), 53.9 (CH_2N), 32.0 (C-6), 30.4 (CH_2CH_2N), 19.8 (CH_2CH_3), 13.2 (CH_3). FABMS: m/z 299 (40, $[M + Na]^+$), 277 (100, $[M + H]^+$). Anal. Calcd for $C_{11}H_{20}N_2O_4S$: C, 47.81; H, 7.29; N, 10.14. Found: C, 47.65; H, 7.14; N, 9.99%.

5-*N*,6-*S*-(*N*′-β-D-Glucopyranosyliminomethylidene)-6-thionojirimycin (38). Column chromatography, eluent 6 : 1 : 1 → 6 : 3 : 1 CH_3CN–H_2O–NH_4OH. Yield: 104 mg (70%). $[\alpha]_D$ −15.5 (*c* 0.97, H_2O). R_f 0.39 (6 : 3 : 1 CH_3CN–H_2O–NH_4OH). ^1H NMR (500 MHz, D_2O) δ 5.70 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.37 (d, 1 H, $J_{1',2'}$ = 8.3 Hz, H-1′), 3.87 (m, 2 H, H-5, H-6′a), 3.74 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.9 Hz, H-3), 3.72 (dd, 1 H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{5',6'b}$ = 5.4 Hz, H-6′b), 3.60 (dd, 1 H, H-2), 3.52 (m, 3 H, H-6a, H-3′, H-5′), 3.46 (t, 1 H, $J_{4,5}$ = 9.9 Hz, H-4), 3.44 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.9 Hz, H-4′), 3.36 (t, 1 H, $J_{2',3'}$ = 8.3 Hz, H-2′), 3.17 (t, 1 H, $J_{6a,6b} = J_{5,6b}$ = 10.3 Hz, H-6b). ^{13}C NMR (125.7 MHz, D_2O) δ 168.2 (CN), 93.2 (C-1′), 77.6 (C-5′), 76.1 (C-3′), 75.8 (C-1), 74.4 (C-2′), 74.0 (C-4), 72.6 (C-3), 71.0 (C-2), 69.6 (C-4′), 60.8 (C-6′), 59.9 (C-5), 30.7 (C-6). FABMS: m/z 405 (15, $[M + Na]^+$). Anal. Calcd for $C_{13}H_{22}N_2O_9S$: C, 40.83; H, 5.80; N, 7.33. Found: C, 4.83; H, 5.83; N, 7.32%.

5-*N*,6-*S*-(*N*′-Phenyliminomethylidene)-6-thionojirimycin (39). Column chromatography, eluent 80 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 81 mg (70%). $[\alpha]_D$ −38.0 (*c* 0.5, H_2O). R_f 0.20 (9 : 1 CH_2Cl_2–MeOH). ^1H NMR (500 MHz, CD_3OD) δ 7.22 (t, 2 H, Ph), 7.00 (t, 1 H, Ph), 6.88 (d, 2 H, Ph), 5.72 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.82 (td, 1 H, $J_{4,5} = J_{5,6b}$ = 9.5 Hz, $J_{5,6a}$ = 6.5 Hz, H-5), 3.71 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.45 (dd, 1 H, H-2), 3.35 (dd, 1 H, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.29 (t, 1 H, H-4), 3.00 (dd, 1 H, H-6b). ^{13}C NMR (125.7 MHz, CD_3OD) δ 162.3 (CN), 152.6–123.0 (Ph), 77.8 (C-1), 76.3 (C-4), 74.8 (C-3), 73.4 (C-2), 61.1 (C-5), 31.8 (C-6). FABMS: m/z 319 (75, $[M + Na]^+$). Anal. Calcd for $C_{13}H_{16}N_2O_4S$: C, 52.69; H, 5.44; N, 9.45. Found: C, 52.50; H, 5.13; N, 9.29%.

5-*N*,6-*S*-(*N*′(*N*,*N*-Bis(2-hexanamidoethyl)aminoethyl)iminomethylidene)-6-thionojirimycin (52). Column chromatography, eluent 60 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 64 mg (90%). $[\alpha]_D$ −15.5 (*c* 1.0, H_2O). R_f 0.41 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). ^1H NMR (500 MHz, D_2O) δ 5.62 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 3.85 (m, 1 H, H-5), 3.74 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.56 (dd, 1 H, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6a}$ = 6.5 Hz, H-6a), 3.45 (m, 6 H, CH_2NCS, CH_2NHCO), 3.44 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.25 (m, 2 H, CH_2NCS), 3.18 (m, 4 H, CH_2CH_2NHCO), 3.17 (t, 1 H, $J_{5,6b}$ = 10.5 Hz, H-6b), 2.16 (t, 4 H, $^3J_{H,H}$ = 7.5 Hz, CH_2CONH), 1.47 (m, 4 H, CH_2CH_2CONH), 1.18 (m, 8 H, CH_2), 0.76 (t, 6 H, $^3J_{H,H}$ = 7.0 Hz, CH_3). ^{13}C NMR (125.7 MHz, D_2O) δ 178.3 (CO), 162.9 (CN), 75.9 (C-1), 74.3 (C-4), 72.6 (C-3), 71.3 (C-2), 60.5 (C-5), 54.4 (CH_2CH_2NCS), 54.0 (CH_2CH_2NHCO), 48.0 (CH_2NCS), 35.7 (CH_2NHCO), 35.6 (CH_2CONH), 31.1 (C-6), 30.6 (CH_2), 25.0 (CH_2CH_2CONH), 21.7 (CH_2CH_3), 13.2 (CH_3). FABMS: m/z 568 (30, $[M + Na]^+$), 546 (40, $[M + H]^+$). Anal. Calcd for $C_{25}H_{47}N_5O_6S$: C, 55.02; H, 8.68; N, 12.83. Found: C, 55.09; H, 8.66; N, 12.86%.

