(~100%): NMR (90 MHz/$CDCl_3$) δ 12.26 (s, 1), 12.08 (s, 1), 7.70 (s, 1), 7.85-7.40 (m, 3), 6.25 (s, 1), 3.20-1.33 (m, 6), 1.17 (t, J = 7 Hz, 3); high-resolution mass spectrum: calcd for $C_{20}H_{16}O_4$, 320.1048; found, 320.1042.

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Registry No. (±)-**2**, 78821-97-3; **3**, 481-39-0; (±)-**4**, 82247-48-1; (±)-**5**, 82247-49-2; (±)-**6**, 82247-50-5; (±)-**6**, regioisomer, 82247-51-6; (±)-**7**, 82247-52-7; (±)-**8**, 82265-50-7; (±)-**9**, 82247-53-8; **10**, 82247-54-9; *trans*-(±)-**11**, 82265-51-8; *cis*-(±)-**12**, 82247-55-0; **13**, 80926-96-1; **17**, 82247-56-1; (±)-**18**, 82247-57-2; (±)-**19**, 82247-58-3; (±)-**20**, 82247-59-4; **21**, 82247-60-7; *trans*-(±)-**22**, 82247-61-8; **23**, 82265-52-9; **24**, 82247-62-9; *cis*-(±)-**25**, 82247-63-0; (±)-**27**, 78821-96-2; 1,3-cyclohexanedione, 504-02-9; triethylamine, 121-44-8; 1,5-dihydroxynaphthane, 83-56-7.

Resolution of Conglomerates with the Assistance of Tailor-made Impurities. Generality and Mechanistic Aspects of the "Rule of Reversal". A New Method for Assignment of Absolute Configuration

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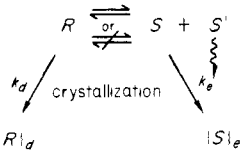
Abstract: A new general and efficient method for kinetic resolution of racemic conglomerates by crystallization in the presence of "tailor-made" additives is described. The process is explained in terms of stereoselective adsorption of the resolved additive at the surface of the growing crystals of the enantiomer of the same absolute configuration, resulting in a drastic decrease in their rate of growth and thus allowing preferential crystallization of the opposite enantiomer ("rule of reversal"). Some empirical resolutions reported in the literature are rationalized through this mechanism, and appropriate additives for the resolution of new systems are designed and successfully applied. The crystallization of the conglomerates (*R*,*S*)-glutamic acid hydrochloride (Glu·HCl), (*R*,*S*)-threonine (Thr), (*R*,*S*)-(*p*-hydroxyphenyl)glycine *p*-toluenesulfonate (pHpgpTs), and (*R*,*S*)-asparagine hydrate (Asn·H_2O) in the presence of other amino acids, used as additives, has been studied in particular. It is demonstrated that the additives are occluded in the bulk of the homochiral crystal in typical amounts of 0.5-1.5%, while they are not found in the bulk of the crystals of the antipode. The possible role of the additives in nucleation and dissolution of the affected crystals is considered. A new method for the assignment of absolute configuration of chiral molecules is proposed.

In recent years there has been an impressive advance in the analytical techniques for separation of enantiomers, mainly in the field of gas and liquid chromatography.[1] However these techniques are not suitable yet for large-scale applications, and thus industrial resolution of racemic mixtures is still mainly performed "Pasteur-like" through fractional crystallization of conglomerates or diastereoisomers.[2] Although these methods have been in use for more than 100 years, we feel that they are still in more of a state-of-the-art category as compared to well-established science. In the precipitation of a conglomerate, the only parameter that has thus far been exploited for achieving separation of the two enantiomorphs is the delay imposed on crystallization by the nucleation step. When seed crystals are supplied, this delay is eliminated, and kinetic resolution may be accomplished. This method is therefore not applicable to systems in which there is a low barrier to spontaneous nucleation or to systems where the two enantiomorphous crystals twin easily. In these cases the two enantiomers crystallize simultaneously, even in the presence of nuclei of one type only.

We present a general approach to the kinetic resolution of conglomerates by the introduction of selective inhibitors that delay the growth of one of the enantiomorphs. This novel approach can be furthermore efficiently coupled with the existing technologies, resulting in improved resolutions.

Scheme I[a]



[a] S' = impurity stereochemically similar to S. In the absence of S', $k_d = k_l$; in the presence of S', $k_d \gg k_l$.

The Rule of Reversal. In a previous study on generation and amplification of optical activity in closed symmetrical systems, we have encountered an interesting phenomenon of asymmetric induction on the crystallization of nonchiral photopolymerizable dienes in chiral crystals.[3] The inducing agents were the enantiomerically pure topochemical dimers, trimers, and oligomers of these same dienes (see Table I). In all the experiments performed, the enantiomorphic crystal with an absolute configuration opposite to that of the one in which the additive was generated crystallized in excess. We established that the additive, which is stereo-

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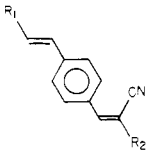
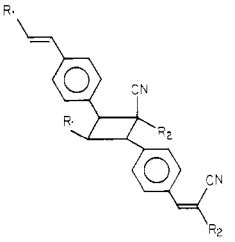
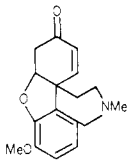
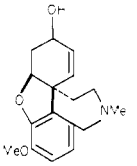
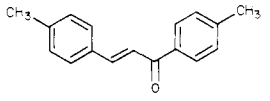
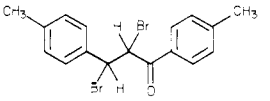
Table I. Resolutions of Conglomerates Accomplished in Our Laboratory, Designed on the Basis of the Rule of Reversal

conglomerate[a]	chiral additive	enantiomer precipitating first in excess	ref
phenylenediacrylates	phenylenediacrylates, dimers from *d* crystals[c,d]	phenylenediacrylates, *l* crystals[c]	4a,b
			
Thr	(*S*)-Glu, (*S*)-Gln, (*S*)-Asn, (*R*)-Cys,[b] (*S*)-Phe, (*S*)-His, (*S*)-Lys, (*S*)-Asp	(*R*)-Thr	12, present study
Glu·HCl	(*S*)-Lys, (*S*)-Orn, (*S*)-His, (*S*)-Ser, (*S*)-Thr, (*R*)-Cys,[b] (*S*)-Tyr, (*S*)-Leu	(*R*)-Glu	present study
Asn·H_2O	(*S*)-Glu, (*S*)-Asp, (*S*)-Ser, (*S*)-Gln, (*S*)-Lys, (*S*)-Orn, (*S*)-His	(*R*)-Asn	12, present study
pHpgpTs	(*S*)-Pg, (*S*)-Tyr, (*S*)-pMpg,[a] (*S*)-Phe, (*S*)-Dopa, (*S*)-MeDopa	(*R*)-pHpg	present study
His·HCl	(*S*)-Trp, (*S*)-Phe	(*R*)-His	unpub results
phenylhydracrylic acid	(*S*)-phenyllactic acid	(*R*)-phenylhydracrylic acid	unpub results

[a] For identification of the notations, see footnote *a*, Table VI. [b] All *S* amino acids are L amino acids, following the D,L notation, with the exception of (*S*)-Cys, which corresponds to D-Cys. [c] The chirality of the crystals is arbitrarily denoted *d* and *l*. [d] The resolution has been performed in seven monomers of the family with different R_1 and R_2 ester groups.

