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TOTAL SYNTHESIS OF VIBRIOBACTIN

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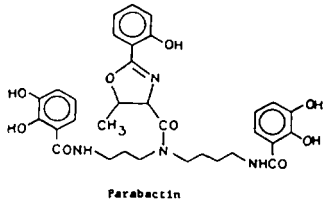
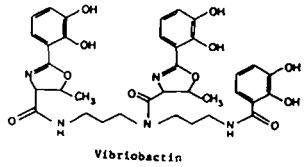
Abstract - The first synthesis of the natural product N-[3-(2,3-dihydroxybenzamido) propyl]-1,3-bis[2-(2,3-dihydroxyphenyl)-trans-5-methyl-2-oxazoline-4-carboxamido]propane (vibriobactin) is described. The synthesis illustrates the problems and solutions involved in the asymmetric functionalization of norspermidine, a triamine which consists of a symmetrical methylene backbone. Finally, the synthesis provides an independent and definitive proof of Neiland's suggested structure of vibriobactin.

Due to the critical role siderophores play in microbial growth processes and the potential they offer as therapeutic agents in the treatment of iron overload diseases, they have received substantial attention from inorganic,[1] organic[2] and biochemists.[3] These natural iron chelators fall largely into two major groups, the hydroxamates and the catecholamides, with the latter demonstrating the highest affinity for iron e.g. enterobactin. Although the catecholamides vary substantially in overall structure, the systems predicated on polyamine backbones seem to be more widespread than initially thought.

Since Tait's isolation and identification of several spermidine-derived catecholamide iron chelators from Paracoccus denitrificans,[4] a number of other similar siderophores have been identified and synthesized. Analogous siderophores, agrobactin and agrobactin A, have been isolated by Neilands from Agrobacterium tumifaciens B_6 cultures.[5] In earlier reports we described the facile synthesis of parabactin[6] (Figure 1), agrobactin A,[7] and more recently, the



Parabactin



Vibriobactin

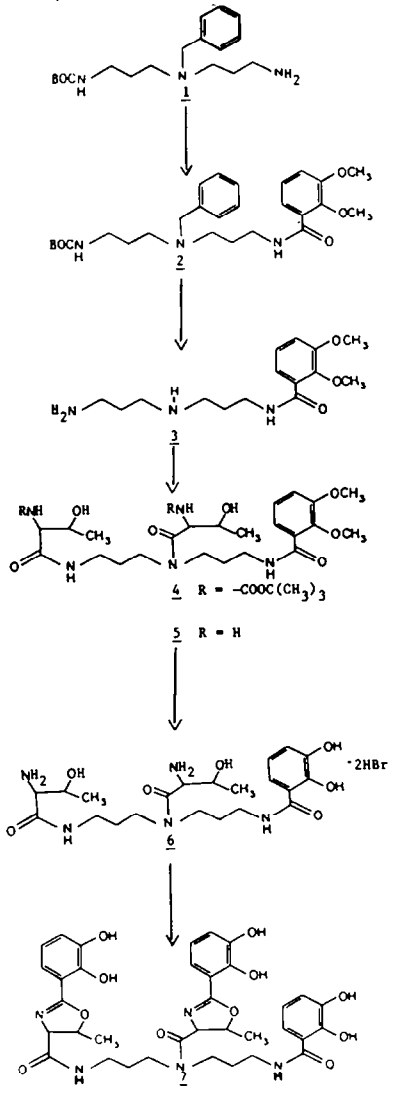
Figure 1

total synthesis of agrobactin.[8] These siderophores all contain the triamine spermidine with its terminal nitrogens acylated to give a bis-[2,3-dihydroxybenzamide] arrangement and have a substituted open or cyclized threonyl group at the N^4 position. The symmetrical spermidine analog N-(3-aminopropyl)-1,3-diaminopropane (norspermidine), which occurs only rarely in nature, has recently been found to be the backbone for a novel siderophore isolated by Neilands[9] from low

iron cultures of Vibrio cholerae. The iron ligand has been identified as N-[3-(2,3-dihydroxybenzamido)propyl]-1,3-bis[2-(2,3-dihydroxyphenyl)-trans-5-methyl-2-oxazoline-4- carboxamido]propane and has been given the trivial name vibriobactin (Figure 1). Unlike the spermidine siderophores vibriobactin has no symmetry with respect to the terminal acyl groups. This suggested the application of our polyamine reagents[10] which allows for the simultaneous primary and secondary amine acylation of triamines.

