Total Synthesis of Acinetoferrin

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The total synthesis of a novel siderophore, acinetoferrin, is described. The key transformation involves the tandem oxidation and acylation of the N_3-amino group of N_1-BOC propane diamine prior to coupling with the external carboxyls of citric acid. A "one-pot−two-step" reaction converted a primary amine (RNH_2) into a *O*-benzoyl hydroxamate (i.e., RN(OOCPh)COR′) in good yield (68%). These studies demonstrated the utility of the *O*-benzoyl protecting group in the synthesis of α,β-unsaturated hydroxamic acids.

Introduction

Iron is an essential micronutrient required by mammals and most other terrestrial lifeforms. Accessing iron is problematic as it exists mainly as insoluble ferric hydroxide polymers in the biosphere. Microorganisms have evolved a group of low molecular weight, virtually ferric ion specific chelating agents (i.e., siderophores) to solubilize and transport iron within their environment. Interestingly, the ability of these ligands to transport ferric ion has found clinical use in the treatment of iron-overload diseases.[1]

In this "across-kingdom" application, the siderophores utilized for iron internalization in microorganisms are found to promote the clearance of iron from humans. Indeed, thalassemia patients have been treated successfully with deferrioxamine B (DFO) isolated from *Streptomyces pilosus* for the past 35 years.[1−6] However, a major difficulty in iron chelation therapy is patient compliance with the regimen involving triweekly infusions of DFO. Since patients would be more receptive to an orally administered drug, research has now focused on the development of orally active iron chelators.

A number of siderophores contain a citric acid component,[7] as seen in schizokinen (**1**)[8] isolated from *Bacillus megaterium*, aerobactin (**2**)[9] isolated from *Aerobacter* strains, and nannochelin A (**3**)[10] isolated from *Nannocystis exedens* (Figure 1). This class of siderophores is of interest as structurally modified derivatives of **1**−**3** may be used as suitable oral vectors, while limiting the proliferation of pathogenic bacterial strains, which grow via citrate siderophores.[11]

Recently, acinetoferrin **4** was isolated from *Acinetobacter haemolyticus* bacteria by Okujo et al.[12] This novel chelator was shown to consist of a rather unusual *trans*-2-octenoylhydroxamic acid appended to a citric acid framework.[12] Acinetoferrin represents an interesting synthetic target because of its unusual 2(*E*)-octenoylhydroxamic acid moiety and because previous synthetic strategies using *O*-benzyl-protected hydroxamates are not readily applicable.[13−16] Removal of such an *O*-benzyl group from an acinetoferrin precursor via hydrogenolysis would compromise the octenoylhydroxamate functionality by a competing hydrogenation process. Attempts at alternative reduction methods (EtSH, $BF_3{\cdot}Et_2O$) by Pattenden et al. with a tris-*O*-benzyl-protected nannochelin A precursor gave only a 30% yield of **3**.[17] Recognizing the structural similarity of acinetoferrin and nannochelin A, we hoped to apply our previous method[18] for the synthesis of α,β-unsaturated hydroxamic acids to the synthesis of acinetoferrin.

Results and Discussion

Acinetoferrin consists of two *N*-(3-aminopropyl)-*N*-(hydroxy)-2(*E*)−octenamide fragments attached via the primary amino group to the terminal carboxyl groups of citric acid by amide bonds (Figure 1).[12] Our strategy involved the construction of a *N*-(3-aminopropyl)-*N*-(hydroxy)-2(*E*)-octenamide precursor via a tandem oxidation-acylation of the primary amino group of a protected

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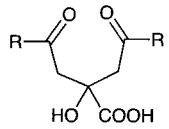


iron chelators

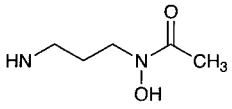
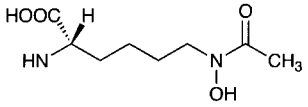
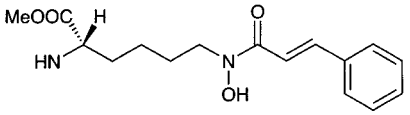
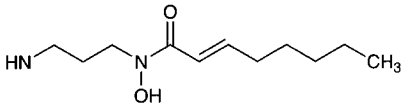
Name	Microorganism	R
schizokinen, **1**	*Bacillus megaterium*	
aerobactin, **2**	*Aerobacter* strains	
nannochelin A, **3**	*Nannocystis exedens*	
acinetoferrin, **4**	*Acinetobacter haemolyticus*	

Figure 1. Iron chelators predicated upon a citric acid component.

propanediamine derivative prior to coupling with the external carboxyls of citric acid.

