182. Synthesis of Fused Triazoles as Probes for the Active Site of Retaining β-Glycosidases: From Which Direction Is the Glycoside Protonated?

by **Tom D. Heightman**, **Monica Locatelli**[1]), and **Andrea Vasella***

Laboratorium für organische Chemie, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

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The structure of the β-glycosidase inhibitors **1**–**7** and **10**–**13** suggests that protonation of *O*–C(1) (the glycosidic O-center) of the substrate by a carboxy group of the retaining β-glycosidases does not occur in the plane perpendicular to the ring of the glycon (β-side; 'from the top'), but in the plane of the ring ('from the side'). The triazoles **17** and **18** have been prepared in six steps from the L-xylofuranose **21**. They possess a CH group instead of the N-center of the related tetrazoles **4** and **5**, corresponding to the glycosidic O-atom, and a very similar structure, both in solution and in the solid state. Unlike the tetrazoles, however, which are good-to-medium inhibitors of retaining β-glycosidases, the triazoles do not inhibit the β-glycosidases from sweet almonds, snail, and bovine liver, and only slightly inhibit the β-glucosidase from *Caldocellum saccharolyticum*. This is in keeping with the proposed direction of protonation in the plane of the saccharide ring and with modelling studies, docking **4** into the active site of the white clover cyanogenic β-glucosidase and **6** into the *E. coli* β-galactosidase and the *Lactococcus lactis* 6-phospho-β-galactosidase.

Introduction. – Despite their diversity, glycosidases may be classified into four groups, according to the configuration of the substrate (α or β) and the stereoselectivity of the hydrolysis (retaining or inverting), suggesting the conservation of characteristic features of their active sites. The mechanism of the glycosidase-catalyzed hydrolysis has been intensely debated [1–8]. *Koshland*'s mechanism for retaining enzymes is still generally accepted [9]: the exocyclic O-atom is protonated by an enzymatic carboxylic-acid residue (AH), and the developing oxycarbenium cation is stabilized by a carboxylate group (B^-), which may or may not form a covalent link to the anomeric center, leading to an intermediate which is attacked by a H_2O molecule (deprotonated by the carboxylate A^-) to generate a hemiacetal (*cf. Fig. 1*).

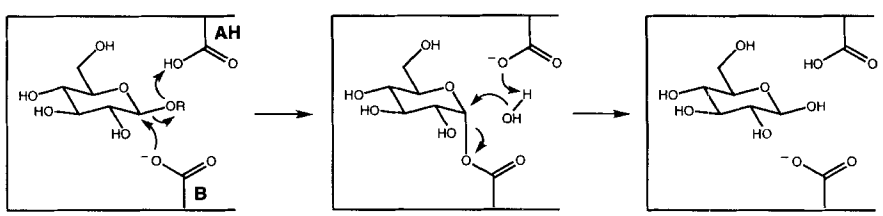
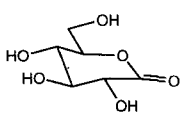


Fig. 1. Koshland's *mechanism for retaining β-glycosidases* (proceeding *via* a covalent intermediate)

[1]) *Erasmus* student November 1995–July 1996, State University of Milan.

Until recently, the evidence for this mechanism, implying a protonation 'from the top' (β-face), *i.e.*, perpendicular to the saccharide ring plane, was based upon the crystal structure of lysozyme [10], and the validity of the mechanism was extended to other glycosidases on the basis of irreversible inhibitor labelling [11] [12], point mutation studies [13], and kinetic isotope effects [14].

Reversible, competitive inhibitors of β-glycosidases have been designed to mimic the putative transition state by a six-membered ring in a flattened half-chair conformation with a positive charge as in the cationic intermediate. Established inhibitors of *exo*-β-glycosidases broadly form three classes, having either one or both of these features. The lactone **1** [15–17], lactam **2** [18], the lactone oxime **3** [19], the tetrazoles **4–6** [20–22], and the imidazole **7**[2]) [23] have a planar anomeric center but are uncharged (at a pH of *ca.* 4–7); 1-deoxynojirimycin **8** [24] and isofagomine **9** [25] [26] have a basic atom at or adjacent to the anomeric center, but are not appreciably flattened [27]; and the lactam oxime **10** [28] [29], the amidine **11**, the amidrazone **12** [29–31], and the guanidine **13** [32] have both features.



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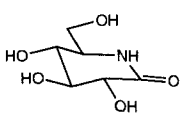

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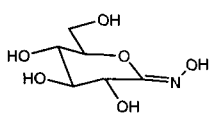

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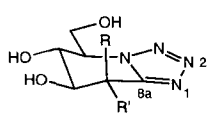

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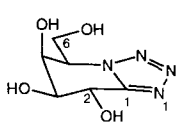
5 R = OH, R' = H



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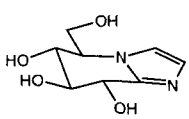

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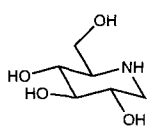

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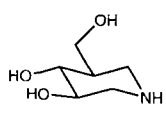

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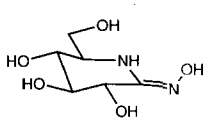

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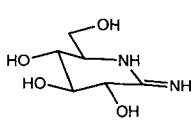

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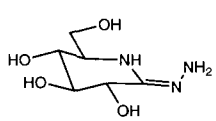

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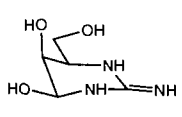

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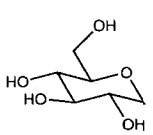

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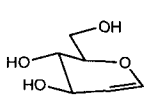

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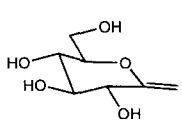

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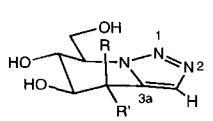

17[a]) R = H, R' = OH
18 R = OH, R' = H

[a]) Systematic numbering. [b]) Arbitrary (carbohydrate) numbering.

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[2]) Possibly, the imidazole is protonated at lower pH values, and should be classified into the third group, depending upon the pH value at which the inhibition is measured.

Among these inhibitors, those which are nonbasic (**1**–**7**[2])) cannot bind by adventitious ionic interactions. However, they all possess a heteroatom (N or O) at the glycosidic position, which suggests an important enzyme-inhibitor interaction at this position. Indeed, the inhibitors **14**–**16** lacking this heteroatom bind more weakly even than the substrate [33–35], although the absence of a heteroatom is not the only difference between **1**–**7** and **14**–**16**. Assuming that the inhibitors **1**–**7** (and **10**–**13**) bind in the same orientation as the substrates, it seems highly probable that the acidic residue (AH) of the enzyme donates a H-bond to this heteroatom, mimicking the protonation of the glycoside O-atom during hydrolysis. The only lone pair in **1**–**7** and **10**–**13** available for such an H-bond is in the plane of the saccharide ring (*Fig. 2, a*). Hence, either the carboxylic-acid residue AH is sufficiently mobile to donate a proton either in the ring plane, or perpendicular to it (*Fig. 2, a* and *b*); or both the inhibitor binding and glycosyl hydrolysis involve protonation in the ring plane (*Fig. 2, a* and *c*), in contrast to *Koshland*'s proposal[3]). We have described the strong-to-moderate inhibition (K_I between 1.5 and 160 μM) of various β-glycosidases by the tetrazoles **4** and **5**, and demonstrated their transition-state analogue character [20] [21]. Replacement of two of the N-atoms in **4** by CH moieties as in the analogous imidazole **7** leads to stronger inhibition [23], which is consistent with the proposed H-bond donation to the N-atom in the glycosidic position, since the energy of the doubly occupied, nonbonding σ-orbital is lower in the imidazole than in the tetrazole. We, therefore, expected the analogous triazoles, in which only one of the N-atoms is replaced by CH, to show inhibition intermediate between that of the imidazole and tetrazole, except when the CH group is in the glycosidic position, preventing the proposed H-bond. In this case, inhibition should be abolished or severely impaired.

