FULL PAPER

Fungicide precursor racemization kinetics for deracemization in complex systems

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Abstract: In the racemization area, the keto-enol equilibrium is a major player when it comes to racemizing α-chiral carbonyl compounds. The racemization kinetics in the co-crystal induced deracemization of a fungicide precursor is complex as next to the racemization catalyst, which is a base, an acidic co-former is used to ensure the crystallization of the co-crystal. Here we show that understanding of the racemization kinetics of the target compound is of key importance for optimization of the co-crystallization based deracemization process. The racemization rates in solution as a function of solvent and base concentration were determined by measuring the decreasing enrichment of the chiral ketone due to racemization over time, using a polarimeter set-up with a continuous recycling loop through the polarimeter cell. The established racemization kinetics model aligns with the experimental data giving access to the intrinsic racemization rate constant. The proposed mechanism is first order with respect to the enantiomeric excess of the target compound and the base-catalyst concentration. The solvent is shown to strongly affect the racemization constant, with protic solvents increasing this rate substantially due to hydrogen-bond stabilization of the enolate. Finally, we observed the presence of the chiral acid co-former to alter the reaction mechanism albeit remaining first order with respect to the enantiomeric excess. Though more complex, the mechanism still followed Arrhenius law, providing key information on the impact of temperature.

Introduction

In the world of enantiopure compound production, deracemization methods have gained increasing attention due to their high efficiency, relatively low cost and easy upscaling compared to other methods like asymmetric synthesis.[1] A deracemization process combines a resolution and a racemization process, allowing to go well beyond the 50% maximum yield limitation of the resolution processes. Based on this combination, methods such as Dynamic Kinetic Resolution, Viedma Ripening and Crystallization Induced Diastereomeric Transformation (CIDT) have already been developed for a large variety of compounds.[2-7] However, these methods all have their limits. Dynamic Kinetic Resolution requires having a synthesis intermediate undergo a chemical reaction[5], Viedma ripening demands a conglomerate forming crystal compound[8] and CIDT a salt forming compound.[3] In other words, not every, compound can be deracemized with these methods, and one is continuously on the lookout for new deracemization methods that enlarge the deracemization application area.

In this context, we recently developed a novel deracemization method based on a co-crystallization approach: Co-crystallization induced deracemization.[9] We applied it on the compound (R,S)-4,4-dimethyl-1-(4-fluorophenyl)-2-(1H-1,2,4-triazol-1-yl)-pentan-3-one [(R,S)-BnFTP] a racemic, non-salt and non-conglomerate forming compound, that makes a suitable conglomerate co-crystal with the chiral compound 3-phenylbutyric acid (PBA). Initial trials showed how a continuous set-up can lead to a deracemization of the racemate in 4 days[9]. The set-up of the process is similar to the one developed for temperature cycling Viedma ripening by Coquerel et Al.[7] As this process combines crystallization aspects with a racemization reaction, optimization of the system requires full understanding of the underlying thermodynamics and kinetics of all physical and chemical processes involved.

In this contribution we focus specifically on the racemization kinetics, which in the case of BnFTP are based on a keto-enol equilibrium, and are influenced by the base catalyst as well as the acid co-former used, PBA. Knowledge of the racemization rate and kinetics is crucial in developing an efficient deracemization process. Indeed, it was shown by R. Oketani et al.[10] that, for their system, productivity was directly correlated to the product of racemization rate *k* and solubility; with *k* governing the deracemization time. Over the years, there have been an extensive number of kinetic studies on the keto-enol equilibrium mostly on acetophenone or acetyl heterocycle derivatives in water[11-18] with only a few studies on molecules similar to BnFTP[19,20] and an even more limited number in organic solvents.[21] Every kinetic study carried out on the enolization of carbonyls was assessed by either deuteration, racemization or halogenation,[13] the latter one being the most used.[11-

[18,22-24] Here we use the racemization approach, conducted with a polarimeter set-up that continuously samples the racemizing mixture to the polarimeter cell, using a recycle loop connected to the reactor in order to measure directly the enantiomeric excess of the compound during the racemization reaction. Such a set-up allows for collection of a data point every 5 seconds, leading to a substantial amount of data, particularly useful for rapid racemization processes. Such a method is quite uncommon for these types of studies but was recently shown to produce easily accessible and high-quality data.[25,26]

We report here the kinetics of racemization of BnFTP in toluene and in presence of a base (diazabicyclo[5.4.0]undec-7-ene). The experimental data are compared to a kinetic model in order to determine the racemization rate. Then, the influence of the solvent on the racemization rate is studied. Finally, the impact of the presence of an acid to the system is assessed.

Results and Discussion

KINETIC MODEL OF RACEMIZATION

In order to study the kinetics of the racemization reaction, we assume an underlying racemization mechanism corresponding to a typical enolization. The overall mechanism can be cut in elementary steps as shown in figure 1.

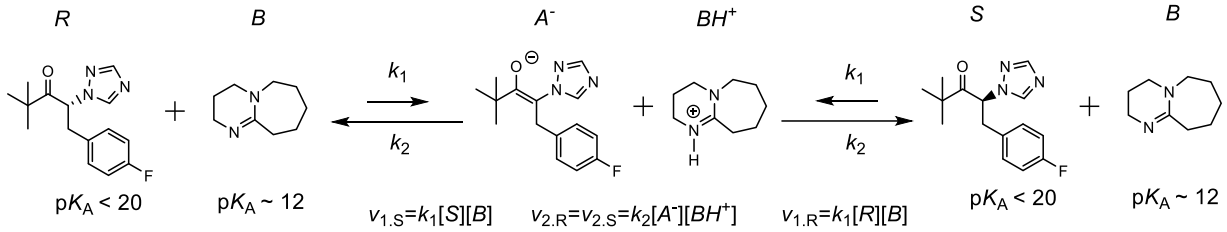

Figure 1. *Acid-basic equilibrium between BnFTP and DBU is in favor of the keto-form of BnFTP due to the lower pK_A value of DBU compared to BnFTP. S and R stands for respectively the (S)-enantiomer of BnFTP and (R)-enantiomer in the mathematical model. B stands for the base, A^- for the enolate species and BH^+ the protonated base in the mathematical model.*

