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Total Synthesis of Petrobactin and Its Homologues as Potential Growth Stimuli for *Marinobacter hydrocarbonoclasticus*, an Oil-Degrading Bacteria

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A modular synthesis was developed to access petrobactin, a catechol-containing siderophore isolated from *Marinobacter hydrocarbonoclasticus*. A range of petrobactin homologues with differing dihydroxybenzamide motifs and in one case an increased number of carbons in the polyamine backbone were also synthesized. As such, these systems represent new isomeric probes to study iron transport properties in *M. hydrocarbonoclasticus*. The synthesis of petrobactin and its homologues and the first biological study of how these agents influence the growth of *Mycobacter hydrocarbonoclasticus* are reported. New synthetic methods were developed to overcome issues (imide formation) encountered in earlier syntheses. Both the ^{1}H and ^{13}C NMR of petrobactin were consistent with the recently revised structure showing that petrobactin in fact contains a 3,4-dihydroxybenzene motif rather than a 2,3-dihydroxybenzene motif. The preliminary biological studies suggested that using the native petrobactin **1b** for *M. hydrocarbonoclasticus*-specific growth stimulation may be a poor strategy for oil-spill cleanup.

Introduction

Oil spills by ocean-faring oil tankers not only result in loss of valuable energy resources but also have disastrous environmental consequences. After the March 1989 Exxon-Valdez oil spill, both Exxon and the EPA have experimented with a shoreline cleanup technique involving bioremediation, which used bacteria to degrade oil into harmless water, carbon dioxide, and fatty acids. Exxon's pilot project involved spraying fertilizers on 74 miles of the more than 900 miles of oiled shoreline in Alaska in the hopes of spurring growth of naturally occurring bacteria that eat oil.[1] The fertilizers appear to have accelerated natural biodegradation of the oil without harmful side effects such as eutrophication, toxicity to sensitive organisms, and release of untreated oil.[1−5] Bioremediation can also be performed by introducing genetically engineered microbes into the water, as was done experimentally in the 1990 Mega Borg spill in the Gulf of Mexico.[2−5]

Another strategy for bioremediation is the development of new growth stimulants for bacteria based upon siderophores. Unlike mammals, which use high molecular weight proteins for iron transport, bacteria use low molecular weight, iron-specific ligands (siderophores) to acquire iron (a growth-limiting nutrient) from their environment. While iron mainly exists as insoluble ferric hydroxide polymers in the biosphere, siderophores solubilize this essential micronutrient and allow for its use by bacteria. Over the course of time, bacteria have generated a variety of siderophore architectures containing iron-binding motifs, which typically include catechols, *N*-hydroxyamides, and α-hydroxycarboxylic acids. Different bacteria often produce their own unique siderophore to sequester iron(III) and presumably have an uptake pathway to facilitate its import.[6] Indeed, one may be able to specifically feed certain bacteria in the presence of others by using a native siderophore−iron complex.

Recently a new siderophore, petrobactin **1a**, was isolated and characterized from an oil-degrading marine bacterium, *Marinobacter hydrocarbonoclasticus*,[7] by Butler et al.[8] Although there are many examples of sidero-

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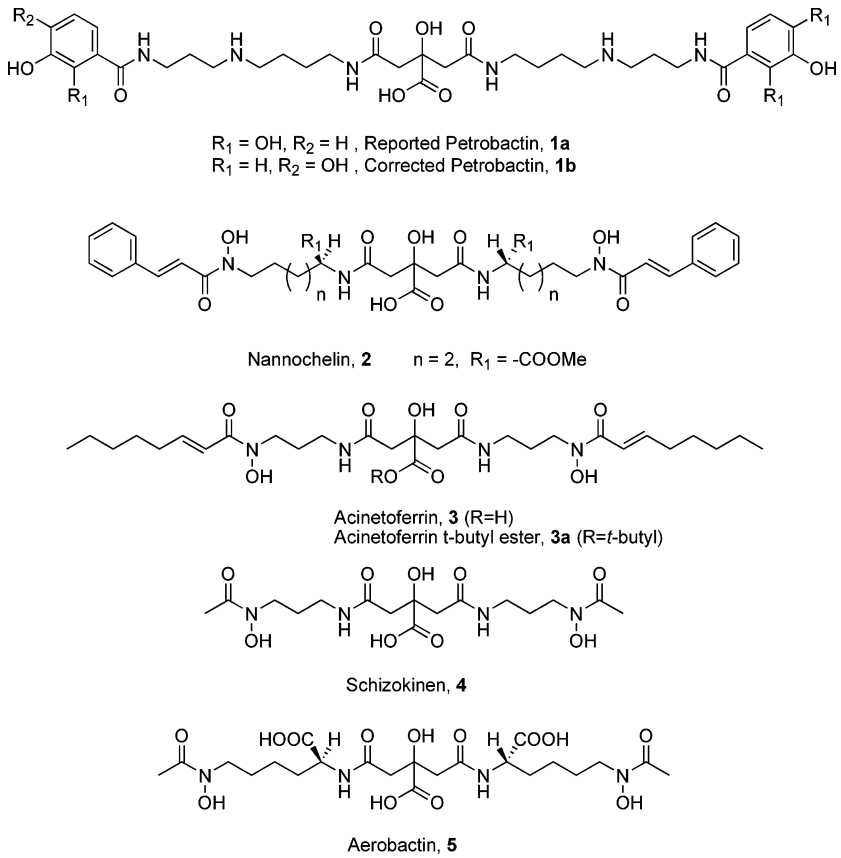

FIGURE 1. Structure of citrate-containing chelators and the reported petrobactins, **1a**,**b**.

phores from soil bacteria, there are only a few examples of marine-based siderophores.[9−12] Upon inspection, **1a** is comprised of three subunits: a 2,3-dihydroxybenzoyl unit, a spermidine unit, and a citric acid moiety. The citric acid component is a common feature found in a number of other siderophores such as nannochelin A **2** from *Nannocystis exedens*,[13] acinetoferrin **3** from *Acinetobacter haemolyticus*,[14] schizokinen **4** from *Bacillus megaterium*,[15] and aerobactin **5** from *Aerobacter* strains.[16]

Utilizing earlier insights from the synthesis of nannochelin A **2** and acinetoferrin **3**, a modular synthesis was developed to access this catechol-containing siderophore. However, during our efforts to access a family of petrobactin derivatives including petrobactin **1a**, Bergeron et al.[17] described its total synthesis and further reported that the structure assigned to it by Butler et al.[8] was incorrect. These authors went on to synthesize the corrected structure of petrobactin, which was reported as **1b**.[17] In this report, we describe our synthesis of petrobactin and its homologues as well as the first biological study of how these agents influence the growth of *M. hydrocarbonoclasticus*.

