Review

Covalent inhibitors of glycosidases and their applications in biochemistry and biology

Brian P Rempel and Stephen G Withers

Glycosidases are important enzymes in a number of essential biological processes. Irreversible inhibitors of this class of enzyme have attracted interest as probes of both structure and function. In this review we discuss some of the compounds used to covalently modify glycosidases, their use in residue identification, structural and mechanistic investigations, and finally their applications, both in vitro and in vivo, to complex biological systems.

Keywords: affinity label/glycoside hydrolase/mechanism-based inactivator

Glycosidase classification and mechanism

Glycosidases are widespread enzymes that are responsible for the hydrolytic cleavage of glycosidic bonds in contexts ranging from primary metabolism through to glycoprotein glycan assembly. As some measure of their importance to biology, around 1–3% of the average genome is dedicated to carbohydrate-active enzymes, many of which are glycosidases (Davies et al. 2005). Not surprisingly, therefore, their function or dysfunction has been implicated in a number of different disease states, leading to an interest in inhibitors of glycosidases as potential therapeutics (Asano et al. 2000; Asano 2003a; Butters et al. 2003; de Melo et al. 2006). There is also interest in this class of enzymes for industrial and biotechnological applications.

Glycosidases can be classified into a number of sequence-related families (Henrissat 1991; Henrissat and Bairoch 1993), which can be found at http://www.cazy.org. Enzymes within a sequence-related family catalyze the cleavage of the glycosidic bond by the same mechanism and share a similar overall structural fold (as reviewed in Sinnott 1990; Rye and Withers 2000; Zechel and Withers 2000; Withers 2001; Vasella et al. 2002; Davies et al. 2005). The two most commonly employed mechanisms used by glycosidases to effect glycosidic bond cleavage with overall inversion or retention of anomeric stereochemistry are shown schematically below (Figure 1).

Inverting glycosidases (1a) effect bond cleavage through the action of two carboxylic acid residues (Asp or Glu), typically located at least 6 Å apart on opposite sides of the active site (Zechel and Withers 2000). Of the two carboxylic acids, only one is deprotonated in the enzyme’s resting state and acts as a general base, removing a proton from the incoming nucleophile (typically water under the normal glycosidase mechanism) during its attack at the anomeric carbon. The other carboxylic acid acts as a general acid residue, protonating the departing aglycone oxygen atom and assisting in its departure from the anomeric center. The bond-making and bond-breaking steps proceed through a single, concerted oxocarbenium ion-like transition state in which the developing positive charge at the anomeric carbon is partially stabilized by electron donation from the ring oxygen. The truncated sugar product is a hemi-acetal that initially has the opposite configuration at the anomeric center to that of the starting material; hence, the glycosidase is termed “inverting.”

Noteworthy features regarding this mechanism include a single oxocarbenium ion-like transition state and the lack of any covalent enzyme intermediate formed during the course of catalysis. Most retaining glycosidases (1b), as with the inverting glycosidases, also have a pair of essential carboxylic acid residues (Asp or Glu) located on opposite sides of the enzyme active site, but they are normally closer together at ~5.5 Å apart (Zechel and Withers 2000). One of the residues functions as a general acid in the first mechanistic step by donating a proton during the departure of the aglycone. In the same step, the second, deprotonated carboxylate acts as a nucleophile, attacking the anomeric carbon in a reaction that also proceeds through an oxocarbenium ion-like transition state. This step, referred to as the glycosylation step, leads to the formation of a covalently linked glycosyl-enzyme intermediate that has an anomeric configuration opposite to that of the starting material. The second step of this reaction, the deglycosylation step, involves the hydrolytic breakdown of the glycosyl-enzyme intermediate. The carboxylate that first acted as an acid catalyst now acts as a base by abstracting a proton from the incoming nucleophile, normally a water molecule. The water molecule attacks the anomeric center of the sugar, and the carboxylate residue departs via a second oxocarbenium ion-like transition state. The product thus obtained is a hemi-acetal that initially has the same anomeric configuration as the starting material. This mechanism differs from that of inverting glycosidases by the formation of a covalently bound glycosyl-enzyme intermediate, and hence proceeds through two oxocarbenium ion-like transition states.

There are currently two other known glycosidase mechanisms that are substantially different from the inverting/retaining glycosidases described above. Glycoside hydrolase family 18, 20, 56, 84, and 85 enzymes utilize a double-displacement mechanism in which the catalytic nucleophile is not an enzymatic carboxylate, but instead is the oxygen of the substrate acetamide group (Knapp et al. 1996; Mark et al. 2001; Macauley et al. 2005). Glycoside hydrolase family 4 and 109 enzymes utilize a
**Irreversible labeling agents of glycosidases**

Fig. 1. (A) Mechanism for an inverting \(\beta\)-glucosidase. (B) Mechanism for a retaining \(\beta\)-glucosidase. Note that the proton delivered during general acid catalysis is delivered in either a *syn* or *anti-*fashion, depending on the specific enzyme.

Enzyme inhibitors can be divided, broadly, into two classes: noncovalent and covalent, with members of each class having different applications. Noncovalent inhibitors of glycosidases bind reversibly and have the greatest potential as therapeutics. These have been extensively studied and reviewed (Legler 1990; Asano et al. 2000; Lillelund et al. 2002; Asano 2003a,b; Butters et al. 2003; de Melo et al. 2006), and are not the intended subject of this review. Covalent inactivators of glycosidases are a class of molecules that ablate the enzyme activity through the formation of a covalent bond between the enzyme and some functionality on the inactivator (Legler 1990; Withers and Aebersold 1995). The bond is typically formed by the attack of an enzyme-based nucleophile onto an electrophilic portion of the inactivator, leading to covalent attachment of the inactivator. This attachment leads to loss of activity, most often because it either physically blocks access to the enzyme active site or modifies an active site residue that is critical for catalysis.

Irreversible glycosidase inhibitors can be used for many different purposes. One of the earliest and still most prevalent uses is in the identification of active site residues. Mutation of the identified residues followed by kinetic analysis of mutants modified at that position can confirm their function in either catalytic or structural roles. Covalent inactivators have

unique NAD\(^+\)-dependent redox elimination/addition sequence (Yip and Withers 2006). Neither of these mechanisms will be discussed in further detail in this review.

**Glycosidase inhibition: an overview**

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also seen use in studying the catalytic mechanism(s) by which glycosidases function, both through kinetic and structural examination. Highly specific probes have been used to selectively inactivate a target enzyme or enzyme activity in complex biological systems while observing the effect of this “deletion” on the organism. This has been further extended to the design of specific probes for the discovery and characterization of novel enzymes.

