# Synthesis of Nornicotine Analogues To Use as Haptens for Immunoassays[1]

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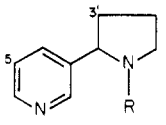
Analogues of N′-substituted nornicotines 1–6 were synthesized with functional groups that can be covalently linked to macromolecules for antibody production. The key intermediate, *trans*-3′-(hydroxymethyl)nornicotine (17) was prepared from 3-pyridinecarboxaldehyde (8) by a four-step synthesis. From 5-bromo-3-carboxypyridine (29), 5-bromonicotine (36) and 5-bromo-*N*′-nitrosonornicotine (37) were also obtained. As shown by $^1$H NMR and high-pressure LC, the analogues of *N*′-nitrosonornicotine crystallize preferentially as the *N*′-nitroso *E* isomer.

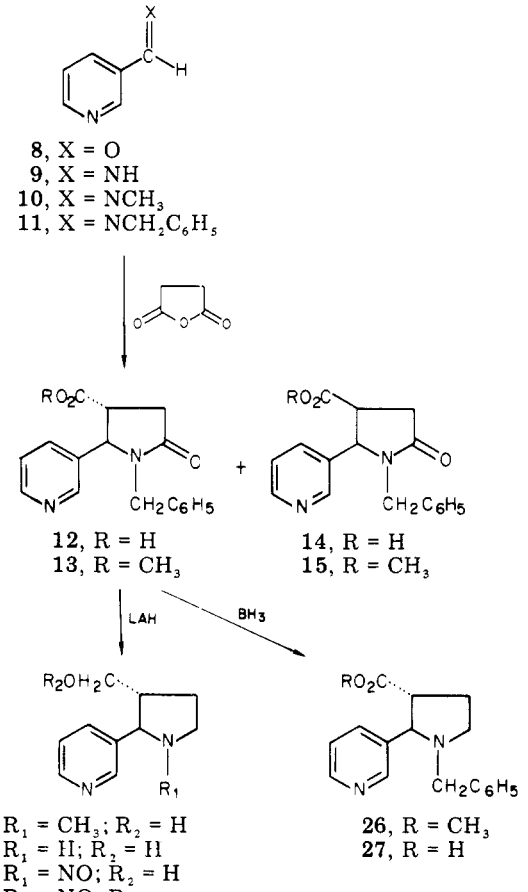
Several analogues of nornicotine (1) with a N-substituted pyrrolidine ring are present in tobacco and tobacco smoke.[3] These alkaloids 1–6 are very likely derived from the major tobacco alkaloid nicotine (7).[4,5] One of these, *N*′-nitroso-



1, R = H
2, R = NO
3, R = CHO
4, R = $COCH_3$
5, R = $COC_5H_{11}$
6, R = $CO_2CH_3$
7, R = $CH_3$

nornicotine (2), is carcinogenic in rats,[6,7] mice,[4,8] and hamsters.[9] An analytical method that could detect compounds 1–6 at the nanogram level would permit detection and estimation of these compounds in the physiological fluids and tissues of experimental animals and smokers. Radioimmunoassays offer the advantages of sensitivity and specificity as well as ease of assay.[10] They have been applied to the detection and estimation of nicotine (7),[11] *N*′-nitrosonornicotine (2),[12] and several metabolites including cotinine,[11] 4-oxo-4-(3-pyridyl)-*N*-methylbutyramide,[13] and the nicotinamide adenine nucleotide analogues of nicotine and cotinine.[14] Before immunoassays for small molecules can be developed, however, it is usually necessary to prepare suitable derivatives having functional groups by which they can be linked to macromolecules for immunization.[15] Nornicotine analogues with substituents in the 3′ and 5 position were synthesized to use as haptens for the development of radioimmunoassays for compounds 1–6.

Scheme I



8, X = O
9, X = NH
10, X = $NCH_3$
11, X = $NCH_2C_6H_5$

12, R = H
13, R = $CH_3$

14, R = H
15, R = $CH_3$

16, $R_1$ = $CH_3$; $R_2$ = H
17, $R_1$ = H; $R_2$ = H
18, $R_1$ = NO; $R_2$ = H
19, $R_1$ = NO; $R_2$ = succ
20, $R_1$ = $CH_2C_6H_5$; $R_2$ = H
21, $R_1$ = $CH_2C_6H_5$; $R_2$ = succ
22, $R_1$ = H; $R_2$ = succ
23, $R_1$ = $COCH_3$; $R_2$ = H
24, $R_1$ = $COCH_3$; $R_2$ = succ
25, $R_1$ = $COC_5H_{11}$; $R_2$ = H

26, R = $CH_3$
27, R = H

While several syntheses of nornicotine 1 have been published,[16-21] only the procedure of Hellmann and Dieterich[22]

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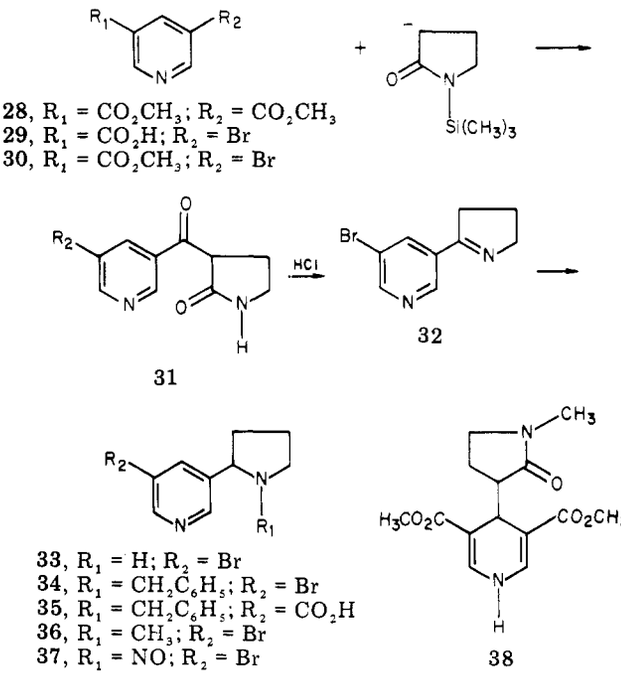
has led to the preparation of a nornicotine derivative, 5′-carboxynornicotine, which has a functional group suitable for linkage to a protein. Our first approach was to prepare the key intermediate *trans*-3′-(hydroxymethyl)-nornicotine (**17**), which could be coupled to a macromolecule through the hydroxyl group (Scheme I). We have synthesized *trans*-3′-(hydroxymethyl)nicotine (**16**) from *N*-methyl-3-pyridinaldimine (**10**) according to the procedure of Cushman and Castagnoli.[23] Like nicotine (**7**),[24] the tertiary amine **16** was demethylated by treatment with sodium nitrite at pH 5.4–6.0. This reaction gave many side products and the *N*′-nitrosonornicotine **18** was isolated by PTLC. Reaction of the *N*-nitrosamine **18** with succinic anhydride gave the hemisuccinate **19** isolated as a semisolid. In the $^1$H NMR spectrum, two doublets centered at 5.12 and 5.53 ppm, with a relative intensity of 3:7, were attributed to the *N*′-nitroso *Z* and *E* isomers, respectively. This ratio is close to the *Z*:*E* ratio observed for *N*′-nitrosonornicotine (**2**).[20]

A second synthesis of the intermediate **17** began with *N*-benzyl-3-pyridinaldimine (**11**) prepared from benzylamine and 3-pyridinecarboxaldehyde (**8**). By refluxing **11** with succinic anhydride in xylene, a mixture of the trans and cis acids **12** and **14** was obtained. This condensation was expected to be stereoselective since Cushman and Castagnoli[23] had reported that the corresponding *N*-methylimine **10** gave exclusively the trans isomer upon treatment with succinic anhydride. When treated with a methanolic $H_2SO_4$ solution, the crude mixture of acids **12** and **14** gave the corresponding methyl esters **13** and **15** in a ratio of 23:2. Crystallization of the condensation product gave the pure trans acid **12** which was used for the synthesis of the key intermediate **17**. Reduction of the ester **13** with $LiAlH_4$ gave the *N*′-benzylnornicotine **20**. Hydrogenolysis of **20** in acetic acid at 70 °C gave **17** in good yield (79%). When the hemisuccinate **21** was debenzylated under the same conditions, the nornicotine hapten **22** was also obtained in good yield (86%).

