Total Synthesis of the Siderophore Danoxamine

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The total synthesis of the linear trihydroxamate siderophore, Danoxamine, is described. Danoxamine is a siderophore component of the naturally occurring siderophore−drug conjugates Salmycin A−D. The synthesis of Danoxamine features a series of coupling reactions involving *N*-(5-benzyloxypentyl)-*O*-benzylhydroxylamine being linked by a succinoyl linker to *N*-(benzyloxy)-1,5-pentanediamine. Two more succinoyl linkers and another *N*-(benzyloxy)-1,5-pentanediamine were used in coupling reactions to afford the fully protected siderophore. The linear tetrabenzyl-protected trihydroxamate was deprotected to afford the natural product Danoxamine.

Introduction

Iron is one of the most important micronutrients to the viability of microorganisms because of its use in physiological redox chemistry.[1] However, in vivo free iron is of such low concentration (10^{-18} M) that bacteria are unable to proliferate.[2] To overcome the low availability of iron, many microorganisms synthesize and utilize low molecular weight iron-chelating compounds called siderophores. These iron-chelating agents are highly specific for the binding and sequestering of iron.[3,4] After a siderophore has chelated iron, the ferric complex is later reassimilated by the microorganism. This novel iron-acquiring mechanism has led to the use of siderophores as "Trojan Horse" drug delivery systems.[1] A series of naturally occurring siderophore−aminoglycoside conjugates, Salmycin A−D, were recently isolated from the *Streptomyces violaceus*, DSM 8286, strain of bacteria.[5] These isolated compounds showed antibacterial activity of 0.01 μg/mL (10 nM) against *Staphylococci* and *Streptococci*, including effectiveness against resistant strains of these bacteria. The siderophore portion of Salmycin A and C had been previously reported as the siderophore Danoxamine (**1**).[6] The development of a flexible synthesis of Danoxamine is key to the preparation of the natural products Salmycin A−D and further development of siderophore−drug conjugates.

Danoxamine is composed of a linear series of three hydroxamic acids, one of three common iron-binding constituents found in siderophores. The concept of siderophore−drug conjugates as "Trojan Horse" delivery systems has been previously demonstrated[1,7−20] but the potential of linear hydroxamate systems as the siderophore component has not been thoroughly investigated. During earlier studies, it became apparent that having a siderophore−drug system in which the siderophore, or carrier portion of the conjugate, could release the drug once inside the cell, was key. Vértesy et al. in their original report of the isolation and characterization of the Salmycins alluded to the possibility that the Salmycin drug component was being actively cleaved from the siderophore Danoxamine. The authors did not elaborate on the exact method of release. However, the possibility exists that after the release of iron from the siderophore by a ferric reductase, the resulting free hydroxamic acid could cyclize to form a six-membered ring, thus participating in the liberation of the drug from the siderophore (Scheme 1).

To investigate this possible release mechanism as well as the further biological action of the Salmycins and other Danoxamine−drug conjugates, a route had to be developed to prepare significant quantities of the natural product Danoxamine.

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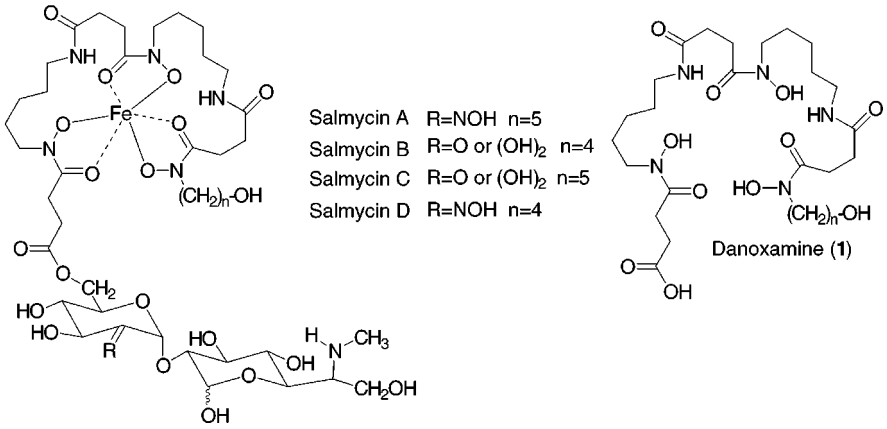
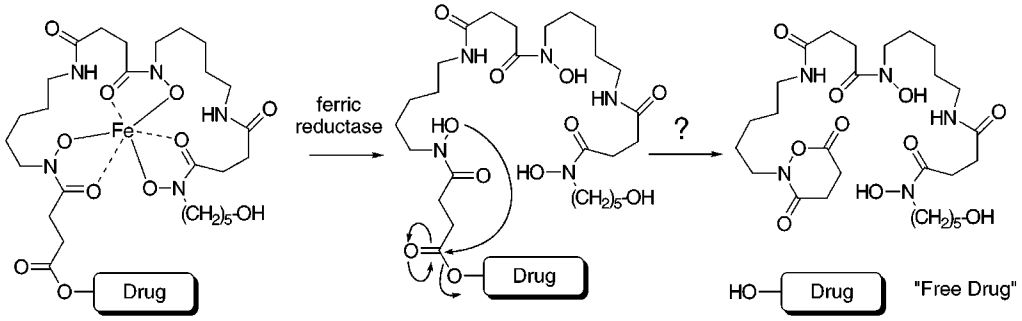
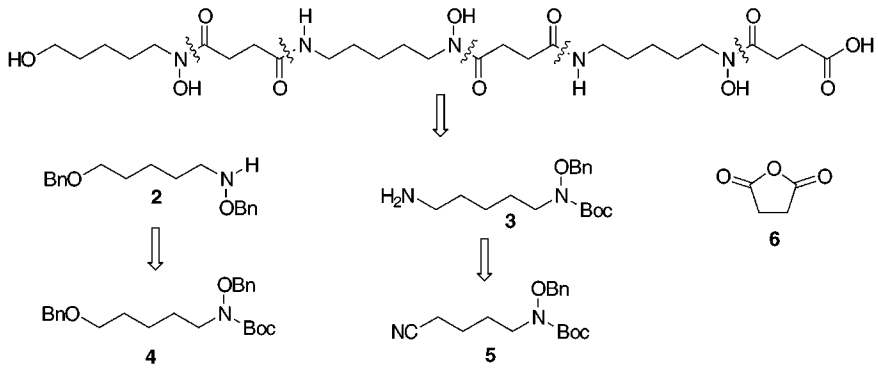


