as an oil (this compound contains a 30% amount of the lactol form): IR (film) 3380, 1725, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 5 Hz), 5.93 (s, 1 H), 9.76 (t, 1 H, J = 1.5 Hz). Anal. Calcd for $C_{15}H_{23}NO_3$: C, 67.89; H, 8.74; N, 5.28. Found: C, 68.01; H, 8.70; N, 5.19.

5α-Hydroxy-2β-(5-pentylisoxazol-3-yl)cyclopentane-1α-*cis*-hept-2-enoic Acid (36). By use of the procedure outlined for the synthesis of **26**, the acid **36** was obtained as an oil in 60% yield by starting from **35**: IR (film) 1700, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3 H, J = 5 Hz), 4.5 (m, 1 H), 5.5 (m, 2 H), 5.9 (s, 1 H), 6 (br s, 2 H). Anal. Calcd for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01. Found: C, 68.68; H, 8.99; N, 3.92.

5α-Hydroxy-2β-(3-oxo-*trans*-1-octenyl)cyclopentane-1α-*cis*-hept-2-enoic Acid (38). By use of the procedure outlined for the synthesis of **15**, the acid **38** was obtained as an oil in 70% yield by starting from **36**: IR (film) 1705, 1670, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H, J = 5 Hz), 4.5 (m, 1 H), 5.5 (m, 2 H), 6 (br s, 2 H), 6.2 (d, 1 H, J = 16 Hz), 6.8 (dd, 1 H, J = 16, 7.5 Hz). Anal. Calcd for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.30; H, 9.65.

Registry No. 1, 78199-90-3; 2, 78199-91-4; 3, 78199-92-5; 4, 78217-49-9; 5, 78199-93-6; 6, 78199-94-7; 7, 78199-95-8; 8, 78199-96-9; 9, 78199-97-0; 10, 78199-98-1; 11, 78199-99-2; 12, 78200-00-7; 13, 78200-01-8; 14, 78200-02-9; 15, 41692-81-3; 16, 78200-03-0; 17, 78200-04-1; 18, 78200-05-2; 19, 78200-06-3; 20 (epimer 1), 78200-07-4; 20 (epimer 2), 78200-08-5; 21 (epimer 1), 78246-84-1; 21 (epimer 2), 78246-85-2; 22, 78200-09-6; 23, 78200-10-9; 24, 78200-11-0; 25, 78200-12-1; 26, 78200-13-2; 28, 78200-14-3; 31, 78200-15-4; 32, 78200-16-5; 33, 78200-17-6; 34 (epimer 1), 78200-18-7; 34 (epimer 2), 78246-86-3; 35, 78200-19-8; 36, 78200-20-1; 38, 78200-21-2; 2-methylcyclopent-2-en-1-one, 1120-73-6; butyl 5-oxocyclopent-1-ene-1-heptanoate, 52477-97-1; butyl 5-oxocyclopent-1-ene-1-acetate, 78200-22-3; 2-allylcyclopent-2-en-1-one, 51557-85-8; 1-heptyne, 628-71-7; 1-heptene, 592-76-7.

Flexible Synthesis of Polyamine Catecholamides

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A scheme is developed for the high-yield synthesis of polyamine catecholamides from the secondary *N*-benzylamines N^5-benzylhomospermidine, N^4-benzylspermidine, and *N*,*N*-bis(3-aminopropyl)benzylamine. These amines are first selectively acylated at the N-terminal positions with 2,3-dimethoxybenzoyl chloride, and the benzyl groups are removed by hydrogenolysis. The resulting diamides are then either demethylated to produce the corresponding bis(catecholamides) or secondary N-acylated and then demethylated. The secondary N-acylations were effected with either 2-hydroxyhippuric acid, *N*-(2,3-dimethoxybenzoyl)glycine, *N*-(2,3-dimethoxybenzoyl)-4-aminobutyric acid, or *N*-(2,3-dimethoxybenzoyl)-β-alanine. Six hexacoordinate and three tetracoordinate catecholamide iron ligands with polyamine backbones of differing lengths were generated by using this procedure. The approach offers a flexible method for optimizing the chelate effect in polyamine catecholamide ligands.

In recent years, a great deal of attention has been focused on the development of new iron chelators.[1-4] The reason for this is probably closely related to the absence of a suitable therapeutic device for the removal of iron from patients suffering toxic iron overload.[5,6] Both natural and synthetic chelators have been considered with most of the synthetic sequestering agents closely modeled after natural products.[7,8] However, a satisfactory drug still has not been developed.[9]

In 1975, Tait reported the isolation of a siderophore, N^4-[*N*-(2-hydroxybenzoyl)threonyl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (I), and its precursor, N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (II), from *Paracoccus denitrificans*[10] (Chart I), both of which showed potential as therapeutic iron-clearing devices.[10,11] These compounds were shown to remove iron from transferrin, the body's serum iron binding protein, as well as from cultured fibroblasts, Chang cells.[11] However, because of the difficulty in isolating these amides, workers were unable to run even the simplest animal studies, i.e., toxicity and iron-clearing experiments.

