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Total synthesis and structure revision of petrobactin

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Abstract—The total synthesis and the revised structural assignment of petrobactin, a siderophore isolated from the marine bacterium *Marinobacter hydrocarbonoclasticus*, is reported. The key step in the synthesis involved condensation of N^1-(2,3-dibenzoyloxybenzoyl)-N^4-benzylspermidine with 1,3-di-(*p*-nitrophenyl)-2-*tert*-butyl citrate. Proton NMR spectra of the synthesized product compared with those reported for the natural product revealed that the compound did not contain 2,3-dihydroxybenzoyl moieties as published; instead, the splitting pattern suggested 3,4-dihydroxybenzoyl fragments. The 3,4-dihydroxybenzoyl analogue was accessed via a similar route; the proton and carbon-13 NMR spectra of this compound were consistent with those reported for natural petrobactin.

1. Introduction

Iron serves as a prosthetic for many different redox systems, including enzymes[1] and cytochromes,[2] which are essential for life itself. Although iron comprises about 5% of the earth's crust, it is not easily accessible to living systems. In the biosphere, iron primarily exists as Fe(III), which forms highly insoluble ferric hydroxide polymers under physiological conditions, $K_{sp} = 2 \times 10^{-39}$, corresponding to a solution concentration of free Fe(III) of approximately 1×10^{-19} M in this pH range.[3,4] Because of this insolubility, most life forms have developed specific ligands to sequester and manage the metal. The bacteria assemble relatively low-molecular-weight, virtually ferric iron-specific, chelators, siderophores, which they secrete into the surroundings.[5–8] These ligands sequester Fe(III) and render it utilizable by the microorganism.

In humans, a number of disease states have been identified in which iron transport and storage systems are overwhelmed with excess metal; significant damage to tissues and organs often occurs.[9,10] Left untreated, these diseases can be fatal; the management of these iron overload syndromes requires chelation therapy. Interestingly, the treatment of choice for iron overload is a hydroxamate siderophore, desferrioxamine,[11–14] but because of the poor efficiency of the drug and the required time-consuming (10 to 12 h/day, 5 or 6 days/week) infusion of the drug, patient compliance is a problem.[15–17] Both synthetic ligands[18] and natural products[19] have been targeted in the search for more efficient and orally deliverable therapeutic agents. The current paper focuses on the total synthesis of a natural product ligand, petrobactin, which is a candidate in these investigations.

Petrobactin is a siderophore that was isolated from an oil-degrading marine bacterium, *Marinobacter hydrocarbonoclasticus*.[20,21] It is a hexacoordinate ligand and forms a 1:1 complex with Fe(III). Although not confirmed by its synthesis, the initially reported structure of petrobactin (Fig. 1) suggested that the compound was predicated on a citrate bis-spermidine backbone, the 2,3-dihydroxybenzoyl

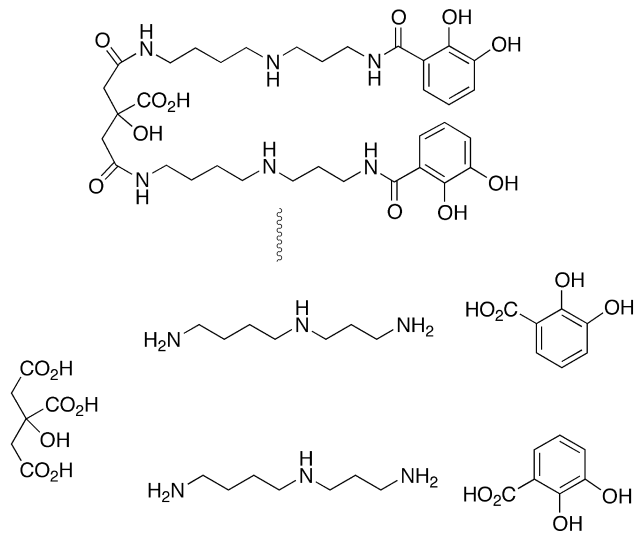

Figure 1. Retrosynthetic schematic of the original structure of petrobactin.

Keywords: siderophore; *Marinobacter hydrocarbonoclasticus*; synthesis; citrate; spermidine.