Figure 1.

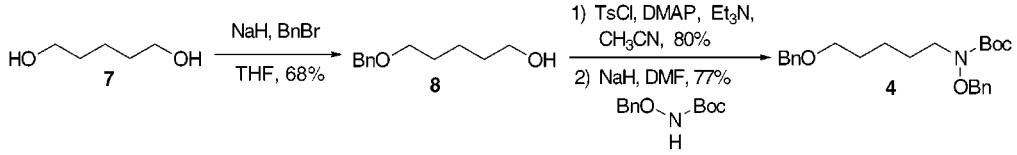
Scheme 1. A Possible Drug Release Mechanism



Scheme 2. Retrosynthetic Analysis of Danoxamine



Scheme 3. Synthesis of Protected Alcohol Component



Results

Danoxamine, similar to the siderophore Desferal,[21,22] is a linear sequence of repeating units which, if cleaved at the hydroxamic acid and amide bonds, requires the synthesis of the major components **4** and **5** (Scheme 2).

The synthesis of **4** (Scheme 3) was begun by the monoprotection of one of the hydroxyl groups of 1,5-pentanediol (**7**). Treatment of the diol with NaH and BnBr in THF afforded monoprotected diol **8**. It was found that a two-step procedure worked best to form key intermediate **4**. The alcohol was converted to the tosylate followed by purification. Then the tosylate was treated with the anion generated from BOC-NHOBn to afford intermediate **4**.[21,23−25]

With intermediate **4** in hand, the synthesis of amine **3** (Scheme 4) was accomplished using Bergeron's protocol.[26]

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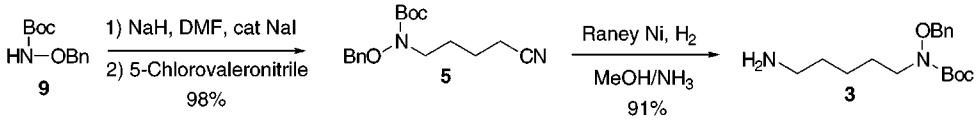
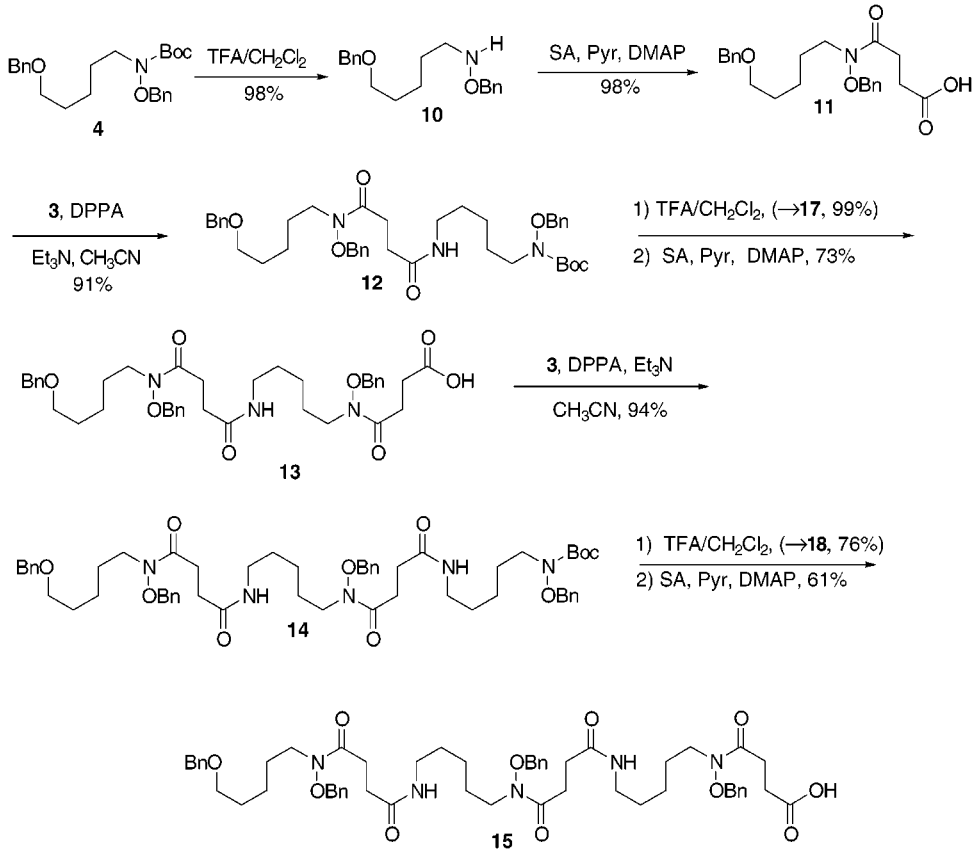
Scheme 4. Synthesis of the Amine Component



