Total Asymmetric Transformations at Interfaces with Centrosymmetric Crystals: Role of Hydrophobic and Kinetic Effects in the Crystallization of the System Glycine/α-Amino Acids†

I. Weissbuch,* L. Addadi,* L. Leiserowitz,* and M. Lahav*

Contribution from the Department of Structural Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel. *Received June 15, 1987*

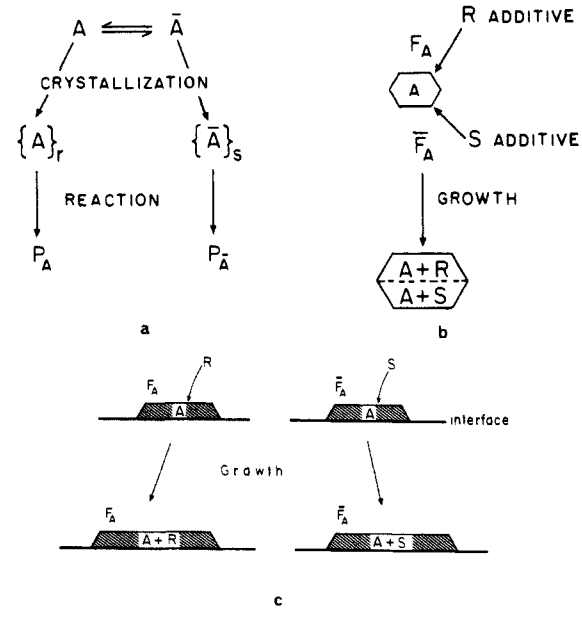
Abstract: A model for generation and amplification of optical activity, using the centrosymmetric crystals of glycine as substrates for total separation of occluded α-amino acids into enantiomeric territories, is described. The principle is based on the enantioselective occlusion of these additives through the enantiotopic (010) and (0$\bar{1}$0) faces of glycine crystals. Such crystals, when floating at the air/solution interface, and if correctly oriented, may incorporate only one of the two enantiomeric additives present in solution. We demonstrate that complete (010) or (0$\bar{1}$0) orientation may be induced by both kinetic and "hydrophobic" effects. The former is achieved through inhibition of nucleation and growth of the say (010) oriented crystals [(010) face toward air], by (*S*) amino acids in solution, while the latter is due to induction of (0$\bar{1}$0) orientation by (*S*) hydrophobic amino acids. By symmetry the enantiomers induce opposite orientation. The two effects are investigated separately, and possible mechanisms are proposed. Combination of the two effects, which operate in the same direction, allows total orientation of glycine crystals and thus triggering of amplification starting with a solution containing leucine with an enantiomeric excess as low as 6%. The relevance of such a mechanism to other systems and to nucleation in general is discussed.

The origin of optical activity in molecules of living matter has puzzled and intrigued scientists since its discovery.[1] A number of hypotheses have been formulated for the explanation of this phenomenon. In one of these,[2,3] an initially symmetrical isolated system is spontaneously transformed into an asymmetrical one by an overall process composed of two distinct steps: a rare random event, which introduces asymmetry via statistical fluctuations from the internally racemic state, followed by an efficient autocatalytic feedback process, which both preserves the asymmetry generated in the first step and propagates it to the whole system.

In the past, chiral crystals composed of nonchiral molecules have been used as systems for achieving such "total asymmetric transformations" (Scheme Ia).[4-7] Enantiotopic surfaces of centrosymmetric crystals composed of nonchiral molecules have been exploited in recent studies to achieve asymmetric syntheses. These include heterogeneous reactions[8] and topochemical photodimerizations in solid solutions of nonchiral guest and host molecules.[9,10] Here, we shall demonstrate that one may accomplish separation of enantiomeric territories and therefore generation and amplification of optical activity by enantioselective occlusion of chiral additives through chiral surfaces of centrosymmetric crystals of glycine.

Recent studies on growth and dissolution of organic crystals in the presence of tailor-made additives[11,12] have shown that the latter may be adsorbed at and occluded only through specific crystal faces and indeed only at specific crystallographic sites on each face. Adsorption depends on the molecular structure of the additive and the structure of the various crystal faces. Such a process results in anisotropic distribution of the additive occluded inside the host crystal, in reduction in crystal symmetry, and in changes in the crystal morphology due to inhibition of growth at the affected faces. Following these observations it was concluded that correctly designed chiral additives of opposite handedness may be occluded enantioselectively inside centrosymmetric crystals, each through one of a pair of enantiotopic faces (Scheme Ib).[13] As long as such a crystal exposes both enantiotopic faces F_A and $\bar{F}_A$ to the solution, the crystal will grow in directions perpendicular to both faces at equal rates. Equal amounts of the two guest enantiomers (*R*) and (*S*) will be occluded inside the host crystal upon growth and will be segregated into the two crystal halves. This process transforms the centrosymmetric single crystal of the pure host into two enantiomorphous halves (A + R) and (A + S) coherently compounded together. The resolution of racemic threonine inside the centrosymmetric monoclinic crystals of (*R,S*)

Scheme I



† Dedicated to the memory of Prof. I. Tabushi.

(1) (a) Mason, S. F. *Nouv. J. Chim.* **1986**, *10*, 739. (b) Mason, S. F. *Nature (London)* **1984**, *310*, 546.
(2) Wald, G. *Ann. N.Y. Acad. Sci.* **1957**, *69*, 352.
(3) Prelog, V. *Science* **1976**, *193*, 17.
(4) Havinga, E. *Biochem. Biophys. Acta* **1954**, *13*, 171.
(5) (a) Pincock, R. E.; Perkins, R. R.; Ma, A. S.; Wilson, K. R. *Science* **1971**, *174*, 1018. (b) Pincock, R. E.; Wilson, K. R. *J. Am. Chem. Soc.* **1975**, *97*, 1474. (c) Pincock, R. E.; Bradshow, R. P.; Perkins, R. R. *J. Mol. Evol.* **1974**, *4*, 67.
(6) Van Mil, J.; Addadi, L.; Gati, E.; Lahav, M. *J. Am. Chem. Soc.* **1982**, *104*, 3429.
(7) Evans, S. V.; Garlbay, M. G.; Omkaram, N.; Scheffer, J. R.; Trotter, J.; Wireko, F. *J. Am. Chem. Soc.* **1987**, *109*, 5648.
(8) (a) Chenchaiah, P. C.; Holland, H. L.; Richardson, M. F. *J. Chem. Soc., Chem. Commun.* **1982**, 436. (b) Chenchaiah, P. C.; Holland, H. L.; Munoz, B.; Richardson, M. F. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1775.
(9) Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; Valda, M.; Weisinger, Y.; Zbaida, D.; Leiserowitz, L.; Lahav, M. In *Stereochemistry of Organic and Biorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; Workshop Conferences Hoechst, 1987; Vol. 17, p 127.