For the synthesis of the *N*′-acetylnornicotine hapten **24**, the nornicotine **17** was first treated with acetic anhydride at 100 °C. Without purification, the ester group of the resulting ester amide was selectively hydrolyzed with aqueous sodium hydroxide. The hemisuccinate **24** which was prepared by reacting **23** with succinic anhydride shows in the $^1$H NMR spectrum a downfield shift of the 3′-($OCH_2$) doublet to 4.25 ppm. By a similar sequence of reactions and by using hexanoic anhydride, the *N*′-hexanoylnornicotine **25** was prepared from **17**.

A synthesis of nicotine[20] was adapted to the preparation of 5-carboxy analogues of **2**–**6** (Scheme II). Condensation of the carbanion of 2-pyrrolidinone with 3,5-bis(carbomethoxy)pyridine (**28**) did not give the expected nicotinoylpyrrolidinone.[20,25] For example, 3-lithio-1-methyl-2-pyrrolidinone adds to the activated but sterically hindered position 4 of the pyridine ring, giving the dihydropyridine **38**. These results are comparable to the

Scheme II




**28**, $R_1 = CO_2CH_3$; $R_2 = CO_2CH_3$
**29**, $R_1 = CO_2H$; $R_2 = Br$
**30**, $R_1 = CO_2CH_3$; $R_2 = Br$

**31** **32**

**33**, $R_1 = H$; $R_2 = Br$
**34**, $R_1 = CH_2C_6H_5$; $R_2 = Br$
**35**, $R_1 = CH_2C_6H_5$; $R_2 = CO_2H$
**36**, $R_1 = CH_3$; $R_2 = Br$
**37**, $R_1 = NO$; $R_2 = Br$

**38**

treatment of the dimethyl ester **28** with $LiAlH_4$ which has been reported to reduce the pyridine ring rather than the ester group.[26,27]

We considered starting the synthesis of analogues of 5′-carboxynornicotine with 5-bromo-3-carboxypyridine (**29**) and subsequently carboxylating the pyridine moiety. Condensation of 3-lithio-1-(trimethylsilyl)-2-pyrrolidinone with 5-bromo-3-(carbomethoxy)pyridine (**30**) gave the acyl lactam **31** in fair yield (48%). Rearrangement of **31** occurred by refluxing in HCl to yield 5-bromomyosmine (**32**).[28] 5-Bromonornicotine (**33**) which was obtained by $NaBH_4$ reduction of 5-bromomyosmine (**32**) shows a long-range coupling ($J$ = 0.54 Hz) between H(4) and H(2′) in the $^1$H NMR spectrum.

While *N*′-nitrosonornicotine (**2**) has been isolated as an oil,[20,24] its 3′-hydroxymethyl analogue **18** does crystallize preferentially as the *N*-nitroso *E* isomer. As previously shown, monitoring of the $Z \rightarrow E$ configurational changes as a function of time could be achieved by $^1$H NMR[29] or high-pressure LC.[30,31] Assuming a first-order reversible reaction,[32] we have measured the initial rate constant [$k$(0 °C) = $7.6 \times 10^{-6}$ s$^{-1}$, $k$(26 °C) = $4.7 \times 10^{-4}$ s$^{-1}$] and estimated the *Z*:*E* ratio in the crystal by extrapolation to the time of dissolution. For 3′-(hydroxymethyl)-*N*′-nitrosonornicotine (**18**) this ratio was found to be 0.08 and is probably dependent on the rate and temperature of crystallization. At equilibrium this ratio increased to 0.48. The activation energy for the conversion of *E* to *Z* isomers of **18** was 25.7 kcal/mol and was comparable to values observed for *N*′-nitrosonornicotine (**2**)[31] and other nitrosamines.[33] Ten minutes after dissolution of **18** in $D_2O$, $^1$H

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NMR shows an intense doublet at 5.42 ppm which was assigned to H(2′) of the *E* isomer. At equilibrium, a second doublet observed at 5.04 ppm was assigned to H(2′) of the *Z* isomer. Since the dihedral angle defined by the N–H and adjacent C–H bonds is approximately 60°, the H(2′) and H(5′) protons are shielded when they are syn to the nitrosyl group.[29] As determined by $^1$H NMR and high-pressure LC with reverse phase, *Z*:*E* ratios in crystallized 5-bromo-*N*′-nitrosonornicotine at equilibrium at pH 7.0 are identical (0.42). The *Z* isomer is more polar and consequently has a smaller retention volume (57.6 mL) than the *E* isomer (73.6 mL).

Metalation of 5-bromonornicotine (**33**) was achieved in low yield after benzylation of the pyrrolidine nitrogen. Development of radioimmunoassays for nornicotine analogues will be the subject of other communications.[34]

## Experimental Section

Melting points were taken for samples in capillary tubes with a Thomas-Hoover apparatus (calibrated thermometer). The IR and UV spectra were determined with Perkin-Elmer Model 567 and Beckman DB-G spectrophotometers, respectively. The $^1$H NMR spectra were recorded with either a Varian A-60A or Bruker WH-90 equipped with a Digilab FT accessory and with $(CH_3)_4Si$ for $CDCl_3$ solutions or $(CH_3)_3Si(CH_2)_3SO_3Na$ for $D_2O$ solutions as an internal standard. The low-resolution mass spectra were recorded on a Du Pont 491 instrument, and high resolutions were obtained by using a CEC 110B double-focusing instrument with photoplate recording.

UV spectra were recorded in 95% $C_2H_5OH$. The TLC and PTLC (preparative thin-layer chromatography) analyses were run on silica gel F-254 plates which were purchased from EM Laboratories. Before use the plates were developed twice with $CH_2Cl_2$:$CH_3OH$ (1:1). After migrations, bands containing the desired compounds were extracted many times with $CH_2Cl_2$:$CH_3OH$ (10:1) or AcOEt:$CH_3OH$ (10:1). PTLC was carried out with 2-mm thick plates which were purchased from EM Laboratories. Florisil (100–200 mesh) furnished by Fisher was used for column chromatography. Elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. All crystallizations were carried out under a nitrogen atmosphere.

