Synthesis of Novel Citrate-Based Siderophores and Siderophore-β-Lactam Conjugates. Iron Transport-Mediated Drug Delivery Systems

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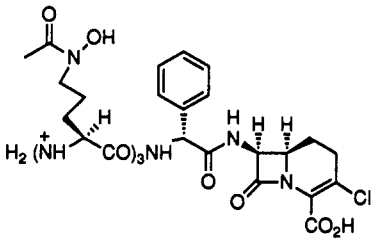
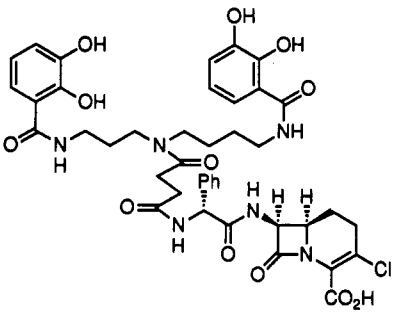
The synthesis of analogs of arthrobactin (**5**), a microbial iron chelator, and its imide **8** are described. The differentially protected citric acid residue **31** served as the key intermediate in making conjugates having a generalized structure **14** with two representative carbacephalosporin units, **15** and **16**. Both conjugates, **49** and **51**, showed antibiotic activity, while conjugate **51** obtained from β-lactam **16** bearing a phenylglycyl side chain was shown to be more effective.

The microbial evolution of elaborate systems for acquiring extremely insoluble ($K_{sp} \sim 10^{-38}$ M at physiological pH), yet essential, iron features the biosynthesis and use of highly specific and efficient ferric ion sequestering agents.[1] These low molecular weight compounds, known as siderophores, primarily utilize hydroxamic acids,[2-5] catechols,[6] α-hydroxy acids, or combinations thereof[1b,c] as the chelating ligands. The extracellularly formed iron complexes of particular siderophores interact with specific receptors in the outer cell membrane and then enter the cell through an active iron transport mechanism.[7] One of our goals is to use this iron complex recognition and assimilation process to smuggle antibiotics into pathogenic bacteria.[8] Preliminary studies, in our laboratory, have successfully demonstrated this concept with some siderophore-β-lactam conjugates.[9] For example, both hydroxamate-containing albomycin-like conjugates (**1**) and catechol-containing spermexatol-like conjugates (**2**) have shown significant and species-selective antimicrobial activity.[10] Because citrate-based siderophores such as aerobactin (**3**) are often used for high affinity iron transport by microbes, syntheses and studies of related conjugates, as described in this paper, was anticipated to provide additional insight and support of the viability of siderophore-mediated drug delivery.

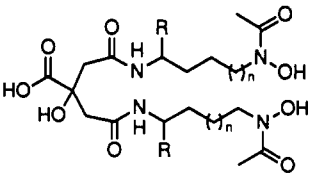
A number of citrate-based siderophores have been reported and several have been synthesized, including aerobactin, schizokinen (**4**), schizokinen A (**7**), and arthrobactin (**5**).[11,12] The synthesis of awaitin A (**6a**), a proposed member of the citrate-based siderophores, also has been described.[13] Recently, a structurally related family of citrate siderophores, nannochelins A, B, and C (**9**) were isolated[14a] from the culture of *Myxobacteria*



1, albomycin-like conjugate



2, spermexatol-like conjugate



3, aerobactin; R=CO_2H, n=2
4, schizokinen; R=H, n=0
5, arthrobactin; R=H, n=2
6a, awaitin A; R=CO_2H, n=1
b, awaitin B; R=H, n=1
c, awaitin C; R=CO_2H, n=0

[⊕] Abstract published in *Advance ACS Abstracts*, December 1, 1993.

(1) (a) Winkelman, G., Ed. *Handbook of Microbial Iron Chelates;* CRC: Boca Raton, FL, 1991. (b) Hider, R. C. *Structure Bonding* **1984**, *58*, 1-25. (c) Winkelman, G.; van der Helm, D.; Neilands, J. B., Eds. *Iron Transport in Microbes, Plants and Animals, VCH*: New York 1987.

(2) Emery, T. In *Microbial Iron Metabolism*; Neilands, J. B., Ed.; Academic Press: New York, 1974; Chapter 5.

(3) Emery, T. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1971**, *35*, 135.

(4) Maehr, H. *Pure Appl. Chem.* **1971**, *28*, 603.

(5) Neilands, J. B. In *Inorganic Biochemistry*; Eichorn, G., Ed.; Elsevier: New York, 1973.

(6) Rosenberg, H.; Young, I. G. In *Microbial Iron Metabolism*; Neilands, J. B., Ed., Academic Press: New York, 1974; Chapter 3.

(7) Mazanake, B. F.; Muller-Matzanke, G.; Raymond, K. N. *Iron Carriers and Iron Proteins;* Loehr, T. M., Ed.; VCH Publishers: New York, 1989; pp 1-121.

(8) For a review on the general principles involved in drug delivery, see: *The Pharmacological Basis of Therapuetics;* Gilman, A. G., Goodman, L. S., Gilman, A., Eds.; MacMillan Publishing Co.: New York, 1980; pp 1-55.

(9) (a) Miller, M. J.; Malouin, F. *Acc. Chem. Res.* **1993**, *26*, 241-249. (b) Miller, M. J. *Chem. Rev.*, **1989**, *89*, 1563-1579.

(10) (a) Minnick, A. A.; McKee, J. A.; Dolence, E. K.; Miller, M. J. *Antimicrob. Agents Chemother.* **1992**, *36*, 840. (b) Brochu, A; Brochu, N.; Nicas, T. I.; Parr, T. R., Jr.; Miller, M. J.; Malouin, F. *Antimicrob. Agents Chemother.* **1992**, *36*, 2166.

(11) Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 3096.

(12) (a) Lee, B. H.; Miller, M. J. *J. Org. Chem.* **1983**, *48*, 24, (b) Milewska, M. J.; Chimiak, A.; Glowacki, Z. *J. Prakt. Chem.* **1987**, *329*, 3, 447.

(13) Steinbaugh, B. M.S. Thesis, University of Notre Dame, Notre Dame, 1985.

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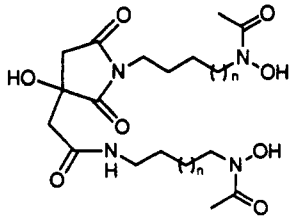
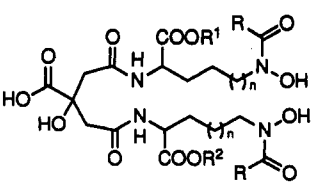


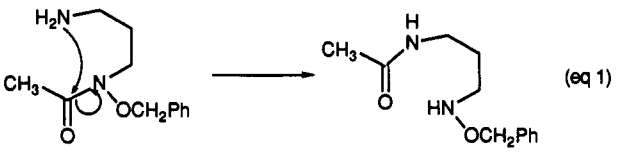
7, schizokinen A; R=H, n=0
8, imide of arthrobactin, n=2
(arthrobactin A)



9, nannochelins;
R=-CH=CHPh, n=2
R^1 and/or R^2 = H and/or Me

nannocystis, and a synthesis[14b] has been reported. Despite this synthetic precedent, several points needed to be considered in the design, synthesis, and study of appropriate citrate-based siderophore antibiotic conjugates. The choice of the siderophore was based on ease of synthesis and recognition by microbes of preliminary interest. β-Lactam derivatives were picked as the first antibiotics for conjugation since the mode of recognition, transport, and activity of the resulting conjugates could be directly compared to our previous siderophore–β-lactam conjugates.[9,10] The position and mode of attachment of the antibiotic was also critical since it was essential to retain effective iron chelation for microbial recognition of the resulting complexed conjugate. Thus, for initial studies, we planned to synthesize and study conjugates of carbacephalosporin antibiotics and arthrobactin (**5**), one of the more easily synthesized citrate-based siderophores.

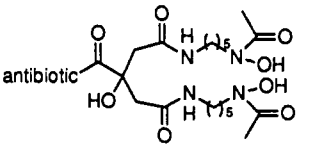
The primary synthetic advantage to the choice of arthrobactin as the siderophore component was the fact that its constituent 1-amino-5-(*N*-acetylhydroxyamino)-pentane derivatives are stable, whereas the shorter 1-amino-3-(*N*-acetylhydroxyamino)propane constituents of the related schizokinen are prone to transacylation (eq 1).[12a]



(eq 1)

10 **11**

Three types of conjugates (**12–14**) were considered on the basis of potential positions and modes of attachment of the antibiotic to arthrobactin. Direct coupling to the free central carboxyl group of the citrate group as in structure **12** seemed obvious and was anticipated to be attempted by straightforward extension of our previous synthesis of arthrobactin.[12a] The primary concern was that modification of the central carboxyl group of the citrate would alter the natural mode of iron chelation through the combined ligation with this carboxylic acid, the adjoining α-hydroxyl group, and the two pendant hydroxamic acids. Modification of the carboxyl group to introduce a third hydroxamate, as in **13**, with an extended functionalized chain for conjugation was anticipated to alleviate the altered chelation problem of **12** but was of concern since such a significant structural change might alter siderophore recognition. For these reasons and because a number of natural hydroxamate-containing siderophores contain variable acyl groups on the chelating hydroxamates, we also considered extension of the acyl chain of one of the hydroxamates of arthrobactin with inclusion of functionality for conjugation to the antibiotic as shown in generalized structure **14**, realizing that such a modification altered the symmetry of the siderophore and ultimately might result in the formation of diastereomeric iron complexes.



