A Versatile Synthesis of Deferrioxamine B

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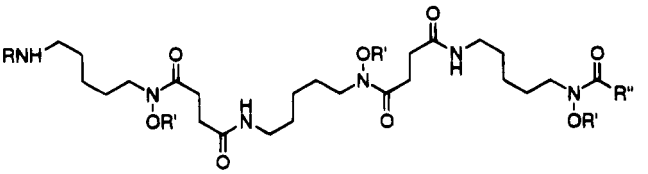
A new and versatile route to *N'*-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]-hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide, deferrioxamine B (DFO), is described. The key improvement over the two prior routes was replacement of the nitrile in **2** with a *tert*-butoxycarbonyl-protected amino terminus. Elimination of the nitrile improved the kinetics of hydrogenation in that the benzyl groups of **12** were cleaved more rapidly than saturation of the cyano group of **2**, and a potential overreduction byproduct (**4**) was thus avoided. *N*-(Benzyloxy)-1,5-diaminopentane (**6**) was selectively protected at the primary amino site with a *tert*-butoxycarbonyl (BOC) group, providing **7**. This was reacted at the (benzyloxy)amine with succinic anhydride to produce carboxylic acid **8**, which was in turn acylated regiospecifically with diamine **6** at the primary amine to give (benzyloxy)amine **9**. The previous two steps were reiterated to afford DFO reagent **11**. This synthon allows for modification of DFO at either end of the molecule, thus providing more flexibility in accessing DFO analogues than any prior route. Acetylation of **11**, followed by hydrogenolysis and *tert*-butoxycarbonyl group removal, furnished DFO.

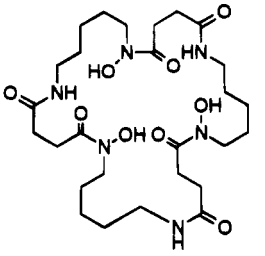
Introduction

Iron is essential for virtually all forms of life. Although it is one of the most abundant elements in the earth's crust,[1] it exists in the biosphere largely as insoluble ferric hydroxide ($K_{sp} = 2 \times 10^{-39}$).[2] Microorganisms produce a group of low molecular weight chelators, or siderophores,[1,3–6] for the purpose of acquiring iron from the environment. Although there are some notable exceptions, such as desferrithiocin[7] and rhizobactin,[8] most siderophores fall into two structural classes: the catecholamides or the hydroxamates.[1,9] While the hexacoordinate catecholamides, e.g. parabactin[3] and vibriobactin,[4] are predicated on the substituted triamines spermidine and norspermidine, respectively, the hydroxamates are frequently derived from the diamines putrescine or cadaverine or from their biochemical precursors ornithine or lysine.[9] The most widely studied chelator, deferrioxamine B (DFO),[6] is a linear trihydroxamate ligand containing alternating succinic acid and *N*-hydroxycadaverine units in its backbone (Chart 1), which forms a 1:1 hexacoordinate, octahedral complex with iron(III) with a formation constant[10] of 3×10^{30} M^{-1}. Interestingly, DFO is one of at least nine hydroxamates, including DFOs D_1, E, and G (Chart 1), that have been isolated from a single microorganism, *Streptomyces pilosus*.[5]

Chart 1



DFO	R = R' = H, R" = Me
DFO D_1	R = Ac, R' = H, R" = Me
DFO G	R = R' = H, R" = $(CH_2)_2CO_2H$
1	R = H, R' = CH_2Ph, R" = $(CH_2)_2CO_2H$



DFO E (Nocardamine)

In contrast to microorganisms, higher life forms (eukaryotes) have developed rather sophisticated iron storage and transport systems, utilizing proteins, e.g. ferritin and transferrin, respectively.[11] Iron metabolism in primates is characterized by a highly efficient recycling process[12–15] with no specific mechanism for eliminating this transition metal. Because it cannot be effectively

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cleared, the introduction of "excess iron" [16-18] into this closed metabolic loop leads to chronic overload and ultimately to peroxidative tissue damage. Although phlebotomy can often be used to remove excess iron,[19] there are iron overload syndromes secondary to chronic transfusion therapy, e.g. aplastic anemia and thalassemia,[20] in which the only alternative is chelation therapy.