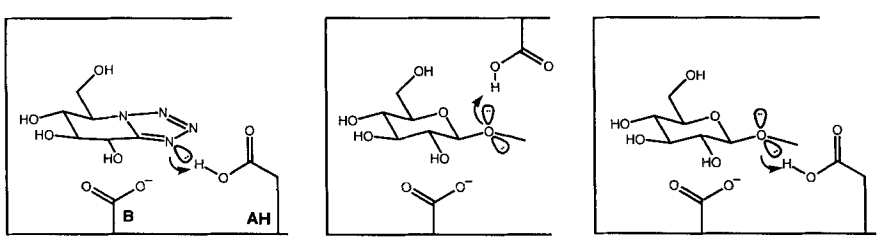


Fig. 2. *Proposed directions of protonation of* a) *inhibitors* **1**–**7**; b) *substrate perpendicular to the ring plane* (after *Koshland*); c) *substrate in the plane of the ring*

[3]) The structures of the hexosaminidase inhibitors **19** [22] and **20** [36] are also in accordance with H-bonding in the ring plane, but the orientations of the catalytic carboxylic-acid residues of lysozyme [10] and the probable anchimeric assistance by the acetamido group of other β-glycosaminidases [37] [38] make it unlikely that the mechanism discussed is valid for these enzymes.

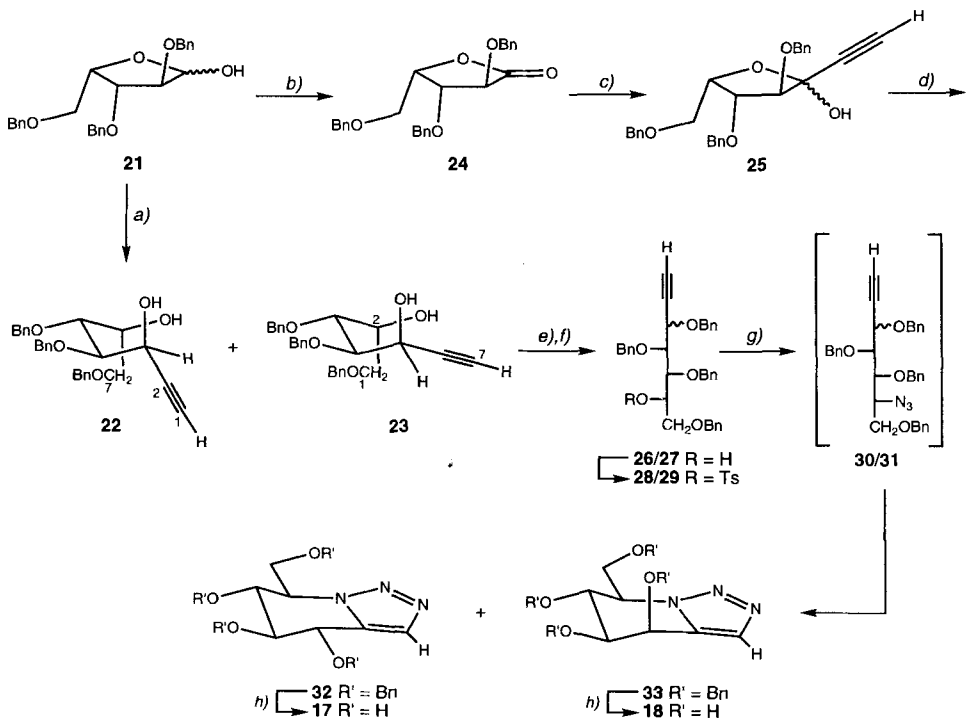
19 **20**

We report the synthesis of the appropriate *gluco*- and *manno*-configurated fused 1,2,3-triazoles **17** and **18**, and a comparison of their inhibition of commercially available glycosidases with that of tetrazoles **4** and **5**. Molecular-modelling studies (Macromodel V4.5, MM3* force field) suggest that the triazoles **17** and **18** should have a structure almost identical to the tetrazoles **4** and **5**, respectively, aside from the CH replacement.

Synthesis and Enzyme Studies. – The synthesis of the tetrazoles **4** and **5** by intramolecular 1,3-dipolar cycloaddition of an azido-nitrile [20] [21] suggests a similar preparation of the triazoles **17** or **18** from an azido-alkyne. This intermediate should be available from the tri-*O*-benzyl-L-xylofuranose **21** [23] (*Scheme*) *via* the L-*ido*- and D-*gluco*-diols **22**/**23**[4]), which have the correct configuration to give the D-*gluco*- and D-*manno*-configurated azido-alkynes **30**/**31** by activation and nucleophilic displacement of HO–C(6)/HO–C(2)[4]).

Ethynylation of the hemiacetal **21** was only satisfactory using a large excess of ethynylcerium(III) dichloride [39], yielding 86% of the L-*ido*- and D-*gluco*-diols **22** and **23** in a ratio of 45:55. Smaller amounts of the Ce reagent, or the analogous ethynylmagne-

Scheme



a) $CH{\equiv}CCeCl_2$, THF, −78→ + 23°; 86%. *b*) $CrO_3 \cdot Py$, CH_2Cl_2, 23°, 98%. *c*) $Me_3SiC{\equiv}CLi$, THF, −78°; 70%. *d*) $NaBH_4$, MeOH, 0→23°; 82%. *e*) NaH, BnBr, THF, reflux; 60%. *f*) TsCl, Py, DMAP, 50°; 87%. *g*) NaN_3, DMSO, 110°; 31% of **32** and 40% of **33**. *h*) H_2, Pd/C, MeOH, AcOH, 23°, 4 d; 83% for **17** and 78% for **18**.

[4]) IUPAC Nomenclature requires that the diols **22** and **23** be numbered from opposite ends.

sium or -lithium [40] reacted only sluggishly under a variety of conditions; the prolonged reaction led to decomposition. On a larger scale, the diols **22**/**23** were synthesized by oxidation of the hemiacetal **21** to the lactone **24**, addition of (trimethylsilyl)ethynyl-lithium, and reduction of the resulting hemiketals **25** with $NaBH_4$. This yielded 56% of **22**/**23** in a ratio of *ca.* 1:1. The separation of the diastereoisomers was postponed to the stage of the protected triazoles **32** and **33**; an analytical sample of **22** and **23** was obtained by HPLC. The ratio of the diastereoisomers **22**/**23** was estimated by ^{1}H-NMR from the integrals of the CH≡C and HO–C(6)(**22**)/HO–C(2)(**23**)[4]) signals at 2.49/2.53 and 2.99/2.66 ppm, respectively.