The synthesis begins with N^4-benzyl-N^1-(t-butoxycarbonyl)norspermidine (1), one of the reagents we have recently developed for the selective functionalization of triamines.[10] This amine was acylated with 2,3-dimethoxybenzoyl chloride in the presence of triethylamine to give the trisubstituted norspermidine (2) in quantitative yield (Figure 2). The functionalized

Figure 2



norspermidine was then deprotected in a stepwise fashion to give the monoacylated norspermidine (3). The order of deprotection is unimportant and intermediates need not be isolated. The t-butoxycarbonyl (BOC) protecting group was removed by brief exposure to trifluoroacetic acid and the N^4-benzyl group was hydrogenolized over palladium chloride in methanolic HCl to give the dihydrochloride salt of the monoacylated norspermidine (3) in 93% yield from two steps. The salt was converted to the free amine by exposure to aqueous base and then bis-acylated in DMF, using the activated ester of (L)-N-tert-butoxy-carbonylthreonine to give the unsymmetrically substituted norspermidine 4 in quantitative yield. As expected on brief exposure of compound 4 to trifluoroacetic acid it collapsed to carbon dioxide, isobutylene and N^1,N^4-bis[(L)-threonyl]-N^7-(2,3-dimethoxybenzoyl)norspermidine (5) in excellent yield (93%). Exhaustive demethylation employ-

ing BBr_3 in methylene chloride afforded the dihydrobromide salt of the completely deprotected norspermidine (6), in moderate yield (63%). The final step required cyclization of each of the threonyl groups with an imidate to give the proper bis-oxazoline configuration. This was readily accomplished by exposure of 6 to an excess of ethyl 2,3-dihydroxybenzimidate[8] in refluxing methanol to give vibriobactin (58%). The product was identical with the naturally occurring compound in its 300 MHz ^1H NMR.

DISCUSSION

The major problem in the synthesis of vibriobactin is related to the disposition of the ligand's acyl groups. A 2-(2,3-dihydroxyphenyl)-trans-5-methyl-2-oxazoline-4-carboxamido group is fixed to both a primary and a secondary amine nitrogen. This asymmetry in structure sets the boundary conditions for the reaction scheme. It suggests that one should either begin with a mono primary amino protected norspermidine or a secondary and primary amine protected norspermidine. The monoprotected triamine would allow for initial fixing of the threonyl groups while the diprotected triamine would allow for initial attachment of the 2,3-dihydroxybenzoyl group. Our polyamine reagents previously described[10] allow for either approach. We chose the latter route in order to avoid the problems associated with acylation of the intermediate bis-threonyl or bis-oxazoline compounds. Of course, one could begin with norspermidine itself and hope for selective monoacylation of its primary amino nitrogen. It is probably unreasonable to hope that such a procedure would proceed in even reasonable yield. Our methodology is of course applicable to both the homospermidine and spermidine analogues of vibriobactin. Although we originally designed these reagents for the synthesis of simple alkyl and acyl polyamines, it is clear that the systems are applicable to complicated natural products. Finally, it is important to point out that our 300 MHz ^1H NMR is identical with Neiland's reported signals, thus providing an independent and definitive proof of structure of vibriobactin.

EXPERIMENTAL

^1H NMR spectra were recorded on a Varian T-60 or a Nicolet NB-300, and unless otherwise noted, in $CDCl_3$ solutions with chemical shifts given in parts per million downfield from an internal tetramethylsilane standard. The IR spectra were recorded on a Beckman Acculab 1 Spectrophotometer. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

N^4-benzyl-N^1-(t-butoxycarbonyl)norspermidine(1). Prepared as previously described.[10]

N^4-benzyl-N^1-(t-butoxycarbonyl)-N^8-(2,3-dimethoxybenzoyl)norspermidine(2). A solution of 1 (1.98 g, 6.2 mmol) and triethylamine (0.7 g, 6.8 mmol) in 30 mL of CH_2Cl_2 was treated dropwise with a solution of 2,3-dimethoxybenzoyl chloride (1.36 g, 6.8 mmol) in 10 mL CH_2Cl_2. After stirring 20 h the solvent was stripped and the residue taken up in the ethyl ether, filtered, and evaporated in vacuo to give 2.98 g (99%) of crude 2. Chromatography on silica gel eluting with 5% MeOH/$CHCl_3$ gave pure 2 as a viscous hygroscopic oil: ^1H NMR δ 1.43 (s, 9H), 1.70 (m, 4H), 2.44 (m, 4H), 2.97-3.60 (m, 6H), 3.76 (s, 3H), 3.94 (s, 3H), 5.20 (br, 1H), 6.9-7.60 (m, 8H), 7.80 (br, 1H); IR (neat) 3350 (m), 1710 (s), 1650 (s), 1455 (s), 1360 (s), 1250 (s), 750 (m), 700 (m) cm^{-1}.

