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A Much Improved Synthesis of the Siderophore Enterobactin[1]

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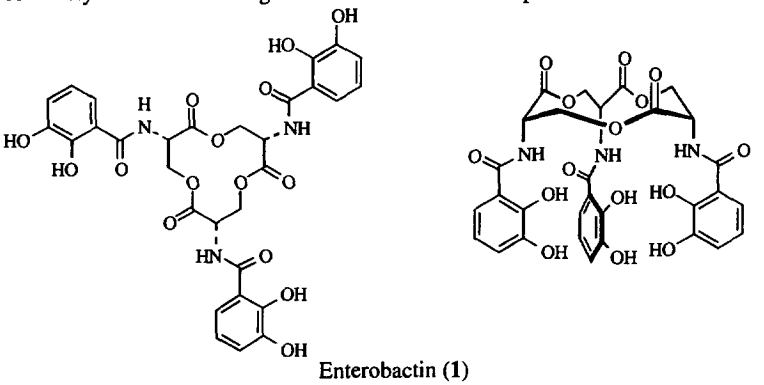
Abstract: Enterobactin, the cyclic trimer of *N*-(2,3-dihydroxybenzoyl)-L-serine has been efficiently prepared. A simple and high yield procedure has been developed for construction of *N*-protected serine trilactone as the key intermediate: methyl *N*-trityl-L-serinate and 2,2-dibutyl-1,3,2-dioxastannolane were refluxed in xylene to produce the triolide as the only lactone product in 85% yield. © 1997, Elsevier Science Ltd. All rights reserved.

The siderophore enterobactin (**1**)[2] is produced by *E. coli* and many other gram negative bacteria[3] to solubilize environmental Fe(III) and transport it into the bacterial cell. 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). This important molecule is of considerable interest in that it exhibits the largest binding constant for ferric ion among all natural substances (log K_{ML}= 49).[4] 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, 6]



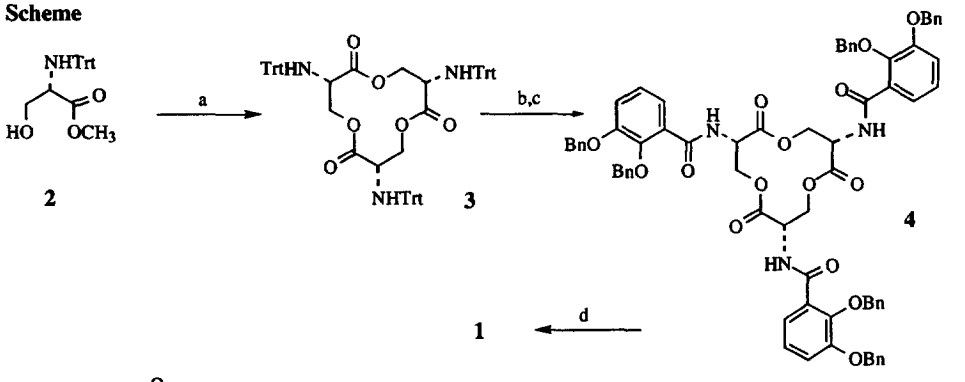
Enterobactin (**1**)

Enterobactin is of interest for structural studies[5] and for probing the details of the biological processes which recognize and transport ferric enterobactin into the bacterial cell.[7] The enterobactin triserine lactone backbone is additionally attractive for the preparation of hybrid synthetic ligands which incorporate the triserine lactone nucleus with binding units other than *N*-2,3-dihydroxybenzoyl.