***N*-Benzyl-3-pyridinaldimine (11).** Freshly distilled benzylamine (57.3 g, 0.53 mol) was added to a solution of 3-pyridinecarboxaldehyde (**8**; 53.5 g, 0.50 mol) in benzene (100 mL) at 0 °C. The mixture was stirred with molecular sieves 3 Å (35 g) at room temperature for 24 h. The molecular sieves were removed by filtration, and the filtrate was concentrated in vacuo. Distillation of the residue gave the imine **11** (77.9 g, 79%) as a colorless oil: bp 130–145 °C (0.2 mm); UV (EtOH) $\lambda_{max}$ 257 nm ($\epsilon$ 1370), 261 (1455), 269 (1097); IR (neat) $\nu_{max}$ 1640 cm$^{-1}$ (C=N); $^1$H NMR ($CDCl_3$) $\delta$ 4.75 (d, $J$ = 1.5 Hz, N–$CH_2$), 7.08 (dd, 5-pyH), 7.25 (s, $C_6H_5$), 7.97 (dt, $J$ = 8.0 and 2.0 Hz, 4-pyH), 8.16 (t, $J$ = 1.5 Hz, CH=N), 8.54 (dd, $J$ = 5.0 and 2.0 Hz, 6-pyH), 8.89 (d, $J$ = 2.0 Hz, 2-pyH); MS *m*/*e* (rel intensity) 196 (M$^+$, 34), 169 (37), 118 (24), 105 (11), 91 (100).

Anal. Calcd for $C_{13}H_{12}N_2$: C, 79.55; H, 6.16; N, 14.28. Found: C, 79.42; H, 6.27; N, 14.37.

**1-Benzyl-*trans*-4-carboxy-5-(3-pyridyl)-2-pyrrolidinone (12).** A solution of the imine **11** (19.6 g, 0.1 mol) in xylene (25 mL) was refluxed with succinic anhydride (10.0 g, 0.1 mol) under $N_2$ for 24 h. The brownish oil obtained after decantation of xylene was dissolved in 5% NaOH (100 mL) and washed with $CHCl_3$ (2 × 100 mL). The aqueous solution was decolorized with charcoal and the pH adjusted to 4.7 with $H_3PO_4$ to precipitate a mixture of **12** and **14** as a white solid (18.8 g, 63%). Crystallization from EtOH gave pure **12**: UV (EtOH) $\lambda_{max}$ 259 nm (sh, $\epsilon$ 1590), 264 (1760), 271 (sh, 1300); IR (KBr) $\nu_{max}$ 1680 (lactam C=O), 3400 cm$^{-1}$ (OH); $^1$H NMR ($D_2O$) $\delta$ 2.76–3.27 (m, 3-$H_2$, 4-H), 4.40 (d, $J$ = 9.1 Hz, N–$CH_2$), 5.03 (d, $J$ = 5.9 Hz, 2-H), 7.23 (m, $C_6H_5$), 7.81 (m, 5-pyH), 8.27 (dt, $J$ = 8.2 and 2.0, 4-pyH), 8.54 (m, 2,6-pyH); MS *m*/*e* (rel intensity) 296 (M$^+$, 63), 205 (17), 192 (7), 150 (67), 146 (51), 118 (26), 104 (40), 91 (100).

Anal. Calcd for $C_{17}H_{16}O_3N_2$: C, 68.90; H, 5.44; N, 9.46. Found: C, 68.70; H, 5.50; N, 9.38.

**1-Benzyl-*trans*-4-(carbomethoxy)-5-(3-pyridyl)-2-pyrrolidinone (13).** The acid **12** (11.2 g, 37.8 mmol) was stirred with molecular sieves 3 Å (5 g) in 2 N methanolic $H_2SO_4$ for 40 h. The filtered solution was neutralized with 8% $NaHCO_3$ (250 mL) and extracted with $CHCl_3$ (7 × 100 mL). Evaporation of the solvent left the ester **13** as a colorless oil (6.7 g, 57%): UV (EtOH) $\lambda_{max}$ 264 nm ($\epsilon$ 2570), 273 (sh, 1904); IR (neat) $\nu_{max}$ 1685 (lactam C=O), 1735 cm$^{-1}$ (ester CO); $^1$H NMR ($D_2O$) $\delta$ 3.03 (m, 4-$H_2$), 3.36 (q, $J$ = 8.2 and 5.9 Hz, 3-H), 3.66 (s, $OCH_3$), 3.93 (d, $J$ = 15.0 Hz, N–$CH_2$) 4.60 (d, $J$ = 5.2 Hz, 2-H), 7.30 (m, 5-H, $C_6H_5$), 7.73 (dt, $J$ = 8.2 and 2.0 Hz, 4-pyH), 8.32 (m, 2-pyH), 8.47 (m, 6-pyH); MS *m*/*e* (rel intensity) 310 (M$^+$, 23), 279 (3), 251 (5), 233 (1), 219 (100).

Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.84; N, 9.03. Found: C, 69.68; H, 5.94; N, 9.22. The picrate was obtained from ethanol and crystallized in $CH_2Cl_2$–EtOH; mp 90–93 °C.

***trans*-3-(Hydroxymethyl)-2-(3-pyridyl)pyrrolidine (*trans*-3′-(Hydroxymethyl)nornicotine, 17).** A solution of the benzylamine **20** (3.6 g, 13.4 mmol) in glacial AcOH (20 mL) was stirred under hydrogen (1 atm) with Pd/C (10%, 180 mg) at 70 °C for 10 h. The catalyst was collected by filtration on Celite and the acetic acid codistilled with cyclohexane. The residual oil **17** (1.9 g, 79%) was used as such in the next steps. An analytical sample was obtained by PTLC (AcOEt/$CH_3OH$/$NH_4OH$, 65/35/11): UV (EtOH) $\lambda_{max}$ 259 nm (sh, $\epsilon$ 2040), 265 (2210), 270 (sh, 1750); IR neat $\nu_{max}$ 3250 cm$^{-1}$ (OH, NH); $^1$H NMR ($D_2O$) $\delta$ 1.47–2.75 (m, 4-H), 3.60 (d, $J$ = 6.1 Hz, –$CH_2O$), 3.85 (t, $J$ = 8.1 Hz, 5-H), 4.95 (d, $J$ = 5.4 Hz, 2-H), 7.47 (m, 5-pyH), 7.87 (dt, $J$ = 6.8 and 2.0 Hz, 4-pyH), 8,50 (m, 2,6-pyH); MS *m*/*e* (rel intensity) 179 (20), 178 (M$^+$, 52) [$C_{10}H_{14}N_2O$, calcd 178.11061, found 178.11278], 177 (34), 161 (31), 159 (21), 150 (36), 120 (100), 119 (5), 118 (39).

**1-Nitroso-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine (*trans*-3′-(Hydroxymethyl)-*N*′-nitrosonornicotine, 18) (from 16).** A solution of the nicotine **16** (1.4 g, 5.93 mmol) in $H_2O$ (15 mL, pH adjusted to 3.0 with dilute HCl) and 12 mL of pH 3 buffer (0.2 M $Na_2HPO_4$ and 0.1 M citric acid) was stirred with $NaNO_2$ (2.05 g, 29.7 mmol) at 90 °C for 6 h under nitrogen. The pH of the reaction mixture was adjusted to 5.4 with citric acid and the reaction pursued at 90 °C (6 h) after addition of $NaNO_2$ (1 g). The cold reaction mixture was saturated with NaCl, adjusted to pH 10–12 with cautious addition of NaOH pellets, and extracted with $CHCl_3$ (4 × 50 mL). Concentration of the dried ($Na_2SO_4$) organic phase gave a yellow oil that was purified by PTLC (AcOEt, 5 migrations). Extraction of the band at $R_f$ 0.30–0.40 with $CH_2Cl_2$:$CH_3OH$ (1:1) gave the nitrosamine **18** as a colorless oil (111 mg, 9%). Analytically pure **18** was obtained by crystallization from $CH_2Cl_2$–cyclohexane: mp 106–107 °C; UV (EtOH) $\lambda_{max}$ 246 nm ($\epsilon$ 4300), 259 (sh, 3800), 264 (sh, 3400); IR (KBr) $\nu_{max}$ 1045, 1440 (N–NO), 3200 cm$^{-1}$ (OH); $^1$H NMR ($D_2O$) $\delta$ 1.81–2.51 (m, 4-$H_2$), 2.64 (m, 3-H), 3.67 (d, $J$ = 5.3 Hz, $CH_2O$), 3.56–4.29 (m, 5-$H_2$), 5.04 (d, $J$ = 8.5 Hz, 2-H of *Z* isomer), 5.42 (d, $J$ = 8.5 Hz, 2-H of *E* isomer), 7.51 (q, $J$ = 7.9 and 4.9 Hz, 5-pyH), 7.85 (dt, $J$ = 9.5 and 2.0 Hz, 4-pyH), 8.58 (m, 2,6-pyH); MS *m*/*e* (rel intensity) 207 (M$^+$, 4), 189 (19), 136 (100), 135 (97), 127 (42), 120 (59).