The deprotonation and re-protonation rate constants k_1 and k_2 are identical for both enantiomers, as the base used is non-selective to the enantiomers. The reaction rate of each species is then given by:

$$\frac{d[S]}{dt} = k_2[A^-][BH^+] - k_1[S][B] \quad (1)$$

$$\frac{d[R]}{dt} = k_2[A^-][BH^+] - k_1[R][B] \quad (2)$$

$$\frac{d[A^-]}{dt} = \frac{d[BH^+]}{dt} = k_1[S][B] - 2k_2[A^-][BH^+] + k_1[R][B] \quad (3)$$

At any moment, the sum of the concentration of (R)-BnFTP, (S)-BnFTP and enolate is equal to the starting concentration of BnFTP, S_0.

$$[A^-] + [R] + [S] = S_0 \quad (4)$$

Similarly, at all times the overall concentration of base and protonated base equals the starting concentration B_0,

$$[B] + [BH^+] = B_0 \quad (5)$$

In our model system, formation of the BnFTP enolate (A^-) is thermodynamically not favored as the pK_A of DBU ($pK_A \approx 12$)[28] is lower than that of the acid in the α position of the ketone ($pK_A < 20$)[29] (figure 1). Consequently, at all times during the reaction, we only expect small concentrations of A^- and BH^+ to be present. Consequently, we can assume the steady state principle[30] for the enolate and the conjugated base:

$$\frac{d[A^-]}{dt} = \frac{d[BH^+]}{dt} \cong 0 \quad (6)$$

Combining the steady state principle (6) with eqs. (2), (1) and (3) the rate equation for both the (S)- and (R)-enantiomer can be expressed as:

$$\frac{d[S]}{dt} = \frac{1}{2}k_1[B]([R] - [S]) \quad (7)$$

$$\frac{d[R]}{dt} = \frac{1}{2}k_1[B]([S] - [R]) = -\frac{d[S]}{dt} \quad (8)$$

Subtracting both results in:

$$\frac{d[S]}{dt} - \frac{d[R]}{dt} = k_1[B]([R] - [S])$$

$[R] + [S]$ is constant and approximately equal to the starting concentration S_0 of BnFTP as the concentration of the enolate species A^- can be neglected compared to that of the other species present. Dividing the previous equation by $[R]+[S]$ leads to

$$\frac{d(E)}{dt} = -k_1[B]E \quad (9)$$

$$\text{with } E = \frac{[S] - [R]}{[R] + [S]}$$

The rate kinetics are thus expected to be first order with respect to the enantiomeric excess E and first order with respect to the concentration $[B]$ of free base, which remains constant during the time of the experiment. The concentration in free base $[B] \cong B_0$ and can be incorporated in an apparent rate constant yielding an overall 1st order rate equation:

$$\frac{d(E)}{dt} = -k'E \quad (10)$$

$$\text{with } k' = k_1B_0$$

which integrates to

$$\ln(E) = -k't + \ln(E_0) \quad (11)$$

When an additional acid with a pKa ≈ 4-5 is also present in the environment, this model no longer holds as DBU is expected to be fully protonated (ΔpKa > 4, quantitative reaction). The carboxylate is not expected to be strong enough to deprotonate BnFTP, leading us to suggest a

coupled acid/carboxylate action (figure 2), provided the acid is in excess compared to DBU.

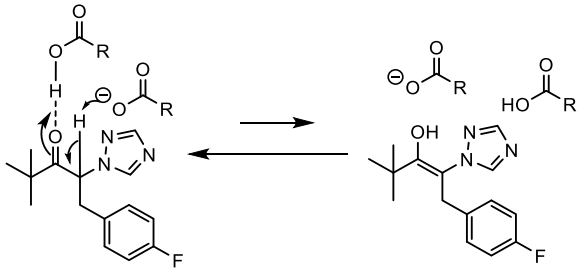

Figure 2. *Assumed mechanism explaining the enolization of BnFTP in the presence of an excess of additional acid.*

This mechanism can be seen as a base-assisted enolization in an acidic medium., there remain few instances of enolization carried out in presence of an amino salt. As well, no kinetic studies, to best of our knowledge, were carried out on such systems.

MODEL VALIDATION

First order verification

Taking a standard experiment (500.2mg of (S)-BnFTP, 50mL of toluene, 100µL of DBU), an excellent linearization of the data [the end of each experiment (generally, when the enantiomeric excess goes under 0.2) was not considered as the polarimeter is not accurate enough at lower E values] is obtained when the natural logarithm of the enantiomeric excess is plotted against time (Figure 3). This agrees with a first order equation with respect to the enantiomeric excess *E* as shown by equation 15. All experiments involving BnFTP and DBU show such typical first order behavior (see SI).

