Heating the *E*-isomer of silylated 1,2-diethynylethene **7** for 20 h at 120 °C did NOT lead to polymerization. This monomer was recovered unchanged.[5,10]

The transformation of **2** into **5** under mild conditions is a unique example of an uncatalyzed carbon–carbon bond-forming reaction. We are confident that examples of selective polymerization of double bonds in **2** and **4** will render considerable impulse to the development of new (conducting) hydrocarbon polymers and polymeric nets. A macromolecular blend[1] as intimate as two polyacetylene strands anchored to one polyethylene backbone (conceivable from the desilylated structure **5**) seems sterically possible in CPK models.

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(9) The presence of silyl groups is essential for the selectivity of the polymerization. Similar experiments with 1,1-diethynylethene derivatives **2** in which R is *tert*-butyl, 1-adamantyl, or phenyl invariably led to destruction of the triple bonds (IR).

(10) Anionic polymerization by adventitious basic initiators is unlikely because of the preparative history of monomers **2** and **4**. $BF_3{\cdot}(OEt)_2$ and *n*-butyllithium in THF do *not* promote polymerization of **2**. Although the trimethylsilyl function is base-sensitive, we will pursue the possibility of an ionic polymerization in the near future.

Enzymatic Resolution of Racemic Amines: Crucial Role of the Solvent

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Asymmetric esterifications or transesterifications catalyzed by hydrolytic enzymes in nonaqueous media are emerging as a method of choice for facile kinetic resolutions of racemic alcohols, acids, and their derivatives.[1] In order to broaden this promising methodology, in the present study we have extended its applicability to another class of organic compounds with great synthetic utility[2]—chiral amines.

Our experimental strategy was based on the ability of lipases and other hydrolases to catalyze the reaction between carboxylic esters and amines in anhydrous organic solvents.[3] With the objective of finding a stereoselective enzyme, we tested the protease subtilisin Carlsberg and lipases from *Candida cylindracea*, *Pseudomonas* sp., *Mucor* sp., porcine pancreas, and *Chromobacterium viscosum* as catalysts[4] of the reaction between trifluoroethyl butyrate and the enantiomers of α-methylbenzylamine in octane.[6] To our disappointment, for no enzyme was any substantial enantioselectivity observed: the ratio of the initial rates for *S* and *R* isomers (v_S/v_R) was found to be[7] 1.4, 0.88, 0.94, 1.0, 1.5, and 0.66, respectively.

Table I. Enantioselectivity of Subtilisin in the Acylation of α-Methylbenzylamine as a Function of the Solvent[7]

solvent	initial reaction rate (v), mM/h		v_S/v_R
	S-amine	*R*-amine	
toluene	0.38	0.40	0.95
cyclohexane	1.1	0.87	1.3
acetonitrile	1.8	1.4	1.3
octane	1.3	0.9	1.4
carbon tetrachloride	0.71	0.46	1.5
ethyl acetate	1.4	0.88	1.6
butyl ether	0.24	0.13	1.8
pyridine	8.3	3.3	2.5
dimethylformamide	0.66	0.23	2.9
tetrahydrofuran	2.6	0.75	3.5
tert-amyl alcohol	4.5	1.1	4.1
3-methyl-3-pentanol	23	3.0	7.7

Table II. Preparative Resolution of Racemic Amines via the Acylation with Trifluoroethyl Butyrate Catalyzed by Subtilisin in Anhydrous 3-Methyl-3-pentanol[a]

racemic amine	product[b]	$[\alpha]^{25}_D$	ee, %
α-methylbenzylamine	*S*-amide[c]	−103° (*c* 2, MeOH)	85[c]
1-(1-naphthyl)ethylamine	*S*-amide[c]	−49.9° (*c* 2, MeOH)	98[d]
1-(1-naphthyl)ethylamine	*R*-amine	+54.8° (*c* 2, EtOH)	99[e]
1-methyl-3-phenylpropylamine	*S*-amide[f]	−25.2° (*c* 1, MeOH)	90[g]
2-aminoheptane	*S*-amide[c]	−4.6° (*c* 1, MeOH)	63[h]
α-methyltryptamine	*R*-amine[i]	−32.3° (*c* 1, MeOH)	95[e]
α-methyltryptamine	*S*-amide[j]	−2.7° (*c* 1, MeOH)	99[j]
1,3-dimethylbutylamine	*S*-amide[k]	+5.5° (*c* 2, MeOH)	59[h]
1-cyclohexylethylamine	*S*-amide[c]	−21.9° (*c* 1, MeOH)	88[c]
phenylalaninamide	*S*-amide[c]	+13.3° (*c* 2, MeOH)	80[c]
1,2,3,4-tetrahydro-1-naphthylamine	*S*-amide[l]	−85.1° (*c* 1, MeOH)	88[m]

