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A computer program for analysis, simulation and optimization of asymmetric catalytic processes proceeding through two consecutive steps. Type 1: asymmetrization–kinetic resolutions

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Abstract: A computer program for the treatment of the kinetics of asymmetric catalytic reactions proceeding through two consecutive steps was developed. This allows analysis, simulation and optimization of processes consisting of a sequential (i) asymmetrization of a bifunctional prochiral or *meso*-compound in a first step, followed by (ii) kinetic resolution of the chiral intermediate in a second step. A case study shows that — provided that the kinetics of both steps are matching — step two may considerably contribute to the asymmetrization reaction. © 1997 Elsevier Science Ltd

Introduction

Catalytic single-step asymmetrization reactions have emerged as one of the major sources for the synthesis of chiral molecules[1–3] (Scheme 1). The most striking advantages of this method are the independence of the enantiomeric composition of the chiral product (*P*+*Q*) from the conversion of the reaction which allows a 100% theoretical yield to be accrued. The selectivity of the reaction is solely governed by the so-called selectivity factor (α), which is equal to the ratio of the apparent first-order rate constants k_1 and k_2.[4] The latter govern the relative speed by which the prochiral (or *meso*) starting material (*S*) is transformed through mirror-image reaction pathways into enantiomeric products *P* and *Q*. It is obvious that only relatively high values of α lead to products having a preparatively useful enantiomeric composition. For instance, an α-value of about 40 is required to cross the threshold of an e.e. of 95%, and only virtually absolute specificities (i.e. α>200) lead to e.e.s of >99%. Nevertheless, such high selectivities are sometimes achievable, in particular by using biocatalysts.[1] For the numerous cases where insufficient α-values are observed, selectivity enhancement is required,[5] which is feasible via modification of the catalyst, the substrate or the 'environment', such as reaction medium,[6] temperature,[7] pH, etc. The majority of these latter techniques have in common that the effects are generally not predictable since they are largely empirical in nature and thus require trial-and-error experiments. In contrast to the kinetic resolution of a racemate,[2,8] the enantiomeric composition of *P*/*Q* in a single-step asymmetrization cannot be controlled through the kinetics, i.e. by stopping the reaction at the appropriate degree of conversion. On the contrary, in racemate resolutions, this latter technique has proven to be highly predictable.[9]

One approach for the optimization of asymmetrization reactions based on the kinetics has proven to be highly flexible. It is applicable to substrates carrying two reactive functional groups, which allows the reaction to proceed through two steps in the same reaction vessel (Scheme 1).[10] On the one hand, both reactive groups may be chemically identical, but stereochemically different — i.e. prochiral or *meso*-substrates — but they also may be of different nature. Furthermore, both steps may not only be catalyzed by a single but also by means of two different (bio)catalyst(s).

Due to the presence of two reactive groups in the starting material, the reaction proceeds via two consecutive steps. As long as the first step is considered alone, the enantiomeric purity of the product

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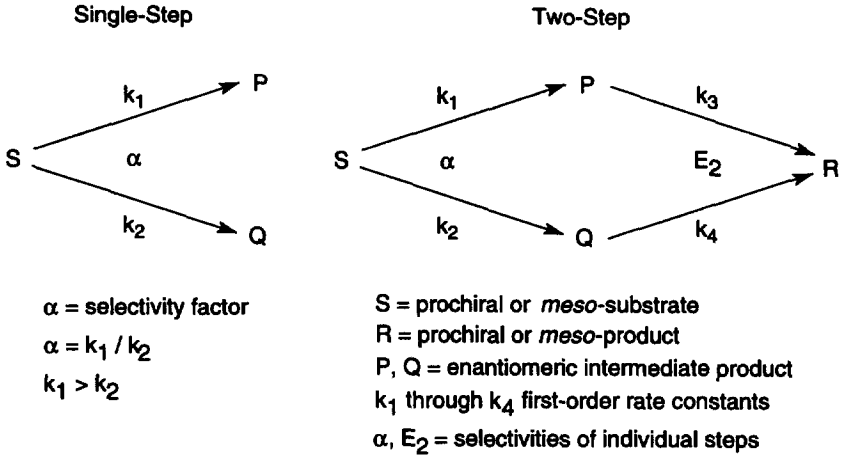

Scheme 1. Single-step asymmetrization and sequential two-step asymmetrization/kinetic resolution (type-1 sequence).