Table II. Resolutions of Conglomerates in the Presence of Chiral Additives Described in the Literature and in Accordance with Our Rule of Reversal

conglomerate[a]	chiral additive	enantiomer precipitating first in excess	ref
Glu	(*S*)-Asp, (*S*)-Leu	(*R*)-Glu	9
Glu	(*S*)-Glu Me	(*R*)-Glu	10
$Cu(Asp)_2$	(*S*)-Glu, (*S*)-Ala	(*R*)-$Cu(Asp)_2$	6, 12
$NaNH_4$ tartrate	D(+)-malic acid	D(−)-$NaNH_4$ tartrate	8
narwedine	(−)-galanthamine	(+)-narwedine	11
			
p,p'-dimethylchalcone	dibromo-*p,p'*-dimethylchalcone from *d* crystals[b]	*p,p'*-dimethylchalcone from *l* crystals	7
			

[a] Table I, footnote *a*. [b] Table I, footnote *c*.

chemically similar to the crystal from which it had been generated, is adsorbed stereoselectively (in amounts of 1–2%) on that same enantiomorph, thereby inhibiting its growth.[4] Consequently, the enantiomorph of opposite chirality precipitates in excess. It is in fact known that adsorption of small amounts of impurities at the surface of growing crystals may decrease their crystallization rates by several orders of magnitude.[5]

A natural extension of this hypothesis leads to the formulation of a general method for the resolution of enantiomers crystallizing in the form of conglomerates. This process is illustrated in Scheme I, where *S'* is a resolved additive with stereochemistry similar enough to that of the unwanted enantiomer, *S*, to be adsorbed on its surface, but different enough to disturb its further growth, once adsorbed. For convenience, we named this phenomenon the "rule of reversal".

A literature search has revealed a number of resolutions performed in the presence of small amounts of additives that could easily fit our scheme: these are reported in Table II. In all these examples observed in the course of studies in different fields of science from amplification of optical activity by crystallization[6-8] to industrial resolution[9,10] to total synthesis of natural products,[11] there exists a marked resemblance, both stereochemical and structural[4] between the additive and the substrate enantiomer remaining in solution. Attempted resolutions, in which there was no stereochemical resemblance between the chiral additive and the substrate, were reported to fail. In some papers explanations were suggested for the observed effects, such as chiral seeding[8] or interactions in solution.[6,9] In the resolution of the alkaloid

(4) At the present stage, the impurity is being selected empirically by introducing a small change in the molecule that composes the enantiomorph, the growth of which we wish to suppress. Studies on resolution that consider both crystal structure as well as crystal morphology of each system are under current investigation. Once these studies are completed, we shall be able to define more precisely the term "stereochemical resemblance" for each given system.

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(6) Harada, K.; Tso, W. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2859.
(7) Green, B. S.; Heller, L. *Science (Washington, D.C.)* **1974**, *185*, 527.
(8) Ostromisslenky, I. *Chem. Ber.* **1908**, *41*, 3035.
(9) Purvis, J. L. U.S. Patent 2 790 001, 1959.

Table III. Resolution of (*R,S*)-Threonine (900 mg in 3 mL of H_2O) in the Presence of Resolved Amino Acids as Additives, under Various Crystallization Conditions, at Room Temperature

			1st crop			2nd crop[a]	
expt	impurity	concn of impur, mg/mL	crystal yield, %	$[\alpha]_D$, deg	ee, config	$[\alpha]_D$, deg config; yield, %	conditions
1	(*S*)-Asn	40	13	+25.8	92.1 (*R*)		standing, 2 days, no seeds
2	(*S*)-Asn	27	15	+25.1	89.6 (*R*)		standing, 2 days, no seeds
3	(*S*)-Asn	13	28	+1.3	4.6 (*R*)		standing, 2 days, no seeds
4	(*S*)-Asn	30	48	0.0	0.0	0	shaking, 2 days, no seeds
5	(*R*)-Asn	30	15	−25.2	90.0 (*S*)		standing, 2 days, no seeds
6	(*S*)-Glu	30	15	+26.4	94.3 (*R*)	−8.0 (*S*) (28)	standing, 2 days, no seeds
7	(*S*)-Glu	15	18	+25.6	91.4 (*R*)	−25.4 (*S*) (15)	standing, 2 days, no seeds
8	(*R*)-Glu	30	15	−26.5	94.8 (*S*)		shaking, seeds *S*, 60, 30 °C
9	(*R*)-Cys·HCl[b]	40	7	+26.1	93.3 (*R*)		standing, 1 day, no seeds
10	(*R*)-Cys·HCl	27	12	+19.2	68.6 (*R*)		standing, 1 day, no seeds
11	(*R*)-Cys·HCl	13	22	+9.7	34.6 (*R*)		standing, 1 day, no seeds
12	(*R*)-Cys·HCl	3	28	0.0	0.0		standing, 1 day, no seeds
13	(*S*)-Asp	33	16	+19.0	67.8 (*R*)		standing, 2 days, no seeds
14	(*S*)-His·HCl	33	29	+11.7	41.8 (*R*)		standing, 1 day, no seeds
15	(*S*)-Lys·HCl	40	29	+19.6	70.0 (*R*)	−26.2 (*S*) (13)	standing, 2 days, no seeds
16	(*S*)-Phe	30	24	+14.6	52.1 (*R*)	−14.4 (*S*) (23)	standing, 2 days, no seeds

[a] The second filtration from the mother liquor was performed after 7 additional days. [b] Table I, footnote *d*.

(+)-narwedine in the presence of the resolved precursor (−)-galanthamine (Table II), Barton and Kirby[11] invoked the possibility of adsorption of the impurity on the growing crystals of the substrate. However, in the absence of a unifying theory, all these examples remained as separate curiosities and were not exploited further.

We present here experiments aimed at elucidating further the role played by the impurity in general and in the crystal growth of the two enantiomorphs in particular. The resolution of four amino acids, glutamic acid hydrochloride (Glu·HCl), asparagine hydrate (Asn·H_2O), (*p*-hydroxyphenyl)glycine *p*-toluenesulfonate (pHpgpTs), and threonine (Thr), were studied in the presence of other resolved amino acids used as impurities. Further, the selectivity and distribution of the resolved additive in the enantiomorphous crystals are analyzed. Once the stereochemical correlation between the molecular structure of the additive and the crystal structure of the substrate and its role in resolution are understood, we can place in a common frame all the above examples as well as devise appropriate impurities for the kinetic resolution of new systems.[12] Some successful resolutions performed on the sole basis of this hypothesis are summarized in Table I.