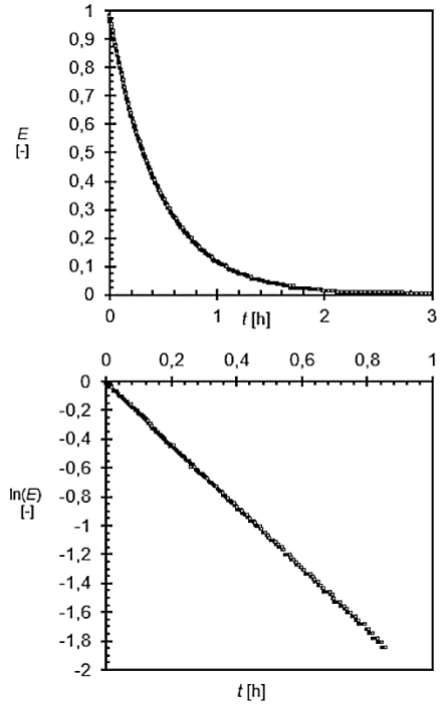

Figure 3. *Top: the evolution of the enantiomeric excess E versus time t for a racemization with 10 mg/mL of (S)-BnFTP in toluene using 100µL of DBU. Bottom: the linearization obtained through equation 15 for the same experiment gave a R^2 of 0,9997. The slope (with the linear model error) is -2.129 ± 0.003h^{-1}.*

In the suggested model, the observed rate constant *k'* is independent of the BnFTP starting concentration S_0. This was verified by a series of experiments, where only the concentration in BnFTP was varied (Figure 4).

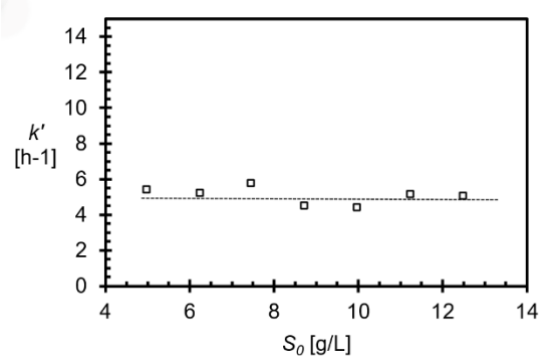

Figure 4. *The observed rate constant k'=5.01±0.48 h^{-1} does not significantly vary with the starting concentration S_0 of BnFTP using 250µL of DBU in toluene. The horizontal dashed line represents the mean value 5.01 h^{-1}. Standard deviation is 0.48 h^{-1}.*

As an excellent linearization is obtained for each individual experiment, the error on each *k'* resulting from the modeling is very small. There is however a stronger variability between different experiments (as shown by figure 6). The average value of *k'* is 5.01 h^{-1} with a standard deviation of 0.48 h^{-1}. As a result, for any experiment, the standard deviation is expected to be around 10%. This latter represents the experimental variability between different samples and one should therefore consider an experimental error of about 0.48 h^{-1} on the values of the rate constants presented here. This error may come from the error on the exact concentration of DBU (error on the volume of added DBU and on the volume of solvent).

Influence of DBU concentration on racemization rate

We then investigated the order with respect to DBU. Since DBU is a catalyst and not consumed during the process, its concentration remains constant over time with $[B] \cong B_0$ as mentioned above. To study the rate order with respect to DBU, we therefore determined the rate constant $k'=k_1B_0$ dependence on DBU concentration. The evolution of the enantiomeric excess of these experiments over time is given in figure 6. This figure shows an increase in the racemization rate as the concentration of DBU increases. Figure 5 shows the *k'* values as a function of base concentration B_0. Linearized data is shown in the SI. The almost perfect linear relation obtained between *k'* and B_0 confirms first order in DBU as predicted by our model and the value of the intrinsic deracemization rate constant *k* in toluene is 157.7 $L.mol^{-1}.h^{-1}$ or 2.63 $L.mol^{-1}.min^{-1}$. Brevegleri et al. measured a rate constant of the same order (9.44 $L.mol^{-1}.min^{-1}$ at 25°C in acetonitrile) for a similar compound, N-(2-methyl-benzylidene)-phenylglycine amide (NMPA) in a more polar solvent[31].

Thus, the kinetic model we propose for the racemization reaction of BnFTP in presence of DBU is confirmed by the experimental data. It is a first-order kinetic model similar to the one developed and validated by Brevegleri et al. and in accordance with literature.[32]

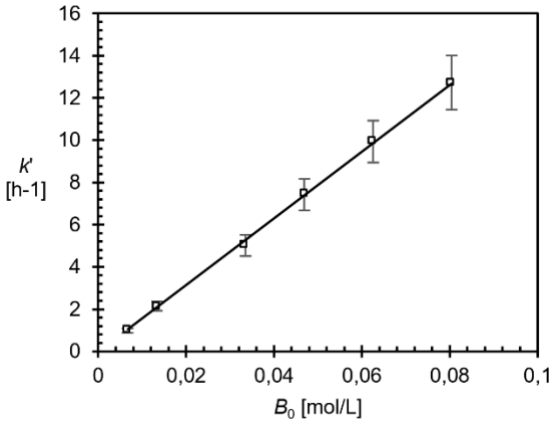

Figure 5. *Linear evolution of the observed rate constant k' with the base concentration B_0 (error bars show the standard deviation). The linear regression gave the following equation: $k' = 157.7B_0$ with a R^2 of 0.9992. The linear model error on the slope is ±1.1 $L.mol^{-1}h^{-1}$.*

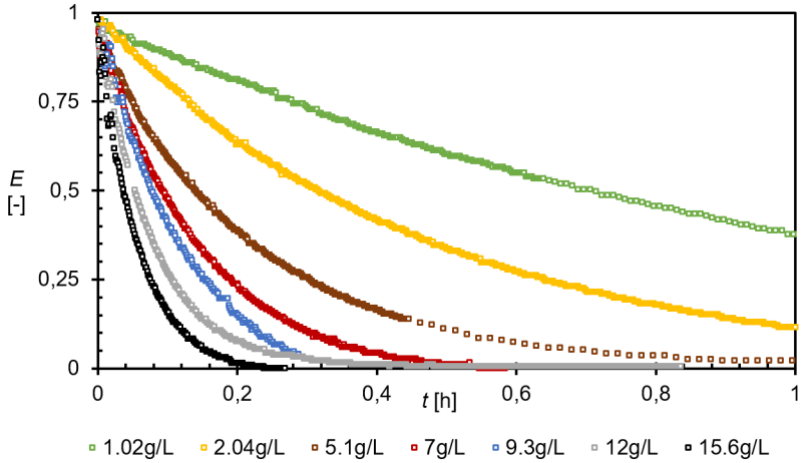

Figure 6. *Enantiomeric excess E over time of (S)-BnFTP in the reaction mixture for different concentrations B_0 of base catalyst, which is indicated by the labels. The higher the concentration of the base, the faster the racemization. For all experiment, the concentration of BnFTP is 10 mg/mL.*

