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# SYNTHESIS, SIDEROPHORE, AND ANTIMICROBIAL EVALUATION OF A SPERMIDINE-BASED TRICATECHOLATE SIDEROPHORE AND CARBACEPHALOSPORIN CONJUGATE

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**Abstract**: The synthesis of a novel spermidine-based tricatecholate compound and the corresponding carbacephalosporin conjugate are described. Interestingly, both compounds exhibited significant siderophore (growth-promoting) activity for several strains of *E. coli*.

We have recently reported the synthesis and antimicrobial activity of a spermidine-based catechol siderophore-carbacephalosporin conjugate (**3**).[1] The design of conjugate **3** was derived from the previous synthesis of spermexatol [2] (**1**), a new type of spermidine-based siderophore.[2] Spermexatol [2], which contains both hydroxamate and catechol ligands, closely resembles the natural spermidine-containing iron chelators, parabactin (**4**) and agrobactin (**5**).[3] Spermexatol [2] was shown to substitute for natural siderophores in mutant strains of *E. coli* and *Vibrio cholerae*.[2] Attachment of a β-lactam antibiotic to the spermexatol nucleus, as shown with conjugate **3**, resulted in the inhibition of microbial growth. This type of iron transport-mediated drug delivery has shown promise with catechol conjugate **3**[1] as well as other catechol and hydroxamate siderophore-based β-lactam conjugates.[4]

In the initial design of conjugate **3**, the antibiotic component was attached to the central portion of the spermidine base by a succinic acid spacer. Thus, this conjugate formally contained only two bidentate ligands and its ability to complex and chelate iron with 1:1 stoichiometry was uncertain. Although natural examples of quadradentate iron chelators containing both hydroxamate (rhodotorulic acid)[5] and catechol ligands (bis(2,3-dihydroxybenzoyl)-L-lysine)[6] exist, we wished to study the effect of providing an additional bidentate ligand to provide a hexacoordinate molecule capable of forming a more common 1:1 complex with ferric ion.

The best example of a tricatecholate siderophore is enterobactin (**6**).[7] The iron binding and growth promoting abilities of enterobactin have been investigated extensively.[8] Many synthetic enterobactin analogs have been produced, including carbocyclic analogs[9] (e.g. MECAM), and a lysine-based dipeptide analog.[6] Linear and cyclic spermidine-based tricatechol derivatives also

have been reported,[10] but no indication of their growth promoting ability was given. Furthermore, in the earlier reported[10] tricatecholate spermidine compounds, no functionality suitable for attachment of an antibiotic was included. In this communication, we report the synthesis of a novel spermidine-based tricatechol iron chelator **7** and describe its growth promoting effects with *E. coli*. In addition, the corresponding conjugate **8** with a carbacephalosporin β-lactam antibiotic is described and evaluated for biological activity.

