(CN) cm^{-1}. Anal. Calcd for $C_{28}H_{27}N_3 \cdot 0.2H_2O$: C, 82.20; H, 6.75; N, 10.27. Found: C, 82.00; H, 6.74; N, 10.24.

1-Benzyl-2,6-dicyano-2-(α-hydroxybenzyl)piperidine (1g and 1h). A 5:2 THF/hexane solution (14 mL) of lithium diisopropylamide (6 mmol) was added drop by drop to a THF (20 mL) solution of 1-benzyl-2,6-dicyanopiperidine (1.13 g, 5.0 mmol) at −78 °C over 15 min. Benzaldehyde (0.68 g, 6.30 mmol) was then added, and stirring was continued for 1 h at −78 °C. Workup and purification by column chromatography on silica gel (benzene/ethyl acetate (10:1)) gave 1.36 g (80%) of a 7:3 mixture of two stereoisomers, **1g** (the more polar) and **1h** (the less polar), as a colorless oil. The isomers were separated by preparative TLC on silica gel (benzene/ethyl acetate (10:1)).

1g: R_f = 0.28 (benzene/ethyl acetate (10:1)); colorless crystals; mp 148–149 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 1.37–1.58 (m, 2 H, $-CH_2CH_2CH_2-$), 1.63–2.00 (m, 4 H, $-CH_2CH_2CH_2-$), 2.68 (d, J = 3.6 Hz, 1 H, O*H*), 3.82 (dd, J = 2.6, 7.1 Hz, 1 H, C*H*CN), 3.87 (d, J = 14.2 Hz, 1 H, $PhCH_aH_b$), 5.04 (d, J = 14.2 Hz, 1 H, $PhCH_aH_b$), 5.10 (d, J = 3.6 Hz, 1 H, C*H*OH), 7.26–7.45 (m, 8 H, Ar*H*), 7.45–7.52 (m, 2 H, Ar*H*); IR (KBr) 3450, 2225, 1488, 1446, 1052, 746, 700 cm^{-1}. Anal. Calcd for $C_{21}H_{21}N_3O$: C, 76.10; H, 6.38; N, 12.67. Found: C, 76.10; H, 6.44; N, 12.63.

1h: R_f = 0.53 (benzene/ethyl acetate (10:1)); colorless crystals; mp 210–212 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 1.37–1.47 (m, 1 H), 1.60–1.72 (m, 1 H), 1.72–1.88 (m, 3 H), 1.95–2.05 (m, 1 H) (the preceding 6 H: $-(CH_2)_3-$), 3.46 (s, 1 H), 3.92 (d, J = 13.5 Hz, 1 H, $PhCH_aH_b$), 3.95 (m, 1 H), 4.54 (d, J = 13.5 Hz, 1 H, $PhCH_aH_b$), 5.36 (s, 1 H, C*H*OH), 7.36–7.46 (m, 8 H, Ar*H*), 7.56–7.60 (m, 2 H, Ar*H*); IR (KBr) 3500, 2220, 1446, 1057, 756, 700 cm^{-1}. Anal. Calcd for $C_{21}H_{21}N_3O$: C, 76.10; H, 6.38; N, 12.67. Found: C, 76.36; H, 6.46; N, 12.42.

Decyanization of (4-Bromophenyl)-1-morpholinoacetonitrile (1a) by Borane. Typical Procedure. Borane–THF complex (1.1 M THF solution, 2.0 mL, 2.2 mmol) was added to a THF (5 mL) solution of **1a** (281 mg, 1.0 mmol) at room temperature. The solution was stirred for 40 h at that temperature. Water (1.0 mL) and 1,4-diazabicyclo[2.2.2]octane (680 mg, 6.0 mmol) were then added, and the mixture was stirred for 14 h at room temperature. The mixture was diluted with dichloromethane (10 mL), dried ($MgSO_4$), and then concentrated under reduced pressure. The oily residue was purified by column chromatography on silica gel (EtOAc/benzene (1:1)) to give 1-[(4-bromophenyl)methyl]morpholine (**2a**; 249 mg, 97%) as colorless crystals: mp 82–83 °C (lit.[12] mp 83–84 °C).

