Bentley for the implementation of NAMOD (Beppu, Y. *QCPE* **1979**, *13*, 370), the computer program used for producing the stereo drawings for this paper.

Registry No. **3**, 82830-22-6; **4**, 82830-23-7; **5**, 82830-24-8; **6**, 82830-25-9; **7**, 82830-33-9; **8**, 82830-26-0; **9**, 78025-77-1; **10**, 94236-24-5; **11**, 94347-78-1; **12**, 78025-89-5; **13**, 82830-27-1; **14**, 82830-28-2; **15**, 82830-29-3; **16**, 82830-30-6; **17**, 82830-31-7; **18**, 78025-76-0; **19**, 94236-25-6; **20**, 94236-26-7; **21**, 78025-88-4; DHFR, 9002-03-0.

Synthesis and Dihydropteridine Reductase Inhibitory Effects of Potential Metabolites of the Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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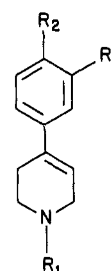
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a nigrostriatal neurotoxin which can cause irreversible parkinsonism in humans and primates by selective destruction of neurons in the substantia nigra. It is possible that MPTP could be metabolized by hydroxylation of the phenyl ring and/or aromatization of its nitrogen-containing ring. Hydroxylated derivatives of 4-phenyl-1,2,3,6-tetrahydropyridine, 4-phenylpiperidine, and 4-phenylpyridine were synthesized and tested in vitro as inhibitors of dihydropteridine reductase (DHPR) from human liver and rat striatal synaptosomes. It was found that all hydroxy derivatives were about 100–10 000 times more inhibitory than MPTP to DHPR. The inhibitory potency of the hydroxylated derivatives increased with the number of hydroxyl substitutions present on the phenyl ring (catechol > phenol) and with oxidation of the nitrogen-containing ring (pyridine > tetrahydropyridine > piperidine).

It is well-known that 4-phenyl-4-(acyloxy)piperidine analgesics can be converted into 4-phenyl-1,2,3,6-tetrahydropyridines (TPYs) by hydrolysis[1] and dehydration[1-3] of the tertiary alcohols, chemically connecting these analgesics[3,4] with tetrahydropyridines. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (**3**, MPTP)[3] and its analogues have been the subject of intense investigation in recent months because of reports that MPTP, a contaminant in an illegally manufactured drug,[5] produced persistent parkinsonian symptoms in individuals who injected the crude drug[5] and in a laboratory worker who was exposed to high levels of MPTP and its analogues.[6] It was found that MPTP produces similar persistent pathological and neurochemical changes in rhesus monkeys[7] but not in guinea pigs or rats.[8] However, some investigators have recently observed neurotoxic effects of MPTP in rats[9,10] and mice.[11] Given these results, it is intriguing to speculate whether the toxic effects of MPTP in humans might be related to the formation and/or clearance of metabolites.[12] Hydroxylation of the phenyl ring of **3**, or aromatization of the heteromoiety, or both, could produce a series of metabolites of potential physiological importance.

In this paper we report an efficient synthesis of the catecholic TPYs **9** and **13**, the piperidine analogues **19** and **21**, and the pyridine **24**, which is the most highly oxidized of the compounds prepared. The recent report that a quaternary 1-methyl-4-phenylpyridinium salt (MPP^+) was found as the metabolite of **3**[13] prompted the preparation of the quaternary salts **25–28** for biological evaluation.



no.	R_1	R_2	R_3
1	H	H	H
2	H	Cl	H
3	CH_3	H	H
4	CH_3	Cl	H
5	CH_3	OH	H
6	CH_3	OH	OCH_3
7	H	OH	H
8	H	OH	OCH_3
9	CH_3	OH	OH
10	$COCH_3$	$OCOCH_3$	OCH_3
11	$COCH_3$	OH	OCH_3
11a	CHO	OH	OCH_3
12	$COCH_3$	OH	OH
13	H	OH	OH
14	$COCH_3$	OCH_3	OCH_3
15	H	OCH_3	OCH_3

At present, no simple model exists to evaluate the neurotoxic effects of such compounds. However, the ef-

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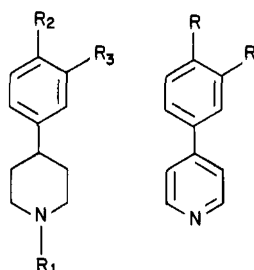
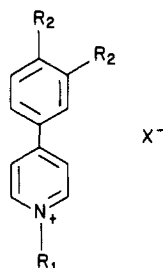


no.	R_1	R_2	R_3	no.	R
16	CH_3	H	H	22	H
17	$COCH_3$	OH	OCH_3	23	OCH_3
18	$COCH_3$	OH	OH	24	OH
19	H	OH	OH		
20	CH_3	OH	OCH_3		
21	CH_3	OH	OH		



no.	R_1	R_2	X
25	CH_3	OH	Br
26	CH_3	H	Br
27	CH_3	H	CH_3OSO_3
28	CH_2=$CHCH_2$	H	Br

fects of potential MPTP metabolites on dihydropteridine reductase (DHPR) have been assessed.[12] This enzyme catalyzes the conversion of dihydrobiopterin to tetrahydrobiopterin, the required cofactor for enzymatic hydroxylation of L-tyrosine to L-dopa. Hence DHPR plays an essential role in regeneration of tetrahydrobiopterin, an important effector molecule in regulating the enzymatic hydroxylation of aromatic amino acids. This reaction represents the rate-limiting step in dopamine synthesis.

Chemistry. TPY **1**, **2** are commercially available as hydrochloride salts and **3** as the free base. TPY **4**[14] was prepared from **2** by N-carbethoxylation and reduction of the carbamate with LAH. The hydroxy-substituted TPY **5**[2] and the *O*-methyl ether **6** were obtained in the condensation of phenol and guajacol, respectively, with 1-methyl-4-piperidone in acetic acid saturated with hydrogen chloride[2] and could be separated from the 4,4-diaryl-substituted byproducts by crystallization or chromatography. A similar procedure also afforded the secondary amine analogues **7** and **8** when 4-piperidone was used in the condensation reaction. The yields of 4-aryl-substituted TPYs were considerably better with 4-piperidone than with 1-methyl-4-piperidone, and a synthesis of **6** by N-methylation of **8** proved to be a better route for making this compound.