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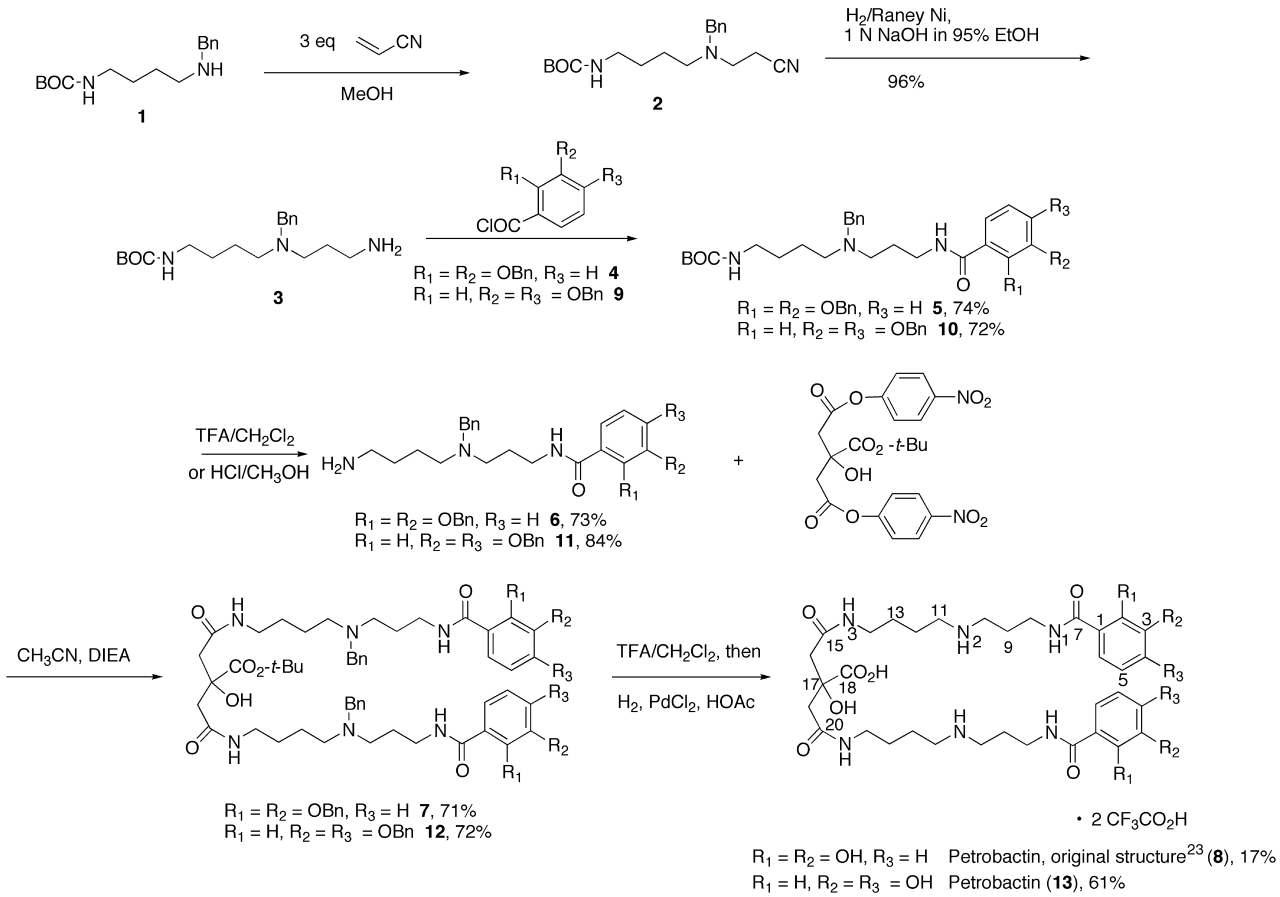

Scheme 1. Total synthesis of petrobactin: generation of the 2,3- and 3,4-dihydroxybenzoyl compounds.

moieties providing four of the requisite six donor groups for Fe(III). This latter functional group is found in a number of well-characterized iron chelators, most notably L-parabactin, which is isolated from *Paracoccus denitrificans*.[22] Interestingly, petrobactin participates in a photolytic ligand-to-metal charge-transfer reaction, leading to decarboxylation and likely reduction of Fe(III) to Fe(II).[23] This occurs in solution when exposed to sunlight. Such Fe(III)-mediated photolytic decarboxylations of α-hydroxy carboxylic acids are well-known.[24,25] Although this may play a role in how the ligand processes the metal, further investigation is needed. The availability of either radiolabeled ligand or greater quantities of the compound would help address some of these issues. Of course, facile access to relatively large amounts of the chelator is requisite if any iron clearance studies are planned in animals.

The current study focuses on synthetic approaches to petrobactin and related compounds. Comparison of the proton NMR of a 2,3-dihydroxybenzamide model compound with the published assignment[23] suggested that the bidentate donor fragment was not, in fact, a 2,3-dihydroxybenzoyl moiety. Closer review of the spectra of the isolated material revealed that the distinct splitting in the aromatic region characteristic of a 2,3-dihydroxybenzoyl fragment was absent. Interestingly, the splitting pattern more nearly approximated that found in 3,4-dihydroxybenzoyl-substituted systems. Total synthesis of both the 2,3- and 3,4-dihydroxybenzoyl compounds unequivocally demonstrates that petrobactin utilizes a 3,4-dihydroxybenzoyl fragment, rather than a 2,3-dihydroxybenzoyl donor. No hexadentate iron chelator from a natural source containing the 3,4-dihydroxybenzamide moiety has been reported previously, in spite of the fact that 3,4-dihydroxybenzoic acid binds iron(III) more tightly than its 2,3-isomer at physiological pH.[26]

2. Results and discussion

2.1. Synthetic design considerations

From a retrosynthetic standpoint (Fig. 1), the original structure of petrobactin[23] is defined by a central citrate moiety, two spermidine fragments, and two terminal 2,3-dihydroxybenzoyl groups. From the perspective of synthetic design, the asymmetry of spermidine, that is, its 3-aminopropyl and 4-aminobutyl limbs, suggests a selectively protected spermidine fragment.[27] Substitution at the terminal carboxyls of citrate warrants a central citric acid fragment protected at the central carboxyl.

2.2. Synthesis of 'petrobactin', original structure

The key step in the synthesis of what was initially reported as petrobactin (**8**, Scheme 1) involved the condensation of the citrate triester 1,3-di-(*p*-nitrophenyl)-2-*tert*-butyl citrate[28] with N^1-(2,3-dibenzyloxybenzoyl)-N^4-benzyl-

Table 1. ^{13}C and ^{1}H resonances for the synthetic 2,3-dihydroxy compound (**8**)

