Racemisation in Chemistry and Biology

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Dedicated to Prof. Dr. Jan B.F.N. Engberts on the occasion of his 80th birthday.

Frontispiece text: When chemistry meets biology, as here with a child stumbling upon the enantiomers of cetirizine in the sea, stereochemistry often governs the outcome. In aqueous environments, the process of racemisation will therefore be important and can be facilitated in a range of ways including by base, acid or enzymatic catalysis. The limited information concerning racemisation under each of these conditions is surveyed and critiqued. Tools to guide prediction of racemisation risk are presented.
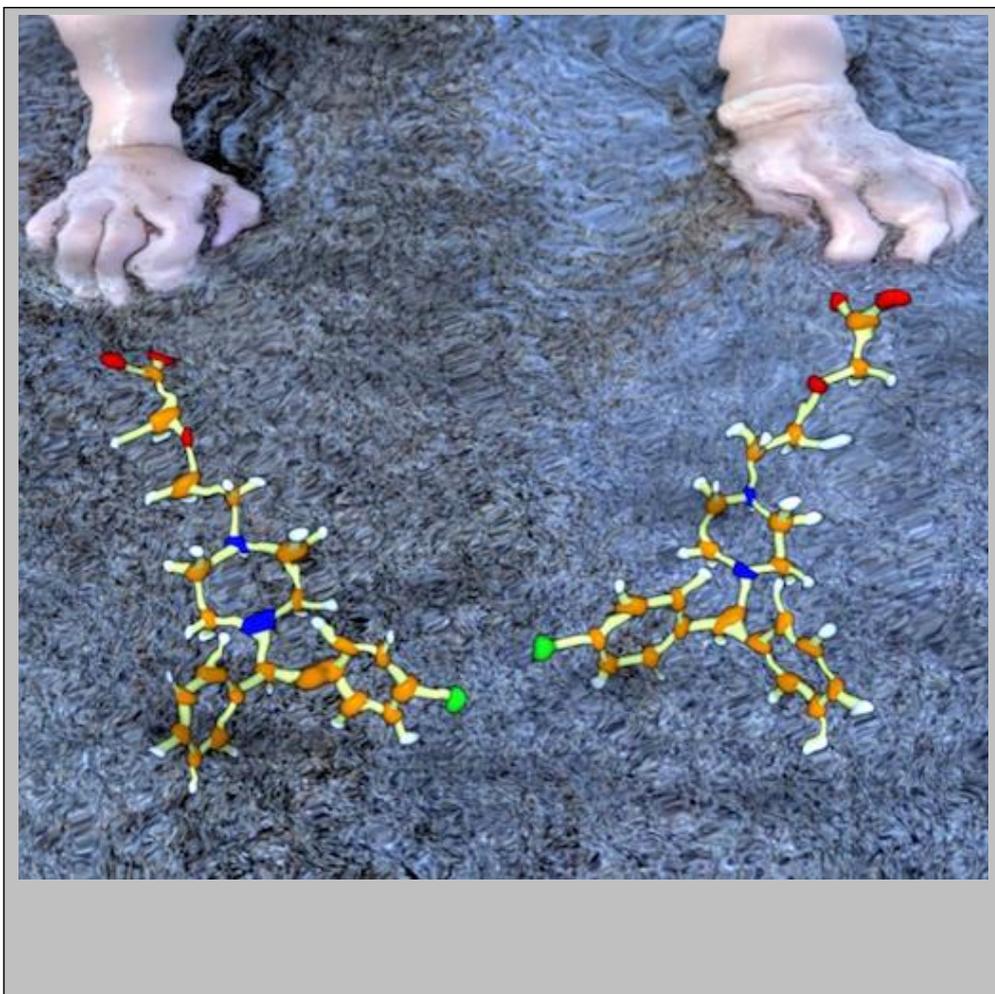
Abstract: The two enantiomers of a compound often have profoundly different biological properties and so their liability to racemisation in aqueous solutions is an important piece of information. We have reviewed the available data concerning the process of racemisation in vivo, in the presence biological molecules (e.g. racemase enzymes, serum albumin, cofactors and derivatives) and under purely chemical but aqueous conditions (acid, base and other aqueous systems). Mechanistic studies are described critically in light of reported kinetic data. The types of experimental measurement that can be used to effectively determine rate constants of racemisation in various conditions are discussed and the data they provide is summarised. The proposed origins of enzymatic racemisation are presented and suggest ways to promote the process that are different from processes taking place in bulk water. Experimental and computational studies that provide understanding and quantitative predictions of racemisation risk are also presented.

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1. Introduction

Molecular symmetry, chirality and stereochemistry are defining concepts for organic chemistry. Appreciation of the relevance of chirality to many applications of molecules, particularly in biological systems, has been steadily growing over time. As a consequence, racemization has also become a subject of increasing concern. Although racemisation and related processes also occur in other media (See, e.g., the work by Blackmond on the origin of chirality which mainly focusses on enantiomerisation in solids), we here focus on racemisation in aqueous solutions and in particular under conditions with some resemblance to physiological conditions.

Racemisation can be necessary and desirable. For instance, in biological systems, reductions in the levels of racemase activity and hence of D-Ser have been linked with the pathophysiology of Alzheimer's disease.[1] Racemisation also underpins dynamic kinetic resolutions, which can be a very effective and efficient route to high yields of single enantiomers.[2,3] Racemisation of amino acids also allows dating of biological samples in amino acid dating.[4,5] However, racemisation is more often undesirable and has been linked with the formation of cataracts when proteins in the lens that are responsible for maintaining clarity undergo stereochemical scrambling and other transformations.[6,7] Racemisation can also cause what would otherwise be safe drugs to convert into toxic or ineffective forms.[8] As a consequence, the Food and Drug Administration (FDA) and other regulatory bodies require unambiguous data to be provided concerning the configurational stability of chiral drugs.[9,10] Racemisation may also feed into so-called "Chiral Pollution" by agrochemicals[11] and drugs.[12]

It has become clear to us over recent years that there have been significant steps forward in our knowledge about, and understanding of, racemisation in several arenas and we wish to draw these together to provide an overview of the strategies for employing or avoiding racemisation used by chemists and by nature. It is however also clear that the scope of these studies remains narrow and the evidence base smaller than is required for a full understanding, both in terms of available kinetic data and mechanistic studies. In this light, we have attempted to provide a comprehensive critical review of the available studies in order to provide a stimulus to others to expand our knowledge. It is also hoped that, by describing (in less depth) some of the key ideas about racemisation in biology, we will promote the transfer of ideas between chemical and biological sciences. Finally, we are also keen to address an attitudinal problem that we have encountered too frequently amongst chemists; because it is easy to post-rationalise a racemisation problem using the concepts of physical organic chemistry, this does not mean that chemists are reliable at predicting these problems in advance. We hope that this review will highlight some of the interesting and challenging problems that chemists should look out for and provide guidance on how to address these challenges.

a) Racemisation and enantiomerisation

Racemisation is a statistical, macroscopic and irreversible process in which half of an enantiopure quantity of compound is transformed into the opposing enantiomer (Scheme 1, top). It is complete when the enantiomeric excess (e.e.) of the sample under analysis is reduced to 0%.

Scheme 1. Top: The process of racemisation. Bottom: The process of enantiomerisation.

Racemisation clearly has to be a macroscopic process because racemisation of a single molecule is impossible. Nevertheless, it is useful to consider the link between microscopic (molecular) processes and macroscopic racemisation. For cases where chirality is based on a stereogenic carbon atom, the molecular processes leading to racemisation often involve formation of an achiral carbocation, an achiral carbanion intermediate or an achiral free radical. Subsequent non-enantiospecific reformation of the stereogenic centre then leads to a racemic mixture. One could call this type of process "racemisation by loss of enantiomeric memory". Typical molecular processes of this kind that lead to macroscopic racemisation are discussed in Sections 2d and 2i.

Enantiomerisation is also related to racemisation but is a microscopic process involving reversible conversion of one molecule of an enantiomer into its mirror image (Scheme 1, bottom). Repeated enantiomerisation eventually results in racemisation. It is often said that enantiomerisation of one molecule effectively reduces the e.e. of the whole system by two molecules and therefore the rate constant for racemisation is twice that of enantiomerisation. More precisely, for a system undergoing enantiomerisation, the observed rate constant k_{rac} for approaching the dynamic equilibrium (that is the racemic mixture) is the sum of the two mechanistic rate constants k_{enant} that together define the corresponding equilibrium constant of 1.[13] For the example in Scheme 1, the rate constant for racemisation k_{rac} is therefore 2 x k_{enant}.

Under certain conditions, racemisation through loss of enantiomeric memory and racemisation resulting from repeated enantiomerisation can therefore be distinguished because the rate constant for racemisation is either equal to or exactly double the rate constant for the underlying process (See, e.g., Section 2c).

Many compounds have more than one stereogenic center in them. In these molecules, the formal process of racemisation involves the inversion of all of the stereocenters. Such molecules are more likely to undergo the related process of diastereoisomerisation or epimerisation in which not all of the stereogenic centers invert. An example of epimerisation is provided by studies of RS10085.[16] The process of epimerisation will not be discussed in this article.

b) Why is racemisation important for drug discovery?

Racemisation would only be important if two enantiomers have biological properties that differ enough to cause measurably different effects in complex biological systems such as a human being. Recent large-scale surveys of measured data have therefore sought to identify which biological properties are most strongly influenced by inversion of chirality. The measured differences between enantiomers in the Astrazeneca and Novartis company databases were analysed by two different statistical approaches (both attempting to distinguish the additional variation between enantiomers from the natural variation between repeat measurements).[17,18] The results of the two surveys are summarized in Table 1. As expected, solubility and logD are unaffected by inversion of stereochemistry. Somewhat surprisingly, given their cellular basis and the involvement of active uptake and efflux processes, the permeability assays (Caco2 and MDCK) do not show a difference between enantiomers. This general lack of sensitivity in permeability is supported by studies investigating trans-dermal delivery of ketoprofen which found no measurable difference between the enantiomers or racemate.[19] The lack of discrimination between enantiomers for the active processes is in line with recent structural findings suggesting that P-glycoprotein efflux transporter PGp presents a large open (undiscriminating) cavity to the cell interior into which substrates can move. This cavity then rearranges to extrude the substrates from a much more constrained cavity towards the cell exterior.[20]

On the other hand, metabolism and in vivo pharmacokinetics are influenced by chirality. Inhibition of most of the cytochrome P450 isoforms is also strongly influenced by the stereochemistry with the exception of isoform 3A4. Isoform 3A4 has a markedly weaker dependence and this likely reflects the fact that 3A4 is another system that employs a large, open and flexible cavity.[21]

Table 1. A range of biological properties of molecules and the degree to which they are affected by inversion of stereochemistry.[17,18]

Property	Astrazeneca data[a]	Novartis data[b]
Solubility	-	-
logD	-	n.m.
Permeability/efflux (Caco2)	-	-
Permeability/efflux (MDCK)	-	n.m.
Plasma protein binding	+	n.m.
Metabolism (microsomes)	+	+++
Metabolism (hepatocytes)	+	n.m.
CYP 1A2 inhibition	++	n.m.
CYP 2C9 inhibition	++	n.m.
CYP 2C19 inhibition	+++	n.m.
CYP 2D6 inhibition	+++	n.m.
CYP 3A4 inhibition	+	-

hERG block	++	+
In vivo pharmacokinetics (Volume of distribution)	++	n.m.
In vivo pharmacokinetics (Clearance)	++	n.m.
Isocitrate dehydrogenase (protein target)	n.m.	+++

a) The variance due to chiral inversion has been categorised as - = 0; + = <0.02; ++ = 0.02-0.1; +++ = >0.1. b) - = p>0.05. For cases where the p-value is less than 0.05, the Kolmogorov-Smirnov D values have been categorised as + = <0.1; ++ = 0.1-0.4; +++ = >0.4. n.m. = not measured.

The differences in properties extend to those molecules that contain chiral axes instead of stereogenic centres. In a recent report, the P atropisomer of the phosphoinositide 3-kinase inhibitor **1** was found to be a markedly better inhibitor than its enantiomeric alternative (the M atropisomer).[22] In addition, **1** has a lower in vitro clearance than its enantiomer in tests using human hepatocytes or microsomes; its enantiomer is particularly liable to metabolism by aldehyde oxidase.

1   2   3
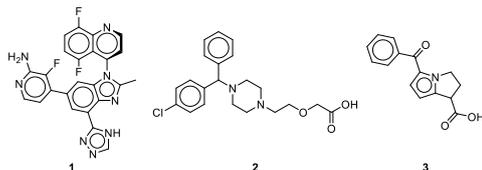
The consequences of the differences in medicinal chemistry properties of enantiomers are nicely demonstrated through the resulting differences in pharmacokinetics aas studied in detail for **2** and **3**. A study of the plasma concentration-time curves for ketorolac (**3**) revealed markedly different profiles for the two enantiomers following intravenous injection of racemate to healthy volunteers.[24] The in vivo clearance of *S*-**3** was found to be 10.99 L min^{-1} while that for *R*-**3** is 3.24 L min^{-1}. This leads to higher concentrations of *R*-**3** being achieved.

