show that the dipolar shifts are inconsistent with only axial anisotropy. Thus eq 5 predicts shifts in opposite direction for δ-CH_3 and γ_2-CH_3, although comparable shifts in the same direction are observed. The pattern of dipolar shifts require a substantial rhombic anisotropy. The orientation of the g tensor and the g values obtained by Hori[47] for metMbCN at 20 K fail to account qualitatively for the observed pattern, suggesting that the magnetic axes differ at 20 and 298 K. This is not surprising since variable-temperature ESR has demonstrated that the magnetic axes change with temperature for two related Mb derivatives.[49,50] While the observed dipolar shifts can, in principle, provide both the components of the susceptibility tensor as well as the location of the magnetic axes, for such an analysis to be meaningful, it is necessary to assign unambiguously other amino acid side chains that exhibit sizable dipolar shifts and whose orientation can be ascertained from relaxation data.

It may be noted that the large upfield dipolar shift for γ_1-CH dictactes that the rhombic axes are aligned approximately so as to cause upfield dipolar shifts for pyrrole II and IV on which are appended 3-CH_3 and 8-CH_3. The nature of the rhombic geometric factor thus requires that the rhombic dipolar shift be negative (downfield) in the region of pyrrole I and III on which are located 1-CH_3 and 5-CH_3. Thus even the well-characterized asymmetry of the heme methyl hyperfine shifts, which have been generally interpreted[12,48,51] in terms of a completely scalar or contact interaction involving the raising of the orbital degeneracy of the porphyrin e_3 π orbitals, may have significant contribution from rhombic dipolar shifts.

Studies aimed at effecting sufficient unambiguous assignments of other purely dipolar-shifted amino acid side chain signals to determine the susceptibility tensor are in progress in this laboratory.

Acknowledgment. We thank Dr. J. Lecomte for expert assistance with the protein coordinate calculations and are grateful for the cooperation and assistance provided by the University of California, Davis Nuclear Magnetic Resonance Facility, and the Computer Graphics Laboratory at the Unversity of California, San Francisco. This work was supported by grants from the National Science Foundation, CHE-81-08766, and in part, from the National Institutes of Health, HL-16087.

Registry No. Ile, 73-32-5; iron, 7439-89-6.

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Stereochemical Studies on the Reactions Catalyzed by the PLP-Dependent Enzyme 1-Aminocyclopropane-1-carboxylate Deaminase

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Abstract: The stereochemical course of 1-aminocyclopropane-1-carboxylate deaminase which catalyzes the fragmentation of the cyclopropane substrate to α-ketobutyrate and ammonia has been unraveled with the help of substrates stereospecifically labeled with deuterium and/or tritium, and this has afforded important information about the process occurring at the active site during enzymatic conversion. These results can be summarized as follows: (1) ring cleavage is regiospecific and only occurs between the *pro-S* and the α-carbon of ACPC; (2) β-H abstraction is *pro-R* stereospecific and the reprotonation at β-C is mediated by the same enzyme base which is partially shielded and located at the *si* face relative to α-C; (3) preferred conformation of the β,γ-olefinic PLP-*p*-quinoid α-anion complex is cisoid and the geometry of the terminal double bond, if trisubstituted, favors *E*, while the major conformation of the nascent intermediate, aminocrotonate, is *Z* (defined as relative to the amino group); (4) protonation at C-4 is mediated by a different enzyme base which is not shielded and is situated at the *si* face with respect to α-C.

The unusual cyclopropanoid amino acid 1-aminocyclopropane 1-carboxylic acid (ACPC) is a natural product found in many plants.[1] Recent studies had implicated it as a precursor of ethylene, a fruit-ripening and growth-regulating plant hormone.[2] The mechanism of this physiologically significant and commercially important fragmentation process is as yet unknown but under examination.[3]

ACPC is formed biosynthetically from methionine presumably via *S*-adenosylmethionine catalyzed by a pyridoxal 5'-phosphate (PLP) linked enzyme (ACPC synthase) which appears to carry out a formal internal γ-displacement reaction initiated by an α-anion equivalent. An alternate route for biological breakdown of ACPC, besides the plant-mediated fragmentation to ethylene, is a cleavage reaction producing α-ketobutyrate **2** and ammonia carried out by certain bacteria. Pseudomonads which can grow on ACPC as sole nitrogen source have been isolated. From these bacteria the PLP-containing enzyme ACPC deaminase, mediating the reaction delineated by eq 1, has been purified to homogeneity.[4] This enzyme will also catalyze the ring-opening process of 2-

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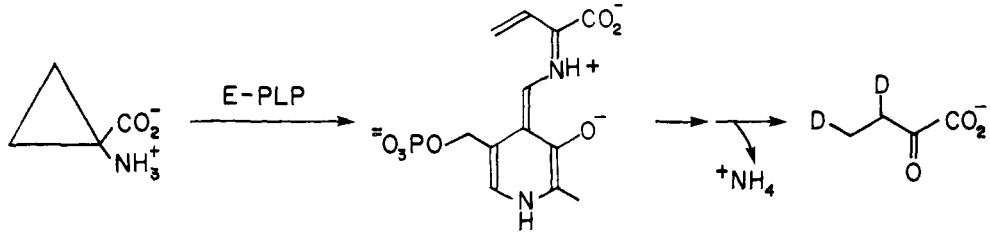

(1)

ethyl-ACPC **3** known as coronamic acid, a constituent of the natural phytotoxin coronatine,[5] and several other monoalkylated ACPC. Thus, the alkyl-substituted cyclopropane amino acid may as well be the physiologically relevant natural substrate. However, of the four possible diastereomers of coronamic acid,[6] only the 1*S*,2*S* isomer can be utilized and is processed regiospecifically to the linear α-keto-*n*-caproic acid.[7]

The deaminase is a PLP-linked enzyme with unique capacity to break the cyclopropane ring. This formal cleavage of the C_α–C_β bond is only precedented for a few other PLP enzymes such as serine transhydroxymethylase or δ-aminolevulinate synthetase. However, reactions catalyzed by those enzymes proceed through the retro-aldol type cleavage, a mechanism that does not seem immediately available in the ACPC case.

In our preliminary mechanistic work on the ACPC deaminase catalyzed reaction, it has been noted that incubation in ^2H_2O led to the incorporation of solvent deuterium atoms into C-3 and C-4 positions of α-ketobutyrate product. Carbon-3 of the product becomes chiral upon monodeuteration and by in situ coupled enzymatic reduction to (2*S*)-2-hydroxybutyrate, a 3*R*/3*S* deuterated product ratio of 72/28, reflecting incomplete stereospecificity at this locus has been found.[8] This labeling pattern is consistent with the intermediacy of a PLP-stabilized vinylglycine α-anion I during catalysis and this contention is supported by vinylglycine turnover study; namely, (2*R*)-vinylglycine can indeed be processed by ACPC deaminase in ^2H_2O to the same 3,4-dideuterio-2-ketobutyrate.

In an attempt to gain further insight into the early steps of ACPC ring fragmentation process, we have undertaken stereochemical studies. ACPC has two enantiotopic β-methylene groups, each of which bears two diastereotopic methylene protons. We anticipated that the enzyme, as a chiral catalyst, should be able to distinguish each level of prochiral center. In a joint effort with the groups of Prof. Richard Hill of Georgia and Prof. Duilio Arigoni of Zürich we have recently examined the interaction of (*R*)-(−)-[2,2-$^2H_2$]-ACPC and (*S*)-(+)-[2,2-$^2H_2$]-ACPC with enzyme where the dideuterio substituents provide the probe of scrutiny of the ability of the enzyme to distinguish the two enantiotopic methylene groups of the ACPC cyclopropanoid skeleton.[9] We noted that (*S*)-(+)-[2,2-$^2H_2$]-ACPC gives [4,4-$^2H_2$]-α-ketobutyrate only, while (*R*)-(−)-[2,2-$^2H_2$]-ACPC yields [3-$^2H_1$]-α-ketobutyrate exclusively, thereby demonstrating that ACPC deaminase can unerringly distinguish the enantiotopic centers and establishing the regiospecificity of C_α–C_β bond fragmentation as cleavage between α-C and *pro-S* β-methylene carbon.[9] The question of regiospecificity of cyclopropane cleavage is only the first of seven stereochemical problems in ACPC deaminase catalysis as noted in Scheme I. Additional questions include the following: (2) which diastereotopic hydrogen from the *pro-R* methylene group is abstracted; (3) what is the conformation (cisoid or transoid) of the conjugated ketimine π-complex in the *p*-quinoidal vinylglycine–PLP species; (4) what is the configuration (*E* or *Z*) of the terminal 3,4 double bond (e.g., when R = deuterium or alkyl); (5) what is the chirality of the C-4 methyl group in the 2-ketobutyrate product, namely, the steric course of C-4 protonation (*re* or *si*);[10] (6) what is the configuration (*E* or *Z* referring to the substituent at C-3 with respect to amino group) of the resulting enamino acid protonated in the active site; and (7) what face (*re* or *si*) of the enamine double bond is exposed for C-3 protonation to yield the observed product. We report experiments relevant to these issues in this paper: determination of answers to 2 and 5 allows interpretation of the stereochemical questions 3, 4, 6, and 7.

Results

Configuration of the Enamine Double Bond in the Aminocrotonate Intermediate. In a previous report using ACPC with deaminase in ^2H_2O we had noted that the enzyme exhibits a stereochemical preference of 72/28 for *R*/*S* distribution in incorporation of a solvent hydrogen species at C-3 in 2-ketobutyrate.[8] The lack of complete stereospecificity could devolve from either the unequal participation of two basic groups at opposite faces of the planar enamino acid intermediate IV in the active site or the existence of two rotamer populations of the aminocrotonate in the active site toward the enzyme–BH^+ about to deliver the proton to C-3. In this latter case the bound aminocrotonate would be a mixture of *Z* and *E* isomers (IVB and IVC) and would so offer *re* or *si* face[10] to a single stationary EBH^+. Selectivity as assayed by *R*/*S* configuration of 3-$^2H_1$ in the 2-ketobutyrate product would derive from excess of one aminocrotonate isomer in the active site environment. In turn, the proposed two conformational isomers of the aminocrotonate may have been inherited from the β,γ-olefinic PLP-*p*-quinoid α-anion complex, which could be present in both the cisoid (IIIA) and transoid (IIIB) conformation at the active site as shown in Scheme I. Protonation at C-4 in that complex would lead to *Z* (IVB) or *E* (IVC) isomers of aminocrotonate. It may possibly be that only one conformation (cisoid or transoid) of the β,γ-olefinic PLP-*p*-quinoid complex is present, and, consequently, only one aminocrotonyl–PLP complex is generated. It would be the nascent aminocrotonate that, upon hydrolysis, flipped over to a 7:3 mixture before being quenched by protonation. The observed 7:3 distribution strongly suggests the protonation actually occurs within the chiral active site microenvironment.

Distinction between the one-base and the two-base mechanism was achieved by careful examination of ^1H NMR spectra of the derivatized enzymatic products isolated from incubation with various isotopic labeled substrates in H_2O or unlabeled analogues in ^2H_2O. As noted in Table I, unlabeled ACPC in ^2H_2O yields product having 1.0 ^2H at C-4 but only 0.85 ^2H at C-3 reproducibly. This indicates a 15% *internal return* of substrate hydrogen (from the *pro-R* methylene group) to C-3 of the 2-ketobutyrate product. With tetradeuterio ACPC **4** in H_2O, the ratio of product protons at C-4/C-3 is 1.0/0.81, again a 19% *internal return*, this time of a deuterium from the substrate *pro-R* methylene group to C-3 in the product. We have previously noted similar internal return in our communication on enzyme fragmentation of (*R*)-[2,2-$^2H_2$]- and (*S*)-[2,2-$^2H_2$]-ACPC.[9] Only the (*R*)-[2,2-$^2H_2$]-ACPC isomer is regiospecifically cleaved to yield product having deuterium at

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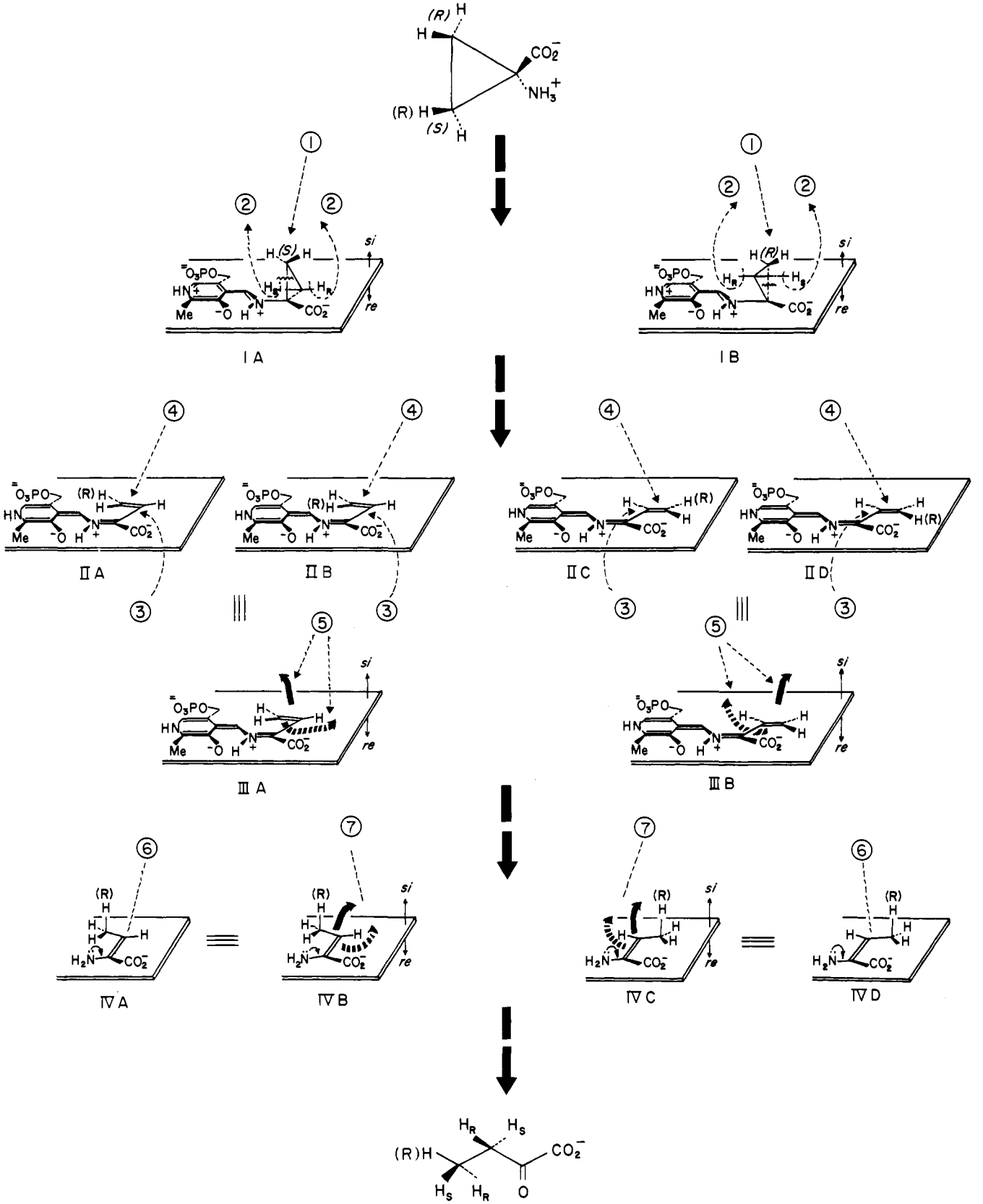
(10) In order to avoid complication arising from valence change ($sp^3 \leftrightarrow sp^2$) of focal centers (e.g., C-3 and C-4) in various intermediates at different stages, *re* and *si* face, have been constantly defined as relative to the α-C of the *initial* substrate–PLP aldimine π-complex in this paper.