Atom	Found DMSO ^{13}C	Found DMSO ^{1}H mult (J in Hz)	Found DMSO:D_2O ca 4:1 ^{1}H mult (J in Hz)
C1 (C29)	115.06	na	na
C2 (C30)	146.21	na	na
C3 (C31)	149.40	na	na
C4 (C32)	118.83	6.92 dd (1.3, 7.8)	6.92 dd (1, 7.8)
C5 (C33)	117.98	6.69 dd (7.8, 8.1)	6.71 dd (7.8, 8.1)
C6 (C34)	117.22	7.26 dd (1.3, 8.1)	7.18 dd (1, 8.1)
C7 (C28)	169.90	na	na
C8 (C27)[a]	36.16	3.36 m	3.26 t (6.6)
C9 (C26)[a]	26.01	1.86 m	1.83 m
C10 (C25)[a]	44.74	2.92 m	2.86 m
C11 (C24)[a]	46.47	2.92 m	2.86 m
C12 (C23)[a]	25.78	1.42 m	1.39 m
C13 (C22)[a]	22.96	1.55 m	1.52 m
C14 (C21)[a]	37.67	3.04 m	3.01 t (6.8)
C15 (C20)	169.52	na	na
C16 (C19)	43.30	2.50 d (14.5)	2.46 d (14.6)
–	–	2.58 d (14.5)	2.56 d (14.6)
C17	73.44	na	na
C18	174.99	na	na
N1 (N6)	na	8.86 t (5.6)	ex
N2 (N5)	na	8.33 br m	ex
N3 (N4)	na	7.97 t (5.6)	ex
OH[b]	na	no	no
OH[c]	na	9.20 s	ex
OH[d]	na	5.52 br s	ex
OH[e]	na	no	no

br=broad, d=doublet, dd=doubled doublet, ex=exchanged, m=multiplet, mult=multiplicity, na=not applicable, no=not observed, nr=not reported, s=singlet, t=triplet.

[a] ^{1}H chemical shifts are the center of the observed multiplet.

[b] OH of C2 and C30, not observed within spectral width of −2 to 11 ppm.

[c] OH of C3 and C31, tentative assignment.

[d] OH of C17.

[e] Carboxylic OH of C18.

spermidine (**6**). The assembly of the spermidine reagent (**6**) began with cyanoethylation of N^1-benzyl-N^4-(*tert*-butoxycarbonyl)-1,4-diaminobutane (**1**)[29] to afford nitrile **2** in quantitative yield. Exposure of **2** to hydrogen over Raney nickel reduced the nitrile to provide N^4-benzyl-N^8-(*tert*-butoxycarbonyl)spermidine (**3**) in 96% yield. This amine was acylated at the N^1-position with 2,3-dibenzyloxybenzoyl chloride (**4**),[30,31] producing the fully protected 'side fragments' N^1-(2,3-dibenzyloxybenzoyl)-N^4-benzyl-N^8-(*tert*-butoxycarbonyl)spermidine (**5**) in 74% yield. Removal of the *tert*-butoxycarbonyl group of **5** with trifluoroacetic acid (TFA) generated the synthon N^1-(2,3-dibenzyloxybenzoyl)-N^4-benzylspermidine (**6**) in 73% yield.

Coupling of the amine (**6**, 2 equiv.) with the citrate triester was accomplished in acetonitrile and *N*,*N*-diisopropylethylamine (DIEA), providing the protected skeleton of the target ligand (**7**) in 71% yield. The *tert*-butyl ester **7** was cleaved to the corresponding acid, 1,3-bis[N^8-(N^1-2,3-dibenzyloxybenzoyl-N^4-benzyl)spermidinyl] citrate, with TFA in CH_2Cl_2. Finally, the six benzyl protecting groups were removed by hydrogenolysis over $PdCl_2$ to provide the putative siderophore (**8**) in 17% calculated yield for the two steps. The synthetic sequence proceeded efficiently except for the unmasking of **7** to chelator **8**. A major byproduct, which was separated by HPLC, resulted from cyclization of **8** to the five-membered imide on the basis of NMR analysis. This instability is inherent in citrate-derived amides such as the siderophores rhizoferrin[32] and acinetoferrin.[33]

2.3. Comparison of the proton NMR spectrum of the synthetic product with that of the reported molecule; synthesis of the 3,4-dihydroxy compound

Unfortunately, the 500-MHz ^{1}H NMR spectrum of the 2,3-dihydroxy synthetic product (**8**) differed significantly from that reported for the siderophore.[23] The observed aromatic proton resonances and their associated coupling constants for the synthetic product were 6.69 ppm (7.8 and 8.1 Hz), 6.92 ppm (1.3 and 7.8 Hz) and 7.26 ppm (1.3 and 8.1 Hz). The two larger coupling constants for the 6.69 ppm resonance were consistent with two ortho couplings,[34] confirming assignment of this resonance to position 5 of the aromatic rings. The two remaining resonances displayed both a small meta and a large ortho coupling, indicating that these protons flank the 5 position. Comparison of the observed shifts with those predicted for a model compound, *N*-methyl-2,3-dihydroxybenzamide,[‡] permitted assignment of the 7.26 ppm resonance to position 6 and the 6.92 ppm resonance to position 4. With the aromatic protons assigned, the aromatic ^{13}C resonances were easily assignable from the $^{1}H-^{13}C$ HMQC spectrum. Table 1 lists observed ^{1}H and ^{13}C resonances and their assignments for the synthetic 2,3-dihydroxy product (**8**).

Chemical shifts and coupling constants as listed for the

[‡] ChemNMR Pro ver. 1.0 as implemented in ChemDraw Pro ver. 4.5, CambridgeSoft Corp, Cambridge, MA

Table 2. Comparison of reported petrobactin[23] and synthetic petrobactin ^{13}C and ^{1}H resonances