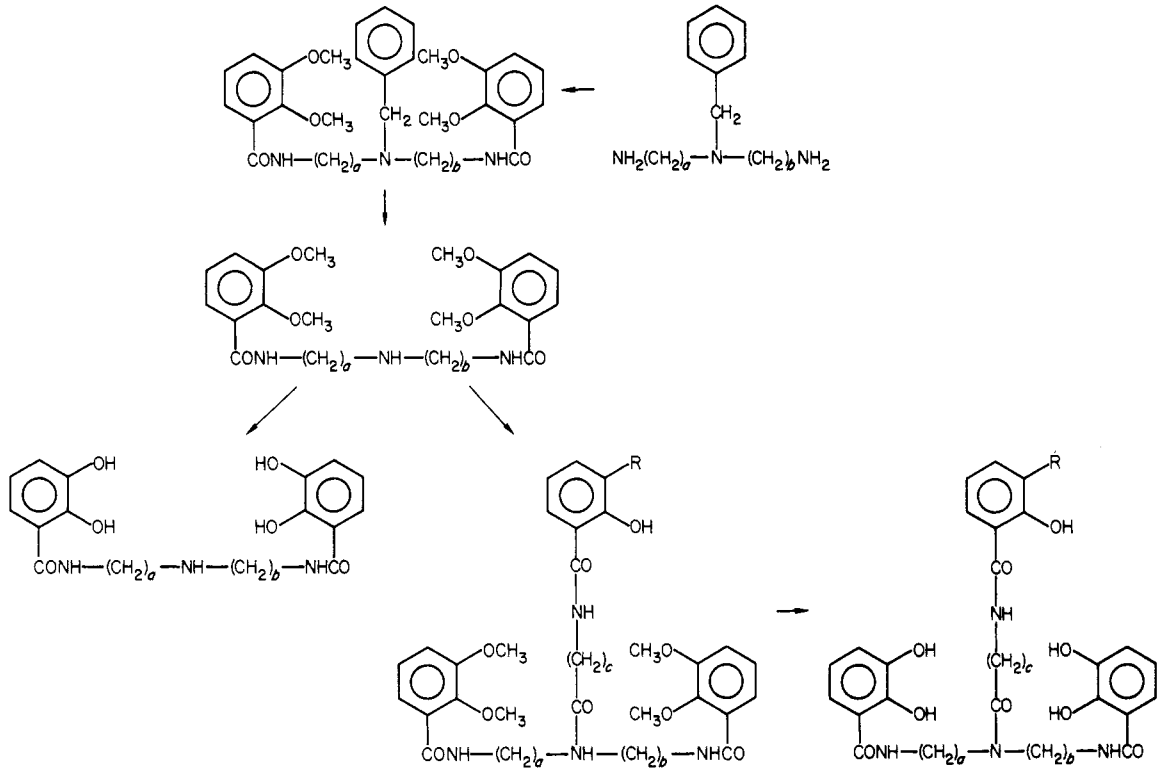
In an earlier paper, we reported on a high-yield synthesis of compound II and demonstrated it to be less toxic than aspirin and to be absorbed across intestinal walls, i.e., a potential orally effective chelator.[7] We have since shown it to be more effective than deferrioxamine at clearing iron from iron overloaded rats.[12] These results encouraged us to consider the development of a general synthesis of both compound I and II analogues.

It is now clear from Neilands' work[13] that in Tait's original isolation procedure, he hydrolyzed the oxazoline ring of compound III, (*N*-[3-(2,3-dihydroxybenzamido)propyl)]-*N*-[4-(2,3-dihydroxybenzamido)butyl]-2-(2-hydroxyphenyl)-5-methyloxazoline-4-carboxamide to pro-

(1) Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 5371.
(2) Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2722.
(3) Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2728.
(4) Venuti, M. C.; Rastetter, W. H.; Neilands, J. B. *J. Med. Chem.* **1979**, *22*, 123.
(5) Smith, S. R. *Ann. N. Y. Acad. Sci.* **1964**, *119*, 766.
(6) Modell, C. B.; Beck, *J. Ann. N. Y. Acad. Sci.* **1974**, *232*, 201.
(7) Bergeron, R. J.; Streiff, R. R.; Burton, P. S.; McGovern, K. A.; St. Onge, E. J. *J. Med. Chem.* **1980**, *23*, 1130.
(8) Peterson, J.; Falk, K. E.; Leong, S. A.; Klein, R. P.; Neilands, J. B. *J. Am. Chem. Soc.* **1980**, *102*, 7715.
(9) Patients with primary hemochromatosis undergoing the currently acceptable deferrioxamine therapy still generally die in their second decade.
(10) Tait, G. T. *Biochem. J.* **1975**, *146*, 191.
(11) Jacobs, A.; White, G. P.; Tait, G. T. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 1626.
(12) Manuscript in preparation.
(13) Peterson, T.; Neilands, J. B. *Tetrahedron Lett.* **1979**, 4805.

0022-3263/81/1946-4524$01.25/0

Scheme I. Synthesis of Hexacoordinate and Tetracoordinate Catecholamides of Differing Length Backbone

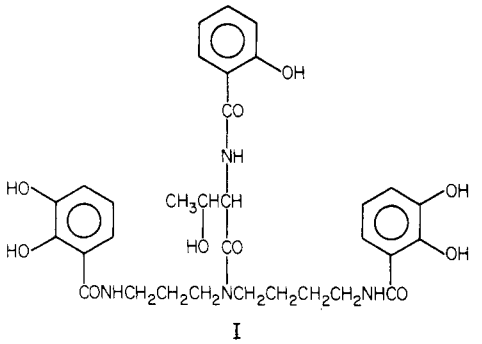


duce compound I. Under the acidic conditions employed in the original isolation, oxazoline hydrolysis would have been very rapid. However, it is clear that compound I is still very effective at removing iron from Chang cells.[11] This is particularly interesting in view of Neilands' suggestion as to the role of compound III's oxazoline nitrogen in iron chelation. If he is correct, it seems unlikely that the α-hydroxyethyl group of the threonine siderophore I plays a significant role in sequestering iron. This implies that the α-hydroxyethyl moiety is singularly unimportant in Tait's iron-clearing experiments.

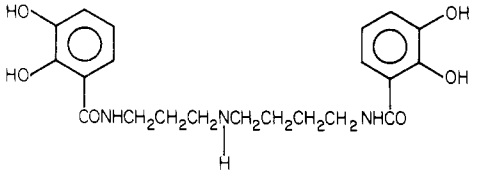
In this paper, we report on a general synthesis of analogues of compounds I and II. Although the scheme adheres to systems with triamine backbones, it allows us to vary the length of the carbon skeleton separating the chelation sites as well as the nature of the sites themselves. In terms of ligand sites, three basic types of ligands are described: those with two N-terminal catecholamides, those with three catecholamide sites, and those with two N-terminal catecholamides and a third secondary *N*-salicyloyl site (see Chart II). The distances between the N-terminal catecholamides are varied only by two methylene units, one methylene shorter than the spermidine catecholamide and one methylene longer. We chose this small variance over the spermidine backbone because the natural product with the spermidine bridge was already quite an effective iron-binding and -clearing device. The distances between the N-terminal catecholamides and the coordination site on the secondary nitrogen were varied by again changing the number of methylene bridges as well as the nature of the third site. In this instance, the methylene bridges varied from one to three methylenes, and the sites were either catecholates or salicylates. The syntheses of these hexacoordinate ligands are of such a high yield and flexible nature that almost any combination of ligand sites and distances can be generated following this scheme.

