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PAPER

Intramolecular cyclization of alkoxyaminosugars: access to novel glycosidase inhibitor families†

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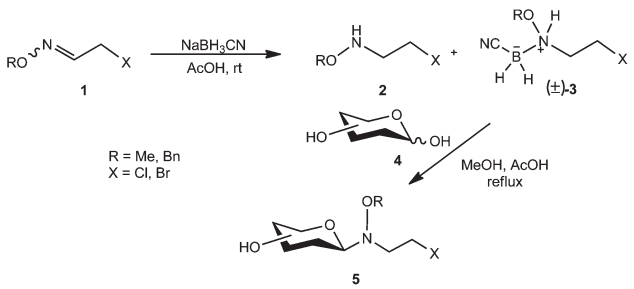
We report the synthesis of two novel families of iminosugars as glycosidase inhibitors involving an intramolecular cyclization between an *N*-alkoxyamino group and a latent aldehyde of a reducing sugar as the key step. Using this methodology we have prepared the hitherto unknown bicyclic polyhydroxylated *N*-(methoxy, benzyloxy)anhydroazepanes and *N*-benzyloxy-D-xylonojirimycin; all these novel compounds turned out to be moderate β-glucosidase inhibitors in a pH-dependent manner.

Introduction

Glycoconjugates, either natural or synthetic, exert a great variety of biological activities, including many of therapeutic interest, where nature of the sugar and its linkage strongly modulate the pharmacology and pharmacokinetic properties of the molecule.[1,2]

The importance of carbohydrates in the borderline between Chemistry, Biochemistry and Medicinal Chemistry has stimulated the development of a vast number of synthetic[3] and biosynthetic[4,5] tools for carrying out glycosylation reactions in which the sugar appendages are incorporated into different scaffolds in chemo- and stereoselective fashions.[3]

Among the arsenal of processes devoted for the preparation of neoglycoconjugates it is worth mentioning the one known as neoglycorandomization,[6,7] a robust methodology which involves a chemoselective ligation of fully unprotected reducing sugars with an alkoxyamine-appended aglycon without any prior activation.[8] This process has received great attention and has been successfully applied to the preparation of glycorandomized libraries of compounds with notable biological activities, such as antitumoral agents[9,10] and antibiotics.[11] In this context, we have recently reported[12] the preparation of functionalized *N*-alkoxyamines **2** by reduction of *O*-alkyloximes **1** with sodium cyanoborohydride in glacial acetic acid (Scheme 1); in this reaction we isolated the first examples of zwitterionic alkoxyamino cyanoboranes **3** as the major components.



Scheme 1 Neoglycosylation reaction from *O*-alkyl oximes.[12]

Boronated alkoxyamines turned out to be excellent cyanoborane transfer agents, and also good glycosyl acceptors in neoglycorandomization reactions with reducing unprotected carbohydrates **4** to afford neoglycosides **5**.

To our knowledge, no intramolecular reaction involving an alkoxyamino group and a latent aldehyde moiety has been reported up to the moment.

Results and discussion

Herein we describe the synthesis of carbohydrate-derived *N*-alkoxyamines and their intramolecular cyclization involving a latent aldehyde of a reducing sugar under acidic conditions in order to afford unprecedented polyhydroxylated *N*-alkoxyanhydroazepanes and reducing polyhydroxylated *N*-alkoxypiperidines, two novel families of iminosugars with potential glycosidase inhibition properties.

Following the procedure previously developed in our research group,[12] we accomplished the preparation of galactose-derived

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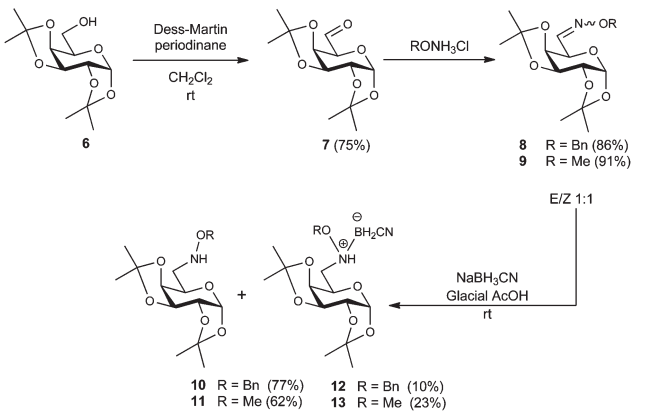
† Electronic supplementary information (ESI) available: Crystallographic data of compound **12**, ^{1}H and ^{13}C NMR of new compounds. CCDC 864830. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25213a

alkoxyamines **10**, **11** starting from their *O*-alkyloxime counterparts **8**, **9** (Scheme 2).

Oxidation of commercially available di-*O*-isopropylidene-protected galactopyranose **6** with Dess–Martin periodinane afforded[13] aldehyde **7** in good yield; condensation of crude **7** with *O*-benzyl and *O*-methyl hydroxylamine hydrochlorides furnished *O*-benzyl and *O*-methyloximes **8** and **9**, as a non-resolved mixture of *E* and *Z* isomers in a 1 : 1 ratio, as deduced from NMR spectra (Scheme 2). The assignment of both isomers is based on the deshielding observed for H-6 of the *E* isomer (*ca.* 0.7 ppm for both, methyl and benzyl derivatives); a similar situation was found for a D-*xylo*-configured oxime.[12]

Both *O*-alkyloximes were reduced using $NaBH_3CN$ in glacial acetic acid to furnish a separable mixture of the D-*galacto*-configured alkoxyamines **10** and **11** (77 and 62% yields, respectively), together with their zwitterionic cyanoborane adducts **12** and **13** as minor compounds (10 and 23% yields, respectively). The outcome of this reaction clearly indicates that the ratio of the aminocyanoborane adducts is strongly affected by the nature of the alkoxyamino moiety; thus boron-containing derivatives **3** (Scheme 1), where the cyanoborane is located on unbranched haloalkyl alkoxyamines, are the major components of the $NaBH_3CN$-mediated reduction. On the contrary, **12** and **13** are minor compounds in the mixtures due to the higher steric hindrance of the pyranose moiety; these compounds are highly polar, with a lower chromatographical mobility on TLC than the alkoxyamino counterparts **10** and **11** (see Experimental), and they are only slightly soluble in CH_2Cl_2 and very soluble in MeOH.



Scheme 2 Preparation of D-*galacto*-configured *N*-alkoxyamines and their boronated counterparts.

The zwitterionic structure of adduct **12** was confirmed by X-ray diffraction; Fig. 1 depicts the ORTEP drawing of **12** showing thermal ellipsoids at the 50% probability level. From the ORTEP it can be deduced that **12** has the *S* configuration at the N-atom, showing a *gauche* relationship between the B–CN bond and N–C6 and N–O bonds. The fact that the crystal structure of **12** only shows the *S* isomer might be due to a selective crystallization from the diastereoisomeric mixture in solution. Tentatively, we suggest that the major isomer in solution corresponds to the *S* derivative.