Results

Resolution. In Tables III–VI some results on resolution of the four above-mentioned substrates, crystallized in the presence of a variety of other amino acids and under various conditions, are reported. Only a few examples are given for each system, chosen to illustrate their general behavior and the specific aspects that we refer to in the discussion. Most experiments were performed on all four systems with all the impurities and with consistent results. The reported enantiomeric excesses are the highest observed for each system in a number of experiments executed in parallel; the lowest were never lower by more than 20%.

Crystallization was carried out by cooling supersaturated filtered solutions (supersaturation 2, i.e., twice the concentration of saturation, was used typically) of the substrate in the appropriate solvent (water for Thr and Asn·H_2O, 5 N HCl for Glu·HCl, and 0.5 N *p*-toluenesulfonic acid for pHpgpTs) containing additive in concentrations well below its solubility limit under the same conditions. When necessary, the pH of the solution was adjusted after addition of the additive, so that it corresponded in each case to that of the blank crystallizations performed in the absence of additive. In the case of Glu·HCl, the presence of seed crystals was necessary to induce crystallization; thus, 0.5 mg of (*R,S*)-Glu·HCl seeds were introduced into the cooled supersaturated solutions. In the other cases, parallel crystallizations were performed with or without seeds, as specified in the tables. In all cases, crystals that have the same morphology as the pure compound, namely, thick bars for Thr, plates for Glu·HCl, prisms for Asn·H_2O,[13] and thin needles for pHpgpTs, appear first. After a substantial delay, crystals of different morphology precipitate. Both the delay and the extent of morphological change are functions of the concentration of the impurity in solution and are in direct correlation to the efficiency of the resolution. If crystallization is interrupted before the appearance of the modified habit, the enantiomeric purity of the separated crystals is almost quantitative, even when racemic seeds are used. When efficient impurities are used in large amounts, the crystals of modified morphology, which turn out to be composed of the affected enantiomer, appear in the cases of Thr and Glu·HCl in the form of agglomerates of powder (see tables). This phenomenon stresses visually in a dramatic way the fact that their growth has been almost totally suppressed. It is evident from the tables that not all the impurities checked have the same degree of efficiency. In all cases, however, when crystallization is left to proceed for a long time or its rate is enhanced by vigorous stirring, racemic mixtures of crystals are obtained, thus confirming the exclusively kinetic nature of the resolution process. The order of precipitation can be inverted by using additives of opposite chiralities.

Preferential Adsorption. It is inherent to the proposed mechanism of these resolutions that the additive (S') must be adsorbed on $\{S\}_l$ crystals of the substrate in larger amounts than on $\{R\}_d$ crystals. To confirm this point, we conducted the following experiments: Enantiomerically pure substrate amino acid was crystallized in the presence of racemic impurity in solution. After crystallization, decantation, and drying, the content of adsorbed impurity was analyzed by a recently developed HPLC method for the determination of the enantiomeric composition of mixtures of amino acids based on the use of the chiral additive Cu(*N,N*-di-*n*-propyl-(*S*)-alanine)$_2$ in the aqueous mobile phase.[14] Some results of these analyses are given in Tables VII–X. Alternatively,

(10) Fike, H. L. U.S. Patent 2 937 200, 1960.
(11) Barton, D. H. R.; Kirby, G. W. *J. Chem. Soc.* **1962**, 806. See also: Harada, K.; Iwasaki, T. *Chem. Lett.* **1972**, 1057.
(12) For preliminary results, see: Addadi, L.; van Mil, J.; Lahav, M. *J. Am. Chem. Soc.* **1981**, *103*, 1249.
(13) For a detailed discussion on morphological changes as a function of structure of impurity, see: Addadi, L.; Gati, E.; Lahav, M. *J. Am. Chem. Soc.* **1981**, *103*, 1251. Addadi, L.; Berkovitch-Yellin, Z.; Domb, N.; Gati, E.; Lahav, M.; Leiserowitz, L. *Nature (London)* **1982**, *296*, 21. Berkovitch-Yellin, Z.; Addadi, L.; Idelson, M.; Leiserowitz, L.; Lahav, M. *Ibid.* **1982**, *296*, 27. Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; Lahav, M.; Leiserowitz, L.; Weinstein, S. *J. Am. Chem. Soc.* **1982**, *104*, 2075.
(14) Weinstein, S. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 218. Weinstein, S.; Engel, M. H.; Hare, P. E. *Anal. Biochem.* **1982**, *121*, 370.

Table IV. Resolution of (*R,S*)-Glutamic Acid Hydrochloride (1 g/5 mL of 5 N HCl) in the Presence of Other Resolved Amino Acids as Additives and with *R,S* Seeds at Room Temperature

		concn of impur, mg/mL	crystals yield, %	1st crop			2nd crop		time, days	
expt	impurity			$[\alpha]_D$, deg	ee config	morphology	$[\alpha]_D$, deg (config) yield, %	morphology	1st, filtn	2nd filtn[a]
1	(*S*)-Orn[b]	5	15	−23.0	93.5 (*R*)	thick + thin plates	+19.4 (*S*) (18)	thin plates prevalently	1	3
2	(*S*)-Orn	10	22	−23.0	93.5 (*R*)	thick + thin plates	+23.2 (*S*) (8)	thin plates prevalently	2	3
3	(*S*)-Orn	20	17	−24.6	100 (*R*)	thick plates	+17.9 (*S*) (7)	powder + thick plates	2	11
4	(*S*)-Orn	40	13	−24.8	100 (*R*)	thick plates		powder + plates	2	11
5	(*S*)-Lys	5	15	−23.7	96.3 (*R*)	thick + thin plates	+20.0 (*S*) (16)	thin plates prevalently	1	3
6	(*S*)-Lys	10	15	−23.9	97.1 (*R*)	thick plates	+11.4 (*S*) (12)	thin + thick plates	1	3
7	(*S*)-Lys	20	16	−24.1	97.9 (*R*)	thick plates	+8.9 (*S*) (12)	thin + thick plates	1	10
8	(*S*)-Lys	40	19	−24.0	97.6 (*R*)	thick plates	+10.9 (*S*) (18)	powder + plates	5	10
9	(*S*)-His	5	20	−3.9	15.8 (*R*)	thick + thin plates			2	
10	(*S*)-His	10	12	−23.7	96.3 (*R*)	thick plates			2	
11	(*S*)-His	40	19	−24.4	99.2 (*R*)	thick plates	+5.5 (*S*) (11)	thick + thin plates	2	8
12	(*S*)-His	160	20	−24.4	99.2 (*R*)	thick plates			10	
13	(*S*)-Ser	40	3	−14.0	56.9 (*R*)	thick + thin plates			1	
14	(*R*)-Cys[b]	10	19	0	0	plates			1	
15	(*R*)-Cys	20	15	−10.7	43.5 (*R*)	plates	+8.0 (*S*) (20)	plates	1	10
16	(*R*)-Cys	40	12	−15.0		thick + thin plates			1	
17	(*R*)-Cys	100	16	−24.0	97.5 (*R*)	thick plates	+23.5 (*S*) (14)	thin plates	2	10
18	(*S*)-Leu	5	12	−9.5	38.6 (*R*)	plates	+4.8 (*S*) (25)	plates	1	3
19	(*S*)-Leu	20	11	−23.8	96.7 (*R*)	thick plates	+8.9 (*S*)	plates + bars	2	8
20	(*S*)-Tyr	20	25	0	0	plates			1	
21	(*S*)-Tyr	100	14	−19.8	80.0 (*R*)	plates			10	

