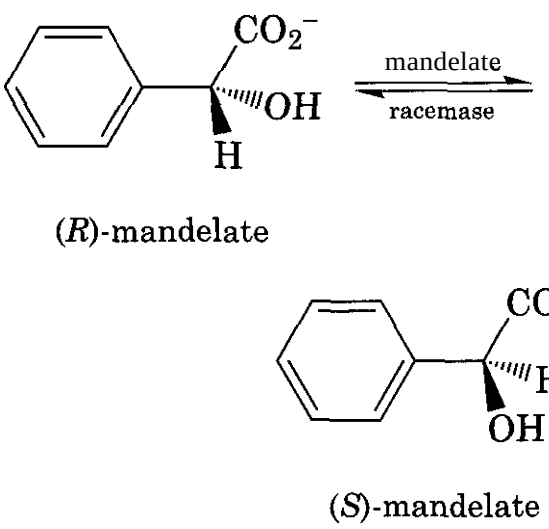
extremely popular for the inactivation of proteases. By incorporating part of the sequence of the physiological substrate into the halomethyl ketone, it was possible to obtain selective inactivation of individual proteases (177). This selective inactivation also meant that chloromethyl ketones became widely used as probes for the binding requirements and chemically reactive residues in the active sites of serine proteases, in particular. Replacement of the chloromethyl ketone moiety by a diazomethyl group provided a specific inactivation of cysteine proteases (172). The use of TPCK has not been restricted to chymotrypsin, as elegantly demonstrated in a recent report on the inhibition of human aldehyde dehydrogenase (178). As a group, proteases remain major targets for therapeutic intervention, and peptide-based affinity labels are still playing a major role in drug design (179).

The interconversion of (*R*)-mandelate and (*S*)-mandelate is catalyzed by mandelate racemase (Equation 17.49). The reaction can be reversibly inhibited by the substrate analog atrolactate (**67**), (Fig. 17.31). Because of its structural similarity to both (67) and the substrate and, given the reactivity of the epoxide group to nucleophiles, (*R*,*S*)-α-phenylglycidate (68) was synthesized as a potential affinity label of mandelate racemase. The compound was found to be an irreversible inhibitor, fitting all the criteria described in section 3.1.1 (180). Later it was established that (*S*)-α-phenylglycidate (*S*-αPGA) did not irreversibly inactivate the enzyme, binding noncovalently and with less affinity than *R*-αPGA (69). As shown in Figure 17.31, the epoxide ring of *R*-αPGA potentially is subject to attack at either of two carbons. Attack at the distal endocyclic carbon atom of (69) (path a) will result in the formation of (**70**), whereas attack at the a-carbon (path b) will yield (71). The crystal structure of the inactivated complex revealed that nucleophilic attack of the ϵ-amino group of Lys166 resulted in adduct (**72**), which is consistent with attack on the distal carbon of the epoxide ring (181). This structure confirmed the original design premise of Fee et al. (180), wherein it was thought that the distal oxirane carbon occupied the position similar to the a-proton in mandelate. Therefore, on binding of the a-phenylglycidate to the enzyme, the electrophilic epoxide group would be subject to attack by the nucleophile responsible for a-proton abstraction in the normal catalytic cycle. Further confirmation is provided by the X-ray structure of (*S*)-atrolactate bound to the racemase (181), which reveals that Lys166 has been pushed away by the a-methyl group of (*S*)-atrolactate (which is positionally equivalent but much larger than the a-proton in (*S*)-mandelate). In both structures the positions of the remaining active-site residues are almost identical.



(17.49)

Perhaps the best-known affinity labeling reagent is aspirin (73)(Fig. 17.31), a member of the class of drugs known as the nonsteroidal anti-inflammatory drugs (NSAIDS), and whose activity was initially reported to result from its inhibition of prostaglandin biosynthesis (182, 183). Prostaglandins are involved in the inflammatory response and can cause headache and vascular pain in humans.

Prostaglandin synthase, which catalyzes the first step in the arachidonic acid cascade, is a heme protein and possesses two activities. As illustrated in Equation 17.50, a cyclooxygenase activity is used in the conversion of arachidonic acid to the bicyclic endoperoxide PGG_2, whereas a peroxidase activity catalyzes the subsequent reduction of PGG_2 to prostaglandin H_2. The latter serves as a branch point in the production of various prostaglandins as well as thromboxane A_2 and prostacyclin (PGI_2).

Aspirin (acetylsalicylicacid) was ultimately confirmed as an inhibitor of prostaglandin synthetase (184). Incubation of [*acetyl*-^{3}H] aspirin showed that one acetyl group was in-