(*P*/*Q*) is independent of the conversion. On the other hand, this does not apply for step two, which consists of a kinetic resolution of the enantiomeric mixture *P*+*Q*. As a consequence, for the whole process the enantiomeric composition of *P*/*Q* becomes a function of the conversion.

The strength of such a sequential process lies in the fact that the second step may contribute to the chiral selection process by removing the 'errors' from *P*/*Q*, which occurred during step one due to insufficient selectivity. As a consequence, an enhanced enantiomeric purity of the chiral product *P*/*Q* may be achieved by stopping the reaction at an appropriate degree of conversion, albeit at the expense of a reduced chemical yield. The latter may be circumvented by recycling of the prochiral (or achiral) materials *S* and *R*.

Due to the fact that the product (*P*+*Q*) from the asymmetrization (step one) represents the starting material of the kinetic resolution (step two), the kinetics of the overall process becomes very complex. Although the principles of such a process were recognized some time ago[11] widespread practical application has been hampered by the lack of a simple procedure for the handling of the kinetics. These facts have prompted us to develop computer programs 'SeKiRe'.[12] The features of these programs along with selected case-studies are described in this paper.[13,14]

It has to be emphasized that the programs are not applicable to processes where the starting material consists of a more complex *meso*/DL-mixture.[15–19] Such reactions require exquisite stereoselectivities, which cannot easily be met. Likewise, sequences proceeding through four consecutive steps (the 'double-*meso*-trick')[20] or via a combination of enantiotopic group and diastereotopic face selectivity[21] are out of scope. Such processes are generally impeded by analytical problems due to the similarity of the large number of intermediate species involved. It is not surprising that preparative examples for such reactions are rare.

Applicability

Sequential asymmetrization/kinetic resolution has been frequently employed, in particular for biocatalyzed reactions.[22] Thus, the hydrolysis of prochiral (or *meso*) diesters[23–30] and the reverse reaction — i.e. the esterification of a prochiral or *meso*-diol via acylation[28,30–32] or alcoholysis of an ester[27] — was frequently successful under catalysis of a protease, esterase or lipase. In addition, also cases where two different biocatalysts were employed to effect each of the individual steps are known: for instance, hydrolysis of a prochiral 1,3-dinitrile catalyzed by a nitrile hydratase furnished the corresponding *mono*-carboxamide, which in turn was further converted by an amidase.[33] Similarly, in a whole-cell biotransformation, prochiral 1,3-dichloropropan-2-ol was transformed into epichlorohydrin

(by a halohydrin epoxidase), which was subsequently hydrolyzed by an epoxide hydrolase to yield 3-chloropropan-1,2-diol as the final product.[34–36] From the non-biocatalytic series, a system employing a metal complex for the synthesis of chiral glycine derivatives by enantioselective proton exchange has been reported.[22]

It is surprising to note that in the majority of studies reported to date the reactions were conducted according to a typical 'black-box approach' and that the possibility to improve the enantiomeric composition of the product was completely neglected. Occasionally, optimization was attempted by trial-and-error[32] or via numeric simulation,[31] and relative rate constants were determined only by two research groups.[23,28–30]

Computer program features

Abbreviations

S=Bifunctional prochiral or *meso*-substrate, *P* and *Q*=intermediate product enantiomers, *R*=final prochiral or *meso*-product; α=selectivity constant for the asymmetrization reaction, i.e. step one ($\alpha=k_1/k_2$); E_2=selectivity constant for the kinetic resolution, i.e. step two (Enantiomeric Ratio); k_1 through k_4 (k_i)=first-order rate constants; t_i=time; [S_0]=substrate concentration at start (t_0); [*S*]=substrate concentration at t_i; [*P*+*Q*], [*P*], [*Q*], [*R*]=concentration of chiral product, product enantiomer *P* and *Q*, and final (achiral) product at t_i, respectively; e.e.$_{P/Q}$=enantiomeric excess of chiral product (positive and negative e.e.-values indicate enantiomer *P* or *Q* being in excess, respectively); *c*=conversion [%] {defined as the fraction of final reaction product formed from starting material ([*R*]/[S_0])}.