1,2,6-Tribenzylpiperidine (2f). Decyanization of **1f** gave a 78:22 mixture of *R*,R** and *R*,S** isomers: colorless oil; ^1H NMR (500 MHz, $CDCl_3$; *R*,R** isomer) δ 1.2–1.5 (m, 3 H), 1.5–1.7 (m, 3 H) (the preceding 6 H, $-(CH_2)_3-$), 2.61 (dd, J = 8.8, 13.2 Hz, 2 H, $PhCH_aH_b$), 2.96 (dd, J = 5.8, 13.2 Hz, 2 H, $PhCH_aH_b$), 3.05–3.15 (m, 2 H, −NC*H*−), 3.87 (d, J = 14.3 Hz, 1 H, $PhCH_aH_bN$), 3.90 (d, J = 14.3 Hz, 1 H, $PhCH_aH_bN$), 6.9–7.3 (m, 13 H, Ar*H*), 7.31 (t, J = 7.7 Hz, 1 H, Ar*H*), 7.47 (d, J = 7.4 Hz, 2 H, Ar*H*); ^1H NMR (500 MHz, $CDCl_3$; *R*,S** isomer) δ 2.42 (dd, J = 10.5, 13.2 Hz, 2 H, $PhCH_aH_b$), 2.85–2.9 (m, 2 H, −NC*H*−), 3.01 (dd, J = 3.3, 13.2 Hz, 2 H, $PhCH_aH_b$), 4.02 (s, 2 H, $PhCH_2N$); IR (KBr) 3050, 2860, 2930, 1495, 1450, 1030, 740, 730, 700 cm^{-1}. Anal. Calcd for $C_{26}H_{29}N \cdot 0.4H_2O$: C, 86.09; H, 8.28; N, 3.86. Found: C, 86.14; H, 8.05; N, 3.85.

1-Benzyl-2-(α-hydroxybenzyl)piperidine (2g). Similarly, **1g** afforded a 91% yield of an 89:11 mixture of (*R*,S**)- and (*R*,R**)-**2g**.[13] **1h** gave 91% yield of a 65:35 mixture of (*R*,S**)- and (*R*,R**)-**2g**: a colorless oil; ^1H NMR (270 MHz, $CDCl_3$; *R*,S** isomer) δ 1.15–1.75 (m, 7 H, $-(CH_2)_3-$ and O*H*), 2.6–2.75 (m, 2 H, $-NCH_2CH_2-$), 2.9–3.05 (m, 1 H, −NC*H*−), 3.86 (d, J = 13.2 Hz, 1 H, $PhCH_aH_b$), 3.97 (d, J = 13.2 Hz, 1 H, $PhCH_aH_b$), 4.80 (d, J = 9.9 Hz, 1 H, C*H*OH), 7.2–7.3 (m, 6 H, Ar*H*), 7.3–7.4 (m, 4 H, Ar*H*); ^1H NMR (270 MHz, $CDCl_3$; *R*,R** isomer) δ 0.9–1.1 (m, 2 H), 2.0–2.15 (m, 1 H), 2.4–2.5 (m, 1 H) (the preceding 4 H, $-(CH_2)_3-$), 3.28 (d, J = 13.4 Hz, 1 H, $PhCH_aH_b$), 4.42 (d, J = 13.4 Hz, 1 H, $PhCH_aH_b$), 5.35 (d, J = 3.2 Hz, 1 H, C*H*OH); ^{13}C NMR ($CDCl_3$; *R*,S** isomer) δ 23.74, 23.84, 25.51, 53.20, 58.11, 65.69, 70.25, 125.71, 126.73, 127.11, 128.04, 128.40, 128.98, 138.57, 141.35; ^{13}C NMR ($CDCl_3$; *R*,R** isomer) δ 18.91, 18.98, 20.94, 45.15, 56.61, 64.17, 70.44, 127.24, 127.31, 127.56, 127.74, 128.20, 128.87, 139.10, 142.63; IR (neat) 3360, 2940, 1450, 1048, 700 cm^{-1} (for the 65:35 mixture). Anal. Calcd for $C_{19}H_{23}NO \cdot 0.1H_2O$: C, 80.58; H, 8.26; N, 4.95. Found: C, 80.62; H, 8.19; N, 4.96.