Scheme I



C-3; there is a 27% internal return. The same 27% internal transfer is seen in racemic mix of (*R*,*S*)-[2,2-$^2H_2$]-ACPC (**5**), produced in a carbene insertion reaction on *N*-acetyldehydroalanine methyl ester (**6**)[11] from $^2H_2$-diazomethane followed by pyrolysis[12] (Scheme II).

All the above data are consistent with the interpretation that enzymatic removal of one of the two diastereotopic hydrogens from the *pro-R* methylene group of ACPC and reprotonation at C-3 in the aminocrotonate at the active site is mediated by a single active site base.[13] The base is partially shielded (15–27%) from

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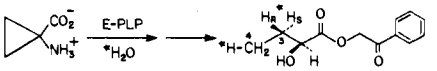
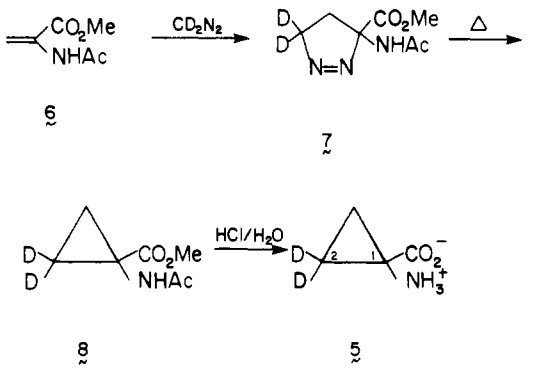
Table I. Relative Ratios of Internal Returns, Proton Contents of 3-Methylene and 4-Methyl Groups, and Conformation of Immediate Precursor of α-Ketobutyrates Isolated from Various Enzymatic Incubations[a]



substrate	incubation solvent	4-Me/3-CH_2	internal return, %	H_{3R}/H_{3S}[b]	E/Z[c]
ACPC	^2H_2O	2/1.15	15	28/72	20/80
ACPC-d_2 (rac)	^2H_2O	2/1.19	19	33/67	25/75
ACPC-d_4	H_2O	1/0.81	19	60/40	40/60
ACPC-d_2 (rac)	H_2O	4/2.73	27	52/48	48/52
ACPC-d_2 (+)	H_2O	1/2		50/50	
ACPC-d_2 (−)	H_2O	3/0.73	27	56/44	44/56

[a] Rection conditions are described in the text and/or Experimental Section. The α-ketobutyrates formed were reduced in situ with L-lactate dehydrogenase and isolated as phenacyl (2*S*)-2-hydroxybutyrates. [b] Ratios were determined by integration of the corresponding signals,, δ 2.01 (3-H_S), 1.86 (3-H_R), and 1.09 (Me), in 250-MHz (or 270-MHz) proton NMR. The doublet of doublets resonance at δ 5.46 (phenacyl methylene) was employed as an internal integration standard. [c] The *E* and *Z* conformation of the enamine double bond in aminocrotonate refer to the substituent at C-3 with respect to amino group.

Scheme II



6 7 8 5

exchange with solvent hydrogens. It is likely that this net internal return mediated by the enzyme base is overall suprafacial. The increase of internal return of racemic ACPC-d_2 from 19% in ^2H_2O to 27% in H_2O may reflect a solvent deuterium isotope effect in proton/deuteron transfer to and from the shielded active site base, but this is only supposition at present. In addition, we have also determined there are no significant V_{max} isotope effects ($V_H/V_D \leq 1.2$) for the tetradeuterio and various dideuterio ACPC species.

Given suprafacial internal return mediated by a single enzyme base, yet a mix of *R* and *S* configurations at C-3 found in the deuterated ketobutyrate products, there must be a population of two conformations (*E* + *Z*) around the C_α–C_β bond of the aminocrotonate intermediate. The question of whether this enzyme base is disposed *si* or *re* as *defined relative to the initial substrate–PLP aldimine π-complex*[10] is now relevant to assessing whether the *Z* or *E* aminocrotonate yields the major 3*R* product (72%). We have previously noted that ACPC deaminase will process the *D* isomer (2*R*) but not the *L* isomer of vinylglycine to α-ketobutyrate.[8] By precedent with all other enzymatic conversions catalyzed by PLP-dependent enzymes in which only one side of the substrate–PLP complex is exposed for enzymatic manipulation,[14] the *si* disposition of the abstractable α-H in (*R*)-vinylglycine–PLP complex suggests it is now the *si* face of the π-system being exposed in the active site (Figure 1). Such an asymmetric binding domain is expected to be preserved and utilized in all reactions catalyzed by ACPC deaminase regardless of the variation of the substrate structures. The active-site base removing the 2*R* proton in vinylglycine could be the one mediating

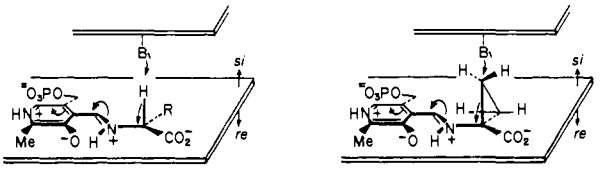


Figure 1. The *si* orientation of C_α–H bond and enzyme base B_1 in the initial substrate coenzyme aldimine complex at the active site. This asymmetric binding domain is expected to be utilized in all reactions catalyzed by ACPC deaminase.

the internal transfer in ACPC fragmentation. However, analysis of [2-$^2H_1$]vinylglycine (**9**), on conversion to 2-hydroxybutyrate (coupled assay), indicated no detectable deuterium was incorporated at C-3 even though we view that the reaction flux from (2*R*)-vinylglycine and ACPC mesh at the β,γ-olefinic PLP-*p*-quinoid α-anion stage. Thus there are at least two active-site bases (designated B_1 for removal of 2-H of (2*R*)-vinylglycine and B_2 for the one showing internal return from *pro-R*-CH_2 of ACPC to C-3 of ketobutyrate). In fact, a third active-site base is mandated for C-4 chemistry as noted later. It is not known if B_1 and B_2 are both on the *si* face with respect to the initial substrate–PLP complex in ACPC deaminase although such is the case in another PLP enzyme involved in γ-replacement amino acid chemistry, namely, cystathionine γ-synthase.[15] Thus, we assume provisionally the same arrangement with ACPC deaminase. This assumption would imply *si* face protonation at C-3 of aminocrotonate and also *si* face abstraction of the *pro-R* methylene hydrogen of ACPC. This would not only answer problem 7 of Scheme I but also implicate that the *Z* conformer (IVA) of aminocrotonate predominates at the active site. Given 72% 3*R* product from ACPC in ^2H_2O and the observed 15% internal transfer, simple algebra shows 80% of the reaction flux goes via the *Z*-aminocrotonate and 20% by the *E* isomer.[16] For tetradeuterated ACPC in H_2O, Table I shows the *R*/*S* preference dropped to 60/40, while racemic dideuterated ACPC **5** gave 3*R*/3*S* chirality ratios of 52:48 and 67:33 in incubations in H_2O

(13) It has been pointed out in editorial review that "the data regarding internal return is, indeed, consistent with a one-base mechanism, but it also a consistent with a two-base mechanism in which transfer of the abstracted proton to a second base prior to its return occurs. In this case both bases would be on the same side of the molecule". This is a more complex explanation than the single-base idea.

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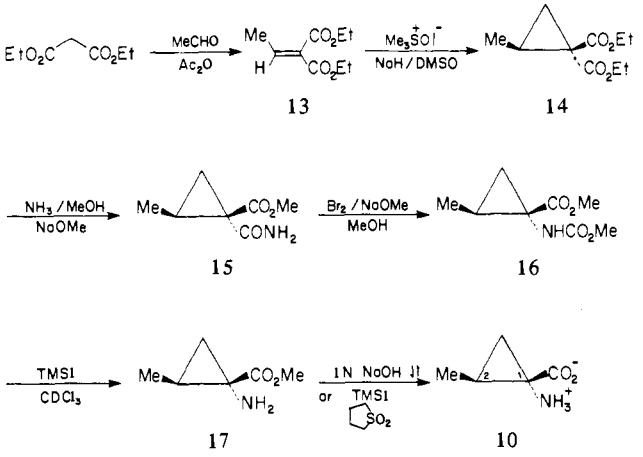
(16) Let the percentage of IVB be *X* and of IVC be (1 − *X*) and let the ratio of $EnzBH^+/EnzBD^+$ be $Y/(1-Y)$. C-3 protonation of IVB, occurring at the *si* face of this planar intermediate, should yield products having either deuterium (if protonated by $EnzBD^+$) labeled at 3-H_R ($X(1-Y)$) or no deuterium (if protonated by $EnzBH^+$) incorporated (XY). Similarly, both monodeuterated (labeled at 3-H_S, $(1-X)(1-Y)$) and unlabeled ($(1-X)Y$) species should be formed during C-3 protonation of IVC. Since product analysis by proton NMR had shown a proton ratio of 3-H_R/3-H_S of 28/72 and a 15% internal hydrogen transfer, the two following equations can be easily formulated.

$$XY + (1-X)Y + (1-X)(1-Y) = H_R = 28(1.15)$$

$$XY + X(1-Y) + (1-X)Y = H_S = 72(1.15)$$

Therefore, $X = 80\%$ and $Y = 15\%$.

Scheme III

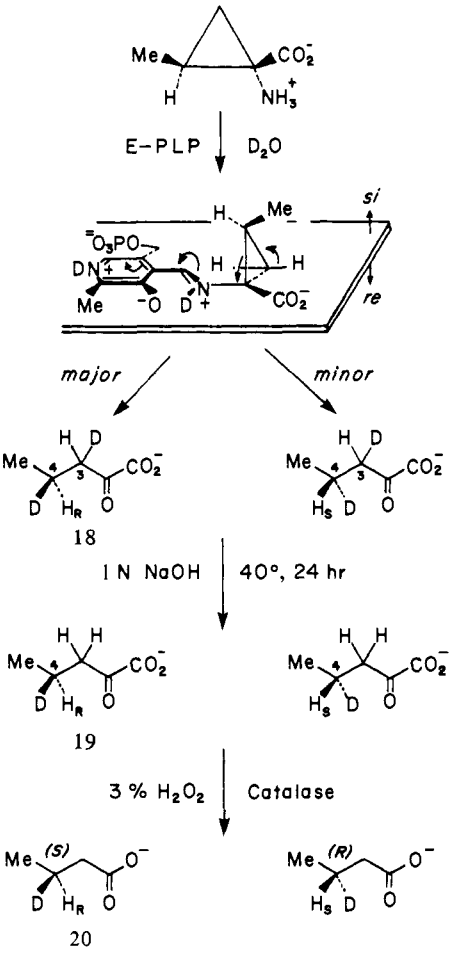


and ^2H_2O, respectively. These compute to an alteration of preferred *Z* conformation from 80% for ACPC in ^2H_2O to 75% with racemic dideuterio ACPC in ^2H_2O. In H_2O the result of tetradeuterio ACPC **4** implies the *Z* population is 60% and that of racemic dideuterio ACPC is 52%. One interpretation of the change from 75–80% *Z* in ^2H_2O to 52–60% *Z* in H_2O might be a reflection of a slightly altered enzyme conformation in H_2O vs. ^2H_2O. (In all examples the incubations were run to completion, no V_{max} deuterium effects were seen, and so the data should be devoid of bias from kinetic isotope effects), but there is the possibility of solvent deuterium isotope effect on partition between H or ^2H transfer to enamine vs. rotational interconversion between *E* and *Z* enamines.

Incubation of (1*S*,2*S*)-Monomethyl-ACPC in ^2H_2O: Stereochemistry at C-4 of the Product. To determine the stereochemical outcome at C-4 in the 2-keto acid product an assay for chirality determination had to be developed. With ACPC itself one would need a sample having deuterium specifically labeled at H_R or H_S of the *pro-S* methylene group; incubation in ^3H_2O would then afford product suitable for chiral methyl analysis. In order to avoid a complicated synthesis, we have instead used two alternate substrates, a single isomer of monomethyl ACPC **10** and the **Z** and **E** isomers of [4-$^2H_1$]vinylglycine (**11** and **12**). The 2-methyl-ACPC, with the methyl trans to the amino group, was prepared as the racemate, 1*S*,2*S* and 1*R*,2*R*, via cyclopropane formation from addition of an oxosulfonium ylide[17] to diethyl ethylidenemalonate as noted in Scheme III. The key factor in the synthesis is the introduction of the amine functionality trans, not cis, to the methyl group. The enzyme was known to accept only the 1*S*,2*S* diastereomer of the natural 2-ethyl-ACPC[7] and so the product synthesized as racemate would suffice, but it was crucial to confirm the relative configuration of the racemate structure. The mechanistic expectation was that in the diethyl 2-methylcyclopropane-1,1-dicarboxylate intermediate **14** the sterically less hindered carboxyl group would be selectively ammonolyzed to give the monoester/monoamide product **15**[18] which would then be transformed to 2-methyl-ACPC without effect on the configuration at C-2. This assignment has been supported by the similar response pattern found for the 2-H signal of both 2-methyl-ACPC and authentic 2-ethyl-ACPC (coronamic acid)[18] in the ^1H NMR aromatic solvent (pyridine) induced shift experiment.[19] Exhaustive incubation of the synthetic 2-methyl-ACPC confirmed 50% utilization by deaminase in keeping with the result on 2-ethyl-ACPC that only the 1*S*, 2*S* diastereomer was processed;[7] we assume then the deaminase also recognizes only (1*S*,2*S*)-2-methyl-ACPC (K_m = 12.5 mM, V_{max} = 16.4% of ACPC).