The presence of a hydroxy group at C-4′ and a methoxy group at C-3′ in **6** was suggested by NMR comparison in neutral and alkaline medium and was later found to be correct by a single-crystal X-ray analysis of the *N*-formyl analogue of **11** (compound **11a**),[15] obtained from **8** with ethyl formate in DMF.

Catechol **9** was prepared from **6** by ether cleavage with a 1:1 mixture of 48% hydrobromic acid and acetic acid under reflux. The catechol **13** could not be obtained from **8** by similar treatment but could be produced by the following sequence of reactions: N,O-acetylation of **8** to **10**, mild alkaline hydrolysis of **10** to **11**, O-demethylation of **11** to **12** with boron tribromide in chloroform, and N-deprotection of **12** with refluxing 2 N hydrochloric acid afforded **13** as its hydrochloric salt.

The *N*-acetyl TPY **11** proved to be a useful synthon for connecting TPYs with the corresponding piperidines and pyridines. Catalytic reduction of TPYs to piperidines with Raney Ni in methanol was reported.[1] TPY **11** similarly afforded **17** when reduced over Adam's catalyst in acetic acid. Cleavage of the methoxy group in **17** was accomplished with boron tribromide in chloroform. Further hydrolysis of **18** with refluxing 2 N hydrochloric acid afforded **19** as the hydrochloride salt. On the other hand, O-methylation of **11** with dimethyl sulfate yielded the amide **14**, which after deprotection with refluxing 2 N hydrochloric acid afforded **15**, which was smoothly aromatized to pyridine **23** with Pd/C in refluxing xylene.[16] Cleavage of both methoxy groups by refluxing **23** with 1:1 mixture of 48% hydrobromide acid and acetic acid afforded catecholic pyridine **24**, which was isolated in the form of a hydrobromide salt. The quaternary methobromide **25** was prepared from **24** with methyl bromide in methanol, the methobromide **26** from 4-phenylpyridine **22** with methyl bromide in acetone, and the methosulfate **27** from **22** with dimethyl sulfate in acetone. Treatment of **22** with allyl bromide similarly afforded the allyl bromide **28**.

1-Methyl-4-phenylpiperidine (**16**)[17] was prepared by reduction of **3** with Pt in the acetic acid and isolated as a hydrochloride salt.

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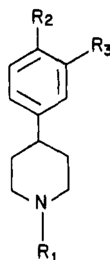
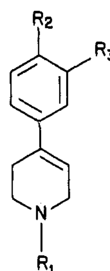
Table I. Inhibition of Dihydropteridine Reductase by 4-Phenylpiperidines[a]



no.	R_1	R_2	R_3	compound	I_{50}, M human liver	I_{50}, M rat striatal synaptosomes
16	CH_3	H	H	1-methyl-4-phenylpiperidine	2.3×10^{-2}	1.2×10^{-2}
17	$COCH_3$	OH	OCH_3	1-acetyl-4-(3′-methoxy-4′-hydroxyphenyl)piperidine	2.7×10^{-5}	4.4×10^{-5}
18	$COCH_3$	OH	OH	1-acetyl-4-(3′,4′-dihydroxyphenyl)piperidine	5.2×10^{-6}	3.1×10^{-6}
19	H	OH	OH	4-(3′,4′-dihydroxyphenyl)piperidine	4.4×10^{-5}	3.8×10^{-5}
20	CH_3	OH	OCH_3	1-methyl-4-(3′-methoxy-4′-hydroxyphenyl)piperidine	1.9×10^{-5}	2.0×10^{-5}
21	CH_3	OH	OH	1-methyl-4-(3′,4′-dihydroxyphenyl)piperidine	4.2×10^{-5}	6.0×10^{-5} [b]

[a] Human liver enzyme (6 mU or 60 ng of protein) or rat striatal synaptosomes (6.5 mU or 75 μg twice-washed P_2 protein) was incubated for 10 min with each compound at 25 °C. Residual enzyme activity was assayed at 50 μM of each substrate to obtain I_{50} values and at different $qDMPH_2$ concentrations (20–50 μM) while the concentration of NADH was kept constant (50 μM), to obtain K_i values. At least six and three concentrations of the test compounds were incubated with the enzyme preparations to obtain I_{50} and K_i values, respectively.[25] [b] A noncompetitive inhibitor of rat striatal synaptosomal enzyme ($K_i = 7.4 \times 10^{-5}$ M).

Table II. Inhibition of Dihydropteridine Reductase by 4-Phenyl-1,2,3,6-tetrahydropyridines[e]



no.	R_1	R_2	R_3	compound	I_{50}, M human liver	I_{50}, M rat striatal synaptosomes
1	H	H	H	4-phenyl-1,2,3,6-tetrahydropyridine (PTP)	1.2×10^{-2} [a]	6.6×10^{-3} [a]
2	H	Cl	H	4-(4′-chlorophenyl)-1,2,3,6-tetrahydropyridine	3.4×10^{-3} [a]	4.0×10^{-3} [a]
3	CH_3	H	H	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	3.0×10^{-3} [a]	4.6×10^{-3} [a]
					7.2×10^{-3} [b]	8.4×10^{-3} [b]
4	CH_3	Cl	H	1-methyl-4-(4′-chlorophenyl)-1,2,3,6-tetrahydropyridine	2.7×10^{-3} [a]	6.4×10^{-3} [a]
5	CH_3	OH	H	1-methyl-4-(4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine	3.0×10^{-6} [a,c]	2.6×10^{-6} [a]
6	CH_3	OH	OCH_3	1-methyl-4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine	9.3×10^{-6} [a]	5.8×10^{-6} [a]
7	H	OH	H	4-(4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine	5.9×10^{-6} [a]	3.4×10^{-6} [a]
8	H	OH	OCH_3	4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine	7.2×10^{-6} [a]	1.0×10^{-5} [a]
9	CH_3	OH	OH	1-methyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,6-tetrahydropyridine	3.4×10^{-6} [a]	2.5×10^{-6} [a,d]
					1.4×10^{-6} [b]	1.2×10^{-6} [b]
11	$COCH_3$	OH	OCH_3	1-acetyl-4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine	5.0×10^{-6}	9.1×10^{-5}
12	$COCH_3$	OH	OH	1-acetyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,6-tetrahydropyridine	2.8×10^{-7}	2.5×10^{-7}
13	H	OH	OH	4-(3′,4′-dihydroxyphenyl)-1,2,3,6-tetrahydropyridine	3.6×10^{-6} [a]	1.9×10^{-6} [a]
14	$COCH_3$	OCH_3	OCH_3	1-acetyl-4-(3′,4′-dimethoxyphenyl)-1,2,3,6-tetrahydropyridine	1.1×10^{-3}	2.3×10^{-3}