Atom	Literature DMSO ^{13}C	Synthetic found DMSO ^{13}C	Literature DMSO ^{1}H (J in Hz)	Synthetic found DMSO ^{1}H mult (J in Hz)	Synthetic found DMSO:D_2O ca. 4:1 ^{1}H mult (J in Hz)
C1 (C29)	125.37	125.36	na	na	na
C2 (C30)	148.47	115.04	na	7.28 d (1.9)	7.22 d (2.1)
C3 (C31)	144.86	144.84	na	na	na
C4 (C32)[a]	114.81	148.45	6.76 (10)	na	na
C5 (C33)[a]	118.95	114.79	7.18 (2, 10)	6.76 d (8.2)	6.76 d (8.3)
C6 (C34)	115.07	118.93	7.28 (2)	7.18 dd (1.9, 8.2)	7.15 dd (2.1, 8.3)
C7 (C28)	166.65	166.65	na	na	na
C8 (C27)[b]	36.15	36.13	3.27	3.28 m	3.26 t (6.6)
C9 (C26)[b]	26.23	26.21	1.80	1.80 m	1.78 m
C10 (C25)[b]	44.77	44.76	2.90	2.90 m	2.86 m
C11 (C24)[b]	46.45	46.44	2.90	2.90 m	2.86 m
C12 (C23)[b]	26.03	26.00	1.43	1.42 m	1.40 m
C13 (C22)[b]	22.98	22.95	1.55	1.55 m	1.52 m
C14 (C21)[b]	37.69	37.67	3.04	3.04 m	3.01 t (6.8)
C15 (C20)	169.54	169.53	na	na	na
C16 (C19)	43.32	43.30	2.50	2.50 d (14.5)	2.47 d (14.3)
–	–	–	2.58	2.59 d (14.5)	2.56 d (14.3)
C17	73.47	73.45	na	na	na
C18	175.02	174.98	na	na	na
N1 (N6)	na	na	8.31	8.30 t (5.7)	ex
N2 (N5)[b]	na	na	8.41	8.37 m	ex
N3 (N4)	na	na	8.01	7.98 t (5.6)	ex
OH[c]	na	na	nr	9.10 s	ex
OH[d]	na	na	nr	9.53 s	ex
OH[e]	na	na	nr	5.53 br s	ex
OH[f]	na	na	nr	no	no

br=broad, d=doublet, dd=doubled doublet, ex=exchanged, m=multiplet, mult=multiplicity, na=not applicable, no=not observed, nr=not reported, s=singlet, t=triplet.

[a] Literature values reflect correction to the apparently transposed $^{1}H-^{1}H$ J values listed in Table 1 of Ref. 23.

[b] ^{1}H chemical shifts are the center of the observed multiplet.

[c] OH of C3 and C31.

[d] OH of C4 and C32.

[e] OH of C17.

[f] Carboxylic OH of C18.

aromatic region of the natural product, however, were 6.76 ppm (2 Hz), 7.18 ppm (2 and 10 Hz), and 7.28 (10 Hz) and were assigned to positions 4, 5 and 6, respectively.[23] But the observation of one large and one small coupling constant suggests two protons ortho to one another (at 7.18 and 7.28 ppm) with an isolated proton (6.76 ppm) meta to the 7.18 ppm resonance and para to the 7.28 ppm resonance. Chemical shift predictions[§] of the model compound *N*-methyl-2,4-dihydroxybenzamide, together with the reported coupling data, suggested a 2,4-dihydroxy arrangement. This result seemed anomalous—as nonadjacent hydroxyl positioning would be unlikely for an efficient iron chelator. Upon review of a plot trace of the ^{1}H 500 MHz spectrum of the natural product, it was apparent that the coupling constants for the 6.76 and 7.28 ppm resonances had been transposed in the initial report.[23] When the chemical shifts were matched to the correct coupling constants and shift-structure correlations were reconsidered, 3,4-dihydroxy substitution provided the best fit.

To definitively settle the question, the assembly of the 3,4-dihydroxy petrobactin analogue (**13**) was initiated. Simply substituting 3,4-dibenzyloxybenzoyl chloride (**9**)[30,35] for 2,3-dibenzyloxybenzoyl chloride in the synthetic route (Scheme 1) provided the target compound. Yields were similar to those obtained for the 2,3-dihydroxy compound, except for the final step, in which direct HPLC purification of the crude product afforded **13** in much greater yield (61%).

As shown in Table 2, both the ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra of the synthetic 3,4-dihydroxybenzoyl compound were identical to those of the naturally occurring siderophore. The major impurity present in the crude synthetic petrobactin was also isolated during final HPLC purification. Its retention time was longer than that of synthetic petrobactin, suggesting a less polar structure; proton NMR and HRMS analyses were consistent with the imide structure **14** (Fig. 2). The proton NMR spectrum clearly showed a loss of symmetry as evidenced by

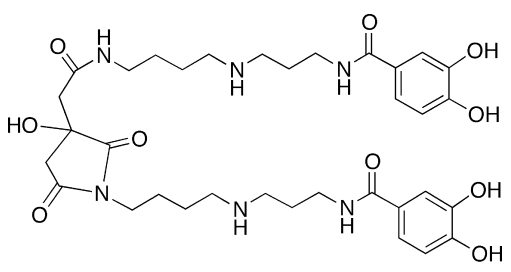

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Figure 2. Structure of the imide (**14**) formed upon cyclization of **13**.

[§] ChemNMR Pro ver. 1.0 as implemented in ChemDraw Pro ver. 4.5, CambridgeSoft Corp, Cambridge, MA

replacement of the two citrate doublets of synthetic petrobactin (2.50 and 2.59 ppm) with four one-hydrogen doublets (2.59, 2.70, 2.83 and 2.87 ppm).[33] Additionally, the high resolution mass spectrum indicated a parent (plus hydrogen) ion of 701 *m/z*, consistent with the loss of a molecule of water from synthetic petrobactin in forming the cyclic imide **14**. The kinetics of the petrobactin cyclization are currently under investigation. A better understanding of this reaction will help increase the overall efficiency of the synthetic route.

3. Conclusion

The structure of petrobactin has been revised and confirmed by its synthesis and that of its 2,3-dihydroxy analogue. Thus, the first preparation of a naturally occurring hexacoordinate chelator containing 3,4-dihydroxybenzamides has been accomplished. The method described here offers efficient access to petrobactin as well as analogues differing in the arrangement of the aromatic hydroxyl groups. Additionally, the spermidine reagent **6** provides a useful synthon for producing the purported photoinduced oxidative breakdown product, 1,5-bis[N^8-(N^1-2,3-dihydroxybenzoyl)spermidinyl]-3-oxoglutarate[23] and its 3,4-dihydroxy isomer.

