

## REVIEW

## Organic Stereochemistry

Part 8<sup>1)</sup>

## Prostereoisomerism and the Concept of Product Stereoselectivity in Biochemistry and Xenobiotic Metabolism

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This eighth review continues and ends our *Series on Organic Stereochemistry*. It focuses on a major concept in biochemistry, and in drug and xenobiotic metabolism, namely, that of *product stereoselectivity*. This concept describes the well-known fact that, when a metabolic reaction creates a *new stereogenic element in a substrate molecule* (most often a stereogenic center from a center of prochirality), the *two stereoisomeric metabolites* will generally be produced at different rates.

In this *Part*, we begin by discussing *relationships between groups or atoms* of same constitution within intact molecules (*topic relationships*, see *Part 1, Fig. 1.12*). Internal comparison of these groups by symmetry operations allows a simplified description of molecular structure. Also, the *molecular environments* of constitutionally identical groups are examined. If these environments are stereoisomeric, the molecule is said to possess elements of prostereoisomerism. *Mislow* has given a classification of steric relationships of groups in intact molecules [1–3], and the presentation that follows is based on this classification.

When applied to endogenous biochemistry and to drug metabolism, the concept of prostereoisomerism appears under the name of *product stereoselectivity*, namely the differential formation of two stereoisomeric metabolites from a single prochiral substrate. As such, the present *Part* is the matching piece to *Part 7* dedicated to *substrate stereoselectivity*.

To repeat what has been stated in *Part 7*, the discrimination between substrate and product stereoselectivities owes much to the pioneering work of *Vladimir Prelog*, Nobel Laureate and a founding father of modern stereochemistry. Beginning in the mid-1950s and for many years thereafter, he investigated the stereoselective reduction of xenobiotic ketones in microorganisms, leading him to *conceptualize* a clear discrimination between substrate and product stereoselectivity (e.g., [4–6]). And given the complexity of the natural world, there are cases where stereoisomeric substrates show distinct product stereoselectivities (*substrate–product stereoselectivity*), as will be duly discussed.

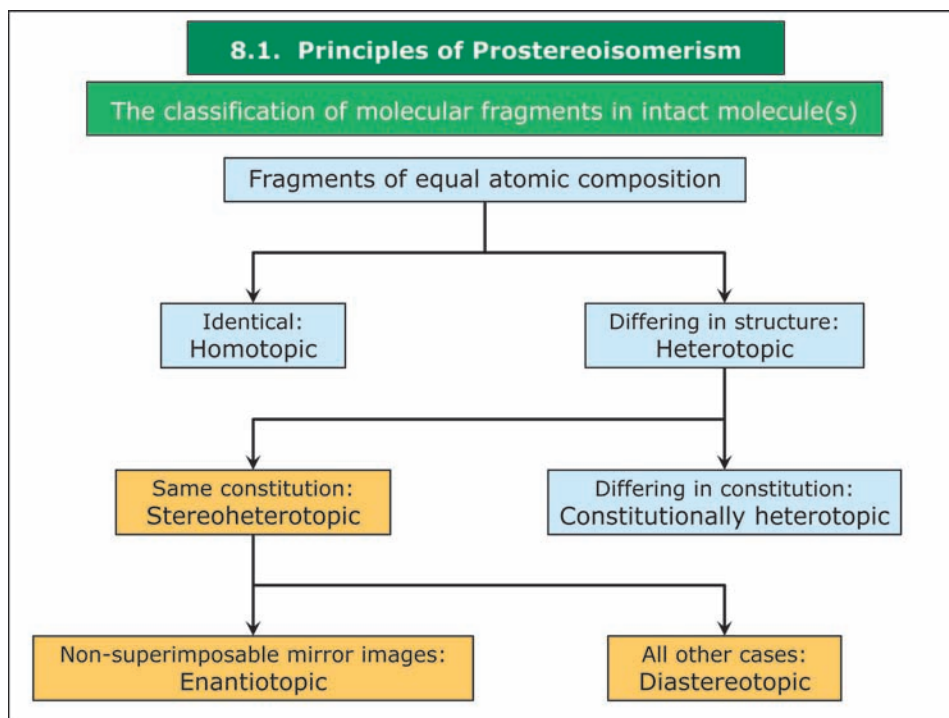
<sup>1)</sup> For the other *Parts*, see *Helv. Chim. Acta* **2013**, *96*, 1–3.

## Part 8. Prostereoisomerism and the Concept of Product Stereoselectivity in Biochemistry and Drug Metabolism

- 8.1. Principles of Prostereoisomerism
- 8.2. Endogenous Biochemistry
- 8.3. Product Stereoselectivity in Drug Metabolism
- 8.4. Substrate–Product Stereoselectivity in Drug Metabolism

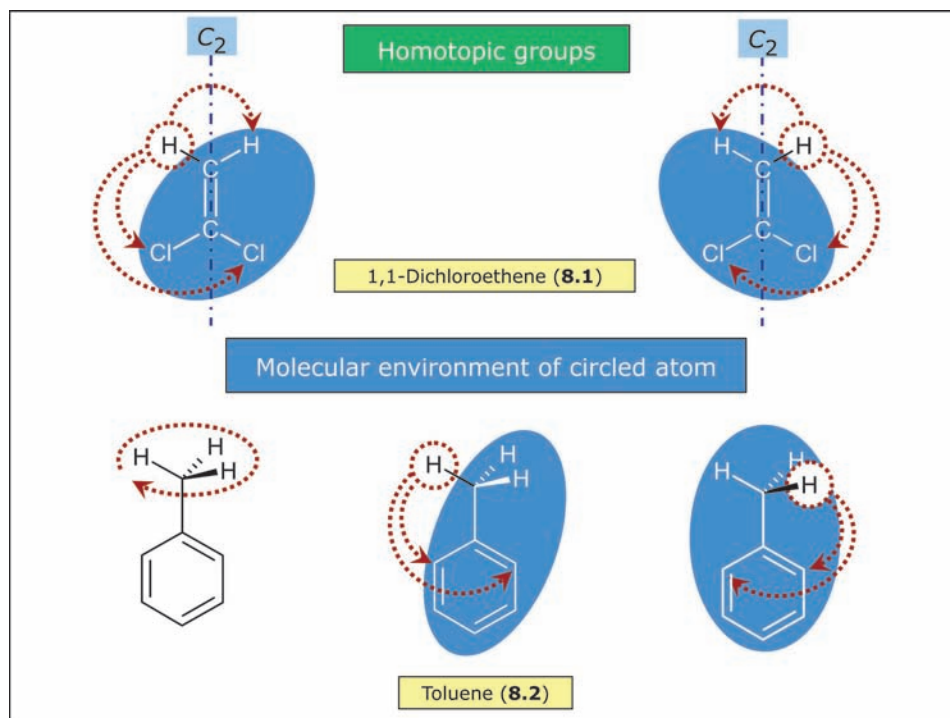
**Fig. 8.1.** The *content of this Part* is summarized here and logically begins with the principles of prostereoisomerism. To some, the concept of prostereoisomerism and its principles appear rather abstract and of limited practical value. This cannot be true given the important applications of the concept in spectroscopy, synthetic chemistry, and (in our context) biochemistry. Indeed, and for reasons that will soon become apparent, enzymes have the remarkable capacity to discriminate not only between *stereoisomeric substrates* (substrate stereoselectivity, see *Part 7*), but also between *stereoheterotopic groups or fragments* in molecules.

Following the overview of principles, three sections will serve to illustrate product stereoselectivity by presenting a variety of relevant examples. First, we shall look at *endogenous metabolism*, which involves the anabolism (synthesis) and catabolism (degradation) of endogenous compounds [7–9]. This will be followed by product stereoselectivity in the *metabolism of drugs and other xenobiotics* [10–19], covering both *functionalization (hydrolysis and redox) reactions* [20] and *conjugations* [21]. The last section deals with the sometimes confusing cases where stereoisomeric substrates show distinct product stereoselectivities, *i.e.*, cases of *substrate–product stereoselectivity*.



**Fig. 8.2.** This Figure takes up the classification of molecular fragments from where we left it in Fig. 1.12 (Part I). There, we saw two types of relationships between fragments (*i.e.*, groups or atoms), namely *topic relationships* which consider fragments in intact molecules, and *morphic relationships* which arise when considering fragments isolated from the rest of the molecule.

*Topic relationships* are at the heart of *prostereoisomerism*, and we begin by returning to the broader context outlined in Part I. Fragments of the same atomic composition can be identical according to any criterion, in which case they are *homotopic*, as exemplified in the next Figure. When there is some element in their *structure* that differentiates them, they will be *heterotopic* and can be subclassified further. Our interest among heterotopic fragments is in *stereoheterotopic fragments*, namely fragments which have identical constitution but whose *molecular environment* is *non-superimposable*. Such fragments are either *enantiotopic* or *diastereotopic* [22–24], as we shall see in the next Figures.



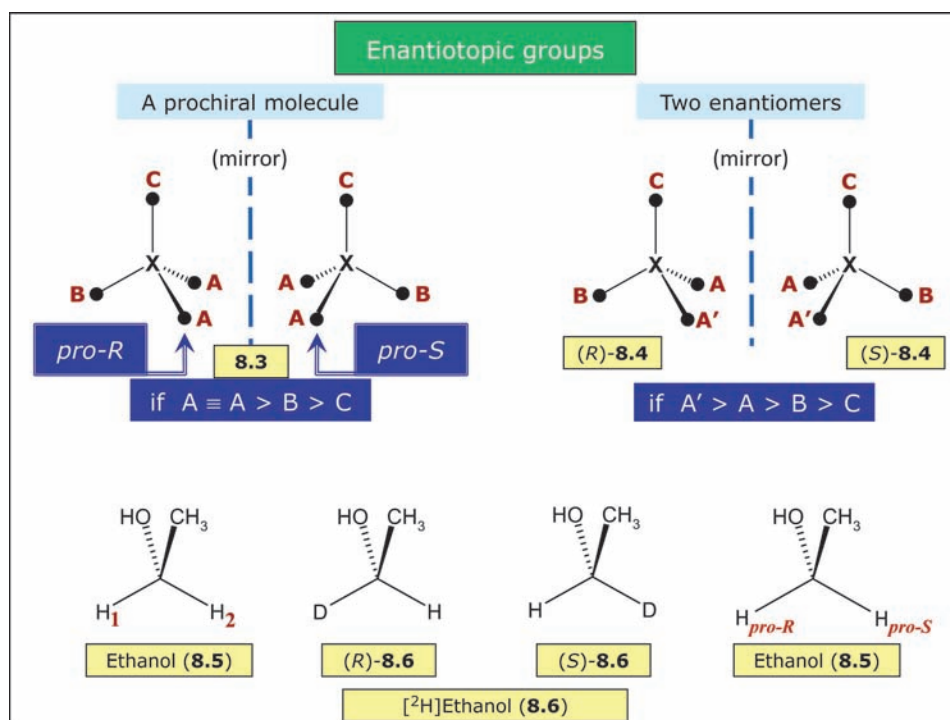
**Fig. 8.3.** When classifying fragments of equal atomic composition, the word ‘*structure*’ used above is too vague and modest in operational context. The accompanying explanation directs the reader’s attention to the molecular environment of the fragments as illustrated here for *homotopic fragments* and later for *stereoheterotopic* ones.

When considering the two H-atoms of *1,1-dichloroethene* (8.1), we see that the molecular environment (in the light blue oval) of the left-hand H-atom is identical in every aspect to the molecular environment of the right-hand H-atom [25–27]. In more concrete terms, these two H-atoms are *homotopic* because their respective molecular environment is a space in which the intramolecular distances (dotted arrows) from each of the two H-atoms to the other atoms are pairwise identical. However, the molecular environment is sometimes a tedious criterion of equivalence, which can be gainfully complemented by simple *symmetry considerations*. These show that *fragments are homotopic* if they can be interconverted by rotation about an *axis of symmetry*  $C_n$  ( $\infty > n > 1$ ). In *1,1-dichloroethene*, rotation about the  $C_2$  axis interchanges the two H-atoms and results in a structure indistinguishable from the original one. It is easy to see that this compound contains two other homotopic groups, namely the two Cl-atoms.

In *toluene* (8.2), rotations of  $120^\circ$  about the C(4)–C(7) axis interchange the three H-atoms of the Me group. But these atoms can be considered as equivalent only if free rotation of the Me group is assumed. Indeed, if *rotation is fast* relative to the time scale of observations or intermolecular interactions, ‘free rotation’ is effective, and the three H-atoms appear equivalent. If, in contrast, *methyl rotation is slow* in the time scale of

observations, equivalence is lost. Assuming, for example, toluene (**8.2**) to be ‘frozen’ in the depicted conformation, it is apparent that the H-atom located in the plane of the phenyl ring experiences a different molecular environment than the two out-of-plane H-atoms. Toluene is an interesting model in this aspect, because it shows that the time factor is of importance to the concept of group equivalence.

Besides criteria of symmetry or molecular environment, the *substitution criterion* can also be useful to assess equivalence of groups. In this procedure, the groups under consideration are *replaced in turn by a test group*, e.g., a protium ( $^1\text{H}$ ) atom is replaced by a deuterium ( $^2\text{H}$ ) atom. The resulting structures are examined for identity. If they are indistinguishable (superimposable by rotation and translation), the groups are equivalent. Thus, substituting, in turn, the two H-atoms in 1,1-dichloroethene (**8.1**) with a  $^2\text{H}$ -atom does not generate isomers but twice the same compound.



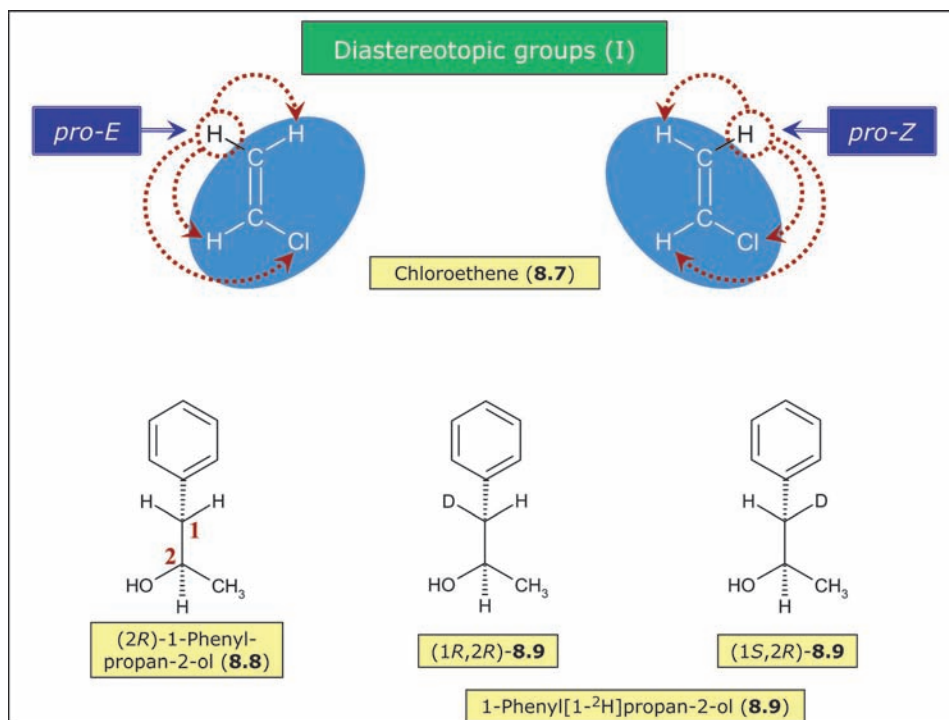
**Fig. 8.4.** Heterotopic fragments and groups having the same constitution are said to be *stereoheterotopic* and can be further separated into *enantiotopic* and *diastereotopic* fragments or groups. Here, we turn our attention to *enantiotopic fragments and groups*, which are the foundation stones of *prostereoisomerism* [1–3][22–31].

