to give an analytically pure product (0.26 g, yield 30%). Melting point and chromatography results are shown in Table I.

Method B. Reduction of Selenious Acid. Selenious acid (0.4 g, 3.1 mmol) was dissolved in 25 mL of water and $NaBH_4$ (~0.5 g) was added in small portions. After heating to reflux, a clear solution was obtained. *N,N*-Dimethylaminoethyl chloride hydrochloride (0.95 g, 6.6 mmol) was added and the mixture was heated to reflux for 1 h and then cooled to room temperature. Following treatment with 0.4 g of NaOH and extraction with 30 mL of chloroform, the chloroform layer was separated, dried over anhydrous sodium sulfate, and filtered. The condensed residue was treated with 1 mL of concentrated HCl and 20 mL of absolute ethanol. After refrigeration overnight, the precipitate was filtered and the product was washed with 10 mL of petroleum ether. After drying, 0.52 g of product was obtained (yield 50%). The IR and NMR spectra for this product were the same as those for the product prepared by method A.

Other Tertiary Diamines, 2-4. Method B using the appropriate substituted aminoethyl chlorides was employed for the syntheses of all of the tertiary diamines. The yields and melting points are shown in Table I.

Bis[β-(*N,N,N*-trimethylammonio)ethyl] Selenide Diiodide (5). Selenious acid (1.29 g, 10 mmol) was dissolved in 50 mL of water and $NaBH_4$ (~1.5 g) was added in small portions. Upon heating to reflux, a clear solution was obtained. *N,N*-Dimethylaminoethyl chloride hydrochloride (2.9 g, 21 mmol) was added, and the resulting solution was refluxed for 1 h. The reaction mixture was cooled to room temperature, treated with 0.4 g of NaOH, and extracted with chloroform (2 × 30 mL). The combined extracts were condensed and the residue was dissolved in 5 mL of methyl iodide and 20 mL of ethanol. Upon standing at room temperature for 5 min, white crystals precipitated. The mixture was refrigerated for 16 h, and the white crystals were collected by filtration and washed with petroleum ether (10 mL) to give 4.4 g of compound **5** (yield 86%).

Se-75 Labeled Tertiary and Quaternary Diamines, 1-5. The desired amount of [^{75}Se]selenious acid was mixed with cold selenious acid and the sequence of method B was carried out until the chloroform extract condensation. The condensed residue was dissovled in saline and the solution was filtered thru a 0.22-μm filter to sterilize the solution. The specific activities ranged from 10 to 1000 μCi/mg, and the yields ranged from 30 to 70% based on radioactivity. For compound **5** the chloroform extract was treated with 1 drop of methyl iodide and 1 mL of absolute ethanol, and the solution was condensed to dryness, dissolved in saline, and filtered thru a 0.22-μm filter. The radiochemical yield was 42%.

Distribution Coefficients. The radioactive compound (0.05-0.2 μCi) was mixed with 1 mL of 1-octanol and 1 mL of phosphate buffer (0.1 M) at the desired pH. This mixture was counted, vortexed for 3 min, and then placed in a water bath shaker at 37 °C for 2 h. After centrifugation (3000 rpm × 5 min), the 1-octanol layer was separated, aliquoted, and counted. The distribution coefficient was calculated by the following equation:

$$\text{distribution coefficient} = \frac{\text{total counts in 1-octanol}}{\text{initial counts} - \text{total counts in 1-octanol}}$$

Organ Distribution Studies. Sprague-Dawley male rats (220-300 g) were injected intravenously (femoral vein) with 0.2 mL of solution (0.5-2.0 μCi) under light ether anesthesia. At different time periods after injection, animals were killed by removing the heart under ether anesthesia, and organs of interest were excised and counted in a well counter. Percent dose was determined by comparison of tissue counts to suitably diluted aliquots of the injected material prepared by injecting a dose into a volumetric flask at the time of administration to the rats using the same syringe. Total activities in blood and muscle were calculated by assuming that they are 7 and 40% of the body weight, respectively. All of the biodistribution data are presented as percent dose per organ (Table II). Percent dose per gram of each organ or tissue can be obtained by dividing percent dose per organ by organ weight (heart, 0.7-1.0 g; lungs, 1.4-1.8 g; liver, 8-10 g; kidneys, 1.8-2.0 g; brain, 1.5-1.8 g).

