conditions to those described in Figure 4. These magnetic data are very similar to recent experimental results reported by other groups on complexes of Cu_2 dimers.[26] Theoretical fit using the BB model to the temperature-dependent molar susceptibility data in this case yields fit parameters $J = -380\ cm^{-1}$ and $J' = -85\ cm^{-1}$. Clearly, the most striking feature is that the field-dependent magnetization does not display a hysteresis loop in this system, the straight line obtained being representative of a more traditional paramagnetic behavior.

Our results dramatically underscore the potential afforded by supramolecular chemistry for the design of molecular nanostructured assemblies with desirable physical properties, while emphasizing how the composition of a material is not the only feature one must consider when designing a phase that exhibits molecular magnetism. Future work will focus on the modularity of this system and on chemical modification of the components: substituting the metal; changing the coordinated ligand; substituting the bdc ligand; incorporation of different guest molecules. We expect a significant effect on magnetic properties as it has already been shown that simply varying the apical coordinated ligand has a measurable effect on the magnetism exhibited by the SBU used in our study.[26]

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[1] R. Feynman, *Eng. Sci.* **1960**, 22 – 36.
[2] B. Moulton, M. J. Zaworotko, *Chem. Rev.* **2001**, *101*, 1629 – 1658.
[3] E. Manousakis, *Rev. Mod. Phys.* **1991**, *63*, 1 – 62.
[4] K. R. Dunbar, *J. Solid State Chem.* **2001**, *159*, 251 – 252.
[5] E. Coronado, J. R. Galan-Mascaros, C. J. Gomez-Garcia, V. Laukhin, *Nature* **2000**, *408*, 447 – 449.
[6] S. R. Batten, B. F. Hoskins, B. Moubaraki, K. S. Murray, R. Robson, *Dalton Trans.* **1999**, 2977 – 2986.
[7] S. S. Y. Chui, S. M. F. Lo, J. P. H. Charmant, A. G. Orpen, I. D. Williams, *Science* **1999**, *283*, 1148 – 1150.
[8] I. Syozi, *Prog. Theor. Phys.* **1951**, *6*, 306 – 308.
[9] S. A. Bourne, J. Lu, A. Mondal, B. Moulton, M. J. Zaworotko, *Angew. Chem.* **2001**, *113*, 2111 – 2113; *Angew. Chem. Int. Ed.* **2001**, *40*, 2169 – 2171.
[10] D. W. Breck, *Zeolite Molecular Sieves: Structure, Chemistry and Use*, Wiley-Interscience, New York, **1974**.
[11] J. Lu, A. Mondal, B. Moulton, M. J. Zaworotko, *Angew. Chem.* **2001**, *113*, 2171 – 2174; *Angew. Chem. Int. Ed.* **2001**, *40*, 2113 – 2116.
[12] B. Moulton, J. Lu, A. Mondal, M. J. Zaworotko, *Chem. Commun.* **2001**, 863 – 864.
[13] H. Abourahma, A. W. Coleman, B. Moulton, B. Rather, P. Shahgaldian, M. J. Zaworotko, *Chem. Commun.* **2001**, 2380 – 2381.
[14] D. E. Bergbreiter, M. Morvant, B. Chen, *Tetrahedron Lett.* **1991**, *32*, 2731 – 2734.
[15] O. Kahn, *Molecular Magnetism*, VCH, Weinheim, Germany, **1993**.
[16] J. S. Miller, A. J. Epstein, *MRS Bull.* **2000**, *25*, 21 – 28.
[17] K. Awaga, T. Okuno, A. Yamaguchi, M. Hasegawa, T. Inabe, Y. Maruyama, N. Wada, *Phys. Rev. B* **1994**, *49*, 3975 – 3981.
[18] A. P. Ramirez, *J. Appl. Phys.* **1991**, *70*, 5952 – 5955.
[19] M. G. Townsend, G. Longworth, E. Roudaut, *Phys. Rev. B* **1986**, *33*, 4919 – 4926.
[20] Intensity data for **1** were collected at 173 K on a Bruker SMART-APEX diffractometer using $Mo_{K\alpha}$ radiation ($\lambda = 0.7107$ Å): $Cu_6C_{123.08}H_{54}N_{9.37}O_{30.73}$, 2536.69 g mol^{-1}, trigonal, space group $P\bar{3}c1$, $a = b = 18.6523(11)$, $c = 19.8313(18)$ Å, $V = 5975.1(7)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.410$ g cm^{-3}, $\mu = 1.128$ mm^{-1}, $F(000) = 2556$, $2\theta_{max} = 50.0°$. Final residuals (for 265 parameters) were $R1 = 0.0491$ for 2112 reflections with $I > 2\sigma I$, and $R1 = 0.0764$, $wR2 = 0.1577$, GoF = 0.985 for all 3520 data. Residual electron density was 0.753 and -0.574 e Å^{-3}. Highly disordered solvent molecules in the hexagonal channels were modeled as a group of variable-occupancy carbon atoms. CCDC-165791 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
[21] R. W. Jotham, J. A. Marks, S. F. A. Kettle, *Dalton Trans.* **1972**, 428 – 438.
[22] M. Kato, H. B. Jonassen, J. C. Fanning, *Chem. Rev.* **1964**, *64*, 99 – 148.
[23] B. Bleaney, K. D. Bowers, *Proc. R. Soc. London A* **1952**, *214*, 451 – 465.
[24] S. H. Sun, C. B. Murray, D. Weller, L. Folks, A. Moser, *Science* **2000**, *287*, 1989 – 1992.
[25] H. Tamura, K. Shiraishi, H. Takayanagi, *Phys. Status Solidi B* **2001**, *224*, 723 – 725.
[26] X. X. Zhang, S. S. Y. Chui, I. D. Williams, *J. Appl. Phys.* **2000**, *87*, 6007 – 6009.

