Biol Metals (1991) 4:62-69

BIOLOGY OF METALS

The design, synthesis and study of siderophore-antibiotic conjugates

Siderophore mediated drug transport

Marvin J. Miller, Julia A. McKee, Albert A. Minnick, and E. Kurt Dolence

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA

Summary. The use of conjugates of microbial iron chelators (siderophores) and antibiotics for illicit transport of antibiotics into cells is a potentially powerful method for the rational design of therapeutic agents. The structural complexity of most natural siderophores has impeded progress in this area. Described here are the design, syntheses and preliminary biological studies of several siderophore-β-lactam antibiotic conjugates. Both hydroxamic-acid-based and catechol-based conjugates with and without amino acid spacers to carbacephalosporins were synthesized and demonstrated to be effective inhibitors of *Escherichia coli* X580. Mutant selection was noted for each class of conjugates. Mutants selected from exposure of the *E. coli* to the hydroxamate conjugates were susceptible to the catechol conjugates and vice versa. Combinations of hydroxamate- and catechol-carbacephalosporin conjugates were most effective inhibitors of *E. coli* X580.

Key words: Siderophore - Iron transport - Antibiotic - β-Lactam

Introduction

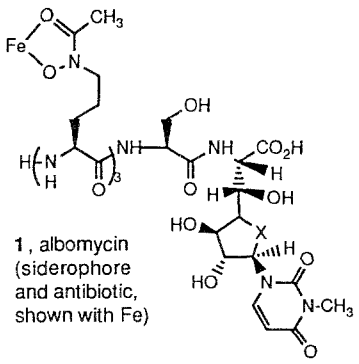
Antibiotic therapy is one of the greatest contributions of science to humanity. β-Lactam antibiotics, typically the penicillins and cephalosporins, represent some of the most effective antimicrobial agents ever discovered and developed. In fact, penicillin and related antibiotics have been credited with nearly half of the >20-year increase in the average person's lifespan since the 1920's (Christensen 1989). Extensive use of antibiotics has promoted the develoment of microbial resistance to them and threatens our therapeutic advantage. Of the several modes of resistance employed by microbes (Waxman and Strominger 1982), the permeability problem, or inability of some β-lactam antibiotics to enter the cell by the usual passive diffusion processes, is especially significant since these drugs can be effective only after they have entered the microbial cell. Thus, the development of methods to facilitate active transport of antibiotics into microbial cells is an important therapeutic goal. Conjugation of antibiotics to siderophores, which are natural iron chelators utilized by many microbes for sequestering and cellular transport of physiologically essential iron, is a conceptually attractive approach to the possible development of processes for active transport of antibiotics (Miller 1989). This paper describes our recent efforts to synthesize, characterize and study the biological properties of siderophore-β-lactam antibiotic conjugates.

Siderophore-antibiotic conjugates can be schematically represented with a simple chemical link between the two components as shown here. As will be illustrated, the link can be a simple direct

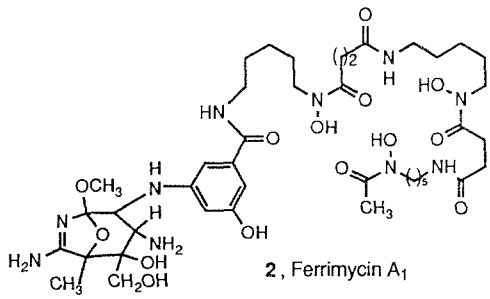
Siderophore —{Linker}— Drug

attachment of the drug to the siderophore or a spacer, typically an amino acid, may be used to bridge the drug with the siderophore. In fact, natural siderophore-antibiotic combinations have been discovered. Albomycin **(1)** and ferrimycin A_1 **(2)** both contain a trihydroxamic-acid-based iron-chelating component and a covalently attached antimicrobial agent that exerts its activity after being actively carried into the cell by the iron transport system (Neilands and Valenta 1985). Early semisynthetic attempts to prepare siderophore-antibiotic conjugates focused on derivatives of ferrioxamine B and ferricrocin, since, besides the iron-chelating hydroxamate groups, these siderophores contain a single additional functionality available for drug attachment. Thus, derivatization of the primary amine of ferrioxamine B and the primary hydroxyl group of ferricrocin produced sulfonamides **3** and **4**, of which only **4a, b** displayed minimal antimicrobial activity against *Staphylococcus aureus*. More recently, significant effort has been directed at modification of peripheral functionality of β-lactam antibiotics, especially cephalospo-

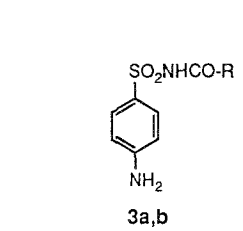
Offprint requests to: M. J. Miller



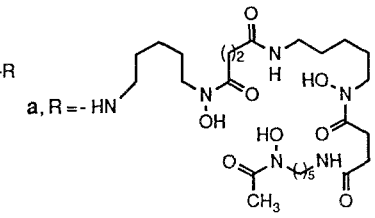
1, albomycin (siderophore and antibiotic, shown with Fe)



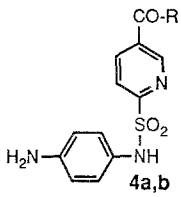
2, Ferrimycin A_1



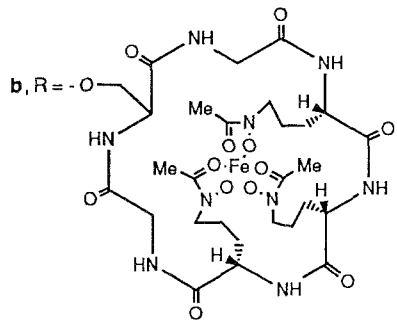
3a,b



a, R = - HN



4a,b



b, R = - O

ferricrocin (- = H)

rins, to incorporate catechol and even hydroxamate side chains with the anticipation that they will bind iron and utilize siderophore transport processes to enter microbial cells (Miller 1989; Silley et al. 1990). However, the structural complexity and multiple chemical functionality of most siderophores have severely limited the chemical preparation of siderophore-antibiotic conjugates. Consequently, no real structure/activity relationship studies have been possible. Efficient synthetic organic methods are now available so that the total synthesis of complex siderophores and antibiotic conjugates is now a feasible approach for exploring the potential for siderophore-mediated drug transport. Thus, we decided to initiate a synthetic program to test the concept of siderophore-mediated drug delivery.

Materials and methods

Biological testing procedure

Growth curve procedure. The preformed iron complexes of the respective siderophore conjugates **16a, b** were prepared by mixing equivalent volumes of stock solutions of the test compound and ferric chloride hexahydrate; the mixtures were added to sterile Luria broth, with and without ethylenediamine di-(*o*-hydroxyphenyl acetic acid) (EDDA), or to Müller-Hinton broth by filtration through an Acro-Disc 0.2-μm filter assembly to give solutions 10 μM in each of the conjugates. Immediately, 20 μL of a 25-h-old trypticase soy broth culture of *E. coli* X580 (Eli Lilly and Co.) was added. The culture flasks were then shaken at 37° C at 300 rpm. Aliquots were removed every 2 h and the culture turbidity measured at 600 nm. Since the spermidine-based conjugates **22** and **24a–d** are not soluble in water, they were predissolved in HPLC-grade dimethylformamide before addition to sterile Luria broth (with and without EDDA) to give either 1.0 mM or 10 mM stock solutions of the test compound. Aliquots (0.1 mL) of these stock solutions were added to the culture broth (100 mL) to give 1.0 μM and 10 μM final concentrations respectively. Immediately, 20 μL of a 23–25-h-old culture of *E. coli* in Luria broth was added to the Luria broth. All flasks were shaken at 300 rpm at 37° C. A sample (1 mL) of test solution was removed every 2 h and the turbidity of this aliquot was recorded at 600 nm.