Scheme 5. First Generation Synthesis of Danoxamine



Nitrile **5** was prepared by treating 5-chlorovalernitrile with the anion generated from BOC-NHOBn. Raney nickle was then used to selectively reduce the nitrile to the amine without clevage of the NO bond to afford amine **3**. With the syntheses of both intermediates **3** and **4** completed, the synthesis of Danoxamine could be accomplished by repetitive couplings using succinic acid linkers between the components in a manner similar to that used in the preparation of other linear trihydroxamate siderophores.[21]

Brief exposure of protected hydroxylamine **4** to TFA afforded free hydroxylamine **10**. The amine was treated with succinic anhydride (SA) in pyridine to afford acid **11**. This acid was coupled to amine **3** using diphenylphosphoryl azide (DPPA) as the activating agent.[27] Protected hydroxylamine **12** could then be cycled through the same procedures to generate the next segment. The BOC protecting group was again removed by brief exposure to acid followed by acylation with succinic anhydride to generate the desired acid. This acid was treated with DPPA and amine **3** to afford **14**. The BOC protecting group was removed by brief exposure to acid and subsequently acylated with succinic anhydride to afford a protected form, **15**, of the siderophore Danoxamine. The protected form was prepared in eight steps from **4** with an overall yield of 28%. Furthermore, each step was easily scalable to afford 12 g of **13**.

The last deprotection and acylation steps, conversion of **14** to **18** and **18** to **15**, posed a major challenge, as the purification of these intermediates was very difficult. Often several purification techniques including regular and reverse phase chromatography as well as size exclusion chromatography were needed. The lower yields of these last steps (76 and 61%, respectively) are a result of these challenging separations. After exposure of **15** to H_2 and Pd/C to remove the benzyl protecting groups, a minor impurity was evident from spectral analysis. Extensive purification techniques and different deprotection conditions failed to produce a clean sample of Danoxamine.

Previous syntheses of linear hydroxamate siderophores have also utilized disalt **16** rather than *N*-protected hydroxylamine **3**.[22] This route was investigated since the previously described deprotection failed to afford pure samples of Danoxamine. After preparation of disalt **16**,[22,28] standard peptide coupling conditions, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP), triethyl-

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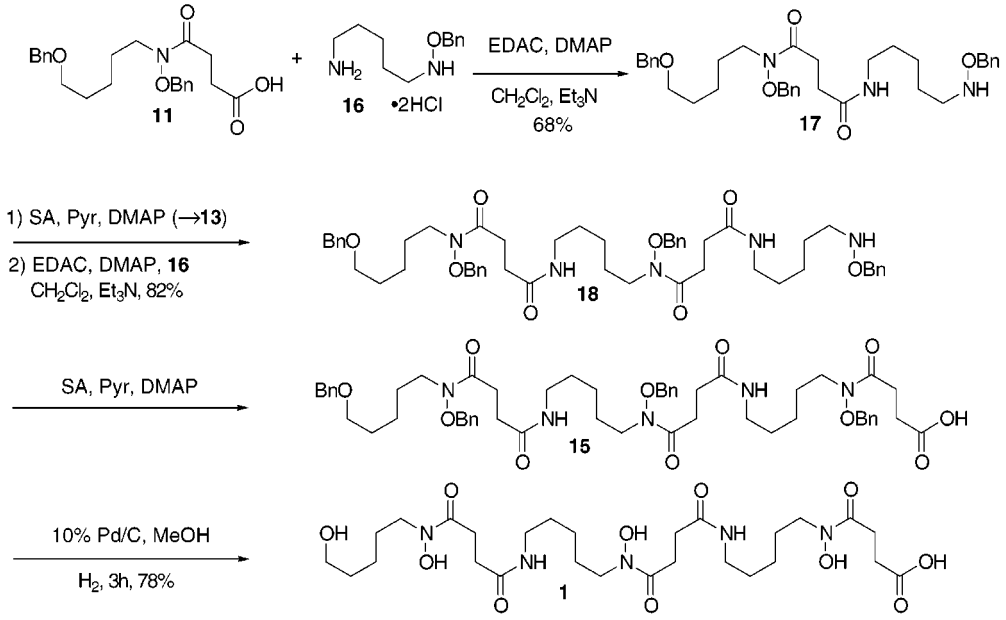
Scheme 6. Second Generation Synthesis of Danoxamine