5-*N*,6-*S*-(*N*′-8′-Aminooctyliminomethylidene)-6-thionojirimycin hydrochloride (53). Column chromatography, eluent 6 : 1 : 1 CH_3CN–H_2O–NH_4OH. Yield: 41 mg (91%). $[\alpha]_D$ −7.0 (*c* 1.0, H_2O). R_f 0.49 (6 : 3 : 1 CH_3CN–H_2O–NH_4OH). ^1H NMR

(500 MHz, D_2O, 313 K) δ 5.76 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.46 (m, 1 H, H-5), 3.92 (m, 2 H, H-3, H-6a), 3.78 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-2), 3.71 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.61 (t, 1 H, $J_{6a,6b} = J_{5,6b}$ = 10.0 Hz, H-6b), 3.57 (t, 2 H, $^3J_{H,H}$ = 7.0 Hz, CH_2N), 3.12 (t, 2 H, $^3J_{H,H}$ = 7.5 Hz, CH_2NH_2), 1.79 (m, 4 H, CH_2), 1.48 (m, 8 H, CH_2). ^{13}C NMR (125.7 MHz, D_2O, 313 K) δ 173.0 (CN), 76.6 (C-1), 73.3 (C-4), 71.9 (C-3), 70.8 (C-2), 63.6 (C-5), 49.2 (CH_2N), 39.8 (CH_2NH_2), 31.4 (C-6), 28.2, 28.1, 28.0, 26.9, 27.8, 25.7 (CH_2). FABMS: m/z 348 (30, $[M + H]^+$). HRFABMS: m/z 348.194640; calcd. for $C_{15}H_{30}N_3O_4S$: 348.195704. Anal. Calcd for $C_{15}H_{30}ClN_3O_4S$: C, 46.92; H, 7.88; N, 10.94; S, 8.35. Found: C, 46.57; H, 7.69; N, 10.61; S, 8.04%.

5-*N*,6-*S*-(*N*′-4′-Aminobutyliminomethylidene)-6-thionojirimycin hydrochloride (54). Column chromatography, eluent 10 : 1 : 1 → 6 : 1 : 1 → 6 : 3 : 1 CH_3CN–H_2O–NH_4OH. Yield: 35 mg (82%). $[\alpha]_D$ −11.3 (*c* 1.0, H_2O). R_f 0.22 (6 : 3 : 1 CH_3CN–H_2O–NH_4OH). ^{1}H NMR (500 MHz, D_2O) δ 5.61 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.32 (m, 1 H, H-5), 3.76 (m, 2 H, H-3, H-6a), 3.62 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-2), 3.56 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.46 (m, 3 H, H-6b, CH_2N), 3.00 (t, 2 H, $^3J_{H,H}$ = 7.1 Hz, CH_2NH_2), 1.73 (m, 4 H, CH_2). ^{13}C NMR (125.7 MHz, D_2O) δ 175.7 (CN), 79.0 (C-1), 75.7 (C-4), 74.2 (C-3), 73.1 (C-2), 66.0 (C-5), 50.6 (CH_2N), 41.5 (CH_2NH_2), 33.9 (C-6), 27.7, 26.5 (CH_2). FABMS: m/z 314 (40, $[M + Na - HCl]^+$), 292 (90, $[M + H - Cl]^+$). Anal. Calcd for $C_{11}H_{22}ClN_3O_4S$: C, 40.30; H, 6.76; N, 12.82; S, 9.78. Found: C, 39.95; H, 6.47; N, 12.49; S, 9.41%.

5-*N*,6-*S*-(*N*′-4′-(Adamantane-1-carbonylamino)butylimino-methylidene)-6-thionojirimycin (55). Column chromatography, eluent 40 : 10 : 1 CH_2Cl_2–H_2O–MeOH. Yield: 58 mg (99%). $[\alpha]_D$ −7.1 (*c* 1.0, H_2O). R_f 0.28 (40 : 10 : 1 CH_2Cl_2–H_2O–MeOH). ^{1}H NMR (500 MHz, D_2O, 313 K) δ 5.73 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.38 (m, 1 H, H-5), 3.91 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.87 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 7.5 Hz, H-6a), 3.74 (dd, 1 H, $J_{2,3}$ = 9.5 Hz, H-2), 3.67 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.53 (m, 3 H, H-6b, CH_2N), 3.33 (t, 2 H, $^3J_{H,H}$ = 6.7 Hz, CH_2NHCO), 2.14 (bs, 3 H, CH), 1.92 (m, 6 H, CCH_2) 1.83 (m, 6 H, $CHCH_2$), 1.67 (m, 2 H, CH_2). ^{13}C NMR (125.7 MHz, D_2O, 313 K) δ 182.0 (CO), 173.0 (CN), 76.6 (C-1), 73.5 (C-4), 72.0 (C-3), 70.9 (C-2), 63.1 (C-5), 49.3 (CH_2N), 40.8 (*C*CONH), 38.8 (CH), 38.6 (CH_2NHCO), 36.1 (CCH_2), 31.4 (C-6), 28.0 ($CHCH_2$), 25.9, 25.6 (CH_2). FABMS: m/z 476 (20, $[M + Na]^+$), 454 (10, $[M + H]^+$). Anal. Calcd for $C_{22}H_{35}ClN_3O_5S$: C, 58.25; H, 7.78; N, 9.26; S, 7.07. Found: C, 58.17; H, 7.66; N, 9.05; S, 6.84%.