General chemical methods. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 727B or Perkin-Elmer 1420 spectrophotometer (TF refers to thin films). ^{1}H-NMR spectra were obtained on a General Electric GN-300 spectrometer. Chemical shifts are reported relative to tetramethylsilane. ^{13}C-NMR spectra were obtained on a General Electric GN-300 spectrometer. Carbon NMR references were the center peak of deuterochloroform (77.0 ppm), the center peak of deuteroacetone (29.8 ppm), or the center peak of deuteromethanol (49.0 ppm). Electron-impact mass spectra, chemical-ionization mass spectra, and fast-atom-bombardment mass spectra were recorded on an AEI Scientific Apparatus MS 902 or Du Pont DP 102 spectrometer. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ). Radial chromatography was performed using a Chromatotron® model 7924 purchased from Harrison Research Inc. (Palo Alto, CA). Flash silica gel chromatography was performed using Merck silica gel 60. All organic solvents were dried and purified by standard methods (Gordon and Ford 1972). The term 'dried' refers to the drying of an organic solvent over anhydrous magnesium sulfate.

The synthesis of hydroxamate conjugates **16a, b** and catechol conjugate **22** have been described (Dolence et al. 1990; McKee, Sharma and Miller, unpublished results).

*7β-[(*N*-tert-Butoxycarbonyl)-D-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (19a).* To a suspension of **18** (0.500 g, 1.29 mmol, Eli Lilly and Co.) in 3 mL anhydrous methylene chloride under nitrogen was added triethylamine (0.131 g, 1.29 mmol). Compound **17a** (0.324 g, 1.21 mmol) and (EEDQ, 2-ethopy-1-ethopycarbonyl-1,2-dihydroquinoline); 0.414 g, 1.67 mmol) were added and the solution was stirred for 5 min, then 1 mL of anhydrous dimethylformamide was added. The solution was stirred for 18 h and then diluted with ethyl acetate and washed with 0.5 M-HCl, brine, dried, filtered, and concentrated. The product was purified by radial chromatography eluting with 50% ethyl acetate/50% hexanes to provide 0.549 g

(73%) of an off-white foam. The foam was crystallized from ethyl acetate/ether: mp 174–175° C; $[\alpha]_D^{27} = -30.5°$ ($c = 1.0$, $CHCl_3$); infrared (KBr) $\nu/cm^{-1} = 3320, 2970, 1770, 1720, 1670$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.40 (m, 10 H, C1 CHC$\underline{H}_2$ and C$(CH_3)_3$), 1.70 (m, 1 H, C1 C$\underline{H}$CH$_2$), 2.45–2.58 (m, 2 H, allylic CH_2), 3.79–3.86 (m, 1 H, C$\underline{H}$CH$_2$), 5.27–5.43 (m, 4 H, benzylic NC$\underline{H}$CO, NC$\underline{H}$CO, and benzylic H), 5.83 (d, $J = 6.6$ Hz, 1 H, NH), 7.25–7.40 (m, 5 H, aromatic H), 7.52 (br s, 1 H, NH), 7.57 (d, $J = 8.7$ Hz, 2 H, aroamtic H), 8.16 (d, $J = 8.7$ Hz, 2 H, aromatic H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ/ppm = 21.46, 28.19, 31.59, 52.45, 58.41, 66.14, 80.26, 122.83, 123.62, 126.91, 128.38, 128.71, 128.85, 131.58, 137.76, 142.10, 147.72, 154.90, 159.80, 164.70, 171.10; MS (positive-ion FAB, glycerol) $m/z = 586$ (M + 1), 530 (M + 1-*t*Bu). Anal. calcd for $C_{28}H_{29}N_4O_8Cl$: C, 57.49; H, 5.00; N, 9.58. Found C, 57.25; H, 5.12; N, 9.51.

*7β-[(*N-tert-*Butoxycarbonyl)-4-hydroxy-D-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (19b)* was prepared from **18** (0.5 g, 1.29 mmol) and **17b** (0.344 g, 1.29 mmol) in the same manner as **19a.** The resulting oil was purified by radial chromatography eluting with 50% ethyl acetate/50% hexanes to provide 0.494 g (64%) of **19b** as a yellow foam. The product resisted all attempts at crystallization: $[\alpha]_D^{25} = 8.0°$ ($c = 1.0$, $CHCl_3$); infrared (KBr) $\nu/cm^{-1} = 3380$ (br), 1770, 1670; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.39 (m, 10 H, CHC$\underline{H}_2$ and C$(CH_3)_3$), 1.75 (m, 1 H, CHC$\underline{H}_2$), 2.45–2.60 (m, 2 H, allylic CH_2), 3.75–3.90 (m, 1 H, C$\underline{H}$CH$_2$), 5.15 (br s, 1 H, benzylic NC$\underline{H}$CO), 5.24–5.42 (m, 3 H, benzylic H and NC$\underline{H}$CO), 5.80 (d, $J = 5.7$ Hz, 1 H, NH), 6.60 (d, $J = 8.1$ Hz, 2 H, aromatic H), 7.09 (d, $J = 8.1$ Hz, 2 H, aromatic H), 7.54 (m, 3 H, aromatic H and NH), 8.14 (d, $J = 8.7$ Hz, 2 H, aromatic H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ/ppm = 21.60, 28.18, 31.60, 52.50, 58.25, 66.17, 77.29, 80.65, 115.86, 122.67, 123.61, 126.94, 128.35, 128.68, 132.04, 142.09, 147.61, 155.24, 156.62, 159.87, 164.88, 171.93; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol) $m/z = 601$ (M + 1), 545 (M + 1-*t*Bu). Anal. calcd for $C_{28}H_{29}N_4O_9Cl$: C, 55.86; H, 5.02; N, 9.31. Found: C, 55.76; H, 5.11; N, 9.08.

*7β-[(*N-tert-*Butoxycarbonyl)-L-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (19c)* was synthesized in the same manner as **19a** in 83% yield after crystallization from ethyl acetate/ether: mp 182–183° C; $[\alpha]_D^{27} = +46.6°$ ($c = 1.0$, $CHCl_3$); infrared (KBr) $\gamma/cm^{-1} = 3310$, 2980, 1770, 1670 (br), 1520; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.40 (m, 10 H, CHC$\underline{H}_2$ and C$(CH_3)_3$), 1.55–2.00 (m, 2 H, CHC$\underline{H}_2$), 2.55–2.70 (m, 2 H, allylic CH_2), 3.80–3.87 (m, 1 H, C$\underline{H}$CH$_2$), 5.09 (t, $J = 5.4$ Hz, 1 H, C7 NC$\underline{H}$CO), 5.18 (m, 1 H, benzylic NC$\underline{H}$CO), 5.34 (ABq, $J = 13.2$ Hz, 2 H, benzylic H), 5.70 (d, $J = 7.2$ Hz, 1 H, NH), 7.26 (s, 5 H, aromatic H), 7.58 (d, $J = 9.0$ Hz, 2 H, aromatic H), 8.17 (d, $J = 8.7$ Hz, 2 H, aromatic H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ/ppm = 21.83, 28.18, 31.66, 52.53, 58.31, 58.68, 66.16, 80.15, 122.70, 123.60, 127.02, 128.45, 128.79, 128.86, 132.00, 136.87, 142.09, 147.73, 155.00, 159.78, 164.81, 171.30; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol) $m/z = 586$ (M + 1), 530 (M + 1-*t*Bu). Anal. calcd for $C_{28}H_{29}N_4O_8Cl$; C, 57.49; H, 5.00; N, 9.58. Found C, 57.41; H, 5.11; N, 9.39.