The importance of racemisation for specific drugs has also been emphasised in a review that details the properties of drugs and their enantiomers.[25] While many have enantiomers that are viewed as inactive, some have toxic or dangerous enantiomers. In both cases, racemisation is harmful either by reducing the dose of active compound or by leading to exposure to harmful compounds even when only the safer enantiomer has been taken.

Racemisation is also important in drug discovery because it can determine whether chiral switching is likely to be an economically attractive proposition. Chiral switching is when an improved single enantiomer (with ongoing patent protection) replaces a racemate (whose patent protection is expired). A marketing case for the single enantiomer is unlikely to be viable if it rapidly racemises. By way of example, a detailed package of studies was undertaken to understand the effect of switching the selective H1-receptor antagonist racemic cetirizine (**2**) to a single enantiomer, levocetirizine (L-**2**). A decision tree was proposed in which the differences between the pharmacokinetics, pharmacodynamics, interactions with other drugs and rate of racemisation are all considered.[23] L-**2** has a higher apparent affinity for the H1 receptor and similar pharmacokinetics to the racemate (and the enantiomer dextrocetirizine). When healthy volunteers were given doses of either 2.5 mg of L-**2** or 5 mg of racemic **2**, after 32 hours those who had taken L-**2** still had a measurable benefit while those who took the racemate did not. Following dosing with levocetirizine, none of the enantiomer dextrocetirizine was found in urine supporting a lack of in vivo racemisation (in line with results in pH 7.4 buffer and in plasma). The authors advocated for a change from racemate to single enantiomer based on this extensive package of data. This example highlights the non-linear effects of dosing mixtures of compounds such as racemates and Table 1 shows that this can arise through changes in a wide range of biological properties that combine in ways that make the prediction of human pharmacokinetics and pharmacodynamics extremely challenging.

In an approach opposite to a chiral switch, one could imagine that administering the racemate of a rapidly racemising drug could lead to naturally enriching the drug as the required enantiomer as a result of selective binding to the target (Cf. dynamic kinetic resolution). Unfortunately, the mass balance of drug-target interactions typically involves a large systemic excess of the drug relative to the target, meaning that this approach is unlikely to be efficient.

For the reasons above, racemisation is important in drug discovery. This fact is further highlighted by the link between racemisation risk and failure in clinical trials. When quantitative calculations (described below) are applied to identify molecules that are at risk of racemisation, it was found that such molecules are less likely than others to proceed from one stage of clinical trials to the next.[26] This is despite the known trend for molecules with a chiral centre to be more likely to succeed than those that lack one.[27]

2. Kinetics and mechanisms of racemisation

In this article, we limit discussion to mechanisms that are relevant in aqueous media; this precludes some possibilities and introduces others, as will be discussed below. Racemisation in aqueous conditions is subject to a number of influences, particularly acid or base catalysis, either of which might be general or specific. The observed rate laws form kinetic signatures for the possible reaction mechanisms (Figure 1).
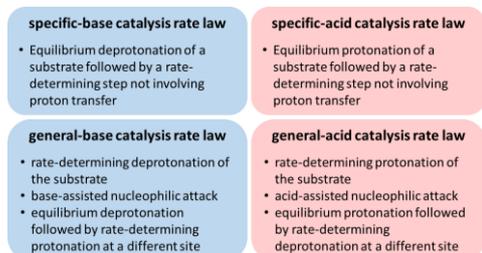
Figure 1. Typical reaction mechanisms for the different rate laws for acid and base catalysis.

In compiling this review, it has become clear that the mechanistic details of the racemization of some molecules has been inadequately or incorrectly classified, despite the long-accepted links between rate laws and mechanisms shown in Figure 1. We therefore provide some critical descriptions of many of these, describing first reactions subject to acid catalysis and then those subject to base catalysis. Finally, racemisation via other mechanisms or uncharacterised processes is described.

a) Mechanisms of racemisation giving rise to acid catalysis

Stereogenic carbons bearing a hydroxyl group have the possibility of specific- or general-acid catalysed racemisation. Protonation of a hydroxyl group enhances its ability as a leaving group, allowing it to depart leaving behind a carbocation (Scheme 2). Protonation in these cases is typically an equilibrium process before the rate-determining step, resulting in a specific-acid-catalysis rate law. The carbocation can be trapped by water, reintroducing a hydroxyl group. Systems in which the carbocation is stabilised are most likely to undergo this type of reaction. Certain molecules also contain nucleophilic groups that can 1) trap the carbocation in an S_N1 fashion which would lead to racemisation of the stereogenic center once the trapping group leaves and water returns to reintroduce the hydroxyl or 2) directly displace the protonated OH which would lead ultimately to retention of stereochemistry upon readdition of water.

In aqueous solutions, protonation of groups other than hydroxyl can only lead to racemisation if the carbocation and leaving group are held within the solvent cage so that they can reform a bond more quickly than the carbocation is intercepted by water. For racemisation to occur, the reaction must proceed in a way that nevertheless permits the group that left to attack either face of the carbocation. In general this is unlikely (See Section 2.f.iii), but the case of stereocenters in ring systems is a special one because the group that leaves can remain part of the molecule and can therefore react intramolecularly (Scheme 2).
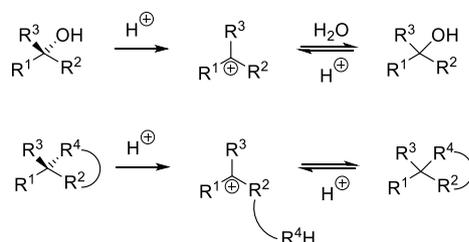
Scheme 2. Generic mechanisms for acid catalysed racemisation.

The examples above typically result in specific-acid-catalysis rate laws. General-acid catalysis occurs less frequently (See Table 5) because racemisation involving rate-determining protonation or involving acid-assisted nucleophilic attack are less likely. We note, however, that a mechanism involving equilibrium protonation followed by rate-determining deprotonation at a different site will also give rise to a general-acid catalysis rate law. This type of behaviour is anticipated, e.g., at neutral pH for stereogenic centres adjacent to carboxylates or next to amines with a pK_a below 7 (Scheme 3).
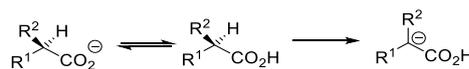
Scheme 3. Generic mechanism involving equilibrium protonation followed by rate-determining deprotonation at a different site leading to a general-acid catalysis rate law

b) Examples of racemisation reactions displaying acid catalysis

We here review aqueous racemisation reactions for which kinetic data indicate acid catalysis, separating the examples into those showing specific-acid catalysis and those showing general-acid catalysis.

i) Specific-acid catalysis

A good example of the acid-catalysed racemisation of a hydroxyl group is provided by the racemisation of catecholamines such as adrenaline (**4**, Scheme 4) in 1.0 M hydrochloric acid. Here the carbocation intermediate is significantly stabilised by the adjacent electron-rich aromatic ring which is required to overcome the destabilisation of the positive charge resulting from the adjacent ammonium cation.[28] For comparison, 1-phenylethanol racemises slightly faster than **4**. Removal of the π-electron donating p-OH slows down the racemisation by three orders of magnitude.
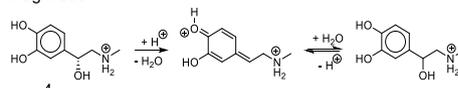
Scheme 4. Racemisation of **4**.

The anti-arrhythmic Ibutilide (**5**, Scheme 5) can also undergo racemization via formation of a carbocation. The alternative direct intramolecular displacement of the protonated hydroxyl group by the amine would give a cyclic intermediate with inversion that would in turn be subject to attack by water yielding the alcohol in its original configuration. It has been shown that both racemisation and formation of the cyclic ammonium ion proceed via the carbocation, but that racemisation through repeated enantiomerisation resulting from direct attack of water on the stereogenic centre plays an important role as well. [29]
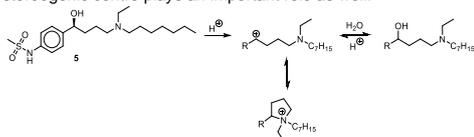
Scheme 5. Racemisation of **5**.

ii) General-acid catalysis

An example of reactions going through the general-acid catalysis rate law as a result of a mechanism involving equilibrium protonation followed by rate-limiting deprotonation elsewhere is presented by tautomerisation reactions, such as shown in Scheme 6. [30]
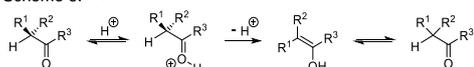
Scheme 6. Acid catalysis of racemisation via tautomerisation.

Racemisation of 9-hydroxyrisperidone **6** is a specific example of this type of reactivity. Compound **6** could undergo acid-catalysed racemisation via protonation and elimination of the alcohol or via protonation at nitrogen (Scheme 7).[31] The latter was proposed as the most likely route for racemisation because of the electron-withdrawing groups adjacent to the carbocation that would form if the alcohol were to leave.
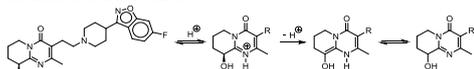
Scheme 7. Racemisation of **6**.

c) Mechanisms of racemisation giving rise to base catalysis

A common base-catalysed racemisation process is one in which a stereocenter of type $R^1R^2R^3CH$ is deprotonated to yield a flat anion (Scheme 8). This anion can then reprotonate from either face.
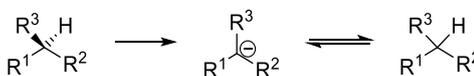
Scheme 8. General mechanism of base-catalysed racemisation.

The details of the deprotonation and reprotonation can significantly influence the outcome and at the extremes can lead to racemisation, enantiomerisation or indeed retention of configuration as demonstrated by Cram et al., albeit not in aqueous solutions.[32]

These reactions can be illuminated by investigating the rate at which the proton at the stereocenter is exchanged for a deuterium if the reaction is performed in deuterated buffers (or deuterium with proton if starting with a deuterated molecule). There are four contrasting scenarios for the rate constants for deuteration k_{deut} and for racemisation k_{rac}:

k_{deut}/k_{rac} is 1 when a purely racemising process takes place and deuteration occurs equally on both faces of an intermediate anion. This involves an S_E1 mechanism in which deprotonation leads to a planar carbanion, with complete separation of the departing proton (i.e. the proton is not retained inside the solvent shell ready to reprotonate). There are many structural features that can stabilise or destabilise these carbanionic intermediates and these are discussed below.

k_{deut}/k_{rac} is 0.5 when the process takes place exclusively with inversion. This involves an S_E2 mechanism in which deprotonation occurs on one face of the stereogenic carbon with simultaneous reprotonation on the opposite face. This push-pull mechanism involves a greater preorganisation and therefore entropic barrier than the S_E1 mechanism. Many of the groups that stabilise carbanions and therefore facilitate the S_E1 mechanism can also facilitate the S_E2; the transition state for this reaction involves significant anionic character at the stereogenic center.

k_{deut}/k_{rac} tends to infinity if the process takes place exclusively with retention (deuteration takes place but there is no stereochemical scrambling)

k_{deut}/k_{rac} tends to zero when racemisation takes place with no deuteration, in which case a mechanism not involving proton transfer is in operation.

Specific-base catalysis is less likely because loss of proton often leads to immediate loss of chirality, apart from for examples such as the deprotonation of a hydroxide in molecules such as chlortalidone (**7**, Scheme 12) where the deprotonated form then undergoes fragmentation leading to an achiral intermediate.

d) Examples of racemisation reactions displaying base catalysis

As for acid catalysis above, we review aqueous racemisation reactions for which kinetic data indicate base catalysis, separating the examples into those showing specific-base catalysis and those showing general-base catalysis.

i) Specific-base catalysis

As mentioned above, specific-base catalysis is likely to be less common than general-base catalysis. Examples do, however, occur.