Impact of the solvent nature

7 solvents were then selected to study the impact the nature of the solvent has on the racemization rate. Figure 7 shows a strong impact of the solvent on the racemization rate for experiments performed with 9.7 g/L BnFTP and a catalyst concentration of 5.1g/L. All the experiments result in the typical behavior of the enantiomeric excess *E* following eq. 15. Ethanol and propanol yield the fastest racemization rate by far. Moreover, a 10-fold difference can be observed between the lowest and highest rate constant (respectively for THF and ethanol). At first sight, the rate constant shows no clear relation with the dielectric constant. For instance, acetonitrile, the solvent with the highest dielectric constant, only gave the fourth highest rate constant *k*' (table 1). Nevertheless, out of these solvents, 3 are protic (Ethanol, Propan-1-ol and Butan-1-ol) and 4 are not (THF, Toluene, Acetone and Acetonitrile). Among the protic solvents, the most polar ones lead to higher racemization rate (figure 8). Similarly, solvent polarity seems to increase racemization rate also for non-protic solvents, even though the effect is less pronounced (figure 8).

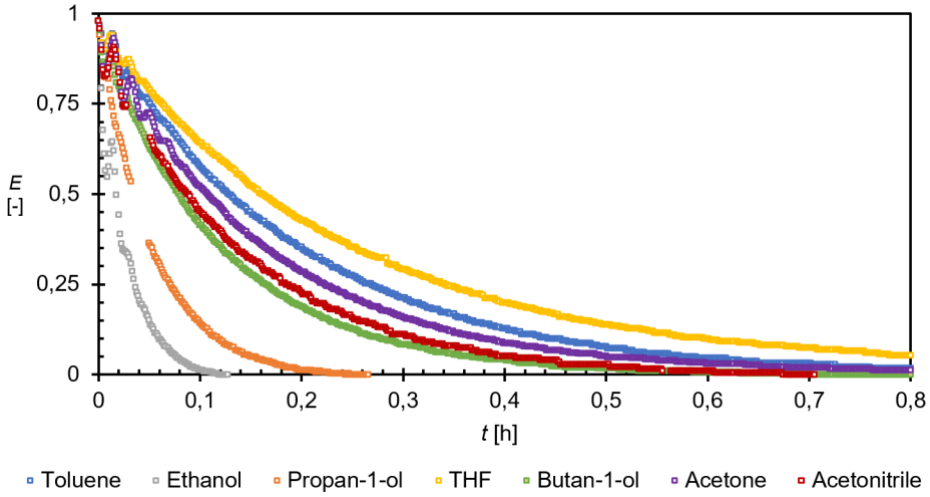

Figure 7. *Time evolution of the enantiomeric excess E of BnFTP in different solvents (grey for ethanol, orange for propan-1-ol, green for butan1-ol, red for acetonitrile, purple for acetone, blue for toluene and yellow for THF) using the same base concentration (5.1g/L).*

Overall racemization kinetics of our system seem therefore enhanced when using protic solvents with dielectric constant ε>18, contrary to the system studied by Breveglieri et al.[31] that showed a strong enhancement of the kinetics when using acetonitrile compared to isopropanol (which has a similar dielectric constant as propan-1-ol). In comparison, the rate constant in propan-1-ol is significantly higher than in acetonitrile. This highlights the importance of the nature of the compound for the enolization kinetics and illustrates that solvent selection in this case will have to be performed on a case dependent basis.

The impact of proticity on racemization kinetics can be explained by the stabilization of the enolate. Protic solvents can stabilize the negatively charged enolate through assisted hydrogen bonds. We therefore expect a lowering of the activation energy for the formation of these species as illustrated in figure 7.

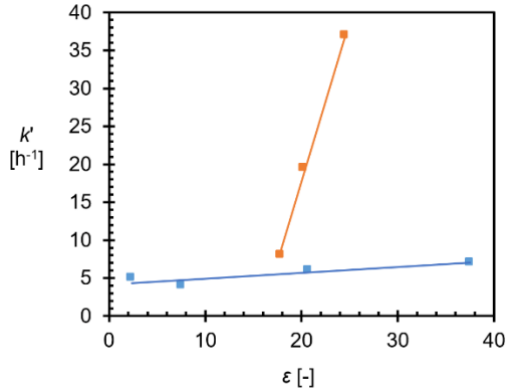

Figure 8. *Observed rate constant k' as a function of the dielectric constant of the solvent. Aprotic solvent are plotted in blue while protic ones in orange. For protic solvents, a small increase in ε induces a high increase in k' while for aprotic solvents, a high increase in ε only slightly increases k'.*

Table 1. *Value of the observed rate constant k' with its experimental error for every studied solvent, compared with their dielectric constant.*

Solvent	Dielectric Constant[33,34]	Protic solvent	k' [h^{-1}]	Linear model error on k' [h^{-1}]	k [$L.mol^{-1}h^{-1}$]	Linear model error on k [$L.mol^{-1}h^{-1}$]
THF	7.58	No	3.88	±0.01	116	±1
Toluene	2.38	No	5.01	±0.01	150	±1
Acetone	20.7	No	5.89	±0.01	176	±1
Acetonitrile	37.5	No	7.07	±0.02	211	±3
Butan-1-ol	17.8	Yes	8.03	±0.01	240	±1
Propan-1-ol	20.3	Yes	19.50	±0.05	582	±8
Ethanol	24.6	Yes	37.02	±0.46	1105	±70

