The species responsible for the 1914/1806 cm^{-1} bands is likely to be of the same nature as those observed from the interaction of NO with Fe^{2+}–Y[10,11] or with Fe(II) on SiO_2.[12,13] They have been assigned previously to a surface dinitrosyl, but the results here imply that a reinterpretation of these earlier studies might be appropriate. The unique feature of the present work which has allowed us to distinguish so clearly between the di- and trinitrosyl has been (1) the deposition technique used which permitted the formation of only one NO–Fe species having intense bands in the 1800–1920-cm^{-1} region and (2) the extremely narrow half-width of the infrared bands which has permitted us for the first time to just resolve the four expected components of a mixed isotopic trinitrosyl surface species. Most importantly, the present results establish that on supported iron surfaces, three rather than two coordination sites can in principle be available for catalysis.

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3-Halovinylglycines. Efficient Irreversible Inhibitors of *E. coli* Alanine Racemase

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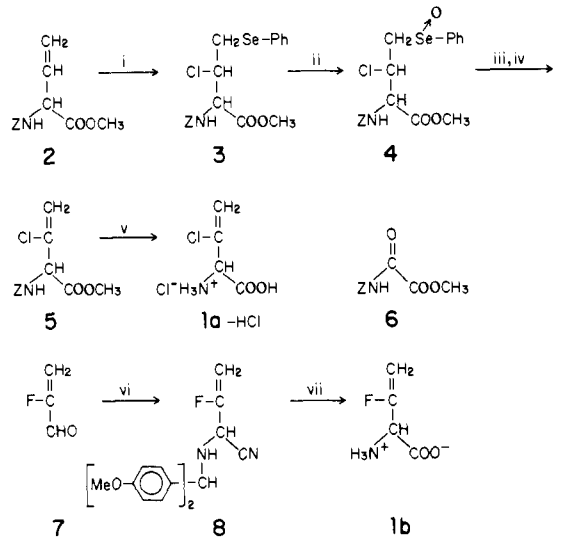
We wish to report that 3-halovinylglycines **1** are a new class of potent mechanism-based irreversible inactivators of alanine racemase. Our results with *Escherichia coli B* alanine racemase indicate that nearly every catalytic event results in irreversible inactivation of the enzyme. In contrast, mechanism-based inactivation by previously studied β-substituted alanines is much less efficient, requiring approximately 800 turnovers with this enzyme to produce one irreversible event.[1]

Alanine racemases are prime targets for design of antibiotics because they are unique to bacteria and are essential for production of D-alanine, required in cell wall biosynthesis.[2] The utility of this approach has been established by 3-fluoro-D-alanine, which is a potent, broad spectrum, orally active antibiotic.[3] However, safety issues unrelated to its mechanism of action have hindered the clinical application of 3-fluoro-D-alanine. Consequently, the search has continued for new agents with this mechanism of action. We now describe studies with 3-halovinylglycines which are particularly effective inhibitors of bacterial alanine racemase.

Our syntheses of 3-chlorovinylglycine [2-amino-3-chlorobutenoic acid, **1a**] and 3-fluorovinylglycine [2-amino-3-fluorobutenoic acid, **1b**] are outlined in Scheme I.[4] D-, L-, and DL-3-chlorovinylglycine were obtained from the corresponding *N*-(benzyloxycarbonyl)-vinylglycine methyl ester (**2**) which in turn was derived from the corresponding D-, L-, or DL-methionine.[5] Extension of this methodology to 3-fluorovinylglycine was not successful, and it was

Scheme I[a]



[a] (i) Ph–SeCl, CH_3CN, 0–25 °C, 2 h;[8] (ii) O_3, CCl_4, −20 °C; (iii) CCl_4, 1 equiv of pyridine, reflux; (iv) silicagel chromatography, elution with EtOAc: hexanes = 1:4, 15–20% yield from **2**;[9] (v) 6 N HCl reflux 80 min, 80–90% yield; (vi) 4,4′-dimethoxybenzhydrylamine, 4 Å molecular sieves, CH_2Cl_2 followed by TMS–CN, 25 °C;[7] (vii) 6 N HCl, reflux 2 h followed by Dowex (H^+) chromatography, elution with 3% pyridine in water, 25–30% yield from **7**.