4. Experimental

4.1. General details

All reactions were performed under a nitrogen atmosphere at ambient pressure unless otherwise indicated. Acrylonitrile and trifluoroacetic acid (TFA) were freshly distilled prior to use. Percent NH_4OH solutions are expressed as volume solutions of 30% (by weight) NH_4OH. Except where stated, ^{1}H NMR spectra were obtained at 300 MHz in $CDCl_3$ and ^{13}C NMR at 75 MHz in $CDCl_3$; chemical shifts (δ) are given in ppm downfield from tetramethylsilane (TMS) as standard.

4.2. Synthesis of 'petrobactin', original structure (8)

4.2.1. *N*-Benzyl-*N*-(2-cyanoethyl)-*N'*-(*tert*-butoxycarbonyl)-1,4-butanediamine (2). Acrylonitrile (4.03 g, 76 mmol) was added to a solution of **1**[29] (6.75 g, 24.2 mmol) in MeOH (50 mL), and the mixture was stirred overnight at 50–53°C. The resulting solution was cooled to room temperature. Removal of volatiles under reduced pressure gave **2** (8.02 g, quantitative) as a colorless oil. ^{1}H NMR δ 1.44 (9H, s, $(CH_3)_3C$), 1.48–1.56 (4H, m, $CH_2-CH_2-CH_2-CH_2$), 2.41 (2H, t, J=6.81 Hz, CH_2-CH_2-CN), 2.48–2.56 (2H, m, $N-CH_2-CH_2$), 2.78 (2H, t, J=6.81 Hz, CH_2-CH_2-CN), 3.03–3.22 (2H, m, $CO-NH-CH_2$), 3.60 (2H, s, CH_2-Ar), 4.60 (1H, br m, NH), 7.25–7.38 (5H, m, Ar); ^{13}C NMR δ 16.3 (CH_2-CN), 24.4 ($NH-CH_2-CH_2-CH_2$), 27.6 ($NH-CH_2-CH_2$), 28.4 ($(CH)_3C$), 40.2 ($NH-CH_2$), 49.2 (CH_2-CH_2-CN), 53.3 ($NH-CH_2-CH_2-CH_2-CH_2$), 58.4 (CH_2-Ar), 79.0 ($(CH)_3C$), 118.9 (CN), 127.3 (ArCH), 128.3 (ArCH), 128.6 (ArCH), 138.7 (ArC), 156.0 (CO). HRMS: found $(M+H)^+$, 332.2338. $C_{19}H_{30}N_3O_2$ requires (M+H) 332.2338. Analysis calcd for $C_{19}H_{29}N_3O_2$: C, 68.85; H, 8.82; N, 12.68. Found: C, 68.76; H, 8.73; N, 12.76.

4.2.2. N^4-Benzyl-N^8-(*tert*-butoxycarbonyl)spermidine (3). Raney Ni (4.45 g, 50% slurry in water) was added to a solution of **2** (6.63 g, 20.0 mmol) in EtOH (25 mL) and 1N NaOH (190 mL) in 95% EtOH in a Parr bottle; the slurry was shaken overnight under a hydrogen atmosphere maintained at 2–3 atm. The mixture was filtered, solids were washed with 95% EtOH (50 mL), and the combined filtrate was concentrated under reduced pressure. Flash chromatography of the residue, eluting with 3% concentrated NH_4OH in MeOH, gave **3** (6.42 g, 96%) as a colorless oil. ^{1}H NMR δ 1.44 (9H, s, $(CH_3)_3C$), 1.45–1.60 (8H, m, $NH-CH_2-CH_2-CH_2-CH_2$ and $CH_2-CH_2-NH_2$), 2.38–2.48 (4H, m, $N-CH_2$ and CH_2-NH_2), 2.70 (2H, t, J=6.81 Hz, $N-CH_2-CH_2-CH_2-NH_2$), 3.05–3.15 (2H, m, $NH-CH_2$), 3.53 (2H, s, CH_2-Ar), 4.68 (1H, br s, CO–NH), 7.15–7.25 (5H, m, Ar). HRMS: found $(M+H)^+$, 336.2648. $C_{19}H_{34}N_3O_2$ requires (M+H) 336.2651. Analysis calcd for $C_{19}H_{33}N_3O_2 \cdot 0.5H_2O$: C, 66.24; H, 9.95; N, 12.20. Found: C, 66.22; H, 9.93; N, 12.20.

4.2.3. N^1-[2,3-Bis(benzyloxy)benzoyl]-N^4-benzyl-N^8-(*tert*-butoxycarbonyl)spermidine (5). A solution of oxalyl chloride (7.28 g, 57.3 mmol) in toluene (25 mL) was added dropwise to an ice-bath cooled slurry of 2,3-bis(benzyloxy)-benzoic acid[30,31] (4.85 g, 14.5 mmol) and dimethylformamide (DMF) (0.2 mL) in toluene (50 mL); the mixture was stirred for 1 h. The ice bath was removed; stirring was continued at rt for 1 h. Volatiles were removed under high vacuum, and **4** was dissolved in CH_2Cl_2 (50 mL) and added dropwise to an ice-bath cooled solution of **3** (3.24 g, 9.66 mmol) and DIEA (13.75 g, 106 mmol) in CH_2Cl_2 (125 mL). The mixture was allowed to warm to rt and was stirred overnight. The solution was washed with water (2×150 mL) and brine (2×150 mL), dried over Na_2SO_4, and filtered; solvents were removed under reduced pressure. Flash chromatography of the residue, eluting with 1:1.5 hexane/EtOAc, gave **5** (4.66 g, 74%) as a pale yellow oil. ^{1}H NMR δ 1.36–1.41 (4H, m, $NH-CH_2-CH_2-CH_2-CH_2$), 1.43 (9H, s, $(CH_3)_3C$), 1.45–1.58 (2H, m, $N-CH_2-CH_2-CH_2-NH$), 2.28–2.37 (4H, m, CH_2-N-CH_2), 2.94–3.06 (2H, m, $OCO-NH-CH_2$), 3.24–3.34 (2H, m, $Ar-CO-NH-CH_2$), 3.48 (2H, s, $N-CH_2-Ar$), 4.68 (1H, br m, OCO–NH), 5.02 (2H, s, $O-CH_2-Ar$), 5.08 (2H, s, $O-CH_2-Ar$), 7.10–7.50 (17H, m, Ar), 7.66–7.73 (1H, m, Ar), 7.92 (1H, br t, J=5.28 Hz, Ar–CO–NH). HRMS: found $(M+H)^+$, 652.3735. $C_{40}H_{50}N_3O_5$ requires (M+H) 652.3750. Analysis calcd for $C_{40}H_{49}N_3O_5$: C, 73.70; H, 7.58; N, 6.45. Found: C, 73.44; H, 7.69; N, 6.45.

