Chemo- and Enzyme-Catalyzed Reactions Revealing a Common Temperature-Dependent Dynamic Solvent Effect on Enantioselectivity

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Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

The enantiomeric ratio E of enzyme-catalyzed (*Candida antarctica* lipase and lipase PS) and chemo-catalyzed (L-proline-based diamines) acylation reactions of 1-(naphthalen-2-yl)ethanol, 2-phenylpropanol, and 2-benzylpropane-1,3-diol is dependent on solvent and temperature. Plots of ln E *vs.* $1/T$ showed the presence of inversion temperatures (T_{inv}). The T_{inv} values for the bio-catalyzed and the chemo-catalyzed reactions are fairly in agreement, and correspond as well to the T_{NMR} values obtained by variable-temperature ^{13}C-NMR experiments on the substrates in the same solvent of the resolution. This result demonstrates that clustering effects in the substrate solvation manage the chemical and the enzymatic enantioselectivity, and, moreover, that the solute–solvent cluster is always the real reacting species in solution for chemical as well as for enzymatic reactions.

Introduction. – The role of solvent in condensed-phase chemical reactions is of pivotal importance for determining reaction rates in simple transformations to understanding the complex behavior of proteins in a liquid environment. The solvent has often a decisive effect on issues of structure, energetics, and reactivity, and cannot, in general, be regarded as a negligible perturbation [1]. Solvent effects are closely related to the intermolecular solute–solvent interactions that constitute the solvation process. A number of experimental, theoretical, and simulation studies have been devoted to the elucidation of a molecular view of solvation processes [2]; however, research in this field is still far from being an exhaustive interpretation of the experimental results, especially concerning solvent effects on stereoselectivity.

In our recent studies of the solvent and temperature effects on the stereoselectivity of a number of different reactions, we have tried to explain the well-known phenomenon of the so-called inversion temperature (T_{inv}), which has been observed by several authors in temperature-dependent studies on stereoselectivity (for a review, see [3a]; for a different interpretation of T_{inv}, see [3b]). Inversion temperatures have indeed been found in a large number of completely different reactions, such as asymmetric *Sharpless* dihydroxylation [4], enantioselective epoxidation [5], diastereoselective reduction [6], and, by our group, in nucleophilic additions [7], *Diels–Alder* reactions [8], and in enzymatic resolutions [9]. In our explanation, the T_{inv} phenomenon depends on the reagent solvation and, therefore, on the complex microscopic interactions between solvent and solute molecules, resulting in a modification of the free energy of the reagents and, as consequence, in a modification of their reactivity and stereoselectivity. In particular, the solvation of a molecule leads

to the formation of a solute–solvent cluster with a more-defined three-dimensional structure than generally assumed. The reactivity and the stereoselectivity of a molecule in solution depends on the structure of this supramolecular solute–solvent cluster, which, in turn, depends on the temperature; thus, the same molecule in the same solvent at different temperatures may show a different reactivity and stereoselectivity. The T_{inv} represents the temperature at which two supramolecular solute–solvent clusters are in equilibrium. At temperatures higher than T_{inv}, a supramolecule possessing a given stereoselectivity is present; at temperatures lower than T_{inv}, there is another supramolecule with a different stereoselectivity. Because of the complexity of solvation processes, it is difficult to formulate a detailed microscopic model of these supramolecules. However, their interconversion could involve a solvent reorganization with transfer of solvent molecules between the supramolecules and the bulk or an internal rearrangement of the solvation cluster. Thus, in our explanation, the true reacting species of a molecule in solution at a given temperature is not the molecule itself but its supramolecular solute–solvent cluster [10].

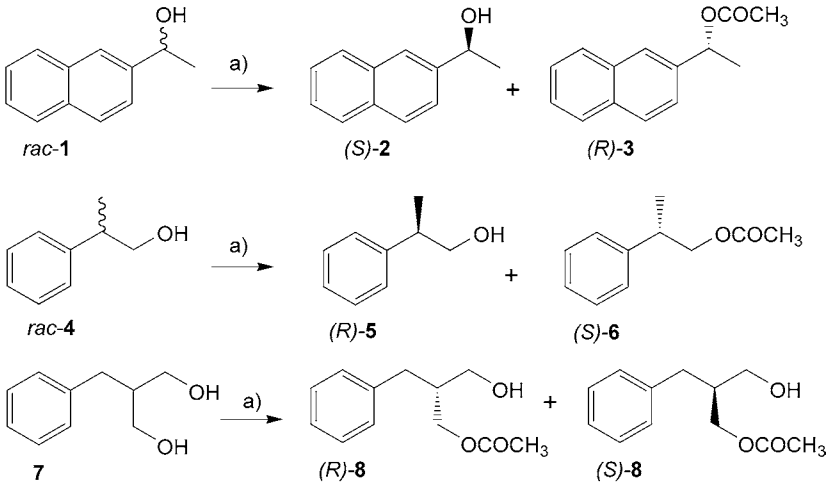
In this paper, we present our results of a comparative study on chemo- and bio-catalyzed kinetic resolution of 1-(naphthalen-2-yl)ethanol, 2-phenylpropanol, and of the asymmetric acylation of 2-benzylpropane-1,3-diol; all reactions revealed phenomena of temperature-dependent solvent effects of the starting compounds. Worthwhile to note is that the chemo- and the enzyme-catalyzed reactions showed fairly similar inversion temperatures.