5-*N*,6-*S*-(*N*′-11-Azido-3,6,9-trioxaundecyliminomethylidene)-6-thionojirimycin (56). Column chromatography, eluent 70 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 43 mg (78%). $[\alpha]_D$ −13.0 (*c* 1.0, H_2O). R_f 0.57 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). ^{1}H NMR (500 MHz, D_2O) δ 5.55 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.73 (m, 14 H, H-3, H-5, OCH_2), 3.54 (dd, 1 H, $J_{2,3}$ = 9.8 Hz, H-2), 3.45 (m, 2 H, CH_2N), 3.45 (dd, 1 H, $J_{6a,6b}$ = 10.7 Hz, $J_{5,6a}$ = 6.3 Hz, H-6a), 3.39 (t, 1 H, $J_{4,5}$ = 9.7 Hz, H-4), 3.35 (m, 2 H, CH_2N_3), 3.05 (t, 1 H, $J_{5,6b}$ = 10.7 Hz, H-6b). ^{13}C NMR (125.7 MHz, D_2O) δ 166.1 (CN), 78.3 (C-1), 77.0 (C-4), 75.3 (C-3), 73.9 (C-2), 73.4, 73.1, 72.1, 72.0, 71.8 (OCH_2), 62.1 (C-5), 56.0 (CH_2N_3), 52.7 (CH_2N), 45.7 ($CH_2CH_2N_3$), 33.1 (C-1). FABMS: m/z 444 (15, $[M + Na]^+$). HRFABMS: m/z 444.154344; calcd. for $C_{15}H_{27}N_5O_7NaS$: 444.152890. Anal. Calcd for $C_{15}H_{27}N_5O_7S$: C, 42.75; H, 6.46; N, 16.62; S, 7.61. Found: C, 42.51; H, 6.55; N, 16.46; S, 7.39%.

5-*N*,6-*S*-(*N*′-11-Adamantane-1-carbonylamino-3,6,9-trioxaundecyliminomethylidene)-6-thionojirimycin (57). Column chromatography, eluent 80 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–H_2O–MeOH. Yield: 72 mg (99%). $[\alpha]_D$ −6.2 (*c* 1.0, H_2O). R_f 0.51 (40 : 10 : 1 CH_2Cl_2–H_2O–MeOH). ^{1}H NMR (500 MHz, D_2O, 313 K) δ 5.76 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.21 (m, 1 H, H-5), 3.90 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.84 (m, 12 H, OCH_2), 3.79 (dd, 1 H, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 7.1 Hz, H-6a), 3.73 (dd, 1 H, H-2), 3.67 (m, 2 H, CH_2N), 3.63 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.52 (t, 2 H, $^3J_{H,H}$ = 5.5 Hz, CH_2NHCO), 3.42 (t, 1 H, $J_{5,6b}$ = 11.2 Hz, H-6b), 2.15 (bs, 3 H, CH), 1.94 (m, 6 H, CCH_2) 1.85 (m, 6 H, CH_2). ^{13}C NMR (125.7 MHz, D_2O, 313 K) δ 182.0 (CO), 163.0 (CN), 76.4 (C-1), 74.0 (C-4), 72.5 (C-3), 71.2 (C-2), 69.9, 69.8, 69.7, 69.3, 69.2 (OCH_2), 61.9 (C-5), 51.1 (CH_2N), 40.8 (*C*CONH), 39.1 (CH_2NHCO), 38.7 (CH), 36.1 (CCH_2), 31.2 (C-6), 27.9 (CH_2). FABMS: m/z 580 (100, $[M + Na]^+$), 558 (5, $[M + H]^+$). Anal. Calcd for $C_{26}H_{43}N_3O_8S$: C, 55.99; H, 7.77; N, 7.53; S, 5.75. Found: C, 55.68; H, 7.58; N, 7.32; S, 5.39%.

General procedure for the synthesis of 5-thioureido-α-D-galactofuranose derivatives 58 and 59. A solution of 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose[16] **12** (350 mg, 1.43 mmol) in MeOH (7 mL) was hydrogenated at atmospheric pressure for 1 h using 10% Pd/C (123 mg) as catalyst. The suspension was filtered through Celite and concentrated. The resulting residue was dissolved in pyridine (8 mL), Et_3N (0.96 mL, 6.9 mmol, 5.6 eq) and the corresponding isothiocyanate (1.5 mmol, 1.2 eq) 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 using the eluent indicated in each case.