Covalent glycosidase inactivators can be shown to be active site-directed, even in the absence of any structural knowledge of the enzyme, by incubation of the inactivator in the presence and absence of a known active site-binding noncovalent inhibitor. Under these conditions, a reduction or ablation of irreversible inhibition should be observed if it is indeed active site-directed, as both inhibitors are competing for the same region of the enzyme. The kinetics or extent of inactivation can usually be studied by incubating a solution of inactivator plus enzyme and removing enzyme aliquots at various time points. Residual enzymatic activity in these aliquots can be assayed using a known substrate to reveal the degree of inactivation as a function of time. Analysis of the data can reveal a time-dependent irreversible loss of enzyme activity, and the kinetic parameters of inactivation can be calculated by straightforward mathematical manipulations of the data (Kitz et al. 1965; Mosi and Withers 2002; Wicki et al. 2002). No general statements can be made about the rapidity of inactivation with any class of compounds, as the rate constant for inactivation is highly dependent on both the inactivator and the specific enzyme being investigated. The time necessary to completely ablate the activity for a given enzyme and inhibitor pair can range from milliseconds to weeks, so interested readers are encouraged to consult the original articles for more details on rate constants or rates of inactivation.

Irreversible inhibitors can be divided into two general categories: affinity labels and mechanism-based inhibitors. An affinity label is any molecule that contains a region designed to impart specificity for a given protein and a reactive functionality that will irreversibly covalently modify a neighboring region of the protein (Fersht 1999). These affinity labels can be further subdivided into two classes: labels that are inherently reactive as a consequence of their chemical bonding (Fersht 1999) and labels that require external activation, such as photo-affinity labels (Vodovozova 2007). By contrast, a mechanism-based inhibitor is a substrate analog that is stable toward spontaneous decomposition, but upon activation by the enzymatic catalytic machinery, it produces a species that reacts to form a covalent bond to the enzyme (Legler 1990; Withers and Aebersold 1995). In this review we will first discuss affinity labels and then mechanism-based inhibitors as covalent inactivators of glycosidases, with special attention being paid to their applications in biochemistry and biology.

**Affinity labels**

**Photoreactive affinity labels**

The application of photoaffinity (PA) probes toward glycosidase labeling has not received a great deal of attention. This class of probe generally consists of compounds containing a specificity moiety that is attached to a diazirine or aryl azide. These functional groups can be photolyzed to generate a carbene or nitrene, respectively. This photolysis results in the generation of a highly reactive electrophile; thus, the site of enzymatic labeling is often nonspecific and difficult to predict.

One of the early examples of a PA probe applied to glycosidase labeling was the use of a diazirine as the photoreactive group linked through a C-glycosidic linkage to a galactotopyranosyl residue as the specificity tag (Figure 2) (Kuhn and Lehmann 1987; Kuhn, Lehmann, Jung 1992). This compound (2a) was found to be a modest inhibitor and was used to label *Escherichia coli* lacZ β-galactosidase upon irradiation. Only moderate levels of inactivation were observed. A radiolabeled version of the inactivator was prepared and used to tag the enzyme, followed by treatment with trypsin and HPLC purification of radioactive peptides to localize sequences containing the radiolabel. While the specifically labeled amino acids were not identified, two short polypeptides were isolated and sequenced, and on this basis proposed to be located close to the active site. With the later determination of the three-dimensional structure of the enzyme by X-ray crystallography (Jacobson et al. 1994; Juers et al. 2000, 2001), it is possible to reexamine this labeling result and see that, while one of the two peptides does indeed lie very close to the active site, the other one is near the surface of the protein, away from the active site. This may be a consequence of the low labeling efficiency observed and the low binding affinity of the probe for the enzyme active site.

A PA probe for human lysosomal hexosaminidases A and B, which are responsible for ganglioside degradation, was synthesized and tested (Kuhn, Lehmann, Sandhoff 1992; Liessem et al. 1995). The thioglycoside (2b) was found to only be a modest inhibitor of enzyme activity, and the diazirine-derived carbene was not a very efficient protein label. Despite these drawbacks, the labeling experiment was still successful in identifying a catalytic glutamate in hexosaminidase B (Liessem et al. 1995), a residue that was subsequently shown through biochemical (Hou et al. 2001), bioinformatic (Fernandes et al. 1997), and structural (Mark et al. 2003) studies to act as the general acid/base during catalysis. This is an example in which a covalent inactivator of a glycosidase was used to obtain structural, mechanistic, and
sequence information prior to a three-dimensional structure becoming available (Mark et al. 2003; Lemieux et al. 2006). One feature of note is that, although enzymes in this family (GH family 20) are retaining enzymes, they do not use an enzymatic carboxylate as the nucleophile. Rather, the amide oxygen on the N-acetyl portion of the substrate acts as the nucleophile. As a result, many covalent labeling strategies discussed below that rely on the specific enzymatic nucleophile in the double-displacement mechanism would not be applicable to this class of enzymes.

Another subclass of PA labeling involves the use of a putative transition state analog as opposed to a substrate analog to increase the affinity of the PA probe prior to irradiation and reaction. In both cases described, an aryl azide was used as the photoreactive portion of the probe (Figure 2, 2c and 2d). A PA probe for sialidases (2e) has been used to label a bacterial (Vanderhorst, Mancini 1990) and two mammalian (Vanderhorst, Rose 1990; Kopitz et al. 1997) sialidases, using a similar concept, where the photoreactive moiety is linked to a tight-binding noncovalent sialidase inhibitor, 2,2-didehydro-2-deoxy-N-acetyl-neuraminic acid (DANA). A radioactive125I nucleus was incorporated to assist in the identification of the labeled protein in complex mixtures or in a multisubunit enzyme complex. More recently, an N-alkylated derivative of 1-deoxynojirimycin with an aryl azide appended to the end of the N-alkyl chain (2d) was used to inhibit and label human α-glucosidase I in a complex microsomal mixture of proteins. The exact site of labeling was not determined, although the authors were able to localize the label to the highly conserved polypeptide spanning residues 582–598 that is thought to make up part of the substrate-binding site (Romaniouk and Vijay 1997).