[a] Data reported in ref 12. [b] Values obtained by using 10 μM qBH_2 and 50 μM NADH for assaying enzyme activities. [c] A noncompetitive inhibitor of human liver enzyme ($K_i = 2.8 \times 10^{-6}$ M). [d] A noncompetitive inhibitor of rat striatal synaptosomal enzyme ($K_1 = 3.3 \times 10^{-6}$ M). [e] Note: see Table I for experimental details.

Biochemical Evaluations. Compounds were tested as potential inhibitors of DHPR by using the procedures previously published.[12] Enzyme preparations were obtained from human liver as described previously[18,19] and from rat striatal synaptosomes, prepared by the method of Gray and Whittaker.[20] Each inhibitor was preincubated with DHPR for 10 min at 25 °C and then the reaction rate was determined by the method of Nielsen et al.[21] by recording the decrease in NADH absorbance at 340 nm of a Gilford Model 250 spectrophotometer equipped with a Model 6051 recorder. IC_{50} values, which are defined as the inhibitor concentrations which give 50% inhibition of enzyme activity, were determined, and the dissociation constants of the enzyme–inhibitor complexes (K_i values) were calculated from plots of the reciprocal of apparent maximal velocity vs. inhibitor concentration. Routinely,

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Table III. Inhibition of Dihydropteridine Reductase by 4-Phenylpyridines[a]

22-24

25-28

no.	R	compound	I_{50}, M human liver	I_{50}, M rat striatal synaptosomes
22	H	4-phenylpyridine	2.4×10^{-3}	1.9×10^{-3}
23	OCH_3	4-(3′,4′-dimethoxyphenyl)pyridine	5.3×10^{-3}	2.0×10^{-3}
24	OH	4-(3′,4′-dihydroxyphenyl)pyridine	3.8×10^{-7}	2.8×10^{-7}

no.	R_1	R_2	X	compound	I_{50}, M human liver	I_{50}, M rat striatal synaptosomes
25	CH_3	OH	Br	1-methyl-4-(3′,4′-dihydroxyphenyl)pyridinium bromide	6.3×10^{-7}	1.6×10^{-6} [b]
26	CH_3	H	Br	1-methyl-4-phenylpyridinium bromide (MPP^+)	1.0×10^{-2}	1.2×10^{-2}
27	CH_3	H	CH_3OSO_3	1-methyl-4-phenylpyridinium methosulfate	5.6×10^{-3}	3.4×10^{-3}
28	$CH_2{=}CHCH_2$	H	Br	1-allyl-4-phenylpyridinium bromide	9.4×10^{-3}	7.4×10^{-3}

[a] Note: see Table I for experimental details. [b] A noncompetitive inhibitor of rat striatal synaptosomal enzyme ($K_i = 1.9 \times 10^{-6}$ M).

the synthetic dihydropterin, 2-amino-6,7-dimethyl-4-hydroxydihydropteridine ($qDMPH_2$), was used as a substrate. However, the natural substrate, (6*R*)-dihydrobiopterin (qBH_2), was also tested with selected inhibitors for purposes of comparison.

The results of these studies demonstrate that MPTP, its nor derivative, and the corresponding 4′-chloro derivatives do not inhibit DHPR at lower than mM concentrations, but all hydroxy derivatives were 100–10000 times more active than MPTP (Tables I–III). The inhibitory potency of the hydroxylated derivatives increased with the number of hydroxyl substitutions present on the phenyl ring (catechol > phenol) and with oxidation of the nitrogen-containing ring (pyridine > tetrahydropyridine > piperidine). Compounds with an *N*-acetyl substitution gave greater inhibition of DHPR than their corresponding *N*-methyl derivatives (compare compounds **9** and **12** and compounds **18** and **21**). The inhibition produced by the hydroxylated nor compounds was approximately equivalent to or slightly less than that of their corresponding *N*-methyl derivatives, and the charged and uncharged pyridine derivatives had approximately equal potency. Each compound inhibited rat synaptosomal DHPR to approximately the same extent or slightly more than human liver DHPR, and tests with the natural substrate (qBH_2) gave about twofold greater inhibition than tests with the synthetic substrate ($qDMPH_2$). The experimental values of I_{50} and K_i were essentially identical in all compounds compared, a result which is expected for noncompetitive inhibitors.[22]

Conclusions. Hydroxylated derivatives of MPTP and its analogues are potent inhibitors of dihydropteridine reductase and hence can be added to a sizable group of phenolic or catecholic compounds, including tyrosine metabolites,[23] catecholamines,[24] tetrahydroisoquinolines,[18] catechol estrogens,[25] and aporphines,[26] which have recently been shown to inhibit this enzyme. Many of these compounds are also known to inhibit tyrosine hydroxylase[28-31] and, in the case of apormorphine and its analogues, to act as dopaminergic agonists.[31] These results raise the possibility that hydroxylated derivatives of MPTP may also inhibit tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, or bind to dopaminergic receptors, although recent studies by Kula et al.[32] showed no effect of MPTP or its *N*-propyl analogue on the binding of ^{3}H-labeled spiperone and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene in membrane preparations from calf caudate nucleus. However, the neurotoxicity of MPTP in calves has not yet been established, and some species (e.g., rats[33]) are relatively resistant to MPTP effects.