Regioselective 3-*O*/6-*O*-benzoylation of the diols **22**/**23** failed [41], but *O*-benzylation (BnBr, NaH, refluxing THF) gave 60% of the desired monoalcohols **26**/**27** (*Scheme*). The position of the OH group is deduced from the coupling between HO–C(6) and H–C(6)(**26**)/HO–C(2) and H–C(2)(**27**), and the disappearance of the two distinct HO–C(3)(**22**)/HO–C(5)(**23**) signals at 3.47 and 3.34 ppm. The tosylates **28**/**29**, obtained in 87% yield from **26**/**27**, were heated in DMSO with excess NaN_3 at 110° for 24 h. The intermediate azidoalkynes **30**/**31** were not observed by TLC, a 1:1 mixture of the tetra-*O*-benzyl triazoles **32** and **33** being produced directly. The diastereoisomers were readily separated by flash chromatography (FC) and isolated in 31 and 40% yields, respectively. The formation of a triazole is evident from the H–C(3) (systematic numbering) resonance at 7.57 and 7.60 ppm, respectively. Hydrogenolytic debenzylation of **32** or **33** gave the crystalline *gluco*- and *manno*-triazoles **17** and **18** in 83 and 78% yields, respectively.

The configurations of **22**/**23** were tentatively assigned by performing the synthesis on a 45:55 mixture, which gave **32**/**33** in a ratio of 45:55. The ^3J(H–C(3),OH) and the ^3J(H–C(5),OH) coupling constants (carbohydrate numbering) are very similar for **22** and **23**, and in keeping with intramolecular H-bonds. The H–C–C–H couplings suggest the preferred conformation indicated in the *Scheme*; in agreement with this, the J(4,5) and J(3,4) values of **22** and **23**, respectively, are quite similar to each other. The smaller value for **23** is in keeping with a slight rotation around the C(3)–C(4) bond to alleviate the interactions between the alkynyl and the C(2) substituents.

The X-ray crystal structure of **17** shows clearly its $^6H_5$ conformation (*Fig. 3*), almost identical to the one of **4**. The torsion angle C(7)–N(8)–C(3a)–C(4) in **17** is −5.7°, which compares well with the value of −3.7 to 4.5° for the equivalent angle in **4** (systematic numbering). The bond lengths of **17** are all within 0.022 Å of those of **4** except for C(3)–C(3a), which is 0.063 Å longer than the equivalent C(8a)–N(1) bond in **4**. The H_2O molecule observed in the crystal structure of **17** is also detected in the combustion analysis. In D_2O and in CD_3OD solution **4** and **17** have the same half-chair ($^6H_5$) conformation, as indicated by the H,H coupling constants (*Table 1*). The solution conformation of **18** in D_2O is also $^6H_5$, again close to that of **5**, as indicated by the coupling constants (*Table 1*). The conformation of both **5** and **18** changes to S_5 on going from D_2O to CD_3OD.

The results of the inhibition studies show that for all the enzymes tested, except one, glycosyl hydrolysis was unaffected by concentrations up to 8 mM of the triazoles **17** or **18** (*Table 2*). The hydrolyses were repeated and cross-checked by adding the corresponding tetrazole **4** or **5** (final concentration 1 mM) to cuvettes containing enzyme, substrate, and triazole, which immediately caused the expected inhibition. For the β-glucosidase from *Caldocellum saccharolyticum*, **17** showed an IC_{50} of 2 mM, which is higher than the K_M of the substrate (1.5 mM) and *ca.* 400 times the IC_{50} of the tetrazole **4** (0.005 mM).

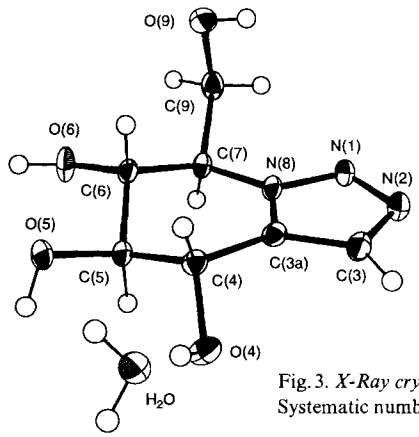


Fig. 3. *X-Ray crystal structure of* **17**. Systematic numbering for the fused rings.

Table 1. *Coupling Constants* J [Hz] *of Tetrazoles* **4** *and* **5** *and Triazoles* **17** *and* **18** *Measured in* D_2O *and* CD_3OD. The conventional carbohydrate numbering is used for convenience.

	4		**17**		**5**		**18**	
	D_2O	CD_3OD	D_2O	CD_3OD	D_2O	CD_3OD	D_2O	CD_3OD
$J(2,3)$	8.4	8.4	8.9	8.7	4.1	3.9	4.2	3.7
$J(3,4)$	9.5	9.6	10.1	9.6	9.0	7.4	10.4	7.4
$J(4,5)$	8.5	8.7	9.3	8.7	7.5	4.8	9.0	5.3
$J(5,6)$	2.3	2.2	2.3	2.5	2.8	[a])	2.8	3.1
$J(5,6')$	2.5	2.7	2.6	2.2	2.5	[a])	3.1	5.0
$J(6,6')$	12.1	12.8	12.6	11.8	12.5	[a])	14.0	11.2

[a]) Coupling constant not calculated due to overlap of the signals.

Table 2. *Comparison of Glycosidase Inhibition by Tetrazoles and Triazoles*

Enzyme	Inhibitor	K_M/mM	K_I/mM[a])
β-Glucosidase (sweet almonds)	**4**	2.4	0.08[b])
	17	2.4	[c])
β-Glucosidase (*C. saccharolyticum*)	**4**	1.5	0.005[b])
	17	1.5	2.0[b])
β-Mannosidase (snail)	**5**	0.49	0.16
	18	0.53	[c])
β-Galactosidase (bovine liver)	**4**	0.063	0.0015
	17	0.080	[c])

[a]) K_M values are for 2-nitrophenyl β-D-glucopyranoside with **4** and **17**, and for 2-nitrophenyl β-D-mannopyranoside with **5** and **18**. [b]) IC_{50}. [c]) No inhibition at 8 mM.

Discussion. – The very low inhibitory activity of both triazoles **17** and **18** strongly suggests that N(1) (systematic numbering) of the tetrazoles **4** and **5**, and equally the heteroatom of **1**–**7** and **10**–**13** corresponding to the glycosidic O-atom, accepts a H-bond in the ring plane from an acidic residue of the enzyme[5]), inviting a reinterpretation of previous observations.

[5]) A H-bond donation to N(2) of the tetrazole **4** has been observed in the X-ray crystal structure of a ternary phosphorylase-tetrazole-phosphate complex [42].

The orientation of the two catalytic residues, one in the ring plane and one perpendicular to it, is compatible with the opening of conduritol epoxides [11] [43] and cyclophellitol [12], as the lone pair of the epoxide O-atom extends rather in the ring plane, towards the site occupied by the carboxylic-acid group AH in the proposed orientation. The observations of the slow protonation of D-glucal (**15**) from the α-face and D-*gluco*-heptenitol (**16**) from the β-face by β-glucosidase from sweet almonds may also be rationalized [34] [35]. If the acidic Glu AH of the enzyme is situated in the ring plane of the substrate, protonation on either face perpendicular to the ring plane is unfavorable; the D-glucal protonation is ascribed to the action of the Glu (BH) which normally acts as the nucleophile (B^-), and the D-*gluco*-heptenitol protonation to a more strained conformation of the acidic Glu AH. In both cases, the hydrolysis is far less efficient than glycosyl hydrolysis, as reflected in the greatly diminished rates.

