124.9, 137.2, 149.2 (C=N), 150.9.

Bis(dimethylglyoximato)(pyridine)(cyclohexylmethylidene)cobalt Complex (6e). Reaction of 5 mmol of **5** with 4.5 mmol of **1e** gave after workup 1.05 g (51%) of **6e** as an orange, powdery solid: mp 150-152 °C dec; MS, *m/z* 464 (MH^+, 20), 463 (M^+, 11), 385 (MH^+ - py, 86), 384 (M^+ - py, 100), 368 (7), 359 (18), 290 (45); IR (KBr) 3110 (w), 3050 (w), 2920 (s), 1600 (w), 1560 (s), 1490 (w), 1440 (s), 1360 (m), 1270 (m), 1230 (s), 1080 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.3 (m, 4, CH_2), 1.45 (m, 2, CH_2), 2.08 (m, 2, CH_2), 2,10 (s, 12, dmg Me), 2.20 (m, 2, CH_2), 5.36 (s, 1, C=CH), 7.32 (m, 2, β-py), 7.71 (m, 1, γ-py), 8.67 (m, 2, α-py); ^{13}C NMR ($CDCl_3$) δ 12.2 (dmg Me), 27.1, 29.06, 30.05, 30.50, 40.0, 125.0, 137.3, 145.1, 149.45 (C=N), 149.70.

Reaction of 5 with Vinyl Triflate 3*Z*. Formation of 7*Z*. Treatment of 400 mg (1.5 mmol) of pure triflate **3*Z*** with 1.6 mmol of **5** (freshly generated from 380 mg of $CoCl_2 \cdot 6H_2O$, 370 mg of dimethylglyoxime, 126 mg of pyridine, 200 mg of NaOH, and 17 mg of $NaBH_4$ in 6 mL of CH_3OH/H_2O) and workup according to the above general procedure gave 420 mg (58%) of **7*Z*** as an orange, microcrystalline product: mp 170-175 °C dec; NMR showed no other product and no **7*E*** could be detected by either ^1H or ^{13}C NMR; IR (KBr) 3110, 3040, 2930, 1600, 1560, 1445, 1290, 1230, 1085, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88 (s, 12, dmg Me), 1.96 (d, 3, Me), 4.98 (m, 1, C=CH), 7.0-7.2 (m, 7), 7.70 (m, 1, γ-py), 8.47 (m, 2, α-py); ^{13}C NMR ($CDCl_3$) δ 12.0 (dmg Me), 33.0 (Me), 124.99, 127.44, 128.21, 137.54, 144.43, 145.56, 149.42, 149.96. See the text and Table I for assignment of olefin stereochemistry.

Reaction of 5 with Vinyl Triflate 3*E*. Formation of a Mixture of 7*Z* and 7*E*. Treatment of 170 mg (0.66 mmol) of pure triflate **3*E*** with 0.70 mmol of **5** and workup as above gave 180 mg (56%) of a *mixture* of **7*E*** and **7*Z*** (66:34). The mixture could not be separated by chromatography, HPLC, or crystallization. All spectra were recorded on the mixture of product isomers (**7*E*** and **7*Z***). ^1H NMR [$CDCl_3$, **7*E*** only (by substraction of signals for **7*Z***)] δ 2.10 (dmg Me), 2.15 (d, 3, Me), 6.46 (m, 1, C=CH), 7.15-7.40 (m, 7), 7.74 (m, 1, γ-py), 8.72 (m, 2, α-py); ^{13}C NMR ($CDCl_3$, mixture of **7*E*** and **7*Z***) δ 12.0 (dmg Me), 17.6, 33.0, 124.99, 125.14, 125.74, 127.44, 127.55, 128.21, 137.54, 140.03, 144.43, 144.91, 145.56, 149.38, 149.42, 149.86, 149.90, 149.96. See the text and Table I for assignment of olefin stereochemistry.

Reaction of 5 with Vinyl Triflate 4*Z*. Formation of 8*Z* and 8*E*. Treatment of 250 mg (1.22 mmol) of pure isomeric **4*Z*** with 1.33 mmol of **5**, as above, and workup gave 300 mg (59%) of a mixture of **8*E*** and **8*Z*** (55:45). The mixture could not be separated by chromatography, HPLC, or crystallization. All spectra were obtained on the mixture and compared to the spectra of the product mixture from the reaction of pure **4*E***. See the text and Table I for assignment of olefin stereochemistry.

Reaction of 5 with Vinyl Triflate 4*E*. Formation of 8*Z* and 8*E*. Treatment of 125 mg (0.61 mmol) of pure isomeric **4*E*** with 0.7 mmol of **5**, as above, and workup gave 155 mg (60%) of a mixture of **8*E*** and **8*Z*** (88:12). The mixture could not be separated by chromatography, HPLC, or crystallization. All spectra were obtained on this mixture and compared to the spectra of the product mixture from the above reaction of pure **4*Z***. See the text and Table I for assignment of olefin stereochemistry. IR (KBr, 88:12 mixture of **8*E*** and **8*Z***) 3105, 3060, 3020, 3000, 2900, 2850, 1600, 1550, 1445, 1370, 1230, 990 cm^{-1}. For **8*E***: ^1H NMR ($CDCl_3$, recorded for 88:12 mixture of **8*E*** and **8*Z***) δ 1.47 (m, 3, Me), 1.66 (m, J = 6.72 Hz, 3, Me), 2.10 (s, 12, dmg Me), 4.97 (m, J = 6.72 Hz, 1.55 Hz, 1, C=CH), 7.30 (m, 2, β-py), 7.70 (m, 1, γ-py), 8.65 (m, 2, α-py); ^{13}C NMR ($CDCl_3$) δ 12.21 (dmg Me), 14.61 (Me), 19.54 (Me), 121.10, 124.95, 137.23, 149.42, 149.78. For **8*Z***: ^1H NMR ($CDCl_3$, recorded on a 55:45 mixture of **8*E*** and **8*Z***) δ 1.65 (m, 3, Me), 1.76 (m, J = 7.1 Hz, 3, Me), 2.10 (s, 12, dmg Me), 4.70 (m, J = 7.1, 1.70 Hz, 1, C=CH), 7.32 (m, 2, β-py), 7.70 (m, 1, γ-py), 8.68 (m, 2, α-py); ^{13}C NMR ($CDCl_3$, **8*E*** and **8*Z*** jointly) δ 12.21 (dmg Me), 14.61 (Me), 14.97 (Me), 19.54 (Me), 31.05 (Me), 121.10, 124.95, 124.98, 125.67, 137.23, 149.30, 149.42, 149.76, 150.15.