5-(*N*′-Butylthioureido)-5-deoxy-1,2-*O*-isopropylidene-α-D-galactofuranose (58). Column chromatography, eluent 20 : 1 CH_2Cl_2–MeOH. Yield: 344 mg (72%). $[\alpha]_D$ +21.9 (*c* 1.0, CH_2Cl_2). R_f 0.46 (10 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3356, 2933, 1375, 1066 cm^{-1}. UV (CH_2Cl_2) 250 nm (ε_{mM} 15.7). ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 6.59 (bs, 2 H, NH), 5.80 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H-1), 4.69 (m, 1 H, H-5), 4.66 (dd, 1 H, $J_{2,3}$ = 2.8 Hz, H-2), 4.23 (dd, 1 H, $J_{3,4}$ = 6.6 Hz, H-3), 3.96 (m, 1 H, H-4), 3.95 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 3.6 Hz, H-6a), 3.83 (dd, 1 H, $J_{5,6b}$ = 4.4 Hz, H-6b), 3.42 (m, 2 H, CH_2N), 1.62 (m, 2 H, CH_2CH_2N), 1.52, 1.39 (2 s, 6 H, CMe_2), 1.40 (m, 2 H, CH_2CH_3), 0.96 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$, 313 K) δ 182.5 (CS), 113.4 (CMe_2), 104.6 (C-1), 86.6 (C-2), 77.2 (C-4), 75.8 (C-3), 63.9 (C-6), 55.0 (C-5), 44.2 (CH_2N), 30.8 (CH_2CH_2N), 27.7, 27.0 (CMe_2), 20.0 (CH_2CH_3), 13.6 (CH_3). FABMS: m/z 357 (52, $[M + Na]^+$), 335 (100, $[M + H]^+$). Anal. Calcd for $C_{14}H_{26}N_2O_5S$: C, 50.28; H, 7.84; N, 8.38. Found: C, 50.14; H, 7.63; N, 8.25%.

5-Deoxy-1,2-*O*-isopropylidene-5-(*N*′-phenylthioureido)-α-D-galactofuranose (59). Column chromatography, eluent 40 : 1→ 20 : 1 CH_2Cl_2–MeOH. Yield: 350 mg (69%). $[\alpha]_D$ +4.0 (*c* 0.53, CH_2Cl_2). R_f 0.28 (20 : 1 CH_2Cl_2–MeOH). UV (CH_2Cl_2) 264 nm (ε_{mM} 12.7). IR (KBr) ν_{max} 3370, 2929, 1383, 1064 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, 313 K) δ 8.10 (bs, 1 H, N′H), 7.46–7.24 (m, 5 H, Ph), 6.92 (d, 1 H, $J_{5,NH}$ = 7.7 Hz, NH), 5.71 (d, 1 H, $J_{1,2}$ = 4.4 Hz,

H-1), 4.93 (m, 1 H, H-5), 4.64 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, H-2), 4.21 (dd, 1 H, $J_{3,4}$ = 7.7 Hz, H-3), 3.96 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 3.0 Hz, H-6a), 3.85 (m, 2 H, H-6b, H-4), 2.67, 1.84 (2 bs, 2 H, OH), 1.35, 1.27 (2 s, 6 H, CMe_2). ^{13}C NMR (125.7 MHz, $CDCl_3$, 313 K) δ 181.3 (CS), 136.1–124.9 (Ph), 114.1 (CMe_2), 104.3 (C-1), 86.9 (C-2), 84.7 (C-4), 75.8 (C-3), 63.6 (C-6), 55.4 (C-5), 27.7, 27.0 (CMe_2). FABMS: m/z 377 (100, $[M + Na]^+$), 355 (15, $[M + H]^+$). Anal. Calcd for $C_{16}H_{22}N_2O_5S$: C, 54.22; H, 6.26; N, 7.90. Found: C, 54.21; H, 5.98; N, 7.75%.

General procedure for the synthesis of (4*S*)-4-(L-threofuranos-4′-yl)-2-amino-2-thiazoline derivatives 60 and 61. The 2-amino-2-thiazolines **60** and **61** were prepared from the corresponding thioureido derivatives **58** and **59** following the general procedure above described.

(4*S*)-2-Butylamino-4-[(4′*S*)-1′,2′-*O*-isopropylidene-β-L-threofuranos-4′-yl]-2-thiazoline (60). Column chromatography, eluent 80 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 163 mg (60%). $[\alpha]_D$ −29.6 (*c* 1.0, CH_2Cl_2). R_f 0.50 (70 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3361, 2924, 1615, 1375, 1063 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.87 (bs, 1 H, NH), 4.56 (dd, 1 H, $J_{2,3}$ = 1.3 Hz, H-2), 4.53 (m, 1 H, H-5), 4.25 (dd, 1 H, $J_{3,4}$ = 5.2 Hz, H-3), 4.06 (t, 1 H, $J_{4,5}$ = 5.2 Hz, H-4), 3.41 (d, 2 H, $J_{5,6}$ = 7.8 Hz, H-6), 3.21 (m, 2 H, CH_2N), 1.54 (m, 2 H, CH_2CH_2N), 1.51, 1.24 (2 s, 6 H, CMe_2), 1.35 (m, 2 H, CH_2CH_3), 0.91 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.2 (CN), 113.4 (CMe_2), 104.6 (C-1), 87.6 (C-2), 86.6 (C-4), 74.8 (C-3), 68.7 (C-5), 46.0 (CH_2N), 34.0 (C-6), 31.6 (CH_2CH_2N), 27.4, 26.5 (CMe_2), 19.8 (CH_2CH_3), 13.5 (CH_3). FABMS: m/z 339 (30, $[M + Na]^+$), 317 (100, $[M + H]^+$). Anal. Calcd for $C_{14}H_{24}N_2O_4S$: C, 53.14; H, 7.65; N, 8.85. Found: C, 53.16; H, 7.56; N, 8.78%.