*7β-[(*N-tert-*Butoxycarbonyl)-4-hydroxyl-L-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (19d)* was synthesized in the same manner as **19a.** After purification by radial chromatography eluting with 50% ethyl acetate/50% hexanes, compound **19d** was obtained in 64% yield as a yellow foam. The foam was crystallized from ethyl acetate/ether to give a white solid: mp 155–157° C; $[\alpha]_D^{25} = +34.9°$ ($c = 1.99$, $CHCl_3$); infrared (KBr) $\nu/cm^{-1} = 3340$, 1770, 1680, 1520; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.40 (s, 9 H, C$(CH_3)_3$), 1.60–1.90 (m, 2 H, CHC$\underline{H}_2$), 2.59 (m, 2 H, allylic CH_2), 3.60–3.90 (m, 1 H, C$\underline{H}$CH$_2$), 5.03 (m, 1 H, benzylic NC$\underline{H}$CO), 5.18 (t, $J = 5.4$ Hz, 1 H, NC$\underline{H}$CO), 5.33 (AB quartet, $J = 13.5$ Hz, 2 H, benzylic H), 5.88 (br s, 1 H, NH), 6.59 (d, $J = 8.1$ Hz, 2 H, aromatic H), 7.00 (d, $J = 8.1$ Hz, 2 H, aromatic H), 7.55 (d, $J = 8.4$ Hz, 2 H, aromatic H), 7.65 (br s, 1 H, NH), 7.85 (br s, 1 H, OH), 8.13 (d, $J = 8.7$ Hz, 2 H, aromatic H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ/ppm = 21.82, 28.22, 31.67, 52.59, 57.79, 58.45, 66.23, 80.37, 115.86, 122.51, 123.62, 127.79, 128.31, 128.80, 132.58, 142.07, 147.63, 155.30, 156.50, 159.82, 165.18, 172.17; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol) $m/z = 601$ (M + 1), 545 (M + 1-*t*Bu). Anal. calcd. for $C_{28}H_{29}N_4O_9Cl$: C, 55.86; H, 5.02; N, 9.31. Found C, 55.63; H, 5.23; N, 9.12.

*7β-[[*N^4-*Succinamido*-N^1,N^8-*bis(2,3-dibenzyloxybenzoyl)spermidine]-D-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (23a).* To a solution of compound **19a** (0.250 g, 0.427 mmol) in 1.0 mL of anhydrous methylene chloride under nitrogen at 0° C was added 1.5 mL of trifluoroacetic acid; the resulting solution was stirred for 1.5 h at which time TLC showed no remaining starting material. The solution was concentrated and any remaining trifluoroacetic acid was removed by repeated evaporations from benzene and hexanes. The residue was dissolved in 10 mL of water and the *N*-hydroxysuccinimide active ester of **20b** (0.475 g, 0.487 mmol) in 10 mL of ethyl acetate was added, quickly followed by $KHCO_3$ (0.128 g, 1.28 mmol). The mixture was stirred for 20 h. The solution was diluted with ethyl acetate and washed with 5% $KHCO_3$, water, brine, dried, filtered and concentrated to afford 0.617 g of an off-white foam. The conjugate **23a** was purified by radial chromatography on a 4-mm plate, eluting with methanol/$CHCl_3$ (1:50), then methanol/$CHCl_3$ (1:20) to provide 0.449 g (78%) of a white foam: mp 69–72° C; $[\alpha]_D^{25} = -24.9°$ ($c = 1.1$, $CHCl_3$); infrared (KBr) $\nu/cm^{-1} = 3380$, 1775, 1735, 1650 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.20–1.60 (m, 7 H, CH_2), 1.75 (m, 1 H, CH_2), 2.20–2.90 (m, 6 H, CH_2, including allylic CH_2), 2.90–3.40 (m, 7 H, CH_2), 3.55 (m, 1 H, CH_2), 3.81 (m, 1 H, CH_2C$\underline{H}$), 5.00–5.19 (m, 8 H, benzylic H), 5.20–5.40 (m, 4 H, benzylic H, benzylic NC$\underline{H}$CO, and NC$\underline{H}$CO), 5.49 (d, $J = 6.9$ Hz, 1 H, NH), 6.85–7.60 (m, 32 H, aromatic H), 7.60–7.68 (m, 1 H, aromatic H), 7.92–8.15 (m, 4 H, aromatic and NH), 8.57 (m, 1 H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, all signals at 25° C reported) δ/ppm = 21.33, 24.80, 26.48, 26.57, 27.41, 28.51, 28.71, 28.83, 31.68, 31.70, 31.79, 36.80, 37.24, 38.79, 42.90, 44.57, 45.08, 47.06, 52.65, 58.03, 58.83, 65.95, 66.03, 71.02, 71.16, 76.05, 76.17, 76.31, 76.41, 116.57, 116.90, 117.00, 122.76, 123.04, 123.08, 124.21, 124.26, 124.33, 126.82, 126.99, 127.53, 127.57, 127.72, 127.76, 127.92, 128.11, 128.16, 128.25, 128.45, 128.56, 128.63, 128.77, 128.01, 130.27, 130.60, 136.25, 136.36, 136.39, 136.87, 136.89, 142.17, 146.35, 146.40, 146.68, 147.53, 147.58, 151.59, 159.90, 160.01, 164.84, 165.10, 165.21, 165.28, 171.08, 171.13, 171.76, 172.05, 172.26, 172.33; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol/dimethylformamide) $m/z = 1345$ (M + 1). Anal. calcd for $C_{76}H_{74}N_7O_{14}Cl$: C, 67.87 H, 5.55; N, 7.29. Found C, 68.01; H, 5.51; N, 7.29.