Test for the Isomerization of Starting Vinyl Triflates 3 and 4 under the Reaction Conditions. A 2-fold excess of *each* pure, isomeric vinyl triflate **3*E*** and **3*Z***, and 4E and 4Z was treated with **5** exactly as above. After the reaction was over, the unreacted starting triflates were analyzed on an analytical GC using a 0.125 in. × 6 ft, 10% UCW-982 on 80/100 Chromosorb W, column for **3*E*** and **3*Z*** and a 0.125 in. × 6 ft, 10% QF-1 on 100/120 Chromosorb W, column for **4*E*** and **4*Z***. In no case was any isomerization of the starting triflate observed.

Test for the Stability of the Isomeric Product Vinyl-Cobaloxime Complexes. Each of the cobaloxime products (0.1 mmol), **7*E***, **7*Z***, **8*E***, and **8*Z*** (or mixture of products, see above) from the reaction of the individual pure isomeric vinyl triflates **3** and **4** was refluxed for 2 h in a 1/1 CH_3OH:$CHCl_3$ mixture. After removal of the solvent, the residue was reanalyzed by ^1H NMR. No change was observed in any product ratios. Likewise, each of the previously isolated product mixtures was subjected to reaction with additional fresh **5**, $[Co(dmgH)_2py]^-$, under the general reaction conditions. Reisolated products, after standard workup, showed no change in isomer ratios by ^1H NMR analyses. Hence, all product ratios observed are the result of the actual reactions and not due to workup, prior, or post isomerization.

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Stereochemical Mechanism of Iodoacetic Acid Mediated Decomposition of L-Methionine to L-Homoserine Lactone

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Abstract: (2*S*,3*S*,4*S*)-, (2*R*,3*R*,4*R*)-, (2*S*,3*R*,4*R*)-, and (2*R*,3*S*,4*S*)-[3,4-$^2H_2$]methionine and (2*S*,3*S*,4*R*)-, (2*R*,3*R*,4*S*)-, (2*S*,3*R*,4*S*)-, and (2*R*,3*S*,4*R*)-[3,4-$^2H_2$]methionine were synthesized from (*E*)-[$^2H_2$]ethylene and (*Z*)-[$^2H_2$]ethylene, respectively, and were utilized to determine the stereochemical mechanism of the iodoacetic acid mediated decomposition of methionine to homoserine lactone. Additionally, a stereochemical mechanism for the conversion of protected tosyl derivatives of L-homoserine derivatives, chiral at the C-4 position due to isotopic substitution, to their corresponding methionine derivatives by reaction with sodium methanethiolate is also proposed.

Investigation of the stereochemical reaction mechanisms of enzymes responsible for the interconversion of four-carbon amino acids has necessitated the syntheses of regio- and stereospecific deuteriated four-carbon amino acids.[1-3] The key step in the synthesis of (4*S*)- and (4*R*)-[4-^2H]-L-methionine employs the direct displacement of a tosylate anion from an appropriately

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protected L-homoserine derivative, stereospecifically deuteriated at the C-4 position,[4,5] with sodium methanethiolate. The assignment of the stereochemistry at the C-4 position of the methionine assumes that the displacement of the tosylate by the methanethiolate group occurs by an S_N2-type reaction mechanism which results in an inversion of configuration at the C-4 position during the transformation of the homoserine derivative to the methionine derivative.

Previous investigators[6] have determined the stereochemistry at the C-4 position of chiral methionine of unknown stereochemistry by converting their (4*R*)- and (4*S*)-[4-^{2}H]-L-methionine into (4*S*)- and (4*R*)-[4-^{2}H]-L-homoserine lactone, respectively, by treating the methionines with iodoacetic acid to form (carboxymethyl)methionine iodides, which are subsequently thermally decomposed to homoserine lactones and then hydrolyzed to homoserine for ^{1}H NMR analysis. It was assumed that the thermal decomposition of (carboxymethyl)methionine iodide to homoserine lactone occurs by an internal S_N2-type reaction in which the C-1 carboxylate anion of the methionine derivative attacks the chiral C-4 methylene carbon to give a homoserine lactone of opposite stereochemistry at the C-4 position relative to that of the starting methionine. An extensive literature search[7-9] failed to establish any direct precedent demonstrating that this decomposition occurs by an internal S_N2-type displacement.

Since the use of stereochemical probes to study reaction mechanisms is totally dependent on the correct labeling assignment in both the substrate and the product, it is absolutely imperative that configurations be known with 100% certainty. Furthermore, knowledge of the stereochemical events involved with the interconversion between methionine and homoserine and vice versa, stereospecifically deuteriated at the C-4 position, is critical not only in the two examples cited above but in the analysis of the stereochemical events of other reaction mechanisms in which either of these two pivotal four-carbon amino acids serves as a crucial intermediate in a more complex synthesis or as the ultimate stereochemical standard in a degradation scheme of a more complex molecule. We report here unequivocal evidence supporting a direct internal S_N2-type displacement mechanism for the thermal decomposition of (carboxymethyl)methionine iodide to homoserine lactone and proof that the stereochemical mechanism for the conversion of protected tosyl derivatives of L-homoserine derivatives, chiral at the C-4 position due to isotopic substitution, to their corresponding methionine derivatives by reaction with sodium methanethiolate also occurs via a direct S_N2-type displacement mechanism.