A computational study previously showed the importance of the solvents' ability to interact through hydrogen bonding with carbonyls [those results were drawn for isomer pairs in which the enol cannot form an internal hydrogen bond] in stabilizing the enol form. They also showed that if two solvents are able to hydrogen bond with the solute, then the difference in stability would be due to the difference of the dipolar moment between the enol and keto-form. Hence, the dielectric constant of the solvent is the determining criterion explaining the difference in stability.[35] Similarly, we observed that a more polar solvent increases the stability of the intermediate enolate, when comparing solvents with similar hydrogen bonding capacity (figure 9). Furthermore, out of these two effects, the formation of hydrogen bonding seems to have a stronger effect, as the rate constants for isopropanol compared to acetone, though similar in polarity, show a four-fold increase while for propanol a three-fold increase is observed compared to acetonitrile. In the case of Breveglieri et al., the non-stabilizing effect of hydrogen bonding when using NMPA,[31] could be explained by a disruption in the

mesomeric effect of the imine group, in the β position of the carbonyl, caused by hydrogen bonding of its nitrogen atom with the alcohol group of the solvent. Resulting in its lone pair no longer being available in the resonance. This likely explains their seemingly contradicting results.

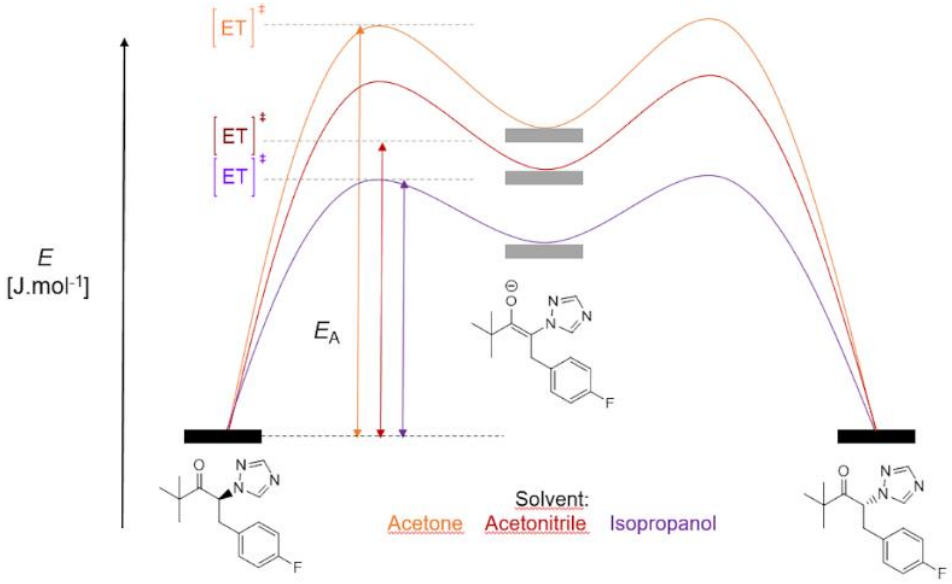

Figure 7. *Hypothetical energetic diagram for BnFTP racemization in acetone, acetonitrile and isopropanol. On the left the molecule of (S)-BnFTP, on the right that of (R)-BnFTP and in the middle the enolate species. Each curve is color-coded according to the solvent.*

Influence of (R)-3-Phenylbutyric acid on racemization

The deracemization process recently developed by us, occurs in presence of (R)-3-Phenylbutyric acid as a co-former. The presence of this acid is expected to significantly impact racemization kinetics as it interacts with the base DBU. To estimate this effect on the racemization, 6 experiments were carried out in toluene with one equivalent of (R)-3-Phenylbutyric acid (compared to (R)-BnFTP) varying the concentration of the racemization catalyst DBU. For 3 experiments, the acid is in excess compared to DBU and for the remaining 3, DBU is in excess. All experiments were carried out in toluene, the solvent used in the deracemization process[9]. The evolution of the enantiomeric excess over time is given in figure 10. All linearization are given in the SI.

A significant drop in racemization rate is observed compared to the experiment without the acid. However, linearization of the data according to first order kinetics remains possible for all experiments, suggesting a similar mechanism. Regarding the evolution of the observed rate constant as a function of DBU concentration, the first observation is that the experiment with 7g/L of DBU gave faster kinetics than the one with 8g/L. Indeed, two cases are to be considered:

- Excess of acid
- Excess of base.

When DBU is in sufficient excess, the observed rate constants are similar to the ones expected for an experiment without acid taking into account the concentration of unprotonated DBU. Indeed, the observed rate constant *k'* obtained for these experiments yields a value of 2.30 h^{-1} for 1.31eq and 0.64h^{-1} for 1.16eq. Using the intrinsic rate constant *k* of 157.7 $L.mol^{-1}.h^{-1}$ from the experiment without the acid, this would imply a DBU concentration of respectively 2.22 (0.32 eq.) and 0.55 (0.09 eq) $g.L^{-1}$. This is approximately the excess amount of DBU present (respectively 0.31eq and 0.16eq), suggesting that for these experiments, the reaction occurs via the proposed racemization mechanism, but with an apparent reduced concentration of DBU available for the racemization, therefore reducing the racemization rate.