(4*S*)-4-[(4′*S*)-1′,2′-*O*-Isopropylidene-β-L-threofuranos-4′-yl]-2-phenylamino-2-thiazoline (61). Column chromatography, eluent 50 : 1 → 20 : 1 CH_2Cl_2–MeOH. Yield: 150 mg (52%). $[\alpha]_D$ −86.3 (*c* 1.0, CH_2Cl_2). R_f 0.30 (20 : 1 CH_2Cl_2–MeOH). IR (KBr) ν_{max} 3361, 2962, 1621, 1375, 1015 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ 7.29–6.99 (m, 5 H, Ph), 5.90 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 4.56 (dd, 1 H, $J_{2,3}$ = 0.75 Hz, H-2), 4.36 (m, 1 H, H-5), 4.22 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, H-3), 3.94 (dd, 1 H, $J_{4,5}$ = 8.1 Hz, H-4), 3.43 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 7.5 Hz, H-6a), 3.19 (dd, 1 H, $J_{5,6b}$ = 6.8 Hz, H-6b), 1.51, 1.33 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, CD_3OD) δ 163.0 (CN), 147.7-122.0 (Ph), 114.1 (CMe_2), 106.6 (C-1), 89.5 (C-2), 88.9 (C-4), 77.1 (C-3), 66.6 (C-5), 33.3 (C-6), 27.5, 26.5 (CMe_2). FABMS: m/z 359 (50, $[M + Na]^+$), 337 (100, $[M + H]^+$). Anal. Calcd for $C_{16}H_{20}N_2O_4S$: C, 57.12; H, 5.99; N, 8.33. Found: C, 56.93; H, 5.75; N, 8.21%.

Synthesis of 5-*N*,6-*S*-(*N*′-iminomethylidene)-6-thiogalactonojirimycin derivatives 62 and 63. The isothioureas **60** and **61** were prepared from the corresponding 2-amino-2-thiazoline precursor (**60** and **61**) following the method B in general procedure above described.

5-*N*,6-*S*-(*N*′-Butyliminomethylidene)-6-thiogalactonojirimycin (62). Column chromatography, eluent 60 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 31 mg (86%). $[\alpha]_D$ −5.5 (*c* 0.6, H_2O). $[\alpha]_{546}$ −7.3 (*c* 0.6, H_2O). R_f 0.21 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). ^{1}H NMR (500 MHz, D_2O) δ 5.61 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.51 (t, 1 H, $J_{5,6}$ = 9.0 Hz, H-5), 4.05 (bs, 1 H, H-4), 3.93 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 1.8 Hz, H-3), 3.83 (dd, 1 H, H-2), 3.49 (d, 2 H, H-6a, H-6b), 3.34 (t, 2 H, $^3J_{H,H}$ = 6.8 Hz, CH_2N), 1.61 (m, 2 H, CH_2CH_2N), 1.33 (m, 2 H, CH_2CH_3), 0.89 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (125.7 MHz, D_2O) δ 170.4 (CN), 76.0 (C-1), 69.1 (C-3), 68.9 (C-4), 67.4 (C-3), 61.8 (C-5), 50.0 (CH_2N), 30.6 (CH_2CH_2N), 27.4 (C-6), 19.3 (CH_2CH_3), 12.9 (CH_3). FABMS: m/z 299 (55, $[M + Na]^+$), 277 (100, $[M + H]^+$). Anal. Calcd for $C_{11}H_{20}N_2O_4S$: C, 47.81; H, 7.29; N, 10.14. Found: C, 47.69; H, 7.17; N, 9.94%.

5-*N*,6-*S*-(*N*′-Phenyliminomethylidene)-6-thiogalactonojirimycin (63). Column chromatography, eluent 80 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 23 mg (60%). $[\alpha]_D$ −25.5 (*c* 0.5, H_2O). R_f 0.55 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). ^{1}H NMR (500 MHz, D_2O) δ 7.38–7.01 (m, 5 H, Ph), 5.74 (bs, 1 H, H-1), 4.23 (bt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ = 9.0 Hz, 6.5 Hz, H-5), 4.04 (bs, 1 H, H-4), 3.95 (bd, 1 H, $J_{2,3}$ = 9.3 Hz, H-3), 3.88 (bd, 1 H, H-2), 3.30 (bt, 1 H, $J_{6a,6b}$ = 9.0 Hz, H-6a), 3.20 (bt, 1 H, H-6b). ^{13}C NMR (125.7 MHz, D_2O) δ 173.9 (CN), 149.0–122.5 (Ph), 75.8 (C-1), 69.7 (C-3), 68.8 (C-4), 67.9 (C-2), 59.2 (C-5), 26.7 (C-6). FABMS: m/z 322 (100, $[M + Na + 3H]^+$), 297 (10, $[M + H]^+$). Anal. Calcd for $C_{13}H_{16}N_2O_4S$: C, 52.69; H, 5.44; N, 9.45. Found: C, 52.69; H, 5.38; N, 9.38%.

