Stereospecific Synthesis of 2,3,6-Trisubstituted Piperidines: An Efficient Total Synthesis of (±)-Pumiliotoxin C[1]

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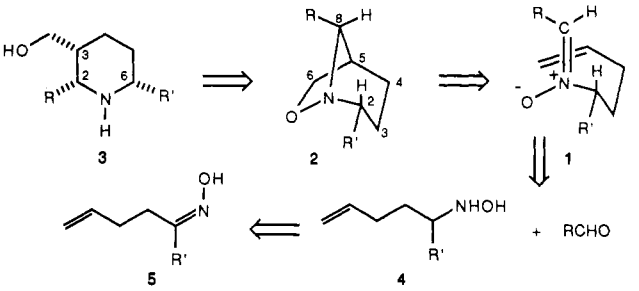
Abstract: The intramolecular cycloaddition of *N*-(1-alkyl-4-pentenyl)nitrones provides a highly regio- and stereoselective route to *all-cis*-2,6-disubstituted-3-(hydroxymethyl)piperidines **3** by way of the bicyclic isoxazolidines **2**. In demonstration studies, the 2,6-dimethyl analogue was transformed into 2*r*,3*c*,6*c*-trimethylpiperidine (**9**) and into *N*-*tert*-butoxy-2*c*,6*c*-dimethylpiperidine-3*r*-carboxaldehyde (**10**). Facile and complete epimerization of the latter into the all-equatorial, 2t,3r,6t aldehyde **11** was achieved, thus giving entry into the diastereomeric 2,3,6-trisubstituted piperidines. These protocols can give rise to short and efficient syntheses not only of the piperidines but of bicyclic nitrogen heterocycles depending on the nature of the substituents. An additional stereogenic center was introduced into the 3-(1-hydroxyalkyl) side chain by employing trans and cis isomers of *N*-(1,5-disubstituted-4-pentenyl)nitrones. The methodology was used to rapidly assemble a piperidine containing four stereogenic centers starting from 2-pentanone and methyl phenyl ketone. This intermediate **20** was then easily converted into (±)-pumiliotoxin C (**12**) by way of an intramolecular alkylation with inversion of configuration. The highly convergent total synthesis was accomplished in 14 steps and 14% overall yield with no attempt at optimization.

The intramolecular cycloaddition of nitrones, first described in 1959,[2] enjoys considerable favor as a versatile key reaction for the synthesis of natural and unnatural products.[3] Although much of our work has involved nitrones with unsaturation in the nitrone carbon substituent, we,[4] and others,[5,6,7] have been interested in the *N*-alkenyl variation. It occurred to us that an *N*-(1-alkyl-4-pentenyl)nitrone **1** could provide a very facile route to 2*c*,6*c*-disubstituted-3*r*-(hydroxymethyl)piperidines **3** as drawn in retrosynthetic format in Scheme I. The regiochemistry shown is quite general for intramolecular cycloadditions of both *C*-5-hexenyl- and *N*-4-pentenylnitrones, namely, C–C bond formation leading preferentially to a six-membered over a seven-membered ring. The relative configuration of the substituent R is determined by the stereochemistry at the nitrone C–N double bond in **1** (acyclic aldonitrones are invariably *Z* as shown), and that of R′ is expected to be endo because of its equatorial orientation in the transition state leading to cycloadduct **2**. Derivation of the precursor hydroxylamine **4** from the oxime **5** is reliable, and the component aldehydes are readily synthesized. Thus the route to compounds **3** is efficient and convergent. The choice of groups R and R′ could also lead to quinolizidine, indolizidines, hydroindoles, and, as will be demonstrated, decahydroquinolines of known configuration. Enantioselective syntheses are possible with chiral, nonracemic hydroxylamines.[6b,7b]

By way of demonstrating the generality of this protocol, the known *N*-(1-methyl-4-pentenyl)hydroxylamine (**4**, R′ = CH_3) was prepared by sodium cyanoborohydride reduction of the oxime of 5-hexen-2-one (**5**, R′ = CH_3), which was, in turn, generated either by oximation of the ketone or by allylation of dilithioacetone oxime. Condensation of **4** (R′ = CH_3) with acetaldehyde, isovaleraldehyde, or benzaldehyde in benzene at room temperature in the presence of sodium sulfate, followed by filtration and heating at

Scheme I



reflux, gave, in each case, the product isoxazolidine **2** [R′ = CH_3; R = CH_3, $(CH_3)_2CHCH_2$, and C_6H_5, respectively]. These crude products consisted of over 93% of a single regioisomer. Purification and subsequent distillation gave isolated yields of 62%, 73%, and 74%, respectively.

The ^1H NMR spectrum of the crude nitrone **1** (R = R′ = CH_3) prior to cyclization showed the aldehydic proton as a quartet at δ 7.0, which is only consistent with the *Z* configuration, as the corresponding *E* isomer should show this proton at lower field. The structure and stereochemistry for the cycloadduct **2** (R = R′ = CH_3) was evident from the following data. Microanalysis of the oxalate salt of **2** (R = R′ = CH_3) supported the molecular formula $C_8H_{15}NO$, and the low-resolution mass spectrum showed M^+ at *m/e* = 141. Isoxazolidine **2** (R = R′ = CH_3) is easily distinguished from the regioisomeric cycloadduct by its ^1H NMR spectrum, which showed *two methylene protons* attached to the oxygen-bearing carbon atom at δ 3.90, a conclusion readily confirmed by the partially decoupled ^{13}C NMR spectrum. The C-2 proton of **2** (R = R′ = CH_3) appeared as a multiplet with a width at half-height of about 20 Hz, which is more consistent with an axial rather than an equatorial orientation. This stereochemical assignment was verified in the piperidine derivatives **3** as described below.

A fourth stereogenic center can be introduced stereospecifically without difficulty into the bicyclic isoxazolidine products at C-6 by use of a 1,5-disubstituted *N*-4-pentenylnitrone. Alkylation of dilithioacetone oxime with *trans*-cinnamyl bromide gave (*E*)-6-phenylhex-5-en-2-one oxime, which was reduced to *N*-[(*E*)-1-methyl-5-phenyl-4-pentenyl]hydroxylamine (**6**). Reaction with acetaldehyde in the usual manner gave the pure bicyclic isoxazolidine **7** (68%). The exo orientation of the phenyl group at C-6 is required by the stereospecific nature of the cycloaddition reaction, and it was confirmed by the observation of a singlet at δ 5.08 in the ^1H NMR spectrum corresponding to an endo benzylic proton. No coupling was expected or observed with the adjacent