Other affinity labels
The other major classes of affinity labels are those wherein the reagents possess a functional group that is inherently chemically reactive (Figure 3). The first five compounds discussed, 3a–e, despite their clever design, have not seen wide use as glycosidase-labeling agents. This is principally due to their instability toward spontaneous hydrolysis, as discussed below. The first three molecules (3a–c), all C-glycosides, act as covalent glycosidase inactivators by the attack of an enzymatic nucleophile onto the highly reactive diazomethyl group which is activated by protonation (Marshall et al. 1981; Bemiller et al. 1993). While 3a/3b and 3c have different modes of activation, they all generate highly reactive carbon species that inactivate the enzyme by undergoing rapid attack by an enzymatic nucleophile (Figure 4). However, all were hydrolytically
unstable at pH <7, limiting the range of glycosidases susceptible to inactivation. Glycosylmethyl-triazenes 3a and 3b were used to inactivate a variety of retaining β-glycosidases, and also surprisingly showed very weak inactivation of some retaining α-glycosidases (Marshall et al. 1981). Notably, the inactivation of E. coli lacZ β-galactosidase was studied in more detail, and the site of labeling was determined to be Met-500 (Sinnott and Smith 1976; Fowler et al. 1978; Sinnott and Smith 1978). Although the glycosylmethyl-triazenes were tested against two inverting β-glycosidases, no inactivation was observed (Marshall et al. 1981). Galactosyl-diazomethyl ketone 3c was shown to be an irreversible inactivator of the β-galactosidase from A. oryzae, and the inactivation was shown to be active site directed. The same compound failed to label E. coli lacZ β-galactosidase, the only other enzyme against which it was tested (Bemiller et al. 1993). A family of glucosylthio-hydroquinones (generally represented by compound 3d) has also been prepared and evaluated as irreversible inhibitors of two very well studied retaining β-glycosidases, the β-glucosidase from Agrobacterium sp. (Abg) and the xylanase from Cellulomonas fimii (Schnabelrauch et al. 1994). These Michael-type acceptors inactivated Abg reasonably well, although they were poorer inactivators of the xylanase from Cellulomonas fimii. They were found to be hydrolytically unstable in buffered solution and hence could be tested in water alone. This hydrolytic instability has thus severely limited the study of this class of compound and precluded identification of the site of labeling. Another electrophilic reagent, the glucosyl-sulpho-hydroquinone (3e) was shown to label almond β-glucosidase (Shulman et al. 1976). Although the inactivator was shown to be active site directed and irreversible, the inactivator efficiency was not particularly high, and it was also unstable under the inactivation conditions. Owing to these drawbacks, no active site residue could be identified using this inactivator. Finally, the N-bromoacetyl glycosylamines and bromoketone C-glycosides (represented by the general structures 3f–h) have been used to label and inactivate several glycosidases (Naider et al. 1972; Black et al. 1993; Keresztesy et al. 1994; Tull et al. 1996; Howard and Withers 1998a,b; Chir et al. 2002; Kiss et al. 2002; Vocablo et al. 2002; Jager and Kiss 2005). Unlike the classes of compounds 3a–e discussed above, the N-bromoacetyl glycosylamines and bromoketone C-glycosides have typically proven to be sufficiently stable toward spontaneous decomposition to be useful as labeling agents when care is taken to select the proper inactivator, as described below. The N-bromoacetyl glycosylamines of general structure 3f have proven useful as probes that often label the acid/base catalytic residue in retaining β-glycosidases (Keresztesy et al. 1994; Tull et al. 1996; Chir et al. 2002; Vocablo et al. 2002), although compounds in this class have also been observed to label other residues, including an active site methionine (Met-500) in E. coli lacZ β-galactosidase (Naider et al. 1972). Interestingly, this is the same methionine as that which was labeled by the galactosylmethyl triazene described above (Sinnott and Smith 1976, 1978; Fowler et al. 1978). In a second case in which this reagent was employed, the authors propose that the catalytic nucleophile is the site of labeling on the basis of the pH dependence on the rate of inactivation and the pH rate profile of enzymatic substrate hydrolysis (Jager and Kiss 2005). However pH/rate profiles can be notoriously difficult to interpret (Knowles 1976), so in the absence of a structural study and a detailed kinetic analysis of both wild-type and site-directed mutants (see Vocablo et al. 2002 for a good example of this type of analysis), caution should be exercised. The model enzyme Abg also did not label cleanly and, while inactivation was shown to be active site directed, the enzyme was found to incorporate one, two, or three N-acetyl-glucosaminyl moieties during the course of the inactivation experiment as determined by ESI-MS (Black et al. 1993). This result again emphasizes the danger in relying on kinetic data alone. Thus, while the N-bromoacetyl glycosylamines have proven valuable in the labeling and identification of the acid/base catalyst in some retaining β-glycosidases, assignments made on this basis should be verified by other means. The bromoketone C-glycosides were introduced primarily in response to the need for a covalent inactivator directed toward the catalytic acid/base residue in retaining α-glycosidases (Howard and Withers 1998a,b). Bromoketone C-glycosides were chosen as labels for retaining α-glycosidases because the equivalent N-bromoacetyl α-glycosylamines are very difficult to synthesize. While bromoketone C-glycosides were also synthesized with the aim to label the acid/base residue in retaining β-glycosidases (3g), they did not meet with much success and showed no advantages over the corresponding N-bromoacetyl glycosylamines (Howard and Withers 1998a). However, bromoketone C-glycoside 3h was synthesized and shown to be an active site-directed inactivator of yeast α-glucosidase, a well-studied retaining α-glycosidase (Howard and Withers 1998b). The residue covalently modified by the inactivator aligned nicely (Howard and Withers 1998b) with the catalytic acid/base residue that had been previously identified in other family 13 glycosidases (Qian et al. 1994; Svensson 1994; Knegtel et al. 1995). Unfortunately, while bromoketone C-glycosides such as 3h are available, a version of this analog with an equatorial hydroxyl at C-2 (gluco-configuration) rapidly underwent intramolecular cyclization, precluding the use of such reagents as general affinity labels.

**Mechanism-based inactivators**

In contrast to affinity labels, a mechanism-based inactivator (MBI) is a molecule that is chemically inert until activated by the catalytic machinery of the enzyme. In the context of glycosidases, this activation typically comes in one of two ways, either the hydrolytic cleavage of a glycosidic bond releases a reactive aglycone that labels the enzyme at some location, or the enzymatic nucleophile attacks a center activated by the general acid.