Prior enterobactin syntheses have revolved around the preparation of the tri-L-serine nucleus as the key intermediate. Two approaches have been used. Corey[8] and Rastetter[9] both used the stepwise

linking of appropriately protected and/or activated serine derivatives to construct linear serine trimers which were then macrocyclized to the tri-L-serine lactone nucleus. Very nice chemistry was developed, but the large number of steps used in the production of the 12-membered serine trilactone detracted from the overall synthesis: the protection and deprotection steps outnumbered those which formed the trilactone macrocycle. Shanzer and Libman[10] formed the triserine lactone nucleus **3** by an efficient cyclooligomerization of *N*-tritylserine ß-lactone with the aid of an organotin template.[11] The Shanzer strategy is attractive because of its directness and economy of steps; yet it included low yield transformations: the ß-lactonization of *N*-tritylserine (26% yield); and trimerization of the *N*-tritylserine-ß-lactone to macrocyclic triserine lactone **3** using 2,2-dibutyl-1,3,2-dioxastannolane as a template, a remarkable transformation even at 22% yield. We have reported a synthesis of enterobactin which produces trilactone **3** in four steps from *N*-t-BOC-serine in an overall 54% yield with the intermediacy of serine lactones following generally the Shanzer approach. While it does increase the overall yield of the trilactone ten-fold and is amenable to scale-up, it is nevertheless experimentally tedious.[12] The present report details an exceptionally efficient (81% yield from commercial methyl *N*-trityl-L-serinate) and experimentally facile synthesis of enterobactin (Scheme), where the synthesis of the trilactone nucleus becomes trivial.

Scheme



a) Bu_2Sn(O,O) , xylene, Δ; b) HCl, EtOH; c) 2,3-dibenzyloxybenzoyl chloride, Et_3N; d) H_2, Pd-C

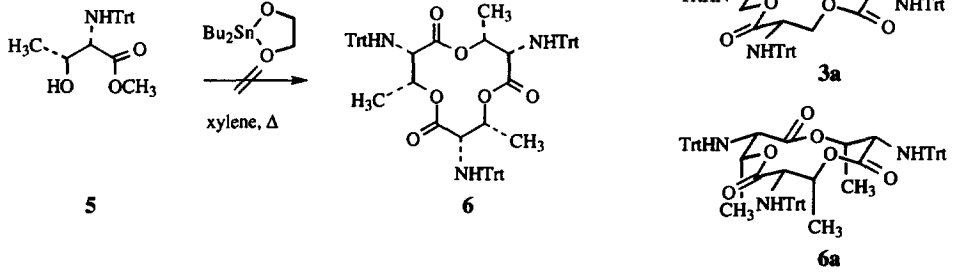
The present work avoids the low-yield and tedious preparation of ß-lactone intermediates. Furthermore, we and others have observed that ß-hydroxy acid derivatives (ß-lactones[10-12, 18] and methyl esters[13]) are cyclooligomerized to mixtures of macrocyclic lactones through thermodynamically controlled processes, with the trimer as the more stable product. Methyl *N*-trityl-L-serinate[14, 15] (**2**), produced in 95% yield from commercial methyl L-serinate, is cyclooligomerized to L-serine trilactone **3** with exceptional efficiency in refluxing xylene by the action of 2,2-dibutyl-1,3,2-dioxastannolane[16] as template. The course of the reaction was readily monitored by changes in the carbonyl region of the ^{13}C NMR spectrum. After 3 hours the reaction mixture included starting methyl ester **2** (δ 173.85 ppm in $CDCl_3$), small amounts of triolide **3** (172.33 ppm), and five other carbonyl-containing species (173.48, 173.16, 173.07, 172.92, 171.93 ppm), presumably higher oligomers. After 12 hours the same seven carbonyl signals remained but with an increase in the relative amount of trimer **3** at the expense of the other six. After 24 hours the only significant carbonyl species present was the triolide **3**. A sample run is illustrative: 5.00 g (13.8 mmol) methyl *N*-trityl-L-

serinate and 0.41 g (1.38 mmol) 2,2-dibutyl-1,3,2-dioxastannolane was reacted in 150 mL dry xylene at reflux for 24 h in a soxhlet extractor containing dry 4Å molecular sieves. Evaporation of the solvent and flash chromatography on silica gel with 2:1 CH_2Cl_2:hexanes gave 3.87 g (85%) of tris(*N*-trityl-L-serine) trilactone (**3**) which was identical to an authentic sample[12]: 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 Hz, J= 4.5 Hz, 3H), 3.30 (m, J=10.8 Hz, J=4.5 Hz, J=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. The overall yield for the conversion of commercial methyl L-serinate to the L-serine trilactone **3** was 81%. We have also prepared the triserine lactone **3** in refluxing toluene in the same concentrations as above, in similar yield but with longer reaction times (48 hours).