Experimental Section

All melting points were obtained on a Mel-temp apparatus and are uncorrected. All literature melting point values are for the nondeuteriated compounds. ^{1}H NMR spectra were recorded on either a Bruker 360-MHz NMR spectrometer or an IBM 270-MHz NMR spectrometer in $CDCl_3$ or D_2O. Samples dissolved in $CDCl_3$ are reported in ppm downfield from tetramethylsilane, while samples dissolved in D_2O are reported in ppm downfield from the sodium 3-(trimethylsilyl)propanoate signal. The proton homonuclear-correlated spectra (^{1}H-^{1}H COSY) of the deuteriated *N*-tritylhomoserine lactones were recorded on an IBM 270-MHz NMR instrument utilizing the Bruker software program COSY.AU with the following parameters: spectral width, $SW_2 = 0.5 \times SW_1 = 3000$ Hz; pulse width, P1 = P2 = 11.2 ms (90°); relaxation delay, $D_1 = 3$s; data matrix, $SI_2 = 0.5 \times SI_1 = 1$K; number of experiments, NE = 128; number of scans, NS = 32; concentration ≈ 0.03 M. Elemental analyses were determined by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4%.

The (*Z*)-[$^2H_2$]- and (*E*)-[$^2H_2$]ethylene were purchased from either MSD Isotopes or Cambridge Isotopes Laboratories. DMF was stirred over KOH for 24 h and distilled from BaO. All other organic solvents were of reagent grade and were used without further purification.

The (4*S*)- and (4*R*)-*N*-(*tert*-butyloxycarbonyl)-[4-^{2}H]-L-homoserine *tert*-butyl esters were prepared as previously described.[5]

Solvents were evaporated in vacuo with a rotary evaporator (water-aspirator vacuum) at 40 °C unless stated otherwise. Medium-grade silica gel (Merck, 70–230 mesh) was used for column chromatography. TLC plates (silica, Analtech) were visualized by ultraviolet irradiation from a Mineralight short-wave UV lamp, by iodine chamber visualization, or by spraying with an ethanolic solution of ninhydrin.

***N*-(*tert*-Butyloxycarbonyl)-*O*-tosyl-(4*S*)-[4-^{2}H]-L-homoserine *tert*-Butyl Ester.** To a magnetically stirred solution of *N*-(*tert*-butyloxycarbonyl)-(4*S*)-[4-^{2}H]-L-homoserine *tert*-butyl ester[5] (0.65 g, 2.8 mmol) in dry pyridine (3 mL) cooled to 0 °C was added solid *p*-toluenesulfonyl chloride (0.48 g, 2.5 mmol) all in one portion. The mixture was stirred at 0 °C for 4 h and at 25 °C for 20 h. The reaction mixture was poured into ice water. The precipitate was filtered and recrystallized from methanol/water to give the desired tosylate as a white, tacky solid (0.92 g, 92%): mp 58–59 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 18 H, NCOO-*t*-C_4H_9, O-*t*-C_4H_9), 2.17 (m, 2 H, CH_2C^2HHOTs), 2.45 (s, 3 H, CH_3), 4.08 (m, 1 H, $CH_2C^2H_SH_ROTs$), 4.13 (m, 1 H, 2-C*H*), 5.02 (d, 1 H, N*H*), 7.35 and 7.79 (d and d, 4 H, aromatic *H*).

***N*-(*tert*-Butyloxycarbonyl)-*O*-tosyl-(4*R*)-[4-^{2}H]-L-homoserine *tert*-Butyl Ester.** The other diastereomeric *N*-(*tert*-butyloxycarbonyl)-(4*R*)-[4-^{2}H]-L-homoserine *tert*-butyl ester[5] (0.7 g, 3 mmol) was tosylated with *p*-toluenesulfonyl chloride (0.6 g, 3.1 mmol) as described above to give the title compound as a white solid (0.95 g 87%): mp 59–60 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 18 H, NCOO-*t*-C_4H_9, O-*t*-C_4H_9), 2.17 (m, 2 H, CH_2C^2HHOTs), 2.45 (s, 3 H, CH_3), 4.09 (m, 1 H, $CH_2C^2H_RH_SOTs$), 4.13 (m, 1 H, 2-C*H*), 5.02 (d, 1 H, N*H*), 7.35 and 7.79 (d and d, 4 H, aromatic *H*).

(4*R*)-[4-^{2}H]-L-Methionine. A solution of N-(*tert*-butyloxycarbonyl)-*O*-tosyl-(4*S*)-[4-^{2}H]-L-homoserine *tert*-butyl ester (0.92 g, 2.1 mmol) and $NaSCH_3$ (0.44 g, 6.3 mmol) in ethanol (10 mL) was heated at reflux for 24 h. The solvent was removed in vacuo and the residue was heated at reflux for 4 h in ethanol (10 mL) which had been saturated with dry HCl gas. After removal of the solvent, the residue was dissolved in H_2O (5 mL) and applied to a column of Dowex 50 W (100–200 mesh, NH_4^+ form). The column was first washed with water (200 mL) and then eluted with 1 N NH_4OH. The ninhydrin-positive fractions were pooled and freeze-dried. The residue was recrystallized from ethanol/water to give the title methionine as a slightly yellow solid (0.23 g, 75%): mp 270–273 °C; ^{1}H NMR (D_2O) δ 2.13 (s, 3 H, SCH_3), 2.13 (m, 2 H, 3-CH_2), 2.68 (d, 1 H, 4-$C^2H_RH_S$), 4.10 (t, 1 H, 2-C*H*).

(4*S*)-[4-^{2}H]-L-Methionine. In a manner analogous to that described above, *N*-(*tert*-butyloxycarbonyl)-*O*-tosyl-(4*R*)-[4-^{2}H]-L-homoserine *tert*-butyl ester (0.90 g, 2 mmol) was reacted with $NaSCH_3$ (0.42 g, 6 mmol) and then heated at reflux with ethanolic HCl to give the target methionine as an off-white solid (0.22 g, 75%): mp 273–276 °C; ^{1}H NMR (D_2O) δ 2.13 (s, 3 H, SCH_3), 2.13 (m, 2 H, 3-CH_2), 2.69 (d, 1 H, 4-$C^2H_SH_R$), 4.11 (t, 1 H, 2-C*H*).