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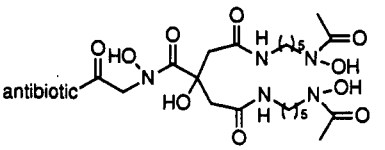

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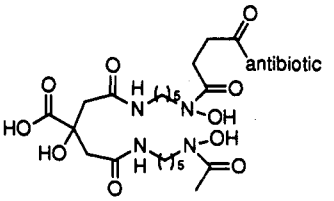
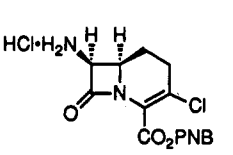


14

β-Lactam antibiotics, including the parent carbacephem core (**15**) and LY 163892[15] (**16c**, Lorabid, Eli Lilly and Co.) which contains a D-phenylglycine side chain, were considered ideal candidates for use as the antibiotic component since any activity of the eventually formed arthrobactin-derived conjugates could be directly compared to previous siderophore–carbacephalosporin conjugates.[9,10] Also, the carbacephalosporins do not contain sulfur and were compatible with planned hydrogenolytic removal of all protective groups at the end of the synthesis.



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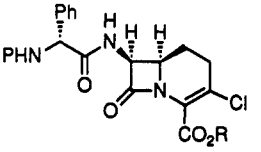


16a, P=BOC, R=PNB
16b, P=H, R=PNB
16c, P=H, R=H

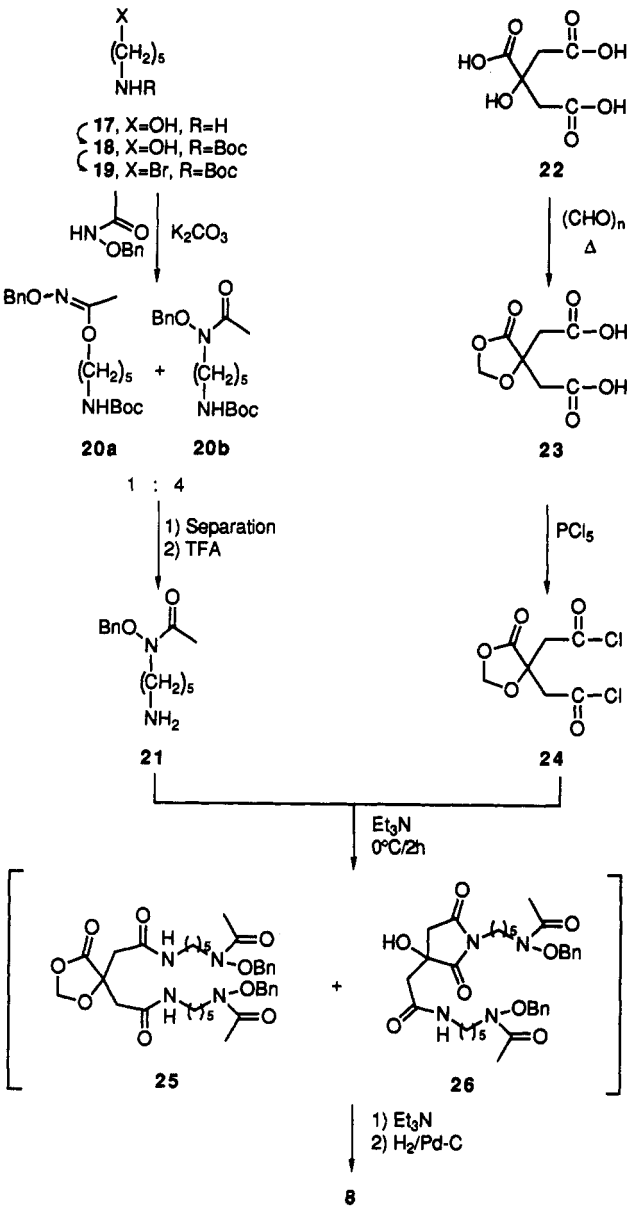
Results and Discussion

Our previous synthesis of arthrobactin[12a] was repeated to determine if direct coupling of an antibiotic or an alternate iron chelating spacer/linker to the central carboxyl group could provide forms of **12** or **13**. The synthetic sequence involved the preparation and coupling of two residues of protected 1-amino-5-(*N*-acetylhydroxyamino)pentane **21** to the two terminal carboxyl

(14) (a) Kunze, B.; Trowitzsch-Kienast, W.; Hofle, G.; Reichenbach, H. *J. Antibiot.* **1992**, *45*(No. 2), 147. (b) Bergeron, R. J.; Phanstiel, O.; IV. *J. Org. Chem.* **1992**, *57*, 7140.

(15) (a) Jones, R. N.; Barry, A. L. *J. Antimicrob. Ther.* **1988**, *22*, 315. (b) Jorgensen, J. H.; Redding, J. S.; Maher, L. A. *Antimicrob. Agents Chemother.* **1988**, *32*, 1477. (c) Howard, A. J.; Dunkin, K. T. *J. Antimicrob. Ther.* **1988**, *22*, 445. (d) Bodurow, C. C.; Boyer, B. D.; Brennan, J.; Bunnell, C. A.; Burks, J. E.; Carr, M. A.; Doecke, C. W.; Eckrich, T. M.; Fisher, J. W.; Gardner, J. P.; Graves, B. J.; Hines, P.; Hoying, R. C.; Jackson, B. G.; Kinnick, M. D.; Kochert, C. D.; Lewis, J. S.; Luke, W. D.; Moore, L. L.; Morin, J. M., Jr.; Nist, R. L.; Prather, D. E.; Sparks, D. L.; Vladuchick, W. C. *Tetrahedron Lett.* **1989**, 30, 2321.

Scheme I



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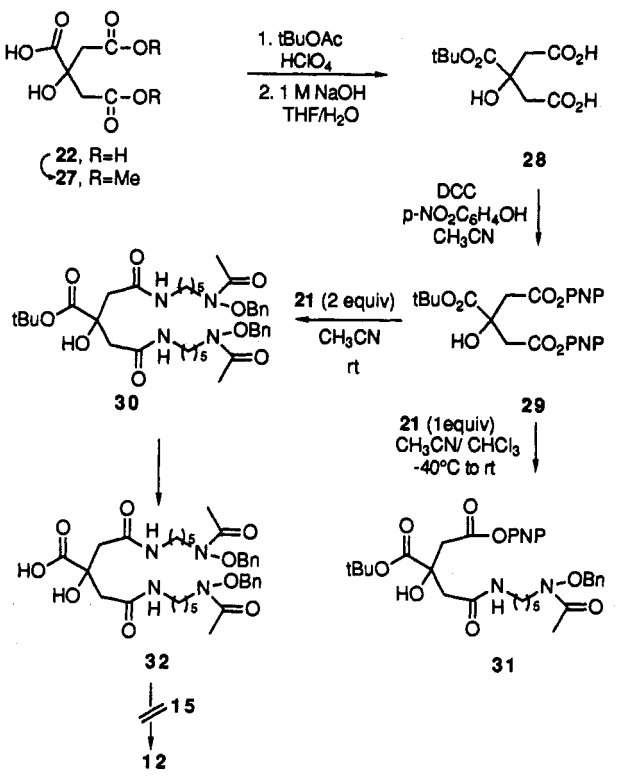
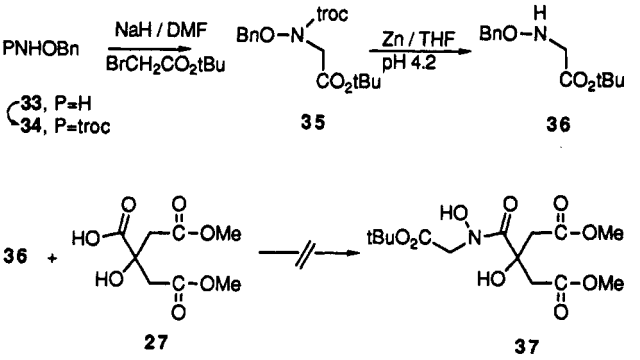


