145. Ethylene Biosynthesis

Part 10[1])

Synthesis and Study of Racemic, (1*R*,2*S*)-, and (1*S*,2*R*)-1-Amino-2-(hydroxymethyl)cyclopropanecarboxylic Acid

by **Michael C. Pirrung***, **Steven E. Dunlap**[2]), and **Uwe P. Trinks**[3])

Department of Chemistry, Stanford University, Stanford, California 94305, USA

(23.VI.89)

The preparation of optically active 1-amino-2-(hydroxymethyl)cyclopropanecarboxylic acid has been achieved by a route involving cycloalkylation of dimethyl malonate with epichlorohydrin and subsequent *Hofmann* rearrangement. The reaction of epichlorohydrin with nucleophiles may occur at either electrophilic site, epoxide or halide. Based on the absolute configuration of the starting materials and the lactones obtained, it has been shown that the initial step of the cycloalkylation occurs at the epoxide moiety. The 1-amino-2-(hydroxymethyl)-cyclopropanecarboxylic acid, an analogue of the precursor to the plant growth hormone ethylene, is to be used in affinity purification techniques and in generation of antibodies.

Introduction. – In the preceding decade, a tremendous amount of information concerning the biosynthesis of ethylene in higher plants has been accumulated. The intermediates in the pathway from methionine have been elucidated [2], and the factors involved in the regulation of the biosynthesis have been studied [2] [3]. The enzyme responsible for the production of 1-aminocyclopropanecarboxylic acid (ACC) from *S*-adenosylmethionine, ACC synthase, has been purified, and the complete steric course of the ACC production has been determined [4]. Monoclonal antibodies have been generated for greater resolution of its turnover *in vivo*, and results have been obtained using them which support the idea that ethylene biosynthesis is under transcriptional control through ACC synthase [5]. The degradation of ACC has been studied biosynthetically using isotopically labeled [6] and alkylated [7] derivatives, and a number of chemical models have been put forward [8]. The one goal which has eluded researchers in this area is the isolation of the ethylene-forming enzyme (EFE). The most elemental system in which valid ethylene production has been demonstrated is the pea vacuole system of *Guy* and *Kende* [9].

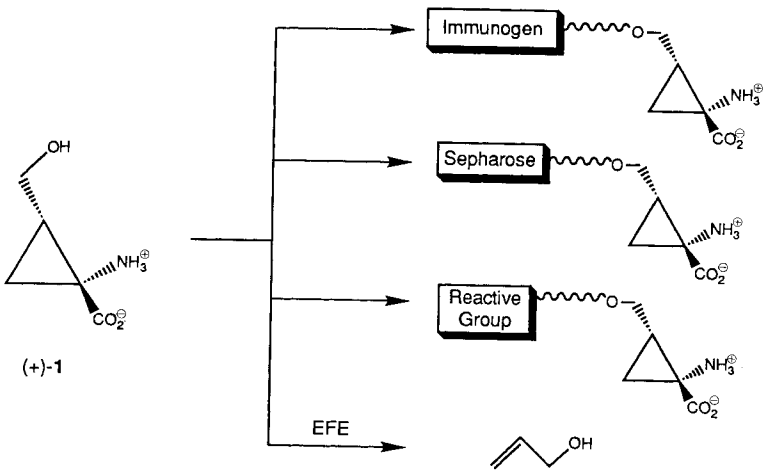
It is clear that for our mechanistic understanding of the oxygen-dependent enzymatic conversion of ACC to ethylene to advance beyond its current level [10], the isolated enzyme is required. Toward that end, the development of biological selection agents for mutagenesis experiments [11] or ligands for affinity purification techniques [12] are important goals. Further, the availability of ACC antibodies would facilitate studies on

[1]) Part 9: [1].

[2]) Present address: Department of Chemistry, USAF Academy, Colorado Springs, Colorado 80840, USA.

[3]) Present address: *Ciba-Geigy AG*, CH-4002 Basel.

Scheme 1



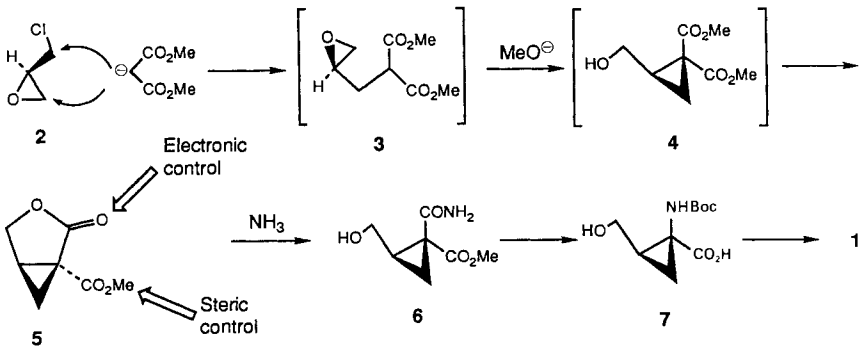
regulation of ethylene biosynthesis using immunoassay and immunocytological methods [13]. In this report, the design and preparation of a molecule necessary to achieve these goals is described *(Scheme 1)*.

Results. – The synthesis of 1-amino-2-(hydroxymethyl)cyclopropanecarboxylic acid (**1**) was chosen because in a protected form, **1** could be linked through its free OH group to a chromatographic support, a reactive functional group, or an immunogenic carrier protein, and because it would be processed by the *in vivo* ethylene-forming enzyme to produce allyl alcohol. The known cytotoxic properties of the latter [14] would presumably promote the death of cell lines which maintain the ability to produce ethylene from ACC, and conversely be *ahimsic* toward those mutants lacking this power. The relative configuration of **1** chosen was based on ample precedent [7] that (1*R*,2*S*)-2-alkyl-ACC's[4]) perform best as substrates. The production of substituted olefins from substituted ACC's is similarly well precedented [7]. The configuration is of no importance for the performance of **1** as a hapten, however.

The synthetic plan was based on a classical route to cyclopropane amino acids, the *Hofmann* rearrangement of cyclopropane-1,1-dicarboxylates [15] (see *Scheme 2*). The (hydroxymethyl)cyclopropane-1,1-dicarboxylate **4** would be expected to be preferentially in the lactonic form, and indeed lactone **5** has been reported by *Temnikova et al.* as a product of the condensation of epibromohydrin and dimethyl malonate with NaOMe as base [16]. However, these workers had earlier misassigned the structure of their product and were unable to fully explain the ^{1}H-NMR spectrum. They also reported that the reaction proceeds only in poor yield under drastic conditions with epichlorohydrin (**2**). In the event, treatment of dimethyl malonate with freshly generated NaOMe, addition of epichlorohydrin (**2**), and refluxing for 16 h leads to the crystalline lactone **5** in 45% yield on a 10–50-g scale. Particularly in the optically active series where greater difficulties were encountered (see below), the concentration at which this reaction is conducted is important. The maximum differential solubility of the malonate ion and NaCl must be main-

[4]) The corresponding configuration of (+)-**1** is also (1*R*,2*S*).