Analysis

Sequential asymmetrizations–kinetic resolutions can be analyzed based on experimental data and the four first-order rate constants (k_i) governing the kinetics of the process can be calculated. The following input data can be used: [*P*+*Q*], [*S*], [*R*], e.e.$_{P/Q}$. At least three values out of the given four are required, with [S_0] being known. Due to inaccuracies emerging from analytical procedures and deviations of the (actual) kinetics from the (theoretical) assumptions (e.g. due to inhibition, etc., see below), at least three but preferably four or five sets are recommended for reliable results. This option provides an overall picture of the process.

Simulation

Starting from assumed or calculated relative rate constants, the following parameters can be plotted versus time or versus conversion (*c*): [*P*+*Q*], [*S*], [*R*], [*P*], [*Q*], e.e.$_{P/Q}$. It has to be emphasized that, due to mathematical reasons, the following combinations for k_is are not allowed: $k_3=k_1+k_2$ and $k_4=k_1+k_2$. However, if such processes are to be simulated, a slight deviation of the k_is avoids these problems (e.g. taking 999 instead of 1000, etc.). Based on the above assumed rate constants, single sets of data consisting of *c*, [*S*], [*R*], [*P*], [*Q*], [*P*+*Q*] and e.e.$_{P/Q}$ at a certain moment (t_i) can be obtained by using the 'single value' option. This feature is designed for the modification of a given process, e.g. acceleration or deceleration of individual reactions by altering the reactions conditions.[37]

Optimization

Based on the four relative rate constants — either obtained from experimentally determined data (*Analysis*) or assumed (*Simulation*) — the maximum obtainable e.e.$_{P/Q}$ can be calculated with matching data for t_i, [*S*], [*R*], [*P*], [*Q*]. By using this option, the optimum point of harvest for the chiral product in a given process can be determined.

Theory

General Remarks

The following assumptions were made:

(1) The specific activity of the enzyme remains constant during the whole period of the reaction, implying that no enzyme deactivation caused by pH, temperature, chemical or mechanical stress occurs.
(2) Absence of inhibition.
(3) Spontaneous (non-biocatalyzed) reactions can be neglected.
(4) All reactions are irreversible.
(5) For the *Analysis* option, the substrate must always be in excess.[38]

Mathematics

With some variations, the mathematics of such processes have been elaborated before.[22,39] The descriptors used below were chosen according to Wang *et al.*,[23] who investigated the hydrolysis of alkyldiol diacetates using porcine liver esterase and porcine pancreatic lipase. The apparent first-order rate constants k_1 through k_4 can be related to the *k*inetic constants of the enzyme as $k_1+k_2=k_{cat(S)}/K_S$, $k_3=k_{cat(P)}/K_P$ and $k_4=k_{cat(Q)}/K_Q$, where $k_{cat(P)}$, $k_{cat(S)}$ and $k_{cat(Q)}$ are turnover numbers and K_S, K_P and K_Q are their respective Michaelis–Menten constants. Since for practical applications, the relative rate constants (k_i) are more meaningful than the corresponding Michaelis–Menten constants — the former immediately provide a picture of the selectivities at first glance — this program was written for the calculation of the values of all k_i.

A sequential asymmetrization–kinetic resolution sequence can be described by the following differential equations:

$$\frac{dS}{dt} = -(k_1 + k_2)S \tag{1}$$

$$\frac{dP}{dt} = -k_3P + k_1S \tag{2}$$

$$\frac{dQ}{dt} = -k_4Q + k_2S \tag{3}$$

$$\frac{dR}{dt} = k_3P + k_4Q \tag{4}$$

After integration of Eqs 1–4 and assuming that the concentration of S is S_0 and the concentration of all other components is nil at the beginning of the reaction (t_0), Eqs 5–7 are obtained, where S, P, Q and R are the concentrations at the time t.