The *generic compound 8.3* features a central, tetravalent atom (generally a C-atom), and the four substituents A, A, B, and C, these groups being assumed to be achiral. First, there is no simple axis of symmetry here, and the two groups A can be

interchanged only by an operation of rotation–reflection ( $S_n$ ) to provide a structure indistinguishable from the original. These two groups are, therefore, *stereoheterotopic*. Second, when replacing in turn one of the two A groups with an achiral group A' ( $A' \neq A$ ), the resulting generic structure **8.4** exists as a pair of enantiomers, as shown. And third, the *molecular environments* of the two groups A are enantiomeric, since the two groups A see the other groups A, B, and C in a *clockwise sequence* for one, and in a *counterclockwise sequence* for the other. In such cases, the stereoheterotopic groups are specified as being *enantiotopic*; the group A having a clockwise environment is designated as *pro-R*, while the other is *pro-S*.

A well-known example is provided by the CH<sub>2</sub> H-atoms of *ethanol* (**8.5**), arbitrarily labeled as H<sub>1</sub> and H<sub>2</sub> in the left-hand side representation of the molecule. The environment of the H<sub>1</sub>-atom is clockwise (OH > Me > H), while that of H<sub>2</sub> is counterclockwise, implying that the former is *pro-R* and the latter *pro-S*. This is also seen when replacing H<sub>1</sub> or H<sub>2</sub> by a <sup>2</sup>H-atom to obtain, respectively, the (*R*)- and (*S*)-enantiomer of the chiral compound [<sup>2</sup>H]*ethanol* (**8.6**). Labeling two enantiotopic groups with the subscripts *pro-R* and *pro-S* (see representation on the right-hand side of the *Figure*) has also been proposed [28].

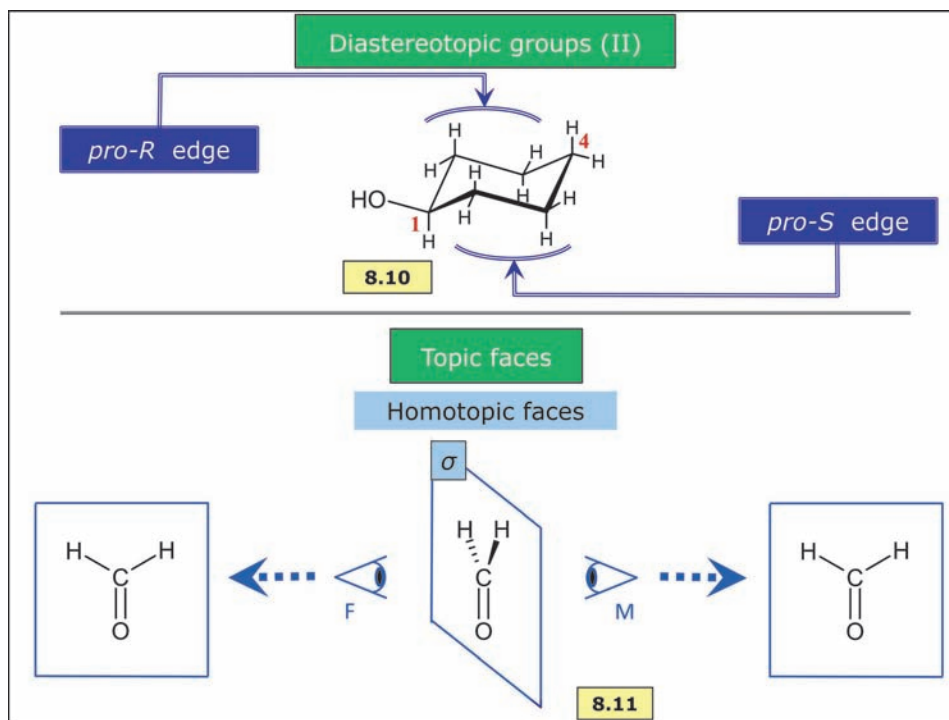
A center bearing enantiotopic groups is obviously achiral, but is at the same time quite different from a non-stereogenic center carrying equivalent groups. It nevertheless took insightful chemists to progressively discover and clarify the concept of prochirality and more generally prostereoisomerism [1–6][22–31]. This concept has now reached a very high level of significance not only in biochemistry as illustrated later, but also in organic synthesis and spectroscopy.



**Fig. 8.5.** *Diastereotopic fragments and groups* are differentiated from enantiotopic ones using the same three criteria presented above. Diastereotopic fragments reside in *diastereoisomeric environments*, cannot be interchanged by any *symmetry operation*, and, upon *replacement* by chiral or achiral groups, lead to diastereoisomeric structures.

The presence of enantiotopic groups in a molecule necessarily implies an element (*e.g.*, a center) of prochirality, whereas diastereotopic groups imply prostereoisomerism, either as an element of prochirality or of *proachirality*. In *chloroethene* (**8.7**), the two geminal H-atoms are *diastereotopic*; for example, their molecular environment is diastereoisomeric, and replacement of one H-atom affords the new compound in either the (*E*)- or (*Z*)-configuration. Note that no element of chirality exists in chloroethene. The two diastereotopic H-atoms are designated as *pro-E* and *pro-Z*, and the C-atom carrying the two H-atoms is a center of *proachirality*.

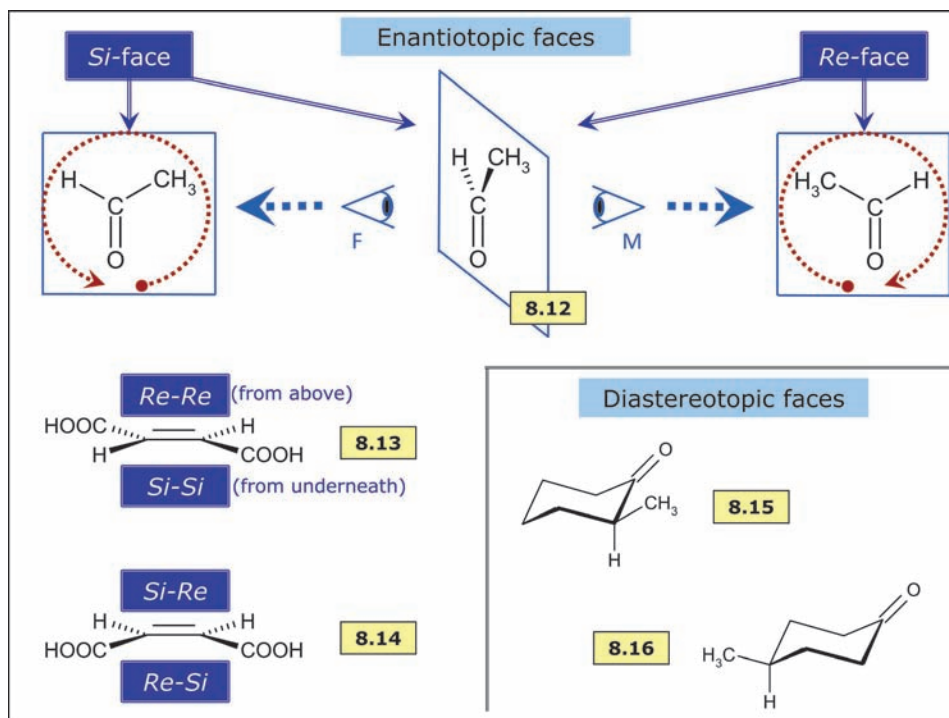
In (*2R*)-1-phenylpropan-2-ol (**8.8**), C(2) is a stereogenic center, whereas C(1) is a center of prochirality as it carries two *stereoheterotopic H-atoms*. Replacement of one of these by a <sup>2</sup>H-atom yields 1-phenyl[1-<sup>2</sup>H]propan-2-ol (**8.9**) and transforms C(1) into a stereogenic center. Due to the presence of the original stereogenic center C(2), compound **8.9** exists as two diastereoisomers, more precisely as a pair of *epimers*. In other words, the two H-atoms in compound **8.8** are indeed designated as *pro-R* and *pro-S*, and they are *diastereotopic groups* at a center of prochirality.



**Fig. 8.6.** Cyclohexanol (**8.10**) combines the concepts of prochirality and proachirality, thus affording an example more complex than the previous ones. Cyclohexanol is achiral, but it contains five centers of prochirality, namely C(1), C(2), C(3), C(5), and C(6). The two stereoheterotopic ligands at C(1) are the two edges of the ring. Substitution at one of the two C-atoms in the proximal edge confers the (*S*)-configuration to C(1), and this edge is, therefore, designated as the *pro-S edge*. The distal edge is thus the *pro-R edge*. But the edge C-atom undergoing substitution is also transformed into a stereogenic center, meaning that its two H-atoms are *diastereotopic*, as are the two edges of the ring. C(4) tells another story, because 4-substituted cyclohexanol derivatives are achiral due to their plane of symmetry cutting through C(1) and C(4). C(4) is thus a *proachirality* center and carries two *diastereotopic H-atoms*.

Intramolecular relationships can sometimes also be defined relative to *faces* instead of fragments or groups. For example, *formaldehyde* (**8.11**) shows the two faces characteristic of C=O groups. The *Figure* demonstrates that its two faces, as seen respectively by observers F and M, are indistinguishable and hence *homotopic*.

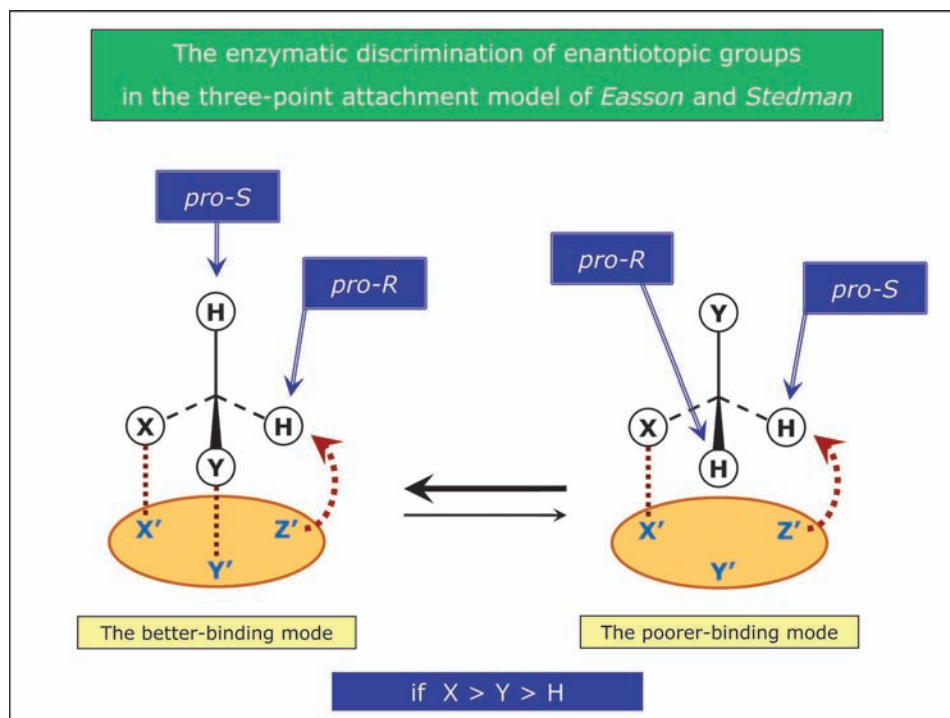




**Fig. 8.7.** The concept of prochirality can also be applied to trigonal centers, *i.e.*, to faces of planar moieties in suitable molecules. Here, we first consider *enantiotopic faces*. In acetaldehyde (**8.12**), for example, the two faces of the molecule are not equivalent but *enantiotopic*. Indeed, observer F sees the substituents on the trigonal center (O > Me > H) in a counterclockwise path, whereas observer M sees them clockwise. The face seen in a clockwise path is labeled as the *Re-face* (from the Latin *rectus*, right), and the other face is known as the *Si-face* (from the Latin *sinister*, left) [22–26].

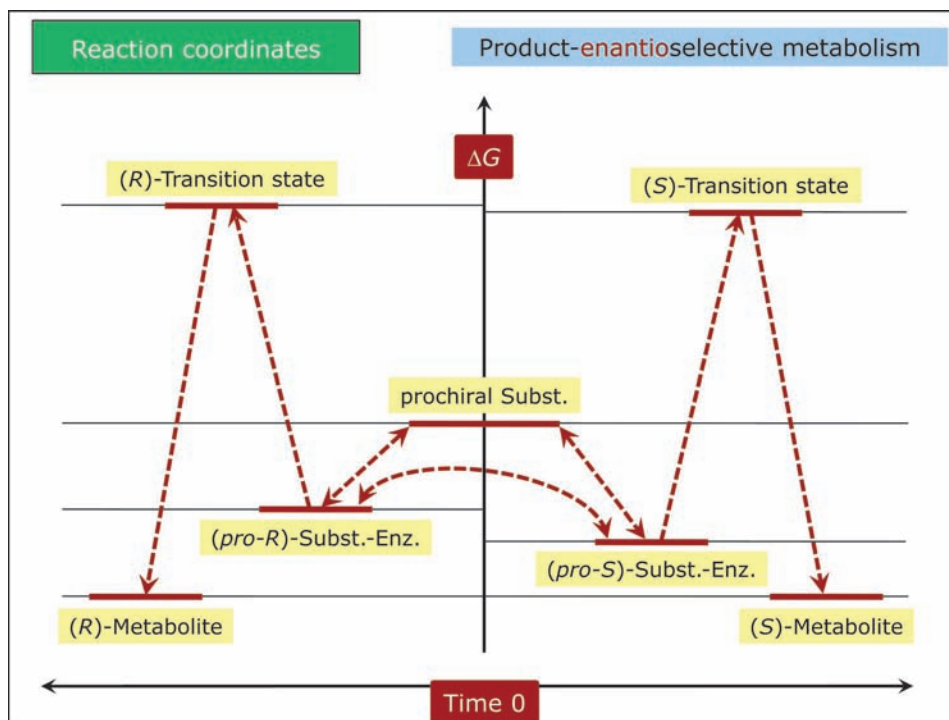
In the case of a C=C bond, the *Re* and *Si* convention is used separately for both ends. Thus, the (*E*)-configured fumaric acid (**8.13**) has a *Re-Re face* and a *Si-Si face*. In contrast, the (*Z*)-configured maleic acid (**8.14**) has two equivalent *Re-Si faces* due to the presence of a  $C_2$  axis in the plane of the molecule [31].

*Diastereotopic faces* also exist. While cyclohexanone itself has two equivalent faces, its monosubstituted derivatives such as **8.15** and **8.16** have two diastereotopic faces. This is exemplified by the chiral 2-methylcyclohexanone (**8.15**), where reduction of C(1) or any substitution at C(3), C(4), C(5), or C(6) creates a new stereogenic center and, hence, diastereoisomerism. 4-Methylcyclohexanone (**8.16**) presents a slightly more complicated example given that the molecule is achiral but, as we saw in Fig. 8.6 for cyclohexanol, any substitution at the edges creates two stereogenic centers and, hence, diastereoisomerism.