Anal. Calcd for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 57.83; H, 6.38; N, 20.18.

**1-Nitroso-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine (18) (from 17).** To a solution of the crude amine **17** (1.20 g, 6.7 mmol) in AcOH:$H_2O$ (1:1, 6 mL) at 0 °C was added $NaNO_2$ (1.86 g, 27.0 mmol). The mixture was stirred at 4 °C for 24 h and then made basic with 20% NaOH. Extraction with $CH_2Cl_2$ (4 × 50 mL) and purification by PTLC (AcOEt, 4 migrations) gave pure **17** (367 mg, 26%) with properties identical with the previous sample.

**1-Nitroso-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine Hemisuccinate (19).** A solution of the nitrosamine **18** (209 mg, 1 mmol) in $C_6H_6$:$CH_2Cl_2$ (1:1, 10 mL) was refluxed overnight with succinic anhydride (125 mg, 1.25 mmol). The hemisuccinate **19** was purified by PTLC (AcOEt:$CH_3OH$, 20:1, 2 migrations) and isolated as a colorless semisolid (76 mg, 24%):

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UV (EtOH) $\lambda_{max}$ 242 nm ($\epsilon$ 4960), 262 (sh, 3305); IR (neat) $\nu_{max}$ 3400 (OH acid), 1735 (CO ester), 1430 $cm^{-1}$ (N–NO); $^1H$ NMR ($D_2O$) $\delta$ 1.77–2.67 (m, 4-$H_2$), 2.52 (m, $CH_2CH_2$ hemisuccinate), 2.93 (m, 3-H), 3.61–4.66 (m, 5-$H_2$), 4.29 (d, $J$ = 6.2 Hz, $-CH_2O$), 5.12 (d, 0.30 H, $J$ = 8.5 Hz, 2-H of *Z* isomer), 5.53 (d, 0.70 H, $J$ = 8.5 Hz, 2-H of *E* isomer), 7.77 (m, 5-pyH), 8.17 (dt, $J$ = 8.3 and 2.0 Hz, 4-pyH), 8.66 (m, 2,6-pyH); MS *m/e* (rel intensity) 277 ($M^+$ – NO, 16), 207 (7), 159 (100), 131 (33).

**1-Benzyl-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine (20).** Lithium aluminum hydride (5.50 g, 146 mmol) was added in small portions with stirring to a solution of the ester **13** (11.3 g, 36 mmol) in ether (400 mL) at 0 °C. The mixture was stirred at room temperature for 24 h, cooled to 0 °C, and hydrolyzed by cautious addition in successive order of 5 mL of $H_2O$, 5 mL of 15% NaOH, and 15 mL of $H_2O$. The white precipitate, collected by filtration, was washed repeatedly with $CH_2Cl_2$. Evaporation of the dried ($Na_2SO_4$) filtrate yielded the pyrrolidine **20** as a yellow oil (9.1 g, 92%): UV (EtOH) $\lambda_{max}$ 217 nm ($\epsilon$ 3150), 260 (sh, 3900), 264 (4030), 270 (sh, 3150); IR (neat) $\nu_{max}$ 3300 $cm^{-1}$ (OH); $^1H$ NMR ($CDCl_3$) $\delta$ 1.80 (m, 4-$H_2$), 2.23 (m, 3-H), 3.20 (m, 5-$H_2$, $NCH_2$), 3.64 (d, $J$ = 5.5 Hz, $CH_2O$), 7.25 (s, $C_6H_5$), 7.36 (q, 5-pyH), 7.90 (dt, $J$ = 7.9 and 2.0 Hz, 4-pyH), 8.56 (m, 2,6-pyH); MS *m/e* (rel intensity) 269 (10), 268 ($M^+$, 62), 267 (20), 220 (8), 219 (10), 192 (5), 191 (47), 190 (100), 177 (44).

Anal. Calcd for $C_{17}H_{20}N_2O$: C, 76.08; H, 7.51; N, 10.44. Found: C, 75.89; H, 7.74; N, 10.52. The dipicrate was obtained as yellow needles from EtOH and crystallized from $CH_2Cl_2$:EtOH; mp 133–136 °C.

**1-Benzyl-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine Hemisuccinate (21).** A solution of the benzylamine **20** (3.0 g, 11.2 mmol) in $C_6H_6$ (10 mL) was refluxed with succinic anhydride (1.12 g, 11.2 mmol) for 1.5 h. PTLC (AcOEt:$CH_3OH$, 10:1, 1 migration) of the reddish residue obtained by concentration of the reaction mixture gave **21** (2.12 g, 51%) as a colorless semisolid: UV (EtOH) $\lambda_{max}$ nm 257 (sh, $\epsilon$ 2380), 262 (2510), 267 (sh, 1920); IR (neat) $\nu_{max}$ 3450 (OH), 1715 $cm^{-1}$ (C=O ester); $^1H$ NMR ($D_2O$) $\delta$ 1.71–2.57 (m, 4-$H_2$), 2.57 (m, $CH_2CH_2$ hemisuccinate), 2.91 (m, 3-H), 3.65 (q, $J$ = 8.0 Hz, 5-$H_2$), 4.17 (d, $J$ = 5.0 Hz, $CH_2O$), 4.37 (d, $J$ = 9.4 Hz, $NCH_2$), 7.32 (m, $C_6H_5$, 5-pyH), 7.97 (dt, $J$ = 8.2 and 2.0 Hz, 4-pyH), 8.50 (m, 2,6-pyH); MS *m/e* (rel intensity) 368 ($M^+$, 100), 367 (35), 291 (31), 290 (77), 277 (93), 268 (46), 251 (62), 235 (22), 210 (15), 209 (15).

Anal. Calcd for $C_{21}H_{24}O_4N_2$: C, 68.47; H, 6.57; N, 7.60. Found: C, 68.25; H, 6.80; N, 7.49.

***trans*-3′-(Hydroxymethyl)nornicotine Hemisuccinate (22).** A solution of the *N*-benzylamine **21** (1.39 g, 3.77 mmol) in acetic acid (25 mL) was stirred with Pd/C (10%, 50 mg) at 70 °C under hydrogen (1 atm) for 1 h. After filtration of the catalyst on Celite, codistillation of the filtrate with cyclohexane to remove the acetic acid left **21** as a semisolid (905 mg, 86%): UV (EtOH) $\lambda_{max}$ 260 nm ($\epsilon$ 1800), 267 (sh, 1400); IR (neat) $\nu_{max}$ 3400 (OH acid, NH), 1730 $cm^{-1}$ (CO ester); $^1H$ NMR ($D_2O$) $\delta$ 1.46–2.09 (m, 4-$H_2$), 2.40 (m, $CH_2CH_2$ hemisuccinate), 2.91 (t, $J$ = 9.0 Hz, 3-H), 3.65 (q, 5-$H_2$), 4.17 (d, $J$ = 7.2 Hz, $-CH_2O$), 7.67 (m, 5-pyH), 8.09 (dt, $J$ = 9.0 and 2.0 Hz, 4-pyH), 8.68 (m, 2,6-pyH); MS *m/e* (rel intensity) 278 ($M^+$, 3), 178 (16), 177 (10), 161 (14), 160 (8), 150 (24), 147 (10), 132 (16), 120 (37), 119 (100).

