isoenzymes of PDE from canine ventricular muscle was carried out essentially as described by Hidaka et al.[19] Cyclic nucleotide PDE assays were performed as described by Hidaka et al.[20] The effect of 41 and milrinone on the PDE I and III were tested at a low concentration (0.4 μM) without calmodulin and a stimulating concentration of calcium. The concentration of agents required to inhibit isoenzymes 50% (IC_{50}) is showed in Table III. The selectivity of each drug for the PDE III against PDE I was determined from the ratio of the IC_{50} for PDE I to that for PDE III.

Acknowledgment. We thank the members of the Analytical Department, Central Research Institute, Mitsui Toatsu Chemicals Inc., for the NMR and elemental analyses.

Registry No. 1, 4746-97-8; **2a**, 103319-00-2; **2b**, 103319-07-9; **2c**, 103319-04-6; **2d**, 117960-27-7; **2e**, 67019-51-6; **2f**, 117960-44-8; **2g**, 117960-45-9; **3a**, 103319-01-3; **3b**, 103319-08-0; **3c**, 103319-15-9; **4a**, 117960-46-0; **4b**, 117960-47-1; **4c**, 117960-48-2; **5d**, 117960-52-8; **5e**, 66336-47-8; **5f**, 117960-53-9; **5g**, 117960-54-0; **6**, 556-48-9; **7h**, 18068-06-9; **7i**, 66227-43-8; **8a**, 103319-02-4; **8b**, 103319-09-1; **8c**, 103319-05-7; **8d**, 117960-49-3; **8e**, 5309-16-0; **8f**, 117960-50-6; **8g**, 117960-51-7; **8h**, 13482-23-0; **8i**, 66227-44-9; **9a** (R^1 = CH_3), 103319-03-5; **9b** (R^1 = CH_3), 103319-10-4; **9c** (R^1 = CH_3), 103319-06-8; **9d** (R^1 = CH_3), 117960-55-1; **9e** (R^1 = CH_3), 117960-56-2; **9f** (R^1 = CH_3), 117960-57-3; **9g** (R^1 = CH_3), 117960-58-4; **9h** (R^1 = CH_3), 103319-11-5; **9i** (R^1 = CH_3), 117960-60-8; **9j** (R^1 = CH_3), 103319-13-7; **9** (R^1 = Me, R^5, R^6, R^8 = H, R^7 = Me), 103319-12-6; **9** (R^1 = Me, R^5, R^6, R^8 = H, R^7 = Et), 103319-12-6; **9** (R^1 = Me, R^5, R^6, R^8 = H, R^7 = *t*-Bu), 117960-59-5; **9** (R^1 = Me, R^5, R^6, R^7, R^8 = H), 874-23-7; **10a**, 117960-64-2; **12**, 117960-66-4; **13**, 17012-30-5; **14**, 103318-87-2; **15**, 117960-28-8; **16**, 103318-93-0; **17**, 117960-29-9; **18**, 103318-86-1; **19**, 117960-30-2; **20**, 103318-88-3; **21**, 117960-31-3; **22**, 117960-32-4; **23**, 103318-83-8; **24**, 103318-85-0; **25**, 103318-84-9; **26**, 117960-33-5; **27**, 117960-34-6; **28**, 53661-31-7; **29**, 117960-35-7; **30**, 103318-90-7; **31**, 103318-91-8; **32**, 103318-92-9; **33**, 117960-36-8; **34**, 115883-28-8; **35**, 115883-30-2; **36**, 117960-37-9; **37**, 117960-38-0; **38**, 107189-97-9; **39**, 107190-00-1; **40**, 115883-40-4; **41**, 107189-96-8; **42**, 115883-34-6; **43**, 117960-39-1; **44**, 115883-37-9; **45**, 115883-41-5; **46**, 115883-38-0; **47**, 115883-39-1; **48**, 16232-45-4; **49**, 117960-40-4; **50**, 117960-41-5; **51**, 117960-42-6; **52**, 117960-43-7; 4-bromopyridine, 1120-87-2; 3-bromopyridine, 626-55-1; 2-bromopyridine, 109-04-6; 4-bromo-*N*,*N*-dimethylaniline, 586-77-6; 1-bromo-4-methoxybenzene, 104-92-7; *p*-bromo-*N*,*N*-dimethylbenzylamine, 586-77-6; *p*-(methoxymethyl)bromobenzene, 1515-88-4; *N*-acetylimidazole, 2466-76-4; 2-cyanoacetamide, 107-91-5; 4-methylcyclohexanone, 589-92-4; 4-ethylcyclohexanone, 5441-51-0; 4-*tert*-butylcyclohexanone, 98-53-3; 4-phenylcyclohexanone, 4894-75-1; cyclohexanone, 108-94-1; 2-propanoylcyclohexanone, 32316-46-4; 2-methylcyclohexanone, 583-60-8; 2-acetyl-6-methylcyclohexanone, 78456-49-2; 3-methylcyclohexanone, 591-24-2; 2-acetyl-5-methylcyclohexanone, 14698-76-1; 2-acetyl-3-methylcyclohexanone, 14580-53-1; 3-(4-pyridyl)cyclohexanone, 115444-30-9; 2-acetyl-5-(4-pyridyl)cyclohexanone, 117960-61-9; 2-propanoyl-4-(4-pyridyl)cyclohexanone, 115883-26-6; 2-pentanoyl-4-(4-pyridyl)cyclohexanone, 117960-62-0; 2-(1-hydroxyethyl)-4-(4-pyridyl)cyclohexanone, 117960-63-1; ethyl cyanoacetate, 105-56-6; 3-chloro-4-cyano-1,7-dimethyl-5,6,7,8-tetrahydroisoquinoline, 117960-65-3; acetoacetamide, 5977-14-0; acetaldehyde, 75-07-0.

(19) (a) Hidaka, H.; Yamaki, T.; Ohiai, Y.; Asano, T.; Yamabe, H. *Biochim. Biophys. Acta* **1977**, *484*, 398. (b) Hidaka, H.; Asano, T. *Biochim. Biophys. Acta* **1976**, *429*, 485.
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(21) (a) Basu, U.; Banerjee, B. *Justus Liebigs Ann. Chem.* **1935**, *516*, 243. (b) Ban, Y.; Seo, M. *Chem. Pharm. Bull.* **1964**, *12*, 1296.
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Spermexatin and Spermexatol: New Synthetic Spermidine-Based Siderophore Analogues

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Syntheses of hexanediamine-based dihydroxamate (Hexamate), spermidine-based trihydroxamate (Spermexatins), and spermidine-based mixed siderophore analogues (Spermexatols) are described. Key intermediates include the *N*-hydroxysuccinimide esters of various hydroxamic acids, e.g., malonohydroxamate, succinohydroxamate, and glutarohydroxamate. These intermediates were synthesized, characterized, and incorporated as the ligating chains on spermidine. Also, mixed iron chelating compounds (Spermexatols) with both catechol and hydroxamic acid side chains were synthesized. The reagent carbobenzoxyimidazole was employed to distinguish between the primary and secondary amino groups of spermidine. The ability of these iron chelators to stimulate microbial growth is also described.

Spermidine (1,8-diamino-4-azaoctane), **1**, has been found to be an essential component of a wide variety of naturally occurring biologically active compounds.[1] Its derivatives are of considerable biological interest because of their potent antibiotic[2] and antineoplastic[3] properties. Spermidine-based microbial iron chelators (siderophores), agrobactin and parabactin, are well known and are being studied for their potential uses in the iron chelation therapy of patients suffering from Cooley's anemia[4] and anticancer treatment. These catecholates have been shown to be quite effective in deferration of mammalian cell lines.[5] Since the isolation[6] of agrobactin (**2a**), parabactin (**2b**), and the norspermidine-derived vibriobactin (**2c**), a

[†] University of Notre Dame.
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[§] Recipient of a NIH Career Development Award, 1983–1988.

(1) (a) Ellestad, G. A.; Cosulich, D. B.; Broschard, R. W.; Martia, J. H.; Kunstmann, P.; Morton, G. V.; Lancaster, J. E.; Fulmore, W.; Lovell, F. M. *J. Am. Chem. Soc.* **1978**, *100*, 2515. (b) Mahler, H. R.; Green, G. *Ann. N.Y. Acad. Sci.* **1970**, *171*, 783. (c) Weisner, K.; MacDonald, P. J.; Bankiewicz, C. *J. Am. Chem. Soc.* **1953**, *75*, 6348.
(2) (a) Hlavka, J. J. *J. Antibiot.* **1978**, *31*, 477. (b) Also see ref 1a.
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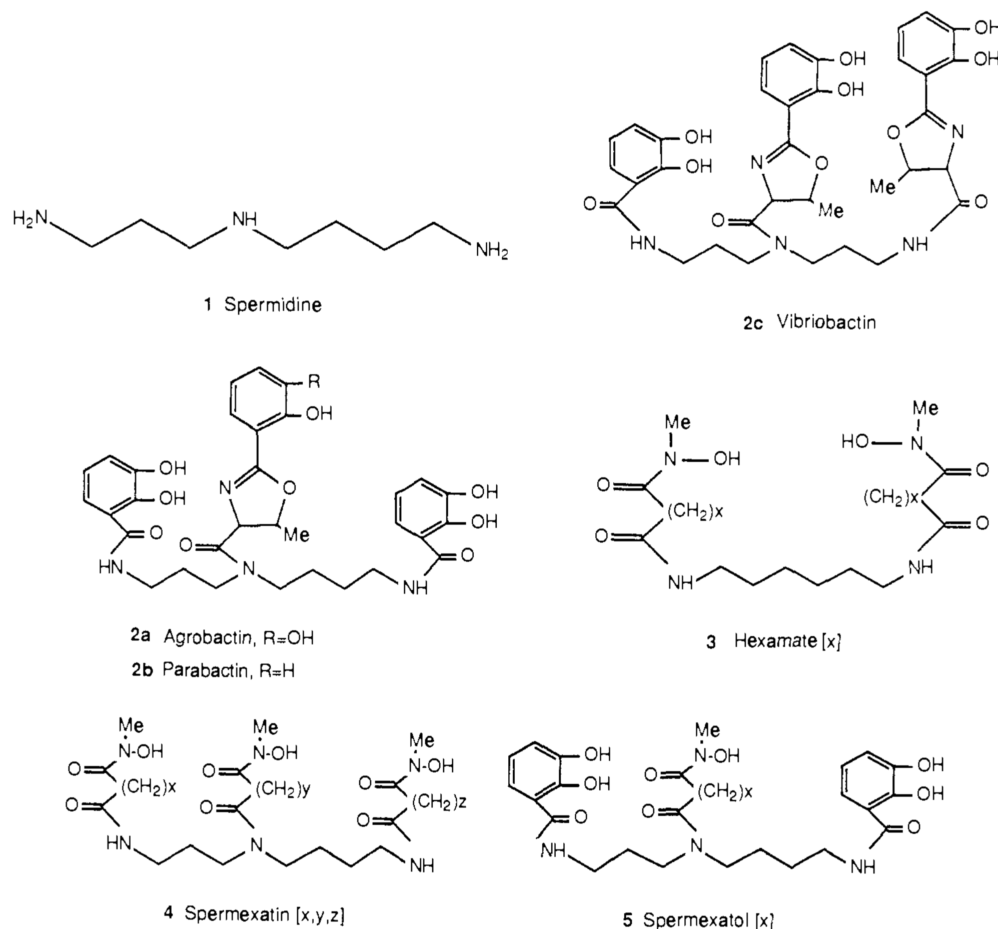
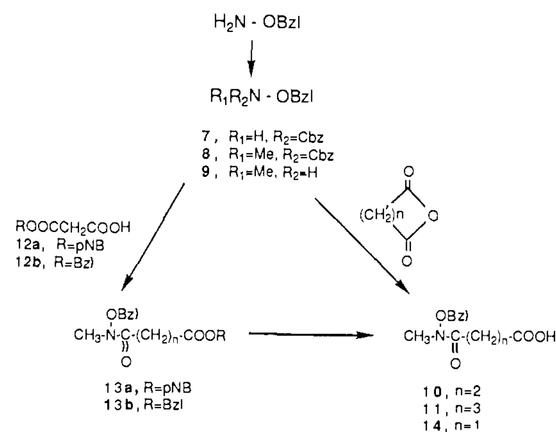


Figure 1.

number of workers have confirmed their structures by NMR studies[6a] and reported their syntheses.[7] All of the naturally occurring spermidine siderophores contain two or more catechol moieties and a heterocyclic component for binding to Fe(III). None of the naturally occurring or synthetic analogues of spermidine-based siderophores are known to contain hydroxamic acid ligands. While the catechols are stronger iron chelators at high pH, the hydroxamates maintain iron complexation more readily at lower physiological pH. Unlike catechols, secondary hydroxamates are less susceptible toward oxidation.[8] Trihydroxamate analogues of rhodotorulic acid have been prepared in our laboratory and have shown potential utility in iron chelation therapy.[9] Here we report the synthesis and biological activity of a hexanediamine-based dihydroxamate (hexamate), **3**, spermidine based trihydroxamates (spermexatins), **4**, and spermidine-based mixed siderophores (spermexatols), **5**[10] (Figure 1).