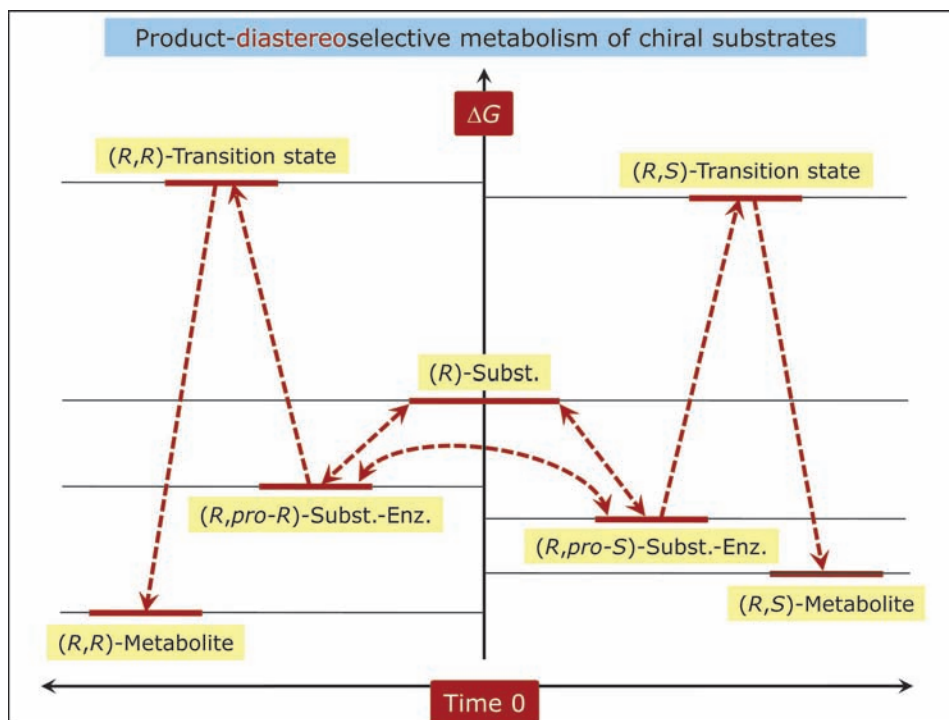
**Fig. 8.8.** The first section in *Part 5* was dedicated to conceptual models of chiral recognition in biology and pharmacology, with *Fig. 5.5* presenting the three-point attachment model of *Easson and Stedman* [32]. Interestingly, this schematic way of depicting the discrimination between two enantiomeric ligands can also be applied to explain the *discrimination between enantiotopic and, more generally, stereoheterotopic groups* in product-stereoselective enzymatic reactions.

This application is illustrated here with a *prochiral substrate* featuring two haptophoric groups X and Y, and two enantiotopic H-atoms as potential targets for attack by the reactive group Z' in the catalytic site. In our depiction, the substrate can bind to the enzyme with two or only one of the X and Y groups, interacting with the complementary functionalities X' and Y' in the enzymatic binding site. This results in *two binding modes* (i.e., two distinct enzyme–substrate complexes) whose fast *interconversion* is the rule, with one binding mode being preferred. The key point, however, is the fact that the two binding modes do not expose the same enantiotopic H-atom to catalytic attack (the red arrow). The energetically preferred binding mode exposes the *pro-R* H-atom, while the other mode exposes the *pro-S* H-atom.



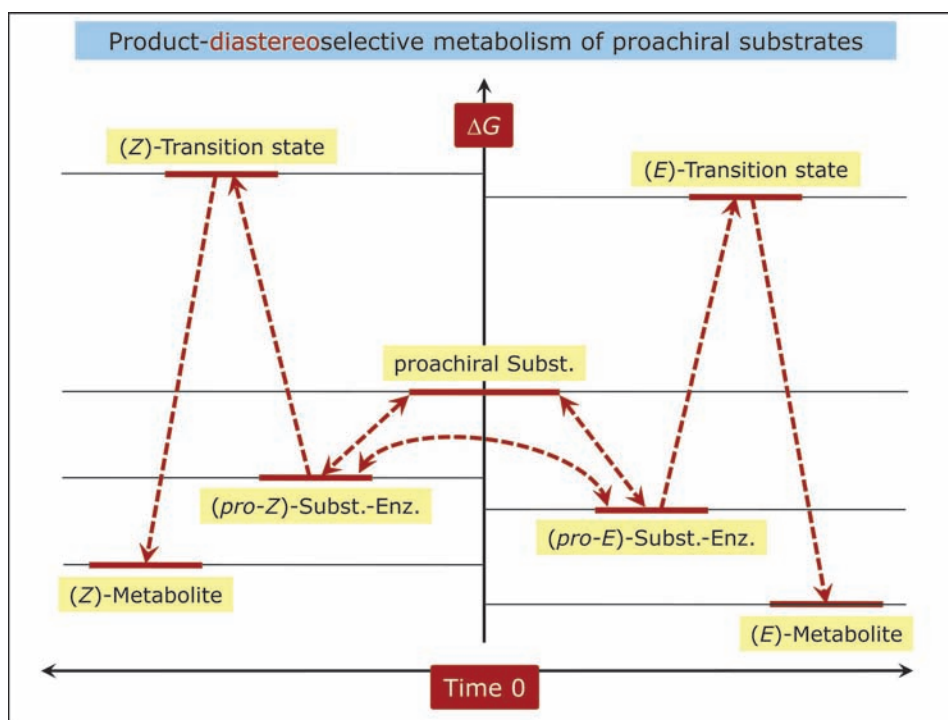
**Fig. 8.9.** In *Part 7*, we described substrate-stereoselective metabolism in terms of *reaction coordinates*. A similar *thermodynamic argument* allows insight into product-stereoselective reactions [19][33]. *Product-enantioselective reactions* result from the metabolic discrimination of enantiotopic groups in a single, prochiral substrate molecule (labeled here ‘*prochiral Subst.*’). This phenomenon is of classical occurrence in biochemistry, but was recognized only in 1948 when *Ogston* [34–37] accomplished a conceptual breakthrough by proposing that ‘*it is possible that an asymmetric enzyme which attacks a symmetrical compound can distinguish between its identical groups.*’ ‘*Identical groups*’ as understood by *Ogston* are now designated as enantiotopic groups.

The present *Figure* deals with a prochiral substrate that forms two distinct interconvertible enzyme–substrate complexes labeled as (*pro-R*)-*Subst.-Enz.* and (*pro-S*)-*Subst.-Enz.*, respectively. As discussed in the previous *Figure*, these two complexes have different energy levels (plotted as *Gibbs energy* = ‘free energy’ =  $\Delta G$ ), with the *pro-S* complex being arbitrarily assigned as the low-energy one. The two transient complexes then undergo the *catalytic step* and pass through *transition states* designated as (*R*)-*Transition state* and (*S*)-*Transition state*. The difference in energy between these two transition states is expected to be minute or vanishingly small, as it depends essentially on the activation energy of the same reaction occurring on two enantiotopic target groups whose sole difference is their very slightly different environments. The reaction ends with the formation of the enantiomeric (*R*)-*Metabolite* and (*S*)-*Metabolite*.



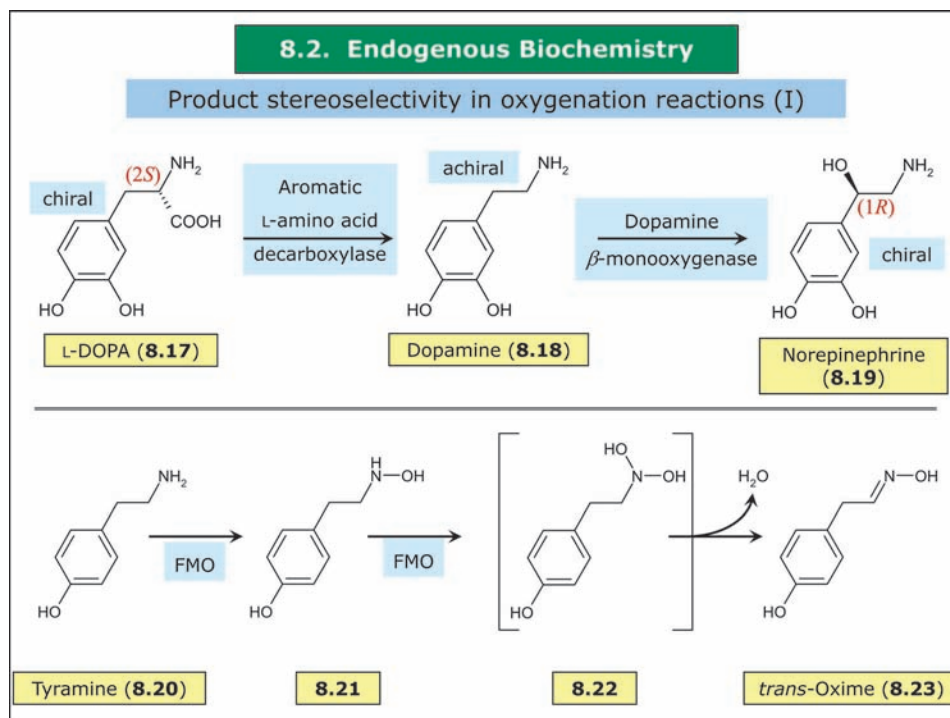
**Fig. 8.10.** A similar argument is applicable to the case of *product diastereoselectivity* examined here. It involves a substrate containing an element of chirality (e.g., a stereogenic center) and an element of prochirality such as two diastereotopic groups or faces. Furthermore, only one of the two enantiomers of the chiral substrate is under investigation, in our example the (*R*)-substrate. The case of two enantiomers, each producing two epimeric metabolites, is one of substrate–product stereoselectivity to be discussed later.

Again two binding modes are assumed in this *Figure*, each exposing one of the two diastereotopic groups or faces to enzymatic attack. The two complexes are thus labeled as (*R,pro-R*)-*Subst.-Enz.* and (*R,pro-S*)-*Subst.-Enz.*, the latter being arbitrarily defined here as the lower-energy one. The enzymatic reaction then reaches the corresponding transition states (*R,R*)-*Transition state* and (*R,S*)-*Transition state*, with the latter being arbitrarily assumed to be of slightly lower energy. These transition states then evolve toward the final, *diastereoisomeric* (*R,R*)-*Metabolite* and (*R,S*)-*Metabolite*, respectively.



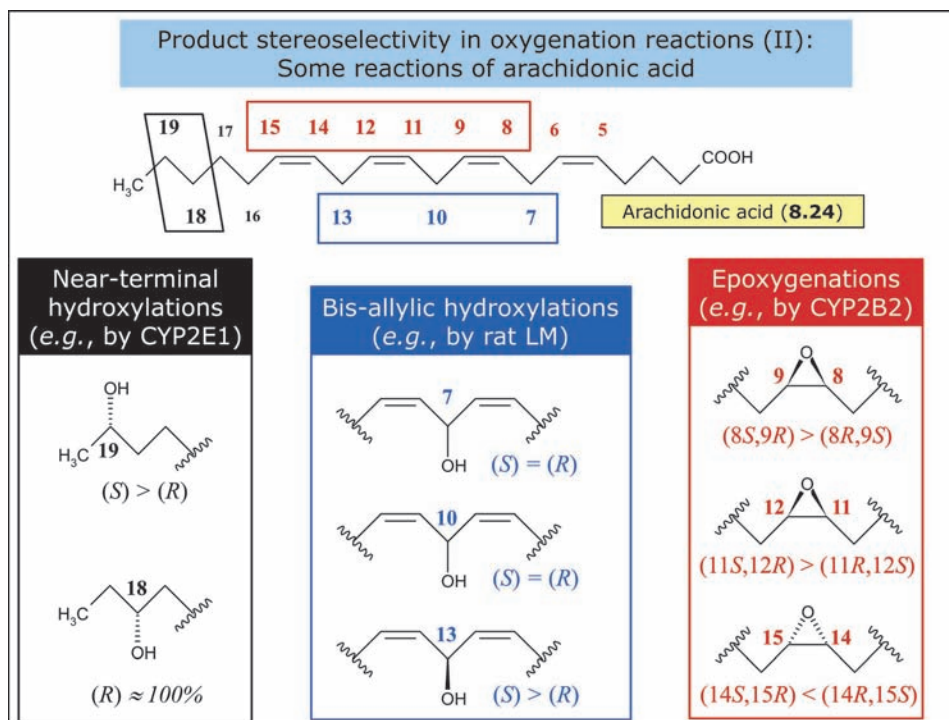
**Fig. 8.11.** *Prochiral substrates* present a case of product diastereoselectivity different from the one schematized in the previous *Figure*. Here, we encounter the case of substrates that contain *two diastereotopic groups* as enzymatic targets. As discussed earlier, these two groups are labeled as *pro-E* and *pro-Z*.

Assuming two binding modes, two substrate-enzyme complexes can be formed and are labeled here as *(pro-Z)-Subst.-Enz.* and *(pro-E)-Subst.-Enz.*, respectively. As in the previous examples, these two complexes have somewhat different energy levels, as have the subsequent *(E)-Transition state* and *(Z)-Transition state*. The final products are diastereoisomers, namely the *(Z)-Metabolite* and the *(E)-Metabolite*. Reasonably, the latter is considered to be the lower-energy one.



**Fig. 8.12.** Moving to the *biochemistry of endogenous compounds* [38], we will examine a few selected examples of product stereoselectivity in oxygenation and reduction reactions. L-Dihydroxyphenylalanine (L-DOPA; **8.17**) is the immediate precursor of the essential neurotransmitter *dopamine* (**8.18**), itself a precursor of noradrenaline (*norepinephrine*; **8.19**). L-DOPA is also a major drug in treating symptoms of *Parkinson's* disease. We note in passing that the decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase (EC 4.1.1.28) is *substrate-enantioselective* [39][40]. The resulting product, dopamine (**8.18**), is achiral but contains two centers of prochirality, namely C(1) and C(2). The former is the target considered here, as its hydroxylation by *dopamine β-monoxygenase* (EC 1.14.17.1) is highly *product-enantioselective* in forming the physiological (1*R*)-noradrenaline [41].

The second example again involves an achiral substrate oxidized with almost complete product diastereoselectivity to one of two possible (*E*)- and (*Z*)-diastereoisomers. *Tyramine* (**8.20**) is an important biological amine inactivated among other reactions by *N*-oxygenation catalyzed by the *flavin-containing monoxygenase* (FMO; EC 1.14.13.8) [42]. When incubated with human liver microsomes [43], **8.20** underwent two consecutive FMO-catalyzed reactions of *N*-hydroxylation to form first the hydroxylamine **8.21**, then the intermediate *N,N*-dihydroxyamine **8.22**. The latter is unstable and dehydrates rapidly; but under abiotic conditions this reaction would lead to a mixture of the *cis*- and *trans*-oxime. The fact that only the *trans*-oxime **8.23** was produced was a determining evidence to indicate that the oxime was formed prior to enzyme–product dissociation, *i.e.*, steric constraints within the enzymatic cavity forced the formation of only *trans*-oxime.