Recent studies by Phillips and Kaufman[34] have shown that the natural cofactor, (6*R*)-tetrahydrobiopterin (BH_4), not only acts as a cofactor in the hydroxylation of phenylalanine to tyrosine but also strongly inhibits the activation of phenylalanine hydroxylase by serving as a negative effector molecule. If BH_4 has a similar dual role in the regulation of tyrosine hydroxylase, the inhibition of DHPR by hydroxylated metabolites of MPTP could initially reduce the availability of BH_4, resulting in activation of tyrosine hydroxylase and increased dopamine synthesis and turnover. Mytilineou and Cohen[35] have suggested that

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increased intraneuronal turnover of dopamine would lead to elevated levels of hydrogen peroxide, which is a product of the oxidative deamination of dopamine and can produce cellular toxicity. Also, MPTP is metabolized in vitro to MPP^+ and presumably hydrogen peroxide by monoamine oxidase, a process which is blocked by MAO-B-specific inhibitors.[13b] Current studies in our laboratories are designed to test these hypotheses.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are corrected. NMR spectra were recorded with a Varian HR-220 spectrometer or a JEOL FX-100 spectrometer with $(CH_3)_4Si$ as the internal reference. Chemical-ionization mass spectra were obtained on a Finnigan 1015 D spectrometer with a Model 6000 data collection system and electron-ionization mass spectra were obtained with a V.G. Micromass 7070F spectrometer (70 eV). Thermospray LC/MS spectra were performed by Dr. A. L. Yergey, Laboratory of Theoretical and Physical Biology, NICHD, NIH. Elemental analysis were performed by the Section on Microanalytical Services and Instrumentation, Laboratory of Chemistry, NIADDK, NIH.

Compounds 1 and 2 as hydrochloride salts and 3 and 22 as free bases were purchased from Aldrich Chemical Co. Compound 5 was obtained as described in ref 2.

1-Methyl-4-(4′-chlorophenyl)-1,2,3,6-tetrahydropyridine (4). A mixture of 2 (0.83 g, 4.3 mmol), methyl chloroformate (0.76 g, 0.62 mL, 8.0 mmol), and anhydrous potassium carbonate (1.6 g, 12.0 mmol) in 15 mL of dry acetone was refluxed with stirring for 14 h. The mixture was evaporated to dryness, dissolved in 100 mL of water, and extracted four times with chloroform. The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure and the residue (1.0 g of yellow oil) was purified by short-column chromatography (SiO_2, CH_2Cl_2) to yield the carbamate (0.64 g) as an colorless oil: EIMS, *m/e* 252 ($M^+ - 1$); ^1H NMR ($CDCl_3$) δ 3.7 (s, 3 H, NCH_3). This material was reduced with $LiAlH_4$ (0.25 g) in THF (20 mL, reflux, 10 h). The product was isolated after usual workup as a solid and then was converted to the hydrochloride salt of 4 and crystallized from isopropyl ether–ethanol to yield 0.58 g (55%) of colorless crystals: mp 171–172 °C; CIMS, *m/e* 208 ($M^+ + 1$); ^1H NMR ($CDCl_3$) of the free base 4 δ 7.30 (m, 4 H, arom), 6.05 (m, 1 H, H-5), 3.10 (m, 2 H, H-2), 2.65 (t, 2 H, H-6, J_{5-6} = 5 Hz), 2.55 (m, 2 H, H-3), 2.40 (s, 3 H, CH_3). Anal. ($C_{12}H_{15}NCl_2$) C, H, N.

1-Methyl-4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (6). Compound 6 was obtained in 35% yield in a manner similar to 8 from 1-methyl-4-piperidone and guaiacol: CIMS, *m/e* 220 ($M^+ + 1$); ^1H NMR (Me_2SO-d_6) δ 9.00 (br s, 1 H, OH), 6.95 (d, 1 H, H-2′, $J_{2'-6'}$ = 2 Hz), 6.83 (dd, 1 H, H-6′, $J_{5'-6'}$ = 8 Hz, $J_{2'-6'}$ = 2 Hz), 6.76 (d, 1 H, H-5′, $J_{5'-6'}$ = 8 Hz), 5.99 (m, 1 H, H-5), 3.80 (s, 3 H, OCH_3), 2.98 (d, 2 H, H-6, J_{5-6} = 2 Hz), 2.53 (m, 2 H, H-2), 2.45 (m, 2 H, H-3), 2.28 (s, 3 H, NCH_3); ^1H NMR (Me_2SO-d_6 + 1 N KOH in CH_3OH) δ 6.80 (d, 1 H, H-2′), 6.73 (dd, 1 H, H-6′), 6.45 (d, 1 H, H-5′). Hydrobromide salt: mp 221–223 °C (from ethanol–acetone). Hydrochloride salt: mp 250–252 °C (from ethanol–acetone). Anal. ($C_{13}H_{18}NO_2Cl$) C, H, N, Cl.

4-(4′-Hydroxyphenyl)-1,2,3,6-tetrahydropyridine (7). Gaseous HCl was passed for 3 min through solution of 15.36 g (0.1 mol) of 4-piperidone monohydrate and 9.4 g (0.1 mol) of phenol in 50 mL of glacial acetic acid. The reaction mixture was then heated on a steam bath for 10 min, and HCl was passed again for 2 min. The reaction mixture was then kept at room temperature. After 2 days the crystalline material which had separated was filtered, washed with cold 2-propanol, and dried, yielding 6.73 g (32%) of colorless crystals of 7·HCl: mp 275–278 °C. The mother liquid after concentration under reduced pressure gave a second crop of crystalline 7·HCl (2.87 g). Total yield: 45%.

A small sample of the hydrochloride salt was converted to the free base by dissolving in water and neutralizing with sodium hydroxide, affording colorless crystals of 7: mp 226–228 °C; CIMS, *m/e* 176 ($M^+ + 1$); ^1H NMR (Me_2SO-d_6) δ 7.30 (d, 2 H, H-2′, 6′, $J_{2'-3'} = J_{5'-6'}$ = 8 Hz), 6.80 (d, 2 H, H-3′,5′, J = 8 Hz), 5.98 (s, 1 H, H-5), 5.70 (br s, 1 H, OH), 3.9 (br s, 1 H, NH), 3.60 (s, 2 H, H-6), 3.16 (t, 2 H, H-2, J_{2-3} = 6 Hz), 2.58 (m, 2 H, H-3). Anal. ($C_{11}H_{14}NOCl$) C, H, N, Cl.