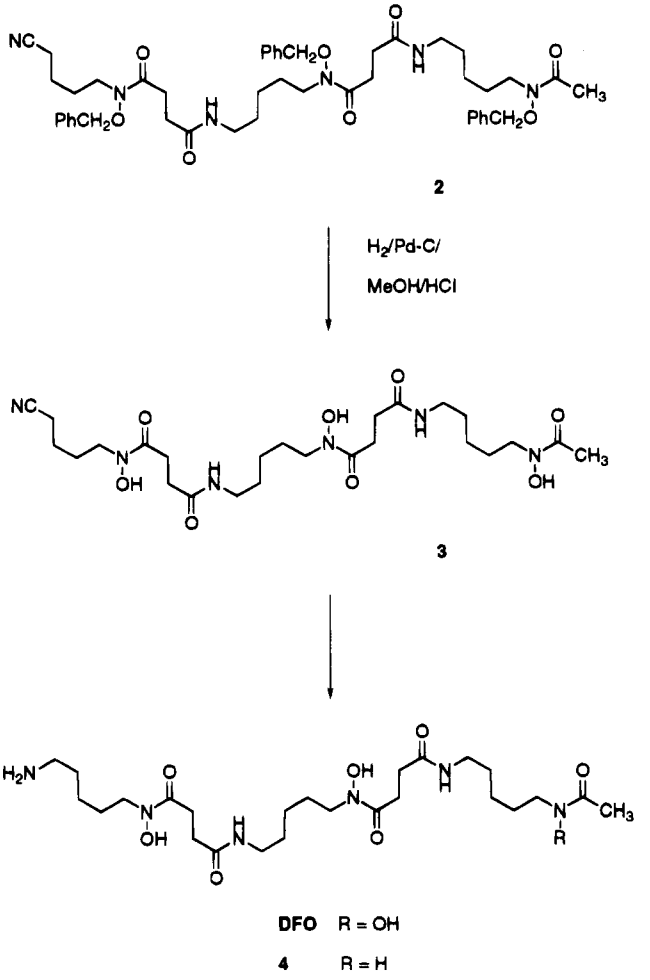
While considerable effort has been invested in the development of new therapeutics for managing thalassemia and other iron overload diseases,[21] the subcutaneous infusion of the mesylate salt of DFO, Desferal, is still the protocol of choice. Although the drug's efficacy and long term tolerability are well documented, it suffers from a number of shortcomings associated with low efficiency, a short half-life in the body and marginal oral activity. The drug must therefore be given by continuous infusion over long periods of time, resulting in serious problems with patient compliance.[22] Thus, the search continues for an orally active drug, or at least for a more efficient therapeutic iron-clearing agent. In keeping with this pursuit, new synthetic methods for accessing iron chelators are of considerable importance.

Results and Discussion

Two syntheses of DFO have been reported from these laboratories,[23,24] both of which allowed access to analogues which were not obtainable by the earlier approach of Prelog.[25] Both of these methodologies focused on the key intermediate **2** (Scheme 1), in which the three hydroxamates are protected by benzyls and the primary amine is masked as a nitrile. The first synthesis of DFO[23] began with the somewhat tedious production of 4-cyanobutanal,[26] a moderately unstable aldehyde. The synthesis of **2** then moved from the *N*-acetyl to the primary amino end of the molecule, or east to west.

The second scheme[24] began with *N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine,[27] which is crystalline, stable, and available in a single step from commercial reagents. *N*-Alkylation with 5-chlorovaleronitrile (NaH, DMF, NaI) gave *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-*N*-(4-cyanobutyl)-hydroxylamine, a triprotected *N*-hydroxy-1,5-pentanediamine reagent which was elaborated to DFO precursor **2** in the opposite direction, that is, west to east. Because the acetyl terminus was not attached until near the end of the synthesis, this route was more suitable for the production of DFO analogues containing other acyl groups.

Scheme 1. Hydrogenation of Nitrile Intermediate 2 in the Previous DFO Syntheses. Trihydroxy Nitrile Intermediate 3 and Overreduced Byproduct, Amide 4.



In both DFO preparations, catalytic hydrogenation of **2** (10% Pd–C, MeOH, HCl, 1 atm) simultaneously cleaved the benzyl protecting groups and saturated the nitrile to a primary amine to furnish DFO. While the benzyl moieties were cleaved in less than 0.5 h, resulting in underreduced intermediate **3** (Scheme 1), the cyano function required longer times for saturation. Prolonged reduction times generated not only DFO but also a troublesome product of overreduction, the acetamide **4** (Scheme 1). The kinetics of reduction could be followed by HPLC[28] (Figure 1) and by ^{1}H NMR in that the acetamide peak (δ 1.96) of **4** was upfield from the acetyl signal of DFO (δ 2.14). On the basis of these studies, 4 h was found to be the optimum time for exposure to hydrogen. Nevertheless, it was still necessary to remove overreduced product **4**.