In 1990 Milewska et al. reported the conversion of the free N^{δ}-amine of N^{α}-(benzyloxycarbonyl)-L-ornithine *tert*-butyl ester to an *O*-benzoyl-protected hydroxamate using benzoyl peroxide (BPO) followed by acetyl chloride.[19] As one strategy to access acinetoferrin involved the oxidation and acylation of a propanediamine derivative, this tandem sequence seemed a worthwhile approach. Indeed, this transformation was employed in our earlier synthesis of nannochelin A,[18] albeit in 25% yield.

As shown in Scheme 1, the reaction of 1,3-propanediamine (**5**) and di-*tert*-butyl dicarbonate afforded 3-(*tert*-butoxycarbonylamino)propylamine (**6**) in 81% yield. Compound **6** was dissolved in a biphasic carbonate buffer (pH = 10.5) and reacted with 2 equiv of BPO dissolved in CH_2Cl_2 at room temperature. This oxidation step generated the desired benzoyloxy amine **7** and benzamide **8**. The mixture was then acylated with *trans*-2-octenoyl chloride to give the desired *N*-(3-(*tert*-butoxycarbonylamino)propyl)-*N*-(benzoyloxy)-2(*E*)-octenamide (**9**) in 68% isolated yield. This is a significant increase in yield, when compared to our earlier efforts with **3**, and was realized only after a careful study of the initial oxidation step.

The mono-oxidation of primary amines is difficult at best as the products are often over-oxidized by the reaction conditions.[20] Early studies with **6** using benzoyl peroxide as the oxidant gave the desired benzoyloxy amine **7** and identified two competing pathways: N-oxidation and N-acylation.[21] We discovered that a biphasic medium (CH_2Cl_2/aqueous buffer solution) favored the formation of the benzoyloxy amine **7** at the expense of the benzamide **8**.[21] Indeed, a careful study of the benzoyl peroxide mediated oxidation of **6** revealed a dramatic dependence on the pH of the aqueous phase and the molar ratio of the amine and BPO. To date, the highest yield of **7** (72%) was obtained with equal volumes of a pH 10.5 carbonate buffer solution and 0.1 M amine **6** in CH_2Cl_2 using 2 equiv of benzoyl peroxide (versus the amine) at room temperature.

While the selective coupling of amino substrates with the terminal carboxyls of citric acid can be achieved utilizing either 2-substituted-1,3-bis-activated esters of citric acid or anhydromethylene citric acid,[8,22] the reported imide formation associated with the latter approach prompted the use of the former coupling methodology. The selective activation of the 1,3 carboxyls of citric acid was accomplished by the formation of the bis-activated ester: 2-*tert*-butyl-1,3-di-*N*-(hydroxy)succinimidyl citrate, **10**.[8]

Synthesis of *tert*-butylacinetoferrin (**11**) required the coupling of the functionalized propylamine fragment to the activated citric acid framework. Previous experience revealed that higher yields were obtained in the condensation step if the benzoyl group was removed prior to amide formation.[18] Therefore, **9** was treated with 10% NH_3/MeOH solution at −23 °C to give the "free" hydroxamate **12** (89%). Treatment of **12** with trifluoroacetic acid (TFA) at 0 °C gave the *N*-(3-aminopropyl)-*N*-(hydroxy)-2(*E*)-octenamide, TFA salt (**13**). Condensation of **13** with a solution of the bis *N*-hydroxysuccinimide (NHS) ester **10**[8] and triethylamine (TEA) in 1,4-dioxane gave *tert*-butylacinetoferrin (**11**) in 86% yield. Finally, treat-

