Synthesis of a 2,5-Diaryloxazoline as a Potential Platelet-Activating Factor Antagonist

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2,5-Bis-(3,4,5-trimethoxyphenyl)-2-oxazoline (**5**, MDL 100,270) was designed as a potential platelet-activating factor (PAF) antagonist on the basis of computer modeling comparison studies with known PAF antagonists. An efficient, four-step synthesis of oxazoline **5** was developed, starting from 3,4,5-trimethoxybenzaldehyde (**6**). Oxazoline **5** was found to inhibit the PAF-induced aggregation of human platelets with an IC_{50} value of 708 μM.

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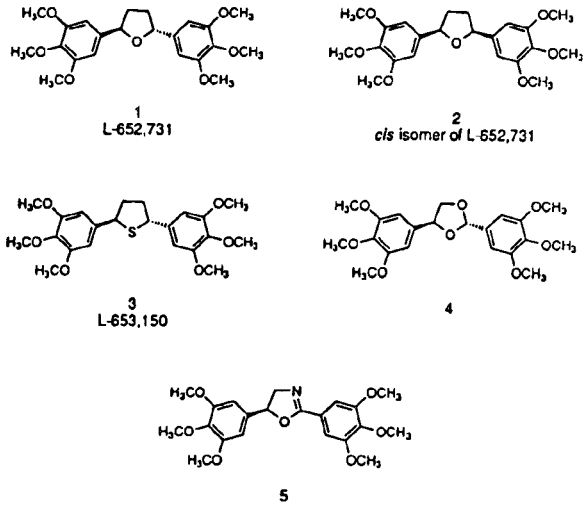
Platelet-activating factor (PAF) is a charged phospholipid which mediates many biological and physiological activities including platelet aggregation and blood pressure lowering. Disease states in which PAF may play a role as an exquisitely sensitive and potent mediator include asthma, gastric ulceration, inflammatory conditions such as rhinitis, psoriasis and endotoxic shock, and ischemic events such as stroke [1-5].

Several classes of heterocyclic compounds, both naturally occurring and synthetic, which are uncharged and not obviously related to the structure of PAF are antagonists of PAF [6,7]. One of the most potent of these classes of antagonists is the 2,5-diaryltetrahydrofurans, and the most potent agent in this class was found to be (-)-*trans*-(2*S*,5*S*)-2[3-[(2-oxopropyl)sulfonyl]-4-*n*-propoxy-5-(3-hydroxypropoxy)phenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran [8]. This specifically substituted compound is a successor to related compounds MK 0287, which has undergone human clinical testing [9], and L-652,731 (compound **1**), an initial member of the 2,5-diaryltetrahydrofuran family.

Several specific analogs of **1** bearing the same substitution pattern in the aryl rings have been prepared (Scheme I), including *cis*-isomer **2** [8,10], tetrahydrothiophene analog **3** [6] and 1,3-dioxolane analog **4** [11]. Computer modeling studies of our proposed synthetic target, oxazoline **5**, with tetrahydrofurans **1** and **2** showed that energy minimized versions of **1** and **5** could occupy nearly the same space (Figure I), and that a similar fit of **2** and **5** (Figure II) was not as precise. *Trans*-isomer **1** is significantly more potent than *cis*-isomer **2** as a competitive PAF receptor antagonist [10].

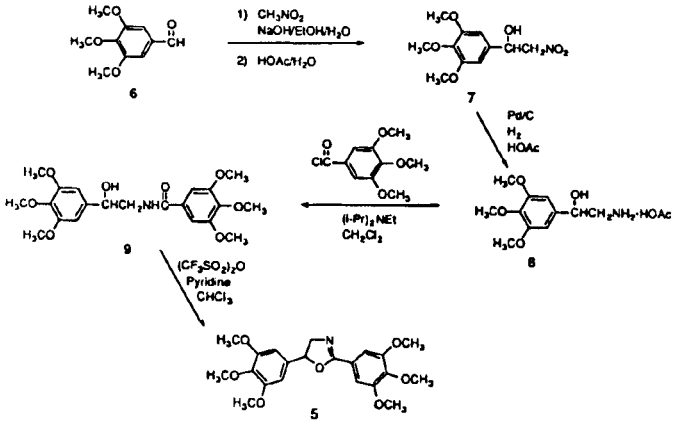
Hydroxyamide **9** (Scheme II) appeared to be a logical precursor to the desired oxazoline **5**. This compound has previously been reported in a U.S. Patent [12]. Ethanolamine **8** was also reported in this same patent, but the exact method of preparation was not specified. General methods cited for its preparation involved (i) conversion of 3,4,5-trimethoxyacetophenone to its α-bromo derivative, displacement of bromide by azide, and reduction of the resulting α-azido ketone with lithium aluminum hydride [13]; and (ii) reduction of the cyanohydrin of 3,4,5-trimethoxybenzaldehyde [14] with lithium aluminum hydride [13].

