Hz), 2.36–1.41 (7 H, m), 1.84 (3 H, d, J = 1.2 Hz).

To a solution of the above selenide (395 mg, 0.985 mmol) in CH_2Cl_2 (10 mL) containing pyridien (0.1 mL) were added at 0 °C three 0.1-mL portions of 15% hydrogen peroxide at 15-min intervals. The reaction mixture was stirred at 0 °C for 1 h, quenched with CH_2Cl_2 (40 mL), and washed successively with saturated $NaHCO_3$, brine, and water. The organic layer was dried over Na_2SO_4 and evaporated in vacuo to give crude (R)-3-methyl-7-(3-butenyl)-7,7a-dihydro-1,6-dioxa-s-indacen-5(8H)-one: IR (neat) 1740, 1630, 1585, 1515, 900 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.95 (1 H, q, J = 1.8 Hz), 5.85–5.31 (1 H, m), 5.04 (1 H, s), 5.01–4.74 (2 H, m), 4.36–4.05 (1 H, m), 3.00–2.84 (2 H, m), 2.15–0.85 (5 H, m), 1.68 (3 H, d, J = 1.8 Hz).

The crude product was dissolved in dry benzene (15 mL) under Ar, and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) (268 mg, 1.18 mmol) was added in benzene (9 mL). The mixture was stirred at room temperature for 40 min and refluxed for 1 h. The residue was passed through a short pad of alumina with CH_2Cl_2 as the eluent and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel by using hexane/ethyl acetate (9:1) to give **11** (91 mg, 38%) as colorless viscous oil: IR ($CHCl_3$) 1750, 1640, 1370, 1090 cm^{-1}; 270-MHz ^{1}H NMR ($CDCl_3$) δ 8.07 (1 H, d, J = 0.5 Hz), 7.53 (1 H, q, J = 1.2 Hz), 7.43 (1 H, t, J = 1.2 Hz), 5.87–5.81 (1 H, m), 5.56 (1 H, dd, J = 8.2, 3.0 Hz), 5.13–5.00 (2 H, m), 2.29 (3 H, d, J = 1.2 Hz), 2.27–2.17 (2 H, m), 2.25–2.21 (2 H, m); MS, m/z (relative intensity) 242 (M^+, 11.9) 200 (M^+ – C_3H_6, 100); high resolution MS calcd for $C_{15}H_{14}O_3$ 242.0942, found 242.0946; $[\alpha]^{26}_D$ +70.79° (c 1.26, $CHCl_3$).

(*R*)-4-Oxo-5,6,9,10-tetradehydro-4,5-secofuranoeremophilane-5,1-carbolactone (1). Cuprous chloride (21.1 mg, 0.21 mmol) and palladium chloride (7.1 mg, 0.04 mmol)) were suspended in DMF (1 mL) containing a trace of H_2O. The mixture was stirred vigorously under oxygen atmosphere until absorption of oxygen ceased (about for 3 h). Then **11** (52 mg, 0.21 mmol) dissolved in DMF (2 mL) was added and the mixture was stirred vigorously under oxygen at room temperature for 2 h. The reaction mixture was poured into 3 N HCl, extracted with ether (3 × 30 mL), and washed successively with saturated $NaHCO_3$ and brine (2 × 10 mL). The solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel using hexane/ethyl acetate (3:1) to give **1** (52 mg, 94%) as colorless crystals: mp 105–106 °C (ether/n-hexane); UV λ_{max} (ether) 228 nm (log ϵ 4.64), 248 (3.81), 263 sh (3.61), 295 (3.41), 304 sh (3.27); IR (CCl_4) 1705, 1760 cm^{-1}; 270-MHz ^{1}H NMR ($CDCl_3$) δ 8.06 (1 H, d, J = 0.8 Hz), 7.54 (1 H, q, J = 1.3 Hz), 7.47 (1 H, t, J = 0.8 Hz), 5.58 (1 H, ddd, J = 8.5, 3.0, 0.8 Hz), 2.81–2.70 (1 H, m), 2.61–2.45 (2 H, m), 1.98–1.84 (1 H, m), 2.29 (3 H, d, J = 1.3 Hz), 2.16 (3 H, s); MS, m/z (relative intensity) 258 (M^+, 15.6), 200 (M^+ – C_3H_6O, 100); high resolution MS calcd for $C_{15}H_{14}O_4$ 258.0891, found 258.0890; $[\alpha]^{27}_D$ +75.6° (c 1.05, $CHCl_3$). Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.74; H, 5.47. Found: C, 69.88; H, 5.58.

Probing Ergot Alkaloid Biosynthesis: Synthesis and Feeding of a Proposed Intermediate along the Biosynthetic Pathway. A New Amidomalonate for Tryptophan Elaboration

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The total synthesis of the diastereomeric amino acids **2** and their N-trideuteriomethyl analogues has been carried out. These compounds represent possible intermediates along the biosynthetic pathway from 4-(γ,γ-dimethylallyl)tryptophan (**1**) to the ergot alkaloids (e.g., **3a**). The synthetic scheme features the preparation of an (indolylvinyl)metallic reagent from 4-ethynylindole via a hydrostannylation/metal–metal exchange sequence, as well as the preparation of dimethyl [N-methyl-N-[(2,2,2-trichloroethoxy)carbonyl]amino]malonate, a new amidomalonate reagent for tryptophan elaboration. Incorporation experiments with *Claviceps sp.* SD58 followed by GC–MS analysis of the major alkaloid, elymoclavine, showed that neither diastereomer of **2**-d_3 is an ergot alkaloid precursor.

The ergot alkaloids represent a pharmacologically interesting class of natural products that find important clinical use and that consequently still command the attention of both synthetic and medicinal chemists.[1]

In spite of the considerable efforts that have been devoted to understanding the biosynthesis of the ergot alkaloids, a number of unsolved problems remain.[2a] Tryptophan was established as a precursor to the ergot alkaloids by Mothes et al. in 1958,[2b] and Floss et al. showed that the L isomer is incorporated with almost complete retention of the α hydrogen and the amino nitrogen.[2c] Further studies gave support to the idea that R-mevalonic acid is then incorporated into the ergot alkaloids by way of dimethylallyl pyrophosphate.[2d] An enzyme-catalyzed reaction between tryptophan and dimethylallyl pyrophosphate thus provides 4-(γ,γ-dimethylallyl)tryptophan (DMAT, **1**) as the first pathway-specific intermediate in ergot biosynthesis. While other studies have convincingly demonstrated that DMAT is converted to elymoclavine

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(1) Floss, H. G. *Tetrahedron* **1976**, *32*, 873.
(2) (a) Floss, H. G.; Anderson, J. A. In *The Biosynthesis of Mycotoxins*; Steyn, P. S., Ed.; Academic Press: New York, 1980; pp 17–67. (b) Mothes, K.; Weygand, F.; Gröger, D.; Grisebach, H. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1958**, *13*, 41. (c) Floss, H. G.; Mothes, U.; Günther, H. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1964**, *19*, 784. (d) Gröger, D.; Mothes, K.; Simon, H.; Floss, H. G.; Weygand, F. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1960**, *15*, 141. (e) Gröger, D.; Erge, D.; Floss, H. G. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1966**, *21*, 827. (f) Fehr, T.; Acklin, W.; Arigoni, D. *J. Chem. Soc., Chem. Commun.* **1966**, *801*. (g) Floss, H. G.; Hornemann, U.; Schilling, N.; Kelley, K.; Gröger, D.; Erge, D. *J. Am. Chem. Soc.* **1968**, *90*, 6500. (h) Fehr, T., Ph.D. Thesis, ETH Zürich, Switzerland, 1967. Pachlatko, P., Ph.D. Thesis, No. 5481, ETH Zürich, Switzerland, 1975.