The scheme calls for four high-yield steps: (1) acylation of the appropriate secondary N-benzylated triamine with 2,3-dimethoxybenzoyl chloride, (2) debenzylation of the resulting diamide, (3) acylation at the secondary nitrogen,

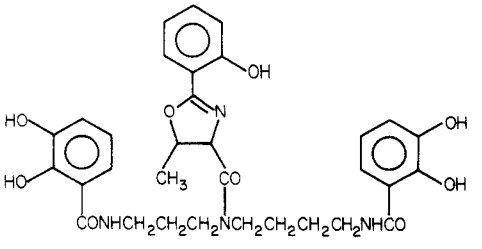
Chart I. Siderophores N^4-[*N*-(2-Hydroxybenzoyl)threonyl-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (I), N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine (II), and *N*-[3-(2,3-Dihydroxybenzamido)propyl]-*N*-[4-(2,3-dihydroxybenzamido)butyl]-2-(2-hydroxyphenyl)-5-methoxazoline-4-carboxamide (III)



I

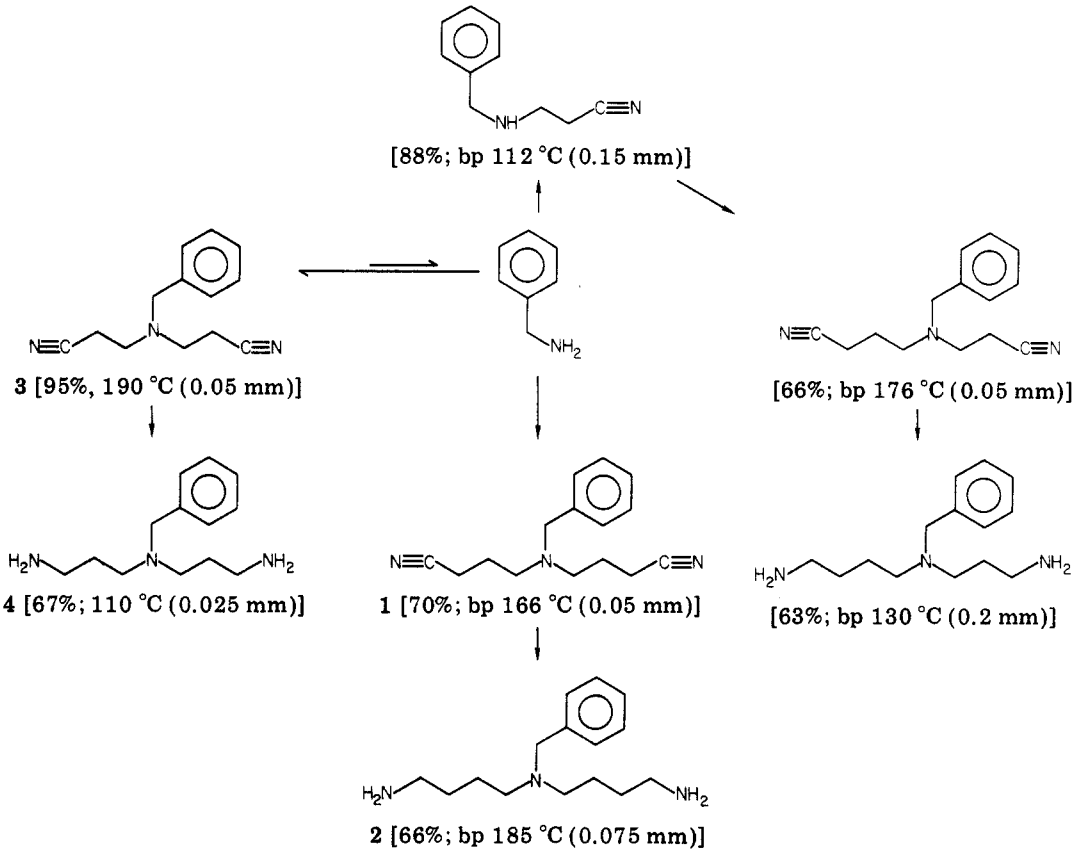


II



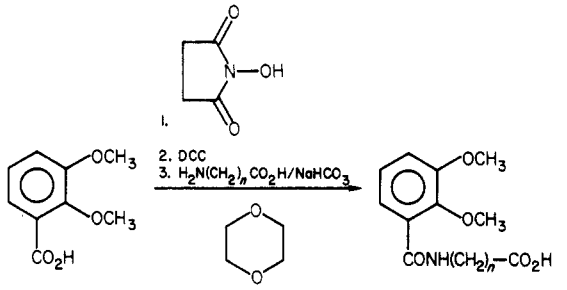
III

Scheme II. Synthesis of Benzylated Triamines



and (4) removal of the methyl protecting groups. Each of these steps proceeds in excess of 95% yield (Scheme I). The sequence, of course, demands the appropriate secondary N-benzylated amine. In an earlier paper, we described the preparation of N^4-benzylspermidine as a reagent for selectively di-N^1,N^8-acylating spermidine.[14] We have now extended this concept to two other amines, N^5-benzylhomospermidine and N,N-bis(3-aminopropyl)-benzylamine. The synthesis of each of these additional reagents can be accomplished in two steps (Scheme II). The latter N-benzyl-protected amine is generated in 64% overall yield by first dicyanoethylation of benzylamine followed by reduction of the dinitrile with lithium aluminum hydride in the presence of aluminum chloride in diethyl ether. The dicyanoethylation is effected in 95% yield by reacting benzylamine with a 3 molar excess of acrylonitrile in the presence of hydroquinone in a sealed tube at 140 °C for 7 days. In the absence of the quinone, the yields are typically around 15–30% with enormous amounts of polymer side products. The N^5-benzylhomospermidine is also prepared in two steps. Benzylamine is dialkylated in 70% yield with 4-chlorobutyronitrile in butanol in the presence of K_2CO_3 at 115 °C for 22 h. This dinitrile was also reduced with lithium aluminum hydride in the presence of aluminum chloride. Each of the secondary N-benzylated amines is now condensed with 2,3-dimethoxybenzoyl chloride in methylene chloride in the presence of triethylamine to produce the corresponding bis(catecholamides) in excess of 95% yield. The acylations are run at room temperature for 20 h in the presence of a slight molar excess of 2,3-dimethoxybenzoyl chloride. The excess acyl chloride is removed by adding 3-(dimethylamino)propylamine to the reaction mixture

Scheme III. Synthesis of N-(2,3-Dimethoxybenzoyl) Amino Acids[a]



[a] The value of n = 1–3.

prior to the acid wash.[15] These catecholamides are next quantitatively debenzylated in acetic acid over $PdCl_2$, and the resulting N-terminal 2,3-dimethoxybenzamides are either demethylated with BBr_3 in methylene chloride or first acylated at the secondary N-position and the products then demethylated.