Scheme I



Chemistry

The selection of the ligating side chains on spermidine was important in order to synthesize the artificial siderophores. A model building study indicated that the ligating side chains chosen here in hexamates, spermexatins, and spermexatols (**3**, **4**, **5**, x, y, z = 1, 2, or 3) would effectively bind to Fe(III) without strain.[11] Thus, hydroxamates **10**, **11**, and **14** were considered to be suitable intermediates for the syntheses of the side chains to be incorporated on spermidine. These ligating side chains all contain a com-

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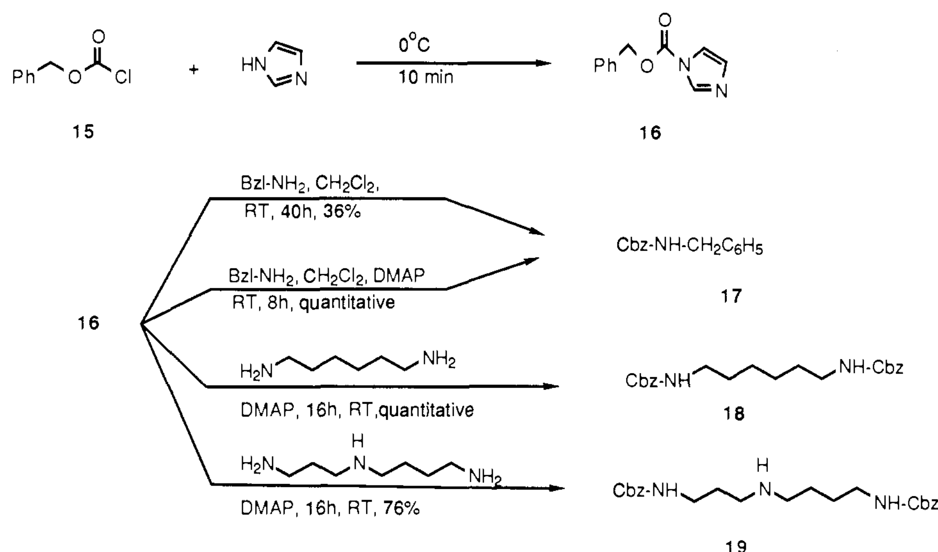
(10) For the nomenclature of the compounds, see Figure 1.
(11) (a) Winkelman, G. *FEBS Lett.* **1979**, *97*, 43. (b) McArdle, J. B.; Sofen, S. R.; Cooper, S. R.; Raymond, K. N. *Inorg. Chem.* **1978**, *17*, 3075.

Scheme II



mon N-methylhydroxylamine component. A suitably protected form (**9**) of the hydroxylamine was prepared from O-benzylhydroxylamine. The amino group of O-benzylhydroxylamine, **6**,[12] was protected by reaction with Cbz-Cl to provide **7** in 93–98% yield (Scheme I). N-Methylation of hydroxamate **7** provided compound **8** in 98% yield. Removal of the Cbz group of **8** with 33% HBr/AcOH provided the key component **9** in 93% yield. The desired hydroxamate side chains **10** and **11** were prepared in 84–94% yields by the treatment of **9** with succinic anhydride and glutaric anhydride, respectively, in refluxing THF. For the preparation of malonohydroxamate **14**, protected hydroxylamine **9** was acylated with malonomonoester **12a** or **12b** with N,N'-carbonyldiimidazole (CDI) to give the corresponding hydroxamate esters **13a** or **13b**. The acid **14** was prepared by the removal of p-nitrobenzyl (pNB) or benzyl (Bzl) groups of **13a** or **13b**, respectively, with aqueous sodium sulfide.[13]

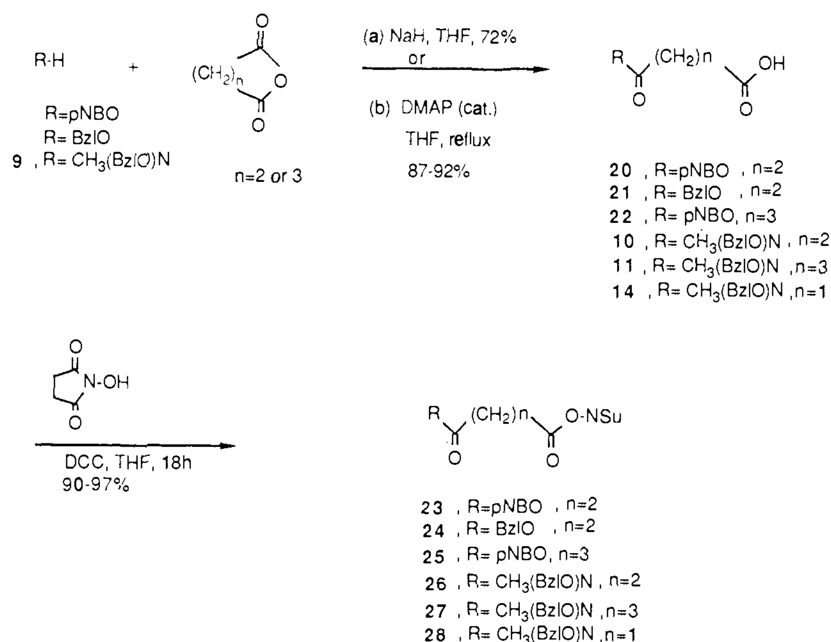
As described later, triacylation of spermidine with acids **10**, **11**, or **14** gave representative spemidine-based trihydroxamates with symmetrical substitution. However, as illustrated by structures **4** and **5**, we also wanted to prepare siderophore analogues with different ligating groups at the central and terminal nitrogens of spermidine. Thus, a synthetic approach was required to distinguish between the primary and the secondary amino groups of spermidine. The less sterically hindered primary amino groups, however, can be acylated selectively.[7a] Ganem and co-workers[14] have reported selective terminal acylation. Fujita and co-workers[7a] protected both primary amino groups of spermidine with a Cbz group with a thiazolidinethione derivative. Selective bisacylation of spermidine and other linear triamines with CDI activated carboxylic acids has also been described.[15] Apparently use of less sterically hindered carboxylic acids results in nonselective acylation of spermidine.

We prepared carbobenzoxyimidazole (Cbz-Im, **16**) in quantitative yield by using Cbz-Cl (**15**) and imidazole.[16] Subsequent model reactions of Cbz-Im indicated that it was a suitable reagent to selectively protect an unhindered primary amino group in the presence of hindered primary and secondary amino groups (Scheme II). The reaction of primary amines and Cbz-Im proceeded in the presence of a catalytic amount of DMAP. Cbz-Im, however, did not afford the corresponding Cbz protected derivative with *sec*-butylamine both in the presence and absence of DMAP. Indeed, with Cbz-Im, the primary amino groups of spermidine were protected by Cbz groups to provide di-Cbz-spermidine (**19**) in good yield. The ready availability of di-Cbz-spermidine (**19**) allowed us to consider incorporation of a variety of acyl groups at the central nitrogen. Thus, N-hydroxysuccinimide esters of several different dicarboxylic acids (**23–28**) were prepared (Scheme III). The precursors of the succinimide esters **10**, **11**, **14**, **20–22**, were prepared in two different ways. In separate reactions, p-nitrobenzyl alcohol (pNB-OH), benzyl alcohol (Bzl-OH) and N-methyl-O-benzylhydroxylamine **9** were treated with succinic anhydride or glutaric anhydride in the presence of NaH (method a) or in the presence of a catalytic amount of DMAP (method b). The latter method provided better yields of the respective products. The carboxylic acids were then converted to the corresponding N-hydroxysuccinimide esters in high yields by treating the acids with N-hydroxysuccinimide and DCC. These active esters were used in the synthesis of spermexatins by either indirect or direct methods as described next.

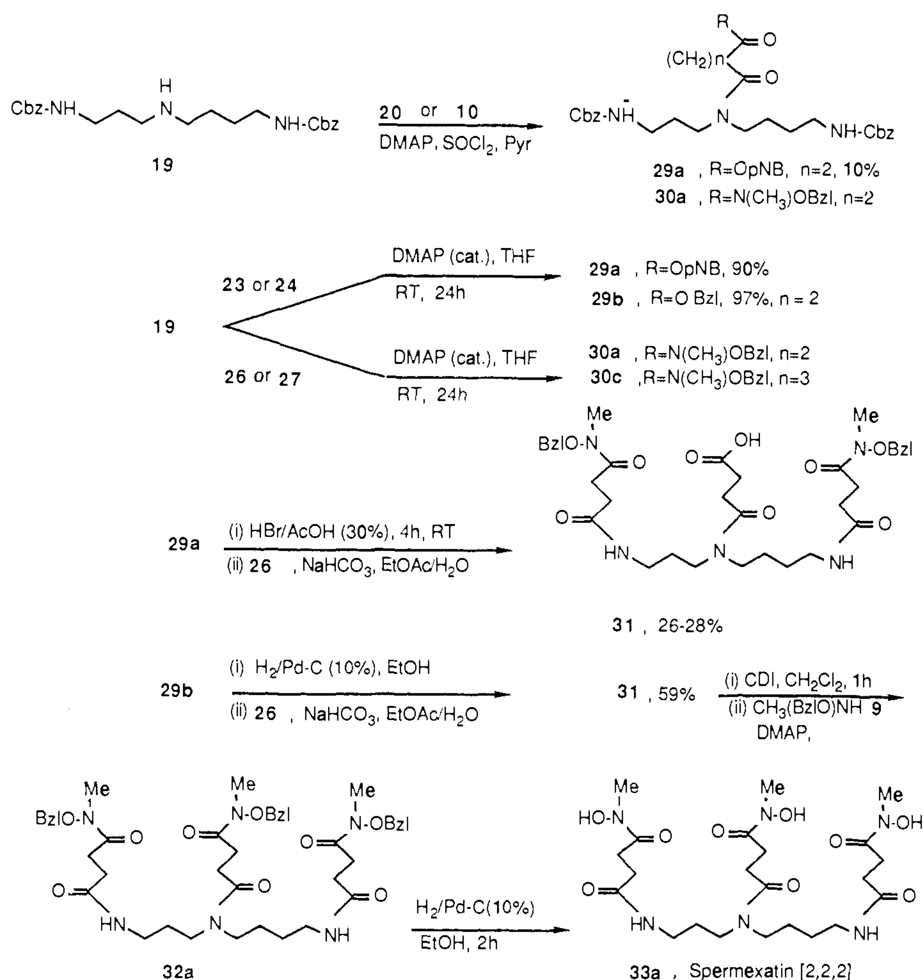
The indirect method (Scheme IV) involved the treatment of diprotected spermidine **19** with acids **20** or **10** in the presence of stoichiometric amounts of $SOCl_2$ and pyridine[17] to give **29a** or **30a**, respectively. However, better results were obtained when **19** was treated with the corresponding N-hydroxysuccinimide esters **23**, **24**, or **26**. Compounds **29a** and **29b** differ from **30a** in that **30a** has a protected hydroxamate side chain at the central nitrogen of spermidine. Compound **30a** was also prepared with different condensing agents from **19** and **10** in rather low yields, i.e. 1,3-dicyclohexylcarbodiimide (DCC) (15%), thiazolidinethione (18%), 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) (17%), and CDI (14%). Analogue **30b** with three methylene units in the side chain was synthesized in a similar manner from active ester **27**. Spermidine derivative **29a** was converted to **31** by two

