A Novel Approach to Anellated Carbasugar Derivatives, Using Intramolecular 1,3-Dipolar Cycloaddition Reactions of Sugar-Derived Nitrones

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Dedicated to Prof. Dr. Richard Neidlein on the occasion of his 70th birthday

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Intramolecular 1,3-dipolar cycloaddition of the intermediate nitrones **5** and **6** to double bonds stereoselectively yielded the benzylated anellated carbasugar derivatives **7** and **8**. The disaccharide analogue **14** was synthesized similarly. Deprotection afforded the anellated carbasugars **9**, **10**, and **15**, which were investigated as potential inhibitors of glycosidases. This paper also describes the theoretical and experimental evaluation of these compounds as ligands for different enzymes, using automatic docking procedures and enzyme assays, respectively.

Introduction

In the past decade, growing attention has been paid to ascertainment of the mechanism of glycosidases and their interaction with corresponding substrates. In the context of the possible use of inhibitors of these enzymes as therapeutic agents for several diseases which are accompanied by a change in carbohydrate metabolism, a great many substances have been synthesized during the last few years.[1] Some of these compounds are potential therapeutic agents for the treatment of infectious diseases like influenza or AIDS and of different types of diabetes mellitus and cancer.[2] Recently, the great influence of bioorganic chemistry, enzyme crystallography, and synthetic organic chemistry in the investigation of the structural properties of enzyme−ligand complexes of glycosylhydrolases has been reported upon.[3]

Disaccharide analogues are one subject of intensive research, in view of their potential as inhibitors of glycosidases.[1a] The use of 1,3-dipolar cycloadditions in the synthesis of C-disaccharides and corresponding compounds is well documented.[4] Nitrile oxides have mainly been used in such reactions. In this paper we report the stereoselective synthesis of furo-anellated and substituted amino carbasugars, using intramolecular 1,3-dipolar cycloaddition of nitrones derived from aldoses. These products could, thanks to their conformational properties, be viewed as potential inhibitors of glucosidases. The theoretical and experimental appraisal of these derivatives as potential inhibitors of different glucosidases is described.

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Results and Discussion

Starting from D-glucose (**1**), it was possible to synthesize the carbasugar derivative **2** in a well-documented way,[5] which was also applied to the preparation of different classes of carbasugars (Scheme 1).[6]

From the inositol derivative **2**, we were able to synthesize the ester **3** by means of a carbene insertion reaction based on a procedure reported by Noels et al.[7] The authors used the reaction of carbenes with alcohols for the preparation of substituted ethers. Treatment of the allylic alcohol **2** with an excess of ethyl diazodicarboxylate in the presence of a catalytic amount of rhodium(II) acetate dimer furnished the ester **3** in 72% yield. In this preparation step it was very important to use a prolonged reaction time, as side reactions gave other products if the reagents were mixed too rapidly. The use of a syringe pump, allowing slow addition over 10 h, gave only a minor amount of decomposed products. The reduction of compound **3** using diisobutylaluminium hydride (DIBAL-H) in toluene at −78 °C yielded the aldehyde **4** in 74% yield. The aldehyde **4** must be used relatively quickly because of its easy decomposition, even at −20 °C.

The reaction between the carbasugar derivative **4** and either *N*-benzyl- or *N*-methylhydroxylamine in toluene at 20 °C furnished the intermediate nitrones **5** and **6**, respectively. These underwent spontaneous intramolecular 1,3-dipolar cycloaddition to afford the tricyclic compounds **7** and **8**, respectively.[6] The presence of the (*S*) configuration at C-1 of **5** and **6** led to a stereoselective cyclization, yielding only the substituted (2a*S*,4a*S*,5*S*,6*S*,7*R*,7a*R*,7b*R*)-5,6,7-tris(benzyloxy)octahydro-2*H*-furo[4,3,2-*c*,*d*][1,2]benzisoxazoles **7** and **8**, respectively. Their structures could be determined using NMR spectroscopic methods and by X-ray crystallographic study of compound **8**.[9] In Figure 1 the molecular structure is displayed. A bowl-shaped geometry of the tricyclic skeleton, the result of the furo anellation in the carbasugar **8**, was found, and in particular a twist-boat con-

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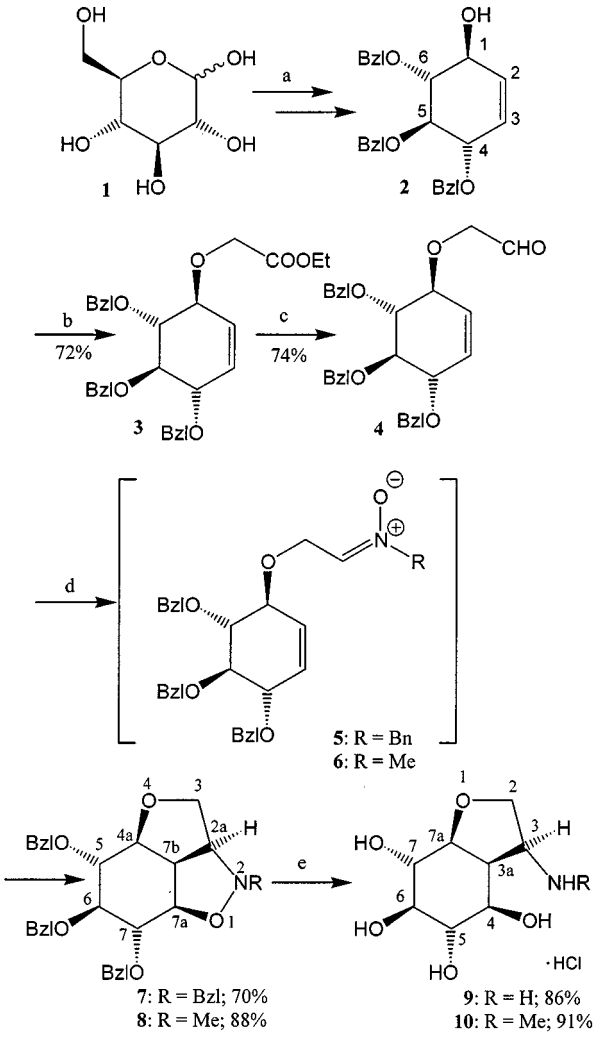