The present synthesis of DFO is both direct and versatile. This new route not only contains the advantages of the second synthesis but also (1) eliminates the problems associated with nitrile reduction by replacing the western terminus of **2** with a *tert*-butoxycarbonyl (BOC)-protected amine, (2) streamlines the building of the DFO chain by capitilizating on the electronic differ-

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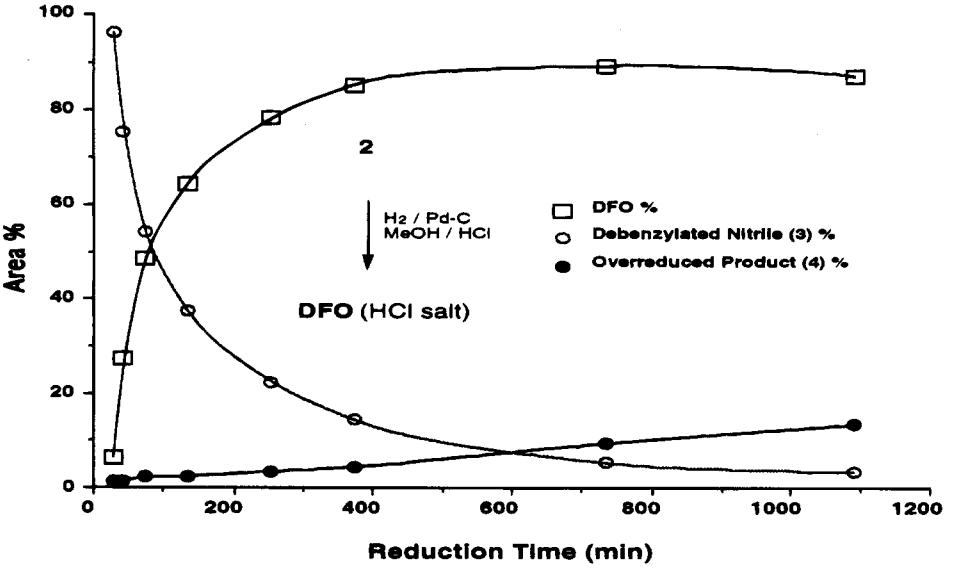

Figure 1. Nitrile reduction vs hydroxamate overreduction during hydrogenation of **2** to DFO.

ences between a primary amine and an *N*-(benzyloxy)amine, and (3) allows for the convenient modification of either terminus of the chelator.

As in the second route, the backbone of DFO was constructed by a series of acylations such that the acetyl function was attached at the end of the synthesis, that is, west to east. Construction of the siderophore began with *N*-(benzyloxy)-*N*-(*tert*-butoxycarbonyl)-1,5-pentanediamine (**5**) (Scheme 2), which was obtained from Raney nickel hydrogenation of the nitrile of the triprotected *N*-hydroxycadaverine reagent.[29] Unlike the nickel-catalyzed hydrogenation of the first route,[23] no special precautions were needed to prevent base-promoted side reactions. Exposure of **5** to trifluoroacetic acid (TFA) and then HCl provided *N*-(benzyloxy)-1,5-pentanediamine dihydrochloride (**6**), which is also available by alkylation of *O*-benzylhydroxylamine with *N*-(5-bromopentyl)phthalimide, followed by hydrazinolysis.[30]

Replacement of the nitrile of **2** with a BOC-protected amine could be readily achieved if diamine **6** could be selectively functionalized using a BOC reagent at the primary amine. Synthesis of the macrocyclic dihydroxamate alcaligin[31] was predicated on just such a regioselective acylation of a primary amine over a (benzyloxy)-amine. *N*-(Benzyloxy)-1,5-diaminopentane **6** as its free amine was treated with 2-[[(*tert*-butoxycarbonyl)oxy]-imino]-2-phenylacetonitrile (BOC-ON, 1 equiv) in THF at 0 °C, cleanly providing *N*-(benzyloxy)-*N'*-(*tert*-butoxycarbonyl)-1,5-pentanediamine (**7**). Both steric[32] and electronic[33,34] factors explain the high regioselectivity of this transformation.