(12) Compound 6 was prepared as described in Chimiak, A.; Kolasa, T. *Bull. Acad. Polon. Sci.* **1974**, 22, 195.
(13) Lammert, S. R.; Ellis, A. I.; Chauvette, R. R.; Kukolja, S. *J. Org. Chem.* **1978**, *43*, 1243.
(14) (a) McManis, J. S.; Ganem, B. *J. Org. Chem.* **1980**, *45*, 2041. (b) Chantrapromma, K.; Ganem, B. *Tetrahedron Lett.* **1981**, *22*, 23.
(15) Joshua, A. V.; Scott, J. R. *Tetrahedron Lett.* **1984**, *25*, 5725.
(16) Staab, H. A. *Angew. Chem. Int. Ed. Engl.* **1962**, *1*, 351.
(17) Matsuda, F.; Yanagiya, M.; Matsumoto, T. *Tetrahedron Lett.* **1982**, *23*, 4043.

Scheme III



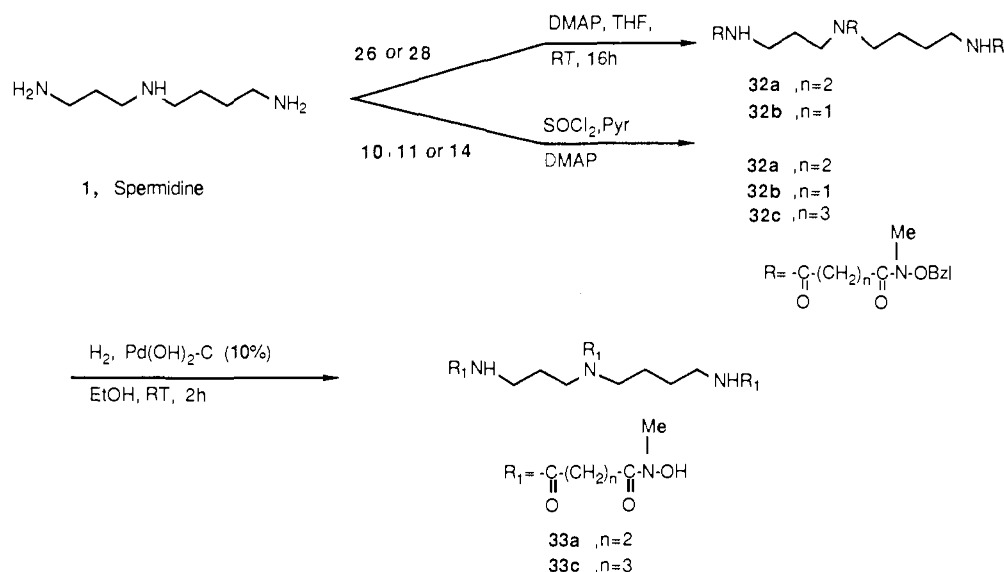
Scheme IV



routes. In the first case, treatment of **29a** with 33% HBr in acetic acid removed both of the Cbz groups and the *p*-nitrobenzyl ester. The resulting crude hydrobromide salt was then treated with active ester **26** in the presence of $NaHCO_3$ to give carboxylic acid **31**. On the other hand, compound **29b**, upon catalytic hydrogenation followed by the treatment with **26**, also provided the same acid **31**. Acid **31** was converted to the corresponding acylimidazole with CDI. Subsequent treatment with hydroxylamine **9** gave protected trihydroxamate **32a** in good yield. Spermexatin [2,2,2] (**33a**) was obtained from **32a** upon catalytic hydrogenation.

Symmetrical spermexatins can also be prepared more directly in just two steps (Scheme V). Spermidine was

Scheme V



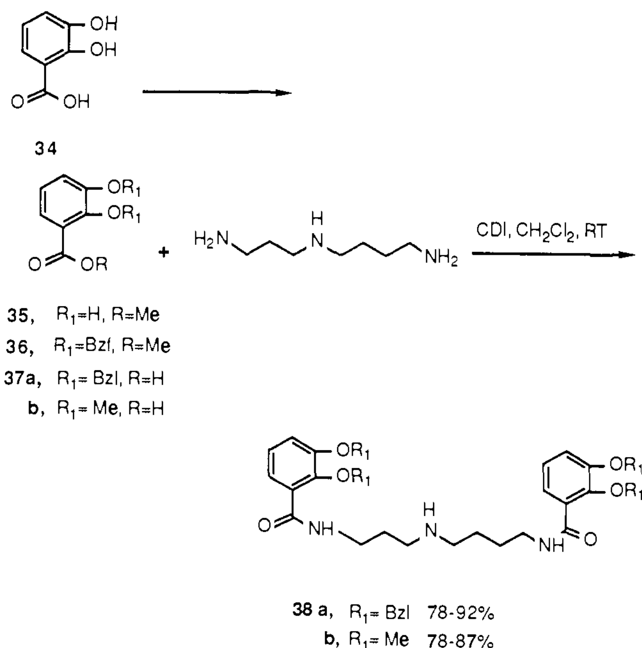
separately treated with active esters **26** and **28** in the presence of a catalytic amount of DMAP to provide triacylated derivatives **32a** and **32b**. Protected spermidine-based trihydroxamates **32a–c** were also prepared from the acid hydroxamates **10**, **11**, and **14** by converting the carboxylic acids to acid chlorides in the presence of $SOCl_2$/pyridine[17] or oxalyl chloride. These acid chlorides were then used to triacylate spermidine to give **32a–c**. The symmetrical spermexatins **33a** and **33c** were obtained upon catalytic hydrogenation in good yields. Although the direct synthetic method employs fewer steps to spermexatins, it lacks the flexibility offered by the indirect route. By use of the indirect route many unsymmetrical spermexatin analogues can be prepared. Potentially one can use the intermediate compounds **30a,b** and vary the ligating side chain lengths such that the chain length on the central nitrogen of spermidine differs from that of the primary amino groups. The carboxylic acid **31** could be an important intermediate for the synthesis of semisynthetic antibiotics.[18]

The analogues with different side chain lengths (see structure **4**, Figure 1) were not prepared. However, their preparation will be of interest in the future.

A new synthetic mixed siderophore, spermexatol [2] (**42**) was also prepared. Spermexatol [2] contains both catechol and hydroxamate ligating side chains. Its synthesis also required preparation of appropriate forms (i.e. **37a,b**) of commercially available 2,3-dihydroxybenzoic acid (**34**). Compound **37a** has been prepared and reported earlier.[6a] However, we employed a different, but simple, method of its preparation. The methyl ester **35** was synthesized from **34** by treatment with $SOCl_2$ in methanol in 87% yield. The hydroxyl groups of **35** were protected as benzyl ethers in 94% yield with benzyl bromide in the presence of anhydrous K_2CO_3 and a catalytic amount of NaI to afford **36**. The resulting methyl ester was saponified with 1 N KOH to provide acid **37a** in 93% yield. The alternative methyl ether protected catechol **37b** was prepared in a

(18) (a) Ohi, N.; Aoki, B.; Shinozaki, T.; Moro, K.; Noto, T.; Nehashi, T.; Okazaki, H.; Matsunaga, I. *J. Antibiot.* **1986**, *39*, 230. (b) Ohi, N.; Aoki, B.; Shinozaki, T.; Moro, K.; Noto, T.; Nehashi, T.; Okazaki, H.; Matsunaga, I. *J. Antibiot.* **1986**, *39*, 242. (c) Watanabe, N.; Nagasu, T.; Katsu, K.; Kitoh, K. *Antimicrob. Agents Chemother.* **1987**, *31*, 497. (d) Sharma, S. K.; Miller, M. J., unpublished.

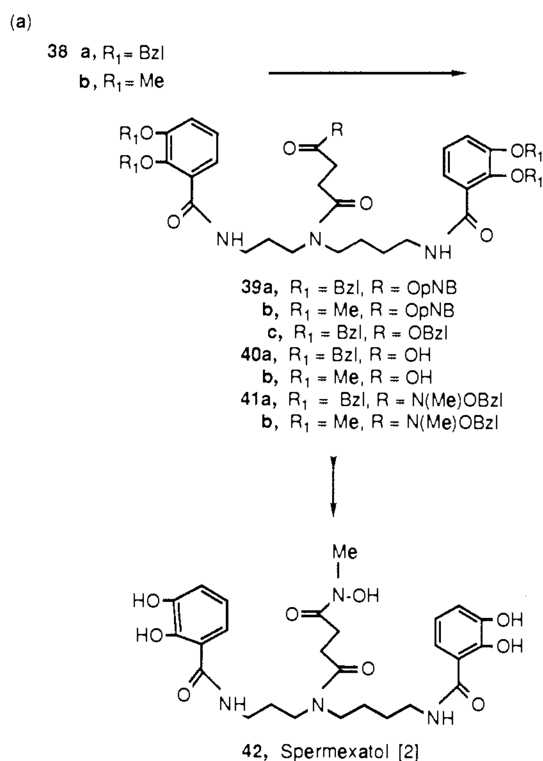
Scheme VI. Synthesis of Diacylated Spermidine

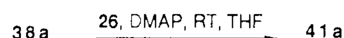


similar manner. Both compounds **37a,b** were important intermediates in the synthesis of spermexatol [2] (Scheme VI). Separate treatment of **37a** and **37b** with commercially available spermidine in the presence of *N,N'*-carbonyldiimidazole (CDI) gave diacylated spermidines **38a** and **38b** in 78–92% yields, respectively.[15]

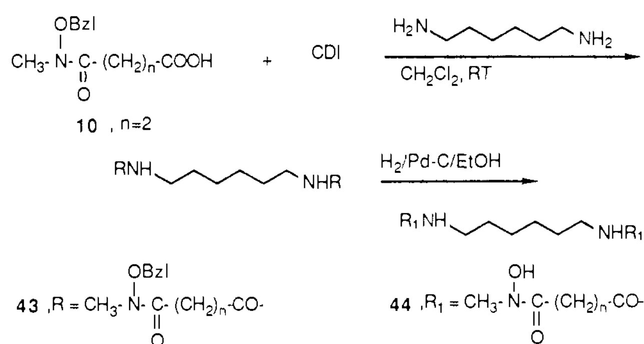
The diacylated spermidines **38a** and **38b** were further acylated at the central nitrogen with the *N*-hydroxysuccinimide esters **23** or **24** and a catalytic amount of DMAP to provide esters **39a–c** in good yields (Scheme VIIa). The pNB esters **39a** and **39b** were hydrolyzed to the corresponding acids upon treatment with Na_2S in moderate yields. The resulting carboxylic acids **40a** and **40b** were then treated with CDI followed by the reaction with hydroxylamine **9** to give protected spermexatol [2] **41a** and **41b**, respectively, in good yields. Finally, spermexatol [2] **42** was prepared from the benzylated precursor **41a** upon catalytic hydrogenation in 92% yield. Although, this route requires many steps to prepare spermexatol [2], like acid **31**, the intermediates **40a,b** can also be used for the synthesis of semisynthetic antibiotics.[18]

Scheme VII





Scheme VIII



Similar to the synthesis of spermexatins via a direct route, benzylated spermexatol [2] **41a** was also prepared in 82% yield from **38a** by treatment with active ester **26** (Scheme VIIb).