(4*S*)-[4-^{2}H]-L-Homoserine Lactone Hydrochloride. To an aqueous solution of iodoacetic acid (75 mg, 0.4 mmol/3 mL) in a flask wrapped with aluminum foil was added (4*R*)-[4-^{2}H]-L-methionine (25 mg, 0.16 mmol). The reaction mixture was stirred magnetically at 45 °C for 14 h and then washed with ethyl acetate (2 mL × 3) to remove excess iodoacetic acid. After the pH of the aqueous layer was adjusted to 3.0 with 0.1 N NaOH, the water was removed in vacuo. The residue was dissolved in *dry* dimethylformamide (3 mL) and the solution was heated at 90 °C for 2 h. The DMF was removed in vacuo with a rotary evaporator equipped with a vacuum pump. The residue was dissolved in water (1 mL) and applied to a cation ion exchange column (Dowex 50 × W 200, H^+ form, 10 mL). The ninhydrin-positive fractions were combined and lyophilized to yield the desired lactone as a white solid (10 mg, 43%): ^{1}H NMR (D_2O) δ 2.43 (m, 1 H, 3-CH_SH_R), 2.80 (m, 1 H, 3-CH_RH_S), 4.43 (m, 1 H, 2-C*H*), 4.62 (d, J = 9 Hz, 1 H, 4-$C^2H_SH_R$).

(4*R*)-[4-^{2}H]-L-Homoserine Lactone Hydrochloride. The title compound was obtained utilizing the same procedure as described above for the degradation of the (4*R*)-[4-^{2}H]-L-methionine. The (4*R*)-[4-^{2}H]-L-homoserine lactone was obtained as a white solid (52%): ^{1}H NMR (D_2O) δ 2.43 (m, 1 H, 3-CH_SH_R), 2.80 (m, 1 H, 3-CH_RH_S), 4.42 (m, 1 H, 2-C*H*), 4.45 (d, 1 H, 4-$C^2H_RH_S$).

Methanesulfenyl Chloride. Methanesulfenyl chloride was prepared as described by Golding et al.[10] Dimethyl disulfide (9.4 g, 0.1 mol) was cooled to −20 °C in a CCl_4/dry ice slurry bath with magnetic stirring

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and sulfuryl chloride (14.3 g, 0.1 mol) was added slowly dropwise during a period of 2 h. The reaction mixture was allowed to warm to 25 °C over a 2-hour period. After 1 h at 25 °C, the resultant reaction mixture was distilled, with a water aspirator vacuum (120 mmHg, 25 °C) set-up with the receiving flasks cooled to 78 °C via dry ice/acetone, to give the title compound (7.0 g, 85%) as an orange oil: ^{1}H NMR ($CDCl_3$) δ 2.91 (s, 3 H, CH_3). This compound was used immediately after distillation and no attempt was made to store it.

(1*S*,2*R*)- and (1*R*,2*S*)-[1,2-$^2H_2$]-1-Chloro-2-(methylthio)ethane. Into a stirred solution of freshly distilled methanesulfenyl chloride (6 g, 7.3 mmol) in dichloromethane (30 mL) in a two-necked flask, equipped with fritted gas dispersion tube (Ace Glass Cat. No. 9435-08) in one neck and a thick-walled balloon secured around the other neck with a rubber band, was bubbled (*E*)-[$^2H_2$]ethylene in small bursts until no further absorption of gas occurred. In most runs the solution was colorless due to the use of a slight excess of ethylene. The dichloromethane was evaporated under reduced pressure (water aspiration) at 20 °C and the title compound was carefully distilled (50 mmHg, 70 °C) to give a colorless oil (4.73 g, 58%): ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3 H, SCH_3), 2.79 (d, J = 7.0 Hz, 1 H, SC^2HH), 3.60 (d, J = 7.2 Hz, 1 H, C^2HHCl).

(1*R*,2*R*)- and (1*S*,2*S*)-[1,2-$^2H_2$]-1-Chloro-2-(methylthio)ethane. The (*Z*)-[$^2H_2$]ethylene was reacted with methanesulfenyl chloride (6.0 g, 7.3 mmol) in a manner analogous to that described above to give the other diastereomeric ethane as a colorless oil (4.5 g, 55%): ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3 H, SCH_3), 2.80 (brs, 1 H SC^2HH), 3.60 (brs, 1 H, C^2HHCl).

(3*R*,4*R*)- and (3*S*,4*S*)-[3,4-$^2H_2$]-D,L-Methionine. A solution of sodium ethoxide was prepared by dissolving sodium (0.97 g, 42 mmol), under a nitrogen atmosphere, in absolute ethanol (35 mL). To the above solution was added diethyl acetamidomalonate (9.16 g, 33.7 mmol), and the reaction mixture was heated under reflux for 30 min. After cooling of the reaction mixture to 25 °C, (1*S*,2*R* + 1*R*,2*S*)-[1,2-$^2H_2$]-1-chloro-2-methylthioethane (4.73 g, 42.2 mmol) was added and the reaction mixture was heated under reflux for 12 h. The solvent from the reaction mixture was removed in vacuo and the residue was heated at reflux with 6 N HCl (55 mL) for 14 h. The water from the resultant reaction mixture was removed in vacuo with a rotary evaporator equipped with a vacuum pump. The residue was dissolved in water (5 mL) and applied to a cation ion exchange column (Dowex 50 × 200, NH_4^+ form, 200 mL). The column was eluted with water (1000 mL) followed by 1 N NH_4OH (200 mL). The fractions containing the ninhydrin-active fractions were freeze-dried to give the title compound as a slightly yellow, fluffy solid (3 g, 47.6%): mp 274–276 °C; ^{1}H NMR (D_2O) δ 2.13 (m, 4 H, SCH_3 and 3-C^2HH), 2.65 (d, 1 H, SC^2HH), 3.87 (d, 1 H, 2-CH).

