Thiooligosaccharides as Tools for Structural Biology

Hugues Driguez*[a]

Oligosaccharides in which at least one glycosidic oxygen atom is replaced with a sulfur atom can be routinely synthesized and act as competitive inhibitors of various glycoside hydrolases. Recent studies using both X-ray crystallography and other biophysical techniques provide structural insight into binding, recognition, and the catalytic mechanism of action of these enzymes.

KEYWORDS:

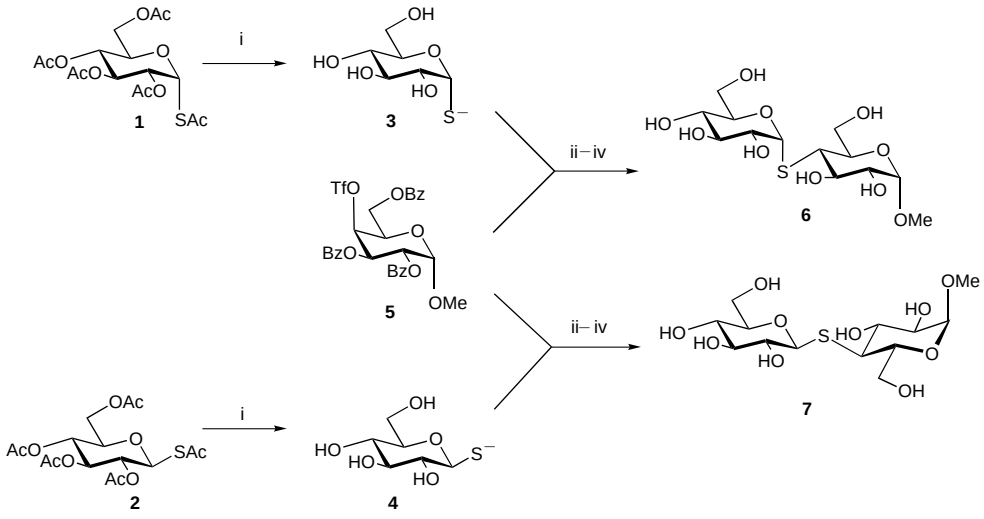
carbohydrates · enzyme catalysis · hydrolases · protein structures · thiooligosaccharides

1. Introducion

In the late sixties, the first three-dimensional structure of an enzyme was determined in Phillips' laboratory.[1, 2] Important features of how enzymes work became clear. Crystallographic studies of hen egg-white lysozyme showed a cleft, on the surface of the protein, into which *N*-acetylchitooligosaccharides bind. Two acidic amino acid residues, essential for the hydrolysis of the substrate, lie within this groove. Modeling studies predicted that distortion of the pyranosyl ring away from its standard $^4C_1$ conformation into a half-chair conformation, as required for the transition state postulated by Koshland,[3] would be a feature of the reaction mechanism. This distortion was demonstrated experimentally when the structure of a complex between lysozyme and tetra-*N*-acetylchitotetrose lactone was solved.[4] These important pieces of information were, however, obtained on the basis of the structure of the enzyme complexed to either oligosaccharide products or putative partial transition-state mimics, but not substrate analogues. Indeed, a further thirty years of work failed to reveal the structure of an enzyme with an oligosaccharide spanning the catalytic center. Only a hydrolytically inert substrate analogue could show the first steps of the binding and recognition events. The challenge for organic chemists was to design and synthesize oligosaccharides that could be potential inhibitors of glycanases and mimic the various binding modes.

Such oligosaccharides should conserve the global geometry of the natural substrate, but contain a glycosidic linkage resistant to enzymatic cleavage. At the time, alkyl or aryl 1-thio-β-D-galactopyranosides were known as competitive inhibitors for the β-galactosidase from *Escherichia coli*.[5] It was therefore proposed to develop the synthesis of oligosaccharides in which the heteroatom(s) of the scissile bond(s) is (are) sulfur atom(s).

In 1978, a general method for the preparation of this class of nonnatural compounds was developed.[6] This method takes advantage of the fact that sulfur is less basic and more nucleophilic than oxygen. Deacetylation of peracetylated 1-thioglucoses **1** or **2** gave the corresponding 1-thiolates **3** or **4**, respectively, which reacted by an S_N2-type mechanism with the acylated acceptor **5** to give, after subsequent *O*-acetylation/*O*-deacetylation steps, the expected thiodisaccharides **6** or **7** in 34 and 52% yield, respectively (Scheme 1). Several methods for the



Scheme 1. *First synthesis of methyl 4-thio-α-maltoside **6** and cellobioside **7**. i) MeONa (1.1 equiv) in MeOH; ii) HMPA; iii) Ac_2O in pyridine; iv) MeONa (catalytic amount) in MeOH (**6** and **7**: 34 and 52% yield, respectively, over three steps). Bz = benzoyl, HMPA = hexamethyl phosphoramide, Tf = trifluoromethanesulfonyl.*

[a] *Dr. H. Driguez*
Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS)
Affiliated with Université Joseph Fourier, Grenoble
B.P. 53, 38041 Grenoble cedex 9 (France)
Fax: (+33) 476-547-203
E-mail: Hugues.Driguez@cermav.cnrs.fr

syntheses of thiooligosaccharides, oligosaccharides having at least one glycosic bond that contains a sulfur atom, have now been published.[7] These successful approaches have clearly demonstrated that the syntheses of *S*-linked oligosaccharides are, at least in most cases, quite simple and that this class of compounds is easier to prepare than the equivalent *C*-glycosides, another class of hydrolytically inert oligosaccharide mimics.[8] Furthermore, *S*-linked oligosaccharides have an additional benefit over their *C*-linked counterparts when used as enzyme inhibitors: The interglycosidic sulfur atom may act as a hydrogen bond acceptor which, as in the natural substrate, could play an important role in binding of the ligand.

The first step in carbohydrate – protein interactions involves substrate recognition, which is primarily dependent on the overall conformation of the oligosaccharide. It was important to determine how closely thiooligosaccharides, these nonnatural substrate analogues, represent the natural compounds in aqueous solution. This has been achieved through a combination of NMR spectroscopy, molecular mechanics calculations, and X-ray structure analysis. Conformational studies of 4-thiomaltose,[9, 10] 4-thiogalabiose,[11] *S*-α-L-fucosyl-(1$\rightarrow$3)-3-thio-D-acetylglucosamine,[12] α,α-thiotrehalose,[13] and thiogangliosides[14] have all resulted in the consistent observations that the thiolinkage provides a high degree of flexibility between glycosyl units and that these molecules possess more conformers than their natural analogues. The results obtained for free compounds in solution suggest that in interactions with proteins, thiooligosaccharides can easily change their conformation to enable a better fit in the catalytic site. The only X-ray crystallographic structure of a thiodisaccharide, that of methyl 4-thio-α-maltoside, revealed—as expected—that the C–S bond is longer than the corresponding C–O bond by about 0.4 Å, but also that the spatial distance between the interglycosidic carbon atoms of the two residues (i.e. C1 of the second glucosyl residue and C4 of the first) is only 0.35 Å longer in the thio analogue.[15] In this Concept article the syntheses of thiooligosaccharides, which have proven useful for structural studies of some cellulases and enzymes acting on α-glucans, are described.