Scheme IV shows the method for determination of chirality of the center of interest on enzymatic processing of (1*S*,2*S*)-2-

Scheme IV

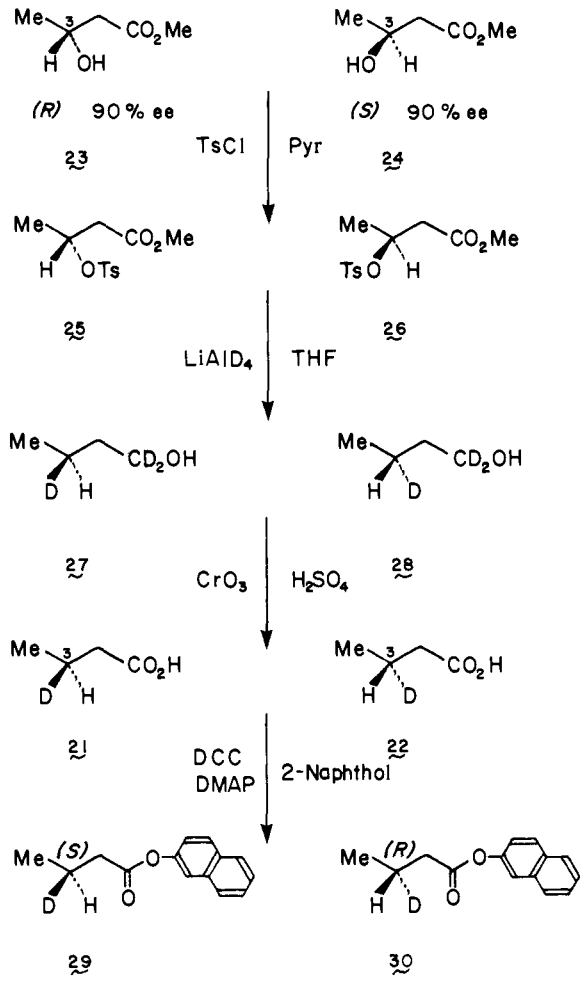


methyl-ACPC in ^2H_2O. As expected solvent deuteriums were incorporated at both C-3 and C-4 of the 2-ketopentanoate product **18**. The deuterium at C-3 was subsequently exchanged out in 1 N NaOH (protic solution) at 40 °C for 24 h. Decarboxylation to [3-$^2H_1$]butyrate (**20**) was smoothly achieved by treatment with 3% H_2O_2 followed by catalase. While we had contemplated using optical rotation to determine the chirality at C-3,[20] we foresaw difficulties with low absorptivity and only milligram quantities of enzymatic product. Fortunately our efforts on circular dichroism of the 2-naphthoyl ester of the enzymic [3-$^2H_1$]butyrate were successfull. It substantiated our expectation that asymmetric perturbation arising from a chiral center (whose chirality is solely due to isotopic substitution) two bonds away from the absorbing chromophore, is sufficient to induce a detectably useful Cotton effect. This has also been proved to be more sensitive than ORD.

Crucial to this result was the availability of stereospecifically deuterated butyrates of known absolute configuration and of proven enantiomeric purity. The synthetic samples of (3*S*)- and (3*R*)-[3-$^2H_1$]butyrates (**21** and **22**) were prepared as in Scheme V starting from commercially available (3*R*)- and (3*S*)-3-hydroxybutyrate methyl esters (**23** and **24**), interconvertible by a modified Mitsunobu reaction.[21] The purity of each commercial hydroxy ester was determined by NMR analysis of its Mosher ester, prepared by reaction with 1-methoxy-1-(trifluoromethyl)-phenylacetic acid (MTPA).[22] The enantiomeric purity was determined to be 90% for each enantiomer. The CD measurements of the 2′-naphthoyl[3-$^2H_1$]butyrate esters in methanol gave a negative absorption at 276 nm with $\Delta\epsilon = -0.36$ for enzymatic product and $\Delta\epsilon = -0.48$ for the (3*S*)-[3-$^2H_1$]butyrate standard (no more than 90% pure as starting material). The predominant

(17) Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
(18) Ichihara, A.; Shiraishi, K.; Sakamura, S.; Nishiyama, K.; Sakai, R. *Tetrahedron Lett.* **1977**, 269.
(19) Dean, F. M.; Varma, R. S. *Tetrahedron* **1982**, *38*, 2069.
(20) Bücklers, L.; Ronchi, Umani,; Retey, J.; Arigoni, D. *Experientia* **1970**, *26*, 931.
(21) Galynker, J.; Still, W. C. *Tetrahedron Lett.* **1982**, 4461.
(22) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

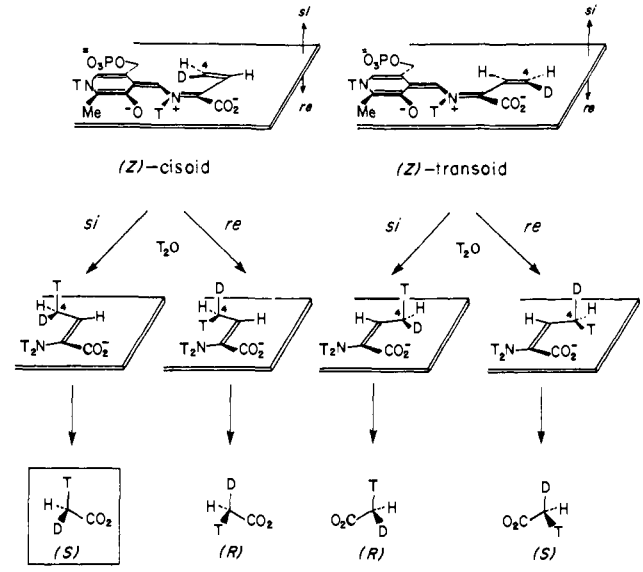
Scheme V



chirality of enzymatic product is thus 3*S* and so 4*S* in the 2-ketopentanoate product. The less than 100% chiral purity (ca. 80% optical pure) of the enzymatic product could reflect some loss of chiral purity during sample manipulation but could as easily (and more likely) represent the conformational heterogeneity of the α,β-olefinic PLP-*p*-quinoidal species, which, as noted earlier, could be present in both cisoid (IIA/IIB) and transoid (IIC/IID) conformations (Scheme I), as progenitors of the aminocrotonate geometric mix (80/20, vide supra). Protonation of cisoid and transoid forms of IIIA and IIIB from a stationary enzyme base would yield a mix of 4R and 4S species. It is worth noting that there is no internal return detected at C-4 in the 2-methyl-ACPC, ACPC, or vinylglycine incubations. The enzymatic base producing stereoselective 4*S*-chiral outcome is *distinct* from the enzyme base carrying out suprafacial, internal transfer to C-3 and also *distinct* from the base pulling off the α-H from (2*R*)-vinylglycine. This enzyme then needs an inventory of three different active-site bases (one for C-2, one for C-3, and one for C-4 acid–base chemistry).

Incubations of (*Z*)-[4-$^2H_1$]Vinylglycine and (*E*)-[3,4-$^2H_2$]-Vinylglycine in ^3H_2O: Chirality at C-4 of Product. Experiments to determine the stereochemical outcome at C-4 of the keto acid product were also performed with vinylglycine. The C-4 methyl of the ketobutyrate product must bear H, ^2H, and ^3H to be analyzable for chirality. The deuterium was introduced in the synthesis of (*Z*)-[4-$^2H_1$]- and (*E*)-[3,4-$^2H_2$]vinylglycine (**11** and **12**), reported by us previously.[15] Enzymatic incubations in ^3H_2O produced 2-ketobutyrate with all three isotopes of hydrogen at C-4. Kuhn–Roth degradation produced [2,2,2-H,$^2H_1$,$^3H_1$]acetates. The labeled acetates derived from the two parallel enzymatic incubations were purified from any contaminating propionate by conversion to the *p*-phenylphenacyl ester, thin-layer chromatography, saponification, and finally crystallization to provide the chiral sodium acetate. The tritiated sodium acetates were mixed

Scheme VI



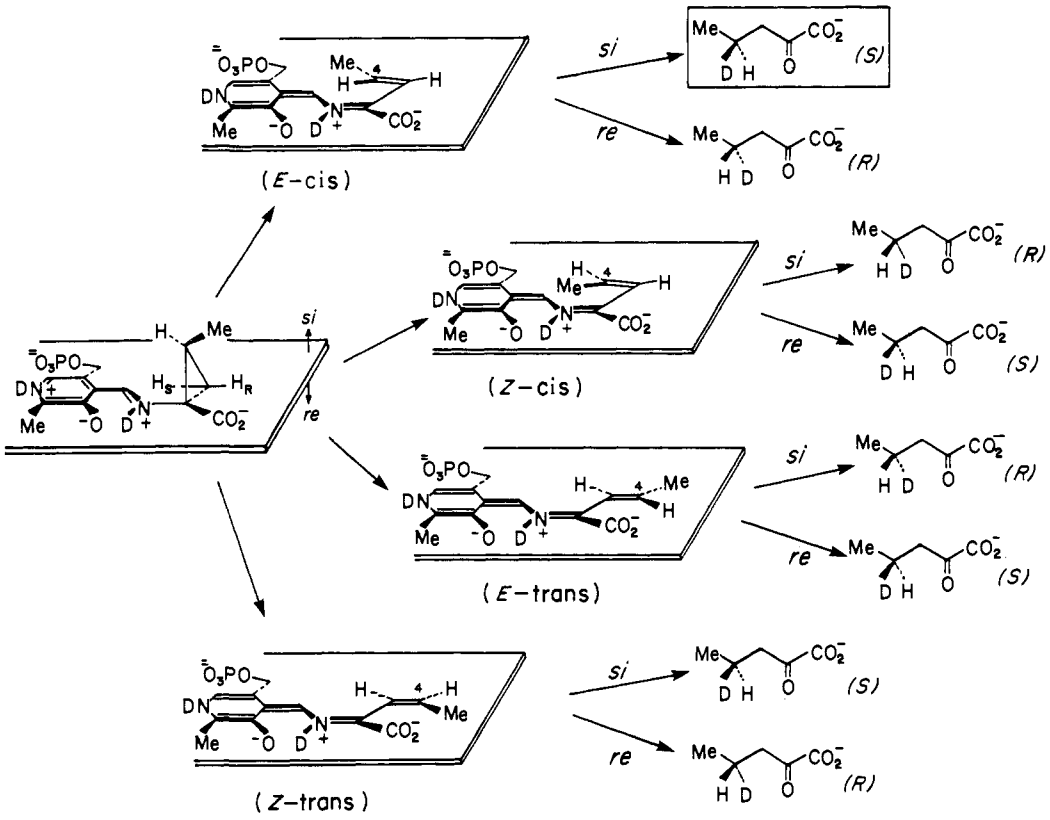
with [U-^{14}C]acetate to give a $^3H/^{14}C$ ratio of 4.5 and then analyzed by the standard malate synthase/fumarase chiral methyl methodology[23] kindly carried out by the group of Prof. Heinz Floss at Ohio State. It was found that, of the tritium label in (2*S*)-malate derived from (*Z*)-[4-$^2H_1$]vinylglycine (**11**), 37.7% (F = 37.7) was retained after conversion into fumarate by fumarase, indicating an excess of (*S*)-acetate in this sodium acetate sample. Enantiomerically pure (*S*)-acetate gives an F value of 21 and (*R*)-acetate gives an F value of 79, on the basis of k_H/k_D of 3.7–3.8 in the malate synthase reaction.[24] The F value of 37.7 indicates an 71/29 split of (*S*)-acetate/(*R*)-acetate and thereby (4*S*)-ketobutyrate/(4*R*)-ketobutyrate from (*Z*)-[4-$^2H_1$]vinylglycine. The less than 100% stereoselectivity but favoring *S* configuration at C-4 is consistent with the stereochemical result obtained with 2-methyl-ACPC (**10**).

The data from (*E*)-[3,4-$^2H_2$]vinylglycine (**12**) tend in the same direction, but are less clear-cut. The F value of the derived tritioacetate is 55.7, which is less than the anticipated F of 62.3 expected as a complement to the F of 37.7 seen above. Yet it is clearly different from a racemic F of 50 and is in the expected direction. Thus, vinylglycine having the opposite double bond configuration (*E*) gives (*R*)-acetate predominantly, and the data suggest a 60/40 split (from F = 55.7) of *R*/*S* acetate and so (4*R*)-/(4*S*)-ketobutyrate. All these results support the view that protonation at C-4 may proceed from two different conformations, cisoid and transoid, of the β,γ-olefinic PLP-*p*-quinoid intermediate, IIIA and IIIB (Scheme I).

Consider now the result with the (*Z*)-[4-$^2H_2$]vinylglycine **11** as example of the four possible pathways for C-4 protonation (depicted in Scheme VI), only the *Z*-cis-*si* and *Z*-trans-*re* routes will lead to the formation of (*S*)-acetate. Since the immediate product of this protonation sequence is the aminocrotonyl-PLP which, upon hydrolysis to give aminocrotonate in the active site, has been shown to have a preferred *Z* geometry around its double bond (when generated from both ACPC and vinylglycine, see Table I), only the cisoid conformation will yield the favored (*Z*)-aminocrotonate. Derived from the known configurations of starting vinylglycine (4*Z*) and degradation product acetate (*S*), the solvent hydrogen incorporated at C-4 has to be introduced from the *si* face *relative to the initial substrate–PLP aldimine π-complex*[10] (answer to stereochemical question 5). The same stereochemical arguments lead to the common conclusion for the (*E*)-[3,4-$^2H_2$]vinylglycine as well.[25]

(23) Floss, H. G. *Methods Enzymol.* **1982**, *87*, 126.
(24) Lenz, H.; Eggerer, H. *Eur. J. Biochem.* **1976**, *65*, 237.

Scheme VII

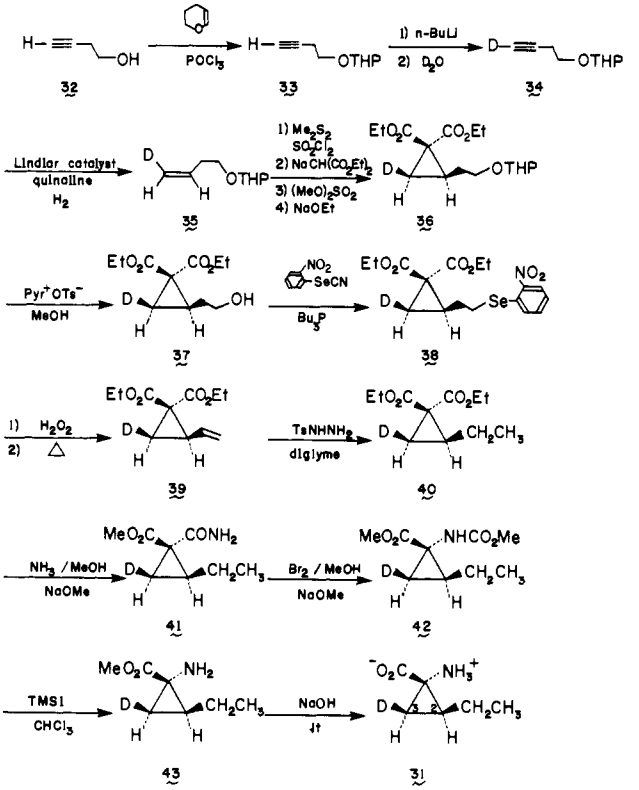


In order to completely define the steric course of this part of the reaction, the configuration of the terminal double bond in the β,γ-olefinic PLP-*p*-quinoid complex derived from monoalkylated ACPC substrates, e.g., (1*S*,2*S*)-2-methyl-ACPC, should also be considered. Scheme VII shows the possible stereochemical modes for the C-4 protonation reaction in the 2-methyl-ACPC case. Four of the eight can be eliminated because the chirality at C-4 of the ketopentanoate is *S*, as determined above on the decarboxylated product (3*S*)-butyrate. With the knowledge of the favored cisoid conformation of the fully conjugated β,γ-olefinic PLP-*p*-quinoid and the *si* face addition now defined from the vinylglycine results, it is apparent that the configuration of the terminal disubstituted double bond can be assigned as *E* in the predominant conformer (answer to stereochemical problem 4). Since this assignment uses information gathered from incubations of several substrates in solvents of varied isotopic composition, no effort is made to offer a more quantitative assessment of the *E*/*Z* ratio in the 2-alkyl-ACPC conversions.