(1) Presented at the 186th Meeting of the American Chemical Society, Washington, DC, August 28–September 2, 1983; *Abstracts of Papers*, ORGN 123. See also Balasubramanian, N. *Diss. Abstr. Internat.* **1983**, *44*, 799-B.
(2) LeBel, N. A.; Whang, J. J. *J. Am. Chem. Soc.* **1959**, *81*, 6334.
(3) For recent general reviews see: (a) Confalone, P. N.; Huie, E. M. *Org. React.* **1988**, *36*, 1–173. (b) Padwa, A. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A.; Ed.; Wiley-Interscience: New York, 1984; Vol. 2, Chapter 12, pp 277–406. (c) Balasubramanian, N. *Org. Prep. Proced. Int.* **1985**, *17*, 23.
(4) Stampwala, S. S. M.S. Thesis, Wayne State University, March 1975.
(5) Lumma, W. C., Jr. *J. Am. Chem. Soc.* **1969**, *91*, 2820.
(6) (a) Oppolzer, W. *Angew. Chem., Int. Ed. Eng.* **1977**, *16*, 10. (b) Oppolzer, W.; Petrzilka, M. *Helv. Chim. Acta* **1978**, *61*, 2755. (c) Oppolzer, W.; Siles, S.; Snowden, R. L.; Bakker, B. H.; Petrzilka, M. *Tetrahedron Lett.* **1979**, 4391. (d) *Ibid. Tetrahedron* **1985**, *41*, 3497.
(7) (a) Holmes, A. B.; Swithenbank, C.; Williams, S. F. *J. Chem. Soc., Chem. Commun.* **1986**, 265. (b) Smith, A. L.; Williams, S. F.; Holmes, A. B.; Hughes, L. R.; Lidert, Z.; Swithenbank, C. *J. Am. Chem. Soc.* **1988**, *110*, 8696.

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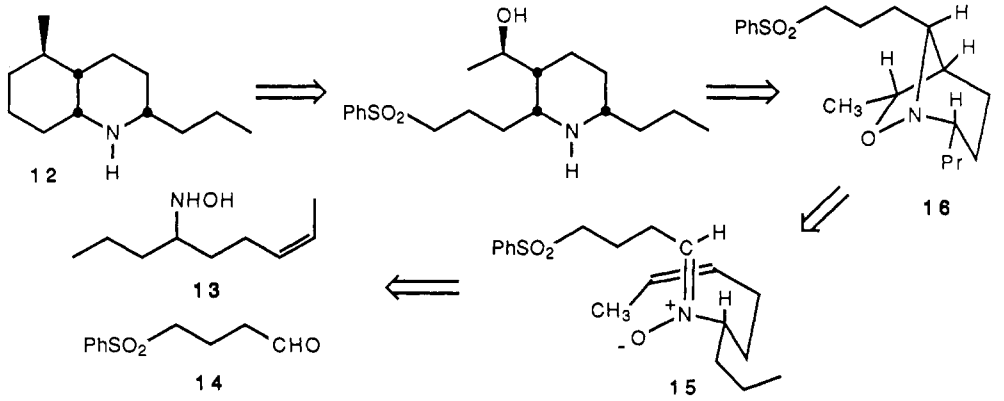
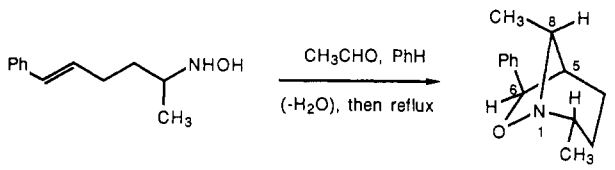
Scheme II



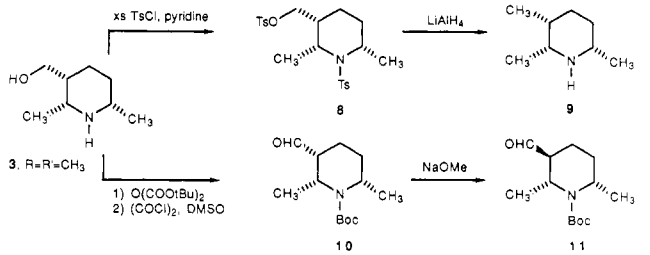
bridgehead proton at C-5 because of the unfavorable dihedral angle, and apparently little or no long-range coupling occurs with the endo C-8 proton. Furthermore, the C-8 methyl signal in **7** occurs at δ 0.85, an upfield shift of about 13 Hz from that in **2** (R = R′ = CH_3) due to shielding by the *exo-oriented* benzene ring.



Reductive cleavage of **2** (R′ = CH_3) with zinc dust in 80% acetic acid (89%), with lithium aluminum hydride in a mixture of THF and diglyme at reflux (74%), with aqueous titanium trichloride (76%), or with hydrogen over a palladium on carbon catalyst (82%) gave 2*c*,6*c*-dimethyl-3*r*-(hydroxymethyl)piperidine (**3**, R = R′ = CH_3). The spectral data were all consistent with the structure and stereochemistry assigned. Specifically, the ^1H NMR spectrum shows the two methyl groups as doublets at δ 1.01 and 0.92 with coupling constants of 6.6 and 6.2 Hz, respectively. The C-2 proton appeared as a doublet of quartets at δ 2.81 (J = 6.6 and 3.0 Hz), which is consistent with the expected axial proton and equatorial methyl at C-2. The equatorial orientation of the C-6 methyl is supported by the signal of the C-6 proton which occurs as wide multiplet at δ 2.57 (J = 12.1, 6.1, and 2.3 Hz), which requires that this proton be axial.

Ditosylation of amino alcohol **3** (R = R′ = CH_3) with excess *p*-toluenesulfonyl chloride in pyridine gave the crude *N*,*O*-ditosylate **8**, which was then heated under reflux with excess lithium aluminum hydride in a mixture of THF and diglyme to give 2,3,6-trimethylpiperidine (**9**). The 2r,3c,6c stereochemistry of **9**, a known compound[8] but for which no spectral data were available, could be confirmed by ^{13}C NMR. Eight carbon resonance lines were observed, three of which were due to methyl carbons. On the basis of literature values for the known *cis*-2,3- and *cis*-2,6-dimethylpiperidines,[9] the lines at 54.3 ppm and 52.5 ppm can be assigned to the equatorially substituted C-2 and C-6 carbons, and C-3 with an axial methyl appears at 34.6 ppm. The *N*,*N*-dimethylammonium iodide of this *all-cis*-trimethylpiperidine had a melting point which is in agreement with the literature value. Therefore, the stereochemistry of the cycloadduct **2** (and also of **7**) is fully confirmed.

As further proof of stereochemistry, amino alcohol **3** (R = R′ = CH_3) was protected at nitrogen by treatment with di-*tert*-butyl dicarbonate to give the Boc derivative which was then oxidized under Swern conditions[10] to the Boc aldehyde **10**. Epimerization with sodium methoxide gave complete conversion to the more stable, all-equatorial 1-(*tert*-butoxycarbonyl)-2*t*,6*t*-dimethylpiperidine-3*r*-carboxaldehyde (**11**). This simple transformation represents a convenient stereoselective synthesis of another diastereomeric 2,3,6-trisubstituted piperidine.



Having established facile stereochemical control at four stereogenic centers, three within and one external to a piperidine ring system, we sought a target molecule for suitable demonstration. Pumiliotoxin C (**12**), one of the physiologically active alkaloids belonging to the family *Dendrobatidae* and localized in the defensive skin secretions of the Central and South American "arrow poison frogs", was chosen.[11] Several total synthesis of pumiliotoxin C have been detailed,[12] however, we saw a distinct advantage to the efficient approach outlined in Scheme II, which employs the simple advanced intermediates hydroxylamine **13** and aldehyde **14**.