It has been suggested that the strong binding of lactone-type compounds to β-glycosidases is due to a 'dipolar interaction' between the nucleophilic carboxylate B^- and the C–heteroatom double bond [1] [6][6]). Although this suggestion is compatible with the poor inhibition by **17** and **18**, as compared with that by **4** and **5**, there is little convincing evidence from other examples.

To examine whether an enzymatic proton donation in the ring plane is reasonable, molecular modelling was performed using X-ray data for three retaining *exo*-β-glycosidases taken from the *Brookhaven Protein Data Base*. The tetrazole **4** was docked into the active site of the white clover cyanogenic β-glucosidase [44], and **6** into the *E. coli* β-galactosidase (*Fig. 4*) [45] and the *Lactococcus lactis* 6-phospho-β-galactosidase [46] in an orientation with the nucleophilic Glu B^- residue positioned beneath C(1) (carbohydrate numbering) of the inhibitor and the aglycon region facing the opening of the binding pocket. The location of **6** in the 6-phospho-β-galactosidase was aided by the sulfate ion bound in the putative binding site of the phosphate moiety. The acidic Glu residue AH in these enzymes is positioned in the ring plane of the inhibitor so as to protonate the lone pair of N(1). This binding model is supported by three further interactions between these enzymes and the inhibitors: H-bonds between HO–C(2) and Asn, and HO–C(3) and His, and a hydrophobic interaction between a Trp residue and

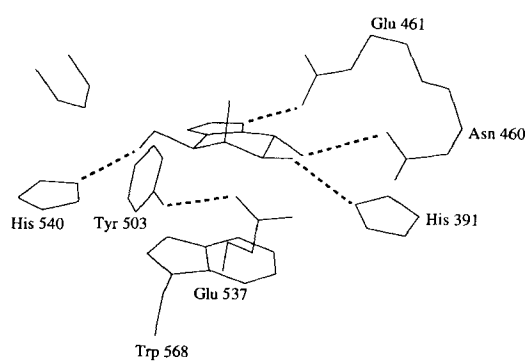


Fig. 4. *Model of the complex between* E. coli *β-galactosidase and tetrazole* **6**

[6]) We thank Prof. *Stephen Withers* for stressing this alternative explanation.

the C(4) region of the inhibitor (*Table 3*). A strikingly similar array of Glu, Asn, and His side chains is also evident in *Withers'* recent X-ray crystal structure of a complex between a catalytically competent hydrolysis intermediate and the *endo-β*-xylanase/glucanase from *C. fimi* (also retaining) [47], and in the structure of uncomplexed human *β*-glucuronidase [48], for which X-ray coordinates have not yet been released.

Table 3. *Proposed Active Site Residues Conserved in the Modelled Complexes with* **4** *and* **6**

Enzyme	*β*-Galactosidase	*β*-Glucosidase	6-Phospho-*β*-galactosidase
Source	*E. coli*	White clover	*Lactococcus lactis*
Family [49]	2	1	1
Nucleophile	Glu 537	Glu 397	Glu 375
Proton donor	Glu 461	Glu 183	Glu 160
HO–C(2) contact[a])	Asn 460	Asn 182	Asn 159
HO–C(3) contact[a])	His 391	His 137	His 116
Hydrophobic contact	Trp 568	Trp 446	Trp 421
Reference	45	44	46

[a]) Carbohydrate numbering.

The observation of similar orientations in the enzymes discussed of not only the catalytic Glu, but also of the Asn, His, and Trp residues relative to the bound inhibitor/substrate, suggests that this binding orientation is quite general among retaining *endo-* and *exo-β*-glycosidases. Location of the glycon binding site in these enzymes should facilitate the identification of likely aglycon binding sites, known to vary considerably from one enzyme to another, and the design of more potent inhibitors.

We thank Dr. *Bernd Schweizer* for the X-ray structure of **17**, and the *Swiss National Science Foundation* and *F. Hoffmann-La Roche AG*, Basel, for generous support.

Experimental Part

General. Enzymes were purchased from *Sigma Chemical Co.* and used without further purification. Solvents were distilled before use. Normal workup implies distribution of the crude product between the indicated org. solvent and H_2O, drying of the org. layer ($MgSO_4$), filtration, and evaporation of the filtrate. TLC: *Merck* silica gel *60F-254* plates; detection by heating with 5% vanillin in conc. H_2SO_4 or with 'mostain' (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6H_2O$, 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Merck 60* (0.04–0.063 mm). M.p.: uncorrected. ^{1}H- (300 MHz, if not indicated otherwise) and ^{13}C-NMR (75 MHz, if not indicated otherwise): chemical shifts δ in ppm and coupling constants J in Hz. FAB- and CI-MS: 3-nitrobenzyl alcohol and NH_3 as matrix, resp., unless indicated otherwise.

*2,3,5-Tri-*O*-benzyl-*L*-xylono-1,4-lactone* (**24**). A soln. of **21** (prepared from commercially available L = xylose by methylation, benzylation, and demethylation [23]; 5.00 g, 11.9 mmol) in CH_2Cl_2 (50 ml) was added to a stirred soln. of pyridine·$2CrO_3$ (48.2 mmol) in CH_2Cl_2 (100 ml) at 23°, followed immediately by Ac_2O (4.50 ml, 47.6 mmol). After 3 h, the soln. was transferred to a column of silica which was eluted with AcOEt. The first fraction was evaporated: **24** (4.80 g, 98%). Colorless crystals. M.p. 62–64°. $[\alpha]_D^{25} = -94.2$ ($c = 1.0$, $CHCl_3$) ([38] for D-enantiomer: m.p. 63–64°, $[\alpha]_D^{25} = +95.6$).

*4,5,7-Tri-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-*L*-ido-heptitol* (**22**) *and 1,3,4-Tri-*O*-benzyl-6,6,7,7-tetradehydro-6,7-dideoxy-*D*-gluco-heptitol* (**23**). *a*) A soln. of 0.5M CH≡CMgCl in THF (100 mmol) was added to a stirred suspension of $CeCl_3$ (25.1 g, 101 mmol) in THF (200 ml) at −78°. After 1 h, a soln. of **21** (2.00 g, 4.76 mmol) in THF (20 ml) was added dropwise. After 1 h, the soln. was warmed slowly to 23° and stirred for a further 4 h, then cooled to 0° and treated with sat. aq. NH_4Cl soln. Normal workup (Et_2O, H_2O, brine, $MgSO_4$) followed by FC (Et_2O/hexane 1:1) gave **22**/**23** *ca.* 45:55 as colorless oil (1.82 g, 86%). Pure samples of **22** and **23** were obtained by HPLC (Et_2O/hexane 1:1, *Spherisorb 5 W*).

b) A soln. of **24** (7.41 g, 17.7 mmol) in dry THF (200 ml) was added dropwise to a stirred soln. of $Me_3SiC{\equiv}CLi$ (35.4 mmol) in THF (200 ml) under Ar at −78°. After 1 h, the soln. was treated with sat. aq. NH_4Cl soln. and warmed to 23°. Normal workup (Et_2O, H_2O, brine, $MgSO_4$) and evaporation gave crude **25** (5.51 g, 70%). A stirred soln. of **25** (5.51 g, 12.4 mmol) in MeOH (200 ml) at 0° was treated with $NaBH_4$ (5.04 g, 133 mmol) and warmed to 23°. After 5 h, the soln. was cooled to 0° and brought to *ca.* pH 2 with 1M aq. HCl. Normal workup (AcOEt, H_2O, brine, $MgSO_4$) followed by FC (Et_2O/hexane 7:3) gave **22**/**23** *ca.* 1:1 (4.53 g, 82%).