Results and Discussion. – Traditionally, kinetic resolutions of racemic alcohols have been performed with enzyme catalysts that react selectively with only one of the two enantiomers [11]. Lipase-catalyzed solvolysis and transesterification is, in fact, a powerful approach to obtain optically active alcohols. More recently, several groups have achieved nonenzymatic kinetic resolutions with chiral acylation catalysts. *Oriyama et al.* have recently reported the utility of proline-based ligands as catalysts for kinetic resolution [12] and asymmetric acylation of *meso*-diols [13].

We used these two well-established techiques to compare the enantioselectivities of enzymatic (bio-catalyst) and nonenzymatic (chemo-catalyst) acylation reactions of two racemic alcohols and one prochiral diol by varying the reaction solvent and the temperature. We selected as substrates 1-(naphthalen-2-yl)ethanol (*rac*-**1**), 2-phenylpropanol (*rac*-**4**), and 2-benzylpropane-1,3-diol (**7**), as enzymes, two lipases, *Candida antarctica* lipase (CAL) and lipase PS (*Amano*), as chemocatalysts, three proline-based diamines **9**, **10**, and **11**, and two solvents, THF and *t*-BuOMe (*Scheme 1*), after a preliminary screening of the stability of the enantioselectivity with the conversion, adequate reaction times, and enantioselectivity values. Enantioselective transterification with lipases were carried out with vinyl acetate as the acylating agent, whereas AcCl was used in the diamine-catalyzed reactions. We then compared the temperature dependence of enantioselectivity between the reaction with the chemo-catalyst and the corresponding enzymatic reaction.

Resolution of 1-(Naphthalen-2-yl)ethanol (*rac*-**1**; *Scheme 2*). The enzymatic resolution of *rac*-**1** was carried out by incubating the substrate (0.33 mmol) in THF (3 ml) with CAL (7000 U/g, 50 mg) or lipase PS (30000 U/g, 100 mg), and vinyl acetate (1.32 mmol). The reaction mixture was maintained under vigorous magnetic stirring

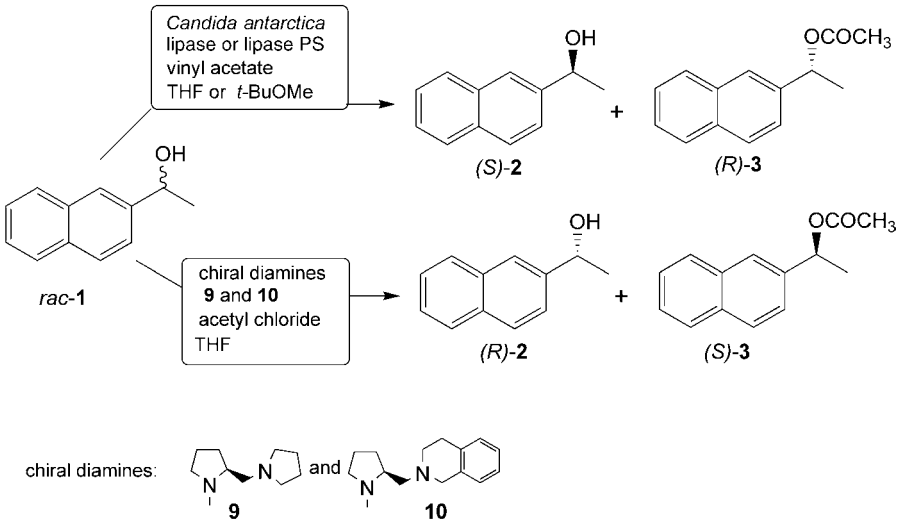
Scheme 1



a) Bio-catalyst: lipase PS (*Amano*) or from *Candida antarctica*; chemo-catalyst: L-proline-derived chiral diamines; acylating agent: vinyl acetate or acetyl chloride; solvent: THF or *t*-BuOMe.

and thermostatted at the desired temperature. The reactions were repeated at different temperatures in the range of $-20°$ to $60°$[1]). The progress of the reaction was monitored by GC. The reactions were terminated at close to 45% of esterification by removal of the enzyme by microfiltration (0.20-μm filter units). The chemo-catalyzed resolution

Scheme 2



[1]) At temperatures lower than $-20°$, the reaction shows too-long reaction times and too-low conversions.

was carried out on 0.33 mmol of the *rac*-**1** in THF (3 ml) with 0.17 mmol (0.5 equiv.) of chiral diamine **9** and **10**, and then AcCl (0.24 mmol). The progress of the reaction was monitored by GC. The reaction was quenched with phosphate buffer (pH 7) and extracted with CH_2Cl_2. The reactions were repeated at different temperatures in the range $-60°$ to $60°$. The enantiomeric excess (ee) values of acetates **3** and alcohols **2** were directly determined on the crude by GC chiral column (see *Exper. Part*).