Results and Discussion

Synthesis. The initial target **1a** contained a 2,3-dihydroxybenzoyl unit attached to spermidine via an amide linkage. The other end of the polyamine was attached via amide bonds to the terminal carboxyl groups of citric acid. Spermidine is an unsymmetrical 3,4-triamine and the strategy required regiospecific control of amine reactivity. This issue was initially addressed by sequential construction of the polyamine chain with use of an amino-alcohol approach.[18−20] The strategy began with the generation of 2,3-bis(dibenzyloxy)benzoic acid **7a**[21−23] (92%) from commercially available **6a**. As shown

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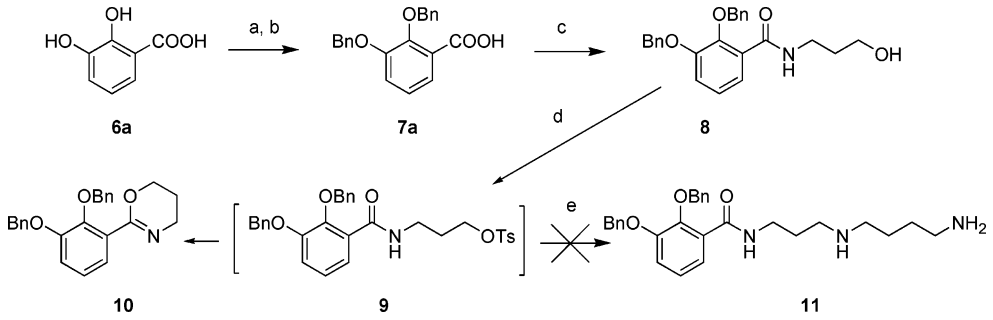
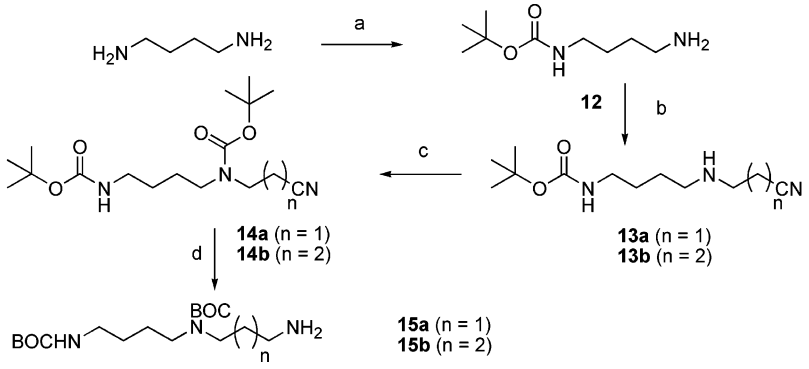
SCHEME 1[a]



[a] Reagents: (a) BnBr, K_2CO_3, acetone; (b) NaOH, MeOH; (c) 3-aminopropanol, HOBt, DCC; (d) TsCl, pyridine; (e) 1,4-diaminobutane.

SCHEME 2[a]



[a] Reagents: (a) BOC_2O (0.33 equiv), TEA, MeOH; (b) $BrCH_2CH_2CN$ or $BrCH_2CH_2CH_2CN$, K_2CO_3, CH_3CN; (c) BOC_2O (1.5 equiv), TEA, MeOH; (d) H_2, Ra Ni, EtOH.

in Scheme 1, condensation of 3-aminopropanol and **7a** with use of HOBt/DCC gave the desired amide **8** (85%). Previous experience[19,20] suggested that polyamine **11** could be made via tosylate **9** and 1,4-diaminobutane. However, tosylation of **8** led mainly to the 1,3-oxazine **10** rather than tosylate **9**. Similar cyclizations have been reported in other activated benzamide derivatives.[23] Therefore, another route was selected.

The synthesis of **15a** (Scheme 2) has been described by Nakanishi and Hu.[24] The mono-BOC protection of diaminobutane gave the amine **12** in a good yield (71%). The addition of bromopropionitrile gave **13a** (70%) and the protection of the newly formed secondary amine with di-*tert*-butyl dicarbonate gave **14a** (91%). Finally, the reduction of the nitrile with Raney Ni gave bis-BOC-protected polyamine **15a** (91%).

Initial attempts to couple acid **7a** with **15a** by using HOBt/DCC did not give the desired amide **17a**. This was interesting as amide **8** was previously made by this method (Scheme 1). As shown in Scheme 3, successful coupling to **15a** was achieved via acid chloride **16a** and provided amide **17a** in 86% yield.[17,21]

Several methods were tried for the Boc deprotection of **17a** to form the diaminoamide **18a** (i.e., the TFA salt of **11**). Under the typical BOC-removal conditions (4 N aq HCl, 100% TFA or 50% TFA/CH_2Cl_2), partial deprotection of the *O*-benzyl groups was observed by ^{1}H NMR. This phenomenon was monitored via the two benzylic methylene singlets at δ 5.1 and δ 5.2, which were converted to three singlets of similar intensity at δ 5.15, δ 5.12, and δ 5.10 after Boc deprotection. A thermal Boc-deprotection method showed that **17a** was stable at 180 °C under argon, but formed complex products at 220 °C. Finally, diaminoamide **18a** was generated by using 10% TFA/CH_2Cl_2 and no *O*-debenzylation was observed.

In earlier work with acinetoferrin **3**,[14] the synthesis of the activated citrate derivative **19** (Figure 2) was achieved with *N*-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC), albeit in 20% isolated yield of **19**.