amine with dicholoromethane as the solvent, were used to build upon acid **11** (Scheme 6) which was already available from the previous synthesis of Danoxamine.

After coupling **11** with **16**, crude protected hydroxylamine **17** was obtained. Further purification provided material which had identical spectra to the sample obtained from the first synthesis of Danoxamine. This intermediate was then acylated with succinic anhydride (SA) and subjected to the coupling conditions again to afford protected hydroxylamine **18**. A final reaction of **18** with succinic anhydride provided protected Danoxamine **15**. The purification of these intermediates was much more straightforward, and after standard regular phase chromatography, the samples were easily obtained without impurities. Furthermore, the new route reduced by two the number of steps required to prepare Danoxamine starting from **4**. The benzyl protecting groups were removed from **15** under standard hydrogenolysis conditions to afford the natural product Danoxamine (**1**) in 78% yield.

The proton (300 MHz) and carbon (75 MHz) spectra (Tables 1 and 2, in the Supporting Information) were essentially identical to the literature values for the natural product.[6]

Conclusion

The siderophore Danoxamine has been prepared and the spectral properties were found to match those of the isolated natural product. This synthesis was designed so that in the future, modifications to the route can easily provide all the siderophores (Figure 1, $n = 4$ and $n = 5$) needed to synthesize the Salmycin series. Furthermore, Danoxamine and other linear hydroxamate siderophores prepared by this method can be utilized to generate new siderophore−drug conjugates for investigation of their biological properties.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR spectra were measured at 300 or 500 MHz. All chemical shifts (δ) are reported relative to tetramethylsilane, residual chloroform, residual DMSO, or residual methanol as an internal reference for spectra measurement. Silica gel flash column chromatography was performed using Silica Gel 60 (30−70 mm irregular particles). Radial chromatography was performed using Kieselgel 60 PF254 gipshaltig. Reverse phase chromatography was performed on Whatman LRP-2 C-18 silica gel (37−53 mm particles). 1,5-Pentanediol and 5-chlorovaleronitrile were obtained from Aldrich and used without further purification.

***N*-(5-Benzyloxypentyl)-*N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine (4).** 5-(Benzyloxy)-1-pentanol[29] (2.91 g, 15 mmol) was dissolved in CH_3CN (15 mL). To this solution were added Et_3N (3.1 mL, 22 mmol), *p*-toluenesulfonyl chloride (4.29 g, 22 mmol), and catalytic DMAP. The reaction was stirred under Ar at room temperature for 10 h. All the volatile solvents were removed under reduced pressure. The resulting solid was dissolved in EtOAc and washed with 5% $NaHCO_3$, 1 N HCl, and brine. All the aqueous components were combined and washed with EtOAc. The organic layers were combined and dried, and the volatile solvents were removed under reduced pressure. The resulting material was purified by column chromatography (hexanes:EtOAc, 2:1). The fractions containing the desired tosylate were collected, and the volatile solvents removed under reduced pressure to yield 4.38 g (80%) of an oil. This material was then dissolved in freshly distilled DMF (10 mL). BOC-NHOBn[30] (2.95 g, 13 mmol) was dissolved in DMF (40 mL) and NaH (0.503 g, 13 mmol, 60% dispersion) was added. After stirring at room temperature for 20 min, the DMF solution of the tosylate was added dropwise. The reaction was allowed to stir at room temperature for 18 h at which time TLC showed no more starting material. To quench the reaction, 5% $NaHCO_3$ was added. The solution was then extracted with EtOAc. The combined organic layers were then washed with brine and dried (Na_2SO_4), and the volatile material was removed under reduced pressure to yield **4** as an oil. The crude oil was purified by column chromatography to yield a clear oil (3.86 g, 77%): IR (film, cm^{-1}) 1703; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27−7.42 (m, 10H), 4.82 (s, 2H), 4.49 (s, 2H), 3.45 (t, J = 6.6 Hz, 2H), 3.40 (t, J = 7.2 Hz, 2H), 1.67−1.34 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.60, 138.60, 135.68, 129.35, 128.45, 128.40, 28.33, 127.60, 127.47, 81.14, 76.90, 72.88, 70.23, 49.59, 29.45, 28.33, 26.90, 23.44; HRMS (FAB) calcd for $C_{24}H_{34}O_4N$ (M + H) 400.2488, found 400.2458.

