Enterobactin and Enantioenterobactin

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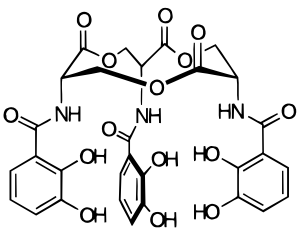
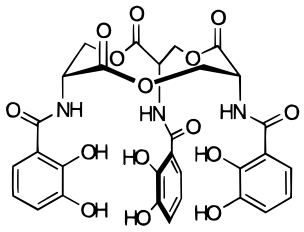
In response to biochemical requirements for iron, and the great insolubility of environmental ferric ion, microorganisms secrete siderophores (from the Greek: *sideros* = iron, *pherein* = to carry), small molecules capable of high affinity binding and transport of Fe(III). Enterobactin (**1**),[1] produced by *E. coli* and many other Gram-negative bacteria,[2] exhibits the largest binding constant (log K_{ML}= 49) for ferric ion of any natural substance.[3] It is composed of three L-serine residues linked head to tail into a trilactone platform to which are attached three pendant *N*-(2,3-dihydroxybenzoyl) groups. The catechol oxygens define the hexadentate binding site for Fe(III). Although many synthetic analogs have been prepared, none exhibit greater ferric ion complex stability constants than enterobactin. Enantioenterobactin (**2**) is the synthetic antipode prepared from D-serine.[4]



Enterobactin (**1**)

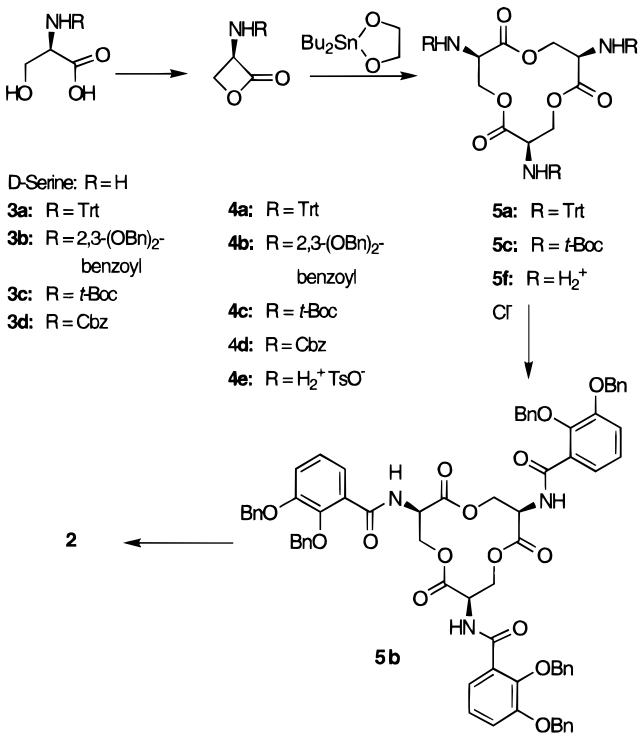


Enantioenterobactin (**2**)

Enterobactin is a particularly intriguing ligand and binds Fe(III) more strongly than other tricatechol ligands. The triserine lactone backbone appears to be a strongly contributing factor in the ligand's efficacy, allowing considerable preorganization of the free ligand and relatively strain-free binding of the ferric ion in the complex.[5]

Enterobactin and enantioenterobactin have been of interest for structural studies[5] and as probes for elucidation of details of the biological processes which recognize and transport ferric enterobactin into the bacterial cell and subsequently make iron available for cellular biochemistry.[6] In addition, the enterobactin triserine lactone backbone is itself attractive as a platform for the preparation of hybrid synthetic ligands which incorporate the serine trilactone nucleus with binding units other than catechol. We present here an efficient synthesis of enterobactin and enantioenterobactin.