Scheme 1. a) See ref.[7]; b) N_2CHCO_2Et, $Rh_2(OAc)_4$; CH_2Cl_2, 20 °C, 10 h; c) DIBAL-H, −78 °C, 2 h; d) RNHOH, toluene, 20 °C, 5 h; e) H_2, Pd/C, ethanol, HCl, 20 °C, 24 h

formation for the six-membered ring. These structural properties were also found in several complexes formed by glycosidases with their substrates.[3]

The NMR spectroscopic data for the compounds **7** and **8** also confirmed this conformation in solution. Comparable results were found by H. G. Aurich and co-workers during their studies on the synthesis of 2,6-dioxa-3-azatricyclo-[5.3.1.$0^{4,11}$]undecanes and 2-oxa-3,6-diazatricyclo-[5.3.1.$0^{4,11}$]undecanes.[10] Catalytic hydrogenation of **7** and **8**, using palladium on activated charcoal, furnished the target compounds **9** (86% yield) and **10** (91% yield), respectively.

We were now curious about the extension of this methodology into the synthesis of unusual disaccharide analogues. As in the case of acarbose,[11] the oligomerisation of the carbocyclic moiety valienamine resulted in a much stronger binding of the inhibitor with glucosidases.

Starting with the readily available aldehyde **11**,[12] it was possible to prepare the aldoxime **12** by treatment with hydroxylamine.[13] Without further purification, compound **12** was reduced almost quantitatively by sodium cyanoborohydride[14] to yield the *N*-substituted hydroxylamine **13**. Treatment of compound **13** with the aldehyde **4** using the method already outlined furnished the crystalline disaccharide analogue **14** in 75% yield. The subsequent catalytic hydrogenation of **14** gave the deprotected derivative **15** in 80% yield (Scheme 2).

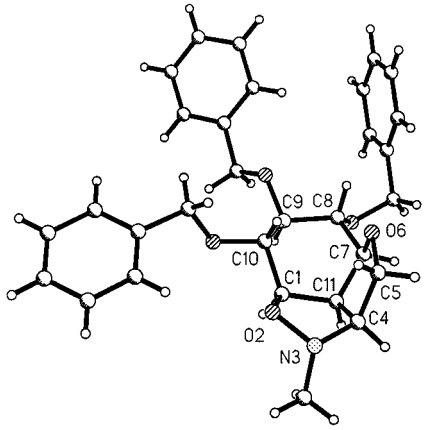
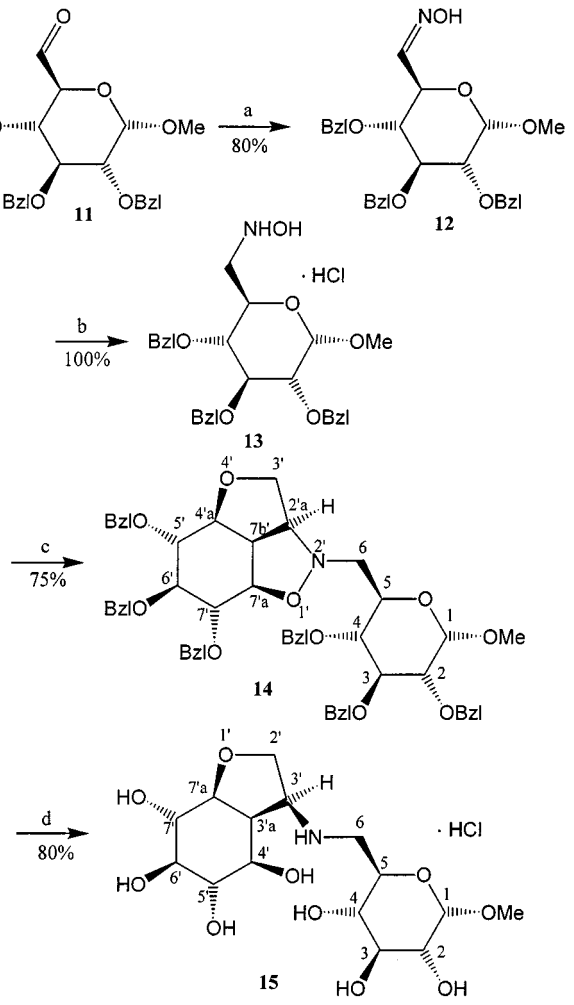


Figure 1. X-ray molecular structure of **8**;[9] selected bond lengths [Å] and angles [°]: O2−N3 1.446(3), O2−C1 1.451(3), O6−C5 1.430(4), O6−C7 1.442(4), N3−C4 1.459(4), N3−C12 1.459(4), C1−C10 1.510(4), C1−C11 1.534(4), C10−C9 1.524(4), C9−C8 1.521(4), C8−C7 1.521(4), C7−C11 1.515(4); N3−O2−C1 109.3(1), C5−O6−C7 108.5(2), O2−N3−C4 102.8(2), C10−C1−C11 114.7(2), C8−C9−C10 110.4(2), C9−C8−C7 114.5(2), O6−C7−C11 104.3(2), C11−C7−C8 117.0(2), N3−C4−C5 112.2(3), N3−C4−C11 106.9(2), C5−C4−C11 103.2(2), H1A−C1−C11−H11A −1.2, H4A−C4−C11−H11A 25.4, H7A−C7−C5−H8A −89.4, H8A−C8−C9−H9A 147.0, H9A−C9−C10−H10A 175.3, H10A−C10−C1−H1A 165.1