Synthesis, Absorption, and Toxicity of N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine, a Potent Iron Chelator

Raymond J. Bergeron,* Philip S. Burton, Kathy Ann McGovern, Erica J. St. Onge,

Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610

and Richard R. Streiff

Medical Service, Veterans Administration Hospital, Gainesville, Florida 32602 and College of Medicine, University of Florida, Gainesville, Florida 32610. *Received March 7, 1980*

A synthesis of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine, a potent iron chelator, is developed employing N^4-benzylspermidine as the starting material. Both the toxicity and the absorption properties of this compound are evaluated, further supporting its potential as a clinical iron chelator.

Phlebotomy therapy for the management of hemochromatosis or other conditions characterized by iron overload with normal red blood cell production has been very successful.[1] However, for patients who are anemic, with limited bone marrow reserve, iron chelation therapy is the only option.

The drug most widely used in iron chelation therapy, desferrioxamine B (Desferal), a hydroxamate siderophore isolated from *Streptomyces pilosus*,[2] has had limited success. Unfortunately, it cannot be taken orally and unless patients are subjected to extended infusion therapy they cannot be kept in negative iron balance. For these reasons, workers have been searching for new, clinically useful iron chelators. To date, most of these efforts have focused on siderophores, natural iron chelators, or synthetic models thereof.[3-6]

In a recent paper[7] focused on the evaluation of isoniazid pyridoxal hydrazone as a therapeutic iron chelator, Jacobs et al. also pointed out the potential of N^1,N^8-bis(2,3-di-

(1) E. R. Weintraub, *N. Engl. J. Med.*, **275**, 169 (1966).
(2) R. S. Smith, *Ann. N.Y. Acad. Sci.*, **119**, 776 (1964).
(3) R. C. Hider, J. Silver, J. B. Nielands, I. E. Morrison, and L. U. Rees, *FEB Lett.*, **102**, 325 (1979).
(4) J. B. Nielands, S. A. Ong, and T. Peterson, *J. Biol. Chem.*, **254**, 1860 (1979).
(5) T. Peterson, J. B. Nielands, *Tetrahedron Lett.*, **50**, 4805 (1979).
(6) J. Leong and J. B. Nielands, *J. Bacteriol.*, **124**, 823 (1976).
(7) T. Hoy, J. Humphrys, A. Jacobs, A. Williams, and P. Pouka, *Br. J. Haematol.*, **43**, 3 (1979).

0022-2623/80/1823-1130$01.00/0

hydroxybenzoyl)spermidine as a chelator. They cited the major problem with this 2,3-dihydroxybenzoic acid derivative as being its lack of accessibility. The compound, a metabolic product of *Micrococcus denitrificans*,[8] is difficult to isolate and purify, even in milligram quantities, thus rendering toxicity and absorption studies impractical.

In spite of the above accessibility problems, Jacobs and Tait still managed to generate some fascinating data on the system.[9] They found that this spermidine derivative decreased ^{59}Fe incorporation in Chang cells and inhibited ferritin synthesis and iron uptake by the ferritin protein. Furthermore, at equimolar concentrations, this chelator is seven times more effective than desferrioxamine in removing iron from transferrin. It should be pointed out that this value is only a lower limit, as the authors mention that the N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine used in the studies was impure.