A loss of chiral purity was observed for the Eg5 inhibitor *R*-litronesib (**8**) during storage in a liquid formulation.[33] This racemisation is base-catalysed, despite the stereogenic centre being quaternary. Studies revealed that the racemisation depends on pH but does not depend on buffer identity, i.e. specific-base catalysis. On the basis of this observation, a mechanism involving equilibrium deprotonation of the sulphonamide was proposed which is followed by a multi-step ylid-mediated process featuring a relay of ionized groups (Scheme 9).
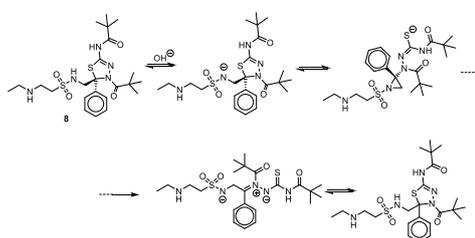
Scheme 9. Racemisation of **8**.

ii) General-base catalysis

An important discussion for racemisation involving general base catalysis is whether the reaction proceeds through the S_E1 or the S_E2 mechanism. There are two cases in which the S_E2 process has been proposed. The first case is the racemisation of some 5-substituted hydantoins (**9**) for which an observed k_{deut}/k_{rac} of 0.5 was found (Table 2).[34] Subsequent analysis has cast doubt on this result.[35]
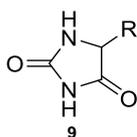
Table 2. Rate constants of H/D exchange and racemisation of hydantoins (**9**).[34]

R	k_{deut} (h^{-1})[a]	k_{rac} (h^{-1})[a]	k_{rac} (h^{-1})[b]
Ph	11.3	21.1	23.7
CH$_2$Ph	0.060	0.094	0.119

[a] determined in a mixture of D$_2$O phosphate buffer (pD 7.4, 0.1M, I = 0.22) and (d^6)DMSO in proportion 1:1 (*v*/*v*) at 50 °C.
[b] determined in a mixture of phosphate buffer (pH 7.4, 0.1M, I = 0.22) and DMSO in proportion 1:1 (*v*/*v*) at 50 °C

We note that the ratio k_{deut}/k_{rac} of 0.5 was found when comparing deuterated with non-deuterated buffer and that the ratio k_{deut}/k_{rac} is different when comparing racemisation and deuteration in the same deuterated buffer. Taken together with results from other studies this casts doubt on whether the S_E2 mechanism is really active here, leaving the S_E1 mechanism as the most likely mechanism.[35–37]

The second case involves the fast racemisation of thiohydantoin **10** which under acidic conditions has also been proposed to occur via an S_E2 mechanism on the basis of a comparison between rate constants for racemisation and deuteration (Scheme 10).[37]
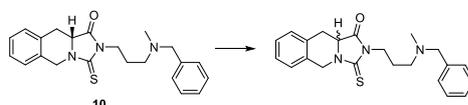
Scheme 10. Racemisation of thiohydantoin **10**.

Amfepramone (**11**, Table 4) racemisation rates were measured over a pH range of 5.5 to 8 and with varying phosphate buffer concentrations (as well as in human plasma).[38] Racemisation rate constants increased with increasing pH and with increasing buffer concentrations, but neither increase was linear. There is no obvious site for equilibrium deprotonation, making general-base catalysis resulting from rate-determining deprotonation the most likely mechanism for racemisation. This interpretation does not explain the non-linear behaviour, however. Compound **11** might also undergo equilibrium protonation on the nitrogen, followed by rate-determining deprotonation of the stereogenic centre. This mechanism can be ruled out in the pH range studied because general-acid catalysis was not observed.

Rate-determining deprotonation has also been proposed for racemisation of the fungicide agrochemical triadimefon (**12**).[39] Although buffer concentrations were not varied and general-base catalysis was therefore not proven, the site of H/D exchange, the kinetic isotope effect, and the ratio $k_{rac} / k_{H/D}$ were all in line with this hypothesis.
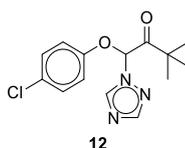
e) Reactions displaying both acid and base catalysis

When racemisation is studied over a wide range of pH, substrates often display pH-dependent racemisation mechanisms, potentially combining acid catalysis, base catalysis and uncatalysed racemisation. For systems displaying both acid and base catalysis but no uncatalysed reaction, a V-shaped pH-rate profile is found. For systems displaying acid catalysis, uncatalysed reaction and base-catalysed reaction, a U-shaped

pH-rate profile is found. Example compounds for which multiple catalytic regimes have been identified are reviewed here.

The pain medication ketorolac (**3**) was studied at 80 °C in a range of buffers with pHs spanning 1 to 11.[40] A U-shaped pH-rate curve (Figure 2) suggests maximum stability at intermediate pHs and supports both acid and base catalysed racemization. The pH-independent region extends from pH 2 to 8 and remarkably, considering that the pK_a of ketorolac is 3.5, shows no break at pH 3.5.
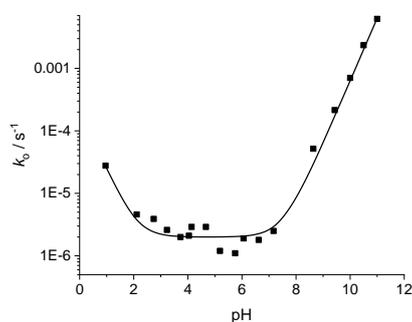
Figure 2. pH-rate profile for racemisation of ketorolac at 80 °C (Reproduced using data from Ref. [40])

Working at pH > 3.5, the authors observed general-acid catalysis when using buffers involving anionic bases but general-base catalysis when using the neutral base tromethamine (better known as TRIS). The authors attributed these observations to neutral bases not experiencing electrostatic repulsion with the carboxylate group, leading to general-base catalysis (Scheme 11a) and general-acid catalysis for anionic bases (Scheme 11b). We propose an alternative mechanism, involving equilibrium protonation of the carboxylate followed by rate-determining deprotonation of the stereogenic centre (Scheme 11c) which would similarly lead to a general-acid catalysis rate law. This proposal also explains why formate buffer can act either as general-acid or general-base catalyst depending on the pH at which the reaction is studied; at low pH, **3** is fully protonated so equilibrium protonation no longer plays a role and the rate law reverts to simple general-base catalysis below pH 3.5.
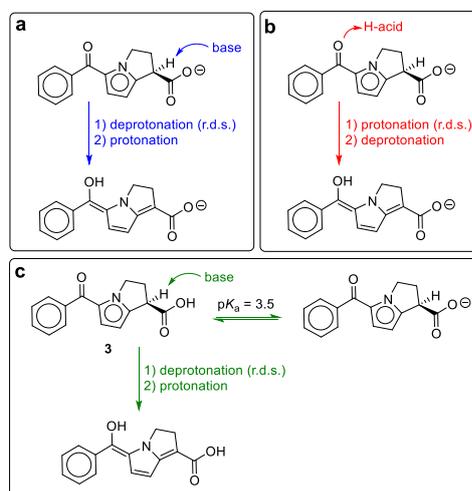
Scheme 11. Racemisation of **3**.

Chlortalidone, **7**, has both acid and base-catalysed routes of racemisation open to it (Scheme 12).[41] This leads to a V-shaped pH-rate curve for racemisation (Figure 3).
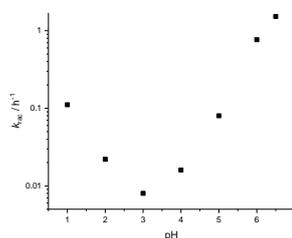
Figure 3. Dependence of rate constant of racemisation of **7** on pH (Reproduced using data reported in Ref. [41])

Under acidic conditions, the reaction is proposed to proceed via a carbocation that can add water. This cationic intermediate can also lead to formation of an imine.[42] The base-catalysed mechanism for racemisation is proposed to involve the hydroxyl group being deprotonated, promoting the fragmentation of the adjoining C-N bond, thereby opening the ring. The ring-opened form is stabilised by formation of a carbonyl and delocalised anion. The ring can then reclose via attack on either face of the carbonyl in the ring-opened form. The proposed mechanisms are reasonable and should result in specific-acid and specific-base catalysis rate laws. The literature does not report data for different buffer concentrations, so these expected rate laws cannot be confirmed.
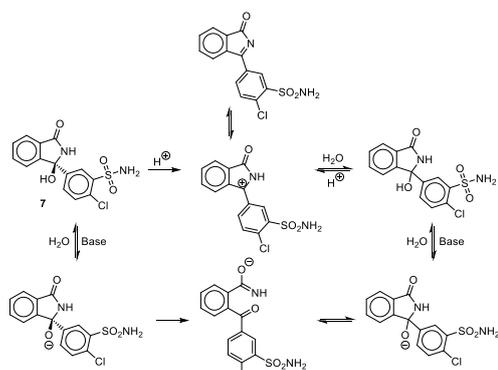
Scheme 12. Racemisation of **7** under acid- or base-catalysed conditions.

Several mechanisms for racemisation of oxazepam (**13**) have been proposed, including formation of an achiral ring-opened iminoaldehyde form (Scheme 13) from protonated **13**. [43] Kinetic data,[44,45] however, show that racemisation of **13** is inhibited at low pH, display a wide pH-independent range which does not involve general-acid catalysis, and a hydroxide-catalysed regime which ends in a plateau, attributed to deprotonated oxazepam not being a good substrate for hydroxide-catalysed deprotonation. Similarly, **13** (Scheme 13) can undergo base-catalysed racemisation via deprotonation, ring opening and ring reclosing.[45] This mechanism would give rise to a specific-base catalysis rate law, as observed. We note, however, the occurrence of kinetic equivalence between neutral **13** reacting with hydroxide ($k_{OH} \times [OH^-]$) and deprotonated **13** reacting via an alternative mechanism (k_{O^-}). Scheme 13 is in agreement with the kinetic data, and with previously determined pKa values,[46] but does not explain by which mechanism racemisation of neutral **13** occurs.
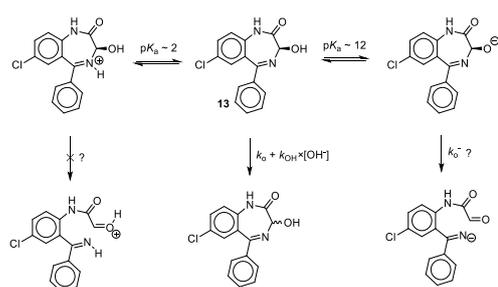
Scheme 13. Racemisation of **13** under acid- or base-catalysed conditions.

Meluadrine (**14**, Scheme 14) undergoes racemisation with a complex dependence on pH (Figure 4).[47] The reaction was studied at a range of pHs and temperatures and this permitted fitting a model to the data to obtain values for the dissociation constants for the phenol and amine and to compute rate constants for the racemisation of each of the species and finally to obtain activation energies that were in the 23 – 27 kcal/mol range. The range of possible mechanisms for racemisation (Scheme 14) is consistent with the observed variation of rate constants and, at low pH, the mechanism is analogous to the data and mechanism discussed for adrenaline **4** above.
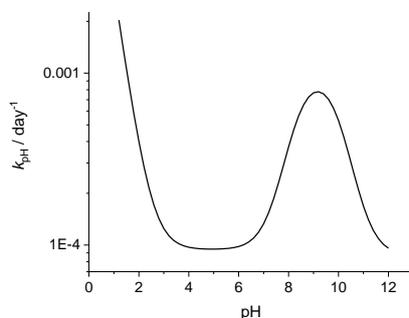
Figure 4. pH dependence of the rate constant for racemisation of **14** at 40 °C, simulated using the rate constants as reported.[47,48]
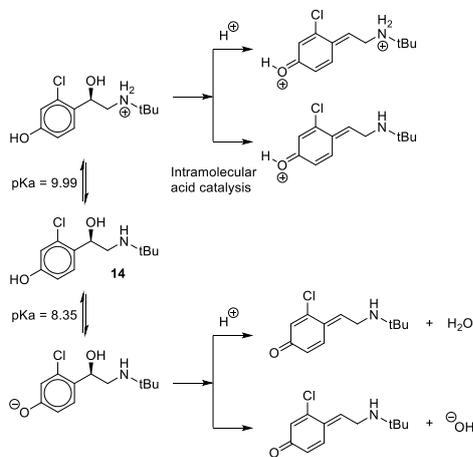
Scheme 14. Racemisation of **14**.

The rate of racemisation of mandelic acid (**15**, Table 5) was found to be faster than that for deuterium incorporation in D_2O under

acid conditions and this was attributed to the reaction proceeding via acid-catalysed dehydration.[49] At higher pHs, the two processes proceed at the same rate and therefore share the enol form as a common intermediate.

