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An Efficient Synthesis of Cobactin T, a Key Component of the Mycobactin Class of Siderophores

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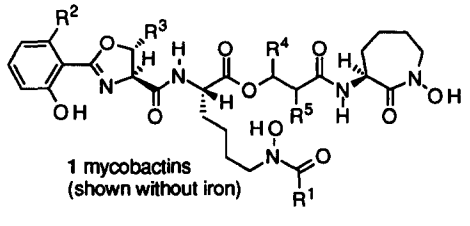
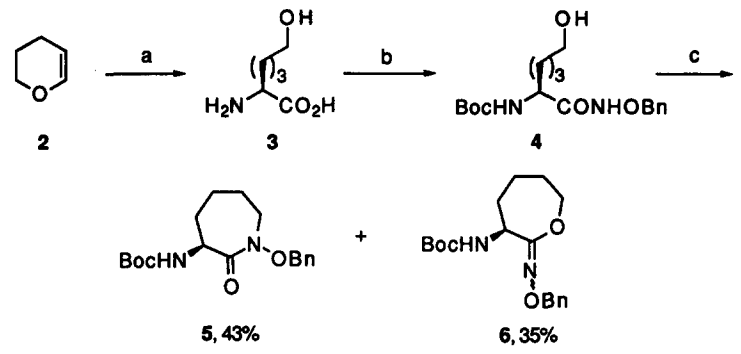
Abstract: N^{α}-Cbz-L-lysine *t*-butyl ester was oxidized by dimethyldioxirane to give nitrone **8c**, which was converted to azopine derivative **15**. Subsequent coupling and deprotection reactions afforded an efficient synthesis of cobactin T (**19**).

Mycobactins **1**[1] are a family of siderophores (microbial iron chelators) isolated from various mycobacteria and essential for growth of pathogenic strains such as *M. tuberculosis*.[2,3] These compounds are of particular interest because of their structural complexity and their roles in microbial iron metabolism. Current resistance of tuberculosis against classic drug therapy[4,5] has prompted us to develop a synthesis of natural mycobactins and analogs for the investigation of iron metabolic mechanisms and mycobactin-drug conjugates for better drug delivery. The synthesis of cobactin T (**19**) was first reported over a decade ago,[6] but the methodology utilized enzymatic resolution to synthesize intermediate L-ε-hydroxynorleucine (**3**) (Scheme 1) and subsequent cyclization was complicated by *N*- vs *O*- selectivity (**4** → **5** + **6**). We report here a novel synthesis of cobactin T, a key component of the mycobactins.