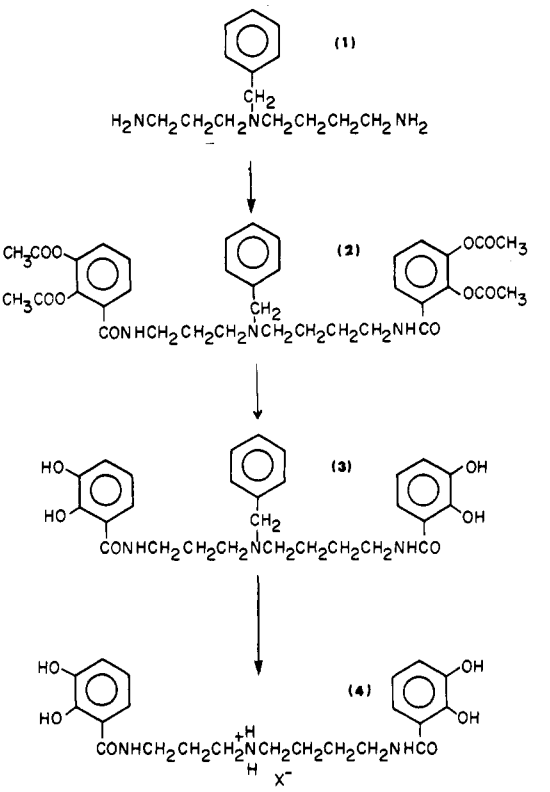
We felt that the iron clearing properties of this compound recommended it for synthetic evaluation. Our boundary conditions for the synthesis were, of course, high yield and a minimal number of steps. Also, it was particularly important that purification procedures be limited to simple techniques applicable to reasonably large quantities of materials.

In this paper, we describe a facile, high-yield synthesis and purification of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine. Some preliminary toxicity and intestinal absorption studies are presented as well; the toxicity studies involve intraperitoneal injection of the drug into mice, while the intestinal absorption studies employ the everted sac method of Wilson et al.[10]

In an earlier paper, we described the synthesis of a reagent, N^4-benzylspermidine, which could be used in the production of N^1,N^8-bis(acyl)spermidines.[11] This compound is a stable, distillable liquid which can be easily N^1,N^8-acylated in high yield with a variety of different acylating agents. In this particular instance, we reacted 2,3-diacetoxybenzoyl chloride with N^4-benzylspermidine to produce N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)-spermidine hydrochloride. Although the 2,3-hydroxyl groups of 2,3-dihydroxybenzoic acid have been protected by a number of methods, including methylation,[12] benzylation,[13] methylenedioxy[14] and sulfite formation,[14] we have found acetylation to work best for this synthesis. This 2,3-diacetoxy compound can be synthesized quite easily and in much higher yields than the other masked 2,3-dihydroxybenzoyl compounds. Furthermore, the acetate groups can be removed under mild conditions and in high yield.

Acetylation of 2,3-dihydroxybenzoic acid is carried out in 98% yield with acetic anhydride in the presence of a catalytic amount of sulfuric acid.[11] The resulting stable crystalline diacetate is converted to the corresponding acid chloride, also a stable crystalline material, in 94% yield by reaction with phosphorous pentachloride. This acid chloride, when reacted with N^4-benzylspermidine in methylene chloride with N^1,N^8-bis(dimethylamino)-naphthalene serving as a "proton sponge", results in an 80% yield of N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)-spermidine hydrochloride. There are two possible routes to N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine from this compound: debenzylation in glacial acetic acid over palladium followed by deacetylation, or deacetylation followed by debenzylation in trifluoroacetic acid. We have determined that the latter is the more desirable procedure.

Scheme I



When N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine is first debenzylated,[15] attempted deacylation of the resulting N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine under both acidic and basic conditions results in some N^4-acetylation. Whether this acetylation is intermolecular or intramolecular remains unclear and is currently under investigation. However, if the deacylation is carried out in methanol/methoxide with the benzyl protecting group still intact, N^4-acetylation does not occur. The hydrogenolysis of the resulting N^4-benzyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine to N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine can then be effected smoothly in trifluoroacetic acid over palladium. Both the deacylation and the debenzylation steps are virtually quantitative and the intermediate deacylated product does not have to be isolated. The synthesis is summarized in Scheme I.