[a] Number of days after first filtration. [b] See Table I, footnote *b*.

racemic substrate was crystallized in the presence of enantiomerically pure (S) impurity, and single crystals of $\{R\}_d$ and $\{S\}_l$, distinguishable by their morphologies and specific rotations, were examined by the HPLC technique. These experiments allowed us to establish quantitatively both the relative amount of additive adsorbed on the crystals and its absolute configuration. In all four systems, the additive of the same absolute configuration as that of the substrate was found in crystals of the latter in amounts of 0.5–3% (with one exception, Asp with Asn·H_2O in which a true solid solution is formed up to 10–12%), while the enantiomer of opposite absolute configuration was present in much smaller amounts, if at all.

The next question to be asked concerns the distribution of the adsorbed additive in the crystals, namely, whether it is located only at the surface of the crystal or is occluded within it. The following experiments were therefore performed with Glu·HCl, Asn·H_2O, and Thr: Single crystals (2–5 mg) of the *R* or *S* amino acid, crystallized in the presence of additive, were gradually dissolved in successive batches of pure water (0.3 mL), and the various dissolved fractions were examined separately by HPLC. With Thr we noticed that single crystals from (*R,S*)-Thr, which have the same crystal structure as pure (*S*)-Thr (powder diffraction), are composed of (*R*)- and (*S*)-Thr in variable ratios. This indicates twinning between the two enantiomorphs, a quite commonly encountered effect in orthorhombic crystals.[15] The HPLC analysis was therefore carried out on single crystals from separate batches of (*R*)- and (*S*)-Thr crystallized in the presence of an impurity.

The results of these analyses for the four systems are shown in Tables VII–X. In all the experiments the crystals contained in their interiors only impurities of the same absolute configuration as their own at approximately constant concentrations of 0.2–1.5%. The bulk of the other enantiomorphic crystal was free from the impurity. Generally, the concentration of impurity in the external layer was higher, with poor stereoselectivity. Such experiments could not be performed on pHpgpTs because suitable crystals were not available.

Seeding and Twinning. The above-mentioned evidence that (*R*)- and (*S*)-Thr crystals undergo twinning implies that (*S*)-Thr crystals can serve as seeds for the crystallization of both enantiomers; this explains why resolution of (*R,S*)-Thr by seeding is not efficient, and requires large amounts of seeds to achieve even relatively small yields of pure compounds. To examine the influence of an impurity on such a system, we ran parallel experiments on the resolution of (*R,S*)-Thr in the presence of the following: (a) *S* seed crystals, (b) *S* seed crystals and (*R*)-glutamic acid as additive, (c) *R* seed crystals, (d) *R* seed crystals and (*S*)-Glu, and (e) *R,S* seed crystals and (*S*)-Glu.

The results, reported in Figure 1, show that the enantiomeric purity of the Thr crystals obtained decays rapidly with increasing crystallization times when only seeds are used, while it is maintained for many hours when (*R*)-Glu was added, independent of the chirality of the seeds, although *S* seeds yield a systematically higher enantiomeric excess than *R* seeds. We have also observed that the mode of preparation of the seeds affects the resolution (see Experimental Section), indicating that the nature of the seed influences the twinning capacity.

Growth of Single *R* and *S* Crystals of Asn·H_2O in the Presence of (*S*)-Serine under Equilibrium Conditions. To isolate the effect of the impurity in the crystal growth step, we performed the following experiment: Six seed crystals of Asn·H_2O, three *R* and three *S* and weighing (*R*): (1) 15 mg, (2) 5 mg, and (3) 5 mg and (*S*) (1) 28 mg, (2) 4 mg, and (3) 4 mg (this crystal had fallen

(15) Goldsmidt, V. M. *Z. Kristallogr. Mineral.* **1915**, *55*, 123. Furberg, S.; Hasel, O. *Acta Chem. Scand.* **1950**, *4*, 1020. Green, B. S.; Knossow, M. *Science (Washington, D.C.)* **1981**, *214*, 795. Berkovic, G. E.; Ludmer, Z. *J. Chem. Soc., Chem. Commun.* **1981**, 768.

Table V. Resolution of (*R*,*S*)-Asparagine (300 mg/3 mL of H_2O) in the Presence of Resolved Amino Acids as Additives at Room Temperature and under Various Experimental Conditions

expt	impur	concn impur, mg/mL	1st crop				2nd crop			time, days			
			crystal yield, %	$[\alpha]_D$, deg	ee config	morphology	$[\alpha]_D$, deg (config) yield, %	morphology	1st filtn	2nd filtn[a]	seeds	conditions
1	(*S*)-Asp	16.6	14	−29.9	98.0 (*R*)	prisms	+9.2 (*S*) (15)	prisms + plates	2	3	*R*,*S*	standing
2	(*S*)-Asp	6.6	17	−23.7	77.7 (*R*)	prisms and plates			4			standing
3	(*S*)-Asp	3.3	38	−9.6	31.4 (*R*)	prisms and plates	+2.4 (*S*) (16)	prisms + plates	4	3		standing
4	(*R*)-Asp	6.6	6	+30.5	100 (*R*)	prisms			6			standing
5	(*S*)-Glu	16.6	22	−26.0	85.2 (*R*)	prisms and polyhedra	+19.5 (*S*) (10)	polyhedra + prisms	4			standing
6	(*R*)-Glu	17.0	14	+24.2	79.3 (*S*)	prisms and polyhedra			6			standing
7	(*S*)-Ser	25.0	21	−15.8	51.8 (*R*)	prisms and octahedra	+30.0 (*S*) (10)	octahedra	4	3	*R*,*S*	standing
8	(*S*)-Ser	16.6	33	−17.1	56.1 (*R*)	prisms and octahedra			1		*R*,*S*	standing
9	(*S*)-Ser	8.3	43	−8.8	28.8 (*R*)	prisms and octahedra			1		*R*,*S*	standing
10	(*S*)-Lys·HCl	33.3	25	−14.7	48.2 (*R*)	prisms and octahedra	+30.0 (*S*) (10)	octahedra	7	3		standing
11	(*S*)-Lys·HCl	8.3	53	−7.7	25.2 (*R*)	prisms			1		*R*,*S*	standing
12	(*S*)-His·HCl	16.6	48	−21.3	69.8 (*R*)	prisms and octahedra			1		*R*,*S*	standing
13	none		43	0	0	prisms			2			standing
14	(*S*)-Glu	17.0	34	0	0	powder			2			stirring

[a] Number of days after first filtration.