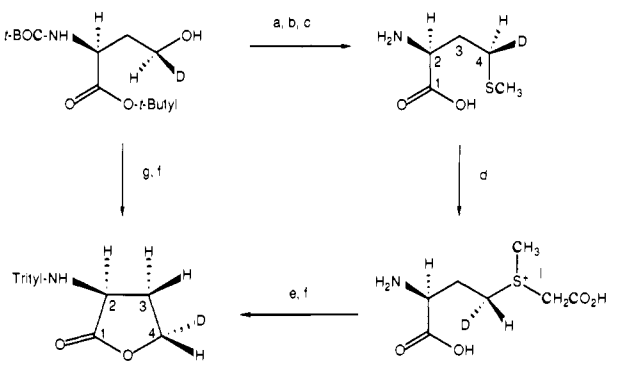
(3*S*,4*R*)- and (3*R*,4*S*)-[3,4-$^2H_2$]-D,L-Methionine. The (1*R*,2*R*)- and (1*S*,2*S*)-[1,2-$^2H_2$]-1-chloro-2-(methylthio)ethane (3.0 g, 26 mmol) was condensed with the sodium salt of diethyl acetamidomalonate (4.99 g, 23 mmol), and the crude diethyl acetamidomalonate derivative was hydrolyzed directly in 6 N HCl as previously described to give the target methionine (1.5 g, 43%): mp 273–275 °C; ^{1}H NMR (D_2O) δ 2.14 (m, 4 H, SCH_3 and 3-C^2HH), 2.65 (d, 1 H, S-C^2HH), 3.88 (d, 1 H, 2-CH).

(3*R*,4*S*)- and (3*S*,4*R*)-[3,4-$^2H_2$]-*N*-Trityl-D,L-Homoserine Lactone. To a solution of iodoacetic acid (150 mg, 0.8 mmol) in H_2O (5 mL) was added (3*R*,4*R*) and (3*S*,4*S*)-[3,4-$^2H_2$]-D,L-methionine (50 mg, 0.34 mmol). The reaction mixture was stirred at 45 °C overnight and the excess iodoacetic acid was removed by extracting with ethyl acetate (5 mL × 2). The aqueous layer was adjusted to pH 3 with 1 N NaOH and the water was removed in vacuo. The residue was suspended in dry DMF (10 mL) and the suspension was heated at 100 °C for 2 h. The solvent was removed in vacuo. The residue was applied to an ion-exchange column (Dowex 50 × 200, H^+ form, 20 mL) and the column was eluted with water (200 mL) followed by 1 N HCl (100 mL). Fractions containing homoserine lactone hydrochloride were combined and dried in vacuo.

To the above crude homoserine lactone dissolved in chloroform (5 mL) were added triphenylmethyl chloride (0.16 g, 0.6 mmol) and triethylamine (0.20 g, 2.0 mmol). The reaction mixture was stirred at 25 °C overnight. After removal of the solvent, the residue was chromatographed on a silica gel column (hexane/ethyl acetate = 9:1) to give the tritylated product, which was recrystallized from diethyl ether/chloroform (4:1) by the dropwise addition of petroleum ether to yield the target compound as colorless crystals (20 mg, 18%): ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 0.5 H, 3-CH_RH_S), 1.61 (m, 0.5 H, 3-CH_SH_R), 2.90 (brs, 1 H, N*H*), 3.40 (d, 1 H, 2-CH), 3.80 (d, 0.5 H, 4-CH_RH_S), 4.13 (s, 0.5 H, 4-CH_SH_R), 7.24 and 7.54 (m, 15 H, aromatic *H*).

(3*S*,4*S*)- and (3*R*,4*R*)-[3,4-$^2H_2$]-*N*-Trityl-D,L-homoserine Lactone. Similarly the compound (3*S*,4*R*)- and (3*R*,4*S*)-[3,4-$^2H_2$]-D,L-methionine (100 mg, 0.67 mmol) was converted, via a (carboxymethyl)methionine derivative, to the title lactone in 30% yield (22 mg) after recrystallization: ^{1}H NMR ($CDCl_3$) δ 1.18 (m, 0.5 H, 3-CH_RH_S), 1.61 (m, 0.5 H, 3-CH_SH_R), 2.90 (brs, 1 H, N*H*), 3.40 (d, 1 H, 2-CH), 3.80 (d, 0.5 H, 4-CH_RH_S), 4.13 (d, 0.5 H, 4-CH_SH_R), 7.24 and 7.54 (m, 15 H, aromatic *H*).

Scheme I[a]



[a] (a) *p*-Toluenesulfonyl chloride, pyridine, 25 °C, 6 h; (b) sodium methanethiolate, ethanol, heat at reflux, 16 h; (c) 6 N hydrochloric acid, heat at reflux, 16 h; (d) iodoacetic acid, water, 45 °C, 16 h; (e) DMF, 90 °C, 2 h; (f) trityl chloride, triethylamine chloroform, 25 °C, 16 h; (g) hydrochloric acid, ethanol, heat at reflux, 3 h.