(Benzyloxy)amine **7** was heated with succinic anhydride in pyridine, giving carboxylic acid **8**. Thus this route to DFO is even more streamlined than before[24] in that the (benzyloxy)amine nitrogen need not be deprotected before addition to succinic anhydride. Treatment of acid **8** with 1,1'-carbonyldiimidazole (CDI)[35] in CH_2Cl_2 generated the *N*-acylimidazole *in situ*, and a solution of **6** as the free diamine in CH_2Cl_2 was slowly added at 0 °C, resulting in *N*-(benzyloxy)amine **9**. CDI was an especially convenient amide-forming reagent in that the imidazole byproduct was washed out during workup. As was the case in the protection of diamine **6** by BOC, acylation occurred at the primary amine end of **6** with a high degree of selectivity.[36-38]

The latter two steps were repeated to generate DFO reagent **11**. That is, *N*-(benzyloxy)amine **9** was converted to acid **10** with succinic anhydride in pyridine. Next, regiospecific acylation of diamine **6** with acid **10** gave tris-[*N*-(benzyloxy)amine) **11**. In addition to providing DFO in three transformations, **11** is a versatile DFO reagent in which the eastern end of DFO can be modified with a wide range of acylating agents. For example, radiolabeled DFO can be made by coupling [^{3}H]- or [^{14}C]acetic acid with **11**.

Reaction of **11** with acetyl chloride (NEt_3/CH_2Cl_2) produced fully protected DFO (**12**). The benzyl groups of **12** were cleaved by hydrogen under mild conditions (10% Pd–C/CH_3OH, 1 atm, rt) within 20 min to give *N*-(*tert*-butoxycarbonyl)DFO (**13**), which was identical by a high-field ^{1}H NMR spectrum to an authentic sample. Thus catalytic unmasking of the hydroxamate esters of **12** with no special precautions was completed in 20 min with no detectable nitrogen–oxygen bond cleavage. This result can be compared to the last step of the first two routes using Figure 1, that is, hydrogenation of tris(*N*-benzyloxy) nitrile **2**. Since the benzyl groups were cleaved faster than the nitrile was saturated, the molecule was greater than 90% in the form of the intermediate trihydroxy nitrile **3** after 20 min. Most of the cyano

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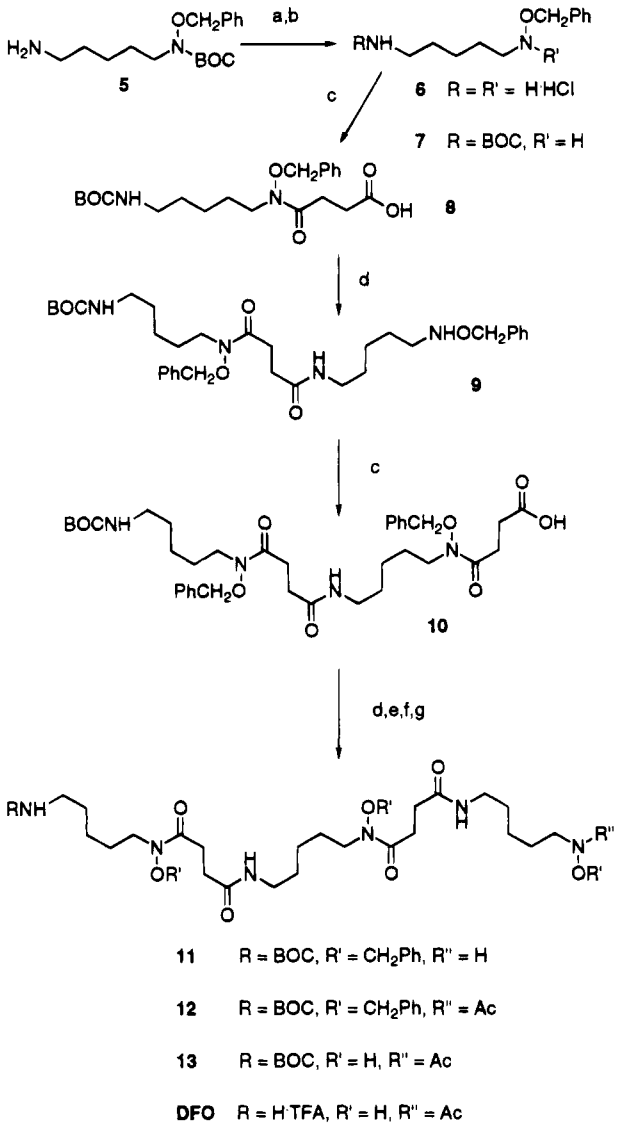
Scheme 2.[a] A Versatile Synthesis of Deferrioxamine B (DFO)



[a] Reagents: (a) TFA, HCl; (b) BOC-ON (1 equiv)/NEt_3/THF; (c) succinic anhydride/pyr (d) 1,1'-carbonyldiimidazole/CH_2Cl_2, **6** (free amine); (e) AcCl/NEt_3/CH_2Cl_2; (f) H_2/10% Pd–C/MeOH; (g) TFA.

function was saturated, as well, after 4 h, resulting in DFO (77%) along with intermediate **3** (21%) and overreduced product **4** (2%). After 12 h, DFO (88%) was accompanied by only 4% of nitrile **3** but 8% of overreduced product **4**.