Using the X-ray study of compound **8** as a basis, we performed molecular dynamics calculations to assist in ascertaining the conformation of the bicyclic compounds **9** and **10** and the disaccharide analogue **15**. Using the CERIUS2 program system and the well-known CFF91 forcefield implemented in the OPEN FORCEFIELD module,[15] we were able to calculate minimum-energy conformations of **9**, **10**, and **15**. These calculations indicated the formation of a hydrogen bond between the amino group and C(4)−OH. The formation of this bond is responsible for the fixing of the rigid ring structure in the bicyclic system. The conformation of the carbocyclic moiety in these compounds indicated conservation of the twist-boat form typical of the structure of ligands found in the active site of glycosylhydrolases.[3] For these structures we evaluated the possible formation of complexes between the carbasugars **9**, **10**, and **15** and different glucosidases, using the program FlexX for calculations on receptor−ligand complexes.[16] This program uses a second generation generic algorithm for automatic docking and optimization of receptor−ligand complexes, based on the method of the de novo ligand design programme system LUDI.[16] The scoring function[17] has several useful advantages. Firstly, it is able to deal with more than atom pair interactions, and hence allows the consideration of atomic groups as interaction partners. Furthermore, as it is an additive function, it is able to calculate partial molecular assemblies quickly.[17] The overall accuracy for the fine-tuning of docked ligands is good.[18] Calculation was possible, on



Scheme 2. a) $NH_2OH{\cdot}HCl$, pyridine, CH_3OH, reflux, 2 h; b) $NaCNBH_3$, CH_3OH, pH = 3, 20 °C, 10 min; c) **4**, Et_3N, toluene, 20 °C, 12 h; d) H_2, Pd/C, ethanol, HCl, 20 °C, 24 h

the basis of the FlexX program, for different kinds of receptor−ligand complexes, with results comparing very well with experimental data. Using X-ray structural data for the α-amylase from *Bacillus subtilis*[19] (family 13;[20] PDB-code: 1BAG.pdb) and the cyanogenic β-glucosidase from *Trifolium repens*[21] (family 1;[20] PDB-code: 1CBG.pdb), we were able to calculate the structure of the lowest-energy complexes of the compounds **9**, **10** and **15** in the active site of these enzymes. Due to sequence homologies of the enzymes used for the calculations and for the experimental investigations the results are comparable. The structure of the complex of the ligand **10** bound in the active site of *Trifolium repens* β-glucosidase is shown in Figure 2.

The ligand binds to the enzyme with an estimated energy of −18.342 kJ/mol. The formation of five hydrogen bonds between the atoms $O\varepsilon_1$ and $O\varepsilon_2$ of the glutamic acid residues Glu 183 and Glu 397 is mainly responsible for the binding to the enzyme. The interaction between the hydroxy groups of the carbocyclic ring system and the catalytically active amino acid residues indicates that the bicyclic compound **10** could serve as a potential ligand for a glucosidase.

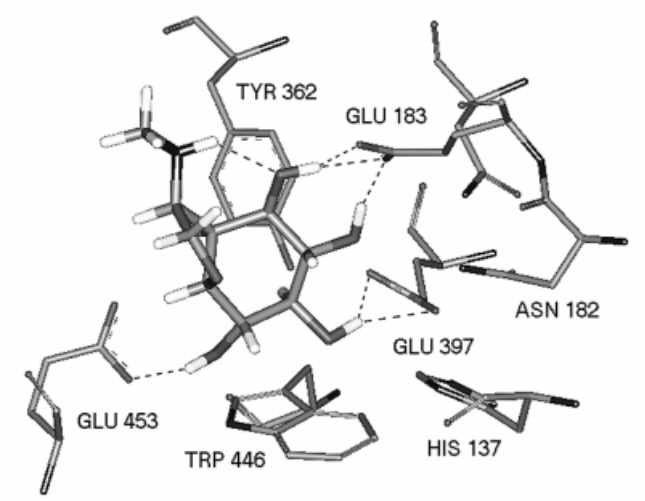


Figure 2. Lowest-energy conformer of compound **10** in the active site of β-glucosidase from *Trifolium repens* (only the active site is shown; hydrogen bonds are calculated)

The calculation of the structure of the receptor−ligand complex with the α-amylase from *Bacillus subtilis* also highlights the possibility that compounds **9**, **10**, and **15** might be able to form stable complexes with this enzyme. In Figure 3, the complex of the disaccharide analogue **15** and the active site of the enzyme 1BAG is displayed. In addition to the formation of a hydrogen bond with Asp 176, the bond to Asp 269 is also predicted. The energy of the complex was calculated as −23.24 kJ/mol for **15** and −22.89 kJ/mol for **10**. In all the calculated complexes the formation of hydrogen bonds confirms the observations made in the X-ray crystallographic study of the enzyme-substrate complex.[19]

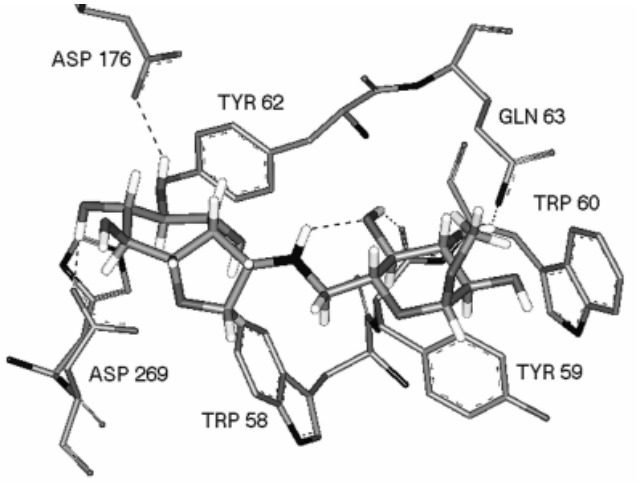


Figure 3. Lowest-energy conformer of compound **15** in the active site of α-amylase from *Bacillus subtilis* (only the active site is shown; hydrogen bonds are calculated)

In order to perform preliminary evaluation of the potential of the newly synthesized compounds as inhibitors of glucosidases, we investigated enzyme assays. Using enzymes from related families, it is also possible to evaluate the theoretical results (Table 1).