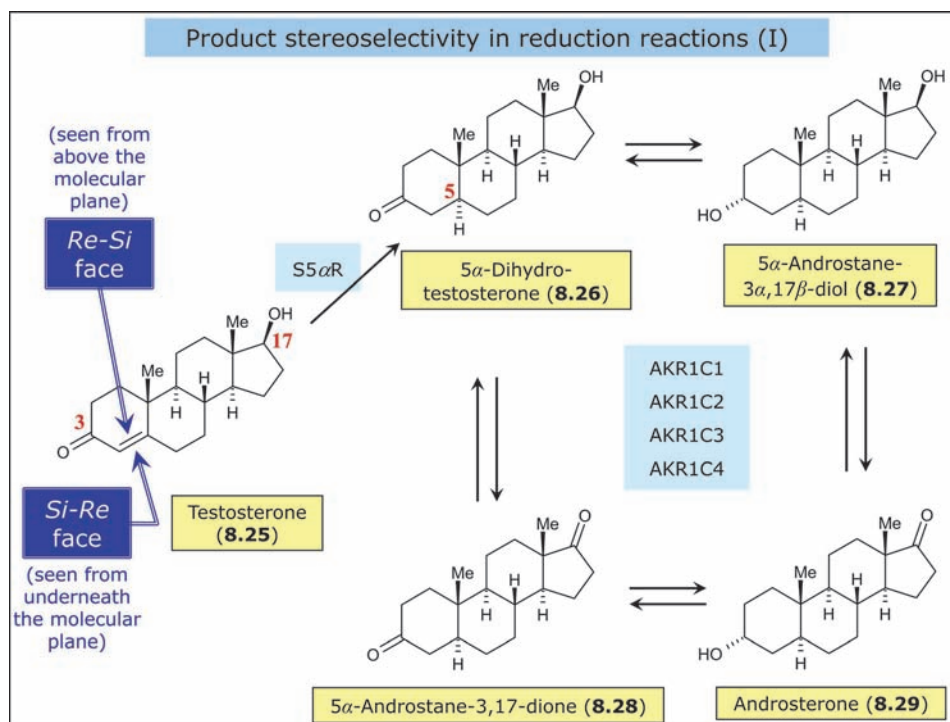


**Fig. 8.13.** Arachidonic acid (AA; (5Z,8Z,11Z,14Z)-icoso-5,8,11,14-tetraenoic acid; **8.24**) is an important ( $\omega-6$ )-polyunsaturated fatty acid and the substrate of a variety of biotransformation reactions to form some physiologically important metabolites. Here, we focus on *cytochrome P450-catalyzed monooxygenations* and have selected a few examples to emphasize the diversity of its metabolism [44]. Three major types of CYP-catalyzed oxidations are known and will be discussed in turn, namely hydroxylations at the terminal C(16)- to C(20)-atoms, hydroxylations at the three bis-allylic positions C(7), C(10), and C(13), and epoxidations of the four C=C bonds.

*Hydroxylations at C(16)–C(20)* are highly regioselective for the various individual CYPs investigated [45] [46]. Since C(20) is not a center of prochirality, its hydroxylation will not be considered here. The three other positions can all undergo hydroxylation, and it was shown, for example, that CYP2E1 is *product-regioselective* for C(18) and C(19), with complete *product enantioselectivity* at C(18) and partial product enantioselectivity at C(19).

*Hydroxylations at the bis-allylic positions* are more complex, since the resulting metabolites are rearranged by C=C bond and OH-group migrations to form *dienols* (i.e.,  $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}(\text{OH})-$ ) [44]. Some investigations have nevertheless succeeded in determining the absolute configuration of the initial metabolites. Thus, induced rat liver microsomes (which are rich in CYP2B and CYP2C) showed a modest product enantioselectivity in 13-hydroxylation, but none in 7- and 10-hydroxylations [47].

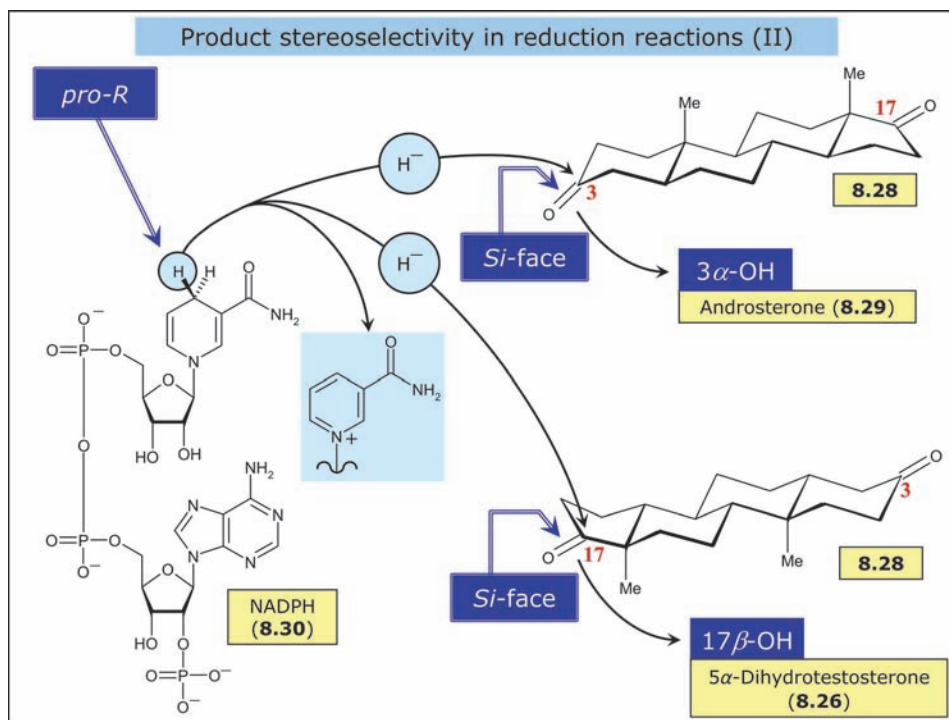
The third major oxidation reaction undergone by AA is the *epoxidation of its C=C bonds*. Again, there is a CYP-dependent product regioselectivity (low 5,6-epoxidation) plus product enantioselectivity, as exemplified here for the case with CYP2B2 [44][48].



**Fig. 8.14.** Steroid hormones offer valuable examples of product stereoselectivity, particularly in their reactions of reduction as illustrated here. Thus, the active male hormone *testosterone* (8.25) contains two double bonds, the keto group at C(3) and the  $\Delta^{4,5}$ -bond, both of which can be reduced. The *reduction of the C=C bond* is catalyzed by *steroid 5 $\alpha$ -reductase* (EC 1.3.99.5) in a highly *product-diastereoselective* reaction which generates the more potent *5 $\alpha$ -dihydrotestosterone* (8.26) [49–53]. This reaction involves an attack by the hydride anion (see next Fig.) on C(5) from underneath the molecular plane.

*5 $\alpha$ -Dihydrotestosterone* (8.26) in turn is a substrate of reductions (hydrogenations) and oxidations (dehydrogenations) at its 3-oxo and 17 $\beta$ -OH groups, respectively. These reactions yield *5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol* (androstane diol; 8.27), *5 $\alpha$ -androstane-3,17-dione* (androstane dione; 8.28), and later *3 $\alpha$ -hydroxy-5 $\alpha$ -androstane-17-one* (androsterone; 8.29). The reader will have noted that the hydrogenation reactions create a new stereogenic center and are thus *product-diastereoselective*, whereas dehydrogenation reactions are *substrate-diastereoselective*. These reactions are catalyzed by enzymes in the *aldo-keto reductase superfamily* (AKRs) [53–56], specifically by human AKR1C1 (EC 1.1.1.149), AKR1C2 (EC 1.1.1.213), AKR1C3 (EC 1.1.1.239), and AKR1C4 (EC 1.1.1.50). These enzymes use *NADPH* or *NADH* as the hydride donor; the first three are *A-specific*, while the last is *B-specific* (see next Fig.).



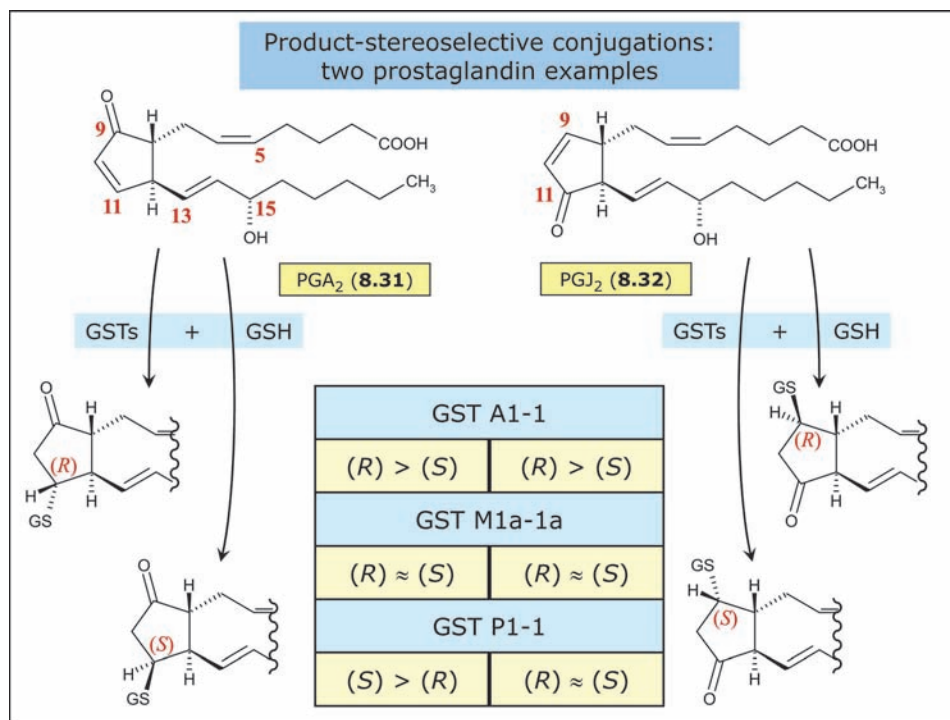


**Fig. 8.15.** This *Figure* outlines the mechanism of product-stereoselective *ketosteroid reduction* by AKRs. Their cofactor *NADPH* (8.30) is shown here with emphasis on its (reduced) dihydronicotinamido moiety. Inspection of its two H-atoms at C(4) reveals that they are enantiotopic, or more accurately *diastereotopic*, given other stereogenic centers in this complex molecule. Keto reduction involves the transfer of one of these two H-atoms as a *hydride anion* ( $H^-$ ), leaving behind an oxidized nicotinamido moiety with a positive charge.

AKR1C1 – AKR1C3 transfer the *pro-R* *H-atom* and are called *A-specific*, whereas AKR1C4 transfers the *pro-S* *H-atom* and is called *B-specific* [54–57]. The carbonyl C-atom is the electrophilic target of H-transfer; the negative charge ends up at the O-atom (now an alcoholate O-atom) which is neutralized by a proton supplied by the medium.

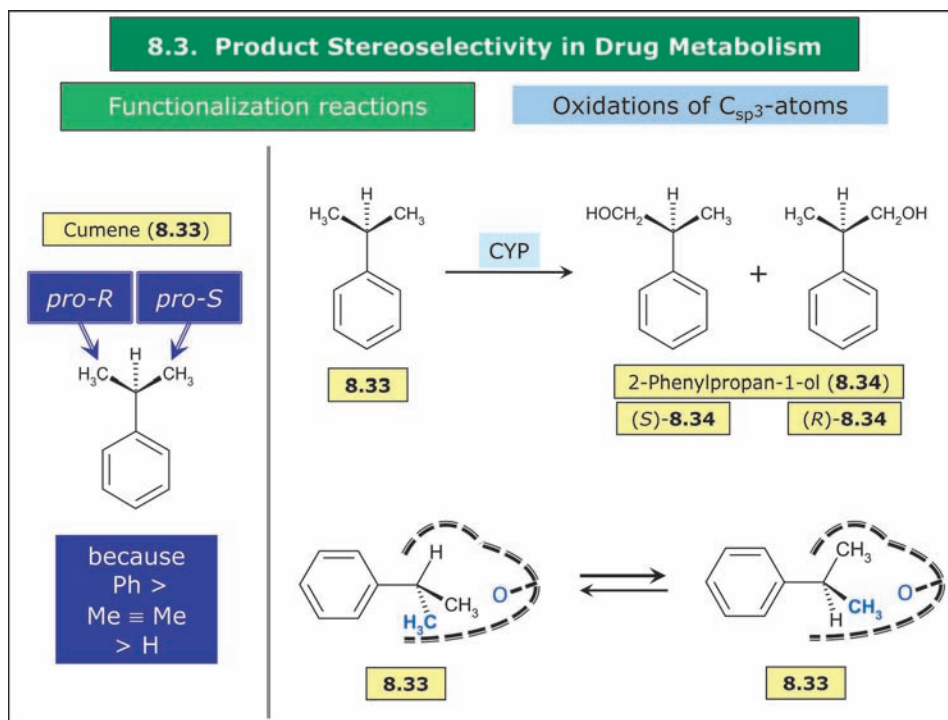
As we saw in the previous *Figure*, *androstenedione* (8.28) can be reduced to androsterone (8.29) by  $3\alpha$ -reduction, or to dihydrotestosterone (8.26) by  $17\beta$ -reduction. This implies *two distinct binding modes* in the enzymatic cavity, as shown. These bring either the C(3)=O or the C(17)=O group in close proximity of the dihydronicotinamide moiety of NADPH, with the *Si-face* exposed to attack by the  $H^-$  anion.

While the details of dehydrogenation reactions mediated by these enzymes are outside our scope, we simply note that the above reaction is reversible and that  $NAD(P)^+$  pulls out a  $H^-$  anion from the C-atom carrying the OH group.



**Fig. 8.16.** Clear cases of product stereoselectivity in the conjugation of endogenous compounds are comparatively rare in the literature. One example is the addition of the tripeptide *glutathione* (GSH) to *stereoheteropic faces* in  $\alpha,\beta$ -unsaturated carbonyl compounds, a reaction catalyzed by *glutathione S-transferases* (GSTs) [58].

*Prostaglandin A<sub>2</sub>* (PGA<sub>2</sub>; **8.31**) and *prostaglandin J<sub>2</sub>* (PGJ<sub>2</sub>; **8.32**) contain such a reactive  $\alpha,\beta$ -unsaturated C=O moiety which not only is a target of GSH conjugation but also accounts for the capacity of these compounds to inhibit cell proliferation. Various purified GST isozymes of human origin, among others GST A1-1, GST M1a-1a, and GST P1-1, were shown to catalyze the addition of GSH at C(11) for **8.31** and at C(9) for **8.32**. A new stereogenic center is thus created in the target molecule, with some markedly different product diastereoselectivities between the three enzymes [59].



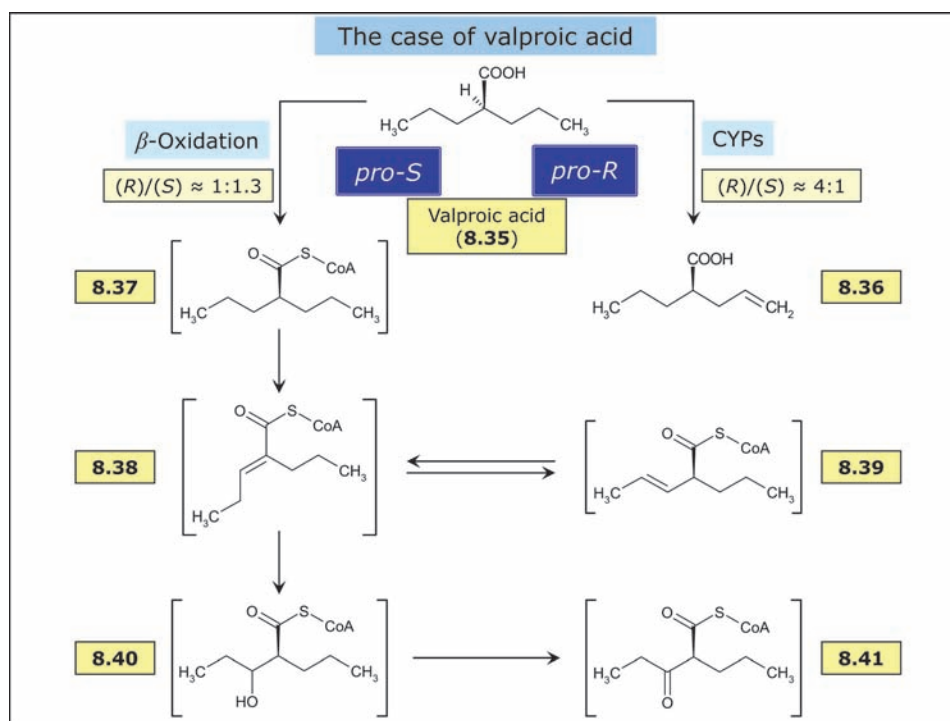
**Fig. 8.17.** We now move from endogenous biochemistry to *xenobiotic metabolism* and begin with functionalization reactions (redox reactions and hydrolyses). A particularly apt and instructive example is the cytochrome P450-catalyzed methyl hydroxylation of *cumene* (2-phenylpropane; **8.33**) [60]. This molecule is prochiral as it contains the motif Ph > Me ≡ Me > H, with the two Me groups being the target of the investigated hydroxylation reaction. The resulting metabolite is 2-phenylpropan-1-ol (**8.34**).