*7β-[[*N^4-*Succinamido*-N^1,N^8-*bis(2,3-dibenzyloxybenzoyl)spermidine]-4-hydroxy-D-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (23b).* To a solution of **19b** (0.175 g, 0.291 mmol) in 1.0 mL of anhydrous methylene chloride under nitrogen was added 1.0 mL of trifluoroacetic acid and the solution was stirred at room temperature for 1 h. The solution was concentrated and any excess trifluoroacetic acid was removed by repeatedly evaporating with benzene and hexanes. The oily residue was suspended in 1.0 mL of anhydrous methylene chloride under nitrogen. Triethylamine (46 mL, 0.330 mmol) was added, quickly followed by **20b** (0.256 g, 0.292 mmol) and EEDQ (0.072 g, 0.292 mmol). The solution was stirred for 36 h, then diluted with 100 ml of ethyl acetate. The solution was washed with three portions of 1 M HCl, water, brine, dried, filtered and concentrated to give 0.391 g of a yellow foam. The product was purified by radial chromatography on a 2-mm plate, eluting with methanol/ethyl acetate (1:30) to provide 0.158 g (40%) of an off-white foam: mp 100–102° C; $[\alpha]_D^{25} = -23.6°$ ($c = 0.47$, $CHCl_3$); infrared (KBr) $\nu/cm^{-1} = 3330$, 1770, 1730, 1645 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.20–1.60 (m, 7 H, CH_2), 1.75 (m, 1 H, C1 CH_2), 2.20–2.60 (m, 6 H, CH_2 including allylic CH_2), 2.65–3.60 (m, 8 H, CH_2), 3.75 (m, 1 H, CH_2C$\underline{H}$), 5.00–5.15 (m, 8 H, benzylic H), 5.16–

5.40 (m, 5 H, benzylic H, NC<u>H</u>CO, N<u>H</u>, and benzylic NC<u>H</u>CO), 6.65 (m, 2 H, aromatic H), 7.00–7.15 (m, 4 H, aromatic H), 7.20–7.70 (m, 24 H, aromatic H), 7.95–8.15 (m, 4 H, aromatic H and NH), 8.35–8.50 (m, 1 H, NH), 8.62 and 8.56 (two s, 1 H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, all signals at 25° C reported) δ/ppm = 21.32, 25.82, 26.55, 27.35, 28.60, 31.27, 31.69, 37.36, 38.92, 39.34, 42.96, 42.98, 45.05, 45.24, 47.01, 47.05, 52.61, 57.42, 57.48, 58.77, 65.92, 66.00, 71.09, 71.17, 76.09, 76.18, 76.33, 76.42, 115.76, 116.79, 116.83, 117.05, 117.09, 117.13, 122.76, 122.95, 123.00, 124.35, 124.39, 126.84, 126.14, 127.54, 127.57, 127.74, 128.15, 128.47, 128.53, 128.55, 128.61, 128.63, 128.70, 128.74, 130.48, 130.87, 136.31, 136.34, 142.17, 146.74 (m), 147.56, 151.58, 151.61, 157.08, 159.86, 159.97, 164.90, 165.42 (m), 171.72, 171.93, 172.50, 172.57; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol) m/z = 1360 (M + 1). Anal. calcd for $C_{76}H_{74}N_7O_{15}Cl$: C, 67.08; H, 5.48; N, 7.20. Found C, 66.76; H, 5.52; N, 6.88.

7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dibenzyloxybenzoyl)spermidine]-L-phenylglycylamino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl) carboxylate] (23c) was synthesized in the same manner as **23a** to afford 0.607 g of a yellow oil. The oil was purified by radial chromatography on a 4-mm plate, eluting successively with $CHCl_3$, methanol/$CHCl_3$ (1:50), then methanol/$CHCl_3$ (1:20) to provide 0.357 g (62%) of a light yellow foam: mp 71–73° C; $[\alpha]_D^{25} = +14.6°$ (c = 0.82, $CHCl_3$); infrared (TF) ν/cm^{-1} = 3400–3275 (br), 1775, 1730, 1650, 1575 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.15–1.65 (m, 6 H, CH_2), 1.70–1.95 (m, 2 H, CHC<u>H</u>$_2$), 2.30–2.75 (m, 6 H, CH_2, including allylic CH_2), 2.78–3.50 (m, 8 H, CH_2), 3.75 (m, 1 H, CH_2C<u>H</u>), 4.98–5.37 (m, 11 H, benzylic H and C7 NC<u>H</u>CO), 5.50 (dd, J = 4.8 and 7.2 Hz, 1 H, benzylic NC<u>H</u>CO), 6.80–6.95 (m, 1 H, NH), 7.05–7.15 (m, 4 H, aromatic H), 7.25–7.70 (m, 28 H, aromatic H), 7.95–8.05 (m, 1 H, NH), 8.07–8.30 (m, 4 H, aromatic and NH); ^{13}C-NMR (75 MHz, $CDCl_3$, all signals at 25° C reported) δ/ppm = 21.72, 21.79, 24.84, 25.69, 26.50, 26.57, 27.27, 28.52, 28.66, 31.51, 31.59, 31.62, 36.93, 37.18, 38.87, 39.23, 42.92, 45.92, 45.07, 45.31, 47.05, 52.34, 57.76, 57.86, 58.50, 58.57, 66.00, 71.05, 71.13, 76.15, 76.21, 76.30, 76.40, 116.60, 116.70, 116.86, 116.90, 122.80, 122.98, 123.01, 123.54, 124.34, 126.84, 127.03, 127.55, 127.59, 128.17, 128.30, 128.47, 128.57, 128.61, 128.69, 128.70, 128.81, 128.84, 130.48, 136.23, 136.33, 136.39, 136.74, 142.13, 146.45, 146.68, 147.55, 147.57, 151.56, 151.62, 159.97, 164.78, 164.98, 165.14, 165.25, 170.97, 171.86, 171.86, 171.99, 172.17, 172.21; MS (positive-ion FAB, *m*-nitrobenzyl alcohol/glycerol/chloroform) m/z = 1345 (M + 1). Anal. calcd for $C_{76}H_{74}N_7O_{14}Cl \cdot 2H_2O$ C, 66.10; H, 5.69; N, 7.10. Found: C, 65.83; H, 5.53; N, 7.09.

7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dibenzyloxybenzoyl)spermidine]-4-hydroxy-L-phenylglycyl-amino]-1-carba-3-chloro-3-cephem-[4-(4-nitrobenzyl)carboxylate] (23d) was synthesized in the same manmer as **23b** in 30% yield after chromatography to give a white foam: mp = 91–93° C; $[\alpha]_D^{25} = +19.8°$ (c = 0.94, $CHCl_3$); infrared (KBr) ν/cm^{-1} = 3340, 1775, 1730, 1640; ^{1}H-NMR (300 MHz, $CDCl_3$) δ/ppm = 1.20–1.60 (m, 7 H, CH_2), 1.70–1.90 (m, 2 H, CHC<u>H</u>$_2$), 2.45–2.60 (m, 5 H, CH_2 including allylic CH_2), 2.80–3.35 (m, 8 H, CH_2), 3.70 (m, 1 H, CH_2C<u>H</u>), 5.00–5.30 (m, 12 H, benzylic H, C7 NC<u>H</u>CO, and benzylic NC<u>H</u>CO), 5.42 (m, 1 H, NH), 6.68 (d, J = 8.1 Hz, 2 H, aromatic H), 7.00–7.20 (m, 4 H, aromatic H), 7.25–7.50 (m, 22 H, aromatic H), 7.55 (m, 2 H, aromatic H), 7.95–8.10 (m, 4 H, aromatic H and NH), 8.36 (br s, 1 H, NH), 8.77 (br s, 1 H, NH); ^{13}C-NMR (75 MHz, $CDCl_3$, all signals at 25° C reported) δ/ppm = 21.73, 25.76, 26.43, 26.55, 27.31, 28.37, 31.06, 31.09, 31.65, 37.04, 37.23, 39.03, 39.32, 43.13, 45.33, 47.11, 52.38, 57.10, 58.50, 65.93, 71.14, 76.14, 76.26, 76.34, 76.42, 115.80, 116.90, 116.98, 117.04, 122.76, 122.97, 123.50, 124.33, 126.76, 126.86, 127.54, 127.57, 128.10, 128.14, 128.47, 128.55, 158.60, 128.69, 131.05, 131.13, 136.27, 136.30, 142.13, 146.68 (m), 151.59, 151.63, 157.13, 159.88, 164.96, 165.26, 165.42, 171.56, 171.61, 171.80, 172.37; MS (positive ion FAB, *m*-nitrobenzyl alcohol/glycerol) m/z = 1360 (M + 1). Anal. calcd for $C_{76}H_{74}N_7O_{15}Cl$: C, 67.08; H, 5.48; N, 7.20. Found C, 66.86; H, 5.58; N, 7.13.