Scheme 2



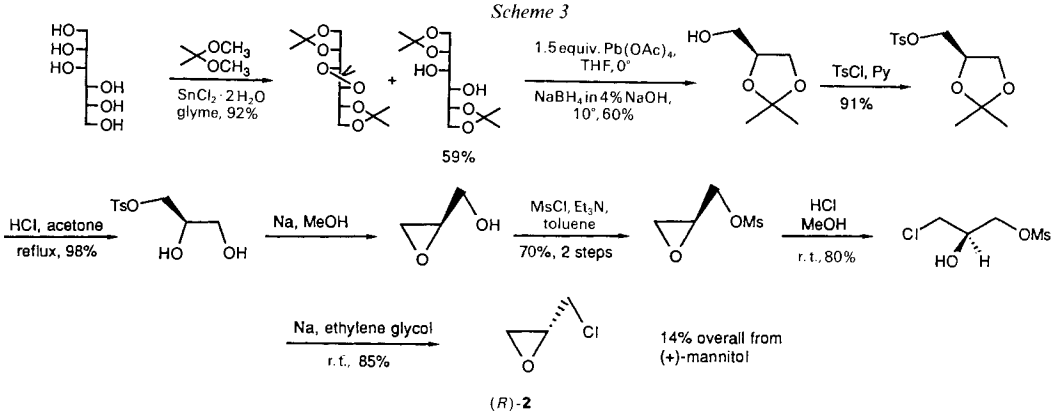
tained to prevent chloride attack on the doubly activated cyclopropane. Presumably, the cyclocondensation proceeds through the intermediacy of epoxymalonate **3** which cyclizes to **4** in accordance with ring-closure rules and other precedents [17].

In previous syntheses of ACC derivatives from cyclopropane-1,1-dicarboxylates, the *cis* configuration of the alkyl and amino functions which is desired for ethylene-biosynthesis studies has required more involved synthetic routes. Direct aminolysis or hydrazinolysis and subsequent *Hofmann/Curtius* degradation leads eventually, for steric reasons, to the *trans* configuration. In our case, however, the ester function, which is *cis* to the alkyl group and in principle more hindered, is involved in a lactone linkage and hence is electronically much more susceptible to nucleophilic attack. This is borne out in its reaction with anhydrous NH_3 in MeOH, which leads to the crystalline hydroxyamide **6** in quantitative yield. Acetylation with Ac_2O/pyridine sets the stage for the *Hofmann* rearrangement. Oxidation is conducted with lead tetraacetate (2 equiv.) [18] in *t*-BuOH. This reaction requires 0.5 to 3 h depending on scale and quality of reagent, and so was carefully followed by TLC. Non-aqueous workup provides the *tert*-butoxycarbonyl-(Boc)-protected compound and some minor impurities which are removed in the subsequent hydrolysis step (1N NaOH in MeOH). This two-step sequence gives **7** (recrystallized) in 60% yield. Cleavage of the Boc group with anhydrous HCl in MeOH gives the free amino acid **1** in 91% yield, after ion-exchange chromatography and recrystallization. In the racemic series, this corresponds to an overall yield of 50% from the bicyclic lactone (±)-**5**. Despite the fact that **1** is a cyclopropylmethanol bearing a donor group, it seems quite stable toward *Grob* fragmentation. This may have to do with the internal buffering effect of the amino acid.

The preparation of optically active (+)-**1** requires optically active epichlorohydrin which has previously been prepared [19] from (+)-D-mannitol. This procedure affords material which is solvent-free but not necessarily H_2O-free. Since the presence of small amounts of H_2O causes complete failure in the reaction with dimethyl malonate, modification was made to the literature route.

In our hands, the bis-acetonide of D-mannitol is always accompanied by minor amounts of tris-acetonide [20] (*Scheme 3*). They are easily quantified by GC and used in the glycol cleavage reaction as a mixture, since the tris-acetonide is unreactive. Direct reduction leads to (*S*)-glycerol 1,2-acetonide in 60% yield. Flash distillation from tris-acetonide and subsequent fractional distillation affords optimal yields in this step. By tosylation, hydrolysis, cyclization, and mesylation, (*S*)-oxirane-2-methyl methanesulfonate is obtained after bulb-to-bulb distillation (100–110°/0.9 Torr) [19]. The opening of its epoxide moiety is conducted with anhydrous HCl in MeOH

Scheme 3



in order to provide H_2O-free 3-chloro-2-hydroxypropyl methanesulfonate. Ring closure to (*R*)-epichlorohydrin (14% (*R*)-**2**; overall yield) is accomplished in freshly dried ethylene glycol under Ar.

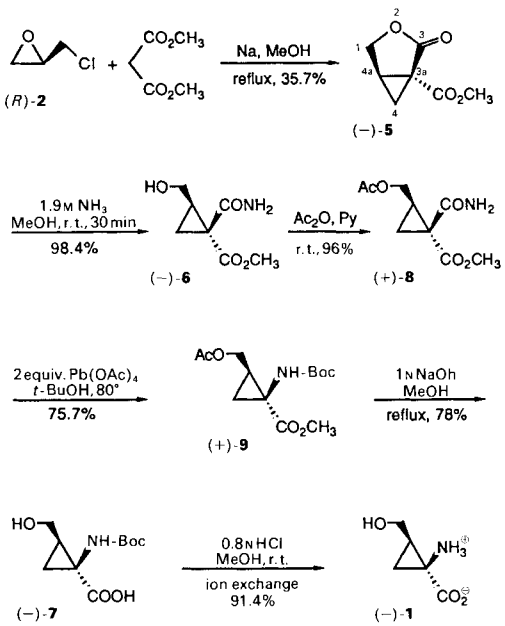
In the optically active series, a new issue arises in the synthesis. Direct displacement of the chloride by malonate might occur to produce an epoxymalonate of the same configuration as the starting epichlorohydrin (see *Scheme 2*). Alternatively, nucleophilic attack at the epoxide could produce an intermediate chlorohydrin salt which cyclizes to the epoxymalonate of the opposite configuration. It is thus conceivable that from a single enantiomer of **2** either enantiomer of **5** might be obtained in high optical purity, depending on the preferred electrophilic site. The issue of the locus of reaction of oxirane-2-methanol derivatives with nucleophiles has been considered in detail in connection with the synthesis of β-adrenergic receptor-blocking drugs [21]. In general, soft nucleophiles react first at the epoxide. Organometallic reagents have recently been included in this group [22].