Scheme 1



The reactions outlined in Scheme 1 use D-serine for preparation of enantioenterobactin (**2**); the reactions with L-serine produce enterobactin (**1**) from intermediates enantiomeric to **3**−**5**. The triserine lactone nucleus **5a** has been prepared generally following the approach developed by Shanzer and Libman,[7] which involves β-lactonization of *N*-tritylserine (**3a**)[8,9] and its subsequent trimerization to the macrocyclic lactone with the aid of an organotin template.[10] This strategy is attractive because of its directness and economy of steps relative to other syntheses,[4,11] yet it includes some low yield transformations, the lactonization of *N*-tritylserine (**3a**) with diisopropylcarbodiimide in the presence of 4-(dimethylamino)pyridine (DMAP) to **4a** (23−26% yields) and trimerization of the *N*-tritylserine β-lactone (**4a**) to macrocyclic triserine lactone (**5a**) using 2,2-dibutyl-2,1,3-dioxastannolane as a template, a remarkable transformation even at 20−23% yield. We have significantly increased the yields of these reactions using methodology which also allows for the preparation of considerable quantities of the triserine nucleus **5a**. The overall yield of enantioenterobactin or enterobactin from commercial starting materials has been increased 10-fold.

Results and Discussion

The formation of serine β-lactones appears to be very sensitive to the identity of the serine N-protecting group.

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The carbodiimide/DMAP procedure converts *N*-tritylserine to β-lactone **4a** in modest yield, yet fails or leads to extremely low yields of lactone when the protecting group is *t*-Boc or Cbz.[7a] Vederas has reported efficient syntheses of β-lactones under Mitsunobu conditions,[12] and this methodology is useful for the conversion of *N*-*t*-Boc- and Cbz-protected serines **3c** and **3d** to lactones **4c** and **4d**. On the other hand, *N*-tritylserine **3a** does not cyclize to the lactone under these Mitsunobu conditions.

A more efficient synthesis of enantioenterobactin (or enterobactin) would avoid or reduce the number of protection/deprotection steps: that is, the reaction sequence **3b** → **4b** → **5b** → **2**, where the N-protecting group in **3b** is the benzyl-protected catechol which appears in the final enantioenterobactin product. Unfortunately we have been unsuccessful in converting *N*-[2,3-bis(benzyloxy)benzoyl]-D-serine (**3b**) to the β-lactone **4b** under a host of variations of either the carbodiimide or Mitsunobu procedures. The predominant reaction course here is the decarboxylation of **3b** to produce the enamine.[12e]

A slight modification of the Vederas[12b] procedures has allowed us to make *N*-*t*-Boc β-lactone **4c** in high yield (84%) from commercial *N*-*t*-Boc-D-serine (**3c**). These reactions are amenable to scale up (the lactonization **3c** → **4c** can routinely be run on 8−9 g of starting material to recover product in 81−84% yield). This compares very favorably with other methods, which produce the β-lactones in low yields which further decrease as the amount of material to be cyclized is increased.

The organotin-mediated trimerization of serine β-lactones also exhibits a sensitivity to the identity of the N-protecting group. Attempts at trimerization of *N*-*t*-Boc β-lactone **4c** to the triserine lactone **5c** were unsuccessful, a particularly discouraging result in that **4c** can be produced in exceptionally high yield. It may be that the urethane carbonyl in this protecting group is too good a ligand for tin, in as much as none of the template effects of the stannylene acetal were observed. Likewise, we have been unsuccessful in oligomerizing β-lactones **4d** bearing the *N*-(benzyloxycarbonyl) protecting group. Since *N*-tritylserine β-lactone (**4a**) has been trimerized by Shanzer,[7] an interchange of N-protecting groups was done in good yield (80%) by removal of the *t*-Boc group in **4c** with triflouroacetic acid and toluenesulfonic acid,[12b] followed by tritylation of the intermediate β-serine tosylate salt (**4e**) to produce **4a**.