The triserine lactone platform **3** was converted into enterobactin as reported before by detritylation to the trisammoniumtrilactone salt with dry HCl,[12, 17] and formation of hexabenzylenterobactin **4** (79% yield) by reaction with 2,3-dibenzyloxybenzoyl chloride. Hydrogenolysis on Pd-C quantitatively produced enterobactin (**1**) identical to authentic material.[10, 12]

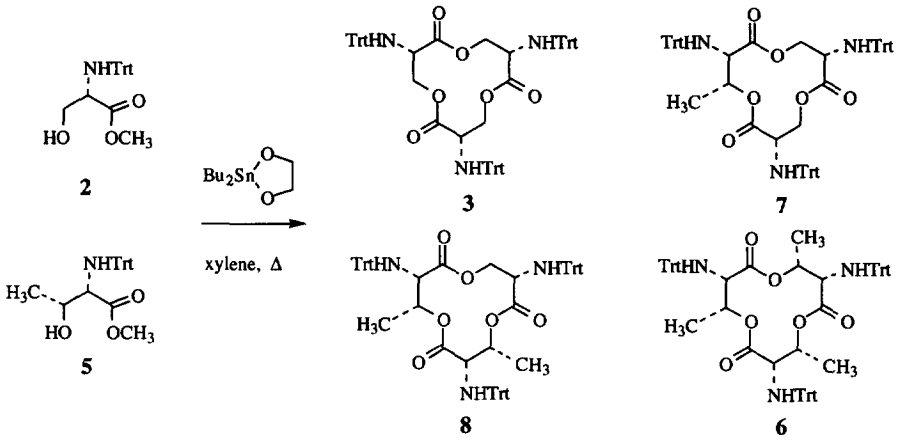
We also sought to prepare the threonine analog of enterobactin for use as a biological probe. In contrast to the ready cyclooligomerization of methyl *N*-trityl-L-serinate, we have been unable to effect similar reaction with methyl *N*-trityl-L-threoninate (**5**). Reaction of **5** with the stannylene acetal template in refluxing xylene for 24 hours resulted not in the formation of the trimer **6** (or any other cyclic oligomer), but rather materials which had suffered detritylation. At shorter reaction times the reaction mixture consisted of unreacted methyl *N*-tritylthreoninate (**5**) and detritylated compounds which were not further characterized. Running the reaction in a lower-boiling solvent (toluene) resulted in recovery of only starting material **5** even after 96 hours of reflux.

It is unclear why the course of the reaction with the threonine derivative **5** should be so different from the serine analog **2**, but it may be related to the relative stabilities of the products. In tris(*N*-trityl-L-serine) trilactone (**3**), the molecule adopts conformation **3a**[11, 12] which places the three bulky *N*-trityl groups in pseudo equatorial positions. In the corresponding threonine trilactone, the similar conformation (**6a**) would require that the three additional methyls be pseudo axial, engendering destabilizing interactions relative to **3a**.



Our inability to produce the trithreonine lactone **6** prompted us to investigate the incorporation of threonine residues into mixed cyclic oligolides. A 50:50 mixture of methyl *N*-trityl-L-serinate (**2**) and methyl *N*-trityl-L-threoninate (**5**) was refluxed 24 hours in xylene in the presence of 10 mole percent 2,2-dibutyl-1,3,2-dioxastannolane. Surprisingly, we did not observe the presence of any oligolide

(such as **7** or **8**) which incorporated threonine, after chromatography on silica gel. The all-serine triolide **3** was isolated as the only tritylated lactone product in 65% yield based on available serine. Seebach has reported the preparation of oligolides (mixolides) from mixtures of different ß-hydroxy acids; however all those triolide products bore substituents only in equatorial positions.[18]



We are currently preparing hybrid ligands by attaching a variety of non-catechol binding units to the enterobactin triserine lactone nucleus **3** that has become readily available through the present work.

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