**1-Acetyl-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine (23).** A mixture of the crude nornicotine **17** (1.08 g, 6 mmol) and acetic anhydride (3 mL) was stirred at 100 °C, for 2 h. The reaction at 0 °C was made basic (pH 9) by addition of 20% NaOH and stirred for 24 h at 4 °C. The amide **23** was extracted with $CH_2Cl_2$ and purified by PTLC (AcOEt:$CH_3OH$, 20:1, 5 migrations): colorless oil (234 mg, 17%); UV (EtOH) $\lambda_{max}$ 260 nm (sh, $\epsilon$ 2150), 265 (2380), 271 (sh, 1770); IR (neat) $\nu_{max}$ 3340 (OH), 1640 $cm^{-1}$ (CO amide); $^1H$ NMR ($D_2O$) $\delta$ 2.16 (s, $COCH_3$), 1.83–2.50 (m, 3-H, 4-$H_2$), 3.65 (d, $J$ = 7.0 Hz, $-CH_2O$), 3.87 (t, $J$ = 6.2 Hz, 5-$H_2$), 4.99 (d, $J$ = 3.8 Hz, 2-H), 7.49 (m, 5-pyH), 7.77 (dt, $J$ = 8.1 and 2.0 Hz, 4-pyH), 8.47 (m, 2,6-pyH); MS *m/e* (rel intensity) 220 ($M^+$, 31), 205 (16), 189 (100), 177 (50), 159 (9), 147 (30). The picrate was crystallized from aqueous ethanol; mp 141–142 °C.

Anal. Calcd for $C_{18}H_{19}N_5O_9$: C, 48.10; H, 4.26; N, 15.58. Found: C, 48.17; H, 4.41; N, 15.58.

**1-Acetyl-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine Hemisuccinate (24).** A solution of the acetylpyrrolidine **23** (535 mg, 2.43 mmol) in $C_6H_6$:$CH_2Cl_2$ (1:1, 20 mL) was refluxed with succinic anhydride (343 mg, 3.43 mmol) and pyridine (4 drops) overnight. The pure hemisuccinate **24** (580 mg, 75%) was obtained by PTLC ($CH_2Cl_2$:$CH_3OH$, 10:1): UV (EtOH) $\lambda_{max}$ 259 nm (sh, $\epsilon$ 2390), 265 (2660), 270 (sh, 1980); IR (neat) $\nu_{max}$ 1640 (CO amide), 1730 (CO ester), 3400 $cm^{-1}$ (OH acid); $^1H$ NMR ($D_2O$) $\delta$ 2.16 (s, $COCH_3$), 1.85–2.56 (m, 3-H, 4-$H_2$), 2.57 (m, $CH_2CH_2$ hemisuccinate), 3.93 (t, $J$ = 7.0 Hz, 5-H), 4.25 ($J$ = 5.3 Hz, $-CH_2O$), 4.97 (d, $J$ = 5.3 Hz, 2-H), 7.84 (m, 5-pyH), 8.26 (dt, $J$ = 7.9 and 2.0 Hz, 4-pyH), 8.62 (m, 2,6-pyH); MS *m/e* (rel intensity) 320 ($M^+$, 26), 277 (100), 236 (9), 220 (17), 205 (7), 189 (78), 177 (13), 159 (32).

**1-Hexanoyl-*trans*-3′-(hydroxymethyl)-2-(3-pyridyl)-pyrrolidine (25).** The crude nornicotine **17** (1.69 g, 9.5 mmol) was treated with hexanoic anhydride at 100 °C for 1 h. To the mixture at 0 °C was added 20% NaOH (5 mL), and the resulting suspension was stirred at 4 °C overnight. The clear solution was extracted with $CH_2Cl_2$ (3 × 5 mL), and the amide **25** was obtained as an oil by PTLC (AcOEt:$CH_3OH$, 50:1): 356 mg, 14%; UV (EtOH) $\lambda_{max}$ 259 nm ($\epsilon$ 1590), 264 (1760), 271 (1300); IR (neat) 1640 (CO lactam), 3360 $cm^{-1}$ (OH); $^1H$ NMR ($D_2O$) $\delta$ 0.86 (t, $J$ = 5.9 Hz, $CH_3$), 1.14–2.62 [m, 4-$H_2$, $(CH_2)_4$], 3.65 (d, $J$ = 6.2 Hz, $-CH_2O$), 3.90 (t, $J$ = 7.0 Hz, 5-$H_2$), 5.01 (d, $J$ = 5.3 Hz, 2-H), 7.41 (m, 5-pyH), 7.75 (dt, $J$ = 7.6 and 2.0 Hz, 4-pyH), 8.45 (m, 2,6-pyH); MS *m/e* (rel intensity) 276 ($M^+$, 33) [$C_{16}H_{24}N_2O_2$, calcd 276.18377, found 276.18666], 275 (17), 245 (58), 233 (29), 221 (13), 220 (69), 219 (41), 205 (40), 190 (36), 189 (100), 187 (9).

**1-Benzyl-*trans*-3-(carbomethoxy)-2-(3-pyridyl)pyrrolidine (26).** A solution of borane in THF (70 mL, 72.8 mmol) was added dropwise with stirring to a solution of the pyrrolidinone **13** (10.5 g, 33.8 mmol) in dry THF (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 h and then diluted with $H_2O$. After evaporation of the solvent in vacuo the residue was extracted with $CHCl_3$ (5 × 100 mL). Evaporation of the dried ($MgSO_4$) organic extract yielded a yellow oil that was purified on a Florisil column (25 × 2.5 cm). Elution with $C_6H_6$ gave the pyrrolidine **26** as a colorless oil (5.0 g, 50%): UV (EtOH) $\lambda_{max}$ 242 nm ($\epsilon$ 3080), 263 (2990); IR (neat) $\nu_{max}$ 1730 $cm^{-1}$ (C=O ester); $^1H$ NMR ($CDCl_3$) $\delta$ 2.23 (m, 4-$H_2$), 3.17 (t, $J$ = 11.9 Hz, 5-$H_2$), 3.40 (s, $OCH_3$), 3.91 (d, $J$ = 8.4 Hz, $NCH_2$), 4.87 (d, $J$ = 2.0 Hz, 2-H), 7.24 (s, $C_6H_5$), 7.35 (m, 5-pyH), 8.03 (dt, $J$ = 7.9 and 2.0 Hz, 4-pyH), 8.50 (m, 2,6-pyH); MS *m/e* (rel intensity) 296 ($M^+$, 7), 281 (6), 265 (10), 218 (24), 205 (100), 119 (49).