The synthesis of hydroxylamine **13** involved alkylation of the lithio derivative of the *E* isomer of 2-pentanone *N*,*N*-dimethylhydrazone (**17**) with (*Z*)-1-bromo-2-butene to give hydrazone **18**. The oxime dianion procedure used for the model studies described above was not employed in this case because the oxime proved to be a 3:1 mixture of stereoisomers. Oxidative hydrolysis of **18** gave (*Z*)-7-nonen-4-one (**19**) in excellent yield. This ketone was converted to the oxime, which was then reduced with sodium cyanoborohydride to give hydroxylamine **13**. The overall yield from 2-pentanone was 63%. It was also possible to directly convert **18** to the oxime of **19** by exchange with a small excess of hydroxylamine hydrochloride, which improved the overall yield somewhat.

Aldehyde **14** was easily obtained in two steps from methyl phenyl sulfone by alkylation of its lithio derivative with 2-(2-bromoethyl)-1,3-dioxolane followed by acid-catalyzed hydrolysis.

(8) Isin, Zh. I.; Sokolov, D. V. *Izv. Akad. Nauk Kaz. SSR, Ser. Khim.* **1974**, *24*, 78.
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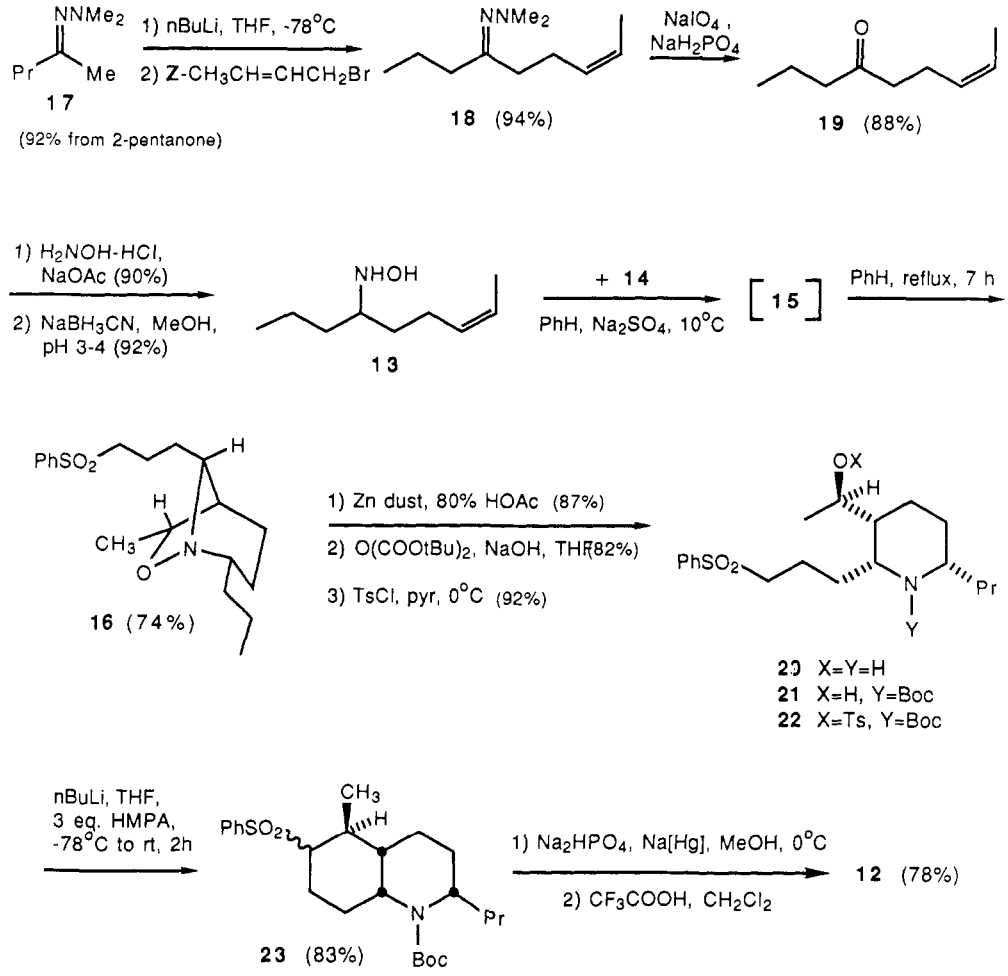
(11) Daly, J. W.; Spande, T. F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Wiley: New York, 1986; Vol. 4, Chapter 1, pp 1–274.
(12) (a) Overman, L. E.; Jessup, P. J. *J. Am. Chem. Soc.* **1978**, *100*, 5179. (b) Oppolzer, W.; Frosti, W.; Weber, H. P. *Helv. Chim. Acta* **1975**, *58*, 590. (c) Hattori, K.; Matsumura, Y.; Miyazaki, T.; Maruoka, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1981**, *103*, 5719. (d) Masamune, S.; Reed, L. A.; Davis, J. T.; Choy, W. *J. Org. Chem.* **1983**, *48*, 4441. (e) Abe, K.; Tsugoshi, T.; Nakamura, N. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 335. (f) Bonin, M.; Royer, J.; Grierson, D. S.; Husson, H.-P. *Tetrahedron Lett.* **1986**, *27*, 1569. (g) Schultz, A. G.; McCloskey, P. J.; Court, J. J. *J. Am. Chem. Soc.* **1987**, *109*, 6493.

Scheme III



Equimolar amounts of **13** and **14** were added to benzene containing anhydrous sodium sulfate, and the mixture was stirred for 10 h (Scheme III). Filtration and concentration gave the crude nitrone **15**, which was then heated in benzene at reflux for another 10 h. Workup and flash chromatography gave pure bicyclic isoxazolidine **16** in 74% yield. The compound was pure by GC analysis. The single most important feature of the ^{1}H NMR spectrum of **16** was a doublet of quartets at δ 4.12 corresponding to a single *exo*-proton coupled to the *endo*-methyl group (J = 7.0 Hz) and the vicinal bridgehead hydrogen (J = 2.5 Hz). Reduction of **16** with 80% zinc dust in acetic acid at 65 °C produced the amino alcohol **20**. Both the ^{1}H and ^{13}C NMR spectra of **20** nicely supported the all-cis 2,3,6-trisubstituted piperidine basic structure. It is worthwhile to restate that in our experience this is the best general method for reduction of isoxazolidines to 1,3-amino alcohols. What is required at this stage is conversion of the hydroxyl into a good leaving group followed by intramolecular alkylation *with inversion of configuration* to give the decahydroquinoline nucleus with the proper stereochemistry of pumiliotoxin C at C-5.

Compound **20** was transformed into its *N*-Boc derivative **21**, which was then converted to the tosylate **22**. The key cyclization with inversion was performed with *n*-butyllithium in THF at −78 °C to which was added 3 equiv of HMPA and then with warming to room temperature, and the penultimate isolated intermediate **23** was obtained in quite respectable yield. Omission of the HMPA, or the utilization of other bases, led to poor yields of desired product and substantial amounts of E2 byproduct.[13]

Deprotection of **23** by sequential removal of the phenylsulfonyl (sodium amalgam) and *tert*-butoxycarbonyl (trifluoroacetic acid) groups gave (±)-pumiliotoxin C (**12**), whose high-resolution mass spectrum confirmed the molecular formula and whose hydrochloride showed a ^{13}C NMR spectrum consistent with the literature values.[12a]

The facility of this intramolecular nitrone approach can be best appreciated by the fact that this total synthesis from the common starting materials 2-pentanone and methyl phenyl sulfone required only 14 steps and gave an overall yield of 14% without optimization.