prepared from 2-fluoroacrolein (**7**)[6] by a modified Strecker synthesis.[7]

Incubation of homogeneous *E. coli B* alanine racemase[10] with D-chlorovinylglycine, L-chlorovinylglycine, or DL-fluorovinylglycine results in irreversible inactivation of the enzyme as demonstrated by the inability of the enzyme to regain catalytic activity after prolonged dialysis. The inactivation kinetics are characterized by rapid, pseudo-first-order irreversible inhibition of 70% of the enzyme. The second-order rate constant that describes this initial inactivation for D-chlorovinylglycine (122 ± 14 M^{-1} s^{-1}) is comparable to the corresponding rate constant for 3-fluoro-D-alanine (93 ± 13 M^{-1} s^{-1}). Irreversible inhibition of the remaining enzyme is described by a first-order rate constant that is independent of inhibitor concentration, halogen, and stereochemistry ($1.2 \pm 0.4 \times 10^{-4}$ s^{-1}). A complete description of the inactivation kinetics has been obtained and will be published later.

Partition ratios were determined in parallel experiments for 3-fluoro-D-alanine and D-chlorovinylglycine (Figure 1). The value of the *x*-intercept represents the inhibitor concentration required to inactivate irreversibly all of the enzyme in the incubation mixture. As can be appreciated from a comparison of the two plots, the *x*-intercept for D-chlorovinylglycine is approximately 300-fold lower than that for 3-fluoro-D-alanine. Assuming a partition ratio of 800 for 3-fluoro-D-alanine,[1] the results from three independent experiments indicate that 2.1 ± 0.6 molecules of D-chlorovinylglycine are required to inactivate 1 molecule of enzyme. The partition ratio that is measured for L-chlorovinylglycine

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(2) For recent reviews, see: (a) Neuhaus, F. C.; Hammes, W. P. *Pharmacol. Ther.* **1981**, *14*, 265. (b) Walsh, C. T. *Tetrahedron* **1982**, *38*, 871. (c) Walsh, C. T. *Ann. Rev. Biochem.* **1984**, *53*, 493.
(3) Kollonitsch, J.; Barash, L.; Kahan, F. H.; Kropp, H. *Nature (London)* **1973**, *243*, 346.
(4) Synthesis of DL-chlorovinylglycine by another route has been described by Borcsok and Abeles (Borcsok, E.; Abeles, R. H. *Arch. Biochem. Biophys.* **1982**, *213*, 695) who reported that it is not an inhibitor of the pyridoxal-dependent enzyme, cystathionine synthase. We are indebted to Dr. Abeles for suggesting that this type of compound also be evaluated for alanine racemase inhibition.
(5) (a) Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817. Chiral **2** has also been obtained from glutamic acid. (b) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425. (c) Barton, D. H. R.; Crich, D.; Yolande, H.; Potier, P.; Thierry, J. *Tetrahedron* **1985**, *19*, 4347. For a recent synthesis of racemic **2**, see: Castelhano, A. L.; Horne, S.; Billedeau, R.; Krantz, A. *Tetrahedron Lett.* **1986**, *27*, 2435.
(6) Buddrus, J.; Nerdel, F.; Hentschel, P.; Klamann, D. *Tetrahedron Lett.* **1966**, 5379.
(7) Procedure of Greenlee, W. J. *J. Org. Chem.* **1984**, *49*, 2632. R. D. Hoffsommer in our laboratory had previously obtained **1b** in trace acounts from **7** via 3-fluorovinylglycolic acid utilizing Baldwin's route to racemic vinylglycine (Baldwin, J. E.; Haber, S. B.; Hoskins, C.; Kruse, L. I. *J. Org. Chem.* **1977**, *42*, 1239).
(8) Procedure of Raucher, S. *Tetrahedron Lett* **1977**, 3909.
(9) *N*-Benzyloxycarbamoyl methyl oxamate **6**, mp 89–90°, a byproduct of the ozonization of **3**, is also produced on CrO_3 based oxidation of Z-Ser-OMe and Z-Thr-OMe (Statchulsky, A. V. *Tetrahedron Lett.* **1982**, *23*, 3789). Ozone oxidation at the α-carbon of **4**, analogous to the O_3 oxidation of acetals (DesLongchamps, P.; Moreau, C. *Can. J. Chem.* **1971**, *49*, 2465), followed by fragmentation would yield **6**.
(10) The enzyme used in these studies was purified from *E. coli* B (MB 1967) that was grown in a medium containing L-alanine as the sole carbon source. The enzyme was purified to homogeneity in 35% yield by using a procedure that will be reported separately.