Data for **22**: R_f (AcOEt/hexane 1:2) 0.22. $[\alpha]_D^{25} = +1.0$ (*c* = 0.83, $CHCl_3$). IR ($CHCl_3$): 3557*m*, 3414*m*, 3306*s*, 3090*m*, 3067*m*, 3007*m*, 2869*m*, 1952*w*, 1877*w*, 1812*w*, 1605*w*, 1497*m*, 1454*s*, 1398*m*, 1357*m*, 1261*m*, 1105*s*, 1028*s*, 912*w*. ^{1}H-NMR ($CDCl_3$): 2.49 (*d*, *J* = 2.8, H–C(1)); 2.99 (*d*, *J* = 6.6, HO–C(6)); 3.40 (*dd*, *J* = 10.4, 6.9, H–C(7)); 3.47 (*d*, *J* = 8.6, HO–C(3)); 3.51 (*dd*, *J* = 10.4, 7.3, H′–C(7)); 3.71 (*dd*, *J* = 7.3, 2.1, H–C(5)); 3.83 (*dd*, *J* = 7.3, 2.8, H–C(4)); 4.06–4.13 (*m*, H–C(6)); 4.43 (*d*, *J* = 13.1, PhC*H*); 4.48 (*d*, *J* = 12.5, PhC*H*); 4.50 (*d*, *J* = 13.1, PhC*H*); 4.66 (*d*, *J* = 12.5, PhC*H*); 4.67–4.69 (*m*, H–C(3)); 4.75 (*d*, *J* = 12.5, PhC*H*); 4.88 (*d*, *J* = 12.5, PhC*H*); 7.21–7.39 (15 arom. H); irrad. at 2.99→4.10 (*td*, *J* = 7.1, 2.1); irrad. at 4.10→3.71 (*d*, *J* = 10.4), 3.51 (*d*, *J* = 10.4), 2.99 (br. *s*). ^{13}C-NMR ($CDCl_3$): 61.50 (*d*); 68.25 (*s*); 68.98 (*d*); 71.25 (*t*); 73.53 (*t*); 74.78 (*t*); 75.10 (*t*); 77.89 (*d*); 81.02 (*d*); 83.78 (*d*); 128.00–128.28 (several *d*); 138.29 (2*s*); 138.37 (*s*). FAB-MS: 893 (30, $[2M + H]^+$), 447 (100, $[M + H]^+$), 181 (42). Anal. calc. for $C_{28}H_{30}O_5$ (446.54): C 75.31, H 6.77; found: C 75.27, H 6.84.

Data for **23**: R_f (AcOEt/hexane 1:2) 0.22. $[\alpha]_D^{25} = -34.8$ (*c* = 0.68, $CHCl_3$). IR ($CHCl_3$): 3566*m*, 3306*m*, 3090*m*, 3067*m*, 3008*m*, 2868*m*, 1952*w*, 1877*w*, 1812*w*, 1605*w*, 1497*m*, 1455*m*, 1398*m*, 1356*m*, 1261*m*, 1070*s*, 1028*s*, 913*w*. ^{1}H-NMR ($CDCl_3$): 2.53 (*d*, *J* = 2.8, H–C(7)); 2.66 (*d*, *J* = 6.6, HO–C(2)); 3.34 (*d*, *J* = 8.6, HO–C(5)); 3.44 (*dd*, *J* = 10.4, 5.9, H–C(1)); 3.50 (*dd*, *J* = 10.4, 8.0, H′–C(1)); 3.81 (*t*, *J* = 5.9, H–C(4)); 3.97 (*dd*, *J* = 5.9, 3.5, H–C(3)); 4.03–4.09 (*m*, H–C(2)); 4.45 (*s*, PhCH_2); 4.62 (*d*, *J* = 12.5, PhC*H*); 4.66 (*d*, *J* = 12.8, PhC*H*); 4.66–4.72 (*m*, H–C(5)); 4.76 (*d*, *J* = 13.1, PhC*H*); 4.80 (*d*, *J* = 12.1, PhC*H*); 7.27–7.39 (15 arom. H); irrad. at 2.53 → change at 4.66–4.72; irrad. at 2.66 → change at 4.03–4.09; irrad. at 4.69 → 2.53 (*s*), 3.34 (*s*), 3.81 (*d*, *J* = 5.9). ^{13}C-NMR ($CDCl_3$): 62.65 (*d*); 70.25 (*d*); 71.38 (*t*); 73.58 (*t*); 74.10 (*t*); 74.83 (*d*); 74.93 (*t*); 79.43 (*d*); 80.41 (*d*); 82.96 (*s*); 128.24–128.84 (several *d*); 138.11 (2*s*); 138.25 (*s*). FAB-MS: 893 (39, $[2M + H]^+$), 447 (100, $[M + H]^+$), 181 (72).

*3,4,5,7-Tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-*L*-ido-heptitol* (**26**) *and 1,3,4,5-Tetra-*O*-benzyl-6,6,7,7-tetradehydro-6,7-dideoxy-*D*-gluco-heptitol* (**27**). A stirred soln. of **22**/**23** *ca.* 1:1 (850 mg, 1.90 mmol) in dry THF (10 ml) was added to a stirred suspension of NaH (48.9 mg, 2.13 mmol) in THF (10 ml) and heated at reflux for 1 h, then cooled to 23°. The soln. was treated with BnBr (230 µl, 1.90 mmol) and heated at reflux for 2 h. After cooling to 23°, the soln. was treated with MeOH (1 ml). Normal workup (Et_2O, H_2O, brine, $MgSO_4$) followed by FC (Et_2O/hexane 2:3) gave **26**/**27** *ca.* 1:1 (613 mg, 60%). Colorless oil. R_f (AcOEt/hexane 1:2) 0.51. IR ($CHCl_3$): 3567*m*, 3305*m*, 3090*m*, 3067*m*, 3008*s*, 2926*m*, 2868*m*, 1952*w*, 1876*w*, 1811*w*, 1605*w*, 1497*m*, 1454*s*, 1392*m*, 1353*m*, 1248*m*, 1070*s*, 1028*s*, 909*m*. ^{1}H-NMR ($CDCl_3$): 2.54 (*d*, *J* = 6.2, 0.5 H, OH); 2.57 (*d*, *J* = 2.2, 0.5 H, CH≡C); 2.59 (*d*, *J* = 2.2, 0.5 H, CH≡C); 2.62 (*d*, *J* = 5.6, 0.5 H, OH); 3.44 (*d*, *J* = 5.9, 1 H, CH_2OBn); 3.47 (*d*, *J* = 5.9, 1 H, CH_2OBn); 3.84 (*dd*, *J* = 5.6, 3.4, 0.5 H); 3.89 (*t*, *J* = 5.4, 0.5 H); 3.93–4.05 (*m*, 2 H); 4.42–4.96 (*m*, 9 H); 7.19–7.39 (*m*, 20 H). ^{13}C-NMR ($CDCl_3$): 69.82 (*d*); 70.31 (*d*); 70.86 (*t*); 71.15 (*t*); 71.31 (*t*); 71.36 (*t*); 73.37 (2*t*); 74.63 (*t*); 75.08 (2*t*); 75.36 (*t*); 76.21 (*d*); 76.38 (*d*); 78.54 (*d*); 79.16 (*d*); 80.39 (*s*); 80.79 (*s*); 81.26 (*d*); 81.34 (*d*); 127.24–128.74 (several *d*); 137.40 (*s*); 137.76 (*s*); 138.39 (2*s*); 138.53 (2*s*); 138.68 (*s*). CI-MS: 537 (1, $[M + H]^+$), 201 (3), 181 (15), 91 (100). Anal. calc. for $C_{35}H_{36}O_5$ (536.67): C 78.33, H 6.76; found: C 78.08, H 6.77.