Experimental Section

General. Reactions were monitored by analytical thin-layer chromatography (TLC) with silica gel plates (0.25 mm, EM Reagents). Flash chromatography was carried out on silica gel 60 (EM Reagents, 230–400 mesh) following the procedure described by Still.[14] Gas chromatography (GC) was performed on a Hewlett-Packard Model 5750 research chromatograph equipped with a flame ionization detector.

^{1}H NMR spectra were obtained at 60 MHz with a Varian Model T-60 spectrometer and at 300 MHz with a Nicolet superconducting spectrometer equipped with a Model 1180 data processor and a Model 293A programmable pulser. In general, only resonances of diagnostic value are reported. ^{13}C NMR were determined on a JEOL JNM-FX60 Fourier transform spectrometer operating at 15 MHz. Chemical shifts are reported in ppm downfield from internal or external tetramethylsilane. Mass spectra (EI) were obtained with a AEI-MS-902 mass spectrometer.

Anhydrous sodium sulfate was used to dry organic extracts except where noted.

***N*-(1-Methyl-4-pentenyl)hydroxylamine (4, R′ = CH$_3$).** This compound was prepared by essentially the procedure reported by House and

(13) With *n*-BuLi/THF/−78 °C, no intramolecular alkylation occurred, and starting material could be recovered. When NaH and KH were used as bases in DMSO at about −20 °C, the major product was a 3-ethylidenepiperidine from an E2 process. When lithium hexamethyldisilazide was used in toluene at −78 °C, low yields of the desired product were detected, as well as starting materials and other byproducts.

(14) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Lee.[15] All of the alkenylhydroxylamines should be prepared and handled under an inert atmosphere.

(*E*)-6-Phenyl-5-hexen-2-one Oxime. To a solution of 1.46 g (50 mmol) of acetone oxime in 70 mL of dry THF, maintained under an argon atmosphere, was added 66 mL (100 mmol) of 1.55 M *n*-butyllithium in hexane. The mixture was cooled to −78 °C, and a solution of 9.85 g (50 mmol) of cinnamyl bromide (Aldrich) in 10 mL of dry THF was added dropwise. Stirring was continued for 2 h; then the solution was allowed to warm to room temperature and was stirred for 2 h. The mixture was poured into ice-cold water, the organic layer was separated, and the aqueous layer was extracted with several 50-mL portions of dichloromethane. The combined organic layers were dried, and the solvents were removed to give 7.56 g (80%) of a yellow solid: mp 75–78 °C; IR (neat) 3250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.03 (1 H, br s, O*H*), 7.23 (5 H, br s), 6.30 (1 H, s) 6.19 (2 H, br t), 2.4 (4 H, m), 1.86 (3 H, s).

***N*-[(*E*)-1-Methyl-5-phenyl-4-pentenyl]hydroxylamine (6).** From 0.378 g (2 mmol) of the oxime, 0.138 g (2.1 mmol) of sodium cyanoborohydride and 1 mg of methyl orange in 5 mL of methanol at pH 3–4 reduction in the usual manner[15] gave 0.363 g (95%) of the hydroxylamine **6** as an oil: IR (neat) 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (2 H, br s, O*H*, N*H*), 7.33, (5 H, br s), 6.30 (2 H, m, C*H*=C*H*), 3.05 (1 H, m, C*H*–NH), 2.36–2.20 (4 H, m), 1.53 (3 H, d, CH_3).

2-*endo*,8-*exo*-Dimethyl-7-oxa-1-azabicyclo[3.2.1]octane (2, R = R′ = CH_3). To a solution of 1.15 g (10 mmol) of the hydroxylamine **4** (R′ = CH_3) in 10 mL of benzene containing 5 g of anhydrous Na_2SO_4 at 0–10 °C was added dropwise with stirring 0.44 g (10 mmol) of freshly distilled acetaldehyde. This mixture was stirred at room temperature for 10 h; then it was filtered and concentrated on a rotary evaporator to give crude nitrone **1** (R = R′ = CH_3): ^1H NMR ($CDCl_3$) δ 7.0 (1 H, q, J = 5 Hz, C*H*=N^+), 5.8–5.6 (1 H, m, C*H*=CH_2), 5.0–4.8 (2 H, m, CH=CH_2), 3.8–3.7 (1 H, m, C*H*–N^+), 1.47 (3 H, d, J = 5 Hz, CH_3CH=N^+), 0.90 (3 H, d, J = 6 Hz, CH_3CH–N^+). The nitrone was dissolved in 25 mL of benzene, and the solution, under argon, was heated at reflux for 10 h. Removal of the solvent on a rotary evaporator gave the crude isoxazolidine **1** (R = R′ = CH_3), which was purified by flash chromatography with a mixture of EtOAc and hexane (1:2) as eluent. The appropriate fractions were identified by TLC and then combined and distilled to give 0.87 g (62%) of product, bp 41–43 °C (0.1 mmHg): ^1H NMR ($CDCl_3$) δ 3.90 (2 H, m, OCH_2), 3.13 (1 H, q, J = 6.5 Hz, C*H*–N), 2.88 (1 H, m, N–C*H*), 2.4 (1 H, m, bridgehead *H*), 1.18 (3 H, d, J = 6.5 Hz, C-8 CH_3), 1.08 (3 H, d, J = 6.6 Hz, C-2 CH_3); ^{13}C NMR ($CDCl_3$) 70.93, 65.73, 60.59, 41.95, 28.89, 25.44, 20.31, and 17.84 ppm; LRMS, *m/e* 141 (M^+).

The **hydrogen oxalate** was prepared and recrystallized from a mixture of ethanol and ether, mp 155–157 °C. Anal. Calcd for $C_{10}H_{17}NO_5$: C, 51.94; H, 7.41; N, 6.06. Found: C, 51.72; H, 7.61; N, 6.14.

8-*exo*-Isobutyl-2-*endo*-methyl-7-oxa-1-azabicyclo[3.2.1]octane [2, R = $(CH_3)_2CHCH_2$, R′ = CH_3]. The same procedure was used with 1.15 g (10 mmol) of the hydroxylamine **4** (R′ = CH_3) and 0.86 g of freshly distilled isovaleraldehyde, which reacted to give crude isoxazolidine as a colorless liquid after bulb-to-bulb distillation (bp 95–105 °C at 10–15 mmHg). GC analysis (6′ silicone SE 30 column, isothermal at 100 °C) showed two components in the ratio 93:7. Flash chromatography using a mixture of EtOAc and hexane (1:5) gave nearly pure product. If desired, final purification could be easily effected by treatment of an ethereal solution of the isoxazolidine dropwise with a saturated ethereal solution of oxalic acid unitl no further precipitate was formed. The salt was collected, recrystallized from a mixture of ethanol and ether, and then treated with ice-cold aqueous sodium carbonate solution. The free base was extracted with dichloromethane; the extract was dried and concentrated under reduced pressure to give 1.33 g (73%) of pure **3** [R = $(CH_3)_2CHCH_2$, R′ = CH_3], bp 37–38 °C (0.1 mmHg): ^1H NMR ($CDCl_3$ δ 3.85 (2 H, m, OCH_2), 3.1–2.7 (2 H, m), 2.35 (1 H, m, bridgehead *H*), 1.12 (3 H, d, J = 7.0 Hz, C-2 CH_3), 0.92 (6 H, d, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$) 71.60, 69.40, 41.40, 29.36, 29.24, 26.18, 25.00, 22.54, and 17.59 ppm; HRMS, calcd for $C_{11}H_{21}NO$ 183.1623, found 183.1617.

