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Supporting information:

(Marianne Reist, Pierre-Alain Carrupt, Eric Francotte, and Bernard Testa. Chiral inversion and hydrolysis of thalidomide: Mechanisms and catalysis by bases and serum albumin, and chiral stability of teratogenic metabolites.)

Detailed calculations to estimate the rate constants of chiral inversion and hydrolysis

Determination of rates of chiral inversion by chiral HPLC of enantiomers undergoing simultaneous hydrolysis

For an optically active compound that simultaneously undergoes racemization and hydrolysis, the following kinetic model can be postulated:

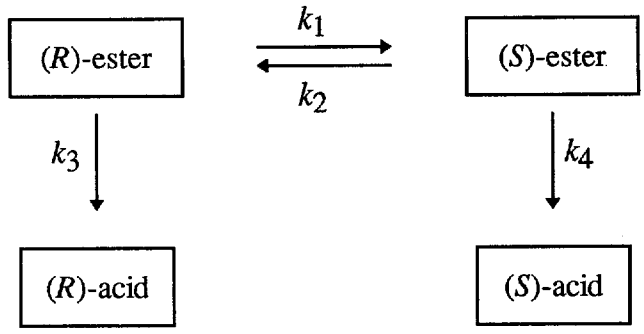

Figure S.1 Kinetic model for simultaneous racemization and hydrolysis. k_1 and k_2: rate constants of chiral inversion, k_3 and k_4: rate constants of hydrolysis.

If hydrolysis is a non-stereoselective reaction (i.e., $k_3 = k_4 = k_{hyd}$), the following rate equations can be applied for the determination of rate constants by chiral HPLC:

1.) $$\frac{d[1]}{dt} = k_2 \cdot [2] - (k_1 + k_{hyd}) \cdot [1] \qquad \text{(S.1)}$$

2.) $$\frac{d[2]}{dt} = k_1 \cdot [1] - (k_2 + k_{hyd}) \cdot [2] \qquad \text{(S.2)}$$

where [1] and [2] represent the concentrations of (*R*)-substrate and (*S*)-substrate respectively. The calculations were made using Laplace transform.

The transformed equations are:

1.) $$p \cdot x_1 + c_1 = k_2 \cdot x_2 - (k_1 + k_{hyd}) \cdot x_1$$

2.) $$p \cdot x_2 + c_2 = k_1 \cdot x_1 - (k_2 + k_{hyd}) \cdot x_2$$

2.) in 1.) $$x_1 = \frac{k_2 \cdot c_2 + c_1(p + k_2 + k_{hyd})}{k_1 k_2 - (p + k_1 + k_{hyd})(p + k_2 + k_{hyd})}$$

Decomposition in simple elements:

$$k_1 k_2 - (p + k_1 + k_{hyd})(p + k_2 + k_{hyd}) = 0$$

$$\rightarrow p_1 = -k_{hyd} \quad \text{and} \quad p_2 = -k_{hyd} - k_1 - k_2$$

$$x_1 = \frac{k_2 \cdot c_2 + c_1(p + k_2 + k_{hyd})}{(p - p_1) \cdot (p - p_2)} = \frac{a_1}{p - p_1} + \frac{a_2}{p - p_2}$$

$$\rightarrow a_1 = \frac{k_2}{k_1 + k_2} \cdot (c_1 + c_2) \quad \text{and} \quad a_2 = \frac{c_1 k_1 - c_2 k_2}{k_1 + k_2}$$

$$x_1 = \frac{k_2}{k_1 + k_2} \cdot \frac{c_1 + c_2}{p + k_{hyd}} + \frac{c_1 k_1 - c_2 k_2}{k_1 + k_2} \cdot \frac{1}{p + k_1 + k_2 + k_{hyd}}$$

Inverse transformation:

1.) $$[1]_t = \frac{k_2}{k_1 + k_2} \cdot (c_1 + c_2) \cdot e^{-k_{hyd} t} + \frac{c_1 k_1 - c_2 k_2}{k_1 + k_2} \cdot e^{-(k_1 + k_2 + k_{hyd}) t}$$

2.) $$[2]_t = \frac{k_1}{k_1 + k_2} \cdot (c_1 + c_2) \cdot e^{-k_{hyd} t} + \frac{c_2 k_2 - c_1 k_1}{k_1 + k_2} \cdot e^{-(k_1 + k_2 + k_{hyd}) t}$$

at $t = 0$:

$$[1] = \frac{k_2}{k_1 + k_2} \cdot (c_1 + c_2) + \frac{c_1 k_1 - c_2 k_2}{k_1 + k_2} = c_1 \qquad \text{(initial concentration of } (R)\text{-ester)}$$

$$[2] = \frac{k_1}{k_1 + k_2} \cdot (c_1 + c_2) + \frac{c_2 k_2 - c_1 k_1}{k_1 + k_2} = c_2 \qquad \text{(initial concentration of } (S)\text{-ester)}$$

$$\Rightarrow \frac{[1]_t - [2]_t}{[1]_t + [2]_t} = \frac{k_2 - k_1}{k_1 + k_2} + 2 \frac{c_1 k_1 - c_2 k_2}{(k_1 + k_2)(c_1 + c_2)} \cdot e^{-(k_1 + k_2) t} \qquad \text{(S.3)}$$

These calculations show that the change in the weighted difference of the two enantiomers (i.e., the decrease of the enantiomeric excess) only depends on the rate constants of chiral inversion k_1 and k_2 (Equation S.3). Hence, the rate of chiral inversion of an optically active substance that is simultaneously subject to non-stereoselective hydrolysis can be determined according to Equation S.3, independently of its hydrolysis.