The enantioselectivity was expressed as the enantiomeric ratio, E, calculated from the ee value of the substrate (ee_s) and product (ee_p) by the relationship [14]:

$$E = \ln[(1 - ee_s)/(1 + ee_s/ee_p)] / \ln[(1 + ee_s)/(1 + ee_s/ee_p)] \quad (1)$$

By studying the temperature dependence of E, the differential activation parameters $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ were evaluated according to *Eqn.* 2 [15]:

$$\ln E = -(\Delta\Delta G^{\ddagger}/RT) = -(\Delta\Delta H^{\ddagger}/RT) + (\Delta\Delta S^{\ddagger}/R) \quad (2)$$

From plots of ln E *vs.* $1/T$, data were then analyzed by least-squares fitting to *Eqn. 2* to obtain linear correlations (*Fig. 1*). The $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values were obtained from slopes and intercepts of the linear plots (*Table*). For each data set, we applied a residual analysis to evaluate the number of linear trends and to ascertain unambiguously the presence of an inversion temperature.

The enzymatic resolution of *rac*-**1** with lipase PS in THF and with CAL in *t*-BuOMe showed the highest enantiomeric ratio with a linear temperature dependence and with the best E value at low temperature ($-20°$). With CAL, as catalyst in THF, an inversion

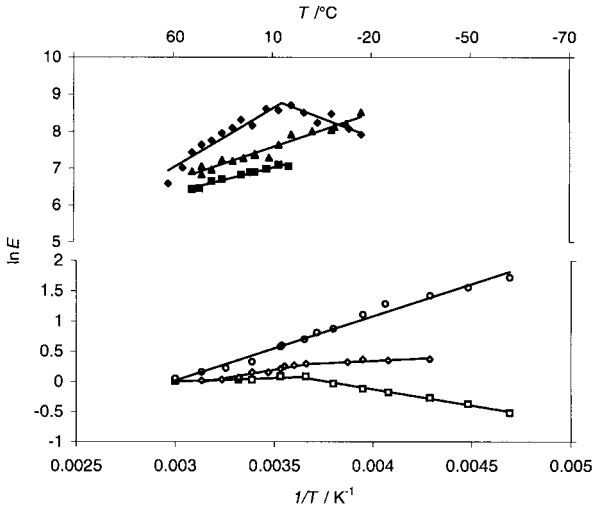


Fig. 1. Eyring *plot for the enantioselective acetylation of 1-(naphthalen-2-yl)ethanol* (*rac*-**1**) *with* Candida antarctica *lipase in THF* (black diamond) *and in* t-*BuOMe* (black triangle), *with lipase PS in THF* (black square), *with chiral diamine* **9** *in THF* (white diamond), *with* **10** *in THF* (white square), *and with* **11** *in THF* (white circle)

Table. *Differential Activation Parameters for Enzyme-Catalyzed and Chiral-Diamine-Catalyzed Enantioselective Acylation of Alcohols* rac-**1**, rac-**4**, and **7** *in THF and* t-*BuOMe*

Substrate	Solvent	Catalyst	T_{inv} [°]	$T > T_{inv}$		$T < T_{inv}$	
				$\Delta\Delta H^{\neq}$ [kcal/mol]	$\Delta\Delta S^{\neq}$ [cal/mol]	$\Delta\Delta H^{\neq}$ [kcal/mol]	$\Delta\Delta S^{\neq}$ [cal/mol]
1	*t*-BuOMe	CAL	–	-3.6 ± 0.2[a])	2.4 ± 0.7	–	–
1	THF	CAL	+9	-6.3 ± 0.6	-5 ± 1	$+4.0\pm0.9$	$+32\pm3$
1	THF	Lipase PS	–	-2.7 ± 0.2[a])	$+4\pm1$	–	–
1	THF	**9**	−1	-1.1 ± 0.1	-3.5 ± 0.5	-0.32 ± 0.06	-0.6 ± 0.2
1	THF	**10**	+2	-0.2 ± 0.1	-0.7 ± 0.4	$+1.08\pm0.05$	$+4.1\pm0.2$
1	THF	**11**	–	-2.1 ± 0.1[a])	-6.3 ± 0.3	–	–
4	*t*-BuOMe	Lipase PS	−19	-0.73 ± 0.06	-0.8 ± 0.2	-0.02 ± 0.06	$+2.0\pm0.3$
4	*t*-BuOMe	**10**	−8	$+0.51\pm0.05$	$+1.5\pm0.2$	-0.15 ± 0.04	-1.02 ± 0.15
4	*t*-BuOMe	**11**	0	-0.80 ± 0.03	-2.5 ± 0.1	-0.20 ± 0.01	-0.32 ± 0.04
4	THF	Lipase PS	+21	-1.5 ± 0.2	-2.9 ± 0.5	-0.38 ± 0.02	$+0.8\pm0.1$
4	THF	**10**	+22	$+0.19\pm0.02$	$+0.5\pm0.1$	$+0.43\pm0.02$	$+1.2\pm0.1$
4	THF	**11**	+22	-0.43 ± 0.07	-1.4 ± 0.2	-0.66 ± 0.02	-2.2 ± 0.1
7	*t*-BuOMe	Lipase PS	+11	-7 ± 1	-16 ± 4	-0.7 ± 0.5	$+5\pm2$
7	THF	Lipase PS	−5	-3.6 ± 0.3	-7 ± 1	$+2.6\pm0.7$	$+16\pm3$
7	THF	**11**	−7	-0.02 ± 0.01	-0.20 ± 0.02	-0.11 ± 0.01	-0.51 ± 0.04