The **hydrogen oxalate** showed mp 172–173 °C. Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 56.92; H, 8.68; N, 5.37.

2-*endo*-Methyl-8-*exo*-phenyl-7-oxa-1-azabicyclo[3.2.1]octane (2, R = C_6H_5, R′ = CH_3). With the procedure described above, 1.15 g (10 mmol) of the hydroxylamine **4** (R′ = CH_3) and 1.06 g of freshly distilled benzaldehyde were reacted to give crude isoxazolidine as a pale yellow liquid after bulb-to-bulb distillation. Both TLC and GC showed a single component. Final purification was effected by the oxalate preparation–regeneration procedure to give 1.50 g (74%), bp 95–97 °C (0.2 mmHg), of isoxazolidine **3** (R = C_6H_5, R′ = CH_3): ^1H NMR ($CDCl_3$) δ 7.4 (5 H, br s), 4.2 (1 H, s, N–C*H*Ph), 3.82 (1 H, d, *endo*-O–C*H*), 3.55 (1 H, br t, *exo*-O–C*H*), 3.1–2.5 (2 H, m, N–C*H* and bridgehead *H*), 1.25 (3 H, d, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 140.40, 127.86, 126.30, 125.72, 72.70, 70.69, 61.20, 43.53, 29.30, 26.05, and 20.59 ppm; HRMS, calcd for $C_{13}H_{17}NO$ 203.1310, found 203.1309.

The **hydrogen oxalate** melted at 166–168 °C.

2-*endo*,8-*exo*-Dimethyl-6-*exo*-phenyl-7-oxa-1-azabicyclo[3.2.1]octane (7). The same procedure was followed with 0.191 g (1 mmol) of the hydroxylamine **6** and 0.044 g (1 mmol) of freshly distilled acetaldehyde to give crude isoxazolidine as a pale yellow liquid after bulb-to-bulb distillation. Purification by flash chromatography using a mixture of EtOAc and hexane (1:4) as eluent gave after distillation 0.147 g (68%) of the colorless liquid product, bp 73 °C (0.1 mmHg): ^1H NMR ($CDCl_3$) δ 7.3 (5 H, br s), 5.08 (1 H, s, *endo*-O–C*H*Ph), 3.1–2.7 (3 H, m), 1.25 (3 H, d, J = 6.5 Hz), 0.85 (3 H, d, J = 6.0 Hz); HRMS, calcd for $C_{14}H_{19}NO$ 217.1467, found 217.1464.

2*c*,6*c*-Dimethyl-3*r*-(hydroxymethyl)piperidine (3, R = R′ = CH_3). To a suspension of 1.32 g (0.20 mol) of zinc dust in 9 mL of 80% aqueous acetic acid at 65 °C was added dropwise with stirring a solution of 0.500 g (3.5 mmol) of isoxazolidine **2** (R = R′ = CH_3) in 1 mL of 80% aqueous acetic acid. The reaction progress was monitored by TLC. After 3 h the mixture was filtered; the filtrate was diluted with water and extracted several times with 20-mL portions of dichloromethane. The combined organic layer was washed successively with sodium bicarbonate solution and brine. Drying and concentration on a rotary evaporator gave 0.427 g (85%) of the amino alcohol as a colorless oil: IR (neat) 3280 cm^{-1} (very broad); ^1H NMR ($CDCl_3$) δ 3.80 (2 H, q, CH_2OH), 3.46 (variable, 2 H, br s, O*H* and N*H*), 3.2–2.5 (2 H, br m), 1.19 (3 H, d, J = 6.7 Hz), 1.01 (3 H, d, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) 63.09, 54.71, 52.24, 37.04, 30.02, 29.56, 21.96, and 18.97 ppm; HRMS, calcd for $C_8H_{17}NO$ 143.1310, found 143.1307.

2*c*,3*c*,6*c*-Trimethylpiperidine (9). The amino alcohol **3** (R = R′ = CH_3) (0.286 g, 2 mmol) in 2 mL of pyridine was treated with 1.14 g (6 mmol) of *p*-toluenesulfonyl chloride for 10 h. The mixture was poured into ice–water and extracted with five 20-mL portions of dichloromethane. The organic layer was dried and concentrated on a rotary evaporator to give the *N,O*-ditosylate **8**: ^1H NMR ($CDCl_3$) δ 8.2–7.6 (8 H, m), 4.05 (2 H, m, CH_2OTs), 2.9 (2 H, m), 2.2 (3 H, s, ArCH_3), 2.1 (3 H, s, ArCH_3), 1.11 (3 H, d, J = 6 Hz), 1.01 (3 H, d, J = 6 Hz).

Crude **8** was reacted with 0.37 g (10 mmol) of $LiAlH_4$ in 15 mL of dry THF and 5 mL of diglyme at reflux temperature for 24 h. The mixture was diluted with ether, and 1 mL of water, 1 mL of 15% aqueous NaOH, and 3 mL of water were added successively; the solution was then filtered through a bed of anhydrous Na_2SO_4. Concentration gave 190 mg (75% for two steps) of *all-cis*-trimethylpiperidine (**9**): bp 38 °C (10 mmHg) (lit.[9] bp 148–149 °C at 700 mmHg); IR (neat) 3250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.7 (1 H, br s, N*H*), 2.8–2.65 (2 H, m, C*H*–N–C*H*), 2.2–1.7 (5 H, m), 1.1 (3 H, d, J = 6.6 Hz, C-2 CH_3), 1.01 (3 H, d, J = 6.8 Hz, C-6 CH_3), 0.91 (3 H, d, J = 7.1 Hz, C-3 CH_3); ^{13}C NMR ($CDCl_3$) 54.3, 52.8, 34.6, 33.6, 31.2, 22.1, 22.4, and 12.2 ppm.

The ***N,N*-dimethylammonium iodide** was prepared, mp 271–273 °C (lit.[9] mp 273–274 °C).

1-(*tert*-Butoxycarbonyl)-2*c*,6*c*-dimethyl-3*r*-(hydroxymethyl)-piperidine. To a solution of 0.143 g (1 mmol) of amino alcohol **3** (R = R′ = CH_3) in 5 mL of THF and water (2:1) was added 1.4 mL of 1 N NaOH followed by 0.300 g (1.4 mmol) of di-*tert*-butyl dicarbonate (Aldrich). After 2.5 h of stirring, the mixture was poured into water and extracted with several portions of dichloromethane. The combined organic layers were dried and concentrated on a rotary evaporator to give 0.176 g (75%) of the *N*-Boc alcohol: IR (neat) 3620, 3440, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.4 (1 H, br m,), 3.8 (1 H, br m), 3.5 (2 H, d), 2.7 (1 H, br s), 2.0–1.2 (5 H, m), 1.4 (9 H, s), 1.15 (3 H, d, J = 7.0 Hz, C-6 CH_3), 1.05 (3 H, d, J = 7.0 Hz, C-2 CH_3); ^{13}C NMR ($CDCl_3$) 155.09, 64.78, 47.17, 45.68, 42.10, 29.95, 28.39, 20.66, 16.76, and 15.39 ppm.

