mL). After 7 days at room temperature, phenyl isocyanate (0.08 mL) was added, the solution was kept for 3 days, and water (0.2 mL) was then added. The mixture was stirred for 18 h, the diphenylurea collected, the fitrate evaporated, and the residue digested with cyclohexane (4 × 10 mL). The digests were combined and concentrated to 5 mL, and the precipitate was collected. This solid was purified by preparative TLC (ethyl acetate–toluene, 2:3) and recrystallization of the main band from cyclohexane and then from diethyl ether. The urethane (1, R = CONHPh, R^1 = Ac, 115 mg) separated from diethyl ether as minute needles: mp 79–82 °C; $[\alpha]^{21}_D$ +84° (*c* 1.19, $CHCl_3$); ^1H NMR δ 1.16 (3 H, H_{14}), 1.84 (3 H, H_{16}), 2.16 (3 H, Ac), 2.22 ($H_{4\beta}$, $J_{4,4}$ = 15.0 Hz, $J_{3,4\beta}$ = 11.2 Hz), 2.35 ($H_{4\alpha}$, $J_{3,4\alpha}$ = 4.4 Hz), 3.14 (H_{13}), 3.18 (H_{13}, $J_{13,13}$ = 4.2 Hz), 3.85 (H), 3.93 (H_2, $J_{2,3}$ = 4.3 Hz), 4.29 (H_{15}), 4.58 (H_{15}, $J_{15,15}$ = 12.0 Hz), 4.84 (H_{11}, $J_{10,11}$ = 5.7 Hz), 4.85 (H_7), 5.25 (H_3), 6.52 (H), 6.61 (H_{10}), 7.10–7.34 (5 H). $C_{24}H_{27}NO_8$ requires: C, 63.0; H, 5.95; N, 3.1. Found: C, 63.1; H, 6.0; N, 3.0.

Acetylation of 3-*O*-Acetyl-DON. To the acetate (339 mg, 1 mmol) in methylene chloride (20 mL) were added acetic acid (65 mg, 1.13 mmol), DCC (210 mg, 0.98 mmol), acetic anhydride (0.05 mL, 53 mmol), and DMAP (35 mg), and the solution was stirred overnight. The precipitated urea was filtered off and washed with methylene chloride, and the combined filtrate and washings were washed with ice-cold hydrochloric acid (1.5 N, 3 × 10 mL), sodium hydrogen carbonate solution (2 × 15 mL), and water. Recovery afforded a crystalline residue (380 mg), which was recrystallized from diisopropyl ether, giving needles, mp 117 °C,[5] of 3,15-di-*O*-acetyl-DON (1, R = R^1 = Ac).

Hydrolysis of 3,15-Di-*O*-acetyl-DON. (a) 3,15-Di-*O*-acetyl-DON (0.136 g, 0.36 mmol) in methyl alcohol (45 mL) was treated with triethylamine (5 mL) and kept at room temperature for 24 h. The solvents were evaporated, and the residue (0.11 g) recrystallized from ethyl acetate gave DON (0.098 g, mp 153 °C).

(b) 3,15-Di-*O*-acetyl-DON (47 mg, 0.12 mmol) in methyl alcohol (2 mL) was treated with ammonium hydroxide solution (1 N, 2 mL). Chromatography of the reaction mixture by HPLC, and by TLC, after 25 min revealed none of the starting material and a 1:1:1 mixture of DON, 3-*O*-acetyl-DON, and 15-*O*-acetyl-DON. The reaction mixture was evaporated, and the residue was absorbed from cyclohexane–chloroform (1:9) on silica gel (1.9 × 20 cm). The column was eluted with toluene–ethyl acetate (1:1), and fractions (20 mL) were collected. The 3-acetate (12 mg, mp 170–172 °C) was collected in fractions 30–35 and the 15-acetate (12 mg, mp 139–140 °C) in fractions 38–42, and vomitoxin (10 mg, mp 153 °C) was eluted with ethyl acetate–methyl alcohol (5:1).