[a]) There is no T_{inv}, thus only one set of differential parameters have been calculated.

temperature was clearly detected at 9°, at which the highest enatioselectivity ratio was reached.

Nonlinear *Arrhenius* plots of the rate data from enzymatic systems have been already reported [16]. However, we observed, for the first time, that nonlinear plots of ln *E vs.* $1/T$ from enzymatic enantioselective reactions consist of the union of two sharply intersecting straight lines, thus identifying an inversion temperature. An *Eyring* plot featuring a T_{inv}, even in the case of enzymatic reactions, indicates the presence of two different substrate–solvent clusters: one above and one below the T_{inv}.

In the enantioselective resolution with chiral diamines, only with pyrrolidine **9** and isochinoline **10** derivatives is there a T_{inv}; with the isoindoline derivative **11**, we obtained the highest enantioselective ratio but no T_{inv}. According to our interpretation, only one solute–solvent cluster is present over all the T range whenever no T_{inv} was observed in an *Eyring* plot. It is worthwhile to note that the T_{inv} values obtained with the two chiral diamines in THF compared with that obtained with CAL in THF, are in a range of 10°.

For $T > T_{inv}$, the differential activation enthalpies differ from each other but have the same negative sign, whereas the sign of the entropy contribution, $\Delta\Delta S^{\neq}$ varies with the enzyme and the solvent. The reactions with CAL and diamine **10** in THF showed a switchover in the activation parameter on going from $T > T_{inv}$ to $T < T_{inv}$, associated to an inverted enantiomeric preference for $\Delta\Delta H^{\neq}$ and $\Delta\Delta S^{\neq}$.

Resolution of 2-Phenylpropan-1-ol (*rac*-**4**; *Scheme 3*). The enzymatic resolution of *rac*-**4** was carried out by incubating the substrate (0.66 mmol) in THF or *t*-BuOMe (10 ml) with lipase PS (30000 U/g, 40 mg), and vinyl acetate (2.64 mmol), and then according to the same protocol as for *rac*-**1**. The chemo-catalyzed resolution was carried

out on 1 mmol of the *rac*-**4** in THF (10 ml) with a catalytic amount (15 µl) of chiral diamine **10** or **11**, 0.5 mmol of (i-Pr)$_2$NEt (*Hünig* base), and then AcCl (0.5 mmol). The *Hünig* base, as a proton sponge, increased the reaction rate; otherwise, with a stoichiometric amount of chiral diamine, the reaction was too slow. The reaction was quenched with HCl (1M) until pH 5 and extracted with Et_2O. The ee values were directly determined on the crude: the ee values of acetates **6** by GC on chiral column and the ee values of alcohols **5** by HPLC (*Chiracel OF*; see *Exper. Part*). The absolute configurations of the products were confirmed by comparison with the commercially available (*R*)-2-phenylpropanol and its (*R*)-*O*-acetate.