Scheme III

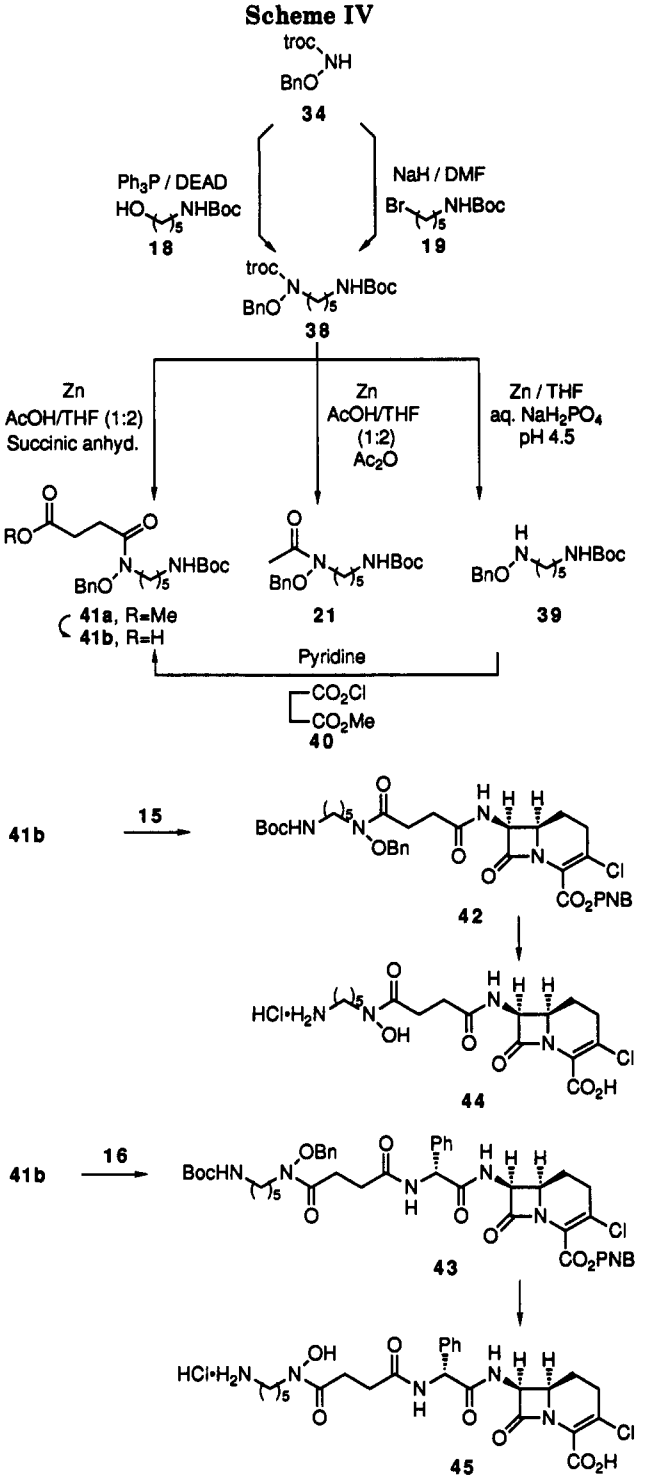


groups of a citric acid residue. The preparation of **21** proceeded without incident as reported earlier[12a] (Scheme I), including formation of a separable 1:4 mixture of O- and desired N-alkylated hydroxamates (**20a** and **20b**) during the key hydroxamate alkylation step. As expected, no transacylation of **21** occurred during the TFA-mediated deprotection of **20b**. Preparation of anhydromethylene citryl chloride **24**, followed by reaction with **21** in the absence of excess base gave protected arthrobactin, **25**, as expected.[12a] Performance of the coupling reaction in the presence of triethylamine gave a mixture of **25** and imide **26**. Use of excess triethylamine provided only the imide, which upon treatment with H_2/Pd–C gave the imide of arthrobactin, arthrobactin A (**8**, so designated by analogy to schizokinen A, **7**). All attempts to open imide **26** or protected arthrobactin **25** with carbacephalosporin nucleus **15** failed to provide a conjugate structurally related to **12**, with direct coupling through the central carboxyl group of arthrobactin. Thus, alternative syntheses were considered.

Use of a *tert*-butyl group to protect the central carboxyl group of the citric acid moiety was anticipated to minimize formation of imides during synthesis of the arthrobactin core and allow subsequent deprotection under acidic conditions, which also do not favor cyclization to the imide form. Thus, dimethyl citrate (**27**, Scheme II) was esterified with *tert*-BuOAc in the presence of $HClO_4$ as described earlier.[12b] Saponification of the resulting triester provided diacid **28**, which was converted to di-*p*-nitrophenyl ester **29** with DCC. Treatment of **29** with 2 equiv of amine **21** in CH_3CN afforded differentially protected arthrobactin **30**. Use of only 1 equiv of amine **21** provided a mixture of half ester amide **31** (30%), diamide **30**, and recovered **29**. However, an improved yield (55%) of **31** was obtained when the reaction was carried out in a mixed ($CHCl_3$/CH_3CN) solvent system at −40 °C to rt.

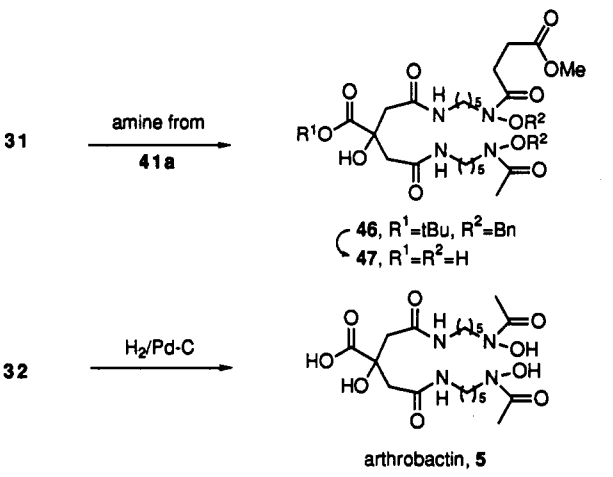
Removal of the *tert*-butyl group from **30** proceeded smoothly to provide desired mono acid **32** in quantitative yield. Interestingly, all attempts to couple **32** with carbacephalosporin nucleus **15** failed under standard peptide coupling conditions that were effective in the preparation of earlier conjugates. Similarly, attempted preparation of precursors **37** of trihydroxamate **13**, by coupling of *tert*-butyl *N*-(benzyloxy)glycinate (**36**) to dimethyl citrate (**27**) were ineffective (Scheme III). Thus, while esters of the central carboxyl group of citrate derivatives were easily prepared, reactions with relatively nonnucleophilic amine derivatives appear to be sluggish. Our attention then focused on the synthesis of conjugates

Scheme IV



related to generalized structure **14**, again anticipating that this type of structure would inherently minimize structural effects required for iron chelation and potentially maximize molecular recognition and microbial transport properties. Compound **31**, with all three of the acyl groups fully differentiated, was ideally suited for the preparation of the desired conjugates. All that was needed was an *N*-hydroxy 1,5-pentanediamine with an appropriate linker for attachment of the antibiotic. Scheme IV summarizes the syntheses of the linker-containing pentanediamine derivative **41** and carbacephalosporin adducts (**42–45**). The carbacephalosporins were attached to **41** before reaction with **31** to allow separate biological studies of **44** and **45**, since several β-lactams bearing single, bidentate iron chelating groups recently have been shown to be effective drugs.[16]

Scheme V



To this end, readily available troc *O*-benzylhydroxylamine (**34**) was alkylated with bromide **19** in the presence of NaH in DMF to provide the desired N-alkylated product **38** exclusively without any O-alkylation. Alternatively, direct reaction of **34** with alcohol **18** provided **38** in over 95% yield. The troc group was then removed by reduction with zinc under buffered conditions to give hydroxylamine **39**. As a control, the troc group of **38** was removed with zinc/acetic acid in the presence of acetic anhydride to give **21**, which was identical to that prepared earlier (Scheme I). Reaction of **39** with mono acid chloride **40** of methyl succinate gave **41a**. Saponification of the methyl ester then afforded desired acid **41b**. More efficiently, reductive removal of the troc group of **38** by brief treatment with freshly activated zinc in the presence of succinic anhydride provided **41b** in near quantitative yield, without loss of the Boc group. Separate coupling of **41b** with carbacephalosporin ester **15** and Lorabid ester **16** was effected with either 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride/1-hydroxybenzotriazole hydrate (EDC/HOBT) (method A) or 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) (method B) to provide fully protected β-lactam derivatives **42** and **43**. Simultaneous removal of the Boc, benzyl, and *p*-nitrobenzyl (PNB) protecting groups under acidic hydrogenolysis conditions (H_2–Pd/C in DMF/HCl/H_2O) gave the desired monohydroxamate-substituted carbacephalosporins **44** and **45**, respectively. Samples of arthrobactin (**5**) and the corresponding succinate derivative **47** were obtained by complete deprotection of coupled products **32** and **46**, respectively (Scheme V).

To complete the synthesis of the desired conjugates **49** and **51**, specific forms of generalized structure **14**, the Boc groups of **42** and **43** were removed with TFA and the resulting crude amines were separately coupled to citrate **31**, giving protected conjugates **48** and **50**, respectively (Scheme VI). Complete deprotection with 5% aqueous DMF, concentrated HCl, and 10% Pd–C[17] furnished arthrobactin–carbacephem conjugates **49** and **51**.

(16) (a) Ohi, N.; Aoki, B.; Shinozaki, T.; Moro, K.; Noto, T.; Nehashi, T.; Okazaki, H.; Matsunaga, I. *J. Antibiot.* **1986**, *39*, 230. (b) Ohi, N.; Aoki, B.; Moro, K.; Kuroki, T.; Sugimura, N. Noto, T.; Nehashi, T.; Matsumoto, M.; Okazaki, H.; Matsunaga, I. *J. Antibiot.* **1986**, *39*, 242. For a more detailed listing, see ref 10 in Miller, M. J.; Malouin, F.; Dolence, E. K.; Gasparski, C. M.; Ghosh, M.; Guzzo, P. R.; Lotz, B. T.; McKee, J. A.; Minnick, A. A.; Teng, M. Iron Transport-Mediated Drug Delivery. *Royal Society of Chemistry Special Publication*, No. 119, **1993**, Ed. P. H. Bentley, R. Ponsford, in Recent Advances in the Chemistry of Anti-Infective Agents. pp 135–159.

(17) Dolence, E. K.; Minnick, A. A.; Lin, C.-A.; Miller, M. J. *J. Med. Chem.* **1991**, *34*, 968.