Thiazolinedione **16** (Table 4) is particularly sensitive to racemisation, even racemising in ethanol-hexane eluents during HPLC.[50] In aqueous solutions, a non-linear relationship with pH was found for the racemisation rate of related pioglitazone **17** (Table 4),[51] with the reaction proceeding faster at higher pH. The data suggest the reaction is subject to base-catalysed racemisation. The effect of buffer concentration was not studied for these compounds, but it is likely that base-catalysed racemisation proceeds via general-base catalysis forming an anion (likely of an aromatic type) in the rate-determining step. The observation by Welch et al. that racemisation in aqueous solutions cannot be stopped with addition of acid might suggest that **16** can also racemise via ring-opening in an acid-catalysed fashion.[50]

f) Other mechanisms

There are several other mechanisms by which compounds can be racemised and several are briefly reviewed here.

i) Tautomerism

Tautomerism can provide a low energy alternative to either a carbocation or carbanion. Translation of protons around a molecule may involve exchange with the solvent or direct transfer and can be acid or base catalysed, as shown for **6**. Ring chain tautomerism, as exemplified by the racemisation of **13**, is also a viable mechanism for racemisation. Tautomers and racemising enantiomers are both challenging problems for those trying to generate electronic representations of chemical structures, such as SMILES strings.[52] When placed in water, exchange between tautomeric forms is often facile and involves the molecule rapidly exchanging between forms that have different connectivity between the atoms, each of which would be represented by a different structural representation. The same problem occurs for molecules that can rapidly racemise; the two enantiomers can be represented by a different SMILES string. When considering interactions with a protein, both stereoisomers must be generated and evaluated, just as all tautomers must, regardless of which structural representation is provided as input by the user.

ii) Latent symmetry

There are some molecules that can undergo racemisation or enantiomerisation when the group that is exchanged is neither proton nor hydroxyl (the groups available in water). They can do this when the group that is exchanged is present in the molecule already. An example is provided by the molecule allantoin (**18**, Scheme 15) in which the attack of the NH_2 of the acyclic urea on the amide carbonyl can lead to urea exchange, which also corresponds to racemisation through repeated enantiomerisation.
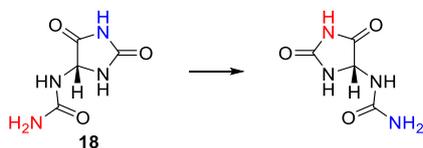
Scheme 15. Racemisation of **18**.

iii) Racemisation through heterolysis

A mechanistic probe of carbocation-mediated racemisation involving a leaving goup that is not hydroxide was provided by studies of the racemisation and solvolysis of the pentafluorobenzoate **19** in 50:50 (v/v) trifluoroethanol-water (Scheme 16).[53] This racemisation is presumed to proceed through the loss of the benzoate to form an ion pair involving carbocation **20** and the pentafluorobenzoate anion. The ion pair can either separate to form free ions, be intercepted by solvent or return to the starting material. If returning to starting material, in principle, the benzoate could attack each face with equal probability leading to racemisation. However, it was found that the microscopic rate constant for racemisation is actually six times lower than for [18]O scrambling and very similar to the microscopic rate constant for separation to free ions. These observations indicate that, most of the time, when the benzoate counterion does reform a bond to the carbocation, it does so with retention. This supports a view in which the ion pair intermediate in the solvent cage is held together quite rigidly and so attack from the two faces of the carbocation is not equally likely.
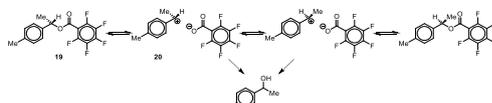
Scheme 16. Racemisation of **19**.

iv) Rotation about single bonds

Molecules that have resolvable enantiomers by virtue of hindered rotation about single bonds undergo racemisation when rotation around that bond occurs. This type of racemisation is intimately related to atropisomerism and has been studied computationally.[15,54] Calculations on **1** using the MMFF force field and at the B3LYP/6-31G** level complemented variable temperature NMR measurements that suggest a barrier to rotation of 35 kcal/mol.[22] Removal of the methyl on the imidazole ring was computed to lower the barrier to about 17 kcal/mol consistent with the observation of free rotation. Another combined experimental and computational study of **21** and **22** revealed the significant effect of ring expansion on racemisation rate (Table 3).[55]

Table 3. Computed[a] and measured barriers and half lives for racemisation of **21** and **22**.[55]

R	Computed barrier (kcal/mol)		Experimental $t_{1/2}$ (s)	
	21	**22**	**21**	**22**
H	16.9	20.5	0.2	1×10^2
Me	24.2	31.2	5×10^4	8×10^9

a) M06-2X/6-311++G(2d,3p)//B3LYP/5-31+G(d,p)

21   22
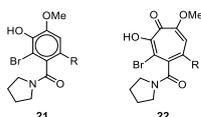
Tan and Paton have shown that the hole-catalyzed racemization of atropisomeric biaryls occurs via a two-step mechanism in a process controlled by frontier molecular orbitals.[56]

v) Mechanism not known

The stereochemical scrambling of vitamin C (**23**) was detected upon storage (in this case, the enantiomer is presumed to be biologically inert and to be rapidly excreted).[57] The process of racemisation was monitored through measurement of optical rotation. This showed that the rotation decreased from 25.55°C to 0°C over the course of 63 days stirring at room temperature (or 6 days at 80-90°C). This observation supported either complete racemisation or degradation to an achiral product (Scheme 17). NMR and IR analysis combined with quantum mechanical calculations supported the reactions shown.
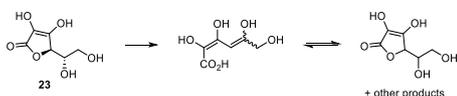
Scheme 17. Racemisation of **23**.

The racemisation of **24**, which occurs alongside hydrolysis, was studied using a triple-column HPLC technique.[58] The hydrolysis product is only observed at low pH. A common ring-opened intermediate for the two processes was proposed (Scheme 18).
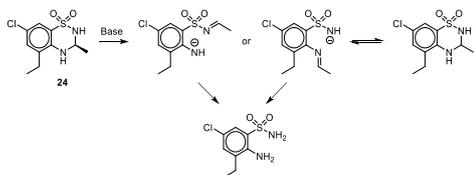
Scheme 18. Racemisation of **24**.

For epimerisation of related compound **25**, the same research group proposed a slightly more elaborate pH-dependent mechanism (Scheme 19).[59]
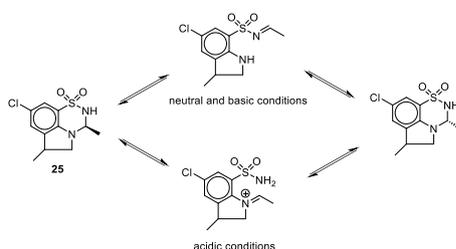
Scheme 19. Epimerisation of **25**.

This mechanism does not explicitly demonstrate the protonation and deprotonation processes. The proposed intermediate is in agreement with the observation that methylation of the sulfonamide-N in this class of compounds leads to inhibition of racemisation at neutral to high pH and an acid-catalysis rate law.[60] The mechanism is also in line with the observation of a lack of H/D exchange on the stereogenic centre in 50-50 CD_3OD-D_2O which indicates that there must be at least one pathway to racemisation that does not involve deprotonation of the stereogenic centre.[61] In general, the kinetics for racemisation for this class of compounds do not show a clear pH dependence.[58,61,62] The effects of changes in buffer concentration were not studied. In addition, there is a potential discrepancy between these data and the data published by Blaschke and co-worker whose kinetic data show a far stronger pH dependence of this reaction, potentially suggesting a specific-base catalysis rate law.[63] As a result, there is currently insufficient data to conclude with certainty what the detailed mechanism of racemisation is.

Similarly, a non-fragmenting quaternary ammonium ionization tag for mass spectrometric sequencing of peptides, based on the N-spiro proline residue (**26**), was found to quickly racemise and undergo H/D-exchange in 1% trimethylamine in D_2O.[64]

26
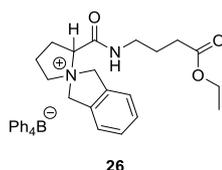
No kinetic data were reported, but it is likely that this reaction proceeds via general-base catalysis.

g) Racemisation of nicotine under pyrolysis conditions

Although most biological racemisations take place in aqueous conditions, the racemisation of one molecule (nicotine, **27**) under extreme heating and in the vapour phase has received a great deal of attention. In normal tobacco, **27** is predominantly the *S* enantiomer with estimates ranging from 0.03 % to about 0.2 to 0.3 % of *R*-**27** present.[65–67] However, in tobacco smoke, this

increases to about 3%. The variation between tobacco types was studied by GCMS and it was found that the *R* enantiomer was consistently below 0.5 % of total **27** in eight different brands and then 2.6 – 3.6 % in the smoke. Subsequent pyrolysis of *S*-**27** revealed that it is stable up to about 400°C and that decomposition, including racemization, occurred in the 450 to 550 °C range and up to 22 % of *R* was detected at the top of this temperature range. A similar effect occurred when tobacco was pyrolysed. It was suggested that a burning cigarette will contain zones at varying temperatures, with those adjacent to the burning area being in the 200 to 600°C range, enough to drive the observed racemization. Others have supported this view.[68] The racemization is presumed to proceed via homolysis of the CH bond at the stereogenic centre leading to a planar radical. Other studies had shown that when pure *S*-**27** is pyrolysed up to 1000°C that no *R*-**27** is observed, suggesting that the racemization in tobacco may rely on other material that is present in the tobacco.[69] It is likely that the heterogeneity of tobacco accounts for much of the divergence between measured amounts of racemization in each of the samples studied.

27
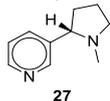
h) Racemisation in dynamic kinetic resolution

Methods have been developed that permit the stereoselective transformation of one enantiomer present in a mixture of enantiomers. This process is intrinsically inefficient because even if it works perfectly, any of the other enantiomer remains as waste. One way to avoid this waste is to simultaneously facilitate racemisation of the starting material. In this way, all of the material can be funnelled through the desired reaction and can yield one stereoisomer exclusively. This is dynamic kinetic resolution and is one of the situations in which racemisation is desirable.[2,3,19,70] If working with amino acids, efficiently racemising derivatives include: hydantoins, oxazolones and thioesters.[71] The topic has been reviewed elsewhere and here a few of the more recent and relevant publications are described, particularly those that have sought to understand the racemisation process. These resolutions often use enzymes in which case the racemization has to be compatible with predominantly aqueous solutions.

In a combined experimental and computational study, the computed deprotonation energies of N-protected thioester amino acid derivatives **28a-h** were compared with the measured rates of racemisation in DMSO-d6 – CD$_3$OD (5:1 v/v).[71] The base in this case was 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) present at 0.5 equivalents. Racemisation was followed by polarimetry and rate constants were found to correlate with computed enthalpies for proton transfer to either of the two bases at the B3LYP/6-311+G** level of theory (Figure 5). H/D exchange of more reactive substrates was also followed in i-PrOH in the presence of 0.5 equivalents of trioctylamine, but these data were not correlated

with computed data. Thioesters were demonstrated to be far more racemisation-prone than oxoesters.
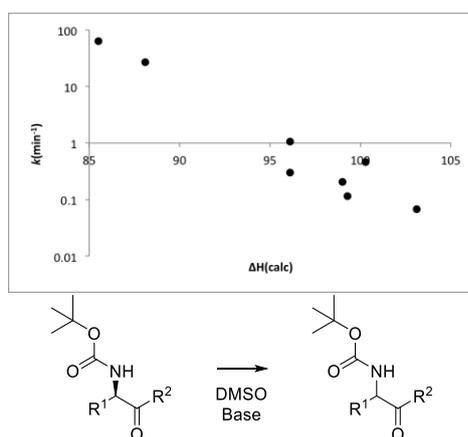
Figure 5. Rate constant for racemisation plotted against computed enthalpy of deprotonation for compounds **28** (**a**: R^1 = Ph, R^2 = SEt; **b**: R^1 = CH$_2$Ph, R^2 = SEt; **c**: R^1 = CH$_2$Ph, R^2 = SEt; **d**: R^1 = CH$_2$Ph, R^2 = SCH$_2$Ph; **e**: R^1 = CH$_2$CH$_2$Ph, R^2 = SEt; **f**: R^1 = CH$_2$CH$_2$Ph, R^2 = SCH$_2$Ph; **g**: R^1 = CH$_2$CH$_2$CH$_2$Me, R^2 = SEt; **h**: R^1 = CH$_2$CH$_2$CH$_2$Me, R^2 = SCH$_2$Ph

An attempted dynamic kinetic resolution of benzoin with lipases required rapid racemisation and so solvent combinations were investigated, using a zirconium-containing silicate, Zr-TUD-1, as a heterogeneous catalyst for racemisation.[72] The addition of small amounts of water to solutions in 2-methyltetrahydrofuran rapidly diminished the racemisation activity whereas the decrease was much less marked in toluene.

A biologically inspired racemization was established for the funneling of unwanted *R*-nornicotine (**29**) through to racemic nornicotine (Scheme 20).[73] This employed pyridoxal (or pyridoxal phosphate, a known cofactor, see also Section 4b). Attempts to use a smaller mimic for pyridoxal, such as formaldehyde, butyraldehyde, benzaldehyde salicylaldehyde and 4-pyridinecarboxaldehyde all saw no racemization but pyridoxal caused racemization to 2.3 % e.e. after 3 hours at 100˙C in pH 4.5 buffer. This observation suggests that both the pyridine nitrogen and adjacent OH are required. Performing the reaction in D$_2$O led to deuteration of all of the hydrogens on the pyrrolidine ring supporting the rapid formation and isomerization of enamines, imines and iminiums. The pH dependence saw the most effective reaction at about pH 5. A mechanism was proposed going via the intermediacy of an achiral iminium intermediate (Scheme 20).
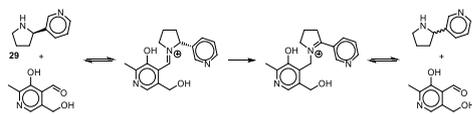
Scheme 20. Racemisation of **29** catalysed by pyridoxal.