Substrate Distortion by a β-Mannanase: Snapshots of the Michaelis and Covalent-Intermediate Complexes Suggest a $B_{2,5}$ Conformation for the Transition State

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More than 6000 glycosidase sequences and related open reading frames are currently known. They have been classified into some 85 families based upon amino acid sequence similarities[1] providing a rich context in which to explore variations in glycosidase mechanism. Experimental demonstration of pyranoside ring conformations along the reaction pathway may assist the design of transition state analogues both as therapeutic agents and mechanistic probes. Here we report the three-dimensional structures of the Michaelis complex and covalent glycosyl – enzyme intermediate for a family-26 β-mannanase, both of which display conformational features never previously seen on any glycosidase. When viewed in light of published work on mannosidase inhibition, this work suggests that the transition state for mannoside

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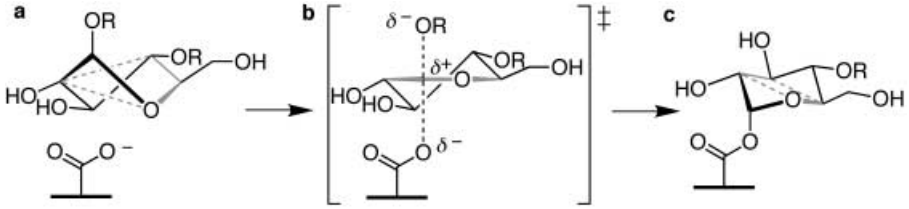
hydrolysis by family-26 enzymes adopts a $B_{2,5}$ boat conformation.

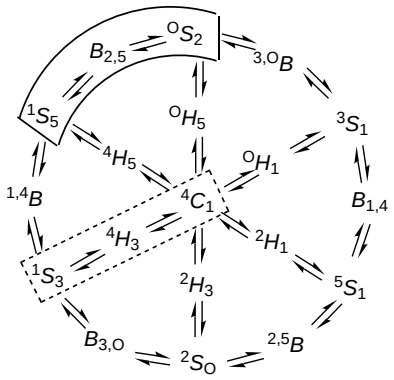
Catalysis by retaining β-glycosidases involves the formation (glycosylation) and subsequent hydrolysis (deglycosylation) of a covalent intermediate flanked by oxocarbenium-like transition states.[2] Substrate distortion, first proposed for hen egg white lysozyme,[3] has been unambiguously observed in the high-resolution crystal structures of Michaelis complexes for two structurally unrelated endoglucanases (from glycoside hydrolase families 5 and 7)[4a,b] and a hexosaminidase (family 20).[4c] For these enzymes, which work on *gluco*-configured substrates, the unhydrolyzed ligands adopt $^1S_3$ skew-boat pyranoside conformations (or a closely related ^4E envelope) in the -1 subsite (Scheme 1a) whilst the subsequent covalent intermediates have been observed as undistorted $^4C_1$ chairs (Scheme 1c).[5]