0002-7863/88/1510-0561$01.50/0

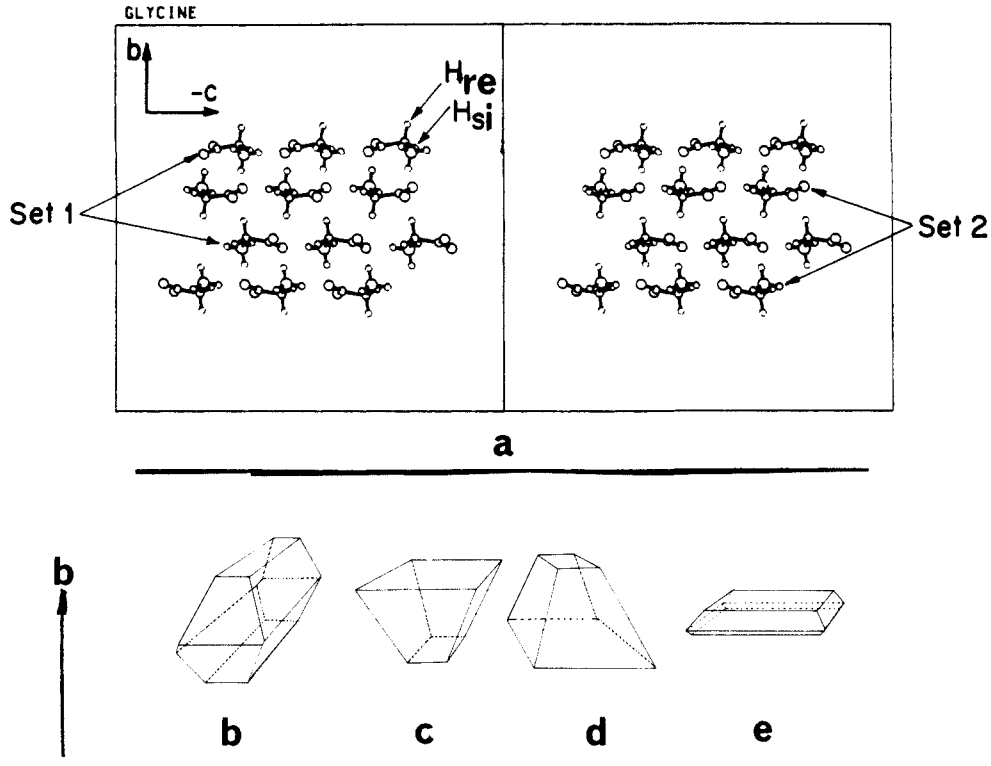


Figure 1. (a) Stereoscopic view of the packing arrangement of the α-form of glycine along the *a* axis. The layer of molecules in set 1 has the C–H_{re} bond directed along $+b$ and C–H_{si} in the *ac* plane; in set 2 C–H_{si} is directed along $-b$ and C–H_{re} lies in the *ac* plane. (b–e) Morphologies of glycine crystals. (b) pure c, d, e grown in the presence of (*R*), (*S*), and (*R*,*S*) α-amino acids, respectively.

serine,[14] of racemic glycyl-leucine inside glycyl-glycine,[15] and of the racemic α-amino acids inside α-glycine[16] are representative examples of such a process.

Following this mechanism one expects that if one set of the enantiotopic faces of the host crystal, say $\bar{F}_A$, does not contact the solution during crystal growth, only the (*R*) enantiomer from the racemic mixture present in solution will be occluded in the crystal (Scheme Ic). Thus, the centrosymmetric host crystal will be transformed into a chiral mixed crystal (A + R). The absolute structure of this enantiomorph will depend upon which face, F_A or $\bar{F}_A$, was exposed to the solution. Since crystals frequently grow at interfaces, such as glass/solution or air/solution, a large variety of centrosymmetric crystals in nature may transform, upon growth in the presence of appropriate additives, into a mixture of enantiomorphous crystals. Thus, in principle, such systems may be exploited for induction of net chirality, provided an efficient feedback process exists to replicate the orientation of the first crystal at the interface to the whole batch. This can be achieved if, for example, the small excess of (*S*) generated in solution by occlusion of (*R*) into the first crystal is able to induce orientation of the next crystal again with F_A exposed to solution. The final result of this process will be a true spontaneous resolution of the racemic mixture. Here we present studies along these lines, and as a model we use the resolution of α-amino acids in the presence of crystals of glycine.[17]

Segregation of α-Amino Acids Inside Crystals of Glycine Grown at Interfaces. We have demonstrated recently[16] that the centrosymmetric plate-like crystals of glycine, when grown from a solution containing racemic mixtures of other α-amino acids, adsorb during growth the (*R*) enantiomers at the (010) face and the (*S*) enantiomers at the (0$\bar{1}$0) face. This happens because an (R)-α-amino acid can easily occupy an (010) surface site of any glycine molecule belonging to set 1, with its side chain emerging from the surface (Figure 1a). An (*S*)-amino acid, on the other hand, cannot be adsorbed at any site at the (010) face, because its side chain would need to lie in the closed-packed *ac* plane in set 1 or would have to point toward the bulk of the crystal in set 2. By symmetry an (*S*) additive can be adsorbed at the (0$\bar{1}$0) face. A small fraction of the α-amino acids (0.02–0.2%) is subsequently occluded into the bulk of the growing glycine crystals. Owing to the enantioselective adsorption, the occlusion leads to segregation of the enantiomers along the symmetry *b* axis.