[a] All reaction mixtures were stirred vigorously under the following conditions (in the order of entries from top to bottom): racemic amine concentrations (M)—0.2, 0.5, 0.42, 0.2, 0.2, 0.2, 0.2, 0.24, 0.2, 0.12, and 0.2; trifluoroethyl butyrate concentrations (M)—0.2, 0.5, 0.56, 0.4, 0.4, 0.4, 0.4, 0.48, 0.4, 0.12, and 0.2; subtilisin concentrations (mg/mL)—5, 2, 6, 10, 10, 10, 12, 12, 4, and 8; solvent volumes (mL)—50, 20, 15, 30, 30, 25, 25, 25, 25, 25, and 30; temperatures (°C)—30, 30, 30, 30, 45, 45, 30, 45, 30, 45, and 30; reaction times (hours)—25, 22, 141, 64, 54, 142, 69, 96, 101, 23, and 52. [b] The products were obtained as follows (in the order of entries from top to bottom): degrees of conversion (%)—35, 33, 57, 38, 36, 54, 39, 30, 31, 42, and 36; isolated yields (%)—33, 26, 46, 32, 32, 30, 25, 27, 29, 35, and 32; melting points (°C)—39–41, 126–127, oil, 44–45, oil, 126–127, 74–75, oil, 83–85, 174–175, and 77–78; purities by GC (%)—100, 99, 99 (contains some solvent), 100, 99, 100, 100, 99, 100, 99, and 100. All products were purified by silica gel column chromatography. The identity of all products was confirmed by high-resolution mass spectrometry and ^1H NMR and, whenever possible, by comparison with authentic samples. [c] Determined by comparison of $[\alpha]^{25}_D$ with that of the chemically butyrylated authentic *S*-amine. [d] Determined by HPLC on a chiral column (Regis's Pirkle 1A). [e] Determined by capillary GC following acylation with the Mosher acid chloride (Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543). [f] Concluded from comparison with its acetyl analogue (Yamamoto, Y.; Oda, J.; Inouye, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3744). [g] The ee of the unreacted amine was determined as outlined in footnote *e*, and then the amine was chemically butyrylated, and its $[\alpha]^{25}_D$ was compared with that of the amide product of the enzymatic reaction. [h] Determined by partial acid hydrolysis, followed by the procedure outlined in footnote *e*. [i] Determined from the literature data (Repke, D. B.; Ferguson, W. J. *J. Heterocycl. Chem.* **1976**, *13*, 775). [j] See footnotes *c* and *i*. [k] Determined from the analysis of $[\alpha]^{25}_D$ of the unreacted amine which was compared to the literature data (Mazur, R. H. *J. Org. Chem.* **1970**, *35*, 2050). [l] Determined as in footnote *k*, except that the literature reference was: Ghislandi, V.; Vercesi, D. *Farmaco, Ed. Sci.* **1971**, *26*, 474. [m] Determined as described in footnote *g*, except that $[Eu(tfc)_3]$ was used as the NMR shift reagent (Goering, H. L.; Eikenberry, J. N.; Koremer, G. S. *J. Am. Chem. Soc.* **1971**, *93*, 5913).

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(7) 100 mg/mL (except for 10 mg/mL subtilisin) enzyme suspensions in anhydrous octane containing 200 mM trifluoroethyl butyrate and 100 mM amine were shaken at 45 °C and 250 rpm; the reactions were followed by capillary gas chromatography on the basis of the appearance of the amide product. No appreciable aminolysis reaction was detected without enzymes.

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In the aforementioned experiment, as well as in all other studies of this sort,[1] the solvent was selected solely on the basis of such criteria as maximal enzymatic conversion rates and reactant solubilities; the possibility of affecting the enzyme enantioselectivity by the solvent was not considered. Encouraged by our recent findings,[8] we proceeded to examine the dependence of v_S, v_R, and their ratio (the enantioselectivity factor) on the reaction medium for one of the enzymes tested above, subtilisin. As seen in Table I, subtilisin's enantioselectivity factor is a strong function of the solvent and varies from near unity for toluene, octane, or cyclohexane to 7.7 for 3-methyl-3-pentanol. An even greater effect was observed for another chiral amine, 1-(1-naphthyl)ethylamine, for which the v_S/v_R ratio under the same conditions was 1.6 in octane and 22 in 3-methyl-3-pentanol.

The marked enantioselectivity expressed by subtilisin in 3-methyl-3-pentanol is of a magnitude that should be sufficient for kinetic resolutions of racemates.[9] Therefore, we employed subtilisin (a readily commercially available enzyme) in this solvent for preparative resolution of numerous racemic amines. Inspection of Table II reveals that the enzyme invariably exhibits a profound *S* selectivity and consequently optically active amides and amines have been prepared on a millimole scale with good, in most cases, enantiomeric excess.