We accomplished the deprotection of **10** using aqueous TFA for 4 days at rt to furnish transient 6-benzyloxyamino-6-deoxy-galactose **14**, which turned out to be an interesting substrate to study neoglycosylation reactions. NMR analysis of the crude

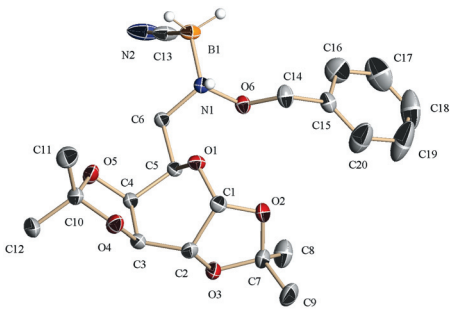
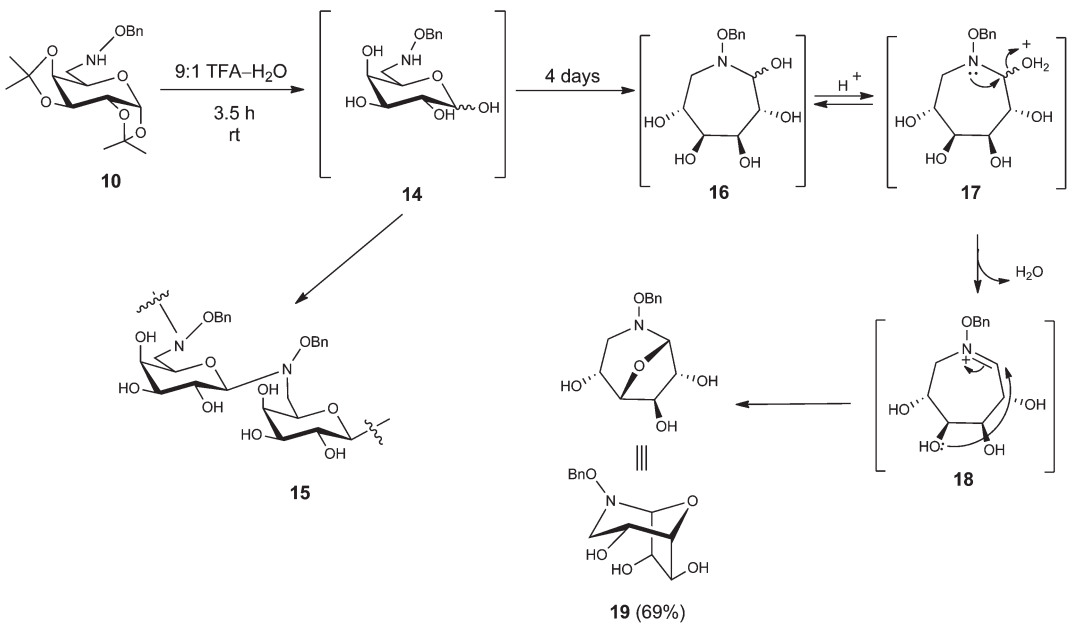


Fig. 1 ORTEP drawing of **12**.



Scheme 3 Intermolecular neoglycosylation reaction *vs.* intramolecular cyclization for compound **10**.

reaction revealed that signals corresponding to the anomeric mixture of the expected fully unprotected derivative **14** disappeared at acidic medium giving access to a new single compound.

Such unexpected transformation can be explained considering the simultaneous presence of an alkoxyamino group and a latent aldehyde in **14**; thus, the neoglycosylation reaction involving such functional groups in acidic medium could furnish an oligomeric or polymeric structure, like **15**. Alternatively, an intramolecular cyclization to afford polyhydroxylated azepane **16** could take place instead (Scheme 3).

Spectroscopical data showed the new compound to be oxazabicyclo-octane **19**, the first example of a polyhydroxylated *N*-alkoxyanhydroazepane. These results suggest that intermolecular neoglycosylation reaction was not favoured for unprotected substrate **14**. Nevertheless, azepane itself **16** was not detected; compound **16** probably evolved to bicyclic derivative **19** *via* an acid-catalyzed dehydration at anomeric position of protonated compound **17**, and subsequent nucleophilic attack of OH-5 on C-2 of the transient iminium cation **18** (Scheme 3).

The only structure similar to **19** found in the literature was reported by Wong and co-workers[14] as a transient intermediate (8-oxa-2-aza-4,6,7-trihydroxybicyclo[3.2.1]octane) in the reduction of 6-azido-D-galactopyranose, which could not be isolated, and was only detected by NMR spectroscopy. Such compound readily evolved in solution under reduction conditions to the corresponding tetrahydroxylated azepane.

Cyclization involving hydroxyl groups on C-3 or C-4 on derivative **18** would lead to highly strained bicyclic compounds (bearing unstable three and four-membered rings), and such compounds are not detected; furthermore, cyclization through hydroxyl group on C-6 is not either favoured, probably due to repulsive 1,3-diaxial interactions involving the hydroxyl group on C-4 and the aminomethylene bridge in compound **20** (Scheme 4).

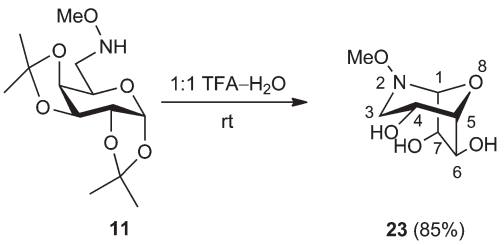
In order to confirm the presence of the fully unprotected 6-alkoxyamino derivative **14** as an intermediate in the formation of **19**, we accomplished the acidic deprotection of **10** for a shorter time, followed by per-acetylation under standard conditions (Scheme 5) to give **21** as a non-resolved mixture of α/β anomers in a 1 : 3.3 ratio. Subsequent deprotection of **21** using methanolic NaOMe allowed removal of the *O*-acetyl groups to furnish *N*-acetyl derivative **22** as an α/β mixture. *E*/*Z* stereoisomers for the acetamido group due to the restriction of free rotation around the N–C bond were detected by NMR.

N-Methoxyamine **11** was deprotected (Scheme 6), yielding to the corresponding azabicyclo-octane **23** in excellent yield (85%).

The bicyclic structure of compounds **19** and **23** was evidenced from their ^{1}H NMR data; four-bond long-range couplings (Table 1) between protons H-1 and H-3$_{eq}$ (1.3 Hz), H-1 and H-5 (0.5 Hz), H-3$_{eq}$ and H-5 (2.1 and H 1.9 Hz) *via* a planar W pathway is in agreement with the chair conformation of the 1,3-oxazinane rings. Another four-bond long-range coupling was also found for H-5 and H-7 (1.0 Hz); these couplings are also supported by 2D-COSY experiments. The structure of these bicyclic compounds was further confirmed by 2D-NOESY experiments, showing a strong correlation between H-3ax and H-6. Furthermore, coupling constants found for both compounds also discard the possible 2,6-anhydroazepane structure **20**, which could have been obtained by cyclization involving hydroxyl on C-6.

Bicyclic anhydroazepanes **19** and **23** exhibited an extraordinary high stability, in contrast with the few related compounds reported so far in the literature;[14] such stability could maybe be explained considering the reduced basicity of *N*-alkoxyamines, which reduces the facility of undergoing a ring-opening reaction.

Stimulated by these results, we also attempted the preparation of 6-membered *N*-alkoxyiminosugars starting from D-*xylo*-

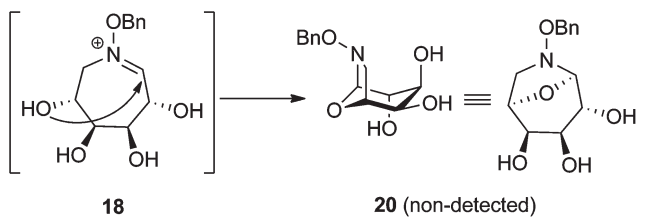


Scheme 6 Synthesis of *N*-methoxyanhydroazepane **23**.