$$S = S_0e^{-(k_1+k_2)t} \tag{5}$$

$$P = S_0\frac{k_1}{k_3 - (k_1 + k_2)}[e^{-(k_1+k_2)t} - e^{-k_3t}] \tag{6}$$

$$Q = S_0\frac{k_2}{k_4 - (k_1 + k_2)}[e^{-(k_1+k_2)t} - e^{-k_4t}] \tag{7}$$

Equations 6 and 7 have also been derived from non-biocatalytic systems.[22] Due to the absence of side reactions, such as decomposition, etc., the sum of the concentrations of all components at any time is constant, and Eq. 8 is derived:

$$R = S_0 - S - P - Q \tag{8}$$

The time, when P and Q have reached a maximum is obtained as follows: derivations of Eqs 6 and 7 versus time t (dP/dt and dQ/dt) has to be zero. Thus the concentration of P and Q, respectively, reaches a maximum after a time of $t_{P\text{-opt}}$ and $t_{Q\text{-opt}}$:

$$t_{P-opt} = \frac{1}{k_3 - (k_1 + k_2)}\ln\frac{k_3}{k_1 + k_2} \tag{9}$$

$$t_{Q\text{-opt}} = \frac{1}{k_4 - (k_1 + k_2)} \ln \frac{k_4}{k_1 + k_2} \tag{10}$$

Limits: in order to construct a diagram, it is necessary to calculate the e.e.$_{P/Q}$ value for t approaching zero. Using the rules of l'Hôpital one gets:

$$\lim_{t \to 0} \text{e.e.}_{P/Q} = \frac{k_1 - k_2}{k_1 + k_2} \tag{11}$$

Calculation of k_is: the input data (i.e. t_i, [S], [P], [Q], [P+Q], [R] and/or the e.e.$_{P/Q}$) are internally converted to a data table containing t_i, [S] and [P+Q]. Most reliable results were obtained by using three to four independent sets of data at a conversion below ~30%. It should be mentioned that the last set of input data should be determined at a conversion of <60%. For all further calculations the principle of the minimum of the sum of the errors squared is used. The k_i are obtained within three steps:[40] (i) linear regression (Eq. 5) to obtain the sum of k_1 and k_2; (ii) k_2 is obtained via determination of k_1 and k_3 (Eq. 6) and from (i); (iii) determination of k_4 (Eq. 7).

Using the principle of the minimum of the sum of errors squared, dF/dk_1 and dF/dk_3 simultaneously have to be nil, which is equivalent to finding the minimum of a three-dimensional plane (with F standing for the sum of errors). This has been accomplished by starting somewhere in the positive quadrant, and — like a ball rolling to the minimum — the gradient was followed 'downhill', in order to make F smaller. The following equations were used:

$$\frac{dF}{dk_1} = 2 \sum (P_{\text{calc.}} - P_{\text{meas.}}) \left\{ \frac{P_{\text{calc.}}}{k_1} + \frac{S_0 k_1}{k_3 - (k_1 + k_2)} [(-t)e^{-(k_1+k_2)t}] + \frac{S_0 k_1}{[k_3 - (k_1 + k_2)]^2} [e^{-(k_1+k_2)t} - e^{-k_3 t}] \right\} \tag{12}$$

For $P_{\text{calc.}}$ see Eq. 6.

$$\frac{dF}{dk_3} = 2 \sum \left\{ (P_{\text{calc.}} - P_{\text{meas.}}) \left[\frac{k_1 S_0 (e^{-k_3 t} - e^{-(k_1+k_2)t})}{(k_3 - k_1 - k_2)^2} + \frac{k_1 S_0 (te^{-k_3 t})}{k_3 - (k_1 + k_2)} \right] \right\} \tag{13}$$

Starting from a $k_{1(\text{old})}$ and $k_{3(\text{old})}$ and by following the gradient, the next $k_{i(\text{new})}$ are obtained by

$$k_{1(\text{new})} = k_{1(\text{old})} + \frac{dF}{dk_1} \frac{\text{Distance}}{\sqrt{\left(\frac{dF}{dk_1}\right)^2 + \left(\frac{dF}{dk_3}\right)^2 + \left[\left(\frac{dF}{dk_1}\right)^2 + \left(\frac{dF}{dk_3}\right)^2\right]^2}} \tag{14}$$

where Distance is an empirical value used to set the size of the steps needed for the gradual approach towards the respective k_i-minimum. An analogous equation was used for $k_{3(\text{new})}$.

For the determination of k_4 the error-function has to be minimised, and, as a consequence, dF/dk_4 has to be nil.

$$\frac{dF}{dk_4} = 2 \sum \left\{ (Q_{\text{calc.}} - Q_{\text{meas.}}) \left[\frac{k_2 S_0 (e^{-k_4 t} - e^{-(k_1+k_2)t})}{(k_4 - k_1 - k_2)^2} + \frac{k_2 S_0 (te^{-k_4 t})}{k_4 - (k_1 + k_2)} \right] \right\} \tag{15}$$

For $Q_{\text{calc.}}$ see Eq. 7.