**MBIs with reactive aglycones**

To date, there have only been two classes of molecules that rely on enzymatic cleavage of a glycosidic bond to release an activated aglycone, a difluoroalkyl glucoside (Figure 5, 5a) and a series of related compounds featuring an activated phenylmethyl aglycone (5b–e). After enzymatic cleavage of 5a, the initially formed α,α-difluoro-alcohol rapidly decomposes with the release of a molecule of HF and generation of a reactive acyl fluoride. This acylating agent then reacts with a nucleophilic residue in the enzyme, irreversibly inhibiting it (Figure 6). The only example of this class of MBI to date (Halazy et al. 1989) was found to inactivate yeast α-glucosidase rapidly and irreversibly, although it acted as a substrate rather than an inactivator for the sucrase-isomaltase enzyme from rat’s small intestine. This lack of inactivation likely arises from the fact that
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Fig. 5. Structures of mechanism-based inactivators with reactive aglycones.

Fig. 6. Mechanism of generation of reactive aglycone from difluoroalkyl glucoside 5a.

Fig. 7. Mechanism of generation of reactive aglycone from activated phenylmethyl glycosides.

the reactive aglycone, once released, has no inherent affinity for the enzyme active site and is free to diffuse out where it may react nonselectively.

Molecules of general structure 5b–e which feature an activated phenylmethyl aglycone have been studied as glycosidase inactivators for a number of enzymes (Driguez et al. 1992; Briggs et al. 1995; Zhu et al. 1998; Ichikawa and Ichikawa 2001; Tsai et al. 2002; Kurogochi et al. 2004; Hinou et al. 2005; Lu et al. 2005; Lo et al. 2006) (e.g., 5b) and para (Tsai et al. 2002; Lo et al. 2005; Shie et al. 2006) (5c) substitutions of the difluoromethyl group on the aromatic ring have been investigated, with a variety of different glycone portions being investigated, including β-glucosides (Tsai et al. 2002; Lo et al. 2005; Shie et al. 2006), a β-galactoside (Kurogochi et al. 2004), a β-xyloside (Lo et al. 2006), a β-N-acetyl-glucosaminide, and α-sialosides (Driguez et al. 1992; Hinou et al. 2005; Lu et al. 2005). Compounds such as 5d have incorporated a reporter group (often dansyl or biotin) attached via a linker to an amide or ester off the phenyl ring. This class of MBI has had limited success, mostly owing to the fact that the activated quinone methide species released has no specific affinity for the target glycosidase. Consequently, it may well diffuse out of the active site and label a remote residue or other nearby proteins (Bolton et al. 1997; Lo et al. 2005). This property renders such reagents essentially useless for proteomic analysis. Indeed, no reports in which one specific active site residue has been selectively labeled, have appeared, although in some cases labeling outside the active site can be quite selective (Hinou et al. 2005). In most cases, the stoichiometry and/or site of labeling were not thoroughly investigated.

It is interesting to note that a natural product, salicortin (5e), has been isolated which was shown to fragment upon enzymatic hydrolysis to generate an activated quinone methide (Claussen et al. 1990), and that this quinone methide was shown to be an inactivator of the β-glucosidase Abg (Zhu et al. 1998). It was shown to be a repeat-attack MBI, with multiple alkylations around the enzyme active site occurring before inactivation was observed. This is an interesting demonstration of the parallel discovery of rationally designed and naturally isolated compounds that may operate through similar mechanisms. This suggests that...
Fig. 8. General mechanism of inactivation by an epoxide \((X = O)\) or an aziridine \((X = \text{NH})\).

Fig. 9. Structures of aziridine-based inactivators.

Fig. 10. Structures of epoxide-based inactivators.

one avenue for the development of new covalent inactivators of glycosidases may be through examination of natural products and mimicking their functionality.

**Epoxide- and aziridine-based inactivators**

Aziridines and epoxides have been utilized to label the catalytic nucleophile in a number of retaining glycosidases. When incorporated with a functionality that imparts specificity toward the active site, they typically are activated by proton donation from one catalytic carboxylic acid and are attacked by the other to covalently label the enzyme active site (Figure 8). Of these, the glycosyl-aziridines have received considerably less attention than the epoxides (discussed below), and examples in the literature appear to be restricted to those shown in Figure 9. Aziridine-based inhibitors enjoy the theoretical advantage of having a higher initial, noncovalent affinity for a glycosidase active site owing to the positively charged (when protonated) nitrogen atom, which should help direct the inactivator toward the negatively charged active site. The spiroaziridines \(9a\) and \(9b\) were tested against a few different enzymes and shown to be only very weak inactivators of a single \(\beta\)-glucosidase and a single \(\alpha\)-glucosidase, respectively, possibly because of the steric demands imposed at the anomeric center (Kapferer et al. 2003). Spiroaziridine \(9c\) was shown to be a potent inactivator of one \(\alpha\)-galactosidase, although it showed no activity against other enzymes (Tong and Ganem 1988). The other glycosyl-aziridine that has been tested is the conduritol aziridine \(9d\) (Caron and Withers 1989), an aza-analog of conduritol B-epoxide (CBE, discussed below). It was found to be a modestly potent irreversible inactivator of both the \(\beta\)-glucosidase \(A_{\beta}\) and the yeast \(\alpha\)-glucosidase, showing slightly higher activity against the \(\alpha\)-glucosidase.

Considerably more work has been done using epoxides as the active electrophile. The general structures for the three classes of epoxides that have been studied are shown in Figure 10. One of the earliest classes of glycosyl-epoxides to be studied is that of the exo-alkyl epoxide glycosides, of general structure \(10a\). These have been employed to label a catalytic carboxylate in a variety of different enzymes (Legler and Bause 1973; Shulman et al. 1976; Clarke and Strating 1989; Hoj et al. 1991, 1992; Keitel et al. 1993; Macarron et al. 1993; Havukainen et al. 1996; Sulzenbacher et al. 1997). The labeled residue has often been the catalytic nucleophile, and this has been definitively proven in some cases through the solution of the three-dimensional X-ray crystallographic structure (Keitel et al. 1993; Havukainen et al. 1996; Sulzenbacher et al. 1997). These X-ray structures show a covalent bond between the residue known to act as the catalytic nucleophile and the alkyl chain of the inactivator. In one case, it was noted that the residue labeled depended on the chain length of the inactivator: a 2,3-epoxypropyl \(\beta\)-D-xyloside labeled the catalytic nucleophile as expected, while a 3,4-epoxybutyl \(\beta\)-D-xyloside labeled the catalytic acid/base residue in a retaining 1,4-xylanase from *Trichoderma reesei* (Havukainen et al. 1996). This unusual result highlights the flexibility of the alkyl chains and their ability to shift the reactive epoxide into different orientations depending on the specific interactions with the enzyme active site. Molecular dynamics simulations allowed a theoretical rationale for this observation to be offered (Laitinen et al. 2000). A similar result was found in a systematic study of exo-alkyl epoxide glycosides of different chain lengths and different epoxide stereochemistries. It was found that the two different enzymes studied were inactivated by epoxides of different configurations and chain lengths, suggesting that this class of inactivator can be somewhat tailored toward a desired enzyme (Hoj et al. 1991).