Secondary N-Acylation and Demethylation

The secondary nitrogens of the catecholamides described above were acylated with either 2-hydroxyhippuric acid, 2,3-dimethoxybenzoylglycine, N-(2,3-dimethoxybenzoyl)-4-aminobutyric acid, or N-(2,3-dimethoxybenzoyl)-β-alanine. While 2-hydroxyhippuric acid is commercially available, the latter three acids had to be synthesized.

All three of the secondary-nitrogen acylating agents were generated from the N-hydroxysuccinimidyl active ester of 2,3-dimethoxybenzoic acid and the appropriate amino acid

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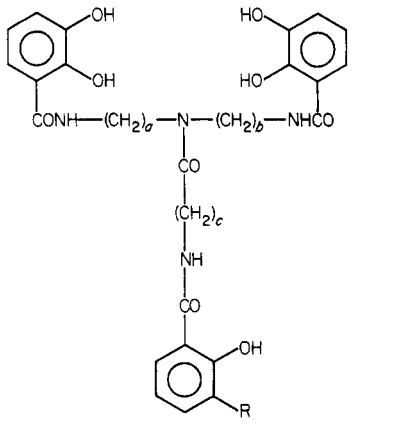
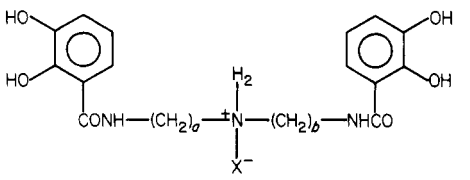
Chart II. Hexacoordinate Catecholamides Generated via Synthesis Indicated in Scheme I



	a	b	c	R
14	3	4	1	H
15	3	3	1	H
16	4	4	1	H
23	3	4	1	OH
24	3	4	2	OH
25	3	4	3	OH



	a	b
26	3	3
27	3	4
28	4	4

(Scheme III). The active ester of 2,3-dimethoxybenzoic acid was generated in dioxane at 15 °C by reacting the acid with *N*-hydroxysuccinimide in the presence of DCC.[16] The dicyclohexylurea was removed by filtration and an aqueous bicarbonate solution of the amino acid added to the filtrate. The resulting mixture was then reacted at room temperature for 27 h, and the products were isolated. Following this procedure, *N*-(2,3-dimethoxybenzoyl)-glycine, *N*-(2,3-dimethoxybenzoyl)-4-aminobutyric acid, and *N*-(2,3-dimethoxybenzoyl)-β-alanine were generated in 91%, 84%, and 82% yields, respectively. Again, with DCC these acids were condensed with the secondary nitrogen of the terminally diacylated amines. Only 2-hydroxyhippuric was activated with trifluoroacetic anhydride. We have now shown that when 2-hydroxyhippuric acid is reacted with trifluoroacetic acid the active acylating agent is 2-(2-trifluoroacetoxyphenyl)-5-oxazolone not the open anhydride. However it is likely this compound is generated through the anhydride. All of the condensations proceeded with yields in excess of 80%. Finally, each of the products was demethylated in nearly quantitative yield with BBr_3 in methylene chloride (Scheme I), and the resulting catecholamides purified by chromatography on Sephadex LH-20.

Discussion

In considering the design of a general synthesis for potential therapeutic acyclic polyamine catecholamide iron ligands, we considered Raymond's stereochemical studies rather closely. He points out that the enterobactin iron-(III) complex has the catechols disposed about the metal in a distorted octahedron. Our synthesis was designed to allow the chelating functionality to adopt a geometry similar to the distorted octahedron described for the enterobactin analogue. Simple CPK model building and bond distance calculations suggested that the compounds synthesized in this paper can adopt the necessary geometry. This is not to say that they are the best possible ligands; however, because of the flexibility of the synthesis, the ligand can be easily modified until the desired sequestering agent is obtained. What will, of course, be interesting is the significance of the variously sized methylene bridges in chelation, i.e., their effect on the entropy component of binding.

The synthesis offers the opportunity of varying both the distance between chelation sites as well as the number and kind of sites. It is not limited to the triamines described, as almost any combination of acrylonitrile and/or halo nitriles can be used in the dialkylation of benzylamine. This means the distances between sites are as variable as the starting materials are accessible. Furthermore, the secondary N-benzylated amines described are all stable and produced in high yield. The condensation of each of these *N*-benzyl amines with 2,3-dimethoxybenzoyl chloride proceeds in high yield, and the products are easily isolated on silica gel.

Although it is possible to terminally N-acylate the simple triamines themselves by employing bulky, sterically hindered acylating agents, this represents a very specific solution to the problem. The most general solution is one which employs a secondary N-protected triamine and does not set any boundary conditions on the acylating agents. The only restriction with the *N*-benzyl polyamines described is that the terminal nitrogens, of course, end up with the same acyl groups. However, the secondary nitrogen, once debenzylated, can be acylated with other kinds of acyl groups. This means that any single ligand can have two different chelating sites at varying distances or three identical sites at varied distances from each other. The systems then can be tailored to the metal. In fact, at the writing of this paper, we have extended the synthesis to octacoordinate ligands simply by annealing two of the bis-N-terminal 2,3-dimethoxybenzoylated amino amides with glutaryl dichloride.

Conclusion

The synthesis described represents a high-yield method for the generation of tetracoordinate, hexacoordinate, and octacoordinate acyclic catecholamide ligands. With the ability to easily vary both the distance between the ligands' sites as well as the nature of the sites, the ligands can be tailored to a variety of metals.

Experimental Section

Materials. All reagents were purchased from Aldrich Chemical Co., and, except where noted, used without further purification. Na_2SO_4 was used as a drying agent unless otherwise specified. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Boiling points are also uncorrected. ^1H NMR samples were prepared in $DCCl_3$ with chemical shifts given in parts per million downfield from an internal Me_4Si standard unless otherwise indicated. The spectra were recorded on a Varian T-60 and/or a JOEL FX-100 spectrometer. The infrared spectra were recorded on Perkin-Elmer 257 and Beckman 4210 spectrophotometers; liquid samples were run neat on salt plates while solids were prepared in KBr. Elemental analyses were performed by Galbraith Laboratories. Preparative thin-layer chromatography was done on Analtech 20 × 20 cm silica gel GF plates.