Scheme 3

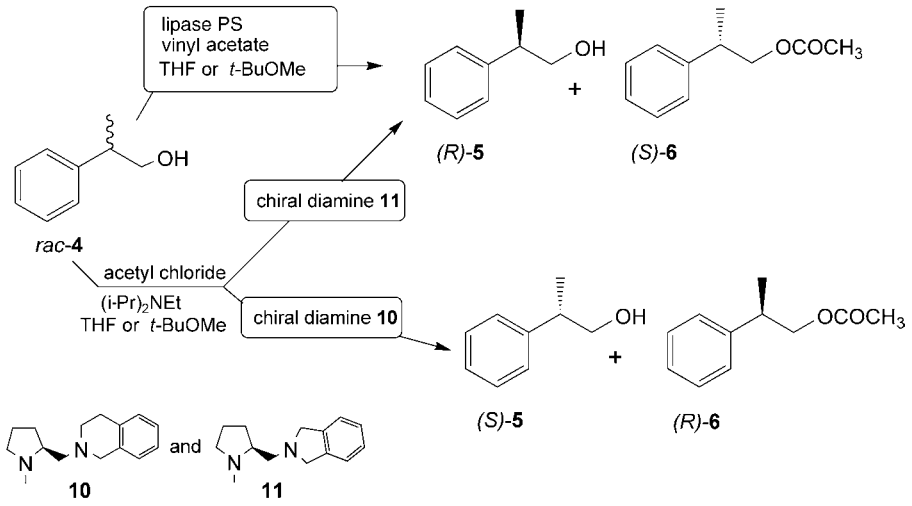


Fig. 2 shows the data of ln *E vs.* 1/*T* for the bio- and chemo-catalyzed acetylation of *rac*-**4**. The enantioselectivity of the resolution of *rac*-**4** is low for both the enzymatic and the chemo-catalyzed reactions; however, due to the high reliability and consistency of experimental data, we can clearly observe the presence of inversion temperatures in all catalyst–solvent pairs[2]). The T_{inv} values observed in THF occurred at higher *T* than those observed in *t*-BuOMe. As observed for *rac*-**1**, the T_{inv} values depend on the solvent and, in the same solvent, they are fairly similar for the enzymatic and the chemo-catalyzed reactions. Quite interesting is the temperature behavior observed with the two chiral diamines that exerted opposite temperature trends. This allows the preference for the opposite enantiomer depending on the catalyst. Analyzing the thermodynamic data, it results that this behavior derives from an opposite sign in the enthalpy contribution ($\Delta\Delta H^{\neq}$) at $T > T_{inv}$ for both diamines, and, in THF, even at $T < T_{inv}$.

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[2]) We reported several cases showing high variations in enantio- or diastereoselectivity with temperature and, in some cases, an inversion of the preferred stereoisomer with *T* (see, *e.g.*, [10] and [7]).

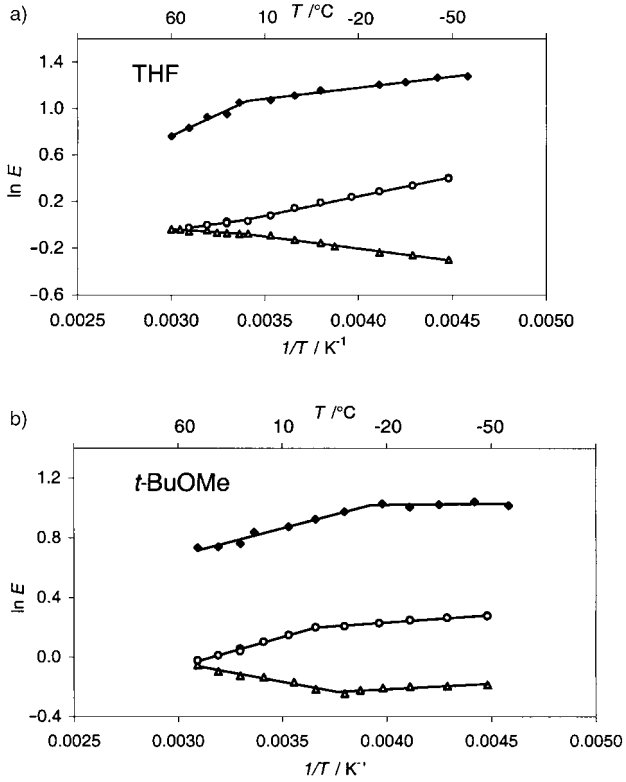

Fig. 2. Eyring *plot for the enantioselective acetylation of 2-phenylpropanol* (*rac*-**4**) a) *in THF with lipase PS* (black diamond), *with chiral diamine* **10** (white triangle), *and with* **11** (white circle), b) *in* t-*BuOMe with lipase PS* (black diamond), *with chiral diamine* **10** (white triangle), *and with* **11** (white circle)

Asymmetric Acylation of 2-Benzylpropane-1,3-diol (**7**; *Scheme 4*). The bio-catalyzed acylation of **7** (0.66 mmol) was performed in THF (10 ml) with lipase PS (30000 U/g, 40 mg) and vinyl acetate (2.64 mmol). The chemo-catalyzed reaction was performed on 1 mmol of substrate **7** with a catalytic amount of the chiral diamine **11** (15 µl), 0.5 mmol of *Hünig* base, and AcCl (0.5 mmol). In both protocols, the progress of the reaction was followed by GC, monitoring also the formation of the diacetate and ensuring its amount to be less than 1%. The ee values of the product **8** was determined by chiral HLPC (*Chiracel OD*). The absolute configuration was determined by comparison of HPLC retention times with the reported data[3]).

Fig. 3 shows the data of ln *R/S vs.* 1/*T*, where *R/S* is the enatiomeric ratio of the product **8**. The temperature dependence of the enantioselective acylation showed the presence of inversion temperatures for both the bio- and the chemo-catalyzed reactions that have similar values for the reaction with lipase PS and the diamine **11** in THF (*Table*).