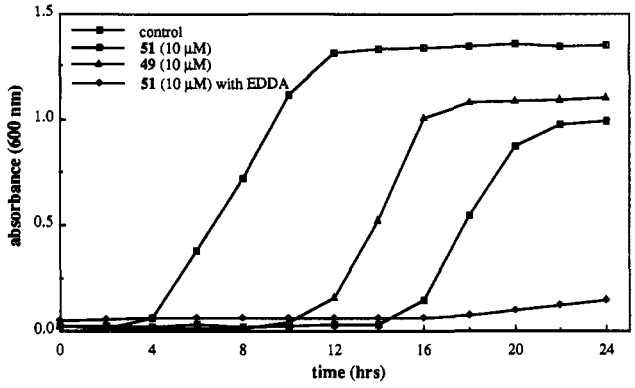
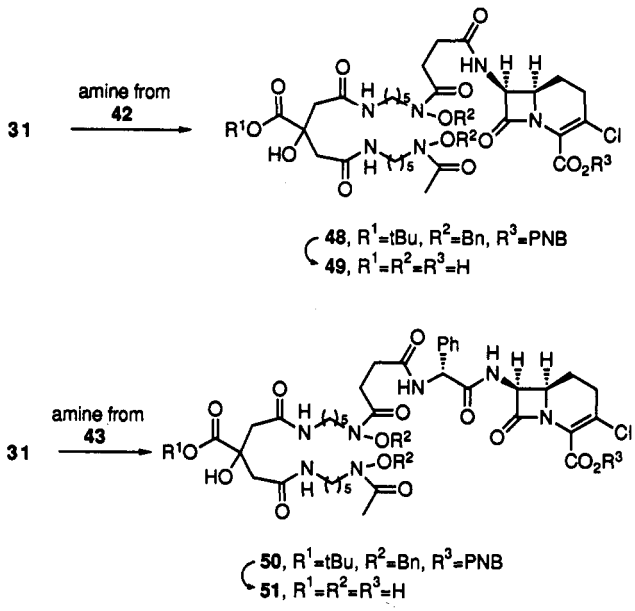


Figure 1. Effects of conjugates **49** and **51** in Luria broth on the growth rate of *E. coli* X580 in the presence and absence of 100 μg/mL of ethylenediaminebis(*o*-hydroxyphenylacetic acid) (EDDA).

Scheme VI



Preliminary biological studies of conjugates **49** and **51** are summarized in Figure 1. Thus, when incubated with *Escherichia coli* X580,[18] both **49** and **51** caused delayed growth even in iron sufficient media, with Lorabid derivative **51** apparently more effective. Under iron-deficient conditions induced by the addition of ethylenediamine bis(*o*-hydroxyphenylacetic acid) (EDDA),[10] essentially no growth was observed. While detailed biological studies of **49**, **51**, **42**, and **43** are being initiated, these preliminary results suggest that conjugates **49** and **51** act in a manner similar to previously synthesized and studied conjugates **1** and **2**,[9,10] that is, the conjugates appear to be recognized and actively transported through the outer membrane of wild-type *E. coli* X580 and the antibiotic components of the conjugates then inhibit microbial growth. Selection of resistant mutants missing the outermembrane receptor for the siderophore component of **49** and **51** presumably is responsible for the microbial growth eventually observed. The apparent critical need for a full set of iron assimilation mechanisms is suggested by the even more effective growth inhibition under the iron-deficient conditions induced by EDDA. The planned detailed antimicrobial assays with wild-type and previously characterized and newly selected mutants, coupled with outermembrane protein analysis of the selected mutants and other studies described for **1** and **2**,[9] are anticipated to further elucidate the mode of action of the new conjugates described here. Especially, it will be interesting to determine if the recognition and transport proteins used by **49** and **51** are different from those used by **1** and **2**, as expected.

(18) A gift from Eli Lilly and Company, Indianapolis, IN.

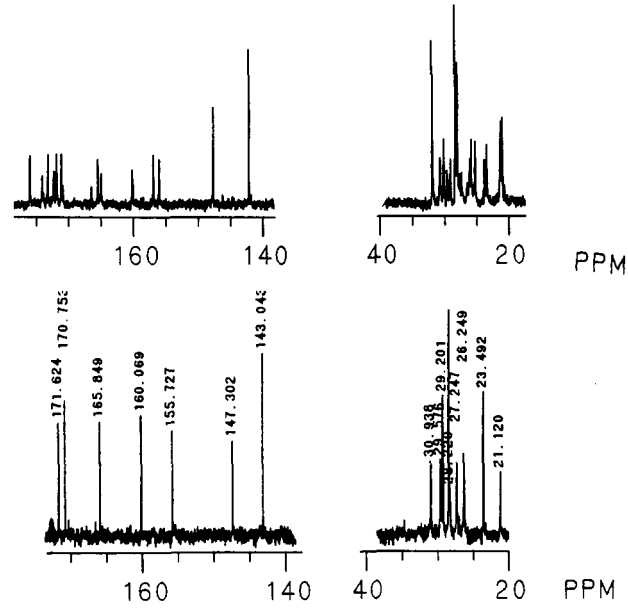


Figure 2. Partial ^{13}C NMR of protected hydroxamate fragment **43** in $CDCl_3$ (top) and 20% DMSO-d_6 in $CDCl_3$ (bottom).

Experimental Section

Instruments and general methods used have been described earlier.[19] However several of the ^1H and ^{13}C NMR spectra were noteworthy in that they were unexpectedly complex when recorded in $CDCl_3$. Use of DMSO-d_6 as the NMR solvent or cosolvent with $CDCl_3$ gave straightforward, easily interpreted spectra (Figure 2). Peak assignments for ^{13}C NMR were made with the assistance of distortionless enhanced polarization transfer (DEPT) pulse program[20] for all protected siderophores and conjugates.

Solvents used were dried and purified by standard methods.[21] The term "dried" refers to the drying of an organic layer over anhydrous magnesium or sodium sulfate. All reactions were performed under nitrogen atmosphere.

2-Hydroxy-1,2,3-propanetricarboxylic acid 1,3-dimethyl ester[12b] and (4-oxo-1,3-dioxolan-5-ylidene)diacetyl chloride[12a] (**24**) were prepared by the literature reported methods except that the products were purified by recrystallization from hot water and by vacuum distillation, respectively.

Coupling Reaction of Amine 21 with Acid Chloride 24.[11a] A solution of 1-[(*tert*-butoxycarbonyl)amino]-5-[acetyl(benzyloxy)-amino]pentane[12a] (**20b**, 0.58 g, 1.66 mmol) in CH_2Cl_2 (2.0 mL) was stirred with anhydrous TFA (2.0 mL) at 0 °C to rt. The reaction was complete after 30 min (monitored by TLC). The volatile components were removed by rotary evaporation and the residue was partitioned between $CHCl_3$ (25 mL) and saturated $NaHCO_3$. In one occasion, the $CHCl_3$ layer was concentrated to check the purity of the free amine: ^1H NMR ($CDCl_3$) δ 1.10–1.79 (m, 6H), 2.07 (s, 3H), 2.62 (m, 2H), 3.62 (t, J = 6.5 Hz, 2H), 4.80 (br s, 1H), 4.85 (s, 2H), 7.42 (m, 5H). However, for the coupling reaction the $CHCl_3$ layer containing free amine was dried (K_2CO_3) and chilled to 0 °C (ice bath) without isolation. Et_3N (0.23 mL, 1.66 mmol) was added followed by dropwise addition of anhydromethylene citryl chloride (**24**, 0.20 g, 0.83 mmol) in $CHCl_3$

(19) Teng, M.; Miller, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 548.
(20) Doddrell, D. M.; Bendall, M. R. *J. Magn. Reson.* **1981**, *45*, 8.
(21) Gordon, A. J.; Ford, R. A. *The Chemist's Companion-a Handbook of Practical Data, Techniques, and References*; John Wiley and Sons: New York, 1972; pp 408–456.

(5 mL). After the solution was stirred for 5 min at 0 °C, it was warmed to room temperature and stirred an additional 40 min. Next, the solution was washed with 0.5 M citric acid (aq), H_2O, 0.5 M $NaHCO_3$, and finally brine. The organic layer was dried and evaporated to leave an oil as a mixture of **25** and **26**. The presence of two singlets at δ 2.55 (<4H) and 5.49 (<2H) in the ^1H NMR of this mixture was indicative of the partial deprotection of the central carboxylic group: MS (FAB) *m/z* at 639 and 669 (MH^+).

The above mixture of products was stirred at rt with excess Et_3N in $CHCl_3$ to provide imide **26** exclusively. IR (neat) 3400–3200 (br), 2920, 1780, 1705, 1660 (br) cm^{-1}; ^1H NMR showed complete disappearance of the two singlets at δ 2.55 and 5.49; ^1H NMR ($CDCl_3$) δ 1.15–1.40 (m, 4H), 1.45–1.75 (m, 6H), 1.85–2.05 (m, 2H), 2.07 (s, 3H), 2.09 (s, 3H), 2.55–2.95 (m, 4H), 3.23 (m, 2H), 3.47 (br t, 2H), 3.63 (br q, 4H), 4.79 (s, 2H), 4.81 (s, 2H), 6.10 (br s, 1H), 7.10 (br s, 1H), 7.37 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 23.4, 23.5, 26.2, 26.6, 28.4, 38.2, 39.0, 41.5, 42.2, 44.54 (m), 72.9 (quaternary), 76.0, 76.0, 128.5, 128.8, 129.0, 134.0, 134.1, 169.6, 172.3 (br), 174.3, 178.2; MS (FAB) *m/z* 639 (MH^+).