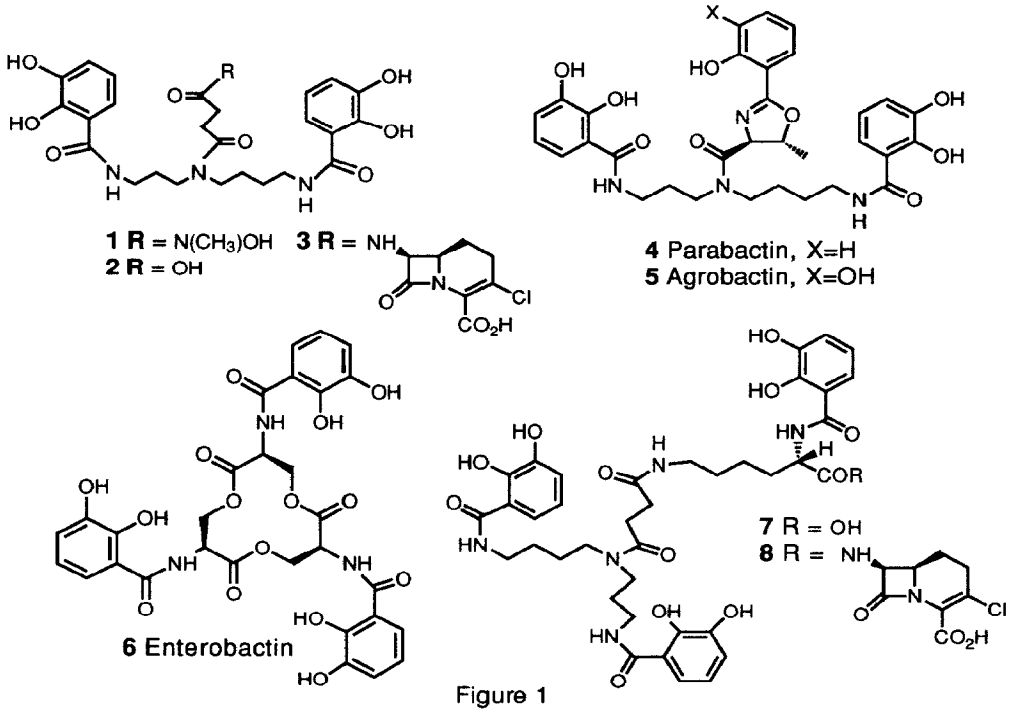
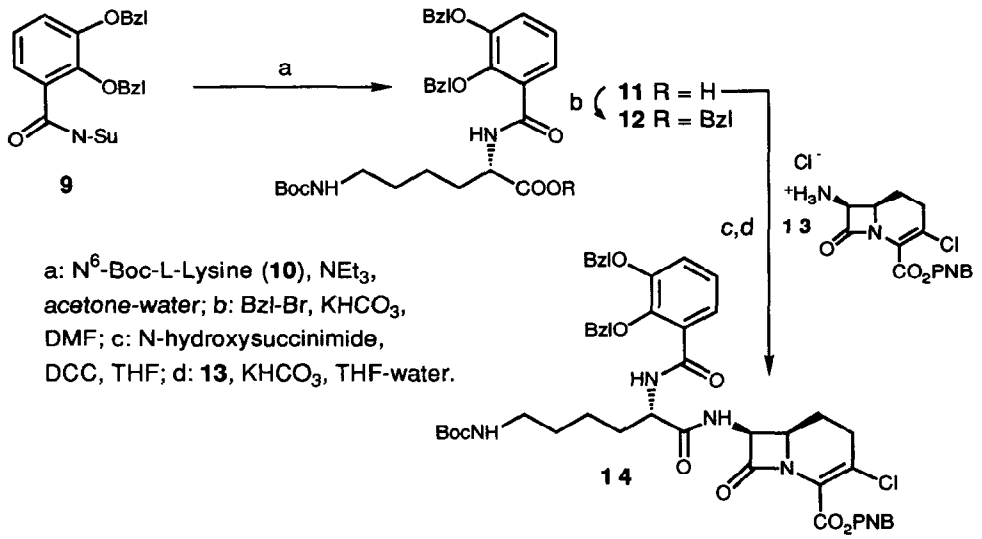



Figure 1

The N-hydroxysuccinimide active ester of bis(2,3-dibenzyloxy)benzoic acid[6] (**9**) was combined with $N^6$-Boc-L-Lysine (**10**, Sigma) and triethylamine to produce the monocatecholate compound **11** in 60% yield as shown in Scheme 1. Compound **11** was esterified using benzyl bromide to give benzyl ester **12** in 52% yield. Monocatecholate β-lactam conjugate **14** was produced under N-hydroxysuccinimide and DCC coupling conditions from carboxylic acid **11** and β-lactam **13**[11] in 74% yield following silica gel chromatography.