Our first task is to define the *pro-R* and *pro-S* groups in the substrate molecule, keeping in mind the IUPAC definition: ‘A stereoheterotopic group *c* (as in tetrahedral *Xabcc*) is described as *pro-R* if, when it is arbitrarily assigned CIP priority over the other stereoheterotopic group *c*, the configuration of the thus generated [stereogenic] centre is assigned the stereodescriptor R. The other group *c* is then described as *pro-S*’ [22][23]. This definition leads to the assignment shown on the left hand side of the Figure; the original publication did not follow this definition.

Using induced rat liver microsomes rich in CYP2B and CYP2C, there was a *ca.* twofold preference for the formation of (*R*)-2-phenylpropan-1-ol. The reader will have noted that the formation of (*R*)-2-phenylpropan-1-ol results from the *hydroxylation of the pro-S Me group of cumene*. This apparent discrepancy is due to the fact that hydroxylation does more than give preference to one previously enantiotopic group over the other, it also alters the relative priority of the Ph group, the priority sequence now being CH<sub>2</sub>OH > Ph > Me > H.

There is yet more to learn from this study, since the use of carefully <sup>2</sup>H-labeled substrates also led to a determination of the *equilibrium constant between the two binding modes* (i.e., the two substrate–enzyme complexes) schematized in the lower

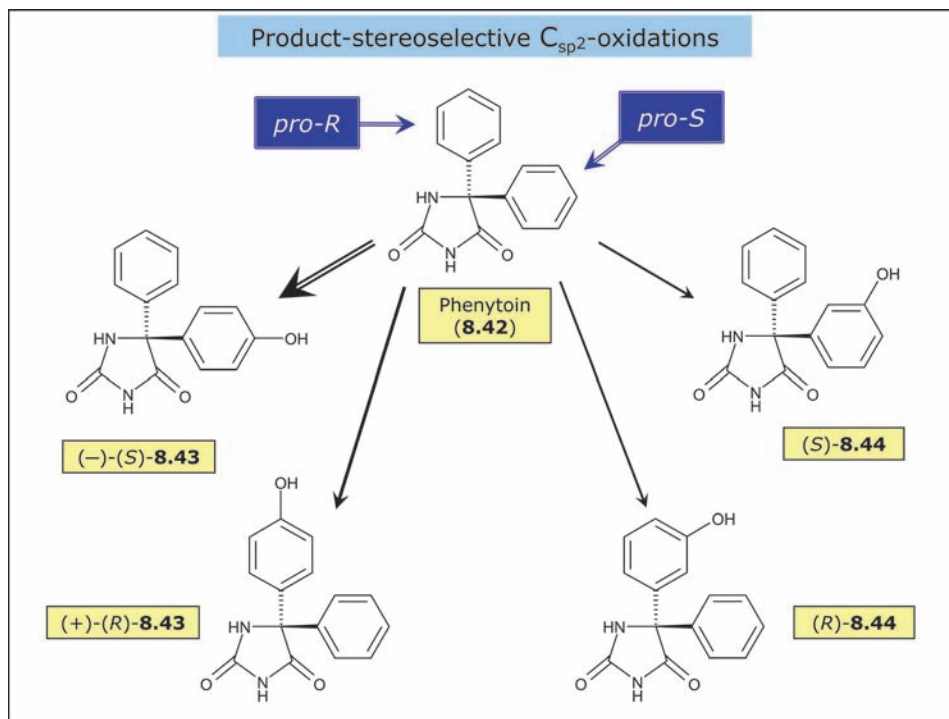
right part of the *Figure*. Under the same biological conditions as above, there was again an approximately twofold preference for positioning the preferred target in closer proximity to the catalytic group, implying that the product enantioselectivity of the reaction had its origin in the relative energies of the binding modes of the substrate, as suggested in *Fig. 8.9* [60].



**Fig. 8.18.** The anti-epileptic drug *valproic acid* (VPA; **8.35**), despite its apparent structural simplicity, undergoes a wealth of metabolic reactions several of which have marked toxicological significance [61]. The compound features two enantiotopic propyl moieties which are the target of *two oxidation pathways* differing both in the enzymatic systems involved and in their product enantioselectivity [62–64]. We summarize here results obtained with carefully labeled VPA substrates incubated with primary cultures of rat hepatocytes where both pathways are present and active [62].

On the one hand, a number of cytochromes P450 have been shown to catalyze C(4),C(5)-didehydrogenation, the reaction occurring with a marked preference for the *pro-R propyl group* to yield preferentially the reactive and hepatotoxic (*R*)-*4-ene-VPA* (**8.36**). On the other hand, VPA (**8.35**) is recognized by mitochondria as a fatty acid and undergoes *β-oxidation*, the pathway of physiological chain-shortening of fatty acids by sequential removal of  $C_2$  units [58]. The reaction begins with the formation of a *VPA-Coenzyme A conjugate* **8.37**, followed by C(2),C(3)-didehydrogenation to (*E*)-*2-ene-VPA-CoA* (**8.38**). The latter is in equilibrium with (*E*)-*3-ene-VPA-CoA* (**8.39**) and undergoes C=C bond hydration to *3-hydroxy-VPA-CoA* (**8.40**), itself a precursor of *3-*

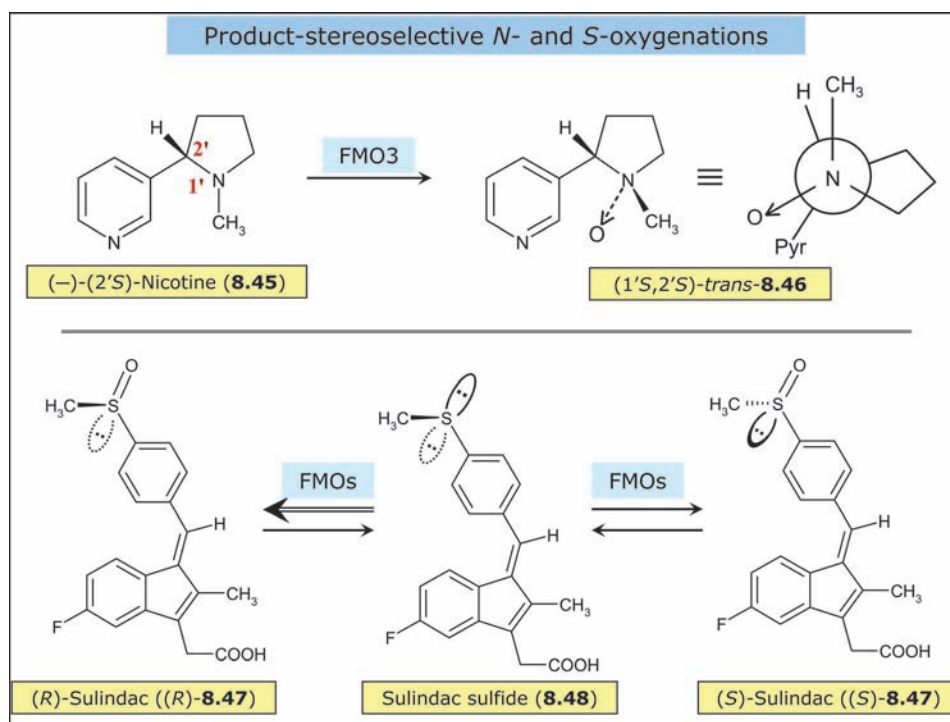
*oxo-VPA-CoA* (**8.41**). When isolated after hydrolytic removal of coenzyme A and extraction, the four metabolites **8.38**, **8.39**, **8.40**, and **8.41** in deconjugated form showed a modest product enantioselectivity toward the *pro-S* *propyl* group. This was consistent with the four metabolites sharing a common metabolic origin, the low level of enantioselectivity being explained by a partial isomerization accompanying the  $\mathbf{8.38} \rightleftharpoons \mathbf{8.39}$  equilibrium [62].



**Fig. 8.19.** The anti-epileptic drug *phenytoin* (**8.42**) offers a rich example of *product-enantioselective aryl oxidation*. The molecule is prochiral as its C(5) carries two enantiotopic Ph rings. Its major metabolic pathways are oxidations of these phenyl rings to yield dihydrodiols (which will not be considered here) and two phenols, namely 5-(4-hydroxyphenyl)-5-phenylhydantoin (4'-HPPH; **8.43**) and 5-(3-hydroxyphenyl)-5-phenylhydantoin (3'-HPPH; **8.44**) [65–67]. The amounts of 4'-HPPH produced in humans administered phenytoin predominated severalfold over those of 3'-HPPH, and comparable results were obtained in incubations with human liver microsomes [67]. The formation of 4'-HPPH is catalyzed by cytochromes P450 belonging to the CYP2C subfamily, but these enzymes do not appear to be involved in the formation of 3'-HPPH.

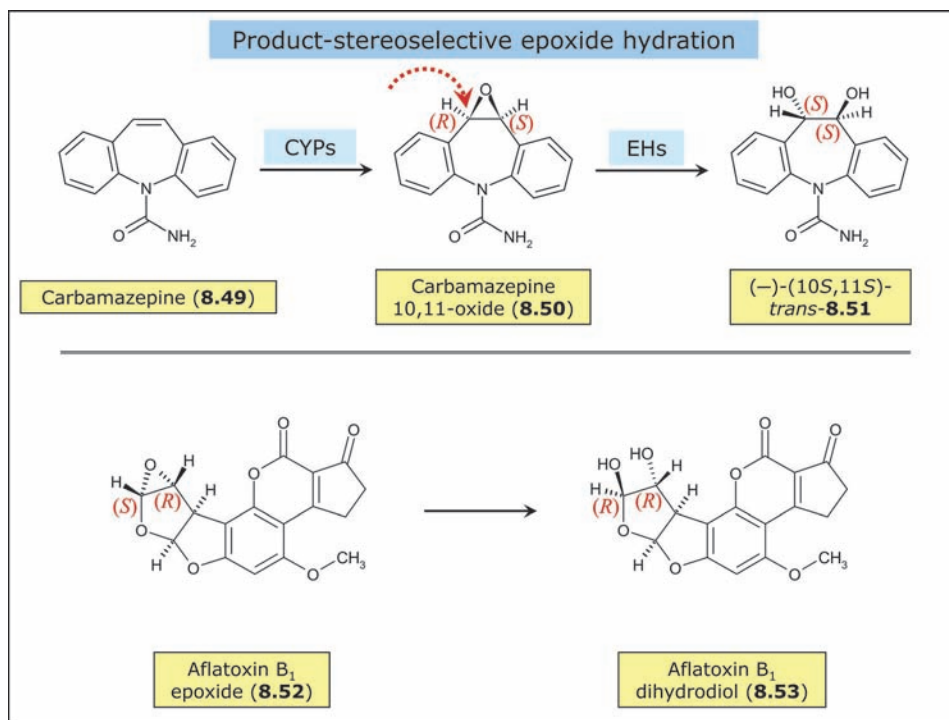
Beside this *product regioselectivity*, phenytoin (**8.42**) is also well-known to exhibit *product enantioselectivity* in the reaction of 4'-hydroxylation, but not in 3'-hydroxylation. Indeed, depending on conditions, the (–)-(S)-4'-HPPH metabolite predomi-

nated by a factor of 2 or markedly more over its enantiomer, as indicated by the relative thickness of the arrows in the *Figure*. CYP2C9 is the enzyme accounting for this preference in humans [66][67]. In contrast to 4'-HPPH, there was no apparent enantioselectivity in the production of 3'-HPPH.



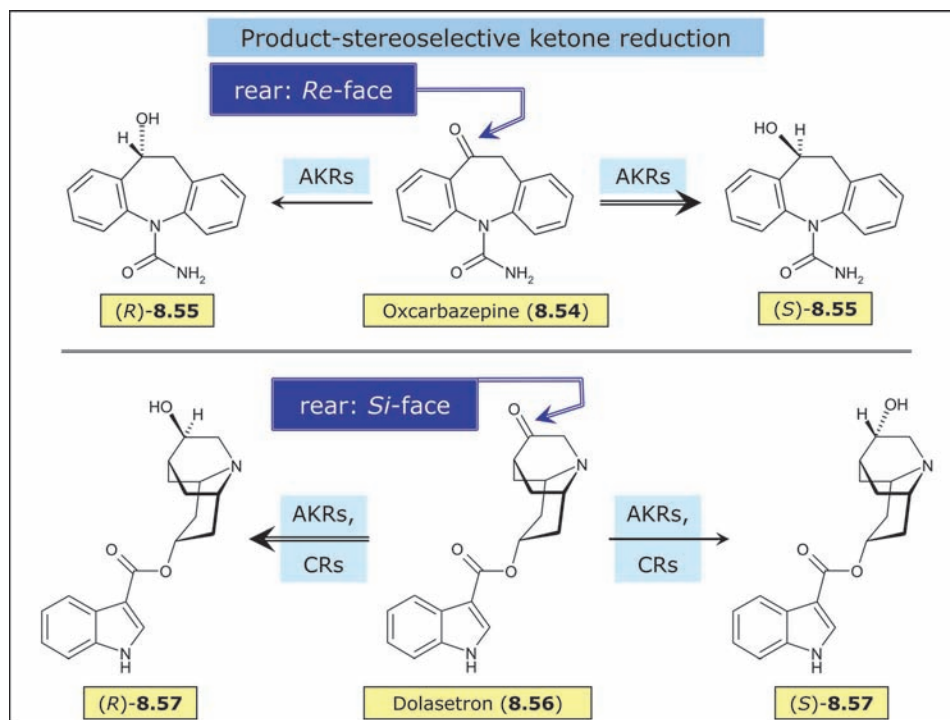
**Fig. 8.20.** Oxygenation reactions of tertiary amines and sulfides are catalyzed by flavin-containing monooxygenases (FMOs) or CYPs. When the necessary structural conditions are met in the substrates, a new and stable stereogenic center (see *Part 2*) is created, and two enantiomers or two epimers are produced. For example, the natural (-)-(2'S)-nicotine (8.45) undergoes *N*-oxygenation as a significant pathway [68]. The reaction is catalyzed mainly by FMO3 in humans and shows product stereoselectivity with a high, if not exclusive, formation of (1'S,2'S)-trans-nicotine *N*-oxide (8.46). The product stereoselectivity appears to be less marked in other species or under the catalysis of other FMOs.

Many *prochiral thioethers*, mostly model compounds, have been investigated for the product stereoselectivity of their *S*-oxygenation [69]. A medically relevant example of *S*-oxygenation is provided by *sulindac sulfide* (8.48), a metabolite of the anti-inflammatory drug *sulindac* (8.47). This latter is a chiral sulfoxide and is used as the racemate, and one of its routes of biotransformation is reduction to sulindac sulfide. What interests us here is the *re-oxygenation of the S-atom* to yield sulindac. The reaction is catalyzed by FMOs and shows a marked product enantioselectivity toward (*R*)-sulindac [70].