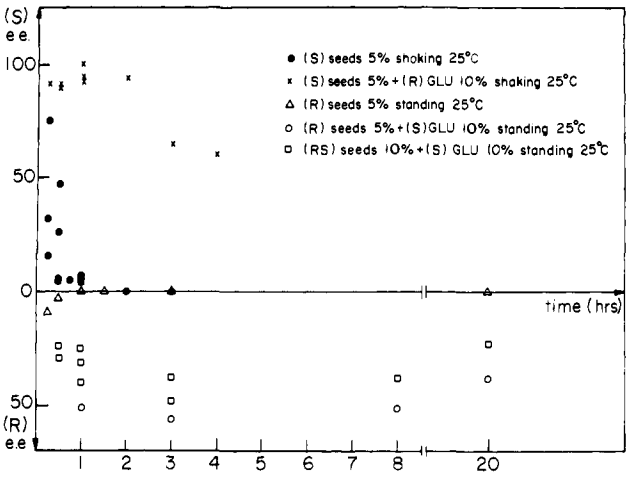

Figure 1. Resolution of (*R*,*S*)-Thr in the presence of (*R*)-, (*S*)-, or (*R*,*S*)-Glu seeds under various conditions.

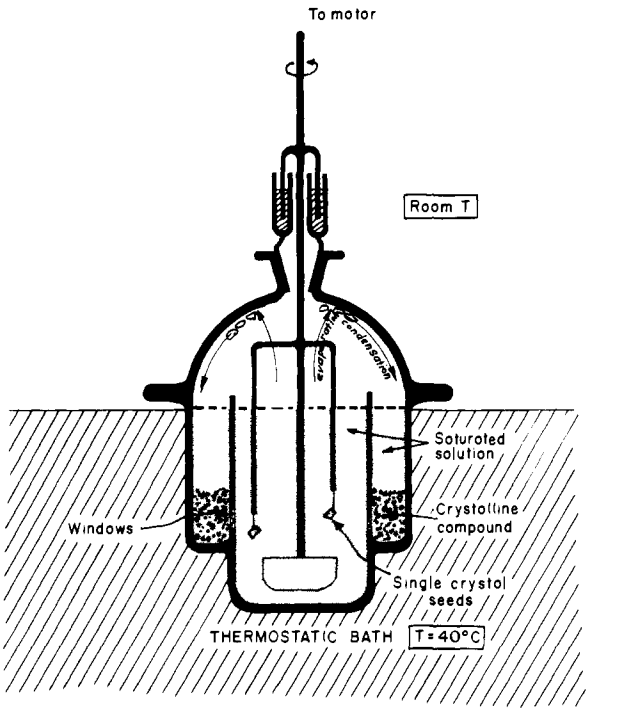

Figure 2. An apparatus for growing enantiomorphous single crystals simultaneously under near-equilibrium conditions.

down accidentally after a few days but had started growing again), were suspended in a single-crystal growth apparatus similar to the one described by Forno[16] and appropriately designed for growth under constant temperature near equilibrium conditions (Figure 2). The crystals were grown in the presence of (*S*)-serine in solution (20 mg/mL) over a period of 45 days. After the experiment was completed the crystals weights were (*R*): (1) 866 mg, (2) 424 mg, and (3) 372 mg and (*S*): (1) 54 mg, (2) 26 mg, and (3) 2 mg (Figure 3). The striking differences in the relative sizes of the *R* and *S* crystals confirm unambiguously the correctness of our hypothesis.

Discussion

Mechanistic Aspects. From the results just described it can be concluded that kinetic resolution of a variety of racemic mixtures of compounds of different chemical nature, crystallizing in the form of conglomerates, can be efficiently accomplished via addition in solution of resolved "tailor-made" additives.

Studies carried out on the crystallization of Glu·HCl, Thr, and

(16) Forno, C. *J. Cryst. Growth* **1974**, *21*, 61.

Table VI. Resolution of (*R,S*)-(*p*-Hydroxyphenyl)glycine *p*-Toluenesulfonate (pHpgpTs) (350 mg/mL, 0.5 N pTs Acid) in the Presence of Resolved Amino Acids as Additives and under Various Experimental Conditions

		impur	1st crop			2nd crop			
expt	impur[a]	concn, mg/mL	yield, %	$[\alpha]_D$, deg	ee (config)	$[\alpha]_D$, deg (config) yield, %	seeds[b]	time, min	conditions
1	none		14	−31.2	46.4 (*R*)	+30.9 (*S*)	*R*	40	25 °C, standing
2	(*S*)-TyrpTs	52.5	12	−66.1	98.2 (*R*)	+28.0 (*S*)	*R*	40	25 °C, standing
3	(*S*)-TyrpTs	52.5	26	−17.1	23.9 (*R*)	+15.2 (*S*)	*R*	180	25 °C, standing
4	(*S*)-TyrpTs	52.5	21	−44.3	65.9 (*R*)		*R*	30	30 °C, shaking
5	none		51	−4.8	7.1 (*R*)		*R*	30	30 °C, shaking
6	(*R*)-PgpTs	52.5	17	+52.2	77.6 (*S*)		*R,S*	45	25 °C, standing
7	(*R*)-PgpTs	52.5	17	+51.0	76.1 (*S*)	+27.9 (*R*) (17)	*R,S*	120	25 °C, standing
8	(*R*)-PgpTs	52.5	19	+53.9	80.2 (*S*)	+49.6 (*R*) (16)	*S*	60	25 °C, standing
9	(*R*)-pMpgpTs	52.5	9	+50.0	74.2 (*S*)		*R,S*	55	25 °C, standing
10	(*R*)-pMpgpTs	52.5	18	+39.0	58.1 (*S*)	−31.1 (*R*) (12)	*R,S*	120	25 °C, standing
11	(*R*)-pMpgpTs	52.5	25	+50.4	80.3 (*S*)	−34.7 (*R*) (14)	*R*	120	25 °C, standing
12	(*S*)-DopapTs	52.5	27	−26.6	39.5 (*R*)		*R*	30	30 °C, standing
13	(*S*)-MeDopapTs	52.5	35	−18.5	27.5 (*R*)		*R*	30	30 °C, standing
14	(*S*)-PhepTs	52.5	27	−26.6	39.5 (*R*)		*R*	30	30 °C, standing

[a] (*S*)-TyrpTs indicates the *p*-toluenesulfonate of tyrosine, etc. Pg stands for phenylglycine, pMpg stands for *p*-methoxyphenylglycine, and Dopa and MeDopa stands for 3,4-dihydroxyphenylalanine and its α-methyl derivative, respectively. [b] The seeds were introduced into the solution at 40 °C.