Subsequent coupling of **19** to a primary amine (80%) gave a 16% yield for the tandem activation and coupling

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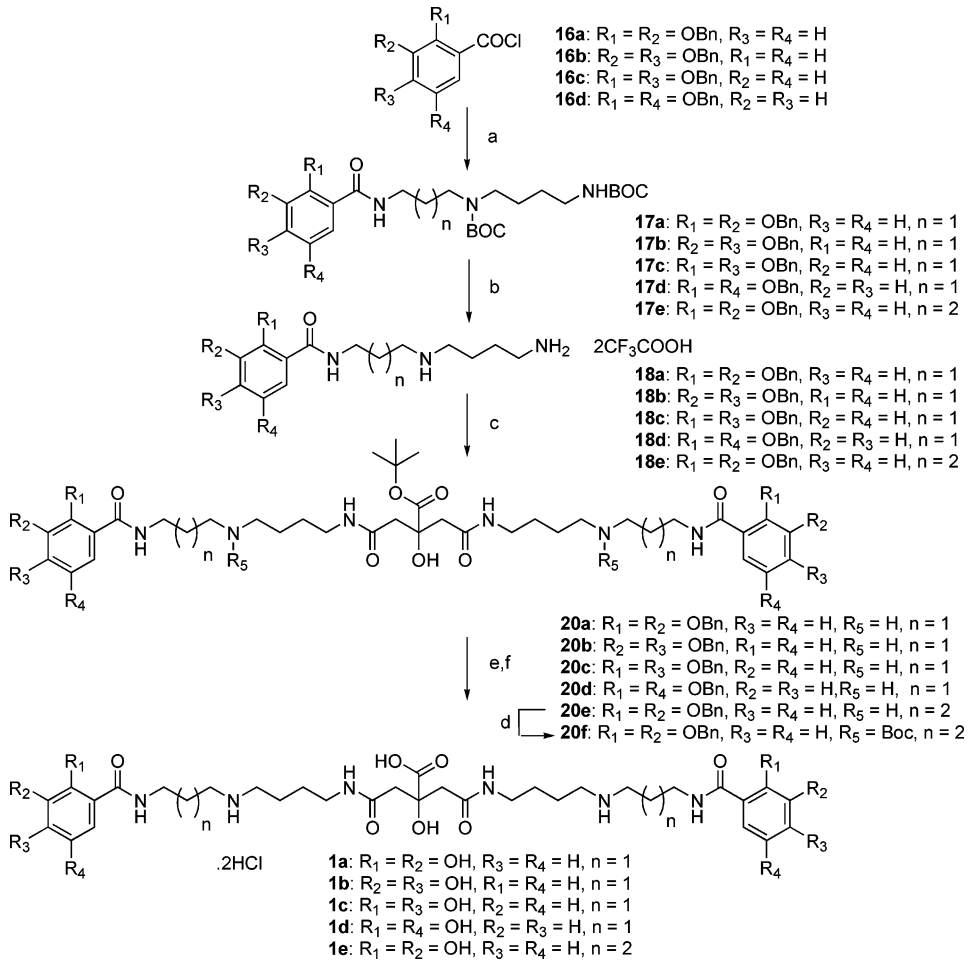
SCHEME 3[a]



[a] Reagents: (a) **15a** or **15b**, NaOH, CH_2Cl_2; (b) 10% TFA, CH_2Cl_2, (c) **19**, TEA, dioxane, CH_2Cl_2; (d) BOC_2O, TEA, MeOH; (e) HCl/AcOH (1:2); (f) H_2, Pd/C, EtOH.

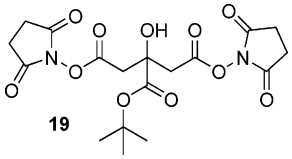


FIGURE 2. Bis NHS ester **19**.

sequence. In more recent work, a number of different reagent combinations were tried to improve this transformation, including HOBt/DCC, HOBt/EDC, NHS/EDC, and NHS/DCC. In each reaction the activated ester was not isolated, but was used directly in the next step to provide **20a**. Ironically, the most efficient reagent was the original NHS and DCC combination. Indeed, significantly higher yields were obtained for the two-step process, when **19** (Figure 2) was not isolated, but used directly in the subsequent coupling step. Therefore, the condensation of a solution of the TFA salt (**18a**) and triethylamine with a solution of crude **19**[14,15] gave the corresponding *tert*-butylpetrobactin **20a** (57%).

The final two steps involved the removal of the *tert*-butyl group and the deprotection of the benzyl ethers to give petrobactin **1a**. In their synthesis of petrobactin, Bergeron et al. reported the formation and isolation of an imide of petrobactin (15%), **21a**, alongside the natural product.[17] This compound was formed either during the removal of the *tert*-butyl group with a solution of 20% TFA in dichloromethane or during their removal of the benzyl groups under acidic reductive conditions ($PdCl_2$/HOAc/water).

In our synthesis of petrobactin, we also encountered the imide and have developed ways to minimize its formation. Previously, the removal of the *tert*-butyl group from a related citrate system, *tert*-butyl acinetoferrin **3a**, had been achieved by using TFA (100%) at room temperature without imide formation.[14] Moreover, the imide was only observed by treating acinetoferrin **3** with refluxing TFA for 3 h. Therefore, TFA at mild temperatures (0−25 °C) seemed to be the most reasonable approach.[14] However, even though TFA facilitated the deprotection of the *tert*-butyl ester group in **20a**, it also generated the respective ammonium salts, which contain the undesirable counterion (CF_3COO^-) for later biological studies. A method was needed that would obviate the need for a later anion exchange step and also produce a salt form of petrobactin that could be used for biological testing.

Several methods were tested involving different solutions of HCl and glacial acetic acid. A solution of 6 M HCl in glacial acetic acid removed both protecting groups: the *tert*-butyl ester and the *O*-benzyl groups.

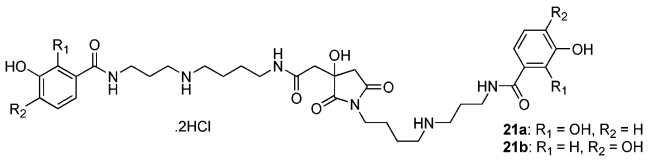


FIGURE 3. The imides of petrobactin, **21a** and **21b**.