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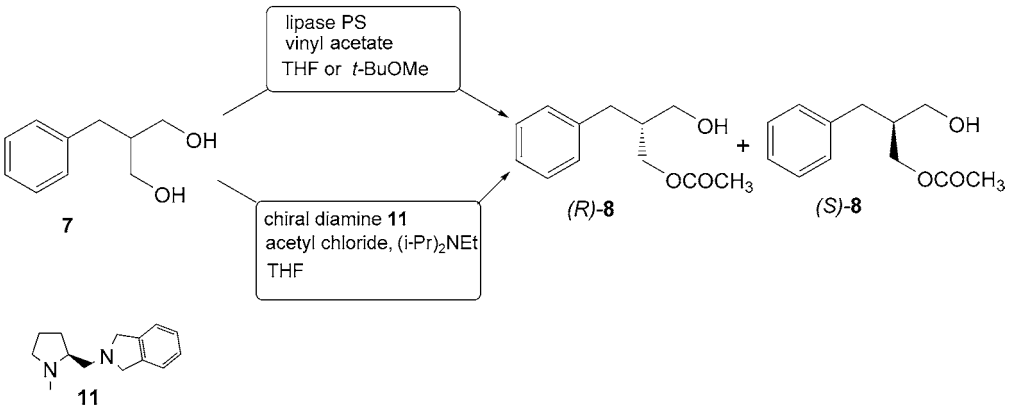
[3]) See the experimental section of [21].

Scheme 4



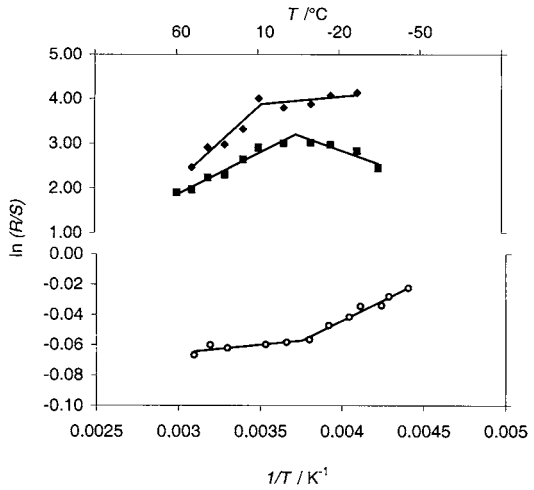


Fig. 3. *Enantioselective acylation of 2-benzylpropane-1,3-diol* (**7**) *with lipase PS in THF* (black square) *and in* t-*BuOMe* (black diamond), *and with chiral diamine* **11** *in THF* (white circle)

Data in the *Table* clearly show that the values of thermodynamic parameters and of T_{inv} are characteristic for each substrate–solvent couple and not of the catalyst of the enantioselective acylation. This result supports our T_{inv} interpretation related to the presence of solute–solvent clustering phenomena, which are dependent on temperature. The temperature-dependence studies on stereoselective processes disclosed the presence of these phenomena, but they are independent of the reactions.

To confirm this in our studies, we have already used variable-temperature (VT) ^{13}C-NMR spectroscopy [17], because chemical shifts and coupling constants are affected by intermolecular interactions with the solvent[4]).

[4]) For solvent effects on chemical shift, see, *e.g.*, [18].

We then conducted VT ^{13}C-NMR experiments with substrates *rac*-**1**, *rac*-**4**, and **7** in (D_8)THF to determine the dependence of chemical shifts on temperature. All spectra, after full assignments, showed a unique set of signals in the temperature range explored, ensuring the presence of a population-weighted average of rapidly interconverting conformers. In *Figs. 4*, *5*, and *6*, plots of selected chemical shifts of substrates *vs.* the absolute temperature are shown. By residual statistical analyses [19], a breaking point (T_{NMR}) in the linear variation of chemical shift *vs. T* could be detected. The correlation with two straight lines is always better than that with a single line. Each substrate showed more than one C-atom with a nonlinear behavior of δ *vs. T*, and with very similar T_{NMR} values. A direct comparison shows a close correspondence between the T_{inv} obtained in bio- and chemo-catalyzed reaction in THF, and the T_{NMR} values. Small differences between their numerical values could account for the fact that T_{NMR} refers to the ground state of the reactant, and T_{inv} to the two diastereoisomeric transition states.

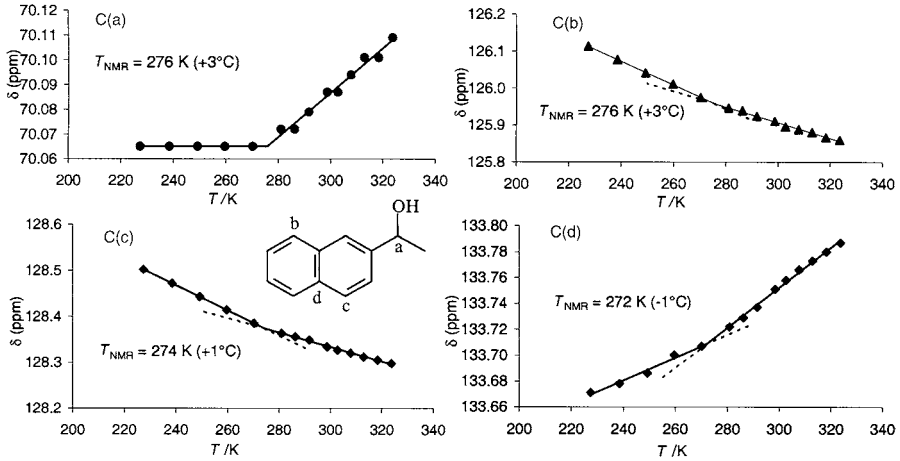


Fig. 4. *Variable-temperature ^{13}C-NMR: chemical shifts for four C-atoms of 1-(naphthalen-2-yl)ethanol* (*rac*-**1**) *in* (D_8)*THF and the corresponding* T_{NMR} *values*