Case studies

The applicability of a given sequential asymmetrization–kinetic resolution for preparative purposes — and the possibility to optimize such a process — is determined by two factors: (i) the chemical and (ii) optical yield of the chiral material P+Q. These parameters are governed by the rate constants k_1 through k_4, which provide the answer to the question whether the overall process will be successful. By consulting the plots of yield [P+Q] versus e.e.$_{P/Q}$ generated from the computer program the optimum

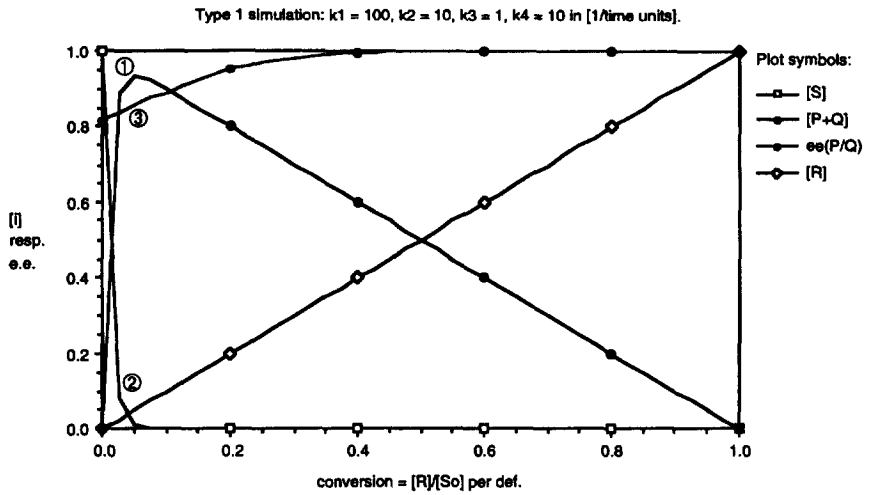

Figure 1. Case I, step one ten times faster than step two, matching selectivities.

point of harvest of $P+Q$ can be determined. The merits and limits of this method are illustrated at hand of selected case studies.

Six different cases were selected for the illustration of Type-1 sequences (Scheme 1). In four cases (Case I–IV) the ratio of the reaction rate of the first step (k_1+k_2) versus the second step (k_3+k_4) is one by ten or vice versa. For Cases V and VI, both steps were chosen to be equally fast because this phenomenon is frequently encountered in practice. In a similar manner, the individual selectivites of each step (denoted as α and E_2) were set to 1:10 (i.e. $\alpha=E_2=10$). For all cases, the lowest relative rate constant was arbitrarily set to 1. In general, it can be stated that the ratio of reaction rates of the first and the second step, expressed as $[(k_1+k_2)/(k_3+k_4)]$ has a major impact on the chemical yield of $P+Q$, whereas the symmetry of the reactions — in other words, with either matching or non-matching selectivities — ($k_1>k_2/k_3<k_4$ or $k_1>k_2/k_3>k_4$, respectively) determines its optical purity.

Case I: $k_1=100$, $k_2=10$, $k_3=1$, $k_4=10$

The fact that the first step of the reaction is ten times faster than the second leads to a high accumulation of chiral intermediate product ($P+Q$) with a maximum of chemical yield (~94%) being reached at an early stage of the reaction (~5% $[R]/[S_0]$, Figure 1, (1)). Consequently, the amount of remaining (non-chiral) substrate $[S]$ and final reaction product $[R]$ is low at this point (~3% each, (2)). The optical purity of P/Q is in an acceptable range (~85%) already at the very beginning of the reaction (3). At this stage, this value is mainly determined by the selectivity of the first reaction ($\alpha=10$) since the second step is significantly slower than the first. Due to the fact that Q is the preferred enantiomer over P in the second step ($k_4>k_3$), the $e.e._{P/Q}$ is gradually enhanced when the reaction proceeds, because Q is converted faster into R than P. In other words, α and E_2 are 'matching' each other by contributing both to the $e.e._{P/Q}$ and the 'errors' which occurred during the selection process in step one (i.e. Q) are selectively removed from the $P+Q$ mixture during the second step. As a consequence, the $e.e._{P/Q}$ is ~85% when the chemical yield has reached its maximum and gradually climbs towards 100% beyond this point. Overall, Case I clearly represents an optimum case — i.e. the first reaction being faster than the second, with matching selectivities ($k_1>k_2$ and $k_4>k_3$).