The cyclic trimerization of **4a** to **5a** using 2,2-dibutyl-2,1,3-dioxastannolane as a template in refluxing chloroform or carbon tetrachloride produces the tritylserine trilactone **5a** in 20−23% yield, along with higher cyclic oligomers, as reported earlier.[7] Roelens has observed that a mixture of the stannylene acetal and dibutyltin dichloride increases the conversion of propiolactone to the corresponding triolide from 25% to 54% relative to stannylene acetal alone.[13] Yet we saw no corresponding increase in the production of **5a** when **4a** was subjected to these reaction conditions. However, we did observe a remarkable increase in the production of macrocyclic trimer **5a** (81% yield) when **4a** was oligomerized in refluxing xylene for an extended time.[14] Organotin-mediated cyclic oligomerization of β-lactones appears to be thermodynamically controlled, with the trimer as the more stable product: after 24 h the reaction mixture consisted largely of the tetrolide and pentolide, while after 110 h the major product was the triolide **5a**. With the improvements in these two key transformations, the overall yield for the conversion of commercial *N*-*t*-Boc-D-serine to the serine trilactone **5a** was 54%.

The triserine lactone platform **5a** was converted into enantioenterobactin by detritylation to trisammonium-trilactone salt **5f** with dry HCl, and formation of the hexabenzylenantioenterobactin **5b** (79% yield) by reaction with 2,3-bis(benzyloxy)benzoyl chloride. Hydrogenolysis on Pd−C produced enantioenterobactin (**2**).

Experimental Section

Melting points were recorded on a digital capillary melting point apparatus and are reported uncorrected. NMR spectra were recorded at either 300 or 400 MHz for ^{1}H and at 75 or 100 MHz for ^{13}C. *J* values are given in Hz. Solvents were dried by standard methods and used just after distillation. All reactions were run under a dry nitrogen or argon atmosphere and were stirred magnetically, except as noted. Separations were done by flash chromatography or by centrifugally accelerated radial thin layer chromatography on silica gel. Glassware was dried in a 140 °C oven for a minimum of 4 h. The following entries describe the largest scale at which we have run these reactions.

***N*-(*t*-Boc)-D-Serine β-Lactone (4c).** A solution of 12.560 g (48.00 mmol) of dry triphenylphosphine (*in vacuo* 72 h over P_2O_5) in 300 mL of anhydrous acetonitrile was cooled to −65 °C (dry ice/*m*-xylene) and stirred 15 min with a mechanical stirrer. Dimethyl azodicarboxylate (DMAD)[15] (5.28 mL, 48.0 mmol) was added dropwise over 10 min. After 20 min a solution of *N*-(*t*-Boc)-D-serine (8.800 g, 42.88 mmol) in 100 mL of anhydrous acetonitrile was added dropwise to the reaction mixture over 30 min. The reaction mixture was stirred for an additional 1.5 h at −65 °C, allowed to slowly warm to room temperature, and subsequently evaporated *in vacuo*. The residue was chromatographed on silica gel with 85:15 hexanes/ethyl acetate, followed by 70:30 hexanes/ethyl acetate to separate 6.760 g (84%) of **4c** as white crystals: mp 118.9−119.7 °C (lit.[12a,e] for L enantiomer mp 119.5−120.5 °C); IR (CH_2Cl_2 cast) 3356, 1835, 1678, 1529, 1289, 1104 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.59, (d, 1 H), 5.10 (m, 1 H), 4.45 (m, 2 H), 1.46 (s, 9 H); ^{13}C NMR (CD_2Cl_2) δ 170.0, 155.1, 81.5, 66.6, 59.9, 28.2.

D-3-Amino-2-oxetanone *p*-Toluenesulfonic Acid Salt (4e).[12e] A mixture of N-(*tert*-butoxycarbonyl)-D-serine β-lactone (**4c**) (6.650 g, 35.5 mmol) and anhydrous *p*-toluenesulfonic acid (6.541 g, 38.0 mmol, dried 3 days in vacuo over P_2O_5) was cooled in an ice bath for 15 min. Anhydrous trifluoroacetic acid (80 mL) was added over 10 min (stirring was initiated when possible). The pale yellow solution was stirred at 0 °C for 15 min, followed by removal of the trifluoroacetic acid at the rotary evaporator, maintaining the temperature below 30 °C. The syrup was further evaporated at the vacuum pump (~0.2 mm) for 1 h. In a glovebag, the resulting solid was triturated with anhydrous diethyl ether, filtered and washed with ether, and then dried under reduced pressure (0.2 mm) overnight to give 8.941 g (97%) of salt **4e**: ^{1}H NMR (300 MHz, DMF-d_7) δ 7.64−7.7 (d, 2 H), 7.15 (d, 2 H, J = 8), 5.54 (dd, 1 H, J = 4.6, 6.5), 4.74 (m, 1 H), 4.66 (m, 1 H), 2.3 (s, 3 H).