***N*-(*tert*-Butoxycarbonyl)-*N*-(4-cyanobutyl)-*O*-benzylhydroxylamine (5)** was synthesized by a published proce-

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(30) Lee, B. H.; Miller, M. J. *J. Org. Chem.* **1983**, *48*, 24−31.

dure[26] with the minor modification of increasing the 5-chlorovaleronitrle to 1.2 equiv to afford an improved yield of 98%.

***N*-(5-Aminopentyl)-*N*-(*tert*-butoxycarbonyl)-*O*-benzylhydroxylamine (3)** was synthesized by a published procedure[26] in 91% yield.

***N*-(5-Benzyloxypentyl)-*O*-benzylhydroxylamine (10).** Trifluoroacetic acid (TFA, 15 mL) was added to a solution of **4** (1.50 g, 3.75 mmol) in CH_2Cl_2 (20 mL). The resulting solution was stirred at room temperature for 10 min at which time TLC analysis indicated that the reaction was complete. TFA and CH_2Cl_2 were removed under reduced pressure. Saturated aqueous $NaHCO_3$ was added to the oil, and the product was extracted into $CHCl_3$. The organic phases were combined and washed with H_2O, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to yield hydroxylamine **10** (1.101 g, 98%: IR (film, cm^{-1}) 3263; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36−7.25 (m, 10H), 5.54 (broad s, 1H), 4.70 (s, 2H), 4.49 (s, 2H), 2.46 (t, J = 6.44 Hz, 2H), 2.93 (t, J = 7.04 Hz, 2H), 1.67−1.34 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.58, 137.97, 128.31, 127.72, 127.57, 127.45, 76.16, 72.86, 70.20, 52.05, 29.60, 27.15, 23.90; HRMS (FAB) calcd for $C_{19}H_{26}O_2N$ (M + H) 300.1964, found 300.1950.

***N*-(Benzyloxy)-*N*-(5-benzyloxypentyl)succinamic Acid (11).** Hydroxylamine **10** (2.90 g, 9.67 mmol), succinic anhydride (1.452 g, 14.5 mmol), and a catalytic amount of DMAP was added to a flask containing pyridine (30 mL). The solution was heated at 95 °C for 3 h under argon, cooled to room temperature, and stirred at room temperature for 3 days. The pyridine was removed under vacuum, and the residue was dissolved in a minimal amount of chloroform and filtered. The chloroform was removed under reduced pressure, and the resulting oil was purified by chromatography (3 to 10% ethanol in $CHCl_3$) to give a yellow oil (3.803 g, 98%): IR (film, cm^{-1}) 1730, 1700; ^1H NMR (300 MHz, $CDCl_3$) δ 9.790 (bs, 1H), 7.25−7.40 (m, 10H), 4.83 (s, 2H), 4.48 (s, 2H), 3.64 (t, J = 6.90 Hz, 2H), 3.45 (t, J = 6.30 Hz, 2H), 2.58−2.76 (m, 4H), 1.56−1.72 (m, 4H), 1.32−1.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.53, 173.17 (broad), 138.47, 134.31, 129.11, 128.93, 128.68, 128.28, 127.57, 127.43, 76.24, 72.77, 70.05, 45.53 (broad), 29.24, 28.70, 27.21, 26.58, 23.33; HRMS (FAB) calcd for $C_{23}H_{30}O_5N$ (M + H) 400.2124, found 400.2121.

17-(*tert*-Butoxycarbonyl)-1,6,17-tris(benzyloxy)-7,10-dioxo-6,11,17-triazaheptadecane (12). Acid **11** (5.08 g, 12.7 mmol) was dissolved in distilled CH_3CN (50 mL). The resulting solution was cooled to 0 °C under argon, and diphenylphosphorazide (DPPA, 3.02 mL, 14.0 mmol) and triethylamine (3.90 mL, 28.0 mmol) were added to the flask. Amine **3** (4.31 g, 14.0 mmol) dissolved in CH_3CN (10 mL) was added dropwise to the reaction. The solution was stirred in an ice bath at 0 °C for 5 h and then allowed to warm to room temperature. After stirring at room temperature for 14 h, H_2O was added and the solution was extracted with EtOAc. The organic layers were combined and washed with brine, dried (Na_2SO_4), and filtered, and the was solvent removed under reduced pressure to yield a yellow oil. The oil was purified by radial chromatography (100% hexanes to 100% EtOAc) to give a yellow oil (7.99 g, 91%): IR (film, cm^{-1}) 3320, 1650; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$) δ 7.26−7.42 (m, 15H), 5.98 (t, J = 4.5 Hz, 1H), 4.837 (s, 2H), 4.813 (s, 2H), 4.477 (s, 2H), 3.624 (t, J = 6.3, 2H), 3.44 (t, J = 6.6 Hz, 2H), 3.40 (t, J = 7.2 Hz, 2H), 3.20 (q, J = 6.9 Hz, 2H), 2.77 (t, J = 5.70, 2H), 2.44 (t, J = 6.3 Hz, 2H), 1.15−1.75 (m + s, 21H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8 (broad) 172.16, 156.56, 138.55, 135.600 134.39, 129.34, 129.12, 128.88, 128.66, 128.45, 128.34, 128.31, 127.56, 127.46, 81.18, 76.87, 76.32, 72.84, 70.10, 49.34, 45.59 (broad), 39.40, 30.87, 29.34, 29.22, 28.32, 28.26, 26.72, 24.04, 23.43; HRMS (FAB) calcd for $C_{40}H_{56}N_3O_7$ (M + H) 690.4118, found 690.4142.