Another example of racemization in mixed aqueous systems is the synthesis (via dynamic kinetic resolution) of *R*-bupivacaine, **30**.[74] Ethylene glycol with 10% v/v of water at 138°C for 9 hours was found to give complete racemization.

The non-steroidal anti-inflammatory drug naproxen has a eutomer (the *S*-enantiomer **31**) that is 28 fold more active than the distomer. Enzymatic resolution is an attractive option for preparing **31** in a stereoselective fashion and therefore racemization protocols were sought. A membrane bioreactor was considered in order to permit harsh conditions for racemization in a way that would not cause degradation of the enzyme.[75] On one side of the membrane is an aqueous solution containing enzyme dispersed in isooctane and on the other is sodium hydroxide in methanol dispersed in isooctane (Figure 6). This bioreactor system was able to prepare a single enantiomer of **31** in about 60% yield.
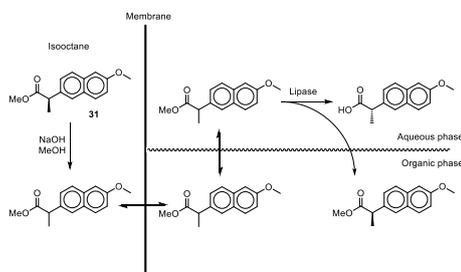
Figure 6. Chemical overview of a membrane bioreactor featuring racemisation of methyl esters of **31**.

3. Techniques for studying racemisation and kinetic data

Typically, the mechanistic studies discussed above were supported by kinetic data. We here discuss how such kinetic data can be acquired and bring together experimentally determined rate constants for racemization under aqueous conditions.

a) Experimental techniques for studying racemisation

Several techniques have been used to study racemisation and developing new techniques has often been a key precursor to new discoveries in racemisation.

Nuclear magnetic resonance (NMR) spectroscopy has been a popular tool for studying racemisation, particularly for centers of type $R^1R^2R^3CH$ that undergo isotopic exchange of hydrogen and deuterium.[34,79–84] Mass spectrometry can also be used for this type of racemisation.[82] In both cases, the rate of appearance of deuterated substrate is monitored and related to the rate of racemisation, if the mechanism is well understood. NMR can also be used to monitor racemisation by the addition of a chiral shift reagent to provide differentiation between enantiomers[53] as long as the chiral shift reagent does not affect the racemisation reaction. Variable temperature NMR experiments can be used to study atropisomerism and racemisation of chiral axes.[22,55]

Circular dichroism (CD) is intrinsically linked to the chirality of species being studied. A signal is only present when there is an excess of one enantiomer. Thus, the process of racemisation can be monitored by the disappearance of a CD signal. The technique requires the compound to have a suitable chromophore in the UV-visible region of the spectrum. This technique can be applied without the need for isotopically labelled buffers and therefore avoids any solvent kinetic isotope effects induced by the forced use of deuterated buffers. Alternatively, the ability to use both non-deuterated and fully deuterated buffers provides convenient direct access to the solvent kinetic isotope effects. CD has been successfully applied to measure the racemisation of several systems.[85–87] Analogous to CD spectroscopy, the process of racemization can also be monitored using the rotation of plane-polarized light using polarimeters and measurements such as $[\alpha]_D$.[57,71]

Chiral chromatographic methods have proved of significant utility for studying racemization. This includes gas chromatography for studying systems such as nicotine in the vapour phase.[65] Liquid chromatographic techniques such as chiral HPLC are used widely for studying stereochemistry and racemisation.[33,38,50,88] Typical approaches involve either full separation of the enantiomers or peak shape analysis for compounds racemising in the HPLC eluent.[10,89] A liquid chromatography method has been adapted to include an artificial membrane intended to mimic cell membranes, an environment that many drugs partition into.[90] This was intended to ensure that the measurements more closely mimic the in vivo environment. A modified liquid chromatographic method saw the use of supercritical liquids.[91]

A triple column technique was developed to study racemisation in stopped flow-type conditions.[58] The first column is chiral and used to separate enantiomers while the third is also chiral and is used to analyse stereoisomers. The second column is achiral and is used to expose the compounds to conditions that may promote racemisation. This technique was used to study the racemisation of **24**, which occurs alongside hydrolysis (vide supra). It should be noted that on-column racemisation kinetics are typically affected by the stationary phase and are not solely representative of the kinetics in the HPLC eluent.

Capillary electrophoresis employing chiral modifiers can also be used to study racemisation. For example, the use of cyclodextrins as chiral modifiers in capillary electrophoresis has been explored and used to investigate the racemisation of adrenaline (particularly concerned with its use as an additive to local anaesthetics).[92] As a further example, on-column racemisation capillary electrophoresis employing chiral cyclodextrin additives was performed in a quest to find analogues of thalidomide (**32**) that are less likely to racemise but the two molecules considered

(**33** and **34**) actually racemised more quickly than thalidomide itself (Figure 7).[10]

ΔG‡(pH 8.0) =101.7 kJ/mol

ΔG‡(pH 8.0) = 96.8 kJ/mol

ΔG‡(pH 9.3) = 93.0 kJ/mol   ΔG‡(pH 9.3) = 91.5 kJ/mol

Figure 7. Free energies of activation for racemisation of **32**, **33** and **34**.

b) Kinetic data for racemisation of synthetic molecules in aqueous conditions

Racemisation has been known about as a problem for synthetic molecules when placed in biological media for a long time. It is therefore surprising how few relevant measurements have been made of the rate of racemisation. We have summarized data of sufficient quality in Tables 4 and 5 for compounds racemising via general-base catalysis and for compounds racemising via other mechanisms, respectively. In Table 4, those compounds that likely proceed via the intermediacy of a carbanion have been processed to provide a consistent set of second-order rate constants k_{gb} for general-base catalysis, where possible. This entails considering the protonation state of the molecule that is most likely to undergo rapid racemisation (e.g., for compounds with a basic amine adjacent to the stereocentre, the base will likely be protonated whereas for those with a carboxylic acid, this will be neutral). The rate constant is corrected for the concentration of this "active" protonation state. The rate constant is also corrected for the active component of the buffer and for changes in temperature using the Eyring equation in order to obtain values for racemisation at 37 ℃ using $\Delta^{\ddagger}H^{\circ}$ of 22.53 kcal mol^{-1}.[34]

The harmonised rate constants collected in Table 4 allowed the development of a predictive model for racemisation kinetics (see Section 5).

Table 4. Rate constants measured in aqueous (or predominantly aqueous) conditions for racemisation reactions proceeding via general-base catalysis.

Compound	Ref	$k_{measured}$ [a]		Mechanism (k_{gb} for general-base-catalysed racemisation via carbanion at 37˚C[b])	Reaction conditions
Amfepramone **11**	[80]	[phosphate]/M 6.7×10^{-4} 1.3×10^{-3} 2.0×10^{-3} 1.3×10^{-2} 6.7×10^{-2} 0.133 0.2	k_{deut} (s^{-1}) 150×10^{-9} 2.7×10^{-6} 3.3×10^{-6} 31×10^{-6} 160×10^{-6} 360×10^{-6} 650×10^{-6}	R^{1}R^{2}R^{3}CH-GBC (4.06×10^{-3} M^{-1}s^{-1})	D$_2$O, pD 7.4, 37˚C, I = 0.43 M
Cathinone **35**	[80]	[phosphate]/M 6.7×10^{-2} 0.1 0.133 0.167 0.2	k_{deut} (s^{-1}) 47×10^{-6} 65×10^{-6} 81×10^{-6} 98×10^{-6} 130×10^{-6}	R^{1}R^{2}R^{3}CH-GBC (8.35×10^{-3} M^{-1}s^{-1})	D$_2$O, pD 7.4, 37˚C, I = 0.43 M
Ketorolac **3**	[40]	[phosphate]/M 0.04 0.02 0.004	k_{rac} (s^{-1}) 5.0×10^{-9} 2.8×10^{-9} 1.3×10^{-9}	R^{1}R^{2}R^{3}CH-GBC (7.27×10^{-4} M^{-1}s^{-1})[c]	H$_2$O, pH 7.2, 25˚C
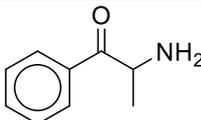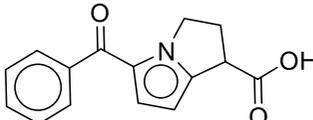
Structure	Ref	Rate	Mechanism	Conditions
Thiazolinedione **16**	[50]	$k_{rac} = 9.6 \times 10^{-4}$ s^{-1} (from reported half life of 0.2 hours)	R^{1}R^{2}R^{3}CH-GBC likely	H$_2$O:DMSO 1:1, presence of buffer not reported; temperature not reported
Pioglitazone **17**	[51]	$k_2 = 1.93 \times 10^{-3}$ M^{-1}s^{-1} d	R^{1}R^{2}R^{3}CH-GBC likely (3.72 $\times 10^{-2}$ M^{-1}s^{-1}) d	H$_2$O, pH 7.4, 37˚C, 25 mM phosphate buffer
Thalidomide **32**	[93]	$k_2 = 6.6 \times 10^{-3}$ M^{-1}s^{-1}	R^{1}R^{2}R^{3}CH-GBC (8.34 $\times 10^{-5}$ M^{-1}s^{-1})	H$_2$O, pH 7.4, 37˚C, phosphate buffer, I = 0.8 M
5-Methylhydantoin **36**	[34]	$k_2 = 1.4 \times 10^{-5}$ M^{-1}s^{-1}	R^{1}R^{2}R^{3}CH-GBC (1.83 $\times 10^{-5}$ M^{-1}s^{-1})	H$_2$O:DMSO 1:1, pH 7.4, 37˚C, phosphate buffer, I = 1.1 M
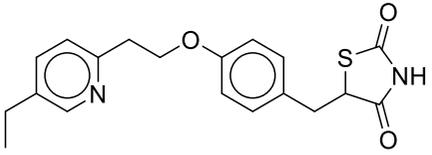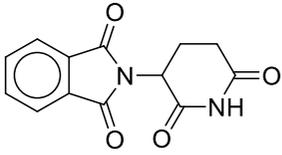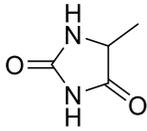
17
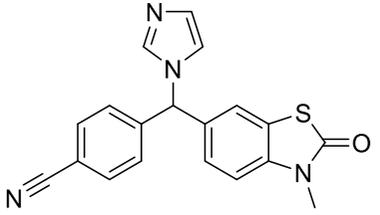
Structure	Ref	Kinetic data	Rate constant	Conditions
37	[88]	$k_{rac} = 9.7 \times 10^{-8}$ s^{-1}	R^{1}R^{2}R^{3}CH-GBC (3.33 x 10^{-5} M^{-1}s^{-1}) ^{d}	H$_2$O:EtOH 9:1, pH 7.4, 37˚C, 0.05 M phosphate buffer
Clopidogrel **38**	[81]	[Phosphate]/M k_{rac} (s^{-1}) 0.1 0.20 x 10^{-6} 0.2 0.21 x 10^{-6} 0.3 0.22 x 10^{-6}	R^{1}R^{2}R^{3}CH-GBC (2.46 x 10^{-6} M^{-1}s^{-1})	H$_2$O:MeOH 1:1, pH 7.4, 37˚C, phosphate buffer, I = 0.78 M
39	[85,94]	e	R^{1}R^{2}R^{3}CH-GBC (R=H: 1.64 x 10^{-5} M^{-1}s^{-1} R=p-OH: 6.01 x 10^{-6} M^{-1}s^{-1} R=p-Me: 1.03 x 10^{-5} M^{-1}s^{-1} R=p-F: 2.20 x 10^{-5} M^{-1}s^{-1} R=p-Cl: 7.47 x 10^{-5} M^{-1}s^{-1} R=m-F: 1.40 x 10^{-4} M^{-1}s^{-1} R=m-Cl: 1.60 x 10^{-4} M^{-1}s^{-1} R=p-CF$_3$: 5.15 x 10^{-4} M^{-1}s^{-1})	H$_2$O, pH 7.4, 37˚C, phosphate buffer, I = 1.0 M
40	[85,94]	e	R^{1}R^{2}R^{3}CH-GBC (7.87 x 10^{-6} M^{-1}s^{-1})	H$_2$O, pH 7.4, 37˚C, phosphate buffer, I = 1.0 M