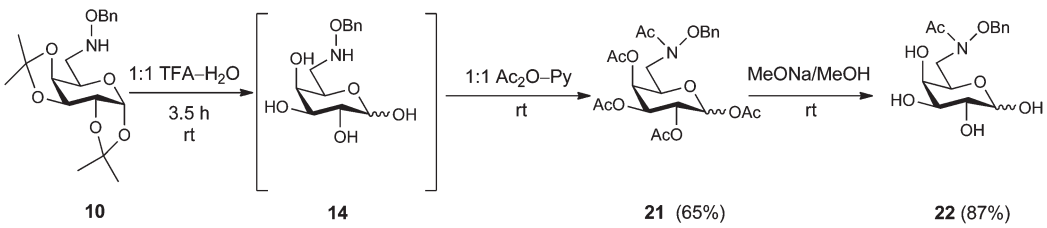
Table 1 Selected NMR data for compounds **19** and **23**[a]

Compound	H-1	$^4J_{1,3eq}$	$^4J_{1,5}$	H-3eq	$^4J_{3eq,5}$	H-3ax	$J_{3ax,4}$
19	4.96	1.3	0.5	3.29	2.1	2.98	11.2
23	5.02	1.3	0.5	3.34	1.9	2.99	11.1

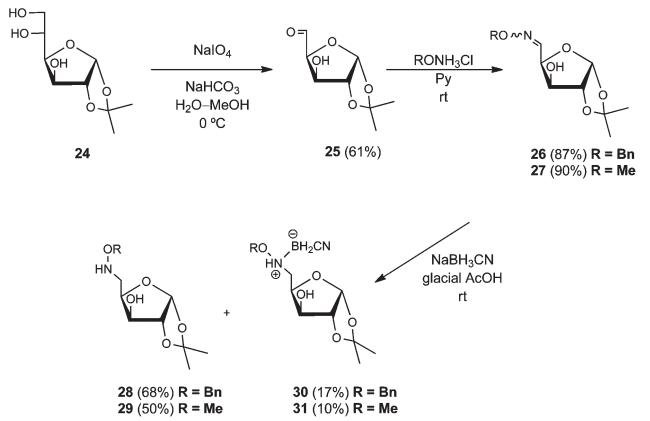
[a] δ in ppm, J in Hz.



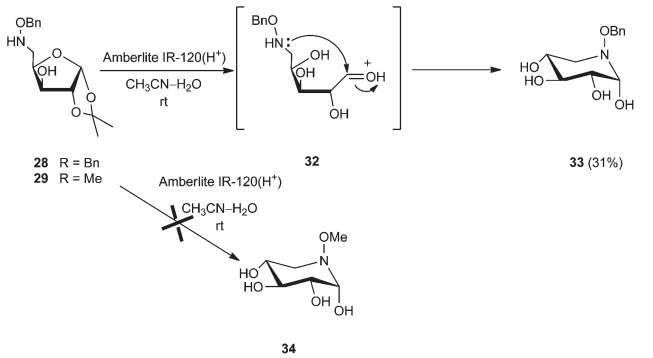
Scheme 4 Another potential intramolecular cyclization on iminium ion **18**.



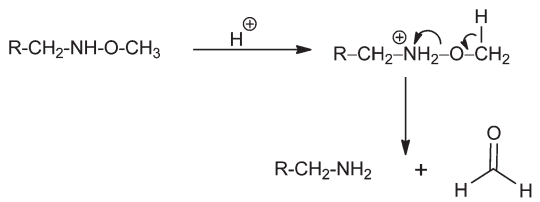
Scheme 5 Trapping of 6-benzyloxyamino galactopyranose as transient intermediate in intramolecular cyclization.



Scheme 7 Synthesis of D-*xylo*-configured 5-alkoxyamines and their cyanoboronated adducts.



Scheme 8 Synthesis of *N*-benzyloxy-D-xylonojirimycin **33**.



Scheme 9 Proposed degradation for the attempted *N*-methoxy-D-xylonojirimycin **34**.

configured aldehyde **25**, readily obtained from commercially-available mono-*O*-isopropylidene-D-glucofuranose **24** by oxidative degradation of the side chain with $NaIO_4$ (Scheme 7).[15]

Treatment of **25** with *O*-benzyl and *O*-methylhydroxylamine in pyridine furnished *O*-alkyloximes **26**[12] and **27** from good to almost quantitative yields, and as a 2 : 1 *E*/*Z* ratio. Reduction of such alkyloximes with $NaBH_3CN$ in glacial acetic acid afforded the expected alkoxyamines **28** and **29**,[12] together with their cyanoboronated adducts **30** and **31**, as minor compounds, in a similar fashion as already observed for D-*galacto* derivatives **12** and **13**.

The conversion of D-*xylo*-configured derivatives **28** and **29** into iminosugars involves the removal of the protective group under acidic conditions. Nevertheless, the deprotection reaction turned out to be more tricky than previously anticipated; standard treatment with aqueous TFA under the same conditions used for the deprotection of **10** and **11** gave complex mixtures. Some other procedures used for the removal of isopropylidene moieties, such as treatment with AcOH–H_2O, NaI/$CeCl_3$/SiO_2 or $(CF_3CO)_2O$/I_2/$FeCl_3$ also furnished very complex mixtures. The extensive decomposition observed under all these conditions came from the N–O bond cleavage, followed by subsequent side-reactions.

To our delight, treatment of *N*-benzyloxy derivative **28** with acidic Amberlite IR-120(H^+) resin under very mild conditions (room temperature for 2 h) led to the formation of *N*-benzyloxy-D-xylonojirimycin **33** *via* an intramolecular cyclization reaction involving the benzyloxyamino group and the latent aldehyde in the transient fully unprotected intermediate **32** (Scheme 8). *N*-Benzyloxy-piperidine **33** was obtained in a 31% yield, after crystallization from the crude reaction medium; it is the first example of a reducing *N*-alkoxyiminosugar. ^{1}H NMR spectrum of **33** showed it to exist exclusively as the α-anomer in solution, as suggested by the small value of the $J_{2,3}$ coupling constant (3.0 Hz); such observation can be explained considering the fact that iminosugar derivatives exhibit stronger anomeric effects than carbohydrates bearing an endocyclic oxygen atom, due to the stronger electron-donating effect of the nitrogen atom when compared with oxygen.[16]

Furthermore, ^{1}H NMR spectra of the crude product did not show any oligomers, so discarding any intermolecular neoglycosylation reaction, and no bicyclic derivatives, indicating that in this case, fused bicyclic structures with an anhydro scaffold are not stable, unlike their D-*galacto* counterparts.

We also tried to extend this strategy to methoxyamino derivative **29**; nevertheless, attempts of deprotection under acidic conditions failed, including the mild conditions derived from the use of the Amberlite IR-120(H^+) resin. In all the cases an extensive decomposition was observed, and the expected compound **34** was observed only as a minor compound, which in turn easily decomposed in solution. NMR spectra proved the lability of the methoxyamino functionality under acidic conditions, and suggested that N–O bond cleavage takes part even prior to the isopropylidene removal.

We suggest that the degradation of the methoxyamino group of **29** occurs by protonation of the amino group, followed by heterolytic cleavage of the N–O bond, releasing the corresponding amino compound and formaldehyde (Scheme 9).

A similar situation, although to a lesser extent, was also observed in the preparation of benzyloxy derivative **33**, as the crude reaction of the acidic deprotection released a smell of benzaldehyde.

Enzymatic inhibition studies

We have evaluated the inhibitory properties of unprotected iminosugar derivatives **19**, **23** and **33** against nine commercially available glycosidases and glycogen phosphorylase type b from rabbit muscle; K_i values are depicted in Table 2.

These data indicate that bicyclic derivative **19**, despite having a very different structure compared with enzyme substrates, exhibits a moderate inhibition against β-glucosidase, although seven times stronger than methoxy counterpart **23**. These results could

Table 2 Inhibitory activities of compounds **19**, **23** and **33**[a] at pH 6.8

Compound enzyme	**19**	**23**	**33**
α-Glucosidase (Baker's yeast)	N.I.[b]	N.I.	N.I.
α-Glucosidase (rice)	N.I.	N.I.	N.I.
β-Glucosidase (almonds)	660	4810	N.I. 146[c]
β-Galactosidase (*E. coli*)	N.I.	N.I.	N.I.
β-Galactosidase (*Aspergillus niger*)	N.I.	N.I.	N.I.
α-Galactosidase (green coffee beans)	N.I.	N.I.	N.I.
α-Manosidase (*Jack beans*)	N.I.	N.I.	N.I.
β-Manosidase (*Helix pomatia*)	N.I.	N.I.	N.I.
α-L-Fucosidase (bovine kidney)	N.I.	N.I.	N.I.
Glycogen phosphorylase b (rabbit muscle)	447	N.I.	—[d]

[a] K_i expressed in μM; [b] No inhibition; [c] At pH 5.0; [d] Not tested.

be explained considering favourable hydrophobic interactions involving the benzyl residue and hydrophobic residues of the enzyme active site. Furthermore, the existence of three hydroxyl groups on **19** might enable hydrogen-bonding interactions with polar motifs of the active site of the enzyme.