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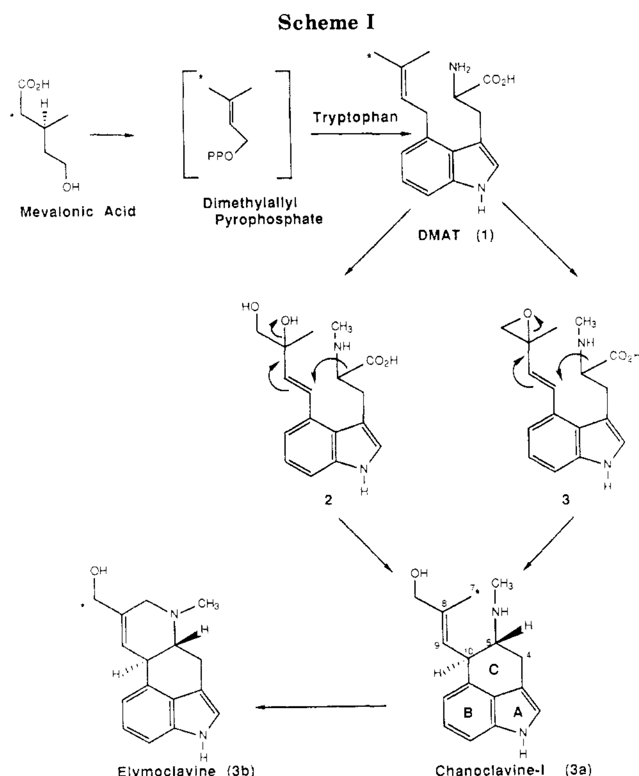
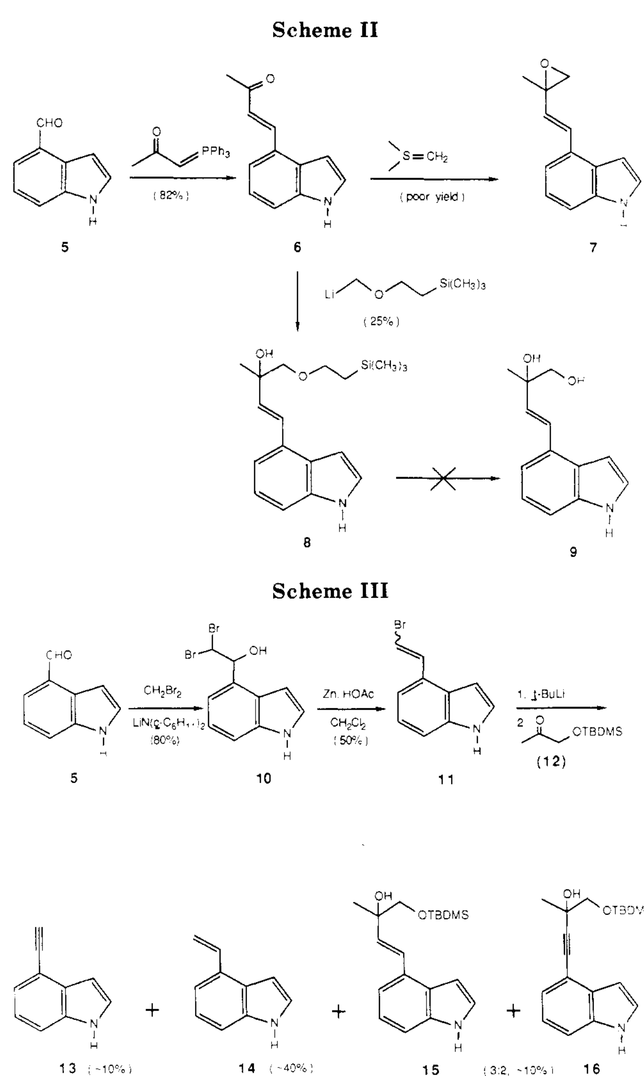
Scheme I



Scheme II

Scheme III



by way of chanoclavine I and agroclavine,[2e] the mechanism of C-ring formation of chanoclavine I remains at best somewhat tenuous. A cis–trans isomerization reaction apparently occurs in the formation of chanoclavine I from dimethylallyl pyrophosphate, for C-2 of mevalonate becomes the *trans*-methyl group of dimethylallyl pyrophosphate, while this carbon becomes the *cis*-methyl group of chanoclavine I (Scheme I).[2f,g]

Observations by Arigoni's group that deoxychanoclavine I, nordeoxychanoclavine I, and 4-(4-hydroxy-3-methyl-2-butenyl)tryptophan are not intermediates in the biosynthesis of the ergot alkaloids[2h] suggest that hydroxylation of either of the methyl groups in the isoprene unit can not be the first step after formation of DMAT and that oxidation of the *cis*-methyl group of the isoprene unit must precede C-ring formation.[2a] These studies together with observations by Floss and co-workers regarding the high specific incorporation of $[^{15}N\text{-}CD_3]$DMAT into elymoclavine[3] led to the suggestion that the diol **2** might serve as a likely precursor to chanoclavine I by way of an S_N2'-like ring closure coupled with a decarboxylation reaction.[4] Arigoni had also suggested the possibility that the epoxide **3** (modified to include the *N*-methyl group) might serve as an alternative reactive intermediate in C-ring synthesis.[5] Since the N-methylation step precedes C-ring formation, it is unlikely that pyridoxal phosphate catalysis is involved in the decarboxylation–ring closure process. The mechanistic details of this C–C bond-forming step would thus appear open to considerable speculation.

In order to probe the possibility that **2** is an actual intermediate in ergot alkaloid biosynthesis, we have designed a total synthesis approach to the diastereomeric forms of this molecule. The synthesis of these compounds and their *N*-trideuteriomethyl analogues is detailed herein along with the results of the incorporation studies.

Synthesis Scheme. From earlier investigations into the total synthesis of the ergot alkaloids, 4-formylindole (**5**) has proven to be a useful starting material for the construction of a variety of 3,4-disubstituted indoles.[6] This material was again chosen as the starting point for our synthesis of **2** (a diastereomeric mixture). Elaboration of the appendage at the 4-position of the indole ring was planned first, followed by introduction of the amino acid chain at C-3. A large body of earlier work in the tryptophan area provides certain useful guidelines in choosing methods for transforming a 4-substituted indole to its tryptophan derivative.[7] Of course, some decision would have to be rendered regarding the stage at which the *N*-methyl substituent would be affixed.