Preparation of 15-*O*-Acetyl-DON from Crude Extracts of *F. culmorum* Cultures. The gum [50 g, 30–40% (1, R = H, R^1 = Ac)] from the methanol phase of the methanol–petroleum ether–water partition of the extract from 100 L of *F. culmorum* culture fluid[3] was dissolved in *tert*-butyl methyl ether (400 mL), the filtered solution treated with diisopropyl ether (400 mL), and the solution kept at −15 °C for 48 h. In about one in four cases, the solution required seeding with a crystal of the acetate. The crystalline precipitate [ca. 12 g 80–85% (1, R = H, R^1 = Ac)] was collected, the mother liquors were evaporated to about 200 mL, when a voluminous precipitate [ca. 6 g, 85–88% (1, R = H, R^1 = Ac)] was obtained. This was collected and combined with the crystals.

This material (10 g) was dissolved in methanol (200 mL) and ammonium hydroxide (1.5 N, 110 mL) added. The solution was kept at room temperature (ca. 24 °C) overnight, when the bulk of the methanol was evaporated, and then *tert*-butyl alcohol was added until a homogeneous solution was obtained. This solution was lyophilized and the resulting powder heated at 40 °C (0.01 mm) for 18 h to remove traces of ammonium acetate. The residue (8 g, 80–90% DON) was dispersed in methylene chloride (200 mL) by stirring for 2–3 h at room temperature. The dispersion was treated with DMAP (80 mg) and then dropwise, over 2 h, with a solution (50 mL) of DCC (6.1 g), acetic acid (1.7 g), and acetic anhydride (0.04 g) in methylene chloride. The mixture was stirred for 15 h, when a further 5 mL of the DCC–acetic acid solution and DMAP (40 mg) were added. The mixture was then stirred for 24 h and filtered, the filtrate evaporated, and the residue taken up in ethyl acetate (25 mL). A small quantity of dicyclohexylurea was filtered off, and the filtrate (12.5 mL) was diluted with toluene (12.5 mL). The solution was applied to a silica gel column (Merck "for TLC", 20 × 6.2 cm, made up in toluene), which was then eluted with ethyl acetate–toluene (1:1). In most cases, the eluate was discarded until a pale yellow band was eluted from the column (in other cases, the first 550 mL was discarded). The following fractions [ca. 15 mL (750 drops)] were collected: fractions 9–12 inclusive contained the 3,15-diacetate, fractions 31–40 the 3-acetate, and fractions 50–75 the 15-acetate. Fractions 50–75 were combined and evaporated, and the crystalline residue (2.56 g), mp 140–141 °C, >99%, 15-*O*-acetyl-DON (ca. 60% conversion on the calculated amount of 3-acetate used), was collected with the aid of a little diethyl ether.

Under these conditions, the 15-acetate is usually in the form of very fine needles that are easily dispersed in air. **Suitable precautions** should therefore be taken to avoid contamination of the laboratory and its staff.

Registry No. 1 (R = H, R^1 = Ac), 50722-38-8; 1 (R = R^1 = Ac), 56676-60-9; 1 (R = Ac, R^1 = H), 88337-96-6; 1 (R = *t*-$BuSiMe_2$, R^1 = Ac), 115032-19-4; 1 (R = *t*-$BuSiMe_2$, R^1 = H), 115032-20-7; 1 (R = *t*-$BuSiMe_2$, R^1 = THP), 115032-21-8; 1 (R = H, R^1 = THP), 115032-22-9; 1 (R = Ac, R^1 = THP), 115032-23-0; 1 (R = THP, R^1 = H), 115032-24-1; 1 (R = THP, R^1 = Ac), 115032-25-2; 1 (R = Ph_3C, R^1 = Ac), 115032-26-3; 1 (R = Ph_3C, R^1 = H), 115032-27-4; 1 (R = CONHPh, R^1 = Ac), 115032-28-5; DON, 51481-10-8.

