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THE TOTAL SYNTHESIS OF DESFERRIOXAMINES E AND G

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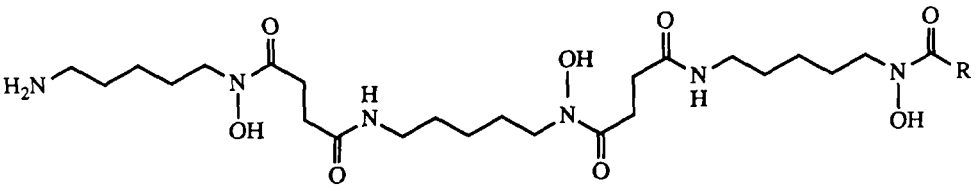
Abstract: The total syntheses of the hexacoordinate amino acid, 32-amino-5,16, 27-trihydroxy-4,12,15, 23, 26-pentaoxo-5,11,16, 22, 27-pentaazadotriacontanoic acid (desferrioxamine G) and the corresponding macrocyclic lactam 1,12, 23-trihydroxy-1,6,12,17, 23, 28-hexaazacyclotritriacontane-2, 5,13,16, 24, 27-hexone (desferrioxamine E, nocardamine) are reported. The synthetic route utilized here is predicated on the efficient formation and selective transformations of O-benzyl-N-(*tert*-butoxycarbonyl)-N-(4-cyanobutyl)hydroxylamine 4, a key reagent in our previous syntheses of bisucaberin and desferrioxamine B. The O-benzyl protected trihydroxamate nitrile acid 9, which is constructed from 4 by a series of selective deprotections and regiospecific acylations, is hydrogenated under mild conditions (Pd, dilute HCl) to give desferrioxamine G directly. Reduction of the nitrile group of 9 leads to amino acid 10, which is cyclized to generate the 33 membered ring, 1,12, 23-tribenzylnocardamine 11. Unmasking the hydroxamates in the final step affords the natural product, nocardamine. Synthetic methodology is now in place for accessing all of the natural product hydroxamate siderophores isolated from *Streptomyces pilosis.*

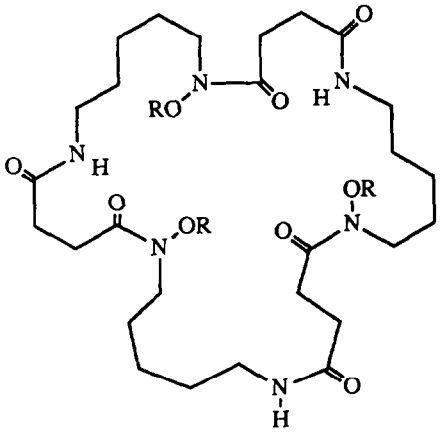
Siderophores, a group of low molecular weight iron chelators, are produced by microorganisms for the purpose of accessing iron, which exists largely in the insoluble ferric state and would be otherwise inaccessible. Although a substantial number of these chelators have been isolated and characterized, they fall largely into two structural classes: the catecholamides and the hydroxamates.[1] Interestingly, many of the ligands of both structural types are predicated on polyamine backbones. For example, the hexacoordinate catecholamides parabactin[2] and vibriobactin[3] are built on a spermidine and norspermidine backbone, respectively. Hydroxamates are frequently derived from the polyamines, 1,3-diaminopropane, putrescine or cadaverine or from their biochemical precursors ornithine or lysine.[1]

The siderophores isolated from *Streptomyces pilosis*, and the subject of this paper, desferrioxamines A-I, consist of a collection of both acyclic and cyclic ligands. These compounds have either repeating putrescine or cadaverine units in their backbones. The parent and most well known of these chelators, desferrioxamine B 1,[4] (Figure 1) is a linear trihydroxamate ligand, which forms a very stable hexacoordinate, octahedral complex[5] with iron (III), $K_f = 1 \times 10^{30}\ M^{-1}$. Although desferrioxamine B will bind a number of different +3 cations, e.g. Al(III), Ga(III), Cr(III), it exhibits a high specificity for iron(III);



Desferrioxamine B R=CH_3 1

Desferrioxamine G R=$(CH_2)_2CO_2H$ 2



11 R=CH_2Ph

3 R=H, Desferrioxamine E (Nocardamine)

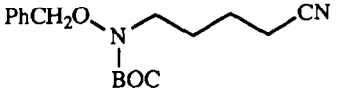


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Figure 1

its mesylate salt Desferal has been employed in the treatment of several iron overload diseases, e.g. thalassaemia.[6] However, the drug's short half life in the body and the fact that patients must be continuously infused has compelled investigators to continue the search for better therapeutic iron chelators.