Although hexadentate siderophores and analogues are generally more effective iron chelators, the dihydroxamate, rhodotorulic acid, has been shown to be an effective deferration agent[19] and analogues deserve further study.[9,19] Rhodotorulic acid is a dihydroxamate siderophore and has been previously synthesized in our laboratory.[20] We have prepared hexamate [2] as a simple analogue of rhodotorulic acid. Hexamate [2] is a synthetic siderophore analogue with a 1,6-hexanediamine backbone containing two symmetrical hydroxamic acid side chains (Figure 1). Acid **10** was converted to its acylimidazole with CDI and was further treated with 1,6-hexanediamine to provide the diacylated product **43** in 79–95% yield. The benzyl groups of **43** were removed by catalytic hydrogenation to provide hexamate [2], **44**, in high yield (Scheme VIII). Similarily, hexamate [1] and [3] may be prepared from 1,6-hexanediamine and acids **14** and **11**, respectively.

Biological Results and Discussion

The siderophore analogues were tested in a bioassay for their ability to stimulate growth of wild type and siderophore mutants of *Vibrio cholerae* and *Escherichia coli* in a low-iron medium (Table I). The mutant strains Lou1510 and UT2300 are receptor mutants defective in transport of vibriobactin and enterobactin, respectively, and were included to determine whether these receptors were involved in transport of the analogues.

Analogue **33a** was able to substitute for vibriobactin. However, it produced smaller zones of stimulation, which may be due to its lower solubility. This compound did not stimulate growth of the vibriobactin transport mutant, suggesting that it is recognized by the same receptor as vibriobactin. Similarily, spermexatol (**42**) was utilized in a manner similar to enterobactin, stimulating growth of RW ($FepA^+$) but not UT 2300 ($FepA^-$) *E. coli*. Spermexatol also stimulated growth of *V. cholerae* but did not require the vibriobactin receptor.

The trihydroxamate **33c** stimulated growth of all four strains and was independent of a functional catechol receptor for transport. It is likely that this compound is recognized by other siderophore receptors, such as the hydroxamate ferrichrome receptor, which are found in these species. However, hexamate [2] (**44**) did not stimulate the growth of any of the strains tested.

In conclusion, we have reported the synthesis of spermidine-based synthetic siderophore analogues and have demonstrated their ability to substitute for natural siderophores in microbial systems. However, the stability contants of these analogues with Fe(III) were not determined. Further studies of the biological activity and potential therapeutic use of these analogues are in progress.

Experimental Section

General Methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on Perkin-Elmer 727B or Perkin-Elmer 1420 spectrophotometers. Proton NMR spectra were obtained on Varian EM-390 or Magnachem A-200 spectrometers. Chemical shifts are reported in ppm relative to tetramethylsilane (δ units). Mass spectra were recorded on an AEI Scientific Apparatus MS 902, Finnigan MAT Model 8430 or Du Pont DP 102 spectrometer. Field-desorption mass spectra were obtained by Dr. John L. Occolowitz (Eli Lilly and Co.). Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ.

***N*-Carbobenzoxy-*O*-benzylhydroxylamine (7).** *O*-benzylhydroxylamine (53 g, 0.43 mol) and K_2CO_3 (198.1 g, 1.45 mol) were dissolved in water (200 mL), and to it a solution of Cbz-Cl (62 g, 0.364 mol) in ethyl acetate was added slowly with stirring at room temperature. The reaction mixture was refluxed overnight. The reaction mixture was made acidic with 3 N HCl and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, dried ($MgSO_4$), and filtered, and the solvent was evaporated to yield a white solid and recrystallized from ethyl acetate/hexane to give 98% yield (91.6 g) of the product: mp 64–66 °C; ^1H NMR ($CDCl_3$) δ 4.86 (s, 2 H), 5.18 (s, 2 H), 7.36 (s, 10 H); IR (KBr) 3700–3100 (broad), 1705 cm^{-1}. Anal. ($C_{15}H_{15}NO_3$) C, H, N.

***N*-Carbobenzoxy-*N*-methyl-*O*-benzylhydroxylamine (8).** Compound **7** (10 g, 39 mmol), anhydrous K_2CO_3 (8.3 g, 60 mmol), and methyl iodide (24.42 g, 172 mmol) were dissolved in anhydrous acetone and refluxed for 12 h. The reaction mixture was filtered, and the acetone was evaporated. The resulting slurry was dis-

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solved in ether, washed with water (5 × 50 mL), dried ($MgSO_4$), and filtered. The solvent was removed to provide the product as a colorless oil in quantitative yield (10.5 g): ^1H NMR ($CDCl_3$) δ 3.06 (s, 3 H), 4.82 (s, 2 H), 5.18 (s, 2 H), 7.32 (m, 10 H); IR (neat) 1720 cm^{-1}; mass spectrum (EI), *m/e* 271 (M^+), 181.91. Anal. ($C_{16}H_{17}NO_3$) C, H, N.

***N*-Methyl-*O*-benzylhydroxylamine (9).** Compound 8 (4 g, 14.76 mmol) and 33% HBr/AcOH (30 mL) were stirred at room temperature for 4 h. The solvent was removed on a rotary evaporator and by freeze-drying. The resulting residue was washed well with ether followed by decantation, and then a solution of 5% $NaHCO_3$ was added to it. The product was extracted with ethyl acetate, dried ($MgSO_4$), and filtered, and the solvent was evaporated to give a light yellow oil in 93% yield (1.88 g): ^1H NMR ($CDCl_3$) δ 2.56 (s, 3 H), 4.64 (s, 2 H), 6.32 (s, 1 H, NH), 7.30 (s, 5 H); IR (neat) 3280, 1040, 1020 cm^{-1}; mass spectrum (EI), *m/e* 137 (M^+), 91.

***N*-Methyl-*N*-(benzyloxy)succinamide (10).** Hydroxylamine **9** (1.5 g, 11 mmol) and succinic anhydride (1.1 g, 11 mmol) were dissolved in dry THF and refluxed for 8 h. The solvent was removed under vacuum, and the resulting slurry was dissolved in ethyl acetate, dried ($MgSO_4$), and filtered, and the solvent was removed to provide a colorless oil. Product **10** was crystallized from ethyl acetate/ether to provide 87% yield (2.5 g) of the desired compound: mp 179–181 °C; ^1H NMR ($CDCl_3$) δ 2.56 (m, 4 H), 3.18 (s, 3 H), 4.82 (s, 2 H), 7.38 (s, 5 H), 10.78 (s, 1 H, COOH); IR (KBr) 3300–2350 cm^{-1}; mass spectrum (FD), *m/e* 237 (M^+), 238 (M + 1). Anal. ($C_{12}H_{15}NO_4$) C, H, N.

***N*-Methyl-*N*-(benzyloxy)glutaramide (11)** was prepared in a manner similar to that for **10** and obtained as a colorless oil in 90% yield: ^1H NMR ($CDCl_3$) δ 1.98–1.70 (q, 2 H), 2.50–2.24 (tt, 4 H), 3.16 (s, 3 H), 4.78 (s, 2 H), 7.34 (s, 5 H); IR (neat) 3450–2750, 1725, 1640 cm^{-1}; mass spectrum (CI), *m/e* 251 (M^+), 252, 342 (M + benzyl). Anal. ($C_{13}H_{17}NO_4$) C, H, N.

***p*-Nitrobenzyl Ester of *N*-Methyl-*N*-(benzyloxy)malonamide (13a).** *p*-Nitrobenzyl malonate **12a** (0.873 g, 3.65 mmol) and CDI (0.592 g, 3.65 mmol) were dissolved in dry methylene chloride and stirred at room temperature for 15 min under nitrogen. To this a solution of **9** (0.5 g, 3.65 mmol) in dry CH_2Cl_2 was added slowly, and the resulting mixture was stirred for 6 h. The solvent was removed in vacuo, and the solid residue was taken up in ethyl acetate, washed with 5% $NaHCO_3$ and 10% citric acid, water, dried ($MgSO_4$), and filtered. The solvent was removed to provide a white crystalline solid, which was recrystallized from ethyl acetate/hexanes in 78% yield (1.02 g): mp 105.5–106.5 °C; ^1H NMR ($CDCl_3$) δ 3.26 (s, 3 H), 3.48 (s, 2 H), 4.84 (s, 2 H), 5.22 (s, 2 H), 7.35 (m, 5 H), 7.47 (d, 2 H), 8.16 (d, 2 H); IR (KBr) 1740, 1640, 1520, 1360 cm^{-1}; mass spectrum (Cl), *m/e* 359 (M + 1), 236 (M − p-NO_2Bzl). Anal. ($C_{18}H_{18}N_2O_6$) C, H, N.

Benzyl ester of *N*-methyl-*N*-(benzyloxy)malonamide (13b) was prepared in a manner similar to that for **13a** as a colorless oil in 88–98% yield, which was purified by column chromatography by elution with ethyl acetate/hexanes (2:1): ^1H NMR ($CDCl_3$) δ 3.20 (s, 3 H), 3.45 (s, 2 H), 4.78 (s, 2 H), 5.12 (s, 2 H), 7.13 (s, 5 H); IR (neat) 1740, 1670 cm^{-1}; mass spectrum (CI), 314 (M + 1), 404 (M + benzyl).

***N*-Methyl-*N*-(benzyloxy)malonamide (14).** A solution of *p*-nitrobenzyl ester **13a** (5.00 g, 13.96 mmol) in 30 mL of THF and 15 mL of water was cooled in an ice bath (0–5 °C). To this a solution of $Na_2S\cdot 9H_2O$ (3.36 g, 13.96 mmol) in 15 mL of water was added. The mixture was stirred at room temperature for 2 h. The solution was made acidic with 1 N HCl, the THF was evaporated, and the solution was extracted with ethyl acetate. The solution was made basic, and the ethyl acetate layer was discarded. The solution was made acidic again and extracted with ethyl acetate. The organic layer was washed with water, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to provide the product as a light yellow oil in quantitative yield (3.08 g): ^1H NMR ($CDCl_3$) δ 3.24 (s, 3 H), 3.40 (s, 2 H), 4.84 (s, 2 H), 7.36 (s, 5 H), 10.48 (br, COOH); IR (neat) 3500–2550 (broad), 1740, 1640–1660 (broad) cm^{-1}; mass spectra (CI), *m/e* 214 (M + 1), 314 (M + benzyl).