4-(3′-Methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (8). A solution of guajacol (6.2 g, 0.05 mmol) and 4-piperidone monohydrate (11.7 g, 0.10 mol) in glacial acetic acid (50 mL) was stirred at room temperature while HCl gas was passed through for 3 min. The reaction mixture was then heated on a steam bath for 10 min, and then HCl was passed through the solution for another 2 min. After 2 days standing at room temperature, the reaction mixture was concentrated under reduced pressure and the residue dissolved in 100 mL of water. The solution was washed with chloroform, made alkaline with concentrated sodium hydroxide, washed with chloroform, adjusted to pH 8 with 37% HCl, and extracted four times with chloroform–2-propanol (5:1). The combined organic extracts were dried (Na_2SO_4), evaporated to dryness, and crystallized from ethanol to yield product 8 (8.2 g, 81%) as orange-pink crystals. A small analytical sample obtained after two more crystallizations from ethanol gave almost colorless crystals: mp 186–188 °C; EIMS, *m/e* 205 (M^+); ^1H NMR (Me_2SO-d_6) δ 6.75 (m, 3 H, arom), 6.00 (m, 1 H, H-5), 3.75 (s, 3 H, OCH_3), 3.30 (m, 2 H, H-6), 2.85 (t, 2 H, H-2, J_{2-3} = 6 Hz), 2.24 (m, 2 H, H-3). Hydrochloride salt (from methanol): mp 229–231 °C. Anal. ($C_{12}H_{16}NO_2Cl$) C, H, N, Cl.

1-Methyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,6-tetrahydropyridine Hydrobromide (9·HBr). The hydrobromide salt of 6 (1.02 g, 3.4 mmol) was dissolved in 20 mL of a 48% HBr–AcOH (1:1) mixture and refluxed under an argon atmosphere for 2 h. After evaporation to dryness under reduced pressure, it was taken up in chlorobenzene and evaporated twice to remove excess HBr. The residue was crystallized from ethanol, yielding 830 mg (86%) of 9·HBr as colorless crystals: mp 239–241 °C; CIMS, *m/e* 205 ($M^+ + 1$); ^1H NMR (Me_2SO-d_6) of the free base δ 6.85 (s, 1 H, H-5′), 6.70 (s, 2 H, H-2′,6′), 5.90 (d, 1 H, H-5, J_{5-6} = 5 Hz), 3.00 (d, 2 H, H-3, J_{2-3} = 5 Hz), 2.60 (m, 2 H, H-2), 2.40 (m, 2 H, H-3), 2.30 (s, 3 H, NCH_3). Anal. ($C_{12}H_{16}NO_2Br$) C, H, N, Br.

1-Acetyl-4-(3′-methoxy-4′-acetoxyphenyl)-1,2,3,6-tetrahydropyridine (10). To a solution of 8 (5.0 g, 24 mmol) in 100 mL of dry pyridine were added 25 mL of acetic anhydride and 250 mg of DMAP. The solution was kept overnight at room temperature and then evaporated under reduced pressure. The residue was dissolved in chloroform, washed with dilute hydrochloric acid and brine, dried (Na_2SO_4), and evaporated to dryness under reduced pressure to produce 6.56 g (93%) of an almost colorless crystalline product, which was used for the next step without further purification.

An analytical sample was crystallized from acetone–isopropyl ether to yield colorless crystals: mp 119–121 °C; EIMS, *m/e* 289 (M^+); ^1H NMR ($CDCl_3$) δ 6.90 (m, 3 H, arom), 5.95 (m, 1 H, H-5), 4.15 (m, 2 H, H-6), 3.82 (s, 3 H, OCH_3), 3.70 (m, 2 H, H-2), 2.50 (m, 2 H, H-3), 2.30 (s, 3 H, $OCOCH_3$), 2.06 (ss, 3 H, $NCOCH_3$). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

1-Acetyl-4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (11). The crude product 10 (6.0 g, 21 mmol) was dissolved together with 3.0 g of potassium carbonate in 100 mL of methanol–water (1:1) and the reaction mixture was stirred overnight at room temperature. It was then concentrated under reduced pressure to approximately 50 mL and extracted three times with chloroform. The combined extracts were dried (Na_2SO_4) and evaporated to dryness, and the crude product was purified by short-column chromatography (silica gel, $CHCl_3$–MeOH, 98:2) and crystallized from ethyl acetate to yield 4.05 g of 11 as colorless crystals: mp 149–152 °C; EIMS, *m/e* 247 (M^+); ^1H NMR (Me_2SO-d_6) δ 9.00 (br s, 1 H, OH), 6.90 (m, 3 H, arom), 6.00 (m, 1 H, H-3), 4.05 (m, 3 H, H-6), 3.80 (s, 3 H, OCH_3), 3.60 (m, 2 H, H-2), 2.45 (m, 2 H, H-3), 2.05 (ss, 3 H, $NCOCH_3$). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

1-Formyl-4-(3′-methoxy-4′-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (11a). A solution of 8 (1.0 g, 4.9 mmol) in DMF (10 mL) and ethyl formate (20 mL) was refluxed for 2 days. The reaction mixture was evaporated under reduced pressure, dissolved in chloroform, washed with sodium carbonate solution, dried, and

(36) Karle, J.; Karle, I. L. *Acta Crystallogr.* **1966**, *21*, 849.
(37) Sheldrick, G. M. SHELXTL, microcomputer programs for structure determination, University of Göttingen, West Germany, 1980.

evaporated to dryness. The residue was crystallized from ethyl acetate to yield **11a** (830 mg, 73%) as colorless crystals: mp 173–175 °C; EIMS, *m/e* 233 (M^+); ^1H NMR ($CDCl_3$) δ 9.00 (s, 1 H, OH), 8.15 (ss, 1 H, CHO), 7.00 (s, 1 H, H-2′), 6.80 (m, 2 H, H-5′,6′), 6.05 (m, 1 H, H-5), 4.05 (m, 2 H, H-6), 3.85 (s, 3 H, OCH_3), 3.65 (m, 2 H, H-2) 2.45 (m, 2 H, H-3). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