While the N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine is isolated and purified as its trifluoroacetate salt, because of the toxicity of trifluoroacetate, the salt was exchanged for the hydrochloride by dissolving it in 20% hydrochloric acid and removing the water under vacuum. This exchange reaction was again quantitative and certainly underscores the remarkable acid stability of this compound; desferrioxamine, the current drug used in treatment of iron overload, is extremely acid labile.[16] Furthermore, chromatography of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine

(8) G. P. Tait, *Biochem. J.*, 146, 191 (1975).
(9) A. Jacobs, G. P. White, and George P. Tait, *Biochem. Biophys. Res. Commun.*, 74, 1626 (1977).
(10) T. H. Wilson and G. Wiseman, *J. Physiol.*, 123, 116 (1954).
(11) R. J. Bergeron, M. A. Channing, P. S. Burton, and K. A. McGovern, *J. Org. Chem.*, 45, 1589 (1980).
(12) H. Albrecht and K. Bernhard, *Helv. Chim. Acta*, 30, 627 (1947).
(13) M. C. Venuti, W. H. Rastetter, and J. B. Nielands, *J. Med. Chem.*, 22, 123 (1979).
(14) N. H. Perkin, Jr., and V. M. Trijojus, *J. Chem. Soc.*, 2925 (1926).
(15) W. H. Hartung and R. Siminonoff, *Org. React.*, 7, 263 (1953).
(16) W. F. Anderson, M. C. Hiller, Eds., *DHEW Publ. (NIH) (U.S.)* NIH 77-994 (1977).

Table I. Transport of N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine Hydrochloride Across the Intestinal Wall

animal	upper[a] (proximal)	middle[a]	lower[a] (distal)
1	42 ± 8	40 ± 12	44 ± 10
2	33 ± 2	35 ± 10	32 ± 13
3	32 ± 6	44 ± 5	50 ± 5

[a] The transport values are reported as the percent of chelator inside the intestinal sac relative to the original chelator concentration in the bathing solution. Each value represents the average of five measurements, the indicated error being the standard deviation of those measurements. Upper, middle, and lower represent the segment of the intestine used, starting from the proximal end.

hydrochloride on Sephadex LH-20 eluting with an ethanol/benzene gradient revealed the compound to be in excess of 97% pure. The N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine hydrochloride isolated in this way was homogeneous on paper chromatography. The compound had an R_f of 0.4 when developed with ammonium formate/formic acid/water on Whatman No. 14 paper as reported by Tait.[2] The NMR spectrum in trifluoroacetic acid revealed the expected signals in parts per million (relative to Me_4Si): 2.13 (br m, 6 H), 3.50, 3.86 (2 br overlapping m, 8 H), 6.63–9.30 (overlapping m, 10 H). Since the phenolic protons are in the fast exchange limit with the trifluoroacetic acid protons, they are buried under the trifluoroacetic acid proton peak. Tait's degradation studies of the N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine isolated from *Micrococcus dentrificans* did not indicate the correct ratio of 2,3-dihydroxybenzoic acid to spermidine. He attributed this to the impurity of the compound.[8] Our NMR spectral data indicate the correct ratio of aromatic to methylene protons. The ultraviolet and infrared data, although not nearly as revealing, were also in agreement with Tait's data (see Experimental Section).

Biological Testing. Our biological studies have focused on the toxicity and absorption properties of the chelator; however, we wish to point out these are only preliminary observations. The toxicity of the chelator was evaluated by injecting Swiss white mice intraperitoneally with suspensions of the compound in sterile isotonic saline. This indicates an $LD_{50} > 800$ mg/kg. The in vitro system used to estimate absorption was the standard everted intestinal sacs method, developed by Wilson and Wiseman.[10] This is a highly reproducible system for studying the absorption of various substances and the data correlates well with intact animal studies.[17] It allows for more precise control of the substances being studied than is possible for intact animals.