Case II: $k_1=100$, $k_2=10$, $k_3=10$, $k_4=1$

Case II is very much related to Case I — the first step faster than the second — but the enantiopreference of the second step is inverted ($k_3>k_4$). As a consequence, the maximum chemical yield of $[P+Q]$ is not significantly altered as compared to Case I (~78% at ~17% of $[R]/[S_0]$, Figure 2, (1)).

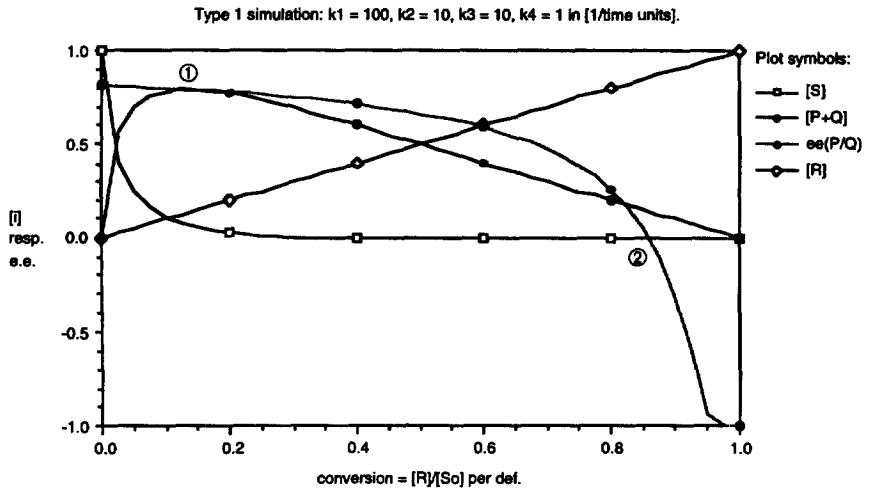

Figure 2. Case II, step one ten times faster than step two, non-matching selectivities.

On the contrary, the function of e.e.$_{P/Q}$ versus the reaction coordinate becomes disadvantageous by constantly dropping during the course of the reaction. This is due to the unfavourable 'non-matching' selectivities: whereas α leads to *accumulation* of P, E_2 causes gradual *depletion* of the latter. As a consequence, the enantiomer being in excess switches from P to Q at the later stage of the reaction (Figure 2, (2)). Nevertheless, Case II still leads to acceptable results: ~78% maximum yield of $[P+Q]$ having an e.e. of ~80% (1). (Negative e.e.$_{P/Q}$ values indicate Q is in excess.)

Case III: $k_1=10$, $k_2=1$, $k_3=10$, $k_4=100$

Case III shows matching selectivites, but the velocity of the reaction rates is inverted—i.e. step one is slower than step two. As a consequence, the chiral product $[P+Q]$ is faster transformed into R than it is formed from S and the chemical yield of $[P+Q]$ remains low throughout the whole process showing a maximum of ~35% at ~30% of conversion (Figure 3, (1)). On the other hand, the e.e.$_{P/Q}$ is excellent due to the matching selectivities (~97% e.e. at $[P+Q]_{max}$, (2)). Reactions following Case III may be acceptable when the chemical yield is of minor importance. (Negative e.e.$_{P/Q}$ values indicate Q is in excess.)

Case IV: $k_1=10$, $k_2=1$, $k_3=100$, $k_4=10$

Case IV shows a worst-case scenario with step one being slower than step two and non-matching selectivities. As a consequence, chemical yields remain very low ($[P+Q]_{max}<10\%$ at a conversion of ~10%, Figure 4, (1)) and the e.e.$_{P/Q}$ begins to drop considerably right from the start (~65% e.e.$_{P/Q}$ at $[P+Q]_{max}$ (2)). Furthermore, the enantiomer being in excess switches from P to Q during the course of the reaction (3). It is obvious that such processes cannot be used for preparative applications. (Negative e.e.$_{P/Q}$ values indicate Q being in excess.)