Scheme I



Our preparation of hydroxyamide **9** and the conditions which were developed for its conversion to oxazoline **5** are shown in Scheme II. The anion of nitromethane was prepared with ethanolic sodium hydroxide and condensed with 3,4,5-trimethoxybenzaldehyde (**6**) to afford nitro alcohol **7** [15]. Reduction of **7** with 10% palladium on carbon in acetic acid gave amino alcohol **8** as the acetic acid salt [16]. Acylation of **8** with 3,4,5-trimethoxybenzoyl chloride gave hydroxamide **9**. Treatment of **9** with *p*-toluenesulfonyl chloride and pyridine in chloroform [17] provided only a 22% yield of oxazoline **5**, along with recovered starting material **9** in 28% yield. Two additional products were isolated which were tentatively assigned as the *O*-tosylate of **9** (less than 1%) and a corresponding product in which the *O*-tosyl group was displaced by chloride ion (8%).

Scheme II



PS-2 personal computer. Superimpositions were done by computationally fitting the structures by finding the best fit for the two structures using seven homologous atoms in the two structures. The seven atoms chosen for this fit procedure were the five atoms in the five-membered rings plus the first atoms in each aryl substituent. Following this fitting procedure, the broken line structure was moved vertically down to a small extent from the solid line structure to provide the offset overlay shown in Figure I. In a similar manner Figure II was created, wherein the solid line is structure **5** and the broken line structure is the *cis* isomer of L-652,731.

Our conclusion regarding the overlays in Figures I and II was that one energy minimized version of oxazoline **5** compared quite favorably with a minimized version of either L-652,731 or its *cis* isomer, and that **5** and L-652,731 were more structurally similar than **5** and the *cis* isomer of L-652,731. Prior to making these comparisons

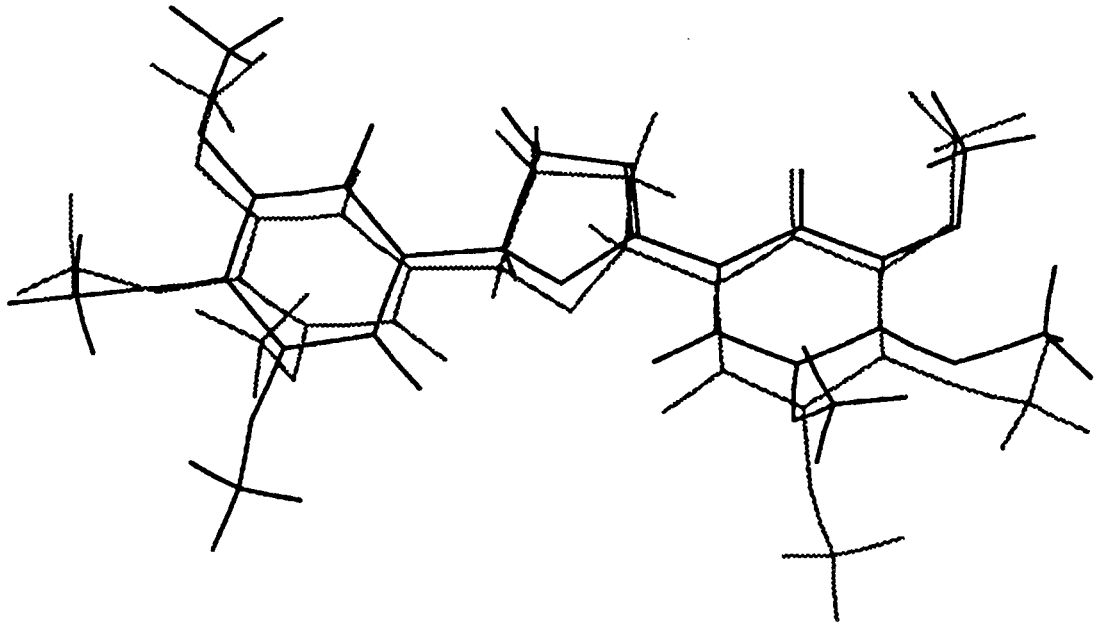


Figure I. Slightly Offset Overlay of Minimized Energy Conformations of Oxazoline **5** (Solid Line) and L-652,731 (Broken Line).