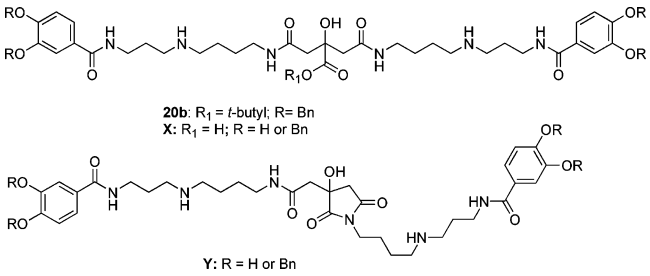
However, these conditions also perturbed other functionalities (benzamide linkage) within the structure. A second solution of 4 M HCl in glacial acetic acid proved to be less severe and left the rest of the molecule intact, although along with the removal of the *tert*-butyl, the ^{1}H NMR revealed 50% deprotection of the *O*-benzyl groups. As these were to be removed in the next step, hydrogenation of the resultant crude with H_2 gas and 10% Pd/C in ethanol provided petrobactin **1a** in a yield of 53%.

^{1}H NMR of the crude prior to isolation of **1a** revealed the formation of petrobactin ethyl ester (5%). While a time-course study revealed that the ethyl ester was suppressed to <1% after a 2-h hydrogenation period, further studies found that performing the hydrogenation (H_2 gas, 10% Pd/C in ethanol) in the presence of water (10 vol %) stopped the formation of the ethyl ester altogether. Having developed a reasonable entry to these systems, we synthesized the remaining derivatives, **1b**−**e**. Compound **1e** required the use of a di-BOC-protected 4,4-triamine: N^5,N^9-di(*tert*-butoxycarbonyl)-homospermidine **15b**. The synthesis of **15b** is shown in Scheme 2. The addition of bromobutyronitrile to the mono-BOC-protected diaminobutane **12** gave **13b** (61%) and the protection of the newly formed secondary amine gave **14b** (87%). Reduction of the nitrile with Raney Ni gave the bis-BOC-protected polyamine **15b** (95%).

The Imide Issue. The imide byproduct is typically formed during the acid treatment of *tert*-butyl citrate derivatives and has been observed by many investigators during siderophore synthesis.[14,25−28] In our studies with petrobactin, formation of the imide was quantified by ^{1}H NMR integration of two doublets at δ 2.79 (1H) and δ 2.65 (1H), corresponding to diastereotopic protons of the citrate methylene group present in the imide impurity. Subsequent comparison of these integrations to that of the doublet at δ 2.59 (2H, corresponding to the citrate methylene groups of linear petrobactin) provided the necessary quantification. For example, in the synthesis of **1b**, the imide was formed in 12% yield with 4 M HCl/AcOH.

A further study was conducted to observe how changing the concentration of HCl during the removal of the *tert*-butyl group from **20b** effected the formation of the imide **21b** (Table 1).

TABLE 1. Conversion of 20b to Acyclic Diamide X and Imide 21b with Different HCl Concentrations



acid conditions	**X**/**Y** ratio
4 M HCl in acetic acid	7.4
1M HCl in acetic acid	8.8
0.2M HCl in acetic acid	14.6
0.1 M HCl in acetic acid	8.9
4 M HCl in acetonitrile	9.8

As shown in Table 1 decreasing the amount of HCl decreased the formation of imide **Y**. Since the conversion of **20b** was typically 100%, the optimal conditions were 0.2 M HCl in acetic acid. Therefore, the imide could be suppressed to ≤5% by this new method. Ironically, this may be a moot point altogether as similar linear citrate diamide systems have been shown to cyclize to their imide form in MeOH at room temperature (79%).[14]

The synthesis of **1b**−**e** has provided a range of petrobactin homologues with differing dihydroxybenzamide motifs and in one case an increased number of carbons in the polyamine backbone. As such, these systems represent new isomeric probes to study iron transport properties in *M. hydrocarbonoclasticus*.

A preliminary study of the growth stimulation ability of these architectures upon *M. hydrocarbonoclasticus* was conducted. Two systems, **1a** (the 2,3 isomer) and **1b** (3,4 isomer), were chosen as they represent petrobactin derivatives with vicinal phenolic OH groups found to be important in iron chelation.[6]

Biological Evaluation. *M. hydrocarbonoclasticus* was cultured in standard marine broth using the protocol of Gauthier.[7] The assay is described in detail in the Experimental Section. Siderophores **1a** and **1b** were dosed and the absorbance at 490 nm measured as a function of time. The time zero absorbance (an average of three measurements) was subtracted from each successive data point within each dosing series to allow for direct comparisons within and between the two siderophore data series.

As shown in Figure 4, the 2,3-isomer **1a** provided a dose-dependent effect on the growth of *M. hydrocarbonoclasticus*. Since absorbance is directly related to bacterial growth, the trend toward higher absorbance as a function of increasing time and siderophore concentration is consistent with growth stimulating activity for **1a**. In this regard, increasing doses of **1a** (e.g., 100 and 500 μM **1a**) provided a 1.1- and 1.4-fold respective increase in bacterial growth after 12 h (compared to the control with no added siderophore). Therefore, while high doses of non-native siderophore **1a** (e.g. 100 or 500 μM) provided some enhancement, it was not dramatic.

The native chelator **1b** (i.e., the 3,4-isomer) provided little to no growth enhancement even at very high concentrations (500 μM) of added siderophore **1b**. Ironically,