7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine]-D-phenylglycylamino]-1-carba-3-chloro-3-cephem-4-carboxylic acid (24a). To a solution of **23a** (0.154 g, 0.1145 mmol) in 1.0 mL of HPLC-grade dimethylformamide and 50 mL of distilled deionized water was added 30 μL of concentrated HCl (300 mol%) and 0.031 g of 10% palladium on carbon. This mixture was exposed to hydrogen at atmospheric pressure for 42 h. The catalyst was removed by filtration and the solvents were removed under high vacuum. The amber residue was repeatedly dissolved in methanol and evaporated in an attempt to remove the residual dimethylformamide but ^{1}H-NMR still showed traces despite this attempt to remove it. The product was obtained as an amber oil in 100% yield: infrared (TF) ν/cm^{-1} = 2980 (br), 1765, 1650, 1540; ^{1}H-NMR (300 MHz, CD_3OD) δ/ppm = 1.50–2.00 (m, 8 H, CH_2), 2.50–3.10 (m, 6 H, CH_2, allylic H obscured by residual dimethylformamide), 3.35–3.50 (m, 8 H, CH_2), 3.82 (m, 1 H, CH_2C<u>H</u>), 5.38 (m, 1 H, NC<u>H</u>CO), 5.50 (m, 1 H, benzylic NC<u>H</u>CO), 6.72 (m, 2 H, aromatic H), 6.97 (d, J = 8.1 Hz, 2 H, aromatic H), 7.30 (m, 1 H, NH), 7.20–7.55 (m, 7 H, aromatic H); ^{13}C-NMR (75 MHz, CD_3OD, all signals at 25° C reported) δ/ppm = 20.91, 22.88, 25.92, 26.97, 27.52, 28.40, 28.67, 29.39, 29.56, 31.86, 32.22, 33.37, 37.81, 37.95, 38.80, 40.03, 44.37, 46.59, 46.64, 53.83, 59.24, 59.54, 59.62, 67.95, 116.64, 118.73, 119.63, 119.68, 121.06, 123.82, 125.72, 125.76, 128.89, 128.94, 129.39, 129.76, 130.25, 131.20, 131.45, 138.09, 138.25, 140.11, 147.10, 150.23, 165.42, 166.15, 171.40 (m), 173.29, 173.72, 174.14, 174.93. The product gave a positive ferric chloride test; MS (positive-ion FAB, *m*-nitrobenzyl alcohol/glycerol) m/z = 849 (M + 1).

7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine]-4-hydroxy-D-phenylglycyl-amino]-1-carba-3-chloro-3-cephem-4-carboxylic acid (24b) was obtained from **23b** (0.130 g, 0.0955 mmol) in the same manner as **24a** as an amber oil in 100% yield: infrared (TF) ν/cm^{-1} = 3000 (br), 2450, 1765, 1650, 1550; ^{1}H-NMR (300 MHz, CD_3OD) δ/ppm = 1.50–1.95 (m, 8 H, CH_2), 2.45–3.05 (m, 6 H, CH_2, allylic CH_2 obscured by residual dimethylformamide), 3.40–3.50 (m, 8 H, CH_2), 3.80 (m, 1 H, CH_2C<u>H</u>), 5.25–5.40 (m, 2 H, NC<u>H</u>CO and benzylic NC<u>H</u>CO), 6.65–6.80 (m, 4 H, aromatic H), 6.96 (d, J = 7.8 Hz, 2 H, aromatic H), 7.20–7.40 (m, 4 H, aromatic H); ^{13}C-NMR (75 MHz, CD_3OD, all signals at 25° C reported) δ/ppm = 20.88, 20.95, 22.92, 25.94, 25.98, 26.98, 27.55, 28.34, 28.40, 29.11, 29.37, 29.61, 31.83, 32.29, 37.84, 37.97, 39.95, 39.83, 40.06, 44.37, 44.51, 45.44, 46.72, 52.14, 53.89, 54.18, 58.84, 58.97, 59.66, 67.95, 116.57, 116.68, 118.74, 119.69, 121.08, 122.86, 123.87, 125.55, 130.26, 130.30, 131.52, 147.12, 150.21, 150.28, 158.70, 164.77, 165.46, 166.25, 171.43 (m), 173.29, 173.82 (m), 174.14, 174.96 (m). The product gave a positive ferric chloride test; MS (positive-ion FAB, *m*-nitrobenzyl alcohol/glycerol/acetic acid) m/z = 865 (M + 1).

7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine]-L-phenylglycylamino]-1-carba-3-chloro-3-cephem-4-carboxylic acid (24c) was obtained from **23c** in the same manner as **24a** as an amber oil in 100% yield: infrared (TF) ν/cm^{-1} = 2900 (br), 1760, 1650, 1540 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) δ/ppm = 1.50–2.10 (m, 8 H, CH_2), 2.40–3.00 (m, 6 H, CH_2, allylic H obscured by residual dimethylformamide), 3.20–3.50 (m, 8 H, CH_2), 3.80 (m, 1 H, CH_2C<u>H</u>), 5.25–5.37 (m, 1 H, NC<u>H</u>CO), 5.45 (br s, 1 H, benzylic NC<u>H</u>CO), 6.65–6.77 (m, 2 H, aromatic H), 6.95–7.00 (m, 2 H, aromatic H), 7.20–7.50 (m, 7 H, aromatic H); ^{13}C-NMR (75 MHz, CD_3OD, all signals at 25° C reported) δ/ppm = 20.90, 23.18, 25.94, 26.88, 27.50, 27.71, 28.34, 28.74, 29.43, 29.45, 31.79, 31.90, 33.35, 37.80, 37.95, 39.84, 40.10, 44.36, 45.40, 46.50, 51.99, 53.84, 59.14, 59.42, 67.94, 116.68, 118.74, 119.67, 121.06, 126.28, 127.54, 128.89, 128.93, 129.36, 129.76, 130.26, 130.37, 131.31, 138.05, 147.13, 150.21, 150.31, 164.74, 165.17, 166.14, 167.20, 171.38 (m), 173.20, 173.70, 174.19, 174.80, 174.89. The product gave a positive ferric chloride test; MS (positive-ion FAB, *m*-nitrobenzyl alcohol/glycerol) m/z = 849 (M + 1).