Table 1. Inhibitory constants (K_I [μM]) found for compounds **9**, **10**, and **15** (GHF − glycosidase hydrolase family[20])

Enzyme	**9**	**10**	**15**
α-glucosidase (brewer's yeast; GHF 13), pH = 6.8	> 1000	> 1000	> 1000
α-glucosidase (brewer's yeast; GHF 13), pH = 6.0	510	540	550
β-glucosidase (sweet almonds; GHF 1), pH = 6.8	> 1000	> 1000	> 1000
β-glucosidase (sweet almonds; GHF 1), pH = 6.0	–	–	257
β-glucosidase (*Caldocellum sacc.*; GHF 1), pH = 6.8	> 1000	> 1000	> 1000
β-glucosidase (*Caldocellum sacc.*; GHF 1), pH = 6.0	> 1000	> 1000	184

The inhibitory constants show that the carbasugar derivatives **9**, **10**, and **15** are only poor inhibitors of glucosidases. Weak inhibition could be observed, especially at lower pH values. The contrast between these observations and the calculated data might be the result of differences in the secondary structures of the enzymes assumed for the calculation and those present in the assays. It is noteworthy that the disaccharide analogue **15** binds significantly better than the monomeric derivatives **9** and **10**. This can be explained by the polyvalent substitution pattern of **15**. The substitution of oxygen in the furan ring in **9**, **10**, and **15** by nitrogen might give more active inhibitors of glycosidases. Further investigations related to this are currently in progress.

Conclusion

In conclusion, we have developed a novel approach to the synthesis of anellated carbasugar derivatives. Making use of easily available precursors, it was possible to prepare bicyclic compounds stereoselectively, using intramolecular cycloaddition reactions with nitrones. The methodology could be used to prepare a variety of different bicyclic compounds. Using a specially substituted *N*-pyranosylhydroxylamine, it was also possible to prepare a disaccharide analogue in only a few steps.

Experimental Section

General Remarks: All solvents were dried according to standard procedures and were freshly distilled prior to use. Reactions were monitored by TLC on silica gel F_{254} plates (Merck) with detection by UV light or by charring with sulfuric acid. − Melting points were determined with a Boetius melting point apparatus and are corrected. − Specific rotations were measured using a Polar LμP machine (IBZ Messtechnik); specific rotation values are given in units of 10^{-1}deg cm^2 g^{-1}. − Infrared spectra were recorded with a Nicolet 205 FT-IR spectrometer. − ^{1}H NMR (250.133 MHz and 300.133 MHz) and ^{13}C NMR (62.896 MHz and 75.466 MHz) spectra were obtained with Bruker AC 250 and ARX 300 instruments, respectively. ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to the solvent signal. − Mass spectra were recorded with an ADM 402/3 spectrometer. − For column chromatography, Merck silica gel 60 (63−200 mesh) was used. − Elemental analyses were carried out with a Leco CHNS-932.

Ethyl 2-[(1*S*,4*S*,5*R*,6*S*)-4,5,6-Tris(benzyloxy)cyclohex-2-enyloxy]acetate (3): To a solution of **2**[5] (0.21 g, 0.5 mmol) and rhodium(II) acetate dimer (4.4 mg) in anhydrous dichloromethane (2 mL) was added, over the course of 10 h by means of a syringe, a solution of ethyl diazoacetate (0.09 g, 0.75 mmol) in anhydrous dichloromethane (10 mL). After completion of the reaction (TLC monitoring) the solution was concentrated at 20 °C under vacuum and the residue was purified using column chromatography to give **3** (0.18 g, 72%) as a colourless oil. − R_f = 0.45 (heptane/ethyl acetate, 2:1). − $[\alpha]_D^{22}$ = +64.98 (c = 0.5, $CHCl_3$). − IR (capillary): $\tilde{\nu}$ = 1752 cm^{-1}. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.25 (t, 3 H, J = 7.1 Hz, CH_3CH$_2$), 3.71 (dt, 2 H, J = 8.0 Hz, $^3J_{1,2}$ = $^3J_{3,4}$ = 10.5 Hz, 1-H, 4-H), 4.18 (q, 2 H, J = 7.1 Hz, CH$_3$CH_2), 4.10−4.30 (m, 4 H, 5-H, 6-H, PhCH_2), 4.68 (d, AB, 2 H, PhCH_2), 4.77−4.98 (m, 4 H, OCH_2COOEt, PhCH_2), 5.77 (ddt, 2 H, $^3J_{2,3}$ = 1.8 Hz, 2-H, 3-H), 7.22−7.37 (m, 15 H, Ph). − ^{13}C NMR (62 MHz, $CDCl_3$): δ = 14.2 (*C*H$_3$CH$_2$), 60.9 (CH$_3$*C*H$_2$), 68.7, 72.4, 75.4 (3 × Ph*C*H$_2$), 80.2 (C-5), 81.5 (C-6), 83.7, 83.8 (C-1, C-4), 127.6, 127.6, 127.7, 127.8, 127.9, 128.0 (3 × *o*,*m*,*p*-*C*$_6$H$_5$CH$_2$), 128.4, 128.4 (C-2, C-3), 138.3, 138.6, 138.6 (3 × *i*-*C*$_6$H$_5$CH$_2$), 170.5 (*C*OOEt). − MS (CI); *m*/*z* (%): 503 (10) [M + H]$^+$. − $C_{31}H_{34}O_6$ (502.60): calcd. C 74.08, H 6.82; found C 73.95, H 6.80.