***N*-Methyl-*N*-(benzyloxy)malonamide (14) from 13b** was prepared in a similar manner to that for **14** from *p*-nitrobenzyl ester **13a** in 78–88% yield. The product gave similar spectral data as described above.

Carbobenzoxyimidazole (16). Cbz-Cl (5 g, 29.33 mmol) in dry CH_2Cl_2 was cooled to 0 °C (ice bath), and a solution of imidazole (4 g, 58.75 mmol, 2 equiv) in dry CH_2Cl_2 was added to it slowly under nitrogen. The reaction mixture was allowed to warm up to room temperature and further stirred for 10 min. The reaction mixture was diluted with CH_2Cl_2 and washed with 10% citric acid, dried ($MgSO_4$), and filtered, and the solvent was evaporated to give a colorless oil: 5.78 g (97% yield); ^1H NMR ($CDCl_3$) δ 5.38 (s, 2 H), 8.26–7.00 (m, 8 H); IR (neat) 1770 cm^{-1}. Anal. Calcd for ($C_{11}H_{10}N_2O_2$): N, 13.86. Found: N, 14.06.

***N*-Carbobenzoxybenzylamine (17).** Carbobenzoxyimidazole (**16**) (0.4 g, 1.97 mmol), benzyl amine (0.426 g, 3.98 mmol), and a catalytic amount of DMAP (5% w/w) were dissolved in methylene chloride at room temperature under nitrogen. The reaction mixture was stirred for 8 h. The solvent was evaporated, and the resulting oil was taken up in ethyl acetate, washed with 10% citric acid and water, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to give a light yellow oil in quantitative yield (0.495 g), which required no further purification: ^1H NMR ($CDCl_3$) δ 4.24 (d, 2 H), 5.06 (s, 2 H), 5.60 (br, 1 H), 7.25 (m, 10 H).

N^1,N^8-Dicarbobenzoxyhexanediamine (18) was prepared in a similar manner to that for **17** (reaction time 16 h) and obtained as a white solid, which was recrystallized from ethanol/water in quantitative yield: mp 113–115 °C; ^1H NMR ($CDCl_3$) δ 1.64–1.20 (m, 10 H), 3.42–3.04 (m, 4 H), 4.96–4.74 (br, 2 H), 5.12 (s, 4 H), 7.38 (s, 10 H).

N^1,N^{10}-Dicarbobenzoxyspermidine (19) was prepared in a similar manner to that for **17** (reaction time 16 h) and obtained as a white solid, which was recrystallized from benzene/ether in 76% yield: mp 104–105 °C (lit.[7a] mp 104.5–105 °C); ^1H NMR ($CDCl_3$) δ 1.36–1.87 (m, 7 H), 2.44–2.80 (m, 4 H), 3.02–3.41 (m, 4 H), 5.07 (s, 4 H), 7.33 (s, 10 H).

***p*-Nitrobenzyl Monoester of Succinic Acid (20). Method a.** Sodium hydride (0.79 g, 60% in oil, 32.65 mmol) was washed twice with dry hexane under nitrogen atmosphere and then suspended in dry THF. To this suspension was added a solution of *p*-nitrobenzyl alcohol (5 g, 32.65 mmol) in dry THF in one portion at 0–5 °C under nitrogen. The reaction mixture was stirred for 5 min, and a solution of succinic anhydride (5 g, 32.65 mmol) in dry THF was added slowly with stirring. The reaction mixture was further stirred for 8 h, and the solvent was removed. The resulting residue was dissolved in ethyl acetate, washed with 5% $NaHCO_3$, and the organic phase was discarded. The basic layer was acidified with 1 N HCl and again extracted with ethyl acetate, dried ($MgSO_4$), and filtered, and the solvent was removed on a rotary evaporator to provide a white solid, which was recrystallized from ethyl acetate/hexanes: 5.92 g (72%), mp 96–97 °C; ^1H NMR ($CDCl_3$) δ 2.74 (s, 4 H), 5.26 (s, 2 H), 7.54 (d, 2 H), 8.24 (d, 2 H), 10.96 (br, 1 H, COOH); IR (KBr) 3700–2700, 1730, 1695, 1530, 1350 cm^{-1}; mass spectrum (CI), *m/e* 254 (M + 1), 236 (M − OH). Anal. ($C_{11}H_{11}NO_6$) C, H, N.

Method b. Succinic anhydride (8.2 g, 82 mmol), *p*-nitrobenzyl alcohol (15 g, 98 mmol), and DMAP (catalytic amount, 5% w/w) were dissolved in anhydrous THF under nitrogen. The reaction mixture was refluxed overnight (12 h). The solution was made acidic with 1 N HCl, and THF was evaporated. The residue was taken up in ethyl acetate and washed with 1 N HCl. The organic layer was made basic with 5% $NaHCO_3$ and washed with ethyl acetate, and organic layer was discarded. The basic layer was acidified with 1 N HCl and extracted with ethyl acetate, dried ($MgSO_4$), and filtered, and the solvent was removed under vacuum to provide a white solid, which was recrystallized from ethyl acetate/hexane to provide 18.2 g (87%) of the product.

Benzyl monoester of succinic acid (21) was prepared in a similar manner to that for **20** by method b and obtained as white crystalline solid, which was recrystallized from ethyl acetate/hexanes to provide pure product in 91% yield: mp 56–57 °C; ^1H NMR ($CDCl_3$) δ 2.64 (s, 4 H), 5.22 (s, 2 H), 7.34 (s, 5 H); IR (KBr) 3700–2800, 1725, 1702 cm^{-1}; mass spectrum (CI), *m/e* 208 (M^+), 209 (M + 1). Anal. ($C_{11}H_{12}O_4$) C, H.

***p*-Nitrobenzyl monoester of glutaric acid (22)** was prepared from *p*-nitrobenzyl alcohol and glutaric anhydride in a similar manner to that for **20** by method b. Recrystallization from ethyl acetate/hexanes provided an 85% yield of the product: mp 71–72 °C; ^1H NMR ($CDCl_3$) δ 2.10–1.93 (q, 2 H), 2.55–2.42 (two merging

triplets, J = 7Hz, 4 H), 5.22 (s, 2 H), 7.53 (d, 2 H), 8.21 (d, 2 H); IR (KBr) 3680–2800, 1720, 1705 cm^{-1}; mass spectrum (CI), m/e 268 (M + 1), 250 (M -OH). Anal. ($C_{12}H_{13}NO_6$) C, H, N.

Succinimido Ester of *p*-Nitrobenzyl Succinate (23). To a mixture of compound **20** (10 g, 39.5 mmol) and *N*-hydroxysuccinimide (5 g, 43.45 mmol) in dry THF was added a solution of DCC (8.97 g, 43.45 mmol) in THF slowly under nitrogen at 0–5 °C (ice bath). The reaction mixture was stirred at 0–5 °C for 3 h and then allowed to warm up to room temperature. The mixture was further stirred for 15 h. The solvent was evaporated, the residue was taken up in benzene, and dicyclohexylurea was removed by filtration. The solvent was removed under vacuum to provide a light yellow solid, which was recrystallized from ethyl acetate/hexanes to provide 12.81 g (93%): mp 98–99 °C; ^{1}H NMR ($CDCl_3$) δ 3.14–2.80 (m, 8 H, includes succinimide CH_2), 5.25 (s, 2 H), 7.53 (d, 2 H), 8.21 (d, 2 H); IR (KBr) 1820, 1785, 1730, 1650 (broad), 1520, 1345 cm^{-1}. Anal. ($C_{15}H_{14}N_2O_8$) C, H, N.

Succinimido ester of benzyl succinate (24) was prepared from **21** in a similar manner to that for **23** as a white solid, which was recrystallized from ethyl acetate/hexanes in 92% yield: mp 94–95 °C; ^{1}H NMR ($CDCl_3$) δ 3.06–2.52 (m, 8 H), 5.10 (s, 2 H), 7.32 (s, 5 H); IR (KBr) 1815, 1780, 1715, 1650 cm^{-1}; mass spectrum (CI), m/e 306 (M + 1).

Succinimido ester of *p*-nitrobenzyl glutarate (25) was prepared from **22** in a similar manner to that for **23** as a white solid, which was recrystallized from ethyl acetate/hexanes in 83% yield: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 2.19–2.01 (q, 2 H), 2.58 (t, 2 H), 2.69 (t, 2 H), 2.85 (s, 4 H), 5.23 (s, 2 H), 7.52 (d, 2 H, J = 8 Hz), 8.22 (d, 2 H, J = 8 Hz); IR (KBr) 1805, 1766, 1720, 1550, 1380 cm^{-1}; mass spectrum (CI), m/e 365 (M + 1). Anal. ($C_{16}H_{16}N_2O_8$) C, H, N.

Succinimido ester of *N*-methyl-*N*-(benzyloxy)succinamic acid (26) was prepared from **10** in a similar manner to that for **23** as a white crystalline solid and recrystallized from EtOH/H_2O in 97% yield: mp 83–84 °C; ^{1}H NMR ($CDCl_3$) δ 2.92–2.64 (m, 8 H), 3.20 (s, 3 H), 4.84 (s, 2 H), 7.52 (s, 5 H); IR (KBr) 1815, 1785, 1735, 1645 (broad) cm^{-1}; mass spectrum (CI), m/e 335 (M + 1), 220 (M – ONSu).

Succinimido ester of *N*-methyl-*N*-(benzyloxy)glutaramic acid (27) was prepared from **11** in a similar manner to that for **23** in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 1.85–2.03 (q, 2 H), 2.38–2.55 (t, 2 H), 2.55–2.66 (t, 2 H), 2.71 (s, 4 H), 3.16 (s, 3 H), 4.80 (s, 2 H), 7.37 (s, 5 H); IR (neat) 1825, 1765, 1735 (broad), 1655 cm^{-1}.

Succinimido ester of *N*-methyl-*N*-(benzyloxy)malonamic acid (28) was prepared from **14** in a similar manner to that for **23** and was crystallized from EtOH/water to provide a 61% yield: ^{1}H NMR ($CDCl_3$) δ 2.77 (s, 4 H), 3.21 (s, 3 H), 3.59 (s, 2 H), 4.88 (s, 2 H), 7.39 (s, 5 H).

***N*^{4}-((*p*-Nitrobenzyl)succinoyl)-*N*^{1},*N*^{8}-dicarbobenzoxyspermidine (29a) from 20.** Compound **20** (0.35 g, 1.38 mmol), $SOCl_2$ (0.18 g, 1.52 mmol), and pyridine (0.164 g, 2.07 mmol) were dissolved in dry methylene chloride under nitrogen at room temperature. The reaction was stirred for 10 min, and the solvent was evaporated. The resulting residue was dissolved in dry methylene chloride, and to it a solution of **19** (0.515 g, 1.24 mmol) and DMAP (0.253 g, 2.07 mmol) in methylene chloride was added slowly. The reaction mixture was stirred for 16 h. After the usual acid/base workup and further purification by column chromatography (2% EtOH in ethyl acetate), the product was obtained as a light yellow oil in 10% yield (0.18 g): ^{1}H NMR ($CDCl_3$) δ 1.84–1.24 (m, 6 H), 2.98–2.58 (m, 4 H), 3.58–2.98 (m, 8 H), 5.14 (s, 2 H), 5.28 (s, 2 H), 7.48 (s, 10 H), 7.68 (d, 2 H), 8.50 (d, 2 H); IR (neat) 3320, 1715, 1640, 1520, 1345 cm^{-1}; mass spectrum (CI), m/e 650 (M + 1).