After removal of the Boc group from **12** with trifluoroacetic acid in methylene chloride at 0°C, the oily residue was stirred with the N-hydroxysuccinimide active ester of the tetrabenzyl protected spermidine derivative (**15**)[2] and triethylamine to afford compound **16** in 57% yield (Scheme 2).

Scheme 1

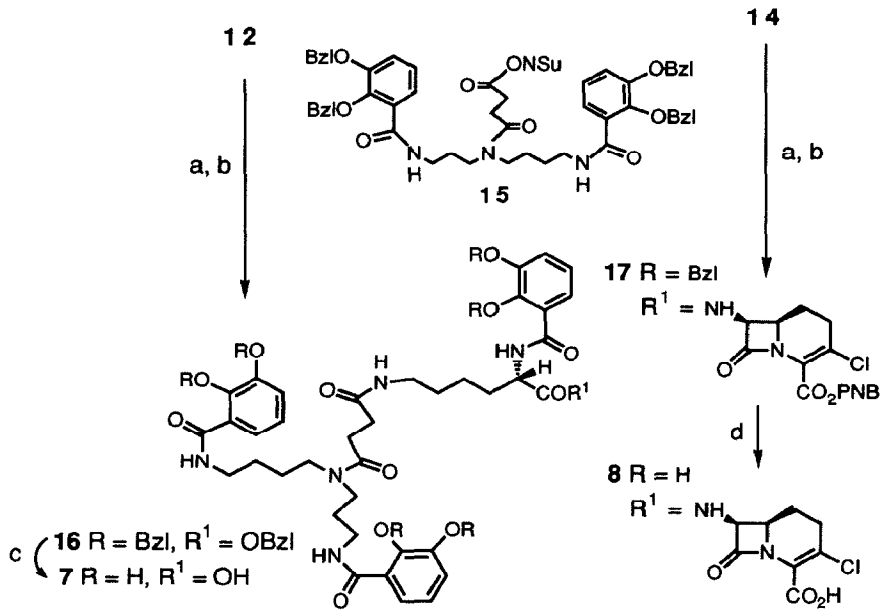



a: $N^6$-Boc-L-Lysine (**10**), $NEt_3$, acetone-water; b: Bzl-Br, $KHCO_3$, DMF; c: N-hydroxysuccinimide, DCC, THF; d: **13**, $KHCO_3$, THF-water.

The benzyl protecting groups of **16** were easily removed with hydrogen in the presence of 10% Pd-C to give tricatecholate siderophore **7** in quantitative yield. Protected conjugate **17** was produced from **14** and **15** in the same manner as **16** in 25% yield. The low yield of the coupling reaction may be attributed to the repeated chromatography that was necessary during purification of **17**. Fully deprotected **8** was obtained quantitatively under the hydrogenation conditions previously used to retain the double bond and the chlorine atom in the carbacephalosporin nucleus (10% Pd-C, 300 mole% of concentrated HCl in 5% aqueous DMF).[4] Confirmation that the double bond and the vinylic chlorine were retained in the β-lactam nucleus was achieved by carbon-13 NMR and mass spectrometric analysis.[12]

Initial screening of β-lactam conjugate **8** by incubation with β-lactam hypersensitive *E. coli* X580[13] provided unexpected results. Unlike most of the other siderophore-carbacephalosporin conjugates that have been synthesized and tested,[4] compound **8** did not exhibit antimicrobial effects against *E. coli* X580. It was initially suspected that this conjugate was not recognized by any of the iron transport proteins, as compound **8** is larger than most natural siderophores. Alternatively, due to its hydrophobic nature, conjugate **8** may have been insoluble in the aqueous testing media, resulting in no observed biological activity. As with the other[1,4a] spermidine-based conjugates tested in our laboratory, the stock solution of compound **8** was prepared with DMF and

Scheme 2




a: TFA, $CH_2Cl_2$; b: **15**, $NEt_3$, THF; c: $H_2$, Pd-C, MeOH; d: $H_2$, Pd-C, HCl, 5% aq. DMF.

added directly to the test media.