The synthesis of (−)-**5** from (*R*)-epichlorohydrin ((*R*)-**2**) proceeds in lower yield (36%) than in the racemic series despite stringent precautions to exclude moisture. When these precautions are not observed, *none* of the desired lactone is obtained. A strong (96.5:3.3) preference for one of the two electrophilic sites is demonstrated by the high enantiomeric excess (93.4%) obtained in the condensation. This value was determined at the stage of (−)-**7** by the *Mosher* method [23]. That the epoxide served first as the electrophile in an indirect displacement *(Scheme 2)* is shown by comparison of the optical rotation of (−)-**5** to lactonized cyclopropane-1,1-dicarboxylates of known absolute configuration prepared by *Meyers* [24]. The assignment of the (3a*S*,4a*R*) configuration to lactone (−)-**5** is thereby possible. On aminolysis of this lactone, amide ester (−)-**6** is obtained as an oil, in contrast to the racemic series. Subsequent steps in the sequence are performed as described above to give (−)-**7** and (−)-**1** *(Scheme 4)*.

The synthesis of (+)-**5** is accomplished from (*S*)-epichlorohydrin ((*S*)-**2**) which was obtained from commercially available (*R*)-oxirane-2-methyl tosylate by chlorohydrin formation and ring closure analogous to *Scheme 3*. The use of (*S*)-**2** in a route differing from *Scheme 4* only in that each compound is of the opposite enantiomeric series provides (+)-**1** in 27% overall yield.

The racemic amino acid (±)-**1** was studied for its ability to inhibit ethylene production from ACC in mung bean hypocotyl segments [7]. *Dixon* analysis [25] at an ACC concen-

Scheme 4



tration of 0.5 mM gives an IC_{50} of 0.6 mM *(Fig.)*. This strong binding, comparable to that of natural substrate, indicates that **1** will prove a useful tool to deliver reagents to ACC-binding proteins, remove them from protein mixtures, or elicit an immune response.

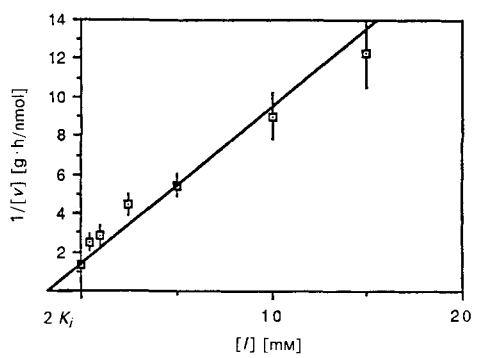


Figure. Dixon *plot* (1/*v vs. I*) *of competitive inhibition of the ethylene-forming enzyme by* **1**, *measured at* [ACC] = 0.5 *m*M (K_m). [*v*] = nmol ethylene produced per gram plant material and hour. [*I*] = *m*M **1**.

Financial support was provided by a *Presidential Young Investigator Award* of the *National Science Foundation* (CHE 84-51324) and matching contributions of *Pfizer* and *Rohm & Haas*. Fellowship support from the *Alfred P. Sloan Foundation (M.C.P.)*, the *Swiss National Science Foundation (U.P.T.)*, and the *USAF Academy (S.E.D.)* is gratefully acknowledged. Dr. *William Brown* made valuable experimental contributions.

Experimental Part

General. Unless otherwise noted, all materials were obtained from commercial suppliers. Solvents were purified by distillation. Abs. solvents were purified as follows by distillation: Et_2O and THF from Na/benzophenone under N_2 immediately prior to use, MeOH from Mg and then again from CaH_2, MeCN from P_2O_5/Na_2CO_3 under N_2, DMF and pyridine from 4 Å sieves, and all other solvents from CaH_2 followed by storage under Ar and over molecular sieves. Reagents were purified as follows: dimethyl malonate, *t*-BuOH, Et_2N, and mesyl chloride were distilled form CaH_2. $Pb(OAc)_4$ was washed with dry Et_2O under dry Ar and dried at r.t./0.2 Torr for 4 d prior to use. (*R*)-Epichlorohydrin ((*R*)-**2**) was dried over sieves prior to use. NH_3/abs. MeOH (*ca.* 1.9M) was prepared as follows: abs. MeOH was cooled in an ice-bath under Ar and NH_3 (g) was bubbled into saturation; the NH_3 content was determined by weight. 1N HCl/abs. MeOH was prepared by adding acetyl chloride dropwise to ice-cooled abs. MeOH under dry N_2 with vigorous stirring, 1N NaOH/abs. MeOH by dissolving NaOH pellets in abs. MeOH under dry N_2 at r.t., and 1N HCl/sat. NaCl soln. from a sat. aq. NaCl soln. by adding conc. HCl. Flash chromatography: *Merck* 0.042–0.063 mm grade silica gel ('Kieselgel' *60*). TLC: *Merck* silica gel *60 F-254* precoated plates. Cation exchange chromatography: *Dowex 50W-X8*, 4% cross-linked, dry mesh 100–200 (H^+-form), freshly activated by eluting with 10 times the column volume of 2N HCl and then with H_2O until neutral. GC: *Hewlett-Packard-5890A* gas chromatograph, equipped with a *Hewlett-Packard-3390A* reporting integrator; *J&W Scientific SE-54* 15 m × 0.25 mm capillary column; unless otherwise stated, the following temp. program was used: initial temp. 100° (initial time 1.0 min), ramp rate 30°/min, final temp. 275° (final time 5 min). M.p.: *Haake-Buchler* capillary melting point apparatus; uncorrected. B.p.: uncorrected. IR spectra: *Perkin-Elmer-1310* spectrometer; 5–10% soln. (200-μm path); film, or 20% mixture in KBr (pellets *ca.* 200 μm thick). ^{1}H-NMR spectra: *Varian-XL-400* or *Nicolet-NT-300* spectrometer; tetramethylsilane (for $CDCl_3$) or the residual protons of the solvent as internal standard; chemical shifts in ppm on the δ scale, coupling constants in Hz. ^{13}C-NMR spectra: at 99.7 MHz on *Varian-XL-400* instrument; solvent signal as internal reference; the multiplicity was determined using the attached proton test (APT) technique. GC/MS: *Hewlett-Packard-5995* gas chromatograph/mass spectrometer, *J&W Scientific SE-54* 25 m × 0.20 mm capillary column (cross-linked methylsilicone, 4600 plates/m, solvent delay 2.1 min; *m/z* (rel. intensity in %) of peaks > 10% are reported. NMR spectra were obtained on instruments provided by *NSF* equipment grants CHE 8109064 and 8414329. Mass spectra were obtained through the *Bioorganic, Biomedical Mass Spectrometry Resource* (*A. L. Burlingame*, Director), supported by NIH Division of Research Resources Grant RR01614.