2. Thiocellooligosaccharides as substrate analogues for cellulases

Cellulases are bacterial or fungal glycoside hydrolases that catalyze the degradation of cellulose, the major constituent of plant biomass. They belong to at least 20 distinct families of glycoside hydrolases, as determined from a comparison of their amino acid sequences.[16]

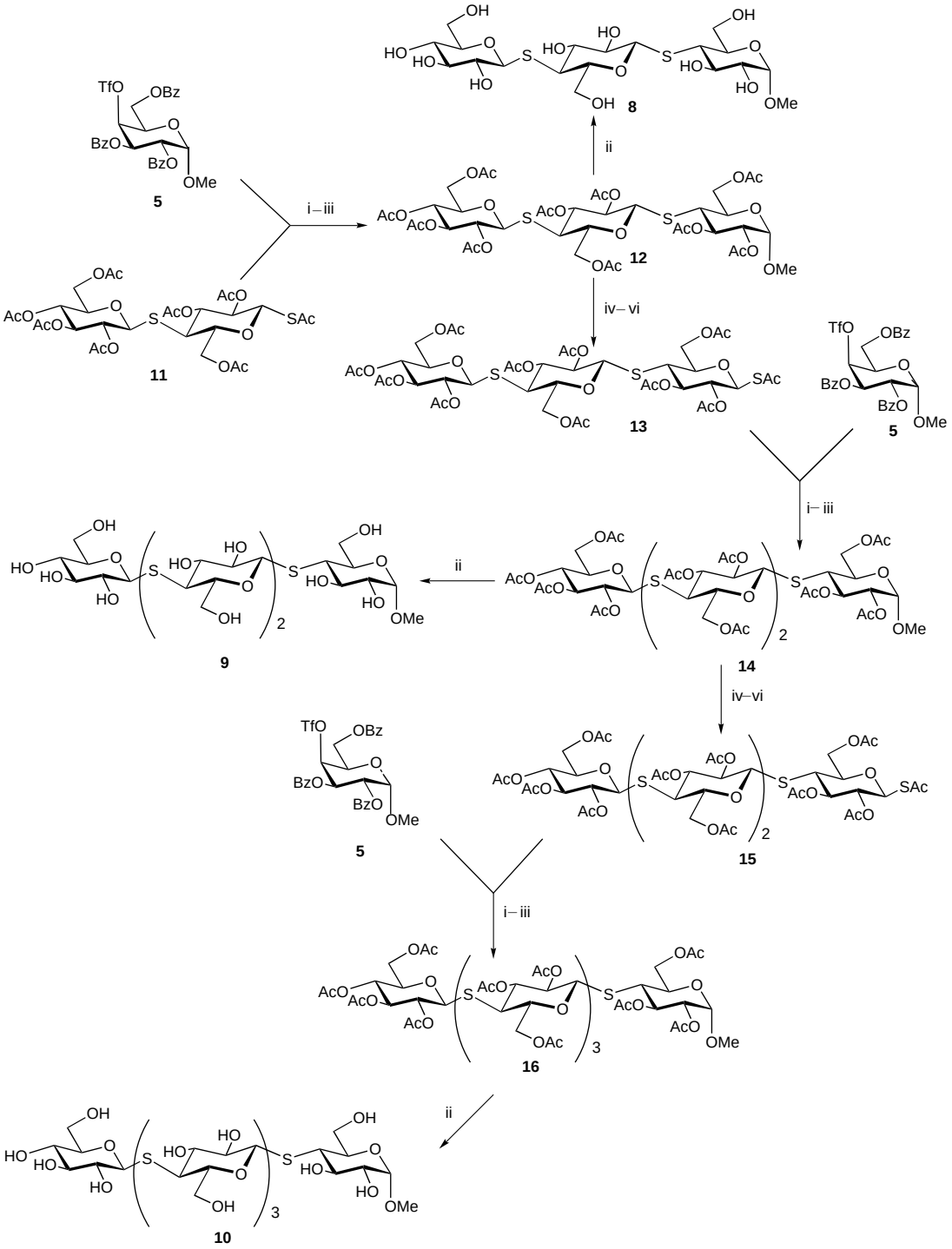
2.1. Substrate distortion at subsite –1 is critical for retaining cellulases

Methyl $4^{I},4^{II}$-dithio-, $4^{I},4^{II},4^{III}$-trithio-, and $4^{I},4^{II},4^{III},4^{IV}$-tetrathiocellooligosaccharides (**8**, **9**, and **10**, respectively) were obtained by using a stepwise synthesis with elongation from the reducing end of the oligosaccharide as shown in Scheme 2.[17] This stepwise strategy was the only possibility for the synthesis: A blockwise approach was precluded since it has been shown that displacement of a trifluoromethanesulfonate (triflate) group of the corresponding β anomer of the acceptor **5** gave the expected coupling product in very low yield due an elimination of the trifluoromethanesulfonyl (triflyl) group.[18] The tetrasaccharide **9** and the pentasaccharide **10** were subsequently found to be excellent inhibitors for numerous cellulases.[17] A group of crystallographers in York (UK) solved the structure of the endoglucanase Cel7B from *Fusarium oxysporum* (formerly known as EGI) complexed with methyl $4^{I},4^{II},4^{III},4^{IV}$-tetrathiocellopentaoside **10**.[19] The electron density map revealed, for the first time, the same oligosaccharide spanning the point of enzymatic cleavage from the -2 to $+1$ subsites (cleavage occurs between subsites -1 and $+1$, donor and acceptor subsites are labeled with $-$ and $+$ signs, respectively).[20] Moreover, the conformation of the sugar unit in the -1 subsite was shifted from the normal $^{4}C_{1}$ chair into a $^{1}S_{3}$ skew-boat, with a quasi-axial orientation for the scissile glycosidic bond (Figure 1). The ring distortion of an internal unit of an oligosaccharide substrate to fit into the active site was demonstrated for the first time. Such a conformational change presents several advantages. The observed distortion induces a movement (ca. 8 Å) of the sugar unit which has to fit into the $+1$ subsite, and since this change of conformation occurs only when the $+1$ subsite is occupied, it is postulated that the substrate has to be flexible to accommodate the kink found around the catalytic point (i.e., where bond cleavage or formation occurs) in the active site of many glycanases.