Brief exposure of *N*-(*tert*-butoxycarbonyl)DFO (**13**) to trifluoroacetic acid (TFA) at 0–5 °C for 20 min resulted in the formation of DFO as its trifluoroacetate salt, along with isobutylene and carbon dioxide. The high-field ^1H NMR spectrum of the synthetic chelator was identical to that of Desferal, except for the latter's methanesulfonate singlet. Moreover, the synthetic natural product was free of overreduced byproduct **4**, as the singlet at δ 1.96 from the acetamide was absent.

In the literature ^1H NMR spectra of Desferal or DFO·HCl in d_6-DMSO[39] or d_6-DMSO/D_2O,[40] respectively, the acetyl absorption is a singlet. However, the 300 MHz

Figure 2. Conformations of DFO in D_2O: trans (*E*) and cis (*Z*).

proton spectra of both synthetic DFO·TFA and Desferal showed an identical but more complex acetyl region in D_2O. Specifically, there were two peaks, one at δ 2.14 and one at δ 2.11, of approximately 4:1 relative intensity. Furthermore, the succinic acid-bridge portion of the spectra was more complex than predicted. A published spectrum[41] of Desferal in D_2O displayed the same pattern; however, the chemical shift was listed as δ 2.14 without assignment of the minor peak at δ 2.11.[39] The solvent dependence of the DFO spectrum warranted further study. The complexity of the high-field spectrum in D_2O could arise from *E*- and *Z* isomers of the terminal acetylhydroxamate due to hindered rotation about the carbon–nitrogen bond, which has been observed in other hydroxamates.[42]

This phenomenon was confirmed by a variable temperature ^1H NMR study of Desferal in D_2O. The two acetyl peaks collapsed to a singlet upon heating the sample, with a coalescence temperature (T_c) of 43 °C. The frequency difference ($\Delta\nu$ = 15.36 Hz) and respective populations at δ 2.14 and 2.11, p*A* (0.78) and p*B* (0.22), which were estimated from the integration of the clearly resolved singlets, were determined at 10 °C. The methods of Kessler[43] and Sandstrom[44] for the determination of the free enthalpy of activation (ΔG^*) from unequally populated singlets were used to calculate ΔG^* for each population, where ΔG_B^* = 16.4 and ΔG_A^* = 17.2 kcal/mol, respectively. These values are in excellent agreement with the literature value (ΔG^* = 16.2 kcal/mol) for the isomerization of an *N*-formyl-*N*-alkylhydroxylamine.[45]

On the basis of studies of *N*-methylacetylhydroxamate,[42] with its acetyl *E* isomer at δ 2.13 and *Z* isomer at δ 2.11, the major peak of DFO's acetyl (δ 2.14) is most probably the *E* (trans) isomer, and the smaller peak at δ 2.11 the *Z* (cis) form (Figure 2). The high proportion of *E* over *Z* conformation could be at least partly due to the use of D_2O, a polar solvent.[45] Intermolecular hydrogen bonding of DFO in the *E* form to the protic solvent could diminish the significance of the intramolecular hydrogen bond in the *Z* form.

In summation, replacement of the nitrile of **2** with a terminal amine, protected by BOC, already in place from the beginning of the synthesis increased the overall efficiency of the DFO preparation. Also, the order of the last two steps of this synthesis (Scheme 2) is not important. Moreover, if the last step were carried out with methanesulfonic acid in dioxane,[46] Desferal could be accessed directly.

Using this methodology, additional hydroxamate siderophores and analogues could be prepared more ef-

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ficiently than by any other published route. As mentioned before, DFO synthon **11** can provide a variety of DFO analogues. The *N*-(benzyloxy) terminus can be coupled with a variety of acylating agents, followed by removal of the BOC protecting group from the terminal primary amine, with the option of further acylation. These would be valuable for determining structure–activity relationships in the search for chelators with a longer clearance time and perhaps oral viability. Also other siderophores from *S. pilosus* could be synthesized more efficiently than before. For example, BOC (benzyloxy)amine **11** could be heated with succinic anhydride (pyr) and then stirred with TFA to give ω-amino acid **1** (Chart 1), which is the acyclic precursor to macrocyclic DFO E (nocardamine) synthesized in this laboratory.[47] In that route, **1** was prepared from the corresponding ω-cyano carboxylic acid by hydrogenation (Raney nickel) in only 21% yield due to cleavage of the chain by alkaline reaction conditions. Hydrogenation of **1** under mild conditions (10% Pd–C/CH_3OH/20 min) would give DFO G, a trihydroxamate ω-amino acid (Chart 1). If DFO reagent **11** were reacted with TFA, both termini of the resulting ω-amino *N*-(benzyloxy)amine could be derivatized with the same acyl group. For example, subsequent treatment of the diamine with acetyl chloride (2 equiv, NEt_3/CH_2Cl_2) and then hydrogenolysis of the benzyl protecting groups would generate the neutral DFO, D_1.[25]