***N,N*-Bis(3-cyanopropyl)benzylamine (1).** Compound 1 was prepared as previously described.[19]

(16) Van Brussel, W.; Van Sumere, C. F. *Bull. Soc. Chim. Belg.* 1978, *87*, 791.

N^5-Benzylhomospermidine (2). Compound 2 was prepared as previously described.[19]

N,N-Bis(2-cyanoethyl)benzylamine (3). Benzylamine (107.15 g, 1.0 mol), acrylonitrile (150.2 g, 3.00 mol), and hydroquinone (3.0 g, 27.2 mmol) were added to a 2-L Carius tube which was sealed and heated at 140 °C for 7 days. Subsequent distillation provided 202.62 g (95%) of the desired product: bp 190 °C (0.05 mm); ^{1}H NMR δ 2.13–2.53 (m, 4 H), 2.57–3.00 (m, 4 H), 3.60 (s, 2 H), 7.13 (s, 5 H). Anal. Calcd for $C_{13}H_{15}N_3$: C, 73.21; H, 7.09; N, 19.70. Found: C, 73.29; H, 6.94; H, 19.65.

N,N-Bis(3-aminopropyl)benzylamine (4) was prepared and purified as described for 2: yield 4.33 g (67%); bp 110 °C (0.025 mm); ^{1}H NMR δ 1.00 (s, 4 H), 1.25–1.84 (m, 4 H), 2.30–2.84 (m, 8 H), 3.45 (s, 2 H), 7.15 (s, 5 H). Anal. Calcd for $C_{13}H_{23}N_3$: C, 70.54; H, 10.47; N, 18.98. Found: C, 70.37; H, 10.56; N, 18.96.

N^4-Benzyl-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine Hydrochloride (5). A solution of N^4-benzylspermidine (8.60 g, 36.5 mmol) and triethylamine (8.87 g, 87.7 mmol) in 500 mL of CH_2Cl_2 was stirred at 0 °C under N_2. Dropwise addition of 2,3-dimethoxybenzoyl chloride[17] (15.39 g, 76.7 mmol) in 100 mL of CH_2Cl_2 was completed over 1 h and the reaction mixture allowed to warm slowly to room temperature. After 18 h, the reaction mixture was cooled to 0 °C, and 3-(dimethylamino)-propylamine[14] (10 mL, 79.5 mmol) in 100 mL of CH_2Cl_2 was added slowly. After the mixture was stirred for 2 h, the reaction vessel was again cooled to 0 °C, 150 mL ice-cold 3 N HCl added, and the mixture stirred an additional 15 min. The organic phase was then washed with ice-cold 3 N HCl (3 × 100 mL), dried, filtered, and evaporated to yield 21.45 g (98%) of product, a white hydroscopic solid.

An analytical sample was dissolved in MeOH and NaOMe added to obtain pH 11. After being stirred 30 min, the mixture was reduced in vacuo. The resulting solid was redissolved in CH_2Cl_2, washed with water, dried, filtered, and evaporated. Silica gel chromatography (10% MeOH/CH_2Cl_2) of the resulting amine gave a tan oil: ^{1}H NMR δ 1.32–1.98 (m, 6 H), 2.22–2.68 (m, 4 H), 3.13–3.82 (m, 6 H), 3.83 (s, 12 H), 6.78–8.05 (m, 13 H); IR ($CHCl_3$) 3390, 2920, 1650, 1305, 1075 cm^{-1}. Anal. Calcd for $C_{32}H_{41}N_3O_6 \cdot H_2O$: C, 66.07; H, 7.45; N, 7.22. Found: C, 66.30; H, 7.18; N, 7.19.

N^4-Benzyl-N^1,N^7-bis(2,3-dimethoxybenzoyl)bis(3-aminopropyl)amine hydrochloride (6) was prepared and purified in the same manner as for 5: 97% yield; ^{1}H NMR δ 1.55–2.03 (m, 4 H), 2.27–2.72 (m, 4 H), 3.22–3.68 (m, 6 H), 3.67 (s, 6 H), 3.72 (s, 6 H), 6.77–7.95 (m, 13 H); IR ($CHCl_3$) 3390, 2940, 1660, 1305, 1305, 1050 cm^{-1}. Anal. Calcd for $C_{31}H_{39}N_3O_6$: C, 67.74; H, 7.15; N, 7.64. Found: C, 67.64; H, 7.20; N, 7.47.

N^5-Benzyl-N^1,N^9-bis(2,3-dimethoxybenzoyl)bis(4-aminobutyl)amine hydrochloride (7) was prepared and purified in the same manner as for 5: 97% yield; ^{1}H NMR δ 1.48–1.85 (m, 8 H), 2.20–2.65 (m, 4 H), 3.08–3.63 (m, 6 H), 3.77 (s, 12 H), 6.78–8.02 (m, 13 H); IR ($CHCl_3$) 3380, 2940, 1675, 1300, 1085 cm^{-1}. Anal. Calcd for $C_{33}H_{43}N_3O_6$: C, 68.61; H, 7.50; N, 7.27. Found: C, 68.43; H, 7.39; N, 7.12.

N^1,N^8-Bis(2,3-dimethoxybenzoyl)spermidine (8). To a solution of 5 (7.31 g, 12.2 mmol) in 50 mL of glacial acetic acid was added $PdCl_2$ (0.5 g, 2.8 mmol). The reaction was stirred at room temperature until hydrogen was no longer taken up. The reaction mixture was then filtered and evaporated, and the residue was dissolved in 50 mL of MeOH. The solution was adjusted to pH 11 with NaOMe. After being stirred 30 min, the mixture was reduced in vacuo. The resulting solid was redissolved in 100 mL of CH_2Cl_2, washed with cold water (2 × 50 mL), dried, filtered, and evaporated to give 5.65 g (98% yield) of the desired product as a light tan oil. An analytical sample was purified by silica gel chromatography (10% MeOH/CH_2Cl_2): ^{1}H NMR δ 1.38–2.02 (m, 7 H), 2.45–2.87 (m, 4 H), 3.18–3.70 (m, 4 H), 3.83 (s, 12 H), δ 6.78–8.37 (m, 8 H); IR ($CHCl_3$) 3390, 3010, 1645, 1525, 1260 cm^{-1}. Anal. Calcd for $C_{25}H_{35}N_3O_6$: C, 63.41; H, 7.45; N, 8.87. Found: C, 63.55; H, 7.33; N, 8.89.