**CBE: mechanistic and structural studies**

CBE (10b) is one example of a very well characterized MBI of retaining glycosidases. There already is an excellent review
of much of the early work on use of the conduritol epoxides and conduritol bromo-epoxides (Legler 1990), thus only a few examples will be discussed here. CBE was used very early on to examine both the glycosidase active sites present in mammalian sucrase-isomaltase. It was shown to be an effective inactivator of hydrolytic activity (Quaroni et al. 1974) and a tritiated version of the inactivator was used to label and identify catalytic carboxylates in both the sucrase and isomaltase active sites through peptic digestion and subsequent sequencing of radioactively labeled peptides (Quaroni et al. 1974; Quaroni and Semenza 1976). The enantiomer responsible for the inactivation behavior was identified and the opening of the epoxide ring by the enzyme was shown to be completely stereospecific and consistent with the general mechanism shown in Figure 8 (Braun et al. 1977).

This general strategy of using a radioactive derivative of CBE highlights one of the most important applications for covalent inactivators of glycosidases: the alkylation, and subsequent identification of active site residues. Until bioinformatics approaches permitted the prediction of active site residues (Henrissat et al. 1991; Henrissat and Bairoch 1993), these types of experiments were one of the few ways of identifying candidate active site residues. Even with the predictive abilities of bioinformatics, experimental verification of those predictions using such reagents as CBE remains an active area of research (see Febbraio et al. 1997; Hrmova et al. 1998; Li et al. 2001 for some examples). Once a putative residue is revealed, however, a highly regarded strategy involves the creation of variant proteins in which the purported active site residue is mutated, followed by detailed mechanistic analysis of mutants so generated (see Ly and Withers 1999; Vocadlo et al. 2002 for an example of this approach, reviewed in Ly and Withers 1999).

Interestingly, owing to the element of symmetry present in CBE, this reagent has also been used as an inactivator of some retaining α-glucosidases (Hermans et al. 1991; Iwanami et al. 1995; Kimura et al. 1997; Okuyama et al. 2001). While the epoxide in CBE should preferentially open in a trans-diaxial manner, it is able to orient itself well enough in the active site of some retaining α-glucosidases to also label the catalytic nucleophile in these enzymes (Figure 11). Particularly noteworthy was the successful trapping and identification of the nucleophile in human lysosomal α-glucosidase (Hermans et al. 1991). Since the first X-ray crystallographic structure of a homologous enzyme was only recently solved (Lovering et al. 2005), studies such as these have been important in identifying active site residues. However, the structural symmetry of CBE that permits it to act as an inactivator of both α-glucosidases and β-glucosidases also allows it to bind in some enzyme active sites in more than one mode, thus reacting with residues other than the catalytic nucleophile. Three important examples of this come from the enzymatic labeling of a carboxylate using the CBE analog conduritol C-epoxide to label E. coli lacZ β-galactosidase (Herrchen and Legler 1984) and CBE itself to label human lysosomal glucocerebrosidase (GCase) (Dinur et al. 1986), and almond β-glucosidase (Legler and Harder 1978). In all three cases, the labeled residue was mistakenly identified as the enzymatic nucleophile, and the nucleophile was later correctly identified with a more specific class of reagent, the 2-deoxy-2-fluoro glycosides (discussed below) (Gebler et al. 1992; Miao et al. 1994; He and Withers 1997).

### CBE: biological applications

One of the most important uses of CBE has been to study mammalian retaining β-glucosidases, and in particular human lysosomal GCase. GCase is normally responsible for the hydrolytic cleavage of the β-glucosyl residue in β-glucosyl ceramide (GlcCer), the final step in the degradation of glycosphingolipids. A deficiency in this enzyme is known to cause accumulation of the substrate, leading to a pathological condition known as Gaucher’s disease (Zhao and Grabowski 2002; Butters 2007).

An X-ray crystal structure of CBE covalently bound to the GCase enzyme has been solved (Premkumar et al. 2005), which gives some insight into the biochemical basis for some forms of Gaucher’s disease (Dvir et al. 2003; Brumstein et al. 2006, 2007; Lieberman et al. 2007).

CBE has been shown to be a selective inactivator of human GCase while not affecting the activity of other known mammalian β-glucosidases (Daniels et al. 1980). This is an important feature that is exploited in the enzymatic assays for GCase activity in liver homogenates (Daniels et al. 1980), as well as in efforts to discover and characterize novel mammalian β-glucosidases since CBE can be added to crude enzyme mixtures to selectively inactivate GCase, allowing other β-glucosidase activities to be examined. This approach allowed the characterization of a non-lysosomal β-GCase activity belonging to β-glucosidase 2 (Boot et al. 2007). This had not been possible previously owing to the enzyme’s instability (Vanweely et al. 1993). CBE has also been helpful in characterizing the activity of a broad specificity β-glucosidase by selectively knocking out GCase activity in cell homogenates (Hay et al. 1998). Interestingly, another β-glucosidase activity that can cleave 6′-acetylamo-4-methylumbelliferin β-D-glucosides was shown to be insensitive to CBE, confirming that it was not GCase (Mikhaylova et al. 1996).
The selectivity of CBE for GCase has also been exploited in the selective ablation of GCase activity in both cell culture and in animal models to answer interesting biochemical and biological questions about the role of the enzyme and its substrate. CBE allows for the selective inhibition of mammalian GCase and permits the accumulation of unprocessed substrate GlcCer. In one example, multi-drug resistance in cancer cells was mimicked in normally drug-sensitive cancer cells by treatment with CBE. This has lent support to the hypothesis that increased levels of GlcCer correlate with increased drug resistance in cancer cells (Morjani et al. 2001). Gaucher’s cells have also been observed to have an altered redox state and increased levels of reactive oxygen species, an observation that could be directly attributed to GCase activity since healthy fibroblasts treated with CBE demonstrated the same behavior (Degunuto et al. 2007). Finally, the ability to selectively turn-off GCase activity in mammalian systems using CBE has been exploited in the characterization of the important role that glycosphingolipids play in skin biochemistry (Takagi et al. 1999; Holleran et al. 2006). CBE was used to help localize GCase activity to the stratum corneum layer of the skin, which is known to play a crucial role in maintaining the skin’s ability to prevent excessive water loss and prevent absorption of foreign substances (Takagi et al. 1999). It has been suggested that proper GCase activity in the stratum corneum is important for maintaining the lipid balance for proper function (Holleran et al. 2006).