1,6,17-Tris(benzyloxy)-7,10-dioxo-6,11,17-triazaheptadecane (17). Method A. Trifluoroacetic acid (TFA, 4 mL) was added to solution of BOC-protected hydroxylamine **12** (0.902 g, 0.94 mmol) in CH_2Cl_2 (10 mL). The reaction was stirred at room temperature for 15 min. TFA and CH_2Cl_2 were removed under reduced pressure, and saturated $NaHCO_3$ was added to the oil. This solution was extracted with $CHCl_3$. The organic layers were combined and washed with brine, dried ($MgSO_4$), and filtered, and the solvent removed under reduced pressure to yield a yellow oil. This oil was purified by column chromatography (5% $CH_3OH/CHCl_3$) to afford deprotected **17** (0.771 g, 99%) as an oil: IR (film, cm^{-1}) 3300, 1640; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30−7.38 (m, 15H), 6.03 (t, J = 4.80, 1H), 4.84 (s, 2H), 4.69 (s, 2H), 4.48 (s, 2H), 3.63 (4, J = 6.8, 2H), 3.44 (t, J = 6.5, 2H), 3.20 (q, J = 6.7, 2H), 2.91 (t, J = 7.1, 2H), 2.78 (t, J = 6.3, 2H), 2.45 (t, J = 6.6, 2H), 1.27−1.70 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.70 (broad), 172.12, 138.52, 137.93, 134.35, 129.08, 128.85, 128.64, 128.30, 127.70, 127.55, 127.42, 76.30, 76.13, 72.81, 70.06, 51.88, 45.55 (broad), 39.35, 30.87, 29.42, 29.32, 28.26, 26.97, 26.67, 24.42, 23.39; HRMS (FAB) calcd for $C_{35}H_{48}N_3O_5$ (M + H) 590.3594, found 590.3593.

Method B. The acid **11** (430 mg, 1.08 mmol), amine **16**[22] (348 mg, 1.24 mmol), DMAP (7.0 mg, 0.05 mmol), CH_2Cl_2 (20 mL), and Et_3N (330 mL, 2.37 mmol) were combined in a round-bottomed flask. To this solution, under argon, was added EDC·HCl (248 mg, 1.29 mmol). The reaction was stirred at room temperature and followed by TLC (3% *i*-PrOH in 3:1 EtOAc:hexanes). After 3 h, CH_2Cl_2 was added to the reaction and 5% $NaHCO_3$ was used to wash the organic layer. Brine was used to further wash the organic layer which was then dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (3% *i*-PrOH in 3:1 EtOAc:hexanes) to afford a white solid (431 mg, 68%). The spectra matched those from the previous preparations of this material.

5,16,21-Tris(benzyloxy)-4,12,15-trioxo-5,11,16-triazahenicosanoic Acid (13). *O*-Protected hydroxylamine **17** (0.77 g, 1.31 mmol), succinic anhydride (0.20 g, 1.96 mmol), and a catalytic amount of DMAP were dissolved in pyridine (30 mL). The solution was heated at 90−95 °C for 2.8 h under argon, cooled to room temperature, and stirred at room temperature for 12 h. Pyridine was removed under reduced pressure. The resulting oil was taken up in ether and extracted with saturated $NaHCO_3$ and 20% $KHCO_3$. The aqueous layers were combined and washed with ether. The aqueous layer was then acidified with 6 N HCl to pH ∼ 3.0 and extracted with $CHCl_3$. The organic layers were combined, washed with H_2O, dried, and filtered, and the solvents were removed under reduced pressure. The resulting oil was purified by radial chromatography to yield the corresponding acid as a thick oil (0.66 g, 73%). The same reaction conditions were later used to prepare **13** on a 12 g scale, but only confirmatory spectra were obtained on this preparation: IR (film, cm^{-1}) 3330, 1730, 1655; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42−7.25 (m, 15H), 6.84 (broad s, 1H), 4.84 (s, 2H), 4.82 (s, 2H), 4.48 (s, 2H), 3.76 (t, J = 5.6, 2H), 3.62 (t, J = 7.1, 2H), 3.44 (t, J = 6.5, 2H), 3.23 (q, J = 5.4, 2H), 2.83 (t, J = 6.75, 2H), 2.66 (s, 4H), 2.53 (t, J = 6.90, 2H), 1.70−1.20 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.67 (broad), 173.98, 173.74, 172.38, 138.41, 134.33, 134.10, 129.12, 128.83, 128.58, 128.21, 127.48, 127.36, 76.31, 76.02, 72.71, 69.95, 45.39, 44.31, 39.28, 30.60, 29.16, 28.55, 28.28, 28.03, 26.86, 26.48, 26.40, 23.48, 23.22; HRMS (FAB) calcd for $C_{39}H_{52}N_3O_8$ (M + H) 690.3754, found 690.3732.