2-[(1*S*,4*S*,5*R*,6*S*)-4,5,6-Tris(benzyloxy)cyclohex-2-enyloxy]acetaldehyde (4): To a solution of **3** (0.25 g, 0.5 mmol) in anhydrous toluene (5 mL) at −78 °C was added dropwise a 1 M solution of DIBAH in toluene (0.55 mL). The mixture was stirred for 30 min at −78 °C. After addition of methanol (0.2 mL) at −78 °C, the reaction mixture was allowed to warm up to 20 °C. The suspension was treated with ice-cold 1 N hydrochloric acid (5 mL), the phases were separated and the aqueous phase was extracted with ethyl acetate (20 mL). The combined organic phases were washed with brine, dried with Na_2SO_4 and concentrated under vacuum. The residue was chromatographed on silica gel to give **4** (0.17 g, 74%) as a colourless oil. − R_f = 0.30 (toluene/ethyl acetate, 5:1). − IR (capillary): $\tilde{\nu}$ = 1733 cm^{-1}. − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.72 (ddd, 2 H, $^3J_{2,3}$ = $^3J_{5,6}$ = 10.1 Hz, 2-H, 5-H), 4.08−4.14 (m, 1 H, 5-H/6-H), 4.19−4.26 (m, 1 H, 5-H/6-H), 4.22 (d, 2 H, $^3J_{CHO,\ CH_2}$ = 0.8 Hz, OCH_2CHO), 4.70 (d, AB, 2 H, PhCH_2), 4.85 (d, AB, 2 H, PhCH_2), 4.90 (d, AB, 2 H, PhCH_2), 5.77 (dd, 2 H, $^3J_{2,3}$ = 1.6 Hz, $^3J_{1,2}$ = $^3J_{3,4}$ = 10.1 Hz, 2-H, 3-H), 7.22−7.37 (m, 15 H, Ph), 9.61 (t, 1 H, $^3J_{CHO,\ CH_2}$ = 0.8 Hz, CHO). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 72.5 (*C*H$_2$CHO), 75.4, 75.5, 76.1 (3 × Ph*C*H$_2$), 80.0 (C-5), 81.4 (C-6), 83.4, 83.7 (C-1, C-4), 127.0, 127.6, 127.7, 127.8, 127.9, 127.9 (3 × *o*,*m*,*p*-*C*$_6$H$_5$CH$_2$), 128.4, 128.5 (C-2, C-3), 138.2, 138.4, 138.6 (*i*-*C*$_6$H$_5$CH$_2$), 200.2 (*C*HO). − MS (CI); *m*/*z* (%): 458 (15) [M]$^+$. − $C_{29}H_{30}O_5$ (458.55): calcd. C 75.96, H 6.59; found C 74.87, H 6.62.

(2a*S*,4a*S*,5*S*,6*S*,7*R*,7a*R*,7b*R*)-2-Benzyl-5,6,7-tris(benzyloxy)octahydro-2*H*-furo[4,3,2-*cd*][1,2]benzisoxazole (7): To a solution of **4** (0.46 g, 1 mmol) in anhydrous toluene (5 mL) was added a solution of *N*-benzylhydroxylamine [0.12 g, 1.1 mmol, prepared from the hydrochloride using triethylamine (0.1 mL)] in anhydrous dichloromethane (2 mL). The reaction mixture was stirred at 20 °C for 2 h. Removal of the solvents under reduced pressure furnished a res-

idue, which was chromatographed to give **7** (0.4 g, 70%) as a colourless wax. – R_f = 0.45 (toluene/ethyl acetate, 5:1). – $[\alpha]_D^{22}$ = –86.14 (c = 1.0, $CHCl_3$). – IR (capillary): $\tilde{\nu}$ = 3028 cm^{-1}, 2877, 1733, 1604, 1496, 1445, 1360. – ^1H NMR (250 MHz, $CDCl_3$): δ = 3.43 (ddd, 1 H, $^3J_{4a,7b}$ = 6.0 Hz, $^3J_{2a,7b}$ = 8.0 Hz, 7b-H), 3.60 (dd, 1 H, $^3J_{5,6}$ = 6.6 Hz, 5-H), 3.64 (dd, 1 H, 4a-H), 3.77 (dd, 1 H, J = 7.8 Hz, 3-H), 3.82 (dd, 1 H, $^3J_{2a,3}$ = 2.1 Hz, 2a-H), 3.92 (dd, 1 H, J = 2.7 Hz, 2-H), 3.97 (dd, 1 H, 6-H), 4.07 (dd, 1 H, $^3J_{7,7a}$ = 3.2 Hz, $^3J_{7a,7b}$ = 6.0 Hz, 7a-H), 4.35 (d, AB, 2 H, PhCH_2), 4.62 (d, AB, 2 H, PhCH_2), 4.78 (2 × d, 4 H, 2 × PhCH_2), 4.83 (d, AB, 2 H, PhCH_2), 7.22–7.40 (m, 15 H, Ph), 7.55–7.60 (m, 5 H, Ph). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 49.1 (C-7b), 61.5 (PhCH_2N), 71.8 (C-2a), 71.9 (C-3), 73.4, 74.0, 74.1 (3 × PhCH_2), 79.5 (C-7a), 80.8 (C-4a, C-7), 81.2 (C-5), 82.2 (C-6), 127.0, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.4, 129.3 (*o*,*m*,*p*-$C_6H_5CH_2$), 136.7, 138.0, 138.8, 138.9 (*i*-$C_6H_5CH_2$). – MS (CI); *m*/*z* (%): 563 (10) $[M]^+$. – $C_{36}H_{37}NO_5$ (563.69): calcd. C 76.71, H 6.62, N 2.48; found C 76.62, H 6.46, N 2.50.

(2a*S*,4a*S*,5*S*,6*S*,7*R*,7a*R*,7b*R*)-5,6,7-Tris(benzyloxy)-2-methyloctahydro-2*H*-furo[4,3,2-*c*,*d*][1,2]benzisoxazole (8): Compound **4** (0.46 g, 1 mmol) and *N*-methylhydroxylamine (0.05 g, 1.1 mmol) were treated according to the procedure described for **7**. The residue was purified on silica gel to give **8** (0.43 g, 88%) as colourless crystals, m.p. 86 °C (methanol). – R_f = 0.33 (toluene/ethyl acetate, 2:1). – $[\alpha]_D^{22}$ = –86.14 (c = 1.0, $CHCl_3$). – IR (capillary): $\tilde{\nu}$ = 2870 cm^{-1}, 1605, 1496, 1454, 1365. – ^1H NMR (250 MHz, $CDCl_3$): δ = 2.67 (s, 3 H, Me), 3.41 (ddd, 1 H, $^3J_{7a,7b}$ = 6.0 Hz, $^3J_{2a,7b}$ = 6.8 Hz, 7b-H), 3.61 (dd, 1 H, $^3J_{6,7}$ = 6.6 Hz, 7-H), 3.69 (dd, 1 H, 7a-H), 3.87 (dd, 1 H, J = 9.4 Hz, 2-H), 3.91 (dd, 1 H, $^3J_{2,2a}$ = 2.6 Hz, 2a-H), 3.98 (dd, 1 H, J = 2.3 Hz, 2-H), 4.01 (dd, 1 H, $^3J_{6,7}$ = 6.6 Hz, 6-H), 4.33 (dd, 1 H, $^3J_{4a,5}$ = 6.8 Hz, $^3J_{4a,7b}$ = 8.3 Hz, 4a-H), 4.64 (d, AB, 2 H, PhCH_2), 4.77 (s, 2 H, PhCH_2), 4.83 (d, AB, 2 H, PhCH_2), 7.20–7.40 (m, 15 H, Ph). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 44.6 (C-7b), 49.0 (Me), 72.6 (C-2), 74.0, 74.2, 74.3 (3 × PhCH_2), 79.6 (C-2a), 80.6 (C-4a), 81.1 (C-7a), 81.5 (C-5), 82.2 (C-7), 82.2 (C-6), 127.4, 127.7, 127.9, 127.9, 128.2, 128.4 (*o*,*m*,*p*-$C_6H_5CH_2$), 138.1, 138.8, 138.9 (*i*-$C_6H_5CH_2$). – MS (CI); *m*/*z* (%): 488 (8) $[M + H]^+$. – $C_{30}H_{33}NO_5$ (487.59): calcd. C 73.90, H 6.82, N 2.87; found C 74.08, H 6.80, N 2.89.