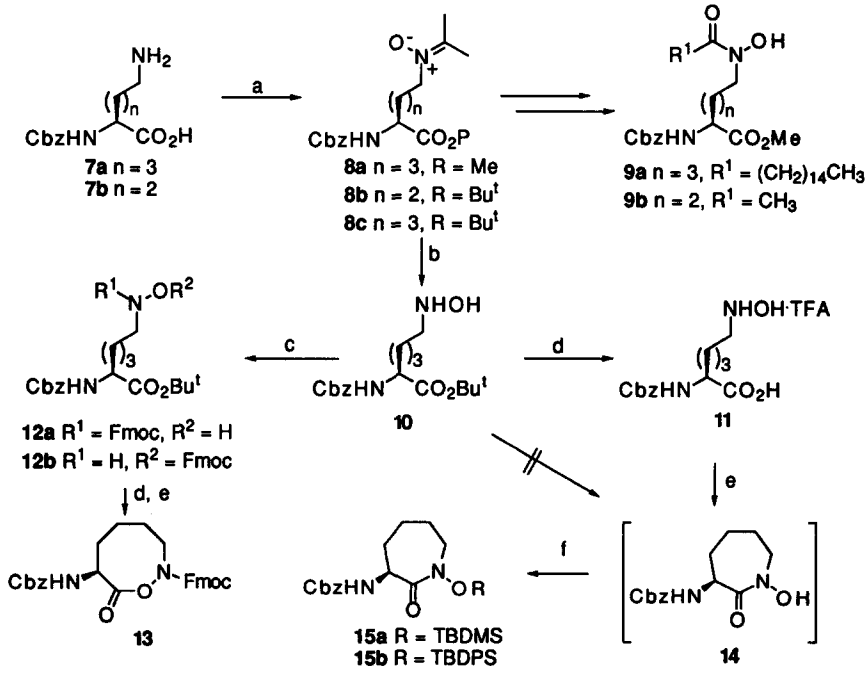




a) i. H_3O^+; ii. CN^-; iii. $(NH_4)_2CO_3$; iv. OH^-/H_2O; v. enzymatic resolution; b) i. $(Boc)_2O$; ii. $PhCH_2ONH_2$; c) PPh_3/DEAD.

Scheme 1

Dimethyldioxirane[7,8] was employed to oxidize N^{α}-Cbz-L-lysine methyl ester to the corresponding nitrone **8a** (Scheme 2).[9] Subsequent hydroxylamine exchange and acylation provided lysine-based hydroxamic acid **9a**, an important synthetic intermediate of the mycobactins. This method was also used to make ornithine-based hydroxamic acid **9b**, which is an iron chelating component of many siderophores.[10-12] We next describe the application of the dimethyldioxirane oxidation to the synthesis of cobactin T.

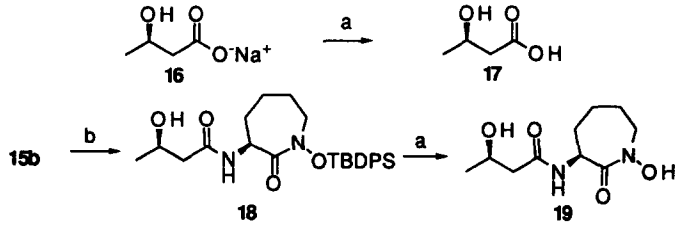


a) i. $AcOBu^t/HClO_4$, rt, or $SOCl_2$/MeOH; ii. dimethyldioxirane, acetone, -78 °C, 60% of **8c** from **7a**; b) i. NH_2OH·HCl, MeOH, 40 °C, 10 min; ii. $NaHCO_3$; c) FmocCl/$NaHCO_3$, 62% of **12a** from **8c**; d) i. TFA, CH_2Cl_2, rt, 1 h; e) DCC, DMAP, DMAP·HCl, $CHCl_3$, reflux, 3 h, 39% of **13** from **12a**; f) TBDMSCl or TBDPSCl/imidazole, DMF, 35 °C, overnight, 54% of **15b** from **8c**.

Scheme 2

N^{α}-Cbz-L-lysine (**7a**) was stirred in $AcOBu^t$ in the presence of $HClO_4$ in a sealed flask overnight to give the corresponding *t*-butyl ester. Subsequent oxidation by dimethyldioxirane provided nitrone **8b** in 60% yield.[9] Treatment of nitrone **8c** with NH_2OH·HCl, followed by basic workup afforded hydroxylamine **10**. The cyclization reaction was the most difficult step in this synthesis. Initial efforts to cyclize hydroxylamine **10** through direct amination with DMAP and $AlMe_3$[13,14] were unsuccessful. After removal of the *t*-butyl group, coupling reagents such as DCC, EDC [1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide], and EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) were utilized to promote cyclization of acid **11**, but in no case gave the required cyclic lysine **14**. Next, we decided to selectively protect the hydroxy group of hydroxylamine **10**. Treatment of hydroxylamine **10** with FmocCl[15] afforded a ferric chloride negative product **12**, which was first regarded as *O*-protected compound **12b**. Compound **12** was then stirred in TFA and methylene chloride at room temperature for 1 h, after removal of TFA and methylene chloride, the resulting acid was cyclized using DCC, DMAP, and DMAP·HCl[16] in a dilute chloroform solution to give the oxazocane **13** in moderate yield. The structure of oxazocane derivative **13** was verified by X-ray crystallography. This also indicated the isolated product **12** was **12a**, not **12b**.