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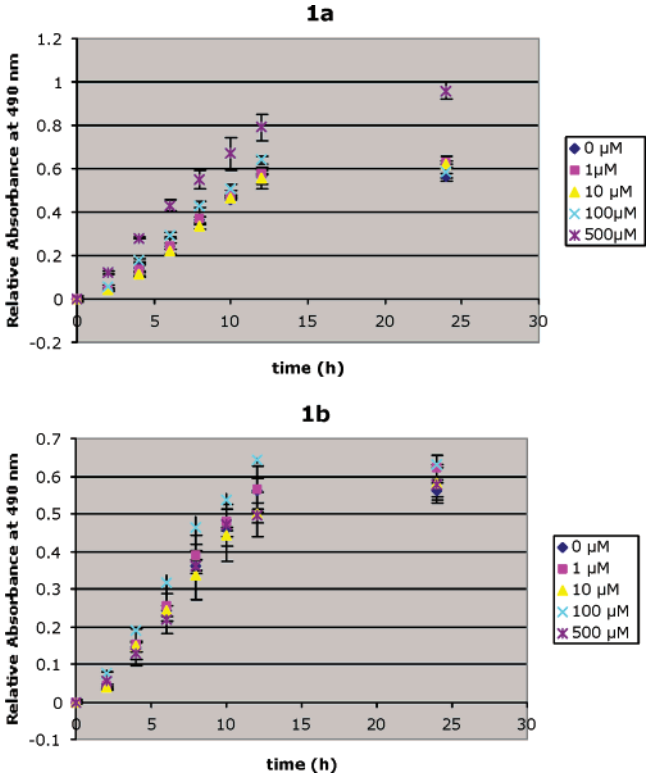

FIGURE 4. Growth curves of *M. hydrocarbonoclasticus* in the presence of added siderophores: 2,3-petrobactin isomer **1a** (top) and 3,4-petrobactin isomer **1b** (bottom). Data are normalized to a starting "zero" absorbance value by subtracting the time zero absorbance value from subsequent data points within each series. The data shown are the average of triplicate measurements. With a few exceptions, the typical error was less than 4%.

the native architecture **1b** provided virtually no advantage in terms of growth stimulation. While the absorbance of both **1a** and **1b** is comparable at the respective 100 μM dosing after 12 h, this parallel trend in their growth stimulation profile is inconsistent especially at higher concentrations (e.g. 500 μM). Therefore, the bacteria seem to be relatively insensitive to additional native siderophore **1b**, while it responded modestly to the non-native **1a**.

This result could be explained by the fact that *M. hydrocarbonoclasticus* can synthesize its own **1b** as needed and that the uptake of the iron−**1b** complex may be transport limited. In this regard, additional **1b** may not provide growth stimulation via an already saturated uptake process. Indeed, since **1b** is the native siderophore for *M. hydrocarbonclasticus*, there are likely optimal conditions for the uptake of its iron complex. Moreover, this uptake may be tightly regulated as found in other biosystems.[29,30]

On the other hand, non-native siderophores such as **1a** may simply be supplying "solubilized" iron for utilization by the bacteria by another uptake pathway, which evokes a growth response. Although further study is needed, this is a "first glimpse" into the specificity of how marine bacteria utilize isomeric iron binding ligands (**1a**, **1b**) for their growth processes. Indeed, to the best of our knowledge, these are the first examples of how petrobactin analogues effect *M. hydrocarbonoclasticus* growth.

Conclusion

In summary, the total synthesis of petrobactin **1b** showed that the structure previously assigned to it (**1a**) by Butler et al. was incorrect and reconfirmed the revised 3,4-dihydroxybenzene structure of **1b**.[17] A modular synthesis was developed that allowed facile access to a number of petrobactin homologues, which differed in both the polyamine and the dihydroxybenzene components. New synthetic methods were developed to overcome the issues (imide formation) encountered in earlier syntheses. The preliminary biological studies suggested that using the native petrobactin **1b** for *M. hydrocarbonoclasticus*-specific growth stimulation may be a poor strategy for oil-spill cleanup. Future work will continue to evaluate the role of siderophores in the growth processes of oil-degrading bacteria and will be reported in due course.

Experimental Section

Biological Evaluation. *Marinobacter hydrocarbonoclasticus* bacteria was grown for 24 h on a shaker at 32°C using the methods of Gauthier.[6] Siderophore stock solutions were prepared in water to the desired concentrations and filtered through a 0.2 μM filter to ensure sterility. The siderophore stock solution was then added to sterile tubes containing only marine broth. These tubes were then inoculated with 200 μL of bacterial culture. All tubes were placed on the shaker for 24 h at 32 °C. Three tubes were taken out at the respective time intervals and vortexed, and the absorbance of a sample (200 μL) was detected at 490 nm on a microplate reader.

For the synthetic details and characterization of 3,4-petrobactin (**1b**), 2,4-petrobactin (**1c**), 2,5-petrobactin (**1d**), and 2,3-homopetrobactin (**1e**) and other intermediate compounds such as dibenzyloxybenzoic acid derivatives (**7b**,[31] **7c**,[31] and **7d**[32,33]), **13b**, **14b**, **15b**, **16b**−**d**,[17,21,34] **17b**, **17c**, **17d**, **17e**, **18b**, **18c**, **18d**, **18e**, **20b**, **20c**, **20d**, and **20e**, including detailed NMR data, please see the Supporting Information.

2,3-Petrobactin (1a). The petrobactin *tert*-butyl ester **20a** (0.3 g, 0.26 mmol) was dissolved in glacial acetic acid (5 mL) and to this was added a mixture of concentrated HCl (12 M, 9 mL) and glacial acetic acid (13 mL). The solution was stirred at room temperature for 45 min. The HCl and glacial acetic acid were removed in vacuo and coevaporation with benzene (3 × 10 mL) and chloroform (3 × 10 mL) removed any residual acids to give a white foam. ^{1}H NMR showed complete removal of the *tert*-butyl group and removal of 50% of the benzyl groups. This compound was used in the next step without further purification. The free acid amine salt was dissolved in EtOH (30 mL) and 10% Pd/C (75 mg) was added and the reaction

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stirred under H_2 at ambient pressure for 2 h. The mixture was filtered through a bed of Sephadex to facilitate removal of the palladium/charcoal and the solids further washed with EtOH. The filtrate was concentrated in vacuo and subsequent co-evaporation with $CHCl_3$ gave a white foam. The crude solid was purified by column chromatography, using LH-20 Sephadex (20 g, 10% CH_3CN/0.1% HCl/water), to give **1a** as a white foam (0.112 g, 53%). R_f 0.44 (35% CH_3CN/0.1% TFA/water, reverse phase silica). ^{1}H NMR (500 MHz, DMSO) δ 8.98 (t, 2H), 8.70 (s, 4H), 8.04 (t, 2H), 7.32 (dd, 2H), 6.92 (dd, 2H), 6.68 (dd, 2H), 3.36 (q, 4H), 3.03 (q, 4H), 2.89 (m, 8H), 2.58 (d, 2H), 2.50 (d, 2H), 1.89 (quin, 4H), 1.59 (quin, 4H), 1.42 (quin, 4H); ^{13}C NMR (300 MHz, DMSO) δ 174.8, 169.8, 169.4, 149.5, 146.1, 118.8, 117.9, 117.2, 114.8, 73.5, 46.5, 44.6, 43.3, 37.7, 36.1, 26.2, 25.7, 23.0; HRMS (FAB) *m*/*z* calcd for $C_{34}H_{51}N_6O_{11}$ $(M + H)^+$ 719.3610, found 719.3624. Anal. Calcd for $C_{34}H_{52}N_6O_{11}Cl_2 \cdot 1.3H_2O$: C, 50.01; H, 6.75; N, 10.29. Found: C, 49.77; H, 6.62; N, 10.06.