N^1,N^7-Bis(2,3-dimethoxybenzoyl)bis(3-aminopropyl)amine (9) was prepared and purified in the same manner as for 8: 98% yield; ^{1}H NMR δ 1.63–2.12 (m, 4 H), 2.53–2.95 (m, 4 H), 3.27–3.83 (m, 5 H), 3.85 (s, 12 H), 6.90–8.25 (m, 8 H); IR ($CHCl_3$) 3390, 3020, 1655, 1515, 1260 cm^{-1}. Anal. Calcd for $C_{24}H_{33}N_3O_6 \cdot H_2O$: C, 60.36; H, 7.39; N, 8.80. Found: C, 60.10; H, 7.29; N, 8.56.

N^1,N^9-Bis(2,3-dimethoxybenzoyl)bis(4-aminobutyl)amine (10) was prepared and purified in the same manner as for 8: 99% yield; ^{1}H NMR δ 1.30–1.87 (m, 8 H), 2.30 (s, 1 H), 2.48–2.82 (m, 4 H), 3.10–3.60 (m, 4 H), 3.80 (s, 12 H), 6.73–8.33 (m, 8 H); IR ($CHCl_3$) 3385, 3000, 1655, 1525, 1275 cm^{-1}. Anal. Calcd for $C_{26}H_{37}N_3O_6$: C, 64.31; H, 7.27; N, 8.65. Found: C, 64.46; H, 7.20; N, 8.86.

N^4-[N-(2-Hydroxybenzoyl)glycyl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (11). Trifluoroacetic anhydride (5.17 g, 24.6 mmol) was added to a suspension of 2-hydroxyhippuric acid (1.53 g, 7.9 mmol) in 35 mL of CH_2Cl_2, and the resulting mixture was stirred under N_2 for 2 h. The solution was evaporated in vacuo and the 2-(2-trifluoroacetoxyphenyl)-5-oxazolone redissolved in 35 mL of CH_2Cl_2. After the mixture was cooled to −78 °C, triethylamine (2.0 g, 19.8 mmol) in 20 mL of CH_2Cl_2 was added followed by the dropwise addition of 8 (3.10 g, 6.5 mmol) in 25 mL of CH_2Cl_2. The mixture was allowed to warm slowly to room temperature. After 40 h, the reaction vessel was cooled to 0 °C, and the mixture was washed with ice-cold 3% (w/v) aqueous HCl (3 × 30 mL), dried, filtered, and evaporated. The residue was dissolved in 100 mL of MeOH and the pH adjusted to 9 with NaOMe under N_2. After the mixture was stirred 30 min, methanolic HCl was added at 0 °C to yield a pH of 3, and the solution was reduced in vacuo. The residue was redissolved in 100 mL of CH_2Cl_2, washed with cold H_2O (2 × 75 mL), dried, filtered, and evaporated. The residue was chromatographed on silica gel (5% MeOH/EtOAc), yielding 4.05 g (95% yield) of the product as a white solid: ^{1}H NMR δ 1.33–2.13 (m, 6 H), 3.08–3.70 (m, 8 H), 3.71–4.03 (m, 12 H), 4.03–4.32 (d, 2 H), 6.48–8.42 (m, 13 H), 12.12 (s, 1 H); IR (KBr) 3420, 1640, 1245, 1060, 750 cm^{-1}. Anal. Calcd for $C_{34}H_{42}N_4O_9$: C, 62.76; H, 6.51; N, 8.61. Found: C, 62.62; H, 6.62; N, 8.55.

N^4-[N-(2-Hydroxybenzoyl)glycyl]-N^1,N^7-bis(2,3-dimethoxybenzoyl)bis(3-aminopropyl)amine (12) was prepared and purified in the same manner as for 11: 93% yield; ^{1}H NMR δ 1.53–2.27 (m, 4 H), 3.10–3.77 (m, 8 H), 3.87 (d, 12 H), 4.17 (s, 2 H), 6.53–8.40 (m, 13 H), 12.12 (s, 1 H); IR (KBr) 3420, 1640, 1255, 1060, 780 cm^{-1}. Anal. Calcd for $C_{33}H_{40}N_4O_9$: C, 62.25; H, 6.33; N, 8.80. Found: C, 62.06; H, 6.41; N, 8.70.

N^4-[N-(2-Hydroxybenzoyl)glycyl]-N^1,N^9-bis(2,3-dimethoxybenzoyl)bis(4-aminobutyl)amine (13) was prepared and purified in the same manner as for 11: 95% yield; ^{1}H NMR δ 1.40–1.93 (m, 8 H), 3.03–3.67 (m, 8 H), 3.80 (s, 12 H), 4.13 (s, 2 H), 6.60–8.10 (m, 13 H), 12.15 (s, 1 H); IR (KBr) 3430, 1640, 1235, 1075, 750 cm^{-1}. Anal. Calcd for $C_{35}H_{44}N_4O_9$: C, 63.24; H, 6.67; N, 8.43. Found: C, 63.16; H, 6.72; N, 8.39.

N^4-[N-(2-hydroxybenzoyl)glycyl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (14). To a solution of BBr_3 (17.0 g, 67.9 mmol) in 75 mL of CH_2Cl_2 at 0 °C was added 11 (3.41 g, 5.2 mmol) in 75 mL of CH_2Cl_2 dropwise under N_2. The reaction mixture was allowed to warm slowly to room temperature. After 18 h, the reaction vessel was cooled to 0 °C, and 75 mL of ice-cold H_2O was added slowly with vigorous stirring. After continued stirring for 2 h, the crude product was collected by filtration and washed thoroughly with water. The resulting solid was dissolved in MeOH and evaporated, the process repeated several times, yielding 3.02 g (97% yield) of product.