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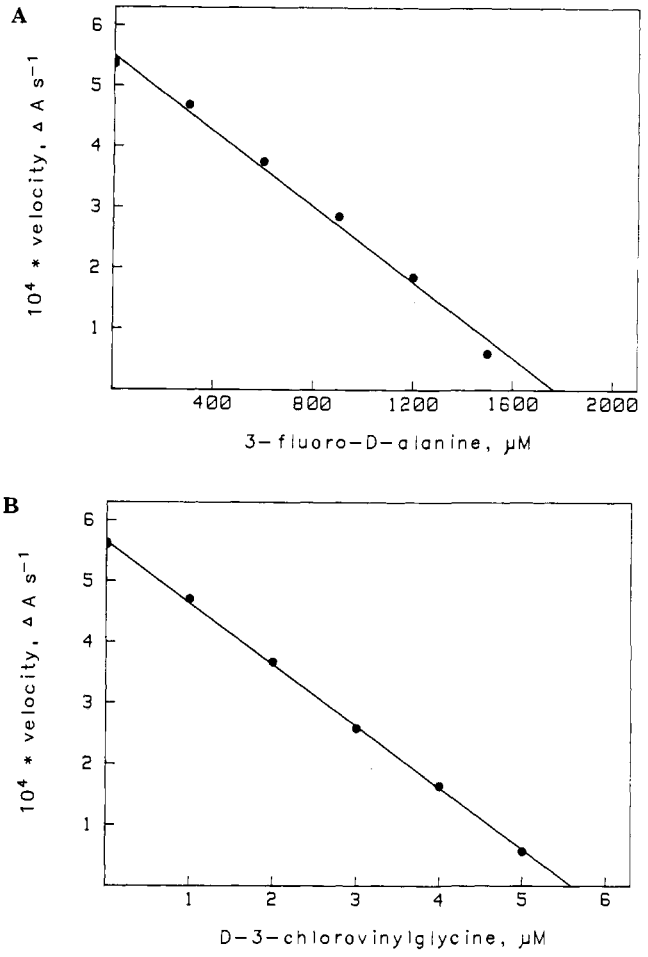

Figure 1. Comparison of partition ratios of D-fluoroalanine (A) and D-chlorovinylglycine (B). The two inhibitors were incubated with identical enzyme solutions in 0.1 M HEPES, pH 8.00 at 25 °C. Inactivation of the enzyme was allowed to proceed to >99% completion of the decomposition of the inhibitor, as calculated from the overall rate constants for inactivation determined under the experimental conditions. The remaining enzymatic activity was measured by using the coupled spectrophotometric assay (D-alanine to L-alanine direction) described by Wang and Walsh.[1] The plots show the residual activity as a function of total inhibitor concentration.

(16 ± 9) is slightly larger than that for the D-isomer.

The fluorovinylglycines are 100-fold less reactive than the chlorovinylglycines. Availability of enzyme has precluded an accurate determination of the partition ratio; an upper limit of 40 has been established for the racemate.

Consistent with the greater efficiency and more complex kinetics, there is physical evidence that the halovinylglycines follow a different mechanism of inhibition than established for their simpler homologues, the nonvinylic β-substituted alanines. Denaturation of the enzyme–inhibitor complex by heat gives quantitative release of free pyridoxal phosphate, as identified by its absorbance spectrum. This contrasts with the Schnackerz adduct of aminoacrylate and pyridoxal phosphate found for nonvinylic β-substituted alanines.[11] Consequently, irreversible inhibition must proceed by a different mechanism, which is currently under study.