(3*S*,3a*S*,4*R*,5*S*,6*R*,7*S*,7a*S*)-3-Aminooctahydrobenzo[*b*]furan-4,5,6,7-tetraol Hydrochloride (9): To a solution of **7** (0.28 g, 0.5 mmol) in ethanol (25 mL) and 1 N aqueous HCl (2 mL) was added palladium on charcoal (50 mg). The suspension was stirred under hydrogen (1 bar) for 48 h at 20 °C. The mixture was filtered through Celite and the residue lyophilized to give **9** (0.11 g, 86%) as a colourless, amorphous solid. – $[\alpha]_D^{22}$ = –11.52 (c = 1.0, D_2O). – ^1H NMR (300 MHz, D_2O): δ = 3.06 (dt, 1 H, $^3J_{3a,7a}$ = 5.7 Hz, $^3J_{7,7a}$ = 8.4 Hz, 7a-H), 3.44 (dd, 1 H, $^3J_{2,3}$ = 6.1 Hz, $^2J_{2,2}$ = 9.7 Hz, 2-H), 3.81 (dd, 1 H, $^3J_{3,3a}$ = 5.1 Hz, $^3J_{2,3}$ = 6.1 Hz, 3-H), 4.00–4.30 (m, 7 H, 2-H, 3a-H, 5-H, 6-H, 7-H). – ^{13}C NMR (75 MHz, D_2O): δ = 50.5 (C-3a), 53.9 (C-3), 71.0 (C-2), 72.8, 75.4, 76.2, 77.6 (C-4, C-5, C-6, C-7), 82.8 (C-7a). – Positive FAB-MS; *m*/*z* (%): 205 (25) [M – Cl]$^+$.