Acyl-Substituent Effects on Ester Aminolysis

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Carbonyl-displacement reactions are a prevalent class of organic and biochemical reactions. Structure–reactivity relationships have been used extensively in mechanistic investigations of these acyl-transfer reactions.[1] For example, the dependence of the rate constants on the basicity (pK_a) of the nucleophile and of the leaving group has been useful in characterizing these reactions in terms of changes in effective charge on reactants in progressing from the ground state to the transition state.[2] Polar aliphatic acyl substituent effects can also be useful in describing the structure of the transition state in carbonyl-displacement reactions.[3,4] Unlike the effects of substituents on the nucleophiles (β_{nuc}) and on the leaving group (β_{lg}), the effects of substituents on the acyl group (ρ^*_{acyl}) depend not just on the charge development but also on the hybridization change upon reaching the transition state.[2,3] By comparing the effects of acyl substituents on the rate of acyl transfers from *p*-nitrophenol to hydroxide and to thiolate with calibrating equilibria (i.e., hydroxide or thiolate addition to a series of substituted aldehydes), we have shown that the magnitude of the Hammett–Taft reaction constant ($\rho^*_{acyl} \sim 3$) is influenced nearly equally by the development of a negative charge on the carbonyl oxygen and by saturation of the carbonyl bond.[3] In these reactions with negatively charged nucleophiles the transition state resembles the anionic tetrahedral intermediate.[3] To further investigate the potential use of the Hammett–Taft reaction constant as an index of transi-

(1) (a) *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978. (b) Jencks, W. P. *Cold Spring Harbor Symp. Quant. Biol.* **1971**, *36*, 1–11. (c) Kirsch, J. F. In *Advances in Linear Free Energy Relationships*; Chapman, N. B., Shorter, J., Eds.; Plenum: New York, 1972; pp 369–340.

(2) Williams, A. *Acc. Chem. Res.* **1984**, *17*, 425–430.

(3) Shames, S. L.; Byers, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 6170–6177.

(4) Bruice, T. C.; Hegarty, A. F.; Felton, S. M.; Donzel, A.; Kundu, N. G. *J. Am. Chem. Soc.* **1970**, *92*, 1372–1378.

0022-3263/88/1953-3862$01.50/0

tion-state structure, we have examined acyl-substituent effects on the rate of acyl transfer to uncharged nucleophiles (amines), because this reaction involves a transition state of zero net charge.

Experimental Section

Materials. *p*-Nitrophenyl Esters. The *p*-nitrophenyl esters of acetic acid and trimethylacetic acid were obtained from Aldrich Chemical Co. The esters of propionic acid, butyric acid, and *N*-carbobenzoxyglycine were obtained from Sigma Chemical Co. Most of the other *p*-nitrophenyl esters were prepared as described previously.[3] *p*-Nitrophenyl cyanoacetate was prepared by condensation of cyanoacetic acid (Aldrich) and *p*-nitrophenol in the presence of dicyclohexylcarbodiimide following the general procedure of Holmquist and Bruice.[5] The ester was purified by flash chromatography (silica gel) with ethyl acetate–hexane (2:1) to yield a white solid with mp 102–103 °C. Elemental anal. (Galbraith Laboratories, Inc., Knoxville, TN). Calcd for $C_9H_6N_2O_4$: C, 52.45; H, 2.94; N, 13.60. Found: C, 52.15; H, 2.96; N, 13.55. *p*-Nitrophenyl methoxyacetate was prepared from methoxyacetyl chloride (Aldrich). The acyl chloride (10 g, 0.09 mol) was added to 250 mL of ice-cold ethyl acetate containing *p*-nitrophenol (10 g, 0.07 mol). Triethylamine (~10 mL) was added, and the solution was magnetically stirred in an ice bath for 0.5 h. After coming to room temperature, the reaction mixture was filtered to remove the precipitate and the ethyl acetate was removed by rotary evaporation. The residue was dissolved in ether and acidified with HCl gas to precipitate remaining traces of the triethylamine. After filtration, the volume of the ether solution was reduced to ~25 mL by rotary evaporation. Crystals formed after standing overnight in the cold (0 °C). The crystals were filtered and washed with petroleum ether. The melting point was found to be 56–58 °C. Anal. Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.28; H, 4.37; N, 6.72. The purity of the *p*-nitrophenyl esters was also checked by monitoring the change in absorbance at 400 nm following basic hydrolysis in 0.1 M NaOH. In all cases the purity was greater than 96%.

The amines and buffers were obtained from Sigma and used without further purification. Reaction solutions were prepared in glass distilled deionized water and titrated to the desired pH with either NaOH or HCl.

Methods. Spectrophotometric measurements were carried out with a Beckman 3600 spectrophotometer with a variable temperature control accessory set at 26 °C. This instrument was used for most of the kinetic measurements, but for the few very rapid reactions (with rate constants > 1 min^{-1}), a Dionex Model D-110 stopped-flow spectrophotometer, interfaced with a Biomation 810 transient recorder and a strip-chart recorder, was used. Potentiometric titrations and pH measurements were carried out on a Metrohm (Brinkmann) combititrator.