(+)-(S)-2,2-Dimethyl-1,3-dioxolane-4-methanol. To D-mannitol (25.0 g, 0.137 mol), 1,2-dimethoxyethane (glyme; 60 ml) and 2,2-dimethoxypropane (20 ml), stirred vigorously with a mechanical stirrer, $SnCl_2 \cdot 2\,H_2O$ (50 mg, 0.22 mmol) was added and refluxed for 3 h. After returning to r.t., a white precipitate was removed by filtration. The filtrate contained 83% of bis-acetonide and 17% of tris-acetonide by GC and was evaporated at 35°/0.1 Torr: 34.5 g. This mixture, containing 25.6 g (97.6 mmol) of bis-acetonide, was added to 150 ml of THF and cooled with an ice/H_2O bath. $Pb(OAc)_4$ (43.3 g, 97.6 mmol) was added portionwise within 15 min while maintaining the temp. < 10°. The transfer was completed under dry N_2. The ice-bath was removed and the mixture stirred for 1.5 h. Pinacol (1.15 g, 9.75 mmol) was added and the mixture stirred for 10 min and filtered through *Celite*. The cake was washed twice with THF. To the filtrate was added dropwise over 3.5 h a soln. of $NaBH_4$ (7.453 g, 0.197 mol) in 150 ml of 4% NaOH soln. with vigorous stirring while maintaining the temp. < 10°. After stirring for 30 min in the cold and 90 min at r.t., solid NH_4Cl was added until the pH was 8. The gray precipitate was removed by filtering through *Celite*, and most of the THF was evaporated. The aq. phase was saturated with NaCl and extracted with AcOEt. The combined extracts were washed with 5% NaOH/sat. NaCl soln., dried ($MgSO_4$), and concentrated. Flash distillation (70–80°/11 Torr) followed by distillation through a 10-cm *Vigreux* column (90.5°/15 Torr) afforded 15.47 g (60%).

Methyl (3aS,4aR)-3,3a,4,4a-Tetrahydro-3-oxo-1H-cyclopropa[c]furan-3a-carboxylate ((−)-**5**). To abs. MeOH (30 ml) under dry N_2, cooled to 0° in an ice-bath, was added Na (900 mg, 39.2 mmol) in 3 portions over 15 min. The ice-bath was removed after complete dissolution, and dimethyl malonate (5.1 ml, 5.95 g, 45.0 mmol) was added at once. (*R*)-Epichlorohydrin ((*R*)-**2**; 3.90 g, 40.0 mmol) was added dropwise with vigorous stirring over 10 min at r.t. After additional 5 min at r.t., the soln. became cloudy and was heated at reflux for 16 h. The resulting suspension was cooled to r.t., the precipitated salts were filtered, and the filtrate was evaporated: yellow oil/white solid. This residue was taken up in Et_2O (50 ml) and stirred for 5 min. The Et_2O layer was decanted and the remaining polymer dissolved in H_2O (40 ml) and extracted with Et_2O (3 × 40 ml) and CH_2Cl_2 (3 × 40 ml). The combined org. phase was dried (Na_2SO_4) and evaporated. The resulting semisolid was chromatographed with Et_2O. Recrystallization from Et_2O at −78° gave white plates (1.798 g, 29.4%). Evaporation of the mother liquor and two recrystallizations gave another 386 mg, together 2.18 g (35.7%) of (−)-**5**. GC: t_R 3.20 min. TLC: R_f 0.5

(Et_2O). M.p. 46–47°. $[\alpha]_D^{25} = -163.8 \pm 0.2$ ($c = 1.32$, CH_2Cl_2). IR ($CHCl_3$): 3020*w* (br.), 2960*w*, 2910*w*, 2410*w*, 1780*s*, 1770*s*, 1720*m*, 1440*m*, 1380*m*, 1370*w*, 1315*m*, 1270*m*, 1200*w*, 1115*m*, 1090*m*, 1045*m*, 1000*m*, 970*w*, 930*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 4.37 (*dd*, $J = 9.5, 4.8$, 1 H–C(1)); 4.20 (*dd*, $J = 9.5, 1.5$, 1 H–C(1)); 3.82 (*s*, CH_3O); 2.76 (*dddd*, $J = 8.1, 5.5, 4.8, 1.5$, H–C(4a)); 2.10 (*dd*, $J = 8.1, 4.8$, 1 H–C(4)); 1.42 (*dd*, $J = 5.5, 4.8$, 1 H–C(4)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.5, 167.3 (2 *s*, C(3), $COOCH_3$); 67.0 (*t*, C(1)); 52.9 (*q*, CH_3O); 29.3 (*s*, C(3a)); 28.0 (*d*, C(4a)); 20.9 (*t*, C(4)). HR-MS: 156.0423 (8.8, M^+, calc. 156.0423, error < 0.1 μ (0.6 ppm)), 126.0319 (92.5, $M^+ - CH_2O$), 125.0234 (63.6, $M^+ - CH_2OH$). GC/MS (4.96 min): 156 (13.1, M^+), 127 (9.0), 126 (*100*, $M^+ - CH_2O$), 125 (54.5), 124 (11.5), 108 (18.0), 100 (23.7), 98 (25.3), 97 (14.1), 95 (14.1), 83 (39.7), 69 (35.1), 68 (38.2), 59 (60.6), 55 (16.6), 54 (12.5), 53 (97.1), 52 (10.3), 51 (11.4), 45 (12.7), 42 (14.5), 41 (44.9). Anal. calc. for $C_7H_8O_4$: C 53.85, H 5.16; found: C 53.68, H 5.11.