Initially, we attempted to carry out an epoxide-forming reaction on the α,β-unsaturated ketone **6**, a compound easily prepared from **5** by reaction with acetonylidenetriphenylphosphorane. While exposure of this enone to dimethylsulfonium methylide[8] provided some of the desired epoxide **7**, the yield was quite dismal. The addition of [[2-(trimethylsilyl)ethoxy]methyl]lithium[9] to **6**, on the

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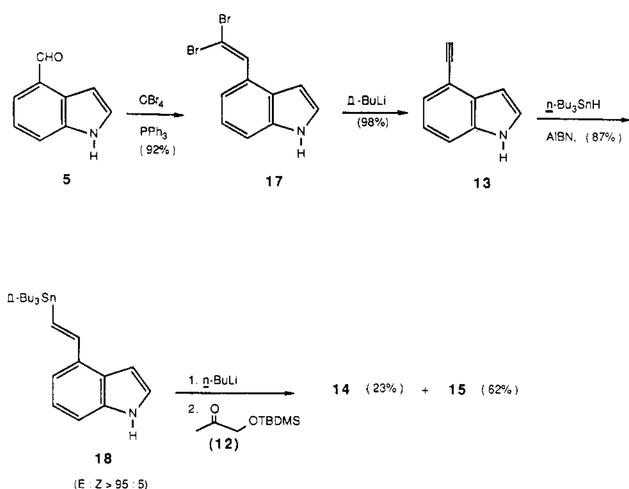
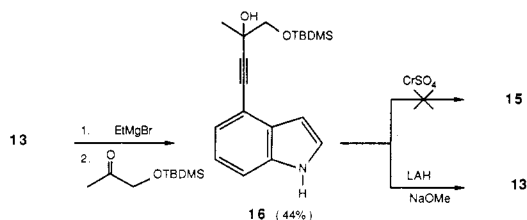
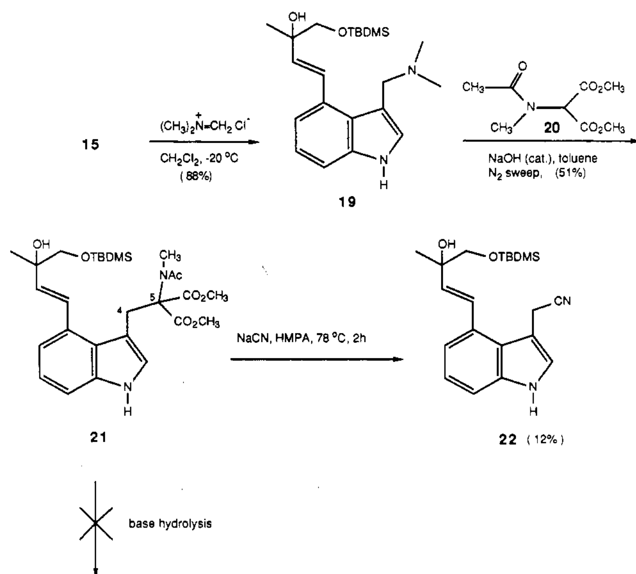
Scheme IV



Scheme V



Scheme VI



other hand, proceeded in moderate yield, but removal of the oxygen protecting group from **8** to generate the free diol **9** could not be achieved (Scheme II).

Faced with these difficulties, we decided to install the C-4 appendage in a more piecemeal fashion. The preparation of an indolylvinylmetallic reagent followed by its addition to a protected derivative of hydroxyacetone appeared reasonable. Accordingly, **5** was treated with (dibromomethyl)lithium,[10] and the resulting alcohol **10** was submitted to a zinc/acetic acid reduction to provide an E/Z mixture of vinyl bromides **11**.[11] On halogen–metal exchange and trapping with [(*tert*-butyldimethylsilyl)oxy]acetone (**12**), **11** was transformed to a potpourri of products **13–16**. As seen in Scheme III, the desired olefin **15** was formed as a mixture in but 6% yield.

Attempts were therefore made to generate the same vinylmetallic reagent by a metal–metal exchange process. 4-Formylindole was reacted with (dibromomethylene)triphenylphosphorane (from CBr_4, Ph_3P)[12] and the resulting dibromoolefin **17** treated in turn with *n*-butyllithium to deliver the acetylene **13** in 90% overall yield. Hydrostannylation of the alkyne provided a >95:5 mixture of the (*E*)- and (*Z*)-vinylstannanes **18** in 87% yield. Metal–metal exchange (*n*-BuLi, THF, −78 °C)[13] followed by reaction with the (silyloxy)acetone **12** provided the desired C-4 functionalized indole **15** in a workable yield of 62% (Scheme IV).

4-Vinylindole (**14**) generated as a side product in this reaction is presumably formed through proton abstraction from the (silyloxy)acetone in lieu of addition. Attempts to reduce the amount of this undesired product through variation of the metal counterion, solvent, reaction temperature, etc., were unsuccessful.

While we also investigated the addition chemistry of the magnesium and lithium acetylides of **13** with (silyloxy)acetone **12**, we were unable to effectively transform the triple bond of the propargylic alcohol product **16** to a double bond using standard reducing agents (Scheme V).[14]

Thus, the hydrostannylation route appeared to offer the best solution to the elaboration of the substituent at the 4-position of the indole ring.

To affix the amino acid appendage, we decided to use a fairly conventional amidomalonate displacement reaction on the corresponding gramine derivative of **15**.[7] Due to the fairly sensitive functionality present in **15**, the use of a preformed iminium salt was required for gramine formation. The reaction of **15** with dimethylmethyleneammonium chloride in methylene chloride at −20 °C provided **19** in 88% yield.[15] While its reaction with dimethyl (*N*-acetyl-*N*-methylamino)malonate (**20**)[16] using sodium hydroxide as a catalyst in toluene at reflux temperature with a nitrogen sweep[17] gave **21** in a modest 51% yield, all attempts (e.g., NaOH, MeOH, H_2O) to cleave the *N*-acetyl group with concomitant ester saponification and decarboxylation led primarily to rupture of the C-4/C-5 (ergot numbering) bond, since **20** was detected in the reaction mixture by TLC and NMR analyses. None of the desired acid **2a/2b** could be isolated. Exposure of **21** to sodium cyanide[18] also proved unsuccessful, for the same fragmentation reaction intervened resulting in formation of the indoleacetonitrile **22** in 12% yield. Finally, attempts to cleave the *N*-acetyl group from **21** with triethyloxonium tetrafluoroborate also proved unsatisfactory (Scheme VI).[19]

We were thus led to consider the preparation of several other (*N*-methylamido)malonate derivatives in which the nitrogen protecting group could be removed under neutral reaction conditions. The two compounds **24** and **26** were thus prepared in which the (trichloroethoxy)carbonyl group replaced the *N*-acetyl protecting group on the ni-

(9) The [[2-(trimethylsilyl)ethoxy]methyl]lithium was first prepared in these laboratories by Kirk Sorgi using procedures analogous to those described by Still: Still, W. C. *J. Am. Chem. Soc.* **1978**, *100*, 1481.
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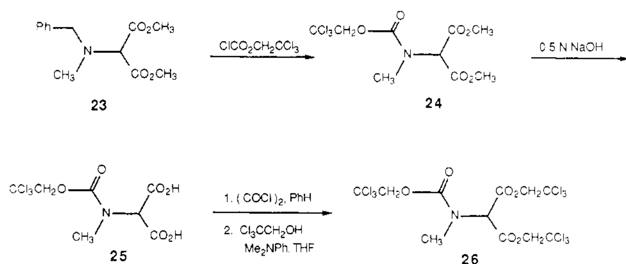
(19) Kozikowski, A. P.; Ishida, H. *J. Am. Chem. Soc.* **1980**, *102*, 4265.