Kinetic Measurements. The stock ester solutions (~5 mM) were made up in acetonitrile (distilled from P_2O_5). The ionic strength of the reaction mixture was maintained at 1.0 M with NaCl. The reactions were initiated by the addition of 5 μL of the ester solution to 1 mL of a thermally equilibrated solution of the other reactants. The pH was maintained either with the amine nucleophiles, when the desired pH was within the buffering capacity of the amine, or with 0.01 M CAPS [3-(cyclohexylamino)propanesulfonic acid], PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], or BICINE [*N*,*N*-bis(2-hydroxyethyl)glycine]. Hydrolysis of the esters, catalyzed by these buffers, was negligible ($k_2 \leq 0.02$ M^{-1} min^{-1}) under the conditions employed in this study. The rates of reaction with the amines were measured by observing the absorbance (400 nm) due to *p*-nitrophenoxide. The reactions were carried out under pseudo-first-order conditions (amine in excess) and found to follow first-order kinetics for at least 3 half-lives. Rate constants were evaluated graphically and determined at least in triplicate for each reaction.

For the pH dependence of the reactions with amines, the kinetic parameters were estimated by the median method of Cornish-Bowden and Eisenthal.[6] The nonparametric 95% confidence intervals for the parameters were calculated by the method of Cornish-Bowden et al.[7] The equation characterizing the pH dependence was expressed in the following form:

$$k_{obsd} - k_h = \frac{k^{lim}(1/a_{H^+})}{(1/K_a) + (1/a_{H^+})} \quad (1)$$

where k_{obsd} is the observed first-order rate constant at a given amine concentration and k_h is the rate constant for ester hydrolysis in the absence of amine. k^{lim} is the limiting rate constant for the reaction with amine in the completely unprotonated form. Plots of ($k_{obsd} - k_h$) vs free amine concentration were found to be linear over the concentration range employed in these studies (typically 4–20 mM).

Results

The Taft plot for the specific base catalyzed hydrolysis of *p*-nitrophenyl esters is linear[8] over the range of acyl substituents examined ($-0.3 \leq \sigma^* \leq 1.3$). The cyanoacetate ester, which can undergo an ElcB hydrolysis,[9] does not deviate from this Taft plot because the reaction was measured at a pH several units below the pK_a corresponding to ionization of the CH group in the ester. The slope of $\rho^* = 2.65$ under our experimental conditions (26 °C, μ = 1.0 M) and can be compared to $\rho^* = 2.87$ at an ionic strength of 0.5 M.[3]

Amines are good nucleophiles in acyl transfers involving *p*-nitrophenyl esters. For example, the second-order rate constant for the reaction of glycine with *p*-nitrophenyl acetate [k_2 = 140 (±5) M^{-1} min^{-1}] is about 18% of that for the hydroxide reaction [k_{OH} = 794 (±20) M^{-1} min^{-1}]. The pH dependence for the reaction of glycine with the *p*-nitrophenyl esters indicates that only the unprotonated amine is reactive and the kinetically determined pK_a's [9.69 (±0.09) with *p*-nitrophenyl acetate and 9.3 (±0.2) with *p*-nitrophenyl chloroacetate] are not significantly different from the potentiometrically determined pK_a value of 9.62 (±0.02). The second-order rate constants for the reaction of unprotonated glycine with these esters were identical (±3%) in H_2O and D_2O. This is consistent with the expected nucleophilic rather than a general base-catalytic role of the amine in these reactions. Solvent isotope effects for reactions of imidazole and *N*-methylimidazole were also examined for the less reactive esters ($\sigma^* \leq 0.1$) and found to be ≈1.

Aminolyses of *p*-nitrophenyl esters are less sensitive to acyl-substituent effects than is alkaline hydrolysis. The Taft plot for the reaction of glycine with a series of *p*-nitrophenyl esters is illustrated in Figure 1. The ρ^* value for this reaction is 1.6. Plotting the logarithm of the limiting second-order rate constant for the reaction of glycine versus the logarithm of the saponification rate constant yields the following correlation equation:

$$\log k_2^{gly} = 0.68 \log k_{OH} - 0.05 \quad (2)$$
$$n = 11,\ r = 0.97$$

The pivalate (trimethylacetate) ester (not included in this correlation) shows a negative deviation (by a factor of 11). This deviation is also seen in the Taft plot for aminolyses with other amines but is not seen in the Taft plot for reaction with hydroxide.