Table VII. Relative Content of Impurity in Powders of pHpgpTs Crystallized in the Presence of 15% of Other Amino Acids (HPLC Measurements)

		% impur in crystld powder		chirality of
expt	impur in soln	*R*	*S*	pHpgpTs
1	(*R,S*)-PgpTs	2.1	0.04	*R*
2	(*R*)-PgpTs	3.1		*R*
3	(*R,S*)-PgpTs	2.2	0.0	*R*
4	(*R*)-PgpTs	0.0		*S*
5	(*S*)-TyrpTs		~0.1	*S*
6	(*R*)-TyrpTs	0.0		*S*
7	(*S*)-TyrpTs		0.0	*R*
8	(*R,S*)-TyrpTs	~0.1	~0.1	*R*
9	(*R*)-pMpgpTs	4.7		*R*
10	(*R*)-pMpgpTs	0.0		*S*

Table VIII. Relative Content of Impurity in Powder and in Successive Dissolution Layers from Single Crystals of (*S*)-Thr Doped with (*R*)- or (*S*)-Glu (HPLC Analysis)

		concn of impur in soln, mg/mL	% impur in crystals		
expt	impur		1st dissln layer	2nd dissln layer	3rd dissln layer
1	(*R*)-Glu	30.0	0.27	0.0	0.0
2	(*R*)-Glu	7.5	0.08	0.0	0.0
3	(*S*)-Glu	7.5	1.0	0.45	0.4
4	(*S*)-Glu	15.0	0.9	0.41	
5	(*S*)-Glu	15.0	1.2	0.45	0.45
6	(*S*)-Glu	30.0	3.1[a]		
7	(*S*)-Glu	30.0	5.3[a]		

[a] Since the crystals turned into powder due to the effect of the impurity, progressive dissolution is not possible. A large part of the impurity is probably mechanically adsorbed onto the powder.

Asn·H_2O in the presence of other resolved amino acids (*S'*) as impurities have demonstrated that on crystallization the precipitating crystal of the *S* enantiomer adsorbs preferentially the *S'* impurity, whereas the crystals of the *R* enantiomer do not, and vice versa. Small amounts of *S'* that are found on the surface of the {*R*} crystals, after filtration, could be easily removed by washing the crystals with fresh solvent, indicating that this enantiomer is adsorbed only mechanically on the surface. On the other hand, we have demonstrated that *S'* is distributed almost uniformly throughout the host {*S*} crystal.

The experiment of simultaneous growth under controlled conditions of three {*S*} and three {*R*} crystals of Asn·H_2O from a saturated solution of the substrate containing (*S*)-serine as an additive clearly demonstrates the dominant role played by the impurity in the growth of the substrate crystals.

Table IX. Relative Content of Impurity in Subsequent Dissolution Layers from Single Crystals of (*S*)- and (*R*)-Glu·HCl Doped with Other (*S*)-Amino Acids (HPLC Analysis)

		concn of impur in soln, mg/mL	% impur in crystals			crystal chirality, Glu·HCl
expt	impur		1st dissln layer	2nd dissln layer	3rd dissln layer	
1	(*S*)-Lys[a]	40	1.5	1.3	1.5	*S*
2	(*S*)-Lys	40	0.9	0.6	1.2	*S*
3	(*S*)-Lys	20	0.5	0.5	0.5	*S*
4	(*S*)-Lys	20	1.2	0.6		*S*
5	(*S*)-Lys	40	0.2	0.0	0.0	*R*
6	(*S*)-Orn[a]	40	1.8	1.6		*S*
7	(*S*)-Orn	20	1.3	0.3		*S*
8	(*S*)-Orn	20	0.7	0.3	0.7	*S*
9	(*S*)-Orn	40	0.1	0.0	0.0	*R*
10	(*S*)-His	40	0.9	0.6		*S*
11	(*S*)-His	20	0.3	0.3	0.4	*S*
12	(*S*)-His	20	0.5	0.5	0.4	*S*
13	(*S*)-His	20	0.3	0.0	0.0	*R*
14	(*S*)-Ser	40	0.4	0.3	0.3	*S*
15	(*S*)-Ser	40	0.6	0.5	0.4	*S*
16	(*S*)-Ser	20	0.3	0.2	0.2	*S*
17	(*S*)-Ser	20	0.2	0.1	0.1	*S*
18	(*S*)-Ser	20	0.0	0.0	0.0	*R*

[a] In the presence of a large concentration of impurity, the crystal plates almost turn into powder (Table IV). More than one crystal was subjected to dissolution in each measurement. The various solutions therefore probably do not reflect exactly the bulk/surface composition.

Inspection of the results of adsorption–resolution shows a correlation between the concentration of the impurity in solution, the amount of impurity occluded in the bulk of the crystal, and the resolving power of the impurity. The same behavior is exhibited by all the various impurities but with different efficiencies, revealing the importance of the nature of the interference brought about by the adsorbed molecule at the surface of the crystal. One clear example is given by the system Glu·HCl in which two groups of additives can be defined: (i) Lys, Orn, and His, which are very effective even at concentrations in solution as low as 5 mg/mL for Lys and Orn and 10 mg/mL for His, and (ii) Ser, Thr, Tyr, and Cys, which are effective only at high concentrations, although, as can be seen from Table IX, the amount of various impurities of the first group in the glutamic acid crystal is of the same range 0.1–1.8% as found for the other systems. All three amino acids of the first group are protonated at the nitrogen of the side chain in 5 N HCl. This would create a layer of charged groups at the

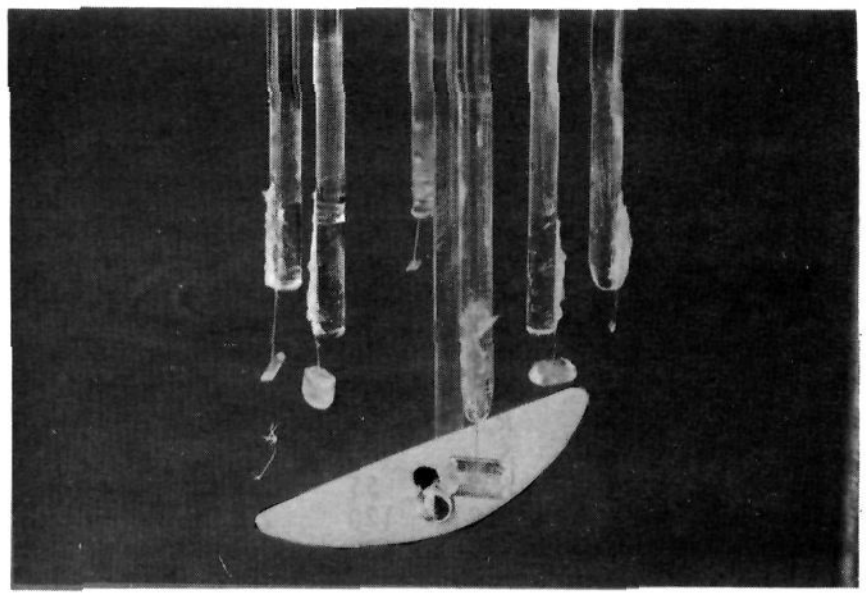

Figure 3. Three *R* and three *S* crystals of Asn·H_2O grown in the apparatus described in Figure 2 in the presence of (*S*)-serine for 45 days. The large crystals are of (*R*)-Asn·H_2O whereas the small ones are (*S*)-Asn·H_2O. The first crystal on the right fell down accidentally after a few days but started growing again on the remaining seeds.