The consequences of this deformation for the mechanism of action of retaining hydrolases acting on β-glycosides are also very important: i) The axial attack by the catalytic amino acid (the general nucleophile) to establish the glycosyl – enzyme intermediate, with the concomitant departure of the axial leaving group, has favorable steric and stereoelectronic implications; ii) the interglycosidic atom of the scissile bond is in the proximity of the second catalytic amino acid (the general acid/base) to become protonated.

2.2. A new class of inhibitors for β-D-glycoside hydrolases

The most recently published example of the use of thiooligosaccharides in structural biology had its origin in an astonishing observation also made by crystallographers in York, who studied complexes of *Bacillus agaradherans* β-1,4-endoglucanase, Cel5A, with oligosaccharides.[21] Initially, *p*-bromophenyl 1-thio-β-cellobioside was prepared as an inhibitor of Cel5A by the $S_{N}2$-type condensation of α-acetobromocellobiose with *p*-bromobenzenethiolate in a manner similar to that already described for the corresponding *p*-nitrophenyl 1-thio-β-D-glucoside.[6] While the ^{1}H NMR spectrum (300 MHz) of this bromophenyl derivative was in agreement with the expected compound, the structure of Cel5A in complex with this compound revealed the presence of *p*-bromophenyl 1-thio-α-cellobioside together with the expected β isomer in the active site.[42] The very efficient trapping of the α anomer, obviously a very minor contaminant in the original preparation of the β compound, suggests that the α-cellobioside must be a much stronger inhibitor than its corresponding β anomer. To understand and quantify the extent of inhibition with this unusual substrate analogue of cellulose, the mixed α/β-



Scheme 2. *Synthesis of methyl 4-thio-α-cellodextrins* ***8*** *–* ***10****. i) Cysteamine, dithioerythritol in HMPA; ii) MeONa in MeOH; iii) Ac_2O in pyridine (****12****,* ***14****,* ***16****: 35, 90, and 55 % yield, respectively, over three steps); iv) H_2SO_4, AcOH in Ac_2O; v) HBr/AcOH in CH_2Cl_2; vi) Bu_4NSAc in toluene (****13****,* ***15****: 57 and 70 % yield, respectively, over three steps).*

thiocellodextrin **17** was designed and synthesized (Scheme 3).[22] The two different experimental conditions (reagents and solvents) used for the establishment of the thio linkages in **20** and **23** were justified by the orientation of the aglycon of the acceptors **19** and **22**, respectively.[18] Compound **17** was found to be a good competitive inhibitor of Cel5A with a K_i value of 100 μM. It is noteworthy that the equivalent methyl 4-thio-β-cellobiosyl-(1 $\rightarrow$4)-β-cellobioside[23] is not an inhibitor for this enzyme. Compound **17** therefore binds at least two orders of magnitude better than the substrate analogue, which mimics natural cellodextrins, thus explaining the initial trapping of *p*-bromophenyl 1-thio-α-cellobioside. The three-dimensional structure of Cel5A in complex with the mixed α/β-thiocellodextrin **17** was subsequently determined by X-ray crystallography at 1.85 Å resolution. This compound makes the expected interactions in the -3 and -2 subsites, which places the 1,2-*cis*

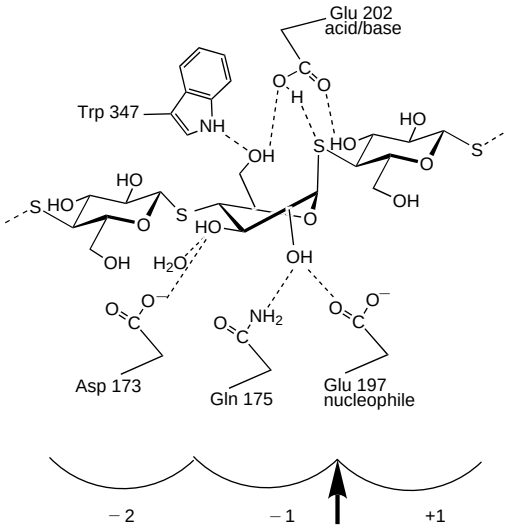


Figure 1. *Skew-boat conformation of the − 1 glucosyl unit in the thiocellodextrin* ***10*** *bound in the active site of the retaining cellulase Cel7B from* Fusarium oxysporum. *The arrow indicates the point of cleavage.*

linkage (1,2-*cis* = α-1,4-*S*-linkage between Glc2 and Glc3) between the − 2 and − 1 subsites and allows the inhibitor to bind in the + 1 subsite. The *O*-glycosidic bond, however, was not hydrolyzed because the *O*-glucosyl unit "by-passed" the − 1 subsite without interacting with the catalytic amino acids. The specific location of the α-thio linkage of this compound obviates any need for substrate distorsion around the catalytic center, as observed for the all-β-linked analogues; instead, binding energy from numerous subsites spanning either side of the catalytic center is used (Figure 2). This mode of binding seems to be quite general, since compound **17** acts as a competitive inhibitor on a number of cellulases.[22] This serendipitous study may lead to the development of a new class of β-D-glycoside hydrolase inhibitors.