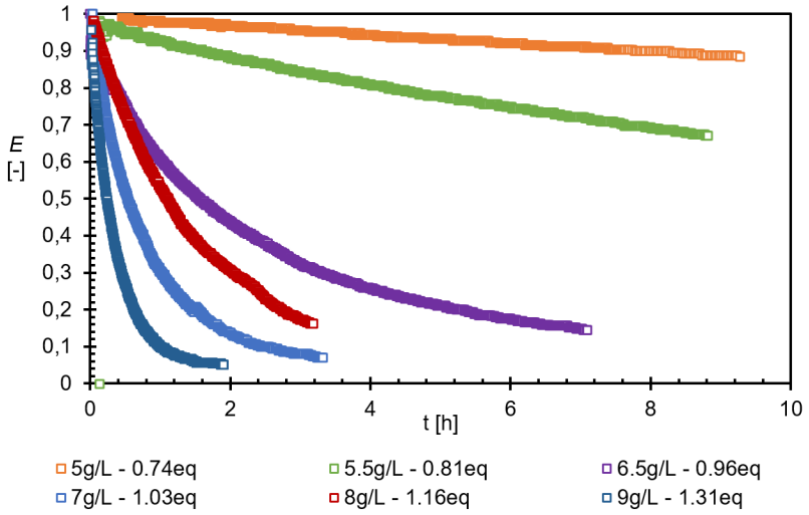

Figure 8. *Time evolution of the enantiomeric excess E of BnFTP in presence of 1 equivalent of 3-Phenylbutyric acid, with different base concentration. In the caption, next to the concentration of base is given its equivalent compared to the acid.*

When the acid is in excess, the value of k' can no longer be correlated using the same relation. Indeed, the mechanism appears to be different regarding the relation between DBU's concentration and the observed rate constant. In figure 11, it can be seen that the evolution can be linearly fitting by expressing the natural logarithm of k' as a function of the concentration of DBU. Moreover, it can be seen that the value obtained with a very small excess of DBU fits the same correlation. As a result, the mechanism is more complex when the acid is in excess. The explanation for the switch between these two cases is simply a matter of dominant mechanism. When the acid is in excess, all DBU is protonated and the mechanism cannot be the same. When DBU is in excess, the mechanism appears to follow that without the acid considering only the concentration of free DBU. In the case of a small excess of base, the mechanism with the acid appears to be kinetically deciding, probably because the concentration of free DBU is too low compared to the concentration of carboxylate/ammonium.

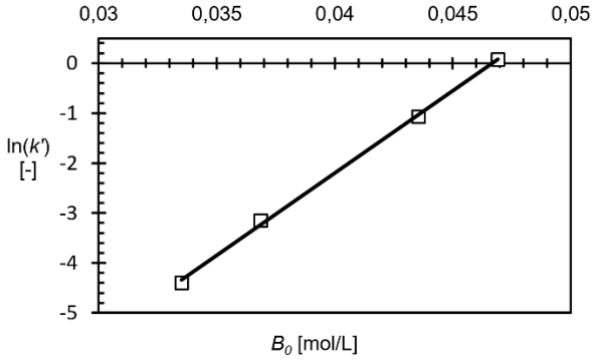

Figure 9. *Linear evolution of the natural logarithm of the observed rate constant ln(k') with the base concentration B_0. The linear regression gave the following equation: ln(k') = 329.7B_0 – 15.4 with a R^2 of 0.999. The linear model error on the slope and intercept is respectively ±7.3 L.mol^{-1}h^{-1} and ±0.3 h^{-1}. The intercept of -15.4 suggest the kinetics of racemization of BnFTP in toluene in presence of the acid but without DBU are very low (2.1,10^{-7} h^{-1}) and can be neglected.*

Though first order to the enantiomeric excess, the mechanism when the acid is in excess, is complex as shown by varying concentrations of DBU. An exponential relationship between the observed rate constant and the concentration of DBU suggests a rather complicated system since no simple mechanism can explain this evolution of the observed constant rate with the base. Consequently, the mechanism is more complex with possible complexation equilibriums in play. Within the scope of this study, the evolution of the observed rate constant with base concentration was a fruitful information for the optimization of the CoISD process. For the same reason, its relationship with temperature was preferentially studied as opposed to the establishment of a full kinetic model explaining empirical observations that would require collecting data on every possible parameter like concentration of acid, concentration of BnFTP…
To do so, a different set-up, adapted to heating was used (see SI). BnFTP was shown to be stable over the range of studied temperature. Five experiments were carried out at different temperatures with a base concentration of 6.8 g/L (0.29eq) to obtain the observed rate constant (SI).

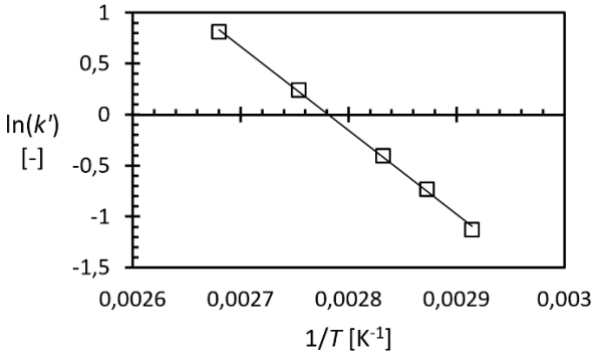

Figure 10. *Linear evolution of the natural logarithm of the observed rate constant ln(k') with the inverse of the temperature 1/T. The linear regression gave the following equation: ln(k') = -8225/T + 22,89 with a R^2 = 0,9979. The linear model error on the slope is respectively ±219 L.mol^{-1}h^{-1}.*

The evolution of the logarithm of the observed rate constant *k'* for each experiment with the inverse of the temperature gave a

straight line (figure 12) showing that despite its complexity, the mechanism of racemization with an excess amount of acid still responds to an Arrhenius law and the activation energy of the reaction is about 68.4 KJ.mol^{-1}. Extrapolating from this data, at 20°C, the observed rate constant is of 8.9x10^{-3} h^{-1}. The activation energy is in the range of other similar racemization reactions like that of L-glutamic acid, catalyzed by salicylaldehyde derivatives, ranging from 54 to 67 KJ/mol.[36]