1-(*tert*-Butoxycarbonyl)-2*c*,6*c*-dimethylpiperidine-3*r*-carboxaldehyde (10). To a solution of 0.1 mL (1.1 mmol) of oxalyl chloride in 3 mL of dichloromethane under argon at −78 °C was added dropwise 0.17 mL (2.2 mmol) of dry DMSO.[10] After 2 min, a solution of the *N*-Boc alcohol (0.122 g, 0.5 mmol) in 1 mL of dichloromethane was added within 5 min. After 15 min more, 0.70 mL (5 mmol) of triethylamine was added, stirring was continued for an additional 5 min at −78 °C, and the mixture was allowed to warm to room temperature. After a water quench and normal workup, concentration of the dried extracts on a rotary evaporator gave 0.116 g (93%) of N-protected aldehyde **10** as a colorless oil: IR (neat) 2710, 1730, and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.33 (1 H, br s), 4.65 (1 H, dq), 4.25 (1 H, br m), 2.4 (1 H, m), 1.7 (4 H, m), 1.4 (9 H, s), 1.14 (3 H, d, C-6 CH_3), 1.03 (3 H, d, C-2 CH_3); ^{13}C NMR ($CDCl_3$) 201.74, 154.64, 79.53, 52.56, 45.55, 45.35, 29.32, 29.10, 20.53, 17.08, and 13.64 ppm; LRMS, *m/e* 241 (M^+). The compound showed one component on GC using a Varian Model 6000 chromatograph with

(15) House, H. O.; Lee, L. F. *J. Org. Chem.* **1976**, *41*, 863.

a flame ionization detector. The column was an 8′ OV-275 on Chromosorb W at 170 °C; retention time = 16.9 min.

1-(*tert*-Butoxycarbonyl)-2*t*,6*t*-dimethylpiperidine-3*r*-carboxaldehyde (11). The aldehyde **10** (60 mg, 0.25 mmol) was stirred with 2 mL of a 0.01 M solution of sodium methoxide in methanol at room temperature. After 3 h, the mixture was neutralized with carbon dioxide. Evaporation of the methanol followed by extraction into dichloromethane, drying, and concentration gave the epimeric aldehyde **11**: IR (neat) 2710, 1730, and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.43 (1 H, br s), 4.7 (1 H, dq), 4.2 (1 H, br s), 2.32 (1 H, m), 1.5 (9 H, s), 1.25–1.10 (6 H, overlapping d, C-6 CH_3 and C-2 CH_3). The compound showed one component on GC under the conditions described above, and the retention time was 18.3 min, 1.4 min longer than that of aldehyde **10**.

(*Z*)-7-Nonen-4-one *N*,*N*-Dimethylhydrazone (18). To a solution of 6.4 g (50 mmol) of 2-pentanone *N*,*N*-dimethylhydrazone (**17**)[16] in 100 mL of dry THF under argon was added dropwise with stirring 39 mL (50 mmol) of 1.55 M *n*-butyllithium in hexane at −78 °C. The solution was stirred at this temperature for 20 min, and 8.1 g of (*Z*)-1-bromo-2-butene[17] in 5 mL of THF was added. The mixture was allowed to warm to room temperature over 3 h and poured into cold brine. The organic layer was separated, and the aqueous layer was extracted with five 30-mL portions of dichloromethane. After drying and concentration, the residue was distilled to give 8.6 g (94%) of **18**, bp 97–98 °C (10 mmHg): IR (neat) 1635 and 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.43 (2 H, m, *cis*-C*H*=C*H*), 2.36 (6 H, s), 1.67 (3 H, d), 1.17 (3 H, t); HRMS, calcd for $C_{11}H_{22}N_2$ 182.1783, found 182.1783.

(*Z*)-7-Nonen-4-one (19). A solution of the hydrazone **18** (5.49 g, 30 mmol) in 400 mL of methanol and 90 mL of 1.0 N pH 7 phosphate buffer at room temperature was allowed to react with a solution of 14.01 g (2.2 equiv) of sodium periodate. After 5 h, the mixture was filtered, diluted with water, and extracted with three 50-mL portions of dichloromethane. The ketone **19** was obtained by drying, concentration, and distillation to give 3.69 g (88%), bp 172 °C: IR (neat) 1700, 1630 (sh), and 725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.45 (2 H, m, *cis*-C*H*=C*H*), 2.6–2.1 (6 H, m), 1.63 (3 H), 0.90 (3 H, t); HRMS, calcd for $C_9H_{16}O$ 140.1201, found 140.1201.

(*Z*)-7-Nonen-4-one Oxime. The oxime was prepared in the usual manner from 8.32 g (0.12 mol) of hydroxylamine hydrochloride, 8.32 g (0.12 mol) of sodium acetate, and 5.6 g (0.04 mol) of ketone **19** in a mixture of water and ethanol. There was obtained 5.58 g (90%) of oxime as an oil, bp 122 °C (12 mmHg): IR (neat) 3250 (br), 1635, and 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.0 (1 H, br s, O*H*), 5.46 (2 H, m, *cis*-C*H*=C*H*), 1.67 (3 H, dd), 0.93 (3 H, t); HRMS, calcd for $C_9H_{17}NO$ 155.1310, found 155.1313.

(*Z*)-*N*-(1-Propyl-4-hexenyl)hydroxylamine (13). The oxime of (*Z*)-7-nonen-2-one (1.55 g, 10 mmol) was reduced with 0.64 g (10 mmol) of sodium cyanoborohydride in the usual manner, maintaining the pH at 3–4 by dropwise addition of 50% methanolic HCl. Workup and concentration on a rotary evaporator gave 1.42 g (92%) of crude **13** which was used directly for reaction with aldehyde **14**: ^1H NMR ($CDCl_3$) δ 7.30 (2 H, br s, O*H*, N*H*), 5.36 (2 H, m, *cis*-C*H*=C*H*), 2.9 (1 H, m, C*H*–NH).

2-[3-(Phenylsulfonyl)propyl]-1,3-dioxolane. To a solution of 6.24 g (40 mmol) of methyl phenyl sulfone in 100 mL of dry THF was added dropwise with stirring 26 mL (40 mmol) of 1.55 M *n*-butyllithium in hexane at 0 °C. After 20 min, the temperature was lowered to −78 °C, and a solution of 7.48 g (40 mmol) of 2-(2-bromoethyl)-1,3-dioxolane[18] in 10 mL of THF was added. The mixture was allowed to warm to room temperature over 3 h and was poured into cold, saturated aqueous ammonium chloride. After the usual workup, distillation gave 8.9 g (88%) of a viscous oil, bp 59 °C (0.04 mmHg): IR (neat) 1310 and 1155 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0 (2 H, m), 7.7 (3 H, m), 4.9 (1 H, t, O–C*H*–O), 3.93 (4 H, br s), 3.2 (2 H, t, CH_2SO_2), 1.8 (4 H, m).