An analytical sample was preadsorbed on Sephadex LH-20, and eluted with an ethanol/benzene gradient (5–50% v/v); spectral properties were identical with those reported in the literature.[18]

N^4-[N-(2-Hydroxybenzoyl)glycyl]-N^1,N^7-bis(2,3-dihydroxybenzoyl)bis(3-aminopropyl)amine (15) was prepared and purified in the same manner as for 14: 95% yield; ^{1}H NMR (acetone-d_6) δ 1.40–2.22 (m, 6 H), 3.27–4.24 (m, 6 H), 4.40–4.86 (s, 2 H), 6.42–8.19 (m, 13 H); IR (KBr) 3375, 1635, 1545, 1280, 790 cm^{-1}. Anal. Calcd for $C_{29}H_{32}N_4O_9$: C, 59.99; H, 5.56; N, 9.65. Found: C, 60.02; H, 5.76.

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N^4-[N-(2-Hydroxybenzoyl)glycyl]-N^1,N^9-bis(2,3-dihydroxybenzoyl)bis(4-aminobutyl)amine (16) was prepared and purified in the same manner as for 14: 94% yield; ^{1}H NMR (acetone-d_6) δ 1.41–2.20 (m, 8 H), 3.25–4.25 (m, 8 H), 4.45–4.87 (s, 2 H), 6.41–8.25 (m, 13 H); IR (KBr) 3380, 1640, 1545, 1275, 780 cm^{-1}. Anal. Calcd for $C_{31}H_{36}N_4O_9$: C, 61.18; H, 5.96; N, 9.21. Found: C, 60.85; H, 6.06; N, 9.05.

N-(2,3-Dimethoxybenzoyl)glycine (17). By use of the procedure of Van Brussel and Van Sumere,[16] a mixture of 2,3-dimethoxybenzoic acid (2.21 g, 12.1 mmol) and N-hydroxysuccinimide (1.68 g, 14.6 mmol) in 35 mL of dioxane was stirred at 15 °C under N_2. Dropwise addition of DCC (3.02 g, 14.6 mmol) in 30 mL of dioxane was completed over 30 min, and the reaction allowed to warm slowly to room temperature. After 13 h, the reaction mixture was filtered and the precipitate washed with 30 mL of cold dioxane. A mixture of glycine (1.21 g, 16.1 mmol) and sodium bicarbonate (1.35 g, 16.1 mmol) in 50 mL of H_2O was added to the filtrate and the resulting mixture stirred at room temperature. After 27 h, the solvent was reduced to one-third its original volume in vacuo. Concentrated HCl was added at 0 °C to a pH of 2, and the resulting solid was collected by filtration. Recrystallization from H_2O yielded 2.64 g (91% yield) of the desired product: mp 134.0–134.5 °C; ^{1}H NMR (CD_3OD) δ 3.83 (s, 3 H), 3.88 (s, 3 H), 4.10 (s, 2 H), 6.95–7.63 (m, 3 H); IR (KBr) 3345, 2960, 1719, 1637, 1575 cm^{-1}.

Anal. Calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.89. Found: C, 55.40; H, 5.61; N, 5.81.

N-(2,3-Dimethoxybenzoyl)-β-alanine (18) was prepared and purified as described for 17: 2.6 g (82%); mp 104–105 °C (EtOAc/petroleum ether); ^{1}H NMR δ 2.65 (t, 2 H), 3.63 (m, 2 H), 3.82 (s, 6 H), 6.87–7.20 (m, 2 H), 7.40–7.67 (dd, 1 H), 7.75–8.02 (m, 1 H), 9.65 (s, 1 H); IR (KBr) 3360, 2960, 1735, 1650, 1557 cm^{-1}. Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.02; H, 6.08; N, 5.49.

4-[(2,3-Dimethoxybenzoyl)amino]butyric acid (19) was prepared and purified as described previously for 17: 1.3 g (84%); mp 71.5–72.5 ($CHCl_3$/petroleum ether); ^{1}H NMR 1.90 (m, 2 H), 2.43 (t, 2 H), 3.45 (q, 2 H), 3.82 (s, 6 H), 6.85–7.10 (m, 2 H), 7.45–7.70 (dd, 1 H), 7.77–8.03 (m, 1 H), 8.58 (br s, 1 H); IR (KBr) 3345, 3960, 1719, 1637, 1575 cm^{-1}. Anal. Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.58; H, 6.43; N, 5.22.

N^4-[4-[(2,3-Dimethoxybenzoyl)amino]butyryl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (22). To a solution of 8 (650 mg, 1.4 mmol) and 19 (345 mg, 1.3 mmol) in 15 mL of dry methylene chloride under N_2 was added DCC (270 mg, 1.3 mmol) in 10 mL of dry $MeCl_2$. The course of the reaction was monitored by TLC (10% MeOH/$CHCl_3$), and, subsequentially, Et_3N (45 μL) was added to effect complete deprotonation of the amine. After 48 h the DCU precipitate was filtered and washed with methylene chloride, and the filtrates were concentrated to afford 1.1 g of crude product as a yellow solid. Further purification was effected by preparative TLC using a gradient system of $CHCl_3$/benzene/MeOH (10:10:1 → 10:10:2) to afford 755 mg (80%) of product as a puffy light yellow hygroscopic solid: ^{1}H NMR δ 1.60 (m, 6 H), 1.92 (m, 2 H), 2.40 (m, 2 H), 3.07–3.73 (m, 10 H), 3.85 (s, 18 H), 6.85–7.17 (s, 6 H), 6.43–7.70 (dd, 3 H), 7.77–8.18 (m, 3 H); IR (KBr) 3350, 1645, 1275, 1015, 765 cm^{-1}. Anal. Calcd for $C_{38}H_{50}N_4O_{10}$: C, 63.14; H, 6.97; N, 7.75. Found: C, 63.17; H, 7.03; N, 7.77.

N^4-[N-(2,3-Dimethoxybenzoyl)glycyl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (20) was prepared and purified in the same manner as for 22: 80% yield; ^{1}H NMR δ 1.43–2.20 (m, 6 H), 3.10–3.68 (m, 8 H), 3.83 (m, 18 H), 4.23 (d, 2 H), 6.77–9.17 (m, 12 H); IR ($CHCl_3$) 3010, 1630, 1453, 1255, 987 cm^{-1}. Anal. Calcd for $C_{36}H_{46}N_4O_{10}$: C, 62.23; H, 6.67; N, 8.06. Found: C, 62.09; H, 6.67; N, 7.96.