Conclusions. – We have studied the temperature dependence of enantiomeric ratio of bio-catalyzed (CAL and lipase PS) and chemo-catalyzed (L-proline-based diamines) acylation reactions of 1-(naphthalen-2-yl)ethanol (*rac*-**1**), 2-phenylpropanol (*rac*-**4**), and 2-benzylpropane-1,3-diol (**7**), in THF and *t*-BuOMe. We observed the presence of dynamic solvation effects related to the presence of inversion temperatures in plots of ln *E vs.* 1/*T* [5]). The T_{inv} values of the bio-catalyzed and the chemo-catalyzed reactions are fairly in agreement. We then connected the T_{inv} values obtained in the resolutions with the T_{NMR} values obtained in VT ^{13}C-NMR experiments on the substrate–solvent couple in THF (*Fig. 7*). T_{inv} and T_{NMR} are independent experimental results of the same phenomenon related to dynamic solvation clustering. The results obtained in the

[5]) All our data clearly show a better two-line correlation of nonlinear *Eyring* plot than one-line, or than a curve.

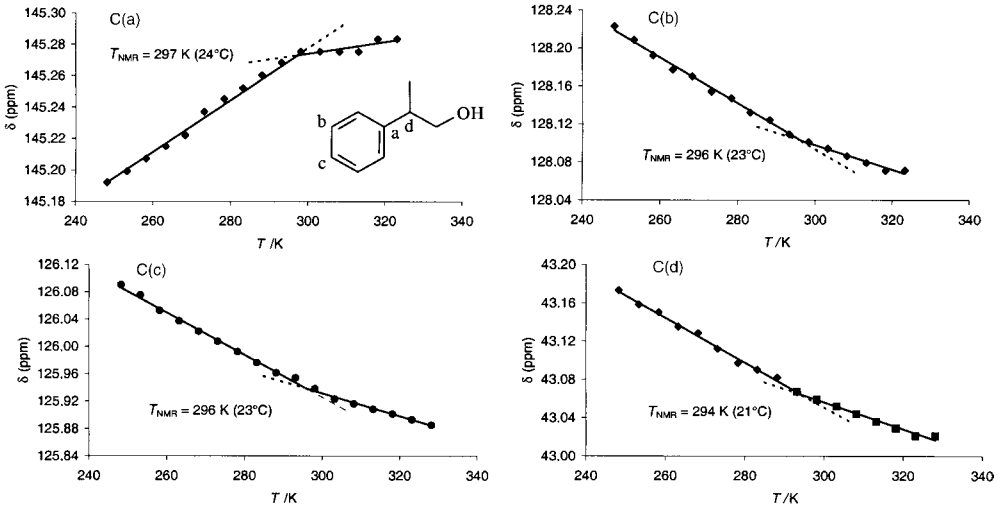


Fig. 5. *Variable-temperature* ^{13}*C-NMR: chemical shifts for four C-atoms of 2-phenylpropanol* (*rac*-**4**) *in* (D_8)*THF and the corresponding* T_{NMR} *values*

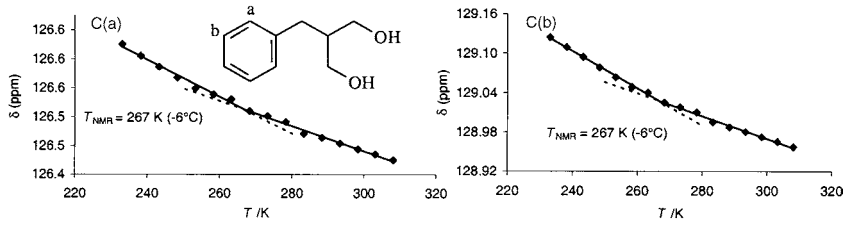


Fig. 6. *Variable-temperature* ^{13}*C-NMR: chemical shifts for two C-atoms of 2-benzylpropane-1,3-diol* (**7**) *in* (D_8)*THF and the corresponding* T_{NMR} *values*

present study clearly confirm that the T_{inv} is a quite general phenomenon independent of the particular reaction system, including the nature, *i.e.*, enzyme or chiral diamine, of the catalyst, but tightly related to the nature of the substrate, in this case the starting alcohol, and the particular reaction solvent used. This means that the solute–solvent clusters are the real reacting species in solution, even in enzymatic reactions, implying that solvent molecules should be present in the transition state of enzymatic reactions.