***N*-Trityl-D-serine β-Lactone (4a).** A solution of 9.240 g (35.7 mmol) of β-lactone tosylate salt **4e** in 100 mL of dry CH_2Cl_2 was cooled to 0 °C, followed by the addition of 19.9 mL of freshly distilled Et_3N over 5 min. A solution of 14.88 g (53.6 mmol) of trityl chloride and 50 mL of CH_2Cl_2 was added over 15 min. The mixture was reacted for an additional 30 min at 0 °C and then warmed to room temperature and stirred 6 days. The reaction mixture was then passed through a 2 in silica gel plug

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(15) The more readily commercially available diethyl azodicarboxylate (DEAD) can be used in place of DMAD, although chromatograpic separation of the product is generally easier with the latter.

with CH_2Cl_2. The solvent was removed at the rotary evaporator and the residue recrystallized from hexanes to give 7.271 g of **4a**. The mother liquor was evaporated and the residue flash chromatographed with 92:8 hexanes/ethyl acetate to give an additional 2.229 g of **4a** (combined yield 81%): mp 192−194 °C [lit.[7] mp 193−195 °C for L-enantiomer]; IR (CH_2Cl_2 cast) 3324, 3063, 1814, 1594, 1487, 1446, 1214, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (m, 9H), 7.28 (m, 6H), 4.61 (m, J = 4.7, 5.6, 11.5 Hz, 1H), 3.55 (pseudo t, J = 5.6 Hz, 1H), 3.12 (q, J = 5.6, 4.7 Hz, 1H), 2.69 (d, J = 11.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 172.0, 145.0, 128.4, 128.3, 127.0, 70.8, 70.6, 64.5.

Tris(*N*-tritylserine) Trilactone (5a). *N*-Trityl-D-serine β-lactone (**4a**) (1.000 g, 3.04 mmol) and 90 mg (0.3 mmol) 2,2-dibutyl-2-stanna-1,3-dioxolane[16] were refluxed in 15 mL of dry *m*-xylene for 110 h. After being cooled to room temperature, the reaction mixture was filtered through a 2 in. plug of silica gel with 300 mL of CH_2Cl_2. The solvent was removed at the rotary evaporator and the residue separated (in two ca. half-gram batches) by centrifugally accelerated radial thin layer chromatography with 2:1 CH_2Cl_2:hexanes to give 0.806 g (81%) of **5a** which was identical to that prepared along the lines of Shanzer:[7] mp > 260 °C; IR (Nujol) 1745, 1600 cm^{-1}; 1H NMR (CD_2Cl_2) δ 7.4−7.6 (m, 18 H), 7.2−7.35 (m, 27 H), 3.90 (pseudo t, J = 10.8 Hz, 3H), 3.44 (q, J = 10.8, 4.5 Hz, 3H), 3.30 (m, J = 10.8, 4.5, 10.8 Hz, 3H), 2.58 (d, J = 10.4 Hz, 3H); ^{13}C NMR (CD_2Cl_2) δ 172.6, 145.8, 128.9, 128.3, 127.0, 71.4, 66.6, 55.1. Anal. Calcd for $C_{66}H_{57}N_3O_6$: C, 80.22; H, 5.81; N, 4.25. Found: C, 80.43; H, 5.69; N, 4.19.