**Fig. 8.21.** Carbamazepine (**8.49**) is a major anti-epileptic drug which gives rise to well over 30 metabolites [71][72]. Thus, the symmetrical meso-10,11-epoxide **8.50** and the 10,11-dihydrodiol **8.51** are urinary metabolites in humans and rats given the drug. Epoxide hydrolases (EHs; EC 3.3.2.9 and 3.3.2.10) open the oxirane ring by adding a molecule of H<sub>2</sub>O to the molecule, and mechanistic investigations have shown the enzymatic attack to occur from the rear on the (*R*)-configured C-atom of the epoxide. As a result, the dihydrodiol is *dissymmetric*, with the (-)-(10*S*,11*S*)-trans-isomer predominating over its (10*R*,11*R*)-enantiomer. Since the pharmacologically active 10,11-epoxide is suspected to contribute to unwanted effects, the EH-catalyzed hydration of the epoxide is as a reaction of detoxification.

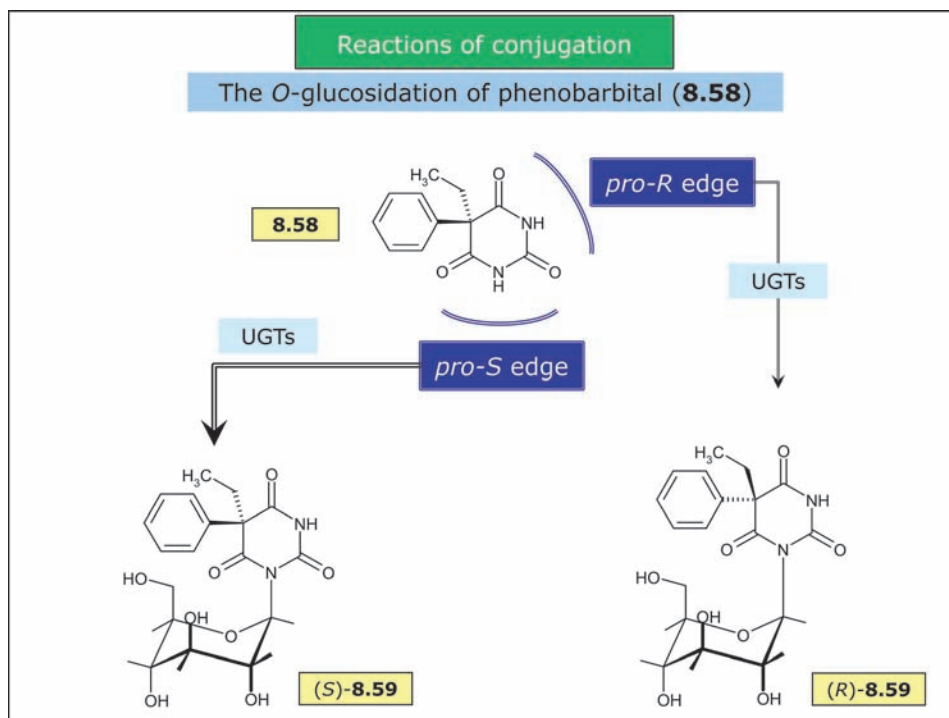
Moving to *toxic compounds*, we encounter the infamous aflatoxin B<sub>1</sub>, a mycotoxin considered to be a major cause of human liver cancer in some parts of the world [73][74]. Oxidation of aflatoxin B<sub>1</sub> at the C(8)=C(9) bond, mainly by CYP3A4, produces the *exo*-8,9-epoxide **8.52** as a major metabolite, and the *endo*-8,9-epoxide as a minor one. In contrast to its unreactive and nontoxic *endo*-diastereoisomer, the *exo*-8,9-epoxide is *highly reactive and genotoxic*. It reacts extremely rapidly with H<sub>2</sub>O by proton-catalyzed and H<sub>2</sub>O-catalyzed hydrolysis, yielding the (8*R*,9*R*)-dihydrodiol **8.53** as the predominant product. Thus, aflatoxin B<sub>1</sub> *exo*-8,9-epoxide is possibly the most reactive oxirane of biological relevance, so reactive in fact that EH does not seem to play a role in its hydration. What we can conclude from epoxide hydrolysis is that its stereochemical outcome is determined by both the 3D structure of the substrate and the reaction mechanism, namely enzymatic or nonenzymatic [75].



**Fig. 8.22.** A number of drugs and other xenobiotics contain a C=O group, and numerous data have been accumulated on their bioreduction by *carbonyl reductases* (CRs; EC 1.1.1.184) and *aldo-keto reductases* (AKRs; see Fig. 8.14), including aldehyde reductases [76–78]. The potent anti-epileptic drug *oxcarbazepine* (8.54) is a close analog of carbamazepine (8.49). Its main route of metabolism in humans is C=O reduction by cytosolic AKRs to the *biologically active* monohydroxy derivative 8.55. A marked product enantioselectivity occurs in humans, with the (*S*)-configured *alcohol* predominating severalfold over its (*R*)-enantiomer [79]. This implies that the H<sup>-</sup> anion transferred from NAD(P)H attacks the C=O group from its *Re-face* (*i.e.*, from the rear in the Fig.). This enantioselectivity, however, is without pharmacological impact, as both enantiomers of 8.55 are equally active.

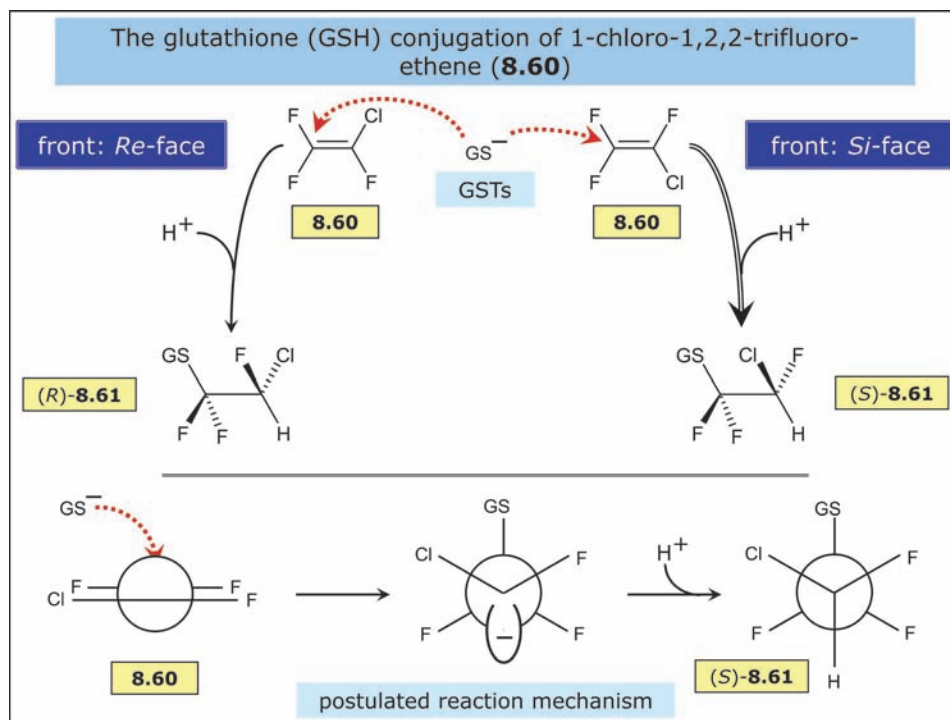
Our next example is the anti-emetic 5-HT<sub>3</sub> receptor antagonist *dolasetron* (8.56), which, among a variety of biotransformations, is rapidly and extensively reduced to the alcohol 8.57, with almost exclusive production of the (*R*)-configured *metabolite* in humans, or when incubated with various human AKRs and CRs [80–82]. In other words, the H<sup>-</sup> anion preferentially attacks the C=O group from its *Si-face* (*i.e.*, again from the rear in the Fig.). Note that reduced dolasetron 8.57 proved to be *ca.* 40 times more active than its parent drug, which is thus almost a prodrug.





**Fig. 8.23.** The literature also contains a limited number of product-stereoselective *conjugation reactions*, two of which are presented in this and the following *Figure*. An unusual conjugation is that of *O- or N-glucosidation*, a reaction catalyzed by *UDP-glucuronosyltransferases* (UGTs) in which glucose rather than glucuronic acid is the coupling agent [58]. A number of barbiturates have been shown to be substrates of *N-glucosidation* [83–86], undergoing only minimal or marginal *N-glucuronidation*. The reaction is of marked interest in our context, as the two target N-atoms in barbiturates are present on *enantiotopic edges*. For example, hexobarbital (see *Fig. 8.27*) is an *N*-methylbarbiturate and, therefore, occurs as two enantiomers.

A case in point is *phenobarbital* (**8.58**), whose conjugation to the *N-glucosides* **8.59** showed a strong and consistent preference for the *pro-S edge* under a number of different biological conditions [83–85]. The C(5)-atom of phenobarbital is rendered chiral by *N-glucosidation*, but the two resulting *N-glucosides* are *epimers* rather than enantiomers given the chirality of glucose (five stereogenic centers).



**Fig. 8.24.** There are a number of examples of substrate enantioselectivity in *glutathione* (GSH) conjugations catalyzed by *glutathione S-transferases* (GSTs), but only few instances of product stereoselectivity are well-documented [58]. Here, we have selected the example of the industrial xenobiotic *1-chloro-2,2,2-trifluoroethene* (**8.60**) to illustrate the creation of a stereogenic center during a reaction of glutathione addition to an *haloalkene* [87]. Since the tripeptide glutathione ( $\gamma$ -Glu-Cys-Gly) is itself chiral, two stereoisomers produced by the reaction will be *epimers*.

In incubations with *microsomal* (*i.e.*, membrane-bound) GSTs [88], (*2-chloro-1,1,2-trifluoroethyl*)glutathione (**8.61**) was produced with a marked product stereoselectivity favoring the *(S)*-configured conjugate. This was explained by the glutathionyl anion attacking the molecule regioselectively at its  $\text{CF}_2$  group and stereoselectively from its *Si*-face. This initial reaction of addition led to the formation of an *intermediate carbanion* whose protonation to form **8.61** was assumed to be facilitated by the enzyme in order to occur before carbanion inversion. The regioselectivity and stereoselectivity of the reaction were tentatively explained by the orientation of the substrate in the catalytic site. Interestingly, the two enantiomers of **8.61** were produced in equal proportions, when *cytosolic GSTs* were used to catalyze the same reaction, implying less constraints in the binding mode of the substrate in these soluble enzymes.

8.4. Substrate–Product Stereoselectivity in Drug Metabolism	
Main cases of metabolic stereoselectivities	
Case 1: (R) and (S) → achiral	If (R) ≠ (S) : Substrate enantioselectivities
Case 2: (R) → (R) and (S) → (S)	
Case 3: Prochiral → (R) + (S)	If (R) ≠ (S) : Product enantioselectivity
Case 4: (R) → (S) + (R) and (S) → (R) + (S)	If (R) ≠ (S) : Substrate–product enantioselectivity
Case 5: (R) → (R,R) + (R,S) and (S) → (S,S) + (S,R)	If (R) ≠ (S) : Substrate–product stereoselectivity

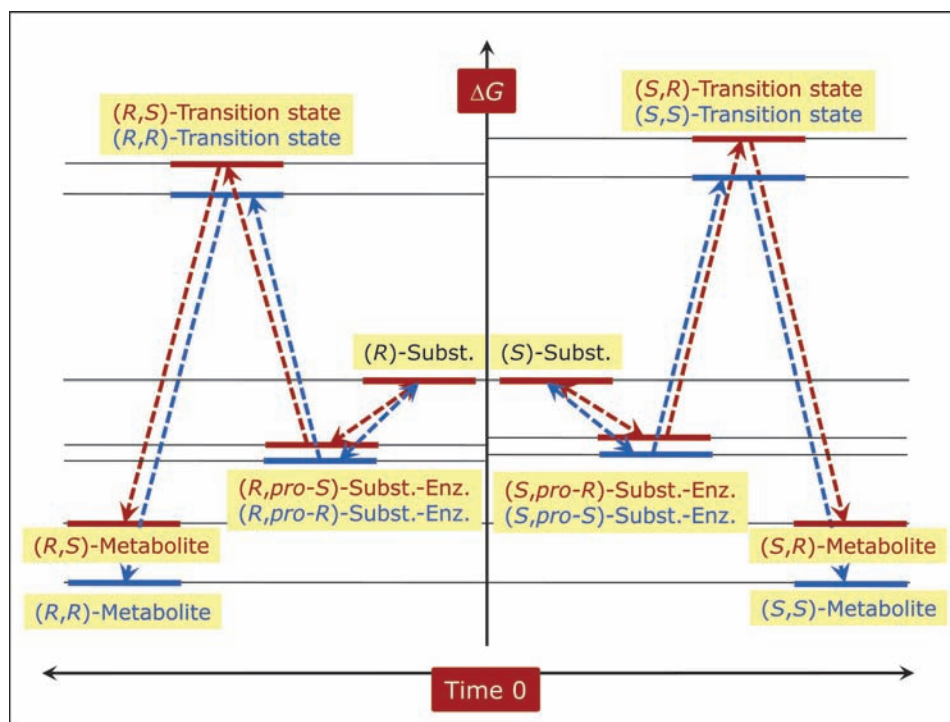
**Fig. 8.25.** This last *Section* deals with stereochemical issues encountered regularly in the drug-metabolism literature. Specifically, we present and illustrate here the concept of *substrate–product stereoselectivity* in the metabolism of foreign compounds, namely the fact that the *outcome of a product-stereoselective reaction may differ between two stereoisomeric substrates*. To facilitate understanding, the *main cases of metabolic stereoselectivities* are compiled (left panel) and *classified* (right panel), with the symbols '(R) ≠ (S)' meaning '(R) and (S) reacting differently or being formed at different rates'.

*Case 1* is the straightforward loss of chirality. *Case 2* occurs when the metabolic reaction leaves the stereogenic center untouched. *Case 3* is the classical product enantioselectivity whereby a prochiral substrate yields two enantiomeric metabolites, as illustrated earlier.

*Case 4* represents a rather fuzzy situation in which the metabolic reaction involves *inversion of the sense of chirality* in some or all substrate molecules, provided, of course, both enantiomeric substrates react differently in quantitative or qualitative terms. This situation, which we will illustrate below with oxiranes and ibuprofen, involves enantiomeric substrates generating enantiomeric metabolites, hence its label as *substrate–product enantioselectivity*.