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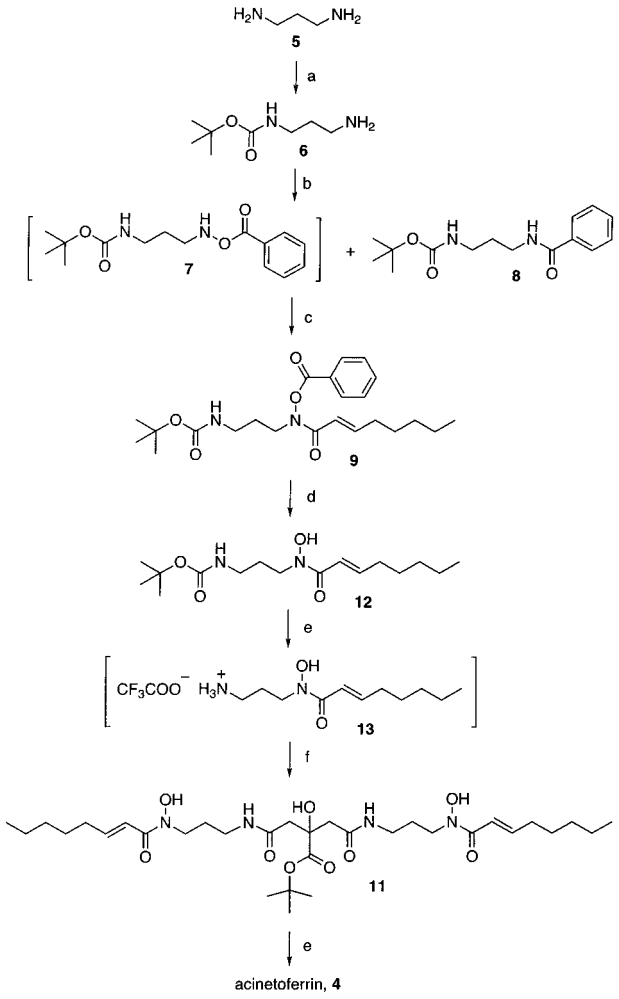
Scheme 1. Synthesis of Acinetoferrin (4)[a]



[a] Reagents: (a) di-*tert*-butyl dicarbonate, 10% TEA/MeOH; (b) benzoyl peroxide (2 equiv)/CH_2Cl_2, pH = 10.5 aqueous sodium carbonate buffer, rt; (c) *trans*-2-octenoyl chloride, CH_2Cl_2; (d) 10% NH_3/MeOH; (e) TFA, 0 °C; (f) TEA, **10**, dry dioxane, 15 °C.

ment of **11** with TFA gave acinetoferrin, (**4**) in 97% yield (see Scheme 1).

The R_f values of **4** in several solvent systems (as determined by TLC) were identical with those reported for the natural product.[12] Although we were unable to obtain an authentic sample, the mass spectrum and the proton NMR spectrum of **4** in CD_3OD of the synthetic material were in complete agreement with the published spectra.[12] In addition, 2-D NMR experiments confirmed the C−H assignments made by Okujo.[12] The ^{1}H NMR spectrum of **4** (in CD_3OD) revealed both *Z* (Peak at δ 3.70) and *E* (peak at δ 3.65) rotamers of acinetoferrin (**4**) (see also ref 12), whereas in DMSO-d_6 these isomers are detected at δ 3.55 and 3.48, respectively. These signals were assigned to the methylene group adjacent to the hydroxamic acid nitrogen in structure **4** (e.g., C**H**$_2$N(OH)-COR).[12] The rotamers are generated from the hindered rotation about the hydroxamic acid carbon−nitrogen bond (Figure 2) and do not pertain to the *E*-alkene moiety. The *E* and *Z* rotamers were assigned by data obtained from earlier model systems[23,24] and were observed in both CD_3OD and DMSO-d_6 with the *Z* isomer