Incubations with 2-Ethyl-[3-$^2H_1$]-ACPC: Stereochemistry of β-Proton Abstraction. The remaining stereochemical question to be answered in ACPC deaminase catalysis is the stereoselectivity of diastereotopic proton abstraction from the *pro-R* methylene group of ACPC in the fragmentation reaction. This proton is the one that undergoes partial conservation and internal transfer to C-3 of the keto acid product.

In order to resolve the problem, 2-ethyl-ACPC containing deuterium with known configuration at C-3 was prepared. Since the enzyme is known to process only the 1S,2S diastereomer and cleave with exclusive regiospecificity to yield the straight-chain keto acid product, incubation of 2-ethyl-[3-$^2H_1$]-ACPC (**31**) should reveal the stereochemistry of β-proton abstraction simply by analysis of the deuterium content at C-3 of the 2-ketohexanoate product (reduced in situ by enzymatic coupling to 2-hydroxy-

Scheme VIII



hexanoate).

This compound, **31**, with deuterium at C-3 cis to the 2-ethyl group, was prepared as shown in Scheme VIII. The relative cis configuration assigned relies on the stereochemical outcome of three reactions in the synthesis: (a) hydrogenation of **34** in the presence of Lindlar catalyst yields the cis double bond;[26] (b) the

(25) It is conceivable that if two bases were involved rather than one in the C-4 protonation step, namely, one on either side of a cisoid (or transoid) intermediate, the results could be accomodated by protonation of the β,γ-olefinic PLP-*p*-quinoid anion to different extents by the two bases.

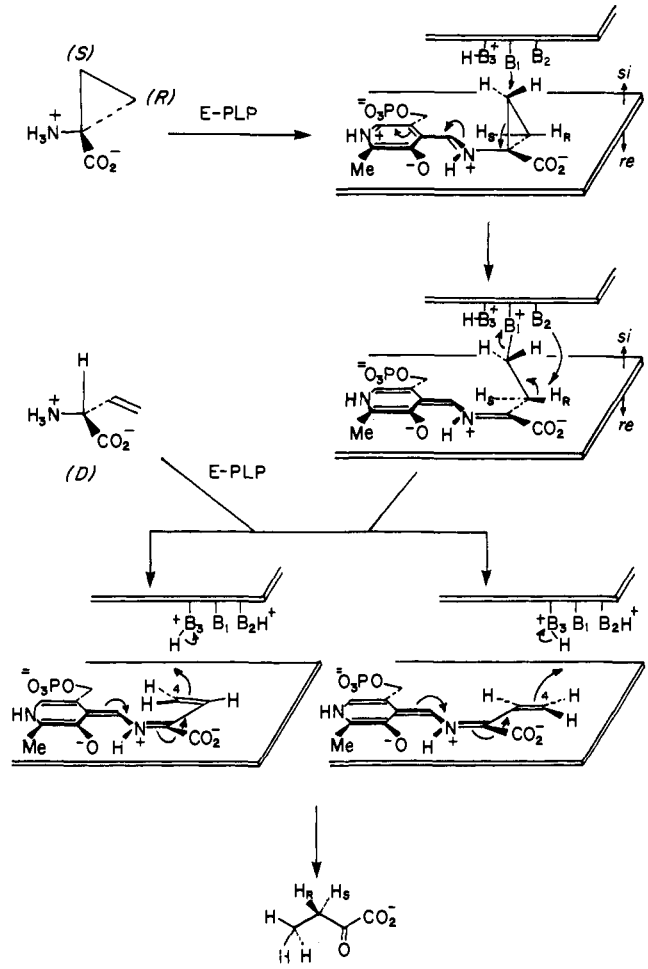
sulfur-mediated cyclopropanation results in retention of the original geometry of the olein;[27] and (c) methanolic ammonolysis selectively converts the less hindered ester group into an amide.[18] All three outcomes are precedented. Since in cyclopropane derivatives the J_{trans} is never larger than the J_{cis} in accord with the Karplus rule,[28] the cis configuration assignment is supported by the large vicinal coupling constant (8.0–10.2 Hz) observed between the 2H and 3H in each cyclopropane intermediate of Scheme VIII. Whereas, in the corresponding epimeric analogues, a smaller coupling constant of 6–8 Hz was generally observed. Nuclear Overhauser effect difference spectroscopy performed on compound **39** was also used to probe the through-space relationship between 2H and 3H. Irradiation at the frequency of the 3-H_{trans} proton (1.41 ppm) caused a 7% enhancement of the 2-H signal, while irradiation at the latter signal (2.45 ppm) produced an NOE of 6.2% on the 3-H resonance, clearly demonstrating through-space proximity of these two protons and thereby defining the orientation of ^2H at C-3 cis to ethyl and also cis to carboxyl, since the synthesis yields only the 1*S*,2*S* and 1*R*,2*R* diasteromers.

However, monodeuteration at C-3 was not complete; by proton NMR integration of the 3-H_{cis} signal relative to other well-spaced proton resonances in various intermediates, the deuterium content was estimated to be 75%. By mass spectroscopic analysis on compound **31** of Scheme VIII, this molecule was 78% monodeuterated and the remainder was undeuterated material.[29] The 22% residual protium at the C-3 *pro-R* locus did complicate the eventual product analysis.

First, undeuterated 2-ethyl-ACPC (**3**) was incubated with ACPC deaminase in ^2H_2O to evaluate the extent of internal return in the product of this six-carbon substrate. The resulting (2*S*)-2-hydroxyhexanoate phenacyl ester had a $3H_R$:$3H_S$ ratio of 46:54 and a 64% internal return of ^1H as estimated from its NMR integration (deuterium content at C-3 is 0.36 atoms). These data indicated the preferred cisoid conjugated *p*-quinoidal-PLP species and the nascent (*Z*)-2-enamine acid as well carry ca. 68% of the reaction flux. The very high percentage of internal β-hydrogen return (64% vs. 15% in ACPC itself) could reflect a more effective shielding of the responsible EBH^+ group by the sterically more bulky ethyl substituent. In any case the large internal return suggested that during the incubation of (1*S*,2*S*,3*R*)-2-ethyl-[3-$^2H_1$]-ACPC even if the deuteron were abstracted, very little might be lost to solvent and most would be returned to C-3 of the product.

In the event of the enzymatic conversion of the specifically labeled 2-ethyl-[3-$^2H_1$]-ACPC (**31**) in H_2O to completion (50% utilization of the racemate) with in situ coupled reduction to yield (2*S*)-2-hydroxyhexanoate, 1.3 hydrogens were found at C-3 by proton NMR analysis, in an *R*:*S* ratio of 35:65. Corresponding mass spectroscopy measurement indicated 65% of the hydroxyhexanoate phenacyl ester was monodeuterated. The loss of 16.7% ((78–65)/78) of the substrate deuterium content on conversion to product (78% → 65% monodeuterated) is small but real and consistent with the anticipated large amount of internal return. That cleavage occurs between the C_3–^2H bond, not the C_3–H bond, is further supported by the fact that C_3–H cleavage would have produced product with the initial 78% monodeuteration level. The

Scheme IX



amount of internal return, up from the 64% for H transfer, to 83% (65/78) for ^2H transfer follows a similar pattern noted in the case of ACPC itself, that internal H transfer is 15% while internal ^2H transfer is up to 27% (Table I). This interpretation would define the stereoselectivity of β-H abstraction from 2-ethyl-ACPC as *pro-R* proton removal. By analogy for ACPC itself, it is the H_R proton at the *pro-R* methylene group that is removed in the fragmentation.

It may be appropriate in the future to examine the β-H abstraction step by using unsubstituted ACPC to lower the possibility of β-H internal transfer. This will necessitate stereo- and regiospecific preparation of (2*R*)-[2-$^2H_1$]-ACPC and (2*S*)-[2-$^2H_1$]-ACPC, as yet unrealized.

Discussion

1-Aminocyclopropane-1-carboxylate deaminase is an unusual PLP enzyme able to fragment the cyclopropane ring of its substrate. The mechanism of this initial step is not yet defined, although we[8] and Honma[4] have noted that it can be construed as an intramolecular variant of the γ-elimination category of PLP enzymes. This bears formal analogy to the ACPC-forming reaction via a separate PLP-linked enzyme, ACPC synthase, which, in plants, can be written as an intramolecular γ-replacement reaction on the substrate *S*-adenosylmethionine. As such it is conceivable, but unproven, that ACPC deaminase catalysis proceeds by enzyme nucleophile addition to the cyclopropane–PLP aldimine complex followed by β-proton abstraction in the typical β,γ-elimination manifold as shown to yield the key intermediate, β,γ-olefinic fully conjugated PLP *p*-quinoid, which is also the common point of entry of (2*R*)-vinylglycine into the reaction flux (Scheme IX).

The stereochemical results presented here and in the earlier full paper[8] and communication[9] now permit a detailed evaluation of the stereochemical constraints on this enzyme-catalyzed process.

(26) ^1H NMR of the reduced olefinic product **35** exhibited a characteristic terminal H_{trans} doublet at δ 4.95 (J = 10.3 Hz). However, some residual H_{cis} resonance (δ 5.01, d, J = 17.7 Hz, ~25% as estimated by its NMR integration) was also observed. Since 78% of the amino acid **31** derived from this olefin had been shown by MS analysis to be monodeuterated and the rest, 22%, was undeuterated material, the presence of this minor species (~25% and should be undeuterated) should be due to the unexpected hydrogen exchange prior to reduction and not due to the lack of sterochemical control in the Lindlar reduction of terminal acetylenes (also see ref 29).

(27) Ohishi, J. *Synthesis* **1980**, *12*, 690.

(28) Jackman, L. M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry"; Pergamon Press: Oxford, New York, 1972; pp 286.

(29) The presence of unlabeled material in this sample was further confirmed by inverse-gated ^1H- and ^2H-decoupled ^{13}C NMR (D_2O, D1 = 5, PW = 5, RD = O and O2 = 4500), in which the C-3 signal (-CHD) appearing at 17.0 ppm was accompanied by a minor peak at 17.3 ppm (C-3 of $-CH_2$ species). The 0.28 ppm upfield shift of the $-CHD$ signal relative to $-CH_2$ peaks falls in the range of the α-shift typical for a monodeuterated species.

Several points are noteworthy. First, the enzyme has at least two and probably three active-site basic groups involved in proton transfers from substrates to intermediates and nascent products. The basic group shown as B_2 in Scheme IX abstracts the H_R proton from the *pro-R* methylene of the ACPC–PLP π-complex and later returns it suprafacially to the *si* face[10] of the predominant *Z* conformer of aminocrotonate in the active site. The enzymic base B_3, delivering a solvent proton to the *si* face[10] at C-4 of the β,γ-olefinic terminus of the *p*-quinoidal intermediate is also on the *si* face of the extended π-system. Finally the group B_1 is involved in proton abstraction at C-2 from (2*R*)-vinylglycine without any internal transfer to C-3 or C-4 of the 2-ketobutyrate product. Knowledge that the regiospecificity of ACPC cleavage is between C_α and the *pro-S* C_β and orientation of (2*R*)-vinylglycine is to place its carboxyl group in line with that of ACPC in the PLP substrate–coenzyme aldimine complex suggests that the B_1 group should also be on the *si* face since the α-H of (2*R*)-vinylglycine is exposed in that direction. (It is conceivable that B_1 and B_3 are identical and, thus, mandates *si* face disposition, but, then B_1 (=B_3) would have to be very flexible, e.g., Glu or Lys, to swing between C-2 and C-4 in order to conduct deprotonation at C-2 of (2*R*)-vinylglycine and protonation at C-4 of β,γ-olefinic PLP-quinoidal complex.) It appears then that all three bases are located on the *si* face of the substrate–PLP system and the protons involved in transformation are also located on the *si* face of the substrate–PLP system; the overall proton transfers are thus suprafacial. We have reached a similar conclusion for the bacterial enzyme cystathionine-γ-synthase which catalyzes both γ-elimination and γ-replacement of *O*-succinylhomoserine.[15] A second feature of ACPC deaminase catalysis, partial and suprafacial internal return of substrate hydrogen to product, was also seen with cystathionine-γ-synthase where up to 55% internal transfer of ^3H from C-2 of *O*-succinyl-(2*S*)-homoserine to C-3 of ketobutyrate ensues.[15] Since in the 2-ethyl-ACPC case 83% internal return is noted when deuterium is being transferred, this sequestering of active-site protons from bulk solvent may be a general feature of PLP enzymes that carry out transformations at substrate γ-carbons.

Several lines of stereochemical evidence point to the fact that one of the proposed intermediates, the fully conjugated β,γ-olefinic PLP-*p*-quinoid α-anion, can rotate around its C_α–C_β bond and also implicate that the cisoid conformer of this π-complex and thus the *Z* isomer of the nascent aminocrotonate carry most (70–78%) of the reaction flux in the experiments conducted in ^2H_2O, but less (52–60%) in H_2O. Our previous stereochemical studies on cystathionine-γ-synthase, again, indicated that the identical β,γ-olefinic *p*-quinoid-PLP species formed in the active site of that enzyme was also the cisoid isomer.[15] This consistence suggests the presence of similar preferential conformational forces in these two PLP γ-displacement enzymes.

A proposal for the overall stereochemical course of ACPC deamination in ^2H_2O is presented in Scheme X, showing an 80/20 split in ring fragmentation to cisoid and transoid forms of the β,γ-olefinic *p*-quinoid α-anion (these may or may not have *interconverted* to this 4/1 ratio), followed by progression with a 15% internal retention of the H_R from the *pro-R* methylene of ACPC on B_2H^+ in the active site. The cisoid β,γ-olefinic *p*-quinoid intermediate protonated on the *si* face gives the (*Z*)-aminocrotonate while the other 20% of the flux via the transoid β,γ-olefinic *p*-quinoid complex which is also protonated by the same enzyme base B_2H^+ produces the (*E*)-aminocrotonate. The distribution of deuterated ketobutyrates in such a manifold would be as follows: (a) all of the product molecules contain one deuterium at C-4 (prochiral unless an alkyl ACPC had been used wherein 80% would be 4*S* and 20% 4*R*); (b) 68% of the product molecules would have another deuterium at C-3 (3*R*-$^2H_1$) and 17% would also have the deuterium at C-3 but as 3*S*-^2H product. This scheme accounts for the observed 3-H_R/3-H_S product distribution of 0.32 H in the H_R locus and 0.83 H in the H_S locus. This distribution leads to the observed H_R/H_S ratio of 28/72 noted in Table I and $H_R + H_S$ = 1.15 H, both facts that have been verified by proton NMR.