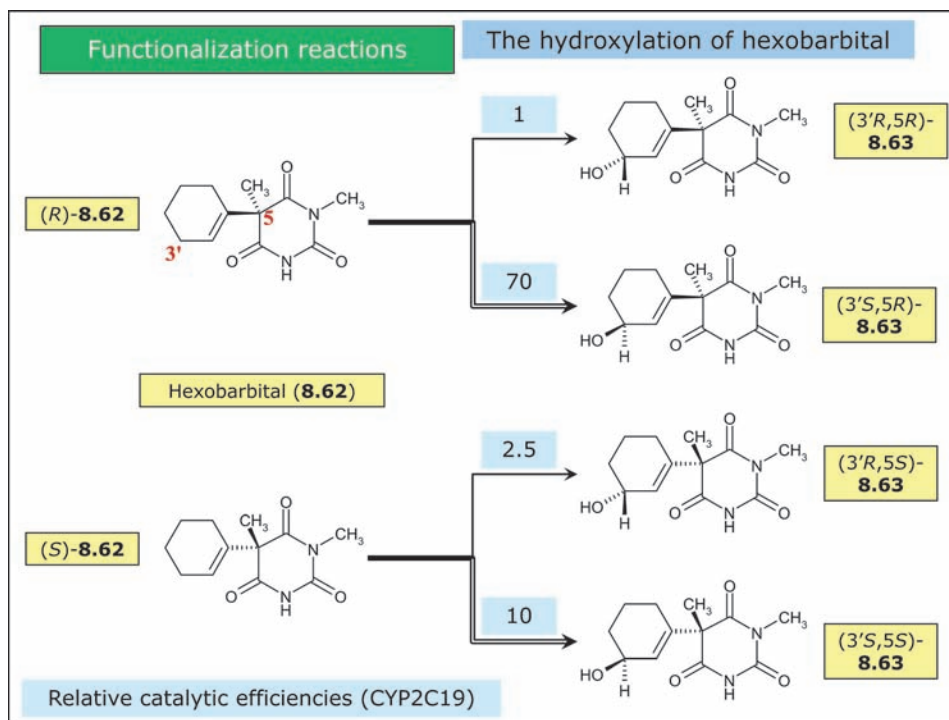
*Case 5* schematizes the classical situation of two enantiomeric substrates also containing a prochirality center whose *dissymmetrization* in a functionalization reaction produces four metabolites. Each of these four stereoisomeric metabolites has one enantiomer and two diastereoisomers, hence the label of *substrate–product*

*stereoselectivity*. Also belonging to *Case 5* are reactions of *conjugation* such as glucuronidation where a conjugating moiety containing one or more stereogenic centers is coupled to the enantiomeric substrates. Such a reaction generates only two epimeric metabolites.



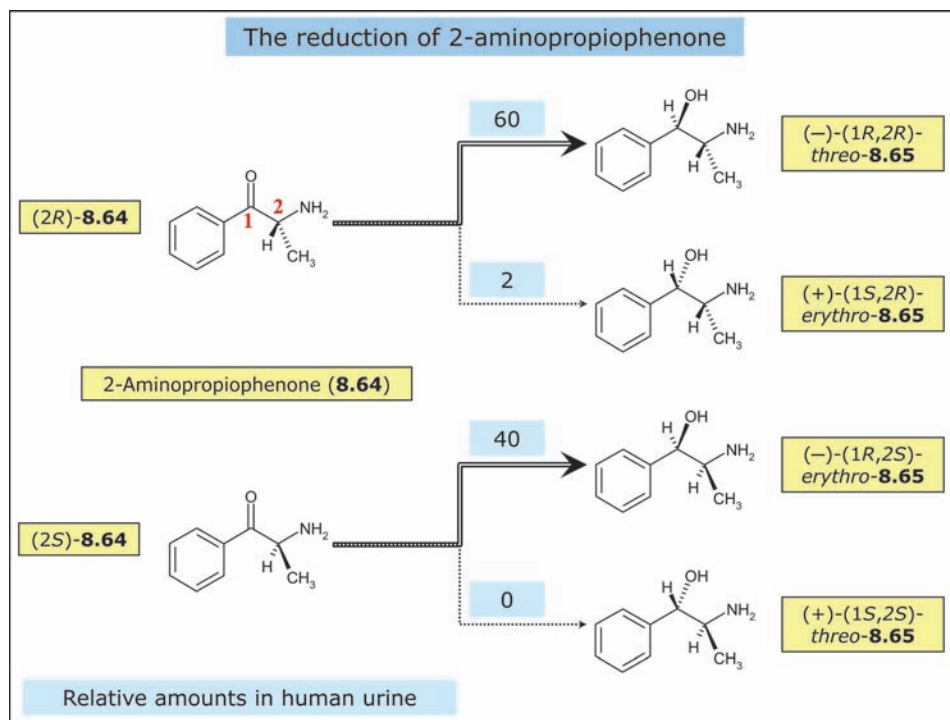
**Fig. 8.26.** *Substrate–product stereoselectivity* (the classical *Case 5* in the previous *Fig.*) is explained here taking advantage of the same type of *reaction coordinates* we used to describe substrate-stereoselective (*Part 7*) and product-stereoselective reactions (*Figs. 8.9–8.11*) [19][33]. The plot is more complicated than the previous ones, since two enantiomeric substrates are considered simultaneously, each yielding two epimeric metabolites. The reaction path leading to the *cis*-configured (*R,S*)- and (*S,R*)-metabolites is described by red arrows, whereas blue arrows mark the path to the *trans*-configured (*R,R*)- and (*S,S*)-metabolites.

The four enzyme–substrate complexes are diastereoisomeric to each other (and have slightly different energy levels) due to the added chirality of the enzymatic binding site. Interconversion may occur within each pair of complexes, but this is not represented here. The four transition states are diastereoisomeric for the same reason. In contrast, each metabolite has an enantiomer in the other pair, and is a diastereoisomer (here, an epimer) to the two other metabolites.



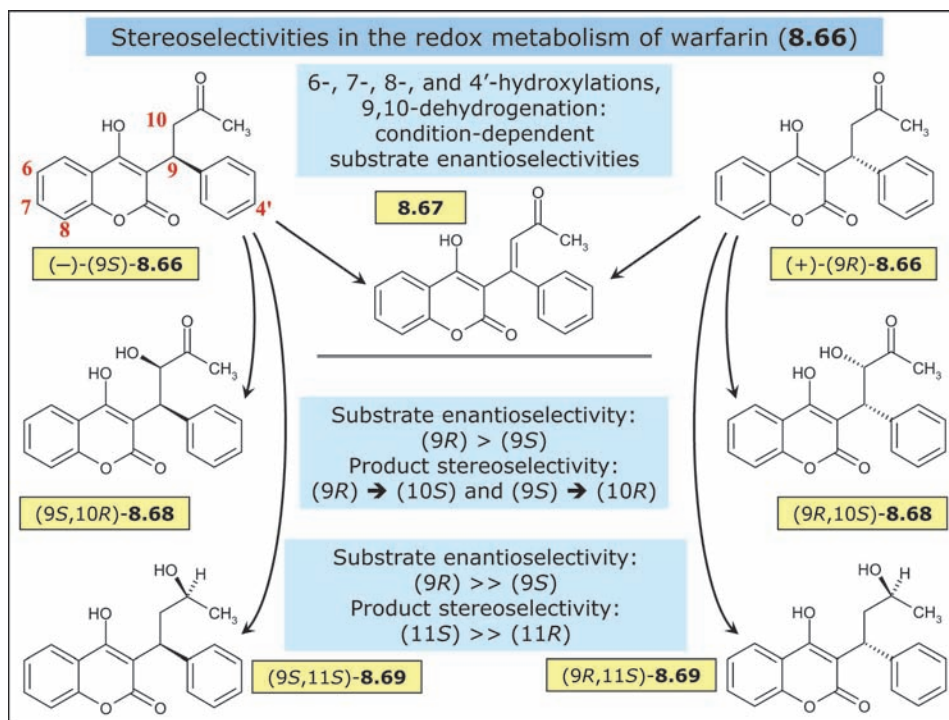
**Fig. 8.27.** Hexobarbital (**8.62**) offers a rather straightforward example of substrate–product stereoselectivity pertaining to *Case 5*. As alluded to earlier, the drug is *chiral* by virtue of the loss of a plane of symmetry caused by *N*-methylation. Hexobarbital is a well-known substrate of CYP2C19-catalyzed hydroxylation at C(3'), an allylic position, to yield 3'-hydroxyhexobarbital (**8.63**). Incubations of the separate enantiomers with recombinant CYP2C19 revealed (*R*)-hexobarbital to be a markedly better substrate than its (*S*)-enantiomer [89]. They also revealed a strong *product stereoselectivity* greatly favoring formation of (3'*S*,5*R*)-**8.63** and (3'*S*,5*S*)-**8.63** over their respective epimers at C(3'). In other words, the formation of the (3'*S*)-diastereoisomers was favored for both enantiomeric substrates, a first example of *substrate–product stereoselectivity (Case 5)*. The quantitative data shown are the relative  $V_{\max}/K_M$  values.

The use of artificial mutants of CYP2C19 demonstrated the key role of some residues in determining the observed substrate–product stereoselectivities. For example, replacing Glu300 with either Ala or Val inverted the observed substrate enantioselectivity.



**Fig. 8.28.** 2-Aminopropiophenone (**8.64**) is a metabolite of the appetite depressant amfepramone (a.k.a. diethylpropion). The compound contains a center of asymmetry at its C(2), and its (*S*)-enantiomer is known as *cathinone*, the main active alkaloid in khat (*Catha edulis* [90]), a plant of abuse. Cathinone is inactivated metabolically by deamination, while its extensive *keto reduction* leads to active *norephedrines* (**8.65**).

A small study in humans administered racemic 2-aminopropiophenone allowed the substrate–product stereoselectivity of the reaction of reduction to be assessed [91]. Under conditions of acidic urinary pH, basic compounds such as ethylphenylamines do not undergo kidney tubular reabsorption, and their rate of urinary excretion reflects their blood concentration. The *in vivo* reduction of 2-aminopropiophenone produced almost exclusively (1*R*,2*R*)-*threo*-**8.65** ((–)-norpseudoephedrine) and (1*R*,2*S*)-*erythro*-**8.65** ((–)-norephedrine). The reaction showed a strong *product stereoselectivity* in that C(1) was reduced almost exclusively to acquire an (*R*)-configuration. In contrast, there was a modest *substrate enantioselectivity*, since the rate of reduction of the (*R*)-enantiomer was only slightly greater than that of the (*S*)-form. These two examples of stereoselectivity resulted in a *substrate–product stereoselectivity* where the absolute configuration at the newly created stereogenic center was almost independent of the pre-existing configuration at C(2). Comparable results have recently been obtained with close analogs [92].

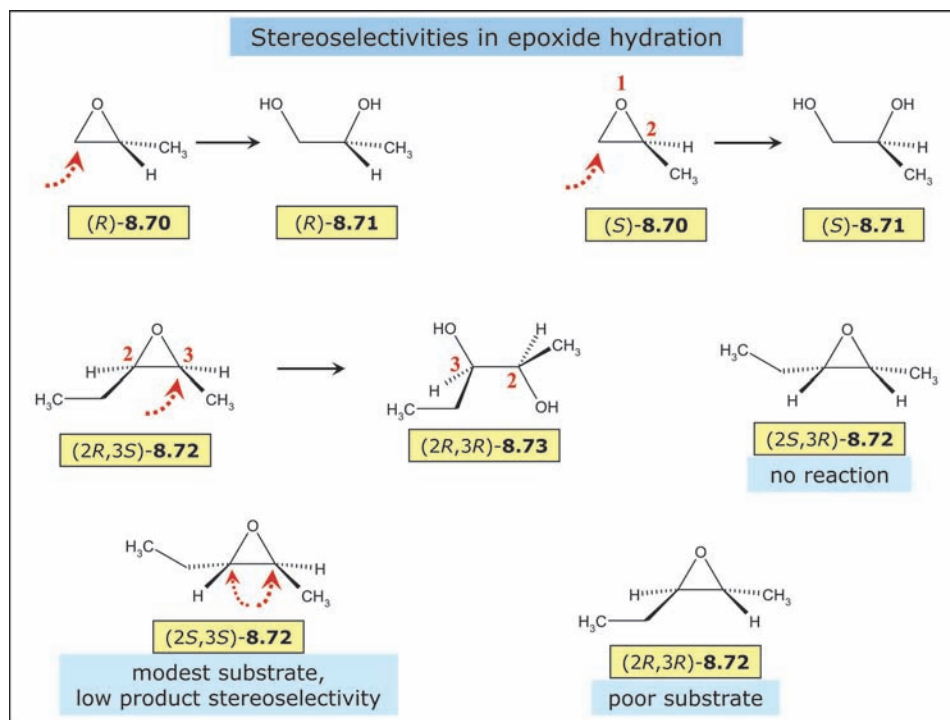


**Fig. 8.29.** The anticoagulant drug warfarin (**8.66**) is metabolized by various routes, including several CYP-catalyzed oxidations [76][93–98]. First, **8.66** undergoes various substrate-enantioselective reactions, most notably *aryl oxidations* (e.g., 6-, 7-, 8-, and 4'-hydroxylations) which produce phenols without affecting the stereogenic center (*i.e.*, *Case 2*). A further reaction is a side-chain oxidation (a *9,10-dehydrogenation*) which yields the achiral metabolite *9,10-dehydrowarfarin* (**8.67**), in other words, an example of *Case 1*. The two enantiomers of warfarin (**8.66**) are metabolized at different rates in these reactions, but the observed *substrate enantioselectivities* are strongly influenced by biological and experimental conditions. In humans, for example, the more active (*S*)-warfarin is oxidized faster than its (*R*)-enantiomer.

In addition to the above reactions, warfarin (**8.66**) also undergoes *alkyl hydroxylation at C(10)* to form *10-hydroxywarfarin* (**8.68**), a metabolite featuring two stereogenic centers. The enzyme involved is mainly CYP3A4, and human liver microsomes markedly favor (*R*)-warfarin as a substrate. More importantly, (*R*)-warfarin furnished almost exclusively (*9R,10S*)-**8.68**, whereas (*S*)-warfarin furnished a large excess of (*9S,10R*)-**8.68** [95], clearly a case of high *substrate–product stereoselectivity* (*Case 5*).

The last reaction examined in this *Figure* is a *keto reduction* catalyzed by cytoplasmic carbonyl reductases, which produces the so-called *warfarin alcohol* (**8.69**) [99]. This reaction also has the potential to be *product-diastereoselective*, since it transforms the *prochiral keto group* into a stereogenic alcohol center. Indeed, reduction preferentially affords the (*S*)-alcohol. Furthermore, the reaction is also strongly

substrate-enantioselective, since (*R*)-warfarin is by far the preferred substrate. As a result of these preferences, a large excess of the (*9R,11S*)-alcohol is produced, implying a marked *substrate–product stereoselectivity* in this reaction (*Case 5*).

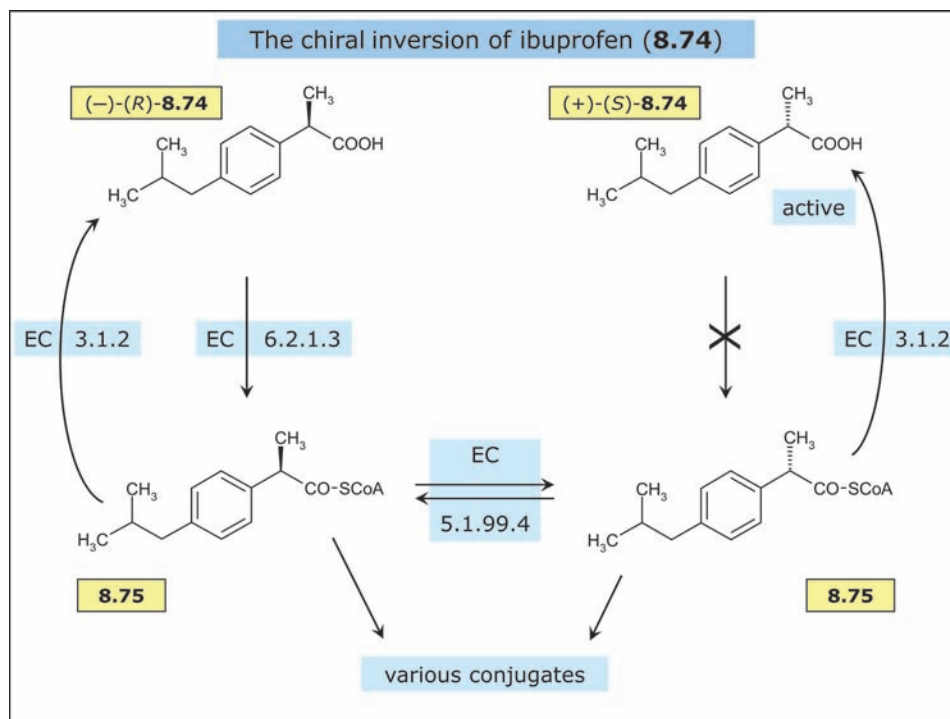


**Fig. 8.30.** Other examples of metabolic reactions combining substrate and product stereoselectivity can be found in the hydrolytic ring opening of epoxides catalyzed by *epoxide hydrolases* [75]. As in other enzymatic reactions, the product regioselectivity (when more than one potential target position exist) will depend on both the binding mode of the substrate and the reactivity of the target site. When the chiral monoalkyl-substituted *2-methyloxirane* (**8.70**) was used as model substrate, both enantiomers readily formed *propane-1,2-diol* (**8.71**) [100]. Furthermore, catalytic attack always occurred at the unsubstituted C(3), a reaction that did not create a new stereogenic center and implied retention of configuration at C(2). Thus, (*R*)-**8.70** was hydrated almost exclusively to (*R*)-**8.71**, and (*S*)-**8.70** to (*S*)-**8.71**, a situation we have classified as *Case 1* (see Fig. 8.25). However, had inversion of the sense of chirality (known as ‘chiral inversion’) occurred due to attack at C(2), the reaction would have been classified under *Case 4*. This example is instructive, since it shows the *fuzziness of the classification system* in Fig. 8.25.