(11) (a) Roise, D.; Soda, K.; Yagi, T.; Walsh, C. T. *Biochemistry* **1984**, *23*, 5195. (b) Badet, B.; Roise, D.; Walsh, C. T. *Biochemistry* **1984**, *23*, 5188. This mechanism had been developed also by Metzler and colleagues for L-aspartate transaminase (Likos, J. J.; Ueno, H.; Feldhaus, R. W.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4377) and for glutamate decarboxylase (Ueno, H.; Likos, J. J.; Metzler, D. E. *Biochemistry* **1982**, *21*, 4387).

Supplementary Material Available: The physical properties and analytical data for compounds [D]-**1a**-HCl, [L]-**1a**-HCl, [D,L]-**1a**-HCl, and [D,L]-**1b** and the separation of the latter into its diastereomers (1 page). Ordering information is given on any current masthead page.

Inversion of the Cyclotribenzylene Cone in a Columnar Mesophase: A Potential Way to Ferroelectric Materials

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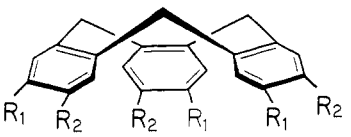
Certain hexaesters **3** of cyclotricatechylene (**1**) form thermotropic columnar mesophases,[1-4] in which the cone-shaped cyclotribenzylene units stack in parallel columns, at a distance of ~4.8 Å, like in the crystal structure of cyclotriveratrylene (**2**).[2] In a



1 $R_1 = R_2 = OH$

2 $R_1 = R_2 = OCH_3$

3 $R_1 = R_2$ = O-CO-alkyl, O-CO-aryl

4 R_1 = H, R_2 = OH

mesophase column, the cones are embedded into one another with the same orientation, making the column axis *polar*. Such mesophases might therefore be ferroelectric, if all the columns adopted the same polarization direction within a macroscopic domain, as sketched in Figure 1.[2,3] However, the high viscosity of these materials severely restricts the freedom of the molecules to turn upside down, and hence this mechanism would not easily allow orientation of the cones (e.g., in an electric field, since the cones may have a dipole moment along their axis).

An interesting property of the cyclotribenzylene structure is the possibility of *conformational inversion* of the nine-membered ring system, which takes place in solution over a barrier of ca. 27 kcal/mol;[5] it is therefore very slow at room temperature but may become fast on heating (the half-life of a given cone conformer is about 1 month at 20 °C, a few minutes at 100 °C, and less than 0.1 s at 200 °C). If the inversion process could still occur at a sufficient rate in a mesophase such as those of **3**, one would expect, by means of an external field, to polarize all the cones, hence the columns, in the same direction, *without need for any rotation of the molecules*; only an umbrellalike inversion of their cores is required.

A simple way to assess whether this process is feasible is to make use of optically active cyclotriveratrylenes; in effect inversion of a cone generates its mirror image structure, and the racemization rate therefore provides a direct measurement of the phenomenon. Such studies cannot be performed with hexaesters of type **3**, where $R_1 = R_2$, which are achiral (C_{3v}). In contrast, cyclotriveratrylenes

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(2) Levelut, A.-M.; Malthête, J.; Collet, A. *J Physique* **1986**, *47*, 351-357.
(3) Zimmermann, H.; Poupko, R.; Luz, Z.; Billard, J. *Z. Naturforsch., A: Phys., Phys. Chem., Kosmophys.* **1985**, *40A*, 149-160.
(4) Zimmermann, H.; Poupko, R.; Luz, Z.; Billard, J. *Z. Naturforsch., A: Phys., Phys. Chem., Kosmophys.* **1986**, *41A*, 1137-1140.
(5) Canceill, J.; Collet, A.; Gottarelli, G. *J. Am. Chem. Soc.* **1984**, *106*, 5997-6003.

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