Hexabenzylenantioenterobactin (7). HCl in dry ethanol[18] (0.642 mL of a 1.133 N solution) was added to a solution of 200 mg (0.202 mmol) of tris(*N*-tritylserine) trilactone **5a** and 1.5 mL of dry ethanol and the mixture refluxed for 2 min in a preheated oil bath. The mixture was cooled and the solvent removed at reduced pressure. Under a steady stream of dry N_2, the residue was washed 4 × 1 mL of dry diethyl ether and the supernatant removed with a Pasteur pipette equipped with a cotton filter at the tip. The residue was suspended in 5.0 mL of dry THF and cooled to 0 °C for 0.5 h. Solutions of 2,3-bis(benzyloxy)benzoyl chloride[17] (436 mg, 1.236 mmol) in 1 mL of THF and 0.26 mL of triethylamine (1.5 × 1.236 mmol) in 1 mL of THF were added simultaneously dropwise over 10 min. The mixture was stirred at 0 °C an additional 15 min, at rt for 30 min, and then passed through a 1 in. plug of silica gel with 50 mL of CH_2Cl_2. The solvent was removed at reduced pressure and the residue separated by centrifugally accelerated radial thin layer chromatography. Elution with 2:1 CH_2Cl_2/hexanes yielded 70 mg of unreacted triserine lactone **5a**. Elution with 1:4 EtOAc/CH_2Cl_2 provided 126 mg of hexabenzylenantioenterobactin (**7**) (51% conversion; 79% yield accounting for recovered starting material) of as a foam:[4] IR ($CHCl_3$) 1755, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.48 (d, 3H), 7.65 (m, 3H), 7.08−7.45 (m, 36H), 5.15 (half of AB q, J = 11 Hz, 3H), 5.11 (s, 6H), 5.04 (half of AB q, J = 11 Hz, 3H), 4.91 (m, 3H), 4.09 (m, 6H). Anal. Calcd for $C_{72}H_{63}N_3O_{15}$: C, 71.45; H, 5.25; N, 3.47. Found: C, 71.27; H, 5.05; N, 3.31.

Enantioenterobactin (2). All glassware and the stirbar were first washed in a KOH−2-propanol bath, rinsed with distilled water, and then soaked in 1 M HCl for 24 h, rinsed, and then washed in a RBS 35[19] solution for 24 h, rinsed with distilled water and then with deionized water, and then air dried at room temperature. Ethanol (1.8 mL) was added dropwise to a solution of 74 mg of hexabenzylenantioenterobactin (**7**) and 0.3 mL ethyl acetate, and the mixture became heterogeneous. After addition of 34 mg of 10% Pd−C ethyl acetate was added dropwise until the solution became clear. The mixture was then hydrogenated (110 psi) for 24 h at room temperature. The product mixture was gravity filtered through acetone-saturated fluted filter paper containing also a plug of cotton and washed with 30 mL of acetone. Evaporation of the solvent at 35 °C and drying in vacuo gave 38 mg (100%) enantioenterobactin as a pale tan glassy residue. Further evaporation *in vacuo* for 5 days yielded a tan solid: mp 202−203 °C (lit.[4,7,11] mp 202−203 °C); ^1H NMR (acetone-d_6) δ 8.6 (d, 3H), 7.3 (d, 3H), 7.0 (d, 3H), 6.78 (t, 3H), 5.15 (m, H), 4.78 (m, 6H), 3.17 (b, 5H); ^{13}C NMR (DMSO-d_6) δ 169.4, 169.0, 148.5, 146.3, 118.9, 118.5, 118.5, 116.3, 51.6, 63.8; IR (KBr) 3700−2500, 1745, 1634, 1580, 1530, 1455, 1340, 1260, 1170 cm^{-1}.

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(18) Anhydrous ethanolic HCl is conveniently prepared by adding freshly distilled acetyl chloride (1.6 mL, 22.5 mmol) to 25 mL of anhydrous ethanol, followed by titration with a standard NaOH solution, using 0.25% phenolphthalein in ethanol as indicator.
(19) RSB-35 is a surface active agent marketed by Pierce Chemical Co.