Crystallographic Data. $C_{13}H_{15}NO_3$ (M_r 233.29). Crystal, clear plate, approximately 0.35 × 0.35 × 0.05 mm, orthorhombic, space group *Pbca*; unit cell, *a* = 6.563 (2) Å, *b* = 21.964 (9) Å, *c* = 16.016 (6) Å, *V* = 2308 (1) Å^3, *Z* = 8, d_{calcd} = 1.34 g/cm^{-3}. A Nicolet R3 diffractometer was used with graphite monochromated Mo Kα radiation (λ = 0.71069 Å). A total of 3311 reflections were measured, 1497 unique, 983 considered observed [$I_{obsd} > 3\sigma(I_{obsd})$]. The structure was solved with direct methods[36] as implemented in the program SHELXTL.[37] Least-squares refinement of the model gave crystallographic agreement factors of R = 0.088, R_w = 0.067 for 983 reflections, and a goodness-of-fit parameter of 1.60. Coordinates and thermal parameters for the molecule have been deposited with the Crystallographic Data Centre, Cambridge University, University Chemical Lab, Cambridge CB2 1EW, England.

1-Acetyl-4-(3′,4′-dihydroxyphenyl)-1,2,3,6-tetrahydropyridine (12). A solution of **11** (2.5 g, 10 mmol) in 50 mL of chloroform was cooled to 0 °C, and 10 g (3.8 mL, 40 mmol) of boron tribromide was added dropwise with stirring. The reaction mixture was stirred 15 min at 0 °C and another 15 min at room temperature. Crushed ice (50 g) was added, the chloroform layer separated, and the aqueous layer extracted three times with chloroform–2-propanol (5:1). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure, giving 2.11 g (89%) of a bright pink solid. The product was purified by column chromatography (SiO_2, $CHCl_3$–MeOH, 95:5), and crystallized from isopropyl ether–ethanol to yield colorless crystals: mp 185–187 °C; CIMS, *m/e* 234 (M^+ + 1); ^1H NMR (Me_2SO-d_6) 8.85 (br s, 2 H, OH), 6.75 (m, 3 H, arom), 4.90 (br s, 1 H, H-5), 4.02 (br s, 2 H H-6), 3.55 (m, 2 H, H-2), 2.40 (m, 2 H, H-3), 2.01 (ss, 3 H, $NCOCH_3$). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

4-(3′,4′-Dihydroxyphenyl)-1,2,3,6-tetrahydropyridine Hydrochloride (13·HCl). Compound **12** (600 mg, 4.3 mmol) was refluxed with 20 mL of 2 N HCl for 2 h. The reaction mixture was evaporated under reduced pressure, taken up in chlorobenzene, and evaporated twice to remove excess HCl. The hydrochloride salt was crystallized from ethanol to yield 420 mg (72%) of slightly yellow, fine crystals: mp 219–222 °C; EIMS, *m/e* 191 (M^+); ^1H NMR (Me_2SO-d_6) δ 9.20 (br m, 4 H, OH, ^+NH), 6.88 (s, 1 H, H-5′), 6.66 (s, 2 H, H-2′,6′), 5.90 (br s, 1 H, H-5), 3.63 (m, 2 H, H-6), 3.22 (m, 2 H, H-2), 2.54 (m, 2 H, H-3). Anal. ($C_{11}H_{14}NO_2Cl$) C, H, N, Cl.

1-Acetyl-4-(3′,4′-dimethoxyphenyl)-1,2,3,6-tetrahydropyridine (14). Compound **11** (700 mg, 2.8 mmol) was dissolved in 40 mL of acetone, 2.5 g of anhydrous potassium carbonate was added, and a solution of dimethyl sulfate (0.5 mL, 0.55 mmol) in 10 mL of acetone was added dropwise, with stirring, under argon atmosphere. The mixture was stirred for 3 h at room temperature, evaporated to dryness, dissolved in 50 mL of water, and extracted three times with chloroform. The combined chloroform extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure, and the residue was crystallized from isopropyl ether–acetone, yielding 670 mg (91%) of colorless crystals: mp 90–92 °C; EIMS, *m/e* 261 (M^+); ^1H NMR ($CDCl_3$) δ 6.84 (m, 3 H, arom), 5.92 (m, 1 H, H-5), 4.12 (m, 2 H, H-6), 3.88 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.65 (m, 2 H, H-2), 2.50 (m, 2 H, H-3), 2.12 (ss, 3 H, $NCOCH_3$).

4-(3′,4′-Dimethoxyphenyl)-1,2,3,6-tetrahydropyridine (15). A solution of **14** (650 mg, 2.5 mmol) in 30 mL of 2 N HCl was refluxed for 2 h. The reaction mixture was cooled, neutralized with 2 N NaOH, and extracted three times with chloroform. The combined chloroform extracts were dried (Na_2SO_4), evaporated to dryness, and chromatographed on silica gel ($CHCl_3$), giving 650 mg (85%) of **15** as a colorless oil: CIMS, *m/e* 220 (M^+ + 1); ^1H NMR ($CDCl_3$) δ 6.90 (m, 3 H, arom), 6.05 (m, 1 H, H-5), 4.90 (s, 3 H, OCH_3), 4.88 (s, 3 H, OCH_3), 3.55 (m, 2 H, H-6), 3.10 (t, 2 H, H-2, J_{2-3} = 6 Hz), 2.45 (m, 2 H, H-3). Hydrochloride salt of **15**: mp 193–196 °C (from isopropyl ether–methanol). Anal. ($C_{13}H_{18}NO_2Cl$) C, H, Cl.