N-Benzyloxy polyhydroxylated piperidine **33** showed no inhibition against the tested glycosidases at pH 6.8. Given that *N*-alkoxyamines are less basic than the parent amines, we also tested compound **33** against β-glucosidase at a lower pH (5.0) in order to increase the concentration of its protonated form, which might better interact with the carboxylic residues of the enzyme active site. Under these conditions, a remarkable increase in inhibition was observed, changing from no inhibition at pH 6.8 to exhibit a moderate K_i value (146 μM) at pH 5.0, 4.5 times the activity shown by **19**. *N*-Benzyloxy piperidine **33** might at lower pH values lose the pseudo-anomeric hydroxyl group to furnish a transient iminium cation resembling an oxocarbenium ion of the enzyme substrate that could interact with the active site of the enzyme; this would explain the absence of activity at higher pH values. Moreover, the absence of an exocyclic hydroxymethyl group on C-6 of **33** would explain a relatively low activity against β-glucosidase. Bicyclic oxabicyclo-octanes **19** and **23** when tested at lower pH, showed a decrease in activity; in this case the highly stable structure of both compounds precludes at pH 5.0 the formation of azepane-based iminium cation **18**, whose conformational flexibility would presumably allow a stronger interaction with the enzyme active site and thus, higher activity.

Moreover, anhydroazepane **19** also displayed moderate inhibition of glycogen phosphorylase b (K_i 447 μM), an interesting target for controlling the level of sugar in blood.[17]

Conclusions

In conclusion, using acid catalysis, we have carried out the first examples of intramolecular cyclizations between *N*-alkoxyamino groups and latent aldehydes of reducing sugars to afford novel iminosugar derivatives. Following this methodology, we accessed two novel families of iminosugars: polyhydroxylated *N*-alkoxyanhydroazepanes and reducing *N*-benzyloxy-D-xylono-jirimycin. Such compounds turned out to be moderate β-glucosidase inhibitors in a pH-dependent manner, and one of them also a moderate inhibitor of glycogen phosphorylase type b.

Experimental

General procedures

Optical rotations were measured with a Jasco P-2000 polarimeter and IR spectra (KBr disks) were obtained with FT-IR Bomem MB-120 and FT-IR JASCO-410 spectrophotometers. ^{1}H (300 and 500 MHz) and ^{13}C (75.5 and 125.7 MHz) NMR spectra were recorded on Bruker Avance-300 and Avance-500 spectrometers at rt. The assignments of ^{1}H and ^{13}C signals were confirmed by homonuclear COSY and heteronuclear 2D correlated spectra, respectively. Mass spectra (CI and LSI) were recorded on Micromass AutoSpec-Q mass spectrometer with a resolution of 1000 or 10 000 (10% valley definition). For LSI spectra, ions were produced by a beam of xenon atoms and Cs^+ ions, respectively, using thioglycerol as matrix and NaI as additive. TLC was performed on aluminium pre-coated sheets (E. Merck Silica Gel 60 F_{254}); spots were visualized by UV light, by charring with 10% H_2SO_4 in EtOH or with 3% ninhydrin in EtOH. Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm). Microanalyses were performed at the Instituto de Investigaciones Químicas "Isla de la Cartuja" US-CSIC, using an elemental analyzer *LECO Truspec CHN/CHNS*.

Enzymatic assays were carried out in a Hitachi U-2900 spectrophotometer. Commercially-available α-glucosidase (baker's yeast), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*), β-galactosidase (*Aspergillus oryzae*), α-mannosidase (*Jack beans*), β-mannosidase (*Helix pomatia*), α-L-fucosidase (bovine kidney) and glycogen phosphorylase type b (rabbit muscle) were used as received, without further purification. Assays were accomplished as described previously.[18] Each glycosidase assay was performed by preparing ten 2-mL samples in PS cuvettes containing 0.1 M phosphate buffer (pH 6.8) and the appropriate substrate solution. The concentration of the substrates ranged from 0.25 to 4.0 K_m. Water or inhibitor solution plus water were also added up to a constant volume of 1.9 mL for the K_m or the K_i measurement, respectively. Reaction was started by adding 0.1 mL of dilute enzyme solution at 25 °C and the formation of the *p*-nitrophenolate was monitored for 2 min by measuring the increase of absorbance at 400 nm.

Initial rates were calculated from the slopes of each reaction and were used to obtain two Hanes plots ([S]/*V* *vs.* [S]), one with and another without inhibitor. Inhibition constants (K_i) were obtained from the formula $K_i = [I]/(K_m'/K_m - 1)$, were [I] is the inhibitor concentration in the cuvette and K_m and K_m' are the enzymatic Michaelis–Menten constants in the absence and in the presence of the inhibitor, respectively.

Glycogen phosphorylase activities were assayed in the direction of glycogen synthesis[19] by using commercial glycogen phosphorylase b (rabbit muscle, 10 μg ml^{-1}) at 30 °C in the presence of 1% oyster glycogen, α-D-glucose-1-phosphate, 1 mM AMP, with or without inhibitors at pH 6.8 (50 mM triethanolamine/HCl, 1 mM dithiothreitol and 1 mM EDTA

buffer). P_i concentration was determined using the method developed by Taussky and Shorr.[20] Hanes plots were built using the initial velocity from each reaction, as indicated above for glycosidases.

(*E*- and *Z*)-1,2:3,4-Di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose-6-*O*-benzyloxime (8*E* and 8*Z*)

To a solution of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose **7** (270 mg, 1.05 mmol) in pyridine (4 mL) was added *O*-benzylhydroxylamine hydrochloride (200 mg, 1.25 mmol, 1.2 equiv.), and the mixture was stirred at rt for 5 h. Then it was concentrated to dryness and the residue was dissolved in EtOAc (50 mL) and washed with H_2O (3 × 50 mL); the organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography (1 : 5 EtOAc–hexane) to give **8** as a 1 : 1 *E*/*Z* diastereoisomeric mixture, as deduced from ^{1}H NMR: 340 mg, 86%; R_F 0.31 (1 : 2 EtOAc–hexane 1 : 2); $[\alpha]_D^{23}$ −69 (*c* 0.7, CH_2Cl_2); IR ν_{max} 2989, 2929, 1641, 1451, 1375, 1256, 1211, 1070, 1014, 896, 872, 701 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 − 7.19 (m, 10H, Ar-H), 5.49, 5.48 (2d, 1H each, $J_{1,2}$ = 4.6 Hz, H-1), 5.09 (m, 2H, PhCH_2), 5.04 (s, 2H, PhCH_2), 4.27 (m, 2H, H-2), 1.49, 1.44, 1.39, 1.27 (4s, 24H, C(CH_3)$_2$); **8*E***: δ 7.42 (d, 1H, $J_{5,6}$ = 6.8 Hz, H-6), 4.85–4.56 (m, 1H, H-3), 4.37 (dd, 1H, $J_{4,5}$ = 1.9 Hz, $J_{5,6}$ = 6.8 Hz, H-5), 4.21 (dd, 1H, $J_{3,4}$ = 7.8 Hz, H-4); **8*Z***: δ 6.72 (d, 1H, $J_{5,6}$ = 4.5 Hz, H-6), 4.91 (dd, 1H, $J_{4,5}$ = 1.8 Hz, $J_{5,6}$ = 4.5 Hz, H-5), 4.85–4.56 (m, 2H, H-4, H-3); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 137.6, 137.3, 128.4, 128.2, 127.9 (Ar-C), 109.7, 109.6, 108.9 (×2) (*C*(CH_3)$_2$), 96.2 (C-1), 76.4, 76.1 (Ph*C*H$_2$), 70.7, 70.5 (C-3), 70.3, 70.2 (C-2), 26.13, 26.0, 25.9, 25.0, 24.9, 24.4, 24.3 (C(*C*H$_3$)$_2$); **8*E***: δ 148.2 (C-6), 73.2 (C-4), 66.7 (C-5); **8*Z***: δ 149.5 (C-6), 71.3 (C-4), 63.9 (C-5); LSI-MS *m*/*z* 364 ([M + H]$^+$, 15%); HRLSI-MS calcd for $C_{19}H_{26}NO_6$ ([M + H]$^+$): 364.1760, found: 364.1787.