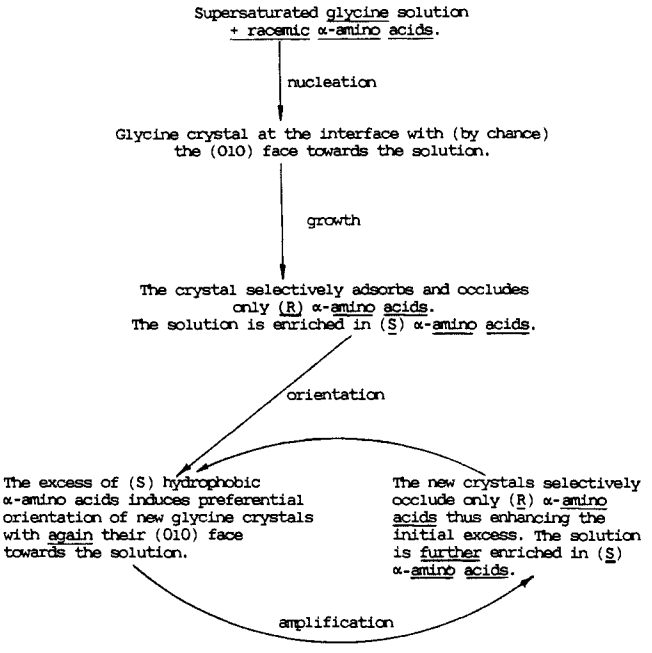
The process of guest adsorption on the {010}[18] faces is accompanied by a change in crystal morphology due to inhibition of growth along the *b* axis. Thus, the bipyramidal crystals of pure glycine (Figure 1b) transform into pyramids with well-developed basal (010) or (0$\bar{1}$0) faces when grown in the presence of the resolved (*R*)- and (*S*)-α-amino acids, respectively (Figure 1c,d), while they grow as {010} plates in the presence of racemic mixtures of the α-amino acids (Figure 1e).

When such growing crystals of glycine rest on the glass bottom or float on the water surface only one of the two enantiotopic {010} faces will be in contact with the solution so that only one of the enantiomers will be ultimately occluded into the crystals as in Scheme Ic.[17] Consequently the overall symmetry of the crystals will be decreased from the centrosymmetric $P2_1/n$ of the pure host to the chiral $P2_1$ of the mixed crystal. In the absence of an outside chiral agent, the number of growing glycine crystals exposing their (010) or (0$\bar{1}$0) faces toward the amino acid solution will be equal, and thus no overall generation of asymmetry is expected. On the other hand, one single crystal of glycine exposing say the (010) face toward the solution will occlude enantiose-

(10) Vaida, M., work in progress.
(11) Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; van Mil, J.; Shimon, L. J. W.; Lahav, M.; Leiserowitz, L. *Angew. Chem.* **1985**, *23*, 346.
(12) Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I.; Lahav, M.; Leiserowitz, L. *Top. Stereochem.* **1986**, *16*.
(13) For the sake of clarity we confine ourselves to triclinic or monoclinic centrosymmetric crystals, but the method is applicable to any system expressing enantiotopic faces.
(14) Weissbuch, I.; Shimon, L.; Addadi, L.; Berkovitch-Yellin, Z.; Lahav, M.; Leiserowitz, L. *Isr. J. Chem.* **1985**, *25* (3–4), 353.
(15) Weissbuch, I.; Berkovitch-Yellin, Z.; Leiserowitz, L.; Lahav, M. *Isr. J. Chem.* **1985**, *25* (3–4), 362.
(16) Weissbuch, I.; Addadi, L.; Berkovitch-Yellin, Z.; Gati, E.; Weinstein, S.; Lahav, M.; Leiserowitz, L. *J. Am. Chem. Soc.* **1983**, *105*, 6615.
(17) Weissbuch, I.; Addadi, L.; Berkovitch-Yellin, Z.; Gati, E.; Lahav, M.; Leiserowitz, L. *Nature (London)* **1984**, *310*, 161.

(18) {010} refers to all symmetry related faces, i.e., (010) and (0$\bar{1}$0), while (010) refers only to the specific face.

Scheme II



lectively the (*R*) enantiomers, thus enriching the aqueous solution with (*S*). If this small excess can induce preferential orientation of the further growing crystals of glycine again with the (010) face pointing toward the solution, replication will ensue by cascade mechanism finally leading to a total separation of enantiomeric territories (Scheme II). We provide experimental results that allow us to achieve such a separation, by an efficient control of the orientation of the glycine crystals at interfaces. This was accomplished through the exploitation of two distinct effects: a hydrophobic one and a kinetic one. The former, to which we have already addressed ourselves in a preliminary study,[17] operates at the air/water interface and is confined to hydrophobic amino acids. The latter applies to all α-amino acid additives, hydrophilic ones included, and operates both at the glass/water and at the air/water interfaces. The two effects will be first examined separately, and we shall see how they can act in unison for the efficient amplification of optical activity at the air/water interface. We shall also provide results that bear upon the general mechanism of crystal nucleation.

Results

1. Kinetic Effects. In previous studies on the crystallization of conglomerates kinetic resolution of the enantiomorphs was accomplished by addition of small amounts of appropriate optically pure additives.[19,20] This resolution is by virtue of the strong inhibition of crystal growth, and probably of crystal nucleation, of that enantiomorph which interacts with the resolved additive. Following the above analysis, glycine crystals growing at an interface and exposing either of the two enantiotopic {010} faces to the solution are formally equivalent to the conglomerate systems, and they are therefore expected to behave in an analogous way: the resolved (say *R*) α-amino acid will selectively impede growth or even preclude nucleation of crystals pointing with the (010) face toward solution. In order to test these possibilities we studied growth of crystals of glycine at glass/water or air/water interfaces, in the presence of different amino acids in solution.

1.1. Growth of Glycine Crystals at Glass/Solution Interfaces in the Presence of Resolved Histidine, Alanine, and Threonine. Orientation by Kinetic Effect. In the first set of experiments, crystals of glycine were grown at the glass bottom of the vials in the presence of varying amounts (0.5–10.0% w/w) of resolved (*S*)- or (*R*)-histidine or (0.5–15.0% w/w) (*S*)-ala or (*S*)-thr. At lower additive concentrations, both pyramids, lying on the basal {010} face, and {010} plates were observed. Under these conditions of growth, pyramidal glycine crystals lying on a side face (i.e., {110} or {011}) also appeared. The platelike crystals become thinner and fewer with increasing additive concentration. At the highest concentrations of histidine used, the platelike crystals disappeared and only pyramids lying on their basal face {(010) with (*R*)- and (0$\bar{1}$0) with (*S*)-histidine} or side faces were observed at the glass bottom of the beaker (Table I). However, since only a small fraction of the glycine crystals were attached with their (010) or (0$\bar{1}$0) face to the glass bottom, experiments providing reliable statistics could not be performed. In addition, many of the crystals probably nucleated in the bulk of the solution may have fallen during the process of crystallization and complicate the overall statistics. The {010} orientation of the crystals was determined here and in all the following experiments, by four independent methods: three of them, i.e., crystal morphology, enantiomeric HPLC analysis of the occluded additive and use of a selective dye indicator (see Experimental), are performed on the whole batch while the fourth, X-ray diffraction, is applied to single crystals. Complete orientation of the crystals of glycine at the glass/water interface in the presence of resolved hydrophobic α-amino acids could not be attained, presumably because of the low solubility of the latter.