Table X. Relative Content of Impurity in Successive Dissolution Layers from Single Crystals of (*S*)- or (*R*)-Asn·H_2O Doped with Other *S* Amino Acids (HPLC Analysis)

			% impur in crystals			
expt	impur	concn of impur in soln, mg/mL	1st dissln layer	2nd dissln layer	3rd dissln layer	crystal chirality, Asn·H_2O
1	(*S*)-Glu	16.6	3.8	1.3	0.7	*S*
2	(*S*)-Glu	16.6	1.9	1.9	2.0	*S*
3	(*S*)-Glu	6.6	0.7	0.5	0.5	*S*
4	(*S*)-Glu	6.6	1.5	0.3	0.3	*S*
5	(*S*)-His	33.3	6.0	0.7	0.9	*S*
6	(*S*)-His	33.3	2.4	1.1	1.1	*S*
7	(*S*)-His	16.6	0.9	0.3	0.4	*S*
8	(*S*)-His	16.6	0.9	0.7	0.7	*S*
9	(*S*)-His	16.6	2.4	0.1	0.0	*R*
10	(*S*)-His	16.6	0.5	0.0	0.0	*R*
11	(*S*)-Asp	6.6	12.3	12.5	11.7	*S*
12	(*S*)-Asp	6.6	9.3	9.8	10.6	*S*
13	(*S*)-Asp	3.3	7.2	4.4	4.7	*S*
14	(*S*)-Asp	3.3	3.2	3.2	3.2	*S*
15	(*S*)-Asp	16.6	0.0	0.0	0.0	*R*
16	(*S*)-Asp	16.6	1.9	0.7	0.0	*R*

surface of the crystal, resulting in repulsion of the oncoming molecules. The same is not true for the additives of the second group.

All the mechanistic information acquired from both chemical and crystallographic approaches, however, does not allow us to predict quantitatively the resolution efficiency of new impurities yet, since this depends on a large number of parameters such as the solubility of the impurity in the solvent of crystallization, interaction with the substrate molecules in solution, and the crystal structure of the substrate and its overall morphology.[13] Nevertheless, it can provide useful guidelines for selection of additives that will act as efficient inhibitors for resolution.

A New Method for the Assignment of Absolute Configuration. The preferential adsorption experiments have demonstrated that there is a clear correlation between the absolute configurations of the crystallizing molecules and that of the impurity adsorbed. This correlation, together with the morphological changes concomitant with the adsorption, can be used therefore for the assignment of the absolute configuration of chiral molecules. The method is not limited to materials undergoing spontaneous resolution since it can be applied to each crystalline enantiomer separately. Like any other empirical method, its reliability depends on its successful application to a large number of compounds. So far we have tested about 30 compounds of different chemical nature, and these followed the rule without exception.

Open Questions on Nucleation and Dissolution. The role played by the impurity in stereoselective inhibition of nucleation, if any, still remains to be clarified. It is reasonable to assume that the structure of the aggregates of molecules in solution, prior to crystallization, is similar to that of the crystal itself. Therefore, in principle, one may expect the chiral tailor-made impurity to affect the two enantiomeric nuclei stereoselectively, preventing one of them from growing to the critical size for crystal growth. The role of the impurity in induced nucleation at the surface of existing seeds may be even more complicated. The experiments carried out in the present work cannot tell conclusively whether any influence on nucleation indeed exists. Some hints, however, are seen in the experiments conducted in crystallization of (*R*,*S*)-Thr with *R*, *S*, or *R*,*S* seeds and in the presence or absence of (*S*)- or (*R*)-Glu. First, the rapid decay of the enantiomeric excess in the absence of impurity has already been interpreted as due to nucleation and twinning, thus indicating that indeed such nucleation is prevented by the additive. Furthermore, the observation that during the first hours of crystallization in the presence of impurities (Figure 1) the enantiomeric excess does not decrease, or even increases, indicates that the effect of the impurity on the growth of the crystal is optimal during that period. Hence, the differences in the initial enantiomeric excess on crystallization (90% for *S* seeds, 35% for *R* seeds, and 25% for *R*,*S* seeds) in the presence of the impurity is due exclusively to the nature of the seeds. This in turn means that the ratio between the number of nuclei of affected and unaffected enantiomer is not increased, or is even decreased during that time, not withstanding the increasing gap in supersaturation of the two enantiomers.

It is self-evident that such a method for inhibition of nucleation might be very important not only in resolution of conglomerates but also in any process in which two competing crystalline forms must be separated.

With the help of tailor-made impurities, it is possible, in principle, to control not only the relative rate of crystallization but also the relative rate of dissolution of enantiomorphic crystals, since growth surfaces are dissolution surfaces as well and the process at equilibrium is microscopically reversible at any instant. This means that the affected enantiomorph should dissolve slower than the unaffected one.[17] Thus, if a racemic conglomerate of $\{R\}_d$ and $\{S\}_l$ crystals is introduced into an undersaturated solution containing *S′* impurity, its partial dissolution should leave a crystalline residue containing the $\{S\}_l$ enantiomer in excess. In a preliminary communication[12] we have reported some results of such dissolution experiments in the systems Thr, Asn·H_2O, and $Cu(Asp)_2$ that were consistent with the expectations, although the effect was smaller than that observed upon crystallization. We have furthermore found that preadsorption of the impurity on the substrate crystals enhanced this effect. However, the same experiments carried out on polycrystalline samples of Glu·HCl and pHpgpTs did not show any preferential dissolution, and in some instances even opposite results were obtained. This is probably due to other parameters, such as dislocations at the surface of the crystals, that give rise to opposite effects, overwhelming the one we anticipate. Comparative studies on rates of dissolution of single crystals grown in the presence and absence of impurities are under current investigation and hopefully will afford a better understanding of the role of impurities on dissolution.

Experimental Section

All amino acids were commercial analytical grade materials; their purity was checked in the limits of the sensitivity of the HPLC method ($<10^{-5}$ mg), and they were used without further purification. *p*-Methoxyphenylglycine (pMpg) was prepared by methylation of pHpg by following a known procedure.[18] Specific rotations were measured with a Perkin-Elmer 141 polarimeter in a 1-dm cell at 25 °C.