*3,4,5,7-Tetra-*O*-benzyl-1,1,2,2-tetradehydro-1,2-dideoxy-6-*O*-(toluene-4-sulfonyl)-*L*-ido-heptitol* (**28**) *and 1,3,4,5-Tetra-*O*-benzyl-6,6,7,7-tetradehydro-6,7-dideoxy-2-*O*-(toluene-4-sulfonyl)-*D*-gluco-heptitol* (**29**). A soln. of **26**/**27** *ca.* 1:1 (850 mg, 1.58 mmol), TsCl (3.66 g, 19.2 mmol) and 4-(dimethylamino)pyridine (DMAP; 20 mg) in dry pyridine was stirred at 50° for 12 h. The soln. was cooled to 0° and treated with sat. aq. $NHCO_3$ soln. After 30 min, the soln. was worked up as usual (Et_2O, H_2O, brine, $MgSO_4$). FC (AcOEt/hexane 1:5) gave **28**/**29** *ca.* 1:1 (1.05 g, 87%). Colorless oil. R_f (Et_2O/hexane 1:2) 0.33. IR ($CHCl_3$): 3300*m*, 3011*m*, 2956*m*, 2867*m*, 1952*w*, 1600*m*, 1494*m*, 1456*m*, 1361*m*, 1261*s*, 1178*s*, 1094*s*, 1027*s*, 911*m*. ^{1}H-NMR ($CDCl_3$): 2.33 (*s*, 1.5 H), 2.35 (*s*, 1.5 H, *Me*C_6H_4); 2.54 (*d*, *J* = 2.1, 0.5 H), 2.57 (*d*, *J* = 2.4, 0.5 H, CH≡C); 3.30 (*dd*, *J* = 12.8, 5.5, 0.5 H), 3.40 (*dd*, *J* = 12.8, 5.5, 0.5 H, CH_2OBn); 3.63 (*dd*, *J* = 12.4, 3.8, 0.5 H), 3.69 (*dd*, *J* = 12.8, 3.5, 0.5 H, CH_2OBn); 3.78 (*dd*, *J* = 7.6, 4.2, 0.5 H); 3.82 (*dd*, *J* = 7.6, 4.2, 0.5 H); 4.10 (*dd*, *J* = 7.3, 4.2, 0.5 H); 4.19–4.61 (*m*, 7.5 H); 4.79–4.95 (*m*, 3 H); 7.09–7.35 (*m*, 22 arom. H); 7.66–7.70 (*m*, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 21.42 (2*q*); 68.07 (*t*); 68.22 (*t*); 68.55 (*d*); 70.14 (*t*); 70.22 (*d*); 70.99 (*t*); 72.39 (*t*); 72.79 (*t*); 73.70 (*t*); 74.28 (*t*); 74.53 (*t*); 74.96 (*t*); 76.08 (*d*); 76.25 (*d*); 76.53 (*d*); 76.98 (*d*); 78.72 (*d*); 79.34 (*d*); 79.94 (*s*); 80.79 (*s*); 81.43 (*d*); 81.63 (*d*); 127.34–129.49 (several *d*); 133.63 (*s*); 133.70 (*s*); 137.12 (*s*); 137.22 (*s*); 137.49 (2*s*); 137.75 (*s*); 137.82 (2*s*); 137.85 (*s*); 144.23 (2*s*). FAB-MS: 691 (1, $[M + H]^+$), 281 (40), 181 (75), 91 (100). Anal. calc. for $C_{42}H_{42}O_7S$ (690.86): C 73.02, H 6.13; found: C 72.99, H 6.21.

*(4*S*,5*S*,6*R*,7*R*)- and (4*R*,5*S*,6*R*,7*R*)-4,5,6-Tris(benzyloxy)-7-[(benzyloxy)methyl]-4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a]pyridine* (**32** and **33**, resp.). A soln. of **28**/**29** 45:55 (2.00 g, 2.91 mmol) and NaN_3 (3.77 g, 57.9

mmol) in DMSO (100 ml) was stirred at 110° for 4 d. Evaporation followed by normal workup (Et_2O, H_2O, brine, $MgSO_4$) and FC (Et_2O/hexane 9:1) gave **32** (444 mg, 31%) and **33** (571 mg, 40%) both as colorless oils.

Data for **32**: R_f (AcOEt/hexane 1:2) 0.32. $[\alpha]_D^{25} = +14.1$ ($c = 0.75$, $CHCl_3$). IR ($CHCl_3$): 3090*w*, 3067*w*, 3008*m*, 2961*m*, 2926*m*, 2870*m*, 1952*w*, 1877*w*, 1810*w*, 1603*w*, 1497*m*, 1454*m*, 1363*m*, 1327*m*, 1261*m*, 1094*s*, 1027*s*, 911*w*. ^{1}H-NMR ($CDCl_3$): 3.99 (*dd*, $J = 8.4$, 7.8, H–C(5)); 4.27 (br. *t*, $J \approx 8.4$, H–C(6)); 4.36–4.48 (*m*, H–C(7), CH_2C(7)); 4.60 (*d*, $J = 10.9$, PhC*H*); 4.75 (*d*, $J = 7.8$, H–C(4)); 4.84 (*s*, PhCH_2); 4.87, 4.88 (inner lines of *AB*, PhCH_2); 4.90 (*d*, $J = 10.9$, PhC*H*); 7.18–7.37 (*m*, 20 arom. H); 7.57 (*s*, H–C(3)). ^{13}C-NMR ($CDCl_3$): 61.23 (*d*); 66.33 (*t*); 73.27 (*d*); 73.37 (*t*); 73.45 (*t*); 73.89 (*t*); 75.19 (*t*); 75.31 (*d*); 82.65 (*d*); 127.69–128.98 (several *d*); 131.60 (*d*); 131.96 (*s*); 135.02 (*s*); 137.38 (*s*); 137.56 (*s*); 137.81 (*s*). FAB-MS: 1123 (6, $[2M + H]^+$), 562 (100, $[M + H]^+$), 454 (9), 364 (4). Anal. calc. for $C_{35}H_{35}N_3O_4$ (561.68): C 74.84, H 6.28, N 7.48; found: C 74.92, H 6.27, N 7.64.