The reactions of these esters with amines less basic than glycine were also examined. As was the case with glycine, the potentiometric pK_a's and the kinetically apparent pK_a's

(5) Holmquist, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1969**, *91*, 2982–2985.
(6) Cornish-Bowden, A.; Eisenthal, R. *Biochem. J.* **1974**, *139*, 721–730.
(7) Cornish-Bowden, A.; Porter, W. R.; Trager, W. F. *J. Theor. Biol.* **1978**, *74*, 163–175.
(8) $\log k_{OH} = 2.65\sigma^* + 2.75$; $n = 14$, $r = 0.97$. The interval estimator of the slope (90% confidence) is 2.32–2.98.
(9) (a) Inoue, M.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 1644–1653. (b) Inoue, M.; Bruice, T. C. *J. Org. Chem.* **1986**, *51*, 959–963.

Table I. Parameters for Taft Plot[a]

amine	pK_a[b]	ρ^*	c[c]	n	r
glycine	9.62 (±0.02)	1.62 (±0.42)	1.91	10	0.967
glycine methyl ester	7.90 (±0.05)	1.03 (±0.27)	1.26	8	0.942
N-methylimidazole	7.38 (±0.05)	1.55 (±0.12)	1.42	10	0.983
imidazole	7.18 (±0.05)	1.64 (±0.13)	1.50	5	0.995

[a] $\log k_2 = \rho^*\sigma^* + c$; 26 °C, μ = 1.0 M. [b] Potentiometric value. [c] c = log k_2 calculated for *p*-nitrophenyl ester of acyl group with $\sigma^* = 0$. Units of k_2 are M^{-1} min^{-1}.

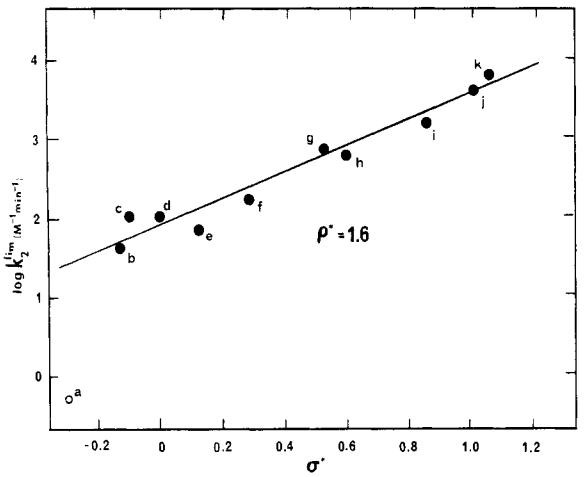

Figure 1. Taft plot for the reaction of various *p*-nitrophenyl esters with glycine (26 °C, μ = 1.0 M). The various acyl substituents are (a) $(CH_3)_3C$, (b) $CH_3(CH_2)_2$, (c) CH_3CH_2, (d) CH_3, (e) $Br(CH_2)_3$, (f) $(CH_3O)_2P(O)CH_2CH_2$, (g) CH_3OCH_2, (h) $C_6H_5CH_2OC(O)NHCH_2$, (i) ICH_2, (j) $BrCH_2$, (k) $ClCH_2$. The interval estimator of the slope (90% confidence) is 1.22–2.04.

(determined with *p*-nitrophenyl acetate) were identical.