When compared to thermal racemization of axial or planar chiral compounds, the activation energy remains of the same order (75 to 116 KJ/mol).[37-40] In those cases, the molecules are generally stable at room temperature and racemize upon heating. Similarly, with our system, we have the same result with low kinetics at room temperature, increasing sufficiently upon heating to induce racemization. Consequently, a temperature of 90°C was necessary to obtain an effective racemization. Indeed, when comparing the rate constants at 70°C and 90°C, there is a 4-fold effect, justifying the necessity of high temperature. Consequently, for the deracemization reaction used in our recent development[8], a two-vessel set-up is required with the racemization running at 90°C in one vessel, and the crystallization in the other vessel. A possible one-pot process would have been feasible using an excess amount of DBU at room temperature, but this latter is not compatible with the crystallization conditions. Indeed, an excess of DBU would result in all the acid being deprotonated, making it impossible for the co-crystal to form since the acidic proton of the acid is necessary for the formation of the co-crystal (hydrogen bond).

Conclusion

The kinetics of base induced BnFTP racemization were studied and the racemization reaction was shown to be first order with respect to the base as well as BnFTP, confirming the proposed enolization racemization mechanism. Protic-polar solvents such as ethanol and 1-propanol lead to fast racemization showing a relatively high racemization rate constant. Addition of a chiral acid strongly decreases the kinetics of the racemization due to the reaction of the base with the acid, yielding an ammonium salt species. Racemization with this ammonium salt as a base displays a seemingly more complex mechanism, with an apparent exponential evolution of the rate constant with respect to the base concentration. Consequently, a full understanding of this system demands further studies. However, the mechanism does remain first order with respect to the enantiomeric excess and was shown to follow the Arrhenius law. This study of BnFTP racemization kinetics provides key information for the future optimization of the deracemization process we recently introduced, like the interest of using protic solvents to increase racemization kinetics, as well as the importance of the base concentration and temperature on racemization kinetics.

Experimental Section

MATERIAL

(R,S)-BnFTP was synthesized from commercially available chloropinacolone (purchased from VWR, ≥95%), 1H-1,2,4-triazole (purchased from Carl Roth, ≥99%), 4-fluorobenzyl chloride (purchased from Fluorochem) and NaH (60% suspension in mineral oil, purchased from Acros) with a 2-reaction synthesis as specified elsewhere[8]. The racemization catalyst, the base 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), was purchased from Sigma Aldrich (≥99%) and used as such. All the solvents used were commercially available and of analytical grade. Finally, the acid added to the system, (S)-3-phenylbutyric acid, was commercially purchased as racemic from Sigma Aldrich (98%) and then resolved[9] by using either (S)- or (R)-phenylethylamine,[27] purchased from VWR (≥98%).

POLARIMETRIC ANALYSIS

The polarimeter used for all the measurement was a Rudolph Autopol IV automatic polarimeter, used in its continuous mode with a 5-second measurement interval. All the measurements were carried out at a wavelength of 589nm –since this wavelength gave the highest rotary power out of the 4 available. The temperature of the cell was measured continuously using a temperature probe. During every experiment, the evolution of the optical rotation α was measured and linked to the evolution of the enantiomeric excess *E* using Biot's law.

Racemization in absence of the co-former

Biot's law gives the expression of the optical rotation *α*. In our case, we consider two enantiomers of molecule whose concentration is the product of the molecule's concentration c_m by the weight ratio of the enantiomer with respect to the other one x_S and x_R:

$$100\alpha = [\alpha] l S_0 (x_S - x_R) \quad (12)$$

With α the optical rotation of the sample in °, *[α]* the specific rotation of (S)-BnFTP in the solvent of the experiment, *l* the path length in dm, S_0 the concentration of BnFTP in g/100mL x_s and x_R the enantiomer weight fraction of respectively (S)- and (R)-BnFTP so that $x_S + x_R = 1$

By definition, the enantiomeric excess *E* of a substance of (S) is the difference between the weight fraction of the (S)-enantiomer and that of the (R)-enantiomer, divided by their sum.

$$E = \frac{x_S - x_R}{x_S + x_R} \quad (13)$$

Equation (3) can then be obtained from (1) and (2), putting in relation the enantiomeric excess of a solution of two enantiomers and the optical rotation observed.

$$E = \frac{100\alpha}{[\alpha] l S_0} \quad (14)$$

For each experiment, the value of the specific rotation of (S)-BnFTP was estimated using the starting value of the optical rotation taken right upon the addition of DBU. Doing so, the change of specific rotation due to the presence of DBU is implicitly taken into account. As the starting E, l and c_m are known, the specific rotation can be estimated through equation (3). All subsequent optical rotation values were then converted to an equivalent enantiomeric excess according to this equation.