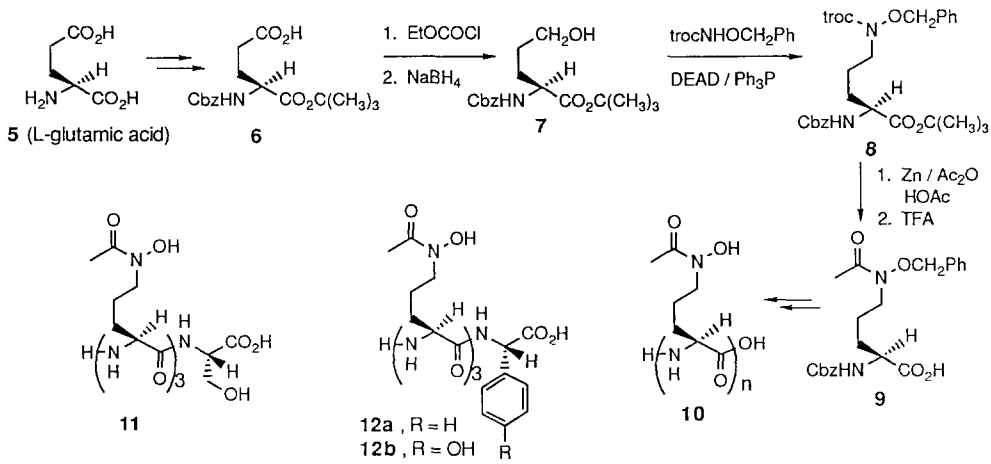
7β-[[N^4-Succinamido-N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine]-4-hydroxy-L-phenylglycyl-amino]-1-carba-3-chloro-3-cephem-4-car-

boxylic acid (24d) was prepared from **23b** in the same manner as compound **24b**. The product was obtained as an amber oil in 100% yield: infrared (TF) $\nu/cm^{-1} = 3000$ (br), 2450, 1755, 1590, 1540; ^{1}H-NMR (300 MHz, CD_3OD) δ/ppm = 1.50–2.10 (m, 8 H, CH_2), 2.20–3.10 (m, 6 H, CH_2, allylic CH_2 obscured by residual dimethylformamide), 3.20–3.55 (m, 8 H, CH_2), 3.75 (m, 1 H, CH_2C<u>H</u>), 5.30 (m, 2 H, NC<u>H</u>CO and benzylic NC<u>H</u>CO), 6.60–6.90 (m, 4 H, aromatic), 6.95 (m, 2 H, aromatic H), 7.24 (m, 4 H, aromatic H); ^{13}C-NMR 75 MHz, CD_3OD, all signals at 25° C reported) δ/ppm = 20.86, 23.25, 25.48, 25.96, 26.82, 27.53, 27.65, 28.34, 28.74, 29.45, 31.80, 31.88, 33.34, 37.85, 37.97, 39.88, 40.12, 44.39, 45.42, 46.57, 53.90, 54.24, 58.82, 58.87, 59.44, 67.98, 116.58, 116.74, 118.78, 119.70, 122.28, 122.30, 125.56, 128.12, 128.67, 129.46, 130.25, 131.27, 147.15, 150.18, 150.123, 150.29, 158.69, 164.78, 165.21, 166.13, 171.40 (m), 173.76, 174.25, 174.83, 174.88; The product gave a positive ferric chloride test; MS (positive-ion FAB, *m*-nitrobenzyl alcohol/glycerol/acetic acid) $m/z = 865$ (M + 1).

Results and discussion

Because albomycin is a superb natural example of the combination of a siderophore and antimicrobial agent, we initiated our synthetic efforts with the synthesis of the constituent iron binding amino acids and peptide component of albomycin. This effort was aided by the recent unambiguous determination of the structure of albomycin by Benz (Benz et al. 1982) and their subsequent chemical, enzymatic and synthetic work. Our synthesis of the required chelating amino acid N^5-acetyl-N^5-hydroxy-L-ornithine, **10** ($n = 1$), and related peptides required careful synthetic elaboration from L-glutamic acid under nonracemizing conditions as shown in Scheme 1 (Miller 1989; Dolence et al. 1990). In order to determine the minimal structure required for microbial iron transport activity, the constituent amino acid **10** ($n = 1$), the corresponding dipeptide **10** ($n = 2$) and tripeptide **10** ($n = 3$) were tested for their ability to serve as microbial transport agents. Of these compounds, only the tripeptide iron complex was an effective siderophore for selected strains of *E. coli* and *Shigella flexneri* under iron-deficient conditions, indicating the apparent need for an effective hexadentate iron chelator in this hydroxamate-based series. Subsequent elaboration to tetrapeptides **11, 12a, 12b** and larger peptides indicated that a considerable variety of carboxy-terminal extensions of the essential chelating tripeptide **10** ($n = 1$) could be tolerated in the microbial transport system (Miller 1989; Dolence et al. 1991). These encouraging results prompted us to pursue the goal of attaching an antibiotic to the tri-N^5-acetyl-N^5-hydroxy-L-ornithinyl peptide framework.

The choice of an antibiotic for the conjugates was determined by the need for a suitable functional group to be attached to the C-terminal carboxyl group of the tripeptide and the compatibility of all of the functional groups with the synthetic manipulations required. Carbacephalosporin derivatives were an ideal choice. Carbacephalosporins (forms of **13**) are effective synthetic β-lactam antibiotics (Kiyoshi and Okachi 1989; Bodurow et al. 1989) which contain a peripheral amino group for direct coupling to the carboxyl group of tripeptide **10** ($n = 3$). One synthetic advantage of these compounds is that, in contrast to the more classical penicillins and cephalosporins, the carbacephalosporins do not contain a sulfur which was anticipated to cause catalyst poisoning during planned hydrogenolytic deprotection of the final siderophore-antibiotic conjugates. The syntheses of the conjugates are summarized in Scheme 2 (Dolence et al. 1990). As indicated in Fig. 1, incubation of the preformed iron complexes of conjugates **16a, b** with β-lactam-hypersensitive *E. coli* X580 resulted in significant delay of the observed microbial growth (Dolence et al. 1990). The eventual growth noted after extended periods required further study. We were initially concerned that the antibiotic conjugates might not be stable under the incubation conditions due to dissociation of the conjugate or to hydrolysis of the important β-lactam. However, preincubation of the conjugates in the fermentation broth for over 24 h before microbial inoculation provided identical results, demonstrating compound stability. Subsequent isolation and study of the bacteria that did eventually populate the medium indicated that a mutant of the parent strain had been selected. Reincubation of these survivors with the same conjugates **(16a, b)** produced no delayed growth, indicating that the selected bacteria were resistant to the antibiotic conjugates. In a separate study, the growth of these selected bacteria un-



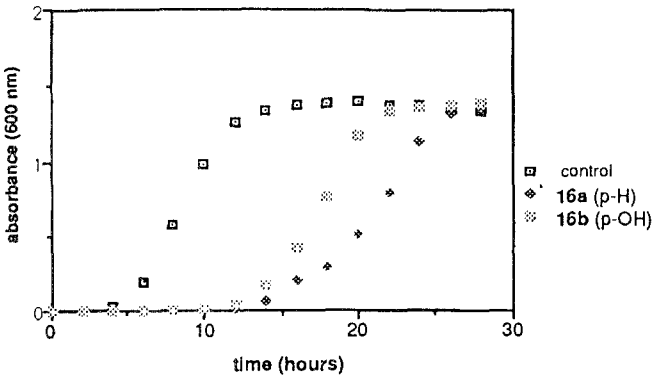


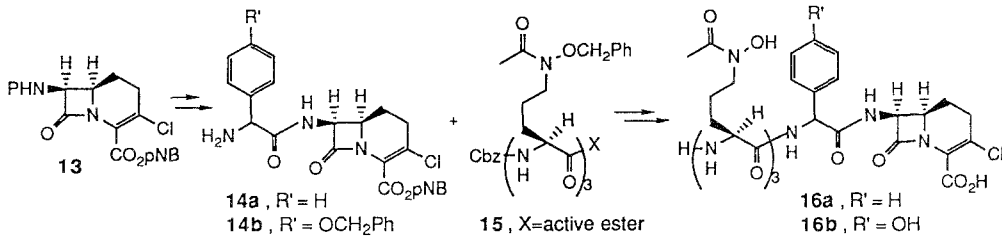
Fig. 1. The effect of the preformed Fe(III) complexes of siderophore-carbacephalosporin conjugates **16a** and **16b** (10 μM) in Müller-Hinton broth on the growth rate of *E. coli* X580