Data for **33**: R_f (AcOEt/hexane 1:2) 0.20. $[\alpha]_D^{25} = -49.7$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3089*w*, 3067*w*, 3007*m*, 2926*m*, 2870*m*, 1952*w*, 1812*w*, 1604*w*, 1497*m*, 1454*m*, 1364*m*, 1327*m*, 1261*m*, 1097*s*, 1028*m*, 912*m*. ^{1}H-NMR ($CDCl_3$): 3.88 (*dd*, $J = 8.4$, 3.4, H–C(5)); 4.06 (*dd*, $J = 9.6$, 3.4, CH–C(7)); 4.21 (*dd*, $J = 9.6$, 5.6, CH′–C(7)); 4.44 (*d*, $J = 12.1$, PhC*H*); 4.48 (*d*, $J = 11.9$, PhC*H*); 4.51 (*d*, $J = 12.1$, PhC*H*); 4.58 (*td*, $J = 5.6$, 3.4, H–C(7)); 4.62–4.66 (*m*, 4 PhC*H*); 4.71 (*dd*, $J = 8.1$, 5.6, H–C(6)); 4.82 (*d*, $J = 3.1$, H–C(4)); 4.83 (*d*, $J = 11.2$, PhC*H*); 7.22–7.37 (*m*, 20 arom. H); 7.60 (*s*, H–C(3)). ^{13}C-NMR ($CDCl_3$): 61.37 (*d*); 66.51 (*d*); 68.35 (*t*); 71.10 (*t*); 72.52 (*t*); 72.99 (*d*); 73.29 (*t*); 74.15 (*t*); 77.55 (*d*); 127.70–128.59 (several *d*); 132.23 (*d*); 132.29 (*s*); 137.26 (*s*); 137.54 (*s*); 137.69 (*s*); 137.78 (*s*). FAB-MS: 1123 (4, $[2M + H]^+$), 900 (10), 652 (4), 562 (100), 454 (7), 91 (65). Anal. calc. for $C_{35}H_{35}N_3O_4$ (561.68): C 74.84, H 6.28, N 7.48; found: C 74.78, H 6.47, N 7.43.

*(4*S*,5*S*,6*R*,7*R*)-4,5,6,7-Tetrahydro-7-(hydroxymethyl)[1,2,3]triazolo[1,5-a]pyridine-4,5,6-triol* (**17**). A soln. of **32** (350 mg, 0.620 mmol) in MeOH (10 ml) at 23° was treated with AcOH (1 ml) and 10% Pd/C (35.2 mg) and hydrogenated at 6 bar for 4 d. The suspension was filtered through *Celite* and the solid residue washed with MeOH (20 ml). Evaporation of the filtrate followed by FC (AcOEt/MeOH 19:1) gave **17** as a colorless oil which crystallized *i.v.* (104 mg, 83%). Recrystallization from EtOH gave crystals for X-ray analysis. R_f (AcOEt/MeOH 3:1) 0.44. M.p. 168–169°. $[\alpha]_D^{25} = -57.5$ ($c = 0.90$, H_2O). IR (KBr): 3439*vs* (br.), 1659*m*, 1650*m*, 1643*m*, 1556*w*, 1434*m*, 1384*m*, 1317*m*, 1114*m*, 1067*m*, 1031*m*, 911*w*, 853*w*. ^{1}H-NMR (500 MHz, D_2O): 3.74 (*dd*, $J = 10.1$, 8.9, H–C(5)); 4.00 (*dd*, $J = 10.1$, 9.4, H–C(6)); 4.11 (*dd*, $J = 12.7$, 2.1, CH–C(7)); 4.31 (*dtd*, $J = 9.3$, 2.3, 0.8, H–C(7)); 4.49 (*dd*, $J = 12.6$, 2.6, CH′–C(7)); 4.69 (*dt*, $J = 8.8$, 0.9, H–C(4)); 7.76 (*d*, $J = 1.0$, H–C(3)). ^{1}H-NMR (CD_3OD): 3.64 (*dd*, $J = 9.6$, 8.7, H–C(5)); 4.04 (*dd*, $J = 9.6$, 8.7, H–C(6)); 4.14 (*dd*, $J = 11.8$, 2.2, CH–C(7)); 4.19 (*dtd*, $J = 8.7$, 3.1, 0.9, H–C(7)); 4.55 (*dd*, $J = 11.8$, 2.5, CH′–C(7)); 4.61 (*dt*, $J = 8.7$, 0.9, H–C(4)); 7.68 (*d*, $J = 0.9$, H–C(3)). ^{13}C-NMR (D_2O): 60.50 (*t*); 65.77 (*d*); 68.44 (*d*); 69.53 (*d*); 77.02 (*d*); 134.19 (*d*); 140.82 (*s*). FAB-MS: 202 (55, $[M + H]^+$). Anal. calc. for $C_7H_{11}N_3O_4 \cdot H_2O$ (219.19): C 38.36, H 5.98, N 19.17; found: C 38.57, H 5.99, N 19.04.

*(4*R*,5*S*,6*R*,7*R*)-4,5,6,7-Tetrahydro-7-(hydroxymethyl)[1,2,3]triazolo[1,5-a]pyridine-4,5,6-triol* (**18**). As described for **17**, with **33** (370 mg, 0.659 mmol): **18** as a colorless oil which crystallized *i.v.* (102 mg, 78%). R_f (AcOEt/MeOH 3:1) 0.41. M.p. 155–156°. $[\alpha]_D^{25} = -103.9$ ($c = 1.0$, H_2O). IR (KBr): 3425*vs* (br.), 1682*w*, 1660*m*, 1651*m*, 1644*m*, 1632*m*, 1455*m*, 1384*m*, 1338*m*, 1261*m*, 1117*s*, 1072*s*, 1030*m*, 989*m*, 901*w*, 864*w*, 830*w*. ^{1}H-NMR (D_2O): 4.01 (*dd*, $J = 10.4$, 4.2, H–C(5)); 4.19 (*dd*, $J = 14.2$, 2.8, CH–C(7)); 4.35 (*dt*, $J = 9.0$, 2.8, H–C(7)); 4.43 (*dd*, $J = 10.4$, 9.0, H–C(6)); 4.53 (*dd*, $J = 13.8$, 3.1, CH′–C(7)); 5.14 (*d*, $J = 4.2$, H–C(4)); 7.85 (*s*, H–C(3)). ^{1}H-NMR (CD_3OD): 3.94 (*dd*, $J = 7.5$, 3.7, H–C(5)); 4.12 (*dd*, $J = 11.2$, 3.1, CH–C(7)); 4.32 (*m*, H–C(7)); 4.39 (*dd*, $J = 11.2$, 5.0, CH′–C(7)); 4.45 (*dd*, $J = 7.5$, 5.3, H–C(6)); 5.01 (*d*, $J = 3.7$, H–C(4)); 7.72 (*s*, H–C(3)). ^{13}C-NMR (D_2O): 61.54 (*t*); 63.37 (*d*); 66.72 (*d*); 67.62 (*d*); 73.25 (*d*); 135.70 (*d*); 139.21 (*s*). FAB-MS: 202 (55, $[M + H]^+$).