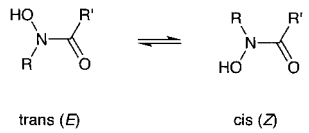


Figure 2. *E* and *Z* rotamers of a typical hydroxamic acid.

predominating. The preference for *Z* isomers in these solvents has been observed by Brown and co-workers with other hydroxamic acids.[23]

During our scale-up of **4**, we observed a facile cyclization in the presence of TFA to give the imide **14**. This phenomenon was temperature dependent. Workup at room temperature gave **4**, whereas elevated temperatures during TFA removal gave **14**. The ^{1}H NMR spectrum of **14** (in CD_3OD) is shown in Figure 3. A sample of **4** in CD_3OD was 79% converted to **14** upon standing at room temperature for 9 months. Similar cyclizations were recently observed with another citric acid based chelator, rhizoferrin,[25] and other amide containing systems.[26−28]

In summary, the synthesis provided both proof of structure of the natural product and a method, which should allow facile access to a variety of acinetoferrin homologues with different amino fragments. The mild, yet selective, method of converting a primary amine into a protected hydroxamate should be of immense value to medicinal chemists interested in synthesizing functionalized hydroxamic acids.

Experimental Section

Materials and Methods. Lipophilic Sephadex LH-20 was obtained from the Sigma Chemical Company. Silica gel 60 (70−230) mesh was purchased from EM Science, Darmstadt, Germany. Solvents were freshly distilled prior to use. Reagents were purchased either from the ACROS Chemical Company or the Aldrich Chemical Co. and were used without further purification. *trans*-2-Octenoic acid was purchased from Pfaltz and Bauer, Inc. All solutions are expressed in volume % unless otherwise indicated. Ammonia-containing solutions were prepared by measuring out concentrated aqueous NH_4-OH in the listed volume %.

Acinetoferrin (4). TFA (2 mL) was added dropwise to **11** (34 mg, 5.3 μmol) at 0 °C. After the addition was complete, the stirred solution was allowed to warm to room temperature. TLC (7% EtOH/$CHCl_3$) showed that no starting material remained after 1 h. The volatiles were removed under high vacuum and gave a yellow oil, which was eluted on LH-20 Sephadex (3.5 g) with 10% EtOH/toluene to give acinetoferrin (**4**) (30 mg, 97%). **4**: R_f = 0.31 (benzene:acetic acid:water (125:72:3, matched lit. value),[12] R_f = 0.38 in 25% MeOH/$CHCl_3$; ^{1}H NMR (500 MHz) (CD_3OD) δ 6.84 (dt, 2H, olefinic), 6.61 (d, 2H, olefinic), two triplets at 3.70 and 3.65 (2.1:1 integration ratio respectively, total integration for both signals 4H, CH_2NO), 3.20 (m, 4H, CH_2NCO), 2.71 (d, 2H, CH_2), 2.64 (d, 2H, CH_2), 2.24 (dt, 4H, $CH_2C{=}C$), 1.82 (m, 4H, CH_2) 1.48 (m, 4H, CH_2), 1.34 (m, 8H, CH_2), 0.90 (t, 6H, CH_3); ^{1}H NMR (600 MHz) (DMSO-d_6) δ 9.83 (s, 1H, COOH), 7.96 (s, 2H), 6.70 (dt, 2H, olefinic), 6.51 (d, 2H, olefinic), two triplets at 3.55 and 3.48 (2.3:1 integration ratio respectively, total integration for both signals 4H, CH_2NO), 3.02 (m, 4H, CH_2NCO), 2.50 (dd, 4H,