**1-Benzyl-*trans*-3-carboxy-2-(3-pyridyl)pyrrolidine (27).** Sodium hydroxide (1 N, 20 mL) was added to a solution of the ester **26** (2.09 g, 7.0 mmol) in THF (5 mL) at 0 °C. After being stirred overnight at 4 °C, the clear reaction mixture was acidified (pH 4) with dilute HCl and extracted with $CH_2Cl_2$ (4 × 50 mL). Evaporation of the dry extract left an oil that was purified by PTLC (AcOEt, 1 migration). The pyrrolidine **27** crystallized from AcOEt (1 g, 50%): mp 80–82 °C; UV (EtOH) $\lambda_{max}$ 237 nm ($\epsilon$ 3300), 262 (3070), 270 (sh, 2920); IR (KBr) $\nu_{max}$ 3400 $cm^{-1}$ (OH); $^1H$ NMR ($D_2O$) $\delta$ 2.50 (m, 4-H), 3.85 (t, $J$ = 8.5 Hz, 5-H), 4.45 (d, $J$ = 11.1 Hz, N–$CH_2$), 4.86 (d, $J$ = 5.4 Hz, 2-H), 6.68 (m, 5-pyH), 8.06 (dt, $J$ = 7.1 and 2.0 Hz, 4-pyH), 8.44 (m, 2,6-pyH); MS *m/e* (rel intensity) 282 ($M^+$, 20) [$C_{17}H_{18}N_2O_2$, calcd 282.13682, found 282.13650], 265 (6), 256 (6), 238 (2), 296 (16), 204 (66), 191 (100).

**5-Bromo-3-(carbomethoxy)pyridine (30).** Esterification of 5-bromo-3-carboxypyridine[35] (**29**; 100 g, 0.49 mol) was carried out in boiling 2 N methanolic $H_2SO_4$ (400 mL) containing molecular sieves 3 Å (20 g). After the removal of the drying agents by filtration, the filtrate was made alkaline with 16% $NaHCO_3$ (500 mL). The ester **30** was extracted with $CHCl_3$ (4 × 50 mL), filtered on a Florisil column (30 × 3.5 cm, elution with $CH_2Cl_2$), and crystallized from $CH_2Cl_2$–hexane: 48.5 g, 45%; mp 94–95 °C (lit.[36] mp 96–97 °C, lit.[37] mp 98–99 °C); IR (KBr) $\nu_{max}$ 1725 $cm^{-1}$ (C=O ester); $^1H$ NMR ($CDCl_3$) $\delta$ 3.96 (s, $OCH_3$), 8.44 (t, 2.0 Hz, 4-H),

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8.85 (m, 5-H), 8.95 (m, 2-H); MS *m/e* (rel intensity) 217 ($M^+$, 77), 215 ($M^+$, 77), 186 (95), 184 (100).

**3-(5-Bromo-3-pyridoyl)-2-pyrrolidinone (31).** Under dry $N_2$, *n*-BuLi in hexane (93 mL, 205 mmol) was added to a cold solution (dry ice:acetone bath) of redistilled diisopropylamine (27.5 g, 272 mmol) in ether (100 mL). After the addition of 1-(trimethylsilyl)pyrrolidin-2-one[20] (34 g, 216 mmol) and 5-bromo-3-(carbomethoxy)pyridine (**30**; 29.2 g, 135 mmol), the reaction mixture was stirred at room temperature overnight and then poured into $H_2O$ (300 mL). The organic phase was washed with $H_2O$ and the combined aqueous phases were neutralized to pH 7.0 with concentrated HCl. Extractions with $CHCl_3$ (10 × 100 mL) and crystallization from $CHCl_3$:hexane gave the acyl lactam **31** (17.46 g, 48% from the ester **30**): mp 162–163 °C; UV (EtOH) $\lambda_{max}$ 234 nm ($\epsilon$ 5780), 285 (2820); IR (KBr) $\nu_{max}$ 3200 (NH), 1705 (C=O), 1675 $cm^{-1}$ (C=O lactam); $^1H$ NMR ($D_2O$) $\delta$ 2.15–3.16 (m, 4-$H_2$), 3.52 (q, $J$ = 13.4 and 5.6 Hz, 5-$H_2$), 4.34 (q, $J$ = 9.0 and 6.0 Hz, 3-H), 6.42 (s, NH), 8.52 (t, $J$ = 2.0 Hz, 4-pyH), 8.56 (m, 6-pyH), 9.22 (m, 2-pyH); MS *m/e* (rel intensity) 270 ($m^+$, 100), 269 (100), 268 ($M^+$, 50), 267 (90), 266 (75), 242 (100), 241 (72), 240 (74), 239 (98).

**5-Bromomyosmine (32).** A solution of the acyl lactam **31** (13.8 g, 51.3 mmol) in concentrated HCl (50 mL) was refluxed overnight and made alkaline (pH 12) by addition of concentrated KOH. After extraction with $CHCl_3$ (10 × 50 mL) and filtration on a Florisil column (6 × 1 cm), the residue was crystallized from $CHCl_3$:hexane to give **32** (11.32 g, 98%): mp 98–99 °C; UV (EtOH) $\lambda_{max}$ 237 nm ($\epsilon$ 7540), 286 (5330); IR (KBr) $\nu_{max}$ 1620 $cm^{-1}$ (N=C); $^1H$ NMR ($CDCl_3$) $\delta$ 2.05 (m, 4-$H_2$), 2.88 (m, 5-$H_2$), 4.10 (m, 3-$H_2$), 8.36 (t, $J$ = 2.0 Hz, 4-pyH), 8.71 (m, 6-pyH), 8.89 (m, 2-pyH); MS *m/e* (rel intensity) 226 ($M^+$, 80), 225 (59), 224 ($M^+$, 83), 223 (51), 198 (98), 196 (100).

Anal. Calcd for $C_9H_9N_2Br$: C, 48.02; H, 4.03; N, 12.45; Br, 35.50. Found: C, 48.20; H, 4.21; N, 12.16; Br, 35.29.

**5-Bromonornicotine (33).** A solution of 5-bromomyosmine (**32**; 1 g, 4.44 mmol) in AcOH:$CH_3OH$ (1:1, 14 mL) at 0 °C was stirred under $N_2$. Sodium borohydride (granules, 300 mg, 7.9 mmol) was added over a 3-h period. The clear mixture was left at room temperature overnight and made alkaline (pH 9.0) with 20% NaOH. Evaporation of the dried ($K_2CO_3$) methylene chloride (4 × 50 mL) extract left **33** as a colorless liquid (970 mg, 96%): UV (EtOH) $\lambda_{max}$ 275 nm ($\epsilon$ 3360); IR (neat) $\nu_{max}$ 3300 $cm^{-1}$ (NH); $^1H$ NMR ($CDCl_3$) $\delta$ 1.10–2.30 (m, 3,4-$H_2$), 2.55 (s, NH), 3.05 (q, 5-$H_2$), 4.13 (t, $J$ = 8.0 Hz, 2-H), 7.93 (td, $J$ = 2.0 and 0.54 Hz, 4-pyH), 8.48 (m, 2,6-pyH); MS *m/e* (rel intensity) 229 ($M^+$, 4), 227 ($M^+$, 34), 228 (30), 226 (41), 225 (40), 200 (41), 199 (98), 198 (68), 197 (100).

Anal. Calcd for $C_9H_{11}N_2Br$: C, 47.59; H, 4.88; N, 12.34; Br, 35.19. Found: C, 47.31; H, 4.88; N, 12.19; Br, 35.28.