(*E*- and *Z*)-1,2:3,4-Di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose-6-*O*-methyloxime (9*E* and 9*Z*)

To a solution of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose **7** (258 mg, 1.00 mmol) in pyridine (3 mL) was added *O*-methylhydroxylamine hydrochloride (100 mg, 1.20 mmol, 1.2 equiv.), and the mixture was stirred at rt for 12 h. Then it was concentrated to dryness and the residue was purified by column chromatography (1 : 15 → 1 : 7 EtOAc–hexane) to give **9** as a 1.25 : 1 *E*/*Z* diastereoisomeric mixture, as deduced from ^{1}H NMR: 261 mg, 91%; R_F 0.75 (2 : 1 EtOAc–hexane); $[\alpha]_D^{23}$ −129 (*c* 1.0, CH_2Cl_2); IR ν_{max} 1694, 1314, 1260, 1064, 999, 866, 797, 718, 630 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89, 3.85 (2s, 3H each, OCH_3), 1.55, 1.45, 1.33, 1.32 (4s, 3H each, C(CH_3)$_2$); **9*E***: δ 7.38 (d, 1H, $J_{5,6}$ = 6.8 Hz, H-6), 5.53 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1), 4.62 (dd, 1H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 7.9 Hz, H-3), 4.41 (dd, 1H, $J_{4,5}$ = 2.0 Hz, H-5) 4.33 (dd, 1H, H-2), 4.26 (dd, 1H, H-4); **9*Z***: δ 6.71 (d, 1H, $J_{5,6}$ = 4.6 Hz, H-6), 5.55 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1), 4.90 (dd, 1H, $J_{4,5}$ = 1.9 Hz, H-5), 4.60 (dd, $J_{2,3}$ = 2.6 Hz, $J_{3,4}$ = 6.6 Hz, H-3), 4.52 (dd, 1H, $J_{4,5}$ = 2.0 Hz, H-4), 4.32 (dd, 1H, H-2); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 96.3 (C-1, C-1), 26.3, 26.2, 26.1, 26.0, 25.1, 25.0, 24.6, 24.5 (C(*C*H$_3$)$_2$); **9*E***: δ 147.8 (C-6), 109.8 (*C*(CH$_3$)$_2$), 73.4 (C-4), 70.8 (C-3), 70.4 (C-2), 66.7 (C-5), 62.4 (OCH_3); **9*Z*** δ 148.9 (C-6), 109.1 (*C*(CH$_3$)$_2$), 71.4 (C-4), 70.7 (C-3), 70.3 (C-2), 63.9 (C-5), 61.9 (OCH_3); LSI-MS *m*/*z* 310 ([M + Na]$^+$, 60%); HRLSI-MS calcd for $C_{13}H_{21}NNaO_6$ ([M + Na]$^+$): 310.1267; found: 310.1247; Anal. calcd for $C_{13}H_{21}NO_6$: C, 54.35; H, 7.37; N, 4.88, found: C, 54.37; H, 7.37; N, 5.06.

6-Benzyloxyamino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (10) and *N*-benzyloxy-*N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)amine-cyanoborane (12)

To a solution of (*E*- and *Z*)-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose-6-*O*-benzyloxime **8** (383 mg, 1.05 mmol) in AcOH (2 mL) at 0 °C was added $NaBH_3CN$ (140 mg, 2.23 mmol, 2.2 equiv.), and the corresponding mixture was stirred at rt for 1.5 h. Then it was concentrated to dryness and the residue was dissolved in EtOAc (60 mL) and washed with H_2O (3 × 50 mL); the organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography (1 : 10 → 1 : 2 EtOAc–hexane) to give a separable mixture of **10** and **12**.

Eluted first was **10**: 295 mg, 77%; R_F 0.51 (1 : 2 EtOAc–hexane); $[\alpha]_D^{23}$ −16 (*c* 0.8, CH_2Cl_2); IR ν_{max} 3275, 2984, 1607, 1454, 1377, 1308, 1254, 1170, 997, 907, 799, 620 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H, Ar-H), 5.46 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 4.65 (s, 2H, PhCH_2), 4.52 (dd, 1H, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 7.9 Hz, H-3), 4.24 (dd, 1H, H-2), 4.13 (m, 1H, H-5), 4.05 (dd, 1H, $J_{4,5}$ = 1.6 Hz, H-4), 3.02 (m, 2H, H-6a, H-6b), 1.44, 1.36, 1.26 (×2) (4s, 3H each, 4C(CH_3)$_2$); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 138.2, 128.4, 128.3, 127.8 (Ar-C), 109.4, 108.9 (*C*(CH$_3$)$_2$), 96.5 (C-1), 76.0 (Ph*C*H$_2$), 72.1 (C-4), 71.0 (C-3), 70.9 (C-2), 64.1 (C-5), 52.4 (C-6), 26.1 (×2), 25.2, 24.5 (C(*C*H$_3$)); LSI-MS *m*/*z* 366 ([M + H]$^+$, 15%); HRLSI-MS calcd for $C_{19}H_{28}NO_6$ [M + H]$^+$: 366.1917, found: 366.1916.

Eluted second was **12**:‡ 42 mg, 10%, R_F 0.27 (1 : 2 EtOAc–hexane); $[\alpha]_D^{24}$ −15 (*c* 0.8, CH_2Cl_2); IR ν_{max} 3320, 3057, 2985, 2434, 1685, 1459, 1377, 1303, 1254, 1168, 1002, 896, 753, 695 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.33 (m, 5H, Ar-H), 6.82 (d, 1H, $J_{NH,H-6}$ = 8.2 Hz, NH), 5.54 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 5.23, 4.96 (2d, 1H each, $J_{H,H}$ = 9.9 Hz, PhCH_2), 4.63 (dd, 1H, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 7.9 Hz, H-3), 4.37 (dd, 1H, H-2), 4.29 (m, 1H, H-5), 4.12 (dd, 1H, $J_{4,5}$ = 1.7 Hz, H-4), 3.52 (m, 2H, H-6a, H-6b), 2.00 (brs, 2H, BH_2), 1.42, 1.38, 1.34, 1.32 (4s, 3H each, C(CH_3)$_2$); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 132.8, 129.4, 129.2, 128.9 (Ar-C), 110.1, 109.4 (*C*(CH$_3$)$_2$), 96.3 (C-1), 74.2 (Ph*C*H$_2$), 70.8 (×2), 70.7 (C-2, C-3, C-4), 62.0 (C-5), 57.0 (C-6), 26.1, 26.0, 25.0, 24.3 (C(*C*H$_3$)); Anal. calcd for $C_{20}H_{29}BN_2O_6$: C, 59.42; H, 7.23; N, 6.93, found: C, 59.35; H, 7.24; N, 6.79.

‡Crystal data for **12**: $C_{20}H_{29}BN_2O_6$, *M* = 404.26, monoclinic, *a* = 8.5632(3) Å, *b* = 11.8122(4) Å, *c* = 10.9084(4) Å, α = 90.00°, β = 91.6900(10)°, γ = 90.00°, *V* = 1102.91(7) Å^3, *T* = 173(2) K, space group *P*2(1), *Z* = 2, μ(MoKα) = 0.089 mm^{-1}, 15 535 reflections measured, 3469 independent reflections (R_{int} = 0.0188). The final R_1 values were 0.0339 ($I > 2\sigma(I)$). The final w$R(F^2)$ values were 0.0863 ($I > 2\sigma(I)$). The final R_1 values were 0.0368 (all data). The final w$R(F^2)$ values were 0.0889 (all data). The goodness of fit on F^2 was 1.033.