Methyl (1R,2R)-1-Carbamoyl-2-(hydroxymethyl)cyclopropanecarboxylate ((−)-**6**). A soln. of (−)-**5** (469 mg, 3 mmol) in 1.9M NH_3/abs. MeOH was stirred at r.t. (TLC monitoring). After 15 min, the soln. was evaporated within 10 min at r.t./0.2 Torr. The remaining clear oil was taken up in CH_2Cl_2 (10 ml) and Et_2O (10 ml) and evaporated to a colorless thick oil (634 mg). Chromatography on silica gel with AcOEt yielded 511.4 mg (98.4%) of (−)-**6** as a colorless thick oil which solidified partially upon standing. TLC: R_f 0.25 (AcOEt). $[\alpha]_D^{25} = -14.0 \pm 0.2$ ($c = 0.68$, CH_2Cl_2). IR (film): 3400*s* (br.), 3320*s* (br.), 3200*m* (br.), 3000*w*, 2940*m*, 2910*w*, 2880*w*, 1705*s*, 1650*s*, 1595*m*, 1430*s*, 1395*s*, 1280*s*, 1230*m*, 1185*m*, 1140*m*, 1055*m*, 1020*m*, 960*w*, 910*m*, 880*w*, 830*w*, 790*w*, 725*s*, 640*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.23 (br. *s*, NH); 6.00 (br. *s*, NH); 3.97 (*dd*, $J = 12.7$, 3.7, 1 H, CH_2–C(2)); 3.74 (partially superimposed with 3.71, *m*, 1 H, CH_2–C(2)); 3.71 (*s*, CH_3O); 3.17 (br. *s*, OH); 2.27 (*dddd*, $J = 9.7$, 8.3, 8.1, 3.7, H–C(2)); 1.90 (*dd*, $J = 8.1$, 4.5, 1 H–C(3)); 1.82 (*dd*, $J = 9.7$, 4.5, 1 H–C(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.4, 170.4 (2 *s*, $COOCH_3$, $CONH_2$); 59.6 (*t*, CH_2–C(2)); 52.6 (*q*, CH_3O); 35.5 (*d*, C(2)); 32.0 (*s*, C(1)); 20.1 (*t*, C(3)). HR-MS: 173.0706 ($C_7H_{11}NO_4$, calc. 173.0688, error < 1.8 μ (10 ppm)), 156.0419 ($M^+ - NH_3$, calc. 156.0423, error < -0.4 μ (−2.6 pm)). MS: 173 (0.09, M^+), 156 (12.1, $M^+ - NH_3$), 141 (11.9), 130 (15.1), 127 (10.7), 126 (*100*, $M^+ - NH_3 - CH_3$).

(1RS,2RS)-1-Carbamoyl-2-(hydroxymethyl)cyclopropanecarboxylate ((±)-**6**). In the same way, (±)-**5** (7.81 g, 50 mmol) was treated with 300 ml of 1.9N NH_3/abs. MeOH. Crystallization from CH_2Cl_2/AcOEt 1:1 gave white needles (8.09 g, 93%). M.p. 82.5–83.5°.

Methyl (1R,2R)-2-(Acetoxymethyl)-1-carbamoylcyclopropanecarboxylate ((+)-**8**). To a soln. of (−)-**6** (466 mg, 2.69 mmol) in 3 ml of abs. pyridine under dry N_2 at r.t., Ac_2O (283 μl, 306 mg, 3 mmol) was added by syringe at once and stirred at r.t. for 22 h. Abs. MeOH (60 μl, 1.5 mmol) was then added and the mixture stirred for additional 4 h. AcOEt (50 ml) and then 1N HCl/sat. NaCl soln. (50 ml) were added. The org. layer was separated and the aq. layer extracted with AcOEt (4 × 25 ml). The combined org. phase was dried (Na_2SO_4) and evaporated to a clear colorless oil which solidified at r.t./0.2 Torr (12 h) to give (+)-**8** (557.7 mg, 96%) as a white solid. Two recrystallizations of 480 mg of (+)-**8** gave 364 mg of colorless crystals of an anal. pure sample. GC: t_R 4.44 min. TLC: R_f 0.2 (Et_2O), 0.7 (AcOEt). M.p. 71.5–72.5°. $[\alpha]_D^{25} = +48.9$ ● 0.2 ($c = 0.98$, $CHCl_3$). IR ($CHCl_3$): 3630*w*, 3460*m*, 3370*w*, 3320*w*, 2970*w*, 2920*w*, 1710*s* (sh), 1700*s*, 1650*s*, 1555*m*, 1425*m*, 1370*m*, 1355*m*, 1290*m*, 1250*m* (sh), 1200*s* (br.), 1130*s*, 1015*m*, 960*w*, 910*w*, 880*w*, 830*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.28 (br. *s*, NH); 5.62 (br. *s*, NH); 4.48 (*dd*, $J = 5.6, 11.9$, 1 H, CH_2–C(2)); 4.14 (*dd*, $J = 8.9, 11.9$, 1 H, CH_2–C(2)); 3.72 (*s*, CH_3O); 2.25 (*dddd*, $J = 9.7, 8.9, 8.0, 5.6$, H–C(2)); 2.05 (*s*, CH_3CO); 1.94 (*dd*, $J = 8.0, 4.3$, 1 H–C(3)); 1.82 (*dd*, $J = 9.7, 4.3$, 1 H–C(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.4, 170.9 (2 *s*, $COOCH_3$, $CONH_2$); 61.7 (*t*, CH_2–C(2)); 52.6 (*q*, CH_3O); 31.4 (*d*, C(2)); 31.1 (*s*, C(1)); 20.9 (*q*, CH_3CO); 20.0 (*t*, C(3)). HR-MS: 215.0799 (calc. 215.0794, error < 0.5 μ (2.3 ppm)), 215.0799 (0.09, M^+), 184.0612 (1.96, $M^+ - CH_3O$), 155.0556 (51.3, $M^+ - CO_2CH_3$). GC/MS: 184 (1, $M^+ - CH_3O$), 156 (5), 155 (18), 130 (10), 123 (10), 117 (46), 113 (12), 53 (13), 44 (21), 43 (*100*, CH_3O^+), 41 (11). Anal. calc. for $C_9H_{13}NO_5$: C 50.23, H 6.09, N 6.51; found: C 50.16, H 6.16, N 6.39.

(1S,2R)-Methyl 2-(Acetoxymethyl)-1-{N-[(tert-butoxy)carbonyl]amino}cyclopropanecarboxylate ((+)-**9**). To a soln. of (+)-**8** (296 mg, 1.37 mmol) in abs. *t*-BuOH (7 ml) at 70° under dry N_2, $Pb(OAc)_4$ (1.22 g, 2.75 mmol, 2 equiv.) was added at once and the brown mixture stirred at reflux (TLC (Et_2O) monitoring). After 30 min (reaction complete), the mixture was heated to reflux for another 30 min and cooled to r.t. Et_2O (50 ml) and $NaHCO_3$ (500 mg) were added, the mixture evaporated, and the residue taken up in Et_2O (50 ml). The brown suspension was filtered through 1 cm of silica gel with 200 ml of Et_2O. The filtrate was evaporated to yield 407 mg of a yellow oil. Chromatography on silica gel gave 298.1 mg (75.7%) of (+)-**9**, containing $< 5\%$ (NMR) of unreacted (+)-**8**. GC: t_R 4.90 min. TLC: R_f 0.9 (Et_2O), 0.28 (Et_2O/hexanes 1:1). $[\alpha]_D^{25} = +3.6 \pm 0.2$ ($c = 0.98$, CH_2Cl_2). IR ($CDCl_3$): 3420*w* (br.), 2980*w*, 2960*w*, 2940*w*, 1720*s* (br.), 1600*w*, 1480*m*, 1440*w*, 1370*m*, 1350*w*, 1265*m* (sh), 1245*s*, 1200*w*, 1165*m*, 1090*w*, 1080*w*, 1060*w*, 1035*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.52 (br. *s*, NH); 4.34 (br. *dd*, $J = 4.1, 11.7$, 1 H, CH_2–C(2)); 4.00 (br. *dd*, $J = 11, 11$, 1 H, CH_2–C(2)); 3.71 (*s*, CH_3O); 2.08 (*s*, CH_3CO); 2.03 (*m*, H–C(2)); 1.80 (br. *m*, 1 H–C(3)); 1.46 (*s*, *t*-Bu); 1.11 (br. *m*, 1 H–C(3)). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.7,