Macrophage cells are the cell type most aberrant in Gaucher’s disease, and as a result many investigators have used CBE to mimic this aberrant “Gaucher’s cell” phenotype. CBE-treated macrophages do in fact display the altered morphology of the Gaucher’s cell (Yatziv et al. 1988), and this alteration can be exacerbated by exposure to liposomes (Das et al. 1987) or red blood cells (Schueler et al. 2004) to artificially increase the GlcCer storage levels. The authors argued that this would better mimic the natural phagocytic role that macrophages play in the clearance of red and white blood cells (Schueler et al. 2004). CBE-induced storage of GlcCer in macrophages has also been used to study altered sphingolipid metabolism (Trajkovic-Bodnevec et al. 2004), which was shown to arise from the increased activity of cytidylylphospho-

Cyclophellitol: mechanistic and biological applications

Cyclophellitol (10c) is another example of the parallel development between the discovery of bioactive natural products and rational design of MBIs for glycosidases. Although CBE has proven to be a useful inactivator, its inherent symmetry allows it to inactivate both α-glucosidases and β-glucosidases as discussed above. Thus, it had been proposed that a CBE derivative that possessed a hydroxymethyl group analogous to the C5 hydroxymethyl group in glucose would be a more selective and potentially also a more potent inactivator of β-glucosidases (Caron and Withers 1989). Indeed, before any report detailing the total synthesis of cyclophellitol was published, the compound was isolated from a mushroom, Phellinus sp., and shown to be a β-glucosidase inhibitor (Atsumi, Iinuma 1990; Atsumi, Umezawa 1990). It was subsequently demonstrated to be a specific, active site-directed inactivator of two β-glucosidases, Abg and almond (Withers and Umezawa 1991). Surprisingly, it was not until recently that an X-ray crystal structure showing an inac-

A diastereomer of cyclophellitol, 1,6-epi-cyclophellitol (10d), has also been synthesized and tested as an inactivator of α-glucosidases (Atsumi, Nosaka, Ochi 1993; Tai et al. 1995). Compound 10d was found to be a potent irreversible inactivator of yeast α-glucosidase and also of the α-mannosidase from jack beans (Tai et al. 1995). 1,6-Epi-cyclophellitol was shown to be an inhibitor of experimental metastasis, whereas cyclophellitol itself was not shown to be active under the same conditions. This definitively shows that one of the enzyme activities important for metastasis is that of an α-glucosidase, and not a β-glucosidase. This distinction was suspected but unproven using noncovalent inhibitors such as castanospermine, since they demonstrated some inhibitory activity against both retaining α- and β-glucosidases (Hadjwigerfangmeier et al. 1989). The use of
of a specific MBI allows the unequivocal ablation of a single enzyme activity, which is often challenging in a complex enzyme mixture.

Cyclophellitol has also been used, in the same manner as CBE, as a small-molecule inhibitor of mammalian GCase in vivo to induce a Gaucher-like state in both cell culture and an animal model (Atsumi S, Inuma H 1990; Atsumi et al. 1992; Atsumi, Nosaka, Iinuma 1993). Cyclophellitol was shown to be a more potent induction agent for the production of the so-called small molecule-induced “Gaucher Mouse” (Atsumi et al. 1992). The administration of cyclophellitol to mice also caused an increase in the level of glucosylsphingosine detected in various organs, leading to the suggestion that this cytotoxic substance may play a role in the pathology of Gaucher’s disease (Atsumi, Nosaka, Iinuma 1993). However, other than these reports, and one other report on the use of cyclophellitol to probe the structural and dynamic aspects of a β-glycosidase by tryptophan emission studies (Bismuto et al. 1999), cyclophellitol (10c) and 1,6-epi-cyclophellitol (10d) have not been used in the wide variety of applications of their predecessor.

Activated fluorinated glycosides

General summary
The activated 2-deoxy-2-fluoro (Figure 12, 12a), 5-fluoro (12b), and 2-deoxy-2,2-difluoro (12c) glycosides represent the most specific class of MBI known for glycosidase activity. Inactivation by these species invariably derives from labeling the enzymatic nucleophile of a retaining glycosidase; there have been no examples to date of any other residue being labeled by this class of reagent (Mosi and Withers 2002; Wicki et al. 2002). The activated 2-deoxy-2-fluoro glycosides (12a) were the first to be introduced and were found to be specific inactivators of a variety of retaining β-glycosidases (Withers et al. 1987, 1988). The 2-deoxy-2-fluoro substitution leads to a destabilization of both the glycosylation and deglycosylation transition states in the retaining glycosidase catalytic mechanism, thereby slowing both the formation of the glycosyl-enzyme intermediate and its hydrolysis. This destabilization of the oxocarbenium ion-like transition state arises both from inductive effects of fluorine and from the removal of hydrogen-bonding interactions ordinarily formed with the OH-2 on the pyranose ring. The addition of an activated leaving group (often a dinitrophenolate, or fluoride) accelerates the glycosylation step, leading to the accumulation of the covalent glycosyl-enzyme intermediate (Figure 13). This species formed can be moderately stable, with observed lifetimes ranging from seconds to months.

The activity of enzymes inactivated in this fashion can be recovered either through hydrolysis of the covalent glycosyl-enzyme intermediate, or by transglycosylation onto a suitable acceptor substrate to restore a catalytically competent enzyme (Mosi and Withers 2002; Wicki et al. 2002). As a consequence of this recovery of activity, this class of molecule is formally better described as being a very slow enzymatic substrate rather than a true inactivator. However, the trapped intermediates are usually sufficiently long lived that for all practical purposes, these reagents function as inactivators and will generally be referred to as such in the context of this review.