Desferrioxamine B is the only one of the series of hydroxamate iron(III) chelators, desferrioxamines A-I,[7] that has been studied as a therapeutic iron chelator. This may be related to the problems associated with the separation of these ligands. In addition to basic desferrioxamine B, there are three other structural types produced by the bacterium which require consideration: the amino acids e.g. desferrioxamine G, the neutral compounds e.g. desferrioxamine E, and the acidic desferrioxamine H. We will describe the synthesis of the former two compounds by methods which can easily be applied to generation of desferrioxamine H.

The structure of desferrioxamine E, or nocardamine, 3 was confirmed to be a 33 membered ring containing three hydroxamate and three secondary amide functional groups through X-ray analysis of ferrioxamine E, its 1:1 complex with ferric ion.[8] The conformation is such that the hydroxamate coordination sites are situated at a maximum distance around the ring. The hexacoordinate siderophores desferrioxamine G 2,[9] the open chain analogue of nocardamine 3, and desferrioxamine E (nocardamine) 3 consist of three alternating succinic acid and N-hydroxycadaverine [1-amino-5-(hydroxyamino)pentane] units (Figure 1).

Interestingly nocardamine 3 does not sensitize tumor cells to macrophage promoted cytolysis[10] as does bisucaberin, a 22 membered dihydroxamate analogue of nocardamine; no explanation for this contrast has been proposed. Clearly a study of structure-activity relationships here would be of great value, a study which demands synthetic methods for access to key analogues. Furthermore the structure of desferrioxamine G 2 affords the opportunity to prepare a variety of potential prodrugs, i.e. desferrioxamine G functionalized at either the primary amine or carboxylic acid terminus. Terminal monofunctionalization of desferrioxamine G would still result in a charged molecule with reasonable water solubility. A convenient synthetic route to siderophores 2 and 3 and their homologues would permit these issues to be addressed.

Results And Discussion

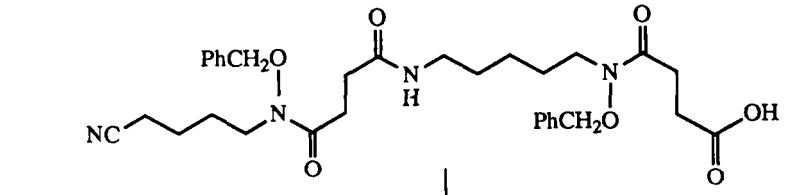
Although ferrioxamine G and the methyl ester of desferrioxamine G and have been synthesized,[11] the route is simply not useful for efficient access to these compounds. The conversion of ferrioxamine G to the corresponding cyclic complex, ferrioxamine E, has also been achieved but in only 4% yield.[9] Recently cyclic and linear trihydroxamates, which are analogous to chelators 2 and 3, respectively, have been synthesized. The macrocyclic ring of the nocardamine analogue, in which the carbonyl and hydroxamate groups are transposed, was formed by cyclization of the N-hydroxysuccinimide ester of the O,O',O"-tribenzyl amino acid under high dilution.[12]

The total syntheses of siderophores 2 and 3 are depicted in Scheme 1. The construction of acyclic nitrile-acid 9 is a further and important example of the applicability of the intermediates and methodologies developed in our total syntheses of desferrioxamine B 1[13] and bisucaberin[14] to the production of additional hydroxamate ligands.