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A Tabtoxinine-Related Metabolite from *Pseudomonas Syringae* pv. *tabaci*[1]

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Many pathogenic bacteria excrete metabolites that are toxic to their hosts and play an important role in the virulence mechanisms underlying the corresponding diseases.[2] An understanding of the biosynthesis of these compounds and the self-protection mechanisms evolved by the producing bacteria may aid in the development of new and better forms of treatment of bacterial infections. The biosynthetic pathways are typically revealed through studies on a set of mutants deficient in toxin production/excretion where, ideally, each mutant is altered at only a single genetic locus. The best way of obtaining such mutants, is through gene disruption by transposon mutagenesis, with transposons (Tn# (# stands for n = 1, 2, 3, ...)) being DNA sequences that can insert themselves somewhat randomly into other DNA sequences, but once resident in a cell generally prevent further entry of identical transposons.[3] These transposons carry one or more antibiotic resistance genes, which allow for the selection of mutated bacteria on antibiotic-containing media, and the genes interrupted by a given transposon can be identified via hybridization with a radioactive probe. However, identification of the *functions* of the affected genes requires the identification/structural elucidation of metabolites that accumulate due to the biosynthetic blocks that were introduced. The tools of organic chemistry and molecular biology thus become very much complementary. We are using such a combined approach to unravel the virulence mechanisms of *Pseudomonas syringae* pv. *tabaci*, the cause of wildfire disease in tobacco, and report here on the structural elucidation of N^6-acetyl-5-hydroxy-5-(hydroxymethyl)lysine, **1**, from a Tn*5*-generated Tox^- mutant. **1** is chemically closely related to tabtoxinine,[4] **2**, which itself is obtained from tabtoxinine β-lactam,[4] **3**, by acid hydrolysis. **3** has been identified as the actual wildfire toxin;[5] however, *P. syringae* pv. *tabaci* excretes it as a nontoxic dipeptide with serine or threonine, the so-called tabtoxin,[6,7] from which **3** is liberated in planta by peptidases.[8,9] As judged by our chromatographic assays, 20 Tox^+ isolates of *P. syringae* pv. *tabaci* from diverse tobacco growing areas around the world produce **1** as a minor component (at 5% of the amount of **3**) whereas nine naturally occurring Tox^- strains do not. **1** therefore is

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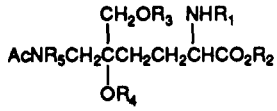
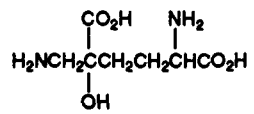
Table I. Comparison of ^{13}C NMR Chemical Shift Data for N^6-Acetyl-5-hydroxy-5-(hydroxymethyl)lysine, 1, and Tabtoxinine, 2

resonance	1	2
COOH	175.33	175.26
CONH	174.69	171.98
C-2	55.40	53.33
C-3	24.78	24.96
C-4	29.97	33.03
C-5	74.70	75.06
CH_2-N	44.45	46.32
CH_2-O	64.80	
CH_3	22.56	

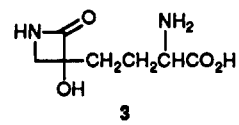
likely to be an intermediate in the biosynthetic pathway of 3 rather than a shunt metabolite thereof.