The corresponding transition state, deduced from kinetic isotope effects and inhibitor design, features considerable oxocarbenium character as the anomeric carbon becomes sp^2 hybridized. Such hybridization is best supported if C5, O5, C1, and C2 form a planar array at or near the transition state. The resulting conformation for the transition state is believed to



Scheme 1. Substrate conformations along the glycosylation step of family-5 and -7 retaining cellulases: a) Michaelis complex ($^1S_3$), b) transition state ($^4H_3$), c) covalent intermediate ($^4C_1$). Planes containing four atoms within the pyranoside ring are indicated with gray solid and dashed lines.

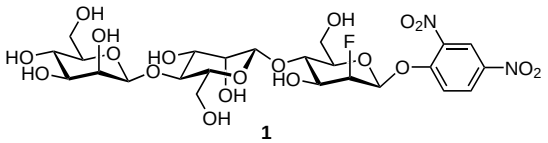
be the $^4H_3$ half chair (Scheme 1b), close in conformational space to the $^1S_3$ skew-boat observed crystallographically.[4] This implies a $^1S_3 \rightarrow {}^4H_3 \rightarrow {}^4C_1$ pseudorotational itinerary for the glycosylation step of the retaining mechanism (Scheme 2). Other glycoside conformations can, however, also place the C5, O5, C1, and C2 atoms within a plane, such as the $^3H_4$ half chair and both $^{2,5}B$ $B_{2,5}$ boats.[6] Indeed, family-11 xylanases display a covalent xylobiosyl–enzyme intermediate in a $^{2,5}B$ boat conformation,[7] suggesting that a similar conformation is



Scheme 2. Partial map of pyranoside ring interconversions (adapted from Stoddart[23]) indicating the proposed cellulase (dashed line) and mannanase (solid line) glycosylation step pathways.

also adopted at the transition state for this class of enzymes, as had previously been suggested for yeast α-glucosidase on the basis of kinetic isotope effects.[8]

Mannanase 26A (Man26A) from *Pseudomonas cellulosa* is a retaining *endo*-β-(1,4)-mannanase whose general acid–base catalyst and nucleophile have been identified as E212 and E320, respectively.[9] The three-dimensional structure of the native enzyme has also been reported.[10] A mechanism-based inactivator of mannanases, 2,4-dinitrophenyl 2-deoxy-2-fluoro-β-mannotrioside (**1**),[11] was used to probe the reaction coordinate of the enzyme. Incubation of wild-type Man26A with **1** failed to trap the covalent intermediate, which instead



1

slowly hydrolyzed as indicated by a steady-state release of dinitrophenolate over time. We followed the example of previous trapping studies[5] and further slowed the deglycosylation step by eliminating base catalysis with the mutation E212A. Indeed, the intermediate was shown to accumulate on this mutant by ESI mass spectrometry (data not shown). Incubation of crystals of the E212A mutant with **1** for short times, however, resulted in the formation of a Michaelis complex of unhydrolyzed substrate,[12] analogous to trapping of such species on other systems.[4] The structure of the resultant Michaelis complex was determined by X-ray crystallography to 1.6 Å resolution. Unhydrolyzed **1** occupied subsites -3 to $+1$ with the DNP glycosidic linkage spanning the -1 and $+1$ subsites. While the -3 and -2 mannopyranoside rings are observed in normal $^4C_1$ chair conformations, the -1 mannopyranoside ring was clearly distorted from a $^4C_1$ chair to a $^1S_5$ skew boat, a conformation never before observed in a glycosidase active site (Figure 1a, b).