Table I. Orientation of Glycine Crystals at the Glass/Solution Interface, as Induced by Resolved his, ala, and thr

additive				
config	concn, (w/w glycine)	crystal habit	% {010} orientation	face in contact with glass
(*S*)-his	0.5–9	pyramids + plates	0–40	(0$\bar{1}$0) (010)
(*S*)-his	10	pyramids	>95	(0$\bar{1}$0)
(*S*)-ala	0.5–10	pyramids + plates	0–20	(0$\bar{1}$0) (010)
(*S*)-ala	10–15	pyramids + plates	30–60	(0$\bar{1}$0) (010)
(*S*)-thr	1–12	pyramids + plates	0–15	(0$\bar{1}$0) (010)
(*S*)-thr	12–18	pyramids + plates	20–30	(0$\bar{1}$0) (010)

1.2. Growth of Crystals of Glycine at the Air/Solution Interface. In contrast to the crystals grown on the glass bottom, all the crystals of glycine grown at the air/solution interface in the presence of hydrophobic α-amino acids exposed an {010}, i.e., (010) or (0$\bar{1}$0), face to air, be the crystal platelike or pyramidal. We shall refer to these as (010) or (0$\bar{1}$0) oriented, respectively, depending upon which face is exposed to air (Scheme III).

In the presence of a resolved hydrophobic α-amino acid, such as leucine, valine, and α-amino butyric or α-amino octanoic acid, well-developed floating pyramids appeared long before crystallization occurs in the bottom. When (*R*)-α-amino acids were used, the pyramids were (010) oriented. By symmetry, (*S*) acids induced enantiomorphous (0$\bar{1}$0) oriented pyramids.

In the presence of the racemic additives, the interface is covered with {010} plates of both orientations. All (0$\bar{1}$0) oriented crystals have occluded the (*R*)-α-amino acids while the (010) oriented crystals have occluded the (*S*) acids (Scheme III). The concentration dependence of the inhibiting effect of racemic additives was studied for leucine since this amino acid induces a large number of floating crystals with a threshold value of 0.1% (w/w of glycine). At this lowest additive concentration, the floating crystals are enantiomorphous pyramids showing thus no macroscopic evidence for the kinetic retardation of growth by leucine. By increasing the leucine concentration within the specific range 0.1–2.8% (w/w) an evident morphological change of the floating glycine crystals was observed, from well-developed truncated pyramids, through thick plates, and eventually very thin plates to powder.

Crystallization experiments using racemic α-amino octanoic acid, which bears a relatively long aliphatic side chain, yielded

(19) Addadi, L.; Berkovitch-Yellin, Z.; Domb, N.; Gati, E.; Lahav, M.; Leiserowitz, L. *Nature (London)* **1982**, *296*, 21.
(20) Addadi, L.; Weinstein, S.; Gati, E.; Weissbuch, I.; Lahav, M.; Leiserowitz, L. *J. Am. Chem. Soc.* **1982**, *104*, 4610.

Scheme III

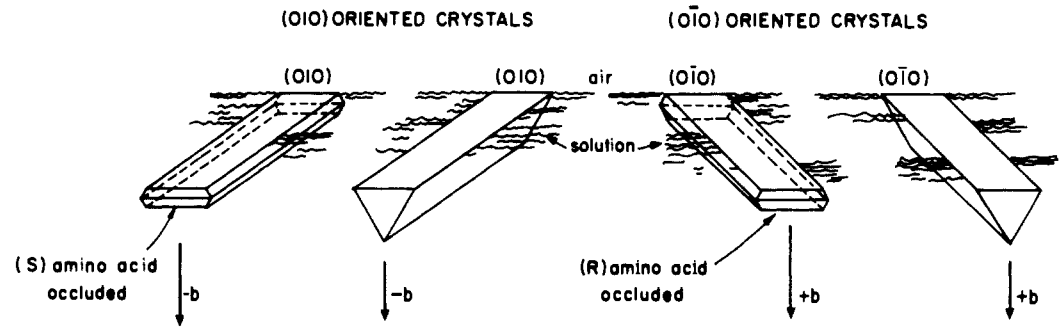


Table II. Orientation by Kinetic Effect of Floating Glycine Crystals in the Presence of Resolved Hydrophilic α-Amino Acids and 1% (*R,S*) Leucine

(*S*) additive concn, % (w/w gly)	% orientation[a] of floating crystals	face exposed to air	%ee[b] of occluded leucine
0	0	(010) + (0$\bar{1}$0)	0
1% alanine	70-75	(0$\bar{1}$0)	60
2% alanine	80	(0$\bar{1}$0)	80
3% alanine	100	(0$\bar{1}$0)	90
1% serine	~0	(010) + (0$\bar{1}$0)	~0
2% serine	70	(0$\bar{1}$0)	70
3% serine	90	(0$\bar{1}$0)	90
4% serine	100	(0$\bar{1}$0)	>95

[a] Checked by X-ray. [b] HPLC enantiomeric analysis of occluded leucine provides an independent check of orientation, following the mechanism in Scheme III.

{010} floating pyramids at concentrations as low as 0.03% w/w. At concentrations of 0.7% w/w the inhibition is so pronounced that only very thin plates *with large ac faces* are observed.

The above results suggest that the hydrophobic α-amino acids must play an additional role in orienting the glycine crystals. This effect should be intrinsically different from the kinetic one operating in the resolution of conglomerates and in the orientation of glycine crystals at the glass/solution interface in the presence of histidine. We differentiated between the two effects via a series of kinetic measurements on the crystallization of glycine in the presence of a mixture of racemic hydrophobic α-amino acids, and varying amounts of resolved hydrophilic α-amino acids such as alanine, serine, histidine, etc. The racemic hydrophobic additive is used only to induce crystallization at the air interface (Table II). We used 1% w/w of (*R,S*)-leu and increasing amounts of (*S*)-ala (1-3%) and (*S*)-ser (1-4%). When 1% of (*S*)-ala or (*S*)-ser were used, two types of crystal plates were formed at the interface, thick (0$\bar{1}$0) oriented and thin (010) oriented. These two types of plates appear as parallelograms in their two-dimensional projection at the interface and are therefore enantiomorphous.[21] The number of thick plates is larger than that of thin ones, which display a distinctly larger surface area because of inhibition of growth along the $-b$ direction. These results clearly indicate that when the (0$\bar{1}$0) face is exposed to solution, it interacts with the resolved ala or ser and thus growth along the $-b$ direction is strongly inhibited. When 3% ala or 4% ser was present only the thick (0$\bar{1}$0) oriented plates were observed. At these concentrations, the kinetic effect of (*S*)-ala or (*S*)-ser combined with 0.5% of (*S*)-leu was so strong that the inhibition was complete.