4-(Phenylsulfonyl)butanal (14). A solution of 2.52 g (10 mmol) of 2-[3-(phenylsulfonyl)propyl]-1,3-dioxolane in 10 mL of a mixture of acetone and water (3:1) containing a few drops of concentrated HCl was stirred for 3 h at room temperature. The acetone was removed; aqueous $NaHCO_3$ was added, followed by extraction with dichloromethane. After drying and concentration, the residue was flash chromatographed (EtOAc/hexane, 6:1). After concentration, 1.69 g (80%) of crude sulfone aldehyde **14** was obtained, which was used directly for reaction with **13**: IR (neat) 2720, 1720, 1305, and 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.6 (1 H, s, C*H*O), 7.85 (2 H, m), 7.73 (3 H, m), 3.2 (2 H, t, CH_2SO_2), 2.7 (2 H, br t, CH_2CHO).

The **(2,4-dinitrophenyl)hydrazone** was prepared and recrystallized from 95% ethanol, mp 183–184 °C. Anal. Calcd for $C_{16}H_{16}SO_6N_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.60; H, 4.23; N, 14.24.

6-*endo*-Methyl-8-*exo*-[3-(phenylsulfonyl)propyl]-2-*endo*-propyl-7-oxa-1-azabicyclo[3.2.1]octane (16). In a manner similar to that employed with the model compounds, 1.42 g (9 mmol) of hydroxylamine **13** was condensed with 1.90 g (9 mmol) of aldehyde **14** in benzene at 0–10 °C to give crude nitrone **15**: ^1H NMR ($CDCl_3$) δ 8.0 (2 H, m), 7.7 (3 H, m), 7.26 (1 H, br s, C*H*=N^+), 5.45 (2 H, m, C*H*=C*H*), 4.05 (1 H, m, N^+–C*H*). The nitrone was heated in benzene under argon for 10 h. Workup and flash chromatography (EtOAc/hexane, 3:1) followed by distillation gave 2.54 g (74%) of pure isoxazolidine **16** as a clear liquid, bp 47 °C (0.01 mmHg): ^1H NMR ($CDCl_3$) δ 7.9 (2 H, m), 7.6 (3 H, m), 4.1 (1 H, dq, OC*H*CH_3), 3.4–3.0 (2 H, br m, CH_2SO_2), 2.9–2.3 (2 H, m, C*H*–N–C*H*), 1.23 (3 H, d, J = 7 Hz, CH_3CH), 0.88 (3 H, m, CH_2CH_3); ^{13}C NMR ($CDCl_3$) 139.56, 133.58, 129.29, 128.06, 79.91, 72.64, 65.62, 60.29, 56.13, 47.30, 37.23, 31.38, 30.27, 25.40, 21.50, 19.36, and 14.09 ppm; HRMS, calcd for $C_{19}H_{29}NO_3S$ 351.1868, found 351.1870.

The **hydrogen oxalate** was prepared and recrystallized from 95% ethanol, mp 143.5 °C. Anal. Calcd for $C_{21}H_{31}NO_7S$: C, 57.13; H, 7.08; N, 3.17. Found: C, 56.88; H, 7.25; N, 2.98.

(2*RS*,3*SR*,6*SR*)-2-[3-(Phenylsulfonyl)propyl]-3-[(1′*RS*)-1′-hydroxyethyl]-6-propylpiperidine (20). The cycloadduct **16** (0.351 g, 1 mmol) was reduced with 0.390 g (6 mmol) of zinc dust in 5 mL of 80% acetic acid in the usual manner, and the reaction progress was monitored by TLC. After workup and flash chromatography (EtOAc/hexane, 1:3), there was obtained 0.310 g (87%) of amino alcohol **20**: ^1H NMR ($CDCl_3$) δ 8.0 (2 H, m), 7.6 (3 H, m), 4.02 (3 H, br m), 3.13–2.8 (4 H, br m), 1.2 (3 H, d, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 139.49, 133.71, 129.36, 128.06, 71.66, 61.27, 57.63, 56.20, 40.41, 39.71, 33.20, 32.68, 28.06, 25.30, 20.14, 18.71, and 14.16 ppm; LRMS, m/e 353 (M^+).

The **hydrogen oxalate** was prepared and recrystallized from 95% ethanol, mp 156 °C.

(2*RS*,3*SR*,6*SR*)-1-(*tert*-Butoxycarbonyl)-2-[3-(phenylsulfonyl)propyl]-3-[(1′*RS*)-1-hydroxyethyl]-6-propylpiperidine (21). The amino alcohol **20** (1.059 g, 3 mmol) was treated with di-*tert*-butyl dicarbonate (0.916 g, 4.2 mmol) by the procedure described earlier to give 1.143 g (82%) of hydroxy carbamate **21**: ^1H NMR ($CDCl_3$) δ 7.98 (2 H, m), 7.55 (3 H, m), 4.7 (1 H, br s, O*H*), 4.2 (1 H, m), 3.5–3.2 (4 H, br m), 2.5–0.8 (28 H, m); ^{13}C NMR ($CDCl_3$) 155.63, 139.47, 133.43, 129.14, 127.91, 79.41, 67.98, 60.25, 56.10, 49.80, 48.24, 36.88, 28.50, 27.92, 22.40, 20.84, 20.19, 18.05, and 14.02 ppm; LRMS, m/e 453 (M^+).

(2*RS*,3*SR*,6*SR*)-1-(*tert*-Butoxycarbonyl)-2-[3-(phenylsulfonyl)propyl]-3-[(1′*RS*)-1′-[(*p*-tolylsulfonyloxy)ethyl]-6-propylpiperidine (22). To a cooled solution of 0.906 g (2 mmol) of Boc derivative **21** in 3 mL of pyridine was added 0.571 g (3 mmol) of *p*-toluenesulfonyl chloride. The mixture was kept at 0 °C for 16 h and then poured into cold water and extracted with dichloromethane. After drying, concentration on a rotary evaporator gave 1.116 g (92%) of the tosylate **22**, mp 49–51 °C: ^1H NMR ($CDCl_3$) δ 8.0–7.2 (9 H, m), 4.4–3.9 (3 H, br m), 3.0 (2 H, br m), 2.47 (3 H, s, ArCH_3), 1.40 (9 H, s); ^{13}C NMR ($CDCl_3$) 155.22, 144.63, 139.37, 134.68, 133.26, 129.69, 129.04, 127.80, 127.21, 80.50, 79.46, 55.88, 49.38, 46.20, 36.65, 28.20, 27.48, 24.44, 20.66, 19.86, 19.82, 17.48, and 13.77 ppm. Anal. Calcd for $C_{31}H_{45}NO_7S_2$: C, 61.26; H, 7.46; N, 2.31. Found: C, 60.93; H, 7.93; N, 2.47.