HPLC Analyses. Samples (20 μL) were injected onto a reversed-phase column (25 cm × 4.6 mm) self-packed with 5-μm Nucleosil C18 (Ma-

(17) Cabrera, N.; Vermilyea, D. A. "Growth and Perfection of Crystals"; Wiley: London, 1958; Chapter 5.
(18) Isumiya, N.; Nagamatsu, A. *Bull. Chem. Soc. Jpn.* **1952**, *25*, 256.

cherey Nagel); mobile-phase composition; aqueous solution of cupric acetate (4×10^{-3} M) and *N,N*-di-*n*-propyl-(*S*)-alanine (8×10^{-3} M) at pH 5.3–5.5. For the analysis of pHpgpTs, 7% acetonitrile was added to the eluant to decrease the retention time. The flow rate was 30 mL/h. Separation was monitored by fluorometry following post-column derivatization with *o*-phthaldialdehyde. Derivatizing reagent: A solution of boric acid (30 g) and EDTA (2.5 g) in 1 L of water was adjusted to pH 9.5 with potassium hydroxide pellets; a solution of 0.5 g of *o*-phthaldialdehyde and 0.5 mL of thioethanol in 10 mL of methanol was added to the previous one. The flow rate of the reagent solution was 50 mL/h. Quantities as low as 10^{-5} mg can be detected in the system. Since the detector response to the various amino acids is not constant, calibration experiments for each series of runs were conducted for the appropriate amino acids. Obviously, the ratio of *R*:*S* enantiomers of the same amino acid is independent of the detector response. The error of the method, as applied here, is smaller than the scatter in content of the impurity in different crystals of the same batch.

Crystallization for Resolution Experiments. Threonine (Thr). At least three parallel batches of 900 mg of (*R,S*)-Thr, one for each different set of conditions, were suspended in 3 mL of deionized water, either alone or with the desired amounts of the *S* or *R* impurity, and the suspensions were heated with stirring until complete dissolution occurred. The solutions were left to cool spontaneously to 40 °C and filtered. When necessary, after filtration, the pH was corrected to the value of the pH of the blank experiments by addition of concentrated KOH. The solution was either left standing or stirred at room temperature, as specified in the tables. After partial crystallization, the solutions were decanted and the crystals dried on filter paper. A second crop was collected from the mother liquors. Specific rotations were measured in deionized water (c = 2–4%).

In the experiments conducted with seeding, 5% seeds of the desired enantiomer or 10% of the *R,S* seeds were added to the filtered solutions at 40 °C, and the seeded solutions were transferred to a thermostated bath at 25 °C in which they were left standing or shaking. Various batches were run in parallel, and the crystallized material was collected after various periods of time (15, 30, 60, 120 s, etc.). Seeds were prepared from batches of commercially available (*S*)-Thr (Sigma) and (*R,S*)-Thr (Ajinomoto). *R* seeds were prepared by resolution of (*R,S*)-Thr in the presence of (*S*)-Glu, following our own procedure. After filtration, the seed crystals were washed with water and recrystallized once more. Their chemical and enantiomeric purities were checked by specific rotation and HPLC.

Glutamic Acid Hydrochloride (Glu·HCl). Crystallization was carried out from supersaturated solutions of 1 g of (*R,S*)-Glu·HCl in 5 mL of 5 N HCl. The supersaturated filtered solutions were seeded at room temperature with 0.5 mg of (*R,S*)-Glu·HCl powder. Workup was as for Thr. Specific rotations were measured in 1 N HCl (c = 2–5%).

Asparagine Hydrate (Asn·H_2O). Crystallizations were carried out from supersaturated solutions of 300 mg of Asn·H_2O in 3 mL of H_2O with or without the addition of racemic Asn·H_2O seeds (0.5 mg). Workup was as for Thr. Specific rotations were measured in 5 N HCl (c = 1–2%).

(*p*-Hydroxyphenyl)glycine *p*-Toluenesulfonate (pHpgpTs). The *p*-toluenesulfonic acid salt of pHpg was prepared by crystallization from 1.5 M solutions of pHpg in aqueous 2 N *p*-toluenesulfonic acid.[19] Resolution was performed by crystallization of (*R,S*)-pHpgpTs supersaturated solutions (350 mg/mL of 0.5 N pTs) without seeds or in the presence of 0.5% (w/w) seeds of (*S*)-, (*R*)- or (*R,S*)-pHpgpTs. Workup was as for Thr. Specific rotations were measured in H_2O (c = 2%).

Crystallization for Preferential Adsorption Experiments. Thr. Supersaturated solutions of 450 mg of (*S*)-Thr in 3 mL of H_2O containing variable amounts of the *R* or *S* impurity (see Table VIII) were crystallized as described above. When well-formed crystals were obtained, single crystals were subjected to progressive dissolution in successive aliquots of 0.3 mL of H_2O, and the dissolved fractions from each crystal were examined separately by HPLC. When powders were obtained, as a result of the morphological effect of the impurity, they were dissolved directly and analyzed.

Glu·HCl. Single crystals of (*R*)- and (*S*)-Glu·HCl, obtained from the resolution experiments and distinguishable by morphology, were subjected to progressive dissolution as described for Thr. The HPLC analysis unambiguously confirmed the chirality of the substrate. In those cases in which the affected enantiomorph crystallizes in the form of very thin plates, a few crystals were subjected to dissolution together (see Table IX).

Asn·H_2O. Single crystals of (*R*)- or (*S*)-Asn·H_2O (3–5 mg), obtained from the resolution experiments and distinguishable by their morphology, were subjected to progressive dissolution as described for Thr. The HPLC analysis unambiguously confirmed the chirality of the substrate crystals.

pHpgpTs. Crystallization was performed from a supersaturated solution of 3 g of (*R*)-pHpgpTs in 10 mL of 0.5 N pTs containing 0.9 g of an *R,S* impurity. Agglomerates of thin needles were thus obtained, which, after filtration and drying, were subjected to dissolution as described for Thr and examined by HPLC.

Single-Crystal Growth under Controlled Conditions. An apparatus was built similar to that described by Forno[16] (Figure 2) for growth of single crystals at constant temperature but modified to allow for the growth of six crystals simultaneously (Figure 3). It consists of a jar with two concentric compartments linked through six sinter-glass (*N*3) windows that permit the circulation of the solution but not of the crystalline material contained in the external chamber. Both compartments are filled with saturated solution. The seed crystals, directly grown on nylon fishing line, are suspended from a six-armed glass axis connected to a motor spinning at 10 rpm and inverting the sense of rotation every 30 s. The jar is immersed into a thermostated bath at 40 °C up to the level of the internal solution. Solvent evaporates from the surface of the internal compartment, condenses on the cooler lid (at room temperature), and flows back into the external compartment. The circulation of solvent creates a constant condition of slight supersaturation in the internal compartment and slight undersaturation in the external compartment, causing transfer of material from the outside to the seed crystals.

Three seed crystals of (*S*)-Asn·H_2O grown in the presence of 6.6 mg/mL of (*S*)-Ser and three of (*R*)-Asn·H_2O grown from pure water solution having similar sizes were chosen and were introduced into the apparatus containing 1.8 L of a saturated solution of (*R,S*)-Asn (after 3 weeks of conditioning) and 6.6 g/L of (*S*)-Ser. The crystals were grown for 45 days.

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Registry No. (*R,S*)-Thr, 80-68-2; Glu·HCl, 15767-75-6; (*R,S*)-Asn, 3130-87-8; (*R,S*)-pHpgpTs, 57084-62-5.

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