Experimental Section

General Remarks. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer 283B spectrophotometer; polystyrene served as the reference and all bands were reported in wavenumbers (cm^{-1}). Ultraviolet spectra were recorded on a Perkin-Elmer 554 spectrophotometer. Circular dichroism measurements were performed on Professor Koji Nakanishi's Jasco J-40 spectropolarimeter (Columbia University) in methanol solutions. Mass spectra were obtained with a Varian MAT 212 spectrometer with a direct inlet probe. All mass spectra were recorded with an electron beam energy of 70 eV. Field-desorption mass spectra (FD-MS) were measured on a Varian MAT 731 mass spectrometer with an emitter current of 5 mA. ^1H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker WM-250 or Bruker WM-270 spectrometer. Chemical shifts were reported in parts per million on the δ scale relative to internal standards (tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate or appropriate solvent peaks). ^1H NMR spectra were usually run with 16K data points over a 4000-Hz range giving an acquisition time of 2.7 s and a relaxation delay of 4.5 s to ensure the complete relaxation of all protons. In NMR descriptions br = broad, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet, and *J* = coupling constant in Hz. Assignments labeled with * or + may be interchangeable. The high-performance liquid chromatography (HPLC) was performed on a Waters Model M-45 solvent delivery system equipped with a Waters 650 automatic gradient controller, a Waters 440 absorbance detector, a HP 3390 A integrator, and a Du Pont C_{18} column (25 cm × 4.6 mm). Flash chromatography was accomplished in columns of various diameters with Merck Sharp and Dohme (230–400 mesh) silica gel by elution with the solvents reported. Analytical and semipreparative thin-layer chromatography (TLC) were performed on Merck silica gel 60 G-254 plates (25 mm) and developed with the solvents mentioned. TLC spots were visualized either with UV light or by dipping into the staining solutions of either vanillin/ethanol/H_2SO_4 (1:98:1) or iodoplatinate (10 mL of 5% aqueous platinum chloride, 5 mL of HCl, 240 mL of 2% aqueous KI) or phosphomolybdic acid (7% EtOH solution) and then heating. Solvents, unless otherwise specified, were reagent grade and distilled once prior to use.

Enzymes. 1-Aminocyclopropane-1-carboxylic acid deaminase, isolated from *Pseudomonas sp.* ACP, was purified to homogeneity according to the procedure by Honma and Shimomura.[4] This enzyme (M_r = 110000) consists of three identical subunits exhibiting a characteristic pyridoxaldimine absorbance at 418 nm. Homogeneous preparations typically display an A_{278}/A_{415} ratio of 6 to ~7. L-Lactate dehydrogenase (rabbit skeletal muscle) and catalase (canine liver) were purchased from Boehringer-Mannheim and Sigma, respectively. Amino acid racemase (*Pseudomonas striata*) was a generous gift of Dr. Kenji Soda (Kyoto University).

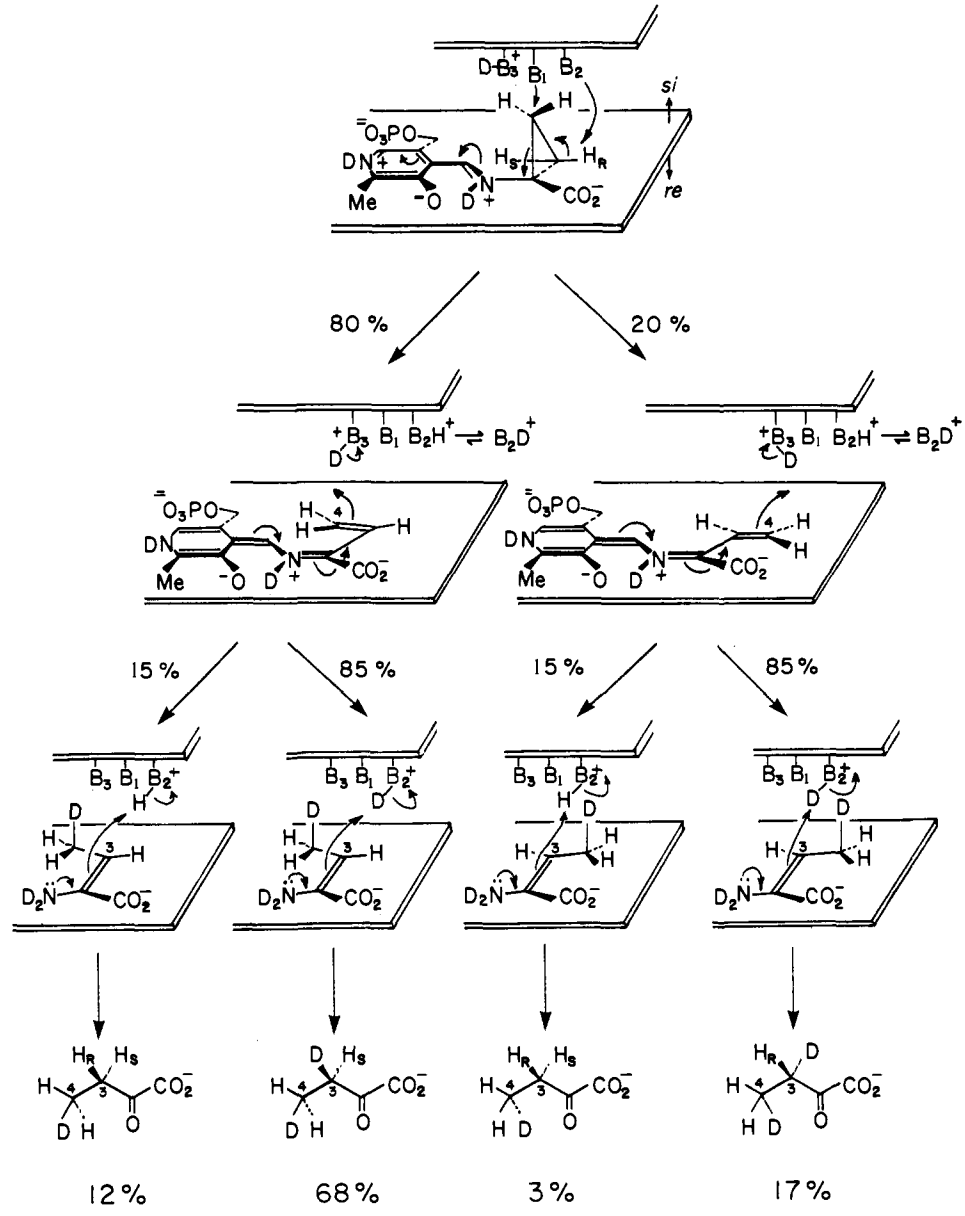
Enzyme Assays. The continuous assay of α-keto acid formation from substrate ACPC and its analogues was accomplished by in situ reduction of the keto acid product to the corresponding L-hydroxy acid in the presence of NADH and L-lactate dehydrogenase. A standard 1.0-mL assay, as described before,[8] was performed in potassium pyrophosphate buffer (50 mM, pH 8.5) at 37 °C, containing substrate, 10–20 μg of ACPC deaminase, 300 μg of lactate dehydrogenase, and 210 μg of NADH. Loss of NADH absorbance at 340 nm was monitored.

Preparation of Substrates. (1) 1-Aminocyclopropane-1-carboxylate (1) was prepared in a manner analogous to that used in the synthesis of [2,2,3,3-$^2H_4$]-ACPC (**4**) as described below in paragraph 3, mp 234–236 °C [lit. mp 254 °C]. Anal. Calcd for $C_4H_7NO_2$: C, 47.52; H, 6.98; N, 13.85. Found: C, 47.51; H, 7.15; N, 13.77.

(2) 1-Amino-2-ethylcyclopropane-1-carboxylate (2-Et-ACPC) (**3**) was synthesized according to the reported procedure.[18]

(3) Synthesis of 1-Amino[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylate (4). Ethyl 1-Cyano[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylate. A hot solution of 1.1 g of sodium (48 mmol) in 20 mL of absolute ethanol was stirring during the addition in a thin stream from a separatory funnel of 5.4 g (48 mmol, 5.1 mL) of ethyl cyanoacetate. This mixture was then added dropwise over ca. 15 min into a solution of 9.6 g (50 mmol, 4.4 mL) of [1,1,2,2,-$^2H_4$]-1,2-dibromoethane in 20 mL of ethanol. Another 1.1 g of sodium in 20 mL of ethanol was added immediately afterward. Stirring and refluxing were continued both during the addition and for another 2 h. The reaction was then allowed to proceed overnight (16 h) at room temperature. The white precipitate was removed by filtration and the burgundy red filtrate was concentrated under reduced pressure. The thick slurry was taken up in diethyl ether and washed with water. The aqueous fraction was separated and extracted with ether several times. The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. The resulting red oil was purified by vacuum distillation (0.1 mmHg, ca. 85 °C) to give a colorless liquid (1.68 g) in 24% yield. ^1H NMR ($CDCl_3$) δ 4.20 (2 H, q, 7.0,

Scheme X



$H_R / H_S = 0.32H / 0.83H = \boxed{22/78}$

$H_R + H_S = \boxed{1.15H}$

OCH_2), 1.35 (3 H, t, 7.0, Me); IR (neat, thin film) 2240 (C≡N), 1735 cm^{-1} (C=O, ester).

1-Carboxy[2,2,3,3-$^2H_4$]cyclopropane-1-carboxamide. The cyanocarboxylic ester was stirred in 6 mL of 20% NaOH solution at 70 to ~90 °C for 1.5 h during which time the reaction mixture became a homogeneous, light red solution. This solution was cooled to room temperature and treated with 4 mL of 30% H_2O_2 solution in small portions. Vigorous gas evolution was observed. After it was stirred at 70 °C for 30 min, the reaction mixture was cooled to room temperature and the pH of this solution was adjusted to 2 with concentrated HCl and glacial acetic acid. The desired product precipitated as a white solid which was collected and recrystallized from hot water. Yield 83%; mp 190–191 °C.

1-Amino[2,2,3,3-$^2H_4$]cyclopropane-1-carboxylate. A suspension of amido ester (1.0 g, 7.5 mmol) in 4 mL of water was chilled to 0 °C and treated sequentially with bromine (1.3 g, 0.43 mL, 8.4 mmol) and an ice-cold solution of NaOH (1.5 g) in 6 mL of water. The reddish-yellow reaction mixture became homogeneous and gas evolution continued for ca. 1 min. After it was stirred at 0 °C for an additional 5 min, the reaction mixture was treated with a solution of 0.6 g of NaOH in 5 mL water and allowed to stand at 70 °C (ca. 14 h). Additional gas evolution was observed after warming and continued for 10 min. The reaction mixture was neutralized to pH 7 with oxalic acid. The white precipitate was removed by filtration and the filtrate was applied to a Dowex-50 (H^+) column (1.5 × 8 cm). The initial aqueous washing was discarded and the subsequent NH_4OH (2 ~3 N) eluate was collected and concentrated in vacuo. The solid residue was crystallized from aqueous ethanol to give fine white crystals of **4** (mp 257–258 °C) in 26% yield. MS (HFB/isobutyl ester derivative), *m/e* 357 (M^+), 301 $(M - 56)^+$. Anal. Calcd for $C_4{}^2H_4H_3NO_2$: C, 45.67; H and ^2H, 10.55; N, 13.32. Found: C, 45.51; H and ^2H, 10.76; N, 13.71.

(4) Synthesis of 1-Amino[2,2,-$^2H_2$]cyclopropane-1-carboxylate (5). 3-Acetamido-3-carbomethoxy[5,5-$^2H_2$]pyrazolin (7). To a mixture of 5.6 g of 30% NaOD in D_2O, 16.7 g of carbitol-*d*, and 10 mL of anhydrous ether maintained at 70 °C was added dropwise a solution of 4.5 g of Diazald in 40 mL of anhydrous ether over a period of 1 h. The resulting [$^2H_2$]diazomethane was distilled into a test tube containing 10 mL of anhydrous ether and chilled at 0 °C. Two portions of 10 mL of ether were added to rinse out the distillation apparatus, and distillation was continued until the yellow color in the receiving tube grew no more intense. The yellow [$^2H_2$]diazomethane solution was then treated with

a solution of methyl 2-acetamidoacrylate[11] (**6**) (700 mg, 5 mmol) in 1 mL of methylene chloride. After standing at room temperature for 10 h, the yellow reaction mixture was quenched with 2 drops of formic acid. The cloudy colorless solution was concentrated under a stream of nitrogen with gentle heating. Without further purification, the crude product which gave one major spot on TLC (silica gel, 10% MeOH/$CHCl_3$, R_f 0.53) was used directly in the next reaction. ^1H NMR ($CDCl_3$) δ 3.81 (3 H, s, OMe), 2.05 (3 H, s, OAc), 1.31 (2 H, bs, $-CH_2$).

Methyl 1-Acetamido[2,2,-$^2H_2$]cyclopropane-1-carboxylate (8). Pyrolysis of the crude product **7** obtained from the preceding reaction was carried out in a test tube without adding cosolvent at 90–95 °C for 2 h. Evolution of nitrogen gas was noted during this period. The crude reaction mixture was purified by column chromatography (silica gel, 1% MeOH/$CHCl_3$) to afford the desired product which was recrystallized from chloroform/hexane (mp 86–87.5 °C). Total yield for these two steps was 13%. ^1H NMR ($CDCl_3$) δ 6.31 (1 H, brs, NH), 3.70 (3 H, s, OMe), 2.00 (3 H, s, OAc), 1.59 (2 H, s, 3-H's); MS, *m/e* 159 (M^+), 127 $(M - 32)^+$, 117 $(M - 42)^+$, 102 $(M - 57)^+$.

1-Amino[2,2,-$^2H_2$]cyclopropane-1-carboxylate (5). Compound **8** was refluxed in 2 mL of 4 N HCl solution for 4 h. The reaction mixture was concentrated under a stream of nitrogen gas with heating to a moist solid. The crude product was loaded onto a pipette column of Dowex-50 (H^+) cation exchange resin (1.5 mL) and, after being washed with water, was eluted with 2N aqueous ammonia solution. The ammonium hydroxide eluate was collected and concentrated. The solid residue was recrystallized from hot ethanol to yield the desired compound as white needles (mp 265–266 °C dec). Anal. Calcd for $C_4{}^2H_2H_5NO_2$: C, 46.59; H and ^2H, 8.80; N, 13.58. Found: C, 45.92; H and ^2H, 9.01; N, 12.98.