Results and Discussion

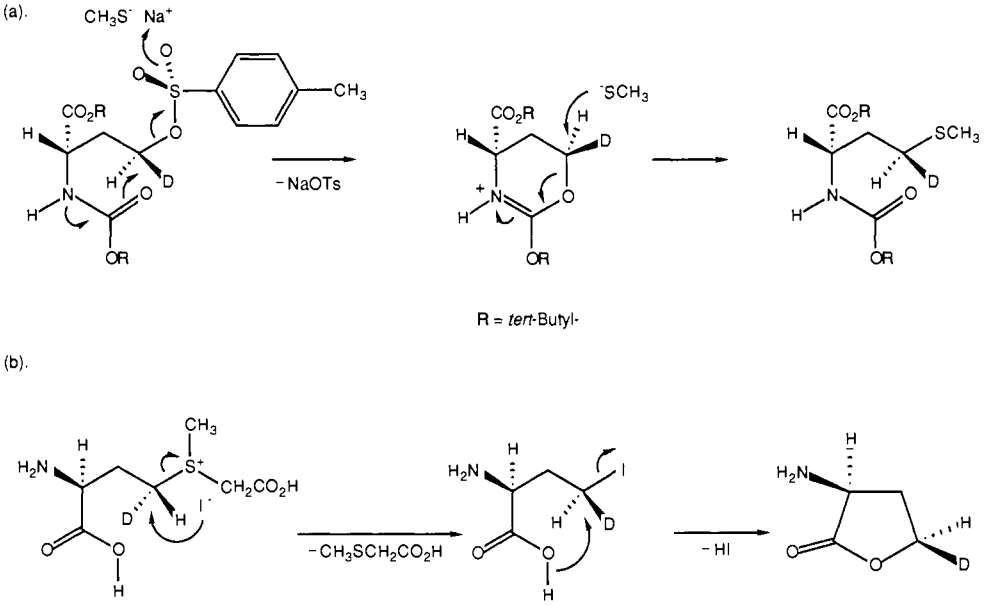
The tosylates of (4*S*)- and (4*R*)-*N*-*tert*-(butyloxycarbonyl)-[4-^2H]-L-homoserine *tert*-butyl esters[5] are transformed into (4*R*)- and (4*S*)-[4-^2H]-L-methionine, respectively, by the direct displacement of the tosylates with sodium methanethiolate (Scheme I). After deprotection, the methionines are converted back into their corresponding homoserine lactones by treatment with iodoacetic acid followed by thermal decomposition in anhydrous DMF as previously described.[6] The configuration at the C-4 position of homoserine lactone is determined by measurement of the ^{1}H NMR spectrum of the *N*-trityl derivative, a compound in which the chemical shift values of all the hydrogens are well separated and known unequivocally.[11,12] The configuration at the C-4 position of the final homoserine lactones is the same as in the original homoserine derivatives from which they are derived. These results *strongly* suggest that the stereochemical events of both reactions at the C-4 center have taken place by two S_N2-type reaction mechanisms, i.e. two inversions of configuration.

Chemically, it is possible that the same homoserine lactones could have been obtained by a set of two reactions in which the stereochemical events of both reactions have occurred with retention of configuration or apparent retention of configuration, i.e., two inversions of configuration. For example, one could envision that retention of configuration in the conversion of the homoserine *O*-tosylate derivative into methionine could be caused by neighboring participation of the relatively nucleophilic oxygen atom of the *tert*-butyloxycarbonyl protecting group of the C-2 nitrogen with the C-4 carbon atom (Scheme IIa). The mechanism of the degradation of these chiral methionines back to their homoserine lactones, via (carboxymethyl)methionine iodides, is most likely an internal S_N2-type reaction in which the carboxylate anion of the methionine derivative attacks the C-4 position. However, there are several other stereochemical reaction mechanisms which could be postulated that would give rise to overall retention of configuration at the C-4 center during the degradation step, such as an initial S_N2 attack of the iodide counterion at the chiral methylene carbon (adjacent to the sulfonium center) followed by a subsequent S_N2-type attack of the carboxylate anion of the methionine derivative to displace the iodide (Scheme IIb).

In order to distinguish between the various possible mechanisms discussed above, it is necessary that an independent method for the synthesis of either C-4 chirally labeled methionine or homoserine be utilized to ascertain the nature of the steric events of either of the two transformations. An excellent method for

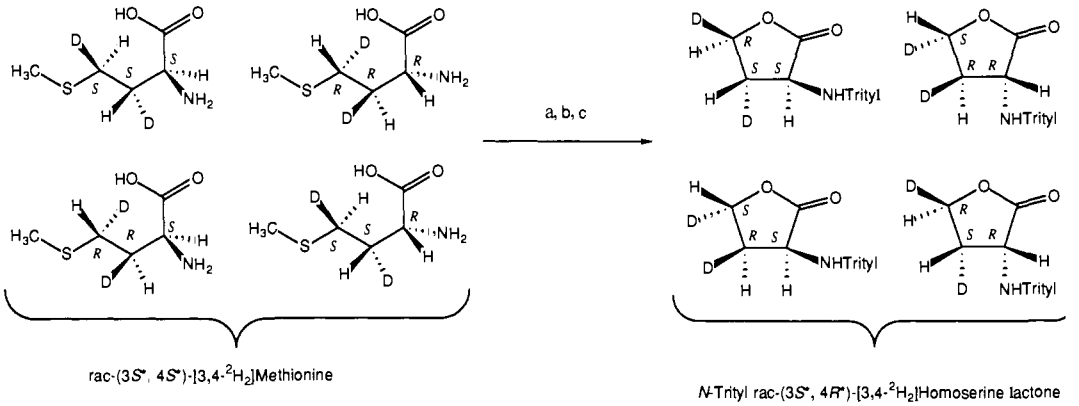
(11) Baldwin, J. E.; North, M.; Flinn, A. *Tetrahedron* **1988**, *44*, 637–642.
(12) Son, J.-K.; Kalvin, D.; Woodard, R. W. *Tetrahedron Lett.* **1988**, *29*, 4045–4048.

Scheme II[a]



[a] (a) Alternate stereochemical mechanism for the conversion of *N*-(*tert*-butyloxycarbonyl)-*O*-tosyl-(4*S*)-[4-^{2}H]-L-homoserine *tert*-butyl ester to *N*-(*tert*-butyloxycarbonyl)-(4*S*)-[4-^{2}H]-L-methionine butyl ester. (b) Alternate stereochemical mechanism for the conversion of the carboxymethyl iodide derivative of (4*R*)-[4-^{2}H]-L-methionine to (4*R*)-[4-^{2}H]-L-homoserine lactone hydrochloride.