Inhibition Studies. The inhibition of β-glycosidases by **17** was measured using concentrations of **17** of 8.0, 2.0, 0.5, 0.2, or 0.05 mM using 4-nitrophenyl β-D-glucopyranoside as substrate, with continuous 4-nitrophenolate detection at 400 nm (for β-glucosidases from sweet almonds: 100 mM K_2HPO_4/KH_2PO_4 buffer, pH 6.8, 37°; for β-glucosidase from *Caldocellum saccharolyticum:* 80 mM Na_2HPO_4/NaH_2PO_4 buffer, 80 mM NaCl, pH 6.8, 55°; for β-galactosidase from bovine liver: 50 mM Na_2HPO_4/NaH_2PO_4 buffer, 1 mM $MgCl_2$, 0.1% BSA (bovine serum albumin), pH 7, 37°). The inhibition of β-mannosidase from snail by **18** was measured according to *Withers'* protocol (50 mM citrate buffer, pH 4.5, 25°, 4-nitrophenyl β-D-mannopyranoside as substrate, quenching of reactions after 5 min using 200 mM borate buffer, 4-nitrophenolate detection at 400 nm) using concentrations of **18** of 8.0, 2.0, 0.5, or 0.1 mM.

REFERENCES

[1] G. Legler, *Adv. Carbohydr. Chem. Biochem.* **1990,** *48,* 319.
[2] M. L. Sinnott, *Chem. Rev.* **1990,** *90,* 1171.
[3] R. W. Franck, *Bioorg. Chem.* **1991,** *20,* 77.
[4] Y. Nishimura, *Stud. Nat. Prod. Chem.* **1992,** *10,* 495.
[5] M. Sinnott, *Bioorg. Chem.* **1993,** *21,* 34.
[6] G. Legler, *Naturwissenschaften* **1993,** *80,* 397.
[7] G. Legler, *Carbohydr. Res.* **1993,** *250,* vii.
[8] J. D. McCarter, S. G. Withers, *Curr. Opin. Struct. Biol.* **1994,** *4,* 885.
[9] D. E. Koshland, *Biol. Rev.* **1953,** *28,* 416.
[10] D. C. Phillips, *Sci. Am.* **1966,** *215,* 78.
[11] G. Legler, *Hoppe-Seyler's Z. Physiol. Chem.* **1970,** *351,* 25.
[12] S. G. Withers, K. Umezawa, *Biochem. Biophys. Res. Commun.* **1991,** *177,* 532.
[13] B. Svensson, M. Søgaard, *J. Biotechnol.* **1993,** *29,* 1.
[14] M. L. Sinnott, in 'Enzyme Mechanisms', Eds. M. I. Page and A. Williams, Royal Society of Chemistry, London, 1987, p. 259.
[15] J. Conchie, G. A. Levvy, *Biochem. J.* **1957,** *65,* 389.
[16] E. T. Reese, F. W. Parrish, M. Ettlinger, *Carbohydr. Res.* **1971,** *18,* 381.
[17] G. A. Levvy, S. M. Snaith, *Adv. Enzymol.* **1972,** *36,* 151.
[18] T. Niwa, S. Inouye, T. Tsuruoka, Y. Koaze, T. Niida, *Agric. Biol. Chem.* **1970,** *34,* 966.
[19] D. Beer, A. Vasella, *Helv. Chim. Acta* **1986,** *69,* 267.
[20] P. Ermert, A. Vasella, *Helv. Chim. Acta* **1991,** *74,* 2043.
[21] P. Ermert, A. Vasella, M. Weber, K. Rupitz, S. G. Withers, *Carbohydr. Res.* **1993,** *250,* 113.
[22] T. D. Heightman, P. Ermert, D. Klein, A. Vasella, *Helv. Chim. Acta* **1995,** *78,* 514.
[23] K. Tatsuta, S. Miura, S. Ohta, H. Gunji, *J. Antibiot.* **1995,** *48,* 286.
[24] I. Inouye, T. Tsuruoka, T. Niida, *J. Antibiot.* **1966,** *19,* 584.
[25] T. M. Jespersen, W. Dong, M. R. Seirks, T. Skrydstrup, I. Lundt, M. Bols, *Angew. Chem.* **1994,** *106,* 1858.
[26] W. Dong, T. Jespersen, M. Bols, T. Skrydstrup, M. Sierks, *Biochemistry* **1996,** *35,* 2788.
[27] A. Linden, R. Hoos, A. Vasella, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1994,** *50,* 746.
[28] R. Hoos, A. B. Naughton, W. Thiel, A. Vasella, W. Weber, K. Rupitz, S. G. Withers, *Helv. Chim. Acta* **1993,** *76,* 2666.
[29] G. Papandreou, M. K. Tong, B. Ganem, *J. Am. Chem. Soc.* **1993,** *115,* 11682.
[30] B. Ganem, G. Papandreou, *J. Am. Chem. Soc.* **1991,** *113,* 8984.
[31] M. K. Tong, G. Papandreou, B. Ganem, *J. Am. Chem. Soc.* **1990,** *112,* 6137.
[32] J.-H. Jeong, B. W. Murray, S. Takayama, C.-H. Wong, *J. Am. Chem. Soc.* **1996,** *118,* 4227.
[33] R. A. Field, A. H. Haines, E. J. T. Chrystal, *Biorg. Med. Chem. Lett.* **1991,** *1,* 667.
[34] E. J. Hehre, D. S. Genghof, H. Sternlicht, C. F. Brewer, *Biochemistry* **1977,** *16,* 1780.
[35] E. J. Hehre, C. F. Brewer, T. Uchiyama, P. Schlesselmann, J. Lehmann, *Biochemistry* **1980,** *19,* 3557.
[36] T. Aoyama, H. Naganawa, H. Suda, K. Uotani, T. Aoyagi, T. Takeuchi, *J. Antibiot.* **1992,** *45,* 1557.
[37] A. C. Terwisscha van Scheltinga, K. H. Kalk, J. J. Beintema, B. W. Dijkstra, *Structure* **1994,** *2,* 1181.
[38] I. Tews, A. Perrakis, A. Oppenheim, Z. Dauter, K. S. Wilson, C. E. Vorgias, *Nature Struct. Biol.* **1996,** *3,* 638.
[39] M. Suzuki, Y. Mikura, S. Terashima, *Chem. Pharm. Bull.* **1986,** *34,* 1531.
[40] E. Calzada, C. A. Clarke, C. Roussin-Bouchard, R. H. Wightman, *J. Chem. Soc., Perkin Trans. 1* **1995,** 517.
[41] J. G. Buchanan, A. D. Dunn, A. R. Edgar, *J. Chem. Soc., Perkin Trans. 1* **1975,** 1191.
[42] E. P. Mitchell, S. G. Withers, P. Ermert, A. T. Vasella, E. F. Garman, N. G. Oikonomakos, L. N. Johnson, *Biochemistry* **1996,** *35,* 7341.
[43] G. Legler, *Hoppe-Seyler's Z. Physiol. Chem.* **1968,** *349,* 767.
[44] T. Barrett, C. G. Suresh, S. P. Tolley, E. J. Dodson, M. A. Hughes, *Structure* **1995,** *3,* 951.
[45] R. H. Jacobsen, X.-J. Zhang, R. F. DuBose, B. W. Matthews, *Nature (London)* **1994,** *369,* 761.
[46] C. Wiesmann, G. Beste, W. Hengstenberg, G. E. Schulz, *Structure* **1995,** *3,* 961.
[47] A. White, D. Tull, K. Johns, S. G. Withers, D. R. Rose, *Nature Struct. Biol.* **1996,** *3,* 149.
[48] S. Jain, W. B. Drendel, Z.-W. Chen, F. S. Mathews, W. S. Sly, J. H. Grubb, *Nature Struct. Biol.* **1996,** *3,* 375.
[49] B. Henrissat, *Biochem. J.* **1991,** *280,* 309.