These experiments prove that orientation of the glycine crystals at an interface can be induced by high concentrations of the additives, through kinetic effects.

2. Hydrophobic Effect. 2.1. We pointed out above that addition of resolved hydrophobic α-amino acids to the supersaturated solutions of glycine orients the glycine crystals grown at the interface. This type of orientation was obtained at concentrations as low as 0.1% for leucine and 0.01% for α-amino octanoic acid,

(21) They become superimposable only upon rotation around an axis in the plane of the interface.

Table III. Orientation of the Floating Glycine Crystals Induced by Resolved Hydrophobic Additives

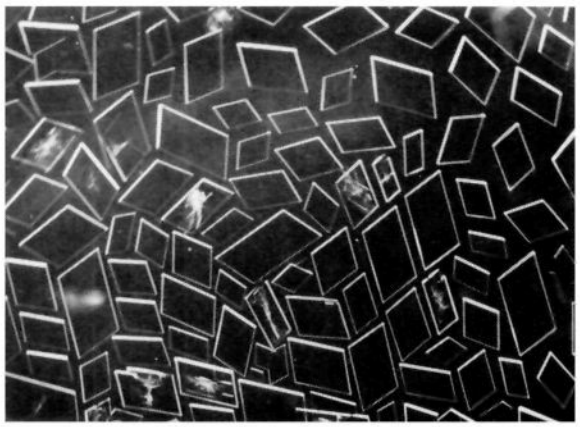
additive (config)	concn, % (w/w glycine)	crystals habit	exclusive crystal orientation (face exposed to air)
(*R*)-leu	0.1-3.0	pyramids	(010)
(*S*)-leu	0.1-3.0	pyramids	(0$\bar{1}$0)
(*R*)-α-aminooctanoic acid	0.01-1.0	pyramids	(010)
(*S*)-α-aminobutyric acid	1.0	pyramids	(0$\bar{1}$0)
(*S*)-phe	0.5-2.0	pyramids	(0$\bar{1}$0)
(*R*)-ala	1-15	plates + pyramids	(0$\bar{1}$0) (010)
(*S*)-ser	1-12	plates + pyramids	(010) (0$\bar{1}$0)
(*S*)-his	1-3	plates + pyramids	(010) (0$\bar{1}$0)

when kinetic inhibition by these additives could not have prevented the appearance of crystals with opposite orientation. We shall refer to this as the "hydrophobic effect". Table III summarizes some of the results.

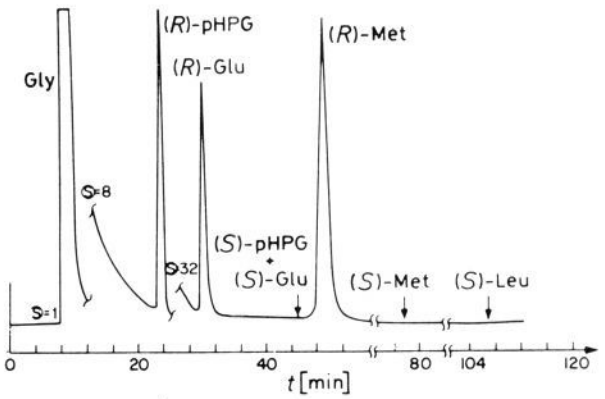
The ability of resolved hydrophobic α-amino acids to induce a specific orientation of the floating glycine crystals, even in the presence of a large excess of hydrophilic α-amino acid of opposite absolute configuration, was next checked. Some representative examples of crystal growth of glycine in the presence of 1% say (*R*)-leucine and 1-15% (*S*) hydrophilic α-amino acids are given in Table IV. In all these experiments platelike glycine crystals all of the same orientation were obtained, in contrast to the results obtained (Table V) when mixtures of only hydrophilic α-amino acids of opposite absolute configuration were used. This orientation was always induced by the hydrophobic resolved additive, no matter how strong the kinetic effect of the hydrophilic acids.

In order to further differentiate between the hydrophobic effect in nucleation and the kinetic effect in the process of crystal growth, comparative measurements of growth in the *b* direction of platelike seeds of glycine were carried out. Pairs of such seeds were placed at the solution/air interface with opposite {010} faces in contact with a slightly supersaturated glycine solution (~2.5%) containing different additives. The crystal thickness in the *b* direction before and after the experiment was measured, under an optical microscope (×50) (Table VI). It is immediately apparent that 1.5% w/w of (*S*)-histidine has an effect on crystal growth in the $-b$ direction larger than that of 0.25% w/w (*R*)-leu in the $+b$ direction. Yet when glycine is grown in the presence of this additive mixture all crystals are (010) oriented, as dictated by leucine (see Table IV, experiment 2, 3). Similar results were obtained for leu-ser (see Table IV, experiment 1). These results once again show that the hydrophobic effect plays a dominant role in the orientation of glycine crystals.

2.2. Resolution of Hydrophilic α-Amino Acids with Crystals of Glycine. The hydrophobic orientation effect (vide supra) can be used for novel resolution of α-amino acids via occlusion inside glycine crystals grown in the presence of small amounts of optically pure leucine. Resolution was performed for all non-hydrophobic α-amino acids. Here we demonstrate the simultaneous resolution of (*R,S*)-*p*-hydroxyphenylglycine, (*R,S*)-methionine, and (*R,S*)-glutamic acid. These have been selected for ease of presentation (Figure 2).



a



b

Figure 2. (a) Platelike crystals of glycine floating at the air/water interface, as grown in the presence of 1% w/w (*S*)-leucine and racemic *p*-hydroxyphenylglycine (0.5%), racemic glutamic acid (1%), and racemic methionine (0.5%). All the crystals float with their (0$\bar{1}$0) face exposed to air and exhibit the same enantiomorphous morphology. (b) HPLC enantiomeric analysis of the racemic additives occluded inside the crystals of glycine grown as in (a). Only (*R*) enantiomers of pHPG, glu, and met are found.