4.2.4. N^1-[2,3-Bis(benzyloxy)benzoyl]-N^4-benzylspermidine (6). A solution of TFA (10 mL) in CH_2Cl_2 (20 mL) was added dropwise to an ice-bath cooled solution of **5** in CH_2Cl_2 (20 mL) and water (0.05 mL). The mixture was stirred for 5 min, then concentrated under reduced pressure at 3–5°C. The yellow residue was dissolved in CH_2Cl_2 (250 mL) and washed with satd. Na_2CO_3 (2×200 mL), water (2×100 mL), and brine (2×100 mL), dried over Na_2SO_4, and filtered; solvents were removed under reduced pressure. ^{1}H NMR analysis of a 5-mg sample indicated 36% conversion to **6**. The remaining residue was retreated as

above, stirring for 15 min after TFA addition. Flash chromatography of the residue remaining after the second workup, eluting with 3% concentrated NH_4OH in MeOH, gave **6** (2.10 g, 73%) as a colorless oil. ^1H NMR δ 1.26–1.51 (8H, m, $NH_2-CH_2-CH_2-CH_2$ and $CH_2-CH_2-NH-CO$), 2.26–2.39 (4H, m, $NH_2-CH_2-CH_2-CH_2-CH_2-N$), 2.59 (2H, t, J=6.59 Hz, $N-CH_2-CH_2-CH_2-NH$), 3.26–3.35 (2H, m, $CO-NH-CH_2$), 3.45 (2H, s, $N-CH_2-Ar$), 5.02 (2H, s, $O-CH_2-Ar$), 5.16 (2H, s, $O-CH_2-Ar$), 7.10–7.50 (17H, m, Ar), 7.64–7.73 (1H, m, Ar), 7.92 (1H, br t, J=5.28 Hz, $Ar-CO-NH$). HRMS: found $(M+H)^+$, 552.3216. $C_{35}H_{42}N_3O_3$ requires (M+H) 552.3226. Analysis calcd for $C_{35}H_{41}N_3O_3\cdot 1H_2O$: C, 73.78; H, 7.61; N, 7.38. Found: C, 73.68; H, 7.37; N, 7.24.

4.2.5. *tert*-Butyl 4-[[4-[[3-[[2,3-Bis(benzyloxy)benzoyl]-amino]propyl](phenylmethyl)amino]butyl]amino]-2-[2-[[4-[[3-[[2,3-bis(benzyloxy)benzoyl]amino]propyl](phenylmethyl)amino]butyl]amino]-2-oxoethyl]-2-hydroxy-4-oxo-butanoate (7). A solution of **6** (1.07 g, 1.94 mmol) in CH_3CN (20 mL) was added dropwise to a solution of 1,3-bis(*p*-nitrophenyl)-2-(*tert*-butyl) citrate[28] (0.40 g, 0.82 mmol) in CH_3CN (40 mL) with ice-bath cooling. A solution of DIEA (0.25 g, 1.97 mmol) in CH_3CN (8 mL) was added dropwise; the mixture was stirred for 2 h and then stored overnight at 0 to −5°C. The solvents were removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (300 mL), washed with satd. $NaHCO_3$ (6×150 mL) and brine (150 mL), dried over Na_2SO_4, and filtered; solvents were removed under reduced pressure. Flash chromatography of the residue, eluting with 1:2:15 MeOH/acetone/$CHCl_3$, gave **7** (0.77 g, 71%) as a yellow oil. ^1H NMR δ 1.43 (9H, s, $(CH_3)_3C$), 1.43–1.58 (12H, m, $CH_2-CH_2-CH_2-CH_2$ and $CH_2-CH_2-CH_2$), 2.28–2.36 (8H, m, CH_2-N-CH_2), 2.49 (2H, d, J=14.9 Hz, $CHH-CO$), 2.65 (2H, d, J=14.9 Hz, $CHH-CO$), 3.03–3.18 (4H, m, $CH_2-CO-NH-CH_2$), 3.25–3.34 (4H, m, $Ar-CO-NH-CH_2$), 3.43 (4H, s, $N-CH_2-Ar$), 5.02 (4H, s, $O-CH_2-Ar$), 5.15 (4H, s, $O-CH_2-Ar$), 5.55 (1H, br s, OH), 6.83 (2H, t, J=5.4 Hz, $CH_2-CO-NH$), 7.10–7.50 (34H, m, Ar), 7.63–7.71 (2H, m, Ar), 7.94 (2H, br t, J=5.5 Hz, $Ar-CO-NH$). Analysis calcd for $C_{80}H_{94}N_6O_{11}$: C, 73.03; H, 7.20; N, 6.39. Found: C, 72.91; H, 7.31; N, 6.38.