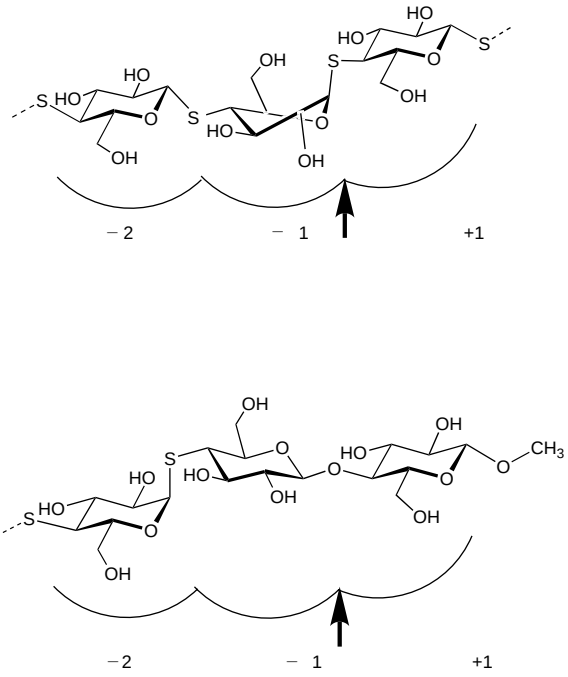
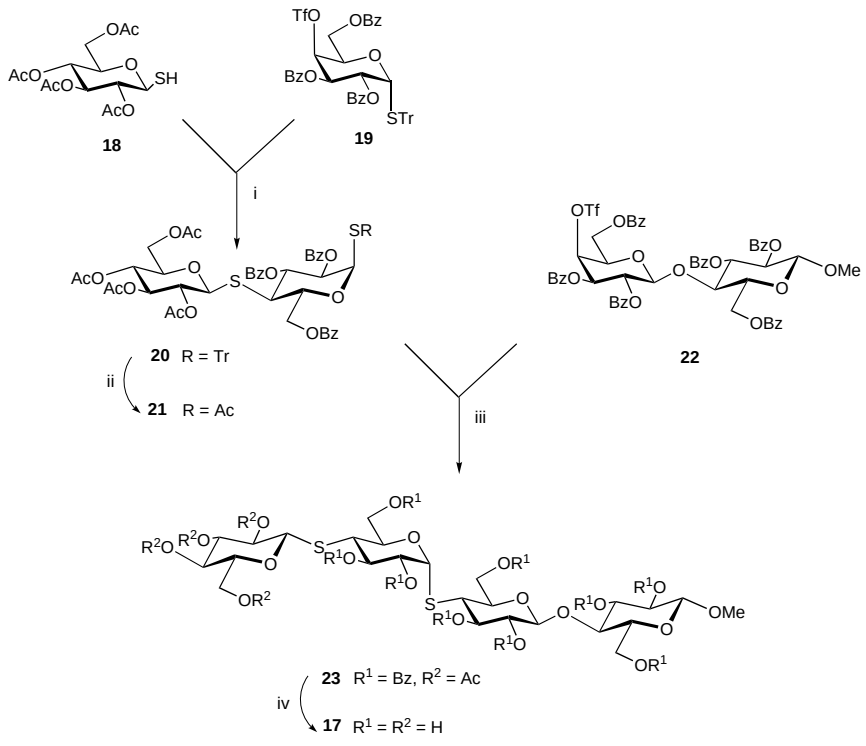


Figure 2. *Comparison of the binding modes of mixed α/β-thiocellodextrin bypassing the − 1 subsite of the active site of retaining cellulases (bottom) and a distorted β-thiocellodextrin in this subsite (top).*



Scheme 3. *Synthesis of mixed α/β-thiocellodextrin* ***17****. i) Cysteamine, dithioerythritol in HMPA (70% yield); ii) Et_3SiH, CF_3CO_2H in CH_2Cl_2, then Ac_2O in pyridine (91% yield); iii) Et_2NH in DMF (62% yield); iv) MeONa in MeOH (99% yield). Tr = triphenylmethyl.*

3. 1,2-*cis*-Thiooligosaccharides as substrate analogues for α-glucan-active enzymes

It has been known for decades that glycogen and starch serve as sources of energy in animal, bacterial, and plant cells. However, the mode of recognition and action of the enzymes responsible for the metabolism of these polysaccharides are still unclear. Together with maltogenic enzymes (classified into *endo*- and *exo*-acting enzymes), phosphorylases and glycosyl transglycosylases are involved in the degradation and transformation of (1 → 4)- and (1 → 6)-α-D-glucans. In the following section, the structure and mechanism of three of these enzymes are discussed on the basis of an analysis of protein – inhibitor complexes.

3.1. Structure of cyclodextrin glycosyltransferase complexed with a β-cyclodextrin analogue

Natural cyclodextrins (CDs) are cyclic molecules produced from starch or its (1→4)-α-oligomers by the bacterial enzyme cyclodextrin glycosyltransferase (CGTase), a member of glycoside hydrolase family 13.[16] The predominant CDs consist of six, seven, or eight α-D-glucosyl units and are denoted α-, β-, and γ-CD, respectively. Notably, branched molecules bearing one or two glucosyl units at the C6 atom, and much larger rings have also been reported.[24] CGTases catalyze the reaction:

$$Glc_n \rightleftharpoons Glc_{n-x} + CD_x$$

where Glc_n is a linear or branched α-glucan chain with $n \geq 8$, and CD_x is an α-, β-, or γ-CD molecule. Each CD can then be considered either as a product or a substrate of this reversible reaction.[25] Biochemical studies have demonstrated that a mechanism by which the α configuration at the anomeric carbon center of the bond to be cleaved (or formed) is retained, involving a double-displacement process, is employed by CGTase. The first step is the cleavage of the glycosidic bond of the donor molecule (Glc_n or CD_x) with the formation of a glycosyl-enzyme intermediate; in a second step, the nucleophilic attack of this intermediate by the 4-hydroxy group of a terminal glucosyl unit of Glc_n or of the acceptor molecule Glc_{n-x} leads to the formation of CD_x or Glc_n, respectively. Previously, an efficient synthesis of *S*-α-glucosyl-6-thio-cyclomaltoheptaose (**24**) was achieved,[26] as exemplified in Scheme 4. The leaving entity of the acceptor molecule **26** was an iodine atom easily introduced into native β-CD (**25**). Almost a decade later, crystals of the inactive mutant Glu257Ala of CGTase from *Bacillus circulans* strain 8 were soaked with compound **24**, and the structure of the enzyme–substrate complex was refined at a resolution of 2.4 Å by crystallographers in Freiburg (Germany).[27] While difference electron density maps revealed the complete cyclic molecule bound in the active site of the enzyme, no electron density was observed for the exocyclic glucosyl unit (Figure 3). This lack of density probably results both from a high mobility of the exocyclic glucosyl residue and "statistical disorder", that is, different binding modes of compound **24** in the active site,