Table IV. Orientation of Floating Glycine Crystals by Hydrophobic Effect in the Presence of Mixtures of Hydrophobic and Hydrophilic α-Amino Acids

	hydrophobic additives		hydrophilic additive		exclusive crystal orientation
expt	(config)	% (w/w gly)	(config)	% (w/w gly)	(face exposed to air)
1	(*R*)-leu	1	(*S*)-ser	1–15	(010)[a]
2	(*R*)-leu	1	(*S*)-his	1–2	(010)
3	(*R*)-leu	0.5	(*S*)-his	1–2	(010)
4	(*S*)-leu	1	(*R*)-thr	1–6	(0$\bar{1}$0)
5	(*S*)-α-aminobutyric acid	1	(*R*)-ala	1	(0$\bar{1}$0)
6	(*R*)-α-aminooctanoic acid	0.03	(*S*)-ser	1–10	(010)
7	(*R*)-α-aminooctanoic acid	0.1	(*S*)-ala	1–10	(010)
8	(*S*)-leu	1	(*R*)-ala	1–5	(0$\bar{1}$0)
9	(*S*)-α-aminobutyric acid	1	(*R*)-ser	1	(0$\bar{1}$0)
10	(*S*)-phe	1–1.5	(*R*)-ala	1–3	(0$\bar{1}$0)
11	(*R*)-phe	1–2	(*S*)-ala	1–3	(010)

[a] For each type of experiment, the thickness of the floating plate-like crystals decreased on increase of the concentration of the hydrophilic additive.

Table V. Orientation of Floating Glycine Crystals in the Presence of Mixtures of Hydrophilic α-Amino Acids

hydrophilic additives w/w (glycine) (config)	crystal habit and orientation (face to exposed to air)
1–2% (*R*)-ala + 1–2% (*S*)-his	(010) plates + (0$\bar{1}$0) plates
3% (*R*)-ala + 3% (*S*)-ser	(010) plates + (0$\bar{1}$0) plates
1–2% (*R*)-ser + 1–2% (*S*)-his	(010) plates + (0$\bar{1}$0) plates
12% (*R*)-ser + 1.5–3% (*S*)-his	(010) plates + (0$\bar{1}$0) plates

2.3. Proposed Explanation of the Hydrophobic Effect. HPLC analysis of oriented crystals of glycine, grown in the presence of (*R*)-leu, reveals only very small amounts, if any, of the additive at the surface of the crystals. This implies that leucine has played its orienting role at a very early stage of the crystallization process. A number of possible mechanisms may account for this effect. It is logical to assume that the hydrophobic side chains of the additive will preferentially emerge from the water interface toward air, while the polar head groups will be immersed in water. Precritical embryos of glycine may interact enantioselectively with the polar heads of leucine. This will result in the formation of small nuclei whose to-be-developed enantiotopic faces (010) and (0$\bar{1}$0) differ from one another in their structural and physical properties. Following the mechanism demonstrated in crystal growth, the (*R*)-leucine molecules will be adsorbed at the (010) "face" of the nucleus, with the hydrophobic side groups emerging from these surfaces. The adsorbed molecules will transform this face into a hydrophobic one that will be preferentially located at the air/water interface thus yielding a net effect of (010) orientation.

An alternative possibility involves spontaneous organization of the hydrophobic amino acids at the interface to yield nucleating domains. Surface tension measurements performed on saturated solutions of glycine show, as expected, a negative solute adsorption at the interface. When hydrophobic α-amino acids (such as leucine) are added, the surface tension (γ) decreases with the increase of the concentration of the additive, and $\Delta\gamma$ is even larger than that measured for the same concentrations of leucine dissolved in pure water (as described in the Experimental Section, Table VII). This implies that in the supersaturated solutions of glycine leucine is more concentrated at the air interface than in the bulk. The packing of leucine and other resolved hydrophobic α-amino acids in their own crystal structures is characterized by layers in which the head groups are hydrogen bonded in a way almost identical with that of α-glycine.[22] If the excess leucine at the

Table VI. Relative Growth in the *b* Direction of (010) and (0$\bar{1}$0) Oriented Glycine Seed Crystals Grown at the Air/Solution Interface in the Presence of Different α-Amino Acid Additives

additive concn, (config) % (w/w glycine)	seed orientation (face exposed to solution)	relative growth in *b* direction
none	(0$\bar{1}$0)	10
	(010)	10
0.25% (*S*)-leu + 0.25% (*R*)-ser	(0$\bar{1}$0)	3.0
	(010)	6.5
0.25% (*S*)-leu + 1.5% (*R*)-ser	(0$\bar{1}$0)	3.3
	(010)	2.6
0.25% (*S*)-leu + 2.5% (*R*)-ser	(0$\bar{1}$0)	3.1
	(010)	2.0
0.25% (*R*)-leu + 1.5% (*S*)-his	(010)	3.0
	(0$\bar{1}$0)	1–1.2
0.1% (*R*)-leu + 0.9% (*S*)-leu	(010)	4.0
	(0$\bar{1}$0)	1.0

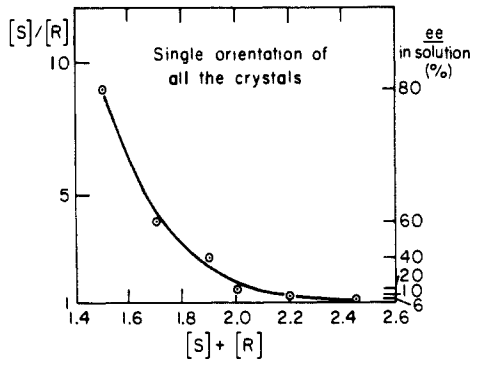


Figure 3. Correlation between the initial leucine enantiomeric ratio and the total concentration needed for the complete (0$\bar{1}$0) orientation of the floating glycine crystals.

interface spontaneously assembles in domains of structure akin to that of their own crystal, it is conceivable that these domains may act as nucleating centers for oriented growth of the glycine crystals. This hypothesis is supported by recent studies on the epitaxial crystallization of glycine underneath compressed and non-compressed monolayers of α-amino-palmitic and stearic acids.[23]

A third mechanism takes into consideration the fact that, in the bulk of the solution, nuclei that have adsorbed the hydrophobic additive should have a lower surface tension and should be thus more stable than the pure glycine nuclei.[24] In other words, one may argue that the hydrophobic α-amino acids should promote glycine nucleation by lowering the critical size of the affected nuclei, because the interaction between glycine molecules inside the nucleus and those between glycine molecules and the α-amino acid head groups of leucine will be almost the same, while the interactions with the water environment will be less favorable. Those nuclei that are close to the air/solution interface will finally adhere to it with the hydrophobic chains pointing outwards.

All three proposed mechanisms would be in agreement with the fact that a much larger number of crystals form very rapidly at the interface (relative to the same solutions without additive) when hydrophobic amino acids are present in solution.