The parameters for the Taft plots are summarized in Table I. The similarity in reactivity of glycine and *N*-methylimidazole, with respect to sensitivity to acyl-substituent effects, is also evident when the logarithms of the respective rate constants are plotted against each other (not shown). The slope of this line, m = 0.97 [interval estimator (90% confidence) = 0.87–1.07], is the ratio of the ρ^* values for the reactions with *N*-methylimidazole and with glycine. Although in this study the reaction of imidazole was only examined with five esters [R = $(CH_3)_3C$, $CH_3(CH_2)_2$, CH_3, $(CH_3O)_2P(O)CH_2CH_2$, and $ClCH_2$], its reactivity with each of these was found to be very similar to that of *N*-methylimidazole. The correlation equation is

$$\log k_2^{\text{N-MeIm}} = 0.94 \log k_2^{\text{Im}} + 0.01 \qquad (3)$$

$$n = 5,\ r = 0.999$$

Thus the ρ^* values for the reactions with glycine, *N*-methylimidazole, or imidazole are all ~1.6, which is about half the magnitude of the ρ^* value for the saponification reaction but slightly higher than the value for the reaction of glycine methyl ester. The ρ^* value with this amine, however, is less reliable (r = 0.942) than the others.

Discussion

Acyl-transfer reactions involve, in addition to proton transfers, heavy-atom reorganization (i.e., bond making, bond breaking, and changes in the hybridization of the acyl carbon). The Taft reaction constant, ρ^*_{acyl}, is sensitive to these changes. An interpretation of this value in terms of transition-state structure is simplified if it is known whether the rate-limiting step involves *formation* or *breakdown* of the tetrahedral intermediate. For example, in the alkaline hydrolysis or thiolysis (with *N*-acetyl-L-cysteine) of *p*-nitrophenyl esters, the rate-limiting step is formation of the tetrahedral intermediate. The ρ^*_{acyl} value for these reactions is ~3.[3] The magnitude of this value indicates a transition-state structure resembling that of the anionic tetrahedral intermediate. As was the case for the hydrolysis and thiolysis reactions, the rate constants for the aminolysis of the *p*-nitrophenyl esters are correlated reasonably well to the single-parameter Hammett–Taft equation based on the polar acyl substituent constant.

For the reaction of either glycine or *N*-methylimidazole with the 10 acyl-substituted *p*-nitrophenyl esters, the ρ^* value is 1.6 (Table I). This sensitivity constant is 60% of that obtained for the alkaline hydrolysis. This lower sensitivity confirms the results of Holmquist and Bruice[10] obtained with *o*-nitrophenyl esters. This is also consistent with a transition state resembling a zwitterionic tetrahedral intermediate in the aminolysis reaction versus a transition state resembling an anionic tetrahedral intermediate in the alkaline hydrolysis. By comparing the equilibrium ρ^* value (1.7) for aldehyde hydration (or thiohemiacetal formation) with the ρ^* value (1.3) for gem diol ionization, we concluded that the ρ^* value for formation of an anionic tetrahedral intermediate is influenced nearly equally by bond saturation (~60%) and electrostatic (~40%) effects.[3] Thus, the aminolysis ρ^* value is in quantitative as well as qualitative agreement with the value expected for a reaction involving a transition state of zero net charge.

Imidazole is well-known as a nucleophilic catalyst for hydrolysis of nitrophenyl esters.[10-14] The similarity of the rate constants for the reactions of imidazole and *N*-methylimidazole with various *p*-nitrophenyl esters (eq 3) suggests a similarity in transition-state structure for the reaction with each of these nucleophiles. Wolfenden and Jencks[15] have shown that acyl-*N*-methylimidazolium (1-acetyl-3-methylimidazolium) is a good nondissociating model for the acylimidazolium ion. The similarity in the ρ^* values for the reactions of glycine, imidazole, and *N*-methylimidazole is consistent with a transition-state structure resembling that of a zwitterionic tetrahedral intermediate. Thus, in the imidazole reaction, if there is any proton abstraction from the zwitterionic tetrahedral intermediate, it must occur after the rate-limiting step.

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Registry No. CAPS, 1135-40-6; PIPES, 5625-37-6; BICINE, 150-25-4; $(CH_3)_3CC(O)O$-*p*-$C_6H_4NO_2$, 4195-17-9; $CH_3(CH_2)_2C(O)O$-*p*-$C_6H_4NO_2$, 2635-84-9; $CH_3CH_2C(O)O$-*p*-$C_6H_4NO_2$, 1956-06-5; $CH_3C(O)O$-*p*-$C_6H_4NO_2$, 830-03-5; $Br(CH_2)_2C(O)O$-*p*-$C_6H_4NO_2$, 78939-58-9; $(CH_3O)_2P(O)CH_2CH_2C(O)O$-*p*-$C_6H_4NO_2$, 78939-55-6; $PhCH_2OC(O)NHCH_2C(O)O$-*p*-$C_6H_4NO_2$, 1738-86-9;

(10) Holmquist, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1969**, *91*, 2985–2993.
(11) Johnson, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237–330.
(12) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969.
(13) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 675–681.
(14) Gresser, M. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 6970–6980.
(15) Wolfenden, R.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4390–4393.