1-Acetyl-4-(3′-methoxy-4′-hydroxyphenyl)piperidine (17). To a solution of **11** (1.62 g, 6.6 mmol) in 50 mL of glacial acetic acid was added 200 mg of PtO_2 (Adam's catalyst), and the reaction mixture was stirred overnight under a hydrogen atmosphere. The catalyst was filtered off, the solution was evaporated under reduced pressure, and the residue was dissolved in chloroform, washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated under reduced pressure. The product was crystallized from ethyl acetate to yield 1.47 g (90%) of colorless crystals: mp 153–156 °C; CIMS, *m/e* 250 (M^+ + 1); ^1H NMR ($CDCl_3$) δ 6.70 (m, 3 H, arom), 5.75 (s, 1 H, OH), 4.75 (m, 1 H), 3.90 (m, 1 H), 3.85 (s, 3 H, OCH_3), 3.10 (m, 1 H), 2.60 (m, 2 H), 2.10 (s, 3 H, $NCOCH_3$), 1.80 (m, 4 H, H-3,5). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

1-Acetyl-4-(3′,4′-dihydroxyphenyl)piperidine (18). Compound **18** was obtained in 77% yield as colorless crystals by O-demethylation of **17** (2.68 g, 10.7 mmol) with BBr_3 as described for the conversion of **11** to **12** affording colorless crystals: mp 172–174 °C (from isopropyl ether–ethanol); CIMS, *m/e* 236 (M^+ + 1); ^1H NMR (Me_2SO-d_6) δ 8.65 (ss, 2 H, OH), 6.55 (m, 3 H, arom), 4.45 (m, 1 H), 3.85 (m, 1 H), 3.02 (m, 1 H), 2.42 (m, 2 H), 1.99 (s, 3 H, $NCOCH_3$), 1.48 (m, 4 H, H-3,5). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

4-(3′,4′-Dihydroxyphenyl)piperidine Hydrochloride (19·HCl). Compound **18** was converted to 68% yield to **19** as described for the conversion **12** to **13**. The hydrochloride salt of **19** was isolated as bright pink, fine crystals: mp 268 °C dec; CIMS, *m/e* 194 (M^+ + 1); ^1H NMR (Me_2SO-d_6) δ 8.80 (m, 4 H, OH, $^+NH_2$), 6.55 (m, 3 H, arom), 3.40–2.40 (m, 5 H, H-2,4,6), 1.75 (m, 4 H, H-3,5). Anal. ($C_{11}H_{16}NO_2Cl$) C, H, N.

1-Methyl-4-(3′-methoxy-4′-hydroxyphenyl)piperidine (20). **(a) By Catalytic Reduction of 6.** Compound **6** was converted in 81% yield to **20** in a similar way as described for the conversion of **11** to **17**. The product, isolated as the hydrobromide salt, was obtained as colorless crystals: mp 210–211 °C (from acetone–ethanol); CIMS, *m/e* 222 (M^+ +]); ^1H NMR (Me_2SO-d_6) of the free base δ 8.60 (br s, 1 H, OH), 6.60 (m, 3 H, arom), 3.72 (s, 3 H, OCH_3), 2.80 (m, 4 H, H-2,6), 2.15 (s, 3 H, NCH_3), 1.89 (m, 1 H, H-4), 1.64 (m, 4 H, H-3,5). Anal. ($C_{13}H_{20}NO_2Br$) C, H, N, Br.

(b) By N-Methylation and Reduction of 8. The hydrochloride salt of **8** (120 mg, 0.5 mmol) was dissolved in 10 mL of 20% acetic acid, to which 150 mg of sodium acetate, 0.2 mL of 37% aqueous formaldehyde solution, and 100 mg of 10% Pd/C were added, and the reaction mixture was stirred for 12 h under a hydrogen atmosphere. The catalyst was filtered off, and the solution neutralized with $NaHCO_3$ and extracted five times with chloroform. The combined chloroform extracts were dried (Na_2SO_4) and evaporated under reduced pressure, and the product was converted to the hydrobromide salt and crystallized from ethanol–acetone, giving 128 mg (85%) of **20·HBr** as colorless crystals: mp 209–211 °C; identical with the material prepared under (a).

1-Methyl-4-(3′,4′-dihydroxyphenyl)piperidine Hydrobromide (21·HBr). Compound **20** was converted with 88% yield to **21** in a manner similar to the conversion of **6** to **9**. The hydrobromide salt of **21**: mp 208–210 °C; CIMS, *m/e* 208 (M^+ + 1); ^1H NMR (Me_2SO-d_6) of the free base δ 8.5 (br s, 2 H, OH), 6.45 (m, 3 H, arom), 2.83 and 2.72 (ss, 4 H, H-2,6), 2.12 (s, 3 H, NCH_3), 1.87 (m, 1 H, H-4), 1.56 (m, 4 H, H-3,5). Anal. ($C_{12}H_{18}NO_2Br$) C, H, N, Br.

4-(3′,4′-Dimethoxyphenyl)pyridine (23). A solution of **15** (1.0 g, 4.6 mmol) in 50 mL of o-xylene was refluxed for 12 h with 200 mg of 10% Pd/C, passing argon through the solution. The catalyst was filtered off and the filtrate evaporated under reduced pressure. The product was converted to the hydrobromide salt and crystallized from acetone, giving 1.1 g (81%) of **23·HBr** as colorless crystals: mp 245–247 °C; EIMS, *m/e* 215 (M^+); ^1H NMR ($CDCl_3$) of the free base δ 8.65 (d, 2 H, H-2,6; J_{2-3} = J_{5-6} = 5 Hz), 7.45 (d, 2 H, H-3,5, J = 5 Hz), 7.20 (m, 2 H, H-2′,5′), 6.95 (d, 1 H, H-5′, $J_{5'-6'}$ = 9 Hz), 3.98 and 3.99 (ss, 6 H, OCH_3). Anal. ($C_{13}H_{14}NO_2Br$) C, H, N, Br.

4-(3′,4′-Dihydroxyphenyl)pyridine Hydrobromide (24·HBr). Compound **23** was converted into **24** by refluxing in HBr–AcOH as described for the convertion of **6** to **9**, giving bright yellow crystals (72% yield): mp 206–209 °C (ethanol); EIMS, *m/e* 187 (M^+); ^1H NMR (Me_2SO-d_6) of the free base δ 9.2 (br s, 2 H, OH), 8.50 (d, 2 H, H-2,6, J_{2-3} = J_{5-6} = 5 Hz), 7.50 (d, 2 H, H-3,5,

J = 5 Hz), 7.10 (m, 2 H, H-2',6'), 6.80 (d, 1 H, H-5', $J_{5'-6'}$ = 8.5 Hz). Anal. ($C_{11}H_{10}NO_2Br$) C, H, N.