(5) *DL*-[2-$^2H_1$]Vinylglycine (9) was prepared by incubation of vinyl glycine with amino acid racemase (*Pseudomonas striata*) in deuterated 2-(cyclohexylamino)ethanesulfonic acid (CHES) buffer (100 mM, pD 8.9) at room temperature for 15 h. The reaction mixture was transferred to a Dowex-50 (H^+) column (1.5 × 7 cm) and the column was washed with water (30 mL) followed by 1 N NH_4OH (30 mL). A light yellow solid was obtained upon removal of aqueous ammonia; mp 234 °C dec. Complete replacement of the α-H with ^2H was demonstrated by the disappearance of the NMR signal at 4.29 ppm (d, 7.3) characteristic for α-H in vinylglycine. ^1H NMR (D_2O) δ 6.05 (1 H, dd, 17.0, 10.5, 3-H), 5.55 (1 H, d, 17.0, H_E), 5.54 (1 H, d, 10.5, H_Z).

(6) Synthesis of 1-Amino-2-methylcyclopropane-1-carboxylate (2-Me-ACPC) (10). Diethyl Ethylidenemalonate (13). A mixture of 50 g (0.31 mol) of ethyl malonate, 50 g (0.49 mol) of acetic anhydride, and 28.5 g (0.65 mol) of acetaldehyde, enclosed in a hydrogenation bomb, was mixed and heated at 100 °C for 20 h without shaking. The bomb content was transferred with the aid of benzene and was distilled through a Vigreux column under atmospheric pressure to remove diethyl malonate and ethylidene diacetate until the temperature of the vapor reached 140 °C. The residual solution was further distilled under diminished pressure to afford compound **13** as a colorless liquid in 79.5% yield; bp 100–106 °C (10 mm) [lit. bp 115–118 °C (17 mm)]. ^1H NMR ($CDCl_3$) δ 7.08 (1 H, q. 7.3, olefinic H), 4.30 (2 H, q, 7.3, OCH_2), 4.23 (2 H, q, 6.8, OCH_2), 1.95 (3 H, d, 7.3, olefinic Me), 1.32 (3 H, t, 7.3, Me), 1.28 (3 H, t, 6.8, Me).

Diethyl 2-Methylcyclopropane-1,1-dicarboxylate (2). To a dry mixture of 9.6 g (0.044 mol) of trimethyloxosulfonium iodide (prepared from dimethyl sulfoxide and methyl iodide)[17] and 2.12 g (0.044 mol, 57% in oil dispersion) of sodium sulfoxide. After frothing ceased, the grey-white suspension was cooled to 15 °C, and to this mixture was added with stirring a solution of 7.4 g (0.04 mol) of diethyl ethylidenemalonate (**13**) in 25 mL of dimethyl sulfoxide in one portion. The stirring was continued for 10 min at 15 °C, overnight at room temperature and 4 h at 50–60 °C. After it was cooled and 400 mL of ice–water was added to it, the mixture was extracted with ether, and the combined ether fractions were washed with brine, dried over anhydrous sodium sulfate, and evaporated to give a pale yellow oil which was purified by flash chromatography (silica gel 160 g, benzene) to afford compound **14** in 86% yield. ^1H NMR ($CDCl_3$) δ 4.16 to ~4.00 (4 H, m, 2X OCH_2), 1.79 (1 H, tq, 8.3, 6.4, 2-H), 1.24 (2 H, m, 3-H's), 1.17 (3 H, t, 6.8, Me), 1.13 (3 H, t, 6.8, Me), 0.98 (3 H, d, 6.4, 2-Me); ^{13}C NMR ($CDCl_3$) 169.3 (C=O), 166.9 (C=O), 60.1 (2X OCH_2), 33.4 (C-1), 21.2 (C-2), 20.6 (C-3), 13.1 (2X Me), 12.4 (Me).

1-Methoxycarbonyl-2-methylcyclopropane-1-carboxamide (15). Compound **14** (1.91 g, 9.55 mmol) was dissolved in a solution of ammonia in methanol (20 mL of a solution saturated at 0 °C) containing catalytic amounts (20 mg) of sodium methoxide. The reaction mixture was kept stirring at room temperature for 5 days. Excess solvent was evaporated in vacuo and the crude product was resuspended in water followed by extraction into chloroform solution, which, after concentration and purification by flash chromatography (silica gel 110 g, $CHCl_3$), afforded 81% yield of compound **15**, mp 75–76 °C. ^1H NMR ($CDCl_3$) δ 7.96 (1 H, brs, NH), 6.71 (1 H, brs, NH), 3.65 (3 H, s, OMe), 1.83 (1 H, m, 2-H), 1.75 (1 H, dd, 7.7, 3.9, 3-H), 1.47 (1 H, dd, 7.3, 3.9, 3-H), 1.11 (3 H, d, 5.9, 2-Me); ^{13}C NMR ($CDCl_3$) 171.5 (C=O), 171.0 (C=O), 51.5 (OMe), 32.4 (C-1), 28.3 (C-2), 22.3 (C-3), 12.4 (2-Me).

Methyl 1-Methoxycarbonylamino-2-methylcyclopropane-1-carboxylate (16). To a solution of sodium methoxide in methanol (7 mL) prepared from 345 mg (15 mmol) of sodium was added, dropwise with vigorous stirring, 800 mg (5 mmol) of bromine at −45 °C.[30] After all the bromine color was discharged, a solution of 785 mg (5 mmol) of compound **15** in 5 mL of methanol was added slowly while maintaining the temperature at ca. −45 °C. The temperature was then raised to room temperature and the clear solution was further heated to 55 °C. After 30 min, the flask was cooled to room temperature, and the solution was made slightly acidic with acetic acid. All solvent was removed under reduced pressure. The white residue was mixed with water and extracted with ether. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated. The residual syrup was purified by flash chromatography (silica gel 100 g, $CHCl_3$) to afford compound **16** in 35% yield. ^1H NMR ($CDCl_3$) δ 5.47 (1 H, brs, NH) 3.69 (3 H, s, OMe), 3.65 (3 H, s, OMe), 1.53 (1 H, m, 2-H), 1.49 (1 H, m, 3-H), 1.28 (1 H, m, 3-H), 1.21 (3 H, d, 6.4, 2-Me); ^{13}C NMR ($CDCl_3$) 172.0 (O—C=O), 157.3 (N—C=O), 52.2 (2X OMe), 39.1 (C-1), 26.2 (C-2), 24.1 (C-3), 12.0 (2-Me).

Methyl 1-Amino-2-methylcyclopropane-1-carboxylate (17). To a solution of 104 mg (0.56 mmol) of the carbamate ester **16** in 10 mL of chloroform, sealed in a reaction vessel under argon, was added dropwise 280 mg (1.40 mmol) of trimethylsilyl iodide with stirring.[31] The reaction was then heated to 50 to ~60 °C and the progress of the reaction was monitored by ^1H NMR spectroscopy. When the reaction was complete (usually within 1 to ~2 h), 3 to ~4 equiv of methanol were added and the volatile components were removed under reduced pressure. The brown residue was taken up in chloroform and extracted with water. The combined aqueous fractions which contained most of the protonated compound **17** were washed with chloroform and concentrated in vacuo. The desired product was isolated in 85% yield. ^1H NMR (D_2O) δ 3.84 (3 H, s, OMe), 1.80 (1 H, m, 2-H), 1.57 (1 H, m, 3-H), 1.52 (1 H, m, 3-H), 1.24 (3 H, d, 6.8, 2-Me).

1-Amino-2-methylcyclopropane-1-carboxylate (10). To a solution of compound **17** (130 mg, 1 mmol) in 5 mL of tetramethylene sulfone was added 400 mg (2 mmol) of trimethylsilyl iodide. This mixture was allowed to react for 2 days at 50–60 °C. The reaction was quenched with water and the product was purified by Dowex-50 (H^+) column chromatography (0.8 × 5 cm) by elution with 1 N NH_4OH after a water wash. Compound **10** was isolated after recrystallization from hot ethanol/H_2O as white needles in 39% yield. ^1H NMR (D_2O) δ 1.52 (1 H, m, 2-H), 1.33 to ~1.24 (2 H, m, 3-H's), 1.19 (3 H, d, 5.9, 2-Me); ^{13}C NMR (D_2O) 173.9 (C=O), 40.2 (C-1), 19.7 (C-2), 17.5 (C-3), 11.1 (2-Me). Compound **10** could also be prepared by dissolving and refluxing of compound **17** in a 1 N NaOH solution for 4 h. The resulting light yellow solution was cooled and loaded onto a Dowex-50 (H^+) column. The column was eluted with water followed by 2 N aqueous ammonia. The second eluate (NH_4OH) was concentrated in vacuo to afford the desired amino acid. Yield, 55%; mp 183–184 °C (dec). Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.93; H, 7.72; N, 12.10.

(7) (*Z*)-*DL*-[4-$^2H_1$]- (11) and (*E*)-*DL*-[3,4-$^2H_2$]vinylglycine (12), prepared as previously described,[15] were gifts of Dr. M. N. T. Chang.

(8) Synthesis of (3*S*)-[3-$^2H_1$]Butyric Acid (21). (3*R*)-Methyl 3-(*p*-Toluenesulfonyl)butyrate (25). To a pyridine solution (5 mL) of (*R*)-(−)-methyl 3-hydroxybutyrate (**23**)[32] (2.36 g, 20 mmol) was added *p*-toluenesulfonyl chloride (4.19 g, 22 mmol), and the resulting reaction mixture was kept stirring at room temperature overnight. The white precipitate was removed and the filtrate was concentrated under reduced pressure. Purified by flash chromatography (silica gel 65 g, benzene), the tosylate ester was isolated in 82.4% yield (mp 48–49 °C). ^1H NMR ($CDCl_3$) δ 7.80 (2 H, d, 8.2, aromatic H's), 7.35 (2 H, d, 8.2, aromatic H's), 4.98 (1 H, m, 3-H), 3.60 (3 H, s, OMe), 2.75 (1 H, dd, 15.9, 6.5, 2-H), 2.53 (1 H, dd, 15.9, 6.6, 2-H), 2.46 (3 H, s, tosyl Me), 1.36 (3 H, d, 6.0, Me); ^{13}C NMR ($CDCl_3$) 168.9 (C=O), 144.2 (subs-C), 133.4 (*p*-C), 129.2 (*o*-C), 127.0 (*m*-C), 75.4 (C-3), 51.0 (OMe), 40.5 (C-2), 20.7 (tosyl Me), 20.1 (Me).

(3*S*)-[1,1,3-$^2H_3$]Butanol 27. A solution of (3*R*)-methyl-3-tosylbutyrate (**25**) (4.35 g, 16 mmol) in 10 mL of dry THF was added slowly with vigorous stirring to a suspension of lithium aluminum deuteride (626 mg, 14.9 mmol) in 20 mL of dry THF at 0 °C. When the addition was

(30) Radlick, P.; Brown, L. R. *Synthesis* **1974**, 290.
(31) Jung, M. E.; Lyster, M. A. *J. Chem. Soc. Chem. Commun.* **1978**, 315.
(32) Both compounds **23** and **24** were purchased from Aldrich.

complete and the initial reaction subsided, the grey milky slurry was refluxed for 3 h. The excess hydride was decomposed by addition of 1.34 mL of ethyl acetate slowly with stirring. This was followed by 16 mL of 6 N HCl. An ice-water bath was used to maintain the temperature below 35 °C. The mixture was diluted with saturated NH_4Cl solution and extracted with ether several times. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo at room temperature to afford the desired alcohol in 76% yield. ^1H NMR ($CDCl_3$) δ 1.52 (2 H, d, 7.4, 2-H's), 1.36 (1 H, m, 3-H), 0.91 (3 H, d, 7.3, Me).

(3*S*)-[3-$^2H_1$]Butyric Acid (21). To an acetone solution (20 mL) of (3*S*)-[1,1,3-$^2H_3$]butanol (**27**) (0.77 g, 10 mmol) was added dropwise chromic oxidizing reagent prepared from chromium trioxide (1.34 g), sulfuric acid (1.2 mL), and H_2O (2.5 mL). A considerable amount of green precipitate was formed and the addition was continued until the orange color of the oxidizing reagent persisted for ca. 20 min. Temperature was controlled under 35 °C during the course of oxidation and excess of oxidizing reagent was titrated back with a few drops of isopropyl alcohol. The reaction mixture was filtered and concentrated at room temperature. The residual green oil was diluted with saturated NaCl solution and extracted with ether which, after concentration by means of a gentle stream of nitrogen at ice-chilled temperature, yielded the deuterated butyric acid as a light yellow oil. ^1H NMR ($CDCl_3$) δ 2.31 (2 H, d, 7.6, 2-H's), 1.57 (1 H, m, 3-H), 0.91 (3 H, d, 7.9, Me).

(3*S*)-Naphthoyl [3-$^2H_1$]Butyrate (29). A mixture of (3*S*)-[3-$^2H_1$]-butyric acid (**21**) (89 mg, 1 mmol), 2-naphthol (288 mg, 2 mmol), and a catalytic amount of (*N*,*N*-dimethylamino)pyridine (6 mg, 0.049 mmol) was stirred in 2 mL of dry DMF for 10 min. To this solution was added *N*,*N*′-dicyclohexylcarbodiimide (206 mg, 1 mmol) at 0 °C, and the reaction mixture was kept stirring at room temperature for 3 h. Diluted with ether and quenched with water, the reaction mixture was extracted with ether several times. The combined organic extracts were dried ($MgSO_4$), concentrated, and separated by flash chromatography (silica gel 6 g, 5% EtOAc/benzene). Further purification performed by HPLC (C_{18} column, 30% H_2O/MeOH) afforded the analytically pure sample suitable for CD measurement. ^1H NMR ($CDCl_3$) δ 7.87 to ~7.21 (7 H, m, aromatic H's), 2.40 (2 H, d, 7.3, 2-H's), 1.81 (1 H, m, 3-H), 1.07 (3 H, d, 7.4, Me); CD (MeOH) $\Delta\epsilon_{276}$ −0.48.

(9) Synthesis of (3*R*)-[3-$^2H_1$]Butyric Acid (22) and Its Naphthoyl Ester (30). Conversion of (*S*)-(+)-Methyl-3-hydroxy butyrate (**32**)[32] into (3*R*)-[3-$^2H_1$]butyric acid (**22**) was accomplished by the same methodology employed in the synthesis of its 3*S* analogue. The spectroscopic data of each intermediate and the final product are listed below.

(3*S*)-Methyl 3-(*p*-Toluenesulfonyl)butyrate (26) (mp 48–49 °C): ^1H NMR ($CDCl_3$) δ 7.79 (2 H, d, 8.4, aromatic H's), 7.35 (2 H, d, 8.4, aromatic H's), 4.96 (1 H, m, 3-H), 3.58 (3 H, s, OMe), 2.73 (1 H, dd, 15.9, 6.6, 2-H), 2.53 (1 H, dd, 15.9, 6.5, 2-H), 2.44 (3 H, s, tosyl Me), 1.35 (3 H, d, 6.1, Me); ^{13}C NMR ($CDCl_3$) 169.5 (C=O), 144.6 (subs-C), 133.6 (*p*-C), 129.6 (*o*-C), 127.5 (*m*-C), 75.6 (C-3), 51.6 (OMe), 41.0 (C-2), 21.4 (tosyl Me), 20.7 (Me).