(3*S*,3a*S*,4*R*,5*S*,6*R*,7*S*,7a*S*)-3-(Methylamino)octahydrobenzo-[*b*]furan-4,5,6,7-tetraol Hydrochloride (10): Compound **8** (0.24 g, 0.5 mmol) was hydrogenated for 48 h, according to the procedure described for the preparation of **9**. The mixture was filtered through Celite to furnish a colourless solid, which was recrystallized from ethanol to give **10** (0.1 g, 91%) as colourless crystals; m.p. 225 °C (ethanol). –$[\alpha]_D^{22}$ = +3.06 (c = 1.0, D_2O). – ^1H NMR (300 MHz, D_2O): δ = 2.88 (s, 3 H, Me), 3.22 (dt, 1 H, $^3J_{3a,7a}$ = 5.6 Hz, $^3J_{7,7a}$ = 8.4 Hz, 7a-H), 3.49 (dd, 1 H, $^3J_{2a,3}$ = 6.4 Hz, $^2J_{2,2}$ = 10.2 Hz, 2-H), 3.77 (dd, 1 H, $^3J_{3,3a}$ = 5 Hz, $^3J_{2,3}$ = 6.4 Hz, 3-H), 4.06 (dd, $^3J_{2,3}$ = 8.2 Hz, 2-H), 4.12–4.24 (m, 6 H, 3a-H, 4-H, 5-H, 6-H, 7-H). – ^{13}C NMR (75 MHz, D_2O): δ = 36.5 (Me), 43.1 (C-3a), 63.9 (C-3), 71.0 (C-2), 72.8, 75.1, 77.1, 79.9 (C-4, C-5, C-6, C-7), 84.6 (C-7a). – Positive FAB-MS; *m*/*z* (%): 220 (18) $[M - Cl]^+$. – $C_9H_{18}ClNO_5$ (255.69): calcd. C 42.28, H 7.10, N 5.48; found C 41.99, H 6.95, N 5.52.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-hydroxyimino-α-D-glucopyranoside Hydrochloride (12):[13] To a solution of **11**[12] (0.23 g, 0.5 mmol) in a mixture of pyridine (3 mL) and methanol (15 mL) was added hydroxylamine hydrochloride (0.04 g, 0.55 mmol). The suspension was stirred at 60 °C for 1 h. After cooling to 20 °C and concentration in vacuum, **12** (0.19 g, 80%) was obtained as a colourless, amorphous solid, which was used in the next step without further purification. – R_f = 0.36 (toluene/ethyl acetate, 5:1). – ^1H NMR (250 MHz, $CDCl_3$): δ = 3.38 (s, 3 H, Me), 3.45 (dd, 1 H, J = 4.6 Hz, 2-H), 3.53 (dd, 1 H, J = 3.6 Hz, $^3J_{4,3}$ = 9.6 Hz, 4-H), 4.02 (dd, 1 H, $^3J_{3,2}$ = 8.9 Hz, $^3J_{3,4}$ = 9.6 Hz, 3-H), 4.25 (ddd, 1 H, $^4J_{3,5}$ = 0.5 Hz, $^3J_{5,6}$ = 6.4 Hz, 5-H), 4.58 (d, AB, 2 H, PhCH_2), 4.62 (d, 1 H, $^3J_{1,2}$ = 1.7 Hz, 1-H), 4.79 (d, AB, 2 H, PhCH_2), 4.90 (d, AB, 2 H, PhCH_2), 7.16–7.40 (m, 16 H, 3 × Ph, 6-H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 55.5 (Me), 68.3 (C-5), 73.5, 74.9, 75.9 (3 PhCH_2), 79.5 (C-4), 79.6 (C-2), 81.4 (C-3), 98.3 (C-1), 127.7, 127.8, 128.0, 128.0, 128.1, 128.4 (*o*,*m*,*p*-$C_6H_5CH_2$), 137.8, 138.0, 138.6 (*i*-$C_6H_5CH_2$), 148.6 (C-6). – Positive FAB-MS; *m*/*z* (%): 478 (14) $[M + H]^+$.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-hydroxyamino-α-D-glucopyranoside Hydrochloride (13):[14] To a solution of sodium cyanoborohydride (0.06 g, 0.11 mmol) in methanol (5 mL) was added a solution of **12** (0.24 g, 0.5 mmol) in methanol (5 mL). This mixture was treated at –10 °C with 6 N HCl in methanol (2 mL), maintaining the pH value of the solution at 3 using a pH meter. The mixture was stirred at 20 °C for 1 h and concentrated under vacuum to furnish **13** (0.24 g, 100%) as a colourless, hygroscopic solid. The product was used in the next step without further purification. – R_f = 0.20 [toluene/ethyl acetate (0.1% triethylamine), 1:1]. – ^1H NMR (250 MHz, [D_6]DMSO): δ = 3.35–3.40 (m, 2 H, 6a-H, 6b-H), 3.38 (s, 3 H, Me), 3.46 (dd, 1 H, $^3J_{2,3}$ = 8.9 Hz, 2-H), 3.50 (dd, 1 H, J = 3.5 Hz, $^3J_{3,4}$ = 9.6 Hz, 4-H), 3.79 (dd, 1 H, 3-H), 3.93–4.03 (m, 1 H, 5-H), 4.65 (d, AB, 2 H, PhCH_2), 4.67 (d, 1 H, $^3J_{1,2}$ = 2.7 Hz, 1-H), 4.70 (d, AB, 2 H, PhCH_2), 4.83 (d, AB, 2 H, PhCH_2), 7.22–7.40 (m, 15 H, 3 × Ph), 11.02–11.10 (br. s, 1 H, NH), 11.68–11.80 (br. s, 1 H, OH). – ^{13}C NMR (62 MHz, [D_6]DMSO): δ = 51.7 (C-6), 55.4 (Me), 64.3 (C-5), 71.7, 74.0, 74.6 (3 × PhCH_2), 78.7 (C-4), 79.5 (C-2), 80.9 (C-3), 97.1 (C-1), 127.6, 127.8, 127.8, 127.9, 128.4, 128.4 (*o*,*m*,*p*-$C_6H_5CH_2$), 138.2, 138.6, 138.8 (*i*-$C_6H_5CH_2$). – MS, CI; *m*/*z* (%): 480 (13) $[M + H]^+$.

Methyl 2,3,4-Tri-*O*-benzyl-6-deoxy-6-{(2a*S*,4a*S*,5*S*,6*S*,7*R*,7a*R*,7b*R*)-5,6,7-tris(benzyloxy)octahydro-2*H*-furo[4,3,2-*c*,*d*]-1,2-benzisoxazol-2-yl}-α-D-glucopyranoside (14): To a solution of **4** (0.46 g, 1 mmol) in anhydrous toluene (5 mL) was added a solution of the free base of **13** [0.53 g, 1.1 mmol, prepared from the hydrochloride **13** and triethylamine (0.2 mL)] in dichloroethane (5 mL). The mixture was stirred at 20 °C for 24 h. Concentration of the suspension and column chromatography furnished a colourless solid, which was recrystallized from ethanol to give **14** (0.69 g, 75%) as colourless crystals. – M.p. 110 °C (ethanol). – R_f = 0.55 (toluene/ethyl acetate, 2:1). – $[\alpha]_D^{22}$ = –8.11 (c = 0.5, $CHCl_3$). – ^1H NMR (250 MHz, $CDCl_3$): δ = 2.88 (dd, 1 H, $^2J_{6a,6b}$ = 13.5 Hz, 6-H), 3.17 (dd, 1 H, 6-H), 3.26–3.36 (m, 1 H, 7b′-H), 3.33 (s, 3 H, Me), 3.48 (dd, 1 H, $^2J_{3'a,3'b}$ = 9.7 Hz, 3′a-H), 3.57–3.70 (m, 3 H, 2-H, 3-H, 4-H), 3.59 (dd, 1 H, 3′b-H), 3.72–3.85 (m, 2 H, 7a′-H, 7′-H), 3.88 (dd, 1 H,

J = 2.4 Hz, J = 6.4 Hz, 2a′-H), 3.95 (t, 1 H, J = 2.5 Hz, 5-H/6′-H), 3.98−4.0 (m, 1 H, 5-H/6′-H), 4.25 (dd, 1 H, $^3J_{4a',5'}$ = 6.4 Hz, $^3J_{4a',7b'}$ = 8.4 Hz, 4a′-H), 4.52 (d, 1 H, J = 3.4 Hz, 5′-H), 4.63 (d, 1 H, $^3J_{1,2}$ = 1.4 Hz, 1-H), 4.64 (d, AB, 2 H, PhCH_2), 4.73 (d, AB, 2 H, PhCH_2), 4.78 (d, AB, 2 H, PhCH_2), 4.81 (d, AB, 2 H, PhCH_2), 4.86 (d, AB, 2 H, PhCH_2), 4.94 (d, AB, 2 H, PhCH_2), 7.20−7.35 (m, 30 H, 6 × Ph). − ^{13}C NMR (75 MHz, $CDCl_3$): δ = 49.3 (C-7b′), 56.0 (Me), 58.2 (C-6), 69.9 (C-5), 73.3 (C-3′), 73.6, 73.8, 74.2, 74.7, 75.4, 76.1 (6 × PhCH_2), 79.0, 79.7, 80.1, 80.7, 81.4, 82.0, 82.3, 84.3 (C-2, C-3, C-4, C-4a′, C-5′, C-6′, C-7′, C-7a′), 98.1 (C-1), 128.1, 128.2, 128.3, 128.4, 128.5, 128.5, 128.6, 128.6, 128.7, 128.8, 128.9, 129.1, 129.2 (*o*,*m*,*p*-$C_6H_5CH_2$), 138.8, 138.9, 139.1, 139.4, 139.5, 139.5 (*i*-$C_6H_5CH_2$) − Positive FAB-MS; *m*/*z*: 920 [M^+] − $C_{57}H_{61}NO_{10}$ (920.11): calcd. C 74.41, H 6.68, N 1.52; found C 74.12, H 6.71, N 1.60.