Results

Suspension of the intestinal sacs in varying concentrations of the chelator ($1.5–6.8 \times 10^{-4}$ M in K–R buffer at pH 7.4) resulted in substantial transport of the chelator across the intestinal membrane. The chelator concentration inside the sac was approximately 40% of that outside. The concentration of the chelator in the sac relative to the original concentration of the chelator in the bathing solution was found to be independent of the concentration of the chelator in the bathing solution. Furthermore, the transport of the material across the intestinal mucosa was independent of the anatomic area from which the intestinal segment was removed. The results are summarized in Table I.

Discussion

The key intermediate, N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine, can be converted to the final product, N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine, in either of two ways. The N^4-benzyl protecting group can first be removed by hydrogenolysis over palladium and the acetoxy groups hydrolyzed, or, alternatively, the acetoxy groups can be hydrolyzed followed by hydrogenolysis of the benzyl group. Both procedures employ basic hydrolysis of the acetoxy protecting groups simply because acid hydrolysis is slow and incomplete. The major problem with removing the acyl protecting groups under basic conditions is oxidation of the 2,3-dihydroxybenzoyl moiety, primarily to the corresponding quinone. Although this is minimized by running the reaction in degassed solvents under nitrogen, it is difficult to eliminate.

The synthesis of N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine described herein presents a significant advantage over the alternative approach. In the synthesis described, the potential generation of oxidized impurites, such as quinones, is minimized, since any quinone produced by the basic methanolysis of N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine is converted back to the corresponding catechol upon reductive removal of the benzyl group by hydrogenolysis. Furthermore, of the two N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine precursors, N^4-benzyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine is more easily isolated and purified than N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine. Although the acyl groups of N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine can be removed in basic methanol or basic methanol/water mixtures, there is some minor N^4-acylation as a result of either intra- or intermolecular O to N transacylation. This transacylation is not a problem with our sequence, since the hydrogenolysis is not effected until the last step, thus leaving the N^4-nitrogen protected during hydrolysis.

The final product, N^1,N^8-bis(2,3-dihydroxybenzoyl)-spermidine trifluoroacetate, is easily converted to the hydrochloride salt. This exchange is effected by dissolving the trifluoroacetate salt in 20% hydrochloric acid and lyophilizing the resulting mixture. While both the hydrochloride and trifluoroacetate salts are transported across the intestinal membranes, our toxicity studies indicate the hydrochloride salt to be substantially less toxic. These results are particularly exciting in view of Jacobs' and Tait's demonstration of the ability of this catecholamide to remove iron from biological systems.

Clearly, N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine binds iron quite tightly; however, the exact nature of the chelate remains to be defined. It is certainly well known that Fe(III) forms very stable hexacoordinate complexes with catechol-type chelates. This leads to the assumption that a 3:2 chelate to iron complex would form. This stoichiometry is certainly not unreasonable in view of the rhodotorulic acid–iron complex described by Raymond et al.[18] We are currently evaluating the thermodynamics of this binding.

Conclusion

The synthesis described provides a facile high-yield route to N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine hydrochloride, a potent iron chelator. Preliminary data show this compound to be both nontoxic and well absorbed through the intestinal mucosa. Furthermore, the absorption seems to be independent of which part of the intestine

(17) I. H. Rosenberg, *N. Engl. J. Med.*, **280**, 985 (1969).

(18) K. N. Raymond and C. J. Carrano, *J. Am. Chem. Soc.*, **100**, 5371 (1978).

is used. The synthetic accessibility of this compound coupled with its acid stability, low toxicity, and its ability to remove iron from biological systems qualify it for serious consideration in chelation therapy. Most importantly, its acid stability and absorption properties emphasize its potential as an effective oral iron chelator. We are currently measuring its ability to sequester and remove iron from iron-overloaded rats.