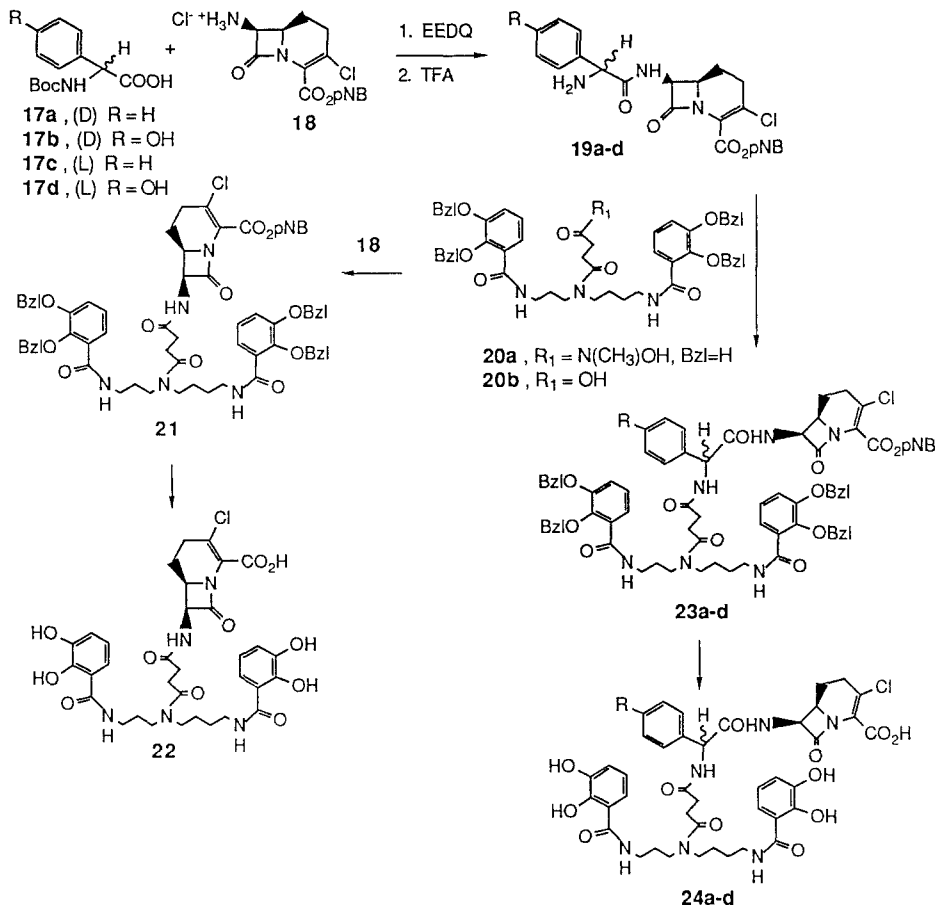
der iron-deficient conditions could not be stimulated by addition of ferrichrome, indicating that they no longer utilized the trihydroxamate-based iron transport system and needed to rely on other processes for iron assimilation. Thus, it appeared that the parent *E. coli* X580 recognized the hydroxamate-based conjugates, transported them into the cells and then were susceptible to the attached antibiotic components. Whether the antibiotics were released intracellularly has not yet been determined. Studies of the outer-membrane profiles of the mutants have been initiated.

To help verify that the conjugates indeed had utilized the trihydroxamate-based iron transport system to deliver the antibiotic, we decided to synthesize and test catechol-based antibiotic conjugates **22** and **24a–d** (Scheme 3). Such conjugates were anticipated to exert antimicrobial activity against the parent strain of *E. coli* and the mutants selected from exposure to the hydroxamate-antibiotic conjugates **16a, b** since they might rely on an alternative catechol-based iron transport system. The design of the catechol conjugates was based on our previous design, syntheses, and biological evaluation of the spermexatols **20a** (Sharma et al. 1989). Spermexatols contain two catechols and one hydroxamate group which serve as a mixed ligand for binding iron. They were shown to be effective spermidine-based analogues of the natural siderophores agrobactin, parabactin and vibriobactin by substituting for the natural siderophores in iron-transport-deficient mutants of *Vibrio cholerae* and *E. coli*. The design of catechol conjugates **22** and **24a–d** relied on the attachment of the antibiotic component to the central portion of the spermidine base. Thus, these initial conjugates formally contained only two bidentate ligands and we were concerned about their ability to complex and transport iron with 1:1 stoichiometry.

Three classes of spermidine-based catechol-carbacephalosporin conjugates were prepared and studied. In the first instance, carbacephalosporin **18** was directly attached to the dipodal chelator **20b** to give **22** (McKee, Sharma and Miller, unpublished results). The other classes of compounds incorporated D- or L-phenylglycine or D- or L-*p*-hydroxyphenylglycine spacers as introduced in structures **19a–d.** The synthesis of conjugates **24a–d** have not been reported previously, but closely follow the synthesis of the spermexatols. Thus, as in the synthesis of the directly linked conjugate **22,** the free carboxyl group of tetrabenzyl-protected intermediate **20b** (Sharma et al. 1989) was directly coupled to the free amino group of the phenylglycyl or *p*-hydroxyphenylglycyl carbacephalosporin *p*-nitrobenzyl esters **(19a–d)** to give the fully protected conjugates **23a–d** by standard peptide methodology described in Methods. Interestingly, the resulting fully protected conjugates displayed unusual NMR spectral characteristics, notably substantial doubling of peaks in both the ^{1}H and ^{13}C spectra. Fearing that the cephalosporin or amino acid components may have racemized during or after the coupling reaction, the protected conjugates were subjected to careful HPLC analysis which proved them to be diastereomerically pure. Since we had noted similar trends due to restricted conformational isomerism in related work on perbenzyl-protected catechol systems, we decided to deprotect the coupled products to the desired free conjugates and determine their diastereomeric integrity at the final stage. The same careful conditions used for deprotection of the hydroxamate-carbacephalosporin conjugates (**16a, b,** Dolence et al. 1990) was again effective for the deprotection of **23a–d** to the final test compounds **24a–d,** which were clearly diastereomerically pure as shown chromatographically and by both ^{1}H and ^{13}C NMR analysis.