The key to the synthesis of desferrioxamine G 2 and desferrioxamine E (nocardamine) 3 (Scheme 1) is the availability of the appropriately protected N-hydroxydiamine 4 (Figure 1). N-(*tert*-Butoxycarbonyl)-N-benzyloxy-1,5-diaminopentane 6 and nitrile-acid 5[14] are coupled using dicyclohexylcarbodiimide to form cyano compound 7 in 44% yield (Scheme 1). Addition of a methylene chloride solution of intermediate 7 to trifluoroacetic acid at 0 °C gives N-(benzyloxy)amine 8 in quantitative yield along with carbon dioxide and isobutylene. Attachment of the third succinate unit to 8 is accomplished with succinic anhydride/pyridine to produce nitrile acid 9 in 78% yield. Carboxylic acid 9 is the intermediate from which both hexacoordinate natural products can readily be generated. Hydrogenation (Pd catalyst, 1 atm) of 9 under weakly acidic conditions, which are used to generate desferrioxamine B,[13] removes the benzyl protecting groups and saturates the nitrile group to produce desferrioxamine G 2 as its hydrochloride salt.

To complete the synthesis of macrocycle 3, nitrile acid 9 is subjected to mild catalytic conditions (Raney nickel, methanolic ammonia) to give 0,0',0"-tribenzyl amino acid 10. A solution of acyclic precursor 10 (2.6 mM in DMF) is cooled to 0 °C, and diphenylphosphoryl azide (the Yamada reagent)[15,16] is added, followed by stirring for four days at 0 °C, to provide 0,0',0"-tribenzylnocardamine 11 in 54% percent yield, far superior to the previous 4% cyclization yield. The generation of such a large ring (33) using routine reaction conditions further illustrates the usefulness of this lactamization method. Finally, catalytic removal of the O-benzyl groups of 11 under mild conditions generates nocardamine 3 in quantitative yield. This synthetic material was identical by 300 MHz NMR and TLC to a sample of natural 3.[17]

Currently, we are applying this methodology to the synthesis of analogues and prodrugs of desferrioxamine G 2. Moreover the flexibility of our synthetic methodology provides easy access to the entire series of desferrioxamine compounds, both cyclic and linear. For example desferrioxamine B 1 could be prepared by an alternate route to our earlier method[13] using Scheme 1 simply by replacing amine 6 with N-acetyl-N-benzyloxy-1,5-diaminopentane. This new synthetic segment could be made from reagent 4 (Figure 1) by *tert*-butoxycarbonyl group removal (trifluoroacetic acid), acetylation and hydrogenation of the cyano group. This N-acetyl-N-hydroxycadaverine reagent could also be readily elaborated to desferrioxamine H, a tetracoordinate carboxylic acid chelator.[18] Finally macrocyclic desferrioxamine D_2 differs from desferrioxamine E only in the replacement of one of the N-hydroxycadaverine units with an N-hydroxyputrescine segment.[19] Alkylation of N-(*tert*-butoxycarbonyl)-O-benzylhydroxylamine with 4-chlorobutyronitrile followed by Raney nickel reduction would give N-(*tert*-butoxycarbonyl)-N-(4-aminobutyl)hydroxylamine, which could be carried through the appropriate steps in Scheme 1 to yield desferrioxamine D_2.



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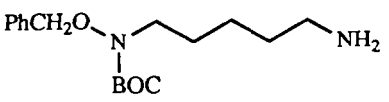

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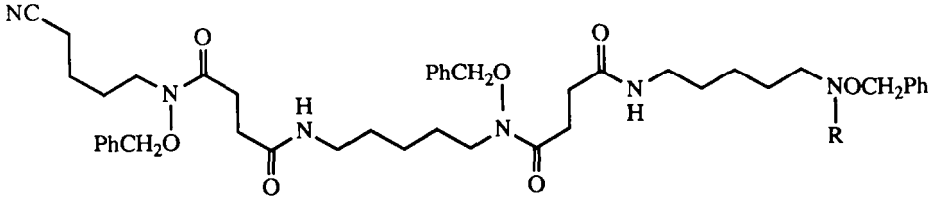
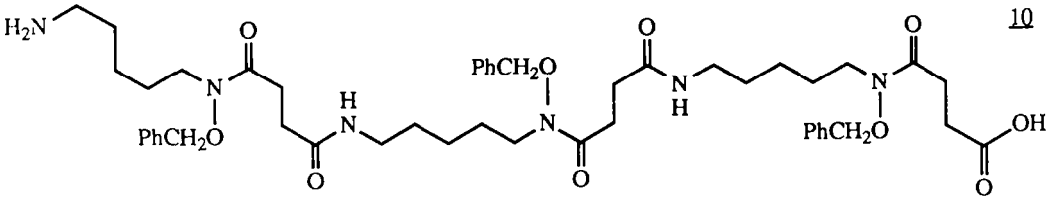