Our findings demonstrate that screening is not the only route to enantioselective enzymes, for a nonselective enzyme in one solvent may become a useful selective catalyst in another. The scope and mechanism of this phenomenon are under investigation.

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Protochlorophyllide Reductase. 1. Determination of the Regiochemistry and the Stereochemistry of the Reduction of Protochlorophyllide to Chlorophyllide

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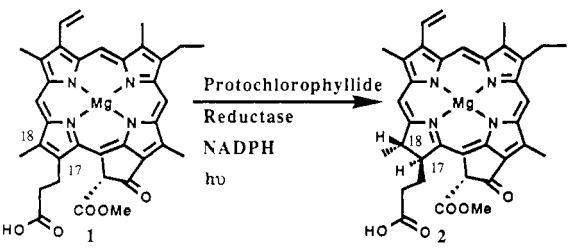
It has been estimated that over 1 billion tons of chlorophyll are synthesized in the biosphere each year.[1] Although the chlorophyll biosynthetic pathway has been the subject of intense investigation over the past 50 years, much remains to be discovered.[2,3] One promising area of investigation is the remarkable color change, from yellow to green, that occurs when dark-grown plants are exposed to light. This "greening reaction" involves the reduction of protochlorophyllide **1** to chlorophyllide **2** and is a key regulatory step both in the biosynthesis of chlorophyll and in the development of the chloroplast[4-6] (eq 1). This reaction is catalyzed by the



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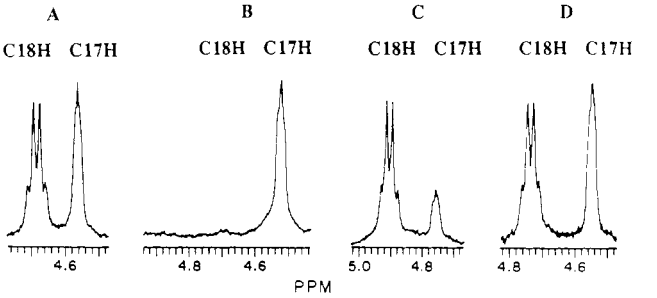

Figure 1. Partial NMR spectrum (400 MHz) showing the C17 and the C18 protons of pheophorbide: (a) control sample formed in protiobuffer, (b) sample formed in deuteriated buffer, (c) sample formed with [(4*S*-^{2}H)NADPH] as substrate, and (d) sample formed with [(4*R*-^{2}H)-NADPH] as substrate.

enzyme protochlorophyllide reductase and is of interest because it is one of only two known light-requiring enzymatic reactions.[7] The enzyme has been purified from oats,[8] barley,[9] wheat,[10] and squash.[11] It is an unstable membrane-bound protein of 37 000 D molecular weight.[8] The quantum yield for protochlorophyllide reduction in barley seedlings is 0.4[12] and the action spectrum for the reaction in corn seedlings follows the absorption spectrum of protochlorophyllide.[13] As the first step in our mechanistic investigation of this important reaction, we have determined the regiochemistry and the stereochemistry of the reduction catalyzed by the oats enzyme. On the basis of this information, we propose a model for the active site of the enzyme.

Enzymatic reactions were run by using a modification of the Griffiths procedure.[14] The reaction mixture consisted of 34 μM protochlorophyllide, 0.75-1.5 mM NADPH, and etioplast membranes (2-4 mg of protein/mL) suspended in 50 mL assay buffer. Protochlorophyllide was isolated from etiolated oat seedlings.[14] [(4*R*-^{2}H)NADPH] was prepared by the alcohol dehydrogenase-catalyzed reduction of $NADP^+$ by 2-propanol-d_8 (from *Thermoanaerobium brockii*).[15] [(4*S*-^{2}H)NADPH] was prepared by the glucose-6-phosphate dehydrogenase catalyzed reduction of $NADP^+$ by glucose-6-phosphate-1-*d*.[16] Etioplast membranes were isolated from 200 g of 7 day old etiolated oat seedlings.[14] These preparations typically contained 60% protochlorophyllide reductase and had a specific activity of 0.25 nmol/mg/flash (averaged over 23 flashes). After 23 flashes (1 flash/40 s) the reaction was quenched with acetone and extracted with ether. The chlorophyllide was then demetalated with hydrochloric acid (20 mM) and the resulting pheophorbide purified by HPLC. The purified product (70-120 μg) was analyzed by ^{1}H NMR.

The region of the ^{1}H NMR spectrum showing the C17 proton and the C18 proton resonances of pheophorbide is shown in Figure 1A.[17] The position of these signals varied between experiments, presumably due to variations in the pheophorbide concentration.[18,19] The relative integration (C18H:C17H) for the control sample was 1:0.8. The level of deuterium incorporation was

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