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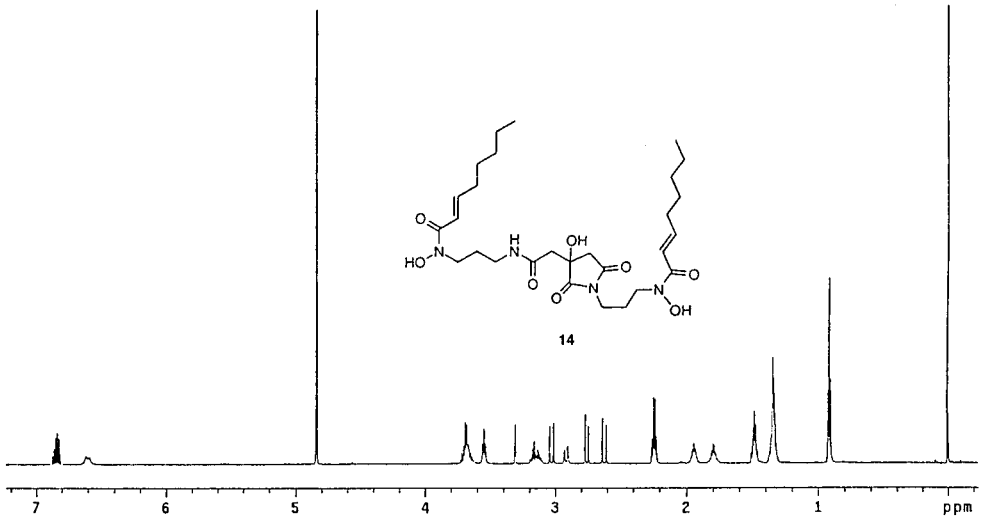

Figure 3. ^{1}H NMR spectrum of **14** in CD_3OD.

CH_2), 2.19 (dt, 4H, $CH_2C{=}C$), 1.63 (m, 4H, CH_2) 1.41 (m, 4H, CH_2), 1.28 (m, 8H, CH_2), 0.86 (t, 6H, CH_3); high-resolution mass spectrum (FAB), theory for ($C_{28}H_{49}N_4O_9$) M + 1 = 585.3500, found M + 1 = 585.3507.

3-(*tert*-Butoxycarbonylamino)propylamine (6). 1,3-Diaminopropane (**5**) (11.16 g, 150 mmol) was dissolved in 350 mL of a 10% TEA/MeOH solution. A solution of di-*tert*-butyl dicarbonate (10.9 g, 50 mmol) and MeOH (20 mL) was added to this mixture with vigorous stirring. The mixture was refluxed for 2 h. The *tert*-butoxycarbonylation was complete as evidenced by thin-layer chromatography (TLC) using 4% MeOH/$CHCl_3$. The mixture was concentrated and subjected to flash column chromatography to give the mono-BOC amine **6** (7.06 g, 81%); R_f = 0.24 (4% NH_3/MeOH); ^{1}H NMR ($CDCl_3$) δ 4.95 (br s, 1H, NH), 3.21 (m, 2H, CH_2NBOC), 2.72 (t, 2H, CH_2N), 1.58 (m, 2H, CH_2), 1.40 (s, 9H, *tert*-butyl).

***N*-(3-(*tert*-Butoxycarbonylamino)propyl)benzamide (8).** The byproduct, **8**, was also isolated (0.67 g, 8%) during the synthesis of **9**. **8:** R_f = 0.29 in 40% ethyl acetate/hexane; ^{1}H NMR ($CDCl_3$) δ 7.85 (d, 2H, aromatic H), 7.44 (m, 3H, aromatic H), 4.92 (broad s, 1H, NH), 3.51 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 1.43 (s, 9H, *tert*-butyl); high-resolution mass spectrum (FAB): theory for ($C_{15}H_{23}N_2O_3$) M + 1 = 279.1709, found M + 1 = 279.1703.