28-(*tert*-Butoxycarbonyl)-1,6,17,28-tetrakis(benzyloxy)-7,10,18,21-tetraoxo-6,11,17,22,28-pentaazaoctacosane (14). Acid **13** (248 mg, 0.36 mmol) was dissolved in CH_3CN (8 mL). The solution was cooled to 0 °C, triethylamine (110 mL, 0.78 mmol) and DPPA (127 mL, 0.43 mmol) were added, and after stirring for 10 min, amine **3** (122 mg, 0.40 mmol) was added. The reaction was stirred at 0 °C for 5 h and then at room temperature for 3 days. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (5% $CH_3OH/CHCl_3$) to yield a white solid (349 mg). The product was recrystallized (hexanes−EtOAc) to yield white crystals (327 mg, 94%): mp = 63−64.5 °C; IR (film, cm^{-1}) 3326, 1659; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42−7.23 (m, 20H), 6.40−6.24 (m, 2H), 4.85 (s, 2H), 4.84 (s, 2H), 4.81 (s, 2H), 4.47 (s, 2H), 3.68−3.56 (m, 4H), 3.44 (t, J = 6.4 Hz, 2H), 3.39 (t, J = 7.0 Hz, 2H), 3.23−3.16 (m, 4H), 2.80 (broad s, 4H), 2.51−2.45 (m, 4H), 1.22−1.72 (m and s, 27H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.97 (broad), 173.58 (broad), 172.13, 172.03, 156.52, 138.49, 135.54, 134.32, 129.31, 129.11, 128.87, 128.84, 128.65,

128.63, 128.43, 128.36, 128.29, 127.53, 127.44, 81.14, 76.83, 76.27, 72.80, 70.05, 49.32, 45.50 (broad), 44.65 (broad), 39.39, 39.28, 30.69, 30.59, 29.32, 29.21, 28.28, 28.11, 27.99, 26.71, 26.34, 24.04, 23.54, 23.40; HRMS (FAB) calcd for $C_{56}H_{77}N_5O_{10}$ (M^+) 979.5670, found 979.5702.

1,6,17,28-Tetrakis(benzyloxy)-7,10,18,21-tetraoxo-6,11,-17,22,28-pentaazaoctacosane (18). Method A. BOC-protected hydroxylamine **14** was dissolved in CH_2Cl_2 (2.5 mL), and TFA (2.0 mL) was added to the flask. After 20 min, TLC analysis indicated the reaction was over so the volatile materials were removed from the reaction flask under reduced pressure, and the resulting oil was dissolved in CH_2Cl_2. A saturated solution of $NaHCO_3$ was added to the solution, and the pH of the aqueous layer was adjusted to 10. The layers were separated, and the aqueous layer was washed with CH_2Cl_2. The organic layers were combined, dried ($MgSO_4$), and filtered, and the solvents were removed under reduced pressure. The resulting oil was purified by column chromatography (3% *i*-PrOH, 1% NH_4OH in EtOAc), and the white solid was then recrystallized to afford a white amorphous solid (739 mg, 76%): mp = 75−76 °C; IR (KBr window, cm^{-1}) 3354, 1672, 1647; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40−7.26 (m, 20H), 6.33 (broad s, 2H), 4.848 (s, 2H), 4.840 (s, 2H), 4.696 (s, 2H), 4.470 (s, 2H), 3.69−3.56 (m, 4H), 3.44 (t, J = 6.6 Hz, 2H), 3.21 (t, J = 6.3 Hz, 2H), 3.17 (t, J = 6.0 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.86−2.74 (m, 4H), 2.52−2.44 (m, 4H), 1.70−1.22 (m, 18H). ^{13}C NMR (125 MHz, DMSO) δ 172.85 (broad), 170.49, 138.68, 138.56, 134.93, 129.21, 129.19, 128.57, 128.44, 128.15, 128.05, 127.97, 127.30, 127.23, 75.40, 75.04, 71.75, 69.43, 51.24, 44.39 broad), 38.46, 38.35, 29.71, 29.09, 28.77, 28.73, 27.21, 26.54, 26.24, 26.14, 24.14, 23.49, 22.86; HRMS (FAB) calcd for $C_{51}H_{69}N_5O_8$ (M^+) 879.5146, found 879.5121.