Experimental Section

Samples of authentic **13** and Desferal were generously provided by Ciba-Geigy, Basel, Switzerland. Reagents were purchased from Aldrich Chemical Co. and were used without further purification. Fisher Optima grade solvents were routinely used, and DMF was distilled. Organic extracts were dried with sodium sulfate. Glassware was presoaked in 3 N HCl for 15 min, rinsed with distilled water and ethanol, and dried, and distilled solvents were used in the presence of hydroxamates. Silica gel 60 (70–230 mesh) obtained from EM Science (Darmstadt, Germany) or silica gel 32–63 (40 μM "flash") from Selecto, Inc. (Kennesaw, GA) was used for column chromatography. Proton NMR spectra were run at 90 or 300 MHz in $CDCl_3$ (not indicated) or D_2O with chemical shifts given in parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt, respectively. Coupling constants (J) are in hertz. Variable-temperature NMR studies were run at 600 MHz. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

***N*-(Benzyloxy)-1,5-pentanediamine Dihydrochloride (6).** Trifluoroacetic acid (TFA, 30 mL) was added to a solution of **5**[29] (19.4 g, 63.0 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The solution was stirred at rt for 30 min under nitrogen, and excess TFA was removed by rotary evaporation. The residue was carefully basified with cold 1 N NaOH (50 mL) and then 50% NaOH (23 mL) until pH >14 and extracted with $CHCl_3$ (3 × 60 mL). After solvent removal, the residue was taken up in EtOH (100 mL) and treated with concentrated HCl (12 mL) with cooling. After solvent removal, recrystallization from absolute EtOH generated 14.4 g of **6** (82%) as a white solid: mp 179–181 °C (lit. mp[30] 170–172 °C); NMR (D_2O) δ 1.3–1.9 (m, 6 H), 2.95 (t, 2 H, J = 7), 3.22 (t, 2 H, J = 7), 5.1 (s, 2 H), 7.45 (s, 5 H). Anal. Calcd for $C_{12}H_{22}Cl_2N_2O$: C, 51.25; H, 7.89; N, 9.96. Found: C, 51.33; H, 7.94; N, 9.90.

***N*-(Benzyloxy)-*N'*-(*tert*-butoxycarbonyl)-1,5-pentanediamine (7).** NaOH (1 N, 100 mL) was added to **6** (6.01 g, 21.4 mmol), followed by extraction with ether (5 × 50 mL). After a brine wash (50 mL), solvent was removed to give 4.39 g of **6** as the free amine. BOC-ON (5.10 g, 20.7 mmol) in distilled THF (100 mL) was added to **6** (free amine, 21.1 mmol) in THF (165 mL) at 0 °C over 30 min. The reaction was stirred (0 °C to rt) for 1 day and concentrated *in vacuo*. Cold 1 N NaOH (100 mL) was added, followed by extraction with EtOAc (4 × 100 mL). The organic layer was washed with cold 1 N NaOH (3 × 80 mL), cold 0.5 M citric acid (100 mL), and brine (2 × 100 mL). Removal of solvent gave 6.38 g (quantitative) of **7**, which was used directly in the next step, as a liquid: NMR δ 1.2–1.7 (m + s, 15 H), 2.66–3.23 (m, 4 H), 4.5 (br s, 1 H), 4.67 (s, 2 H), 7.16–7.34 (m, 5 H). An analytical sample was purified on a silica gel column, eluting with 15% EtOAc/$CHCl_3$. Anal. Calcd for $C_{17}H_{28}N_2O_3$: C, 66.20; H, 9.15; N, 9.08. Found: C, 66.13; H, 9.10; N, 9.05.