In order to try and determine if the synthetic siderophore and conjugate were soluble enough for biological assimilation and were capable of being recognized by any iron transport receptor proteins in *E. coli*, compounds **7** and **8** were tested for growth promoting activity with *E. coli* X580, and two isogenic *E. coli* strains (RW193 and RWB18). The RW193 strain of *E. coli* K-12 is able to use both catecholate and hydroxamate siderophores, but is unable to synthesize enterobactin. The RWB18 strain is a mutant derived from RW193, which lacks an outer membrane protein implicated in ferrienterobactin uptake.[14]

As the Table shows, tricatecholate conjugate **8** did act as growth promoter for all of the strains of *E. coli*. The conjugate was not as good a growth promoter as the corresponding tricatecholate siderophore (compound **7**), but it does appear that the conjugate was able to provide iron to the microbes. The tricatecholate spermidine siderophore (**7**) exhibited better growth promotion than the bis-catecholate siderophore (**2**),[1] implying that a 1:1 complex indeed may be more suitable for receptor recognition than a 3:2 siderophore-iron complex. Compound **7** was not as effective a growth promoter as the natural siderophore, enterobactin. Interestingly, all of the

synthetic spermidine catecholate compounds were growth promoters for the RWB18 strain as well as the RW193 strain. This growth promoting ability with the RWB18 may indicate that the enterobactin receptor is not required for the uptake of these compounds in this strain of *E. coli*. It is not apparent why β-lactam conjugate **8** was not toxic to *E. coli* X580. Although the amide linkage is similar to that used for our previously reported hydroxamic acid and catechol-based β-lactam conjugates,[4] the lack of antimicrobial activity of **8** may be an indicator of the need for a release mechanism to cleave the antibiotic from the carrier siderophore. Another possibility is that the β-lactam cannot reach its site of action due to the bulkiness of the tricatecholate carrier.

TABLE 1. GROWTH PROMOTION OF CATECHOL SIDEROPHORES WITH STRAINS OF *E. coli* [15]

| | Zones of Stimulation of Growth in mm[a] | | |
|---|---|---|---|
| | *E. coli* X580 | *E. coli* RW193 | *E. coli* RWB18 |
| Enterobactin (**6**)[16] | 38 (24)[b] | - | - |
| **6** / Fe (III) (1:1) | 40 (30) | - | - |
| **2** | 0 | 22 | 24 (16) |
| **2** / Fe (III) (3:2) | 16 | 20 | 24 (18) |
| **7** | 20 | 24 | 24 (16) |
| **7** / Fe (III) (1:1) | 22 | 28 (22) | 24 (16) |
| **8** | 0 | 18 | 16 (10) |
| **8** / Fe (III) (1:1) | 12 | 18 (12) | 20 (12) |

[a] Zones of stimulation were measured as the diameter of growth after incubation of the plates upside down at 37°C for 24 hours.
[b] Compounds were tested at 25 nmoles and 5 nmoles (5 nmole measurements in parenthesis).

The enhanced growth promoting ability of the tricatecholate siderophore **7**, compared to the quadradentate siderophore **2** indicates that these tricatecholate spermidine derivatives merit additional study. Modifications of the β-lactam conjugate **8** with respect to the linkage of the antibiotic to the iron chelating moiety are under consideration and may provide a conjugate with antimicrobial activity.