2,3-Bis(benzyloxy)benzoic Acid (7a).[21,23] A solution of 2,3-dihydroxybenzoic acid **6a** (0.31 g, 2.0 mmol), benzyl bromide (2.07 g, 12 mmol), and $K_2CO_3 \cdot 1.5H_2O$ (5 g) in acetone (40 mL) was refluxed for 1 day under nitrogen. After filtration, the solution was concentrated in vacuo to give the crude product as a clear oil. The crude product was dissolved in methanol (120 mL) and an aqueous solution of 5 M NaOH (30 mL) was added. The mixture was refluxed for 3 h and the solution concentrated in vacuo to remove the methanol. The residue was dissolved in water and extracted twice with hexane. Then the water phase was acidified with 3 N HCl to pH 2 and filtered to give the product **7a** as a white solid (3.11 g, 93%). R_f 0.62 (40% acetone/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.2 (br s, 1H), 7.70 (d, 1H), 7.4−7.2 (m, 12H), 5.23 (s, 2H), 5.19 (s, 2H); HRMS (FAB) *m*/*z* calcd for $C_{21}H_{18}O_4Na$ $(M + Na)^+$ 357.1097, found 357.1116.

2,3-Bis(benzyloxy)-*N*-(3-hydroxypropyl)benzamide (8). A solution of 2,3-bis(benzyloxy)benzoic acid **7a** (0.424 g, 1.3 mmol), HOBt (0.027 g, 0.2 mmol), and DCC (0.308 g, 1.5 mmol) in CH_2Cl_2 (20 mL) was stirred for 30 min at room temperature. 3-Amino-1-propanol (0.10 g, 1.3 mmol) was added dropwise over 3 min and the mixture stirred overnight. The solution was filtered to remove some of the dicyclohexyl urea (DCU). The filtrate was concentrated in vacuo and the residue purified by flash column chromatography (40% acetone/hexane) to give the product **8** as a clear oil (85%). R_f 0.40 (40% acetone/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (br s, 1H, NH), 7.72−7.20 (m, 13H), 5.16 (s, 2H), 5.08 (s, 2H), 3.48 (t, 2H), 3.40 (m, 2H), 2.80 (br s, 1H, OH), 1.53 (m, 2H); ^{13}C NMR δ 166.6, 151.9, 147.1, 136.5, 129.03, 129.01, 128.95, 128.9, 128.5, 127.9, 126.9, 124.7, 123.5, 117.4, 76.9, 76.8, 71.6, 58.9, 36.0, 32.9; HRMS (FAB) *m*/*z* calcd for $C_{24}H_{26}NO_4$ $(M + H)^+$ 392.1862, found 392.1862.

4-Methoxybenzenesulfonic Acid 3-(2,3-Dibenzyloxybenzoylamino)propyl Ester (9)/2-(2,3-Bis(benzyloxy)phenyl)-5,6-dihydro-4*H*-[1,3]oxazine (10). The amido alcohol **8** (1.07 g, 2.74 mmol) was dissolved in dry pyridine (15 mL) and stirred at 0 °C for 10 min. *p*-Toluenesulfonyl chloride (TsCl, 1.10 g, 5.5 mmol) was added in small portions over 30 min. The mixture was stirred for an additional hour at 0 °C. The reaction flask was then placed in a refrigerator (0−5 °C) overnight. The mixture was poured into 100 mL of ice water, and a viscous liquid typically precipitated. After decanting off the upper aqueous layer, the remaining viscous liquid was dissolved in methylene dichloride and washed with deionized water several times. The organic layer was concentrated in vacuo and the residue purified by flash column chromatography (30% acetone/hexane) to give an unwanted side product **10** (60%). R_f 0.29 (30% acetone/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.00 (m, 13H), 5.12 (s, 2H), 5.08 (s, 2H), 4.20 (t, 2H), 3.51 (t, 2H), 1.90 (m, 2H); ^{13}C NMR δ 156.6, 152.4, 147.1, 138.2, 137.1, 131.3, 128.7, 128.5, 128.4, 128.1, 127.9, 127.7, 124.2, 122.3, 116.2, 75.9, 71.5, 65.5, 43.1, 22.2; HRMS (FAB) *m*/*z* calcd for $C_{24}H_{24}NO_3$ $(M + H)^+$ 374.1756, found 374.1750.

(4-Aminobutyl)carbamic Acid *tert*-Butyl Ester (12). 1,4-Diaminobutane (4.4 g, 0.05 mol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 110 mL). A solution of di-*tert*-butyl dicarbonate (3.63 g, 0.017 mol) in methanol (10 mL) was added dropwise to this mixture with vigorous stirring. The mixture was stirred at room temperature overnight. The methanol and TEA were removed in vacuo to yield an oily residue that was dissolved in dichloromethane (100 mL) and washed with a solution of 10% aq sodium carbonate (2 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered, the solvent removed in vacuo, and the oily residue purified by flash column chromatography (1:10:89 NH_4OH:MeOH:$CHCl_3$) to give the product **12** as a clear oil (2.23 g, 71%). R_f 0.38 (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72 (br s, 1H, NHCO), 3.12 (q, 2H, CH_2), 2.70 (t, 2H, CH_2), 1.57−1.30 (m, 13H, 2 × CH_2, 3 × CH_3).