Anal. Cal. for $C_{27}H_{39}N_3O_5 \cdot H_2O$: C, 64.39; H, 8.21; N, 8.34: Found: C, 64.53; H, 7.88; N, 8.16.

N^1-(2,3-dimethoxybenzoyl)norspermidine (3). To a dry 25 mL flask containing 2 (1.26 g, 2.59 mmol) and a stir bar under nitrogen was added trifluoroacetic acid (10 mL). After stirring 1 h the solution was evaporated in vacuo to a light brown oil. The oil was taken up in CH_2Cl_2 (100 mL) and extracted with saturated $NaHCO_3$ (2 x 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo to a volume less than 2 mL. This solution was diluted with methanol (25 mL) and treated with 10 drops of concentrated HCl and $PdCl_2$ (0.10 g). The suspension was stirred under an atmosphere of H_2 for 24 h at which time it was filtered through sintered glass and the filtrate evaporated in vacuo. The resulting hygroscopic white solid was recrystallized with EtOH/Et_2O to give 0.89 g (93%) of pure 3·2 HCl as a hygroscopic white solid.

Anal. Cal. for $C_{15}H_{27}N_3O_3Cl_2$: C, 48.92; H, 7.39; N, 11.41. Found: C, 48.68; H, 7.40; N, 11.34.

The title compound was obtained as the free amine by taking the solid up in H_2O (50 mL) and adding saturated K_2CO_3 (40 mL). The cloudy suspension was extracted with CH_2Cl_2 (5 x 50 mL), the combined organic layers dried over Na_2SO_4, and evaporated in vacuo to give N^1-(2,3-dimethoxybenzoyl)norspermidine (3): ^1H NMR δ 1.30 (br, 3H), 1.75 (m, 4H), 2.70 (m, 6H), 3.9 (quar, 2H), 3.83 (s, 6H), 6.98 (m, 2H), 7.55 (m, 1H), 8.00 (br, 1H); IR 3350 (br), 1650 (s), 1580 (s), 1265 (s), 805 (m), 750 (s) cm^{-1}.

(L)-N-hydroxysuccinimido-N-(tert-butoxycarbonyl)threonate. Prepared as described previously.[2]

N^1,N^4-bis[(L)-N-tert-butoxycarbonylthreonyl)]-N^7-(2,3-dimethoxybenzoyl)norspermidine (4). To a solution of N1-(2,3-dimethoxybenzoyl)norspermidine (0.43 g, 1.46 mmol) in dry DMF (16 mL) was added a solution of freshly prepared (L)-N-hydroxysuccinimido-N-tert-butoxycarbonylthreonate (1.013 g, 3.20 mmol) in DMF (16 mL). After stirring for 68 h the solvent was removed under vacuum at 25°C and the residue taken up in CH_2Cl_2 (50 mL). This solution was washed with 5% K_2CO_3 (3 x 25 mL), distilled water (25 mL), dried over Na_2SO_4 and evaporated in vacuo to give 1.015 g (99%) of the title compound. This crude product was purified on silica gel eluting with 10% EtOH/$CHCl_3$ to give (4) as a white puffy solid which tenaciously held solvent: ^1H NMR δ 1.20 (d, 6H), 1.41 (s, 18H), 1.84 (m, 4H), 3.42 (m, 8H), 3.84 (s, 3H), 3.90 (s, 3H), 3.90-4.64 (m, 6H), 5.62 (m, 2H), 6.94-7.60 (m, 3H), 8.05 (br, 1H); IR ($CHCl_3$), 3425 (br), 1705 (s), 1660 (s), 1650 (s), 1500 (s), 1400 (m), 1375 (s), 1220 (s), cm^{-1}.

Anal. Cal. for $C_{33}H_{55}N_5O_{11}$: C, 56.80; H, 7.94; N, 10.04. Found: C, 56.53; H, 7.97; N, 9.91.