3. The Amplification Step. The hydrophobic resolved α-amino acids are advantageous for such a study with respect to the hydrophilic ones because both the kinetic inhibitory effect and the stabilization of the nuclei by hydrophobic effect act in the same direction for orientation of the growing floating crystals of glycine: the presence of an excess of (*R*)-amino acids will favor (010) oriented nucleation at the surface while preventing at the same time the growth of (0$\bar{1}$0) oriented crystals from the solution. For this reason, we next studied the crystallization of glycine in the presence of partially enriched mixtures of (*R*,*S*)-leucine at various concentrations. Figure 3 summarizes some results that correlate the initial enantiomeric ratio of (*R*,*S*)-leucine with the total concentration needed to obtain complete orientation of the floating glycine crystals. The results show that complete say (010) orientation could be attained at an enantiomeric excess as low as 6% (i.e., 53% (*R*)-leu/47% (*S*)-leu), with a total concentration of leucine of 2.4% w/w of glycine. In a regime of high enantiomeric excess, in solution, e.g., a total concentration of leucine as low as 1.5% was sufficient. When leucine was introduced at an ee lower than 6%, its total concentration had to be raised above 2.4% in order to obtain complete orientation. The glycine crystals under these conditions are so thin as to make a correct evaluation of their degree of orientation very difficult. The limit imposed to the minimum enantiomeric excess of the additive appears to be thus dictated more by technical difficulties than by intrinsic limitations of the system.

The possibility of inducing a complete orientation of the glycine crystals at the solution/air interface in the presence of such a low enantiomeric excess of leucine (6%) provides strong evidence for the feasibility of our proposed model for the spontaneous generation and amplification of optical activity under prebiotic conditions as depicted in Scheme II. Spontaneous fluctuations of some parameter of the system, like temperature or concentration, can promote the appearance of the first glycine crystal in contact with a solution containing other, hydrophobic amino acids. The enantioselective occlusion of the additives into the first crystal will generate a small enantiomeric excess of the antipodes in solution. The above results suggest that this excess might be sufficient to trigger amplification such that all subsequent glycine crystals will be oriented in the same way. The amino acids occluded in the crust are all enantiomerically pure. The solution still contains both enantiomers, but its enantiomeric excess will increase with continued crystallization. This can eventually lead, in principle, to a complete separation of territories.

Concluding Remarks

We have presented a new model for achieving total separation of racemic α-amino acids into enantiomeric territories, and we have proposed a number of explanations to the observed effects. All proposed mechanisms lead to the conclusion that the same enantioselectivity rules which apply to the mature glycine crystal hold for their nuclei as well. This implies, although indirectly, that the nuclei have a structure very similar to that of the crystal. The stereochemical parameters of the system and the enantioselectivity provide us in this case with a convenient tool to glean information on the nucleation process at the structural and molecular level, information that is not easily available by other means. In any case, the experiments described here show in an unequivocal way that the addition of small amounts of chiral additives with appropriate structure may either catalyze or inhibit and even prevent formation of nuclei in an enantioselective way, providing us with novel routes to the amplification of optical activity, which remain to be explored further.

The mechanism of amplification of optical activity through enantioselective adsorption at enantiotopic faces may be relevant, in principle, to any crystalline structure displaying an enantiopolar axis. This is a symmetry constraint that can be met by organic or inorganic crystalline materials belonging to crystal classes such as triclinic, monoclinic, or tetragonal.[25] A wide choice of materials meet these conditions, among them are gypsum (space group $P2_1/c$), kaolinite ($P\bar{1}$), talc ($C2/c$), montmorillonite (2/m), chlorite ($C2/m$), etc., which must have been abundant in the prebiotic world. Thus reports of asymmetric induction in centrosymmetric crystals, which have given rise to controversy on symmetry grounds, may have been based on the same principles.[26] Furthermore the system considered here, glycine/α-amino acids, may in itself be relevant to the origin of optical activity since it involves compounds that are among the simplest building blocks in life.

Experimental Section

All amino acids were commercial analytical grade materials; their purity was checked in the limits of sensitivity of HPLC ($\sim 10^{-6}$ mg) and they were used without further purifications.

Crystallization Experiments. In a typical experiment 10 g of glycine were dissolved by heating together with the appropriate amounts of additives (see Tables) in 30 mL of double distilled water (33% supersaturation at 25 °C). The hot (~90 °C) solutions were filtered through cotton wool in crystallizing dishes (30-mL volume, 60-mm diameter) in 3 × 10 mL batches. Crystallizations were performed in an air thermostat at 27 ± 1 °C in a range of 1–15 h. In the presence of hydrophobic amino acids the time of appearance of floating crystals (up to 2 h) was much shorter than that with hydrophilic ones. When the crystals had reached a mature size (mm), they were removed from the interface, dried, counted, and measured. The average number of floating crystals was at

(22) Benedetti, E.; Pedone, C.; Sirigu, A. *Acta Crystallog.* **1972**, *B29*, 730.
(23) Landau, E. M.; Levanon, M.; Lahav, M.; Leiserowitz, L.; Sagiv, J. *Nature (London)* **1985**, *318*, 353.
(24) Garside, J. In *Biological Mineralization and Demineralization*; Nancollas, G. H., Ed.; Springer-Verlag: Berlin, 1982; p 23.
(25) Orthorhombic crystals do not display enantiopolar axes, but they display enantiopolar directions. The constraint in this case is more stringent and the symmetry conditions to be respected have been analyzed elsewhere.[12]
(26) Bondy, S. C.; Harrington, M. E. *Origins of optical activity in Nature*; Walker, D. C., Ed.; Elsevier: Amsterdam, 1979; p 141.

Table VII. Surface Tension, γ [dyn/cm], of Leucine Solutions at 20 °C as a Function of Concentration and Cosolute

concn, mol/L	aqueous solution	saturated glycine solution (22.8 g/100 mL)
0	72.8	75.7
0.05	72.1	72.1
0.075	71.3	70.0
0.1	70.0	69.2

least 100/batch in the presence of hydrophobic additives, while with hydrophilic ones all the crystals appeared at the bottom of the crystallizer.