6-Deoxy-1,2:3,4-di-*O*-isopropylidene-6-methoxyamino-α-D-galactopyranose (11) and *N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-*N*-methoxyamine-cyanoborane (13)

To a solution of (*E*- and *Z*)-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose-6-*O*-methyloxime **9** (236 mg, 0.82 mmol) in AcOH (4 mL) at 0 °C was added $NaBH_3CN$ (129 mg, 2.06 mmol, 2.5 equiv.), and the corresponding mixture was stirred at rt for 12 h. Then it was concentrated to dryness and the residue was purified by column chromatography (1 : 5 → 1 : 2 EtOAc–hexane) to give a separable mixture of **11** and **13**.

Eluted first was **11**: 147 mg, 62%; R_F 0.43 (1 : 2 EtOAc–hexane); $[\alpha]_D^{26}$ −17 (*c* 1.0, CH_2Cl_2); IR ν_{max} 2991, 2928, 1459, 1381, 1245, 1201, 1173, 1061, 993, 891, 862 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.54 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1), 4.62 (dd, 1H, $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 7.8 Hz, H-3), 4.32 (dd, 1H, H-2), 4.18 (m, 1H, H-4), 4.15 (m, 1H, H-5), 3.13 (dd, 1H, $J_{5,6a}$ = 3.8 Hz, $J_{6a,6b}$ = 14.0 Hz, H-6a), 3.54 (s, 3H, OCH_3), 3.05 (dd, 1H, $J_{5,6b}$ = 8.6 Hz, H-6b), 1.55, 1.44, 1.34 (×2) (4s, 3H each, $C(CH_3)_2$); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 109.6, 108.0 ($C(CH_3)_2$), 96.6 (C-1), 72.3 (C-4), 71.1 (C-3), 70.9 (C-2), 64.1 (C-5), 61.7 (OCH_3), 52.1 (C-6), 26.2, 26.1, 25.3, 24.6 ($C(CH_3)_2$); LSI-MS *m/z* 290 ($[M + H]^+$, 100%); HRLSI-MS calcd for $C_{13}H_{24}NO_6$ ($[M + H]^+$): 290.1580, found: 290.1560.

Eluted second was **13**, obtained as a non-resolved 10 : 1 diastereoisomeric mixture: 61 mg, 23%; R_F 0.26 (1 : 2 EtOAc–hexane); $[\alpha]_D^{25}$ +23 (*c* 0.8, CH_2Cl_2); IR ν_{max} 3021, 2435, 1381, 1254, 1254, 1213, 1164, 1098, 1071, 1007, 909, 879, 852, 766 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) major diastereoisomer: δ 6.46 (brd, 1H, $J_{NH,H6}$ = 2.3 Hz, NH), 5.52, (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 4.65 (dd, 1H, $J_{2,3}$ = 2.3 Hz, $J_{3,4}$ = 7.8 Hz, H-3), 4.38 (dd, 1H, H-2), 4.29 (m, 1H, H-5), 4.14 (dd, 1H, $J_{4,5}$ = 1.7 Hz, H-4), 3.85 (s, 3H, OCH_3), 3.43–3.37 (m, 2H, H-6a, H-6b), 1.53, 1.43 1.35, 1.33 (4s, 3H each, $C(CH_3)_2$); minor diastereoisomer: δ 6.71 (brd, 1H, $J_{NH,H}$ = 8.0 Hz, NH), 5.49 (d, 1H $J_{1,2}$ = 4.9 Hz, H-1), 4.60 (dd, 1H, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 7.9 Hz, H-3), 4.36 (dd, 1H, H-2), 4.11 (m, 1H, H-5), 4.20 (dd, 1H, $J_{4,5}$ = 1.9 Hz, H-4), 3.82 (s, 3H, OCH_3), 3.48–3.33 (m, 2H, H-6a, H-6b), 2.00 (brs, 2H, BH_2), 1.56, 1.50, 1.47, 1.46 (4s, 3H each, $C(CH_3)_2$); ^{13}C-NMR (75.5 MHz, $CDCl_3$) major diastereoisomer: δ 110.3, 109.6 ($C(CH_3)_2$), 96.3 (C-1), 71.0 (C-2), 70.8 (C-3, C-4), 62.1 (C-5), 61.0 (OCH_3), 56.7 (C-6), 26.0, 25.9, 25.1, 24.4 ($C(CH_3)_2$); minor diastereoisomer: δ 109.7, 108.9 ($C(CH_3)_2$), 96.0 (C-1), 71.2 (C-4), 70.7 (C-3), 70.6 (C-2), 63.8 (C-5), 59.4 (OCH_3), 56.4 (C-6), 26.1 (×2), 25.2, 24.6 ($C(CH_3)_2$); LSI-MS *m/z* 351 ($[M + Na]^+$, 40%); HRLSIMS calcd for $C_{14}H_{25}BN_2NaO_6$ ($[M + Na]^+$): 351.1703, found: 351.1714.

(1*S*,4*R*,5*S*,6*R*,7*R*)-2-Benzyloxy-8-oxa-2-azabicyclo[3.2.1]octane-4,6,7-triol (19)

A solution of 6-benzyloxyamino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **10** (508 mg, 1.39 mmol), in a 1 : 1 TFA–H_2O mixture (1.2 mL) was stirred at rt 4 days; after that it was concentrated to dryness and the residue was purified by column chromatography (5 : 1 EtOAc–MeOH) to give **19**: 256 mg, 69%; R_F 0.6 (5 : 1 EtOAc–MeOH); $[\alpha]_D^{23}$ +52 (*c* 0.6, MeOH); IR ν_{max} 3350, 1643, 1493, 1454, 1197, 1046, 969, 745, 702 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.30–7.26 (m, 5H, Ar-H), 4.96 (ddd, 1H, $J_{1,5}$ = 0.5 Hz, $J_{1,3e}$ = 1.3 Hz, $J_{1,7}$ = 5.9 Hz, H-1), 4.70, 4.67 (2d, 1H each, $J_{H,H}$ = 11.3 Hz, $PhCH_2$), 4.15 (d, 1H, $J_{5,6} \approx 0$ Hz, $J_{6,7}$ = 2.4 Hz, H-6), 4.14 (ddd, 1H, $J_{5,7}$ = 1.0 Hz, $J_{6,7}$ = 2.4, $J_{1,7}$ = 5.9 Hz, H-7), 4.07 (ddd, 1H, $J_{4,5}$ = 4.4 Hz, $J_{3e,4}$ = 6.0 Hz, $J_{3a,4}$ = 11.2 Hz, H-4), 3.91 (m, 1H, H-5), 3.29 (dddd, 1H, $J_{1,3e}$ = 1.3 Hz, $J_{3e,5}$ = 2.1 Hz, $J_{3e,4}$ = 6.0 Hz, $J_{3a,3e}$ = 14.9 Hz, H-3e), 2.98 (dd, 1H, $J_{3a,4}$ = 11.2 Hz, $J_{3a,3e}$ = 14.9 Hz, H-3a); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 138.8, 129.9, 129.3, 128.9 (Ar-C), 92.6 (C-1), 86.0 (C-5), 81.3 (C-7), 77.3 (C-6), 76.0 ($PhCH_2$), 62.5 (C-4), 53.9 (C-3); LSI-MS *m/z* 290 ($[M + Na]^+$, 18%); HRLSI-MS calcd for $C_{13}H_{17}NNaO_5$ ($[M + Na]^+$): 290.1004, found: 290.0995.