The activated 5-fluoro glycosides (12b) operate using a very similar mechanism to that of the activated 2-deoxy-2-fluoro glycosides. One structural difference lies in the replacement of a hydrogen atom by fluorine, as opposed to the replacement of a hydroxyl group with fluorine as seen with the 2-fluoro glycosides. This change would be expected to be more strongly destabilizing to both glycosylation and deglycosylation transition states, on the basis of the larger change in electronegativity arising from replacement of hydrogen with fluorine. However, the replacement of an oxygen atom with fluorine in the 2-fluoro glycosides also strongly attenuates the hydrogen-bonding interactions at that position, which have generally been shown to be very important in both the glycosylation and deglycosylation transition states. No such loss of hydrogen-bonding interactions is incurred by 5-fluoro substitution. The combined effect of these two competing factors is that, in the context of inhibitory activity against retaining β-glycosidases, the 5-fluoro glycosides tend to have both higher glycosylation and higher deglycosylation rates than the analogous 2-deoxy-2-fluoro glycosides. Indeed they often function as slow substrates for which the second step (deglycosylation) is rate limiting; thus, the intermediate accumulates. Kinetically, this is revealed in very low Km values (if monitored

Fig. 12. Structures of activated (A) 2-deoxy-2-fluoro glycosides; (B) 5-fluoro glycosyl fluorides and (C) trinitrophenyl 2-deoxy-2,2-difluoro glucoside. R = F, dinitrophenol; TNP = trinitrophenyl.

Fig. 13. Mechanism of inactivation of a β-glucosidase by 2-deoxy-2-fluoro-β-D-glucosyl fluoride.
as a substrate) or apparent very tight binding (low \( K_m \) values) if monitored as a “reversible inhibitor.” For a more detailed discussion, see Mosi and Withers (2002). Interestingly, and in contrast to what is found with the 2-deoxy-2-fluoro glycosides, the appropriately activated 5-fluoro glycosides are capable of inactivating retaining \( \alpha \)-glycosidases. Kinetic, mechanistic, and structural studies have confirmed that this inactivation is indeed due to the accumulation of a stable 5-fluoroglycosyl-enzyme species (Numao et al. 2003). By contrast, the 2-deoxy-2-fluoro \( \alpha \)-glycosides act as slow substrates with \( \alpha \)-glycosidases since the deglycosylation step is faster than glycosylation. The origin of this selectivity, based on the site of fluorination, is not entirely clear, although it is thought to be related to the relative distribution of partial positive charge between the anomeric carbon and the ring oxygen in the transition state of the reaction (Zechel and Withers 2000).

A third class of fluorinated sugars, that of the activated 2-deoxy-2,2-difluoro glycosides, has also been used to trap the covalent glycosyl-enzyme intermediate in retaining \( \alpha \)-glycosidases, although there have only been three reports to date on the use of this class of inactivator (Braun et al. 1995; Hart et al. 2000; Zhang et al. 2008).

### Activated fluorinated glycosides: mechanistic and structural studies

The use of activated fluorinated glycosides to identify and label the catalytic nucleophile in retaining glycosidases has been thoroughly reviewed previously (Withers and Aebersold 1995; Mosi and Withers 2002; Wicki et al. 2002) and will not be covered in detail here. In brief, the current strategy typically employed to identify the catalytic nucleophile involves inactivation of the target glycosidase followed by proteolysis, peptide localization, and sequencing by HPLC/MS using collision-induced fragmentation to identify the labeled residue. Important examples of the use of activated 2-deoxy-2-fluoro glycosides in correcting the identities of the enzymatic nucleophiles in \( E. coli \) lacZ \( \beta \)-galactosidase (Gebler et al. 1992), GCcase (Miao et al. 1994), and almond \( \beta \)-glucosidase (He and Withers 1997) through the use of the appropriately configured glycoside have been published. Another noteworthy example of an important application is the identification, using an activated 5-fluoro-glucosyl fluoride, of a novel \( \alpha \)-glucosidase from an acidophilic archaeon in which the catalytic nucleophile was shown to be a threonine rather than the more typically seen glutamate or aspartate (Ferrer et al. 2005). This use of a different nucleophile is an interesting example of the evolutionary adaptation presumably necessary for the enzyme to function under the harsh conditions in which the organism survives. Similarly, the catalytic nucleophile in sialidase enzymes responsible for cleavage of anionic sialic acid residues has been trapped and identified as a tyrosine (Watts et al. 2003; Watts and Withers 2004). A further example of the use of a 2-deoxy-2-fluoro glycoside was in trapping the covalent glycosyl-enzyme intermediate of myrosinase (Cottaz et al. 1996). Interestingly, the aglycone in the 2-deoxy-2-fluoroglucotropaeolin is the same as the aglycone in the natural substrate, sinigrin (McCarter et al. 1997). In this case the natural-leaving group is relatively reactive, and indeed the enzyme has evolved without a general acid/base catalytic residue, and a bound ascorbate functions as the base catalyst (Burmeister et al. 2000). In a few special, other cases, a “natural” sugar-leaving group was shown to be sufficient to render the 2-fluoroglycoside useful in trapping of the intermediate. Necessary conditions for this behavior were evaluated in that paper (McCarter et al. 1997).

Another important application for the activated 2-deoxy-2-fluoro and 5-fluoro glycosides is in the study of the trapped glycosyl-enzyme intermediates by X-ray crystallography and protein NMR spectrometry to gain mechanistic insights into this class of enzymes. Many examples, in particular X-ray crystallographic structures, of the glycosyl-enzyme intermediate exist, so only a few notable examples will be mentioned here. One of the most significant examples was the crystallization of the covalently bound glycosyl-enzyme intermediate of hen egg-white lysozyme using 2-acetamido-2-deoxy-\( \beta \)-D-glucopyranosyl-(1\( \rightarrow \)4)-2-deoxy-2-fluoro-\( \beta \)-D-glucopyranosyl fluoride and a mutant enzyme in which the catalytic acid/base residue had been removed by site-directed mutagenesis (Vocadlo et al. 2001). This report was key in establishing a new paradigm in the understanding of glycosidase mechanisms, refuting the ion-pair intermediate mechanism proposed by Phillips (1967) and supporting the double-displacement mechanism first proposed by Koshland (1953). Activated 2-deoxy-2-fluoro and 5-fluoro glycosides have also been used in conjunction with X-ray crystallography to gain mechanistic insights into the conformations of the sugar ring during the course of enzymatic catalysis. X-ray crystallographic structures have been solved for the covalent glycosyl-enzyme intermediate in both \( \alpha \)-retaining (Numao et al. 2003; Lovering et al. 2005) and \( \beta \)-retaining (Ducros et al. 2002; Davies et al. 2003) glycosidases using activated 5-fluoro and 2-deoxy-2-fluoro glycosides, respectively. These types of studies of the structures of the resting enzyme, Michaelis, intermediate and product complexes have allowed a mapping of the conformations adopted by the pyranose ring during the course of catalysis and have led to hypotheses regarding the conformation of the sugar ring at the enzymatic transition state. Mechanistic studies such as these may permit the rational design of tighter binding inhibitors that are more selective for one class of enzymes over another, based on knowledge of the transition state (Gloster, Meloncelli 2007).