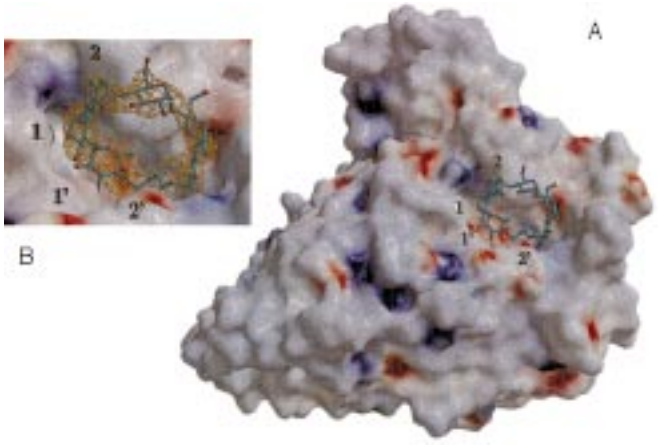
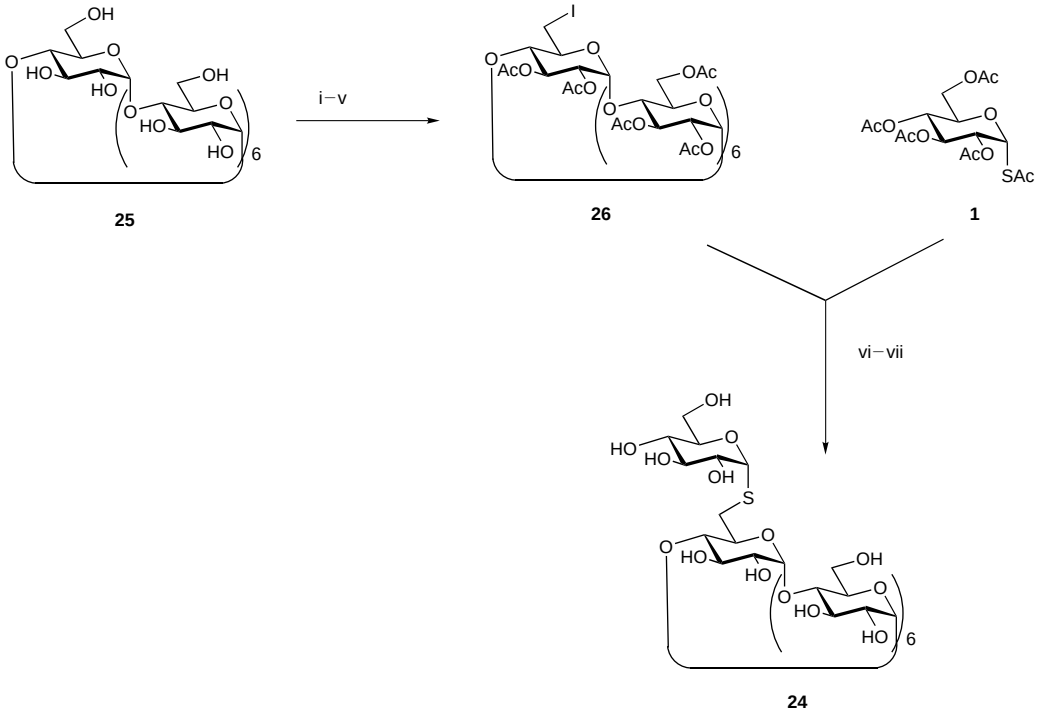


Figure 3. *Surface representation of CGTase from* Bacillus circulans *strain 8. A: Wild-type enzyme with a β-CD molecule bound in the active site. B: Close-up view of the active site of the mutant Glu257Ala. The subsite designations 2, 1, 1′, 2′ in this picture correspond to +2, +1, −1, −2 used in the present work. (Reproduced from ref. [27] with permission.)*



Scheme 4. *Synthesis of 6-S-α-D-glucopyranosyl-6-thiocyclomaltoheptaose (**24**). i) Ph_3CCl in pyridine; ii) Ac_2O in pyridine; iii) HBF_4 in H_2O/MeCN; iv) MsCl in pyridine; v) NaI in DMF (34% overall yield from **25**); vi) cysteamine, dithioerythritol in HMPA; vii) MeONa in MeOH, then NaOH in water (80% yield).*

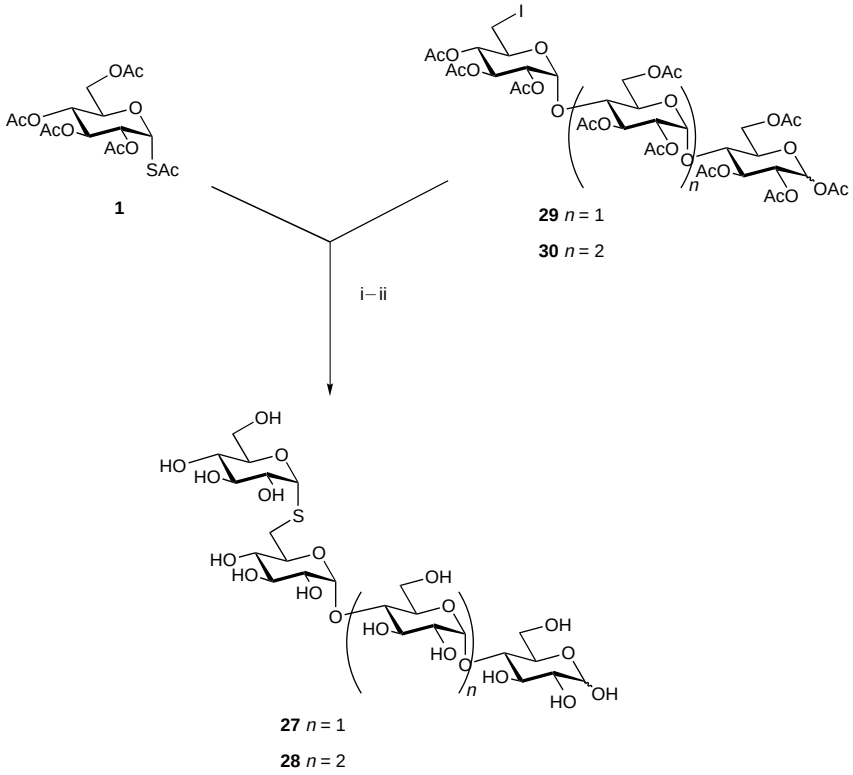
leading to the distribution of the exocyclic glucosyl residue over two, and possibly more, subsites. From an earlier biochemical analysis[28] and for steric reasons (as shown in the crystallographic study[27]), the branch is probably located in subsites -3 or -4. The binding of β-CD in the active site involved polar interactions with the protein only between the -1 and $+3$ subsites, and these tight binding lead to a ring distortion for the glucosyl unit in the -1 subsite. Another interesting feature of this complex is the possible "template effect" played by Tyr 195 during the ring formation, as suggested by various site-directed mutagenesis studies of CGTase and the absence of a residue with an aromatic side chain in α-amylases at this position.[29]