Methyl 6-Deoxy-6-{(3*S*,3a*S*,4*R*,5*S*,6*R*,7*S*,7a*S*)-4,5,6,7-tetrahydroxyoctahydrobenzo[*b*]furan-3-ylamino}-α-D-glucopyranoside Hydrochloride (15): Compound **14** (0.15 g, 0.16 mmol) was hydrogenated under hydrogen (1 bar) in ethanol (10 mL) and 1 N aqueous HCl (2 mL) over 48 h at 20 °C in the presence of palladium on charcoal (0.05 g). Filtration through Celite and concentration under reduced pressure furnished a colourless hygroscopic solid, which was lyophilized to give **15** (0.05 g, 80%) as a colourless amorphous solid. − $[\alpha]_D^{22}$ = +51.67 (c = 1.0, D_2O). − ^{1}H NMR (250 MHz, D_2O): δ = 3.15 (dt, 1 H, $^3J_{6',7'}$ = 8.2 Hz, 7′-H), 3.17−3.42 (m, 2 H, 5-H, 6-H), 3.26 (dd, 1 H, $^2J_{2'a,2'b}$ = 12.5 Hz, 2′a-H), 3.41 (s, 3 H, Me), 3.58 (dd, 1 H, J = 3.6 Hz, J = 9.6 Hz, 6-H), 3.60−3.68 (m, 3 H, 2′b-H, 2-H, 4-H), 3.86−4.24 (m, 7 H, 3-H, 3′-H, 3a′-H, 4′-H, 5′-H, 6′-H, 7a′-H), 4.03 (d, 1 H, $^3J_{1,2}$ = 1.6 Hz, 1-H). − ^{13}C NMR (62 MHz, D_2O): δ = 42.4 (C-3a′), 52.2 (C-6), 58.5 (Me), 63.6 (C-7a′), 69.7 (C-5), 71.2 (C-2′), 72.9, 73.8, 74.5, 74.9, 75.4, 77.4, 80.0, 84.3 (C-2, C-3, C-3′, C-4, C-4′, C-5′, C-6′, C-7′), 102.4 (C-1). − Positive FAB-MS; *m*/*z*: 382 [M − Cl]$^+$.

Computational Calculations: All calculations were carried out with a Silicon Graphics O2/R5000 workstation. Forcefield calculations were made using the CERIUS2 program system, with the CFF91 forcefield[15] implemented in the OPEN FORCEFIELD module. Docking calculations were made using the program FlexX (version 1.5).[16] The default triangle mode was used for the calculation of receptor−ligand complexes. After the initial calculation of all complexes, the FlexX internal optimization procedure was used for the refinement of structural data.

General Enzyme Assay Procedure: The enzymes α-glucosidases (*brewer's yeast*, *sweet almonds*) and β-glucosidase (*Caldocellum saccharolyticum*) were purchased from SIGMA and used without further purification. K_I values were determined using six inhibitor concentrations, by taking the slopes from Lineweaver−Burk plots and plotting against inhibitor concentrations (straight line fitting). − a) Inhibition of α-glucosidase (*brewer's yeast*): Experiments were performed at 25 °C, using 0.05 M NaH_2PO_4/Na_2HPO_4 buffer solutions and 4-nitrophenyl α-D-glucopyranoside as the substrate. Measurements were started on addition of the enzyme. Data were collected over 12 min (1 min each). − b) Inhibition of β-glucosidase (*Caldocellum saccharolyticum*) and β-glucosidase (*sweet almonds*): Experiments were performed in similar manner to a), except that 4-nitrophenyl β-D-glucopyranoside was used for the measurements.

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[9] Crystal structure analysis of **8**: Siemens P4 four-circle diffractometer, Mo-K_α radiation, graphite monochromator, crystal size 0.84 × 0.46 × 0.14 mm, T = 293(2) K, $C_{30}H_{33}NO_5$, M = 487.57, colourless plates, monoclinic, a = 8.530(2), b = 11.766(2), c = 13.664(2) Å, β = 106.59(2)°, V = 1314.3(4) Å^3, Z = 2, space group $P2_1$, $\rho_{calcd.}$ = 1.232 Mg m^{-3}, μ = 0.083 mm^{-1}, F(000) = 520, data collection range: 4.66° ≤ 2Θ ≤ 44.00°, −8 ≤ h ≤ 8, −12 ≤ k ≤ 12, −14 ≤ l ≤ 14, 3652 reflections collected, 3215 independent and 2931 observed [I > 2σ (I)], R_1 = 0.0425. wR_2 = 0.1071, GOF(F^2) = 1.033; max./min. residual electron density: +0.136/−0.128 e·Å^{-3} The weighting scheme was calculated according to $w^{-1} = \sigma^2(F_o^2) + (0.0501P)^2 + 0.1345P$ with $P = (F_o^2 + 2F_o^2)/3$. − The structure was solved with direct methods (G. M. Sheldrick, *SHELXS-86*, Universität Göttingen, **1986**). All non-hydrogen atoms were refined anisotropically, hydrogen atoms introduced at theoretical positions and refined using the riding model. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-135526. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033 or E-mail: deposit@ccdc.cam.ac.uk].

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