$ICH_2C(O)O$-*p*-$C_6H_4NO_2$, 31252-85-4; $BrCH_2C(O)O$-*p*-$C_6H_4NO_2$, 19199-82-7; $ClCH_2C(O)O$-*p*-$C_6H_4NO_2$, 777-84-4; *p*-nitrophenyl cyanoacetate, 80256-92-4; *p*-nitrophenyl methoxyacetate, 31252-86-5; glycine, 56-40-6; glycine methyl ester, 616-34-2; *N*-methylimidazole, 616-47-7; imidazole, 288-32-4; cyanoacetic acid, 372-09-8; methoxyacetyl chloride, 38870-89-2.

Highly Stereocontrolled Synthesis of the Four Individual Stereoisomers of Statine

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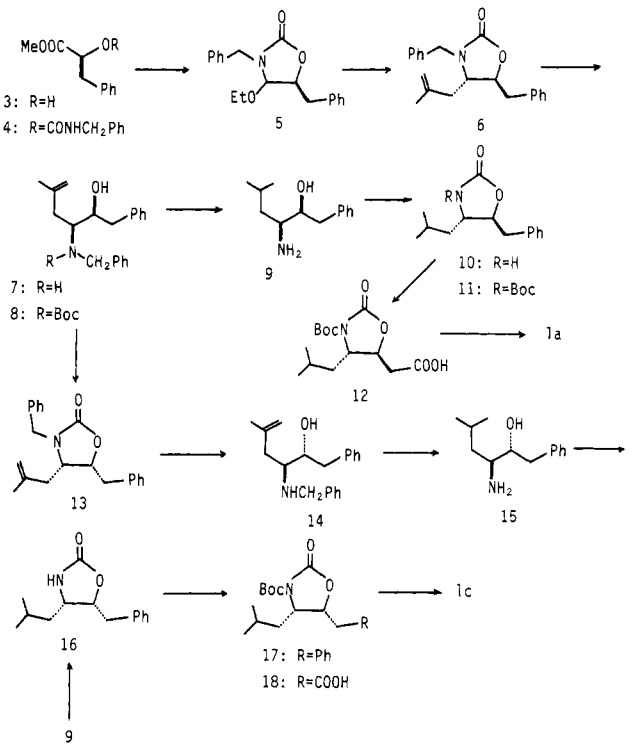
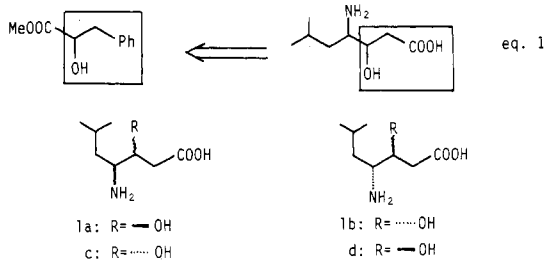
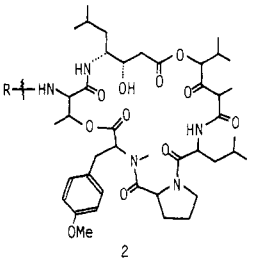
Pepstatin[1] is a natural peptide having the structure Iva-Val-Val-Sta-Ala-Sta, wherein statine is (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**1a**). Incorporation of statine into appropriate peptide sequences has led to the discovery of potent human renin inhibitors.[2] Several methods for synthesis of statine have been reported.[3-5] The most practical methods among them appear to be those based on the aldol condensation of a N-protected form of (*S*)-leucinal (as the source of C_3 and C_4) with an acetic acid derivative (e.g., metalated, as the source of C_1 and C_2).[3] In the reported examples based on this approach, however, the 3*R*,4*S* isomer **1c** must be separated from (3*S*,4*S*)-statine (**1a**) by somewhat laborious column chromatography. Methods[4] for an asymmetric synthesis of (3*S*,4*S*)-statine of high enantiomeric purity starting from L-leucine have also been reported. Recently, the 3*S*,4*R* isomer **1d** of statine,[6] the isomer derived from D-leucine, has received considerable attention as a key component of the didemnins (**2**),[7,8] compounds with significant antitumor and antiviral activity. We have now developed a new method for synthesis of all four stereoisomers of statine with high enantiomeric purity. The 3*R*,4*S* and 3*S*,4*R* isomers were prepared by diastereoconversion of the 3*S*,4*S* and 3*R*,4*R* isomers, respectively, through an improved method involving cyclocarbamation.[9] Our synthetic strategy utilizes an α-hydroxyphenylpropionic acid ester, both enantiomers of which are readily available, as the synthon for the β-hydroxy carboxylic acid moiety (eq 1), and a highly diastereoselective isobutenylation[10] as the source of the 4-isobutyl group in the final products **1**. The results of these studies are described in this paper.