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Structure	Ref		$R^1R^2R^3CH$-GBC	Conditions
41	[85,94]	e	$R^1R^2R^3CH$-GBC (2.74×10^{-3} $M^{-1}s^{-1}$)	H_2O, pH 7.4, 37˚C, phosphate buffer, I = 1.0 M
42	[85,94]	e	$R^1R^2R^3CH$-GBC (5.62×10^{-5} $M^{-1}s^{-1}$)	H_2O, pH 7.4, 37˚C, phosphate buffer, I = 1.0 M
43	[35,85]	e	$R^1R^2R^3CH$-GBC (R=phenyl: 7.16×10^{-5} $M^{-1}s^{-1}$ R=p-fluorophenyl: 8.92×10^{-5} $M^{-1}s^{-1}$ R=p-methoxyphenyl: 5.84×10^{-5} $M^{-1}s^{-1}$ R=2-tetrahydrofuranyl: 1.47×10^{-4} $M^{-1}s^{-1}$ R=OH: 2.81×10^{-4} $M^{-1}s^{-1}$ R=m-pyridyl: 1.23×10^{-4} $M^{-1}s^{-1}$ R=2-N-methylpyrrolyl: 7.92×10^{-5} $M^{-1}s^{-1}$)	H_2O, pH 7.4, 25˚C, phosphate buffer, I = 1.0 M
44	[35,85]	e	$R^1R^2R^3CH$-GBC (R=phenyl: 4.30×10^{-5} $M^{-1}s^{-1}$ R=p-fluorophenyl: 3.89×10^{-5} $M^{-1}s^{-1}$ R=p-methoxyphenyl: 3.14×10^{-5} $M^{-1}s^{-1}$ R=m-pyridyl: 4.19×10^{-4} $M^{-1}s^{-1}$)	H_2O, pH 7.4, 25˚C, phosphate buffer, I = 1.0 M
45	[35,85]	e	$R^1R^2R^3CH$-GBC (26.9×10^{-3} $M^{-1}s^{-1}$)	H_2O, pH 7.4, 25˚C, phosphate buffer, I = 1.0 M

45				
 45	[34]	$k_{rac} = 2.56 \times 10^{-3}\,s^{-1}$	$R^1R^2R^3$CH-GBC	H_2O:DMSO 1:1, pH 7.4, 40˙C, 0.1 M phosphate buffer
 46	[85,95]	e	$R^1R^2R^3$CH-GBC (R=H: $2.81 \times 10^{-3}\,M^{-1}s^{-1}$ R=phenyl: $5.14 \times 10^{-3}\,M^{-1}s^{-1}$ R=CH$_2$-indolyl: $2.43 \times 10^{-3}\,M^{-1}s^{-1}$ R=CH$_2$CH$_2$SMe: $9.41 \times 10^{-3}\,M^{-1}s^{-1}$)	H_2O, pH 7.4, 37˙C, phosphate buffer, I = 0.9 M
 47	[85,95]	e	$R^1R^2R^3$CH-GBC ($7.66 \times 10^{-2}\,M^{-1}s^{-1}$)	H_2O, pH 7.4, 37˙C, phosphate buffer, I = 0.9 M
 48	[96]	$k_{rac} = 4.81 \times 10^{-5}\,s^{-1}$ (from reported half life of 4 hours)	$R^1R^2R^3$CH-GBC	H_2O, pH 7.4, 37 °C, 0.01 M phosphate buffer
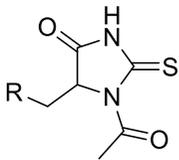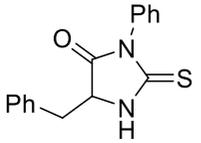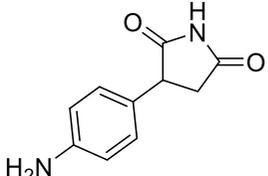
Compound	Ref	k_{rac}	Mechanism	Reaction conditions
49	[96]	$k_{rac} = 6.42 \times 10^{-5}\,s^{-1}$ (from reported half life of 3 hours)	$R^1R^2R^3CH$-GBC	H_2O, pH 7.4, 37 °C, 0.01 M phosphate buffer
Econazole **50**	[96]	$k_{rac} = 7.29 \times 10^{-5}\,s^{-1}$ (from reported half life of 2.64 hours)	$R^1R^2R^3CH$-GBC	H_2O, pH 7.4, 37 °C, 0.01 M phosphate buffer

a. Rate constants are as reported for the indicated reaction conditions.
b. Rate constants in brackets are extracted second-order k_{gb} for general-base-catalysed racemisation by phosphate via a carbanion at 37˚C. These rate constants have been corrected to represent the hypothetical process involving fully protonated substrate, with concentrations corrected to represent only the basic component of the buffer where necessary, and corrected for temperature changes using the Eyring equation where necessary.
c. Rate constant not corrected for temperature but reported as at 25˚C.
d. Assuming no uncatalysed reaction.
e. Second-order rate constants were determined explicitly in the original reference.

Table 5. Rate constants measured in aqueous (or predominantly aqueous) conditions for racemisation reactions not proceeding via general-base catalysis.

Compound	Ref	$k_{measured}$	Mechanism	Reaction conditions

		k_{rac}	Mechanism	Conditions
 9-hydroxyrisperidone **6**	[31]	$k_{rac} = 4.47 \times 10^{-5}\ s^{-1}$	GAC (imine-enamine tautomerism)	H_2O, pH 2.5, 37˚C, 0.01 M phosphate buffer
 Ibutilide **5**	[29,97]	NA	AC (dehydration and/or intramolecular attack)	H_2O, several pH and temperature
 Adrenaline **4**	[28]	$k_{rac} = 6.83 \times 10^{-6}\ s^{-1}$	AC (dehydration)	H_2O, 1.0 M HCl, 30˚C
 Allantoin **18**	[98]	NA	Intramolecular attack and/or $R^1R^2R^3CH$-GBC	H_2O, neutral pH, phosphate buffer
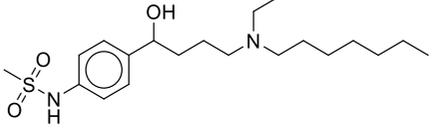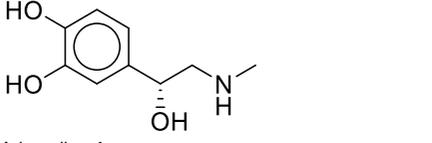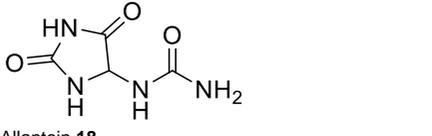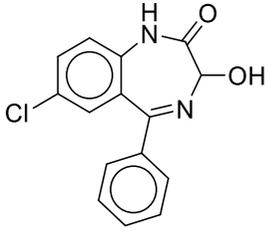
Oxazepam **13**	[45]	$k_{rac} = 3.85 \times 10^{-3}$ s^{-1}		H$_2$O, pH 7.5, 23°C, 0.1 M Tris-HCl buffer
Chlorthalidone **7**	[41]	$k_{rac} = 4.24 \times 10^{-4}$ s^{-1}	AC (dehydration) BC (via ring opening)	H$_2$O, pH 6.5, 22°C, Briton-Robinson buffer
Thioridazine **51**	[99]	No racemisation observed		H$_2$O, pH 5, 7 and 8.5, 4°C, 0.05M phosphate buffer
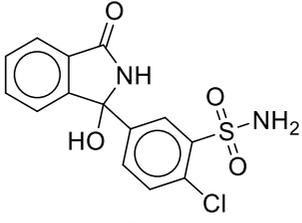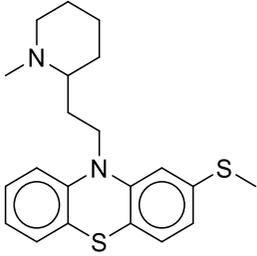
Mandelic acid **15**	[49]	$k_{rac} = 3.54 \times 10^{-5}$ s^{-1}	multiple reaction pathways	H$_2$O, 2.42M HCl, 138.4˚C
52	[4]	R=CH$_2$OH: 15.5×10^{-6} s^{-1} R=CH$_2$OtBu: 10.0×10^{-6} s^{-1} R=CH(CH$_3$)OH: 3.98×10^{-6} s^{-1} R=CH(CH$_3$)OtBu: 2.08×10^{-6} s^{-1} R=CH$_2$CH$_2$SMe: 3.08×10^{-6} s^{-1} R=CH$_2$Ph: 2.35×10^{-6} s^{-1} R=CH$_3$: 1.95×10^{-6} s^{-1} R=CH(CH$_3$)CO$_2$H: 4.31×10^{-6} s^{-1} R=CH$_2$CO$_2$H: 4.00×10^{-6} s^{-1} R=CH$_2$CH(CH$_3$)CO$_2$H: 1.46×10^{-6} s^{-1} R=CH$_2$CH$_2$CO$_2$H: 1.04×10^{-6} s^{-1} R=CH$_2$CH$_2$CH$_2$CO$_2$H: 0.63×10^{-6} s^{-1} R=cyclic-CHCH$_2$CH$_2$CONH: 0.19×10^{-6} s^{-1}		
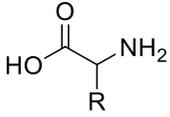
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4. Biological Racemisation

a) Racemisation in plasma, serum albumin solutions and in the presence of cyclodextrins and liposomes

The racemisation of thalidomide (**32**) has been studied in a range of environments in order to obtain a detailed understanding of the toxicity of this most infamous of chiral molecules.[93,100] Studies in the 1980s measured the half life for racemisation in pH 7.4 0.067 M phosphate buffer at 37˚C to be 160 minutes (2.7 hours),[100,101] in reasonable agreement with the kinetic data by Testa et al.[93] In plasma, by contrast, racemisation was *complete* in 2 hours.[100] The racemisation of each enantiomer separately was also studied and revealed that in phosphate buffer the half life is 260 – 290 minutes whereas in human citrated plasma the (+) enantiomer has a half life of 11.5 minutes and the (-) enantiomer 8.3 minutes suggesting a more rapid racemisation for the (-) enantiomer, as was also the case in rabbit plasma. Analogously, in defatted human serum albumin made up in phosphate buffer, the (+) enantiomer had a half-life of 18.5 minutes and the (-) enantiomer 9.5 minutes. Processes may be operational in plasma that do not occur in the serum albumin or citrated plasma; in both of these media, processes that do not occur in buffer alone lead to faster racemisation. For the imidazole-containing aromatase inhibitor, **21**, there was found to be no change in racemisation half life on going from buffer solutions to bovine serum albumin solutions.[88] The racemisation of thiazolinedione **16** in human plasma and in dog plasma was found to occur within minutes.[50] Similarly, racemisation of (+)-econazole administered to rats occurs rapidly with a half life of 1.24 hours, i.e. approximately two times faster than in 0.01 M phosphate buffer.[96] Human serum albumin slowed down racemisation of ethiazide and epimerisation of etoposide but accelerated epimerisation of carbenicillin,[102] showing that serum albumins do not necessarily exert analogous effects on all chiral pharmaceuticals.

The effect of adding cyclodextrins on racemisation of **11** was also investigated with the expectation that formation of inclusion complexes would have a protective effect. The cyclodextrins actually exerted a rather mixed effect with α-, β- and γ-cyclodextrin causing faster racemization. Acetylated and hydroxypropylated versions of some of the cyclodextrins also had the same effect. By contrast, the sulfobutylether-derivatised and methylated forms of β-cyclodextrin had a protective effect. It was speculated that this derivative benefits from electrostatic effects that favour formation of the inclusion complex.

Racemisation of **7** was found to be retarded by liposomes.[41] The data were analysed in terms of what is essentially a pseudophase model[103] showing that racemisation is almost 7-fold slower in the liposomes than free in solution.

b) Enzymes to catalyse racemisation

In the vast majority of cases, naturally occurring amino acids are found in the L-form. Nevertheless, in certain cases, D-amino acids are also found. The strategies used in nature to achieve racemisation can shed light on approaches that chemists could consider, especially in enzymatic dynamic kinetic resolutions that are likely to require racemisation in conditions compatible with the enzymes that undertake the chiral resolution. Given that all enzymes are themselves chiral, they are unlikely to achieve rigorous racemization to a 50:50 mixture of R:S.