(3*R*)-[1,1,3-$^2H_3$]Butanol (28): ^1H NMR ($CDCl_3$) 1.51 (2 H, d, 7.4, 2-H's), 1.35 (1 H, m, 3-H), 0.90 (3 H, d, 6.7, Me).

(3*R*)-[3-$^2H_1$]Butyric Acid (22): ^1H NMR (CD_3OD) δ 2.25 (2 H, d, 7.4, 2-H's), 1.58 (1 H, m, 3-H), 0.94 (3 H, d, 7.4, Me).

(3*R*)-Naphthoyl [3-$^2H_1$]Butyrate (30): ^1H NMR ($CDCl_3$) δ 7.85 to ~7.21 (7 H, m, aromatic H's), 2.26 (2 H, d, 7.3, 2-H's), 1.81 (1 H, m, 3-H), 1.07 (3 H; d, 7.4, Me); CD (MeOH) $\Delta\epsilon_{276}$ +0.46.

(10) Synthesis of 1-Amino-2-ethyl[3-$^2H_1$]cyclopropane-1-carboxylate (31). 2-(But-3′-yn-1′-yloxy)tetrahydropyran (33). Phosphorus oxychloride (1.43 g, 9.33 mmol) was added to a mixture of 3-butyn-1-ol (150 g, 2.14 mol) and 2,3-dihydropyran (210 g, 2.5 mol). The mixture rapidly became warm and was cooled in an ice bath to moderate the reaction. After 2 h at room temperature, potassium hydroxide solution was added and the product was extracted into ether. The combined ether fractions were washed with brine, dried with anhydrous magnesium sulfate, and concentrated. Purification by distillation afforded a colorless liquid; yield 95.3%. ^1H NMR ($CDCl_3$) δ 4.49 (1 H, dd, 3.4, 3.1, ketalic H), 3.76–3.61 (2 H, m, ring OC*H*, side-chain OC*H*), 3.44–3.33 (2 H, m, ring OC*H*, side-chain OC*H*), 2.33 (1 H, dd, 7.2, 6.9, —C*H*—C≡), 2.32 (1 H, dd, 7.0, 6.9, —C*H*—C≡), 1.87 (1 H, t, 2.6, *H*—C≡), 1.70–1.39 (6 H, m, ring CH_2's); ^{13}C NMR ($CDCl_3$) 98.0 (d, ketalic C), 80.8 (s, C—*C*≡C), 68.9 (d, H—*C*≡C), 64.9 (t, side-chain OCH_2), 61.3 (t, ring OCH_2), 19.4 (t, C≡C—*C*), 30.0 (t), 25.0 (t), and 18.8 (t) (all ring CH_2's).

[4′-$^2H_1$]-2-(But-3′-yn-1′-yloxy)tetrahydropyran (34). 2-(But-3′-yn-1′-yloxy)tetrahydropyran (**33**) (314 g, 2.04 mol) was added dropwise to a solution of *n*-butyllithium (1.14 L, 2.4 mol, 2.1 M solution in hexane) in anhydrous ether (500 mL) at 0 °C. A gel formed gradually upon addition of the acetylenic THP ether at this temperature, but slowly liquified to a homogeneous, viscous solution after stirring at room temperature for a few hours (2–3 h). The resulting light yellow solution was kept stirring at room temperature overnight and then chilled with an ice bath and quenched with D_2O (99.8% of D, 600 mL). The ether layer was separated and the aqueous fraction was extracted with anhydrous ether several times. The organic extracts were combined and dried over anhydrous $MgSO_4$. Concentration followed by distillation under reduced pressure afforded 64% yield of the deutered product. Deuterium content was greater than 95% as revealed by proton NMR. ^1H NMR ($CDCl_3$) δ 4.55 (1 H, dd, 3.4, 3.1, ketalic H), 3.82–3.68 (2 H, m, ring OC*H*, side-chain OC*H*), 3.50–3.37 (2 H, m, ring OC*H*, side-chain OC*H*), 2.38 (2 H, dd, 7.1, 7.0, CH_2—C≡), 1.75–1.36 (6 H, m, ring CH_2's); ^{13}C NMR ($CDCl_3$) 98.4 (ketalic C), 80.6 (t, J = 6.6 Hz, C—*C*≡C), 68.8 (t, J = 38.0 Hz, D—*C*≡C), 65.2 (side-chain OCH_2), 61.8 (ring OCH_2), 19.6 (C=C—*C*), 30.2, 25.2, 19.0 (all ring CH_2's); MS(EI) 115, 101, 85, 67, 56, 41.

[4′-$^2H_1$]-2-(But-3′-en-1′-yloxy)tetrahydropyran (35). Compound **34** (158 g, 1.02 mol) obtained from the preceding experiment was reduced to the corresponding olefin in four batches. Each time 39.5 g (0.255 mol) of the acetylenic deuterated THP ether was mixed with freshly distilled quinoline (0.53 mL) and Lindlar catalysts (0.93 g) in ethyl acetate (250 mL). Hydrogenation was carried out at 4.06 atm (45 psi) at room temperature in a Parr Hydrogenator. The hydrogen uptake was stopped after 25 min (at 25 psi), at which point 1 equiv had been consumed. The catalyst was filtered off through a thick pad of Celite and the light yellow filtrates from the four batches were combined and concentrated. The desired product was isolated as a colorless oil by vacuum distillation. The yield was 87.9%. However, on the basis of ^1H NMR integration, the deuterium content of this compound was only 75%. ^1H NMR ($CDCl_3$) δ 5.75 (1 H, m, DHC=C*H*R), 5.01 (0.25 H, d, 17.7, *c*-*H*HC=C*H*R), 4.95 (1 H, d, 10.3, *t*-D*H*C=CHR), 4.53 (1 H, dd, 3.7, 3.1, ketalic H), 3.84–3.67 (2 H, m, ring OC*H*, side-chain OC*H*), 3.47–3.33 (2 H, m, ring OC*H*, side-chain OC*H*), 2.30 (1 H, t, 6.8, allylic H), 2.27 (1 H, t, 6.8, allylic H), 1.81–1.44 (6 H, m, ring CH_2); ^{13}C NMR ($CDCl_3$) 134.9 (=*C*—R), 115.7 (t, J = 23.9 Hz, D—*C*=C), 98.4 (ketalic C), 66.5 (side-chain OCH_2), 61.8 (ring OCH_2), 25.3 (allylic C), 33.9, 30.4, and 19.2 (all ring CH_2's); MS(EI) 115, 101, 85, 67, 56, 41.

Diethyl 2-(Tetrahydropyranyl)hydroxyethyl[3-$^2H_1$]cyclopropane-1,1-dicarboxylate (36). To a solution of dimethyl disulfide (39.6 mL, 0.439 mol) in dry methylene chloride (250 mL) was added sulfuryl chloride (35.3 mL, 0.439 mol) at −78 °C. After being stirred for 10 min, the deuterated 3-buten-1-ol THP ether **35** (138 g, 0.879 mol) was added dropwise to this yellow solution at −78 °C. Stirring was continued for another 10 min, and then the solvent and sulfur dioxide were removed under reduced pressure at room temperature. The residual brown oil was transferred by the aid of a small amount of anhydrous ether to a solution of diethyl sodiomalonate which was prepared from diethyl malonate (154.9 g, 0.967 mol) and sodium ethoxide (0.967 mol, 22.2 g of sodium) in dry metehanol (880 mL), and the mixture was heated under reflux for 4 h. After removal of the solvent from this mixture, the residual brown oil together with a considerable amount of white precipitate were suspended in water and extracted with ether. The combined ether extracts were washed with calcium chloride solution, dried over anhydrous $MgSO_4$, and concentrated in vacuo. TLC of this crude product revealed the presence of one major sulfur-containing component which gave the characteristic color (white) upon treatment with iodoplatinate solution. To this crude material was added dimethyl sulfate (82.5 mL, 0.872 mol) at 0 °C, and the resulting mixture was kept stirring at room temperature for 36 h. Sodium ethoxide (1.76 mol, 40.5 g of sodium and 1000 mL of dry ethanol) solution was added to the resulting viscous dark brown syrup, and the mixture was refluxed for 20 min. The solvent was removed and the residue was extracted with ether, washed with calcium chloride solution, dried over anhydrous $MgSO_4$, and concentrated. The brown oil was then purified by flash chromatography (three times with silica gel 200 g, 20% EtOAc/petroleum ether) to give the desired product in 36.4% yield. ^{13}C NMR ($CDCl_3$) 169.3 (C=O), 167.2 (C=O), 97.5 (ketalic C), 65.4 and 65.2 (side-chain OCH_2), 61.0* and 60.8* (OCH_2's), 60.4* (ring OCH_2), 33.0 (C-1), 29.9 (ring CH_2), 28.2 (side-chain *C*-C-O), 24.9$^+$ (C-2), 24.4$^+$ (ring CH_2), 19.7 (t, C-3), 18.6 (ring CH_2), 13.3 (Me's).

Diethyl 2-(Hydroxyethyl)[3-$^2H_1$]cyclopropane-1,1-dicarboxylate (37). A solution of 49.4 g (0.157 mol) of the THP ether **36** and 7.5 g (0.03 mol) of pyridinium toluenesulfonate (PPTS) in 500 mL of methanol was allowed to stand at room temperature under argon. The progress of the reaction was monitored by TLC. After completion, methanol was removed under reduced pressure at room temperature, and the residual light brown oil was purified by flash chromatography (twice with silica gel 160 g, 1.5% MeOH/$CHCl_3$). Compound **37** was isolated in 71.5% yield as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 4.15 (4 H, m, OCH_2's), 3.67 (2 H, t, 6.3, -CH_2OH), 2.52 (1 H, br s, O*H*), 1.93 (1 H, m, 2-H), 1.64 (1 H, m, C*H*-C-O), 1.48 (1 H, m, C*H*-C-O), 1.37 (1 H, d, 9.0,

3-H_S), 1.33 (>0.3 H, 3-H_R), 1.25 (3 H, *t*, 7.1, Me), 1.22 (3 H, t, 7.1, Me); ^{13}C NMR ($CDCl_3$) 170.6 (s, C=O), 168.2 (s, C=O), 61.6 and 61.4 (t, OCH_2's), 33.7 (s, C-1), 31.6 (t, *C*-C-O), 25.0 (d, C-2), 20.0 (t, J = 25.3, C-3), 14.0 and 13.9 (q, Me's).

Diethyl 2-(*o*-Nitrophenylseleno)ethyl[3-$^2H_1$]cyclopropane-1,1-dicarboxylate (38). A solution of compound **37** (25.2 g, 0.109 mol) in tetrahydrofuran (500 mL) containing *o*-nitrophenylselenocyanate[33] (29.72 g, 0.131 mol) under argon was treated with tributylphosphine (32.6 mL, 0.131 mol) dropwise at room temperature.[34] The internal temperature of the mixture rose slightly at the beginning but cooled back to room temperature as the reaction proceeded. After the reaction was stirred at room temperature overnight, the solvent was removed under reduced pressure. Flash chromatography (benzene) of the residual brown oil on silica gel (160 g), afforded the seleno derivative **38** as a yellow oil in 78.5% yield. ^1H NMR ($CDCl_3$) δ 8.18 (1 H, d, 8.3), 7.44 (2 H, m), and 7.25–7.19 (1 H, m) all aromatic H's, 4.22–4.04 (4 H, m, OCH_2's), 2.99–2.88 (2 H, m, CH_2-Se), 1.96 (1 H, m, 2-H), 1.81 (1 H, m, C*H*-C-Se), 1.67 (1 H, m, C*H*-C-Se), 1.37 (1 H, d, 8.8, 3-H_S), 1.35 (residual 3-H_R, partially burried under 3-H_S), 1.20 (3 H, t, 7.4, Me), 1.19 (3 H, t, 7.3, Me); ^{13}C NMR ($CDCl_3$) 169.7 (C=O), 167.7 (C=O), 133.4 (aromatic *C*-NO_2), 128.8 (sub-aromatic *C*), 128.1, 126.2, and 125.7 (all aromatic C's), 61.3 (OCH_2's), 34.0 (C-1), 27.8* (*C*-C-Se), 27.7* (C-2), 24.5 (*C*-Se), 20.1 (t, J = 25.2, C-3), 14.0 (Me), 13.8 (Me).

Diethyl 2-Vinyl[3-$^2H_1$]cyclopropane-1,1-dicarboxylate (39). To a solution of 35.5 g (85.5 mmol) of the preceding selenide (**38**) in 800 mL of tetrahydrofuran at 0 °C was added dropwise 46.6 mL of 30% hydrogen peroxide. After addition was complete, the reaction mixture was allowed to warm up to room temperature and stirred for another 3 h. The reaction was then quenched with 150 mL of ice–water and the mixed solvents were removed under reduced pressure in a warm water bath (ca. 40–50 °C). The products were isolated by extraction of the remaining aqueous layer with several portions of chloroform. The combined organic extracts were washed with brine and dried over anhydrous $MgSO_4$. Filtration followed by removal of the solvent provided the crude products. This dark brown oil was purified by flash chromatography (first $CHCl_3$, second benzene) to afford the vinyl cyclopropane diester **39** as a light yellow oil in 45.6% yield. ^1H NMR ($CDCl_3$) δ 5.32 (1 H, m, *H*RC=), 5.17 (1 H, dd, 7.1, 2.0, *t*-HRC=CH*H*), 5.01 (1 H, dd, 9.8, 2.0, *c*-HRC=C*H*H), 4.09 (4 H, m, OCH_2's), 2.45 (1 H, t, 8.4, 2-H), 1.50 (0.25 H dd, 6.8, 4.2, 3-H_R), 1.41 (1 H, d, 8.9, 3-H_S), 1.16 (3 H, t, 7.3, Me), 1.15 (3 H, t, 7.4, Me); ^{13}C NMR ($CDCl_3$) 169.2 (C=O), 167.0 (C=O), 132.9 (R*C*H=CH_2), 118.1 (RCH=*C*H_2), 61.3 (OCH_2), 61.1 (OCH_2), 35.7 (C-1), 30.7 (C-2), 19.7 (t, J = 25.1, C-3), 13.9 (Me), 13.8 (Me).

Diethyl 2-Ethyl[3-$^2H_1$]cyclopropane-1,1-dicarboxylate (40). To a solution of compound **39** (7.9 g, 37.1 mmol) in 40 mL of diglyme was added *p*-toluenesulfonyl hydrazide (13.8 g, 74.2 mmol). This mixture was refluxed at 170 °C for 3 h. After being cooled down to room temperature, the resulting brown solution was diluted with water followed by ether (2×) and chloroform (2×) extraction. The combined organic extracts were dried and concentrated. Removal of the excess diglyme was effected by distillation under reduced pressure, and the residual brown syrup was further purified by flash chromatography (silica gel 160 g each, first hexane, second 5% EtOAc/Hex). Compound **40** was isolated as a colorless oil. Yield 70.9%; ^1H NMR ($CDCl_3$) δ 4.14 (4 H, m, OCH_2), 1.80 (1 H, m, 2-H), 1.37 (2 H, m, side-chain CH_2), 1.29 (1 H, d, 8.8, 3-H_S), 1.23 (3 H, t, 7.2, Me), 1.20 (3 H, t, 6.9, Me), 0.95 (3 H, t, 6.9, side-chain Me), signal of 3-H_R was buried under the methyl resonances; ^{13}C NMR ($CDCl_3$) 170.4 (s, C=O), 168.2 (s, C=O), 61.2 (t, OCH_2), 61.1 (t, OCH_2), 34.1 (s, C-1), 29.7 (d, C-2), 22.0 (t, side-chain CH_2), 20.4 (t, J = 25.3, C-3), 14.0 (q, Me), 13.9 (q, Me), 13.1 (q, Me).