The activity of the catechol-carbacephalosporin conjugates **22** and **24a–d** against our test strain of *E. coli* X580 is summarized by the representative data shown for compound **22** in Fig. 2. In the initial studies with conjugate **22,** with the direct linkage of the spermidine-based biscatechol to the carbacephalosporin, no attempt was made to deferrate the media. As with the hydroxamate-based antibiotic conjugates **16a, b,** incubation of conjugate **22** with the *E. coli* resulted in a delay in observed growth compared to the control; reincubation of **22** with the bacteria that eventually did





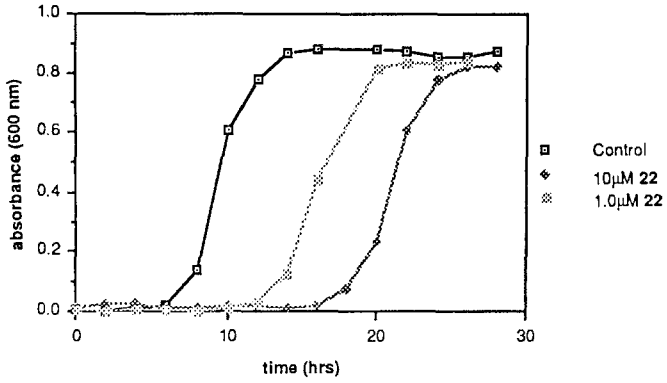


Fig. 2. The effect of catechol siderophore-carbacephalosporin conjugate **22** (1.0 μM and 10 μM) on the growth of *E. coli* X580 in Luria broth

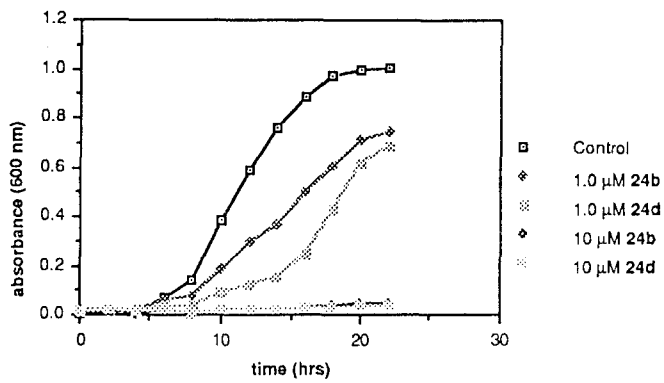


Fig. 3. The effect of catechol-siderophore-carbacephalosporin conjugates **24b** and **24d** (1.0 μM and 10 μM) on the growth rate of *E. coli* X580 in Luria broth supplemented with 100 μg/mL EDDA

grow resulted in no delay in observed growth. This result again suggested that during the first incubation, a mutant of the parent *E. coli* strain was selected that was resistant to **22.** Furthermore, the bacteria resistant to the hydroxamate-based siderophore conjugates **16a, b** were found to be susceptible to the catechol-based conjugate **22,** further indicating that the initial hydroxamate-conjugate-induced mutants lacked the usual hydroxamate siderophore transport system and relied extensively on a catechol-mediated iron transport process.

Initial tests also demonstrated that separate combinations of hydroxamates **16a** or **16b** with catechol **22,** at half of the normal test concentrations of each, so as to give an identical concentration of the β-lactam, were more potent than either type of conjugate used alone.

Incubation of the phenylglycine-containing catechol conjugates **24a-d** with *E. coli* X580 gave results with notable delay of observed growth similar to that observed with the parent compound **22.** Interestingly, tests of the conjugates at 10 μM with the same organism

under iron-deficient conditions, induced by deferration of the media with EDDA (100 μg/mL), led to complete inhibition of observed growth (Fig. 3, data shown for **24b** and **24d** only since the data obtained for the other two catechol conjugates was essentially the same). At 1.0 μM, growth was eventually detectable within 10–20 h, with conjugates **24c** and **24d,** containing the L-amino acids, being more effective at delaying eventual growth. This result provides the first indication that intracellular release of the antibiotic from the conjugate may be important, since the L-isomers would be more prone to proteolytic cleavage of the siderophore-drug linkage. While this is speculative and further studies are needed to confirm a release process, preincubation of all conjugates with the media before inoculation with *E. coli* had no effect on the growth curves, suggesting that the conjugates are stable under the incubation conditions in the absence of *E. coli.*

The syntheses and preliminary biological studies of iron chelator-antibiotic conjugates **16a, b, 22,** and **24a–d** indicate that covalent attachment of antimicrobial agents to siderophores and analogs may indeed provide a viable method for the rational design of drug delivery agents. These same conjugates also hold considerable potential as biological tools for the selection of iron-transport-deficient mutants which will be useful for the elucidation of physiologically essential processes associated with iron assimilation and metabolism.

Acknowledgements. We gratefully acknowledge the financial support of the National Institutes of Health. Earlier studies in our group by Dr Chia-En Lin and Dr Sushil K. Sharma and stimulating interactions with Dr Shelley M. Payne (University of Texas at Austin), Dr Thalia Nicas (Eli Lilly and Co.) and Dr Francois Malouin (University of Laval, Canada) are sincerely appreciated. The gift of the carbacephalosporins from Eli Lilly and Co. was most instrumental in facilitating these studies.

References

Benz G, Schroder T, Kurz J, Wunsche C, Karl W, Steffens G, Pfitzner J, Schmidt D (1982) Konstitution der Desferriform der Albomycine δ_1, δ_2, ε. Angew Chem Suppl 1322–1325

Bodurow CC, Boyer BD, Brennan J, Bunnell CA, Burks JE, Carr MA, Doecke CW, Eckrich TM, Fisher JW, Gardner JP, Graves BJ, Hines P, Hoying RC, Jackson BG, Kinnick MD, Kochert CD, Lewis JS, Luke WD, Moore LL, Morin Jr JM, Nist RL, Prather DE, Sparks DL, Vladuchik WC (1989) An enantioselective synthesis of loracarbef (LY163892/KT3777) Tetrahedron Lett 30:2321–2324

Christensen BG (1989) Tienam – from natural product to antibiotic. Chem Br 371–374

Dolence EK, Minnick AA, Miller MJ (1990) N^5-Acetyl-N^5-hydroxy-L-ornithine-derived siderophore-carbacephalosporin β-lactam conjugates: iron transport-mediated drug delivery. J Med Chem 33:461–464

Dolence EK, Lin C-E, Miller MJ, Payne SM (1991) Synthesis and siderophore activity of albomycin-like peptides derived from N^5-acetyl-N^5-hydroxy-L-ornithine. J Med Chem 34:0000

Gordon AJ, Ford RA (1972) The chemist's companion – a handbook of practical data, techniques and references. John Wiley and Sons, New York

Kiyoshi S, Okachi R (1989) In vitro and in vivo antibacterial activity of KT3777, a new orally active carbacephem. J Antibiot 42:1844–1853

Miller MJ (1989) Synthesis and therapeutic potential of hydroxamic-acid-based siderophores and analogues. Chem Rev 89:1563–1579

Neilands JB, Valenta JR (1985) Iron-containing antibiotics. In: Sigel H (ed) Metal ions in biological systems. Marcel Dekker, New York, vol. 19, chap 11

Sharma SK, Miller MJ, Payne SM (1989) Spermexatin and spermexatol: new synthetic spermidine-based siderophore analogues. J Med Chem 32:357–367

Silley P, Griffiths JW, Monsey D, Harris AM (1990) Mode of action of GR69153, a novel catechol-substituted cephalosporin, and its interaction with the *tonB*-dependent iron transport system. Antimicrob Agents Chemother 34:1806–1808

Waxman DJ, Strominger JL (1982) β-Lactam antibiotics: biochemical mode of action. In: Morin RB, Gorman M (eds) Chemistry and biology of β-lactam antibiotics. Academic Press, New York, vol 3, pp 209–285