	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
4	H	Me	H	H	H
5	H	Me	Ac	Ac	H
6	Ac	Me	Ac	Ac	H
7	Dns	H	H	H	H
8	Tms	Tms	Tms	Tms	Tms



2



3

Tn5 mutagenesis was carried out using standard molecular biological procedures,[3,10] and toxin deficient mutants were selected by screening for lack of inhibition zone production on a lawn of *Escherichia coli*,[11] for inability to produce a chlorotic halo in a tobacco leaf assay,[12] and for absence of tabtoxin and tabtoxinine β-lactam in a chromatographic assay.[5] Two toxin-deficient mutants, PT039 and PT243, were isolated. PT039 overproduced 1 and was grown in 1 L of a chemically defined medium.[12] The culture filtrate was passed over a cation-exchange column, and the extracted material eluted with 4% aqueous ammonia. Protein and polysaccharides were removed by methanol precipitation. After chromatography on silica gel and Sephadex LH-20, 7 mg of pure 1 were obtained.

Accurate mass measurement of 1 by FAB-MS established an elemental composition of $C_9H_{18}N_2O_5$ and two double bond equivalents. H/D exchange with D_2O revealed six acidic hydrogen atoms. These could be subdivided into carboxy, amino, and hydroxyl hydrogens as follows. Esterification with methanolic HCl shifted the mass of the molecular ion by 1 × 14 units, thus revealing one carboxy function, which in turn accounted for one double bond equivalent. Acetylation of the methylated sample shifted the mass of the molecular ion by up to 3 × 42 units, revealing three additional amino/hydroxyl functions. Of these, one was shown to be an amino function by dansylation of the underivatized metabolite, which shifted the molecular ion from m/z 235 to 468 (Dns − H = 233 units). Together these functionalities accounted for five acidic hydrogens (COOH, 2 OH, and NH_2). Only one spot was seen upon TLC analysis of the products formed after acid hydrolysis and dansylation of the metabolite.[13] Its position did not correspond to that of any of the common amino acids. Hydrolysis shifted the mass of the metabolite to 193 (−42 units), and subsequent dansylation to 426 (+233 units) and 659 (+2 × 233 units). These data were consistent with an additional CH_3CONH group, which could also account for the sixth acidic hydrogen and the second double bond equivalent. This then excluded a ring structure or multiple carbon–carbon bonds.

Table II. Comparison of ^1H NMR Chemical Shift and Multiplicity Data for N^6-Acetyl-5-hydroxy-5-(hydroxymethyl)lysine, 1, and Tabtoxinine, 2

resonance	1	2
C-2	3.66 (t, J = 6 Hz)	4.30 (t, J = 6 Hz)
C-3	1.86 (m)	2.28 (m)
C-4	1.52 (m)	2.09 (m)
CH_2-N, $CH_{a,b}$-N	3.22 (s)	3.42 (d, J = 13 Hz)
		3.64 (d, J = 13 Hz)
CH_2-O	3.38 (s)	
CH_3	1.95 (s)	

Table III. Characteristic Fragment Ions of 1 and Derivatives Observed in FAB-CA-B/E Linked Scan Analysis

	M + H$^+$	a	b	c	d
1	235	217	158	140	94
4	249	231	172	154	94
5	333	273	214	154	94
6	375	315	256	196	136
7	468	450	391	373	327