Case V: $k_1=10$, $k_2=1$, $k_3=1$, $k_4=10$

Cases V and VI have been selected for being quite probable, i.e. both steps of the sequential reaction are equally fast. As may be expected, the scenario from Case V (matching selectivities) represents an intermediate situation between Cases I and III—acceptable results when considering ~78% yield (1) and ~95% e.e.$_{P/Q}$ (2) at $[P+Q]_{max}$ (Figure 5).

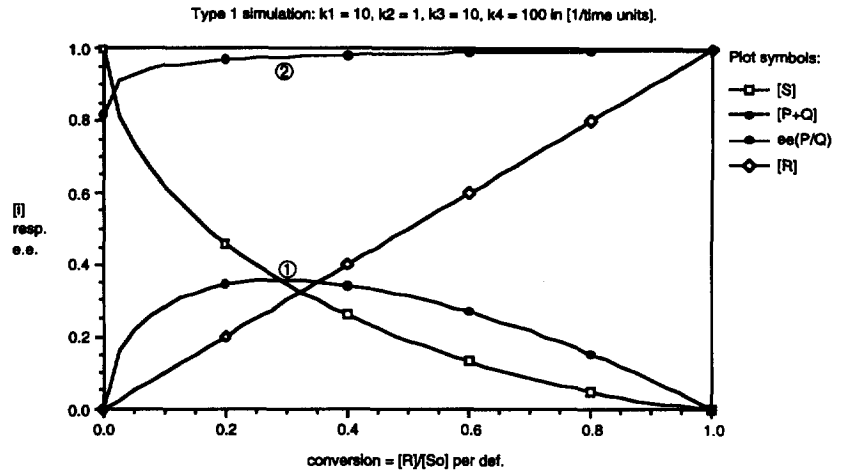

Figure 3. Case III, step one ten times slower than step two, matching selectivities.

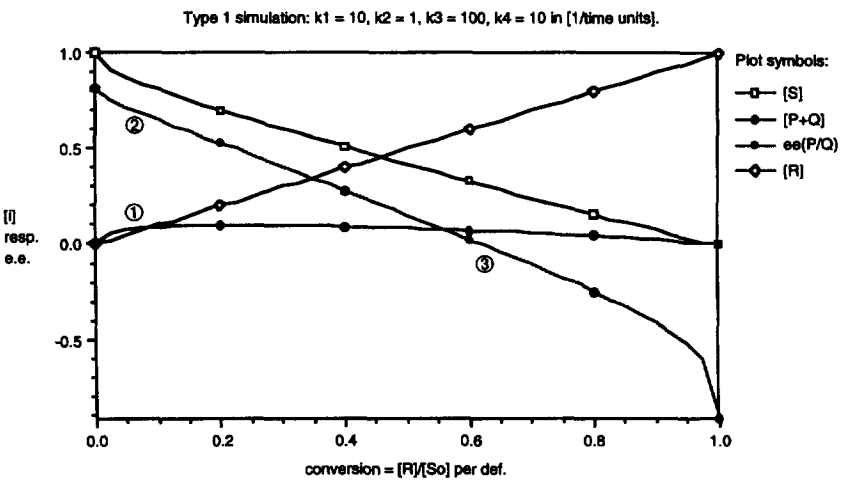

Figure 4. Case IV, step one ten times slower than step two, non-matching selectivities.

Case VI: $k_1=10$, $k_2=1$, $k_3=10$, $k_4=1$

Related to Cases II and IV, both the chemical and optical yields (Figure 6, (1) and (2), respectively) are considerably depleted due to non-matching selectivities. Again, the enantiomer being in excess switches from *P* to *Q* during the late stage of the reaction (3).