Conditions which cleanly converted hydroxyamide **9** to 2,5-bis-(3,4,5-trimethoxyphenyl)-2-oxazoline (**5**) were developed using a more reactive sulfonating reagent. Treatment of **9** with trifluoromethanesulfonic anhydride and pyridine in chloroform gave a 59% yield of **5** [18].

In Figure I are shown energy minimized structures of oxazoline **5** and L-652,731. Oxazoline **5** is the structure which is graphically represented by the solid line while L-652,731 is the broken line structure. The structures were drawn and minimizations and overlays were performed using ALCHEMY III software [19] installed on an IBM

it was not obvious to us that a disubstituted five-membered ring with two sp^2 centers (*e.g.*, **5**) would structurally resemble a disubstituted, all-tetrahedral five-membered ring (*e.g.*, L-652,731).

Oxazoline **5** was evaluated for inhibition of PAF-induced aggregation of human platelets in platelet-rich plasma (Table I). Alprazolam was used as a positive control for this study. IC_{50} values of *ca* 700 μM and 30 μM were measured for oxazoline **5** and alprazolam, respectively. Both compounds inhibited the aggregation response in dose-dependent fashion.

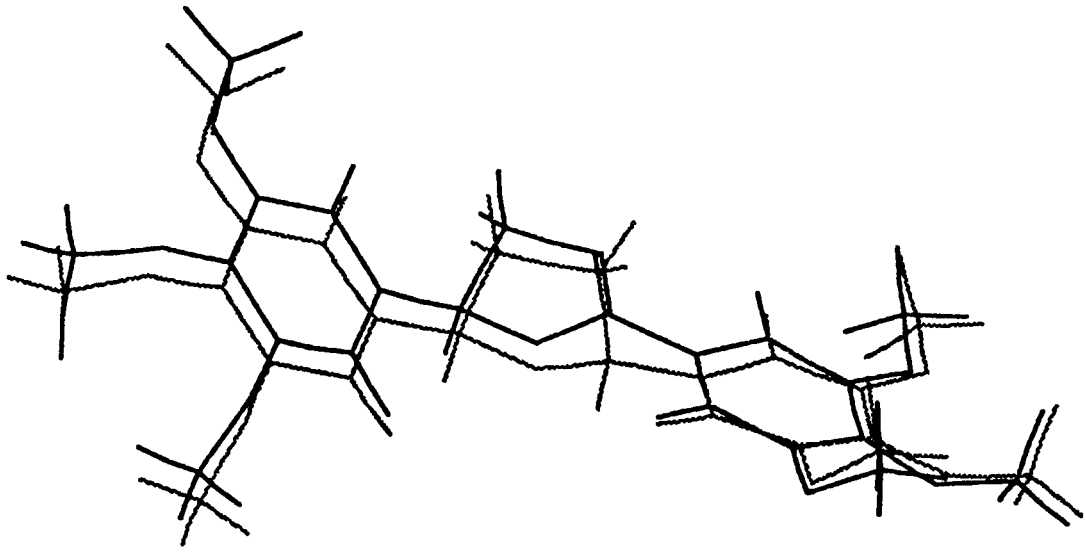


Figure II. Slightly Offset Overlay of Minimized Energy Conformations of Oxazoline **5** (Solid Line) and *cis*-L-652,731 (Broken Line).

Table I

Inhibition of PAF-Induced Aggregation of Human Platelets by Oxazoline **5** and Alprazolam

Compound	IC_{50} (μM) [a]
Oxazoline **5** (MDL 100,270)	708 ±39
alprazolam	28 ±0.2 [b]

[a] Platelet aggregation studies were performed in platelet-rich human plasma. See "Platelet Aggregation Assay" in the Experimental. [b] This value is similar to the IC_{50} value of *ca* 5 μM reported by E. Kornecki, Y. H. Ehrlich and R. H. Lenox, *Science*, **226**, 1454 (1984).