^{13}C NMR spectroscopy confirmed nine carbon atoms (Table I). Two resonances were observed in the carboxylic acid/carbamide region as expected. The seven resonances for the aliphatic carbon atoms could be subdivided into one quaternary, one tertiary, four secondary, and one primary carbon atom using the DEPT pulse sequence. These assignments were also consistent with the ^1H NMR data (Table II). ^1H homonuclear correlation spectroscopy (COSY) established a connectivity of CH_2–CH_2–CH and two isolated CH_2 groups. Comparison with the NMR data obtained for 2 under identical conditions, indicated that 1 had indeed a tabtoxinine backbone with, however, two modifications. The first was the acetylation of probably the ε-amino function, since the COSY spectrum could be interpreted as showing a weak ^5J coupling between CH_3CO and one of the isolated methylene groups. The second modification was the apparent replacement of the carboxyl group at the quaternary carbon by a CH_2X group. Since this reduced the degree of chirality at the quaternary carbon center and thereby the degree of magnetic nonequivalence for the geminal protons at the aminomethyl group, it explained why in the ^1H NMR spectrum of 1 the methylene groups gave rise to a singlet, whereas in 2 and other α-hydroxy-α-(aminomethyl)carbonyl systems they give rise to a pair of doublets.[4,14]

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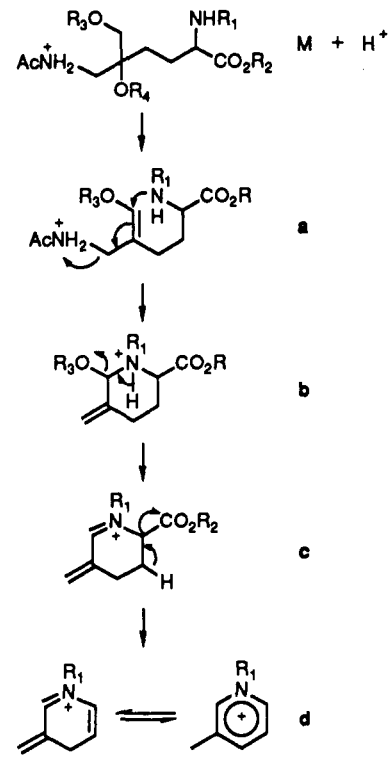
Unambigous positional assignment of the functional groups, especially the acetylamino function, was expected to come from collision activation and B/E linked scan analysis on the $[M + H]^+$ ions of 1 and its derivatives. However, while a consistent fragment pattern emerged (Table III, discussed below), it involved a cyclic rearrangement that was not readily interpreted. 1 was therefore converted to its Tms_5 derivative, 8, to allow comparison with the reported data for the Tms_5 derivative of 2.[4] The most useful information from the EIMS analysis of 8 came from observation of the α-cleavage products at the α-amino group, namely $TmsNH{=}CHCOOTms^+$ and $M^+ - CO_2Tms$, which proved that the ϵ- and not the α-amino group was acetylated. 1 was also corroborated by the sequential loss from the molecular ion of (i) $AcNTmsCH_2$, TmsOH, and TmsOH plus CO (the latter from the carboxylic acid function) and of (ii) TmsOH and $TmsOCH_2$. However, a major ion at m/z 288 that according to linked scan studies arises from m/z 477 ($M^+ - CO_2Tms$), remains unexplained at the present time. Interestingly, an ion at m/z 288 is likewise present in the reported EIMS spectrum for Tms_5-tabtoxinine for which it also has not been explained.[4] We intend to clarify this fragmentation in the context of our analysis of four other potential tabtoxinine intermediates. Since there is no δ-carboxyl function in 1, it is not able to undergo the McLafferty rearrangement involving the δ-carboxyl and ϵ-aminomethyl groups that has been found for 2.[4]