Table I. GC-Mass Spectral Analysis of Elymoclavine from Feeding Experiments with Labeled Materials Using *Claviceps sp.*, Strain SD 58

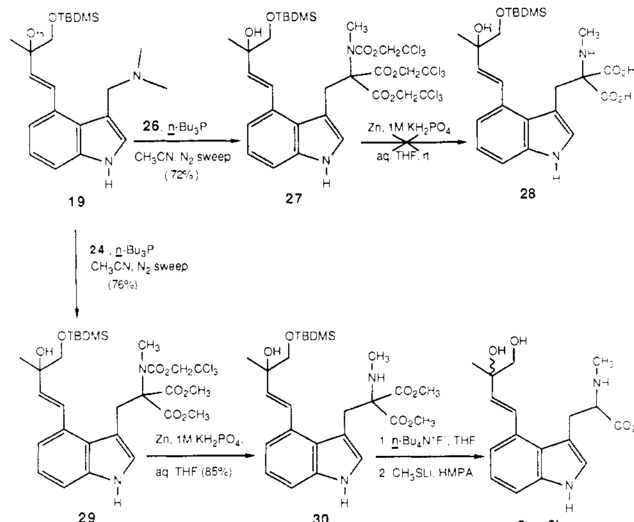
compound analyzed	relative abundance of isotopic species (%) m/z 253	m/z 254[a]	m/z 255	m/z 256	m/z 257	m/z 258	incorporation (%)
elymoclavine (**3b**)	100	49	7	1			
3b from **2a**-d_3	100	54	8				0
3b from **2b**-d_3	100	61	9	1			0
3b from $^{13}C^2H_3$-methionine	100	49	14	8	76	35	43

[a] Molecular ion.

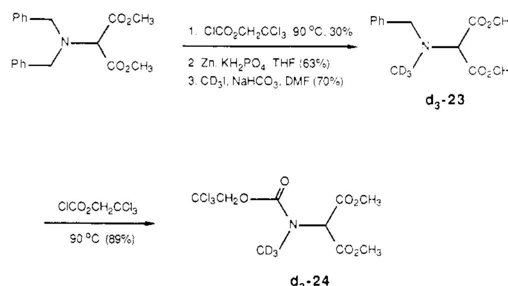
Scheme VII



Scheme VIII



Scheme IX



trogen atom. Additionally, in the case of **26** the methyl groups of the ester residues were replaced by trichloroethyl groups. Both of these new reagents were prepared from the known (*N*-benzyl-*N*-methylamino)malonate **23**.[16] Accordingly, treatment of this aminomalonate with (2,2,2-trichloroethoxy)carbonyl chloride delivered **24**,[20] which was saponified to the corresponding diacid and converted to the diacid chloride with oxalyl chloride. Reaction of the diacid chloride with 2,2,2-trichloroethanol then afforded reagent **26** (Scheme VII).

While **26** reacted with the gramine derivative **19** in the presence of tri-*n*-butylphosphine as catalyst[21] to furnish the (indolylmethyl)malonate **27**, subsequent attempts to cleave simultaneously the nitrogen and acid protecting groups as indicated in Scheme VIII failed to provide **28**.

Likewise, the amidomalonate **24** could be coupled with gramine **19** by the tri-*n*-butylphosphine method to furnish **29**. Desilylation of **29** using tetra-*n*-butylammonium fluoride resulted, however, in the formation of a complex mixture from which **24** could again be isolated in low yield. Treatment of **29** with zinc and 1 M potassium dihydrogen phosphate[22] in THF led, on the other hand, to the N-deprotected aminomalonate **30** in an acceptable 85% yield. On exposure of **30** to tetra-*n*-butylammonium fluoride to effect desilylation and then to lithium methylmercaptide in HMPA[23] to bring about ester cleavage and decarboxylation, a 1:1 mixture of the desired diastereomeric amino acids **2a** and **2b** was formed. This mixture could be separated readily by reverse-phase preparative TLC. Other methods that were examined for the conversion of the ester **30** to the acids **2a**/**2b** proved less satisfactory (Scheme VIII).

The *N*-trideuteriomethyl analogues of **2a** and **2b** were accordingly synthesized in an identical fashion by substituting *N*-(trideuteriomethyl)-**24** for **24** in Scheme VIII. The sequence of reactions employed to prepare **24**-d_3 is shown in Scheme IX.

Feeding Studies. The deuteriated samples of **2a** and **2b** were fed to replacement cultures of *Claviceps sp.*, strain SD58 in phosphate buffer, which were incubated for 2 days. For comparison a parallel culture was incubated with D,L-mevalonic acid, L-tryptophan, and L-[$^{13}C^2H_3$]-methionine. The total alkaloids were then isolated by solvent partitioning and chromatographed on a column of alumina,[3] and the fraction of the major alkaloid, elymoclavine (**3b**), was subjected to GC–MS analysis. The results are summarized in Table I. Under electron-impact conditions **3b** gives a very strong M − 1 peak (m/z 253), which together with the molecular ion allows ready analysis of the isotopic composition. The samples of **3b** derived from **2a**-d_3 and **2b**-d_3 showed essentially the same mass spectrum as the unlabeled reference compound, indicating no significant incorporation. Although negative results of feeding experiments always have to be interpreted with caution, the control experiment showing substantial incorporation of the methyl group of methionine demonstrates that the biosynthetic system was working properly. In a variety of analogous experiments[1,2] precursors of similar structure and complexity (e.g., DMAT, the corresponding amine, *N*-Me-DMAT, and the corresponding amine, and various tricyclic and tetracyclic alkaloids) have been successfully used in feeding experiments with the ergot fungus. The past results have always been clearcut: compounds that are intermediates were efficiently incorporated (25–50% specific incorporation); those that are not were not significantly incorporated (less than 1–2%

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specific incorporation). Hence we conclude that there are no permeability barriers to such compounds and that compounds **2a** and **2b** are probably not intermediates on the biosynthetic pathway to ergot alkaloids.

In summation, the chemistry described herein provides a reliable method for preparing a proposed intermediate in ergot alkaloid biosynthesis. All of the steps with the exception of the final ester cleavage/decarboxylation reaction proceed in good yield. Biological evaluation of the labeled material showed that the compound is not, however, a biosynthetic intermediate. Additional efforts to prepare and to test other potential intermediates derived from DMAT (1) are underway.