Table II

Inhibition of PAF-Induced Rabbit Platelet Aggregation by Standard Compounds

Compound	IC_{50} μM[a]
CV 3988	25.9 [b]
triazolam	11.1 [c]
alprazolam	41.2 [c]
WEB 2086	0.34 [d]
kadsurenone	3.3 [e]
L-652,731	1.6 [f]

[a] Platelet aggregation studies were performed in platelet-rich rabbit plasma. Values are averages of 2-6 determinations. [b] Z. Terashita, S. Tsushima, T. Yoshioka, H. Nomura, Y. Inada and N. Nishikawa, *Life Sci.*, **32**, 1975 (1983). [c] E. Kornecki, Y. H. Ehrlich and R. H. Lenox, *Science*, **226**, 1454 (1984). [d] J. Casals-Stenzel, G. Muacevic and K. H. Weber, *J. Pharmacol. Exp. Ther.*, **241**, 974 (1987). [e] T. Y. Shen, S.-B. Hwang, M. N. Chang, T. W. Doebber, M.-H. T. Lam, M. S. Wu, X. Wang, G. Han and R. Li, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 672 (1985). [f] T. Biftu, N. F. Gamble, T. W. Doebber, S.-B. Hwang, T.-Y. Shen and J. Snyder, *J. Med. Chem.*, **29**, 1917 (1987).

In Table II is shown a collection of PAF antagonists and their inhibitory IC_{50} values for PAF-induced aggregation of rabbit platelets in platelet-rich plasma. These values are included to relate alprazolam to other known PAF antagonists and to the activity of oxazoline **5**.

In summary, we have described an efficient synthesis for 2,5-bis-(3,4,5-trimethoxyphenyl)-2-oxazoline (**5**, MDL 100,270). Computer modeling studies in which **5** was compared with known PAF antagonists suggested it was a reasonable target compound. In a PAF-induced human platelet aggregation model, oxazoline **5** was found to inhibit aggregation with an IC_{50} value of 708 μM.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Analyses (tlc) were performed with Merck DC-f_{254} silica gel plates, with visualization by alkaline permanganate or uv irradiation. Flash chromatography was performed with Merck silica gel 60 (0.040-0.063 mm). The nmr spectra were recorded on a Varian VXR-300, Gemini-300 or EM-390 spectrometer. The ir spectra were recorded on a Perkin-Elmer Model 1800 or Mattson Galaxy 5020 FT-IR spectrophotometer. The ms data were collected on a Finnigan MAT 4600 or MAT TSQ-700 spectrometer.

2-Nitro-1-(3,4,5-trimethoxyphenyl)ethanol (**7**).

To a rapidly stirred solution of 3,4,5-trimethoxybenzaldehyde (10.0 g, 51.0 mmoles) and nitromethane (10 ml, 0.18 mmoles) in 95% ethanol cooled in an ice-water bath was added 10% aqueous sodium hydroxide (21.4 ml, 53.5 mmoles). After 45 seconds, 2% aqueous acetic acid (162 ml) was added and the reaction mixture stirred for an additional one hour at ice bath temperatures. Suction filtration gave the known [15] compound

7 (8.31 g, 63%) as an off-white solid, (lit [15] mp 109°); ^{1}H nmr (90 MHz, deuteriochloroform): δ 6.56 (s, 5H, aryl), 5.46-5.21 (m, 1H, CHO), 4.69-4.36 (m, 2H, CH_2), 3.79 (s, 9H, 3 x OCH_3), 3.50 (d, 1H, OH); ms: (70 eV, electron impact) m/z 258 (MH^+, 20), 257 (M^+, 100), 210 (35), 197 (88), 169 (62), 154 (30), 151 (32), 138 (34).

2-Amino-1-(3,4,5-trimethoxyphenyl)ethanol, Acetic Acid Salt (**8**).

A solution of **7** (4.00 g, 15.55 mmoles) in acetic acid (90 ml) was added to 10% palladium on charcoal catalyst (1.0 g) and the mixture hydrogenated under 40 psi of hydrogen in a Parr apparatus for 23 hours. The catalyst was removed by filtration through a bed of filter aid-magnesium sulfate and the filtrate concentrated *in vacuo* to give **8** (quantitative) as a colorless oil which solidified to give a white solid upon standing; ^{1}H nmr (90 MHz, deuteriochloroform): δ 8.95 (br s, exchangeable protons), 6.59 (s, 2H, aryl), 4.94 (br d, 1H, J = 12 Hz, CHO), 3.80 and 3.79 (pr s, 9H, 3 x OCH_3), 3.18 (br d, 1H, J = 12 Hz, 1/2 CH_2N), 3.05 (br t, 1H, J = 12 Hz, 1/2 CH_2N), 1.95 (s, CH_3CO_2); ms: (chemical ionization, methane) m/z 228 (MH^+, 73), 210 (100). The hydrochloride, oxalate and hydrogen oxalate salt analogs of **8** have been similarly prepared [16].