General procedure for the synthesis of 5,6-di-*N*-(*N*′-iminomethylidene)-α-D-(*galacto*)glucofuranose derivatives 66–69. A solution of **64**[1] or **65**[2] (0.73 mmol) and the corresponding amine (1.5 eq) in DMF (15 mL) was heated at 70 °C under Ar for 18 h and concentrated. The resulting residue was purified by column chromatography using the eluent indicated in each case. Excepting **66**, the 5,6-di-*N*-(*N*′-iminomethylidene)-α-D-glycofuranose derivatives were obtained as the corresponding hydrochloride salt by freeze-drying from a solution of hydrochloric acid (pH 5).

5,6-Diamino-5,6-di-*N*-(*N*′-butyliminomethylidene)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (66). Column chromatography, eluent 80 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 166 mg (76%). $[\alpha]_D$ −45.4 (*c* 1.0, CH_2Cl_2). R_f 0.49 (70 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3284, 2959, 1673, 1375, 1075 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ 5.95 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.53 (d, 1 H, H-2), 4.35 (m, 1 H, H-5), 4.26 (dd, 1 H, $J_{4,5}$ = 4.9 Hz, $J_{3,4}$ = 3.0 Hz, H-4), 4.20 (d, 1 H, H-3), 3.82 (dd, 1 H, $J_{6a,6b}$ = 9.9 Hz, $J_{5,6a}$ = 7.0 Hz, H-6a), 3.78 (t, 1 H, $J_{5,6b}$ = 9.9 Hz, H-6b), 3.23 (t, 2 H, $^3J_{H,H}$ = 7.0 Hz, CH_2N), 1.60 (m, 2 H, CH_2CH_2N), 1.48, 1.32 (2 s, 6 H, CMe_2), 1.42 (m, 2 H, CH_2CH_3), 0.98 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 160.2 (CN), 113.0 (CMe_2), 106.6 (C-1), 86.8 (C-2), 82.5 (C-4), 75.4 (C-3), 55.6 (C-5), 46.1 (C-6), 43.6 (CH_2N), 32.2 (CH_2CH_2N), 27.2, 26.4 (CMe_2), 20.8 (CH_2CH_3), 13.9 (CH_3). FABMS: m/z 300 (100, $[M + H]^+$). Anal. Calcd for $C_{14}H_{25}N_3O_4$: C, 56.17; H, 8.42; N, 14.04. Found: C, 55.96; H, 8.65; N, 13.94%.

5,6-Diamino-5,6-di-*N*-(*N*′-benzyliminomethylidene)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-glucofuranose hydrochloride (67). Column chromatography, eluent 90 : 10 : 1 → 40 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 175 mg (65%). $[\alpha]_D$ −15.4 (*c* 1.0, CH_2Cl_2). R_f 0.63 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3292, 2985, 1669, 1378, 1074 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ 7.43–7.33 (m, 5 H, Ph), 5.95 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.52 (d, 1 H, H-2), 4.45 (s, 2 H, CH_2Ph), 4.38 (ddd, 1 H, $J_{5,6b}$ = 10.1 Hz, $J_{5,6a}$ = 6.7 Hz, $J_{4,5}$ = 4.8 Hz, H-5), 4.26 (dd, 1 H, $J_{3,4}$ = 3.0 Hz, H-4), 4.17 (d, 1 H, H-3), 3.85 (dd, 1 H, $J_{6a,6b}$ = 10.2 Hz, H-6a), 3.80 (t, 1 H,

H-6b), 1.48, 1.32 (2 s, 6 H, CMe_2). ^{13}C NMR (75.5 MHz, CD_3OD) δ 160.3 (CN), 137.5–128.4 (Ph), 113.0 (CMe_2), 106.6 (C-1), 86.9 (C-2), 82.5 (C-4), 75.5 (C-3), 55.8 (C-5), 47.3 (CH_2Ph), 46.2 (C-6), 27.2, 26.4 (CMe_2). FABMS: m/z 334 (100, $[M + H - Cl]^+$). Anal. Calcd for $C_{17}H_{24}ClN_3O_4$: C, 55.21; H, 6.54; N, 11.36. Found: C, 55.15; H, 6.56; N, 11.29%.