7 R=BOC

8 R=H

9 R=$(CH_2)_2CO_2H$

2 Desferrioxamine G



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11 R=CH_2Ph

3 R=H, Desferrioxamine E (Nocardamine)

Scheme 1

Experimental

All reagents were purchased from Aldrich Chemical Company and were used without further purification. Sodium sulfate was employed as a drying agent, and Fisher Optima grade solvents were routinely used. Melting points are uncorrected. Silica gel 60 (70-230 mesh), obtained from EM Science, Darmstadt, West Germany, was used for column chromatography. Preparative layer chromatography was carried out on silica gel GF plates (2 mm thick) purchased from Analtech, Newark, DE. NMR spectra were recorded on a Varian EM-390 or a Nicolet NT-300 instrument and, unless otherwise noted, were run in $CDCl_3$ with chemical shifts given in parts per million downfield from an internal tetramethylsilane standard (coupling constants are in hertz). Chemical shifts are reported with HOD (δ 4.8) as the standard for samples run in D_2O. Mass spectra were carried out on a Kratos MS 80 instrument. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

5,16-bis(Benzyloxy)-20-cyano-4,12,15-trioxo-5,11,16-triazaeicosanoic acid (5) and O-benzyl-N-(5-aminopentyl)-N-(*tert*-butoxycarbonyl)hydroxylamine (6) were prepared by methods developed in this laboratory.[14]

27-[N-(*tert*-Butoxycarbonyl)-N-(benzyloxy)amino]-6,17-bis(benzyloxy)-7,10,18,21-tetraoxo-6,11,17,22-tetraazaheptacosanenitrile (7). Acid 5 (8.42 g, 14.2 mmol), amine 6 (4.67 g, 15.2 mmol) and 4-dimethylaminopyridine (DMAP, 0.20 g, 1.6 mmol) in CH_2Cl_2 (180 mL) were cooled to 0 °C, and a solution of 1,3-dicyclohexylcarbodiimide (DCC, 3.04 g, 14.7 mmol) in CH_2Cl_2 (70 mL) was added over 17 min. The mixture was stirred (0 °C to RT) for 4 days. The mixture was filtered through Celite, and the filtrate concentrated to give 16.47 g of material, to which 5% $NaHCO_3$ (100 mL) was added. Product was extracted with $CHCl_3$ (3x100 mL), and the organic extract was washed with 5% $NaHCO_3$ (100 mL) and water (100 mL) then evaporated to give 16.53 g of material, which was run through a short column (20% EtOH/$CHCl_3$). A fraction was chromatographed on silica gel (6% EtOH/$CHCl_3$) to afford 5.47 g of 7 as an oil (44%). NMR δ 1.1-1.8 (s+m, 25 H), 2.13-2.85 (m, 10 H), 2.95-3.71 (m, 10 H), 4.76 and 4.80 (2 s, 6 H), 6.15 (br s, 2 H), 7.32 (s, 15 H). Anal. calcd. for $C_{49}H_{68}N_6O_9$: C, 66.49; H, 7.74; N, 9.50. Found: C, 66.44; H, 7.75; N, 9.49.