N^1,N^4-bis[(L)-threonyl]-N^7-(2,3-dimethoxybenzoyl)norspermidine (5). To 5 mL of TFA was added 4 (0.300 g, 0.43 mmol), followed by stirring at room temperature for 0.5 h. The solvent was then stripped under vacuum, the residue dissolved in CH_2Cl_2 (20 mL) and the solvent again removed. This was repeated two times. Finally, drying under high vacuum in the presence of P_2O_5 gave a white solid which was purified by column chromatography on LH-20 and eluting with 10% EtOH/C_6H_6 to give 0.301 g (96%) of the bis-trifluoracetic acid salt of 5: ^1H NMR (CD_3OD) δ 1.19-2.42 (m, 10H), 3.02-4.58 (overlapping multiplets with methyl singlet (6H) at 3.92, 18H total), 6.90-7.28 (m, 2H); IR (KBr), 3600-2500 (br), 1750-1600 (br), cm^{-1}.

Anal. Cal. for $C_{27}H_{41}N_5O_{11}F_6$: C, 44.69; H, 9.65; N, 5.70. Found: C, 44.51; H, 5.70; N, 9.57.

The bis-trifluoroacetic acid salt of 5 (0.65 g, 0.93 mmol) was taken up in water (10 mL) and treated with Na_2CO_3 (0.3 g, 2.8 mmol) and stirred 5 minutes. The solution was then frozen and lyophilized to give a white solid mixed with Na_2CO_3. The product was isolated by putting the solids on a LH-20 column and eluting with 18% EtOH/C_6H_6 to give 0.45 g (97%) of 5 as a glassy solid: ^1H NMR δ 0.97 (m, 6H), 1.81 (m, 4H), 2.52 (br, 6H), 2.95-4.48 (overlapping, 20H), 6.92-8.10 (m, 5H); IR ($CHCl_3$) 3200 (br), 1658 (s), 1650 (s), 1645 (s) cm^{-1}.

Anal. Cal. for $C_{23}H_{29}N_5O_7$: C, 55.52; H, 7.90; N, 14.07. Found: C, 55.35; H, 7.92; N, 14.01.

N^1,N^4-bis[(L)-threonyl]-N^7-(2,3-dihydroxybenzoyl)norspermidine dihydrobromide (6). To a 1 M solution of BBr_3 (14.5 mL, 14.49 mmol) in CH_2Cl_2 (15 mL) under N_2 at 0°C was added 5 (0.390 g, 0.785 mmol) in CH_2Cl_2 (15 mL). After 2 h the reaction mixture was allowed to warm to room temperature and stirred an additional 20 h at which time it was cooled to 0°C and cautiously treated dropwise with H_2O (20 mL). After the two phase mixture was stirred vigorously for 3 h the aqueous layer was removed and evaporated in vacuo at 25°C to give 0.480 g (97%) of light brown solid. This residue was taken up in MeOH (20 mL) and concentrated several times. The resulting brown solid was easily purified on Sephadex LH-20 eluting with 20% EtOH/Benzene to give 0.300 g (63%) of pure 6: ^1H NMR δ (CD_3OD) 1.28 (m, 6H), 1.91 (m, 4H), 3.28-4.38 (overlapping multiplets, 12H), 6.72 (hex, 1H), 6.95 (d, 1H), 7.28 (quar, 1H); IR (Nujol) 3300 (br), 1670 (s), 1640 (s), 1630 (s) cm^{-1}.

Anal. Cal. for $C_{21}H_{37}N_5O_7Br_2$: C, 39.95; H, 5.91; N, 11.09. Found: C, 39.83; H, 6.00; N, 11.03.

N-[3-(2,3-dihydroxybenzamido)propyl]-1,3-bis[2-(2,3-dihydroxyphenyl)-trans-5-methyl-2-oxazoline-4-carboxamido]propane (Vibriobactin) (7). To a solution of 6 (0.108 g, 0.171 mmol) in dry, degassed methanol (10 mL) was added ethyl 2,3-dihydroxybenzimidate (0.189 g, 1.13 mmol, 6.6 equiv.). The mixture was heated at reflux under nitrogen for 33 h. The solution was concentrated and dissolved in ethanol then dry packed on Sephadex LH-20 (1.32 g). Column chromatography on LH-20, eluting with 15% EtOH/Benzene afforded 0.07 g (58%) of 7 as a tan glass. The spectral characteristics were identical with those reported in the literature.[9]

Ethyl 2,3-dihydroxybenzimidate. Prepared as described previously.[8]

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