N-[2-Hydroxy-2-(3,4,5-trimethoxyphenyl)ethyl]-3,4,5-trimethoxybenzamide (**9**).

To a stirred solution of **8** (15.55 mmoles) and diisopropylethyl amine (8.13 ml, 46.65 mmoles) in dichloromethane (100 ml) under argon and cooled in an ice-water bath was slowly added a solution of 3,4,5-trimethoxybenzoyl chloride (3.59 g, 15.55 mmoles) in dichloromethane (15 ml). After 15 minutes the reaction was allowed to warm to room temperature. Thirty minutes after reaching room temperature the reaction mixture was concentrated, the residue dissolved in 10 ml of dichloromethane and loaded onto a silica gel column (7.5 x 14 cm). Flash chromatography, eluting with ethyl acetate gave **9** (6.31 g, 96%) as a white crystalline solid, mp 169-174° (lit [12] mp 152-154°); tlc R_f= 0.28 (ethyl acetate); ir (potassium bromide): 3410, 3334, 2940, 2840, 1636, 1582, 1538, 1532, 1502, 1464, 1414, 1334, 1236, 1132, 996 cm^{-1}; ^{1}H nmr (300 MHz, deuteriochloroform): δ 6.98 (s, 2H, aryl), 6.63 (s, 2H, aryl), 6.60 (br t, 1H, NH), 4.90 (dd, J = 8, 3 Hz, CHO), 3.89 and 3.84 and 3.83 (three s, 18H, 6 x OCH_3), 3.95-3.82 (buried m, 1H, 1/2 CH_2N), 3.49 (ddd, 1H, J = 13, 8, 5 Hz, 1/2 CH_2N); ^{13}C nmr (75 MHz, deuteriochloroform): δ 168.5 (CON), 153.3, 153.2, 141.1, 137.6, 137.3, 129.4, 104.4, 102.6, 73.9, 60.9, 60.8, 56.3, 56.1 and 48.1; ms: (70 eV, electron impact) m/z 421 (M+, 3), 403 (3), 225 (55), 224 (47), 210 (100), 195 (70).

Anal. Calcd. for $C_{21}H_{27}NO_8$: C, 59.85; H, 6.46; N, 3.32. Found: C, 59.71; H, 6.50; N, 3.14.

2,5-Bis-(3,4,5-trimethoxyphenyl)-2-oxazoline (**5**).

To a stirred solution of **9** (0.84 g, 2.00 mmoles) in chloroform (35 ml) under argon and cooled to -20° was added pyridine (0.40 ml, 5.00 mmoles) followed by triflic anhydride (0.42 ml, 2.50 mmoles). After 20 minutes, the reaction mixture was washed with saturated aqueous sodium bicarbonate (2 x 25 ml) followed by brine (25 ml), dried (sodium sulfate) and concentrated. The residue was flash chromatographed (5 x 16 cm silica gel column) eluting with ethyl acetate/hexane (4:1, v/v) to give crude **5**. Crude **5** was dissolved in dichloromethane, charcoaled and crystallized from dichloromethane-hexane to yield 0.48 g (59%) of **5** as an off-white solid, mp 126-128°; tlc R_f = 0.32 (ethyl acetate-hexane 4:1); ir (potassium bromide): 3432, 2943, 2841, 1641, 1587, 1506, 1465, 1416, 1352, 1244, 1235, 1128 and 1004 cm^{-1}; ^{1}H nmr (300 MHz, deuteriochloroform): δ 7.25 (s, 2H, aryl), 6.58 (s, 2H, aryl), 5.59 (dd, 1H, J = 8.4, 10.1 Hz, CHO), 4.47 (dd, 1H, J = 10.1, 14.9, 1/2 CH_2N), 4.01 (dd, 1H, J = 8.4, 14.9 Hz, 1/2 CH_2N), 3.91 and 3.87 and 3.86 (three s, 18H, 6 x OCH_3); ^{13}C nmr (75 MHz, deuteriochloroform): δ 163.8, 153.6, 153.1, 140.9, 138.0, 136.2, 122.7, 105.4, 103.0, 81.7, 63.1, 60.9, 60.8, 56.22, 56.18; ms: (70 eV, electron impact) m/z 403 (M^+, 53), 208 (17), 207 (100), 195 (45), 192 (17).

Anal. Calcd for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47. Found: C, 62.50; H, 6.60; N, 3.52.

Pharmacological Evaluation.