Deprotection of Imide 26. Synthesis of the Imide of Arthrobactin (Arthrobactin A, 8). Imide **26** (120 mg, 0.188 mmol) was dissolved in THF/H_2O (2:1, 15 mL) and treated with 10% Pd/C (120 mg). The reaction mixture was stirred at room temperature for 3 h under 1 atm of H_2 and then filtered. Removal of solvent afforded **8** as a glassy solid (83 mg, 96%): ^1H NMR (D_2O) δ 1.13–1.31 (m), 1.33–1.69 (m), 2.04 (s, 3H), 2.06 (s, 3H), 2.65–2.97 (m, 4H), 2.98–3.15 (m, 2H), 3.44 (t, *J* = 6.6 Hz, 2H), 3.53 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (D_2O) δ 19.2, 23.0, 25.3, 25.4, 26.3, 27.8, 38.7, 39.0, 41.4, 42.0, 47.5, 47.6, 53.1, 72.6, 170.7, 173.4, 177.3, 180.1; MS (FAB) *m/z* 459 (MH^+); HRMS (FAB) calcd for $C_{20}H_{34}N_4O_8Na_1$ (MNa^+) 481.2274, found 481.2263.

2-Hydroxy-1,2,3-propanetricarboxylic Acid 2-*tert*-Butyl Ester (28).[11b] A solution of 1,3-dimethyl citrate (**27**, 18 g, 75 mmol), 150 mL of tert-butyl acetate, and 1 mL of 70% $HClO_4$ was placed in a Paar bottle and left on a mechanical shaker for 24 h. After venting, the reaction mixture was diluted with ether, and the organic phase was washed with water, saturated $NaHCO_3$, and brine. After drying and removal of solvent, the residue was purified by column chromatography eluting with EtOAc/hexanes (1:1). The triester was obtained as a colorless oil: IR (neat) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (s, 9H), 2.59 (s, 4H), 3.41 (s, 6H).

2-Hydroxy-1,2,3-propanetricarboxylic acid 2-*tert*-butyl 1,3-dimethyl triester (5.5 g, 20 mmol) was dissolved in a mixture of 100 mL of THF/H_2O (1:1) with 1 M NaOH (3 equiv) and stirred for 3 h at room temperature. After removal of the THF, the water solution was acidified to pH 4 and extracted with EtOAc. The solid so obtained was recrystallized from EtOAc/hexanes to afford **28** (3.9 g, 80%), mp 120–122 °C (lit.[11b] mp 123–124 °C): ^1H NMR (acetone-d_6) δ 1.49 (s, 9H), 2.89 and 2.90 (2d, AB coupling, *J* = 15.6 Hz, total 4H), 4.39 (s, 1H), 10.9 (br s).

2-Hydroxy-1,2,3-propanetricarboxylic Acid 2-*tert*-Butyl 1,3-bis(*p*-nitrophenyl) Triester (29). Diacid **28** (2.48 g, 10 mmol) and *p*-nitrophenol (2.92 g, 21 mmol) were dissolved in dry acetonitrile (35 mL). The solution was cooled in an ice bath, and DCC (4.33 g, 21 mmol) in a small amount of acetonitrile was added all at once. The reaction mixture was stirred at 0 °C for 30 min and then at rt for 5 h. The dicyclohexylurea was filtered off, and the residue was chromatographed on silica gel eluting with CH_2Cl_2. The product was recrystallized from ethyl acetate/hexanes to provide **29** (2.8 g, 58%), mp 162–164 °C: IR (KBr) 3230 (br), 1760 (br), 1720, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51 (s, 9H), 3.13 and 3.22 (2d, *J* = 15.3 Hz, total 4H), 4.13 (s, 1H), 7.30 (d, *J* = 9.0 Hz, 2H), 8.30 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 27.8, 43.6, 73.1, 84.6, 122.4, 125.3, 145.5, 154.8, 166,9, 171.8; Anal. Calcd for $C_{22}H_{22}N_2O_{11}$: C, 53.88; H, 4.52; N, 5.71. Found C, 53.66; H; 4.75; N, 5.74.

Preparation of Diamide 30. To the solution of double active ester **29** (329 mg, 0.672 mmol) in CH_3CN was slowly added a $CHCl_3$ solution of amine **21**, freshly generated by the brief exposure of **20b** (336 mg, 1.34 mmol) to TFA/CH_2Cl_2 (1:1). The reaction mixture was stirred for 2 h, washed with a saturated solution of $NaHCO_3$ and brine, and dried. Removal of solvent and radial chromatography eluting first with ethyl acetate and then 5% *i*-PrOH/EtOAc afforded **30** (450 mg, 94%) as a clear oil: IR (neat) 3310 (br), 1730, 1650 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21–1.38 (m, 4H), 1.41–1.75 (m containing s at 1.45, 17H), 2.09 (s, 6H), 2.56 and 2.68 (2d, AB coupling, *J* = 14.9 Hz, 4H), 3.19 (q, *J* = 6.1 Hz, 4H), 3.63 (t, *J* = 6.2 Hz, 4H), 4.81 (s, 4H), 6.98 (t, *J* = 5.6 Hz, 2H), 7.38 (s, 10 H); ^{13}C NMR ($CDCl_3$) δ 20.3, 23.7, 26.3, 27.6, 28.6, 39.0, 43.5, 44.7 (m), 73.9, 76.0, 82.3, 128.5, 128.8, 129.0, 134.2, 172.2, 172.4; MS (FAB) *m/z* 827 (MH^+).

Complete Deprotection of Diamide 30: Synthesis of 5 (Arthrobactin).[12a] A solution of diamide **30** (150 mg, 0.23 mmol) in anhydrous CH_2Cl_2/TFA (1:1, 4 mL) was stirred at 0 °C to rt until complete consumption of the starting material was evident (TLC, 30 min). The volatile components were removed by rotary evaporation, and the residue was dissolved in EtOAc. The organic layer was washed once with H_2O, dried, and evaporated to provide the free acid in nearly quantitative yield: ^1H NMR ($CDCl_3$) δ 1.20–1.40 (m, 4H), 1.50–1.80 (m, 8H), 2.14 (s, 6H), 2.8–3.0 (m, 4H), 3.32 (q, *J* = 6.3 Hz, 4H), 3.72 (t, *J* = 6.9 Hz, 4H), 4.84 (s, 4H), 5.30 (s, 1H), 7.43 (m, 10 H), 7.99 (br s, 3H).

Acid **32**, without further purification, was dissolved in THF/water (2:1, 9 mL) and treated with 10% Pd–C (30 mg). The solution was stirred under 1 atm of hydrogen for 3 h, filtered, and evaporated. The residue was partitioned between deionized distilled H_2O/EtOAc (1:1, 10 mL). The aqueous phase, after dilution with MeOH (5 mL), was passed through a short reverse phase (C-18) column and lyophilized to furnish **5** as a hygroscopic off-white solid: $FeCl_3$ positive (red-purple); ^1H NMR (D_2O) δ 1.12–1.32 (m, 4H), 1.34–1.66 (m, 8H), 2.04 (s, 6H), 2.59 and 2.77 (2d, AB coupling, *J* = 14.6 Hz, 4H), 3.08 (t, *J* = 5.7 Hz, 4H), 3.52 (t, *J* = 6.5 Hz, 4H); MS (FAB) *m/z* 477 (MH^+), 499 (MNa^+); HRMS(FAB) calcd for $C_{20}H_{36}N_4O_9Na$ (MNa^+) 499.2380, found 499.2388.

Preparation of Half Ester–Amide 31. As in the case of **30**, the free amine generated from the brief treatment of **20b** (0.350 g, 1 mmol) in TFA/CH_2Cl_2 (1:1) was treated immediately with a cold (−40 °C) solution of active ester **29** (0.490 mg, 1 mmol) in a mixed solvent system (1:1, $CHCl_3$/CH_3CN; 200 mL). Removal of solvent and flash chromatography of the residue using 1% *i*-PrOH/EtOAc provided **31** (329 mg, 55%) as a pale yellow oil: IR (neat) 3400–3100 (br), 1765, 1730, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22–1.39 (m, 2H), 1.42–1.75 (m containing s at 1.49, 13H), 2.10 (s, 3H), 2.64 and 2.73 (2d, AB coupling, *J* = 14.5 Hz, 2H), 3.03 and 3.16 (2d, AB coupling, *J* = 15.5 Hz, 2H), 3.25 (m, 2H), 3.68 (m, 2H), 4.81 (s, 2H), 6.39 (q, *J* = 6.6 Hz, 1H), 7.28 (m, 2H), 7.37 (m, 5H), 8.24 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.3, 23.5, 26.2, 27.6, 28.4, 39.2, 43.0, 44.3, 44.5(m), 73.5, 76.1, 83.4, 122.3, 125.0, 128.6, 128.9, 129.0, 134.1, 145.2, 154.9, 167.3, 169.1, 172.2, 172.4; HRMS (FAB) calcd for $C_{30}H_{40}N_3O_{10}$ (MH^+) 602.2713, found 602.2711.