The level of D-amino acids for Ser, Ala, Pro, Glu and Asp present in proteins in eubacteria (both gram positive and negative), archaea and eukaryotes was measured.[104] Most amino acids were present in the D configuration at below 1 %. D-Pro was present at less than 1 % in all organisms whereas D-Asp was present above this level in most of them. D-Ala accounted for above 5% of the Ala present in several of the gram-negative bacteria and one of these also had above 20 % of D-Glu. D-Glu was elevated to 7 % in one of the gram-positive strains that also saw 2 % of D-Ser. The results suggest that the tendency to have significant concentrations of D-amino acids changed as eubacteria evolved towards archaea and eukaryotes. The unnatural enantiomer of alanine, D-Ala, is required in the construction of the peptidoglycan component of the cell wall of bacteria suggesting that an alanine racemase is likely to be essential.[105] The absence of a homolog of alanine racemase in humans has seen this class of enzymes studied intensely as a potential source of a breakthrough in antibacterial research, including in drug resistant strains of C. difficile and against strains of B. anthracis that are potential biological weapons.[106,107] Indeed, S. coelicolor engineered to lack alanine racemase required the exogenous addition of D-Ala. Methods to study the racemase activity in S. Lavendulae and E. Coli using circular dichroism have been developed.[105,108] However, alternative routes for the production of D-Ala take over when alanine racemase is mutated in M. smegmatis[109] and in Chlamydia pneumonia.[110]

Alanine racemase employs a so-called two-base mechanism in which an active site Lys and Tyr are the key catalytic residues.[111] Hydrogen kinetic isotope effects (KIE) changing the isotope at C_α as well as the isotope in the solvent were measured (Figure 8). These revealed a divergence between isotope effects for the two enantiomers; the KIE for D-Ala reduced in D_2O, whereas that for L-Ala did not and solvent isotope effects for the D to L transformation were higher than those for the reverse reaction. These differences were interpreted as supporting a two-step mechanism through a planar anionic intermediate (Scheme 21) that can protonate on either face. If Tyr 265 is the proton source then L-Ala is formed, whereas if Lys 39 is the proton source D-Ala is formed.
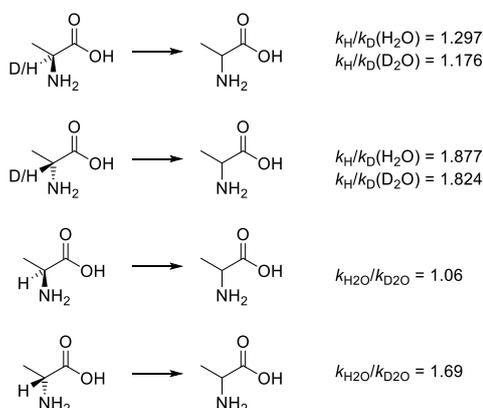
Figure 8. Hydrogen kinetic isotope effects on reactions catalysed by alanine racemase.

$k_H/k_D(H_2O) = 1.297$
$k_H/k_D(D_2O) = 1.176$

$k_H/k_D(H_2O) = 1.877$
$k_H/k_D(D_2O) = 1.824$

$k_{H2O}/k_{D2O} = 1.06$

$k_{H2O}/k_{D2O} = 1.69$
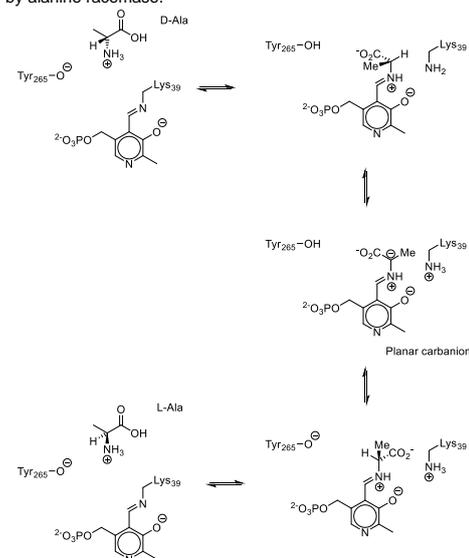
Scheme 21.

The effect of the pyridoxal cofactor on the carbon acidity of glycine was studied to understand its effect in the racemase enzymes.[112] The pKa of the C_α in several protonation states of the cofactor-glycine conjugate reveal that the most acidic form is the one in which both nitrogens, the carboxylic acid and the phenol are all protonated (Figure 9).[112] Deprotonation of the carboxylic acid and phenol make the C_α less acidic. However, the crystal structure of the enzymes that use pyridoxal are interpreted as being best able to stabilise the form in which the carboxylic acid and phenol are deprotonated. It is therefore suggested that it is this form that is the key intermediate in these reactions. QM/MM studies (AM1 parameterised to reproduce higher levels of theory was used for the QM part) using phenol as a model for

a key catalytic site tyrosine suggest that the pKa for deprotonation of alanine at C_α is lowered by about 22 units by the presence of the pyridoxal and its surroundings.[113] Further computational studies investigated the reaction of alanine racemase in gas phase, water, with PLP in its unprotonated and protonated form and in the enzyme.[114] These calculations (Table 6) suggested that enzyme catalysis arises from approximately equal contributions from the co-factor and interactions with the enzyme.

pK$_a$ = 6   pK$_a$ = 11   pK$_a$ = 17
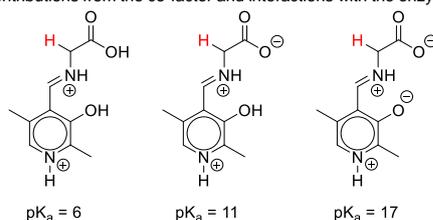
Figure 9. Acidity of the C_α proton in a range of protonation states of the glycine complex with pyridoxal.

Table 6. Computed and experimental free energy barriers to the uncatalysed, pyridoxal- and alanine racemase-catalysed racemisations of alanine.

System	ΔG‡(computed)	ΔG‡(experiment)[111,115]
Uncatalyzed	32.7 kcal/mol	30.4 kcal/mol
Pyridoxal (nitrogen protonated)	-	25.5 kcal/mol
Pyridoxal (nitrogen unprotonated)	26.3 kcal/mol	>25 kcal/mol
Alanine Racemase	18.6 kcal/mol	12.8 kcal/mol

Serine racemase is active in human and other mammals and its product, D-Ser, plays a role in neurotransmission and memory.[116] Like alanine racemase, it employs a pyridoxal co-factor. QM/MM methods employing AM1 parameterised to reproduce DFT energetics and geometries were used to study the action of this racemase, which acts in a dimeric form (Table 7). A steered molecular dynamics simulation followed the reaction coordinate for racemisation and revealed barriers in excellent agreement with experiment. These support the co-factor being present in its non-protonated form and the reaction proceeding in a way that sees the hybridisation of C_α go from sp3 to sp2 and back again, in line with an intermediate carbanion. The residues responsible for protonating each of the two faces are Lys 56 and Ser 84. These play roles in Serine racemase that are analogous to those for Lys39 and Tyr 265 in Alanine racemase, shown in Scheme 21.

Table 7. Computed and experimental free energy barriers to the enantiomerisation reactions catalysed by serine racemase.

Reaction direction	ΔG‡(computed)	ΔG‡(experiment)[117]
L-Ser to D-Ser	20.7 kcal/mol	19.1 kcal/mol
D-Ser to L-Ser	17.4 kcal/mol	18.3 kcal/mol

Racemase (epimerase) enzymes that do not require pyridoxal include the diaminopimelic acid epimerase found in M.

tuberculosis and proline racemase.[118] It was found (via mutation and chemical transformation) that there are two key cysteine residues responsible for the stereochemical scrambling in these enzymes (Scheme 22). A computational study investigated the mechanism of action of proline racemase as well as the process that operates in aqueous solution.[119] QM/MM calculations using AM1 parameterised to reproduce higher levels of theory suggested that neither the uncatalysed nor catalysed reaction involve a stable anionic intermediate because the barrier to reprotonation is negligible. The catalytic effect of the enzyme amounts to 14 kcal/mol of barrier lowering thanks to a tight network of hydrogen bonds that are poised to stabilise the transition state of the reaction. Neighbouring residues stabilise the system during movement of the negative charge from one Cys thiolate to another. A definitive explanation for the surprising acidity of the thiols was not provided.
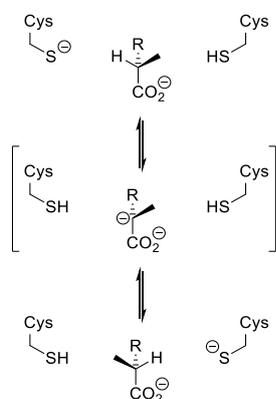
Scheme 22.

The antibiotic molecule D-cycloserine is produced by several strains of Streptomyces. A key step in its formation is stereochemical inversion, likely in intermediate **53**, and a putative enzyme for this (DcsC) was identified and isolated (Scheme 23).[120] It was surprising that the K_M and k_{cat} values for the L to D process (110 mM and 158 s^{-1}) differed significantly from those for the D to L process (17 mM and 29 s^{-1}). A double base thiol-thiolate mechanism was supported by the observation of irreversible inhibition by an epoxide (**54**) and by site-directed mutagenesis of key cysteines.[120,121]
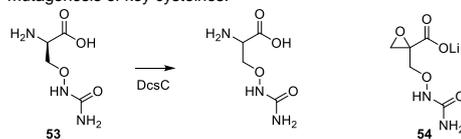
Scheme 23.

In order to understand the action of mandelate racemase, which racemises mandelic acid, the uncatalyzed reaction in aqueous buffers was studied alongside reactions with a range of additives that support active site glutamate and histidine residues acting in their protonated forms (Figure 10).[122]
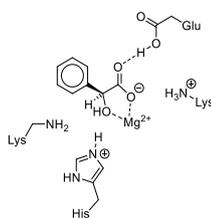
Figure 10. Proposed active site protonation states for mandelate racemase.

c) Racemisation (or epimerisation) of proteins and amino acids

The uncatalysed racemisation of amino acids is generally very slow and this can be a useful feature to facilitate the dating of proteinaceous material.[4,5] Consequently, the influence of the sidechain on the rate of racemization is worth understanding.[4] These studies support the cyclization of acidic sidechains to yield readily racemising lactams (see below). The alcohols in serine and threonine elevate rate constants by being both electron withdrawing and hydrogen bond donors (interacting with base, water or terminal carboxylates). The tert-butyl ethers of Ser and Thr have lower rate constants for racemisation supporting the hydrogen bonding effect; these ethers are in turn faster racemising than Ala, supporting the importance of the electron-withdrawing oxygen. The increased rate constant for Met may also be caused by a through-space interaction; thioethers have been shown to be able to act like weak hydrogen bond donors, albeit with strict geometrical restrictions.[123] Phe has a higher rate constant for racemisation than Ala due to the inductive effect of the aromatic ring.

Computational studies predicting the free energy of deprotonation of amino acids and substituted amino acids using B3LYP/6-311G** and the IEFPCM solvation model achieved a good correlation with measured rate constants (Figure 11).[124] The authors recommend the use of gas phase calculations because the addition of the IEFPCM solvation did not improve the correlation but did slow down the calculations. A special mechanism for racemisation of aspartic acid and asparagine residues has been proposed that proceeds via succinimide intermediates (Scheme 24).[125] The proposed succinimide intermediate **55** is computed to be 13 kcal/mol more acidic than the open chain structure. This mechanism also suggests that the formation of D-Asp could be from L-Asn, rather than L-Asp. Recent results have suggested that anionic intermediates that have the possibility of aromatic character can enhance racemisation liability and it is noteworthy that the keto-enol tautomer of **55** would fall into this category.[85] Other studies have cast doubt on the relevance of cyclic species favouring a homolysis via a radical.[126]

28
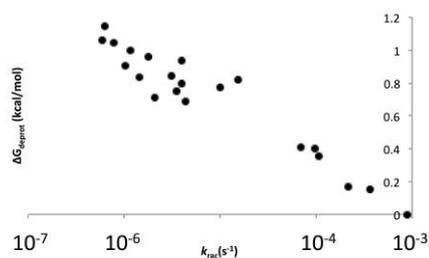
Figure 11. Computed deprotonation energies plotted against rate constants for racemisation for amino acids.
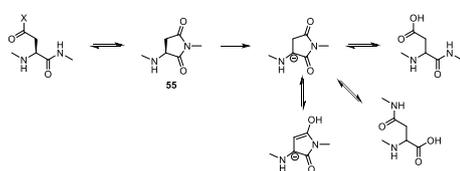
Scheme 24.

Stereochemical scrambling of the amino acids in proteins has also been proposed as a method to validate the origin of various natural compounds.[127] For instance, being able to prove the source of balsamic vinegars is important to prevent counterfeit products reaching the market, particularly those that circumvent the expensive aging process. In this context, racemisation of proline to form D-Pro is particularly prominent in vinegars. The percentage of D-amino acid was measured over time under heating in both red wine vinegar and aqueous acetic acid giving the rate constants shown in Table 8; Pro was found to be slowly racemizing. An enzyme-mediated process by residual microbial racemases was suggested to explain this. Racemisation in the presence of sugars (glucose and fructose are present in balsamic vinegars) was also observed, presumably via Schiff base formation and this too might explain the rapid racemisation of Pro, which, uniquely among the amino acids, can form an iminium that cannot shed a proton to become a neutral imine.

Table 8. Rate constants for racemisation in red wine vinegar and aqueous acetic acid.