As with the $^1S_3$ conformations observed previously for *gluco*-configured substrates, the $^1S_5$ conformation forces the scissile glycosidic bond into an axial position, consistent with stereoelectronic expectations, while simultaneously allowing unrestricted nucleophilic attack by E320 at the opposite face of the anomeric carbon. The remarkable feature of the $^1S_5$ conformation, however, is its subtle accommodation of both the C2 fluoro and C5 hydroxymethyl substituents of the mannopyranoside ring within favorable pseudo-equatorial positions (Scheme 3a). The $^1S_3$ conformation achieves the same stability with *gluco*-configured substrates, which have the opposite configuration at C2. Thus in each case the conformation strikes an ideal balance of reactivity and conformational stability of the C2 and C5 substituents. It is noteworthy that isoquinuclidine **2** (Scheme 4), a potent β-mannosidase inhibitor, adopts a $^{1,4}B$ conformation that is

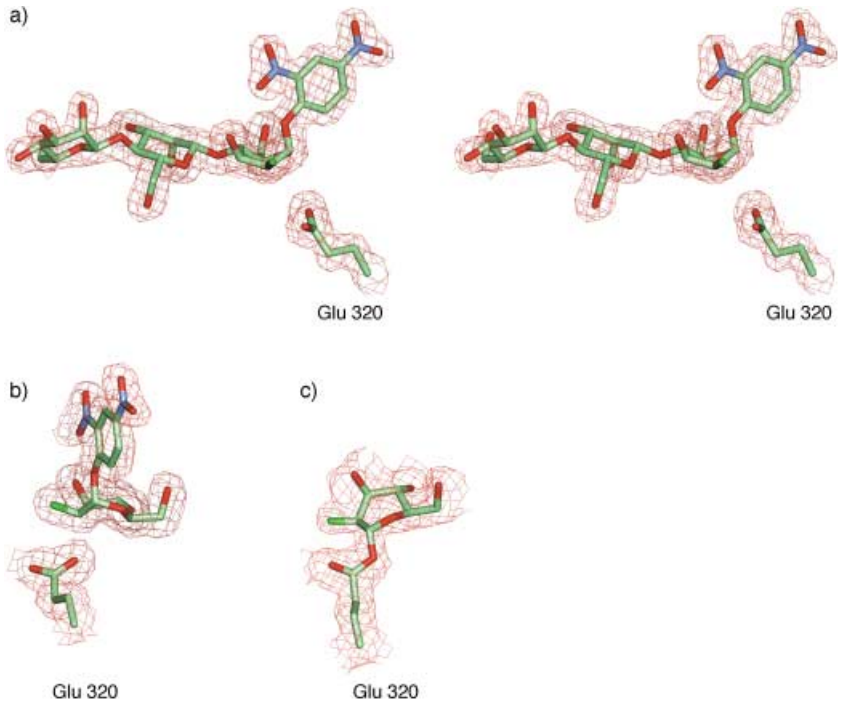
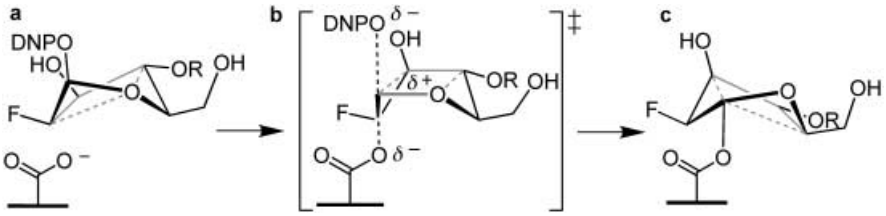
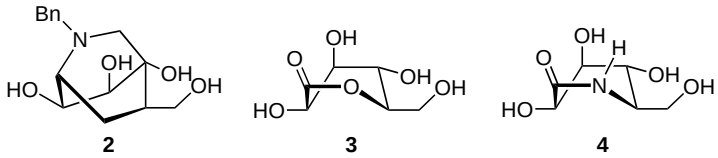


Figure 1. a) Crystal structure of the Man2A E212A complex with unhydrolyzed **1**, Michaelis complex side profile. The map is a maximum-likelihood-weighted $2F_{obs} - F_{calc}$ map contoured at 0.6 electrons Å^{-3} and is shown in divergent (wall-eyed) stereo. b) Front profile of the Michaelis complex. c) Front profile of the covalent 2-fluoromannotriosyl–enzyme intermediate; only the trapped intermediate is shown (for clarity), although electron density reveals that some unhydrolyzed substrate remains with partial (~50%) occupancy.



Scheme 3. Substrate conformations along the glycosylation step of Man26A E212A. a) Michaelis complex ($^1S_5$), b) transition state ($B_{2,5}$), c) covalent intermediate ($^OS_2$). Gray lines indicate planes containing four atoms within the pyranoside ring.