171.2 (2 *s*, $COOCH_3$, CH_3CO); 156.3 (*s*, *t*-BuO*C*O); 80.2 (*s*, $(CH_3)_3C$); 63.0 (*t*, CH_2–C(2)); 52.2 (*q*, CH_3O); 38.2 (*s*, C(1)); 28.2 (*q*, $(CH_3)_3C$); 26.2 (*d*, C(2)); 21.0 (*q*, CH_3CO); 20.6 (*t*, C(3)). HR-MS: no M^+ ($C_{13}H_{21}NO_6$, calc. 287.1365), 231.0746 ($[M - C_4H_8]^+$, calc. 231.0743, error < 0.3 μ (1.3 ppm)). GC/MS: 232 (2), 231 (25, $M^+ - C_4H_8$), 171 (10), 154 (15), 128 (26), 127 (48), 114 (12), 112 (35), 86 (13), 68 (61), 67 (15), 61 (11), 59 (15), 57 (96), 55 (10), 54 (13), 43 (*100*, CH_3CO^+), 42 (16), 41 (89).

(1S,2R)-1-{N-[(tert-Butoxy)carbonyl]amino}-2-(hydroxymethyl)cyclopropanecarboxylic Acid ((–)-**7**). Amide (+)-**8** (1.97 g, 9.13 mmol) was oxidized with $Pb(OAc)_4$ (8.1 g, 18.3 mmol, 2 equiv.) in *t*-BuOH (35 ml) as described above (reflux time 2 h). After evaporation, the mixture was filtered through 1 cm of silica gel with 400 ml of Et_2O and the filtrate evaporated: yellow oil (3.50 g). This was directly dissolved in 50 ml of 1N NaOH/abs. MeOH heated at reflux for 1 h, and then allowed to cool to r.t. The yellow soln. was filtered to remove insoluble material (176 mg) and evaporated. The residue was taken up in H_2O (100 ml), the pH adjusted to 4.0 using a pH-meter and 1N HCl, and this soln. extracted with AcOEt (6 × 50 ml). The pH was readjusted with 1N HCl after every two extractions. The combined org. phase was dried ($MgSO_4$) and evaporated, and the resulting crystals were washed with AcOEt (2 × 2 ml). Recrystallization from AcOEt/hexanes (r.t. → –20°) and drying at r.t./0.2 Torr for 24 h yielded 1.17 g (55.4%) of colorless crystals. From the mother liquor and the AcOEt washings were obtained another 84.4 mg after two recrystallizations, total 1.25 g (59.2% over two steps). M.p. 148° (dec). $[\alpha]_D^{25} = -38.0 \pm 0.2$ (*c* = 2.55, MeOH). ^{1}H-NMR (400 MHz, CD_3CN): like that of (±)-**7** (see below). The optical purity was determined by converting (–)-**7** into its methyl ester with diazomethane and then into its (–)-MTPA-amide with (–)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid ((–)-MTPA) and DCC [26]. A diastereoisomeric excess of 93.4% was measured by ^{19}F-NMR.

(1RS,2SR)-1-{N-[(tert-Butoxy)carbonyl]amino}-2-(hydroxymethyl)cyclopropanecarboxylic Acid ((±)-**7**) *from* (±)-**5**. A soln. of (±)-**5** (7.81 g, 50 mmol) in *ca.* 2M NH_3/abs. MeOH (300 ml) was stirred at r.t. for 30 min, after which the solvent was evaporated at r.t./0.2 Torr. The residue was taken up in CH_2Cl_2 (50 ml) evaporated: 10.0 g of colorless solid (±)-**6**.

To a soln. of (±)-**6** (10 g) in abs. pyridine (50 ml) at r.t. under dry N_2, Ac_2O (5.2 ml, 5.6 g, 55 mmol) was added dropwise with stirring within 5 min. The mixture was stirred at r.t. for 18 h, then abs. MeOH (1 ml, 25 mmol) added, and the mixture stirred for another 12 h. The solvent was distilled off at 30°/0.5 Torr to a dry ice trap. The resulting clear liquid was taken up in AcOEt (50 ml) and evaporated and the colorless solid dried at r.t./0.2 Torr for 12 h: give 11.42 g of (±)-**8**.

To a soln. of (±)-**8** (11.42 g) in abs. *t*-BuOH (200 ml) at 70° under dry N_2, $Pb(OAc)_4$ (44.3 g, 100 mmol) was added in one portion and the brown mixture stirred at 70–80° for 3 h. After cooling to r.t., the mixture was evaporated, taken up in Et_2O (300 ml), and filtered through 2 cm of silica gel with 1500 ml of Et_2O. The filtrate was evaporated: 26.8 g of (±)-**9** as a yellow oil.