5-(Benzyloxy)-11-(*tert*-butoxycarbonyl)-4-oxo-5,11-diazaundecanoic Acid (8). Pyridine (80 mL) was added to **7** (6.38 g, 20.7 mmol) and succinic anhydride (3.11 g, 31.1 mmol), and the reaction was heated under nitrogen at 80–86 °C for 1.5 h. Solvent was removed under high vacuum, and the residue was dissolved with ether (150 mL) and washed with saturated $NaHCO_3$ (4 × 50 mL). The aqueous portion was extracted with ether (2 × 50 mL), cooled to 0 °C, acidified with cold 1 N HCl (350 mL), and extracted with $CHCl_3$ (6 × 100 mL). A water wash (100 mL), solvent removal, and flash column chromatography, eluting with 6% CH_3OH/CH_2Cl_2 gave 7.46 g (88%) of **8** as a solid: mp 93–94 °C; NMR δ 1.2–1.8 (m + s, 16 H), 2.62 (s, 4 H), 2.9–3.2 (m, 2 H), 3.51–3.76 (m, 2 H), 4.80 (s, 2 H), 7.33 (s, 6 H). Anal. Calcd for $C_{21}H_{32}N_2O_6$: C, 61.75; H, 7.90; N, 6.86. Found: C, 61.48; H, 7.90; N, 6.79.

7,18-Bis(benzyloxy)-1-(*tert*-butoxycarbonyl)-8,11-dioxo-1,7,12,18-tetraazaoctadecane (9). 1,1'-Carbonyldiimidazole (CDI, 0.592 g, 3.65 mmol) was added to **8** (1.42 g, 3.48 mmol) in dry CH_2Cl_2 (90 mL). After stirring for 1 h, the solution was cooled to 0 °C, and **6** (free amine, 0.74 g, 3.55 mmol) in CH_2Cl_2 (65 mL) was added by cannula over 5 min. After the solution was stirred for 12 h (0 °C to rt), solvent was removed by rotary evaporation. Dilute brine (200 mL) was added, followed by extraction with EtOAc (4 × 100 mL). The organic phase was washed with 100 mL portions of cold 0.5 M citric acid, 5% $NaHCO_3$, and brine. Solvent removal led to 2.2 g (quantitative) of **9** as an oil, which was directly used in the next step. An analytical sample was chromatographed on silica gel, eluting with 3.5% EtOH/$CHCl_3$: 300 MHz NMR δ 1.1–1.7 (m + s, 21 H), 2.3–3.3 (m, 10 H), 3.60 (t, 2 H, J = 7), 4.6 (br s, 1 H), 4.67 (s, 2 H), 4.80 (s, 2 H), 6.0 (br s, 1 H), 7.25–7.40 (m, 10 H). Anal. Calcd for $C_{33}H_{50}N_4O_6$: C, 66.19; H, 8.42; N, 9.36. Found: C, 66.27; H, 8.40; N, 9.33.

5,16-Bis(benzyloxy)-22-(*tert*-butoxycarbonyl)-4,12,15-trioxo-5,11,16,22-tetraazadocosanoic Acid (10). A mixture of **9** (2.2 g, 3.48 mmol), succinic anhydride (0.55 g, 5.5 mmol), and pyridine (70 mL) was heated at 81 °C for 2 h. Solvent was removed *in vacuo*, and cold 1 N HCl (100 mL) was added. Extraction was carried out with $CHCl_3$ (6 × 75 mL), followed by a water wash (100 mL). Solvent removal and flash column chromatography, eluting with 7% MeOH/CH_2Cl_2, afforded 1.98 g (81%) of **10** as an oil: 300 MHz NMR δ 1.2–1.7 (m + s, 21 H), 2.53 (t, 2 H, J = 7), 2.67 (s, 4 H), 2.78–2.86 (m, 2 H), 3.02–3.11 (m, 2 H), 3.22 (q, 2 H, J = 6), 3.56–3.77 (m, 4 H), 4.7 (br s, 1 H), 4.83 (s, 2 H), 4.86 (s, 2 H), 6.73 (br s, 1 H), 7.38 (s, 10 H). Anal. Calcd for $C_{37}H_{54}N_4O_9$: C, 63.59; H, 7.79; N, 8.02. Found: C, 63.45; H, 7.81; N, 8.07.

1-(*tert*-Butoxycarbonyl)-7,18,29-tris(benzyloxy)-8,11,-19,22-tetraoxo-1,7,12,18,23,29-hexaazanonacosane (11). CDI (0.188 g, 1.16 mmol) was added to a solution of **10** (0.76 g, 1.09 mmol) in dry CH_2Cl_2 (45 mL). The solution was cooled to 0 °C after 1 h, and **6** (free amine, 0.240 g, 1.15 mmol) was added by cannula in CH_2Cl_2 (60 mL) over 5 min. The reaction was stirred at 0 °C to rt for 1 day. Solvent was removed *in vacuo*, and dilute brine (100 mL) was added to the residue. Extraction was carried out with EtOAc (5 × 75 mL), followed by washing with 50 mL of cold 0.5 M citric acid, 5% $NaHCO_3$, water, and brine. Solvent removal gave 0.87 g (90%) of **11**, which was used directly in the next reaction: NMR δ 1.1–1.8 (m + s, 27 H), 2.3–3.7 (m, 20 H), 4.5 (br s, 1 H), 4.67 (s, 2 H), 4.82 (s, 4 H), 6.2 (br s, 1 H), 7.2–7.4 (m, 15 H). An analytical sample of **11** was obtained from silica gel column chromatog-