***N*-(3-(*tert*-Butoxycarbonylamino)propyl)-*N*-(benzoyloxy)-2(*E*)-octenamide (9).** A solution of benzoyl peroxide (14.52 g, 60 mmol) and 225 mL of CH_2Cl_2 was added dropwise at room temperature to a vigorously stirred mixture of **6** (5.22 g, 30 mmol) and 300 mL of a carbonate buffer solution (at pH 10.5). The buffer solution was prepared by combining 222 mL of 0.75 N aqueous $NaHCO_3$ and 78 mL of 1.5 N aqueous NaOH. The starting material was consumed after 4 h as shown by TLC (4% NH_3/MeOH). Column chromatography (40% ethyl acetate/hexane) was used to separate *N*-(benzoyloxy)-3-(*tert*-butoxycarbonylamino)propylamine (**7**) and the benzamide **8** (e.g., R_f = 0.43 and 0.29, respectively). A characterization of **7** is included for completeness. **7**: ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 2H, aromatic H), 7.58 (m, 1H, aromatic H), 7.47 (m, 2H, aromatic H), 4.81 (broad m, 1H, NH), 3.22 (m, 4H, CH_2), 1.82 (m, 2H, CH_2), 1.41 (s, 9H, *tert*-butyl); high-resolution mass spectrum (FAB), theory for ($C_{15}H_{23}N_2O_4$) M + 1 = 295.1658, found M + 1 = 295.1645. However, further studies showed that the next step could be accomplished without isolation of **7**. After the first reaction was complete, a solution of *trans*-2-octenoyl chloride (4.81 g, 30 mmol) in 30 mL of CH_2Cl_2 was added dropwise over 15 min. Note: the acid chloride was generated from *trans*-2-octenoic acid using refluxing thionyl chloride and was distilled under vacuum prior to use. The disappearance of **7** was monitored by TLC (40% ethyl acetate/hexane). After the acylation was complete, the organic layer was separated and the remaining water layer was washed twice with additional CH_2Cl_2 (2 × 100 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the crude product. The crude amide **9** was subjected to flash column chromatography, eluting with 30% ethyl acetate/hexane, to give the *N*-(3-(*tert*-butoxycarbonylamino)propyl)-*N*-(benzoyloxy)-2(*E*)-octenamide **9** (8.58 g: 68%) as a colorless oil. **9**: R_f = 0.33 in 30% ethyl acetate/hexane; ^{1}H NMR ($CDCl_3$) δ 8.12 (d, 2H, aromatic), 7.71 (t, 1H, aromatic H), 7.54 (t, 2H, aromatic H), 7.03 (dt, 1H, olefinic), 6.05 (d, 1H, olefinic), 5.20 (broad, 1H, NH), 3.92 (t, 2H, CH_2-NO), 3.24 (m, 2H, CH_2), 2.15 (m, 2H, $CH_2C{=}C$), 1.82 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.53−1.15 (m, 13H, *tert*-butyl and 2 CH_2), 0.83 (t, 3H, CH_3). Anal. Calcd for $C_{23}H_{34}N_2O_5$: C, 66.01; H, 8.19; N, 6.69. Found: C, 65.83; H, 8.17; N, 6.61.

2-*tert*-Butyl-1,3-di-*N*-(hydroxy)succinimidyl Citrate (10). The bis NHS ester **10** was prepared by a previous method (see ref 8 and an improved purification method in ref 18).

Acinetoferrin *tert*-Butyl Ester (11). Triethylamine (3.5 g, 34.7 mmol) was added dropwise to a mixture of **10** (1.25 g, 2.83 mmol) and **13** (1.82 g, 6.2 mmol) in 60 mL of dry dioxane at 15 °C under nitrogen. The solution was allowed to warm to room temperature and stirred overnight. The volatiles were removed under high vacuum and the residue was chromatographed using pretreated silica gel. Before use the silica gel was made "iron free" by washing with methanol:acetone:10M HCl (45:45:10), followed by a 10 wt % Na_2CO_3 solution, and then rinsed with deionized water until pH 7 and air-dried. Eluting the column with 7.5% MeOH/$CHCl_3$ gave the *tert*-butyl ester of acinetoferrin (**11**) (1.56 g, 86%). **11**: R_f = 0.27 in 7% MeOH/$CHCl_3$; ^{1}H NMR (600 MHz) ($CDCl_3$) δ 9.38 (broad s, 1H), 7.46 (s, 2H), 6.85 (m, 2H, olefinic), 6.60 (d, 2H, olefinic), two multiplets at 3.74 and 3.68 (1:1 integration ratio respectively, total integration 4H, CH_2), 3.22 (broad s, 4H, CH_2), 2.63 (dd, 4H, CH_2), 2.19 (dt, 4H, CH_2), 1.84 (m, 4H, CH_2), 1.46 (s, 9H, *tert*-butyl), 1.44 (m, 4H, CH_2), 1.29 (m, 8H, CH_2), 0.88 (t, 6H, CH_3); high-resolution mass spectrum (FAB): theory for ($C_{32}H_{56}N_4O_9$) M + 1 = 641.4126, found M + 1 = 641.4143. Anal. Calcd for $C_{32}H_{56}N_4O_9$: C, 59.98; H, 8.81; N, 8.74. Found: C, 59.69; H, 8.69; N, 8.51.