3.2. Motion of the two modules of glucoamylase upon substrate analogue binding

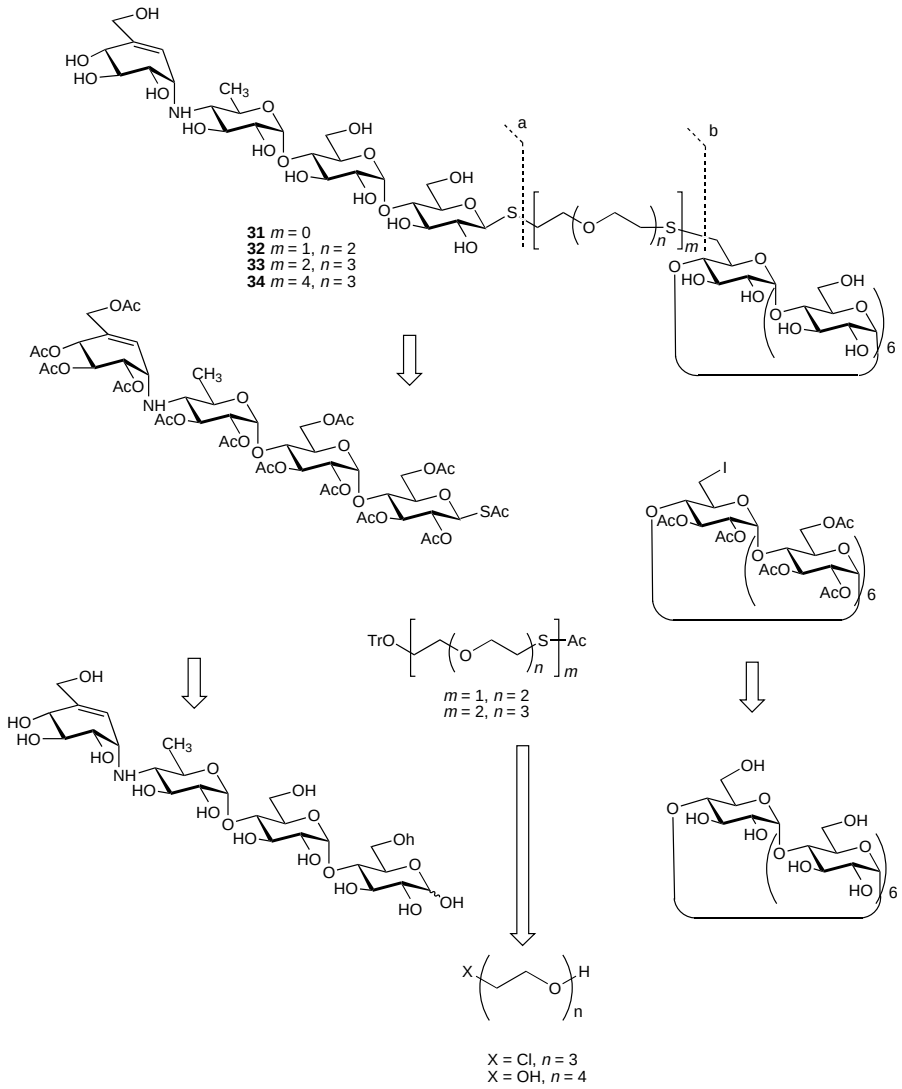
Glucoamylases (GAs) catalyze the hydrolytic release of β-D-glucose from nonreducing ends of starch and related oligo- and polysaccharides. Most GAs possess a starch-binding module (SBM) which is separated from the catalytic module (CM) by a glycopeptide linker of variable length.[30] Removal of the binding module by proteolytic cleavage[31] reduces the activity of the *Aspergillus niger* GA on insoluble starch, but not on soluble substrates. 6^{ω}-*S*-D-Glucopyranosyl-6^{ω}-thiomaltooligosaccharides **27** and **28** were synthesized as inhibitors for GAs (Scheme 5).[32] A transglycosylation reaction catalyzed by CGTase was employed for the preparation of the acceptors **29** and **30**, which were then condensed under standard conditions with the donor **1**. It was also shown that compounds **27** and **28** bind to the SBM and modulate the activity of GA on starch.[32] This suggested the possibility of an interaction between the two modules during starch hydrolysis. To obtain new insights into the structure–activity relationships of GA, heterobidentate ligands were designed that bind to the two modules simultaneously. It was well-known that acarbose is a potent inhibitor of GA and that cyclodextrin may mimic the binding of amylose on two sites of the starch-binding module, but cannot fit into the active site.[33] These observations suggested that one could mimic the interaction of an amylose chain with both modules of GA by using probes made of acarbose that was tethered to a cyclodextrin. Thio linkages were chosen to connect the linkers to the acarbose and cyclodextrin moieties, but notably this choice was not based on the resistance of these linkages to hydrolysis, but on their ease of establishment (Scheme 6).[34]

First, the thermodynamics of ligand binding to full-length GA, GA-CM, and GA-SBM were studied by using isothermal titration calorimetry.[35] It was found that ΔH^0 of binding of the four heterobidentate ligands **31**–**34** (Scheme 6) were, within experimental error, equal to the sum of ΔH^0 of binding of free acarbose and β-cyclodextrin to both modules. This result showed that the catalytic and starch-binding sites are in close proximity in solution and furthermore indicated a considerable flexibility of the linker region.

Then, the hydrodynamic dimensions of full-length GA, GA-CM, and GA-SBM were examined by quasi-elastic light scattering experiments. It was assumed that isolated SBM and CM both possess spherical shapes and the two-module GA featured a dumbbell form, so it is more appropriately considered as an ellipsoid whose minor semi-axis length equals approximately the hydrodynamic radius of CM. The hydrodynamic dimensions found for GA (157 Å) as well as the isolated CM (60 Å) were in good agreement with the values previously determined by other techniques (141 Å and 60–65 Å, respectively).[36, 37] It was also shown that within experimental error the dimensions of GA did not change in the presence of cyclodextrin or acarbose alone or when both ligands were added simultaneously. However, in the presence of the bifunctional ligands **31**–**33** a dramatic drop in the hydrodynamic radius of GA was observed (to 124 Å), whereas with the longest probe, **34**, an intermediate value of 145 Å was found. It seems that the presence of a bound substrate mimic may stabilize a closed conformation of GA. If this motion of the two modules occurs with the natural substrate, it may aid the processivity of the enzyme: GA sticks to the starch chain by way of its SBM, and the "to-and-fro" movement of CM allows the cleavage of several glucosyl residues located at the nonreducing end of this chain before the release of the enzyme. This concept is currently under