Nakamura et al. observed that lipases recognize not only the structure of the substrate, but also that of solvent, assuming that a solvent influences enzymatic reactions as a molecule rather than as a bulk medium [20]. *Klibanov* and co-workers stated that the solvent dependence of enzymatic prochiral selectivity can be attributed primarily to changes in the relative solvation modes of the substrate in the transition state [21]. Our interpretation does not contrast with these observations, but we affirm, moreover, that the solute–solvent cluster is the real reacting species in solution, in chemical as well as in enzymatic reactions.

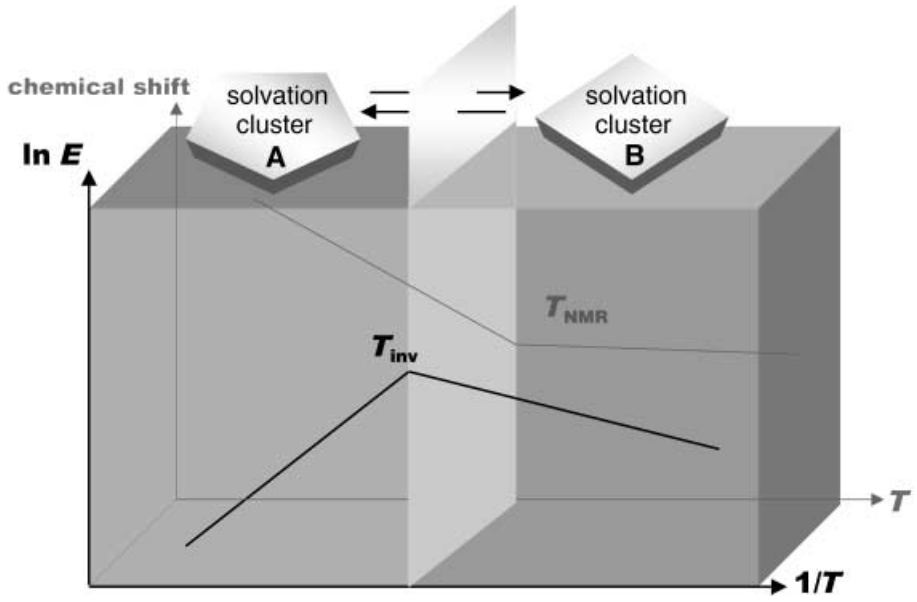


Fig. 7. *The* T_{inv} *in an* Eyring *plot and the* T_{NMR}, *as the interconversion temperatures between two supramolecular solute–solvent clusters*

Experimental Part

General. THF and *t*-BuOMe were dried by distillation from sodium-benzophenone and stored over molecular sieves (4 Å). (D_8)THF was purchased from *Aldrich*. Lipase PS and *Candida antarctica* lipase were kindly furnished by *Amano Enzyme Inc*. and *Enzyme Europe*, resp. 2-Phenylpropanol (**4**) is commercial, 1-(naphthalen-2-yl)ethanol (**1**) was obtained in 98% yield by reduction of 1-(naphtalen-2-yl)ethanone with $NaBH_4$ (1.2 equiv.) in EtOH/THF, 2-benzylpropane-1,3-diol (**7**) was obtained in 85% yield by reduction of commercial diethyl benzylmalonate with $LiAlH_4$. Chiral diamines **9**, **10**, and **11** were obtained starting from L-proline and the corresponding secondary amine according to the procedure reported in [22].

Reactions with enzymes were performed in screw-capped vials. To set and maintain temp. in the range of ± 1°, a *Julabo FT901* immersion cooler in *Dewar* container filled with EtOH, an oil bath with H_2O cooling, or a water bath with a *Techne TE-10D Tempunit* thermoregulator was used, depending on the chosen temp. The temp. was referred to the interior of the reaction apparatus.

^{13}C-NMR Spectra: *Varian Gemini-300* and *Mercury-400* instruments with a 5-mm probe. The temp. was calibrated from differences in the chemical shift of a MeOH sample [23]; all chemical shifts have been quoted relative to (D_8)THF signals.

Determination of the ee Values. The ee values of products were determined directly on the crude as follows: ee of alcohols **2** and acetates **3** with a GC chiral column (*Megadex 5*, 25 m × 0.25 mm × 0.25 μ, $T = 155°$), absolute configurations established by comparison with reported data [24]; ee of alcohols **5** by HPLC (*Daicel Chiracel OF*, 25 cm × 0.46 cm, hexane/*i*-PrOH 94 : 6, 0.5 ml/min, λ 214 nm) and of acetate **6** by GC (*Megadex 5*, 25 m × 0.25 mm × 0.25 μ, $T = 90°$), absolute configurations established by comparison with the commercially available (*R*)-2-phenylpropan-1-ol and its (*R*)-*O*-acetate; ee of **8** by HPLC (*Daicel Chiracel OD*, 25 cm × 0.46 cm, hexane: *i*-PrOH 95 : 5, 0.7 ml/min, λ 214 nm), absolute configuration determined by comparison with the data reported in [21]. The average standard deviation for the ee measurements was less than 1%.

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