A 2,3-dialkyl-substituted oxirane such as *2-ethyl-3-methyloxirane* (**8.72**) contains two different substituents and two stereogenic centers. Epoxide hydrolase-catalyzed hydration led to *pentane-2,3-diol* (**8.73**), with marked differences between the four



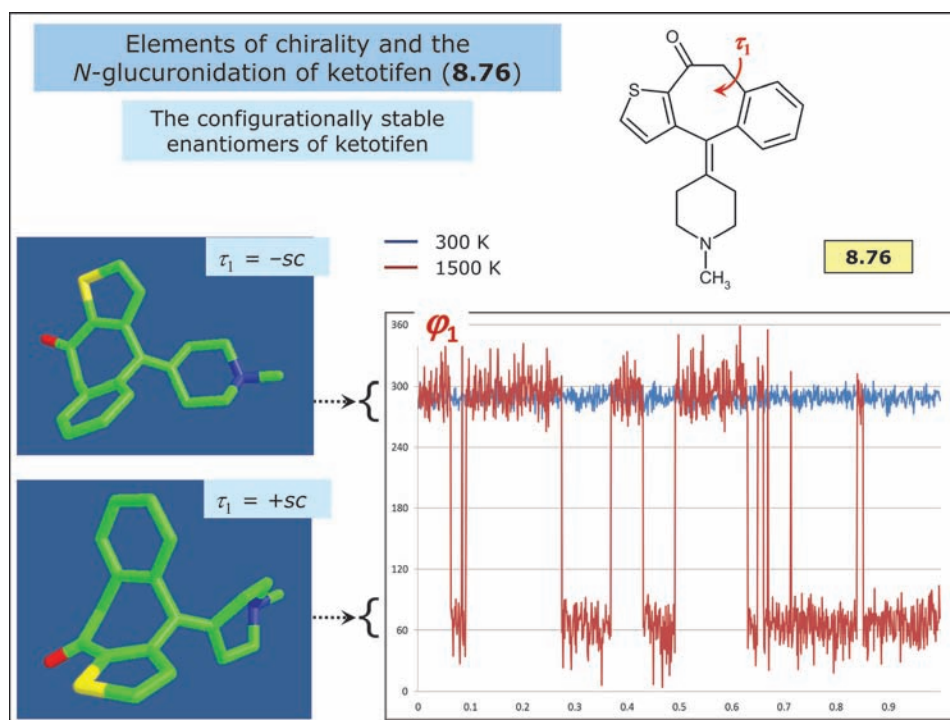
stereoisomeric substrates. Indeed, *cis*-(2*R*,3*S*)-2-ethyl-3-methyloxirane ((2*R*,3*S*)-**8.72**) was the only good substrate and yielded (2*R*,3*R*)-*threo*-pentane-2,3-diol ((2*R*,3*R*)-**8.73**), while its enantiomer, (2*S*,3*R*)-**8.72**, gave no reaction. In contrast, the two *trans*-isomers (2*S*,3*S*)- and (2*R*,3*R*)-2-ethyl-3-methyloxirane were modest and poor substrates, respectively. The former reacted with a low product stereoselectivity, since it gave (2*R*,3*S*)-*erythro*-pentane-2,3-diol (attack according to the thick red arrow) in slight excess over the (2*S*,3*R*)-*erythro*-pentane-2,3-diol (thin red arrow). In other words, we have here an example close to *Case 4* due to the substrate-dependent, differential enzymatic attack at two positions.



**Fig. 8.31.** An intriguing metabolic reaction is the inversion of the sense of chirality of some non-steroidal anti-inflammatory 2-arylpropanoic acids (*i.e.*, profens), the most studied of which is *ibuprofen* (**8.74**) [101–104]. The enzymology and mechanism of this reaction are now well understood and begin with the formation of an *acyl-coenzyme A intermediate*, **8.75**, a reaction catalyzed by long-chain acyl-CoA ligase (EC 6.2.1.3) [18][105]. This reaction is *substrate-enantioselective* in that it shows a marked or almost exclusive preference (depending on animal species) for the inactive (–)-(R)-*ibuprofen* [106]. In other words, the ligase reacts almost only with (R)-*ibuprofen* to form its *acyl-CoA conjugate* **8.75**. Once formed, this intermediate is the substrate of an *inversion of sense of chirality* catalyzed by 2-methylacyl-CoA 2-epimerase (EC 5.1.99.4;  $\alpha$ -methylacyl-CoA racemase), a peroxisomal and mitochondrial enzyme [107–109]. In

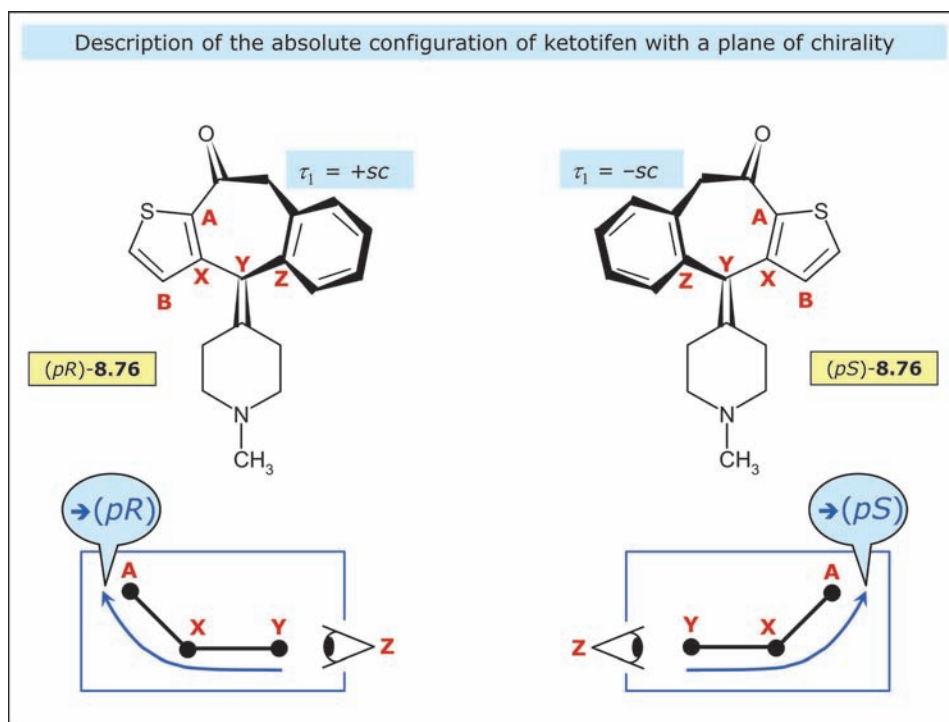
strictly correct terms, this reaction is one of *epimerization*, since coenzyme A is itself chiral. As a result, the ibuprofenoyl moiety now exists in the (*R*)- and (*S*)-forms, and acyl-CoA thioesterases (EC 3.1.2) act on both (*R*)-ibuprofenoyl-CoA and (*S*)-ibuprofenoyl-CoA to liberate the corresponding ibuprofen enantiomer. In the metabolic scheme shown here, (*S*)-ibuprofen is thus an outcome but not an entry point; in contrast, (*R*)-ibuprofen is both.

How are we to classify this reaction of inversion? Because only (*R*)-ibuprofen is a substrate, *substrate enantioselectivity* is obvious. And since (*R*)-ibuprofen is partly converted to (*S*)-ibuprofen, the reaction is one of *product enantioselectivity*. By combining the two approaches, we can conclude that this metabolic pathway of inversion affecting ibuprofen and other profens [110][111] is an example of *substrate–product enantioselectivity (Case 4)*.



**Fig. 8.32.** We conclude this *Part* with an intriguing and currently poorly solved example, namely the *N*-glucuronidation of the anti-asthmatic and anti-allergic drug *ketotifen* (**8.76**) [112]. This molecule occurs as *two enantiomers* due to *a*) the non-planarity and stereogenicity of its tricyclic system, and *b*) its configurational stability under ambient and physiological conditions (*cf.* [113][114] and refs. cit. therein). The *configurational stability* of ketotifen enantiomer is ascribed to a high-energy barrier caused by the two non-junction  $C_{sp^2}$ -atom in the central ring, while the relative flexibility of this ring is due to the intracyclic  $CH_2$  group.

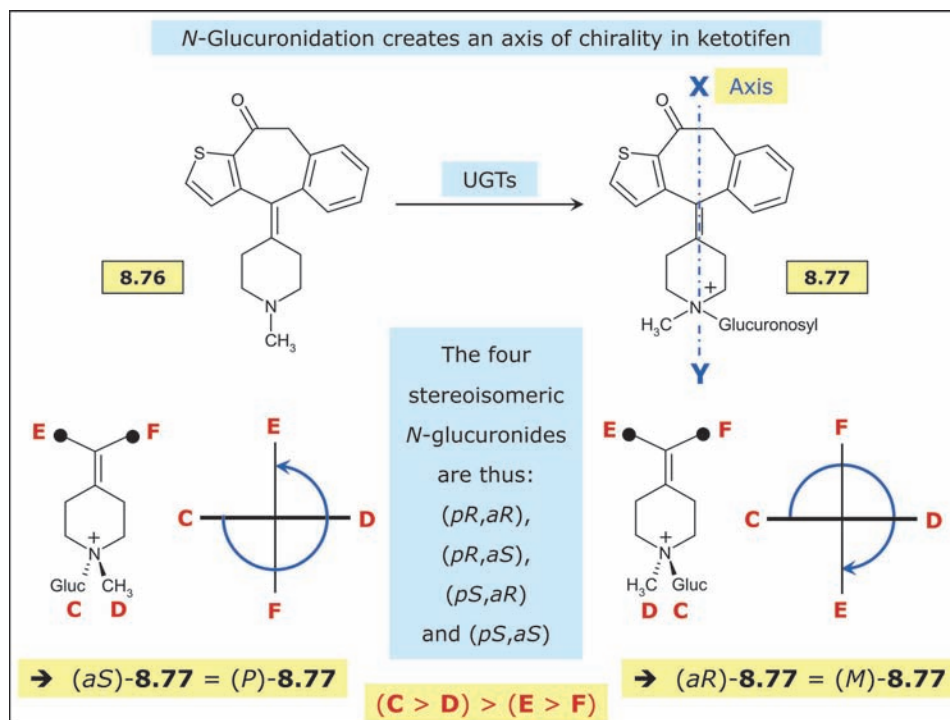
Using the same approach as used for *telenzepine* in *Part 6*, we have submitted one enantiomer of ketotifen (described by  $\tau_1 = -\text{synclinal}$ ) to 1-ns molecular dynamics (MD) simulations at temperatures of 300 and 1500 K<sup>2)</sup>. As shown by the *blue trace*, this enantiomer remained configurationally stable during the entire simulation at 300 K, in agreement with experimental results. At 1500 K (*red trace*), in contrast, at least 14 events of ring reversal were seen. As observed for *telenzepine* (*Part 6*), the two ketotifen enantiomers are best described as *conformational clusters* given the observed fluctuations of  $\tau_1$  around  $+60^\circ$  and  $-60^\circ$ .



**Fig. 8.33.** Having documented the configurational stability and flexibility of the ring system of ketotifen, we must face the task of finding an adequate configurational descriptor for its two enantiomers. Using axial chirality appears unrealistic given that the rotating bond characterized by  $\tau_1$  does not feature four fiducial atoms able to define an axis of chirality.

<sup>2)</sup> *Technical note:* One enantiomer of ketoprofen ( $\tau_1 = -sc$ ) was submitted to 1-ns molecular dynamics (MD) simulations at 300 and 1500 K, monitoring the variations of the  $\tau_1$  torsion angle. The simulations had the following characteristics: *a)* *Newton's* equation was integrated every fs; *b)* the temperature was maintained at  $1000 \pm 10$  K by means of *Langevin's* algorithm; *c)* *Lennard–Jones (L–J)* interactions were calculated with a cut-off of 10 Å, and the pair list was updated every 20 iterations; *d)* a frame was stored every ps, yielding 1000 frames; and *f)* no constraints were applied to the systems. Two representative enantiomeric structures were minimized by a PM6 semi-empirical method.

Resorting to planar chirality appears as a more promising approach. In *Part 3*, we saw that a *plane of chirality* exists, when there are four coplanar atoms (labeled there and here as A, B, X, and Y), plus an out-of plane atom labeled Z, called the *pilot atom*. This approach has also been illustrated in *Part 6* with telenzepine, a close analog of ketotifen. Here, we see that the conditions for planar chirality are indeed fulfilled and allow the two enantiomers (*i.e.*, the two conformational clusters) to be labeled as (*pR*)- and (*pS*)-ketotifen, respectively.



**Fig. 8.34.** Moving now to *biotransformation*, a major metabolic reaction of ketotifen in humans is the *N-glucuronidation* of its tertiary amino group. The reaction is catalyzed by UDP-glucuronosyltransferases, particularly UGT1A3 and UGT1A4 [114], and transforms the molecule into a *quaternary ammonium glucuronide*, **8.77**. The authors of the study [113][114] consistently observed that *four stable stereoisomeric N-glucuronides* were formed in humans and *in vitro* from racemic ketotifen, and two, when each enantiomer was incubated separately with human liver microsomes or some UGTs. The explanation offered at the time was one of a conformational difference in the piperidine ring, an unrealistic hypothesis. In fact, the authors of [113][114] seem to have failed to realize that *an axis of chirality* is created upon the transformation of the tertiary amino group into a quaternary ammonium. Indeed, the *N-glucuronidated* side chain in ketotifen *N-glucuronides* has a structure similar to that of *chiral alkylidene-cycloalkanes*, which contain an axis of chirality when the conditions explained in *Part 3* are

fulfilled. These conditions imply that the *two proximal fiducial groups* be different (here  $C \neq D$ ), as must the *two far fiducial groups* (here  $E \neq F$ ) (see *Part 3*).

The two elements of chirality in ketotifen *N*-glucuronide (*a plane of chirality and an axis of chirality*) allow for the existence of the four stereoisomers listed here, and all four were indeed formed as stated above. However, their configurations were not solved for the reasons explained, and they were casually labeled (*R*)-Gluc<sub>A</sub>, (*R*)-Gluc<sub>B</sub>, (*S*)-Gluc<sub>A</sub>, and (*S*)-Gluc<sub>B</sub>. In terms of stereoselectivities, *substrate enantioselectivity* was marginal, whereas there was an about twofold *product stereoselectivity* favoring the Gluc<sub>B</sub> epimers.

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