1-[(*tert*-Butoxycarbonyl)amino]-5-[(trichloroethoxycarbonyl)(benzyloxy)amino]pentane (38) by Alkylation with Bromide 19. To a washed (hexanes) suspension of NaH (120 mg, 50% dispersion in mineral oil, 2.5 mmol) in dry DMF (5 mL) was added dropwise a solution of carbamate **34** (750 mg, 2.5 mmol) in DMF (5 mL) with ice bath cooling over a 30 min period. The ice bath was removed and the reaction mixture was stirred an additional 15 min at rt. 5-[(*tert*-Butoxycarbonyl)amino]-1-pentyl bromide (**19**; 660 mg, 2.5 mmol) in DMF (5 mL) was added slowly under cold (ice bath) conditions. Toward the end of the addition the ice bath was removed, and the reaction mixture was allowed to stir overnight at rt. The reaction was diluted with an equal volume of ice-cold water and then extracted with EtOAc. The EtOAc layer was washed with water, dried, filtered, and concentrated. The residue was chromatographed (20% EtOAc/hexanes) on silica gel to provide **38** as an oil (910 mg, 80%): ^1H NMR ($CDCl_3$) δ 1.22–1.39 (m, 2H), 1.42–1.52 (m containing s at 1.44, 11H), 1.59–1.72 (m, 2H), 4.88 (q, *J* = 6.4 Hz, 2H), 3.50 (t, *J* = 7.1 Hz, 2H), 4.58 (br s, 1H), 4.83 (s, 2H), 4.92 (s, 2H), 7.34–7.46 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 23.8, 26.6, 28.4, 29.6, 40.4, 49.5, 75.1, 77.2, 95.3, 128.5, 128.8, 129.5, 134.8, 155.1, 155.9; HRMS (FAB) calcd for $C_{20}H_{30}N_2O_5Cl_3$ (MH^+) 483.1220, found 483.1217.

Compound 38 by Alkylation with Alcohol 18. 5-[(*tert*-Butoxycarbonyl)amino]-1-pentanol (**18**; 1.01 g, 5 mmol), triphenylphospine (1.73 g, 6.5 mmol), and *O*-benzyl-*N*-(trichloroethoxycarbonyl)hydroxylamine (**38**, 1.49 g, 5 mmol) were dissolved in THF (35 mL) and treated dropwise with diethyl azodicarboxylate (DEAD) (0.87 mL, 5.5 mmol) in THF (10 mL) over 20 min. After the addition was completed, the mixture was allowed to stir for 16 h at room temperature. Volatile components were evaporated and the residue was chromatographed on a silica gel

column eluting with 20% EtOAc/hexanes to provide **38** as a colorless oil (2.1 g, 95.5%), fully identical with the sample obtained above.

1-((*tert*-Butoxycarbonyl)amino)-5-((benzyloxy)amino)-pentane (39). Compound **38** (0.5 g, 1 mmol) was dissolved in THF (10 mL) and treated with Zn dust (1 g) and KH_2PO_4 (1 M, 2 mL). The mixture was allowed to stir at room temperature for 3 h. The solvent was removed after filtration and the residue was dissolved in ethyl acetate. The ethyl acetate layer was washed with saturated Na_2CO_3 followed by brine. Drying, filtration, and removal of solvent afforded **39** as a colorless oil (290 mg, 94%): ^{1}H NMR ($CDCl_3$) δ 1.22–1.37 (m, 2H), 1.38–1.56 (m, containing s at 1.43, 13H), 2.90 (t, J = 7.0 Hz, 2H), 3.08 (q, J = 6.3 Hz, 2H), 4.64-4.70 (m containing s at 4.68, 3H), 7.25–7.4 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 24.2, 26.8, 28.3, 29.8, 40.3, 51.8, 76.0, 78.8, 127.6, 128.2, 137.8, 155.8; MS (FAB) m/z 309 (MH^+).

Succinate Derivatives 41a and 41b. Compound **39** (0.28 g, 0.91 mmol) was dissolved in acetonitrile (10 mL) and treated with pyridine (0.16 mL, 2 mmol), followed by the acid chloride of monomethyl succinate (0.16 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 12 h. The solvent was removed. The residue was taken up in EtOAc and washed with citric acid (10%), saturated Na_2CO_3, and brine. Drying, filtration, and removal of solvent afforded the methyl ester of **41a** as a clear oil (0.373 g, 97%): IR (neat) 3300 (br), 1735, 1700, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21–1.58 (m, 2H), 1.40–1.53 (m containing s at 1.43, 11H), 1.58–1.70 (m, 2H), 2.60–2.76 (m, 4H), 3.08 (q, J = 6.3 Hz, 2H), 3.64 (t, J = 6.8 Hz, 2H), 3.68 (s, 3H), 4.8 (br s, 1H), 4.85 (s, 2H), 7.39 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 23.6, 26.3, 27.1, 28.2, 29.3, 40.2, 45.0, 51.5, 76.1, 78.7, 128.5, 128.7, 129.0, 134.3, 155.8, 173.0, 173.3; MS (CI) m/z 423 (MH^+); HRMS (EI) calcd for $C_{22}H_{34}N_2O_6$ (M^+ - 56) 366.1790, found: 366.1799.

Ester **41a** was saponified using 3 equiv of 1 M NaOH in (1:1) THF/H_2O to provide the corresponding acid as a crystalline solid after workup. Recrystallization from ethyl acetate/hexanes provided a pure sample of **41b** (0.088 g, 90%), mp 90–91 °C: IR (KBr) 3300 (br), 1705, 1680, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20–1.35 (m, 2H), 1.40–1.53 (m containing s at 1.43, 11H), 1.58–1.70 (m, 2H), 2.60–2.76 (m, 4H), 3.08 (q, J = 6.3 Hz, 2H), 3.64 (t, J = 6.8 Hz, 2H), 4.8 (br s, 1H), 4.85 (s, 2H), 7.39 (s, 5H), 7.6–7.8 (br); ^{13}C NMR ($CDCl_3$/DMSO-d_6) δ 23.6, 26.3, 27.1, 28.4, 28.4, 29.3, 39.9, 45.0 (m), 75.9 (benzylic), 77.9 (quaternary), 128.4, 128.6, 129.1, 134.5, 155.9, 172.9, 174.2. Anal. Calcd for $C_{21}H_{32}N_2O_6$: C, 61.75; H, 7.9; N, 6.86. Found: C, 61.49; H 7.81; N, 6.56.

Succinate 41b Directly from 38. Compound **38** (2 g, 4.1 mmol) was dissolved in 25 mL of a 1:1 mixture of THF and AcOH and treated with freshly activated Zn (2 g) followed by succinic anhydride (1.5 g, 5 mmol). The reaction mixture stirred vigorously at room temperature. After 30 min, TLC analysis showed complete consumption of starting material. After being stirred for another 30 min, the reaction mixture was filtered and concentrated. The solid residue was dissolved in warm benzene and then diluted with one volume of hexanes to afford **41b** with slight contamination of succinic acid. This was dissolved in ethyl acetate, washed repeatedly with water and brine, dried, filtered, and concentrated to give **41b**, fully identical with the sample obtained above by acylation of **39**.

Protected Carbacephalosporin Adduct 42. Method A: 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide Hydrochloride/1-Hydroxybenzotriazole Hydrate (EDC/HOBT). Compound **41b** (98 mg, 0.24 mmol) and carbacephalosporin **15**[18] (0.093 g, 0.24 mmol) were suspended in 2.0 mL of anhydrous methylene chloride under a nitrogen atmosphere. Triethylamine (0.024 g, 0.24 mmol) and HOBT (0.033 g, 0.24 mmol) were added, and the solution was stirred for 5 min. The reaction mixture was then cooled (ice bath) and EDC (0.069 g, 0.36 mmol) and the rest of the Et_3N (0.036 mg, 0.36 mmol) were added. The solution was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, washed with 0.5 N HCl, water, and brine, dried, filtered, and concentrated to provide **42** as a yellow oil. Radial chromatography using MeOH/EtOAc (1:10) provided pure **42** (110 mg, 62%).

Method B: 2-Ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ). To a suspension of HCl salt **15** (0.525 g, 1.35 mmol) in 2.0 mL of CH_2Cl_2 was added Et_3N (188 μL, 1.35 mmol) followed by acid **41b** (0.551 g, 1.35 mmol) and then EEDQ (0.435 g, 1.75 mmol). More CH_2Cl_2 (1 mL) was added. The reaction was stirred for 5 min, and then 1 mL of DMF was added. The reaction mixture was stirred at rt overnight and diluted with EtOAc, and the solution was washed with 0.5 M HCl, water, and brine. The organic phase was dried, filtered, and evaporated to give 890 mg of a foam. This was purified by radial silica gel chromatography eluting with MeOH/EtOAc (1:10) to afford pure **42** (0.580 g, 58%): IR (TF) 3200 (br), 1770, 1650, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20–1.38 (m, 2H), 1.39–1.52 (m containing s at 1.42, 11H), 1.53–1.71 (m, 2H), 1.80–2.10 (m, 2H), 2.43–2.76 (m, 5H), 2.78–2.93 (m, 1H), 3.07 (q, J = 6.3 Hz, 2H), 3.62 (t, J = 5.9 Hz, 2H), 3.85–3.95 (m, 1H), 4.69 (br t, 1H), 4.83 (s, 2H), 5.3–5.45 (m containing 2d at 5.33 and 5.43, AB coupling, J = 13.3 Hz, 3H), 7.11 (br, 1H), 7.62 (s, 5H), 7.61 (d, J = 9 Hz, 2H), 8.21 (d, J = 8.7 Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 21.8, 23.7, 26.2, 27.5, 28.2, 29.4, 29.9, 31.6, 40.2, 45.0 (m), 52.4, 58.5, 66.0, 76.1, 78.8, 122.8, 123.5, 123.7, 128.6, 128.6, 128.8, 129.0, 131.2, 134.1, 142.1, 147.5, 155.9, 159.9, 165.5, 172.7, 173.3; MS (FAB) m/z 742 (MH^+). Anal. Calcd for $C_{36}H_{44}N_5O_{10}Cl$: C, 58.28; H, 5.98; N, 9.45. Found C, 58.22; H, 6.06; N, 9.45.