NMR spectroscopy has been applied to studying mechanistic and dynamic aspects of glycosidases, and the activated 2-deoxy-2-fluoro glycosides have proven to be useful tools in these types of studies. One of the earliest examples was in demonstrating the stereochemistry of the covalent glycosyl-enzyme intermediate formed upon reaction of Abg with an activated 2-deoxy-2-fluoro glucoside using \( ^{19} \text{F} \) NMR spectroscopy (Withers and Street 1988). In another case, the covalent glycosyl-enzyme intermediate of the xylanase from \( \text{Cellulomonas fimi} \) was trapped and studied by NMR spectroscopy (Poon et al. 2007). It was found that flexible portions of the protein became more ordered upon inactivation, and the protein was also observed to be much more stable upon inactivation by 2,4-dinitrophenyl 2-deoxy-2-fluoro-cellobioside. The \( pK_a \) values of the acid/base and nucleophilic catalytic residues of a retaining xylanase, another important aspect of the catalytic machinery, were probed by pH titration of both the free and inactivated enzyme that had been site-specifically \( ^{13} \text{C} \) labeled on the two active site carboxylic acids (McIntosh et al. 1996). \( ^{13} \text{C} \)-NMR spectra of each were recorded at different pH values, and titration curves (chemical shift versus pH) were constructed. The study revealed how the \( pK_a \) value for the acid/base catalytic residue “cycles” to suit its
role at each step through the course of the reaction in response to the local electrostatic environment.

Activated fluorinated glycosides: biochemical applications

The high specificity of the activated 2-deoxy-2-fluoro glycosides for their cognate glycosidase has been exploited in a similar manner to that for which CBE was used in the characterization of mammalian retaining β-glucosidases. The demonstration of a cytosolic pyridoxine-β-glucosidase activity in mammalian cells relied on both CBE and 2-deoxy-2-fluoro-β-glucopyranosyl fluoride to selectively ablate the GCase and broad-specificity cytosolic β-glucosidase activities, respectively, during the purification and characterization of this novel enzyme (McMahon et al. 1997). Another example of exploiting the specificity of MBIs of glycosidases came in the characterization of the two active sites present on mammalian intestinal lactase phlorizin hydrolyase enzyme (Arribas et al. 2000; Day et al. 2000; Mackey et al. 2002). In these reports, activated 2-deoxy-2-fluoro glycosides were used to selectively inactivate either the lactase or phlorizin hydrolysis activities. This ability to selectively knockout one enzymatic activity over the other allows full kinetic characterization of only the one active site. This was previously found to be challenging as the two active sites showed some substrate cross-reactivity. The activated 2-deoxy-2-fluoro glycosides also helped localize the two active sites onto different regions of the polypeptide chain following labeling and sequencing (Arribas et al. 2000), which helped clarify earlier conflicting reports regarding the location of the two active sites (Wacker et al. 1992; Zecca et al. 1998). Activated 2-deoxy-2-fluoro glycosides may also prove useful in live animal studies, as 2-deoxy-2-fluoro-β-D-glucosyl fluoride has already been demonstrated to get into all organs, including the brain, and to selectively label the β-glucosidases in a rat model (McCarter et al. 1994).

The ability to trap a covalent glycosyl-enzyme intermediate using an activated 2-deoxy-2-fluoro glycoside has also been used to explore the aglycone-binding site specificity. It is known that, in some cases, the rate of turnover of the covalent glycosyl-enzyme intermediate formed with a fluorinated sugar can be accelerated by the addition of another acceptor molecule (besides water) which has some affinity for the enzyme’s aglycone-binding site and binds productively (Figure 14) (Withers et al. 1987). This behavior has been used to study the aglycone site interactions in a number of different enzymes (Blanchard and Withers 2001; Hommalai et al. 2005) and could be applicable to studying the aglycone-binding site when nothing is known of an enzyme’s specificity. Indeed the approach has been developed into a high-throughput format for the rapid screening of aglycone specificity and represents the only realistic way of uncovering such information.

Activated 2-deoxy-2-fluoro and 5-fluoro glycosides have also been the basis for new activity-based proteomic profiling (ABPP) probes when conjugated to a reporter group such as biotin through a covalent linker (Vocadlo and Bertozzi 2004; Hekmat et al. 2005; Williams et al. 2006; Stubbs et al. 2008). While this technology is still in the earliest stages of development, the ability to selectively label the desired class of retaining β-glucosidase (either β-galactosidase (Vocadlo and Bertozzi 2004), xylanase (Hekmat et al. 2005; Williams et al. 2006), or β-glucosaminidase (Stubbs et al. 2008)) in a complex protein mixture has already been demonstrated. In one instance, active site peptide “fingerprinting” of the labeled peptides derived from a biologically relevant protein mixture led to the discovery of a new enzyme not previously characterized in the organism, Cellulomonas fimi (Hekmat et al. 2005) while the use of fluorescent tags of different colours on inactivators of xylanases and cellulases allowed facile visual inspection of such enzymes produced in Cellulomonas fimi grown upon different substrates (Hekmat et al. 2007). A novel β-glucosaminidase thought to be involved in antibiotic resistance has also been confirmed to exist in the pathological organism Pseudomonas aeruginosa by ABPP methods using an activated 5-fluoro glycoside (Stubbs et al. 2008). Most recently,
isotopically encoded versions of these reagents have been employed for quantitative proteomic analysis of glycosidase activities on a proteome-wide basis (Hekmat and Withers 2008). This topic has recently been elaborated on elsewhere (Stubbs and Vocadlo 2006), so this review will not focus on the specific details. However, it can be anticipated that a wide variety of biologically relevant applications will arise in the near future from such technologies, and this represents one of the many very important applications that covalent inactivators of glycosidases may find in the future.

Concluding remarks

It can be seen that no one structural feature makes for an ideal general covalent inactivator against all glycosidases. The majority of the compounds reviewed above are only active against retaining glycosidases; no general reagent for the efficient covalent modification or inactivation of inverting glycosidases has been described to date. Many of the MBs described above are restricted to, or show a preference for, inactivation of retaining glycosidases; no general reagent for the efficient con- jority of the compounds reviewed above are only active against

Conflict of interest statement

Neither author has any conflict of interests.

Abbreviations

Abg. *Agrobacterium sp.* β-glucosidase; ABPP, activity-based proteomic profiling; CBE, conduritol B-epoxide; CTP, cytidylyltriphostate; GCase, glucocerebrosidase; GlcCer, β-Glucosyl ceramide; MBI, mechanism-based inactivator; PA, photoaffinity.

References