27-[N-(Benzyloxy)amino]-6,17-bis(benzyloxy)-7,10,18,21-tetraoxo-6,11,17,22-tetraazaheptacosanenitrile (8). A solution of 7 (2.70 g, 3.05 mmol) in CH_2Cl_2 (50 mL) was added over 9 min to trifluoroacetic acid (18 mL) at 0 °C. After stirring for 3 min, the ice bath was removed and stirring continued for 17 min. Solvents were removed on the rotovap, and saturated $NaHCO_3$ (200 mL) was added. Product was extracted into $CHCl_3$ (3x100 mL), and the combined organic layer washed with water (100 mL) and concentrated to give 2.46 g of 8 as an oil (quantitative). NMR δ 1.1-1.8 (m, 16 H), 2.18-3.28 (m, 16 H), 3.58 (t, 4 H), 4.63 (s, 2 H), 4.80 (s, 4 H), 7.2-7.4 (m, 15 H). Anal. calcd. for $C_{44}H_{60}N_6O_7$: C, 67.32; H, 7.70; N, 10.71. Found: C, 67.26; H, 7.74; N, 10.68.

5,16,27-tris(Benzyloxy)-31-cyano-4,12,15,23,26-pentaoxo-5,11,16,22,27-pentaazahentriacontanoic acid (9). A solution of 8 (2.44 g, 3.11 mmol) and succinic anhydride (0.47 g, 4.70 mmol) were heated in pyridine (25 mL) at 101 °C for 1h 52 min. After cooling, pyridine was removed under high vacuum, and saturated $NaHCO_3$ (100 mL) was added. Cooled 6N HCl (40 mL) was added in portions with swirling (external ice cooling) and extracted with $CHCl_3$ (3x100 mL). The combined extracts were washed with water (100 mL) and concentrated to give 2.46 g of material, which was purified by column chromatography (10% $CH_3OH/CHCl_3$) to afford 2.15 g of 9 (78%). NMR δ 1.05-1.8 (m, 16 H), 2.17-3.72 (m, 24 H), 4.80 (s with shoulder, 6 H), 6.5 (br s, 1H), 6.95 (br s, 1H), 7.2-7.35 (m, 15 H); FABMS calcd. for $C_{48}H_{64}N_6O_{10}$: 884, found 885 (M+1). A sample of 9 (0.264 g) was treated with 6 N HCl (10 mL) and extracted with CH_2Cl_2 (3x20 mL). The combined extracts were

washed with water (20 mL) and concentrated to give 0.252 g 9. Anal. calcd. for $C_{48}H_{64}N_6O_{10}$: C, 65.14; H, 7.29; N, 9.50. Found: C, 65.01; H, 7.53; N, 9.46.

32-Amino-5,16,27-trihydroxy-4,12,15,23,26-pentaoxo-5,11,16,22,27-pentaazadotriacontanoic acid (desferrioxamine G) (2). Glassware was soaked in 3 N HCl, rinsed with distilled water and CH_3OH and oven dried; solvents were iron free. Aqueous 0.1 N HCl (3.0 mL, 0.3 mmol) and 10% Pd-C (0.28 g) were added to 9 (0.207 g, 0.234 mmol) in CH_3OH (80 mL). The mixture was stirred under a hydrogen atmosphere for 2 h 20 min, and the catalyst filtered off. Concentration of the filtrate gave 0.14 g of the hydrochloride of 2 (92%) as a glass. NMR (CD_3OD/D_2O) δ 1.18-1.95 (m, 18 H), 2.42-3.37 (m, 18 H), 3.67 (t, 6H). A sample of 2 hydrochloride was passed through a column of AG 50W8-X8 Cation Exchange Resin (Bio-Rad) and eluted with H_2O then 1.4 N NH_4OH to give 2 as an amorphous solid. FABMS calcd. for $C_{27}H_{50}N_6O_{10}$: 618, found 619 (M+1). Anal. calcd. for $C_{27}H_{50}N_6O_{10}$: C, 52.41; H, 8.14; N, 13.58. Found: C, 52.13; H, 8.18; N, 13.52.