1-Methyl-4-(3',4'-dihydroxyphenyl)pyridinium Bromide (25). Compound **24** (free base, 50 mg, 0.27 mmol) was dissolved in 40 mL of methanol, 250 mg of methyl bromide was added, and the reaction mixture was kept at room temperature. The progress of the reaction was monitored by TLC. After 1 week, the reaction mixture was concentrated to dryness and the residue crystallized from ethanol to give 51 mg (68%) of fine yellow crystals: mp 244 °C dec; Thermospray LC/MS, m/e 202 (M^+). Anal. ($C_{12}H_{12}NO_2Br$) C, H, N.

1-Methyl-4-phenylpyridinium Bromide (26). To a solution of **22** (2.0 g, 12.9 mmol) in 50 mL of acetone was added 1.7 g (18 mmol) of methyl bromide and the reaction mixture was kept at room temperature overnight. The crystalline, colorless product, which came out of the solution, was filtered, washed with acetone, and dried to yield 2.50 g (78%) of **26**: mp 168–170 °C; Thermospray LC/MS, m/e 170 (M^+). Anal. ($C_{12}H_{12}NBr$) C, H, N, Br.

1-Methyl-4-phenylpyridinium Methosulfate (27). To a solution of **22** (2.0 g, 12.9 mmol) in 50 mL of acetone was added 1.4 mL (1.86 g, 14.8 mmol) of dimethyl sulfate, and the reaction mixture was kept at 50 °C for 3 h. The product came out of the solution as colorless needles, which were filtered, washed with acetone, and dried, giving 2.94 g (82%) of a crystalline product: mp 165–167 °C; Thermospray LC/MS, m/e 170 (M^+). Anal. ($C_{13}H_{15}NO_4S$) C, H, N, S.

1-Allyl-4-phenylpyridinium Bromide (28). To a solution of **22** (2.0 g, 12.9 mmol) in 50 mL of acetone was added allyl bromide (1.75 g, 14.5 mmol) and the reaction mixture was kept at room temperature overnight. The product crystallized out of solution as almost colorless needles. These were filtered, washed with acetone, and dried, giving 2.64 g (74%) of product: mp 153–156 °C; Thermospray LC/MS, m/e 196 (M^+). Anal. ($C_{14}H_{14}NBr$) C, H, N, Br.

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Registry No. **1**, 10338-69-9; **2**, 30005-58-4; **3**, 28289-54-5; **4**, 5048-08-8; **4**·HCl, 6653-08-3; **5**, 5233-54-5; **6**, 90684-18-7; **6**·HBr, 94427-28-8; **6**·HCl, 94427-29-9; **7**, 90684-15-4; **7**·HCl, 94427-30-2; **8**, 90684-19-8; **8**·HCl, 94427-31-3; **9**, 90684-16-5; **9**·HBr, 94427-32-4; **10**, 94427-33-5; **11**, 94427-34-6; **11a**, 94427-35-7; **12**, 94427-36-8; **13**·HCl, 94427-37-9; **14**, 94427-38-0; **15**, 94427-39-1; **16**, 774-52-7; **17**, 94427-40-4; **18**, 94427-41-5; **19**, 94427-43-7; **19**·HCl, 94427-42-6; **20**, 94427-44-8; **20**·HBr, 94427-45-9; **21**, 94427-47-1; **21**·HBr, 94427-46-0; **22**, 939-23-1; **23**, 39795-63-6; **23**·HBr, 94427-48-2; **24**, 79445-43-5; **24**·HBr, 79445-42-4; **25**, 94427-49-3; **26**, 2589-31-3; **27**, 39795-54-5; **28**, 94427-50-6; ClC(O)OMe, 79-22-1; PhOH, 108-95-2; HC(O)OEt, 109-94-4; HCHO, 50-00-0; $CH_2{=}CHCH_2Br$, 106-95-6; 1-(methoxycarbonyl)-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine, 94427-27-7; 1-methyl-4-piperidone, 1445-73-4; guaiacol, 90-05-1; 4-piperidone, 41661-47-6.

Artificial Siderophores. 1. Synthesis and Microbial Iron Transport Capabilities

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Several di- and trihydroxamate analogues of natural microbial iron chelators have been prepared. The syntheses involved linkage of core structural units, including pyridinedicarboxylic acid, benzenetricarboxylic acid, nitrilotriacetic acid, and tricarballylic acid, by amide bonds to 1-amino-ω-(hydroxyamino)alkanes to provide the polyhydroxamates **1–5**. The required protected (hydroxyamino)alkanes **8**, **16**, and **21** were prepared by different routes. 1-Amino-3-[(benzyloxy)amino]propane di-p-toluenesulfonate (**8**) was prepared from the N-protected aminopropanol **6** by oxidation to the aldehyde, formation of the substituted oxime, and reduction with $NaBH_3CN$ followed by deprotection of the Boc group. The pentyl derivatives **16** and **21** were made by direct alkylation with either benzyl acetohydroxamate or N-carbobenzoxy-O-benzylhydroxylamine. In *Escherichia coli* RW193 most of the analogues behaved nutritionally as ferrichrome. However, in *E. coli* AN193, a mutant lacking the ferrichrome receptor, capacity to use other natural siderophores was retained while response to all analogues was lost.

Iron is the most abundant transition metal and probably the most well-known metal in biological systems. Within the last 20 years several catechol and hydroxamate-containing microbial iron chelators (siderophores) have been isolated and related biochemical studies have contributed significantly to our knowledge of iron metabolism.[1-4] Siderophores are also important models of the development of drugs for the treatment of iron-overloaded patients.[5-7] Thus, the design of therapeutically useful iron chelating agents should also take into account the evolution-derived design of microbial systems. Desferal is the standard iron chelator used for the treatment of iron-storage diseases. Because Desferal must be administered by injection and large doses are needed to mobilize iron faster than it is accumulated,[8,9] the search for alternatives continues.

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