Scheme 5. *Synthesis of 6^{ω}-S-α-D-glucopyranosyl-6^{ω}-thiomaltooligosaccharides **27** and **28**. i) Cysteamine, dithioerythritol in HMPA; ii) MeONa in MeOH (**27** and **28**: 84 and 78% yield, respectively, over two steps).*



Scheme 6. *Retrosynthetic pathway for the preparation of bifunctional ligands **31** – **34**. Broken lines a and b indicate the disconnections of the molecules.*

investigation in studies using probes and glucoamylases with peptide linkers of various lengths.[38]

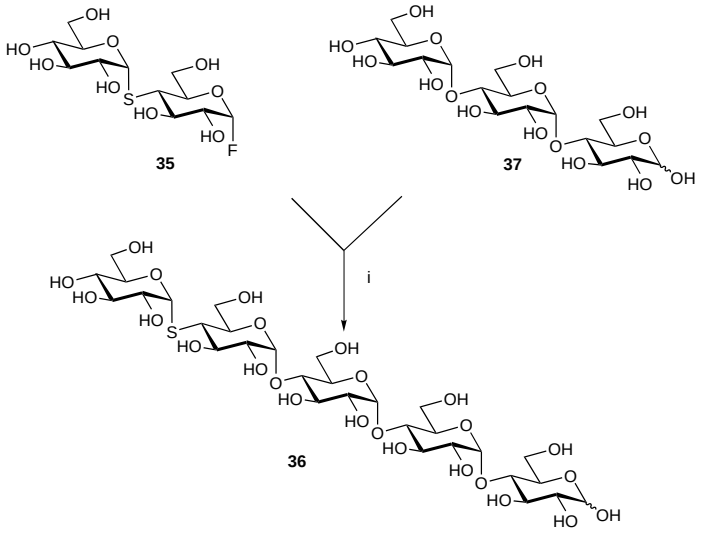
3.3. Recognition and mechanism of action of phosphorylases

Bacterial or mammalian phosphorylases play a critical role in the metabolism of starch since they catalyze the reversible reaction:

$$Glc_n \rightleftharpoons Glc_{n-1} + \text{Glc-1-P}$$

where Glc-1-P is glucose 1-phosphate. The phosphorolytic cleavage occurs from the nonreducing end of maltooligosaccharides. From previous biochemical, molecular modeling, and structural studies[39] it was proposed that the binding site is located at the end of a 20-Å channel consisting of five subsites (-1 to $+4$) with a kink between subsites -1 and $+1$. Modeling studies indicated that a maltooligosaccharide, in its ground-state conformation, could not be accommodated into the groove.[39] Several attempts to cocrystallize the enzyme in the presence of a substrate or transition-state analogue failed to give a productive complex spanning the catalytic point. Thus, the question whether there is a conformational change of the protein or of the substrate remained unanswered.

4-Thio-α-maltosyl fluoride (**35**) was employed as a donor in a transglycosylation reaction catalyzed by *A. niger* α-amylase in aqueous organic medium for the moderate-yield synthesis of the thiomaltooligosaccharide **36**, in which only the terminal scissile bond is a thio linkage (Scheme 7).[40] Protein crystallographers in Oxford (UK) compared the structures of the complexes of maltodextrin phosphorylase (MalP) from *E. coli* bound to the natural substrate and the thio analogue **36**, and also determined the structure of a ternary complex consisting of MalP, thio analogue **36**, and inorganic phosphate (Figure 4).[40] The results showed that the two pentasaccharide molecules were bound in the same manner through the -1 to $+4$ subsites with a twisted conformation across the catalytic site. The thorough comparison of the behavior of *O*- and *S*-linked oligosaccharides demonstrated once more that thiooligosaccharides were good mimics of the natural substrates. The



Scheme 7. *Synthesis of 4^{IV}-S-α-D-glucopyranosyl-4^{IV}-thiomaltotetraose (**36**). i) Taka-α-amylase in MeOH/phosphate buffer (21% yield).*

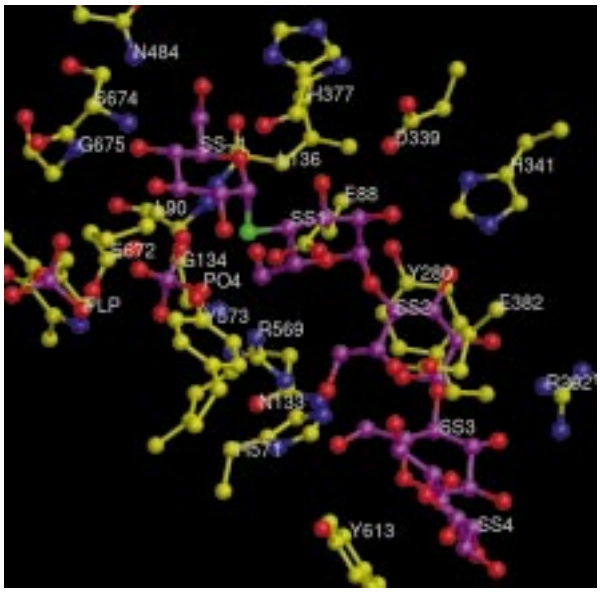


Figure 4. *Structure of the active site of MalP showing the thio compound* ***36*** *and inorganic phosphate. The thio linkage is drawn in green.*

alteration in the torsion angles of the interglycosidic bonds gave a conformation for the maltosyl residue spanning the catalytic point which is close to the second minimum-energy conformer predicted by molecular mechanics calculations.[10] Furthermore, the analysis of the ternary complex provided new insights into the mechanism of action of this enzyme.

4. Conclusion

Thiooligosaccharides have been synthesized for over a quarter of a century, and they represent the largest class of specific inhibitors of glycanases. Six years ago a proceeding entitled "Thiooligosaccharides: toys or tools for the studies of glycanases" was published in order to stimulate the interest in these mimics.[41] Together with the advances in X-ray crytallography, three-dimensional structures of noncovalent enzyme – substrate complexes resulting from the events of substrate recognition and binding can now be easily obtained. Information provided by these structures can further allow the rational design of new and potent inhibitors of glycoside hydrolases.

The author is grateful to all his co-workers who have been involved in the synthesis of thiooligosaccharides; to G. Davies, G. E. Schulz, and L. N. Johnson for using these compounds in their X-ray crystallographic studies; to M. Schülein and B. Svensson for providing cellulases and glucoamylase; and to J. K. Fairweather and B. Henrissat for fruitful discussions. This work was supported by the CNRS and by the European Union Biotechnology Program (contracts BIO4-CT97-2303 and BIO4-CT98-0022).