Analytical Methods for Quantitative Evaluation of Crystal Orientations. Complete orientation of the glycine crystals was established by the following four independent methods:

a. Crystal Coloring. Crystallization experiments were carried out in the presence of very small amounts (0.01% w/w, which does not have any kinetic effect) of the resolved yellow dye N^{ϵ}-2,4-dinitrophenyl-(*S*)-lysine, which is enantioselectively occluded into the (010) oriented glycine crystals, thus imparting to them a yellow color. These crystals are easily differentiated visually from the non-colored ($0\bar{1}0$) oriented counterpart.

b. Enantiomeric HPLC Measurements. Samples (20 μL) were injected onto a reverse-phase column (25 cm × 4.6 mm) self-packed with 5 μm Nucleosil C18 (Macherey Nagel), with a mobile phase composition of an 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.[16] The samples were prepared by dissolving the whole batch of floating crystals, after thorough washing in water, into the appropriate amounts of mobile phase.

c. Morphology of the Crystals. The (010) and ($0\bar{1}0$) oriented glycine crystals assume an enantiomorphous morphology in two-dimensional projection at the interface and are therefore visually differentiable. This enantiomorphism arises from the fact that the β angle is 112° and that within the same batch the ratio of the lengths of the crystals along *a* and *c* is approximately constant.

d. Crystallographic Measurements. The direction of the crystallographic axes and the Miller indexes of the basal {010} faces were assigned by X-ray diffraction measurements on a Siemens diffractometer after the face exposed at the interface was marked.[16]

Methods a–c provide information on the degree of orientation of the entire batch of crystals. Method d which is absolute and unambiguous is only applicable to specimen crystal. Ten percent of each batch containing more than 100 crystals was routinely analized. In addition the few false negatives observed in methods a and c (for example, those crystals which assume a rhombic habit or those which are too thin to occlude sufficient amount of dye) were singularly analyzed by method d.

Growth Experiments. In the experiments of growth from seeds, pairs of seeds of known size (0.5 × 1 mm area, 0.14–0.16 mm thickness) were deposited with opposite ($\pm b$) orientations on the surface of a 2.5% supersaturated glycine solution (300 mL) containing the appropriate additive (Table VI). The solution surface was divided in two compartments by aluminum foil in order to avoid contact between the seeds. Growth of the seeds was performed in closed vessels without stirring in an air thermostat at 26.5 °C, for a period of up to 48 h. The grown crystals were removed and their thickness in the *b* direction remeasured under an optical microscope (×50). The relative growth along *b* is represented by the ratio final:initial thickness.

Amplification Measurements. Crystallizations were performed as described above, both in excess of (*R*) and (*S*) additive. For each enantiomeric excess of the additive used, the appropriate concentration was determined which yields complete orientation of the glycine crystals. In the presence of high concentrations of leucine (>1.5%) of low enantiomeric excess, the floating crystals of glycine are very thin plates. Their orientation was therefore confirmed by all four methods described above.

Surface Tension Measurements. These were performed on a semiautomatic Fisher surface tensiometer by the du Nouy method with use of a platinum iridium ring, for saturated solutions of glycine containing up to 0.1 mol/L of additive.

Acknowledgment. We thank Prof. Z. Berkovitch-Yellin for discussions, E. Gati Shavit for her skillful help, the Israel Academy of Science and Humanities, the U.S.-Israel Binational Foundation, Jerusalem, and the donors of the Petroleum Research Fund, administrated by the American Chemical Society, for financial support. L.A. is a recipient of the Charles S. Revson Career Development Chair.

Thermochemical Study of the Addition of Carbenium Ions to Alkenes

Christian Schade,[1a] Herbert Mayr,*[1a] and Edward M. Arnett[1b]

Contribution from the Institut für Chemie der Medizinischen Universität zu Lübeck, Ratzeburger Allee 160, D-2400 Lübeck, Federal Rupublic of Germany, and Department of Chemistry, Duke University, Durham, North Carolina 27706. *Received June 22, 1987*

Abstract: Scales of carbenium ion stabilities have been obtained from ^{1}H NMR equilibrium studies of mixtures of partly ionized para-substituted diarylmethyl chlorides in CD_2Cl_2/BCl_3 at −70 °C and via calorimetric determination of the heats of ionization (ΔH_i) of the alkyl chlorides ($Ar_2CHCl + BCl_3 \rightarrow Ar_2CH^+BCl_4^-$) in CH_2Cl_2/BCl_3 at −70 °C. The heats of the reactions of diarylmethyl tetrachloroborates with 2-methyl-1-pentene ($Ar_2CH^+BCl_4^- + CH_2{=}C(CH_3)C_3H_7 \rightarrow Ar_2CH\text{-}CH_2C(CH_3)ClC_3H_7 + BCl_3$), which were determined by low-temperature calorimetry, increase from −53.1 kJ/mol for $(H_3CC_6H_4)_2CH^+BCl_4^-$ to −33.0 kJ/mol for the better stabilized $(H_3COC_6H_4)(H_3CC_6H_4)CH^+BCl_4^-$. In contrast, the heats (ΔH_a) of the Lewis acid *catalyzed* additions of the corresponding para-substituted diarylmethyl chlorides to 2-methyl-1-pentene are independent of the para substituents ($\Delta H_a = -86.5 \pm 2.7$ kJ/mol). Similar values of ΔH_a were obtained for the addition of *p*-anisylphenylmethyl chloride to trimethylethylene, styrene, β-methylstyrene, and isoprene. ΔH_a is predominantly determined by the conversion of a π(C=C) into a σ(C—C) bond, and the heats of addition of diarylmethyl tetrachloroborates to alkenes in CH_2Cl_2 are given by the equation $\Delta H_b = (9.5 \pm 6.3\ pK_{R^+})$ kJ/mol.

Most synthetic reactions that yield carbon–carbon bonds proceed via polar mechanisms. Charged and neutral species may be used as synthetic equivalents of a- and d-synthons.[2] One conceivable extreme, the reaction of carbenium ions with carbanions, has recently been investigated in detail.[3] In that system, the heats of reaction only depend on the stabilization energies of both ions (pK_a and pK_{R^+}), since the neutral products are characterized by approximately constant σ(C–C) bond increments. The addition

(1) (a) Medizinische Universität zu Lübeck. (b) Duke University.

(2) (a) Warren, S. *Organic Synthesis: The Disconnection Approach*; Wiley: New York, 1982. (b) Fuhrhop, J.; Penzlin, G. *Organic Synthesis: Concepts, Methods, Starting Materials*; Verlag Chemie: Weinheim, 1984.

(3) (a) Arnett, E. M.; Molter, K. E. *Acc. Chem. Res.* **1985**, *18*, 339. (b) Arnett, E. M.; Chawla, B.; Amarnath, K.; Healy, M.; Molter, K. E. *J. Am. Chem. Soc.* **1985**, *107*, 5288. (c) Arnett, E. M.; Chawla, B.; Amarnath, K.; Whitesell, L. G., Jr. *Energy Fuels* **1987**, *1*, 17.

0002-7863/88/1510-0567$01.50/0