Scheme 4. Observed conformations of mannosidase inhibitors: polyhydroxylated isoquinuclidine (**2**), D-manno-1,5-lactone (**3**), D-manno-1,5-lactam (**4**).

closely related to the high-energy $^1S_5$ conformation described here.[13]

Pre-incubation of the acid–base mutant Man26A E212A with **1** prior to crystallization allowed partial[14] accumulation of a 2-fluoromannotriosyl moiety covalently linked to the nucleophile E320. Intriguingly, the covalently linked −1 mannopyranoside ring was not observed in the expected $^4C_1$ chair but instead adopts a conformation close to the $^OS_2$ skew boat (Figure 1c, Scheme 3c).[15] Together, the intermediate $^OS_2$ and Michaelis $^1S_5$ conformations flank the $B_{2,5}$ boat on the pseudorotational itinerary (Scheme 2). This suggests that the transition state for formation of the mannotriosyl–enzyme intermediate features the $B_{2,5}$ conformation (Scheme 3b). The $B_{2,5}$ boat is also observed for the transition state analogs D-manno-1,5-lactone (**3**)[16] and D-manno-1,5-lactam (**4**),[17] both strong inhibitors of β-mannosidases (Scheme 4). In both cases, the conformation of these mannosyl oxocarbenium-ion mimics is dictated by the configuration at C2 as well as C5 (*gluco*-configured lactones and lactams instead assume $^4H_3$ half chairs),[18] analogous to the $^{2,5}B$ transition state proposed for family-11 xylanases.[7]

These unique substrate conformations appear to be favored by His211 and His143 interacting with the 2- and 3-hydroxyl groups, respectively, of the −1 sugar. Both residues have been shown to play significant roles in catalysis[10] and may well be key determinants in mannanase substrate specificity. As noted above, the $^1S_5$ mannose and $^1S_3$ glucose conformations place the 2-hydroxyl group in similar equatorial positions. Indeed, both mannanases and cellulases from clan GH-A typically possess an Asn in an equivalent position to His211 of Man26A, to interact with the 2-hydroxyl group.[19] In contrast, the 3-hydroxyl group is pseudoaxial in $^1S_5$ mannosides and pseudoequatorial in $^1S_3$ glucosides. Thus His143 is observed in a substantially different position relative to equivalent His residues in cellulases.[4] The discrimination of *gluco*- versus *manno*-configured substrates appears to be transferred along the sugar ring from the 2-hydroxyl to the 3-hydroxyl group as a result of the differing pyranoside ring conformations. Furthermore, the $B_{2,5}$ and ^2S_O conformations may actually permit close interaction between the pseudoequatorial 2-OH and the carbonyl oxygen of the nucleophile as observed for glucosidases acting on *gluco*-configured substrates.[5d, 7c]

The assumption that all glycosidases react through the $^4H_3$ half-chair transition state is incorrect. Indeed, for a mannoside such a conformation results in *syn*-diaxial orientations for the 2-hydroxyl and the leaving group which may instead be alleviated through utilization of a transition state that places the 2-hydroxyl group pseudoequatorial. The $^1S_5$ and ^2S_O conformations for the Michaelis complex and intermediate, respectively, lie adjacent to the $B_{2,5}$ conformation which is also observed for known transition state analogs of β-mannosidases. Together these data lead us to suggest that the $B_{2,5}$ conformation may be adopted in the transition state for the hydrolysis of β-mannosides and that Man26A follows a reaction coordinate that is distinct from that of glucosidases, cellulases, and xylanases.

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Experimental Section

The synthesis of **1** has been described previously.[11] Details of the recombinant plasmid-encoding Man26A mutant E212A have been described,[10] and the expression of the protein in *E. coli* and its subsequent purification were carried out as previously reported.[9]

To obtain the Michaelis complex, a small quantity of powered **1** was added to the mother liquor surrounding a single crystal of Man26A E212A[10] and left for 5 min. The crystal was then transferred rapidly to a cryoprotectant stabilizing solution consisting of the crystal growth solution supplemented with 20% (v/v) glycerol prior to mounting in rayon fibre loops and freezing in a boiling nitrogen stream at 100 K. X-ray diffraction data, to 1.6 Å resolution, were collected at the Daresbury Synchrotron Radiation Source (SRS) on beamline PX14.2 using an ADSC (ADSC, Poway, CA, USA) Quad-4 charge-coupled device (CCD) as detector. Data were processed using the HKL suite of programs.[20] The structure was isomorphous with the native structure described previously[10] and was refined using REFMAC[21] from the CCP4 suite.[22]