Racemization in presence of the co-former

When 1eq of a chiral acid is added to the system, a change both in the overall optical rotation as well as in the specific rotation of (S)-BnFTP will occur, this latter due to intermolecular interactions. Taking this effect into account, the new specific rotation of (S)-BnFTP is called *[α]'*. Furthermore, when DBU is added to the mixture, a carboxylate species is created. Since this carboxylate has a different specific rotation from the acid, this once more impacts the overall optical rotation of the system. This latter was experimentally observed as the optical rotation went from a negative to positive value upon DBU addition to a mixture of (R)-BnFTP and (R)-acid. The overall expression of the optical rotation is a sum of the optical

rotations caused by BnFTP, the acid and the carboxylate with the specific rotation value taking into account the intermolecular interactions:

$$100\alpha = [\alpha]' l S_0 x_{\mathrm{m}} (x_{\mathrm{S}} - x_{\mathrm{R}}) + [\alpha]^A l c_{\mathrm{m}} {x_{\mathrm{m}}}^A + [\alpha]^{A-} l c_{\mathrm{m}} {x_{\mathrm{m}}}^{A-} \quad (15)$$

With *[α]'* the specific rotation of (S)-BnFTP (in presence of the acid) in the solvent of the experiment, c_m the overall mass concentration of the 1:1 BnFTP/acid mixture in g/100mL, x_m the weight fraction of BnFTP compared to the overall BnFTP/acid amount, $[\alpha]^A$ the specific rotation of the acid in the considered system, ${x_m}^A$ the mass fraction of the acid, $[\alpha]^{A-}$ the specific rotation of the carboxylate in the considered system and ${x_m}^{A-}$ the mass fraction of the carboxylate.

With equation (4), a new relation between the enantiomeric excess and the observed optical rotation can be drawn:

$$E = \frac{100\alpha - [\alpha]^A l S_0 {x_{\mathrm{m}}}^A - [\alpha]^{A-} l S_0 {x_{\mathrm{m}}}^{A-}}{[\alpha]' l S_0 x_{\mathrm{m}}}$$

For $E = 0$, $\alpha_{\mathrm{f}} = \frac{[\alpha]^A l S_0 {x_{\mathrm{m}}}^A + [\alpha]^{A-} l S_0 {x_{\mathrm{m}}}^{A-}}{100}$ With α_f the optical rotation at the end of the experiment. This value can be determined by either waiting for full racemization and extrapolating the value of α_f from the experimental data or by experimentally creating a mixture corresponding to the fully racemized mixture containing (R,S)-BnFTP, the acid and the DBU with the exact same concentrations. We chose this latter option for all experiments, as reaction times were very long in some case. This yields a final equation linking the enantiomeric excess E to the *in situ* measured optical rotation *α*.

$$E = \frac{100(\alpha - \alpha_{\mathrm{f}})}{[\alpha]' l S_0 x_{\mathrm{m}}} \quad (16)$$

For each experiment, the enantiomeric excess was extracted from the optical rotation using eq. (5), after *[α]'* was estimated using the corrected onset value [The measured onset value was subtracted by α_f to yield the corrected onset value] of the optical rotation right upon DBU addition, when the enantiomeric excess is still the same (before racemization occurs).

PREPARATION OF COCRYSTAL (R)-BnFTP-(R)-PBA

(R)-BnFTP was resolved from (R,S)-BnFTP by co-crystallization with (R)-3-Phenylbutyric acid yielding the (R)-BnFTP-(R)-PBA co-crystal. The protocol is the following[8]: In a vial, to 1g of (R,S)-BnFTP, 8 mL of Toluene was added. Then, 0.6g of (S)-3-Phenylbutyric acid (1 eq) was added and the mixture was stirred overnight with a magnetic stirrer at room temperature. A white solid in suspension was obtained, filtered over Buchner and washed with cyclohexane. The solid was dried at 50°C for 2h. The cake was analyzed by chiral HPLC (see supporting information) to determine its enantiomeric ratio. An amount of 516.1mg of a white solid (yield of 32%) was obtained, with an enantiomeric excess of 99.3% is obtained.

PREPARATION OF (S)-BnFTP

(S)-BnFTP was obtained by breaking the enantiopure co-crystal (S)-BnFTP-(S)-PBA obtained from the same resolution as to make the co-crystal (S)-BnFTP-(S)-PBA, expect that (R)-PBA was used instead of (S)-PBA. The protocol was the following: For 1g of co-crystal, 15mL of Ethyl acetate were added to achieve full dissolution at room temperature. In parallel, 1.1 equivalent of NaOH was dissolved in 20mL of water. Two successive extractions were performed using 10mL of the NaOH solution. The organic phase was then dried over $MgSO_4$, and evaporated under vacuum. Enantiopure (S)-BnFTP was obtained with a 90% yield.

RACEMIZATION SET-UP

The *in situ* set-up consisted of a polarimeter with a flow through cell. This cell was connected to a 100mL-glass vessel (VWR, 215-1592) containing the racemization solution. A gear pump (ISMATEC, ISM405A-230V) was used for a continuous flow of the solution (figure 1&2). The tubing used was made of Nylon (RS PRO Air Hose Blue Nylon, OD: 8mm, ID: 5.5mm), which is toluene resistant, and resistant to all used solvents. The reaction vessel was located on a stirring plate (IKA RH digital) and the environment stirred with a stirring bar at a speed of 400 rpm. The solution was pumped at a rate of 43.32mL/min, which allowed a stable optical measurement. All experiments were conducted at room temperature with continuous monitoring of the temperature in the cell showing a variation between 22-24°C at maximum.

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Keywords: Racemization • Kinetics • Polarimeter • CoISD • Co-crystallization Induced Spontaneous Deracemization

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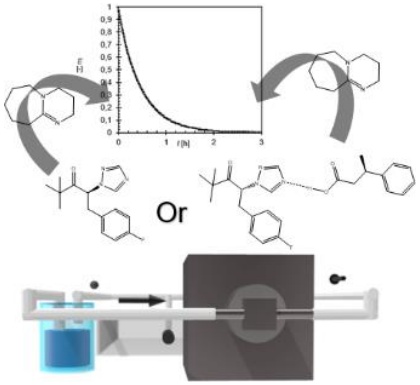

The kinetics of racemization of a fungicide precursor compound were studied in the presence of a base, varying its concentration, the solvent or by adding an acid in the system. Kinetics are first order with respect to the enantiomeric excess and base concentration without the acid. When the acid is present, a decrease in the overall speed of racemization is observed alongside a more complex mechanism.