Human blood was collected from healthy, drug-free, male volunteers into plastic syringes containing 3.8% trisodium citrate (1:10, v:v). Blood was centrifuged at 200 x g for 10 minutes to obtain platelet-rich plasma (PRP).

Aggregation assays were performed using a dual-channel aggregometer (Chrono-log Corp., Model 460-VS, Hamerstown, PA). Solutions of test compounds, prepared in dimethylsulfoxide and diluted to a final concentration in saline, or vehicle were added to PRP and, after incubation for 30 sec, PAF (1 μ*M*) was added. Inhibitory responses were expressed as percent inhibition when compared to a vehicle control value. For each experiment, the standard PAF antagonist alprazolam was used as a positive control. The concentration resulting in 50% inhibition of aggregation (IC_{50}) was calculated by simple linear regression.

REFERENCES AND NOTES

[1] M. Koltai, D. Hosford, P. Guinot, A. Esanu and P. Braquet, *Drugs*, **42**, 174 (1991).

[2] M. Koltai, D. Hosford, P. Guinot, A. Esanu and P. Braquet, *Drugs*, **42**, 9 (1991).

[3] P. Braquet, L. Touqui, T. Y. Shen and B. B. Vargaftig, *Pharm. Rev.*, **39**, 97 (1987).

[4] M. C. Venuti, *Ann. Rep. Med. Chem.*, **20**, 193 (1985).

[5] F. Snyder, *Med. Res. Rev.*, **5**, 107 (1985).

[6] P. Braquet and J. J. Godfroid, *Trends Pharm. Sci.* **7**, 397 (1986).

[7] Interestingly, carbohydrate-derived furan analogs of PAF have recently been reported. See F. Nicotra, L. Panza, G. Russo and L. Zucchelli, *J. Org. Chem.*, **57**, 2154 (1992).

[8] N. N. Girotra, T. Biftu, M. M. Ponpipom, J. J. Acton, A. W. Alberts, T. N. Bach, R. G. Ball, R. L. Bugianesi, W. H. Parsons, J. C. Chabala, P. Davies, T. W. Doebber, J. Doherty, D. W. Graham, S.-B. Hwang, C. H. Kuo, M.-H. Lam, S. Luell, D. E. MacIntyre, R. Meurer, C. D. Roberts, S. P. Sahoo and M. S. Wu, *J. Med. Chem.*, **35**, 3474 (1992).

[9] S. P. Sahoo, D. W. Graham, J. J. Acton, T. Biftu, R. L. Bugianesi, N. N. Girotra, C.-H. Kuo, M. M. Ponpipom, T. W. Doebber, M. S. Wu, S.-B. Hwang, M.-H. Lam, D. E. MacIntyre, T. J. Bach, S. Luell, R. Meurer, P. Davies, A. W. Alberts and J. C. Chabala, *Bioorg. Med. Chem. Letters*, **1**, 327 (1991).

[10] S.-B. Hwang, M.-H. Lam, T. Biftu, T. R. Beattie and T.-Y. Shen, *J. Biol. Chem.*, **260**, 15639 (1985).

[11] E. J. Corey, C.-P. Chen and M. J. Parry, *Tetrahedron Letters*, **29**, 2899 (1988).

[12] W. A. Skinner, J. G. Johansson and H. L. Johnson,

Tranquilizing 3,4,5-Trimethoxybenzamides of Phenyl- and Pyridylalkylamines, U.S. Patent 3,676,447 (July 11, 1972).

[13] G. I. Poos, J. R. Carson, J. D. Rosenau, A. P. Rosowski, N. M. Kelley and J. McGowin, *J. Med. Chem.*, **6**, 266 (1963).

[14] J. Levine, T. E. Eble and H. Fischbach, *J. Am. Chem. Soc.*, **70**, 1930 (1948).

[15] R. A. Heacock, O. Hutzinger and C. Nerenberg, *Can. J. Chem.*, **39**, 1143 (1961).

[16] The hydrogen chloride, hydrogen oxalate and oxalate salt analogs of **8** have been prepared similarly. See R. A. Heacock and O. Hutzinger, *Can. J. Chem.*, **40**, 128 (1962).

[17] J. Attenburrow, D. F. Elliott and G. F. Penny, *J. Chem. Soc.*, 310 (1948).

[18] Cyclodehydration of related hydroxamides (serine and threonine derivatives) was recently reported. See P. Wipf and C. P. Miller, *Tetrahedron Letters*, **33**, 907 (1992).

[19] Tripos Associates, Inc., 1699 S. Hanley Road, Suite #303, St. Louis, Missouri, 63144.