Scheme III[a]



[a] (a) Iodoacetic acid, water, 45 °C, 16 h; (b) DMF, 90 °C, 2 h; (c) trityl chloride, triethylamine, chloroform, 25 °C, 16 h.

following the stereochemical events at one chiral methylene is to establish an adjacent chiral methylene center. The two chiral centers need then only be correlatable in a relative sense, i.e. the absolute stereochemical configuration of neither center is as important in the analysis as is their relative relationship. It was therefore decided to use this type of approach to determine the stereochemical reaction mechanism of the conversion of methionine to homoserine lactone via the intermediate (carboxymethyl)-methionine iodide since the ^{1}H NMR spectrum of the final product *N*-trityl-L-homoserine is significantly easier to analyze than is the ^{1}H NMR of stereospecifically deuterium-labeled methionine[6,11,12] and such methionine derivatives and their configurations have previously been reported.[10] The *rac*-(3*S**,4*S**)-[3,4-$^2H_2$]-methionine[13] and *rac*-(3*S**,4*R**)-[3,4-$^2H_2$]methionine[13] needed are synthesized from (*E*)-[$^2H_2$]ethylene and (*Z*)-[$^2H_2$]ethylene, respectively.[10] These stereochemically complex mixtures of methionine are well-suited for use in solving stereochemical mechanistic problems which involve methionine as substrate,[14-16] even though each mixture contains four stereoisomers, since there is only one hydrogen and one deuterium atom at both the C-3 and C-4 positions and in each of the separately prepared mixtures of the stereoisomers the configuration is always 3*S**,4*S** in one mixture and 3*S**,4*R** in the other. Therefore, it is possible from two experiments, one with each mixture, to extrapolate information about both the stereospecificity and kinetics of the reaction under study.

If the thermal decomposition of the carboxymethyl iodide of the *rac*-(3*S**,4*S**)-[3,4-$^2H_2$]methionine occurs, as would be predicted, with inversion of configuration at the C-4 position, *N*-trityl-*rac*-(3*S**,4*R**)-[3,4-$^2H_2$]homoserine lactone[13] should be formed after derivatization (Scheme III). Conversely the carboxymethyl iodide of *rac*-(3*S**,4*R**)-[3,4-$^2H_2$]methionine should give *N*-trityl-*rac*-(3*S**,4*S**)-[3,4-$^2H_2$]homoserine lactone.[13] Although each stereoisomeric mixture of lactones will contain four isomers, two sets of enantiomers, there is only one composite

(13) The following abbreviations are used to simplify discussion: *rac*-(3*S**,4*S**)-[3,4-$^2H_2$]methionine, (2*S*,3*S*,4*S*)-, (2*R*,3*R*,4*R*)-, (2*S*,3*R*,4*R*)-, and (2*R*,3*S*,4*S*)-[3,4-$^2H_2$]methionine; *rac*-(3*S**,4*R**)-[3,4-$^2H_2$]methionine, (2*S*,3*S*,4*R*)-, (2*R*,3*R*,4*S*)-, (2*S*,3*R*,4*S*)-, and (2*R*,3*S*,4*R*)-[3,4-$^2H_2$]-methionine; *N*-trityl-*rac*-(3*S**,4*R**)-[3,4-$^2H_2$]homoserine lactone, *N*-trityl-(2*S*,3*S*,4*R*)-, -(2*R*,3*R*,4*S*)-, -(2*S*,3*R*,4*S*)- and -(2*R*,3*S*,4*R*)-[3,4-$^2H_2$]homoserine lactone; *N*-trityl-*rac*-(3*S**,4*S**)-[3,4-$^2H_2$]homoserine lactone, *N*-trityl-(2*S*,3*S*,4*S*)-, -(2*R*,3*R*,4*R*)-, -(2*S*,3*R*,4*R*)-, and -(2*R*,3*S*,4*S*)-[3,4-$^2H_2$]-homoserine lactone.

(14) Golding, B. T.; Nassereddin, I. K. *J. Am. Chem. Soc.* **1982**, *104*, 5815-5817.

(15) Ramalingam, K.; Lee, K.-M.; Woodard, R. W.; Bleecker, A. B.; Kende, H. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7820-7824.

(16) Wiesender, R.; Martinoni, B.; Boller, T.; Arigoni, D. *Experientia* **1986**, *42*, 207-209.