The fragmentation for 1 in its protonated form was found to be drastically different from that of the molecular ion radical of its Tms derivative. FAB-CA-B/E linked scan analysis of 1 showed the following characteristic fragmentation pattern: m/z 235 → 217 ($-H_2O$), 172 ($-H_2O$, $-CO_2H$), and 140 ($-H_2O$, -77); m/z 217 → 199 ($-H_2O$), 171 (-46; $-H_2O$, -CO), 140 (-77); m/z 140 → 94 (-46). Corresponding fragments were seen for the methyl, 4, the methyl/acetyl, 5 + 6, and the dansyl, 7, derivatives. The major features were a loss of water or acetic acid to give ion **a**, followed by loss of mass 77 + n acetyl groups (n = 0, 1), resulting in an ion **c** of mass 140 plus derivatizing groups (Table III). Upon methylation, the mass of **c** increased by 14 units, demonstrating that it harbored the carboxy function. Dansylation and methylation/acetylation shifted m/z 140 to 373 and 196, respectively, showing that **c** also had a free amino group. The observed loss of 46 units ($-H_2O$, -CO) from **1c** and **7c** was consistent with the assignment of the carboxy function. As expected, methylation transformed the loss of 46 into a loss of 60 units (m/z 154 →94). The generation of **c** from **a** could not be accounted for by a single bond cleavage but had to involve a hydroxyl function, since the loss of 77 units could be shifted by acetylation, but not by dansylation, to a loss of 119 units.

We rationalize the loss of 77 or 119 units from **a** as a loss of 59 + 18 or 60 units as follows (Scheme I). Loss of the tertiary hydroxyl group as water or acetic acid creates **a** with a double bond at the quaternary carbon, which is then subject to a nucleophilic attack by the α-amino group, leading to **b** via the loss of acetamide (-59 units); **b** ions, although of minor abundance, were indeed confirmed (Table III). The primary hydroxyl group is subsequently lost as water or acetic acid to give **c**, which then looses the carboxy or carboxymethyl group to give the stable **d** ions.

Recently, Anzai et al.[15] suggested that *P. syringae* pv. *tabaci* can be defeated with its own biosynthetic machinery by transfering one of its genes, designated *ttr*, into tobacco and thereby rendering the normally susceptible tobacco plant tabtoxin-resistent. Crude extracts from transformed *Escherichia coli* harboring the *ttr* gene, but not from *E. coli* lacking it, formed new radioactive, but as yet uncharacterized, products from tabtoxin and [^{14}C]acetyl-CoA. Nevertheless, Anzai et al. concluded that the *ttr* gene encodes an enzyme which acetylates tabtoxin and/or 3 and speculated that acetylation probably occurs at the β-lactam amino group. Our study provides the first hard evidence for such an acetylation. Since per experimental design 1 must be a biosynthetic intermediate of 3 or a metabolite thereof, it appears that acetylation takes place early in the biosynthesis of tabtoxin rather than at the level of the final products. If it does, this may explain why *P. syringae* pv. *tabaci* is not affected by its own metabolites. If the *N*-acetyl group stays on until the dipeptide with serine or threonine has formed and only then the β-lactam ring is generated, the cytoplasm of *P. syringae* pv. *tabaci* cells may never be exposed to even low concentrations of toxic 3.

Scheme I. Suggested Fragmentation Pathway for the M + H$^+$ Ions of 1 and 4–7



Experimental Section

General. ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-400 instrument and referenced to the solvents D_2O/DHO at 4.75 or D_2O/CD_3CN at 119.7 ppm, respectively. Mass spectra were obtained on a VG-ZAB-SE instrument using glycerol as the matrix in FABMS and the direct probe inlet for EIMS. Elemental compositions were determined at a mass resolution of 10000 (10% valley definition). B/E linked scan spectra were acquired with a scan rate of 5 s/decade and fragmentation was enhanced by collisional activation with helium in the first field-free region under conditions where the parent ions were 50–90% reduced.