4.2.6. 4-[[4-[[3-[(2,3-Dihydroxybenzoyl)amino]propyl]-amino]butyl]amino]-2-[2-[[4-[[3-[(2,3-dihydroxybenzoyl)amino]propyl]amino]butyl]amino]-2-oxoethyl]-2-hydroxy-4-oxo-butanoic acid, bis(trifluoroacetate) salt (reported petrobactin, 8). TFA (15 mL) was added dropwise to an ice-bath cooled solution of **7** (0.385 g, 0.293 mmol) in CH_2Cl_2 (15 mL) and water (0.15 mL), and the mixture was stirred for 1 h. The ice-bath was removed and stirring continued for 1 h. The mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2 (25 mL) and water (0.15 mL) and reconcentrated (3×). The concentrate was used directly in the next reaction and was dissolved in a solution of glacial HOAc (15 mL) and water (0.75 mL), $PdCl_2$ (163 mg) was added, and the mixture was stirred under H_2 at ambient pressure for 7 h. The mixture was filtered, solids were washed with a solution of glacial HOAc (15 mL) and water (0.75 mL); the combined filtrate and washings were concentrated under reduced pressure. Toluene (15 mL) and water (0.25 mL) were added and removed under reduced pressure (3×) to facilitate removal of excess HOAc. The residue was dissolved in MeOH (25 mL), Sephadex LH-20 (1.35 g) was added, and the mixture was allowed to stand overnight at 0 to −5°C. The solvents were removed under reduced pressure; the residue was loaded onto a wet-packed Sephadex LH-20 (27 g, 2.5×15 cm) column, eluting with 1:8:13 water/EtOH/toluene at 0.25 mL/min. The iron active fractions were combined and concentrated. An aliquot of approximately a third of the resulting solution was passed through an anion exchange column (TFA, 14 mL bed); the iron active fractions were combined and lyophilized to give crude **8** (0.023 g, 25%). Final purification of an analytical sample by HPLC on a C4 column (solvent: 94.4% water, 5.5% MeCN, 0.1% TFA, isocratic elution, detection by uv absorption at 280 nm, R_t=11.0–12.5 min), followed by lyophilization, gave **8** as a white amorphous solid, calculated yield 17%. Detailed ^1H (500 MHz) and ^{13}C (125 MHz) NMR analysis is given in Table 1. HRMS: found $(M+H)^+$, 719.3606. $C_{34}H_{51}N_6O_{11}$ requires (M+H) 719.3616.

4.3. Synthesis of petrobactin (13)

4.3.1. N^1-[3,4-Bis(benzyloxy)benzoyl]-N^4-benzyl-N^8-(*tert*-butoxycarbonyl)spermidine (10). A solution of oxalyl chloride (15.04 g, 120 mmol) in CH_2Cl_2 (50 mL) was added dropwise to an ice-bath cooled slurry of 3,4-bis(benzyloxy)benzoic acid[30,35] (10.02 g, 30 mmol) and DMF (0.4 mL) in toluene (50 mL); the mixture was stirred for 1 h. The ice-bath was removed and stirring continued at rt for 1 h. Volatiles were removed under high vacuum; **9** was dissolved in CH_2Cl_2 (100 mL) and added dropwise to an ice-bath cooled solution of **3** (6.70 g, 20 mmol) and DIEA (28.54 g, 220 mmol) in CH_2Cl_2 (250 mL). The mixture was allowed to warm to rt and was stirred overnight. The solution was washed with water (2×250 mL) and brine (2×250 mL), dried over Na_2SO_4, and filtered; solvents were removed under reduced pressure. Flash chromatgraphy of the residue, eluting with EtOAc, gave **10** (9.32 g, 72%) as a pale yellow solid, mp 97°C. ^1H NMR δ 1.42 (9H, s, $(CH_3)_3C$), 1.58–1.73 (6H, m, $N-CH_2-CH_2-CH_2-CH_2$ and $N-CH_2-CH_2-CH_2-N$), 2.46 (2H, t, J=6.9 Hz, $NH-CH_2-CH_2-CH_2-CH_2-N$), 2.53 (2H, t, J=6.0 Hz, $N-CH_2-CH_2-CH_2-NH$), 3.02–3.09 (2H, m, $OCO-NH-CH_2$), 3.42–3.48 (2H, m, $Ar-CO-NH-CH_2$), 3.52 (2H, s, $N-CH_2-Ar$), 4.58 (1H, br m, OCONH), 5.14 (2H, s, $O-CH_2-Ar$), 5.21 (2H, s, $O-CH_2-Ar$), 6.83 (1H, d, J=8.4 Hz, Ar), 7.01 (1H, d, J=8.4 Hz, Ar), 7.13–7.48 (16H, m, Ar). Analysis calcd for $C_{40}H_{49}N_3O_5$: C, 73.70; H, 7.58; N, 6.45. Found: C, 73.59; H, 7.67; N, 6.54.

4.3.2. N^1-[3,4-Bis(benzyloxy)benzoyl]-N^4-benzylspermidine (11). A solution of **10** (2.5 g, 3.8 mmol) in CH_3OH (41 mL) and concentrated HCl (16 mL) was stirred at rt for 12 h. The mixture was concentrated to dryness. Water (100 mL) was added to the residue, the pH was adjusted to 9 with satd. Na_2CO_3, and the aqueous layer was extracted with EtOAc (3×100 mL). The combined organic extracts were washed with H_2O (2×100 mL), and the solvent was removed in vacuo. Silica gel flash chromatography of the residue, eluting with 3% concentrated NH_4OH in MeOH, gave **11** (1.79 g, 84%) as a white solid, mp 68°C. ^1H NMR δ 1.35–1.70 (8H, m, $NH_2-CH_2-CH_2-CH_2$ and CH_2-CH_2-

NH–CO), 2.46 (2H, t, J=7.8 Hz, NH_2–CH_2), 2.55 (2H, t, J=6.0 Hz, NH_2–CH_2–CH_2–CH_2–CH_2–N), 2.62 (2H, t, J=6.6 Hz, N–CH_2–CH_2–CH_2–NH), 3.44–3.49 (2H, m, CO–NH–CH_2), 3.54 (2H, s, N–CH_2–Ar), 5.13 (2H, s, O–CH_2–Ar), 5.21 (2H, s, O–CH_2–Ar), 6.84 (1H, d, J=8.4 Hz, Ar), 7.02 (1H, d, J=8.4 Hz, Ar), 7.18–7.48 (16H, m, Ar). Analysis calcd for $C_{35}H_{41}N_3O_3{\cdot}0.5H_2O$: C, 74.97; H, 7.55; N, 7.49. Found: C, 74.90; H, 7.39; N, 7.41.