5,16,27-tris(Benzyloxy)-32-amino-4,12,15,23,26-pentaoxo-5,11,16,22,27-pentaazadotriacontanoic acid (10). A solution of 9 (2.11 g, 2.38 mmol) in CH_3OH (40 mL) was introduced to a 250 mL Parr bottle, followed by Raney nickel (W-2 grade, 1.61 g) and concentrated NH_4OH (6 mL). The suspension was cooled to 0 °C, and a gentle stream of NH_3 was bubbled in for 10 min. Hydrogenation at 57-60 psi was carried out for 3 h. The catalyst was filtered off (Celite), the solids were washed with CH_3OH, and solvent was stripped off to give 2.02 g of crude product. Column chromatography (0.4% concentrated NH_4OH/CH_3OH) furnished 0.45 g of 10 as a glass (21%). NMR (d_6-DMSO/D_2O) δ 1.1-1.7 (m, 18 H), 2.1-3.6 (m, 24 H + HOD, DMSO), 4.81 (s, 6 H), 7.33 (s, 15 H). Anal. calcd. for $C_{48}H_{68}N_6O_{10}$: C, 64.84; H, 7.71; N, 9.45. Found: C, 64.65; H, 7.73; N, 9.41.

1,12,23-tris(Benzyloxy)-1,6,12,17,23,28-hexaazacyclotritria-contane-2,5,13,16,24,27-hexone (11). A solution of 10 (0.42 g, 0.47 mmol) in DMF (180 mL) was cooled to 0 °C. Diphenylphosphoryl azide (0.13 mL, 0.60 mmol) was added by syringe, and the solution was stirred at -1 to 8 °C for 4 days. After removal of the solvent *in vacuo*, water (100 mL) was added, followed by extraction with $CHCl_3$ (3x100 mL). After washing the organic layer with water (100 mL), evaporation of the solvent gave 0.64 g of crude product. Purification was achieved by column chromatography (7% then 20% EtOH/$CHCl_3$), followed by preparative layer chromatography (7% EtOH/$CHCl_3$) to give 0.22 g 11 as a glass (54%). NMR δ 1.1-1.75 (m, 18 H), 2.35-2.62 (m, 6 H), 2.63-2.90 (m, 6 H), 3.01-3.30 (m, 6 H), 3.59 (t, 6 H), 4.78 (s, 6 H), 6.8 (m, 3 H), 7.33 (s, 15 H) ; FABMS calcd. for $C_{48}H_{66}N_6O_9$: 870, found 871 (M+1). Anal. calcd. for $C_{48}H_{66}N_6O_9$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.26; H, 7.68; N, 9.61.

1,12,23-Trihydroxy-1,6,12,17,23,28-hexaazacyclotritriacontane-2,5,13,16,24,27-hexone (desferrioxamine E, nocardamine) (3). Iron free glassware was employed (see 2). Macrocycle 11 (0.102 g, 0.117 mmol) was dissolved in CH_3OH (40 mL) and 10% Pd-C (O.11 g) added. The suspension was stirred under a hydrogen atmosphere for 7 h, the catalyst filtered and washed with CH_3OH, and solvent removed to give 0.07 g 3 as a white solid (quantitative). Synthetic 3 was identical to an authentic sample[17] by cospotting on silica gel TLC (11% $CH_3OH/CHCl_3$). A sample was recrystallized from distilled water to give 3: mp 180-181.5 °C, mp (lit) 181-183 °C[20] and 183-184 °C[21]; NMR (d_6-DMSO, 300 MHz) δ 1.12-1.56 (m, 18 H), 2.28 (t, 6 H), 2.53-2.64 (m, 6 H), 2.93-3.05 (m, 6 H), 3.46 (t, 6 H), 7.72 (m, 3 H), 9.62 (s, 3 H); ^{13}C NMR (d_6-DMSO, 75 MHz) δ 23.32, 25.99, 27.66, 28.78, 30.13, 38.50, 39.72 (septuplet, d_6-DMSO standard), 47.09, 171.6 (C=O), 172.2 (C=O); FABMS calcd. for $C_{27}H_{48}N_6O_9$: 600, found 601 (M+1). Anal. calcd. for $C_{27}H_{48}N_6O_9$: C, 53.99; H, 8.05; N, 13.99. Found: C, 53.86; H, 8.03; N, 13.93. The high field NMR spectra were identical to those of authentic 3.[17] The two samples were identical by cospotting on silica gel TLC (11% $CH_3OH/CHCl_3$,

20% EtOH/$CHCl_3$, 20% EtOH/EtOAc).

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