Data for the covalent intermediate were obtained by first pre-incubating Man26A E212A with a stoichiometric quantity of **1** for 30 min at 293 K, prior to crystallization as described.[10] Data were collected to a resolution of 1.65 Å on beamline ID14-2 of the European Synchrotron Radiation Facility, again at 100 K with an ADSC CCD as detector. The structure was refined as described above. Coordinates have been deposited with the Macromolecular Structures Database with accession codes 1gvy (Michaelis complex) and 1gw1 (covalent intermediate). Figure 1 was prepared using QUANTA (Accelrys Inc., San Diego, USA).

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[1] http://afmb.cnrs-mrs.fr/~cazy/CAZY/.

[2] D. L. Zechel, S. G. Withers, *Acc. Chem. Res.* **2000**, *33*, 11–18.

[3] C. C. Blake, D. F. Koenig, G. A. Mair, A. C. North, D. C. Phillips, V. R. Sarma, *Nature* **1965**, *206*, 757–761.

[4] a) G. Sulzenbacher, H. Driguez, B. Henrissat, M. Schülein, G. J. Davies, *Biochemistry* **1996**, *35*, 15280–15287; b) G. J. Davies, L. Mackenzie, A. Varrot, M. Dauter, A. M. Brzozowski, M. Schülein, S. G. Withers, *Biochemistry* **1998**, *37*, 11707–11713; c) I. Tews, A. Perrakis, A. Oppenheim, Z. Dauter, K. S. Wilson, C. E. Vorgias, *Nat. Struct. Biol.* **1996**, *3*, 638–648.

[5] a) D. H. Juers, T. D. Heightman, A. Vasella, J. D. McCarter, L. Mackenzie, S. G. Withers, B. W. Matthews, *Biochemistry* **2001**, *40*, 14781–14794; b) D. J. Vocadlo, G. J. Davies, R. Laine, S. G. Withers, *Nature* **2001**, *412*, 835–838; c) W. P. Burmeister, S. Cottaz, P. Rollin, A. Vasella, B. Henrissat, *J. Biol. Chem.* **2000**, *275*, 39385–39393; c) G. Sulzenbacher, L. F. Mackenzie, K. S. Wilson, S. G. Withers, C. Dupont, G. J. Davies, *Biochemistry* **1999**, *38*, 4826–4833; d) V. Notenboom, C. Birsan, M. Nitz, D. R. Rose, R. A. J. Warren, S. G. Withers, *Nat. Struct. Biol.* **1998**, *5*, 812–818; e) V. Notenboom, C. Birsan, R. A. J. Warren, S. G. Withers, D. R. Rose, *Biochemistry* **1998**, *37*, 4751–4758; f) L. F. Mackenzie, G. Sulzenbacher, C. Divne, T. A. Jones, H. F. Woldike, M. Schülein, S. G. Withers, G. J. Davies, *Biochem. J.* **1998**, *335*, 409–416.

[6] L. Hosie, P. J. Marshall, M. L. Sinnott, *J. Chem. Soc. Perkin Trans. 2* **1984**, 1121–1131.

[7] a) E. Sabini, K. S. Wilson, S. Danielsen, M. Schulein, G. J. Davies, *Acta Crystallogr. Sect. D* **2001**, *57*, 1344–1347; b) E. Sabini, G. Sulzenbacher, M. Dauter, Z. Dauter, P. L. Jorgensen, M. Schulein, C. Dupont, G. J. Davies, K. S. Wilson, *Chem. Biol.* **1999**, *6*, 483–492; c) G. Sidhu, S. G. Withers, N. T. Nguyen, L. P. McIntosh, L. Ziser, G. D. Brayer, *Biochemistry* **1999**, *38*, 5346–5354.

[8] L. Hosie, M. L. Sinnott, *Biochem. J.* **1985**, *226*, 437–446.