***N*-(3-(*tert*-Butoxycarbonylamino)propyl)-*N*-(hydroxy)-2(*E*)-octenamide (12).** A 10% NH_3/MeOH solution (30 mL) was added dropwise to **9** (3.7 g, 8.85 mmol) under nitrogen at −23 °C using a dry ice/CCl_4 bath. The reaction was monitored by TLC (30% ethyl acetate/hexane). After 3 h, the reaction was complete. The mixture was concentrated to give a crude light yellow oil, which was subjected to flash column chromatography on "pretreated" silica gel (see procedure for **11**). The product was eluted with 40% ethyl acetate/hexane to give **12** as a light yellow solid (2.47 g, 89%). R_f = 0.29 in 45% ethyl acetate/hexane. **12**: ^{1}H NMR ($CDCl_3$) δ 6.83 (m, 1H), 6.57 (m, 1H), 5.15 (broad, 1H), 3.67 (t, 2H), 3.09 (m, 2H), 2.13 (m, 2H),

1.75 (m, 2H), 1.53−1.15 (m, 15H, *tert*-butyl and CH_2), 0.83 (t, 3H); high-resolution mass spectrum (FAB), theory for ($C_{16}H_{31}N_2O_4$) M + 1 = 315.2283, found M + 1 = 315.2273.

***N*-(3-Aminopropyl)-*N*-(hydroxy)-2(*E*)-octenamide, Trifluoroacetic Acid Salt (13).** TFA (50 mL) was added dropwise over 1 min to **12** (1.97 g, 6.27 mmol) at 0 °C under nitrogen. The ice bath was removed and the solution stirred for 5 min. TLC (45% ethyl acetate/hexane) showed that the reaction was complete. The volatiles were removed under reduced pressure to give the desired salt **13** (2.06 g). The solid residue was consumed in the synthesis of *tert*-butylacinetoferrin (**11**).

Imide of Acinetoferrin (14). Compound **14** was generated during the removal of TFA under heat and reduced pressure. **14**: R_f = 0.77 in 50% acetone/$CHCl_3$, R_f = 0.64 in benzene:acetic acid:water (125:72:3), and R_f = 0.46 in 15% MeOH/$CHCl_3$; ^{1}H NMR (600 MHz) (CD_3OD) δ 6.84 (m, 2H), 6.62 (d, 2H), 3.69 (m, 4H), 3.57 (t, 2H), 3.17 (m, 2H), 3.05 (d, 1H), 2.93 (d, 1H), 2.77 (d, 1H), 2.63 (d, 1H), 2.24 (m, 4H), 1.96 (m, 2H), 1.80 (m, 2H), 1.49 (m, 4H), 1.36 (m, 8H), 0.93 (t, 6H); ^{13}C NMR (600 MHz) (CD_3OD) δ 14.5, 23.7, 26.2, 27.8, 29.3, 32.7, 33.6, 37.6, 37.7, 43.2, 43.3, 47.0, 47.1, 73.8, 120.5, 120.6, 148.4, 148.5, 168.7, 171.6, 177.0, 180.6; high-resolution mass spectrum (FAB), theory for ($C_{28}H_{47}N_4O_8$) M + 1 = 568.3347, found M + 1 = 568.3347. Anal. Calcd for $C_{28}H_{47}N_4O_8$: C, 59.24; H, 8.34; N, 9.87. Found: C, 59.14; H, 8.16; N, 9.93.

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