Preparation of 29a from 23. A solution of **23** (2.00 g, 7.9 mmol) and DMAP (5% w/w) in dry THF was added to a solution of **19** (3.265 g, 7.9 mmol) in dry THF at room temperature under nitrogen. This was stirred for 18 h, and the solvent was evaporated. The oily residue was taken up in ethyl acetate, washed well with 1 N HCl, 2% NaOH, water, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to provide 4.61 g (90%) of a colorless oil that required no further purification. The spectral data was the same as that obtained from the earlier preparation.

***N*^{4}-(Benzylsuccinoyl)-*N*^{1},*N*^{8}-dicarbobenzoxyspermidine (29b)** was prepared from **19** and **24** in a similar manner to that for **29a** and obtained as a colorless oil in 97% yield: ^{1}H NMR ($CDCl_3$) δ 1.84–1.34 (m, 6 H), 2.78–2.52 (m, 4 H), 3.48–2.96 (m, 8 H), 5.08 (s, 6 H), 7.34 (s, 15 H); IR (neat) 3330 (broad), 1718, 1640 cm^{-1}; mass spectrum (CI), m/e 604 (M^+), 605 (M + 1); R_f 0.61 (ethyl acetate).

***N*^{4}-(*N*-Methyl-*N*-(benzyloxy)succinamoyl)-*N*^{1},*N*^{8}-dicarbobenzoxyspermidine (30a)** was prepared from **19** and the acid chloride ($SOCl_2$, pyridine) of **10** in a similar manner to that for **29a** and obtained as a colorless oil in 67% yield: ^{1}H NMR ($CDCl_3$) δ 2.02–1.36 (m, 6 H), 2.52–3.04 (m, 4 H), 3.16–3.62 (m, 11 H, includes CH_3), 5.04 (s, 2 H), 5.26 (s, 4 H), 7.56 (m, 15 H); IR (neat) 3335 (broad), 1715, 1670 (broad) cm^{-1}.

Spermidine derivatives 30a and *N*^{4}-(*N*-methyl-*N*-(benzyloxy)glutaramoyl)-*N*^{1},*N*^{8}-dicarbobenzoxyspermidine (30c) were prepared in 83% and 87% yields, respectively, from **19** and **26** or **27** in a manner similar to that for **29b** from **24**. Compound **30a**: for the spectral data, see above. Compound **30c**: ^{1}H NMR ($CDCl_3$) δ 2.02–1.36 (m, 8 H), 2.56–2.18 (m, 4 H), 3.68–3.04 (m, 11 H, includes CH_3 singlet), 4.90 (s, 2 H), 5.24 (s, 4 H), 5.64 (br, NH), 6.02 (br, NH), 7.56 (m, 15 H); IR (neat) 3340 (broad), 1720, 1680 (broad) cm^{-1}.

***N*^{4}-Succinoyl-*N*^{1},*N*^{8}-bis(*N*-methyl-*N*-(benzyloxy)succinamoyl)spermidine (31) from 29a.** Compound **29a** (4.6 g, 7.1 mmol) was dissolved in 50 mL of HBr/AcOH (33%) at room temperature and was stirred for 4 h. The solvent was removed on a rotary evaporator and by freeze-drying. The resulting residue was dissolved in water, and $NaHCO_3$ (2.06 g, 24.5 mmol) was added to it. To this a solution of **26** (5.8 g, 17.38 mmol) in ethyl acetate was added at room temperature. The reaction mixture was further stirred for 24 h. After the usual acid/base workup, pure product was obtained as a colorless oil in 28% yield (1.5 g): ^{1}H NMR ($CDCl_3$) δ 1.98–1.34 (m, 6 H), 3.04–2.48 (m, 12 H), 3.66–3.12 (m, 14 H, includes CH_3), 5.06 (s, 4 H), 7.62 (s, 10 H), 8.22 (br, 2 H), 10.22 (br, COOH); IR (neat) 3650–2700, 1720 cm^{-1}; mass spectrum (FD), m/e 684 (M + 1); R_f 0.11 (ethyl acetate).

Compound 31 from 29b. Compound **29b** (0.7 g, 1.16 mmol) was dissolved in a solution of EtOH and AcOH, and to this 10% Pd/C (20% w/w) was added. Hydrogen gas was bubbled through this for 2 h, Pd/C was filtered, and the solution was washed with EtOH. The solvent was removed on a rotary evaporator and by freeze-drying. The resulting residue was dissolved in a solution of $NaHCO_3$ (0.13 g) in water. To this was added a solution of **26** (0.85 g, 2.55 mmol) in ethyl acetate at room temperature, and the mixture was further stirred for 24 h. After the usual acid/base workup, compound **31** was obtained as a colorless oil in 59% yield (0.467 g). The spectral data was the same as obtained from the earlier preparation.

***N*^{1},*N*^{4},*N*^{8}-Tris(*N*-methyl-*N*-(benzyloxy)succinamoyl)spermidine (32a) from 31.** Compound **31** (0.2 g, 0.293 mmol) and CDI (0.048 g, 0.293 mmol) were dissolved in dry methylene chloride under nitrogen at room temperature. The reaction mixture was stirred for 1 h, and to this was added a solution of hydroxylamine **9** (0.04 g, 0.293 mmol) in dry methylene chloride, and the reaction mixture was further stirred for 8 h. The solvent was evaporated, and the oily residue was dissolved in ethyl acetate. The organic phase was washed well with 1 N HCl, water, 1 N NaOH, water, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to provide the product as a colorless oil in 71% yield (0.167 g): ^{1}H NMR ($CDCl_3$) δ 1.36–2.08 (m, 6 H), 2.44–3.04 (m, 12 H), 3.20–3.66 (m, 18 H), 5.02 (m, 6 H), 6.98 (br, 1 H), 7.62 (s, 15 H); IR (neat) 3300 (broad), 1650 (broad) cm^{-1}; mass spectrum (FD), m/e 803 (M + 1), 893 (M + benzyl); R_f 0.05 (ethyl acetate) or 0.13 (ethyl acetate/ethanol, 9:1). Anal. ($C_{43}H_{58}N_6O_9$) C, H, N.

Preparation of 32a Using 26. Spermidine (1 g, 6.88 mmol) was dissolved in dry THF under nitrogen and to this a catalytic amount of DMAP (5% w/w) was added. To this mixture was added slowly a solution of **13** (6.9 g, 20.65 mmol) in dry THF. The reaction mixture was stirred at room temperature for 16 h. The solvent was removed under vacuum, and the residue was taken up in ethyl acetate. The organic layer was washed well with 1 N HCl, water, 1 N NaOH, water, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to give 2.83 g (52%) of **32a** as a viscous colorless oil. No further purification

was necessary, and the same spectral data as above was obtained.

N^1,N^4,N^8-Tris(*N*-methyl-*N*-(benzyloxy)glutaramoyl)sperimidine (32c) was prepared in a similar manner to that for **32a** and obtained as a colorless oil in 63% yield: ^{1}H NMR δ 1.79–1.39 (m, 6 H), 3.53–3.06 (m, 23 H), 4.88 (m, 6 H), 7.38 (s, 16 H, includes NH), 7.61 (br, 1 H, NH); IR (neat) 3500–3100 (broad) cm^{-1}; mass spectrum (CI), *m/e* 761 (M^+), 671, 556, 419.

Preparation of 32a Using Acid Chlorides. Compound **10** (10 g, 42.2 mmol), pyridine (4.34 g, 54.9 mmol), and $SOCl_2$ (5.52 g, 46.4 mmol) were dissolved in dry methylene chloride under nitrogen at −10 °C. The reaction mixture was stirred for 1 h and was added to a solution of spermidine (1.53 g, 10.55 mmol), pyridine (5.00 g, 63.3 mmol), and DMAP (catalytic, 5% w/w) in dry methylene chloride at room temperature. The reaction mixture was further stirred for 18 h. After the mixture was washed with water, 2% NaOH, 1 N HCl, and brine, dried over anhydrous $MgSO_4$, and filtered, the solvent was removed under vacuum to give a dark brown oil. The product was purified by gradient column chromatography [(i) CH_2Cl_2; (ii) 2% CH_3OH in CH_2Cl_2; (iii) 5% CH_3OH in CH_2Cl_2] on silica gel to provide 1.895 g (22%) of product. The product gave the same spectral data as above.

N^1,N^4,N^8-Tris(*N*-methyl-*N*-(benzoyloxy)glutaramoyl)spermidine (32c) and N^1,N^4,N^8-tris(*N*-methyl-*N*-(benzyloxy)malonamoyl)spermidine (32b) were prepared in a similar manner to that for **32a** with acid chlorides of **14** or **11**. Compound **32b**: 56%; ^{1}H NMR ($CDCl_3$) δ 1.66–1.38 (m, 6 H), 2.52–1.81 (m, 18 H), 3.38–3.05 (m, 17 H, includes CH_3), 4.81 (s, 6 H), 6.53 (br, 1 H, NH), 6.92 (br, 1 H, NH), 7.37 (s, 15 H); IR (neat) 3600–3100, 1660 (broad) cm^{-1}; mass spectrum (FD), *m/e* 845 (M + 1), 935 (M + benzyl).

N^1,N^4,N^8-Tris(*N*-methyl-*N*-hydroxysuccinamoyl)spermidine or Spermexatin [2,2,2] (33a). Compound **32a** (1.895 g, 2.36 mmol) and 10% Pd/C (20% w/w) were suspended in ethanol. The reaction mixture was purged with hydrogen gas for 4 h. The reaction mixture was filtered and washed with ethanol. Ethanol was evaporated to give compound **33a** as a viscous colorless oil in 83% yield (1.13 g): ^{1}H NMR (D_2O) δ 2.32–1.44 (m, 6 H), 2.92–2.44 (m, 12 H), 3.46–3.12 (m, 17 H, includes CH_3); mass spectrum (FD), *m/e* 533 (M + 1), 555 (M + Na). Anal. ($C_{22}H_{40}N_6O_9$) C, H, N.

N^1,N^4,N^8-Tris(*N*-methyl-*N*-hydroxyglutaramoyl)spermidine or Spermexatin [3,3,3] (33c). Compound **33c** was prepared from **32c** in a similar manner to that for **33a** and was obtained as a colorless oil in 91% yield: ^{1}H NMR (D_2O) δ 1.62–1.33 (m, 6 H), 1.92–1.64 (m, 6 H), 2.56–2.13 (m, 12 H), 3.40–3.30 (m, 17 H, includes CH_3); IR (neat) 3700–2750, 1680–1610 (broad), 1200 cm^{-1}; mass spectrum of the sample was taken after running the NMR in D_2O and subsequent exchange with H_2O (FD), *m/e* 576 (M + 2), 598 (M + 1 + Na). Anal. ($C_{25}H_{46}N_6O_9$) C, H, N.

Methyl 2,3-Dihydroxybenzoate (35). 2,3-Dihydroxybenzoic acid (**34**) (2 g, 13 mmol) was dissolved in methanol, and $SOCl_2$ (1.1 equiv) was added to it at room temperature slowly with stirring. The reaction mixture was stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated to give a white solid. The product was recrystallized from ethyl acetate/hexanes in 92% yield (1.98 g): mp 72–74 °C; ^{1}H NMR ($CDCl_3$) δ 4.08 (s, 3 H), 5.94 (br, 1 H), 7.74–6.90 (m, 3 H); IR (KBr) 3750–3140 (broad), 1670, 1435 cm^{-1}; mass spectrum (CI), *m/e* 168 (M^+).