Scheme I







2

Condensation of benzyl isocyanate with methyl (*S*)-α-hydroxy-β-phenylpropionate (**3**), obtained by esterification of (*S*)-α-hydroxy-β-phenylpropionic acid,[11,12] yielded the carbamate **4** (Scheme I). Reduction of **4** with diisobutylaluminum hydride followed by treatment with ethanol (pH 1–2, 4 h) yielded the 4-ethoxy derivative **5** as a 1:1 mixture of 4,5-cis and -trans isomers in 95% yield from **4**. Isobutenylation at the 4-position was achieved by treatment of **5** with β-methallyltriphenylstannane ($TiCl_4$, CH_2Cl_2, 0 °C → room temperature, 10 h) to give **6** in 84%

(1) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, H.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259.
(2) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T. Y.; Payne, L. S.; Schorn, T. W.; Lamont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Boparai, A. S. *Nature (London)* **1983**, *303*, 81.
(3) Rich, D. H.; Sun, E. T.; Boparai, A. S. *J. Org. Chem.* **1978**, *43*, 3624. Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016.
(4) Woo, P. W. K. *Tetrahedron Lett.* **1985**, *26*, 2973. Kogen, H.; Nishi, T. *J. Chem. Soc., Chem. Commun.* **1987**, 311.
(5) Morishima, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 115. Kinoshita, M.; Aburaki, S.; Hagiwara, A.; Imai, J. *J. Antibiot.* **1973**, *26*, 249. Danishefsky, S.; Kobayashi, S.; Kerwin, J. F., Jr. *J. Org. Chem.* **1982**, *47*, 1981. Hayan, A. F.; Fehrentz, J. A.; Chapleur, Y.; Castro, B. *Bull. Soc. Chim. Fr.* **1983**, 207. Rague, B.; Fehrentz, J. A.; Guegan, R.; Chapluer, Y.; Castro, B. *Bull. Soc. Chim. Fr.* **1983**, 230. Dufour, M.-N.; Jouin, P.; Poncet, J.; Pontaroni, A.; Castro, B. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1895. Sakaitani, M.; Ohfune, Y. *Tetrahedron Lett.* **1987**, *28*, 3987. Other references are cited therein.
(6) Harris, B. D.; Bhat, K. L.; Joullie, M. M. *Tetrahedron Lett.* **1987**, *28*, 2837.
(7) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857.
(8) Rinehart, K. L., Jr.; Gloer, J. B.; Hughes, R. G., Jr.; Renis, H. E.; McGovern, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science (Washington, D.C.)* **1981**, *212*, 933. Jiang, T. L.; Liu, R. H.; Salmon, S. E. *Cancer Chemother. Pharmacol.* **1983**, *11*, 1.
(9) Kano, S.; Yokomatsu, T.; Iwasawa, H.; Shibuya, S. *Tetrahedron Lett.* **1987**, *28*, 6331.
(10) Kano, S.; Yuasa, Y.; Shibuya, S. *Heterocycles* **1987**, *26*, 373.
(11) Cohen, S. G.; Weinstein, S. Y. *J. Am. Chem. Soc.* **1964**, *86*, 5326 and references cited therein.
(12) Yamada, S.; Koga, K.; Juang, T. M.; Achiwa, K. *Chem. Lett.* **1976**, 927.

0022-3263/88/1953-3865$01.50/0