Experimental Section

^{1}H NMR spectra were recorded on a Varian T-60, Varian EM-360, or Bruker WH-300 spectrometer using tetramethylsilane as the internal standard. Infrared spectra were recorded on a Perkin-Elmer 137 IR spectrophotometer with the polystyrene absorption at 1602 cm^{-1} as the reference. Low-resolution MS were determined on a LKB-9000 instrument. High-resolution MS were determined on a Varian MAT CH-5DF instrument by peak matching.

Gravity column chromatography and flash column chromatography were carried out on E. Merck 0.063–0.200 mm and 0.040–0.063 mm silica gel, respectively. Analytical TLC was performed on E. Merck silica gel 60 F-254 on plastic or aluminum supported plates. Alumina column chromatography was carried out on Woelm neutral alumina, while analytical TLC was performed on E. Merck aluminum oxide F-254 neutral type T 0.20 mm aluminum supported plates. Reverse-phase TLC was carried out on Analtech RPS-F glass plates. Distilled reagent-grade solvents were used for all chromatographic separations.

Benzene, toluene, and hexamethylphosphoramide were distilled from calcium hydride. Diethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Dichloromethane was dried either by passage through a column of activity I neutral alumina or by distillation from P_2O_5. Acetonitrile was distilled from P_2O_5.

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

All compounds described below were judged to be of at least 95% purity as ascertained by high field NMR analysis.

4-(2,2-Dibromoethenyl)indole (17). To a solution of indole-4-carboxaldehyde (1.15 g, 7.92 mmol)[24] in CH_2Cl_2 (240 mL) under a nitrogen atmosphere at salt–ice bath temperatures was added triphenylphosphine (12.5 g, 47.7 mmol). After dissolution of the phosphine had taken place, carbon tetrabromide (7.88 g, 23.8 mmol) was added. After being stirred for 2 h, the resulting pale brown solution was washed with saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was chromatographed on silica gel with 20% ethyl acetate–hexane as eluent to afford 2.2 g (92%) of the title compound as a pale yellow oil. This oil solidified in the refrigerator and gradually darkened in color when left at room temperature: R_f 0.30 (silica gel, 25% ethyl acetate–hexane); mp 39–40 °C; IR (film) 3440, 1490, 1420, 1400, 1325, 1260, 1195, 1155, 1105, 1075, 830, 745 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (br s, 1 H), 7.83 (s, 1 H), 7.53 (br d, 1 H, J = 7.3 Hz), 7.41 (d, 1 H, J = 8.1 Hz), 7.29–7.20 (m, 2 H), 6.59 (m, 1 H); MS (70 eV), m/z 303, 301, 299 (each M$^+$), 222, 220, 195, 193, 141 (base); HRMS calcd for $C_{10}H_7N^{79}Br^{81}Br$ 300.8925, found 300.8917.

4-Ethynylindole (13). To a solution of 4-(2,2-dibromoethenyl)indole (2.20 g, 7.31 mmol) in THF (70 mL) cooled to −78 °C under an argon atmosphere was added n-BuLi (1.55 M in hexane, 18.9 mL, 29.3 mmol). After 1 h saturated aqueous NH_4Cl was added at −78 °C and the resulting mixture was allowed to warm to room temperature. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with 10% ethyl acetate–hexane as eluent to afford 4-ethynylindole (1.01 g, 98%) as a pale brown solid that was recrystallized from hexane: R_f 0.20 (silica gel, 25% ethyl acetate–hexane); mp 66–66.5 °C; IR ($CHCl_3$) 3490, 3300, 3000, 2100, 1490, 1415, 1400, 1340, 1325, 1260, 1100, 1075, 1045, 895, 830 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (br s, 1 H), 7.41 (d, 1 H, J = 8.0 Hz), 7.32 (two pairs of d, 1 H, J = 7.5 Hz), 7.28 (m, 1 H), 7.15 (m, 1 H), 6.74 (m, 1 H), 3.31 (s, 1 H); MS (70 eV), m/z 141 (M$^+$, base), 114, 113, 88, 70, 63; HRMS calcd for $C_{10}H_7N$ 141.0578, found 141.0578.

4-[(*E*)-2-(Tributylstannyl)ethenyl]indole (18). A mixture of 4-ethynylindole (2.57 g, 18.2 mmol), n-Bu_3SnH (5.3 g, 18.2 mmol), and AIBN (40 mg) was heated at 90 °C under a nitrogen atmosphere for 1 h. The resulting pale brown oil was purified by chromatography on aluminum oxide eluting first with 25% ethyl acetate–hexane and then with 50% ethyl acetate–hexane to furnish 6.87 g (87%) of the title compound as a pale brown oil containing <5% of the Z isomer by ^{1}H NMR analysis: R_f 0.32 (aluminum oxide, 25% ethyl acetate–hexane), 0.46 (silica gel, 25% ethyl acetate–hexane); IR ($CHCl_3$) 3500, 2960, 2940, 2870, 1460, 1340, 1080, 985 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.18 (br s, 1 H), 7.31 (d, 1 H, J = 19.6 Hz), 7.31–7.16 (m, 4 H), 7.00 (d, 1 H, J = 19.6 Hz), 6.81 (m, 1 H), 1.8–1.6 (m, 6 H), 1.45–1.3 (m, 6 H), 1.05–0.9 (m, 15 H); mass spectrum (15 eV), m/z 434, 432, 430 (each M$^+$), 377 (base), 375, 373, 143. A pure sample of the Z isomer obtained by chromatography on aluminum oxide displayed the following spectral characteristics: R_f 0.51 (aluminum oxide, 25% ethyl acetate–hexane); IR ($CHCl_3$) 3500, 2960, 2870, 1460, 1340, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (br s, 1 H), 7.98 (d, 1 H, J = 13.6 Hz), 7.34–7.02 (m, 4 H), 6.61 (m, 1 H), 6.32 (d, 1 H, J = 13.6 Hz), 1.5–1.1 (m, 12 H), 1.0–0.6 (m, 15 H); MS (15 eV), m/z 418, 416, 414 (each M$^+$ − CH_3), 376 (base), 374, 372, 143.

***tert*-Butyldimethyl[(2-oxopropyl)oxy]silane (12).** To a solution of acetol (1.48 g, 20.0 mmol), imidazole (1.08 g, 15.9 mmol), and DMAP (20 mg, 0.16 mmol) in DMF (60 mL) under a nitrogen atmosphere at room temperature was added *tert*-butyldimethylchlorosilane (2.0 g, 13.3 mmol). After being stirred for 21 h, the clear solution was diluted with benzene and washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Purification by a short silica gel column (elution with 10% ethyl acetate–hexane) and bulb-to-bulb distillation (oven temperature 100 °C, 20 mmHg) gave *tert*-butyldimethyl[(2-oxopropyl)oxy]silane (1.94 g, 10.3 mmol, 78% based on silane) as a colorless liquid: R_f 0.45 (silica gel, 25% ethyl acetate–hexane); IR (film) 2920, 2850, 1740 (sh), 1715, 1460, 1340, 1250, 1130, 1110, 835, 775 cm^{-1}; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 4.04 (s, 2 H), 2.13 (s, 3 H), 0.91 (s, 9 H), 0.09 (s, 6 H).