[9] D. N. Bolam, N. Hughes, R. Virden, J. H. Lakey, G. P. Hazelwood, B. Henrissat, K. L. Braithwaite, H. J. Gilbert, *Biochemistry* **1996**, *35*, 16195–16204.

[10] D. Hogg, E.-J. Woo, D. N. Bolam, V. A. McKie, H. J. Gilbert, R. W. Pickersgill, *J. Biol. Chem.* **2001**, *276*, 31186–31192.

[11] O. Nashiru, D. L. Zechel, D. Stoll, T. Mohammadzadeh, R. A. J. Warren, S. G. Withers, *Angew. Chem.* **2001**, *113*, 431–434; *Angew. Chem. Int. Ed.* **2001**, *40*, 417–420.

[12] In the wild-type[10] and Michaelis complexes described here a zinc ion binds to the nucleophile and the adjacent His211 and may mask the true interaction of His211 with the 2-substituent. Addition of 2 mM $ZnCl_2$ (as used in the crystallization conditions) to extensively dialyzed wild-type enzyme results in approximately 50% inhibition. It is unlikely that this zinc plays a catalytic function since upon partial trapping of the intermediate the zinc occupancy reduces by 50%, consistent with the occupancy of the trapped intermediate.[14]

[13] E. Lorthiois, M. Meyyappan, A. Vasella, *Chem. Commun.* **2000**, 1829–1830.

[14] The structure reveals "double occupancy" of a covalent intermediate with the nonhydrolyzed substrate. The crystallographically determined occupancy of the covalent intermediate was approximately 0.5, with the remaining 0.5 accounted by unreacted **1**. This double occupancy is also reflected in two positions for His211 which interacts with 2-F in the intermediate.

[15] The substitution of fluorine at the 2-position of mannose is unlikely to promote unusual ring conformations. For example, 2-deoxy-2-fluoro-β-mannosyl fluoride adopts a normal $^4C_1$ chair in solution (S. G. Withers, I. P. Street, *Can. J. Chem.* **1986**, *64*, 232–236) and the anomeric equilibrium of 2-deoxy-2-fluoromannose is virtually identical to that of mannose (J. Adamson, A. B. Foster, L. D. Hall, R. N. Johnson, R. H. Heese, *Carbohydr. Res.* **1970**, *15*, 351–359; L. Phillips, V. Wray, *J. Chem. Soc. B* **1971**, 1618–1624).

[16] a) Z. Walaszek, D. Horton, I. Ekiel, *Carbohydr. Res.* **1982**, *106*, 193–201; b) G. A. Levvy, S. M. Snaith, *Adv. Enzymol.* **1972**, *36*, 151–181; c) G. A. Levvy, A. J. Hay, J. Conchie, *Biochem. J.* **1964**, *91*, 378–384.

[17] Y. Nishimura, H. Adachi, T. Satoh, E. Shitara, H. Nakamura, F. Kojima, T. Takeuchi, *J. Org. Chem.* **2000**, *65*, 4871–4882.

[18] It is remarkable that these $B_{2,5}$ conformations are observed in both protic and aprotic solvents as well as in solution and crystalline states. See ref. [16, 17].

[19] A number of related glycosidases form a clan, "GH-A", of related structures. B. Henrissat, I. Callebaut, S. Fabrega, P. Lehn, J. P. Mornon, G. J. Davies, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7090–7094. Enzymes active on both *manno*- and *gluco*-configured substrates appear to share the same recognition elements, typically an invariant Asn or His, around the 2-OH position although family-1 β-mannosidases appear to use an Asp residue to interact with the 2-hydroxyl group which is implicated in mannoside specificity (See T. Kaper, H. H. van Heusden, B. van Loo, A. Vasella, J. van der Oost, W. M. de Vos, *Biochemistry* **2002**, *41*, 4147–4155).

[20] "Macromolecular Crystallography": Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307–326.

[21] G. N. Murshudov, A. A. Vagin, E. J. Dodson, *Acta Crystallogr. Sect. D* **1997**, *53*, 240–255.

[22] "Collaborative Computational Project Number 4": *Acta Crystallogr. Sect. D* **1994**, *50*, 760–763.

[23] J. F. Stoddart, *Stereochemistry of Carbohydrates*, Wiley-Interscience, Toronto, **1971**.

  1433-7851/02/4115-2827 $ 20.00+.50/0