A mixture of (±)-**9** (26.8 g) and 1.5N NaOH/abs. MeOH (250 ml) was heated to reflux for 1 h. The resulting yellow suspension was allowed to cool to r.t. The insolubles (2.08 g of a yellow powder) were filtered off. The filtrate was evaporated, the residue dissolved in H_2O (250 ml), and the pH adjusted to 4.0 with 1N HCl/sat. NaCl soln. using a pH-meter. The soln. was extracted with AcOEt (6 × 300 ml), readjusting the pH after every extraction. The combined org. phase was dried ($MgSO_4$) diluted with hexanes (50 ml), and evaporated: white crystals/brown oil. The brown oil was washed off the crystals with 10 ml of cold AcOEt, and the crystals were dried to give 5.29 g of white rhombs. The AcOEt filtrate was decolorized with charcoal and evaporated, and the residue recrystallized from AcOEt/hexanes: 927 mg of white crystals. A second recrystallization of the mother liquor gave 120 mg, together 6.332 g (54.8% over four steps) of (±)-**7** as white rhombs. TLC: R_f 0.6 (AcOEt). M.p. 151°. IR (KBr): *3410m*, *3250s*, 3110*m*, 3050*m*, 2990*m*, 2950*m*, 1680*s*, 1550*m*, 1435*m*, 1400*w*, 1375*m*, 1330*m*, 1300*s*, 1260*m*, 1240*m*, 1190*m*, 1100*w*, 1070*w*, 1035*m*, 980*w*, 960*w*, 920*w*, 885*w*, 855*w*, 775*w*, 750*w*, 670*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.14 (br. *s*, NH); 4.01 (*dd*, *J* = 3.2, 12.2, 1 H, CH_2–C(2)); 3.22 (br. *t*, *J* = 11, 1 H, CH_2–C(2)); 2.34 (br. *dd*, *J* = 12, 4, H–C(2)); 1.65 (br. *dd*, *J* = 9.8, 5, H–C(3)); 1.48 (*s*, *t*-Bu); 0.87 (br. *dd*, *J* = 7.7, 5, H–C(3)). ^{1}H-NMR (400 MHz, CD_3CN): 5.98 (br. *s*, NH); 3.79 (*dd*, *J* = 4.0, 12.2, 1 H, CH_2–C(2)); 3.15 (*dd*, *J* = 9.8, 12.2, 1 H, CH_2–C(2)); 2.04 (*dddd*, *J* = 9.8, 9.8, 7.7, 4.0, H–C(2)); 1.48 (*dd*, *J* = 9.8, 5.1, 1 H–C(3)); 1.43 (*s*, *t*-Bu); 0.88 (*dd*, *J* = 7.7, 5.1, 1 H–C(3)). ^{13}C-NMR (100 MHz, CD_3CN): 171.8 (*s*, COOH); 156.6 (*s*, *t*-BuO*C*O); 78.5 (*s*, $(CH_3)_3C$); 59.3 (*t*, CH_2–C(2)); 35.7 (*s*, C(1)); 28.7 (*d*, C(2)); 25.8 (*q*, $(CH_3)_3C$); 17.3 (*t*, C(3)). HR-MS: no M^+ 175.0483 ($[M - C_4H_8]^+$, $C_6H_9NO_5$, calc. 175.0481, error < 0.2 μ (1.1 ppm)). MS: 175 (32.3, $M^+ - C_4H_8$), 158 (15.2), 157 (13.6), 114 (12.2), 113 (11.6), 101 (15.1), 100 (66.9).

(1RS,2SR)-1-Amino-2-(hydroxymethyl)cyclopropanecarboxylic Acid ((±)-**1**). A soln. (prepared at 0°) of (±)-**7** (232 mg, 1 mmol) in 40 ml of 1N HCl/abs. MeOH (prepared from AcCl and MeOH) was stirred for 48 h at 0°. The mixture was evaporated: greenish oil. The oil was taken up in H_2O (3 ml), loaded on a *Dowex* column (H^+-form, 1.5 × 16 cm), and eluted with H_2O until neutral and chloride-free ($AgNO_3$ test). The product was then

eluted with 100 ml of 2N NH_3/H_2O. Evaporation gave a colorless semisolid which was taken up in EtOH (20 ml) and evaporated: white powder. This was dissolved in H_2O (1.5 ml) at 90°, and EtOH (4 ml) was added until the soln. became turbid. After 4 d at −20°, colorless crystals formed which were dried at r.t./0.2 Torr for 24 h: 118 mg (90%) of (±)-**1**. TLC: R_f 0.50 (H_2O/AcOH/BuOH/AcOEt 1:1:1:1). M.p. 202° (dec.). IR (KBr): 3250*s* (br.), 3050*s* (br.), 2700*m* (br.), 1630*s*, 1570*s*, 1480*w*, 1425*s*, 1390*s*, 1270*m*, 1245*m*, 1200*w*, 1155*w*, 1105*w*, 1050*s*, 1005*w*, 935*w*, 905*w*, 850*w*, 795*w*, 770*w*, 690*w* (br.). ^{1}H-NMR (400 MHz, D_2O): 3.96 (*dd*, J = 5, 12.5, I H, CH_2–C(2)); 3.75 (*dd*, J = 7, 12.5, 1 H, CH_2–C(2)); 1.88 (*dddd*, J = 10, 8, 7, 5, H–C(2)); 1.49 (*dd*, J = 10, 7, 1 H–C(3)); 1.20 (*dd*, J = 8, 7, 1 H–C(3)). ^{13}C-NMR (100 MHz, D_2O): 177.4 (*s*, COOH); 60.7 (*t*, CH_2–C(2)); 41.6 (*s*, C(1)); 26.6 (*d*, C(2)); 17.1 (*t*, C(3)). HR-MS ($C_5H_9NO_3$): 131.0569 (M^+, 131.0582, error < −1.3 μ (−10 ppm)). MS: 131 (0.6, M^+), 114 (6.1, $M^+ - H_2O$), 101 (13.4), 100 (*100*, $M^+ - CH_2OH$), 87 (31.9).

(1S,2R)-1-Amino-2-(hydroxymethyl)cyclopropanecarboxylic Acid ((−)-**1**). From 232 mg (1 mmol) of (−)-**7** was produced, upon the above treatment, 120 mg (91.4%) of (−)-**1** as colorless crystals. M.p. 240° (dec.). $[\alpha]_D^{25} = -74.5 \pm 1.0$ (c = 0.184, H_2O).

(1R,2S)-1-Amino-2-(hydroxymethyl)cyclopropanecarboxylic Acid ((+)-**1**). The condensation of (*S*)-epichlorohydrin ((*S*)-**2**) with dimethyl malonate produced (+)-**5** in 50% yield: $[\alpha]_D^{25} = +138.2$ (c = 0.76, CH_2Cl_2). Ammonolysis gave (+)-**6** in quantitative yield: $[\alpha]_D^{25} = +13.5$ (c = 0.42, CH_2Cl_2). Acetylation yielded (−)-**8** (70% yield): $[\alpha]_D^{25} = -47.11$ (c = 0.45, $CHCl_3$). $Pb(OAc)_4$ treatment and hydrolysis (85%) gave (+)-**7**: $[\alpha]_D^{25} = +37.77$ (c = 0.45, MeOH). Hydrolysis and ion-exchange chromatography gave (+)-**1** in 90% yield: $[\alpha]_D^{25} = +73.81$ (c = 0.48, H_2O).

REFERENCES

[1] M.C. Pirrung, U.P. Trinks, *J. Chem. Soc., Chem. Commun.*, in press.

[2] S.F. Yang, N.E. Hoffman, *Annu. Rev. Plant Physiol.* **1984,** *35,* 155.

[3] M.C. Pirrung, J.I. Brauman, *Plant Physiol. Biochem.* **1987,** *25,* 55.