***cis*-1-(*tert*-Butoxycarbonyl)-5β-methyl-6-(phenylsulfonyl)-2α-propyldecahydroquinoline (23).** To a solution of 0.304 g (0.5 mmol) of sulfone tosylate **22** in 5 mL of dry THF at −78 °C was added 0.35 mL (0.5 mmol) of 1.55 M *n*-butyllithium in hexane. The solution immediately turned red, and it was allowed to stir for 0.5 h at −78 °C. Three equivalents (0.3 mL) of dry hexamethylphosphoric triamide was added, and the mixture was allowed to warm to slowly to room temperature. After 5 h the mixture was poured onto ice–water and extracted with five 10-mL portions of dichloromethane. Drying and concentration gave 0.180 g (83%) of **23** as a viscous gum: IR (neat) 1670, 1600, 1320, and 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.87 (2 H, m), 7.6 (3 H, m), 4.2 (2 H, m), 3.82 (1 H, m), 1.45 (9 H, s), 0.94 (3 H, d, CH_3CH), 0.91 (3 H, br s, CH_3CH_2); HRMS, calcd for $C_{24}H_{37}NO_4S$ 435.2443, found 435.2445.

(±)-Pumiliotoxin C (12). To a solution of 0.218 g (0.5 mmol) of sulfone **23** and 2.0 mmol of anhydrous disodium hydrogen phosphate in 5 mL of methanol at 0 °C was added 0.75 g of finely pulverized 6% sodium amalgam. The mixture was stirred for 2 h, poured into water, and extracted with several portions of ether. After drying (anhydrous $MgSO_4$), removal of the ether gave the carbamate: ^1H NMR ($CDCl_3$) δ 4.05 (H, br m), 3.69 (1 H, br m), 3.82 (1 H, m), 1.43 (3 H, d), 0.87 (3 H, t).

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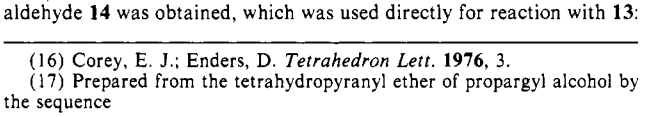
(17) Prepared from the tetrahydropyranyl ether of propargyl alcohol by the sequence



(18) Stowell, J. C.; Keith, D. R. *Synthesis* **1979**, 132.

The crude carbamate was taken up in 5.0 mL of dichloromethane and stirred with 1.0 mL of trifluoroacetic acid for 3 h. The mixture was made basic to pH 11 with aqueous NaOH, the layers were separated, and the aqueous layer was extracted with five 10-mL portions of dichloromethane. Drying and concentration gave 0.076 g (78%) of the free base **12**: ^{1}H NMR ($CDCl_3$) δ 6.81 (1 H, br s, N*H*), 2.97 (1 H, m, C-8a *H*), 2.62 (1 H, br m, C-2 *H*); HRMS, calcd for $C_{13}H_{25}N$ 195.1987, found 195.197.

The **hydrochloride** was prepared and recrystallized from a mixture of isopropyl alcohol and ether (3:1 v/v), mp 231-233 °C (lit.[12a] mp 243-244 °C): IR (KBr) 3400, 2530, 1585, 1480, 1467, 1438, 1390, 1195, 1130, 982, and 960 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.32 (1 H, m), 2.96 (1 H, br m), 0.96 (3 H, t, J = 6.0 Hz), 0.88 (3 H, d, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$-D_2O) 60.1, 58.0, 41.0, 35.0, 34.6, 29.2, 27.4, 25.3, 23.2, 20.7, 19.8, 18.8, and 13.8 ppm (lit.[12a] ^{13}C NMR 60.1, 58.1, 41.0, 35.0, 34.6, 29.2, 27.4, 25.3, 23.3, 20.7, 19.8, 19.2, and 13.7 ppm).

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Crystal Structure of the Covalent Complex Formed by a Peptidyl α,α-Difluoro-β-keto Amide with Porcine Pancreatic Elastase at 1.78-Å Resolution†

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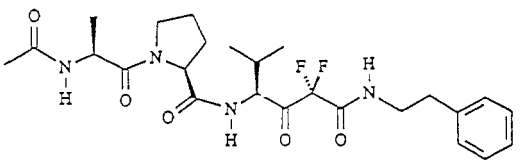
Abstract: The crystal structure analysis of the covalent enzyme-inhibitor complex of porcine pancreatic elastase (PPE) with a peptidyl α,α-difluoro-β-keto amide has shown that the tightly bound inhibitor forms an hemiketal complex with the O$^\gamma$ atom of the catalytic Ser-195 and is stabilized by five intermolecular H bonds and optimal van der Waals' surface interactions. The inhibitor is bound to the enzyme in an antiparallel β-pleated sheet arrangement. The carbonyl oxygen atom of the inhibitor is situated in the "oxyanion hole", hydrogen bonded to the amido nitrogen atoms of Ser-195 and Gly-193. A strong hydrogen bond between His-57 and a fluorine atom also aids in stabilizing the complex. The H-bonding catalytic tetrad of elastase is structurally intact. Covalent attachment of ligand plus active site Ser-195 is based upon contiguous electron density found in the initial, unbiased difference Fourier electron density map. The resulting hemiketal linkage has chemical and structural similarities with the putative tetrahedral intermediate of a productive enzyme-peptide ligand complex. This analysis provides structural evidence for the preferred binding of a novel class of inhibitors of the serine proteinases. Two refinement programs, EREF and TNT, were used to refine the enzyme + inhibitor model.

Elastases (EC 3.4.21.11) are possibly the most destructive enzymes in the body, having the ability to degrade virtually all connective tissue components. Elastases are involved in the pathogenesis of pancreatitis and emphysema.[1,2] They have also been implicated in atheroschlerosis,[3] adult respiratory distress syndrome,[4] rheumatic arthritis,[5] and other disease states.[6]

Peptidyl fluorinated ketones have been shown to be excellent inhibitors of the elastases. Imperiali and Abeles,[7] Kolb,[8] and Trainor[9] have synthesized potent peptidyl difluoromethylene ketone and peptidyl trifluoromethyl ketone inhibitors of HLE[10] and PPE. Kinetic analysis of some of these fluorinated ketones suggests that these compounds are transition-state analogue inhibitors.[7,11,12] The enhanced electrophilicity of the fluorinated ketone carbonyl was expected to facilitate an enzyme-catalyzed addition of the active site serine to the ketone carbonyl, forming a stable hemiketal intermediate.

To establish unequivocally the nature of the complexation of PPE with a peptidyl α,α-difluoro-β-keto amide, (*S*)-*N*-acetyl-L-alanyl-*N*-[3,3-difluoro-1-(1-methylethyl)-2,4-dioxo-4-[(2-phenylethyl)amino]butyl]-L-prolinamide (**1**) was prepared for crystallographic analysis. Additional interactions between the S′ subsites (nomenclature of Schechter and Berger[13]) of the enzyme and the P′ fragments of the peptidyl difluoro ketone inhibitors



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were believed to contribute to the overall tight binding. Subsequent analogues could then be designed for more specific S′-P′ inter-

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(10) Abbreviations: MEO, methoxy; SUC, succinyl; PNA, *p*-nitroaniline; DFK, difluoro ketone; PPE, porcine pancreatic elastase; HLE, human leucocyte elastase; Vaf, a valine residue with a difluoromethyl hemiketal group in place of the carbonyl group ($COCF_2$); Pea, β-ketophenethylamide; rms, root-mean-square; BPTI, bovine pancreatic trypsin inhibitor.

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