4-[(*E*)-3-Hydroxy-3-methyl-4-[(*tert*-butyldimethylsilyl)oxy]-1-butenyl]indole (15). To a solution of **18** (6.53 g, 15.1 mmol, containing <5% of the Z isomer) in THF (150 mL) under a nitrogen atmosphere at −78 °C was added n-BuLi (1.55 M in hexane, 23.4 mL, 36.3 mmol). The reddish solution was stirred for 2.5 h, and then a solution of *tert*-butyldimethyl[(2-oxopropyl)oxy]silane (5.69 g, 30.2 mmol) in ether (50 mL) was added in one portion at −78 °C. After stirring for 1 h, saturated aqueous NH_4Cl was added at −78 °C, and the mixture was allowed to warm to room temperature. The organic layer was decanted, and the aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. Purification by chromatography on silica gel with 10% ethyl acetate–hexane as eluent gave as the first fraction 4-vinylindole (0.50 g, 3.5 mmol, 23%): R_f 0.39 (silica gel, 25% ethyl acetate–hexane). The spectroscopic properties of this compound were identical with those available from the literature.[24]

The second substance to be eluted from the column was the Z isomer of the title compound (90 mg, 0.27 mmol, 2%): R_f 0.32 (silica gel, 25% ethyl acetate–hexane); IR ($CHCl_3$) 3590, 3490, 2950, 2860, 1455, 1400, 1250, 1200, 1080, 925, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.23 (br s, 1 H), 7.31–7.12 (m, 4 H), 6.82 (d, 1 H, J =12.7 Hz), 6.54 (m, 1 H), 5.88 (d, 1 H, J = 12.7 Hz), 3.53 and 3.41 (AB q, 2 H, J = 9.5 Hz), 2.64 (s, 1 H), 1.23 (s, 3 H), 0.88 (s, 9 H), 0.01 (s, 6 H); MS (15 eV), m/z 331 (M$^+$), 274, 186 (base), 182, 141, 130, 74, 59, 45, 31. The final compound to be eluted from the column was the desired product **15** (3.10 g, 9.35 mmol, 62%, colorless oil): R_f 0.29 (silica gel, 25% ethyl acetate–hexane); IR ($CHCl_3$) 3590, 3490, 2950, 2850, 1455, 1440, 1330, 1250, 1080, 965, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.22 (br s, 1 H),

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7.32–7.13 (m, 4 H), 7.06 (d, 1 H, J = 16.0 Hz), 6.77 (m, 1 H), 6.41 (d, 1 H, J = 16.0 Hz), 3.63 and 3.56 (AB q, 2 H, J = 9.7 Hz), 2.78 (br s, 1 H), 1.39 (s, 3 H), 0.91 (s, 9 H), 0.09 (s, 3 H), 0.08 (s, 3 H); MS (15 eV), m/z 331 (M^+), 313, 274, 186 (base), 182, 130, 75, 58, 43; HRMS calcd for $C_{19}H_{29}NO_2Si$ 331.1968, found 331.1962.

3-[(Dimethylamino)methyl]-4-[(*E*)-4-[(*tert*-butyldimethylsilyl)oxy]-3-hydroxy-3-methyl-1-butenyl]indole (19). To a solution of **15** (2.88 g, 8.69 mmol) in CH_2Cl_2 (35 mL) at −20 °C was added dimethylmethyleneammonium chloride (1.63 g, 17.4 mmol). After being stirred for 1 h in a stoppered flask, the reaction mixture was treated with sufficient 5% aqueous NaOH to make the aqueous phase basic, and the resulting mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Hexane was added to the crude product, and the resulting mixture was let stand at 5 °C overnight and then filtered to afford 2.80 g (83%) of the desired gramine derivative as colorless prisms: R_f 0.18 (silica gel, 9:0.9:0.1 chloroform/methanol/concentrated ammonium hydroxide); mp 110–111 °C (hexane); IR ($CHCl_3$) 3590, 3500, 2950, 2850, 2810, 2790, 1455, 1330, 1250, 1070, 1050, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.31 (br s, 1 H), 7.75 (d, 1 H, J = 16.2 Hz), 7.23–7.19 (m, 2 H), 7.11 (m, 1 H), 7.01 (m, 1 H), 6.21 (d, 1 H, J = 16.2 Hz), 3.61 and 3.55 (AB q, 2 H, J = 9.5 Hz), 3.57 (m, 2 H), 2.7 (br s, 1 H), 2.27 (s, 6 H), 1.40 (s, 3 H), 0.91 (s, 9 H), 0.09 (s, 3 H), 0.08 (s, 3 H); MS (15 eV), m/z 388 (M^+), 370, 345, 343, 325, 286, 199 (base), 194; HRMS calcd for $C_{22}H_{36}N_2O_2Si$ 388.2546, found 388.2531. The mother liquor from the crystallization was purified by chromatography on silica gel (elution with 9:0.9:0.1 chloroform/methanol/concentrated ammonium hydroxide) to give an additional 0.16 g (5%) of the gramine as a white solid.

Dimethyl [Methyl[(2,2,2-trichloroethoxy)carbonyl]amino]malonate (24). A mixture of dimethyl (benzylmethylamino)malonate[16] (2.50 g, 9.96 mmol) and 96% 2,2,2-trichloroethyl chloroformate (3.05 g, 13.8 mmol) was heated at 80 °C for 14 h. After cooling, the pale brown reaction mixture was chromatographed on silica gel with 20% ethyl acetate–hexane as eluent to give dimethyl [methyl[(2,2,2-trichloroethoxy)carbonyl]amino]malonate (3.10 g, 9.21 mmol, 92%) as a colorless oil: R_f 0.25 (silica gel, 25% ethyl acetate–hexane); IR (film) 2950, 1745 (sh), 1720, 1430, 1385, 1300, 1140, 1025 cm^{-1}; ^1H NMR ($CDCl_3$, 60 MHz) δ 5.54 and 5.44 (each br s, 1 H), 4.74 (s, 2 H), 3.81 (s, 6 H), 3.10 and 3.07 (each s, 3 H); MS (70 eV), m/z 339, 337, 335 (each M^+), 280, 278, 276 (base), 132, 102, 74, 59; HRMS calcd for $C_9H_{12}NO_6{}^{35}Cl_3$ 334.9730, found 334.9735.