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11. We sincerely appreciate the gift of the carbacephalosporin from Eli Lilly and Company.
12. All new compounds gave satisfactory $^1$H, $^{13}$C, IR, and mass spectral analysis. Characterization data for **$N^6$-($N^4$-Succinamido-$N^1$,$N^8$-bis(2,3-dihydroxybenzoyl)spermidine-[$N^2$-(2,3-dihydroxybenzoyl)]-L-lysine (7)** $FeCl_3$ positive (red-purple); IR (TF) 3380, 1740, 1650 cm$^{-1}$; $^1$H NMR (300 MHz, methanol-$d_4$) δ 1.40-2.10 (m, 13 H, $CH_2$), 2.46 (m, 2 H, $CH_2$), 2.65 (m, 2 H, $CH_2$), 3.10-3.45 (m, 9 H, $CH_2$), 4.58 (m, 1 H, NCHCO), 6.65-6.80 (m, 3 H, aromatic H), 6.90-7.00 (m, 3 H, aromatic H), 7.20-7.25 (m, 2 H, aromatic H), 7.37 (dd, $J$ = 1.5 and 8.1 Hz, 1 H, aromatic H); $^{13}$C NMR (75 MHz, methanol-$d_4$) δ 24.30, 25.94, 26.89, 27.57, 28.34, 29.29, 29.83, 31.92, 32.01, 37.54, 37.95, 39.81, 40.00, 44.29, 46.63, 53.74, 116.50, 116.64, 116.86, 118.48, 118.61, 118.65, 119.46, 119.58, 119.73, 119.79, 147.67, 149.17, 149.71, 150.10, 150.16, 150.21, 150.31, 170.97, 171.34, 171.41, 171.46, 171.59, 173.72, 174.33, 174.76, 175.39; MS (negative ion FAB, glycerol) m/z 780 (M-1).
**7β-[($N^4$-Succinamido-$N^1$,$N^8$-bis(2,3-dihydroxybenzoyl)spermidine)-[$N^2$-(2,3-dihydroxybenzoyl)]-L-lysylamino]-1-carba-3-chloro-3-cephem-(4-carboxylate) (8)** $FeCl_3$ positive (red-purple); IR (TF) 3520-2440 (br), 1760, 1670, 1650 cm$^{-1}$; $^1$H NMR (300 MHz, methanol-$d_4$) δ 1.20-2.00 (m, 12 H, $CH_2$ and C1 $CH_2$), 2.15-3.20 (m, 8 H, $CH_2$ and allylic $CH_2$), 3.30-3.50 (m, 10 H, $CH_2$), 3.90 (m, 1 H, C8 NCH$CH_2$), 4.55 (m, 1 H, NCHCO), 5.33 (m, 1 H, C7 NCHCO), 6.70 (m, 2 H, aromatic H), 6.95 (m, 3 H, aromatic H), 7.25 (m, 3 H, aromatic H), 7.37 (m, 1 H, aromatic H); $^{13}$C NMR (75 MHz, methanol-$d_4$, all signals at 25°C reported) δ 20.59, 24.20, 25.83, 26.95, 27.58, 28.38, 28.73, 29.43, 29.86, 31.67, 32.05, 32.10, 32.38, 33.30, 33.34, 35.43, 36.94, 37.66, 38.04, 39.94, 40.04, 44.35, 44.43, 46.50, 46.77, 53.87, 55.07, 59.60, 68.03, 74.51, 116.71, 116.74, 116.80, 116.83, 118.73, 118.78, 119.64, 119.94, 131.28, 147.04, 147.13, 149.64, 149.87, 150.15, 150.23, 164.72, 164.76, 165.12, 170.98, 171.40, 171.45, 174.73, 174.78, 174.96; MS (positive ion FAB, glycerol/*m*-nitrobenzyl alcohol) m/z 980 (M+1).
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15. Petri dish growth promotion assay procedure: Overnight Luria broth startup cultures were diluted 1:200,000 into sterile Luria agar containing 100 μg/mL of the iron chelator ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDA). An aliquot (5 μL) of the 5.0 mM or 1.0 mM siderophore stock solution (made with 2.8% aq. $NH_4OH$) was placed on sterile filter paper disks. The Petri dishes were incubated upside down at 37°C for 24 hours, then examined for circular zones of stimulation around the filter paper disks. All tests were performed in duplicate.
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