[4-(2-Cyanoethylamino)butyl]carbamic Acid *tert*-Butyl Ester (13a). To a solution of the amine **12** (2.10 g, 11 mmol) in anhydrous acetonitrile (50 mL) was added potassium carbonate (5.14 g) and the suspension was stirred at rt for 10 min. A solution of 3-bromopropionitrile (1.50 g, 11 mmol) in acetonitrile (25 mL) was added and the resulting mixture stirred at 50 °C for 24 h. The mixture was filtered to remove most of the inorganic salts and the acetonitrile was removed in vacuo to give a semisolid residue that was purified by flash column chromatography (1:5:94 NH_4OH:MeOH:$CHCl_3$) to yield the product **13a** as a clear oil (1.88 g, 70%). R_f 0.45 (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.73 (br s, 1H, NHCO), 3.13 (q, 2H, CH_2), 2.92 (t, 2H, CH_2), 2.65 (t, 2H, CH_2), 2.52 (t, 2H, CH_2), 1.52 (m, 4H, 2 × CH_2), 1.44 (s, 9H, 3 × CH_3).

(4-(*tert*-Butoxycarbonylamino)butyl)(2-cyanoethyl)carbamic Acid *tert*-Butyl Ester (14a). The nitrile **13a** (1.88 g, 7.8 mmol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 40 mL). A solution of di-*tert*-butyl dicarbonate (2.55 g, 12 mmol) in methanol (20 mL) was added dropwise to this mixture with vigorous stirring. The methanol and TEA were removed in vacuo to yield an oily residue that was dissolved in dichloromethane (100 mL) and washed with a solution of sodium hydroxide (2.5 M, 2 × 50 mL) and water (50 mL). The organic layer was dried over anhydrous sodium sulfate and filtered, the solvent removed in vacuo, and the oily residue purified by flash column chromatography (40% EtAc/hexane) to give the product **14a** as a clear oil (2.42 g, 91%). R_f 0.4 (40% EtAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (m, 1H, NHCO), 3.45 (t, 2H, CH_2), 3.26 (t, 2H, CH_2), 3.12 (q, 2H, CH_2), 2.58 (m, 2H, CH_2), 1.55 (quin, 2H, CH_2), 1.50−1.38 (m, 20H, CH_2, 6 × CH_3); HRMS (FAB) *m*/*z* calcd for $C_{17}H_{32}N_3O_4$ $(M + H)^+$ 342.2393, found 342.2395.

(3-Aminopropyl)(4-(*tert*-butoxycarbonylamino)butyl)carbamic Acid *tert*-Butyl Ester (15a). The nitrile **14a** (2.30 g, 6.7 mmol) was dissolved in ethanol (100 mL). NH_4OH (10 mL) and Raney nickel (8 g) were added and ammonia gas was bubbled through the solution for 20 min at 0 °C. The suspension was hydrogenated at 50 psi for 24 h. Air was bubbled through the solution and the Raney nickel was removed by filtering through a sintered glass funnel keeping the Raney nickel residue moist at all times. The ethanol and NH_4OH were removed in vacuo and the oily residue dissolved in CH_2Cl_2 and washed with sodium carbonate (10%, aq, 3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and filtered and the solvent removed in vacuo to give the product **15a** as a clear oil without further purification (2.12 g, 91%). R_f 0.4 (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.63 (m, 1H, NHCO), 3.29−3.02 (m, 6H, 3 × CH_2), 2.64 (m, 2H, CH_2), 1.64 (quin, 2H, CH_2), 1.50 (m, 2H, CH_2), 1.47−1.35 (m, 20H, CH_2, 6 × CH_3);^{13}C NMR (300 MHz, $CDCl_3$) δ 156.2, 79.7, 46.8, 40.5, 40.0, 39.4, 28.83, 28.78, 27.8, 26.2,

25.9; HRMS (FAB) *m*/*z* calcd for $C_{17}H_{36}N_3O_4$ $(M + H)^+$ 346.2706, found 346.2718.

2,3-Bis(benzyloxy)benzoyl Chloride (16a).[17,21] A solution of 3,4-bis(benzyloxy)benzoic acid **7a** (1.50 g, 4.5 mmol) in 2:1 dichloromethane/benzene (30 mL) was stirred at 0 °C for 10 min. Anhydrous DMF (3 drops) and oxalyl chloride (3.0 mL) were added in sequence and the mixture was stirred for 30 min at 0 °C. The solution was concentrated in vacuo to give the crude product **16a** that was consumed immediately in the next step.

(4-{[3-(2,3-Bis(benzyloxy)benzoylamino)propyl]-*tert*-butoxycarbonylamino}butyl)carbamic Acid *tert*-Butyl Ester (17a). A solution of amine **15a** (1.66 g, 4.8 mmol) in CH_2Cl_2 (20 mL) was mixed with 10% aqueous NaOH (10 mL). The mixture was stirred at 0 °C for 20 min, then a solution of 2,3-dibenzyloxybenzoic acid chloride **16a** (1.45 g, 4.1 mmol) in CH_2Cl_2 (8 mL) was added dropwise with vigorous stirring. The white cloudy solution was stirred at room temperature for 2 h. The organic phase was separated and the water phase extracted with CH_2Cl_2. The organic phases were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (EtAc/$CHCl_3$ 8:92) to give the product **17a** as a clear oil (2.33 g, 86%). R_f 0.33 (EtAc/$CHCl_3$ 8:92); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (br s, 1H), 7.66 (br s, 1H), 7.38 (m, 10H), 7.13 (m, 2H), 5.14 (s, 2H), 5.08 (s, 2H), 4.62 (br s, 1H), 3.23 (br s, 2H), 3.08 (m, 6H), 1.57 (br s, 2H), 1.40 (m, 22H); HRMS (FAB) *m*/*z* calcd for $C_{38}H_{52}N_3O_7$ $(M + H)^+$ 662.3805, found 662.3800. Anal. Calcd for $C_{38}H_{51}N_3O_7$: C, 68.96; H, 7.77; N, 6.35. Found: C, 68.87; H, 7.93; N, 6.32.