Amino acid	k_{rac} x 10^{-7} (s^{-1}) in red wine vinegar, pH 2.5, 100 °C	k_{rac} x 10^{-7} (s^{-1}) in 5 % AcOH, pH 2.5, 100 °C
Asp	40.28	32.64
Ser	8.89	7.94
Ala	5.14	1.81
Phe	4.44	1.58
Lys	3.47	1.53
Met	3.47	1.39
Tyr	3.06	1.11
Glu	2.92	0.83
Pro	2.78	0.69
Leu	1.67	0.42
Val	0.69	0.01

d) Derivatives and prodrugs

One of the complexities of racemisation in a biological context is that the process can proceed through the transient formation of biological conjugates or metabolites. For instance, the profen drugs (including ibuprofen) are known to undergo racemization in vivo.[128–132] For these compounds, which have a eutomer with the S configuration, racemisation occurs via formation of the Acyl CoA thioesters in which the stabilising carboxylate is transformed into a destabilising thioester group (see below). The thioester can be rapidly hydrolysed to recover the acid after the stereochemical scrambling. Direct racemisation of ibuprofen could only be achieved under non-biological conditions of high pH and temperature.[133]

Some medications are provided as prodrugs, which are molecules that are not themselves active but are transformed into active drugs. For prodrugs, it is possible that racemisation occurs in either the prodrug or the drug. One example is clopidogrel (**38**, Table 4), which is provided as a methyl ester that is hydrolysed in vivo to a carboxylic acid. [134] This in turn can be transformed into acyl CoA thioesters. All of these possibilities were considered in studies of the racemisation of clopidogrel in which hydrolysis was monitored and for which the possibility of conjugates was considered via the addition of hepatocytes.[81] In vivo studies in rat supported the acid being the predominant species present in circulation and that about 4 to 8 % of this is R enantiomer after dosing the S enantiomer. None of the in vitro studies were able to rationalise this finding.

Similarly, in a prodrug, the chirality may not reside in the active drug as in the case of valaciclovir, **56**. Compound **56** is absorbed by the body more readily than acyclovir, **57**, and is swiftly broken down into L-valine and acyclovir by esterases in the body.[135,136] Compound **56** was selected from a series of 18 amino acid ester prodrugs. The prodrugs were administered to rats and the amount of the active metabolite **57** in their urine was determined. It was determined that L-**56** gave the highest amount of excreted **57**. The D- and rac- forms were also tested and gave a lower amount of excreted **57**. The authors suggested that this is likely to be due to a stereoselective transporter assisting absorption.[137]
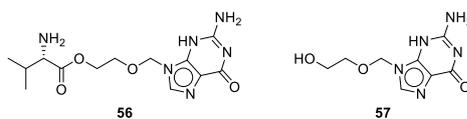
5. Tools to understand and predict racemisation rates.

It will have been noted by the attentive reader that the chemical diversity of the compounds for which the rate of aqueous racemisation has been measured is rather limited. For this reason, several groups have tried to provide rules of thumb and others

29

have used the chemical agnosticism of quantum mechanics to provide methods to predict the likely impact of racemisation.

The stability of carbanions in various molecular structures in aqueous environments is relevant to many biological reactions including decarboxylations and has been studied by the Richard group over the course of several years.[138–147] These studies provide a host of experimental measurements relating to the pK$_a$ of carbon acids and rationales for these observed acidities. Many of the groups stabilise the carbanion by resonance, notably the adjacent carbonyl (**58**), nitrile and aromatic groups (Figure 12).[140] The effect of a range of carbonyl types on the pK$_a$ of the carbanions was summarised (Table 9).[143,146] The differing effects of these groups were rationalised by a combination of electron-withdrawing character and a π-donor (or acceptor) effect. A reversed amide also stabilises carbanions, as assessed by the reduction in pK$_a$ from 18.7 to 14.5 when adding such a group alpha to a ketone (Figure 13, top).[138,143,148] It was proposed that this occurs via the resonance forms shown and is helped by a through-space electrostatic interaction between the anion and the polarized NH bond. An electrostatic effect can be achieved by an adjacent basic amine group (Figure 13, bottom left).[138,144] This change also increased the rate of deuteration 3500-fold for N-protonated glycine methyl ester compared to the rate for ethyl acetate.[139] An imine group has an even larger effect on pKa than protonated amines giving a very large (7 log unit) decrease from 21 to 14.[143,146] The opposite electrostatic effect is in operation for acidic groups such as carboxylates (Figure 13, bottom right) and this leads these to be stabilising groups, reflected in their impact on carbanion pKa values (Table 9).[143,146] Naturally, the amount of stabilisation will be pK$_a$ and pH dependent.
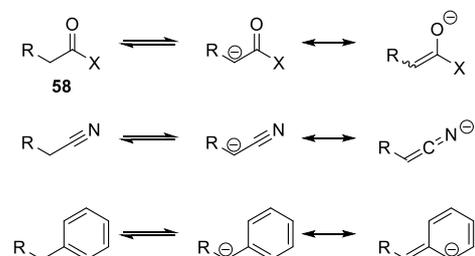
Figure 12. The resonance stabilisation of carbanions leading to increased acidity.

Table 9. pKa values for methyl substituted carbonyls (**58**)

X (R = H)	pKa
H	16.7
Me	19.3
SEt	21.0
OMe	25.6
OH	26.6
NH$_2$	28.4
NMe$_2$	29.4
O$^-$	33.5
C$_6$H$_4$CH$_3$	18.7
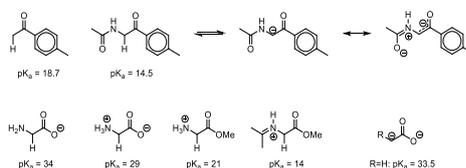
Figure 13. The carbon acid pKas of a range of carbonyl-containing compounds.

The distance dependence of the electrostatic acceleration is emphasised by the contrast between the deuteration rates of **59** and **60** (Figure 14).[144] The cyclic **59** exchanges 500 times more slowly than **60** because the positive charged pyridinium would be in closer proximity to the anion in **60**.

59

pK$_a$ = 11.9
k_{deprot}(OH) = 11 M^{-1}s^{-1}

60

pK$_a$ = 11.0
k_{deprot}(OH) = 5300 M^{-1}s^{-1}
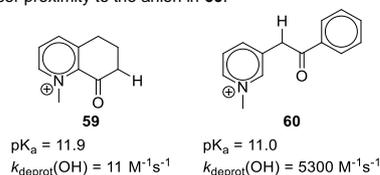
Figure 14. pKa and deprotonation rates of two pyridinium-substituted ketones with similar pKas.

A predictive method of assessing racemisation resulted from a series of papers from the Université de Lausanne. These papers describe studies of the rate of racemisation of a range of compounds in aqueous media and led to a summary of the structure-racemisation relationships for the general-base mediated reaction.[149,150] For compounds of type R^{1}R^{2}R^{3}CH to racemise, there should be two or three of the groups that decrease stability of the chiral center, shown in Table 10. These can be combined only with neutral groups, the presence of any of the groups that increase stability is sufficient to remove the risk of racemisation. It can be seen that the groups that decrease stability of the stereogenic centre do so by stabilising an adjacent negative charge either by delocalisation or by induction and those that increase stability of a stereogenic centre destabilise adjacent negative charges. This approach provides a very simple non-quantitative but useful initial guide to identifying racemisation risk.

Table 10. Groups identified by Testa as increasing or decreasing the stability of an adjacent stereogenic carbon. From Ref. [149]

Decrease stability	Neutral	Increase stability
Ester	Alkyl	Carboxylate
Aryl ketone		Sulfate
Amide		
Hydroxyl		
Amine		
N-linked imine		
Halogens		
Pseudohalogens		
Aromatic groups		
Benzyl groups		
CH$_2$OH		

Computational studies have also been applied to understand and predict racemisation. One of the rare examples of a stereogenic centre in drug molecules that is not a tetrahedral carbon is in proton pump inhibitors such as omeprazole, **61**. Calculations using RHF theory suggested that the barrier for this compound to invert via planarization at sulfur would be 43 kcal/mol which would make it highly unlikely to racemise.[151] Semiempirical AM1 calculations were used to understand the racemisation in acetonitrile of two chiral ketones, **62** and **63**.[152] The aforementioned studies by Servi and co-workers and by Zeng and co-workers correlate the rate constants for racemisation of amino acids with experimental rate constants.[71,124] However, these computational studies provide correlations strictly within compound classes.

61   **62**   **63**
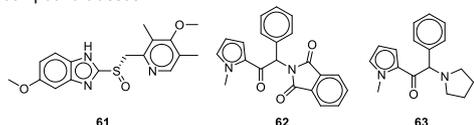
Computational studies have also been prominent for understanding the racemisation of chiral axes. The work of a group at Boehringer-Ingelheim defined many of the key ideas, including the classification of compounds into those that rotate so quickly that rapid equilibration will always occur, those that are so slow that enantiomers would be separable and stable and finally, those in the middle that are likely to require more care. Compounds with barriers at the HF/6-31G** level below ~20 kcal/mol or above ~30 kcal/mol can be developed as single compounds whereas those in between these two extremes are likely to form mixtures with a time dependence that is comparable to storage and dosing of drugs.[15] Variations on this approach have been described subsequently.[22,55]

A quantitative approach to rates of general-base-catalysed racemisation across compound classes was recently described in which two computational and two practical methods complement one another to provide differing levels of speed and throughput and different levels of accuracy.[85] Measurements of the rate of racemisation exploited NMR spectroscopy to follow deuterium incorporation or circular dichroism to follow racemisation directly. These techniques were used to expand the available set of racemisation rate constants. One computational approach considers the contribution of groups adjacent to a carbanion to its stability, this technique can be rapidly applied to sets of many compounds. An alternative uses quantum mechanics to compute the energy of deprotonation (at the B3LYP/6-31+G* level including IEFPCM solvation). In both cases, the deprotonation energy is correlated with measured second-order rate constants (Figure 15) to create a linear free energy relationship (LFER). This LFER permits these rate constants to be predicted from purely computational data. An estimate of the concentration of active basic components of phosphate buffer that is equivalent to that in blood permits the amount of racemisation to be expected in vivo in any given time period to be estimated. A subset of compounds were found to follow a different trend in the group contribution approach – those that feature an anionic intermediate that could be thought of as aromatic and such molecules have an enhanced rate of racemisation.
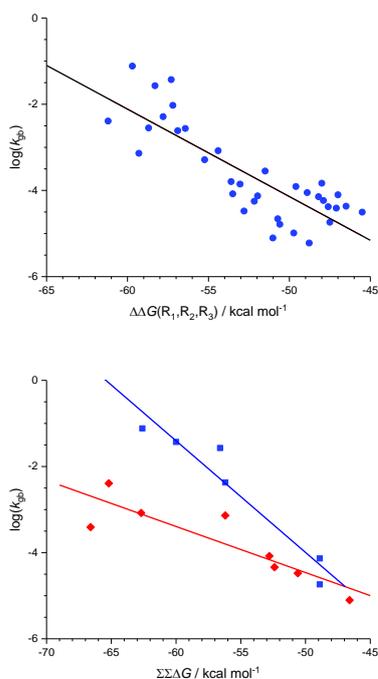
Figure 15. Top) Plot of logarithms of the second-order rate constants for general-base-catalysed racemisation ($\log k_{gb}$) in aqueous conditions plotted against deprotonation free energy computed computed using quantum mechanical calculations and Bottom) Plot of $\log k_{gb}$ against the sum of group contributions to anion stabilisation with compounds that involve an aromatic anion in blue squares and those that do not as red diamonds.

These approaches suggest that there is a strong relationship between the stability of a carbanion and liability towards racemization, as expected for a mechanism involving rate-determining deprotonation.

The correlation provided by this LFER finally provides a quantitative prediction of racemisation risk across compound classes.

6. Summary and Outlook

There is a strong awareness of the importance of chirality in biomolecular recognition and this has led to a steady interest in the process of racemisation. Nevertheless, kinetic and mechanistic studies of racemisation processes under relevant conditions have been few and sets of kinetic data are rarely sufficient for unambiguous mechanistic interpretation. This review summarises kinetic data and its interpretation for a series of compounds for which racemisation has been studied. The review also links such chemical studies with the analogous processes

occurring in biological systems. Probably as a result of limited good quality experimental data, until recently models predicting racemisation risk were limited to individual compound classes, but this situation has now changed. We hope that this review will further stimulate the renaissance of kinetic and mechanistic studies in this important area so that our understanding and future predictive models can be further improved.

Acknowledgements ((optional))

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Entry for the Table of Contents (Please choose one layout)

Layout 1:

REVIEW

Although the importance of racemisation is well known, the process remains poorly studied. In this review, the available data concerning chemical (acid and base) and enzymatic catalysis of racemisation and predictive tools are presented and discussed. The paucity of relevant data is striking.
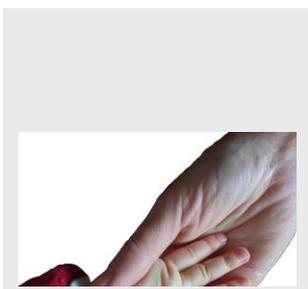
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