In the case of a non-stereoselective inversion of configuration, the two rate constants k_1 and k_2 are the same and Equation S.3 can be simplified.

For $k_1 = k_2 = k_{enant} = 1/2\ k_{rac}$ we find:

$$\frac{[1]_t - [2]_t}{[1]_t + [2]_t} = \frac{c_1 - c_2}{c_1 + c_2} \cdot e^{-2k_{enant}t} = \frac{c_1 - c_2}{c_1 + c_2} \cdot e^{-k_{rac}t} \qquad \text{(S.4)}$$

Consequently, the observed pseudo-first-order rate constants of racemization or enantiomerization can be obtained by plotting the weighted difference of the two enantiomers, i.e., the natural logarithm of the decreasing enantiomeric excess of the enantiomer initially present (pure or in excess) as a function of time according to Equation S.5:

$$\ln\left(\frac{[1]_t - [2]_t}{[1]_t + [2]_t}\right) = -k_{rac} \bullet t = -2k_{enant} \bullet t \qquad \text{(S.5)}$$

where $[1]_t$ is the concentration of the decreasing, initially present enantiomer at time t, $[2]_t$ the concentration of the increasing enantiomer at time t, k_{rac} the observed pseudo-first-order rate constant of racemization and k_{enant} that of enantiomerization.

In the case of a stereoselective inversion of configuration, however, $k_1 \neq k_2$, and Equation S.3 has to be used to estimate the two rate constants k_1 and k_2. If c_1 and c_2 (initial concentrations of (*R*)- and (*S*)-substrate) are 100% and 0% respectively, c_2 is equal to zero, and Equation S.3 can be simplified:

$$\frac{[1]_t - [2]_t}{[1]_t + [2]_t} = \frac{k_2 - k_1}{k_1 + k_2} + 2\frac{k_1}{k_1 + k_2} \cdot e^{-(k_1 + k_2)t} \qquad \text{(S.6)}$$

The rate constants of chiral inversion k_1 and k_2 can be estimated by non linear-regression analysis of the experimentally determined, decreasing weighted differences of the two enantiomers, using Equation S.6.

Discussion of the use of ^1H NMR ($^1H/^2H$ substitution) as a tool to assay the racemization of chiral centers of the type R''R'RC-H

The racemization of chiral centers of the type R''R'RC-H can be assayed by ^1H NMR. The method takes advantage of the fact that inversion of such chiral centers and proton-deuterium exchange share a common mechanism. Briefly, when a compound having a chirally labile C-H group is dissolved in D_2O, the proton is irreversibly replaced by a deuterium, and deuteration can be followed by integrating the signal of the exchanging proton. Assuming a common intermediate for deuteration and either racemization or enantiomerization (i.e., inversion of configuration), the rate of deuteration must reflect that of racemization or enantiomerization respectively.

Four limiting ratios of k_{deut} (rate constant of deuteration) over k_{rac} (rate constant of racemization) can be envisaged: (1) if deuteration occurs with complete retention of configuration, the ratio k_{deut}/k_{rac} approaches infinity (isoinversion); (2) if deuteration takes place with complete racemization, each carbanion is captured from either side with equal probability and k_{deut}/k_{rac} equals unity; (3) if deuteration occurs with inversion of configuration, its rate is half that of racemization (i.e., equal to that of enantiomerization) and the ratio k_{deut}/k_{rac} equals 0.5; (4) if racemization takes place without deuteration, the ratio k_{deut}/k_{rac} approaches zero (isoracemization). For the study of the configurational stability of chiral compounds in physiological fluids (i.e., aqueous media), we shall concentrate on cases 2 and 3. Cases 1 and 4 have only been observed in nondissociating nonpolar solvents, or in aprotic solvents with aprotic bases, respectively. When the rates of deuteration reflect those of racemization (i.e., $k_{deut} = k_{rac}$, case 2) the common reaction mechanism must be of an S_E1 type with a symmetrical intermediate such as a neutral enol or a resonance-stabilized carbanion,

since its deuteration occurs equally from either side and thus the rate of deuteration is identical with that of racemization. When, on the other hand, the rates of deuteration reflect those of enantiomerization (i.e., $k_{deut} = k_{enant} = 1/2\ k_{rac}$, case 3) two possible mechanisms for which isotopic substitution results in inversion of configuration can be suggested: (A) an S_E1 mechanism with a very short-lived asymmetrically solvated carbanion intermediate, and (B) a push-pull S_E2 mechanism.

Once the nature of the common mechanism of $^1H/^2H$ substitution and chiral inversion is known, reliable rates of racemization can be obtained by ^1H NMR. If, in contrast, the common mechanism is unknown, the relative rates of deuteration will nevertheless be of indicative value. Thus the measurement of the $^1H/^2H$ exchange provides a fast method to determine the configurational stability of drug candidates having a chiral center of the type R''R'RC-H. The reaction has the considerable advantage that it can be performed with the racemate. In other words, it is a convenient method to screen chiral drug candidates prior to their resolution.