Experimental Section

Materials. All reagents were purchased from Aldrich Chemical Co. Unless specified otherwise, Na_2SO_4 was used as a drying agent. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Samples for ^1H NMR were prepared in $CDCl_3$ with chemical shifts given in parts per million relative to an internal Me_4Si standard unless stated otherwise. The spectra were recorded on Varian T-60 and XL-100 spectrometers. The infrared spectra were recorded on a Perkin-Elmer 257 grating spectrometer, prepared in KBr. Ultraviolet spectra were recorded on a Beckman 25 spectrophotometer. High-pressure liquid chromatography was carried out on a Waters 100-Å μStyragel column. Elemental analyses were performed by Galbraith, Inc., Knoxville, Tenn.

Biological Methods. White rats (300 g) were sacrificed and the abdomens opened by a midline incision. The small intestine was separated from the mesentery and removed in toto. Most of the fat was removed from the intestine and it was divided into upper, middle, and lower thirds. Each third was everted over a glass rod and rinsed with a 0.9% NaCl solution containing 0.6% glucose. Each third was cut into about five 5-cm segments and one end of each was tied with surgical silk. The open end of each sac was tied around the blunt needle of a syringe. One milliliter of Krebs–Henseleit Ringer–bicarbonate buffer (K–R buffer) at pH 7.4 was injected, and the knot was tightened as the needle was removed. The sac was placed in a 10-mL flask. Then, 8 mL of a solution of the chelator in K–R buffer was added and 95% O_2/5% CO_2 was bubbled through for about 30 s to oxygenate the solution. The stoppered flasks were placed in a Dubnoff incubator at 37 °C and shaken for 1 h. At this point, the intestinal segments were removed and washed thoroughly with distilled, deionized water. Each sac was nicked and its contents were allowed to drain into a test tube. The tubes were centrifuged in a Servall centrifuge at 16000*g* for 15 min to remove any large protein or particulate matter present. Ultraviolet spectra of the sac contents were taken at 330 nm at pH 7.4 against a K–R buffer blank to determine the concentration of the chelator passing through the intestine. To determine if leakage through the sacs' walls via punctures, etc., could be occurring, several of the sacs were suspended in a solution of Blue dextran, a molecule which does not pass through the intestine and which absorbs strongly in the visible spectrum, allowing easy detection. Control sacs were suspended in the buffer solution devoid of the chelator and dye. These experiments were repeated on several animals, amounting to some 45 intestinal segments.

Synthetic Methods. 2,3-Diacetoxybenzoyl Chloride. A solution of the 2,3-diacetoxybenzoic acid (16.41 g, 0.069 mol) in 600 mL of anhydrous EtOH-free chloroform was cooled to 0 °C under N_2, and phosphorous pentachloride (20.14 g, 0.097 mol) was added. The reaction mixture was allowed to warm slowly to room temperature and the reaction continued for 24 h. The solvent was then evaporated, and the crude product was taken up in 900 mL of benzene and washed 3 × 50 mL of aqueous 5% $NaHCO_3$ (w/v). After drying and filtering, the benzene solution was reduced in vacuo to give 16.70 g (94% yield) of the desired product, mp (CCl_4) 76–77 °C. Anal. ($C_{11}H_9O_5Cl$) C, H.