Method B. Acid **13** (252 mg, 0.37 mmol), amine **16** (118 mg, 0.42 mmol), DMAP (2 mg, 0.018 mmol), CH_2Cl_2 (10 mL), and Et_3N (12 mL, 0.86 mmol) were combined in a round-bottomed flask. To this solution, under argon, was added EDC·HCl (84 mg, 0.44 mmol). The reaction was stirred at room temperature and followed by TLC. After 7 h, CH_2Cl_2 and brine were added to the reaction. The organic layer was separated and further washed with 5% $NaHCO_3$ and brine. The organic layer was then dried (Na_2SO_4) and filtered, and the volatile materials removed under reduced pressure. The crude material was purified by column chromatography (3% *i*-PrOH, 1% concentrated NH_4OH in EtOAc) to afford a white solid (334 mg, 82%). The spectra of this material matched those from the previous preparations.

Tetra-*O*-benzyl Danoxamine (15). Protected hydroxylamine **18** (365 mg, 0.415 mmol), from the second generation synthesis, and succinic anhydride (83 mg, 0.829 mmol) were dissolved in freshly distilled CH_2Cl_2 (10 mL). To this solution was added DMAP (2.5 mg, 0.021 mmol), and the reaction was placed in the dark under argon and followed by TLC. After 28 h, CH_2Cl_2 and 1 M HCl were added to the reaction. The organic layer was collected and washed with brine, dried (Na_2SO_4), and filtered, and the volatile materials were removed under reduced pressure to afford a white solid. This material was purified by size exclusion chromatography (Lipophilic Sephadex LH-40, 1:1 MeOH:CH_2Cl_2) and further purified by silica gel chromatography (5% MeOH in CH_2Cl_2) to afford a white solid (247 mg, 61%): mp = 63.0−64.5 °C; IR (thin film on NaCl plate, cm^{-1}) 3328, 1729, 1652; ^1H NMR (500 MHz, DMSO) δ 12.02 (s, 1H), 7.76 (t, J = 5.4 Hz, 2H), 7.46−7.22 (m, 20H), 4.86 (s, 6H), 4.40 (s, 2H), 3.56 (t, J = 6.89 Hz, 6H), 3.37 (t, J = 6.7 Hz, 2H), 2.99 (t, J = 6.3 Hz, 2H), 2.97 (t, J = 6.6 Hz, 2H), 2.61 (t, J = 6.1 Hz, 6H), 2.41 (t, J = 6.6 Hz, 2H), 2.29 (t, J = 6.9 Hz, 4H), 1.58−1.14 (m, 18H); ^{13}C NMR (75 MHz, DMSO) δ 173.73, 172.72 (broad), 170.94, 170.92, 138.67, 134.93, 129.21, 129.16, 128.54, 128.42, 128.13, 127.28, 127.20, 75.38, 71.73, 69.42, 44.57 (broad), 38.34, 29.69, 28.74, 28.71, 28.31, 27.20, 26.82, 26.23, 26.11, 23.46, 22.84; HRMS (FAB) for $C_{55}H_{74}N_5O_{11}$ (M + H) 980.5385, found 980.5358.

Danoxamine (1). Fully protected Danoxamine **15** (63 mg, 0.064 mmol) was dissolved in MeOH (15 mL). To this solution was added 10% Pd/C (31 mg, 5% Pd by wt). The solution was stirred as it was placed under a stream of electrolytically generated hydrogen. After 3 h, only one spot was present on reverse phase TLC (3:1, MeOH:H_2O). The reaction mixture was filtered by passing through a pipet that was 3/4 full of reverse phase (C-18) silica. The solvents were removed under reduced pressure to afford a white solid (31 mg, 78%). This material was further purified by reverse phase (C-18) chromatography (100% H_2O to 1:1 MeOH:H_2O) to afford 25 mg of a white solid: IR (KBr pellet, cm^{-1}) 3424, 3309, 3173, 1634, 1625; ^1H NMR (300 MHz, CD_3OD/DMSO) δ 3.59 (t, J = 6.7 Hz, 6H), 3.54 (t, J = 6.6 Hz, 2H), 3.16 (t, J = 6.9 Hz, 4H), 2.75 (t, J = 7.2 Hz, 6H), 2.55 (t, J = 6.7 Hz, 2H), 2.45 (t, J = 7.2 Hz, 4H), 1.47−1.96 (m, 12H), 1.27−1.42 (m, 6H); ^{13}C NMR (125 MHz, DMSO) δ 174.05, 171.95, 171.90, 171.59, 171.29, 60.58, 47.20, 47.09, 38.40, 32.18, 29.90, 28.80, 28.57, 27.56, 27.09, 26.21, 26.01, 23.47, 22.64; HRMS (FAB) for $C_{27}H_{50}N_5O_{11}$ (M + H) 620.3507, found 620.3538.

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Supporting Information Available: ^1H NMR and ^{13}C NMR for compounds **1**, **4**, **10**, **11**, **12**, **13**, **14**, **15**, **17**, **18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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