5,6-Diamino-5,6-di-*N*-(*N′*-butyliminomethylidene)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-galactofuranose hydrochloride (68). Column chromatography, eluent 100 : 10 : 1 → 80 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 297 mg (75%). $[\alpha]_D$ −31.6 (*c* 1.0, MeOH). R_f 0.49 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3285, 2954, 1673, 1379, 1068 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ 5.93 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.57 (d, 1 H, H-2), 4.31 (m, 1 H, H-5), 4.07 (d, 1 H, $J_{3,4}$ = 2.6 Hz, H-3), 3.89 (dd, 1 H, $J_{4,5}$ = 8.9 Hz, H-4), 3.87 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 10.0 Hz, H-6a), 3.55 (dd, 1 H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.24 (t, 2 H, $^3J_{H,H}$ = 7.0 Hz, CH_2N), 1.60 (m, 2 H, CH_2CH_2N), 1.53, 1.34 (2 s, 6 H, CMe_2), 1.41 (m, 2 H, CH_2CH_3), 0.98 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (75.5 MHz, CD_3OD) δ 160.5 (CN), 114.0 (CMe_2), 107.1 (C-1), 89.9 (C-4), 88.4 (C-2), 76.3 (C-3), 58.2 (C-5), 46.1 (C-6), 43.7 (CH_2N), 32.2 (CH_2CH_2N), 27.4, 26.2 (CMe_2), 20.7 (CH_2CH_3), 13.9 (CH_3). FABMS: m/z 300 (100, $[M + H - Cl]^+$). Anal. Calcd for $C_{14}H_{26}ClN_3O_4$: C, 50.07; H, 7.80; N, 12.51. Found: C, 50.1; H, 8.03; N, 12.30%.

5,6-Diamino-5,6-di-*N*-(*N′*-benzyliminomethylidene)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-galactofuranose hydrochloride (69). Column chromatography, eluent 100 : 10 : 1 → 70 : 10 : 1 CH_2Cl_2–MeOH–H_2O. Yield: 179 mg (55%). $[\alpha]_D$ −62.0 (*c* 1.0, MeOH). R_f 0.43 (40 : 10 : 1 CH_2Cl_2–MeOH–H_2O). IR (KBr) ν_{max} 3389, 2931, 1674, 1367, 1067 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 7.41–7.33 (m, 5 H, Ph), 5.94 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.58 (d, 1 H, H-2), 4.47 (s, 2 H, CH_2Ph), 4.36 (m, 1 H, H-5), 4.09 (d, 1 H, $J_{3,4}$ = 2.6 Hz, H-3), 3.91 (dd, 1 H, $J_{4,5}$ = 9.1 Hz, H-4), 3.90 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.9 Hz, H-6a), 3.58 (dd, 1 H, $J_{5,6b}$ = 5.9 Hz, H-6b), 1.53, 1.34 (2 s, 6 H, CMe_2). ^{13}C NMR (125.7 MHz, CD_3OD) δ 160.5 (CN), 137.5–128.4 (Ph), 114.0 (CMe_2), 107.1 (C-1), 89.9 (C-4), 88.4 (C-2), 76.3 (C-3), 58.3 (C-5), 47.3 (CH_2Ph), 46.2 (C-6), 27.4, 26.2 (CMe_2). FABMS: m/z 334 (100, $[M + HCl]^+$). HRFABMS: m/z 334.176627; calcd. for $C_{17}H_{24}N_3O_4$: 334.176682. Anal. Calcd for $C_{17}H_{26}ClN_3O_5$: C, 52.64; H, 6.76; N, 10.83. Found: C, 52.64; H, 6.64; N, 10.71%.

General procedure for the synthesis of 6-amino-6-deoxy-5,6-di-*N*-(*N′*-iminomethylidene)(*galacto*)nojirimycin derivatives 70–73. The corresponding 2-amino-2-imidazoline precursor **66–69** (0.41 mmol) was treated with TFA-H_2O (9 : 1, 1.7 mL) for 1 h at 0 °C, concentrated under reduced pressure, coevaporated several times with water, treated with NaOH 0.1 N until pH 8, and subjected to column chromatography with the eluent indicated in each case. The corresponding guanidines were isolated as hydrochloride salt, purified by GPC (Sephadex G-10, 1 : 1 MeOH–H_2O), concentrated, and freeze-dried.

6-Amino-5,6-di-*N*-(*N′*-butyliminomethylidene)-6-deoxynojirimycin hydrochloride (70). Column chromatography, eluent 4 : 1 : 1 CH_3CN–H_2O–NH_4OH. Yield: 103 mg (80%). $[\alpha]_D$ +7.7 (*c* 0.8, H_2O). R_f 0.53 (6 : 3 : 1 CH_3CN–H_2O–AcOH). ^1H NMR (300 MHz, D_2O) δ 5.42 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.95 (m, 1 H, H-5), 3.86 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.5 Hz, H-6a), 3.72 (t, 1 H, $J_{2,3} = J_{3,8}$ = 9.5 Hz, H-3), 3.60 (dd, 1 H, H-2), 3.52 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.50 (t, 1 H, $J_{5,6b}$ = 9.5 Hz, H-6b), 3.23 (t, 2 H, $^3J_{H,H}$ = 7.1 Hz, CH_2N), 1.57 (m, 2 H, CH_2CH_2N), 1.35 (m, 2 H, CH_2CH_3), 0.92 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (75.5 MHz, D_2O) δ 156.6 (CN), 74.6 (C-1), 73.5 (C-4), 72.6 (C-3), 71.7 (C-2), 56.3 (C-5), 46.3 (C-6), 42.8 (CH_2N), 30.3 (CH_2CH_2N), 19.3 (CH_2CH_3), 12.9 (CH_3). FABMS: m/z 260 (100, $[M + Cl]^+$). Anal. Calcd for $C_{11}H_{24}ClN_3O_5$: C, 42.11; H, 7.71; N, 13.39. Found: C, 42.21; H, 7.81; N, 13.31%.