Indole 29. To a stirred solution of gramine **19** (112 mg, 0.29 mmol) and the amidomalonate **24** (145 mg, 0.43 mmol) in dry acetonitrile (8 mL) at reflux temperature under a nitrogen atmosphere was added n-Bu_3P (14 μL, 11.4 mg, 0.056 mmol). The reaction mixture was refluxed under a continuous stream of nitrogen for 9 h. After cooling, the solution was evaporated in vacuo and chromatographed on silica gel with 25% ethyl acetate–hexane as eluent to give **29** (149 mg, 0.22 mmol, 76%) as a colorless amorphous solid: R_f 0.23 (silica gel, 33% ethyl acetate–hexane); IR ($CHCl_3$) 3590, 3490, 2950, 2860, 1740, 1705, 1455, 1380, 1330, 1250, 1170, 1080, 835 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.23 (br s, 1 H), 7.35 (d, 1 H, J =15.8 Hz), 7.25–7.07 (m, 4 H), 6.12 (d, 1 H, J = 15.8 Hz), 4.76 (s, 2 H), 3.96 (s, 2 H), 3.68 (s, 6 H), 3.59 and 3.55 (AB q, 2 H, J = 9.4 Hz), 3.01 (br s, 3 H), 2.82 (br s, 1 H), 1.38 (s, 3 H), 0.92 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H); MS (70 eV), m/z 680, 678 (each M^+), 662, 660, 535, 533, 326, 278, 276, 212, 198 (base), 194; HRMS calcd for $C_{29}H_{39}N_2O_7Si^{35}Cl_3$ 660.1592, found 660.1594.

Removal of the Nitrogen Protecting Group from 29. To a vigorously stirred solution of **29** (1.73 g, 2.54 mmol) in THF (85 mL) at room temperature was added activated zinc dust (16.6 g) followed by 1 M aqueous KH_2PO_4 (17 mL). After being stirred for 45 min, the reaction mixture was passed through a Celite pad, and the filter cake was washed with ethanol. The filtrate was evaporated and chromatographed on silica gel with 50% ethyl acetate–hexane as eluent to give a small amount of the dichloroethyl carbamate corresponding to **29** (0.19 g, 0.29 mmol, 11%) as a colorless amorphous solid: R_f 0.44 (silica gel, 50% ethyl acetate–hexane); IR ($CHCl_3$) 3590, 3490, 2950, 2860, 1740, 1705, 1455, 1380, 1330, 1250, 1170, 1080, 835 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.18 (br s, 1 H), 7.34 (d, 1 H, J = 15.6 Hz), 7.25–7.08 (m, 4 H), 6.11 (d, 1 H, J = 15.6 Hz), 5.83 (t, 1 H, J = 5.9 Hz), 4.44 (d, 1 H, J = 5.9 Hz), 3.94 (s, 2 H), 3.69 (s, 6 H), 3.60 and 3.56 (AB q, 2 H, J = 9.3 Hz), 2.94 (br s, 3 H), 2.82 (br s, 1 H), 1.37 (s, 3 H), 0.92 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H); MS (15 eV), m/z 645, 643 (each M^+), 627, 625, 501, 499, 457, 330, 326, 243, 241, 198 (base), 194, 75. The second fraction from the chromatography consisted of the secondary amine **30** (1.09 g, 2.16 mmol, 85%), which was recovered as a slightly discolored viscous oil: R_f 0.21 (silica gel, 50% ethyl acetate–hexane); IR ($CHCl_3$) 3550, 3500, 3350, 2950, 2860, 1735, 1455, 1330, 1245, 1080, 835 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.09 (br s, 1 H), 7.56 (d, 1 H, J = 15.8 Hz), 7.31 (br d, J = 2.4 Hz), 7.22 (m, 1 H), 7.10 (m, 2 H), 6.16 (d, 1 H, J = 15.8 Hz), 3.72 (br s, 2 H), 3.67 (s, 6 H), 3.66 and 3.59 (AB q, 2 H, J = 9.5 Hz), 2.35 (s, 3 H), 1.44 (s, 3 H), 0.93 (s, 9 H), 0.11 (s, 3 H), 0.10 (s, 3 H); MS (70 eV), m/z 504 (M^+), 486, 344 (base) 326, 256, 212, 193; HRMS calcd for $C_{26}H_{38}N_2O_5Si$ 486.2550, found 486.2531.

Desilylation of 30. To a solution of the secondary amine **30** (215 mg, 0.426 mmol) in THF (4 mL) under nitrogen at 0 °C was added a 1 M solution of n-Bu_4NF in THF (0.51 mL, 0.51 mmol). After being stirred at room temperature for 40 min, the pale yellow suspension was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo. Purification by chromatography on silica gel with 5% methanol–chloroform as eluent gave the amino diol (153 mg, 0.392 mmol, 92%) as a pale pink foam, which was crystallized from ether: R_f 0.32 (silica gel, 10% methanol–chloroform), 0.17 (silica gel, ethyl acetate); mp 107–109 °C; IR ($CHCl_3$) 3600, 3490, 3350, 2990, 2950, 1730, 1420, 1330, 1200, 1130, 1075, 1040, 925 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.44 (br s, 1 H), 7.49 (d, 1 H, J = 16.0 Hz), 7.24 (m, 1 H), 7.14 (br d, 1 H, J = 2.6 Hz), 7.10 (br d, 1 H, J = 3.4 Hz), 7.09 (s, 1 H), 6.08 (d, 1 H, J = 16.0 Hz), 3.73 and 3.56 (AB q, 2 H, J = 11.1 Hz), 3.71 (br s, 2 H), 3.68 (s, 3 H), 3.66 (s, 3 H), 3.1 (br m, 3 H), 2.33 (s, 3 H), 1.42 (s, 3 H); MS (15 eV), m/z 390 (M^+), 372, 230 (base), 212, 184, 156; HRMS calcd for $C_{20}H_{26}N_2O_6$ 390.1791, found 390.1764.

3-[4-((*E*)-3,4-Dihydroxy-3-methyl-1-butenyl)-1*H*-indol-3-yl]-2-(methylamino)propanoic Acid (2a and 2b). To a mixture of the above diester (39 mg, 0.1 mmol) and lithium methylmercaptide[23] (27 mg, 0.5 mmol) under an argon atmosphere at room temperature was added HMPA (0.25 mL). The reaction mixture was stirred for 67 h, diluted with 1 mL of water at 0 °C and neutralized with 10% HCl (pH 5). An additional 10 mL of water was added. After washing with dichloromethane, the aqueous layer was evaporated in vacuo. Purification by preparative TLC (silica gel, 20:1:2 2-propanol/concentrated NH_4OH/water) gave a yellow residue. Further purification by preparative TLC (reverse-phase plate, 15% methanol–water) gave in a higher R_f band amino acid **2a** (4 mg, 0.013 mmol, 13% as an amorphous solid): R_f 0.22 (reverse phase, 10% methanol–water); ^1H NMR (D_2O, 300 MHz) δ 7.31 (m, 1 H), 7.22 (d, 1 H, J = 16 Hz), 7.17 (s, 1 H), 7.10 (m, 2 H), 6.15 (d, 1 H, J = 16 Hz), 3.72 (dd, 1 H, J = 9.6, 5.2 Hz), 3.50 (m, 1 H), 3.49 (s, 2 H), 3.16 (dd, 1 H, J = 15.6, 9.6 Hz), 2.42 (s, 3 H), 1.27 (s, 3 H). The band of lower R_f gave amino acid **2b** (4 mg, 0.013 mmol, 13%) as small, colorless prisms after crystallization from water: R_f 0.16 (reverse phase, 10% methanol–water); mp 200–202 °C dec; ^1H NMR (D_2O, 300 MHz) 7.31 (m, 1 H), 7.24 (d, 1 H, J = 16 Hz), 7.12 (m, 2 H), 6.16 (d, 1 H, J = 16 Hz), 3.77 (dd, 1 H, J = 10.1, 4.3 Hz), 3.52 (m, 1 H), 3.50 and 3.47 (AB q, 2 H, J = 11.5 Hz), 3.09 (dd, 1 H, J = 15.6, 10.1 Hz), 2.38 (s, 3 H), 1.24 (s, 3 H). The trideuteriated analogues of **2a** and **2b** were prepared in an identical manner by substituting **24**-d_3 for **24** in the preparation of indole **29**. The diastereomeric amino acids were purified as above to yield **2a**-d_3 as an amorphous solid on precipitation from water and **2b**-d_3 as colorless prisms on recrystallization from water [mp 200 °C dec].