4.3.3. *tert*-Butyl 4-[[4-[[3-[[3,4-Bis(benzyloxy)benzoyl]-amino]propyl](phenylmethyl)amino]butyl]amino]-2-[2-[[4-[[3-[[3,4-bis(benzyloxy)benzoyl]amino]propyl]-(phenylmethyl)amino]butyl]amino]-2-oxoethyl]-2-hydroxy-4-oxo-butanoate (12). A solution of **11** (1.59 g, 2.88 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a solution of 1,3-bis(*p*-nitrophenyl)-2-(*tert*-butyl) citrate[28] (0.59 g, 1.2 mmol) in CH_2Cl_2 (50 mL) with ice-bath cooling. A solution of NEt_3 (0.156 g, 1.42 mmol) in CH_2Cl_2 (10 mL) was added dropwise; the mixture was stirred for 2 h then stored overnight at 0 to −5°C. Workup according to the method described for **7** and flash chromatography of the residue, eluting with 2:10 MeOH/$CHCl_3$, afforded **12** (1.15 g, 72%) as a yellow solid, mp 65°C. ^{1}H NMR δ 1.42 (9H, s, $(CH_3)_3C$), 1.66–1.72 (12H, m, CH_2–CH_2–CH_2–CH_2 and CH_2–CH_2–CH_2), 2.43–2.63 (12H, m, CH_2–N–CH_2, CH–CO, CH–CO), 3.13–3.15 (4H, m, CH_2–CO–NH–CH_2), 3.42–3.43 (4H, m, Ar–CO–NH–CH_2), 3.52 (4H, s, N–CH_2–Ar), 5.13 (4H, s, O–CH_2–Ar), 5.19 (4H, s, O–CH_2–Ar), 6.62 (1H, br s, OH), 6.84 (2H, d, J=8.4 Hz, Ar), 7.08 (2H, d, J=8.4 Hz, Ar), 7.16–7.48 (32H, m, Ar). Analysis calcd for $C_{80}H_{94}N_6O_{11}$: C, 73.03; H, 7.20; N, 6.39. Found: C, 72.65; H, 7.23; N, 6.45.

4.3.4. 4-[[4-[[3-[(3,4-Dihydroxybenzoyl)amino]propyl]-amino]butyl]amino]-2-[2-[[4-[[3-[(3,4-dihydroxyben-zoyl)amino]propyl]amino]butyl]amino]-2-oxoethyl]-2-hydroxy-4-oxo-butanoic acid, bis(trifluoroacetate) salt (petrobactin, 13). TFA (15 mL) was added dropwise to an ice-bath cooled solution of **12** (0.385 g, 0.293 mmol) in CH_2Cl_2 (15 mL) and water (0.15 mL); the mixture was stirred for 1 h. The ice-bath was removed and stirring continued for 1 h. The mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2 (25 mL) and water (0.15 mL) and reconcentrated (3×); this concentrate was used directly in the next reaction. The residue was dissolved in a solution of glacial HOAc (15 mL) and water (0.75 mL), $PdCl_2$ (163 mg) was added, and the mixture was stirred under H_2 at ambient pressure overnight. Filtration and workup were as described for compound **8**. Purification of the crude product was directly accomplished by HPLC on a C4 column in the manner described for compound **8** (R_t=11.0–12.5 min). Lyophilization of the pooled products afforded **13** as a solid in 61% calculated yield. Detailed ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR analysis is given in Table 2. HRMS: found $(M+H)^+$, 719.3606. $C_{34}H_{51}N_6O_{11}$ requires (M+H) 719.3615.

The imide **14** (Fig. 2) was also isolated by HPLC during the petrobactin preparative runs (retention time 17–19 min). Lyophilization gave **14** as a white amorphous solid, calculated yield 15%: ^{1}H NMR (500 MHz, DMSO/D_2O 4:1) δ 1.39–1.47 (2H, m, CH_2–CH_2–CH_2–CH_2–NH–CO–CH_2), 1.52–1.62 (6H, m, CH_2–CH_2–CH_2–CH_2–NH–CO–CH_2 and N$(CO)_2$–CH_2–CH_2–CH_2), 1.79–1.87 (4H, m, N–CH_2–CH_2–CH_2–N), 2.59 (1H, d, J=15.3 Hz, NC(O)–CH–C(OH)), 2.70 (1H, d, J=15.3 Hz, NC(O)–CH–C(OH)), 2.83 (1H, d, J=15.4 Hz, C(OH)–C(O)–N–C(O)–CH), 2.87 (1H, d, J=15.4 Hz, C(OH)–C(O)–N–C(O)–CH), 2.88–2.94 (8H, m, CH_2–NH–CH_2), 2.96–3.07 (2H, m, CH_2–CH_2–CH_2–CH_2–NH–CO–CH_2), 3.28 (4H, m, Ar–CO–NH–CH_2), 3.39–3.43 (2H, m, N$(CO)_2$–CH_2), 6.82 (2H, d, J=8.2 Hz, C(C)–CH–C(OH)–C(OH)–CH–CH), 7.20 (2H, dd, J=2.3, 8.2 Hz, C(C)–CH–C(OH)–C(OH)–CH–CH), 7.27 (2H, d, J=2.3 Hz, C(C)–CH–C(OH)–C(OH)–CH–CH). HRMS: found $(M+H)^+$, 701.3546. $C_{34}H_{49}N_6O_{10}$ requires (M+H) 701.3510.

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