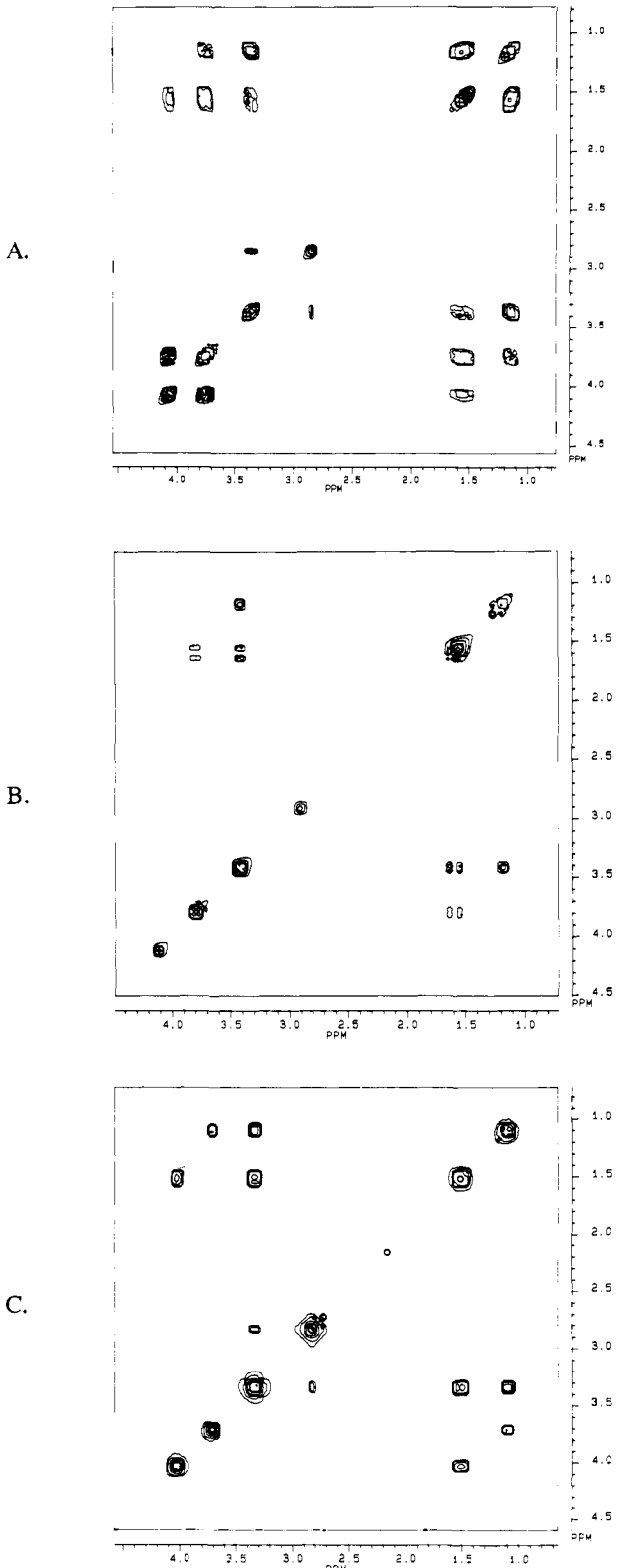


Figure 1. The 270-MHz $^1H-^1H$ COSY of (A) upper panel, unlabeled *N*-trityl-L-homoserine lactone; (B) middle panel, the decomposition product of the *rac*-(3*S**,4*S**)-[3,4-$^2H_2$]methionine derivative after tritylation; (C) lower panel, the decomposition product of the *rac*-(3*S**,4*R**)-[3,4-$^2H_2$]methionine derivative after tritylation.

$^1H-^1H$ COSY spectrum associated with each of the *N*-trityl-D,L-homoserine lactone mixtures which consists of the separate $^1H-^1H$ COSYs of the individual diastereomers present in each mixture.[17] The $^1H-^1H$ COSY spectrum of unlabeled *N*-trityl-L-homoserine lactone is shown in the upper panel of Figure 1. The $^1H-^1H$ COSY spectrum of *N*-trityl-(3*S**,4*R**)-[3,4-$^2H_2$]homoserine lactone (shown in Scheme III), however, will not show $^2J_{HH}$ cross peaks but should show four different types of $^3J_{HH}$ cross peaks, one between the 4*S* hydrogen (δ = 3.8 ppm) and the 3*R* hydrogen (δ = 1.6 ppm) and one between the 2*S* hydrogen (δ = 3.4 ppm) and the 3*R* hydrogen (δ = 1.6 ppm) in the 2*S* member of one diastereomeric pair (i.e. *N*-trityl-(2*S*,3*S*,4*R*)-[$^2H_2$]homoserine lactone) as well as one between the 4*R* hydrogen (δ = 4.1 ppm) and 3*S* hydrogen (δ = 1.2 ppm) and one between the 2*S* hydrogen (δ = 3.4 ppm) and 3*S* hydrogen (δ = 1.2 ppm) in the 2*S* member of the other diastereomeric pair (i.e. *N*-trityl-(2*S*,3*R*,4*S*)-[$^2H_2$]homoserine lactone).[17] However, in *N*-trityl-L-homoserine lactone[12] the *pro*-4 *R* hydrogen (δ = 4.1 ppm) is orthogonal to the *pro*-3 *S* hydrogen (δ = 1.2 ppm); therefore, no coupling between the peaks at 4.1 ppm and 1.2 ppm is observed, as can be seen in Figure 1A. On the basis of arguments similar to those presented above, the $^1H-^1H$ COSY spectrum of *rac*-*N*-trityl-(3*S**,4*S**)-[3,4-$^2H_2$]homoserine lactone will show four cross peaks; one between δ = 4.1 ppm and δ = 1.6 ppm, one between δ = 3.8 ppm and δ = 1.2 ppm, one between δ = 3.4 ppm and δ = 1.6 ppm, and one between δ = 3.4 and 1.2 ppm.

Since the $^1H-^1H$ COSY spectrum of the decomposition product of the *rac*-(3*S**,4*S**)-[3,4-$^2H_2$]methionine derivative, after tritylation, shown in the middle panel of Figure 1, contains the three predicted cross peaks for *rac*-*N*-trityl-(3*S**,4*R**)-[3,4-$^2H_2$]-homoserine lactone and the decomposition product of the *rac*-(3*S**,4*R**)-[3,4-$^2H_2$]-methionine derivative, after tritylation, shown in the lower panel of Figure 1, contains the four predicted cross peaks for *rac*-*N*-trityl-(3*S**,4*S**)-[3,4-$^2H_2$]homoserine lactone, the decomposition of the carboxymethyl)methionine iodide to homoserine lactone occurs with an *inversion of configuration* at the C-4 position.

Furthermore, since the [4-^2H]-L-methionines, chiral at the C-4 position due to deuterium substitution, derived from the (4*S*)- and (4*R*)-[4-^2H]-L-homoserine derivatives were converted back to (4*S*)- and (4*R*)-[4-^2H]-L-homoserine lactone, respectively, by the iodoacetic acid reaction pathway described above, the displacement of the *O*-tosylate anion from the C-4 position of the homoserine derivative by the sodium methanethiolate also proceeds with *inversion of configuration.*

In conclusion, the stereochemistry for the degradation of methionine to homoserine has been firmly established to be inversion of configuration as previously assumed and therefore allows the conclusions reached to stay as reported.[6] In addition, the stereochemistry of the conversion of the readily obtainable tosylates of (4*S*)- and (4*R*)-*N*-*tert*-butyloxycarbonyl)-[4-^2H]-L-homoserine *tert*-butyl esters to their respective methionines has been unequivocally established. Since homoserine and methionine (or its biologically active form (*S*)-adenosyl-L-methionine) serve as the precursor(s) for a number of biologically important compounds derived from the four-carbon portion of the amino acid moiety, the investigation of the stereochemical events involved in these transformations should now be relatively straightforward on the basis of the data presented herein.

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(17) In order to simplify the discussion of the $^1H-^1H$ COSY and the stereochemical position of each of the hydrogens involved, only the 2*S* isomer is discussed. The same arguments apply to the 2*R* isomer, the only difference being the description of the stereochemistry of the hydrogens attached to the C-2, C-3, C-4 position, which are the enantiomers of the ones discussed in the text. The necessity of this footnote to explain both the stereochemical positions and the ^1H NMR is the only drawback to the use of these methionines as stereochemical probes.