Feeding Experiments. *Claviceps sp.*, strain SD58[25] was grown for 5 days in shake culture at 25 °C in 500-mL Erlenmeyer flasks containing 100 mL of medium NL 406.[26] Cultures were then filtered, and the mycelia were washed with water under sterile conditions and resuspended in 100 mL of $^1/_{15}$ M phosphate buffer, pH 7.3. The mycelia were again filtered and washed under sterile conditions and then suspended in 100 mL of $^1/_{15}$ M phosphate

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buffers, pH 7.3, in 500-mL Erlenmeyer flasks. Labeled precursors (expt 1, 14 mg of **2a**-d_3; expt 2, 12 mg of **2b**-d_3 expt 3, 10 mg of L-tryptophan, 20 mg of D,L-mevalonic acid, and 10 mg of L-[$^{13}C^2H_3$]methionine) were each distributed evenly into two replacement cultures, and these were incubated with shaking for 2 days. Alkaloids were then extracted from the alkalinized (NH_4OH) culture filtrate with 1:2 2-propanol/chloroform. The organic extract was evaporated in vacuo and the residue partitioned between 2% aqueous succinic acid and methylene chloride. The aqueous layer was washed twice with CH_2Cl_2, made alkaline (NH_4OH), and extracted 3 times with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated in vacuo. All these operations were carried out in test tubes by using Pasteur pipets for the transfers to avoid contamination with silicone grease or plasticizer. The alkaloid residue was chromatographed on an alumina column (2 g Alumina Neutral containing 20% water, 80–200 mesh, Fisher Scientific; elution with 1:40 $CH_3OH–CH_2Cl_2$). The fraction containing **3b** was subjected to GC–MS analysis (Hewlett-Packard 5970A, SPB-5 capillary column 0.25 mm × 15 m, flow rate 1.0 mL/min, temperature program 4 min 60 °C, then 10 °C/min to 290 °C; **3b**, t_R 24 min). Isotopic composition was calculated from the MS data as described by Biemann.[27]

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Registry No. **1**, 29702-35-0; **2**, 112152-11-1; **2**-d_3, 112152-35-9; **3a**, 2390-99-0; **3b**, 548-43-6; **5**, 1074-86-8; **6**, 112152-12-2; **7**, 112152-13-3; **8**, 112152-14-4; **10**, 112152-15-5; (*E*)-**11**, 112152-16-6; (*Z*)-**11**, 112152-31-5; **12**, 74685-00-0; **13**, 102301-81-5; **14**, 68900-05-0; (*E*)-**15**, 112152-17-7; (*Z*)-**15**, 112152-32-6; **16**, 112152-18-8; **17**, 112152-19-9; (*E*)-**18**, 112152-20-2; (*Z*)-**18**, 112152-33-7; **19**, 112152-21-3; **20**, 112152-22-4; **21**, 112152-23-5; **22**, 112152-24-6; **23**, 5417-21-0; d_3-**23**, 112152-36-0; **24**, 112152-25-7; d_3-**24**, 112152-37-1; **25**, 112152-26-8; **26**, 112152-27-9; **27**, 112152-28-0; **29**, 112152-29-1; **30**, 112152-30-4; **30** (desilylated), 112152-34-8; $HOCH_2COCH_3$, 116-09-6; $ClCO_2CH_2CCl_3$, 17341-93-4; $H_3CCOCH{=}PPh_3$, 1439-36-7; $LiCH_2OCH_2CH_2SiMe_3$, 112152-38-2; $(PhCH_2)_2NCH(CO_2Me)_2$, 112152-39-3; CD_3I, 865-50-9; H-Trp-OH, 73-22-3.

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A Facile Synthesis of Statine and Analogues by Reduction of β-Keto Esters Derived from Boc-Protected Amino Acids. HPLC Analyses of Their Enantiomeric Purity

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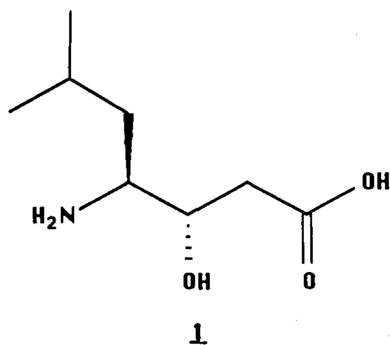
The synthesis of γ-amino-β-keto ester derivatives **3a–e** from *N*-Boc-protected L-amino acids by *N,N'*-carbonyldiimidazole activation and treatment with the magnesium enolate of hydrogen ethyl malonate is described. Racemization during activation, which depended upon the reaction conditions for imidazolide formation, was minimized for **3a–c** but was almost complete in the case of *N*-Boc-*S*-methylcysteine (**2d**). The diastereoselectivity for reducing intermediate **3** with sodium borohydride, potassium tri-*sec*-butylborohydride (K-Selectride), and other reducing agents to the (3*S*,4*S*)- and (3*R*,4*S*)-statine derivatives **4** and **5** was examined. A high selectivity for the *S*,*S* diastereomers, but low reactivity, was observed for the sterically demanding K-Selectride. Diastereomerically pure Boc-(*S*,*S*)-statine ethyl ester (**4a**) (with enantiomeric purity ee = 97%) and its analogue **4b** (*N*-Boc-AHPPA-OEt; ee = 95%) were obtained after $NaBH_4$ reduction in THF–MeOH (98:2) in 27% and 41% total yield. The enantiomeric purity of (*S*,*S*)/(*R*,*R*)-statine and analogues was determined by precolumn derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) followed by HPLC analyses on a silica gel column. The synthesis of a new histidine side-chain analogue of statone is described.

Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**1**), is a constituent of the naturally occurring small peptide pepstatin[1] which is a strong and general inhibitor of aspartic proteinases, e.g., pepsin, renin, and cathepsin D.[2] The importance of the statine stereochemistry for the slow and tight-binding inhibition has been demonstrated by kinetic studies.[3,4d] Because statine has proven useful for developing new inhibitors of aspartic proteinases, numerous syntheses of **1** and its N-protected ester derivatives have been published.[4] Most of these methods proceed via condensation of an N-protected (*S*)-amino aldehyde with a metalated ethyl acetate to form the 3*S*,4*S* and 3*R*,4*S*



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