Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 2007–2014

TETRAHEDRON

Total synthesis and structure revision of petrobactin

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Received 15 November 2002; revised 10 January 2003; accepted 10 January 2003

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

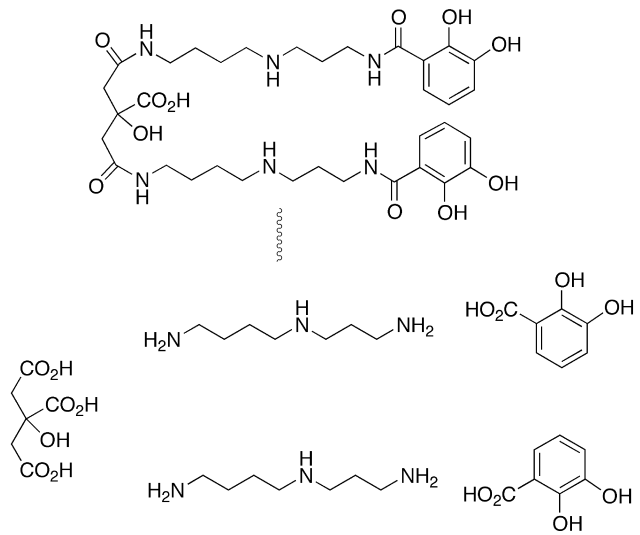

Figure 1. Retrosynthetic schematic of the original structure of petrobactin.

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

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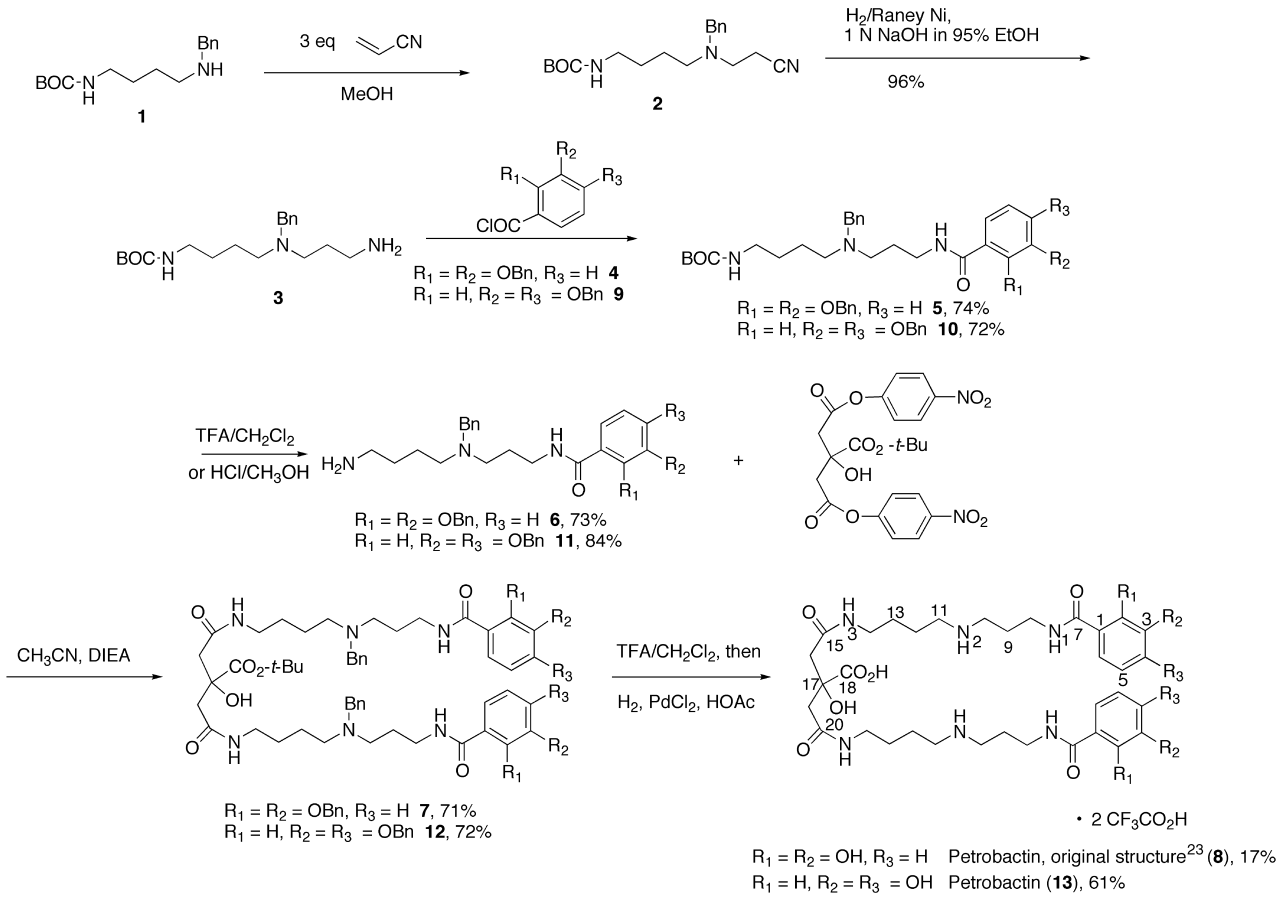
doi:10.1016/S0040-4020(03)00103-0



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-