N^4-[N-(2,3-Dimethoxybenzoyl)-β-alanyl]-N^1,N^8-bis(2,3-dimethoxybenzoyl)spermidine (21) was prepared and purified as described previously for 22: 660 mg (78%); ^{1}H NMR 1.62 (br, 6 H), 2.63 (t, 2 H), 3.02–3.82 (br, 10 H), 3.83 (s, 18 H), 6.75–7.12 (m, 6 H), 7.5 (dd, 3 H), 7.76–8.73 (br, 3 H); IR 3375, 1650, 1255, 1025, 765 cm^{-1}. Anal. Calcd for $C_{37}H_{48}N_4O_{10}$: C, 62.70; H, 6.83; N, 7.90. Found: C, 62.57; H, 7.00; N, 7.87.

N^4-[4-[(2,3-Dihydroxybenzoyl)amino]butyryl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (25). A 1 M boron tribromide solution (12.5 mL, 12.5 mmol) was added to an additional 15 mL of dry CH_2Cl_2 under N_2 and cooled (ice bath). To this was added slowly a solution of 22 (600 mg, 83 mmol) in 25 mL of dry CH_2Cl_2. After the addition was completed, the ice bath was removed and the resulting suspension stirred for an additional 20 h, at which time hydrolysis was effected by cooling the reaction mixture to 0 °C and slowly adding 25 mL of H_2O. The ice bath was once again removed and the suspension stirred for 2 h. The reaction mixture was then filtered and the crude product washed alternately with $MeCl_2$ and H_2O several times. The product was then dissolved in degassed MeOH (25 mL) and concentrated. This process was repeated twice more and then with acetone (twice) to afford 490 mg (92% crude) of the desired compound as a light brown solid.

An analytical sample was prepared on a short silica gel column by eluting with degassed EtOAC: ^{1}H NMR (acetone-d_6) δ 1.67 (m, 6 H), 1.95–2.33 (m, part obscured by solvent peak), 2.40–2.73 (m, 2 H), 3.12–3.70 (m, 10 H), 6.40–7.37 (m, 9 H), 7.70–8.70 (br, 3 H), 13.10 (br, 3 H); IR 3420, 3280, 1652, 1605, 1555, 1277 cm^{-1}. Anal. Calcd for $C_{32}H_{38}N_4O_{10}\cdot H_2O$: C, 58.53; H, 6.14. Found: C, 58.40; H, 6.10.

N^4-[N-(2,3-Dihydroxybenzoyl)glycyl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (23) was prepared and purified in the same manner as for 25: 94% yield; ^{1}H NMR (acetone-d_6) δ 1.47–2.20 (m, 6 H), 3.19–4.30 (m, 8 H), 4.25–4.81 (s, 2 H), 6.42–8.31 (m, 12 H); IR (KBr) 3370, 1635, 1270, 790 cm^{-1}. Anal. Calcd for $C_{30}H_{34}N_4O_{10}$: C, 59.01; H, 5.61; N, 9.18. Found: C, 58.90; H, 5.83; N, 9.01.

N^4-[N-(2,3Dihydroxybenzoyl)-β-alanyl]-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (24) was prepared and purified as described previously for 25: 400 mg (94%); ^{1}H NMR (acetone-d_6) δ 1.70 (m, 6 H), 2.67 (m, 2 H), 3.10–3.74 (m, 13 H), 6.47–7.38 (m, 9 H), 7.76–8.68 (br, 3 H), 13.04 (br, 3 H); IR 3420, 3290, 1655, 1610, 1550, 1275 cm^{-1}. Anal. Calcd for $C_{31}H_{36}N_4O_{10}\cdot H_2O$: C, 57.93; H, 5.96. Found: C, 58.12; H, 6.35.

N^1,N^9-Bis(2,3-dihydroxybenzoyl)bis(4-aminobutyl)amine Hydrobromide (28). To a solution of BBr_3 (6.39 g, 25.5 mmol) in 50 mL of CH_2Cl_2 at 0 °C was added 10 (1.13 g, 2.3 mmol) in 45 mL of CH_2Cl_2 dropwise under N_2. The mixture was allowed to warm slowly to room temperature. After 15 h, the reaction mixture was cooled to 0 °C and 75 mL of ice-cold H_2O added slowly with vigorous stirring. After continued stirring for 2 h, the crude product was collected by filtration and washed well with H_2O and CH_2Cl_2. The resulting solid was crystallized from H_2O to give 1.10 g (93% yield) of the desired product: IR (KBr) 3350, 1635, 1570, 1250 cm^{-1}. Anal. Calcd for $C_{22}H_{30}N_3O_6Br$: C, 51.57; H, 5.90; N, 8.20. Found: C, 51.75; H, 6.01; N, 7.95.

N^1,N^7-Bis(2,3-dihydroxybenzoyl)bis(3-aminopropyl)amine hydrobromide (26) was prepared and purified in the same manner as for 28: 93% yield. Anal. Calcd for $C_{20}H_{20}N_3O_6Br$: C, 49.60; H. 5.41; N, 8.68. Found: C, 49.77; H, 5.40; N, 8.42.

N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine hydrobromide (27) was prepared and purified in the same manner as for 28: 94% yield; characteristics were identical with those reported in the literature.[7]

Registry No. 1, 78217-67-1; 2, 78217-68-2; 3, 782-87-6; 4, 1555-71-1; 5, 78217-69-3; 5 free base, 78217-70-6; 6, 78217-71-7; 6 free base, 78217-72-8; 7, 78217-73-9; 7 free base, 78217-74-0; 8, 78217-75-1; 9, 78217-76-2; 10, 78217-77-3; 11, 78217-78-4; 12, 78217-79-5; 13, 78217-80-8; 14, 76927-62-3; 15, 78217-81-9; 16, 78217-82-0; 17, 78217-83-1; 18, 78217-84-2; 19, 78217-85-3; 20, 78217-86-4; 21, 78217-87-5; 22, 78217-88-6; 23, 78217-89-7; 24, 78217-90-0; 25, 78217-91-1; 26, 78217-92-2; 27, 78217-93-3; 28, 78217-94-4; 4-chlorobutyronitrile, 628-20-6; benzylamine, 100-46-9; N^4-benzylspermidine, 73038-05-8; acrylonitrile, 107-13-1; 2-hydroxyhippuric acid, 487-54-7; [N-[2-(trifluoroacetoxy)benzoyl]glycyl]trifluoroacetic anhydride, 77409-72-4; 2,3-dimethoxybenzoic acid, 1521-38-6; N-hydroxysuccinimide, 6066-82-6.