After all the unsuccessful attempts, we found that treatment of hydroxylamine **11** with DCC, DMAP, and DMAP·HCl provided the desired hydroxamic acid **14**, but still in low isolated yield. This problem was solved by adding silyl protecting groups to decrease the polarity of the product. Intermediate **14** was treated with TBDMSCl or TBDPSCl/imidazole at 35 °C overnight. Aqueous workup followed by chromatographic purification gave azopine **15** in excellent yield.



a) Dowex® 50X8-200 ion-exchange resin, MeOH, 94% of **17** from **16**; 62% of **19** from **18**; b) i. H_2 (1 atm) /Pd-C, MeOH, rt, 2 h; ii. **17**/DCC, DMAP, DMAP·HCl, $CHCl_3$, 40 °C, 20 min, 63% from **15b**.

Scheme 3

(*R*)-3-Hydroxy butyric acid (**17**) was obtained by treatment of the corresponding sodium salt **16** (Aldrich) with Dowex® 50X8-200 ion-exchange resin. After hydrogenolytic removal of the Cbz group from azopine **15b**, the resulting amine was coupled with (*R*)-3-hydroxy butyric acid (**17**) in the presence of DCC, DMAP, DMAP·HCl to provide *O*-TBDPS cobactin T **18** in 63% yield. The TBDPS group was cleaved with 49% HF aqueous solution in acetonitrile.[17] It could also be removed by being stirred with Dowex® 50X8-200 ion-exchange resin in MeOH for a few minutes, a very mild deprotection method,[18] to give cobactin T (**19**) in 62% yield.

In conclusion, we utilized the readily available N^{α}-Cbz-L-lysine (**7a**) as a chiral starting material. Dimethyldioxirane oxidation followed by DCC/DMAP/DMAP·HCl mediated cyclization[16] provided azopine derivative **15** in good yield. Subsequent coupling and deprotection reactions gave an efficient synthesis of cobactin T.

Preparation of Azopine 15b. To a stirred solution of nitrone **8c** (481 mg, 1.23 mmol) in MeOH

(5 mL) was added NH_2OH·HCl (423 mg, 6.14 mmol, 5 eq). The solution was stirred for 10 min at 40 °C. After removal of the solvent, the residue was then taken up in saturated $NaHCO_3$ (10 mL), extracted by CH_2Cl_2, dried, filtered and concentrated to afford hydroxylamine **10**. Hydroxylamine **10** was then stirred in TFA/CH_2Cl_2 (3 mL/3 mL) for 1.5 h at rt, then concentrated to give acid **11**. To a refluxing solution of DCC (1.26 g, 6.14 mmol, 5 eq), DMAP (749 mg, 6.14 mmol, 5 eq) and DMAP·HCl (976 mg, 6.14 mmol, 5 eq) in $CHCl_3$ (70 mL) was added acid **11**/$CHCl_3$ (70 mL) dropwise over 2 h. After refluxing for one more h, the reaction mixture was concentrated, and then taken up in DMF (5 mL), treated with TBDPSCl (800 μL, 3.08 mmol, 2.5 eq) and imidazole (418 mg, 6.14 mmol, 5 eq). After being stirred at 35 °C overnight, the reaction mixture was diluted by the addition of EtOAc, washed with H_2O, and brine, dried, filtered, concentrated, and chromatographed eluting with EtOAc:Skelly B (1:5) to give azopine **15b** (339 mg, 54%), as a clear oil: R_f = 0.19 (EtOAc:Skelly B = 1:5); IR (neat) 3410, 3330, 2930, 2860, 1720, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.75-7.71, (m, 4H), 7.45-7.33, (m, 11H), 6.03, (d, J = 6 Hz, 1H), 5.05, (dd, J_1 = 18 Hz, J_2 = 12 Hz, 2H), 4.05-3.99, (m, 1H), 3.52-3.45, (m, 2H), 1.89-1.05, (m, 15H); ^{13}C NMR ($CDCl_3$) δ 169.49, 155.26, 136.50, 136.09, 135.99, 132.04, 131.57, 130.19, 130.14, 128.36, 127.90, 127.76, 127.49, 127.44, 66.46, 54.17, 53.06, 31.54, 27.30, 26.85, 25.25, 19.48; HRFABMS calcd. for $C_{30}H_{37}N_2O_4Si$ 517.2523, found 517.2568.

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