***N*-[3-(4-Aminobutylamino)propyl]-2,3-bis(benzyloxy)-benzamide, Trifluoroacetic Acid Salt (18a).** A solution of **17a** (2.30 g, 3.48 mmol) in CH_2Cl_2 (50 mL) was stirred at 0 °C for 30 min. A solution of trifluoroacetic acid (9 mL) in CH_2Cl_2 (40 mL) was added dropwise over 1 h with vigorous stirring. The mixture was stirred at room temperature for 2 h. After adding benzene (100 mL), the solution was concentrated to half of the total volume. This process was repeated three more times. The solution was then concentrated in vacuo and the residue used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO−3 drops of D_2O) δ 8.38 (br s, 1H), 7.44 (m, 2H), 7.38 (m, 9H), 7.13 (m, 2H), 5.19 (s, 2H), 5.00 (s, 2H), 3.23 (m, 2H), 2.80 (m, 6H), 1.78 (m, 2H), 1.57 (br s, 4H).

2,3-Petrobactin *tert*-Butyl Ester (20a). In an "iron-free" round-bottom flask 2-*tert*-butyl citrate (0.10 g, 0.4 mmol) was dissolved in anhydrous THF (5 mL). *N*-Hydroxysuccinimide (0.127 g, 1.10 mmol) and dicyclohexylcarbodiimide (DCC, 0.25 g, 1.21 mmol) were added to this solution and the reaction was stirred at room temperature for 3 h producing the activated 1,3-bis-*N*-hydroxysuccinimide ester **19** and a white precipitate. The ^{1}H NMR (acetone-d_6) showed that the signal at ~δ2.8 (dd) corresponding to the two CH_2 groups of 2-*tert*-butyl citrate had disappeared and a new group of signals at ~δ 3.3 (dd) corresponding to the two CH_2 groups in the activated NHS ester **19** had appeared. The solution was concentrated in vacuo giving a white solid residue that was used directly for the next coupling step. The solution of crude **19** in dry dioxane (5 mL) was stirred at 10 °C for 10 min. The solution of crude **18a** (0.63 g, 0.90 mmol, 2.2 equiv) in CH_2Cl_2 (5 mL) was treated with 1.6 mL of TEA at 0 °C. This solution of the free amine of **18a** was added to the solution containing **19** over 2 min. The mixture was then stirred at room temperature overnight. The DCU was removed by filtration and the filtrate concentrated in vacuo. The residue was then dissolved in chloroform and washed twice with saturated aq Na_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (MeOH/$CHCl_3$/NH_4OH 10:88:2) on "iron-free" silica gel to give the product **20a** as a clear oil (0.269 g, 57%). R_f 0.26 (MeOH/$CHCl_3$/NH_4OH 10:88:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (br s, 2H), 7.63 (m, 2H), 7.38 (m, 20H), 7.11 (m, 6H, including NH_2), 5.14 (s, 4H), 5.04 (s, 4H), 3.33 (m, 4H), 3.20 (m, 4H), 2.65−2.45 (m, 12H), 1.60−1.40 (m, 21H); ^{13}C NMR δ 172.9, 169.8, 165.6, 151.9, 146.8, 136.6, 136.5, 129.0, 128.9, 128.5, 127.9, 127.5, 124.7, 123.3, 117.1, 82.9, 76.7, 74.4, 71.5, 49.2, 47.2, 44.5, 39.3, 37.8, 29.5, 28.2, 27.3, 27.1; HRMS (FAB) *m*/*z* calcd for $C_{66}H_{83}N_6O_{11}$ $(M + H)^+$ 1135.6120, found 1135.6083. Anal. Calcd for $C_{66}H_{82}N_6O_{11}\cdot 1.1H_2O$: C, 68.62; H, 7.35; N, 7.28. Found: C, 68.36; H, 7.10; N, 7.65.

Preparation of 20f. A solution of **20e** (0.776 g, 0.66 mmol) in triethylamine−methanol (1:9 v/v, 20 mL) was stirred at 0 °C for 10 min. A solution of di-*tert*-butyl dicarbonate (0.58 g, 2.67 mmol) in methanol (5 mL) was added dropwise over 5 min. The mixture was stirred for one additional hour and the temperature was allowed to warm to room temperature and stirred overnight. The mixture was concentrated in vacuo and the residue dissolved in CH_2Cl_2 and washed several times with deionized water. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (30% acetone/hexane) to give the product **20f** as a clear oil (0.573 g, 63%). R_f 0.48 (50% acetone/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (t, 2H), 7.68 (m, 2H), 7.38 (m, 20H), 7.11 (m, 4H), 7.02 (br s, 2H), 5.14 (s, 4H), 5.04 (s, 4H), 3.22 (m, 8H), 3.05 (br s, 8H), 2.60 (dd, 4H), 1.42−1.30 (m, 43H); ^{13}C NMR δ 172.7, 169.9, 165.3, 155.7, 151.9, 146.9, 136.5, 136.5, 128.9, 128.9, 128.6, 128.5, 127.9, 127.4, 124.7, 123.4, 117.1, 82.8, 79.6, 76.7, 74.4, 71.5, 46.84, 46.78, 44.1, 39.6, 39.4, 28.9, 28.2, 27.1, 26.8, 26.4, 26.0; HRMS (FAB) *m*/*z* calcd for $C_{78}H_{103}N_6O_{15}$ $(M + H)^+$ 1364.6836, found 1364.6752. Anal. Calcd for $C_{78}H_{102}N_6O_{15}$: C, 68.70; H, 7.54; N, 6.16. Found: C, 68.76; H, 7.56; N, 6.11.

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Supporting Information Available: The experimental details for **16b**−**d**[17,21,32] and the characterization (NMR, HRMS, and C, H, and N analyses) of 3,4-petrobactin **1b**, 2,4-petrobactin **1c**, 2,5-petrobactin **1d**, **and** 2,3-homopetrobactin **1e**, **17b**, **17c**, **20b**, **20c**, and **20d**; the ^{1}H NMR spectral data of compounds **7b**, **7c**, **7d**, **13b**, **14b**, **15b**, **17d**, **17e**, **18b**, **18c**, **18d**, **18e**, and **20e**; HRMS of compounds **7b**, **7c**, **7d**, **15b**, **17d**, **17e**, and **20e**; C, H, and N analyses of compounds **17d** and **17e**; the ^{13}C NMR spectra of compounds **15b** and **20e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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