1-(Methoxycarbonyl)-2-ethyl[3-$^2H_1$]cyclopropane-1-carboxamide (41). To a clear solution of 2-ethyl[3-$^2H_1$]cyclopropane diester **40** (5.66 g, 26.3 mmol) in 10 mL of dry methanol was added a methanol solution of ammonia (presaturated at 0 °C) containing catalytic amounts of sodium methoxide (60 mg of sodium). The reaction was allowed to stir at room temperature for 7 days. Excess solvent was evaporated in vacuo and the residual yellow-colored oil was extracted into chloroform solution, which after concentration and purification by flash chromatography (silica gel 100 g, $CHCl_3$) afforded 65.8% yield of the expected product **41** as a white powder, mp 71–72 °C. ^1H NMR ($CDCl_3$) δ 7.93 (1 H, brs, N*H*), 6.86 (1 H, brs, N*H*), 3.59 (3 H, s, OMe), 1.71 (1 H, m, 2-H), 1.45 to ~1.30 (3 H, m, 3-H_S, side-chain CH_2), 0.82 (3 H, t, 7.4, Me), signal of 3-H_R could not be isolated; ^{13}C NMR ($CDCl_3$) 171.8 (C=O), 171.5 (C=O), 51.7 (OMe), 36.0 (C-1), 32.0 (C-2), 21.2 (t, J = 25.3, C-3), 21.0 (side-chain CH_2), 12.8 (Me); MS(EI) 172 (M^+), 154, 141, 131, 123, 117 (base peak).

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Methyl 1-((Methoxycarbonyl)amino)-2-ethyl[3-$^2H_1$]cyclopropane-1-carboxylate (42). A solution of methyl hypobromite[30] was prepared under cooling by dry ice and acetone from bromine (3.32 g, 20.76 mmol) and a solution of sodium (1.19 g, 51.9 mmol) in dry methanol (22 mL). Vigorous stirring was continued until the bromine color was discharged. To this solution at −78 °C was added dropwise the carboxamide **41** (2.97 g, 17.3 mmol) in 20 mL of dioxane–methanol (3:2 (v:v)) and a considerable amount of white precipitate was formed. The milky white mixture was allowed to warm slowly to room temperature and stirred at room temperature overnight. Further heating was conducted at 60 °C for 30 min. The resulting clear yellow solution was cooled back to room temperature and then neutralized with acetic acid to pH 7. The solvent was removed in vacuo, and the residue was taken up into water. The aqueous suspension was extracted three times with ether and once with chloroform. After the organic extracts were combined, washed with brine, dried, and concentrated, the crude product was purified by flash chromatography (silica gel 100 g, $CHCl_3$) to give a 58% yield of compound **42**. ^1H NMR ($CDCl_3$) δ 5.32 (1 H, brs, N*H*), 3.72 (3 H, s, OMe), 3.68 (3 H, s, OMe), 1.72 ~1.50 (4 H, m, 2-H, 3-H_S, side-chain CH_2), 0.96 (3 H, t, 7.3, Me), signal of 3-H_R could not be isolated; ^{13}C NMR ($CDCl_3$) 172.1 (C=O), 157.0 (NHC=O), 52.3 (CO_2CH_3), 52.1 ($NHCO_2$-CH_3), 39.6 (C-1), 33.7 (C-2), 23.3 (t, J = 25.5, C-3), 20.3 (side-chain CH_2), 13.4 (Me).

Methyl 1-Amino-2-ethyl[3-$^2H_1$]cyclopropane-1-carboxylate (43). To a solution of the carbamate ester **42** (1.36 g, 6.73 mmol) in chloroform (30 mL) was added trimethylsilyl iodide[31] (2.40 mL, 16.8 mmol) at room temperature. This deep red solution was kept stirring at 50 °C for 12 h. After the reaction was complete, 3–4 equiv of methanol were added to quench the reaction at 0 °C. The volatile components were removed under reduced pressure. The brown residue was redissolved in chloroform and extracted with water. The combined aqueous extracts were concentrated in vacuo to afford the desired product as a yellow powder. No purification was carried out at this stage. ^1H NMR ($CDCl_3$) δ 4.11 (2 H, brs, NH_2), 3.69 (3 H, s, OMe), 1.96 (1 H, m, 2-H), 1.82 (1 H, d, 10.2, 3-H_S), 1.46 (2 H, m, side-chain CH_2), 1.35 (<0.3 H, m, 3-H_R), 0.82 (3 H, t, 7.3, Me); ^{13}C NMR ($CDCl_3$) 169.1 (C=O), 50.1 (OMe), 38.7 (C-1), 29.9 (C-2), 19.9 (side-chain CH_2), 19.4 (t, J = 25.3, C-3), 13.2 (Me).

1-Amino-2-ethyl[3-$^2H_1$]cyclopropane-1-carboxylate (31). The amine ester **43** (1.08 g, 7.5 mmol) was dissolved in 15 mL of 2 N sodium hydroxide (1.2 g, 30 mmol) solution and refluxed for 3 h. The resulting light yellow solution was cooled to room temperature and loaded onto a Dowex-50 (H^+) column (1.5 × 8 cm). The column was washed with water followed by 2 N ammonium hydroxide solution, and the ammonia eluate was collected and concentrated in vacuo to afford the desired amino acid as white crystals. Recrystallized from hot aqueous ethanol, compound **31** was isolated in 51.4% yield; mp 162–165 °C (dec). ^1H NMR (D_2O) δ 1.64 (1 H, m, 2-H), 1.49 (2 H, m, side-chain CH_2), 1.30 (1 H, d, 8.0, 3-H), 0.93 (3 H, t, 7.0, Me); ^{13}C NMR (D_2O) 173.8 (C=O), 39.3 (C-1), 27.8 (C-2), 19.8 (side-chain CH_2), 17.0 (t, J = 25.3, C-3),[29] 12.7 (Me); MS (EI, 70 eV) 101 (M − Et)$^+$, 88 (*M* − $CH_2CH_2CH_3$)$^+$, 55 (CDH=C=C=O)$^+$, 43 (CDH=C=NH)$^+$; MS (FD, 5 mA) 130 (M)$^+$. Anal. Calcd for $C_6H_{10}{}^2H_1NO_2$: C, 55.36; H and ^2H, 9.29; N, 10.76. Found: C, 54.99; H and ^2H, 9.08; N, 10.50.

Sample Preparation for Chiral Methyl Analysis. Conversion of Vinylglycine 11 and 12 Into α-Ketobutyrate. The vinylglycine samples (ca. 10 mg), stereospecifically deuterated at C-4 position, **11** and **12** were incubated separately with ACPC deaminase (200 μL, 0.41 mg/mL) in 1 mL of potassium pyrophosphate buffer (50 mM, pH 8.5) which had been tritiated by lyophilization and addition of tritium oxide (200 mCi/mL). After standing at 37 °C for 24 h, each of the reaction mixtures was passed through a Dowex-50 (H^+) column (1.5 × 5 cm) to remove the protein and any unreacted starting material. A volume of 75 mL of aqueous eluate was collected and the pH of this solution was adjusted to 9–10 by dropwise addition of 1.0 N NaOH. Removal of the solvent in vacuo yielded the desired product, double-labeled α-ketobutyrate, as white residue. After the product was redissolved in 10 mL of water, a small aliquot was assayed for its ^3H activity which was 6.37 × 10^6 cpm for the product from *E* isomer and 4.26 × 10^6 cpm for the product derived from *Z* isomer.

Degradation of Stereospecifically Double-Labeled α-Ketobutyrate. Kuhn–Roth oxidative degradations of the α-ketobutyrate samples obtained from (*E*)-*DL*-[3,4-$^2H_2$]vinylglycine (**12**) and (*Z*)-*DL*-[4-$^2H_1$]-vinylglycine (**11**) were carried out separately in a solution of 50 mg of potassium dichromate, 3.2 mL of concentrated sulfuric acid, and 10 mL of water. After being refluxed for 16 h under argon, the resulting orange solution was subjected to steam distillation. The pH was adjusted to 8–9 with 0.1 N NaOH and the solvent was removed under reduced pressure. As noticed in previous model studies, the crude products should contain both acetate and propionate. The total activity was 1.56 × 10^6 cpm for

the degradation product yielded from the *E* isomer and was 6.12×10^5 cpm for that obtained from the *Z* isomer.

Derivatization and Separation of the Degradation Products. Redissolved in 2 mL of water, the degradation products were mixed with excess α-bromophenylacetophenone (60 mg) in 40 mL of acetone. The resulting milky white solution was refluxed overnight. After being cooled to room temperature and concentrated in vacuo, the yellow solid residue was purified by preparative TLC ($CHCl_3$). For the sample derived from (*E*)-[3,4-$^2H_2$]vinylglycine (**12**), 4.5 mg of the *p*-phenylphenacyl acetate having a total activity of 2.24×10^5 cpm was obtained; whereas, in the case of (*Z*)-[4-$^2H_2$]vinylglycine (**11**), 8.4 mg of the *p*-phenylphenacyl acetate having a total activity of 1.38×10^5 cpm was collected. Since acetate accounts for only 23% of the total mass in each sample, the specific activity can be estimated to be 2.13×10^5 cpm/mg (1.28×10^7 cpm/mmol) and 7.04×10^4 cpm/mg (4.22×10^6 cpm/mmol) for the expected chiral acetate derived from *E* and *Z* isomer, respectively.

Conversion of Phenylphenacyl Acetate to Acetate. Each of the p-phenylphenacyl acetates was redissolved in 1 mL of acetone and then mixed with 1 g of *p*-toluenesulfonic acid and 10 mL of water. Refluxing was maintained overnight, during which time a considerable amount of brown precipitate was formed. Steam distillation followed by basification to pH 8 with 0.1 N NaOH of the collected distillate was carried out, and the resulting clear solution was concentrated to dryness in vacuo. Total ^3H activity was 2.06×10^5 cpm for the chiral acetate derived from *E* isomer and was 1.24×10^5 cpm for its analogue obtained from the *Z* isomer. To allow more sensitive counting of the $^3H/^{14}C$ ratio in the subsequent analyses, 4.58×10^4 cpm and 2.76×10^4 of [U-^{14}C]acetate were mixed with the acetate samples derived from *E*- and *Z*-[4-^{2}H]-vinylglycine, respectively, to make the $^3H/^{14}C$ ratio 4.5/1 in both cases.

Sample Preparation for C-4 Chirality Determination. Conversion of 1-Amino-2-methylcyclopropane-1-carboxylate (10) to 2-Keto[4-$^2H_1$]pentanoate (19). 2-Me-ACPC (**10**) (12 mg) and ACPC deaminase (200λ, 0.4 mg/mL) were incubated in deuterated potassium pyrophosphate buffer (50 mM, pD 8.8, 3 mL) for 2 days at 37 °C. The reaction mixture was loaded onto a Dowex-50 (H^+) column (1.5 × 5 cm) and the column was then eluted with water. The aqueous eluate was concentrated and the white residue was redissolved in NaOH solution (8 mL) and incubated at 40 °C for 24 h. The resulting clear solution was again loaded on a Dowex-50 (H^+) column 1.5 × 5 cm) and washed with water. The eluate was collected and concentrated in vacuo to afford the 2-keto-pentanoate sodium salt (**19**) as a white solid. ^1H NMR (D_2O) δ 2.93 (d, 7.3, 3-H's, keto form), 1.88 (d, 7.9, 3-H's, hydrated form), 1.65 (m, 4-H, keto form), 1.25 (m, 4-H, hydrated form), 0.95 (d, 7.9, Me).

Decarboxylation of 2-Keto[4-$^2H_1$]pentanoate (19). To a clear solution of 2-keto[4-$^2H_1$]pentanoate (**19**) in 3% H_2O_2 (10 mL, pH adjusted to 7.8 with 0.1 N NaOH) was added 200λ of catalase over a period of 10 min. After being stirred vigorously for 5 h at room temperature, the resulting cloudy solution was subjected to Dowex-50 (H^+) cation exchange chromatography (1.5 × 5 cm) and eluted with water. The aqueous eluate was saturated with sodium chloride and extracted thoroughly with ether. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated to give [3-$^2H_1$]butyric acid (**20**).

Derivatization of Enzymically Produced [3-$^2H_1$]Butyric Acid (20). The residual colorless oil **20** obtained from the preceding reaction was dissolved in DMF (2 mL). To this solution was added 2-naphthol (28.8 mg, 0.2 mmol), (dimethylamino)pyridine (6 mg), and dicyclohexylcarbodiimide (20.6 mg, 0.1 mmol). The reaction mixture was kept stirring at room temperature overnight. After the mixture was diluted with ether and quenched with water, the aqueous layer was separated and further extracted with ether. The pooled ether extracts were dried ($MgSO_4$), concentrated, and purified by HPLC (C_{18} column, 30% H_2O/MeOH). CD (MeOH), $\Delta\epsilon$ (276 nm) = −0.36.

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Synthesis of 1′-Deoxy-1′-fluorosucrose via Sucrose Synthetase Mediated Coupling of 1-Deoxy-1-fluorofructose with Uridine Diphosphate Glucose

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Abstract: 1′-Deoxy-1′-fluorosucrose (**1**) has been synthesized by a sucrose synthetase mediated coupling of 1-deoxy-1-fluoro-D-fructose with UDP-glucose. 1-Deoxy-1-fluoro-D-fructose (**2**) was prepared from 2,3:4,5-di-*O*-isopropylidene-D-fructopyranose via tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) reaction upon the corresponding trifylate followed by hydrolysis. Fluorosucrose **1** is stable to hydrolysis by invertase and is recognized by the sucrose carrier protein.

Sucrose is the predominant carbohydrate transported from photoautotrophic organs to heterotrophic organs in most plant species. Such long-distance transport, in the absence of a fluid pumping mechanism, requires the establishment of a steep sucrose concentration gradient between exporting and importing organs. This gradient is apparently established by a sucrose carrier protein in the membranes of certain cells within leaf vascular tissue and in some other organs, which is capable of moving sucrose across the membrane against a large concentration gradient.[1] Study of this carrier protein and of the physiology of sucrose transport is complicated by the metabolic lability of sucrose, especially its susceptibility to extracellular hydrolysis by invertase. To further

(1) Giaquinta, R. T. *Ann. Rev. Plant Physiol.* **1983**.

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