1,2,3,4-Tetra-*O*-acetyl-6-(*N*-acetyl-*N*-benzyloxy)amino-6-deoxy-α and β-D-galactopyranose (21)

A solution of 6-benzyloxyamino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **10** (102 mg, 0.28 mmol) in a 4 : 1 TFA–H_2O mixture (1 mL) was kept at rt for 3.5 h. Then it was concentrated to dryness, and the residue was dissolved in a 1 : 1 Ac_2O–Py mixture (3 mL) and DMAP (8 mg, 0.065 mmol, 0.23 equiv.) was added. The corresponding solution was kept at rt for 12 h; after that, water was slowly added at 0 °C and concentrated to dryness. The residue was dissolved in EtOAc (40 mL) and washed with H_2O (3 × 30 mL); the organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. The residue was purified by column chromatography (1 : 5 → 1 : 2 EtOAc–hexane) to give **21** as a non-resolved anomeric mixture, as deduced from ^1H NMR: 90.1 mg, 65%; R_F 0.7 (1 : 1 EtOAc–hexane); IR ν_{max} 2934, 1753, 1670, 1433, 1371, 1221, 1055, 954, 740, 701 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 (m, 5H, Ar-H); **21α**: δ 6.35 (brs, 1H, H-1), 5.38 (m, 1H, H-4), 5.30–5.28 (m, 2H, H-2, H-3), 4.82, 4.78 (2d, 1H each, $J_{H,H}$ = 10.6 Hz, $PhCH_2$), 4.42 (t, 1H, $J_{4,5} \approx 0$ Hz, $J_{5,6a} = J_{5,6b}$ = 6.9 Hz, H-5), 3.84–3.82 (m, 1H, H-6a), 3.62 (m, 1H, H-6b), 2.12, 2.07, 2.03, 2.00, 1.98 (5s, 3H each, 5Ac); **21β**: δ 5.63 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 5.31 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2), 5.28 (d, 1H, $J_{3,4}$ = 3.5 Hz, $J_{4,5} \sim 0$ Hz, H-4), 5.03 (dd, 1H, H-3), 4.81 (s, 2H, $PhCH_2$), 4.05 (t, 1H, $J_{5,6a} = J_{5,6b}$ = 6.3 Hz, H-5), 3.96 (m, 1H, H-6a), 3.63 (dd, 1H, $J_{6a,6b}$ = 14.5 Hz, H-6b), 2.13, 2.06, 2.03 (×2), 1.96 (5s, 3H each, Ac); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 134.4, 129.3, 129.2, 128.9 (Ar-C), 20.9 (×2), 20.8, 20.7, 20.4 (Ac); **21α**: δ 170.3, 170.2, 170.0 (×2), 169.1 (C═O), 89.8 (C-1), 76.9 ($PhCH_2$), 67.8 (C-5), 67.7 (C-2, C-3), 65.6 (C-4), 53.5 (C-6); **21β**: δ 170.3, 170.0, 169.5 (×2), 169.2 (C═O), 92.4 (C-1), 77.0 ($PhCH_2$), 71.0 (C-5), 70.1 (C-3), 67.9 (C-2), 67.2 (C-4), 46.6 (C-6); LSI-MS *m/z* 518 ($[M + Na]^+$, 5%); HRLSI-MS calcd for $C_{23}H_{29}NNaO_{11}$ ($[M + Na]^+$): 518.1638, found: 518.1610.

6-(*N*-Acetyl-*N*-benzyloxy)amino-6-deoxy-D-galactopyranose (22)

To a solution of 1,2,3,4-tetra-*O*-acetyl-6-(*N*-acetyl-*N*-benzyloxy) amino-6-deoxy-D-galactopyranose **21** (50 mg, 0.1 mmol) in MeOH (3 mL) was added a 0.5 M methanolic NaOMe solution (0.3 mL, 0.15 mmol) and the mixture was stirred at rt for 45 min. After that, the reaction was neutralized with Amberlite IR-120(H^+) resin, filtered and the filtrate was concentrated to

dryness to give pure **22**: 28.4 mg, 87%; R_F 0.17 (5 : 1 EtOAc–MeOH); $[\alpha]_D^{22}$ +50 (*c* 0.4, MeOH); IR ν_{max} 3530, 2853, 1690, 1504, 1397, 1200, 1043, 967, 734, 701 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) δ 7.42–7.33 (m, 5H, Ar-H), 5.14 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1α), 5.01–4.90 (m, 4H, CH_2Ph), 4.39 (m, 1H, H-1β), 4.33 (m, 1H, H-5α), 4.10–3.88 (m, 5H, H-2α, H-3α, H-4α, H-4β, H-5β), 3.80–3.71 (m, 4H, H-6aα, H-6bα, H-6aβ, H-6bβ), 3.46 (m, 2H, H-2β, H-3β), 2.05 (s, 6H, NAc); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 130.8, 130.7, 130.0, 129.7 (Ar-C), 98.8 (C-1β), 94.3 (C-1α), 77.6, 77.2 (CH_2Ph), 74.9 (C-3β), 73.6 (C-2β), 72.6 (C-5β), 71.5 (C-3α), 71.1 (C-2α), 70.8 (C-4β), 70.3 (C-4α), 68.0 (C-5α), 20.6 (NAc); LSI-MS *m/z* 350 ($[M + Na]^+$, 35%); HRLSI-MS calcd for $C_{15}H_{21}NNaO_7$ ($[M + Na]^+$): 350.1216, found: 350.1228.

(1*S*,4*R*,5*S*,6*R*,7*R*)-2-Methoxy-8-oxa-2-azabicyclo[3.2.1]octane-4,6,7-triol (23)

A solution of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-6-methoxyamino-α-D-galactopyranose **11** (259 mg, 0.89 mmol) in 9 : 1 TFA–H_2O (0.9 mL) was stirred at rt for 4 days. Then it was neutralized with Amberlite IRA-68 resin, filtered and washed with water and methanol. The combined filtrates were concentrated to dryness and the residue was purified by column chromatography (EtOAc → 5 : 1 EtOAc–MeOH) to give **23**: 145 mg, 85%; R_F 0.39 (5 : 1 EtOAc–MeOH); $[\alpha]_D^{23}$ +67 (*c* 0.8, MeOH); IR ν_{max} 3364, 1662, 1444, 1197, 1139, 1046, 1022, 1003, 843, 794, 716 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ 5.02 (ddd, 1H, $J_{1,5}$ = 0.5 Hz, $J_{1,3e}$ = 1.3 Hz, $J_{1,7}$ = 5.9 Hz, H-1, H-1), 4.20 (ddd, 1H, $J_{5,7}$ = 1.0 Hz, $J_{6,7}$ = 2.4 Hz, $J_{1,7}$ = 5.9, H-7), 4.18 (d, 1H, $J_{5,6} \sim 0$ Hz, $J_{6,7}$ = 2.4, H-6), 4.06 (ddd, 1H, $J_{4,5}$ = 4.3 Hz, $J_{3e,4}$ = 6.0 Hz, $J_{3a,4}$ = 11.2 Hz, H-4), 3.94 (m, 1H, H-5), 3.52 (s, 3H, OCH_3), 3.34 (dddd, 1H, $J_{1,3e}$ = 1.3 Hz, $J_{3e,5}$ = 1.9 Hz, $J_{3e,4}$ = 6.0 Hz, $J_{3e,3a}$ = 14.9 Hz, H-3e), 2.99 (dd, 1H, $J_{3a,4}$ = 11.2 Hz, $J_{3e,3a}$ = 14.9 Hz, H-3a); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 92.9 (C-1), 86.0 (C-5), 81.2 (C-7), 77.3 (C-6), 63.3 (C-4), 60.2 (OCH_3), 53.2 (C-3); CIMS *m/z* 192 ($[M + H]^+$, 100%); HRCI-MS calcd for $C_7H_{14}NO_5$ ($[M + H]^+$): 192.0872, found: 192.0866; Anal. calcd for $C_7H_{13}NO_5$: C, 43.98; H, 6.85; N, 7.33, found: C,43.95; H, 7.00; N, 7.20.