N^4-Benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine Hydrochloride (2). A solution of N^4-benzylspermidine (1; 2.80 g, 11.9 mmol) and 1,8-bis(dimethylamino)naphthalene (5.00 g, 23.3 mmol) in 400 mL of CH_2Cl_2 was cooled to 0 °C under N_2. The 2,3-diacetoxybenzoyl chloride (6.00 g, 23.4 mmol) in 250 mL of CH_2Cl_2 was added over a 4-h period. After addition was completed, the reaction vessel was allowed to warm slowly to room temperature with continued stirring under N_2. After 20 h, the reaction mixture was again cooled to 0 °C, washed 3 × 30 mL of ice-cold 1.1% aqueous HCl (w/v) and 3 × 30 mL of ice–H_2O, dried, filtered, and evaporated to give 5.62 g (66% crude yield) of the desired product, a white semicrystalline solid. Yields as high as 80% were obtained when a 20% excess of the acid chloride was used.

An analytical sample was purified by high-pressure liquid chromatography on a 100-Å μStyragel column eluted with THF. This purification demonstrated the crude product was in excess of 95% pure: mp 121–123 °C; ^1H NMR 1.50 (m, 6 H), 2.23 (s, 12 H), 2.93 (m, 4 H), 3.27 (m, 4 H), 3.83 (s, 2 H), 7.27 (m, 14 H) ppm. Anal. ($C_{36}H_{42}O_{10}N_3Cl$) C, H, N, Cl.

N^4-Benzyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine Hydrochloride (3). A solution of N^4-benzyl-N^1,N^8-bis(2,3-diacetoxybenzoyl)spermidine hydrochloride (2; 1.0 g, 1.4 mmol) and sodium methoxide (0.3 g, 5.6 mmol) in 20 mL of dry degassed methanol was allowed to stir under N_2. After 6 h the pH of the solution was adjusted to approximately 2 by addition of HCl saturated methanol. The resulting suspension was filtered through sintered glass and solvent was removed to yield 750 mg (98% crude yield) of the desired compound.

An analytical sample was purified by chromatography on Sephadex LH-20 eluting with absolute ethanol in benzene (5–65%). This purification demonstrated that the crude product was in excess of 98% pure: mp (EtOH) 110 °C dec; ^1H NMR (trifluoroacetic acid, chemical shifts downfield from Me_4Si, calculated relative to internal CH_2Cl_2, 5.28 ppm) 2.05, 2.45 (2 br overlapping m, 6 H), 3.48, 3.81 (two overlapping m, 8 H), 4.51 (m, 2 H), 6.71–9.24 (overlapping m, 14 H) ppm. Anal. ($C_{28}H_{34}O_6N_3Cl$) C, H, N.

N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine Hydrochloride (4). $PdCl_2$ (0.14 g, 0.82 mmol) was added to a solution of N^4-benzyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine hydrochloride (3; 0.4 g, 0.74 mmol) in 20 mL of trifluoroacetic acid, and the resulting suspension was stirred under a hydrogen atmosphere. After 90-h total reaction time, the catalyst was filtered off and solvent removed in vacuo. The resulting crude residue was then twice suspended in 50 mL of 20% aqueous HCl (w/v) and sonicated, the solvent being removed under high vacuum. The resulting white solid was chromatographed on Sephadex LH-20 (1.2 × 35 cm) eluting with anhydrous ethanol in benzene (5–60%). Fractions containing the pure compound were pooled, the solvent was removed, and residue was triturated with deionized water and freeze-dried to yield 370 mg (95%) of the desired product, a white crystalline solid: mp (EtOH) 170 °C dec; ^1H NMR (trifluoroacetic acid; chemical shifts downfield from Me_4Si, calculated relative to internal CH_2Cl_2, 5.28 ppm) 2.13 (br m, 6 H), 3.50, 3.86 (2 br overlapping m, 8 H), 6.63–9.30 (overlapping m, 10 H) ppm; IR 3100 (br m), 1638 (m), 1585 (s), 1537 (s) cm^{-1}. UV spectra of this compound were identical with Tait's data. Anal. ($C_{21}H_{28}N_3O_6Cl \cdot H_2O$) C, H, N.

Acknowledgment. We acknowledge the University of Florida for their support of this work and the research service of the Veterans Administration Hospital.