Methyl 2,3-Bis(benzyloxy)benzoate (36). Compound **35** (1.8 g, 11.08 mmol), anhydrous K_2CO_3 (6.13 g, 44.33 mmol), and NaI (0.1 g) were dissolved in dry DMF under nitrogen at room temperature, and to this a solution of benzyl bromide (3.8 g, 22.16 mmol) in DMF was added. The reaction mixture was stirred for 16 h and then filtered. The filterate was diluted with ether and washed with 2% NaOH and water. The ether layer was dried ($MgSO_4$) and filtered, and the solvent was removed under vacuum to give a white solid. The product was recrystallized from ethyl acetate/hexanes to give a white crystalline solid in 87% yield (3.33 g): mp 55–57 °C; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3 H), 5.20 (s, 4 H), 7.84–7.26 (m, 13 H); mass spectrum (CI), *m/e* 348 (M^+).

2,3-Bis(benzyloxy)benzoic Acid (37a). Compound **36** (3.2 g, 9.25 mmol) was dissolved in a solution of THF/water (1:1), and 20 mL of 1 N KOH was added to it. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was acidified and extracted with ethyl acetate. After the usual acid/base workup, the product was obtained as white fluffy solid and recrystallized from ethanol/water to provide 3.05 g (quantitative): mp 114–116 °C (lit.[21] mp 120 °C); ^{1}H NMR ($CDCl_3$) δ 5.14 (2 s, 4 H), 7.12–7.78 (m, 13 H); IR (KBr) 3650–2700 cm^{-1}; mass spectrum (CI), *m/e* 334 (M^+), 335 (M + 1).

N^1,N^8-Bis(2,3-bis(benzyloxy)benzoyl)spermidine (38a). Compound **38a** was prepared by the method described by Joshua et al.[15] Compound **37a** (7.18 g, 21.63 mmol) and CDI (3.51 g, 21.6 mmol) were dissolved in dry methylene chloride under nitrogen at room temperature. This was stirred for 1 h, and to this was added a solution of spermidine (1.571 g, 10.81 mmol) in dry methylene chloride. The reaction mixture was further stirred overnight. The solvent was evaporated, and the residue was taken up in ethyl acetate, washed with 2% NaOH, water, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to give a yellow oil, and the product was purified by column chromatography on silica gel (5% EtOH in ethyl acetate) as a colorless oil: 7.27 g (87%); ^{1}H NMR ($CDCl_3$) δ 1.12–1.62 (m, 6 H), 2.18–2.64 (m, 4 H), 3.04–3.48 (m, 4 H), 5.10 (2 s, 8 H), 7.02–7.18 (m, 28 H, includes NH); IR (neat) 3480 (broad), 3100, 2940, 1680 (broad) cm^{-1}.

N^1,N^8-Bis(2,3-bis(methyloxy)benzoyl)spermidine (38b) was prepared in a similar manner to that for **38a** and obtained as a colorless oil in 81% yield: ^{1}H NMR ($CDCl_3$) δ 1.22–1.86 (m, 6 H), 2.40–2.78 (m, 4 H), 3.32–3.62 (m, 4 H), 3.82 (m, 12 H), 5.30 (br, 1 H, NH), 7.02–8.44 (m, 8 H, includes 2 NH); IR (neat) 3400–3100 (broad), 1660–1700 (broad) cm^{-1}; mass spectrum (CI), *m/e* 473 (M^+).

N^4-((*p*-Nitrobenzyl)succinoyl)-N^1,N^8-bis(2,3-bis(benzyloxy)benzoyl)spermidine (39a). Compounds **38a** (3 g, 3.88 mmol), **23** (1.50 g, 4.27 mmol), and a catalytic amount of DMAP (5% w/w) were dissolved in dry THF under nitrogen at room temperature. The reaction mixture was stirred for 16 h, and the solvent was evaporated. The residue was taken up in ethyl acetate and washed well with 1 N HCl, water, 2% NaOH, water, and brine, dried ($MgSO_4$), and filtered. The solvent was removed under vacuum to give a yellow oil, which was purified by column chromatography on silica gel (ethyl acetate/hexanes, 3:2) to provide compound **39a** as a light yellow oil in 90% yield (3.53 g): ^{1}H NMR ($CDCl_3$) δ 1.64–1.22 (m, 6 H), 2.75–2.40 (m, 4 H), 3.30–2.90 (m, 8 H), 5.16–4.88 (m, 10 H), 7.22 (m, 4 H), 7.50–7.26 (m, 24 H), 7.65 (br, NH), 8.00 (br, NH), 8.36 (d, 2 H); IR (neat) 3380, 1762 cm^{-1}; mass spectrum (FD), *m/e* 1013 (M + 1), 922 (M − benzyl), 778 (M −233); R_f 0.42 (ethyl acetate). Anal. ($C_{60}H_{60}N_4O_{11}$) C, H, N.

N^4-((*p*-Nitrobenzyl)succinoyl)-N^1,N^8-bis(2,3-bis(dimethyloxy)benzoyl)spermidine (39b) was prepared in a similar manner to that for **39a** from **38b** and **23** and obtained as a light yellow oil in 93% yield: ^{1}H NMR ($CDCl_3$) δ 1.80–1.40 (m, 6 H), 2.72–2.52 (m, 4 H), 3.52–3.20 (m, 8 H), 3.82 (overlapping singlets, 12 H), 5.14 (s, 2 H), 7.20–6.90 (m, 4 H), 7.48 (d, 2 H), 7.64 (d, 2 H), 8.00 (br, 1 H, NH), 8.18 (d, 2 H), 8.32 (br, 1 H, NH); IR (neat) 3362, 1764 cm^{-1}, mass spectrum (FD), *m/e* 709 (M + 1); R_f 0.17 (ethyl acetate).

N^4-(Benzylsuccinoyl)-N^1,N^8-bis(2,3-bis(benzyloxy)benzoyl)spermidine (39c) was prepared in a similar manner to that of **39a** from **38a** and **24** and obtained as a light yellow oil in 93% yield: ^{1}H NMR ($CDCl_3$) δ 1.74–1.12 (m, 6 H), 2.80–2.42 (m, 4 H), 3.52–3.96 (m, 8 H), 5.22 (2 overlapping singlets, 10 H), 7.76–7.18 (m, 29 H), 8.04–7.82 (m, 2 H), 8.22 (br 2 NH); IR (neat) 3392, 1740, 1660 cm^{-1}; mass spectrum (FD), *m/e* 968 (M + 1), 969 (M + 2), 778 (M − 189); R_f 0.42 (ethyl acetate). Anal. ($C_{60}H_{61}N_3O_9$) C, H, N.

N^4-Succinoyl-N^1,N^8-bis(2,3-bis(benzyloxy)benzoyl)spermidine (40a). Compound **39a** (3.4 g, 3.37 mmol) was dissolved in aqueous THF, and the solution was cooled to 0–5 °C (ice bath). To this was added a solution of $Na_2S{\cdot}9H_2O$ (1.62 g, 6.74 mmol) in water in one portion. The reaction mixture was stirred at room temperature for 2 h. The solution was made acidic with 1 N HCl and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under vacuum to give a yellow oil. The product was purified by gradient column chromatography on silica gel [(i) ethyl acetate/hexanes (3:2); (ii)

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Table I. Biological Activity of Siderophore Analogues

	growth of indicator strain in low-iron medium[a]			
	V. cholerae		*E. coli*	
siderophore, mM	Lou 15	1510	RW 193	UT2300
vibriobactin				
50	nd	0	0	0
10	32	0	0	0
1	32	0	0	0
0.2	32	0	0	0
enterobactin				
10	28	0	nd	0
1	22	0	40	0
0.2	0	0	28	0
33a				
50	19	0	12	12
10	17	0	0	0
1	17	0	0	0
0.2	16	0	0	0
33c				
50	12	12	18	16
10	0[b]	0	10	10
1	0[b]	0	0	0
0.2	0[b]	0	0	0
42				
50	36	28	21	0
10	0[b]	0	12	0
1	0[b]	0	0	0
0.2	0[b]	0	0	0

[a] Results are shown as diameter (millimeters) of zone of growth at 18 h around the disk containing the indicated concentration of the siderophore. [b] Small zone of stimulation after 42-h incubation. Compound **44** did not stimulate the growth of all strains tested.

5% methanol in ethyl acetate] to give **40a** as a colorless oil in 64% yield (1.88 g): ^{1}H NMR (CDCl$_3$) δ 1.63–1.18 (m, 6 H), 2.66–2.41 (m, 4 H), 3.32–3.00 (m, 8 H), 5.08 (m, 8 H), 7.15–7.03 (m, 4 H), 7.48–7.23 (m, 20 H), 7.77–7.58 (m, 2 H), 8.15–7.95 (br, 2 NH); IR (neat) 3640–2750, 1722 cm^{-1}. Anal. ($C_{53}H_{55}N_3O_9$) C, H, N.

***N*^{4}-Succinoyl-*N*^{1},*N*^{8}-bis(2,3-bis(methyloxy)benzoyl)-spermidine (40b)** was prepared in 72% yield in a similar manner to that for compound **40a** from **39b**, which was purified by usual acid/base workup and required no further purification: ^{1}H NMR (CDCl$_3$) δ 1.98–1.42 (m, 6 H), 2.68 (m, 4 H), 3.96 (s, 12 H), 7.28 (d, 2 H), 7.82 (d, 2 H), 8.32 (t, br, NH), 8.62 (t, br, NH), 9.12 (br, COOH); IR (neat) 3640–2700, 1726 cm^{-1}; mass spectrum (FD), *m/e* 574 (M$^+$); R_f 0.01 (ethyl acetate).

***N*^{4}-(*N*-Methyl-*N*-(benzyloxy)succinamoyl)-*N*^{1},*N*^{8}-bis-(2,3-bis(benzyloxy)benzoyl)spermidine (41a).** Compound **40a** (0.93 g, 1.07 mmol) and CDI (0.19 g, 1.17 mmol) were dissolved in dry methylene chloride under nitrogen at room temperature. The reaction mixture was stirred for 30 min, and to this was added a solution of **9** (0.146 g, 1.07 mmol) in dry methylene chloride slowly with stirring. The reaction mixture was further stirred for 12 h. The solvent was removed, and the oily residue was dissolved in ethyl acetate. The organic layer was washed with 1 N HCl, H$_2$O, 5% NaOH, H$_2$O, and brine, dried (MgSO$_4$), and filtered. The solvent was evaporated to give a yellow oil, which was purified by column chromatography (10% CH$_3$OH in CH$_2$Cl$_2$) to provide **41a** as a light yellow oil in 81% yield (0.856 g): ^{1}H NMR (CDCl$_3$) δ 1.76–1.12 (m, 6 H), 2.86–2.34 (m, 4 H), 3.50–2.94 (m, 11 H), 4.84 (s, 2 H), 5.08 (s, 8 H), 7.82–6.96 (m, 31 H), 8.02 (t, br, 2 NH); IR (neat) 3380 (br), 1670 (br) cm^{-1}; R_f 0.32 (ethyl acetate).