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raphy, eluting with 5% MeOH/$CHCl_3$. Anal. Calcd for $C_{49}H_{72}N_6O_9$: C, 66.19; H, 8.16; N, 9.45. Found: C, 66.18; H, 8.17; N, 9.42.

1-(*tert*-Butoxycarbonyl)-7,18,29-tris(benzyloxy)-8,11,19,22,30-pentaoxo-1,7,12,18,23,29-hexaazahentriacontane (12). NEt_3 (0.16 mL, 1.15 mmol) was added to **11** (0.87 g, 0.98 mmol) in CH_2Cl_2 (40 mL), and the solution was cooled to 0 °C. Acetyl chloride (0.074 M in CH_2Cl_2, 14 mL, 1.04 mmol) was added by syringe over 4 min, and stirring was continued (0 °C to rt) for 1 day. The aqueous phase was extracted with CH_2Cl_2 (4×), and the organic phase was washed with 40 mL portions of cold 0.25 M citric acid, 5% $NaHCO_3$, and H_2O. Solvent removal and flash column chromatography with 60% acetone/hexane gave 0.79 g (87%) of **12** as an oil: 300 MHz NMR δ 1.23–1.69 (m + s, 27 H), 2.09 (s, 3 H), 2.44–2.53 (m, 4 H), 2.76–2.84 (m, 6 H), 3.03–3.24 (m, 6 H), 3.57–3.68 (m, 6 H), 4.7 (br s, 1 H), 4.81 (s, 2 H), 4.85 and 4.86 (2 s, 4 H), 6.35 (br s, 1 H), 7.38 (s, 15 H). Anal. Calcd for $C_{51}H_{74}N_6O_{10}$: C, 65.78; H, 8.01; N, 9.03. Found: C, 65.89; H, 8.04; N, 8.95.

1-(*tert*-Butoxycarbonyl)-7,18,29-trihydroxy-8,11,19,22,30-pentaoxo-1,7,12,18,23,29-hexaazahentriacontane (13). Pd–C (10%, 227 mg) was added to a solution of **12** (0.346 g, 0.372 mmol) in MeOH (100 mL). The reaction flask was degassed three times with nitrogen; hydrogen gas was introduced at 1 atm. The mixture was rapidly stirred at rt for 20 min. After the reaction flask was evacuated and filled with nitrogen several times, catalyst was filtered through a sintered glass filter (10–15 μm), and solids were washed with MeOH. After solvent removal by rotary evaporation, the residue was passed through a short Sephadex LH-20 column, eluting with MeOH. The band was dried to provide 199 mg (81%) of **13** as an amorphous white solid: 300 MHz NMR (d_6-DMSO) δ 1.12–1.53 (m + s, 27 H), 1.95 (s, 3 H), 2.25 (t, 4 H, J = 7), 2.52–2.60 (m, 4 H), 2.82–3.03 (m, 6 H), 3.43 (t, 6 H, J = 6), 7.4 (m, 1 H), 7.8 (m, 2 H), 9.7 (br s, 3 H). The spectrum was identical to that of an authentic sample of **13**.

Deferrioxamine B Trifluoroacetate (DFO). Compound **13** (19.5 mg, 0.030 mmol) was cooled to 0 °C under argon. TFA (2.4 mL) was added by pipette, and the reaction was stirred at 0 °C for 20 min. Excess TFA was removed under high vacuum at 1–4 °C on the rotary evaporator and then at rt to give 25.1 mg (quantitative) of DFO trifluoroacetate as a glass: 300 MHz NMR (D_2O) δ 1.23–1.77 (m, 18 H), 2.10 and 2.12 (2 unequal s, 3 H), 2.49 (t, 4 H, J = 7), 2.63–2.84 (t and br s, 4 H, J = 7), 2.98 (t, 2 H, J = 7), 3.15 (t, 4 H, J = 7), 3.56–3.70 (m, 6 H). This spectrum was identical to that of Desferal, except for the methanesulfonate singlet in the latter, and to that of DFO hydrochloride, obtained from passing Desferal through a column packed with BioRad Anion Exchange Resin AG1-X8 (hydroxide form).

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