6-Amino-5,6-di-*N*-(*N′*-benzyliminomethylidene)-6-deoxynojirimycin hydrochloride (71). Column chromatography, eluent 4 : 1 : 1 CH_3CN–H_2O–NH_4OH. Yield: 93 mg (65%). $[\alpha]_D$ +4.0 (*c* 1.0, H_2O). R_f 0.29 (4 : 1 : 1 CH_3CN–H_2O–NH_4OH). ^1H NMR (400 MHz, D_2O) δ 7.34 (m, 5 H, Ph), 5.40 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.40 (s, 2 H, CH_2Ph), 3.93 (m, 1 H, H-5), 3.76 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 9.5 Hz, H-6a), 3.66 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.46 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.42 (dd, 1 H, $J_{5,6b}$ = 9.5 Hz, H-6b). ^{13}C NMR (75.5 MHz, D_2O) δ 156.5 (CN), 136.3-127.0 (Ph), 74.4 (C-1), 73.3 (C-4), 72.4 (C-3), 71.4 (C-2), 56.2 (C-5), 46.1 (C-6, CH_2Ph). FABMS: m/z 294 (60, $[M + Cl]^+$). Anal. Calcd for $C_{14}H_{22}ClN_3O_5$: C, 48.35; H, 6.38; N, 12.08. Found: C, 48.19; H, 6.20; N, 11.92%.

6-Amino-5,6-di-*N*-(*N′*-butyliminomethylidene)-6-deoxygalactonojirimycin hydrochloride (72). Column chromatography, eluent 100 : 10 : 0.5 → 100 : 10 : 1 CH_3CN–H_2O–AcOH. Yield: 73 mg (60%). $[\alpha]_D$ +8.0 (*c* 1.0, H_2O). R_f 0.61 (20 : 2 : 1 CH_3CN–H_2O–AcOH). ^1H NMR (500 MHz, D_2O) δ 5.40 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.25 (t, 1 H, $J_{5,6a} = J_{5,6b}$ = 9.7 Hz, H-5), 3.98 (m, 1 H, H-4), 3.82 (dd, 1 H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 3.74 (dd, 1 H, H-2), 3.69 (t, 1 H, $J_{6a,6b}$ = 9.7 Hz, H-6a), 3.56 (t, 1 H, H-6b), 3.15 (t, 2 H, $^3J_{H,H}$ = 7.0 Hz, CH_2N), 1.48 (m, 2 H, CH_2CH_2N), 1.26 (m, 2 H, CH_2CH_3), 0.81 (t, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3). ^{13}C NMR (125.7 MHz, D_2O) δ 155.9 (CN), 74.0 (C-1), 69.2 (C-3), 68.1 (C-4), 67.8 (C-2), 55.7 (C-5), 42.7 (CH_2N), 41.7 (C-6), 30.1 (CH_2CH_2N), 19.2 (CH_2CH_3), 12.9 (CH_3). FABMS: m/z 260 (100, $[M - Cl]^+$). HRFABMS: m/z 260.160864; calcd. for $C_{11}H_{22}N_3O_4$: 260.161031. Anal. Calcd for $C_{11}H_{22}ClN_3O_4$: C, 44.67; H, 7.50; N, 14.21. Found: C, 44.29; H, 7.35; N, 13.93%.

6-Amino-5,6-di-*N*-(*N′*-benzyliminomethylidene)-6-deoxygalactonojirimycin hydrochloride (73). Column chromatography, eluent 50 : 5 : 1 → 20 : 2 : 1 CH_3CN–H_2O–AcOH. Yield: 92 mg (68%). $[\alpha]_D$ +7.0 (*c* 0.8, H_2O). R_f 0.32 (20 : 2 : 1 CH_3CN–H_2O–AcOH). ^1H NMR (300 MHz, D_2O) δ 7.39–7.27 (m, 5 H, Ph), 5.45(d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.41 (s, 2 H, CH_2Ph), 4.28 (td, 1 H, $J_{5,6a} = J_{5,6b}$ = 9.8 Hz, $J_{4,5}$ = 1.4 Hz, H-8a), 3.99 (dd, 1 H, $J_{3,4}$ = 2.7 Hz, H-4), 3.84(dd, 1 H, $J_{2,3}$ = 10.2 Hz, H-3), 3.77 (dd, 1 H, H-2), 3.68 (t, 1 H, $J_{6a,6b}$ = 9.8 Hz, H-6a), 3.55 (t, 1 H, H-6b). ^{13}C NMR (75.5 MHz, D_2O) δ 156.7 (CN), 136.1–127.6 (Ph), 74.8 (C-1), 69.7 (C-3), 68.7 (C-4), 68.4 (C-2), 56.4 (C-5), 46.4 (CH_2Ph), 42.5 (C-6). FABMS: m/z 294 (100, $[M - Cl]^+$). HRFABMS: m/z 294.144663; calcd. for $C_{14}H_{20}N_3O_4$: 294.145381. Anal. Calcd for $C_{14}H_{22}ClN_3O_5$: C, 48.35; H, 6.38; N, 12.08. Found: C, 48.02; H, 6.16; N, 11.76%.

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