Transposon Mutagenesis. Mutants (Tox^-) deficient in toxin production were produced by transposon mutagenesis with Tn*5* as follows. The recipient strain, *P. syringae* pv. *tabaci* 11528R, was subjected to two passages in nutrient broth (NB, 50 mL, 24 and 30 h, respectively, at 24 °C, and 250 rpm on a rotary shaker in the dark) containing rifamycin SV (200 μg/mL). Three milliliters of the final culture were washed four times with NB and suspended in 1 mL of NB. The donor strain, *P. aeruginosa* PA011

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(pM075),[10] was subjected to two passages (18 and 2.5 h at 37 °C) in NB plus carbenicillin (500 μg/mL) and kanamycin sulfate (20 μg/mL). A 0.5-mL aliquot of the final culture was washed four times with NB and suspended in 0.5 mL of NB. Donor and recipient strain were mixed, pelleted by centrifugation, and resuspended in 50 μL of NB. The drop was placed on a NB agar plate having a dry surface and allowed to dry before incubation at 28 °C for 16 h. Bacteria that had grown at that time were removed, suspended in 1 mL of 0.9% NaCl and screened for Tn*5* mutants on NB agar containing rifamycin and kanamycin (28 °C for 2 days). After a second passage on this selective medium, colonies of Tn*5* mutants were obtained at a frequency of 5.2×10^{-3} per donor cell. The colonies were screened for Tox^- mutants employing the method of Gasson,[11] who found that Tox^+ bacteria produce an inhibition zone on a lawn of *E. coli*. Two Tn*5*-generated Tox^- mutants were isolated, PT039 and PT243. Lack of toxin production was verified using a tobacco leaf response.[12]

Quantitative Analysis of Culture Filtrates. Quantitation of **1**, **3**, and tabtoxins in culture filtrates from diverse *P. syringae* pv. *tabaci* isolates was by ion-exchange, Sephadex LH20, and RP chromatography as described by Thomas et al.[5] Identification of **1** for strains other than PT039 was based on cochromatography.

N^6-Acetyl-5-hydroxy-5-(hydroxymethyl)lysine (1). *P. syringae* pv. *tabaci* PT039 was grown in the chemically defined medium of Wooley et al.[12] (1 L, at 20 °C and 250 rpm on a rotary shaker for 3 days). Cells were removed by centrifugation (40000*g* for 15 min), and **1** was extracted from the culture filtrate by passing the latter over a cation-exchange column (Amberlite CG-120, 2 × 29 cm), from which it was then desorbed with 4% aqueous ammonia. The eluate (30 mL) was immediately concentrated almost to dryness by rotatory evaporation at 15–20 °C, and the concentrate was repeatedly diluted with water and concentrated in vacuo to remove the ammonia. The final concentrate (5 mL) was adjusted to 80% methanol and the resulting precipitate removed by centrifugation (20000*g* for 10 min). The supernatant was concentrated to 200 μL in vacuo and applied to preparative silica gel plates (Brinkman silica gel 60 F254, 20 × 20 cm, 2 mm thick). Upon development with butanol–acetic acid–water (3:1:1), **1** migrated as a band with R_f 0.26, was removed with water, concentrated, and finally purified by isocratic chromatography on a Sephadex LH-20 column (2.5 × 80 cm) using MeOH–water (1:1; 0.61 mL/min) for elution. Ten minute fractions were collected, spotted onto a TLC plate, and analyzed for amino acids using ninhydrin; fraction 23 contained 7 mg of pure **1** and was used for the structure elucidation; less pure **1** was also found in fractions 24 and 25. HRFABMS M + H^+ obsd 235.1328, $C_9H_{18}N_2O_5$, calcd 235.1294. D/H exchange of **1** was studied by FABMS using 1 μL of the ^1H NMR sample.

Deacetylation, Dansylation, and Methylation/Acetylation of 1. All derivatizations were carried out on approximately 200 μg of **1**. For deacetylation, **1** was treated for 20 h at 105 °C with 200 μL of 6 N HCl under N_2 in a sealed vial. Dansylation was achieved by dissolving **1** or deacetylated **1** in 200 μL of 0.2 M $NaHCO_3$ and treating it with a 2–4-fold molar excess of dansyl chloride in acetone (ca. 2 mg/mL) for 1 h at 37 °C. FABMS of the dansyl derivatives was only successful after separation from excess danylOH by crude purification with RP-HPLC.[16] Methylation to **4** was by 1 mL of anhydrous 3 N HCl in MeOH for 4 h at 25 °C. Subsequent acetylation to a mixture of **5** and **6** was achieved by treating **4** with 200 μL of acetanhydride/pyridine (1:1) for 16 h at 25 °C.