Deprotection of 42 to Carbacephalosporin Derivative 44. To a solution of **42** (0.050 g, 0.067 mmol) in 1 mL of 5% aqueous DMF (prepared from deionized distilled water and HPLC-grade DMF) were added 17.4 μL (0.202 mmol, 3.0 equiv) of concentrated HCl and 0.010 g (20% w/w) of 10% Pd/C. This mixture was exposed to hydrogen for 24 h at atmospheric pressure followed by filtration of the catalyst. The DMF–water was removed by evaporation under vacuum. The residue was dissolved in deionized distilled water (5 mL) and EtOAc (5 mL). The separated aqueous phase was lyophilized to give **44** as a light amber semisolid in near quantitative yield: $FeCl_3$ positive (red-purple); IR (neat) 3600–3200 (br), 1780, 1660 (br) cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.19–2.10 (m, 8H), 2.35–3.00 (m, 6H), 3.10–3.25 (m, 2H), 3.50–3.65 (m, 2H), 3.85–3.95 (m, 1H), 5.2–5.4 (m, 1H), 7.99–8.25 (m); ^{13}C NMR δ 21.1, 22.9, 24.5, 26.7, 27.2, 28.6, 30.2, 31.7 (allylic), 40.4, 53.5, 59.1, 139.9, 140.8, 158.0, 164.1, 166.7, 173.8, 175.0; HRMS(FAB) calcd for $C_{17}H_{26}N_4O_6Cl$ (MH^+) 417.1541, found 417.1533.

Protected Lorabid Adduct 43. Method A. To a solution of **16a** (0.441 g, 0.754 mmol) in 3.0 mL of anhydrous dichloromethane at 0 °C under nitrogen was added anhydrous TFA (3.0 mL). The reaction was monitored by TLC and, after 30 min, no starting material was left. The solvent was evaporated, and the residue was dissolved in anhydrous benzene, evaporated, diluted with hexanes, and evaporated again to give the TFA salt as an oil. The TFA salt, the acid **41b** (0.308 g, 0.754 mmol), and HOBT (0.204 g, 1.507 mmol) were dissolved in anhydrous CH_2Cl_2 (10 mL) in an ice bath and Et_3N (105 μL, 0.754 mmol) was added. After 5 min, EDC (0.578 g, 3.015 mmol) was added followed by the rest of the Et_3N (420 μL, 3.015 mmol). The solution was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc, washed with 0.5 N HCl, water, and brine, dried, filtered, and concentrated to provide **43** as a yellow oil. Radial chromatography using MeOH/EtOAc (1:10) provided pure **43** (0.396 g, 60%).

Method B (EEDQ). To the TFA salt obtained from Boc protected amine **16a** (0.465 g, 0.795 mmol), as in method A, in 2.0 mL of CH_2Cl_2 was added Et_3N (110 μL, 0.794 mmol) followed by acid **41b** (0.324 g, 0.795 mmol) and then EEDQ (0.256 g, 1.04 mmol). More CH_2Cl_2 (1 mL) was added. The reaction was stirred for 5 min, and then 1 mL of DMF was added. The reaction mixture was stirred at rt overnight and diluted with EtOAc, and the solution was washed with 0.5 M HCl, water, and brine. The organic phase was dried, filtered, and evaporated to give 530 mg of a foam. This was purified by radial silica gel chromatography eluting with MeOH/EtOAc (1:10) to afford pure **43** (0.389 g, 56%): IR (TF) 3200 (br), 1770, 1650, 1515 cm^{-1}; ^{1}H NMR (20% DMSO-d_6/$CDCl_3$) δ 1.10–1.25 (m, 2H), 1.26–1.41 (m containing a s at 1.34, 11H), 1.42–1.71 (m, 4H), 2.40–2.65 (m, 6H), 2.86 (q, J = 6.3 Hz, 2H), 3.59 (m, obscured by residual solvent, 2H), 3.80–3.88 (m, 1H), 4.86 (s, 2H), 5.35–5.45 (m containing s at 5.41), 7.11 (br, 1H), 5.48 (d, J = 7.8 Hz, 1H), 6.74 (t, J = 5.4 Hz, 1H), 7.20–7.45 (m, 10H), 7.70 (d, J = 9 Hz, 2H), 8.21 (d, J = 8.7 Hz, 2H), 8.64 (d, J = 10.8 Hz, 1H, NH), 9.11 (d, J = 10.8 Hz, 1H, NH); ^{13}C NMR (20% DMSO-d_6/$CDCl_3$) δ 21.1, 23.5, 26.3, 27.3, 28.4, 29.2, 29.6, 30.9, 40.0, 44.7 (m), 52.1, 56.6, 57.8, 65.9, 75.6, 77.5, 79.2, 122.8, 123.6, 123.75, 127.4 127.9, 128.5, 128.6, 128.8, 128.9, 129.1, 129.4, 134.9, 138.1, 143.0, 147.3, 155.7, 160.1, 165.9, 170.8,

171.6, 173.5; MS (FAB) m/z 742 (MH^+). Anal. Calcd for $C_{44}H_{51}N_6O_{11}Cl$: C, 60.37; H, 5.87; N, 9.60. Found: C, 60.43, H, 6.17, N, 9.32.

Deprotection of 43 to Lorabid Derivative 45. Following the procedure described for the synthesis of **44**, a solution of **43** (0.093 g, 0.106 mmol) in 1 mL of 5% aqueous DMF was exposed to hydrogen for 24 h in the presence of 27.5 μL (0.319 mmol, 3.0 equiv) of concentrated HCl and 0.019 g (20% w/w) of 10% Pd/C. Workup, as for **44**, furnished **45** quantitatively as a light amber semisolid mass: $FeCl_3$ positive (red-purple); IR (neat) 3500–3000 (br), 1775, 1660 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.18–2.12 (m, 8H), 2.34–2.98 (m, 6H), 3.12–3.26 (m, 2H), 3.51–3.67 (m, 2H), 3.85–3.95 (m, 1H), 5.19–5.41 (m, 2H), 7.20–7.45 (m, 5H), 8.12–9.22 (m); ^{13}C NMR (CD_3OD) δ 21.0, 22.7, 24.7, 27.1, 28.4, 29.1, 30.5, 31.7 (allylic), 40.0, 53.7, 55.1, 56.3, (multiplet containing major peaks at 128.9, 129.3, 129.7), 131.4, 138.1, 140.1, 158.2, 164.2, 164.6, 165.3, 173.2, 174.2; HRMS (FAB) calcd for $C_{25}H_{33}N_5O_7Cl$ (MH^+) 550.2069, found 550.2067.

Succinate Analog of Arthrobactin (47). The TFA salt of the corresponding amine obtained from the brief exposure of **41a** (97 mg, 0.23 mmol) to TFA in anhydrous CH_2Cl_2 (1:1) as before was dissolved in $CHCl_3$ and washed once with saturated $NaHCO_3$ to generate the free amine. The amine solution in $CHCl_3$ was dried over K_2CO_3 and filtered immediately into a solution of half active ester **31** (137 mg, 0.23 mmol) in CH_3CN (50 mL) and stirred overnight at rt. The volatile components were removed and the reaction mixture was dissolved in EtOAc and washed with a 10% Na_2CO_3 solution to remove the phenolic byproduct. Drying and removal of solvent afforded the crude desired product as an oil which was purified by flash chromatography using 30% EtOAc/hexanes as the eluent to provide **46** as a foam (160 mg, 89%): IR (neat) 3200–2900 (br), 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18–1.32 (m, 4H), 1.36–1.50 (m containing a s at 1.39, 13H), 1.52-1.64 (m, 4H), 2.03 (s, 3H), 2.45–2.73 (m, 8H), 3.14 (q, J = 6.4 Hz, 4H), 3.59 (br t containing a s at 3.62, 7H), 4.76 (s, 2H), 4.80 (s, 2H), 5.48 (s, 1H), 6.85 (q, J = 5.7 Hz), 7.33 (br s, 10H); ^{13}C NMR ($CDCl_3$) δ 20.3, 23.7, 26.2, 26.2, 27.1, 27.5, 28.2, 28.6, 38.9, 43.4, 43.6, 44.7 (m), 51.6, 73.9, 76.03, 82.2, 128.5, 128.7, 128.9, 134.2, 169.6, 169.7, 172.1, 172.4, 173.0, 173.3; HRMS (FAB) calcd for $C_{41}H_{61}N_4O_{11}$ (MH^+) 785.4337, found 785.4326.