Summary

A computer program has been developed for the analysis, simulation and optimization of sequential reactions, which consist of (i) an asymmetrization of a bifunctional prochiral or *meso*-compound followed by (ii) kinetic resolution of the chiral intermediate. Selected case studies show that the relative velocity of the first and the second reaction steps has a major impact on the chemical yield of the desired chiral product, whereas the symmetry of the selectivites of both steps—either matching or non-matching—determines its optical purity. A maximum in chemical and optical yields can be accrued in processes, where (i) the first step is faster than the second [$(k_1+k_2)>(k_3+k_4)$] and (ii) where the individual selectivities of both steps are matching each other by both contributing to the chiral

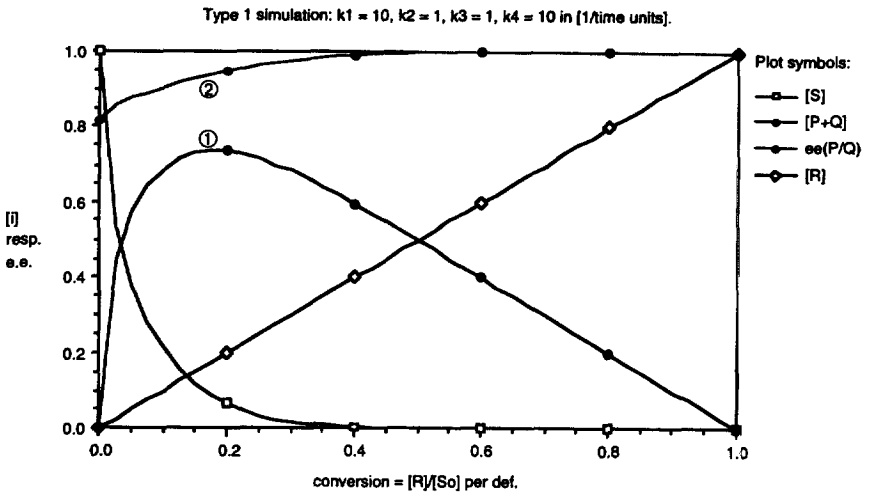

Figure 5. Case V, both steps equally fast, matching selectivities.

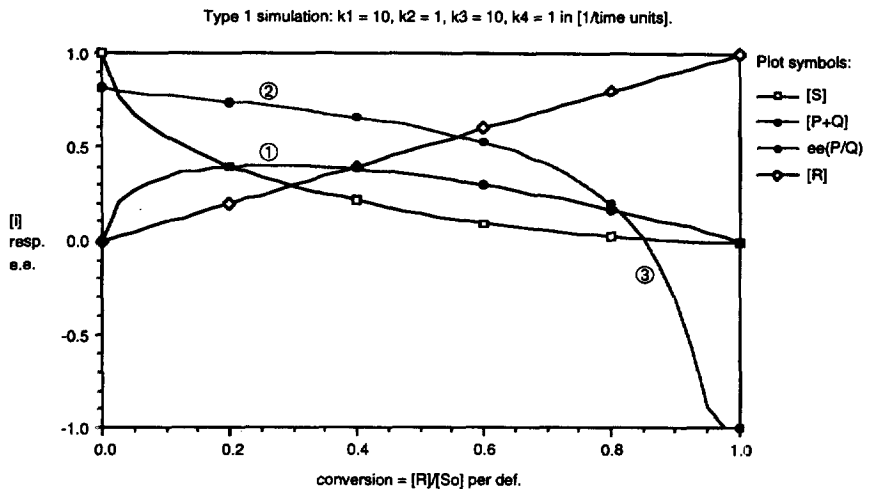

Figure 6. Case VI, both steps equally fast, non-matching selectivities.

selection process ($k_1 < k_2/k_3 > k_4$ or $k_1 > k_2/k_3 < k_4$). In such a scenario the 'errors' which occurred during the incomplete selectivity in step one are sorted out during step two. The application of these programs for the optimization of two-step asymmetrization–kinetic resolution processes is being studied.

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13. For an alternative approach, which requires more elaborate (chiral) analyses see ref. 23: in principle, both of the selectivities (i.e. α and E_2) governing the overall process can be independently determined via two separate experiments: an approximate value for α can be obtained from an experiment which is stopped at a very low conversion — where the second step has not taken effect due to the infinitesimal low concentration of its starting material ($P+Q$). However, this is only feasible as long as step one is considerably faster than step two. Furthermore, the low amount of $P+Q$ formed at this point does not facilitate (chiral) analysis. Next, E_2 can be determined in a separate experiment which uses a racemic substrate mixture of $P+Q$, through the formula derived for single-step kinetic resolutions,[9b] i.e. by determining c {expressed as $[R]/[P+Q]$}and e.e.$_{P/Q}$. However, this requires the availability of racemic $P+Q$ and renders the whole process very time-consuming.
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