**1-Benzyl-2-(5-bromo-3-pyridyl)pyrrolidine (34).** A solution of 5-bromonornicotine (**33**; 1.08 g, 4.7 mmol) and benzyl chloride (0.6 g, 4.7 mmol) in dry DMF (10 mL) was refluxed with $Na_2CO_3$ (0.5 mg, 4.7 mmol) and NaI (25 mg) under $N_2$ for 2 h. The mixture was diluted with water (20 mL) and extracted with $CH_2Cl_2$ (4 × 50 mL). PTLC (cyclohexane:AcOEt, 1:1) of the dried ($MgSO_4$) extract gives the pyrrolidine **34** (1.09 g, 72%); UV (EtOH) $\lambda_{max}$ 229 nm ($\epsilon$ 2470), 270 (sh, 3400), 275 (3630), 280 (sh, 3040); IR (neat) $\nu_{max}$ 1580, 1035, 710 $cm^{-1}$ (pyridine); $^1H$ NMR ($CDCl_3$) $\delta$ 1.81 (m, 3,4-$H_2$), 2.25 (m, 5-$H_2$), 3.17 and 3.77 (2 d, $J$ = 13.0 Hz, benzylic $CH_2$), 3.43 (dd, $J$ = 8.5 and 8.5 Hz, 2-H), 7.25 (s, $C_6H_5$), 7.95 (t, $J$ = 2.0 Hz, 4-pyH), 8.53 (m, 2,6-pyH); MS *m/e* (rel intensity) 319 (78), 318 ($M^+$, 100), 317 (100), 316 ($M^+$, 100), 315 (94), 243 (74), 242 (83), 241 (54), 240 (99).

**1-Benzyl-2-(5-carboxy-3-pyridyl)pyrrolidine (35).** A solution of the pyrrolidine **34** (907 mg, 2.86 mmol) in anhydrous ether (10 mL) was stirred under dry $N_2$ at −77 °C. After the addition of *n*-BuLi:hexane (5 mL, 10 mmol) the mixture was stirred for 2 h. Dry carbon dioxide was bubbled through the mixture for 1 h. After acidification to pH 3 with HCl the mixture was extracted with $CH_2Cl_2$ (4 × 10 mL). The combined dried ($MgSO_4$) extracts were purified by PTLC ($CH_2Cl_2$:$CH_3OH$, 20:1, 3 migrations). The lower band ($R_f$ 0–0.1) was extracted and purified by a second PTLC ($CH_2Cl_2$:$CH_3OH$, 10:1, 1 migration). Extraction of the band at $R_f$ 0.58–0.69 gave the pyrrolidine **35** (59 mg, 7.3%): UV (EtOH) $\lambda_{max}$ 230 nm ($\epsilon$ 3310), 268 (3130); IR (KBr) 2500–3100 (OH), 1710 $cm^{-1}$ (C=O); $^1H$ NMR ($CDCl_3$) $\delta$ 1.82–2.83 (m, 3,4-$H_2$), 3.69 and 4.09 (2 d, $J$ = 13.5 Hz, N-$CH_2$), 3.61–4.16 (m, 2-H, 5-$H_2$), 7.32 (s, $C_6H_5$), 8.68 (t, $J$ = 2.0 Hz, 4-pyH), 9.17 and 9.28 (2 t, $J$ = 2.0 Hz, 2,6-pyH); MS *m/e* (rel intensity) 283 (18), 282 ($M^+$, 100), 281 (71), 239 (22), 205 (65).

**5-Bromonicotine (36).** 5-Bromonornicotine (**33**; 70 mg, 0.31 mmol) was added to a solution of formic acid (97%, 100 mg) and formaldehyde (37%, 100 mg) in water (0.5 mL) and the mixture refluxed for 12 h under $N_2$ and made alkaline with 20% NaOH. Extraction with $CH_2Cl_2$ (5 × 3 mL) and PTLC of the dried ($Na_2SO_4$) organic phases gave **36** as a colorless oil (29 mg, 39%): UV (EtOH) $\lambda_{max}$ 229 nm ($\epsilon$ 1740), 267 (sh, 3004), 275 (3390), 281 (sh, 2740); IR (neat) $\nu_{max}$ 1580, 1020, 710 $cm^{-1}$ (pyridine); $^1H$ NMR ($CDCl_3$) $\delta$ 1.53–2.57 (m, 3,4-$H_2$, 5-H), 2.19 (s, $NCH_3$), 3.00–3.40 (2-H, 5-H), 7.96 (t, $J$ = 2.0 Hz, 4-pyH), 8.60 (m, 2,6-pyH); MS *m/e* (rel intensity) 243 (83), 242 ($M^+$, 87), 241 (96), 240 ($M^+$, 100), 239 (68), 225 (25), 224 (28), 213 (55), 212 (45), 211 (68).

**1-Nitroso-2-(5-bromo-3-pyridyl)pyrrolidine (5-Bromo-*N'*-nitrosonornicotine, 37).** A solution of 5-bromonornicotine (**33**; 190 mg, 0.84 mmol) in $CH_3CO_2H$:$H_2O$ (1:1, 3 mL) was treated with $NaNO_2$ (230 mg, 3.34 mmol) at 0 °C. The mixture was stirred at 4 °C overnight, saturated with $Na_2CO_3$, and extracted with $CH_2Cl_2$ (5 × 3 mL). Purification of the residue by PTLC (AcOEt, 2 migrations) gave **37** as a colorless oil which crystallized on standing at 4 °C (192 mg, 90%): mp 79–81 °C; UV (EtOH) $\lambda_{max}$ 240 nm ($\epsilon$ 6640), 272 (4057), 280 (sh, 3270); IR (KBr) $\nu_{max}$ 1020, 1450 $cm^{-1}$ (N-NO); $^1H$ NMR ($D_2O$, pH 5.4) $\delta$ 1.94–2.67 (m, 3,4-$H_2$), 3.84 (m, 1 H, 5-$H_2$ of *E* isomer), 4.57 (m, 1 H, 5-$H_2$ of *Z* isomer), 5.25 (m, 0.5 H, 2-H of *Z* isomer), 5.79 (m, 0.5 H, 2-H of *E* isomer), 8.02 (t, $J$ = 2.2 Hz, 4-pyH), 8.48 (m, 2,6-pyH); MS *m/e* (rel intensity) 257 ($M^+$, 88), 225 (100), 227 (48), 225 (98).

**1-Methyl-3-[3,5-bis(carbomethoxy)-1,4-dihydro-4-pyridyl]-2-pyrrolidinone (38).** A cold solution (dry ice:acetone bath) of lithium diisopropylamide was prepared from diisopropylamine (1 g, 9.9 mmol) and *n*-butyllithium (5 mL of a 2.0 M solution in hexane, 10 mmol) in ether (25 mL). 1-Methyl-2-pyrrolidinone (991 mg, 10 mmol) and a solution of 3,5-bis(carbomethoxy)pyridine[38] (**28**; 1.95 g, 10 mmol) in dry THF (100 mL) were added dropwise. After the mixture was stirred at room temperature overnight, water (20 mL) was added and the aqueous phase neutralized (pH 7.0) with 10% HCl. The dried ($MgSO_4$) chloroform extract (4 × 50 mL) was evaporated and the residue chromatographed on a Florisil column (27.5 × 3.0 cm). Elution with AcOEt gave the adduct **38** (390 mg, 13%). An analytical sample was crystallized from $CHCl_3$:hexane: mp 195–205 °C; UV (EtOH) $\lambda_{max}$ 279 nm ($\epsilon$ 16 560), 285 (4690); IR (KBr) $\nu_{max}$ 1640 (C=C), 1675 (C=O amide), 1690 (C=O ester), 3240 $cm^{-1}$ (NH); $^1H$ NMR ($CDCl_3$) $\delta$ 2.00 (m, 4-$H_2$), 2.85 (s, $NCH_3$), 3.31 (t, $J$ = 6.0 Hz, 5-$H_2$), 3.70 and 3.73 [2 s, 2($OCH_3$)], 5.30 (t, $J$ = 3.5 Hz, 4-dihydropy $H_2$), 7.68 and 7.67 (2 d, $J$ = 1.4 Hz, 2,6-dihydropy H); MS *m/e* (rel intensity) 294 ($M^+$, 53) [$C_{14}H_{18}N_2O_5$, calcd 294.121 57, found 294.120 78], 263 (41), 236 (100), 197 (92).