***N*^{4}-(*N*-Methyl-*N*-(benzyloxy)succinamoyl)-*N*^{1},*N*^{8}-bis-(2,3-bis(methyloxy)benzoyl)spermidine (41b)** was prepared in a similar manner to that for **41a** from **40b** and hydroxylamine **9** and was obtained as a light yellow oil in 77% yield: ^{1}H NMR (CDCl$_3$) δ 1.98–1.36 (m, 6 H), 2.90–2.38 (m, 4 H), 3.12 (s, 3 H), 3.60–3.22 (m, 8 H), 3.80 (m, 12 H), 4.86 (s, 2 H), 7.12–6.84 (m, 4 H), 7.30 (s, 5 H), 7.70–7.48 (m, 2 H), 8.08 (t, br, NH), 8.40 (t, br, NH); IR (neat) 3380 (broad), 1645 (broad) cm^{-1}; R_f 0.192 (ethyl acetate). Anal. ($C_{37}H_{48}N_3O_9$) C, H, N.

Protected Spermexatol 41a from 38a. Compound **41a** was prepared in a similar manner as that for **39a** from **38a** and **26** in the presence of a catalytic amount of DMAP in 82% yield, and the product gave spectral data similar to that given above for **41a**.

***N*^{4}-(*N*-Methyl-*N*-hydroxysuccinamoyl)-*N*^{1},*N*^{8}-bis(2,3-dihydroxybenzoyl)spermidine or Spermexatol [2] (42).** Compound **41a** (0.34 g, 0.343 mmol) and 10% Pd–C (20% w/w) were suspended in ethanol, and the mixture was purged with hydrogen with stirring for 3 h. The mixture was filtered, and ethanol was evaporated under vacuum to give **42** as a light yellow oil in 90% yield (0.167 g): ^{1}H NMR (Acetone-d_6) δ 1.46–1.83 (m, 6 H), 2.58–2.81 (m, 4 H), 3.06–3.53 (m, 11 H), 6.67 (m, 2 H), 6.92 (d, 2 H), 7.22 (d, 2 H), 7.81 (br, NH). Anal. ($C_{26}H_{34}N_4O_9$) C, H, N.

***N*^{1},*N*^{6}-Bis(*N*-methyl-*N*-(benzyloxy)succinamoyl)hexanediamine (43).** Acid **10** (0.98 g, 4.14 mmol) and CDI (0.67 g, 4.14 mmol) were dissolved in 20 mL of dry methylene chloride under nitrogen at room temperature, and the solution was stirred for 1 h. To this a solution of 1,6-hexanediamine (0.24 g, 2.07 mmol) in methylene chloride was added, and the reaction mixture was further stirred overnight. The reaction mixture was diluted with methylene chloride and washed with 1 N HCl, 2% NaOH, water, and brine, dried (MgSO$_4$), and filtered. The solvent was evaporated to give a white solid, which was recrystallized from ethanol/water to provide **43** in 79% yield (0.90 g): mp 123–124 °C; ^{1}H NMR (CDCl$_3$) δ 1.16–1.54 (m, 8 H), 2.32–2.86 (m, 6 H), 3.06–3.30 (m, 8 H, includes CH$_3$), 4.80 (s, 4 H), 6.10 (br, 2 NH), 7.36 (s, 10 H); mass spectrum (CI), *m/e* 554 (M$^+$).

***N*^{1},*N*^{6}-Bis(*N*-methyl-*N*-hydroxysuccinamoyl)hexanediamine or Hexamate [2] (44).** Compound **43** (1.25 g, 2.26 mmol) and 10% Pd–C (20% w/w) were suspended in ethanol, and hydrogen was purged through it for 2 h. The Pd–C was filtered, and the solvent was removed under vacuum to give a white solid, which was recrystallized from ethanol/water to provide **44** in 71% yield (0.6 g): mp 159.5–160.5 °C; ^{1}H NMR (D$_2$O) δ 1.02–1.61 (m, 8 H), 1.83 (s, 1 H), 2.05 (s, 1 H), 2.21–3.01 (m, 4 H), 2.57–3.01 (m, 4 H), 2.92–3.35 (m, 10 H); mass spectrum (CI), *m/e* 374 (M$^+$). Anal. ($C_{16}H_{30}N_4O_6$) C, H, N.

Bioassays. Biological activity of the siderophore analogues was determined by measuring their ability to stimulate growth of bacterial strains in a low-iron medium. Strains used were *V. cholerae* Lou 15 and the isogenic vibriobactin receptor mutant 1510 and *E. coli* RW 193 (FepA$^+$) and UT2300 (RW 193 FepA$^-$). Bacteria were seeded at a concentration of 10^4/mL into Luira agar containing the iron chelator ethylenediamine bis(*O*-hydroxyphenyl acetic acid) (EDDA). EDDA concentrations were 250 mg/mL for *V. cholerae* and 500 mg/mL for *E. coli*. When seeded at low density, even wild type bacteria are unable to grow except around disks containing Fe or a usable siderophore.[22]

Siderophores were prepared as 50 mM solutions in ethanol (hexamate [2] (44) and **33a** were not fully soluble at this concentration). The solutions were further diluted in ethanol to give 10, 1, and 0.2 mM solutions. Ten milliliters of each solution was placed on a sterile sensi-disk, and the ethanol was allowed to evaporate. Disks were placed on the surface of the seeded plates, and the plates were incubated for 18 h at 37 °C. Ten milliliters of 10 mM FeCl$_3$ on a disk was placed on each plate as a positive control.

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Registry No. 1, 124-20-9; **6**, 622-33-3; **7**, 15255-86-4; **8**, 110272-02-1; **9**, 22513-22-0; **10**, 117679-85-3; 11, 117686-97-2; **12a**, 77359-11-6; **12b**, 40204-26-0; **13a**, 117679-86-4; **13b**, 117679-87-5; 14, 117679-88-6; **16**, 22129-07-3; **17**, 39896-97-4; **18**, 16644-57-8; **19**, 89965-56-0; **20**, 79581-93-4; **21**, 103-40-2; **22**, 117679-89-7; **23**, 117679-90-0; 24, 117679-91-1; **25**, 117679-92-2; **26**, 117679-93-3; **27**, 117679-94-4; **28**, 117679-95-5; **29a**, 117679-96-6; **29b**, 117679-97-7; **30a**, 117679-98-8; **30c**, 117679-99-9; 31, 117680-00-9; **32a**, 117680-01-0; **32b**, 117680-02-1; **32c**, 117680-03-2; **33a**, 117680-04-3;

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33c, 117680-05-4; **35**, 2411-83-8; **36**, 2169-27-9; **37a**, 74272-78-9; **37b**, 1251-38-6; **38a**, 74272-81-4; **34**, 303-38-8; **38b**, 78217-75-1; **39a**, 117680-06-5; **39b**, 117680-07-6; **39c**, 117680-08-7; **40a**, 117680-09-8; **40b**, 117680-10-1; **41a**, 117680-11-2; **41b**, 117680-12-3; **42**, 117680-13-4; **43**, 117680-14-5; **44**, 117680-15-6; P-NBOH, 619-73-8; BzlOH, 100-51-6; $BzlNH_2$, 100-46-9; $H_2N(CH_2)_6NH_2$, 124-09-4; succinic anhydride, 108-30-5; glutaric anhydride, 108-55-4; imidazole, 288-32-4; *N*-hydroxysuccinimide, 6066-82-6.

Synthesis and Antiviral Activity of Phosphonoacetic and Phosphonoformic Acid Esters of 5-Bromo-2′-deoxyuridine and Related Pyrimidine Nucleosides and Acyclonucleosides[†]

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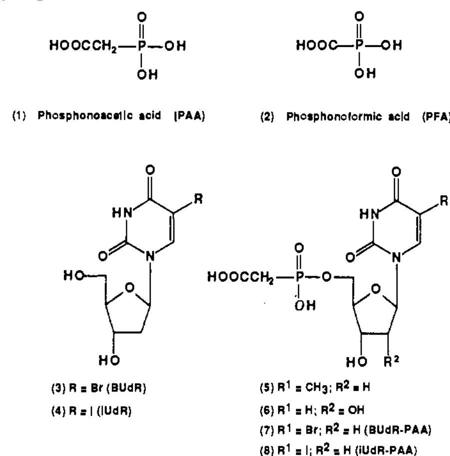
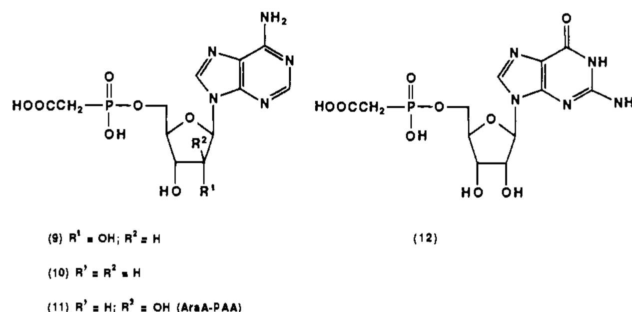
Phosphonoacetic acid (PAA, **1**) was coupled with various acyclonucleosides, 2′-deoxyuridines, cytidines, and arabinosyluracils, with 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) or dicyclohexylcarbodiimide (DCCI) as condensing agents, to give a range of phosphonate esters. The carboxylic ester linkage of PAA to the 5′-position of 5-bromo-2′-deoxyuridine (BUdR, **3**) was achieved via the mixed anhydride formed from (diethylphosphono)acetic acid and trifluoroacetic anhydride. Phosphonoformic acid (PFA, **2**) was coupled with BUdR by using the DCCI method to give the phosphonate ester (**59**). Of these compounds only phosphonate esters in the 2′-deoxyuridine series showed significant activity against herpes simplex virus types 1 and 2. The BUdR-PAA derivative (**7**) and the BUdR-PFA derivative (**59**) were highly active, especially the latter, which was more active than the parent nucleoside BUdR (**3**) against the type 2 virus. The active compounds may exert their effects by extracellular or intracellular hydrolysis to the corresponding antiviral agents, but an intrinsic component of antiviral activity may also be involved.

Phosphonoacetic acid (PAA, **1**) and phosphonoformic acid (PFA, **2**) show good antiviral activity[1,2] against herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2). PAA appears to have a high affinity for bone that may preclude its use in humans[3] whereas PFA (Foscarnet) has been used clinically against HSV-1 and HSV-2.



In an earlier study, PAA had been coupled to the nucleosides adenosine, guanosine, thymidine, uridine, arabinosyladenosine (*ara*-A), 5-bromo-2′-deoxyuridine (BUdR, **3**), and 5-iodo-2′-deoxyuridine (IUdR, **4**) to give the novel compounds **5–12**.[4] Of these compounds, BUdR-PAA (**7**) and IUdR-PAA (**8**) were the most active in the protection



of mice against a systemic infection of HSV-1.[5] These compounds did not inhibit HSV-induced DNA polymerase.[6] In contrast to the naturally occurring nucleoside 5′-monophosphates, these phosphonates were reported[5] to be resistant to the dephosphorylative action of bacterial alkaline and calf intestinal phosphatases. A slow release of nucleoside was observed upon incubation with snake venom 5′-nucleotidase.[5]

We have further investigated the attachment of PAA to BUdR and to the 5′-position of some of the newer antiviral agents that have improved therapeutic ratios. In

[†] Abbreviations used are: PAA = phosphonoacetic acid (1), PFA = phosphonoformic acid (2), BUdR = 5-bromo-2′-deoxyuridine (3), IUdR = 5-iodo-2′-deoxyuridine (4), TPS = 2,4,6-triisopropylbenzenesulfonyl chloride, DCCI = dicyclohexylcarbodiimide, *ara*-A = arabinoadenine, ACG = acycloguanosine, BVDU = (*E*)-5-(2-bromovinyl)-2′-deoxyuridine.

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[§] Biology Group, Roche Products Limited.
[‖] Hoffmann-La Roche Inc.

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