Tms_5 Derivative of 1 (8). Approximately 200 μg of **1** was silylated in a sealed vial with 25 μL of neat BSTFA for 3 h at 100 °C. The Tms_5 derivative proved to be unstable. After contact with moisture, only the Tms_4 derivative could be detected. The Tms_5 derivative was, however, repeatedly observed when drying of the reaction mixture was performed en route to mass analysis, namely, in the roughing vacuum of the mass spectrometer: EIMS *m/z* (rel intensity) M^+, $C_9H_{13}N_2O_5Tms_5$, calcd 594.3192, obsd 594.3188 (28), daughter ions 504, 274, 147; $M^+ - AcNTmsCH_2$, $C_6H_8NO_4Tms_4$, calcd 450.2347, obsd 450.2355 (5); $M^+ - AcNTmsCH_2 - TmsOH$, $C_6H_7NO_3Tms_3$, calcd 360.1847, obsd 360.1856 (70), daughter ions 270, 242, 191, 170; $M^+ - AcNTmsCH_2 - 2\ TmsOH - CO$, $C_5H_6NOTms_2$, calcd 242.1396, obsd 242.1371 (5); $M^+ - TmsOH$, $C_9H_{12}N_2O_4Tms_4$, calcd 504.2691, obsd 504.2708 (2); $M^+ - TmsOH - TmsOCH_2$, $C_8H_{10}N_2O_3Tms_3$, calcd 401.2112, obsd 401.2100 (24); $M^+ - CO_2Tms$, $C_8H_{13}N_2O_3Tms_4$, calcd 477.2820, obsd 477.2841 (14), daughter ions 387, 288, 198, 128; obsd 288.1668 (68), daughter ions 272, 198, 170, 147, 129, 103, 94; TmsNH=CHCOOTms, $C_2H_2NO_2Tms_2$, calcd 218.1055, obsd 218.1059 (8).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra, EI-MS spectrum of Tms_5–1, and EI-B/E-linked scan spectrum of *m/z* 477 of Tms_5–1 (6 pages). Ordering information is given on any current masthead page.

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Nucleophilic Substitution at Nitrogen and Carboxyl Carbon of *N*-Aryl-*O*-pivaloylhydroxylamines in Aqueous Solution: Competition with S_N1 Solvolysis of Model Carcinogens

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It has become apparent that S_N2 attack of aromatic and aliphatic amines, and certain carbon nucleophiles, can occur on the nitrogen of ester derivatives of *N*-arylhydroxylamines (eq 1) under conditions in which solvolysis

$$\underset{\perp}{\text{ArNHOY}} + \text{NuH} \longrightarrow \text{ArNHNu} + \text{HOY} \quad (1)$$

via an S_N1 mechanism is suppressed by low solvent polarity.[1,2] Specifically, the reaction of eq 1 has been demonstrated to occur in the neat aliphatic or aromatic amine as solvent,[1a] in THF[1b] or MeOH,[2] or in mixed-solvent systems of $CHCl_3/EtOH/H_2O$.[1c] How well the S_N2 reaction competes with S_N1 solvolysis in H_2O has not been previously reported. This is of interest because the ester derivatives **1** are models for carcinogenic metabolites of polycyclic aromatic amines, and the environment in which the carcinogens are generated in vivo is an aqueous one.[3] The possibility that acyl transfer (eq 2) may occur in an aqueous environment must also be considered.

$$\underset{\perp}{\text{ArNHOY}} + \text{NuH} \longrightarrow \text{ArNHOH} + \text{NuY} \quad (2)$$

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