(*E*- and *Z*)-1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose-5-*O*-methyloxime (27*E* and 27*Z*)

To a solution of 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-l,4-furanose **25** (425 mg, 2.26 mmol) in pyridine (4 mL) was added *O*-methylhydroxylamine hydrochloride (226 mg, 2.71 mmol, 1.2 equiv.) and the mixture was stirred at rt for 16 h. Then it was concentrated to dryness and the residue was dissolved in EtOAc (50 mL) and washed with H_2O (3 × 30 mL). The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was concentrated to dryness, and the residue was purified by column chromatography (1 : 10 → 1 : 2 EtOAc–hexane) to give **27** as a 2 : 1 *E*/*Z* diastereoisomeric mixture, as deduced from ^{1}H NMR.

Yield: 440 mg, 90%; R_F: 0.71 (**27*E***) and 0.60 (**27*Z***) (1 : 2 EtOAc–hexane); $[\alpha]_D^{26}$ −64 (*c* 1.0, CH_2Cl_2); IR ν_{max} 3398, 2981, 2938, 1381, 1254, 1206, 1158, 1075, 1012, 852, 779, 639 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60–4.55 (m, 2H, H-2); **27*E***: 7.50 (d 1H, $J_{4,5}$ = 5.2 Hz, H-5), 6.00 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1), 4.70 (dd, 1H, $J_{3,4}$ = 2.6 Hz, H-4), 4.36 (t, 1H, $J_{2,3}$ = 2.4 Hz, H-3), 3.90 (s, 3H, OCH_3); **27*Z***: δ 6.83 (d, 1H, $J_{4,5}$ = 3.3 Hz, H-5), 5.98 (d, 1H, $J_{1,2}$ = 4.6 Hz, H-1), 5.06 (t, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 4.60–4.55 (m, 1H, H-3), 3.93 (s, 3H, OCH_3); ^{13}C-NMR (75.5 MHz, $CDCl_3$) **27*E***: δ 146.3 (C-5), 112.2 ($C(CH_3)_2$), 105.2 (C-1), 85.1 (C-2), 77.4 (C-4), 76.3 (C-3), 62.3 (OCH_3), 26.9, 26.4 (C(CH_3)$_2$); **27*Z***: δ 148.5 (C-5), 112.1 ($C(CH_3)_2$), 104.8 (C-1), 85.2 (C-2), 78.0 (C-4), 75.9 (C-3), 62.6 (OCH_3), 27.1, 26.3 (C(CH_3)$_2$); LSI-MS *m/z* 218 ($[M + H]^+$, 70%); HRLSI-MS calcd for $C_9H_{16}NO_5$ ($[M + H]^+$): 218.1031, found: 218.1028; Anal. calcd for $C_9H_{15}NO_5$: C, 49.76; H, 6.96; N, 6.45, found: C, 49.75; H, 7.11; N, 6.19.

5-Deoxy-1,2-*O*-isopropylidene-5-methoxyamino-α-D-xylofuranose (29) and *N*-(5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranos-5-yl)-*N*-methoxyamino-cyanoborane (31)

To a solution of (*E*- and *Z*)-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose-5-*O*-methyloxime **27** (1.12 g, 5.17 mmol) was added $NaBH_3CN$ (877 mg, 13.96 mmol, 2.7 equiv.), and the mixture was stirred at rt for 7 h. Then it was concentrated to dryness and the residue was dissolved in EtOAc (50 mL) and washed with H_2O (3 × 50 mL); the organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography (1 : 5 → 1 : 1 EtOAc–hexane) to give a separable mixture of **29** and **31**.

Eluted first was **29**: 564 mg, 50%; R_F 0.31 (1 : 2 EtOAc–hexane); $[\alpha]_D^{23}$ +130 (*c* 0.7, CH_2Cl_2); IR ν_{max} 3398, 2981, 1567, 1201, 1054, 1006, 976, 849, 789, 635 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 5.86 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.48 (d, 1H, $J_{2,3} \sim 0$ Hz, H-2), 4.10 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-3), 4.32 (m, 1H, H-4), 3.52 (s, 3H, OCH_3), 3.19 (dd, 1H, $J_{4,5a}$ = 4.8 Hz, $J_{5a,5b}$ = 13.5 Hz, H-5a), 3.06 (dd, 1H, $J_{4,5b}$ = 7.3 Hz, H-5b), 1.44, 1.29 (2s, 3H each, C(CH_3)$_2$); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 112.8 ($C(CH_3)_2$), 106.2 (C-1), 87.0 (C-2), 79.1 (C-4), 76.3 (C-3), 61.7 (OCH_3), 51.0 (C-5), 27.2, 26.6 (C(CH_3)$_2$); LSI-MS *m/z* 242 ($[M + Na]^+$, 25%); HRLSI-MS *m/z* calcd for $C_9H_{17}NNaO_5$ ($[M + Na]^+$): 242.1014, found: 242.0992.

Eluted second was **31**: 133 mg, 10%; R_F 0.23 (1 : 2 EtOAc–hexane); $[\alpha]_D^{23}$ −64 (*c* 0.9, CH_2Cl_2); IR ν_{max} 3394, 2991, 2938, 2439, 1459, 1371, 1226, 1075, 1008 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 5.92 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.51 (d, 1H, $J_{2,3} \sim 0$ Hz, H-2), 4.51 (dt, 1H, $J_{3,4}$ = $J_{4,5a}$ = 2.8 Hz, $J_{4,5b}$ = 8.7 Hz, H-4), 4.11 (d, 1H, H-3), 3.58 (dd, 1H, $J_{5a,5b}$ = 14.2 Hz, H-5a), 4.52 (s, 3H, OCH_3), 3.26 (dd, 1H, H-5b), 1.47, 1.30 (s, 3H each, C(CH_3)$_2$); ^{13}C-NMR (75.5 MHz, CD_3OD) δ 113.2 ($C(CH_3)_2$), 106.4 (C-1), 87.2 (C-2), 76.3 (C-4), 76.2 (C-4), 76.1 (C-3), 60.3 (C-5), 56.2 (OCH_3), 27.3, 26.6 (C(CH_3)$_2$); CI-MS *m/z* 259 ($[M + H]^+$, 10%); HRCI-MS *m/z* calcd for $C_{10}H_{20}BN_2O_5$ ($[M + H]^+$): 259.1837, found: 259.1843.

(2*R*,3*R*,4*S*,5*R*)-1-Benzyloxy-2,3,4,5-tetrahydroxypiperidine (33)

To a solution of 5-benzyloxyamino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose **28** (100 mg, 0.32 mmol), in a 2 : 1 CH_3CN–H_2O mixture (3 mL) was added Amberlite IR-120(H^+) resin (135 mg) and the mixture was stirred at rt for 2 h. Then,

the resin was filtered off, and the residue was concentrated to dryness and crystallized from CH_3CN, to give **33** in the α-pyranose form exclusively. Yield: 25.2 mg, 31%; R_F 0.10 (AcOEt); IR ν_{max} 3409, 119, 1061, 1012, 881, 745, 702 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6 and a drop of D_2O) δ 7.31–7.26 (m, 5H, Ar-H), 4.59 (d, 1H, $J_{2,3}$ = 3.0 Hz, H-2), 4.05 (s, 2H, PhCH_2), 3.25–3.22 (m, 2H, H-4, H-5), 3.13 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 2.86 (m, 2H, H-6a, H-6b); ^{13}C-NMR (125.5 MHz, DMSO-d_6 and a drop of D_2O) δ 138.4, 129.4, 129.0, 128.6 (Ar-C), 84.5 (C-2), 74.3 (PhCH_2), 74.0 (C-4), 71.4 (C-3), 69.1 (C-5), 52.1 (C-6); LSIMS *m*/*z* 278 ([M + Na]$^+$ 2%); HRLSI-MS calcd for $C_{12}H_{17}NNaO_5$ [M + Na]$^+$: 278.5632, found: 278.5124.

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