Anal. Calcd for $C_{14}H_{17}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.41; H, 6.37; N, 9.39.

## Kinetics Experiments

A Model 404 liquid chromatograph (Waters Associates, Milford, MA) equipped with a Model 440 UV detector set at 254 nm and a Model U6K injection system was employed. *N*-Nitroso isomer separations were achieved with a 6 mm × 30 cm Microbandapak/$C_{18}$ column (Waters Associates). For 1-nitroso-*trans*-3-(hydroxymethyl)-2-(3-pyridyl)pyrrolidine (**18**), the eluting solvent was $KH_2PO_4$:$CH_3CN$:$H_2O$ (5:1:100) with a flow rate of 2 mL/min. Retention times of the *Z* and *E* isomers were 25.3 and 30.6 min, respectively. For *N'*-nitrosonornicotine (**2**), the eluting solvent was $KH_2PO_4$:$CH_3CN$:$H_2O$ (2.5:5:100), and the retention times were 23.0 min (*Z*) and 27.0 min (*E*). Separation of the two *N*-nitroso isomers of 5-bromo-*N'*-nitrosonornicotine (**37**) was achieved with a mixture of $KH_2PO_4$:$CH_3CN$:$H_2O$ (5:10:100) and with a flow rate of 4 mL/min. Retention times were 29.3 and 37.3 min for the *Z* and *E* isomers, respectively. The *N'*-nitrosamines (2 mg) were dissolved in glass-distilled water (100 mL), and the size of the injections ranged from 10 to 50 $\mu$L.

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**Registry No.** 8, 500-22-1; 11, 71718-88-2; 12, 71718-89-3; 13, 71718-90-6; 13 picrate, 71718-91-7; 14, 71718-92-8; 15, 71749-89-8; 16, 71771-91-0; 17, 71718-93-9; 18, 71718-94-0; 19, 71718-95-1; 20, 71718-96-2; 20 dipicrate, 71718-97-3; 21, 71718-98-4; 22, 71718-99-5; 23, 71719-00-1; 23 picrate, 71785-25-6; 24, 71719-01-2; 25, 71719-02-3; 26, 71719-03-4; 27, 71719-04-5; 28, 4591-55-3; 29, 20826-04-4; 30, 29681-44-5; 31, 71719-05-6; 32, 64319-85-3; 33, 71719-06-7; 34, 71719-07-8; 35, 71719-08-9; 36, 71719-09-0; 37, 71719-10-3; 38, 71719-11-4; benzylamine, 100-46-9; succinic anhydride, 108-30-5; hexanoic anhydride, 2051-49-2; 1-(trimethylsilyl)pyrrolidin-2-one, 14468-90-7; benzyl chloride, 100-44-7; 1-methyl-2-pyrrolidinone, 872-50-4.

# Conversion of Berberine into Phthalideisoquinolines[1]

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Ferricyanide oxidation of berberine (1) yields the dimer oxybis(berberine) whose breakdown with methanolic hydrogen chloride gives 8-methoxyberberinephenolbetaine (12). Hydration of 12 in wet ether furnishes enaminol 14 which can be N-methylated to dehydrohydrastine methyl ester (15). $NaBH_4$ reduction of 15 leads to a 1:2 mixture of (±)-α-hydrastine (9) and (±)-β-hydrastine (6), thus achieving the first conversion of 1 to the hydrastines. Whereas $NaBH_4$ reduction of 14·HCl gives mostly β-norhydrastine (5) together with a little α-norhydrastine (8), similar reduction of *N*-*n*-propyldehydronorhydrastine methyl ester (17) hydroiodide provides only *N*-*n*-propyl-α-norhydrastine (11). Diisobutylaluminum hydride reduction of 5 leads to (±)-epiophiocarpine (22), and the diastereoisomeric (±)-ophiocarpine (23) is formed by $NaBH_4$ reduction of betaine 12. Hydration of 12 in wet THF generates methyl isoanhydroberberilate (26), while methyl anhydroberberilate (32) is obtained through acetic anhydride treatment of betaine 12 to furnish 13-acetoxyoxoberberine (30), followed by reaction with methanolic KOH.

Berberine is the best known, oldest, and most available of the protoberberine alkaloids, having been first isolated in 1826,[2] while its structural elucidation dates to the first decade of this century.[3,4] The protoberberines themselves occupy a prominent position in the biogenetic lineage of other groups of isoquinoline alkaloids, including the phthalideisoquinolines, spirobenzylisoquinolines, rhoeadines, and protopines.[5,6] A biogenetic relationship between protoberberines and phthalideisoquinolines was first suggested by Perkin and Robinson in 1910.[4,7] Their hypothesis has been supported and refined by radio tracer incorporation studies in recent years, involving in particular the feeding of labeled (−)-scoulerine (3).[8] The rational conclusion was thus drawn that it is tetrahydroprotoberberines rather than berberinium salts that act as biogenetic precursors for the phthalideisoquinoline alkaloids.[8]

Interestingly enough, however, at the inception of our studies on berberine (1), the chemical conversion of this alkaloid or of its tetrahydro derivative canadine (2) to its phthalideisoquinoline analogue β-hydrastine (6) still remained to be achieved. Key requirements for such a transformation are the selective oxidation of the protoberberine skeleton at carbons 8 and 13, and the cleavage of ring C with accompanying N-methylation, since *N*-norphthalideisoquinolines are unknown in nature.

Because introduction of an oxygen substituent at position 8 of berberine (1) is trivially accomplished with aqueous alkali, our initial effort was directed toward an alkaline reagent for the selective oxygenation of position 13, necessary for the conversion to the phthalideisoquinoline alkaloid β-hydrastine (6). Since even dilute alkaline permanganate treatment results in drastic oxidation of the berberine system, the use of the mild, alkali-stable one-electron oxidant potassium ferricyanide was investigated. Indeed, on our very first attempt, using potassium ferricyanide followed by aqueous sodium hydroxide, colorless plates of the hitherto unknown dimer oxybis(berberine) were obtained. Although the exact structure of this dimer is rather complex and remains to be elucidated, it is clear from its reactions that it is formed from the condensation of 1 mol of a berberine derivative that has been oxidized at C-8 and C-13 with 1 mol of unoxidized berberine. Significantly, however, both the IR and $^{13}C$ NMR spectra of oxybis(berberine) show no evidence of carbonyl groups.[9]

When a slurry of oxybis(berberine) in methanol was treated with 10% methanolic hydrogen chloride, immediate breakdown of the dimer took place with formation of a red coloration. Reaction workup gave the new compound 8-methoxyberberinephenolbetaine (12) as fine orange needles, together with an equivalent amount of berberine (1) chloride.

It was immediately recognized that the phenolbetaine 12 possesses the requisite oxygen function at C-13 as well as a potential carboxylic acid at C-8 for transformation to β-hydrastine (6). The unmasking of the C-8 carboxyl was achieved by simple hydration whereby a stirring solution

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(9) An X-ray analysis of oxybis(berberine) is presently being attempted. It should be stated that this dimer is not an absolute requirement for the preparation of 8-methoxyberberinephenolbetaine (12) since subsequent to our initial communication[1] 12 was prepared through photoirradiation of berberine in methanol: see ref 13 below.

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