[4] Y.-B. Yu, D.O. Adams, S.F. Yang, *Arch. Biochem. Biophys.* **1979,** *198,* 280; T. Boller, R.C. Herner, H. Kende, *Planta* **1979,** *145,* 293; S. Khanioskouee, J.P. Jones, R.W. Woodard, *Biochem. Biophys. Res. Commun.* **1984,** *121,* 181; R. Wiesendanger, B. Martinoni, T. Boller, D. Arigoni, *J. Chem. Soc., Chem. Commun.* **1986,** 238; K. Ramalingam, K.M. Lee, R.W. Woodard, A.B. Bleecker, H. Kende, *Proc. Natl. Acad. Sci. U.S.A.* **1985,** *82,* 7820; S. Khanioskouee, K. Ramalingam, D. Kalvin, R. Woodard, *Bioorg. Chem.* **1987,** *15,* 92.

[5] A.B. Bleecker, G. Robinson, H. Kende, *Planta* **1988,** *173,* 385.

[6] R.M. Adlington, R.T. Aplin, J.E. Baldwin, B.J. Rawlings, D. Osborne, *J. Chem. Soc., Chem. Commun.* **1982,** 1086; R.M. Adlington, J.E. Baldwin, B.J. Rawlings, *ibid.* **1983,** 290; M.C. Pirrung, *J. Am. Chem. Soc.* **1983,** *105,* 7207; G.D. Peiser, T.-T. Wang, N.E. Hoffman, S.F. Yang, H.-W. Liu, C.T. Walsh, *Proc. Natl. Acad. Sci. U.S.A.* **1984,** *81,* 3059; M.C. Pirrung, *Bioorg. Chem.* **1985,** *13,* 219.

[7] N.E. Hoffman, S.F. Yang, A. Ichihara, S. Sakamura, *Plant Physiol.* **1982,** *70,* 195; M.C. Pirrung, G.M. McGeehan, *Angew. Chem. Int. Ed.* **1985,** *24,* 1044; J.E. Baldwin, R.M. Adlington, G.S. Lajoie, B.J. Rawlings, *J. Chem. Soc., Chem. Commun.* **1985,** 1496; M.C. Pirrung, G.M. McGeehan, *J. Org. Chem.* **1986,** *51,* 2103; J.E. Baldwin, R.M. Adlington, G.S. Lajoie, C. Lowe, P.D. Baird, K. Prout, *J. Chem. Soc., Chem. Commun.* **1988,** 775.

[8] M.C. Pirrung, *J. Am. Chem. Soc.* **1983,** *105,* 7207; J.E. Baldwin, D.A. Jackson, R.M. Adlington, B.J. Rawlings, *J. Chem. Soc., Chem. Commun.* **1985,** 206; M.C. Pirrung, *Biochemistry* **1986,** *25,* 114; H.W. Gardner, J.W. Newton, *Phytochemistry* **1987,** *26,* 621; T. Okamoto, M. Shimada, S. Oka, *Chem. Lett.* **1987,** 817.

[9] M. Guy, H. Kende, *Planta* **1984,** *160,* 281.

[10] M.C. Pirrung, G.M. McGeehan, *J. Am. Chem. Soc.* **1986,** *108,* 5647.

[11] J.D. Watson, J. Tooze, D.T. Kurtz, in 'Recombinant DNA', Ed. W.H. Freeman, New York, 1983, Chapt. 8.

[12] P.D.G. Dean, W.S. Johnson, F.A. Middle, 'Affinity Chromatography: A Practical Approach', IRL Press, Washington, 1985; H. Schott, 'Affinity Chromatography', Dekker, New York, 1984; 'Affinity Techniques', in 'Meth. Enzymol.', Eds. W.B. Jakoby and M. Wilchek, Academic Press, New York, 1974, Vol. 34; J.R. Knowles, *Acc. Chem. Res.* **1972,** *5,* 155; I. Chaiken, M. Wilchek, I. Parikh, Eds., 'Affinity Chromatography and Biological Recognition', Academic Press, New York, 1984; H. Schott, 'Affinity Chromatography: Template Chromatography of Nucleic Acids and Proteins', Dekker, New York, 1985.

[13] F.R. Seiler, P. Gronski, R. Kurrle, G. Luber, H.-P. Harthus, W. Ax, K. Bosslet, H.-G. Schnick, *Angew. Chem. Int. Ed.* **1985,** *24,* 139; J.W. Goding, 'Monoclonal Antibodies: Principles and Practice', Academic Press,

Washington, 1983; J. M. Polak, S. V. Noorden, 'An Introduction to Immunocytochemistry', Oxford–London, 1987.
[14] R. L. Koerker, A. J. Berlinn, F. H. Schneider, *Toxicol. Appl. Pharmacol.* **1976,** *37,* 281.
[15] J. E. Baldwin, R. M. Adlington, B. J. Rawlings, *Tetrahedron Lett.* **1985,** *26,* 481; J. E. Baldwin, R. M. Adlington, B. J. Rawlings, R. H. Jones, *ibid.* **1985,** *26,* 485.
[16] T. I. Temnikova, S. N. Semenova, *Zh. Org. Khim.* **1966,** *2,* 1171.
[17] J. E. Baldwin, *J. Chem. Soc., Chem. Commun.* **1976,** *738;* G. Stork, J. Cohen, *J. Am. Chem. Soc.* **1974,** *96,* 5270; G. Mouzin, H. Cousse, B. Bonnaud, *Synthesis* **1978,** 304.
[18] H. E. Baumgarten, A. Staklis, *J. Am. Chem. Soc.* **1965,** *87,* 1141.
[19] J. J. Baldwin, A. W. Raab, K. Mensler, B. H. Arison, D. E. McClure, *J. Org. Chem.* **1978,** *43,* 4876.
[20] G. J. F. Chittenden, *Carbohydr. Res.* **1980,** *84,* 350.
[21] D. E. McClure, B. H. Arison, J. J. Baldwin, *J. Am. Chem. Soc.* **1979,** *101,* 3666.
[22] J. M. Klunder, T. Onami, K. B. Sharpless, *J. Org. Chem.* **1989,** *52,* 1295.
[23] J. A. Dale, D. Dull, H. Mosher, *J. Org. Chem.* **1969,** *34,* 2543.
[24] A. I. Meyers, J. L. Romino, S. A. Fleming, *J. Am. Chem. Soc.* **1988,** *110,* 7245.
[25] M. Dixon, *Biochem. J.* **1953,** *55,* 170; I. H. Segel, 'Biochemical Calculations', Wiley, New York, 1976.
[26] G. M. McGeehan, Ph. D. Thesis, Stanford University, 1986.