Fully protected siderophore **46** was exposed to TFA/CH_2Cl_2 (1:1) to generate the free acid as in the case of arthrobactin. A suspension of 10% Pd/C (32 mg) in THF/water (2:1, 9 mL) and partially protected siderophore was hydrogenated at atmospheric pressure for 3 h. The catalyst was removed by filtration and the solvent was evaporated. The residue was partioned between EtOAc/water (1:1, 10 mL). The aqueous phase was separated and lyophilized to provide **47** as a yellowish oil: $FeCl_3$ positive (purple); ^1H NMR (CD_3OD) δ 1.10–1.30 (m, 4H), 1.32–1.62 (m, 8H), 2.03 (s, 3H), 2.40–2.70 (m, 8H), 3.11 (br t, 4H), 3.55 (br t, containing s at 3.61, 7H); MS (FAB) m/z 549 (MH^+).

Protected Carbacephalosporin Conjugate 48. The TFA salt of the amine obtained from **42** (0.086. g, 0.116 mmol) after treatment with TFA in anhydrous CH_2Cl_2 (1:1) as before was dissolved in $CHCl_3$ and washed once with saturated $NaHCO_3$. The $CHCl_3$ layer was dried over K_2CO_3, filtered immediately into a solution of the half active ester **31** (0.070 g, 0.116 mmol) in CH_3CN (50 mL), and stirred overnight at rt. The volatile components were removed and the reaction mixture was dissolved in EtOAc and washed with 10% Na_2CO_3 solution to remove the phenolic byproduct. Drying and removal of solvent afforded the desired product as an oil which was purified through preparative layer chromatography using MeOH/EtOAc (1:10) as the eluent to provide **48** as a foam (117 mg, 91%): IR (TF) 3200–2900 (br), 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20–2.00 (m containing s at 1.42, 23H), 2.08 (s, 2H, 3H), 2.40–2.85 (m, 10H), 3.10–3.25 (m, 4H), 3.28–3.48 (m, 4H), 3.75 (m, 1H), 4.80 (s, 2H), 4.86 (s, 2H), 5.22–5.38 (m, 3H), 6.64 (br t, 1H), 6.86 (br t, 1H), 7.40 (m, 10 H), 7.60 (m, 2H), 8.21 (m, 2H); ^1H NMR (20% DMSO-d_6/$CDCl_3$) δ 1.20–1.35 (m, 4H), 1.36–1.52 (m containing a s at 1.42, 13H), 1.53–1.66 (m, 4H), 1.80–2.00 (m, 2H, 2.04 (s, 3H), 2.45–2.78 (m, 10H), 3.08 (q, J = 6.3 Hz, 4H), 3.59 (t, J = 6.0 Hz, 4H), 3.88 (m, 1H), 4.83 (s, 2H), 4.86 (s, 2H), 5.22–5.38 (m containing 2d, AB coupling, 3H), 5.66 (s, 1H), 7.38 (br s, 10H), 7.67 (d containing br, J = 9.0 Hz, 4H), 8.19 (d, J = 9.0 Hz, 2H), 8.69 (d, J = 8.1 Hz, 1H); ^{13}C NMR (20% DMSO-d_6/$CDCl_3$) δ 20.1, 212, 23.4, 26.0, 26.9, 27.3, 28.4, 29.2, 31.1, 38.3, 43.2, 44.5, (m), 51.9, 57.9, 65.4, 73.7, 75.4, 75.5, 80.5, 80.6, 122.6, 123.1, 123.2, 128.1, 128.3, 128.3, 128.8, 128.8, 129.2, 134.2, 142.3, 147.0, 159.6, 165.7, 169.3, 171.1, 172.0, 172.1, 172.5. Anal. Calcd for $C_{58}H_{70}N_7O_{15}Cl$: C, 59.8; H,6.39; N, 8.88. Found: C, 59.66; H, 6.58; N, 8.85.

Deprotection of 48 To Give Carbacephalosporin Conjugate 49. To a solution of **48** (0.078 g, 0.071 mmol) in 1 mL of 5% aqueous DMF (prepared from deionized distilled water and HPLC-grade DMF) were added 18.2 μL (0.212 mmol, 3.0 equiv) of concentrated HCl and 0.016 g (20% w/w) of 10% Pd/C. This mixture was exposed to hydrogen for 24 h at atmospheric pressure followed by filtration of the catalyst. The DMF–water was removed by evaporation under vacuum. The residue was dissolved in deionized distilled water/EtOAc (1:1, 10 mL). The separated aqueous phase was lyophilized and the residue was eluted on a short column of LH-20 Sephadex (3 g) with 10% MeOH/EtOAc to give **49** as a light amber semisolid in near quantitative yield: $FeCl_3$ positive (red-purple); IR (neat) 3500–3300 (br), 1780, 1730, 1660 (br) cm^{-1} ^1H NMR (300 MHz, CD_3OD) δ 1.19–1.39 (m, 4H), 1.40–1.81 (m, 10H), 2.07 (s, 3H), 2.41–3.10 (m, 10H), 3.07–3.19 (m, 4H), 3.56–3.70 (m, 4H), 3.85–3.95 (m, 1H), 5.35–5.48 (m, 1H), 8.99–9.20 (m); ^{13}C NMR (CD_3OD) δ 20.0, 20.1, 22.8, 24.8, 24.8, 27.2, 28.1, 28.9, 29.6, 31.5, 39.9, 43.9, 45.1, 53.8, 59.5, 74.5, 137.1, 139.2, 163.3, 165.3, 166.1, 171.1, 173.8, 177.1; MS (FAB) m/z 733 (MH^+).

Protected Lorabid Conjugate 50. Following the procedure described for the synthesis of **48**, a $CHCl_3$ solution of the free amine, obtained from **43** (0.145. g, 0.166 mmol) after treatment with TFA followed by $NaHCO_3$, was added to a solution of the half active ester (0.100 g, 0.166 mmol) in CH_3CN (50 mL) and stirred overnight at rt. Workup as before and purification through preparative layer chromatography using MeOH/EtOAc (1:10) as the eluent afforded **50** (178 mg, 87%) as a white foam: IR (TF) 3200–2900 (br), 1770 cm^{-1}; ^1H NMR (20% DMSO-d_6/$CDCl_3$) δ 1.16–1.32 (m, 4H), 1.34–1.48 (m containing a s at 1.41, 13H), 1.50–1.66 (m, 5H), 2.04 (s, 3H), 2.45–2.78 (m, 10H), 3.08 (m, 4H), 3.59 (m, 4H), 3.75–3.85 (m, 1H), 4.81 (s, 4H), 5.22–5.42 (m, 3H), 5.58 (d, J = 7.3 Hz, 1H), 5.71 (s, 1H), 7.38 (br m, 15H), 7.65 (d overlapping a br peak, J = 9.0 Hz, 4H), 8.19 (d, 2H, J = 8.4 Hz), 8.45 (br s, 1H), 9.10 (br s, 1H); ^{13}C NMR (20% DMSO-d_6/$CDCl_3$) δ 20.0, 20.8, 23.4, 23.4, 25.9, 26.0 27.3, 28.3, 28.4, 29.6, 31.1, 38.4, 43.2, 44.5 (m), 52.0, 56.6, 57.9, 65.5, 73.7, 80.7, 80.8, 122.6, 123.0, 123.2, 127.1, 127.4, 128.0, 128.1, 128.3, 128.7, 128.7, 129.3, 129.4, 134.2, 137.5, 142.2, 147.1, 159.6, 164.9, 169.3, 170.7, 171.2, 171.5, 172.1, 173.2; HRMS (FAB) calcd for $C_{63}H_{78}N_8O_{16}Cl$ (MH^+) 1237.5224, found 1237.5274. Anal. Calcd for $C_{63}H_{77}N_8O_{16}Cl.H_2O$: C, 60.26; H, 6.35; N, 8.93. Found: C, 60.46, H, 6.41, N, 9.00.

Deprotection of 50 To Give Lorabid Conjugate 51. Following the procedure described for the synthesis of **49**, a solution of **50** (150 mg, 0.121 mmol) in 1 mL of 5% aqueous DMF was hydrogenated in the presence of 0.030 g (20% w/w) of 10% Pd/C and 31.4 μL (0.364 mmol, 3.0 equiv) of concentrated HCl for 24 h. Usual workup, as in the case of **49**, followed by elution on a short column of LH-20 Sephadex (5 g) with 10% MeOH/EtOAc provided **51** as a light amber semisolid in near quantitative yield: $FeCl_3$ positive (red-purple); IR (neat) 3600–3200 (br), 1780, 1730, 1660 (br) cm^{-1}; ^1H NMR (CD_3OD) δ 1.22–1.38 (m, 4H), 1.40–1.80 (m, 10H), 2.08 (s, 3H), 2.45–3.00 (m, 10H), 3.08–3.20 (m, 4H), 3.55–3.65 (br t, J = 6.1 Hz, 4H), 3.82–3.92 (m, 1H), 5.30–5.50 (m, 2H), 7.22–7.45 (m, 5H), 7.85–9.00 (m); ^{13}C NMR (CD_3OD) δ 20.3, 21.0, 22.9, 24.8, 24.9, 27.2, 28.2, 28.9, 29.8, 31.7, 40.1, 45.0, 45.6, 53.9, 59.2, 59.6, 74.5, (multiplet containing major peaks at 129.0, 129.8, 130.5, 131.5), 136.1, 138.2, 163.5, 165.3, 166.1, 171.8, 173.8, 174.3, 177.1; MS (FAB) m/z 866 (MH^+).

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Supplementary Material Available: Copies of ^1H and ^{13}C NMR spectra of **8**, **26**, **30**, **31**, **38**, **39**, **41a/b**, **42–46**, **49** and **51** and copies of ^1H NMR spectra of **5** and **47** (35 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.