[1] C. C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips, V. R. Sarma, *Nature* **1965**, *206*, 757 – 761.
[2] C. C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, V. R. Sarma, *Proc. R. Soc. London B* **1967**, *167*, 378 – 388.
[3] D. E. Koshland, *Biol. Rev.* **1953**, *28*, 416 – 436.
[4] L. O. Ford, L. N. Johnson, P. A. Machin, D. C. Phillips, R. Tjian, *J. Mol. Biol.* **1974**, *88*, 349 – 371.
[5] K. Wallenfels, O. P. Malhotra, *Adv. Carbohydr. Chem.* **1961**, *16*, 239 – 298.
[6] M. Blanc-Muesser, J. Defaye, H. Driguez, *Carbohydr. Res.* **1978**, *67*, 305 – 328.
[7] H. Driguez, *Top. Curr. Chem.* **1997**, *187*, 85 – 116.
[8] F. Nicotra, *Top. Curr. Chem.* **1997**, *187*, 55 – 83.
[9] K. Mazeau, I. Tvaroška, *Carbohydr. Res.* **1978**, *67*, 305 – 328.
[10] K. Bock, J. Œ. Duus, S. Refn, *Carbohydr. Res.* **1992**, *225*, 27 – 41.
[11] U. Nilsson, R. Johansson, G. Magnusson, *Chem. Eur. J.* **1996**, *2*, 295 – 302.
[12] B. Aguilera, J. Jiménez-Barbero, A. Fernández-Mayoralas, *Carbohydr. Res.* **1998**, *308*, 19 – 27.
[13] K. Bock, J. Defaye, H. Driguez, E. Bar-Guilloux, *Eur. J. Biochem.* **1983**, *131*, 595 – 600.
[14] A. Geyer, G. Hummel, T. Eisele, S. Reinhardt, R. R. Schmidt, *Chem. Eur. J.* **1996**, *2*, 981 – 988.
[15] S. Perez, C. Vergelati, *Acta Crystallogr. Sect. B* **1984**, *40*, 294 – 299.
[16] B. Henrissat, *Biochem. J.* **1991**, *280*, 309 – 316.
[17] C. Schou, G. Rasmussen, M. Schülein, B. Henrissat, H. Driguez, *J. Carbohydr. Chem.* **1993**, *12*, 743 – 752.
[18] V. Moreau, J. C. Norrild, H. Driguez, *Carbohydr. Res.* **1997**, *300*, 271 – 277.
[19] G. Sulzenbacher, H. Driguez, B. Henrissat, M. Schülein, G. J. Davies, *Biochemistry* **1996**, *35*, 15 280 – 15 287.
[20] G. J. Davies, K. S. Wilson, B. Henrissat, *Biochem. J.* **1997**, *321*, 557 – 559.
[21] G. J. Davies, M. Dauter, M. Brzozowski, M. E. Bjornvad, K. V. Andersen, M. Schülein, *Biochemistry* **1998**, *37*, 1926 – 1932.
[22] S. Fort, A. Varrot, M. Schülein, S. Cottaz, H. Driguez, G. J. Davies, *ChemBioChem* **2001**, *2*, 319 – 325.
[23] C. Reverbel-Leroy, G. Parsiegla, V. Moreau, M. Juy, C. Tardif, H. Driguez, J.-P. Belaich, R. Haser, *Acta Crystallogr. Sect. D* **1998**, *54*, 114 – 118.
[24] Y. Terada, M. Yanase, H. Takata, T. Takaha, S. Okada, *J. Biol. Chem.* **1997**, *272*, 15 729 – 15 733.
[25] D. French, *Adv. Carbohydr. Chem.* **1957**, *12*, 189 – 260.
[26] S. Cottaz, H. Driguez, *Synthesis* **1989**, 755 – 757.
[27] A. K. Schmidt, S. Cottaz, H. Driguez, G. E. Schulz, *Biochemistry* **1998**, *37*, 5909 – 5915.
[28] S. Kobayashi, H. R. Lee Ashraf, P. Braun, D. French, *Starch/Staerke* **1988**, *40*, 112 – 116.
[29] D. Penninga, B. Stokopytov, H. J. Rozeboom, C. L. Lawson, B. W. Dijkstra, J. Bergsma, L. Dijkhuizen, *Biochemistry* **1995**, *34*, 3368 – 3376.
[30] P. M. Coutinho, P. J. Reilly, *Proteins Struct. Funct. Genet.* **1997**, *29*, 334 – 347.
[31] N. J. Belshaw, G. Williamson, *FEBS Lett.* **1990**, *269*, 350 – 353.
[32] C. Apparu, H. Driguez, G. Williamson, B. Svensson, *Carbohydr. Res.* **1995**, *277*, 313 – 320.
[33] B. W. Sigurskjœld, B. Svensson, G. Williamson, H. Driguez, *Eur. J. Biochem.* **1994**, *225*, 133 – 141.
[34] N. Payre, S. Cottaz, C. Boisset, R. Borsali, B. Svensson, B. Henrissat, H. Driguez, *Angew. Chem.* **1999**, *111*, 1027 – 1030; *Angew. Chem. Int. Ed.* **1999**, *38*, 974 – 977.
[35] B. W. Sigurskjœld, T. Christensen, N. Payre, S. Cottaz, H. Driguez, B. Svensson, *Biochemistry* **1998**, *37*, 10 446 – 10 452.
[36] A. P. Gunning, V. J. Morris, G. Williamson, N. J. Belshaw, G. F. H. Kramer, M. W. Kanning, *Analyst* **1994**, *119*, 1939 – 1942, and references therein.
[37] A. E. Aleshin, L. M. Firsov, R. B. Honzatko, *J. Mol. Biol.* **1994**, *238*, 575 – 591, and references therein.
[38] B. Svensson, personal communication.
[39] M. O'Reilly, K. A. Watson, R. Schinzel, D. Palm, L. N. Johnson, *Nat. Struct. Biol.* **1997**, *4*, 405 – 412.
[40] K. A. Watson, C. McCleverty, S. Geremia, S. Cottaz, H. Driguez, L. N. Johnson, *EMBO J.* **1999**, *18*, 4619 – 4632.
[41] H. Driguez in *Carbohydrate Bioengineering* (Eds.: S. B. Petersen, B. Svensson, S. Pedersen), Elsevier, Amsterdam, **1995**, pp. 113 – 124.
[42] A. Varrot, G. Davies, unpublished results.

Received: August 2, 2000
Revised version: October 25, 2000 [C 110]